TERPENE COMPOSITION OF ESSENTIAL OILS

by

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requirements for the degree of

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ABSTRACT

A review is given of techniques for sampling, extraction, separation and analysis of essential oils, and the effects of each upon the finally determined essential oil composition. It is concluded that presently-available techniques such as steam-distillation, solvent extraction, vapour trapping and even solid-sampling gas chromatographic injection procedures impose so many characteristic effects upon the volatile terpenoids in a plant sample, that it is not practicable to obtain instantaneous measurement of volatiles as they are released to the atmosphere. In addition, many of the well-known techniques and approaches to essential oil analysis lead to many losses and artifacts, and are so protracted as to mitigate against the feasibility of a routine analytical procedure for use in a survey of essential oils.

A recommended routine analytical procedure was developed for use in surveys of essential oils, and its effectiveness is illustrated by the analysis of essential oils from 7 endemic Tasmanian plants.

Details are given of the development of syringe-headspace gas chromatographic analysis, which is a novel method of identifying and monitoring components in vapours from comminuted plant tissue as they are released to the atmosphere at room temperature. The syringe-headspace technique is recommended as a means of directly using verified plant species from a botanical gardens as a source of

reference terpenes, which otherwise may be too unstable to be stored as pure compounds. This technique allows the relative retention time of an authentic terpenoid to be measured in plant material for direct comparison with that of an unidentified compound. It thereby enables the worker to justify spending considerable effort in synthesizing, isolating or purchasing the suspected terpenoid.

Successive injections of vapour from a single sample of comminuted foliage, by the syringe-headspace technique, often exhibit changes in the proportions of some components with respect to one another. Such changes were observed between terpenoids having a common hypothetical precursor according to the biosynthetic scheme by Ruzicka. This technique is a novel means of directly measuring biosynthetic changes. It considerably extends the usefulness of Zavarin's earlier advocated procedure for utilizing 'quantitative co-occurrence', which is a basically different procedure to supplement the results of tracer studies.

Both the routine analytical procedure for analysis of essential oils, and the syringe-headspace technique were used to compare the compositions of terpenoids in 19 species of conifer susceptible to attack by the Woodwasp, *Sirex noctilio*. An investigation was also made of earlier inferred changes in the compositions of essential oils following the wounding of trees of *Pinus radiata*. Widely varying compositions are reported for the first time for oils distilled from bark of felled trees. Variations in oil composition from a single

injured tree trunk ranged from 15.7 to 20.3 percent α-pinene,

54.8 to 68.2 percent β-pinene, 9.0 to 18.3 percent limonene

and 2.5 to 6.7 percent myrcene. The range of compositions

of oils from within and between trees of a species was very

wide, so that in a comparison of each species, no single

insect-attractive optimum composition could be envisaged.

It appeared more likely that *S. noctilio* could be attracted to

one of the 'temporarily-released' components which appeared to

cause qualitative changes in many oils.

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I

INTRODUCTION

Presently-used methods for the detailed examination of an essential oil are often so protracted as to mitigate against the feasibility of a routine analytical procedure for use in a survey of essential oils. In addition, many of the techniques introduce characteristic features, including artifacts, which affect the finally determined oil composition.

To undertake a survey of Tasmanian essential oil-bearing plants a study is first required of the characteristics of each presently-used technique, which might impose some feature upon an oil composition. From those techniques considered less likely to impose a deleterious effect upon an oil, a recommended procedure should be developed, which would have general use for a large number of oils. This concept is contrary to the view that for each oil there must be a particular series of techniques.

A comparative study of the attractiveness of a number of conifers attacked by the Woodwasp, *Sirex noctilio*, would also benefit from the development of a routine analytical procedure. However when searching for an insect attractant it must be remembered that the compound the insect detects will be among the broad mixture of vapours released to the atmosphere. Analyses of solvent extracts or even steam-distillate may not yield this component because of the limitations of each of these techniques.

A technique is therefore required which will enable the identification and measurement of components as they are released to the atmosphere from plant tissues. By such a procedure it may also be possible to detect any 'temporarily-released' component, which might be the insect attractant that renders a tree attractive for a specific period. Madden has shown that upon felling a *Pinus radiata* it immediately becomes attractive and remains so for about 14 days. This worker has postulated the existence of changes in oil composition following various degrees of tree injury.

In the search for an attractive oil vapour composition from *P. radiata* and other conifers, an examination should first be made of any changes in oil composition that might be induced by wounding a tree. This concept is almost unknown, although R. H. Smith has hinted at the possibility of changes following wounding during a naval stores operation upon *P. ponderosa*.

If, as postulated, changes in oil composition can be found in a single wounded tree, then an examination should also be made of any biosynthetic relationships between changes in the proportions of components. Earlier workers have used changes during plant maturation as corroborative evidence for a biosynthetic sequence. Should any changes be found then this would enable Zavarin's principle of quantitative co-occurrence to be immediately extended beyond the study of the naturally-occurring tree-to-tree variations.

CONCEPTS OF ESSENTIAL OIL COMPOSITION

The multitude of techniques that have been used for the analysis of essential oils has led researchers to often report compositions which differ considerably from the compositions of the oils as they exist, both in the extracted state and in living plant tissue. The techniques have appeared over many years and have usually been the best available when introduced. Many have been retained, together with increasing knowledge of their limitations, as arbitrary techniques suitable for specific purposes, e.g. quality control analyses of commercial oils and blends. Some workers have continued to use these earlier limited techniques, e.g. distillation at atmospheric pressure, and have as a result reported both quantitative and qualitative characteristics which cannot be confirmed by other analytical techniques. Similarly, other workers have unquestioningly accepted the earlier literature on essential oil compositions that have been determined using the then available techniques.

More recent techniques, e.g. gas chromatography (GC), have since been used by workers who have often reported essential oil compositions as if the techniques used were infallible. An increasing amount of literature is now appearing which indicates that many workers are ignorant of the limitations of recent sophisticated techniques. It would appear that the enormously-increased amount of information, that may be provided on essential oil compositions by sophisticated techniques, has

blinded many workers to the fact that these techniques may also provide misleading information, e.g. from losses and structural changes in catalytically and thermally unstable components.

A need therefore exists for an examination of the concepts of essential oil composition that result from the use of specific analytical techniques. A detailed study of each of the characteristic distorting influences on essential oil composition that has been reported for each analytical technique would possibly lead to a textbook. This discussion is only intended as an examination of some of the different concepts of essential oil composition, together with an assessment of their validity or use for current areas of essential oil research.

A.   Summary of Techniques for Extraction and Analysis of Essential Oils

The origin of the multiplicity of concepts of essential oil composition results partly from the loose definition of an essential oil. An essential oil is commonly defined [1] as a more or less volatile material extracted from an odorous plant of a single species by a physical process. Essential oils are necessarily obtained from plant and animal material by a variety of processes, biochemical as well as physical. Since an essential oil consists of a complex mixture of components with a wide range of volatilities, each extraction technique may be expected to influence the final composition. The description of the composition of an essential oil is complicated even further when more than one extraction technique may be used with a particular plant species.

It is generally recognized that there are numerous techniques for essential oil extraction, separation and isolation of oil components, and qualitative and quantitative analysis, all of which may influence the determination of the oil composition. However, there are also some techniques preceding the extraction step which must be considered.

Pre-extraction techniques that influence essential oil composition are mostly related to the harvesting process, which for many plants involves fermentation. For a number of other plants no direct fermentation is required to release the essential oil, but there would appear to be enzymatic processes which must be controlled during harvest to both increase oil yield and ensure that the qualitative nature of the oil does not change. Other pre-extraction techniques involve comminution conditions, autoxidation, physiological changes associated with extraction steps, e.g. enfleurage and maceration, storage and drying time of plant material, and effects due to light and time of day when plants are harvested. There is also evidence that sampling of the same plant material from different plants and parts of the plant may sometimes lead to widely different essential oil compositions.

Essential oils have been extracted by dry- and steam-distillation of tree stumps after industrial processing; dry- and steam-distillation of cortical oleoresin obtained by hand-picking, by collection from a stimulated or naturally-flowing open-face cut, or by trapping in sealed vessels inserted into the cortex; by enfleurage, maceration, expression or solvent-extraction of various plant tissues; directly from oil glands

and by solid-sample injection of tissues into a gas chromatograph; and by various methods of distilling foliage and flowers including micro-distillation of individual leaves, steam-distillation of water and oil from a single flask, conventional and dry steam-distillation, and distillation in a cohobation still. The volatilized oil in the atmosphere surrounding plant tissue has been trapped by absorption on an activated material, e.g. carbon; condensing from the air passed through a cold trap; absorbing in non-volatile solvent materials, such as purified fat or GC liquid phase; and by directly sampling headspace vapours in a gas-tight GC syringe, sample loop or cooled pre-column.

Techniques for the separation and isolation of essential oil components have included simple and fractional distillation at atmospheric and reduced pressures, molecular distillation, column and paper chromatography, thin-layer chromatography (TLC), crystallization, liquid-liquid partitioning, acid-neutral-base extraction, GC with packed and capillary columns, and coupled GC-TLC. Numerous other GC techniques have been used, e.g. cooled pre-columns for concentrating trace components, stream-switched auxiliary columns, irreversibly-absorbing column sections, reaction-GC and preparative-GC.

Isolated essential oil components have been characterized by such physical measurements as specific gravity, refractive index, optical rotation, boiling point, solubility and specific absorptions in the ultraviolet (UV) and infrared (IR). These simple measurement techniques could not be expected to lead to any distortion of the finally determined essential oil

composition. By comparison, more sophisticated qualitative techniques which could lead to misleading data, largely through sample-handling or transfer difficulties, include: GC measurements on packed and capillary columns, organoleptic tests, and studies of directly-measured and collected micro-sized fractions by nuclear magnetic resonance (NMR), optical rotatory dispersion (ORD), UV, IR, mass spectrometry (MS), electron paramagnetic resonance (EPR), and measurements of melting point, boiling point, refractive index, optical rotation and specific gravity.

The quantitative composition of an essential oil is determined primarily by GC. Some arbitrary colorimetric and spectrophotometric measurements of the products of various functional group reactions are still used, particularly in industrial applications. These latter arbitrary techniques, which were used before GC methods became available, have provided very precise but not necessarily accurate determinations of esters, acids, alcohols, ketones, lactones and aldehydes. They still form the basis for standardization of quantitative analysis in the essential oil industry.

B.   The effect of various techniques on the composition of
      an essential oil

1.   Pre-extraction techniques

Most of the pre-extraction techniques that influence essential oil composition are features of the commercial harvesting process. Other techniques involved in the sampling of limited amounts of plant material, for the extraction of

essential oils for research purposes, may also lead to differences
in oil composition. The different effects on the composition of
essential oils resulting from the natural variability of plant
material, sampled between and within individual plants, is
discussed separately.

Numerous reports, of the influence of fermentation and
various conditions for comminution and storage of harvested plant
material, would suggest that fermentation and enzymatic processes
are responsible for changes in the composition of essential oils
from a larger number of plants than previously envisaged.
Fermentation and enzymatic processes had earlier been thought
of as being peculiar to such plant materials as bitter almond
and sweet birch, which required enzyme hydrolysis to liberate
benzaldehyde and methyl salicylate, respectively, from the
odourless glycosides. Many of the older techniques for subtly
controlling essential oil odour and yield from certain flowers,
e.g. storage of rose petals in water at 15-18°C, are now
regarded as conditions for the control of fermentation and
enzyme activity.

(i) Fermentation and enzymatic effects

Essential oils are closely related to the characteristic
volatile flavour components in fruits and other vegetable
materials [2], in that they result from enzymatic action upon
other complex components during the maturing stages of plants.
In vitro studies have demonstrated that enzymes may liberate
volatile flavour components from specific precursors [3-5].
Unless enzyme activity is arrested, as in food processing,

less desirable flavour materials may also be generated [6].
In some plants however, the characteristic odour does not
appear until enzymes are released during injury to plant
tissue, e.g. in the genus *Allium* (onion, garlic, leek, chive)
[7]. Special fermentation procedures, e.g. *Aspergillus*
fermentation [8] and fermentation with acid hydrolysis [9],
have also been applied to obtain an additional yield of
essential oil from exhaustively-extracted rose petals.

Fermentation and enzyme activity have been employed
to not only increase the yield of essential oil [8-12], but
have also been found to alter the concentrations of specific
terpene components [13-15]. The concentrations of terpene
alcohols, such as phenethyl alcohol, have been found to
increase during fermentation of rose petals [16]. Tanasienko
et al. [17, 18] have studied the change in terpene alcohol
concentration during the time of fermentation and with
pretreatment of the petals, while others have described the
optimum fermentation conditions that produce oils having higher
terpene alcohol content [19] and 'better odour' [20]. Enzyme
action has been concluded to be the reason for lactarazulene,
verdazulene and other components being found only in the oil
from stored, rather than fresh fruit of *Lactaricus deliciosus*
[21]. Kepner and Maarse [22] have also noted that enzymes may
have been responsible for terpene artifacts as a result of
injury to tissues during processing of Douglas fir needle
samples. 2-Hexenal and cis-3-hexenol isolated by Pauly and

von Rudloff [67] in leaf oil of *Pinus contorta* var *latifolia*,
were similarly thought to be artifacts, formed during the
cutting of the leaves to obtain a high yield of oil. 2-Hexenal
found in Douglas fir needles was similarly described as a
possible artifact by Sakai *et al.* [56], after the suggestion
of Major *et al.* [68], who observed its formation when leaves
of *Ginkgo biloba* were ground in an air atmosphere.

(ii) Effects due to handling of plant material, comminution,
     storage, drying and autoxidation

· The handling of the plant material before oil extraction
is known to affect the final composition because of interrelated
features of comminution, plant storage, terpene autoxidation
and fermentation. When comminution or tissue damage is begun,
oil is released from the glands and becomes exposed to both
fermentation and air, which increases the rate of autoxidation
[1]. Comminution and storage times therefore require close
control.

Storage of plant material, with varying degrees of
tissue damage and oil release, has been shown to result in
changes (increases and decreases) in oil content [23-28] and
composition [23, 24, 26, 27, 29, 30]. Typical of the changes
which have been reported was the loss of cis-2-hexenol and its
acetate during refrigerated storage of green tea leaves [31].
Drying has similarly been found to result in changes in yield
and composition of oils [15, 25, 31-34].

Autoxidation may lead to off-flavour changes in
essential oil composition in both stored plant material as well

as extracted oils, particularly where tissue damage during harvesting has exposed oils to the air. The terpene hydrocarbons are readily autoxidizable, giving peroxides with unpleasant flavours that lead to deterioration of the whole oil [1]. Sato [35] has studied the oxidation of many essential oils including lemon, caraway and lavender. Aromatic waters were shown during storage to diminish in their concentrations of limonene, linalool and menthol [36]. Infrared studies by Guenther have clearly indicated the oxidation products of deteriorated lemon oil, such as trans-carveol, carvone and limonene epoxide [37].

(iii) **Effect of time of harvesting on composition and**
   oil content

Changes during the course of the day in the yield and composition of essential oils in growing plants has led a number of workers to investigate the best time of day for harvesting [38-40]. Lozzi found that oil content in rose blossoms decreased by 50 percent during one hour after they were freshly opened [41]. Baslas [42] recommended harvesting of *Mentha arvensis* plants on a sunny day during the flowering season for high oil yield and menthol content, while Myint and Gale [43] concluded that the highest yield resulted if plants were cut at 10 a.m.

(iv)   Effects dur to post-harvesting physiological activity

Continuation of physiological activity, and production

of oil after harvesting of flowers partly led to the earlier

introduction of particular extraction processes. Enfleurage

was introduced in Grasse (France) by growers who hoped to yield

further amounts of oil from e.g. jasmin and tuberose, which

could be allowed to continue physiological activity for up to

several days while the petals were immersed in a fat chassis.

Steam distillation would otherwise have resulted in poor yields

of oils which would have undergone compositional changes at the

temperature of boiling water. Maceration has been used to

extract oils from violet, rose, lily of the valley and other

plants whose physiological activity ceases rapidly after

harvesting. The petals in this case are quickly extracted

within an hour or two in molten fat. Rose petals are well

known for their tendency to otherwise soon begin fermenting,

and lead to changes in oil composition [1].

(v)   Factors influencing composition of food flavour

       volatiles

The compositions of food flavour volatiles are

particularly susceptible to the steps in food processing

preceding final extraction. The much larger quantities of

food which must be extracted, to yield a minute amount of

flavour concentrate, usually require considerable repetition

of extraction processes, with consequently greater risk of

introducing artifacts and off-aromas from heat damage and

air oxidation. Other interfering contaminants in the flavour

concentrate may be introduced from solvents, containers, tubing, lubricants, etc. [44]. Such a complicated extraction was undertaken by Teranishi *et al.* [45], who yielded 50 ml of isopentane flavour solution from 10 tons of condensate taken from strawberry jam pot stills. The only presently conceivable means of overcoming such interferences and artifact formation would appear to be direct GC analysis of headspace vapours emitted from foods [46].

(vi) Effect of unknown factors upon oil composition

The compositions of oils are subject to variations from one harvest to the next due to the influence of unknown factors. Commercial growers endeavour to maintain harvesting and extraction conditions as uniform as possible, yet considerable variations still occur with some essential oils. Similar variations are experienced from one producer to the other. Table 1 [1] is a list of constituents of bergamot oil, together with minimum, maximum and average percentages in fifty samples.

2.   Extraction techniques

Numerous techniques have been used in order to present essential oils ready for analysis. Many of the techniques impose some characteristic feature upon the final quantitative and/or qualitative analytical data.

Most essential oils have been extracted directly from biological material, but an increasing number of reports

Table 1. The variation in percentage of constituents in 50 samples of bergamot oil. Such differences in composition, due to unknown factors, may be found in oils from one harvest to the next or one producer to the other [1]

	Minimum	Maximum	Average
α -pinene	1.005	1.798	1.387
camphene	0.027	0.099	0.054
β-pinene + sabinene (4:1)	5.266	11.861	7.697
myrcene (alloocimene)	0.616	1.320	0.933
α-phellandrene		0.091	0.062
Δ$_3$-carene	0.096	0.233	0.167
α-limonene	19.411	34.822	28.355
p-cymene	0.399	1.676	0.765
γ-terpinene	4.723	11.758	7.651
terpinolene	0.317	0.830	0.494
octyl acetate		0.121	0.074
aldehyde C$_9$	0.067	0.176	0.180
methyl glycollate	0.066	0.226	0.108
citronellol	0.036	0.163	0.075
linalool	7.070	29.120	16.457
aldehyde C$_{10}$	0.418	0.690	0.542
terpinene-4-ol	0.046	0.093	0.068
dehydrolinalool	0.143	0.200	0.174
linalyl acetate	23.755	35.624	29.726
neral	0.453	0.641	0.532
α-terpineol ,	0.245	0.430	0.309
decyl acetate	0.062	0.156	0.097
geranial	0.472	0.710	0.595
ethyl glycollate		0.429	0.238
terpenyl acetate	0.175	0.537	0.384
citronellol	0.246	0.607	0.341
neryl acetate	0.442	1.189	0.718
geranyl acetate	0.322	0.825	0.525
bergamottene	0.039	0.156	0.077
caryophyllene	0.215	0.408	0.286
humulene	0.161	0.395	0.239
bisabolene	0.094	0.295	0.173
bisabolene isomer	0.473	0.857	0.649

are appearing of analyses of essential oils that have been
sampled from the atmosphere. From a consideration of the
volatilities of essential oil components and the nature of
the biological substrate, it is expected that there would
be basic differences between the composition of an essential
oil in the liquid and vapour states associated with a single
plant species. Further compositional differences may also be
found when considering individual methods of isolating each of
these types of essential oil.

(i) Extraction from biological sources

The analysis of essential oils has been based for
many years on the assumption that an oil should first be
isolated in a purified form from the biological source material.
Recent GC techniques have shown the feasibility of analyzing
some oils directly from plant material. The latter approach
was developed in an attempt to avoid some of the well known
changes in oil composition that result from techniques in
isolating the oil. However direct-GC extraction and analysis
may also be shown to involve some characteristic features,
which alter the composition of the oil as it exists in the
living biological material.

Food flavours may also be considered with more
conventionally-known essential oils, since their analyzed
composition may be affected because of the approach taken
in extracting flavour components. Flavours consist of
terpenoids and other volatiles, which may be analyzed either
directly in the original biological material or following
isolation.

(a) Isolation of essential oil preceding analysis

Extraction of essential oils by the major basic·
techniques in each case involves some disproportionate
isolation of oil components, i.e. whether extracted from
collected-oleoresin, by distillation, solvent extraction,
direct physical removal from oil glands or even micro-scale
isolation and collection on a GC pre-column. Further changes
in composition may subsequently occur in the period between
isolating the essential oil and analysis.

Oleoresin collection

Methods of collecting oleoresin, for subsequent
isolation of essential oil or direct analysis, have been
considered to involve losses due to vapourization of more
volatile terpénes [47]. Oleoresin is obtained from trunks of
Pinus species by either collecting drips from an open-face
gash into an unsealed receptacle or by inserting an air-tight
tube into a hole drilled into the bark [47-49]. Even though
Mirov [47] recognized that the open-face technique allowed
evaporation and loss of compounds possessing a high vapour
pressure, he and fellow collaborators chose to follow this
approach because most turpentines were at that time obtained
by this technique. Smith, however, has preferred collection
of oleoresin with a closed-face microtap [50].

Bannister *et al* [51] noted, in his work, that
oleoresin exuded from wood was exposed to sunlight and the
atmosphere for varying periods before final sealing and
refrigeration. The compositions were subsequently regarded

as only accurate for the samples at the time of analysis in the laboratory, and could not be accepted as estimates of the composition of the volatile fractions which actually emerged from the trees.

Although loss of terpenes, due to their volatility, has been considered to be the principal disadvantage of oleoresin collection, other factors also appear to influence the finally determined essential oil composition. Bannister *et al* [51] noted that of the two terpenes, α- and β-pinene, the former had the higher vapour pressure and would therefore have been expected to be lost more rapidly from an exposed oleoresin. However it was found by these workers that the ratio of α- to β-pinene had in fact increased in older exposed oleoresins from pine trees (Table 2).

Table 2. Percentage composition of volatile fractions of oleoresins from two trees [51]

Tree No.	Sample	α-Pinene	β-Pinene	Δ_3-Carene	Limonene	Unidentified
1	(a) oleoresin from 4 month old wound	58.0	42.0	–	trace	–
	(b) oleoresin from freshly-opened wound	52.5	46.0	–	1.5	–
2	(a)	35.5	64.5	–	trace	trace
	(b)	22.5	71.0	trace	4.5	2.0
	oleoresin from freshly-opened bark blisters	18.5	72.0	2.5	7.0	trace

In conifers other than *Pinus* cortical oleoresin
has been commonly collected from bark blisters and even by
tapping exposed resin ducts [52]. Resin ducts of *Abies*
have been tapped by removing the outermost layers of tissue
with a razor blade, allowing a small amount of oleoresin to
flow. It is usually not practicable to obtain a flow of
oleoresin as found with *Pinus* species.

Analysis of oleoresin from *Pinus* bark blisters has
rarely been reported. It would appear from Table 2 [51]
that the terpene composition may differ considerably from
that of freshly exuded oleoresin.

Zavarin and others have based much of their
chemotaxonomic studies of the genus *Abies* upon the analysis
of terpenes in balsams collected from bark blisters. Balsam
was collected by perforating the upper part of a blister,
containing up to a few ml of honey-like mixture of terpenes,
sesquiterpenes, resin acids, fatty acids both free and as
glycerides, and neutral non-volatiles. Pressure under the
perforation assisted the balsam to flow directly into a vial
containing a few crystals of pyrogallol [53].

No information could be found in the literature
which would have indicated whether terpenes in balsams have
undergone changes during storage or whether bark blister
terpenes are freshly synthesized.

Distillation

Distillation has been used for extraction from
oleoresin or plant material provided the essential oil is
stable enough to prevent the formation of alteration products.
If the stability of the oil is high, it may be extracted by
distillation with water, steam or dry steam. In some
circumstances a less stable oil may be steam distilled at
reduced pressure [1]. No form of distillation is suitable
for the extraction of many delicate oils without the formation
of alteration products. Solvent extraction is usually used
for such oils.

Although some form of steam distillation has been
used for the extraction of essential oils from earliest times,
Mirov's monumental work with *Pinus* turpentines [47] was
undertaken using distillation of oleoresin at reduced pressure,
because it was believed that steam distillation led to the
isolation of turpentines having a different composition. It
was considered that high-boiling compounds such as sesquiterpene
derivatives or diterpenes were frequently not removed from
oleoresin by steam distillation. A time of 3 to 4 hours was
considered necessary to expel most of the turpentine, and that a
much longer and impracticable time would be needed to distil
off the high-boiling compounds.

Mirov [47] usually distilled batches of about 1½ l
of oleoresin from which most of the turpentine was recovered
when conditions had reached ~ 200° at 0.5 to 1.0 mm.

Many *Pinus* oleoresins have since been found to
contain highly volatile hydrocarbons, such as n-heptane, which

could be expected to be lost during distillation, particularly
at reduced pressure. Mirov [47] advised extreme caution when
extracting turpentine from the 8 or so species and
hybrids recognized to contain n-heptane. The distillation
procedure was then made more complex by the introduction of
a second dry ice trap together with dry ice cooling of the
receiver.

Several workers have drawn attention to advantages,
losses of volatiles and the formation of artifacts that result
from distillation at reduced pressure. Wilson compared the
composition of celery oils produced by distillation and thin
film evaporation and found quantitative but no qualitative
differences, with the latter oil having an apparently fresher
flavour [54, 55]. Sakai *et al* [56] did not find all of the
compounds reported in Guenther's summary of early work on Douglas
fir, and considered that some of the reported compounds were
artifacts resulting from the classical isolation and fractional
distillation processes. The camphor found earlier in oil of
cedar (*Thuja occidentalis*) by Wallach [57-60] was similarly
considered by von Rudloff [61] to be an artifact. Karlsen [62]
noted as a general statement that direct heating of biological
material or dry extracts may produce artifacts, yet distillation
was still a useful procedure, since artifacts did not necessarily
interfere with qualitative and quantitative studies of certain
oil components.

Improvements to the earlier reduced-pressure
distillation, in the form of molecular distillation [63, 64]

and thin-film rotary evaporation [65] are now widely accepted
methods of extracting essential oils relatively free of
artifacts. Smith [66] quantitatively compared *Pinus
ponderosa* turpentines extracted from oleoresin samples in a
Hickman molecular still and as ether solutions, and found
approximately the same monoterpene composition for each type
of preparation (Table 3).

Steam distillation is possibly the most commonly
used method of extracting essential oils, and as a result
its effects upon composition have been closely studied.
The method is well known for its greater efficiency for
extracting more volatile essential oil components [47].
Although the bulk of an essential oil is steam distilled and
collected within the first few minutes, depending upon the
nature of the biological material, the last few fractions to
be collected in a batch distillation have been found to contain
a higher proportion of less volatile compounds [17, 18].
Juhasz *et al* [92] extracted the residue after steam distilling
chamomile and reported nearly 0.5 percent of an oil containing
more alcohols and acids than the distilled oil. Von Rudloff
and Sood [69]' reported in their work on leaf oil of *Juniperus
communis,* that the yield of farnesol could be increased by
steam distillation of 800 g batches for a further 3-4 hours
beyond the 5-6 hours used to extract the bulk of the oil.

Steam distillation has also been reported to involve
losses of more volatile components due to their evaporation
during the process. Cermak *et al* [70] concluded in their work
on essential oils in seeds of *Abies* that evaporation of very

Table 3. Monoterpene composition of oleoresin of selected ponderosa pine prepared by ether extract (Ext.) and by molecular distillation (Dis.)

Tree no.	Preparation	Percentages of monoterpenes:						
		α-Pinene	α-Pinene	Δ_3-Carene	Myrcene	Limonene	β-Phellandrene	Unidentified
1	Dis.	5.9	21.0	37.6	19.6	13.2	1.2	1.4
	Ext.	5.4	19.8	38.6	17.3	14.6	1.9	2.4
2	Dis.	9.1	22.1	28.6	15.0	21.9	2.5	0.7
	Ext.	8.0	20.6	29.1	13.4	25.1	1.7	2.0
3	Dis.	11.8	49.2	–	16.5	18.7	3.8	–
	Ext.	10.8	52.8	–	14.9	19.2	2.3	–
4	Dis.	6.9	28.9	19.2	16.9	25.2	1.6	1.2
	Ext.	6.3	29.5	20.1	14.4	26.2	2.2	1.2
5	Dis.	5.6	21.4	26.5	24.1	19.1	1.8	1.6
	Ext.	5.2	21.4	28.2	21.8	20.2	2.0	1.1
6	Dis.	3.8	13.8	26.5	25.5	25.5	3.0	1.9
	Ext.	4.2	14.5	27.7	23.3	27.3	1.4	1.6
7	Dis.	4.4	16.9	52.7	9.5	11.7	2.0	2.8
	Ext.	3.4	15.7	55.5	9.0	12.6	1.1	2.8
8	Dis.	8.0	9.0	66.5	8.2	4.5	0.4	3.3
	Ext.	7.8	9.1	64.4	9.1	5.3	0.7	3.7

22.

volatile compounds during steam distillation led to oils
containing altered ratios of individual compounds. Although
many steam distillation systems may involve losses of volatiles,
an appropriately designed apparatus, with a liquid-air-cooled
cold finger in a closed system boiling at 40°, was shown to
be suitable for 70 to 100% recovery of 0.1 - 0.2 ml samples
of chlorinated derivatives of methane and ethane [73].
Similarly, a micro steam distillation apparatus has been
described for the extraction of essential oil in 0.1 - 1 g
samples of plant material [77].

The direct heating of biological material, as in
steam distillation, may be expected to produce artifacts
resulting from the alteration of some compounds [71].
Johnson and Cain [72] reported that the 0.07 percent salicylic
acid in steam distillate of leaves and twigs of Douglas fir
was probably a product of hydrolysis. Linalyl acetate and
linalool in clary, bigarade and Bergamot leaves were considered
during steam distillation to lead to the formation of racemic
linalool, d-α-terpineol, geraniol, nerol and ocimene [74, 75].
During steam distillation of the leaf oil of *Pinus pinaster* [76]
some alcohols, mainly α-terpineol and linalool seemed to be
formed, yet were absent when the leaf oil was obtained by
solvent extraction.

Heating and autoxidation effects were recently
considered to be the basis for reports of some norterpenoid
components in *Pinus* oils [307]. Other workers also reported
norditerpenes to be artifacts formed from the corresponding
4-axial aldehydes during isolation of oils, e.g. from the

autoxidation of pimara-8(14),15-dien-19-al [308] and torulosal

[309, 310]. Lu *et al* [307] confirmed that longifolene may

autoxidize to longicamphenylone and that nopinene was one of

four similarly-derived products from β-pinene. These workers

concluded that nopinene and longicamphenylone and

18-norpimara-8(14),15-dien-4-ol were very likely to be

artifacts during isolation of oil from *Pinus taiwanensis*.

The effects upon oil composition resulting from

steam distillation have been compared with the effects from

other methods of extracting an oil. Several workers have

compared oils obtained by steam distillation and solvent

extraction [56, 66, 70, 76, 78-80]. Solvent extracted oils

typically contained a higher proportion of less volatile and

even some non-volatile substances, e.g. mace extracted with

carbon dioxide yielded an oil containing 14.5 percent non-

volatile substances [79]. Other authors have compared and

reported quantitative differences between steam distilled oils

extracted directly from oil glands [81, 84], cold- or hand-pressed

oils [82, 83] and oils obtained by molecular distillation [63].

Various methods of recovering an essential oil from

the distilled waters have also been shown to lead to differences

in oil composition. From earliest times many workers have been

content to regard an oil as the material which is not miscible

with the aqueous distillate. Different types of glass receiving

apparatus have even been designed to collect oils which either

float on the water or have a specific gravity greater than that

of water. Other workers have used cohobation stills which

have yielded oils containing higher concentrations of water-soluble components than distilled oils [1].

Zavarin *et al* [85] were content to merely pipette off the oil from the surface of the aqueous distillate from needles of *Pinus ponderosa*. Earlier in their procedure, these workers took various precautions in an attempt to recover an oil that underwent as few compositional changes as possible, i.e. by freezing the needles, pulverizing in the presence of dry ice, steam-distilling for a limited period in a circulating apparatus, and finally storing the oil in the presence of an antioxidant.

More serious attention is now given to the fact that many components of essential oils are water soluble, and that a further extraction process must be used to recover the oil dissolved in distillation waters. Lund *et al* [86] have reported quantitative data for 20 water-soluble aromatic compounds from orange peel. Phenethyl alcohol and citronellol have been shown to be major components in water of rose distillate [87], whereas distillation waters from citronella grass yielded an entirely different oil containing among other compounds 12.1 percent perillaldehyde, 10.7 percent ℓ-carvotanacetone, 8.4 percent phellandral and 2.3 percent furfural [88].

A number of workers have reported differences in the decanted oils and oils extracted from distillation waters. For example decanted rosemary oil had much less optical activity than oil extracted from distillation waters [89]; 50 percent of the oil of cassis leaves is water soluble [90]; the

composition of pentane extracts from distilled rose water was found to be highly heterogeneous [91]; while differences were reported for the composition of decanted oil and oil recovered from the distillation water of *Mentha arvensis* [93]. Procedures recommended for the recovery of oils from distillation waters include: passing the waters through a layer of poroplast material and then extracting the absorbed oil [94, 106, 107]; use of methylene chloride as the best solvent for recovering rose oil [95, 101, 105]; removal from solution by millipore membranes of the MF type [96]; and carbon absorption of the distillate to increase yield of oil in a two-step procedure [97, 108].

Cohobation waters are more efficiently extracted through re-distillation of the aqueous distillate [102, 103], but even these waters should be further extracted to recover an oil with a high proportion of water-soluble steam volatiles. Juhasz *et al* recovered residual oils with a different composition, by absorption on a bed of activated carbon with subsequent extraction by 1,2-dichloroethane [98]. These workers also treated cohobation waters by salting-out to recover oils of lavender and peppermint buds [99], and by charcoal absorption for oils of chamomile, lavender and peppermint [100].

Solvent extraction

Plant materials that do not yield appreciable amounts of essential oil by steam distillation, or which yield oils that undergo qualitative changes in boiling water, e.g. rose, jasmin and violet, are extracted with solvents in an attempt to overcome

these problems. However, solvent extracts often differ

considerably from essential oils (obtained by a distillation

process). The differences are principally due to the presence

of extracted non-volatile plant compounds [79], but may also

occur through losses of more volatile compounds and chemical

changes of certain others.

Some workers appear to have ignored the concentration

of non-volatile compounds in solvent extracts, and have

reported the concentrations of individual terpenes in

evaporated extracts as if they were equivalent to essential

oils. The differences in composition between a raw solvent

extract, a concrete and an absolute are well known. That is,

an absolute contains a considerably smaller concentration of

waxes, colours, etc., than its concrete form. A typical

methylene chloride extract from the bark of *Pinus sylvestris*

contains free and esterified fatty acids, resin acids,

ferulic esters, fatty alcohols, alkanes, sterols, monoterpenes,

sesquiterpenes, diterpenes, norditerpenes, triterpenes and

their oxygenated derivatives [123]. Many of these compounds

would not be found in a steam distilled oil.

Each evaporation step required to concentrate a raw

extract to a concrete, and in turn to an absolute, also

involves partial or complete losses of some more volatile

components [22, 104, 109, 110]. By contrast, oil of *Cedrus*

deodara obtained by extraction was similar to steam distilled

oil [111]. Similarly *Pinus ponderosa* wood yielded an ether

extract with approximately the same monoterpene composition as

obtained from the oleoresin in a molecular still [66],

while the carbon dioxide extract from patchouli had essentially

the same composition as the distilled oil [112]. The recovery

advantages of solvent extraction were particularly apparent

in the case of patchouli, which yielded 2.5 percent of

extracted oil compared with 0.15 to 0.38 percent by

distillation.

In some better documented instances, solvent extraction

has been concluded to yield oils which are both qualitatively

and quantitatively different, even though retaining closely

similar odours. Hefendehl [84] compared microfilm extraction

of external glands of *Mentha piperita* oil with steam

distillation and solvent extraction. Solvent extraction led

to differences in terpenes, sabinene and menthofuran. Steam

distillation gave quantitative differences in cineole, sabinene,

menthol issuers and some ketones. Tarragon carbon dioxide

extract [80] retained a 'native aroma' and the flavour of the

raw material, and its yield was higher than that of the steam

distilled oil. Acid, neutral, and phenolic compounds, which

were not volatile with steam, together with the essential oil

itself, passed into the carbon dioxide extract and formed a

residue comprising about 8 percent of the weight of the extract.

Improvements to the solvent extraction process,

such as the application of ultrasound to break up plant cells,

have speeded the procedure [113] and increased the yield [1],

without affecting the final composition. Other workers have

examined the effects of different solvents on the yield and

composition of rose oil [114]. Liquefied butane [1] and other

highly volatile solvents, such as carbon disulphide [52], pentane [104, 124], propane and liquid carbon dioxide [115] have been used in preference to conventional less volatile solvents, which involve greater losses of components during the evaporation step. Carbon dioxide was considered in comparison with propane to be "the more selective and to result in a product fully retaining the aroma and taste of the botanical" [115]. Carbon dioxide has since been widely used and reported to yield oils both similar [116] and different from distilled oils [79, 117].

Chemical changes during the extraction process have been discussed by some workers [22, 44, 118-121]. In a well authenticated instance, jasmine oil was found to contain methyl jasmonate [118], but not the ketolactone reported earlier by Naves. Naves and Grampoloff [119, 120] however, reaffirmed their previous finding with new data, and the three authors subsequently agreed [121] that the ketolactone actually occurs in the oil of jasmine, while methyl jasmonate could be identified in absolute of jasmine.

Numerous other types of solvent extracts may be produced, depending upon the 'clean-up' procedures used, which may lead to compositions that differ considerably from one another and from the distilled oil. In addition to concretes and absolutes, another commonly produced extract is the acid-free ether-soluble fraction from an oleoresin or balsam [122]. Other workers have reported the compositions of both extracted and distilled oils that have been washed with cold 1 percent caustic soda and/or 0.01N sulphuric acid.

Direct- and micro-extraction from glands

Isolation of some essential oils, by pricking and micromanipulation directly from glandular hairs, has been considered as a means of avoiding thermal and hydrolytic decompositions inherent in other isolation methods. The essential oil isolated in this way from *Mentha aquatica* had some qualitative differences in comparison with the distilled oil [125].

In the original report of the technique by Hefendehl [126], in which oil was extracted from *Mentha piperita* by absorbing and lifting off on a plastic film, it was considered that the oils obtained by this method and by distillation were identical. However it was subsequently shown [84] that extraction with solvents gave different results, particularly in terpenes, sabinene and menthofuran. Steam distillation gave some quantitative differences in cineole, sabinene, and methol isomers, and to some extent the ketones.

Other workers have compared the compositions of oils removed from glands and distilled oils [128]. Millet *et al* [127] found that the oil of orange obtained by puncture of glands contained carotenoids. Quantitative differences only were found in the two types of oils from *Mentha piperita* and *M. aquatica* [81], and from other *Mentha* species [129], while unspecified differences were reported for oils of orange [130], lime [83] and mandarin [82].

Although the apparent advantages of directly puncturing oil glands for removal of oil have interested some

workers, who have even recommended the micromanipulation

technique for commercial production [131], the question

remains as to whether such oils may be subjected to even

greater effects due to air exposure and volatilization of

more volatile components.

Extraction and isolation of oil directly onto a GC column

Trapping of the volatiles from plant material, by

absorption directly onto a GC column, avoids problems

otherwise associated with steam distillation of plant material

prior to GC [132]. The technique usually involves a detachable

and separately heated injector chamber, into which plant

material or oleoresin [53, 135] is inserted, heated, then

flushed onto the main GC column by opening a special loop in

the carrier gas line. This procedure is to be distinguished

from solid-sampling GC, in which the plant material is usually

injected directly onto the main GC column.

The procedure has been used to recover considerably

higher yields of volatiles from lavender blossoms, fennel

fruit, peppermint leaflet, etc. [133]. The technique has also

been used to make feasible the almost direct analysis by

capillary column-GC of volatiles from needles of *Pinus*

sylvestris and *Picea excelsa* [134]. Volatile compounds from

needles were frozen out onto a precolumn, which was then

joined to the capillary column without interrupting the carrier

gas flow. A similar system was used by Grob and Grob [136]

for the analysis of trace components that might be lost during

preliminary extraction, e.g. insecticides in butter extract,

aroma headspace from liquors and auto exhaust gas. Smedman

et al [135] used the Hamilton injector pre-column system as

a parallel method to check on compositional changes which may

have occurred in a solvent extraction procedure for the

analysis of *Abies* balsams. The injector tube system was found

to retain acids and other high-boiling compounds present in

balsams. Hence the volatiles that were flushed onto the main

GC column were almost the same compounds that occurred in a

steam or vacuum distillate.

Stability of essential oil components preceding analysis

Even though many precautions may be taken to prevent

changes occurring in the composition of an oil during extraction,

the oil may immediately undergo minor qualitative and

quantitative changes due to the instability of some compounds

[61]. Investigations have shown that many of the changes are

initiated or accelerated by light, heat [137] and air [138].

The subtle changes in orange oils that subsequently fail to

meet pharmacopeia specifications, yet are indistinguishable

organoleptically, have been known for many years to depend

upon the degree of exposure to light and air [139]. When

protected from light, changes were almost undetectable by

earlier methods; otherwise an orange oil could undergo extensive

changes within 3 months. The time taken for changes to occur

may be from as little as a few hours to several years,

depending upon the nature of the compounds in the oil.

One of the most documented alteration products
is ρ-cymene, which has been shown in lemon oil to form on
standing, not from citral, but from the oxidation of
γ-terpinene [141]. Snajberk *et al* [141] attributed the
presence of ρ-cymen-8-ene and ρ-cymen-8-ol in cortical
oleoresin of *Pseudotsuga menziesii* to the alteration of
terpinolene, which was found earlier to autoxidize to these
compounds [142]. The possibility that 2-hexenal is an
autoxidation product in needle oil of *Pseudotsuga menziesii*
[56] is suggested by the observation [68] that 2-hexenal is
formed when leaves of *Ginkgo biloba* are ground in an air
atmosphere.

In oils of *Pinus* species it has been considered
that the ratio of α- to β-pinene is not affected by UV light
or storage time [144]. Paraffin hydrocarbons were also
considered to be unaffected upon standing. However the α-
and β-phellandrenes are known to be unstable and to readily
polymerize. Marked decreases in β-phellandrene were shown to
occur in oil of *P. coulteri* due to the effects of light and
heat during storage for 2 months [145]. Similarly, β-myrcene
polymerization has been thought to account for its seldom
being found in pines [145], as suggested during the study of
Podocarpus spicatus [146]. Although a number of artifacts
and chemical alterations have been documented, the identity
has not been established of a 1 to 7% component which rapidly
degrades in the steam distillates of *P. coulteri, P. ponderosa,
P. contorta, P. patula, P. montezumae* and *P. muricata* [145].

(b) Direct extraction and analysis of essential oils

It is apparent from the preceding discussion that isolation of an extracted essential oil may involve changes in oil composition. Other techniques have been designed specifically to omit the isolation step, i.e. in which the oil is analyzed immediately upon extraction from the plant material.

Solid-sampling GC

Injection of plant material into a GC and direct analysis of the volatiles, that are driven out of the column by heat and carrier gas, has provided a means of eliminating the changes in oil composition that otherwise result from steam distillation, solvent extraction or during oleoresin exudation [104]. This procedure has enabled gas chromatograms to be produced from the volatiles in only a few mg of sample, yielding analyses more representative of oils as they exist in plant material [148].

Karlsen and Baerheim Svendsen [71] have compared the monoterpene hydrocarbons in needle oil of Norwegian spruce (*Picea excelsa*), determined directly by solid-sampling GC and after extraction by steam distillation. As little as 10 mg of fresh plant material by direct injection enabled the identification of 18 monoterpene hydrocarbons, yet only 16 of the 18 could be found in the steam distillate. Stepanov and Dubovenko [149] also compared direct GC and analysis of the isolated oil, and showed that the concentration of camphene in conifer needle oils was lower (by a factor of 2.6), and those of α-pinene, β-pinene, and Δ_3-carene were higher by

10,40 and 14 percent when the direct method was used.
Further fundamental quantitative differences were found in
directly injected needle material of *Picea obovata,*
Abies sibirica, Pinus sylvestris and *Pinus sibirica.*

Even though the injected plant material is
subjected to a high temperature to drive volatiles onto the
GC column, the percentage evaporation of low-boiling
monoterpene hydrocarbons differs from the higher-boiling
oxygen-containing monoterpenes. It has been claimed however,
that the percentage evaporation of each compound can be
calculated, providing reproducible results for the analysis
of monoterpenes in a 2 mg sample of leaf of rosemary,
Rosemarinus officinalis [150].

A further comparison of the solid-sampling GC
technique with solvent extraction has shown that highly
variable results may be expected from the latter technique
[104]. GC analysis of cold pentane extracts of homogenized
single needles and 0.1 g samples of wood resulted in unequal
loss of monoterpene hydrocarbons during concentration of the
extracts. Roberts therefore preferred the solid-sampling
technique for analysis of pine oleoresin, and questioned the
need to either prepare extracts or even directly inject
exuded oleoresin, which has been the basis of some well-known
studies [151-153].

A particular advantage of solid-sampling GC is the
lack of any apparent thermal degradation of some unstable
compounds. For example α-farnesene in the Dufour gland of

an ant is known to rearrange above 140°C to an allo-farnesene, but GC-MS has shown the same compound to be chromatographed by this technique as found in a pentane glandular extract [110].

Not only does solid-sampling GC enable the study of such small samples as single insect glands [110, 161], but there is no large solvent peak to obscure rapidly eluted compounds that would result from a solvent extract.

Maarse and Kepner [22] considered the advantages of the direct injection techniques of von Rudloff [147] and others [154, 155], i.e. in overcoming both qualitative and quantitative composition changes during isolation and concentration of the oil. However, these workers considered that solvent extraction, with the vacuum transfer technique of Kubeczka [156], would provide a technique comparable with direct-sampling.

In spite of the number of workers who have enthusiastically reported their use of solid-sampling GC, recently reviewed by Rasmussen and Karlsen [157], these techniques lack the versatility that would allow solid-sampling GC to be a recommended and much applied method. A widely accepted technique should allow easy and reproducible sample introduction, together with treatment of the plant material to suit the GC system, i.e. to produce a uniform plant tissue consistency to allow injection under reproducible conditions [158-160]. A further problem that does not appear from the literature to have been studied, is the rate of loss of more volatile terpenoids from the micro-quantity

of plant material that must, in most techniques, be comminuted to render it suitable for injection.

Direct injection GC with glass wool medium

A modification of the direct-injection or solid-sampling principle, by which a basically non-volatile liquid mixture is diffused onto glass wool and inserted into the injector chamber of a GC, then temperature-programmed, has enabled the solid-sampling technique to be used for the analysis of ng/g concentrations of volatiles. The technique is particularly amenable to such difficult materials as oleoresin and vegetable oils, which if injected directly onto a GC column would rapidly block the system or at least alter the eluting properties of the liquid phase. This application of the solid-sampling technique differs from an earlier-described procedure, in which an essential oil is flushed directly from plant material and isolated on a GC column.

A principle advantage of the glass wool injector medium is the ease by which the non-volatile residue of a sample may be removed, i.e. merely by replacing the medium in the injector. In the solid-sampling technique described earlier, plant material residues would accumulate at the head of the main column, necessitating its frequent removal for cleaning purposes.

Dupuy *et al* |162, 163| demonstrated the advantages of the technique by analyzing 10 ng/g concentrations of volatiles in vegetable oils, without the need for a pre-concentration step.

Although a commercial injector block (Hamilton) has been reported in use as a means of confirming the presence or absence of artifacts, the convenience of this modified solid-sampling method has not yet been used to full advantage.

If fully exploited, i.e. by using a gas chromatograph fitted with a separate carrier gas loop through the injector, a glass wool medium enclosing a potentially wide range of plant material sizes could be used for the routine anlaysis of essential oils, without any of the features inherent in distillation, solvent evaporation, tissue damage, or oil storage, which may lead to artifacts or losses of volatiles.

(c) Extraction of food flavour components

The much smaller concentrations of often water-soluble compounds, which constitute the flavour components of foods, necessitate the use of a number of extraction and concentrating techniques to yield an isolate. Consequently, there is a much greater chance of introducing artifacts from solvents, containers, tubing and lubricants, together with off-aromas resulting from heat damage, air oxidation, etc. For this reason a food flavour is often not as clearly defined as the classic concept of an essential oil, since the flavour may be due to compounds which have appeared during a step in the preparation of the food, e.g. cooked food odours distinct from fresh fruit flavours.

Issenberg and Hornstein [44] have discussed the concepts of food flavour and noted that there is good reason in some cases for only considering the flavour as the volatilized headspace components. Examples were described of attempts to obtain food flavour extracts using long sequences of extraction procedures. In some cases, where 10 tons of food eventually yielded a few ml of a solvent extract with a strong flavour, the question was raised as to whether the final extract was meaningful because of the number of artifacts and alteration products which must also have been recovered.

The isolation of meat flavour volatiles by collecting the total volatiles released under high vacuum distillation [164], i.e. by using a one-step extraction and so minimising the chance of altering the flavour composition, has been adopted by Hornstein *et al* [165] as an acceptable procedure. However, even this very simple type of extraction has been found to discriminate against components of lower volatility, which in turn would suggest the need for a further step to extract the aqueous fraction [143].

Among the techniques used to extract food flavours include flash distillation, fractional distillations, liquid-liquid extraction, activated charcoal absorption from aqueous solutions, Soxhlet extraction, freeze-drying and column chromatography.

Despite the fact that the above techniques may cause changes in the composition of a food flavour, the poorly defined nature of the flavour composition may often

enable such changes to be acceptable, i.e. without altering

the basic flavour. Carson and Wong [166] showed that an

almost identical onion flavour could be produced by two

different sequences of extraction steps. In each case the

same disulphides and trisulphides were obtained, but the

accompanying ethanol, normal and iso-propanols differed

quantitatively. Since the alcohols contributed little to

onion flavour, then either of the extraction sequences was

concluded to be satisfactory for extracting onion flavour.

It is concluded that the validity of specific

techniques for extracting food flavours, i.e. that do not

alter the chemical composition of the flavour components,

depends upon the nature of the specific flavour compounds in

a food material. The flavour of some foods has as a result

not been found amenable to any of the usual extraction

procedures, which has led to their identification by direct

GC analysis of headspace volatiles [44].

(ii) Isolation of essential oils from the vapour phase

Since essential oils exist in both the liquid and

vapour phases, consideration should be given to the differences

in composition between each type of terpene mixture.

Numerous reports have appeared of the analysis of

headspace volatiles above foods that are difficult to extract.

Many of these studies have been by workers who have condensed

volatiles from the atmosphere about plant material and subsequently

assumed the composition to correspond to that of the volatiles in

the original food or plant material. In some cases the features

which cause the compositions of vaporized oils to differ from the liquid phase are poorly understood. In addition, the techniques used to isolate and analyze the composition of the volatile mixture may introduce characteristic effects upon the composition.

The composition of the headspace volatiles differs both quantitatively and qualitatively from the total volatiles, i.e. the extractable or liquid-phase volatiles. The composition of the headspace volatiles directly above a food depends upon the vapour pressure of the volatile compounds in their pure state, at the temperature of the food, and on the interactions of these compounds with the matrix. Headspace sampling could therefore result in the omission of trace amounts of high boiling compounds, and conceivably also those compounds that may be important flavour contributors [44]. In other situations some headspace compounds with low volatility, which at low concentrations are easily detected by the olfactory sensory system, may be below the limit of GC detection. It may therefore be advantageous to attempt to concentrate headspace volatiles before analysis without causing changes in composition.

Investigations have been made into the relationship between vapour concentration and the concentration of a flavour component in the liquid substrate. Nawar [167] found the vapour phase concentrations of 2-heptanone in aqueous solutions to be about 20 times higher than in oil solutions. The volatility in skim milk was shown to be similar to aqueous solutions in the concentration range 200 to 6,000 µg/g.

However at concentrations above 2,000 µg/g a nonlinearity or deviation from Henry's Law has been found. Buttery *et al* [168] evolved a method for estimating the volatility of organic flavour compounds in food, and hence their contribution to the aroma, by either calculation or by experimental determination. These workers [169] had earlier confirmed theoretical predictions that higher molecular weight homologues of aldehydes, ketones and esters, up to C_9, in dilute water solutions are actually more volatile than the lower molecular weight homologues. It has also been postulated [22] that the surface structure of new plant growth (Douglas fir needles) may be more permeable to terpenes than that of mature growth, since more α- and β-pinene were found in new than mature, when compared with analysis following extraction by distillation.

Comparison of headspace with extract analyses has resulted in some well documented differences. For example, banana headspace vapour [170] was shown to contain a number of unidentified higher boiling peaks (not thought to be artifacts) that were not detected in banana extracts.

Other workers have used headspace analysis in parallel with steam distillation, or solvent extraction, as a check on formation of artifacts. However it was conceded by Maarse and Kepner [22] that the analysis of oil components by the two methods cannot be cross-related. Although there is expected to be considerably less chance for artifact formation during headspace analysis, the value of this technique as a parallel check on artifact formation is

questionable. In a further misleading instance, two peaks reported in a headspace chromatogram were subsequently shown to be shadows of two major peaks resulting from a dead-space holdup in the GC injector chamber [171].

Headspace volatiles have been analyzed by both direct sampling and in combination with a pre-concentration step.

(a) Direct sampling and analysis of vapour

Direct sampling and GC analysis of the volatiles in the atmosphere of a closed container has been a simple, convenient and sensitive method for the investigation of volatiles which could not have been easily studied by distillation/solvent extraction techniques, e.g. changes in volatiles during ripening of banana species [172], identification of Allium sp. from the proportion of freshly-released volatiles [7], study of hexanal autoxidation product in processed vegetables [46] and odours emitted by cultures of micro-organisms [174]. A common procedure has involved a glass container fitted with a rubber stopper or septum that is penetrated with a syringe needle for sampling [175, 176].

Repeated sampling from a rubber-sealed container has been reported to produce variations in the relative proportions of volatiles [177, 178], but no explanation was given. Absorption of volatiles by the rubber stopper or septum was suggested by Hoff and Feit [158]. Davis [180] has since studied the changes in headspace volatiles due to rubber absorption, and devised a glass stopcock system which eliminated the losses.

Since most headspace analyses appear to have been carried out with probable losses due to some degree of rubber absorption, reservations must be placed upon reports prior to 1970. Among the terpenes β-pinene and limonene were most rapidly absorbed. Compounds with higher molecular weights appeared to be absorbed more rapidly, which correlated with more rapid losses of compounds with longer skeletal chains. Other structures with similar molecular weights were also more readily absorbed, e.g. 1-pentanol > valeraldehyde > hexane.

Table 4. Decrease in concentration (%) of volatile compounds in closed containers [180]

Type	Compound	Closed by rubber stopper 30 min.	Closed by rubber stopper 60 min.	Closed by glass stopcock 60 min.
Alkane	Ethylene	2.0	2.1	– –
	Hexane	7.6	13.8	– –
	Heptane	21.9	30.4	– –
Alcohol	Ethanol	6.9	12.6	– –
	1-Butanol	26.7	36.0	– –
	1-Pentanol	44.0	59.3	– –
Aldehyde	Propional	4.6	8.0	– –
	Valeraldehyde	26.3	37.6	– –
	Heptanal	64.5	77.8	– –
Ketone	Acetone	7.4	10.2	– –
Ester	Ethyl acetate	15.8	24.4	– –
	Butyl acetate	48.3	62.2	– –
Terpenes	β-linene	60.9	75.1	– –
	Limonene	79.5	88.1	– –

Loper and Webster [181] considered that to obtain a representative sample of headspace volatiles was fraught with difficulties. Among such problems included differential absorption of volatiles on glass walls and desorption of contaminants from the container and septum. It was therefore

considered more valid to inject the total headspace. For
the analysis of volatiles from alfalfa flowers these workers
placed the flowers in a 100 ml heated syringe and injected
the 70 ml headspace. The system was however not sensitive
enough for direct analysis and so a cryogenic pre-concentrating
GC trapping loop was used.

Although some investigators have reported headspace
sample bags to be suitable for relatively concentrated
vapours, e.g. of smog [182, 183] and volatiles from plant
sources [184, 185], others have found plastic materials to
sometimes give rise to contaminant volatiles [186]. Problems
due to absorption and diffusion losses were also encountered
with dilute samples of automobile exhaust [208, 209]. When
whole branch segments of *Pinus strobus* were stored in plastic
bags in a freezer, after 14 days an unknown compound appeared
in much larger amounts, and was accordingly regarded as a
degradation product [186].

(b)   Techniques for trapping and concentrating volatiles

Many reports have appeared of the analysis of
volatiles that have first been isolated from the atmosphere,
and loosely described as 'headspace volatiles'. Such
analyses should be distinguished from the direct sampling
and analysis previously described for headspace volatiles,
because the techniques employed to trap and concentrate
have often led to changes in the composition of a volatile
mixture of compounds. Difficulties with earlier cold-
trap and solid adsorbent trapping systems have led to

numerous other attempts to isolate volatiles, which are
present at too low concentrations for the limited sensitivity
of currently available detectors.

Since the olfactory sensory system may often detect
low concentrations of volatiles which cannot be detected by
any current instrument used for headspace analysis, a need
therefore exists for a suitable trapping and concentrating
technique. Numerous trapping techniques have been devised,
particularly for trapping eluted GC fractions for subsequent
identification by MS, IR, etc. [187]. However, many of these
techniques have since been shown to lead to both qualitative
and quantitative changes in the microgram to milligram
quantities of compounds isolated.

A suitably-improved volatiles-trapping technique would
also facilitate the quantitative handling of micro-quantities of
odoriferous compounds. Such investigations include the study of
volatiles from labial glands of bees, from wing scales of
butterflies and from orchids [173].

Cold-trap condensation of volatiles

Although traps cooled with dry ice or liquid nitrogen
have been successfully used to isolate as little as 0.1 µg of
GC fractions with boiling points as low as that of methane [179],
the principle is unsatisfactory for condensing volatiles from
much larger volumes of air. The main difficulty results from
the amount of water that condenses with the sample. The mass
of condensed water usually far exceeds the volatiles [191] and
may lead to a dilute aqueous extract that is difficult to analyze.

Attempts to separate volatiles from the water
have involved the introduction of a further step, which may
lead to changes in the composition of the volatile mixture.
For example some studies of cold-trap condensed air pollutants
[188, 189, 190] included a step in which volatiles were
recovered by fractional distillation. The problems of
distillation were discussed earlier. Others [192] have
recommended drying agents prior to condensation,
e.g. potassium carbonate [210, 211]. Unless a particular
drying agent has been demonstrated to not absorb components
from a mixture of volatiles in a particular application then
this step as well could not be recommended.

Cryogenic sampling, however, is the only technique
presently available which reflects a true sample composition
of the low molecular weight end of the range of airborne
volatiles [196]. With this technique secondary reactions such
as oxidation or polymerization are also minimized.

Trapping in solvents

Bubbling of a stream of air and volatiles through
an appropriate solvent has enabled highly volatile GC fractions
to be condensed for subsequent recording of an IR spectrum [193].
The technique has also been attempted as a means of trapping
volatiles from the atmosphere [191], however later removal of
solvent has led to significant losses of more volatile compounds.
Higher boiling point compounds may however be recovered with
minimum losses.

Trapping on solid absorbents

Absorption of volatiles onto several types of solid absorbents has enabled microquantities of some organic compounds to be trapped for subsequent spectroscopic identification [187], however the recovery from absorbents may be incomplete and even accompanied by qualitative changes. Several writers have discussed the use of different absorbents, including activated charcoal [190, 194, 196], talc and silica [195], and molecular sieves [197].

Of all the absorbents activated charcoal has been most successfully used for trapping many types of compounds, although Rasmussen [190] has reported the recovery of components to be usually incomplete and variable. Some compounds were also reported to undergo chemical alteration catalyzed by charcoal [190]. Intensive investigations of the absorption of volatiles, from atmospheres in undersea and space laboratories, have shown that activated charcoal may be used in conjunction with appropriate desorption techniques to efficiently recover most trapped materials [198]. Among techniques that have been successfully used to quantitatively recover particular volatiles include vacuum desorption [199-201], steam desorption [202, 203] and n-decane extraction, e.g. of chlorinated hydrocarbons [204]. Solvent extraction of charcoal was found to be most effective with carbon disulphide [205]. Grob and Grob [206] demonstrated the value of the technique by absorbing and identifying by GC-MS more than 100 compounds, from C_7 to C_{20}, that had been absorbed from $25m^3$ of air on as little as 25 mg of charcoal, subsequently extracted with

carbon disulphide. For recovery of less volatile materials a combination of charcoal and molecular sieve 5A has been· proposed with heat desorption in vacuum [207].

Less use has been made of other adsorbent trap materials, no doubt because of well-known effects upon mixtures of adsorbed volatiles. Silica gel has been found unsuitable because of the conversion of alcohols to olefins and irreversible adsorption of amines [197]. Molecular sieve 5A under certain conditions has been shown to strongly absorb straight chain molecules but at the same time weakly adsorb all types of more complex molecules [197].

Adsorption on coated GC supports

Ducting of headspace vapours through a GC column has enabled microquantities of volatiles to be trapped and concentrated for subsequent analysis, without some of the changes in composition that accompany trapping by other techniques. Concentration methods based upon the equilibrium of volatile compounds with a liquid stationary phase, have been recommended [212, 213] because solutes could be easily recovered, while the more volatile part of the sample would be lost. The method is not suitable for large volumes of air, which could only be ducted very slowly through conventional GC columns [215]. Short lengths of column, with faster flow rates, have been used to trap toxic organic compounds in air [214], however the method is selective in only trapping certain groups of compounds. Kubeczka [217] demonstrated the

successful use of this technique by trapping the limited amount

of volatiles in the headspace above fungal cultures, using a

short column of 15%SE-30/AW diatomite (60-80), which upon

analysis was found to have trapped a mixture of volatiles

quantitatively and qualitatively similar to a steam distillate

from the culture. Adsorption involving coated GC supports is

not generally applicable [196].

In an attempt to also trap the more volatile

components, particularly on shorter lengths of column, several

workers combined the technique with a cold-trap condensation

step. Hornstein and Crowe [216] passed prepurified nitrogen

over sample in a flask, then through a cooled column in which

volatiles were frozen out. Issenberg and Mysliwy [170]

subsequently trapped volatiles on a capillary column cooled in

liquid nitrogen. In this latter adaptation, which was fraught

with difficulty, five 20 ml vapour samples were injected

into a 30 cm length of coiled capillary column, then allowed

to return to room temperature over a 30 min. period before

beginning a very slow temperature-programmed analysis. Use of

this technique to study the total headspace surrounding alfalfa

flowers [181], where volatiles were trapped on a condensing

coil cooled in dry ice-acetone, only produced a capillary-column

chromatogram in which many of the early-eluting peaks had to

be ignored because of the masking effects of water.

Headspace volatiles have however been successfully

trapped and concentrated on capillary columns, without the

effect of any condensed water upon the finally determined

composition. In the usual cold-trapping procedure, in which a short piece of capillary is cooled in dry ice or liquid nitrogen [218-221], the practical sample size is greatly reduced because of the volume of water that is condensed. Even if the technique is successful in avoiding a water-blockage in the capillary, condensed droplets may still move along the column and destroy the coating film [222]. Grob and Grob [223] have subsequently shown that volatiles may be successfully trapped on a capillary column without the need for freezing the column. These workers showed that the temperature need only be kept to at least 50° below analysis temperature. In a further improvement, Grob and Grob [136] devised a detachable injection block containing a short piece of capillary, which could be coated with an appropriately retentive liquid phase that might differ from the coating in the analytical column.

Other workers have introduced further improvements that have enabled headspace volatiles to be trapped on coated supports without condensing any water. Although packed columns may tolerate more condensed water than capillary columns, they still cannot accommodate the condensation from more than a few ml of air [182, 183, 224]. Kaiser [225] successfully devised a means of preventing the condensation of water by maintaining a temperature gradient along a section of packed GC column. Tyson and Carle [226] designed what might appear to be the ultimate system, in which a 20ℓ air sample containing as little as 0.2 μg/ℓ of volatiles could be cryogenically preconcentrated, separated completely from water in a preparative-scale GC, then ducted into a GC-MS for analysis. The selectivity of

the system, and presumably that of any attempt at trapping

and separating volatiles from condensed water, is indicated

in the recoveries for individual terpenes obtained by Tyson

and Carle. In spite of the poor recoveries of some terpenes,

the overall performance of this system appears to be far

superior to any other yet reported.

Table 5. Efficiency of recovery of selected terpenes
concentrated from the air (0.1 µℓ or 10 µg
terpenes + 200 mg of water in 20ℓ of air)
in the system designed by Tyson and Carle
[226]

	Peak height:		
Component	Direct injection	Processed sample	Recovery (%)
α-Pinene	101	99	98
Camphene	121	133	110
β-Pinene	114	95	83
Myrcene	42	40	95
Δ₃-Carene	50	46	92
Limonene	90	84	93
Linalool	37	33	89
Thujone	27	16	60
Menthone	36	41	114
Isomenthone	51	30	59
Estragol	47	41	87
Bornyl acetate	34	10	30

Adsorption on porous polymer GC supports

Columns of non-polar uncoated porous polyaromatic

polymer beads, e.g. of the Porapak series by Waters Associates,

have enabled organic compounds to be trapped while water is

largely eluted from such a column [191]. The technique has

been used successfully to isolate several previously unidentified

organic components of orange juice headspace [210]. Most

polymers have been used at room temperature to trap volatiles,

which were then desorbed by heating in a GC [215, 227, 228].

By comparison, samples similarly collected on support-bonded

silicones were recovered by solvent extraction with the

attendant disadvantages [229].

Bertsch *et al* [196] have more recently studied the

properties of a particular porous polymer adsorbent, Tenax GC,

from Applied Science Laboratories. Tenax GC is a porous

polymer of 2,4-diphenyl paraphenylene oxide, which may

efficiently trap volatiles and has negligible affinity for

water. The volatiles are then released by heating. By

comparison, styrene divinylbenzene polymers such as Porapak

and Chromosorb 102 may often release artifacts and exhibit

limited thermal stability.

Even though Tenax would appear to supersede presently

available GC supports for isolating headspace volatiles, it

has been found that there is still a degree of reduced adsorption

efficiency, which for some groups of compounds depends upon

the sampling flow-rate conditions [196]. Alkanes, alcohols

and amines were more efficiently trapped than aldehydes, ketones

and phenols. High molecular weight compounds were more easily

retained than low molecular weight substances, i.e. particularly

those smaller than C_5. In addition, each compound has a specific

temperature and sampling flow-rate at which it may be

quantitatively adsorbed.

As for all other headspace trapping materials, the feasibility of Tenax as an adsorbent for a particular group of volatiles should be established beforehand to ensure that it does not introduce any effects upon the final composition of the terpene mixture.

3. Effects of techniques for separation and analysis of oil components

(i) Techniques for preliminary separation of components

Although the advent of newer chromatographic techniques held an initial promise of separating all oil components for identification, thereby allowing the investigator to avoid the already-discussed adverse features of classical methods of isolation and fractional distillation [56], the newer techniques have shown many essential oils to be even more complex than realized.

As a result such a renowned investigator as von Ludloff, who initiated the use of GC column sequences to preparatively separate essential oil components, has recently concluded that many complex terpene mixtures would be better prefractionated by distillation or column chromatography rather than preparative GC alone [230]. Even with the knowledge that distillation and solvent extractions may lead to chemical changes, artifacts and losses of components, some workers have reported using various multi-step gross fractionation systems, e.g. the pre-GC simplification of banana concentrate [231]:

Banana concentrate
1.2 g

Free acids
80 mg

Me esters

Neutral fraction
800 mg

hydrolysis

"Acid fraction"
200 mg

Me esters

Alcohols and
neutral compounds
400 mg
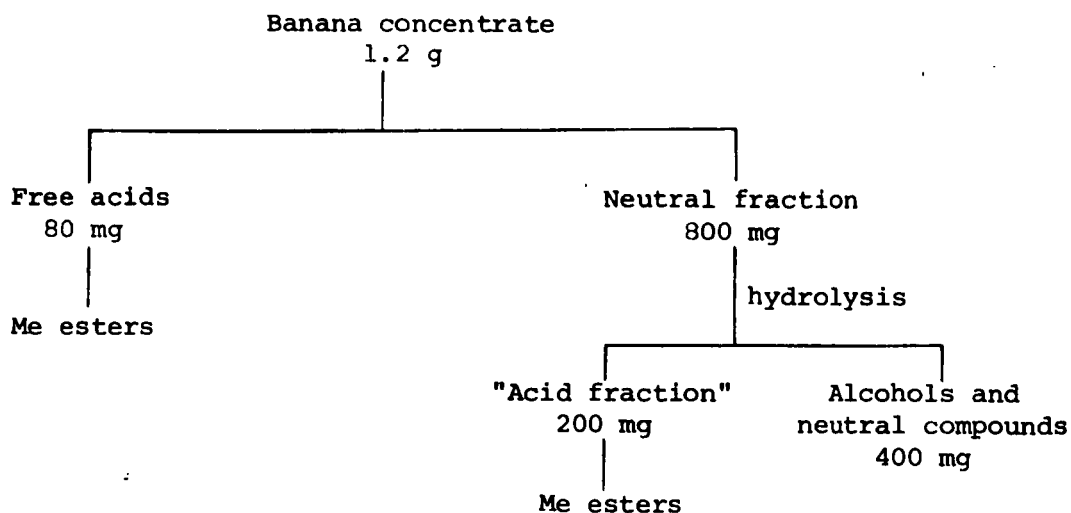
and the fractionation of cotton plant *(Gossypium hirsutum)*

bud oil |232|:

Crude square oil

silica gel/C20M
col. chrom.

Pentane
frac.

2-Chloropropane
frac.

Methanol

α
(hydrocarbons)

β
(midpolar compds.)

γ
(polar compds.)

Prep.
GC/C4M

150°
(terpenes)

175°
(sesqui-
terpenes)

Girard T
chem. extn.

ppt/
filter

γ_S

Glycol/CCl$_4$
partition

β_1
(carbonyls)

β_2
(noncarbonyls)

γ_A
(alcohols)

γ_B
(non-
alcohols)

TLC silica gel/
C_6H_6

Silica gel
col. chrom./
C_6H_6

Distn.

Distn.

β_2

β_2

γ_{A1-5} 6 resid.

γ_{B1} 2 3 4 5

$\beta_2 3$ $\beta_2 1$

Prep.
GC/C20M

Prep.
GC/C20M

low bpt
alcohols

high bpt
alcohols

β-bisabolol

Although some workers have advocated the use of analytical
GC to trace the components separated in each fraction, and
particularly to detect losses and artifacts, it is not clear
from many reports that such precautions are always taken.

Techniques for gross separation of terpenoid
components have been reviewed by Chang [233, 235] and Scott
[234]. However, no report was found in the literature of
any systematic review of the chemical changes to be expected
from each of the main terpenoids subjected to these procedures.

(a) Fractional distillation

The changes in terpenoids as a result of distillation
have not been systematically detailed, but instead precautions
have been recommended when distilling particular groups of
compounds. For example in Mirov's treatise on the composition
of gum turpentines of pines [47], it was recommended that a
preliminary fractionation should "indicate that no appreciable
amounts of sesquiterpene fractions were present and that easily
polymerized terpenes were absent, then the fractionation was
performed under atmospheric pressure". The only recommended
alternative when such compounds existed was to distil at reduced
pressure.

Until much more information becomes available as to
the identities of such complex mixtures of isomers as the
sesquiterpenes, together with further studies on the properties
of terpenoids, it will not be possible to predict the expected
changes of more than a few compounds. The situation is
complicated even further by the fact that the nature of changes
will also depend on intermolecular reactions, and hence some

changes will be specific to particular essential oil mixtures.

While most chemists are familiar with the dark-, coloured, relatively non-volatile residue that remains after a distillation, many workers have been content to refer to this material as a mixture of polymeric products. Slater and Watkins [236] however documented some specific chemical transformations which may occur when expressed lime oil is distilled from or stored in contact with limejuice. In another study it was shown that in the separation of oil of *Schizandrae chinensis* into hydrocarbons and ketones, azulenes were formed during the distillation of the hydrocarbon fraction [237]. Von Rudloff [312] similarly found isothujone to be extensively isomerized to dl-carvotanacetone.

Some workers have preferred instead to use more sophisticated forms of distillation in an attempt to minimize chemical changes. Moshonas [238] used a single pass rotary film molecular still, at 105° and 0.7 to 0.3 mm., to reduce the chance of decomposition of cold-pressed grapefruit peel oil that could occur at higher temperatures or with long exposure to heat. Coleman and Shaw [239] similarly distilled under reduced pressure in a rotary evaporator to yield 3 major fractions from orange aroma oils. In contrast, other workers [240] have distilled up to 150° under reduced pressure, to yield 30 fractions in a search for new trace components.

Since the most efficient fractional distillation may only be used to produce a gross separation of an essential oil into fractions that may still be quite complex, some

workers have preferred to use other preliminary fractionation techniques with considerably better resolution. In the case of citrus oils it was recommended [241] that chromatographic separation had advantages over vacuum distillation and selective solvent extraction, since there is better separation of the oxygenated and hydrocarbon fractions together with the elimination of high temperature chemical changes.

(b) Column chromatography

Prefractionation of more complex terpenoid mixtures, such as conifer leaf oils [230], may be undertaken with even more advantages by column chromatography than preparative GC. Unfortunately the literature is replete with instances of acid-catalyzed isomerization of terpenes on chromatographic adsorbents. Many workers have as a result been deterred by this procedure.

Von Rudloff [230] has demonstrated the indispensibility of column chromatographic prefractionation, and has furthermore concluded after studies with several oils, that isomerization of more reactive terpenes may be largely prevented by the use of deactivated silicic acid as described by Kugler and Kováts [242]. For the less stable terpenes it was recommended that the silicic acid adsorbent should be treated with polyethylene glycol [243]. This technique has been used successfully by other workers, e.g. in the deterpenation of orange oil [244].

Alumina adsorbent has similarly been shown to catalyze terpenoids, e.g. the formation of β-humulen-7-ol when oil of

wild ginger is chromatographed [245], and isomerization of
epoxides to α,β-unsaturated alcohols together with some
side reactions [246].

Other column chromatographic techniques have also
been reported that have conveniently provided terpenoid
prefractionations free of any detectable changes in components.
Murray and Stanley developed a silica gel microcolumn on which
30 to 100 µℓ samples of flavour concentrates may be separated
into hydrocarbons, alcohols, carbonyl compounds and esters
[247], while Palmer successfully separated functional group
classes in coffee flavour on silica gel eluted with Freon
[248]. The separation of olefins, or silver-adductable from
non-adductable fractions, on silver nitrate-impregnated
columns of silicic acid [249, 250] and alumina [240] has been
refined to prevent the conversion of aromatic components to their
nitro derivatives [251]. A further adsorbent material, Florisil,
used with success in the separation of µg quantities of highly
labile pesticide residues, has been largely ignored by essential
oil chemists although used to advantage by several workers [239, 252,
253], including Beroza [254].

(c) Chemical derivative separations

From the numerous derivatives that could be prepared
to assist the separation of particular functional group classes
of compounds, several have been attempted for the gross
fractionation of terpenoids. However Nigam and Levi [263]
have concluded in the case of the Girard reaction for the

isolation of carbonyls, that there are too many labile compounds in essential oils and that the isomerization which results, e.g. of ℓ-menthone, would give rise to misleading data. Similar problems are expected with some other derivatives, e.g. semicarbazones [264].

(ii) Techniques for separation and identification of components

Other separatory techniques used to also identify components may, because of chemical changes in certain compounds, yield completely misleading data and so lead to an incorrect identification. Identifications based upon TLC or GC data should therefore be confirmed where possible by an alternative procedure.

(a) Thin-layer chromatography

TLC has often been used to separate and identify terpenoids as an adjunct to GC, but may produce misleading data due to chemical alteration of some components, or irreversible adsorption of others [255].

Wrolstad and Jennings [256, 257] reported an extensive study of the isomerization of monoterpene hydrocarbons during isolation by TLC on silica gel. Table 6 contains a summary of the changes found by these workers. Other workers have critically reviewed the use of TLC for the examination of essential oils, and similarly noted the isomerization effects [258-260].

Table 6. Monoterpene hydrocarbons that were
chemically changed or remained unchanged
during TLC on silica gel [256]

Terpenes unchanged	Terpenes changed	Alteration products
α-Thujene		α-Thujene
α-Pinene		α-Terpinene
β-Pinene		γ-Terpinene
Myrcene		Limonene
Δ₃-Carene	Sabinene to:	β-Phellandrene
Limonene		Terpinolene
β-Phellandrene		α-Phellandrene*
γ-Terpinene		ρ-Cymene*
Terpinolene	α-Terpinene to	ρ-Cymene
ρ-Cymene	α-Phellandrene to	ρ-Cymene

* α-Phellandrene and ρ-cymene were recovered with the
other alteration products from an induced silica gel
isomerization of sabinene in a non-TLC system.

Some advantages have however been obtained from the
use of impregnated silica gel adsorbents. For example a silver
nitrate-impregnated layer was found to separate sabinene and
β-pinene, which could not be achieved on conventional adsorbent
[261]. Whether the formation of a π-complex with a silver ion
has also provided a means of stabilizing some monoterpene
hydrocarbons and preventing their isomerization or oxidation
has yet to be proven. An even more stable system, enabling
better separations and reproducible results involved a thallium
nitrate-impregnated adsorbent [262].

(b) Gas chromatography

GC techniques have provided some of the most important advances in the analysis of essential oils, and as a result many workers have been led into a sense of unreal confidence in the reliability of their GC analyses. By searching the literature numerous examples may be found of oil component identifications that have been incorrectly reported due to:

unresolved peaks on particular columns;

chemical alteration of components on particular

column materials, and in injector chambers;

and irreversible adsorption and hence losses of components.

Component identification and unresolved GC peaks

Since no single GC column has yet been devised with sufficient resolution to completely separate complex mixtures of terpenes, all reports of essential oil compositions based upon GC analysis should be viewed with this limitation in mind. Although many workers have attempted to overcome identification problems arising from incomplete resolution of components, by using alternative column systems, the inherent limitation still remains to at least some degree.

Further, many workers have failed to correctly use retention time data only as indicators of component identity. Many structurally different components may have identical relative retention times on particular columns, and may therefore be indistinguishable from one another. Reports

are still appearing in which 'identifications' have been
based on retention data from only one column operated at
one temperature.

Rigorous proof of identity of a component resolved
by GC must be obtained by reliable chemical or spectroscopic
methods. Retention data may only be used to tentatively
identify components in oils not previously investigated.
However in the case of previously-studied oils, suitably
obtained retention data may lead to a positive identification
of a component.

Detailed discussions of identification by GC have
been given in comprehensive reviews of the GC of terpenes
[230] and of spectroscopic identification of microquantities of
GC-separated components [187].

The type of column employed to separate oil components
may significantly influence the reliability of identifications
based upon retention data. The high degree of resolution of
the 0.25 mm ID capillary column is presently the best
obtainable, but unfortunately has inherent limitations which
prevent this column from rendering all others obsolete. In
Table 7 a comparison is given of the features of each of the
major types of columns, from which it can be seen that the
very small 5 µg load tolerance per component would greatly
limit the 0.25 mm ID capillary column as a means of separating
components prior to MS identification. Other disadvantages
of capillary columns have been discussed by von Rudloff [230],
e.g. need for use with the sensitive but less reproducible flame-

Table 7. Comparison of GC columns [267]

	Wall-coated open tubular (capillary)			Porous-wall open tubular (support-coated, SCOT)	Packed	
Outside diameter (mm)	−	−	−	−	$3.2(\frac{1}{8}$ in.$)$	$6.4(\frac{1}{4}$ in.$)$
Inside diameter (mm)	0.25	0.50	0.75	0.50	1.7	3.9
Length (commonly used)(m)	90	60	150	30	12	6
Total number of theoretical plates $\times 10^3$	75	35	30	35	20	6
Plates per m.	830	580	200	1,170	1,670	1,000
Sample size (largest component)(μg)	5	20	100	100	1,000	5,000
Carrier flow (ml/min)	1.5	6	10	3	15	60

ionization detector (FID), inherent component selectivity of the

precolumn splitter system, displacement of peaks due to

use of a diluting solvent and the easily-damaged liquid phase

coating. However, if used purely for qualitative purposes

[265] the capillary column is an excellent complement to the

packed columns [230, 266].

Much of the problem of GC separation and

identification is peculiar to each specific oil and should

be considered along with such inseparable factors as column

type, length, liquid phase and solid support. In the case

of peppermint oil, quality criteria which govern its market-

ability have caused workers to search for a suitable column

system that separates the following often-unresolved component

groups:

1,8-cineole and limonene,

menthofuran and menthone,

and menthyl acetate and pulegone.

Packed columns were previously thought to be unsuitable for

these separations in this oil. Many laboratories have as

a result adopted commercially-available Micropack or SCOT

columns. The above separations in peppermint oil were found

to be largely attainable on a 15 m × 0.50 mm ID Carbowax

1540 SCOT column [268]. For the separation of menthofuran

other laboratories currently prefer a SCOT column coated

with FFAP, a modified Carbowax. Von Rudloff [230] has

however advised caution when using FFAP because of a report

that aldehydes may be adsorbed irreversibly [270]. Instead,

von Rudloff has drawn attention to the improved separations

now attainable for peppermint oil using a lower load of

3 percent Carbowax 20M on high-performance Chromosorb G

(DMCS, 100-120 mesh) in a 36 m × 4.5 mm OD packed column.

Since the improved separations on this column closely approach

that of the SCOT columns, von Rudloff has recommended use of

the former largely because of their ready availability.

Figure 1 [230] shows the required separations of peppermint

oil attainable on three well-known liquid phases: sucrose

diacetate hexaisobutyrate (SAIB), polyethylene glycol (PEG

or Carbowax) 20M, and polyethylene glycol adipate (PEGA).

A full discussion of the advantages and limitations

of each major type of liquid phase has been given by von Rudloff

[230]. From Figure 2 also by von Rudloff, several instances

can be seen in which the identities of terpenes could have

been confused with one another if reliance had been placed on

one or even two inappropriate liquid phases.

Improvement in the reliability of terpene

identification has also been obtained by the use of the

appropriate solid support, particularly Chromosorb G HP.

Figure 3 is an illustration of the effects of several solid

supports upon the resolving capabilities of the much-favoured

Carbowax 20M. It can be seen that such components as

tricyclene, sabinene, Δ_3-carene, camphene hydrate, α-terpineol

and borneol could otherwise be indistinguishable from

neighbouring peaks, particularly if present as minor components

appearing as shoulders on major component peaks.

Figure 1. Gas chromatograms of peppermint oil components separated on packed 3.5 m x 4.5 mm OD coiled copper columns [230]. These columns can be seen to separate 1,8-cineole from limonene, menthofuran from menthone, and menthyl acetate from pulegone, which could previously not be achieved on packed columns and necessitated the use of SCOT columns.
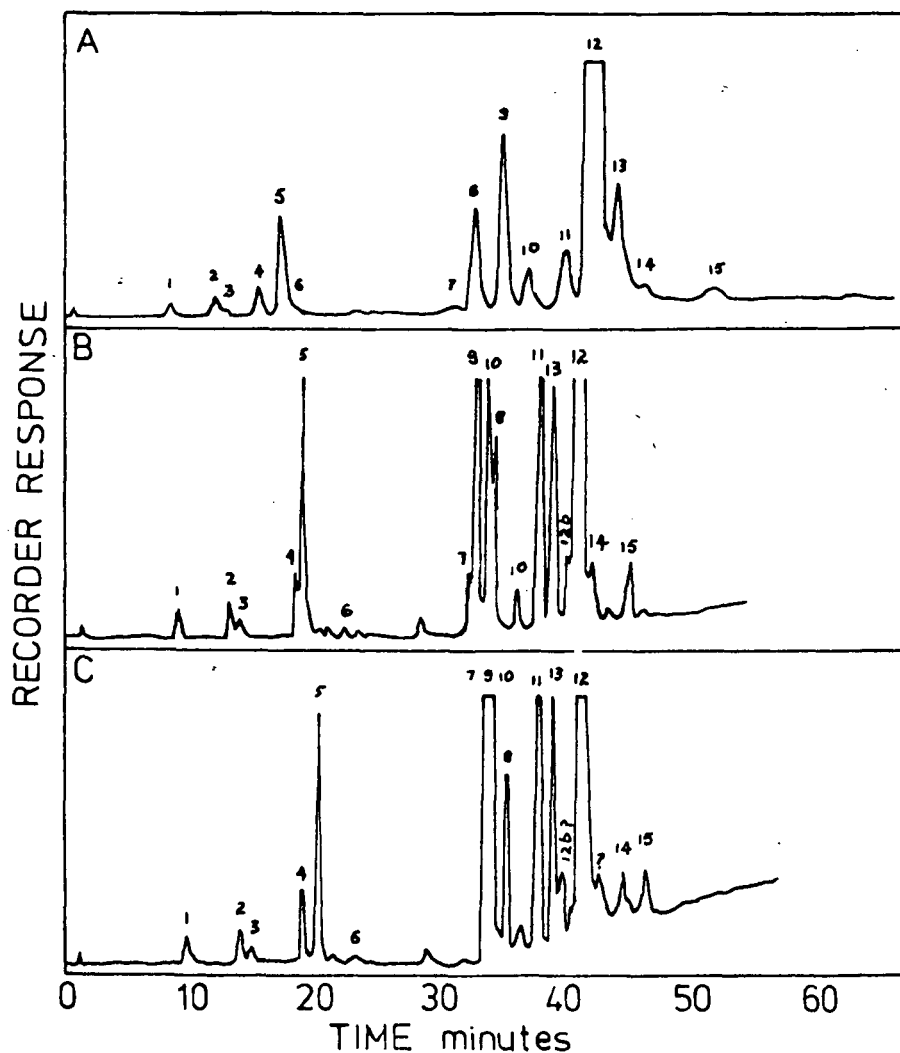
(A) 20% SAIB/Chromosorb W (HMDS, 60-80 mesh), 110-170°C at 1.5°/min;
(B) 3% PEG 20M/Chromosorb G HP (DMCS, 100-120), 50-200°C at 2.9°/min;
(C) 3% PEGA/Chromosorb G HP (DMCS, 100-120), 50-190°C at 2.9°/min.

Terpene identification:

peak 4	limonene	peak 11	neomenthol
5	1,8-cineole	12	menthol
7	sabinene hydrate	12b	caryophyllene
8	menthofuran	13	menthyl acetate
9	menthone	14	pulegone
10	isomenthone		

Figure 2. Gas chromatograms of leaf oil of white spruce *(Picea glauca)* separated on several types of liquid phases [230]. It can be seen that the identities of some terpenes could be confused if reliance had been placed on only one type of column.

Terpene identification:

peak		
1	santene	
2	tricyclene	
3	α-pinene	
4	camphene	
5	β-pinene (+ sabinene)	
6	Δ$_3$-carene	
7	myrcene	
8	α-phellandrene (+ α-terpinene)	
9	limonene	
10	1,8-cineole	
11	β-phellandrene	
12	γ-terpinene	
13	ρ-cymene	
14	terpinolene	
21	camphor	
24	linalool	
25a	camphene hydrate	
25b	bornyl acetate	
26	4-terpinenol	
30b	α-terpineol	
31	borneol	
32	Unidentified	
33	"	
34	"	
34a	"	
34b	"	
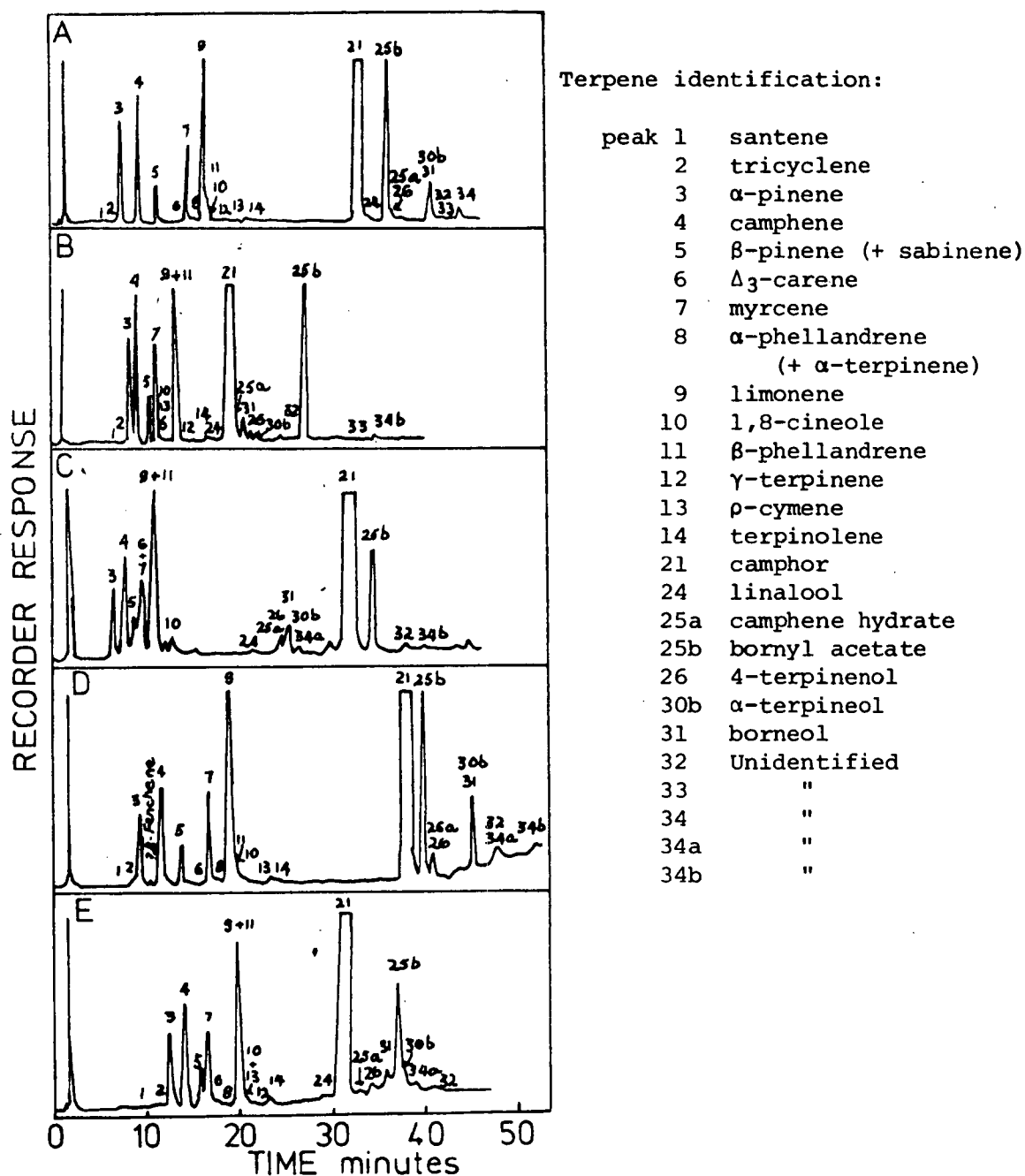
(A) 5% PEG 20M (Aeropak 30, 70-80 mesh), 50-200°C at 2.9°/min;

(B) 5% SE-30 (Gas-Chrom Q, 60-80 mesh), 50-200°C at 2.9°/min;

(C) 5% QF-1 (Gas-Chrom Q, 60-80 mesh), 50-200°C at 2.9°/min;

(D) 3% PEGA (high performance Chromosorb G, 100-120 mesh), 50-190°C at 2.9°/min;

(E) 5% Rapeseed oil (Aeropak 30, 70-80 mesh), 50-170°C at 2.9°/min.

Figure 3. Separation of some terpenes on Carbowax 20M coated on different solid supports [230]. It can be seen that a minor tricyclene shoulder on a major α-pinene peak, sabinene on β-pinene, Δ3-carene on myrcene, camphene hydrate on bornyl acetate or α-terpineol on a borneol peak may not be identified if an inappropriate solid support is used.
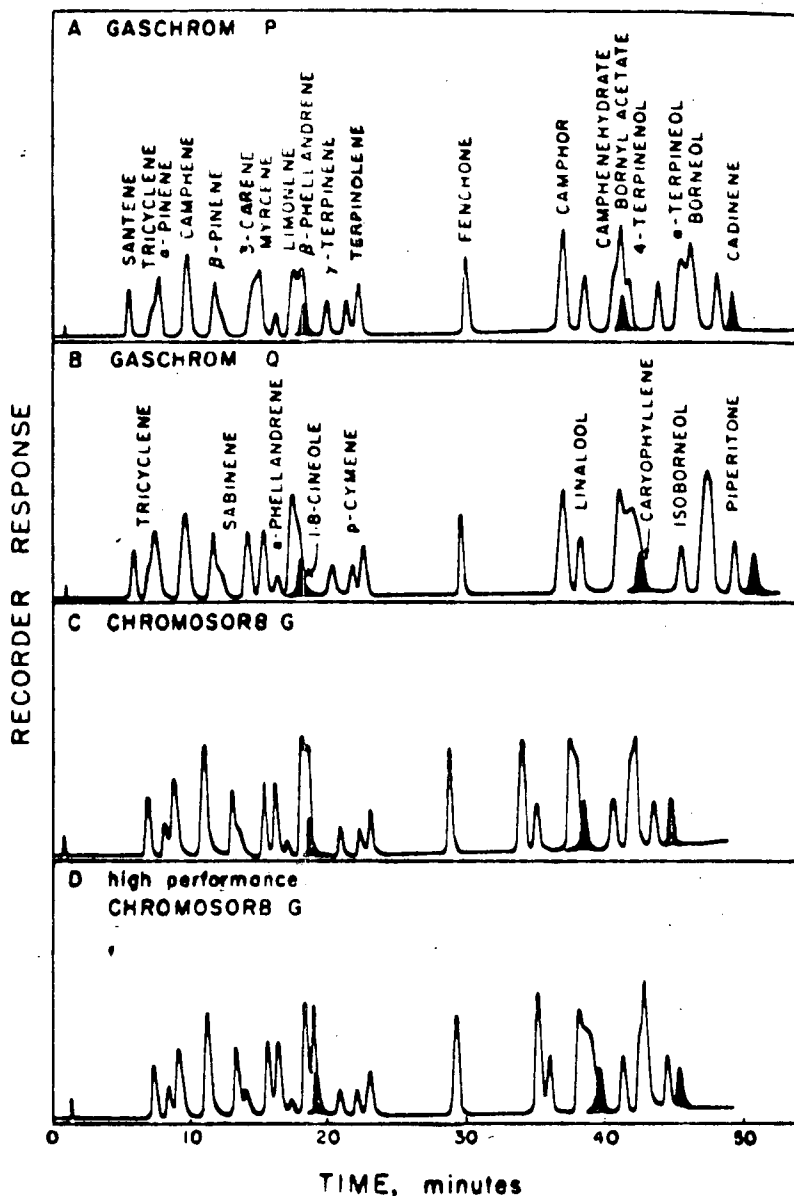
(A) Gas-Chrom P, 60-80 mesh (5% PEG);
(B) Gas-Chrom Q, 60-80 mesh (5% PEG);
(C) Chromosorb G, 70-80 mesh (3% PEG);
(D) High-performance Chromosorb G (AW-DMCS), 100-120 mesh (3% PEG)

from 50-200° at 2.9°/min.

Effect of injector system upon oil composition

Changes in the nature of certain terpenoids, when exposed to catalytic conditions in the injector chamber, may seriously affect the finally determined composition of an oil.

Much of the chemical alteration of components has been attributed to the effects of heat and acidic catalyst material upon labile terpenoids. Heat alone has caused myrcene to polymerize and block a heated needle during on-column preparative-GC [271]. Temperatures above 120-150°C have led to the dehydration of linalool [272] and camphene hydrate [273].

Day and Miller [274] reported the decomposition of terpenes at temperatures above 200 C, and in particular reported that α-terpineol decomposed at much lower temperatures. It was subsequently shown [275] that the decomposition could be avoided by direct injection of the sample onto the top of a packed column. At least part of the decomposition was then attributed to acidity in the sample or from the accumulated degradation products. α-Terpineol was subsequently injected up to 275°C without decomposition.

In spite of the most stringent precautions, such as a removable glass liner, control of temperature and on-column injection, some terpenoids still decompose, e.g. pulegone peroxide [276]. Other workers have described the hydrogenolysis of terpenes in a GC injector port [277].

The actual conditions of operating the injector chamber may also yield misleading compositional data. Although it might be assumed that most workers would heat the chamber to the required temperature, and also inject an appropriately

small sample, there are numerous reports of identifications

based upon an overloaded injector with resultant effects upon

retention data. Overloading at the column inlet will cause

the sample to act as a stationary phase for components eluting

after the major component [278]. Identification of trace

components may then be made difficult by alteration of their

retention data. In other cases workers have incorrectly

identified early-eluted trace components because of the effect

of a solvent peak, particularly when it elutes with a significant

tail.

Chemical changes of components on the GC column

One of the major obstacles encountered in GC analysis

of terpenes is the risk of on-column chemical changes, which

have frequently led to incorrect component identifications.

Most terpenes are acid-sensitive while several decompose on

exposure to higher temperatures [230].

Numerous instances of terpenoid chemical alteration

or irreversible adsorption have been cited or suspected.

It is considered that some components repeatedly identified

in essential oils may actually be artifacts, i.e. products

of degradation or rearrangement.

Much of the problem has also arisen from the

impracticability, or failure in other instances, of many

workers to confirm the identity of the compound that has

eluted from the GC column. It is a desirable practice to

isolate each eluted component responsible for a GC peak and

confirm its identity from a suitable spectrum. However many

of the components in essential oils, particularly the
sesquiterpenes and higher-boiling compounds, exist in numerous
possible isomeric forms, which have not been available as
standards that would make possible their unambiguous GC
identification. Von Rudloff [230] considered that so much
confusion has resulted from reports of sesquiterpene compositions
due to on-column changes, that much of the older work will have
to be reheated to establish the identities of e.g. the cadinenes
and muurolenes.

Instances of chemical alteration and adsorption
may be so subtle as to easily mislead the gas chromatographer.
Irreversible adsorption of minor components in a complex
mixture may not even be noticed. Major components may be
completely removed or diminished in the chromatogram. By
comparison, partial decomposition of a component may be
indicated by a change in peak shape, i.e. by a tail, peak-
broadening or the appearance of artifact peaks. Complete
degradation may result in an entire peak removal, which if
detected may be confused with an irreversible adsorption.
In a further instance a major component may be reported to
be completely unaffected by a particular column system, but
when present in trace proportions may be decomposed,
e.g. linalool and linalyl acetate [230].

From the mass of literature containing recommended
procedures to avoid on-column alteration of components, some
of the more accepted techniques include: avoid acid-washing
of solid supports, silanize support material to block any
active sites, operate at lowest possible column temperatures,

and coat liquid-phase onto solid support with a 3 to 5 percent loading to enable use of a lower operating temperature. Table 8 contains a list of some of the documented changes of essential oil components that have occurred on GC columns.

Table 8. Documented changes during elution of some essential oil components on GC columns

Change	Reference
Caryophyllene oxide both rearranged to an aldehyde and decomposed on a Reoplex 400 polyester column with an acid-washed Chromosorb W solid support.	[269]
Decomposition by labile terpenes may cause a change in peak shape, e.g. of sabinene, sabinene hydrate, camphene hydrate, linalool, linalyl acetate and others.	[279]
Acid-catalyzed terpene changes on Firebrick C-22 solid support	[280]
Sabinene always rearranged on a silicone oil column, while β-pinene only changed occasionally	[281]
Linalool and linalyl acetate both very sensitive to light, heat and acid, and decompose on GC packings under even the mildest conditions. When present in concentrations of less than 1 percent irregular-shaped peaks indicate decomposition even when column has been deactivated and operated below 130° to 150°C.	[230]
Several of the large number of possible stereoisomers of the humulenes, caryophyllenes and clovenes are subject to thermal rearrangements during GC.	[282]
Controversy over α-bergamotene and α-santalene assignments may be due to rearrangement on acid-washed Chromosorb W support.	[230, 283, 284]
Dehydration of elemol leaves doubt as to whether elemene is an artifact or a natural constituent.	[285]
Aldehydes may be irreversibly adsorbed on FFAP liquid phase.	[270]
Germacrene-A readily rearranged to β-elemene and β-selinene	[286]
Hedycaryol readily rearranged to elemol.	[287]
An alkaline Carbowax 20M column irreversibly adsorbed 2,8-p-menthadien-1-ols	[288]

Detailed discussions of chemical alteration of terpenes have been given in comprehensive reviews, e.g. in the GC of essential oils [230] and on GC column technology to prevent changes that would hinder identification of micro-quantities [187].

This consideration alone, of on-column terpene alteration, is justification of the need for spectroscopic identification of eluted components that have not been previously examined in a particular oil. Any purported oil analysis by elution from a single column should therefore be treated only as tentative.

Reaction-GC

Reaction-GC has been recommended as a means of identifying specific terpenes [289]. The technique has recently been discussed in detail as a means of preparing structurally diagnostic and more amenable derivatives of labile compounds [187]. Von Rudloff [230] has since considered the technique to have only limited value for terpenoid identification. By comparison Andersen *et al* [296] have presented highly reproducible GC retention data for common sesquiterpene dehydrogenation products.

Although reaction-GC would appear to be a valuable identification technique, it has only limited use for work with terpenes. Terpenes often yield multiple derivatives, e.g. upon hydrogenation or dehydrogenation, which may not indicate the original carbon skeleton. For example the selenium dehydrogenation of thujopsene gave three compounds [290]:

thujopsene   dihydrocurcumene   isocadalene
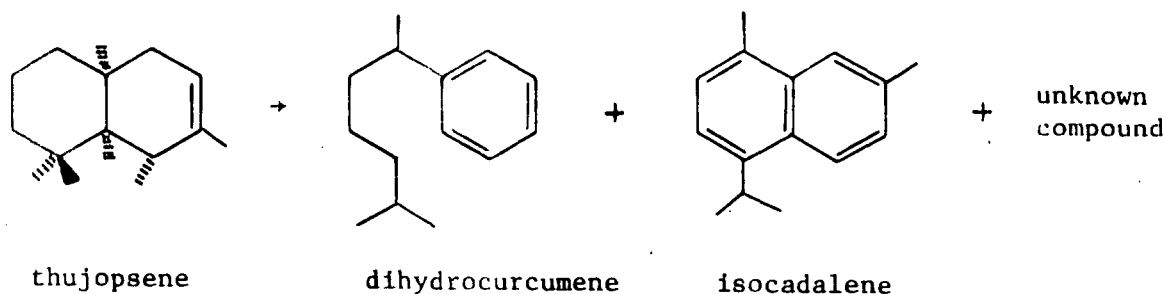
Nevertheless, the reaction may produce a fingerprint characteristic for comparison with available reference compounds [291].

Column abstraction techniques have also been recommended for the identification of terpenes [292, 295], e.g. the removal of primary and secondary terpene alcohols on a boric acid column [293, 294]. Von Rudloff has however warned [230] that on boric acid such tertiary alcohols as linalool are dehydrated and product a number of misleading isomeric hydrocarbons. Whereas in the case of camphene hydrate a single hydrocarbon is produced, i.e. camphene [273].

Spectroscopic identification of GC fractions

From the previous discussions there is little doubt that rigorous proof of identity of a terpenoid component resolved by GC should be obtained by spectroscopic methods. The advantages and limitations of spectroscopic methods of identification of terpenoids could only be adequately reviewed in a massive text. There is however a need to consider some aspects of this field, because the application of spectroscopy to the identification of terpenoids, particularly when studied in microquantities [187], has often led to reports of incorrect identification and structural assignments.

Of all the spectroscopic techniques MS, particularly as GC-MS, is widely regarded as the most valuable technique to be used in conjunction with others for terpenoid identification. Once the potential of MS was realized this technique was widely exploited for terpenoid identification. However, the amount of conflicting data that was soon published on terpene mass spectra [297] led Beynon *et al* [298] to specify that any proposals for terpene fragmentation mechanisms should be accompanied by isotopic labelling data. It was then realized that the study of fragmentation mechanisms could often provide contradictory conclusions to those obtained from a peak comparison identification. A full discussion has been given of the limitations of both methods in their use for terpenoid identification [297]. It was shown in particular that where a preionization rearrangement probably occurs, the use of only one of these approaches may lead to an incorrect conclusion. Contradictory conclusions from the use of both approaches would accordingly show each to be unreliable in such instances.

Although there are many well-documented instances when GC-MS has been used to advantage for separation and identification of terpenes [299, 301, 302], the extensive migration of hydrogen ions and double bond mobility due to electron impact, has imposed real limitations on the value of MS for the detection and position of double bonds in polyisoprenoids [300]. In spite of the cautions that have been advocated when using fragmentation patterns for terpene identification, a study by Thomas and Willhalm [303] has shown

that 32 cyclic terpenes could be distinguished from one another
while only 2 allo-ocimenes exhibited identical fragmentation
patterns. Numerous other instances of misleading MS
fragmentation data were cited in the recent biennial review of
MS by Burlingame *et al* [304].

The use of an IR spectrum in conjunction with NMR
and GC relative retention data usually suffices for
unequivocal identification of the better-known terpenes [230].
A detailed discussion has appeared recently [187], in which
it was shown that the IR identificaion of microquantities of
GC fractions has been misused by some workers who have failed
to realize the limits of the technique. It would appear that
although the use of reference spectra has often been used to
advantage as a means of excluding structural possibilities,
this procedure has been misunderstood by some workers who have
incorrectly considered that the IR spectrum constituted an
unambiguous fingerprint for each molecule.

The principle value of the IR spectrum lies in group
frequency interpretation, which will often lead to a partial
structural characterization that may supplement or confirm
conclusions from MS, etc. In addition, IR may yield structural
information not easily available elsewhere, such as the
specific environment of a carbonyl group, the stereochemistry
involving a hydroxyl moiety, the presence of a gem-dimethyl
system, or the type of aromatic substition.

Specific instances of misleading IR data for the
identification of terpenes have been well-documented. For
example, ozonolysis of an ocimene from lavender oil showed

that it had the b-structure, not the a-structure assumed

from its IR spectrum [305]. The poor resolution of some

sesquiterpene alcohols on GC columns, e.g. the elemol-eudesmol

and cadinal groups, lead to considerable difficulties in their

identification, particularly when the IR spectra are very

similar, e.g. the spectra of a-cadinol and γ-eudesmol [306].

Although the potential advantages of the coupled GC-IR system

has encouraged the development of many quite sensitive

instruments [187], von Rudloff [230] has concluded that the

poor standard of most of these vapour-state spectra would

usually prevent the unequivocal differentiation between

closely related terpenes.

NMR has tended to be used for the identification

of molecular structures that have not previously been studied.

It is not conveniently used for the study of sub-mg quantities,

unless sophisticated computer-based sensitivity-enhancing

techniques are available. This technique has therefore not

been misused as have MS and IR, as to lead to the existence

of a mass of conflicting structural information for the

identification of terpenes. This form of spectroscopy along

with numerous others, such as UV, ORD, laser-Raman, microwave

absorption, ion cyclotron resonance, etc., have been discussed

in detail in a recent text [187].

(iii) Factors affecting quantitation of essential oil

   components

The accepted error for the GC quantitation of oil

components, of as much as several percent for each component,

does not appear to significantly affect the compositions of

most reported oils. Considerable discussion has appeared on

the factors governing the quantitative error in determining

terpenoids, however most published reports have tended to

ignore these factors. Many workers have justified omitting

precautions to improve accuracy because it was considered

that the inherent GC detector error was more significant.

Although the error due to GC is generally acceptable

for original studies of the compositions of new oils, it is

however unacceptable for estimating the market value of

commercial oils that are based upon the contents of some major

components. Standard methods are used in these cases with

accuracy usually to within one percent. Standard specifications

for commercial oils are listed in the "British Standards" (U.K.)

and the Essential Oil Association's "E.O.A. Specifications and

Standards" (U.S.A.). GC is nevertheless the accepted technique

for minor and trace components.

By contrast it is surprising to note that some

workers have assumed an unreal degree of accuracy when using

GC for essential oils. Kugler and Kováts [242] reported the

percentage composition of a citrus oil to 2 and 3 decimal

places. Von Schantz and Ivors [311] and Lu et al [307]

similarly gave compositions of oils to 2 decimal places and

even 4 significant figures.

Von Rudloff [230] considered that his chemotaxonomic studies were adequate with a relative error of 0.1 to 0.3 percent, attainable with electronic integrators. However in reporting the composition of oil of tansy [312], using internal standards and a triangulation technique, he accounted for only 98 ± 2 percent of the oil.

In spite of the often-repeated claims that GC error is far less than 1 percent absolute for major components, together with 0.1 percent accuracy for minor components, several studies would indicate that these beliefs are poorly founded. Tables 9 and 10 illustrate the errors that may be obtained by analysis of oils on different liquid phases, while the error in replication is shown in Table 11. Von Rudloff has noted the absence of systematic studies of the errors in measuring terpenoids [230]. It would appear therefore that some study should be made of factors which might affect the error in quantitation of terpenoids, viz. detector responses for specific terpenes, peak measurement methods (such as triangulation, electronic integration and peak height comparison), the use of internal standards, the effect of carrier gas and hydrogen flow rates on relative molecular response of the flame ionization detector and the effects of different liquid phases. Such systematic studies would indicate the degree of confidence to be held by many workers when reporting the quantitative accuracy of the compositions of many essential oils.

Table 9. *Pinus ponderosa* resin monoterpene composition (%) determined using different columns [66]

Tree	Column	α-Pinene	β-Pinene	Δ_3-Carene	Myrcene	Limonene	β-Phellandrene
1	LAC	6.5	31.4	46.8	8.1	7.1	
	ODPN	5.4	25.9	51.2	7.0	6.3	2.0
2	LAC	5.6	13.8	43.5	14.0	23.1	
	ODPN	4.4	12.9	43.7	13.1	22.3	3.6
3	LAC	7.5	13.9	49.8	12.1	14.1	
	ODPN	5.0	14.6	51.8	11.2	13.1	1.9
4	LAC	8.6	37.4	34.2	10.6	9.2	
	ODPN	7.4	33.8	34.4	9.7	10.0	2.1
5	DDP	1.5	3.9	72.1	9.5	12.2	
	ODPN	2.2	3.3	73.3	7.8	11.1	1.1
6	DDP	5.9	38.3	43.8	11.7	0.4	
	ODPN	7.6	40.0	37.4	11.3	1.4	2.3
7	DDP	5.4	18.2	55.4	9.5	11.0	
	ODPN	6.4	17.5	52.1	7.9	10.7	2.5

Table 10. Estimates of α-pinene as percent total terpenes, using different columns [51]

Tree No.	Benzyl diphenyl	Di-iso decylphthalate	Dinonyl phthalate	Tolyl phosphate	Mean value
			Column		
OK/4	28.2	27.9	27.9	27.5	27.88
MA/6	32.6	32.1	31.4	32.1	32.05
MA/9	46.3	45.4	45.4	45.5	45.65
WU/3	64.8	63.9	64.7	62.6	64.00
WO/5	96.2	96.2	96.3	96.1	96.20
WO/6	96.8	96.8	96.7	96.8	96.78

Percent deviation from mean

OK/4	+1.15	+0.07	+0.07	-1.36
MA/6	+1.89	+0.16	-2.03	+0.16
MA/9	+1.42	-0.55	-0.55	-0.33
WU/3	+1.25	-0.16	+1.09	-2.19
WO/5	nil	nil	+0.10	-0.10
WO/6	+0.02	+0.02	+0.08	+0.02

Table 11. Replicate analyses of a single sample of
turpentine [144] (thermistor detector,
triangulation peak measurement,
di-iso-decylphthalate column)

α-Pinene	Camphene	β-Pinene	Limonene
29.7	1.9	65.2	3.2
29.6	1.6	65.8	3.0
29.6	1.7	65.4	3.4
29.4	1.8	65.6	3.2
29.4	1.9	65.7	2.8
29.4	1.4	65.8	3.4
29.4	1.6	66.0	2.9
29.7	1.6	66.4	2.4
29.1	2.0	65.1	3.8
29.0	1.7	65.8	3.5
29.0	2.0	65.9	3.2
29.1	1.3	66.0	3.7
29.5	1.2	65.7	3.5
28.5	1.5	65.8	4.2

Mean	29.3	1.7	65.7	3.3
Standard deviation	0.34	0.25	0.33	0.45

C. The effect of plant sampling upon the composition of an essential oil

The composition of the essential oil of a particular
plant species has too often been reported following the
mistaken assumption that the botanically-authenticated plant
sample contained an oil that was representative of the species.
In fact the composition of the oil from a species, or from a
single plant, may often vary widely depending upon the method
of sampling the oleoresin or plant material.

The essential oil from a single plant may differ depending upon the organ sampled, its location upon the plant, age and maturity of the plant tissue, the season of the year and daily fluctuations in plant biochemistry. The composition may even vary following the effect of plant injury due to burning, chopping or tissue removal during a previous sampling.

Further variations in composition may be exhibited between individual plants in a population and between different populations. In addition to fundamental clonal variations there may be genotypically different strains or chemotypes, which may be further masked by the subtle effects of hybridism in a population. Many different oil compositions reported for morphologically indistinguishable populations, growing in different regions, have been subsequently shown to be due to such genetic origins. Nevertheless, a number of environmental factors have been identified that may contribute to slight compositional differences, e.g. light, elevation, nutrition and sundry climatic features.

1.   Variations in oil composition within a single plant

(i)   Oils from different plant organs

Apart from the flowers, all oil-bearing organs of a plant are generally thought to contain a similar oil. However some plants are exceptions, e.g. *Cinnamomum zeylanicum*, which yields quite different oils from the leaves, roots and bark. Of the commercial cinnamon oils, oil of cinnamon bark contains 60 to 75 percent cinnamaldehyde and 6 to 15 percent eugenol, whereas the leaf oil contains 80 to 96 percent eugenol [1].

Recent detailed studies have shown that the differences
in oil composition from various tissues are wider than previously
thought. Roberts [313] discussed previous studies of oleoresin
composition from different tissues of *Pinus* sp., and in turn
reported considerable differences between monoterpene
percentages from needles, branch cortex, branch xylem, trunk
xylem and root xylem of *Pinus elliottii* var. *elliottii*
(Table 12). In a similar study of xylem and phloem
monoterpenoids of *Abies lasiocarpa*, Zavarin *et al* [52] noted
definite differences, and concluded that the two types of
oleoresin should be considered separately in chemosystematic
work. A further comparison of monoterpenes in heartwood and
sapwood of several *Pinus* spp. similarly showed fundamental
and often dramatic differences between the various oils
(Table 13) [314-317].

Table 12. Percentages of monoterpene hydrocarbons
in different tissues of 2 groups of
trees of *Pinus elliottii* var. *elliottii*
bearing high- and low-β-phellandrene
contents [313]

Tissue	Percentages of major components:			
	α-Pinene	β-Pinene	β-Phellandrene	Myrcene
(Trees with high β-phellandrene content)				
Needles	21	46	14	17
Branch cortex	20	38	23	18
Branch xylem	13	31	52	2
Trunk xylem	43	34	20	trace
Root xylem	82	17	trace	trace
(Trees with low β-phellandrene content)				
Needles	24	73	trace	trace
Branch cortex	36	62	trace	trace
Branch xylem	58	40	trace	trace
Trunk xylem	59	37	trace	trace
Root xylem	53	46	trace	trace

Table 13. The difference in composition of
monoterpenes (percentage of total)
in woody tissues of 2 *Pinus* spp.

Compound	*Pinus attenuata* [314]		*Pinus lambertiana* [315]	
	Sapwood	Heartwood	Sapwood	Heartwood
n-Heptane	–	trace	–	–
α-Pinene	–	72	14	55
Camphene	14	4	6	trace
β-Pinene	–	1	5	2
Δ_3-Carene	49	1	30	31
α-Phellandrene	–	trace	–	–
Myrcene	6	trace	8	2
Limonene	6	15	10	5
β-Phellandrene	17	trace	trace	–
γ-Terpinene	–	trace	8	trace
Terpinolene	–	7	19	5
Unidentified	8	–	–	–

From the studies cited it must be concluded that when
reporting the composition of an essential oil from a particular
species, that care should be taken to ensure that the oil was
extracted from a single organ or tissue that had been correctly
identified, e.g. whether xylem or phloem.

(ii) Oils from an organ located at different sites on the

plant

Oils extracted from parts of the same organ from
different positions of a plant may exhibit quantitative
differences. Numerous studies of such differences have been
reported for oils obtained particularly from different aspects
of a tree, various heights up a trunk, and even from one
leaf to another.

Von Rudloff [318] began his series of chemosystematic studies in the genus *Picea* with a consideration of the difficulty of obtaining a representative sample of oil from a tree. He found at different sides or heights that the needle oil contained no qualitative and relatively little quantitative differences. However the oil from one leaf to another showed considerable differences, so that to obtain a representative sample several branches had to be collected from different heights of a tree. In a study of individual leaf oils of *Juniperus ashei* [273] he found that the quantitative differences were relatively small for monoterpenes (1 to 5 percent) and more pronounced for sesquiterpenes (5 to 20 percent).

From such studies as that by von Rudloff most workers have concluded that the variations in oil composition in one particular organ, at different positions on a tree, are either negligible or not likely to cause any sampling problems. Similar work by Blight and McDonald [151] confirmed the view that there was little compositional difference between replicate samples taken from the same tree position (Table 14) and from different positions on trees of various *Pinus* spp. (Table 15).

Further investigation by other workers subsequently showed that although there were trees which exhibited little or no such variation in oil composition, there were others that varied considerably. Hanover [319] studied cortical oleoresin from several trees of *Pinus monticola* and found 13.6 and 13.8 percent Δ_3-carene in north- and south-facing branches, respectively. Yet in a further example he found 23.7 and 18.1 percent Δ_3-carene. In a similar study of

Table 14. Concentration of the major component in the
monoterpene fraction of cortical oleoresin
in replicate samples taken from the same
position on trees of two *Pinus* spp. [151]

Species	Major monoterpene	Percentage of monoterpene	Range of values
P. muricata	Δ_3-Carene	86.8 89.0	2.2
	α-Pinene	97.6 98.6	1.0
	Δ_3-Carene	69.0 69.1	0.1
	Δ_3-Carene	80.8 81.5 81.0 81.5	0.7
	Δ_3-Carene	61.0 59.3	1.7
P. montezumae	Δ_3-Carene	87.8 88.1	0.3
	α-Pinene	98.0 98.6	0.6
	α-Pinene	95.1 94.6	0.5
	α-Pinene	98.2 98.5	0.3
	α-Pinene	98.4 98.4	–

Mean range: 0.8 percent

Table 15. Concentration of the major component in
the monoterpene fraction of cortical
oleoresin taken from different sample
positions (height and compass point)
within trees of two *Pinus* spp. [151]

P. *muricata*		P. *radiata*		P. *radiata*	
Position	Percent Δ_3-carene	Position	Percent β-pinene	Position	Percent β-pinene
6 in. NW	91.1	6 in. NW	76.3	6 in. N	83.9
6 in. SE	89.3	6 in. SE	75.0	6 in. S	84.1
4 ft. NW	90.1	4 ft. NW	75.1	3 ft. N	83.0
4 ft. SE	90.1	4 ft. SE	75.1	3 ft. S	83.8
29 ft. NW	91.8	30 ft. NW	74.9	4 ft. N	83.6
29 ft. SE	90.6	30 ft. SE	76.0	4 ft. S	82.8
50 ft. NW	90.6			5 ft. N	83.6
				5 ft. S	84.9
Mean	90.5		75.4		83.7
Standard deviation	0.8		0.59		0.65

Average standard deviation: 0.7 percent

Pinus ponderosa cortical oleoresin Smith [66] found no

compositional differences at various compass points, but small

and consistent differences were noted at various trunk heights,

e.g. 48.3 and 45.9 β-pinene at 3 and 60 ft., respectively.

Smith did however recommend the need for further work to determine the authenticity of the slight increase in β-pinene with a decrease in limonene in going from tree top to base.

A very detailed study of the compositional variation of trunk xylem oleoresin in groups of *Pinus elliottii* var. *elliottii* showed almost dramatic differences. Roberts [313] distinguished high- and low-β-phellandrene trees, and found height changes in all four trees examined. The changes were greater in high- than low-β-phellandrene trees (Table 16).

Table 16.   Percentage composition of monoterpene
            hydrocarbons in xylem oleoresin from
            3 trunk heights [313]

Height above ground (ft.)	α-Pinene	β-Pinene	β-Phellandrene
(Trees with high β-phellandrene content)			
1	71	21	6
16	31	40	26
31	26	40	27
(Trees with low β-phellandrene content)			
1	54	41	trace
16	59	36	trace
31	64	33	trace

In subsequent intensive sampling of trunk xylem from one high-β-phellandrene tree, Roberts demonstrated a reciprocal relationship between α- and β-pinene throughout the trunk length (Figure 4).

Figure 4. Changes in monoterpene hydrocarbon
composition of trunk xylem eleoresin
throughout the height of one high-
β-phellandrene tree of *Pinus elliottii*
var. *elliottii* [313]
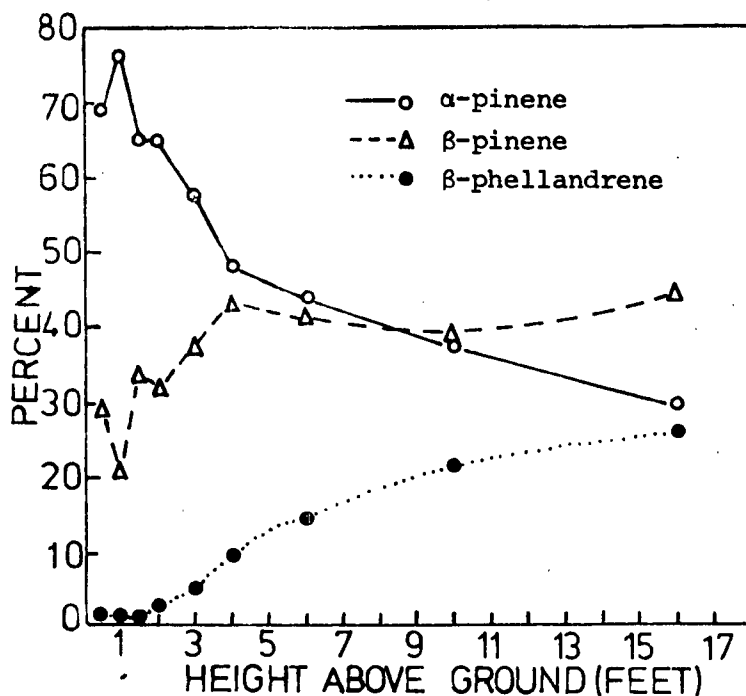
From numerous often contradictory studies of oil

composition in tissues sampled from various positions on a

tree, it would appear that to obtain a representative sample

tissue should be bulked together from several positions.

It should be noted that most inconclusive studies involved

the analysis of oil from either foliage or cortical oleoresin,

while the greatest compositional differences were found in one

of the few systematic investigations of xylem oleoresin.

A need therefore exists for an extension of Roberts' work [313]

on xylem oleoresin to determine the existence of any phenomenal

differences in other populations and species.

(iii) **Oils from tissues of various ages**

There is now sufficient evidence to conclude that
for many species there is a widespread change in oil
composition associated with change in biochemical activity
with growth of tissue. Lesser changes occur over much longer
periods in mature tissues where biochemical activity has almost
ceased. Earlier observations by such workers as Pigulevskii [320]
had been disputed by Smith [321] and others, whose inadequately-
designed investigations [66] had led to contradictory views.
Smith asserted that Pigulevskii's report of the difference in
monoterpene composition of xylem oleoresin in groups of young and
old trees was merely another instance of intraspecific variation.

Essential oils isolated from immature tissues have
often been shown to differ considerably from the composition
of oils from mature tissues. Reitsema *et al* [322] used C^{14}-
labelling techniques to document some of the qualitative
changes of oil in new tissues of *Mentha piperita*. In a
study of oil from *Pinus pinaster* seedlings Funes *et al* [323]
found under various growth conditions that the composition
changed from predominantly α- and β-pinene to an oil containing
a high proportion of other components (Table 17). Other
studies of needle and foliage oil confirmed that younger
tissues of *Thuja plicata* [324], *Pinus ponderosa* [85], *Pinus
elliottii* var. *elliottii* [313] and *Pseudotsuga menziesii* [22]
contained oils with compositions different from those of mature
leaf tissue.

Table 17.   Percentage compositions of oil of *Pinus pinaster* seedlings cultivated under various conditions [323]

Time of growth:	0	3	6	10	15	20	25-hr.
(Cultivated in dark)							
α-Pinene	84.6	92.0	72.0	46.0	36.5	35.1	40.0
β-Pinene	15.3	8.0	28.0	54.0	63.0	58.0	57.8
Sabinene	–	–	–	–	–	1.6	1.0
Δ₃-Carene	–	–	–	–	–	0.3	0.4
Limonene	–	–	–	–	–	1.6	1.2
(Cultivation with 14-hr. illumination)							
α-Pinene	84.6	80.3	94.0	43.0	35.0	33.5	33.1
β-Pinene	15.3	13.2	6.0	57.0	60.0	63.0	63.5
Sabinene	–	–	–	–	2.9	1.4	0.7
Δ₃-Carene	–	–	–	–	8.8	0.9	1.0
Limonene	–	–	–	–	1.7	11.0	1.5
(Cultivation with continuous illumination)							
α-Pinene	84.6	92.0	81.0	45.4	33.5	35.5	35.7
β-Pinene	15.3	8.0	19.0	54.6	66.5	51.4	55.8
Sabinene	–	–	–	–	0.4	2.1	1.3
Δ₃-Carene	–	–	–	–	–	1.3	1.3
Limonene	–	–	–	–	–	3.7	22.3
β-Phellandrene	–	–	–	–	–	0.9	0.7
Terpinolene	–	–	–	–	–	5.0	3.9

Possibly the most detailed study of the oil changes which ensue during development of buds of *Picea mariana*, has been given by von Rudloff [325]. After initially demonstrating the compositional differences between the oils of leaves, buds and twigs (Table 18), von Rudloff documented the considerable changes in major oil components throughout the year as buds developed into new leaves (Figures 5-7).

Table 18. Percentage composition of terpenes in oils of leaves, buds and twigs of *Picea mariana* [325]

Component in oil	Leaves	Buds	Twigs
Santene	5.5	-	0.1
Tricyclene	1.3	-	trace
α-Pinene	8.8	14.5	18.4
Camphene	19.1	0.8	0.9
β-Pinene	1.3	11.9	9.8
Sabinene	0.1	2.5	2.0
Myrcene	2.2	4.0	5.3
Δ3-Carene	0.2	50.5	50.5
Limonene	3.3	0.9	1.6
β-Phellandrene	0.4	0.7	1.8
Terpinolene	0.6	4.7	4.7
1,8-Cineole	0.6	-	-
Camphene hydrate	1.7	trace	trace
Terpinen-4-ol	0.1	0.3	0.8
Borneol	0.9	trace	trace
α-Terpineol	0.5	0.3	0.5
Bornyl acetate	46.5	6.4	1.3
C_{15} hydrocarbons	1.0	0.7	1.1
C_{15} alcohols	2.7	0.4	0.8

Changes in components of cortical oleoresin have similarly been studied [313, 319, 326], although the differences have not always been so noteworthy. Nevertheless young cortical tissue of *Pinus radiata* has been shown [327] to yield an oil which has a composition far different from older oleoresin (Table 19).

It must be concluded that for the above species at least, the composition of the essential oil may for many trees depend upon the age of the tissue sampled. Sampling of foliage
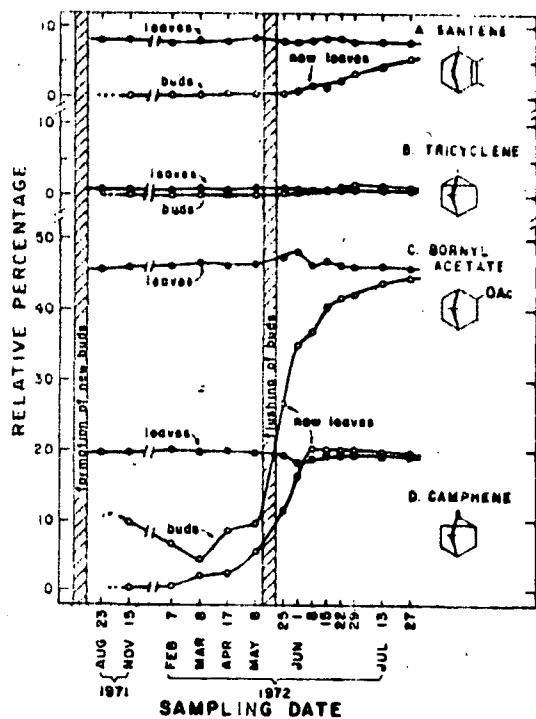
Fig. 5 Change in the relative percentage of (A) santene, (B) tricyclene, (C) bornyl acetate, (D) camphene in the volatile oil of the mature leaves (●), buds and young leaves (O) during the course of a single year (August to July). [325]
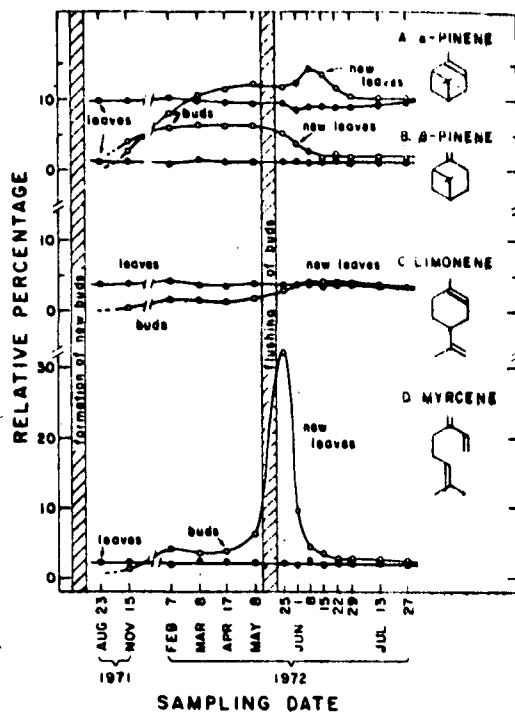
Fig. 6 Change in the relative percentage of (A) x-pinene, (B) β-pinene, (C) limonene, and D) myrcene in the volatile oil of the mature leaves (●), buds and young leaves (O) during the course of a single year (August to July). [325]
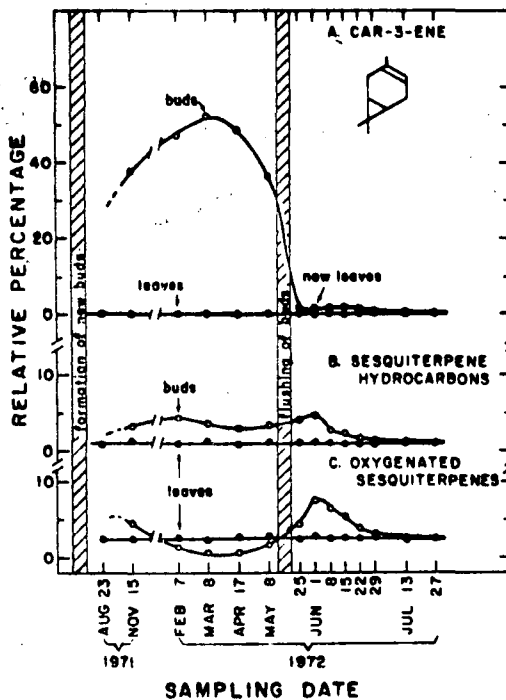
Fig. 7 Change in the relative percentage of (A) car-3-ene, (B) sesquiterpene hydrocarbons and (C) oxygenated sesquiterpenes in the volatile oil of the mature leaves (●), buds and young leaves (O) during the course of a single year (August to July). [325]

Table 19. Percentage monoterpene composition of
cortical oleoresins of *Pinus radiata*
[327]

Major components	Composition of mature blister oleoresin	Composition of young cortical oleoresin
α-Pinene	70.3	33.5
β-Pinene	28.5	8.5
Δ$_3$-Carene	-	16.5
Limonene	0.9	31.6
β-Phellandrene	-	4.8
Terpinolene	0.3	5.1

in particular should therefore be based upon a selection

not of random tissue but of uniformly-aged tissue.

(iv) Short-term variations in oil composition

It has been postulated by some workers that short-

term physiological changes may cause fluctuations in oil

content, and even composition, i.e. at different times of the

day or following the physiological trauma that accompanies

tree injury. The results of investigations are as yet

inconclusive.

(a) Daily changes in oils

Daily periodicity or variations in physiological

activity were shown by Schib to result in considerable

differences in oil yield of *Pinus sylvestris* [328] and leaf

oil of *Salvia officinalis* [329]. A 50 percent rise in oil

yield has also been reported for *Mentha arvensis* sampled at

10 A.M. instead of 6 A.M. [43]. It should be noted that none
of these investigations utilized analytical techniques which
could detect subtle differences in composition.

(b) Injury-induced changes in oil composition

Preliminary studies, and other indirect evidence,
would also suggest that there are changes in composition of
volatiles following tree injury. Madden [331, 332] has
studied the attractiveness of the woodwasp, *Sirex noctilio*
to *Pinus radiata*, and concluded that physiologically stressed
trees probably released a volatile insect attractant. In his
investigations Madden correlated the duration of insect
attack with fairly definite periods following physiological
stress, viz. attack/attractiveness of about 14 days after
felling, attack some 9-12 days after lopping or girdling, and
prolonged attacked if felled. The inference from these
investigations is that the uninjured tree exudes an unattractive
mixture of volatile vapours. The only other report found in
the literature which would support the possibility of changes
in essential oil composition was a preliminary investigation
by Roberts [313]. This latter author noted that the oleoresin
composition of *Pinus elliottii* var. *elliottii* is changed on
the wounded side of a tree but not on the unwounded reverse
side. Although additional work was envisaged no report was found
of any further conclusions.

Further investigations are required to confirm the
possibility of short-term variations, particularly those which

might be due to tree injury and could possibly contribute to an apparently greater variability in composition between individual plants.

2. Variability in oil composition between individual plants

Possibly the greatest differences in essential oil composition are genetically-based intraspecific quantitative variations. Tree-to-tree variations may be much greater than those between the averages from trees growing on different plots [66].

(i) Variations within a small population

Non-genetic factors, such as age, elevation, etc., which have been thought to lead to wide quantitative variations in wood oleoresin for trees on the same plot, have been studied in detail and found not to contribute significantly [66]. Such variations have been reported for oleoresins from numerous *Pinus* species [321] including *P. contorta*, *P. maritima* and *P. pinaster*, *P. muricata*, *P. radiata* and *P. ponderosa*.

The ranges of concentrations of α-pinene in gum turpentine from *Pinus radiata* [144], grown at different sites, are typical of the variations to be found for some other species (Figure 8). Leaf oils similarly exhibit wide tree-to-tree variations (Table 20), as shown in recent studies of *Juniperus scopulorum* [333].

Figure 8. The variation in percentage concentration of α-pinene in gum turpentines of five populations of *Pinus radiata* [144]. Arrows indicate sample means.

α-PINENE CONTENT (percent)
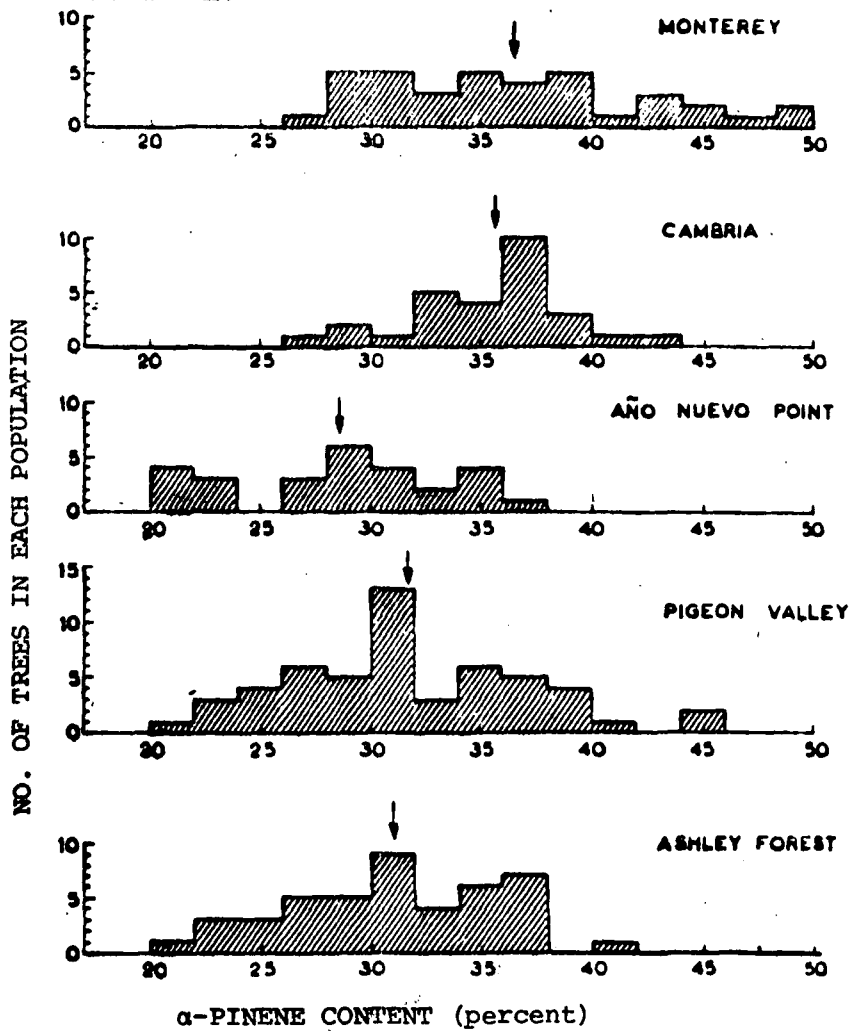
Table 20. Tree-to-tree variation in percentages (excluding aromatic ethers) of major terpene components in foliage oils of 10 *Juniperus scopulorum* trees from a south-western British Columbia district [333]

Tree no.	1	2	3	4	5	6	7	8	9	10
α-Thujene } α-Pinene	3	4.5	6	5	4.5	4.5	6	4	3.5	4.5
Sabinene	34	45	48	32	40.5	37.5	35	40	36.5	50
Myrcene	1	1	1.5	1	1	1	1	1	1	1
Limonene	1.5	1.5	2.5	1.5	2.5	1	1	1	1.5	1.5
Terpinenes	6	6.5	6.5	4.5	5.5	3.5	4	4	4.5	5
Terpinen-4-ol	8	9	8.5	11	15	12	12	8.5	13.5	10
Sabinene hydrates	2	2	0.5	1.5	1	1.5	1.5	2	1	1.5
Citronellol					less than 0.5					
Methyl citronellate	4	3.5	8	5.5	6.5	3.5	4.5	7	5.5	9
Cadinenes	2.5	3	1	2.5	3.5	5	1.5	2	1.5	1.5
Cadinols + acetates					less than 0.5					
Elemol + acetates	37	21.5	15	31	20	25	30	27.5	27.5	10
Aromatic ethers	1	1	6.5	1.5	31.5	29	26	29	10	27.5

It would appear from these few cited examples that the tree-to-tree variation in oil composition could be so great that a representative oil sample might only be obtained if plant tissue were collected from a large number of trees growing on a single plot.

(ii) Variations between populations

The variations in essential oil composition between-trees and between-populations have been variously described as due to one or other, or both, environmental and genetic considerations. Since there are now sufficient studies to substantiate the view that between-population variations are very often less than between-tree variations, it would appear for conifers at least, that genetic variability has a much greater influence on oil composition.

In general, clonal variability may be within ±1.0 percent for α- and β-pinene in Pinus radiata and P. attenuata [151]. Hanover [319] has experimentally confirmed that the effects of a range of environmental factors were barely detectable. Four clones of Pinus monticola were grown at three completely different locations. Variations in percentages of the monoterpenes in each oil were generally within experimental error (Table 21).

Numerous investigations have since been founded upon carefully-documented within-population oil variations to show the existence of genotypes and "chemovars", together with instances of hybridism, particularly among conifers distributed

Table 21. Percentage concentrations of monoterpenes
in 4 clones of *Pinus monticola* grown at
3 completely different environments [319]

Clone No.	Site No.	α-Pinene	β-Pinene	Myrcene	Δ_3-Carene	Limonene
I	1	5.0	0.4	5.0	1.2	16.4
	2	4.6	0.2	4.8	0.8	14.6
	3	5.4	1.4	4.6	2.2	13.2
II	1	4.6	13.4	2.9	12.0	2.3
	2	4.7	14.3	2.2	11.0	1.6
	3	4.4	13.5	2.6	11.0	2.1
III	1	5.5	19.2	3.9	0.5	3.3
	2	7.2	23.5	3.8	0.3	1.7
	3	7.2	20.8	3.8	1.8	3.6
IV	1	5.2	5.4	6.6	0.4	13.4
	2	4.8	5.3	7.6	0.3	10.8
	3	6.0	4.0	6.0	0.8	10.3

throughout New Zealand and North America [52, 144, 151, 334-6].
In each study, variability within a species has been documented
for populations growing at a sufficiently large number of sites.
Von Rudloff's study of *Juniperus* leaf oils [333] is possibly
one of the best documented investigations. From this latter
study, the variability of mean compositions of leaf oils of
each of 10 populations of *J. scopulorum* (Table 22), is seen to
be no greater than the between-tree variation at one site
(Table 20). Some of the variation in Table 22 was also
attributed to a degree of hybridism.

From the few investigations cited it is apparent
that the composition of an oil to be reported from a particular
species, or one of its forms, should only be quoted as
characteristic of the species if a study has first been made
of the range of compositions that are to be found in several
populations.

Table 22. Mean percentages of major components (excluding aromatic ethers) of foliage oils of 10 trees of *Juniperus scopulorum* of each of 10 different collection sites [333]

Tree no.	1a	1b	2	3	4	5	13	14	15	16	17
α-Thujene	1.5	2	1.5	2	1.5	1.5	2	2	2	1.5	1
α-Pinene	2.5	3	2	5	2.5	3	3	2.5	3.5	3.5	3
Sabinene	40.5	44.5	34	34	29	42	38	36	40.5	48	46.5
Myrcene	1	1	1	1	1	1.5	1	1	2	2	2
Limonene	1.5	2	4	11	10.5	9	2	6.5	10.5	2.5	2
Terpinenes	8	4.5	2.5	2.5	3	5.5	3.5	5.5	7.5	7	6
Terpinen-4-ol	11	6	5.5	2.5	3.5	7	4.5	5	8	9	6.5
Sabinene hydrates	2	2	0.5	4	2	2	2.5	1	1.5	2	2
Citronellol	0.5	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methyl citronellate	6	5	2	3.5	1.5	6.5	0.5	0.5	0.5	0.5	0.5
Cadinenes	2.5	2	1	0.5	1.5	2	1.5	1.5	1	0.5	1.5
Cadinols + acetates		less than 0.5					1.5		less than 0.5		
Elemol + acetates	18	23	39	32	41	16	37	35	18	17	23
Aromatic ethers	19	18	6	2	1.5	3.5	0.5	0.5	0.5	0.5	0.5

* No. 1 = Keremeos (a) March 1970, (b) October 1973, No. 2 = Trapp Lake, No. 3 = Dutch Creek, No. 4 = Windermere and No. 5 = Golden, British Columbia; No. 13 = Billings and No. 14 = Garrison, Montana; No. 15 = Cortez, No. 16 = Alamosa and No. 17 = Manitou Springs regions, Colorado.

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D. Concepts of essential oil composition

The composition of an essential oil is an arbitrary concept which has been used to best advantage to characterize the oil isolated from a particular biological source. The concept derives its arbitrary nature from the use of a physical technique to isolate the oil, followed by a host of minor chemical alterations, artifacts and component losses associated with the techniques for isolation and analysis. The limitations of these techniques do not therefore enable more than a rough estimate to be made of the terpenoid composition in either living tissue, or in the atmosphere at a distance from the plant. The composition of the arbitrarily obtained and analyzed oil is however well suited for its commercial evaluation and for chemosystematic studies of plant taxonomy.

1. Static concept of essential oil composition

From historical times there has been a preoccupation with a static concept of essential oil composition, in which the qualitative and quantitative composition differs little for all oils extracted from numerous populations within a species. This view has existed despite many well known instances of variations in the composition of each oil. The limited technology available earlier for the isolation of essential oils has caused most workers to think of oil composition as static rather than dynamic. The actual isolation of an oil with measurable characteristics has tended to lead workers toward the static concept. As a result of this concept many investigations of

the attraction of insects to terpenoids of host plants have been based only upon an extensive program of separation and identification of components, with a subsequent finding of no particularly attractive component(s). The static concept has also severely limited the whole field of investigation into the biosynthesis of terpenoids, which has to this time, except for tracer studies, been based largely upon the co-occurrence of terpenes with respect to one another.

2. Dynamic concept of essential oil composition

A dynamic concept of essential oil composition would allow for the existence of an ever-changing composition, based upon a major genetic control and physiological factors producing different proportions of terpenes within certain limits. The development of suitable technology for the instantaneous measurement of changing terpenoid concentrations in and about tissues, would greatly facilitate investigations of insect attractants. The possibility of a temporarily-produced attractant compound, perhaps influenced by external stimulation, could be studied together with a wide range of attractive proportionate mixtures of terpenoids. The worker interested in terpenoid biosynthesis might by this concept be able to examine relationships of terpenoids changing quantitatively with respect to one another, thereby giving real-time data indicating the biosynthetic origin of each compound. The value of this latter type of study has been realized to a limited extent in investigations of changes in terpenoids during the process of plant maturation.

3. **Subjective versus objective assessment of an essential oil**

In perfume and food flavour evaluation accent is given to organoleptic or subjective methods of assessment, assisted by instrumental or objective means wherever applicable. By contrast, the vast field of investigation into new essential oils has been based almost entirely upon objective methods of examination. The objective techniques for examining a new oil have consequently come to be considered almost as standard procedures, and even to have superseded subjective methods.

From the numerous publications of investigations into terpenoid insect attractants it would appear that workers have attempted to correlate bio-assay or bio-response insect-subjective methods with instrumental objective techniques. Very often an oil or a piece of plant tissue has been exposed to caged insects and their responses noted. Unfortunately these investigations have been handicapped because the insect has been unable to subjectively evaluate the oil vapour under the same conditions that apply in nature.

The absence of techniques for the instantaneous measurement of terpenoid concentrations in and about plant tissue, due in part to a preoccupation with the static concept of oil composition, has led investigators of insect attractants to bypass any concept of the atmospheric vapour composition that is subjectively evaluated by the insect under natural conditions. Consequently, the conditions under which a sample of oil or odorous tissue is exposed to a caged insect could bear only a rough resemblance to the real-life situation. There will be no guarantee that the exposed oil came from the

right plant organ, that the oil was isolated from the tissue
under the right physiological conditions, or that the
composition of the oil had not been altered during the isolation
procedure.

Investigations of insect response to essential oil
vapours should therefore be based upon an appreciation of the
oil composition perceived by the insect in the atmosphere,
rather than upon an arbitrary concept of composition that may
be completely meaningless.

4.   Compositions of essential oils from a plant species

From preceding sections it is possible to distinguish
a variety of essential oil 'compositions' from a single
species, or even from one particular plant, viz:

oil sampled from more than one tree, such as

mean oil composition from several tree populations
(Table 22),

mean oil composition from several trees of a clone
(Table 21),

mean oil composition from several trees of a population
(Table 20);

oil sampled from a single tree, that is

from different organs, i.e. leaves, cortex, xylem,
flowers (Tables 12, 13),

from the same organ at different positions on the
tree (Table 16, Figure 4),

from tissues of various ages (Table 18, Figures 5-7), and

oils produced in a tree experiencing different
physiological conditions (hypothesized);

oil compositions modified by techniques for isolation

and analysis, such as

pre-extraction techniques (fermentation and enzyme

action on plant material, comminution, storage,

drying and autoxidation);

extraction techniques (oleoresin collection method,

distillation, solvent extraction, direct micro-

extraction from glands, trapping directly onto a

GC column and solid-sampling GC),

techniques for separation and analysis of

components (fractional distillation, column

chromatography, chemical derivative separations,

TLC and GC);

and oils sampled in the vapour phase, involving

techniques for direct-sampling and analysis

(container surface effects),

techniques for trapping and condensation

(cold-trap condensation, solvent trapping,

solid adsorbent trapping, and adsorption on

coated and uncoated GC supports).

From this summary it can be seen that there is no

recognized method for instantaneously and accurately measuring

the concentrations of terpenoids in living tissue, which would

make possible the documentation of any rapid changes in oil

composition. Each of the physical techniques for isolating an

oil are time-consuming and hence preclude an instantaneous

or rapid analysis. By contrast, direct-sampling and GC analysis

of terpenoids in the vapour phase may be carried out in an almost instantaneous process. However direct-sampling techniques reviewed in previous sections were all seen to involve a degree of component selectivity, e.g. by loss of components onto container surfaces or into rubber sleeves.

It must be concluded that if essential oil studies are to become more meaningful in the fields of insect attractants and terpene biosynthesis, then a technique is required that provides a more realistic assessment of the oil composition by enabling instantaneous isolation and analysis.

5. Instantaneous analysis: the ultimate concept of

    essential oil composition

Even though a need has been described for instantaneous analysis of terpenoids in and about living tissue, it is not envisaged that this ultimate concept will be fully realized.

Possibly the only type of procedure that might eventually be developed for instantaneous analysis would be a form of direct-sampling of freshly-released vapours. This technique has been of particular value in studies of volatiles released from cultures of micro-organisms grown in actual sample containers. It is not expected therefore that this technique could be directly applicable to large trees.

Changes in terpenoid concentrations in small amounts of plant tissue from large trees could conceivably be studied in a direct-sampling system. Unfortunately the tissue would need to be comminuted to reduce it to a manageable size.

Changes in large plants could therefore only be measured in consecutive portions of tissue. Conversely, changes in small plants might be studied without damaging the plant.

An investigation should therefore be made of direct-sampling procedures, with an attempt to overcome present limitations, and perhaps obtain instantaneous vapour analyses.

A RECOMMENDED ROUTINE ANALYTICAL PROCEDURE

FOR USE IN A SURVEY OF ESSENTIAL OILS

The present widely-held view, that selection and
sequence of techniques for the analysis of an essential oil
is specific to each oil, mitigates against the feasibility
of a routine analytical procedure for use in a detailed
survey of economically-important oil-bearing plants. Many
reports of the detailed analysis of oils indicate that
workers have often spent several months studying a particular
oil. A survey of oil-bearing plants would necessarily require
an analytical procedure that took only a few days for each
oil.

Numerous combinations of techniques have been used
for the separation and analysis of major, trace and difficultly-
separable components. For the analysis of cedar oil, from
Juniperus virginiana, Wenninger *et al* [249] separated several
hydrocarbon fractions from the oil by column chromatography
on alumina, then isolated silver nitrate-adductable and
non-adductable sub-fractions, which were in turn fractionally
distilled and purified by preparative GC before final
spectroscopic and GC identification of sesquiterpenes.
Oxygenated monoterpenes and sesquiterpene hydrocarbons from
the cortical oleoresin of *Abies magnifica* [122] were separated
as above on alumina, fractionally distilled to separate mono-
from sesquiterpenes, again fractionally distilled using a

spinning band column, followed by combinations of preparative GC and silver nitrate-silica gel chromatography to isolate individual components. Von Rudloff used preparative GC alone to separate components of needle oil of *Picea pungens* [330]; whereas for oil of leaves of *Juniperus communis* [69] he used Carbowax 20M-coated silicic acid to separate hydrocarbon, mid- and polar-fractions, which were in turn subjected to preparative GC to isolate individual components for identification, principally by GC and IR. Much of von Rudloff's work with other oils involved a combination of silicic acid-column chromatography and preparative GC pre-fractionation, with identification by IR and GC on dissimilar columns.

Many workers have futilely pursued, as their ultimate goal, the identification and quantitation of the smallest possible traces of components. To pursue this objective invariably requires several fractionation steps, each of which may cause component changes, losses or introduction of artifacts. A point should therefore be set beyond which a search for even smaller traces of components would yield no further significant information.

In a survey of the economic importance of new essential oils, the analytical procedure used should enable positive identification and quantitation of all major components, together with at least a tentative identification of trace components. Such a procedure would therefore be expected to yield qualitative and quantitative data on about

95 percent of the mass of the oil. The procedure should also involve a minimum number of fractionation steps, to both reduce the risk of chemical changes and minimize the time needed for the entire operation.

To formulate a detailed analysis that was feasible for routine use in a survey of essential oils, a study was required of the effectiveness of a simple sequence of techniques, such as column chromatography and preparative GC prefractionation followed by analytical GC and IR identification.

A. Techniques

An examination was made of the separating efficiency of several techniques which could perhaps be combined in a basic system to yield the most qualitative information for the limited work involved.

1. Gas chromatography

(1) Analytical column system

The relative suitabilities for the chromatography of terpenoids were determined for 2 m × 4 mm ID glass columns packed with:

10% DC-200 + 15% QF-1(1:1 mixed bed)/Gas Chrom Q(80-100),

3% OV-1/Chromosorb W HP (80-100),

3% OV-17/Chromosorb W HP (80-100),

3% OV-17/Gas Chrom Q (80-100),

3% OV-17/Gas Chrom Q (100-120),

5% OV-17/Gas Chrom Q (80-100),

3% DEGS/Gas Chrom Q (80-100),

and 5% Carbowax 20M/Gas Chrom Q (80-100).

Each of these columns has been recommended for oil analysis by various authors, however little general preference has been shown to any one in particular, except Carbowax 20M. The suitability of each column was assessed mainly on the basis of the separations of components in commercial Swedish turpentine, eucalyptus flotation oil, bark oil of *Pinus radiata* and leaf oils of *Atherosperma moschatum* and *Eucalyptus viminalis*. These oils represent some of the different types that commonly occur, i.e. oils consisting of complex mixtures of monoterpenes, oils containing a major component, and oils containing numerous oxygenated or sesquiterpene components.

A study was also made of the effect on oil composition as a result of using lower-load silicone liquid phase columns (OV series), particularly in terms of the amount of component degradation that occurred. Various treatments with hexamethyldisilazane (HMDS) and Silyl-8 (Pierce Chemical Company) were attempted for each column.

The most suitable dissimilar column pair was considered to be the higher-load combination:

5% Carbowax 20M/Gas Chrom Q (80-100)

and 5% OV-17/Gas Chrom Q (80-100).

Chromosorb W HP solid support would have been preferred

instead of Gas Chrom Q, but supplies of this material at the

time of the study were limited. Gas Chrom Q (80-100 mesh)

was not a serious disadvantage since the resolution of

certain component groups would only have been marginally

improved by a HP support. (See Figure 3 for the advantage

of high performance columns.)

The lower-load (3%) columns were found in some

instances to degrade sesquiterpenes and oxygenated monoterpenes.

This was apparent when injections of oil onto an OV column

were alternated with Silyl-8 treatments, that successively

reduced the amount of degradation. In some instances a

Silyl-8-improved column was found to deteriorate while

conditioning overnight. The slightly higher 5% load was

therefore adopted in the hope that the extra amount of liquid

phase would reduce the number of active sites indicated on

a 3%-loaded column.

SCOT and 0.25 mm ID capillary columns were also

assessed. The resolution advantages of these columns are

well-known, however it was considered that the greater

stability of the packed columns justified their use instead

of the former. The relative retention volumes of SCOT and

capillary columns were too easily affected by preceding

injections. Furthermore, much of the analytical program

required syringe head-space injections of a mixture of oil

and water vapours. SCOT and capillary columns are entirely unsuited to both headspace injections and water. In conventional oil injections, even the thujone major component of *Thuja plicata* leaf oil was found to temporarily unsettle a capillary column, resulting in altered relative retention times of subsequently-eluted and injected components.

(ii) Relative retention time (RRT) of terpenoids

The effectiveness of the dual dissimilar column system, consisting of injections of a sample on columns each of 5% Carbowax 20M and 5% OV-17/Gas Chrom Q (80-100), is indicated in Table 23. The two columns were injected with terpenoids that had been purified by preparative GC and the identities confirmed by IR, or injected with commercial 'authentic' terpenoids. From the table there is no doubt that many components of oils may be unambiguously identified on the dissimilar columns. It is also feasible in the case of an oil component that is present in too small a proportion to be isolated for its identity to be confirmed by IR, that GC on this dissimilar column system may lead to an almost conclusive identification, particularly for monoterpene hydrocarbons. GC identification of many other components should still however be regarded only as tentative.

The complexity of essential oils is seen, from the degree of overlap of RRT data, to render direct GC analysis not feasible for the numerous minor components. The need for a prefractionation step is therefore indicated.

Table 23. Relative retention times of terpenoids injected as
liquids on two dissimilar columns

Terpenoid	No. of injections*	5% Carbowax 20M/Gas Chrom Q		5% OV-17/Gas Chrom Q	
		RRT	Standard deviation	RRT	Standard deviation
(Relative to α-pinene at 60°)					
Tricyclene	1	0.90		0.89	
Terpene alcohol (1)	1	1.00		1.00	
α-Pinene	53	1.01	±0.03	1.01	±0.02
Camphene	17	1.30	±0.04	1.20	±0.02
β-Pinene	51	1.62	±0.04	1.56	±0.03
Sabinene	2	1.74, 1.75		1.59, 1.58	
Δ_3-Carene	19	2.06	±0.07	1.99	±0.07
Myrcene	55	2.24	±0.05	1.75	±0.03
α-Phellandrene	1	2.27		1.97	
Limonene	57	2.76	±0.06	2.38	±0.04
β-Phellandrene	13	2.93	±0.09	2.54	±0.07
1,8-Cineole	14	3.11	±0.09	2.82	±0.05
γ-Terpinene	15	3.64	±0.09	3.25	±0.06
ρ-Cymene	25	4.28	±0.09	2.80	±0.05
Terpinolene	30	4.58	±0.09	4.10	±0.05
Anisole	1	7.18		1.91	
Fenchone	1	9.79		6.00	
Thujone	5	11.6	±0.5	6.6	±0.1

(Continued)

Table 23 continued

Terpenoid	No. of injections*	5% Carbowax 20M/Gas Chrom Q		5% OV-17/Gas Chrom Q	
		RRT	Standard deviation	RRT	Standard deviation
Isothujone	5	12.6	±0.6	7.1	±0.3
Camphor	1	18.4		10.3	
(Relative to camphor at 130°)					
1,8-Cineole	1	0.39		0.52	
Anisole	1	0.47		0.36	
Fenchone	1	0.65		0.73	
Thujone Isothujone	9	0.74	±0.04	0.77	±0.03
Sesquiterpene(6)	2	0.81, 0.85		1.68, 1.72	
Linalool	9	0.92	±0.02	0.61	±0.03
Linalyl acetate	1	1.00		1.18	
Camphor	5	1.02	±0.02	1.02	±0.03
Cis-βTerpinyl acetate	1	1.22		1.48	
Terpinen-4-ol	18	1.24	±0.04	0.98	±0.04
Bornyl acetate	5	1.27, 1.27 1.19, 1.15, 1.24		1.52, 1.60 1.41, 1.55, 1.66	
Caryophyllene	13	1.28	±0.07	2.60	±0.07
Sesquiterpene(7)	2	1.32, 1.33		2.71, 2.85	
Cis-β-Terpineol	2	1.35, 1.40		0.82, 0.84	
Menthol	1	1.43		0.91	
Isogeranyl acetate	3	1.50, 1.55, 1.61		1.70, 1.70, 1.73	

(Continued)

118.

Table 23 continued

Terpenoid	No. of injections*	5% Carbowax 20M/Gas Chrom Q		5% OV-17/Gas Chrom Q	
		RRT	Standard deviation	RRT	Standard deviation
Pulegone	1	1.55		1.50	
Citronellyl acetate	10	1.58	±0.04	1.97	±0.07
Chavicol methyl ether	13	1.58	±0.03	1.27	±0.05
α-Humulene	8	1.58, 1.59, 1.67, 1.59, 1.50, 1.68, 1.60		3.11, 3.11, 3.39, 3.10, 3.30, 3.22, 3.15	
Isoborneol	1	1.63		0.91	
Citral (one of 2 peaks)	1	1.77		1.45	
α-Terpineol	20	1.78	±0.04	1.09	±0.04
Sesquiterpene(5)	4	1.79, 1.85, 1.96, 1.78		3.93, 4.00 4.22, 3.80	
α-Terpinyl acetate	3	1.84, 1.78, 1.90		2.19, 2.00, 2.05	
Sesquiterpene(1)	1	2.00		1.67	
Piperitone	2	2.00, 2.07		1.67, 1.73	
†Citral (major component of 2 peaks)	2	2.12, 2.10		1.65, 1.68	
Carvone	1	2.20		1.67	
Geranyl acetate	12	2.27	±0.07	2.61	±0.04
Citronellol	13	2.31	±0.06	1.13	±0.04
Anethole	1	3.20		2.00	
Geraniol	1	3.29		1.32	
Safrole	6	3.52	±0.06	2.24	±0.07

(Continued)

† Has IR spectrum of citral.

119.

Table 23 continued

Terpenoid	No. of injections*	5% Carbowax 20M/Gas Chrom Q		5% OV-17/Gas Chrom Q	
		RRT	Standard deviation	RRT	Standard deviation
Carvacrol	1	4.60		2.09	
β-Ionone	1	4.74		4.73	
Thymol	1	5.13		1.86	
Eugenol methyl ether	4	6.62	±0.23	4.32	±0.07
Cinnamic aldehyde	1	7.17		2.57	
Terpene alcohol(4)	1	8.04		7.15	
Eugenol	1	8.71		3.09	
Terpin hydrate	1	9.07		2.27	
Sesquiterpene(8)	1	9.60 (1.05 rel. to thymol;180°)		>>22 (6.70, 6.90 rel. thymol)	
Terpene alcohol(2)	2	9.62, 9.78		7.55, 7.67	
Sesquiterpene(10)	1	10.1		7.30	
Terpene alcohol(3)	2	14.6, 14.7		11.8, 12.3	
Terpene alcohol(5)	4	14.9, 15.1 15.4, 15.2		11.0, 11.0 11.2, 10.9	
Aromatic(?) ether(1)	2	15.7, 15.9		9.68, 10.1	
Vanillin	1	>>21		5.77	
Ethyl vanillin	1	>>28		6.39	

* The number of injections shown represents the number of times during the investigation of more than 30 essential oils, in which a compound has been isolated by preparative GC, identified by IR and by RRT data on each column. Unidentified compounds listed as a numbered terpene alcohol, sesquiterpene, etc., have been repeatedly isolated from endemic Tasmanian plant oils and partly characterized on the basis of IR spectra and chromatographic elution properties.

(a) <u>Preparation of analytical columns</u>

Each empty column was prepared from a 2 m × 4 mm
ID glass U-tube, which was washed with chromic acid, distilled
water, ethanol and diethyl ether, then dried with a stream
of air. Active sites in the tube were then blocked by
rinsing with a solution of 5 percent dimethyl-dichlorosilane
(DMCS) in toluene, then several times with toluene to
remove excess DMCS, finally with ethanol, then dried in a
stream of nitrogen.

To coat the liquid phase onto the solid support
0.5 g of liquid phase was weighed into a 100 ml long-necked
round-bottom flask, to which was added 50 to 75 ml of the
appropriate solvent for the particular liquid phase. When
the liquid phase had completely dissolved, 10 g of solid
support was added. The solvent was then evaporated off in
a gentle stream of nitrogen while the flask was maintained
at about room temperature by rotating over a water bath.
The dried coated support was stored in a dessicator before
use. Care was taken to avoid fracturing particles (and
creating active sites) by not using a stirring rod and merely
rotating the flask.

Columns were packed with the aid of a vacuum.
A 1 cm. plug of DMCS-treated glass wool was first inserted
into the end of the tube to prevent the packing from being
sucked into the vacuum line. Packing was poured in through
a micro-funnel, 5 to 6 cm. at a time, accompanied by gentle
tapping with a pencil. No electric vibrator was used, since
this could fracture particles.

The column was conditioned in the GC oven under the mildest conditions to ensure the creation of an even layer of liquid phase throughout the glass/particle system. The column was fitted with the detector end disconnected, then temperature-programmed from 50 to 200° at 1° per minute and left overnight at 200°. Next day the injector end was topped up with more packing and the column temperature-programmed as before. During the conditioning process over a period of several days the OV-17 column was treated alternatively with 10 $\mu\ell$ injections of Silyl-8 and 0.2 $\mu\ell$ of essential oil terpene alcohol fraction until a reproducible chromatogram could be achieved.

(b) **Analytical GC operation**

Chromatograms leading to RRT data in Table 23 were obtained using a Tracor MT220 instrument fitted with a flame-ionization detector (FID). The following conditions were used for most of the program:

carrier - high-purity N_2, 40 PSI, 80 ml/min;

FID gases - H_2, 15 PSI, 180 ml/min.,

air, 40 PSI, 50 ml/min,

O_2, 40 PSI, 20 ml/min;

inlet - 205°;

column-to-detector transfer - 250°;

detector - 220°;

temperature-program - 50° to 200° at 5°/min;

isothermal runs - 60° or 130°;

attenuation - 4×10^3;

chart speed - 38 cm/hr;

and injection - 0.2 $\mu\ell$.

For the frequent injections of α-pinene and camphor as arbitrary reference compounds a convenient practice was established, in which a sufficiently small peak was obtained by injecting 1 ml of the headspace vapour from the container of each compound. This practice led to a slight difference in absolute retention time when compared with an injection of the compound in liquid form. However since the choice of a reference compound is arbitrary, this practice is valid.

(iii) Quantitation in analytical GC

Various commonly-used methods of routinely measuring the concentrations of oil components were examined. It was concluded that for survey purposes calculations based upon peak height could,with the above column system, give results which were comparable to other methods, viz. electronic integration and peak area measurement (from the product of peak height and peak width at half peak height). No attempt was made to estimate accuracy by comparing these results with that obtainable by the use of internal standards. The systematic use of internal standards to cover the large number of compounds would not have been a feasible routine procedure.

It was considered that if GC was to be used for quantitation, as opposed to classical standard procedures, then an error of at least several percent could be expected for major components. Precision would however by high by GC (Table 11).

In the absence of any common use of internal
standards, and since in most essential oil studies component
concentration has been considered proportional to peak area,
it was decided to accept a similar degree of error that is
also inherent in a method involving peak height measurement.

Justification for this method arises from common
practice in numerous laboratories, where it is considered
that if all peaks in a chromatogram have a closely similar
width at half height, then peak area is nearly proportional
to peak height. To achieve this situation is not possible,
but optimum control of GC operating conditions may lead to
chromatograms having peaks of very nearly the same width.
It is also considered that trace peaks, i.e. less than about
0.5 percent, will be measured with the greatest error since
peak width is somewhat smaller. However it is of little concern
in a survey whether a trace component is reported as 0.5 or
0.3 percent.

In Table 24 the concentrations of components are seen
to be comparable by each technique. The precision in the
determination of the major component of the leaf oil of
Atherosperma moschatum is seen to be probably high by all GC
techniques.

(iv) Preparative GC

Preparative GC was examined as a means of
fractionating an oil into wide fractions for further sub-
fractionation, and also as a technique for isolating sufficient
quantities of pure components for IR identification.

Table 24. Determination of the percentage concentrations
of components of leaf oil of *Atherosperma*
moschatum by 3 GC peak-measuring techniques

Component	Peak height	Concentration calculated proportional to:	
		Product of peak height and width at half peak height	Peak area measured by electronic integration
α-Pinene	1.3	1.2	0.8
Camphene	5.0	4.7	4.0
Myrcene + α-phellandrene	0.9	0.8	0.6
Limonene	0.7	0.7	0.5
ρ-Cymene	0.4	0.3	0.3
Linalool + camphor	18.2	17.0	18.2
Terpinen-4-ol(?) + bornyl acetate(?)	5.7	4.9	4.4
Unidentified	0.7	0.6	0.4
α-Terpineol	3.3	3.1	2.8
Safrole	13.8	12.9	12.1
Eugenol methyl ether	49.5	53.4	55.7
Unidentified	0.4	0.3	0.2

This technique has the potential for use as a means
of simplifying an oil by cutting it into wide fractions for
subsequent study and separations. Von Rudloff successfully
used preparative columns for wide fractionating, e.g. using a
90 × 0.9 cm OD Al column containing 20-25% SE-30/Chromosorb
W(60-80) to separate monoterpene and sesquiterpene hydrocarbons
[337]. It should be noted however that this use of preparative
GC has not been usual for essential oil investigations. An
attempt was made to separate monoterpene and sesquiterpene
hydrocarbons on a 2.1 m × 0.95 cm glass column of 20%
DC-200/Chromosorb W HMDS (60-80). However it was concluded

that even with automatic sampling and programming, preparative GC would only yield several ml· of each efficiently separated fraction if the instrument was allowed to run for several days. This further limited the technique to studies in which many ml of oil are available. Concern has also been felt at the risk of chemical alteration of components due to repeated GC fractionation, particularly at the excessive temperatures required for highly-loaded preparative columns [337].

Preparative GC was confirmed as a convenient and efficient means of isolating or purifying a mg or more of a pure component for IR identification, rather than as a means of systematically fractionating an oil.

(a) Preparative GC operation

The more acceptable technique for isolating purified components involved the use of a conventional preparative column. Trapping of less than 1 μℓ of a component eluted from an analytical column, rarely produced sufficient sample to record an IR spectrum that contained the necessary fine band structures for conclusive identification, i.e. using the "Extrocell" system by RIIC.

Throughout the program a Pye-Unicam Series 105 · Model 15 preparative gas chromatograph was used, fitted with a FID and a 1:99 effluent splitter. Purifications were satisfactory on a 2.1 m × 0.95 cm OD glass column containing 20% DC-200/Chromosorb W HMDS (60-80) under the following conditions:

carrier gas - N_2 65 PSI, 120 ml/min;

detector gases - air 60 PSI, ≈600 ml/min

$\quad\quad\quad\quad\quad\quad\quad$ H_2 12 PSI, ≈40ml/min;

temperature-program - 150° - 240° at 2°/min;

injector - 210°;

sample - 100 or 10 µℓ, depending upon nature of sample;

attenuation - 5 to 20 × 10^2.

Numerous columns have been recommended for preparative

GC. It was considered that an alternative type of separation

should be provided by a column different from the analytical

Carbowax 20M. Apiezon L was suitable for elution of

monoterpenes between 100° and 200°. Sesquiterpenes, however,

were not eluted within a practical time interval or at a

desirably low temperature. Other supports were tested,

such as GC-22 "Super-support" (30-60), and were found

unsuited to essential oils due to their need for a treatment

for the blocking of active sites, unlike Chromosorb W HMDS

(60-80) which has been treated (with HMDS).

2. Column chromatography

(1) Florisil as chromatographic adsorbent

Florisil (pesticide residues grade, 60-100 mesh;

Floridin Co., Pittsburgh, Pa.) [338] was investigated and

confirmed as a suitable adsorbent for the column chromatography

of labile terpenoids, which could otherwise undergo

dehydration, isomerization, polymerization and irreversible

adsorption on other adsorbents, such as alumina and silic acid.

This adsorbent has been used for terpenoids by only a few workers [238, 239, 252]. Its suitability was apparent because of its widespread acceptance for the chromatography of µg quantities of highly labile pesticide residues [339].

Numerous workers have been content to use other adsorbents, despite the well known risk of chemical alteration, e.g. as found with alumina [245, 246] and silicic acid. The only other acceptable adsorbent that has been recommended for terpenoids was silicic acid impregnated with 1 percent Carbowax 20M [242, 243].

It was experimentally confirmed that when activate for at least 5 hr at 130° Florisil could be most efficiently used to quantitatively separate hydrocarbons from oxygenated components, even when a major proportion of a difficultly-separable compound such as cineole was present. Various degrees of activation were also examined, such as activation for 1 hr at 130° and activations at different times followed by deactivation with 6 percent water. Analysis of components before and after elution showed no evidence of any qualitative changes due to chemical alteration of components, even when eluted from used and re-generated Florisil. A comparison of the efficiency of separation of 5,10 and 20 ml portions of oil on 100 g of Florisil showed that 10 ml of a high-cineole oil could be quantitatively fractionated, while a 20 ml portion of low-cineole oil could be similarly eluted. The column was however shown, as for other adsorbents, to be unsuited to sub-fractionation of the hydrocarbon fraction. A solvent

system in which the polarity was incrementally raised was found to sub-fractionate oxygenated compounds, however the separations were not always quantitative.

(a) Experimental

Florisil was initially treated by igniting at 600° for 2 hr., then stored in a sealed container. Before use it was re-activated at 130° for 5 hr and stored as before for no more than 30 days. Batches varied in ability to remain active for longer periods.

The 38 cm. × 38 mm column with a Teflon stop-cock was packed with 100 g of activated Florisil, then with a pre-wetting volume of re-distilled petroleum ether (58°-68°) sufficient to expel all bubbles, fill the column, and just cover the adsorbent surface when the tap was closed. The 10 ml sample was pipetted onto the column with minimum disturbance of the adsorbent surface, then allowed to elute into the column while rinsed with a syringe of pet. ether. Hydrocarbons were completely eluted with 400 ml of pet. ether at 5 ml/min., followed by 400 ml of redistilled diethyl ether or methanol to remove oxygenated components.

Each fraction was evaporated at 20 to 25° to reduce the risk of chemical alteration and evaporative loss of more volatile components. Solvents were removed from eluates in 500 ml evaporating basins on the water bath. A carefully-controlled stream of nitrogen, directed onto each basin, both assisted the rate of evaporation and at the same time kept the

solution at close to room temperature. Care was taken at the end of the evaporation to ensure that there was no rise in temperature. The concentrated extract was then removed from the bath and the nitrogen stream continued, ensuring that the temperature did not drop and cause condensation of water. With care a recovery of 90 to 95 percent of the oil may be achieved.

Used Florisil was recovered by eluting with hot water while in a 100 × 4.5 cm column. Hot water elution was continued until the effluent gave a negative reaction to acidified barium chloride solution. Elution was then continued with alcohol and diethyl ether, after which the Florisil was dried with a stream of air. The adsorbent was subsequently dried on a 100° water bath, then ignited in a furnace at 600° for 2 hr. and stored in a sealed container.

(iii) Argentative column chromatography

The use of silver ions on a column, to induce a further separation of components on the basis of their ability to form π-bonding complexes, was investigated and found to have some potential use for the separation of mono- and sesquiterpenoids. Further work is required if the method is to be used as a routine procedure.

This technique was however found to elute sesquiterpene hydrocarbons within the first few ml of eluent. Unfortunately the mass of terpenoids eluted was too small, thereby preventing the technique being used as a means of separating monoterpene

and sesquiterpene hydrocarbon fractions. It would be more
suitable for the separation of simpler hydrocarbon mixtures,
e.g. as used by Wenninger *et al* [249] to separate sesquiterpenes
into silver nitrate-adductable and non-adductable fractions.
The technique has been reviewed by Norin and Westfelt [340],
who recommended silver nitrate-silicic acid for both TLC plates
and columns for the separation of diterpenoids; while Willner
[341] showed that silver nitrate-Florisil was suitable for the
separation of fatty acid esters.

(a) Experimental

Silver nitrate-Florisil was prepared according to
the method described by Gordon [251] for coating silicic acid.
To prevent the reaction of silver with impurities, Florisil
was first acid-washed with nitric acid, distilled water,
ethanol and ether. 56.1 g of washed Florisil was suspended
in 160 ml of methanol, then added to a solution of 14 g of
silver nitrate in 60 ml. of 50 percent aqueous methanol.
The mixture was rotary evaporated to dryness at 40° to 55°
and 15 mm Hg, then stored in the dark. Before use the
adsorbent was activated at 130° for 5 hr.

The column was packed with 200 g· of adsorbent,
prewet with about 150 ml. of pet. ether, then charged with
10 g. of a terpene hydrocarbon fraction. To collect
sesquiterpene components, free of monoterpenes, it was
necessary to first recover the pre-wetting solvent, then
collect 10 ml. fractions (or even less). Solvent polarity

increments were raised very gradually, i.e. after the

first 400 ml of collected eluent 0.2 percent diethyl ether

in pet. ether was added, which removed all remaining monoterpenoids.

As a check on chemical alteration the column was

finally stripped by eluting with diethyl ether. Evidence of

polymerization was found when a green-coloured non-volatile

effluent was recovered.

(iii)   Summary

A routine procedure for a survey of essential oils

should include a quantitative column-chromatographic separation

of hydrocarbon and oxygenated terpenoids. This step could be

achieved with Florisil adsorbent, which would not be expected

to chemically alter components. Figure 9 illustrates the

separation of eucalyptus flotation oil achieved on Florisil

and silver nitrate-Florisil columns.

3.   Partition between solvents

It was concluded from a study of the partition

of eucalyptus flotation oil between various solvent pairs

that this technique, although convenient, does not provide

sufficiently clear-cut separations as to be useful for

fractionating oils into major fractions. Solvent partition

would appear to be of more value for selectively

concentrating specific terpenoids in simpler mixtures.

Fig. 9(a). Gas chromatogram of eucalyptus flotation oil before fractionation on Florisil. (GC on 5% Carbowax 20M/Gas Chrom Q, temperature program 50° to 200° at 5°/min., 0.2 µℓ sample, attenuation 4 x 10^2).

Sesquiterpenes and oxygenated monoterpenes   Monoterpenes and cineole

Fig. 9(b). **Gas chromatogram** of hydrocarbon fraction of eucalyptus flotation oil after column chromatography on Florisil.

Sesquiterpenes

Monoterpenes
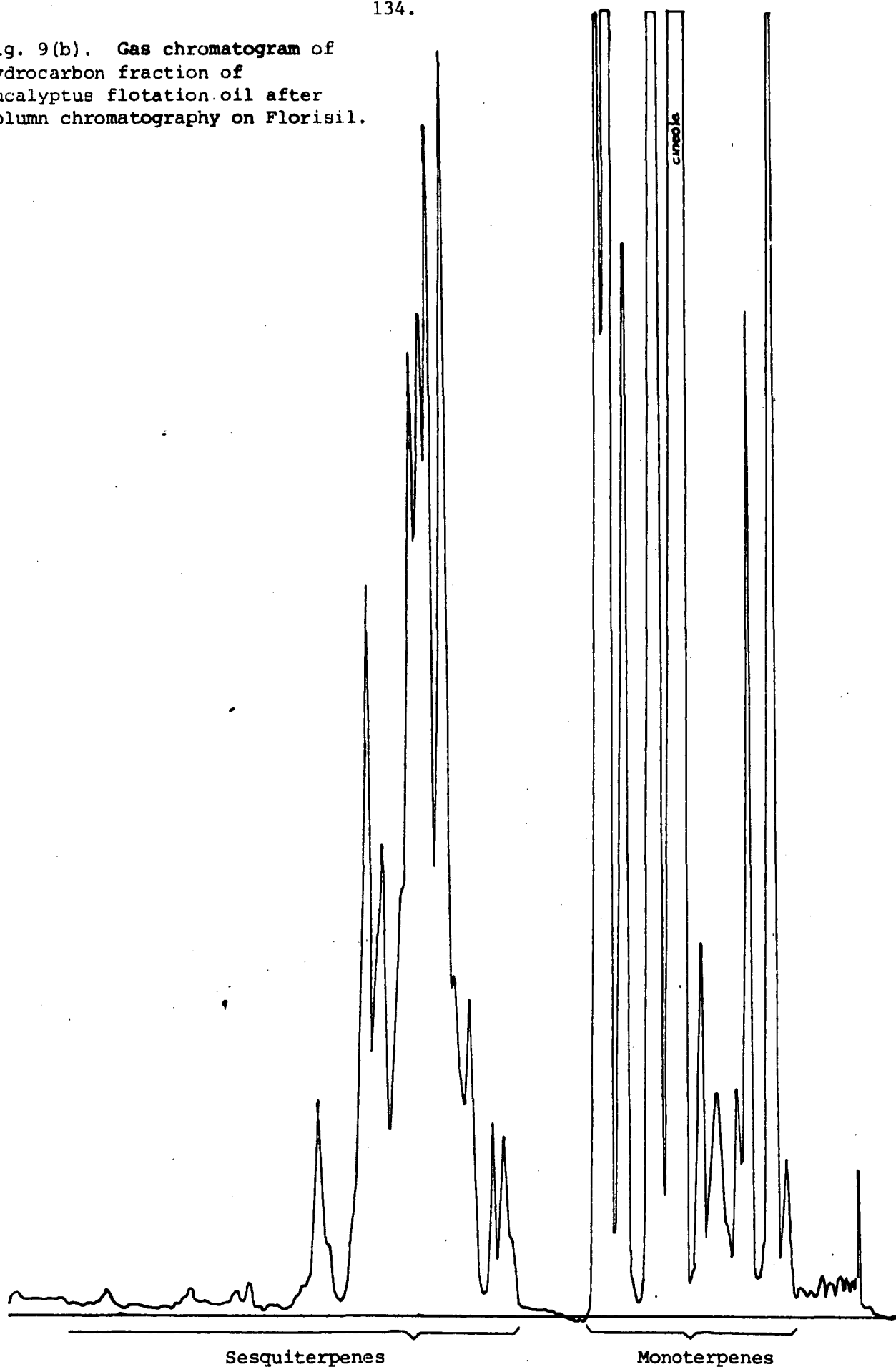
Fig. 9(c). Gas chromatogram
of oxygenated components of
eucalyptus flotation oil
after column chromatography
on Florisil.

Oxygenated monoterpenes
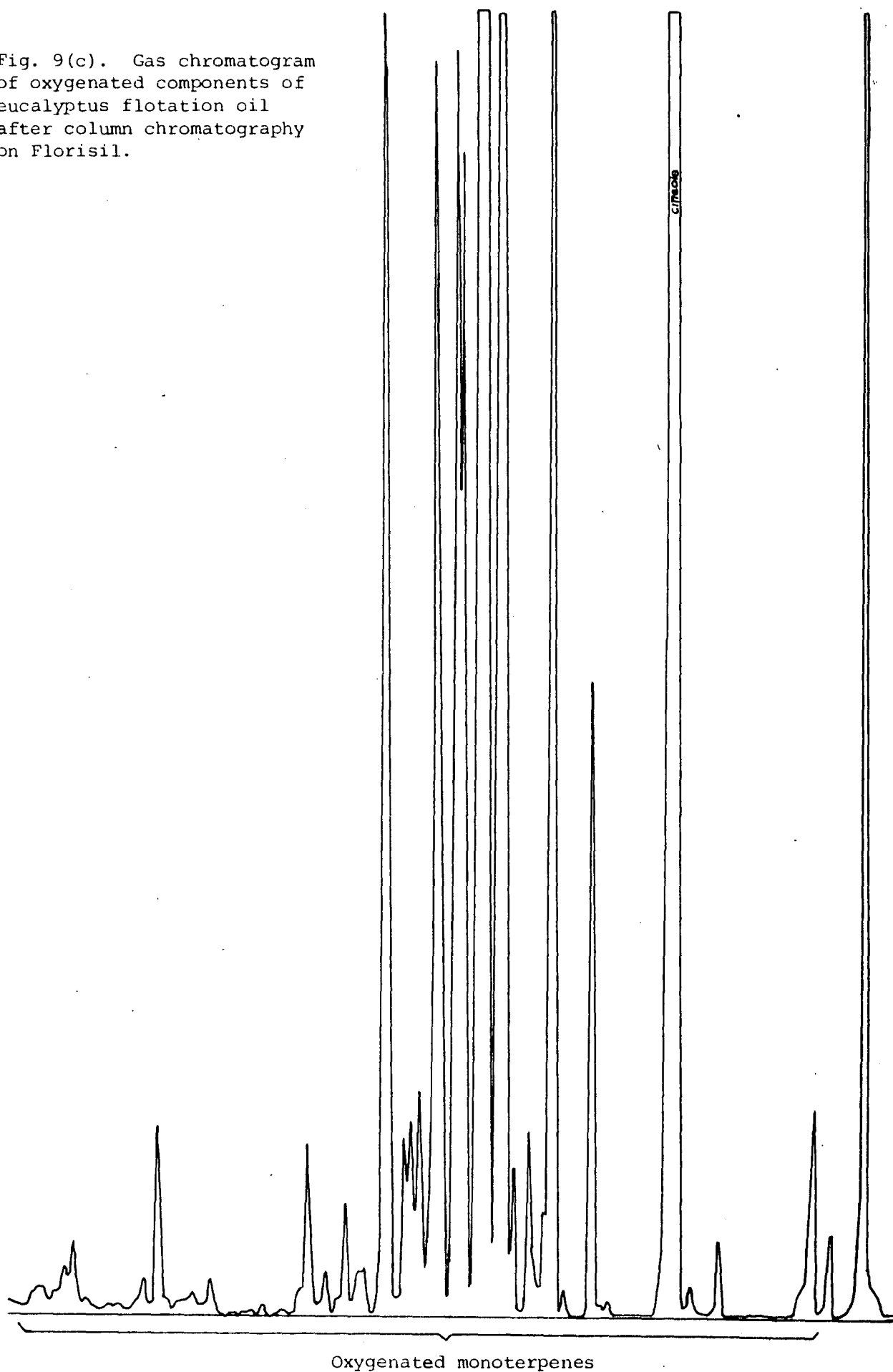
Fig. 9(d). Gas chromatogram
of first few ml of eluent
(mainly sesquiterpenes) from
silver nitrate-Florisil
chromatography of hydrocarbon
fraction of eucalyptus
flotation oil.

Sesquiterpenes
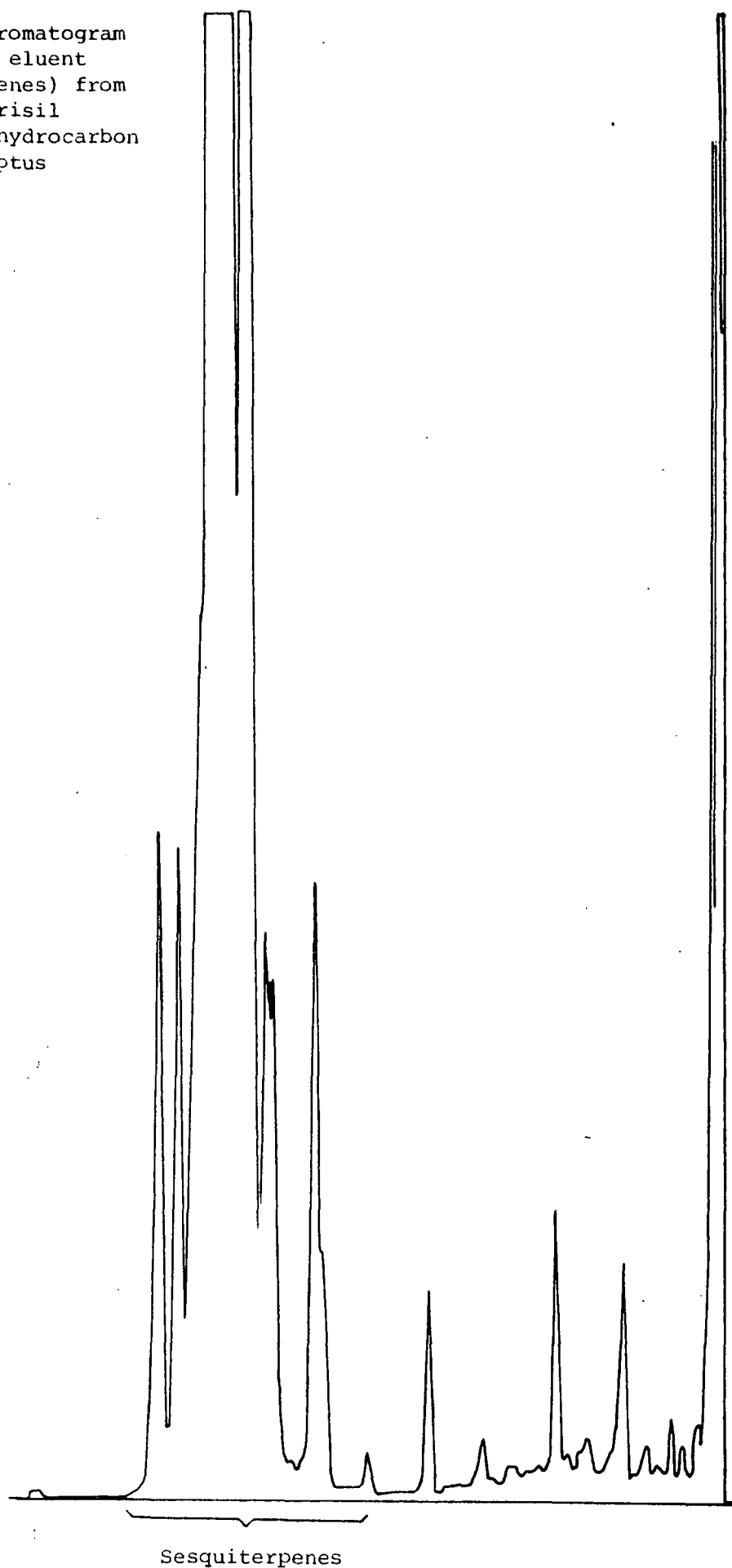
Fig. 9(e). Gas chromatogram of last-eluted components (mainly monoterpenes) from silver nitrate-Florisil chromatography of hydrocarbon fraction of eucalyptus flotation oil.

Alteration products (?)　　　Monoterpenes
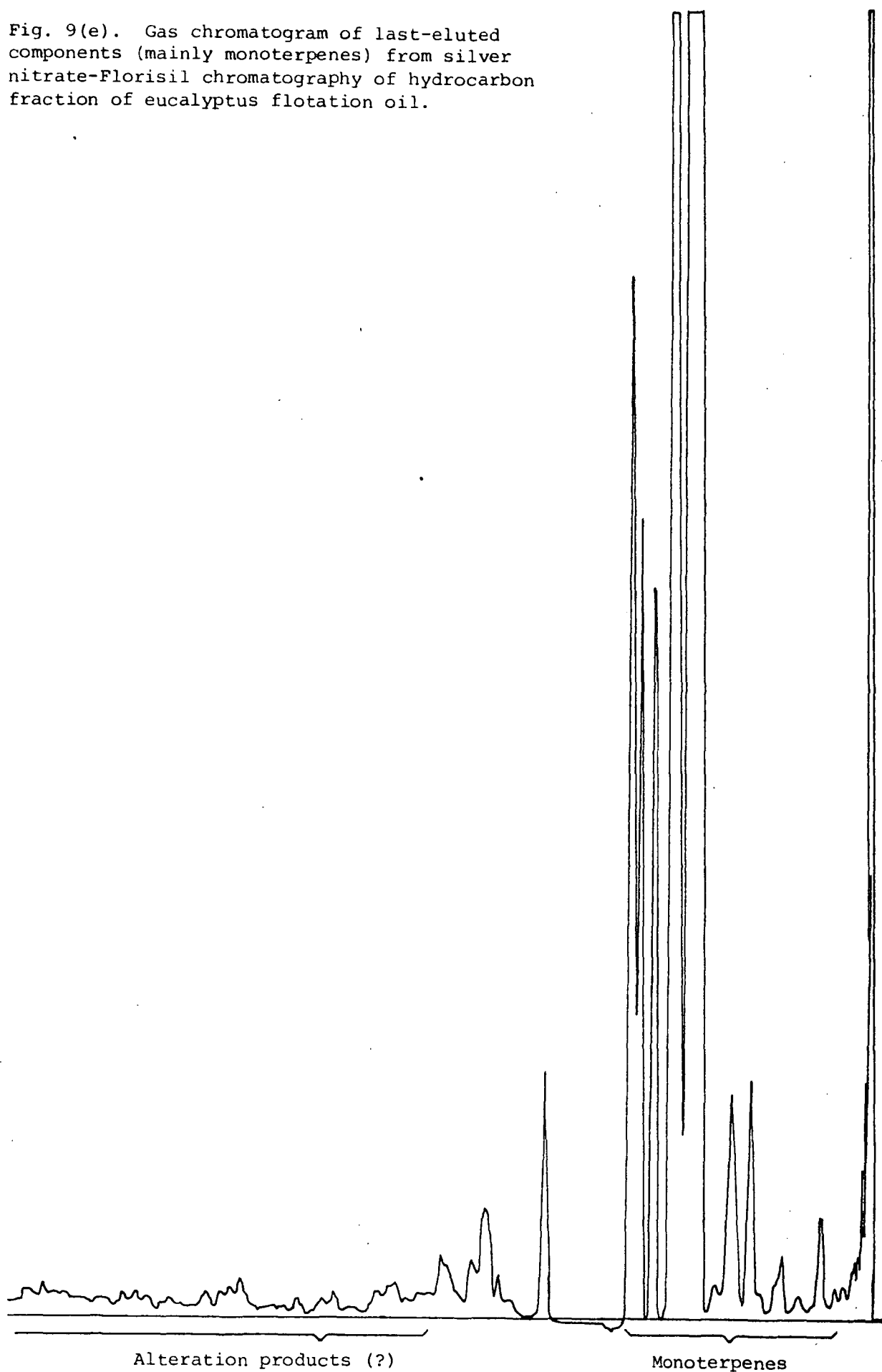
(i) Partition between n-pentane and dimethyl sulphoxide (DMSO)

This solvent pair has been recommended for the deterpenation of citrus oils [342] and was thought to compare favourably with chromatography on silicic acid. Eucalyptus flotation oil was exhaustively partitioned between these two solvents, but it was found that although DMSO selectively removed oxygenated components, cineole was evenly distributed between each solvent. Some hydrocarbons were also extracted into the DMSO layer.

(ii) Partition between n-pentane and acetonitrile (MeCN)

During the partition between these solvents the MeCN layer retained nearly all the oxygenated components after an exhaustive extraction. Unfortunately the MeCN also contained a large number of hydrocarbons. Cineole was evenly partitioned.

It was concluded that this partition would be more suitable for an automatic countercurrent system.

(iii) Other partitions

Partition between n-pentane and 90 percent aqueous methanol, between chloroform and aqueous ethanol and between glycol and carbon tetrachloride [232] gave results similar to that found for n-pentane and MeCN.

4. Fractional distillation

Many workers have used fractional distillation as a means of fractionating an oil to simplify it for subsequent GC investigation. This technique cannot however be recommended

as a routine technique for a survey of new oils, largely because
of the need for a large quantity of each oil, but also
due to the fact that there is too much risk of chemical
alteration of components. GC analysis of each fraction
confirmed that the technique tended only to concentrate
certain components in particular fractions.

(i) Experimental

A distillation unit was constructed with the following
features:

fractionating column, 2.5 × 76 cm , packed with 4 mm

Fenske helices;

column heating system having 2 staggered 450 cm

Nichrome elements (No. 33 B and S, ≈40 Ω/m)

arranged as in the Todd Scientific Company

apparatus, each controlled by a separate rheostat;

still-head with capillary take-off tube and tap to

control the reflux ratio;

Perkin triangle;

and Teflon tap inserts to provide a leak-proof system with

finer control of flow rates.

A 669 g. charge of eucalyptus flotation oil was
fractionally distilled under reduced pressure, from which was
collected 65 1°-fractions (Figure 10). The distillation was
discontinued when the pot residue adopted a black viscous
appearance that is typical of extensive chemical alteration.
Each fraction was analyzed by GC.

Fig. 10.   Fractional distillation of eucalyptus flotation oil.
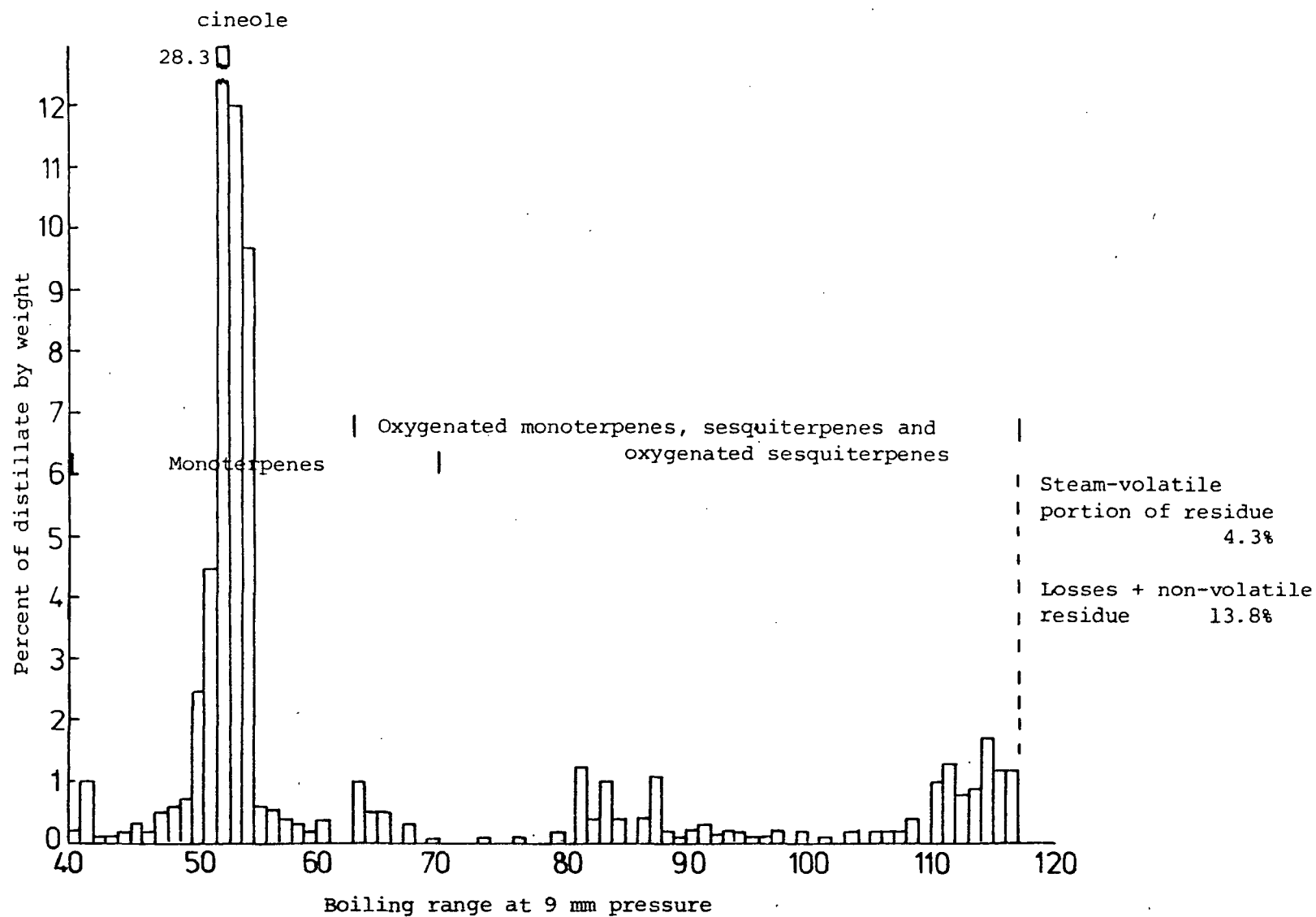
5. Isolation of oil from steam distillate

It was resolved that the mixture of terpenoids and water in steam distillate should be extracted with a solvent in an attempt to recover water-soluble oil components. This procedure contrasts with the practice of numerous workers, including Zavarin and his collaborators [85, 326], who have been content only to pipette the oil off the surface of the aqueous distillate [85]. Other workers have however drawn attention to the losses of water-soluble components [86-91, 93], and the need for procedures for their recovery [94-101, 105-107].

A comparison of the floated and ether-extracted oils from the following steam distillates (Table 25) showed that a further significant yield may be recovered in the ether extract.

Table 25. Percentages of essential oil yields recovered from distillates by removal of the floating layer and by extraction of the aqueous layer with diethyl ether

Essential oil	Percentage of oil yield recovered:	
	Removal of organic layer	Extraction of aqueous layer
Foliage oil of Thuja plicata	0.86 (wet wt.)	0.06
Foliage oil of Pseudotsuga menziesii	0.21	0.05
Cortical oleoresin turpentine of Pinus ponderosa	14.2	1.1

The compositions of the organic layer and ether extract also exhibited differences, particularly in the higher boiling components, possibly oxygenated materials (Figure 11).

Fig. 11(a). High sensitivity
gas chromatogram of oil of
Thuja plicata pipetted off
the surface of the steam-
distillate (GC on 5% Carbowax
20M/Gas Chrom Q; temperature
program 50° to 200° at 5°/min.)
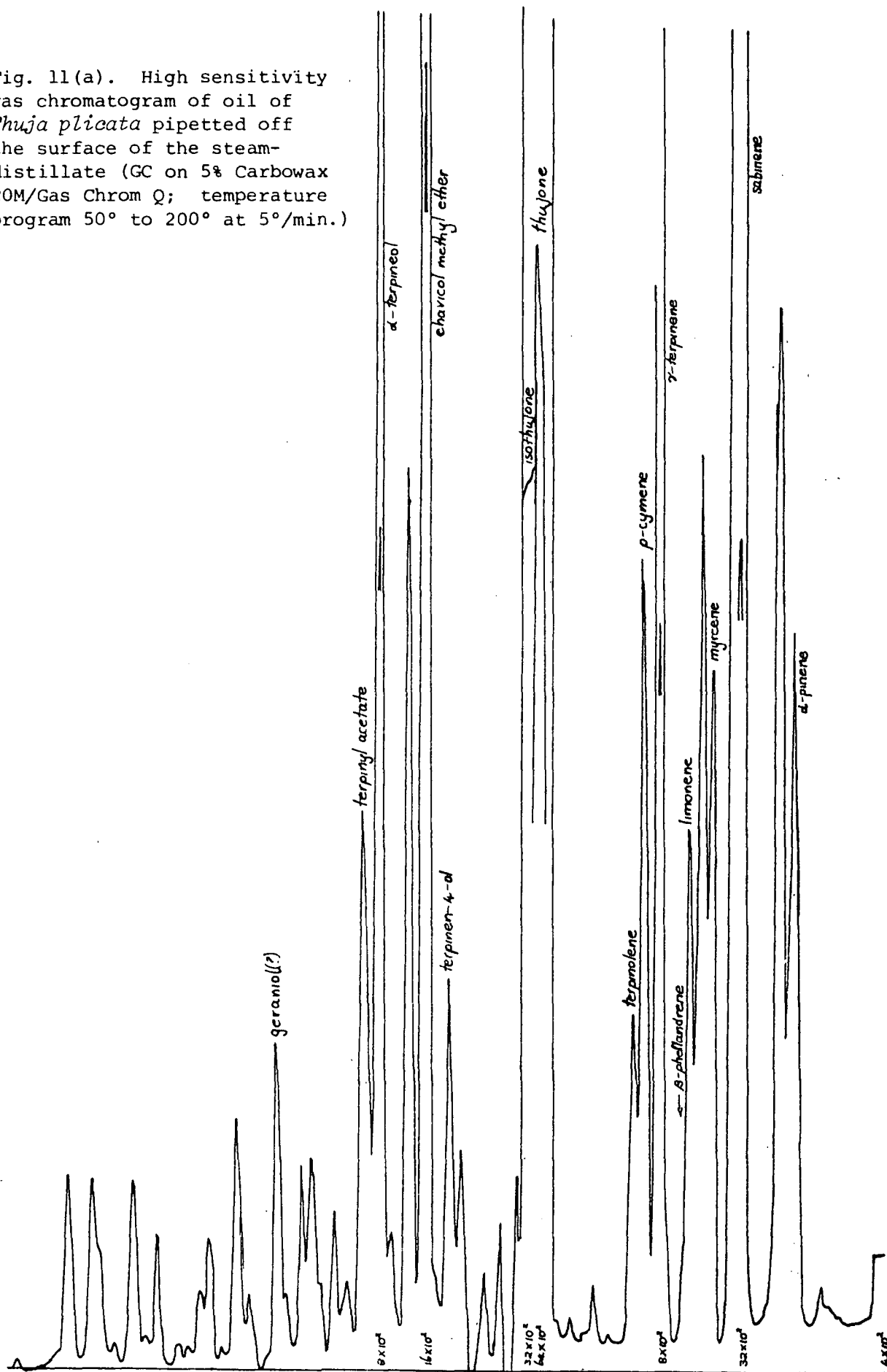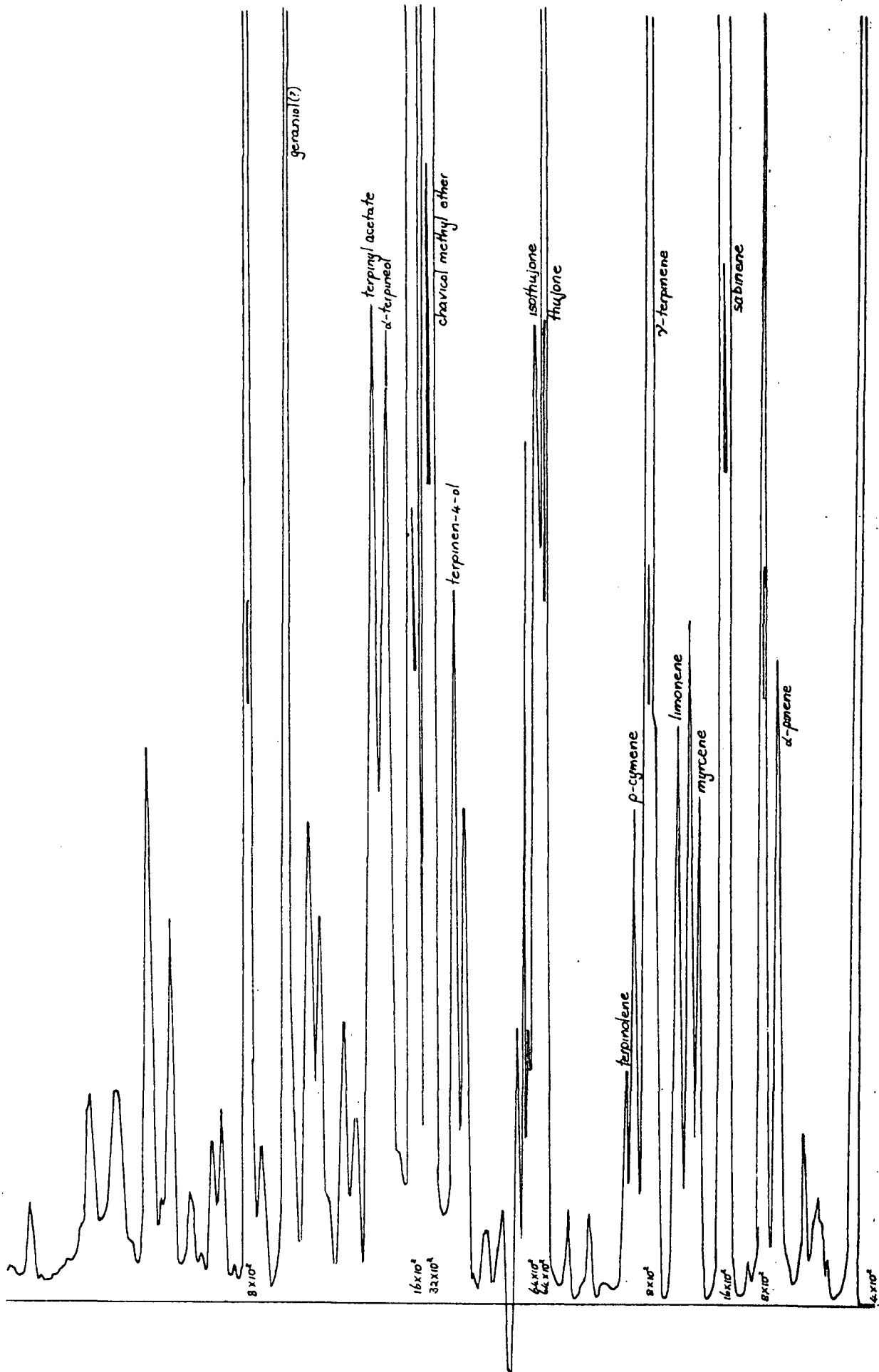
Fig. 11(b). High sensitivity gas chromatogram of oil of *Thuja plicata* after ether extraction from the water layer of the steam-distillate.

6.   Summary

An examination was made of several commonly-used techniques for the investigation of essential oils. A feasible routine analytical procedure for use in a survey of essential oils was found to include:

isolation of oil from steam distillate with the aid

of a solvent in the receiver flask to ensure the

recovery of water-soluble components;

column chromatography on Florisil adsorbent to

separate an oil into hydrocarbon and oxygenated

components;

preparative GC for the isolation of pure components

in sufficient quantity for recording IR spectra;

and   analytical GC on the dissimilar column system,

5% Carbowax 20M/Gas Chrom Q (80-100) and

5% OV-17/Gas Chrom Q (80-100), for identification

and quantitation of components.

Analytical GC should in addition, be used before and after each step in the procedure, to confirm any changes in components due to chemical alteration. This latter precaution should overcome a commonly encountered problem in IR identification of a component isolated by preparative GC. The identity of a component has frequently been reported as identified by IR without confirmation by analytical GC before and after the preparative step.

Although the above procedure is recommended as a convenient and routine series of techniques, with error

acceptable for survey purposes, it is not proposed that this
be used as a standard method for the complete examination of
an oil. A complete examination should perhaps incorporate
this routine procedure as an initial investigation, followed
by a thorough sampling program to determine the ranges of
concentrations to be expected, and even a macro-scale complex
fractionation, incorporating several preparative GC columns
to isolate more difficultly-resolved components for spectroscopic
identification.

B.   Compositions of essential oils of some endemic

      Tasmanian plants

A need exists for a systematic documentation of the
oil compositions of endemic Tasmanian oil-bearing plants.
While much work has been undertaken with respect to alkaloids
and other natural products, essential oils of the Tasmanian
flora have received relatively little attention except for
earlier work by Penfold and co-workers. There exist many
plants which are either restricted entirely to Tasmania, or
grow sparsely in the quite different climate of mainland
Australia.

Since the Tasmanian flora contains many unique
features a systematic search should be made for plants
containing economically-valuable oils.

A systematic study of oil compositions is also
expected to provide data that might assist botanists engaged
in the taxonomy of Tasmanian plants. Many plants are known

to exist in various morphological forms [343-345], which
might with chemotaxonomic data be better grouped into hybrids
and distinct forms.

Essential oil studies in a relatively unexamined
and unique flora have further value, in that corroborative
evidence may become available that could confirm existing
hypotheses regarding the biogenesis and biosynthesis of
terpenoids.

A survey is therefore required, in which oil
compositions are initially documented using a routine method
of analysis. Subsequent analyses of the oils of particular
species should then be undertaken to confirm the ranges of
compositions of chemotaxonomic interest. More detailed in-
depth analyses of minor components should similarly be
carried out which might yield further data of value in studies
of terpene biosynthesis.

1. Composition of foliage oil from Celery-top Pine

   (Phyllocladus aspleniifolius)

(i) Introduction

   Phyllocladus aspleniifolius (Labill.) Hook. f.
(Fam. Podocarpaceae) grows into a tree 6-18 m high, in
temperate rain and wet sclerophyll forests, from sea level
to about 800 m and is the only species of this genus that is
endemic in Tasmania [343]. A principal characteristic of the
mature foliage is the leaf-like cladodes which contain the oil.

   No report was found in the literature of any study
of oil of *P. aspleniifolius*, although Crook *et al* [346]

compared the chemical compositions of woods of ancient
and modern trees. Several early studies have however been
reported for oils of other species, i.e. *P. glaucus* [347],
P. trichomanoides [348] and *P. alpinus* [349-351]. Other
species in the Family Podocarpaceae have been extensively
studied by New Zealand workers.

(ii) Results and discussion

Cladodes and branchlets of several trees of
P. aspleniifolius were collected in mid-summer in the Harz
Mountain National Park in southern Tasmania. The steam-
distilled oil was pale green in colour with an odour of a
mixture of pinene and woody components. The oil was separated
by column chromatography into fractions consisting of hydro-
carbon and oxygenated components. From each of these fractions
components were isolated by preparative GC and identified
mainly by IR spectroscopy. The quantitative composition of
the oil was estimated by analytical GC on a peak height basis.
Analytical GC on dissimilar columns was also used to
tentatively identify components for which IR spectra could not
be recorded. Chromatograms were obtained for the freshly-
distilled oil, for fractions isolated by both column
chromatography and preparative GC, and were then compared in
order to check on the possibility of any detected compounds
being artifacts.

The qualitative and quantitative composition of
components distinguishable in the whole oil, using two dissimilar

columns, is recorded in Table 26. RRT values leading to the

tentative identification of components in hydrocarbon and

oxygenated fractions, and sub-fractions, are listed in

Table 27. Gas chromatograms in Figure 12 illustrate the

complexity of the oil and show a nearly complete separation

of components into hydrocarbon and oxygenated fractions.

Evaluation of each oil fraction by GC indicated a

number of instances of chemical alteration of components.

This was particularly apparent during the preparative GC

isolation of higher-boiling oxygenated components, which

from IR and GC on dissimilar columns, appear to have undergone

-dehydration to yield compounds recognizable as sesquiterpene

hydrocarbons (Table 27). Instances of chemical alteration

during column chromatography on Florisil were not detected

although such changes would have been masked by the amount

of overlap of components in chromatograms of the whole oil.

The oil was found to consist largely of α-pinene

and several higher-boiling components, which eluted on

Carbowax 20M with RRT values generally far higher than most

sesquiterpenes. IR spectra and GC on dissimilar columns were

used to confirm the identities of 6 compounds. A further

2 compounds, with boiling points higher than that of monoterpene

hydrocarbons, were identified by their spectra as sesquiterpene

hydrocarbons, although there was insufficient spectral detail

for more positive identifications. Sesquiterpene (2) was

present in the hydrocarbon fraction, but since it also appeared

in an oxygenated sub-fraction, it is likely to be a dehydration

Table 26. Components distinguishable in the whole
oil of *Phyllocladus aspleniifolius*

	Qualitative RRT data:		Quantitative composition (percent, based on peak height)
	C20M	OV-17	
	(60° isothermal, ref. α-pinene)		(TP 50-220°, 5°/min)
*α-Pinene	1.03	1.01	43.3
Camphene	1.30	1.21	0.3
*β-Pinene	1.63	1.57	2.6
Δ$_3$-Carene	2.07	2.01	t
*Myrcene	2.24	1.77	6.4
Unidentified	2.45		0.3
*Limonene	2.76	2.40	2.9
β-Phellandrene	2.91	2.52	4.2
γ-Terpinene	3.70	3.27	t
ρ-Cymene	4.30	2.81	t
*Terpinolene	4.59	4.09	1.7
(130° isothermal, ref. camphor)			
Linalool	0.90	0.59	0.1
*Caryophyllene ⎱	1.27	2.69	4.7
Terpinen-4-ol ⎰		1.01	1.2
*α-Humulene	1.50	3.30	1.4
*α-Terpineol	1.78	1.09	2.6
Unidentified	2.02		1.6
"	5.53		0.1
"	7.43	6.09	0.5
"	9.85		2.1
"			1.3
(180° isothermal, ref. thymol)			
"	1.88	13.0	17.9
*High-boiling alcohol (?) (Fig. 13)	5.04	20.7	4.8

---

* IR spectrum recorded
t: trace, <0.1 percent

Table 27. RRT values on the dissimilar liquid phases C20M and OV-17, for components found in hydrocarbon and oxygenated fractions, and preparative GC sub-fractions, isolated from oil of *Phyllocladus aspleniifolius*

Component	Hydrocarbon fraction		Hydrocarbon sub-fractions			Oxygenated fraction		Oxygenated sub-fractions		
	C20M	OV-17	No.	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)										
α-Pinene	1.01	1.00	H1	1.03	1.02					
Camphene	1.26	1.18	H2	1.29	1.21					
β-Pinene	1.61	1.57	H3	1.64	1.57					
Δ_3-Carene	2.05	2.00						02	2.10	2.02
Myrcene	2.22	1.75	H2	2.24	1.75	2.26	1.78	01	2.24	1.76
Unidentified	2.46	2.20						02	2.57	2.25
Limonene	2.75	2.36	H6	2.74	2.40	2.76	2.38	03	2.76	2.42
β-Phellandrene	2.89	2.50	H6	2.91	2.54			02	2.90	2.48
1,8-Cineole						3.10	2.82	03	3.06	2.88
γ-Terpinene	3.71	3.26								
ρ-Cymene	4.31	2.79						03	4.37	2.88
Terpinolene	4.58	4.10	H8	4.54	4.10					
Linalool								04	4.96	4.37
(130° isothermal, ref. camphor)										
Linalool						0.93	0.59	04	0.91	0.63
Caryophyllene	1.26	2.62	H9	1.26	2.63			07	1.25	2.60
Terpinen-4-ol						1.26	1.00	05	1.23	0.97
Unidentified								07	1.45	2.88
α-Humulene								09	1.59	3.10
α-Terpineol						1.75	1.10	06	1.79	1.09
Unidentified								010	1.90	3.89
Unidentified								09	2.32	3.77
Unidentified								012	5.52	7.90
Unidentified								011	7.79	5.70
Unidentified (a)	9.76	>22								
Unidentified (b)	13.7	>22								
Unidentified (c)	18.5	>22								
(180° isothermal, ref. thymol)										
Unidentified (a)		7.7								
Unidentified (b)		9.6								
Unidentified (c)		12.4								
High-boiling alcohol(?)						5.27	21.5	014	5.0	18.4

Fig. 12(a).   Low sensitivity gas chromatogram of whole oil of foliage
of *Phyllocladus aspleniifolius* (GC on 5% Carbowax 20M/Gas Chrom Q:
temperature program 50° to 220° at 5°/min;   0.2 μℓ sample, attenuation
8 x 10³).
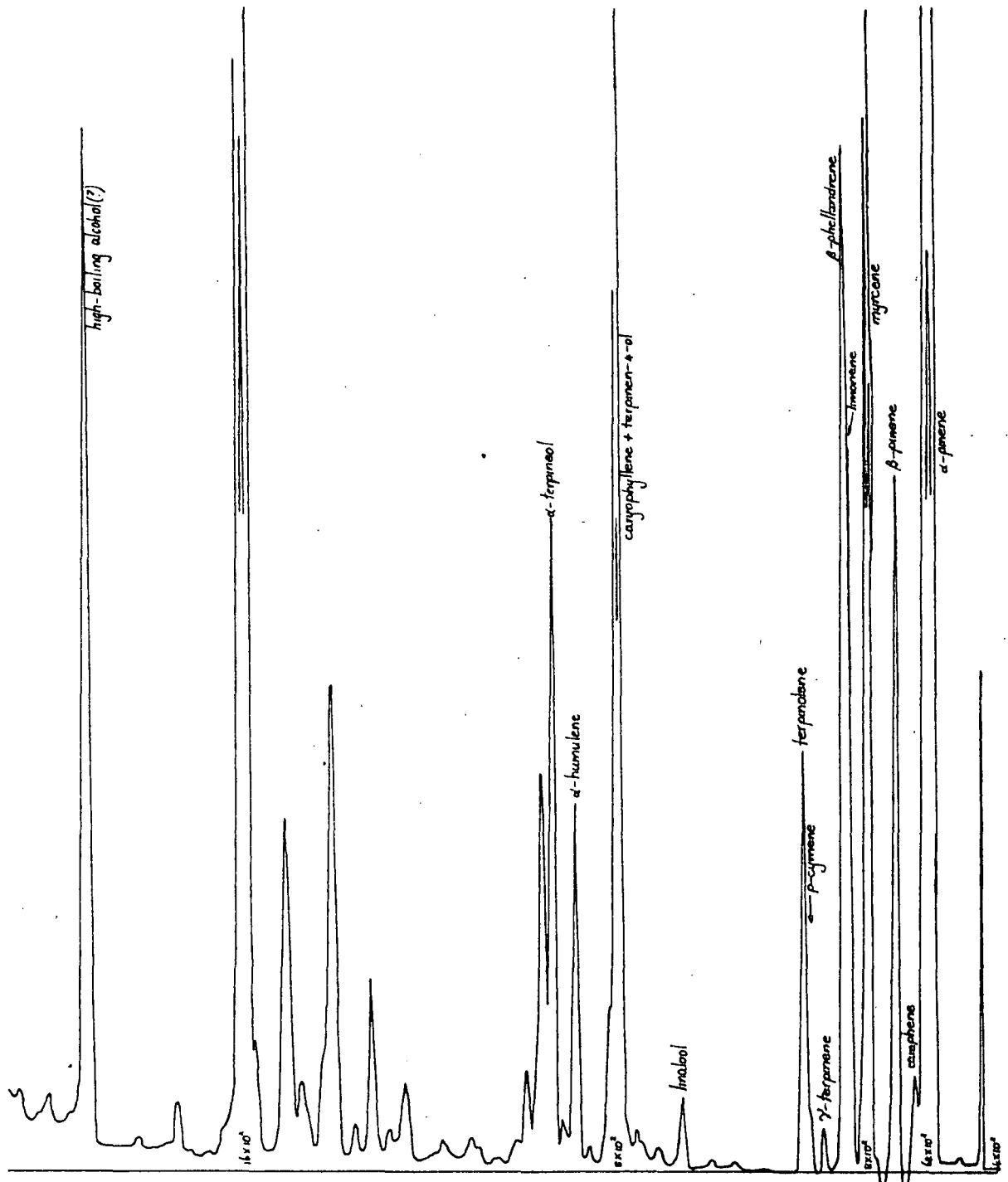
Fig. 12(b). High sensitivity gas chromatogram of whole oil of foliage of *Phyllocladus aspleniifolius* (attenuation 4 x 10^2).
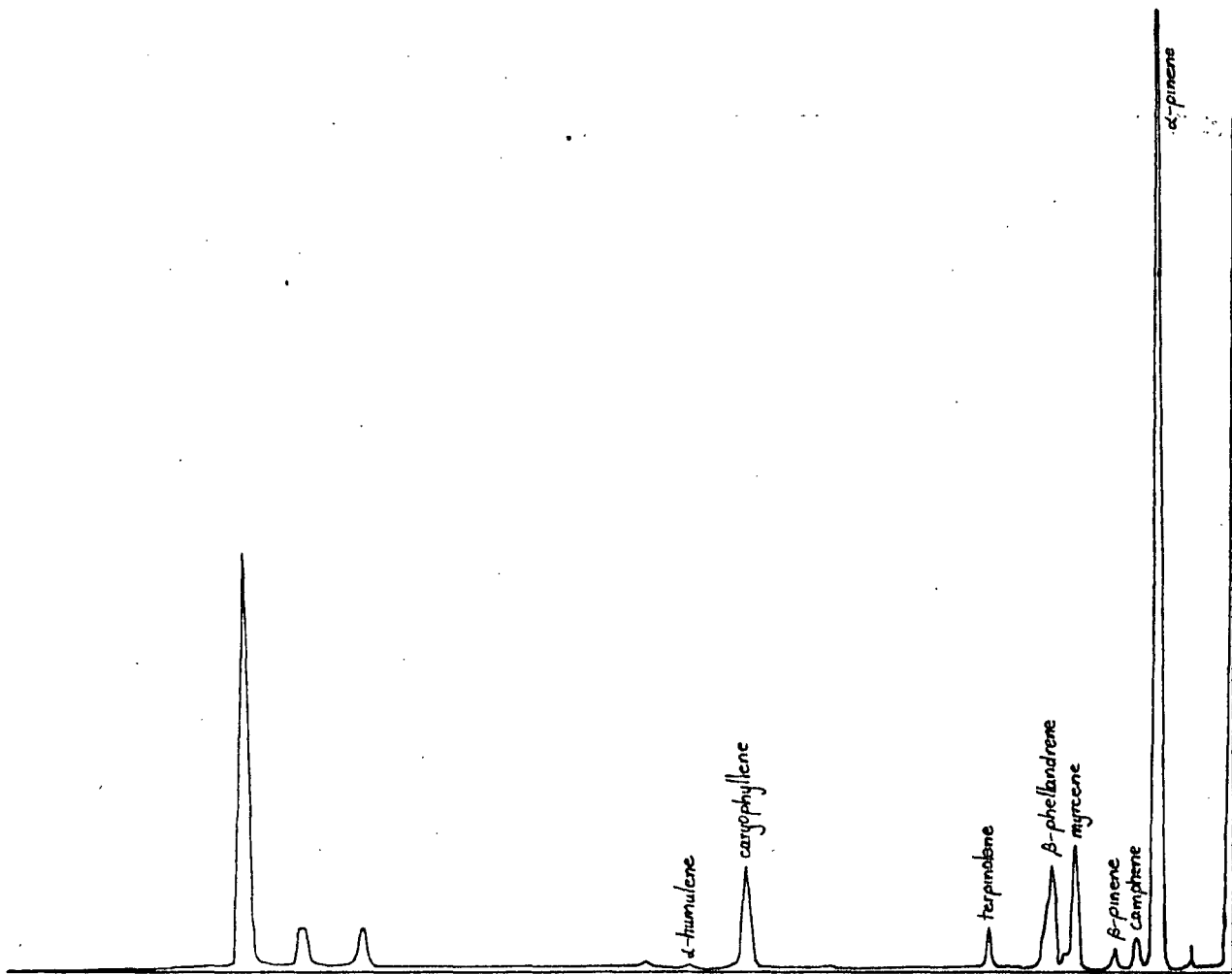
Fig. 12(c). Low sensitivity gas chromatogram of hydrocarbon fraction of oil of *Phyllocladus aspleniifolius* separated on Florisil.
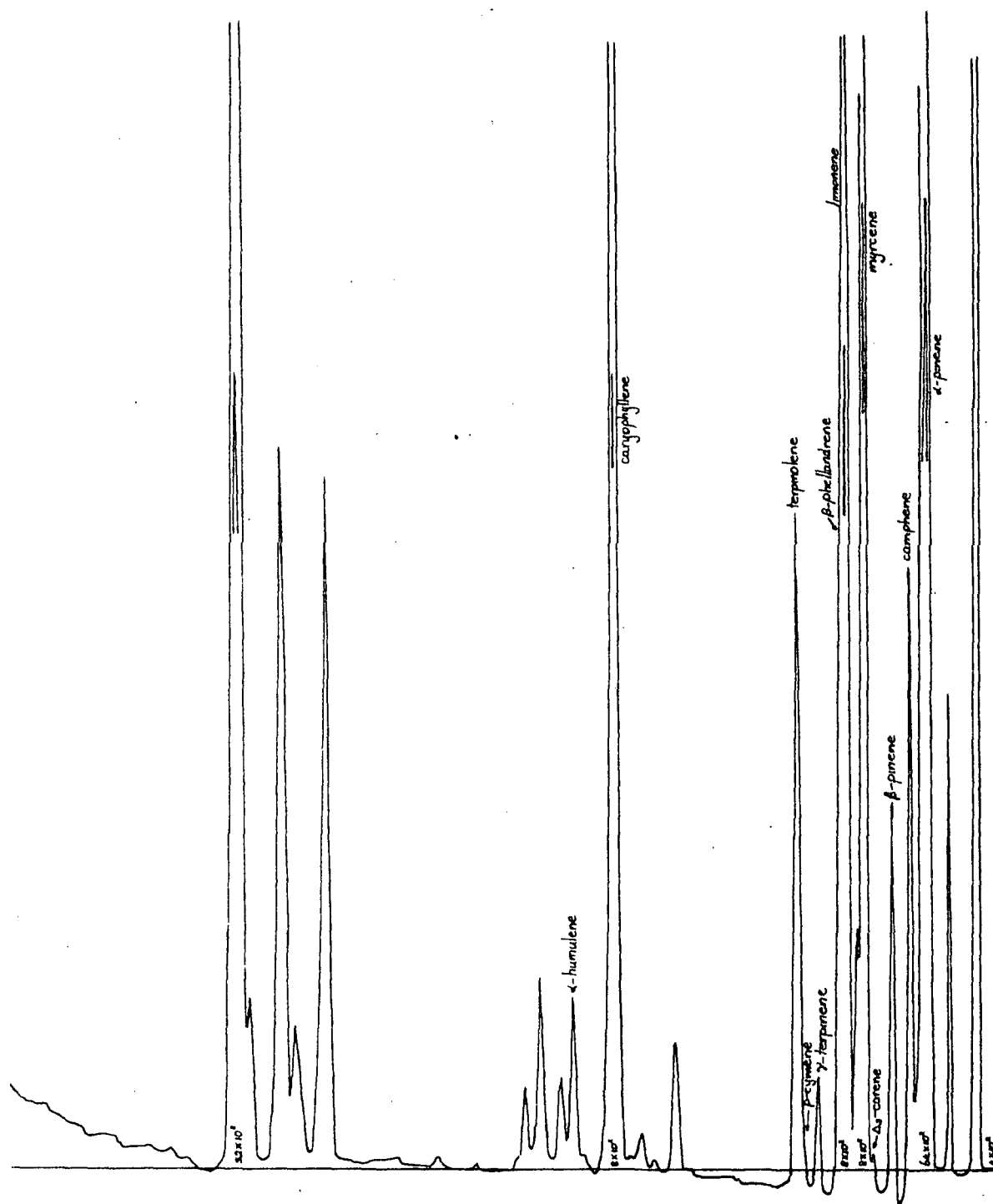
Fig. 12(d). High sensitivity gas chromatogram of hydrocarbon fraction of oil of *Phyllocladus aspleniifolius* separated on Florisil.
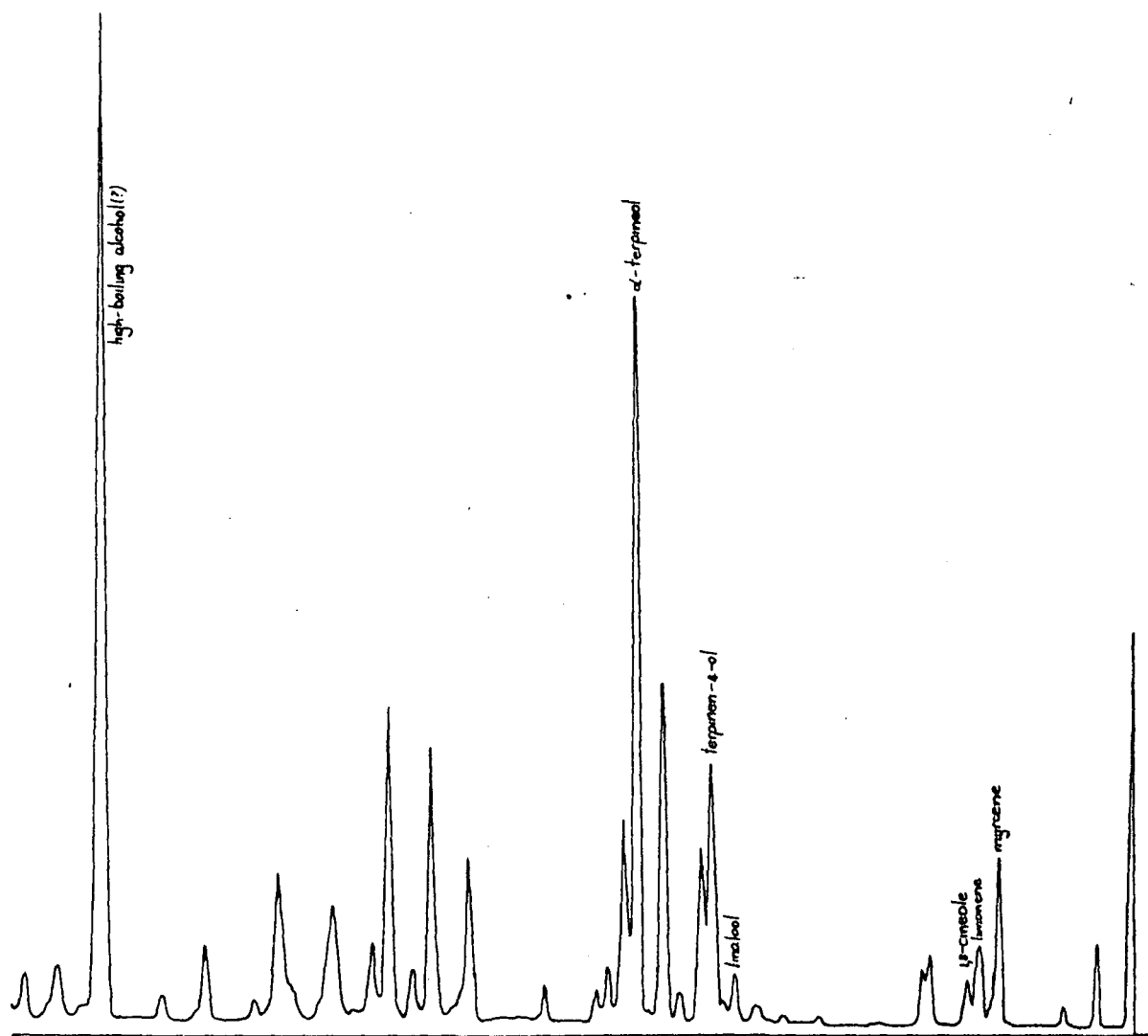
Fig. 12(e).   Low sensitivity gas chromatogram of oxygenated fraction of oil of *Phyllocladus aspleniifolius* separated on Florisil.

156.
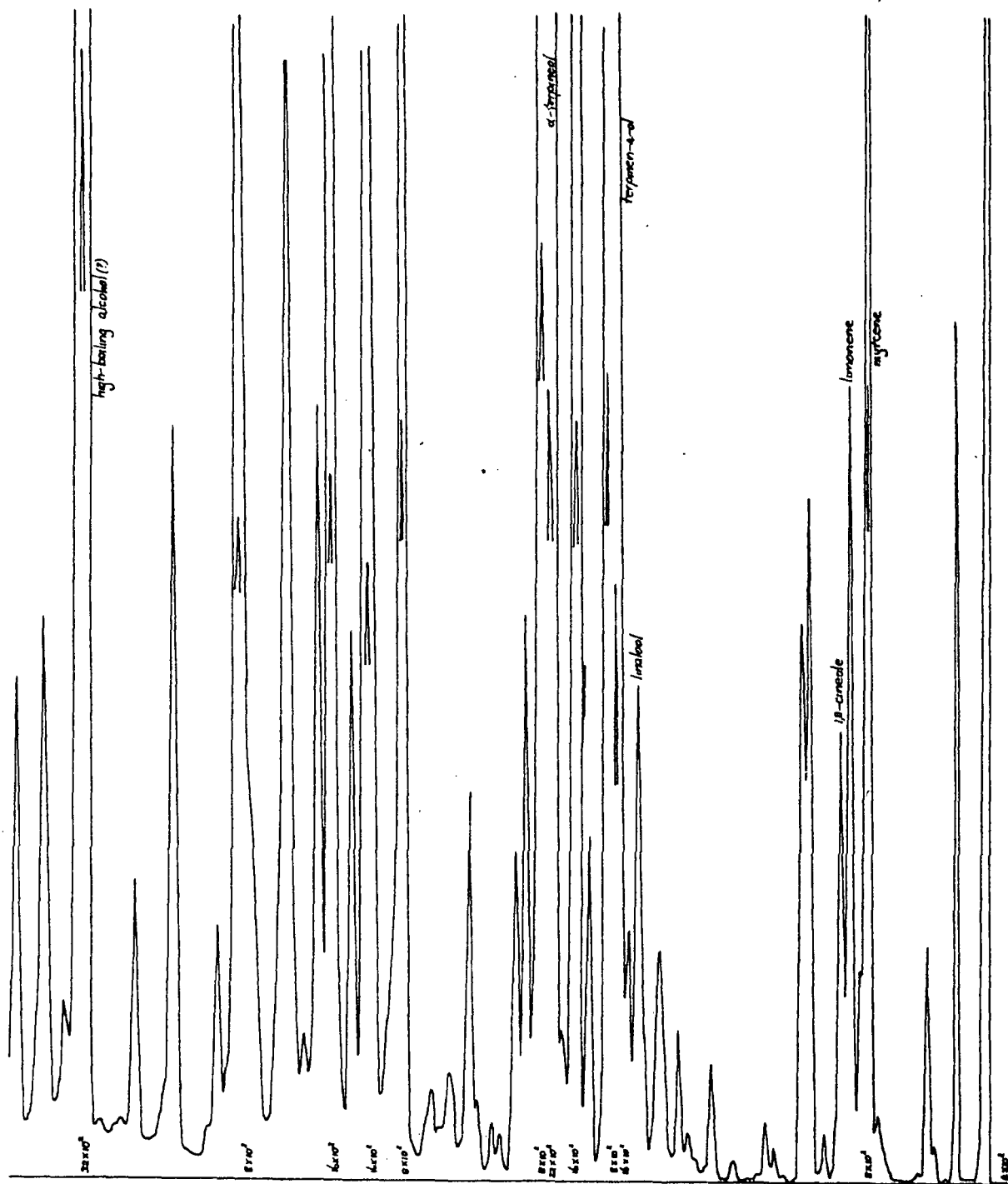
Fig. 12(f). High sensitivity gas chromatogram of oxygenated fraction of oil of *Phyllocladus aspleniifolius* separated on Florisil.

product. Sesquiterpene (4) was similarly partially

identified from its IR spectrum, and because it could only

be found in the original oil and in an oxygenated sub-fraction,

it must also be concluded to be a dehydration product. At

least 7 other components were tentatively identified by GC

on dissimilar columns.

Components that constituted 65 percent of the oil

were at least tentatively identified. The question arises as

to the futility of trying to identify particularly the

sesquiterpenes, some of which appear to be artifacts. Any further

detailed analysis should therefore incorporate analytical GC

on dissimilar columns to monitor artifact formation.

Of the highest-boiling components a compound in the

oxygenated fraction was isolated by preparative GC apparently

without chemical alteration. Analytical GC on dissimilar

columns did not indicate traces of any minor constituents in

this O14 sub-fraction. The IR spectrum indicated the presence

of methyl groups and a terminal methylene. A relatively weak

absorption, with a sharp band at 3,700 cm^{-1} and a broad band

centred around 3,500 cm^{-1}, was the only indication of a

hydroxyl group that might account for the oxygenated nature

of the compound (Figure 13). The NMR spectrum using $CDCI_3$

solvent indicated the presence of $=CH_2$ possibly adjacent to

=CH-. An indistinct series of absorptions in the region 8.2

to 9.3τ indicated approximately 31 protons (Figure 14a).

A further NMR spectrum run with added D_2O was almost identical

except for the absence of a minor band in the complex at 9.09τ.
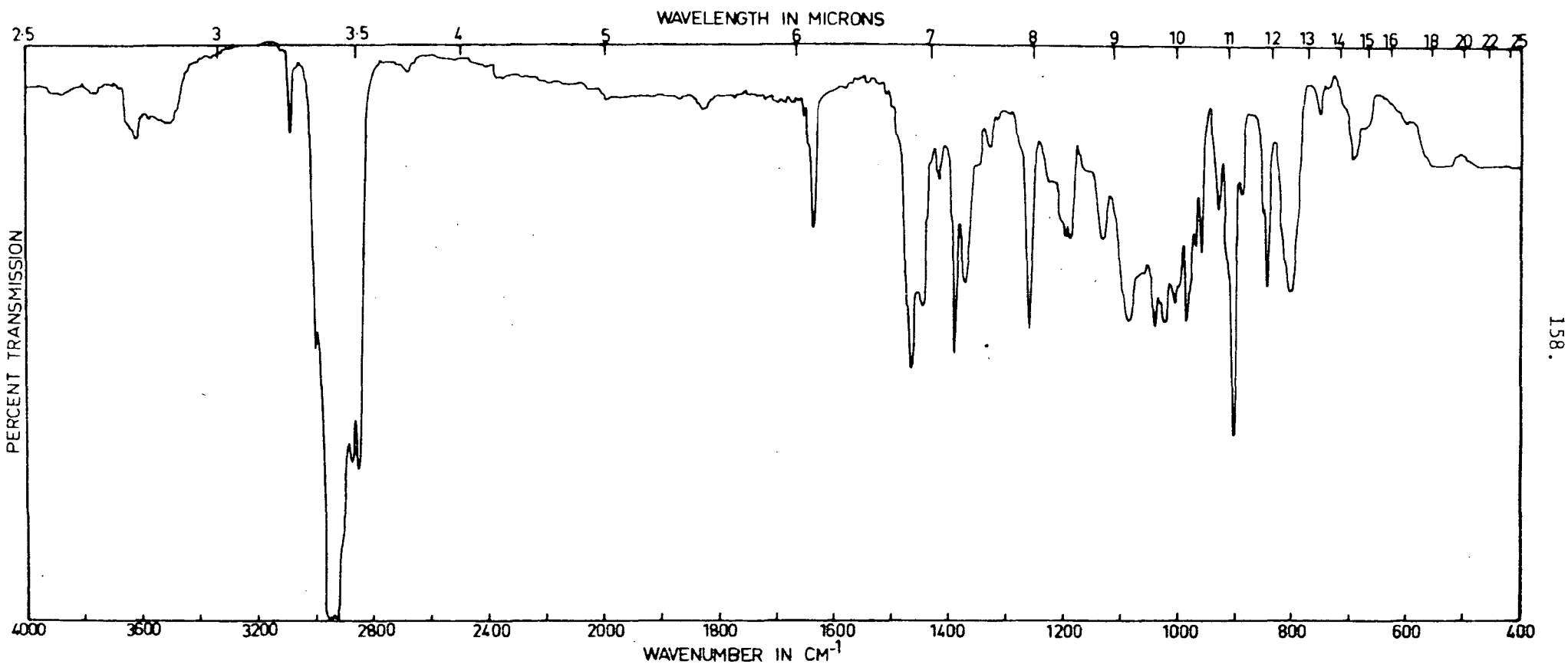
Fig. 13. IR spectrum of the pure compound isolated in the oxygenated sub-fraction O14 of oil of *Phyllocladus aspleniifolius* [RRT$_{thymol}$ 5.04 (C20M) and 18.4 (OV-17); IR spectrum of 0.007 mm film].
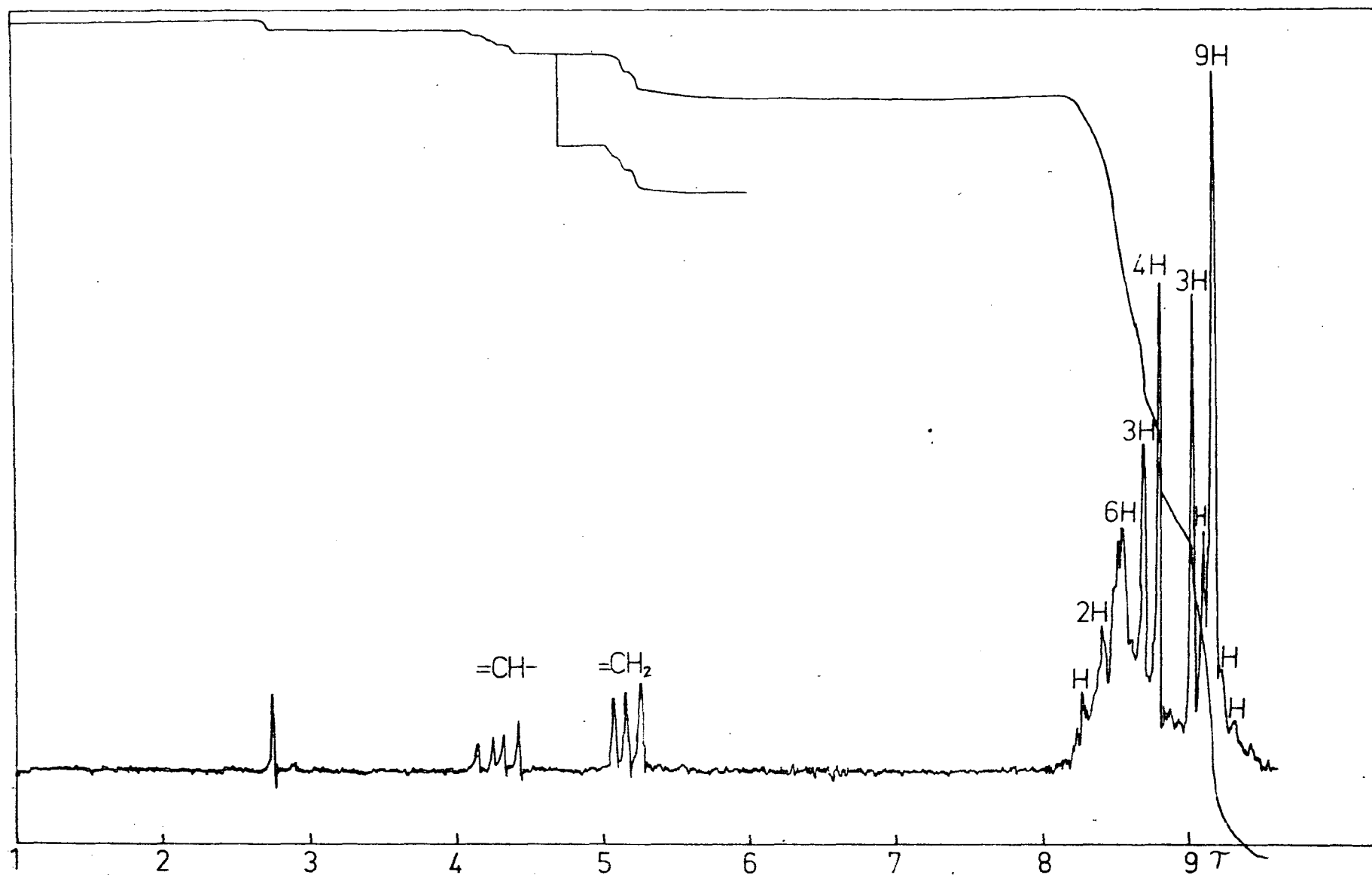
Fig. 14(a). NMR spectrum of the pure compound isolated in the oxygenated sub-fraction O14 of oil of *Phyllocladus aspleniifolius* (CDCl₃ solvent).
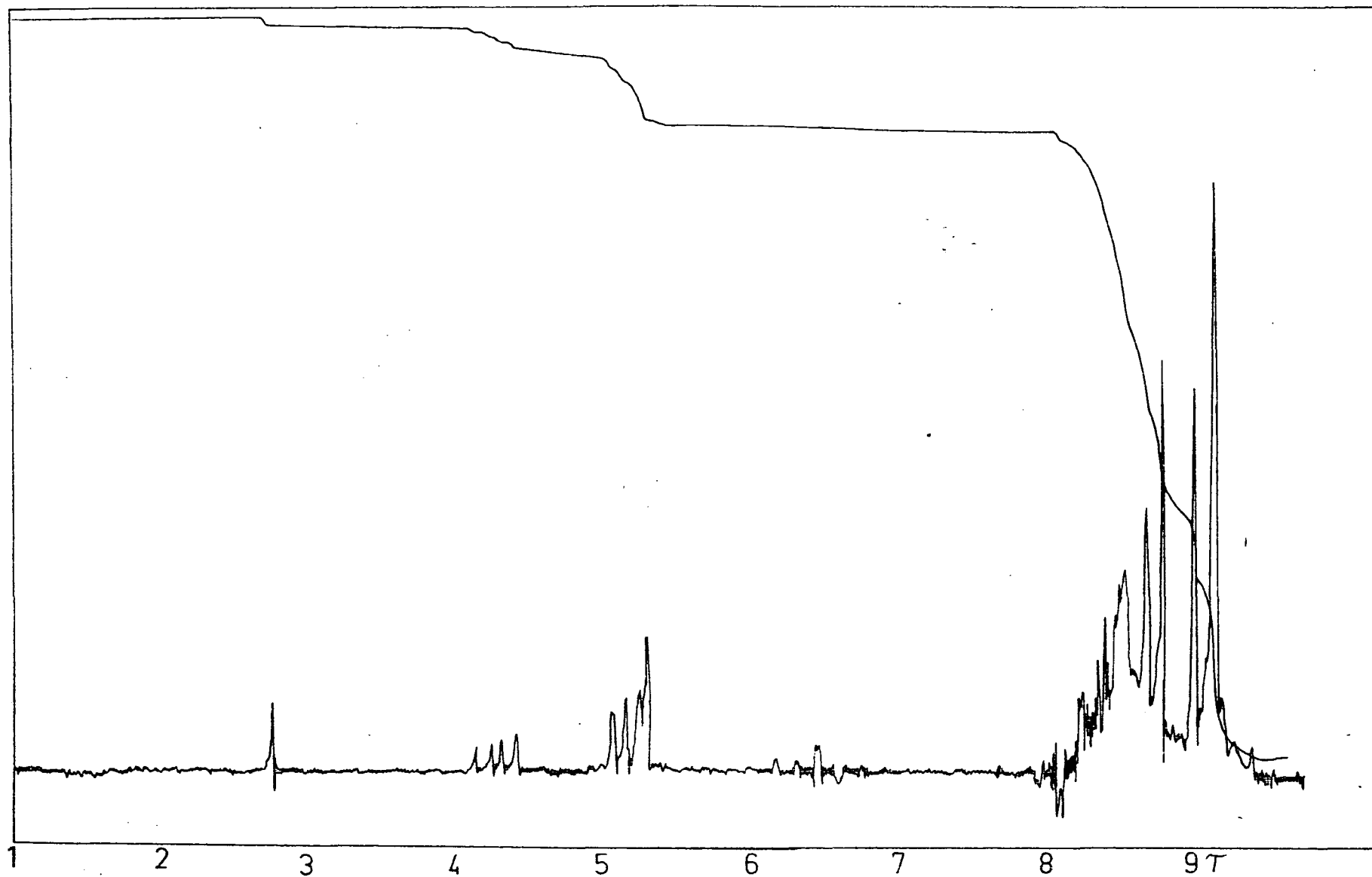
Fig. 14(b).   NMR spectrum of the pure compound isolated in the oxygenated sub-fraction O14 of oil of *Phyllocladus aspleniifolius* (D$_2$O added to CDCl$_3$ solvent).

This feature could not be construed however as clear
evidence of a hydroxyl proton (Figure 14b). Since the
compound has about 34 hydrogen atoms, together with a
degree of unsaturation, it is móst unlikely to be a
sesquiterpene derivative. It must therefore be one of the
more volatile of the much higher-boiling compounds that are not
often encountered in steam-distilled oils.

By comparing the composition of the oil found in
P. aspleniifolius with those reported for other species in
the genus *Phyllocladus*, and others of the family Podocarpaceae
(Table 28), the presence of a major proportion of highest-
boiling components can be readily understood. Although most
of the oils reported were studied in earlier times with
limited techniques, an unsaturated diterpene, phyllocladene,
has been repeatedly identified in steam-distilled oils of
this family. Phyllocladene has been reported as a major
component in oils of each of the *Phyllocladus* species studied,
and in several species of *Dacrydium* and *Podocarpus*. Future
study of the oil of *P. aspleniifolius* should therefore make
provision for the extraction of diterpenoids and oxygenated
high-boiling components which appear to constitute a significant
proportion of this oil.

The biosynthesis of the monoterpenes in this oil,
indicated by their qualitative co-occurrence [352-355], appears
to agree with the hypothesis proposed by Ruzicka [356], and
more recently supported by Zavarin [357] (Figure 15).

Table 28. Components reported in essential oils extracted from species of the Family Podocarpaceae. Although most of the reports appeared before the widespread use of chromatography, oils from this family are generaly characterized by a high proportion of high-boiling compounds, including steam-distillable diterpenoids. Highest-boiling compounds in oil of *Phyllocladus aspleniifolius* (this study) might therefore include diterpenes in common with oils of these other species.

Plant species	Components reported	Reference/date
Phyllocladus glaucus	Phyllocladene	[347] 1959
P. trichomanoides	α-Pinene (31.6%), myrcene + dipentene (3.2%), γ-terpinene, sesquiterpenes, sesquiterpene alc., phyllocladene + other diterpenes	[348] 1948
P. alpinus	Phyllocladene	[349] 1937
	Phyllocladene (30%)	[350] 1938
Dacrydium colensoi [N.B. confused in literature with *D. biforme*]		
	Sesquiterpenes and mainly the unsatd. diterpene dacrene (m.pt. 96°)	[359] 1929
	ℓ-α-Pinene (17%), myrcene (4%), ℓ-limonene (5%), terpinolene (2%), d-longifolene (5%), muurolene (5%), d-β-cadinene (5%) + other sesquiterpenes, diterpenes including d-phyllocladene (35%)(m.pt. 96°)	[358] 1952
D. biforme (?)	Sesquiterpene (40%), oxygenated compd., dacrene (13%) (suggested name)	[360] 1923
	Cadinene + a sesquiterpene, oxygenated compd., dacrene, isodacrene	[361] 1928
	Sesquiterpene structures (2) ascribed	[362] 1954
	Myrcene, β-terpinene, (+)-longifolene, (+)-aromadendrene, (−)-metrosiderene, (+)-δ-cadinene, α-camphorene, oxygenated terpenes, sesquiterpene alc., (+)-phyllocladene	[363] 1955
D. franklinii	Eugenol methyl ether (~90%)	[364] 1924
	Low b.pt. hydrocarbon, eugenol methyl ether (90-95%)	[365] 1929
	Mainly eugenol methyl ether	[366] 1944
	Eugenol, eugenol methyl ether, elemicin, coniferyl aldehyde methyl ether, coniferyl alcohol methyl ether, β-sitosterol, dacriniol, dacrinial, fatty acids, waxes, etc. (in heartwood ext.)	[367] 1967
D. cupressinum	Sesquiterpene and mainly a diterpene (m.pt. 55.5°) that isomerizes to diterpene (m.pt. 92°) on distillation	[368] 1925
	α-Terpene (~20%), sesquiterpene (9.4%), 2 diterpenes	[369] 1932
	Identified myrcene, limonene, α-terpinene, p-cymene, terpinolene; tentatively identified β-pinene, camphene, β-phellandrene	[370] 1974
D. laxifolium	Phyllocladene	[371] 1960

(Continued)

Table 28 continued

Plant species	Components reported	Reference-date
D. kirkii	d-α-Pinene, myrcene, limonene, bornyl acetate, carvone, sobrerol, cadinene, sesquiterpene alcohols, azulenes, phyllocladene	[372] 1947
D. elatum	ℓ-Cedrene, d-cedrol	[373] 1925
Podocarpus dacrydiodes	Terpenes (3%), terpene alcohols (5%), acids + phenols + aldehydes (3%), esters (4%), sesquiterpenes (62%), sesquiterpene alcohols (9%)	[374] 1929
	α-Pinene, β-pinene, oxygenated compds., sesquiterpenes, sesquiterpene alcohols	[375] 1932
	Selin-11-en-4α-ol (m.pt. 94-6°)	[376] 1967
P. ferrugineus	d-α-Pinene (36%), cineole (2%), d-limonene + dipentene (5%), cadinene (12%), diterpene mirene (27%)	[377] 1928
	Mainly ferruginol + diterpenes (in heartwood)	[351] 1971
	Identified myrcene, β- and α-terpinene; tentatively identified β-pinene, sabinene, terpinolene	[370] 1974
P. lambertius	Phyllocladene, isophyllocladene, totarols, 17-isophyllocladenol, other diterpenoids	[378] 1975
P. nivalis	Phyllocladene	[371] 1960
P. spicata	α-Pinene, β-pinene, limonene, cadinene, a sesquiterpene, a sesquiterpene ketone, sesquiterpene alcohols, 2 diterpenes	[380] 1939
	α-Pinene, myrcene, α-terpinene, β-phellandrene, dipentene, γ-terpinene, ρ-cymene, α-terpineol, aromadendrine, a selinene cadinene, kaurene or phyllocladene	[146] 1960
P. hallii	Phyllocladene (m.pt. 96-5°) (first occasion found in a *Podocarpus* sp.)	[381] 1940
P. totara	α-Pinene, terpenes, sesquiterpenes, diterpenes	[374] 1929
	α-Pinene, β-pinene, cadinene, rimuene, dacrene	[382] 1933
	Rimuene	[381] 1940
P. macrophylla	α-Pinene, camphene, β-pinene, cadinene	[383] 1930
	α-Podocarprene, β-podocarprene (names suggested)	[384] 1931

163.

164.
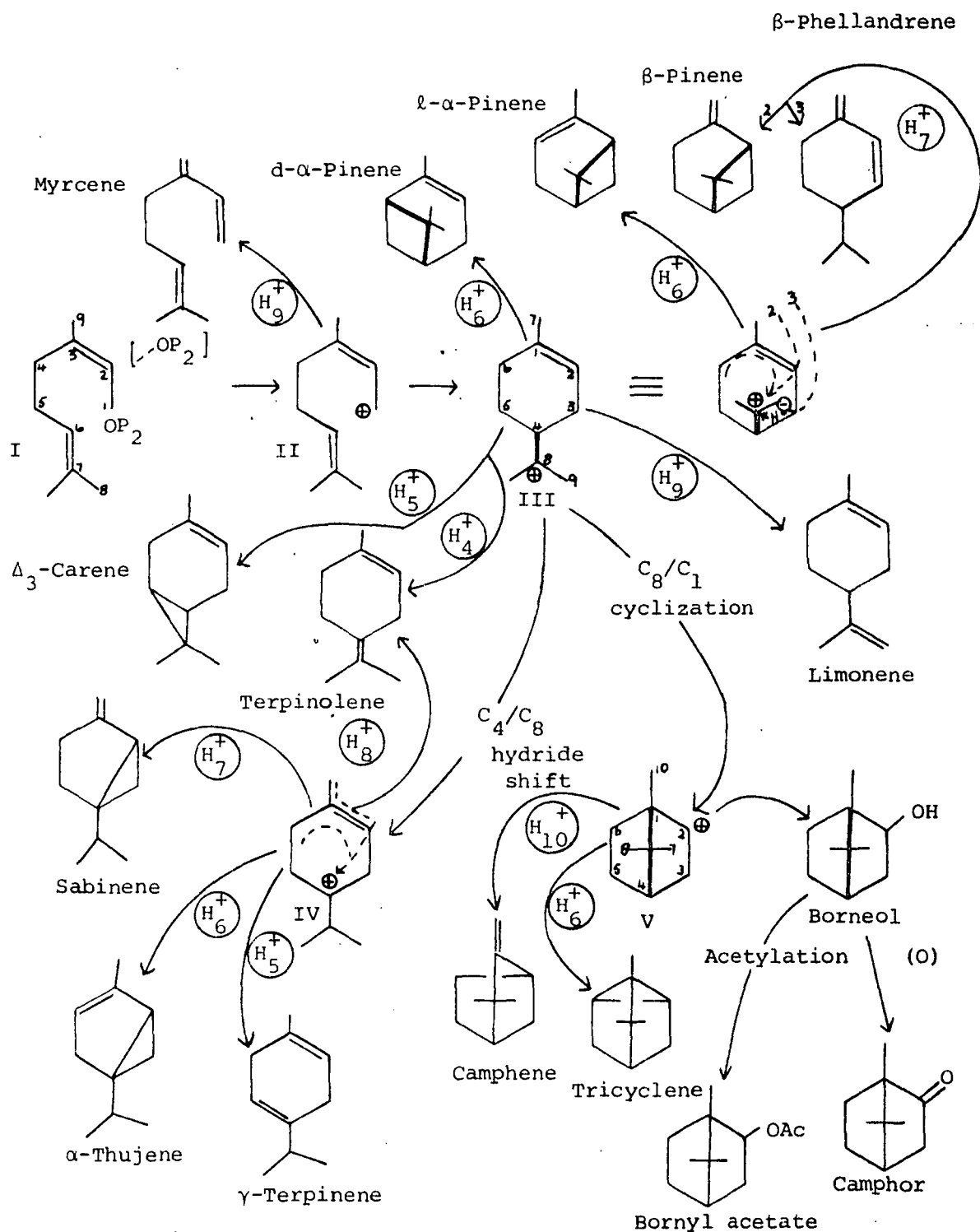
Fig. 15. Hypothesized pathways in the biosynthesis of monoterpenoids.

The biosynthesis of α-pinene as the single major monoterpene, i.e. in the absence of any other major co-synthesized monoterpene, is in accordance with either of the two pathways proposed by Ruzicka that result in this single product through interaction of the positively-charged C_8 with the double bond of the 1-ρ-menthene-8-carbonium ion intermediate (III). The almost complete absence of members of the group consisting of camphene, tricyclene and bornyl acetate, suggests the absence of any cyclization of the 1-ρ-menthene-8-carbonium ion (III) that would have led to a 2-bornane carbonium ion (V) precursor. Similarly, the apparent absence of the sabinene, α-thujene and α-terpinene group suggests the absence of a C_4-C_8 hydride shift in the 1-ρ-menthene-8-carbonium ion (III) that would have led to a 1-ρ-menthene-4-carbonium ion (IV) precursor. Small percentages of α-pinene, α-phellandrene, myrcene, limonene and terpinolene could have been derived individually from the hypothetical ions, II, III and its equivalent form, as indicated in Figure 15. The synthesis of diterpenoids is considered to be well separated from that of monoterpenoids [357], so that no speculation should be made as to any biosynthetic relationship between α-pinene and such a high-boiling component, i.e. providing it is subsequently shown to be a diterpenoid.

(iii) <u>Experimental</u>

(a)   <u>Sampling</u>

A composite sample of 3,020 g of foliage and small branches was collected in mid-summer from a large number of trees along the road to the Harz Mountain National Park in southern Tasmania. The foliage was stored frozen until steam distilled.

(b)   <u>Isolation of oil</u>

Steam distillation.of the foliage, cut into $\simeq 1$ cm pieces, was carried out for 2 hr. The distillate was collected in a separating funnel containing a layer of redistilled diethyl ether over a concentrated solution of sodium chloride. The total distillate was shaken, partitioned, and extracted again with a further small portion of ether. The ether extract was dried by passing rapidly through a column of anhydrous sodium sulphate. The solvent was finally removed by evaporation in a long-necked round-bottom flask. This was achieved as rapidly as possible at almost room temperature with the aid of a fine stream of nitrogen with the flask standing on a 60°-80° waterbath.

The recovered 9.26 g of pale green oil represented a yield of 0.31 percent of the wet foliage.

(c)   <u>Separation</u>

A charge of 8.93 g of oil was added to a column containing 150 g of Florisil that had been activated at 130°

for at least 5 hr. The column had first been pre-wet with

$58^{\circ}-68^{\circ}$ redistilled petroleum ether. A hydrocarbon fraction

was first eluted at 5 ml/min with 400 ml of pet. ether,

followed then by the oxygenated fraction eluted and drained

with 400 ml of diethyl ether.

Evaporation of the solvents, as described

previously, yielded 6.76 g (76%) of hydrocarbons and 1.43 g

(24%) of oxygenated material.

Each fraction was subsequently separated by

preparative GC into a number of sub-fractions for identification

by IR spectroscopy. Preparative GC was carried out with

repeated 10 µℓ injections onto a 2.1 m × 0.95 cm OD glass

column of 20% DC-200/Chromosorb W HMDS (60-80) temperature-

programmed from 150° to 240° at 2°/min.

(d) Qualitative and quantitative analyses

The identity of components was tentatively determined

using the dissimilar column analytical GC system:

2 m × 4 mm ID glass column packed with 5%

Carbowax 20M/Gas Chrom Q (80-100),

and 5% OV-17/Gas Chrom Q (80-100).

RRT values were compared with those of authentic compounds.

Confirmation of identity relied upon additional IR spectroscopic

data from those compounds that were readily isolated pure as

above.

IR spectra were recorded from a few µℓ of a component

when available in sufficient quantity. Spectra containing the

necessary fine detail for identification purposes could in this way be obtained with a 0.007 mm film between sodium chloride plates. Spectra of sub-fractions available in less than a μℓ quantity were recorded in 2% chloroform solution.

Quantitative analysis was based upon the area of a peak on the Carbowax 20M column. It was concluded that if suitable temperature-program conditions were selected, then each peak on this column had a very nearly identical width at half peak height. Quantitative calculations could then be made, based upon peak height comparison alone, and still only have the same degree of error as by peak area.

(e) Check on artifact formation

Before and after each separation step a temperature-programmed chromatogram was recorded in an attempt to document any losses, RRT shifts or artifacts arising from chemical alteration of components. A chromatogram was recorded on the 5% Carbowax 20M column of the whole oil, hydrocarbon and oxygenated fractions, and their sub-fractions. This further ensured that a recorded IR spectrum was in fact of the unaltered GC peak of interest.

(iv) Summary

Components of steam-distilled foliage oil of *Phyllocladus aspleniifolius* were analyzed by chromatographic and spectroscopic methods. Monoterpenoids identified were α-pinene (43.3%), β-pinene (2.6%), myrcene (6.4%), limonene

(2.9%), terpinolene (1.7%) and α-terpineol (2.6%).

Tentatively identified on a dissimilar GC liquid phase

system, consisting of 5% Carbowax 20M and 5% OV-17, were

camphene (0.3%), Δ_3-carene (<0.1%), β-phellandrene (4.2%),

1,8-cineole, γ-terpinene (<0.1%), p-cymene (<0.1%),

caryophyllene (4.7%), α-humulene (1.4%) , linalool (0.1%)

and terpinen-4-ol (1.2%). Two components were detected which

eluted on Carbowax 20M long after sesquiterpenes. One of

these components, which was selectively removed in a fraction

consisting of oxygenated materials, was studied by both IR

and NMR but was not identified. Several instances were found

of dehydration of oxygenated components to sesquiterpene

hydrocarbons.

2.   Composition of leaf oil from King Billy Pine

     (*Anthrotaxis selaginoides*)

(i)   Introduction

          Athrotaxis selaginoids Don (Fam. Taxodiaceae) grows

into a tree, often only 15 m high, on valley slopes in areas

of high rainfall at 800 to 1,300 m above sea level. It is

one of three species of this genus that is native to Tasmania

[343].

          No report was found in the literature of any study

of leaf oil of *A. selaginoides*, although some heartwood

extractives have been identified [385, 386].

(ii) Results and discussion

Foliage was collected in mid-summer from a tree on the shore of Lake Perry in the Harz Mountain National Park in southern Tasmania. The steam-distilled oil was a deep lemon-yellow colour and had an unpleasant burnt woody odour.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 29. RRT values leading to the tentative identification of components in hydrocarbon and oxygenated fractions, and sub-fractions, are listed in Table 30. Gas chromatograms of Figure 16 show the distribution of oil components eluted from a Carbowax 20M column, and indicate the degree of separation of components into hydrocarbon and oxygenated fractions.

The presence of significant amounts of myrcene and limonene in the oxygenated fraction was surprising, because from previous experience these hydrocarbons have been found to be quantitatively separated on Florisil into the hydrocarbon fraction.

The oil was found to consist largely of limonene, several high-boiling oxygenated components, with small percentages of myrcene and linalool. IR spectra of four unidentified components, two sesquiterpenes and two alcohols, are given in Figures 17-20. The two alcohols were eluted from Carbowax 20M with RRT values generally far higher than most monoterpene alcohols, which could indicate that these compounds belonged to the large group of undocumented sesquiterpene alcohols.

Table 29. Components distinguishable in the whole oil of *Athrotaxis selaginoides*

Component	Qualitative RRT data: C20M	OV-17	Quantitative composition (percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50-220°, 5°/min)
Unidentified	1.07		t
"	1.81		t
*Myrcene	2.24	1.74	5.1
*Limonene	2.78	2.39	40.3
γ-Terpinene	3.68	3.27	t
ρ-Cymene	4.27	2.80	t
Terpinolene	4.61	4.13	t
	(130° isothermal, ref. camphor)		
Unidentified	0.50		1.8
"	0.65		0.8
"	0.78		0.4
*Linalool	0.92		5.1
Unidentified	1.23		} 0.8
*Sesquiterpene(7) (Fig. 17)	1.31	2.77	
Unidentified	1.50	3.20	0.4
α-Terpineol	} 1.73	1.06	0.4
*Sesquiterpene(5)		4.04	1.3
Unidentified	2.03	4.04	10.5
"	2.38		0.5
"	2.62		0.3
"	3.01		0.3
*Terpene alcohol(2) (Fig. 19)	} 9.63	7.29	} 10.2
*Sesquiterpene(8)? (Fig. 18)			1.0
Unidentified	12.0		
*Terpene alcohol(3) (Fig. 20)	14.6	11.7	4.0
	(180° isothermal, ref. thymol)		
*Terpene alcohol(2)	} 0.97	2.82	
*Sesquiterpene?(8)		7.27	
Unidentified	1.10		
*Terpene alcohol(3)	1.22	3.62	
Unidentified	1.65		1.3
"	1.85		0.4
"	5.20	18.8	15.0

* IR spectrum recorded
t: trace, <0.1 percent

Table 30. RKT values on the dissimilar liquid phases C20M and OV-17, for components found in hydrocarbon and oxygenated fractions, and preparative GC sub-fractions, isolated from oil of *Athrotaxis selaginoides*

Component	Hydrocarbon fraction		Hydrocarbon sub-fractions			Oxygenated fraction		Oxygenated sub-fractions		
	C20M	OV-17	No.	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)										
α-Pinene	1.00	1.00								
Unidentified	1.11									
Camphene	1.29	1.19								
Δ₃-Carene	2.00	1.98								
Myrcene	2.22	1.76	H1	2.28	1.79	2.25	1.76	O1	2.24	1.73
Limonene	2.80	2.41	H2	2.77	2.40	2.76	2.38	O2	2.77	2.38
γ-Terpinene	3.67	3.25								
ρ-Cymene	4.29	2.79								
Terpinolene	4.57									
(130° isothermal, ref. camphor)										
Linalool						0.93	0.61	O3	0.95	0.61
Sesquiterpene(7)	1.29	2.78	H3	1.30	2.79					
Unidentified	1.51	3.09	H3	1.49	3.14					
Sesquiterpene(5)	1.73	3.77	H4	1.75	3.93					
α-Terpineol						1.78	1.09			
Terpene alcohol(2)						9.74	7.30	O4	9.64	7.48
Sesquiterpene?(6)	9.82		H5	9.84	>22					
Unidentified (a)	11.9									
" (b)	13.5									
Terpene alcohol(3)						14.7	11.8	O5	14.6	11.8
Unidentified (c)	16.8									
" (d)	17.9									
(180° isothermal, ref. thymol)										
Terpene alcohol(2)						0.95	2.67			
Sesquiterpene?(3)		7.23	H5	1.05	6.95					
Unidentified (a)		8.50								
" (b)		9.15								
Terpene alcohol(3)						1.19	3.42			
Unidentified (c)		10.4								
" (d)		11.8								
Unidentified						5.02	18.1			
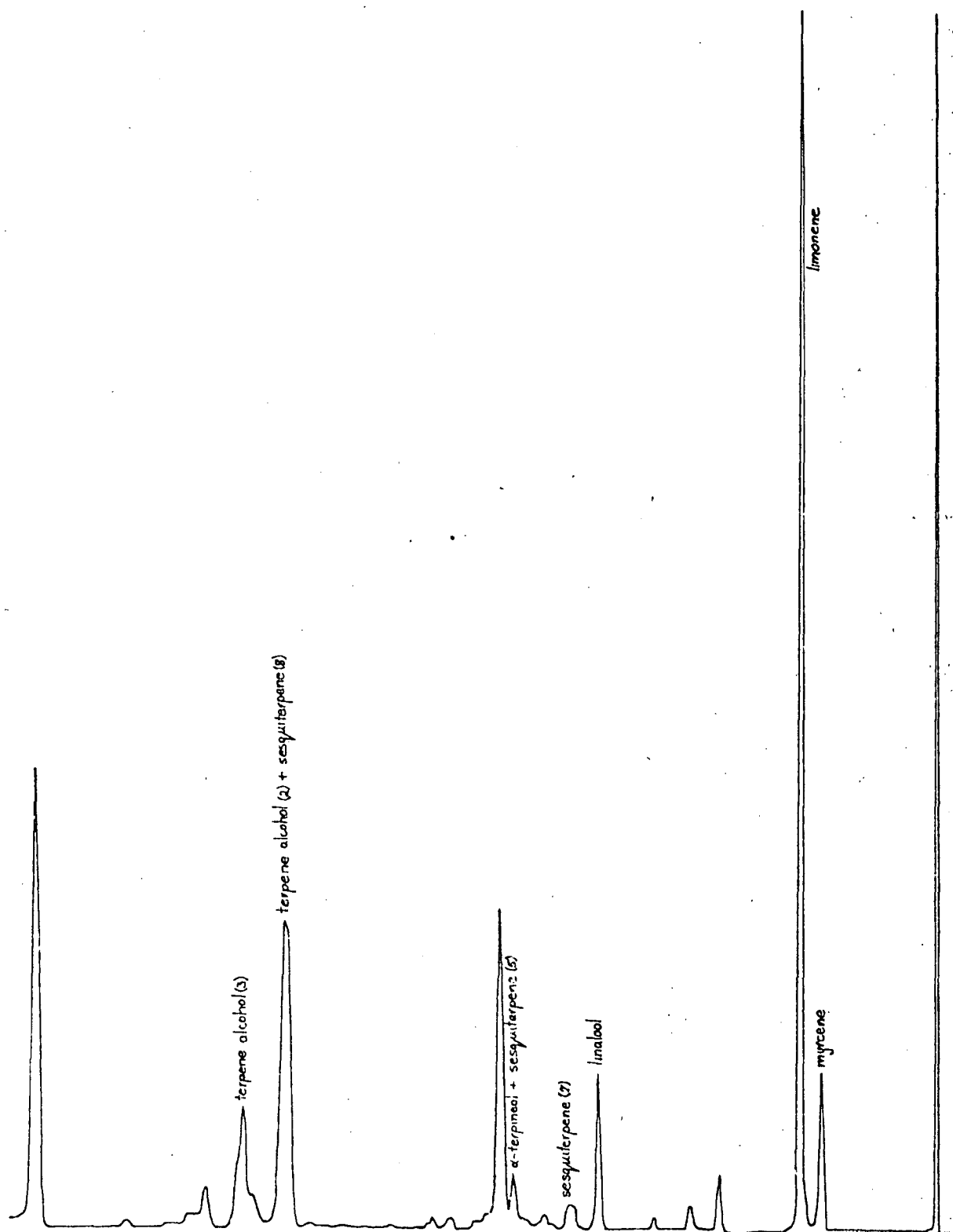
Fig. 16(a). Low sensitivity gas chromatogram of whole oil of foliage
of *Athrotaxis selaginoides* (GC on 5% Carbowax 20M/Gas Chrom Q;
temperature program 50° to 220° at 5°/min; 0.2 µℓ sample;
attenuation 4 x 10³).
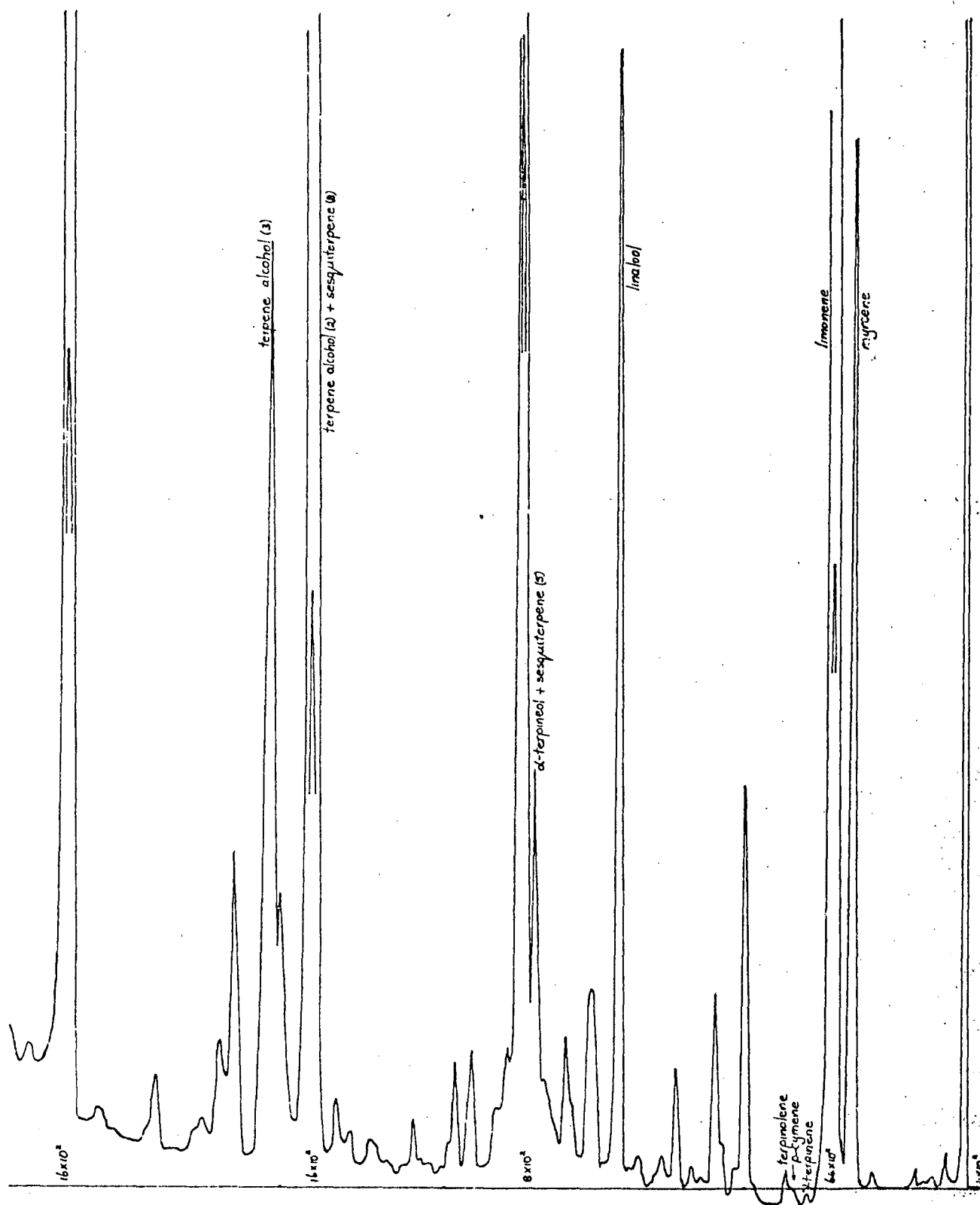
Fig. 16(b). High sensitivity gas chromatogram of whole oil of foliage of
Athrotaxis selaginoides (attenuation 4 x 10^2).
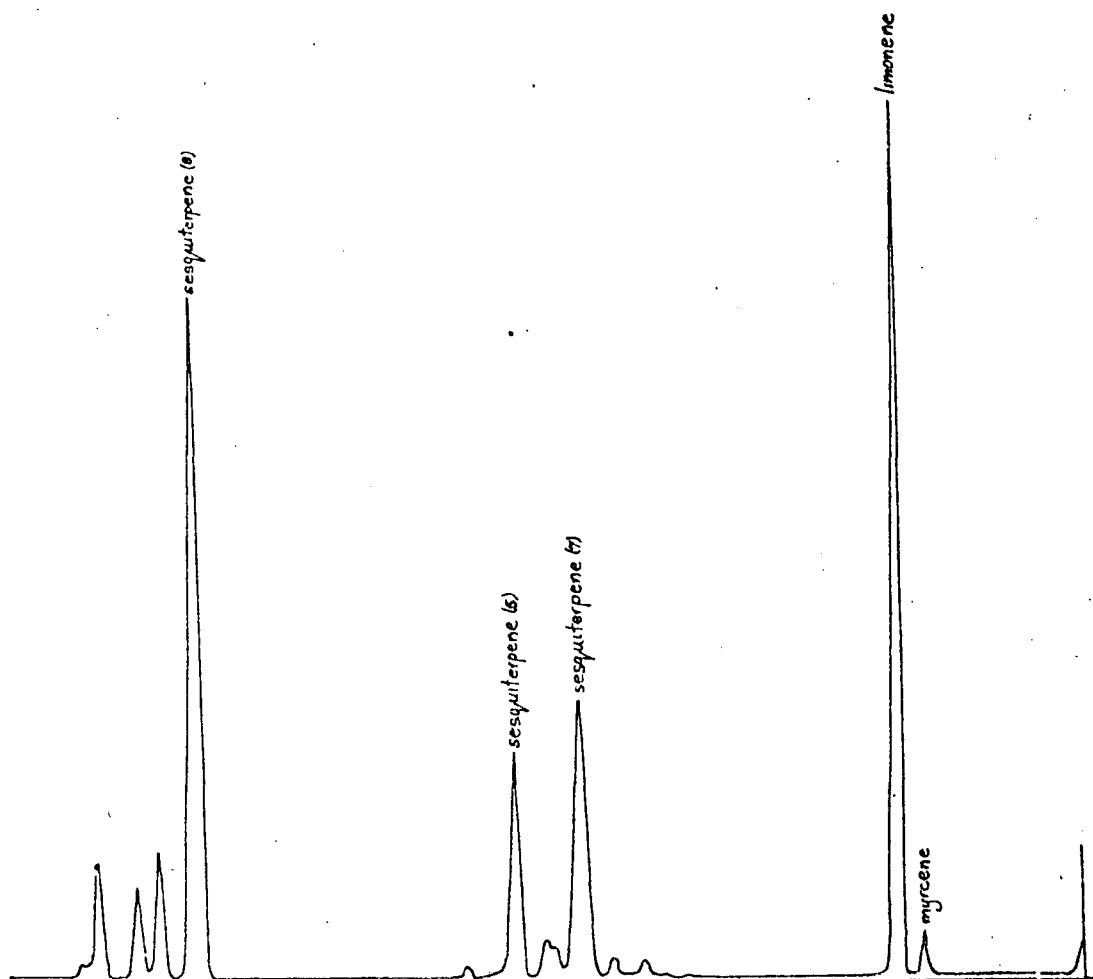
Fig. 16(c). Low sensitivity gas chromatogram of hydrocarbon fraction of oil of *Athrotaxis selaginoides* separated on Florisil.
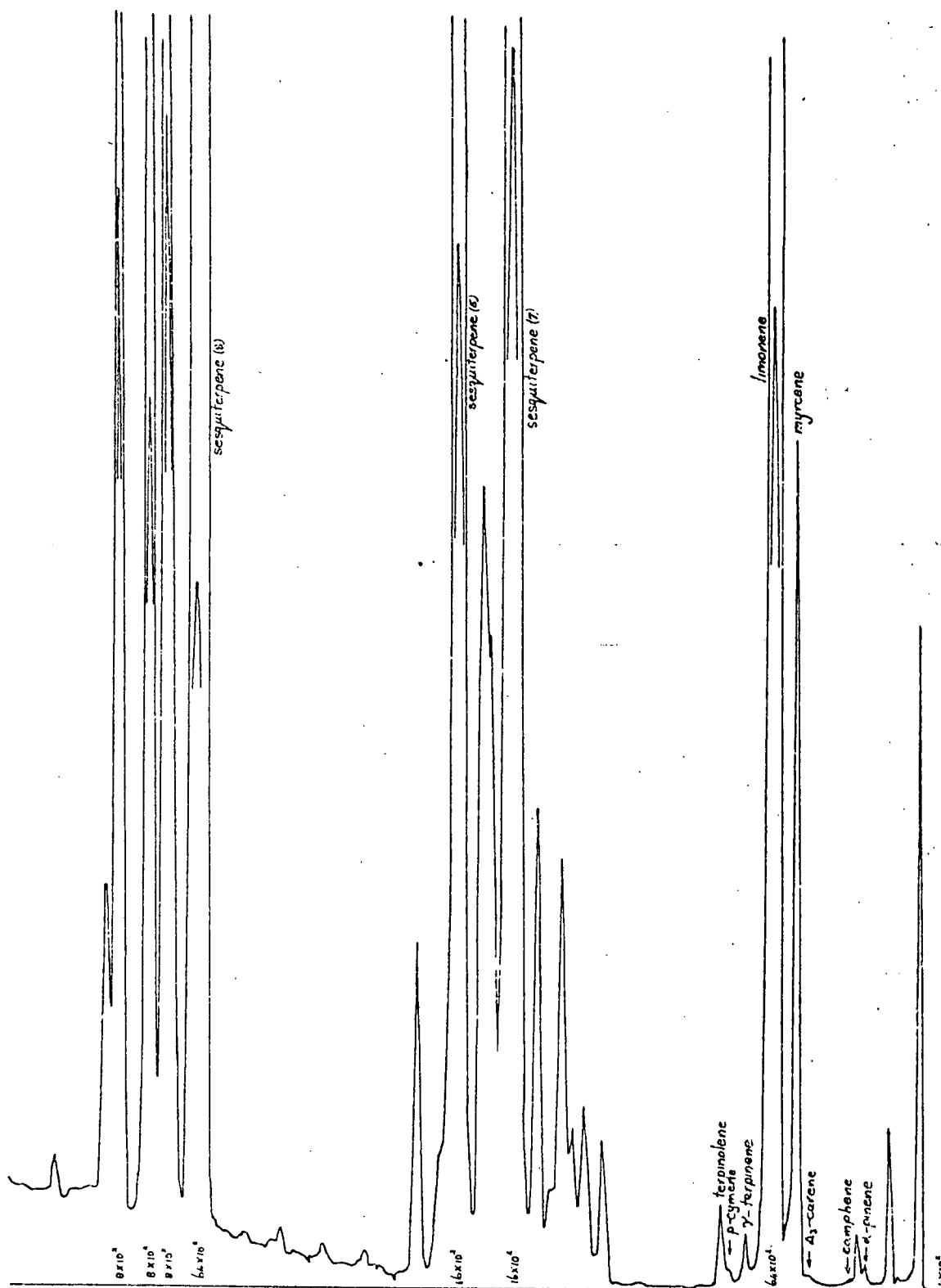
Fig. 16(d).   High sensitivity gas chromatogram of hydrocarbon
fraction of oil of *Athrotaxis selaginoides* separated on Florisil.
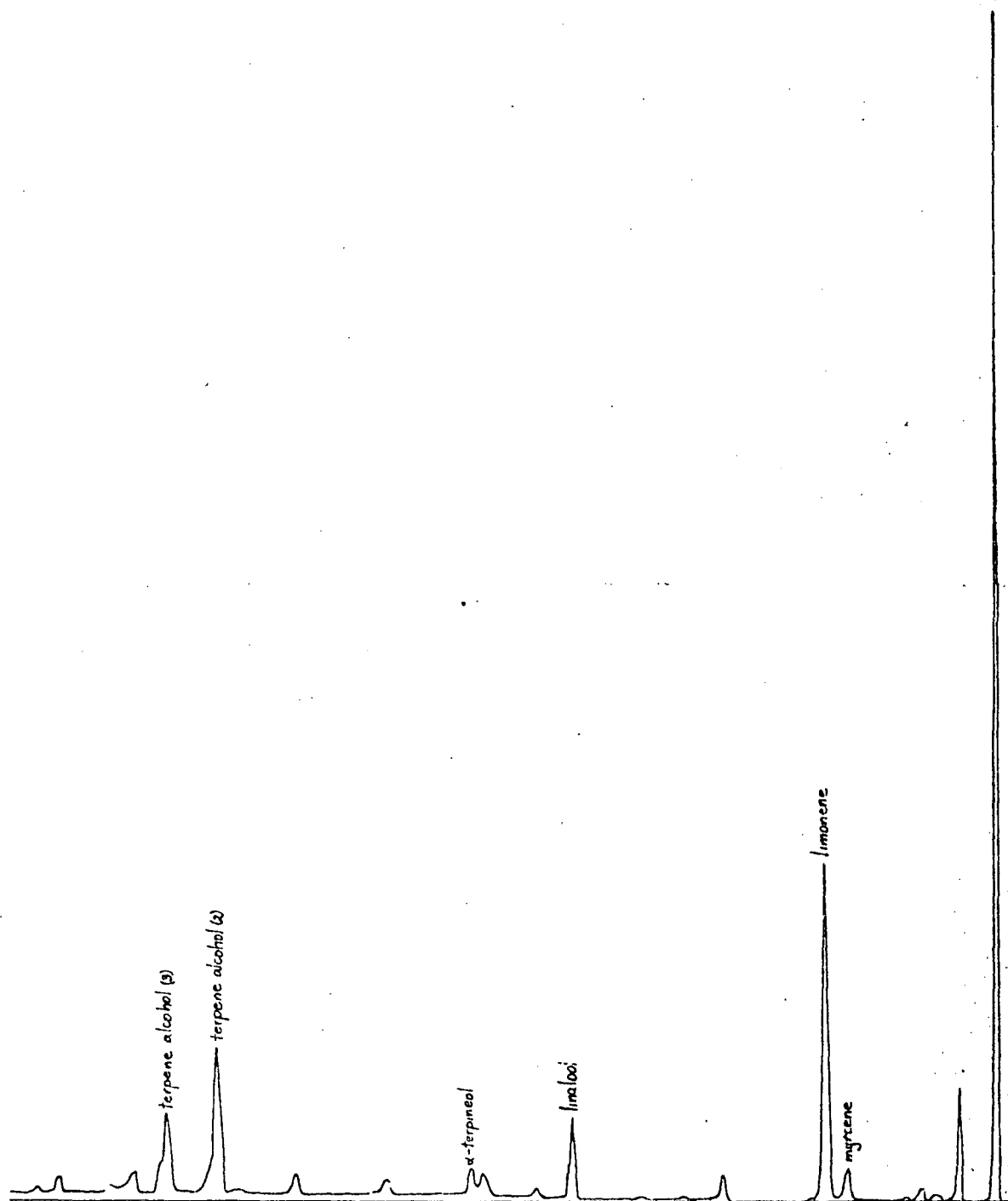
Fig. 16(e).   Low sensitivity gas chromatogram of oxygenated
fraction of oil of *Athrotaxis selaginoides* separated on Florisil.

Fig. 16(f). High sensitivity gas chromatogram of oxygenated fraction of oil of *Athrotaxis selaginoides* separated on Florisil.

WAVELENGTH IN MICRONS

WAVENUMBER IN CM⁻¹
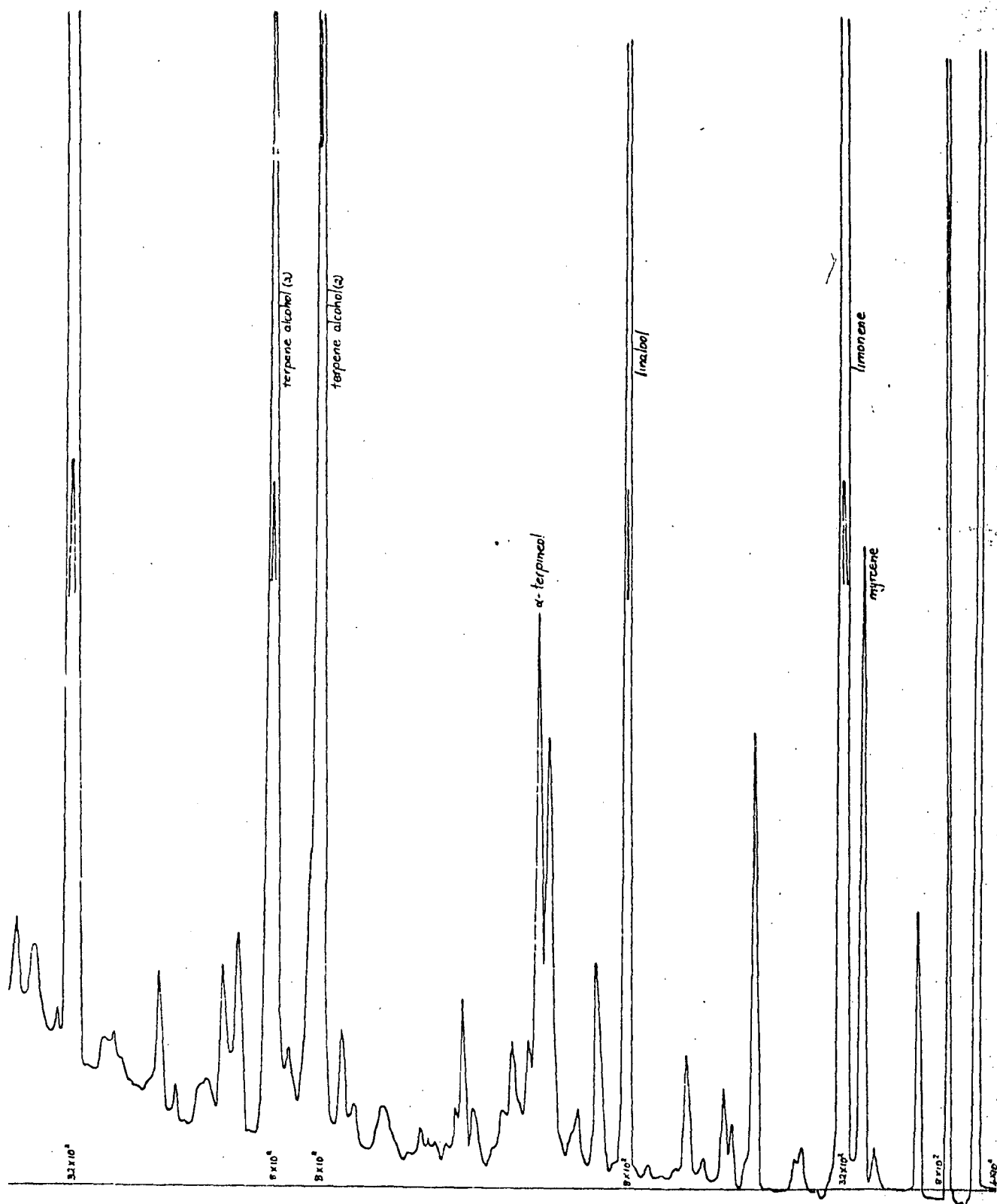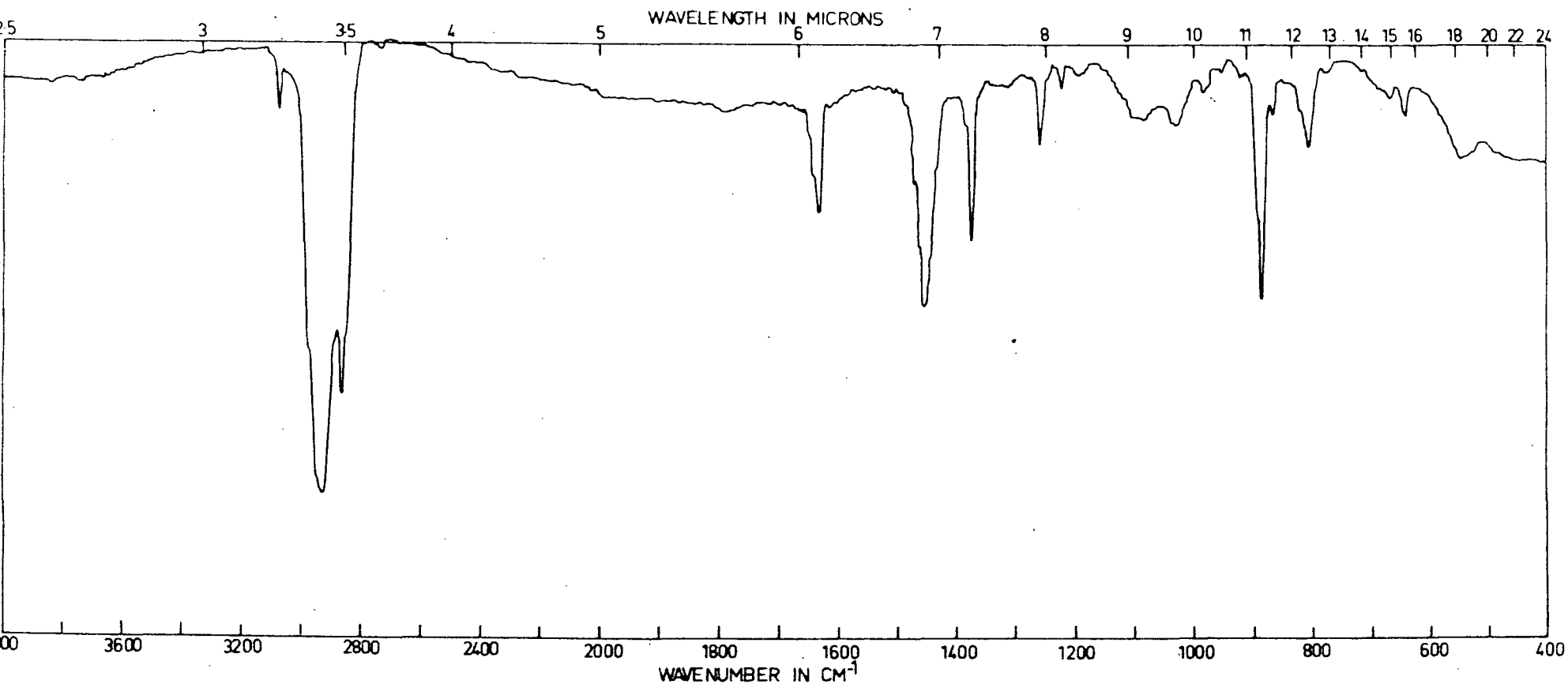
Fig. 17.   IR spectrum of the sesquiterpene isolated (>95% pure) in the hydrocarbon sub-fraction H3 of oil of
Athrotaxis selaginoides [designated as sesquiterpene (7);   RRT$_{camphor}$ 1.30 (C20M) and 2.79 (OV-17);   IR spectrum
of 0.007 mm film].

WAVELENGTH IN MICRONS

WAVENUMBER IN CM⁻¹

PERCENT TRANSMISSION
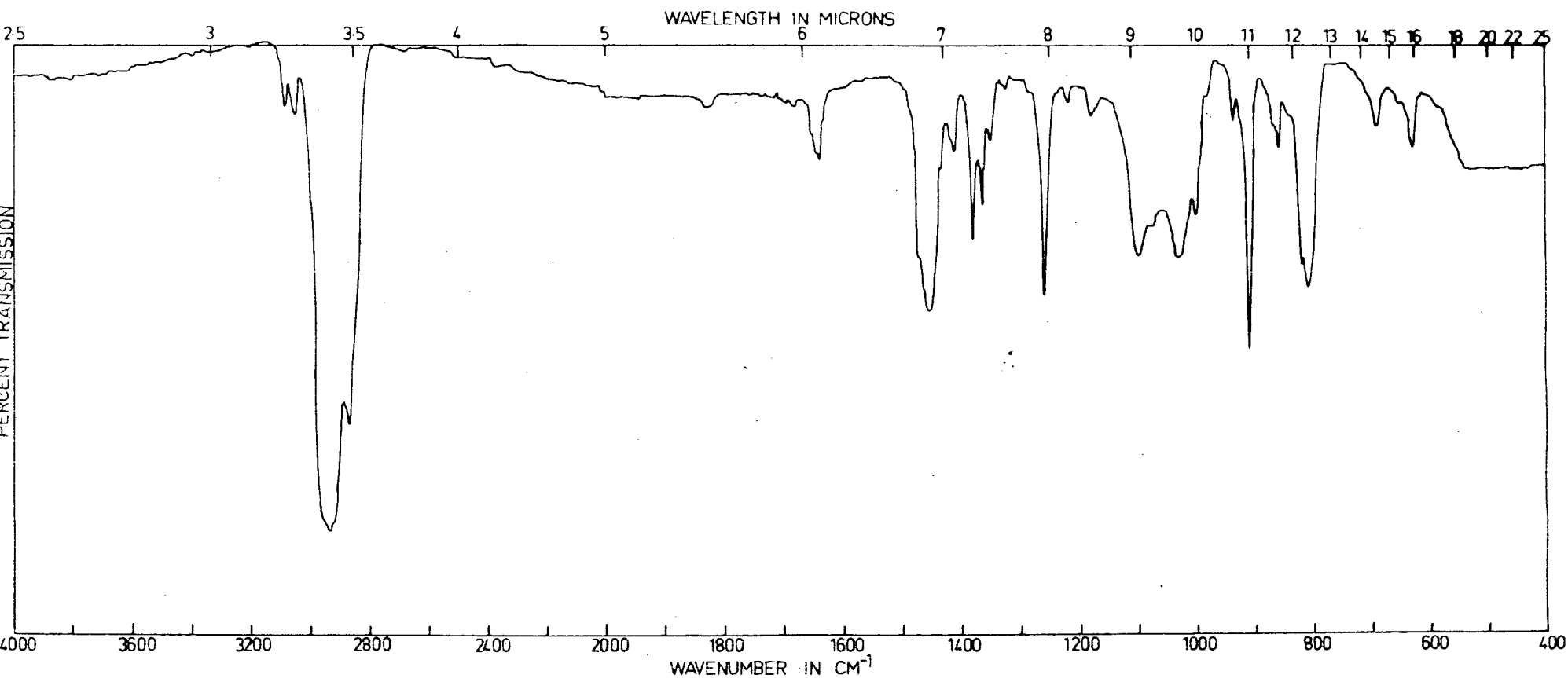
180.

Fig. 18.   IR spectrum of the sesquiterpene (?) isolated pure in the hydrocarbon sub-fraction H5 of oil of
Athrotaxis selaginoides [designated as sesquiterpene? (8); RRT$_{camphor}$ 9.84 (C20M) and >22 (OV-17); RRT$_{thymol}$
1.05 (C20M) and 6.95 (OV-17); IR spectrum from 0.007 mm film].

WAVELENGTH IN MICRONS

WAVENUMBER IN CM^{-1}

PERCENT TRANSMISSION

181.
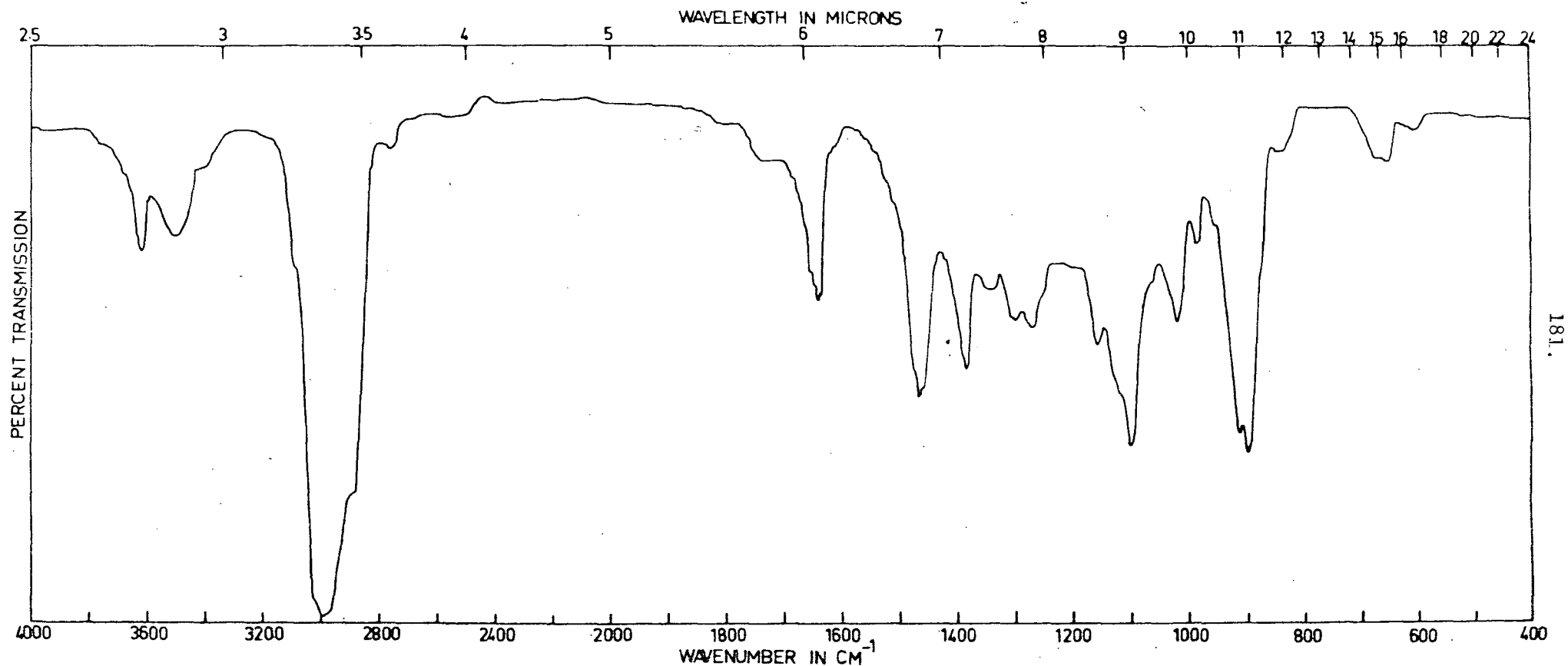
Fig. 19.   IR spectrum of the high-boiling alcohol isolated 95% pure in the oxygenated sub-fraction O4 of oil of *Athrotaxis selaginoides* [designated terpene alcohol (2);   RRT$_{camphor}$ 9.64 (C20M) and 7.48 (OV-17);   RRT$_{thymol}$ 0.95 (C20M) and 2.67 (OV-17);   IR spectrum of solution in CHCl$_3$].

WAVELENGTH IN MICRONS
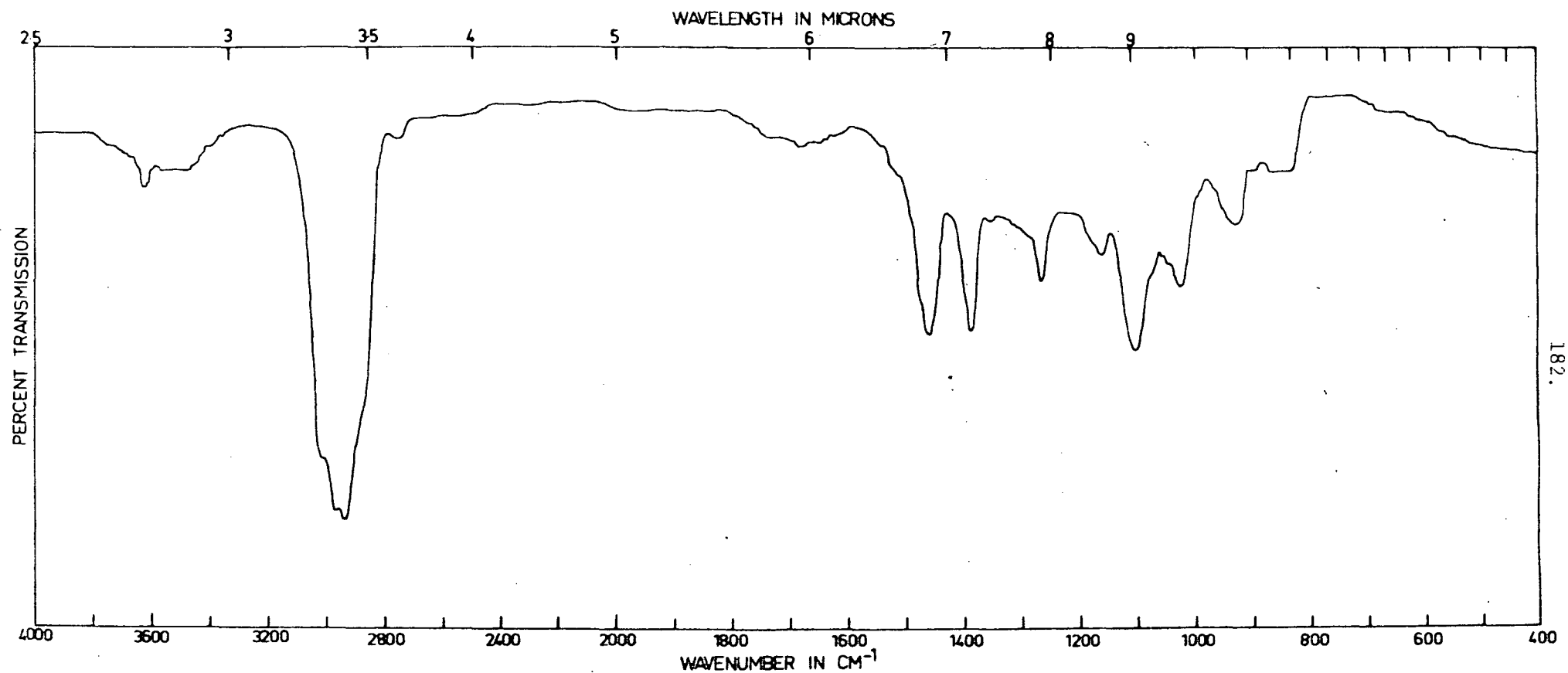
Fig. 20. IR spectrum of the high-boiling alcohol isolated >90% pure in the oxygenated sub-fraction O5 of oil of *Athrotaxis selaginoides* [designated terpene alcohol (3); RRT$_{camphor}$ 14.6 (C20M) and 11.8 (OV-17); RRT$_{thymol}$ 1.19 (C20M) and 3.42 (OV-17); IR spectrum of solution in CHCl$_3$].

Comparison of the IR spectra of sesquiterpenes (7) and (8) with those of compounds previously reported in heartwood extracts, showed that sesquiterpenes (7) and (8) were not among those already identified. In earlier work [386] heartwood terpenoids reported were: cadinene, α-, δ- and an unknown cadinol. More recently Westfelt and Wickberg [385] identified as heartwood constituents: copaene, γ-muurolene, α-muurolene, δ-cadinene and γ-cadinene. Also thought to be present were muurolol, torreyol, α-cadinol and a cadinol existing in two modifications.

The biosynthesis of the monoterpenes in this oil, indicated by the qualitative co-occurrence [352-357] pattern, agrees with Ruzicka's [356] single-product biosynthesis in the case of limonene. That is limonene can be visualized as forming directly by proton loss from C_9 or C_{10} of 1-ρ-menthene-8-carbonium ion (III) (Figure 15). The apparent or near absences of the groups: β-pinene + β-phellandrene, sabinene + α-thujene + γ-terpinene + terpinolene, tricyclene + camphene + borneol, coincide with the absence of each of the respective hypothesized biosynthetic pathways. The presence of similar amounts each of myrcene and linalool, both acyclics, agrees with a C_9 proton loss from carbonium ion (II), which is a cyclization precursor of the 1-ρ-menthene-8-carbonium ion (III).

(iii) <u>Experimental</u>

Details of sampling, isolation of oil, separation

of components and analysis are as previously described. The

oil was isolated from foliage in 0.06 percent wet weight yield.

A 1.05 g. portion of the oil was chromatographed on Florisil,

yielding 0.20 g. of a colourless hydrocarbon fraction and

0.76 g. of a yellow-green oxygenated fraction.

(iv) <u>Summary</u>

Components of steam-distilled leaf oil of *Athrotaxis*

selaginoides were analyzed by chromatographic and spectroscopic

methods. Monoterpenoids identified were myrcene (5.1%),

limonene (40.3%) and linalool (5.1%). Components tentatively

identified by GC on dissimilar columns were: α-pinene (<0.1%),

camphene (<0.1%), Δ_3-carene (<0.1%), γ-terpinene (<0.1%),

ρ-cymene (<0.1%), terpinolene (<0.1%) and α-terpineol (0.4%).

Several high-boiling components were also detected, two of

which were found from IR spectra to be alcohols while a further

two were hydrocarbons.

3.   <u>Composition of foliage oil of *Boronia citriodora*</u>

(i)   <u>Introduction</u>

Boronia citriodora Gunn ex. Hook. f. (Fam. Rutaceae)

grows as a small shrub, with branches 30-60 cm. long. It is

abundant on sub-alpine moors and down to sea level in the

south-west of Tasmania [343]. It has become well known for

its strong lemon scent released when the leaves are crushed.

In 1925 Penfold [387] described the oil as one of the richest citronellol-bearing oils so far reported. He found about 80 percent citronellol in the oil obtained in a yield of up to 0.93 percent. The principal constituents identified were citronellol and its esters, principally the acetate with some valerate, d-α-pinene, sesquiterpene, a paraffin with m.pt. 64-5°, together with small quantities of a phenolic substance and free capric acid.

(ii) Results and discussion

Foliage was collected in mid-summer from the Lake Dobson region of Mount Field National Park in southern Tasmania. The steam-distilled oil was a very pale-green colour and had a strong rose-like odour.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 31. RRT values leading to the tentative identification of components in hydrocarbon and oxygenated fractions, and sub-fractions, are listed in Table 32. Gas chromatograms of Figure 21 show the distribution of oil components eluted from a Carbowax 20M column, and indicate the degree of separation of components into hydrocarbon and oxygenated fractions.

The oil was found to consist largely of citronellyl acetate rather than citronellol as reported earlier [387]. It should not be assumed that this difference in oils was due to the limitations of earlier analytical techniques, but

Table 31. Components distinguishable in the whole
oil of *Boronia citriodora*

Component	Qualitative RRT data:		Quantitative composition (percent, based on peak height)
	C20M	OV-17	
(60° isothermal, ref. α-pinene)			(TP 50-210°, 5°/min)
Tricyclene	0.92	0.91	t
*α-Pinene	1.00	1.00	1.5
*Camphene	1.25	1.21	2.4
Unidentified	1.48		t
β-Pinene	1.57	}1.56	0.7
Sabinene	1.71		
Δ_3-Carene	1.92	2.00	t
*Myrcene	2.21	1.77	1.1
Unidentified	2.42		0.7
*Limonene	2.71	2.37	2.2
β-Phellandrene	2.83	2.49	0.7
Unidentified	3.25		t
*γ-Terpinene	3.63	3.23	1.5
*ρ-Cymene	4.21	2.79	t
*Terpinolene	4.50	4.14	0.2
Unidentified	5.25		t
(130° isothermal, ref. camphor)			
*Thujone	0.70	0.74	6.4
Isothujone?	0.79	0.81	1.5
Linalool	0.94	0.60	3.4
Unidentified			0.7
"			0.7
*Terpinen-4-ol	}1.24	1.00	4.4
*Caryophyllene		2.60	
*Isogeranyl acetate	}1.55	1.70	8.7
*Citronellyl acetate		2.00	48.6
α-Terpineol	1.77	1.14	2.7
Unidentified	1.93		1.1
*Citronellol	}2.30	1.14	}8.7
*Geranyl acetate		2.60	
*Aromatic ether?(1) (Fig. 22)	15.9	10.1	2.2

+ at least 30 other components in trace proportions.

* IR spectrum recorded
t: trace, <0.1 percent

Table 32. RRT values on the dissimilar liquid phases C20M and OV-17,
for components found in hydrocarbon and oxygenated fractions,
and preparative GC sub-fractions, isolated from oil of
Boronia citriodora

Component	Hydrocarbon fraction		Hydrocarbon sub-fractions			Oxygenated fraction		Oxygenated sub-fractions		
	C20M	OV-17	No.	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)										
Tricyclene	0.93	0.88	H1		0.91					
α-Pinene	1.00	1.00	H1	1.00	1.00					
Camphene	1.29	1.21	H2	1.25	1.19					
Unidentified						1.54	1.13			
β-Pinene	1.63	1.53	H2	1.58	1.56					
Δ_3-Carene	1.96	1.98								
Myrcene	2.23	1.74	H2	2.21	1.74					
Unidentified	2.43	2.19								
Limonene	2.73	2.40	H4	2.78	2.41					
Unidentified						2.89	2.46			
β-Phellandrene	2.88	2.53	H4	2.91	2.56					
γ-Terpinene	3.63	3.28	H5	3.61	3.24					
ρ-Cymene	4.27	2.84	H5	4.26	2.81					
Terpinolene	4.54	4.12	H6	4.61	4.07					
Unidentified	5.75									
(130° isothermal, ref. camphor)										
Thujone						0.72	0.74	01	0.70	0.75
Linalool						0.93	0.61	01	0.93	0.62
Terpinen-4-ol						1.29	0.99	03	1.26	0.95
Unidentified								04	1.23	1.85
Caryophyllene	1.26	2.62	H7	1.26	2.43					
Isogeranyl acetate						1.61	1.73	04	1.50	1.70
Citronellyl acetate						1.61	2.04	05	1.52	1.95
								06	1.56	2.00
Unidentified								05	1.70	1.95
α-Terpineol						1.82	1.11	02	1.74	1.12
Unidentified								04	1.81	1.85
"								05	1.96	2.33
Citronellol						2.36	1.11	03	2.26	1.05
Geranyl acetate						2.31	2.64	06	2.26	2.65
Aromatic ether?(1)							10.4	07	15.7	9.86
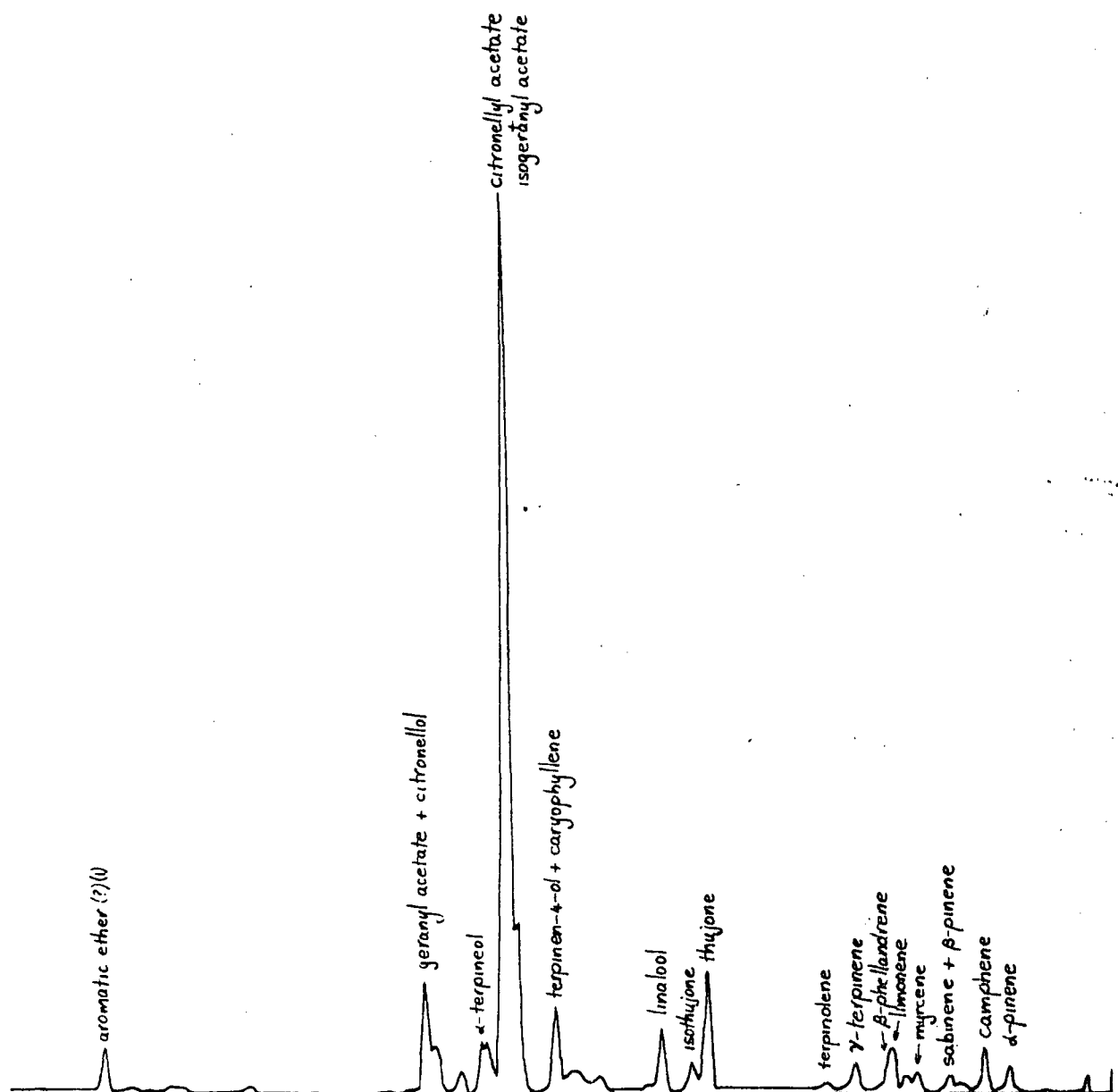
Fig. 21(a). Low sensitivity gas chromatogram of whole oil of foliage of *Boronia citriodora* (GC on Carbowax 20M/Gas Chrom Q; temperature program 50° to 200° at 5°/min, 0.2 μℓ sample; attenuation 6 x 10^2).
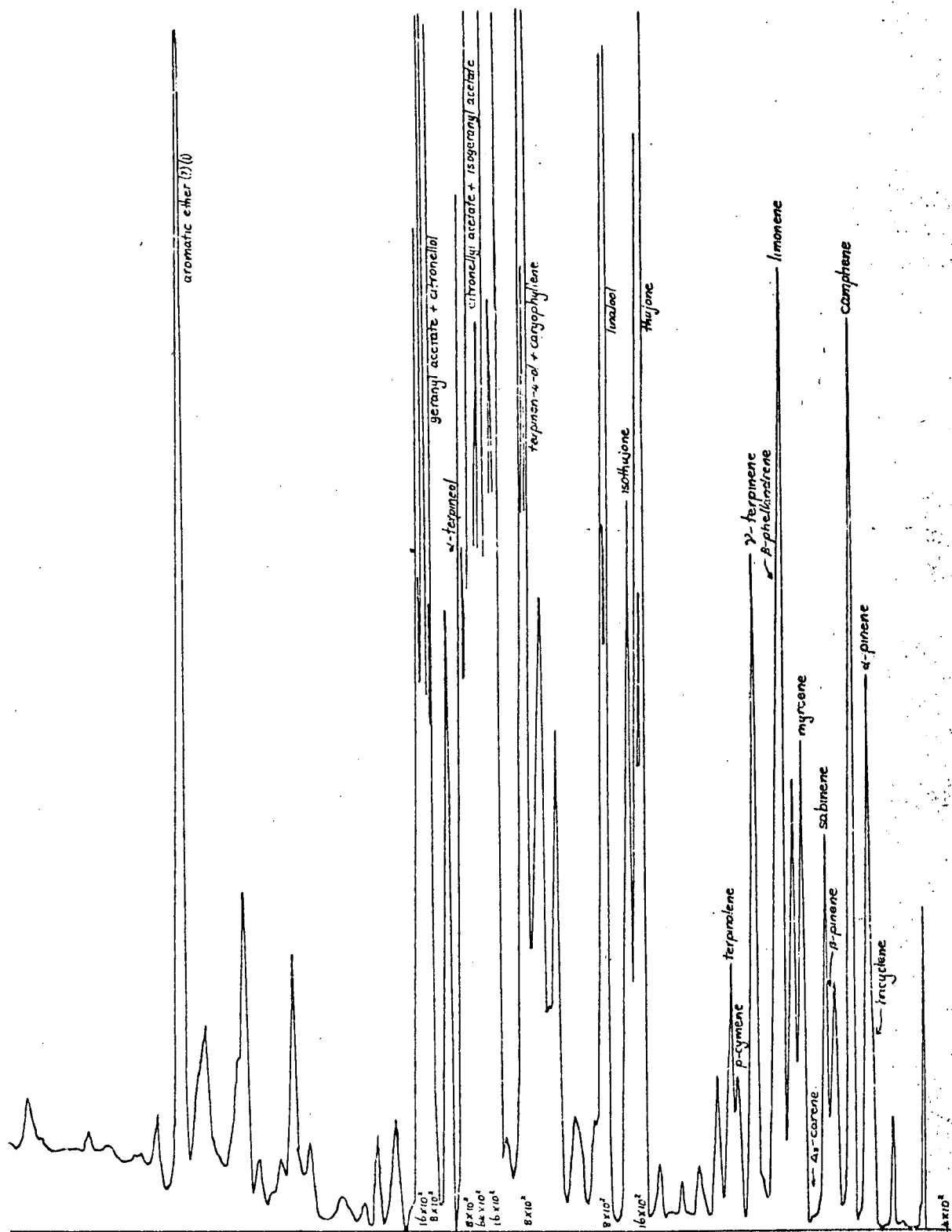
Fig. 21(b).   High sensitivity gas chromatogram of whole oil of foliage
of *Boronia citriodora* (attenuation 4 x 10^3).
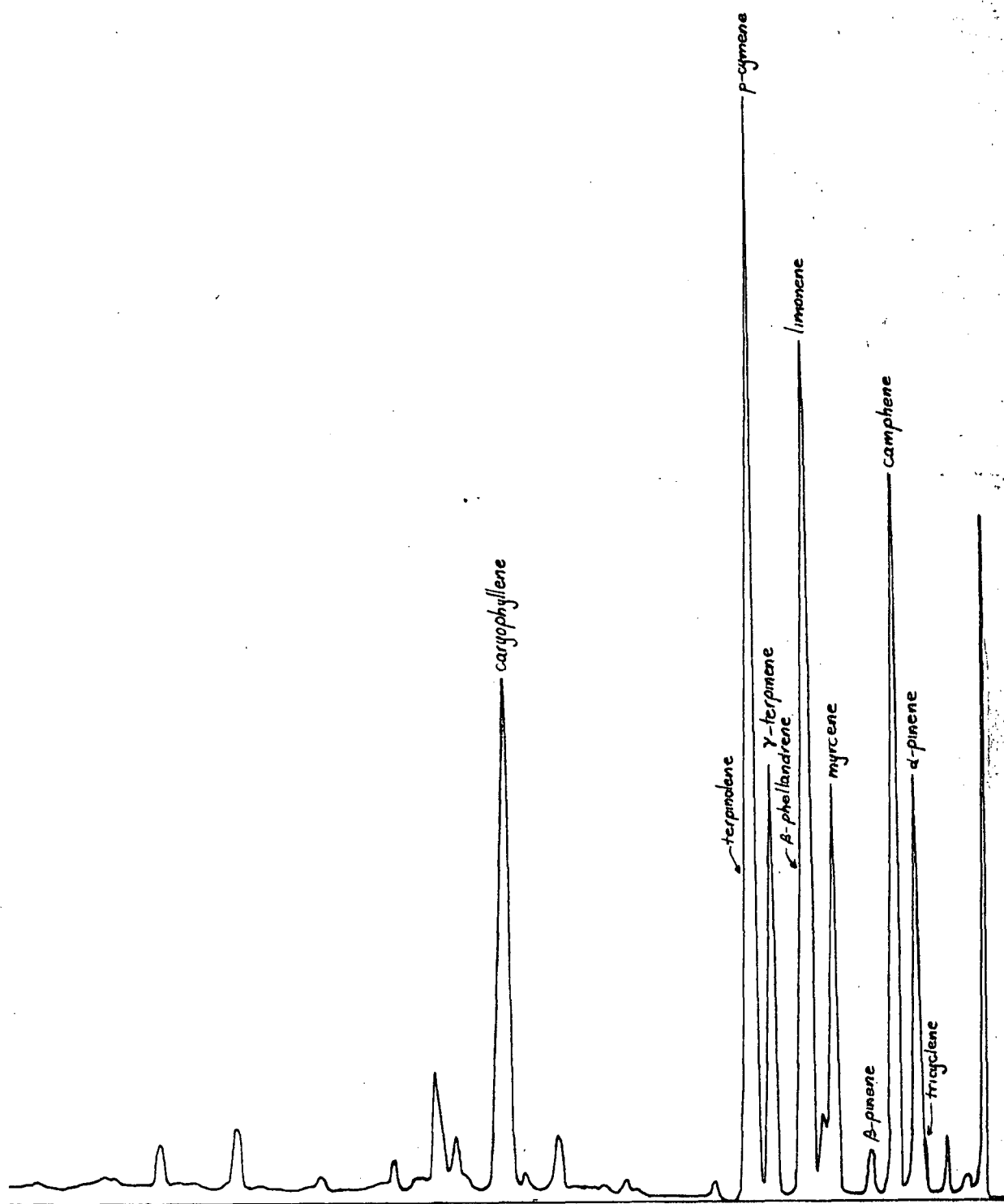
Fig. 21(c).   Low sensitivity gas chromatogram of hydrocarbon fraction of oil of *Boronia citriodora* separated on Florisil.
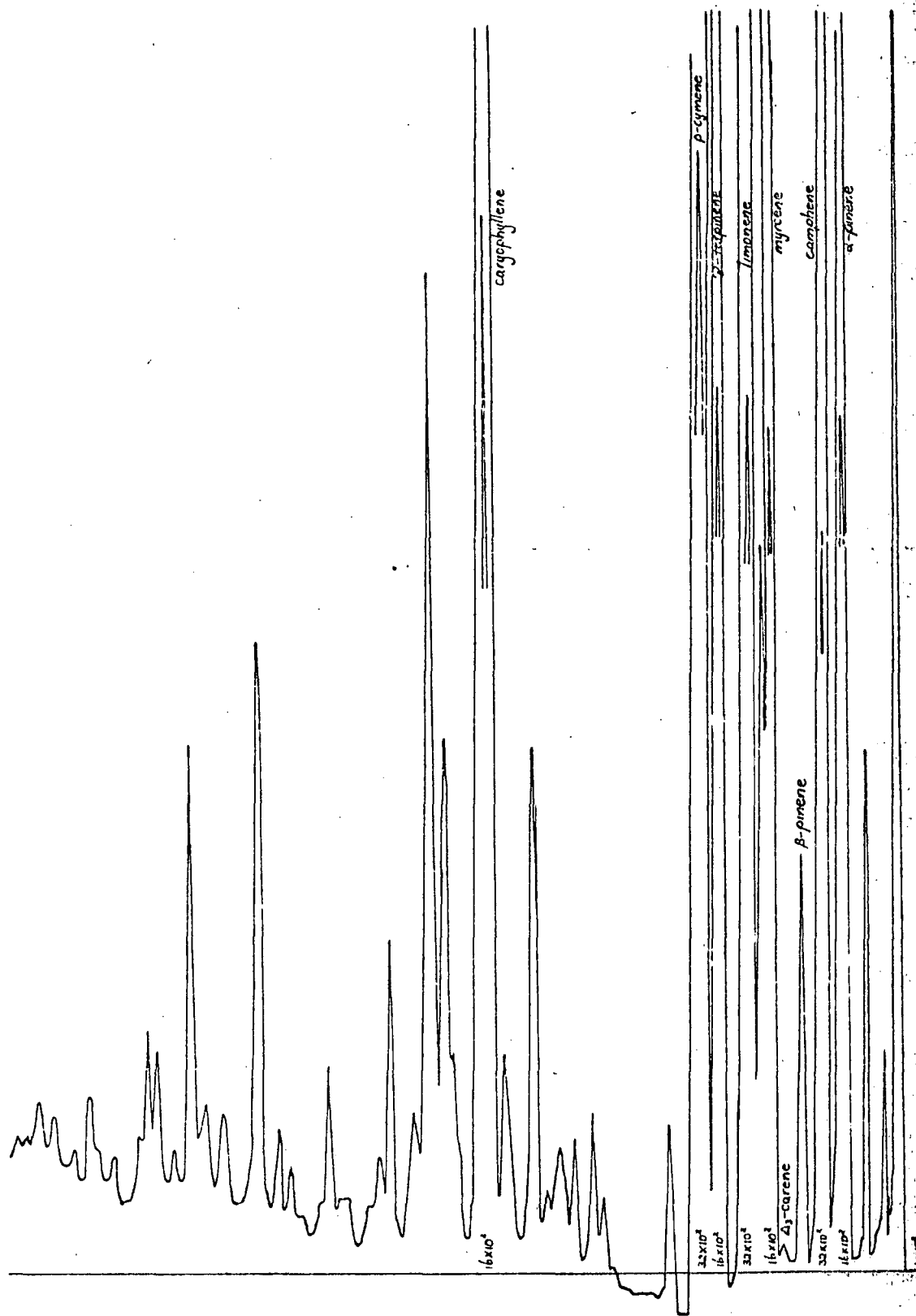
Fig. 21(d).   High sensitivity gas chromatogram of hydrocarbon fraction of oil of *Boronia citriodora* separated on Florisil.
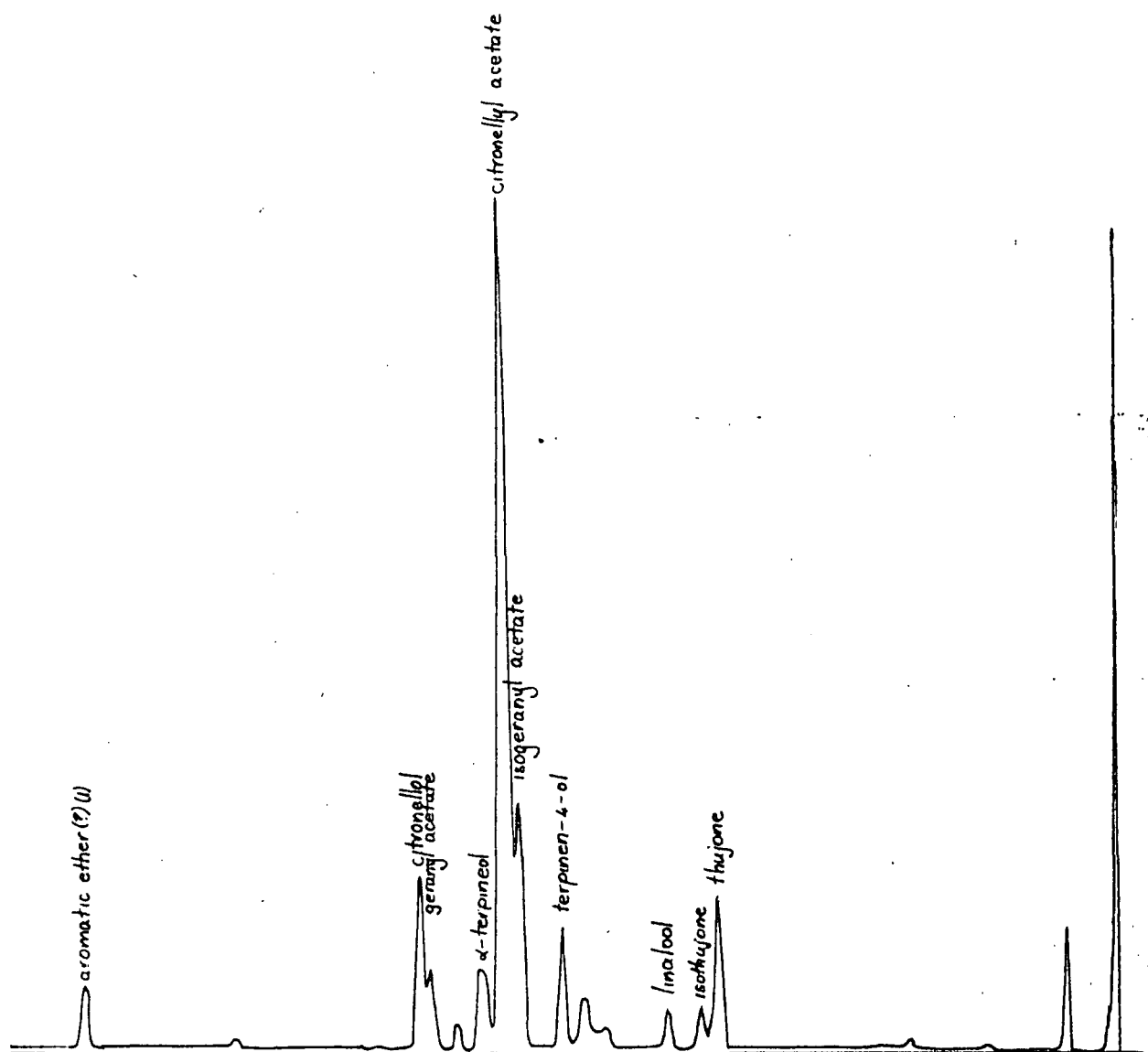
Fig. 21(e).   Low sensitivity gas chromatogram of oxygenated fraction of oil of *Boronia citriodora* separated on Florisil.
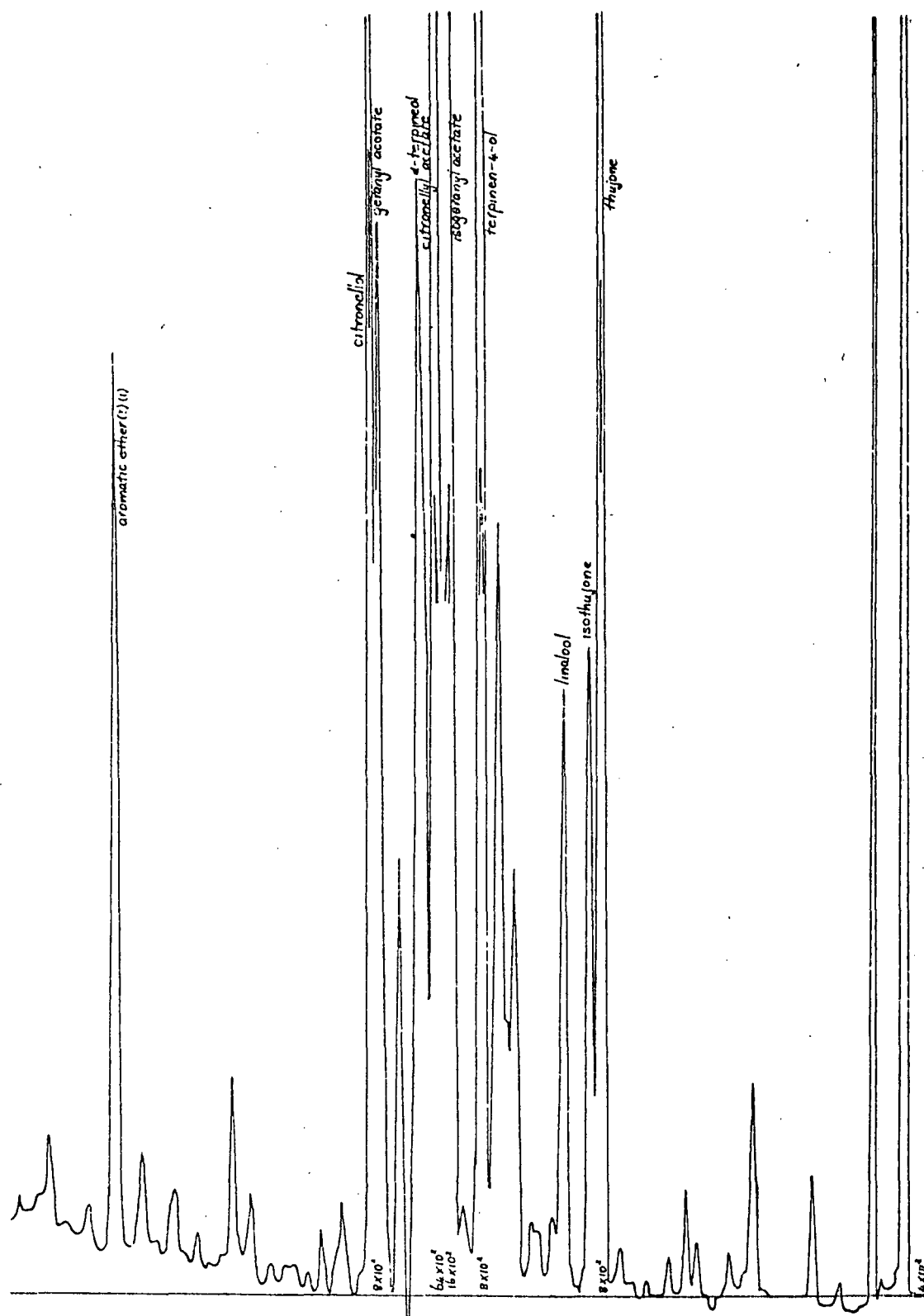
Fig. 21(f). High sensitivity gas chromatogram of oxygenated
fraction of oil of *Boronia citriodora* separated on Florisil.

instead should be the basis for further investigation of
the range of oil compositions that exist in different regions.
An investigation of the seasonal change in composition might
also be shown to result in such differences.

Among the components for which IR spectra were
recorded was a high-boiling ether that was probably aromatic
(Figure 22).

The very minor proportion of monoterpenes in this
oil does not enable any biosynthetic mechanisms to be envisaged,
i.e. based on qualitative co-occurrence of specific terpenes.
If investigations throughout a season, as recommended above,
show that there are considerable changes in oil composition in
this species, then there might be a basis for hypothesizing
biosynthetic mechanisms from quantitative co-occurrence
evidence.

(iii) Experimental

Details of sampling, isolation of oil, separation of
components and analysis are as previously described. The oil
was isolated from foliage in a 0.55 percent wet weight yield.
A 7.20 g portion of oil was chromatographed on Florisil,
yielding 0.22 g of a colourless hydrocarbon fraction and 6.65 g
of a pale-green oxygenated fraction.

WAVELELENGTH IN MICRONS

PERCENT TRANSMISSION

WAVENUMBER IN CM⁻¹

195.
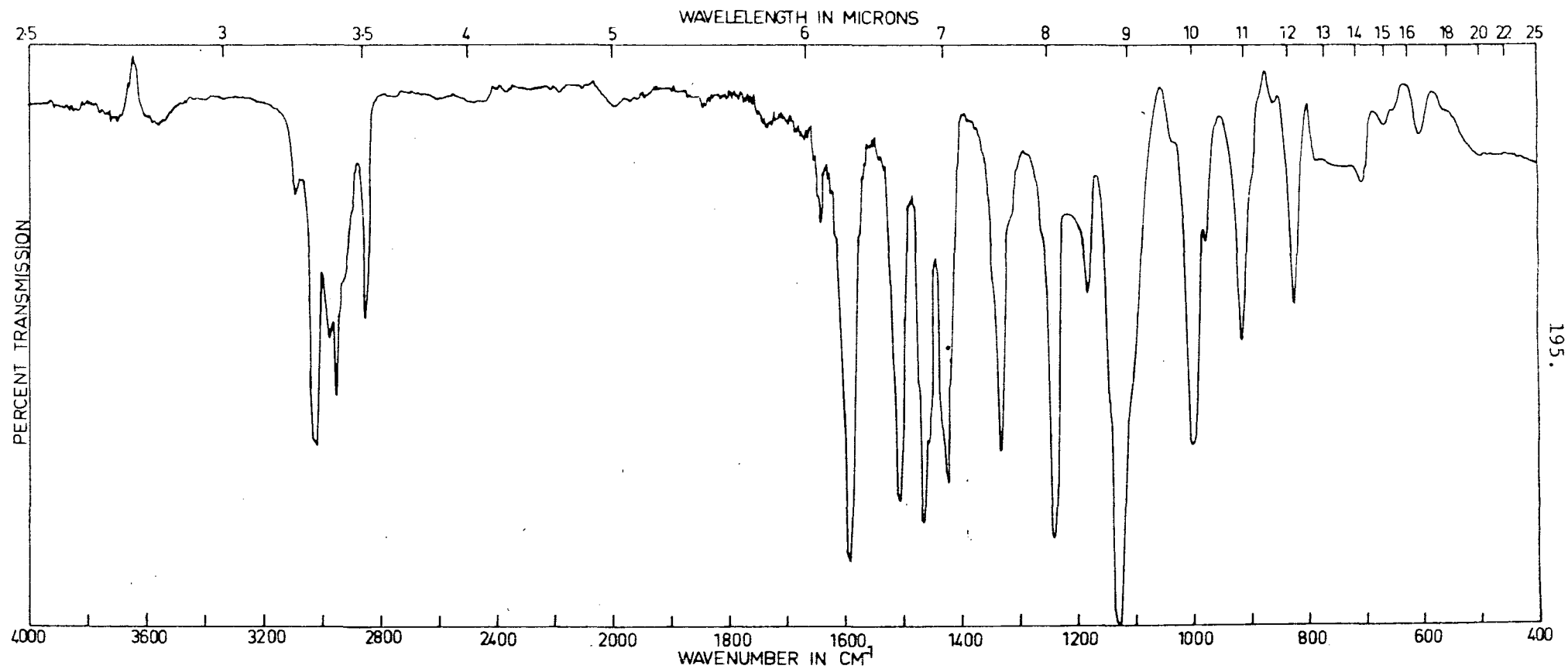
Fig. 22. IR spectrum of the high-boiling ether isolated pure in the oxygenated sub-fraction O7 of oil of *Boronia citriodora* [designated as aromatic ether (1); $RRT_{camphor}$ 15.7 (C20M) and 9.86 (OV-17); IR spectrum of solution in $CHCl_3$].

(iv) Summary

Components of steam-distilled foliage oil of *Boronia citriodora* were analyzed by chromatographic and spectroscopic methods. The following identifications were confirmed: α-pinene (1.5%), camphene (2.4%), myrcene (1.1%), limonene (2.2%), γ-terpinene (1.5%), ρ-cymene (<0.1%), terpinolene (0.2%), thujone (6.4%), terpinen-4-ol + caryophyllene (4.4%), isogeranyl acetate (8.7%), citronellyl acetate (48.6%) and citronellol + geranyl acetate (8.7%). Tentatively identified were tricyclene (<0.1%), β-pinene + sabinene (0.7%), Δ_3-carene (<0.1%), β-phellandrene (0.7%), isothujone (1.5%), linalool (3.4%) and α-terpineol (2.7%). The IR spectrum was recorded for a high-boiling component (2.2%), which appeared to be an aromatic ether.

4. Composition of leaf oil of Woolly Tea-tree
   (Leptospermum lanigerum)

(i) Introduction

Leptospermum lanigerum Sm. (Fam. Myrtaceae) grows often as a much-branched shrub 3-4 m. high, abundantly in damp places such as river banks and ranging from sea level to montane habitats [343].

In 1926 Penfold [388] described "silver" and "green" leaf forms of *L. lanigerum* which exhibited distinctive differences in their oils, viz.

	"silver" leaf	"green" leaf
Oil colour	bright yellow	deep brown
Odour	cinnamon-like	pinene mingled with darwinol and its acid ester
Constituents	16-20% d-α-pinene,	40-60% d-α-pinene,
	60-75% sesquiterpenes (aromadendrene and eudesmene),	small quantities of sesquiterpenes
		40-45% darwinol and its acetate,
	small quantities of sesquiterpene alc. and isovaleric acid ester,	small quantities of sesquiterpene alc. and esters,
	unidentified phenolic substances,	unidentified phenolic substances.
	geraniol,	
	geraniol formate and cinnamate,	
	citral,	
	cineole.	

More recently Hellyer and Pinhey [389] reported the structure
of grandiflorone, a β-triketone, which occurs together with
small amounts of leptospermone and flavesone in essential
oils of two distinct morphological forms of *L. lanigerum*.

(ii) Results and discussion

Foliage was collected in mid-summer from the dense
growth of *L. lanigerum* on the alpine plateau near the summit
of the Harz Mountain National Park in southern Tasmania. The
steam-distilled oil was a green colour with a characteristic
fruity-sweet odour.

The qualitative and quantitative composition of
components distinguishable in the whole oil, using two
dissimilar columns, is recorded in Table 33. RRT values
leading to the tentative identification of components in
hydrocarbon and oxygenated fractions, and sub-fractions,
are listed in Table 34. Gas chromatograms of Figure 23 show
the distribution of oil components eluted from a Carbowax 20M
column, and indicate the degree of separation of components
into hydrocarbon and oxygenated fractions.

The oil was found to consist largely of a mixture
of an unidentified high-boiling alcohol, a further unidentified
oxygenated component, citronellol, geranyl acetate, α-terpineol,
caryophyllene, 1,8-cineole and α-pinene. The composition of
this oil was unlike either of those described earlier by
Penfold [388]. If a comparison were to be drawn, the presence
of a significant proportion (19.9 percent) of a high-boiling
terpene alcohol (Figure 24) and only small quantities of
sesquiterpenes, might enable this oil to be correlated with
that of the "green" leaf morphological form. This oil did not
appear to contain 60 to 75 percent of a mixture of aromadendrene
and eudesmene sesquiterpenes, as in the "silver" leaf oil, but

Table 33. Composition of terpenoids distinguishable
in the whole oil of *Leptospermum lanigerum*

Component	Qualitative RRT data:		Quantitative composition (percent, based on peak height)
	C20M	OV-17	
	(60° isothermal, ref. α-pinene)		(TP 50-210°, 5°/min)
*α-Pinene	} 1.05	0.98	5.5
Unidentified		1.12	2.8
Camphene	1.32	1.20	t
β-Pinene	1.62	1.59	1.2
Δ$_3$-Carene	2.01	2.04	t
Myrcene	2.27	1.80	0.3
Unidentified	2.50	2.22	0.1
*Limonene	2.77	2.42	0.8
*1,8-Cineole	3.09	2.85	16.5
γ-Terpinene	3.71	3.31	0.2
*ρ-Cymene	4.32	2.85	2.0
Terpinolene	4.64	4.17	0.2
Unidentified	7.59		0.1
"	10.2		0.3
(130° isothermal, ref. camphor)			
Linalool	0.94		1.1
Unidentified	1.13		2.0
*Terpinen-4-ol	} 1.28	1.14	} 3.5
*Caryophyllene		~2.8	
*α-Terpineol	1.80	1.14	6.2
*Geranyl acetate	} 2.29	~2.8	13.1
*Citronellol		1.14	
Unidentified	2.65		3.2
"	3.31		0.6
"	3.65		1.5
"	4.96		0.3
"	5.60		0.5
"	8.01		4.4
"	9.96		1.4
"	12.1	9.71	12.2
*Terpene alcohol(5) (Fig. 24)	15.0	11.0	19.9

* IR spectrum recorded

t: trace <0.1 percent

200.

Table 34. RRT values on the dissimilar liquid phases C20M and OV-17,
for components found in hyrdocarbon and oxygenated fractions,
and preparative GC sub-fractions, isolated from oil of
Leptospermum lanigerum

Component	Hydrocarbon fraction		Hydrocarbon sub-fractions			Oxygenated fraction		Oxygenated sub-fractions		
	C20M	OV-17	No.	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)										
α-Pinene	1.00	1.00	H1	1.03	1.03					
Camphene	1.26	1.22	H1	1.30	1.22					
β-Pinene	1.61	1.54	H3	1.61	1.54					
Δ_3-Carene	1.96	1.98								
Myrcene	2.26	1.73								
Unidentified	2.48	2.17	H3	2.43	2.10					
Limonene	2.78	2.37	H3	2.70	2.32					
β-Phellandrene			H3	2.96	2.46					
1,8-Cineole						3.05	2.80	O1	3.08	2.82
γ-Terpinene	3.61	3.22	H3	3.70	3.22					
ρ-Cymene	4.26	2.80	H3	4.22	2.73					
Terpinolene	4.57	4.07								
(130° isothermal, ref. camphor)										
1,8-Cineole						0.41	0.50			
Linalool						0.93	0.60			
Unidentified						1.10	1.13	O2	1.11	1.19
Terpinen-4-ol						1.26	0.99	O2	1.26	1.00
								O3	1.30	1.00
Caryophyllene	1.30	2.64	H6	1.22	2.63					
Unidentified			H6	1.22	2.84					
α-Terpineol						1.78	1.13	O2	1.78	1.10
								O3	1.81	1.10
Geranyl acetate						2.26		O4	2.22	2.64
Citronellol						2.26	1.13	O3	2.33	1.10
Unidentified						12.1	9.70	O5	12.0	9.65
Terpene alcohol(5)						15.1	11.2	O5	~14.8	10.9
								O6	14.9	11.0
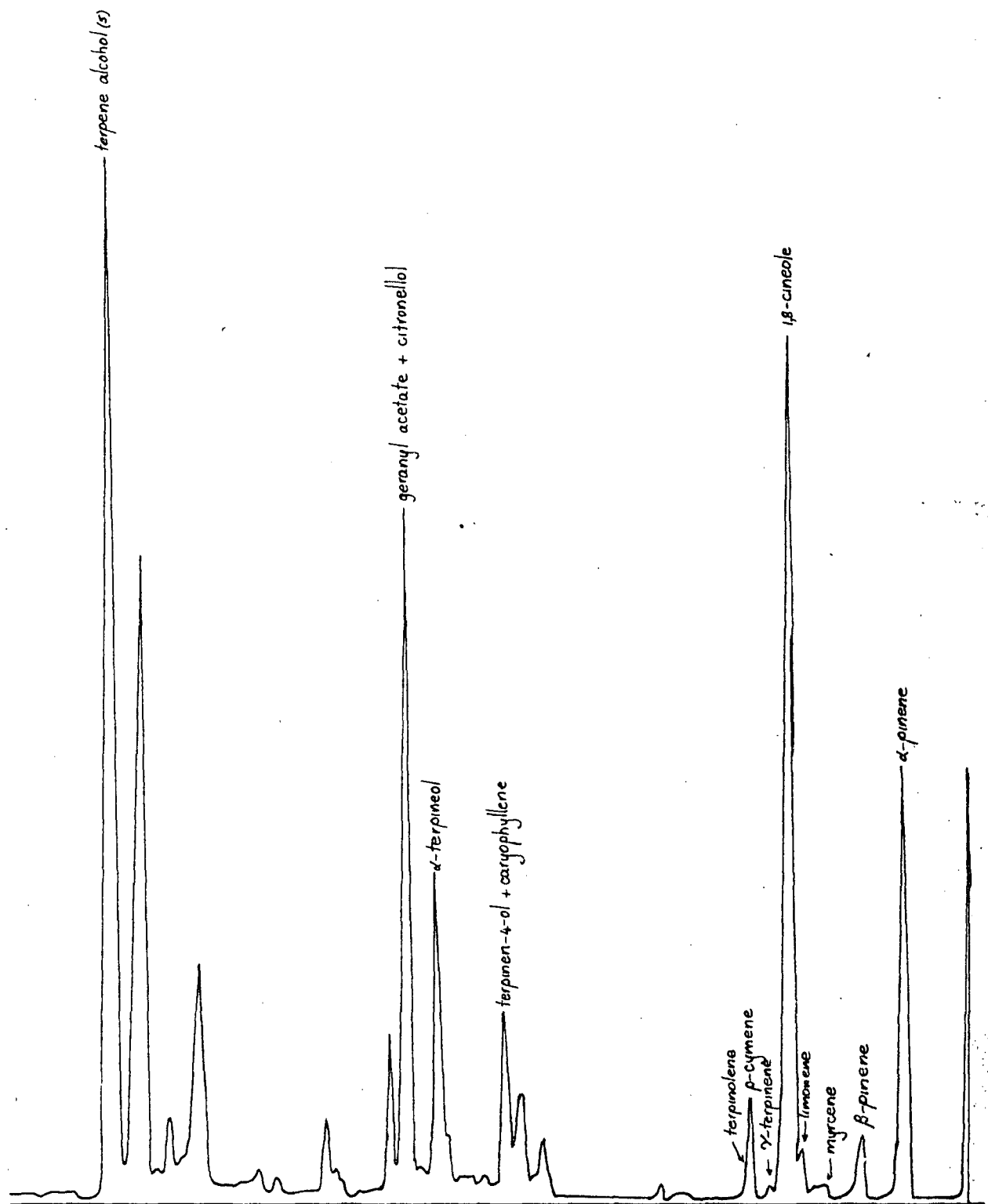
Fig. 23(a).   Low sensitivity gas chromatogram of whole oil of foliage
of *Leptospermum lanigerum* (GC on Carbowax 20M/Gas Chrom Q;   temperature
program 50° to 200° at 5°/min;   0.2 µℓ sample;   attenuation 4 x 10³).
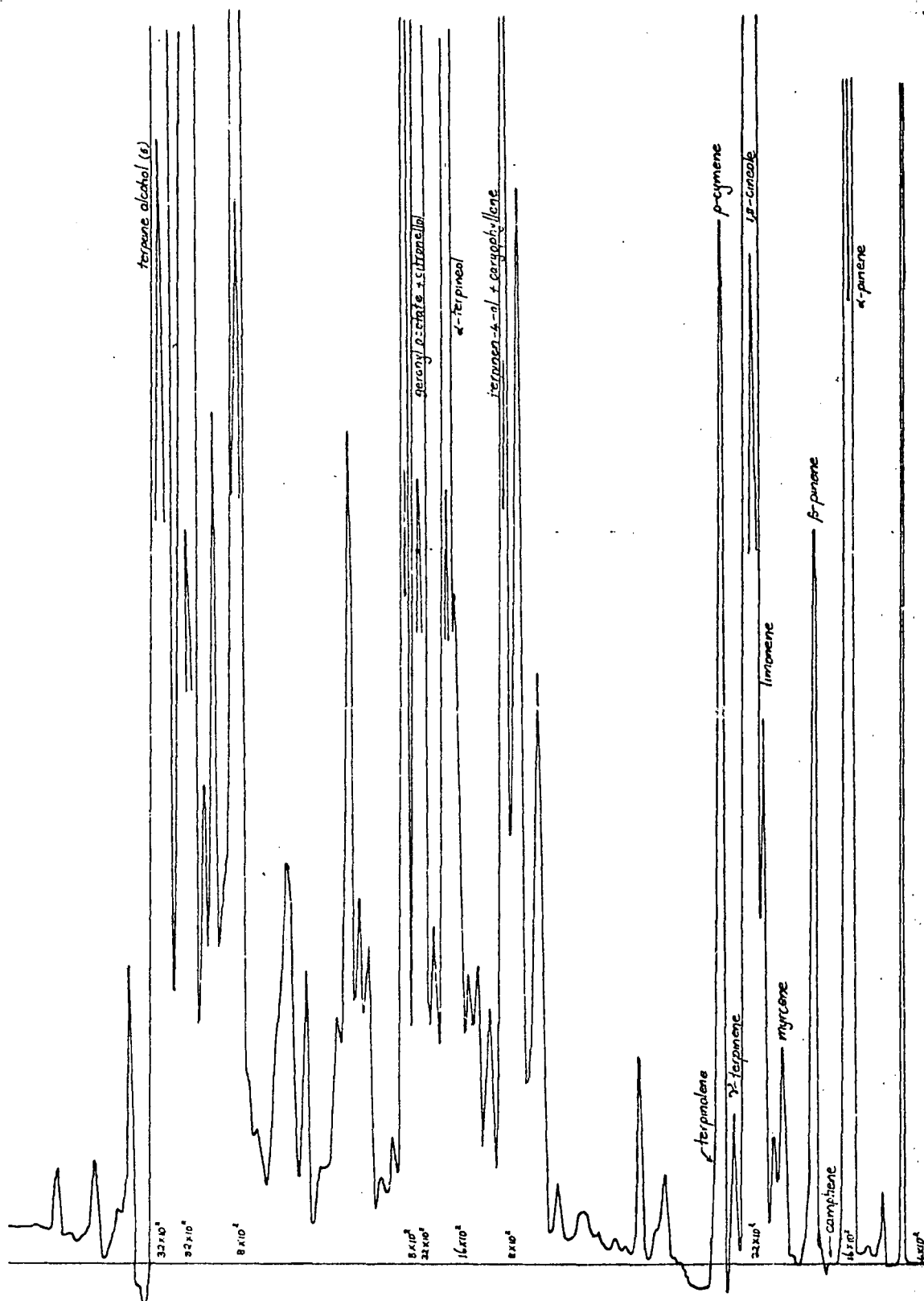
Fig. 23(b). High sensitivity gas chromatogram of whole oil of foliage of *Leptospermum lanigerum* (attenuation 4 x 10²).
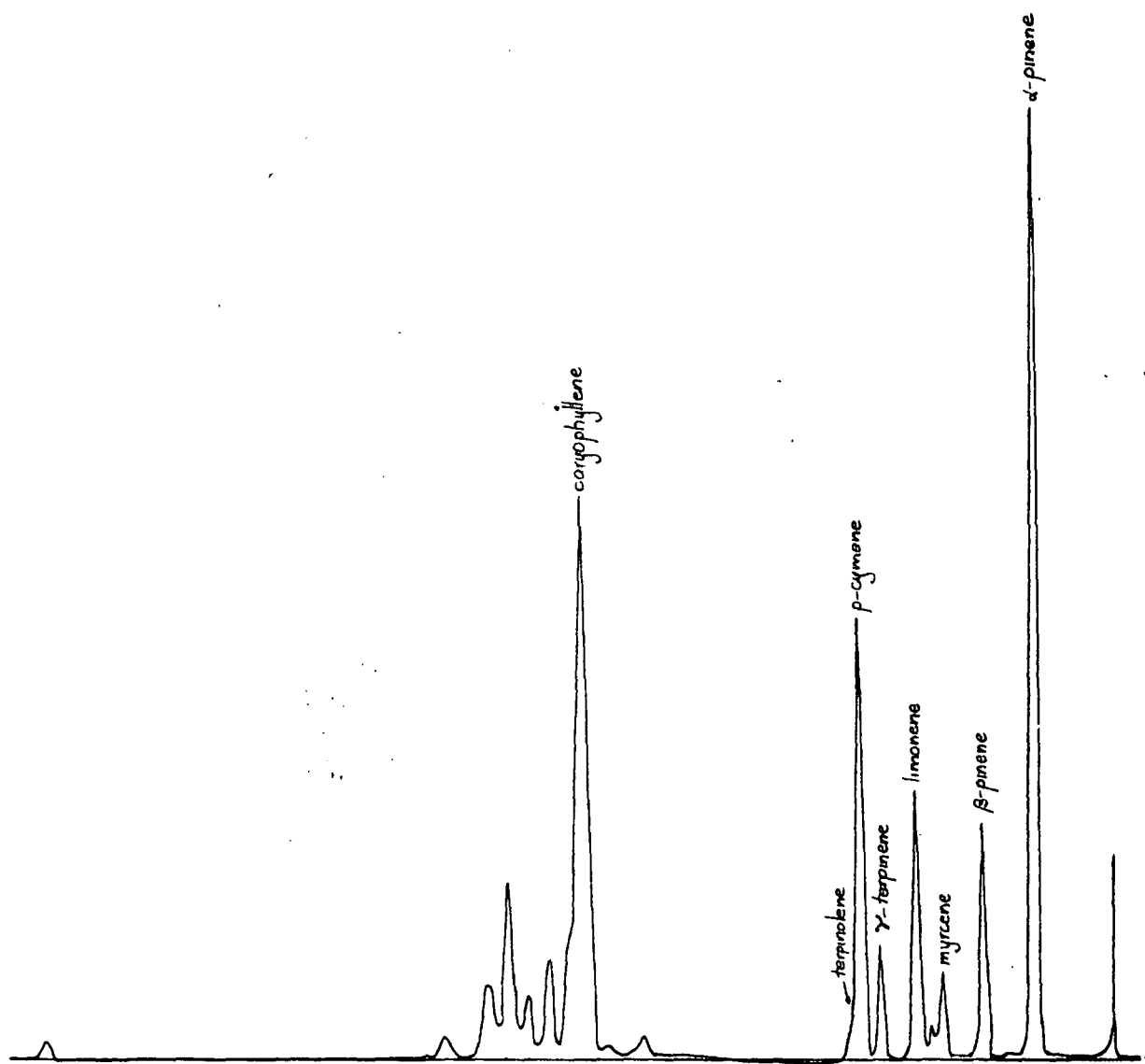
Fig. 23(c). Low sensitivity gas chromatogram of hydrocarbon fraction
of oil of *Leptospermum lanigerum* separated on Florisil.

Fig. 23(d). High sensitivity gas chromatogram of hydrocarbon fraction of oil of *Leptospermum lanigerum* separated on Florisil.

Fig. 23(e). Low sensitivity gas chromatogram of oxygenated
fraction of oil of *Leptospermum lanigerum* separated on Florisil.

Fig. 23(f). High sensitivity gas chromatogram of oxygenated fraction of oil of *Leptospermum lanigerum* separated on Florisil.

WAVELENGTH IN MICRONS

WAVENUMBER IN CM⁻¹

Fig. 24. IR spectrum of the high-boiling alcohol isolated pure in the oxygenated sub-fraction O6 of oil of *Leptospermum lanigerum* [designated terpene alcohol (5); $RRT_{camphor}$ 14.9 (C20M) and 11.0 (OV-17); IR spectrum of solution in $CHCl_3$].

instead contained only a small quantity of caryophyllene.
Similarly, no indication was obtained of the β-triketones
also reported in this oil. It is possible that the major
β-triketone, grandiflorone, might not have been eluted from
any of the GC columns used.

Since this oil appears to differ significantly
from the oils reported in two particular morphological forms
of *L. lanigerum*, there would appear to be a justification for
further study of oil compositions to ultimately determine
whether there are other forms of this species. Further
investigations should also allow for the probability that
the steam-distilled oil may contain high-boiling components
that should be isolated prior to GC, and so avoid omitting
them merely because they were not eluted from a particular
GC column system.

(iii) Experimental

Details of sampling, isolation of oil, separation of
components and analysis are as previously described. The oil
was isolated from foliage in a 0.33 percent wet weight yield.
A 7.47 g portion of the oil was chromatographed on Florisil,
yielding 0.24 g of a colourless hydrocarbon fraction and
6.86 g of a very pale-green oxygenated fraction.

(iv) Summary

Components of steam-distilled leaf oil of

Leptospermum lanigerum were analyzed by chromatographic and

spectroscopic methods. The following identifications were

confirmed: α-pinene (5.5%), limonene (0.8%), 1,8-cineole (16.5%),

ρ-cymene (2.0%), terpinen-4-ol + caryophyllene (3.5%),

α-terpineol (6.2%) and geranyl acetate + citronellol (13.1%).

Tentatively identified were camphene (<0.1%), β-pinene (1.2%),

Δ_3-carene (<0.1%), myrcene (0.3%), β-phellandrene, γ-terpinene (0.2%),

terpinolene (0.2%) and linalool (1.1%). The IR spectrum was

recorded of a high-boiling component (19.9%), which appeared

to be an alcohol.

5. Composition of leaf oil of Christmas Bush

(*Prostanthera lasianthos*

(i) Introduction

Prostanthera lasianthos Labill. (Fam. Labiatae)

grows as a tall erect shrub or small tree 2 to 6 m high.

It is widespread on the banks of streams and the margins of

wet eucalypt forests, from sea level to about 1,000 m [345].

No report was found in the literature of any study

of the oil from this species. However, among other species

of *Prostanthera*, Hellyer [379] has noted the occurrence of

particular sesquiterpene alcohols, i.e. maaliol (60 percent)

in oil of *P. prunellioides* and globulol in oils of *P. sieberi*

and *P. rotundifolia*.

(ii) Results and discussion

Foliage was collected in mid-summer along the Shoobridge Track on Mount Wellington in southern Tasmania. The steam-distilled oil was bright green in colour and had a cineole-like odour. Unlike most other cineole-containing oils including that of *P. rotundifolia*, oil of *P. lasianthos* emitted a pungent, lachrymatous vapour. During the evaporation of solvent from only 5 g of this oil, the pungency could be detected throughout a three-storey building. By comparison, the normal cineole odour from *P. rotundifolia* was only noticeable in the room in which the oil was isolated.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 35. RRT values leading to the tentative identification of components in hydrocarbon and oxygenated fractions, and sub-fractions, are listed in Table 36. Gas chromatograms of Figure 25 show the distribution of oil components eluted from a Carbowax 20M column, and indicate the degree of separation of components into hydrocarbon and oxygenated fractions.

The oil was found to consist largely of 1,8-cineole, ρ-cymene, α-pinene, with smaller quantities limonene and an unidentified higher-boiling hydrocarbon, possibly a sesquiterpene. Only a very small amount (1.7 percent) was found of any individual oxygenated high-boiling component, which corresponded with the expected RRT range of values for sesquiterpene alcohols. No component was detected which

Table 35. Components distinguishable in the whole oil of *Prostanthera lasianthos*

Component	Qualitative RRT data:		Quantitative composition (percent, based on peak height)
	C20M	OV-17	
	(60° isothermal, ref. α-pinene)		(TP 50-200°, 5°/min)
*α-Pinene	1.01	1.00	11.2
Unidentified	1.10	1.12	t
Camphene	1.28	1.19	t
*β-Pinene	1.59	1.54	2.8
Sabinene	1.72		t
Unidentified	1.95		0.2
*Myrcene	2.22	1.73	0.3
α-Phellandrene		1.98	2.5
Unidentified	2.40		t
*Limonene	2.74	2.35	4.2
*1,8-Cineole	3.14	2.81	48.2
γ-Terpinene	3.68	3.22	t
*ρ-Cymene	4.24	2.81	13.4
Terpinolene		4.09	t
(130° isothermal, ref. camphor)			
Terpinen-4-ol	1.26	0.98	2.0
Unidentified		2.85	t
"	1.54		0.3
*α-Terpineol	1.78	1.09	2.7
Unidentified			0.5
"	1.99	3.84	7.7
"	2.10		1.2
"	2.67	1.38	0.2
"	8.33		0.8
"	10.0	7.53	1.7

+ at least 46 other components in trace proportions.

* IR spectrum recorded

t: trace, <0.1 percent

Table 36. P.RT values on the dissimilar liquid phases C20M and OV-17,
for components found in hydrocarbon and oxygenated fractions,
and preparative GC sub-fractions, isolated from oil of
Prostanthera lasianthos

Component	Hydrocarbon fraction		Hydrocarbon sub-fractions			Oxygenated fraction		Oxygenated sub-fractions		
	C20M	OV-17	No.	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
($60°$ isothermal, ref. α-pinene)										
α-Pinene	1.00	1.00	H1	1.00	1.00					
Unidentified						1.10	0.46			
Camphene	1.26	1.19	H1	1.29	1.21					
			H2	1.30	1.20					
β-Pinene	1.61	1.53	H3	1.60	1.56					
			H4	1.62	1.59					
Unidentified						1.95	1.44			
Myrcene	2.25	1.73	H2	2.25	1.76					
α-Phellandrene	2.25	1.95	H4	2.29	1.99					
Limonene	2.77	2.38	H4	2.74	2.37	2.74	2.39			
β-Phellandrene	2.96	2.50	H5	2.96	2.54					
1,8-Cineole						3.09	2.84	O1	3.08	2.84
γ-Terpinene	3.69	3.25								
Unidentified						4.21	2.02	O1	4.23	
p-Cymene	4.34	2.80	H4	4.31	2.82					
Terpinolene	4.61	4.10								
($130°$ isothermal, ref. camphor)										
1,8-Cineole						0.41	0.50			
Unidentified						0.68	0.87			
Terpinen-4-ol						1.24	0.96	O2	1.22	1.04
Unidentified	1.26	2.84								
α-Terpineol						1.76	1.08	O2	1.75	1.14
Unidentified	1.93	3.88								
"						2.63	1.35	O2	2.64	1.27
"						9.55	7.31			

Fig. 25(a).   Low sensitivity gas chromatogram of whole oil of
foliage of *Prostanthera lasianthos* (GC on Carbowax 20M/Gas Chrom Q;
temperature program 50° to 200° at 5°/min;   0.2 µℓ sample;
attenuation 8 x 10^3).

214.

Fig. 25(b).   High sensitivity gas chromatogram of whole oil of
foliage of *Prostanthera lasianthos* (attenuation 4 x 10^2).
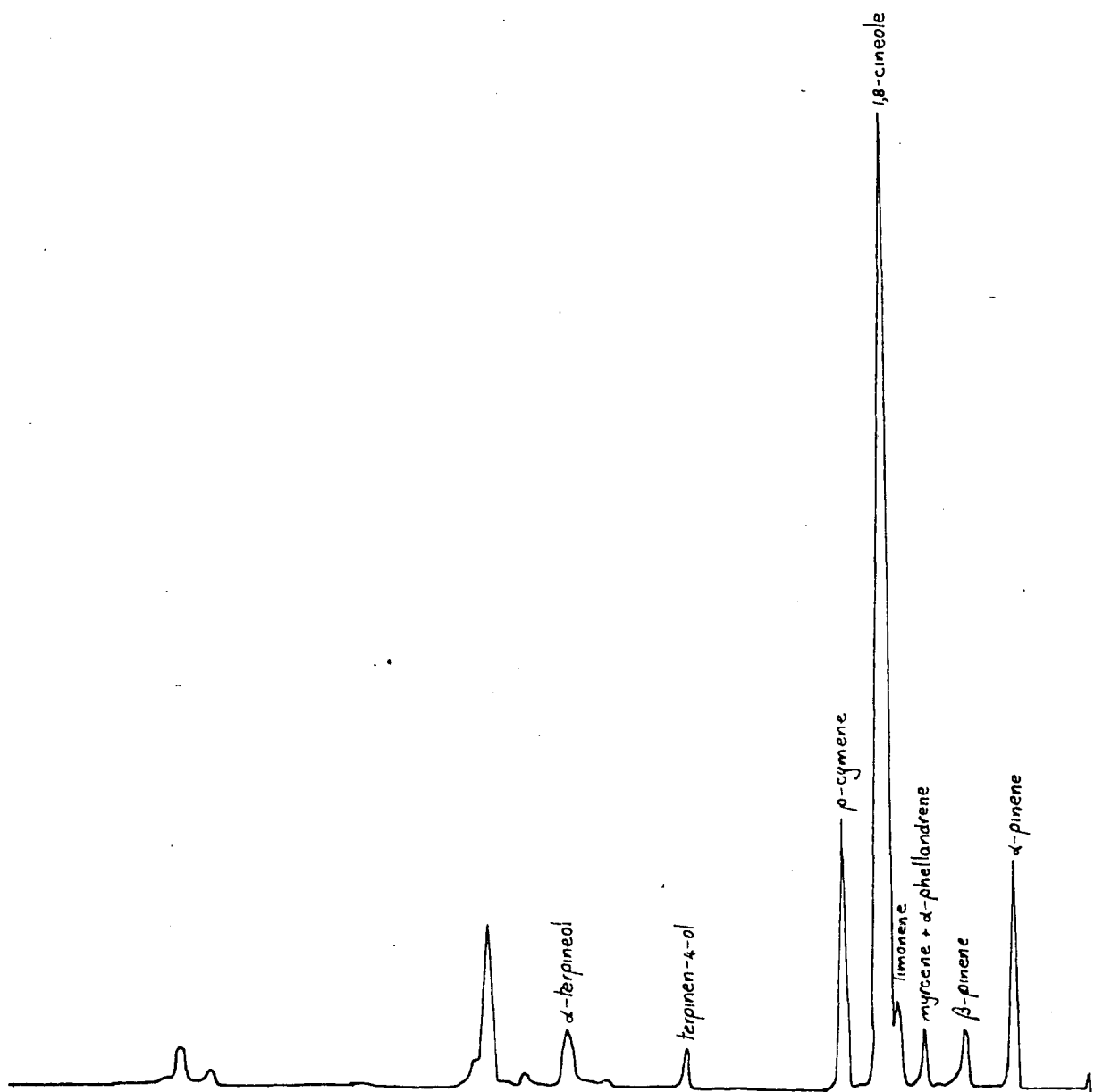
Fig. 25(c). Low sensitivity gas chromatogram of hydrocarbon
fraction of oil of *Prostanthera lasianthos* separated on Florisil.

Fig. 25(d). High sensitivity gas chromatogram of hydrocarbon
fraction of oil of *Prostanthera lasianthos* separated on Florisil.

Fig. 25(e). Low sensitivity gas chromatogram of oxygenated fraction of oil of *Prostanthera lasianthos* separated on Florisil.

Fig. 25(f).   High sensitivity gas chromatogram of oxygenated
fraction of oil of *Prostanthera lasianthos* separated on Florisil.

could account for the pungent and even lachrymatous properties
of this oil.

(iii) <u>Experimental</u>

Details of sampling, isolation of oil, separation
of components and analysis are as previously described. The
oil was isolated from foliage in a 0.11 percent wet weight
yield. A 5.03 g portion of the oil was chromatographed on
Florisil, yielding 0.54 g of a colourless hydrocarbon
fraction and 3.36 g of a pale-green oxygenated fraction.

(iv) <u>Summary</u>

Components of steam-distilled leaf oil of *Prostanthera
lasianthos* were analyzed by chromatographic and spectroscopic
methods. The following identifications were confirmed:
α-pinene (11.2%), β-pinene (2.8%), myrcene (0.3%), limonene (4.2%),
1,8-cineole (48.2%), p-cymene (13.4%) and α-terpineol (2.7%).
Tentatively identified were camphene, sabinene, α-phellandrene
(2.5%), β-phellandrene, γ-terpinene, terpinolene and terpinen-4-ol
(2.0%). No individual component was detected which could account
for the pungent and even lachrymatous properties which accompanied
the basic cineole odour of this oil.

6. Composition of leaf oil of *Prostanthera rotundifolia*

(i) Introduction

Prostanthera rotundifolia R.Br. (Fam. Labiatae)
[345] is a much-branched shrub that grows up to about 2 m
high. The flowers are mostly dense, lilac-coloured terminal
racemes, which make the plant an attractive species for
garden cultivation. *P. rotundifolia* is well-known for its
strong cineole odour from the crushed leaves. It is locally
frequent in northern and eastern regions of Tasmania and on
the Australian mainland.

The only report found in the literature on the oil
of this species was a reference to the occurrence of a
sesquiterpene alcohol, globulol [379].

(ii) Results and discussion

Foliage was collected in mid-summer from five
cultivated shrubs in the grounds of the University of Tasmania.
The steam-distilled oil was green in colour and had a
cineole odour.

The qualitative and quantitative composition of
components distinguishable in the whole oil, using two
dissimilar columns, is recorded in Table 37. RRT values
leading to the tentative identification of components in
hydrocarbon and oxygenated fractions, and sub-fractions, are
listed in Table 38. Gas chromatograms of Figure 26 show the
distribution of oil components eluted from a Carbowax 20M
column, and indicate the degree of separation of components
into hydrocarbon and oxygenated fractions.

Table 37.   Components distinguishable in the whole
oil of *Prostanthera rotundifolia*

Component	Qualitative RRT data:		Quantitative composition (percent, based on peak height)
	C20M	OV-17	
	(60° isothermal, ref. α-pinene)		(TP 50-200°, 5°/min)
α-Pinene	1.00	1.00	1.4
Unidentified	1.12		t
Camphene	1.25	1.20	t
*β-Pinene	1.60	1.56	2.3
Sabinene	1.71		0.7
*Myrcene	} 2.26	1.78	} 3.7
α-Phellandrene		2.00	
Unidentified	2.44		0.3
Limonene	2.75	2.40	1.1
*1,8-Cineole	3.12	2.89	47.0
γ-Terpinene	3.66	3.27	0.3
*p-Cymene	4.29	2.89	23.2
Terpinolene	4.64	4.14	t
(130° isothermal, ref. camphor)			
*Terpinen-4-ol	1.22	0.97	1.5
Chavicol methyl ether	1.60	1.20	1.0
*α-Terpineol	1.76	1.06	4.1
Unidentified	2.49	1.45	t
Geraniol	3.21	1.20	2.3
Unidentified	6.46	8.76	t
*Terpene alcohol(4) (Fig. 27)	8.13	7.57	6.9
Unidentified	9.66	7.57	1.4
Several others unidentified			sum 2.8

* IR spectrum recorded

t: trace, <0.1 percent

Table 38. RRT values on the dissimilar liquid phases C20M and OV-17, for components found in hydrocarbon and oxygenated fractions, and preparative GC sub-fractions, isolated from oil of *Prostanthera rotundifolia*

| Component	Hydrocarbon fraction		Hydrocarbon sub-fractions			Oxygenated fraction		Oxygenated sub-fractions		
	C20M	OV-17	No.	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)										
α-Pinene	1.02	0.99								
Unidentified						1.08	0.47			
"	1.13									
Camphene	1.30	1.18	H2	1.29	1.20					
β-Pinene	1.64	1.55	H2	1.61	1.55					
			H3	1.63	1.59					
Sabinene	1.85									
Δ₃-Carene	2.04		H3	2.08	2.03					
Myrcene	2.25	1.74	H2	2.22	1.74					
α-Phellandrene		1.95								
Unidentified	2.43	2.13	H3	2.37						
"			H2	2.70	2.06	2.69	2.06			
Limonene	2.74	2.34	H3	2.77	2.44					
			H4	2.72	2.36					
1,8-Cineole	3.09	2.85	H3	3.06	2.88	3.10	2.82	O1	3.04	2.81
			H4	3.04	2.79					
γ-Terpinene	3.65	3.22	H4	3.63	3.21					
p-Cymene	4.37	2.85	H3	4.25	2.88					
			H4	4.22	2.79					
(130° isothermal, ref. camphor),										
1,8-Cineole						0.40	0.53			
Terpinen-4-ol						1.25	1.00	O2	1.24	0.97
Chavicol methyl ether						1.60	1.24	O2	1.59	1.19
α-Terpineol						1.77	1.12	O3	1.78	1.09
Unidentified						2.51	1.41	O2	2.44	1.43
Geraniol						3.28	1.24	O3	3.28	1.22
Unidentified						6.66	8.68	O5	6.65	8.47
Terpene alcohol(4)						8.38	7.60	O4	8.42	7.30
Unidentified						9.95	7.60	O4	10.1	7.30

Fig. 26(a). Low sensitivity gas chromatogram of whole oil of foliage of *Prostanthera rotundifolia* (GC on Carbowax 20M/Gas Chrom Q; temperature program 50° to 200° at 5°/min; 0.2 µℓ sample; attenuation 8 x 10³).
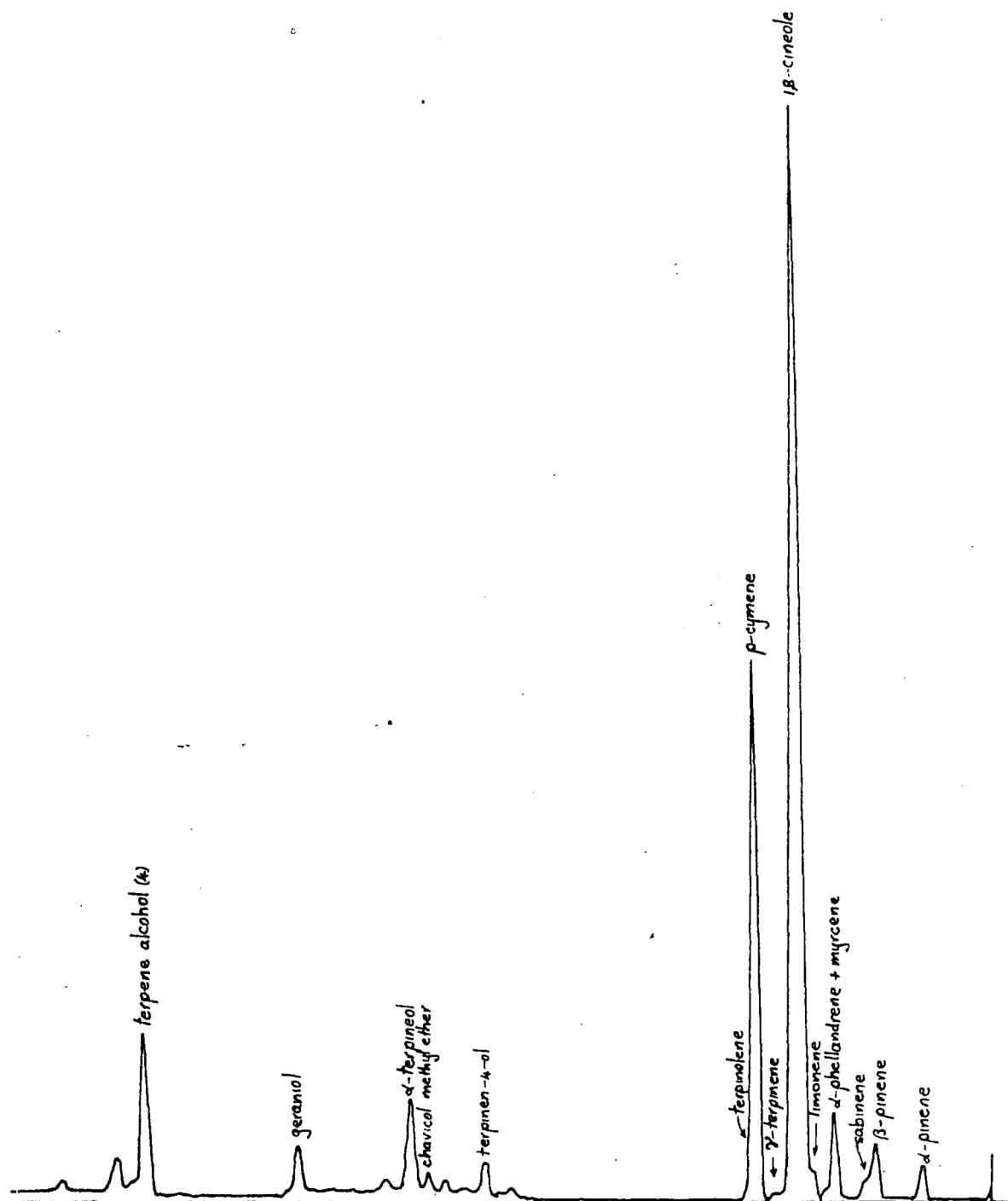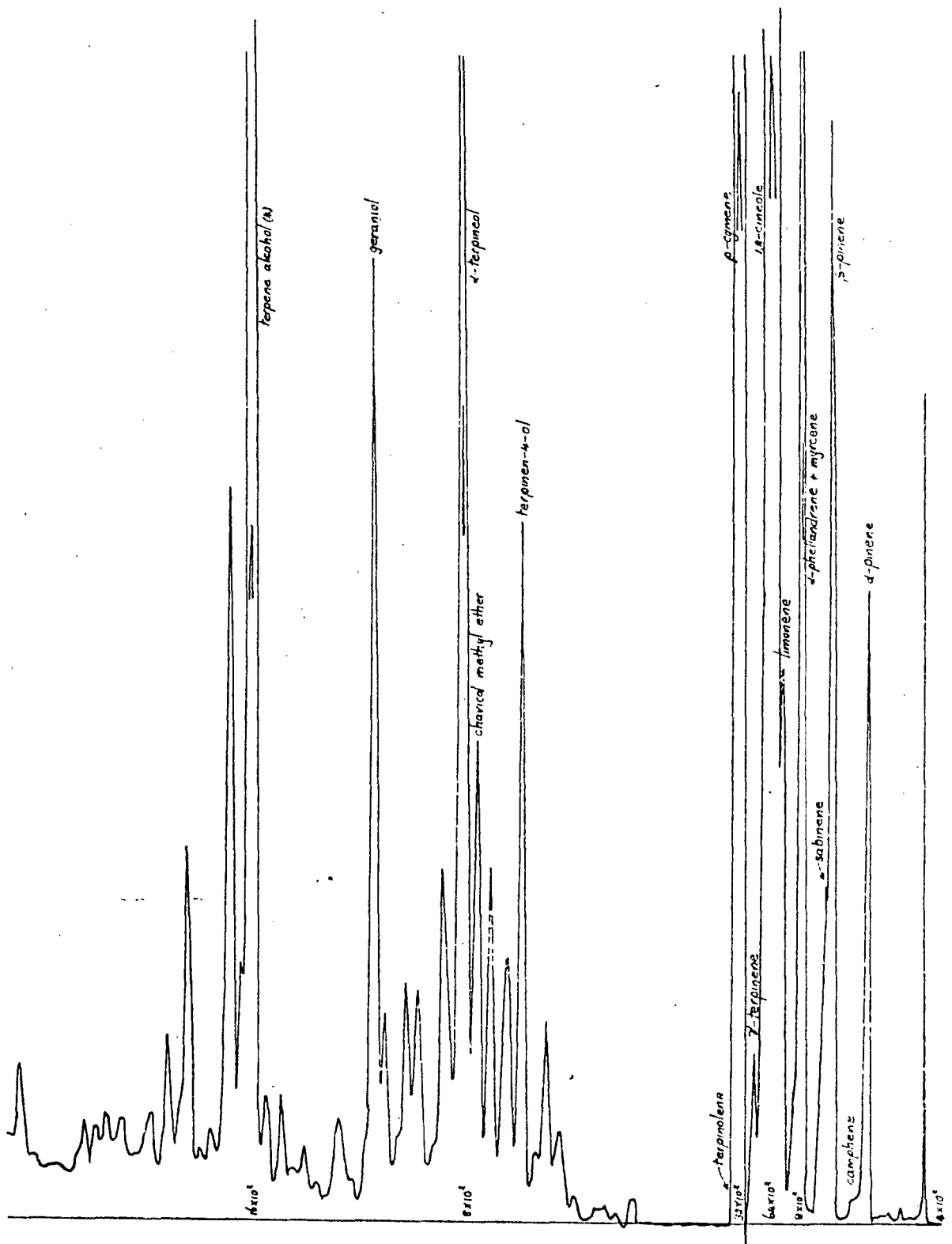
Fig. 26(b). High sensitivity gas chromatogram of whole oil of foliage of *Prostanthera rotundifolia* (attenuation 4 x 10^2).

Fig. 26(c). Low sensitivity gas chromatogram of hydrocarbon fraction of oil of *Prostanthera rotundifolia*, separated on Florisil.
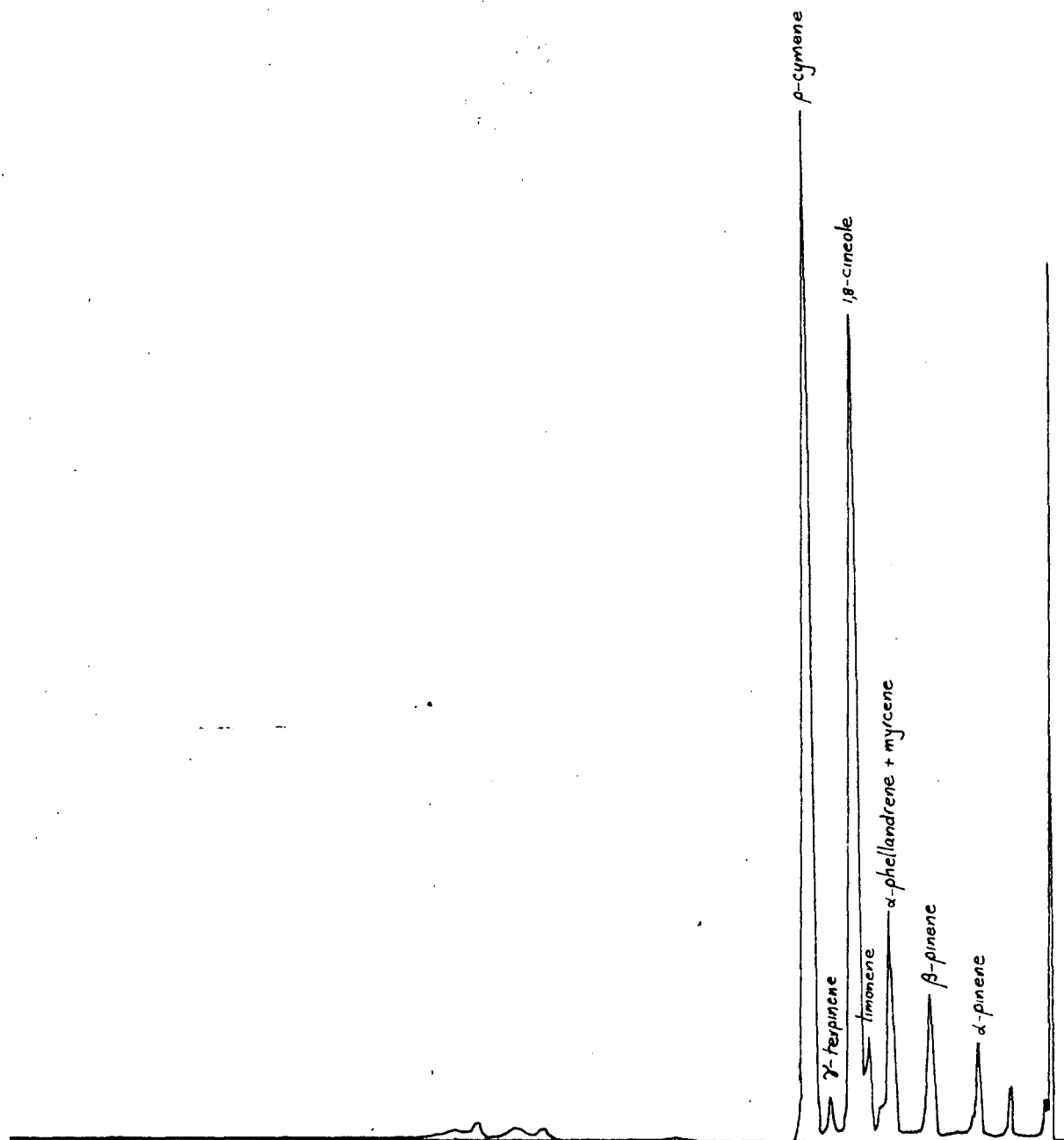
Fig. 26(d). High sensitivity gas chromatogram of hydrocarbon fraction of oil of *Prostanthera rotundifolia* separated on Florisil.
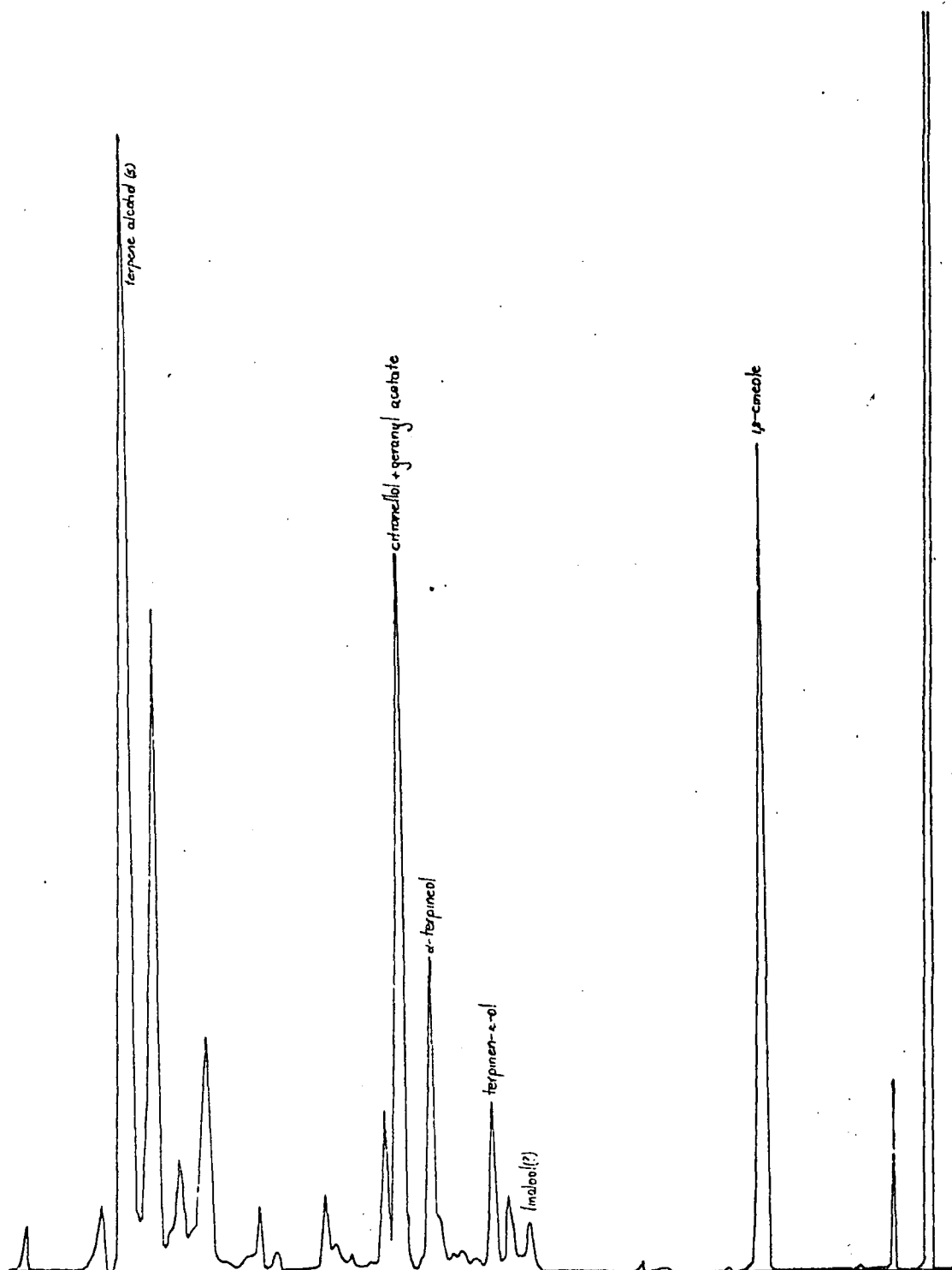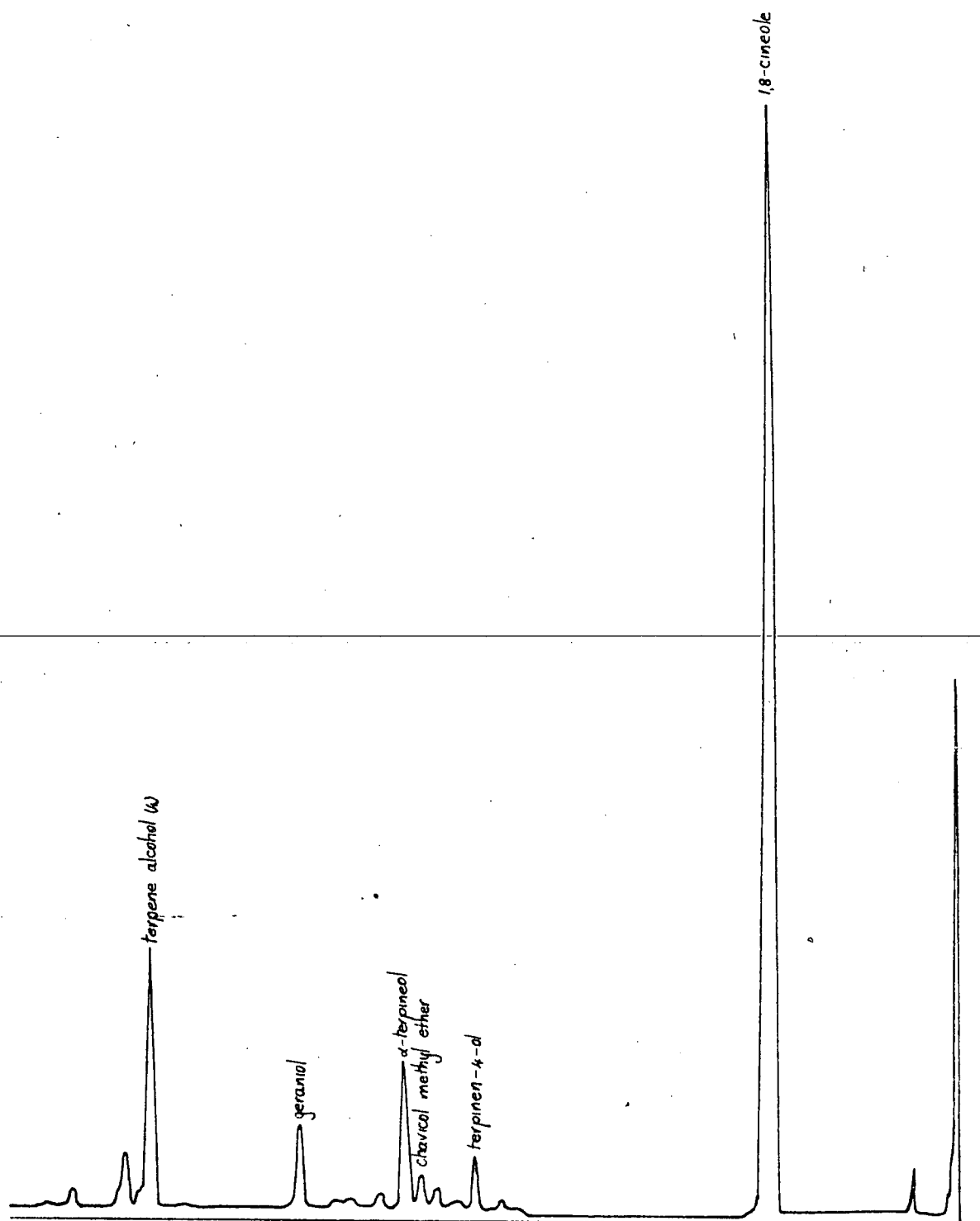
Fig. 26(e).   Low sensitivity gas chromatogram of oxygenated fraction
of oil of *Prostanthera rotundifolia* separated on Florisil.
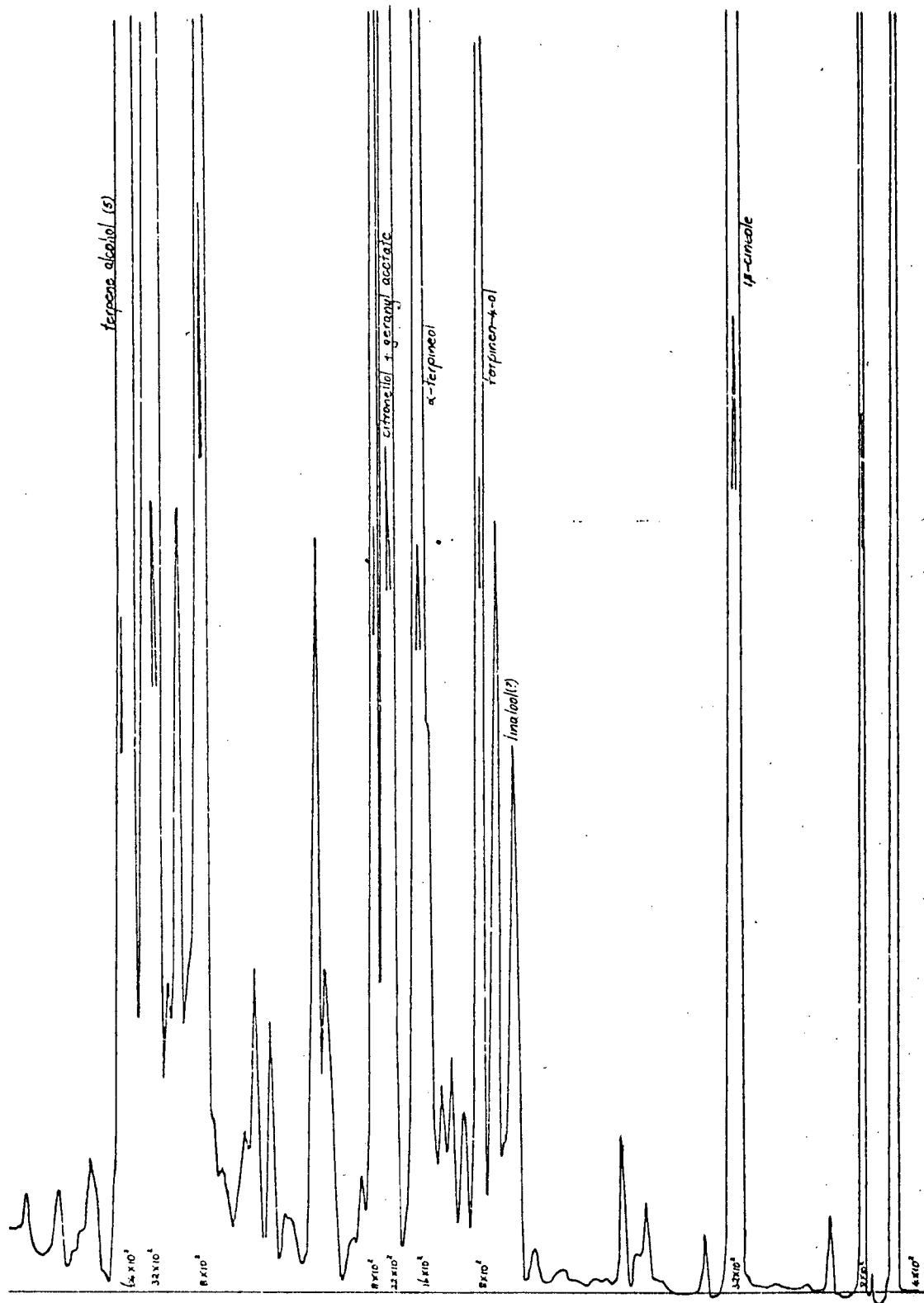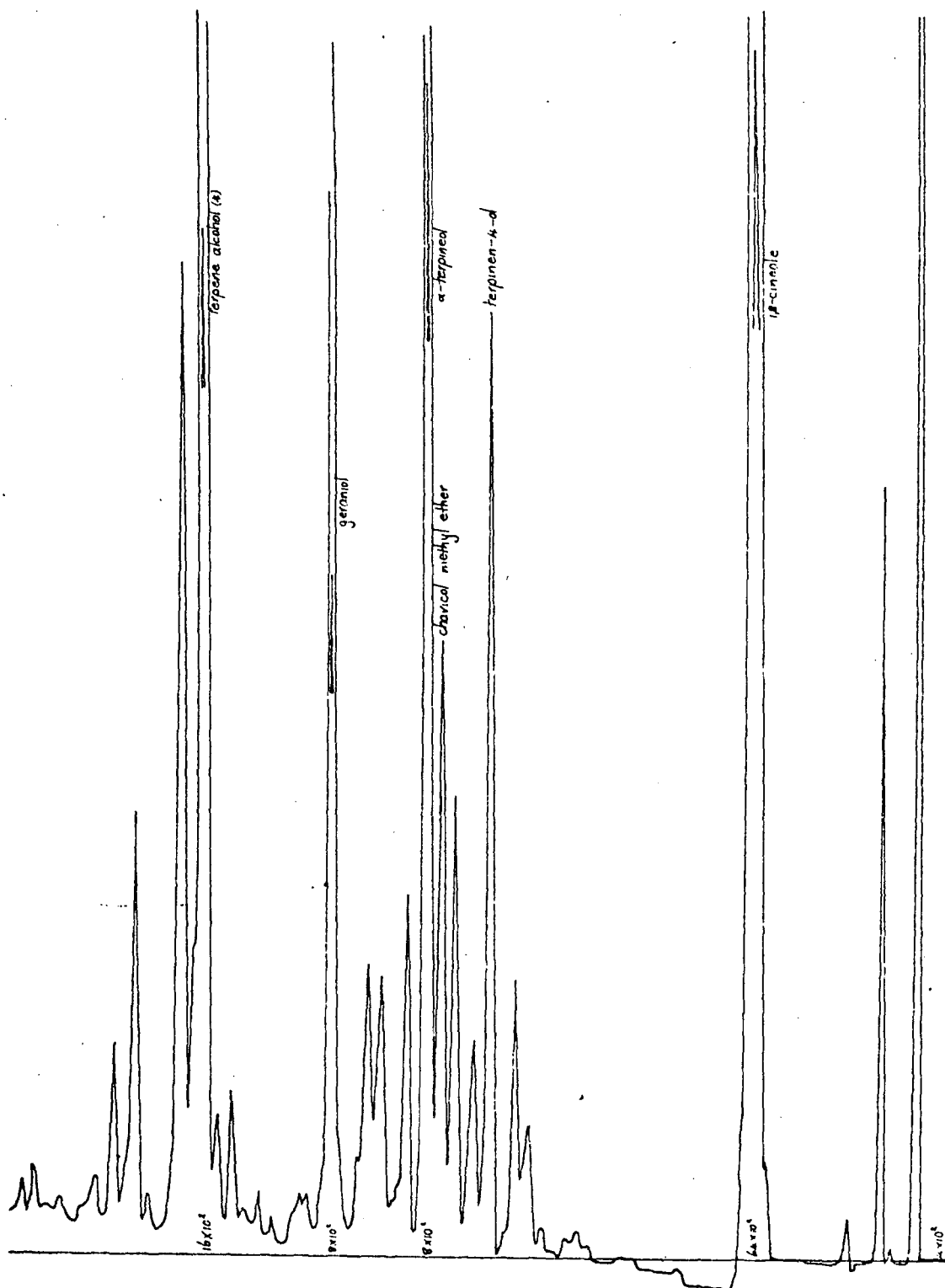
Fig. 26(f).   High sensitivity gas chromatogram of oxygenated fraction of oil of *Prostanthera rotundifolia* separated on Florisil.
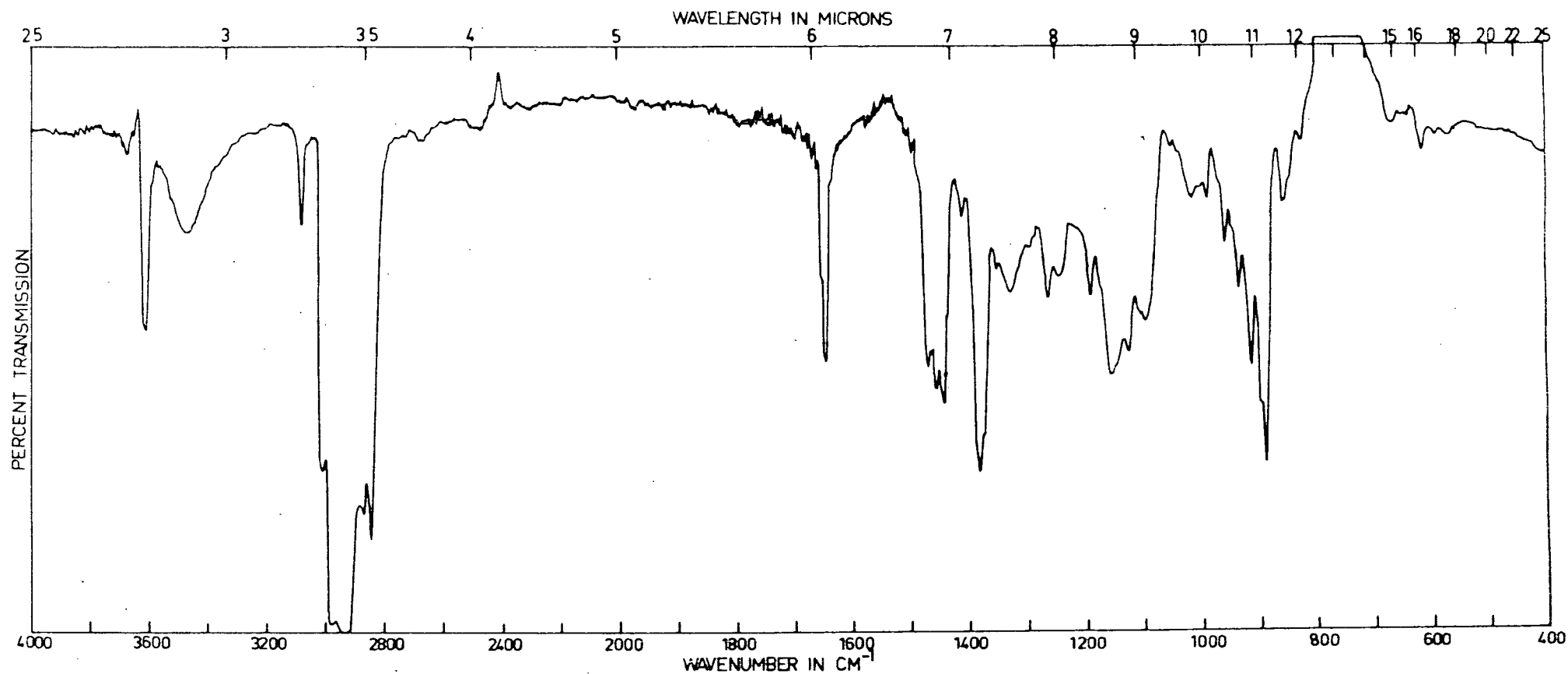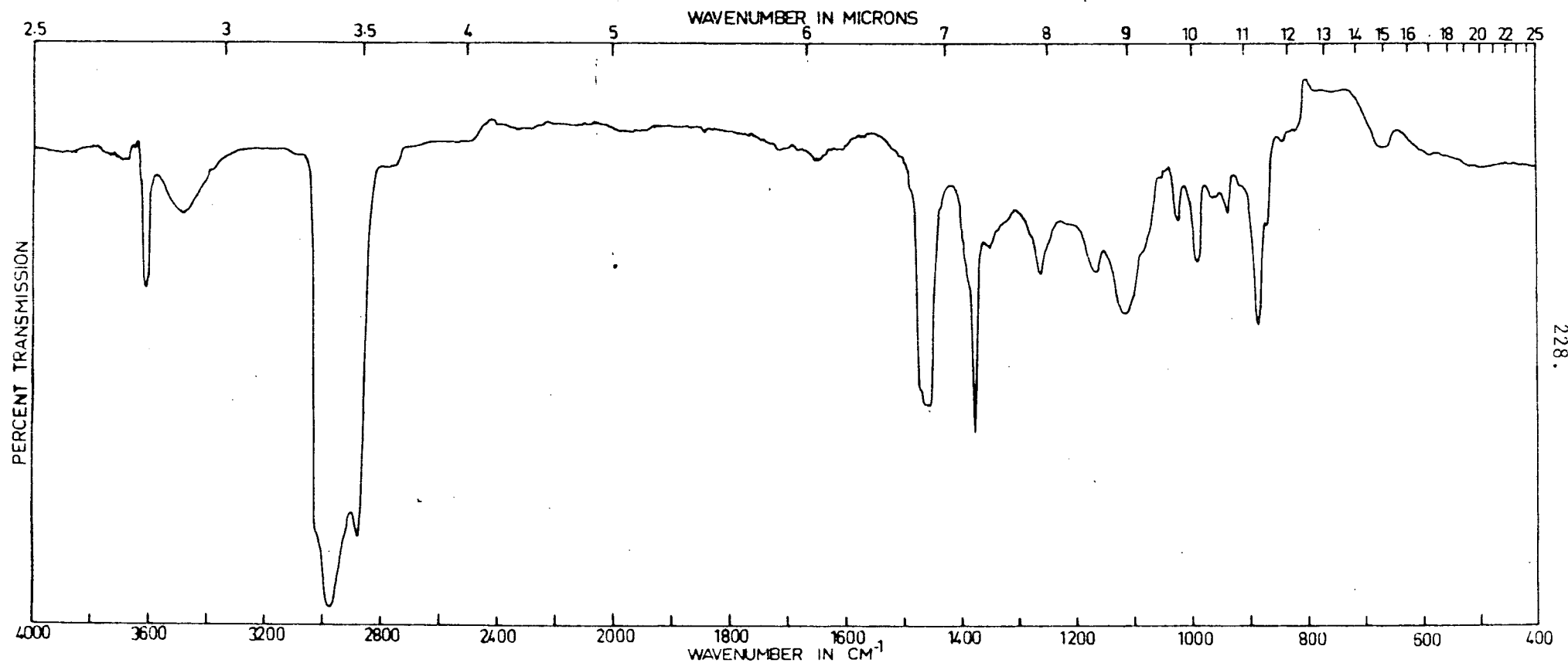
Fig. 27.   IR spectrum of the high-boiling alcohol isolated pure in the oxygenated sub-fraction O5 of oil of *Prostanthera rotundifolia* [designated terpene alcohol (4);   RRT$_{camphor}$ 8.41 (C20M) and 7.30 (OV-17);   IR spectrum of solution in CHCl$_3$].

The oil was found to consist largely of 1.8-cineole and ρ-cymene with small quantities of β-pinene, myrcene, α-phellandrene, terpinen-4-ol, α-terpineol and an unidentified high-boiling alcohol (6.9 percent) (Figure 27). This last component could correspond to the sesquiterpene alcohol, globulol, referred to by Hellyer [379].

Comparison of the oils of P. *rotundifolia* and P. *lasianthos* (Tables 35 and 37) shows some features which could be of interest in subsequent biosynthetic investigations. ρ-Cymene has often been cited as being a product of the chemical alterations of specific monoterpenes, but in these oils it is present as a major component, perhaps as a result of a concerted biosynthetic mechanism leading to its sole production or perhaps as a co-product in the formation of 1,8-cineole. Quantitative co-occurrence investigations should therefore be undertaken with these species to examine the biosynthetic relationship of ρ-cymene to other terpenoids.

(iii) Experimental

Details of sampling, isolation of oil, separation of components and analysis are as previously described. The oil was isolated from foliage in a 1.01 percent wet weight yield. A 10.80 g. portion of the oil was chromatographed on Florisil, yielding 2.23 g of a colourless hydrocarbon fraction and 6.35 g of a pale-green oxygenated fraction.

(iv) <u>Summary</u>

Components of steam-distilled leaf oil of

Prostanthera rotundifolia were analyzed by chromatographic and

spectroscopic methods. The following identifications were

confirmed: β-pinene (2.3%), myrcene, 1,8-cineole (47.0%),

ρ-cymene (23.2%), terpinen-4-ol (1.5%) and α-terpineol (4.1%).

Tentatively identified were α-pinene (1.4%), camphene, sabinene,

Δ_3-carene, α-phellandrene (3.7%), limonene (1.1%),

γ-terpinene (0.3%), terpinolene, chavicol methyl ether (1.0%)

and geraniol (2.3%). The IR spectrum was recorded of a high-

boiling component (6.9%), which appeared to be an alcohol.

7. <u>Composition of leaf oil of Sassafras</u>

    <u>*(Atherosperma moschatum)*</u>

(i) <u>Introduction</u>

Atherosperma moschatum Labill. (Fam. Monimiaceae)

grows into a tree 15 to 45 m high in rain forests of Tasmania

and other Australian states [345]. It is well known for the

aromatic odour released when the leaves are crushed.

The only reference in the literature was an early

study of this oil by Scott [390], who reported having found

pinene (15-20%), camphor (15-20%), safrole (5-10%) and

eugenol methyl ether (50-60%).

(ii) Results and discussion

Foliage was collected in mid-summer from trees growing along the Arve River Road, near Geeveston in southern Tasmania. The steam-distilled oil was a pale yellow-green colour with a rich odour of eugenol, camphor and safrole.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 39. RRT values leading to the tentative identification of components in hydrocarbon and oxygenated fractions, and sub-fractions, are listed in Table 40. Gas chromatograms of Figure 28 show the distribution of oil components eluted from a Carbowax 20M column, and indicate the degree of separation of components into hydrocarbon and oxygenated fractions.

The composition of the oil was found to largely agree with that reported by Scott [390], consisting of eugenol methyl ether, camphor and safrole, with smaller quantities of α-terpineol, bornyl acetate, terpinen-4-ol, linalool and camphene.

The qualitative co-occurrence in this oil of camphene, bornyl acetate and camphor, conforms with Ruzicka's proposal for the biosynthetic pathway [356] for this group of compounds. That is, cyclization of the 1-ρ-menthene-8-carbonium ion (III) (Figure 14) to the 2-bornane carbonium ion (V), which upon stabilization leads to the above group of compounds that all contain the bornane skeleton. Subsequent examination of this oil could also show the existence of borneol which could be obscured by major GC peaks.

Table 39. Components distinguishable in the whole
oil of *Atherosperma moschatum*

Component	Qualitative RRT data:		Quantitative composition (percent, based on peak height)
	C20M	OV-17	
	(60° isothermal, ref. α-pinene)		(TP 50-200°, 5°/min)
*α-Pinene	1.00	1.00	1.3
*Camphene	1.27	1.24	5.0
β-Pinene	1.59	1.58	t
Myrcene	} 2.23	1.76	} 0.9
α-Phellandrene		1.97	
*Limonene	2.68	2.37	0.7
β-Phellandrene	2.95		t
Unidentified	3.36	~2.8	t
*γ-Terpinene	3.68	3.21	t
*ρ-Cyrene	4.18	2.76	0.4
Terpinolene	4.41	4.16	t
(130° isothermal, ref. camphor)			
Unidentified	0.65	0.63	t
*Linalool	0.92	0.63	} 18.2
*Camphor	1.00	1.00	
Terpinen-4-ol	} 1.19	1.00	} 5.7
Bornyl acetate		1.68	
Unidentified	1.58		0.7
*α-Terpineol	1.77	1.05	3.3
Unidentified	2.11		t
*Safrole	3.42	2.26	13.8
*Eugenol methyl ether	6.35	4.37	49.5
Unidentified		3.16	0.4

+ at least 18 other components in trace proportions

* IR spectrum recorded

t: trace, <0.1 percent

Table 40. RRT values on the dissimilar liquid phases C20M and OV-17, for components found in hydrocarbon and oxygenated fractions, and preparative GC sub-fractions, isolated from oil of *Atherosperma moschatum*

Component	Hydrocarbon fraction		Hydrocarbon sub-fractions			Oxygenated fraction		Oxygenated sub-fractions		
	C20M	OV-17	No.	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)										
α-Pinene	1.00	1.00	H1	1.05	0.98					
Camphene	1.27	1.21	H2	1.29	1.20					
β-Pinene	1.64		H2	1.67	1.58					
Myrcene	2.27	1.76	H2	2.29	1.73					
α-Phellandrene	2.27	2.03	H3	2.22	2.00					
Unidentified	2.50	2.82	H3	2.45	2.74					
Limonene	2.77	2.39	H3	2.73	2.36					
Unidentified						2.86	2.19			
β-Phellandrene	3.00	2.55	H4	2.86	2.51					
1,8-Cineole						3.18	2.89			
Unidentified	3.39	2.82	H3	3.47	2.74					
γ-Terpinene	3.74	3.29	H5	3.71	3.23					
Unidentified			H4	3.73	2.90					
p-Cymene	4.32	2.82	H3	4.26	2.79					
Terpinolene	4.59	4.21								
Unidentified			H5	8.43	5.82					
(130° isothermal, ref. camphor)										
1,8-Cineole						0.38	0.47			
Unidentified						0.46				
"						0.69	0.63	O1	0.63	0.60
Linalool						0.92	0.63	O1	0.89	0.60
Camphor						1.00	1.00	O2	1.04	1.00
Terpinen-4-ol						1.19	1.00	O2	1.23	1.00
Bornyl acetate						1.19	1.58	O4	1.19	1.60
Unidentified	1.31	2.84								
"						1.58				
"								O3	1.78	1.00
α-Terpineol						1.81	1.00	O3	1.83	1.05
Unidentified	1.81	3.84	H7	1.81	4.11					
"	2.27	~4.5	H7	2.31	4.74					
Safrole	3.50	2.21	H7	3.58	2.26	3.50	2.16	O4	3.54	2.20
Eugenol methyl ether						6.54	4.26	O5	6.73	4.40
Unidentified						9.77	7.32			

Fig. 28(a). Low sensitivity gas chromatogram of whole oil of foliage of *Atherosperma moschatum* (GC on Carbowax 20M/Gas Chrom Q; temperature program 50° to 200° at 5°/min; 0.2 µℓ sample; attenuation 8 x 10³).
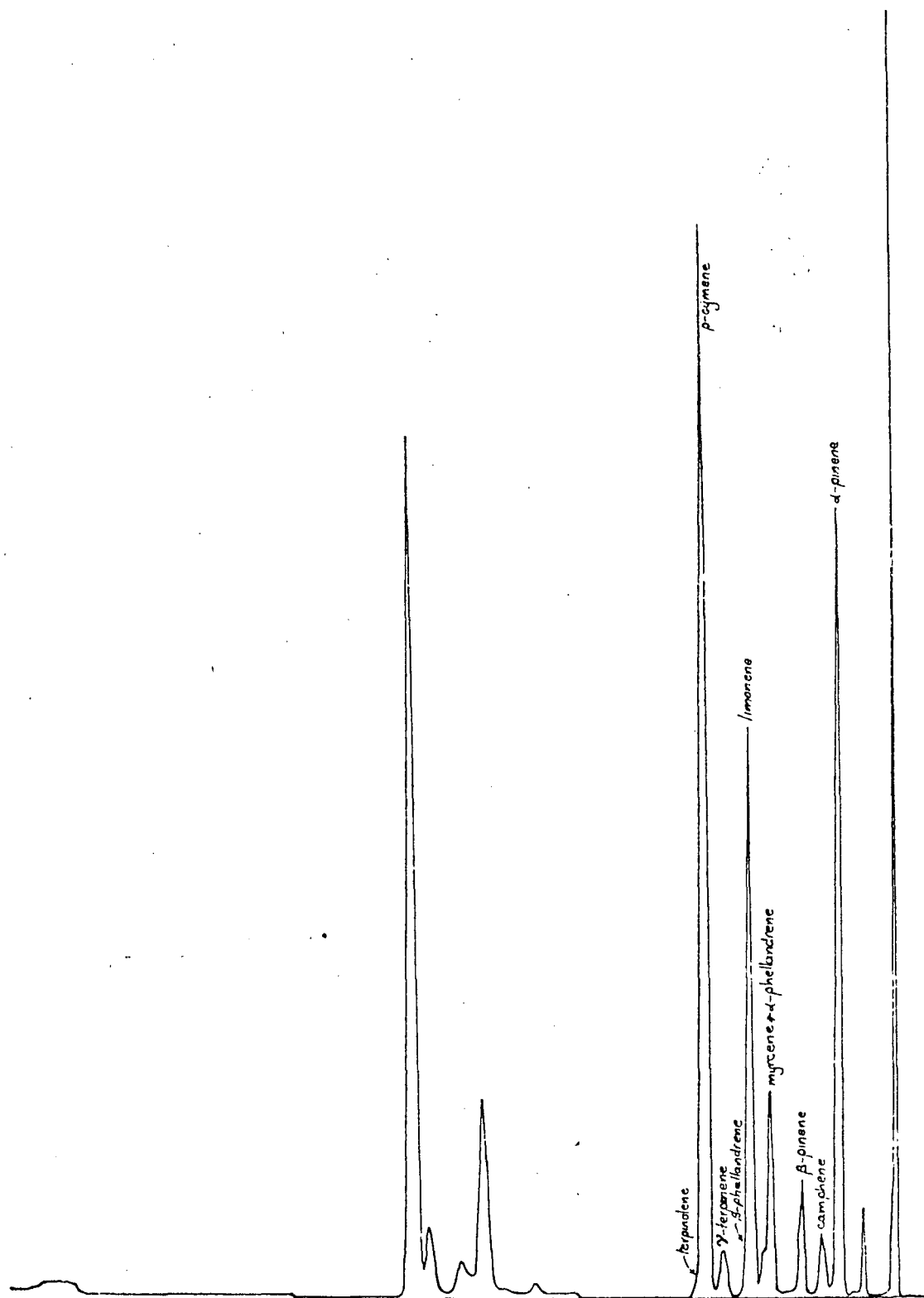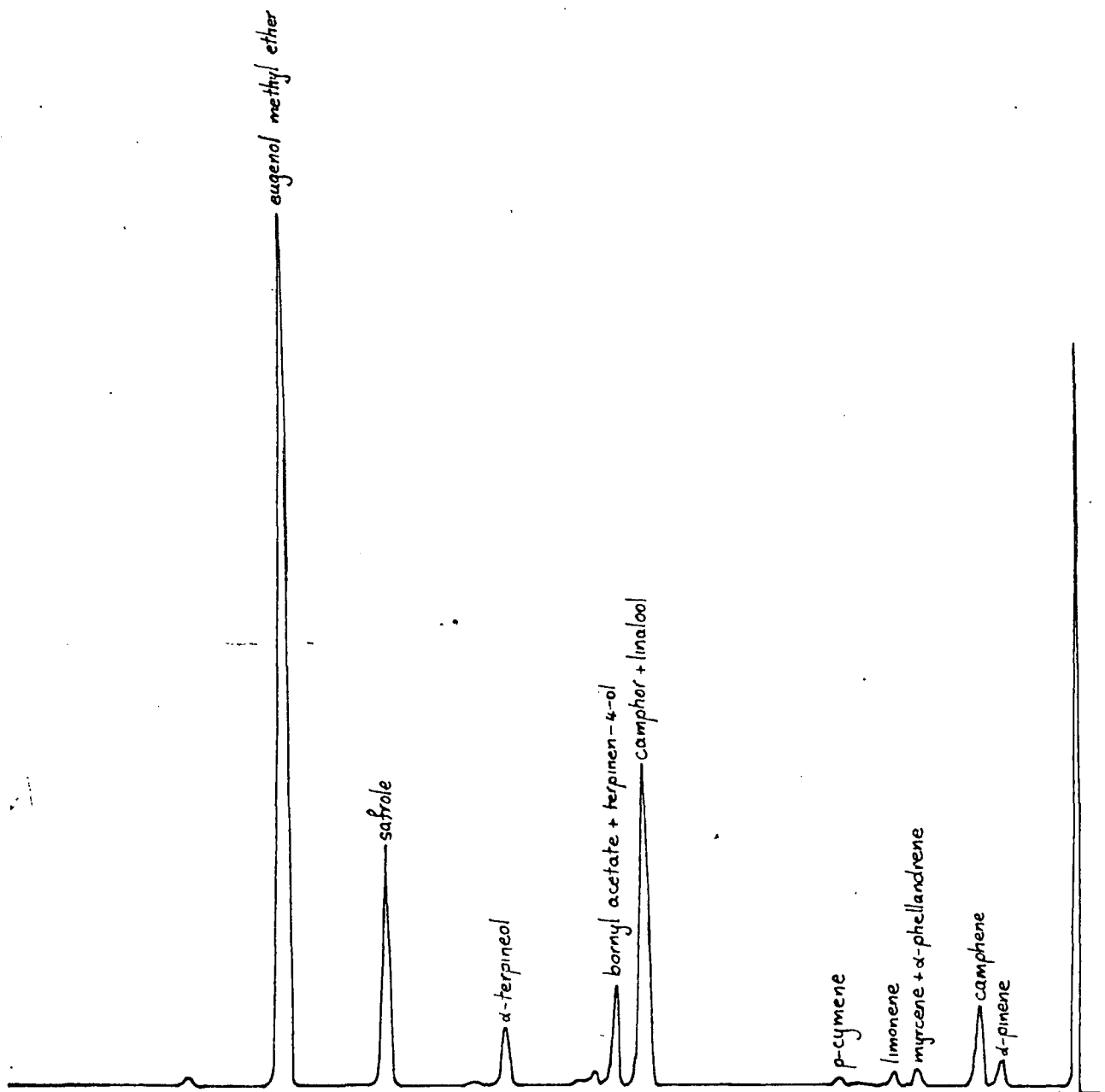
Fig. 28(b). High sensitivity gas chromatogram of whole oil of *Atherosperma moschatum* (attenuation 4×10^2).

235.
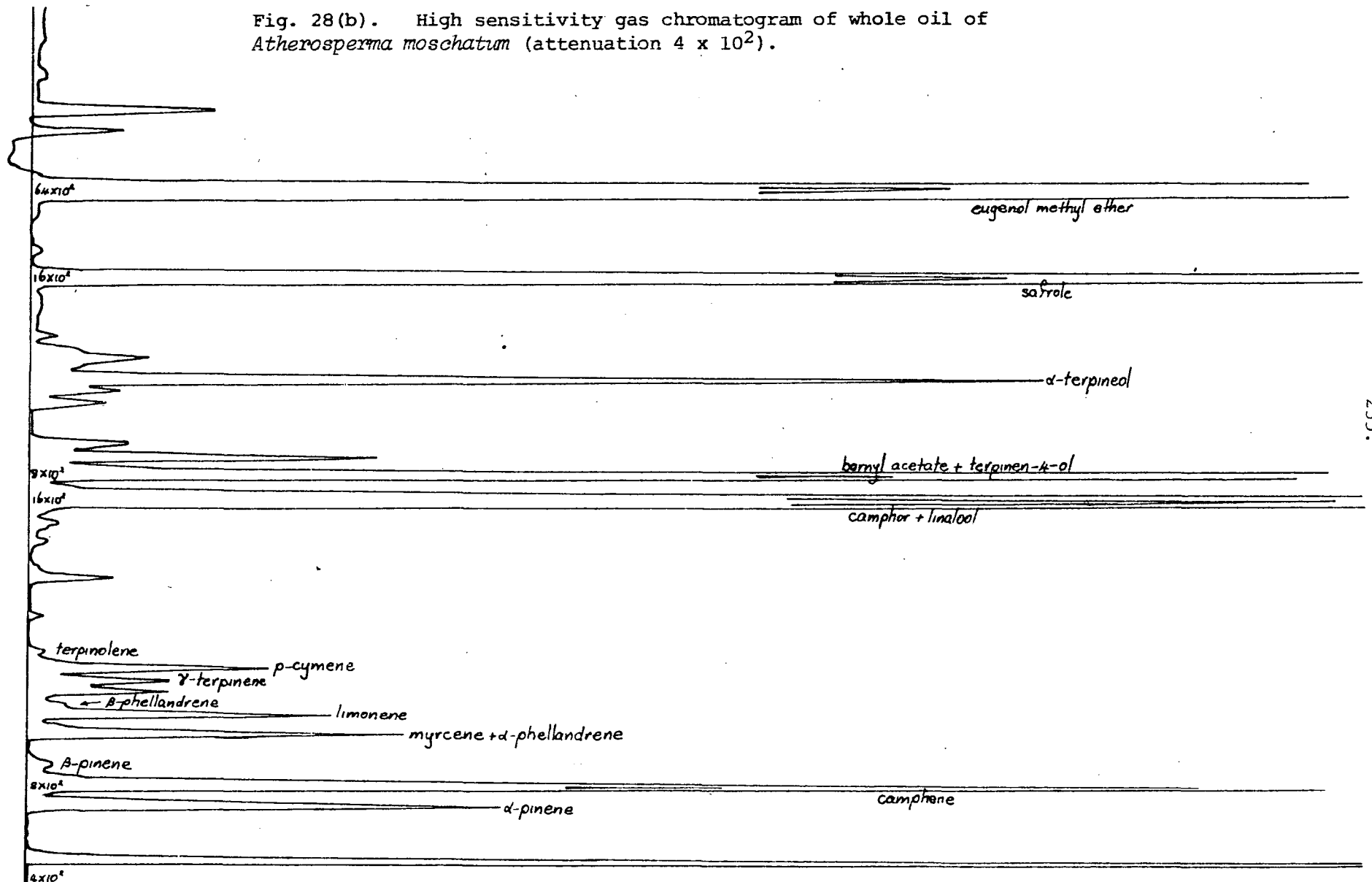
Fig. 28(c): Low sensitivity gas chromatogram of hydrocarbon fraction of oil of *Atherosperma nioschatum* separated on Florisil.
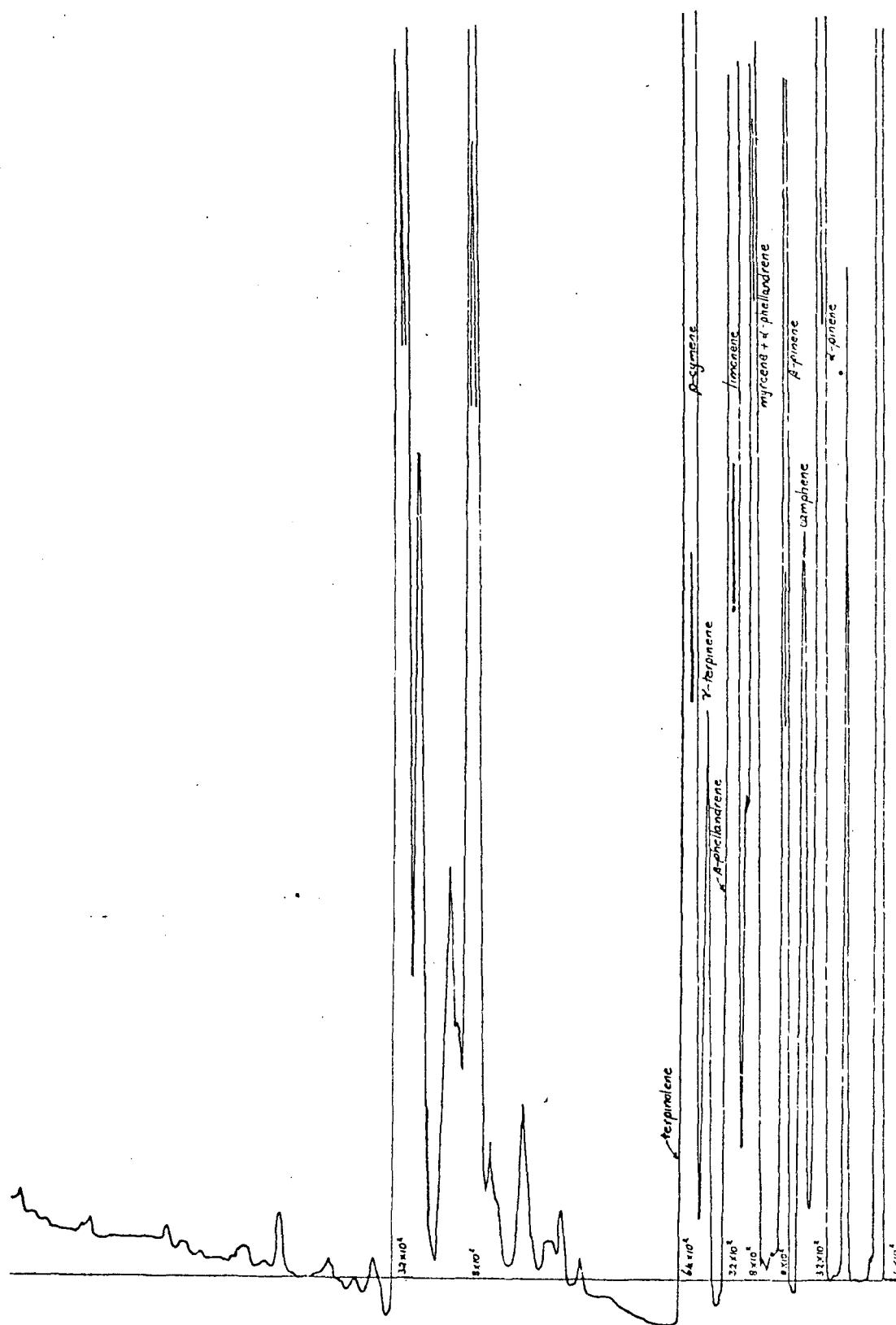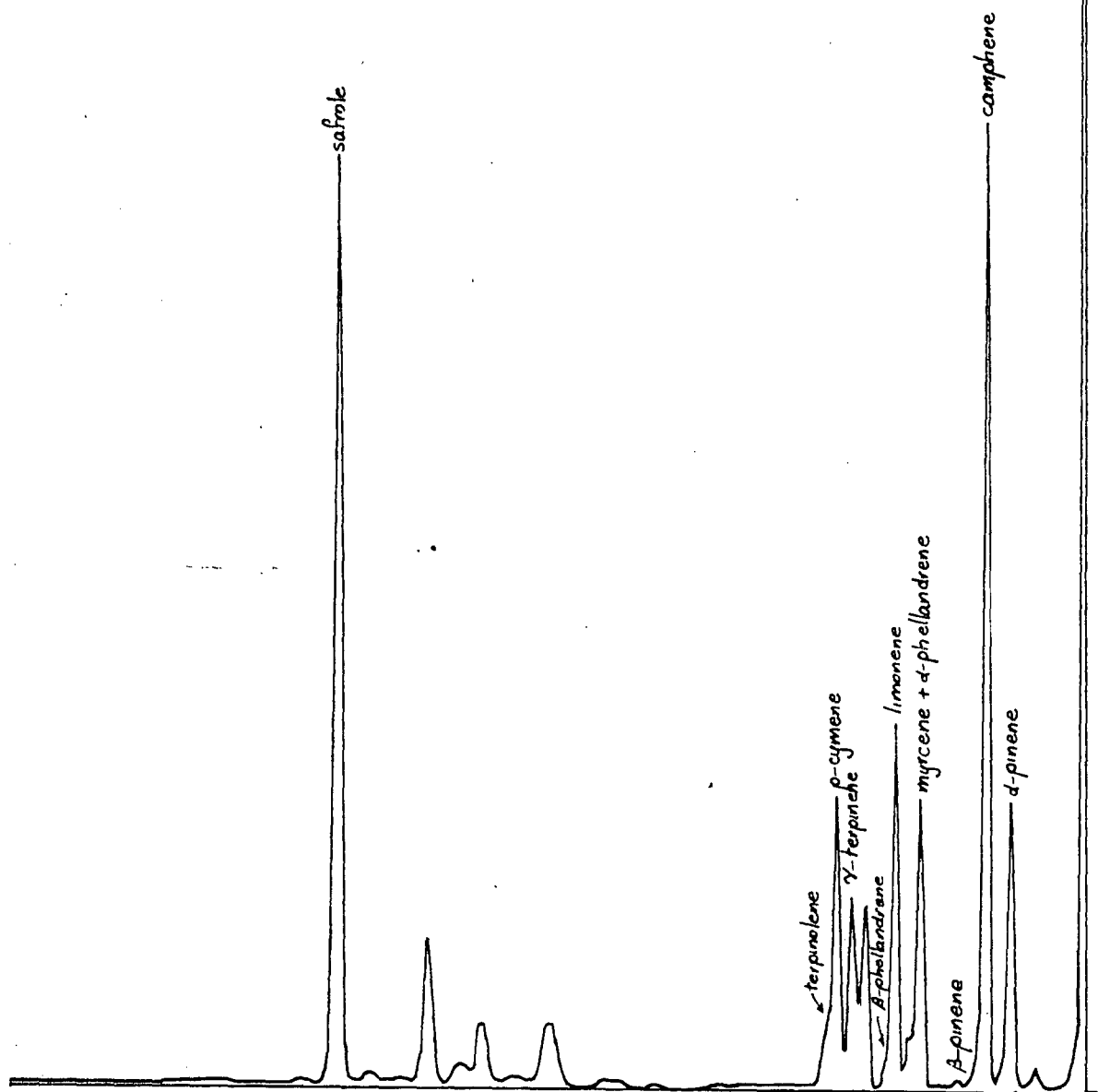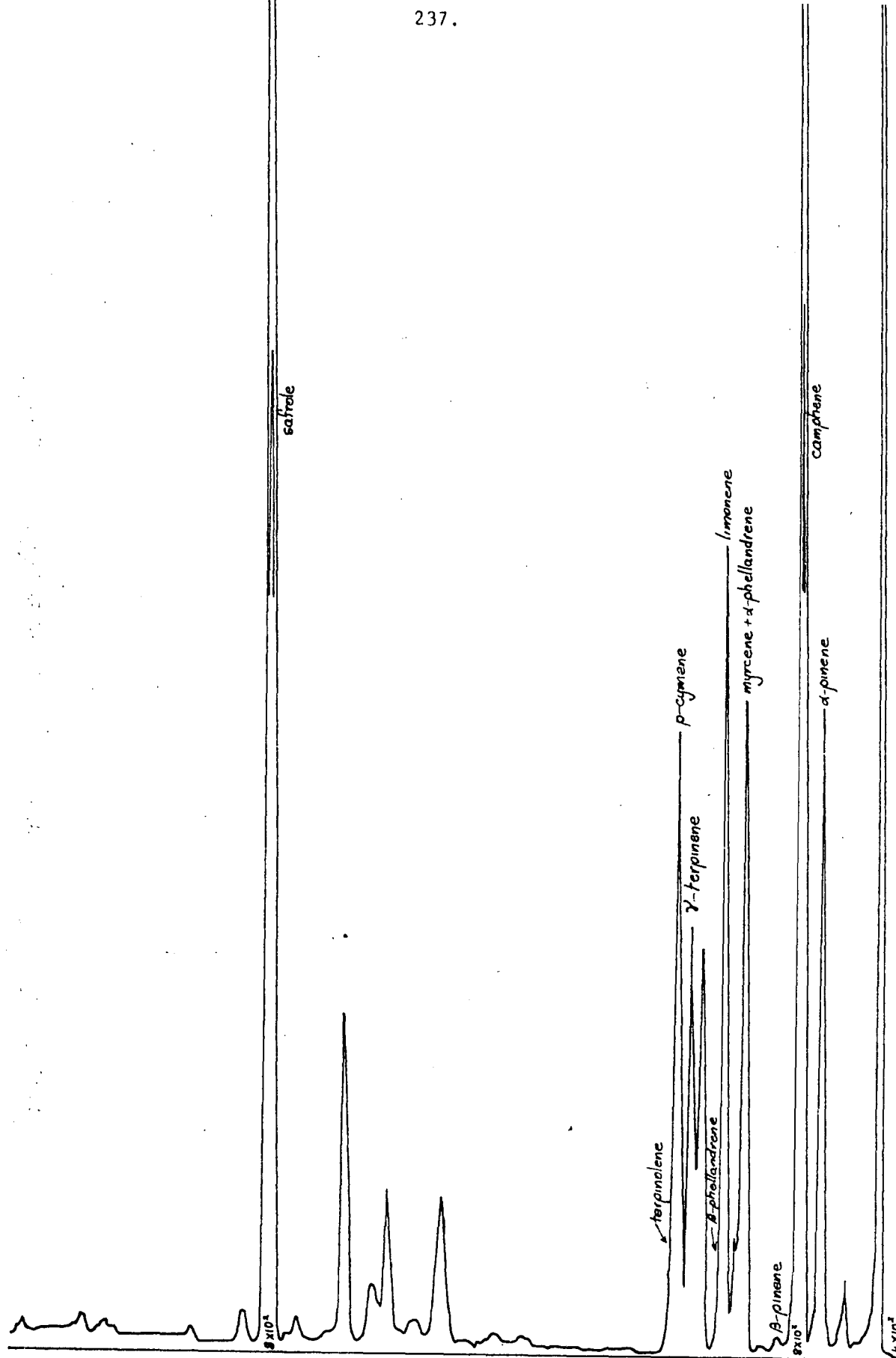
Fig. 28.(d). High sensitivity gas chromatogram of hydrocarbon fraction of oil of *Atherosperma moschatum* separated on Florisil.
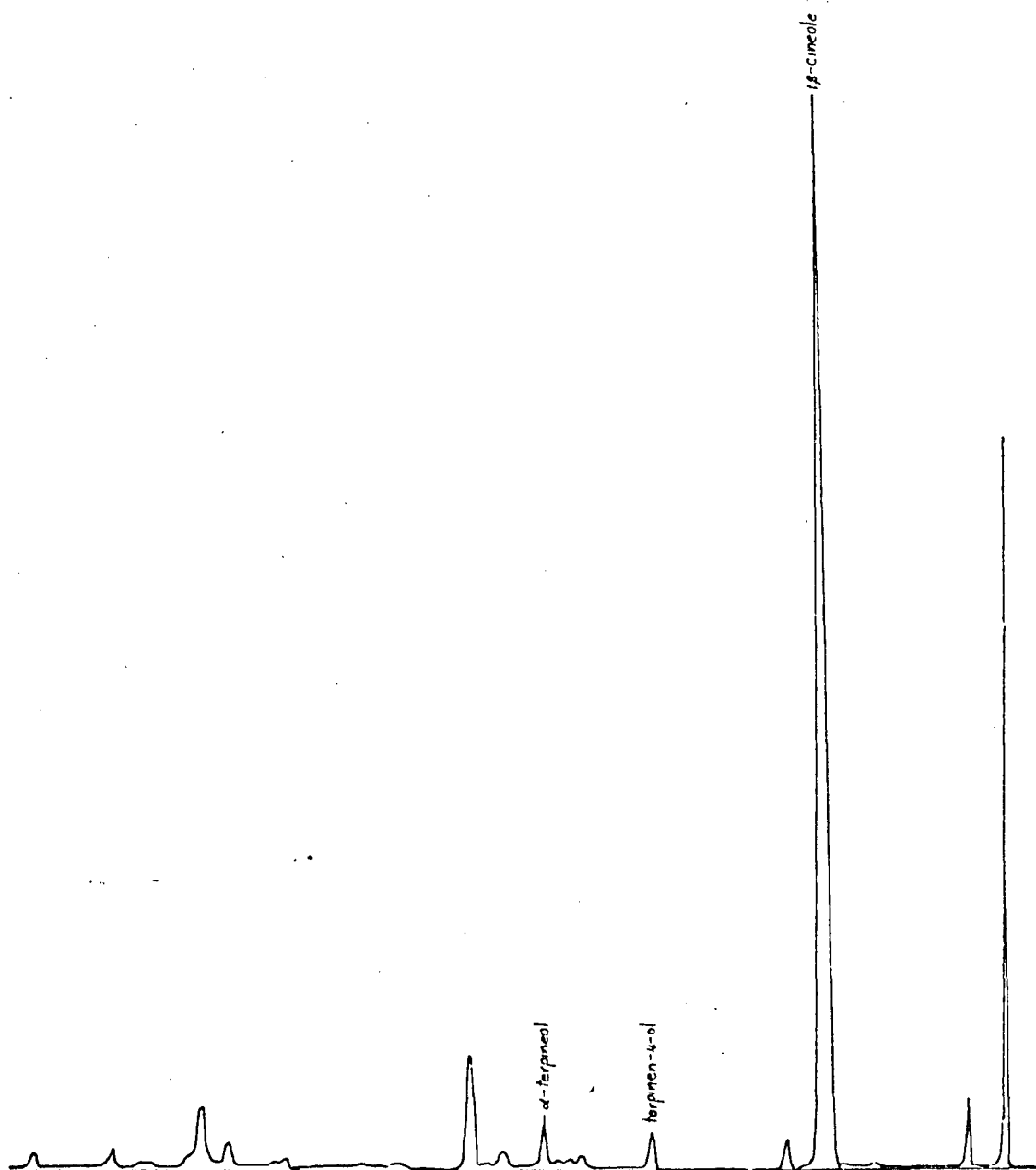
Fig. 28(e).   Low sensitivity gas chromatogram of oxygenated
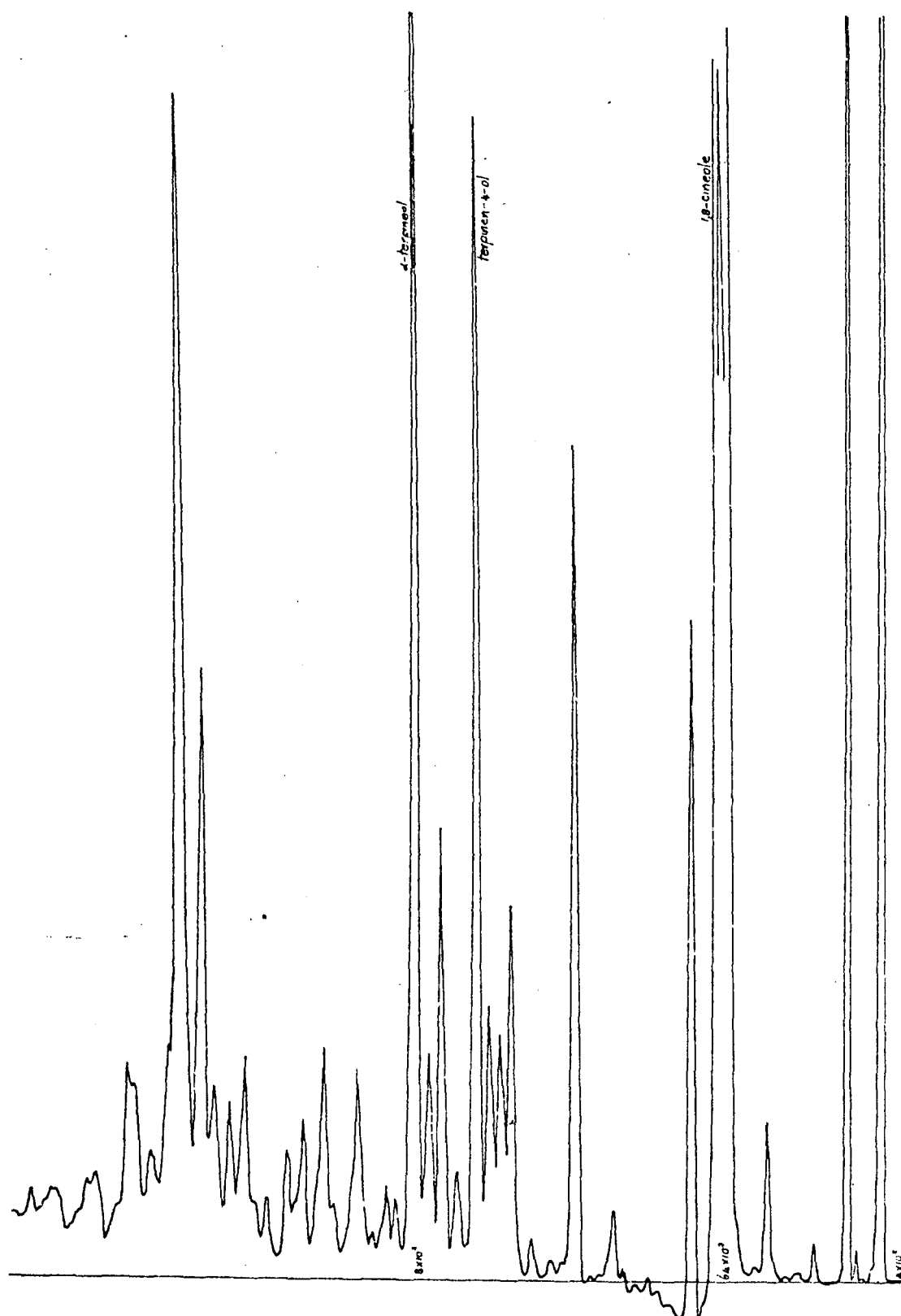fraction of oil of *Atherosperma moschatum* separated on Florisil.
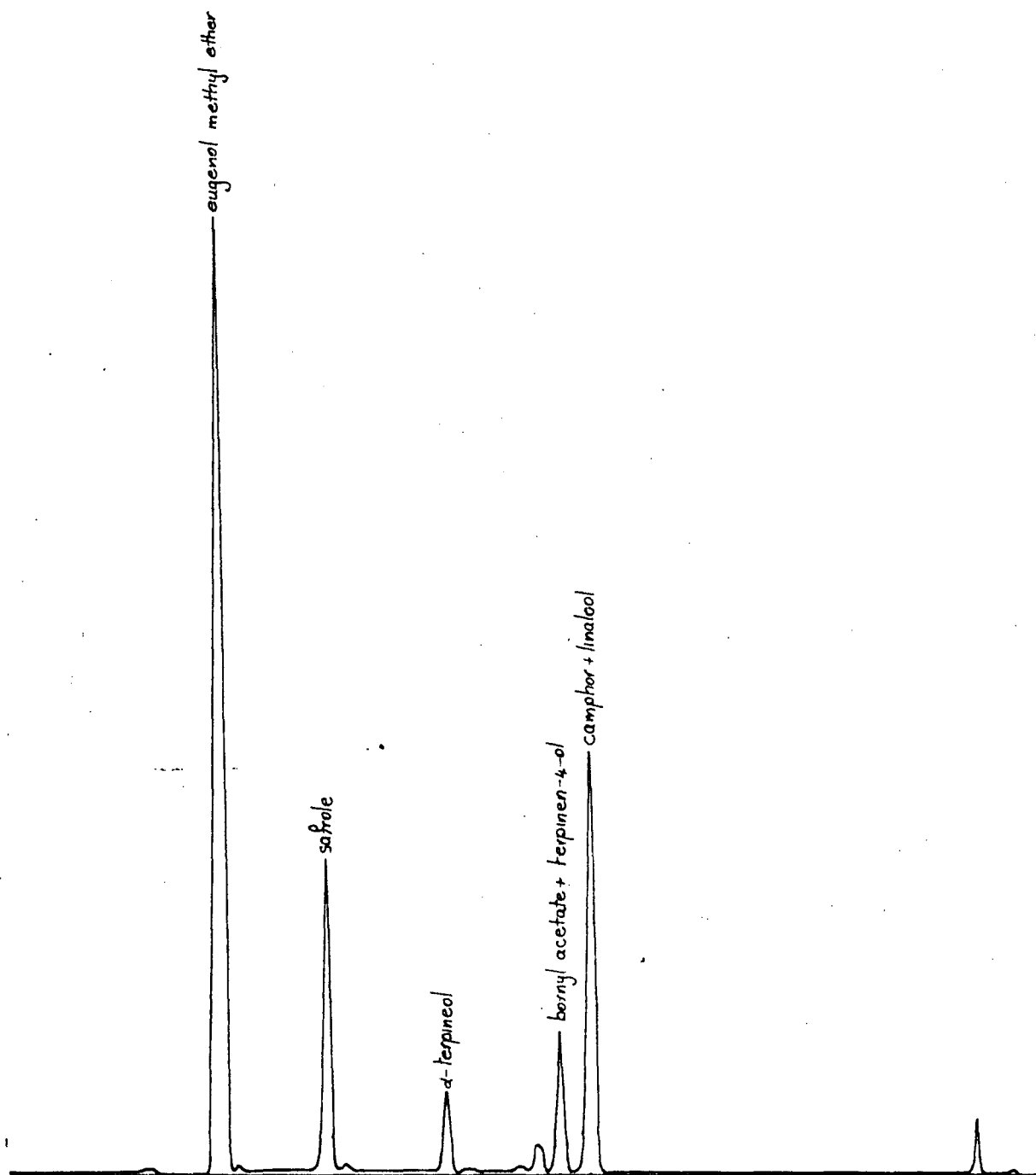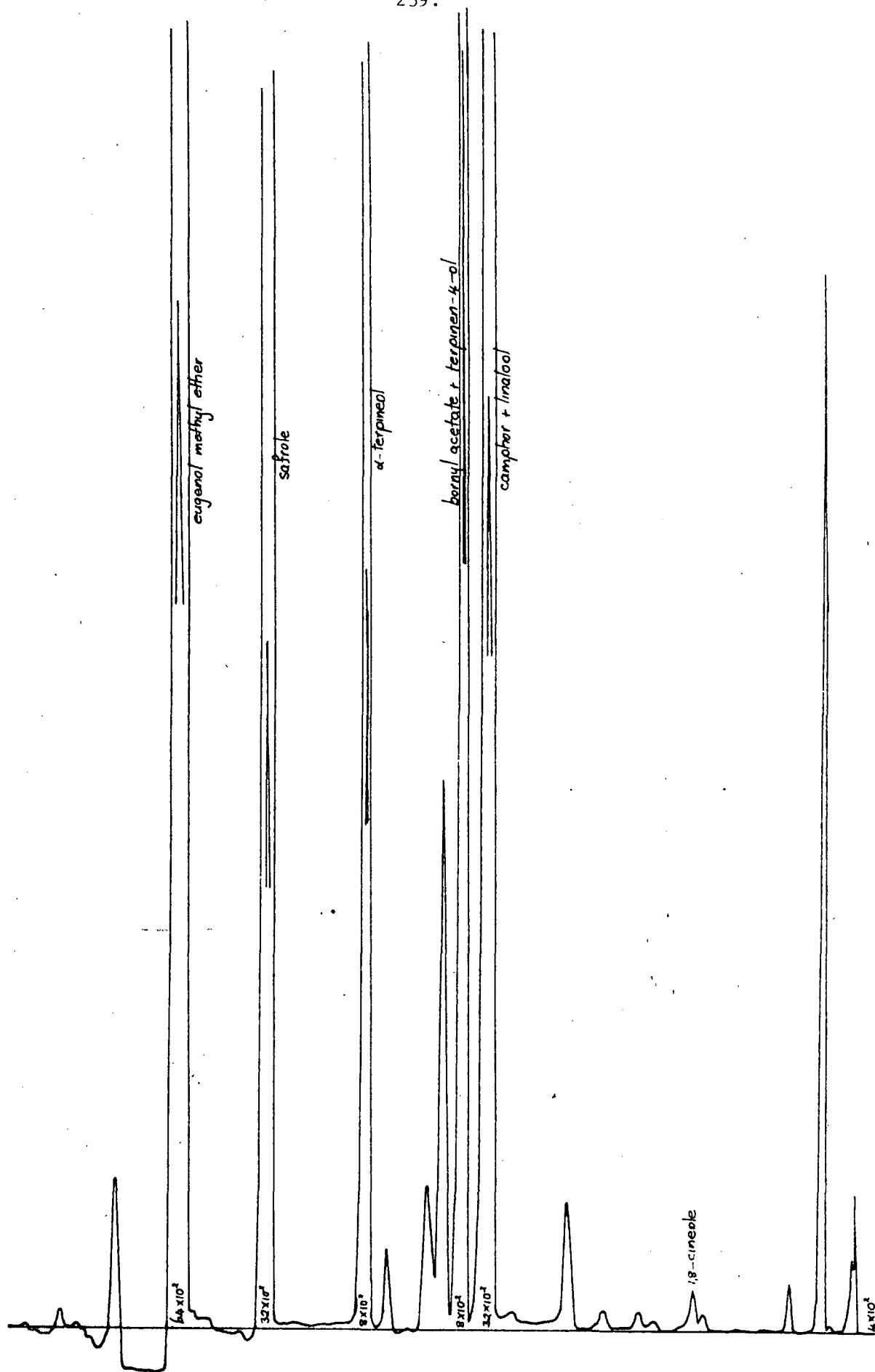
Fig. 28(f). High sensitivity gas chromatogram of oxygenated fraction of oil of *Atherosperma moschatum* separated on Florisil.

(iii) Experimental

Details of sampling, isolation of oil, separation of components and analysis are as previously described. The oil was isolated from leaves in a 1.66 percent wet weight yield. A 10.0 g portion of the oil was chromatographed on Florisil, yielding 0.43 g of a hydrocarbon fraction and 8.20 g of oxygenated fraction.

(iv) Summary

Components of steam-distilled leaf oil of *Atherosperma moschatum* were analyzed by chromatographic and spectroscopic methods. The following identifications were confirmed: α-pinene (1.3%), camphene (5.0%), limonene (0.7%), γ-terpinene (<0.1%), ρ-cymene (0.4%), linalool + camphor (18.2%), α-terpineol (3.3%), safrole (13.8%) and eugenol methyl ether (49.5%). Tentatively identified were β-pinene, myrcene, α-phellandrene, β-phellandrene, 1,8-cineole, terpinolene, terpinen-4-ol and bornyl acetate.

C. Feasibility of a routine analytical procedure for a survey of essential oils

A simple sequence of techniques has been found suitable for the routine analysis of major, most minor and even some trace components, in a survey of essential oils. The procedure involves steam-distillation of the plant material, separation of the oil by column chromatography into hydrocarbon and oxygenated fractions, further separation and isolation of

pure components by preparative GC, with final identification
by IR spectroscopy and analytical GC on dissimilar columns.
Chromatograms were necessary before and after each treatment
of the oil to monitor any chemical alteration of components.

This rapid routine analytical procedure is recommended
as an initial step in the investigation of a new essential oil.
It should then be followed by a more detailed study of the
components in the oil using a more complex macro-scale
fractionation procedure.

SYRINGE-HEADSPACE GC ANALYSIS: A NOVEL APPROACH

IN THE STUDY OF ESSENTIAL OIL COMPOSITION

1. Introduction

A need exists for the measurement of terpenoid vapours at the moment they are released to the atmosphere from plant tissues, and similarly, in the proportions in which they are detected in the air by attracted insects.

The direct measurement of suspected short-term changes of terpenoids with respect to one another, i.e. within a period of a few hours, could be a further technique of value in the study of terpene biosynthesis. As one of the basically different approaches, which are needed for the study of terpene biosynthesis [357], instantaneous or direct measurement has not been attempted because of the absence of a suitable technique for monitoring the individual concentrations of terpenoid vapours as they are released to the atmosphere. Most direct-sampling techniques have involved destruction of the cellular enzyme system, i.e. by heating, solvent extraction, etc. Head-space GC techniques that would have enabled repeated sampling over a period of time have usually allowed only very dilute vapours to be measured. Measurement of the headspace composition in a culture vessel would be confused by the diffusion into the headspace of terpenoid vapours condensed onto the container surface.

Loper and Webster [181] attempted the measurement of volatiles released from flowers of *Medicago sativa*, and tried to overcome the problem of too dilute vapours by using an ice-trap condenser. The condensate was injected onto a capillary column and led to a predictable problem with water vapour. Unfortunately the techniques in this study also involved a 30 min. vapour-equilibration period in a heated chamber. This system would not have enabled direct measurement of released vapours at intervals of less than 2.5 hr. (2 hr. for GC elution), reproducibility of RRT data due to the effect of water in a capillary column, nor would it have allowed measurement of water-masked components eluted within the first 25 minutes. These workers did however detect qualitative and quantitative changes over an 8 hr. period.

The development of a suitable technique could enable the relationships of released terpenoid vapours to be studied with respect to one another, and hence correlations to be inferred as to possible biosynthetic pathways. Previously, the study of time-course changes has been restricted to those occurring during plant maturation [322, 325].

The absence of a suitable technique has also prevented the study of terpenoid vapours in the proportions to one another in which they may be detected by insects attracted to the host plant. In all previous studies there has been some feature, usually a concentrating technique, which changes the terpenoid composition. By contrast, solid-injection GC involves the volatilization of a higher proportion of higher-boiling components,

whereas adsorption onto activated carbon involves a disproportionate trapping and selected release of components during later analysis.

Even though most workers have been satisfied with a technique for studying insect attractants that enabled vapours from attractive and unattractive plants to be examined under standardized conditions, the vapours measured could quite easily have been collected with the exclusion of the actual attractant(s). Many workers have been forced through necessity to compare the attractiveness of steam-distillates, which could be expected to have even completely lost a volatile component, particularly if it is more volatile than α-pinene.

If the insect attractant is a minor component that only occurs for a short period of time, e.g. following tree injury, it might not be found in a steam-distillate, solvent extract, condensed vapour, etc. A technique is therefore required which can also monitor the temporary release of an insect-attractive component in the vapour from plant tissues.

A study is presented of a technique for the analysis of terpenoid vapours at the moment they are released from plant tissues.

2. Principle

Comminuted oil-containing plant tissue is packed into a gas-tight syringe at room temperature, and the terpenoid vapour is immediately injected into the packed column of a gas chromatograph.

3. Experimental

(1) Determination of optimum sample size

From a series of injections of α-pinene headspace vapour onto a 2 m × 4 mm ID packed GC column, using 1, 5, 10 and 50 ml gas-tight syringes, it was found that the largest practicable volume that could be injected was between 8 and 10 ml. Larger volumes involved splitting of the sample leading to a number of peaks from a single component.

The minimum practicable sample of pure α-pinene headspace vapour was obtained with an injection of 0.05 ml, which produced a signal of 40 percent FSD. This value was recorded at an attenuation setting designed to produce a 0.5 percent FSD background on the baseline. The GC and Carbowax 20M column conditions were as described earlier.

A further series of headspace injections, of the vapour from comminuted foliage of a *Thuja* sp., showed that the most sensitive and practical chromatogram was obtained with a 1 g sample of plant tissue (2 ml volume) in a 10 ml syringe (Figure 29), from which an 8 ml injection volume was delivered into the GC column. It was also found that the intensity of the GC peak depended closely upon the volume of air injected through the plant material (Figure 29), rather than upon the amount of plant sample in the syringe (Table 41). With the recognition of this relationship it was apparent that the syringe could where necessary be loaded with a sufficiently small amount of plant sample, i.e. 1.0 g or less, and thereby provide injections with similar sensitivity yet minimal effects
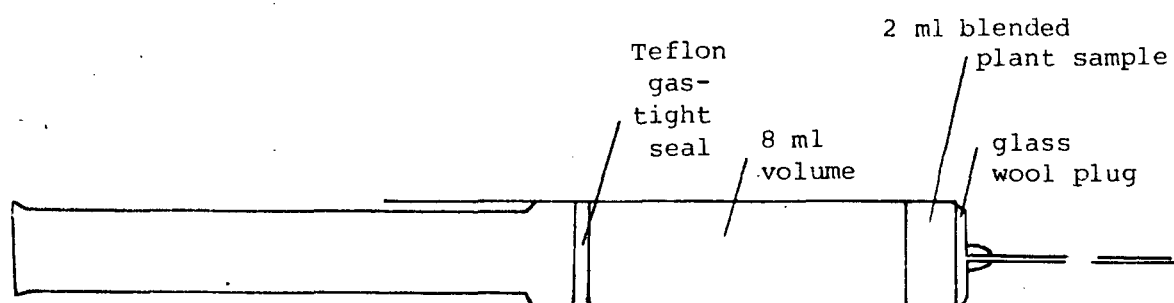
Fig. 29(a).   10 ml gas-tight syringe containing 2 ml (1 g) of blended
plant tissue ready for injection of 8 ml of syringe headspace onto a
GC column.
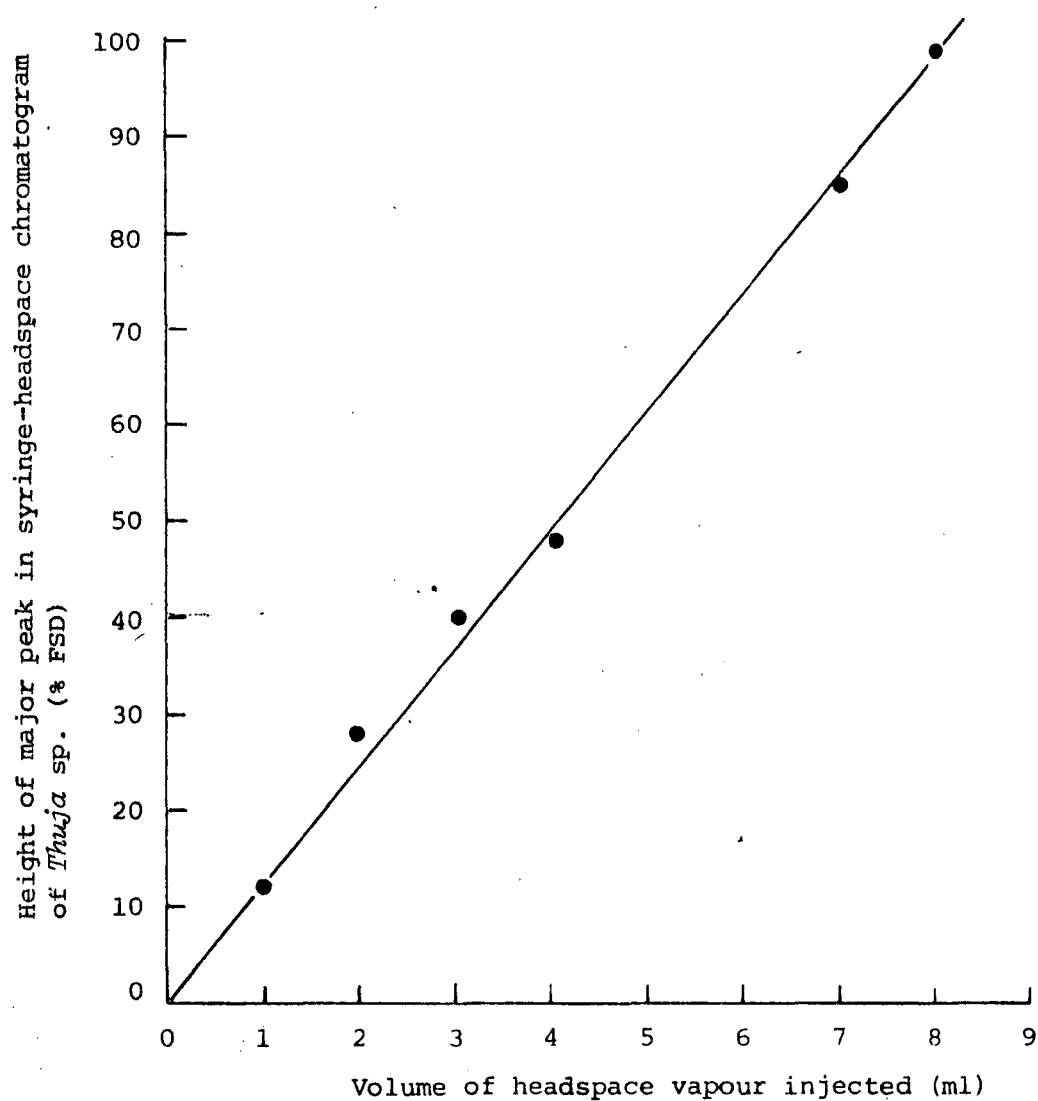
Fig. 29(b).   Relationship between sensitivity of GC peak and the
volume of injected headspace.

due to water vapour. Components down to about 0.1 percent of the total vapour composition were detected in most materials

Table 41. Height of a major GC peak obtained on injecting various volumes of air through different amounts of blended plant material in a 10 ml gas-tight syringe.

Volume injected (ml)	Weight of plant sample (g)	Height of GC peak (%FSD)
1.0	0.1	12
2.0	0.4	28
3.0	0.4	40
4.0	0.4	48
7.0	0.6	85
8.0	1.0	99

(ii) Use of RRT data for tentative identification of terpenoids in syringe-headspace GC analysis

The dual dissimilar column system, 5% Carbowax 20M and 5% OV-17, as described previously for conventional analytical GC, was found to be just as convenient for the identification of components during syringe-headspace GC analysis (Table 42). Each column was injected with syringe headspace vapour samples from plant material that was simultaneously studied by conventional steam-distillation, GC and IR techniques. Standard α-pinene and camphor vapour reference materials were injected as before, enabling a calculation of RRT data.

Table 42. Relative retention times on dissimilar
columns of terpenoids injected using
the syringe-headspace technique.
RRT data for conventional liquid
injections are also given for comparison.

Component	No. of injections	SYRINGE-HEADSPACE GC				LIQUID-INJECTION GC			
		RRT on C20M	SD	RRT on OV-17	SD	RRT on C20M	SD	RRT on OV-17	SD
		(60° isothermal, ref. α-pinene)				(TP 50° to 200°, 5°/min)			
α-Pinene	15	1.00	±0.01	1.00	±0.01	1.01	±0.03	1.01	±0.02
Camphene		1.29		1.18		1.30	±0.04	1.20	±0.02
β-Pinene	14	1.63	±0.02	1.54	±0.02	1.62	±0.04	1.56	±0.03
Δ₃-Carene	7	2.07	±0.07	1.92	±0.04	2.06	±0.07	1.99	±0.07
Myrcene	14	2.30	±0.04	1.72	±0.03	2.24	±0.05	1.75	±0.03
Limonene	10	2.84	±0.04	2.33	±0.04	2.76	±0.06	2.38	±0.04
β-Phellandrene		2.99		2.47		2.93	±0.09	2.54	±0.07
γ-Terpinene		3.86		3.25		3.64	±0.09	3.25	±0.06
Terpinolene		4.89		4.07		4.58	±0.09	4.10	±0.05
Thujone		10.7		6.44		11.6	±0.5	6.6	±0.1
Isothujone		12.1		7.09		12.6	±0.6	7.1	±0.3
		(130° isothermal, ref. camphor)							
Terpinen-4-ol		1.24		1.00		1.24	±0.04	0.98	±0.04
Charicol methyl ether		1.52		1.21		1.58	±0.03	1.27	±0.05
Citronellyl acetate		1.62		1.91		1.58	±0.04	1.97	±0.07

(iii) Compositions of successive injections of syringe-
        headspace vapour

(a)   Quantitative changes in composition

It was found that a single charge of plant material
in a syringe could be used as a source for successive injections
of headspace vapour. Continual saturation of the syringe-
headspace by terpenoid vapours enabled the same charge to be
used repeatedly for several hours without diminution of the
terpenoid source. Figure 30 contains a series of chromatograms
of *Pinus radiata* foliage headspace injected over a 3 hr. period
without loss of sensitivity.

Care should also be taken to ensure that the oil
vapour in a comminuted sample is not allowed to volatilize into
the atmosphere and result in an initial injection of vapour that
is deficient in the more volatile components. A small loss of
vapour will be inevitable. The following table shows the
compositions of initial injections of vapour, found from portions
of comminuted foliage of *Pinus sylvestris*, when injected at
various times following comminution and exposure to the
atmosphere.
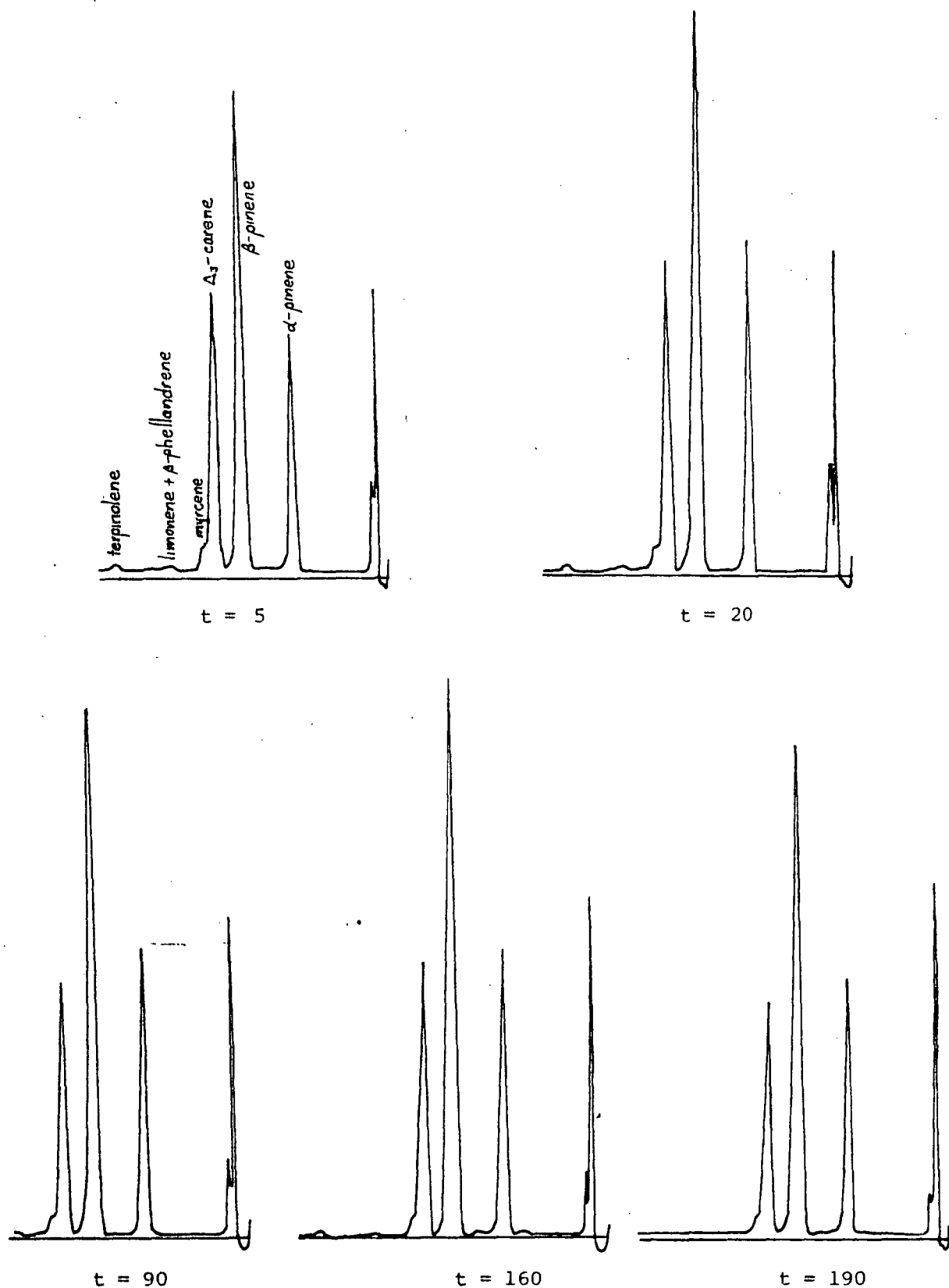
Fig. 30. Chromatograms of successive injections of syringe-headspace vapour over a 3 hr. period without reduction in peak sensitivity (t represents time in minutes since comminution of sample of foliage of *Pinus radiata;* GC on 5% Carbowax 20M/Gas Chrom Q, temperature program from 50° at 5°/min; injection 8 ml headspace through 2 ml comminuted foliage in 10 ml gas-tight syringe; attenuation 16 x 10^2).

Component	Time since homogenization and exposure to atmosphere (mins.):			
	0	150	170	185
Unidentified group	-	0.4%	0.1%	0.3%
α-Pinene	40.9	33.7	32.8	29.5
Camphene	1.1	1.1	1.0	0.9
β-Pinene	3.3	2.9	2.9	2.8
Sabinene	1.4	1.3	1.2	1.5
Δ_3-Carene	39.5	44.6	46.0	47.7
Myrcene	10.3	11.1	11.2	11.8
Unidentified	0.3	0.4	0.3	0.3
Limonene	0.2	0.4	0.3	0.3
β-Phellandrene	0.4	0.6	0.4	0.4
γ-Terpinene	0.2	0.3	0.3	0.3
Unidentified	0.7	1.0	1.0	0.9
ρ-Cymene	-	t	t	0.3
Terpinolene	1.7	2.4	2.6	3.1

It can be seen from this table that there is a greater loss of the more volatile components, with an apparent increase in those that are not as volatile.

Comparison of the monoterpene compositions in chromatograms produced from successive injections of syringe-headspace vapour from a single sample (Table 43) shows that there are changes in the relative proportions of released terpene vapours (Figure 31). A study of the release of monoterpenes from foliage of *Pinus ponderosa* and other species also indicated changes in relative proportions of components in successive injections over several hours. To confirm that the initial compositional changes in the first hour were not the result of experimental error, a further sample of foliage was similarly treated and found to follow the same initial course (Figure 32).

Table 43. Compositions of monoterpenes in successive injections of syringe-headspace vapour over a 3 hr. period, from a single charge of *Pinus radiata* foliage

Time since comminution of sample (min.)	Sum of all peak heights per injection (mm.)	Percentage composition of monoterpenes:		
		α-pinene	β-pinene	Δ_3-carene
5	172	23.8	48.3	27.9
20	207	27.6	46.5	25.9
40	166	27.8	47.7	24.5
55	153	26.9	48.9	24.3
75	163	27.3	49.1	23.6
90	184	27.0	49.3	23.7
115	160	26.9	50.0	23.1
130	155	27.1	50.0	22.9
145	188	26.4	49.9	23.7
160	192	25.6	49.9	24.6
175	179	25.8	49.9	24.4
190	167	26.1	50.0	24.0

Fig. 31.   Graphical representation of changes in proportions of
monoterpenes in successive injections of syringe-headspace vapour
from a single charge of comminuted *Pinus radiata* foliage.   The
total GC response for all monoterpenes also indicates fluctuations
in the amount of oil vapour.
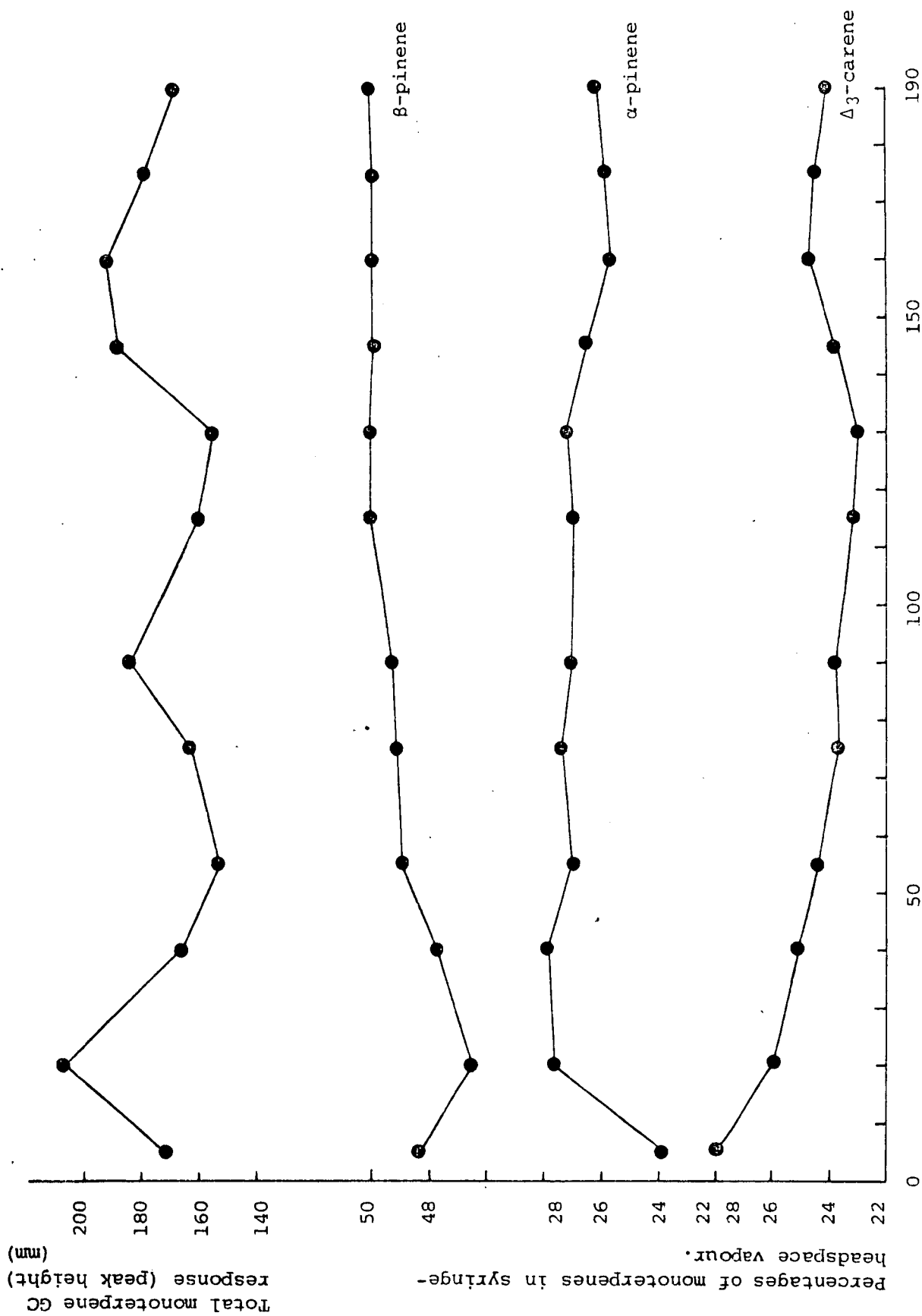
Fig. 32(a). Graphical representation of changes in proportions of monoterpenes in successive injections of syringe-headspace vapour from a single charge of comminuted *Pinus ponderosa* foliage (I). A further sample of foliage from the same tree was similarly found to follow the same initial course of terpene changes (II).
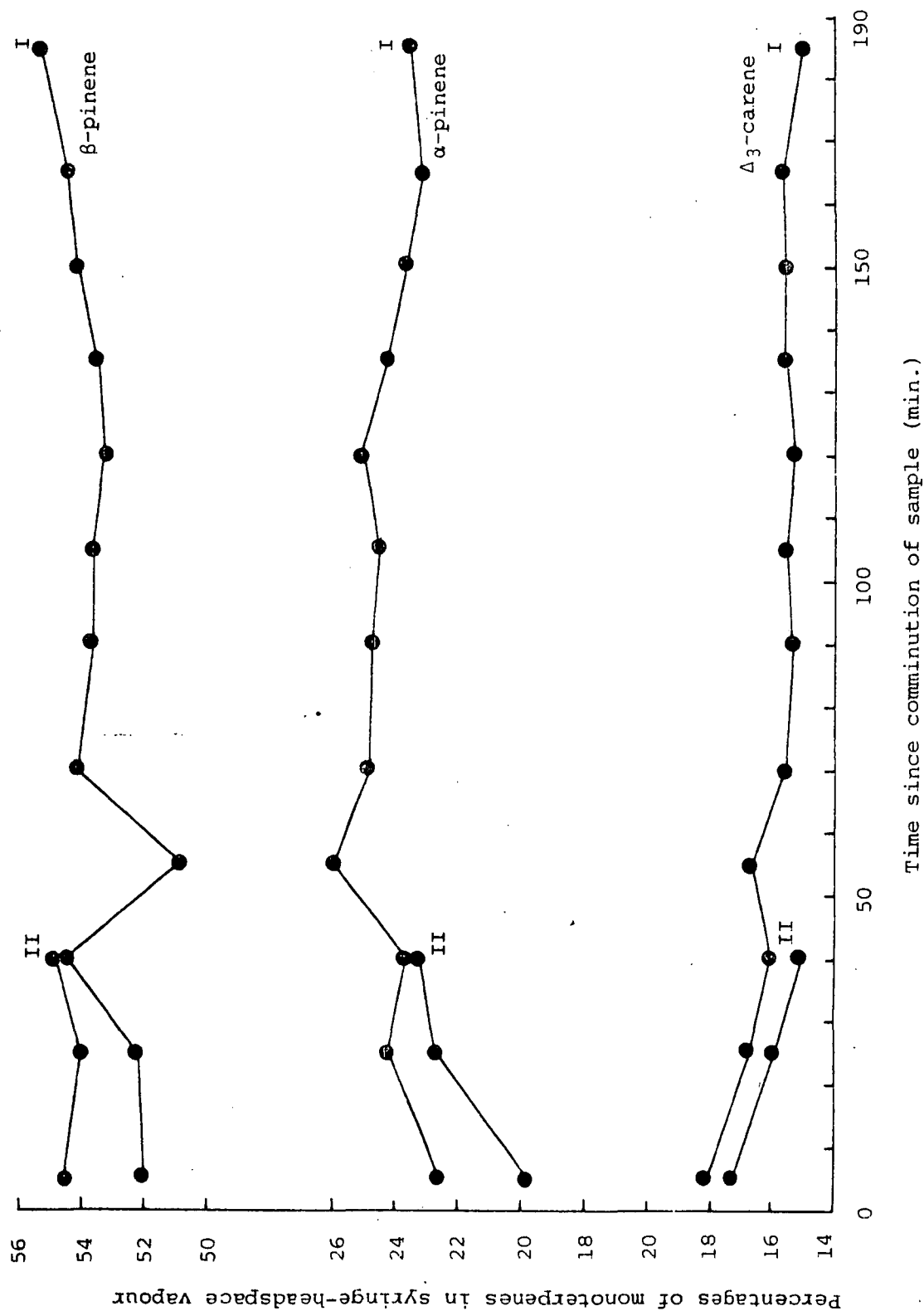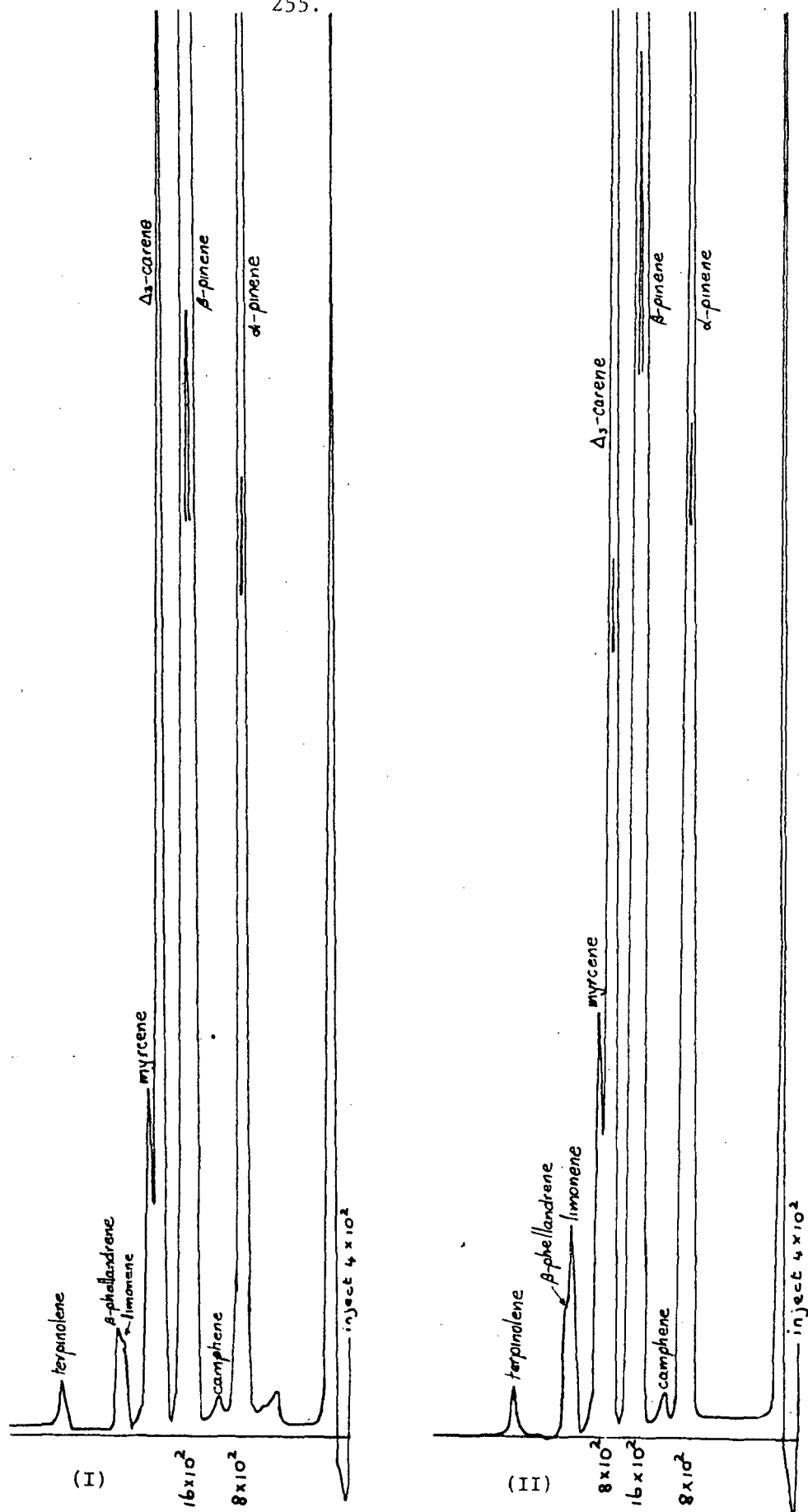
Fig. 32(b).   Gas chromatograms from initial injections of syringe-headspace vapour from foliage of *Pinus ponderosa*.   Each chromatogram (I and II) is from a different sample of foliage from the same tree and corresponds to the graphs shown in Fig. 32(a) (GC conditions as before).

(b) Qualitative changes in composition

Successive injections of syringe-headspace vapour from a single charge of plant material were found to also include apparent qualitative changes in monoterpene composition. Figure 33 shows a series of chromatograms in which a component was present in the initial vapour, following comminution of the foliage tissue, but failed to appear in subsequent injections. Another component, however, made a later appearance.

Table 44 illustrates the use of the syringe-headspace technique to tentatively identify the vapourized components in a sample of *Boronia citriodora* available from a botanical gardens source as a sprig off a 15 cm. seedling. Two sets of quantitative data indicate the composition changes which ensued between the first and second vapour injections.

(iv) Comparison of compositions of syringe-headspace vapour and steam-distillate

A comparison of the terpenoid composition of steam-distilled oil and the syringe-headspace vapour showed, as expected, that the former contained a higher proportion of high-boiling components. Figure 34 is a chromatogram of steam-distilled oil obtained from the remainder of a single sample of comminuted foliage of *Cedrus deodara*. The composition of the syringe-headspace vapour of this sample is indicated in Figure 33.
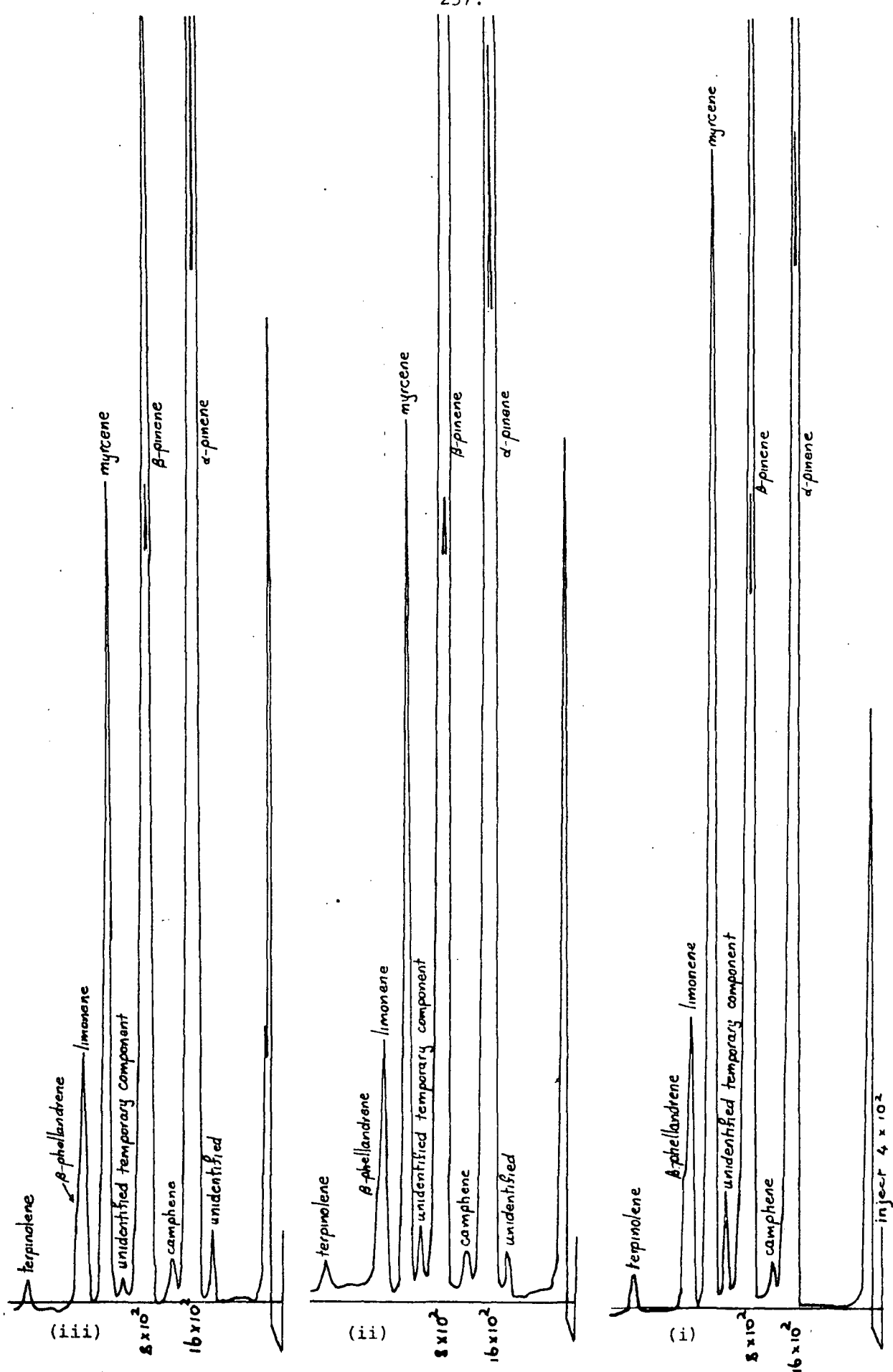
Fig. 33(a). Syringe-headspace chromatograms of foliage of *Cedrus deodara* (tree II). Chromatograms (i) to (iii) were from injections at 15 min. intervals (GC conditions as before). In the initial injection, (i), an unidentified 'temporary component' is seen to eventually disappear.
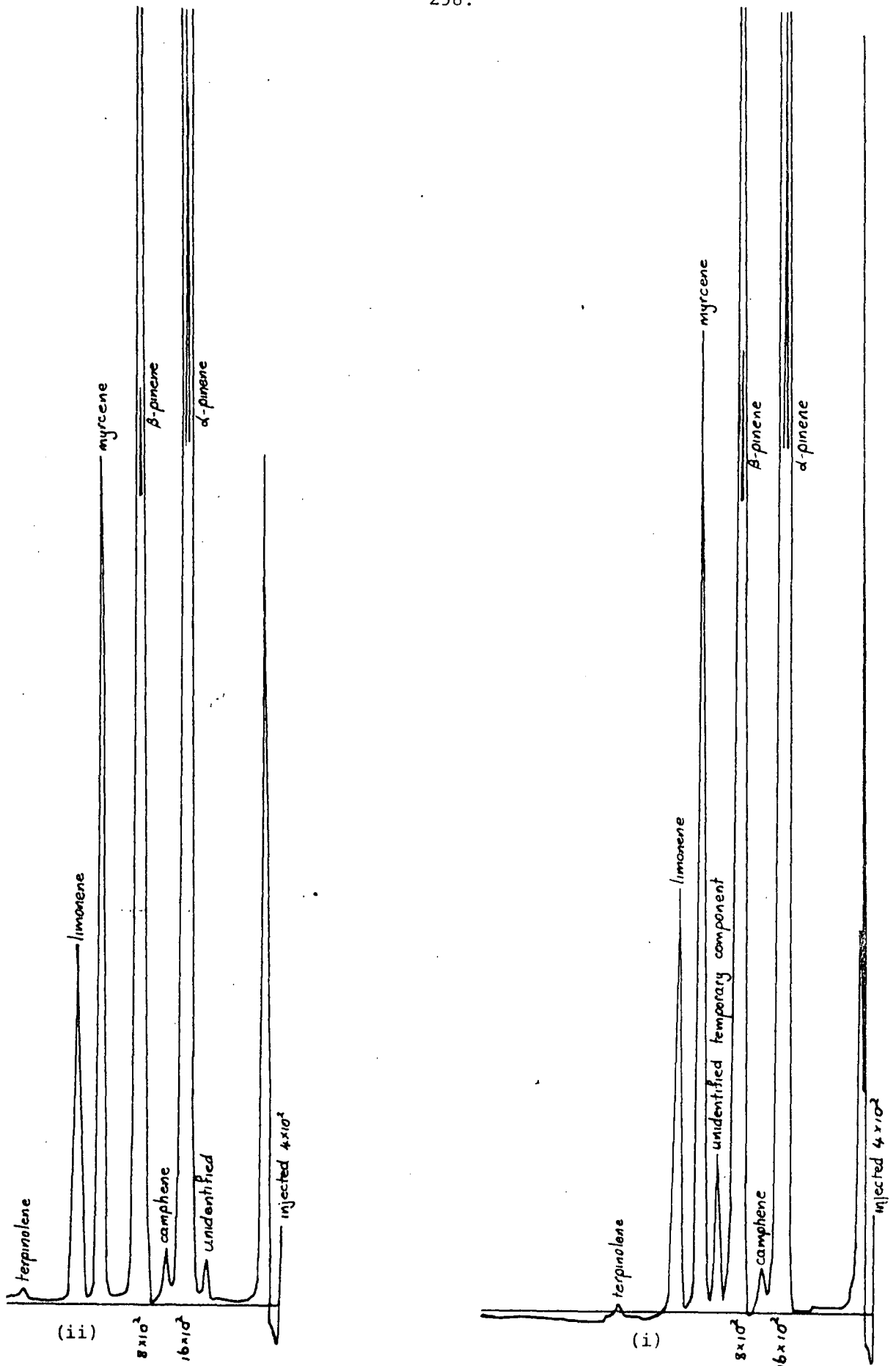
Fig. 33(b). Syringe-headspace chromatograms of foliage of *Cedrus deodara* (tree I). Chromatograms (i) and (ii) were from injections 40 min. apart (GC conditions as before). The unidentified temporary component seen in vapour of tree II is not apparent in tree I after only 40 min.

Table 44. Use of the syringe-headspace technique
to analyse the vapour from a sprig of
Boronia citriodora. The composition
is given of each of the first two
successive injections of vapour to illustrate
the qualitative and quantitative changes that
may occur

Component	Qualitative RRT data		Quantitative composition (percent by peak height)	
	C20M	OV-17	Injection I	Injection II
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min)	
Unidentified	0.65		2.8	t
"	0.77		1.4	t
Tricyclene	0.91	0.93	t	0.6
α-Pinene	1.01	1.00	5.2	7.7
Camphene	1.31	1.19	3.1	6.0
Sabinene (+ β-pinene	1.81	1.53	6.9	8.0
Unidentified (+ Δ3-carene)	2.04	1.96	3.8	0.3
Myrcene	2.35	1.73	3.5	4.4
Limonene	2.85	2.31	37.8	57.5
(130° isothermal, ref. camphor)				
Unidentified	0.55	0.57	t	t
Thujone (+ isothujone)	0.77	0.77	5.6	2.2
Terpinen-4-ol	1.26	1.05	t	t
Caryophyllene	1.40	2.46	8.7	6.6
Citronellyl acetate	1.61	1.90	16.7	5.7
α-Terpineol	1.87		3.1	0.9
Citronellol + Geranyl acetate	2.40		1.4	

Fig. 34.   Chromatogram of steam-distilled oil from portion of comminuted foliage of *Cedrus deodara* (tree I) used to produce the syringe-headspace chromatograms of Fig. 33(b).   The steam-distillate contains a higher proportion of high-boiling components, but no sign of the unidentified temporary component.
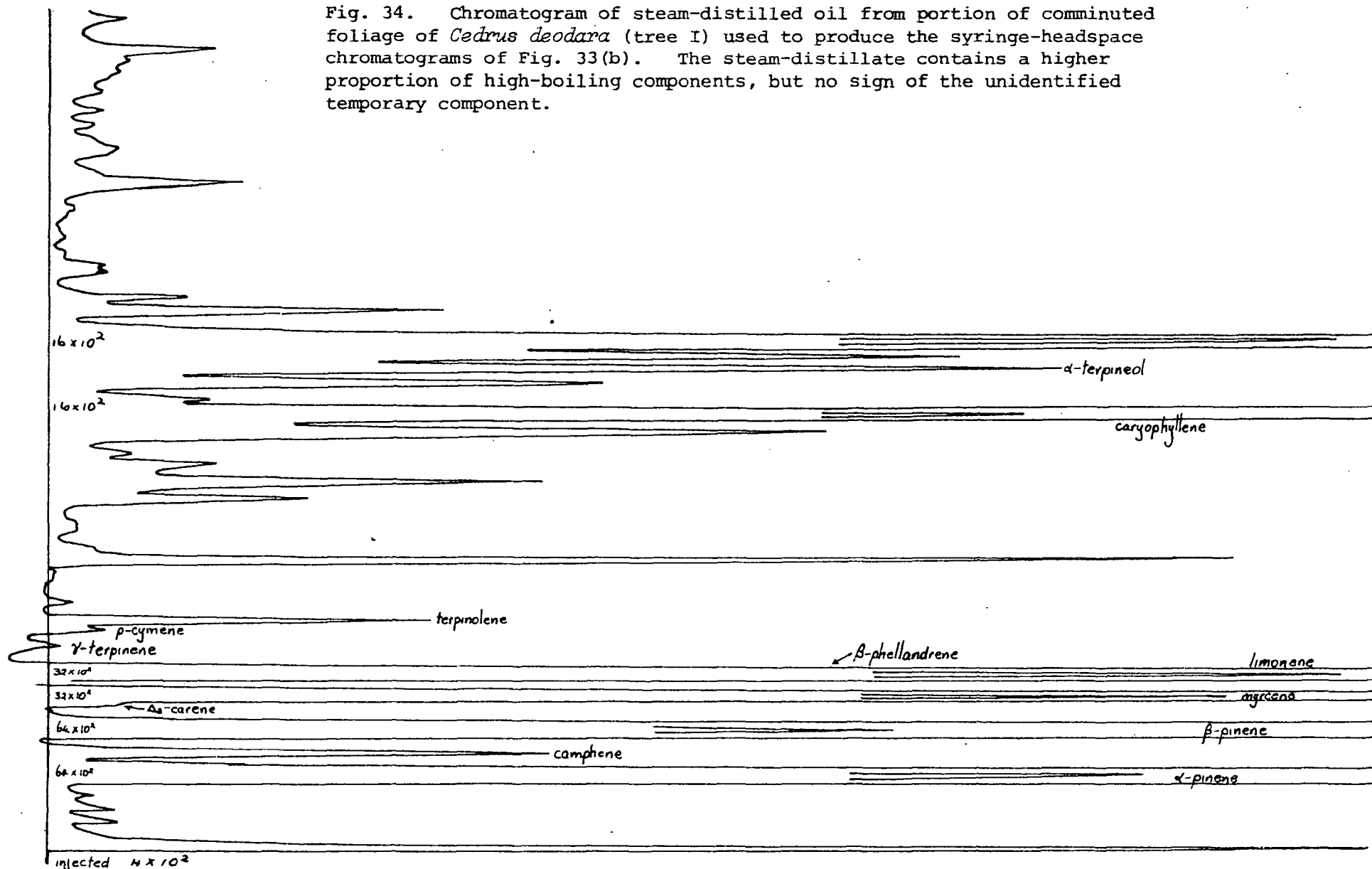
4. . Discussion

Analysis of the monoterpene composition of syringe-headspace vapour would appear to be just as valid a method of estimating the terpenoid composition of plant material as any other procedure, i.e. involving oil isolation or direct-sampling. Since the method involves a direct measure of terpenoids which have volatilized at room temperature, the vapour composition was found, as expected, to contain a much higher proportion of monoterpenoids.

The arbitrary nature of syringe-headspace vapour measurement is similar to that of other procedures. For example, steam-distillation is selective for vapours under a different set of conditions, and as a result yields an oil containing a much higher proportion of more volatile components than would have been isolated in a solvent-extracted oil.

A principle criterion for selection of the appropriate technique for isolating an oil is whether or not it yields an oil composition that contains the component or feature of interest. In the cases of steam-distillation and solvent-extraction, which yield many more terpenoids from a sample of plant material, it is problematical whether a temporarily-produced component will be isolated. It would also be difficult to follow, over a short period, the time course of changes in terpenoid composition using one of these other techniques.

Syringe-headspace GC analysis would appear, with some reservations, to meet present requirements, i.e. to provide analysis of components as they are released to the atmosphere.

Further investigations are however required to determine
whether the oil vapour composition being instantly analyzed
is really the composition experienced by the insect from
uninjured plant tissue. It should be noted that there is
evidence [331, 332] to suggest that some insects are more
attracted to certain injured plant tissues. It should also
be determined whether comminution has an immediate effect upon
oil vapour composition.

The advantage of the technique for qualitative
analysis of components in a sample available only in limited
quantity was illustrated with the analysis of vapour from a
sprig of *Boronia citriodora*. The technique was found in such
samples to be as well suited to the identification of monoterpenes
as the conventional analytical GC of isolated oils. In this
respect the technique would be superior to most solid-sample
injection procedures, since column reproducibility and life is
impaired by the repeated need to dismantle and remove an initial
section of packing that contains accumulation of catalytic
material.

The syringe-headspace technique compared favourably
with the technique of Loper and Webster [181], in which a 70 ml
sample of headspace is concentrated in a condenser, with the
consequent injection of a considerable amount of water vapour.
By injecting 8 ml or less of headspace, no water peak was
produced which obscured the chromatogram.

To determine the significance of the changes of each
monoterpene as a percentage of total monoterpenes, a systematic

study should be made of similar changes in a number of plant species. Care should be taken in such a study to ensure that individual changes, and correlated changes between components, are not arithmetic variations that result from a percentage reduction in one component when another has a percentage increase.

5.   Summary

A technique described as syringe-headspace GC analysis was investigated as a means of instantly analyzing terpenoid vapours at the moment they are released to the atmosphere at room temperature. Plant material is comminuted to the consistency of tobacco, and charged into a gas-tight syringe, from which a few ml of syringe-headspace is injected onto a GC column. The technique was shown to be convenient for the tentative identification of terpenoids on a dissimilar GC column system. Chromatograms are mainly of monoterpenoids. Successive injections of vapour from a single charge of comminuted foliage of *Pinus radiata* and *P. ponderosa*, enabled the measurement over a period of a few hours of changes in monoterpenoid composition. Qualitative changes were also detected in the monoterpenoid vapours of comminuted foliage of *Boronia citriodora* and *Cedrus deodara*.

REFERENCE TERPENES FROM NATURAL SOURCES BY THE

SYRINGE-HEADSPACE GC TECHNIQUE

1. Introduction

Investigations of essential oils are commonly
hindered by problems associated with authentic reference
terpenoids. The availability of pure reference compounds of
known structure and stereochemistry is a factor which limits
the value of the combined GC-MS technique [230]. Quite often
authentic terpenes are found to be mixtures. Many cannot be
stored in a pure state because they are subject to
autoxidation.

The identification of an unknown component in an
essential oil is frequently handicapped by the absence of a
reference terpene with the structure predicted from properties
of the unknown. This is particularly the case when the full
advantages of an instrumental investigation require the worker
to only operate with micro-quantities. In a subsequently more-
detailed investigation, where a large amount of oil has been
fractionated, major components are usually isolated in a pure
state in sufficient quantity to allow identification by both
spectroscopic and classical derivative techniques.

Even in a more-detailed investigation there are also
minor components which must still remain tentatively identified
by GC. Authentic terpene materials will then be required to
provide the basis for excluding alternative possible structures.

The finding of an alternative structure, with the same GC characteristics as the unknown compound, will then justify the investigator spending considerable time in either synthesizing the suspected compound or isolating it from a recognized natural source.

Wrolstad and Jennings [391] have recognized the problem and assisted with the publication of a list of recommended natural sources of monoterpene hydrocarbons. Other lists of natural sources may also be found in any of the national pharmacopoeias.

From the previous description of the syringe-headspace GC technique, in which it was shown that very precise RRT data may be produced, it would appear that this technique could render a collection of plants in a botanic gardens readily accessible as a source of reference terpenes.

A study is presented in which two terpenoids were tentatively identified using recognized plant species as sources of reference terpenoids, that were injected in the syringe-headspace GC technique.

2. Results and discussion

(i) Sabinene from *Juniperus sabina*

β-Pinene in oils of several *Pinus* species was found to be eluted on a 5% Carbowax 20M column often with a small unidentified shoulder component. For example, foliage of *Pinus montezumae* contained in a syringe-headspace chromatogram a β-pinene peak, constituting ~7 percent of the total vapour, with

$RRT_{\alpha-pinene}$ 1.64(C20M) and 1.57(OV-17). The unresolved

shoulder on the β-pinene peak, constituting 1 percent of the

vapour, was recorded with $RRT_{\alpha-pinene}$ 1.75(C20M) and found.

indistinguishable on the OV-17 column. The shoulder component

was well separated on Carbowax 20M from subsequently eluted

Δ_3-carene and myrcene.

An examination of some of the available lists of

terpenoids resolved on Carbowax 20M indicated that the shoulder

peak could have been sabinene. Sabinene is known to occur as

a major component in several oils, including that of *Juniperus*

sabina. Von Rudloff [285] reported 30.5 percent sabinene in oil

of *J. sabina* and listed it as the major monoterpene.

A sprig of *Juniperus sabina* was obtained from a shrub

cultivated in the Royal Botanical Gardens (Tasmania), and was

shown in chromatograms obtained by the syringe-headspace

technique (Figure 35), as previously described, to contain the

expected major monoterpene. Data in Table 45, which shows RRT

values and percentage composition of *J. sabina* syringe-headspace

vapour, was used to tentatively identify the shoulder peak of

Pinus montezumae as sabinene. The RRT values also indicated

that the sabinene shoulder peak was actually unresolved from

β-pinene on OV-17.

(ii) Chavicol methyl ether from *Artemisia dracunculus*

A component isolated repeatedly from oils of *Thuja* and

Pinus species was indicated from its IR spectrum to have

characteristics in common with the structure of chavicol methyl

ether, i.e.

Fig. 35.   Gas chromatogram of syringe-headspace vapour
from foliage of *Juniperus sabina* (GC on 5% Carbowax 20M/
Gas Chrom Q;   temperature program from 50° to 200° at
5°/min;   8 ml headspace injection through ∿2 ml comminuted
foliage in 10 ml gas-tight syringe;   attenuation 4×10^2).
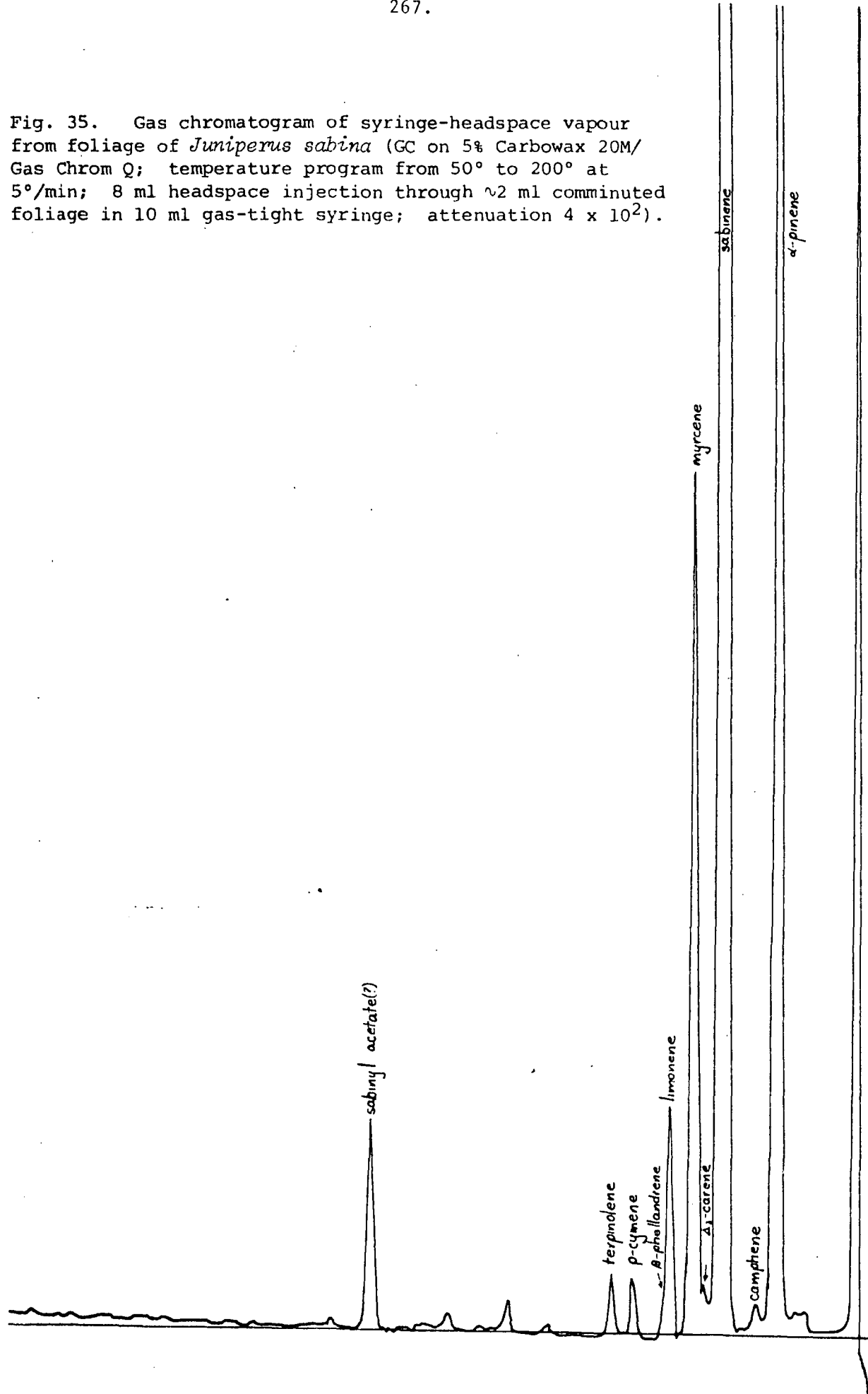
Table 45. RRT data and percentage composition
determined from syringe-headspace GC
analysis of foliage of *Juniperus sabina*

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak area)
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min)
α-Pinene	1.00	0.97	17.4
Camphene	1.28	1.16	0.1
Sabinene	1.77	1.53	72.0
Δ₃-Carene	2.09	1.89	0.1
Myrcene	2.32	1.71	5.8
Limonene	2.83	2.32	1.6
β-Phellandrene	2.96		0.3
p-Cymene	4.07	2.90	0.4
Terpinolene	4.81	4.06	0.4
Unspecified peaks at longer elution times:			0.2
			0.1
			1.5
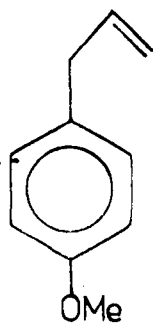
Before arranging for the purchase, isolation or synthesis of authentic chavicol methyl ether, it was decided to obtain further data which would either corroborate or discount the possibility of the unknown having this structure.

From the literature it was learnt that chavicol methyl ether constituted 60 to 75 percent of oil of tarragon from *Artemisia dracunculus* L. [392, 393].

A sprig of authentic *Artemisia dracunculus* was all that could be spared from a clone that had originated from the Royal Botanic Gardens, Kew (U.K.). The RRT values of the major component in syringe-headspace chromatograms (Table 46, Figure 36), showed that the unknown component was not distinguishable from chavicol methyl ether on either of two GC columns.

On the basis of both IR and GC syringe-headspace evidence there was considered to be good justification for spending time in synthesizing pure chavicol methyl ether to compare IR spectra and confirm its identification.

When attempting a tentative identification by the syringe-headspace GC technique, care should be taken to ensure the authenticity of the plant material. In the investigation of the natural source of chavicol methyl ether an initial chromatogram was produced that contained high-boiling components which could have been misleading. Although the plant sample was 'conclusively verified' by one botanist as *A. dracunculus*, a further authority confirmed that the plant was in fact *Artemisia dracunculoides*, which accounted for the misleading chemical composition (Table 47, Figure 37).

Table 46. RRT data and percentage composition
determined from syringe headspace GC
analysis of foliage of *Artemisia dracunculus*
used to tentatively identify chavicol methyl
ether in oil of *Thuja plicata*

Component	Qualitative RRT data		Quantitative composition
	C2OM	OV-17	(percent, based on peak height)
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min)
α-Pinene	1.00	1.00	1.1
Unidentified temporary component	(not measurable)		6.2
Limonene	2.81	2.40	3.4
Unidentified (a)	3.63	2.81	17.6
Unidentified (b)	4.03	2.98	17.1
(130° isothermal, ref. camphor)			
Major component [chavicol methyl ether]	1.53	1.28	54.1
Unidentified (a+b)	0.34	0.42	
Unidentified			0.6

RRT data of component isolated from oil of *Thuja plicata*

(130° isothermal, ref. camphor)

	(C2OM)	(OV-17)
Unknown	1.54	1.26

Conclusion:

The unknown component in oil of *Thuja plicata* is not distinguishable on either GC column from chavicol methyl ether in *Artemisia dracunculus*.

chavicol methyl ether

limonene

unidentified component not detected in subsequent injections
(elutes near Δ₃-carene as in Cedrus deodara)

d-pinene
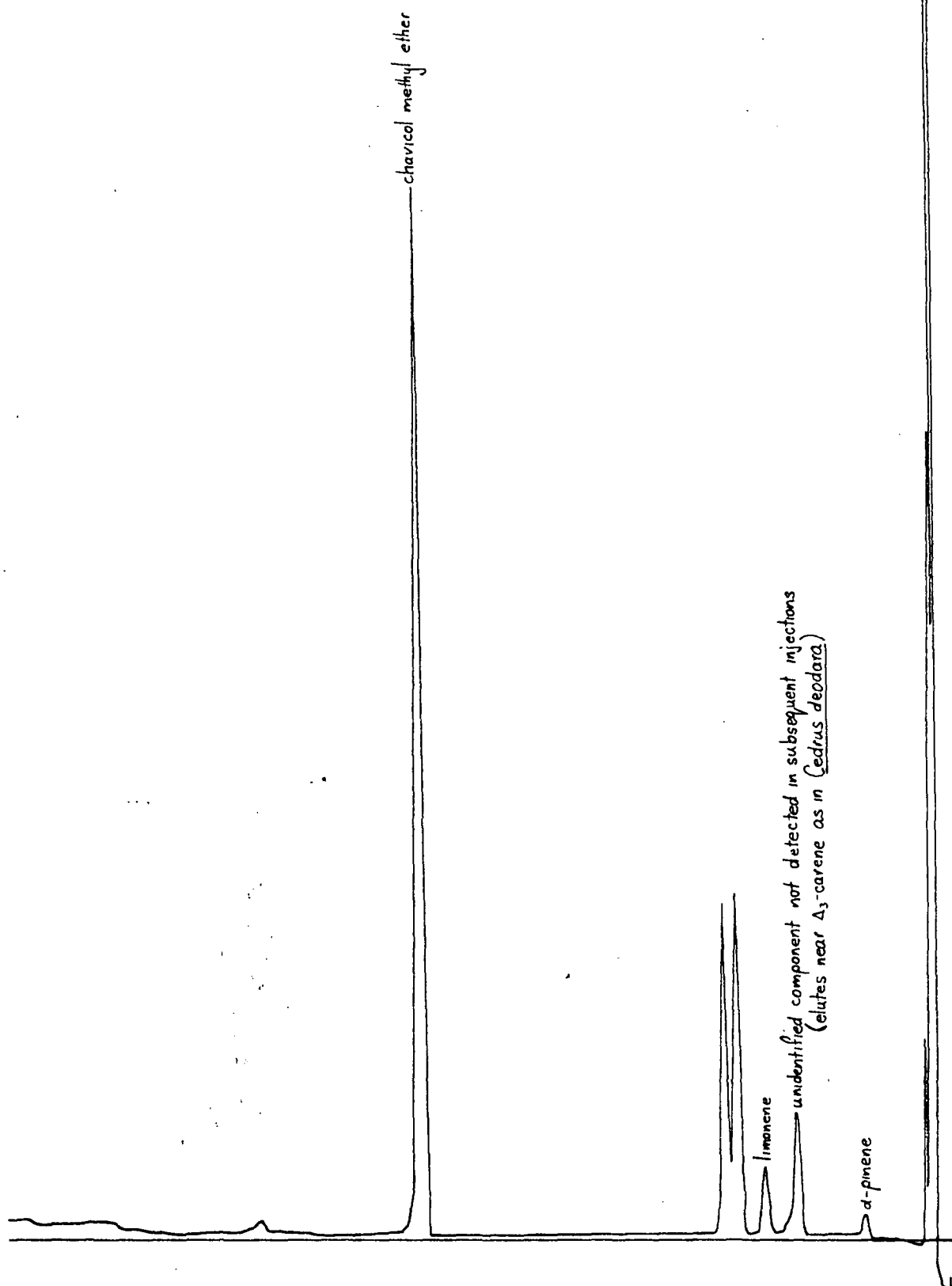
Fig. 36.   Gas chromatogram of syringe-headspace vapour from foliage of
Artemisia dracunculus (GC conditions as before).

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak height)
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min)
Unidentified	0.68	0.56	1.5
Tricyclene		0.91	1.5
α-Pinene	1.00	1.00	9.0
Camphene	1.27	1.19	20.0
β-Pinene	1.61	1.54	2.6
Sabinene	1.71	1.54	4.2
Limonene	2.69	2.35	3.2
β-Phellandrene	2.99	2.54	2.1
ρ-Cymene	4.15	2.82	3.6
Thujone (or isothujone)	12.0	6.85	28.7
Camphor	18.1	10.2	21.5
(130° isothermal, ref. camphor)			
Thujone (or isothujone)	0.74	0.79	
Camphor	1.00	1.00	
Unidentified	1.19	1.63	2.1

3. Summary

Syringe-headspace GC analysis of essential oil vapour released at room temperature from comminuted plant material, is recommended as a means of directly recording RRT data of terpenes in recognized plant sources. A botanical gardens collection may then be used as a source of reference terpenes, which could otherwise be difficult to obtain or store in a pure state. This technique provides GC evidence for the exclusion of numerous structural possibilities, and thereby enables a worker to justify spending considerable effort in synthesizing, isolating or purchasing an authentic terpenoid for direct comparison with an unknown.

Fig. 37.. Gas chromatogram of syringe-headspace vapour from foliage of *Artemisia dracunculoides* (GC conditions as before).
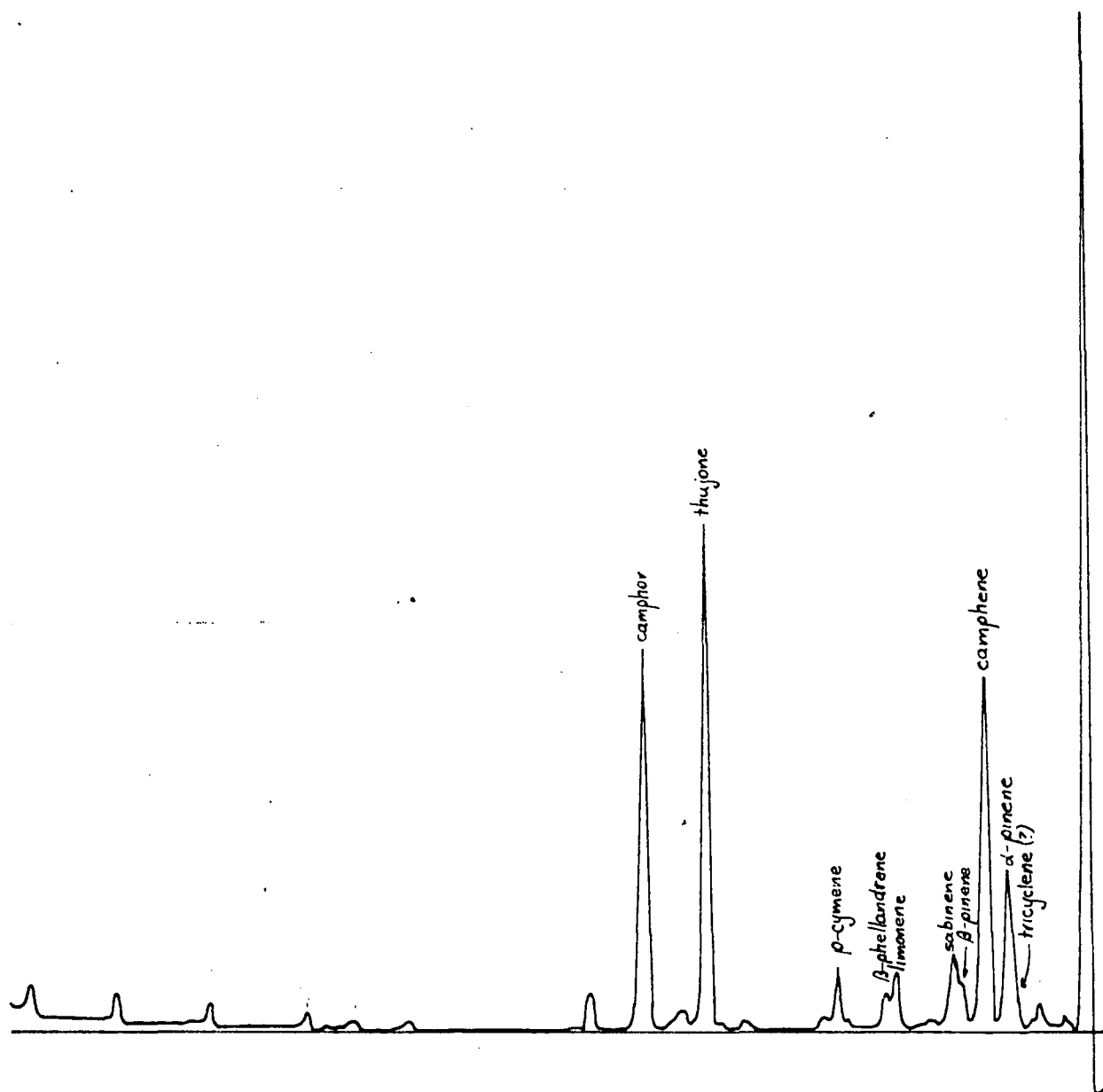
A COMPARATIVE STUDY OF VOLATILE TERPENOIDS OF

WOODWASP-ATTACKED CONIFERS USING CONVENTIONAL AND

SYRINGE-HEADSPACE TECHNIQUES

Introduction

Attack of conifers by the Siricidae is well known,
particularly that of *Pinus radiata* by the Woodwasp, *Sirex
noctilio* F. A comprehensive literature review of world-wide
attacks of conifers by Siricidae has been given by Wolf [394],
who also summarized the results of personal observations in
Belgium of specificity of *S. noctilio* for *Pinus nigra* var
austriaca and *P. sylvestris*. Many of the observations of
attacks reviewed by Wolf were from New Zealand, where a more
recent list has been compiled (Table 48)[395]. In Tasmania,
a description by Mucha [396] of the establishment and spread
of *S. noctilio* only contained reports of attacks on *P. radiata*.

Predisposition of trees to attack by Siricidae has
been given much attention. In Austria and West Germany the
susceptibility of *Picea* spp., *Abies alba* and some *Pinus sylvestris*
was found to be almost entirely confined to trees with logging
and deer barking injuries, or those which had been weakened
physiologically by disturbances of the water balance [397].
Madden showed the timing and duration of attack in Tasmanian
P. radiata to be correlated with the degree of stress undergone
by the host tree [332]. Felling was followed by immediate
attack with susceptibility for about 14 days. Lopping and

Table 48. List of conifer species in which *Sirex noctilio* has been found breeding in New Zealand [395].

Abies Mill.

   A. alba Mill.

   A. nordmanniana (Steven) Spach.

Cedrus deodara (Rox.) G.Don

Larix Mill.

   L. decidua Mill.

   L. leptolepis (Sieb. and Zucc.) A. Murr.

Picea Link.

   P. abies (L.) Karst.

   P. sitchensis (Bong.) Carr.

Pinus L.

   P. attenuata Lemm.

   P. banksiana Lamb.

   P. caribaea Morelet.

   P. contorta Dougl.

   P. echinata Mill.

   P. elliottii Engelm. **var** *elliottii*

   P. lambertiana Dougl.

   P. leiophylla.Schiede and Deppe

   P. montezumae Lamb.

   P. muricata D.Don

   P. nigra Arn. var *austriaca*

              var *laricio*

   P. palustris Mill.

   P. patula Schiede and Deppe

   P. pinaster Ait.

   P. ponderosa Laws. var *scopulorum*

   P. radiata D.Don

   P. strobus L.

   P. sylvestris L.

   P. taeda L.

Pseudotsuga Carr.

   P. menziesii (Mirb.) Franco.

Thuja plicata D.Don

girdling was correlated with attack 9 to 12 days later, but for longer periods than if felled. Some girdled trees even remained susceptible for more than one season. Defoliation by *Dothistroma pini* in New Zealand [399] appeared however to not predispose trees to attack.

The attraction of *S. noctilio* to *P. radiata* was concluded to be the result of an attractant released from the stems of physiologically stressed trees [331]. It was thought that the attractant was released when the supply of soluble solids was limited. Mucosecretion which the female wasp injected into the tree appeared to worsen the initial stress condition and resulted in attacks by more females. Further evidence of differences in tree chemistry of unattacked and woodwasp-attacked *P. radiata* was given by Hillis and Inoue [398], who noted considerable changes in the composition of polyphenols in sapwood, heartwood and knotwood. The release upon injury of larger quantities of ethylene, correlated with polyphenol production, may be a convenient means of distinguishing resistant from susceptible trees [401]. A chemical "mucous test" has also been claimed as a means of determining which trees were resistant to attack [400].

The attraction of insect pests to terpenoids is well-known. Since 1960 more than 150 reports appeared which described attraction of insect species, particularly to monoterpenes of various trees.

A study is presented of changes in terpenoids associated with the wounding of some conifers, together with a comparison

of the terpenoid compositions of essential oils and released
vapours from trees reported to have been attacked by *S. noctilio*.

A.   Results and discussion

(1)   Changes in monoterpene composition after wounding a tree

Several experimental trials were undertaken to confirm
the existence and nature of short-term changes in monoterpene
composition following injury to a tree. Attempts to correlate
terpenoid composition before and after injuring a tree were
thought to be complicated because an initial sampling of bark
would constitute an injury sufficient to render a tree attractive
to *S. noctilio*. Emphasis was therefore given to searching for
changes and attempting to show any influence of the nature of
the injury upon compositional changes.

(i)   Changes within a few hours in steam-distilled oil from

bark removed in a single operation

A single large sheet of bark was removed from the trunk
of a newly-felled *Pinus radiata*, from which representative areas
of bark were sampled and steam-distilled over a 7 hr. period.
Each sample of bark was sliced as quickly as possible and steam-
distilled until no further oil appeared in the distillate.
The efficiency of removal of turpentine from the pot was gauged
by the appearance of the rosin buttons, which lose their
stickyness and become quite brittle once turpentine has been
removed [47].

GC analysis indicated changes of as much as several percent for major components. Some minor components could not be detected in particular oil compositions. Although there was a possibility of qualitative changes occurring, the failure to detect a minor component was not evidence of its absence. Figure 38 contains gas chromatograms from capillary-column separations, that illustrate quite different monoterpene compositions found in bark both newly-removed and removed 4.25 hrs. The full list of data is given in Table 49.

Although quantitative changes were documented, most of the variations were well within the limits of experimental error. A principle feature was the initial difference between oils isolated within the first hour. One group of components increased in this period, i.e. β-pinene, Δ_3-carene and ρ-cymene, while another group appeared to decrease, i.e. α-pinene, camphene, myrcene, β-phellandrene and terpinolene. Little correlation could otherwise be seen between changes in groups of monoterpenes. The changes required verification and more detailed study.

(ii) Changes over a 14-day period in monoterpenes from bark intact on a felled tree

Representative samples of bark were removed daily from a felled *Pinus radiata* and steam-distilled. Several parameters were independently measured in each oil to confirm the existence of differences in composition. In addition other features were measured which might be correlated with changes in the chemistry of a felled tree, i.e. bark moisture content, percentage of
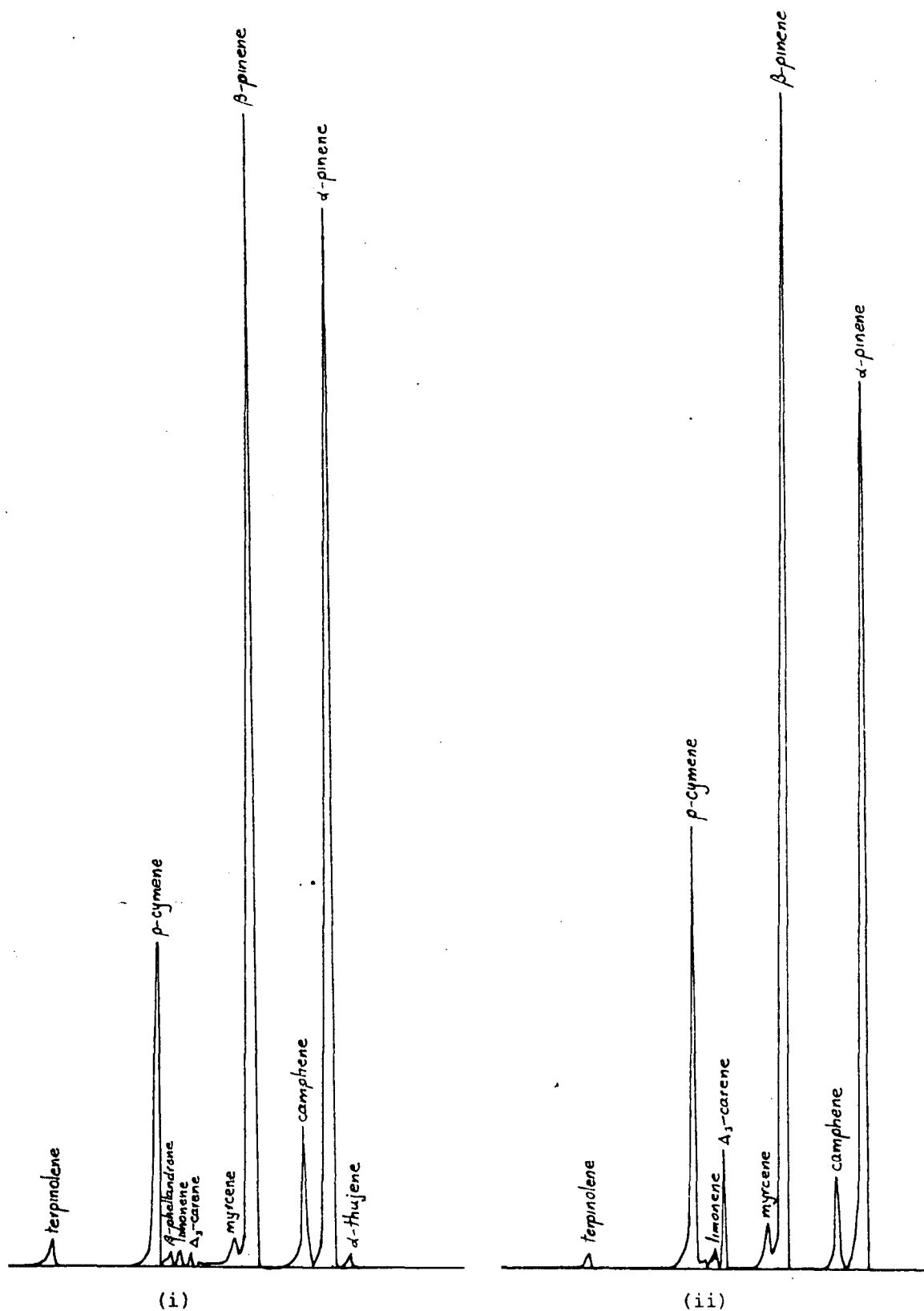
Fig. 38.   GC capillary-column (DC-200) chromatograms of steam-distilled oils from a single sheet of bark of *Pinus radiata*, sampled at 0.67 (i) and 4.25 hrs. (ii) after removal from the log.

Table 49. Compositions of oils steam-distilled from a single sheet of bark of *Pinus radiata* over a 7 hr. period

Time since removal of bark from tree (hrs)	0.67	0.92	2.50	3.17	4.25	5.67	6.67
Yield of turpentine (g/m^2 bark)	3.59	1.57	6.06	4.13	3.52	3.95	2.65
Density of turpentine, d^{20}_{20}	0.86	0.88	0.86	0.89	0.86	0.88	0.85
Monoterpenes (percent, based on peak area):							
α-thujene	0.4	0.4	0.3	0.2	–	–	–
α-pinene	35.0	32.0	31.3	30.3	28.1	30.7	32.3
camphene	5.1	3.2	3.3	4.6	2.9	3.6	3.8
β-pinene	47.2	50.6	50.6	50.1	50.1	49.6	50.1
myrcene	2.3	1.5	2.1	2.0	2.6	1.6	1.9
Δ_3-carene	0.3	1.4	1.4	1.0	2.9	2.5	1.0
limonene	0.5	0.5	0.4	0.6	0.7	0.4	0.4
β-phellandrene	0.3	0.2	0.1	0.4	–	–	–
ρ-cymene	8.1	9.5	9.5	9.9	12.1	11.0	9.5
α-terpinene(?)	–	0.2	0.2	–	–	–	–
γ-terpinene(?)	–	0.1	0.2	–	–	–	0.1
terpinolene(?)	0.9	0.5	0.7	0.9	0.7	0.6	0.9

crystalline rosin and oil yield. The complete record of measurements is given in Table 50.

It would appear from this experimental trial that upon felling a tree there are immediate and complex changes in tree chemistry, including monoterpene composition. Changes in rosin and oil content possibly resulted from a change in physiology that accompanied loss of moisture. Equally significant were wide fluctuations in oil composition. β-Pinene was found to vary by as much as 8 percent, while the ρ-cymene

Table 50. Composition of steam-distilled oils, togther with parameters related to changes in tree chemistry, obtained from bark samples removed daily from a felled *Pinus radiata*

Time bark removed after felling tree (days)	Percent water (of dry bark minus percent oil)	Yield of rosin (percent of dry bark)	Yield of oil (percent of dry bark)	Density of oil (d_{20}^{20})	Refractive index of oil (n_D^{20})	Specific rotation of oil $[\alpha]_D^{22}$	α-Pinene	β-Pinene	Myrcene	Limonene	Unidentified + p-cymene	Unidentified
							Monoterpene percentages in oil: (based on peak area)					
0	143.9	–	0.46	0.8746	1.4780	-22.50	9.3	72.2	1.6	4.3	12.3	0.2
1	124.7	–	0.36	0.8730	1.4767	-22.94	9.4	72.7	1.5	4.5	11.5	0.3
2	122.7	0.27	0.54	0.8712	1.4764	-22.45	9.1	75.0	1.5	4.1	9.8	0.4
			(loss)									
3	88.4	0.11	0.36	0.8798	1.4784	-21.70	9.3	76.4	1.0	3.3	9.7	0.3
4	79.5	0.56	0.54	0.8712	1.4760	-24.17	10.0	74.8	0.6	3.7	10.5	0.3
5	84.7	2.17	0.86	0.8696	1.4751	-28.04	10.6	72.3	0.9	3.7	12.2	0.3
6	84.7	1.39	0.66	0.8712	1.4756	-28.00	10.5	72.3	0.9	3.2	12.9	0.1
7	90.1	2.08	0.87	0.8696	1.4753	-26.91	10.4	72.2	0.8	3.6	12.7	0.3
8	86.9	1.77	0.68	0.8668	1.4747	-28.33	9.9	74.8	0.7	2.8	11.6	0.1
9	76.3	0.89	0.55	0.8684	1.4755	-28.12	10.3	72.7	0.6	3.8	12.5	0.1
10	82.9	1.14	0.57	0.8718	1.4766	-23.15	11.1	78.1	0.4	2.4	7.9	0.2
11	79.3	0.81	0.47	0.8730	1.4772	-23.83	10.3	79.4	0.3	2.2	7.5	0.2
12	80.9	1.25	0.62	0.8718	1.4765	-24.45	10.7	79.7	0.2	2.2	6.9	0.2
13	79.0	0.54	0.41	0.8730	1.4777	-22.87	10.1	80.2	t	2.2	7.3	0.2
14	72.2	0.39	0.50	0.8690	1.4761	-25.40	10.0	79.6	t	2.3	7.8	0.2

t: <0.1 percent

percentage appeared to follow β-pinene in an inverse relationship,
i.e. after taking into account the percentage of a change in the
concentration of β-pinene upon the percentage of each other
component (Figure 39).

The compositional changes documented in this trial
required verification together with any indication that might
be obtained of reproducibility. It was also considered that the
changes should be correlated with various degrees of injury.

(iii) Comparison of the daily changes in oil composition associated
both with daily removal of bark from a felled tree and bark
removal in a single operation

A single large *Pinus radiata* was felled and the bark
sampled from it in two ways. On the same day as felling, half of
the total bark area was removed in representative sections, from
which samples were taken daily and steam-distilled. The remaining
bark on the trunk was left intact and similarly sampled on a
daily basis. The steam-distilled oils were then compared by GC
analysis and measurement of physical properties.

The measurements confirmed that there were quite
different changes in the chemistry and physiology of bark
that had been injured to a different extent during each
procedure, i.e. left intact and removed from a felled tree
(Table 51, Figure 40). Bark considered to be more seriously
injured, by removal as a single sheet rather than left intact
on the log, showed quite large changes much sooner than intact
bark. A more rapid drop in moisture content of removed bark
appeared to be correlated with a sudden drop in yield of oil
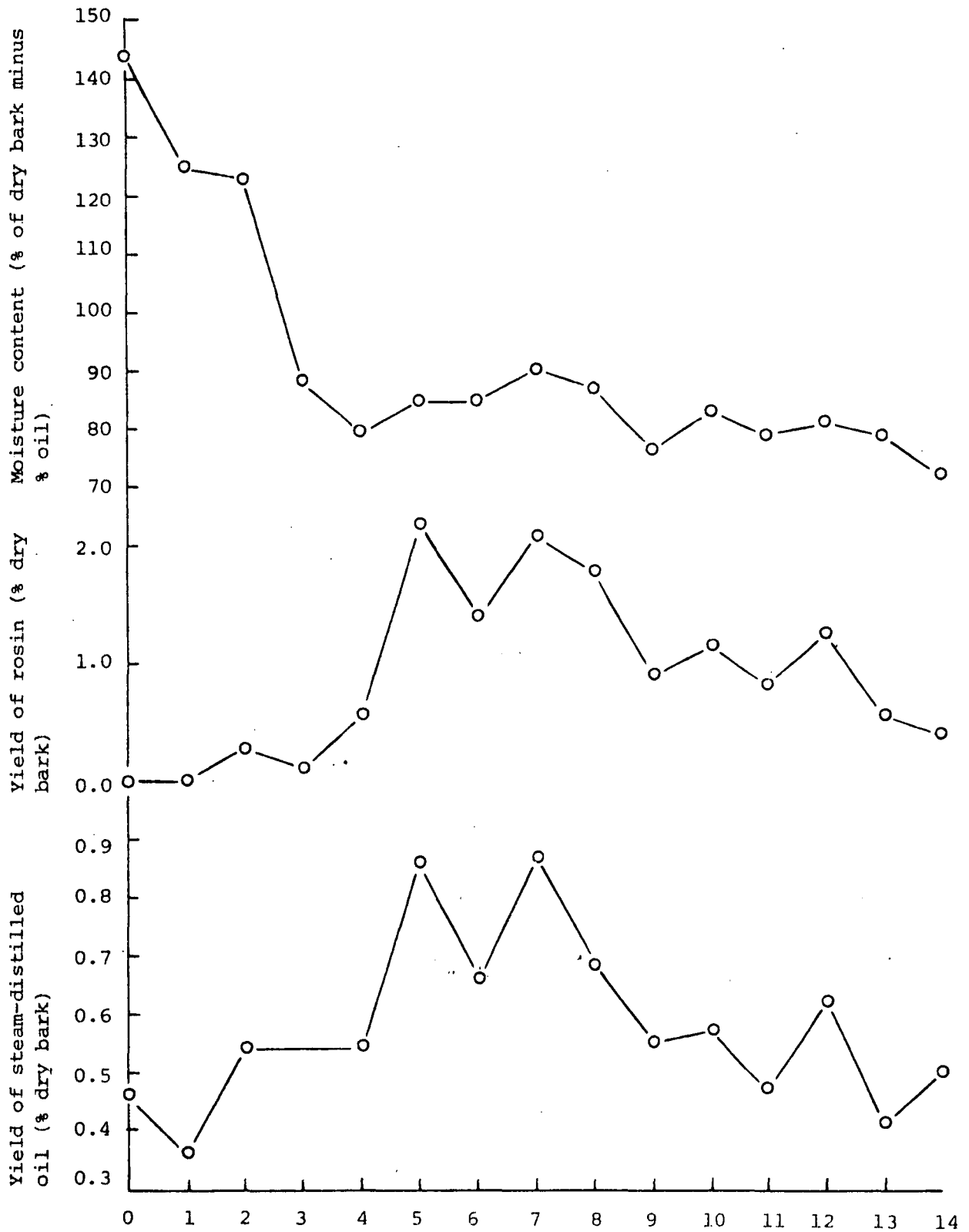
Fig. 39(a).   Changes over a 14-day period in the chemistry of a felled tree of *Pinus radiata*, indicated by moisture, rosin and oil content in bark removed daily.
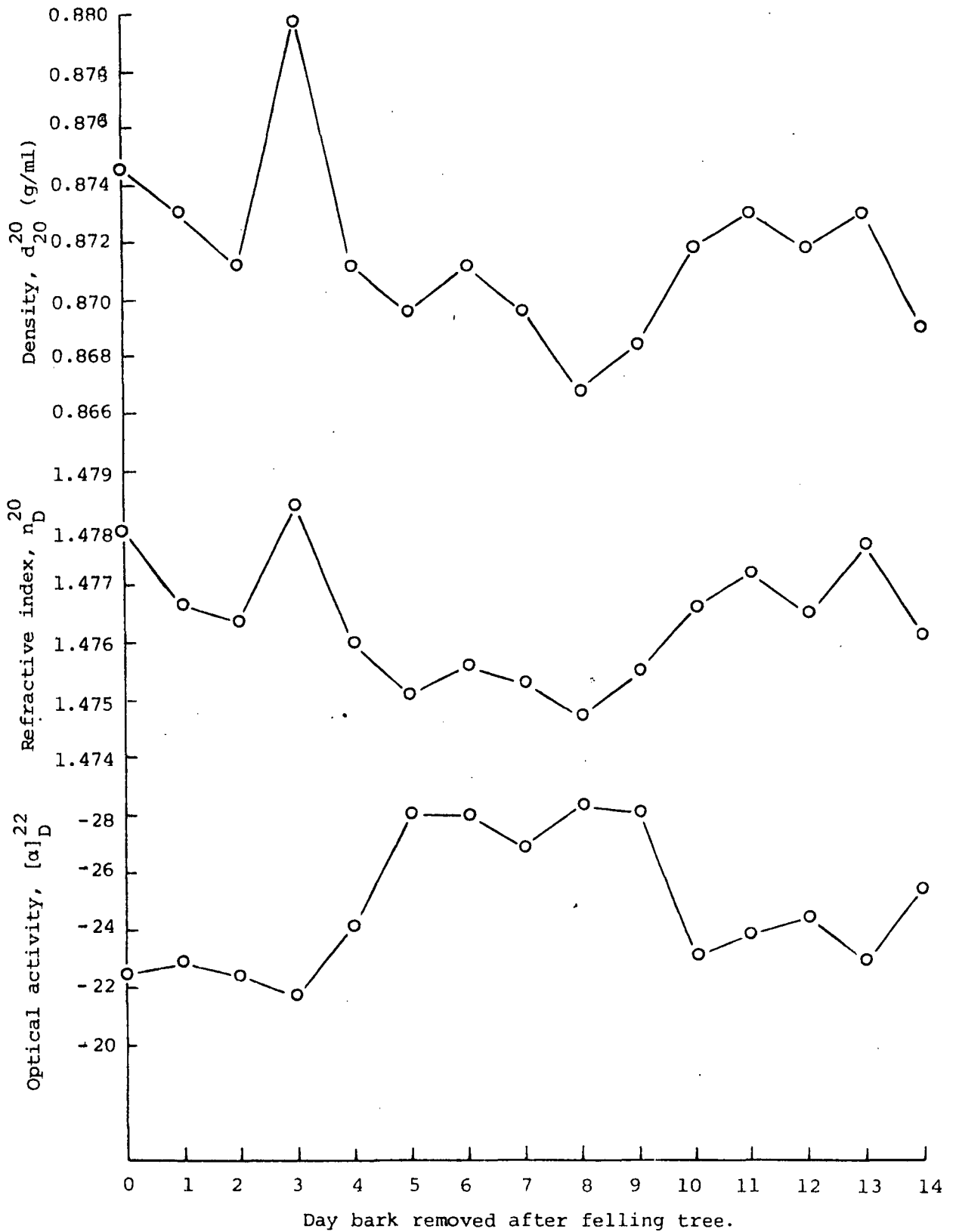
Fig. 39(b). Changes over a 14-day period in the chemistry of a felled tree of *Pinus radiata*, indicated by physical properties of oil steam-distilled daily from the bark.
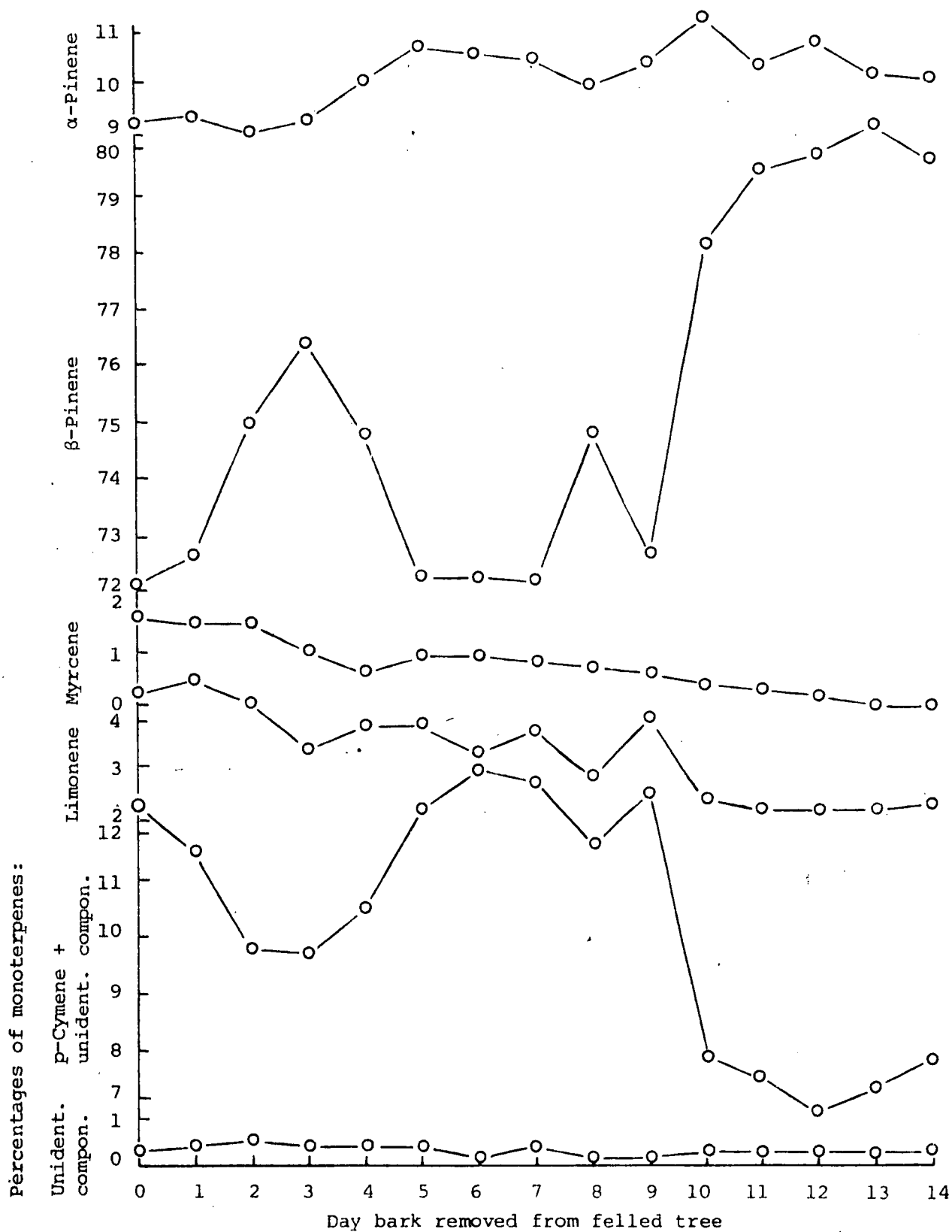
Fig. 39(c). Changes over a 14-day period in the monoterpene composition of oil from daily samples of bark from a felled tree of *Pinus radiata*.

Table 51. Comparison of daily measurements of parameters indicative of tree chemistry in bark removed daily and in a single operation from a felled tree of *Pinus radiata*

Day bark removed from log (I)	Day since single bark removed (II)	% H_2O (of dry bark minus % oil)		Yield of rosin (% of dry bark)		Yield of oil (% of dry bark)		Oil density, d^{20}_{20}		Refractive index of oil, n^{20}_D		Specific rotation, $[\alpha]^{22}_D$	
		I	II	I	II	I	II	I	II	I	II	I	II
0	0	210.4		0.50		0.69		0.8712		1.4776		-22.37	
1	1	204.9	168.3	1.80	0.42	0.66	0.26	0.8680	0.8736	1.4755	1.4772	-25.36	-21.58
2	2	201.7	98.4	1.25	0.22	0.80	0.24	0.8662	0.8800	1.4761	1.4791	-25.30	-20.86
3	3	182.9	97.3	1.38	0.49	0.70	0.26	0.8680	0.8754	1.4761	1.4775	-24.10	-19.92
4	4	170.7	101.2	0.88	0.56	0.49	0.31	0.8674	0.8696	1.4760	1.4760	-24.00	-21.92
5	5	165.2	64.3	0.81	0.05	0.62	0.18	0.8684	0.8736	1.4762	1.4778	-25.47	-21.92
6	6	151.0	87.2	0.95	0.46	0.55	0.33	0.8667	0.8741	1.4756	1.4770	-27.64	-21.53
7	7	144.4	54.8	0.17	0.53	0.67	0.45	0.8690	0.8684	1.4767	1.4758	-25.97	-21.89
8	8	167.8	16.9	0.46	0.82	0.59	0.47	0.8667	0.8662	1.4762	1.4753	-24.87	-21.48
9		182.7		–		0.46		0.8712		1.4773		-21.51	
10		184.8		0.18		0.28		0.8864		1.4807		-21.85	
11		181.3		0.34		0.30		0.8790		1.4855		-21.52	
12		165.5		0.42		0.47		0.8656		1.4760		-24.40	

286.

Table 51 cont.

Monoterpenes in oil (%, based on peak area):

Day bark removed from log (I)	Day since single bark removed (II)	α-Pinene		Camphene		β-Pinene		Myrcene		Limonene + (β-phellandrene)		Unidentified		ρ-Cymene	
		I	II	I	II	I	II	I	II	I	II	I	II	I	II
0	0	17.0		0.3		63.3		4.2		12.2		t		2.8	
1	1	16.7	16.5	0.3	0.3	61.0	67.9	5.3	3.7	13.9	9.7	t	t	2.8	2.0
2	2	17.3	16.9	"	"	58.2	68.3	6.0	3.3	15.4	9.0	"	"	"	2.2
3	3	16.3	19.0	"	"	59.7	65.8	5.5	3.2	15.5	9.6	"	-	"	2.1
4	4	18.3	20.3	"	"	61.8	63.2	4.0	3.4	12.5	9.7	"	0.3	3.2	2.9
5	5	17.0	18.7	"	"	58.1	65.6	5.7	3.5	15.9	9.1	"	0.3	3.1	2.5
6	6	19.2	18.2	"	"	56.3	64.9	6.7	3.7	13.6	10.5	0.3	-	3.6	2.5
7	7	17.3	19.3	"	"	54.8	65.3	6.1	2.5	18.3	9.4	t	-	3.3	3.3
8	8	17.1	20.0	"	"	54.9	64.7	6.6	2.9	17.9	9.5	"	t	3.2	2.6
9		18.0		"		55.7		6.4		16.5		"		"	
10		15.7		"		60.7		5.6		14.9		"		2.8	
11		16.4		"		55.9		6.5		17.7		"		3.2	
12		16.8		"		57.1		6.1		16.5		0.3		3.1	

t: trace, <0.1%
-: not detected
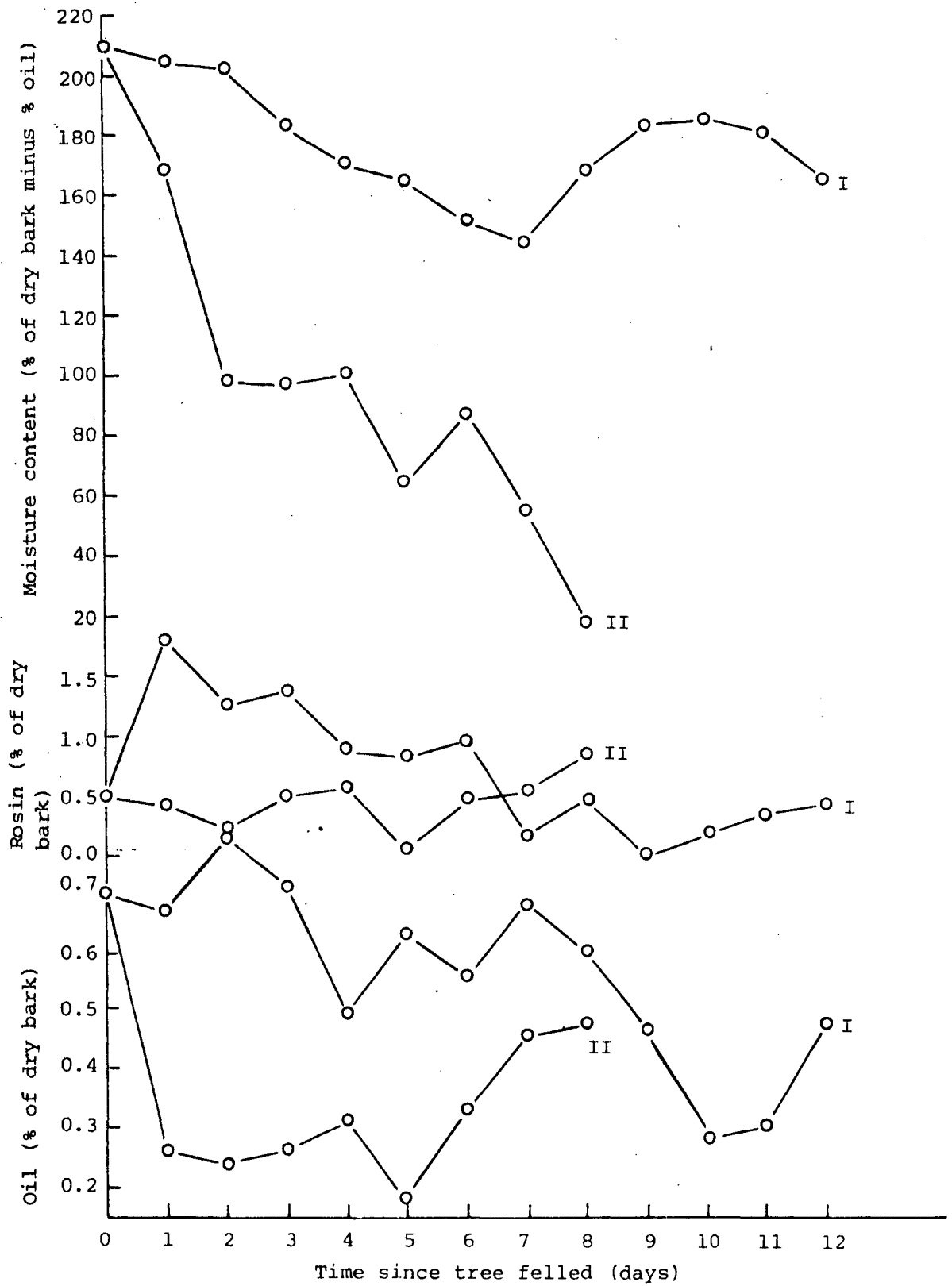
Fig. 40(a). Comparison of the changes in the chemistry of bark removed daily (II) and in a single operation (I) from a felled tree of *Pinus radiata*.
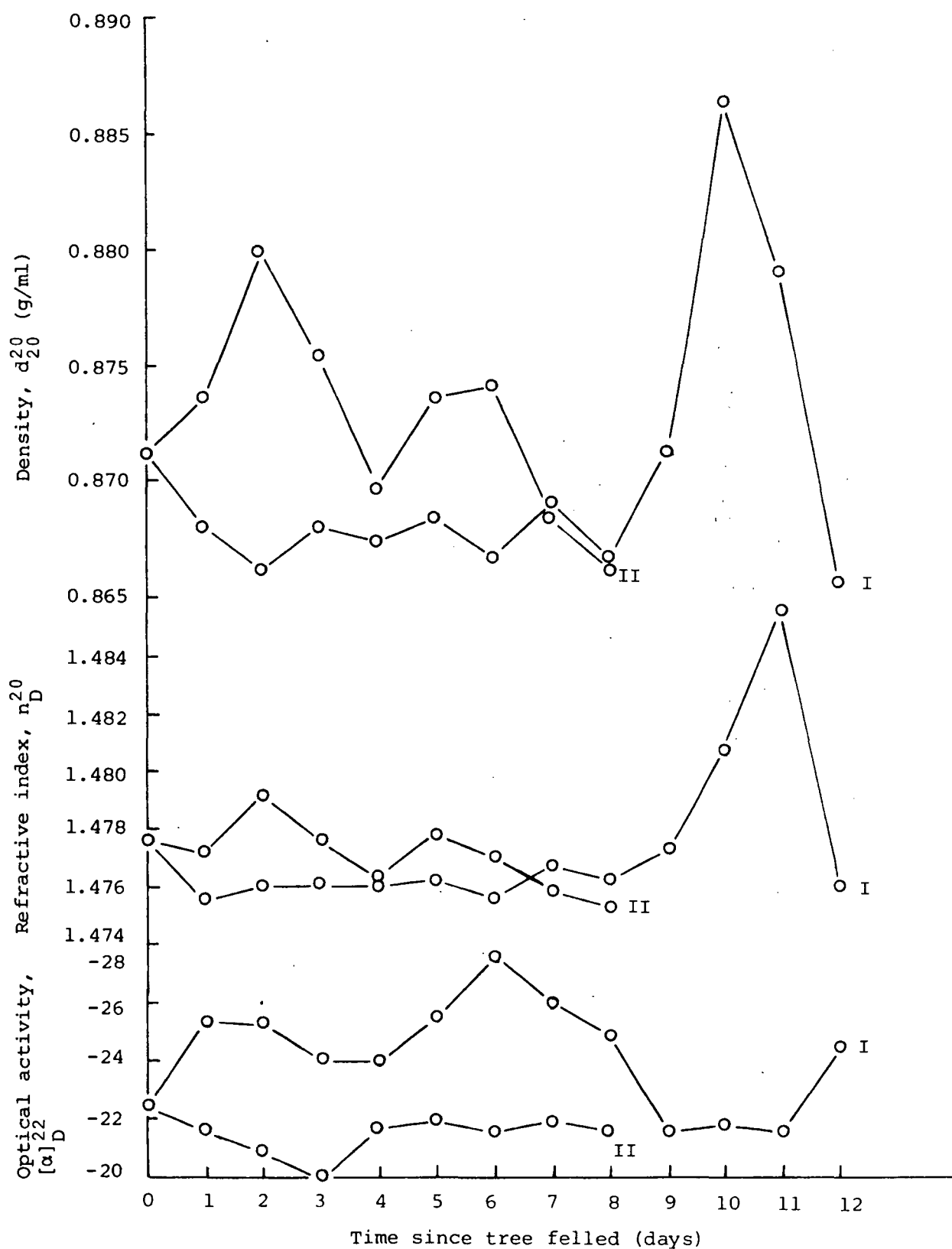
Fig. 40(b). Comparison of the changes in physical properties of steam-distilled oil of *Pinus radiata* bark, removed daily (II) and in a single operation (I) from a felled tree.
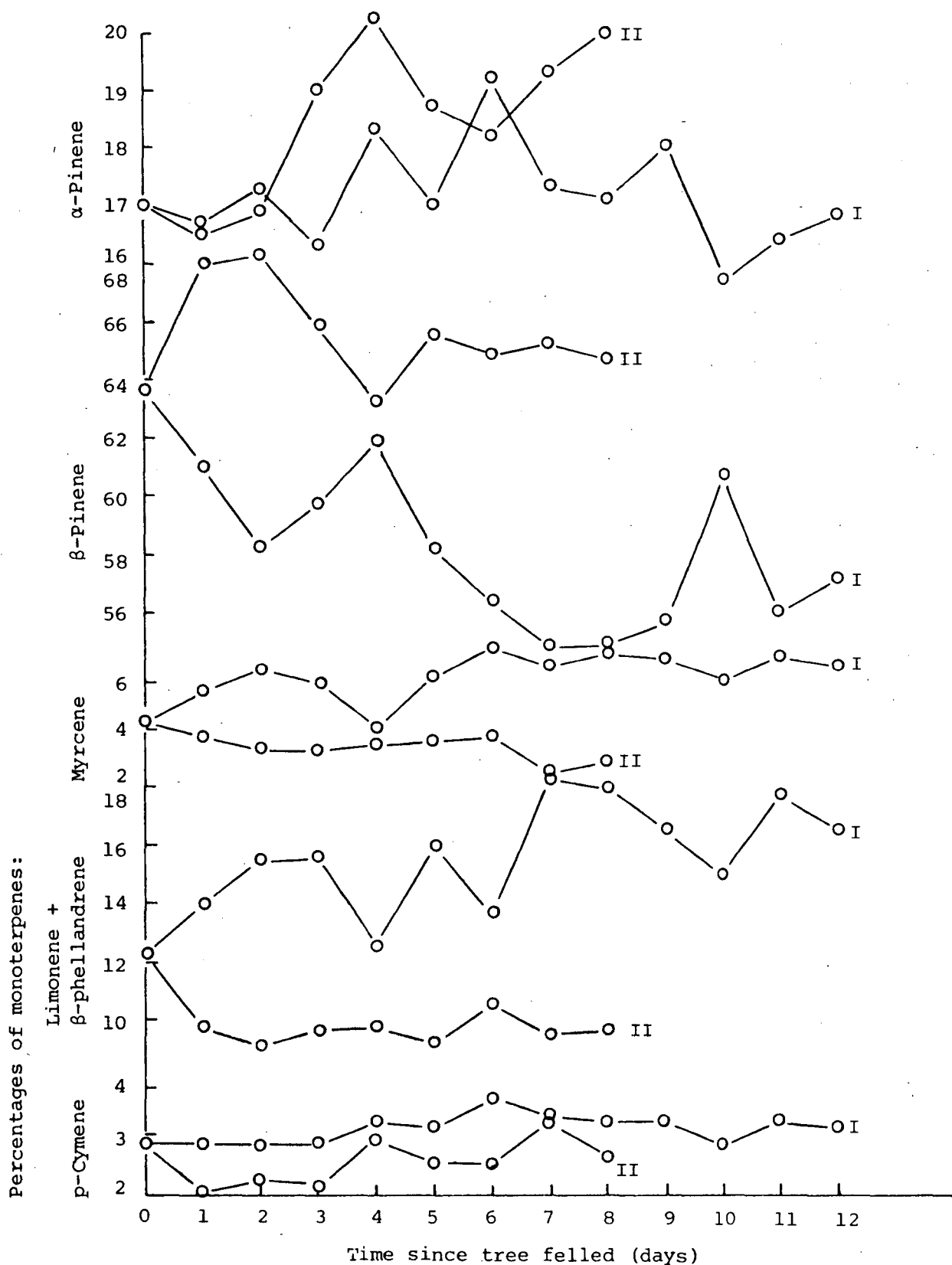
Fig. 40(c). Comparison of the changes in monoterpene composition of steam-distilled oil of bark removed daily (II) and in a single operation (I) from a felled *Pinus radiata*.

and a large initial change in oil properties, particularly

density. Intact bark exhibited large changes much later,

e.g. a large reduction in yield of oil at about day 10 rather

than day 1.

The changes in oil composition were complex and will

require much more investigation before their full significance

can ever be understood. The changes in β-pinene content can be

used to illustrate the complexity. A particular physiological

process in removed bark is considered to have led to an initial

increase in β-pinene content, whereas an entirely different

process in intact bark led to an initial reduction in β-pinene.

As a result the percentage of β-pinene varied in bark oils from

this same tree from 54.8 to 68.3 percent.

Correlations between daily measurements obtained from

intact bark in this and the preceding experimental trial were

barely distinguishable. In each case there was an initial increase

and then a gradual reduction in both oil and rosin yields.

Changes in oil composition were not correlated.

(iv) Changes in oil composition in bark removed from a

standing tree

Bark from several standing trees was sampled and

analyzed over varying periods of time, and although variations

in oil composition were detected, they were often only within

the range of experimental error. A typical series of analyses

is shown in Table 52.

Table 52. Composition and yield of oil steam-distilled from bark sampled from a standing tree of *Pinus radiata*

Time distillation began after first bark removal (hrs)	Yield of oil (percent of dry bark)	Monoterpene percentage in oil (based on peak area):				
		α-pinene	camphene	β-pinene + myrcene	Δ₃-carene	limonene + β-phellandrene
1.2	0.36	23.4	3.2	61.1	3.3	9.0
5.5	0.45	23.8	3.3	59.8	3.8	9.4
24	0.39	25.0	3.4	60.0	2.8	8.8
30	0.50	24.9	3.0	59.7	3.4	9.1
48	0.42	24.7	2.8	59.2	4.2	9.1
53	0.48	25.8	2.2	60.7	3.0	8.3

(v) Conclusions from studies of changes in composition of
     steam-distilled oils of *Pinus radiata*

Wide variations in oil composition may be encountered
in bark oils from the same tree trunk, i.e. after ensuring that
the bark has been representatively sampled. There were
indications that the changes in composition, together with
chemical processes leading to production of rosin and oil, may
be influenced by the degree of injury to the bark. To confirm
whether tree felling and bark sampling indicate specific changes
in oil composition requires further study on a statistical basis.
Documented changes in oil composition were too complex for any
correlations to be seen.

Although many changes in composition were found, it
cannot be assumed that any particular composition or range of
compositions was necessarily attractive to *Sirex noctilio*.
Changes that were documented were still however well within the
range of compositions found for individual trees within a stand.
Further data should be obtained that might indicate the existence
of temporary oil components, which could conceivably also be
attractive to *S. noctilio*. Variations in the composition of
a number of trees (also sampled in different ways) are
presented under the headings for different species attractive
to *S. noctilio*.

(2) Compositions of terpenoids from conifers in which *Sirex*

noctilio has been found breeding in New Zealand

Studies are presented of a number of woodwasp-

attractive conifers, in which oils are investigated using

well-known techniques for the analysis of isolated oils.

Syringe-headspace GC analysis data is also given as complementary

information, to assist in a comparison of the possible

attractiveness of terpenoid vapours released to the atmosphere

from each of these species.

Major changes in the proportions of monoterpenes in

successive injections of vapour may be found at any time, but

are often encountered between the first and second injections.

As previously discussed, it is inevitable that some change in

monoterpene proportions must be expected due to a small loss

of vapour during the few seconds when the comminuted sample is

being transferred to the syringe. However during this interval

there might also be major changes due to interference or

interruption of biosynthetic mechanisms that could occur during

comminution. Until subsequent investigation is carried out,

which might show that the initial change is due partly or

entirely to an evaporative effect during sample handling, then

this initial change will be treated in these studies as possible

changes resulting from wounding of tissue. Every care is

however taken to minimize the loss of terpenes during handling.

The conifer species studied were selected from those

listed in Table 48, depending upon their availability in the

Royal Botanical Gardens (Tasmania). Where possible, data is

given for terpenoid compositions from a number of trees from each species, together with repeated samplings from a single tree to indicate the range of compositions. Some species were only available as small trees, or even seedlings, for which data from the syringe-headspace is all that could reasonably be obtained.

(1) *Abies   nordmanniana (Steven) Spach*.

Oil from Caucasian Fir *(Abies   nordmanniana)* does not appear from the literature to have been subjected to such detailed investigations as other species of this genus. Weissman [402] compared needle oils of three American fir species and reported that *A. nordmanniana* oil contained a much higher concentration of Δ_3-carene than *A. veitchii* and *A. koreana*. Cermak *et al* [30] reported the composition of the seed oil of *A. nordmanniana*, while Chararas and Berton [403] used a galvanic osinopile to study the release of monoterpene vapours thought to have led to a reaction by the insect pest, *Ips sexdentatus*.

Since the introduction of GC a search of the literature has shown that more than 60 papers have been published on oils of *Abies* species. Some of the most significant recent work has been by Zavarin and co-workers who surveyed terpenes in 17 *Abies* balsams [53], reported upon the within tree variation of terpenes of the cortical oleoresin of several *Abies* species [326] and documented the oxygenated monoterpenoids and sesquiterpenes of most North American *Abies* species [135]. Norin [404] has reviewed the chemistry of the genus *Abies* along with other genera of the Order Pinales.

(a) Analysis of the isolated foliage oil

        Foliage was collected from a single tree (tree I)

in the Royal Botanical Gardens (Tasmania) and steam-distilled,

yielding 0.2 percent of oil with a faint green colour. The

absence of suitable flow of oleoresin from the trunk prevented

recovery of a cortical turpentine.

        The qualitative and quantitative composition of components

distinguishable in the whole oil, using two dissimilar columns

as described previously, is recorded in Table 53. RRT values

leading to the tentative identification of components isolated

directly from the whole oil by preparative GC are listed in

Table 54. Gas chromatograms of Figure 41 illustrate the degree

of complexity of this oil.

        The major components found were Δ_3-carene and α-pinene.

The composition was similar to that of other *Abies* species [53]

and differed from *Pinus* oils, in that it contained significant

proportions of the group of terpenoids derived from Ruzicka's

hypothesized 2-bornane carbonium ion precursor [356],

i.e. camphene and bornyl acetate.

        The IR spectrum was recorded of an unidentified

component which eluted from Carbowax 20M and OV-17 at the

same time as α-pinene. It was however separated by preparative

GC on DC-200. Although listed as unidentified, its spectrum

was very similar to that of cis-3-hexenol.

Table 53. Components distinguishable in the whole
oil of *Abies nordmanniana* (tree I)

Component	Qualitative RRT data		Quantitative composition
	C2OM	OV-17	(percent, based on peak height)
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min)
Unidentified	0.83	0.74	0.6
*α-Pinene	1.01	1.01	19.3
*Alcohol (1)(Fig.42)			t
*Camphene	1.30	1.22	5.8
*β-Pinene	1.63	1.57	8.5
*Δ$_3$-Carene	2.11	2.00	22.3
*Myrcene	2.26	1.78	3.7
Unidentified	2.50		0.1
*Limonene	2.76	2.38	7.0
β-Phellandrene	2.92	2.54	3.0
Unidentified	3.29		0.2
γ-Terpinene	3.67	3.29	0.2
ρ-Cymene	4.30	2.81	0.2
*Terpinolene	4.56	4.11	2.5
Unidentified (a)	9.95		1.1
(130° isothermal, ref. camphor)			
Unidentified (a)	0.58		
*Sesquiterpene (6)	0.88	1.74	1.5
Unidentified	0.99		0.2
"			1.2
*Bornyl acetate	1.24	1.66	11.5
*Caryophyllene	1.31	2.66	
Unidentified	1.54	3.53	0.4
*α-Humulene	1.68	3.22	4.7
Unidentified	1.81		0.2
*Unidentified sesquiterpene	2.00	4.02	4.0
Unidentified	5.12		0.2
"	5.51		0.1
"	10.5		1.0
"			0.2

* IR spectrum recorded
t: trace; <0.1 percent

Table 54. RRT values, on the dissimilar liquid phases
C20M and OV-17, for components found in
preparative GC fractions isolated from
oil of *Abies nordmanniana* (tree I)

Component	Preparative GC fraction No.	Column	
		C20M	OV-17
(60° isothermal, ref. α-pinene)			
Alcohol (1)	W1	~1.00	1.00
α-Pinene	W3	1.00	1.00
Camphene	W3	1.29	1.19
	W4	1.29	1.19
β-Pinene	W4	1.64	1.53
	W5	1.62	1.54
Sabinene	W4	1.76	
Δ₃-Carene	W6	2.06	1.94
	W7	2.09	1.97
Myrcene	W4	2.25	1.71
α-Phellandrene	W5	2.28	1.96
	W6	2.27	
Unidentified	W6	2.50	
Limonene	W6	2.74	2.35
	W7	2.79	2.35
β-Phellandrene	W7	2.95	2.49
γ-Terpinene	W7	3.71	3.21
Terpinolene	W8	4.57	4.11
(130° isothermal, ref. camphor)			
Sesquiterpene (6)	W10	0.83	1.73
Unidentified	W11	1.15	2.41
Bornyl acetate	W9	1.15	1.55
Caryophyllene	W11	1.23	2.53
	W12	1.24	2.62
Unidentified	W12	1.46	3.48
α-Humulene	W12	1.60	3.15
Unidentified	W13	1.86	3.90
"	W13	2.18	4.26
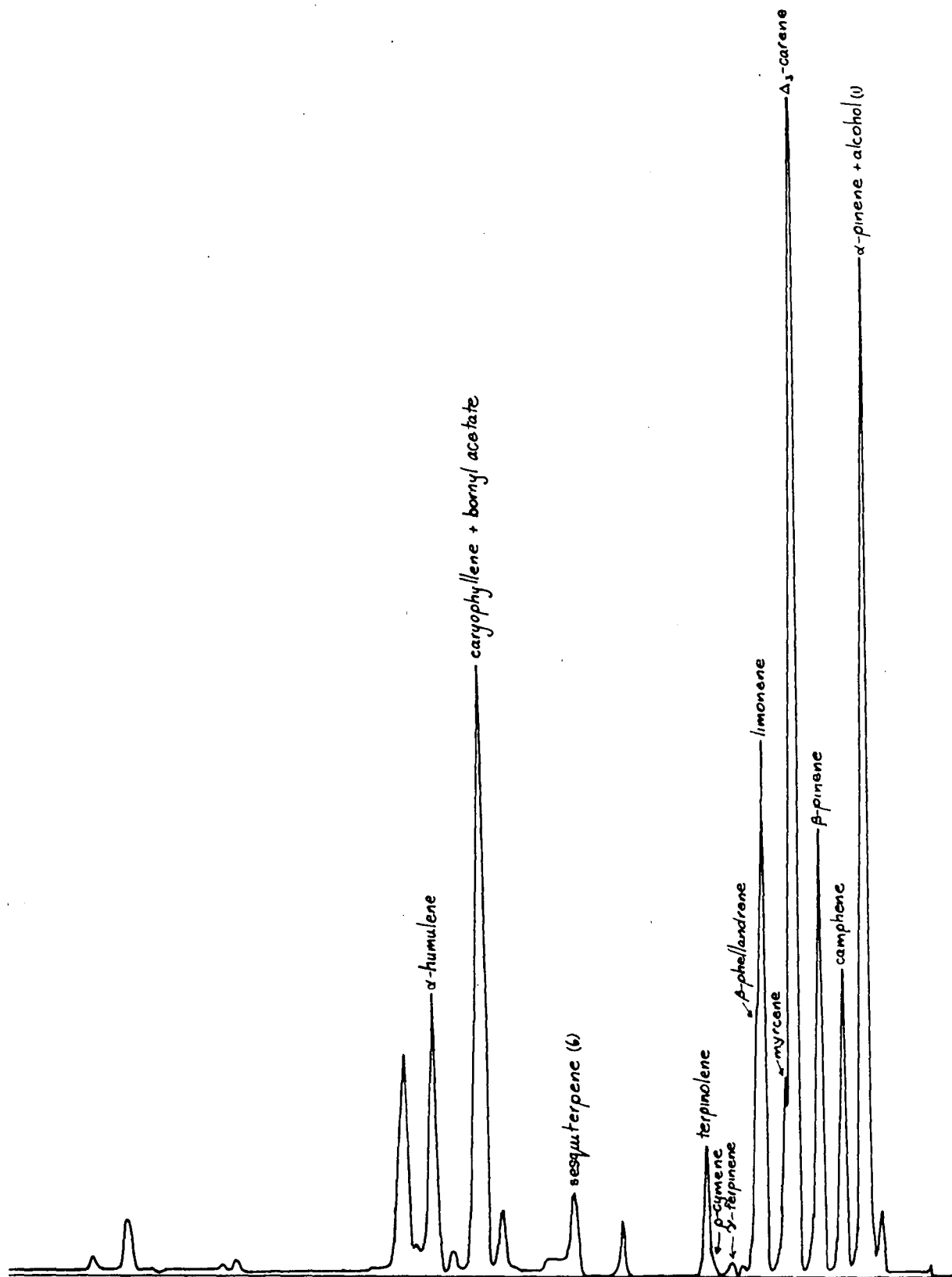
Fig. 41(a).   Low sensitivity gas chromatogram of whole oil of foliage
of *Abies nordmanniana* (GC on 5% Carbowax 20M/Gas Chrom Q;   temperature
program 50 to 220° at 5°/min;   0.2 µℓ sample, attenuation 4 x 10³).

Fig. 41(b). High sensitivity gas chromatogram of whole oil
of foliage of *Abies nordmanniana* (attenuation 4x10^2).
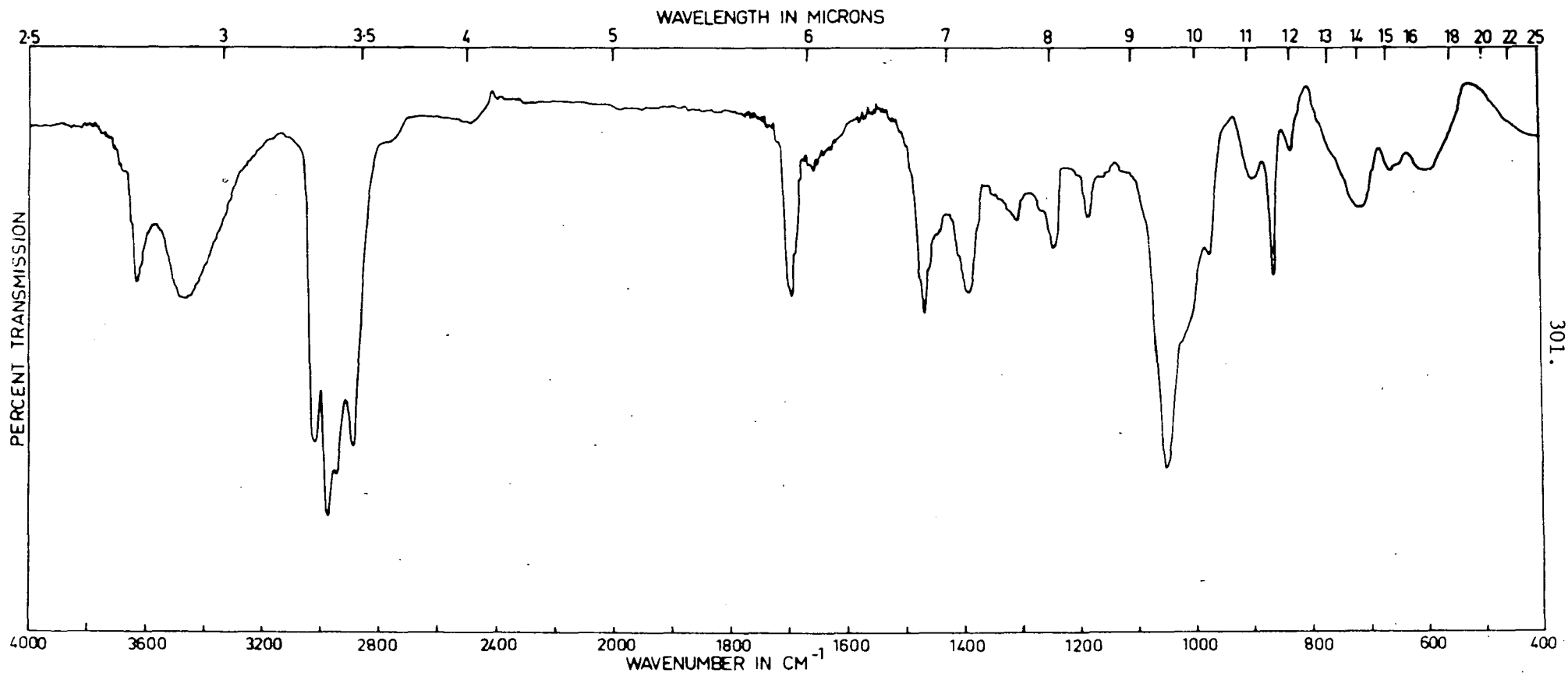
Fig. 42. IR spectrum of the alcohol isolated pure in the fraction Wl of oil of *Abies nordmanniana* [designated terpene alcohol (1); RRT$_{\alpha\text{-pinene}}$ ~100 (C20M) and 1.00 (OV-17); IR spectrum of solution in CHCl$_3$].

(b) <u>Syringe-headspace GC analysis of foliage terpenoids</u>

   <u>(Tree I)</u>

Syringe-headspace GC analysis, as previously described, has enabled further minor volatile components to be detected in a sample of foliage from the tree studied previously, i.e. tree I. The composition and syringe-headspace RRT data are given in Table 55, while Figure 43 contains a gas chromatogram of the syringe-headspace vapour which may be compared with the corresponding chromatogram of oil steam-distilled from the remainder of the same foliage sample.

Table 55. RRT data and percentage composition of volatile terpenoids in foliage of *Abies nordmanniana* (tree I) determined by syringe-headspace GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak height of initial injection)
	C2OM	OV-17	
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min)
Unidentified	0.80	0.75	0.8
Tricyclene	0.90	0.90	2.9
α-Pinene	0.99	1.00	35.5
Camphene	1.28	1.21	6.8
β-Pinene	1.65	1.54	12.5
Δ₃-Carene	2.10	1.94	28.0
Myrcene	2.32	1.75	2.7
Limonene	2.85	2.36	7.0
β-Phellandrene	2.99	2.46	2.9
γ-Terpinene?		-	0.1
Terpinolene	4.89		0.9

Fig. 43(a). Syringe-headspace gas chromatogram of vapour from foliage of *Abies nordmanniana* (tree I) (GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50 to 200° at 5°/min; attenuation 4 x 10^2).
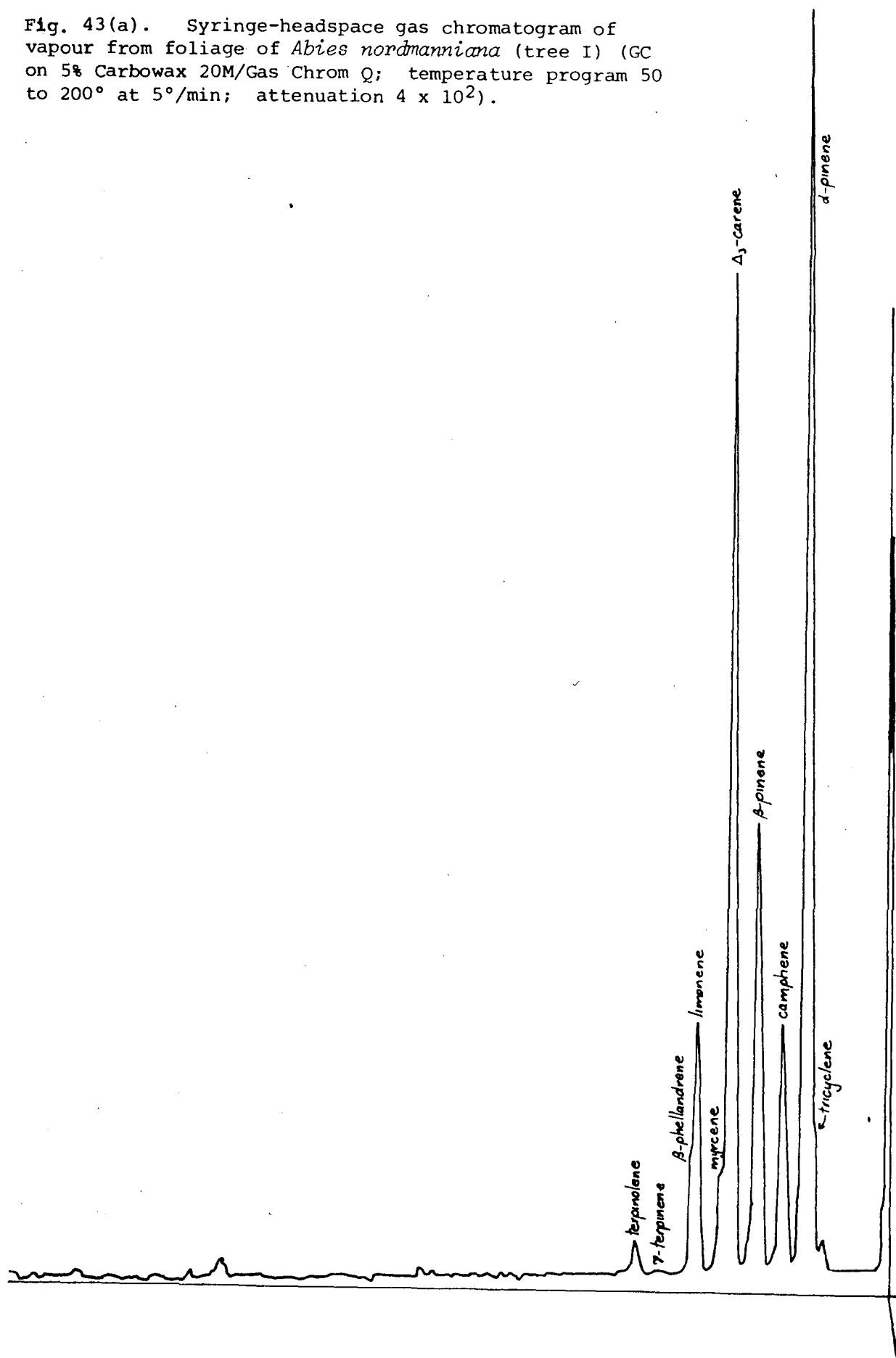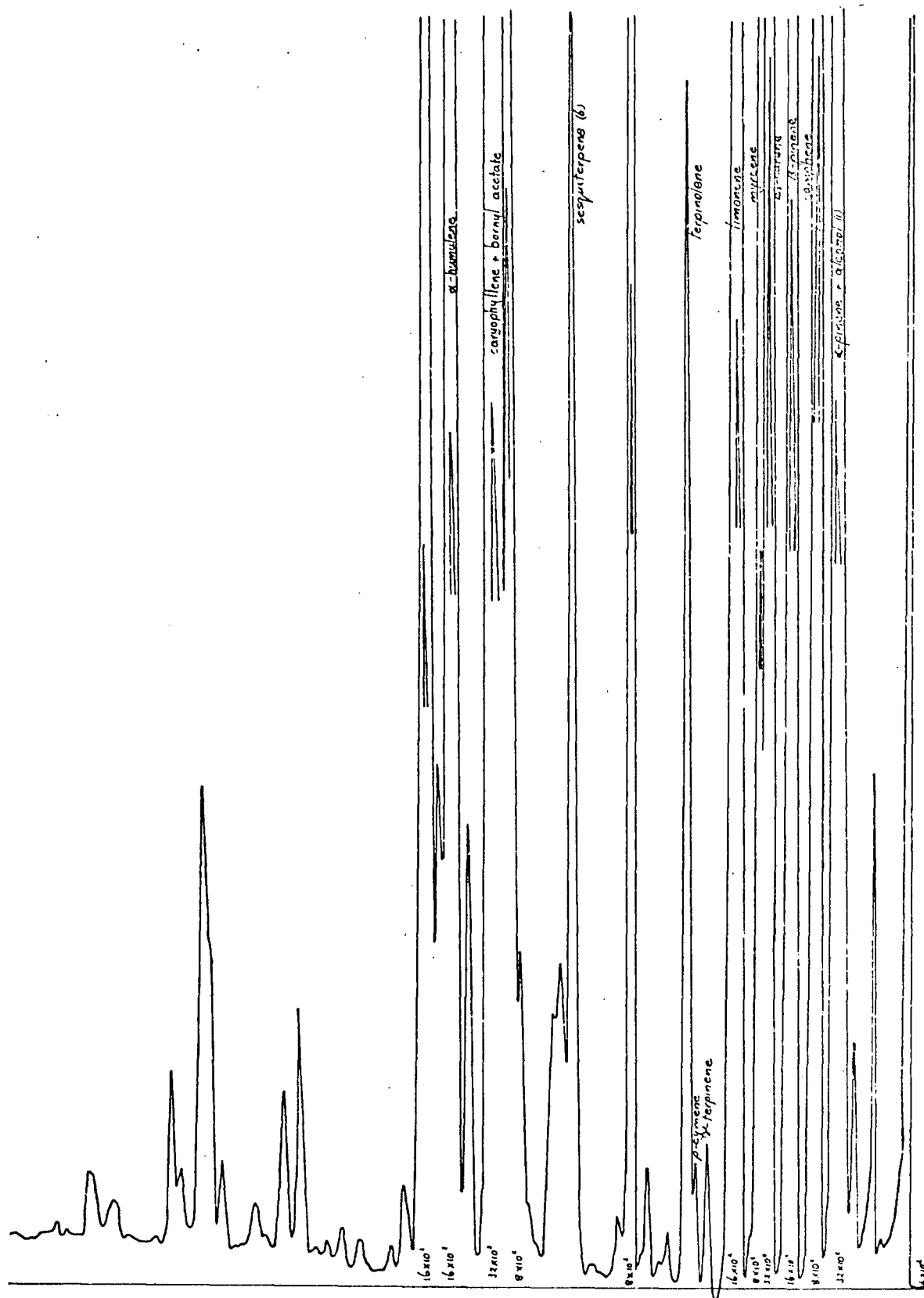
Fig. 43(b). Gas chromatogram of steam-distilled oil from remainder of foliage sample studied by the syringe-headspace technique in Fig. 43(a) (GC conditions as before, attenuation 4 x 10²).

The initially-eluted component, $RRT_{\alpha\text{-pinene}}$ 0.80(C20M)

and 0.75(OV-17), could correspond to santene, noted by Zavarin [53]

in several oils of *Abies* species. The tentative identification

of tricyclene, also reported by Zavarin as a typical component,

has produced a further feature in common with oils of many *Abies*

species.

(c) Composition of successive injections of syringe-headspace

vapour from foliage (Tree I)

Successive injections over a 3 hr. period, of a single

sample of foliage from tree I, resulted in variations of 35.5 to

40.9 percent α-pinene and 23.1 to 28.5 percent Δ_3-carene.

Although only significant peaks are documented in Table 56,

trace components listed in Table 55 were also detected together

with sabinene and ρ-cymene. There was no indication of the

existence of temporary components as found in other species,

such as *Cedrus deodara*.

(d) Composition of syringe-headspace vapour from foliage of

several trees of *A. nordmanniana* (Trees I-VII)

A single injection of syringe-headspace vapour, from

one sample of foliage from each of seven trees, showed

considerable variation in the monoterpene composition of

individual trees of this species (Table 57).

Table 56. Compositions of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Abies nordmanniana* (tree I)

Percentage composition of monoterpenes:
(peak height basis)

Time since comminution of sample (mins.)	Santene?	Tricyclene	α-Pinene	Camphene	β-Pinene	Δ₃-Carene	Myrcene	Limonene	β-Phellandrene	Terpinolene
0	0.6	2.3	36.5	8.7	12.4	27.9	3.0	5.6	2.0	0.9
15	0.9	3.4	39.7	9.2	12.2	24.5	2.6	4.6	2.0	0.8
30	0.9	3.4	40.5	9.5	12.3	23.7	2.5	4.4	2.1	0.7
45	1.0	3.4	40.1	9.3	12.3	23.8	2.5	4.5	2.4	0.8
60	1.0	3.7	40.8	9.5	12.4	23.1	2.5	4.2	2.1	0.6
80	1.1	3.4	40.9	9.5	12.5	23.2	2.5	4.2	2.0	0.7
95	1.0	3.4	40.5	9.6	12.6	23.4	2.5	4.2	2.1	0.7
110	1.1	3.9	40.2	9.4	12.4	23.2	2.5	4.4	2.2	0.7
125	1.0	3.4	40.1	9.4	12.8	23.6	2.6	4.4	2.1	0.7
140	0.9	3.4	40.1	9.7	12.8	23.5	2.5	4.2	2.2	0.7
175	0.9	3.3	39.0	9.5	13.1	24.4	2.6	4.4	2.1	0.7
210	0.7	2.8	35.5	7.9	12.2	28.5	3.0	5.8	2.7	1.1

(e) Composition of syringe-headspace vapour from foliage sampled repeatedly from the same tree (Tree I)

Comparison of the composition of initial vapour injections for each sample of foliage taken from tree I (Tables 55, 56 and 57) indicated a variation of α-pinene from 35.5 to 44.4 percent, and of Δ₃-carene from 22.1 to 28.0 percent.

Table 57. Composition of monoterpenes in initial syringe-headspace injections of vapour
from foliage of several trees of *Abies nordmanniana*

Percentage composition of monoterpenes (peak height basis):

Tree No.	Unidentified + santene?	Tricyclene	α-Pinene	Camphene	β-Pinene	Δ_3-Carene	Myrcene	Limonene	β-Phellandrene	Terpinolene
I	1.1	3.2	44.4	9.3	10.6	22.1	2.5	3.9	2.1	0.7
Steam-distilled oil from remainder of comminuted foliage from I:										
	2.2	1.4	22.4	7.8	13.7	31.1	4.7	12.2		3.8
+ γ-terpinene 0.5 and ρ-cymene 0.4										
II	0.5	1.1	44.8	4.2	9.2	29.2	4.4	3.1	2.6	0.9
III	0.8	2.5	41.3	9.2	22.5	11.8	1.5	9.9		0.4
IV	2.0	1.9	21.5	6.2	22.1	12.9	2.3	17.0	13.7	0.5
V	1.3	1.8	18.0	6.6	20.9	16.3	2.5	17.6	14.4	0.5
VI	1.4	2.5	29.2	8.0	7.7	36.5	2.7	1.2	9.7	1.0
VII	–	–	51.2	0.4	35.9	7.3	–	1.0	4.0	0.1

(f) Summary

Components of steam–distilled foliage oil from a single

tree of *Abies nordmanniana* were analyzed by chromatographic and

spectroscopic methods. The following identifications were

confirmed: α-pinene (19.3%), camphene (5.8%), β-pinene (8.5%),

Δ_3-carene (22.3%), myrcene (3.7%), limonene (7.0%), terpinolene

(2.5%), bornyl acetate and caryophyllene (11.5%) and α–humulene

(4.7%). Tentatively identified were sabinene, α-phellandrene,

β–phellandrene (3.0%), γ-terpinene (0.2%) and ρ-cymene (0.2%).

The IR spectrum was recorded of a trace component which had

features closely resembling those in the spectrum of cis-3-hexenol.

The composition of terpenoids in foliage of *Abies*

nordmanniana, based upon study of a single sample of isolated

oil from one tree was shown however to be not representative of

the species. Further samples of foliage from the same tree

indicated a wide range of compositions, perhaps even as wide as

the range of compositions between individual trees. The major

component in syringe-headspace GC, α-pinene, ranged from 18.0

to 51.2 percent with. Δ_3-carene from 7.3 to 36.5 percent between

7 individual trees. Three samples from one particular tree

ranged from 35.5 to 44.4 percent α-pinene and 22.1 to 28.0

percent Δ_3-carene. However successive injections of vapour from

a single sample over a 3 hr. period also led to a wide range

of variations, i.e. 35.5 to 40.9 percent α-pinene and 23.1 to

28.5 percent Δ_3-carene. Any insect–attractive terpene mixture

would therefore lie within a wide range of compositions. No

evidence was found of any component temporarily present in vapour.

(ii) *Cedrus deodara* (Rox.) G.Don.

Needle oil from *Cedrus deodara* is distinguished from the wood oil, or Himalayan oil [1] of commerce. Grewal and Sadgopal [405] noted the different proportions of phenols, aldehydes and ketones in various oils from this tree, and also commented upon needle oils existing in yellow and green forms. Akimov and Kuznetsov [406] recently compared the proportions of several components in oils of 1 year and 100 year old trees of *C. deodara*, together with oils of *C. atlantica* and *C. libani*.

In 1952 Rao *et al* [407] steam-distilled volatile oil from wood chips and isolated 4-methyl-Δ^3-tetrahydroacetophenone, α- and β-himachalene, atlantones and himachalol. Dev and co-workers have since identified in the alcohol portion of the oil [408] (+)-longiborneol (29 percent), himachalol (41 percent) [409] and allohimachalol (30 percent) [410]. Two further sesquiterpenes, α- and β-himachalene, were subsequently isolated [411]. This was followed more recently by a novel sesquiterpene tetrahydro-γ-pyrone, deodarone [412].

Needle oil has been reported [413] to contain ℓ-α-pinene, ℓ-β-pinene, ℓ-Δ_3-carene, ℓ-limonene, borneol, bornyl acetate, ℓ-α-cadinene and several sesquiterpenes.

(a) Analysis of the isolated needle oil

Needles collected from a single tree (tree I) in the Royal Botanical Gardens (Tasmania) yielded upon steam-distillation 0.2 percent of a faint green coloured oil with a fruity pine odour. Attempts were made to collect oleoresin based upon flow through tubes inserted into the cortex, but

this was unsuccessful. The study was therefore restricted to needle oil.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 58. RRT values leading to the

Table 58. Components distinguishable in the whole
oil of *Cedrus deodara* (tree I)

Component	Qualitative RRT data		Quantitative composition
	C2OM	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 200°, 5°/min)
*α-Pinene	1.00	0.99	36.2
Camphene	1.27	1.18	0.5
*β-Pinene	1.60	1.54	22.8
Δ_3-Carene?		1.99	t
*Myrcene	2.24	1.74	14.9
*Limonene	2.73	2.35	6.8
β-Phellandrene	2.92	2.51	2.9
γ-Terpinene	3.65	3.25	t
ρ-Cymene	4.25	2.76	t
*Terpinolene	4.51	4.06	0.1
(130° isothermal, ref. camphor)			
Unidentified	0.85		0.1
"	0.99		t
"	1.21	2.67	1.3
*Caryophyllene	1.29	2.67	3.1
Unidentified	1.70	3.28	t
"	1.53	2.94	1.6
*α-Terpineol	1.80	1.11	
Unidentified	⎱1.93	1.66	⎱9.1
"	⎰	4.11	⎰
"	2.27	4.74	0.5

* IR spectrum recorded
t: trace, <0.1 percent

tentative identification of components isolated directly from
the whole oil by preparative GC are listed in Table 59. Gas
chromatograms of Figure 44 illustrate the degree of complexity
of this oil.

The major components found were α-pinene, β-pinene
and myrcene, with smaller quantities of limonene, β-phellandrene,
caryophyllene and α-terpineol. This composition differed from
that reported by Chaudhary *et al* [413]. Unlike the oil
examined by these workers no evidence was found of bornyl
acetate, nor was there a significant amount of Δ_3-carene.

(b) Syringe-headspace GC analysis of foliage terpenoids

(Tree I)

Syringe-headspace GC analysis (Table 60, Figure 45)
indicated the presence of a temporarily-released component,
which eluted on Carbowax 20M with a RRT value very close to
that of Δ_3-carene. In addition, further minor components were
detected which eluted before α-pinene. One of these, at
$RRT_{\alpha-pinene}$ 0.81(C20M), could correspond to santene.

(c) Composition of successive injections of syringe-headspace

vapour from foliage (Tree I)

Successive injections of vapour over a 3 hr. period,
from a single sample of foliage from tree I, exhibited not only
a fluctuation of α-pinene from 49.4 to 57.2 percent, but also
some apparently qualitative changes in the vapour composition.
The previously-mentioned component eluting near Δ_3-carene could

Table 59. RRT values, on the dissimilar liquid phases
C20M and OV-17, for components found in
preparative GC fractions isolated from
oil of *Cedrus deodara* (tree I)

Component	Preparative GC fraction No.	Column C20M	OV-17
(60° isothermal, ref. α-pinene)			
α-Pinene	W1	1.02	1.00
Camphene	W1	1.26	1.21
	W2	1.29	1.22
β-Pinene	W2	1.61	1.57
	W3	1.61	1.57
Sabinene	W2	1.74	
Myrcene	W2	2.24	1.76
Limonene	W4	2.77	2.38
β-Phellandrene	W4	2.90	2.51
γ-Terpinene	W4	3.69	
ρ-Cymene	W4	4.31	
Terpinolene	W5	4.55	4.13
(130° isothermal, ref. camphor)			
Unidentified	W14	1.15	2.58
	W15	1.15	2.67
Caryophyllene	W14	1.24	2.58
	W16	1.23	2.45
Unidentified	W15	1.46	3.09
	W16	1.56	3.01
"	W15	1.62	3.33
	W16	1.65	3.31
α-Terpineol	W7	1.75	1.11
Unidentified	W8	2.00	1.56
"	W18	2.04	4.00
"	W18	2.19	4.53
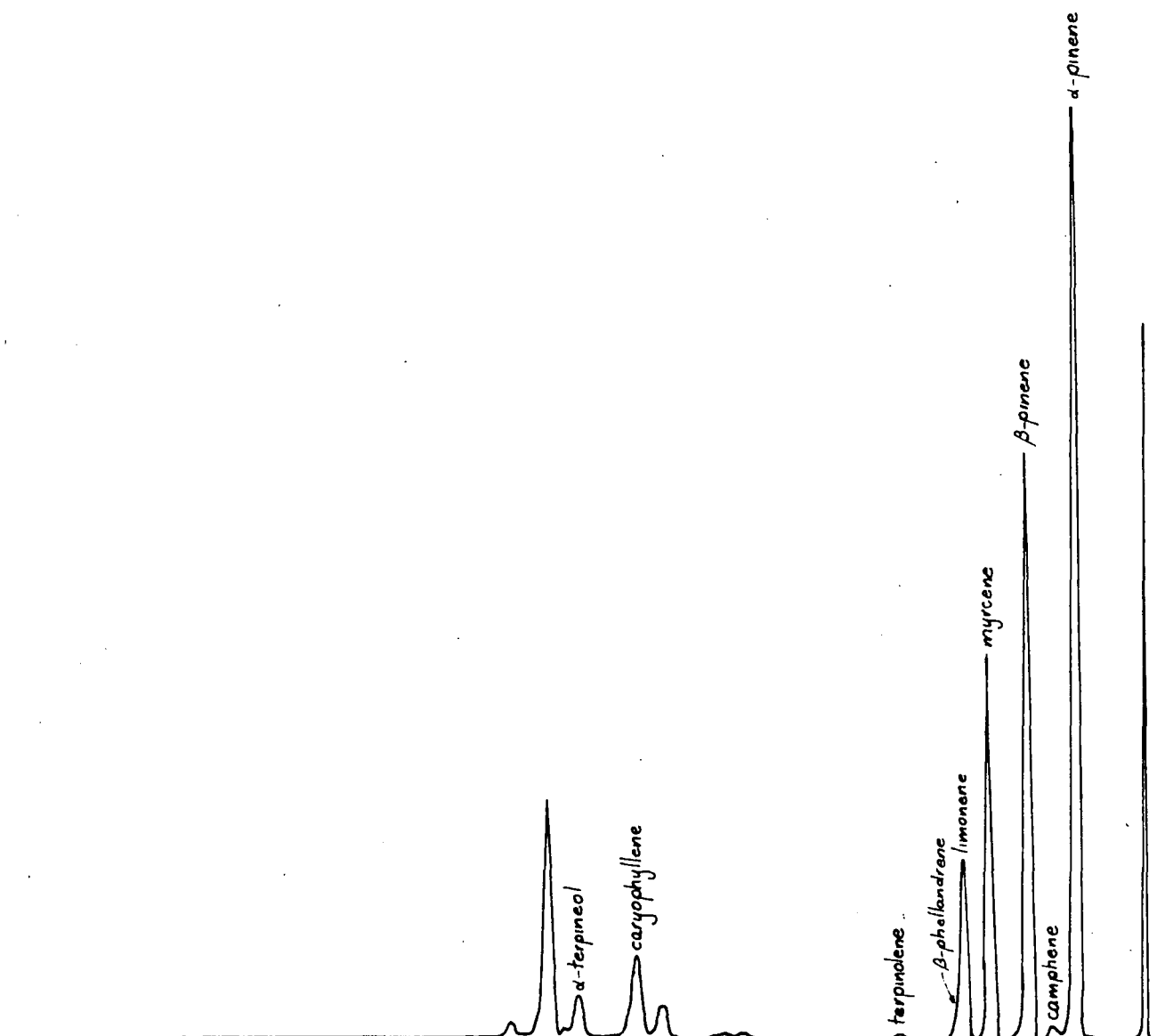
Fig. 44(a). Low sensitivity gas chromatogram of whole oil of foliage of *Cedrus deodara* (GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50° to 220° at 5°/min; 0.2 μℓ sample; attenuation 8 x 10^3).

314.
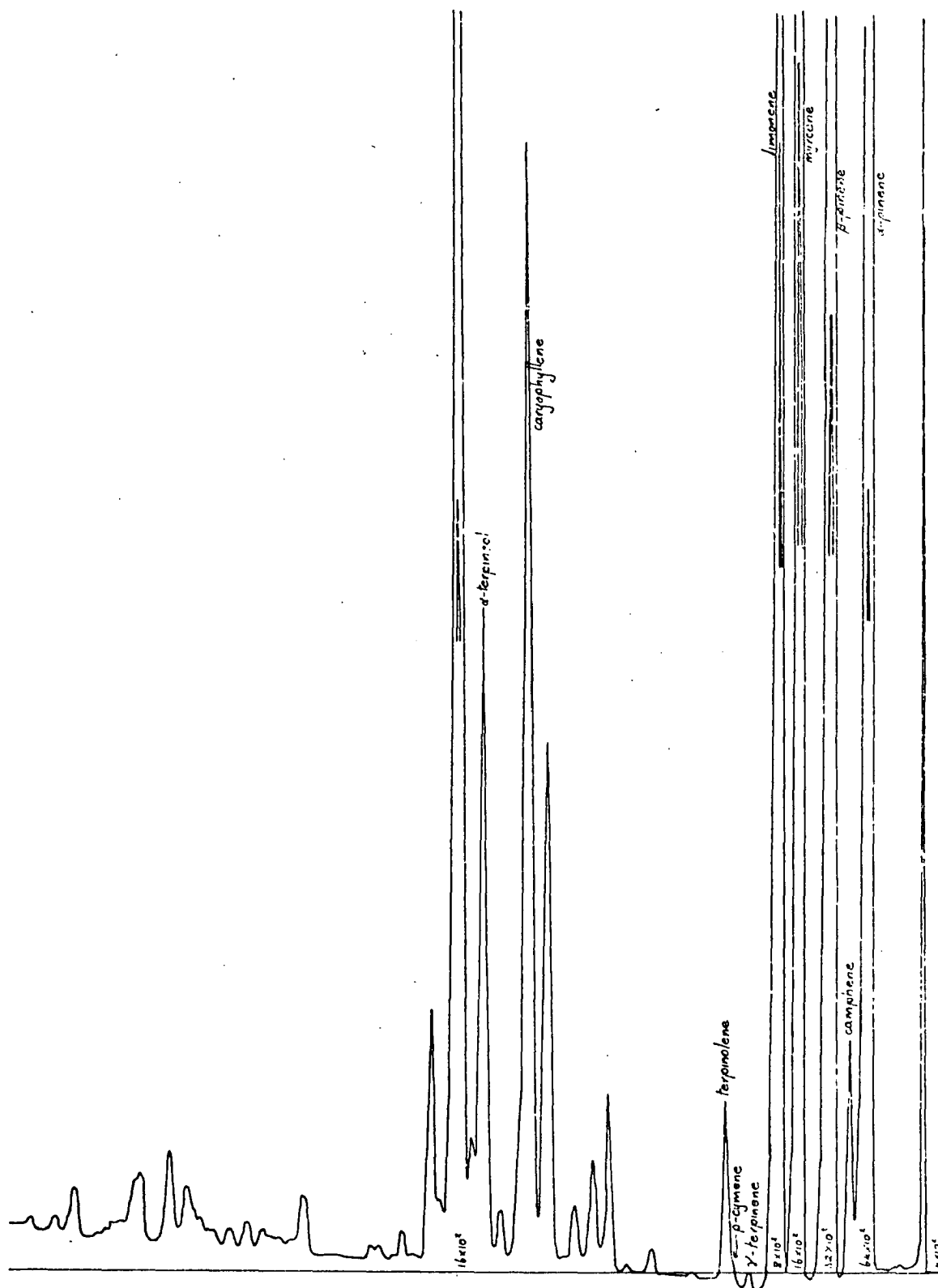
Fig. 44(b).   High sensitivity gas chromatogram of whole oil of
foliage of *Cedrus deodara* (attenuation 4 x 10^2).

Table 60. RRT data and percentage composition of
volatile terpenoids in needles of *Cedrus
deodara* (tree I) determined by syringe-
headspace GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak area of initial injection)
	C20M	OV-17	
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min)
Unidentified		0.46	t
Santene?	0.81		0.2
α-Pinene	1.00	1.00	57.0
Camphene	1.33	1.19	0.3
β-Pinene	1.63	1.53	25.4
Unidentified; detected in initial injection only	∼2.0		1.1
Myrcene	2.33	1.72	10.6
Limonene	2.84	2.33	5.0
β-Phellandrene	2.98	2.48	0.9
(130° isothermal, ref. camphor)			
Unidentified	0.48		0.1
"	0.52		0.1
Caryophyllene	1.36	2.44	0.1
Unidentified	2.03	3.45	0.2

t: trace, <0.1 percent

not be detected after the 35 minute injection, while

γ-terpinene was not detected in some chromatograms (Table 61,

Figure 46). It is possible particularly in the case of

γ-terpinene, that there was a fluctuation in concentration of

this trace component to such a low proportion that it could

not be detected. The non-reappearance of the component

eluting near Δ_3-carene, after an initial reduction from 2.8

percent to a trace proportion, suggests that there might really

be a qualitative change in the vapour due to that component.

Fig. 45.   Syringe-headspace gas chromatogram
of vapour from foliage of *Cedrus deodara*
(tree I) (GC on 5% Carbowax 20M/Gas Chrom Q;
temperature program 50° to 200° at 5°/min;
attenuation 4 x 10^2).   The temporarily-
released component, which did not appear
in later injections, is seen between
β-pinene and myrcene peaks.
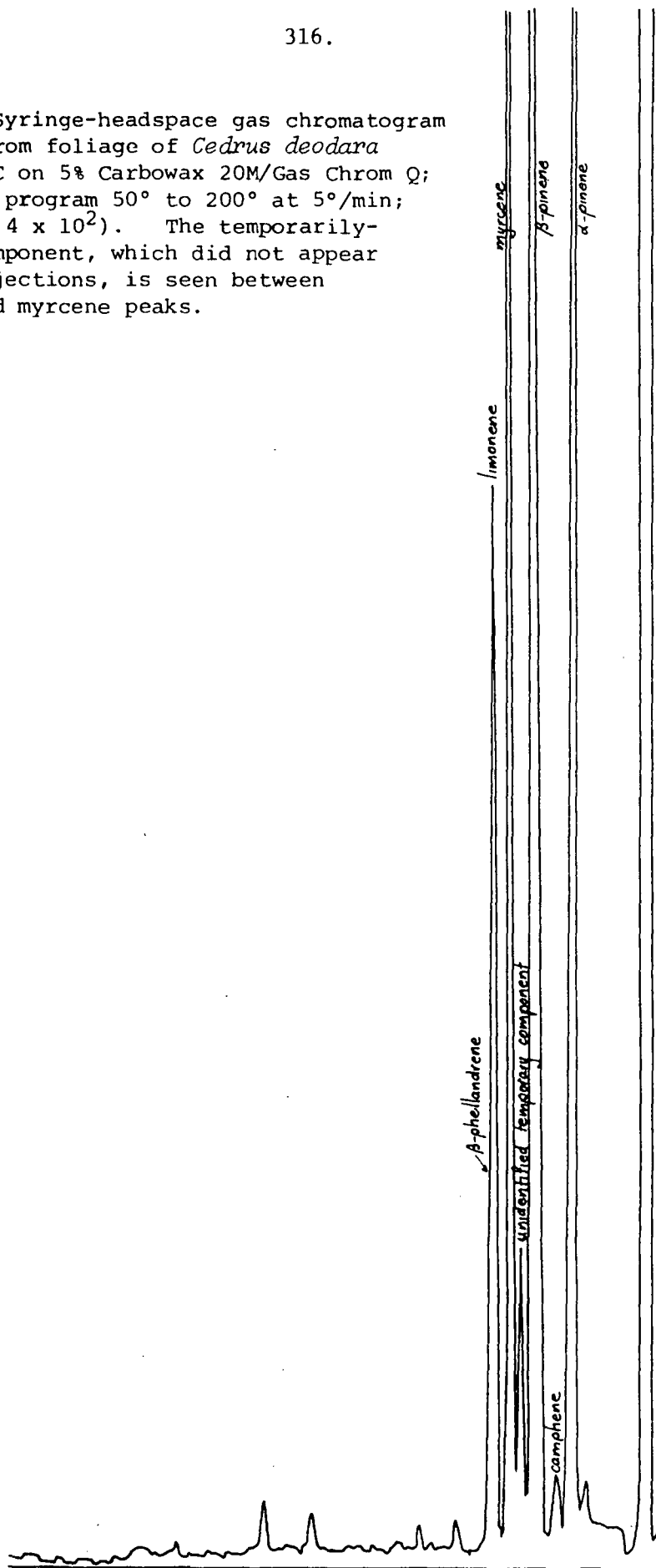
Table 61. Compositions of monoterpenes in successive
injections of syringe-headspace vapour from
foliage of *Cedrus deodara* (tree I)

Percentage composition of monoterpenes:
(peak height basis)

Time since comminution of sample (mins.)	Unidentified + santene?	α-Pinene	Camphene	β-Pinene + Sabinene	Unidentified	Myrcene	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene
0	0.3	49.4	0.4	17.9	2.8	21.3	6.4	1.3	t	0.3
20	0.7	54.9	0.5	18.4	0.3	18.5	5.5	1.1	t	0.2
35	0.9	55.7	0.5	18.2	t	18.3	5.5	1.0	t	t
55	0.9	55.6	0.5	18.2		18.1	5.5	1.0	t	0.2
70	1.0	55.4	0.5	18.3		18.3	5.3	1.0	t	0.2
90	1.0	54.4	0.4	18.8		18.5	5.5	1.0	0.1	0.3
105	1.0	54.2	0.4	18.7		19.0	5.6	1.0	t	0.1
120	0.8	54.6	0.5	18.7		18.7	5.5	1.0		0.1
135	0.9	55.6	0.5	18.4		18.3	5.2	0.8	t	0.2
150	0.8	57.2	0.5	18.2		17.3	4.8	0.9	t	0.2
170	0.3	55.9	0.4	19.1		18.5	5.0	0.8		t
185	0.5	56.1	0.5	18.6		18.3	4.9	0.9		0.2
200	0.6	55.2	0.6	18.6		18.6	5.1	0.9	0.1	0.3

(d) Composition of syringe-headspace vapour from foliage
of several trees of *Cedrus deodara* (Trees I-IV)

The wide variation in monoterpene composition of
the vapour from a single sample of foliage, from each of four
trees, is shown in Table 62. In the vapour sample from each
tree there appeared to be the same temporarily-released
component. The 'temporary component' also appeared to be
produced for different initial periods in foliage samples from
individual trees, although further work would be required to
determine whether this was a tree-dependent characteristic.

Fig. 46. Changes in the percentages of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Cedrus deodara* (Tree I).

318.
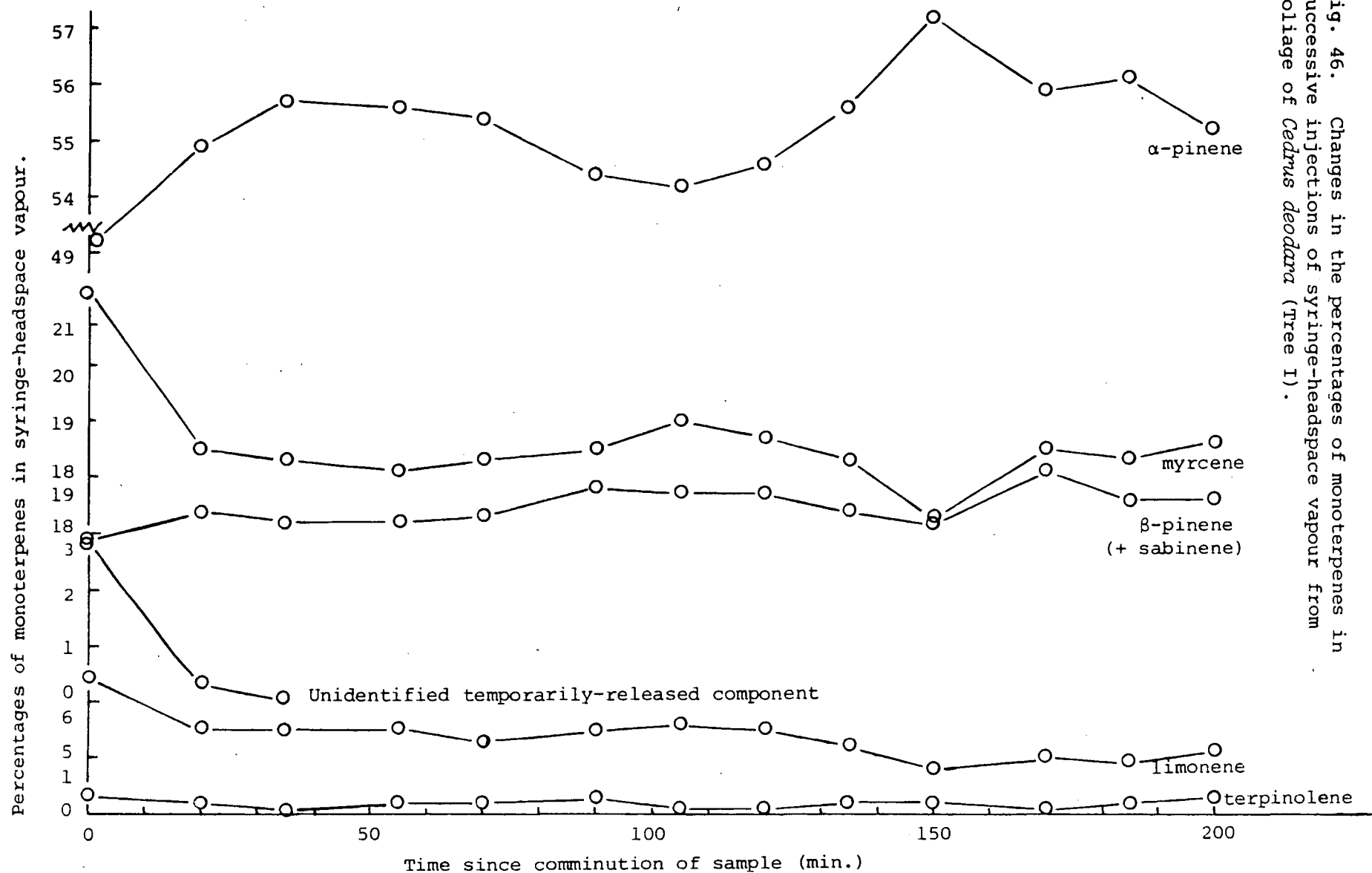
Table 62. Composition of monoterpenes in syringe-headspace injections of vapour from foliage of several trees of *Cedrus deodara*. The compositions of the first three successive injections are shown in each case to illustrate the apparent "disappearance" of the component eluting near Δ_3-carene.

Percentage composition of monoterpenes (peak height basis):

Tree No.	Time since comminution (mins.)	Unidentified + santene?	α-Pinene	Camphene	β-Pinene	Unidentified	Δ_3-Carene	Myrcene	Limonene	β-Phellandrene	Terpinolene
I	0	–	60.5	0.5	21.1	1.8	–	10.9	4.7	t	0.2
	40	0.4	72.5	0.5	16.0	–	–	7.4	3.1	t	0.1
	55	0.5	64.3	0.6	21.0	–	–	9.7	3.9	t	0.1

Steam-distilled oil from remainder of comminuted foliage from tree I

| | | 0.3 | 36.7 | 1.1 | 28.6 | – | 0.2 | 19.7 | 12.4 | t | 0.8 |

+ γ-terpinene 0.1 and ρ-cymene 0.2

Tree No.	Time since comminution (mins.)	Unidentified + santene?	α-Pinene	Camphene	β-Pinene	Unidentified	Δ_3-Carene	Myrcene	Limonene	β-Phellandrene	Terpinolene
II	0	–	59.1	0.6	20.2	1.4	–	14.5	3.7	1.4	0.4
	15	0.6	63.2	0.5	20.2	0.8	–	11.0	3.2	1.3	0.4
	30	0.9	64.4	0.6	20.2	0.3	–	10.1	3.2	1.2	0.4
III	0	0.1	43.8	0.5	15.5	3.3	–	28.2	8.5	0.4	0.3
	15	0.5	48.7	0.4	15.4	3.0	–	24.5	7.3	0.5	0.2
	25	0.8	49.2	0.3	15.9	1.7	–	24.5	7.3	0.7	0.2
IV	0	–	58.4	0.6	25.2	1.0	–	12.8	0.9	1.1	t
	10	0.2	63.9	0.6	23.5	0.4	–	9.9	0.7	0.9	t
	25	0.2	63.9	0.6	23.7	0.1	–	10.0	0.7	0.9	–

t: trace; <0.1 percent
-: not detected

(e)　Composition of syringe-headspace vapour from foliage

　　　sampled repeatedly from the same tree (Tree I)

The composition of the initial injection of vapour from three samples of foliage from tree I varied considerably (Tables 60-63), i.e. from 43.7 to 60.5 percent α-pinene, 16.1 to 25.4 percent β-pinene and 10.6 to 27.1 percent myrcene.

(f)　Composition of syringe-headspace vapour from foliage

　　　sampled daily from a lopped branch (Tree I)

An examination was made over an 18-day period of the monoterpene vapour composition of foliage intact on a lopped branch. Most needles remained intact for 13 days, then rapidly detached within a 24 hour period, after which the analyzed sample consisted of comminuted bark and fine twigs. Fluctuations in monoterpene proportions were encountered in the first 13 days, after which a considerably different composition became apparent. This latter change could however be due to the fundamentally different composition to be expected from the analysis of a different organ, i.e. a change from needles + bark + twigs to bark + twigs. Fundamental differences in composition have recently been reported by von Rudloff [325] after a study of leaves, buds and twigs of *Picea mariana*.

Although fluctuations in monoterpene proportions were discernible, no particular pattern of changes could be seen which could have been correlated with an attractiveness to insects immediately after lopping. Table 63 contains the monoterpene compositions of the first three successive injections of each daily sample. In each sample the same

Table 63. Composition of monoterpenes in syringe-headspace injections of vapour from daily samples of foliage of a lopped branch of *Cedrus deodara*. For each sample the composition is given of the first three successive injections. Until day 13 samples consisted of needles, bark and fine twigs, so that from day 14 the samples of bark and fine twigs only could be correlated with a change in monoterpene composition. The 'temporary component' released on days 14 and 15 from bark and fine twigs ceased to be detected from day 16.

Percentage composition of monoterpenes (peak height basis):

Day No.	Time since comminution (mins.)	Unidentified group	Santene?	α-Pinene	Camphene	Unidentified	β-Pinene	Unidentified	Myrcene	Limonene	β-Phellandrene	γ-Terpinene + others	Terpinolene
0	0	t	0.1	43.7	0.4	–	16.1	3.8	27.1	8.5	–	t	0.3
	15	t	0.3	53.8	0.4	0.1	16.3	3.3	19.1	6.6	t	t	0.2
	30	–	0.4	55.3	0.4	–	17.6	1.5	18.4	6.4	t	–	–
1	0	t	t	49.4	0.4	–	16.0	5.3	22.0	6.7	t	–	0.3
	15	t	0.4	58.1	0.4	0.1	17.5	2.1	15.8	5.4	–	–	0.1
	30	–	0.6	59.7	0.5	0.1	18.0	0.9	14.9	5.3	t	t	0.1
2	0	0.1	0.1	51.9	0.6	0.1	16.4	2.9	21.8	5.9	t	–	0.1
	15	0.1	0.3	58.0	0.5	0.1	16.8	1.7	17.6	4.7	t	–	0.2
	30	0.1	0.5	59.2	0.4	t	17.0	0.7	17.2	4.5	t	0.1	0.2
3	0	0.7	t	48.5	0.4	t	15.9	4.4	23.5	6.3	–	t	0.2
	15	0.4	0.3	56.1	0.3	t	16.3	2.4	19.1	4.9	t	–	0.1
	30	0.3	0.4	58.1	0.4	t	16.5	1.2	18.3	4.8	0.4	–	0.1
4	0	0.1	0.1	58.4	0.5	0.1	18.8	3.2	14.1	4.6	–	–	0.1
	15	–	0.3	62.1	0.6	0.1	19.4	1.7	11.9	4.0	t	t	0.1
	30	–	0.4	63.1	0.5	0.1	19.2	0.9	12.0	3.9	t	t	t
5	0	0.4	0.2	51.9	0.5	0.1	16.3	4.4	20.1	6.1	–	t	0.1
	15	0.2	0.3	60.0	0.4	0.1	17.6	2.0	14.1	5.1	t	–	0.1
	30	–	0.5	61.0	0.5	0.1	18.0	1.0	13.8	5.0	0.4	–	0.1

Table 63 continued

Percentage composition of monoterpenes (peak height basis):

Day No.	Time since comminution (mins.)	Unidentified group	Santene?	α-Pinene	Camphene	Unidentified	β-Pinene	Unidentified	Myrcene	Limonene	β-Phellandrene	γ-Terpinene + others	Terpinolene
6	0	0.1	0.1	54.5	0.5	0.1	18.3	2.6	18.5	5.2	0.9	–	0.2
	15	0.1	0.2	57.2	0.4	0.1	18.6	2.3	16.4	4.7	0.4	–	0.1
	35	0.1	0.4	57.5	0.4	0.1	18.7	1.1	16.4	4.8	0.5	–	0.1
7	0	–	t	50.9	0.4	0.1	16.8	4.2	21.5	6.1	t	–	t
	10	t	0.1	59.5	0.4	0.1	17.1	1.8	16.1	4.9	t	–	0.1
	25	t	0.3	60.9	0.4	0.1	17.6	0.9	15.2	4.6	t	–	0.1
8	0	–	0.4	51.9	0.4	t	17.5	3.6	19.6	6.4	t	–	0.2
	15	–	t	58.8	0.5	t	19.5	0.8	15.2	5.2	–	–	–
	30	–	0.5	60.0	0.6	–	19.9	–	14.2	4.7	–	–	–
9	0	0.9	t	45.7	0.4	0.1	17.5	5.1	23.7	6.4	–	t	0.2
	15	0.3	0.3	58.4	0.4	0.1	16.7	2.2	17.4	4.3	t	–	0.1
	30	0.2	0.5	59.1	0.4	0.1	17.0	0.9	17.4	4.2	0.4	t	0.1
10	0	0.7	t	50.5	0.4	0.1	17.8	3.2	20.5	6.5	t	–	0.4
	15	0.2	t	60.7	0.6	t	18.3	1.6	13.4	5.1	t	–	0.1
	40	0.3	0.3	62.1	0.6	–	18.9	0.4	12.5	4.9	t	t	0.1
11	0	2.6	t	52.3	0.4	–	17.8	3.9	17.0	5.8	t	t	0.2
	15	1.7	t	58.7	0.4	t	18.1	2.4	13.9	4.7	t	–	t
	30	1.7	0.4	59.2	0.6	0.1	18.4	1.0	13.7	4.8	t	–	0.1
12	0	0.2	0.2	46.6	0.2	–	15.6	6.2	23.4	7.4	t	t	0.2
	15	t	0.3	56.3	0.4	0.2	16.8	2.5	17.3	6.1	t	t	0.1
	30	t	0.5	57.5	0.5	0.1	17.3	1.0	16.9	5.9	0.6	t	0.2

Table 63 continued

Percentage composition of monoterpenes (peak height basis):

Day No.	Time since comminution (mins.)	Unidentified group	Santene?	α-Pinene	Camphene	Unidentified	β-Pinene	Unidentified	Myrcene	Limonene	β-Phellandrene	γ-Terpinene + others	Terpinolene
13	0	1.4	t	52.7	0.4	–	18.4	4.0	16.0	7.0	t	–	0.1
	15	0.8	0.2	59.8	0.5	0.1	19.2	1.2	12.3	5.9	t	t	0.1
	35	0.7	0.4	61.2	0.5	0.1	19.4	0.3	11.7	5.5	0.3	–	0.1

(all needles detached; bark and twigs only)

Day No.	Time since comminution (mins.)	Unidentified group	Santene?	α-Pinene	Camphene	Unidentified	β-Pinene	Unidentified	Myrcene	Limonene	β-Phellandrene	γ-Terpinene + others	Terpinolene
14	0	0.2	–	56.6	0.4	t	19.4	0.9	16.6	5.7	–	–	0.1
	15	0.3	0.1	64.9	0.5	0.1	19.8	0.2	9.5	4.6	t	–	0.1
	30	0.5	0.1	65.6	0.6	0.1	20.1	0.1	8.5	4.3	t	–	0.1
15	0	0.1	0.1	64.3	0.5	–	22.2	0.8	6.3	5.6	–	–	0.2
	15	t	–	69.3	0.6	0.1	22.3	t	3.5	4.1	t	–	0.1
	35	0.1	t	68.8	0.6	t	23.1	–	3.3	4.0	–	–	0.1
16	0	0.9	–	68.5	0.6	t	24.7	–	1.3	3.9	t	–	0.1
	15	0.4	–	69.9	0.6	–	24.3	–	1.2	3.5	t	–	0.1
	30	0.3	–	66.4	0.6	0.1	28.0	–	1.1	3.3	t	–	0.1
17	0	0.5	–	68.3	0.5	–	24.5	–	1.6	4.4	–	–	0.2
	15	0.1	t	72.2	0.7	t	22.7	–	1.1	3.1	–	–	0.1
	30	0.1	t	72.1	0.6	t	22.9	–	1.1	3.1	–	t	0.1
18	0	1.2	–	68.0	0.6	–	23.7	–	1.4	5.0	–	–	0.1
	15	0.6	–	71.0	0.7	0.1	22.6	–	1.2	3.9	–	–	0.1
	35	0.6	t	70.6	0.7	0.1	22.8	–	1.2	4.0	–	–	0.1

'temporary component' appeared to decrease in the initial injections. It could be of interest for the study of insect attractiveness to note that the 'temporary component' appeared in the vapour of comminuted bark and twigs on days 14 and 15, but then ceased to be detected. Figure 47 is an illustration of these changes.

(g) Summary

Components of steam-distilled foliage oil from a single tree of *Cedrus deodara* were analyzed by chromatographic and spectroscopic methods. The following identifications were confirmed: α-pinene (36.2%), β-pinene (22.8%), myrcene (14.9%), limonene (6.8%), terpinolene (0.1%), caryophyllene (3.1%) and α-terpineol. Tentatively identified were camphene (0.5%), sabinene, Δ_3-carene, β-phellandrene (2.9%), γ-terpinene and ρ-cymene. The syringe-headspace GC technique indicated the existence of a wide range of monoterpene compositions to be found in successive injections of vapour from a single sample of foliage, in different samples of foliage from the same tree and from different trees. Successive injections of one sample over a 3 hr. period contained from 49.4 to 57.2% α-pinene, while β-pinene and myrcene varied within 1.2 and 4.0%, respectively. Foliage vapour from 4 samples from the same tree contained from 43.7 to 60.5% α-pinene, 16.1 to 25.4% β-pinene and 10.6 to 27.1% myrcene; whereas from 4 different trees the vapour contained from 43.8 to 72.5% α-pinene, 15.4 to 25.2% β-pinene and 7.4 to 28.2% myrcene. The syringe-headspace monoterpene composition of daily samples of foliage from a lopped branch did not exhibit any fundamental change in composition that might be correlated
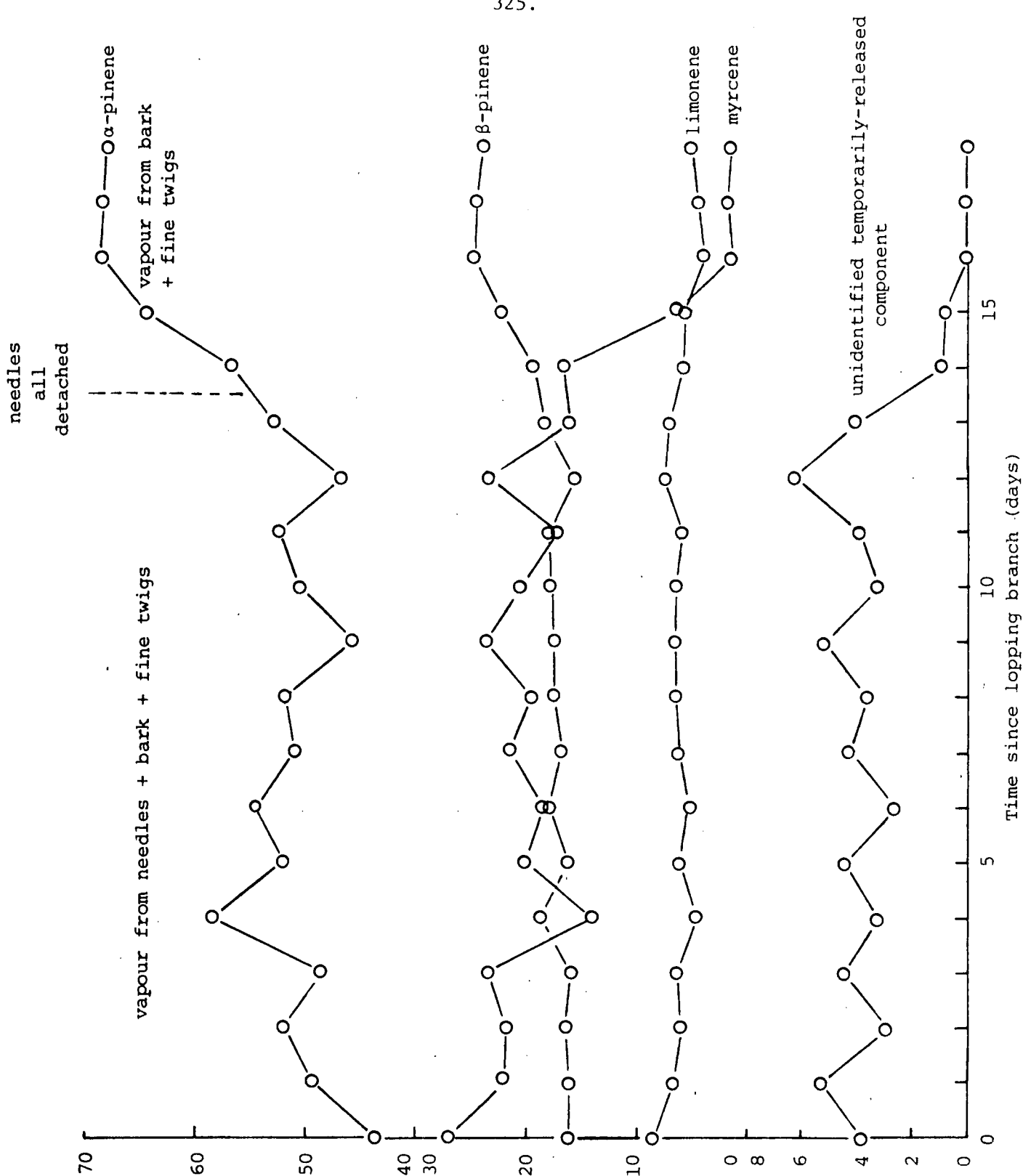
Percentages of monoterpenes in syringe-headspace vapour.

Fig. 47.   Composition of monoterpenes in syringe-headspace initial injections
of vapour from daily samples of foliage of a lopped branch of *Cedrus deodara*.
The 'temporary component' is seen to be released in vapour from bark and fine
twigs on days 14 and 15, but is not detected on further days.   Relationships
between proportions of monoterpenes are apparent in this plot, whereas it
appears in many other plots of quantitative changes that a change in one major
component leads to a percentage inverse effect in the proportion of each other
component.   However in this plot it is clear that a change in α-pinene does
not influence the proportion of limonene or even β-pinene.   The reciprocal
relationship between α-pinene and myrcene could then be attributed to a
feature of the biosynthesis of monoterpenes.   The unidentified 'temporary
component' would therefore also appear to be linked to the biosynthetic
relationship of α-pinene and myrcene.

with attractiveness to *Sirex noctilio* following lopping, although
there was a basic change in the composition of foliage vapour
from the 13th day when all needles became detached. α-Pinene,
myrcene and an unidentified 'temporarily-released component'
exhibited an apparently biosynthetic relationship to one
another. In all syringe-headspace analyses as much as 6.1
percent of the 'temporarily-released component' was found
in the first few successive injections, ranging from 5 to
about 35 minutes, after which it was not detected.

(iii) *Larix decidua* Mill.

The oil of Common or European Larch, *Larix decidua*
Mill., does not appear from reports in the literature to have
been investigated in such detail as has *L. sibirica*. The few
investigations of oil of *L. decidua* since the advent of GC
have dealt almost exclusively with monoterpenoids. Whereas
oils from *L. sibirica*, *L. dahurica* and *L. czekanowskii* have
been distinguished by a high-content of Δ_3-carene [414], this
component was not found in oil from *L. decidua* [415].

Stairs [415] compared the monoterpene composition
of stem oleoresins of *L. decidua* and other species of *Larix*,
and furthermore noted that neither collection season nor growth
rate appreciably influenced the proportions of monoterpenes.
L. decidua was found to contain approximately 84 percent
α-pinene, 16 percent β-pinene, 3 percent limonene, 2 percent
camphene and 1 percent myrcene. The monoterpene compositions
of *L. decidua* and *L. leptolepis* were similar, yet different from

those of other species [415]. *L. laricina* and *L. occidentalis*
had lesser proportions of α-pinene (about 66 percent) and a
small amount of Δ_3-carene (about 9 percent). *L. gmelini*
L. sibirica contained even less α-pinene (about 29 percent),
but more Δ_3-carene (about 44 percent). It is interesting to
note that both *L. decidua* and *L. leptolepis* have been
considered to be attractive to *Sirex noctilio* [395].

A further investigation by Deryuzhkin *et al* [416],
of the oils of *L. decidua, L. sibirica* and *L. dahurica,* led
these workers to conclude however that *L. decidua* was one of
a number of species that contained significant amounts of
Δ_3-carene, i.e. 9.8 to 37.7 percent Δ_3-carene. Other
components identified were α-terpinene, β-phellandrene,
γ-terpinene, terpinolene and santene. Other workers have
subsequently identified in oils of *Larix* spp. minor amounts
of tricyclene, α-phellandrene, cineole, ρ-cymene [417] and
chavicol methyl ether [419]. Gibbard and Schoental [418]
studied wood smokes and wood distillates and reported coniferyl
aldehyde and vanillin from *L. decidua*.

(a) <u>Analysis of the isolated foliage oil</u>

Needle-covered foliage, available in limited quantity
(39 g.) in the Royal Botanical Gardens (Tasmania), yielded upon
steam-distillation 0.6 percent of a light green oil with a
strong woody odour. The study was restricted to foliage oil
because of the small size of the tree which would not have
permitted isolation of oleoresin from the stem. The 0.23 g of

oil obtained from foliage was insufficient for isolation and spectroscopic identification of individual components.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 64. The gas chromatogram (Figure 48) shows that this oil contained a number of very high-boiling components, which accounted for about 50 percent of the total oil mass. The quantitative composition reported in Table 64 is therefore not comparable with data summarized from the literature, because published values were all based upon percentages of monoterpenes.

The major components found were several unidentified high-boiling materials, together with α-pinene and terpinen-4-ol. A smaller quantity of Δ_3-carene agreed with the finding of Deryuzhkin *et al* [416], which linked this oil with those of others containing Δ_3-carene. The components tentatively identified by GC in Table 64 agree with those reported as occurring in *Larix* species [417].

Evidence was found of degradation of high-boiling components, which would lead in the future to some concern as to the validity of any attempt to isolate and identify these materials. Degradation was noted during isothermal elution at 130°, when peak shapes acquired the characteristic asymmetric broadening together with a noisy baseline.

Table 64. Components distinguishable in the whole
oil of *Larix decidua*

Component	Qualitative RRT data C2OM	OV-17	Quantitative composition (percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 240°, 5°/min)
Unidentified	0.55		0.4
"	0.66		1.3
α-Pinene	1.00	1.00	12.9
Camphene	1.28	1.20	0.9
β-Pinene	1.61	} 1.54	2.6
Sabinene	1.75		0.4
Δ₃-Carene	2.02	1.96	2.7
Myrcene	2.22	1.75	1.2
Unidentified	2.48	2.17	0.1
Limonene	2.76	2.39	1.3
β-Phellandrene	2.94	2.52	2.8
1.8-Cineole	3.05	2.80	3.8
γ-Terpinene	3.67	3.29	0.2
ρ-Cymene	4.27	2.80	1.0
Terpinolene	4.59	4.13	0.9
Unidentified	6.22		0.2
	(130° isothermal, ref. camphor)		
Terpinen-4-ol	1.20	1.00	12.9
Chavicol methyl ether	1.56	1.32	5.9
α-Terpineol?	1.77	1.11	?
	(180° isothermal, ref. thymol)		
Unidentified	4.44	13.8	(major)
Sum of unspecified peaks			48.6

Where Δ_3-Carene appears and ρ-Cymene is ρ-Cymene.

330.

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of dormant bark and fine twigs (without needles) confirmed that the monoterpene composition was principally α-pinene and β-pinene with smaller amounts of Δ_3-carene, limonene and β-phellandrene (Table 65).

Table 65. RRT data and percentage composition of volatile terpenoids in vapour from dormant bark and fine twigs of *Larix decidua* determined by syringe-headspace GC analysis

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak area)
	(60° isothermal, ref. α-pinene)		(60° isothermal)
Unidentified	0.66		
"		0.26	
"		0.49	0.2
"		0.75	
α-Pinene	1.00	1.01	81.6
Camphene	1.29	1.17	1.6
β-Pinene	1.64	1.53	11.3
Sabinene	1.79		1.0
Δ_3-Carene	2.09	1.91	1.8
Myrcene	2.31	1.72	0.7
Unidentified	2.51	2.12	t
Limonene	2.84	2.31	0.6
β-Phellandrene	2.99	2.46	1.1
γ-Terpinene	3.84		t
ρ-Cymene	4.44	2.86	0.1
Terpinolene	4.89	4.07	t

t: trace; <0.1 percent
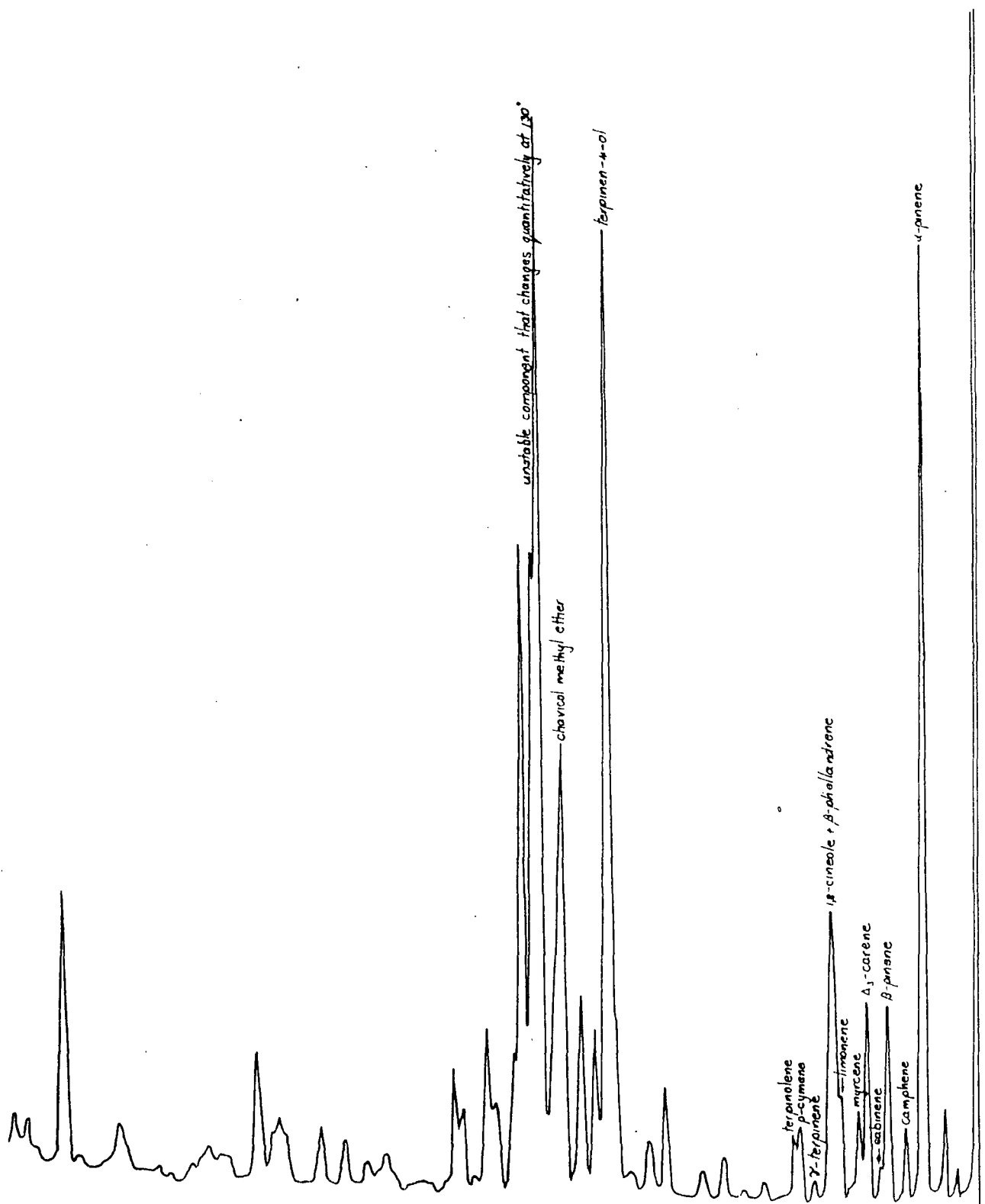
Fig. 48. Low sensitivity gas chromatogram of whole oil of summer foliage of *Larix decidua* (GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50° to 240° at 5°/min; 0.2 μℓ sample, attenuation 8 x 10^2).

(c) Composition of successive injections of syringe-

    headspace vapour from foliage

Successive injections of vapour over a 3 hr. period
from a single sample of dormant foliage, exhibited changes of
68.6 to 75.3 percent for α-pinene and 9.2 to 12.0 percent for
β-pinene (Table 66). There was no indication of the existence
of any 'temporarily-released component' as found in *Cedrus
deodara*.

The remaining portion of comminuted foliage was steam-
distilled to obtain the oil for direct comparison with the
syringe-headspace vapour (Figure 49).

(d) Composition of syringe-headspace vapour from foliage

    sampled repeatedly from the same tree

Although only two samples were taken for study of the
syringe-headspace vapour composition, considerable differences
were documented for the initial injection on each occasion
(Tables 65 and 66). The percentages found for α-pinene were
68.6 and 81.6, β-pinene 12.0 and 11.3, while Δ$_3$-carene was
found to be 6.2 and 1.8 percent.

(e) Summary

Components tentatively identified by GC in steam-
distilled summer foliage oil from a single tree of *Larix decidua*
were: α-pinene (12.9%), camphene (0.9%), β-pinene (2.6%),
sabinene (0.4%), Δ$_3$-carene (2.7%), myrcene (1.2%), limonene (1.3%),
β-phellandrene (2.8%), 1,8-cineole (3.8%), γ-terpinene (0.2%),

Table 66. Compositions of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Larix decidua*

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified + tricyclene?	α-Pinene	Camphene	β-Pinene	Sabinene	Δ$_3$-carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene	ρ-Cymene	Terpinolene
0	0.2	68.6	3.5	12.0	1.3	6.2	2.0	0.1	1.7	3.7	0.1	0.2	0.3
30	0.2	72.8	3.4	10.5	1.1	5.4	1.7	0.1	1.4	3.0	0.1	0.3	0.2
45	0.2	73.0	3.3	10.2	1.2	5.4	1.7	t	1.4	3.0	0.1	0.3	0.3
60	0.2	73.3	3.0	9.9	1.4	5.2	1.7	0.1	1.4	3.0	0.1	0.3	0.3
75	0.2	73.9	3.1	9.9	1.2	5.1	1.7	0.1	1.4	3.0	0.1	0.2	0.2
90	0.1	74.1	3.2	9.5	1.1	5.2	1.6	0.1	1.3	3.0	0.1	0.3	0.3
105	0.2	74.4	3.2	9.7	0.8	5.1	1.6	0.1	1.3	2.9	0.1	0.3	0.3
120	0.1	74.8	3.1	9.4	0.8	5.1	1.6	0.1	1.3	3.0	0.1	0.3	0.3
135	0.1	74.9	3.1	9.4	0.9	5.1	1.6	0.1	1.3	2.9	0.1	0.3	0.2
150	0.1	75.1	3.1	9.4	0.9	5.0	1.6	t	1.3	2.9	0.1	0.3	0.2
165	0.1	75.3	3.0	9.3	0.9	5.0	1.6	t	1.3	2.9	0.1	0.3	0.2
180	0.1	75.2	3.0	9.2	1.0	5.0	1.6	0.1	1.2	3.0	0.1	0.3	0.2

Steam-distilled oil from remaining portion of comminuted foliage:

	7.8	53.3	2.5	9.6	1.1	6.7	2.4	0.4	3.7	10.0	0.5	1.0	1.0

t: trace; <0.1 percent

333.

Fig. 49(a).   Syringe-headspace gas
chromatogram of vapour from dormant
foliage of *Larix decidua* (GC on 5%
Carbowax 20M/Gas Chrom Q;   temperature
program 50° to 140° at 5°/min;
attenuation 4 x 10^2).
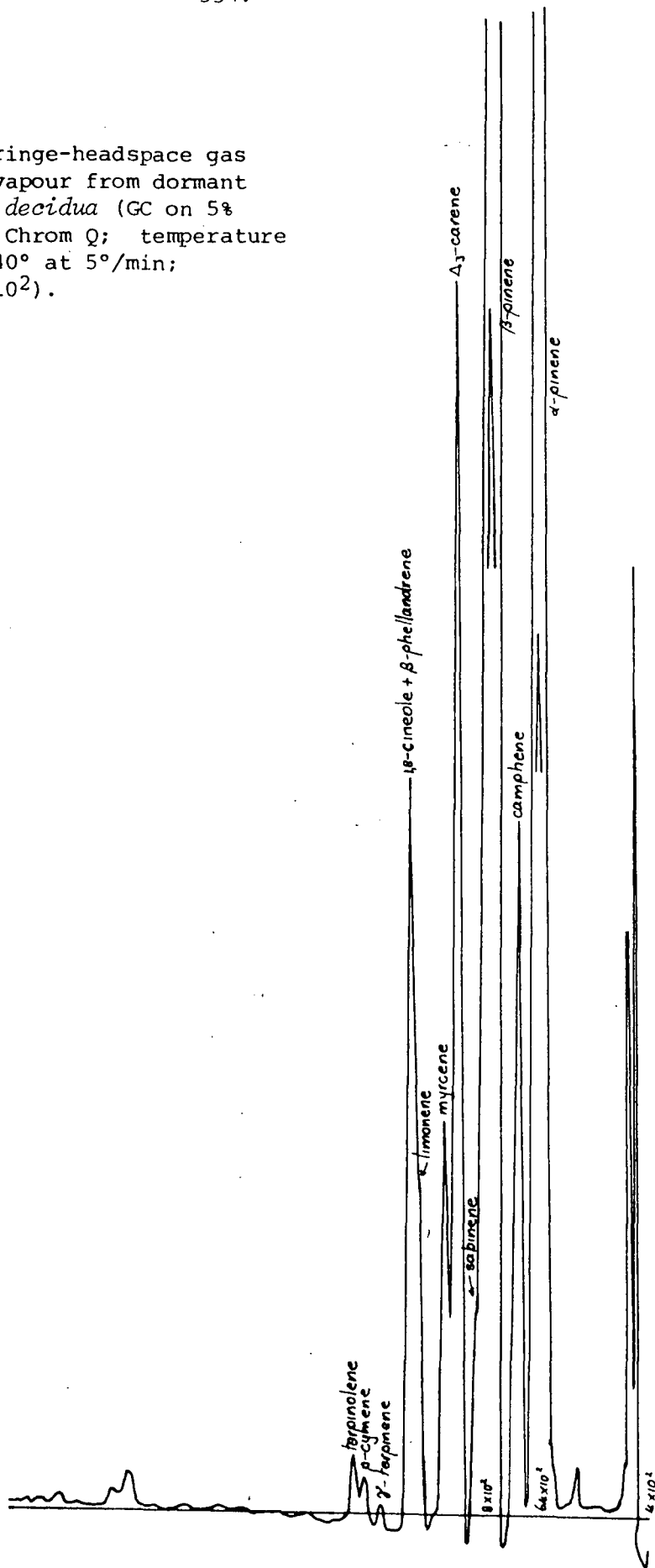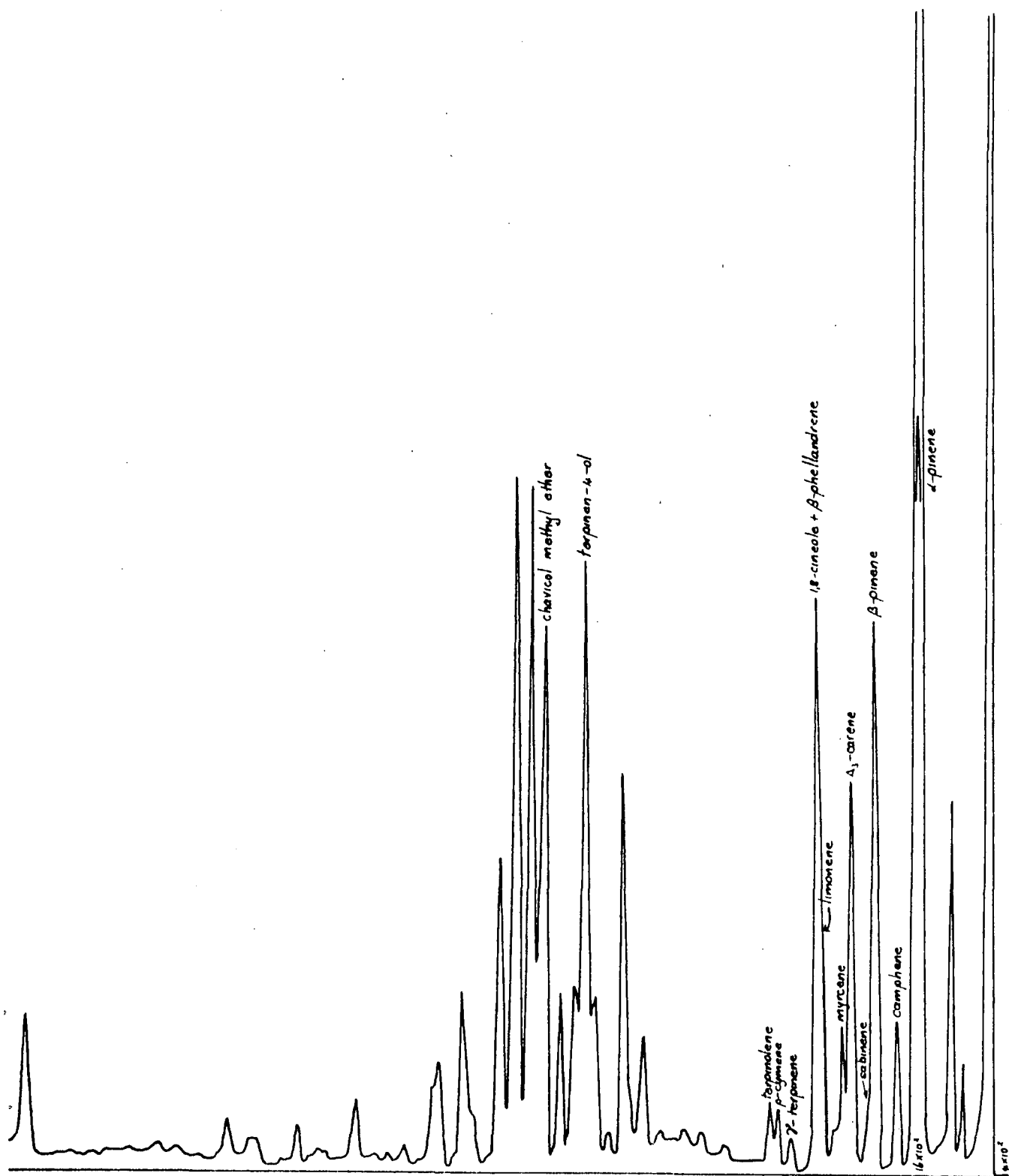
Fig. 49(b).   Gas chromatogram of steam-distilled oil from remainder of dormant foliage sample studied by the syringe-headspace technique in Fig. 49(a), Table 65 (GC conditions as before, attenuation 4 x 10^2).   Fundamental differences exist between this dormant foliage oil and the summer foliage oil of Fig. 48.

ρ-cymene (1.0%), terpinolene (0.9%), terpinen-4-ol (12.9%) and chavicol methyl ether (5.9%). Approximately 50% of the steam-distilled oil was found to be high-boiling components. The syringe-headspace GC technique indicated the existence of a wide range of monoterpene compositions to be found in successive injections of vapour from a single sample of dormant foliage, and also in vapour from different samples of foliage from the same tree. Successive injections over a 3 hr period contained from 68.6 to 75.3% α-pinene and 9.2 to 12.0% β-pinene; whereas from 2 samples of foliage from the same tree the vapour contained from 68.6 to 81.6% α-pinene, 12.0 to 11.3% β-pinene and 1.8 to 6.2% Δ_3-carene.

(iv) *Picea abies* (L.) Karst.

Foliage oil of Common (European or Norway) Spruce, *Picea abies* (L.) Karst. or *P. excelsa* Link, has been studied in considerable detail by several workers since the advent of GC.

Von Schantz [420, 424] and Juvonen [424] compared the composition of oils of *P. abies* with 12 other *Picea* species and showed for each species that there were major quantitative differences between oils steam-distilled from needles, twigs, branches, the trunk and even the roots. Components detected were santene, tricyclene, α-pinene, fenchene, camphene, β-pinene, sabinene, Δ_3-carene, myrcene, α-phellandrene, limonene, β-phellandrene, cineole, γ-terpinene, α-terpinene, cis-β-ocimene, ρ-cymene, terpinolene, fenchone, camphor, bornyl acetate,

β-caryophyllene, chavicol methyl ether, borneol, α-terpineol, δ-cadinene and several unidentified sesquiterpenes. Yankov *et al* [425] confirmed the presence of many of these components. In addition von Schantz and Juvonen [421] detected in *P. abies* and other species the sesquiterpenes: longipinene, elemene, β-caryophyllene, β-humulene, α-muurolene, δ-cadinene and an unidentified sesquiterpene. Other volatile components reported from *P. abies* include longifolene in a bark extract [422], α-terpinyl acetate and perillaldehyde in steam-distillate from spruce gum [423], α-thujene in solid-sample gas chromatograms of volatiles from needle tissue [71, 154] together with relatively high proportions of fenchol, terpinen-4-ol and carvone [419].

The various oils obtainable from a single tree of *P. abies* have been reported to exhibit quite different quantitative compositions [420, 421]. For example the principal components of needle oil were limonene and borneol, twig oil contained large amounts of borneol and camphene, oils from branches and the stem had high contents of limonene and α-pinene, while oil from the roots was high in β-pinene and α-pinene. Table 67 contains a summary of some quantitative differences between these oils.

Table 67. Major quantitative differences (percent)
between oils steam-distilled from various
tissues of *Picea abies* [420, 421]

Oil source:

Component	Needles	Twigs	Branches	Stem	Roots
α-Pinene	(major) 10.0		(major)	(major) 24.9	(major)
Camphene	9.7	(major)			
β-Pinene				19.7	(major)
Δ$_3$-Carene	<1	1-5	<1	<1	1-5
Myrcene					
Limonene	25.3		(major)	(major) 33.4	
β-Phellandrene	14.8			12.5	
Camphor	5.2				
Borneol	(major) 17.5	(major)			
α-Longipinene	0.3	0.3	0.7	0.7	not studied
Elemene	0.5				
β-Caryophyllene	0.5	(detected)	(detected)	(detected)	
β-Humulene	0.5				
α-Muurolene	1.1	(detected)	(detected)	(detected)	
δ-Cadinene	1.4	3.5	0.5	0.6	
Unidentified sesquiterpene	0.6	0.4			

An indication of the quantitative composition can
be obtained by comparison of the data reported by Ohloff [423]:

6% ℓ-2-pinene, 8% ℓ-camphene, 60% ℓ-2(10)-pinene,

10% ℓ-Δ$_3$-carene, 6% dipentene; small amounts of

ℓ-α-phellandrene, ℓ-β-phellandrene, ℓ-limonene,

α-terpinene, terpinolene; 9% of higher-boiling

compounds including ℓ-borneol, ℓ-bornyl acetate,

ℓ-α-terpineol, ℓ-α-terpinyl acetate and perillaldehyde;

and by Bardyshev and Cherches [426]:

> 0.6% santene, 1.5% tricyclene, 10.0% ℓ-α-pinene,
>
> 12.8% ℓ-camphene, 3.5% ℓ-β-pinene, 5.6% Δ$_3$-carene
>
> + myrcene, 7.2% cineole, 12% ℓ-limonene + dipentene,
>
> 1.2% ρ-cymene + ℓ-β-phellandrene, 4% ℓ-camphor,
>
> ℓ-borneol, 15% ℓ-bornyl acetate and 2% of a sesquiterpene
>
> fraction.

These oils were steam-distillates of spruce gum and needles,
respectively. The compositions were obtained by fractional
distillation followed by chemical identification of the isolates.

(a) <u>Analysis of the isolated foliage oil</u>.

A pleasant camphor-smelling, light-green oil was
distilled in 0.3 percent yield from 115 g. of needles and fine
twigs, available in limited quantity from a small tree in the
Royal Botanical Gardens (Tasmania). A study of this species
was restricted to foliage or needle oil because the small size
of the tree would not have permitted isolation of oleoresin
from the stem. The 0.30 g of oil obtained was insufficient
for isolation and spectroscopic identification of components.

The qualitative and quantitative composition of
components distinguishable in the whole oil, using two
dissimilar columns, is recorded in Table 68. Gas chromatograms
of Figure 50 indicate the complexity of this oil, particularly
the existence of a large number of high-boiling components
which constitute a major portion.

The quantitative composition listed in Table 68 is similar to that reported by Bardyshev and Cherches [426]. The components tentatively identified by GC have all been previously documented [419, 420] in this oil.

Table 68. Components distinguishable in the whole oil of *Picea abies*

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak height)
(60° isothermal, ref. -pinene)			(TP 50° to 245°, 5°/min)
Unidentified	0.57		0.1
"	0.71		0.3
Santene?	0.82		1.0
Tricyclene	0.92	0.89	0.9
α-Pinene	0.99	0.99	8.1
Camphene	1.26	1.20	8.4
β-Pinene	1.57	1.56	7.6
Sabinene	1.74		0.5
Δ_3-Carene	2.01	1.97	2.7
Myrcene	2.20	1.76	6.5
Limonene	2.71	2.37	12.9
1,8-Cineole	3.08	2.78	5.4
γ-Terpinene	3.66	3.24	0.2
ρ-Cymene	4.29		0.2
Terpinolene	4.59	4.10	0.7
(130° isothermal, ref. camphor)			
Camphor	1.00	1.05	3.0
Terpinen-4-ol	} 1.23	0.98	} 12.4
Bornyl acetate		1.53	
α-Terpineol	1.81	1.05	} 8.0
Unidentified	1.85	1.60	

Sum of unspecified peaks   21.2
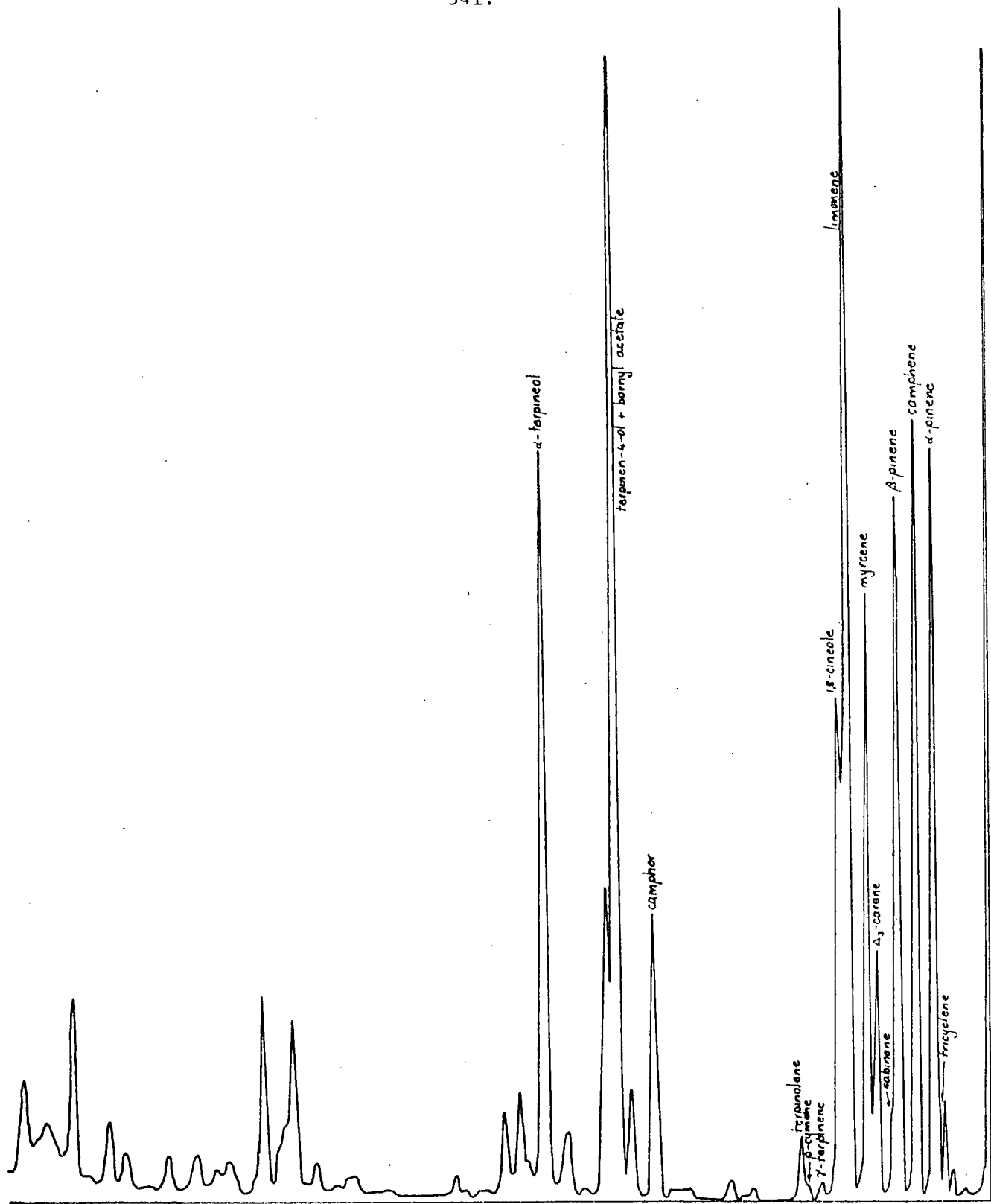
Fig. 50(a). Low sensitivity gas chromatogram of whole oil of foliage of
Picea abies (GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50° to
245° at 5°/min; 0.2 µℓ sample; attenuation 16 x 10^2).
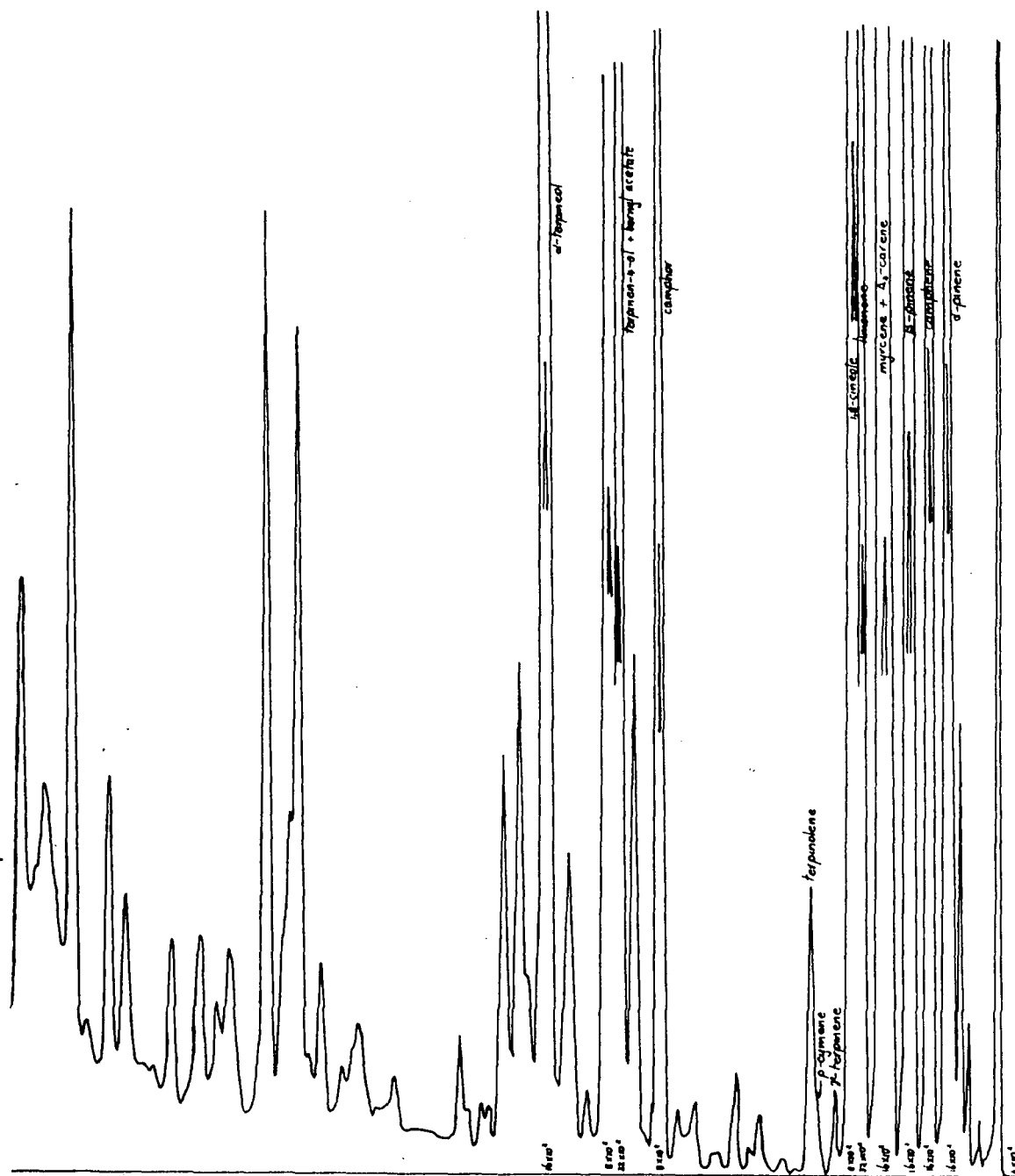
Fig. 50 (b). High sensitivity gas chromatogram of whole oil of foliage
of *Picea abies* (GC conditions as before; attenuation 4 x 10^2).

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from comminuted needles and fine twigs (Table 69, Figure 51) confirmed the existence of the monoterpenes already identified, but also indicated some minor volatiles eluting before α-pinene.

Table 69.   RRT data and percentage composition of volatile terpenoids in vapour from foliage of *Picea abies* determined by syringe-headspace GC analysis

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak height)
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min)
Unidentified	0.63		⎫
Santene?	0.76		⎪
Unidentified		0.29	⎬ 0.2
"		0.41	⎪
"		0.72	⎭
Tricyclene	0.88	0.88	4.0
α-Pinene	0.98	1.00	22.9
Camphene	1.27	1.20	21.3
β-Pinene	1.62	1.52	8.0
Sabinene	1.78		1.5
Δ_3-Carene	2.09	1.90	9.7
Myrcene	2.32	1.71	7.4
Limonene	2.83	2.32	14.7
β-Phellandrene	3.02	2.46	6.7
ρ-Cymene		2.83	0.6
Terpinolene	4.87	4.03	t
(130° isothermal, ref. camphor)			
Terpinen-4-ol and Bornyl acetate	1.23		1.8

t: trace; <0.1 percent

Fig. 51.   Syringe-headspace gas chromatogram
of vapour from foliage of *Picea abies* (GC
conditions as before;   attenuation 4×10^2).
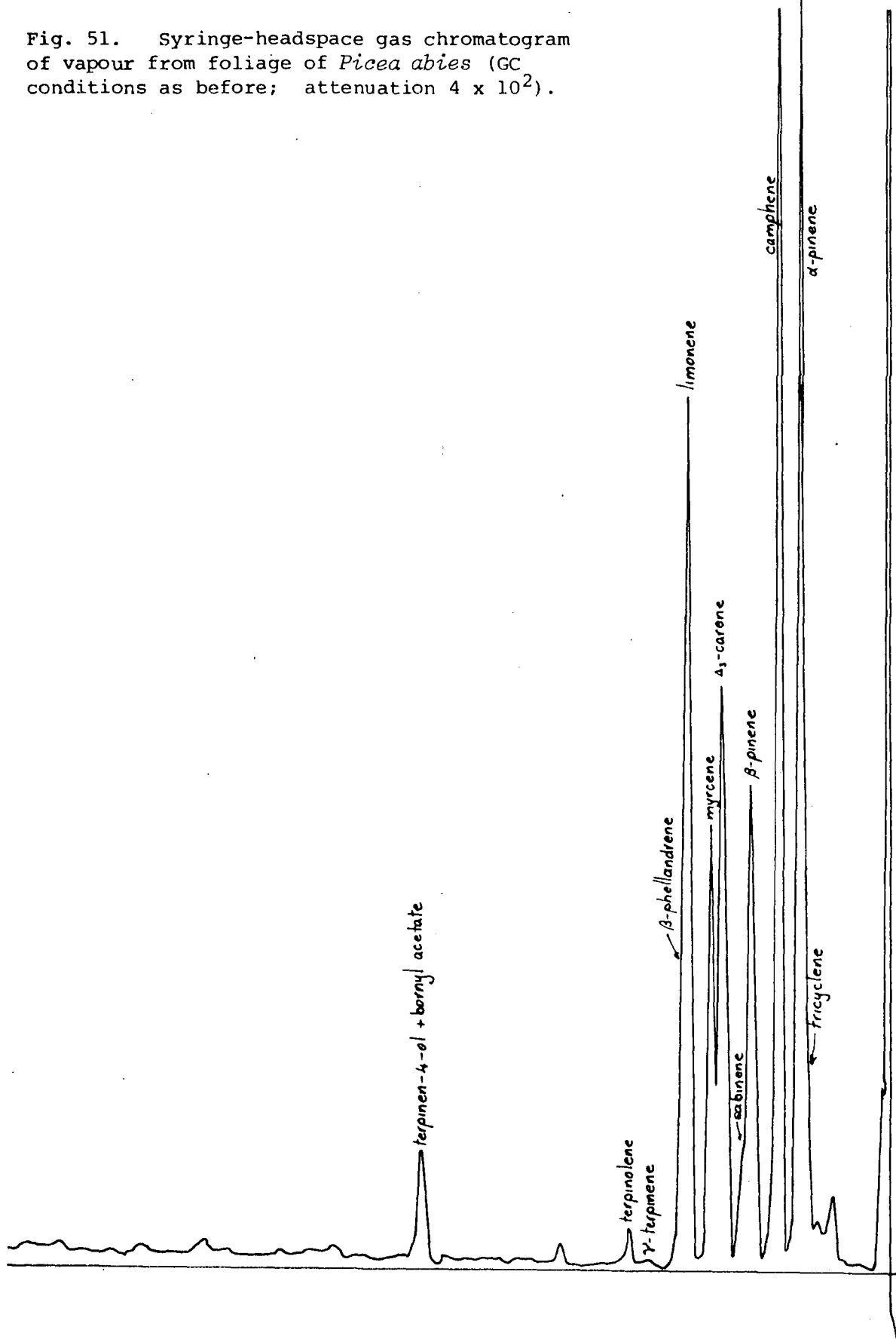
(c) Composition of successive injections of syringe-headspace
vapour from foliage

Successive injections of vapour over a $3\frac{1}{2}$ hr. period,
from a single sample of foliage, exhibited changes of 24.9 to
30.1 percent for α-pinene, 11.8 to 15.3 percent for β-pinene
and 15.9 to 22.6 percent for Δ_3-carene (Table 70). There was
no indication of the existence of any 'temporarily-released
component'. A plot of the proportionate changes in vapour
components (Figure 52) indicated a possible biosynthetic
relationship between camphene and tricyclene.

(d) Composition of syringe-headspace vapour from foliage
sampled repeatedly from the same tree

From the three samples of foliage studied by the
syringe-headspace technique (initial injections of Tables 69
and 70) considerable differences in compositions were found
for some major components, i.e. α-pinene ranged from 22.9 to
27.6 percent, camphene from 9.1 to 21.3 percent, β-pinene from
8.0 to 15.3 percent, Δ_3-carene from 9.7 to 22.6 percent, while
limonene varied from 9.1 to 14.7 percent.

(e) Summary

Components previously identified in foliage oil of
Picea abies and confirmed by GC were: tricyclene (0.9%),
α-pinene (8.1%), camphene (8.4%), β-pinene (7.6%), sabinene (0.5%),
Δ_3-carene (2.7%), myrcene (6.5%), limonene (12.9%),
β-phellandrene and 1,8-cineole (5.4%), γ-terpinene (0.2%),

Table 70. Compositions of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Picea abies*

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (min.)	Unidentified + santene?	Tricyclene	α-Pinene	Camphene	β-Pinene	Sabinene	Δ₃-Carene	Myrcene	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene
(Sample A)												
0	4.9	2.8	24.9	9.1	15.3	4.4	22.6	5.7	9.1	5.6	t	1.2
25	3.5	3.5	30.1	12.5	13.9	3.3	18.9	5.0	8.4	5.1	t	0.9
45	3.6	3.8	30.0	13.4	13.4	3.0	18.1	5.1	8.6	4.9	t	0.9
80	4.6	4.0	30.0	13.1	13.0	3.0	17.4	5.1	8.8	4.9	t	1.0
105	4.6	4.2	28.9	13.8	12.6	3.0	17.2	5.3	9.3	5.5	0.2	0.9
130	5.1	4.1	28.4	14.2	12.4	3.0	16.9	5.3	9.6	5.4	t	0.9
150	5.2	4.0	28.6	15.3	12.0	2.9	16.5	5.2	9.5	5.4	t	0.9
175	5.5	4.2	28.2	15.6	11.9	2.6	16.3	5.5	9.5	5.4	t	0.7
200	5.7	3.9	28.3	16.5	11.8	2.5	15.9	5.2	9.4	4.8	–	0.9
225	5.6	4.1	27.5	15.4	11.8	2.5	16.4	5.5	10.3	5.4	–	0.9
(Sample B)												
0	0.4	3.6	27.6	17.1	11.9	2.4	14.2	9.5	12.7	6.9	–	0.7
45	1.1	4.0	28.6	19.2	10.7	2.1	14.1	8.5	11.0	5.1	0.1	0.5
60	1.3	3.5	30.2	17.5	10.5	2.0	14.6	8.4	11.3	5.6	t	0.6
Steam-distilled oil from remaining portion of comminuted foliage:												
	9.4	0.7	8.5	7.1	8.0	1.3	16.1	9.3	19.7	16.3	0.4	2.8

t: trace; <0.1 percent

Fig. 52. Composition of monoterpenes in successive injections of syringe-headspace vapour from a single sample of foliage of *Picea abies*. A change in the proportion of a major component has not influenced the proportion of camphene, which is seen to steadily increase in concentration along with the concentration of tricyclene. This relationship could be an indication of a biosynthetic link between these components.
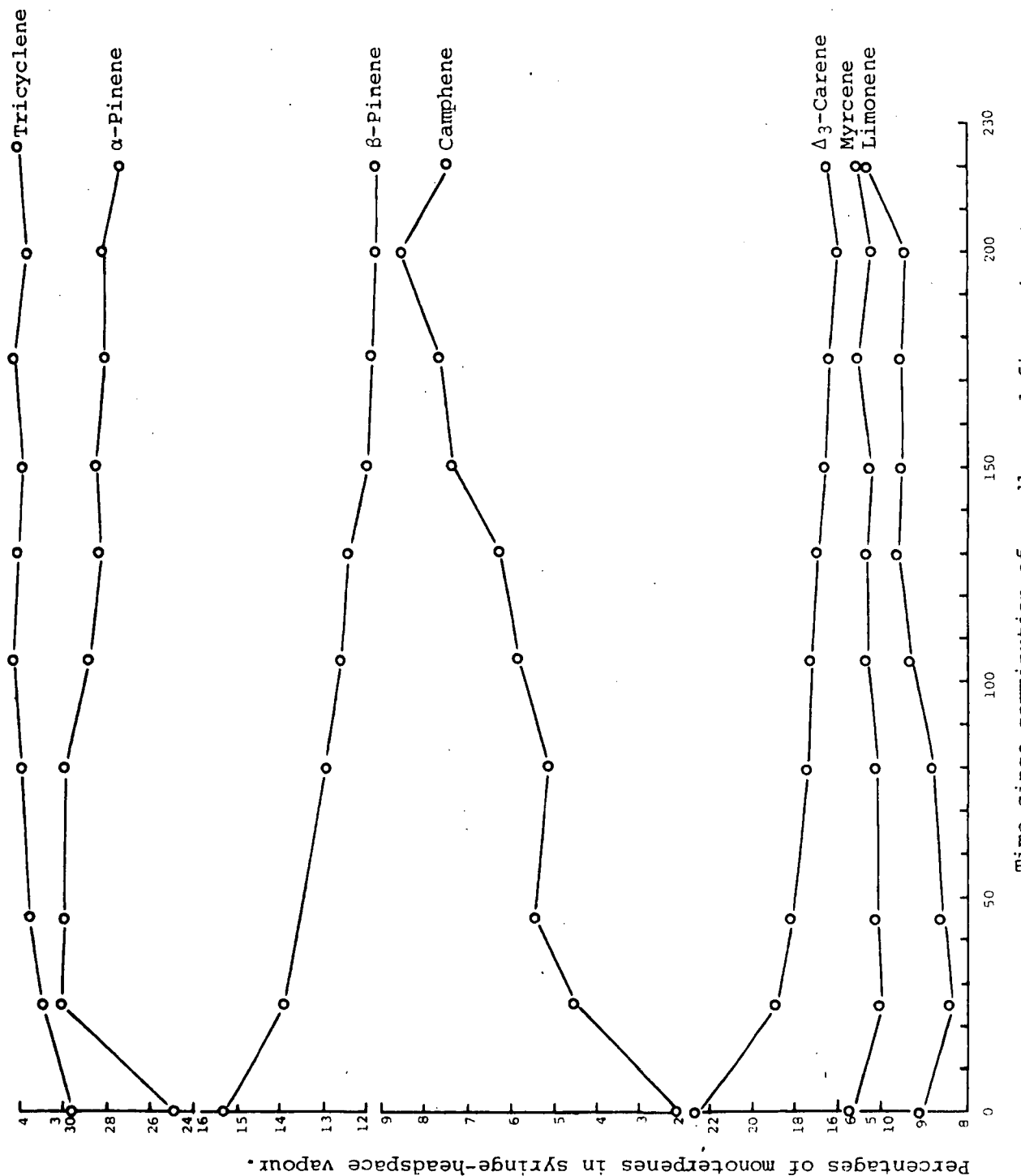
ρ-cymene (0.2%), terpinolene (0.7%), camphor (3.0%),

terpinen-4-ol + bornyl acetate (12.4%) and α-terpineol + an

unidentified component (8.0%). Approximately 20% of the

steam-distilled oil was found to be high-boiling components.

The syringe-headspace GC technique indicated the existence of

a wide range of monoterpene compositions in successive injections

of vapour from a single sample of foliage and also in vapour

from different samples of foliage from the same tree. Over a

$3\frac{1}{2}$ hr. period successive injections contained from 24.9 to 30.1%

α-pinene, 9.1 to 16.5% camphene, 11.8 to 15.3% β-pinene, and

15.9 to 22.6% Δ_3-carene; whereas from 3 samples of foliage from

the same tree the vapour contained from 22.9 to 27.6% α-pinene,

9.1 to 21.3% camphene, 8.0 to 15.3% β-pinene and 9.7 to 22.6%

Δ_3-carene. A possible biosynthetic relationship was exhibited

between tricyclene, camphene, and perhaps α-pinene, which each

varied in an apparently related quantitative manner in a series

of successive injections from a single sample of foliage.

(v) *Pinus attenuata* Lemm.

Turpentine distilled from cortical oleoresin of the

Knobcone Pine, *Pinus attenuata* Lemm., was reported in California,

by Mirov [47] to consist of 98 percent d-α-pinene. Bannister

et al [51] similarly examined steam-distillate of oleoresin,

from trees growing in New Zealand, and reported a mean

composition of 94.2 percent α-pinene, a trace of camphene,

3.2 percent of β-pinene, and a variable Δ_3-carene content of

as much as 10.0 percent. Subsequent investigation by Anderson

et al [314] also showed the existence of n-heptane,

α-phellandrene, myrcene, limonene, β-phellandrene, γ-terpinene
and terpinolene in quite different proportions in volatile
oils from sapwood and heartwood (Table 13). Other studies of
P. attenuata have been made with respect to the biosynthesis
of (+)-α-pinene from labelled mevalonate [427] and the genetic
origin governing α-pinene formation [428].

No report was found in the literature of any study
of the terpenoids released from the foliage.

(a) Analysis of the oil steam-distilled from cortical
     oleoresin

Cortical oleoresin collected by the "tube in the
hole" method [151] from a single tree in the Royal Botanical
Gardens (Tasmania), yielded upon steam-distillation 19.5 percent
of a colourless oil with a pinene odour.

The qualitative and quantitative composition of
components distinguishable in the whole oil, using two dissimilar
columns, is recorded in Table 71. RRT values leading to the
tentative identification of components isolated directly from
the whole oil by preparative GC are listed in Table 72. Gas
chromatograms of Figure 53 clearly illustrate the very small
proportion in this oil of sesquiterpenes and other higher-
boiling components, i.e. approximately 0.1 percent.

The major component in this oil was α-pinene as
reported by Mirov [47] and others [51]. The availability of
better techniques for separation of components in this study
probably led to the recognition of somewhat higher proportions
of minor components, which would tend to reduce the calculated

Table 71. Components distinguishable in the whole
oil from oleoresin of *Pinus attenuata*

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 200°, 5°/min)
*α-Pinene	1.08	1.07	77.4
*Camphene	1.35	1.23	2.8
*β-Pinene	1.67	1.58	9.7
*Δ_3-Carene	2.05	1.98	0.9
*Myrcene	2.23	1.77	1.9
Limonene	2.72	2.39	1.9
*β-Phellandrene	2.89	2.53	5.3
γ-Terpinene?			t
ρ-Cymene?			t
Terpinolene	4.57	4.10	t
(130° isothermal, ref. camphor)			
Terpinolene	0.42	0.54	
Unidentified	0.56	0.85?	t

* IR spectrum recorded
t: trace; <0.1 percent

Table 72. RRT values, on the dissimilar liquid phases
C20M and OV-17, for components found in
preparative GC fractions isolated from whole
oil of *Pinus attenuata* oleoresin

Component	Preparative GC fraction No.	Column	
		C20M	OV-17
(60° isothermal, ref. α-pinene)			
α-Pinene	W1	1.02	1.04
Camphene	W1	1.29	1.24
	W2	1.29	1.21
β-Pinene	W3	1.64	1.58
Sabinene	W2	1.78	1.55
Δ_3-Carene	W4	2.05	1.97
Myrcene	W2	2.25	1.75
Limonene	W5	2.79	2.37
β-Phellandrene	W5	2.95	2.49

Fig. 53(a).   Low sensitivity gas chromatogram
of whole oil from *Pinus attenuata* oleoresin (GC
on 5% Carbowax 20M/Gas Chrom Q;   temperature
program from 50°, 5°/min;   0.2 μℓ sample;
attenuation 16 x 10^3).

Fig. 53(b).   High sensitivity gas chromatogram
of whole oil from *Pinus attenuata* oleoresin (GC
conditions as before;   attenuation 4 x 10^2).

(a)

(b)
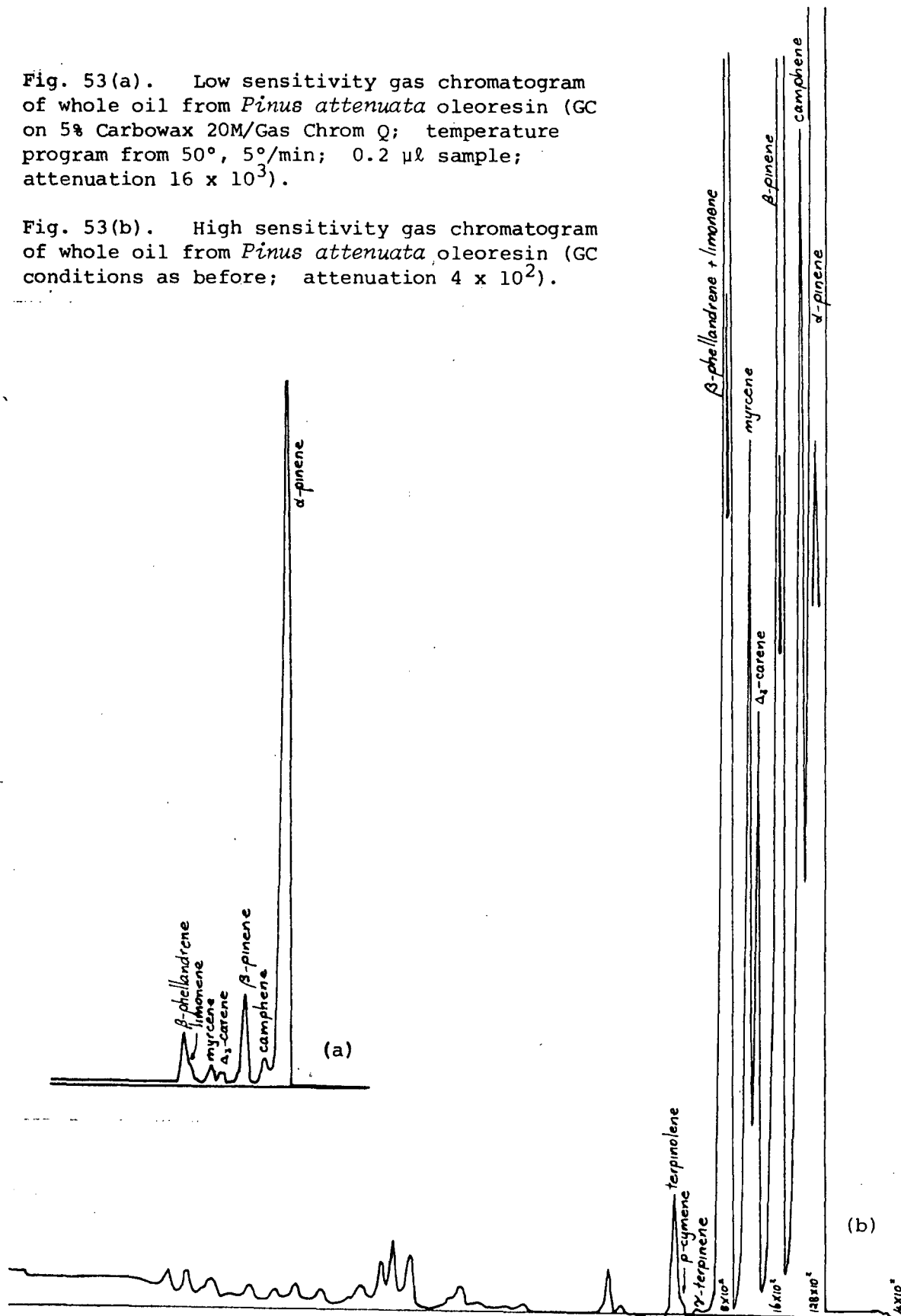
percentage of α-pinene. Whereas Anderson *et al* [314] reported

oil from heartwood to contain 72 percent α-pinene and 15 percent

limonene, the oil in this study from cortical oleoresin contained

77 percent α-pinene, but only 2 percent limonene. A statistical

study of cortical oleoresins would however be required to enable

a distinction to be made between the compositions of the oils

from these two sources.

(b)   Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from

comminuted foliage (Table 73, Figure 54) indicated a

fundamentally different oil vapour composition being

released to the atmosphere from this tree. This oil consisted

not only of α-pinene but also contained a high proportion of

β-pinene (see also Table 74 and Figure 55). A further more

volatile component, possibly santene, was also detected.

(c)   Composition of successive injections of syringe-headspace
      vapour from foliage

Successive injections of vapour over a 3 hr. period,

from a single sample of foliage, exhibited not only a fluctuation

of α-pinene from 51.4 to 57.0 percent, but also some apparently

qualitative changes in the vapour composition. The previously-

mentioned component eluting near Δ_3-carene could only be

detected in vapour injected within approximately 40 minutes

after comminution (Table 74, Figure 55). In Figure 55 an

injection of vapour 40 minutes after the initial injection is

seen to also contain peaks due possibly to santene and a

higher-boiling component.

Table 73.   RRT data and percentage composition of
            volatile terpenoids in foliage of *Pinus*
            attenuata determined by syringe-headspace
            GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak area of 4th successive injection)
	C2OM	OV-17	
(60° isoterhmal, ref. α-pinene)			(60° isothermal)
Santene?	0.79		0.3
α-Pinene	1.00	0.99	50.2
Camphene	1.29	1.18	0.5
β-Pinene	1.63	1.52	40.5
Δ_3-Carene (+ unidentified 'temporary component')	2.08	1.93	2.5
Myrcene	2.31	1.72	1.0
Limonene	2.86	2.34	4.2
β-Phellandrene	3.01	2.48	0.3
Unidentified	4.14		0.4

(d)   Composition of syringe-headspace vapour from foliage

      sampled repeatedly from the same tree

      From the three samples of foliage studied by the

syringe-headspace technique (Tables 73 and 74) considerable

differences in compositions were found for some components,

i.e. 46.4 to 51.4 percent α-pinene, 31.8 to 40.5 percent

β-pinene and 2.5 to 5.8 percent Δ_3-carene.

Fig. 54. Syringe-headspace gas chromatogram of vapour from foliage of *Pinus attenuata* (GC conditions as before; attenuation 4 x 10^2).
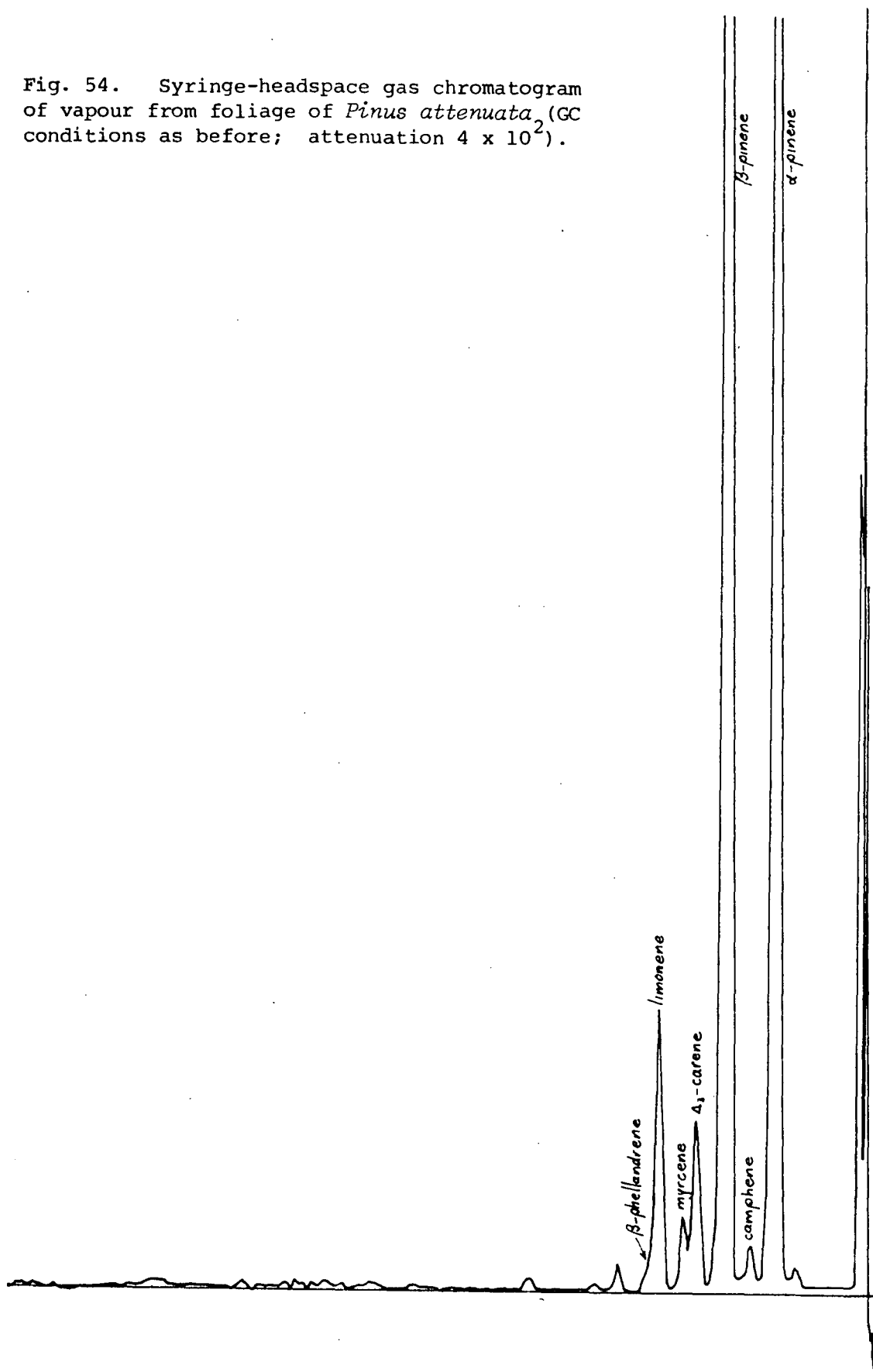
Table 74. Compositions of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Pinus attenuata*

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified + santene?	α-Pinene	Camphene	β-Pinene	Unidentified	Δ₃-Carene	Myrcene	Limonene	β-Phellandrene	γ-Terpinene?	Terpinolene
(Sample A)											
0	0.3	51.4	0.7	31.8	3.8	5.8	1.4	4.0	1.0	0.3	0.3
20	0.4	56.6	0.8	31.0	t	5.7	1.3	3.7	0.9	0.3	0.2
35	0.5	57.0	0.8	30.8	–	5.5	1.2	3.6	0.8	0.3	0.2
55	0.5	56.9	0.8	30.8	–	5.5	1.3	3.7	0.8	0.2	0.2
70	0.6	56.5	0.8	31.4	–	5.3	1.2	3.7	0.8	0.3	0.2
90	0.6	56.9	0.8	31.4	–	5.2	1.2	3.5	0.9	0.3	0.2
105	0.5	56.5	0.8	31.8	–	5.2	1.2	3.6	0.8	0.3	0.2
125	0.5	55.7	0.9	32.5	–	5.3	1.2	3.5	0.9	0.3	0.2
145	0.5	55.6	0.8	32.6	–	5.3	1.2	3.5	0.9	0.3	0.2
160	0.6	55.2	0.7	32.7	–	5.4	1.3	3.7	1.0	0.3	0.2
180	0.4	55.3	0.8	33.0	–	5.2	1.2	3.6	0.9	0.3	0.2
200	0.5	56.3	0.8	32.5	–	5.0	1.2	3.3	0.9	0.3	0.2
(Sample B)											
0	–	46.4	0.7	40.5	3.1	4.8	1.6	5.1	0.9	0.6	0.2
40	0.4	49.3	0.8	39.9	–	3.8	1.3	4.0	1.0	0.4	0.2
60	0.4	49.9	0.8	39.6	–	3.7	1.3	3.8	0.9	0.4	0.2
Steam-distilled oil from remaining portion of comminuted foliage:											
	7.2	34.0	0.8	35.3		6.9	2.0	9.8	1.8	1.2	1.0

+ ρ-cymene (0.1%)
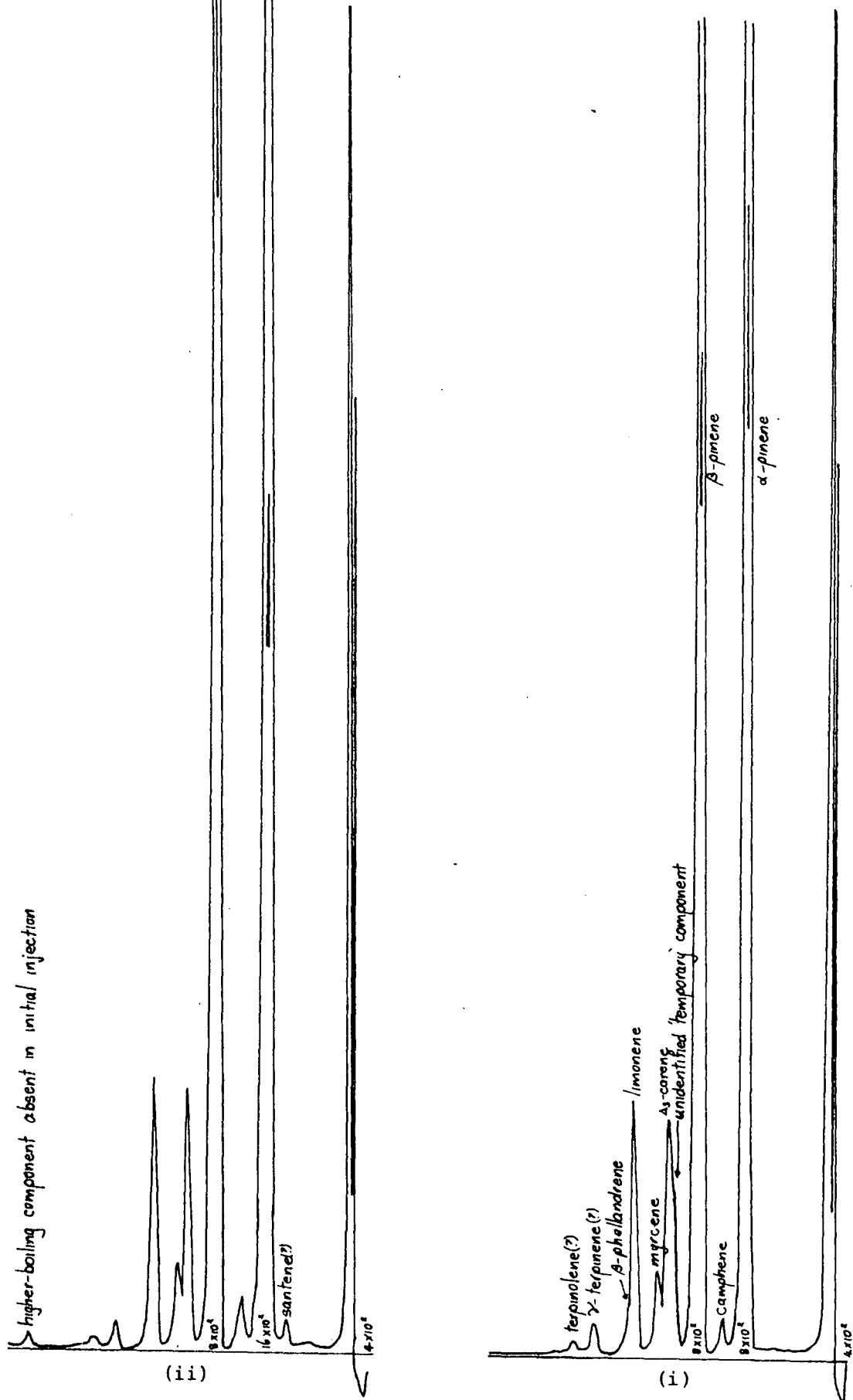
Fig. 55(a).   Syringe-headspace chromatograms of foliage vapour
of *Pinus attenuata*.   In the initial injection (i) an unidentified
'temporary component' appears as a shoulder on the Δ₃-carene peak.
A second injection of vapour 40 mins. later (ii) contains no trace
of the Δ₃-carene-shoulder component, but is seen to contain small
peaks possibly due to santene and a higher boiling component (GC
conditions as before).
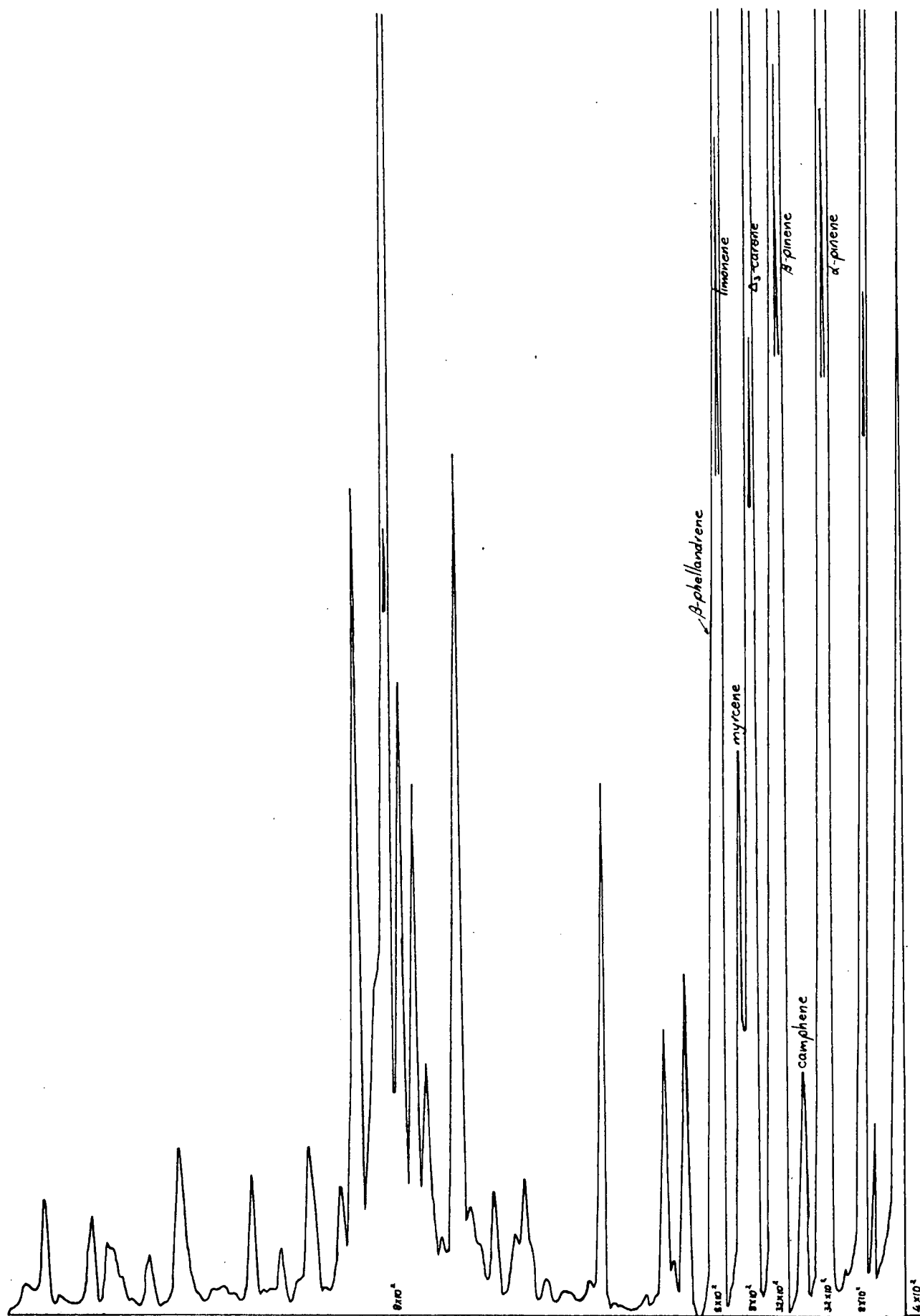
Fig. 55(b). High sensitivity gas chromatogram of oil steam-distilled from remainder of comminuted foliage of *Pinus attenuata* studied by syringe-headspace vapour GC in Fig. 55(a) (GC conditions as before).

(e) <u>Summary</u>

Components of steam-distilled oil from oleoresin of *Pinus attenuata* were analyzed by chromatographic and spectroscopic methods. The following identifications were confirmed: α-pinene (77.4%), camphene (2.8%), β-pinene (9.7%), Δ_3-carene (0.9%), myrcene (1.9%) and β-phellandrene (5.3%). Tentatively identified were sabinene, limonene (1.9%) and terpinolene. The oil from oleoresin contained only about 0.1% sesquiterpenes and other higher-boiling components. By comparison steam-distilled oil from foliage had a basically different composition, i.e. it contained a component eluted before α-pinene (7.2%), α-pinene (34.0%), camphene (0.8%), β-pinene (35.3%), Δ_3-carene (6.9%), myrcene (2.0%), limonene (9.8%), β-phellandrene (1.8%), γ-terpinene (1.2%), ρ-cymene (0.1%) and terpinolene (1.0%). Sabinene and ρ-cymene do not appear from the literature to have been previously found in this oil.

The syringe-headspace GC technique indicated the existence of a wide range of monoterpene compositions to be found in successive injections of vapour from a single sample of foliage, and also from different samples of foliage from the same tree. Successive injections of one sample over a 3 hr. period contained from 46.4 to 51.4% α-pinene, 31.8 to 40.5% β-pinene and 2.5 to 5.8% Δ_3-carene; whereas from 3 samples of foliage from the same tree the vapour contained from 46.4 to 51.4% α-pinene, 31.8 to 40.5% β-pinene and 2.5 to 5.8% Δ_3-carene.

Qualitative changes in foliage vapour composition were also indicated in successive injections from a single sample. A component that eluted close to Δ_3-carene on Carbowax 20M appeared in the initial injection of vapour, but could not be found 40 minutes later in a subsequent injection. Later injections were however found to contain a GC peak possibly corresponding to santene, together with that of an unidentified higher-boiling component.

(vi) *Pinus contorta* Dougl.

Lodgepole Pine or *Pinus contorta* Dougl. is a widespread and morphologically variable North American species, which has been reported by different workers to contain a range of oleoresin monoterpene compositions, in each case with β-phellandrene as the principal component.

Although Mirov [47] found 95 to 100 percent β-phellandrene with 0 to 5 percent α-pinene in oleoresin turpentines of trees recognized in different regions as morphological variants of *P. contorta*, subsequent investigations have indicated other quite different monoterpene compositions. Of the four distinguished varieties [429], Mirov noted that the Pacific coastal form is designated as *Pinus contorta* proper, inland forms of the Sierra Nevada and American Rockies are known respectively as *P. contorta* var. *murrayana* (Grev. & Balf.) Engelm. and var. *latifolia* Engelm., while a scrub-like form in a limited region of the Californian coast is known as var. *bolanderi* [47]. This convenient division into four varieties is however

confused in British Columbia where inland and coastal forms
are 'merged' and known only as *P. contorta*. The confusion
is added to by the recognition that *P. contorta* and
P. banksiana have intercrossed in Alberta.

Mirov [47] preferred as a result to distinguish two
extreme types of turpentines which could conceivably be
described loosely as '*P. contorta* turpentine', i.e. the
turpentine of *P. contorta* proper with a 100 percent
ℓ-β-phellandrene content, whereas monoterpenes of the hybrid
of *P. banksiana* consisted of a mixture of α- and β-pinene with
a smaller amount of ℓ-β-phellandrene.

Volatile oil from cortical oleoresin of 9 trees of
P. contorta var *murrayana* has been reported by Smith to contain
a trace of n-heptane, 6.4 percent α-pinene, 0.5 percent
camphene, 5.7 percent β-pinene, 8.9 percent Δ_3-carene, 2.1
percent sabinene, 0.7 percent α-phellandrene, 3.9 percent
myrcene, 2.4 percent limonene and 69.4 percent β-phellandrene
[430]. These values differ considerably from those reported
earlier in New Zealand for a single tree of *P. contorta* of
unknown origin [145], viz. 2 percent α-pinene, a trace of
camphene, 3 percent β-pinene plus myrcene, 4 percent Δ_3-carene
and 90 percent β-phellandrene.

Monoterpenes isolated from the wood have also been
found to differ considerably from the earlier reported 100
percent ℓ-β-phellandrene content. Findings comparable with
those of Smith [430, 432] have been reported by Drew and
Pylant [431], who however did not detect limonene in the
wood oil but did find 2.1 percent ρ-cymene. Anderson *et al* [314]

subsequently compared the monoterpene compositions of sapwood
and heartwood of *P. contorta* (California) which were found
to be quite different (Table 75). Rowe *et al* [433] have since
isolated among other bark extractives, the sesquiterpenoids,
γ-cadinene and oplopanone.

The leaf oil of *P. contorta* var *latifolia* (Rocky
Mountains) has been shown by Pauly and von Rudloff [67] to
consist principally of ℓ-β-phellandrene (34 percent) and
ℓ-β-pinene (30.5 percent). Other components identified in
smaller proportions were 6.5 percent α-pinene, 3 percent myrcene,
2.5 percent cis-ocimene, 1.5 percent Δ3-carene, 1 percent
terpinolene, 0.3 percent γ-terpinene, 4 percent α-terpineol,

Table 75. Comparison of the percentage monoterpene
compositions in the wood of *Pinus contorta*
(California)[314]

Component	Sapwood	Heartwood
n-Heptane	t	2
α-Pinene	3	1
Camphene	1	1
β-Pinene	1	t
Δ3-Carene	10	12
α-Phellandrene	12	3
Myrcene	2	1
Limonene	15	9
β-Phellandrene	49	71
γ-Terpinene	1	–
Terpinolene	5	–
Unidentified	1	–

t: trace; <0.5 percent

-: not detected

0.5 percent terpinen-4-ol, 0.5 percent chavicol methyl ether, 0.5 percent bornyl acetate, 0.3 percent linalool, 5 percent of a mixture of cadinene isomers, 1.5 percent of cadinol and muurolol isomers and 0.6 percent nerolidol. Tentatively identified were camphene, α-phellandrene, limonene, isopulegol, camphene hydrate, citronellol, bisabolol, and cis- and trans-farnesol.

Pauly and von Rudloff [67] made a special note of two further components in the leaf oil which were thought might have originated when the leaves were cut to assist the steam-distillation process. The components were 1.5 percent hex-2-en-1-al and 1 percent cis-hex-3-en-1-ol.

The monoterpene compositions of heartwood extractives has been proposed as a means of differentiating the woods of *P. contorta* forms and hybrids. Pauly and von Rudloff [67] pointed out that the effects of introgression of *P. contorta* with *P. banksiana* have been detected much further south-west than previously envisaged by Mirov and others. Swan [434] has proposed that heartwoods be distinguished by analysis of the steam-distilled volatiles. *P. contorta* would be distinguished by the oil containing mostly β-phellandrene, whereas *P. contorta* var *latifolia* oil contained slightly less β-phellandrene, although its contents of limonene and α- and β-pinene would be similar to that of *P. contorta*. *P. banksiana* heartwood oil however contained large amounts of α- and β-pinene, so that a *P. banksiana* × *contorta* hybrid would be distinguished by a mixture of β-phellandrene, α- and β-pinene. Zavarin *et al*

[435] have further studied the genetic basis for the formation

of β-phellandrene in cortical oleoresins of these hybrids.

The changes in extractives that occur in the sapwood

of P. *contorta* var *latifolia*, in response to attack by the

bark beetle *(Dendroctonus ponderosae)* and associated micro-

organisms, were studied by Shrimpton [436]. A large increase

in terpene content was noted in conjunction with a decrease in

free sugar level. This worker did not detect any significant

quantitative or qualitative changes in terpenoid composition.

(a) Analysis of the oil steam-distilled from cortical oleoresin

Cortical oleoresin collected as before from a single

tree of P. *contorta* (tree IV) in the Royal Botanical Gardens

(Tasmania), yielded upon steam-distillation 25.4 percent of a

colourless oil with a sweet pinene odour.

The qualitative and quantitative composition of

components distinguishable in the whole oil, using two

dissimilar columns, is recorded in Table 76. RRT values

leading to the tentative identification of components in

hydrocarbon and oxygenated fractions, and preparative GC

fractions from the oil, are listed in Table 77. Gas

chromatograms of Figure 56 show the distribution of components

eluted from a Carbowax 20M column, and indicate the degree of

separation of components into hydrocarbon and oxygenated

fractions.

This oil is seen from Tables 76, 77 and Figure 56

to contain a very small proportion of sesquiterpenes and to

Table 76. Components distinguishable in the whole
oil from oleoresin of *Pinus contorta*
(tree IV)

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 200°, 5°/min)
*α-Pinene	0.99	1.00	11.1
Camphene	1.28	1.21	1.1
*β-Pinene	1.63	} 1.56	7.9
*Sabinene	1.75		3.3
Δ$_3$-Carene	2.07	1.98	6.1
*Myrcene	2.27	1.76	8.5
Unidentified			0.2
*β-Phellandrene	3.15	2.69	60.8
γ-Terpinene	3.69		0.3
ρ-Cymene			0.1
Terpinolene	4.64	4.19	0.4
	(130° isothermal, ref. camphor)		
Chavicol methyl ether	1.59	1.32	0.2
Unidentified	1.75	1.44	0.1

* IR spectrum recorded

consist principally of β-phellandrene. The composition more
closely resembles that reported by Smith [430] for oil from
cortical oleoresin of *P. contorta* var *murrayana*.

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from comminuted
foliage of tree I (Table 78, Figure 57) indicated a fundamentally
different oil vapour composition being released to the atmosphere
from foliage of this tree. This oil consisted not only of
β-phellandrene but also contained a high proportion of β-pinene.
A further more volatile component, possibly santene, was detected
along with a 'temporarily-released component' eluting near
Δ$_3$-carene as found with *Cedrus deodara*.

Table 77. RRT values on the dissimilar liquid phases C20M and OV-17, for components found in hydrocarbon, oxygenated and preparative GC fractions, isolated from steam-distilled oil from oleoresin of *Pinus contorta* (tree IV)

Component	Hydrocarbon fraction		Oxygenated fraction		Preparative GC fractions		
	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)							
α-Pinene	1.02	0.98			W1	1.00	1.00
Camphene	1.29	1.18			W1	1.29	1.20
					W2	1.32	1.19
β-Pinene	1.62	1.54			W2	1.65	1.51
					W3	1.63	1.56
Sabinene					W2	1.76	1.51
Δ₃-Carene	2.05	1.94			W4	2.00	1.97
Myrcene	2.24	1.72	2.30	1.80	W2	2.25	1.73
					W3	2.25	1.76
α-Phellandrene					W3	2.25	2.00
Unidentified	2.45	2.16					
Limonene	2.73		2.80	2.42			
β-Phellandrene	3.01	2.58	2.94	2.56	W4	3.09	2.63
γ-Terpinene	3.66	3.25			W4	3.67	
ρ-Cymene	4.25	2.82	4.35	2.85	W4	4.35	
Terpinolene	4.56	4.09	4.62	4.13			
(130° isothermal, ref. camphor)							
Chavicol methyl ether			1.59	1.32			
Unidentified	1.74	1.39					

Fig. 56(a).   Low sensitivity gas chromatogram of
whole oil from oleoresin of *Pinus contorta* (GC on 5%
Carbowax 20M/Gas Chrom Q;   temperature program 50°
to 200° at 5°/min;   0.2 µℓ sample;   attenuation
8×10^3).
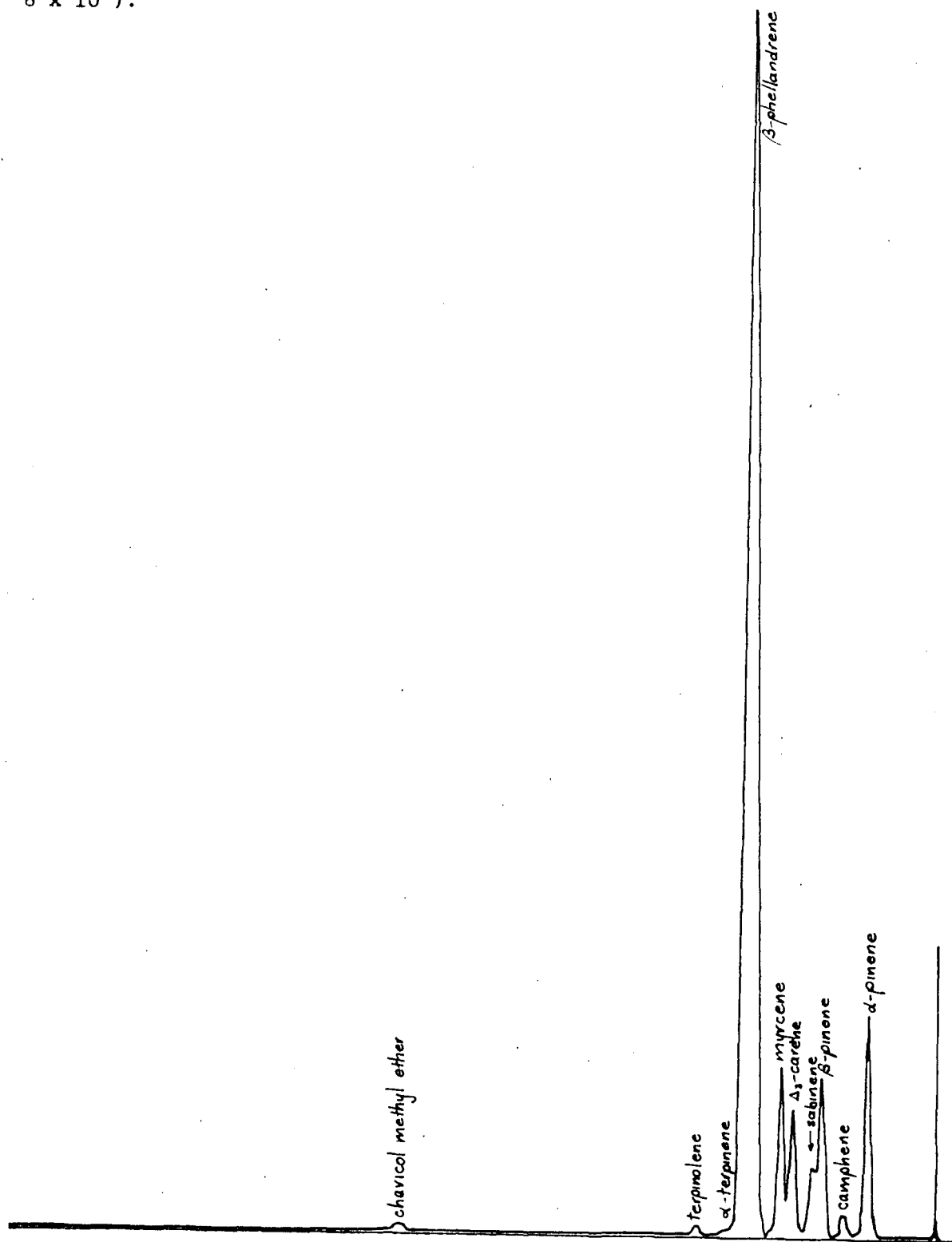
Fig. 56(b).   High sensitivity gas chromatogram
of whole oil from oleoresin of *Pinus contorta*
(attenuation 4×10^2).
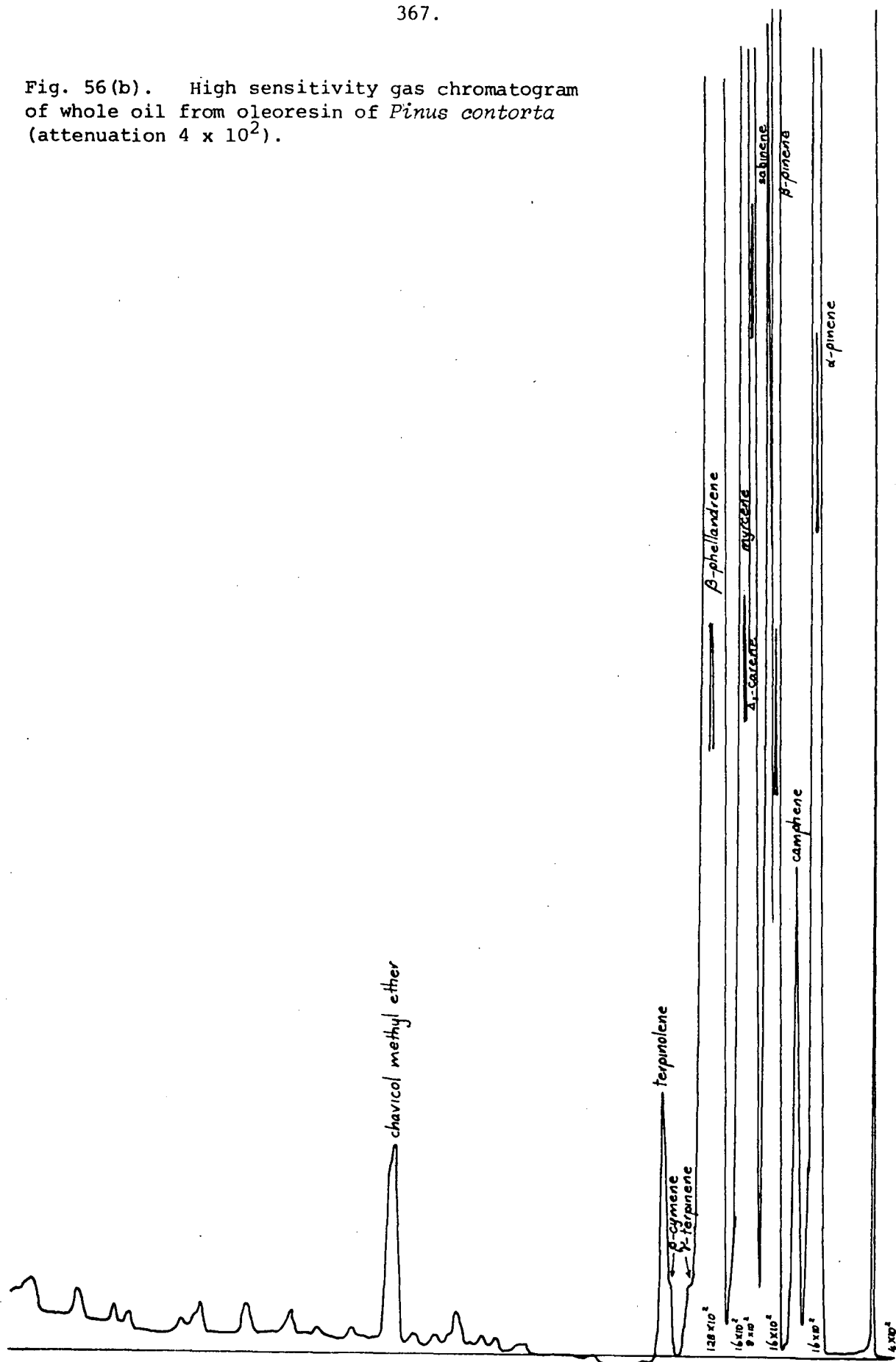
Fig. 56(c). Low sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus contorta* separated on Florisil.
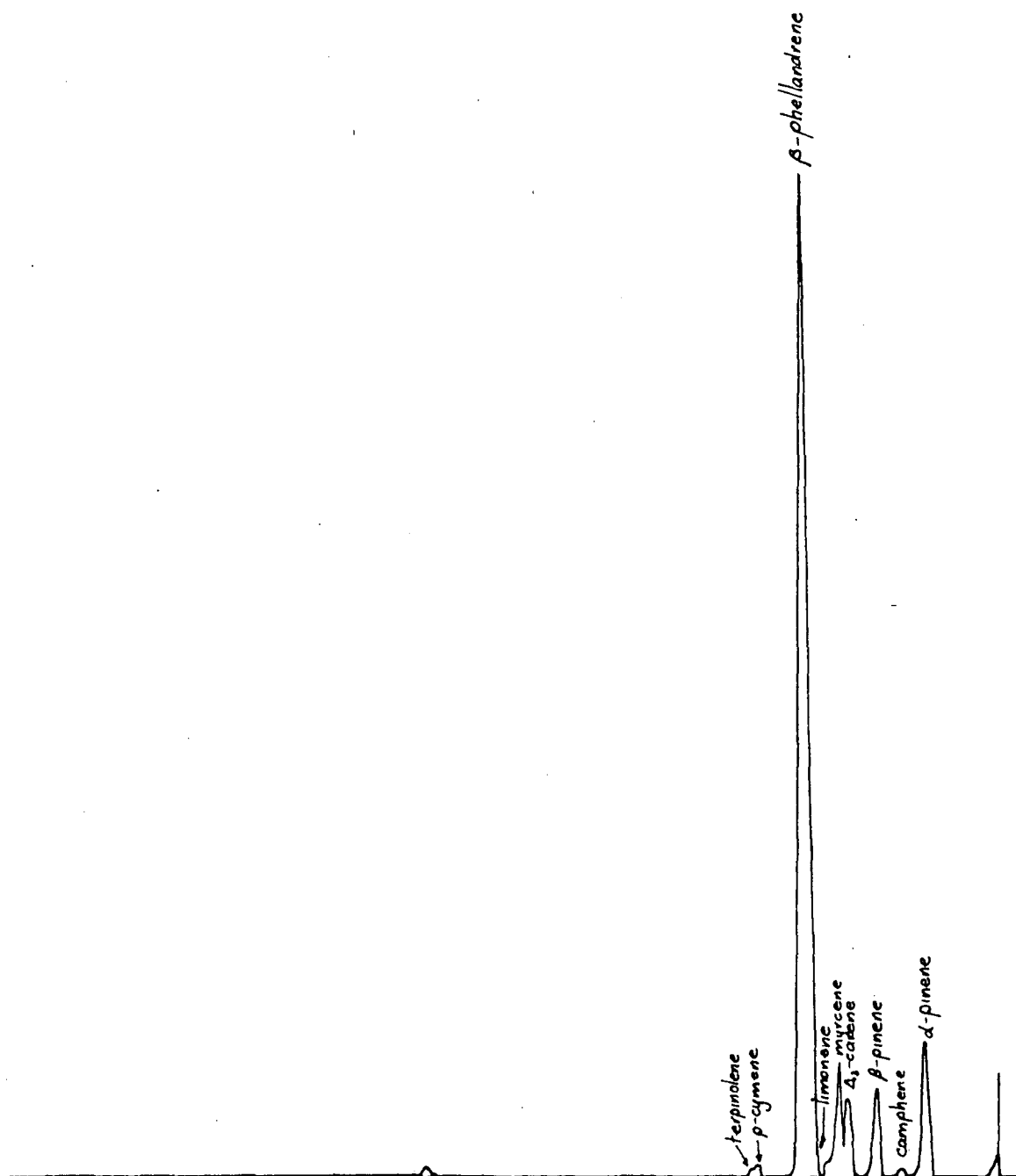
Fig. 56(d).   High sensitivity gas chromatogram
of hydrocarbon fraction of oil from oleoresin of
Pinus contorta separated on Florisil.
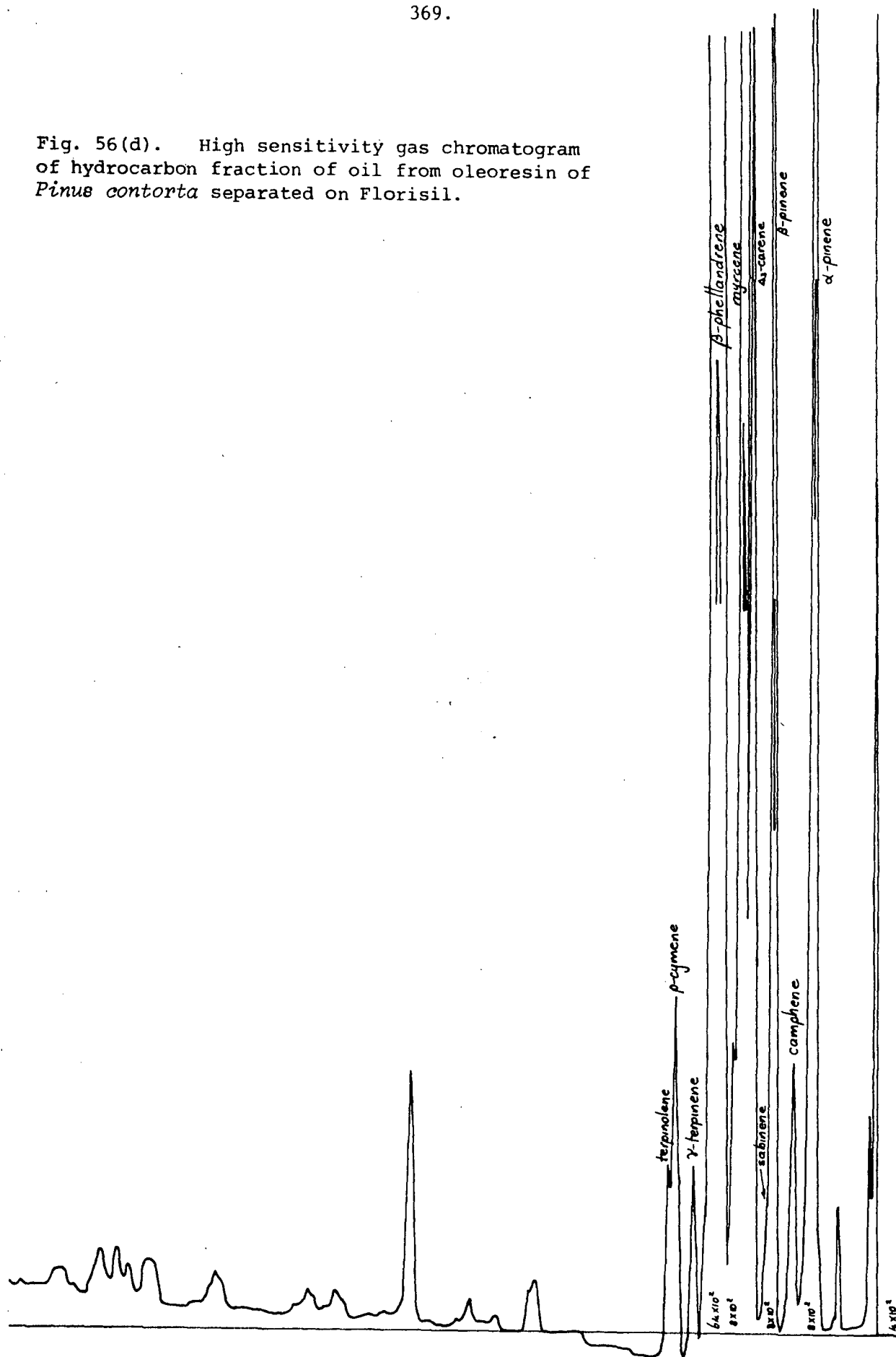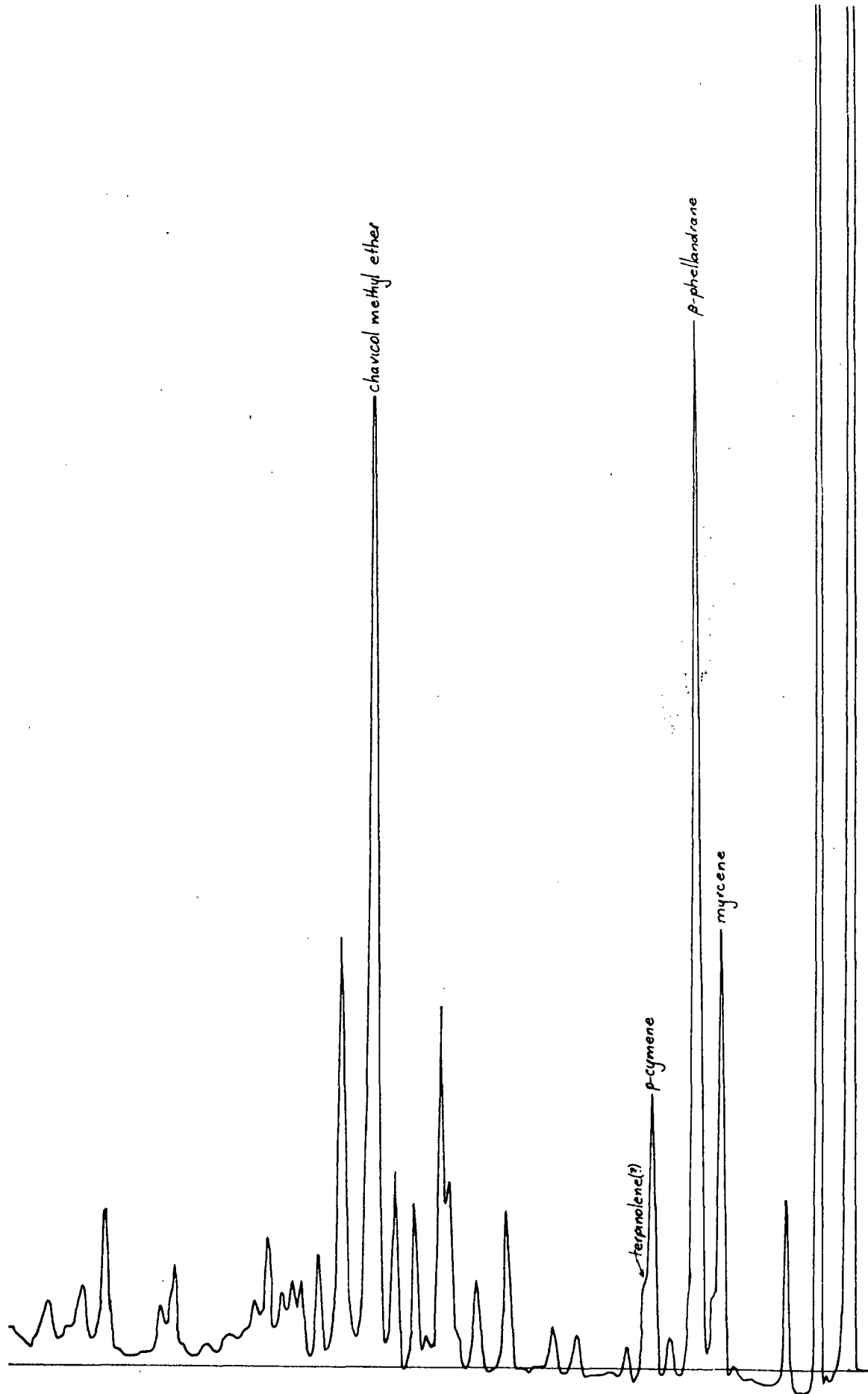
Fig. 56(e). High sensitivity gas chromatogram of oxygenated fraction of oil from oleoresin of *Pinus contorta* separated on Florisil.

Table 78. RRT data and percentage composition of
volatile terpenoids in foliage of *Pinus
contorta* (tree I) determined by syringe-
headspace GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak area of 2nd successive injection)
	C20M	OV-17	
(60° isothermal, ref. α-pinene)			(60° isothermal)
Santene?	0.79		0.3
α-Pinene	1.00	0.98	14.4
Camphene	1.28	1.19	0.7
β-Pinene	1.64	1.52	40.2
Sabinene	1.76	1.52	8.1
Unidentified 'temporary component'	2.00		t
Δ_3-Carene	2.08	1.94	0.9
Myrcene	2.33	1.71	2.1
Unidentified	2.55	2.08	t
β-Phellandrene	3.01	2.47	30.9
γ-Terpinene?			t
Terpinolene	4.89	4.07	2.4

(c) Composition of successive injections of syringe-headspace

vapour from foliage (tree I)

Successive injections of vapour over a 3 hr. period,

from a single sample of foliage, exhibited not only

fluctuations in the proportion of β-phellandrene, α- and β-

pinene, but also some apparently qualitative changes in the

vapour composition. The previously-mentioned component

eluting near Δ_3-carene could only be detected in vapour

injected within approximately 1 hr. after comminution

(Table 79, Figure 58). In Figure 58 an injection of vapour

95 minutes after the initial injection is seen to contain no

trace of the earlier-released component, but to contain an

increased concentration of a peak due possibly to santene.

Fig. 57.   Syringe-headspace gas chromatogram
of vapour from foliage of *Pinus contorta* (GC
conditions as before;   attenuation 4 x 10^2).
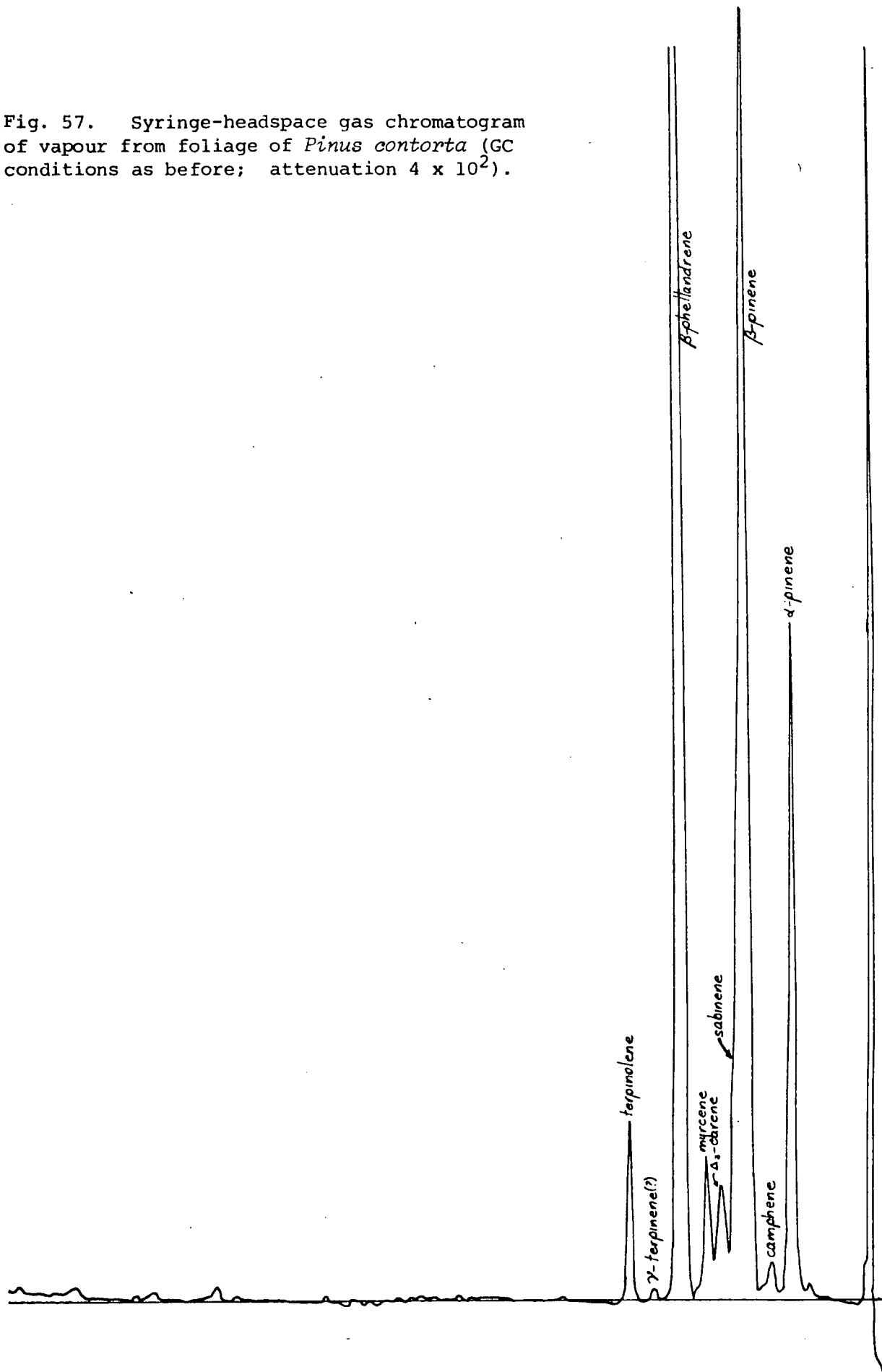
Table 79. Compositions of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Pinus contorta* (tree I)

<u>Percentage composition of monoterpenes (peak height basis)</u>:

Time since comminution of sample (mins.)	Unidentified + santene?	α-Pinene	Camphene	Unidentified	β-Pinene	Sabinene	Unidentified 'temporary-component'	Δ$_3$-Carene	Myrcene	β-Phellandrene + Limonene	Terpinolene
0	0.2	11.2	0.5	0.2	33.4	9.9	6.8	0.5	2.3	32.3	2.7
15	0.8	15.1	0.8	0.5	34.1	10.0	3.4	0.8	2.3	30.1	2.4
35	1.3	15.9	0.8	0.5	35.5	9.9	1.8	0.9	2.2	28.9	2.4
50	1.7	15.8	0.6	0.6	35.8	10.2	1.1	1.1	2.3	28.6	2.2
65	1.7	15.5	0.5	0.2	36.8	10.5	0.7	0.7	2.3	28.9	2.2
80	2.0	17.6	1.0	0.1	31.9	10.3	t	0.7	2.5	31.3	2.5
95	2.0	16.5	0.7	0.1	36.0	10.3	–	0.7	2.4	29.1	2.2
110	2.1	16.1	0.6	–	35.8	10.6	–	0.8	2.3	29.5	2.3
125	2.1	15.5	0.6	–	35.7	10.4	–	0.8	2.4	29.9	2.4
140	2.2	15.4	0.5	–	35.5	10.7	–	0.8	2.3	30.2	2.4
155	2.3	15.3	0.8	0.2	35.9	10.3	–	0.8	2.4	29.7	2.3
170	2.2	14.7	0.7	0.2	35.8	10.5	–	0.8	2.4	30.2	2.5

Fig. 58.   Composition of monoterpenes in successive injections of
syringe-headspace vapour from a single sample of foliage of *Pinus
contorta* (tree I).   An unidentified component is seen in injections
of vapour up to 95 minutes after comminution of the sample.   Throughout
this period there is an increase in the concentration of a peak possibly
due to santene.
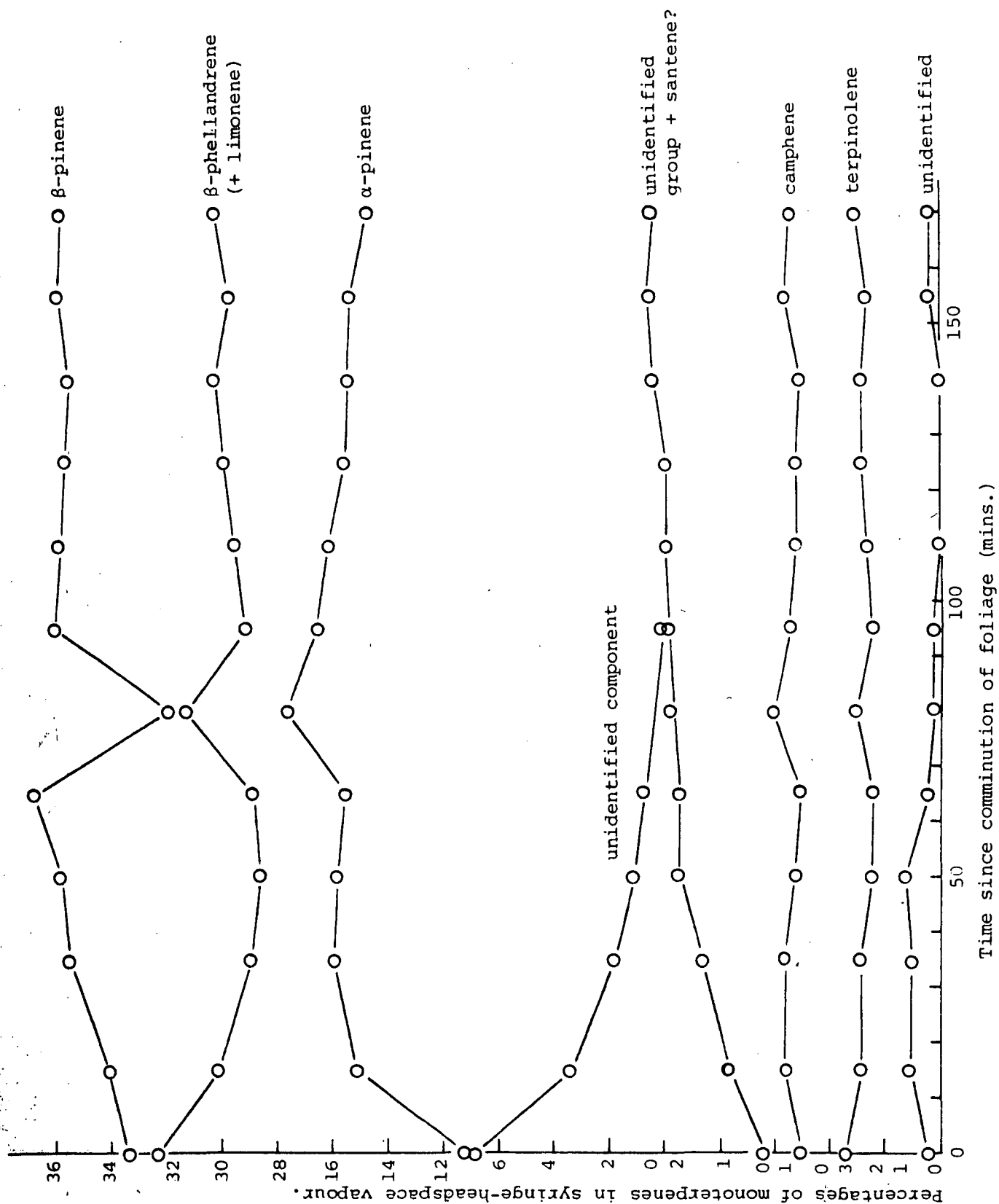
(d) Composition of syringe-headspace vapour from foliage

of several trees of *Pinus contorta* (trees I-IV)

The wide variation in monoterpene composition of the

vapour from a random sample of foliage, from each of four trees,

is shown in Table 80 (trees I-IV). In the vapour of three of

the trees there appeared to be the same temporarily-released

component. The extreme variation in compositions between these

trees, three of which have been botanically identified as

P. contorta proper, may possibly be due to the well-known

variability of this species.

(e) Composition of syringe-headspace vapour from foliage

sampled repeatedly from the same tree (tree I)

The composition of the vapour from three samples of

foliage from tree I varied (Tables 78-80) from 11.2 to 14.4

percent α-pinene, 23.2 to 40.2 percent β-pinene and 30.9 to

40.3 percent β-phellandrene.

A chromatogram of the steam-distilled oil from the

remainder of the comminuted foliage from tree I is given for

comparison (Table 80) in Figure 59.

(f) Summary

Components of the steam-distilled oil from oleoresin

of *Pinus contorta* were analyzed by chromatographic and

spectroscopic methods. The following identifications were

confirmed: α-pinene (11.1%), β-pinene (7.9%), sabinene (3.3%),

myrcene (8.5%) and β-phellandrene (60.8%). Tentatively

identified were camphene (1.1%), Δ_3-carene (6.1%),

Table 80. Compositions of monoterpenes in syringe-headspace injections of vapour from foliage of several trees of *Pinus contorta*. The compositions of the first three successive injections in each case illustrate the apparent "disappearance" of the component eluting near Δ_3-carene.

Percentage composition of monoterpenes (peak height basis):

Tree No.	Time since comminution (mins.)	Unidentified + santene?	α-Pinene	Camphene	β-Pinene	Sabinene	Unidentified	Δ_3-Carene	Myrcene	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene
I	0	–	13.5	0.7	23.2	10.9	3.5	1.5	2.9	t	40.3	0.3	3.2
	35	0.7	15.6	0.9	23.6	11.4	0.9	1.3	3.1	t	39.5	0.2	2.9
	50	0.8	16.1	0.9	23.6	11.4	–	1.3	3.1	t	39.7	0.3	2.9

Steam-distilled oil from remainder of comminuted foliage from I:

| | | 26.3 | 3.4 | 0.2 | 10.2 | 3.1 | 3.3 | 1.9 | 2.1 | | 42.2 | 0.8 | 6.1 |

+ ρ-cymene 0.2

II (var *latifolia*)

	0	0.1	67.0	1.2	27.8	–	0.6	–	0.8	t	2.5	–	t
	15	0.1	68.2	1.4	27.1	–	0.2	–	0.7	t	2.2	–	t
	30	0.1	69.2	1.4	26.4	–	t	–	0.7	t	2.1	–	t
III	0	0.2	30.7	0.7	26.2	–	2.0	–	25.2	6.7	8.2	–	–
	15	0.4	36.1	0.7	27.0	–	0.5	–	22.5	5.8	7.1	–	–
	30	0.5	36.8	0.8	27.2	–	0.1	–	22.3	5.5	6.9	–	–
IV	0	0.1	19.5	0.6	57.4	–	–	5.3	2.2	t	13.7	0.9	0.3
	20	0.3	20.5	0.6	55.3	–	–	6.9	2.1	t	13.3	0.7	0.4
	35	0.4	20.6	0.5	54.8	–	–	7.3	2.0	t	13.2	0.6	0.4

t: trace; <0.1 percent
-: not detected

376.

Labels on chromatogram (i): terpinolene, γ-terpinene, β-phellandrene, myrcene, Δ₃-carene (Δ3-carene), unidentified 'temporary' component, sabinene, α-pinene, β-pinene, camphene

(iii)   (ii)   (i)
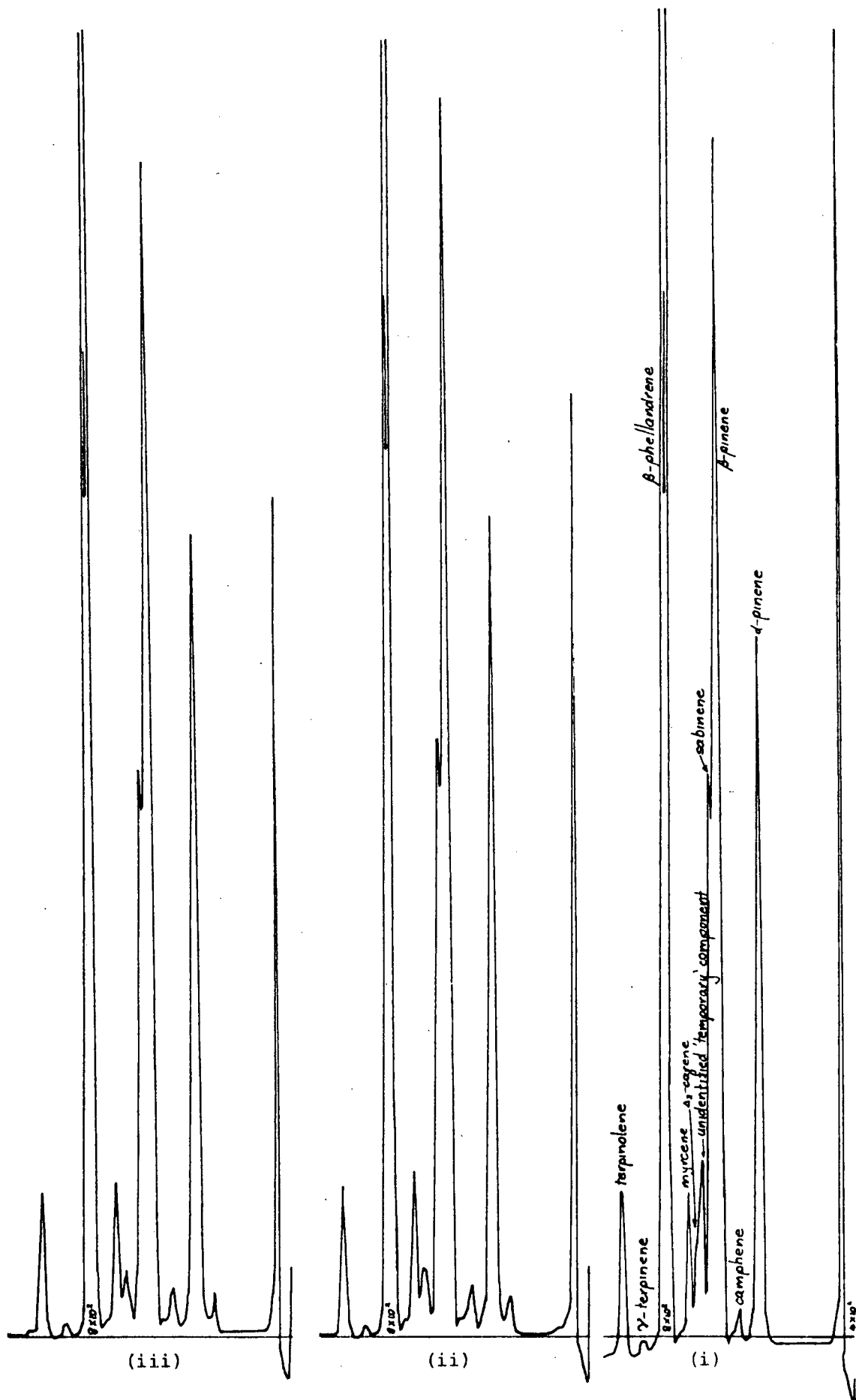
Fig. 59(a). Syringe-headspace chromatograms of foliage vapour of *Pinus contorta* (tree I). In the initial injection (i) an unidentified 'temporary component' appears as a peak overlapping the Δ_3-carene peak. Subsequent injections contain little evidence of this component, but are seen to contain a small peak possibly due to santene
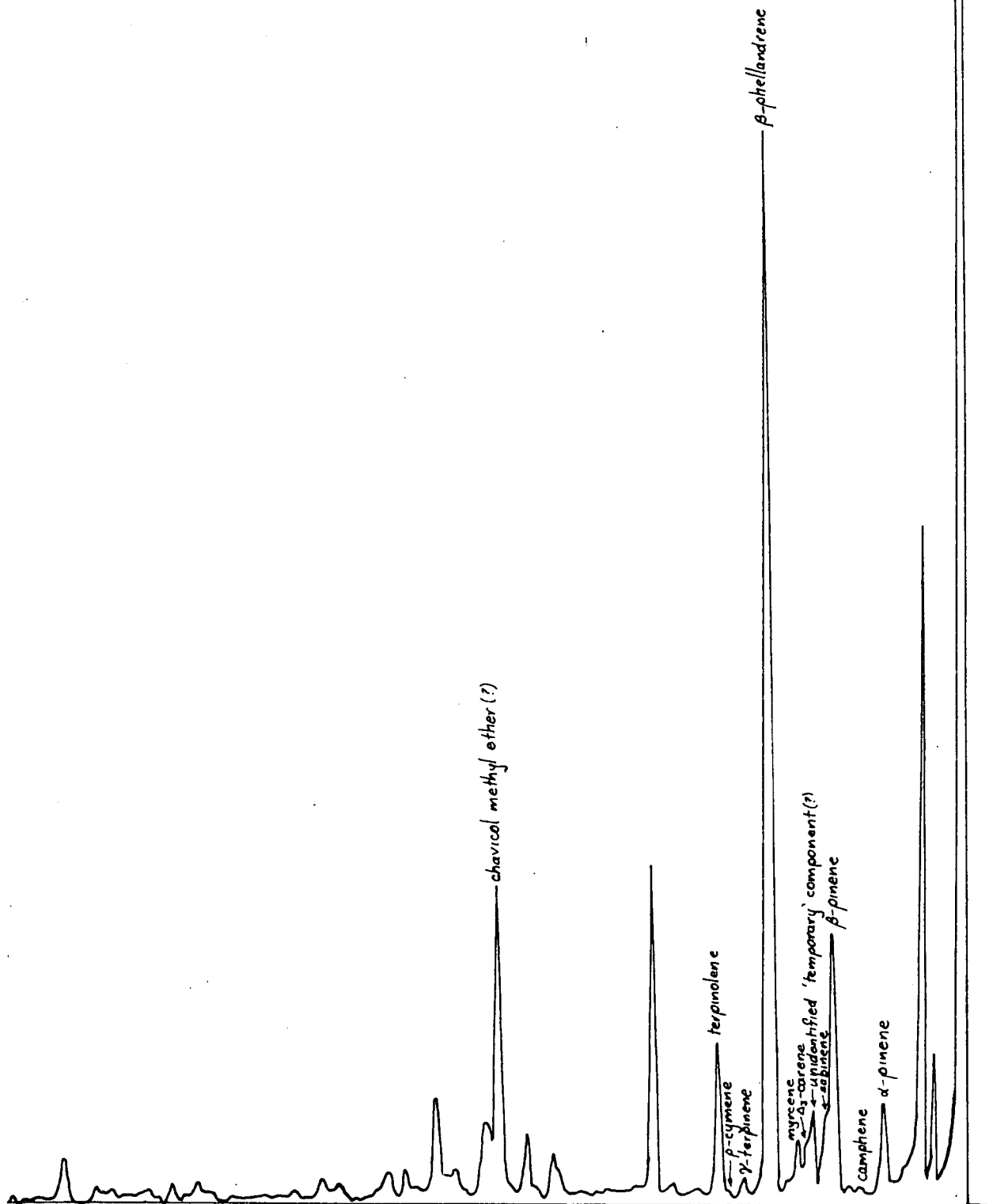
Fig. 59(b). Gas chromatogram of oil steam-distilled from the remainder
of comminuted foliage of *Pinus contorta* studied by syringe-headspace vapour
GC in Fig. 59(a) (GC conditions as before).

γ-terpinene (0.3%), ρ-cymene (0.1%), α-phellandrene,
terpinolene (0.4%) and chavicol methyl ether (0.2%). By
comparison, monoterpenes of the steam-distilled foliage oil
included: a component eluted before α-pinene (26.3%),
α-pinene (3.4%), camphene (0.2%), β-pinene (10.2%),
sabinene (3.1%), an unidentified component (3.3%), Δ_3-carene (1.9%),
myrcene (2.1%), β-phellandrene (42.2%), γ-terpinene (0.8%),
ρ-cymene (0.2%) and terpinolene (6.1%). Each of these components
had been previously reported from *P. contorta* oils.

The syringe-headspace GC technique indicated the
existence of a wide range of monoterpene compositions to be
found in successive injections of vapour from a single sample
of foliage, and also from different samples of foliage from the
same tree. Successive injections of vapour over a 3 hr. period
contained 11.2 to 17.6% α-pinene, 31.9 to 36.8% β-pinene, and
28.6 to 32.3% β-phellandrene; whereas from 3 samples of foliage
from the same tree the vapour contained from 11.2 to 14.4% α-pinene,
23.2 to 40.2% β-pinene and 30.9 to 40.3% β-phellandrene. The
compositions of foliage vapour from 3 different trees were so
widely ranging that it is considered that this feature could be
related to the well-known morphological variability of the
species, i.e. α-pinene varied from 13.5 to 30.7%, β-pinene from
23.2 to 57.4% while β-phellandrene ranged from 8.2 to 40.3%.

An apparently qualitative change in foliage vapour was
indicated when a component (6.8%), eluted near Δ_3-carene on
Carbowax 20M, could not be detected after 90 minutes of successive
injections from the same sample.

(vii) *Pinus echinata* Mill.

Shortleaf Pine or *Pinus echinata* Mill. has been considered a poor source of commercial oleoresin, and consequently its terpenoid composition has not been thoroughly investigated. Using fractional distillation techniques Mirov [47] found the turpentine to consist of 85 percent d-α-pinene and 11 percent ℓ-β-pinene.

(a) Syringe-headspace GC analysis of foliage terpenoids

The limited amount of sample available in the Royal Botanical Gardens (Tasmania) was sufficient to enable a syringe-headspace GC analysis of foliage vapour. The sample consisted of a sprig from a seedling being cultivated to establish *P. echinata* for the first time in these gardens.

The composition of the syringe-headspace foliage vapour consistent with that reported by Mirov, is given in Table 81 and illustrated in Figure 60.

Table 81. RRT data and percentage composition of volatile terpenoids in foliage of a seedling of *Pinus echinata* determined by syringe-headspace GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak area of 4th successive injection)
	C20M	OV-17	
(60° isothermal, ref. α-pinene)			(60° isothermal)
Unidentified	0.62		11.7
α-Pinene	1.00	1.00	74.8
Camphene	1.29	1.17	0.8
β-Pinene	1.64	1.53	9.3
Unidentified	~2.0		t
Myrcene	2.34	1.73	0.7
Unidentified	~2.5		0.1
Limonene	2.85	2.45	0.4
β-Phellandrene	2.99	2.50	2.2

Fig. 60. Syringe-headspace gas chromatogram
of vapour from foliage of a seedling of *Pinus
echinata* (GC conditions as before; attenuation
4×10^2).
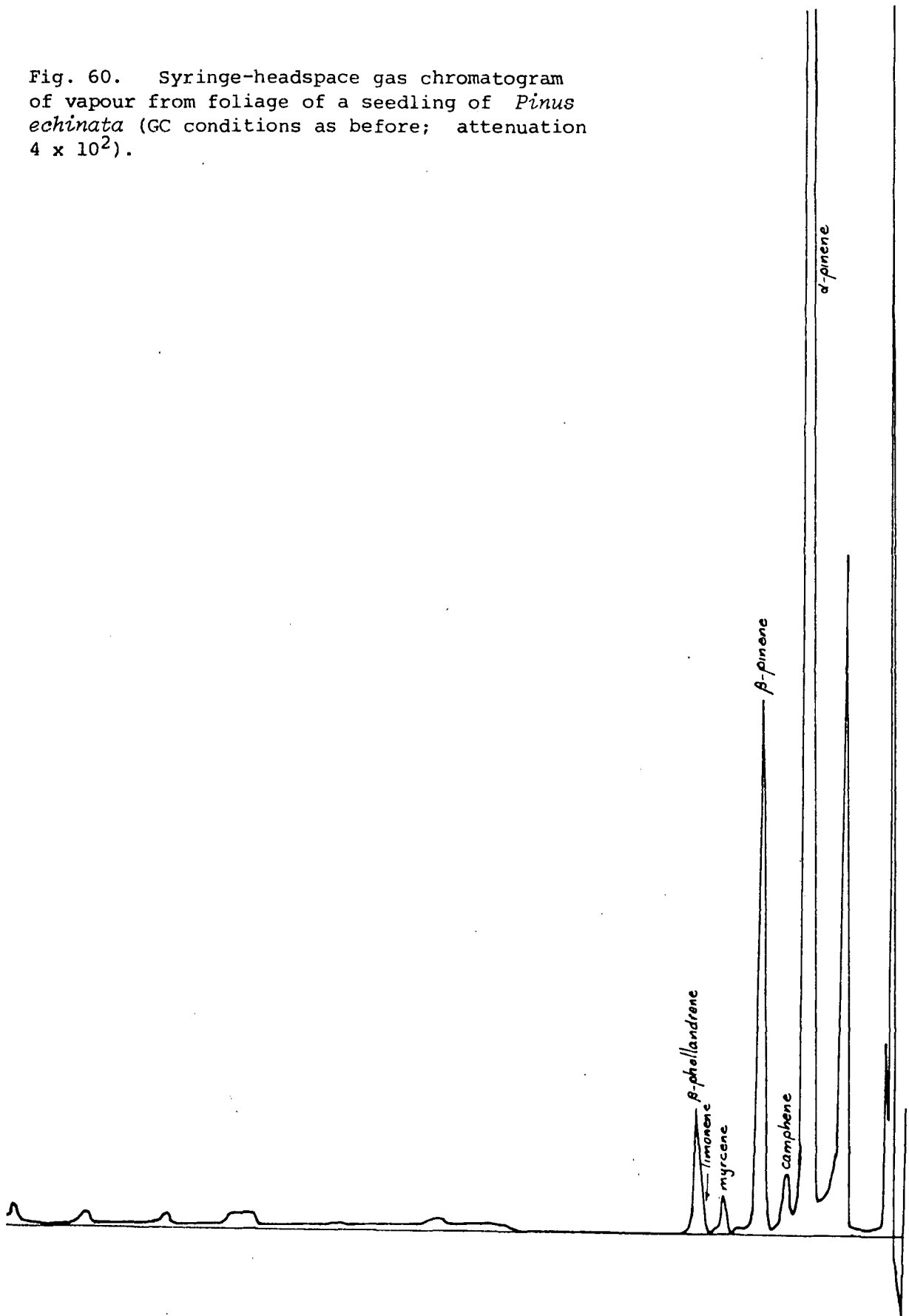
(b) <u>Summary</u>

Components of the syringe-headspace vapour from a single sample of foliage, available from a seedling of *Pinus echinata*, were tentatively identified by GC on two dissimilar columns. Found were an unidentified component that eluted before α-pinene on Carbowax 20M (11.7%), α-pinene (74.8%), camphene (0.8%), β-pinene (9.3%), myrcene (0.7%), limonene (0.4%) and β-phellandrene (2.2%). Camphene, myrcene, limonene and β-phellandrene are reported for the first time in this species.

(viii) "<u>*Pinus elliottii* Engelm</u>."

Mirov [47, 437] has repeatedly outlined the botanical nomenclature of varities within this species which differ considerably in the monoterpene compositions of their oleoresins. Unfortunately some workers have ignored the rules of botanical nomenclature (Art. 25, 8th Int. Bot. Congr., Paris, 1924) and persisted in using the term "*P. elliottii*" when it was possible that var *elliottii*, var *densa* or even *P. caribaea* could have been the true variety or species.

.The variety found attractive to *Sirex noctilio* [395] was *P. elliottii* var *elliottii*.

Slash Pine, or *Pinus elliottii* Engelm. var *elliottii*, was earlier reported to contain in oil from the oleoresin a high proportion of ℓ-α-pinene, e.g. 61 percent, with a lesser proportion of ℓ-β-pinene, e.g. 33.7 percent [47]. These values differed from those of var *densa*, which was found to contain

71 percent ℓ,dℓ-α-pinene and only 3 to 4 percent ℓ-β-pinene.
P. caribaea, a species previously classified under the name
P. elliottii, also yielded an oil from the oleoresin in which
was found about 60 percent ℓ,dℓ-α-pinene and negligible
β-pinene [47].

Mirov *et al* [437] have subsequently confirmed the
composition of oil from oleoresin of var *elliottii* as α-pinene
(56.6 percent), camphene (0.6 percent), β-pinene (35.6 percent),
myrcene (0.3 percent), dipentene (1.5 percent), β-phellandrene
(3.3 percent), ρ-cymene (0.2 percent) and chavicol methyl ether
(0.7 percent). This analysis agrees substantially with that
of New Zealand workers [145], who reported 59.0 percent
α-pinene, 2.0 percent camphene, 34.5 percent β-pinene,
2.0 percent myrcene, a trace of limonene and 2.5 percent
β-phellandrene in a study of an unspecified *P. elliottii.*
Roberts has reported the existence of high- and low-β-phellandrene
trees of var *elliottii* [313], which yielded trunk oleoresins
with widely varying compositions (Table 15, Figure 4) depending
upon the point of sampling.

Oils from needles, branch cortex and xylem tissues
of branches, trunk and roots were also shown by Roberts to be
quite different when sampled from high- and low-β-phellandrene
trees (Table 12)[313]. A further study of needle oil by
Joye *et al* [438] revealed a further quite different composition
with much lower proportions of α- and β-pinene. Needle oil in
this latter study contained 9.3 percent α-pinene, 1.3 percent
camphene, 14.0 percent β-pinene, 4.6 percent limonene, 3.1 percent

β-phellandrene, 3.6 percent ρ-cymene, 0.3 percent trans-
dihydro-α-terpineol, 0.3 percent α-fenchol, 0.7 percent
bornyl acetate, 1.0 percent β-terpineol, 2.3 percent terpinen-4-ol,
5.4 percent caryophyllene, 20.9 percent α-terpineol, 2.0
percent borneol, and 3.6 percent cadinene.

Other workers have investigated the genetic basis
for the formation of β-phellandrene [153, 439], considered to
be related to the resistance of this species to fusiform
rust [440].

(a) Syringe-headspace GC analysis of foliage terpenoids

The limited amount of sample available in the Royal
Botanical Gardens (Tasmania) was sufficient to enable a syringe-
headspace GC analysis of foliage vapour. The sample consisted
of a sprig from a seedling of unspecified "P. elliottii"
being cultivated to establish the Slash Pine for the first time
in these gardens.

The composition of the syringe-headspace foliage vapour
(Table 82, Figure 61) was basically different from that
described [438] for var elliottii. The much higher proportion
of α-pinene to β-pinene could indicate that the "P. elliottii"
seedling was either a variety other than var elliottii or that
there is a wide range of foliage oil compositions to be found in
the species.

Table 82. RRT data and percentage composition of
          volatile terpenoids in foliage of a seedling
          of an unspecified variety of *Pinus elliottii*
          determined by syringe-headspace GC analysis

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak area)
(60° isothermal, ref. α-pinene)			(60° isothermal)
Unidentified	0.61		5.2
α-Pinene	1.00	1.00	78.4
Camphene	1.28	1.19	0.5
β-Pinene	1.61	1.55	6.8
Sabinene	1.79		0.1
Myrcene	2.30	1.74	7.5
Limonene	2.81	2.37	0.5
β-Phellandrene	2.99	2.52	0.9

(b) Summary

Components of the syringe-headspace vapour from
a single sample of foliage, available from a seedling of an
unspecified variety of *Pinus elliottii*, were tentatively
identified by GC on two dissimilar columns. Found were:
an unidentified component that eluted before α-pinene on
Carbowax 20M (5.2%), α-pinene (78.4%), camphene (0.5%),
β-pinene (6.8%), sabinene (0.1%), myrcene (7.5%), limonene
(0.5%) and β-phellandrene (0.9%).

(ix) *Pinus montezumae* Lamb.

Oil from the Montezuma Pine, *Pinus montezumae* Lamb.,
does not appear from a search of the literature to have been
examined since the earlier GC investigations by New Zealand
workers [145, 151]. Mirov [47] noted that this species is

Fig. 61.   Syringe-headspace gas chromatogram
of vapour from foliage of a seedling of an
unspecified variety of *Pinus elliottii* (GC
conditions as before;   attenuation 4×10^2).
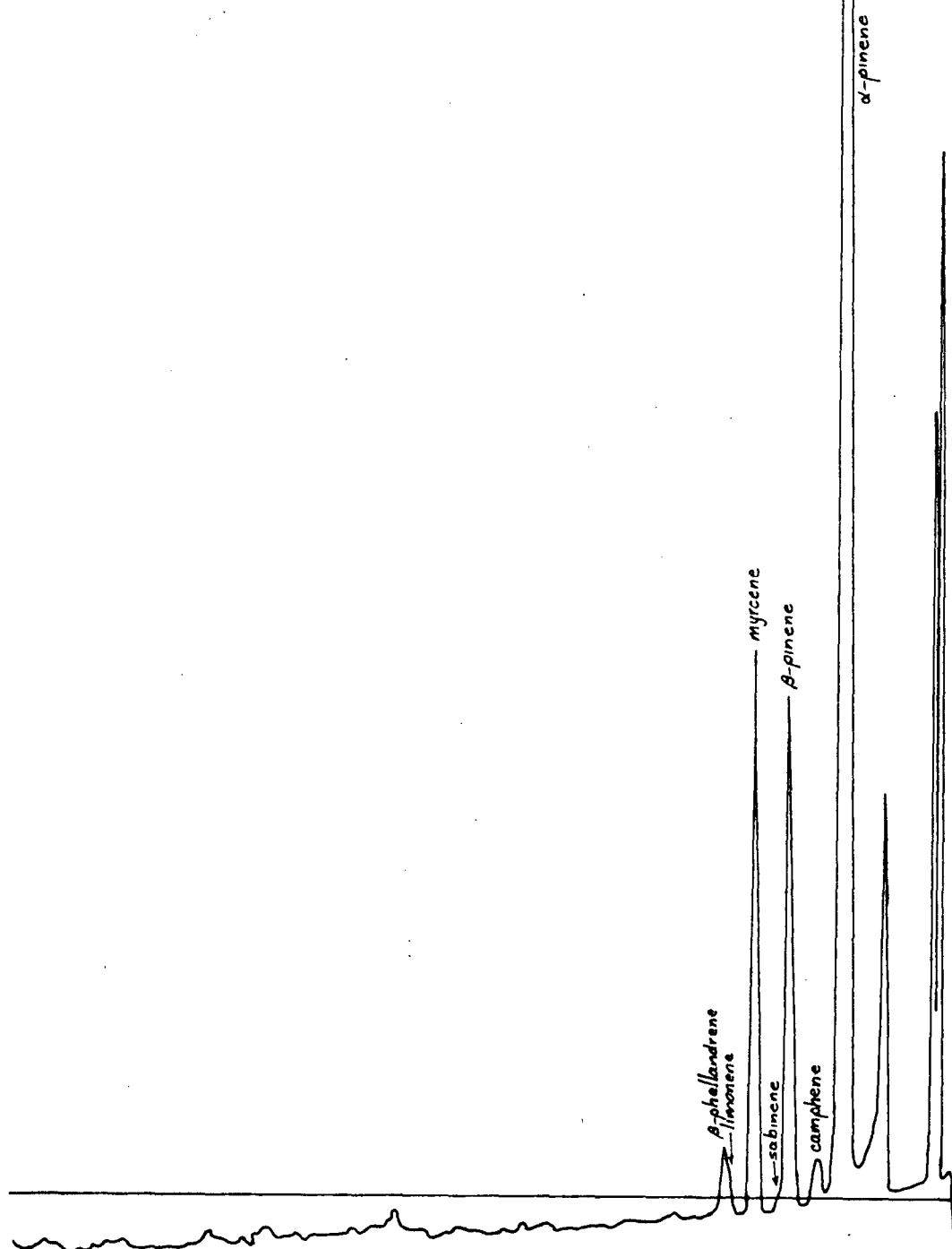
a complex from which several varieties have been subsequently recognized as separate species. The variability, together with suspected hybridism, was given as the reason for chemically distinct turpentines being found.

Mirov described two compositions of oil from the oleoresin [47]. Trees in one district of Mexico yielded an oil repeatedly found to consist of 96 to 98 percent d-α-pinene. However in another locality the oil contained 8 percent n-heptane, 72 percent d,dℓ-α-pinene, 6 to 7 percent ℓ,dℓ-limonene, 8 percent d-longifolene and 1 to 2 percent of oxygenated terpenoids.

An oleoresin oil, bearing no resemblance to either of the above, was examined by Williams and Bannister [145]. This oil contained 8.5 percent α-pinene, 2.0 percent β-pinene, 1.5 percent myrcene, 87.0 percent Δ_3-carene, 1.0 percent β-phellandrene and traces of camphene, limonene and ρ-cymene. Oils from other trees studied by New Zealand workers were however shown to contain 88 to 98 percent α-pinene [151].

(a) Analysis of the oil steam-distilled from cortical oleoresin

Cortical oleoresin collected as before from a single tree in the Royal Botanical Gardens (Tasmania), yielded upon steam-distillation 31.4 percent of a colourless oil having a typical pinene odour with an additional "rose-petal" note.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 83. RRT values leading to the

Table 83. Components distinguishable in the whole
oil from oleoresin of *Pinus montezumae*

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 200° at 5°/min)
*α-Pinene	1.02	1.00	45.8
Camphene	1.27	1.20	0.6
*β-Pinene	1.64	1.59	44.9
Δ₃-Carene	2.00	1.96	t
*Myrcene	2.24	1.75	3.8
*Limonene	2.74	2.40	0.9
*β-Phellandrene	2.85	2.52	1.4
γ-Terpinene	3.63		t
Terpinolene	4.57	4.12	t
	(130° isothermal, ref. camphor)		
Terpinolene	0.45		
*Linalool	0.91	0.59	2.0
Bornyl acetate	1.17	1.61	0.3
*Chavicol methyl ether	1.58	1.28	0.3
α-Terpineol	1.76	1.11	t

* IR spectrum recorded
t: trace; <0.1 percent

tentative identification of components in hydrocarbon and
oxygenated fractions, and preparative GC fractions from the
whole oil, are listed in Table 84. Gas chromatograms of
Figure 62 show the distribution of components eluted from a
Carbowax 20M column, and indicate the degree of separation of
components into hydrocarbon and oxygenated fractions.

This oil is seen from Tables 83, 84 and Figure 62
to consist largely of α- and β-pinene in nearly equal proportions.
The oleoresin of this species is distinguished from that of
other pines by the presence of a significant proportion of
oxygenated monoterpenes, which contribute the "rose-petal" note.

Table 84. RRT values on the dissimilar liquid
phases C20M and OV-17, for components
found in the hydrocarbon, oxygenated
and preparative GC fractions, isolated
from steam-distilled oil from oleoresin
of *Pinus montezumae*

Component	Hydrocarbon fraction		Oxygenated fraction		Preparative GC fractions		
	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)							
α-Pinene	0.99	1.01			W1	0.99	1.01
Camphene	1.26	1.22			W2	1.33	1.23
					W3	1.31	
β-Pinene	1.59	1.58			W2	1.65	1.58
					W3	1.68	1.60
					W4	1.64	1.61
Sabinene					W2	1.77	1.58
Δ_3-Carene	2.01	1.96					
Myrcene	2.19	1.75			W2	2.20	1.75
Limonene	2.73	2.38			W4	2.80	2.40
β-Phellandrene					W4	2.93	2.54
γ-Terpinene	3.63						
ρ-Cymene	4.27	2.76					
Terpinolene	4.59	4.10					
Unidentified (a)	8.10	5.70					
(130° isothermal, ref. camphor)							
Unidentified (a)	0.60						
Linalool			0.97	0.65	W5	0.92	0.60
Bornyl acetate			1.21	1.67			
Chavicol methyl ether			1.59	1.30	W6	1.60	1.29
α-Terpineol			1.80	1.14	W6	1.80	1.12
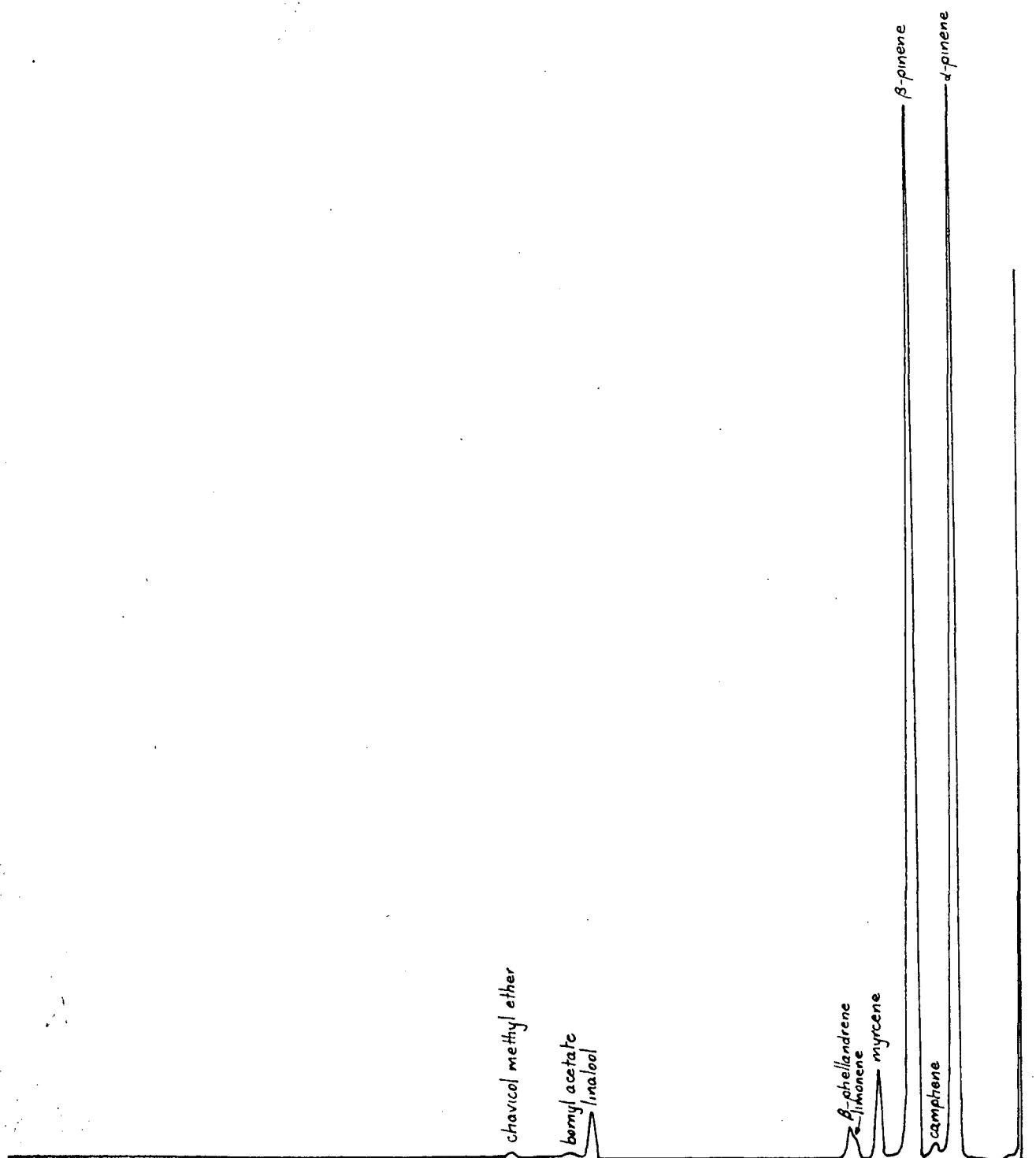
Fig. 62(a).   Low sensitivity gas chromatogram of whole oil from
oleoresin of *Pinus montezumae* (GC on 5% Carbowax 20M/Gas Chrom Q;
temperature program 50° to 200° at 5°/min;   0.2 µℓ sample;
attenuation 8 x 10^3).
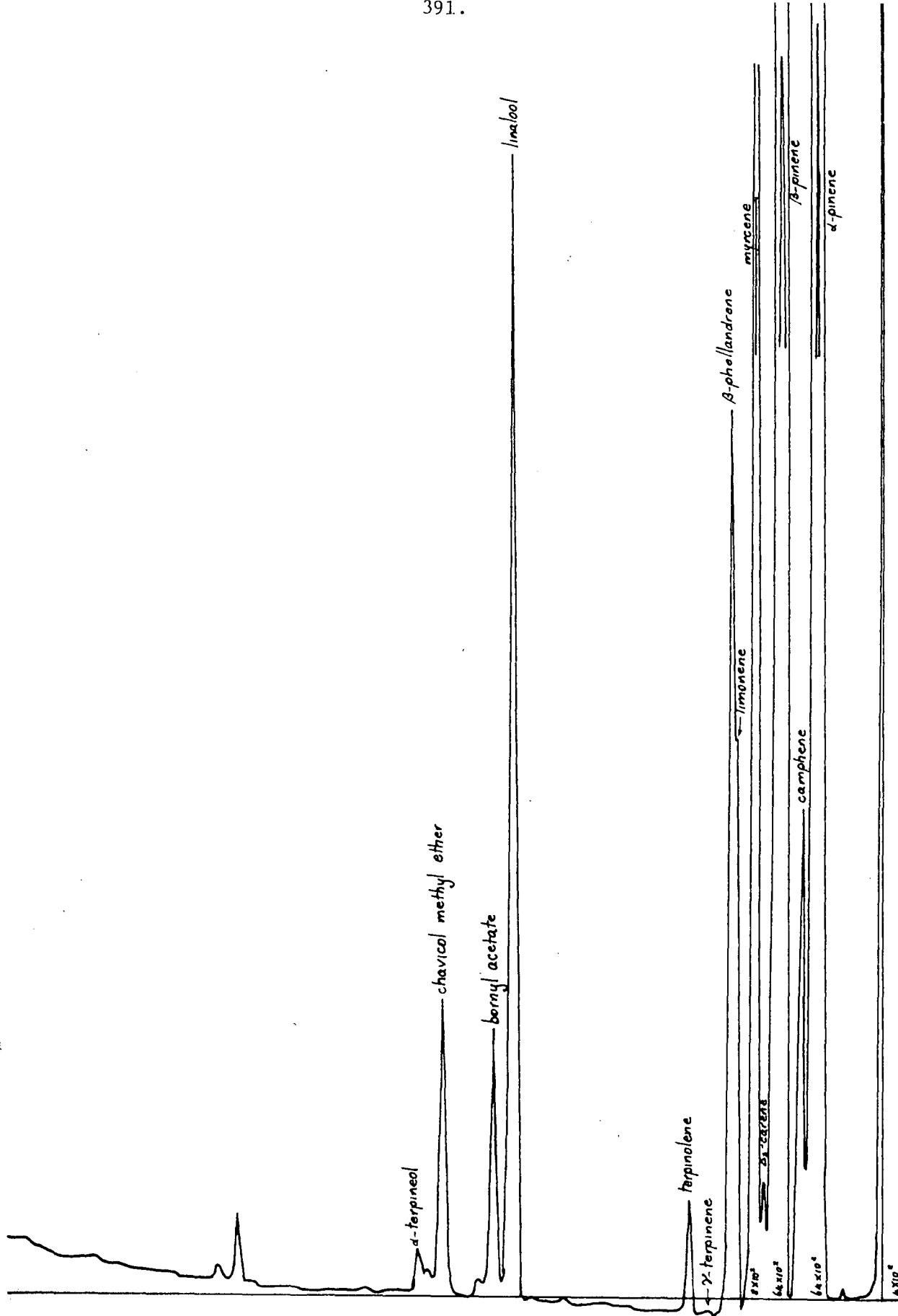
Fig. 62(b). High sensitivity gas chromatogram of whole oil from oleoresin of *Pinus montezumae* (attenuation 4 x 10^2).
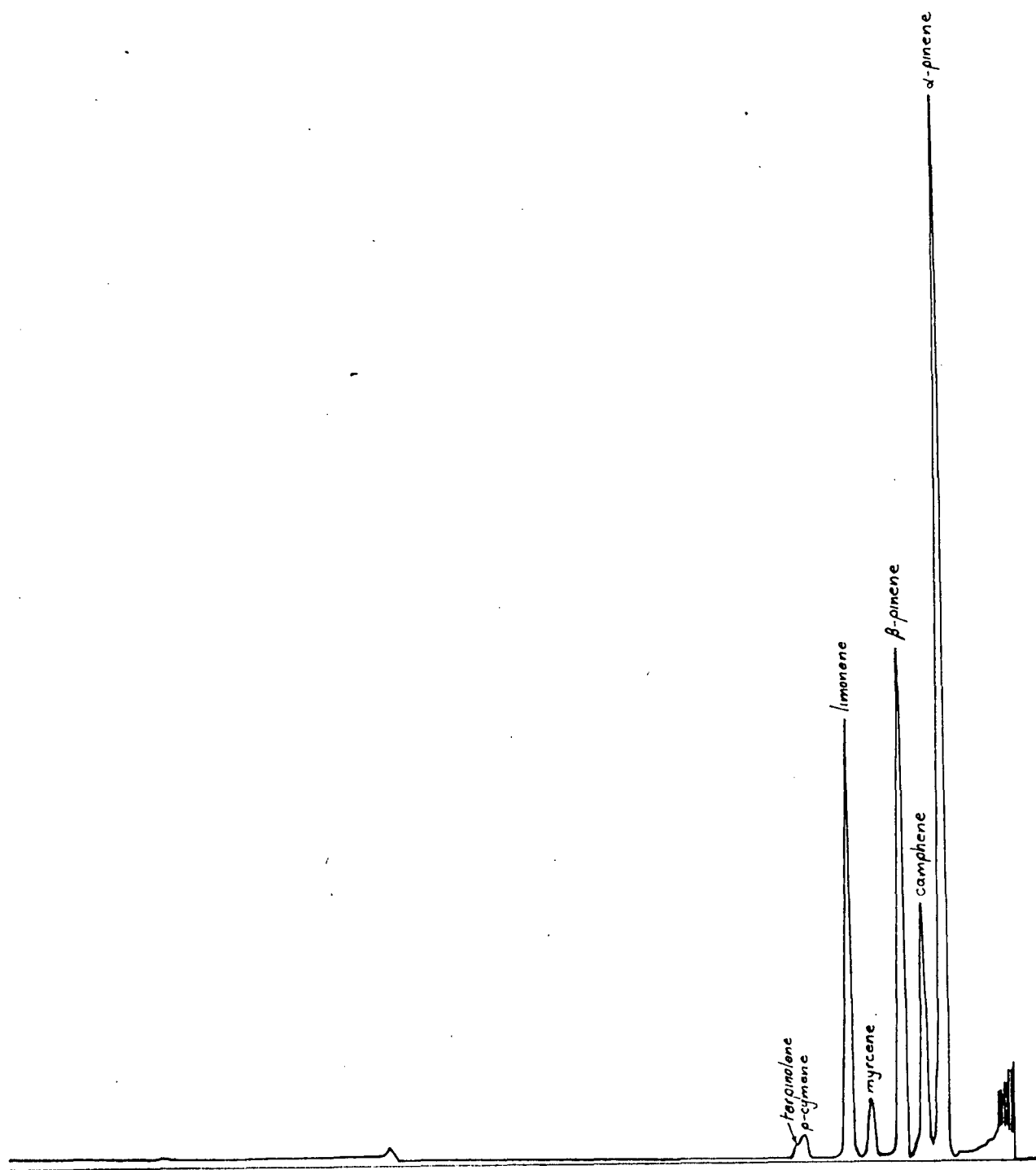
Fig. 62(c). Low sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus montezumae* separated on Florisil.

Fig. 62(d). High sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus montezumae* separated on Florisil.

Fig. 62(e).   Low sensitivity gas chromatogram of oxygenated fraction
of oil from oleoresin of *Pinus montezumae* separated on Florisil.
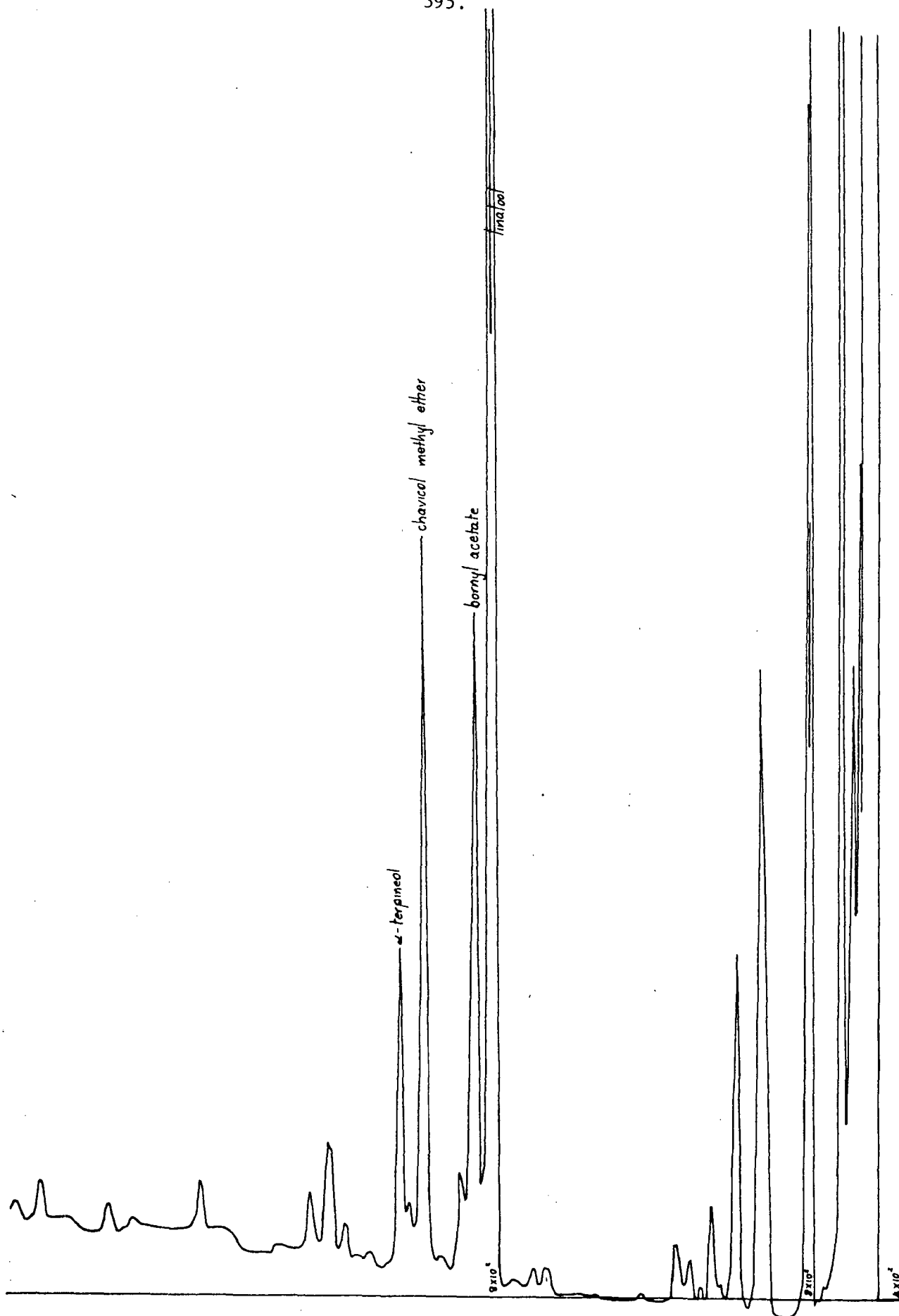
Fig. 62(f).   High sensitivity gas chromatogram of oxygenated fraction
of oil from oleoresin of *Pinus montezumae* separated on Florisil.

The composition of this oil is quite different from any of the previously described oils from this species [47, 145]. This difference is very likely to be due to the well-known variability of the species.

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from comminuted foliage (Table 85, Figure 63) indicated that a fundamentally different oil was being released to the atmosphere from the foliage. The foliage oil consisted principally of α-pinene with a lesser proportion of myrcene. ·Components that elute before α-pinene on Carbowax 20M were also detected. One of these peaks could be due to n-heptane found earlier in this species [47]. A 'temporarily-released component' was also found which eluted near Δ_3-carene as in the case of *Cedrus deodara*.

(c) Composition of successive injections of syringe-headspace
     vapour from foliage

Successive injections of vapour over a 3 hr. period, from a single sample of foliage, exhibited not only a wide initial fluctuation in the content of α-pinene, but also some apparently qualitative changes in the vapour composition. The previously-mentioned component, eluting near Δ_3-carene, could only be detected in vapour injected within approximately 15 minutes after comminution (Table 84, Figure 64). As found with several other species, successive injections of vapour contained an increased concentration of a peak due possibly to santene. In Figure 64 major changes can be seen in at least four components.

Table 85. RRT data and percentage composition of
volatile terpenoids in foliage of *Pinus
montezumae* determined by syringe-headspace
GC analysis

| Composition | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak area)
	(60° isothermal, ref. α-pinene)		(60° isothermal)
n-Heptane?	0.65		1.0
Santene?	0.77		0.5
α-Pinene	0.99	0.99	69.2
Camphene	1.26	1.19	2.4
β-Pinene	1.63	1.54	7.1
Sabinene	1.77		1.4
Unidentified	~2.0		t
Myrcene	2.30	1.76	14.8
Limonene	2.86	2.35	1.0
β-Phellandrene	2.98	2.49	0.4
Unidentified	4.09	2.91	1.3
Terpinolene	4.91	4.06	1.0

(d) Composition of syringe-headspace vapour from foliage

   sampled repeatedly from the same tree

The composition of the vapour, from initial injections
of three samples of foliage from the same tree, varied (Tables
85 and 86) from 55.0 to 69.2 percent α-pinene, 7.1 to 9.6
percent β-pinene and 14.0 to 17.6 percent myrcene.

A chromatogram of the steam-distilled oil, from the
remainder of the comminuted foliage studied by the syringe-
headspace technique (Table 86), is given in Figure 64.

Fig. 63.   Syringe-headspace gas chromatogram
of vapour from foliage of *Pinus montezumae* (GC
conditions as before;   attenuation 4 x 10^2).
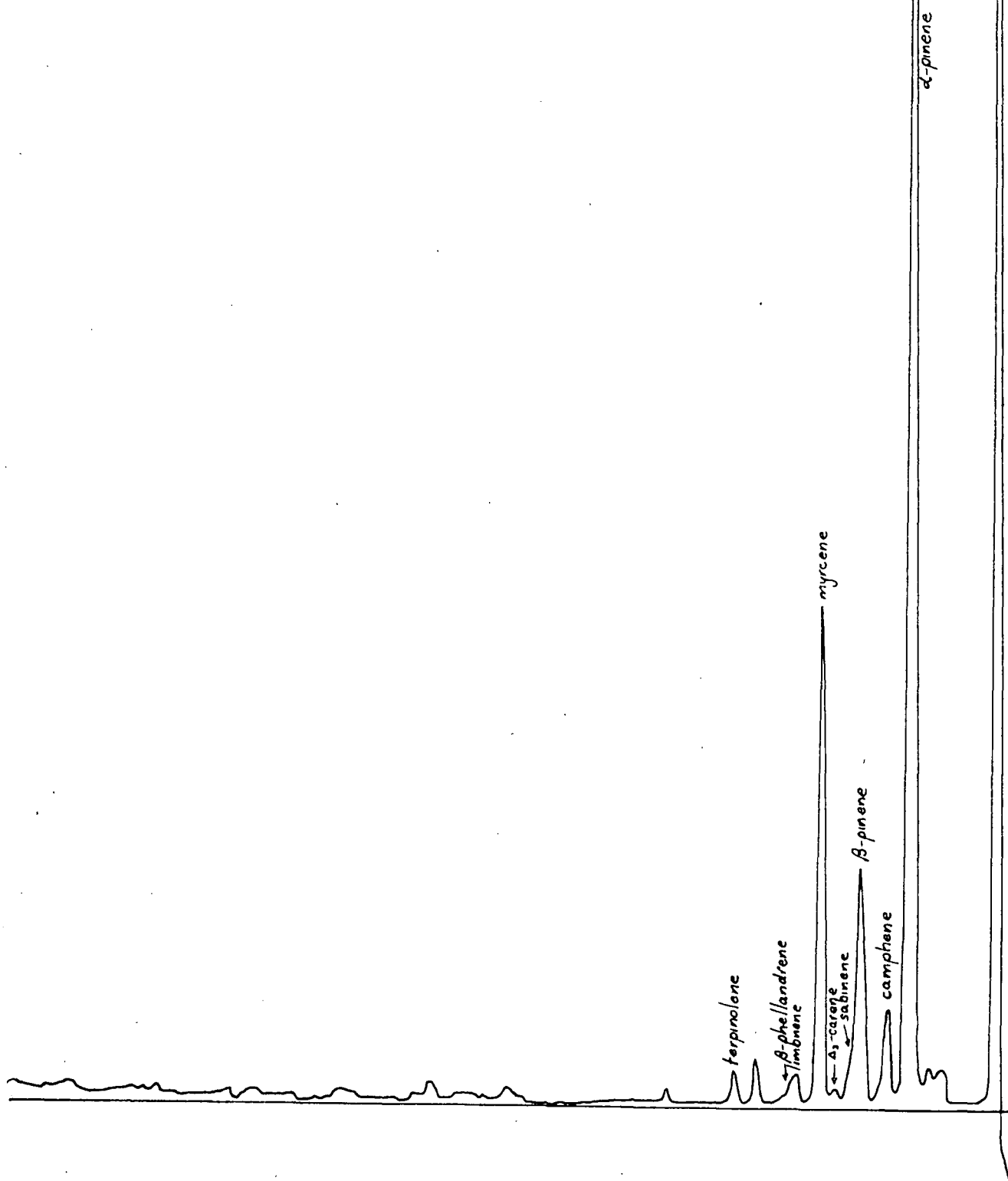
Table 86. Compositions of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Pinus montezumae*

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified + santene?	α-Pinene	Unidentified	Camphene	Unidentified	β-Pinene	Sabinene	Unidentified 'temporary component'	Myrcene	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene
(Sample A)													
0	0.2	55.0	6.4	2.5	0.7	7.2	2.5	2.5	17.6	1.5	0.5	1.7	1.6
15	0.5	68.9	–	3.2	–	6.8	1.4	0.2	15.3	1.1	0.3	1.2	1.0
30	0.7	69.3	–	3.1	–	6.6	1.3	–	15.3	1.2	0.3	1.2	1.0
50	0.6	69.0	–	3.1	–	6.7	1.5	–	15.2	1.3	0.3	1.2	1.0
70	0.9	69.1	–	3.0	–	6.4	1.4	–	15.1	1.3	0.3	1.3	1.2
95	1.1	69.6	–	3.0	–	6.5	1.5	–	14.7	1.1	0.3	1.1	1.0
110	0.8	69.7	–	3.0	–	6.2	1.4	–	14.9	1.3	0.3	1.3	1.1
130	0.9	69.4	–	3.1	–	6.4	1.5	–	15.1	1.2	0.3	1.2	1.0
150	1.0	69.3	–	3.0	–	6.1	1.6	–	15.0	1.4	0.4	1.1	1.1
170	1.2	69.4	–	3.0	–	6.3	1.5	–	14.7	1.2	0.3	1.2	1.2
190	1.5	68.7	–	3.1	–	6.3	1.6	–	14.9	1.4	0.3	1.1	1.1
205	1.6	68.6	–	3.0	–	6.2	1.6	–	15.1	1.5	0.3	1.1	1.1
(Sample B)													
0	–	68.5		1.8		9.6	1.4	1.4	14.0	1.0	0.5	0.9	0.9
40	–	76.8		2.0		8.0	1.7	–	9.0	0.8	0.3	0.7	0.7
55	0.2	77.2		1.8		7.9	1.8	–	8.6	0.9	0.3	0.6	0.8
70	0.2	77.5		2.0		7.8	1.5	–	8.5	0.9	0.3	0.6	0.8
90	–	78.1		2.0		7.6	1.6	–	8.3	0.8	0.3	0.6	0.7
Steam-distilled oil from remainder of comminuted foliage:													
	5.2	51.8		2.2		7.8	1.9		17.7	3.0	1.3	2.9	5.9

including 0.3 percent Δ_3-carene
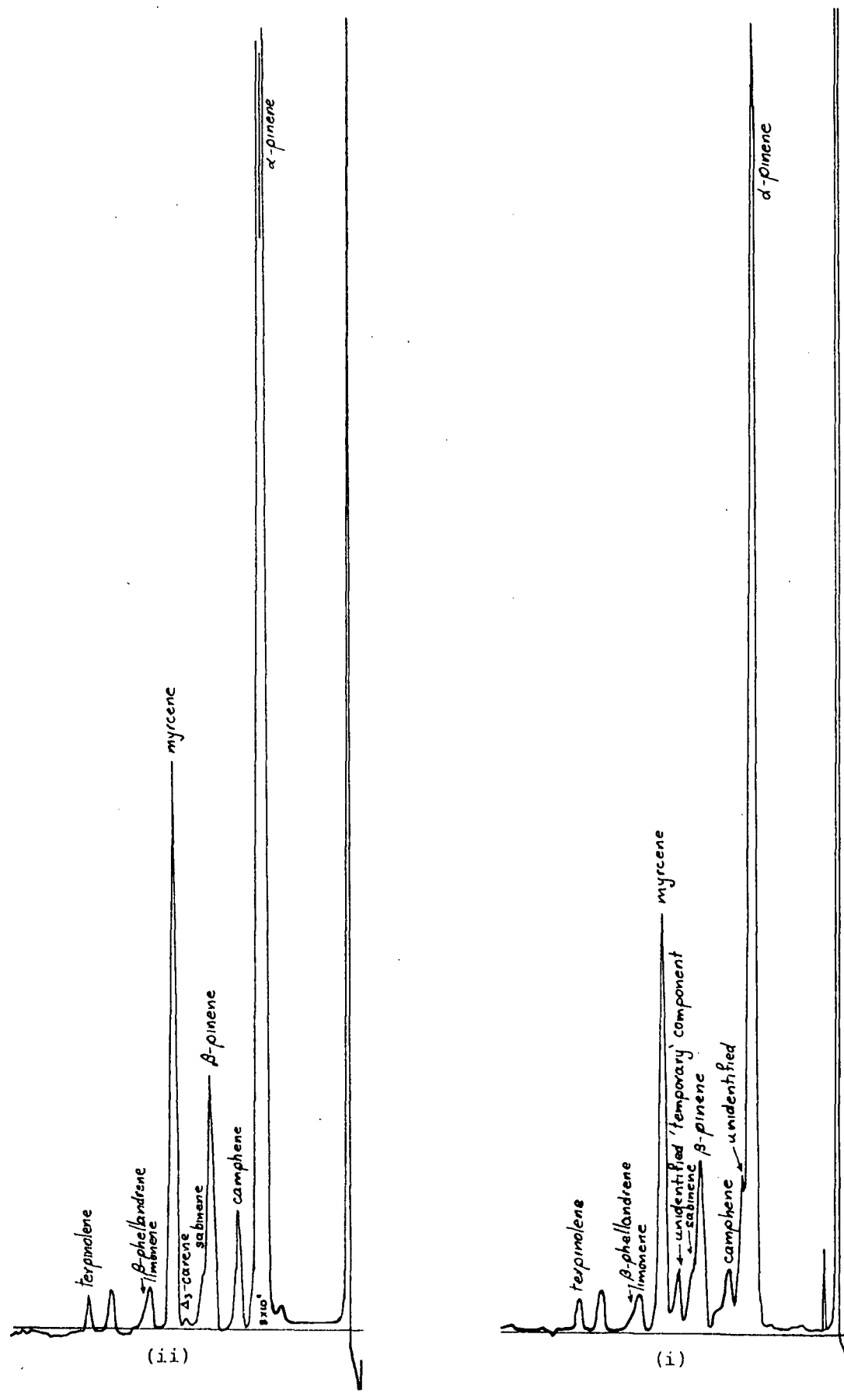
Fig. 64(a). Syringe-headspace chromatograms of foliage vapour of *Pinus montezumae*. Major quantitative and perhaps qualitative changes can be seen in the vapour compositions of the initial injection (i) and a subsequent injection (ii) (15 minutes later) from the same sample of foliage.

Fig. 64(b). Gas chromatogram of oil steam-distilled from the remainder of comminuted foliage of *Pinus montezumae* studied by the syringe-headspace GC technique in Fig. 64(a) (GC conditions as before).

(e) <u>Summary</u>

Components of the steam-distilled oil from oleoresin of *Pinus montezumae* were analyzed by chromatographic and spectroscopic methods. The following identifications were confirmed: α-pinene (45.8%), β-pinene (44.9%), myrcene (3.8%), limonene (0.9%), β-phellandrene (1.4%), linalool (2.0%) and chavicol methyl ether (0.3%). Tentatively identified were camphene (0.6%), sabinene, Δ_3-carene, γ-terpinene, ρ-cymene, terpinolene, bornyl acetate (0.3%) and α-terpineol. The composition of this oil was unlike any of the other oils reported from local variants of this complex species. The following components are reported from this species for the first time: linalool, chavicol methyl ether, sabinene, γ-terpinene, terpinolene, bornyl acetate and α-terpineol.

By comparison, the steam-distilled oil from foliage contained α-pinene (51.8%), camphene (2.2%), β-pinene (7.8%), sabinene (1.9%), Δ_3-carene (0.3%), myrcene (17.7%), limonene (3.0%), β-phellandrene (1.3%), γ-terpinene (2.9%) and terpinolene (5.9%).

The syringe-headspace GC technique indicated the existence of a wide range of monoterpene compositions to be found in successive injections of vapour from a single sample of foliage, and also from different samples of foliage from the same tree. Successive injections of vapour over a 3 hr. period contained from 55.0 to 69.7% α-pinene, 6.1 to 7.2% β-pinene and 14.7 to 17.6% myrcene; whereas from 3 samples of foliage from the same tree the vapour contained from 55.0 to 69.2% α-pinene, 7.1 to 9.6% β-pinene and 14.0 to 17.6% myrcene.

Qualitative changes were indicated in initial and subsequent injections of vapour from the same sample of foliage.

(x) *Pinus muricata* D.Don.

Bishop Pine *(Pinus muricata* D.Don.) was shown by Forde and Blight [441] to exhibit a chemical variability rarely found in other pines. On the basis of the compositions of oils distilled from oleoresins those workers proposed three distinct chemical races. The oil from one race consisted almost entirely of d,dℓ-α-pinene, another contained predominantly d-Δ_3-carene, while oils from a further race consisted of high proportions of ℓ-sabinene and terpinolene. During this investigation sabinene was reported for the first time in the genus *Pinus* [442].

Mirov *et al* [334] subsequently examined populations of *P. muricata* distributed throughout the south-west region of North America and reported a wide chemical diversification possessed by no other *Pinus* species. To indicate the range of oil compositions to be found in this species a summary of the compositions of oils found by these workers in 16 populations is reproduced in Table 87.

Although sample reproducibility of major monoterpenes has been studied in *P. muricata* oleoresin [151], no report was found in the literature of any investigation of foliage oils, which in other species has been found to be highly variable.

Table 87. Percentage compositions of oils from oleoresins of different North American populations of *P. muricata* [334]

Population	No. of trees examined	α-Pinene	Camphene	β-Pinene	Δ₃-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene	α-Thujene
Trinidad	6	97.0-98.5	0.5-1.0	0.0-1.0	-	-	0.5	t-0.5	-	-	-	-
Fort Bragg	11	92.5-97.5	1.0-1.5	1.0-3.0	-	-	0.5-1.0	t-0.5	t-2.0	-	-	-
Van Damme S.P.	8	94.5-97.5	1.0	1.0-2.0	-	0.0-2.0	0.5-1.0	t-0.5	t-0.5	-	0.0-0.5	-
Gualala	6	91.0-99.0	0.5-1.0	0.5-1.5	-	t-4.0	t-0.5	t-0.5	t-1.0	-	t-1.5	-
3.5 km. N of Annapolis	7	90.5-98.0	0.5-1.0	1.0-3.0	-	0.0-3.5	0.5-1.0	t-1.0	t-1.5	-	t-1.5	-
Annapolis	10	2.0-97.5	t-1.0	t-1.5	0.0-84.0	0.0-3.5	0.5-2.5	t-2.5	t-0.5	0.0-0.5	t-9.5	0.0-0.5
2 km. S of Annapolis	3	4.0-5.5	t	t	79.0-83.5	2.0-2.5	2.0-2.5	t	0.5	0.5	7.0-7.5	0.0-t
Stewarts Point	5	4.5-13.5	t	t-0.5	73.0-79.5	3.5-7.0	2.0-2.5	t-0.5	0.5	0.0-0.5	4.0-8.0	-
Fort Ross	8	3.0-18.5	-	t-1.5	71.0-90.5	0.0-4.5	0.5-2.5	t-0.5	t-1.0	0.0-0.5	4.5-6.5	0.0-0.5
Inverness (Tomales Bay)	9	2.5-20.0	0.0-t	t-2.0	69.0-86.0	0.1-6.0	2.0-2.5	t-0.5	0.5	0.5	5.0-7.5	0.0-0.5
Monterey	10	1.0-5.0	t-0.5	t-1.5	84.5-94.5	t-1.0	1.5-3.0	t-0.5	t-1.5	0.0-0.5	t-7.0	0.0-0.5
San Luis Obispo	10	4.5-9.0	t	0.5-1.5	t-1.0	53.5-62.5	0.5-1.5	t-2.0	1.0-1.5	0.5-3.5	25.5-35.0	1.0-2.0
Lompoc		4.0-12.5	t-1.0	1.5-4.0	0.5-2.0	44.0-73.5	0.0-1.5	0.5-1.5	0.5-1.5	t-2.5	8.0-31.0	1.0-11.0
Santa Cruz Is.	10	11.5-54.5	t-2.0	1.5-13.5	0.0-1.0	25.0-62.0	t-1.0	0.0-1.0	0.5-2.0	0.0-2.5	3.5-32.5	t-3.0
Santa Rosa Is.	5	36.5-59.5	0.5-1.5	14.5-20.0	0.0-1.0	8.5-30.0	t-1.0	t-1.5	0.0-1.5	t-2.0	8.5-12.0	t-1.0
San Vicente		5.5-8.5	0.0-1.0	1.5-2.5	0.0-1.0	41.0-62.0	0.0-4.0	t-10.0	1.5-15.0	t-1.0	3.0-30.5	1.0-2.0

404.

(a) Analysis of the oil steam-distilled from cortical oleoresin

Cortical oleoresin collected as before from a single tree of *P. muricata* in the Royal Botanical Gardens (Tasmania), yielded upon steam-distillation 26.9 percent of a colourless oil with a sweet pinene odour.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 88. RRT values leading to the tentative identification of components in hydrocarbon and oxygenated fractions, and preparative GC fractions from the

Table 88. Components distinguishable in the whole oil from oleoresin of *Pinus muricata*

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak height)
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min)
*α-Pinene	1.00	0.98	20.4
Camphene	1.26	1.18	0.1
β-Pinene	1.60	} 1.54	0.2
*Sabinene	1.72		2.7
*Δ₃-Carene	2.14	2.05	53.5
*Myrcene	2.29	1.76	9.9
Unidentified	2.41		0.7
Limonene	2.72	2.39	0.4
β-Phellandrene	2.84	2.54	0.6
*γ-Terpinene	3.60	3.25	0.6
ρ-Cymene	4.26	2.83	0.2
*Terpinolene	4.59	4.10	10.5

(130° isothermal, ref. camphor)

Several minor peaks, too small for accurate and correlated RRT measurement

* IR spectrum recorded

whole oil, are listed in Table 89. Gas chromatograms of Figure 65 show the distribution of components eluted from a Carbowax 20M column, and indicate the degree of separation of components into hydrocarbon and oxygenated fractions.

The monoterpene composition of the oleoresin from this single tree does not resemble that of any of the populations studied by Mirov *et al* [334]. The presence however of significant concentrations of α-pinene, sabinene, Δ₃-carene, myrcene and terpinolene is in keeping with the character of *P. murica*, which has the genetic capability to synthesize oils containing these components in major proportions.

Table 89. RRT values on the dissimilar liquid phases C20M and OV-17, for components found in hydrocarbon, oxygenated and preparative GC fractions, isolated from steam-distilled oil from oleoresin of *Pinus muricata*

Component	Hydrocarbon fraction		Oxygenated fraction		Preparative GC fractions		
	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)							
α-Pinene	1.03	0.99			W1	0.99	1.01
Camphene	1.28	1.18			W1	1.29	1.22
					W2	1.29	1.23
β-Pinene	1.62	1.56			W2	1.60	1.55
Sabinene					W2	1.72	
Δ₃-Carene	2.10	2.01			W3	2.10	2.06
					W4	2.05	1.96
Myrcene	2.27	1.74			W2	2.24	1.76
Unidentified					W3	2.39	
Limonene	2.78	2.38			W3	2.68	2.37
β-Phellandrene	2.92	2.51					
γ-Terpinene	3.66	3.25					
ρ-Cymene	4.25	2.80					
Terpinolene	4.57	4.13			W4	4.58	4.10
(130° isothermal, ref. camphor)							
Geraniol			3.23	1.23			

Fig. 65(a). Low sensitivity gas chromatogram of whole oil from oleoresin of *Pinus muricata* (GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50° to 200° at 5°/min; 0.2 μℓ sample; attenuation 8 x 10³).

Fig. 65(b).   High sensitivity gas chromatogram of whole oil from oleoresin of *Pinus muricata* (attenuation 4×10^2).
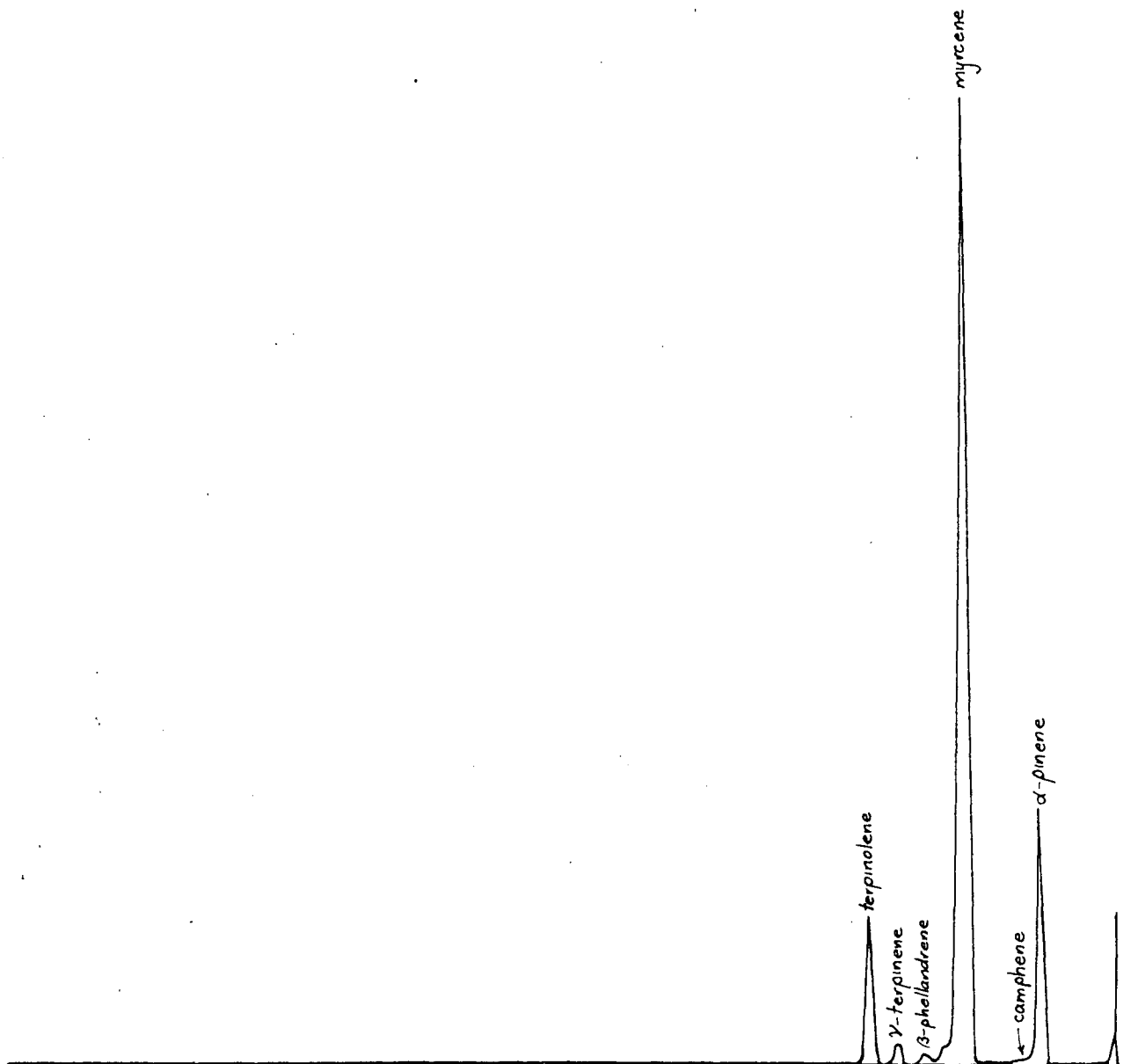
Fig. 65(c). Low sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus muricata* separated on Florisil.
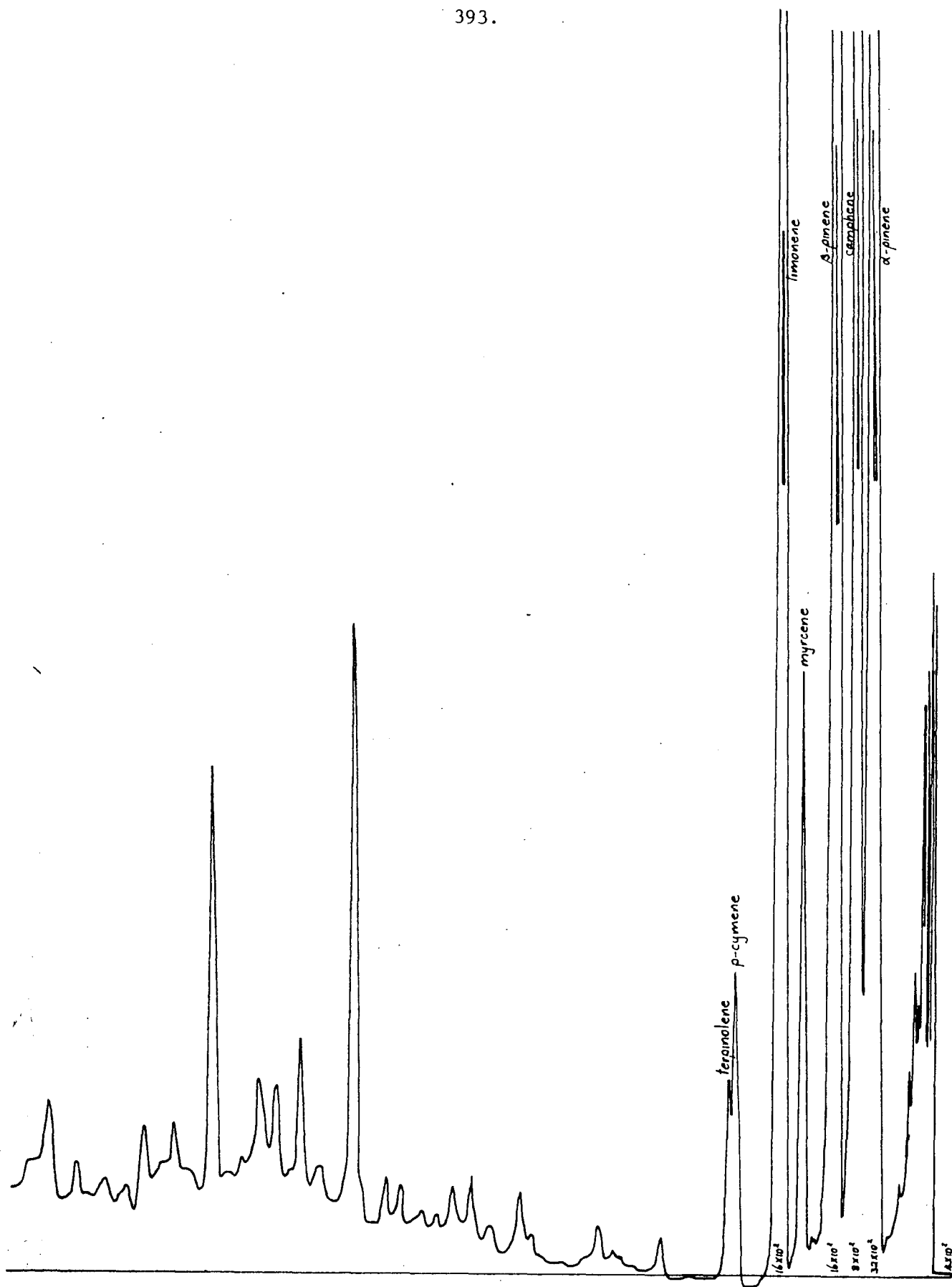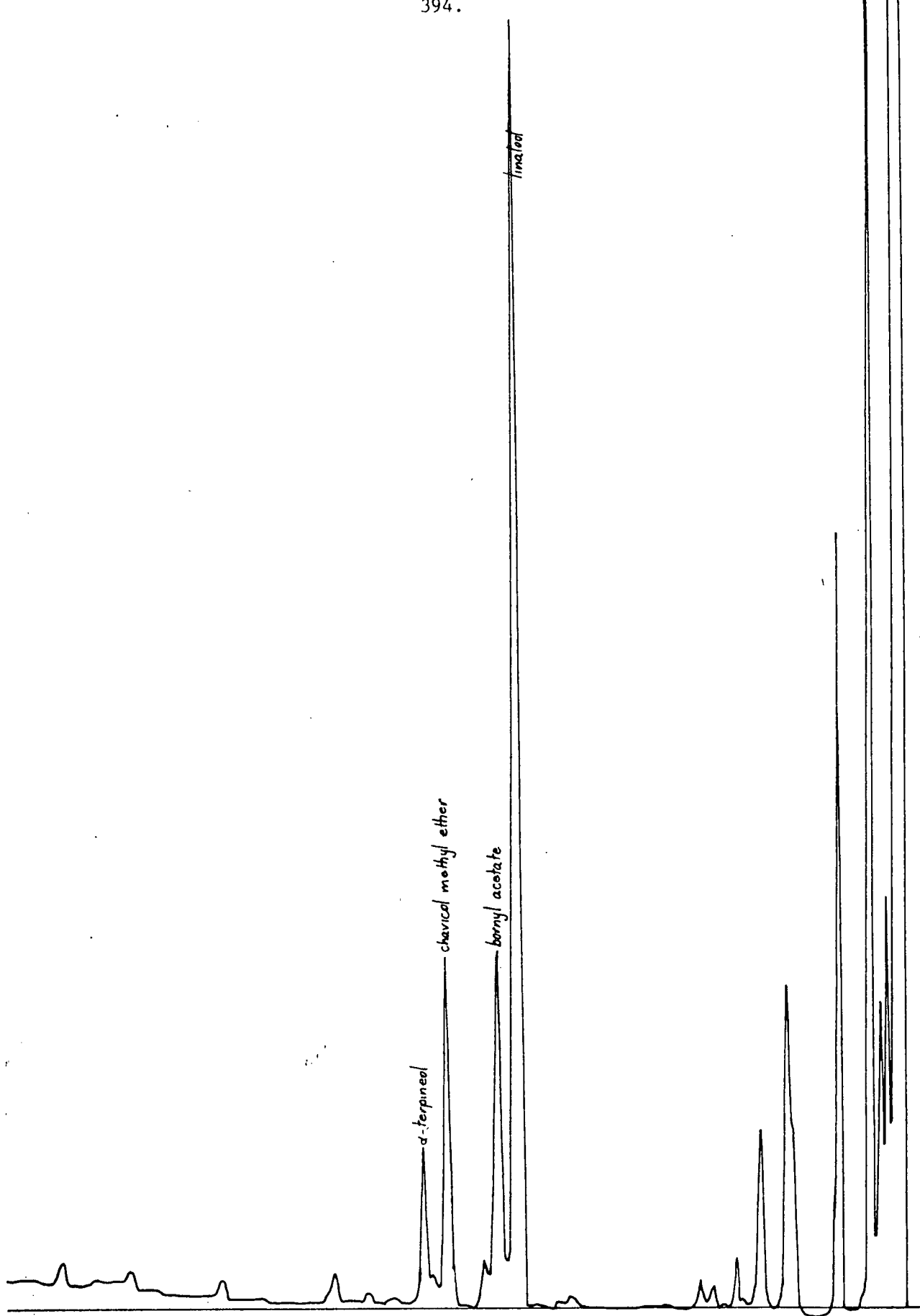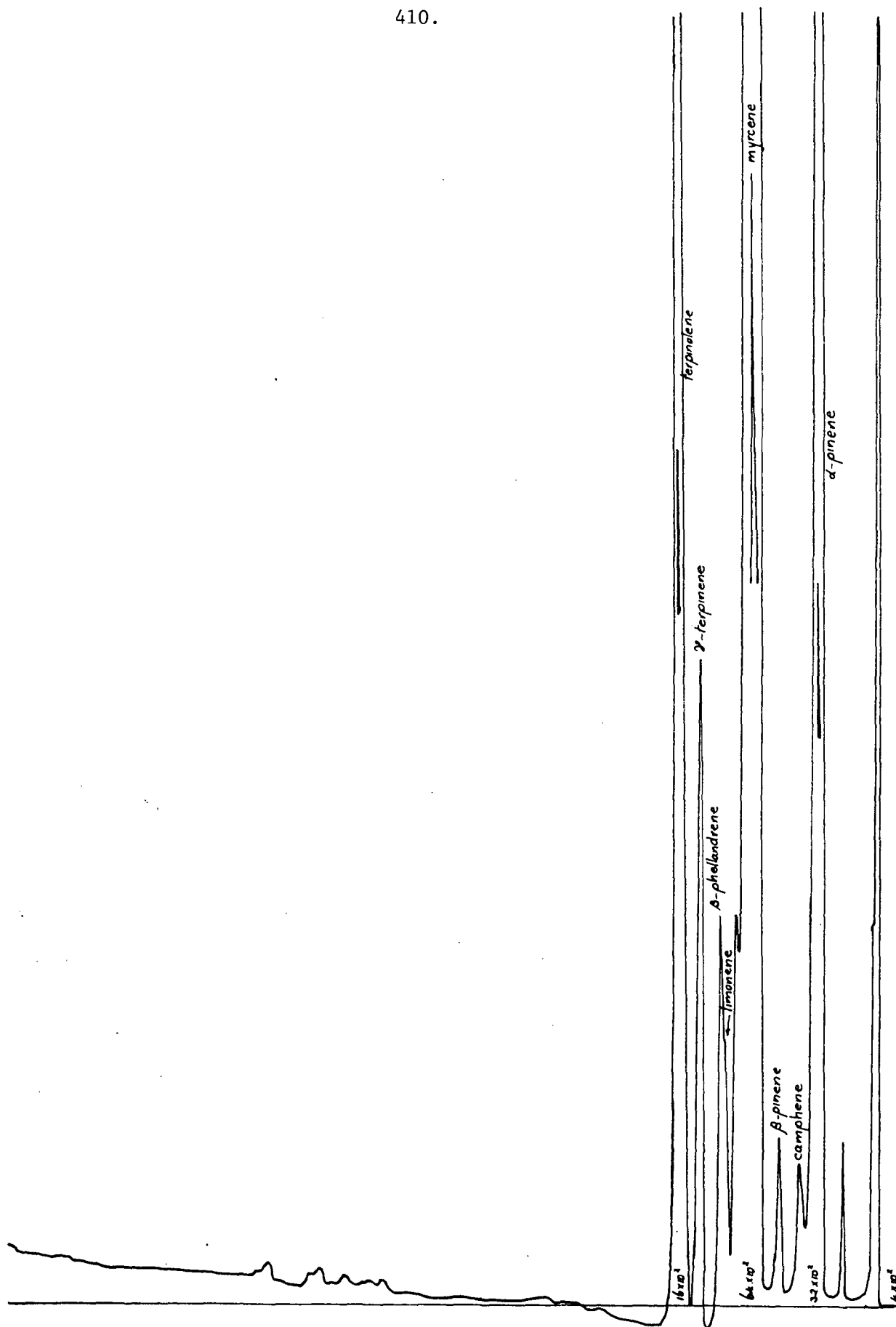
Fig. 65(d). High sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus muricata* separated on Florisil.
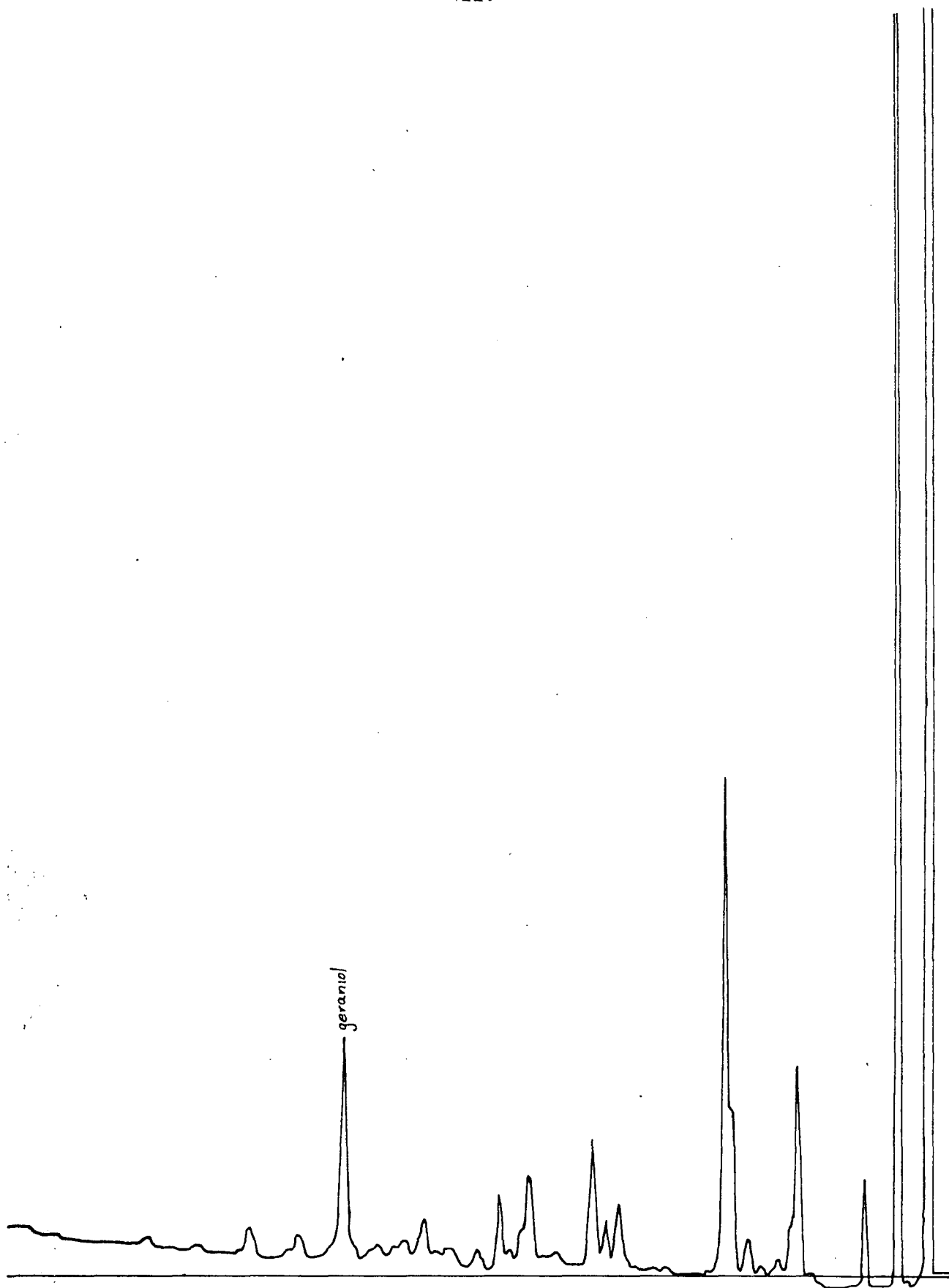
Fig. 65(e). High sensitivity gas chromatogram of oxygenated fraction of oil from oleoresin of *Pinus muricata* separated on Florisil.

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from comminuted foliage (Table 90, Figure 66) indicated the existence of a quite different vapour composition being released to the atmosphere from this tree. This oil contained increased proportions of α- and β-pinene with a lesser amount of Δ_3-carene. A further early-eluting component was detected along with a 'temporarily-released component', eluting near Δ_3-carene as found with *Cedrus deodara*.

Table 90. RRT data and percentage composition of volatile terpenoids in foliage of *Pinus muricata* determined by syringe-headspace GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak area of 4th successive injection)
	C20M	OV-17	
(60° isothermal, ref. α-pinene)			(60° isothermal)
Unidentified	0.77		0.5
"		0.47	t
"		0.79	
α-Pinene	0.99	0.99	72.2
Camphene	1.28	1.18	5.5
β-Pinene	1.62	1.52	8.2
[Unidentified	~2.0; several percent in first injection only]		
Δ_3-Carene	2.07	1.93	7.6
Myrcene	2.29	1.72	1.1
Limonene	2.84	2.35	1.2
β-Phellandrene	2.96	2.46	0.8
Unidentified	4.01	2.91	2.2
Terpinolene	4.83	4.04	0.6

t: trace; <0.1 percent
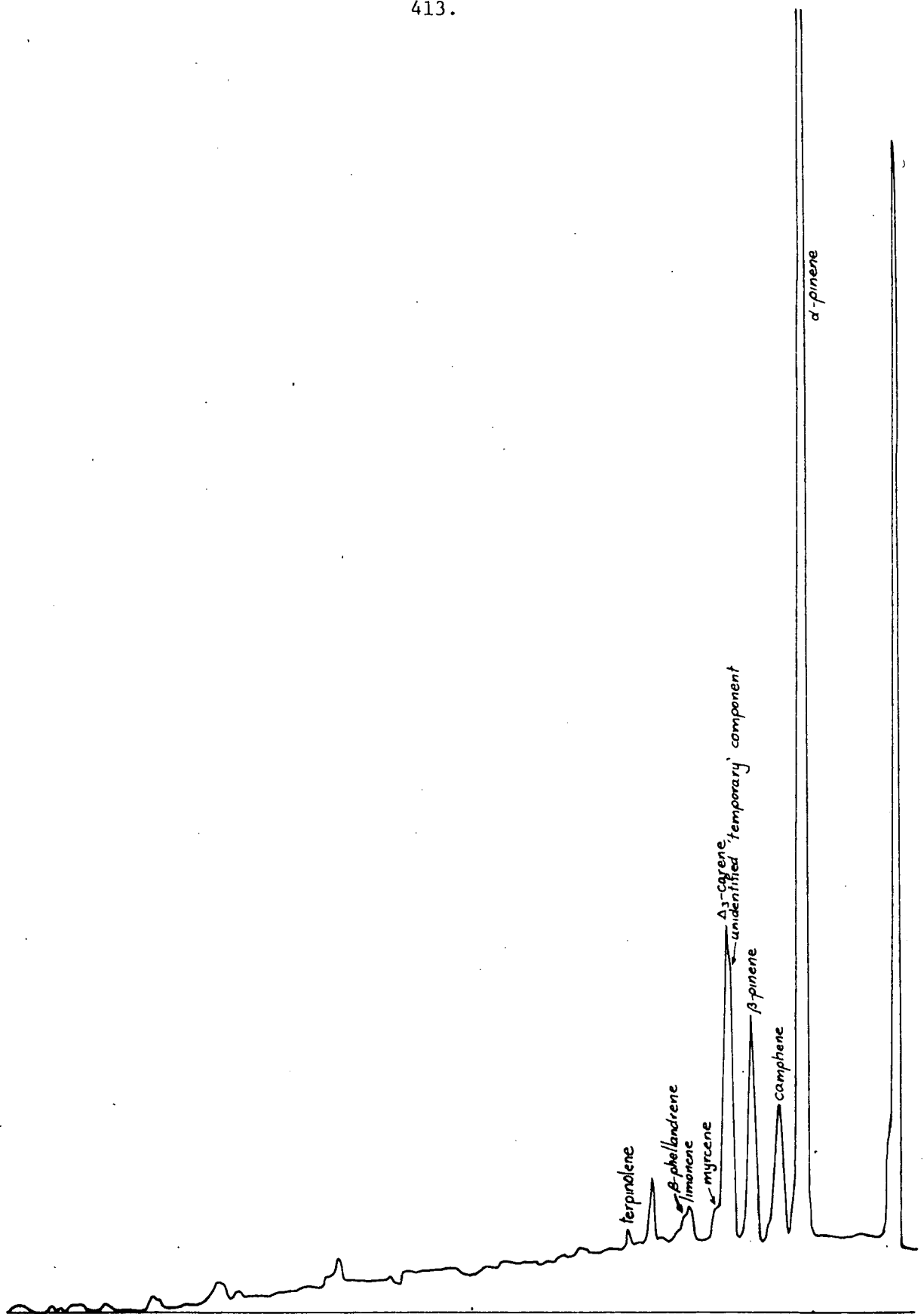
Fig. 66.   Syringe-headspace gas chromatogram of vapour from foliage of
*Pinus muricata* (GC conditions as before;   attenuation 4 x 10^2).

(c) <u>Composition of successive injections of syringe-headspace</u>

<u>vapour from foliage</u>

Successive injections of vapour over a 3 hr. period, from a single sample of foliage, exhibited initial fluctuations in the content of α-pinene and Δ_3-carene, but also some apparently qualitative changes in the vapour composition (Table 91, Figure 67).

(d) <u>Composition of syringe-headspace vapour from duplicate</u>

<u>samples of foliage from the same tree</u>

The composition of the vapour from two samples of foliage from the same tree varied (Tables 90 and 91) from 68.4 to 72.2 percent α-pinene, 8.1 to 8.2 percent β-pinene and 7.6 to 12.4 percent Δ_3-carene.

A chromatogram of the steam-distilled oil from the remainder of the comminuted foliage from (d) is given for comparison (Table 91) in Figure 67.

(e) <u>Summary</u>

Components of the steam-distilled oil from oleoresin of *Pinus muricata* were analyzed by chromatographic and spectroscopic methods. The following identifications were confirmed: α-pinene (20.4%), sabinene (2.7%), Δ_3-carene (53.5%), myrcene (9.9%), γ-terpinene (0.6%) and terpinolene (10.5%). Tentatively identified were camphene (0.1%), β-pinene (0.2%), limonene (0.4%), β-phellandrene (0.6%), ρ-cymene (0.2%) and geraniol. Geraniol has not previously been reported in this oil.

Table 91. Compositions of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Pinus muricata*

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified group	α-Pinene	Camphene	Unidentified	β-Pinene	Sabinene	Δ_3-Carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene(?)	Terpinolene
0	0.3	68.4	5.1	0.4	8.1	0.8	12.4	1.1	t	1.0	0.7	1.1	0.8
35	1.5	70.2	5.8	0.3	7.2	0.7	10.4	0.8	0.1	0.9	0.4	1.0	0.6
55	1.6	71.1	5.5	0.3	7.1	0.6	10.2	0.8	0.1	0.8	0.3	0.7	0.6
70	1.6	71.7	5.4	0.4	7.0	0.5	10.0	0.8	0.2	0.8	0.4	0.8	0.4
85	1.6	72.5	5.4	t	7.0	0.5	10.1	0.7	0.1	0.7	0.2	0.7	0.5
105	1.4	71.8	5.4	0.2	7.1	0.5	10.1	0.8	0.1	0.8	0.5	0.8	0.6
120	1.5	71.8	5.5	0.3	7.1	0.3	10.1	0.8	0.1	0.8	0.5	0.7	0.5
135	1.5	71.4	5.5	0.3	7.0	0.5	10.1	0.8	0.1	0.9	0.6	0.8	0.5
150	1.5	71.7	5.5	0.1	7.1	0.5	10.1	0.7	0.1	0.8	0.5	0.8	0.6
165	1.5	71.8	5.6	t	7.2	0.2	10.2	0.8	0.1	0.9	0.5	0.7	0.5
180	1.6	71.7	5.5	t	7.0	0.4	10.2	0.7	0.1	0.9	0.5	0.8	0.4
195	1.4	71.4	5.6	0.3	7.0	0.5	10.2	0.8	0.1	0.9	0.5	0.8	0.4

Steam-distilled oil from remainder of comminuted foliage:

	12.0	42.6	4.1	0.5	7.0	0.5	14.8	1.1	0.4	2.1	9.9	2.8	2.1
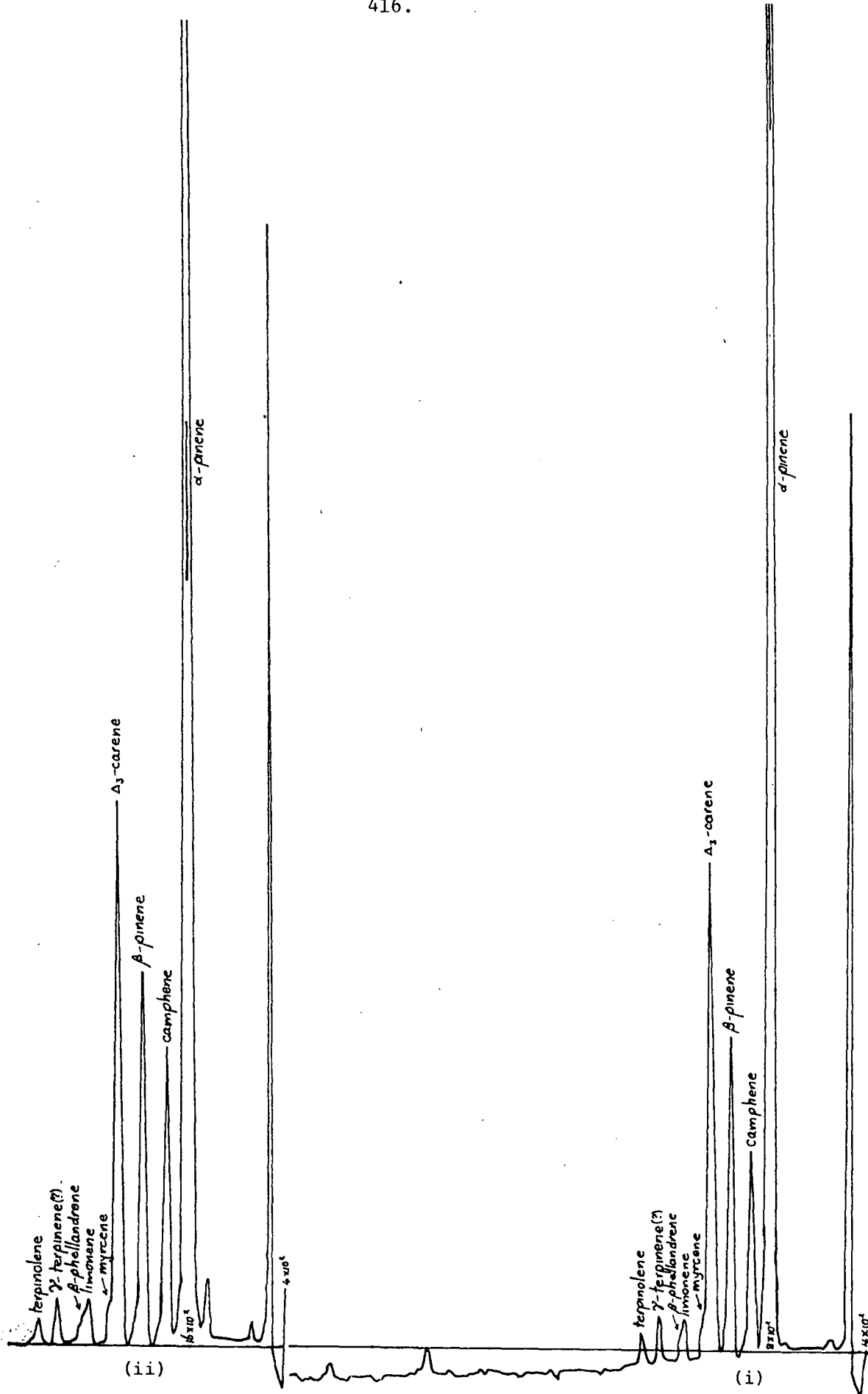
Fig. 67(a).   Syringe-headspace chromatograms of foliage vapour of
Pinus muricata.   Fluctuations in the proportions of α-pinene and
Δ₃-carene may be seen, together with apparently qualitative changes
in the vapour compositions by comparison of the initial injection (i)
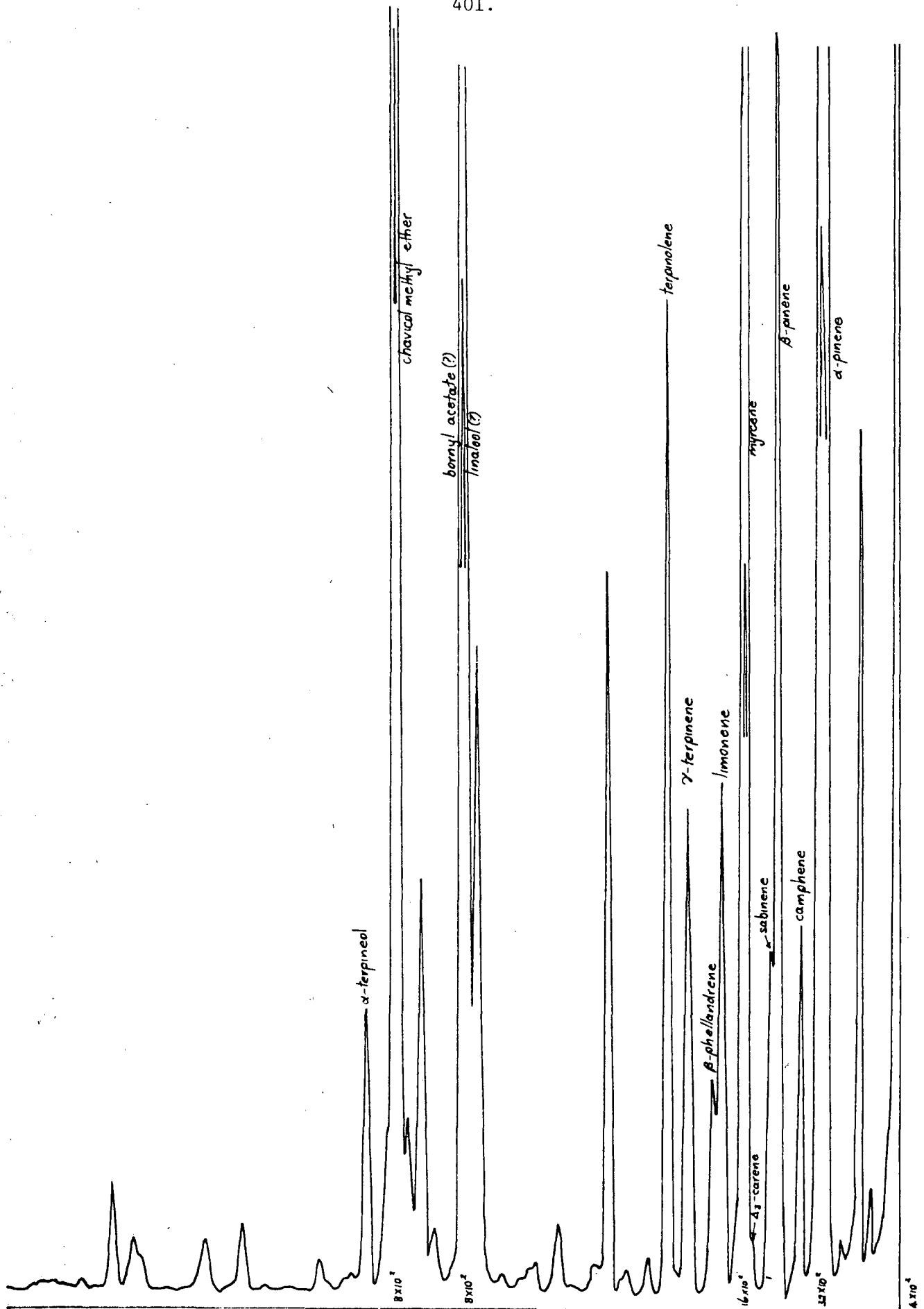and an injection 35 mins. later (same sample of foliage) (ii).
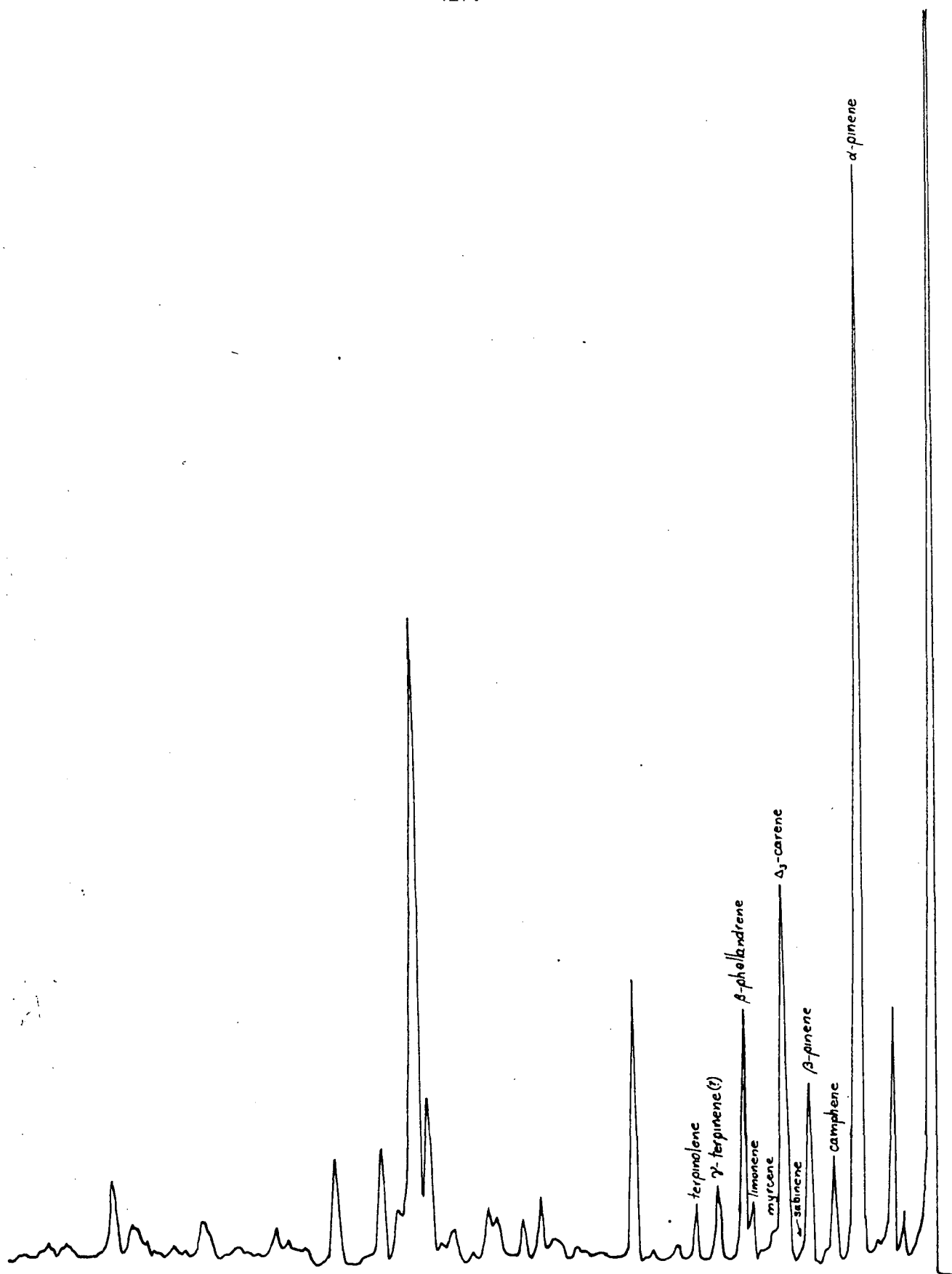
Fig. 67(b). Gas chromatogram of oil steam-distilled from the remainder of comminuted foliage of *Pinus muricata* studied by syringe-headspace vapour GC in Fig. 67(a) (GC conditions as before).

By comparison monoterpenes of the steam-distilled foliage oil included: a component eluted before α-pinene (12.0%), α-pinene (42.6%), camphene (4.1%), β-pinene (7.0%), sabinene (0.5%), Δ_3-carene (14.8%), myrcene (1.1%), limonene (2.1%), β-phellandrene (9.9%), γ-terpinene (2.8%) and terpinolene (2.1%).

The syringe-headspace GC technique indicated the existence of a range of monoterpene compositions to be found in successive injections of vapour from a single sample of foliage, and also from different samples of foliage from the same tree. Successive injections of vapour over a 3 hr. period contained 68.4 to 72.5% α-pinene, 8.1 to 7.0% β-pinene and 10.0 to 12.4% Δ_3-carene; whereas vapour from duplicate samples of foliage from the same tree contained 68.4 to 72.2% α-pinene, 8.1 to 8.2% β-pinene and 7.6 to 12.4% Δ_3-carene.

An apparently qualitative change in foliage vapour was indicated when several percent of a component, eluted near Δ_3-carene on Carbowax 20M, could only be detected in the initial injection of a freshly-comminuted sample.

(ix) *Pinus nigra* Arn. var *austriaca*

Oils of the Austrian or Black Pine, *Pinus nigra* Arn., have been investigated from trees grown throughout Europe and Asia Minor, but unfortunately most workers have not bothered to distinguish the variety. Mirov summarized much of the

early work and concluded that the composition of oil from

oleoresin of most varieties was very similar. For example

P. nigra var *corsicana* (Corsica) was reported to contain

87 percent ℓ-α-pinene, var *austriaca* (France) contained 94

percent, var *austriaca* (Ukraine) contained 85 percent,

var *pallasiana* (Ukraine) contained 87.2 percent, a Spanish

variety (probably *hispanica*) contained 90 percent, whereas

var *nigricans* (Bulgaria) contained 53 percent ℓ-α-pinene and

38 percent ℓ-β-pinene. Low percentages of β-pinene, camphene

and limonene were found in most [47]. Other workers have

found oil from oleoresin of *P. nigra* in Slovenia to contain 78 to

80 percent α-pinene [443], a cultivated variety in New Zealand

contained 93 percent α-pinene [145], while Bardyshev *et al* [444]

reported Bulgarian oil which contained as high as 89.9 percent

α-pinene.

A detailed investigation by Bardyshev *et al* [445], of

components in the oil from oleoresin of Bulgarian *P. nigra*, has

shown the presence of tricyclene (<0.1 percent), α-pinene

(89.9 percent), camphene (2.0 percent), β-pinene (2.0 percent),

myrcene (1.0 percent), Δ_3-carene (<0.1 percent), dipentene

(4.6 percent), β-phellandrene (0.5 percent), γ-terpinene and

ρ-cymene (<0.1 percent), terpinolene (0.1 percent), and a

distillation residue (1.8 percent) consisting of longifolene,

bornyl acetate, terpinen-4-ol, γ-terpineol, borneol, chavicol

methyl ether, α-terpineol, α-terpinyl acetate, verberone

and 19 unidentified sesquiterpenes.

Oil from the steam-distilled needles and foliage of

P. nigra has been reported from Jogoslavia [446] to contain

69.3 percent ℓ-α-pinene, 17.3 percent ℓ-β-pinene and myrcene,

5.7 percent ℓ-limonene, 4.1 percent ρ-cymene, 1.8 percent

camphene and dipentene; whereas needle oil from Turkey [447]

contained 78.8 percent α-pinene, 1.7 percent camphene, 19.5

percent β-pinene and no detectable traces of Δ_3-carene or

limonene. The presence of α-phellandrene has also been

established [425] in the foliage oil.

The attraction of *Orthotomicus erosus*, particularly

by α-pinene, but also by β-pinene, Δ_3-carene and myrcene, was

reported by Chararas and M'Sadda [448]. These workers showed

that the attraction by these monoterpenes caused infestations

of *Pinus halepensis* in the field and also of *P. pinea*,

P. pinaster, *P. nigra* and *P. radiata* under laboratory conditions.

Studies of the biosynthesis of α-pinene in *P. nigra*

var austriaca have been reported by Sandermann [449, 450], and

more recently by Banthorpe and Le Patourel [427].

(a) Analysis of the oil steam-distilled from cortical oleoresin

Cortical oleoresin collected as before from a single

tree of *P. nigra* var *austriaca* (tree IV) in the Royal Botanical

Gardens (Tasmania), yielded upon steam-distillation 27.9 percent

of a colourless oil with a pinene odour. A further tree

identified only as *P. nigra* (tree I) yielded 28.1 percent oil

from the oleoresin.

The qualitative and quantitative composition of

components distinguishable in the whole oil, using two dissimilar

columns, is recorded in Table 92. RRT values leading to the
tentative identification of components in hydrocarbon and
oxygenated fractions, and preparative GC fractions from the
whole oil, are listed in Table 93. Gas chromatograms of
Figure 68 show the distribution of components eluted from a
Carbowax 20M column from injections of the whole oil (of tree IV),
hydrocarbon and oxygenated fractions.

Table 92. Components distinguishable in the whole
oils from oleoresin of 2 trees: a *Pinus
nigra* var *austriaca* (tree IV) and an
unclassified *Pinus nigra* (tree I)

Component	Qualitative RRT data				Quantitative composition	
	(tree IV)		(tree I)		(tree IV)	(tree I)
	C20M	OV-17	C20M	OV-17	(percent, based on peak height)	
(60° isothermal, ref. α-pinene)					(TP 50° to 200°, 5°/min)	
α-Pinene	1.05	1.04	1.04	1.01	*79.9	*84.2
Camphene	1.32	1.20	1.28	1.16	* 4.3	2.3
β-Pinene	1.65	1.57	1.61	1.54	* 3.9	* 2.1
Δ$_3$-Carene			2.06	1.94		t
Myrcene	2.28	1.76	2.25	1.72	* 1.9	* 3.9
Limonene	2.75	2.38	2.76	2.34	* 6.1	* 0.7
β-Phellandrene	2.89	2.50	2.93	2.48	1.9	0.3
γ-Terpinene			3.65			t
Terpinolene			4.55	4.10		t
(130° isothermal, ref. camphor)						
Terpinolene	0.45	0.57	0.44	0.59	t	
α-Terpineol	1.77				t	
Unidentified	1.91	3.67	1.91	3.81	1.9	6.6
"	2.25	4.58			t	

* IR spectrum
t: trace; <0.1 percent

Table 93. RRT values on the dissimilar liquid phases C20M and OV-17, for components found in hydrocarbon, oxygenated and preparative GC fractions, isolated from steam-distilled oil from oleoresin of a tree of *Pinus nigra* var *austriaca* (tree IV) and an unclassified *Pinus nigra* (tree I)

Component	Hydrocarbon fraction		Oxygenated fraction		Preparative GC fractions		
	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(tree IV) (60° isothermal, ref. α-pinene)							
α-Pinene	1.04	1.03			W1	1.05	1.04
Camphene	1.31	1.21			W1	1.26	1.18
					W2	1.31	1.23
					W3	1.26	1.22
β-Pinene	1.63	1.59			W3	1.60	1.57
Sabinene					W2	1.75	1.57
Myrcene	2.27	1.76			W2	2.25	1.75
					W3	2.23	1.75
Limonene	2.77	2.37			W4	2.76	2.38
β-Phellandrene					W4	2.91	2.56
p-Cymene	4.23	2.79					
Unidentified (a)	8.06	5.57					
(130° isothermal, ref. camphor)							
Unidentified (a)	0.61						
Unidentified	1.72		1.73				
(tree I) (60° isothermal, ref. α-pinene)							
α-Pinene	1.02	1.01			W1	1.06	1.03
Camphene	1.27	1.19			W2	1.30	1.21
β-Pinene	1.62	1.54			W3	1.61	1.59
Δ_3-Carene	2.06	1.95			W4	2.05	
Myrcene	2.21	1.72			W2	2.26	1.75
Limonene	2.73	2.35			W5	2.76	2.38
1,8-Cineole			3.13	2.81			
p-Cymene	4.30	2.80	4.36	2.81			
Terpinolene	4.57						
Unidentified (a)	8.21	5.66					
(130° isothermal, ref. camphor)							
Unidentified (a)	0.62	0.72					
Chavicol methyl ether			1.53	1.22			
α-Terpineol			1.77	1.11			
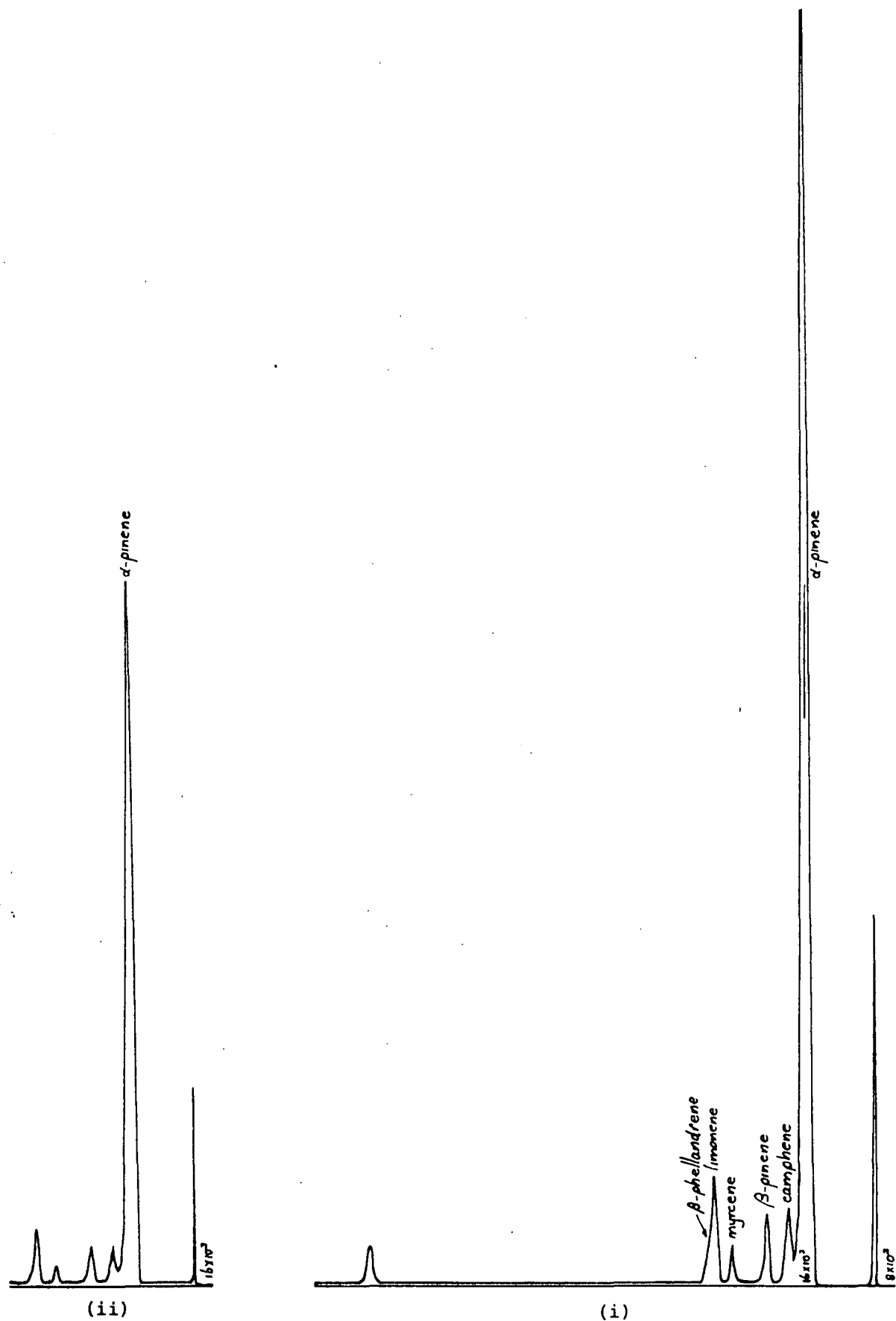
Fig. 68(a).   Low sensitivity gas chromatograms of whole oil from oleoresin of *Pinus nigra* var *austriaca* [GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50° to 140° at 5°/min;   0.2 µℓ sample;   attenuation (i) 8 x 10^3, (ii) 16 x 10^3].
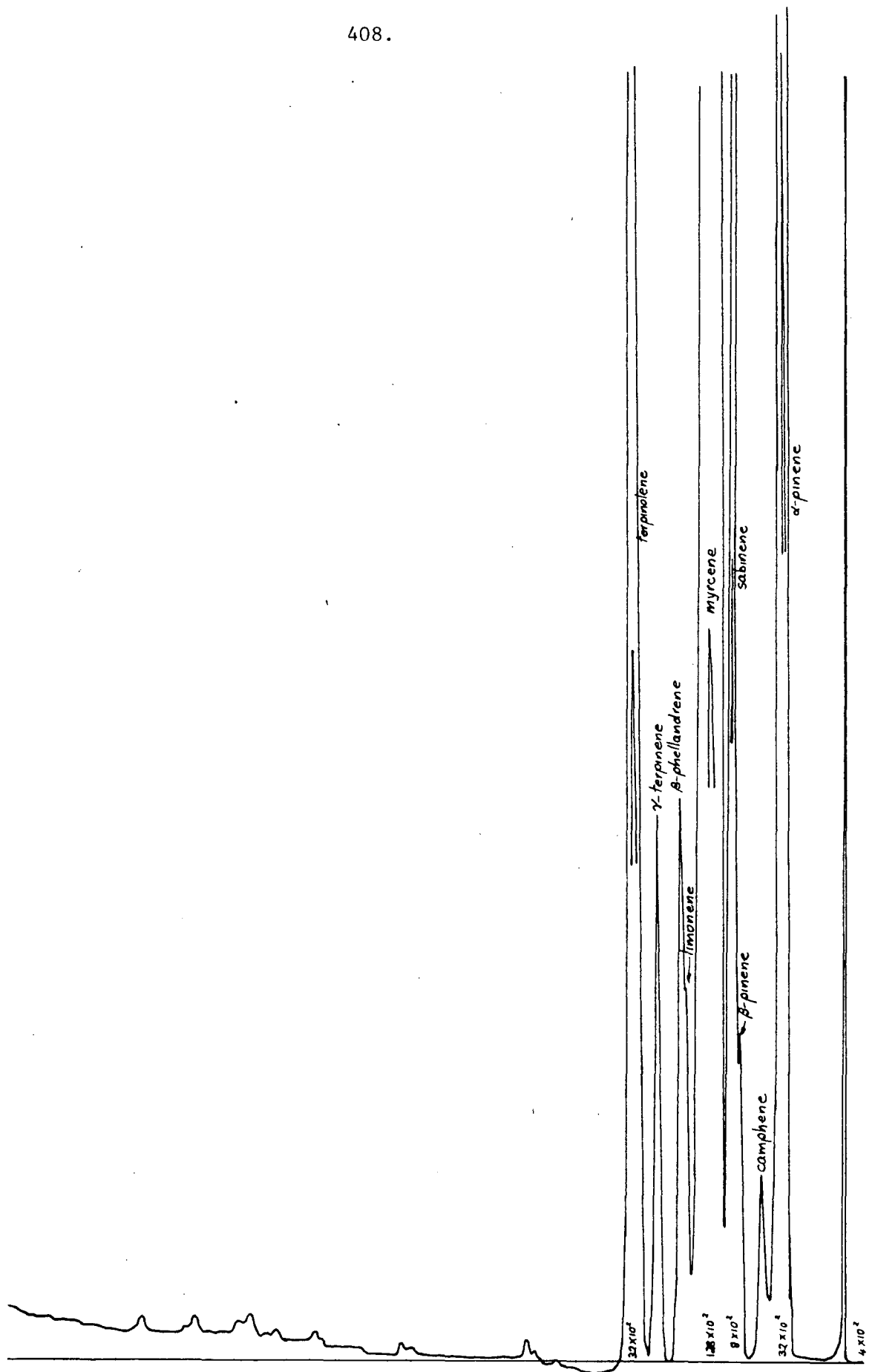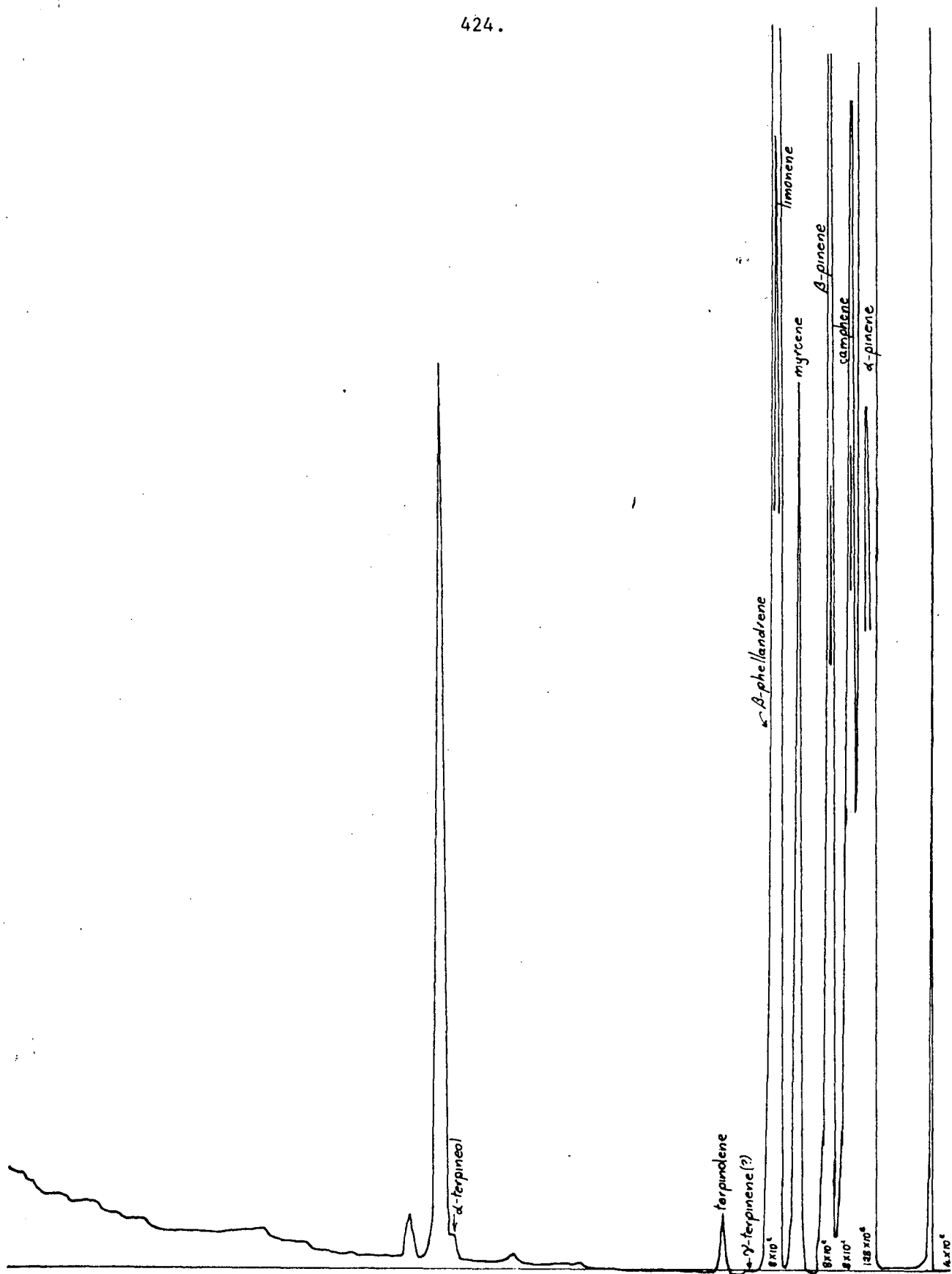
Fig. 68(b).   High sensitivity gas chromatogram of whole oil from oleoresin of *Pinus nigra* var *austriaca* (attenuation 4 x 10²).
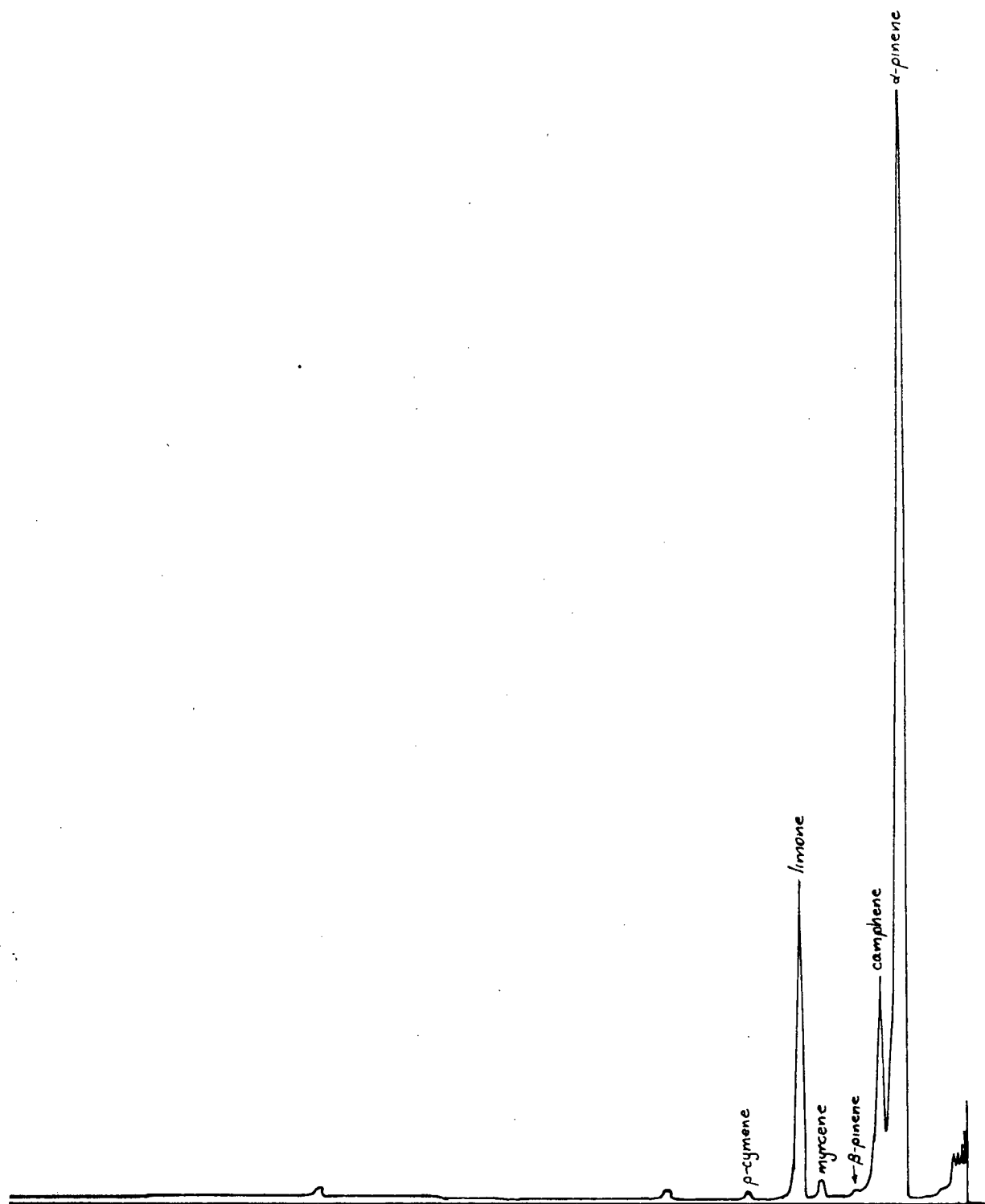
Fig. 68(c).   Low sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus nigra* var *austriaca* separated on Florisil.
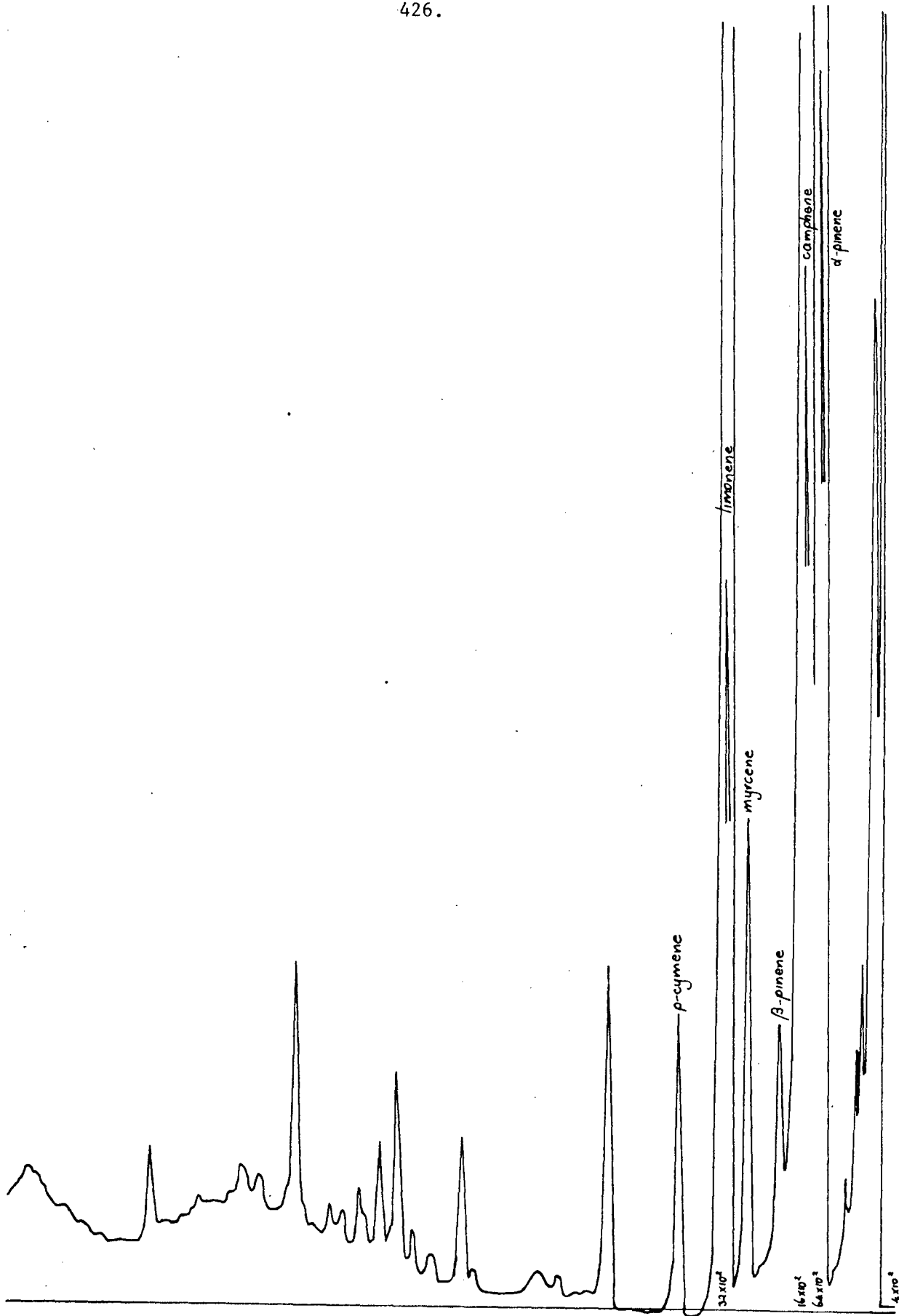
Fig. 68(d).   High sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus nigra* var *austriaca* separated on Florisil.
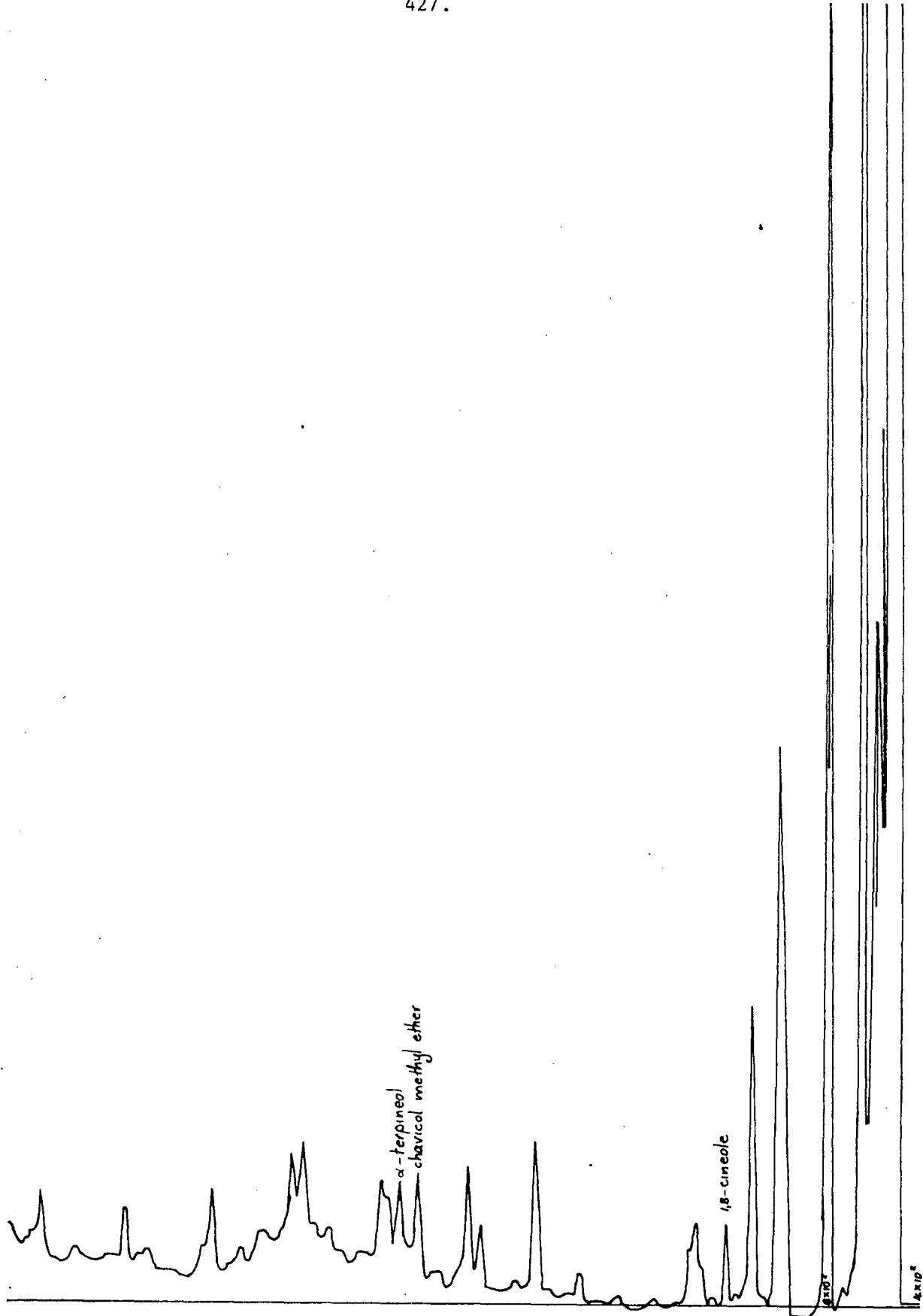
Fig. 68(c). High sensitivity gas chromatogram of oxygenated fraction
of oil from oleoresin of *Pinus nigra* var *austriaca* separated on
Florisil.

The compositions of the oils from oleoresins of both trees are seen from Tables 92, 93 and Figure 68 to be very similar, and also to be typical of the compositions previously reported for this species. The omission of chavicol methyl ether and α-terpineol from the list of components in the oil from var *austriaca* (tree IV) is not an indication of qualitative differences between the two trees. The chromatogram of the oxygenated fraction from tree IV contains numerous peaks from trace components, two of which could be these compounds. The numerous minor peaks shown in the chromatograms were often unable to be correlated on both columns. Components documented in the tables are as a result usually only those which might be tentatively identified from correlated RRT values.

(b) Syringe-headspace GC analysis of foliage terpenoids
     (tree IV)

Syringe-headspace GC analysis of vapour from comminuted foliage of tree IV (Table 94) indicated a similar oil vapour composition being released to the atmosphere from both the foliage and bark of this tree. As in other species however, there was a slightly larger proportion of β-pinene synthesized in the foliage. A further 'temporarily-released component' was detected, which eluted near Δ_3-carene on the Carbowax 20M column. This component is shown in Table 94 to represent 3 percent of the total initially-released monoterpenes in foliage vapour.

Table 94. RRT data and percentage composition of
volatile terpenoids in foliage of *Pinus*
nigra var *austriaca* (tree IV) determined
by syringe-headspace GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak areas of 1st and 2nd successive injections*)	
	C20M	OV-17		
	(60° isothermal, ref. α-pinene)		(60° isothermal)	
			(1st)	(2nd)
Unidentified	0.75		–	0.3
α-Pinene	0.98	1.01	80.5	84.4
Camphene	1.24	1.19	0.9	0.8
β-Pinene	1.59	1.56	9.0	9.0
Unidentified 'temporary component'	1.94		3.0	0.3
Myrcene	2.23	1.74	5.7	4.5
Limonene	2.76	2.35	0.4	0.3
β-Phellandrene	2.90	2.52	0.4	0.3

* 30 mins. between injections

(c) Composition of successive injections of syringe-headspace

vapour from foliage (tree IV)

Successive injections of vapour over a 3 hr. period,

from a single sample of foliage, resulted in only minor

fluctuations in the proportions of monoterpenes. However an

apparently qualitative change was exhibited when the previously-

mentioned component eluting near Δ_3-carene could only be

detected in vapour injected within the first half hour after

comminution (Table 95, Figure 69). This component was not

detected in the steam-distilled oil from the remainder of the

comminuted foliage of either tree IV or I (Tables 95 and 96).

Table 95. Compositions of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Pinus nigra* var *austriaca* (tree IV)

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Sabinene	Unidentified 'temporary component'	Myrcene	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene
0	–	90.3	1.0	4.3	t	0.3	3.5	0.2	0.2	0.1	–
15	–	90.5	0.9	4.4	–	0.3	3.4	0.3	0.3	0.1	–
30	–	91.0	0.9	4.3	–	–	3.4	0.2	0.2	–	–
45	0.1	90.1	1.0	4.6	t	–	3.6	0.2	0.2	0.1	–
60	0.2	90.6	1.0	4.4	t	–	3.1	0.3	0.2	0.2	0.1
90	0.1	89.0	0.9	5.0	t	–	4.0	0.3	0.4	0.3	t
105	0.2	90.3	1.0	4.5	t	–	3.4	0.3	0.3	t	–
120	0.3	90.1	1.0	4.7	t	–	3.2	0.3	0.2	0.2	–
135	0.2	90.2	1.0	4.9	t	–	3.2	0.2	0.2	t	–
150	0.2	89.8	0.8	4.8	0.2	–	3.3	0.3	0.3	0.2	t
170	–	88.1	1.1	5.4	0.4	–	3.8	0.5	0.5	0.1	0.1

Steam-distilled oil from remainder of comminuted foliage

| | 2.4 | 72.7 | 1.2 | 11.2 | 0.3 | – · | 7.9 | 1.5 | 1.5 | 1.4 | 0.1 |
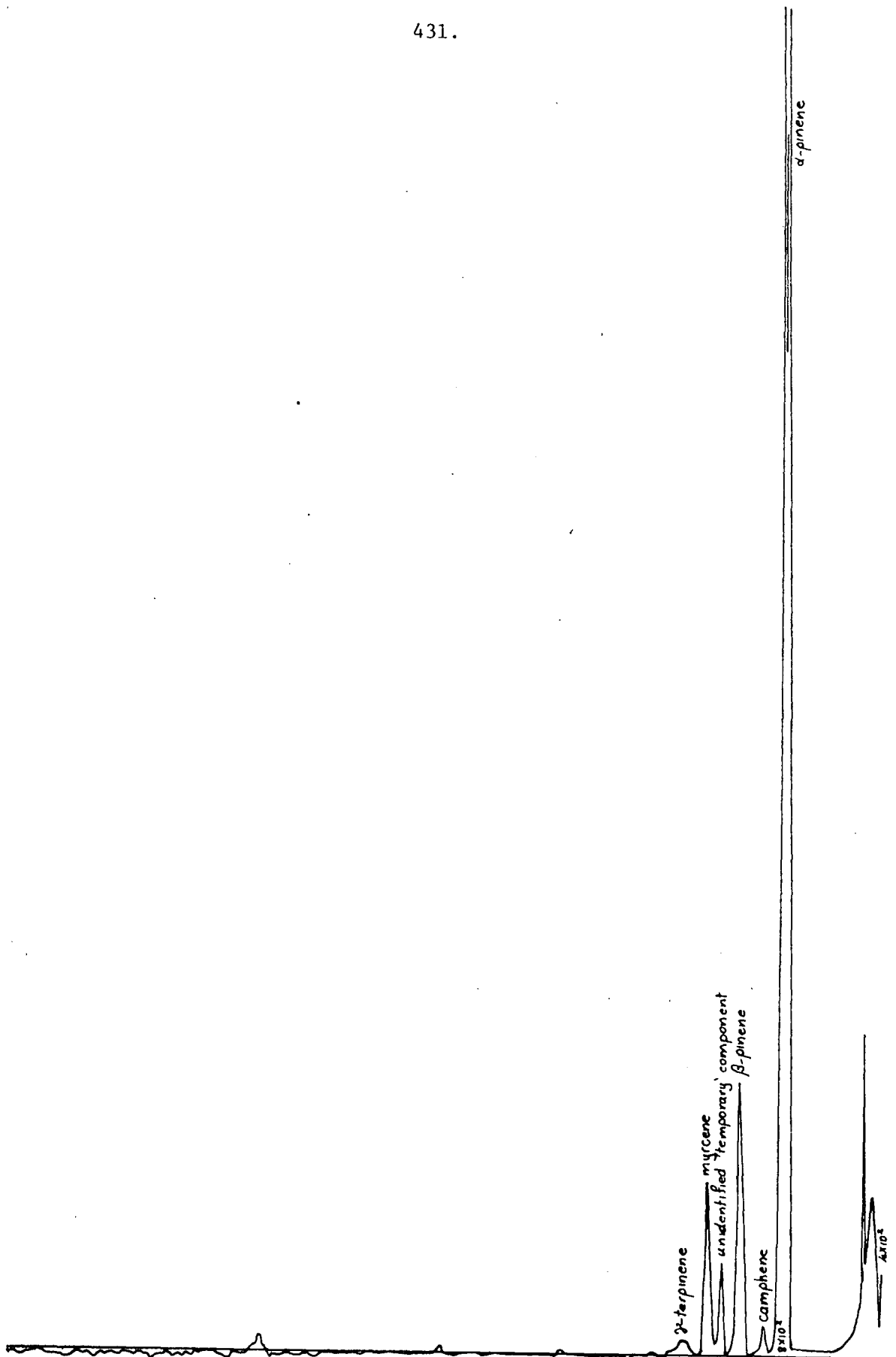
Fig. 69(a). Syringe-headspace chromatogram of foliage vapour of
Pinus nigra var *austriaca* (tree IV).

Fig. 69(b). Gas chromatogram of oil steam-distilled from the remainder of comminuted foliage of *Pinus nigra* var *austriaca* (tree IV) studied by syringe-headspace vapour GC in Fig. 69(a) (GC conditions as before).
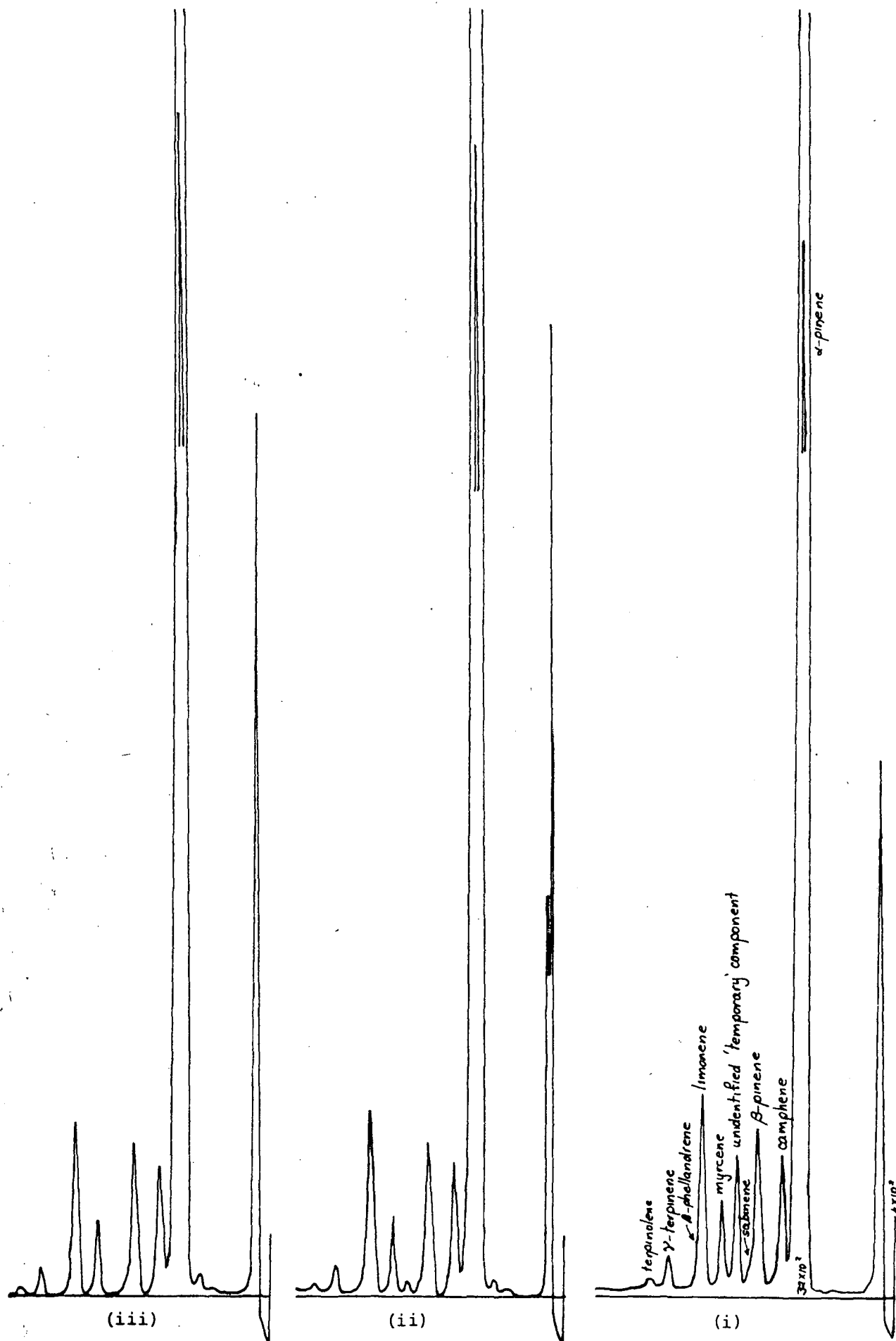
Fig. 69(c).   Syringe-headspace chromatograms of foliage vapour of *Pinus nigra* var *austriaca* (tree IV).   In the initial injection (i) an unidentified 'temporary' component appears as a peak with RRT similar to Δ₃-carene. Subsequent injections (i) and (iii), 20 and 15 mins. apart, contain little and finally no evidence of this component.

(d) <u>Composition of syringe-headspace vapour from foliage of</u>

    <u>several trees of *Pinus nigra* (trees I-IV)</u>

        The wide variation in monoterpene composition of the
vapour from samples of foliage from each of three trees is
shown in Table 96. The extreme variation in vapour composition
between these trees is possibly due to basic differences
between variants of this species. The only trees available
for study were recognized as being morphologically different
and could perhaps also be chemically different.

(e) <u>Composition of syringe-headspace vapour from foliage</u>

    <u>sampled repeatedly from the same tree (tree IV)</u>

        The composition of the vapour from three samples of
foliage from tree IV varied (Tables 94-96) from 80.5 to 91.3
percent α-pinene, while β-pinene varied from 1.7 to 9.0 percent.

(f) <u>Summary</u>

        Components of the steam-distilled oil from oleoresin of
Pinus nigra var *austriaca* were analyzed by chromatographic and
spectroscopic methods. The following identifications were
confirmed: α-pinene (79.9%), camphene (4.3%), β-pinene (3.9%),
myrcene (1.9%) and limonene (6.1%). Tentatively identified
were sabinene, β-phellandrene (1.9%), ρ-cymene, terpinolene and
α-terpineol. Oleoresin from an unspecified variety of *P. nigra*
was shown to have a closely similar composition, while further
components tentatively identified were Δ_3-carene, 1,8-cineole,
γ-terpinene and chavicol methyl ether. From a search of the
literature sabinene does not appear to have been previously
reported in this species.

Table 96. Composition of monoterpenes in syringe-headspace vapour from foliage of several trees of *Pinus nigra*. The compositions of the first three successive injections illustrate the apparent "disappearance" of the component eluting near Δ_3-carene.

Percentage composition of monoterpenes (peak height basis):

Tree No.	Time since comminution (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Sabinene	Unidentified 'temporary component'	Δ_3-Carene	Myrcene	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene
I	*(P. nigra)*												
	0	–	21.5	0.2	59.4	–	–	14.3	1.7	1.2	1.2	t	0.5
	40	0.2	24.0	0.3	58.1	–	–	13.6	1.5	1.0	1.0	–	0.4
	55	0.3	24.3	0.3	58.3	–	–	13.1	1.5	0.9	1.0	t	0.4
	Steam-distilled oil from remainder of comminuted foliage												
		10.9	10.8	0.1	54.0	–	–	16.4	1.9	2.0	2.2	0.3	1.6
II	*(P. nigra* var *tenuifolia)*												
	0	–	81.1	1.1	8.6	–	2.3	–	5.6	0.5	0.5	0.3	–
	15	0.2	84.6	1.0	8.4	–	0.8	–	4.1	0.3	0.3	0.3	–
	30	0.3	85.8	1.0	8.2	–	0.3	–	3.8	0.3	0.3	0.3	–
IV	*(P. nigra* var *austriaca)*												
	0	t	91.3	1.4	1.7	0.2	1.4	–	0.9	2.1	0.4	0.4	0.1
	20	0.2	93.2	1.3	1.6	0.2	0.1	–	0.8	1.8	0.5	0.3	0.1
	35	0.3	93.6	1.3	1.5	0.2	–	–	0.8	1.7	0.4	0.3	0.1

By comparison, monoterpenes of the steam-distilled foliage oil of var *austriaca* included a component eluted before α-pinene (2.4%), α-pinene (72.7%), camphene (1.2%), β-pinene (11.2%), sabinene (0.3%), myrcene (7.9%), limonene (1.5%), β-phellandrene (1.5%), γ-terpinene (1.4%) and terpinolene (0.1%). The unspecified tree of *P. nigra* however contained a much higher proportion of β-pinene in the foliage oil, i.e. component eluted before α-pinene (10.9%), α-pinene (10.8%), camphene (0.1%), β-pinene (54.0%), Δ_3-carene (16.4%), myrcene (1.9%), limonene (2.0%), β-phellandrene (2.2%), γ-terpinene (0.3%) and terpinolene (1.6%).

The syringe-headspace GC technique indicated the existence of a possible qualitative change in foliage vapour composition, when a component eluted near Δ_3-carene on Carbowax 20M, could not be detected after 30 minutes of successive injections of vapour from the same sample. Successive injections from the same sample varied from as little as 3%, i.e. from 88.1 to 91.0% α-pinene. However vapour from three samples of foliage from the same tree ranged in composition from 80.5 to 91.3% α-pinene and 1.7 to 9.0% β-pinene. Considerable differences were also found between the foliage vapour compositions of different varieties of *P. nigra*.

(xii) *Pinus pinaster* Ait.

Earlier investigations of the composition of the oil from oleoresin of French Maritime Pine (*Pinus pinaster* Ait.) have been reviewed by Mirov [47]. The oil usually consisted of a major portion of ℓ-α-pinene, e.g. 63 percent, and a smaller amount of ℓ-β-pinene, e.g. 26.5 percent, together with a tails fraction that had been found to include limonene, caryophyllene, longifolene, cadinene, an isomer of copaene, an azulene, cadinol, pinol and pinol hydrate. Williams and Bannister [145] examined oil from oleoresin grown in New Zealand and reported the composition as 90.0 percent α-pinene, 1.0 percent camphene, 6.5 percent β-pinene, 1.5 percent myrcene, 1.0 percent limonene and a trace of β-phellandrene. Considerable chemical individuality has been indicated in a study of the widely varying optical rotations of oils from different trees [451].

Steam-distilled leaf oil has been shown to contain a higher proportion of oxygenated components [76], as have other younger-aged tissues [452]. Nonterpenoid components have also been reported [453], viz. phenethyl isovalerate and phenethyl 2-methylbutyrate. A detailed investigation of the leaf oil [76] from trees growing in the region of Gironde (France) showed the oil to contain: 0.05 percent hexenal, 0.5 percent cis-hexenol, 10.2 percent α-pinene, 0.22 percent camphene, 16.0 percent β-pinene, 6.1 percent myrcene, 3.5 percent Δ_3-carene, a trace of ρ-cymene, 4.6 percent limonene, 0.5 percent cis-ocimene, a trace of pinol, 0.9 percent terpinolene, 1.5 percent linalool, 0.6 percent α-fenchol,

a trace of trans-pinocarveol, 0.2 percent terpinen-4-ol,
5.8 percent α-terpineol, a trace of dihydrocarveol, 0.2 percent
linalyl acetate, 0.2 percent geraniol, 0.4 percent piperitone,
0.1 percent bornyl acetate, 0.1 percent neryl acetate, 0.4 percent
α-cubebene, 0.3 percent geranyl acetate, 0.7 percent copaene,
0.6 percent eugenol methyl ether, 12.6 percent caryophyllene,
1.8 percent humulene, 1.9 percent phenethyl isovalerate,
6.1 percent phenethyl-2-methylbutyrate, 11.5 percent germacrene D,
1.2 percent γ-muurolene, 1.2 percent α-muurolene, 1.3 percent
γ-cadinene, 5.0 percent δ-cadinene, a trace of nerolidol,
0.1 percent phenethyl 3,3-dimethyl-acrylate, 0.2 percent guaiol,
0.2 percent junenol, 1.0 percent cadinol isomers, 0.9 percent
α-cadinol and 0.3 percent farnesyl acetate. Reservations were
held however when reporting some alcohols, mainly α-terpineol
and linalool, which appeared to be formed during the steam-
distillation process.

In a study of essential oil yield and composition
during maturation of the plant from the seedling stage, an
indication was obtained of different terpene biosynthetic
processes occurring at various stages of development [323, 454].
Oil from seedlings was found to be nearly all α- and β-pinene
(e.g. 84.6 percent α-pinene and 15.3 percent β-pinene at day 0),
the relative proportions of which were completely reversed after
6 to 10 days of growth (e.g. 35.0 percent α-pinene and 60.0
percent β-pinene at day 15), followed by a further reversion to
the mature stage after 60 to 65 days.

The heredity and biosynthesis of monoterpenes in
P. pinaster has been studied by several workers [454-456].
Although other workers were concerned as to the possibility
of α-terpineol and linalool being distillation artifacts [76],
Machado *et al* [457] have since demonstrated the in-vivo
synthesis of these components in *P. pinaster* from C^{14}-labelled
mevalonic acid. These alcohols were also shown to be
interconvertible.

Other workers have investigated the role of
P. pinaster monoterpenes as insect attractants [458, 403, 448].

(a)   Analysis of the oil steam-distilled from cortical oleoresin

Cortical oleoresin collected as before from a single
tree of *P. pinaster* (tree I) in the Royal Botanical Gardens
(Tasmania), yielded upon steam distillation 23.7 percent of
a colourless oil with a pinene odour. Insufficient oil was
obtained to enable a separation of hydrocarbon and oxygenated
fractions.

The qualitative and quantitative composition of
components distinguishable in the whole oil, using two dissimilar
columns, is recorded in Table 97. RRT values leading to the
tentative identification of components isolated directly from
the whole oil by preparative GC are listed in Table 98. Gas
chromatograms of Figure 70 show the distribution of components
eluted from a Carbowax 20M column from injections of the whole
oil (tree I).

This oil is seen from Tables 97, 98 and Figure 70 to
contain a typical high proportion of α- to β-pinene, with only
small amounts of other components, as reported from previous

Table 97. Components distinguishable in the whole
oil from oleoresin of *Pinus Pinaster* (tree I)

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 200°, 5°/min)
*α-Pinene	1.00	1.03	65.8
Camphene	1.24	1.22	1.3
*β-Pinene	1.58	1.56	19.9
*Δ_3-Carene	2.01	1.96	2.3
*Myrcene	2.22	1.74	2.3
*Limonene	2.73	2.39	2.6
β-Phellandrene	2.89	2.52	1.3
γ-Terpinene	3.71		t
Terpinolene	4.64	4.10	0.1
	(130° isothermal, ref. camphor)		
Terpinolene	0.45	0.59	
Caryophyllene	1.22	2.54	4.4

* IR spectrum recorded

t: trace; <0.1 percent

Table 98. RRT values, on the dissimilar liquid phases
C20M and OV-17, for components found in
preparative GC fractions isolated from whole
oil of oleoresin from *Pinus pinaster*

Component	Preparative GC fraction No.	Column	
		C20M	OV-17
	(60°) isothermal, ref. α-pinene)		
α-Pinene	W1	1.01	1.03
Camphene	W2	1.32	1.21
β-Pinene	W3	1.62	1.57
Sabinene	W2	1.79	1.57
Δ_3-Carene	W4	2.04	1.93
Myrcene	W2	2.24	1.75
Limonene	W5	2.84	2.37
β-Phellandrene	W5	2.95	2.49
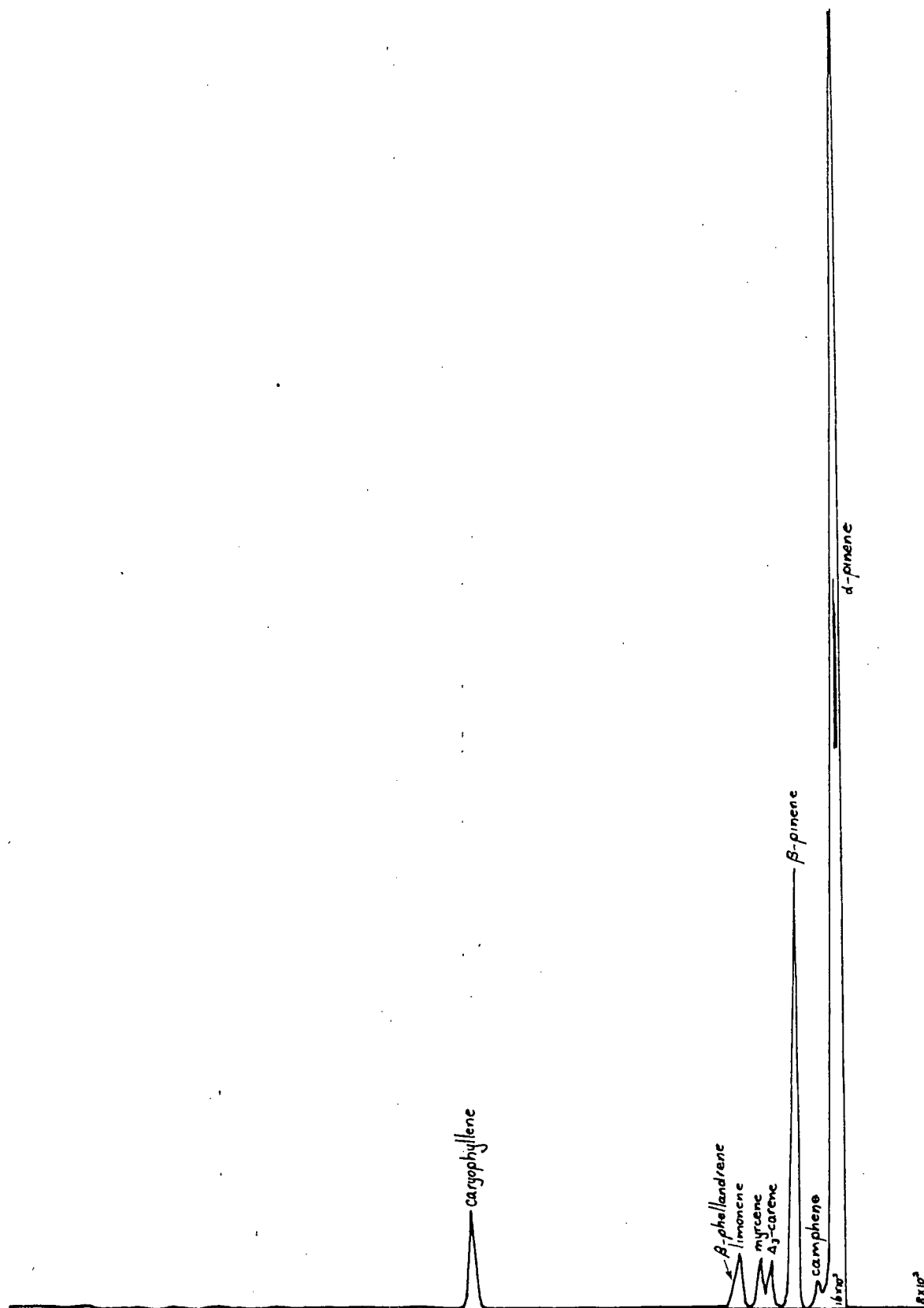
Fig. 70(a). Low sensitivity gas chromatogram of whole oil from oleoresin of *Pinus pinaster* (GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50° to 200° at 5°/min; 0.2 µℓ sample; attenuation 8 x 10³).
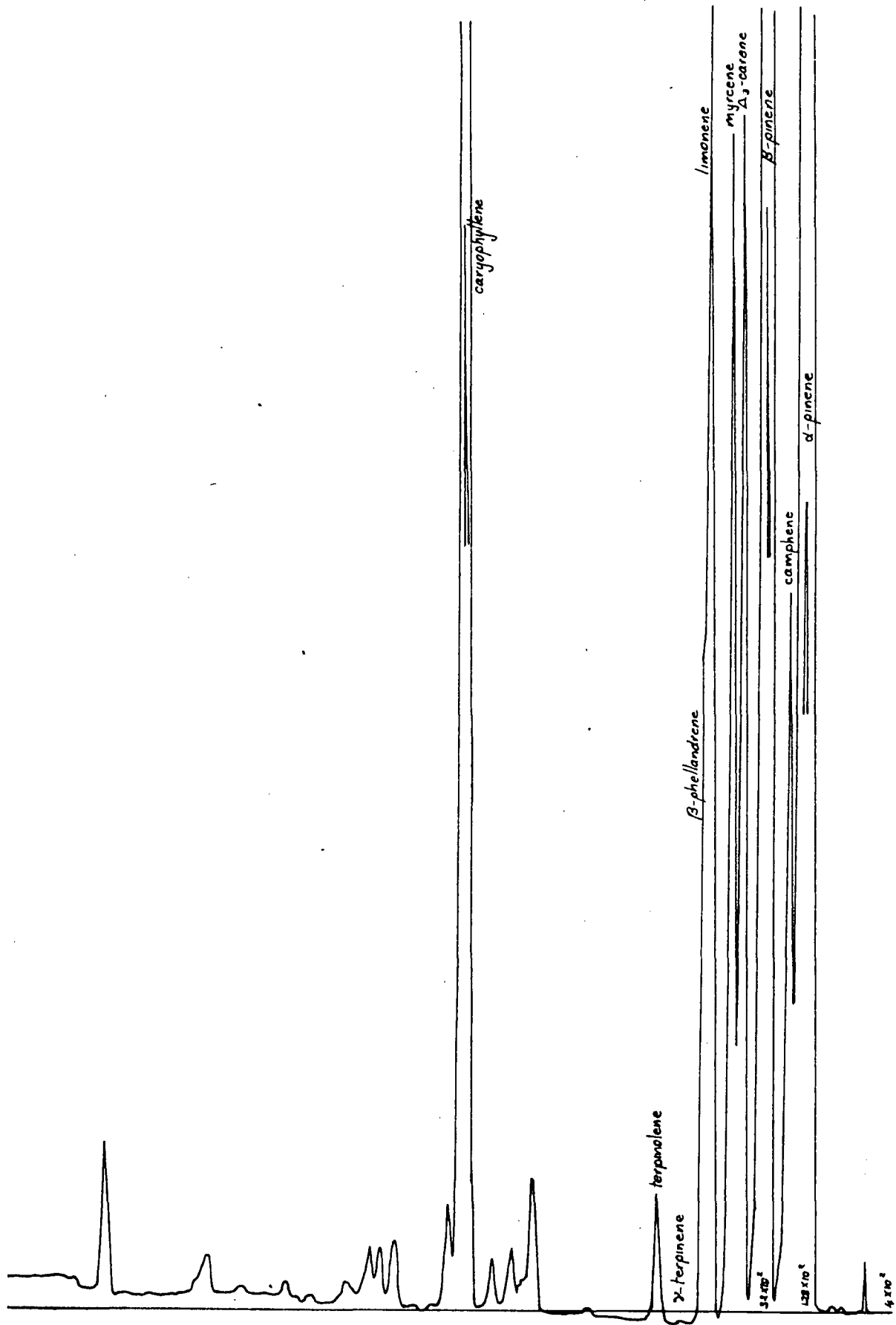
Fig. 70(b).   High sensitivity gas chromatogram of whole oil from oleoresin of *Pinus pinaster* (attenuation 4 x 10^2).

studies. Sabinene did not appear from the literature to have been previously reported. Numerous minor components eluted on Carbowax 20M are shown in Figure 70 which were not correlated with particular peaks on the OV-17 column.

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from comminuted foliage of tree II (Table 99) enabled a tentative identification from RRT data of most components found in the oleoresin oil. Similar analysis of foliage vapour from tree I (Table 100), together with that of the steam-distilled foliage oil (Figure 71) indicated the existence of a similar proportion of α- to β-pinene as found in the oleoresin. However, the foliage

Table 99. RRT data and percentage composition of volatile terpenoids in foliage of *Pinus pinaster* (tree II) determined by syringe-headspace GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak area of 2nd successive injection)
	C20M	OV-17	
(60° isothermal, ref. -pinene)			(60° isothermal)
Unidentified	0.75		0.2
α-Pinene	1.00	1.00	57.0
Camphene	1.25	1.19	0.2
β-Pinene	1.63	1.53	34.8
Unidentified 'temporary component'	1.96		0.2
Myrcene	2.23	1.71	3.3
Limonene	2.84	2.35	3.7
β-Phellandrene	3.00	2.46	0.6
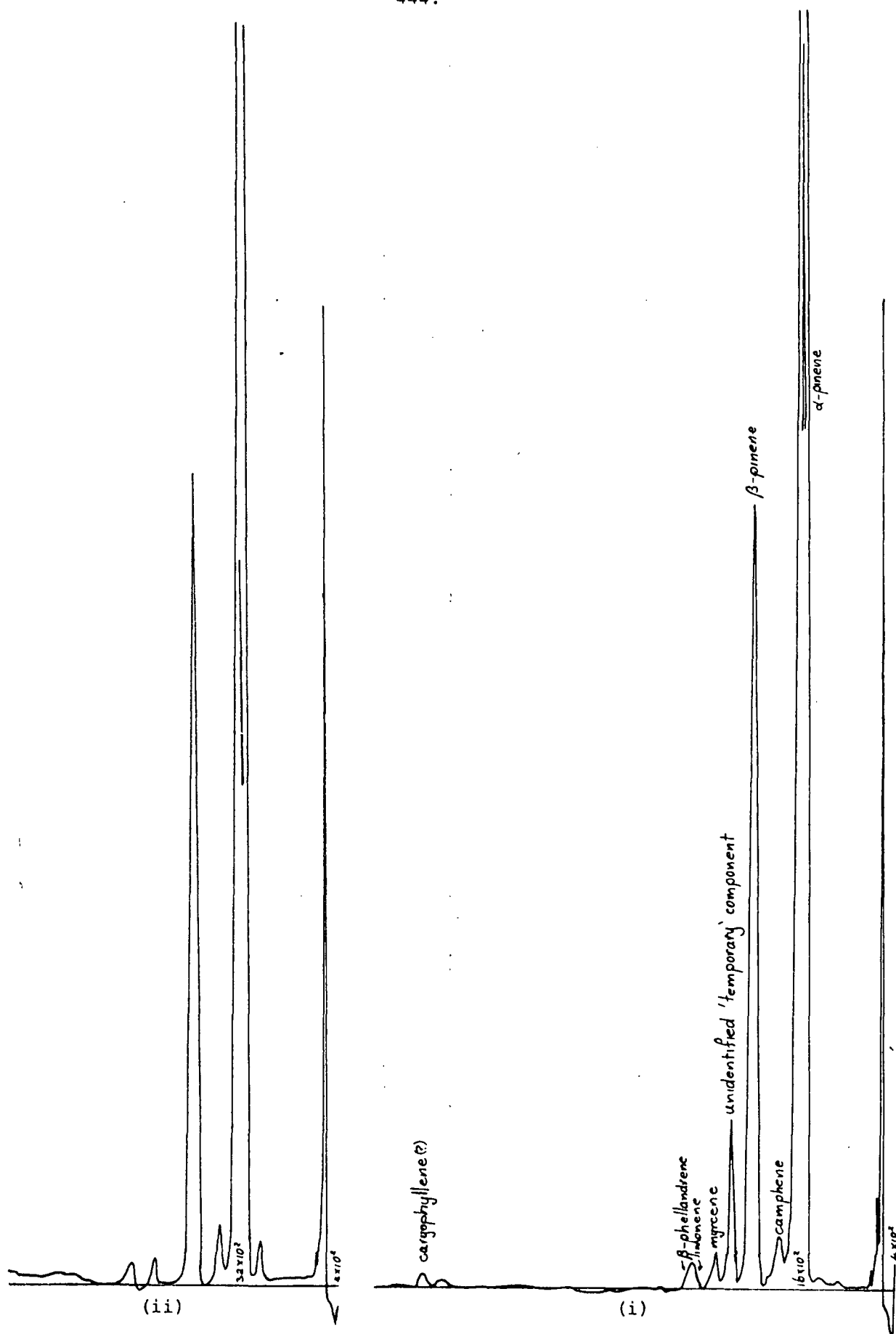
Fig. 71(a). Syringe-headspace chromatograms of foliage vapour of *Pinus pinaster* (tree I). In the initial injection (i) an unidentified 'temporary component' appears as a peak with RRT similar to Δ_3-carene. A subsequent injection 40 mins. later (ii) contains no trace of this component.
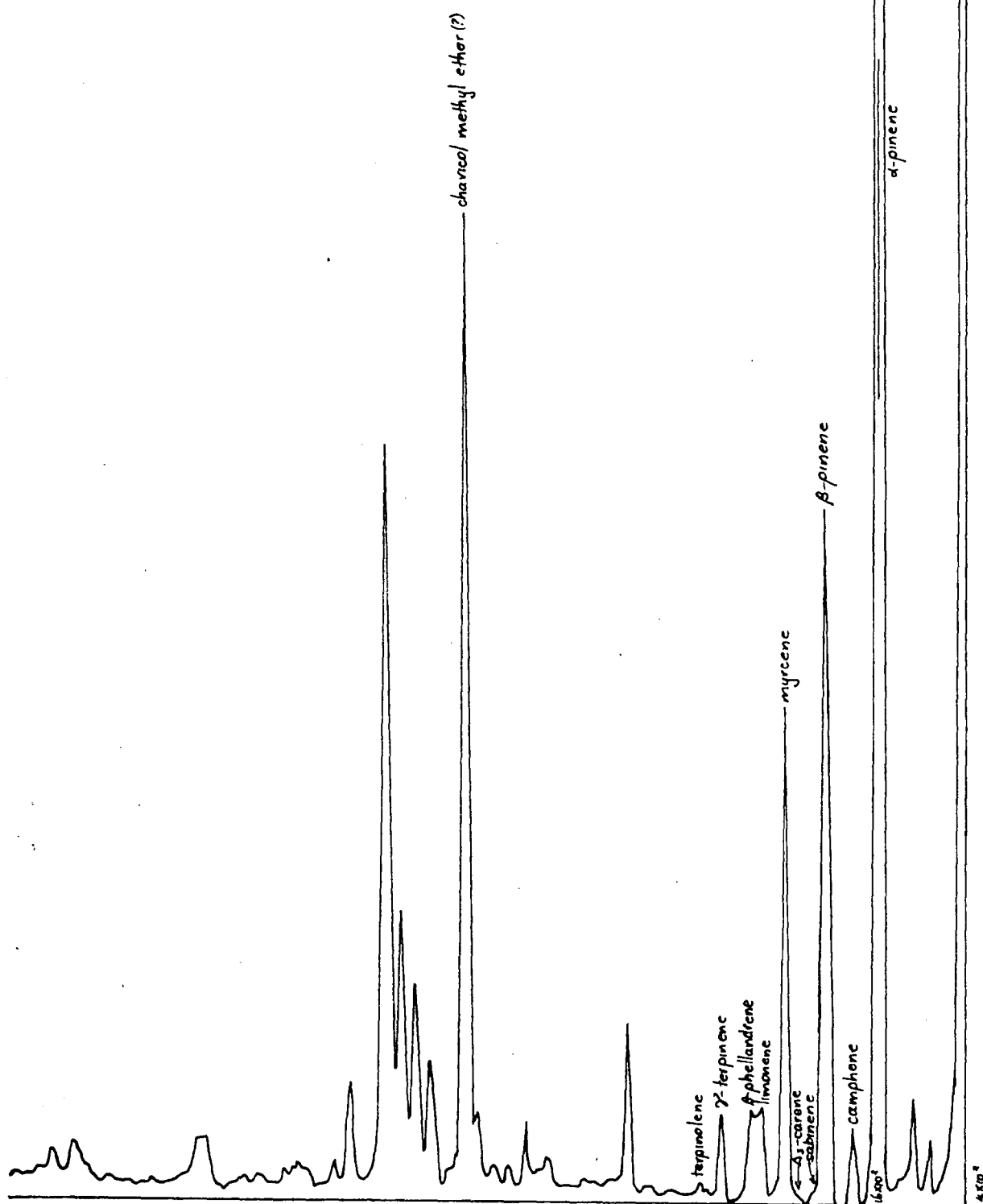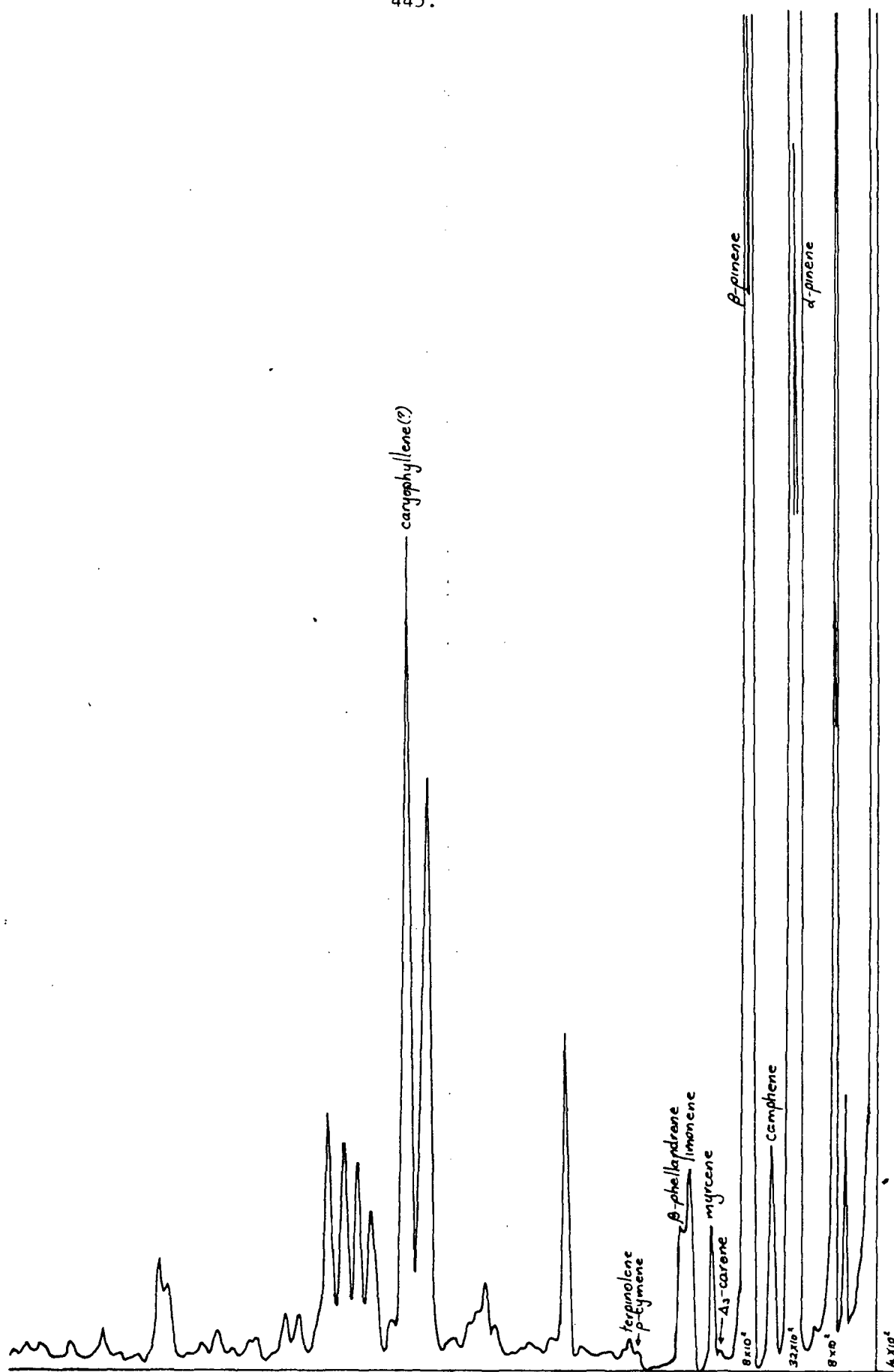
Fig. 71(b). Gas chromatogram of oil steam-distilled from the remainder of comminuted foliage of *Pinus pinaster* (tree I) studied by syringe-headspace vapour GC in Fig. 71(a) (GC conditions as before).

Table 100. Composition of monoterpenes in successive injections of syringe-headspace vapour from foliage of several trees of *Pinus pinaster* (trees I, II)

Time since comminution of sample (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene (+ sabinene) trace	Unidentified	Myrcene	Limonene	β-Phellandrene	Terpinolene
(Tree II)									
0	–	52.0	0.3	39.6	t	3.6	3.3	1.2	0.1
15	0.1	56.9	0.5	35.2	–	3.3	2.9	1.0	t
30	0.2	58.3	0.5	33.8	t	3.3	3.0	1.1	t
45	0.1	58.3	0.6	34.1	0.1	3.1	2.8	0.9	–
60	0.2	58.7	0.5	33.6	–	3.2	2.8	1.0	t
75	0.1	58.6	0.6	33.9	t	3.1	2.8	0.9	t
90	0.2	57.8	0.6	34.6	–	3.1	2.9	0.9	t
105	0.1	58.9	0.5	33.8	t	3.1	2.8	0.8	t
120	0.1	58.8	0.5	34.1	–	3.0	2.8	0.7	–
130	0.1	58.4	0.5	34.3	–	3.1	2.8	0.8	–
145	0.2	58.2	0.5	34.2	–	3.1	2.9	1.0	–
155	0.3	58.4	0.5	33.9	–	3.1	2.9	0.9	–
(Tree I)									
0	0.2	81.7	0.9	12.9	2.8	0.6	0.5	0.5	–
40	0.6	85.3	0.9	12.0	t	0.4	0.4	0.3	–
55	0.7	85.6	0.8	11.9	t	0.4	0.3	0.3	–
Steam-distilled oil from remainder of comminuted foliage from tree I					(Δ_3-Carene)				
	10.4	66.4	1.5	18.3	0.1	1.0	1.4	1.0	t
(Tree II – further sample)					(Unidentified)				
0	0.2	54.9	0.6	37.3	0.2	3.2	2.5	1.1	–
15	0.4	57.8	0.6	34.6	0.3	3.0	2.3	1.0	–
35	0.2	58.4	0.7	34.6	–	3.0	2.1	1.0	–

464.

vapour released to the atmosphere contained a much higher

proportion of α-pinene (Table 100).

An unidentified 'temporarily-released component',

eluting near Δ_3-carene, was detected in initial injections of

vapour from each sample of foliage.

(c) Composition of successive injections of syringe-headspace

vapour from foliage (tree II)

Successive injections of vapour over a $2\frac{1}{2}$ hr. period,

from a single sample of foliage, exhibited some wide initial

fluctuations in the proportion of α- to β-pinene (Table 100).

Subsequent samples also showed the previously-mentioned

'temporarily-released' component, which could not be detected

after 40 minutes from the time of comminution (Figure 71).

(d) Composition of syringe-headspace vapour from foliage

of several trees of *Pinus pinaster* (trees I-III)

The wide variation in monoterpene composition of

the initial injection of vapour from a sample of foliage of

each of three trees can be seen from Tables 100 and 101.   The

proportion of α-pinene ranged from 52.0 to 81.7 percent while

β-pinene ranged from 12.9 to 39.6 percent.

(e) Composition of syringe-headspace vapour from foliage

sampled repeatedly from the same tree (tree II)

The composition of the vapour from three samples of

foliage from tree II varied (Tables 99, 100) from 52.0 to 57.0

percent α-pinene, while β-pinene varied from 34.8 to 39.6

percent.

(f) Composition of syringe-headspace vapour from foliage

   sampled daily from a lopped branch (tree III)

An examination was made over an 18-day period, of

the monoterpene vapour composition of foliage intact on a

lopped branch. Unlike *Cedrus deodara,* which was also studied

on a comparative basis, needles of *P. pinaster* remained

attached throughout the period. Fluctuations in composition

were exhibited for most of the period. However the most

significant change occurred on day 15, when the highest

proportion of β-pinene was encountered. These changes are

shown in Table 101 and Figure 72.

Although fluctuations in monoterpene proportions

were discernible, no particular pattern of changes could be

seen which could have been correlated with an attractiveness

to insects, i.e. immediately after lopping and after a 14-day

period [332]. No similarities could be seen which could be

correlated with any of the compositional changes in *Cedrus*

deodara.

(g) Summary

Components of the steam-distilled oil from oleoresin

of *Pinus pinaster* were analyzed by chromatographic and

spectroscopic methods. The following identifications were

confirmed: α-pinene (65.8%), β-pinene (19.9%), Δ_3-carene (2.3%),

myrcene (2.3%) and limonene (2.6%). Tentatively identified

were camphene (1.3%), sabinene, β-phellandrene (1.3%),

γ-terpinene, terpinolene (0.1%) and caryophyllene (4.4%).

Table 101. Composition of monoterpenes in syringe-headspace injections of vapour from daily samples of foliage of a lopped branch of *Pinus pinaster*. For each sample the composition is given of the first three successive injections, which gives a better indication of the range of vapour compositions encountered in a single sample of foliage.

Percentage composition of monoterpenes (peak height basis):

Day No.	Time since comminution (mins.)	Unknown group	Santene?	α-Pinene	Camphene	Unknown	β-Pinene	Unidentified	Myrcene	Limonene	β-Phellandrene	γ-Terpinene and others	Terpinolene
0	0	t	t	55.2	0.3	t	27.5	2.5	5.7	8.6	t	0.2	–
	15	–	0.2	56.9	0.4	0.1	27.2	1.8	5.2	7.9	–	0.3	–
	30	–	0.4	57.9	0.3	0.1	26.9	1.1	5.3	7.8	–	0.2	–
1	0	0.1	–	50.1	0.3	–	31.4	3.4	5.9	8.5	–	0.3	–
	15	–	t	51.5	0.3	0.1	31.3	3.0	5.7	8.0	–	0.2	–
	30	–	0.1	51.3	0.2	0.1	32.3	2.3	5.7	7.7	–	0.3	–
2	0	t	t	49.9	0.5	0.2	29.0	4.4	6.5	9.2	–	0.3	–
	15	0.1	0.2	53.9	0.4	–	27.7	2.5	6.3	8.8	–	0.2	–
	30	–	0.5	53.8	0.4	0.2	27.2	1.9	6.7	9.0	–	0.3	–
3	0	–	–	49.0	0.3	t	30.6	4.4	6.3	9.1	–	0.3	–
	15	t	0.1	53.0	0.3	0.1	29.3	3.3	5.7	7.9	–	0.3	–
	30	–	0.3	52.7	0.4	0.2	29.3	2.7	6.3	7.9	–	0.3	–
4	0	0.1	–	51.0	0.3	–	30.0	4.5	5.8	8.0	–	0.2	–
	15	0.2	0.2	53.2	0.4	0.1	29.7	3.4	5.6	7.2	–	0.2	–
	40	0.1	0.2	54.2	0.5	0.1	29.9	2.6	5.3	6.9	–	0.2	–

(Continued)

Table 101 continued

Day No.	Time since comminution (mins.)	Unknown group	Santene?	α-Pinene	Camphene	Unknown	β-Pinene	Unidentified	Myrcene	Limonene	β-Phellandrene	γ-Terpinene and others	Terpinolene
						Percentage composition of monoterpenes (peak height basis):							
5	0	t	t	51.0	0.4	0.1	29.8	4.0	6.1	8.5	–	0.2	–
	15	t	0.2	54.1	0.3	0.1	29.1	2.6	5.6	7.9	–	0.2	–
	30	0.1	0.2	54.6	0.2	0.1	29.0	2.0	5.6	7.9	–	0.2	–
6	0	t	t	50.9	0.3	0.1	31.1	3.3	5.8	8.1	t	0.3	–
	15	t	0.1	53.5	0.3	0.1	30.9	2.5	5.4	7.0	0.5	0.2	–
	30	t	0.3	54.7	0.3	0.1	30.5	1.9	5.2	6.7	0.4	0.2	–
7	0	t	t	53.8	0.3	0.1	31.4	3.5	4.5	6.1	t	0.3	–
	10	–	t	57.3	0.3	0.1	30.1	2.5	4.2	5.4	t	0.2	–
	25	–	0.1	57.4	0.3	0.2	30.0	2.2	4.2	5.4	t	0.2	–
8	0	0.1	0.1	56.1	0.3	t	30.3	3.2	4.5	5.2	t	0.2	–
	20	–	0.1	58.2	0.4	0.1	29.9	2.3	4.3	4.6	t	0.1	–
	35	t	0.2	58.0	0.3	0.1	29.5	2.1	4.7	5.0	0.2	0.2	–
9	0	t	t	53.3	0.3	0.1	29.5	3.7	5.4	7.4	–	0.3	–
	15	–	t	55.5	0.3	0.2	28.2	3.5	5.3	6.9	–	0.2	–
	30	t	0.2	56.4	0.3	0.2	28.2	2.8	5.1	6.7	0.3	0.2	–
10	0	0.2	–	53.9	0.4	0.2	28.7	3.7	5.1	7.7	–	0.2	–
	15	0.2	0.1	56.1	0.4	0.1	27.5	3.1	5.2	7.1	0.5	0.4	–
	30	t	0.2	56.6	0.3	0.1	27.8	2.1	5.3	7.3	0.4	0.2	–
11	0	0.6	–	52.6	0.3	t	29.3	4.0	5.4	7.7	–	0.1	–
	15	0.5	0.1	55.7	0.3	0.2	28.1	3.3	5.0	6.6	0.6	0.2	–
	30	0.4	0.2	56.0	0.4	0.3	28.2	2.4	5.1	6.9	0.6	0.2	–

(Continued)

Table 101 continued

Percentage composition of monoterpenes (peak height basis):

Day No.	Time since comminution (mins.)	Unknown group	Santene?	α-Pinene	Camphene	Unknown	β-Pinene	Unidentified	Myrcene	Limonene	β-Phellandrene	γ-Terpinene and others	Terpinolene
12	0	0.2	–	52.9	0.3	0.1	28.4	4.7	5.2	8.0	–	0.3	–
	75	0.2	0.4	58.1	0.2	0.2	27.7	0.7	4.7	6.8	0.9	0.2	–
	90	0.1	0.3	57.7	0.3	0.2	28.0	0.5	4.8	6.9	1.0	0.2	–
13	0	0.1	t	51.5	0.3	0.1	29.2	4.6	5.9	8.1	–	0.3	–
	15	t	0.1	54.8	0.3	0.3	28.9	2.7	5.5	7.1	0.3	0.2	–
	30	t	0.1	55.6	0.3	0.3	28.8	1.8	5.6	7.2	0.6	0.3	–
14	0	t	–	52.5	0.4	0.1	28.7	4.1	5.6	8.4	–	0.3	t
	15	t	0.1	55.0	0.4	0.2	27.8	2.7	5.8	7.9	0.4	0.2	–
	30	t	0.2	55.9	0.3	0.2	27.6	1.6	5.9	8.1	0.7	0.3	–
15	0	t	t	50.4	0.3	–	34.1	2.8	5.8	6.5	–	0.2	t
	15	0.1	0.1	52.8	0.3	0.1	32.7	1.5	6.0	6.2	t	0.1	–
	30	t	0.2	53.5	0.4	0.1	32.4	1.0	6.0	6.2	t	0.2	–
16	0	0.5	t	50.1	0.3	0.1	34.1	4.0	4.6	6.0	t	0.3	–
	15	0.2	t	53.0	0.4	0.4	33.1	2.8	4.6	5.4	0.4	0.2	–
	30	0.1	0.1	53.8	0.3	0.3	33.5	1.6	4.6	5.5	0.7	0.3	–
17	0	0.6	–	55.2	0.3	0.1	27.2	4.2	5.1	7.0	–	0.3	t
	15	0.3	t	58.8	0.3	0.2	27.0	2.0	4.9	6.4	t	0.3	–
	35	0.3	0.2	58.6	0.4	0.3	27.6	1.0	5.0	6.4	0.5	0.2	–
18	0	t	t	55.1	0.3	0.1	28.1	4.0	5.0	7.2	t	0.3	–
	20	0.1	0.1	57.9	0.3	0.5	28.4	1.9	4.4	6.1	0.3	0.2	–
	35	0.1	0.2	58.4	0.3	0.6	28.7	0.9	4.5	6.0	0.6	0.2	t

Fig. 72. Composition of monoterpenes in syringe-headspace initial injections of vapour from daily samples of foliage of a lopped branch of *Pinus pinaster*. No real correlation is discernible between changes in monoterpene composition and cessation of attractiveness to *Sirex noctilio* on the 14th day after lopping the branch. The proportion of α- to β-pinene is not always a reciprocal relationship which follows from an increase in the percentage of one major component. The reciprocal relationship between α-pinene and myrcene might then be attributed to a feature of the biosynthesis of monoterpenes. Limonene and the unidentified 'temporary component' might also be linked biosynthetically to α-pinene, while β-pinene does not appear to be so linked to any other component.
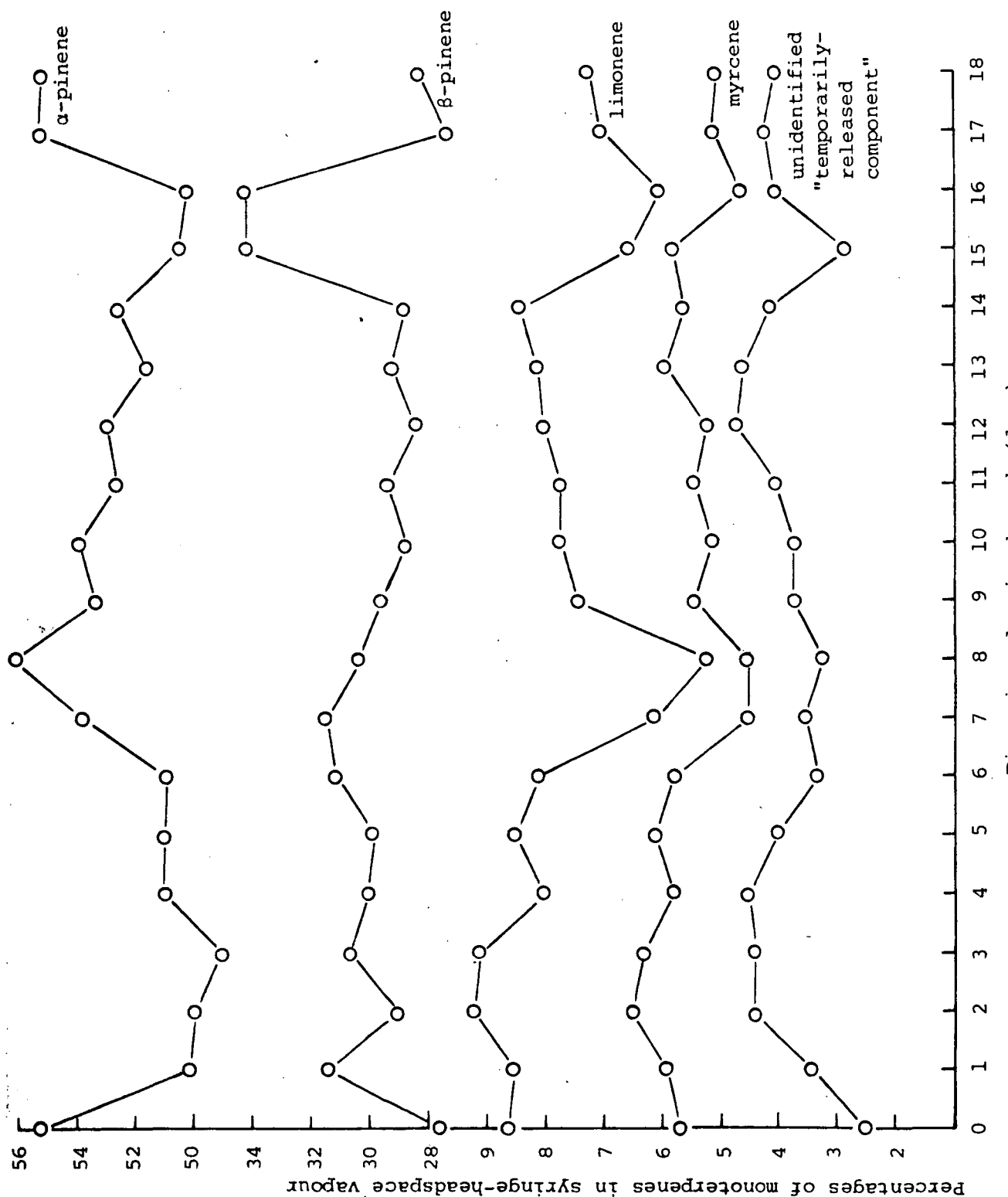
By comparison monoterpenes of the steam-distilled foliage oil included a group of components eluted before α-pinene (10.4%), α-pinene (66.4%), camphene (1.5%), β-pinene and sabinene (18.3%), Δ_3-carene (0.1%), myrcene (1.0%), limonene (1.4%), β-phellandrene (1.0%) and terpinolene. Sabinene did not appear from the literature to have been previously found in this species.

The syringe-headspace GC technique indicated the existence of a wide range of monoterpene compositions to be found in successive injections of vapour from a single sample of foliage, and also from different samples of foliage from the same tree. Successive injections of vapour over a 2½ hr. period contained from 52.0 to 58.9% α-pinene and 33.6 to 39.6% β-pinene; whereas from 3 samples of foliage from the same tree the vapour contained from 52.0 to 57.0% α-pinene and 34.8 to 39.6% β-pinene. The compositions of foliage vapour from 3 different trees were widely ranging, i.e. α-pinene varied from 52.0 to 81.7% while β-pinene varied from 12.9 to 39.6%.

An apparently qualitative change in foliage vapour was indicated when a component, eluted near Δ_3-carene on Carbowax 20M, could not be detected after 40 minutes of successive injections from the same sample.

The syringe-headspace monoterpene composition of daily samples of foliage from a lopped branch did not exhibit any fundamental change which might be correlated with attractiveness to *Sirex noctilio* following lopping. The fluctuations in proportions of components appeared in some cases to be linked biosynthetically to one another, e.g. α-pinene with myrcene and perhaps the unidentified 'temporarily-released component'.

(xiii) *Pinus ponderosa* Laws. var *scopulorum*

The Ponderosa Pine (*Pinus ponderosa*) is distributed throughout western North America and is known to exist in both morphologically and chemically distinguishable forms. The variety *scopulorum* Engelm. is a form that grows in the Rocky Mountains and has been considered on the basis of the chemical composition of oil from oleoresin (60% Δ_3-carene, <20% β-pinene) to have a close relationship to *P. washoensis* [432]. In the Warner Mountains of California, Smith distinguished the high Δ_3-carene (and low β-pinene) content in oleoresin from *P. washoensis* growing at higher elevations, while at lower elevations *P. ponderosa* co-existed with trees having oil composition characteristics that suggested hybridization between the two species [459]. Mirov [47] had earlier considered *P. washoensis* to be a variety or mutant of *P. ponderosa*. No evidence was found in the literature which directly linked var *scopulorum* with *P. washoensis*, nor was any detailed report found which distinguished the composition of the oil of this variety from the wide range encountered among the chemical forms of "the *P. ponderosa* complex" [47].

By comparison, a coastal form of *P. ponderosa* has been reported having an oil composition similar to that of *P. coulteri*. It is interesting that trees of these two species are the only ones normally attacked by the Western Pine Beetle *(Dendroctonus brevicomis)* [47]. Smith observed that trees which were not killed contained significantly greater amounts of myrcene and limonene in the oleoresin [460],

while toxicity of terpenes to this insect varied in the
decreasing order: limonene, Δ_3-carene, myrcene, β-pinene
(β-pinene equivalent to α-pinene) [461]. Silverstein *et al*
[462] studied sex pheromone terpenoids [(-)-2-methyl-6-methylene-
7-octen-4-ol and trans-2-methyl-6-methylene-3,7-octodien-2-ol]
found in frass of *Ips confusus* and *I. latidens* feeding on
P. ponderosa.

Several workers have reported upon the wide variation
in chemical composition of oleoresins throughout the species.
Mirov [47] summarized earlier data on oil compositions of trees
from 12 localities throughout the entire geographical range.
Smith *et al* [463] considered inter-tree diversity in xylem
monoterpene composition both locally and regionally. These
workers studied 927 trees and found at least 25 distinctive
compositional variants among 4 major and 4 minor regional types.
In an earlier study Smith documented the following ranges in
composition of monoterpenes from oils of 64 trees [464]:

	Percentages:		
	(min.)	(max.)	(av.)
Δ_3-carene	trace	82.5	36.2
β-pinene	trace	57.5	26.4
limonene	trace	30.7	14.5
myrcene	4.6	27.5	13.3
α-pinene	1.5	13.3	6.3
β-phellandrene	0.3	3.7	1.8
unknown	0.0	3.1	1.5
camphene	0.0	trace	trace
heptane	0.0	trace	trace

The within-tree constancy of monoterpene composition of *P. ponderosa* has been studied in considerable detail. Smith reported upon the perennial constancy of monoterpene synthesis in wood oleoresin [321]; while in another publication [66] he described the limited variability in oleoresin composition associated with time and position when sampling from a tree, along with other possible factors, e.g. age, elevation, method of propagation, etc. Zavarin and Cobb [465] have since studied the variability of oleoresin exudation pressure, oleoresin yield and propensity to crystallize, since these features could perhaps have been related to the susceptibility of *P. ponderosa* to bark beetles *(D. brevicomis)*.

The monoterpene composition of sapwood has been shown to differ from that of heartwood [466]:

| | Percentage composition in | |
Component	sapwood	heartwood
α-pinene	3	10
β-pinene	12	20
Δ_3-carene	51	37
myrcene	5	10
limonene	24	17
β-phellandrene	t	1
γ-terpinene	2	1
terpinolene	3	4

t: trace; <0.5 percent

Other components reported in oil from oleoresin of *P. ponderosa* have been: n-undecane, cadinene, longifolene, ρ-cymene and an unspecified "d-terpinene" [47], together with a trace of n-nonane [145].

. Foliage oil was examined earlier by Schorger [467], who found 2 percent ℓ-α-pinene, 75 percent ℓ-β-pinene, 6 percent dℓ-limonene, 7 percent borneol and 2 percent bornyl acetate. Zavarin *et al* have since identified in oil collections averaged throughout the year: α-pinene (11.9 percent), β-pinene (70.2 percent), Δ_3-carene (8.0 percent), myrcene (5.0 percent), limonene (1.8 percent), β-phellandrene (2.2 percent), chavicol methyl ether (6.4 percent) and traces of terpinolene. Whereas these workers found considerable variation in oil composition from season to season, in which chavicol methyl ether occasionally amounted to 40 percent of the oil, Cobb *et al* [468] observed a considerable reduction of only this component in oil of trees injured by photochemical atmospheric contamination.

(a) Analysis of the oil steam-distilled from cortical oleoresin

Cortical oleoresin collected as before from a single tree of *P. ponderosa* (tree I) in the Royal Botanical Gardens (Tasmania), yielded upon steam-distillation 15.3 percent of a colourless oil with a sweet pinene odour.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 102. RRT values leading to the tentative identification of components in the oxygenated fraction and preparative GC fractions are listed in Table 103. Gas chromatograms of Figure 73 show the distribution of components eluted from a Carbowax 20M column, and indicate which components separated into the oxygenated fraction.

Table 102. Components distinguishable in the whole
oil from oleoresin of *Pinus ponderosa*
(tree I)

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak height)
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min.)
*α-Pinene	1.00	1.00	10.6
Camphene	1.30	1.21	t
*β-Pinene	1.63	1.59	27.2
*Δ_3-Carene	2.07	2.02	26.9
*Myrcene	2.25	1.77	16.0
*Limonene	2.77	2.42	14.8
γ-Terpinene	3.71	3.26	t
*Terpinolene	4.60	4.09	2.1
(130° isothermal, ref. camphor)			
Unidentified	1.15	2.49	0.5
Chavicol methyl ether	1.56	1.27	0.5
Unidentified	1.87	3.68	1.2

This oil is seen from Tables 102, 103 and Figure 73 to consist principally of β-pinene, Δ_3-carene, myrcene, limonene and α-pinene, with a very small percentage of high-boiling components such as sesquiterpenes. The oil sample is therefore similar to many of those examined by Smith [464].

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from comminuted foliage of tree I (Table 104, Figure 74) indicated a fundamentally different oil vapour composition being released to the atmosphere from foliage of this tree. This

Table 103. RRT values on the dissimilar liquid
phases C20M and OV-17, for components
found in oxygenated and preparative
GC fractions, isolated from steam-
distilled oil from oleoresin of
Pinus ponderosa (tree I)

Component	Hydrocarbon fraction		Oxygenated fraction		Preparative GC fractions		
	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17

(60° isothermal, ref. α-pinene)

Component	Hydrocarbon fraction		Oxygenated fraction		No.	C20M	OV-17
α-Pinene	All components				W1	1.00	1.01
Camphene	polymerized in this				W2	1.26	1.21
β-Pinene	fraction				W4	1.61	1.61
					W2	1.60	}1.52
Sabinene					W2	1.72	
Δ₃-Carene					W3	2.07	2.00
					W4	2.15	2.08
					W5	2.02	1.94
Myrcene					W1	2.32	1.76
					W2	2.23	1.72
					W3	2.25	1.78
Unidentified					W4	2.44	
Limonene					W4	2.72	2.42
					W5	2.75	2.38
ρ-Cymene					W4	4.23	
Terpinolene					W6	4.57	4.05

(Δ₃-Carene W4/W2/W2 rows bracketed to 1.52 for OV-17)

(130° isothermal, ref. camphor)

Component	Oxygenated fraction	
	C20M	OV-17
Chavicol methyl ether	1.60	1.26
Geraniol	3.27	1.26
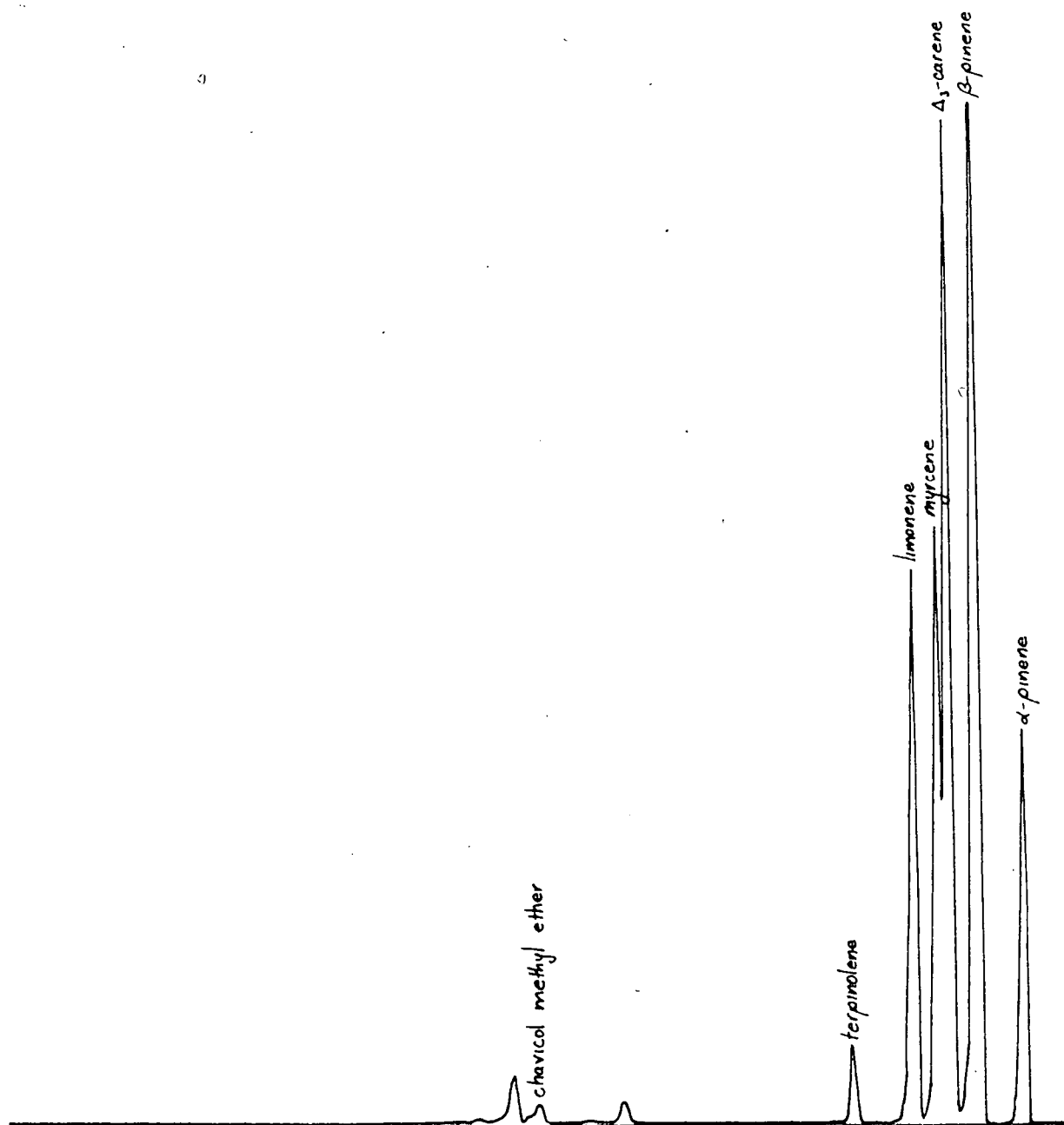
Fig. 73(a).   Low sensitivity gas chromatogram of whole oil from oleoresin
of *Pinus ponderosa* (GC on 5% Carbowax 20M/Gas Chrom Q;   temperature program
50° to 210° at 5°/min;   0.2 µℓ sample;   attenuation 8 x 10³).
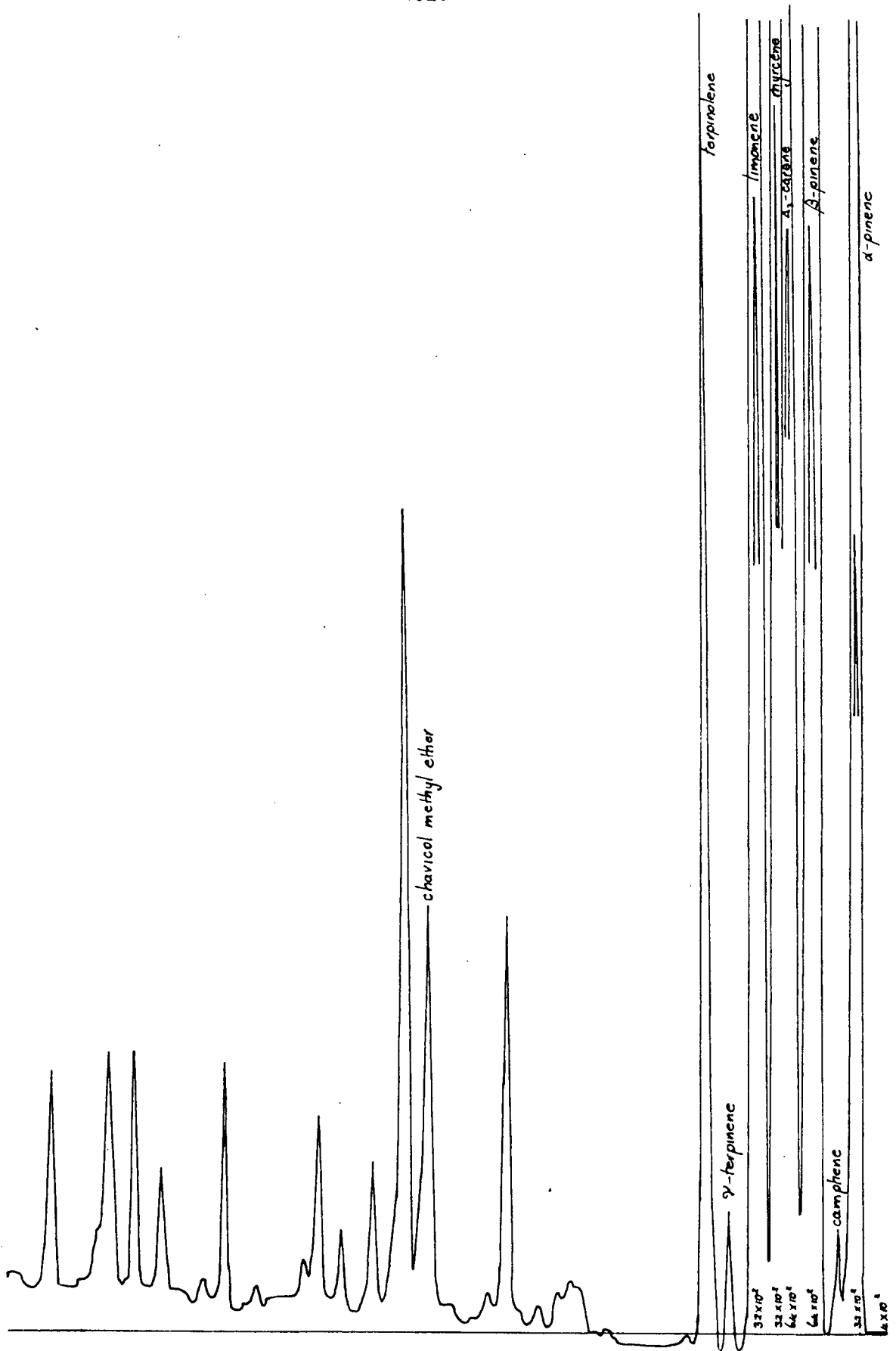
Fig. 73(b). High sensitivity gas chromatogram of whole oil from oleoresin of *Pinus ponderosa* (attenuation 4 x 10^2).
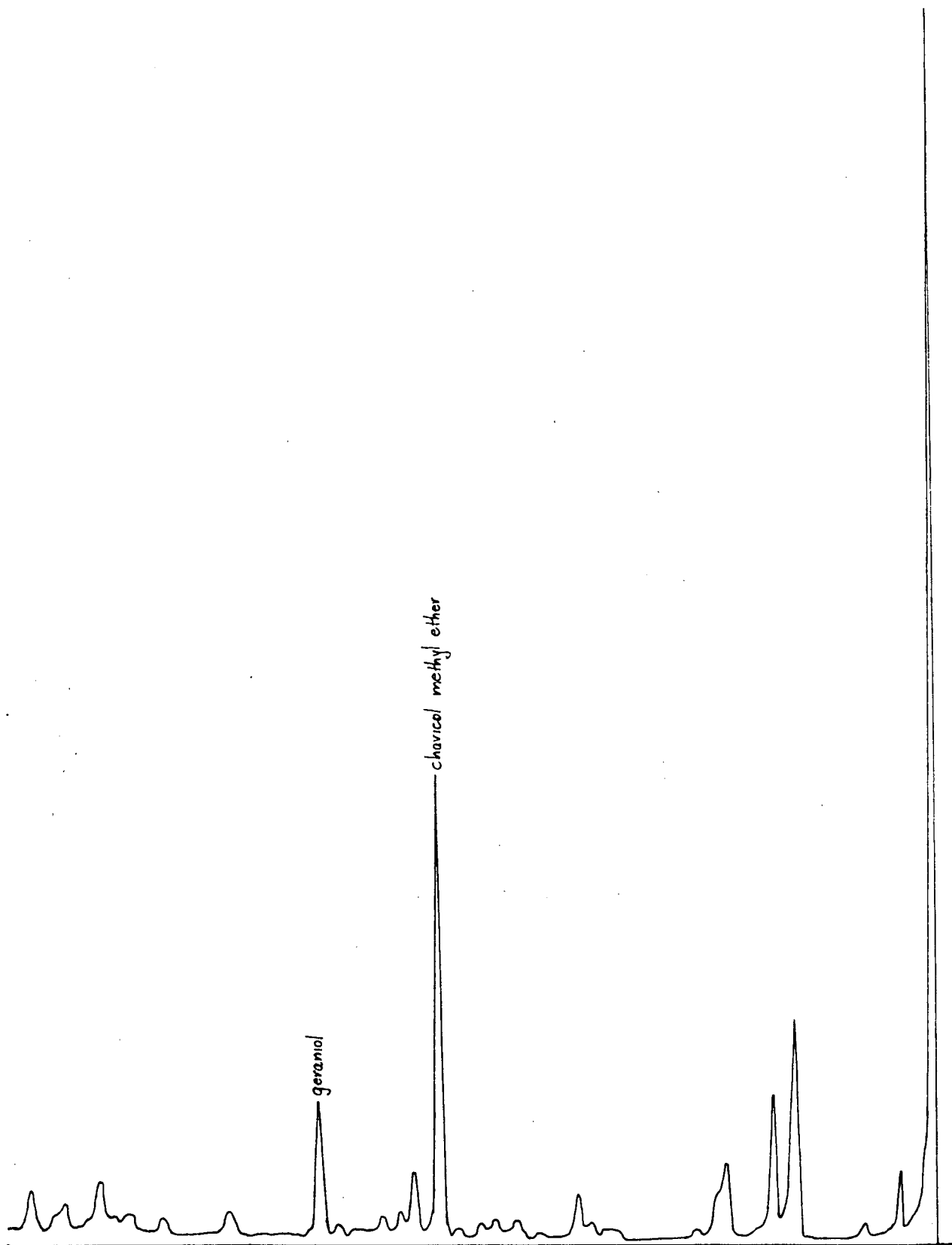
Fig. 73(c). Low sensitivity gas chromatogram of oxygenated fraction of oil from oleoresin of *Pinus ponderosa* separated on Florisil.

Table 104. RRT data and percentage composition of
volatile terpenoids in foliage of *Pinus
ponderosa* (tree I) determined by syringe-
headspace GC analysis

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak area)
(60° isothermal, ref. α-pinene)			(60° isothermal)
α-Pinene	0.99	0.99	17.3
Camphene	1.26	1.17	0.2
β-Pinene	1.63	1.54	58.3
Δ_3-Carene	2.08	1.92	19.0
Myrcene	2.30	1.70	2.6
Limonene	2.85	2.35	1.1
β-Phellandrene	2.96	2.45	1.0
γ-Terpinene?			0.1
Terpinolene	4.86	4.10	0.5

Additional components detected in foliage vapour from tree II.

Unidentified	0.41	0.47	
"	0.50	0.60	
"	0.74		
"	1.53		
Sabinene	1.75		

oil contained, as in foliage of other species, a mugh higher

proportion of β-pinene. Myrcene and limonene proportions were

much less than in oil from oleoresin.

(c) Composition of successive injections of syringe-headspace

vapour from foliage (tree I)

Successive injections of vapour over a 3 hr. period,

from a single sample of foliage, exhibited fluctuations in the

proportions of α-pinene, β-pinene and Δ_3-carene (Table 105

and Figure 32).
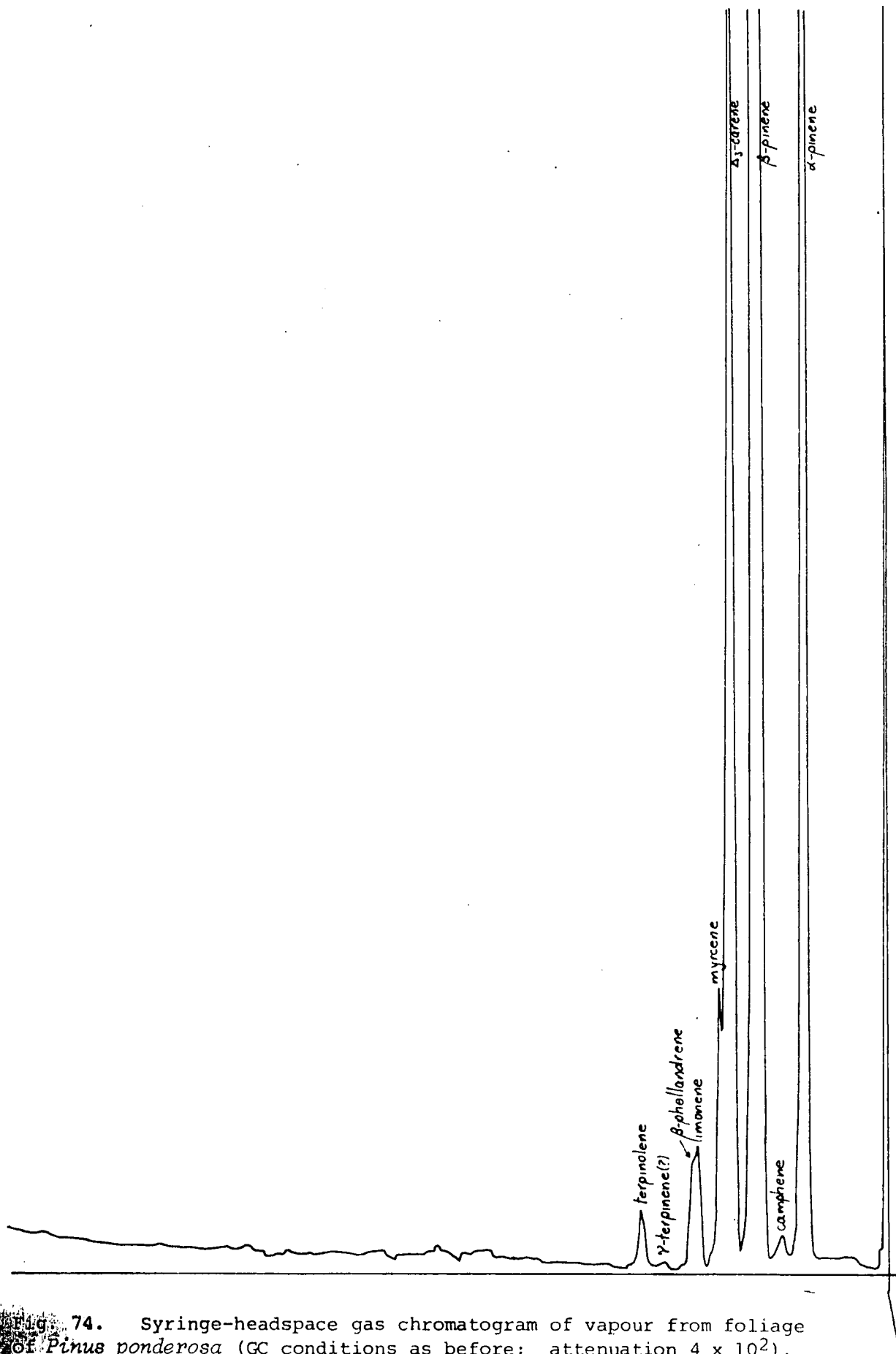
Fig. **74.** Syringe-headspace gas chromatogram of vapour from foliage of *Pinus ponderosa* (GC conditions as before; attenuation 4×10^2).

Table 105. Compositions of monoterpenes in
successive injections of syringe-
headspace vapour from foliage of
Pinus ponderosa (tree I)

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Δ_3-Carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene (+ ρ-cymene)
0	0.4	22.1	0.3	52.2	18.1	4.0	0.1	1.0	1.2	–	0.6
20	0.3	23.4	0.3	52.6	16.8	3.6	0.2	0.8	1.1	0.1	0.5
35	t	23.6	0.3	54.3	16.0	3.4	0.2	0.8	1.0	t	0.5
50	0.3	25.1	0.2	52.0	16.2	3.7	0.1	1.0	1.1	–	0.5
65	0.3	24.5	0.3	53.6	15.5	3.4	0.1	0.8	1.0	–	0.4
85	0.4	24.4	0.3	53.8	15.3	3.6	0.1	0.8	0.9	–	0.4
100	0.4	24.2	0.3	53.6	15.5	3.6	0.2	0.8	1.0	–	0.4
115	0.4	24.7	0.3	53.2	15.3	3.6	0.1	0.8	1.0	–	0.4
130	0.5	24.1	0.3	53.6	15.4	3.7	0.1	0.8	1.0	t	0.4
145	0.6	23.4	0.3	54.2	15.6	3.8	t	0.8	1.0	–	0.4
160	0.5	22.9	0.3	54.5	15.6	3.8	0.1	0.8	1.0	–	0.4
180	0.4	23.6	0.2	55.2	14.9	3.5	0.1	0.7	1.0	–	0.4

(d) Composition of syringe-headspace vapour from foliage of

several trees of *Pinus ponderosa* (trees I-IV)

The wide variation in monoterpene composition of the

vapour from a single sample of foliage, from each of four trees,

is shown in Table 106 (trees I-IV). The extreme variation in

compositions between these trees may possibly be due to the

Table 106. Composition of monoterpenes in syringe-headspace injections of vapour from foliage of several trees of *Ponderosa*. The composition of the first three successive injections in the case of tree II illustrates the apparent "disappearance" of the component eluting near Δ_3-carene.

Percentage composition of monoterpenes (peak height basis):

Tree No.	Time since comminution (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Sabinene	Unidentified 'temporary' component	Δ_3-Carene	Myrcene	Limonene	β-Phellandrene	γ-Terpinene	ρ-Cymene	Terpinolene
I	0	-	19.7	0.3	54.5	-	-	17.3	4.2	2.1	1.2	t	-	0.5
	20	0.1	22.5	0.3	53.7	-	-	15.8	3.8	1.9	1.2	t	-	0.4
	35	t	23.3	0.3	54.7	-	-	15.0	3.6	1.7	1.1	t	-	0.3
	50	t	21.0	0.3	55.6	-	-	15.8	3.8	1.8	1.1	-	-	0.4
Further sample of I														
	0	0.1	19.7	0.3	52.7	-	-	17.1	4.3	4.2	1.1	0.1	-	0.5
	40	0.1	22.5	0.4	52.2	-	-	16.5	3.8	3.2	0.8	t	-	0.4
	55	0.1	22.9	0.3	52.9	-	-	16.1	3.7	2.9	0.7	t	-	0.4
Steam-distilled oil from remainder of comminuted foliage from I														
		42.8	6.4	0.1	30.8	0.5	-	12.1	2.6	3.0	0.6	0.1	0.2	0.7
II	0	1.1	78.8	1.0	1.3	1.2	0.5	-	6.5	0.6	8.3	0.1	0.2	0.3
	15	1.5	82.4	1.1	1.3	1.1	-	-	5.3	0.5	6.3	0.1	0.2	0.2
	35	1.6	82.6	1.1	1.3	1.0	-	-	5.1	0.8	6.1	0.1	0.2	0.1
III	0	0.1	41.1	2.7	5.7	1.8	-	40.6	4.1	0.6	0.7	0.2	0.1	1.9
	20	0.1	45.2	3.2	5.8	1.5	-	36.8	3.8	0.6	0.6	0.2	0.1	1.5
	35	0.2	46.2	3.1	5.9	1.5	-	35.9	3.9	0.6	0.6	0.2	0.1	1.4
IV	0	0.1	68.8	2.9	6.8	1.8	-	1.1	2.1	7.4	8.0	0.4	-	0.5
	20	0.3	72.6	3.1	6.4	1.7	-	0.8	1.8	5.7	6.6	0.3	-	0.4
	35	0.4	73.2	3.1	6.4	1.6	-	0.9	1.8	5.5	6.4	0.3	-	0.4

N.B. Further trace peaks occasionally appear between myrcene and limonene, just before α-pinene (tricyclene?) and just before γ-terpinene.

well-known variability of this species. In the vapour of one
of the trees, which appeared not to contain Δ_3-carene, a
component was detected which eluted with a RRT value close to
that of Δ_3-carene. This component was only detected in the
initial injection and appeared to be temporarily released from
the freshly comminuted foliage tissue (Figure 75).

A chromatogram of the steam-distilled oil from the
remainder of the comminuted foliage from tree I is also given
for comparison in Figure 75.

(e) Composition of syringe-headspace vapour from foliage

sampled repeatedly from the same tree (tree I)

The composition of the vapour from 4 samples of foliage
from tree I varied (Tables 104-106) from 17.3 to 22.1 percent
α-pinene, 52.2 to 58.3 percent β-pinene and 17.1 to 19.0 percent
Δ_3-carene.

(f) Summary

Components of the steam-distilled oil from oleoresin
of *Pinus ponderosa* were analyzed by chromatographic and
spectroscopic methods. The following identifications were
confirmed: α-pinene (10.6%), β-pinene (27.2%), Δ_3-carene (26.9%),
myrcene (16.0%), limonene (14.8%) and terpinolene (2.1%).
Tentatively identified were camphene, sabinene, γ-terpinene,
ρ-cymene, chavicol methyl ether (0.5%) and geraniol. By
comparison, monoterpenes of the steam-distilled foliage oil
included a component eluted before α-pinene (42.8%), α-pinene
(6.4%), camphene (0.1%), β-pinene (30.8%), sabinene (0.5%),
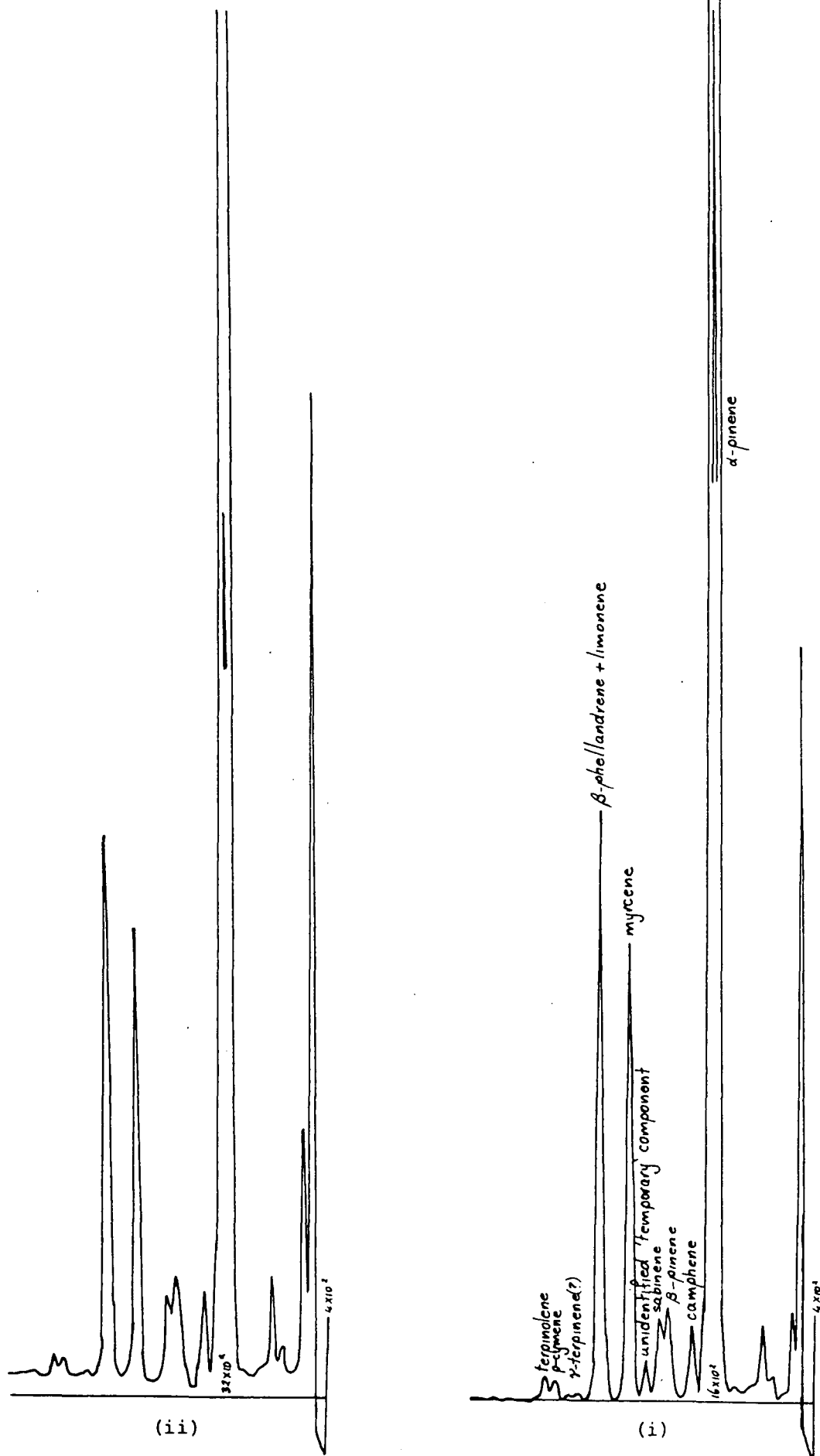
Fig. 75(a). Syringe-headspace chromatograms of foliage vapour of *Pinus ponderosa* (tree II). In the initial injection (i) an unidentified 'temporary component' appears as a peak with RRT similar to Δ_3-carene. A subsequent injection 15 mins. later (ii) contains no trace of this component.

Fig. 75(b). Gas chromatogram of steam-distilled oil from the remainder of comminuted foliage of *Pinus ponderosa* (tree I) studied by syringe-headspace vapour GC in Fig. 32(b) (GC conditions as before).

Δ_3-carene (12.1%), myrcene (2.6%), limonene (3.0%), β-phellandrene (0.6%), γ-terpinene (0.1%), ρ-cymene (0.2%) and terpinolene (0.7%). Sabinene and geraniol do not appear from the literature to have been previously found in this species.

The syringe-headspace GC technique indicated the existence of a range of monoterpene compositions to be found in successive injections of vapour from a single sample of foliage from the same tree. Successive injections of vapour over a 3 hr. period contained from 22.1 to 25.1% α-pinene, 52.0 to 55.2% β-pinene and 14.9 to 18.1% Δ_3-carene; whereas from 4 samples of foliage from the same tree the vapour contained from 17.3 to 22.1% α-pinene, 52.2 to 58.3% β-pinene and 17.1 to 19.0% Δ_3-carene. The compositions of foliage vapour from 4 different trees were so widely-ranging that it is considered that this feature could be related to the well-known morphological variability of the species, i.e. α-pinene varied from 17.3 to 78.8%, β-pinene from 1.3 to 58.3% and Δ_3-carene varied from 0.0 to 40.6%.

An apparently qualitative change in foliage vapour was indicated when a component, eluted near Δ_3-carene on Carbowax 20M, could not be detected in one tree after 15 minutes of successive injections from the same sample.

(xiv) *Pinus radiata* D.Don

Oil from the oleoresin of the mature Monterey Pine *(Pinus radiata)* has from the earliest investigations been shown to consist of highly variable proportions of mainly α- and β-pinene [47]. This is understandable because the species is known to exist as a wide range of morphological variants [327]. Components that have been reported in this oil are: α-pinene, β-pinene, dipentene and camphene [47], together with small amounts of limonene and β-phellandrene [51, 144]. The proportion of α-pinene has been reported [144] to range from 12.6 to 58 percent. Blight, Bannister and co-workers [144, 151, 469] have examined the variation in monoterpene composition of oils from different populations of *P. radiata*.

Younger trees and tissues have since been shown to contain limonene as a major component, together with α- and β-pinenes, camphene, sabinene, myrcene, Δ_3-carene and β-phellandrene [470]. Further monoterpenes subsequently identified in young cortical oleoresin [327] include toluene, α-phellandrene, α-terpinene, γ-terpinene, ρ-cymene and terpinolene.

The genetic basis for the formation of various proportions of α- and β-pinenes in *P. radiata* and hybrids has been investigated by several workers [51, 144, 212, 428]. Others have studied the biosynthesis of monoterpenoids [427, 471-475] and the attractiveness of *P. radiata* to insect pests [331, 332, 398, 401, 448, 476].

(a) Analysis of the oil steam-distilled from cortical oleoresin

Cortical oleoresin collected as before from a single tree of *P. radiata* (tree I) in the Royal Botanical Gardens (Tasmania) yielded upon steam-distillation 33.7 percent of a colourless oil with a pinene odour.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 107. RRT values leading to the tentative identification of components in hydrocarbon and oxygenated fractions, and preparative GC fractions from the whole oil, are listed in Table 108. Gas chromatograms of Figure 76 show the distribution of components eluted from a Carbowax 20M column, and indicate the degree of separation of components into hydrocarbon and oxygenated fractions.

This oil is seen from Tables 107, 108 and Figure 76 to consist largely of α-pinene and β-pinene with small proportions of other monoterpenes as reported earlier. No sesquiterpenes were detected in the high-boiling elution range of the hydrocarbon fraction. Some oxygenated components were detected which do not appear from the literature to have been previously found in *P. radiata*, i.e. terpinen-4-ol, chavicol methyl ether and α-terpineol.

Table 107.   Components distinguishable in the whole
oil from oleoresin of *Pinus radiata*
(tree I)

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 200°, 5°/min.)
*α-Pinene	1.00	1.00	29.6
*Camphene	1.30	1.21	0.4
*β-Pinene	1.65	1.61	66.1
Δ_3-Carene	2.04	1.97	t
*Myrcene	2.22		1.0
*Limonene	2.74	2.39	1.2
*β-Phellandrene	2.87	2.50	1.8
Terpinolene		4.11	t
	(130° isothermal, ref. camphor)		
Unidentified	0.62		t

At least 40 unidentified trace components.

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* IR spectrum recorded
t: trace; <0.1 percent

(b)   Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from
comminuted foliage of tree I (Table 109) indicated the
existence of a different oil vapour composition being
released to the atmosphere from foliage of this tree.   This
oil contained an additional high-proportion of Δ_3-carene.

(c)   Composition of successive injections of syringe-headspace vapour from foliage (tree I)

Successive injections of vapour over a 3 hr. period,
from a single sample of foliage, exhibited initial fluctuations
in the proportions of α- and β-pinene and Δ_3-carene
(Tables 43, 110 and Figure 31).

Table 108. RRT values on the dissimilar liquid
phases C20M and OV-17, for components
found in hydrocarbon, oxygenated and
preparative GC fractions, isolated
from steam-distilled oil from
oleoresin of *Pinus radiata* (tree I)

Component	Hydrocarbon fraction		Oxygenated fraction		Preparative GC fractions		
	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)							
α-Pinene	1.00	1.00			W1	1.00	1.00
Camphene	1.32	1.21			W1	1.33	1.18
					W2	1.30	1.21
β-Pinene	1.68	1.62	1.57	1.59	W2	1.61	1.56
					W3	1.61	1.56
					W4	1.59	1.56
Δ_3-Carene	2.05	1.95					
Myrcene	2.25		2.17	1.77	W2	2.22	1.77
Unidentified	2.45				W1	2.38	
Limonene	2.77	2.36	2.70	2.41	W4	2.77	2.36
β-Phellandrene	2.91	2.46			W4	2.91	2.49
γ-Terpinene	3.73	3.23					
ρ-Cymene	4.36	2.77	4.30	2.87			
Terpinolene	4.64	4.08					
Unidentified			7.22	4.23			
(130° isothermal, ref. camphor)							
Terpinen-4-ol			1.19	0.89			
Chavicol methyl ether			1.54	1.35			
α-Terpineol			1.77	1.11			
Unidentified			1.50				
"			2.62	2.13			
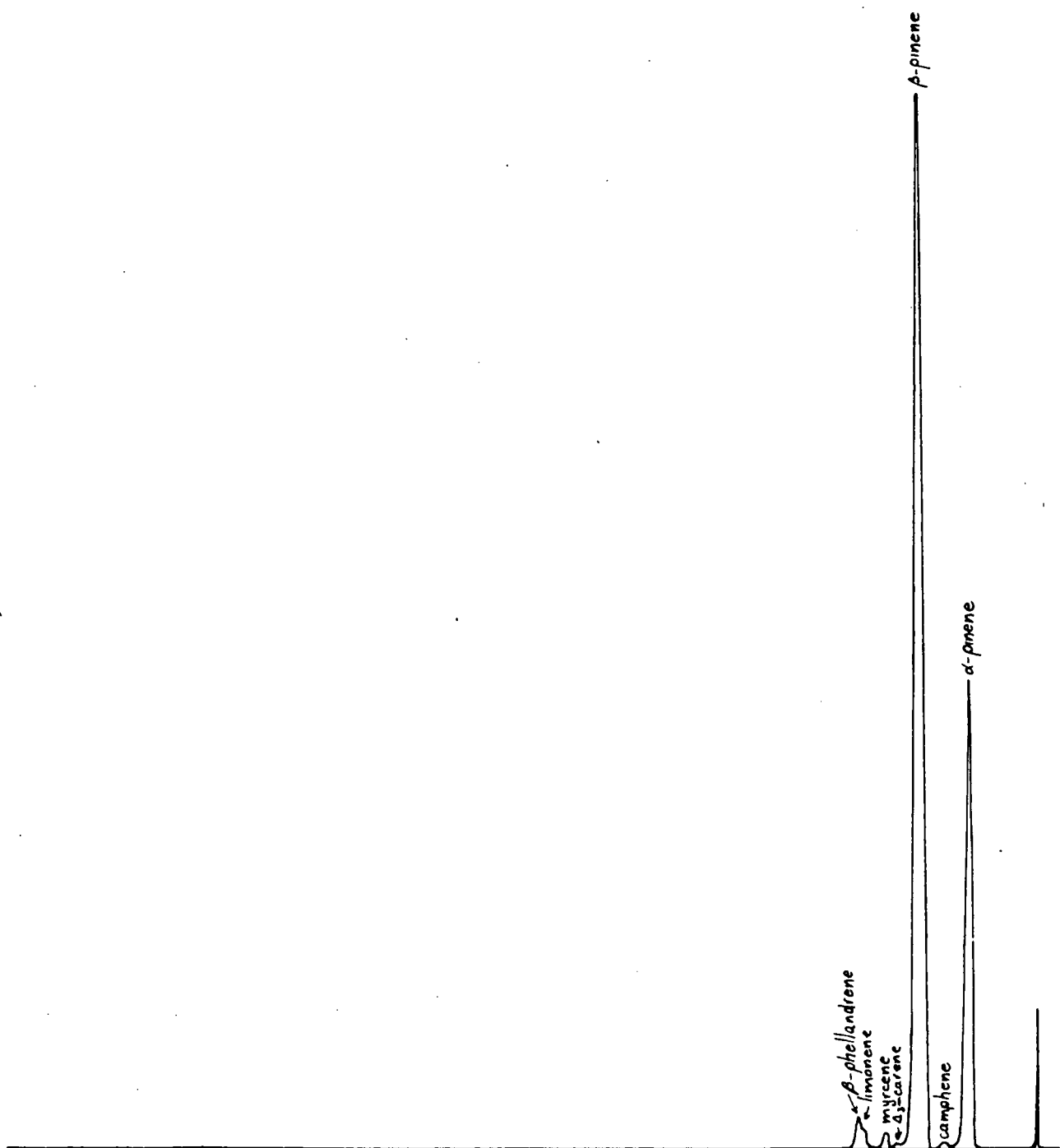
Fig. 76(a). Low sensitivity gas chromatogram of whole oil from oleoresin of *Pinus radiata* (GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50° to 200° at 5°/min; 0.2 μℓ sample; attenuation 8 x 10^3).
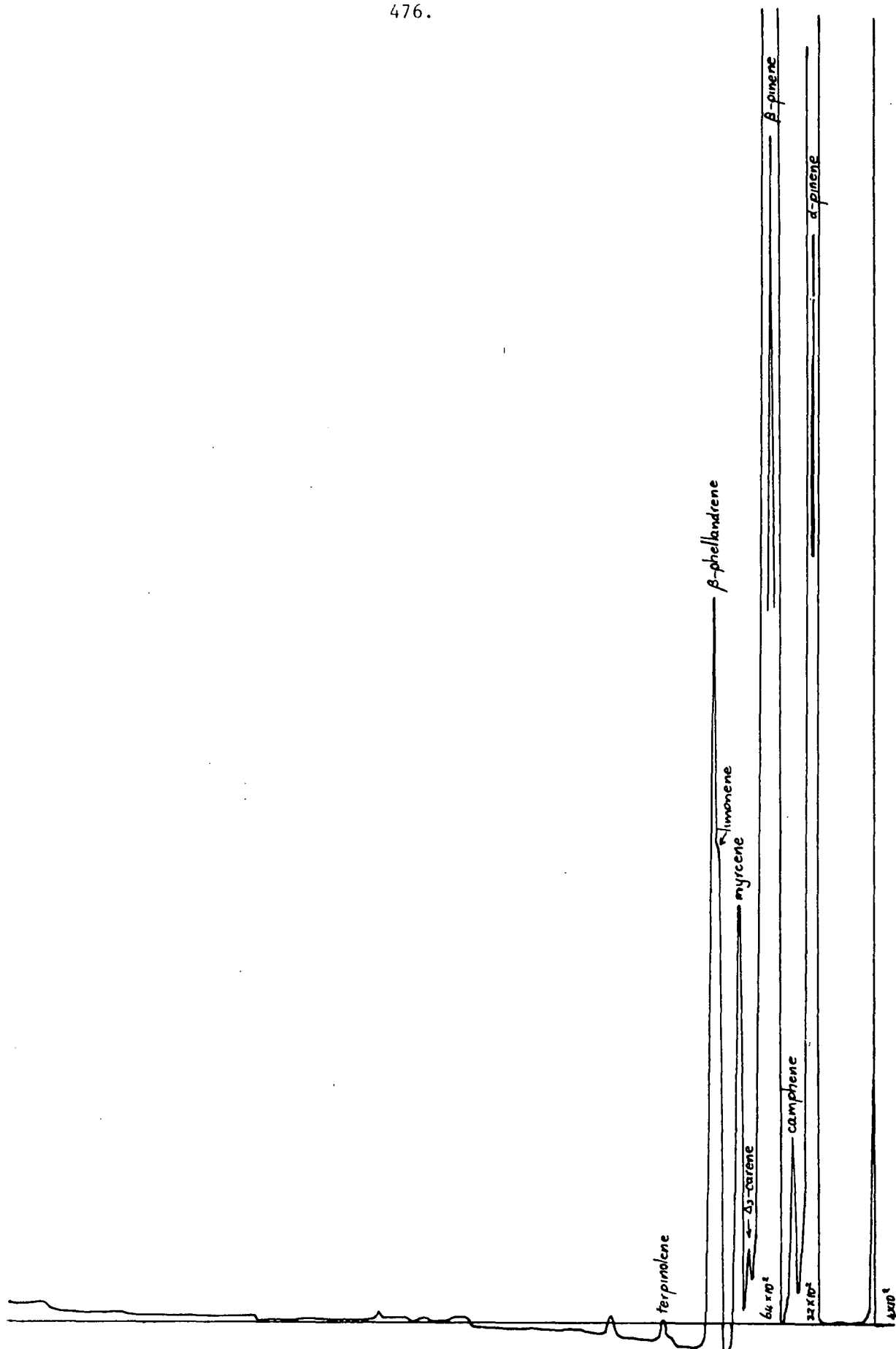
Fig. 76(b). High sensitivity gas chromatogram of whole oil from oleoresin
of *Pinus radiata* (attenuation 4×10^2).
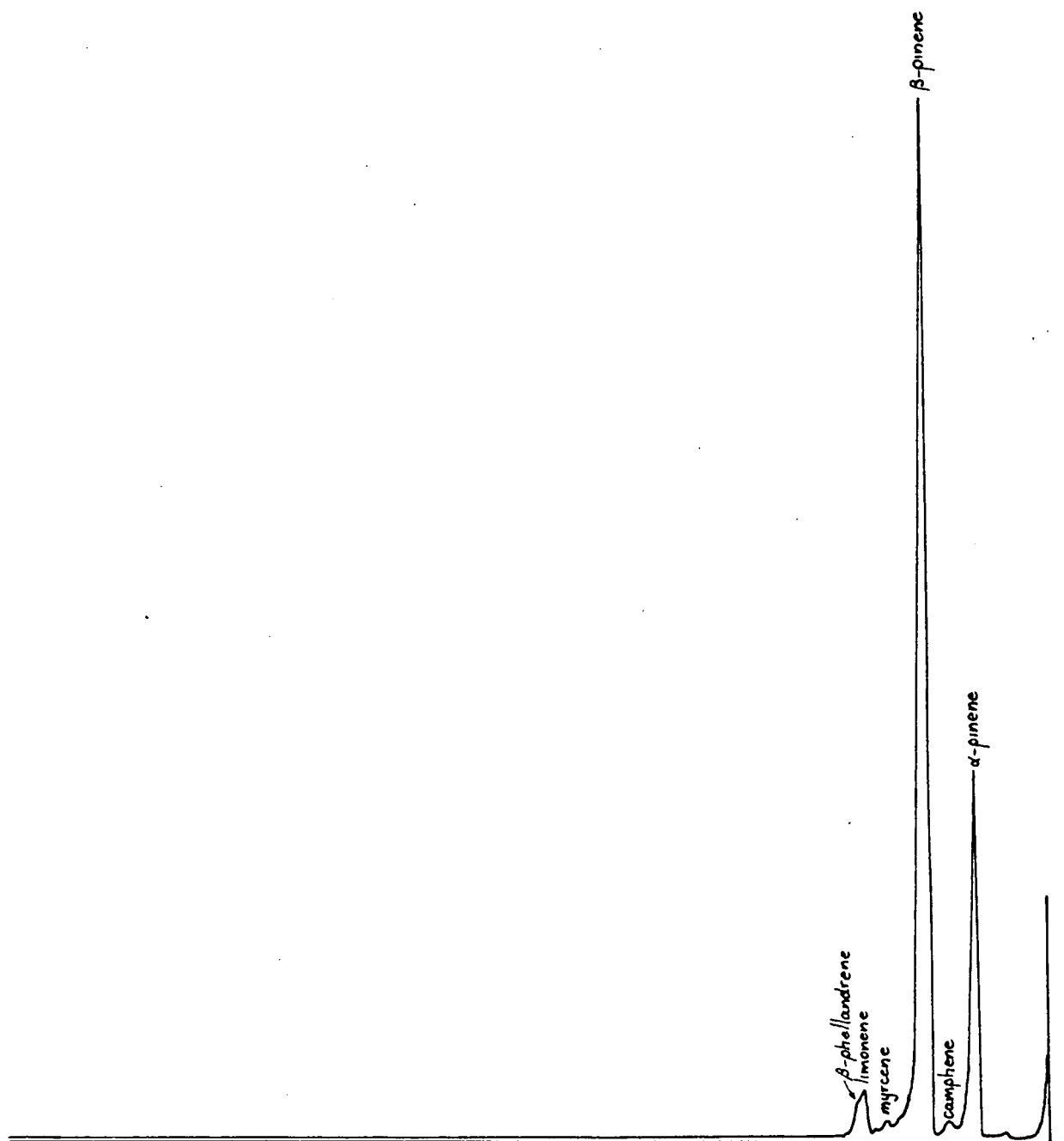
Fig. 76(c). Low sensitivity gas chromatogram of hydrocarbon fractions of oil from oleoresin of *Pinus radiata* separated on Florisil.
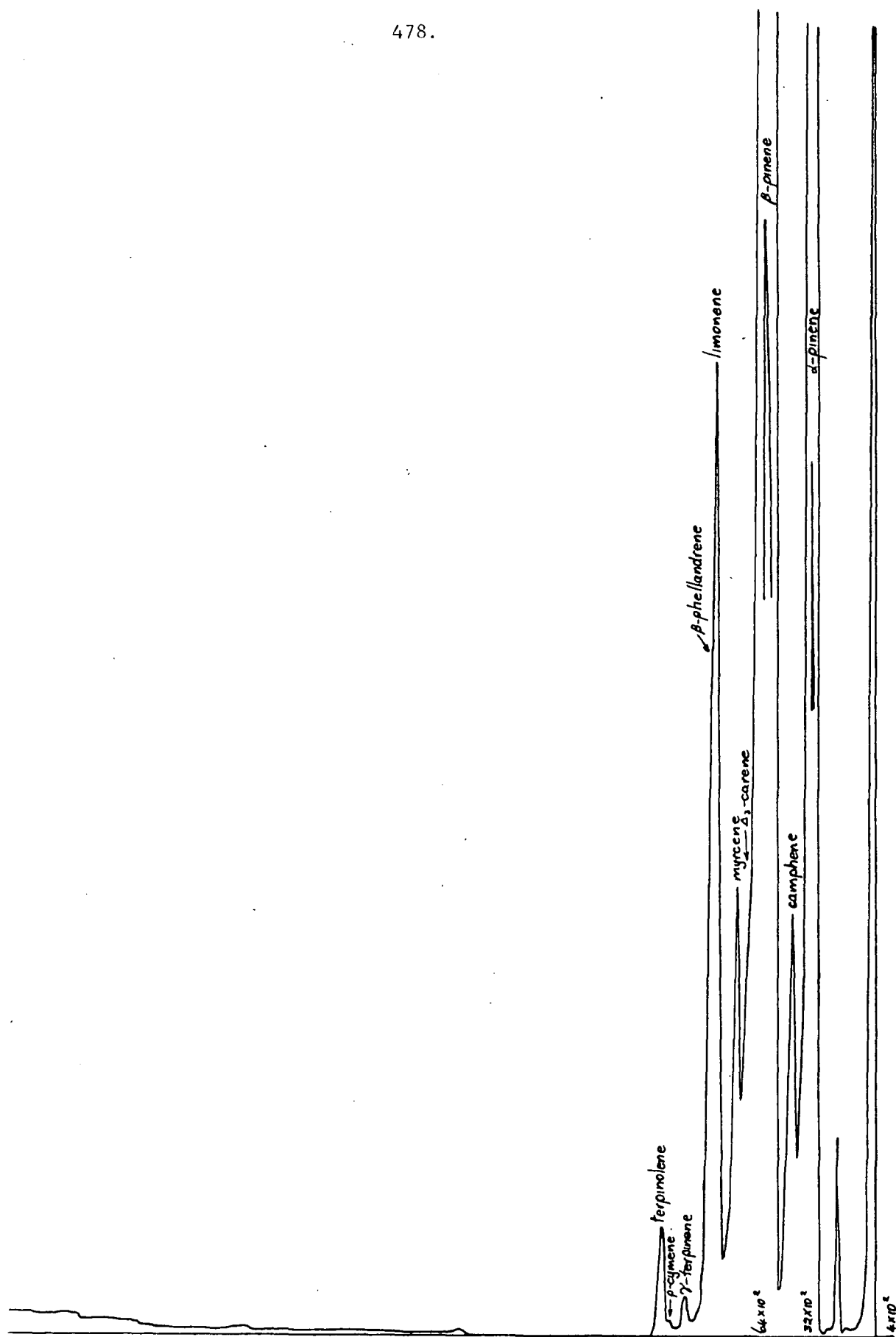
Fig. 76(d). High sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus radiata* separated on Florisil.
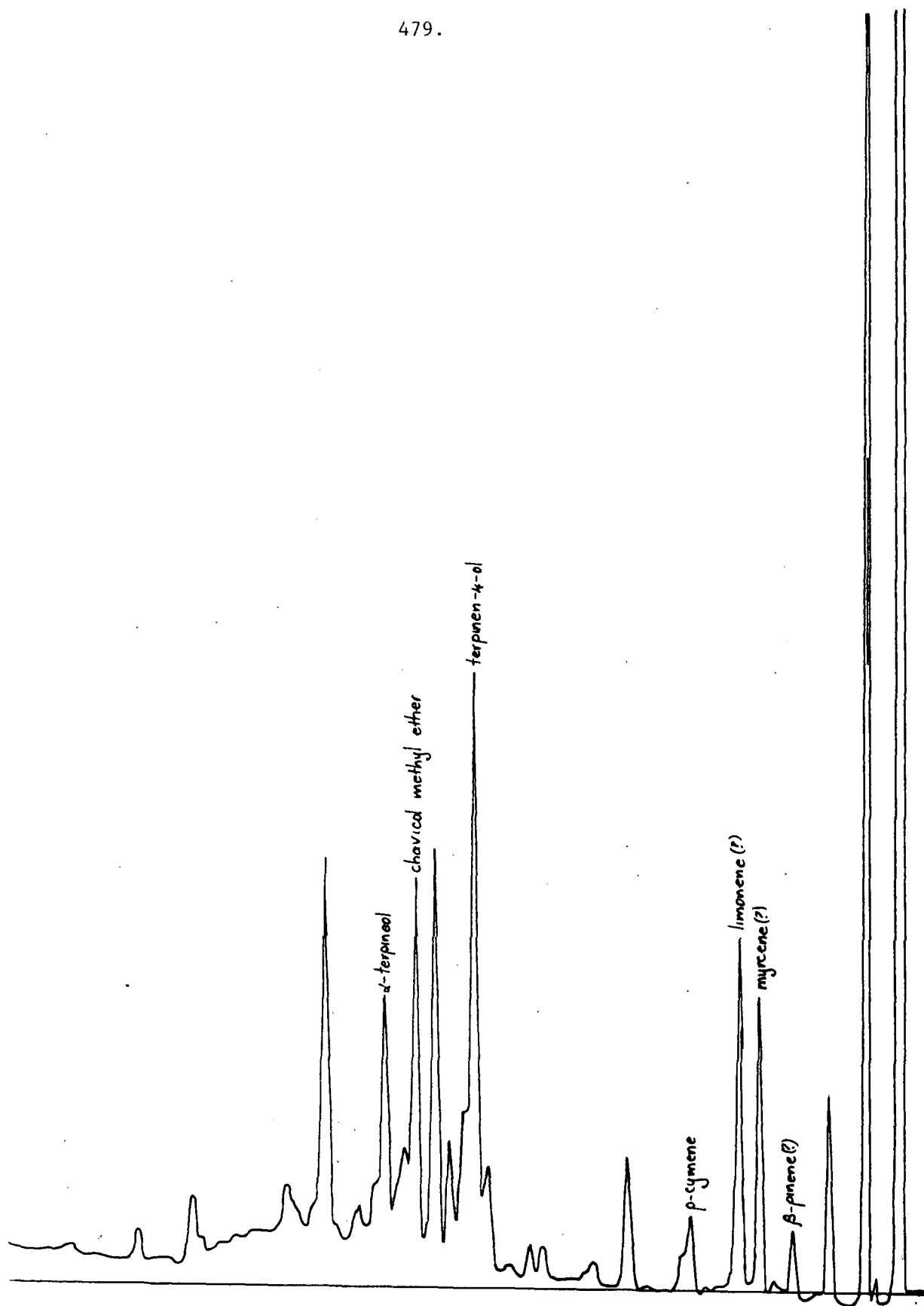
Fig. 76(e).   High sensitivity gas chromatogram of oxygenated fraction of oil from oleoresin of *Pinus radiata* separated on Florisil.

Table 109. RRT data and percentage composition of
volatile terpenoids in foliage of *Pinus
radiata* (tree I) determined by syringe-
headspace GC analysis

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak height)
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min.)
Unidentified	0.75		0.4
"		0.48	
α-Pinene	1.00	1.00	26.3
Camphene	1.26	1.18	t
β-Pinene	1.64	1.57	49.0
Δ_3-Carene	2.10	1.94	20.6
Myrcene	2.32	1.74	2.3
Limonene	2.85	2.36	0.2
β-Phellandrene	3.03	2.50	0.6
Terpinolene	4.93	4.09	0.6

(d) Composition of syringe-headspace vapour from foliage of

several trees of *Pinus radiata* (trees I-IV)

The wide variation in monoterpene composition of the
vapour from a random sample of foliage, from each of four trees,
is shown in Table 111 (trees I-IV). The felled tree foliage
composition described in (f) is not included because it belonged
to a re-growth forest with the possibility of a degree of
hybridism. Trees included in this study were all authentic
P. radiata.

The extreme variation in compositions between these
trees may possibly be due to the variability of this species.

A chromatogram of the steam-distilled oil from the
remainder of the comminuted foliage from tree I is given for
comparison in Figure 77 (and Table 111).

Table 110. Compositions of monoterpenes in successive injections of syringe-headspace vapour from foliage of *Pinus radiata* (tree I)

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified group	α-Pinene	Unidentified	Camphene	β-Pinene	Sabinene	Δ_3-Carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene (+ p-cymene)
(First sample)													
5	–	22.9	–	t	46.3	t	27.1	2.3	–	0.6		–	0.8
20	–	26.8	–	–	45.1	t	25.2	1.9	–	0.5	–	–	0.5
40	t	26.8	–	–	46.4	1.2	23.8	1.8	–	–	–	–	t
55	–	26.0	–	–	47.0	1.6	23.2	1.6	–	t	–	–	0.6
75	–	26.8	–	t	48.2	t	23.2	1.8	–	–	–	–	–
90	–	26.3	–	–	48.0	1.1	23.1	1.6	–	t	–	–	t
115	–	26.1	–	–	48.5	1.2	22.4	1.8	–	t	–	–	–
130	–	26.8	–	–	49.2	t	22.4	1.6	–	–	–	–	–
145	0.5	25.5	–	–	48.4	0.8	23.2	1.6	–	–	–	–	–
160	0.5	24.7	–	t	48.2	t	24.0	1.8	–	0.3	–	–	0.5
175	0.5	25.0	–	–	47.8	1.1	23.4	1.6	–	–	–	–	0.5
190	0.3	25.4	–	t	49.1	t	23.4	1.8	–	–	–	–	–
(Second sample)													
0	0.1	23.0	t	0.1	48.9	–	23.4	2.4	0.1	0.4	0.8	0.2	0.6
20	0.5	26.7	t	0.2	48.1	–	20.7	1.9	0.1	0.3	0.6	0.1	0.6
35	0.7	26.7	t	0.3	48.1	–	20.6	1.9	0.1	0.3	0.6	t	0.5
50	0.7	26.7	t	0.3	48.5	–	20.3	1.8	0.1	0.3	0.7	t	0.6
65	0.8	26.6	t	0.3	48.3	–	20.4	1.8	0.1	0.3	0.7	0.1	0.6
80	0.9	26.0	t	0.3	48.5	–	20.5	1.8	0.1	0.4	0.7	t	0.6
100	0.9	26.0	1.1	0.3	48.8	–	20.3	2.0	0.1	0.2	0.6	0.2	0.6
115	1.0	25.7	1.2	0.2	49.2	–	20.3	2.0	–	0.2	0.6	0.1	0.5
130	0.9	25.8	t	0.3	49.3	–	20.4	1.8	–	0.4	0.6	t	0.5
143	0.8	25.7	t	0.2	49.4	–	20.2	1.8	0.1	0.4	0.6	0.2	0.5
155	0.8	25.3	t	0.2	49.6	–	20.6	1.8	0.1	0.3	0.6	0.1	0.6
170	0.9	25.0	t	0.2	49.8	–	20.7	1.7	0.1	0.3	0.6	t	0.6

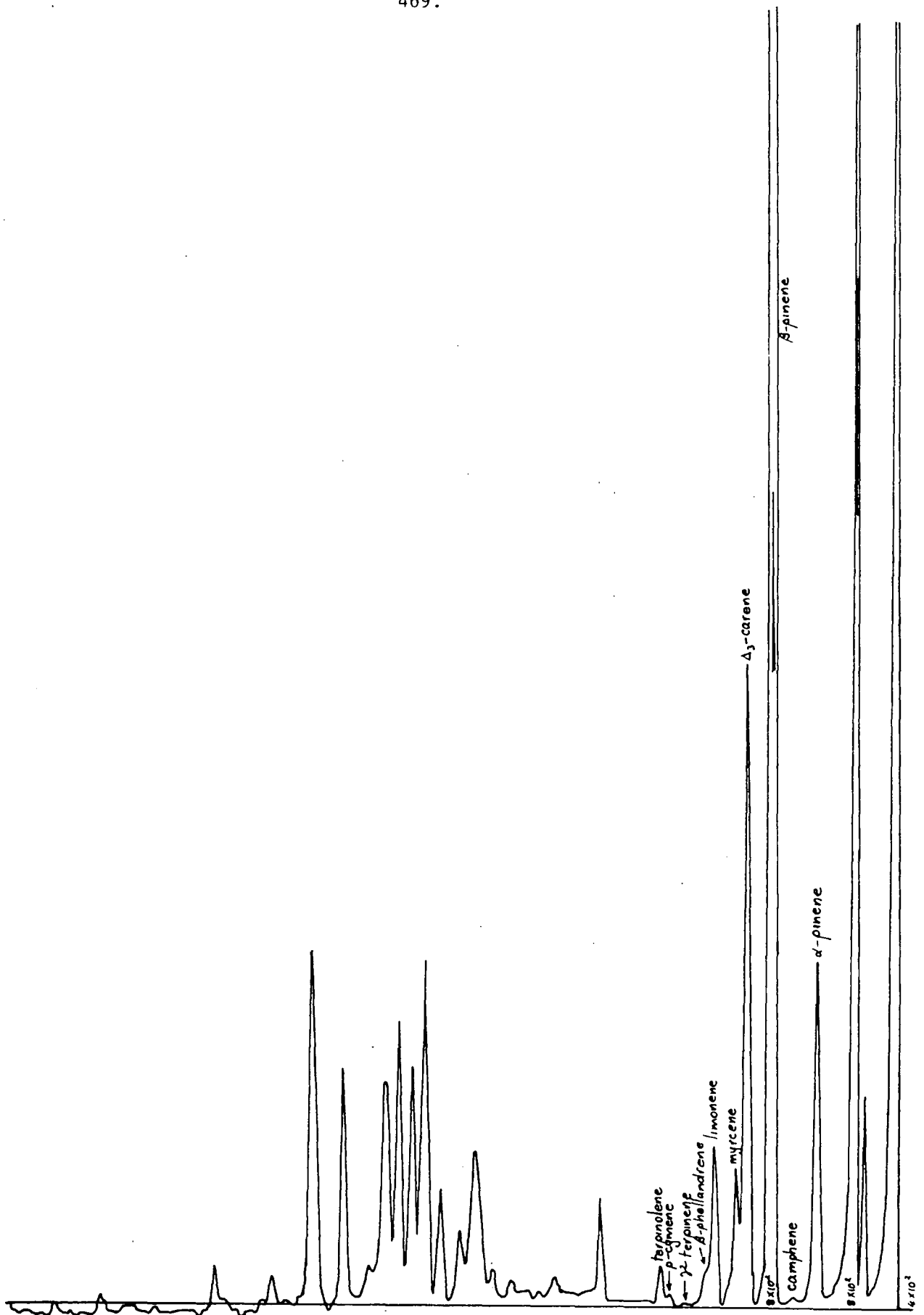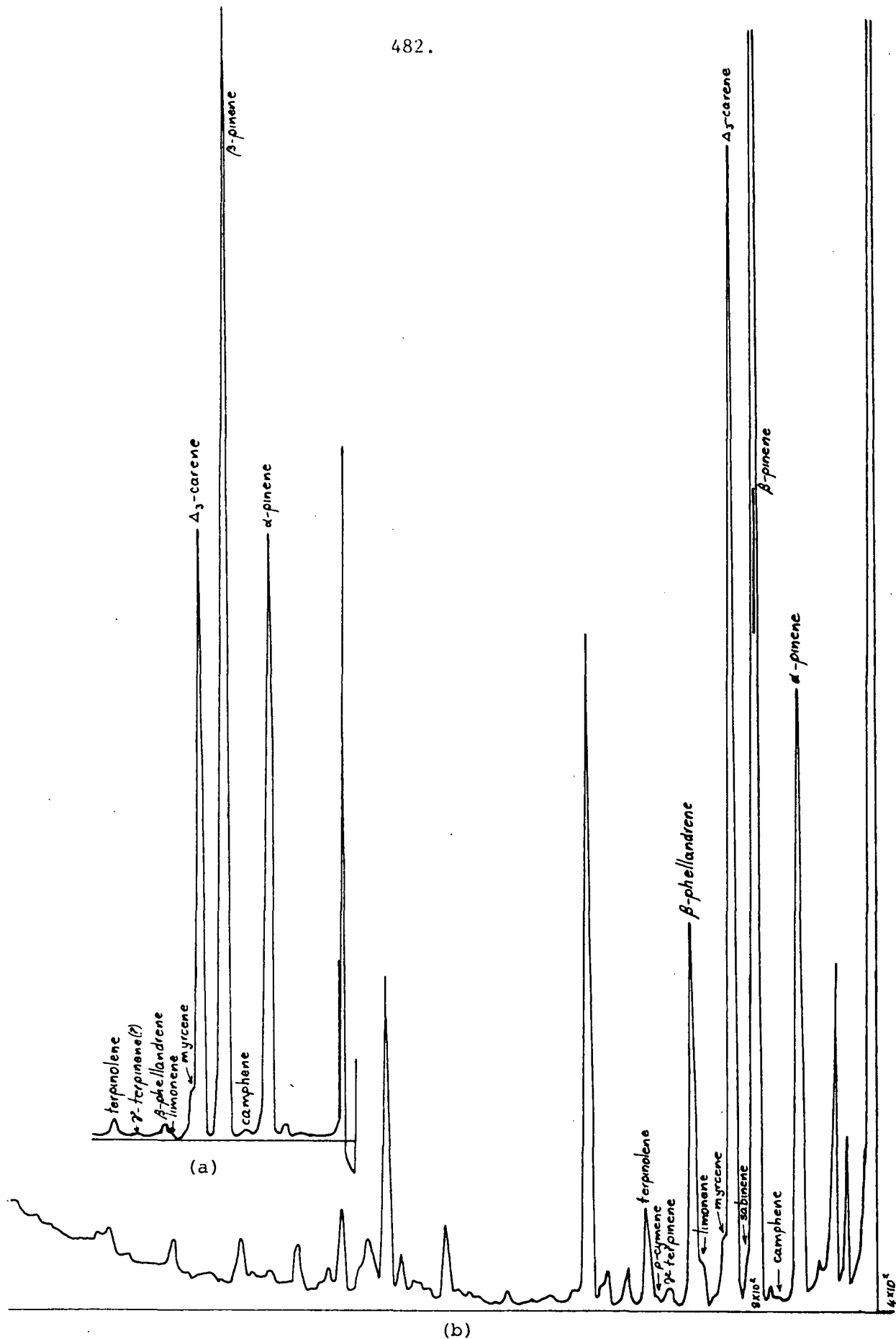Fig. 77(a). Syringe-headspace chromatogram of foliage vapour of *Pinus radiata* (tree I).

(b). Gas chromatogram of oil steam-distilled from the remainder of comminuted foliage of *Pinus radiata* studied by syringe-headspace GC in (a).

Table 111. Composition of monoterpenes in syringe-headspace injections of vapour from foliage of several trees of *Pinus radiata*

Percentage composition of monoterpenes (peak height basis):

Tree No.	Time since comminution (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Sabinene	Δ_3-Carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene
I	0	–	21.7	0.5	48.1	–	26.0	2.0	–	0.3	0.7	t	0.7
	25	0.4	25.3	0.2	45.6	–	25.2	2.0	–	0.2	0.5	t	0.6
	55	0.4	24.2	0.2	47.2	–	24.4	1.9	t	0.3	0.7	t	0.7

Steam-distilled oil from remainder of comminuted foliage from I:

| | | 8.1 | 13.8 | 0.2 | 37.1 | – | 26.5 | 1.6 | 0.2 | 1.0 | 8.7 | 0.4 | 2.2 |

+ ρ-cymene (0.1%)

Tree No.	Time since comminution (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Sabinene	Δ_3-Carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene	Terpinolene
II	0	–	37.7	0.3	35.7	6.7	11.4	5.7	0.1	0.4	0.7	0.9	0.5
	15	0.1	40.3	0.3	35.9	7.0	9.8	4.6	t	0.3	0.6	0.6	0.4
	30	0.1	44.3	0.4	38.8	–	9.7	4.7	0.1	0.3	0.6	0.6	0.4
III	0	t	17.1	0.3	37.0	1.7	7.9	4.6	t	20.9	9.9	0.4	0.2
	15	0.4	19.8	0.3	39.7	1.8	7.3	3.9	t	17.9	8.4	0.4	0.1
	35	0.7	20.7	0.3	41.0	1.7	6.8	3.7	–	17.1	7.5	0.3	0.2
IV	0	0.1	38.2	0.3	28.1	2.3	25.5	3.1	0.2	0.5	0.6	0.2	1.1
	20	0.2	40.3	0.3	28.7	1.9	23.6	2.7	0.1	0.5	0.6	0.1	0.9
	35	0.2	41.2	0.5	28.9	1.7	22.7	2.6	0.2	0.4	0.5	0.3	0.8

t: trace; <0.1 percent

In Figure 78 chromatograms of vapour from earlier injections contain the highest concentrations of the previously-described 'temporarily-released' component, which appears as an early-eluting shoulder on the Δ_3-carene peak.

(e)   Composition of syringe-headspace vapour from foliage
      sampled repeatedly from the same tree (tree I)

The composition of the vapour from four samples of foliage from tree I varied (Tables 109-111) from 21.7 to 26.3 percent α-pinene, 46.3 to 49.0 percent β-pinene and 20.6 to 27.1 percent Δ_3-carene.

(f)   Composition of syringe-headspace vapour from foliage
      sampled daily from a lopped branch

An examination was made over an 18-day period, of the monoterpene vapour composition of foliage intact on a lopped branch. Unlike *Cedrus deodara*, which was also studied on a comparative basis, needles of *P. radiata* remained attached throughout the period. Fluctuations in composition were exhibited throughout the period. The most significant changes occurred about day 13 and day 17 (Table 112, Figure 79). On day 13 proportions of α- and β-pinene were both reduced, while limonene, Δ_3-carene and myrcene increased. This would not appear to be the effect on the overall monoterpene percentage composition of a change in a single component, and could therefore be ascribed to some actual change in the biosynthetic mechanism. Comparison of the oil composition on day 0 with that at the end of the period indicates a definite overall change.
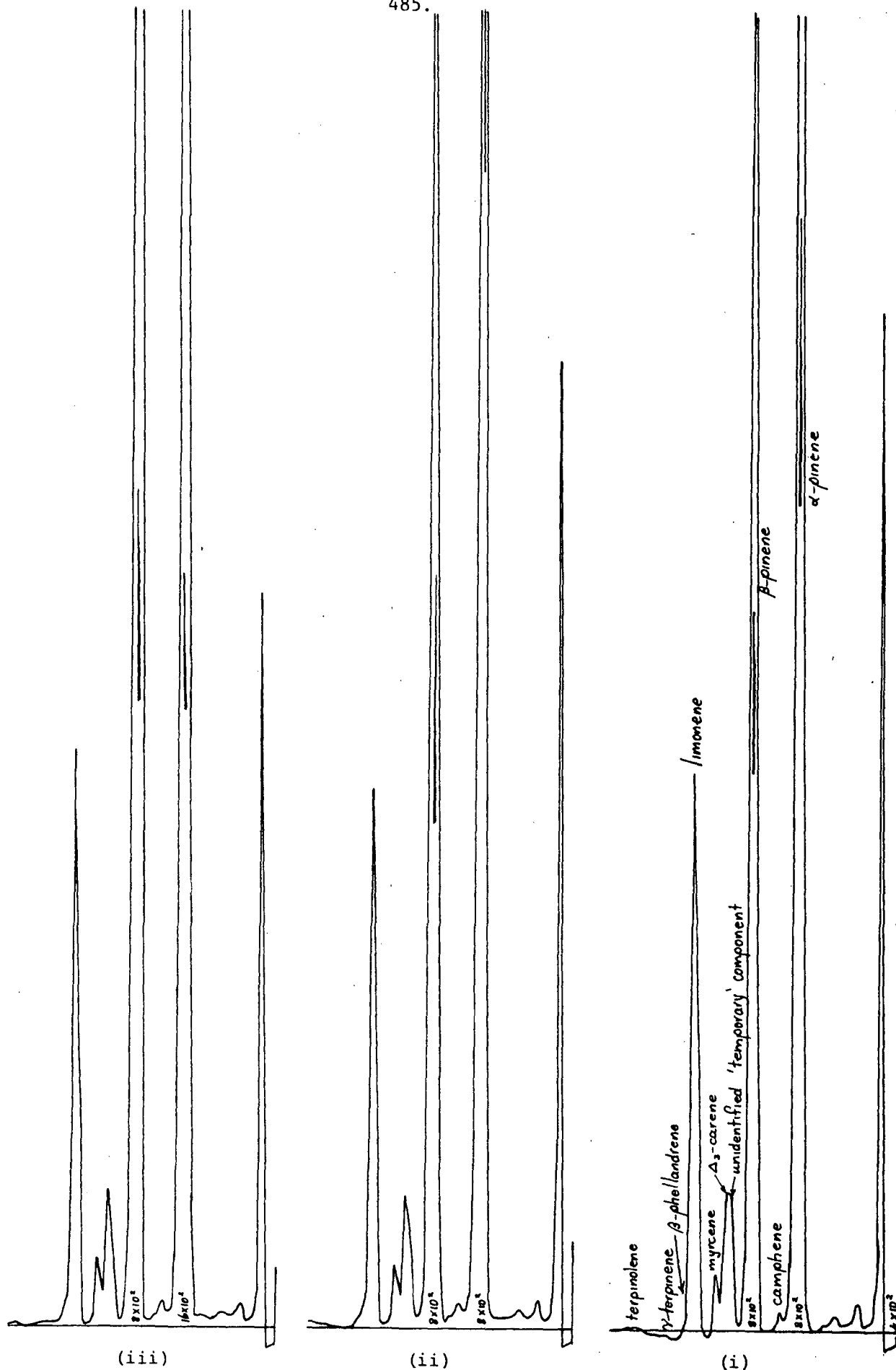
Fig. 78. Syringe-headspace chromatograms of foliage vapour of a re-growth
Pinus radiata with a low content of Δ_3-carene. In the initial injection
(i) an unidentified 'temporary component' appears as a shoulder on the
Δ_3-carene peak. In the initial injection (i) an unidentified 'temporary
component' appears as a shoulder on the Δ_3-carene peak. In subsequent
injections at 15 min. intervals, (ii) and (iii), the shoulder is less

Table 112. Composition of monoterpenes in syringe-headspace injections of vapour from daily samples of foliage of a lopped branch of *Pinus radiata*. For each sample the composition is given of the first three successive injections, which gives a better indication of the range of vapour compositions encountered in a single sample of foliage.

Percentage composition of monoterpenes (peak height basis):

Day No.	Time since comminution (mins.)	Unknown group	Santene?	α-Pinene	Camphene	β-Pinene	Unidentified Δ3-carene shoulder	Δ3-Carene	Myrcene	Limonene	γ-Terpinene and others	Terpinolene
0	0	0.1	-	50.1	0.5	34.2	-	2.7	1.4	11.0	0.1	0.1
	10	-	-	54.4	0.4	33.9	-	2.2	1.0	8.0	0.1	0.1
	25	-	t	54.8	0.5	34.0	-	2.1	1.0	7.5	0.1	0.1
1	0	t	0.3	52.1	0.4	32.0	-	3.2	1.2	10.9	0.1	0.1
	15	0.1	t	55.1	0.3	32.2	-	2.6	1.1	8.5	0.1	0.1
	30	0.1	-	54.3	0.4	33.0	-	2.6	1.0	8.4	0.1	0.1
2	0	0.1	-	50.9	0.3	33.5	1.9	2.7	1.2	11.3	0.1	0.1
	15	0.1	-	53.3	0.4	33.5	1.8	2.6	1.1	9.1	t	-
	30	0.3	0.2	53.5	0.5	33.2	-	2.3	1.1	8.7	0.1	0.1
3	0	0.1	-	50.8	0.3	35.3	-	3.7	1.1	8.8	-	0.1
	15	0.2	t	52.9	0.4	35.3	-	3.2	1.0	7.1	-	0.1
	30°	0.2	t	53.4	0.3	35.4	-	3.0	0.9	6.9	t	t
4	0	0.1	-	51.9	0.3	34.5	-	2.0	1.3	9.8	t	0.1
	15	0.1	-	55.1	0.5	32.8	-	2.0	1.0	8.2	0.1	0.1
	30	0.1	-	55.1	0.5	32.7	-	2.1	1.2	8.2	0.1	0.1
5	0	0.2	t	51.0	0.5	35.8	-	3.4	1.2	8.0	0.1	t
	15	0.1	-	53.8	0.5	35.2	-	2.8	1.0	6.5	0.1	t
6	0	0.2	-	52.6	0.4	32.4	-	2.5	1.2	10.7	-	-
	15	0.3	t	57.5	0.5	31.0	0.8	2.1	0.9	7.8	t	t
	30	0.1	t	59.2	0.5	30.6	0.5	2.0	0.9	6.9	t	-
7	0	0.2	-	52.6	0.2	30.8	1.7	2.4	1.3	12.4	-	-
	10	0.1	-	56.3	0.3	29.6	1.7	2.2	1.1	10.4	-	-
	25	0.2	t	56.1	0.4	30.4	1.4	2.1	1.1	9.7	-	-
8	0	0.2	-	50.6	0.3	35.4	-	2.8	1.1	9.5	t	0.1
	15	0.1	t	57.1	0.3	31.0	-	2.6	1.0	7.7	0.1	0.1
	25	0.2	-	57.8	0.3	30.5	-	2.6	0.9	7.6	t	0.1

Table 112 continued

<u>Percentage composition of monoterpenes (peak height basis):</u>

Day No.	Time since comminution (mins.)	Unknown group	Santene?	α-Pinene	Camphene	β-Pinene	Unidentified Δ₃-Carene shoulder	Δ₃-Carene	Myrcene	Limonene	γ-Terpinene and others	Terpinolene
9	0	0.5	–	52.1	0.3	34.7	1.0	1.3	1.0	10.2	t	–
	15	0.1	–	56.9	0.4	31.7	1.4	2.0	1.0	7.9	–	–
	30	0.1	t	56.4	0.4	31.6	1.2	2.1	1.0	8.4	–	–
10	0	0.2	–	51.8	0.3	36.6	1.5	2.7	1.0	7.3	–	–
	15	t	–	54.1	0.4	36.1	0.4	2.3	0.9	6.2	t	0.1
	30	t	–	54.2	0.5	36.0	0.6	2.4	0.9	6.1	t	t
11	0	0.3	–	50.1	0.3	31.4	1.7	3.7	1.1	11.4	t	–
	15	0.4	t	54.2	0.4	29.0	2.4	3.5	1.2	9.0	–	–
	30	0.4	t	55.6	0.4	29.3	1.4	3.2	0.9	8.9	–	–
12	0	0.1	t	50.1	0.5	36.3	–	2.8	1.2	8.9	0.1	0.1
	15	0.2	–	53.5	0.4	34.4	–	2.7	1.0	7.6	0.1	0.1
	30	0.1	–	52.8	0.5	34.7	–	2.8	1.2	7.7	0.1	0.1
13	0	0.1	–	47.1	0.4	29.4	–	3.8	1.7	17.2	0.1	0.1
	20	0.2	–	51.3	0.4	29.0	–	3.3	1.4	14.4	t	0.1
	35	t	t	51.9	0.3	29.7	–	3.1	1.4	13.6	t	0.1
14	0	0.7	–	49.6	0.4	32.1	3.0	3.1	1.3	12.7	0.1	0.1
	15	0.6	–	55.2	0.3	29.6	1.8	2.5	1.2	10.6	–	0.1
	30	0.4	0.1	54.2	0.3	30.5	0.4	2.4	1.1	11.0	t	t
15	0	0.2	t	50.2	0.3	36.5	–	1.6	1.2	10.1	0.1	t
	15	0.1	t	57.5	0.4	31.3	–	1.5	1.1	8.0	0.1	t
	30	0.2	0.1	46.4	0.4	45.0	–	1.1	0.8	6.0	0.1	0.1
16	0	0.1	–	51.3	0.4	32.5	–	3.3	1.4	10.9	0.1	0.2
	20	0.1	t	53.4	0.4	32.3	–	3.0	1.3	9.4	0.1	0.1
	35	0.1	–	53.5	0.4	32.1	–	2.9	1.2	9.5	0.1	0.1
17	0	0.1	–	50.7	0.4	40.3	–	0.6	1.0	7.0	t	–
	15	0.1	–	54.8	0.4	37.7	–	0.5	0.8	5.7	–	–
	30	0.1	t	54.7	0.5	37.7	–	0.6	0.8	5.6	–	t
18	0	0.3	–	49.0	0.3	38.8	–	0.8	2.3	9.0	t	t
	15	0.4	t	54.2	0.4	36.6	–	0.6	1.5	6.4	–	t
	30	0.3	t	54.5	0.5	36.5	–	0.6	1.3	6.3	t	t

Fig. 79. Composition of monoterpenes in syringe-headspace initial injections of vapour from daily samples of foliage of a lopped branch of *Pinus radiata*. No real correlation is discernible between changes in monoterpene composition and cessation of attractiveness to *Sirex noctilio* on the 14th day after lopping the branch. Reciprocal relationships may be seen for particular components for limited periods of time, e.g. between limonene and Δ_3-carene from day 1 to 10, whereas from day 10 to 18 they follow another related course of changes. Limonene and β-pinene reciprocate throughout the period. Fluctuations in β-pinene are much larger than for α-pinene. Such changes seen in a very complex series of relationships might be linked to the biosynthesis of monoterpenes.

Time since lopping branch (days)

Percentages of monoterpenes in syringe-headspace vapour
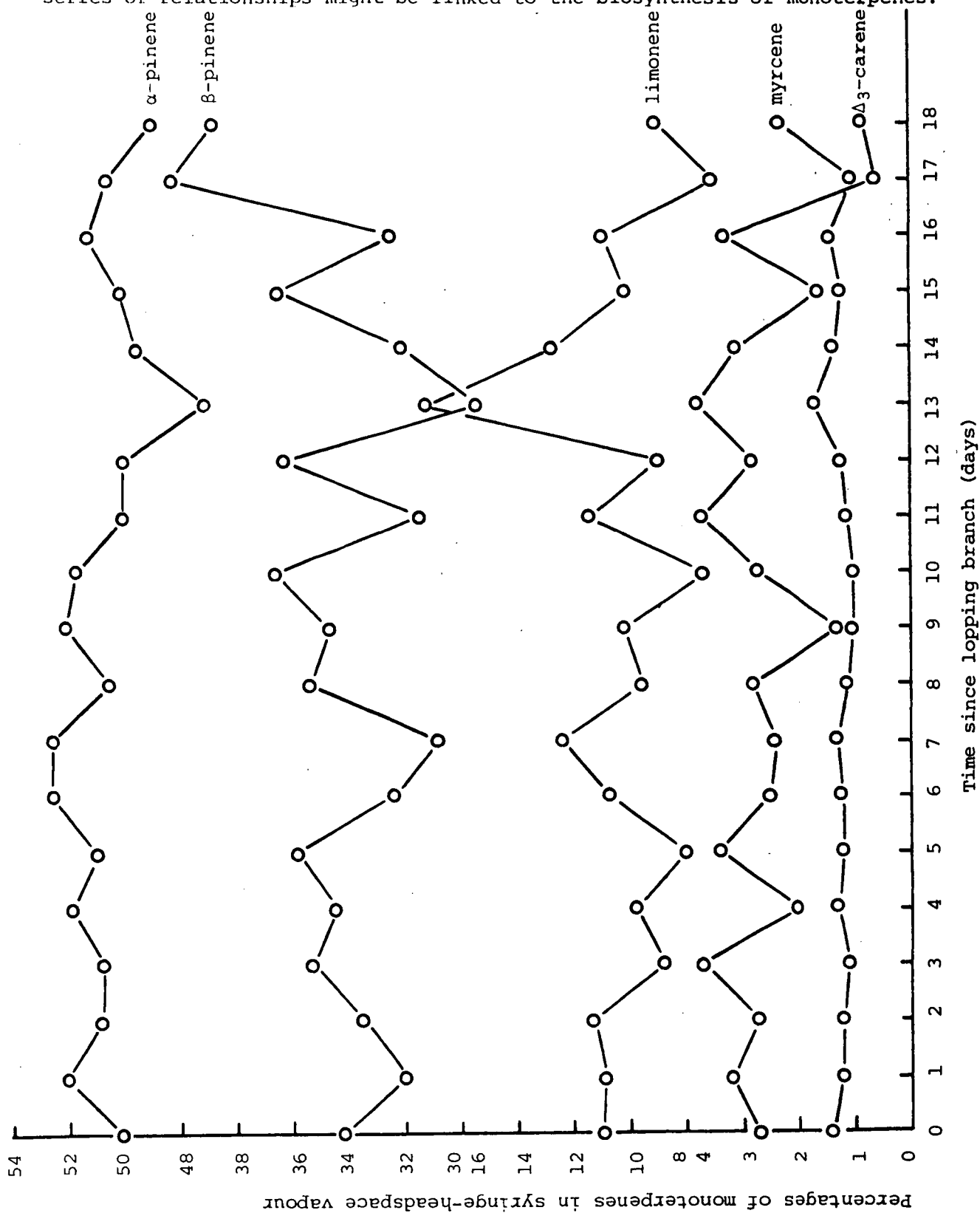
Although fluctuations in the monoterpene composition were discernible, no particular pattern of changes could be seen which could have been correlated with an attractiveness to insects, i.e. immediately after lopping and after a 14-day period [332]. No similarities could be seen which might be correlated with any compositional changes in either *Cedrus deodara* or *Pinus pinaster*.

Such experimental trials should be continued to determine whether an eventual oil composition appears which contains an increased amount of a monoterpene that is toxic to the insect pest, perhaps also in conjunction with a reduction in an attractive component.

(g) Summary

Components of the steam-distilled oil from oleoresin of *Pinus radiata* were analyzed by chromatographic and spectroscopic methods. The following identifications were confirmed: α-pinene (29.6%), camphene (0.4%), β-pinene (66.1%), myrcene (1.0%), limonene (1.2%) and β-phellandrene (1.8%). Tentatively identified were Δ_3-carene, γ-terpinene, ρ-cymene, terpinolene, terpinen-4-ol, chavicol methyl ether and α-terpineol. By comparison monoterpenes of the steam-distilled foliage oil included a group of components eluted before α-pinene (8.1%), α-pinene (13.8%), camphene (0.2%), β-pinene (37.1%), sabinene, Δ_3-carene (26.5%), myrcene (1.6%), limonene (1.0%), β-phellandrene (8.7%), γ-terpinene (0.4%), ρ-cymene (0.1%) and terpinolene (2.2%). No reports were found in the literature of terpinen-4-ol, chavicol methyl ether and α-terpineol having been previously found in this species.

The syringe-headspace GC technique indicated the existence of a range of monoterpene compositions to be found in successive injections of vapour from a single sample of foliage, and also from different samples of foliage from the same tree. Successive injections of vapour over a 3 hr. period contained from 23.0 to 26.7% α-pinene, 48.1 to 49.8% β-pinene and 20.2 to 23.4% Δ_3-carene; whereas from 4 samples of foliage from the same tree the vapour contained from 21.7 to 26.3% α-pinene, 46.3 to 49.0% β-pinene and 20.6 to 27.1% Δ_3-carene. The compositions of foliage vapour from 4 different trees were widely ranging, i.e. α-pinene varied from 17.1 to 38.2%, β-pinene from 28.1 to 48.9%, Δ_3-carene from 7.9 to 27.1%, while limonene varied from 0.3 to 20.9%.

The same 'temporarily-released component', eluting near Δ_3-carene in foliage of other conifers, was found in oil from a regrowth tree. The component was noticeable in oil from this tree because it contained such a minor proportion of Δ_3-carene that the 'temporary component' was resolved on a Carbowax column.

The syringe-headspace monoterpene composition of daily samples of foliage from a lopped branch did not exhibit any fundamental change which might be correlated with attractiveness to *Sirex noctilio* following lopping. The fluctuations in proportions of some components, particularly β-pinene, limonene and Δ_3-carene, were much more rapid than those of α-pinene. Reciprocal and corresponding changes were

observed between certain components for limited periods during the 18-day trial. No conclusions could be drawn as to any possible biosynthetic relationships because any patterns of changes were too complex.

(xv) *Pinus strobus* L.

Oil from oleoresin of the Eastern White Pine *(Pinus strobus)* was reported in the earlier literature [47] to contain 75 percent dℓ-α-pinene, 15 percent ℓ-β-pinene, 4 percent of oxygenated components and 0.3 percent of a tricyclic sesquiterpene. Russian workers have since reported the presence in the oil of camphene, Δ_3-carene, sabinene, myrcene, dipentene, β-phellandrene, γ-terpinene and ρ-cymene [477].

Vapours given off by branch segments stored in containers made of glass, polyurethane foam and rubber, were studied by GC analysis of the internal atmosphere, in which was found a highly variable composition of heptane, α- and β-pinene and limonene [186]. These components were only tentatively identified, while the identities of undecane, camphene, myrcene, Δ_3-carene, octane and monane were speculated after comparison of RRT data with values reported in the literature. These writers found considerable quantitative differences between duplicate analyses, i.e. close agreement was felt to be within ±10 percent for major components. Stored branches were however thought to emit a degradation product that was more volatile than α-pinene. Unfortunately it was not recognized that vapours emitted from the plant tissues would become unevenly distributed in different phases on surfaces within the container, and also be selectively absorbed by some container materials.

Chararas and Berton [403] also studied vapours
released from *P. strobus* and reported a small proportion
of limonene.

(a) Syringe-headspace GC analysis of foliage terpenoids

The limited amount of sample available in the
Royal Botanical Gardens (Tasmania) was sufficient to enable
a syringe-headspace GC analysis of foliage vapour. The
sample consisted of a sprig from a seedling being cultivated
to establish *P. strobus* for the first time in these gardens.

The composition of the syringe-headspace foliage
vapour is consistent with that reported by Mirov [47], and
is given in Table 113 and illustrated in Figure 80.

Table 113. RRT data and percentage composition of
volatile terpenoids in foliage of a
seedling of *Pinus strobus* determined
by syringe-headspace GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak area of 4th successive injection)
	C20M	OV-17	
	(60° isothermal, ref. α-pinene)		(60° isothermal)
Unidentified	0.62		6.7
α-Pinene	1.00	1.00	68.0
Camphene	1.27	1.21	1.2
β-Pinene	1.64	1.55	17.2
Δ_3-Carene	2.01		t
Myrcene	2.33	1.76	5.8
Limonene	2.87	2.37	0.5
β-Phellandrene	3.01	2.52	0.6
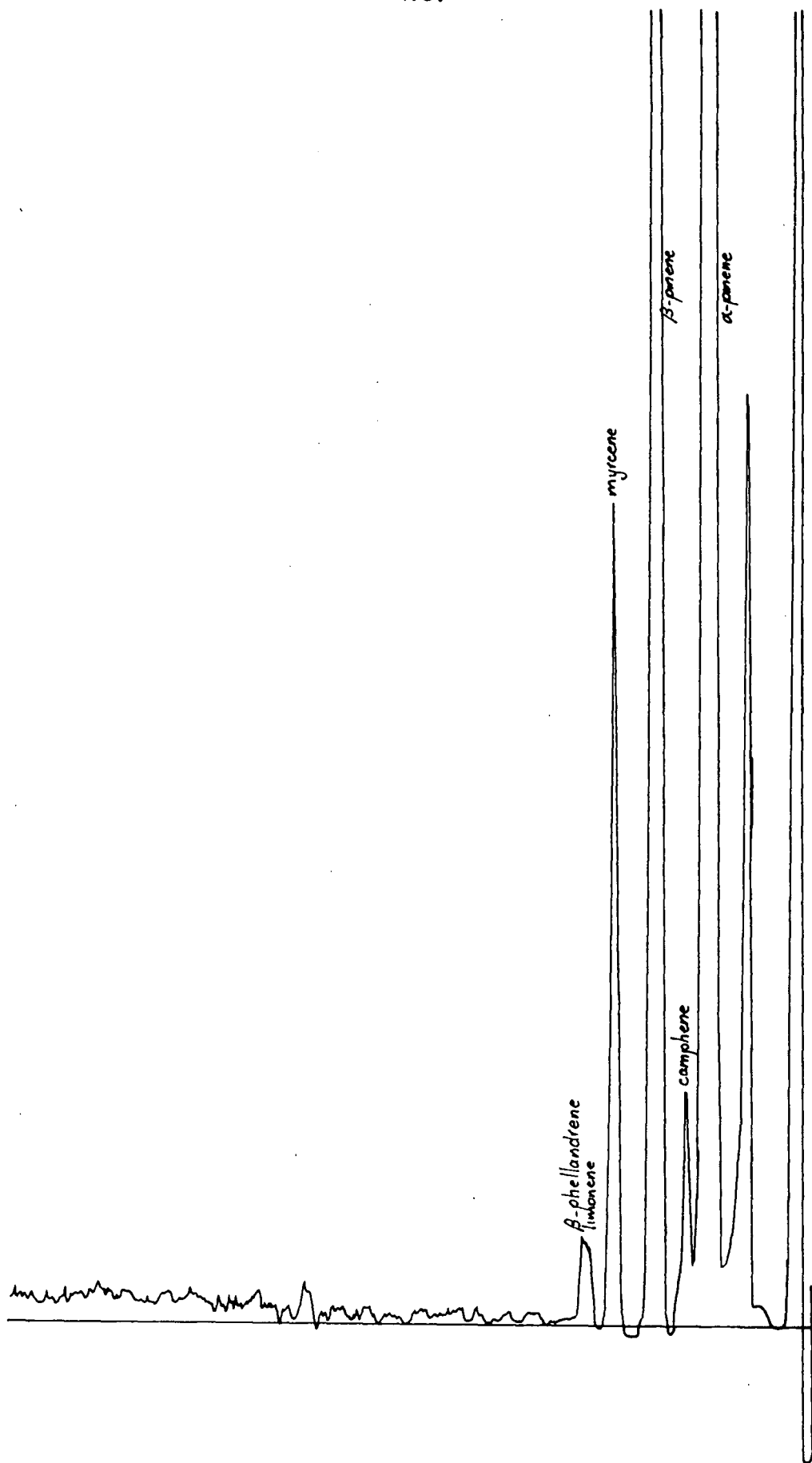
Fig. 80. Syringe-headspace gas chromatogram of vapour from foliage of a seedling of *Pinus strobus* (GC conditions as before; attenuation 2×10^2).

(b) Summary

       Components of the syringe-headspace vapour from a single sample of foliage, available from a seedling of *Pinus strobus*, were tentatively identified by GC on two dissimilar columns. Found were an unidentified component that eluted before α-pinene on Carbowax 20M (6.7%), α-pinene (68.0%), camphene (1.2%), β-pinene (17.2%), Δ_3-carene (trace), myrcene (5.8%), limonene (0.5%) and β-phellandrene (0.6%).

(xvi) *Pinus sylvestris* L.

       The Scotch Pine *(Pinus sylvestris* L.) is a complex of varieties or perhaps species, which grows throughout northern Europe and Asia. The oils from this tree are one of the most economically important and as a result, have been investigated and reported upon more than any other. Since 1950 more than 100 papers appeared on oils of *P. sylvestris*.

       The oleoresin of trees grown in widely distributed regions, i.e. from France, Kazan (Upper Volga region) and the Altai Mountains (southern Siberia), all contained a characteristic proportion of Δ_3-carene [47].

       Oil distilled from cortical oleoresin of trees in the Mongolian People's Republic [478] contained tricyclene (<0.1 percent), α-pinene (86.5 percent), camphene (0.9 percent), β-pinene (5.6 percent), myrcene (1.5 percent), Δ_3-carene (1.6 percent), α-terpinene, dipentene and limonene (2.1 percent), β-phellandrene (1.0 percent), γ-terpinene, ρ-cymene, terpinolene (0.1 percent) and a distillation residue (2.5 percent), which was shown to contain longifolene, bornyl acetate, terpinen-4-ol, γ-terpineol, isoborneol, borneol, chavicol methyl ether, α-terpineol, α-terpenyl acetate, verbenone and numerous unidentified sesquiterpenes. Other components reported by

Bardyshev and co-workers [419, 479] were isobornyl acetate, α-fenchol, camphor, trans-dihydro-α-terpineol, carvone, m-mentha-1,3(8)-diene, trans- and cis-alloocimene, fenchone, β-terpineol and "silveterpineol" (m-menth-6-en-8-ol). Cameron and Sutherland [480] also reported the presence of 1,4-cineole, 1,8-cineole and acetylacetone.

Further sesquiterpenes identified in cortical oleoresin include α- and γ-muurolene [481]; ylangene (copaene), δ- and γ-cadinene [482]; α-longipinene and longicyclene [483]; copaborneol and β-copaene [123]; iso-longifolene, sibirene, β-ylangene and ε-muurolene [484].

It should be noted that Bardyshev and Vedeneev [485] have distinguished different groups of *P. sylvestris* on the basis of monoterpene composition. Two groups have been documented with the following compositions:

	I	II
α-pinene	91.6 - 95.4 percent	49.3 - 76.7 percent
β-pinene	0.8 - 2.9	0.6 - 1.9
Δ_3-carene	0.1 - 0.5	14.2 - 41.2
dipentene	0.5 - 3.2	0.5 - 3.4

Other forms of this species have been characterized [486], in which the oil contains

   <80 percent α-pinene, 10 percent β-pinene, little or
      or no Δ_3-carene;

   40 to 50 percent each of α- and β-pinene, with
      20 to 45 percent Δ_3-carene;

   10 to 40 percent limonene;

   10 to 15 percent β-phellandrene;

and an oil with a high content of terpinolene.

Terpenoids of heartwood extracts have been investigated by several workers. Heartwood extracts were reported to have the same qualitative composition as cortical turpentines, but to contain 5 to 15 percent more α-pinene, a few percent less each of dipentene and β-phellandrene, while the characteristic ratio of α-pinene to Δ_3-carene for a particular tree was maintained in both types of tissue [487, 488]. Westfelt was responsible for isolating several sesquiterpenes in wood extractives [489-493]. Sesquiterpenoids isolated from the wood include α-longipinene, copaene, longifolene, β-ylangene, β-copaene, α-, γ- and ε-muurolene, γ- and δ-cadinene, calamenene, α-calacorene [494] and copaborneol [490]. Other components since found include furfural, 2,5-hexanedione and camphor [495].

Oils from organs of different ages have been shown to have compositions which differ considerably. Pigulevskii and Maksimova [320, 496, 497] examined cortical monoterpenes in branches and stems of various ages and concluded that high proportions of β-phellandrene was a characteristic of younger tissues, and hence was indicative of the biogenetic sequence of monoterpenes [498, 499]. Steam-distilled oils from the following organs were found to contain:

Percentage composition of monoterpenes:

Organ (and age)	α-Pinene	β-Pinene	Myrcene	Δ_3-Carene	β-Phellandrene
Limbs (3 yr. old) from 6-8 yr. old trees	22	13		20	26
Limbs (3 yr. old) from 16-18 yr. old trees	21	3		29	25
Trunks (6-8 yr. old)	42	9.4		7	24
Trunks (16-18 yr. old)	42	2.8		31.7	15.2
Trunks (40 yr. old)	55.3	-		44.6	-

The absence of β-phellandrene in old trees was thought to be due to its transformation to other materials. Poltavchenko *et al* [500] even concluded that tissues of all organs of young *P. sylvestris*, except needles, were characterized by high proportions of monocyclic terpenes such as limonene and β-phellandrene, with lesser proportions of α- and β-pinenes, camphene and Δ_3-carene.

Steam-distilled needle oil from trees grown in Bulgaria has been reported to contain α-pinene (46 percent), camphene (3 percent), β-pinene and myrcene (28 percent), limonene (8 percent), ocimene (3.5 percent) and 10 percent of borneol, bornyl acetate and sesquiterpenes [501]. An earlier paper by Rau and Simonsen [502] also included cadinene as a component of needle oil. Several other volatile components have been found among petroleum ether extractives

of needles [503], and include propanol, tert-amyl alcohol,
isoamyl alcohol, together with butyric, isocaproic, valeric
and caproic acids. Juvonen examined oils from young and old
needle-growth and noted a close inverse relationship between
α-pinene and Δ_3-carene during the growing season [504].
Further studies of seasonal variations in oil composition of
various branchlets [505] and twig whorls [506] led Juvonen to
suggest possible terpene biosynthetic pathways. Among
components identified were α-terpinene and δ-cadinene.

The biogenesis and biosynthesis of terpenes in
P. sylvestris have been studied by several workers [450, 498-500,
505-508]. Others have investigated the attractiveness and
toxicity of oil components to various insect [403, 509-514] and
fungal pests [515-518].

(a) Analysis of the oil steam-distilled from cortical oleoresin

Cortical oleoresin collected as before from a single
tree of *P. sylvestris* in the Royal Botanical Gardens
(Tasmania) yielded upon steam-distillation 27.3 percent of
a colourless oil with a sweet pinene odour.

The qualitative and quantitative composition of
components distinguishable in the whole oil, using two dissimilar
columns, is recorded in Table 114. RRT values leading to the
tentative identification of components in hydrocarbon and
oxygenated fractions, and preparative GC fractions from the
whole oil, are listed in Table 115. Gas chromatograms of
Figure 81 show the distribution of components eluted from a
Carbowax 20M column, and indicate the degree of separation
of components into hydrocarbon and oxygenated fractions.

Table 114. Components distinguishable in the whole
oil from oleoresin of *Pinus sylvestris*

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak height)
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min.)
*α-Pinene	1.05	1.05	56.0
Camphene	1.28	1.23	1.0
*β-Pinene	1.61	1.58	2.4
Sabinene	1.73		1.0
*Δ₃-Carene	2.06	2.02	30.7
*Myrcene	2.22	1.79	3.0
Unidentified	2.44		t
*Limonene	2.75	2.42	0.8
*β-Phellandrene	2.89	2.54	0.8
γ-Terpinene	3.67	3.31	0.1
ρ-Cymene	4.25	2.86	t
*Terpinolene	4.59	4.13	3.4
(130° isothermal, ref. camphor)			
*Chavicol methyl ether	1.62	1.26	0.3
Unidentified	1.92	4.00	0.5

* IR spectrum recorded
t: trace; <0.1 percent

This oil is seen from Tables 114, 115 and Figure 81 to be typical of one of the high-Δ_3-carene group of oils described by Bardyshev and co-workers [485, 486]. No report was found in the literature of geraniol having been previously identified in this oil. It should be noted that the oxygenated monoterpenes and sesquiterpenoid components reported by Bardyshev and co-workers [419, 479] were identified in turpentine distillation residue, which could be expected to have resulted in the complete catalytic conversion of some components.

Table 115. RRT values on the dissimilar liquid
phases C20M and OV-17, for components
found in hydrocarbon, oxygenated and
preparative GC fractions, isolated
from steam-distilled oil from oleoresin
of *Pinus sylvestris*

Component	Hydrocarbon fraction		Oxygenated fraction		Preparative GC fractions		
	C20M	OV-17	C20M	OV-17	No.	C20M	OV-17
(60° isothermal, ref. α-pinene)							
α-Pinene	1.01	1.02			W1	0.98	1.02
Camphene	1.27	1.20			W2	1.30	1.22
β-Pinene	1.62	1.56			W3	1.60	1.56
Sabinene					W2	1.75	1.54
Δ_3-Carene	2.06	1.99			W4	2.06	1.95
					W5	2.06	1.98
Myrcene	2.23	1.76			W2	2.24	1.75
Unidentified	2.45						
Limonene	2.74	2.39			W5	2.79	2.42
β-Phellandrene	2.96	2.57			W5	2.93	2.52
γ-Terpinene	3.68	3.27					
ρ-Cymene	4.31	2.80	4.29	2.80			
Terpinolene	4.60	4.09			W7	4.59	4.11
(130° isothermal, ref. camphor)							
Chavicol methyl ether			1.58	1.30	W8	1.58	1.25
Unidentified	1.95	4.01					
Geraniol			3.23	1.30			
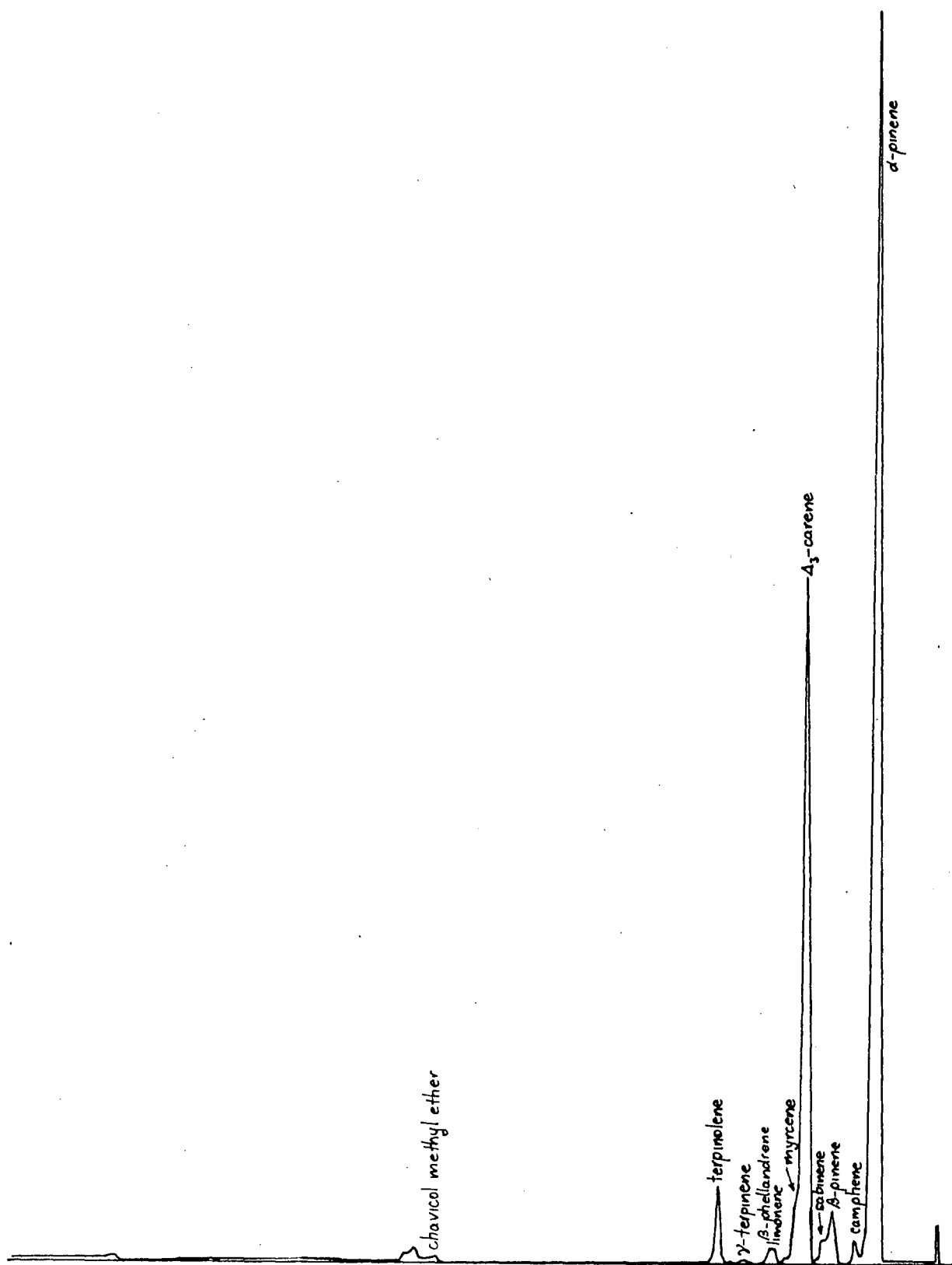
Fig. 81(a). Low sensitivity gas chromatogram of whole oil from oleoresin of *Pinus sylvestris* (GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50° to 200° at 5°/min; 0.2 μℓ sample; attenuation 8 x 10³).
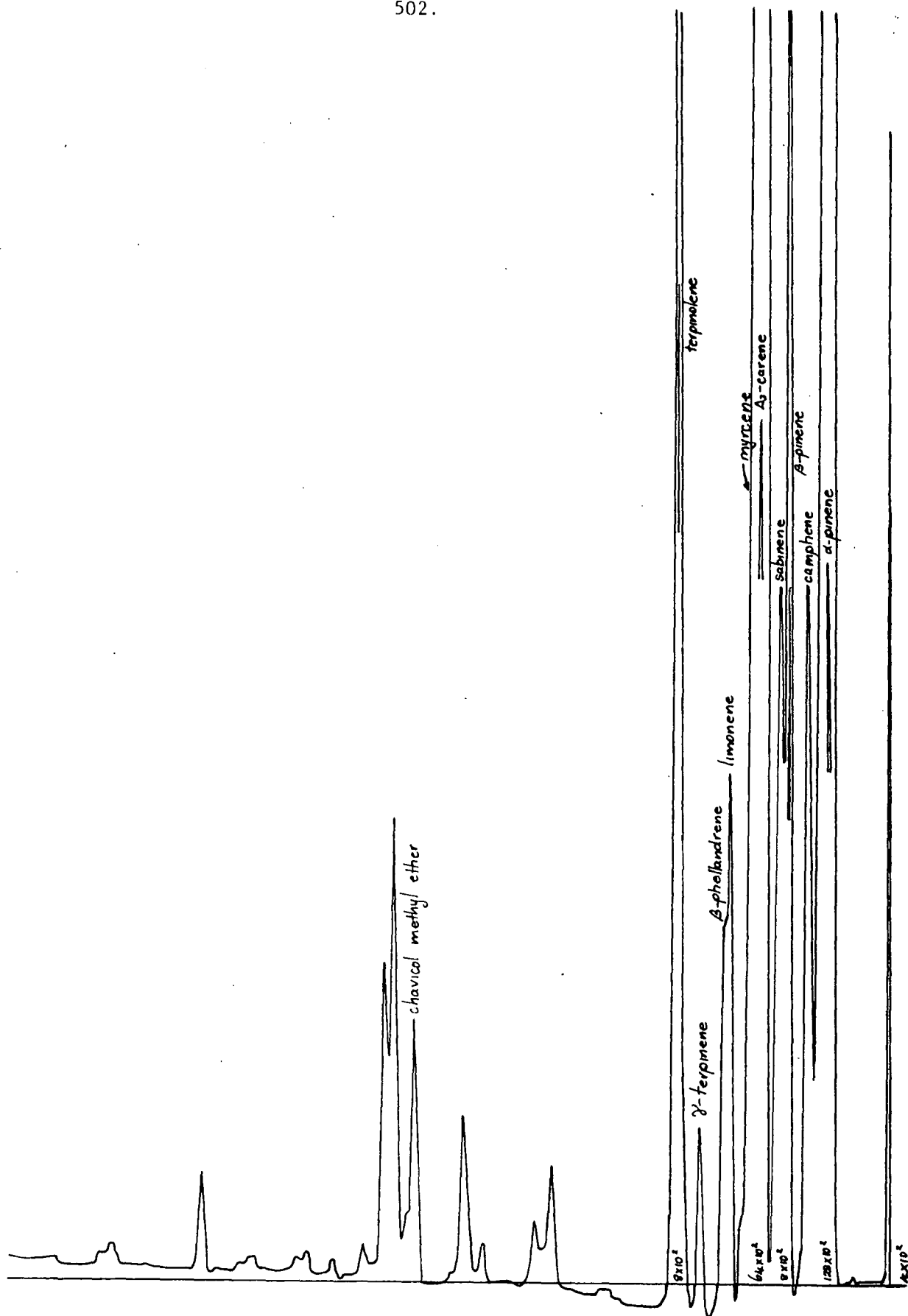
Fig. 81(b).   High sensitivity gas chromatogram of whole oil from oleoresin of *Pinus sylvestris* (attenuation 4 x 10²).
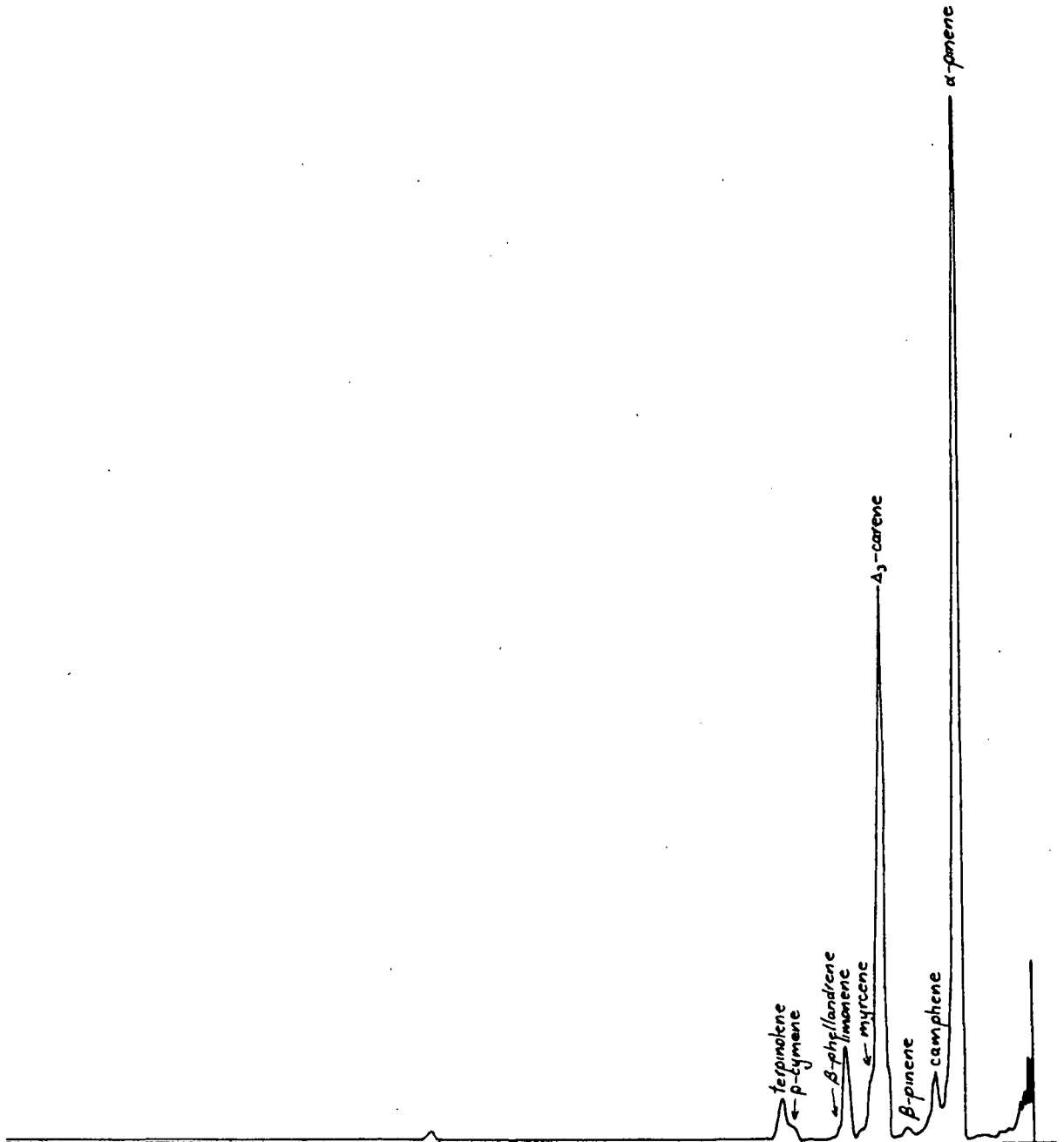
Fig. 81(c). Low sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus sylvestris* separated on Florisil.
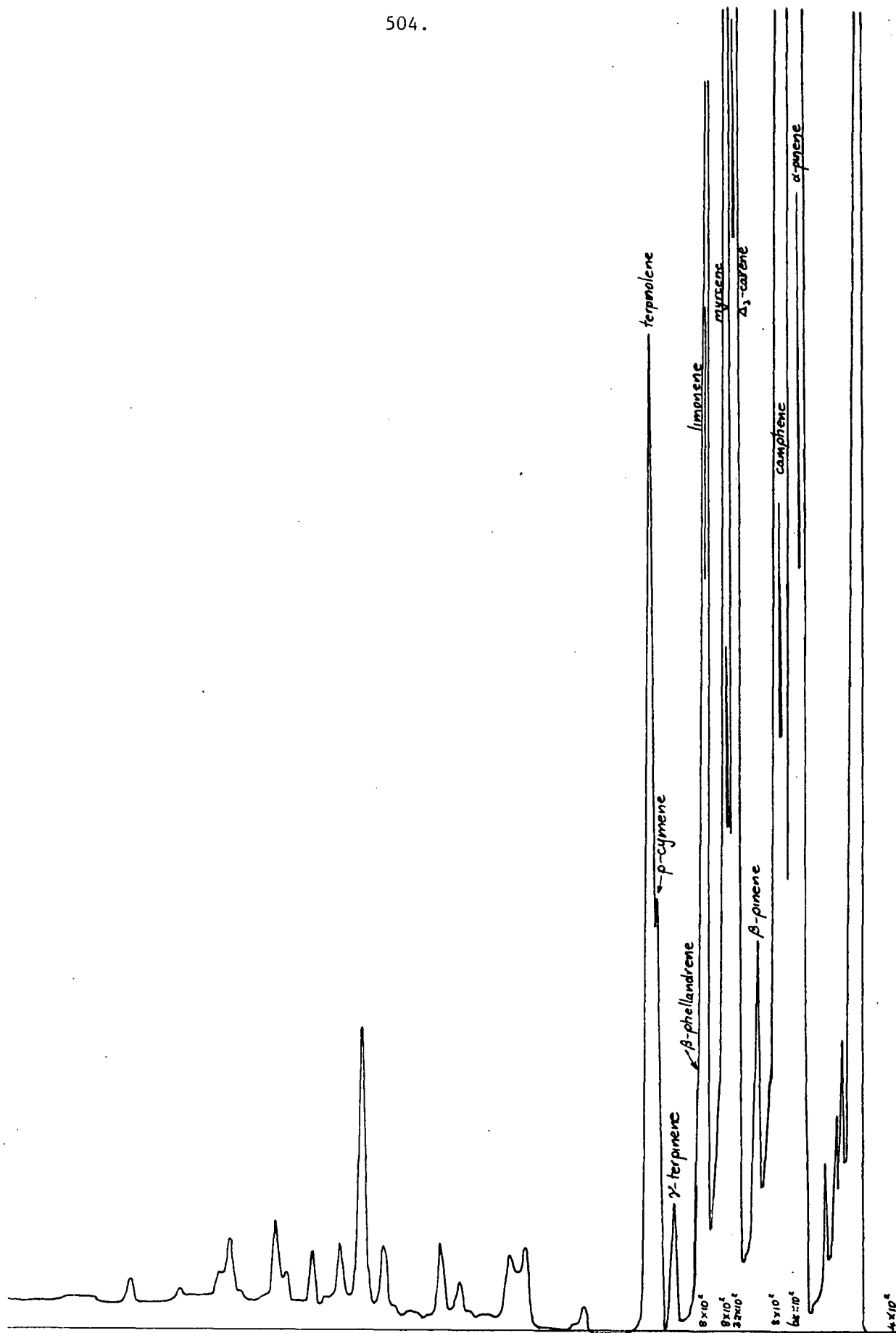
Fig. 81(d). High sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus sylvestris* separated on Florisil.
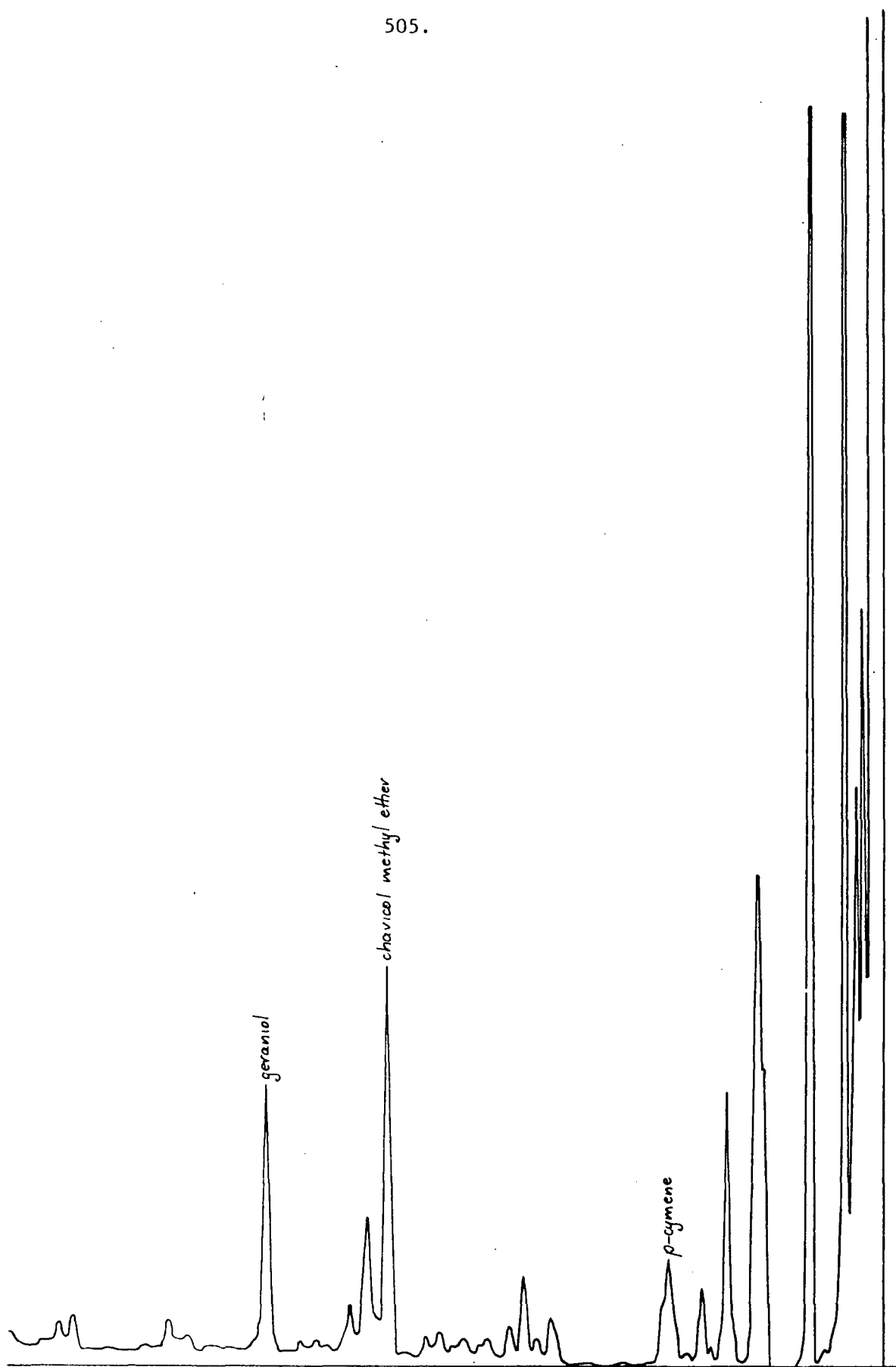
Fig. 81(e).   High sensitivity gas chromatogram of oxygenated fraction
of oil from oleoresin of *Pinus sylvestris* separated on Florisil.

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from
comminuted foliage (Table 116, Figure 82) indicated a quite
different oil vapour composition being released to the
atmosphere from foliage of this tree. This oil consisted
predominantly of Δ_3-carene with large proportions of α-pinene
and myrcene. The steam-distilled oil from a further portion
of the sample of comminuted foliage also contained a significant
proportion of terpinolene.

Whereas foliage oils of some other *Pinus* species
contained higher proportions of β-pinene than in the oil from
cortical oleoresin, this oil exhibited an increase in Δ_3-carene.
The proportion of β-pinene in each steam-distilled oil was
approximately 2 percent.

(c) Composition of successive injections of syringe-

headspace vapour from foliage

Successive injections of vapour over a 2 hr. period,
from a single sample of foliage, exhibited initial fluctuations
in the proportions of α-pinene and Δ_3-carene (Table 117).

A chromatogram of the steam-distilled oil from the
remainder of the comminuted foliage is given for comparison
in Figure 83 (also Table 117).

Table 116. RRT data and percentage composition
of volatile terpenoids in foliage of
Pinus sylvestris determined by
syringe-headspace GC analysis

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 150° at 5°/min.)
Unidentified	0.80		⎫
"		0.51	⎬ t
			⎭
α-Pinene	1.00	1.05	40.9
Camphene	1.30	1.22	1.1
β-Pinene	1.66	1.58	3.3
Sabinene	1.77		1.4
Δ₃-Carene	2.08	1.99	39.5
Myrcene	2.32	1.78	10.3
Unidentified	2.53		0.3
"		2.20	
Limonene	2.87		0.2
β-Phellandrene	3.05	2.54	0.4
γ-Terpinene	3.88		0.2
Unidentified	4.10	2.96	0.7
Terpinolene	4.87	4.08	1.7

t: trace, <0.1 percent

(d) Composition of syringe-headspace vapour from foliage

sampled repeatedly from the same tree

The composition of the vapour from two samples of

foliage (Tables 116 and 117) varied from 40.9 to 41.8 percent

α-pinene, 39.5 to 39.6 percent Δ₃-carene, 10.0 to 10.3

percent myrcene and 2.4 to 3.3 percent β-pinene.
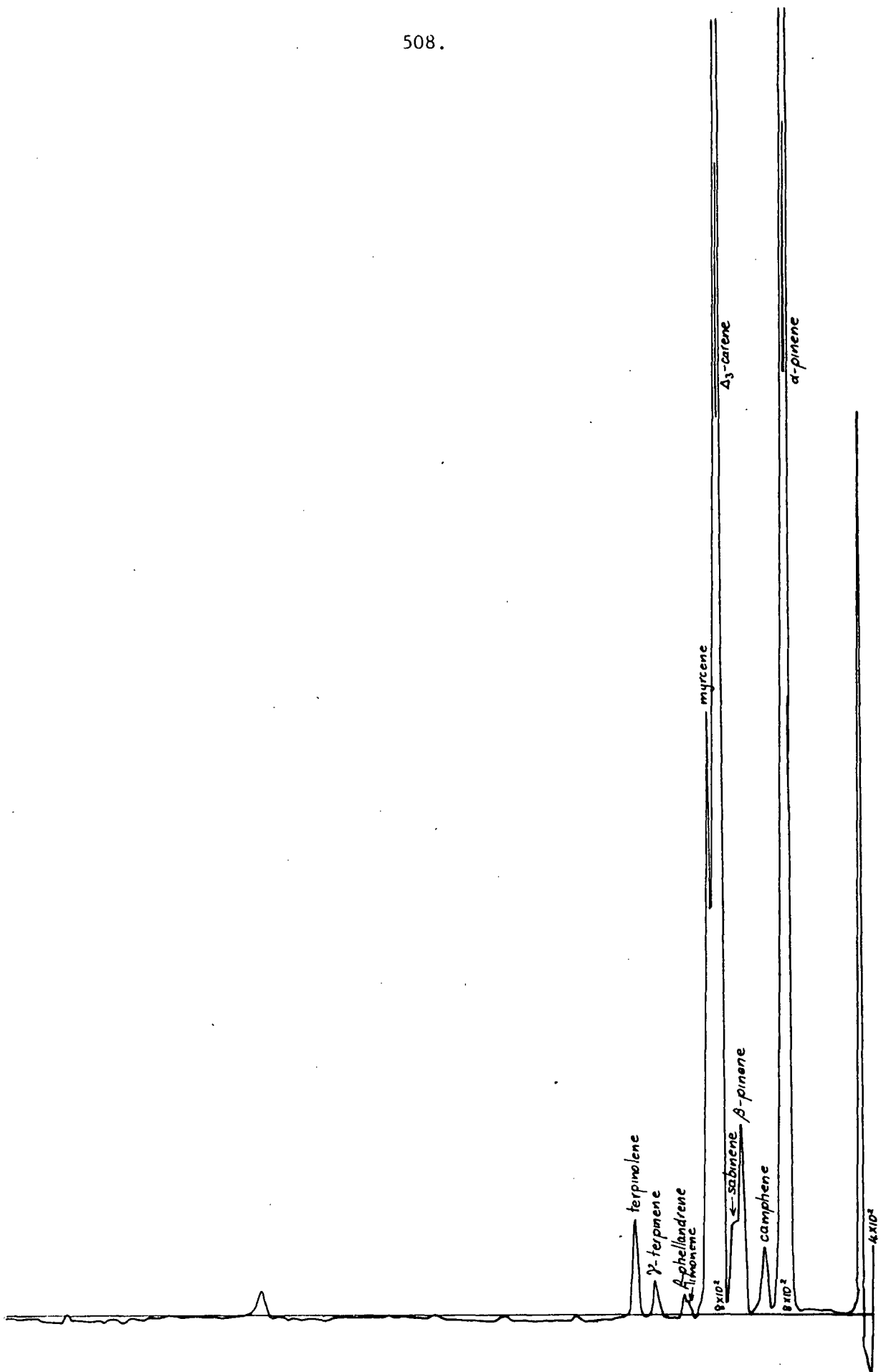
Fig. 82. Syringe-headspace gas chromatogram of vapour from foliage of *Pinus sylvestris* (GC conditions as before; attenuation 4 x 10^2).

Table 117. Composition of monoterpenes in
successive injections of syringe-
headspace vapour from foliage of
Pinus sylvestris

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Sabinene	Δ₃-Carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene	Unidentified	ρ-Cymene	Terpinolene
0	-	41.8	0.8	2.4	1.3	39.6	10.0	0.3	0.3	0.6	0.4	0.6	-	1.7
15	0.2	46.6	0.8	2.4	1.2	37.6	8.8	0.4	0.1	0.2	0.1	0.2	t	1.4
30	0.2	47.4	0.9	2.2	1.1	37.2	8.5	0.4	0.1	0.2	0.2	0.3	-	1.3
45	0.3	46.6	1.1	2.4	1.3	36.9	8.6	0.3	0.2	0.2	0.2	0.3	-	1.4
60	0.3	47.2	0.9	2.3	1.1	36.8	8.4	0.4	0.1	0.2	0.4	0.4	t	1.4
75	0.2	46.7	1.0	2.5	1.2	37.6	8.4	0.4	0.1	0.3	t	0.3	t	1.2
90	0.3	46.2	1.1	2.5	1.2	37.1	8.4	0.4	0.3	0.3	0.1	0.4	0.2	1.4
105	0.5	46.0	1.0	2.2	1.3	37.2	8.5	0.3	0.2	0.4	0.3	0.5	t	1.6
120	0.3	46.2	1.1	2.4	1.0	37.4	8.3	0.6	0.3	0.3	t	0.5	t	1.6
135	0.2	45.9	1.0	2.3	1.4	37.8	8.3	0.4	0.4	0.4	0.2	0.4	t	1.4

Steam-distilled oil from remainder of comminuted foliage:

| | 13.4 | 14.4 | 0.4 | 2.1 | 0.9 | 47.0 | 10.3 | 1.3 | 0.3 | 0.5 | 0.8 | 1.6 | 0.3 | 6.7 |

t: trace; <0.1 percent
-: not detected
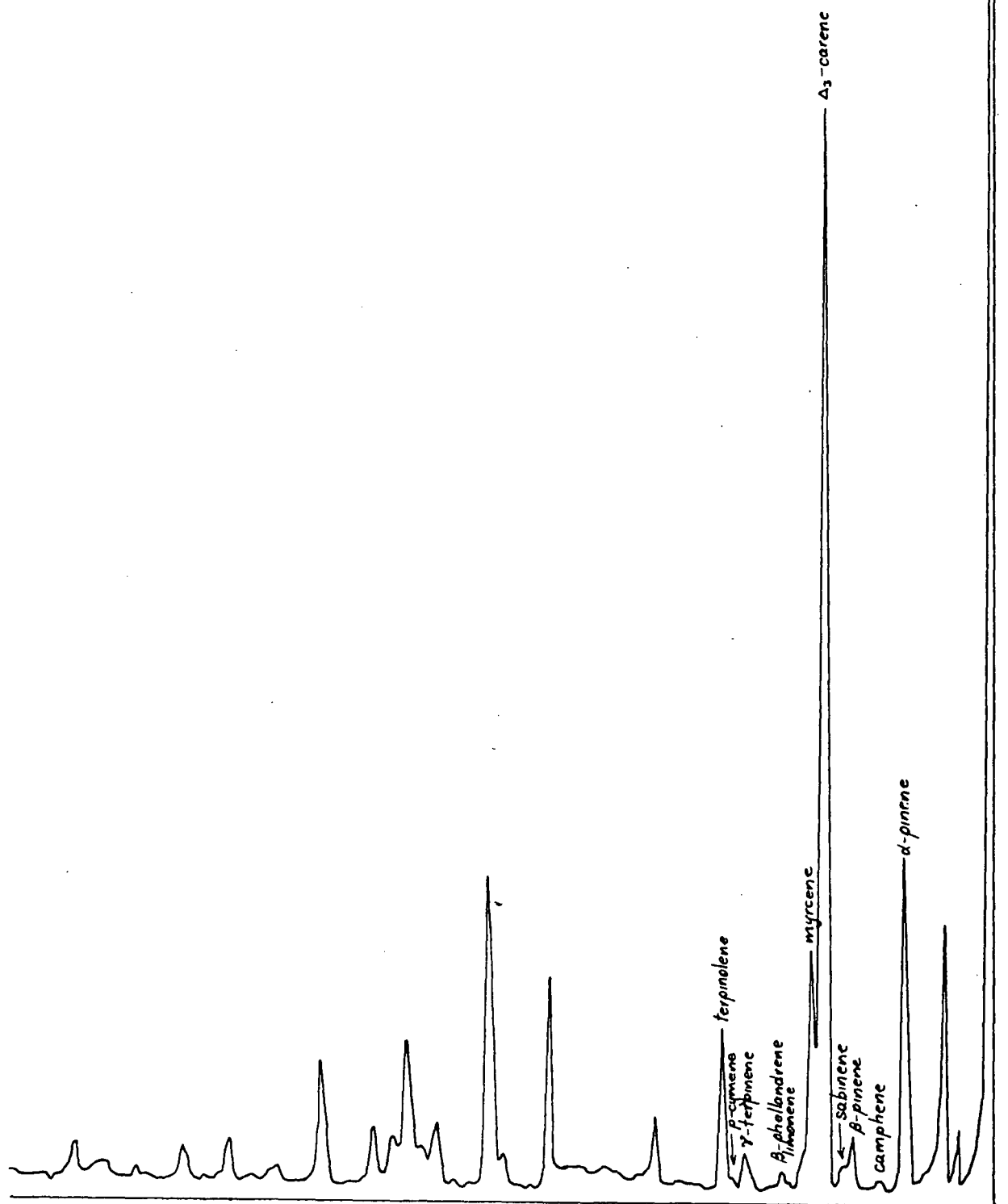
Fig. 83.   Gas chromatogram of steam-distilled oil from the remainder of comminuted foliage of *Pinus sylvestris* studied by syringe-headspace vapour GC in Fig. 82 (GC conditions as before).

(e) <u>Summary</u>

Components of the steam-distilled oil from oleoresin of *Pinus sylvestris* were analyzed by chromatographic and spectroscopic methods. The following identifications were confirmed: α-pinene (56.0%), β-pinene (2.4%), Δ_3-carene (30.7%), myrcene (3.0%), limonene (0.8%), β-phellandrene (0.8%), terpinolene (3.4%) and chavicol methyl ether (0.3%). Tentatively identified were camphene (1.0%), sabinene (1.0%), γ-terpinene (0.1%), ρ-cymene and geraniol. By comparison, monoterpenes of the steam-distilled foliage oil included a component eluted before α-pinene (13.4%), α-pinene (14.4%), camphene (0.4%), β-pinene (2.1%), sabinene (0.9%), Δ_3-carene (47.0%), myrcene (10.3%), limonene (0.3%), β-phellandrene (0.5%), γ-terpinene (0.8%), ρ-cymene (0.3%) and terpinolene (6.7%). Geraniol did not appear from the literature to have been previously found in this species.

The syringe-headspace GC technique indicated the existence of a wide range of monoterpene compositions to be found in successive injections of vapour from a single sample of foliage, although duplicate samples from the same tree emitted monoterpene vapour with closely-similar compositions. Successive injections of vapour over a 2 hr. period contained 41.8 to 47.4% α-pinene, 2.2 to 2.5% β-pinene, 36.8 to 39.6% Δ_3-carene and 8.3 to 10.0% myrcene; whereas from duplicate samples of foliage from the same tree initial vapour injections contained 40.9 and 41.8% α-pinene, 2.4 and 3.3% β-pinene, 39.5 and 39.6% Δ_3-carene, 10.0 and 10.3% myrcene.

(xvii) *Pinus taeda* L.

Oil distilled from oleoresin of the Loblolly Pine *(Pinus taeda* L.*)* has been described in the earlier literature [47] as consisting principally of α- and β-pinene with a smaller proportion of limonene. Sutherland and Wells [519] reported having found d-α-pinene (71 percent), ℓ-β-pinene (22 percent), chavicol methyl ether (1 percent), a fraction containing myrcene, limonene and ρ-cymene (1 percent), together with small amounts of an unsaturated ketone, a sesquiterpene and other unidentified components. Williams and Bannister [145] subsequently examined the oil using GC techniques and reported 87.5 percent α-pinene, 2.0 percent camphene, 3.5 percent β-pinene, 3.5 percent myrcene, a trace of Δ_3-carene, 3.5 percent limonene and a trace of β-phellandrene. The tree-to-tree variation in monoterpene composition of oleoresin has since been found to be widely-ranging [520].

Needle oil of *P. taeda* was examined by Joye *et al* [438], who reported having found 16.4 percent α-pinene, 1.8 percent camphene, 10.2 percent β-pinene, 4.1 percent limonene, 5.9 percent β-phellandrene, 2.9 percent ρ-cymene, 0.2 percent trans-dihydro-α-terpineol, 0.5 percent α-fenchol, 0.8 percent bornyl acetate, 1.2 percent β-terpineol, 2.2 percent terpinen-4-ol, 9.4 percent caryophyllene, 13.6 percent α-terpineol, 0.8 percent borneol and 1.6 percent cadinene.

Studies have been reported of the attractiveness of *P. taeda* monoterpenoids to the insect pests *Dendroctonus* [521], *Blastophagus piniperda* [509] and *Hylobius pales* [522].

The high resistance of the bark of *P. taeda* to decomposition by soil fungi was attributed by Kuhlman [523] to the chemical constituents in the bark.

(a) Analysis of the oil steam-distilled from cortical

oleoresin

Cortical oleoresin collected as before from a single tree of *P. taeda* in the Royal Botanical Gardens (Tasmania) yielded upon steam-distillation 22.1 percent of a colourless oil with a sweet pinene odour.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 118. RRT values leading to the tentative identification of components in hydrocarbon and oxygenated fractions, and preparative GC fractions from the whole oil, are listed in Table 119. Gas chromatograms of Figure 84 show the distribution of components eluted from a Carbowax 20M column, and indicate the degree of separation of components into hydrocarbon and oxygenated fractions.

This oil is seen from Tables 118, 119 and Figure 84 to consist of major proportions each of α-pinene, β-pinene, Δ_3-carene, myrcene and limonene. It does not resemble any oil previously reported from this species, and may indicate the existence of different chemical forms. No reports were found in the literature of sabinene, γ-terpinene, terpinolene, linalool and geraniol having been identified in this species.

Table 118. Components distinguishable in the whole oil from oleoresin of *Pinus taeda*

Component	Qualitative RRT data		Quantitative composition
	C20M	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 200°, 5°/min.)
*α-Pinene	1.00	1.01	8.4
Camphene	1.27	1.21	t
*β-Pinene	1.64	1.61	27.5
*Δ_3-Carene	2.08	2.01	23.6
*Myrcene	2.25	1.78	14.9
*Limonene	2.81	2.43	22.0
γ-Terpinene	3.70	3.28	0.2
ρ-Cymene	4.33	2.80	t
*Terpinolene	4.61	4.10	1.7
	(130° isothermal, ref. camphor)		
Terpinolene	0.44		
Linalool	0.93	0.57	0.4
Unidentified	1.15	2.53	t
*Chavicol methyl ether	1.57	1.26	1.2
α-Terpineol	1.72	1.08	t
Unidentified	1.85	3.78	0.1

* IR spectrum recorded
t: trace, <0.1 percent

Table 119. RRT values on the dissimilar liquid phases C20M and OV-17, for components found in hydrocarbon, oxygenated and preparative GC fractions, isolated from steam-distilled oil from oleoresin of *Pinus taeda*

Component	Hydrocarbon fraction C20M	OV-17	Oxygenated fraction C20M	OV-17	Preparative GC fractions No.	C20M	OV-17
(60° isothermal, ref. α-pinene)							
α-Pinene	0.99	1.01			W1	1.00	1.00
Camphene	1.25	1.22			W2	1.32	1.23
β-Pinene	1.57	1.56			W3	1.65	1.61
					W2	1.65	1.56
Sabinene					W2	1.76	
Δ_3-Carene	2.00	1.97			W3	2.03	1.96
					W4	2.06	1.99
Myrcene	2.19	1.76			W2	2.22	1.75
					W3	2.20	1.79
Unidentified					W4	2.37	
Limonene	2.75	2.41			W4	2.66	2.39
					W5	2.73	2.35
γ-Terpinene	3.64	3.24					
ρ-Cymene	4.29	2.79					
Terpinolene	4.57	4.09			W6	4.70	4.11
(130° isothermal, ref. camphor)							
Linalool			0.91	0.64			
Unidentified	1.20	2.56					
Chavicol methyl ether			1.57	1.31	W7	1.57	1.26
α-Terpineol			1.76	1.09			
Geraniol			3.24	1.31			
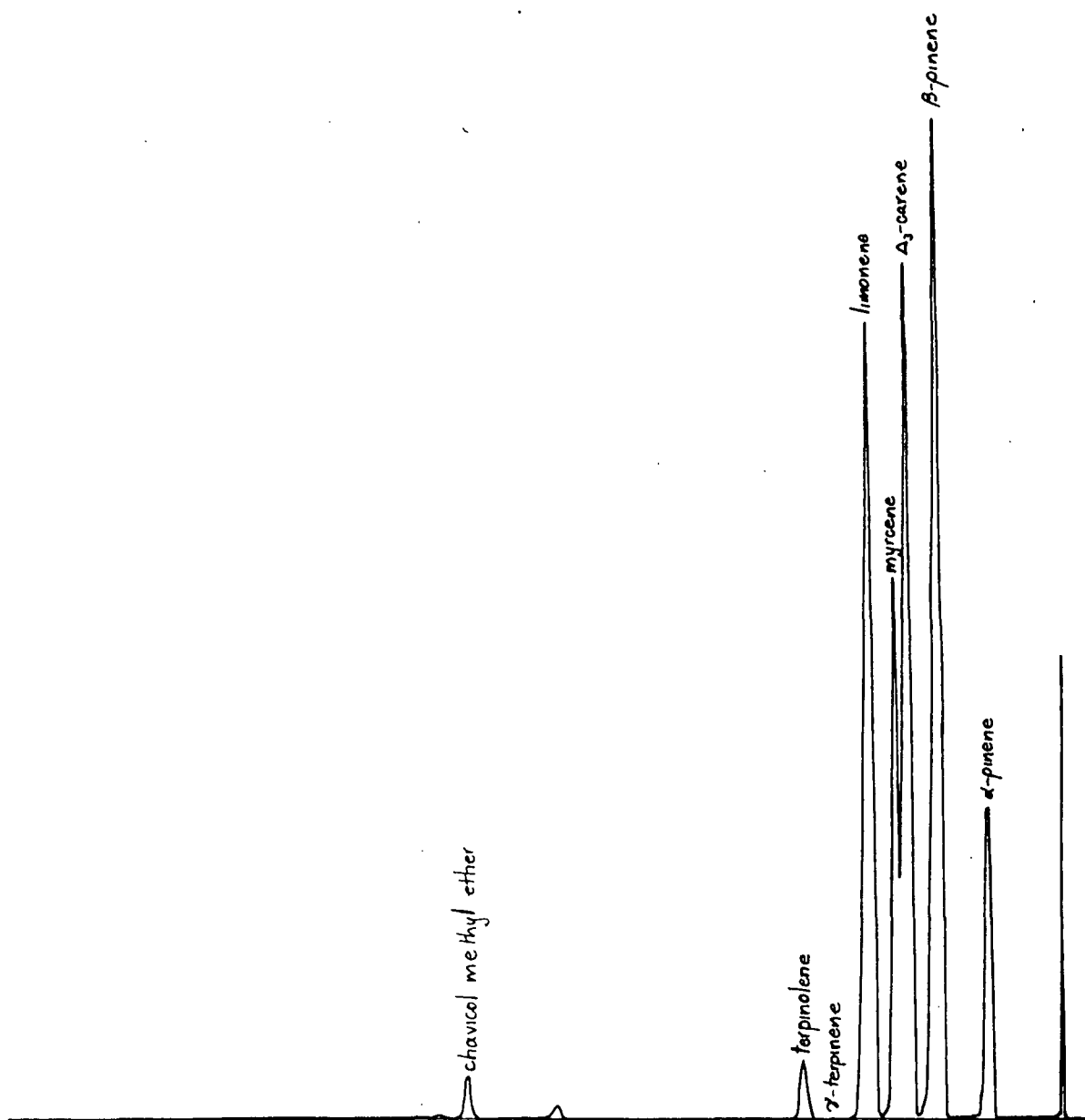
Fig. 84(a). Low sensitivity gas chromatogram of whole oil from oleoresin of *Pinus taeda* (GC on 5% Carbowax 20M/Gas Chrom Q; temperature program 50° to 200° at 5°/min; 0.2 µℓ sample; attenuation 8 x 10³).
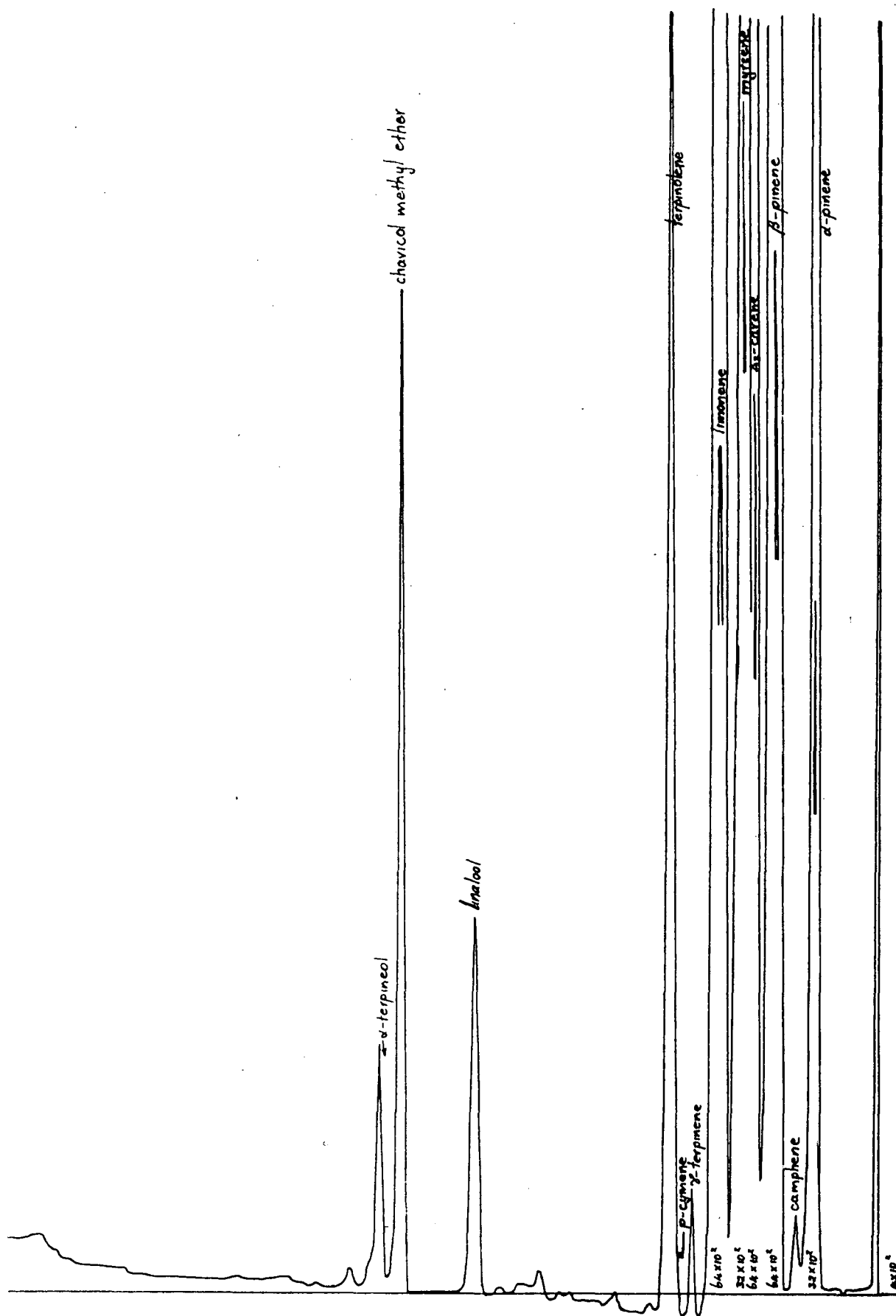
Fig. 84(b).   High sensitivity gas chromatogram of whole oil from oleoresin
of *Pinus taeda* (attenuation 4 x 10^2).
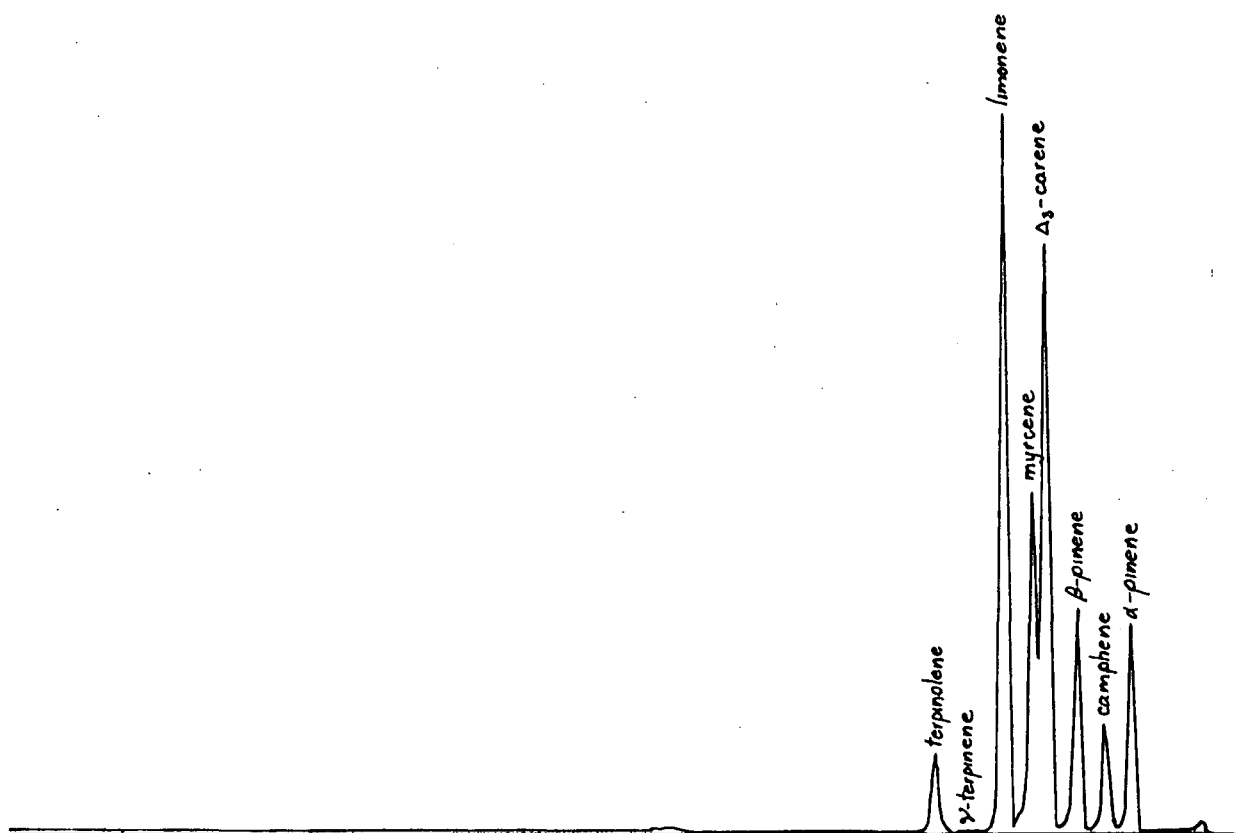
Fig. 84(c). Low sensitivity gas chromatogram of hydrocarbon fraction of oil from oleoresin of *Pinus taeda* separated on Florisil.
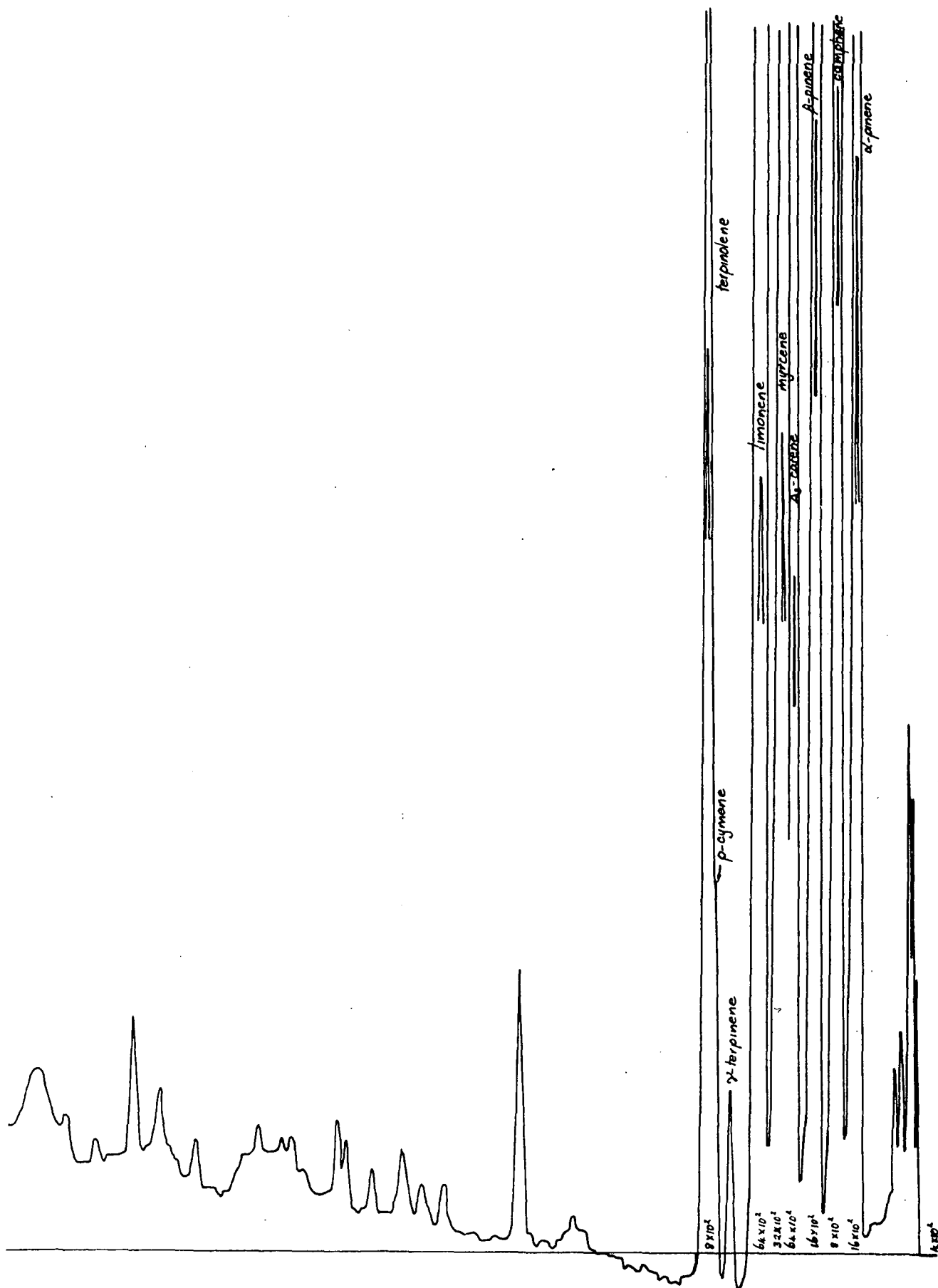
Fig. 84(d). High sensitivity gas chromatogram of hydrocarbon fractions of oil from oleoresin of *Pinus taeda* separated on Florisil.
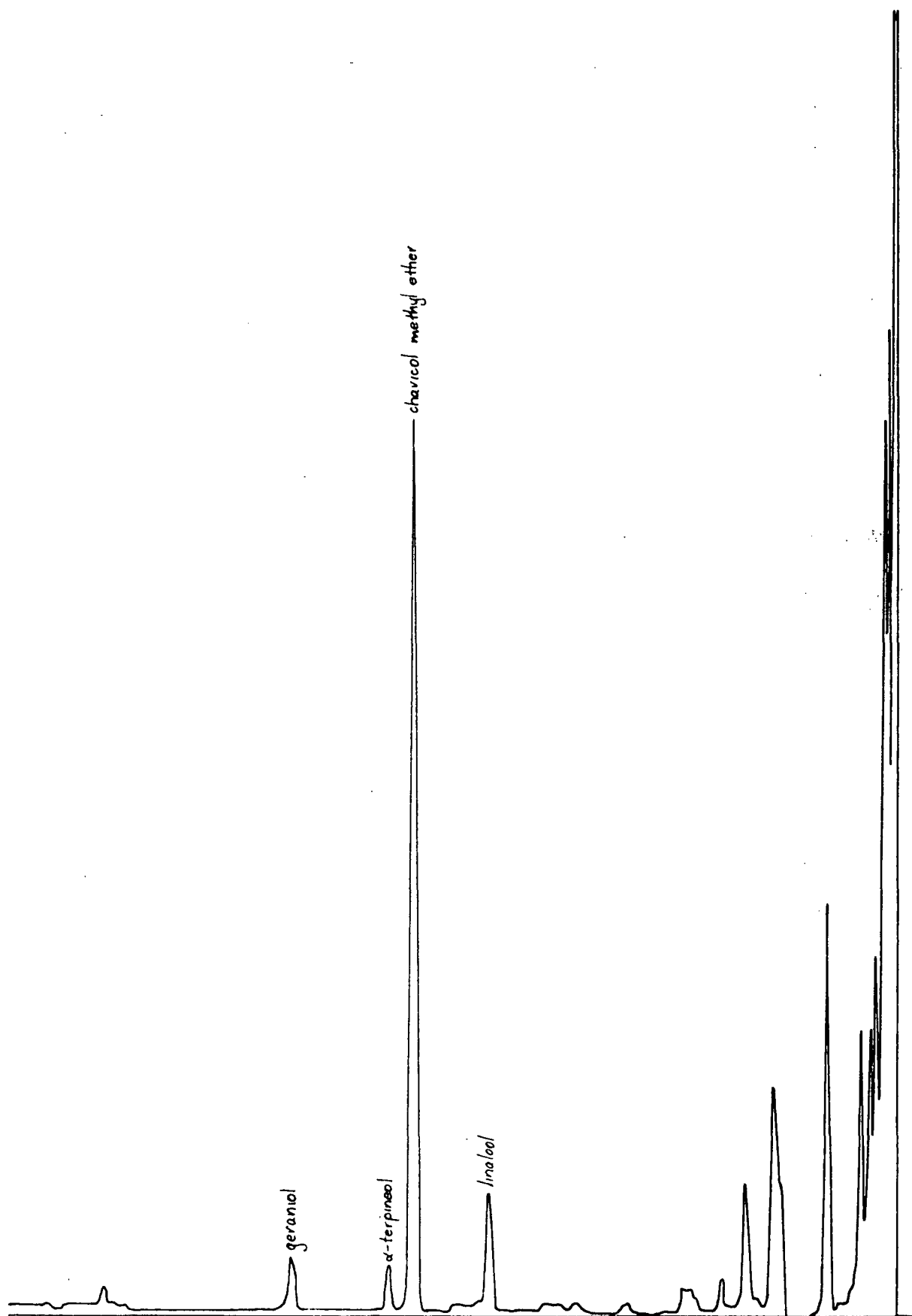
Fig. 84(e). Low sensitivity gas chromatogram of oxygenated fraction of oil from oleoresin of *Pinus taeda* separated on Florisil.
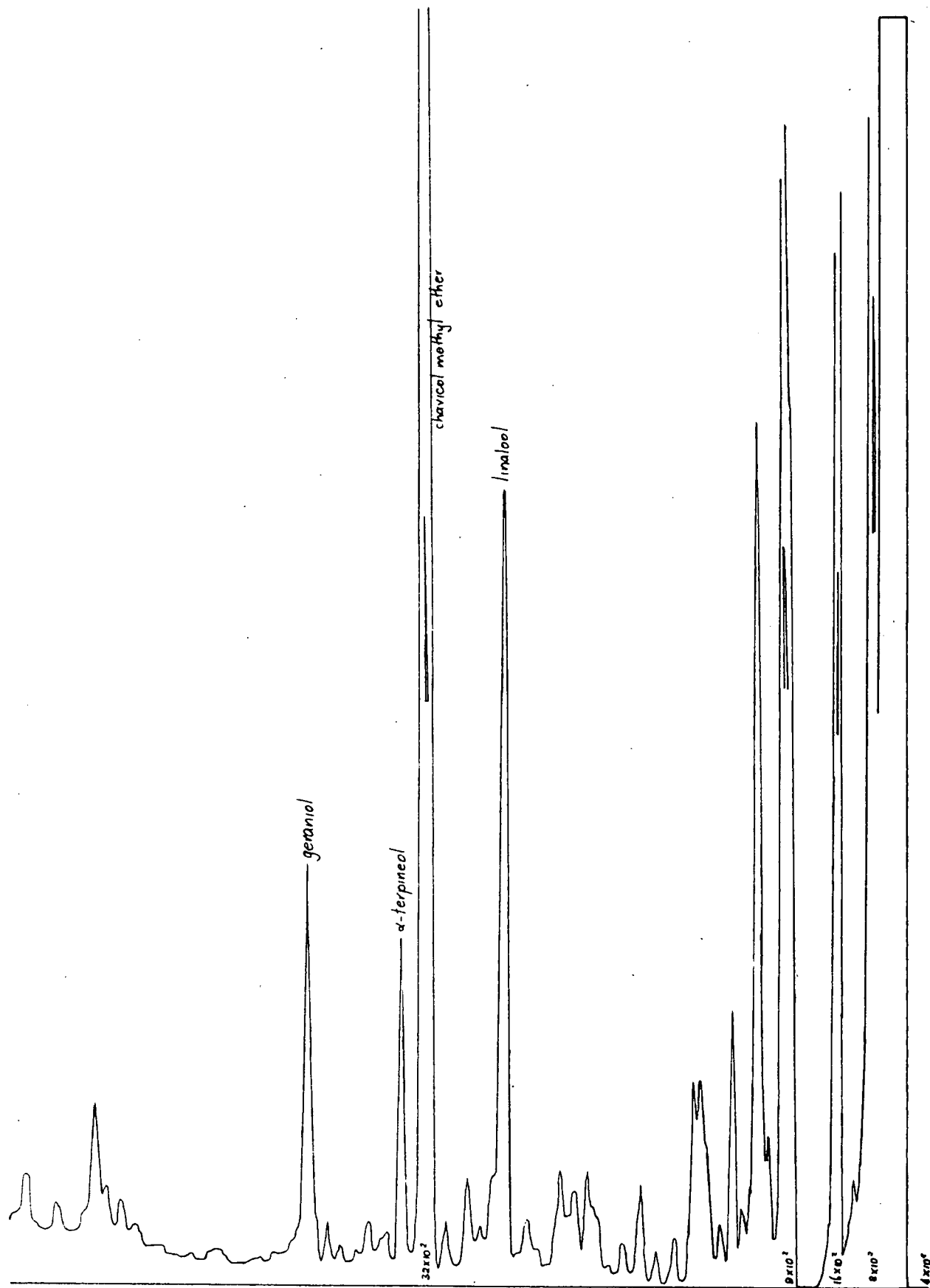
Fig. 84(f).   High sensitivity gas chromatogram of oxygenated fraction
of oil from oleoresin of *Pinus taeda* separated on Florisil.

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from
comminuted foliage (Table 120, Figure 85) indicated a
fundamentally different oil vapour composition being released
to the atmosphere from foliage of this tree. By comparison
with oil from oleoresin, this foliage oil contained an
increased proportion of β-pinene with greatly reduced
proportions of myrcene and limonene.

(c) Composition of successive injections of syringe-
     headspace vapour from foliage

Successive injections of vapour over a 3 hr. period,
from a single sample of foliage, exhibited some initial
fluctuations in the proportions of Δ_3-carene and α- and β-pinene.

Table 120. RRT data and percentage composition
            of volatile terpenoids in foliage
            of *Pinus taeda* determined by
            syringe-headspace GC analysis

Component	Qualitative RRT data		Quantitative composition (percent, based on peak area of 4th successive injection)
	C20M	OV-17	
(60° isothermal, ref. α-pinene)			(60° isothermal)
Unidentified	0.78		⎫
"		0.46	⎬ 0.3
α-Pinene	0.97	1.00	20.0
Camphene	1.29	1.22	0.3
β-Pinene	1.63	1.56	54.8
Δ_3-Carene	2.07	1.97	16.5
Myrcene	2.29	1.75	2.7
Unidentified	2.45		0.3
Limonene	2.86	2.41	3.0
β-Phellandrene	2.99	2.53	1.5
Terpinolene	4.93		0.7
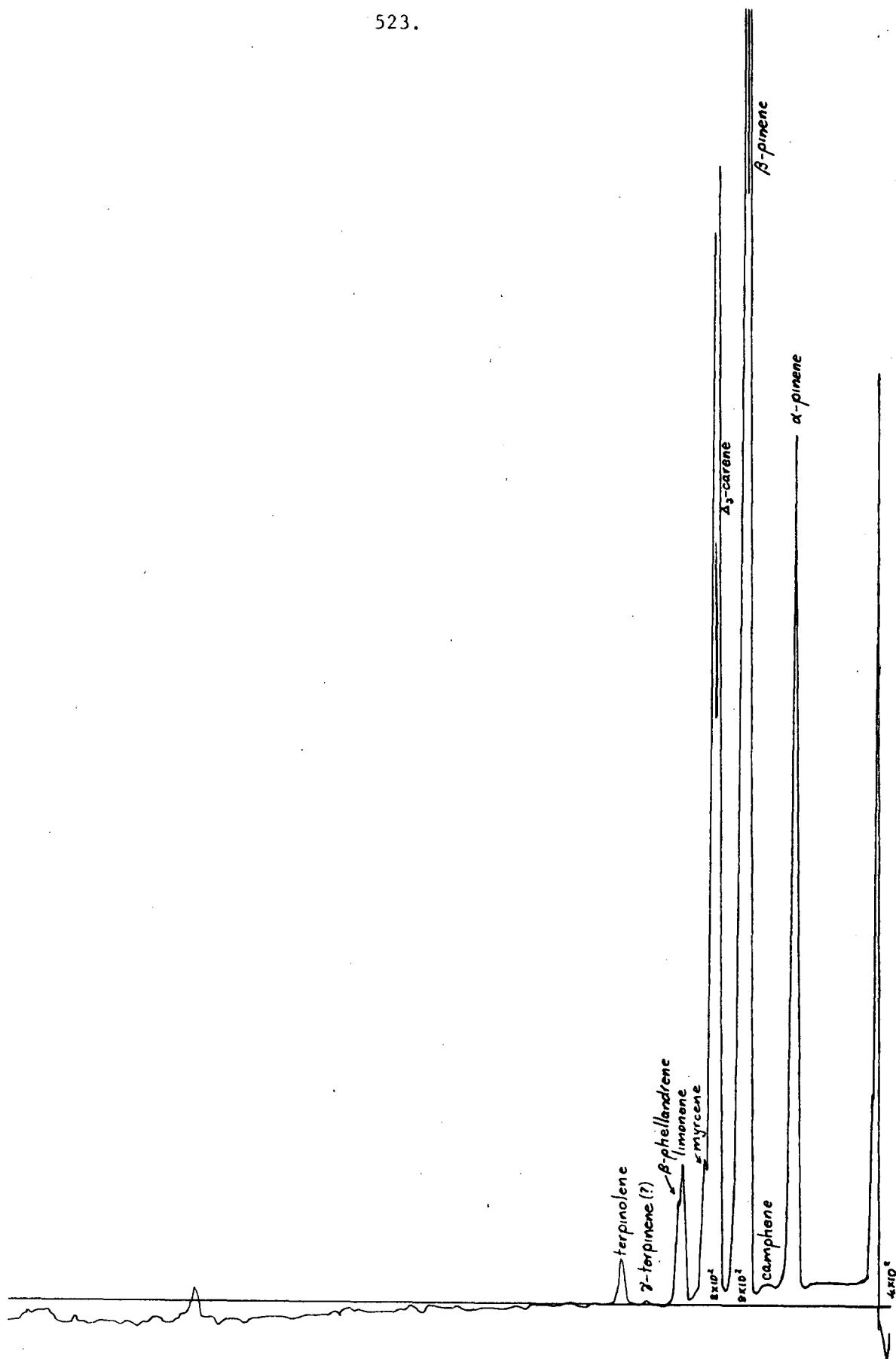
Fig. 85(a). Syringe-headspace gas chromatogram of vapour from foliage of *Pinus taeda* (GC conditions as before; attenuation 4 x 10^2).
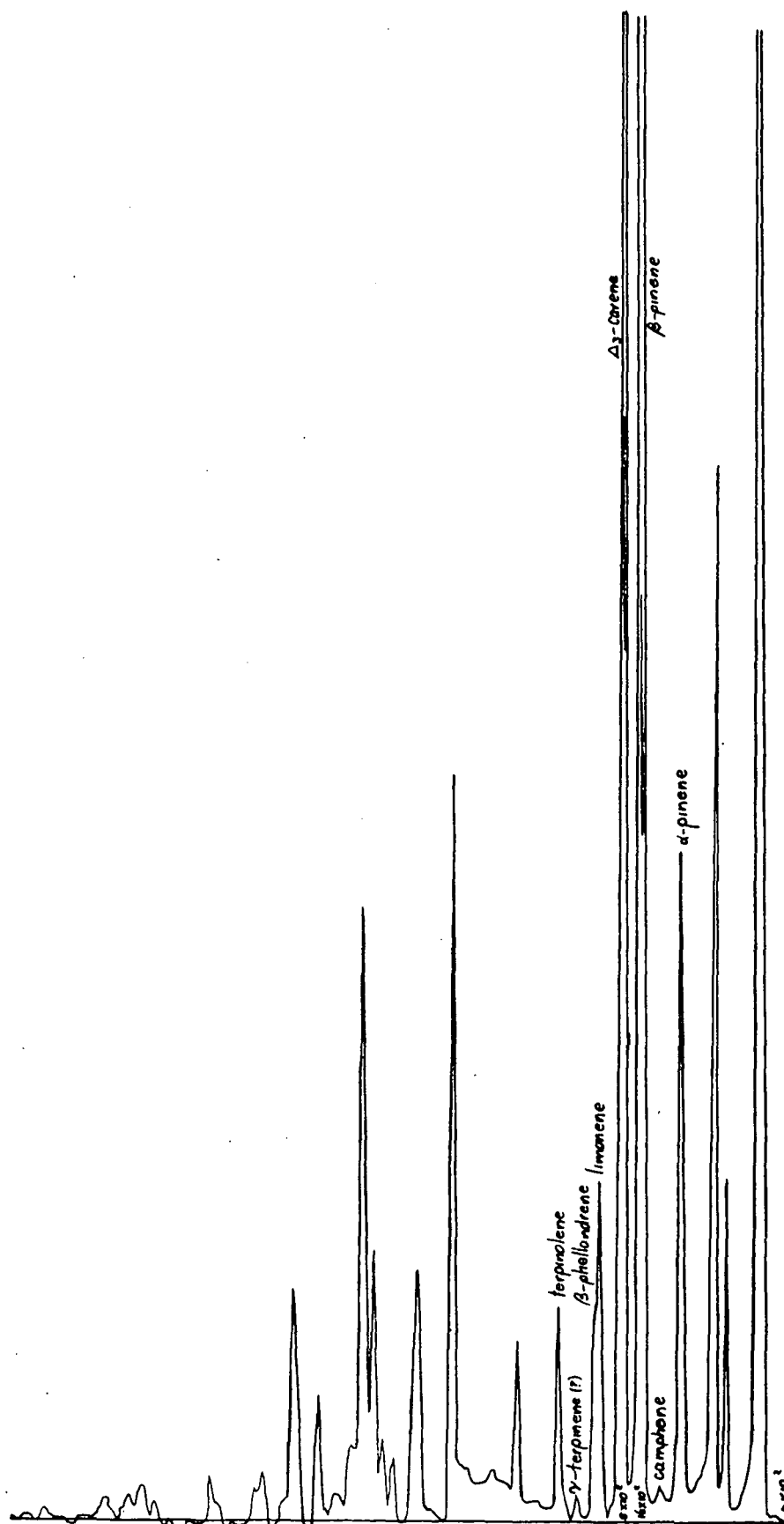
Fig. 85(b). Gas chromatogram of steam-distilled oil from the remainder of comminuted foliage of *Pinus taeda* studied by syringe-headspace vapour GC in Fig. 85(a) (GC conditions as before).

Throughout most of the period fluctuations were only minor.
Whereas changes in proportions of α- and β-pinene appeared
not to be related, there was some indication of a reciprocal
relationship between β-pinene and Δ_3-carene (Table 121).

A chromatogram of the steam-distilled oil from
the remainder of the comminuted foliage is given for
comparison in Figure 85 (see Table 121).

Table 121.   Compositions of monoterpenes in
successive injections of syringe-
headspace vapour from foliage of
Pinus taeda

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Δ_3-Carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene	ρ-Cymene	Terpinolene
0	–	16.1	0.1	48.4	28.4	2.7	0.2	2.6	0.6	0.1	–	0.9
40	0.2	18.5	0.2	49.1	25.5	2.5	0.1	2.3	0.9	0.1	–	0.7
55	0.3	18.7	0.2	49.5	25.1	2.3	0.1	2.2	1.0	t	0.1	0.6
70	0.3	19.2	0.2	50.0	24.4	2.3	t	2.1	1.0	t	0.1	0.5
90	0.1	18.9	0.2	49.9	24.4	2.4	0.1	2.4	1.0	–	–	0.6
105	0.2	18.9	0.3	49.8	24.3	2.5	t	2.3	1.1	–	–	0.6
120	0.2	19.0	0.2	50.2	23.8	2.4	0.1	2.2	1.2	0.1	0.1	0.6
135	0.3	19.1	0.2	49.9	23.7	2.4	0.3	2.2	1.2	0.1	0.1	0.7
150	0.3	19.3	0.2	49.9	23.9	2.4	0.1	2.3	1.0	0.1	0.1	0.6
165	0.3	19.2	0.2	50.3	23.6	2.3	0.1	2.2	1.0	0.1	0.1	0.6
175	0.3	18.8	0.2	50.2	23.6	2.4	0.2	2.4	1.1	0.1	0.1	0.6
190	0.3	18.7	0.2	51.0	23.6	2.4	t	2.2	1.0	t	t	0.6

Steam-distilled oil from remainder of comminuted foliage

| | | 15.3 | 7.3 | 0.1 | 41.3 | 24.7 | 2.6 | 0.1 | 3.8 | 1.9 | 0.3 | 0.2 | 2.3 |

(d) Composition of syringe-headspace vapour from foliage

  sampled repeatedly from the same tree

The compositions of the vapour from duplicate samples of foliage varied from 18.5 to 20.0 percent α-pinene, 49.1 to 54.8 percent β-pinene, 16.5 to 25.5 percent Δ_3-carene, 2.5 to 2.7 percent myrcene and 2.3 to 3.0 percent limonene.

(e) Summary

Components of the steam-distilled oil from oleoresin of *Pinus taeda* were analyzed by chromatographic and spectroscopic methods. The following identifications were confirmed: α-pinene (8.4%), β-pinene (27.5%), Δ_3-carene (23.6%), myrcene (14.9%), limonene (22.0%), terpinolene (1.7%) and chavicol methyl ether (1.2%). Tentatively identified were camphene, sabinene, γ-terpinene (0.2%), ρ-cymene, linalool, α-terpineol and geraniol. By comparison, monoterpenes of the steam-distilled foliage oil included: a component eluted before α-pinene (15.3%), α-pinene (7.3%), camphene (0.1%), β-pinene (41.3%), Δ_3-carene (24.7%), myrcene (2.6%), limonene (3.8%), β-phellandrene (1.9%), γ-terpinene (0.3%), ρ-cymene (0.2%) and terpinolene (2.3%). No reports were found in the literature of sabinene, γ-terpinene, terpinolene, linalool and geraniol having been identified in oils of this species.

The syringe-headspace GC technique indicated the existence of a range of monoterpene compositions to be found in successive injections of vapour from a single sample of foliage, and also from different samples of foliage from the same tree. Successive injections of vapour over a 3 hr.

period contained 16.1 to 19.3% α-pinene, 48.4 to 51.0% β-pinene

and 23.6 to 28.4% Δ_3-carene; whereas from duplicate samples of

foliage the vapour contained 18.5 to 20.0% α-pinene, 49.1 to 54.8%

β-pinene and 16.5 to 25.5% Δ_3-carene.

(xviii) *Pseudotsuga menziesii* (Mirb.) Franco.

Oils from the Douglas Fir or Oregon Pine *(Pseudotsuga*

menziesii) have recently been intensively investigated,

possibly in more detail than any other conifer to this time.

These intensive studies were undertaken to identify components

in oil, both from cortical oleoresin and foliage, and to

correlate variability of oil composition with morphologically

and geographically distinct forms and varieties.

Terpenoid components in the oleoresin or wood were

reported earlier by Guenther [60], Zavarin and Suajberk [53],

Hancock and Swan [525], and Erdtman *et al* [524]. Zavarin and

Suajberk [526] extensively documented the differences and

intergradations in cortical monoterpene compositions of the

North American coastal var *menziesii* and the inland var *glauca*

(Beissn.) Franco. Zavarin *et al* [527] have subsequently

identified many trace sesquiterpenes and oxygenated components.

Markedly different monoterpene compositions in

cortical oleoresins, particularly in the proportions of

sabinene and α-pinene, have been shown to clearly distinguish

the 'pure' coastal var *menziesii,* the inland var *glauca*

and the northern inland var *caesia* Aschers. and Graebn. [526].

A further southern inland (California) variety was also

distinguishable. Table 122 illustrates the ranges of

Table 122. Percentage compositions of monoterpenes in oleoresins of three chemically-distinguishable varieties of *Pseudotsuga menziesii* growing in western North America [526]. (Each documented composition represents an individual population.)

α-Thujene	α-Pinene	Camphene	β-Pinene	Δ₃-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene

Δ_3-Carene

[coastal populations, var *menziesii*]

α-Thujene	α-Pinene	Camphene	β-Pinene	Δ_3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene
0.9	23.0	0.4	7.0	14.5	33.0	2.8	2.2	1.0	15.3
1.5	16.4	0.2	6.6	20.1	30.9	3.1	1.9	1.5	16.9
1.7	16.8	0.2	10.8	17.0	31.7	2.8	1.6	1.1	16.4
1.1	17.0	0.1	8.1	21.8	31.3	2.6	1.5	0.7	16.0
1.1	27.8	0.3	12.7	16.4	23.7	2.7	2.4	1.2	11.7
1.9	21.9	0.2	6.0	20.4	25.9	2.7	2.0	1.2	15.0
0.7	30.6	0.3	11.5	20.4	18.1	2.8	3.4	1.2	11.0
1.1	24.3	0.2	10.2	17.1	28.3	2.6	3.0	1.0	12.4
0.9	25.9	0.2	12.5	17.5	23.6	2.9	2.9	1.6	12.1

[southern inland populations, var *glauca* Schneider]

α-Thujene	α-Pinene	Camphene	β-Pinene	Δ_3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene
—	46.4	1.6	3.8	1.5	t	9.2	35.0	0.8	1.7
—	55.1	0.9	1.3	8.7	t	7.6	25.6	0.1	0.7
—	40.3	1.0	1.4	4.4	0.4	10.7	40.9	0.1	0.8
—	49.8	1.4	8.6	4.9	—	6.4	22.7	3.0	3.2
—	42.7	4.0	5.8	12.5	t	6.8	25.5	1.9	0.8
—	40.9	1.8	4.8	6.2	—	9.4	35.1	1.6	1.4
—	52.9	2.6	4.4	4.1	—	7.5	27.2	1.0	0.4
—	44.3	3.5	5.1	5.5	—	7.8	31.4	1.7	0.6
—	44.0	2.4	3.3	8.7	—	8.5	31.0	0.8	1.3

[northern inland populations, var *glauca* (Beissn.) Franco]

α-Thujene	α-Pinene	Camphene	β-Pinene	Δ_3-Carene	Sabinene	Myrcene	Limonene	β-Phellandrene	Terpinolene
—	68.8	1.2	4.4	9.0	—	3.0	6.9	0.8	5.8
—	68.6	1.7	4.2	5.8	—	4.1	12.2	0.6	2.9
—	58.4	1.1	4.8	6.6	0.6	5.4	17.8	1.2	4.2
—	59.5	1.1	10.4	6.1	0.5	4.1	12.7	3.4	3.3
—	75.9	0.6	8.4	0.9	—	2.3	8.6	2.4	0.9
—	71.4	1.3	8.6	3.3	0.4	3.0	7.8	2.6	1.5
—	68.8	2.0	12.1	1.6	0.2	2.3	7.2	4.1	2.0

monoterpene compositions found in several populations of the first three varieties. Components identified in the oleoresin of trees growing near Fort Bragg on the California coast were shown [527] to consist of α-pinene (36.5 percent), camphene (0.8 percent), β-pinene (26.8 percent), Δ_3-carene (11.3 percent), sabinene (7.9 percent), myrcene (3.0 percent), limonene (4.4 percent), β-phellandrene (3.0 percent), terpinolene (6.3 percent), together with traces of ρ-cymene, ρ-cymen-8-ene (ρ-α-dimethylstyrene), citronellol, linalool, geraniol, nerol, citronellyl acetate, geranyl acetate, neryl acetate, terpinen-4-ol, terpinen-4-ol acetate, methylthymol, isopulegol, borneol, bornyl acetate, camphor, methyl salicylate, anethole, β-farnesene, α-muurolene, γ-muurolene, β_1-, γ- and ε-cadinene, calamenene, sibirene [selina-4(14),5-diene], selina-3,7(11)-diene, α- and δ-guaiene, sativene, cyclosativene, α- and β-copaene, α-cubebene, α- and β-humulene, β-caryophyllene, α- and β-himachalene, longifolene, α-longipinene and longicyclene. Other volatile components reported from the oleoresin or wood include α-terpineol [528]; furfural and citral [60]; Δ_4-carene, α-phellandrene, α-terpinene and 3,8-menthadiene [525]; and the new sesquiterpenoids dihydropseudotsugonal, dihydropseud-otsugonol (reported along with todomatuic acid) [529].

Von Rudloff examined the compositions of needle oils and showed the existence of several chemical variants or races in each markedly different variety of *P. menziesii* [530, 531]. The characteristics of the var *menziesii* (higher β-pinene, sabinene, α- and γ-terpinene, terpinolene and terpinen-4-ol) and var *glauca* (higher santene, tricyclene,

α-pinene, camphene, limonene and bornyl acetate), together with examples of the compositions of oils of intermediate forms are shown in Table 123.

Components of the oil of mature needles of *P. menziesii* reported by Maarse, Kepner, Sakai and co-workers [22, 56] included α-pinene, camphene, β-pinene, Δ_3-carene, myrcene, limonene, 2-hexenal, ethyl caproate, γ-terpinene, ρ-cymene, terpinolene, ethyl caprylate, citronellal, linalool, fenchyl alcohol, bornyl acetate, terpinen-4-ol, β-caryophyllene, citronellyl acetate, α-terpineol, citronellol, geranyl acetate, farnesyl acetate and farnesol. Oil from immature needles was found however to be almost devoid of acyclic oxygenated monoterpenes and cis-ocimene, although these components appear during maturation. Cyclic oxygenated monoterpenes are immediately present in new growth. Other components reported were β-phellandrene, 1,8-cineole, cis-ocimene and sabinene. Von Rudloff subsequently identified [530] santene, tricyclene, α-terpinene, trans-ocimene and α-phellandrene.

Although numerous components have been reported in oils from this species, most workers added a caution regarding the ever-present possibility of some components being artifacts that might arise during isolation and analysis.

The attractiveness of essential oil components, which render this species liable to attack by *Deudroctonus pseudotsugae*, has been investigated by several workers [532, 533].

Table 123. Characteristic percentages of
principal terpenes in the leaf
oils of *Pseudotsuga menziesii*
var *menziesii* (coastal form) and
var *glauca* (Rocky mountain or
inland form) together with two
intermediate forms [531]

Component	Coastal	Coastal Intermediate	Inland Intermediate	Rocky Mountain
Santene	–	0.1-1	1-4	3-5
Tricyclene	–	0.1-1	1-3	2.5-4
α-Pinene	7-15	8-15	12-18	15-20
Camphene	0-0.2	0.3-8	15-25	20-30
β-Pinene	20-35	15-30	5-20	5-10
Sabinene	2-15	2-12	0.5-5	0.1-0.5
α-Terpinene	2-5	1-3	0.1-1.5	0-0.3
Limonene	0.5-1.5	1-3	3-10	5-10
γ-Terpinene	3-8	2-8	0.1-4	0.1-1
Terpinolene	5-20	5-15	1-5	0.5-3
Terpinen-4-ol	5-15	5-15	1-5	0.5-3
α-Terpineol	1-3	1-3	0.5-2	0.2-1
Citronellol	1-5	1-3	0.5-2	0.1-1
Bornyl acetate	0-0.3	0.5-5	15-25	20-30
Citronellyl acetate	2-4	2-6	1-3	0.1-2
Geranyl acetate	1-3	2-5	0.5-2	0.1-1

(a) Analysis of the oil steam-distilled from foliage

Foliage from a single tree of *P. menziesii* (tree V) in the Royal Botanical Gardens (Tasmania) yielded upon steam-distillation 0.26 percent (wet weight) of a pale yellow oil with a sweet fruity odour.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 124.

Since this oil had been shown by previous workers to contain more complex monoterpene and oxygenated fractions than often encountered with other conifers, it was considered advantageous to separate the oil into several fractions using the column chromatographic procedure of Smedman *et al* [135]. A 9.32 g sample of the oil was eluted from a 150 g (2 cm ID) Florisil column with 300 ml of petroleum ether, 150 ml of petroleum ether/benzene (1:iv/v), 100 ml of benzene/diethyl ether (1:1 v/v) and 300 ml of diethyl ether. An 87 percent yield of the sample was recovered in 23 50 ml fractions. Each fraction was analyzed by GC, the results of which enabled similar fractions to be bulked together, evaporated and treated as 8 major fractions (A to H). Individual components were isolated from fractions A to H by preparative GC and identified by IR and analytical GC.

RRT values leading to the tentative identification of components in fractions A to H (and preparative GC sub-fractions) are listed in Table 125. Gas chromatograms of Figure 86 show the order of elution of the complex mixture

Table 124. Components distinguishable in the whole
oil from foliage of *Pseudotsuga
menziesii* (tree V)

Component	Qualitative RRT data		Quantitative composition (percent, based on peak height)
	C20M	OV-17	
(60° isothermal, ref. α-pinene)			(TP 50° to 200°, 5°/min.)
*α-Pinene	1.03	1.00	17.7
Camphene	1.28	1.21	1.2
*β-Pinene	1.66	1.59	34.0
Sabinene	1.77		10.0
Δ$_3$-Carene	2.05	1.98	2.3
Myrcene	2.22	1.78	2.5
Unidentified	2.47	2.19	2.5
*Limonene	2.76	2.38	2.8
β-Phellandrene	2.86	2.48	2.2
Unidentified	3.23		
*γ-Terpinene	3.63	3.24	4.0
*ρ-Cymene	4.24	2.78	0.7
*Terpinolene	4.56	4.09	8.7
(130° isothermal, ref. camphor)			
Unidentified	1.15		0.2
*Terpinen-4-ol	1.24	1.04	2.8
*Citronellyl acetate	1.53	1.99	4.4
α-Terpineol	1.79	1.04	1.1
*Geranyl acetate	2.23	2.62	1.8
*Citronellol		1.13	1.2

* IR spectrum recorded

Table 125. RRT values on the dissimilar liquid phases C20M and OV-17, for components found in fractions A to H (and preparative GC sub-fractions) isolated from steam-distilled oil from the foliage of *Pseudotsuga menziesii* (tree V)

Component	Frac. A C20M	Frac. A OV-17	Frac. B No.	Frac. B C20M	Frac. B OV-17	Frac. C C20M	Frac. C OV-17	Frac. D C20M	Frac. D OV-17	Frac. E C20M	Frac. E OV-17	Frac. F No.	Frac. F C20M	Frac. F OV-17	Frac. G C20M	Frac. G OV-17	Frac. H C20M	Frac. H OV-17
(60° isothermal, ref. α-pinene)																		
α-Pinene	1.01	1.01		1.00	1.00	1.01	1.00											
Unidentified						1.12	1.00											
Camphene	1.26	1.20		1.27	1.21	1.27	1.20											
			B1	1.25	1.21													
β-Pinene	1.62	1.57		1.64	1.59	1.60	1.57											
			B1	1.63	1.58													
			B2	1.56	1.57													
			B3	1.58	1.58													
			B4	1.63	1.58													
Sabinene			B2	1.82		1.81												
Unidentified			B1	1.95														
Δ3-Carene	2.02	1.95		2.04	1.98	2.03	2.03											
			B2	1.97	1.97													
Unidentified			B1	2.12	1.92													
Myrcene	2.23			2.24	1.78	2.22	1.72											
Unidentified	2.48			2.47	2.17	2.42	2.22											
			B2	2.38														
Limonene	2.72	2.40		2.76	2.37	2.75	2.43											
			B2	2.83	2.49													
			B3	2.72	2.42													
			B4	2.78	2.37													

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Table 125 continued

Component	Frac. A C20M	OV-17	No.	Frac. B C20M	OV-17	Frac. C C20M	OV-17	Frac. D C20M	OV-17	Frac. E C20M	OV-17	No.	Frac. F C20M	OV-17	Frac. G C20M	OV-17	Frac. H C20M	OV-17
β-Phellandrene	2.92	2.61		2.96	2.53													
			B3	2.90	2.55													
Unidentified	3.23					3.18												
Unidentified			B2	3.45														
α-Terpinene	3.66	3.28		3.63	3.22	3.59	3.23											
			B3	3.64	3.26													
Unidentified			B1	4.03														
ρ-Cymene		2.85		4.25	2.78	4.25	2.83											
			B2	4.27	2.83													
			B3	4.30	2.84													
			B4	4.26	2.79													
Terpinolene	4.60	4.11		4.57	4.11	4.56	4.10											
			B4	4.64	4.11													
Unidentified	5.31																	

(130° isothermal, ref. camphor)

Component	Frac. A C20M	OV-17	No.	Frac. B C20M	OV-17	Frac. C C20M	OV-17	Frac. D C20M	OV-17	Frac. E C20M	OV-17	No.	Frac. F C20M	OV-17	Frac. G C20M	OV-17	Frac. H C20M	OV-17
Unidentified	0.40	0.56																
Unidentified	0.80	1.83		0.78		0.77												
Linalool													1.00	0.64	1.01	0.69		
Unidentified	1.03	2.27																
Unidentified	1.12	2.42																

535.

Table 125 continued

Component	Frac. A C20M	OV-17	No.	Frac. B C20M	OV-17	Frac. C C20M	OV-17	Frac. D C20M	OV-17	Frac. E C20M	OV-17	No.	Frac. F C20M	OV-17	Frac. G C20M	OV-17	Frac. H C20M	OV-17
Terpinen-4-ol										1.22	0.97		1.24	1.02				
												F1	1.25	0.99				
												F2	1.24	1.03				
Citronellyl acetate										1.61	2.00		1.53	1.95				
												F2	1.54	2.00				
												F3	1.51	1.98				
Unidentified						1.62		1.66	1.42									
α-Terpinyl acetate												F2	1.76	2.00				
α-Terpineol										1.82			1.76	1.10				
												F1	1.77	1.07				
												F2	1.76	1.12				
Unidentified								2.14	3.17									
Geranyl acetate										2.32	2.59		2.26	2.60			2.33	2.67
												F2	2.23	2.67				
												F3	2.22	2.64				
Citronellol															2.33	1.13	2.33	1.18
Unidentified															2.61	0.78		
Geraniol				3.19		3.25							3.24				3.23	1.41
												F1	3.28	1.20				

(180° isothermal, ref. thymol)

Unidentified	1.39	20.2
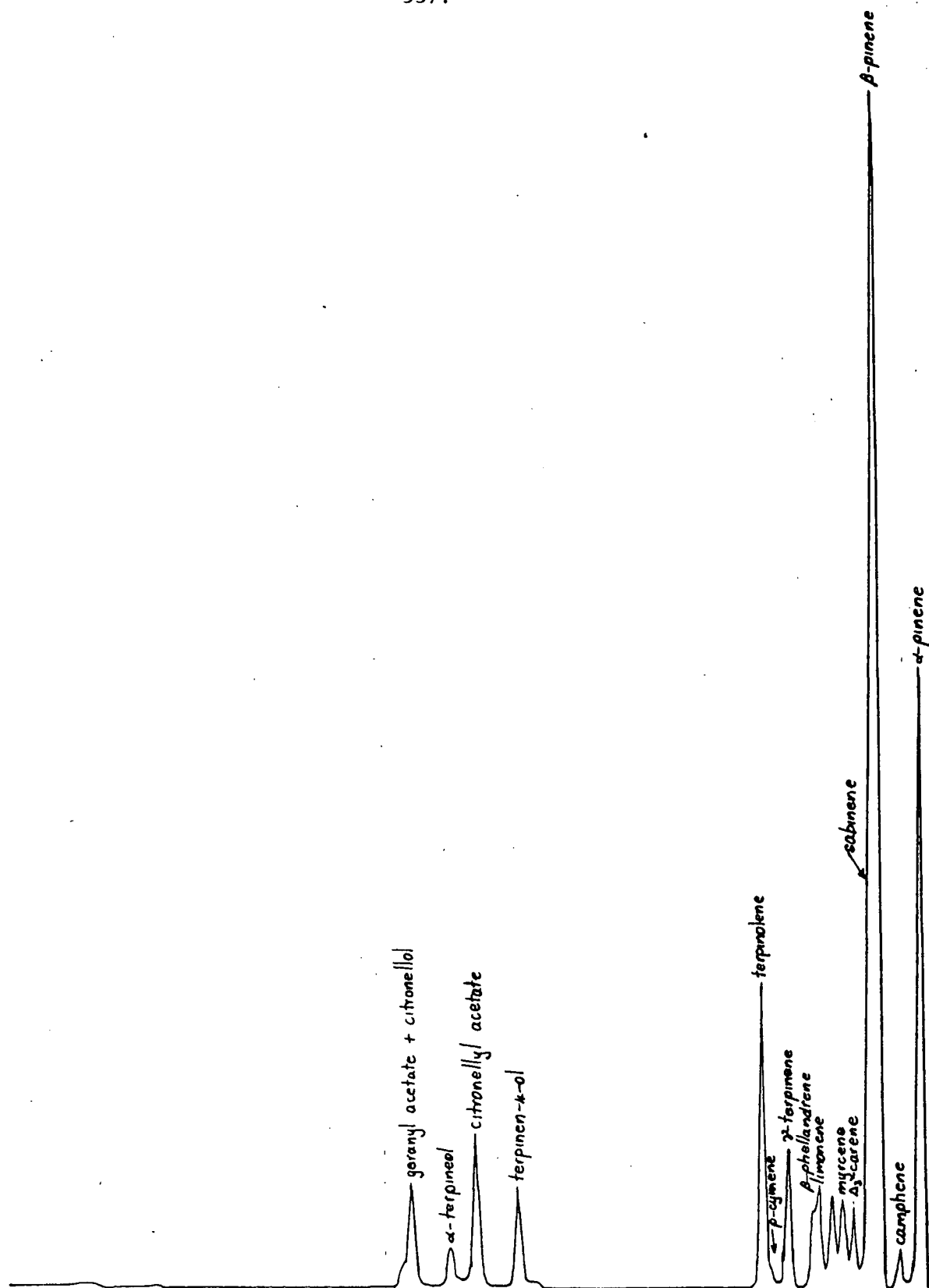
Fig. 86(a).   Low sensitivity gas chromatogram of whole oil of foliage of *Pseudotsuga menziesii* (GC on 5% Carbowax 20M/Gas Chrom Q;   temperature program 50° to 200° at 5°/min;   0.2 µℓ sample;   attenuation 8 x 10³).
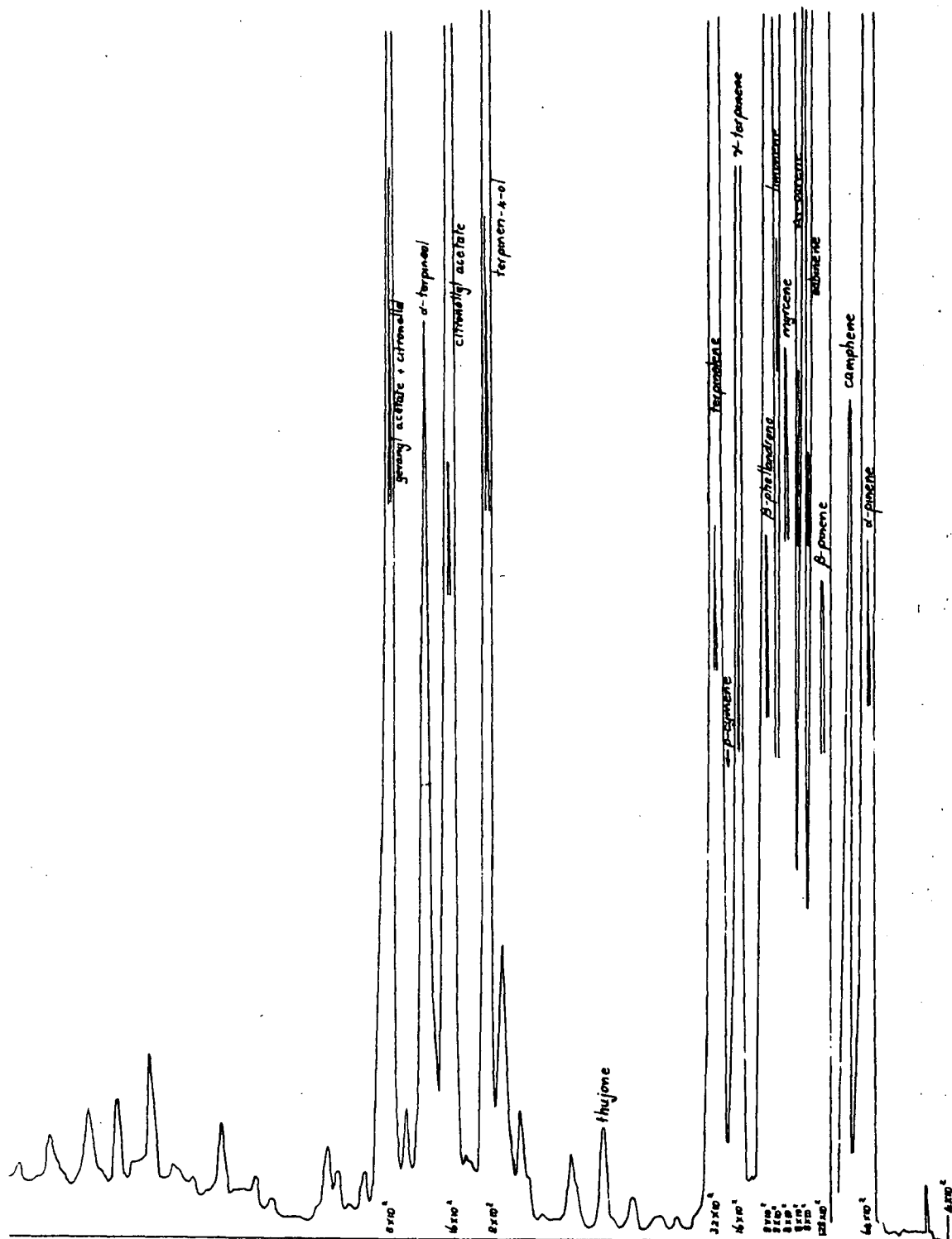
Fig. 86(b).   High sensitivity gas chromatogram of whole oil of foliage of *Pseudotsuga menziesii* (attenuation 4×10^2).
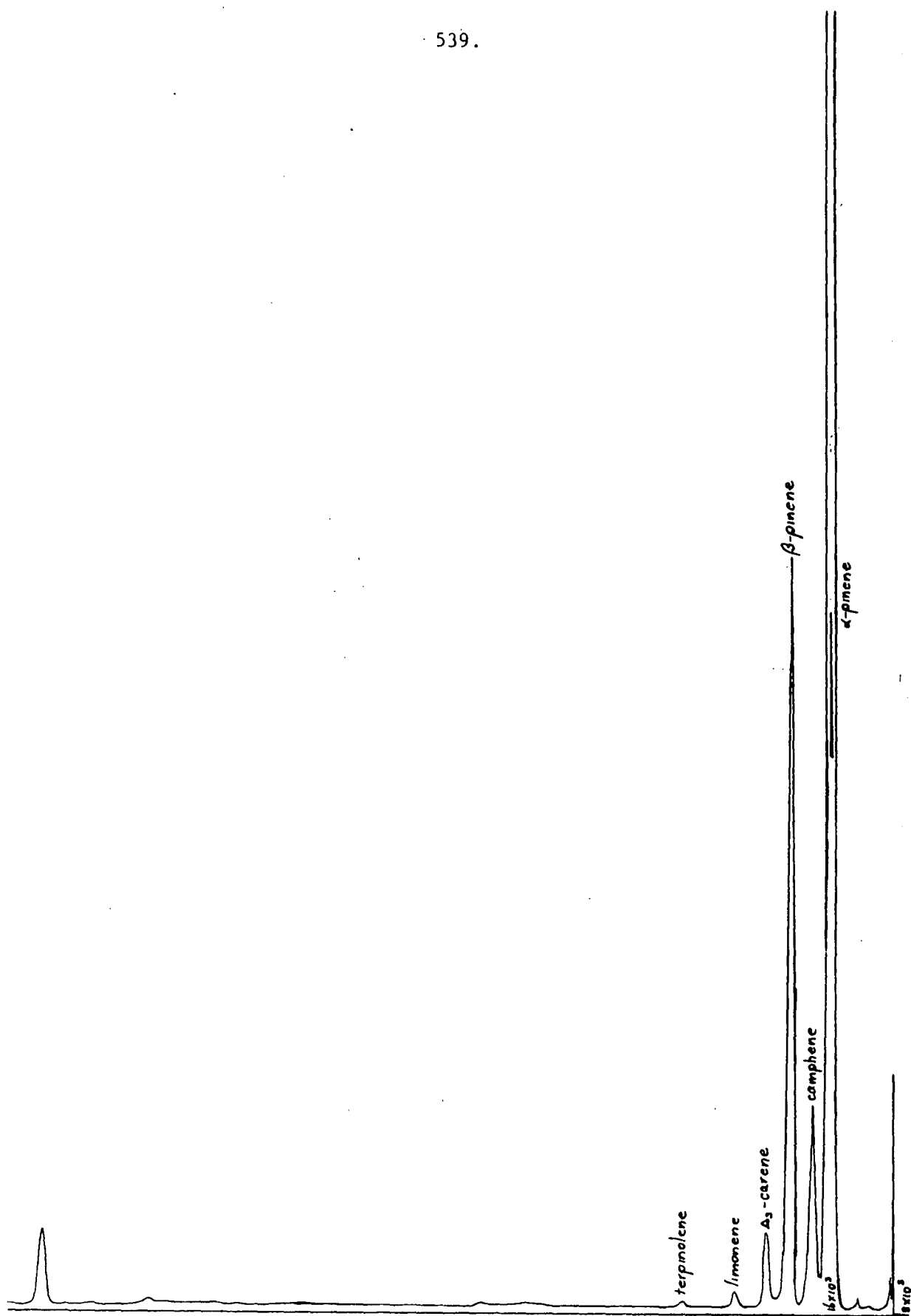
Fig. 86(c).   Low sensitivity gas chromatogram of fraction A of foliage oil
of *Pseudotsuga menziesii* separated on Florisil.

540.
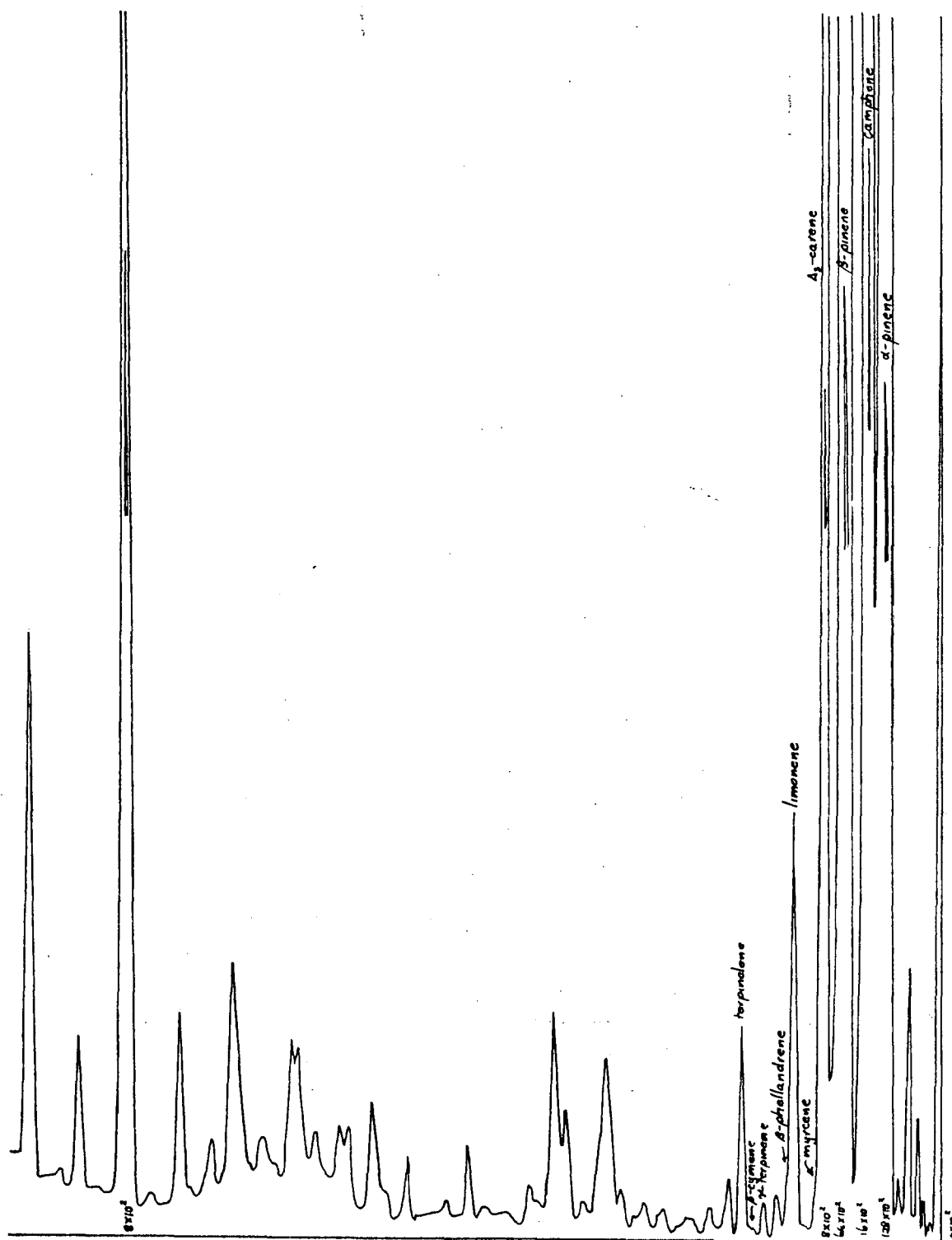
Fig. 86(d).   High sensitivity gas chromatogram of fraction A of
foliage oil of *Pseudotsuga menziesii* separated on Florisil.
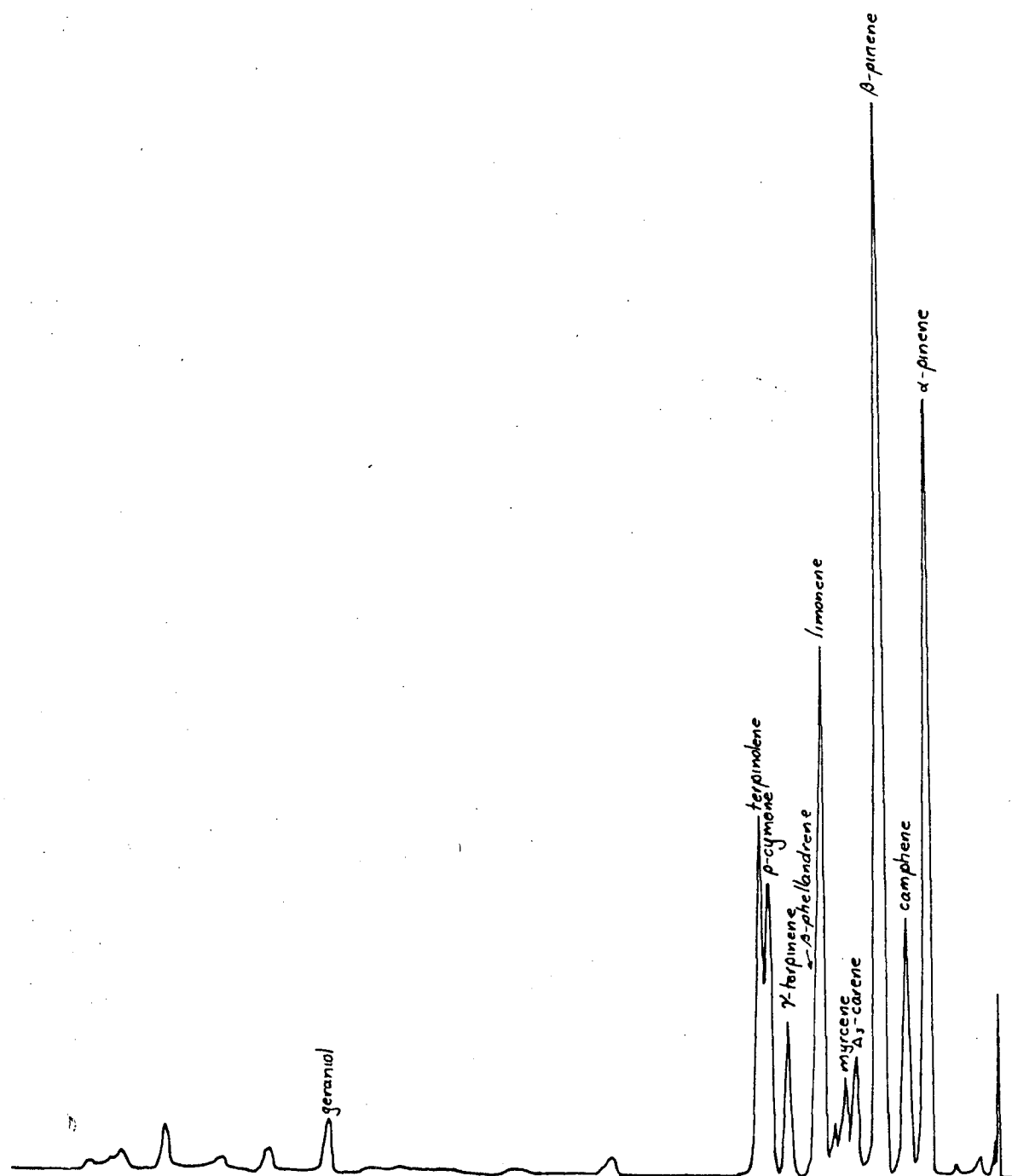
Fig. 86(e). Low sensitivity gas chromatogram of fraction B of foliage oil of *Pseudotsuga menziesii* separated on Florisil.
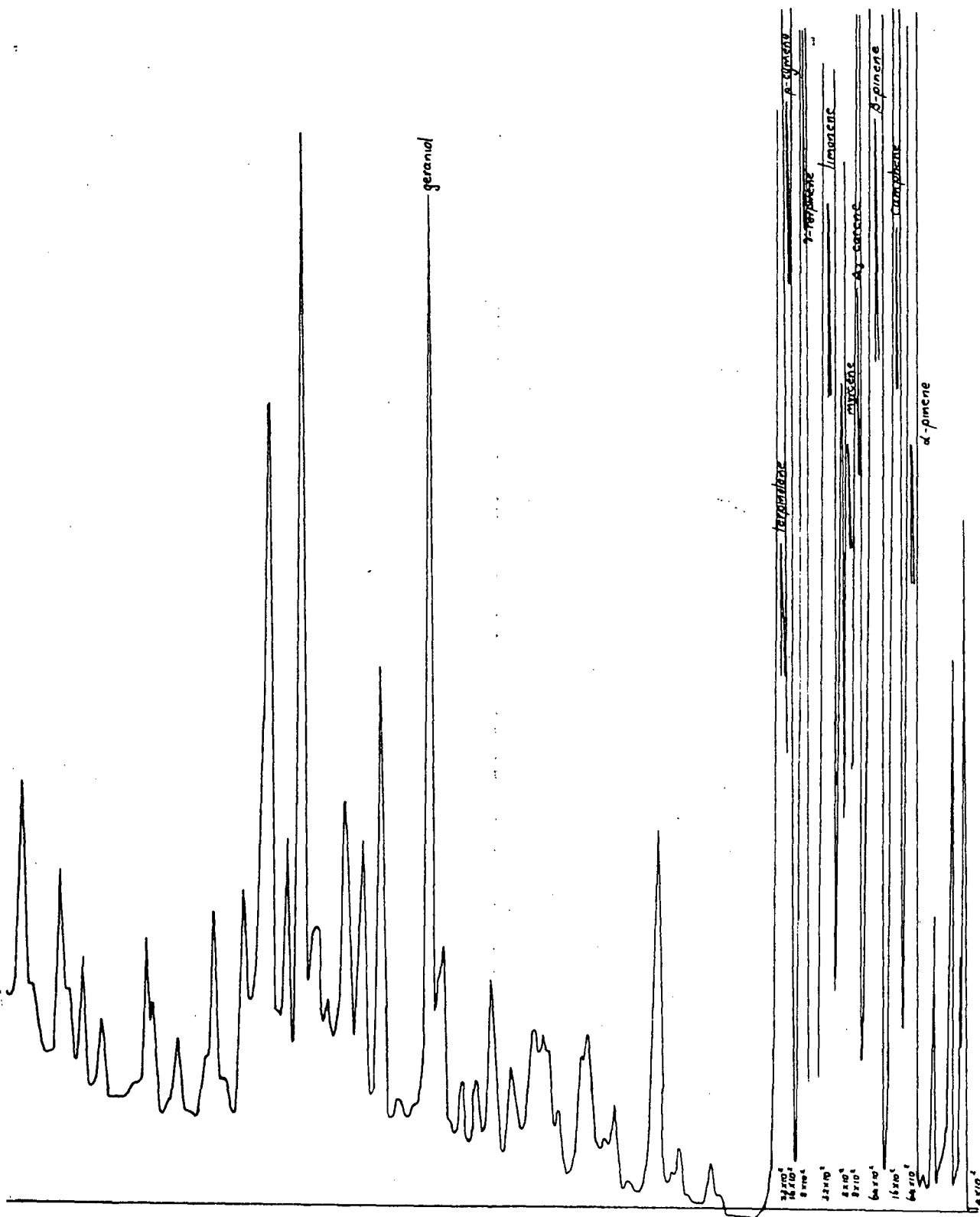
Fig. 86(f).   High sensitivity gas chromatogram of fraction B of foliage oil of *Pseudotsuga menziesii* separated on Florisil.
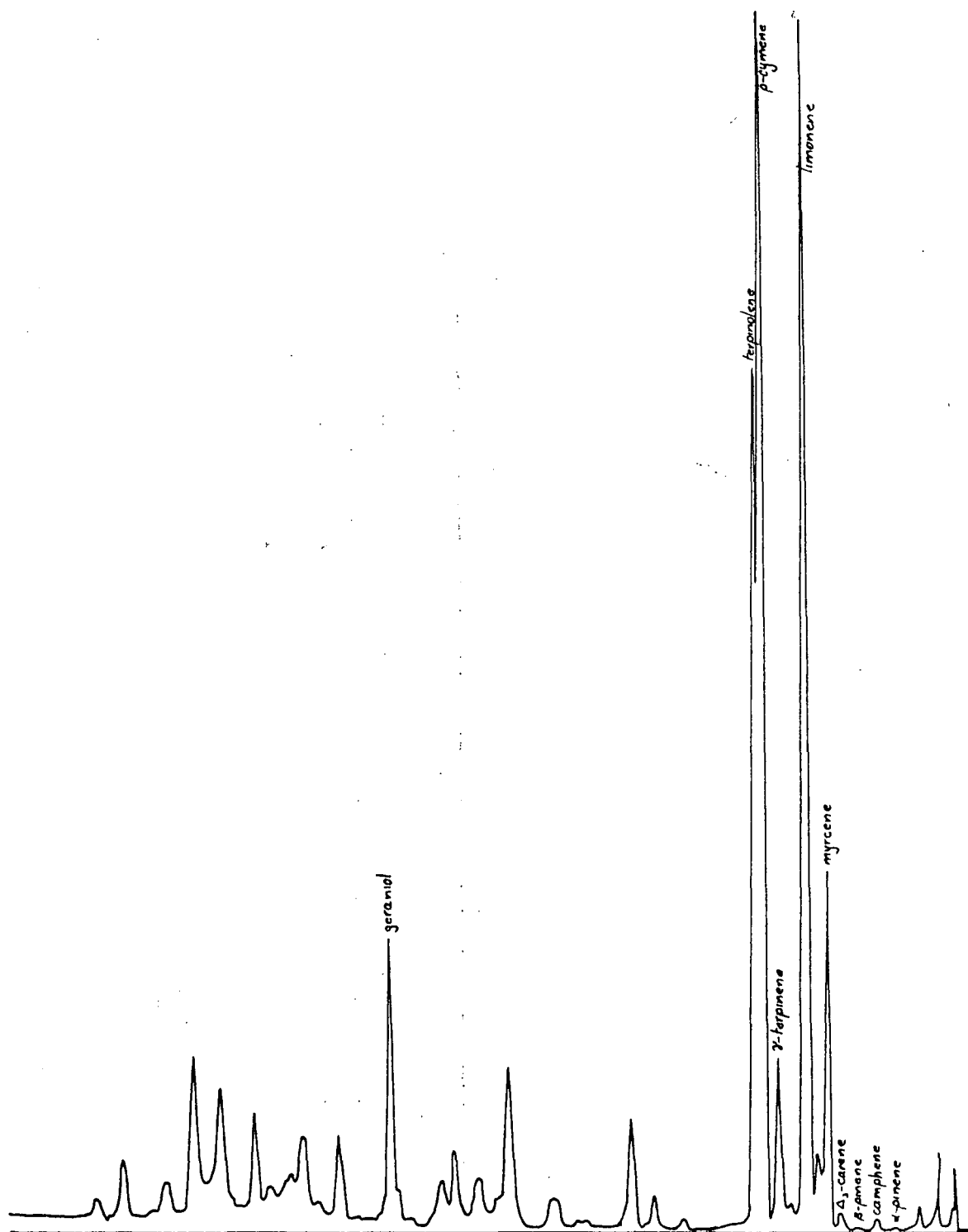
Fig. 86(g).   Low sensitivity gas chromatogram of fraction C of foliage
oil of *Pseudotsuga menziesii* separated on Florisil.
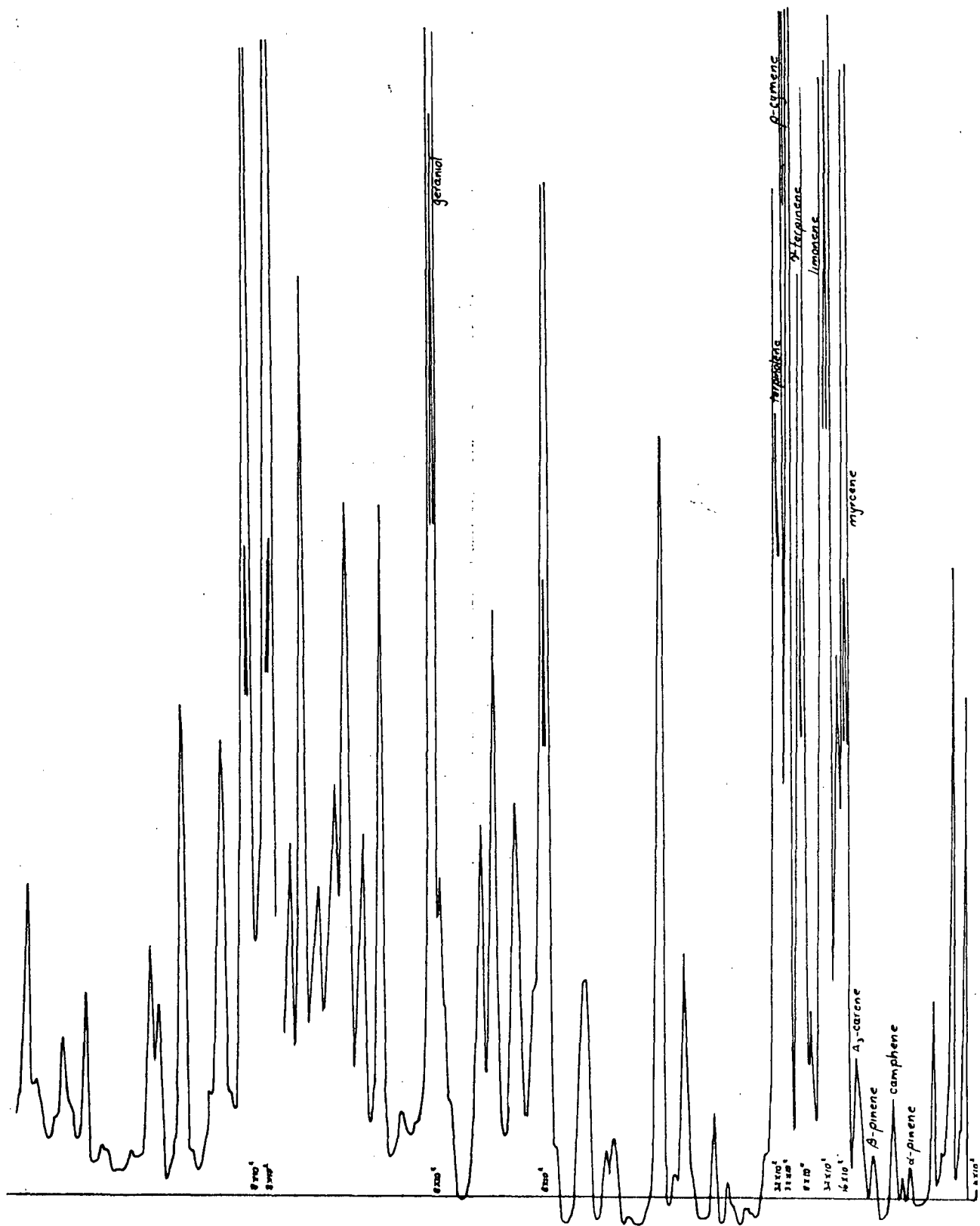
Fig. 86(h).   High sensitivity gas chromatogram of fraction C of foliage oil
of *Pseudotsuga menziesii* separated on Florisil.
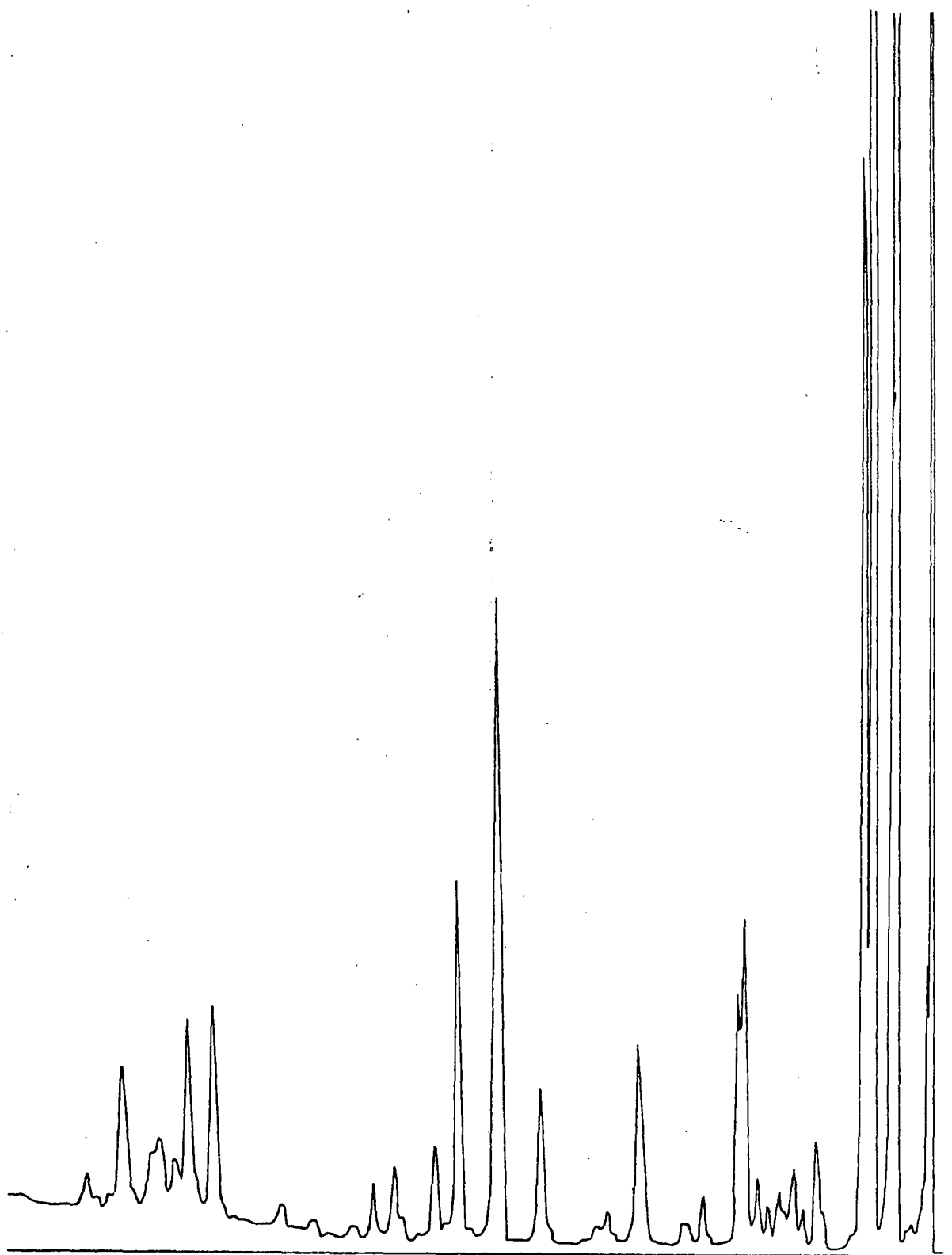
Fig. 86(i).   Low sensitivity gas chromatogram of fraction D of foliage
oil of *Pseudotsuga menziesii* separated on Florisil.
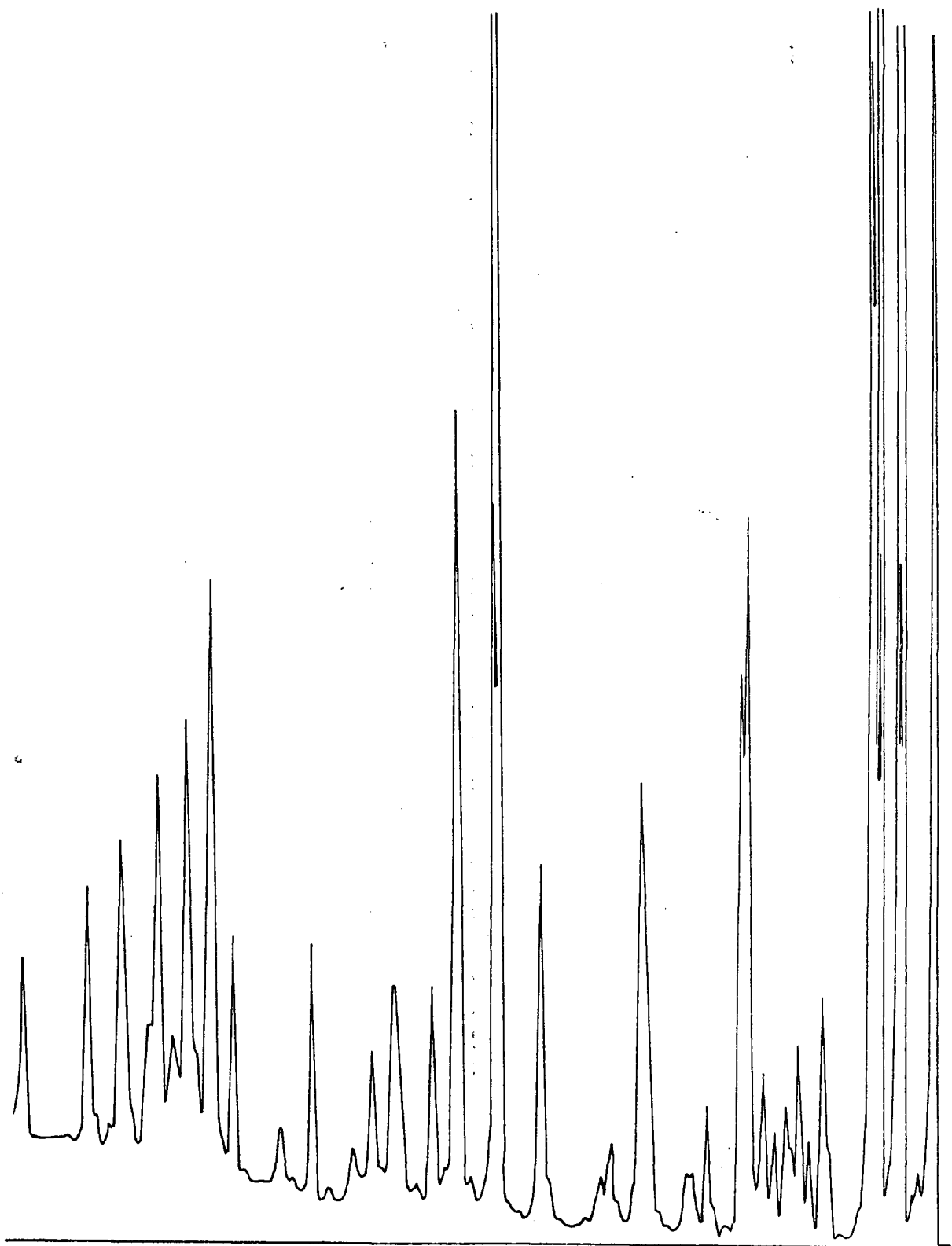
Fig. 86(j).   High sensitivity gas chromatogram of fraction D of foliage
oil of *Pseudotsuga menziesii* separated on Florisil.
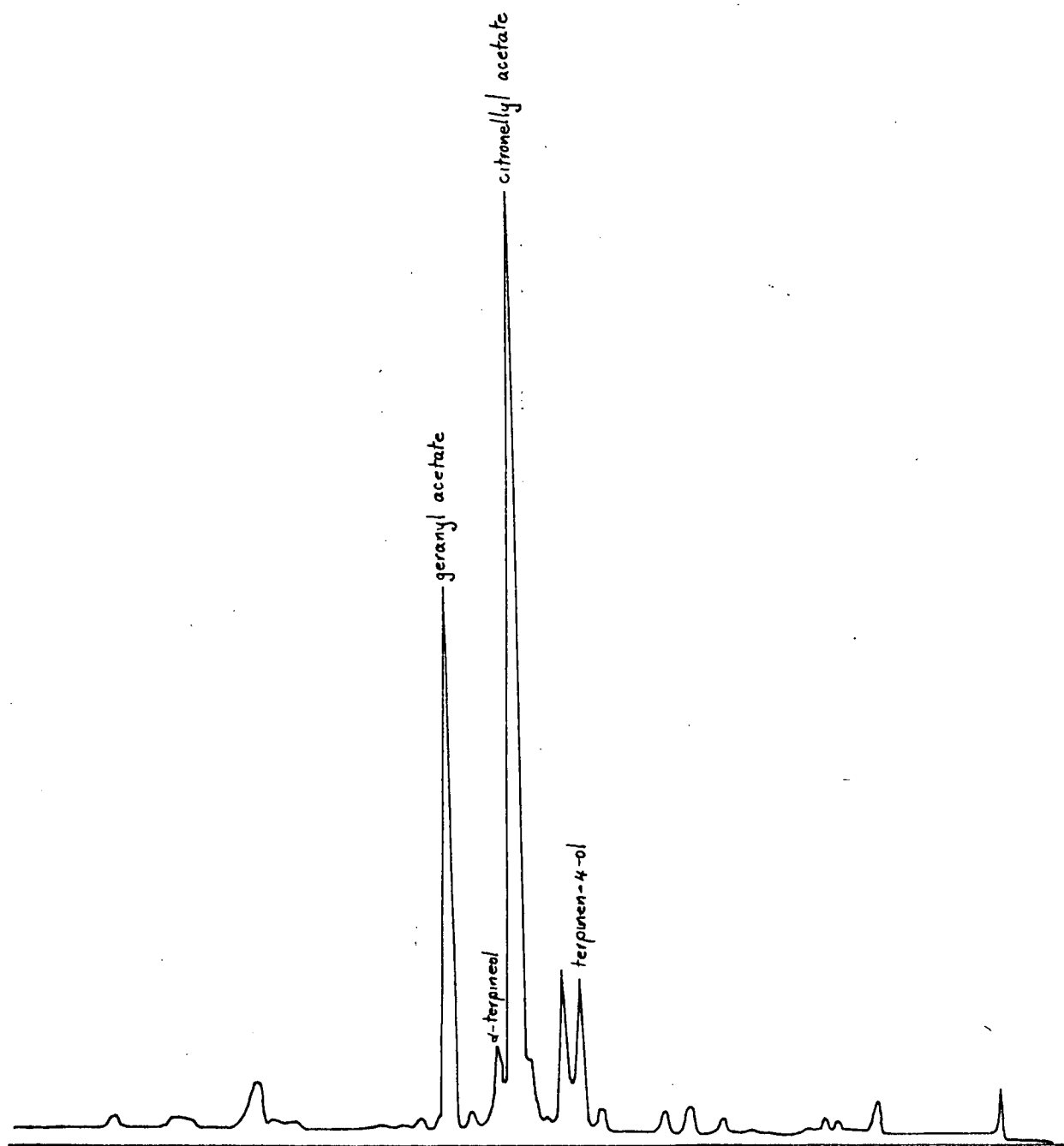
Fig. 86(k).   Low sensitivity gas chromatogram of fraction E of foliage oil of *Pseudotsuga menziesii* separated on Florisil.
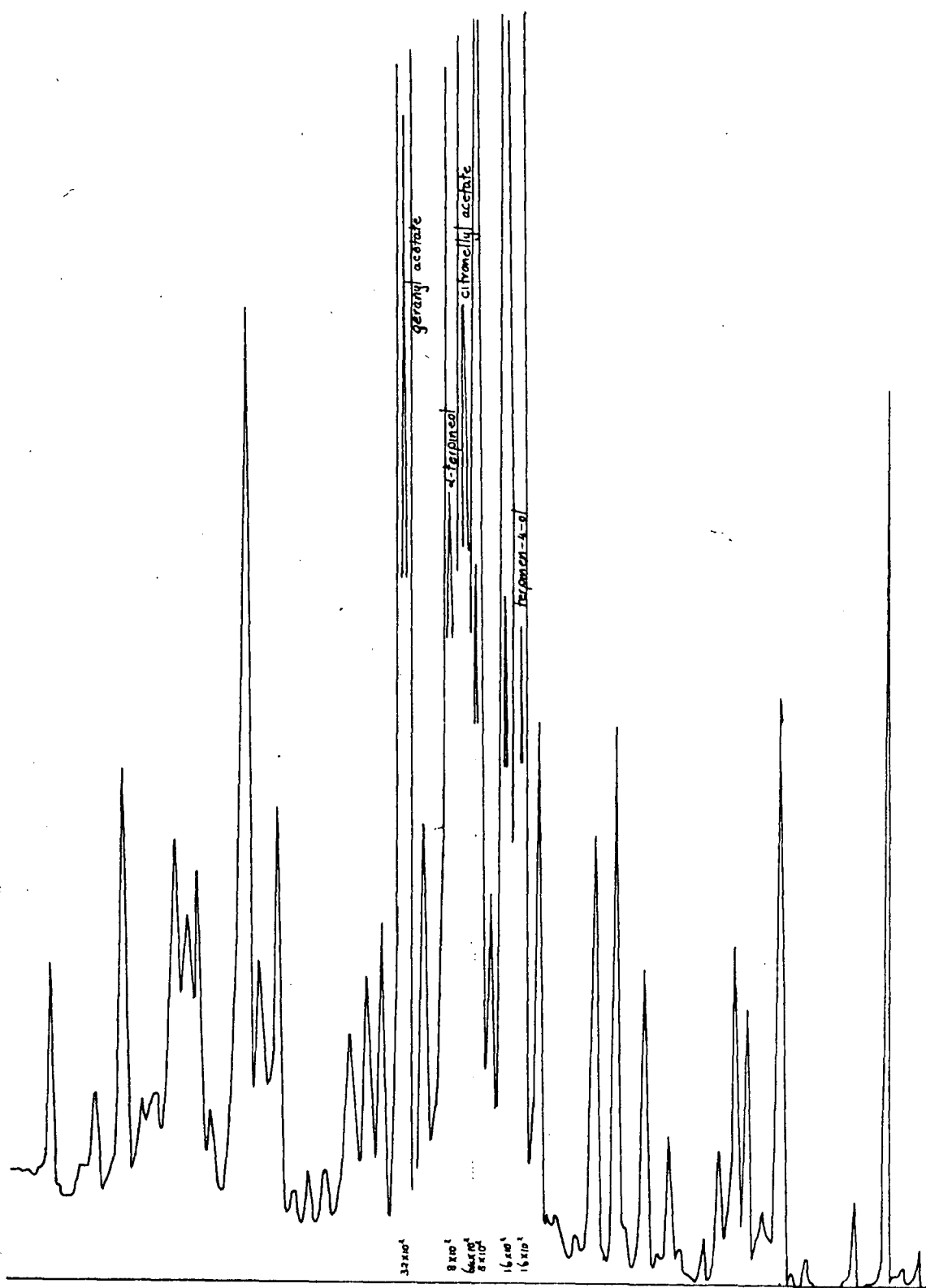
Fig. 86(ℓ).   High sensitivity gas chromatogram of fraction E of
foliage oil of *Pseudotsuga menziesii* separated on Florisil.
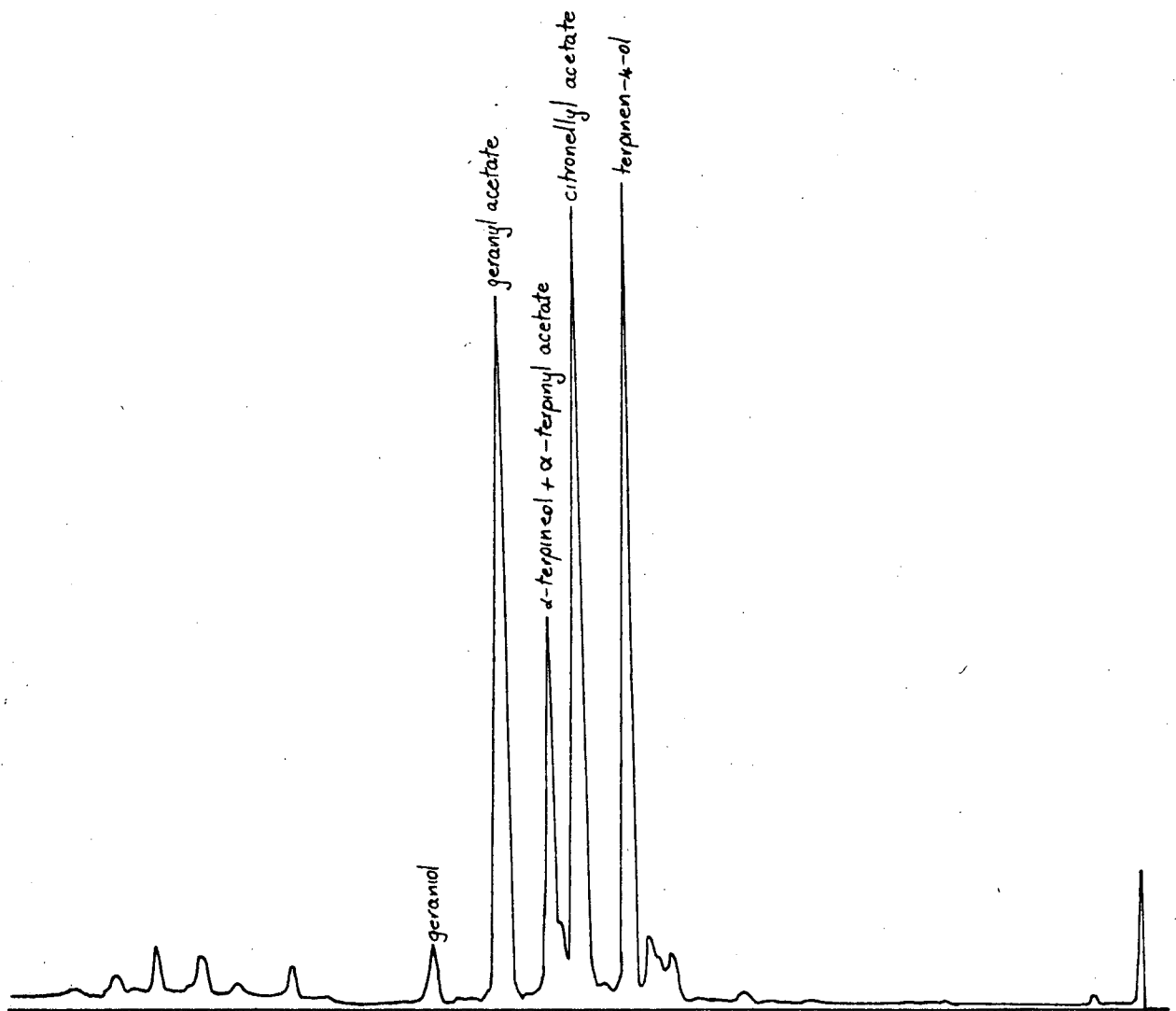
Fig. 86(m).   Low sensitivity gas chromatogram of fraction F of foliage oil
of *Pseudotsuga menziesii* separated on Florisil.
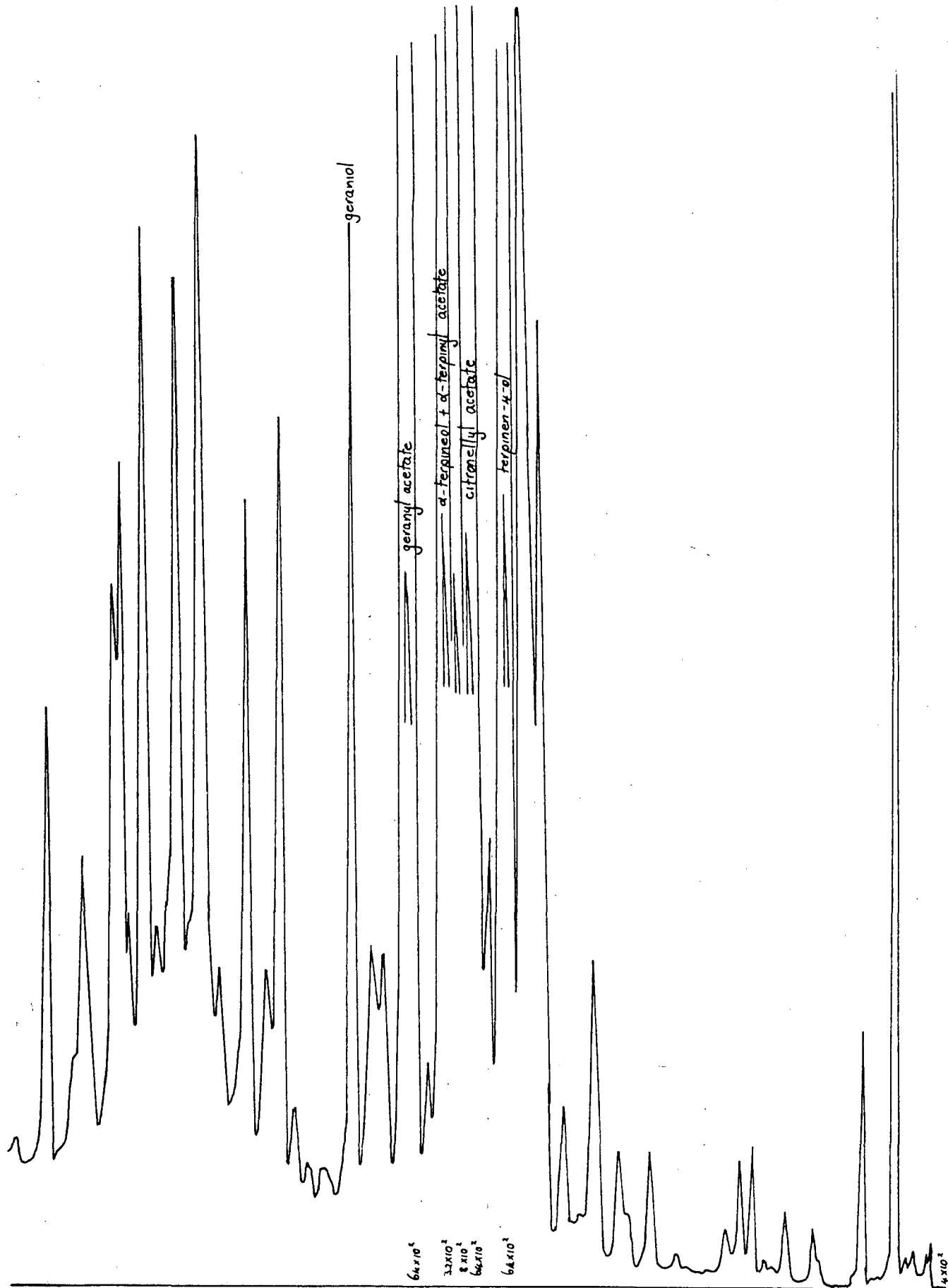
Fig. 86(n).   High sensitivity gas chromatogram of fraction F of foliage oil of *Pseudotsuga menziesii* separated on Florisil.
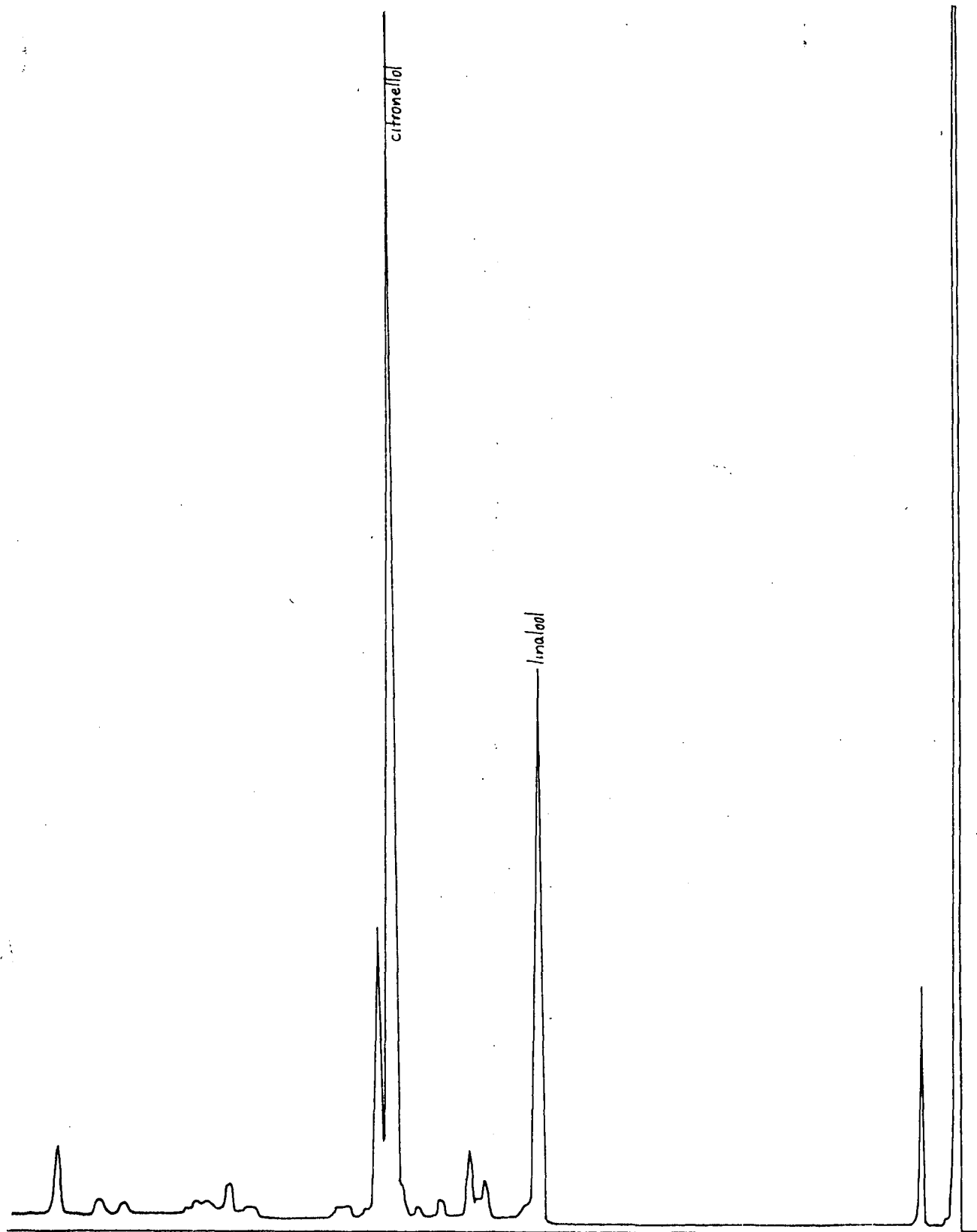
Fig. 86(o). Low sensitivity gas chromatogram of fraction G of foliage of *Pseudotsuga menziesii* separated on Florisil.
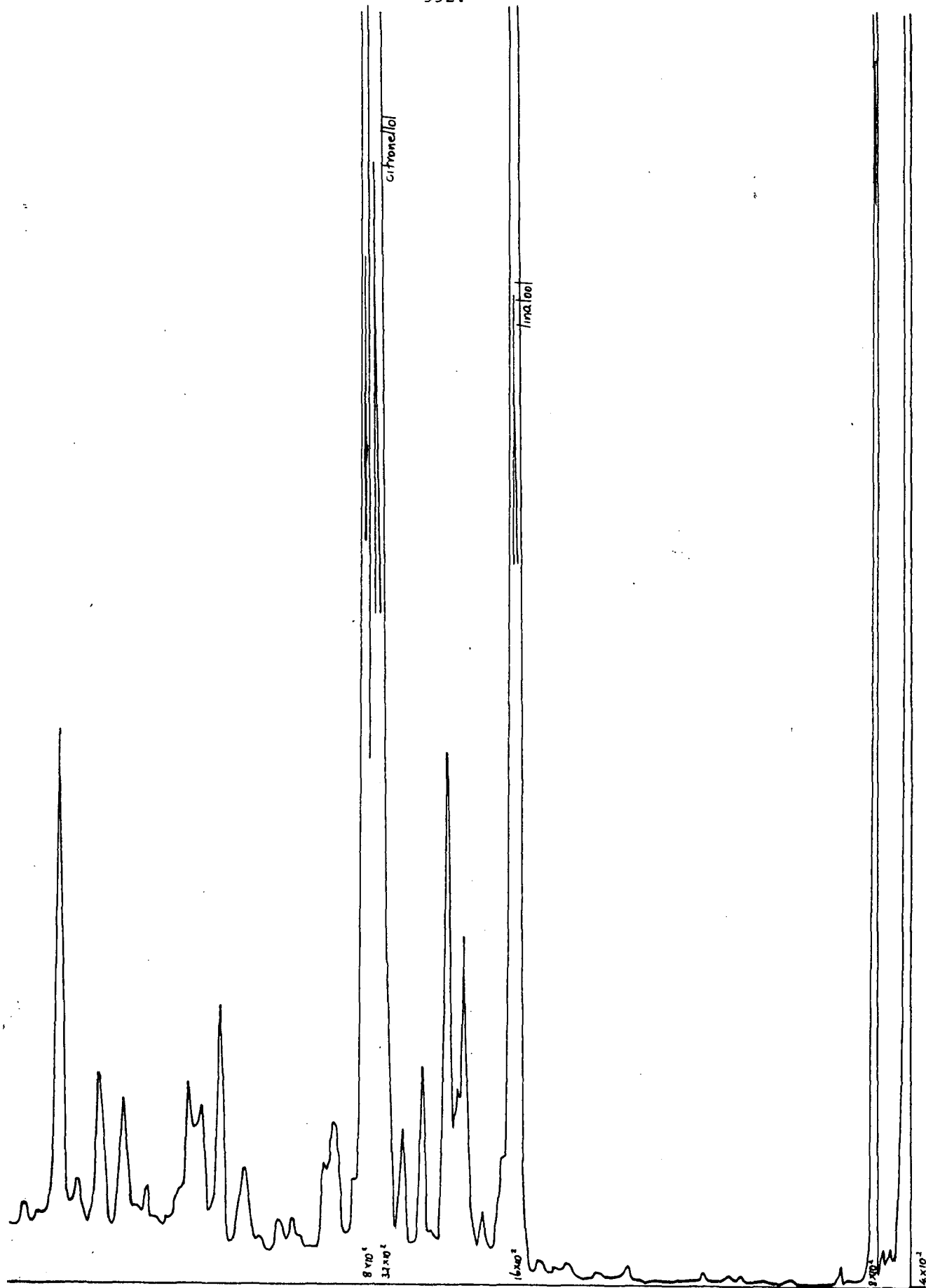
Fig. 86(p).   High sensitivity gas chromatogram of fraction G of foliage of
Pseudotsuga menziesii separated on Florisil.
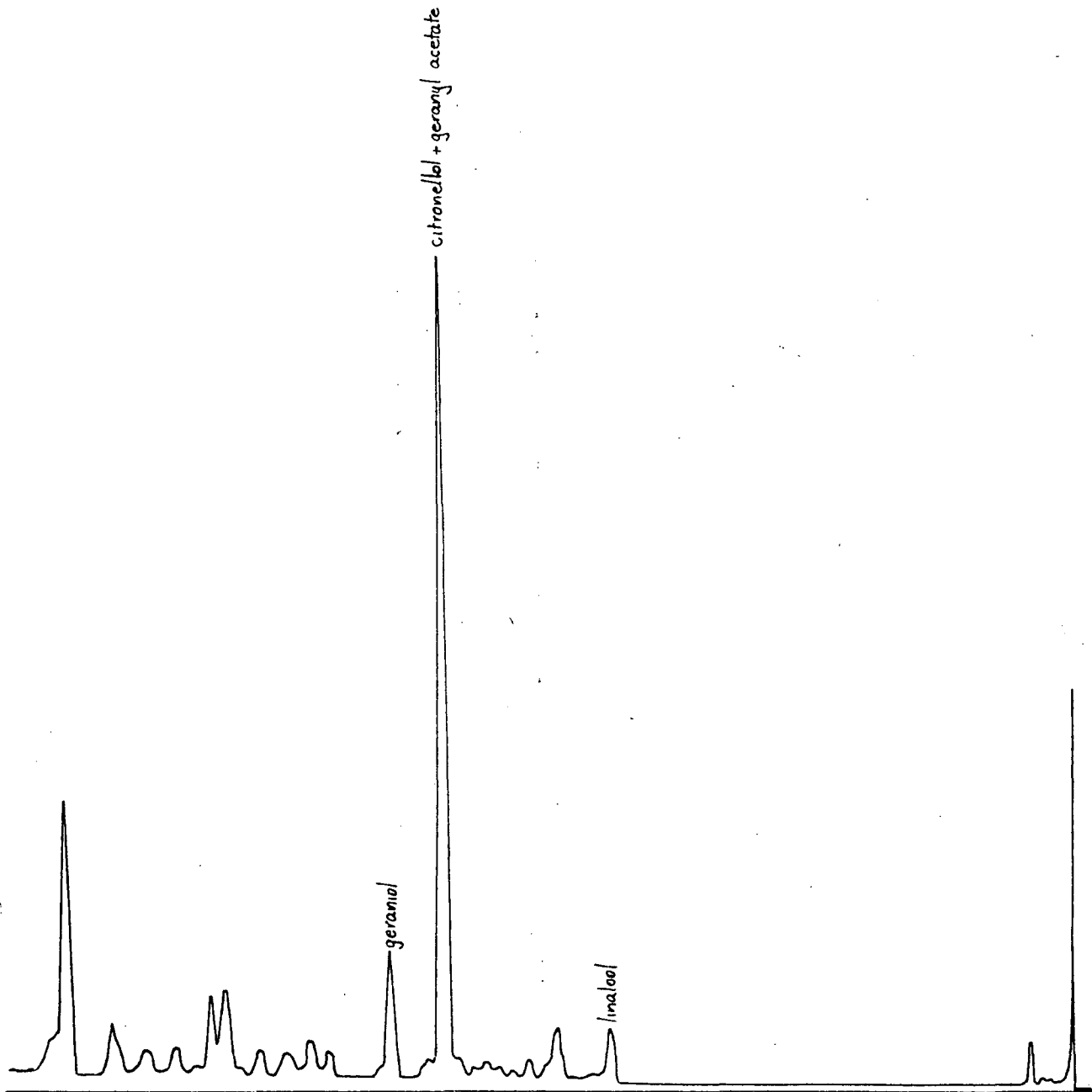
Fig. 86(q).   Low sensitivity gas chromatogram of fraction H of foliage of *Pseudotsuga menziesii* separated on Florisil.
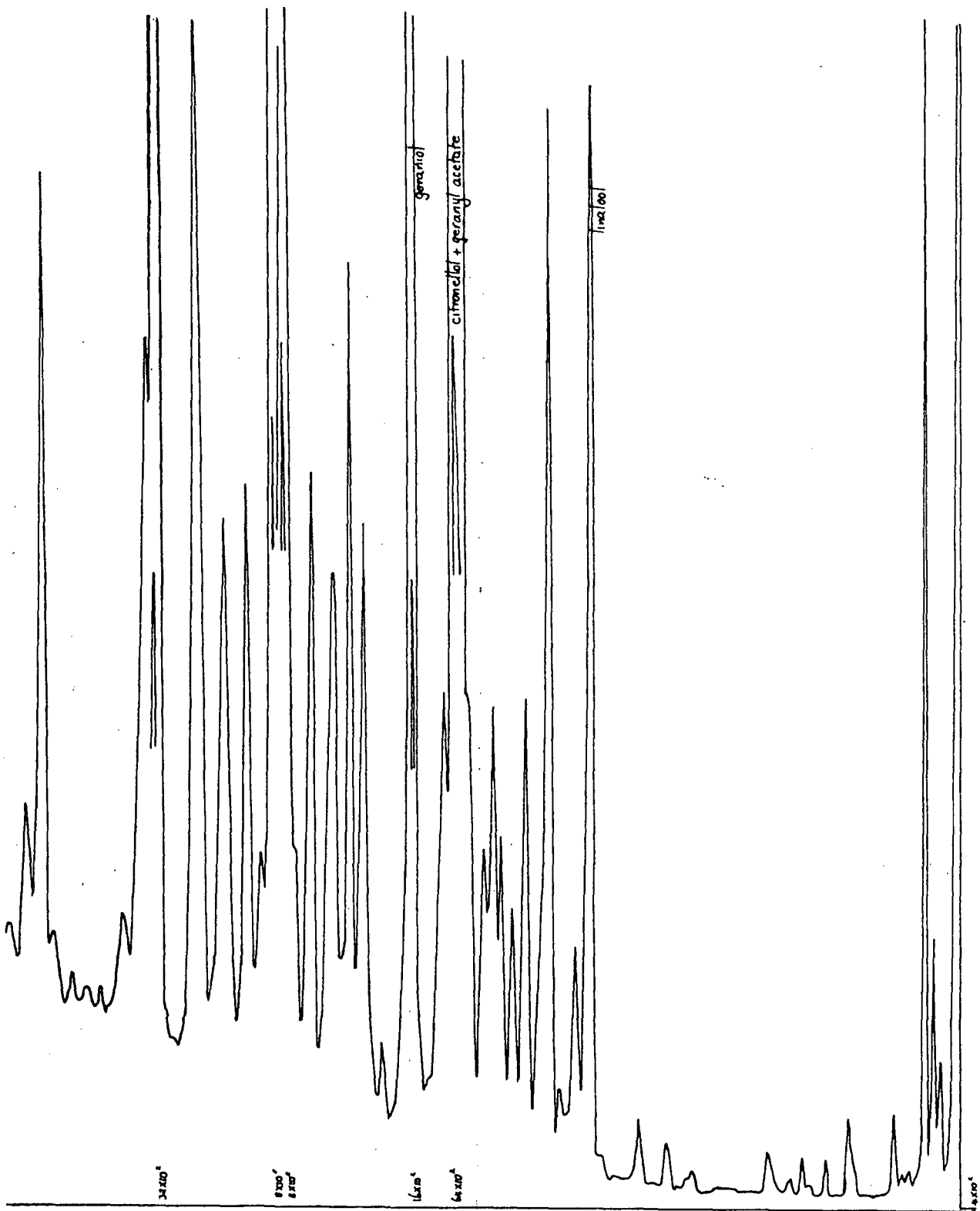
Fig. 86(r).   High sensitivity gas chromatogram of fraction H of foliage of *Pseudotsuga menziesii* separated on Florisil.

of components from a Carbowax 20M column, and indicate the degree of separation of components into the major column chromatographic fractions.

The composition of the foliage oil from this particular tree (V) is seen from Tables 123 and 124 to be within the range of compositions reported by von Rudloff [531] for the coastal variety, i.e. var *menziesii*. Although numerous components have been reported in this oil, it would appear from Figure 86 that there are possibly some hundreds of as yet unidentified components. Geraniol and α-terpinyl acetate did not appear from a search of the literature to have been previously identified in the needle oil, although they were both reported [527] in the cortical oleoresin.

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from comminuted foliage (tree I) similarly enabled the same monoterpene components to be identified (Table 126). Further minor components were detected which eluted before α-pinene on the Carbowax 20M column. A comparison of the syringe-headspace monoterpene composition of tree V (Table 128) with that of the steam-distilled oil, shows the expected higher proportions of more volatile components, i.e. sabinene and α- and β-pinene.

Table 126. RRT data and percentage composition
of volatile terpenoids in foliage of
Pseudotsuga menziesii (tree I)
determined by syringe-headspace GC
analysis

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak area)
	(60° isothermal, ref. α-pinene)		(60° isothermal)
Unidentified	0.77		0.1
α-Pinene	1.00	0.99	16.7
Camphene	1.28	1.18	0.2
β-Pinene	1.64	}1.52	15.7
Sabinene	1.76		56.1
Δ$_3$-Carene	2.09	1.93	2.4
Myrcene	2.31	1.73	0.8
Unidentified	2.55	2.15	0.1
Limonene	2.86	2.35	0.5
β-Phellandrene	2.99	2.48	0.5
γ-Terpinene	3.83	3.24	0.2
Unidentified	3.91		0.3
ρ-Cymene		2.79	0.1
Terpinolene	4.87	4.06	6.5

(c) Comparison of successive injections of syringe-
headspace vapour from foliage (tree I)

Successive injections of vapour over a 3 hr. period,
from a single sample of foliage, exhibited minor fluctuations
in the proportions of monoterpenes, i.e. less than 2 percent
for each major component (Table 127). Indications of a
reciprocal relationship between β-pinene and terpinolene
(Figure 87) with no simultaneous changes in the proportions
of other components, might be the result of a biosynthetic
link between these two structures. Further study should be
undertaken with this technique to document any possible
biosynthetic relationships.

Table 127.   Compositions of monoterpenes in
successive injections of syringe-
headspace vapour from foliage of
Pseudotsuga menziesii (tree I)

Percentage composition of monoterpenes (peak height basis):

Time since comminution of sample (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Sabinene	Δ₃-Carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene	Unidentified	Terpinolene
0	t	15.3	0.3	18.6	46.8	4.2	1.5	0.1	0.7	0.8	0.2	0.4	11.1
15	0.1	16.8	0.3	18.4	46.1	4.5	1.4	0.1	0.6	0.7	0.1	0.4	10.4
30	0.2	16.6	0.3	18.6	46.1	4.6	1.5	0.1	0.6	0.7	0.2	0.4	10.0
45	0.2	16.6	0.3	18.3	46.4	4.6	1.5	0.1	0.6	0.7	0.1	0.4	10.0
65	0.2	16.6	0.3	19.0	46.7	4.6	1.4	0.1	0.7	0.7	0.1	0.4	9.2
80	0.2	16.4	0.3	18.3	46.8	4.5	1.4	0.1	0.6	0.8	0.1	0.5	9.9
95	0.2	16.6	0.4	18.4	46.6	4.6	1.4	0.1	0.7	0.7	0.2	0.4	9.8
110	0.2	16.4	0.3	18.3	47.0	4.6	1.4	0.1	0.7	0.8	0.2	0.5	9.6
125	0.2	16.4	0.3	18.5	47.0	4.6	1.4	0.1	0.6	0.7	0.2	0.5	9.4
140	0.2	16.2	0.3	18.4	47.1	4.5	1.4	0.1	0.7	0.8	0.1	0.5	9.6
165	0.2	15.9	0.3	18.7	47.5	4.5	1.4	0.2	0.6	0.7	0.1	0.5	9.3
180	0.2	16.5	0.3	18.4	46.9	4.5	1.4	0.2	0.7	0.8	0.1	0.5	9.5

(d)   Composition of syringe-headspace vapour from foliage

of several trees of *P. menziesii* (trees I-V)

The wide variation in monoterpene composition of

the vapour from a random sample of foliage, from each of

5 trees, is shown in Table 128.   The compositions of the

vapours from trees I, II and V are distinguished by the

higher proportions of sabinene and terpinolene that were

shown by von Rudloff [531] to be typical of var *menziesii*.

By comparison, vapour from trees III and IV contained much

558.

Fig. 87. Composition of monoterpenes in successive injections of
syringe-headspace vapour from a single sample of foliage of
Pseudotsuga menziesii (tree I).   A possibly reciprocal relationship
between β-pinene and terpinolene, in the absence of similar changes
in the proportions of other components, might be attributed to a
biosynthetic link between these two monoterpenes.
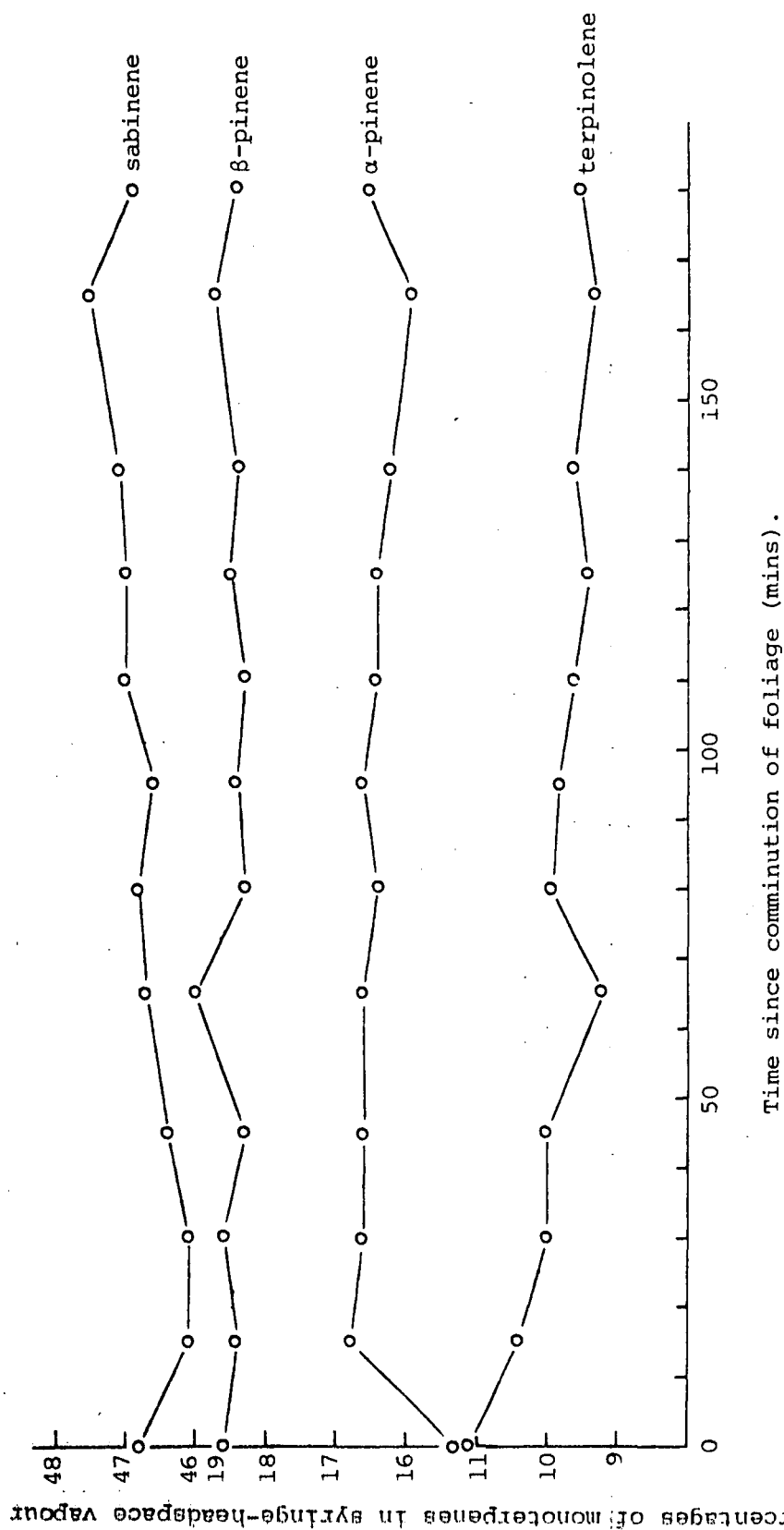
Table 128. Compositions of monoterpenes in
syringe-headspace injections of
vapour from foliage of several
trees of *Pseudotsuga menziesii*

<u>Percentage composition of monoterpenes (peak height basis)</u>:

Tree No.	Time since comminution (mins.)	Unidentified group	α-Pinene	Camphene	β-Pinene	Sabinene	Unidentified	Δ₃-Carene	Myrcene	Unidentified	Limonene	β-Phellandrene	γ-Terpinene	Unidentified	Terpinolene
I	0	[loss]													
	15	–	15.9	0.3	19.9	47.0	–	3.5	1.5	0.2	0.7	0.7	0.5	0.2	9.
	30	0.1	15.8	0.3	20.1	47.0	–	3.5	1.5	0.2	0.7	0.8	0.5	0.2	9.
II	0	0.1	25.4	0.7	48.4	16.6	1.1	1.4	1.5	0.1	0.5	1.0	0.2	0.1	2.
	15	0.1	26.9	0.3	50.2	15.0	0.8	1.3	1.3	0.1	0.4	0.8	0.2	t	2.
	30	0.1	26.6	0.7	50.9	15.4	–	1.3	1.3	0.1	0.4	0.8	0.2	t	2.
III	0	t	32.3	3.5	52.0	2.3	3.2	2.4	1.3	–	1.8	0.9	0.2	–	0.
	15	0.2	36.0	3.7	50.7	1.7	1.5	2.0	1.3	–	1.7	0.8	0.2	–	0.
	30	0.3	36.4	3.8	50.8	2.7	–	2.1	1.2	–	1.6	0.8	0.2	–	0.
IV	0	–	43.7	37.1	6.3	4.7	–	0.3	1.5	t	4.6	t	1.0	–	1.
	15	0.1	41.4	32.1	5.3	3.6	–	0.2	1.1	t	3.2	t	0.7	–	0.
	35	0.1	42.1	32.4	5.2	3.4	–	0.3	1.1	t	3.1	0.3	0.6	–	0.
	(IV also contained ~10 percent tricyclene)														
V	0	0.3	28.2	1.0	47.8	14.4	–	1.9	1.7	0.1	1.2	1.1	0.1	0.2	2.
	40	0.5	30.5	1.1	47.6	13.2	–	1.9	1.5	0.1	1.0	0.9	0.1	0.1	1.
	55	0.5	30.8	1.0	47.2	13.4	–	1.9	1.4	t	1.0	0.9	0.1	0.1	1.

lower proportions of sabinene and terpinolene, and are
further distinguished by the presence of tricyclene and a
higher proportion of camphene (as high as 37 percent in tree
IV). Trees III and IV are therefore seen to more closely
resemble var *glauca*. Figure 88 shows the different gas
chromatograms of vapour from each variety.

(e) Composition of syringe-headspace vapour from foliage
sampled repeatedly from the same tree (tree I)

The composition of the vapour from three samples
of foliage from tree I varied (Tables 126-128) from 15.3 to
16.7 percent α-pinene, 15.7 to 19.9 percent β-pinene, 47.0
to 56.1 percent sabinene and 6.5 to 11.1 percent terpinolene.

(f) Summary

Components of the steam-distilled foliage oil of
Pseudotsuga menziesii were analyzed by chromatographic and
spectroscopic methods. The following identifications were
confirmed: α-pinene (17.7%), β-pinene (34.0%), limonene
(2.8%), γ-terpinene (4.0%), ρ-cymene (0.7%), terpinolene
(8.7%), terpinen-4-ol (2.8%), citronellyl acetate (4.4%),
geranyl acetate (1.8%) and citronellol (1.2%). Tentatively
identified were camphene (1.2%), sabinene (10.0%), Δ_3-carene
(2.3%), myrcene (2.5%), β-phellandrene (2.2%), linalool,
α-terpinyl acetate, α-terpineol (1.1%) and geraniol. Although
known to occur in the oleoresin, neither geraniol nor α-terpinyl
acetate appeared from the literature to have been previously
found in the needle oil.
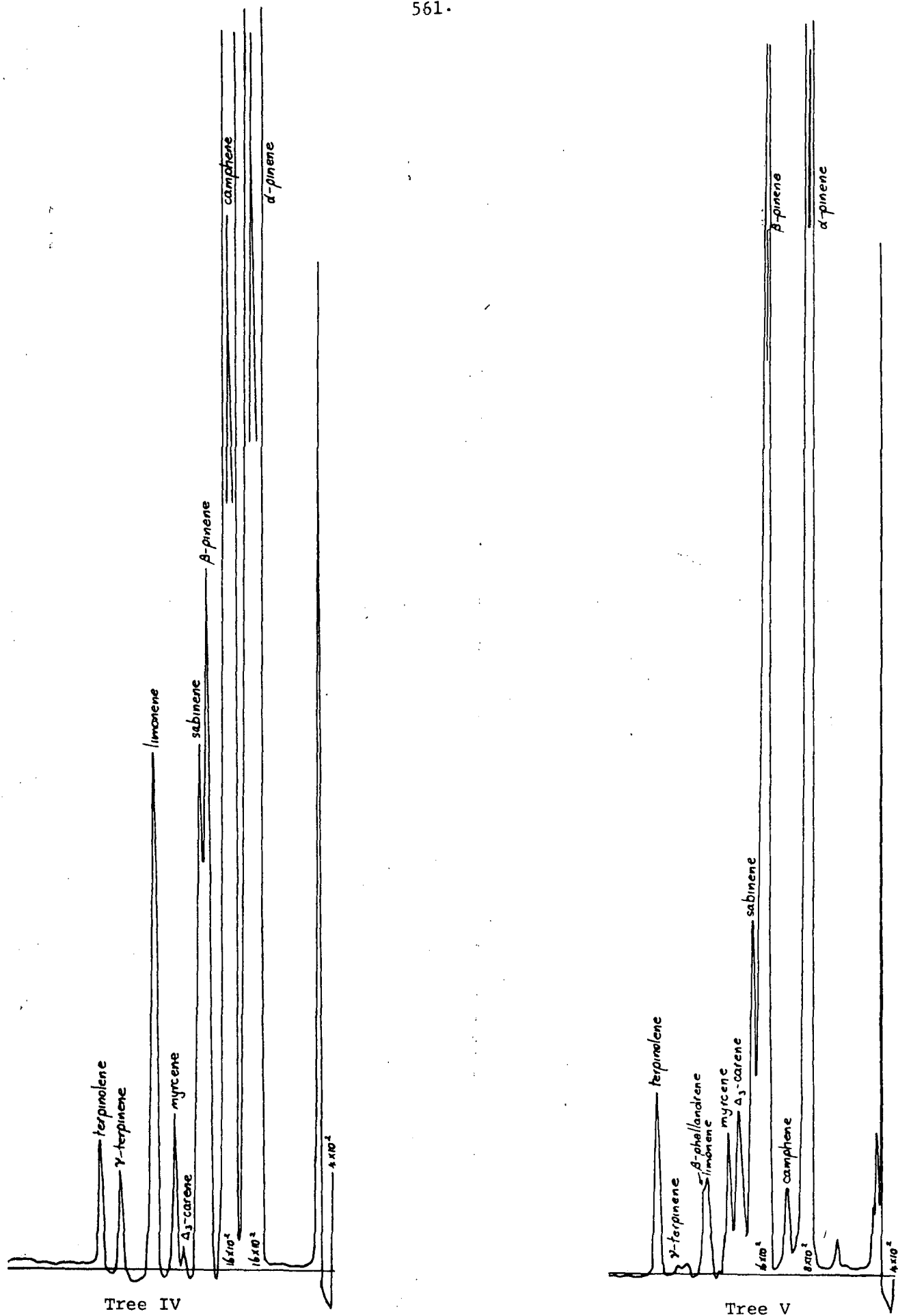
Fig. 88.   Syringe-headspace gas chromatograms of vapour foliage of two apparently different varieties of *Pseudotsuga menziesii* (GC conditions as before;   attenuation 4 x 10²).   Tree V appears to be var *menziesii*, while tree IV more closely resembles var *glauca*.

The syringe-headspace GC technique provided a convenient means of examining the widely different monoterpene vapour compositions released from foliage of several trees. Of 5 trees in the Royal Botanical Gardens (Tasmania) 3 appeared from characteristic monoterpene compositions to belong to the var *menziesii* while 2 were more closely related to the var *glauca*. Successive injections of vapour over a 3 hr. period, from a single sample of foliage, varied within a narrow range, i.e. 15.3 to 16.8% α-pinene, 18.3 to 19.0% β-pinene, 46.1 to 47.5% sabinene and 9.2 to 11.1% terpinolene. Further study of successive injections of vapour may confirm a possible biosynthetic link between β-pinene and terpinolene. The compositions of vapour from 3 samples of foliage from a single tree ranged from 15.3 to 16.7% α-pinene, 15.7 to 19.9% β-pinene, 47.0 to 56.1% sabinene and 6.5 to 11.1% terpinolene.

An apparently qualitative change in foliage vapour was indicated when a component, eluted near Δ_3-carene on Carbowax 20M, could not be detected after 15 minutes of successive injections from the same sample. This phenomenon was only discernible in vapour from trees that contained less Δ_3-carene.

(xix) *Thuja plicata* D.Don

Leaf oil of Western Red Cedar, *Thuja plicata,* is characterized by its major constituent, ℓ-thujone [534, 535]. Optimum conditions for extraction of the leaf oil have been recommended [536]. The chemistry of *T. plicata* wood extractives has been reviewed by Gardner [537], who had earlier isolated thujaplicins and reported the occurrence of β-dolabrin (4-isopropenyltropolone) in the steam-distilled wood oil [538]. The wood oil was reported by Arndt [541] to be toxic to certain insects.

Von Rudloff [324] examined the leaf oil and found by GC and IR techniques that it consisted of approximately 80 percent ℓ-thujone and 8 percent d-isothujone, together with lesser amounts of d-sabinene and possibly Δ_4-carene. Other components reported were d-α-pinene, d-limonene and d-terpinen-4-ol. α-Fenchene, camphene, γ-terpinene, terpinolene, ρ-cymene and 1,8-cineole were tentatively identified by GC data only. Thujyl alcohol, thujyl acetate, borneol, bornyl acetate, fenchone and camphor identified in earlier investigations [534, 535], were not detected by von Rudloff.

Biosynthesis of components in oil of *T. plicata* has been discussed by von Rudloff [324], and more recently by Banthorpe and co-workers [539, 540], who examined the incorporation of ^{14}C into thujone from the $2-^{14}$C mevalonic acid precursor.

(a) <u>Analysis of the oil steam-distilled from foliage</u>

Foliage from a single tree of *T. plicata* in the Royal Botanical Gardens (Tasmania) yielded upon steam-distillation 0.92 percent (wet weight) of a colourless oil with the sweet fruity odour of thujone.

The qualitative and quantitative composition of components distinguishable in the whole oil, using two dissimilar columns, is recorded in Table 129.

The previously established presence in this oil of major proportions of thujone and isothujone were expected to provide considerable analytical difficulty unless these components were first isolated into a major fraction and treated separately. Thujone has a temporary effect on Carbowax liquid phase and would be expected to confuse the identity of components due to alteration of RRT values. Removal of this major component would significantly concentrate others and make their isolation easier. The oil was fractionated as in the case of oil of *Pseudotsuga menziesii*, according to the procedure used by Smedman *et al* [135]. Six major fractions (A to F) were then available for isolation of pure components by preparative GC and identification by IR and analytical GC.

RRT values leading to the tentative identification of components in fractions A to F (and preparative GC sub-fractions) are listed in Table 130. Gas chromatograms of Figure 89 show the order of elution of the complex mixture of components from a Carbowax 20M column, and indicate the degree of separation of components into the major column chromatographic fractions.

Table 129.   Components distinguishable in the whole
oil from foliage of *Thuja plicata*

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 200°, 5°/min.)
Unidentified	0.70	0.49	t
"	0.81	0.55	t
α-Pinene	1.01	1.01	1.2
Unidentified	} 1.28	0.78	1.9
Camphene		1.20	t
*Sabinene	1.76	1.56	13.3
Myrcene	2.23	1.77	0.7
Unidentified	2.44	2.20	0.3
Limonene	2.73	2.39	0.5
β-Phellandrene	2.92	2.50	t
*γ-Terpinene	3.63	3.22	0.4
ρ-Cymene	4.27	2.80	2.9
*Terpinolene	4.60	4.14	0.3
Unidentified	7.67		t
*Thujone	12.1	6.56	57.5
Isothujone	13.1	6.95	11.7
	(130° isothermal, ref. camphor)		
ρ-Cymene	0.45		
Thujone (+ isothujone)	0.80	0.76	
*Terpinen-4-ol	1.14	1.00	0.1
*Chavicol methyl ether	1.35	1.23	6.7
Unidentified	~1.4		0.7
α-Terpineol	1.67	1.06	1.5
Terpinyl acetate	1.87	2.11	0.3
Geraniol	3.31		0.1

* IR spectrum recorded
t: trace; <0.1 percent

Table 130. RRT values on the dissimilar liquid phases C20M and OV-17, for components found in fractions A to F (and preparative GC sub-fractions) isolated from steam-distilled oil from the foliage of *Thuja plicata*

Component	Whole oil Prep. GC No.	C20M	OV-17	Frac. A No.	C20M	OV-17	Frac. B C20M	OV-17	Frac. C No.	C20M	OV-17	Frac. D C20M	OV-17	Frac. E No.	C20M	OV-17	Frac. F C20M	OV-17
(60° isothermal, ref. α-pinene)																	[too complex; no peaks correlated on both columns]	
α-Pinene					1.03	1.03	1.02	1.00										
Unidentified					1.14							1.15	0.49		1.12	0.48		
Unidentified												1.24	0.80		1.21	0.80		
Camphene					1.32	1.23	1.30	1.22										
β-Pinene					1.62	1.57	1.63											
Sabinene	W2	1.74	1.55															
Unidentified						1.40	1.87	1.38										
Myrcene	W2	2.27	1.78		2.25	1.77	2.21	1.76										
α-Phellandrene?						2.00												
Unidentified					2.47	2.18	2.40	2.19										
Limonene					2.77	2.35	2.70	2.38										
β-Phellandrene					2.91	2.52												
γ-Terpinene					3.67	3.24	3.63	3.25										
				A5	3.73	3.29												
p-Cymene					4.30	2.79	4.29	2.84		4.28	2.80							
				A5	4.26	2.82												
Terpinolene					4.59	4.08	4.53	4.09										
				A6	4.59	4.05												
Thujone										11.5	6.63	11.6	6.57		11.7	6.46		
									C1	11.7	6.76							
Isothujone										12.4	6.96	12.6	6.87		12.6	6.79		
									C1	12.7	7.13							
Terpinen-4-ol															>22	10.1		
(130° isothermal, ref. camphor)																		
p-Cymene										0.42	0.50							
										0.72	0.78	0.72	0.78		0.73	0.74		
Thujone (+ isothujone)																		
									C1	0.71	0.76			E3	0.74	0.74		
									C2	0.74	0.79							
Terpinen-4-ol															1.27	0.98		
														E3	1.24	0.98		
Chavicol methyl ether										1.56	1.26							
									C2	1.54	1.26							
α-Terpineol															1.80			
α-Terpinyl acetate												1.81	2.11					
Geraniol															3.23			
Unidentified					9.79	25.1												
Unidentified					11.1	27.3												
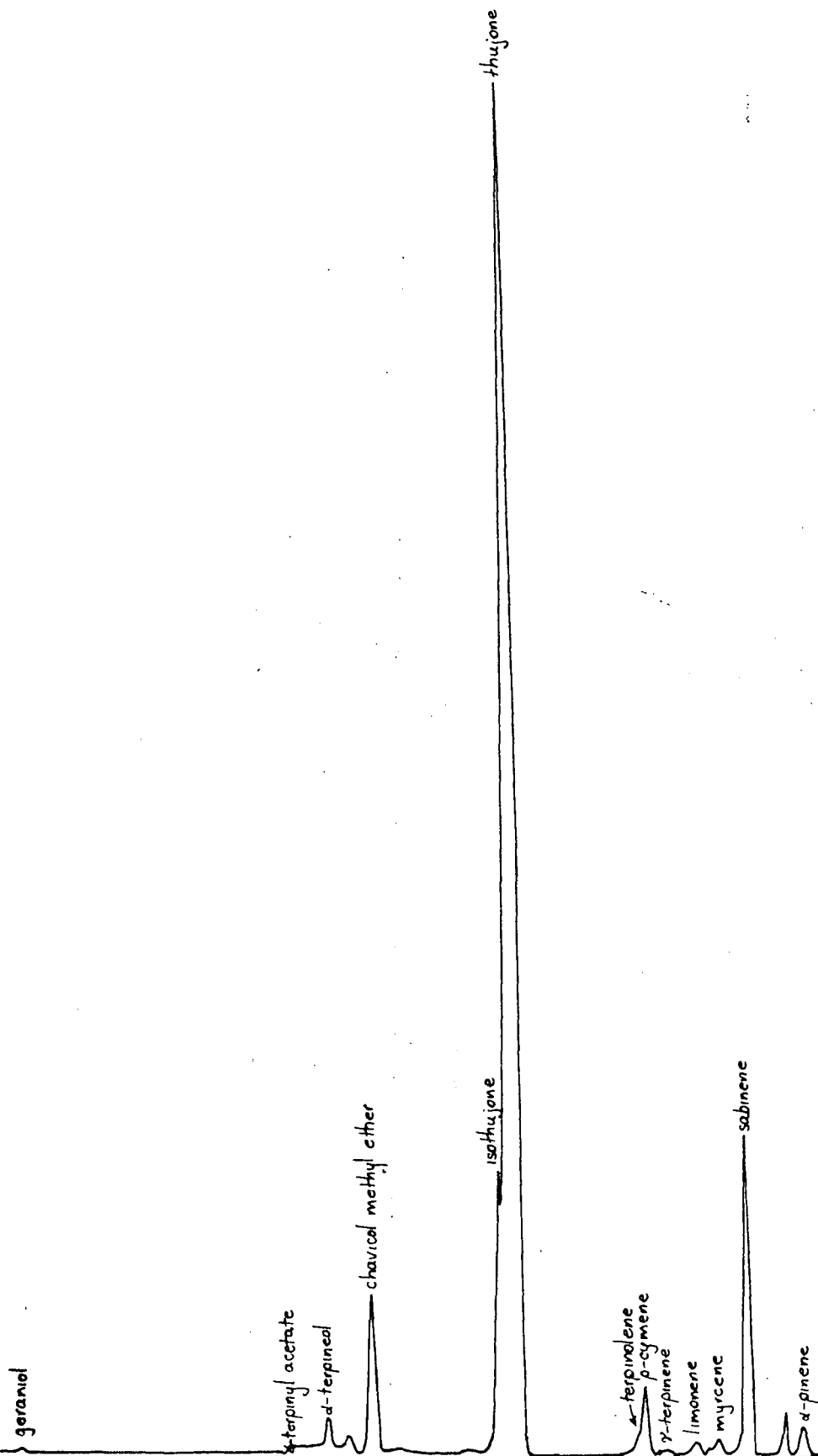
Fig. 89(a).   Low sensitivity gas chromatogram of whole oil of foliage of
Thuja plicata (GC on 5% Carbowax 20M/Gas Chrom Q;   temperature program 50°
to 200° at 5°/min;   0.2 μℓ sample;   attenuation 8 x 10³).
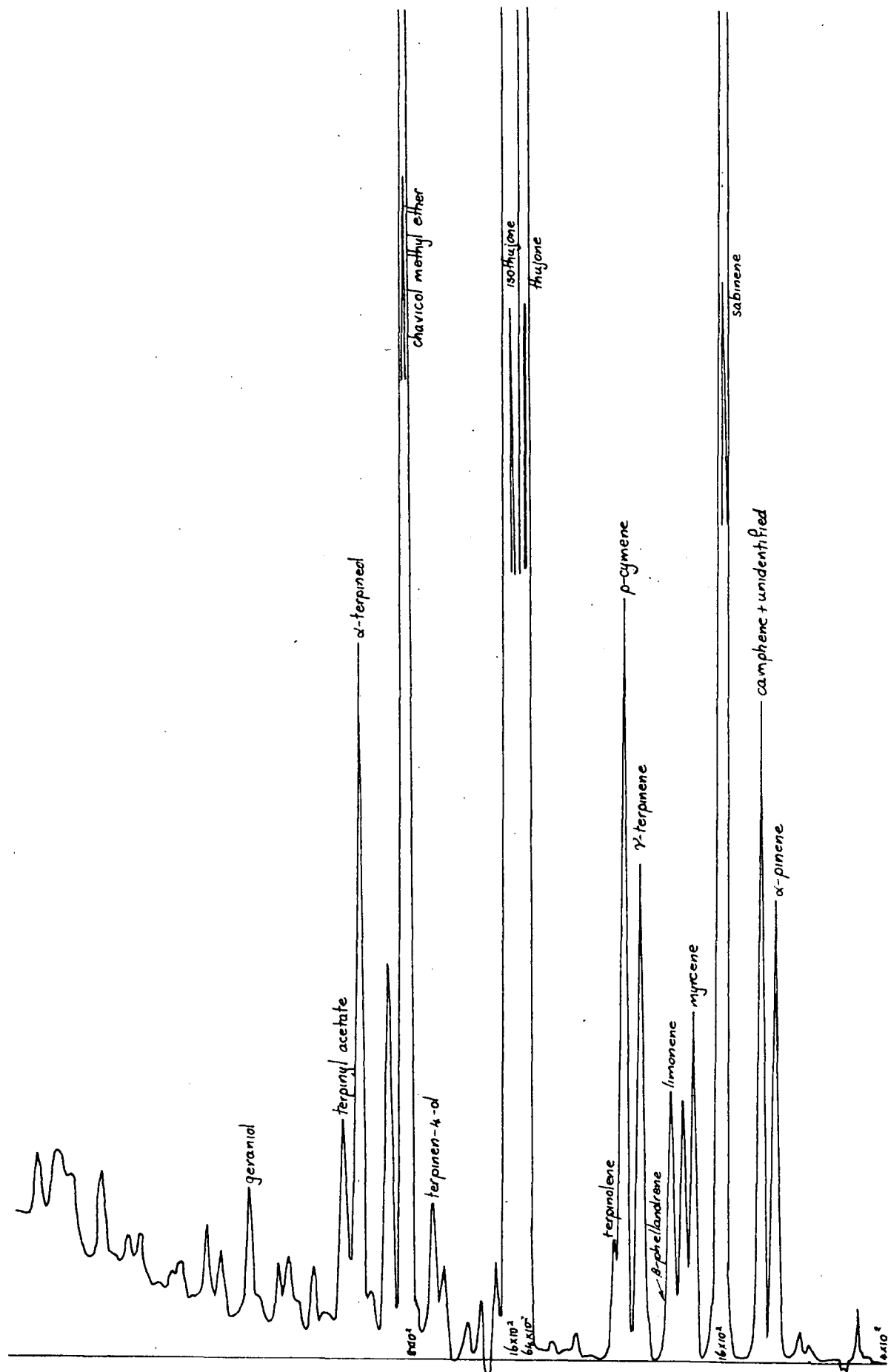
Fig. 89(b).   High sensitivity gas chromatogram of whole oil of foliage
of *Thuja plicata* (attenuation 4 x 10^2).
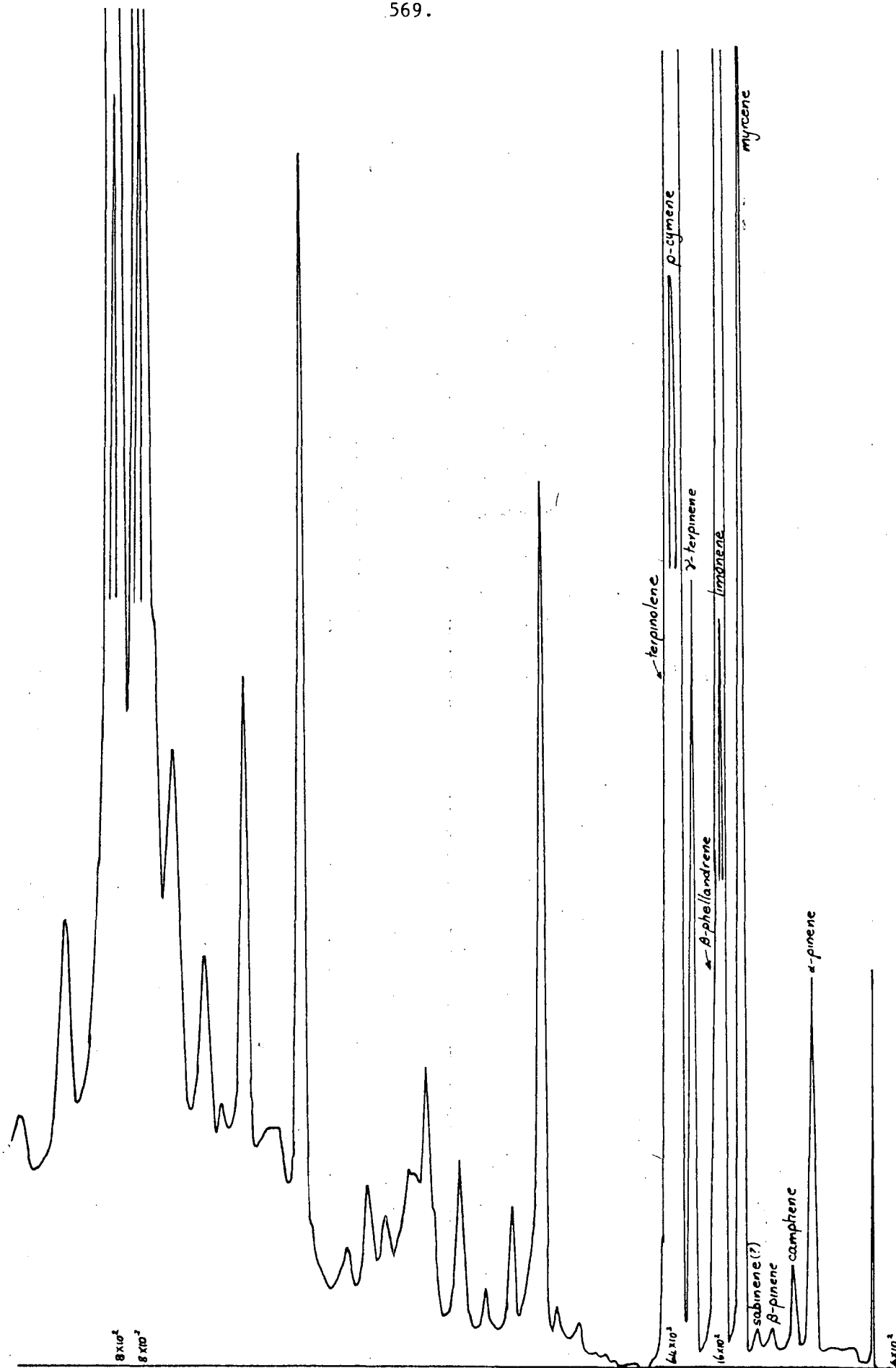
Fig. 89(c). High sensitivity gas chromatogram of fraction A of foliage oil of *Thuja plicata* separated on Florisil.
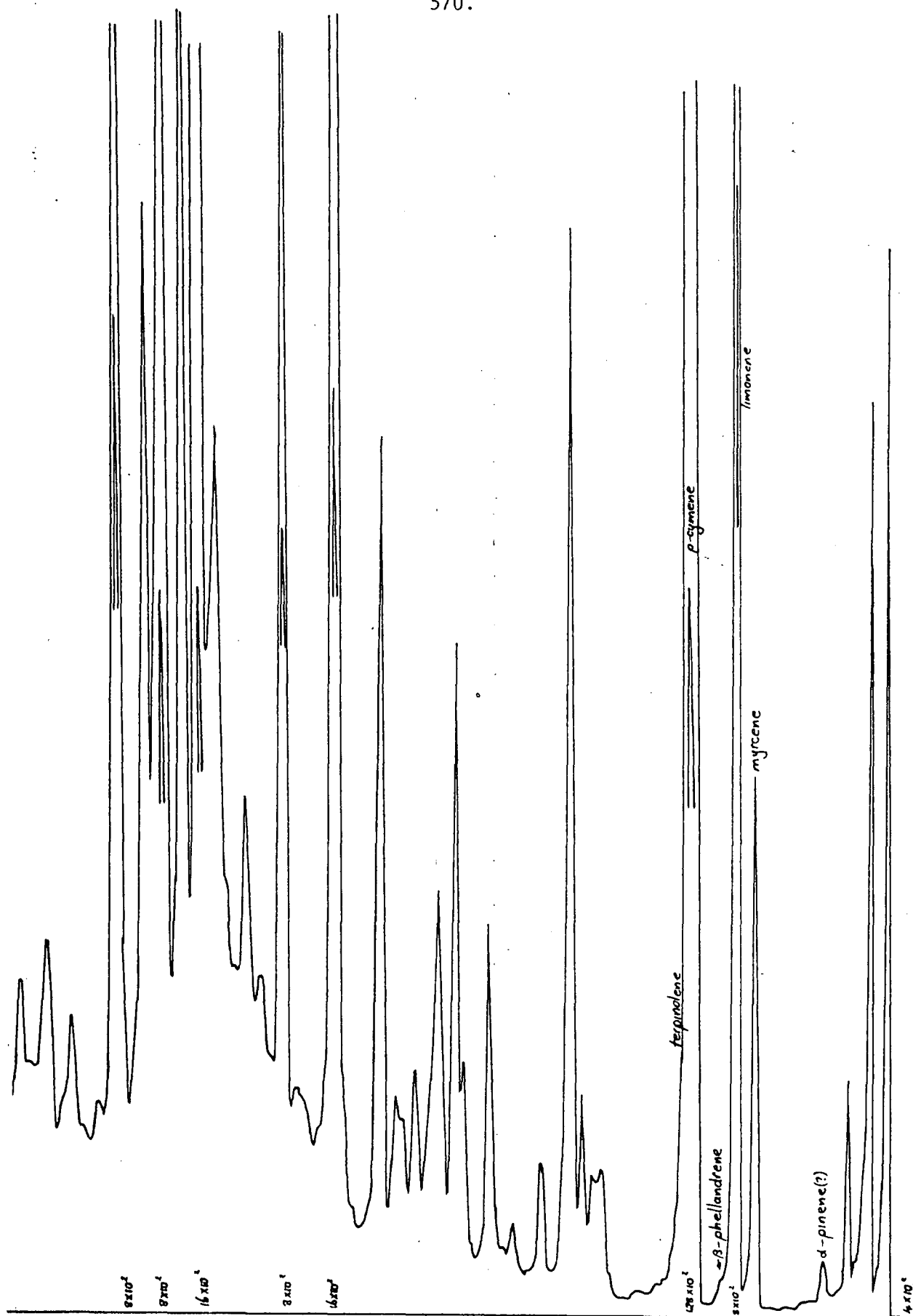
Fig. 89(d). High sensitivity gas chromatogram of fraction B of foliage oil of *Thuja plicata* separated on Florisil.
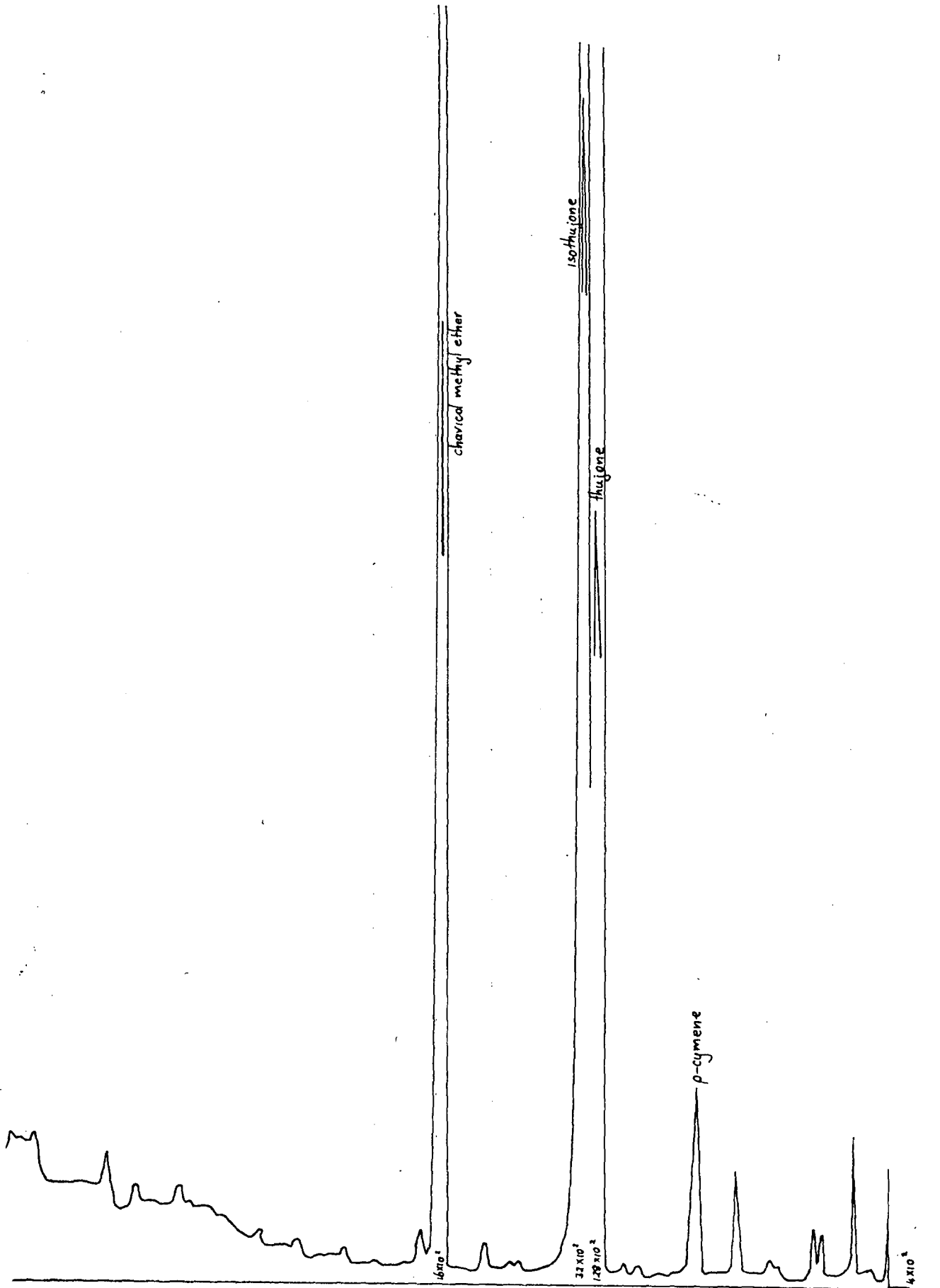
Fig. 89(e).   High sensitivity gas chromatogram of fraction C of foliage oil
of *Thuja plicata* separated on Florisil.
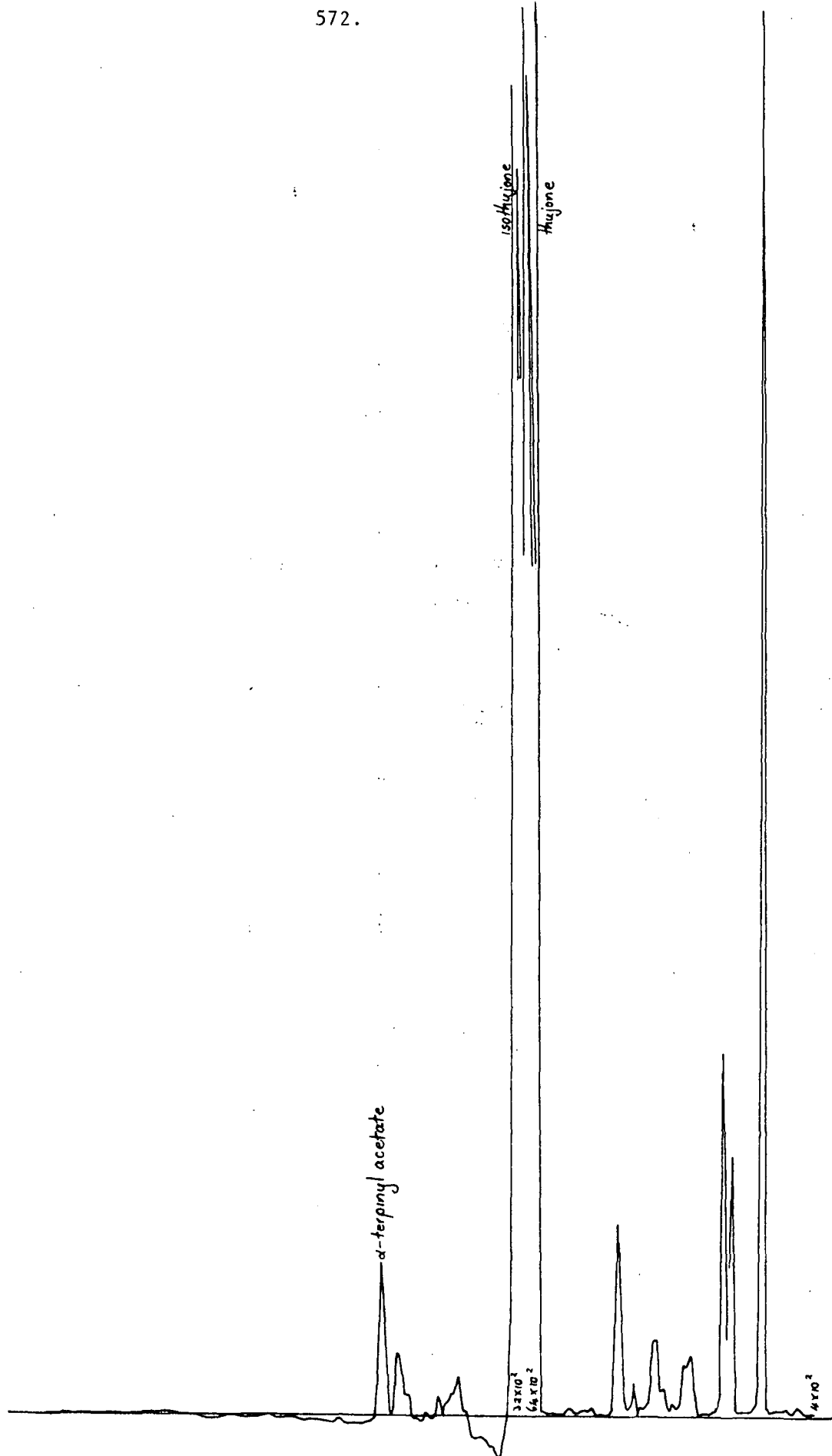
Fig. 89(f).   High sensitivity gas chromatogram of fraction D
of foliage oil of *Thuja plicata* separated on Florisil.
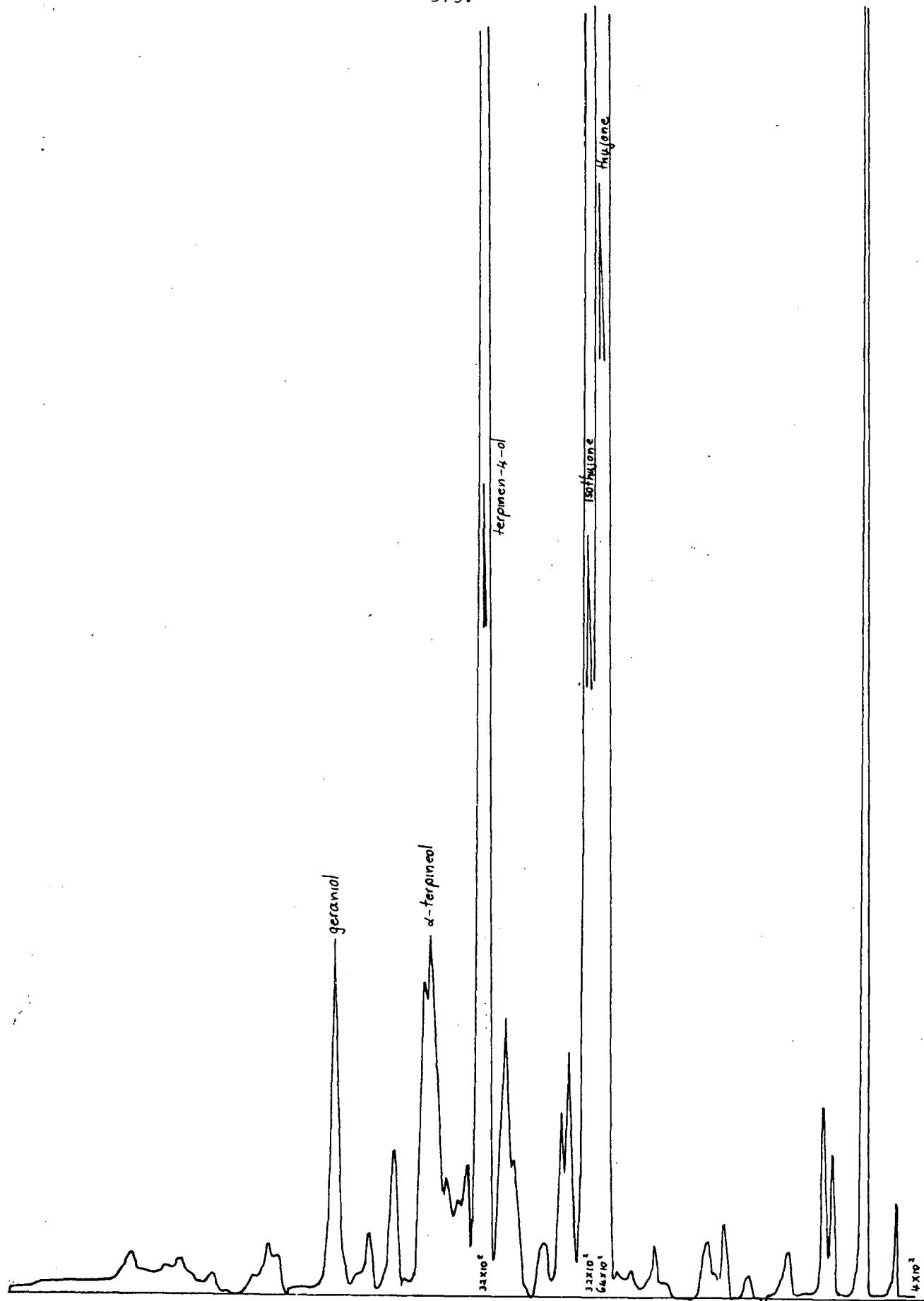
Fig. 89(g).   High sensitivity gas chromatogram of fraction E of foliage oil of *Thuja plicata* separated on Florisil.
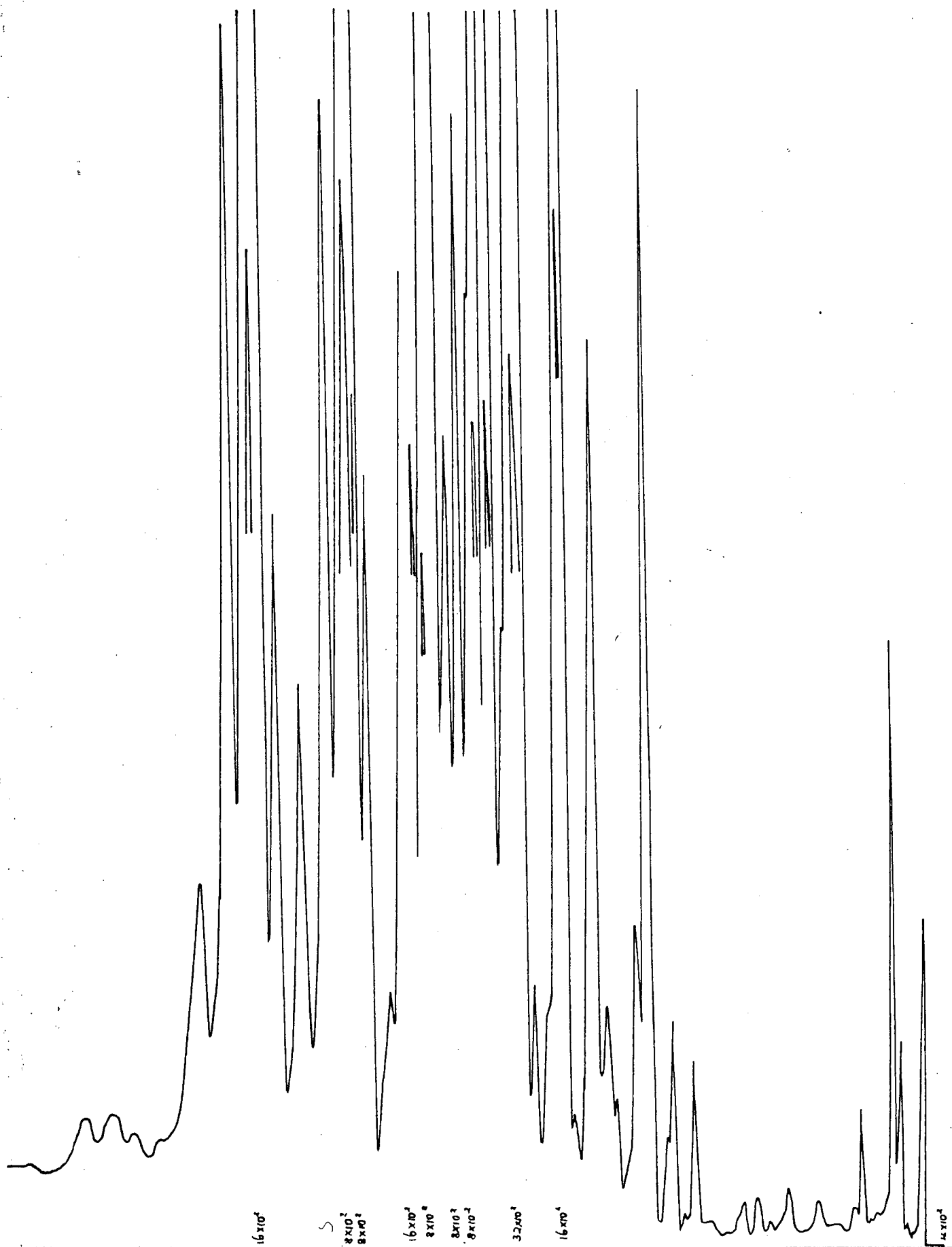
Fig. 89(h).   High sensitivity gas chromatogram of fraction F of foliage
oil of *Thuja plicata* separated on Florisil.

The composition of this oil was found to consist principally of thujone with smaller proportions of isothujone, sabinene, ρ-cymene and chavicol methyl ether. Whereas von Rudloff detected only a few dozen components, this oil is seen from Figure 89 to contain hundreds of components. From Table 130 it can be seen that fractions A and B were hydrocarbons, whereas fractions C to F contained mostly oxygenated compounds. Fractions A and B (Figure 89) contained numerous high-boiling, apparently sesquiterpene components, which are seen from fraction C to have been completely eluted in these two fractions. Fractions C, D and E (Figure 89) were relatively free of the complex of more polar components which in fraction F were so numerous as to prevent any attempt at isolation or correlation of RRT data on both columns.

Whereas von Rudloff only tentatively identified (i.e. identification by RRT data without spectroscopic evidence) γ-terpinene and terpinolene, the identities of these two components were confirmed in this study by IR spectra. The identity was also confirmed of chavicol methyl ether, which had not previously been reported. Other unreported components found, although only identified by RRT data, were β-pinene, myrcene, β-phellandrene, α-terpineol, α-terpinyl acetate, geraniol and possibly α-phellandrene.

(b) Syringe-headspace GC analysis of foliage terpenoids

Syringe-headspace GC analysis of vapour from comminuted foliage similarly enabled the same monoterpene components, thujone and isothujone to be identified (Table 131,

Figure 90). Comparison of the syringe-headspace vapour
composition with that of the steam-distilled oil (Figures 89,
90) shows the expected higher proportions of more volatile
components, i.e. sabinene and α-pinene.

Table 131. RRT data and percentage composition
of volatile terpenoids in foliage of
Thuja plicata determined by syringe-
headspace GC analysis

| Component | Qualitative RRT data | | Quantitative composition |
	C20M	OV-17	(percent, based on peak height)
	(60° isothermal, ref. α-pinene)		(TP 50° to 200°, 5°/min.)
Unidentified	0.74		0.3
α-Pinene	1.00	0.98	11.1
Unidentified	1.16	0.78	⎫
Camphene	1.31	1.17	⎬ 2.5
Sabinene (+ β-pinene)	1.79	1.51	73.7
Myrcene	2.32	1.72	2.7
Limonene	2.85	2.34	⎫ 1.2
β-Phellandrene		2.45	⎬
Unidentified	3.97		0.3
ρ-Cymene (+ terpinolene)	4.52	2.81	0.8
Thujone	10.8	6.39	6.6
Isothujone	12.5		0.5
	(130° isothermal, ref. camphor)		
Thujone	0.71	0.74	
Unidentified	0.99	1.20	0.3
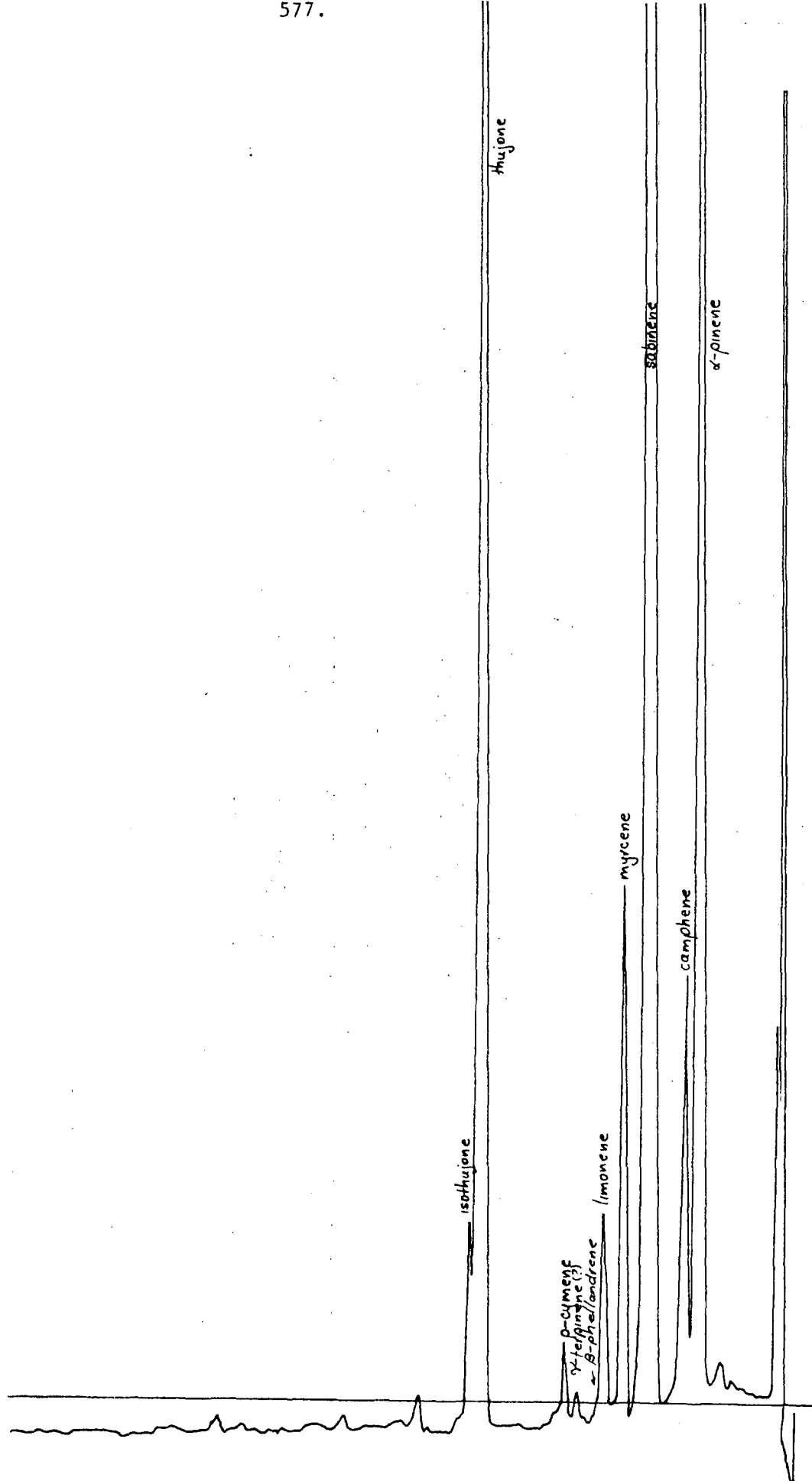
Fig. 90.   Syringe-headspace gas chromatogram of vapour from foliage of
Thuja plicata (GC conditions as before;   attenuation 4 x 10^2).

(c) <u>Comparison of successive injections of syringe-</u>
<u>headspace vapour from foliage</u>

Successive injections of vapour over a 4½ hr. period,
from a single sample of foliage, exhibited minor fluctuations
in the proportions of some components, i.e. less than 4 percent
for each major component (Table 132). Indications of a
reciprocal relationship between thujone and sabinene were
obtained in each series of injections given in Table 132.
Figure 91 illustrates corresponding fluctuations in sabinene
and terpinolene in the absence of similar changes in proportions
of other components. Further study should be undertaken to
document the nature of any detectable biosynthetic
relationship between these components.

(d) <u>Composition of syringe-headspace vapour from foliage</u>
<u>sampled repeatedly from the same tree</u>

The composition of the vapour from three samples of
foliage from the same tree varied (Tables 131, 132) from
11.1 to 12.1 percent α-pinene, 49.5 to 73.7 percent sabinene
and 6.6 to 27.6 percent thujone.

The extremely wide variation in the composition of
the three random samples from this tree could be of value in
a future study of biosynthesis based upon the principle of
quantitative co-occurrence. Von Rudloff found very little
variation in oil composition from different samples of
foliage from the same tree [324].

Table 132. Composition of monoterpenoids in
successive injections of syringe-
headspace vapour from foliage of
Thuja plicata

Percentage composition of monoterpenoids
(peak height basis):

Time since comminution of sample (mins.)	Unidentified group	α-Pinene	Unidentified (+ camphene)	Sabinene (+ β-pinene)	Myrcene	Limonene (+ β-phellandrene)	Unidentified	Thujone	Isothujone
(Sample 1)									
0	0.5	11.8	3.6	49.5	2.6	1.5	1.1	27.6	1.8
35	0.5	13.0	3.7	50.6	2.3	1.3	1.0	25.9	1.6
60	0.4	13.2	3.6	50.5	2.3	1.3	1.0	26.0	1.6
85	0.6	13.1	3.4	49.9	2.3	1.3	1.0	26.8	1.7
105	0.5	12.8	3.4	49.6	2.3	1.3	1.0	27.3	1.7
130	0.4	12.4	3.2	49.9	2.4	1.3	1.0	27.7	1.7
155	0.5	12.9	3.1	50.4	2.3	1.2	1.0	27.0	1.7
180	0.3	12.4	2.9	50.0	2.1	1.2	1.0	28.3	1.7
215	0.3	12.7	2.9	49.9	2.2	1.2	1.0	28.0	1.7
230	0.5	12.4	3.0	49.7	2.3	1.3	1.0	28.0	1.8
255	0.4	12.2	3.0	50.2	2.3	1.3	1.0	27.8	1.8
280	0.4	12.4	2.9	49.5	2.3	1.3	1.1	28.4	1.8
(Sample 2)									
0	0.4	12.1	2.9	53.1	4.1	1.6	0.9	23.4	1.4
25	0.7	13.2	3.8	50.8	3.7	1.3	0.8	24.2	1.4
55	0.9	12.7	3.8	50.9	3.4	1.3	0.8	24.6	1.5
95	0.9	12.0	3.8	50.7	3.3	1.3	0.8	25.6	1.5
120	0.9	11.7	3.8	49.6	3.4	1.4	0.9	26.8	1.5
150	1.4	11.9	3.7	51.1	3.3	1.3	0.8	24.9	1.4
175	1.6	11.6	3.7	49.5	3.3	1.4	0.9	26.4	1.6
200	1.7	11.4	3.5	49.5	3.3	1.3	0.9	26.9	1.6
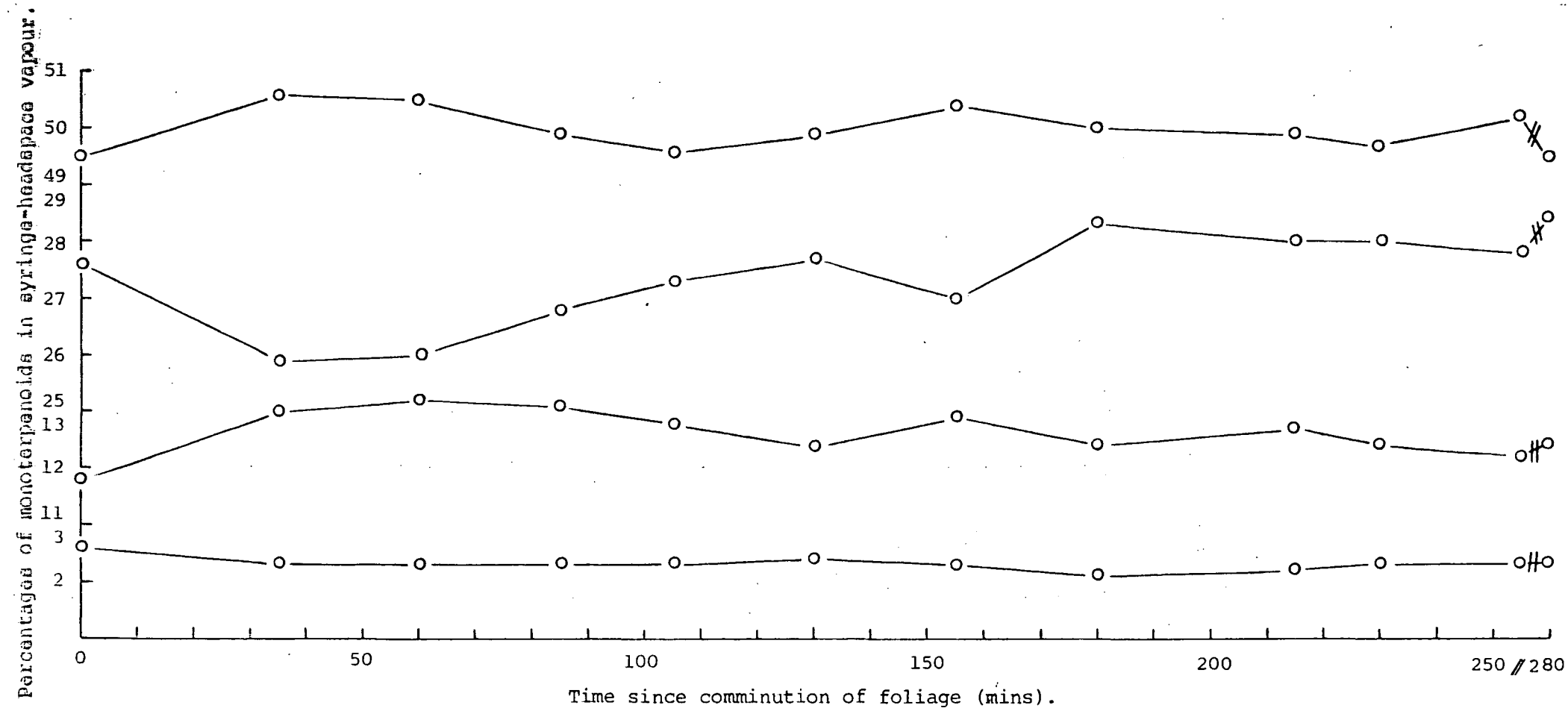
Fig. 91. Composition of monoterpenoids in successive injections of syringe-headspace vapour from a single sample of foliage of *Thuja plicata* (sample 1 of Table 132). A possible reciprocal relationship between sabinene and thujone might be attributed to a biosynthetic link between these components.

580.

(e). Summary

Components of the steam-distilled foliage oil of
Thuja plicata were analyzed by chromatographic and spectroscopic
methods. The following identifications were confirmed:
sabinene (13.3%), γ-terpinene (0.4%), terpinolene (0.3%),
thujone (57.5%), terpinen-4-ol (0.1%) and chavicol methyl
ether (6.7%). Tentatively identified were: camphene,
β-pinene, myrcene (0.7%), limonene (0.5%), β-phellandrene,
ρ-cymene (2.9%), isothujone (11.7%), α-terpineol (1.5%),
α-terpinyl acetate (0.3%), geraniol (0.1%) and possibly
α-phellandrene. Identifications of γ-terpinene, terpinolene
and chavicol methyl ether were confirmed in this oil for the
first time. Components tentatively identified, but not
previously reported, were β-pinene, myrcene, β-phellandrene,
α-terpineol, α-terpinyl acetate, geraniol and possibly
α-phellandrene.

The syringe-headspace GC technique provided a
convenient means of examining the monoterpene vapour
compositions released from foliage to the atmosphere.
Successive injections of vapour over a 3 hr. period, from
a single sample of foliage, varied within a narrow range,
i.e. 11.4 to 13.2% α-pinene, 49.5 to 53.1% sabinene and
23.4 to 26.9% thujone. Further study of successive injections
of vapour may confirm the nature of a possible biosynthetic link
between sabinene and thujone. The compositions of vapour from
3 samples of foliage from a single tree ranged from 11.1 to 12.1%
α-pinene, 49.5 to 73.7% sabinene and 6.6 to 27.6% thujone.

The wide variation encountered in samples from the same tree could be of value in future studies of biosynthesis based upon the principle of quantitative co-occurrence.

B.   Summary of investigations of Woodwasp-attacked conifers

A study was made of earlier inferred changes in the compositions of essential oils that might have resulted from the wounding of a tree and consequent attraction it may have for the Woodwasp, *Sirex noctilio*. As well, 19 species of conifers reported to have hosted breeding females of *S. noctilio*, were examined with respect to both steam-distillable oils and monoterpenoid vapours released to the atmosphere.

Widely varying compositions were detected for monoterpenoids in bark and foliage sampled from injured trees. The variations in oil composition greatly exceeded the well-known differences due to replicate sampling and experimental error. Variations in composition studied over periods of time were often complex and difficult to correlate, e.g. with the 14 day period of attractiveness after felling a tree. Greater variations were obtained in oil from bark tissue injured by stripping from the wood, compared with the changes found in bark still attached to a felled and even a standing tree. Variations in oil composition were accompanied by wide variations in the contents of moisture,

rosin and oil. It was concluded that physiological changes upon wounding may lead to changes in essential oil composition. Some of the variations found in oils from representatively-sampled bark from a single tree trunk were: 0.0 to 2.2 percent rosin (dry bark basis), 0.18 to 0.78 percent oil (dry bark basis), 15.7 to 20.3 percent α-pinene, 54.8 to 68.2 percent β-pinene, 9.0 to 18.3 percent limonene (+ β-phellandrene) and 2.5 to 6.7 percent myrcene.

To confirm whether a particular type of injury leads to specific changes in oil composition would require further study on a statistical basis.

The 19 species of conifer were each studied, where practicable, using conventional oil isolation and terpenoid identification techniques. Oils from cortical oleoresin and foliage were compared. In addition the syringe-headspace GC technique was used to identify more volatile terpenoids released from foliage tissues to the atmosphere at room temperature. This simple technique also facilitated multiple examinations of foliage vapour from the same tree and from several trees of a species. Successive injections of vapour from a single sample of foliage, over a period of several hours, often indicated changes in oil composition perhaps initiated by comminution of the foliage. The variations in composition were however not as wide as previously encountered in the bark oil experiments. Such changes were mostly quantitative, although there were indications of possible qualitative changes, particularly with respect to a component that could constitute up to several percent of the vapour

in initial injections, but could not be detected some minutes later. Components in successive injections often appeared to fluctuate in proportional or reciprocal relationships, that suggested biosynthetic links between one another.

Daily samples of foliage from felled branches of *Cedrus deodara, Pinus pinaster* and *P. radiata* were examined over a period of 18 days using the syringe-headspace GC technique. Considerable variations in vapour composition were detected. A remarkable change in vapour composition was found from day 13 in the foliage of *Cedrus deodara*. The change appeared to be due to the rapid loss of needles at this time. Subsequent injections of a quite different vapour were from very fine twigs. Although the two *Pinus* spp. did not lose all their needles in a short period of time, a notably large fluctuation in vapour composition occurred between days 14 to 16 in *P. pinaster*, and on day 13 in *P. radiata*. Several other fluctuations were documented at different times in each species.

No common quantitative composition was discernible which could be correlated with the attractiveness of each species to *S. noctilio*. In fact a range of monoterpenoid compositions was encountered in each species, i.e. from different samples of foliage, from foliage and bark, and between different trees. Trees of some species were even so diversified in oil composition (quantitatively) that the oils barely resembled one another, e.g. *Pseudotsuga menziesii, Pinus muricata, Pinus montezumae*, etc.

The concept of an attractive terpenoid mixture is difficult to sustain when it is remembered that the flying insect attracted to a specific tree must be detecting a very complex and fluctuating mixture of odours about a forest. From a single tree there are usually different proportions of monoterpenes emitted from foliage and from bark. As well, there are further vapour compositions emitted from different samples of foliage, from buds, young and old needles, bark from twigs and sometimes from bark from different heights up a trunk. The concept of an attractive mixture in insect-attractant studies is favoured by repeated demonstrations of various degrees of attractiveness of different terpenoids.

The indication in this study of the existence of qualitative changes in conifer oils must lend weight to the alternative concept of a specific attractant component. There is however a real difficulty in concluding that two oils are qualitatively different, since it is presently not feasible to demonstrate the absence of a component which may still be present as a minute trace. When a component is present in foliage vapour at a few percent concentration, and is shown to rapidly decrease within a few minutes to a concentration smaller by 2 orders of magnitude, then an "apparently qualitative change" has been detected. Several components were indicated which might cause a qualitative change in an oil vapour. Future study of insect attractants should involve the use of techniques, such as syringe-headspace GC, which may enable 'temporary components' to be indicated.

SYRINGE-HEADSPACE GC STUDY OF TERPENOID

BIOSYNTHESIS: A PRELIMINARY NOTE

1.   Introduction

Most tracer studies have merely demonstrated the
incorporation of mevalonate or acetate in monoterpene
structures.   Experimental data from basically different
approaches is therefore required which may associate
individual monoterpenes with specific biosynthetic routes,
e.g. as outlined in the hypothetical scheme (Figure 15) by
Ruzicka [356].   Results of tracer studies by Sandermann
[449, 542, 543], Banthorpe and co-workers [539, 544] are
sometimes contradictory, and thereby indicate the need for
further investigation involving a basically different
procedure.   In other instances tracer studies can only lead
to ambiguous results.

Zavarin [357] has advocated the utilization of
qualitative and quantitative co-occurrence of natural
compounds to establish biosynthetic hypotheses.   Such a
basically different approach may also provide evidence to
support conclusions from tracer studies.   Banthorpe *et al*
[545] have however regarded this approach as essentially a
pre-tracer technique, which has since been rendered more
ambitious because of its mathematical foundation.   These
workers considered that conclusions from Zavarin's approach
were trivial compared with information from tracer studies.

It was conceded however that the approach enabled deduction of the genetically-controlled formation of a particular precursor, such as a carbonium ion, which would explain the occurrence in oils of terpenoids in fixed ratios.

The concept of qualitative co-occurrence, based upon statistical incidence of particular compounds occurring together in a large number of natural sources, has often been used to intimate a biosynthetic relationship, e.g. of tricyclene, bornylene and bornyl acetate. Quantitative co-occurrence however, relies upon a demonstrated mathematical relationship between proportions of components in particular oils, e.g. the linear relationship between camphene and bornyl acetate or tricyclene in *Abies* species. A proportional relationship between two components indicates a common precursor, e.g. Ruzicka's 2-bornane carbonium ion precursor of camphene and other components with the bornane skeleton.

A particular advantage of the quantitative co-occurrence method was in the distinction of two alternative routes to terpinolene [357]. According to Ruzicka's scheme terpinolene may arise through either the 1-ρ-menthene-8-carbonium ion or the 1-ρ-menthene-4-carbonium precursor. A proportional quantitative relationship between terpinolene and Δ_3-carene in oil of *Abies amabilis* suggested that terpinolene arose via the 1-ρ-menthene-8-carbonium ion. It would otherwise be difficult from a structural point of view to propose the formation of Δ_3-carene from the 1-ρ-menthene-4-carbonium ion.

Unfortunately the data used by Zavarin in the quantitative co-occurrence technique was derived largely from the analysis of many oils that exhibited considerable variation in each of the components of interest. By contrast, the measurement of changing proportions of components in the oil of a particular plant source would, because of its direct nature, appear to constitute more valuable evidence of a biosynthetic relationship. Quantitative changes in oils of developing buds, leaves and twigs might be used to demonstrate biosynthetic links between components. In the development of buds to new leaves of *Picea mariana* [325] santene, tricyclene, camphene and borneol increased as a related group in the oil, from trace to major proportions. Von Rudloff consequently concluded a close biosynthetic relationship between these components.

2. Syringe-headspace GC technique as a means of studying biosynthetic relationships

Successive injections of syringe-headspace vapour from a single sample of foliage has often in this study indicated the existence of quantitative relationships between fluctuating proportions of some components. Care must be taken however to ensure that a spurious relationship is not inferred in a two-component mixture, where a percentage change in one component may be compensated by a similar change in the second component.

Changes in the proportions of monoterpenes have however been found to often be complex, so that a relationship between two components may not always be apparent. In a further situation the relationship may not be exhibited throughout the several-hour period of successive injections.

Relationships appeared to exist between the following components:

Cedrus deodara (daily samples over 18 day period, Fig. 47)

α-pinene inversely with myrcene and unidentified component,

β-pinene inversely with limonene;

Pinus contorta (3 hr. period, Fig. 58)

β-pinene inversely with β-phellandrene;

Pinus pinaster (daily samples over 18 day period, Fig. 72)

α-pinene inversely with myrcene, limonene and unidentified component;

Pinus radiata (3 hr. period, Fig. 31)

α-pinene inversely with Δ_3-carene,

(daily samples over 18 day period, Fig. 79)

β-pinene inversely with limonene and Δ_3-carene (days 10-18),

α-pinene inversely with Δ_3-carene (days 0-10);

Pseudotsuga menziesii (3 hr. period, Fig. 87)

β-pinene inversely with terpinolene(?)

and *Thuja plicata* (4 hr. period, Fig. 91)

sabinene inversely with thujone.

Most of the relationships are in accord with the biosynthetic scheme by Ruzicka (Figure 15). It is particularly significant that each of the above possible relationships occurred in the

presence of other components, which showed no corresponding
fluctuations. In each case it would appear that conversion
of a common precursor to a particular monoterpene product
led to a corresponding decrease in the amount of an alternative
product.

Similar evidence of biosynthetic relationships may
also be obtained from relationships between components in oils
steam-distilled from bark of a wounded tree, e.g.

β-pinene inversely with ρ-cymene and limonene

in daily samples from *Pinus radiata* (Figure 39).

3.   Conclusions

Changes in the proportions of terpenoids in wounded
plant tissue, either foliage or bark, appear to indicate
relationships of biosynthetic origin.

The presence of very complex changes, together with
relationships exhibited during part only of a period of
investigation, indicates the need for detailed study to
exploit the full advantages of this technique.

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