A PRELIMINARY SURVEY OF SELECTED SPECIES OF ENDEMIC PLANTS TO DETERMINE COMMERCIAL CROPPING POTENTIAL FOR ESSENTIAL OILS.

by

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SUMMARY

This work was embarked upon as a preliminary survey of the Tasmanian endemic essential-oil-bearing plants, with a view to locating species which may be suitable for commercial production of oil.

Initially, twenty plant species were selected as having been observed to contain an essential oil. The species were : *Bedfordia salicina, Beyeria viscosa, Callitris tasmanica, Cassinia aculeata, Drimys lanceolata* (also known as *Tasmannia lanceolata*), *Eriostemon virgatus, Kunzea ambigua, Leptospermum glaucescens, Leptospermum lanigerum, Leptospermum scoparium, Melaleuca squamea, Melaleuca squarrosa, Olearia argophylla, Olearia phlogopappa, Phebalium squameum, Prostanthera lasianthos, Senecia linearifolius* and *Zieria arborescens*. Material from these species was taken for steam distillation and solvent extraction, and the oil samples, thus obtained, were analysed by GC-MS. In addition, data on yields of oil were compiled, in order that comparisons between species could be made.

The number of species under investigation was reduced to ten, by excluding those that did not possess a persistent or powerfully pleasant odour. The other major criterion used in determining which species may be of commercial value was oil yield, which was not to be less than 0.1%. The species that were retained were as follows: *B. viscosa, C. tasmanica, D. lanceolata, E. virgatus, E. amygdalina, K. ambigua, L. glaucescens, L. lanigerum, O. phlogopappa* and *P. squameum*.

Comparisons between the estimated yield of these species, on a per hectare basis, with that of commercial essential oil crops, showed that they have the potential to produce similar quantities of oil.

Scanning electron microscope studies of the oil glands of the ten selected species were undertaken. These showed definite similarities in structure, both among themselves and with glands described in the literature.

Small-scale propagation trials were run with the species listed above. Many were found to grow readily form cuttings, whilst others were easily raised from seed, and all of the ten species were propagated by

one or other of these means. However, tissue culture techniques would vastly improve the efficiency of any large scale propagation project.

The GC-MS results showed the preesence of many commercially important compounds, as well as one which had not been cited previously in the literature, namely epi-γ-eudesmol in *Olearia* spp. Compounds which were identified by this method were quantified by determining the area under the relevant peak. In addition, the oils from these species were examined using a gas chromatograph which had been equipped with a splitter, so that the various components could be described as they were vented from the column.

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1. INTRODUCTION

The flavour and fragrance industry today is constantly searching for new sources of interesting essential oils. With the changing "fashions" in flavours and fragrances, and the increasing costs of many of the synthetically produced starting materials and additives, such new sources are always highly desirable. Since there are only so many different impressions which can be created by modification of a particular oil with "standard" additives, the search for novel oil components can be rewarding.

The designation of commercial essential oil is not always an accurate indication of their source or quality. For instance, "Clove Oil" may be genuine clove bud oil or one obtained from clove stems or leaves and rectified to bring its profile and analytical characters nearer to that of the genuine bud oil; or it may be a blend from all these sources. It is only necessary to critically examine a range of samples of the oil to realize the wide spectrum of profiles that exists. In some countries, however, oils that have been sophisticated or extended by addition of synthetic versions of the major compounds must be labelled "imitation".

When dealing with blends, taste and effect required in the end-product determine what grade is used. However, it is fairly certain that any pretensions to quality or authenticity will depend entirely on the amount of genuine oil that is present.

Essential oils have an astonishingly wide and varied application for the flavouring of foods, beverages, confectionery, biscuits and flour confectionery and in tobacco. In addition, they are used in the creation of fragrances for cosmetics, toiletries and industrial deodorants. Many of them are powerful external and internal antiseptics, carminatives etc., of proven medicinal value. There is barely an industry that does not make use of these versatile natural products.

Many of the prime components of an essential oil may be separated and purified to give chemical entities called "isolates". In many instances, these isolates can be produced more economically than an identical compound can be prepared synthetically, although this will depend on the current market value of the starting material, and its availability. Some typical natural isolates are :

Menthol - isolated from Japanese mint (*Mentha arvensis* var *piperascens* D.C.) by freezing.

Eucalyptol (Cineole) - isolated from eucalyptus oil (from *E. globulus* Lab.) by freezing.

Linalool - isolated from oil of Bois de Rose by fractional distillation.

Eugenol - isolated from clove oil by means of alkali and re-distilled.

There are numerous other examples, some of which are available as both natural isolates and synthetic chemicals. In all cases, the isolates exist in the natural material in the same chemical identities as they are recovered. These materials can then be used for the production of further derivatives. For example, *iso*-eugenol from eugenol

Australia has a relatively un-researched collection of essential oil bearing plants, with *Eucalyptus* being the only widely recognised oil producing genus. However, the virtues of *Melaleuca alternifolia* are slowly being accepted and the value of this oil to the medical and pharmaceutical professions is already established. However, there are many families that have oil bearing members, constituting many species which have not been investigated. In Tasmania, the situation is the same; no endemic species has been used as a commercial oil producing crop to date. There are almost one hundred families of native plants in Tasmania. Of these, the following six have the most species containing essential oils :

Compositae (now known as Asteraceae),

Labiatae (now known as Lamiaceae),

Euphorbiaceae,

Myrtaceae,

Rutaceae,

and Umbelliferae (now known as Apiaceae).

To survey the entire collection of endemic flora would indeed be a mammoth task, therefore this study concentrates primarily on common, low-altitude examples of those families abundant in odoriferous members.

2. LITERATURE REVIEW

2.1 THE ECONOMIC SITUATION

2.1.1 ECONOMIC OVERVIEW

The growing impact of economic forces on the world's flavour and fragrance industries has been forcing perfumers and flavourists to reappraise their formulations more critically than ever before. In addition, it is seldom advantageous for the producers to attempt to over-exploit modern equipment in attempts to boost their revenue by acquiring the additional equipment needed for refining or otherwise re-processing of the oil. In spite of a gradual transfer of refining technology away fom the industrialised countries, the fact remains that most blenders prefer their raw materials to be in as basic a form as posible in order to give them the maximum latitude for modifying them in accordance with their specific requirements. Even where they are prepared to purchase ready-modified, deterpenated oils, they remain much more inclined to resort to the major established processors with whose products they have been familiar over a long period of time.

2.1.2 THE USE OF SYNTHETIC COMPOUNDS

At the user end of the essential oils market, the increasing cost of compounding has over the years brought about a very sharp decline in the number of major product manufacturers who undertake their own compounding. The position of the large perfume and flavour compounding companies, particularly multinationals, has been correspondingly strengthened. At the same time, the increasing financial constraints imposed upon end-users by world economic conditions generally have brought about demands for sharp reductions in the cost of perfume and flavour compounds, not only in real terms, but often in actual money terms.

The effect of such demands is that of a progressive reduction on the part of perfumers in their dependence on natural essential oils, particularly the more expensive ones, and an increase in the proportion of relatively cheap synthetic ingredients used to give a desired effect.

Unfortunately, the competitive pressure on natural essential oils from synthetic perfumery and flavouring materials is not moderating, in spite of the long-standing expectation that successive increases in the

world price of petroleum oil would be rapidly and fully reflected in the prices of petroleum-based perfumery and flavouring isolates. As a consequence of the petroleum industry holding down the price of this group of derived products, their competitiveness and comparative price stability compared to natural essential oils has not been affected sufficiently to bring about an appreciable resurgence in the use of natural oils.

Of course, the quality of very many natural essential oils is more variable than that of many synthetics. Natural oils are prone to sharper fluctuations in price levels than are synthetics on account of the effect of crop failures, other unpredictable influences, and the greater lack of knowledge on the part of all concerned of developments elsewhere in the production and marketing chains.

In spite of these adverse forces, however, the volume of natural essential oils traded on the international market is still considerable, and a few influences still work in their favour. Firstly, certain types of oil stubbornly ward off all attempts at synthesis at a reasonable cost; woody type oils such as those of patchouli and vetiver are good examples, but there are many others. Other oils, either by virtue of their being by-products or on account of their being technically simple to produce, remain very cheap in relation to the synthetic competition and continue to hold their own, an obvious example is orange oil.

The relentless advance of legislation in relation to the use of synthetic ingredients in final products also works in favour of natural oils. Health considerations are important, but the underlying public preference for 'natural' products is also a powerful factor (Robbins, 1983).

2.1.3 PROSPECTS FOR SELECTED OILS

The demand for cineole-rich oils (such as that of *Eucalyptus globulus*), particularly those conforming to international and Pharmacopoeia standards, seems buoyant. These oils have been comparatively free from undue price fluctuations since 1975.

While existing suppliers appear to be keeping abreast with demand, it is possible that in the longer term a new producer of an oil of consistently very high cineole content would find a regular market for his product. There has also been some trade interest in eucalyptus oils rich in phellandrene and piperitone, such as *Eucalyptus dives* (Robbins, 1983).

2.2 HISTORY OF AUSTRALIAN ESSENTIAL OILS

In Australia's history, essential oils have played a part even from the earliest establishment on the shores of Port Jackson by Captain Cook. *Eucalyptus piperita* was reputed to be useful in the removal of digestive complaints in the same manner as peppermint oil (Lassak and Murray, 1980). *Eucalyptus globulus* also became an important article of trade. However, the oils reaching Europe were of variable quality, due partly to a confused taxonomy of eucalypt species. It was not fully realized that different species yielded different oils.

J.H.Maiden recognised the vastness of the Australian indigenous flora and the enormous potential it offered for chemical research (Maiden, 1889). By 1920, some 300 plant species had been investigated by Smith and Baker and 40 compounds from Eucalyptus oils alone had been characterised. Much of this work was published in the early 1900's (Baker and Smith, 1910,1920). At about the same time, it was realised that there was an interdependence between chemical constitution and botanical classification of plants.

Separate taxa were established with chemical, and corroborative morphological, evidence. Thus, the numerous cases of wide and apparently qualitative variation in leaf oil composition within groups of morphologically indistinguishable individuals belonging to the same species led, in the 1920's, to the proposal of the existence of "physiological forms".

Subsequent research has shown this phenomenon to be of common, widespread occurrence in the native Australian essential oil bearing flora. Apart from several genera of the Myrtaceae family (*Eucalyptus, Backhousia, Leptospermum* and *Melaleuca*), chemovars have been reported in the Rutaceae (*Boronia, Zieria, Geijera*), Myoporaceae (*Myoporum*) and others.

Lawrence, (1980) investigated the well known existence of chemical types of individual species within the Labiatae family. He defines intraspecific differences as chemical differences that exist in morphologically identical species. A number of apparent variabilities found to exist in the chemical composition of an essential oil can be related to :

a) mis-identification of the plant

b) analysis of mixed populations

c) component mis-identification or

d) ontogenetical dissimilarities.

By the end of the World Wars, research into essential oils had been carried out into *Agonis, Cinnamomum, Daphnandra, Eremocitrus, Santalum* and *Melaleuca*. Terpenoid chemistry was also flourishing. In particular, the phellandrenes and their reactions and biosyntheses were being investigated.

The bactericidal properties of Australian essential oils and essential oil components were first studied in the 1920's by Penfold *et al*, who found that the leaf oil of *Melaleuca alternifolia* was particularily effective. This work was broadened by Dr. Nancy Atkinson, who added the flower oils of *Darwinia citriodora, Agonis linearis* and *Chamaelaucium uncinatum* to the growing list of essential oils.

Research into essential oils decreased from the mid 1960's on, except for studies into the metabolism of eucalyptus oil by koalas and possums; the fungal hydroxylation of piperitone as well as various chemotaxonomic studies of *Eucalyptus, Zieria* and *Prostanthera*.

2.3 AUSTRALIAN ESSENTIAL OIL BEARING FAMILIES AND GENERA

More recently, two families, previously overlooked, were investigated for essential oils. These were the Labiatae and Asteraceae. Over 40 species of *Prostanthera* (Labiatae) were examined for yield and composition and found to reflect the botanical classification of that genus. The section Euprostanthera series Racemosae give high yields of 1,8-cineole rich oils, whereas the Subconcavae series were poor or devoid of oil. Species in the Klanderia section gave oils rich in the rare sesquiterpenoid alcohol maaliol. *Kippistia suaedifolia* F.U.M.(Asteraceae) is a plant rich in the unusual ester $\hat{d}$-perillyl acetate (ca. 65% of the oil) Brophy *et al*, 1980.

Only about 10 of the 80 species of *Prostanthera* have received chemical attention. The work by Lassak in Australia is valuable in re-organising this somewhat confused genus.

The essential oil bearing members of the indigenous Australian flora contain an abundance of commercially useful and structurally interesting isolates. The family Myrtaceae alone contains well over one thousand endemic species spread among such oil rich genera as *Eucalyptus,*

Melaleuca, Leptospermum, Backhousia, Baeckea, Darwinia etc.

Lassak and Southwell attempt to give an updated list of isolates, made necessary through the continuing discovery of important isolates from previously uninvestigated species and the decreasing availability of some commercially useful essential oi components.

The list is restricted to isolates which (1) are either in use commercially (perfumery, medicinal, flavour) or possess commercial potential or (2) form a high percentage of the oil or (3) can be readily separated from associated oil components.

As the natural abundance of many of the listed species is restricted, then commercial exploitation may involve the establishment of plantations. Since essential oils from morphologically identical plant sources may vary, and the existence of chemical variations is often ignored by botanists, it is important to select material (seed or cuttings) for propagation from varieties yielding optimum quantites of the appropriate isolate and then esablish the consistency of the progeny.

In 1977, only 100 tonnes per annum of eucalyptus oils of the cineole type were being produced in Australia, and a few tonnes per annum of the terpinene-4-ol rich oil of *M. alternifolia* were being produced (Lassak and Southwell, 1977).

Lawrence (1980) reviewed the chemical composition of uncommon members of the genus *Mentha*. Among these was *M. diemenica* which is a rare mint found in southern Australia and Tasmania. He found the compositon similar to that of *M. pulegium, M. gallefosser* and *M. japonica*.

The essential oil bearing genus *Zieria* is restricted to eastern Australia except for one species endemic to New Caledonia. In the 40 taxa examined by Southwell and Armstrong,(1980),the major components of the steam distilled essential oil were benzaldehyde, car-3-en-2-one, chrysanthenone, cis-chrysanthenyl acetate, trans-chrysanthenyl acetate, citronellol, dimethoxy-methylene, dioxystyrene, elemicin, farnesol, limonene, methyl eugenol, myristicin, naphthalene, α-pinene, safrole and tetramethoxystyrene. Taxonomic features, were investigated. For instance the type form of *Z. smithii* has methyleugenol (>50%), elemicin (>40%) and safrole (>80%) chemotypes.

The type form of *Z. arborescens* shows a decreasing zierone content (from 50-70% to 0-20%) as its distribution extends north, and the new subspecies *Z. cystades* shows an abrupt fall in chrysanthenone content as the species crosses Bass Strait from Tasmania (40%) to mainland

Australia (<20%).

Southwell, looks at the genus *Zieria*, which is rich in both essential oils and components toxic to stock. About 300 samples from the 40 known taxa were tested for cyanogenic glycosides and distilled for essential oil. Cyanogenic glycosides were present in hazardous quantities in *Z. arborescens, aspalathoides, caducibracteata, cytisoides, fraseri, furfuracea, laevigata, montana, robusta* and *smithii*. The isolation and structural elucidation of methoxylated styrenes represented the first known occurrence of non-terpenoid styrene monomers in essential oils. Other useful components abundant in the oils were benzaldehyde, citronellal, methyl eugenol, limonene, (flavouring) carenone, chrysanthenone, (perfumery) and chrysanthenyl acetate (medicinal) (Southwell, 1980).

2.4 OILS USED FOR IDENTIFICATION AND DIFFERENTIATION OF PLANTS

Brooker and Lassak used the leaf oils of *Eucalyptus brookerana* M.Gray and *E. ovata* Labill. to differentiate between the two species. The oil of *E. ovata* showed 21±7 peaks while *E. brookerana* showed 43±9 peaks on G.L.C. examination. In addition, *E. brookerana* has a high proportion of monoterpenoid compounds, particularily 1,8-cineole, while *E. ovata* is rich in sesquiterpenes (Brooker and Lassak, 1981).

The volatile leaf oils of 27 individual trees of *E. brookerana*, chosen throughout the species distribution and of 13 trees of *E. ovata* from three different sites were examined by G.L.C. The oils of *E. brookerana* were shown to be fairly simple and mostly monoterpenoid in nature. The oils of *E. ovata* were more complex and characterised by comparatively large amounts of sesquiterpenoid alcohols.

In addition, the constituents of the oils varied depending on collection site. For instance, there is a complete and consistent absence of α-phellandrene from all samples of *E. brookerana* of Victorian origin, whereas in Tasmanian samples it became the major constituent. Comparing the two oils, there are striking qualitative differences such as the total detectable absence of β-pinene, piperitone, cuminal, geranial and viridiflorol in *E. brookerana*, yet these are present in *E. ovata*. On the other hand, terpenyl acetate and particularly γ-terpinene, both present in *E. brookerana*, are not in *E ovata*. The most important difference, however, is the complexities of the oils (Brooker and Lassak, 1981).

Carr and Carr describe a correlation between the stomatal size

(length, pole to pole) and diameter of the largest oil glands of two closely related eucalyptus species. The correlation is independent of leaf size and shape. The correlation stems from the fact that both stomata and the largest oil glands have their origin in single epidermal initials (Carr and Carr, 1980).

Maurer endeavours to locate scented scandents and to discover whether any of the Natural Orders offer a peculiar priviledge for fragrance shared by no other. He gives a mastergroup of scented Climbers and Twiners of Magnitude.

NATURAL ORDER	PROTOTYPE GENERA	APPROX. NO OF SPECIES	BASIC ODOUR
Passifloraceae Passion flowers	*Passiflora caerules*	200	Hawthorn
Ranunculaceae Virgin's Bower	*Clematis flammula*	150	Vanilla
Ampelidaceae Wild grape-vine	*Vitis labrusca*	150	Violet leaves
Caprifoliaceae Honeysuckle	*Lonicera fragrantissima*	150	Lilac
Caprifoliaceae Viburnum	*Viburnum fragrans*	100	Heliotrope
Leguminosaea Sweet Pea	*Lathyrus odoratus*	100	Neroli
Oleaceae Jasmin	*Jasmin officinale major*	70	Jasmine
Analiaceae Scented Ivy	*Hedera fragrans*	50	Musk
Rosaceae	*Rosa spp.*	xxx	Rose

(Maurer, 1965)

Gottlieb, (1982) attempts to establish that the natural occurrence of specific biologically active compounds is ecologically and systematicaly conditioned and it should be possible to build a system capable of predicting the existence and the nature of useful chemicals in plant taxa.

Williams and Harborne examined the essential oils of fruits of 124 species of the tribe Caucalideae of the Umbelliferae. The patterns observed were useful for separating the tribe into species (Williams and Harborne, 1972).

2.5 NEW ESSENTIAL OILS AND ISOLATES

The volatile leaf oil of *Melaleuca leucadendron* was investigated by Sood. The odour of this oil is very characteristic : cineole-like with a pepper-like note similar to the oils of Cajeput. It is very strong, and would therefore, be difficult to use io perfumery. Reports state that the yield varies from 0.09 to 0.90% (Sood, 1966).

The search for particular essential oil isolates in the Australian essential oil species includes the work by Hellyer, in which he records the first occurrence of maaliol and elemol in the Australian flora. *Eriostemon myoporoides* D.C. (Rutaceae) and *Prostanthera prunelloides* R.Br. (Labiatae) contained maaliol. *Hedycarya angustifolia* A.Cunn. (Monimiaceae) contained 60% elemol and *Prostanthera sieberi* Benth. (Labiatae) and *P. rotundifolia* R.Br. were found to contain globulol (Hellyer, 1962).

El-Gazzar and Watson extracted essential oils from 22 genera of Labiatae and examined them by G.L.C. Their results support the taxonomic suggestion that there are two major series of genera - one group being oil rich, the other, oil poor. They draw attention to the fact that certain genera of the oil rich group have commercial possibilities. For example, *Plectranthus*. Guenther (1958) has reported relatively high concentrations of essential oils, individually often of considerable chemical complexity, in species from forty-nine genera. However, only four of his list occur in Australia : *Mentha, Plectranthus, Prostathera,* and *Salvia*. Of the 180 genera and 4000 species of Labiatae, only a tiny proportion have yet been exhaustively analysed for useful essential oils, and relatively few have been considered at all (El-Gazzar and Watson, 1970).

Related work in other areas includes that by Corbett and Gibson, (1959) on extractives from the New Zealand Myrtaceae. They list the following of the 28 species of *Leptospermum* to have been examined. *L. scoparium, L. flavescens* var *grandiflorus, L. flavescens* var *microphyllum, L. flavescens* var *leptophyllum, L. odoratum, L. lanigerum, L. loversidgei, L. petersonii* and *L. ericoides*.

Flynn *et al*, 1979, investigated the volatile leaf oils of three species of *Leptospermum* : namely *L. sphaerocarpum, L. lanigerum* var *macrocarpum* and *L. scoparium* var *rotumdifolium*. They found similarities in all three of the essential oils, which supports their botanical relationship. The oils were predominantly monoterpenoid in character, and contained large amounts of α-pinene, 1,8-cineole and terpinen-4-ol.

The only sesquiterpenes present in any significant quantity were the alcohols viridiflorol and the isomeric α-,β- and γ-eudesmols.

The large family Compositae was examined for new sesquiterpenes by Bohlmann. The compounds isolated and reported were: isocamene and modhephene from *Isocama* sp. and *Berkheya* sp. respectively. The same compounds were isolated from *Silphum*, together with further tricyclic hydrocarbons. An additional one is present in *Senecio* sp. Others are described in his paper (Bohlmann, 1980).

Lassak and Southwell, (1980) in a short report on Australian flora, remind us that the Australian indigenous flora includes at least 4,000 species containing volatile essential oils in their leaves, wood, bark, fruits or flowers. A systematic survey of these oil-bearing species aims at the discovery of new essential oils with perfumery or flavouring potential and the identification of new sources of essential oils with established uses. For example, cineole-rich oils (used medicinally); piperitone-rich oils (a raw material used for the manufacture of menthol); terpinene-4-ol rich oils (used both medicinally and as a flavouring agent). Essential oils are also useful in the classification of plant species, using their chemical characteristics as one of the taxonomic criteria (chemotaxonomy). These authors suggest that the volatile oils obtained by steam distillation be characterised in the first instance by their physical constants (refractive index, optical rotation, relative density etc.), infra-red spectra and gas-liquid chromatographic patterns. Individual oil components are tentatively identified by comparing retention times and co-injection with authentic specimens.

Oils which show either commercial potential or taxonomically interesting features are selected for more detailed study. These are investigated by G.C.- M.S. and N.M.R. as well as by classical chemical techniques.

Some 60 samples of foliage, belonging to some 40 species of *Eucalyptus, Melaleuca, Zieria, Eriostemon, Metrosideros, Senecio* and *Baeckea* have been distilled and characterised. Amongst these, *Melaleuca linophylla* from central Australia yielded 1.6 per cent of oil, containing 52 per cent terpinene-4-ol and shows potential as a commercial source of this bactericidal compound. Some Tasmanian and all King Island populations of *E. brookerana* yield substantial amounts of oil (ca 2.5-3 per cent) rich in cineole (55-65 per cent) and may also have commercial potential.

2.6 OIL YIELD AND PLANT SPACING

Experiments on the effects of plant spacing and season of growth on *Melaleuca alternifolia* were performed by Small. He compared the response to three within-row spacings and measured the effects of seasons over several harvests. He found that there was an average increase in leaf and oil yield of 93% (2.9 t/ha and 46 l/ha, respectively) in the highest population (26,908 trees/ha), compared with the lowest (6,727 trees/ha). He concluded that this native is amenable to cultivation for tea tree oil production, with plant spacing being an important factor in management. The optimum population exceeds 27,000 trees per hectare (Small, 1981).

2.7 SECRETORY CELLS

Essential oils may be found throughout the plant cellular tissue or in special cells, glands or ducts located in several parts of the plant, that is, in the leaves, bark, roots, flowers, fruit or seeds, sometimes confined to special structures, sometimes not.

Hardman (1973) states that the type of structure of the secretory tissues is one of the characteristics of a botanical family and may be grouped as follows:

a) oil cells - Graminae, Zingiberaceae, Piperaceae, Magnoliaceae, Myristaceae and Lauraceae
b) schizolysigerous cells - Rutaceae, Myrtaceae
c) oil canals - Burseraceae
d) vittae or secretory oil ducts - Umbelliferae
e) glandular trichomes or hairs - Labiatae.

A thorough review of the location, structure, function and ontogeny of secretory tissues in plants is given by Fahn (1979). In genera of the Myrtaceae, the development of the lacunae in a schizogenous manner is a common phenomenon, whilst the secretory cavities of the Rutaceae develop lysigenouly or schizogenously. A diagrammatic representation of the development of a secretory cavity in *Eucalyptus* is shown in Figure 2.7.I

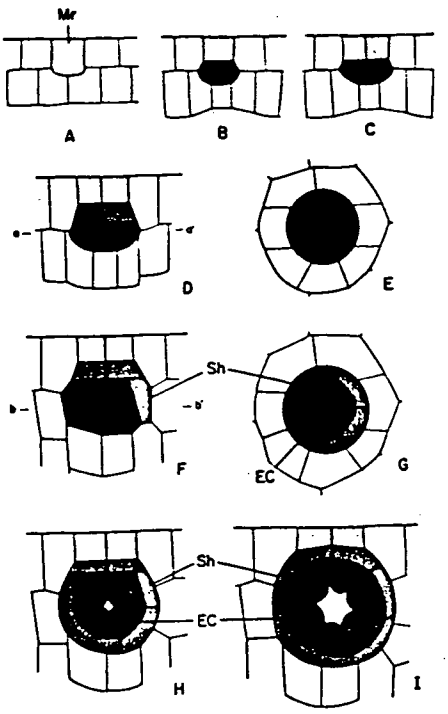


Figure 2.7.I

Diagram showing various stages of *Eucalyptus* secretory cavity development

E is a cross-section of D at the level of a-a'.

G is a cross-section of F at the level of b-b'.

EC, epithelial cell; Mr, meristemoid; Sh, sheath cell.

(Fahn, 1979)

2.8 ESTABLISHMENT OF NATIVES FOR COMMERCIAL PRODUCTION

Work on regeneration of native plants shows that the early successional changes were from annual herbs to shrub stages in which eucalypts were absent. Eucalyptus seedlings were found only where a seed source was present after clearing. Mallee eucalypts require conditions which include low levels of rabbit grazing and minimal competition. It would seem, from this study, that recolonization of cleared land by undisturbed eucalypts is a slow process, in part because of very limited seed dispersal and the absence of the ability to store seed in the soil. These characteristics produce dependence on seeds being held in the canopy and mean that Mallee eucalypts must normally have adults present at the site immediately before disturbance to allow regeneration after cases of total clearing (Onans and Parsons, 1980).

Muralidharan and Ramankutty (1982) confirm Baker and Smith's (1920) observation that *Eucalyptus citriodora* contains an oil rich in citronellal (65-85%). Considerable quantities of leaves can be obtained for distillation bey felling and allowing the sucke shoots to

grow from the stems. However, pruning the plants at ground level was found to be detrimental for regeneration. A spacing of 2m x 2m was recommended for preliminary planting. The study was undertaken to find an optimum spacing and height of pruning for maximum production of leaf and oil from this crop. Results showed a linear increase on the yield of leaf and oil with an increase in plant density and height of pruning. A spacing of 2m x 2m was significantly superior to other spacings. The yield of oil remains proportional to yield of leaf. Similarily, pruning at 3m and 5m above ground level was significantly better than pruning at 1m. There was no significant interaction between spacing and height of pruning, indicating that those factors were independent in their action. Since the maximum yield of leaf and oil were obtained under conditions of 2m x 2m (2500 plants/ha), and 5m height of pruning, the same can be adopted for large-scale cultivation of this crop.

2.9 ATTEMPTS TO INTRODUCE NEW ESSENTIAL OIL PLANTS IN OTHER COUNTRIES

Nepeta transcaucasica Grossh. is a wild plant of the Azerbaijan Soviet Socialist Republic, which was discovered in 1936. The oil contains a large number of components valuable for perfumery : geraniol, nerol, geranyl acetate, citronellol, citronellal and citral. Intraspecific variation exists with three types being characterized by the content of prevalent components. On the basis of research carried out in nurseries by Khilik *et al* (1977), *N. transcaucasica* is recommended to be introduced into the industry as a new and supplemental source for the production of a geraniol-citronellol type oil.

However, its introduction has been hampered by the lack of recommendations on agrotechnics of cultivation : optimum date and scheme of planting, harvest time, reaction to fertilizers etc. Methods of reproduction investigated include softwood cuttings. In addition, mechanical planting, irrigation regime, basal and additional fertilization, chemical weed control, mechanical harvesting and raw material processing was looked at.

Frazao *et al* state that there are more than one hundred annual and perennial species of Portugese flora which have not yet been studied for their essential oils. They give oil yields, chemical composition, growth habits and analyses of the essential oil from *Santolina rosmannifolia* L. and *Foeniculum vulgaris* Mill. (Frazao *et al*, 1977).

2.10 CULTIVATION OF NATIVE PLANTS

2.10.1 PREFERRED CONDITIONS AND FERTILIZATION

Information on the growth habits and preferred situations of Australian natives is relatively scarce. However, *Leptospermum lanigerum* prefers a wet position (Gray, 1967). Frost tolerant species include *M. squamea* and *M. squarrosa* (Verdon, 1973). That is, they tolerate temperatures lower than -7°C. *M. squameum* does well in wet peaty heaths and high elevations.

Olearia argophylla prefers wetter areas and propagates from seed (Gray, 1971).

Simmons (1966) lists plants that can sustain tip and bud damage and recover to grow on in the spring - *B. ramosissima, Eriostemon* spp, *Kunzea ambigua,* Melaleuca's, *Leptospermum scoparium, Prostanthera,* and *Zieria.*

Callitris tasmanica is common in areas of low rainfall and poor soils (Blomberry,1967).

McIntyre,(1976) looks at the use of fertilizers on native plants. He reaffirms the statement that natives can show improved growth with the application of fertilizers. The addition of nitrogen and phosphorus (NP) or nitrogen, phosphorus and potassium (NPK) fertilizers result in plant responses that are quite marked. Fertilization should be done in the Spring or early Autumn or both, that is, just before the two growth peaks during the year. Blood and bone has long been the universal panacea for natives. It is a safe additive but has the disadvantage that it is very low in N P K and can vary widely in these components. Because of this, blood and bone is an uneconomic fertilzer in terms of cost per unit nitrogen, phosphorus and potassium.

2.10.2 SOIL TYPES AND NATIVES

Specht (1976) points out that throughout the Australian landscape there are a number of different soil types, many of which occupy considerable areas and are populated by distinctive native flora. He lists the following examples:-

1. Samphire (or salt-marsh) plants and mangroves are confined to saline soils.
2. Calcicole (limestone adapted) plants are seen growing on highly calcareous soils.
3. Heath plants (also a component of the dry sclerophyll forests) are found on very infertile, non-calcareous, soils, often deep sands, sandstone soils, or leached lateritic soils.
4. A rich flora of grasses and herbs tends to grow on more fertile, sometimes calcareous, soils, which are often loamy or clayey in texture.
5. The best development of rain-forest vegetation in eastern Australia tends to be on deep volcanic soils - non-calcareous and of medium fertility.
6. Swampy peats tend to be very infertile and support a vegetation dominated by heathy plants or sedges.

Studies made on the heathland species *Banksia, Grevillea, Telopea, Melaleuca, Leptospermum, Thryptomene, Boronia, Eriostemon* and others show that the infertile soil on which these species are found are too poor to support the growth of grasses and herbs. Yet, attempts to grow these heath plants on more fertile soils have brought out the following points:

1. Certain of the heath plants are short-lived pioneer species, which grow vigorously on the ashes of a bush fire. These species can be successfully cultivated.
2. In contrast, when a long-lived species is placed in a soil of higher fertility, one of the following effects may occur:
   (a) The seedlings tend to be very sensitive to the higher nutrient level and osmotic burning of the roots may eventuate; nutrient-imbalance may result; root-rotting fungi, stimulated by the increased fertility, may invade the roots; the seedlings may become more frost sensitive.
   (b) If the seedling survives, the tender, rapidly growing shoots appear to loose water at a greater rate than it can be supplied by the roots. The problem is often exacerbated by the invasion of

root fungi which block the passage of water into the plant.

(c) If the heath plant survives, it grows and produces a wealth of flowers, but fertilized plants appear to age faster than unfertilized ones.

2.11 PROPAGATION

2.11.1 GENERAL

Bunker gives guidelines for the propagation of some of the native Australian shrubs and trees from softwood cuttings. He emphasised the need to use juvenile cuttings, and such juvenility needs often to be induced in the stock plants. Stock shrubs are pruned regularily on a four-month rotation. Initially the stock bushes are allowed to grow to a reasonably mature stage, during which time cuttings are taken as required. When they begin to harden off they are pruned severely, cutting the plant back to about one third of its original size. The stock area is maintained with low fertility and regular spraying for mites and leaf spot (*Verucisporum proteacarum*).

After cuttings are collected, they are dipped in a sugar solution. It has been widely known for many years, that these very immature, soft cuttings maintain well on the mist benches after a sugar dip. It is thought that the cuttings absorb some of the solution which tends to make them more turgid.

Cuttings are planted in trays of perlite and peat after being dipped in a 1 to 2 second dip of 4 grams per litre of indole-3-butyric acid and water. The cuttings are kept on heated benches for about two weeks (Bunker, 1982).

McIntyre and Veitch, (1972) state that *Eriostemon australasius* is only one species in a large group in which the seeds will not germinate normally, but do so after a bushfire. Many of these are in the Rutaceae and include *Eriostemon, Boronia, Zieria* and *Phebalium* spp. This group contains many species of great horticultural potential. Some are difficult to grow from cuttings so a method allowing them to be grown from seed is desirable. The method developed was as follows: the testa is scarified around the radicle end of the seed with a scalpel, exposing the endosperm, being careful not to damage the radicle. The seeds are put in small muslin bags and placed in a stream of running water for up to two weeks. Running water prevents fungal and bacterial growth and continuously removes substances which inhibit germination. Germination

usually takes about one week.

Nixon,(1981) explains that the horticultural exploitation of *Eriostemon australasius* is hampered by the fact that it is difficult to propagate. McIntyre and Veitch, (1972) showed that germination of the seed can be increased 25% by chipping the testa and by leaching. Only moderate successes are reported from propagating by cuttings with IBA. Nixon found that chipping increased germination but a long period of leaching is not necessary to remove any inhibitor that is present. With 12 month old seed, misting alone is sufficient.

McIntyre,(1972) outlines the propagation methods used by the National Botanic Gardens for native plants.

Ellyard,(1981) concludes that species respond to hormone formulation as well as concentration. For instance, many Grevilleas are sensitive to NAA and root following the application of IBA. *Prostanthera* species, by contrast, root best following treatment with NAA. Research at the National Botanic Gardens suggests that good results can be expected with many species by employing a 500 ppm IBA/500 ppm NAA mixture.

The effect of fertilization on rooted cuttings of *Grevillea* and *Leptospermum* species is outlined by Higgs,(1970). He found that fertilization adversely affects these species especially if the plants are put too deep into the medium.

A study on the effect of fertilization on the compostion of an essential oil was performed by Franz *et al*,(1983). They found that there was little influence of nitrogen, phosphorus and potassium fertilization on the oil derived from Chamomile.

The series of articles by Payne (1980..,1981,1981) suggests methods of germinating natives seeds, ranging from scarification to leaching and hot water treatments.

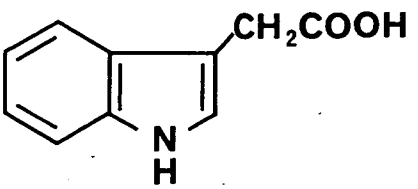
2.11.2 PROPAGATION BY CUTTINGS

Hartmann and Kester,(1975) state that in preparing cuttings it is often recommended that a "heel" (a small slice of older wood) be retained at the base of the cutting in order to obtain maximum rooting. For hardwood cuttings of some plants, this may be true. In quince (*Cydonia oblonga*), considerably better rooting was obtained with the heel type of cutting, probably owing in this case to the presence of preformed root initials in the older wood. Narrow-leaved evergreen cuttings often, but not always, root more readily if a heel of old wood is retained at the base of the cuttings.

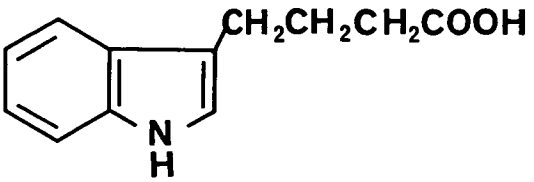
Evergreens usually root most readily if the cuttings are taken after a flush of growth has been completed and wood is partially matured. This occurs, depending upon the species, from spring to late autumn.

Hartmann and Kester offer the following information on propagation by cuttings. Mixtures of root-promoting substances are sometimes more effective than either component alone. For example, equal parts of indolebutyric acid and naphthaleneacetic acid, when used on a number of widely diverse species, were found to induce a higher percentage of cuttings to root and more roots per cutting than either material alone. For general use in rooting stem cuttings of the majority of plant species, naphthaleneacetic acid (NAA), and indolebutyric acid (IBA), particularly the latter, are recommended. See Figure 2.11.I. To determine the best material and optimum concentration for rooting any particular species under a given set of conditions, empirical trials are necessary.

3-indole acetic acid
(beta-indole acetic acid) (IAA)



3-indole butyric acid
(gamma-(indole-3)-butyric acid) (IBA)



1-naphthalene acetic acid
(alpha-naphthalene acetic acid) (NAA)

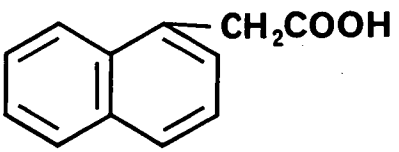


FIGURE 2.11.I

Structural formulae of some growth regulators, (auxins), active in promoting adventitious root development in cuttings.

Wounding, that is, removing a thin slice of bark for about two centimetres from the base of the cutting, exposing the cambium but not cutting deeply into the wood. Basal wounding is beneficial in rooting cuttings in some species, especially those with older wood at the base. Following wounding, callus production and root development are frequently heavier along the margins of the wound. Evidently, wounded tissues are stimulated into cell division and the production of root primordia. This is due, perhaps, to a natural accumulation of auxins and carbohydrates, and to an increase in the respiration rate. In addition, injured tissues from wounding would be stimulated to produce ethylene, which is known to promote adventitious root formation.

Wounded cuttings will absorb more moisture from the medium than unwouded. Greater absorption of applied growth regulators is also possible by the tissues at the base of the cuttings. In stem tissues of some species there is a sclerenchymatic ring of tough fibre cells in the cortex external to the point of origin of adventitious roots. There is

some evidence (Basu, Roy and Bose, 1970), that roots have difficulty penetrating this band of cells. A shallow wound would cut through these cells and perhaps permits outward penetration of the roots.

The rooting medium has three functions :

(a) to hold the cutting in place,

(b) to provide moisture for the cutting and

(c) to permit penetration of air to the base of the cutting.

An ideal rooting medium provides sufficient porosity to allow good aeration, has a high water-holding capacity, and yet is well-drained. For tender, softwood and semi-hardwood cuttings it should be free of harmful fungi and bacteria.

Indolebutyric acid (IBA) and naphthaleneacetic acid (NAA) are the most commonly used rooting hormones. IBA is perhaps the best for general use as it is non-toxic over a wide concentration range and is effective in promoting rooting of a large number of plant species.

Commercial powder preparations can be used in the following way : cuttings are wounded and dipped into the powder. The powder adhering to the cutting after it is tapped lightly is sufficient. Care must then be taken when pushing the cutting into the medium so that the powder is not lost. A preliminary trough is usually created with a blunt tool.

The other major application method is the concentrated solution dip. Here a solution varying from 500 to 10,000 ppm in 50% ethanol is prepared and the base of the cutting is dipped in it for about 5 seconds. The cuttings are then inserted into the rooting medium (Hartmann and Kester, 1975).

Bansal and Nanda, showed that there is a close relationship between rooting and IAA oxidase activity, which indicated that this enzyme is involved in the rooting process. This may explain why the cuttings of *Eucalyptus citriodora* failed to root, since these do not exhibit IAA oxidase activity. Exogenously applied IAA also failed to exhibit IAA oxidase activity perhaps due to the presence of some inhibitor(s) of IAA oxidase. The stem cuttings of *Eucalyptus citirodora* contain such inhibitors as demonstrated by these authors (Bansal and Nanda, 1982).

Specific examples of plants that cannot be readily raised from seed but are easily propagated from cuttings include *Senecio lautus* spp. *maritimus* (National Botanic Gardens, 1976), *Kunzea ambigua* from semi-hardwood cuttings taken in late spring to early autumn (National Botanic Gardens, 1978), *Eriostemon myoporoides* (National Botanic Gardens, 1971). Cuttings of *Olearia phlogopappa* and *Tasmannia purpurascens* also strike easily. In the case of *Phebalium coxii*, where there are complex

dormancy problems with seed, cuttings provide a reasonable method of propagation (National Botanic Gardens, 1982). Ollerenshaw, (1980) states that *Phebalium squamulosum* subsp. *argenteum* cuttings strike readily in 4 to 8 weeks. With *Kunzea* sp. 'Badja Carpet' there is the possibility that, in addition to cuttings, layering may be effective, since roots are observed to develop at nodes in contact with a moist, mulched substrate (Jackson, 1980)

2.11.3 PROPAGATION BY SEED

Grossbechler (1982), investigates the amenabiltity of Australian native species to direct seeding to direct seeding techniques. He states that direct sowing lends itself to automation by using the vacuum plate for sowing as is used in the flower seedling trade. By using the pasteurised growing media, wide spacing and high aeration and light intensity, damping off, mildews and other diseases are virtually never encountered.

The same author also gives details of a method for pregermination treatment which is applicable to all Acacia species. The seed is put into a muslin bag in boiling water for 4 to 7 minutes, then washed in cold water. The seed is then placed between moist blotting paper or hessian in a cool shady location to ensure that the blotting paper or hessian does not dry out.

In two to three days the radicle is produced. These seeds are collected daily and are sown singly per container. Once the radicle appears the seeds have to be sown immediately, since the radicle grows very rapidly, and sowing without damage is difficult. This method is time consuming because the sowing is spread over several days but has the advantage of 100% germination, as seeds with the best germination potential produce the radical first. Crops produced by this method give uniform, healthy plants.

Large seeds such as *Banksia, Hakea, Macrozamia, Acacia* and other Leguminous species can be sown one or two per container by hand. However, with very fine seed, for example, *Eucalyptus, Melaleuca, Callistemon, Leptospermum* etc. single placement is impossible, and other means can be adopted to speed up sowing without unnecessary wasting of seed.

In most Eucalypts the seed is mixed with a considerable amount of chaff, sometimes the two being indistinguishable. Therefore, the percentage of viable seed in the mixture must be considered when

determining sowing rates.

This last point is also made by Mullett,(1982). Her observations are that Eucalypt seed are small, average diameter of about 1mm, angular seeds either three cornered or sickle-shaped. The seeds are always associated with sterile chaff material, which is very difficult to distinguish from the fertile seed. The seed coat appears woody, but is usually quite permeable and brittle. Because the chaffy material resembles the seeds both in size and shape, cleaning is difficult. The sowing material, therefore, contains a considerable proportion of inert matter and amounts sown should be adjusted accordingly. Each fruit capsule contains a number of seeds but the proportion of chaff can be as high as ninety per cent.

Ali and Bird consider the question of seed dormancy. Their report outlines the various types of dormancy mechanisms. The role of the bushfire in overcoming dormancy in many Australian natives is discussed. The obvious physical effect of splitting hard seed coats does not always account for germination, since seeds are often buried, so other factors are involved.

The removal of forest floor litter alters the pH of the area, and the litter may contain a germination inhibitor which would be removed. In addition, the removal of litter lets light, air and moisture enter. The species *Erythrina austalis* (Coral Tree) germinates in the presence of carbonic acid, since the testa is broken down. It is a fact that ash and rain are associated with a rise in carbonic acid, which in turn could well be a side effect of a bushfire.

Germination inhibitors are also a major factor in dormancy, as shown by *Eucalyptus bicostata* which is surrounded by an area free from all growth. In addition, macerated leaves soaked in water will inhibit germination in many Australian native plant seeds (Ali and Bird, 1976).

MacLeod and Reid,(1982) have studied the effects of gibberellic acid (GA_3) on the germination of five species of eucaypts. They found that in the case of *Eucalyptus delegatensis, E. regnans, E. globulus, E. nitens* and *E. obliqua* the total germination was improved by the application of the hormone GA_3. In almost all casees the speed of germination was also increased. The effects were more pronounced in the two cases where the controls gave low values for germination, that is, for *E. delegatensis* and *E. obliqua*. It is interesting to note that these species produce dormant seeds.

Some Australian native species are grown easily from seed, but not from cuttings. These include the conifers such as *Callitris rhomboidea*

(formerly *C. tasmanica*). Donaldson, (1980) observed that *Melaleuca elliptica* is propagated best from seed sown in spring, although cuttings taken in late summer are also successful. Similarily with *Melaleuca lateritia*, where old capsules can be stored dry and warm, causing the release of seed (National Botanic Gardens, 1978). *Prostanthera lasianthos* can be grown from seed (National Botanic Gardens, 1973).

Coode, (1982) studied the genus *Elaeocarpus* in Australia and New Zealand. He found that 8% of *E. augustifolius* and 20% of *E. reticulatus* seeds germinated after 12 months; 30% after 22 months. Filing of the seeds did not improve the germination rate.

2.11.4 GRAFTING

In horticulture, grafting has long been practiced to provide uniform clonal plants on reliable rootstocks. Many desirable plants will not grow well in some areas because their root systems succumb to root diseases, nematodes, etc. This problem is overcome in many cases by grafting the plant onto a reliable rootstock, usually of a species or variety taxanomically very close to itself.

Australian plants are no exception. Over 30 species of *Prostanthera* spp. have been grafted onto *Westringia fruticosa* rootstock. These grafts have mainly been top wedge with some simultaneous cuttings and grafts being used. That is *Prostanthera* sp. is grafted onto a cutting of *W. fruticosa*, and as the cutting roots, the graft takes.

Western Australian *Banksia* spp. have also been grafted onto eastern rootstock with some success. The rootstocks were *B. serrata, B. robur, B. spinulosa, B. ericifolia* and *B. oblongifolia*. The scions included *B. media, B. speciosa, B. baueri, B. lehmanniana* and *B. occidentalis*. Other successful grafts include *Kunzea baxteri* and *K. pomifera* onto *K. ambigua*. All grafts were carried out under sterile conditions. (McIntyre, 1976)

2.11.5 PROPAGATION BY TISSUE CULTURE

A general review of tissue culture principles and techniques is presented in a paper by Murashige, though, as he points out the conclusions of the review are not directly applicable to woody perennials, since most experiments have been carried out with herbaceous genera.

There are four areas in which applications of plant tissue culture

are possible.

1. Production of pharmaceuticals and other natural products.
2. The genetic improvement of crops.
3. The recovery of disease free clones and preservation of valuable germ plasm.
4. The rapid clonal multiplication of selected varieties.

The requirements of the three stages of tissue culturing are described in detail. The first stage is the establishment of an aseptic tissue culture of the plant in question. The next stage produces a rapid increase of organs and other strucures which ultimately give rise to plants. This can be achieved by either inducing adventitious organ or embryo formation or by enhancing axillary shoot initiation. The final stage prepares the tissue culture derived plants for establishment in soil. In addition, a list of plants with demonstrated potential for clonal multiplication through tissue cultures is given (Murashige, 1974).

Other workers have obtained multiple shoots from terminal buds of 20 year old trees of *Eucalyptus citriodora* Hook. on Murashige and Skoog's medium, supplemented with calcium pantothenate, biotin, benzylaminopurine and kinetin. Rooting of shoot cultures was induced by naphthalene acetic acid. A temperature of 15°C with continuous illumination followed by growth in agitated liquid cultures was essential for inducing shoot development in the primary terminal buds. Later subcultures and explants derived from from seedlings did not need this treatment. The authors estimate that over 100,000 plants can be obtained by this method in a year from a single bud of mature *Eucalyptus citriodora* trees (Gupta, Mascarenhas and Jagannathan, 1981).

National Botanic Gardens, (1978) describe the used of tissue culture for Australian native plants. Two possible pathways can be used, as shown, simplified, in Figure 2.11.II.

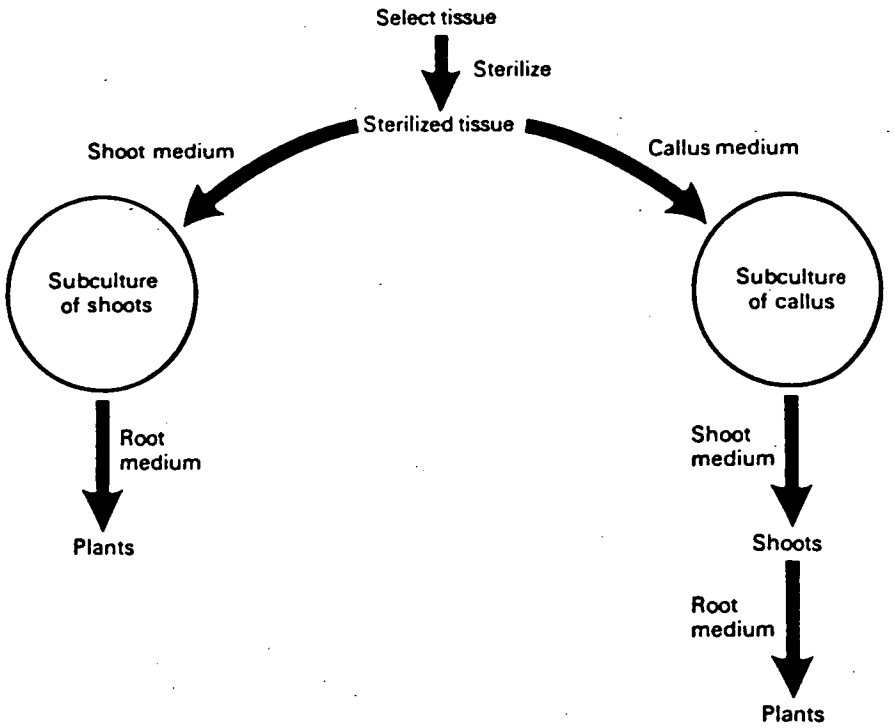


Figure 2.11.II
Two possible pathways in the
propagation of plants by tissue culture.

Research into the propagation of Australian plants has concentrated on the kangaroo paws (*Anigozanthos* spp. and *Macropidia fuliginosa*). While most species in the genus *Anigozanthos* can be readily propagated from seed, some of the hybrids, which have been produced in an effort to increase resistance to inkspot disease or to improve flowering form, cannot be propagated in this way.

In addition research has begun on the tissue culture of native ferns, several terrestrial orchids and the Western Australian pitcher plant (*Cephalotus follicularis*).

The potential use of tissue culture in the propagation of Australian native species remains largely untapped. A number of individuals and nurseries are, however, becoming aware of its merits, and are undertaking research. Thus, tissue culture can be expected to play a rôle of growing significance in the propagation of horticulturally desirable Australian species.

Knosh-Khui *et al*, (1984) relate work on the rapid clonal propagation of *Myrtus communis* L., which is an important ornamental species native to Europe, South America, Australia and New Zealand. The

tips of vegetative shoots were excised for *in vitro* propagation. Optimum shoot proliferation was obtained on a medium containing half-strength Murashige and Skoog salts plus (in mg l^{-1}): nicotinic acid, 0.5; pyridoxine HCl, 0.5; thiamin HCl, 0.1; glycine, 2.0; inositol, 100; sucrose, 30,000; BA, 1.5; NAA, 0.1. The transfer of excised shoots to half-strength MS salts plus vitamins, and supplemented with 0.5 mg l^{-1} BA and either 0.4 or 0.6 mg l^{-1} NAA, resulted in good root development whereupon the plants were successfully transferred to soil.

Gorst *et al*, (1982), have recorded work on *Eucalyptus ficifolia* F. Muell. explants. They found that the morphology of developing roots was dependent upon the structure of the auxin placed in the medium. A phenolic oxygen located between the aromatic ring and side chain induced callus formation. The absence of this oxygen caused root development, and, in this category, α-NAA, β-NAA, IPropA and IPyrA were associated with 100% rooting of explants and root systems with 2 to 3 long roots with lateral. IBA induced many short roots with laterals and root hairs while a stunted root system with minimum lateral developed was produced by IAA.

Plummer and Fossard, (1982) investigated the influence of plant hormones and growth factors on the growth of *Eriostemon australasius* Pers. in tissue culture. Cultures of apical and axillary buds showed adventitious bud formation only in the presence of BA and PBA.

The production of callus of *Callistemon viminalis* from axillary buds derived from nodal tissue was achieved on a medium containing macro- and micro-nutrients, sucrose 0.06M, inositol 300μM, nicotinic acid 20μM, pyridoxine HCl 3μM, thiamine HCl 2μM. BA 5μM and 4CPA 0.1μM provided most vigorous callus development and sprout formation. Rooting was observed on the basal medium, while auxins stimulated root initiation. 4CPA stimulated root initiation, but repressed the growth of roots. IBA stimulated both root initiation and growth at 0.005 to 0.1μM. Shipton, (1982) concluded that IBA at 0.01μM was best for root and shoot growth in this species.

Recently Williams *et al* (1984), reported on *in vitro* shoot multiplication and root formation from mature shoot explants of the woody ornamental species *Dampiera deversifolia* and *Prostanthera rotundifolia*. The most prolific shoot multiplication in both species was given with 0.5 μM BAP + 0.5 μM kinetin in the absence of auxins. Rooting of *Dampiera* was completely inhibited when cytokinins were added, the greatest response being with IBA and NAA at concentrations of either 0.5 or 0.05 μM. Inclusion of NOA caused excess callus formation. *Prostanthera* produced

the most roots with IBA + NOA at 2 μM. Callus formation was low in this species.

2.12 MYCORRHIZAL ASSOCIATIONS

Warcup discusses ectomycorrhizal associations of Australian indigenous plants. Species known to form such associations are *Eucalyptus, Leptospermum, Casuarina* and *Pomaderris.* Soil type may determine whether the plant forms an ectomycorrhizal or endomycorrhizal association. Such is the case with *Leptosperm juniperinum.*

Examination of field plants and plants grown in field soils, resulted in a list of species found to form such associations. It is suggested that all species of *Eucalytpus* are ectomycorrhizal, as well as most Leptospermideae. In addition, all species of *Pomaderris, Cryptandra, Spyridium* and *Trymalium* tested formed ectomycorrhizas, generally of the beech type. Many species of *Acacia* were also found to be ectomycorrhizal. It is noteworthy that a number of non-woody herbs, including *Poranthera microphylla, Brunonia australis, Goodenia glomerata, Stylidium soboliferum* and *S. perpusillum* formed associations. Generally there were patches of sheath, sometimes with a Hartig net, on some of the fine roots of the plant. The smallest plant found to have well-defined sheaths on its roots was *Stylidium perpusillum*, which is an annual 2 to 4 cm high.

In soil of low available phosphate, both typical and loose ectomycorrhizal associations markedly increased plant growth, compared with that of uninoculated plants (Warcup, 1980).

The effect of inoculation of peppermint (*Mentha arvensis* L.) with *Glomerus fasciculatus* in unsterilized soils and differential phosphate availability was studied by Sirohi and Singh. Vesicular-arbuscular (VA) mycorrhiza increase plant uptake of water and nutrients. Specifically, they improve phosphorous nutrition and growth. Peppermint has been shown to be a good host for VA mycorrhiza (Bierrmann and Linderman, 1981), and a strong association is formed. The result is an inc rease in dry matter yield, total P uptake, inorganic and organic P fraction, percent oil and total oil yield. Under P deficiency conditions, oil synthesis is limited, due to a lack of phosphatidal cofactors, such as $NADPH_2$. It is likely that endomycorrhiza can greatly increase the utilization of inorganic and organic P for the synthesis of essential oils in peppermint (Sirohi and Singh, 1983).

2.13 SENSORY MEASUREMENTS

To be perceived as an odorant, a molecule must be volatilized from its source, inhaled into the nasal cavity and dissolved inthe protective mucus layer lining the epithelium which contains the olfactory sensory cells. It is believed that the molecule must then be bound by a protein receptor on hair-like protrusions from the cell. The presumed binding process results in dramatic changes within the cell, initiating olfactory nerve impulses which travel from the sensory cell to the olfactory lobe of the brain. Finally, in what is probably an extremely complex process, the brain interprets the incoming signals by associating them with a previous olfactory experience, in order to assign an odor descriptor. The phenomenon of odorant-receptor binding, the type of neurotransmitters involved in nerve-lobe communication, and the correlation between odor quality and regional neural activity within the olfactory lobe and other parts of the brain are all active areas of research (Labows and Wysocki, 1984).

Pangborn, (1981) states that when properly conducted, sensory measurements result in valid and reliable information. However, it is time-consuming, subject to human variability error, and requires extensive statistical analysis for interpretation. Instrumental analyses, in contrast, are more reproducible and less expensive to run, but are only academic exercises unless they correlate with sensory judgement. Although no instrument or combination thereeof, can duplicate or replace the human senses, it is essential to develop rapid and reliable physical and chemical mehtods for measuring flavour compounds to complement and supplement sensory analyses. During G.L.C. analysis, the effluent can be split, permitting judges to sniff compounds at the exit port. However, there is not always agreement between the G.L.C. and nasal appraisals. The G.L.C. is a separator whose responses are linear wih concentration, whereas the human olfactory system is an integrator whose responses are often a power function with concentration. Human olfaction and G.L.C. vary widely in sensitivity to different molecular specimens. For example, the lower limits of G.L.C. flame ionization detection of acetone is 0.03 ppm, while 500 ppm are needed for smell. On the other hand, vanillin and methyl salicylate are distinct to the nose yet are relatively non-volatile. Despite lack of complete agreement, G.L.C. can be supplemented by sensory measurement by:

a) indicating which compound(s) contribute to the characteristic aroma of the product;

b) describing the quality and relative intensities of individual peaks;

c) establishing the relative importance of the mixture as well as of isolated fractions to the total flavour of the product. That is, the role of aroma compared to taste , texture etc., and

d) determining what no instrument can approximate : the relative pleasantness of individual compounds or of mixture (Wick, 1965).

Compared to taste, olfaction is extremely sensitive to low concentrations of chemical substances and is able to discriminate among them. For instance, it is estimated that a single molecule of butane-1-thiol can stimulate a single olfactory receptor in humans. This sensitivity is remarkable even in comparison with the performance of physical instruments (MacLeod and Morton, 1982). Thus, Kendall and Nielson (1964) found that odour thresholds of human subjects for each of four odorants (anethole, citral, methyl salicylate and safrol) were markedly lower than the minimum concentrations detectable by G.C. with an F.I.D. Furthermore, the nose detected trace contaminants in the odorants at concentrations several orders of ten below the sensitivity of the instrument. This observation helps to explain the dominant role that odours play in delineating a flavour, a role that is further supported by our ability to discriminate among many odours - over 10,000 according to some estimates.

The context in which an odour is perceived can also determine whether an odorant gives a positive or negative response. Methyl mercaptan and isovaleric acid are unacceptable when associated with humans (for example, in breath and axillary odours), but are desirable in certain foods (for example, in cheeses) (Labows and Wysocki, 1983).

3. MATERIALS AND METHODS

3.1 GAS CHROMATOGRAPHY

Gas chromatographic analyses of the oil samples were performed using a Pye-Unicam Series 104 Gas Chromatograph equipped with a flame ionization detector (F.I.D). A splitter was fitted prior to the detector. The splitter allowed 9/10 of the effluent to the exit port, where it could be sniffed, and the remaining 1/10 to the F.I.D.

The samples were injected with a Hamilton microlitre syringe (No. 705, NCH), fitted with a churney adaptor.

The following conditions were used for the analyses:

Glass capillary column : OV101 (50m x 0.5mm)
support coated open tubular (SCOT)
Temperature programme : 70 - 230° C at 4°C per minute
Detector temp : 250°C
Initial injector head temperature : 150°C
Hydrogen pressure : 117 kPa
Air pressure : 76 kPa
Nitrogen flow rate : 2 ml/min (Carrier gas)
Make up gas (Nitrogen) : 25 ml/min

3.2 GAS CHROMATOGRAPHY - MASS SPECTROMETRY

Mass spectra of oil samples wwere obtained with a V.G.- 7070 Mass Spectrometer (V.G. Micromass Ltd. Winsford, England), interfaced to a Pye Unicam 204 Gas Ckromatograph. The column used was an OV101 0.5 mm with a Helium flow rate of 2 ml/min.

3.3 EXTRACTION MATERIAL

Most samples were obtained from one of the three main collection sites. These sites were : a) Mount Wellington slopes with an elevation of about 300m.

b) Fern Tree
and c) Glendevie.

In addition some samples came from Winnaleah and Chimney Pot.

Table 3.3.I shows the origins of the selected species.

In order to secure samples which would be of interest, the

collection area was carefully surveyed for potential oil-producing plants. The presence or absence of oil was established by crushing leaf material and noting any resultant odour. If there was evidence of odoriferous constituents, cuttings were taken. Only young growth was removed, that is, either the current or one year old growth.

TABLE 3.3.I

Sites of origin of species

Bedfordia salicina	Old Farm Road
Beyeria viscosa	The Springs
Callitris tasmanica	Kingston
Callitris tasmanica (hybrid)	Chimney Pot
Cassinia aculeata	The Springs, Glendevie
Drimys lanceolata	Winnaleah, Fern Tree
Eriostemon virgatus	Glendevie
Eucalyptus amygdalina	Glendevie
Eucalyptus pulchella	Glendevie
Kunzea ambigua	Ansons Bay, Blackman's Bay
Leptospermum glaucescens	Glendevie, The Springs
Leptospermum lanigerum	Mt. Wellington, Fern Tree, The Springs
Leptospermum scoparium	Glendevie
Melaleuca squamea	Glendevie
Melaleuca squarrosa	Glendevie
Olearia argophylla	Old Farm Road, Fern Tree
Olearia phlogopappa	Mt. Wellington, Fern Tree
Phebalium squameum	Fern Tree
Prostanthera lasianthos	Mt. Wellington, Fern Tree, Old Farm Road
Zieria arborescens	Old Farm Road

3.4 STEAM DISTILLATION

A modified aluminium pressure cooker (20 litre, S.E.B.), was used for all steam distillation extractions. The modifications consisted of blocking the pressure release outlet, and the fitting of a glass condenser to the centre of the lid. The joint was sealed with silicone sealant.

The condenser was such that the oil remained in the collection arm of the unit and the distillation water was recycled to the distillation vessel. Inside the vessel, a stainless steel screen, supported approximately 10 cm above the water, was used to hold the plant material up above the boiling water. The amount of material extracted in one loading varied depending on the species of plant, but was generally about one kilogram. The running time also varied with the species, as well as with the amount and its moisture content, but all samples had reached exhaustion at the end of 1.5 hours. During each run, the rate of distillation was kept constant at 5 ml/min. Distillation was continued until no oil droplets were visible coming from the condenser into the collection area.

3.5 SOLVENT EXTRACTION

Samples of each species were extracted with petroleum ether (B.P. 40-60°C). An average of 750 g of plant material was macerated with a blender to allow better penetration of the solvent. The crushed herb was placed in a preserving jar, topped up with solvent, and sealed with a rubber ring. The rubber ring was prevented from deterioration by the solvent by placing a film of plastic between it and the pet. ether. The jars were placed on an automatic shaker, and left for 4 to 5 days. After that time the solvent was removed and filtered if necessary, to remove plant debris. A rotary evaporator was used to reduce the volume of the samples, which were then transferred to 50 ml round-bottomed flasks, and taken to dryness. The yield of extract was recorded.

3.6 DETERMINATION OF PHYSICAL CONSTANTS

The oil samples were examined for refractive index and optical rotation values. The optical rotations were performed on a Bellingham and Stanley optical rotation bench. A 10 cm long tube was filled with sample oil and placed in the path of light from a sodium vapour lamp. The rotation of the light was noted.

The refractive indicies were determined using an Atago Abbe refractometer. The standard procedure was followed, wherein a film of oil was placed on the ground glass plate, and the degree of refraction was noted.

3.7 PROPAGATION BY CUTTINGS.

Cutting material was collected from the same sites as for extractions and distillations. In the same manner, only young growth was selected. Cuttings were prepared by pulling off 4 or 5 cm long shoots from the main stems. This process leaves a heel of older tissue at the base of the shoot, which aids in callus development, in much the same way as wounding. The growing tips were removed to promote lateral growth. Punnets were filled with a 50:50 mixture of vermiculite and Clark's composted eucalyptus bark. In each punnet were placed 10 cuttings which had been given one of the following treatments :

Nil
500 ppm IBA
1000 ppm IBA
2000 ppm IBA
4000 ppm IBA
Strike (2.5% Captan fungicide, 0.25% NAA in talc w/w)
Seradix semi-hardwood (0.3% IBA in talc w/w)
Seradix hardwood (0.8% IBA in talc w/w)

A 4000 ppm solution of IBA was prepared by dissolving 0.100 g of IBA in 25 ml of 50% ethanol. The lower concentrations were obtained by the dilution of the 4000 ppm solution with 50% ethanol. The solutions were applied by dipping the end centimetre into the solution for 5 seconds. This allows the uptake of the active constituent but destroys the tissue that has been in contact with the solution.

Both Strike and Seradix are commercially available rooting hormone powders which are applied by dipping the end of the cutting into the powder and shaking off he excess.

The punnets of cuttings were placed under an automatic misting system.

3.8 PROPAGATION FROM SEED.

Dried seeds were obtained from Mr. Walduck (Kingston) at the end of January 1984. Seeds were planted in a 50:50 mixture of sand and peat. Two separate trials were run, one using steam sterilized, the other using unsterilized medium. Table 3.7.I shows the species used in the trial and the number of seeds planted in sterilized and unsterilized media.

TABLE 3.7.I

SPECIES	STERILIZED	UNSTERILIZED
Callitris tasmanica	15	32
Drimys lanceolata	9	15
Eriostemon virgatus	21	200
Eucalyptus amygdalina	45	455
Kunzea ambigua	80	140
Leptospermum glaucescens	65	615
Leptospermum lanigerum	50	500

The trays of seeds were placed in the temperate glasshouse (min. 14° C, max. 30 °C). Trays were equipped with a layer of coarse sand at the bottom on which the punnets were placed. This sand layer was kept moist either by the addition of water by hand, or by inverting a bottle containing water and fungicide so that the neck of the bottle was imbedded in the sand. In that way the sand was constantly moistened as water was taken up by the seed punnets.

3.9 SENSORY EVALUATION

3.9.1 PRELIMINARY REVIEW

Once samples of all steam distilled oils and solvent extracts had been obtained, they were screened for selection for further investigation. The criteria used were as follows:

(a) It was preferable if the extract possessed a pleasant odour, coupled with the quality of being either powerful or persistent, or both.

(b) The yield of oil or concrete was not to be too low; say, less than 0.1%

(c) If possible, the G.C.- M.S. analysis was to demonstrate the presence of commercially useful compounds, either singly in high concentrations, or as an appealing mixture.

(d) The plant from which the extract was derived, should show signs of being amenable to cultivation. For instance, an alpine plant would be disregarded.

3.9.2 DETAILED ASSESSMENT

Of the original number of potential plants, some 10 were selected for detailed investigation. This comprised determination of approximate percentage compostion data, G.C. analysis with a splitter fitted so that individual components of the oils could be smelt, and observation of the behaviour of the extract on a taper. In addition, small-scale propagation trials were performed.

3.10 SCANNING ELECTRON MICROSCOPY

3.10.1 SELECTION OF SPECIMENS

Leaf and/or stem tissue was selected from each species. Transverse sections were cut so that oil gland structures would be seen. Care was taken when cutting the sections, not to draw the blade along the edge surface, but rather to sever the tissue with a single chopping motion. This prevented undue tissue damage.

3.10.2 FIXATION

The samples were fixed in chilled 4% glutaraldehyde in sodium phosphate buffer (0.1 M, pH 7.2). The leaf specimens remained in this solution for 20-24 hours at 4°C. The glutaraldehyde and buffer was taken off by a weak suction. The specimens were then rinsed in the same chilled buffer for 10 minutes. This was followed by 2 more washings in buffer at room temperature, each of 10 minutes duration. The buffer washings were followed by three changes in deionised water.

3.10.3 DEHYDRATION

The specimens were dehydrated immediately after the last deionised water wash. A graded ethanol series was used as shown below in Table 3.10.I.

TABLE 3.10.I

Aqueous Ethanol %(v/v)	Acetone %(v/v)	Immersion Time (min)
25	-	20
50	-	20
60	-	20
70	-	20
80	-	20
90	-	20
95	-	20
100 Dry	-	20
100 Dry	-	20
100 Dry	-	20
50 Dry	50 Dry	15
-	100 Dry	15
-	100 Dry	15
-	100 Dry	

3.10.4 CRITICAL POINT DRYING

During the initial stage of the final acetone wash, the specimens were placed in a boat which was transferred to the critical point dryer (Polaron E-3000). The boat was filled with 100% acetone and the C.P.D. had previously been cooled to 14°C using a circulating water bath.

A preliminary 3 minute flush with liquid carbon dioxide at 14°C was repeated after 3, 10, 60, 80 and 100 minutes. The level of liquid carbon dioxide was then allowed to fall to the top of the specimen boat and the critical point dryer was heated to between 36 and 38°C. After the critical point had passed, the carbon dioxide was gently vented off.

3.10.5 MOUNTING, COATING AND OBSERVATION

The specimens were mounted on electron microscope stubs using conductive paint. They were arranged so that all facets of the sections were accessible for observation in the S.E.M. A Blazers Sputter Coater was used to coat the samples with approximately 20nm of gold. A Phillips 505 Scanning Electron Microscope, set at 15 kV, was used to observe the specimens, and electron micrographs were recorved with a Rolex 120 land camera.

3.11 EPI-γ-EUDESMOL SEPARATION AND CHARACTERISATION

An R401 differential refractometer linked to a Waters Associates high performance liquid chromatograph (HPLC) was used to resolve the α-, β-, and γ- isomers of eudesmol in the oil of *Olearia argophylla*. The method, as described by Hara *et al*, involved the used of a 10 μm argentated column, prepared by cycling a solution of 0.8 g of silver nitrate in 200ml of methanol through the column for 3 hours at 1.5 ml/min. This impregnated the surface of the column with silver nitrate.

The sample size was 15 μl, which was run at 1.5 ml/min using a 2.6% (mol/mol) acetone/n-hexane solvent system. The γ-eudesmol peak was collected at the exit port. Enough runs were completed so as to provide a sufficient quantity for GC-MS, Infra-Red (IR) and Nuclear Magnetic Resonance (NMR) studies. The collected fraction was checked for purity by GC, and found to be 99% pure eudesmol.

An NMR spectrum of the epimer fraction was obtained through the Brisbane NMR Centre. The IR spectrum was determined on a Hitachi 270-30 Infra-Red Spectrometer.

The GC-MS verification was performed on a Hewlett-Packard 5890 GC-MS station. Here the conditons were the same as for work done on the Pye apparatus, except that the small bore OV101 SCOT column was 12 m long, and the temperature programme was isothermal at 145°. Thus, the run was completed in 7 minutes. Two samples were injected. One of *Leptospermum lanigerum* oil, which contains all three eudesmol isomers, the other being a mixture of this oil and the postulated epimer sample. The mass spectra were obtained for the two peaks which were thought to represent the two isomers of γ-eudesmol.

4. RESULTS

4.1 DESCRIPTIONS OF SPECIES

Beyeria viscosa: Pinkwood

Tas. widespread and locally abundant, especially on shaded rocky banks.

Callitris rhomboidea: Oyster Bay Pine

Locally abundant on the east coast from Tasman Peninsula (Fortescue Bay) to Elephant Pass and Flinders Island.

Drimys lanceolata: Mountain Pepper

Highlands, mountains and reaching sea level, except in dry parts are the preferred areas for this species. It appears on cold wet flats after being burnt and is widespread from sea level to sub-alpine regions, in high rainfall areas and montane grasslands.

Eriostemon virgatus:

Locally common in scattered localities incliding the east coast between Oyster Bay and Georges Bay near the west coast and Macquarie harbour, the south-west at Port Davey, the south near Cygnet.

Eucalyptus amygdalina: Black peppermint (*E. salicifolia*)

These are small trees with a mallee habit. Widespread and abundant throughout the eastern half of the State, tolerating shallow and poor acid soils. Replaced by *E. nitida* in the west and extreme south. There are six hybrids.

Kunzea ambigua: (*Leptospermum ambiguum* Sm.)

Locally abundant in sandy heaths at the east coast and in the islands of Bass Strait. Wet scrub, granitic country of the east and north-east and Bass Strait Islands.

Leptospermum glaucescens: (*L. flavescens* and *L. myrtifolium* of Rodway's Tasmanian Flora 1903 and *L. sericeum* W.M.Curtis)

Widespread and abundant in wet peaty heaths. This plant is commonly found to proliferate where competition is not great, such as in clearings, riverbanks and so on.

Leptospermum lanigerum:

Widespread and often in damp places such as river banks and from sea level to montane habitats.

Olearia phlogopappa: (*Olearia gunniana* and *Eurybia gunniana*)

Widespread and abundant from sea level to mountain plateau. A highly variable plant.

var *phlogopappa* : near coasts in S. and S.E.
var *brevipes* : widespread throughout the state
var *microcephala*:
var *augustifolia*: local, collected at Risdon
var *salicifolia* : locally frequent near the north coast
var *supreparda* : frequent on mountains at altitudes of about 1000 m.

This species tends to occur near open areas, such as roadsides and clearings. Its growth is very restricted in dense forest, where it appears very stunted and etiolated.

Phebalium squameum:

1. subsp. *squameum*
2. subsp. *retusum*

1. Common in damp woods. In N.W. grows to 12 m.
2. N.E. region and mountains of central plateau.

This species tends to occur in clusters where conditions are favourable. It will not tolerate deep shade, so is a good competitor where there is sufficient sunlight.

4.2 YIELDS OF ESSENTIAL OILS AND SOLVENT EXTRACTS

The yields of essential oils from all species investigated are arranged alphabetically in Table 4.2.I below. In most cases, the yield of solvent extract is also shown. The values cited for yield on a dry weight basis are derived from the wet weight yields and the dry weights for each species. A compilation of dry weights is presented in Table 4.2.II.

Abbreviations used : S.D. - Steam Distillation
SOL - Solvent Extraction

Table 4.2.I
Yields of Essential Oils
And Concretes

		% YIELD			
	DATE	WET S.D.	DRY S.D.	WET SOL.	DRY SOL.
B. salicina	24/04/83	0.03	0.09	0.97	2.87
	23/07/83	0.08	0.23		
		mean:	0.16		2.87
B. viscosa	24/04/83	0.21	0.45	3.58	7.58
C. tasmanica	26/09/83	0.37	0.65	3.25	5.63
	5/12/83	0.21	0.36		
	20/02/84	0.27	0.47		
		mean:	0.49		
C. tasmanica (hybrid)	20/02/84	0.63	1.10		
C. aculeata	24/04/83	0.04	0.08	1.46	3.01
	4/05/83	0.13	0.22	0.79	1.39
		mean:	0.11		2.20

		% YIELD			
		WET	DRY	WET	DRY
	DATE	S.D.	SOL.	S.D.	SOL.
D. lanceolata	9/09/83	0.37	0.59	0.87	1.37
	9/09/83	0.35	0.56		
	17/02/84	0.44	0.89		
		mean:	0.68		
E. virgatus	4/05/83	0.54	0.96	2.46	4.33
	4/05/83	0.40	0.58		
	19/08/83	0.64	1.43		
		mean:	0.99		
E. amygdalina	4/05/83	0.43	0.94	0.93	2.02
	4/05/83	0.36	0.78		
	19/08/83	1.71	3.73		
		mean:	2.15		
E. pulchella	4/05/83	0.59	1.50	1.29	3.28
	4/05/83	0.77	1.95		
	19/08/83	0.54	1.37		
		mena:	1.61		
K. ambigua	14/08/83	0.63	0.75		
	8/09/83	0.63	0.75		
		mean:	0.75		
L. glaucescens	4/05/83	0.18	0.29	0.72	1.14
	4/05/83	0.12	0.19		
	19/08/83	0.13	0.20		
		mean:	0.23		
O. lanigerum	24/04/83	0.35	0.64	0.83	1.51
	23/07/83	0.63	1.14		
		mean:	0.89		

		% YIELD			
		WET	DRY	WET	DRY
	DATE	S.D.	SOL.	S.D.	SOL.
L. scoparium	4/05/83	0.14	0.26	0.23	0.42
	4/05/83	0.17	0.30		
	4/05/83	0.16	0.29		
	19/08/83	0.29	0.52		
		mean:	0.34		
M. squamea	4/05/83	0.02	0.05	0.32	0.76
	4/05/83	0.02	0.05		
	19/08/83	0.01	0.02		
		mean:	0.04		
M. squarrosa	4/05/83	0.13	0.27	0.85	1.75
	4/05/83	0.14	0.28		
	19/08/83	0.15	0.36		
		mean:	0.30		
O. argophylla	24/04/83			1.30	3.25
	23/07/83	0.03	0.07		
	28/10/83	0.51	1.27		
		mean:	0.66		
O. phlogopappa	23/03/83	0.11	0.27		
	20/02/84	0.13	0.33		
	22/08/84	0.14	0.34		
		mean:	0.31		
P. squameum	21/02/84	0.40	0.73*		
	27/02/84	0.48	0.87#	0.36	0.65"
	27/02/84	0.09	0.16"	0.88	1.60*
P. lasianthos	23/03/83	0.05	0.10		
	24/04/83	0.13	0.28	0.50	1.07
	23/07/83	0.15	0.33		
		mean:	0.24		

* stems and leaves # leaves " stems

		% YIELD			
		WET	DRY	WET	DRY
	DATE	S.D.	SOL.	S.D.	SOL.
S. linearifolius	23/03/83	0.46	1.59		
	24/04/83	0.23	0.81	1.78	6.15
		mean:	1.20		
Z. arborescens	24/04/83	0.13	0.33	2.28	5.77

TABLE 4.2.II

Dry Weights

(Expresseed As Percentage Wet Weight)

Bedfordia salicina	33.6%
Beyeria viscosa	47.3%
Callitris tasmanica	57.7%
Cassinia aculeata	48.5%
Drimys lanceolata	62.9%
Drimys lanceolata	49.7%
Eriostemon virgatus	56.4%
Eucalyptus amygdalina	45.9%
Eucalyptus pulchella	39.4%
Kunzea ambigua	83.5%
Leptospermum glaucescens	62.5%
Leptospermum lanigerum	54.9%
Leptospermum scoparium	55.1%
Melaleuca squamea	42.7%
Melaleuca squarrosa	48.6%
Olearia argophylla	40.1%
Olearia phlogopappa	40.7%
Phebalium squameum	55.1%
Prostanthera lasianthos	46.9%
Senecio linearifolius	29.0%
Zieria arborescens	39.5%

4.3 ESTIMATION OF YIELD PER HECTARE

In the table below, the column headed A represents the average number of plants per hectare. This value was estimated by setting up quadrats of 5m x 5m and counting the number of plants of the particular species under investigation. The procedure was repeated four times for each species, then averaged. The final estimate of number of plants per hectare was then calculated by multiplying the average number per quadrat by 400.

Column B shows the approximate weight of plant material that is harvested from each individual, in grams.

Column C indicates the weight of essential oil, in grams, that can be derived from each individual on a dry weight basis. This figure is obtained from the average dry weight yield as shown in Table 4.2.I, and the value given in Column B.

Column D is the average yield per hectare as calculated by mutliplying the weight of oil per individual (Column C), by the number of individuals per hectare (Column A). The results are expressed in kilograms per hectare.

It should be noted that the weight of essential oil shown in Column C is the yield obtained by steam distillation in all cases except for *Beyeria viscosa*. In the latter instance, the solvent extraction dry weight yield was used, since the yield by steam distillation was disappointing both in quantity and quality.

Table 4.3.I is an extremely conservative estimation of yields obtainable from natural stands, where the total stripping of any one plant is not desirable. In fact, each plant was selectively pruned in such a way as to retain the natural shape of its canopy and only the youngest portions of growth were removed. Therefore, no whole limbs were removed and no plants were destroyed. It is estimated that one third, or less, of the foliage was taken in each case.

Table 4.3.II gives an estimation of potential commercial yields, based on high density plantings, coupled with a mowing-type harvesting technique, where all foliage above a pre-determined height is removed. The columns are labelled in the same way as in Table 4.3.I.

TABLE 4.3.I

	A	B	C	D
Bedfordia salicina	2480	2000	3.20	7.94
Beyeria viscosa	3000	300	22.74	68.22*
Callitris tasmanica	280	1600	17.60	4.93
Cassinia aculeata	1600	2200	4.84	7.74
Drimys lanceolata	2000	300	2.04	4.08
Eriostemon virgatus	2000	500	4.96	9.91
Eucalyptus amygdalina	1600	1780	45.21	72.34
Eucalyptus pulchella	2400	975	19.50	46.80
Kunzea ambigua	1200	300	2.25	2.70
Leptospermum glaucescens	1200	850	1.96	2.35
Leptospermum lanigerum	4080	950	8.46	34.50
Leptospermum scoparium	6400	1575	8.19	52.42
Melaleuca squamea	2800	705	0.28	0.97
Melaleuca squarrosa	400	765	2.37	0.85
Olearia argophylla	1000	1000	12.70	12.70
Olearia phlogopappa	1200	300	0.81	0.97
Phebalium squameum	2400	750	6.53	15.66
Prostanthera lasianthos	3400	750	1.80	6.12
Senecio linearifolius	1800	500	6.00	10.80
Zieria arborescens	8000	500	1.65	13.70

* Solvent Extraction

TABLE 4.3.II

	A	B	C	D
Bedfordia salicina	3000	3000	5	14
Beyeria viscosa	5500	1000	76	417
Callitris tasmanica	2000	10000	110	220
Cassinia aculeata	3000	4000	9	26
Drimys lanceolata	4500	1500	10	45
Eriostemon virgatus	4500	1500	22	97
Eucalyptus amygdalina	2500	12000	448	1119
Eucalyptus pulchella	2500	10000	195	488
Kunzea ambigua	4000	3000	23	90
Leptospermum glaucescens	4000	2500	6	23
Leptospermum lanigerum	4000	2500	22	89
Leptospermum scoparium	4000	3000	16	63
Melaleuca squamea	4000	2500	1	4
Melaleuca squarrosa	4000	2500	8	30
Olearia argophylla	2500	3000	38	95
Olearia phlogopappa	4000	2000	6	22
Phebalium squameum	3500	1500	11	38
Prostanthera lasianthos	4000	2000	5	19
Senecio linearifolius	4700	2000	24	113
Zieria arborescens	8000	3000	10	79

4.4 PHYSICAL PROPERTIES

The refractive indicies and optical rotations of the steam distilled oil samples are shown in Tables 4.4.I and 4.4.II respectively.

TABLE 4.4.I

Refractive Index

SPECIES	n_D^{20}
Bedfordia salicina	1.5082
Callitris tasmanica	1.4658
Callitris tasmanica (hybrid)	1.4629
Cassinia aculeata	1.5025
Drimys lanceolata	1.4765
Eriostemon virgatus	1.4758
Eucalyptus amygdalina	1.4764
Eucalyptus pulchella	1.4852
Kunzea ambigua	1.4790
Leptospermum glaucescens	1.4832
Leptospermum lanigerum	1.4910
Leptospermum scoparium	1.4826
Melaleuca squamea	1.4676
Melaleuca squarrosa	1.4710
Olearia argophylla	1.5022
Olearia phlogopappa	1.4990
Phebalium squameum	1.4766
Prostanthera lasianthos	1.4682
Ziera arborescens	1.4969

TABLE 4.4.II

Optical Rotation

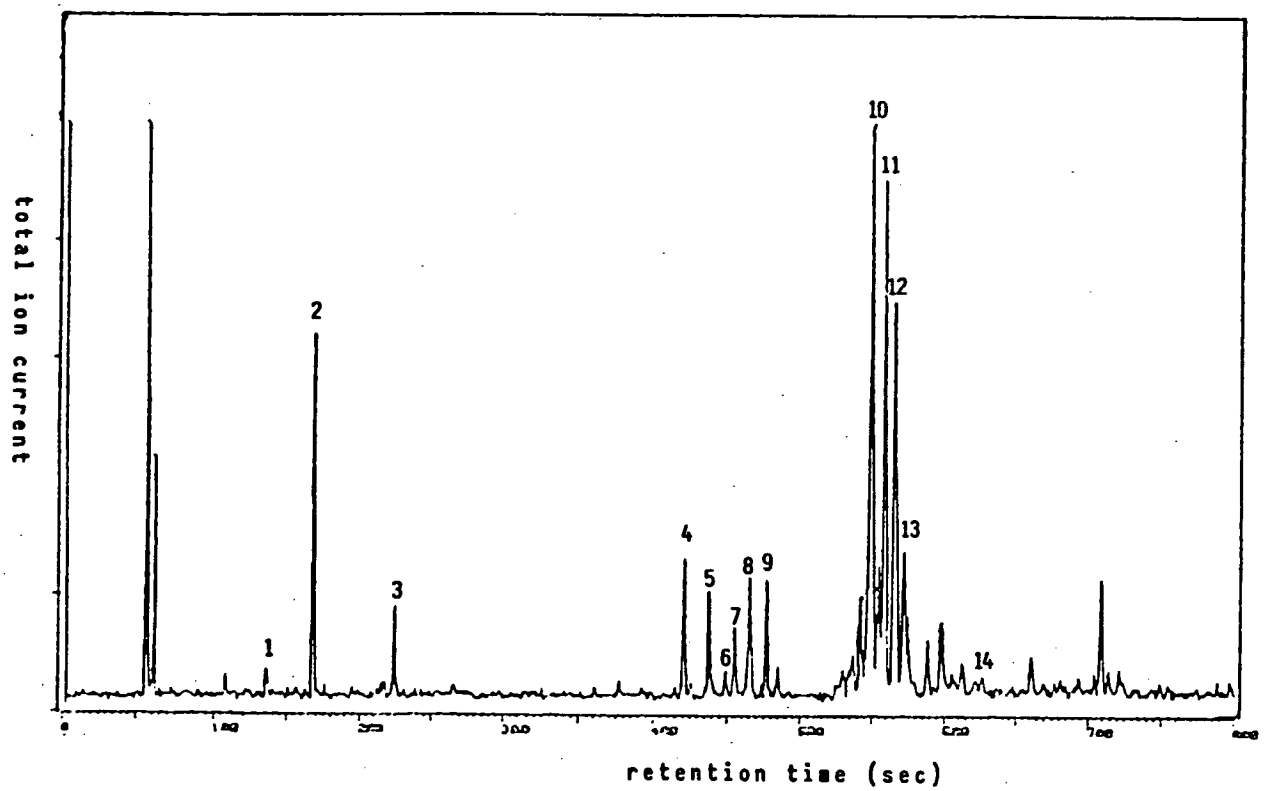
SPECIES	$[\alpha]_D^{20}$
Callitris tasmanica	-8.46°
Drimys lanceolata	+10.65°
Eriostemon virgatus	+29.49°
Eucalyptus amygdalina	-48.98°
Eucalyptus pulchella	-19.36°
Leptospermum glaucescens	+18.26°
Leptospermum lanigerum	+18.77°
Phebalium squameum (leaves)	+29.47°
Phebalium squameum	+24.44°
Prostanthera lasianthos	+4.69°
Zieria arborescens	-52.90°

The list of optical rotation measurements is incomplete. This was caused by the fact that in some cases only a very small volume of oil was available. The oil must completely fill the tube in which the rotation is measured. In addition *Beyeria viscosa* posed the extra problem of crystallization in the tube, due to the presence of camphene. The above table can be added to when sufficient volumes of oils become available.

Values such as those given above can be useful in relating the odour of these new oils to the odour of known compounds. The optical rotation of an oil with a particular major constituent may vary from the rotation of that component. In such a case, the major constituent may be an isomeric form of that substance. In addition, the above figures give another criteria for comparisons between other oils, and synthetically produced oils, though physical measurements of this nature are not as meaningful as organoleptic assessments.

4.5 GAS CHROMATOGRAPHY - MASS SPECTROMETRY

The results of the GC-MS analyses are presented in this section. In addition, the aromagrams of the species which were considered to be of the greatest potential, are given below the GC-MS traces. Here they serve as an aid to correlating the odours noted during the GC run with the compounds identified during analysis.



Bedfordia salicina

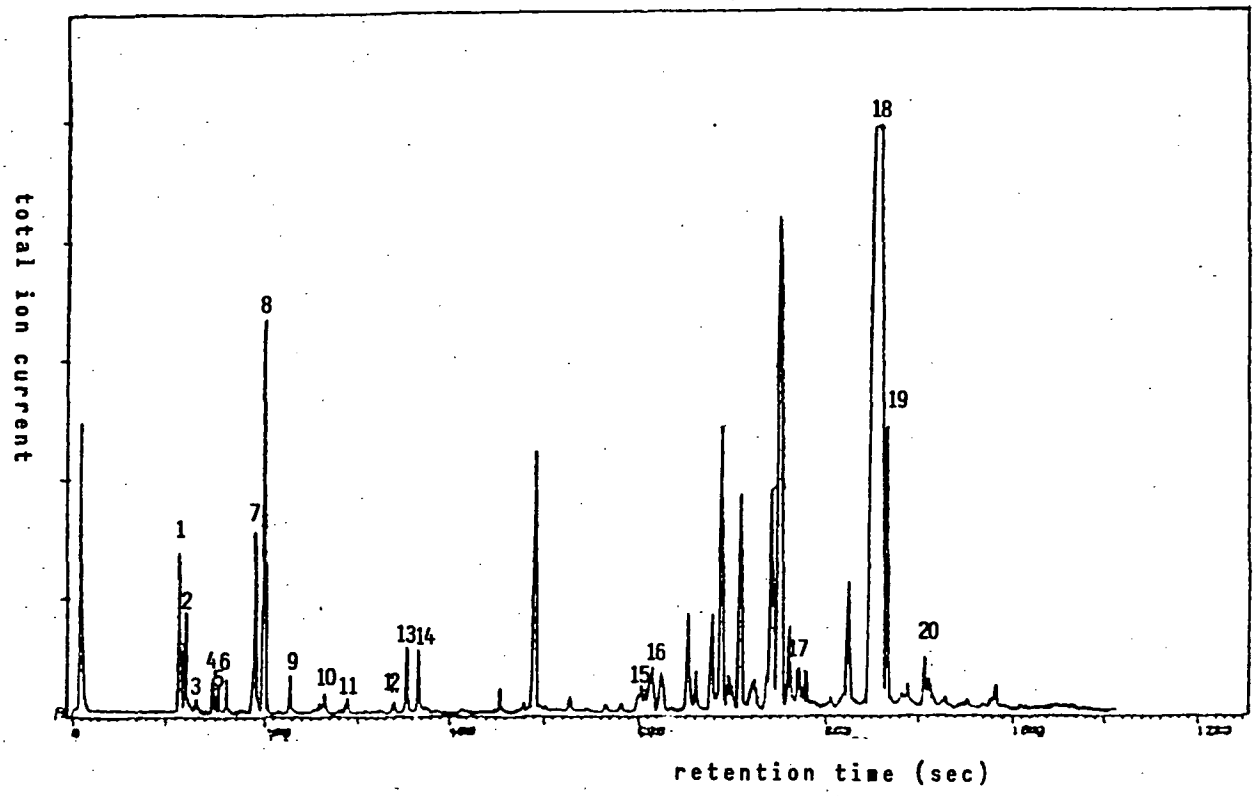
1 cymene	8 α-curcumene
2 linalool	9 sesquiterpene
3 α-terpineol	10
4 caryophyllene	11 globulol/viridiflorol/ledol
5 sesquiterpene	12
6 humulene	13 rosifoliol
7 sesquiterpene	14 β-eudesmol

Figure 4.5.I

Beyeria viscosa

1 α-thujene
2 α-pinene
3 camphene
4 sabinene
5 β-pinene
6 myrcene
7 p-cymene
8 limonene + cineole
9 γ-terpinene
10 linalool
11 perillene
12 pinocamphone type
13 terpinene-4-ol
14 α-terpineol
15 α-copaene
16 sesquiterpene
17 δ-cadinene
18 ledol/globulol/viridiflorol
19 rosifoliol
20 eudesmols

1 Spicey strong Beyeria
2 Violets
3 Sweet, soapy
4 Smokey, floral, sweet
5 Sweet
6 Dry, dusty, sweet
7 Smokey
8 Fresh, biting, spicey
9 Citrus, fresh
10 Beyeria
11 Cool and clear
12 Bushy
13 Floral, Beyeria
14 Fruity
15 Mint-like
16 Strong floral sweet and soapy
17 Shaving cream
18 Faint Beyeria
19 Dry bushy
20 Sweet savory
21 Aftershave
22 Cologne
23 Thick and soapy
24 Strong Beyeria sharp, floral, vaccine
25 Sweet fragrant light
26 Soft and vague
27 Astringent, heady
28 Clear, cooling
29 Light, piercing sweet and strong
30 Strong, pungent lingering
31 Distasteful
32 Pleasant, floral and waxy
33 Lemon
34 Soap, sweet
35 Light, Beyeria tangy
36 Refreshing
37 Rubber
38 Heavier
39 Sweet, spicey pungent, Beyeria
40 Solder
41 Fragrant
42 Dry
43 Soapy, hot, floral
44 Beyeria, light pleasant
45 Burning



Beyeria viscosa

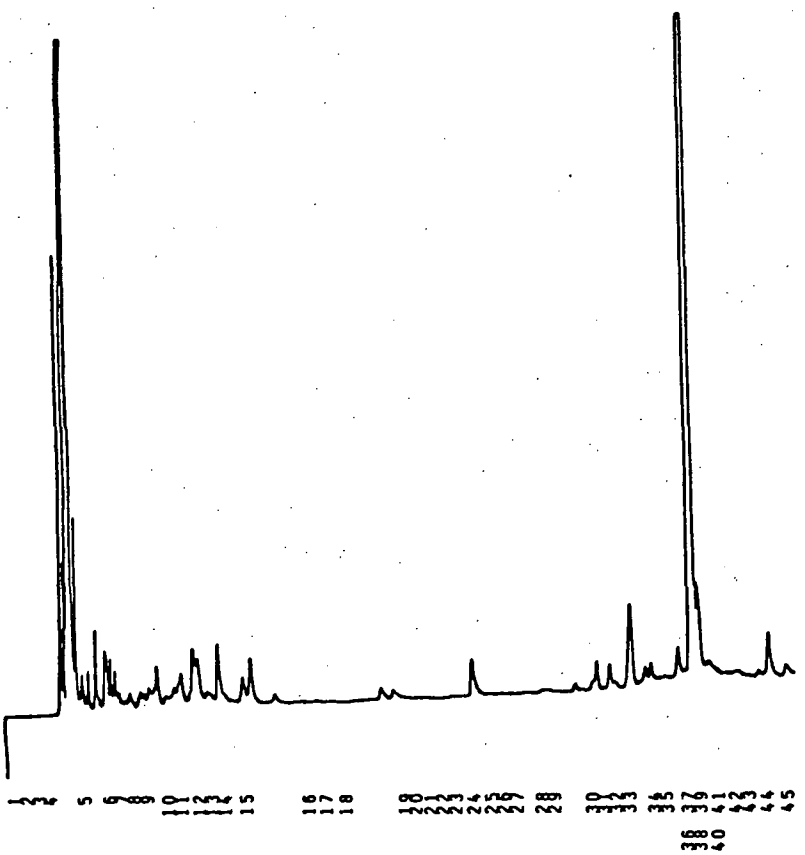
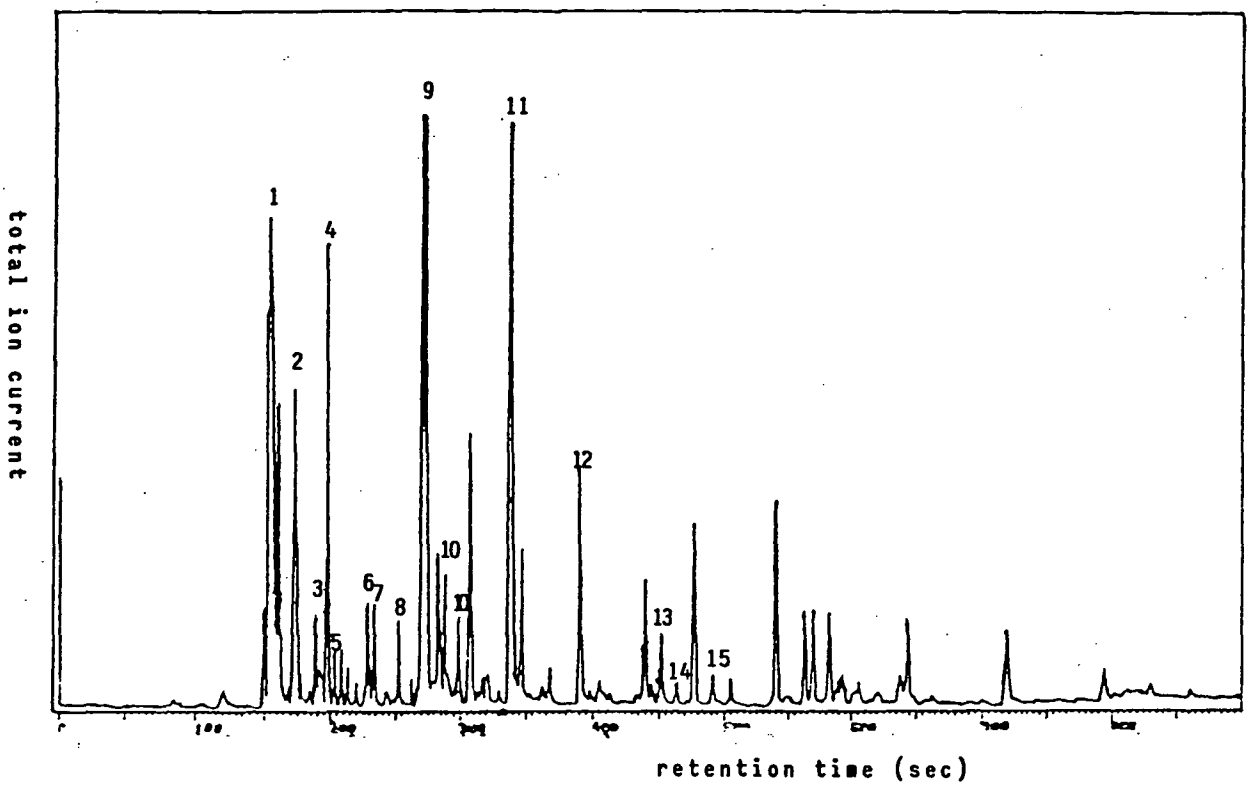


Figure 4.5.II

Callitris tasmanica

1 α-pinene
2 β-pinene
3 Δ-3-ocimene
4 limonene
5 3-methyl butyl butanoate
6 fenchone ?
7 α-terpinolene
8 α-campholene aldehyde
9 citronellal
10 terpenes
11 citronellol
12 anethole ?
13 phenyl ethyl ester
14 citronellyl acetate
15 geranyl acetate



Callitris tasmanica

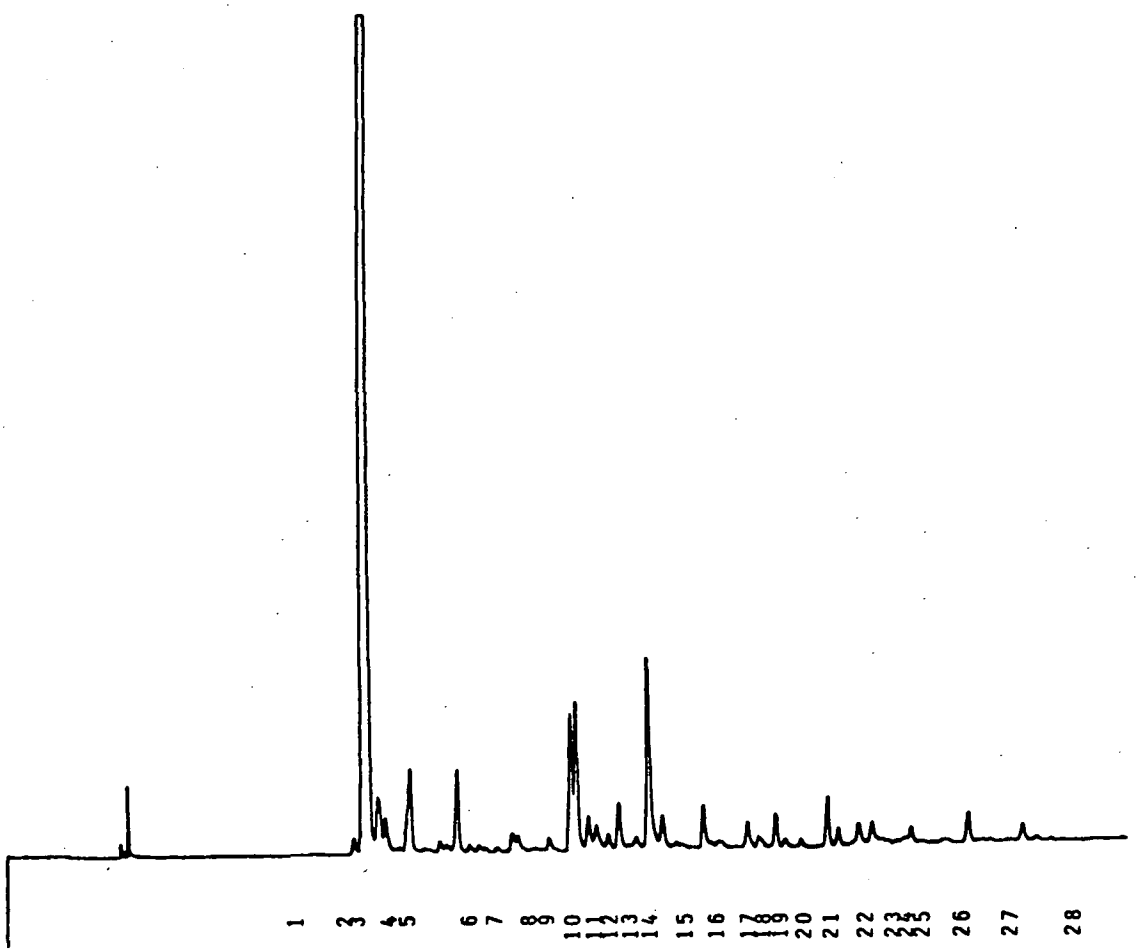
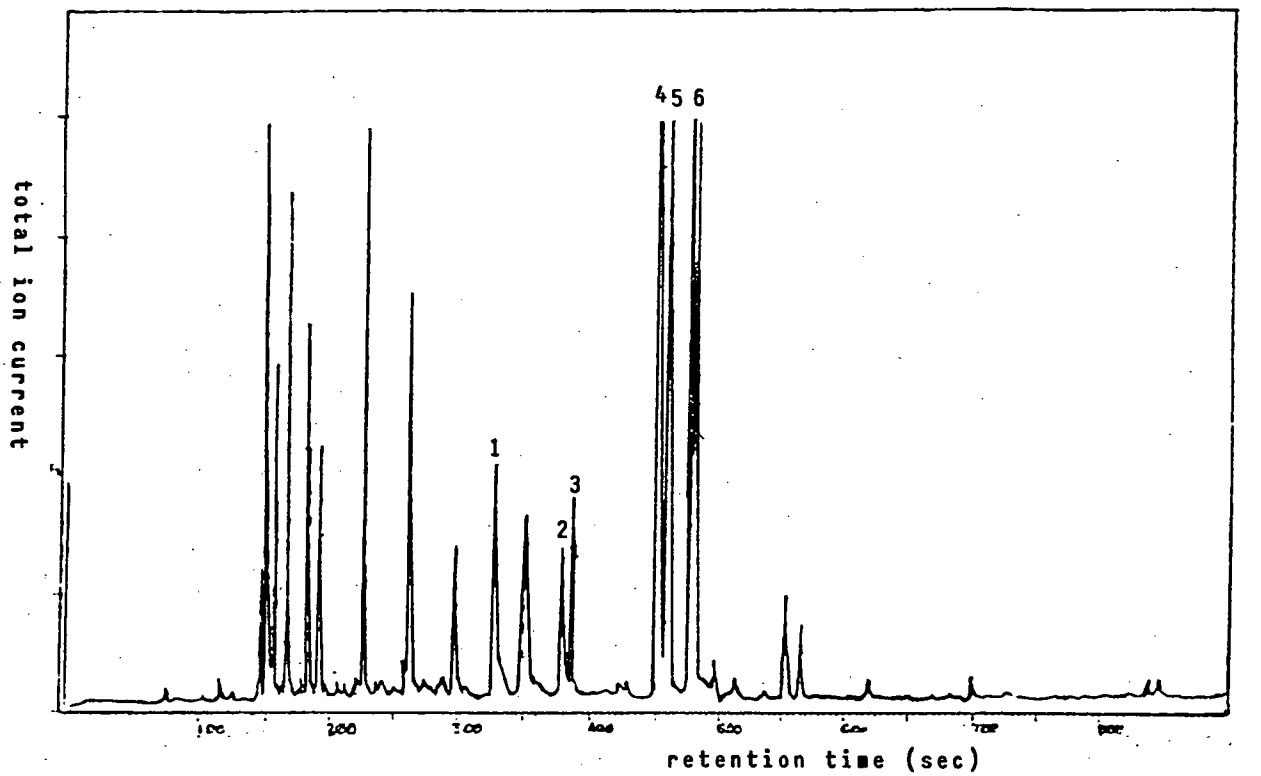


Figure 4.5.III

Callitris tasmanica (hybrid)

1 citronellol
2 anethole ?
3 bornyl acetate
4 citronellyl acetate
5 neryl acetate
6 lavandulyl acetate

1 Spicey, sweet
2 Hot sugar
3 Cool, clear, piney
4 Sweet mint
5 Cool, clear, pine
6 Sweet, slightly floral
7 Peppery, sweet, floral
8 Sweet floral becoming cooling
9 Inky, minty
10 Fruity sweet, cool
11 Cool, clear, cologne
12 Biting teatree
13 Citrus
14 Lavender, violets
15 Woody, citrus
16 Woody pine
17 Geranium, faint
18 Sickly sweet, medicinal
19 Medicinal
20 Faintly sweet, herby
21 Very soapy
22 Astringent
23 Sweet
24 Citrus
25 Cool clear
26 Harsh
27 Light, piney
28 Sweet pine



Callitris tasmanica (hybrid)

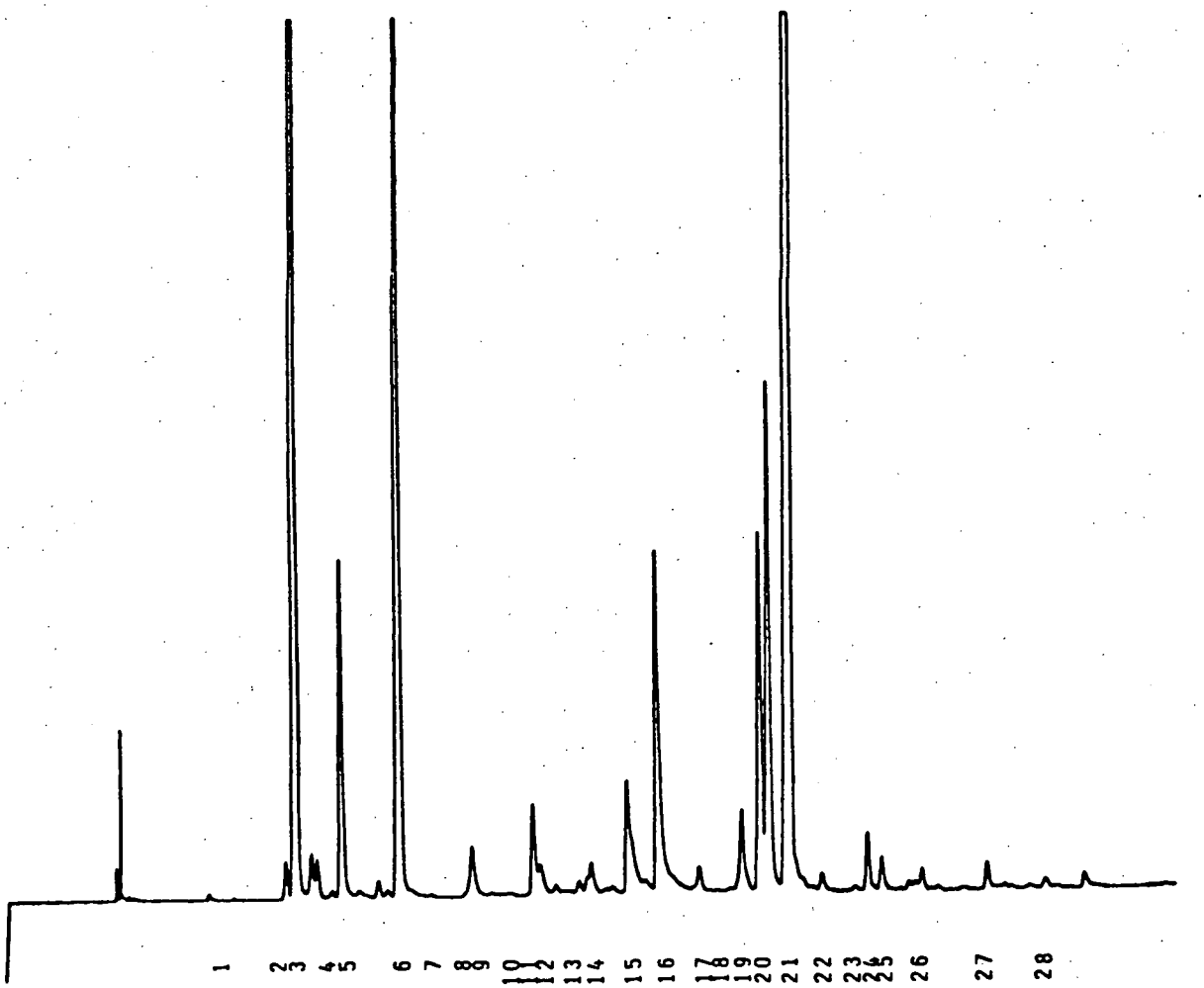
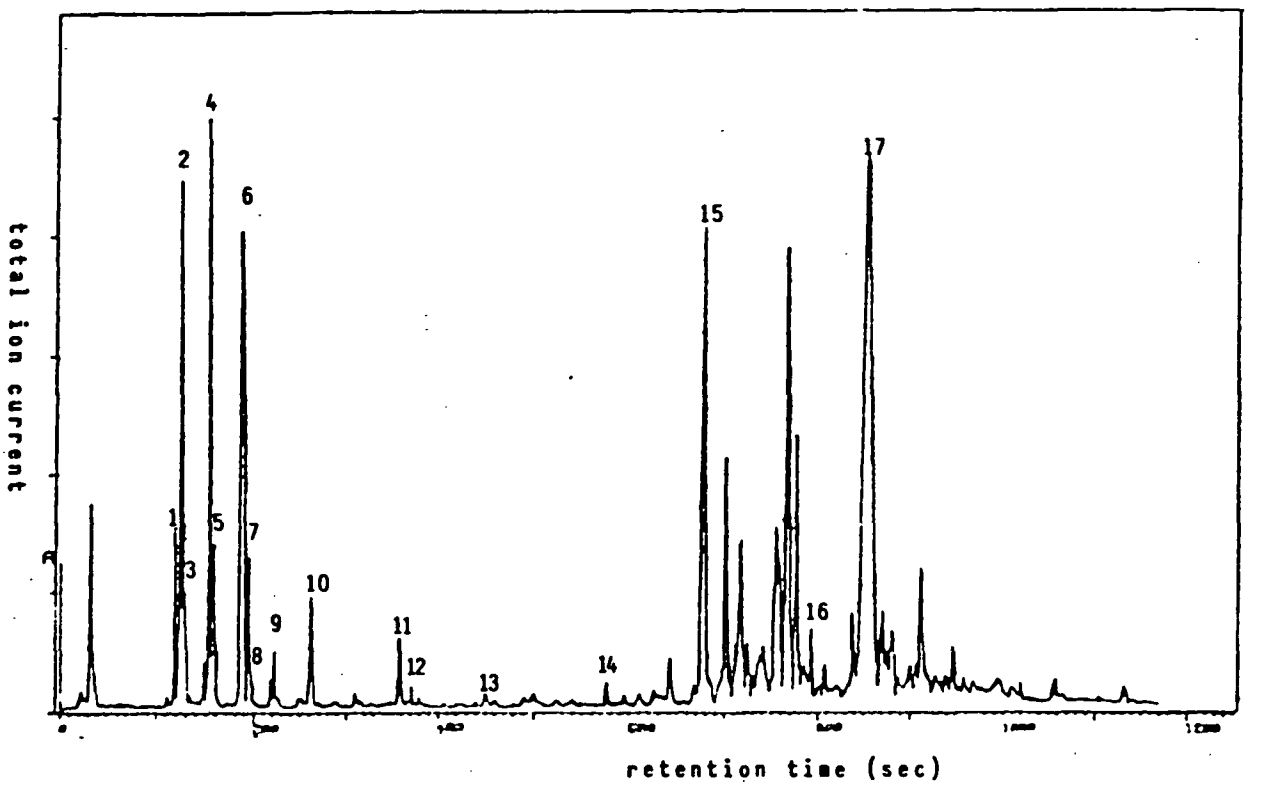


Figure 4.5.IV



Cassinia aculeata

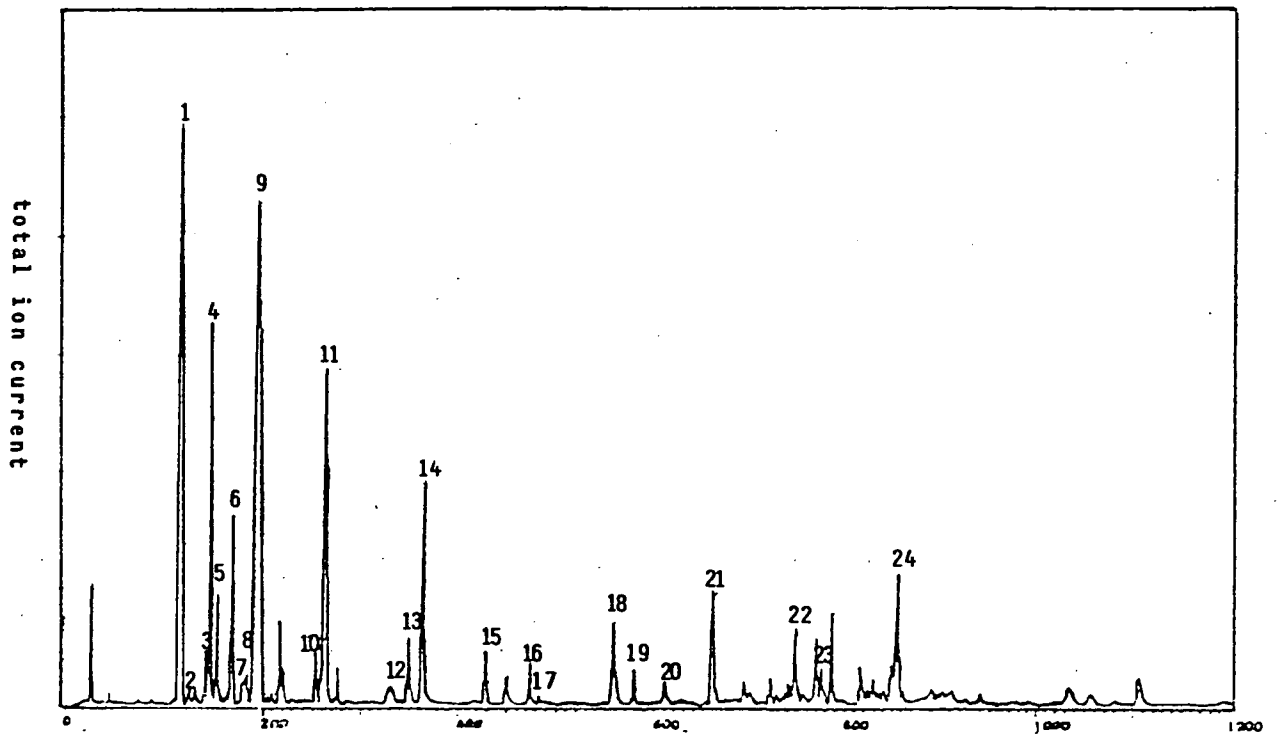
1 α-thujene	9 γ-terpinene
2 α-pinene	10 linalool
3 sabinene	11 terpinene-4-ol
4 β-pinene	12 α-terpineol
5 myrcene	13 nerol
6 p-cymene	14 γ-elemene
7 limonene/ 1,8-cineole	15 β-phellandrene
8 decanol	16 δ-cadinene

Figure 4.5.V

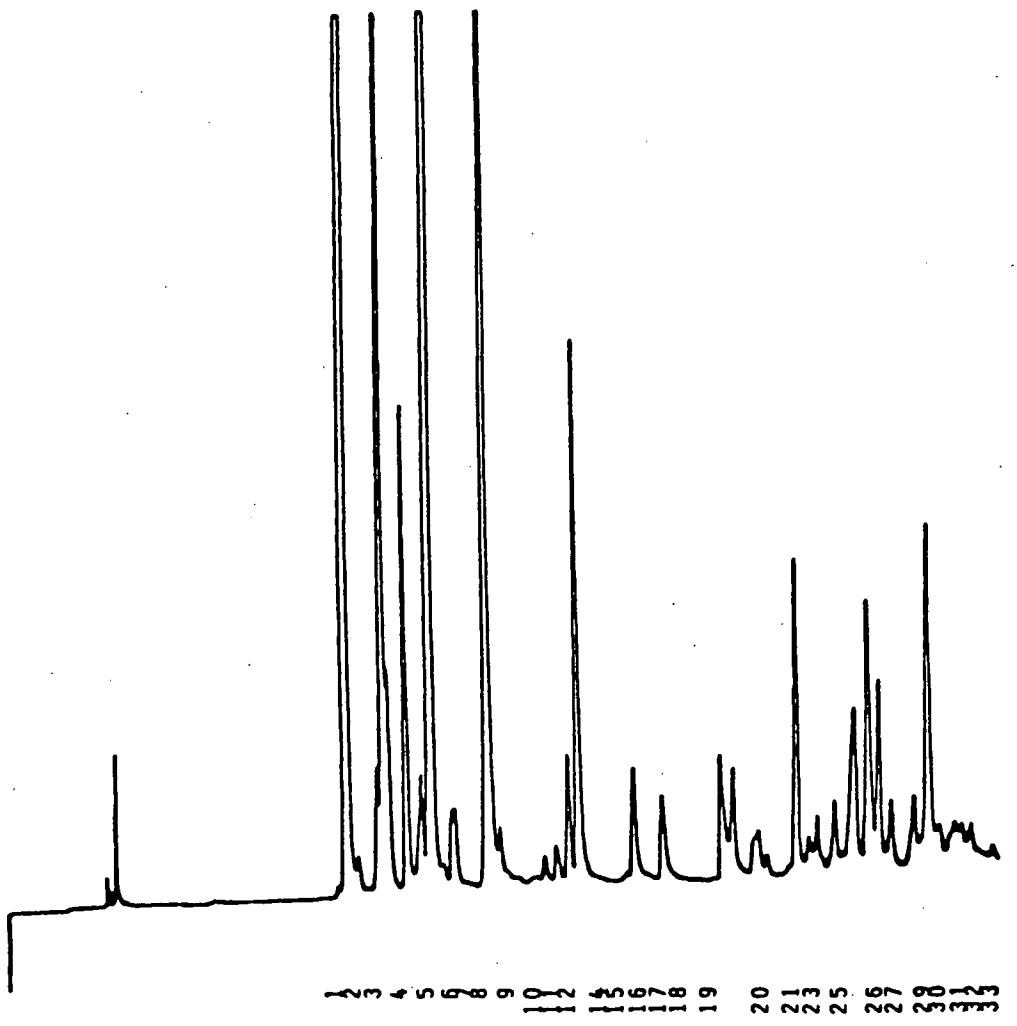
Drimys lanceolata

1 α-pinene
2 camphene
3 sabinene
4 β-pinene
5 myrcene
6 α-phellandrene
7 α-terpinene
8 p-cymene
9 1,8-cineole + limonene
10 terpinolene
11 linalool
12 γ-terpineol ?
13 terpinene-4-ol
14 α-terpineol
15 piperitone
16 safrole
17 sabinyl acetate
18 eugenol
19 copaene
20 methyl eugenol
21 caryophyllene
22 myristicin
23 δ-cadinene
24 guaiol

1 Pine
2 Syrupy sweet
3 Piney clear
4 Sweet cherry medicinal
5 Dry cloves
6 Eucalyptus
7 Bitter sweet
8 Lavender
9 Mint
10 Doughy sweet
11 Fruity
12 Fennel
13 Acrid
14 Lavender, very strong
15 Bandages
16 Marigolds
17 Surface cleaner
18 Faint pleasant
19 Cool clear antiseptic
20 Sweet mint
21 Cool clear pine
22 Violets
23 Stale beer
24 Cider
25 Floral cool
26 Pleasantly sweet
27 Cologne
28 Pleasant
29 Cool, fruity then rose-like
30 Soapy fragrant
31 Light and sweet
32 Soapy
33 Burning, woody



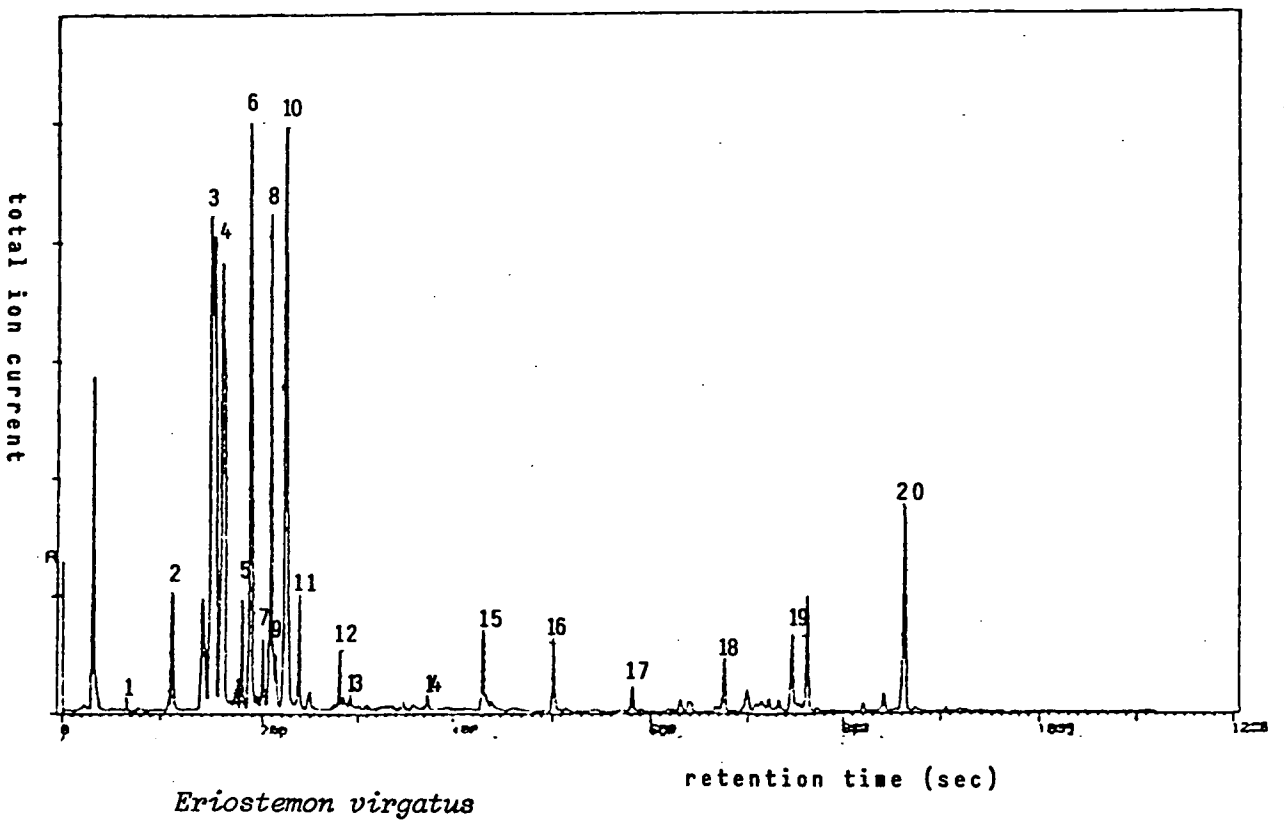
Drimys lanceolata



Eriostemon virgatus

1 iso-propyl butyrate
2 iso-propyl iso-valerate
3 α-pinene
4 camphene
5 β-pinene
6 myrcene
7 α-phellandrene
8 Δ-3-carene
9 ρ-cymene
10 limonene
11 cis-β-ocimene
12 terpinolene
13 iso-pentyl 2-methyl butanoate
14 terpinene-4-ol
15 citronellal
16 bornyl acetate
17 citronellyl acetate
18 β-caryophyllene
19 iso-propyl cinnamate
20 globulol/viridiflorol/ledol

1 Sweet pine solvents
2 Sweet, pine floral
3 Bitter eucalypt
4 Fresh tangy pine
5 Cool minty antiseptic
6 Sweeter woody pine
7 Preserving cucumbers
8 Leafy, musty, forest floor
9 Mushrooms
10 Violets fairly strong
11 Cabbage
12 Fresh pine woody
13 Sweet soapy
14 Almost spicey
15 Nauseating, worrying
16 Sweeter, piney
17 Eriostemon
18 Pea soup
19 Faint vegetable
20 Leatherwood flowers
21 Eriostemon
22 Sweet woody nondescript
23 Lavender
24 Hot peppery
25 Nauseating, sick
26 Cat fur
27 Floral, Calendulas
28 Celery
29 Mouthwatering, camphoraceous
30 Solder, flux, mellowing
31 Woody, Huon pine faint
32 Hot, furry
33 Clean, pine woody
34 Sulphurous
35 Comfortable, sweet piney, resinous



Eriostemon virgatus

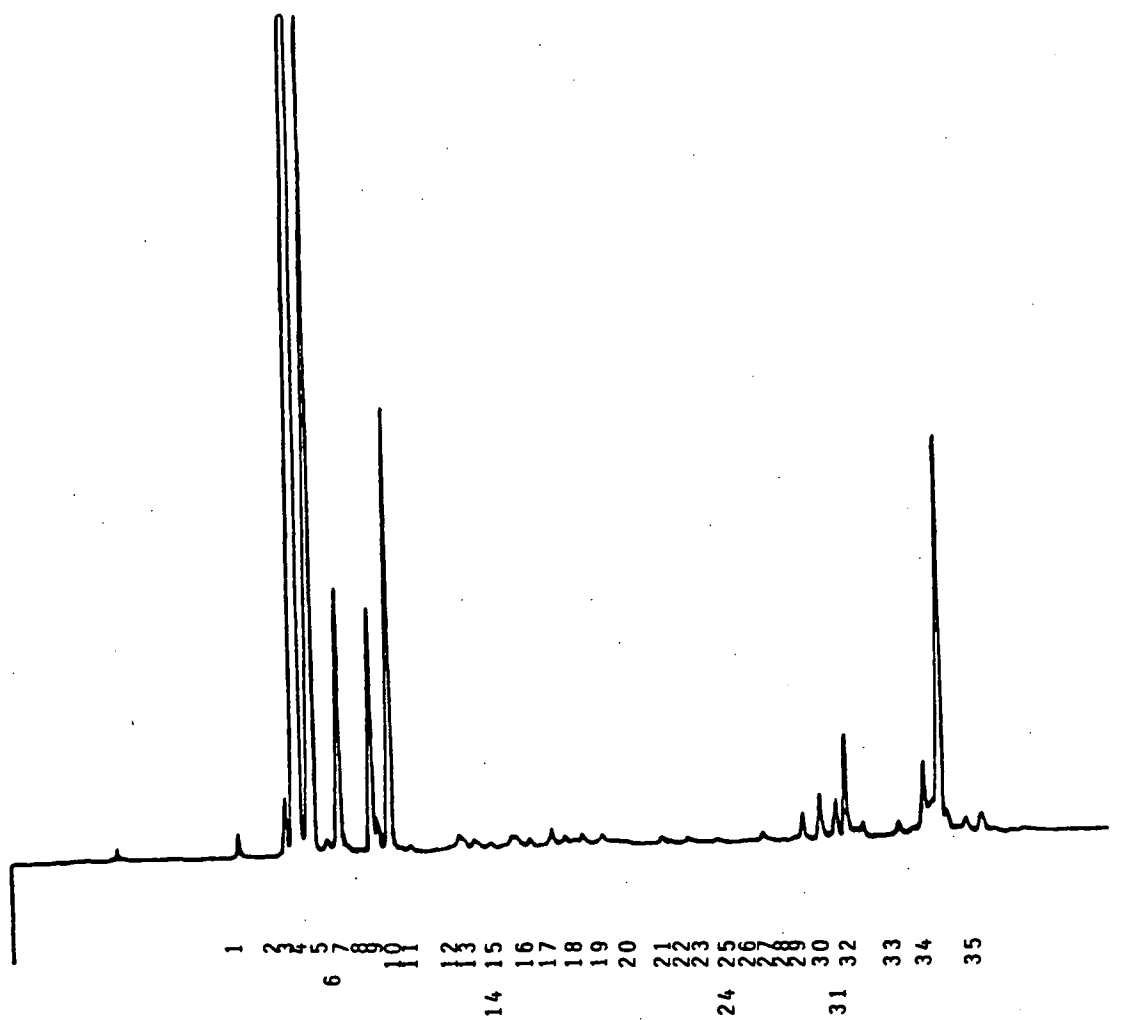
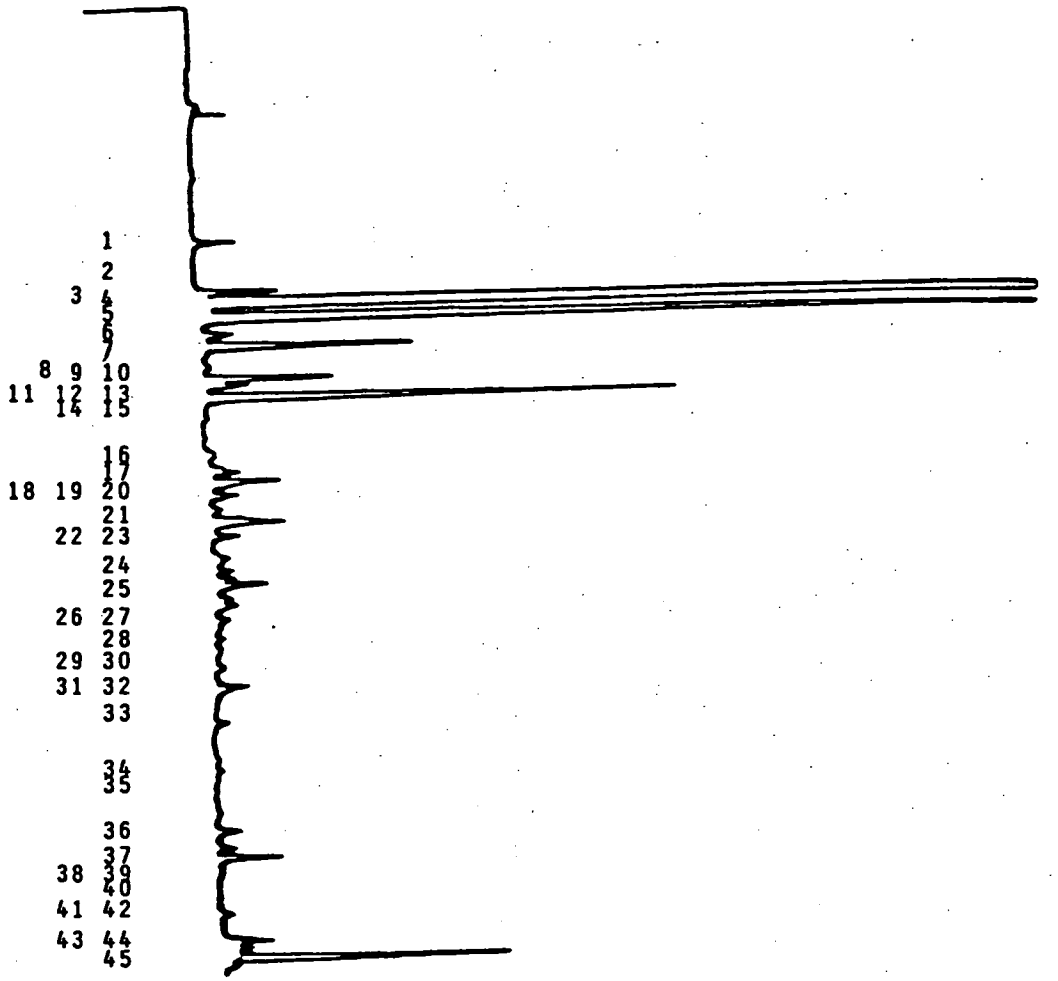


Figure 4.5.VII

Eriostemon virgatus 4-5-83



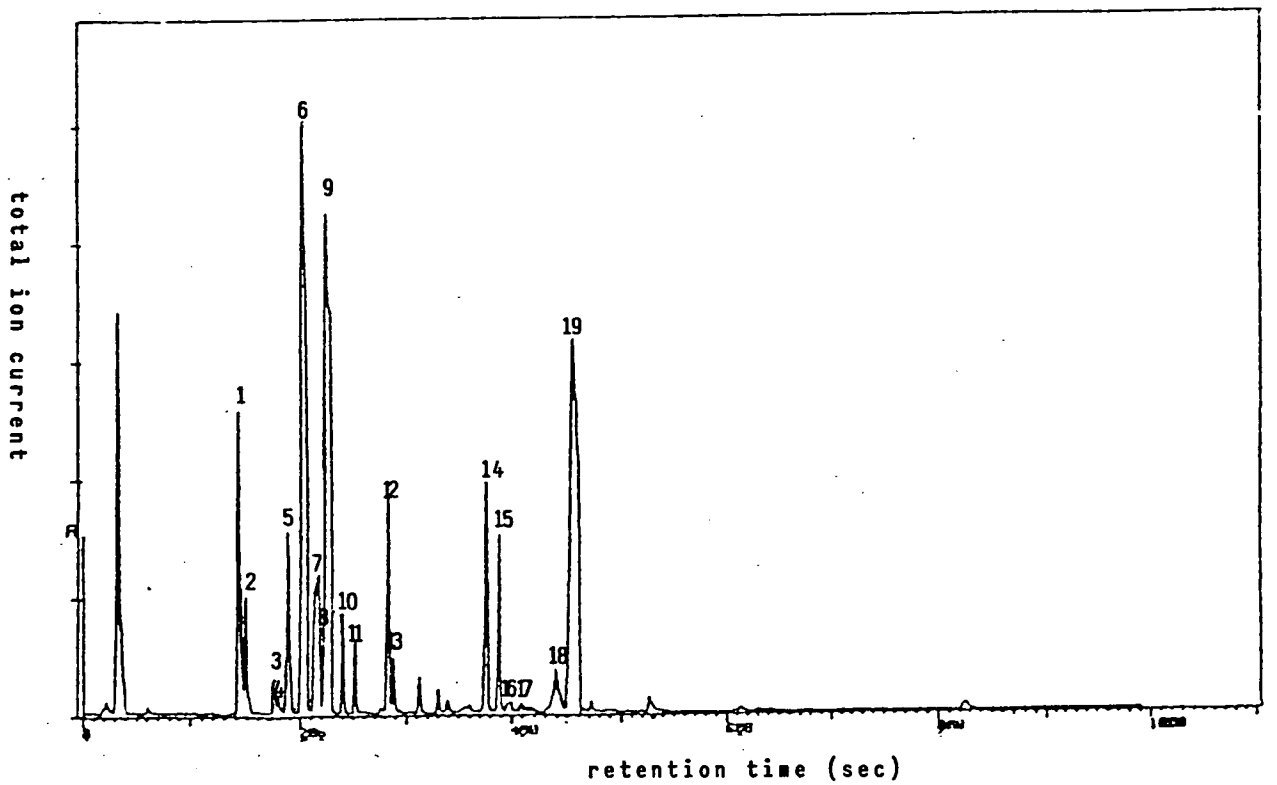
1 Floral
2 Medicinal, sweet
3 Sweet and nutty
4 Clear, sweet, piney
5 Eucalypt
6 Pine
7 Fruity, citrus
8 Heavy, 'Clag'
9 Grass
10 Leafy green
11 Mouldy
12 Violets
13 Heavy smokey, acrid
14 Clear, fresh aftershave
15 Woody, 'chlorine', strong
16 Hot plastic
17 Cashews
18 Mouthwatering, acrid, heavy
19 Piney
20 Sweet
21 Eriostemon
22 More woody, acrid
23 Floral, herby
24 Celery, vegetables
25 Leatherwood
26 Onion
27 Very sweet, woody
28 Tangy
29 Floral sweet
30 Acrid
31 Pleasant, forest
32 Capsicum
33 Celery
34 Violets
35 Minty, solder
36 Musty
37 Damp, leafy, pleasant
38 Lemon, pine
39 Sulphurous
40 Slightly woody, piney sweet
41 Woody, leafy
42 Huon pine, faint
43 Piney woody
44 Clear, sweet, piney resinous

Figure 4.5.VIII

Eucalyptus amygdalina

1 α-thujene
2 α-pinene
3 sabinene
4 β-pinene
5 myrcene
6 α-phellandrene
7 α-terpinene
8 p-cymene
9 1,8-cineole
10 cis-β-ocimene
11 sabinene dihydrate ?
12 terpinolene
13 linalool
14 terpinene-4-ol
15 α-terpineol
16 cis-piperitol
17 trans-piperitol
18 citronellal
19 piperitone

1 Pine
2 Cool, clear, thick
3 Violets
4 Citrus
5 Sweet
6 Burning
7 Cool, light, lemon
8 Cool, light, lemon, apples
9 Characteristic, leafy
10 Heavy, sweet, floral
11 Cooling, eucalypt
12 Cooling odour
13 Dry and dusty
14 Peppermint
15 Minty, apple
16 Cooling, clear, mint but heavy
17 Sweeter, woodier
18 Limes, heavy
19 Fragrant, soapy
20 Eucalypt
21 Sweet, floral
22 Sweet, floral
23 Dry and sweet



Eucalyptus amygdalina

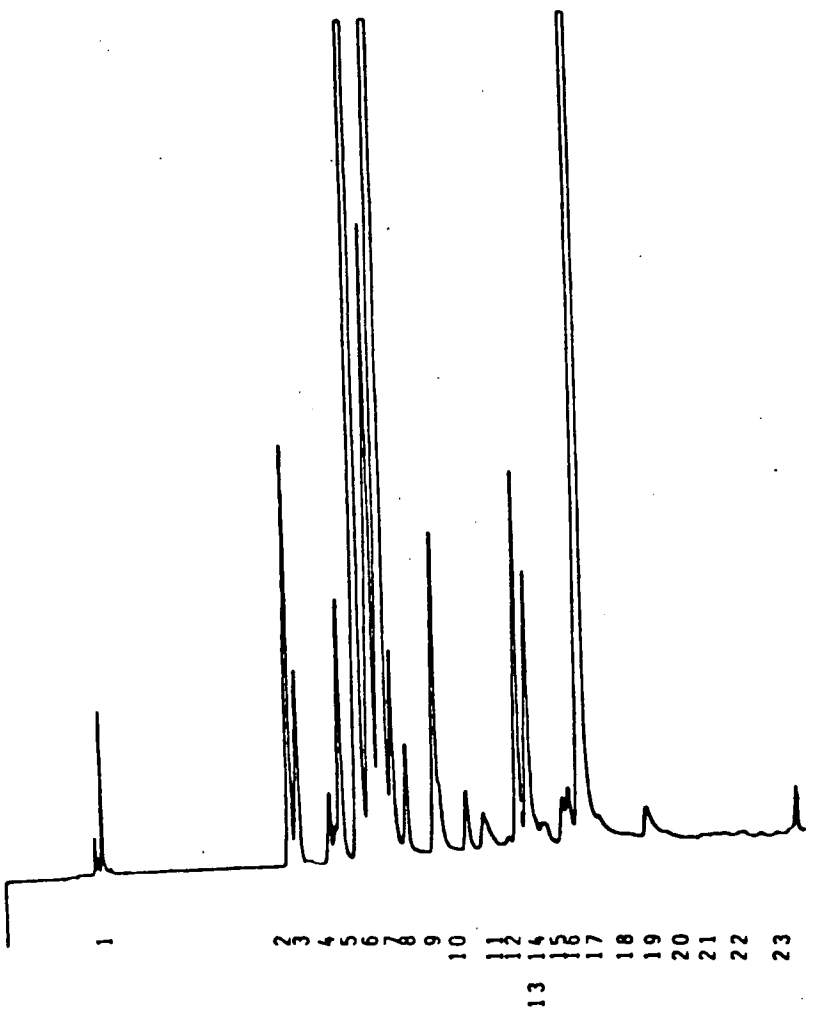
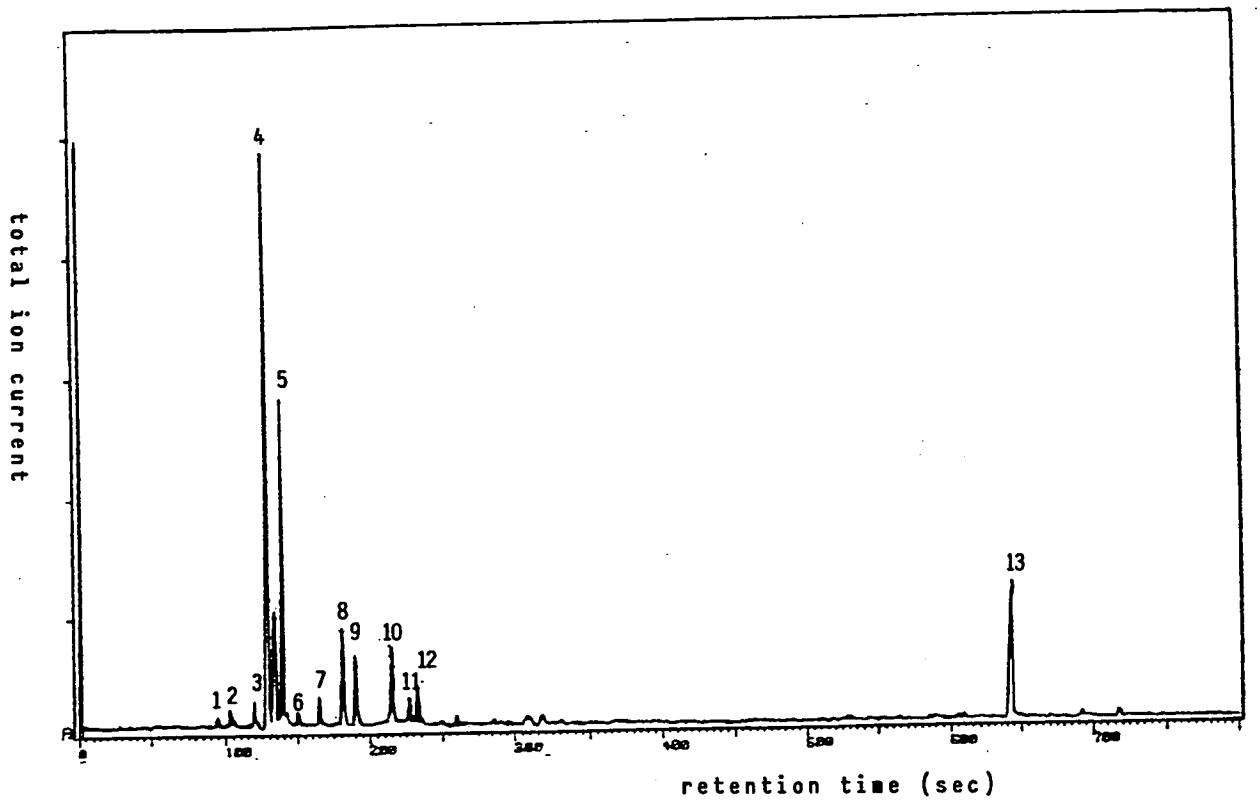


Figure 4.5.IX



Eucalyptus pulchella

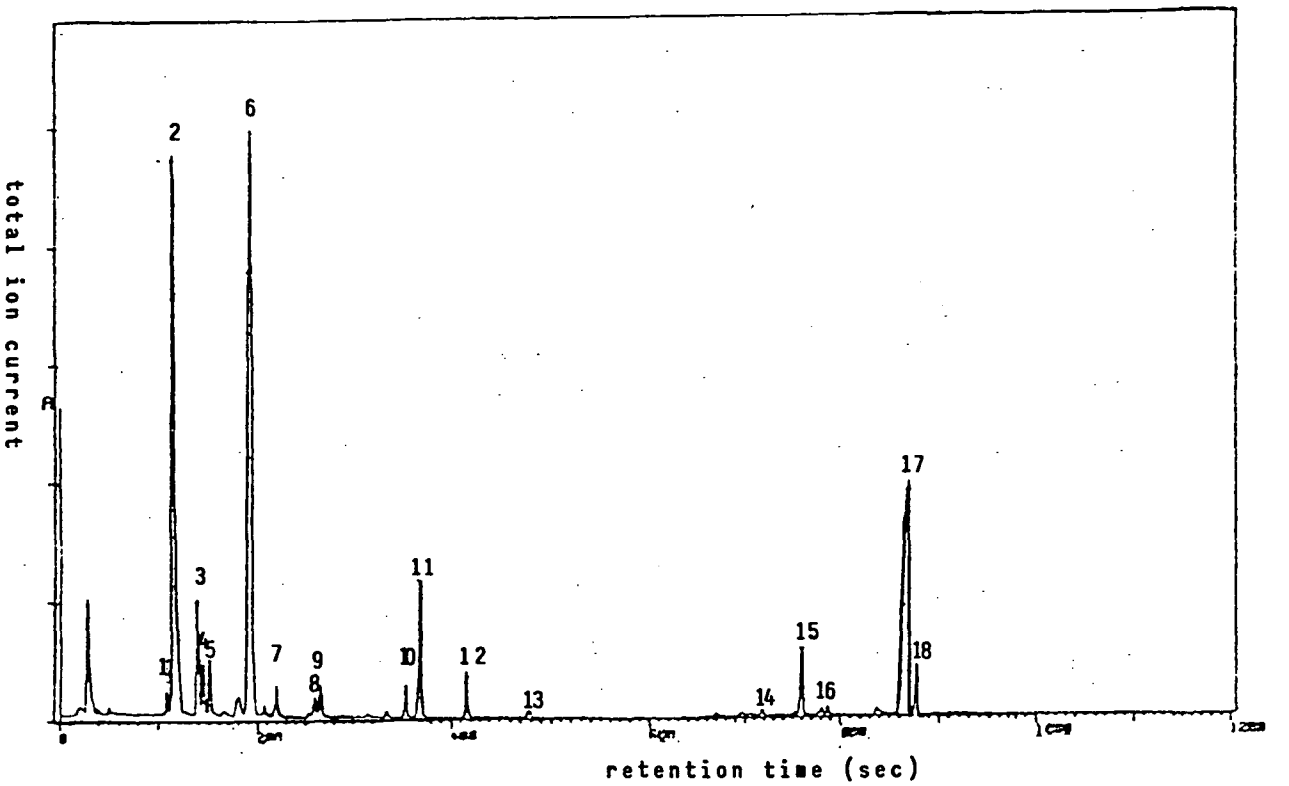
1 iso-butyl iso-butyrate
2 α-thujene
3 myrcene
4 α-phellandrene
5 β-phellandrene
6 γ-terpineol
7 α-terpinolene
8 unknown
9 unknown
10 terpinene-4-ol
11 cis piperitol
12 trans piperitol
13 unusual unknown

Figure 4.5.X

Kunzea ambigua

1 α-thujene
2 α-pinene
3 sabinene
4 β-pinene
5 myrcene
6 1,8-cineole
7 γ-terpinene
8 iso-pentyl 2-methyl-butryate type
9 iso pentyl iso pentanoate
10 terpinene-4-ol
11 α-terpineol
12 citronellol
13 anethole ?
14 β-caryophyllene
15 sesquiterpene
16 δ-cadinene
17 globulol/viridiflorol/ledol
18 ledol

1 Sweet, sweaty, ants, piney
2 Light
3 Light; a bit off
4 Menthol
5 Light 'airy'
6 Mouth-watering
7 Woody, old pine
8 Sweet, stimulating
9 Sweet, herby
10 Clear pine eucalypt
11 Clear and fresh
12 Apple
13 Mandarin
14 Stale, wet paper
15 Citrus
16 Spearmint, chewing gum
17 Floral, citrus
18 Clean, lime
19 Sweet floral
20 Citrus
21 Aniseed
22 Sweet
23 Sweet, vaguely minty
24 Floral, dry, green
25 Sweet, floral, light
26 Petrol
27 Heady



Kunzea ambigua

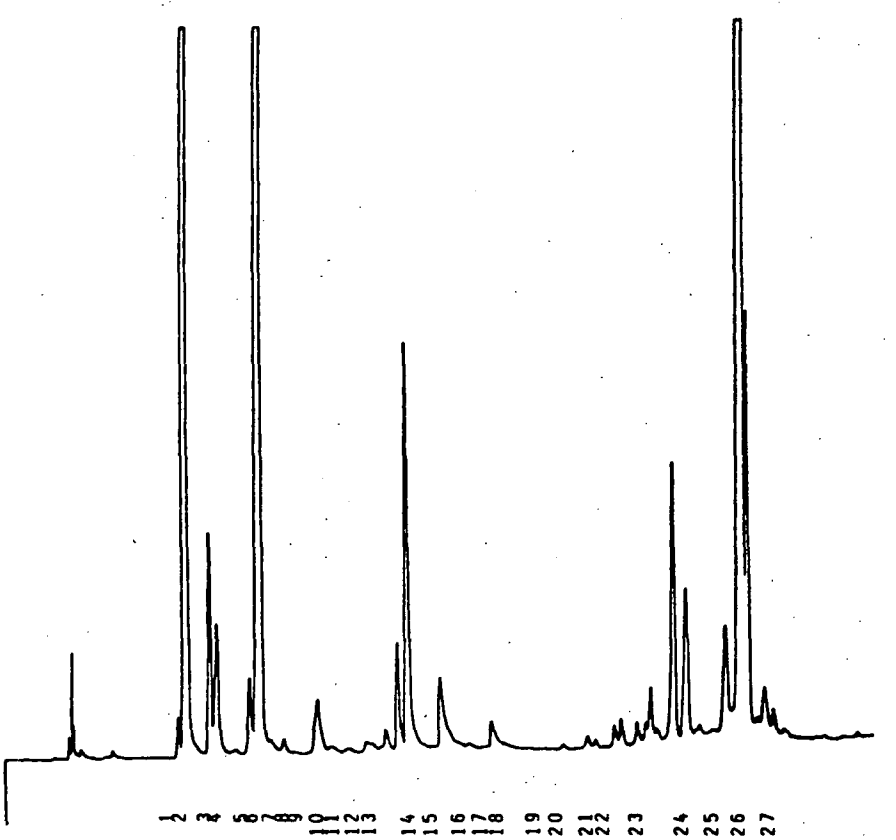
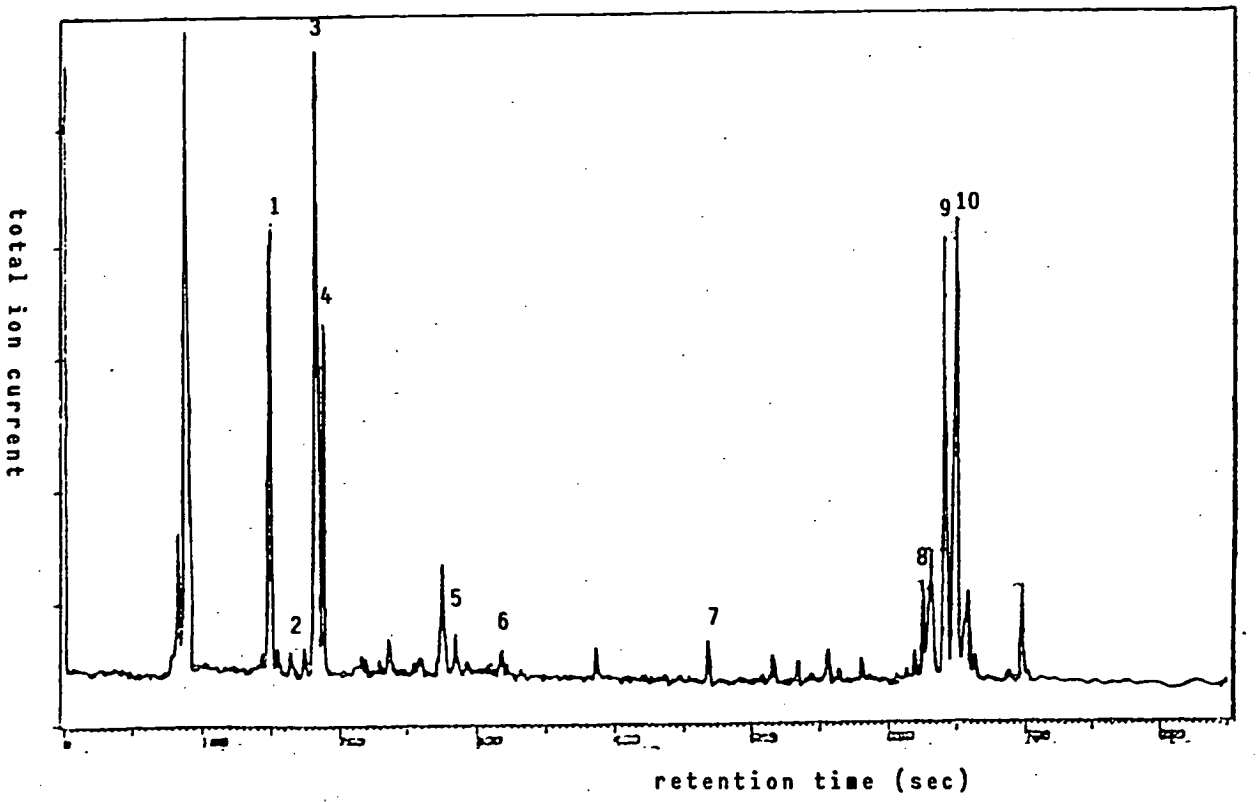


Figure 4.5.XI

Leptospermum glaucescens

1 α-pinene
2 β-pinene
3 p-cymene
4 limonene
5 α-terpineol
6 carvone
7 β-elemene
8 γ-eudesmol
9
sesquiterpene alcohols
10

1 Sickly sweet
2 Faint
3 Fresh citrus
4 Cooling
5 Sweet, dry wine then peppery
6 Sweet and musty
7 Sweet floral
8 Spicey
9 Violets then ashtray
10 Sweet floral
11 Sweet floral
12 Lime, very strong
13 Lime, citrus, sweet
14 Citrus : lemon, orange and lime
15 Sweet
16 Clean, clear
17 Antiseptic and woody
18 Sweet and clear
19 Light and sweet
20 Heavy sweet
21 Sweet leptospermum
22 Menthol
23 Sweet and dry
24 Dry bushy



Leptospermum glaucescens

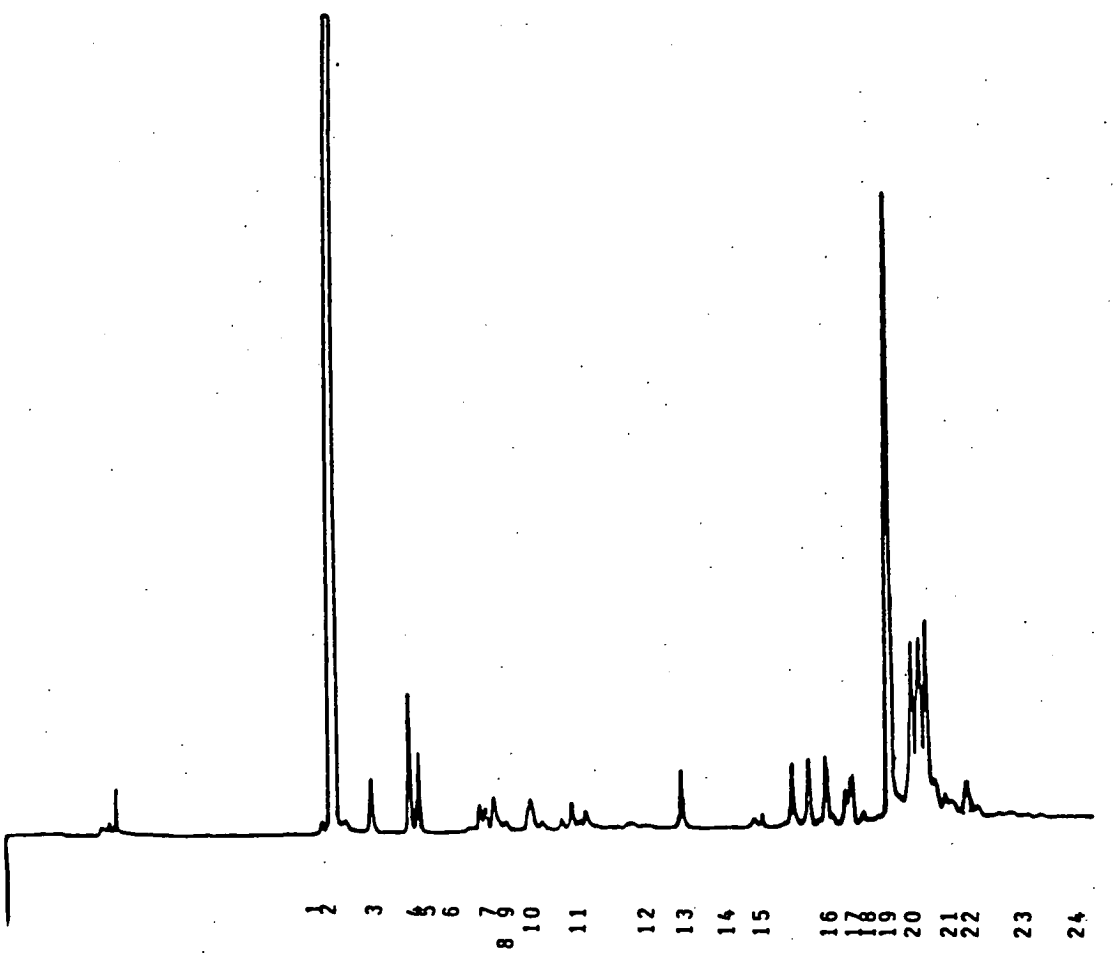
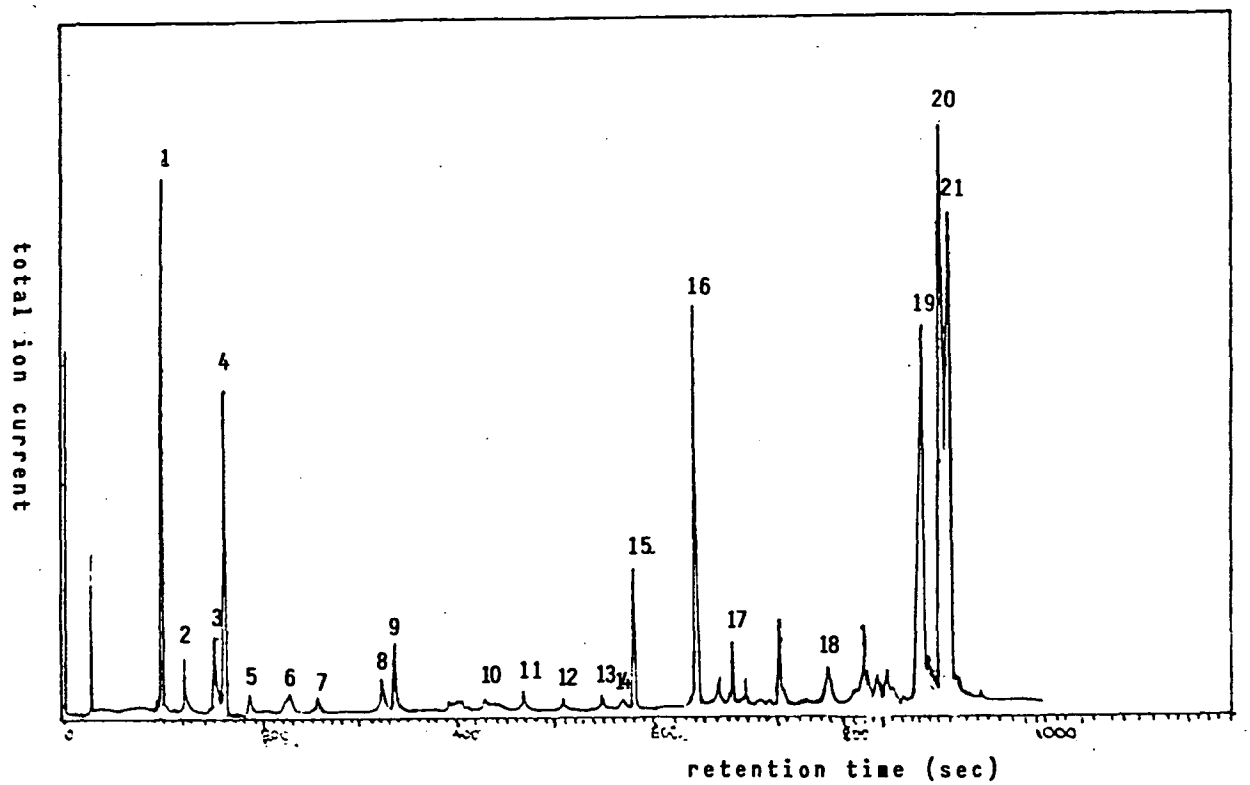


Figure 4.5.XII

Leptospermum lanigerum

1 α-pinene
2 β-pinene
3 ρ-cymene
4 1,8-cineole
5 γ-terpinene
6 linalool
7 hopinone ?
8 terpinene-4-ol
9 α-terpineol
10 nerol ?
11 2-undecanone
12 sabinyl acetate ?
13 citronellyl acetate
14 methyl cinnamate
15 myrtanyl acetate
16 caryophyllene
17 humulene
18 elemol
19 γ-eudesmol
20 β-eudesmol
21 α-eudesmol

1 Vaguely minty
2 Pine
3 Clear
4 Sweet pine
5 Cool, clear sweet
6 Spicey characteristic
7 Pine, cool aftershave
8 Leptospermum
9 Sweeter, floral
10 Terpentine
11 Pine woody
12 Leptospermum
13 Light sweet
14 Citrus
15 Leptospermum
16 Sweet
17 Leptospermum, peppery
18 Menthol
19 Leptospermum
20 Sweet and clear
21 Dry bush
22 Floral
23 Cool, clear spicey
24 Spicey sweet
25 Dry sweet, musty
26 Floral
27 Cool and clean
28 Heavy
29 Cool and biting
30 Sweet



Leptospermum lanigerum

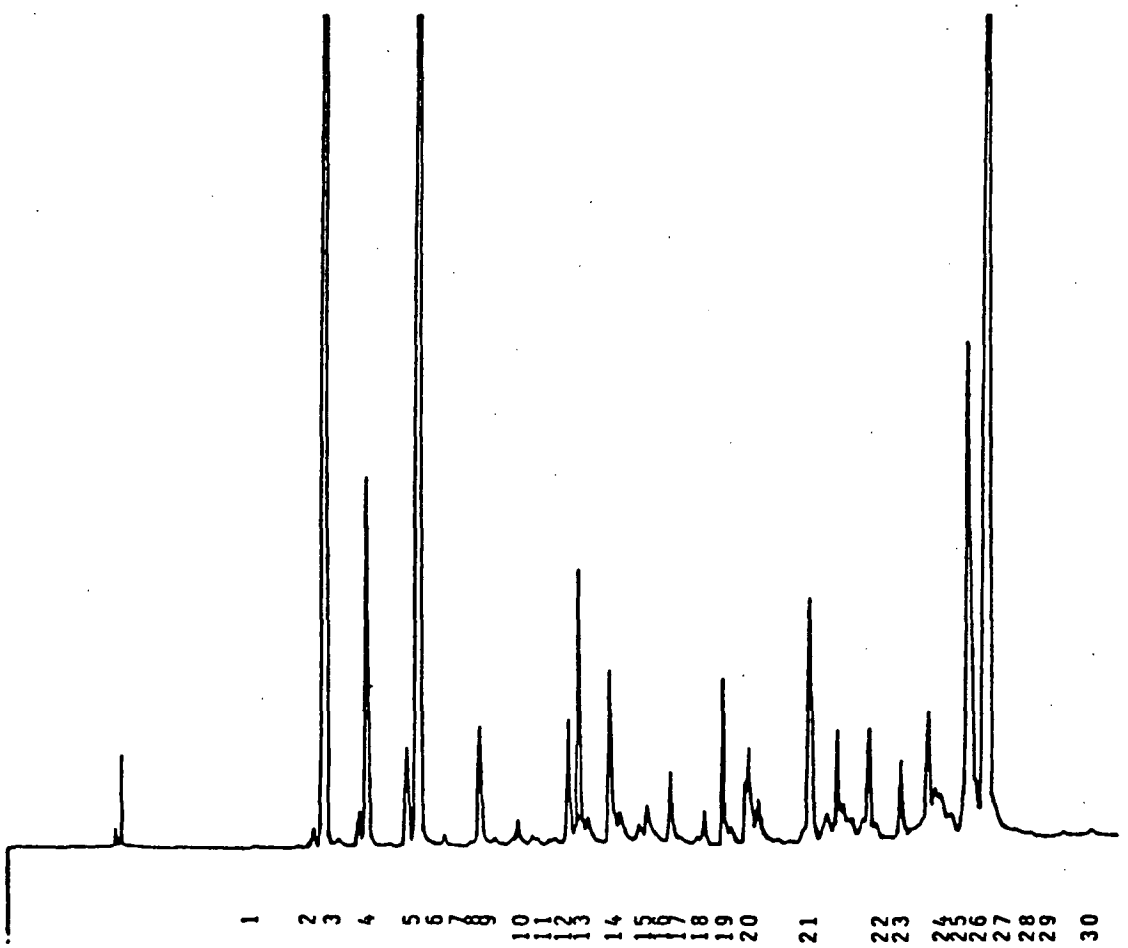


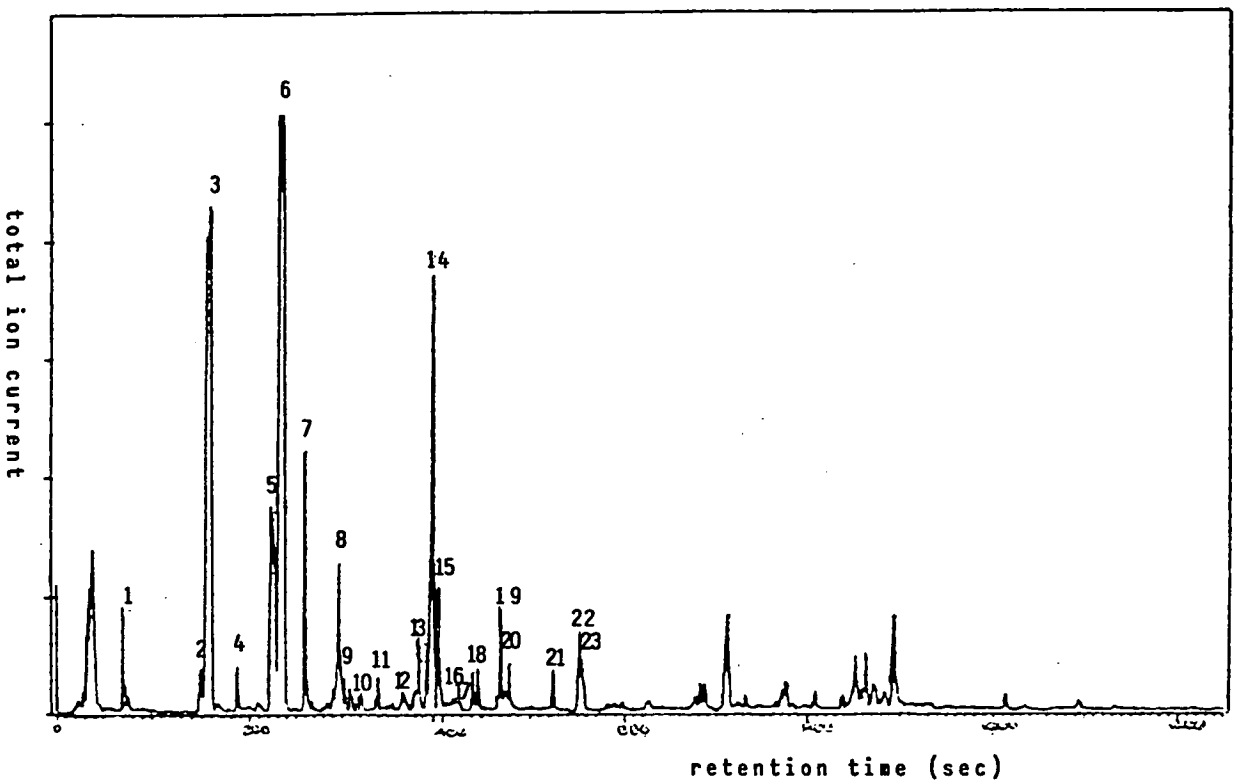
Figure 4.5.XIII

Melaleuca squamea

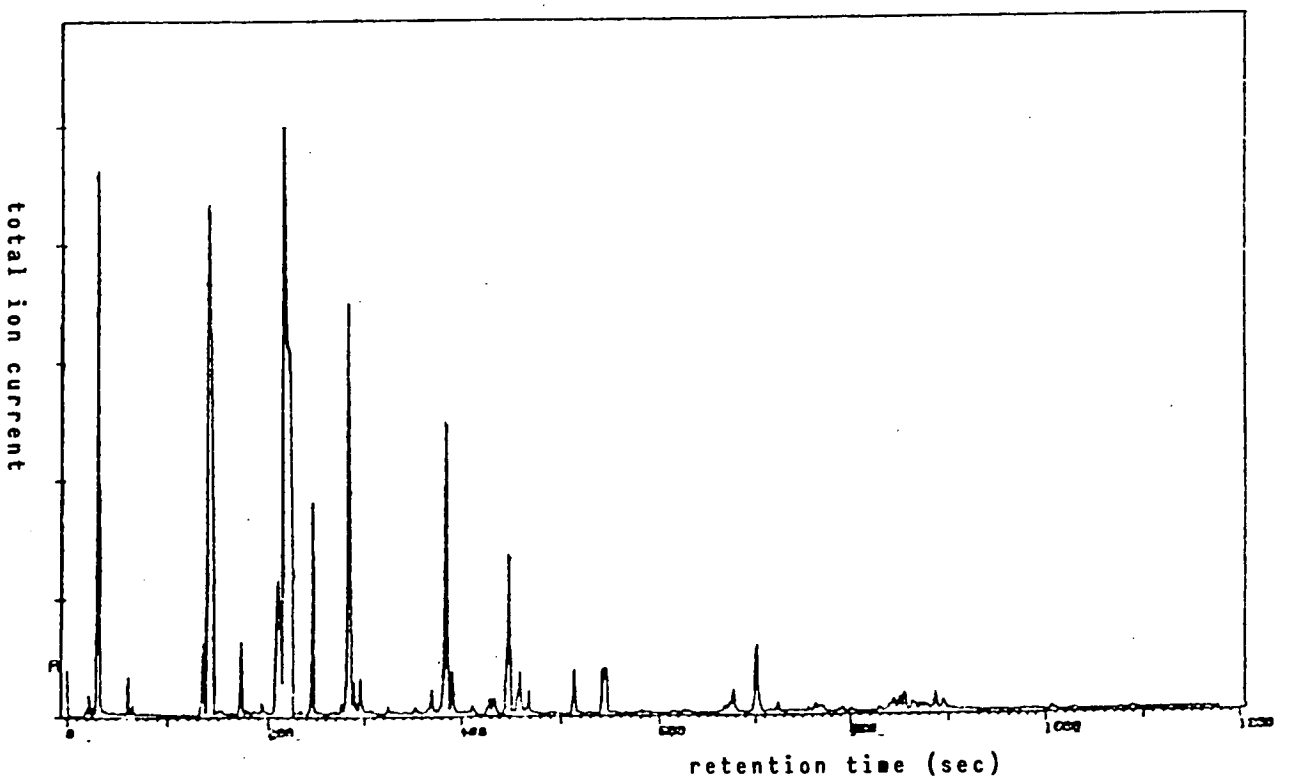
1 hexa-2,3-dione
2 α-thujene
3 α-pinene
4 β-pinene
5 cymene
6 cineole
7 γ-terpinene
8 linalool
9 iso pent-2-me-butanoate type
10 pinocamphone
11 trans pinocarveol ?
12 unknown alcohol
13 terpinene-4-ol
14 α-terpineol
15 myrtenol
16 p-menthen-9-ol
17 hexenyl 2-methyl-butanoate type
18 nerol
19 geraniol
20 neral/geranial
21 phenyl propan-2-one
22 iso-butyl acetate type

Melaleuca squarrosa

SEE ABOVE

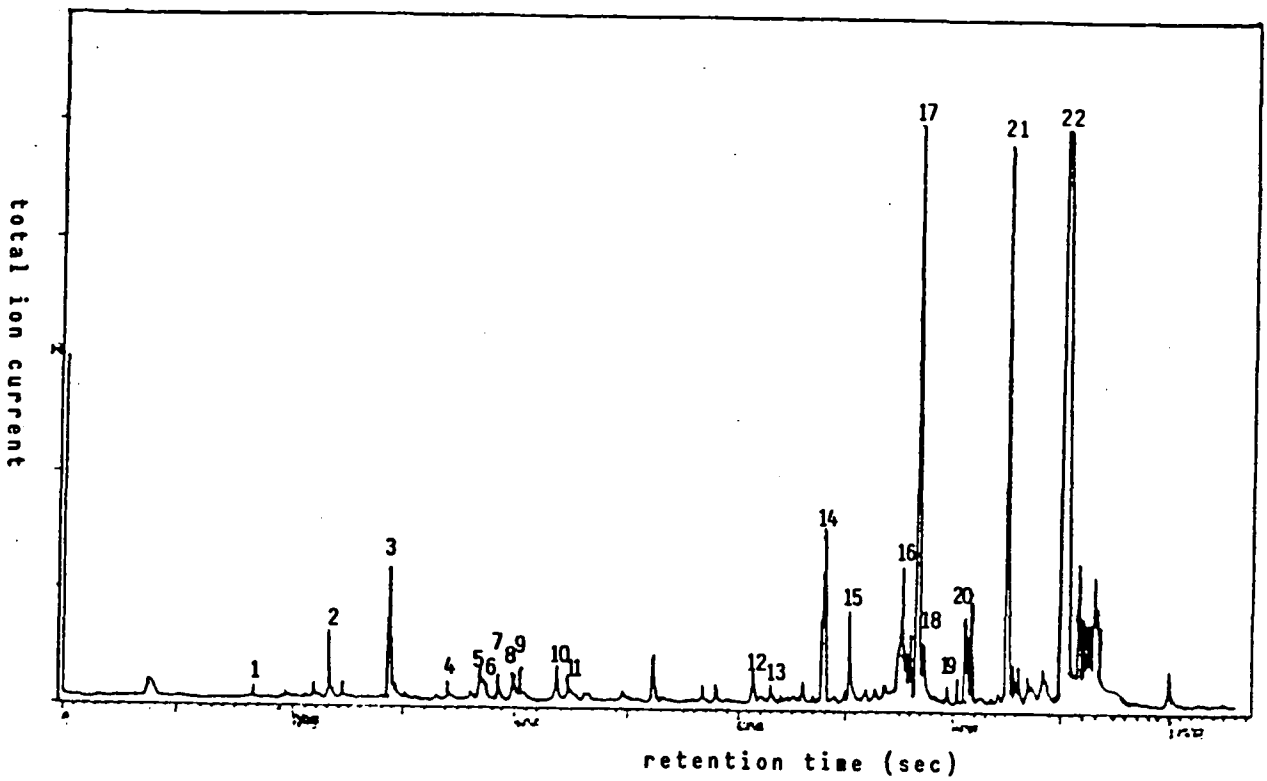


Melaleuca squamea



Melaleuca squarrosa

Figure 4.5.XIV



Olearia argophylla

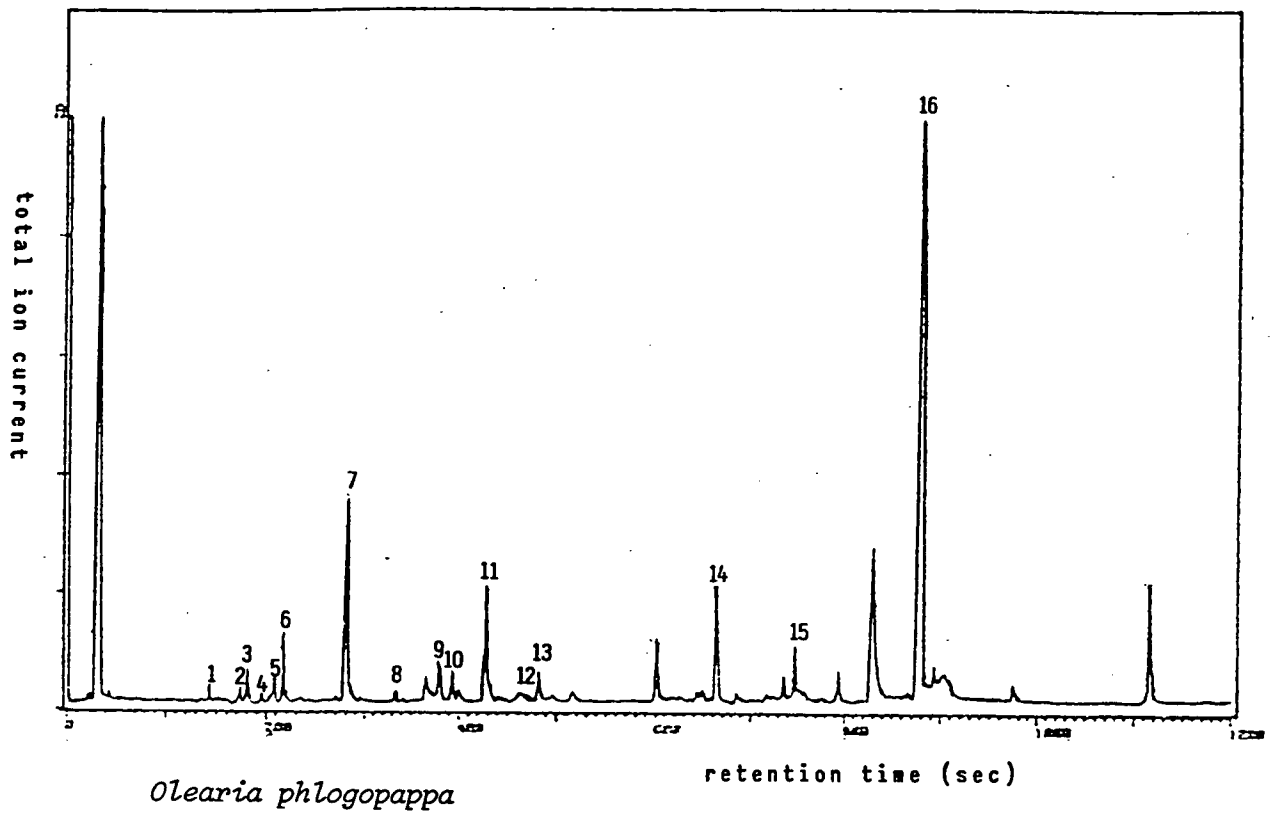
1 α-pinene
2 limonene
3 linalool
4 menthone
5 menthol
6 terpinene-4-ol
7 α-terpineol
8 decanol
9 pentyl iso-valerate type
10 hexenyl pentanoate type
11 n-hexyl isovalerate
12 damascenone
13 α-cubebene ?
14 caryophyllene
15 aromadendrene
16 selinene type
17 sesquiterpene hydrocarbon
18 monoterpene ester
19 δ-cadinene
20 sesquiterpene hydrocarbon
21 unknown common to many natives
22 γ-eudesmol

Figure 4.5.XV

Olearia phlogopappa

1 α-pinene
2 β-pinene
3 myrcene
4 α-phellandrene
5 p-cymene
6 limonene
7 linalool
8 menthone ?
9 α-terpineol
10 undecanal
11 carvone
12 nerol
13 anethole/estragol
14 caryophyllene
15 sesquiterpene + myristicine
16 γ-eudesmol
17 terpenoid type

1 Light and clear
2 Sweet, heavier, fruity
3 Lemon
4 Sweet and musty
5 Eucalypt, citrus
6 Sweet, light, fruity
7 Spicey
8 Dry woody
9 Citrus fruity
10 Sweet fruity
11 Sweet fruity
12 Sweaty
13 Cool and clean
14 Apple cider
15 Floral
16 Bandages, linament
17 Dry mint
18 Clinical, antiseptic
19 Cool, clear and fresh
20 Pine
21 Woody
22 Awful, heady
23 Pungent sassafras
24 Pine
25 Woody



Olearia phlogopappa

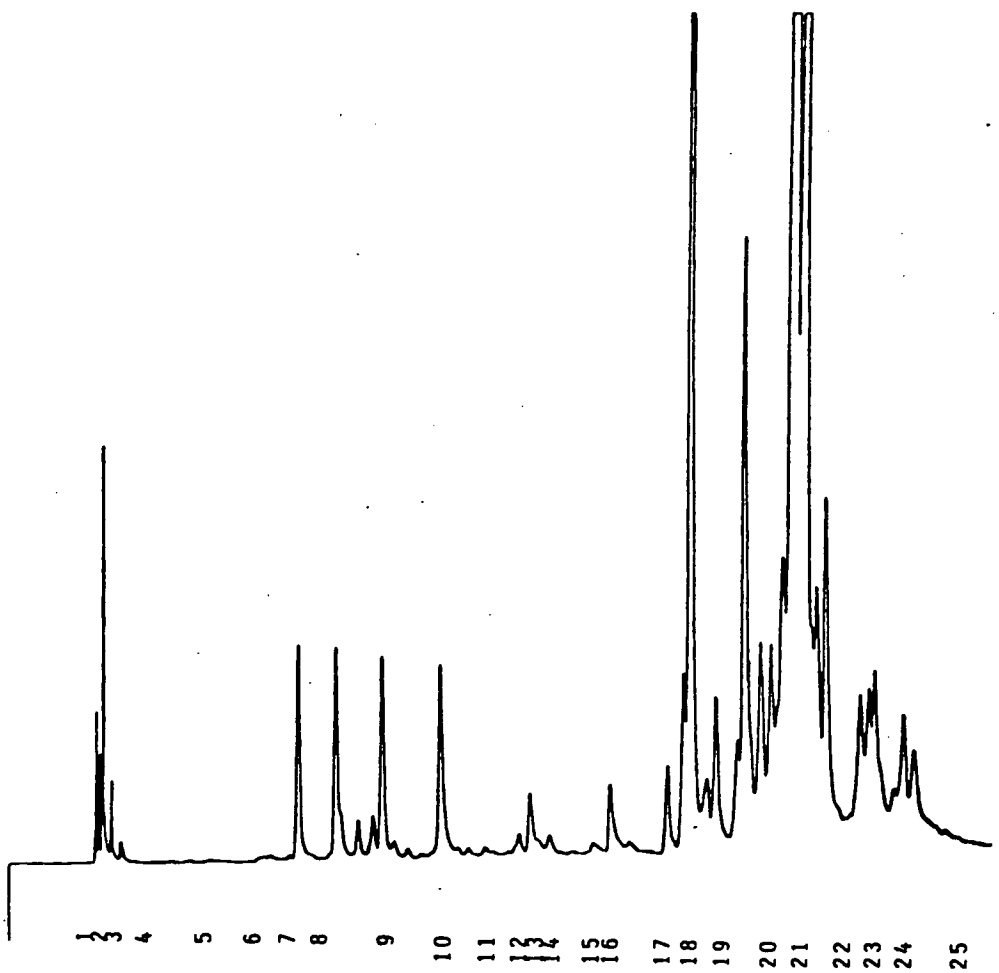
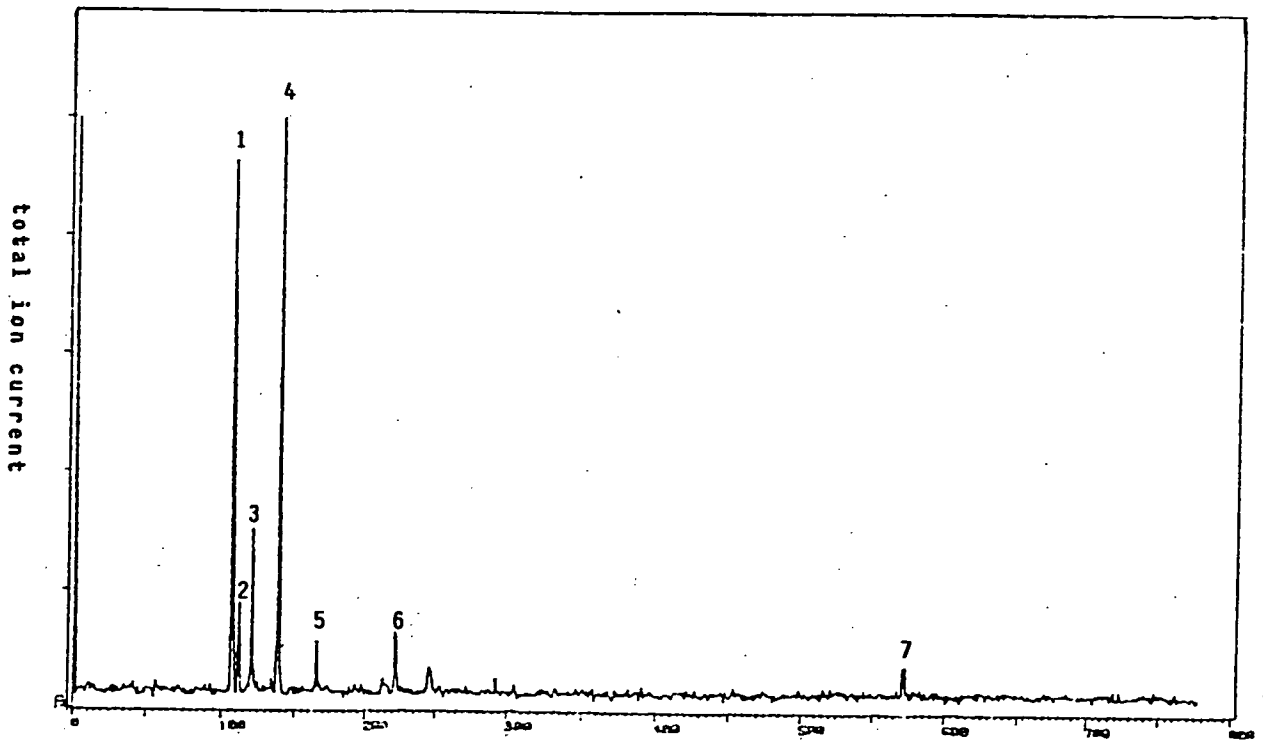


Figure 4.5.XVI

Phebalium squameum

1 α-pinene
2 camphene
3 myrcene
4 limonene
5 linalool
6 α-terpineol
7 sesquiterpene alcohol

1 Faintly spicey
2 Faint, acrid, a bit like putty
3 Cool, clear, fresh, minty
4 Heavier, engines, astringent
5 Sharp and minty
6 Fruity citrus, lemons
7 Tangy
8 Melon
9 Sweet, fruity
10 Sweaty fruity
11 Sticky, fruity
12 Faint, sweet
13 Raspberry
14 Citrus, fruity, orange
15 Sharp, sour-sweet, woody, burning
16 Phebalium
17 Papery, sweet, rose-like
18 Light, clear, violets
19 Fungi
20 Wet carpet
21 Vitamin E
22 Fruity citrus, edible mouth-watering, acrid
23 Faint, burning, off
24 Sweet, soapy, then acrid
25 Spicey, rasping
26 Sweet, faintly minty
27 Old vegetables
28 Sweet, floral
29 Dry and dusty
30 Sweeter
31 Sweet, floral
32 Penetrating, clear
33 Solder, metallic
34 Woody
35 Burning wood
36 Very strong, heavy woody, resinous
37 Spicey wood, like sassafras
38 Spicey, woody, sweet, old Phebalium
39 Faintly peppery
40 Stronger



Phebalium squameum

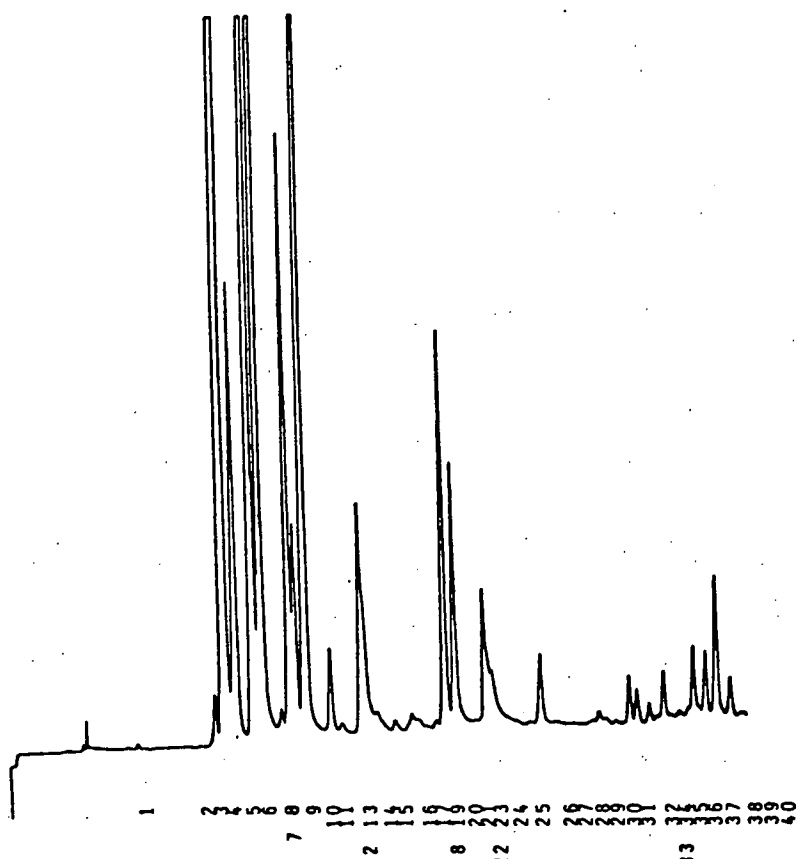


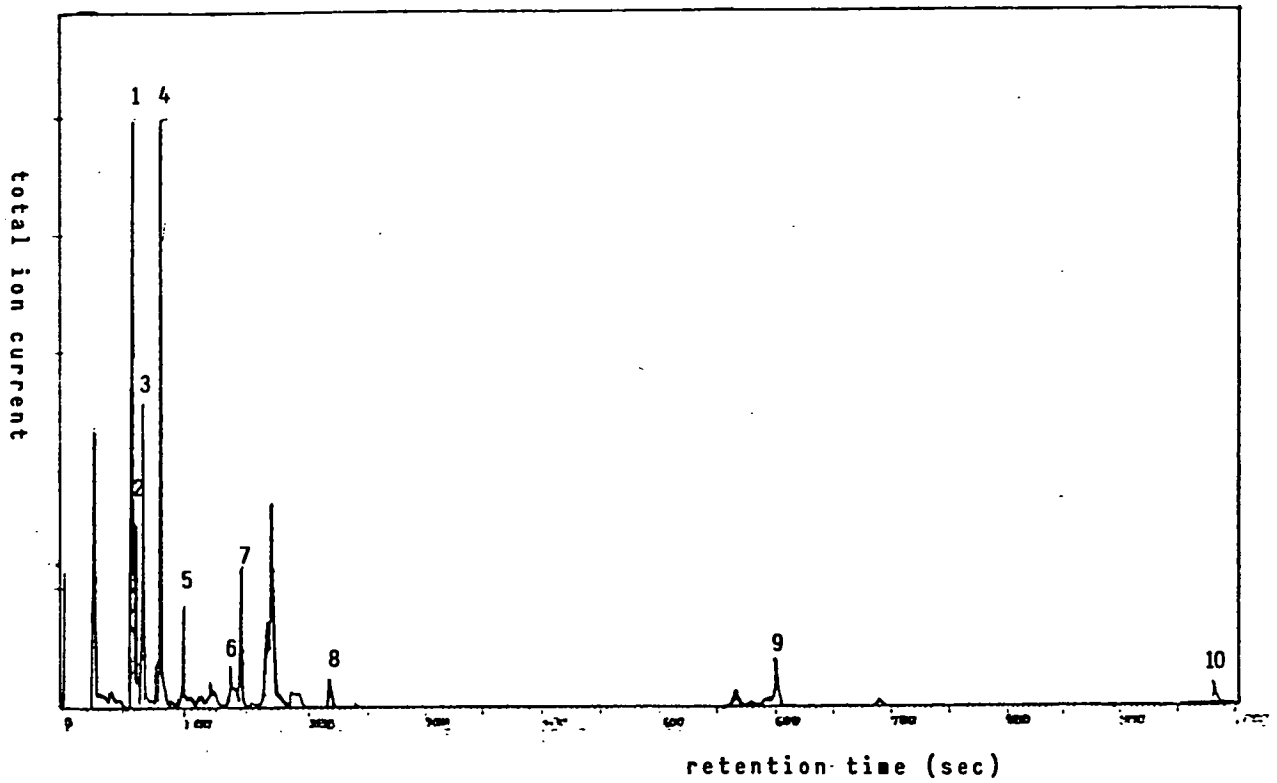
Figure 4.5.XVII

Phebalium squameum (concrete)

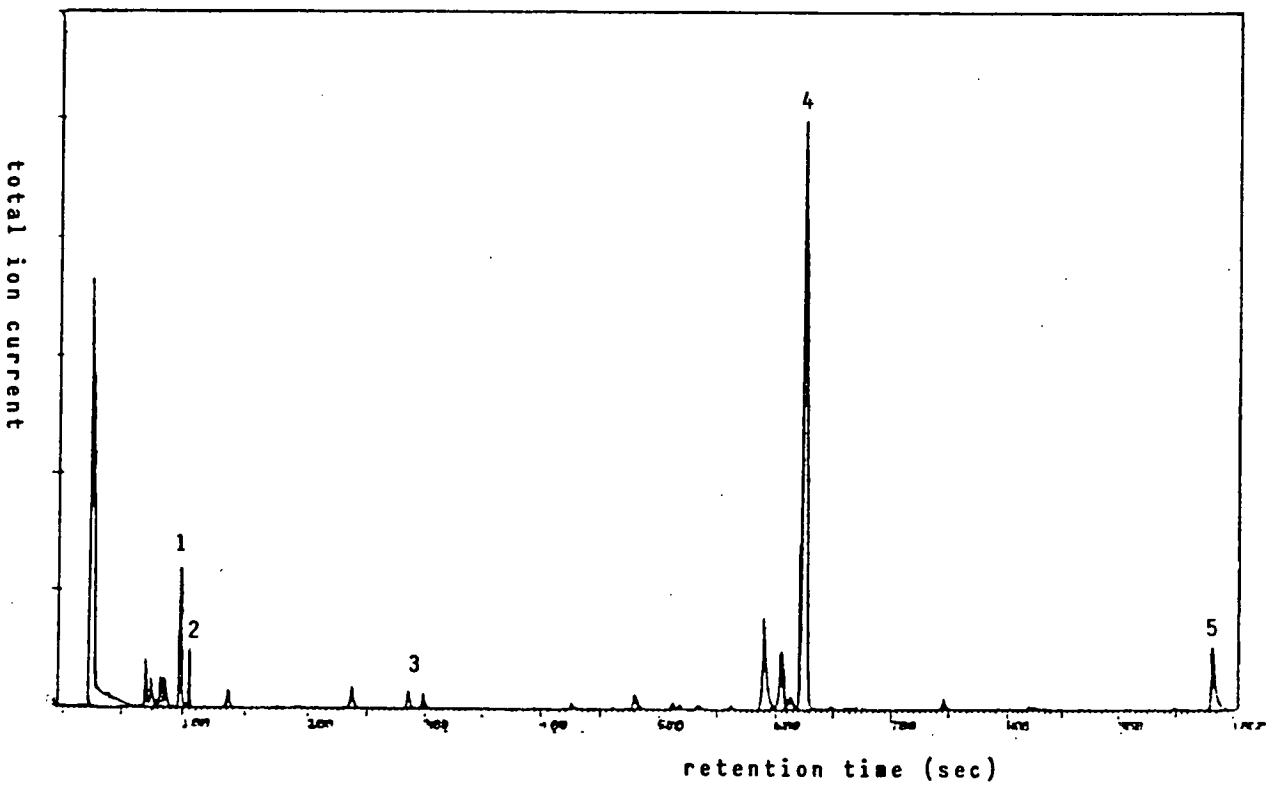
1 α-pinene
2 camphene
3 β-pinene
4 limonene
5 linalool
6 terpinene-4-ol
7 α-terpineol
8 bornyl acetate
9 hedycaryol
10 palmitic acid

Phebalium squameum (stem concrete)

1 Δ-3-carene
2 limonene
3 bornyl acetate
4 hedycaryol
5 palmitic acid

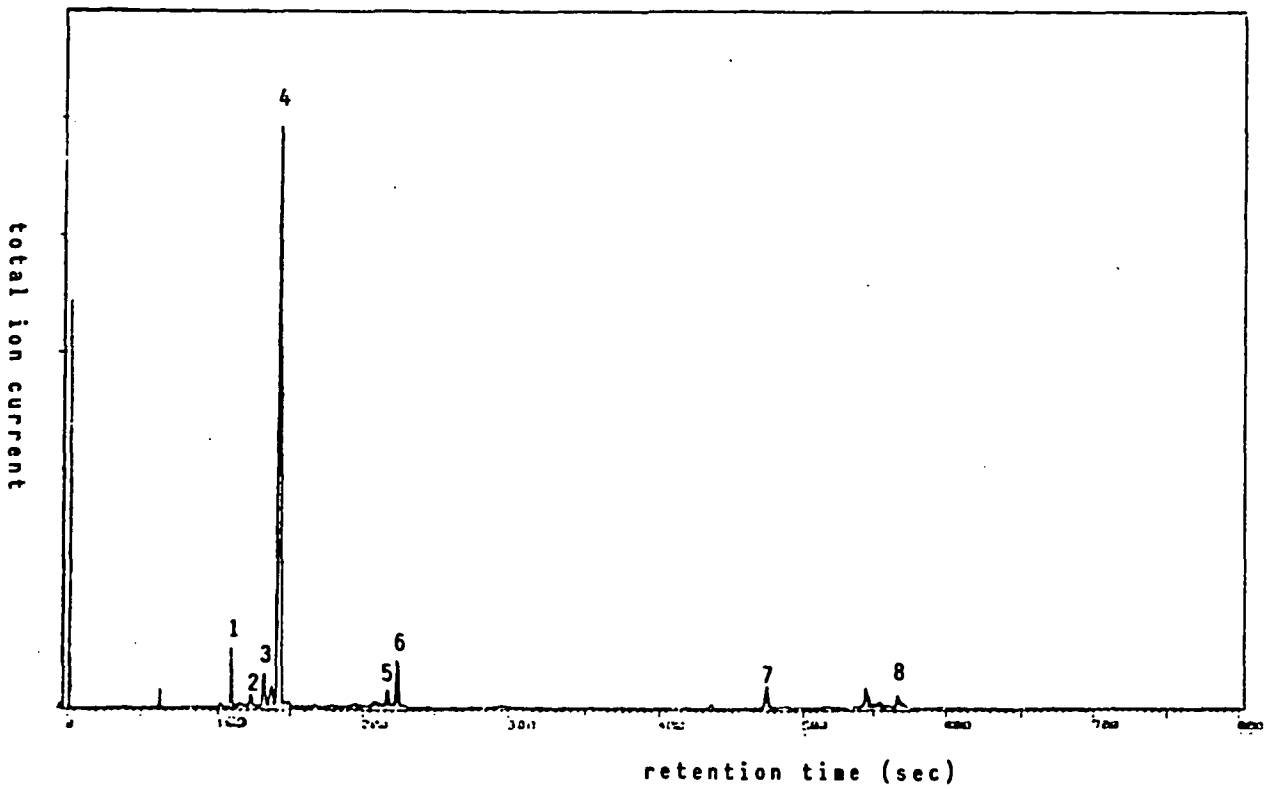


Phebalium squameum (concrete)



Phebalium squameum (stem concrete)

Figure 4.5.XVIII



Prostanthera lasianthos

1 α-pinene
2 β-pinene
3 ρ-cymene
4 1,8-cineole
5 terpinene-4-ol
6 α-terpineol
7 β-selinene
8 rosifoliol

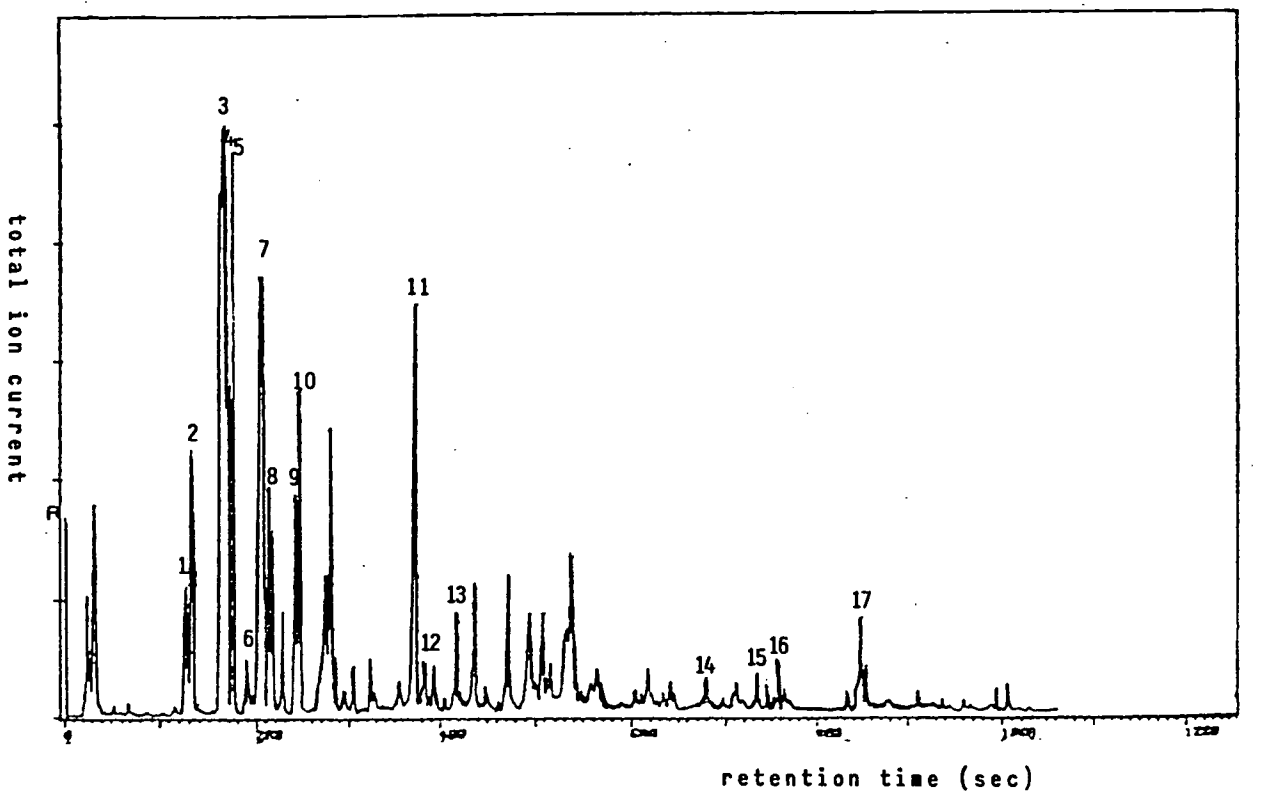
Figure 4.5.XIX

Senecio linearifolius

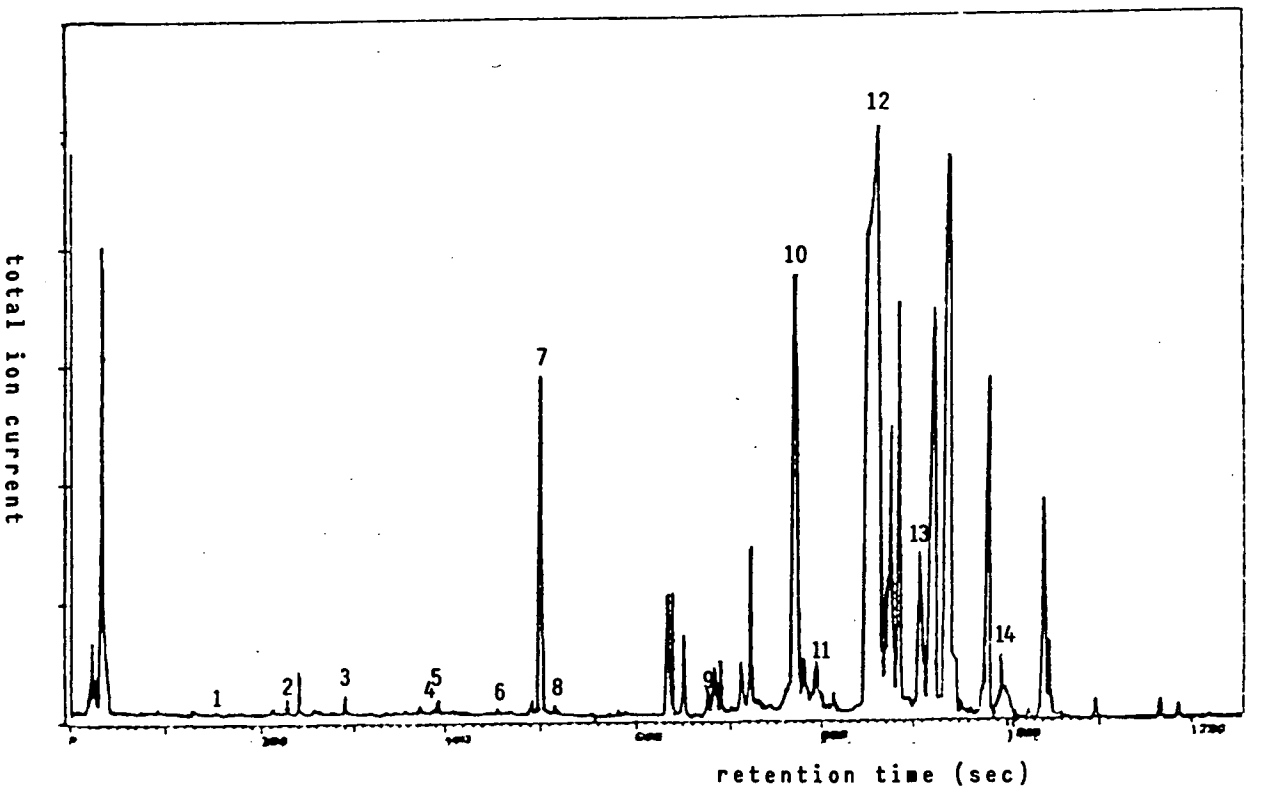
1 α-thujene
2 α-pinene
3 sabinene
4 β-pinene
5 myrcene
6 α-phellandrene
7 ρ-cymene
8 limonene + other monoterpenes
9 terpinene
10 linalool
11 terpinene-4-ol
12 α-terpineol
13 3,7-dimethyl 6-oxo 2-octenol
14 caryophyllene
15 β-phenyl ethyl pentanoate
16 myristicine
17 oxygenated sesquiterpenes

Zieria arborescens

1 monoterpenes/ α-pinene
2 limonene/ cineole-2-one
3 heptyl formate
4 methyl salicylate
5 decan-2-ol
6 piperitone
7 safrole
8 undecan-2-one
9 α-gurjunene
10 myristicine
11 eleminin
12 zierone
13 tetra methoxy styrene
14 silicone



Senecio linearifolius



Zieria arborescens

Figure 4.5.XX

4.6 PERCENTAGE COMPOSTITIONS

The percentage compostitions of the ten selected species are arranged alphabetically in Tables 4.6.I to 4.6.XI.

The method of determining the compostion of each essential oil was reliant on the areas under the peaks, as estimated by an integrator linked to the Gas Chromatograph. MInor resolution errors and varying responses to the compounds under investigation by the F.I.D. were not taken into consideration for the purpose of this study.

Beyeria viscosa	%
α-thujene	0.57
α-pinene	1.64
camphene	0.97
sabinene	11.80
β-pinene	4.60
myrcene	1.17
p-cymene	0.23
limonene	0.94
γ-terpinene	0.73
linalool	1.33
perillene	0.39
pinocamphone type	0.84
terpinene-4-ol	0.37
α-terpineol	0.46
α-copaene	0.11
sesquiterpenes	3.50
δ-cadinene	0.58
ledol/globulol/rosifoliol	36.64
eudesmols	0.32
SUM:	73.19

Callitris tasmanica 21-2-83	%
α-pinene	68.70
β-pinene	2.26
p-cymene	0.43
limonene	1.52
3-methyl butyl butanoate	0.47
α-terpinolene	0.87
α-caryophyllene aldehyde	0.33
citronellal	2.37
citronellol	3.95
SUM:	80.90

%

Callitris tasmanica (hybrid)	26-9-83	8-11-83	SUM	MEAN
α-pinene	18.79	19.98	38.77	19.39
β-pinene	4.21	3.04	7.25	3.63
p-cymene	0.22	1.67	1.89	0.95
limonene	14.43	8.73	23.16	11.58
3-methyl butyl butanoate	0.26	2.74	3.00	1.50
α-terpinolene	1.29		1.29	1.29
citronellal	1.89	2.48	4.37	2.19
citronellol	2.84	2.79	5.63	2.82
bornyl acetate	0.65	1.26	1.91	0.96
citronellyl acetate	4.86	11.23	16.09	8.05
neryl acetate	7.11	8.20	15.31	7.66
lavandulyl acetate	27.37	27.87	55.24	27.62
SUM:	83.92	89.99		

Drimys lanceolata	%
α-pinene	24.25
camphene	0.34
sabinene	0.73
β-pinene	7.62
myrcene	1.29
α-phellandrene	2.96
α-terpinene	1.07
1,8-cineole + limonene	22.89
terpinolene	0.07
linalool	10.43
γ-terpineol	0.41
terpinene-4-ol	0.96
α-terpineol	4.08
piperitone	1.09
safrole	0.99
bornyl acetate	0.03
eugenol	1.17
copaene	1.03
methyl eugenol	0.38
β-caryophyllene	2.24
sesquiterpenes + myristicin	1.30
δ-cadinene	0.57
guaiol	2.94
SUM:	88.84

%

Eriostemon virgatus		19-8-83	SUM	MEAN
iso propyl buterate		0.32	0.32	0.32
iso propyl iso valerate	0.15	0.89	1.04	0.52
α-pinene	48.90	50.05	98.95	49.47
camphene	18.30	13.72	32.02	16.01
β-pinene	5.06	0.39	5.45	2.92
myrcene	6.70	4.12	10.82	5.41
α-phellandrene	0.76	0.16	0.92	0.46
Δ^3-carene	0.17	3.49	3.66	1.83
limonene	0.52	6.45	6.97	3.48
cis-β-ocimene		0.14	0.14	0.14
terpinolene	0.18	0.67	0.85	0.42
iso pentyl-2-methyl butyrate		0.17	0.17	0.17
terpinene-4-ol	0.31	0.60	0.91	0.45
citronellol	1.50	0.50	2.00	1.00
bornyl acetate	0.99	0.73	1.72	0.86
citronellyl acetate	0.27	0.31	0.58	0.29
β-caryophyllene	0.86	0.27	1.13	0.56
iso propyl cinnamate		0.80	0.80	0.80
globulol/viridiflorol/ledol	6.32	7.15	13.47	6.73
SUM:	90.99	90.93		

%

Eucalyptus amygdalina			SUM	MEAN
α-thujene	0.31	2.58	2.89	1.45
α-pinene	5.59	1.34	6.93	3.47
sabinene		0.46	0.46	0.46
β-pinene	0.53	0.21	0.74	0.37
myrcene		1.90	1.90	1.90
α-phellandrene	25.48	22.14	47.62	23.81
1,8-cineole	26.52	27.52	54.04	27.02
cis-β-ocimene		1.69	1.69	1.69
sabinene hydrate		0.99	0.99	0.99
terpinolene	0.58	2.14	2.72	1.36
linalool	0.98	0.79	1.77	0.89
terpinene-4-ol	2.97	2.96	5.93	2.97
α-terpineol	0.50	2.46	2.96	1.48
cis-piperitol	0.16	0.19	0.35	0.18
trans-piperitol with above		0.33	0.33	0.33
citronellol	2.49	0.67	3.16	1.58
piperitone	23.45	20.49	43.94	21.97
SUM:	93.68	88.86		

%

Kunzea ambigua			SUM	MEAN
α-thujene	1.24	0.26	1.50	0.75
α-pinene	28.55	24.12	52.67	26.34
sabinene		1.62	1.62	1.62
β-pinene	1.08	1.34	2.42	1.21
myrcene	0.16	0.13	0.29	0.15
1,8-cineole	21.95	23.09	45.04	22.52
γ-terpinene	0.47	0.20	0.67	0.34
terpineolene	0.29	3.85	4.14	2.07
2,6 di hydro 4,7-dien 2-ol	0.93		0.93	0.93
terpineol-4-ol	1.76		1.76	1.76
α-terpineol	3.58		3.58	3.58
citronellol	2.09	1.37	3.46	1.73
anethole		0.65	0.65	0.65
caryophyllene	0.46	0.51	0.97	0.49
sesquiterpenes	2.94		2.94	2.94
δ-cadinene		0.23	0.23	0.23
globulol/viridiflorol/ledol	26.93	28.09	55.02	27.51
ledol		3.23	3.23	3.23
SUM:	92.43	88.69		

Leptospermum glaucescens	%
α-pinene	53.06
β-pinene	1.41
p-cymene	3.54
limonene	1.96
α-terpineol	0.95
carvone	2.19
β-elemene	1.28
γ-eudesmol	0.82
sesquiterpene alcohol	16.01
SUM:	81.22

%

Leptospermum lanigerum	23-3-83	24-4-83	23-7-83	SUM	MEAN
α-pinene	13.91	17.95	12.73	44.59	14.86
β-pinene	1.18	4.12	1.41	6.71	2.24
cymene	0.49	1.78	2.85	5.12	1.71
1,8-cineole	6.52	16.49	7.61	30.62	10.21
γ-terpinene	trace	0.17	0.10	0.27	0.14
linalool	1.56	1.70	0.86	4.12	1.37
hopinone	trace	0.47	0.57	1.04	0.52
terpinene-4-ol	1.12	1.45	1.20	3.77	1.26
α-terpineol	2.65	3.12	1.97	7.74	2.58
nerol	trace	0.86	0.97	1.83	0.92
undecan-2-one	1.27	1.04	0.52	2.83	0.94
sabinyl acetate	0.57	1.75	0.30	2.62	0.87
citronellyl acetate	0.45	0.22	0.37	1.04	0.35
methyl cinnamate	0.60	trace	trace	0.60	0.60
myrtanyl acetate	1.16	1.55	3.27	5.98	1.99
β-caryophyllene	2.19	3.11	11.13	16.43	5.48
humulene	0.86	1.38	1.03	3.27	1.09
elemol	2.52	2.11	1.80	6.43	2.14
γ-eudesmol	12.84	8.01	13.38	34.23	11.41
β-eudesmol	25.36	19.46	24.70	69.52	23.17
α-eudesmol	1.89	0.08	0.66	2.63	0.88
SUM:	77.14	86.82	87.43		

Olearia phlogopappa	%
α-pinene	0.31
β-pinene	0.25
myrcene	0.50
α-phellandrene	0.27
p-cymene	0.15
limonene	0.02
α-terpineol	1.45
carvone	1.66
β-caryophyllene	0.74
sesquiterpenes + myristicin	9.25
epi-γ-eudesmol	48.59
SUM:	63.19

%

Phebalium squameum			SUM	MEAN
α-pinene	33.32	23.86	57.18	28.59
camphene	4.18	4.48	8.66	4.33
β-pinene	14.73	28.84	43.57	21.79
sabinene	9.79	0.25	10.04	5.02
Δ-3-carene	3.29	1.92	5.21	2.61
1,8-cineole	11.50	13.00	24.50	12.25
α-terpinolene	3.36	3.24	6.60	3.30
bornyl acetate	0.40	0.63	1.03	0.52
terpinene-4-ol	2.82	3.53	6.35	3.18
β-caryophyllene	0.37	0.32	0.69	0.35
α-terpineol	2.25	2.50	4.75	2.38
δ-cadinene	0.85	1.20	2.05	1.03
SUM:	86.86	83.77		

4.7 TAPERS

The following odour profiles have been compiled by assigning an impact value based on the graded scale used by Appell, (1968). A number between 1 and 4 is allocated to the odour on the taper, at any particular time, depending on the impact as indicated below:

1:::Slight
2:::Moderate
3:::Strong
4:::Powerful

The species are listed alphabetically in Table 4.7.I.

TABLE 4.7.I.

TIME	IMPACT	NOTES
Beyeria viscosa		
Initial	2-3	Thick , heady, male perfume. Gives impression of fading rapidly
1hr	3	Stable, thick, green and spicey
2	2-3	
6	2	Subtle, manly odour, green
24	2	Pleasant
Dry Out	2	Still heavy but pleasant,leafy,green. STRONG MASCULINE, SPICEY PLEASANT ALMOST SWEET, WOODY.
Callitris tasmanica		
Initial	3	Piney but floral.Soapy floral, lavender-like, with citrus flower overtones
1hr	2-3	Woody (pine), floral :-harsh roses.
2	2-3	
6	2-3	Very fruity, piney.
24	3	
Dry Out	0	Nil. AFTERSHAVE, SWEET, PINEY, AIR FRESHENER IF STRONGER

TIME	IMPACT	NOTES
Drimys lanceolata		
Initial	4	Spicey, clear, eucalyptus, menthol mouth-watering, bush.
1hr	3-4	Spicey - citrusy component more noticeable. Also fragrant, sweet and clear.
2	3	More peppery.
6	3	Peppery factor more pronounced. Also floral and fruity.
24	2-3	
Dry Out	1-2	Woody and spicey. MEDIUM-STRONG PEPPERY SWEET FLORAL
Eriostemon virgatus		
Initial	2-3	Cool, clear, eucalyptus-like but lemon, light, fragrant,pleasant
1hr	3	Bushy, leafy, clear, fine, mouth-watering.
2	2-3	
6	2	Herby, citrusy, strong, pleasant.
24	1-2	Eucalyptus-like, citrusy, pleasant
Dry Out	1-2	Floral and light. Strawberries! STRONG CITRUSY, LEMON-LIME, SWEETS, FRESH, OPEN-AIR

TIME	IMPACT	NOTES
Eucalyptus amygdalina		
Initial	3	Clear, clean and woody.
1hr	3	Cooling, clear, stronger woody note very pleasant.
2	3	Mellowing.
6	3	Heavier notes coming out - herby, bush-like, clear and clean.
24	0	
Dry Out	1	Woody - piney.
		FAINT, LIGHT AND CLEAR
Kunzea ambigua		
Initial	3	Eucalyptus like, bush like, piney light and clean.
1hr	3	A little spicey, warm, but still fresh.
2	3	
6	3	Boronia-like, without the floral notes being prevalent. Pleasant.
24	2-3	Floral.
Dry Out	2	Woody, sasafrass.
		MODERATELY STRONG, FLORAL, SWEET

TIME	IMPACT	NOTES
Leptospermum glaucescens		
Initial	2	Citrusy, lemon-lime, clear, fresh. Also smells like the bush. Pleasant
1hr	2	Tea-tree, with floral notes, piney
2	2	
6	1-2	Sourish, pleasant, citrus.
24	1	Pleasant, eucalyptusy.
Dry Out	0	Faintly woody.
		MEDIUM STRENGTH, SWEETISH CITRUS, FAINTLY LIMEY, PLEASANT.
Leptospermum lanigerum		
Initial	3	Woody-piney, bush-like, eucalyptusy mouth-watering, strongly clear,clean
1hr	2-3	Strongly green and bushy, almost floral. Fresh.
2	3	
6	3	Strongly tea-tree, fresh, bush, herby.
24	2-3	Tea-tree. Very like bandages
Dry Out	2	Woody (tea-tree)
		STRONG PLEASANT, CLEAN, FRESH, BUSH, ROSE-LIKE

TIME	IMPACT	NOTES
Olearia phlogopappa		
Initial	4	Spicey green-leaf odour with woody notes.
1hr	4	Strong floral, leafy, sasafrass.
2	3-4	Floral.
6	3-4	Sasafrass, floral, light.
24	3	
Dry Out	2-3	Sweet, citrusy, fruity. CINNAMON, SPICEY, STRONG, (could be NUTMEG), WOODY (SASAFRASS)
Phebalium squameum		
Initial	3	Spicey, biting, citrusy.
1hr	3	Spicey, but also clear, cool with heavier, sweeter notes.
2	3	More spicey.
6	3	Spicey, woody (sasafrass?)
24	3	
Dry Out	3	Spicey - sasafrass. NUMBING, ANESTHETIC, MODERATELY STRONG, SPICEY, PEPPERY.

4.8 SCANNING ELECTRON MICROGRAPHS

The oil glands and topographical characteristics of the leaves of the ten selected species were examined by means of the scanning electron microscope. The resulting micrographs are presented here as Figures 4.8.I to 4.8.XI.

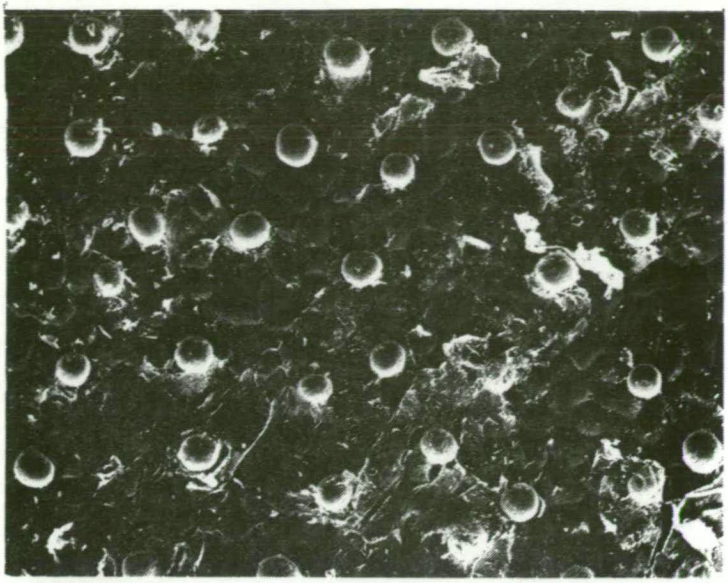
Figure 4.8.I

Beyeria viscosa

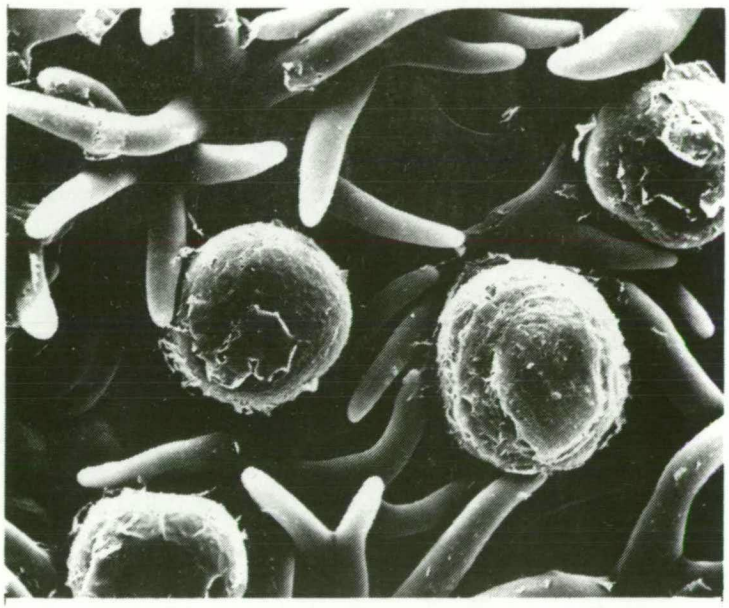
(a) Scanning electron micrograph of the adaxial leaf surface. No trichomes are present, however, prominent oil glands are visible. X 160

(b) Abaxial surface of the leaf. This view is an enlargement of (c), below. The surface is covered in fine hairs, between which, large oil glands are visible. X 920

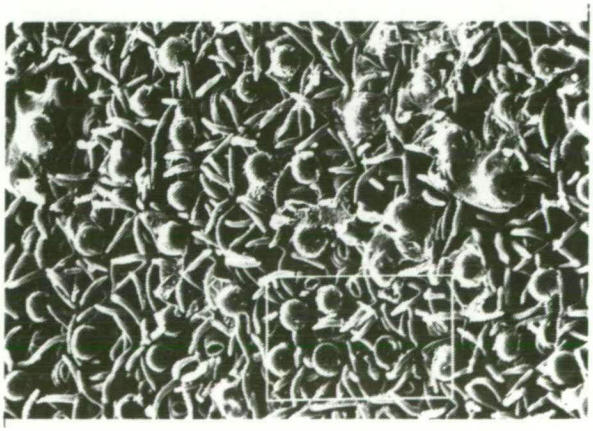
(c) The abaxial leaf surface, showing the location and frequency of oil glands and trichomes. X 160



(a)



(b)



(c)

Figure 4.8.I

Figure 4.8.II

Beyeria viscosa

(a) The adaxial leaf surface of the leaf is extremely shiny and waxy. The oil glands appear as white spots, which are present over the entire surface. A point of interest is the apparent seasonal alteration in leaf morphology. At the onset of the summer flowering period, the leaves take on this shiny globulose character, which presumably coincides with the accumulation of oil. Winter foliage is dull and matt in texture. This suggests that photosynthate is directed to oil synthesis during summer, and this reserve is utilized during winter. X 7

(b) This plate shows a surface view of the leaf, which is covered in obvious grey shaded areas, containing oil. X5

(a)

(b)

Figure 4.8.II

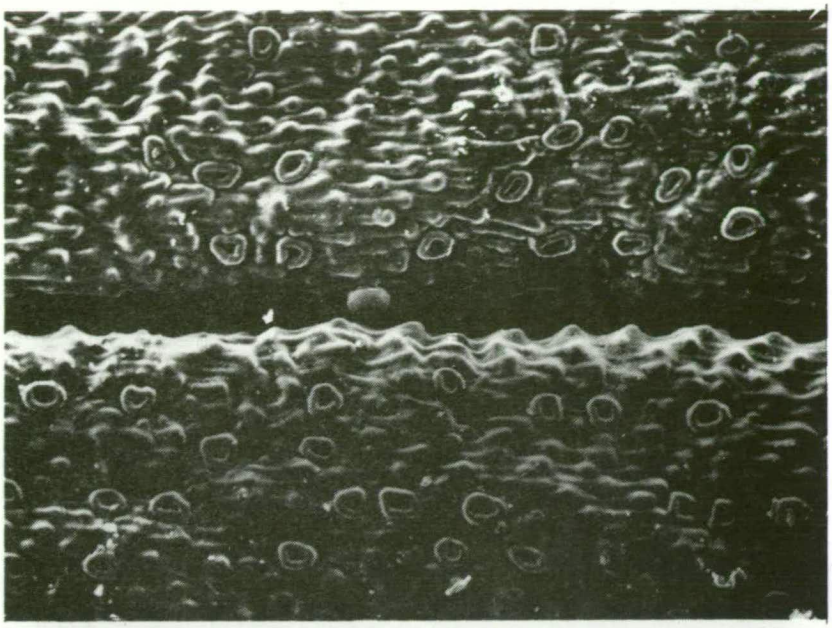
Figure 4.8.III
Callitris tasmanica

The oil glands, or sites of accumulation of oil in *Callitris tasmanica* were not positively identified. However, the series of figures below show that the surface of the needles have an undulated, noded aspect.

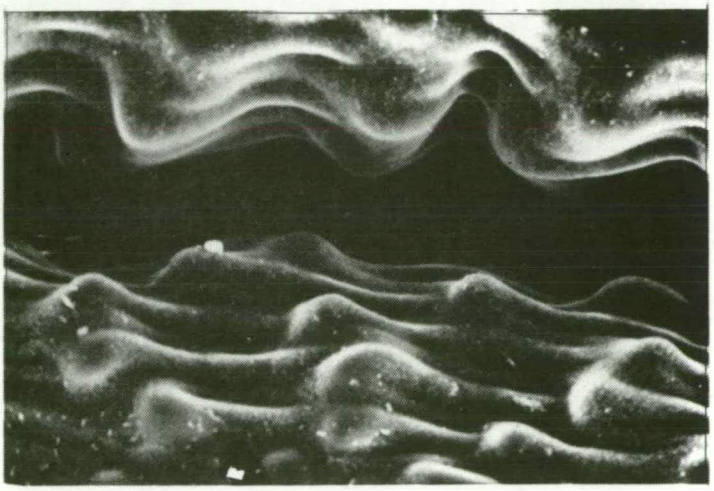
(a) Scanning electron micrograph of the area where two leaf scales meet. The stomata are concentrated here, and the nature of the leaf surface is visible. X 130

(b) This plate is an enlarged view of (a), above. The undulations are seen to be very regular and arranged in rows. X 520

(c) Large, open cells lie beneath the nodules which are visible on the surface. It is postulated that the oil accumulates in these cells. X 1300



(a)



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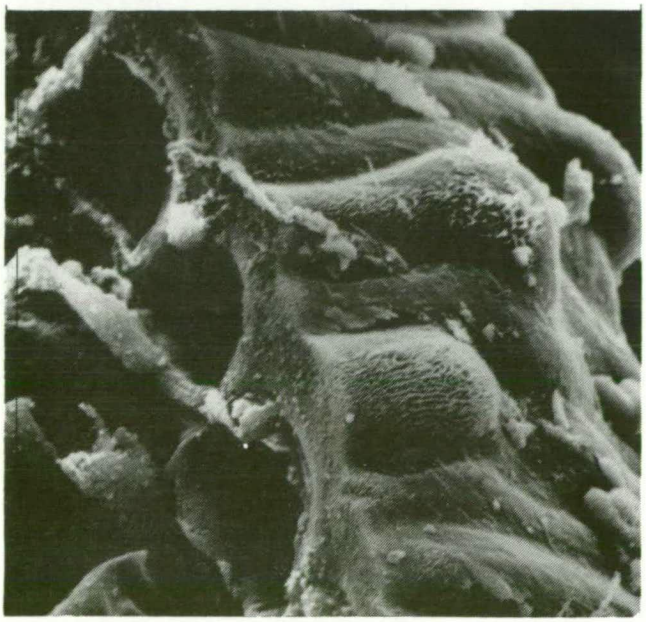


(c)

Figure 4.8.III

Figure 4.8.IV

Drimys lanceolata

This figure is a light microscope view of the adaxial leaf surface. Large dark, dots cover the entire area, and it is here that the essential oil is concentrated. X 30

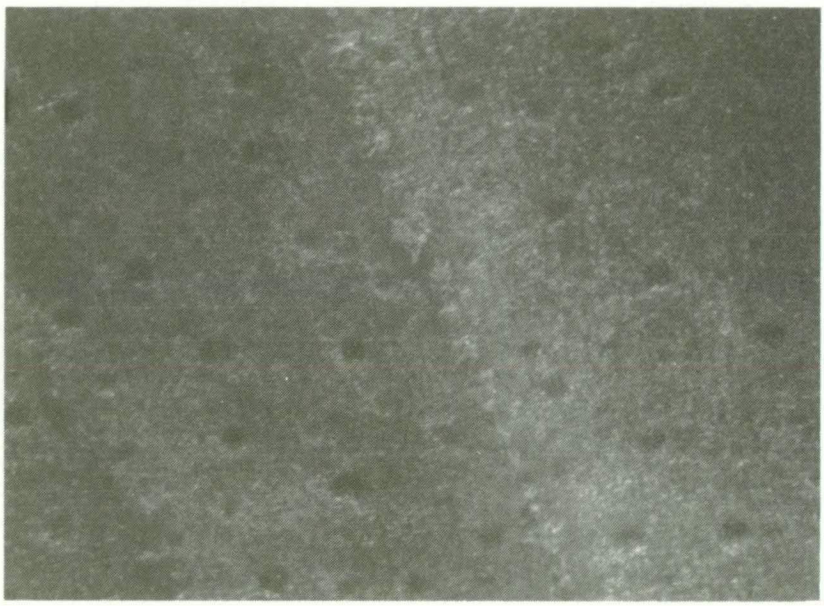


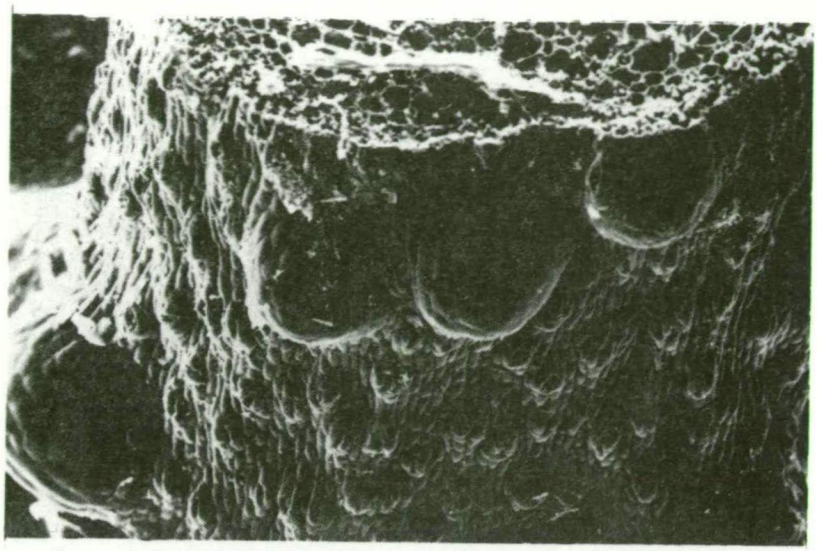
Figure 4.8, IV

Figure 4.8.V

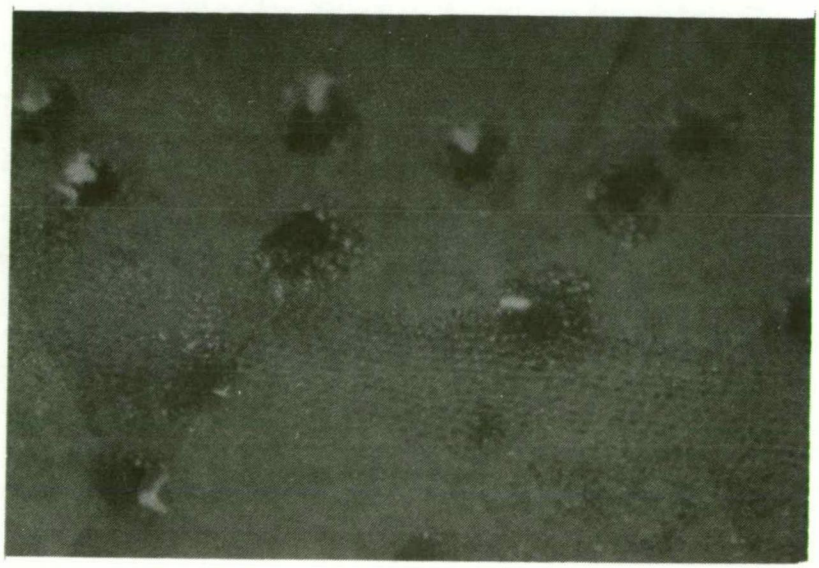
Eriostemon virgatus

(a) Scanning electron micrograph of a section of stem material. Large protuberances are visible, as well as many minor nodules. The accumulation of oil is considered to occur at these sites. X 100

(b) A light microscope image of an adaxial leaf surface is given here. Under the heat of the lights used to take this picture, the oil has exuded slightly from the cells, giving the shiny surface impression, where oil containing cells occur. X 60



(a)



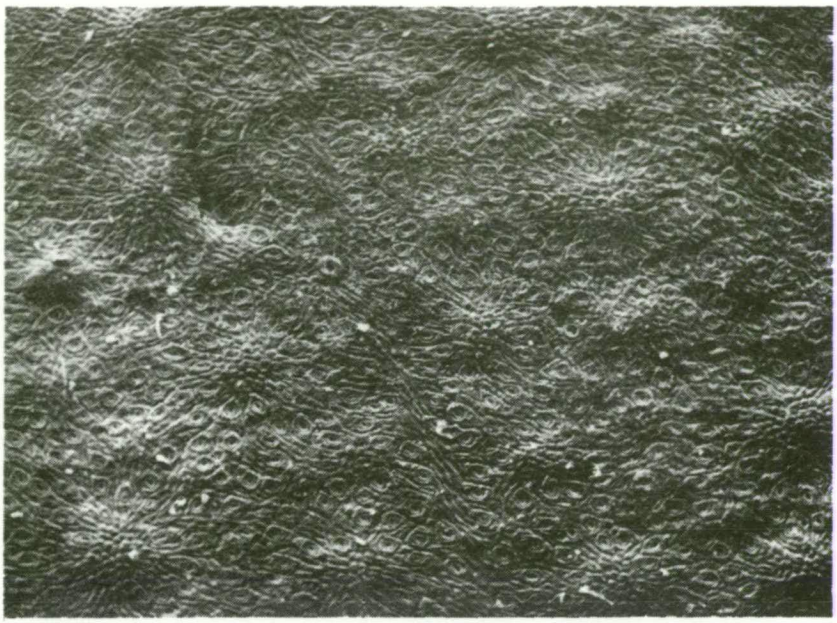
(b)

Figure 4.8.V

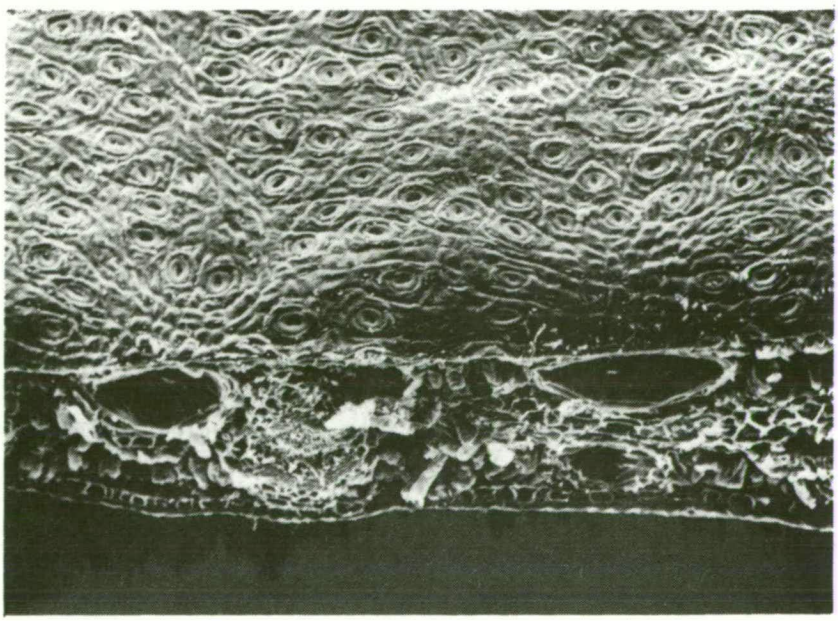
Figure 4.8.VI
Eucalyptus amygdalina

(a) *Eucalyptus amygdalina* has similar adaxial and abaxial leaf surfaces. The undulating character of the topography of the leaf suggests that there are large cavities below the surface. X 49

(b) A transverse section of the leaf tissue shows large cavities beneath the epidermis, which are thought to contain essential oil. X 98



(a)



(b)

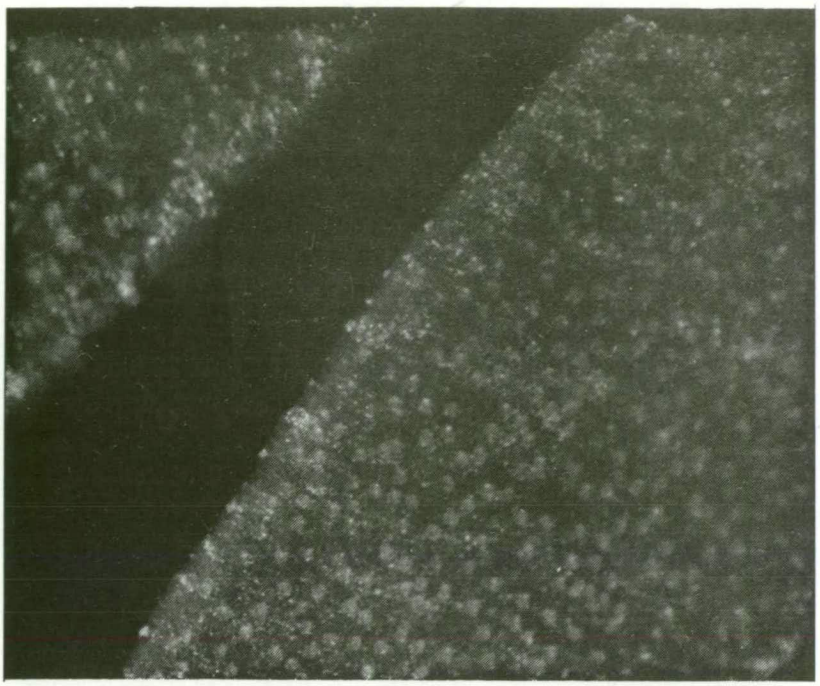
Figure 4.8.VI

Figure 4.8.VII

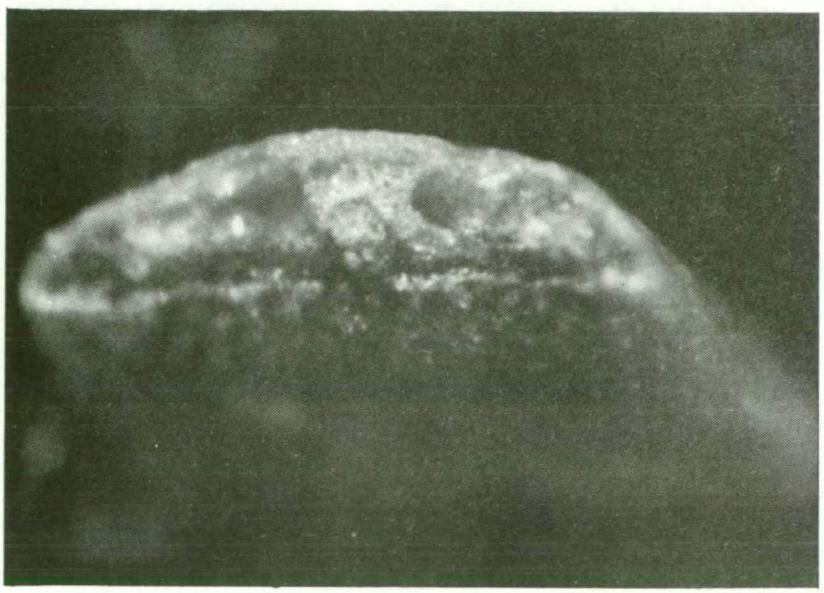
Kunzea ambigua

(a) THis plate shows a light microscope picture of the adaxial leaf surface of *Kunzea ambigua*. The light areas are sites of oil accumulation.
X 11

(b) An attempt is made here to demonstrate the large cavities which lie beneath the leaf surface. Their large size suggests that they contain oil. The cavity to the left of centre is oozing oil, hence, a white image is produced as light is reflected.
X 37



(a)



(b)

Figure 4.8.VII

Figure 4.8.VIII

Leptospermum glaucescens

The leaf surfaces of this species of *Leptospermum* are covered with masses of long, fine hairs. These obscure any view of the actual leaf surface, and possible oil containing structures.
However, it can be postulated that oil is produced in a similar fashion as in *L. lanigerum* and other *Leptospermum* species. X 160

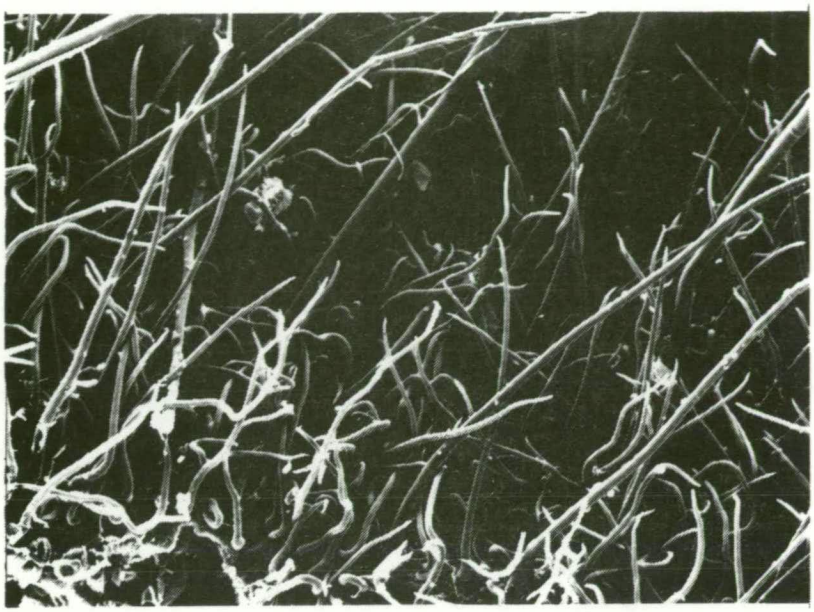


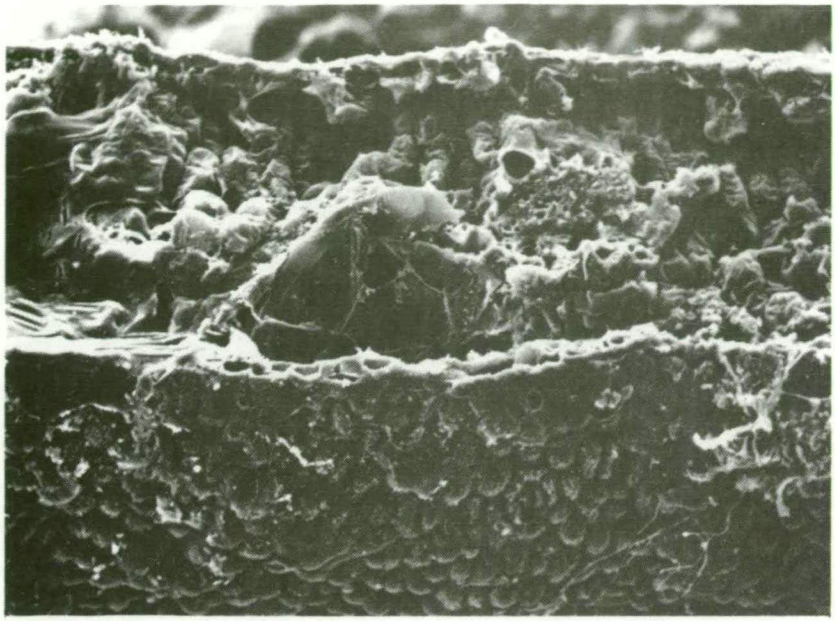
Figure 4.8.VIII

Figure 4.8.IX

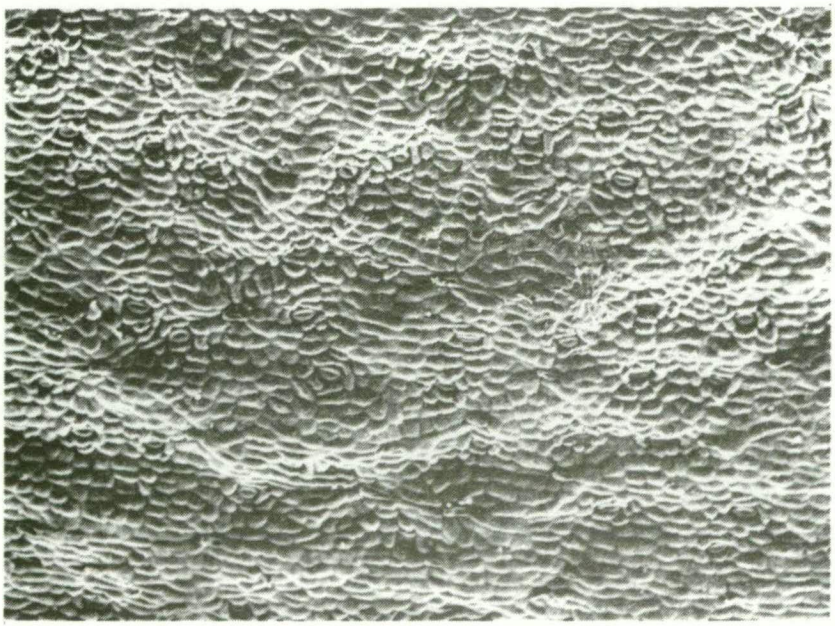
Leptospermum lanigerum

(a) Transverse section showing huge cavities beneath the epidermis, which may be used for the storage of essential oil.
Observations indicated that the cavities below, matched the raised areas which are visible from below.
X 240

(b) Scanning electron micrograph showing the surface cells, stomata and undulating character of the leaf surface.
X 160



(a)



(b)

Figure 4.8.IX

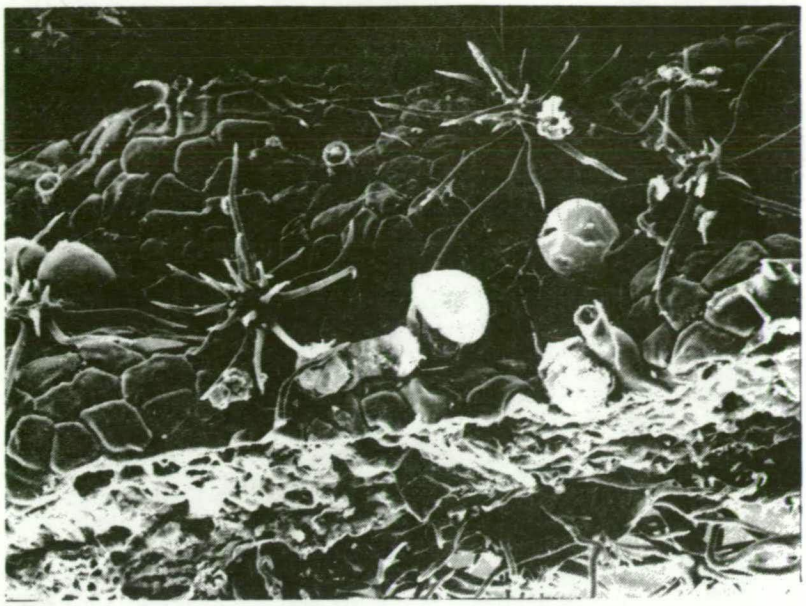
Figure 4.8.X

Olearia phlogopappa

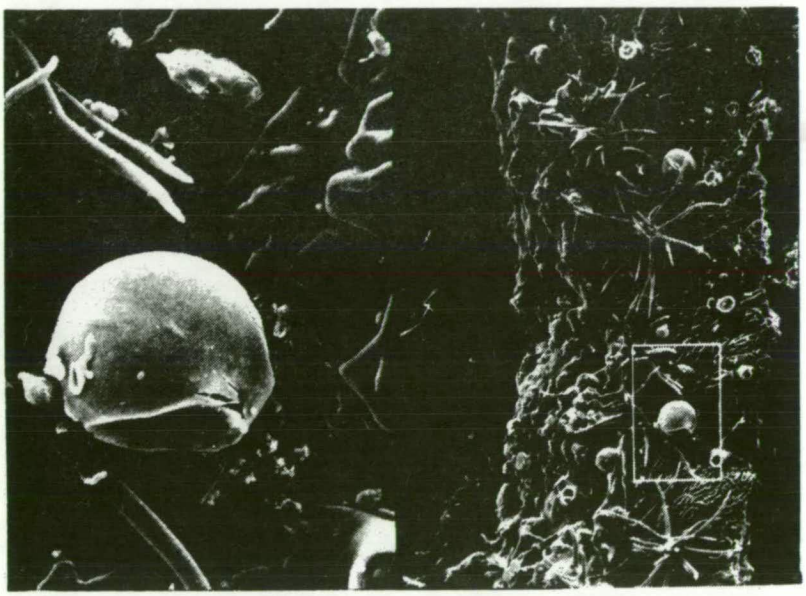
(a) This figure reveals that the rounded oil glands are carried on stout stalks. Some are seen here without the globular structure at their extremity. They are assumed to have been lost during the preparative procedures. X 160

(b) An oil gland is shown here on the right of the split-screen. Its relative location is indicated in the left side of the plate. X 78

(c) Transverse leaf section showing large flattened trichomes and cavities which possibly contain oil. X 245



(a)



(b)

(c)

Figure 4.8.X

Figure 4.8.XI

Rhebalium Squameum

This plate shows a typical leaf surface. The dark areas indicate the location of oil rich cells. They can be seen to cover the entire abaxial surface. X 8

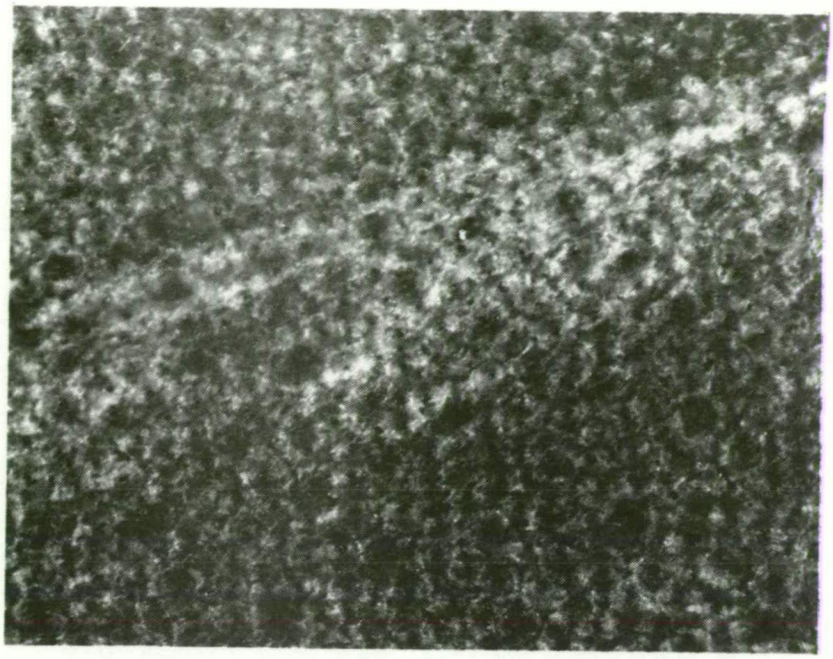


Figure 4.8.XI

4.9 EPI-γ-EUDESMOL IN *OLEARIA* SPP.

The GC-MS traces for *Leptospermum lanigerum* oil and a co-injection of *L. lanigerum* and the epi-γ-eudesmol derived from *Olearia argophylla* oil are presented in Figures 4.9.I and 4.9.II respectively. The two γ-eudesmol peaks in Figure 4.9.II have a relative retention time of 0.296 minutes under the conditions used. Their mass spectra are given in Figure 4.9.III which show that the two peaks have identical mass spectra.

The NMR spectrum of epi-γ-eudesmol is shown in Figure 4.9.IV. Note the presence of the twin methyl peaks at 1.17 and 1.23 δ.

The IR spectrum is given in Figure 4.9.V. This confirms the presence of a double bond and an -OH group within the molecule. Table 4.9.I gives a list of peaks and their % transmittance.

Figure 4.9.VI represents the structural formulae of γ-eudesmol and epi-γ-eudesmol. The NMR data is appended.

Table 4.9.I

epi-γ-eudesmol IR peaks

WN (cm)	% T	WN (cm)	% T
3402.0	33.6#	1205.0	54.1
2930.0	14.4*	1137.0	37.6
1719.0	76.6	1031.0	62.0
1639.0	77.5	979.0	62.5
1511.0	74.6	948.0	51.7
1458.0	32.6"	927.0	42.1
1374.0	33.2	888.0	59.9
1252.0	56.4	843.0	64.4
1224.0	58.3	782.0	73.0
		737.0	65.6

methyl groups

* -OH group

" C=C

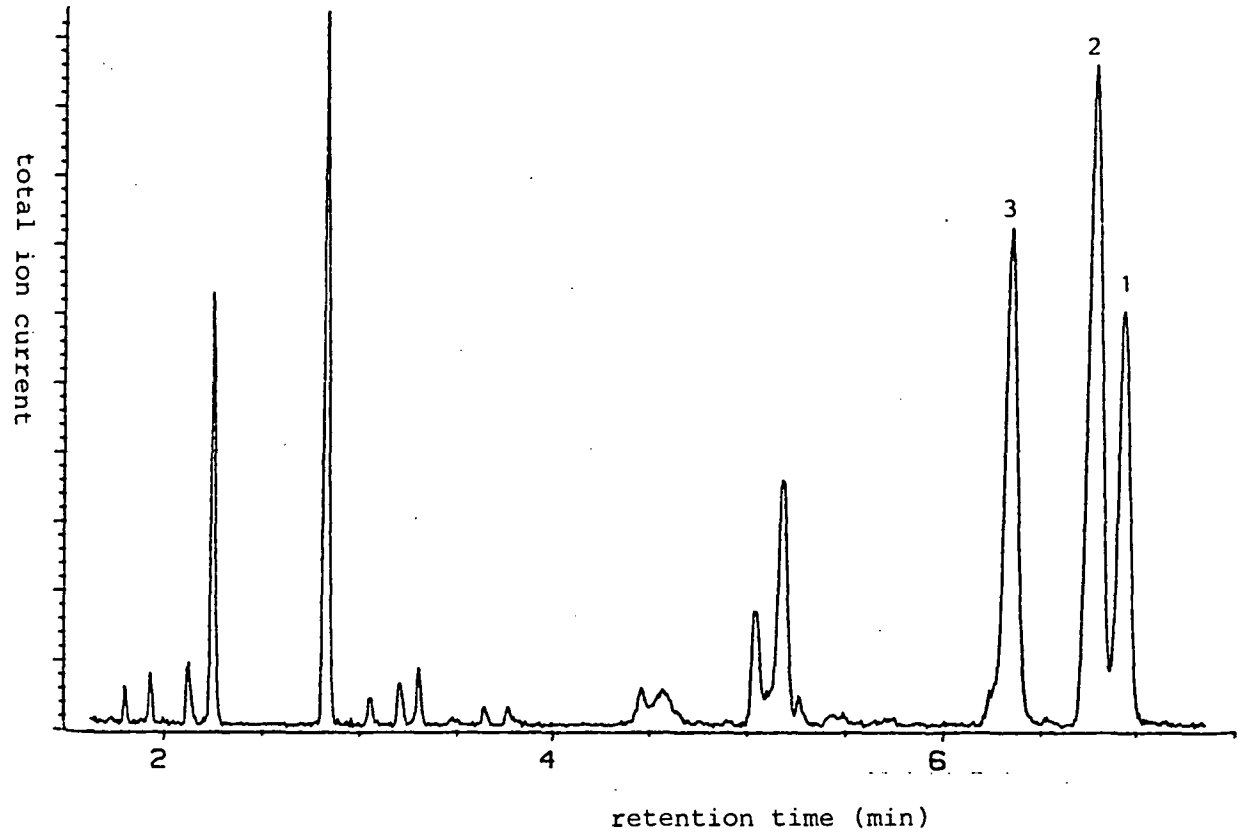


Figure 4.9.I
GC-MS trace of *L. lanigerum* oil

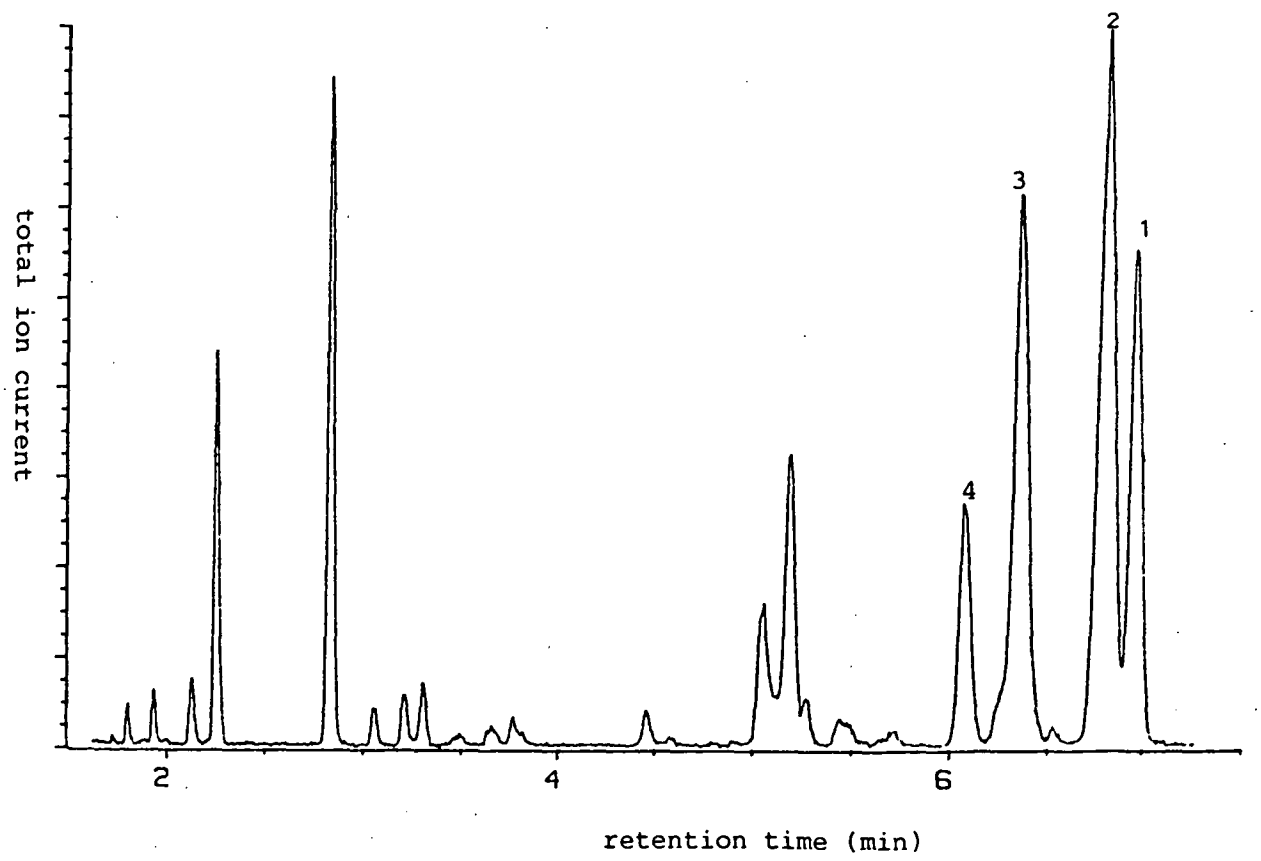


Figure 4.9.II
GC-MS trace of *L. lanigerum* oil with epi-γ-eudesmol

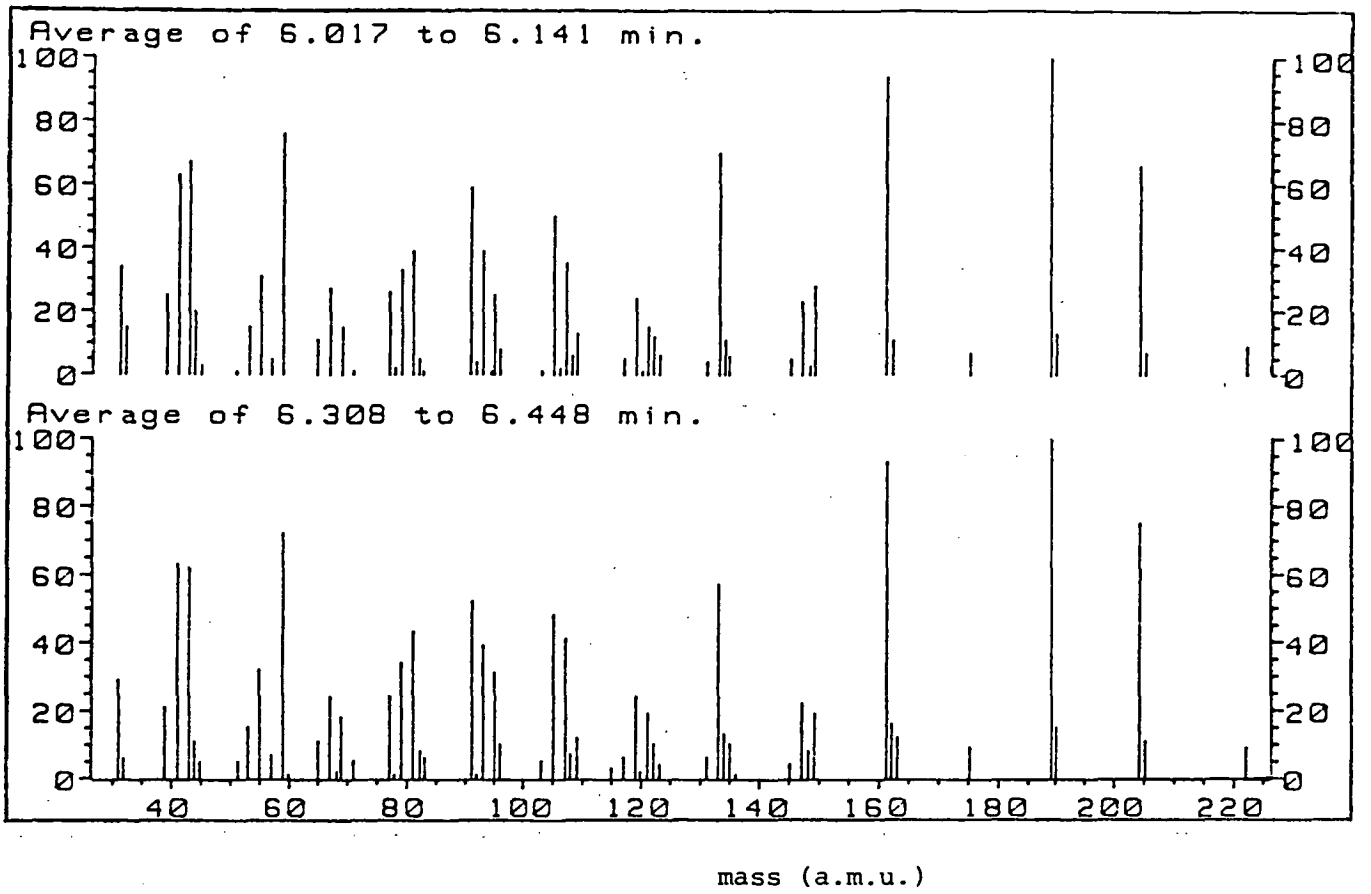


Figure 4.9.III

Mass spectra of epi-γ-eudesmol (top),

and γ-eudesmol (bottom)

(peaks 4 and 3 of Figure 4.9.II, respectively)

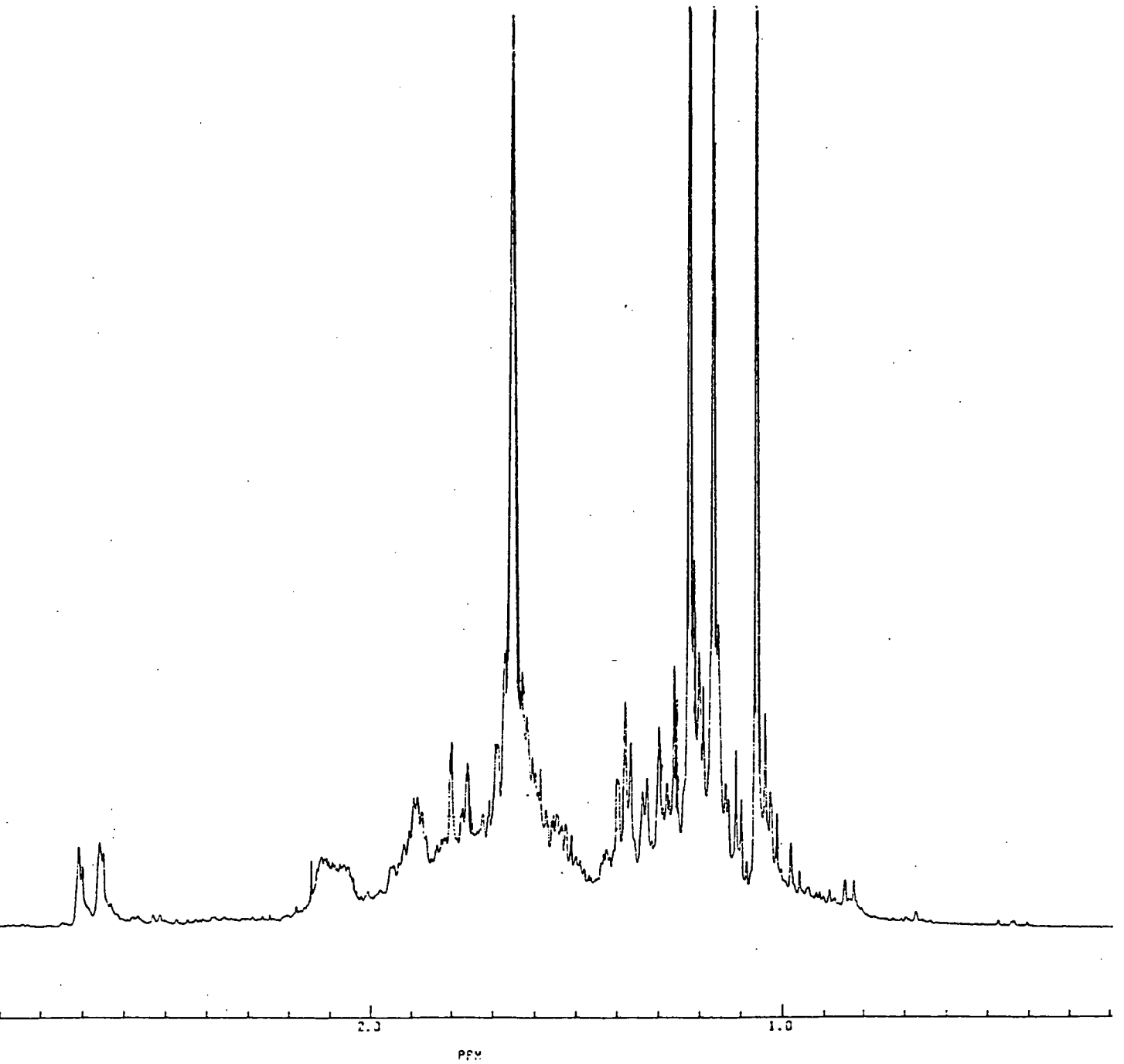


Figure 4.9.IV

NMR spectrum of epi-γ-eudesmol

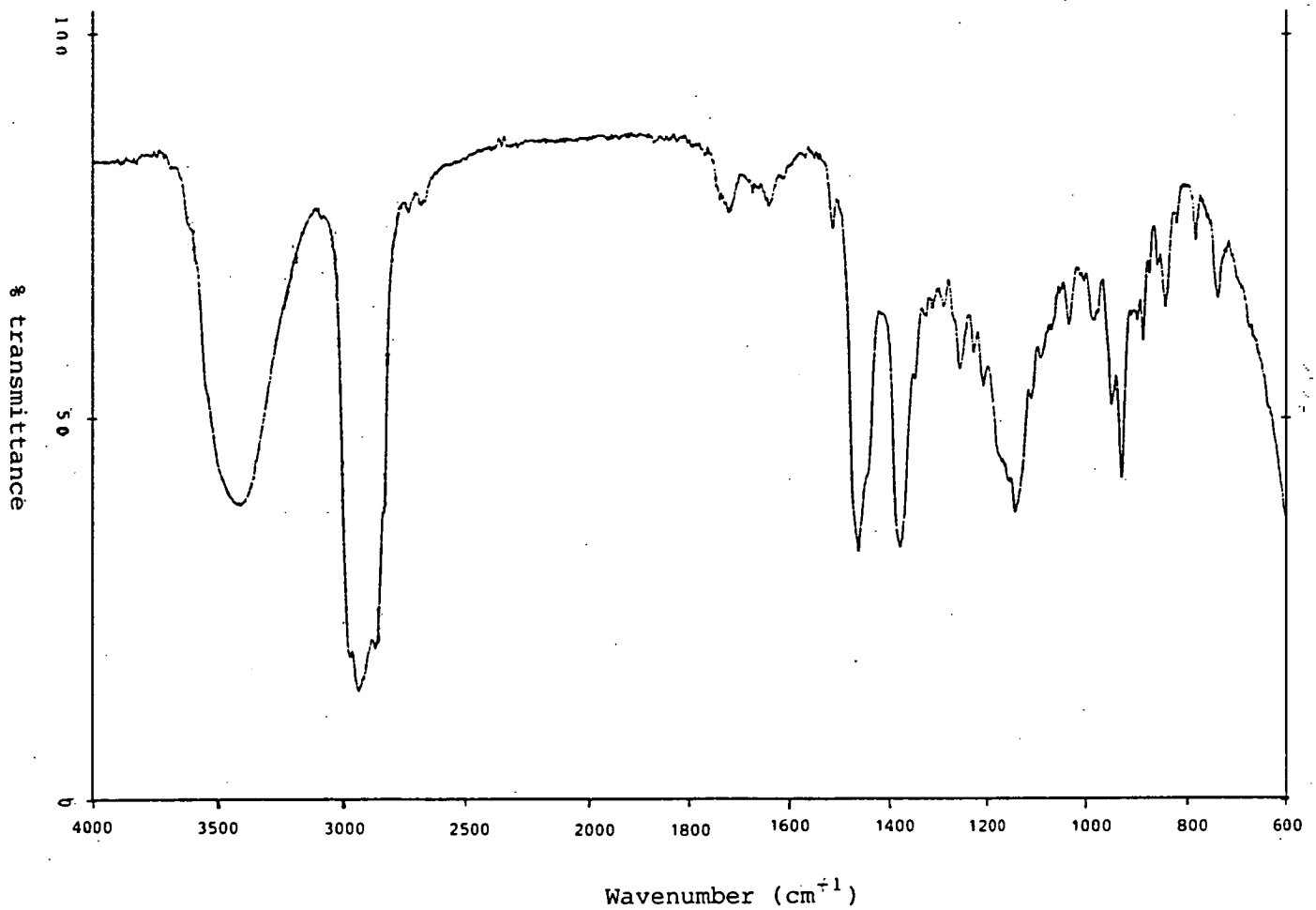
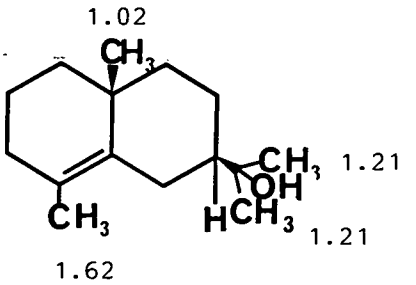


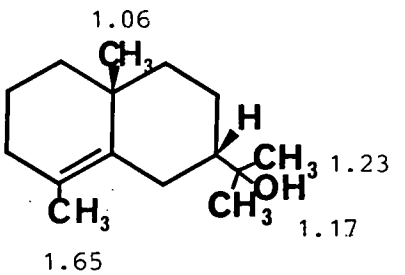
Figure 4.9.V

Infra-Red Spectrum of epi-γ-eudesmol



γ-EUDESMOL

(Hara *et al*, 1980)



EPI-γ-EUDESMOL

Figure 4.9.VI

Structural formulae and NMR data for the eudesmol isomers

4.10 PROPAGATION TRIALS

In order to help assess the suitability of the ten chosen species to commercial applications, their amenability to macro-propagation was tested. Some species were obviously not suitable for propagation by cuttings, so seeds from these species were obtained and their ease of germination was examined by means of small scale trials

4.10.1 CUTTINGS

The results of cutting trials are shown in Table 4.10.I The percentages shown represent the number of cuttings which had 'taken' after a period of 12 weeks. That is, if the root length was observed to be greater than 1.5 cm. However, in most cases, and especially with *Olearia, Beyeria, Phebalium* and *Eriostemon*, the roots generally averaged between 7 and 8 cm.

TABLE 4.10.I

Results of cutting trials

SPECIES	IBA						
	Nil	500 ppm	1000 ppm	2000 ppm	4000 ppm	Strike	Seradix
L. lanigerum	90	30	15	80	20		
L. glaucescens	80					40	45
B. viscosa	95	75	85	70	60		
D. lanceolata	80	40	85	65	70		
O. phlogopappa	100	100	100	70	85	100	100
O. argophylla	70					40	60
E. virgatus	75					90	80
P. squameum	80	100	100	90	100	100	100

Observations : 14-12-83 and 30-1-84 for cuttings planted on 28-10-83, 3-11-83 and 8-11-83.

Table 4.10.I indicates that the species *Olearia* and *Phebalium* are very easy to propagate by cuttings even with no hormone treatment. In fact, *Olearia phlogopappa* gives the lowest strike rate when the I.B.A. levels are highest. The optimum levels of I.B.A. which produce roots on cutting, can be tentatively assigned for the species of interest as follows:

L. glaucescens	Nil
L. lanigerum	Nil
B. viscosa	Nil or 1000 ppm
D. lanceolata	1000ppm
O. phlogopappa	Nil, 500, 1000, Seradix or Strike
E. virgatus	Strike or Seradix
P. squameum	All treatments

4.10.2 VIABILITY TEST

Viability tests were performed on those species which normally produce their seed with an amount of associated infertile chaff. That is, on *Eucalyptus amygdalina, Leptospermum lanigerum* and *Leptospermum glaucescens*. The seeds were soaked in tepid water overnight. Those that remained floating were deemed to be sterile or chaff matter. The results are shown in Table 4.10.II.

TABLE 4.10.II

Percentage viability of seeds

SPECIES	SEEDS TESTED	% VIABILITY
E. amygdalina	212	11.8
L. lanigerum	266	19.5
L. glaucescens	181	21.2

4.10.3 SEED TRIALS

The results of the seed trials are shown in Table 4.10.III, 4.10.IV and 4.10.V.

TABLE 4.10.III

Results of seed trials.

Number Germinated

14-3-1984

	No. planted	No. viable	No. germinated	% germination
C. tasmanica	32	*	7	21.9
D. lanceolata	28	*	0	0
E. amygdalina	80	10	4	40.0
K. ambigua	160	*	120	75.0
L. glaucescens	150	32	12	37.5
L. lanigerum	210	41	15	36.6

This particular trial was useful only in showing that *D. lanceolata* is not easily grown from seed. Scarification may be necessary, or the seed was not freshly harvested. It is more likely that the latter is the case, since many native seeds will not germinate after their prime has passed. It was suspected at the time of sowing that these seeds were collected during the previous season.

TABLE 4.10.IV

Sterilized Media

Results of seed trials.

Percentage germination.

14-4-1984

	No. planted	No. viable	No. germinated	% germination
C. tasmanica	15	*	3	20
D. lanceolata	9	*	0	0
E. virgatus	21	*	1	4.8
E. amygdalina	45	6	0	0
K. ambigua	80	*	36	45
L. glaucescens	65	14	25	100
L. lanigerum	50	10	4	40

In this trial with sterilized media, only *Leptospermum glaucescens*

and *Kunzea ambigua* gave a reasonable germination rate. *Drimys lanceolata* and *Eucalyptus amygdalina* did not germinate at all.

TABLE 4.10.V

Unsterilized Media

Results of seed trials

Percentage germination

14-4-1984

	No. planted	No. viable	No. germinated	% germination
C. tasmanica	32	*	13	40.6
D. lanceolata	15	*	0	0
E. viragatus	200	*	2	1.0
E. amygdalina	455	54	14	25.9
K. ambigua	140	*	140	100
L. glaucescens	615	130	120	92.3
L. lanigerum	500	100	120	100

The use of an unsterilized medium increased the germination of several of the species under investigation. In fact, the only species which did not respond favourably to this treatment were *D. lanceolata* and *E. virgatus*. The first of these can possibly be attributed to the use of stale seed, and the latter to the special conditions that seem to be required for *Eriostemon* to germinate.

Whitehorne and McIntyre (1976), state that seeds of species of the Eastern Rutaceae, which include *Boronia* spp., *Eriostemon* spp., *Zieria* spp., *Phebalium* spp., etc., will not germinate by normal means. They will not germinate if their seed coats are nicked or abraded, so physical dormancy does not operate. In nature they germinate in profusion after a fire has been through the area. These authors suggest a series of treatments to overcome dormancy in these species. Their method for the removal of dormancy is outlined below:

Treatment 1	
Temperature 20-30°C	Non-dormant seeds:
Adequate water	Germination should occur
Oxygen	within 1 to 3 months

When no germination has occurred using Treatment 1 :

Treatment 2	
Scarify	Seeds with Physical dormancy:
Boiling water	Seeds will imbibe water, swell
Nicking seedcoat	and germinate when given
Acid treatment	Treatment 1.

When both Treatments 1 and 2 have failed :

Treatment 3	
Light	Seeds with Chemical dormancy:
Scarification and washing	Following one or more of
Chipping and washing	these treatments seeds may
Removal of seed coat	germinate when subsequently
and washing	given Treatment 1.
GA_3	
Kinetin	
KNO_3	
Ethylene	

5. DISCUSSION

5.1 YIELDS

It can be seen from Table 4.3.II that the species with the most commercial potential on a yield per hectare basis are *Beyeria viscosa, Eriostemon virgatus, Eucalyptus amygdalina, Eucalyptus pulchella, Leptospermum glaucescens, Leptospermum lanigerum, Melaleuca squamea, Olearia argophylla, Phebalium squameum, Senecio linearifolius* and *Zieria arborescens*. Some of these were rejected for further study on the basis of organoleptic properties, while others where included, despite low yield per hectare values.

In order to give some real idea of the yields of the native species cited, a comparison with yields from peppermint, spearmint, parsley, fennel and eucalyptus can be made. The average yield of peppermint and spearmint oils are 0.5 and 0.6 %, respectively. This compares with the yields from *B. viscosa, D. lanceolata, K. ambigua, L. lanigerum, O. argophylla* and *C. tasmanica*, whose yields are also in this range. The percentage oil yield from parsley is of the order of 2 %, as is eucalyptus, while fennel yields around 5 %. The endemic species which have similar yields include *C. tasmanica* (hybrid) (1 %), *E. virgatus* (1 %), *E. amygdalina* (1.5-3 %) and *S. linearifolius* (1 %).

On a per hectare basis, the currently grown essential oil crops yield as follows:

	Kg/Ha
Peppermint	60
Spearmint	80
Fennel	120
Parsley	50
Wormwood	30
Blackcurrant	8

(R. Clark, pers. comm.)

Thus, the estimated yields of *B. viscosa, C. tasmanica, E. amygdalina, K. ambigua* and *L. lanigerum* are all comparable with those that are being obtained from established commercial crops. The possiblity of increasing these yields, through management practices and species development, remains.

Purely on a yield basis, the hybrid *Callitris* species is interesting, since it yields twice as much oil by steam distillation as *C. tasmanica*. Thus, there is evidence that clonal selection of this species could produce a form which has both an acceptable oil and copious yield.

5.2 OIL GLAND MORPHOLOGY

From the observations of leaf tissue of each of the species in this study, there appears to be a group of plants that exhibit similar types of oil producing structures. These are *E. amygdalina, K. ambigua, L. lanigerum, L. glaucescens* (Myrtaceae), *E. virgatus* and *P. squameum* (Rutaceae). The characteristics of the oil producing glands in these plants include large cavities below the epidermis. Although such formations were not directly observed in *E, virgatus* and *P. squameum*, they are considered to exist due to the similarities of the surface morphology of their leaves with the other species examined.

Secretory cavities and ducts occurring below the epidermis, consist of relatively large intercellular spaces, lined by an epithelium of secretory cells. The lumina are formed either schizogenously, i.e. by separation of walls of neighbouring cells, or lysigenously, i.e. by the disintegration of cells in the area where the space develops. The lumina may be of various forms : spherical (secretory cavities), or elongated (ducts). The secretory cells of the ducts and cavities may secrete terpenes only or terpenes together with carbohydrates and other substances.

Cavities secreting lipophilic substances occur in many families, including Myrtaceae, Rutaceae, Myoporaceae, Leguminosae and Hypericaceae (Fahn, 1979). These observations are in accordance with those of Hardmann, (1973), who states that the Rutaceae and Myrtaceae are characterized by having such schizolysigenous oil cells.

The similarity between *Kunzea* snd *Leptospermum* spp. is not surprising, since *K. ambigua* is synonymous with *L. ambiguum* Sm. Likewise, there is a close relationship between *Phebalium* and *Eriostemon; P. squameum* has also been termed *E. squameus* Labill.

The secretory cells of *O. phlogopappa* also consist of large cavities below the surface of the leaf, but these open out into globular structures, held on a short column of cells. This type of arrangement is unique among the samples examined. *Olearia* spp. belong to the family Compositae. Other members of this family also bear glandular trichomes

of this nature. *Cannabis sativa*, for instance, has capitate-stalked glands consisting of a one celled head and a long multicellular stalk. The secreted terpenes collect in the subcuticular space (Fahn, 1979).

The nature of the oil glands in *D. lanceolata* appears to be very similar to that observed in members of the Rutaceae and Myrtaceae. The surface of the leaf exhibits areas of essential oil bearing cells, just as in *Kunzea* and *Phebalium* spp. The arrangement of the cells below the cluster of oil cells was not determined.

5.3 PROPAGATION

The propagation trials demonstrate that seven of the ten selected species can be readily propagated from cuttings. The other three, that is, *Kunzea, Eucalyptus* and *Callitris*, can be derived from a seed base.

Despite the preliminary nature of the cutting trial, and the lack of replicate experiments, *O. phlogopappa* and *P. squameum* can be seen to be readily propagated by cuttings, and need no added rooting hormones for root initiation. The optimum levels of IBA required by the species investigated, as suggested by the tentative results, can be assigned as follows :

O. phlogopappa,	
L. glaucescens,	
L. lanigerum,	
B. viscosa	Nil
D. lanceolata	1000 ppm
E. virgatus	Strike
P. squameum	500 to 1000 ppm, Strike or Seradix.

The treatment of cuttings with fungicides, such as captan in Strike, provides some protection from attack by various fungi. Better survival and improved root quality should, therefore, result. Thielges (1972) showed that cuttings of *Pinus strobus* treated with 5% benomyl and 25% captan in talc gave maximal rooting, and IBA did not increase the response. Therefore, preparations containing fungicides can directly influence the initiation of roots, or promote rooting by preventing the decay of the cutting by fungi. The response of *E. virgatus* to the

Strike treatment may be attributable to the presence of captan. Problems are often encountered with the propagation of native species by cuttings. This is due to low level of endogenous auxin, or the presence of inhibitors, which form in the roots and move upwards. These inhibitors accumulate in the shoots, and ultimately prevent the initiation of roots.

Plants that are easy to propagate by cuttings contain sufficient auxin and the necessary co-factors that enable them to form cuttings under the appropriate conditions. Difficulty in rooting arises when there is a lack of auxin, but this can be overcome by treating the cuttings with an auxin before planting. The last group of plants do not respond to added auxin, since some necessary co-factor is not active.

Many observers have reported that seeds from natives do not germinate well in sterilized media. The results here are in agreement with this observation. The fungi that cause damping off include *Pythium, Rhizoctonia* and *Sclerotinia* species. Sterilization of the soil by heating to 82°C for 30 minutes will kill most harmful bacteria and fungi, as well as nematodes, insects, and most weed seeds. However, partial sterilization with a steam/air mixture at a temperature of 60° C for 30 minutes will destroy all known root pathogens, but does not kill other beneficial soil micro-organisms such as nitrifying bacteria and some actinomycetes. The rapid build up in the populations of these beneficial organisms in the soil after sterilization acts in a highly competitive and antagonistic way to the reintroduction of the fungi that cause damping off.

The preliminary nature of the seed trial prevents the drawing of any definite conclusions concerning the propagation of the species studied by seed. However, with *Leptospermum, Kunzea, Callitris* and *Eucalyptus* species, a trend is discernable, in which germination more successful in unsterilized media.

Some seeds may need a pre-treatment. *Eriostemon*, for example, produces seed which have a very horny endocarp. This would be sufficient to prevent the ingress of water, and thus, germination. Mechanical abrasion, freeze/thaw, attack by soil micro-organisms, passage through a digestive tract or fire are the natural methods by which the barrier presented by the seed coat is broken down. The action of soil bacteria and fungi are most effective under non-sterile conditions.

The seeds of *Leptospermum* species should be carefully chosen for any seed trial. They are produced in large numbers, but many are sterile. The fertile ones are distinguished as being broader and narrowly winged.

Similarily, the seeds of *Eucalypts* are often abortive.

The age of the seeds is also important. *Drimys* sp. seeds are berry-like when fresh. The seeds used in this study were no longer fleshy, and this could explain the poor germination results in this case.

Further studies should be made on the propagation of theses endemic species, either by macro- or micro- methods. It is probable that all these natives are amenable to propagation through tissue culture. The determination of suitable media and hormone concentrations would be an important step, since the rapid production of planting material at a reasonable cost, is vital to the establishment of a fledgling industry of this nature.

5.4 GAS CHROMATOGRAPHY - MASS SPECTROMETRY ANALYSES

The analysis of steam distilled oil samples by gas chromatography coupled with mass spectrometry (GC-MS) resulted in the tentative identification of many of the constituents of these oils. The GC-MS traces are presented in Figures 4.5.I to 4.5.XXII, and identified peaks are listed.

The analyses brought out many points of interest. For instance, the two species of *Eucalyptus* produced very different oils, both in smell and composition. *E. pulchella* contains the α- and β- forms of phellandrene, whereas only α-phellandrene exists in *E. amygdalina*. In addition, the presence of 1,8-cineole was noted in *E. amygdalina*, but not in *E. pulchella*.

Three different extracts of *Phebalium* were examined. That is, oil extracted from stems and leaves by steam distillation, and concretes from stems only and from stems plus leaves. Comparison of the two concretes shows that stems contain an large proportion of hedycaryol and Δ-3-carene, whereas leaves do not. Components of the steam distilled oil include α-pinene and limonene. An interesting observation is the lack of hedycaryol in the steam distilled extract, with the occurence of an unknown sesquiterpene alcolhol eluting in the same region of the GC-MS trace. The absence of hedycaryol may be explained by the fact that, under acid conditions and heating, it undergoes a Cope rearrangement to produce elemol (Jones and Sutherland, 1968). The conditions during steam distillation are thought to be suitable for such a conversion to take place.

Both *Olearia phlogopappa* and *O. argophylla* contain epi-γ-eudesmol as their major constituents, occurring as peaks 16 and 22 respectively. It occurs in concentrations of 48%. This compound was thought to be γ-eudesmol, at first, since their mass spectra were identical. However, co-injection of a sample containing known γ-eudesmol during the G.C.-M.S. run, resulted in two peaks which were differentiable under the conditions used. An epimer form of γ-eudesmol has not been previously reported in any natural extracts.

Examination of the NMR spectrum of epi-γ-eudesmol reveals that there are two peaks representing methyl groups (1.17 and 1.23 δ). The proposed structure of epi-γ-eudesmol is not consistent with this result, since the two methyl groups adjacent to the -OH group are in identical environments. However, it may be that there is hindered rotation in this configuration, which could be tested by NMR analysis, using a higher temperature run. Altering the conditions in this way would cause the peaks to coalesce, if it is a case of hindered rotation at the lower temperature. The remainder of the spectrum is consistent with the structure of epi-γ-eudesmol, as it has been put forward. The infra-red and mass spectra are also in accord with the suggested epimer structure.

The extract of *Zieria arborescens* contains methyl salicylate which is a useful component in medicine and dental flavourings. In addition, zierone and α-gurjenene are present.

Perillene, α-copaene, γ-cadinene and eudesmol were found in *Beyeria viscosa* oil. Of these, γ-cadinene has found commercial uses. It occurs in oil of Cade, and is a suggested constituent in synthetic ylang-ylang oil in concentrations up to 5%.

Two types of *Callitris tasmanica* oil were found. The 'hybrid' contained such compounds as bornyl acetate, (which was also found in the *P. squameum* and *E. virgatus* extracts). Bornyl acetate has the refreshing odour of pines, so it is used largely in cinema and theatre sprays. When used in soap perfumery it has a powerful and persistent odour. Citronellyl acetate, neryl acetate and lavandulyl acetate were also found. The first of these has an odour like a mixture of lime and bergamot, and is used in rose and carnation compounds (<10%). It has a sweetening effect on almost any type of perfume compostion. Neryl acetate has a sweet and floral odour. It is used in artificial neroli and jasmine oils, sweetening a fine perfume bouquet. The occurrence of these components, along with citronellol, account for the sweet, lemony fragrance of the hybrid pine oil.

The other oil lacks bornyl acetate, neryl acetate and lavandulyl

acetate, but contains geranyl acetate. This oil, which comes from a pine that has been cultivated in India since 1885, nominally contains 30% esters, calculated as geranyl acetate (Poucher, 1976). It is particularily suitable for the perfuming of soaps.

Nerol, a useful addition to orange blossom and rose compounds, is found to be present in the *Melaleuca* spp. However, the synthetic production of nerol, along with geraniol and linalool, from myrcene is economically superior to extraction from natural sources. The *Melaleuca* spp. also contained myrtenol, which has a heavy woody pine note. It is mildly camphoraceous and oily. Hay-like overtones are developed when exposed to the air, due to the formation of myrtenal. Myrtenol is used in spruce and pine perfume compounds.

Eugenol and methyl eugenol were found in *D. lanceolata.* Eugenol is the major component of clove oil, where it constitutes some 85% of the oil (Poucher, 1976). Its uses include forming the basis of carnation compounds, soap perfumes and in the commercial production of vanillin. Methyl eugenol has a less powerful odour, but is useful in modifying carnation and lilac bouquets.

The alcohol guaiol is found in *D. lanceolata.* It was first discovered in the oil of guaiac wood, but has no specific use in perfumery (Poucher, 1976).

All of the oils which were investigated, contain some substances which occur in oils of commercial importance. Therefore, a comparison of the oils from the selected natives with other oils, by means of their compositions, can be instructive.

L. lanigerum has a composition fairly similar to that of other *Leptospermum* species. Its major constituens are α-pinene (14 %), 1,8-cineole (10 %), γ-eudesmol (11 %) and α-eudesmol (23 %). This compares with *L. lanigerum* var *macrocarpum* : 22 % α-pinene, 12 % 1,8-cineole, 6 % γ-eudesmol and 7 % α-eudesmol. *L. sphaerocarpum* and *L. scoparium* var *rotundifolium* have a higher α-pinene (30-34 %) and 1,8-cineole (17-35 %) level, with γ-eudesmol (1-2 %) and α-eudesmol (3-5 %) (Flynn *et al*, 1979). The endemic species has a higher γ- and α-eudesmol, and a lower 1,8-cineole concentration. This would influence the aroma of the oil to some extent; 1,8-cineole tending to give a harsher note to the complex.

Beyeria oil contains some 11 % sabinene. This is a major constituent of the terpene fraction of West Indian nutmeg oil (*Myristica* sp.S) Baldry *et al*, 1976 . The nutmeg flavour of East Indian nutmeg oil is not as strong as that of the West Indian variety. Myristicin (which

constitutes 9 % of the oil of *O. phlogopappa*), is present in large amounts in the East Indian nutmeg oils.

The oil of *D. lanceolata* has similarities with citrus oils, where limonene forms a base to which other odours are added. The compounds α-pinene, β-pinene, α-terpineol, α-phellandrene and linalool all occur in citrus oils (Lawrence, 1980 i, ii, iii, 1979). *Drimys* oil has α-pinene (24 %), β-pinene (7 %), myrcene (1.5 %), α-phellandrene (3 %), α-terpinene (1 %), limonene (23 %), linalool (10 %), α-terpineol (4 %) and carophyllene (2 %) as its major constituents.

Lemon oil has less limonene than other types of citrus oils. The esters geranyl and neryl acetate give it full-bodied flavour (Shaw, 1979). One of these, neryl acetate, is a consitiuent of the *Callitris* hybrid oil (11 %). This extract has a citrusy character, which is perhaps due to the presence of neryl acetate, along with 14 % limonene, citronellal and citronellol.

The *Callitris* hybrid also contains 27 % lavandulyl acetate. This ester occurs in lavender oil, but only in concentrations of about 3 % (Lawrence, 1980).

The main contributors to the aroma of Myrtle oil are α-pinene, camphene and 1,8-cineole. *Leptospermum lanigerum* oil has high levels of α-pinene and 1,8-cineole, but no camphene. On the other hand, *Phebalium* extracts contain 26 % α-pinene, 4 % camphene and 12 % 1,8-cineole. The odour of the oil from this species may well be similar to that of Myrtle.

Eucalyptus amygdalina contains piperitone which is the main constituent of the oil of *Cymbopogon jawaracusa* (Khavi grass) (Saeed, 1978). It is also a major component of other species of *Eucalyptus*, such as *E. dives, E. apiculata* and *E. elata* where it constitutes 40-50% of the steam distilled oil (Shaw *et al*, 1959).

According to Guenther (1949), *Eucalyptus amygdalina* var *salicifolia* yields 1.8 % of oil, has an optical rotation of -59.1 to -75.1 and a refractive index of 1.475 to 1.4781. The yield compares well with that obtained in this study. The optical rotation was -48.9 and the refractive index was 1.4764 which lies in the specified range. The similar values for these two physical properities indicates that the oils contain similar optically active constituents. This is borne out by the presence of α-phellandrene, 1,8-cineole (12-24 %) and piperitone in the reference, which closely allies with the major constituents of the present sample :

13 % α-pinene, 24 % α-phellandrene, 14 % 1,8-cineole and 22 %

piperitone.

5.5 POTENTIAL APPLICATIONS

Many of the oils from the species which were examined in this study contain components in excess of 20%. These oils are considered to be potential sources of isolates, and are listed below, along with their major constituents and relative percentages.

Beyeria viscosa	37%	ledol/globulol/rosifoliol
Callitris tasmanica	35-68%	α-pinene
Callitris tasmanica (hybrid)	28%	lavandulyl acetate
Drimys lanceolata	25%	α-pinene
Eriostemon virgatus	50%	α-pinene
Eucalyptus amygalina	24%	α-phellandrene
	21%	piperitone
Kunzea ambigua	26%	α-pinene
	23%	1,8-cineole
	27%	globulol
Leptospermum glaucescens	53%	α-pinene
Leptospermum lanigerum	23%	γ-eudesmol
Olearia phlogopappa	48%	epi-γ-eudesmol
Phebalium squameum	28%	α-pinene
	22%	β-pinene

α-, β- and γ-eudesmol are bicyclic sesquiterpene tertiary alcohols ($C_{15}H_{26}O$), which have a fine sweet woody odour and are used as fixatives in floral and spicey perfumes. A eudesmol will blend well with all types of odours and can be converted into a very useful acetate derivative. This derivative has a floral odour reminiscent of bergamot and helps enhance linalyl acetate odour in a blend. It can replace, modify or intensify the aroma.

α-pinene is a cyclic terpene hydrocarbon of great commercial importance. Its odour is warm, refreshing and pine-like. This compound yields many semi-synthetics such as: ocimene, terpinolene, terpin hydrate, terpineol, camphor, allocimene, verbinone and camphene.

β-pinene has a dry woody, piney odour and is used as a starting material for the manufacture of many industrially important odour chemicals. The most important of these is myrcene, which is sweet and balsamic, which introducing a refreshing character into citrus and spicey colognes. Myrcene lies on the pathway to citral, citronellal, geraniol, hydroxy citronellol, ionones, linalool etc.

In order to determine whether any of the plant species cited here are suitable for commercial purposes, the essential oil samples will be subjected to further quality, economic and organoleptic assessment. On this basis, one species can be selected as the subject of a major study.

All of the oils extracted from the ten selected species may find application in the fragrance industry. In particular, the fresh lingering character of *D. lanceolata, O. phlogopappa* and *P. squameum* would render them useful components of such products as air fresheners, antiseptic and cleansing preparations. The other oils would probably be more suitable for perfumes and as flavouring agents. Of course, the factor that will determine the commercial success of any of these new oils, is the economics involved in establishing large-scale plantings, harvesting techniques and the stability of the price of the product in the market place. The latter is determined by the quality control enforced in production, as well as by the demand for the oil.

Once a particular species has been chosen, the initial steps toward its attaining commercial protential must lie in the study of its natural variability. In order to be successful, a high degree of natural variability is desirable; the aim being to find an individual whose characterisitics are most suited to the purpose at hand. Having located these individual plants, they can be bulked up, or further modified by cross-breeding with other plants that show additional desirable traits. As demonstrated by Hellyer *et al* (1969), even morphologically

indistinguishable types of the one species can vary greatly in essential oil composition. They found that in some trees of *Eucalyptus dives* the oil contained 45% piperitone and 40% hydrocarbon fraction, consisting of α-phellandrene, myrcene, *p*-cymene, γ-terpinene and α-thujene. Other trees yielded an oil with 70% 1,8-cineole and only a small hydrocarbon fraction. The oil from *Eucalyptus amygdalina* may be particularily variable. Trees from different areas where crossbreeding with other species has occurred may yield oils with different characteristics.

One species which has been shown to be highly variable by Boyer, (1980), is *O. phlogopappa*. It varies from a small, woody, much branched shrub 30-40 cm high with leaves having wooly indumentum on both surfaces, leaves elliptical to oblanceolate in shape 0.8-2.0 cm long, to a medium, densely branched shrub to 2 m with elliptical leaves up to 6.5 cm long, having a woolly indumentum of stellate hairs only on the lower surface. The habitats also vary from subalpine shrubberies to sclerophyllous forests to the highly halophylic communities found on coastal sand dunes.

Hellyer and Lassak (1968) examined the chemical variation in *Melaleuca viridiflora* Gaertn. This species occurs as one of two forms : nerolidol and linalool, or 1,8-cineole and viridiflorol. The form *M. viridiflora* Sol. ex Gaertn. is different again, with 75% methyl cinnamate and 20% *trans*-β-ocimene as the main constituents of its essential oil. Variability of this nature can be expected to occur among the endemic *Melaleuca* and *Leptospermum* species.

The *Callitris* sp. are ready examples of naturally variable trees, with the lemon scented type exhibiting a totally different essential oil composition to the 'normal' type. An alteration in composition is also obtainable by using different plant parts as sources of essential oil. The *Phebalium* extracts showed that a large amount of hedycaryol is present in stems, compared to extracts from a predominantly leaf source.

The fact that *Olearia phlogopappa* oil contains some 48% of epi-γ-eudesmol suggests that if this component of the oil is valuable as an extract, then it can be obtained from this species. The entire oil is also very pleasant, so it may find perfumery or other applications in its own stead. *O. phlogopappa* is readily propagated from cuttings during the summer growth period. No doubt it would respond well to clonal techniques as well.

Beyeria viscosa yields a large amount of concrete by solvent extraction. Its unique odour qualities are promising, in that it may find applications either as a component of a male toiletry, or as a

flavouring agent in confectionery. Similarily, *Drimys lanceolata* and *Phebalium squameum* offer unusul notes in their bouquets, which could be applied in a variety of ways.

The clonal propagation of a potential essential oil crop plant will be a major step. The rapid propagation of plants is vital for any large-scale commercial plantings to be undertaken. Clonal techniques also ensure a uniformity of planting material unobtainable by other methods of stock increase. A tight control over the quality of essential oil compostion is therefore possible, however, environmental effects must not be disregarded. Most of the species studied here will lend themselves to micro-propagation; future research into appropriate media and techniques is called for.

The applications of essential oils need not be confined to those that are already established. Admittedly, there is a lucrative market available in the fragrance and flavour industries for a novel essential oil. However, intensive tangential study could reveal that some of the oils discussed herein have a wider range of applications than purely for perfumery. Other areas, such as agricultural pesticides must not be forgotten. Essential oils can be used in many ways for the control of various insect populations. They behave as insecticides, synergists, attractants/repellents, phagostimulants/deterrants, insect growth regulators, chemo-sterilants, etc. There is a long list of essential oils which have been reported to possess an insecticidal property. Also, many pure compounds such as camphene, carvacrol, carvone, 1,8-cineole, citral, citronellol, eugenol, farnesol, geraniol, limonene, linalool, α-phellandrene, and α-pinene have a lethal action against house flies.

Synergists are capable of increasing the biological activity of other active compounds, such as pyrethrin. The most potent of these is piperonyl butoxide, a compound synthesised on a large commercial scale from safrole, derived from safrol oil. However, other oils of this nature are sure to exist in the vast array of natural extracts available.

The oils that are effective as ovicides include peppermint, pine, lemon, lavender, parsley, rosemary, rue and others. Since some of the oils studied contain similar components, they too may be effective ovicides. In particular some of the sweet smelling terpenoids are attractants to insects for oviposition on one hand, and are ovicidal on the other, making them promising insecticides for the future.

The list of insect attractants and repellents is also a long one. Some forty essential oils were known in 1952 which repelled mosquitoes,

march flies and sandflies. Of these, oils from *Dacrydium franklini, Backhousia myrtifolia, Melaleuca bracteata* and *Zieria smithii* are worth noting. *Eucalyptus* and *Caryophyllum* oils also possess similar properties (Saxena and Opender, 1982).

5.6 FUTURE INVESTIGATIONS

Further research, with plants that show potential, should be carried out along the same lines as other species that have recently been introduced to the Tasmanian essential oil industry. Good examples of suitable models include *Boronia megastigma* and peppermint (*Mentha piperita* L.). Some plants may be amenable to improvement through an extensive cloning programme, and large areas may be brought into production only by the refinement of a tissue culture technique. Optimum conditions for the production of high yields may need to be determined. In addition a canopy design for mechanical harvesting may have to be investigated.

Studies on the effect of harvest date, irrigation and nitrogen status on the quality and quantity of oil produced would be valuable, since guidelines for management practices must be established. Oil quality and quantity produced in regrowth will be of particular interest, since the possibility of multiple harvesting, and hence, increased oil yield per annum, may arise, as it did with peppermint. Parallel investigations have been conducted with blackcurrants, parsley and fennel.

The accumulation of such data on one of the selected native species would be particularly valuable if the essential oil contains one major isolate. Cultivation of the crop under specific conditions would allow control of isolate concentration. For other types of oils, where the entire mixture of components is useful, correct environmental conditions maintained.

A preliminary survey, such as this one, always raises more questions, and opens up more new avenues of research, than is anticpated at the outset. Even though only a small fraction of the Tasmanian essence-bearing plants have been taken for this study, it is perhaps not over-ambitious to say that a useful addition has been made to the current knowledge of our endemic species.

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