ALKALOIDS OF ATHROTAXIS SPECIES

by

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DOCTOR OF PHILOSOPHY

graduation 1985

UNIVERSITY OF TASMANIA

HOBART

APRIL, 1984

For my Parents

Except as stated therein, this thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and, to the best of my knowledge and belief, this thesis contains no copy or paraphrase of material previously published or written by another person, except when due reference is made in the text of this thesis.

S. Panichanun

Sirichai Panichanun

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I am especially grateful to Mrs. C. Cooper for typing this thesis. Finally, I would like to thank the University of Tasmania for a Postgraduate Research Scholarship and the Australian Research Grants for financial assistance for this work.

Memorandum

Microanalyses were carried out either by Australian Microanalytical Service, Melbourne, or by Dr. A. Campbell, Chemistry Department, Otago University, Dunedin, New Zealand.

Melting points were determined on a Yanagimoto Seisakusho Micro-Melting Point apparatus and are uncorrected.

Specific optical rotations were measured in chloroform solvent (unless otherwise specified) on a "PEPOL 60" Spectropolarimeter.

Proton magnetic resonance (^{1}H nmr) spectra were recorded on deutero-chloroform solutions with tetramethylsilane as internal standard, at 100 MHz with a Jeol JNM-4H-100 spectrometer unless otherwise specified; the 270 MHz ^{1}H nmr spectra were recorded with a Bruker HX-270 spectrometer. Chemical shifts (δ) are given in ppm, coupling constants are in Hertz (Hz), and peaks are described as singlet (s), doublet (d), quartet (q) or multiplet (m).

The carbon-13 nuclear magnetic resonance (^{13}C nmr) spectra were determined with a Bruker HX-270 spectrometer operating at 67.89 MHz. Chemical shifts (δ) were measured in ppm from internal tetramethylsilane.

Ultraviolet (uv) absorption spectra were recorded on ethanol solutions with a Hitachi-Perkin-Elmer 124 spectrometer, and extinction coefficients are given in parenthesis.

Infrared spectra were recorded on chloroform solutions (unless otherwise specified) with a Beckman IR-33 spectrometer.

Low resolution mass spectra (ms) were run on an EAI Quad 300, employing an inlet temperature of 250° and an electron beam energy maintained at 70 eV High resolution; high resolution mass spectra were run on an A.E.I. MS 902 spectrometer or on a Vacuum General Micromass 7070 F spectrometer using the direct insertion technique. The source temperature of the latter instrument was 200° and that of the former was 150°. In each instrument the electron beam energy was maintained

at 70 eV. Peaks are listed in descending order of m/z ratio.

Thin-layer chromatography (tlc), preparative thin-layer chromatography (ptlc) and column chromatography were performed with Merck silica gel GF_{254} or CAMAG silica gel DSF-5, and the compounds were visualised by spraying with iodoplatinate reagent or by examination under uv light. High-performance liquid chromatography (hplc) was carried out on a 7.7 mm x 25 cm column with octadecyl silane as a stationary phase, and with buffered aqueous acetonitrile for elution at a flow rate 2 ml/min.

Solvents were purified by standard methods. Evaporation of solvents was carried out under reduced pressure.

Due to the small quantities available, elemental analyses on some compounds could not be accomplished. In such cases, high resolution mass spectra were used to determine molecular formulae whenever possible and homogeneity on tlc was used as a criterion of purity.

Abstract

A detailed phytochemical examination of the alkaloid content of three species of the family Taxodiaceae, *Athrotaxis cupressoides* D. Don, *Athrotaxis selaginoides* D. Don, and *Athrotaxis laxifolia* Hook has been undertaken.

A total of twelve alkaloids were isolated; the alkaloids proved to have structures of the homo*erythrina* type, and included five known bases previously isolated from other plants. Spectroscopic and chemical evidence for the structures of the seven new alkaloids are presented in Chapter II.

The synthesis of the homo*erythrina* alkaloid skeleton has been attempted by two different approaches as presented in Chapter III, and the work done on homo*erythrina* alkaloids has been briefly reviewed in Chapter I.

Discaria toumatou and *Discaria pubescens*, members of the family Rhamnaceae, have been investigated. The alkaloids present were found to have the benzylisoquinoline type of structure, and the results appear in Chapter IV.

A preliminary investigation of *Hypserpa veillardii*, a species of the family Menispermaceae, has been made, and the results appear in Chapter V. The known bisbenzylisoquinoline alkaloid limacine was the only base to be isolated.

CHAPTER I

Homo*erythrina* Alkaloids

1. General Introduction
2. Occurrence and Isolation
3. Nomenclature
4. Structural Determination
5. Synthesis
6. Biosynthesis
7. References

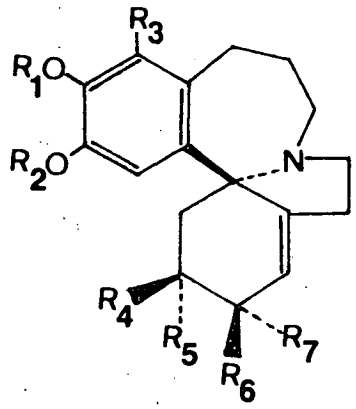
1. General Introduction

The homo*erythrina* alkaloids are a relatively recently established group, the first examples being isolated and identified from *Schelhammera pedunculata* F. Muell in 1968[1,2]. Schelhammerine (I) and schelhammeridine (XI) were the first representatives of the homo*erythrina* alkaloids to be structurally elucidated.

2. Occurrence and isolation

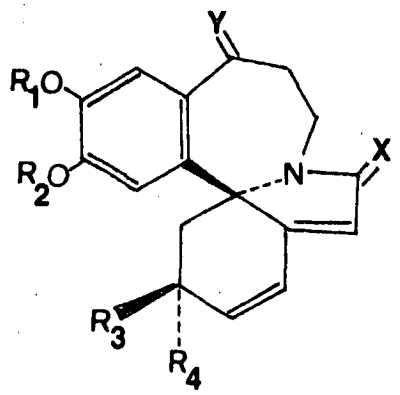
Homo*erythrina* alkaloids have been isolated from all three species of *Schelhammera* (Liliaceae)[2,4]; from the leaves of a species of *Phelline* (Icacaceae)[3]; from the roots and stems of certain species of *Cephalotaxus* (Cephalotaxaceae)[5-7,10,11], particularly *C. wilsoniana* Hayata in which they are the major alkaloids; and from the leaves of a species of *Dysoxylum* (Meliaceae)[13] (see Table I). So far over 20 individual homo*erythrina* alkaloids have been isolated, although the structure of two of them[3,5] remains incomplete because of insufficient material. The alkaloids of known structure are shown in Figs. I, II, III, IV, and V. Within the three genera, the alkaloid profile is fairly distinctive, with only 3-epischelhammericine (IV) occurring in all three.

The alkaloids have been isolated (ether) by alcohol extraction of dried plant material[2-7] or by either extraction of the basified material[3]. The crude mixture was then fractionated by countercurrent distribution, followed by chromatographic purification and recrystallisation.



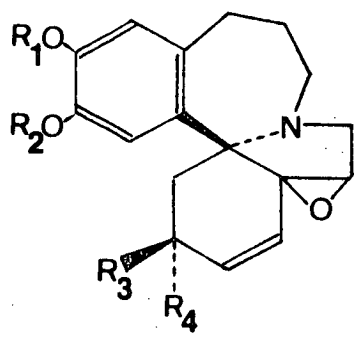
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Ref.
Schelhammerine (I)	$-CH_2-$		H	OCH_3	H	H	OH	1,2
2-Epihomoerythratine (II)	$-CH_2-$		H	H	OCH_3	H	OH	3,4,8,14
Homoerythratine (III)	$-CH_2-$		H	H	OCH_3	OH	H	14
3-Epischelhammericine (IV)	$-CH_2-$		H	H	OCH_3	H	H	2-6,8,12
Schelhammericine (V)	$-CH_2-$		H	OCH_3	H	H	H	2
3-Epi-2,7-dihydrohomo-erysotrine (VI)	CH_3	CH_3	H	OCH_3	H	H	H	6
(VII)	CH_3	CH_3	H	H	OCH_3	H	H	5
Taxodine (VIII)	CH_3	H	H	H	OCH_3	H	H	2,4,5
(IX)	CH_3	H	H	OCH_3	H	H	H	5
O-Methylathrocupressine (X)	CH_3	CH_3	OCH_3	H	OCH_3	H	H	3

Fig. I Homo*erythrina* alkaloids: Δ1(6) alkene series.



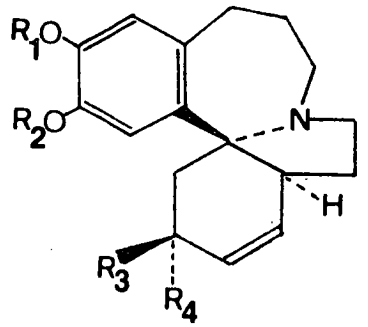
	R_1	R_2	R_3	R_4	X	Y	Ref.
Schelhammeridine (XI)		$-CH_2-$	OCH_3	H	H_2	H_2	1,2
(XII)		$-CH_2-$	OH	H	H_2	H_2	2
3-Epischelhammeridine (XII)		$-CH_2-$	H	OCH_3	H_2	H_2	1,2
11a-Oxoschelhammeridine (XIV)		$-CH_2-$	OCH_3	H	H_2	0	2
8-Oxoschelhammeridine (XV)		$-CH_2-$	OCH_3	H	0	H_2	2

Fig. II - Homo*erythrina* alkaloids: 1,6 diene series



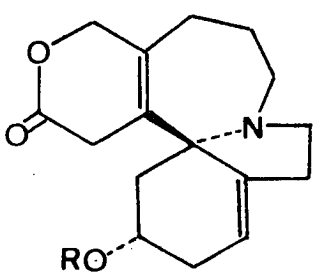
	R_1	R_2	R_3	R_4	Ref.
Wilsonine (XVI)	CH_3	CH_3	OCH_3	H	3
3-Epiwilsonine (XVII)	CH_3	CH_3	H	OCH_3	3,6,7,8
3-Methoxy-15,16-methylenedioxy -6,7-epoxy-C-homoerythrinan-2(1)-ene (XVIII)	$-CH_2-$		H	OCH_3	6,7

Fig. III - Homo*erythrina* alkaloids: epoxy-Δ2(1) series



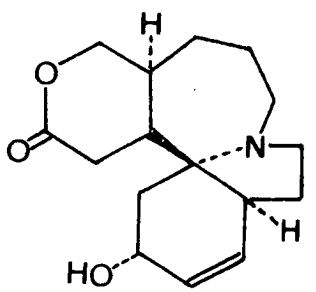
	R_1	R_2	R_3	R_4	Ref.
3-Epi-6,7-dihydrohomoerythraline (XIX)	$-CH_2-$		OCH_3	H	6,7
6,7-Dihydrohomoerythraline (XX)	$-CH_2-$		H	OCH_3	3,8
6,7-Dihydrohomoerysotrine (XXI)	CH_3	CH_3	OCH_3	H	3,8

Fig. IV - Homo*erythrina* alkaloids: 2(1) alkene series

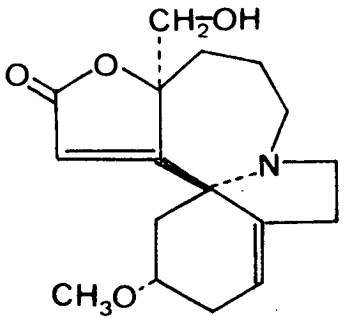


R=H (XXII) Ref. 3

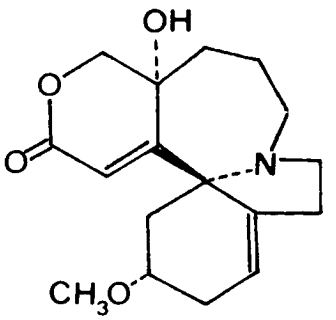
R=CH_3 (XXIII) Ref. 3



Phellibiline (XXIV) Ref. 8,9



Isophellibilidine (XXIV) Ref. 15



Phellibilidine (XXV) Ref. 19

Fig. V - Homo*erythrina* alkaloids: lactone series

Table I

Physical properties of homo*erythrina* alkaloids

Alkaloid	Formula	mp (^{o}C)	$[\alpha]_D^a$	Plant source[b]
Phellibiline (XXIV)	$C_{16}H_{21}NO_3$	-	-	E
(XXII)	$C_{16}H_{21}NO_3$	186-188	+143	E
(XXIII)	$C_{17}H_{23}NO_3$	133	+140	E
Phellibilidine (XXV)	$C_{17}H_{23}NO_4$	160-162	+76	E
Isophellibilidine (XXIV)	$C_{17}H_{23}NO_4$	132	+204	E
8-Oxoschelhammeridine (XV)	$C_{19}H_{19}NO_4$	170-171	+35	A
Ila-Oxoschelhammeridine (XIV)	$C_{19}H_{19}NO_4$	151-153	-47	A
Schelhammeridine (XI)	$C_{19}H_{21}NO_3$	118	-108	A
3-Epischelhammeridine (XIII)	$C_{19}H_{21}NO_3$	131-133	+24	A
Alkaloid 6 (XVIII)	$C_{19}H_{21}NO_4$	126	+63	D
3-Epischelhammericine (IV)	$C_{19}H_{23}NO_3$	169-172[c]	+123	A,B,C,H,F,G,
		170-171[c]	+98	D
Schelhammericine (V)	$C_{19}H_{23}NO_3$	76-77	+122	A
Alkaloid A (XIX)	$C_{19}H_{23}NO_3$	188-189[c]	-100	A
Alkaloid I (XX)	$C_{19}H_{23}NO_3$	260[d]	+75	D

Table I - continued

Alkaloid	Formula	mp (°C)	$[\alpha]_D^a$	Plant source[b]
3-Epischelhammerine (II)	$C_{19}H_{23}NO_4$	182-185	+167	A,I
2-Epihomoerythratine (II)	$C_{19}H_{23}NO_4$	184-185	+172	D
Schelhammerine (I)	$C_{19}H_{23}NO_4$	173-174	+186	A
Homoerythratine (III)	$C_{19}H_{23}NO_4$	176	+75	I
(IX)	$C_{19}H_{23}NO_4$	e	+76	F
Alkaloid B (VIII)	$C_{19}H_{25}NO_3$	152-153	+111	A,C
		150-151	+115	F
Wilsonine (XVI)	$C_{20}H_{25}NO_4$	150-151	-51	G
3-Epiwilsonine (XVII)	$C_{20}H_{25}NO_4$	244 dec[d]	+58	D
(VI)	$C_{20}H_{27}NO_3$	e	+118	F
(VII)	$C_{20}H_{27}NO_3$	e	+122	F,G,H
Alkaloid 2 (XXI)	$C_{20}H_{27}NO_3$	143-145[c]	+72	D
Alkaloid 5 (X)	$C_{21}H_{29}NO_4$	100-101	+91	D

[a]Solvent : chloroform.

[b]*A, S. pedunculata* F. Muell; *B, S. multiflora* R. Br.; *C, S. undulata* R. Br.; *D, P. comosa* Labill; *E, P. billardieri*; *F, C. harringtonia* K. Koch var. *harringtonia*; *G. C. wilsoniana* Hay; *H, D. lenticellare*; *I. P. brachyphylla*

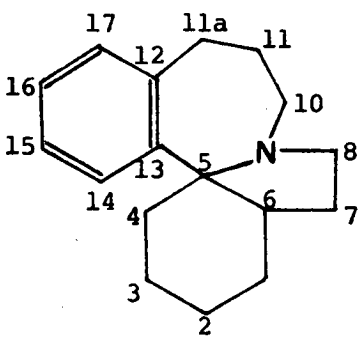
[c]Picrate.

[d]Hydrochloride.

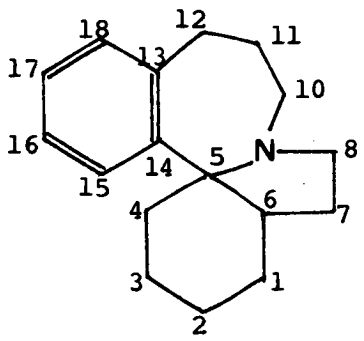
[e]Noncrystalline.

3. Nomenclature

The nomenclature of the homo*erythrina* group constitutes a problem, as only a few of the members have been given trivial names. Since the structures of the homo*erythrina* group parallel those of the *Erythrina* group, Dyke and Quessy decided[16] to refer to those members not previously named as homo analogs of the corresponding *Erythrina* alkaloids. The system of numbering shown in (XXVI) is used when the homo*erythrina* structures are related to *Erythrina* structures, and when this is not possible, the chemical abstracts numbering system shown in (XXVII) is used.



XXVI



XXVII

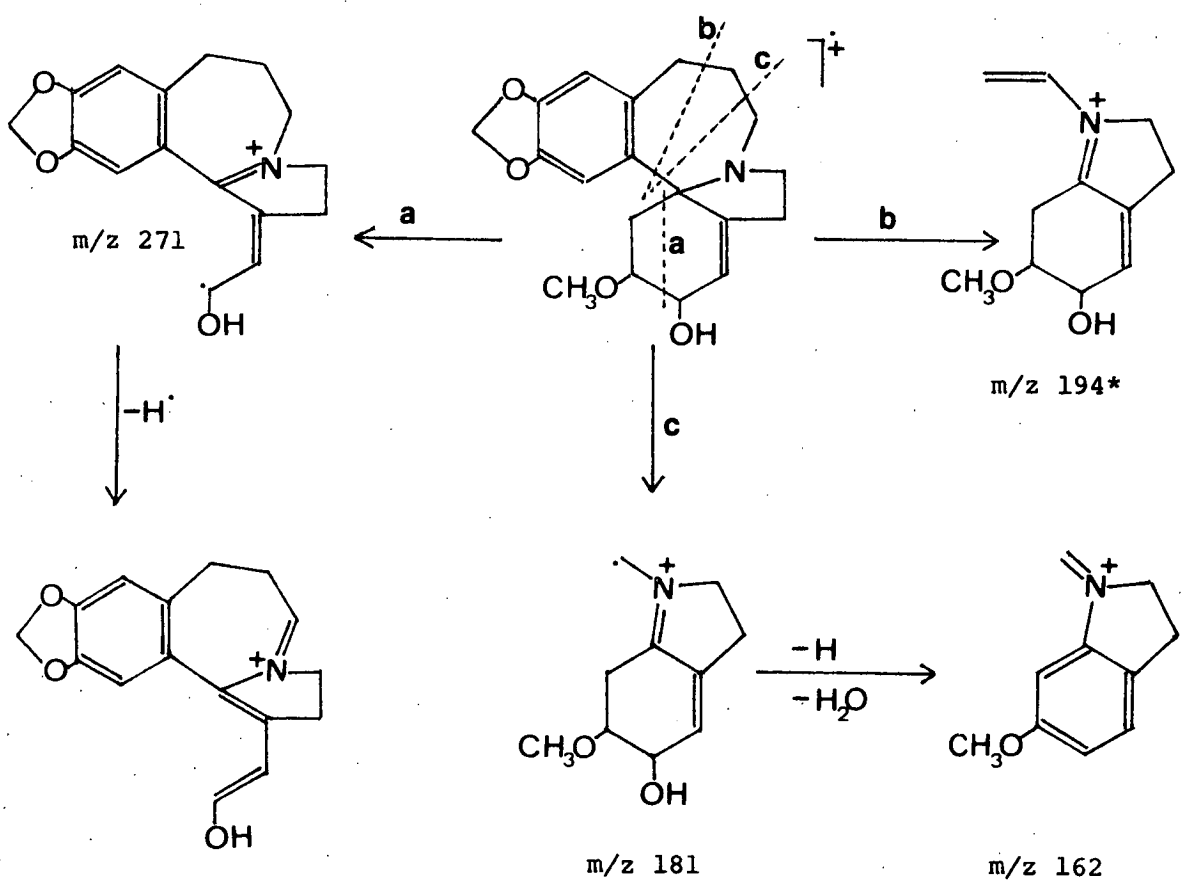
4. Structural determination

The structural determination of the first homo*erythrina* alkaloids obtained from *Schelhammera* spp. was the subject of an elegant series of papers by the CSIRO group[2] in Australia. The structural elucidation was done mainly by nmr and mass spectroscopy and the absolute stereochemistry was confirmed by X-ray analysis of schelhammerine hydrobromide. Details concerning the structural elucidation of the known homo*erythrina* alkaloids have been briefly reviewed by Dyke and Quessy[16].

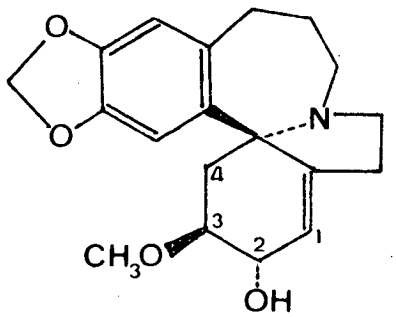
The mass spectroscopic fragmentation of schelhammerine and its analogs is dominated by the cleavage of ring B; the other important pathway involves retro- Diels-Adler fragmentation of ring D (see scheme I).

Scheme I

Fragmentation of schelhammerine



The uv and ir spectra of the homo*erythrina* series are similar to those of the corresponding *Erythrina* series. The uv spectra are useful in differentiating between the 1,6 diene series and the isolated olefine series.



Schelhammerine (I).

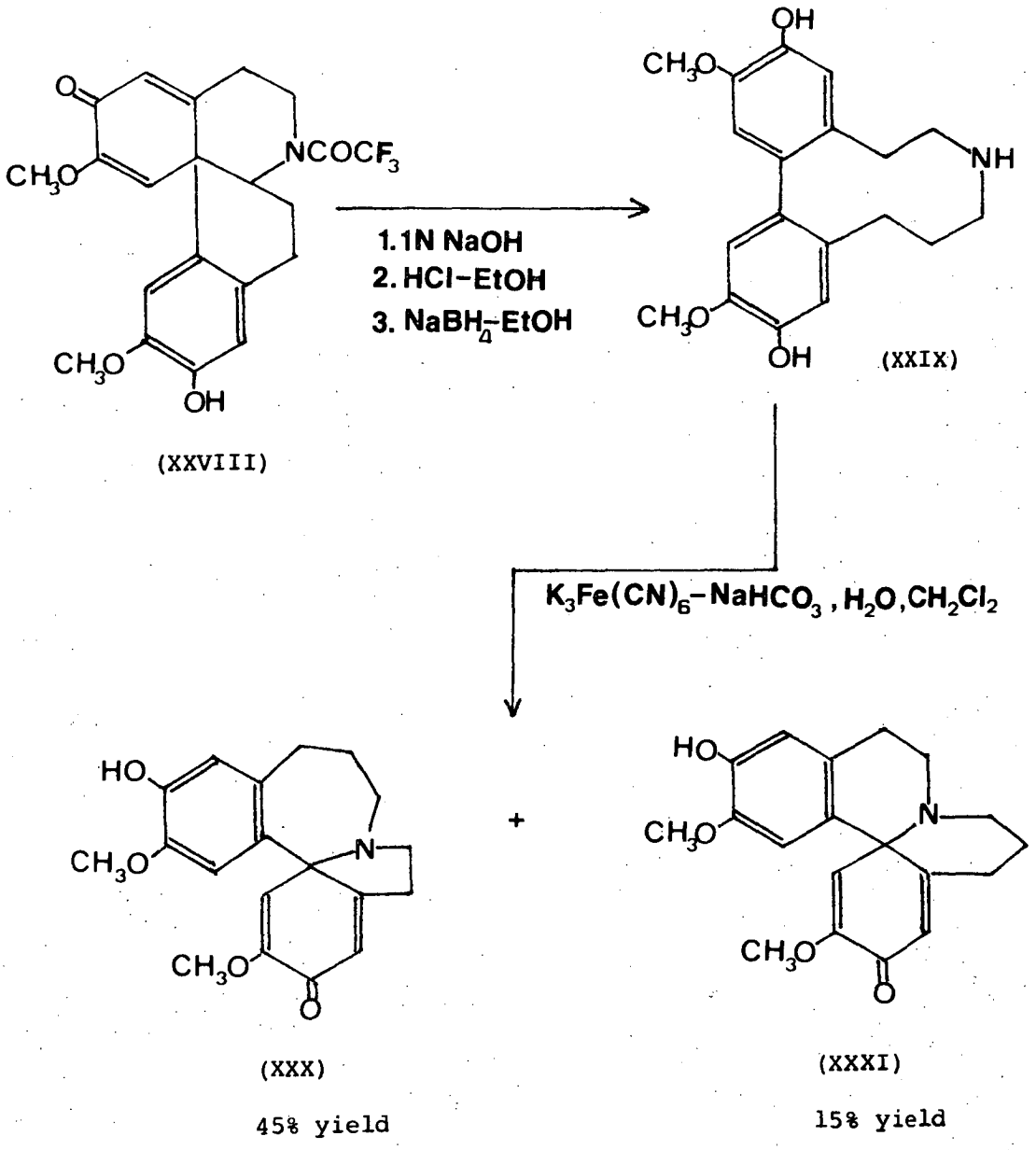
The stereochemistry of schelhammerine was studied by 100 MHz ^1H nmr spectroscopy in benzene-d_6 solution. Evidence for the configuration at C3 and the conformation of the cyclohexene ring was obtained by determination of the coupling constant between $C4_{ax}$-H and $C4_{eq}$-H. A double irradiation experiment was used to assign the resonance signals and measure the coupling constant. The signal from the C1 olefinic proton, which resonated as a doublet at δ 5.58 ($J_{1,2}$ 2.8 Hz), was a convenient starting point for a systematic sequence of double irradiation experiments. The multiplet at δ 4.3 could be assigned to the C2 proton by irradiation of the C1-H doublet, which collapsed the C2-H multiplet at δ 4.3 to a broadened doublet ($J_{2,3}$ 3.0 Hz). Conversely irradiation of the C2-H multiplet collapsed the C1-H doublet to a singlet. By the same technique, a value of 5.0 Hz for $J_{3,4_{eq}}$ and 3.2 Hz for $J_{3,4_{ax}}$ was obtained., which indicated that the proton at C3 was equatorial; however, a value of

3.0 Hz for $J_{2,3}$ did not allow a definite assignment of the stereochemistry at C2, although the value suggested that C2 was also equatorial. A sequence of double resonance experiments indicated a half-chair conformation for the cyclohexene ring.

5. Synthesis

Synthetic work in the homo*erythrina* alkaloid area is proceeding very slowly in several laboratories. Phenolic oxidative coupling plays a key role in a reported synthesis of these alkaloids (see scheme II)[17]. The dienone (XXVIII), available by $VOCl_3$-promoted coupling of the corresponding phenylisoquinoline derivative, was subjected to base-catalysed fragmentation followed by borohydride reduction to give the amine (XXIX) in 76% overall yield. Whereas oxidation of the trifluoroacetyl derivative of (XXIX) led to undesired products, direct oxidation of the free amine (XXIX) gave the dienone (XXX) cleanly in 45% yield, together with compound (XXXI) in 15% yield. An improvement in yield in the oxidation of (XXIX) to (XXX) using modified conditions has been reported by another group of workers[18].

Scheme II.

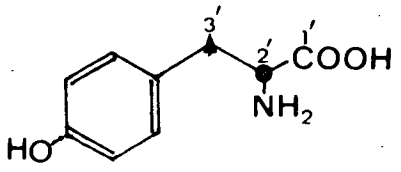

O
NCOCF3
CH3O
CH3O
OH
(XXVIII)
1. 1N NaOH
2. HCl-EtOH
3. NaBH4-EtOH
OH
CH3O
NH
CH3O
OH
(XXIX)
K3Fe(CN)6-NaHCO3, H2O, CH2Cl2
HO
CH3O
N
CH3O
O
(XXX)
45% yield
+
HO
CH3O
N
CH3O
O
(XXXI)
15% yield

6. Biosynthesis

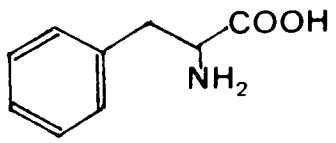
The first two homo*erythrina* alkaloids to be isolated[2] were schelhammerine (I) and schelhammeridine (XI), and since various species of Lilaceae also contain phenethylisoquinoline alkaloids, it was suggested[2,5] that homo*erythrina* alkaloid derivatives are biosynthesised along a pathway analogous to that followed by the *Erythrina* alkaloids (see scheme III). The phenethylisoquinoline (XXXIII) could be derived from tyrosine and phenylalanine; these amino acids are precursors of other phenethylisoquinolines such as colchicine and autumnaline, the biosynthesis of which has been established in broad outline[21]. In these alkaloids C1 is derived from the carboxyl group of phenylalanine, and a plausible precursor of the "phenyl" half of (XXXIII) is the aldehyde (XXXII). A para-para oxidative coupling of (XXXIII) could afford the dienone (XXXIV) which could undergo ring cleavage and reduction to yield the amine (XXXV). The quinone (XXXVI) produced on oxidation could then undergo formation of homo*erythrina* alkaloids. The quinone (XXXVI) is also suggested as the intermediate precursor in the formation of the *Cephalotaxus* alkaloids by Powell[5], Fitzgerald *et al.*[2]. The labelling pattern expected from incorporation of tyrosine labelled at the 2' and 3' positions is indicated in scheme III. In a preliminary study, Battersby[20] has in fact established that (2'-^{14}C) tyrosine, when fed to *Schelhammera pedunculata* plants, yielded schelhammeridine labelled solely at C8. Phenylalanine, cinnamic acid and dopamine were also incorporated into this alkaloid, but no degradations were reported. Derivatives of the amine (XXXV) and the phenethylisoquinoline (XXXIII) have been recently isolated from the leaves of a *Dysoxylum* species[13] which also produces homo*erythrina* alkaloids; these observations provide further evidence to support the biosynthetic pathway of homo*erythrina* alkaloids shown in scheme III.

Scheme III.

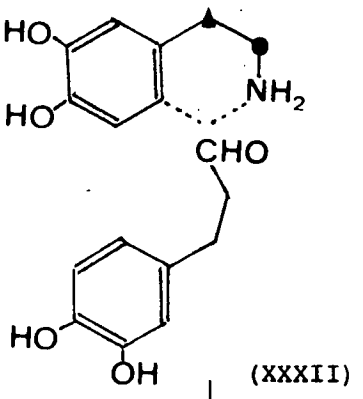
Suggested biosynthetic pathway of the homo*erythrina* alkaloids



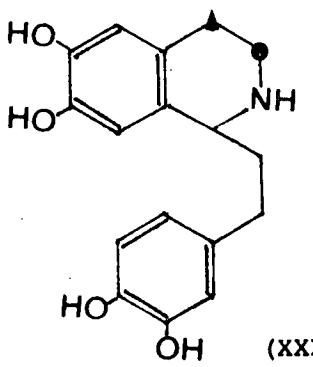
Tyrosine



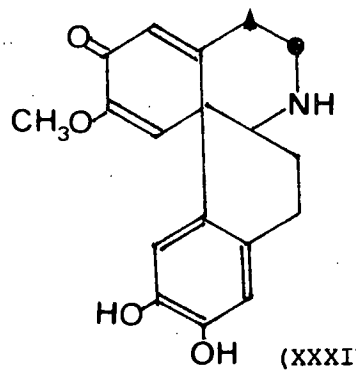
Phenylalanine



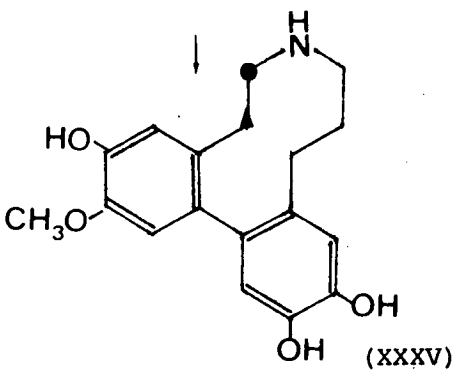
(XXXII)



(XXXIII)



(XXXIV)



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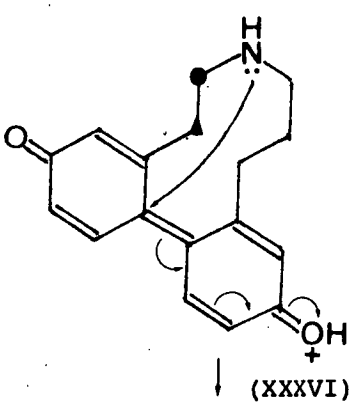

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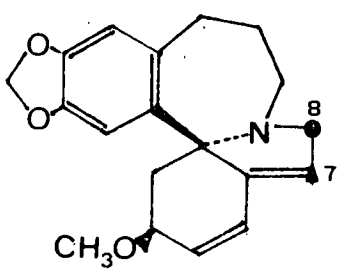
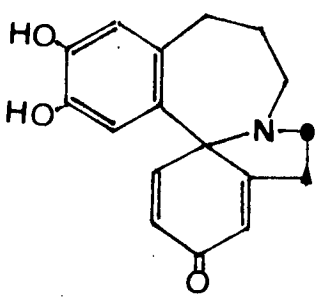


Schelhammeridine



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CHAPTER II

Alkaloids of *Athrotaxis* Species

1. Introduction
2. Results and Discussion
   - 2.1 Alkaloid content of *A. cupressoides*
   - 2.2 Alkaloid content of *A. selaginoides*
   - 2.3 Alkaloid content of *A. laxifolia*
3. Experimental
   - 3.1 *A. cupressoides*
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4. References
5. Spectrum data of new homo*erythrina* alkaloids

1. Introduction.

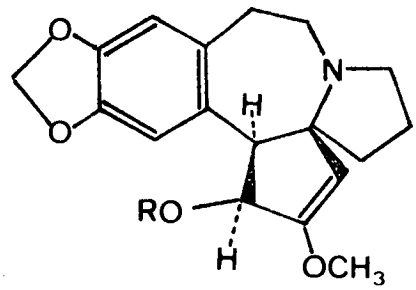
The genus *Athrotaxis* is confined to Tasmania[1,2], and comprises the only representatives in the southern hemisphere of the Taxodiaceae, a rather small family that nevertheless contains some of the largest and tallest trees in the world: *Sequoiadendron gigentum* (Big Tree) and *S. sempervirens* (Californian Redwood) respectively. The family is now largely confined to eastern Asia and north America, but was once much more widely distributed as shown by fossil specimens, one of which (*Metasequoia glyptostrobus*) was found in recent times still growing in China. There are ten genera in the family Taxodiaceae, and the distribution is shown in Table 2.

A. selaginoides D. Don, a native tree known locally as King Billy Pine, is one of the three species of the genus *Athrotaxis*, the other two species being *A. cupressoides* D. Don, whose common name is Pencil Pine, and *A. laxifolia* Hook. The latter species, which occurs as isolated trees, seems intermediate in many characters between *A. selaginoides* and *A. cupressoides*. A hybrid origin for *A. laxifolia* has been postulated, but cytological studies showed no evidence for this[1].

In the course of a survey of Tasmanian plants[3,13] for alkaloids, a moderately strong positive test was observed for *A. selaginoides* and *A. cupressoides*, but *A. laxifolia* gave negative alkaloid tests. Alkaloid occurrences in this family had been recorded in a couple of species (see Table II)[2] but no alkaloids had previously been isolated. Several alkaloids have been isolated from other related families of the conifers, such as Cephalotaxaceae, Taxaceae, and Pinaceae.

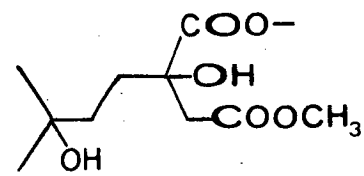
Cephalotaxus and homo*erythrina* alkaloids have been isolated from the Cephalotaxaceae, especially from the genus *Cephalotaxus*[4-8]; the isolation of homo*erythrina* alkaloids from members of this genus has been briefly reviewed in Chapter I. The more important alkaloids[8] are

cephalotaxine (Ia) and harringtonine (Ib), isolated from the plant *C. harringtonia* var. *drupaceae*. Harringtonine has shown significant inhibitory activity against experimental leukemia in mice.

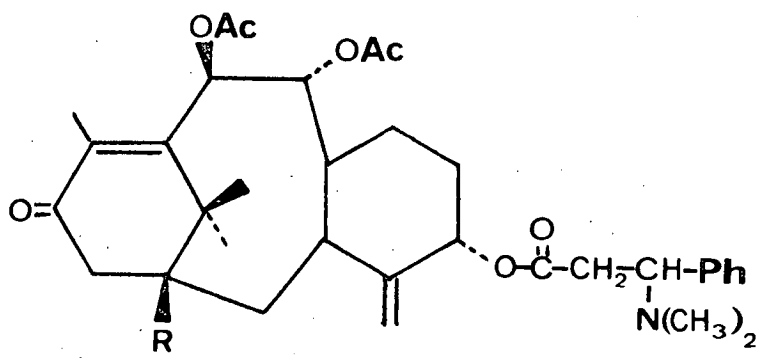


Cephalotaxine (Ia) R = H

Harringtonine (Ib) R =



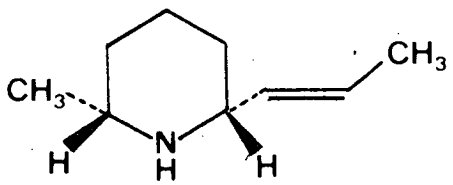
The genus *Taxus* belongs to the family Taxaceae; the *Taxus* species comprise trees, known commonly as the yews, which contain the alkaloid taxine[9], the compound responsible for their toxic properties. Later work[9] showed that taxine is actually a mixture of two compounds, taxine I (IIa) and taxine II (IIb).



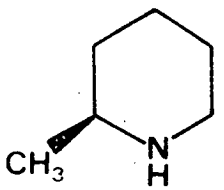
Taxine I (IIa) R = OH

Taxine II (IIb) R = H

Two simple alkaloids have also been isolated from the Pinaceae[11]: pinidine (III) and α-pipecoline (IV), from the tree *Pinus sabiniana* Dougl, and related species. The stereochemistry of pinidine (III) was determined by Hill *et al.*[10].

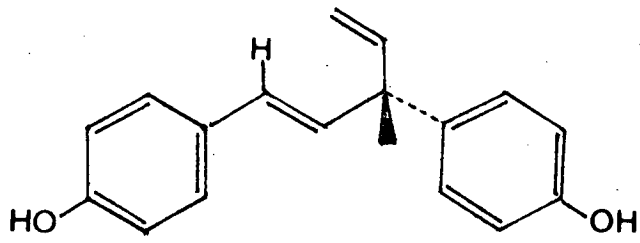


Pinidine (III)

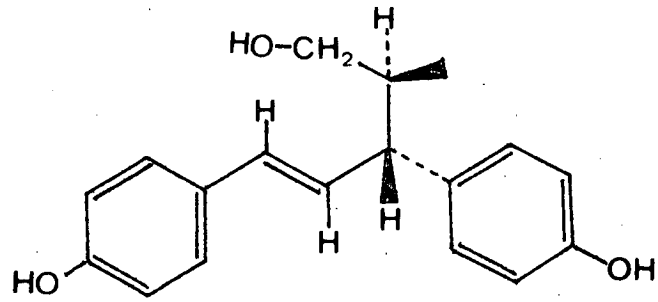


α-Pipecoline (IV)

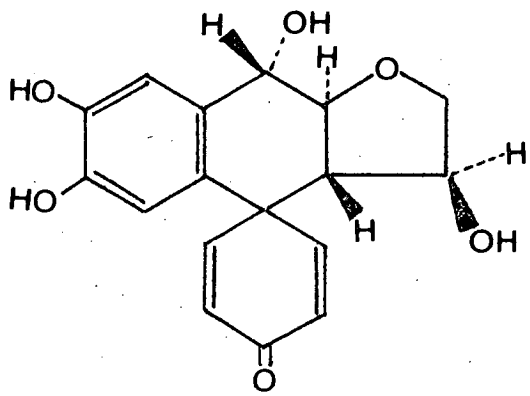
Some chemical work has been done on the heartwood of *A. selaginoides*. The compounds hinokiresinol (Va), agatharesinol (Vb) and a novel C_{17}-phenol, athrotaxin (Vc), were isolated by Daniels *et al.*[12]. Another compound, sugerisinol (Vd), was obtained but it appeared to be an artifact, since none could be detected in small scale extracts.



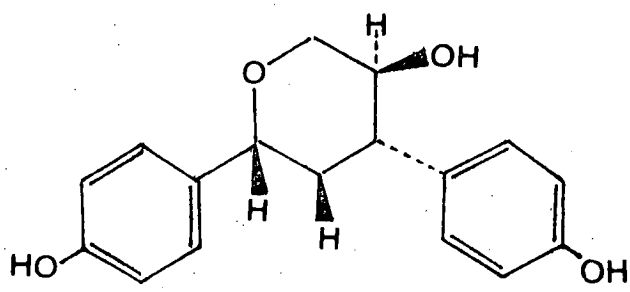
(Va)



(Vb)



(Vc)



(Vd)

TABLE 1 - Alkaloid Survey of the Family Taxodiaceae

BOTANICAL NAME	MONTH, PLACE OF COLLECTION	PART(S)	RESULT	REFERENCE
Athrotaxis selaginoides D. Don	Apr; Private Garden, Hobart	Lf	+	1
(King Billy Pine)	May; Zeehan	Lf,Bk	+	1
Athrotaxis cupressoides D. Don	Feb; Mt. Rufus	Lf	+	1
Athrotaxis laxifolia Hook	Dec; Mt. Read	Lf,Bk	-	1
Cunninghamia lanceolata Hook		Sd	+	3
Taxodium distichum Rich	Oct; U.S.A.	Rw,Rb	+	25
	Sep; U.S.A.	Sb,Fl,Lf,Tw	-	26, 27
Taxodium mucranatum Ten	Sep; Mexico	Lf,St,Fl	-	28
	Feb; Botanical Garden, Hobart	Lf,St,Bk	-	1
Sequoia semperviren Endl	Jun, U.S.A.	Sb,St,Lf,Fl	-	26
Sequoiadendron gigentum Buch	Nov; U.S.A.	Sd	-	28
Metasequoia glyptostrobus Hu & Chang	Jun; U.S.A.	St,Lf	-	26
Cryptomeria japonica D. Don	Apr; New Zealand	St,Lf,Fl	-	25

Lf - Leaf St - Stem Fl - Flower Rb - Root-bark Tw - Twig Sd - Seed

Rw - Root-wood Bk - Bark Sb - Stem-bark

TABLE 2 - Distribution of the Family Taxodiaceae

Genus	Hemispheric Distribution	Place	No. of Species
Sequoia	Northern	Southern Oregon, Northern California	1
Sequoiadendron	Northern	California - very restricted	1
Metasequoia	Northern	Central China - restricted	1
Cunninghamia	Northern	Southeastern China	2
Glyptostrobus	Northern	Southeastern China	1
Cryptomeria	Northern	China, Japan	1
Taiwania	Northern	Formosa	1
Sciadopitys	Northern	Japan	1
Taxodium	Northern	Southern U.S.A., Mexico	3
Athrotaxis	Southern	Tasmania	3

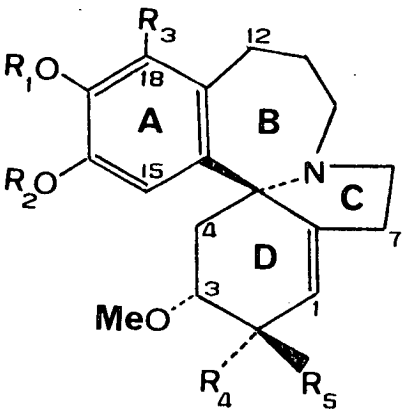
2. Results and discussion

2.1 Alkaloid content of *Athrotaxis cupressoides*.

A. cupressoides Don (Pencil Pine) is a tree that grows above 1000 m in central and western Tasmania. The plant material, twigs and leaves, was collected around the upper reach of the Ouse river near the Great Lake, Tasmania, in May, 1980. The extraction of the fresh plant material was carried out by standard means and had to be completed as soon as possible to avoid a marked diminution in yield of alkaloids: they become undetectable in the plant material after a few days. The crude alkaloids were separated by preparative thin-layer chromatography (ptlc) supplemented by high-performance liquid chromatography (hplc) in cases when the mixture could not be separated by ptlc; a total of eleven alkaloids were isolated.

The alkaloids proved to have structures of the homo*erythrina* type, and include five known bases: taxodine (1), which occurs in *Schelhammera* spp. as alkaloid B[15] and in *Cephalotaxus harringtonia* as alkaloid V[6]; 3-epischelhammericine (2), also a constituent of the two latter plants in which it is designated alkaloid E[15] and alkaloid IV[6] respectively, and of three further plants, *C. wilsoniana*[5], *Dysloxylum lenticellare*[21], and *Phelline comosa*, in which it occurs as alkaloid 4[16]; 0-methylathrocupressine (3), present in *P. comosa* as alkaloid 3[16]; 2-epihomoerythratine (4), found in *S. pedunculata* as alkaloid H[15] and in *P. comosa* as alkaloid 3[16], and finally homoerythratine (5), first isolated from *P. brachyphylla*[17]. The identity of these *Athrotaxis* bases has been confirmed by the agreement of their physical data with reported values, and by a direct comparison with authentic specimens where these were available.

Of the remaining alkaloids, the spectra of 2-hydroxytaxodine (6) showed that it has a phenolic group and two methoxyls which, as in the



1 Taxodine (R_1 = Me, R_2=R_3=R_4=R_5=H)

2 3-Epischelhammericine (R_1-R_2=CH_2, R_3=R_4=R_5=H)

3 O-Methylathrocupressine (R_1=R_2=Me, R_3=OMe, R_4=R_5=H)

4 2-Epihomoerythratine (R_1-R_2=CH_2, R_3=R_5=H, R_4=OH)

5 Homoerythratine (R_1-R_2=CH_2, R_3=R_4=H, R_5=OH)

6 2-Hydroxytaxodine (R_1=Me, R_2=R_3=R_4=H, R_5=OH)

7 2-Hydroxyisotaxodine (R_1=R_3=R_4=H, R_2=Me, R_5=OH)

8 2-Epihydroxyisotaxodine (R_1=R_3=R_5=H, R_2=Me, R_4=OH)

9 Athrocupressine (R_1=R_2=Me, R_3=OH, R_4=R_5=H)

10 (R_1=R_3=R_4=R_5=H, R_2=Me).

11 2-Acetoxytaxodine (R_1=R_3=R_4=H, R_2=Me, R_5=OAc)

12 2-Acetoxyisotaxodine (R_1=R_3=R_4=H, R_2=Me, R_5=OAc)

13 Schelhammerine (R_1-R_2=CH_2, R_3=R_5=H, R_4=OH; MeO▶at C-3)

case of taxodine (1), must be attached to aromatic and aliphatic carbons respectively from their ^1H nmr proton resonances. However, 2-hydroxytaxodine has an extra allylic alcohol group that can readily be removed[16] by conversion to the corresponding chloride followed by LAH reduction. In the process a multiplet signal around δ 4.3, corresponding in chemical shift to a methine proton in the carbinol group of an allylic alcohol, is eliminated from the ^1H nmr spectrum of the product, which proved to be identical with taxodine (1): the alcohol group in 2-hydroxytaxodine must thus be located at C7 or C2. A decision in favour of the latter alternative can be made from mass spectrometry: the ms of 2-hydroxytaxodine (6) corresponds very closely with those of homoerythratine (5)[17], 2-epihomoerythratine (4)[15,16], and schelhammerine (13)[8], all of which have similar structures to (6) with an allylic group at C2 and a methoxyl at C3. On the other hand, there are significant differences in fragmentation pattern as compared to the ms of analogous bases where the alcohol group is attached to C7 instead of C2[16]; moreover, the C1 olefinic protons in the ^1H nmr spectra of the latter bases resonate at δ 5.92 as compared to δ 5.55 for 2-hydroxytaxodine (6). At the same time, this chemical shift for the olefinic proton in (6) gives evidence for the configuration of the hydroxyl group attached to the adjacent C2 position: the corresponding values for the known alkaloids homoerythratine (5) and 2-epihomoerythratine (4), which differ only in the orientation of their C2 hydroxyls, are δ 5.52 and 5.78 respectively. On this basis, the configuration shown in (6) is proposed for 2-hydroxytaxodine with the alcohol group *trans* to the C3 methoxyl as in homoerythratine (5), and this stereochemistry is supported by a comparison of the specific rotation of 2-hydroxytaxodine (6, $+51.5^{\circ}$) with those of homoerythratine (5, $+63.6^{\circ}$) and 2-epihomoerythratine (4, $+171^{\circ}$) measured under comparable conditions.

2-Hydroxyisotaxodine (7) is isomeric with 2-hydroxytaxodine (6), and a spectroscopic comparison of the two alkaloids indicated that they have the same functional groups and closely related structures and configurations. Their ms are almost identical, and furthermore, the C3 methoxyl protons resonate at δ 3.28 in the ^{1}H nmr spectra of both bases, and also in that of the known alkaloid 2-epihomoerythratine (4)[15]. With the epimeric configuration of the methoxyl, a value around δ 2.75 would be expected from analogy with schelhammerine (13)[15], the C3 diastereomer of (4). On Barton oxidation[18], 2-hydroxyisotaxodine (7) gave a conjugated ketone whose ^{1}H nmr spectrum shows a peak at δ 6.00 corresponding to an olefinic proton α to the carbonyl group; a β-proton would be expected to resonate distinctly further downfield[20]. The conjugated ketone was closely similar to, although not identical with that formed by a corresponding oxidation of 2-hydroxytaxodine (6). The alcohol group in (7) must thus be located at C2, and furthermore it must have the same configuration as that in 2-hydroxytaxodine (6) from the optical rotation (+60.7°) of 2-hydroxyisotaxodine (7), and from the chemical shift (δ 5.575) of the olefinic proton in its ^{1}H nmr spectrum. When the allylic hydroxyl was removed from (7), the product (10) proved to be isomeric with taxodine (1) and closely similar to it spectroscopically. The mass spectra of (1) and (10) are virtually indistinguishable; there are, however, small but significant differences in their other spectra, in particular to the chemical shifts of the aromatic singlets in the ^{1}H nmr spectra, and the mixed melting point showed that the two bases are not identical. They evidently differ only in the pattern of substitution in their aromatic rings, and a corresponding difference must also exist between the alkaloids 2-hydroxytaxodine (6) and 2-hydroxyisotaxodine (7). When the C3-H (ca. δ 3.4) and the C12-H protons (ca. δ 2.2) of (7) were separately irradiated, the ^{1}H nmr spectrum revealed distinct nOe effects on the two aromatic singlets

(δ 6.725 and 6.575), which must thus be located at C15 and C18 respectively. The phenolic and methoxyl groups of 2-hydroxyisotaxodine are in consequence attached at C17 and C16, in the reverse arrangement to that of 2-hydroxytaxodine (6), and the structure and stereochemistry of 2-hydroxyisotaxodine is represented by (7).

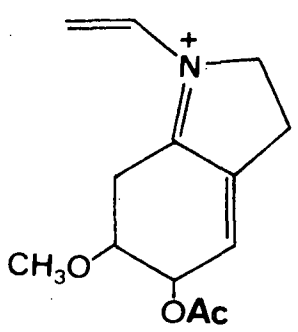
2-Epihydroxyisotaxodine (8) could not be completely separated from 2-hydroxytaxodine (6). It proved to be isomeric but not identical with (6) and 2-hydroxyisotaxodine (7), and on Barton oxidation it gave a conjugated ketone corresponding to that from (7), but different to the ketone from (6). 2-Epihydroxyisotaxodine (8) and 2-hydroxyisotaxodine (7) presumably differ only in the configuration of their allylic alcohol groups, and this difference is borne out by the chemical shifts of their C1 olefinic protons: δ 5.75 and δ 5.575 respectively (cf. 6, 5, and 4 above).

Athrocupressine (9) is a lower homologue of the known alkaloid O-methylathrocupressine (3), to which it shows a general similarity in spectra. However, it has one less methoxyl, but it has instead a phenolic group from the bathochromic shift in its uv spectrum on addition of alkali, and from its positive Gibbs reaction[19]. The latter observation indicates that the phenolic has a free *para* position and is thus attached to C15 or to C18. The second alternative is evidently the correct one, since irradiation of the only aromatic proton in the ^{1}H nmr spectrum of (9) produced an nOe effect on a methine proton resonating around δ 3.3, which must be attached to the aliphatic carbon (C3) bearing the methoxyl group. The protons of the latter group have the same chemical shift (δ 3.28) as those of O-methylathrocupressine (3), and the stereochemistry at C3 for athrocupressine (9) must thus be the same as in (3), since the epimeric configuration of the C3 methoxyl would result in a value around δ 2.75 (cf. 13,8). The specific rotation and the remaining spectroscopic data for athrocupressine are

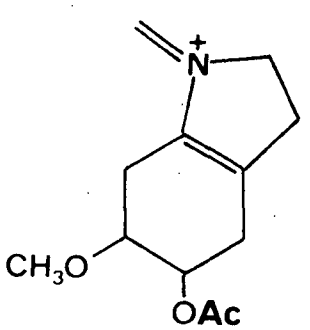
in full accord with the structure and stereochemistry shown in (9).

The alkaloids 2-acetoxytaxodine (11) and 2-acetoxyisotaxodine (12) were obtained in very small amounts only, and could not be satisfactorily separated from one another. Their spectra show a close resemblance to those of 2-hydroxytaxodine (6) and 2-hydroxyisotaxodine (7) except that additional absorptions due to acetate ester groups are present: in particular, a peak at 1730 cm^{-1} and a singlet at 2.11 in their ir and ^1H nmr spectra respectively showed that (11) and (12) are esters of alcohols rather than of phenols. Intense ions in their ms which may be formulated as (14) and (15)[15] indicated that the acetoxy groups are attached at C2. On basic hydrolysis, the alkaloids gave products corresponding to 2-hydroxytaxodine (6) and 2-hydroxyisotaxodine (7), and their structures may thus be represented by (11) and (12) respectively.

Since acetic acid was used in the course of the extraction and purification of the alkaloids, 2-acetoxytaxodine (11) and 2-acetoxyisotaxodine (12) could conceivably be artifacts formed by acylation of (6) and (7) respectively. To test this possibility, a rapid small scale extraction of the fresh plant material was carried out and a crude alkaloid fraction was isolated without the use of acetic acid. The presence of (11) and (12) therein was established by the ms technique of multiple metastable peak monitoring[24].



(14) m/z 236

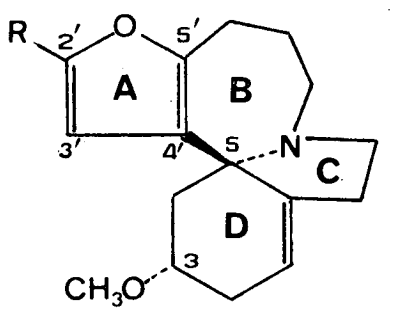


(15) m/z 223

2.2 Alkaloid content of *Athroxis selaginoides*.

A. Selaginoides Don is endemic in Tasmania, where it grows in high rainfall and mountainous country of the centre and west; some specimens attain 40 m in height and are over 1000 years old. The tree is well known in Tasmania under the name of King Billy Pine for its valuable soft-wood timber[1,3,13].

As in the case of *A. cupressoides*, the fresh plant material must be extracted promptly and the alkaloids separated as rapidly as possible to avoid loss. Eight alkaloids were isolated by standard procedures, of which the following homo*erythrina* bases had been found to occur in *A. cupressoides:* taxodine (1)[15,6], 3-epischelhammericine (2)[15,6], homoerythratine (5)[17], 2-hydroxytaxodine (6), 2-hydroxyisotaxodine (7), 2-epihydroxyisotaxodine (8), and athrocupressine (9). The first three have also been reported previously from various other plants.



Selaginoidine (16) R = $-\overset{O}{\overset{\|}{C}}-OMe$

(17) R = $-CH_2-OH$

The spectroscopic data of the remaining base, which was named selaginoidine (16), suggested a close relationship to the homo*erythrina* group: its nmr spectra showed signals corresponding in detail to those

of carbons and hydrogens associated with rings B, C, and D of the homo-*erythrina* bases, and its mass spectrum included peaks derived from fragments of these rings. In particular, a methoxyl proton peak appeared in the ^1H nmr spectrum at δ 3.28, almost identical with the values for taxodine (1) and 3-epischelhammericine (2) which have methoxyl groups attached at C3 *trans* to the 5-14 bond[15]; on the other hand, the C3 methoxyl peak in the spectrum of schelhammericine (13), which has the *cis* arrangement of the methoxy group, appears at δ 2.74[15].

Apart from these similarities to the usual type of homo*erythrina* alkaloid, selaginoidine (16) also shows some differences: its uv spectrum suggested that it has a furan nucleus, and its ir and nmr spectra gave evidence of a methyl ester group, whose presence was confirmed by intense M-58 and M-59 peaks in its mass spectrum, and by its reduction with LAH to a primary alcohol (17) with one less carbon atom. With the exception of the ester carbonyl and the olefinic group of ring D, selaginoidine has only four sp^2 carbons as shown by its ^{13}C nmr spectrum, in comparison to the six aromatic carbons of the homo*erythrina* alkaloids. The four signals, from three quaternary and one methine carbon, correspond in chemical shift to those of a trisubstituted furan nucleus, which is evidently fused on to ring B in place of the usual aromatic ring A of the homo*erythrina* alkaloids. The methine carbon resonates at δ 122.2, and must occupy a β position in the trisubstituted ring[22]. This assignment is supported by the chemical shift of the proton attached to it: the value δ 6.82 in the ^1H nmr spectrum of (16) is in harmony with that expected for C3'-H in a furan bearing a carbomethoxy group at C2' and fused to a saturated ring at C4' and C5'[23]. The signal at δ 6.82 is the only one in the aromatic region, and it undergoes a pronounced nOe effect on irradiation of a broad multiplet around δ 3.5, corresponding to the C3-H of ring D. These two methine

protons must thus be close to one another in space; structure (16) which follows from these observations is in full accord with all the spectroscopic data for selaginoidine. Its specific rotation as compared to those of taxodine (1) and 3-epischelhammericine (2) suggests that (16) may also represent the absolute configuration of selaginoidine.

From a previous investigation of the alkaloids of *A. selaginoides*[13], a mixture of 2-acetoxytaxodine (11) and 2-acetoxyisotaxodine (12) was isolated, but the plant material used in this study was collected at a different period of the year. It is possible that these compounds are produced by the plant at a certain time of year only; on the other hand they could possibly be artifacts, since acetic acid was used in the course of the extraction and purification of the alkaloids. To test this possibility, a small amount of fresh plant material was collected at the same period, in March, as the original study, and the extraction was carried out in the same fashion as for *A. cupressoides* using dilute sulfuric acid only. The presence of (11) and (12) therein was established by the same ms technique of multiple metastable peak monitoring[24] as that used for *A. cupressoides*; from these observations it would seem that these compounds are produced at a certain period of the year only.

2.3 Alkaloid content of *Athrotaxis laxifolia*.

A. laxifolia Hook is a rare species endemic in Tasmania which is intermediate taxonomically between *A. cupressoides* and *A. selaginoides*; it is found associated with these species as isolated trees, and a hybrid origin for it has been postulated, but this has not been substantiated[1]. From the small sample of plant material available, the following alkaloids were isolated: taxodine (1), 3-epischelhammericine (2), homoerythratine (5), 2-hydroxytaxodine (6), 2-hydroxyisotaxodine (7), 2-epihydroxyisotaxodine (8), and selaginoidine (16). The alkaloid content is thus the same as that of *A. selaginoides*, except that no athrocupressine (9) was found in *A. laxifolia*.

3. Experimental

3.1 *A. cupressoides*

Extraction - Twigs and leaves of Pencil Pine were collected around the upper reaches of the Ouse river near Great Lake, Tasmania, in May, 1980, and were immersed as soon as possible after collection in 150 liters of methanol. After three days, the plant material was removed, air dried for one day, then put through a compost shredder. The dry plant material (100 kg) was then percolated with methanol until a test sample gave a negative reaction with Mayer's reagent. The combined extracts were concentrated under reduced pressure at a temperature below 40° to a thick gummy dark brown syrup, which was dissolved in 10 liters of warm glacial acetic acid. The solution was poured in a fine stream into 50 liters of water, which was subjected simultaneously to vigorous agitation with a vibromixer. The dilute acid extract was left to stand overnight, and the precipitate that settled out was filtered off, washed with water until free from alkaloids, then discarded. The washings combined with the acid aqueous solution were evaporated to dryness under reduced pressure at a temperature below 35°. The residue was dissolved in 10 liters of water and again evaporated to dryness: the process of dilution and evaporation was repeated once more to get rid of most of the acetic acid. Finally the residue was dissolved in 10 liters of water and the solution was basified to pH 8-9 with ammonia (d 0.88). The heavy precipitate that formed was left overnight to settle, then filtered off through Hi-Flo Supercel. The dried precipitate was extracted with chloroform until the residue gave a negative Mayer's test, and the filtrate was likewise extracted with chloroform. The combined chloroform solutions were extracted with 5% (w/v) sulfuric acid (30x150 ml) until free from alkaloids. The aqueous acid solution was basified with ammonia (d 0.88) and again thoroughly extracted with chloroform

Voucher specimens of the plant material have been deposited in the collection of dried plants specimens in the Chemistry Dept., University of Tasmania.

(20x150 ml). The combined chloroform extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give 4 g of crude alkaloids.

Isolation, purification, and characterisation of the alkaloids - The crude alkaloid mixture (2.00 g) was separated by ptlc (7% MeOH/$CHCl_3$) into 7 fractions:

Fraction 1 amounted to 0.12 g, and contained two components from tlc (30% EtOAc/C_6H_6). The mixture was separated by triple development ptlc with the same solvent system. The higher *Rf* component (16 mg) proved to be the known homo*erythrina* alkaloid 3-epischelhammericine (2); physical data: $[\alpha]_D^{19}$+106 (C=1.6); λ max: 284 (3800), 219 nm(3900); γ max: 3000, 2920, 2840, 1460, 1210, 1120 cm^{-1}; ^1H nmr: 6.7(s, 1H), 6.6(s, 1H), 5.88(s, 2H), 5.5(bs, 1H), 3.21(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 313 (M^+, 30), 282(35), 255(70), 254(70), 207(20), 178(100), 165(3), 146(32); picrate derivative mp 169-172°, undepressed on admixture with an authentic sample.

The lower *Rf* component (19 mg) was obtained as white needles from hexane, identical with the known homo*erythrina* alkaloid O-methylthrocupressine (3); mp and mixed mp with an authentic sample: 100-101°; $[\alpha]_D^{19}$+91 (C=1.5); λ max: 228 (1160), 275 nm(260); γ max: 3010, 2920, 2850, 1600, 1540, 1450, 1310, 1220 cm^{-1}; ^1H nmr: 6.57(s, 1H), 5.58(bs, 1H), 3.82(s, 6H), 3.28(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z 359(M^+, 22); meas.: 359.2085, calc. for $C_{21}H_{29}NO_4$: 359.2298; 328(35), 301(4), 286(15), 254(10), 178(100), 165(30), 146(25).

Fraction 2 (0.199 g) also contained two components from tlc (5% MeOH/$CHCl_3$); the mixture was separated by double development ptlc with the same solvent system. The component of lower *Rf* (51.7 mg) proved to be a new homo*erythrina* alkaloid, athrocupressine (9), mp 152-153° (Me_2CO), $[\alpha]_D^{19}$+102.23 (C=0.43), λ max: 227 (4204), 280 nm(940); after addition

of a drop of 5% aqueous sodium hydroxide: 237 and 298 nm; γ max: 3500, 3400, 3000, 2920, 2840, 1600, 1490, 1310, 1120, 750 cm^{-1}; ^1H nmr (270 MHz): 6.39(s, 1H), 5.65(bs, 1H), 3.94(s, 3H), 3.82(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm. When the singlet proton at δ 6.39 was irradiated, the proton signal around δ 3.3 showed a nuclear Overhouser effect; m/z: 345(M^+, 25); meas.: 345.1944, calc. for $C_{20}H_{27}NO_4$: 345.0378; 314(30), 278(45), 286(25), 272(20), 178(100), 165(35), 146(35); found: C 69.19; H 8.33; N 4.05; $C_{20}H_{27}NO_4$ requires: C 69.56; H 7.82; N 4.05%. Athrocupressine gave a positive test with Gibb's reagent.

The component of higher *Rf* (20.7 mg) contained two compounds present in very small amounts, insufficient for satisfactory separation. From spectroscopic and chemical data, the mixture appeared to consist of the two isomeric acetyl derivatives (11) and (12); λ max: 270 and 207 nm (no change on addition dil. NaOH); γ max: 3400, 3000, 2920, 2840, 1730, 1680, 1510, 1240 cm^{-1}; ^1H nmr: 6.80(m, 2H), 5.55(m, 2H), 3.92 (s, 1H), 3.84(s, 3H), 3.28(s, 3H), 2.11(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 373(M^+, 40); meas.: 373.1912, calc. for $C_{21}H_{27}NO_5$: 373.1910; 342(35), 341(20), 330(20), 314(75), 313(70), 297(40), 282(42), 272(72), 261(75), 260(70), 260(70), 236(100), 223(60), 176(25), 162(70), 132(72).

Fraction 3 (0.149 g) contained two components from tlc (8% $MeOH/CHCl_3$), which were separated by ptlc with the same solvent system. The component of higher *Rf* was non-alkaloidal, and that of lower *Rf* (0.6, 6.6 mg) proved identical with taxodine (1); mp 150-152° (Me_2CO); $[\alpha]_D^{19}$+111.0 (C= 0.9); λ max: 209 (4720), 230 (1890), 291 nm(861); after addition of a drop of 5% aqueous sodium hydroxide; 210, 250, 298 nm; γ max: 3380, 3000, 2920, 2840, 2500, 2450, 1210 cm^{-1}; ^1H nmr: 6.78(s, 1H), 6.63(s, 1H), 5.52(bs, 1H), 3.87(s, 3H), 3.22(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 315(M^+, 35), meas.: 315.1833, calc.

for $C_{19}H_{25}NO_3$; 315.1834, 284(40), 257(80), 256(79), 240(15), 178(100), 165(39), 146(35), 137(25).

Fraction 4 (0.165 g) appeared to be a mixture from its ^1H nmr spectrum, but a variety of solvent systems failed to separate it by ptlc. The separation was finally achieved by hplc with acetonitrile/water (18:82) buffered to pH 3.0 (0.1M $NH_4H_2PO_4$ and H_3PO_4) for elution. The mixture of alkaloids (15 mg) was dissolved in 1 ml of the solvent system and injected in 200 microliter aliquots; each run required 32.4 min. The separated fractions were bulked and basified with aqueous ammonia (d, 0.88), then extracted with dichloromethane, evaporation of which yielded the two separated components. The minor component (4.9 mg) was identified as 2-epihomoerythratine (4), mp 184-185° (Me_2CO); $[\alpha]_D^{19}$+170 (C=1.4); λ max: 240 (5500), 290 nm(5000); γ max: 3420, 3000, 2920, 2850, 1500, 1460, 1456, 1260 cm^{-1}; ^1H nmr: 6.60(s, 1H), 6.58(s, 1H), 5.92(s, 2H), 5.78(bs, 1H), 4.38(bs, 1H), 3.32(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 329(M^+, 50); meas.: 320.16407, calc. for $C_{19}H_{23}NO_4$: 329.16778; 298(15), 271(65), 255(50), 194(100), 181(30), 162(32).

The major component of fraction 4 (7.8 mg) proved to be homoerythratine (5), mp 178-179° (Me_2CO); $[\alpha]_D^{19}$+63.61 (C=3.63); λ max: 219 (2757), 288 nm(1316); γ max: 3380, 2920, 2840, 1490, 1470, 1240, 1040, cm^{-1}; ^1H nmr: 6.82(s, 1H), 6.65(s, 1H), 5.93(s, 2H), 5.52(bs, 1H), 4.35(m, 1H), 3.3(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 329(M^+, 50), meas.: 329.16407, calc. for $C_{19}H_{23}NO_4$: 329.16778; 298(18), 271(25), 270(15), 255(25), 254(20), 242(10), 194(100), 181(24), 162(25); found: C 69.38, H 7.46, N 4.21; $C_{19}H_{25}NO_4$ requires: C 69.30, H 6.99, N 4.25.

Fractions 5 and 6 appeared from tlc to contain the same two components in different proportions. The combined fractions (0.157 g) were separated by ptlc (5% MeOH/$CHCl_3$, double development), and the

subfraction of lower Rf (10.7 mg) yielded 2-hydroxytaxodine (6), mp 192-193° (Me_2CO); $[\alpha]_D^{19}$+51.47 (C=1.03); λ max: 212 (4062), 230 (1812), 283 nm(750); after addition of 5% aqueous sodium hydroxide: 218, 250, 298 nm; γ max: 3380, 3000, 2920, 2840, 1560, 1448, 1270, 1200, 1150 cm^{-1}; ^1H nmr: 6.86(s, 1H), 6.65(s, 1H), 5.5(s, 1H), 4.3(m, 1H), 3.85(s, 3H), 3.28(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 331(M^+, 60); meas.: 331.1785, calc. for $C_{19}H_{25}NO_4$: 331.1783; 300(18), 273(35), 272(30), 257(38), 256(30), 194(100), 178(35), 162(30).

The component of higher Rf (80 mg) from fractions 5 and 6 was separated by hplc with a solvent system made up of two solutions, A and B: solution A contained acetonitrile/water (1:9) and a buffer solution (0.02M of $NH_4H_2PO_4/H_3PO_4$, pH 2.5, and triethylamine 0.007M), and solution B consisted of THF/acetonitrile (1:9). Solutions A and B were mixed in the ratio 93.5:6.5. The mixture of alkaloids was dissolved in 5 ml of mixed solvent and injected on to the column (maximum volume per injection 225 microliters). The mixture was separated into three subfractions, which were bulked and basified with aqueous ammonia (d 0.88), then extracted with dichloromethane. The first subfractions (53.6 mg) yielded 2-hydroxyisotaxodine (7) which could not be crystallised; $[\alpha]_D^{19}$+60.69 (C=1.0); λ max: 240 (1900), 283 nm(850); after addition of a drop of 5% aqueous sodium hydroxide: 218, 250, 298 nm; γ max: 3400, 3120, 2950, 2870, 1550, 1520, 1450, 1280, 1226, 1050 cm^{-1}; ^1H nmr (270 MHz): 6.725(s, 1H), 6.575(s, 1H), 5.575(bs, 1H), 4.3(m, 1H), 3.85(s, 3H), 3.28(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; when the multiplet at δ 3.4 was irradiated, the singlet at δ 6.725 showed an nOe effect, and when the multiplet at δ 2.2 was irradiated, the singlet at δ 6.575 showed an nOe effect; m/z: 331 (M^+, 60); meas.: 331.1785, calc. for $C_{19}H_{25}NO_4$: 331.1783; 300(18), 273 (75), 272(3), 257(38), 256(30), 194(100), 178(35), 162(30).

The second subfraction (9.3 mg) consisted of (6) and (8) which could not be further separated. The mass spectrum of the mixture showed differences in intensity only as compared with those of (6) and (7). ^{1}H nmr of (8) (after elimination of the proton signals from (6)): 6.675(s, 1H), 6.55(s, 1H), 5.75(bs, 1H), 4.3(m, 1H), 3.75(s, 3H), 3.25(s, 3H) and an unresolved number of protons between 1.5 and 3.5 ppm.

The third subfraction (5.7 mg) was obtained in too small a quantity for adequate study.

Fraction 7 proved to be devoid of alkaloids.

Hydrolysis of 2-acetoxytaxodine (11) and 2-acetoxyisotaxodine (12) - The mixture of alkaloids (11) and (12) (10 mg) from fraction 2 was dissolved in aqueous methanol (1:1, 10 ml) and dilute sodium hydroxide (1%, 2 ml) was added. The solution was refluxed for 4 hours, then cooled to room temperature and neutralised with dilute sulfuric acid (5% w/v). The neutral solution was exhaustively extracted with chloroform, and the extract on evaporation left a residue (5 mg) whose components gave *Rf* values and ms corresponding to those of authentic specimens of 2-hydroxytaxodine (6) and 2-hydroxyisotaxodine (7) respectively.

Oxidation of 2-epihomoerythratine (4) - A sample of (4) (13 mg, 0.04 m mole) was oxidised with μ-oxo bis(triphenyl bismuth dichloride) (40 mg, 0.04 m mole) and potassium carbonate (50 mg) in chloroform (5 ml) at room temperature overnight. After separation by ptlc (5% $MeOH/CHCl_3$), the ketone derivative of (4) was obtained (7 mg), mp 172-173° (Me_2CO); λ max: 230 (9343), 288 nm(1094); γ max: 2910, 2820, 1680, 1470, 1220, 1110, 1020 cm^{-1}; ^{1}H nmr: 6.77(s, 1H), 6.67(s, 1H), 6.1(s, 1H), 6.0(s, 2H), 3.54(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 327(M^+, 65); meas.: 327.14669, calc. for $C_{19}H_{21}NO_4$:

327.14668; 284(50), 269(17), 241(60), 240(100), 211(20), 192(85), 179(10), 160(38), 149(25).

Dehydroxylation of 2-epihomoerythratine (4) - A sample of (4) (13.5 mg, 0.04 m mole) was dissolved in 5 ml of dry benzene and treated with freshly distilled thionyl chloride (2 ml). The reaction mixture was stirred at room temperature for 2 hours, then evaporated to remove excess of thionyl chloride and solvent. The residue from the reaction mixture was redissolved in benzene/THF (3 ml: 100 ml) and treated with LAH (100 mg). The mixture was refluxed overnight, then excess of LAH was destroyed by adding water. The precipitate was removed by filtration, and washed until free from alkaloid. The filtrate was extracted with chloroform (3x50 ml), and the combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was separated by ptlc (7% MeOH/$CHCl_3$) to give a product (10 mg) identical (ir, uv, ^1H nmr, ms) with (2). The mp of the picrate derivative was not depressed on admixture with the corresponding derivative of (2).

Oxidation of homoerythratine (5) - A sample of (5) (13 mg, 0.04 m mole) was oxidised with μ-oxo-bis(triphenyl bismuth chloride) (40 mg, 0.04 m mole) and potassium carbonate (50 mg) in chloroform (5 ml) at room temperature overnight. After separation by ptlc (5% MeOH/$CHCl_3$), a ketone identical (ir, uv, ^1H nmr, ms) with that formed by a corresponding oxidation of (4) was obtained (8 mg).

Dehydroxylation of homoerythratine (5) - A sample of (5) (50 mg, 0.14 m mole) was converted to the corresponding chloride with thionyl chloride (2 ml) in dry benzene (5 ml) as described above. The crude product was reduced with LAH (200 mg) in 3 ml of dry benzene and 10 ml of THF. The product (25 mg) was identical (ir, uv, ^1H nmr, ms) with (2).

Dehydroxylation of 2-hydroxytaxodine (6) - A sample of (6) (28 mg, 0.084 m mole) was converted to the chloride derivative with thionyl chloride (1.5 ml) in dry benzene (3 ml) as described above. The crude product was reduced with LAH (100 mg) in 3 ml of dry benzene and 10 ml of THF. The product (4.1 mg) was identical (ir, uv, ^{1}H nmr, ms) with taxodine (1).

Dehydroxylation of 2-hydroxyisotaxodine (7) - A sample of (7) (28 mg, 0.084 m mole) was converted to the corresponding chloride with thionyl chloride (1.0 ml) in dry benzene (3 ml) as described above. The crude product was reduced with LAH (100 mg) in 3 ml of dry benzene and 10 ml of THF. The product (6 mg) had mp 142-145°; λ max: 219 (115550), 230 (10920), 283 nm(5460); after addition of a drop of 5% aqueous sodium hydroxide: 220, 253, and 295 nm; γ max: 3400, 2920, 2840, 1855, 1500, 1450, 1270, 1210, 1100, 1080 cm^{-1}; ^{1}H nmr: 6.70(s, 2H), 5.55(bs, 1H), 3.8(s, 3H), 3.25(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 315(M^+, 35); meas.: 315.1833, calc. for $C_{19}H_{25}NO_3$: 315.1834; 284(4), 257(80), 256(79), 240(16), 178(100), 165(39), 146(35), 137(25).

Oxidation of 2-hydroxytaxodine (6) - A sample of (6) (28 mg, 0.084 m mole) was oxidised with μ-oxo-bis(triphenyl bismuth dichloride) (0.12 g, 0.12 m mole) and potassium carbonate (50 mg) in chloroform (5 ml) at room temperature overnight. After separation by ptlc (5% MeOH/$CHCL_3$), the ketone derivative of (6) (15 mg) was obtained as a colorless oil; λ max: 230 (12,502), 283 nm(4,167); after addition of a drop of 1% aqueous sodium hydroxide: 237 and 298 nm; γ max: 3500, 3400, 3010, 2920, 2850, 1670, 1580, 1500, 1450, 1350, 1310, 1270, 1210, 1160, 1120, 1050 cm^{-1}; ^{1}H nmr: 6.77(s, 1H), 6.55(s, 1H), 6.05(s, 1H), 3.725(s, 3H), 3.425(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 329(M^+, 30); meas.: 329.1668, calc. for $C_{19}H_{23}NO_4$: 329.1627;

286(35), 271(45), 243(38), 242(40), 228(35), 212(25), 192(50), 160(30), 85(60), 83(100).

Oxidation of 2-hydroxyisotaxodine (7) - A sample of (7) (28 mg, 0.084 m mole) was oxidised with μ-oxo-bis(triphenyl bismuth dichloride) (129 mg, 0.12 m mole) and potassium carbonate (50 mg) in chloroform (5 ml) at room temperature overnight. After separation by ptlc (5% MeOH/$CHCl_3$), the ketone derivative of (7) was obtained (17 mg) as a colorless oil; λ max: 230 (12,500), 283 nm(4,160); after addition of a drop of 1% sodium hydroxide; 237 and 298 nm; γ max: 3500, 3400, 3010, 2920, 2850, 1670, 1570, 1500, 1450, 1310, 1270, 1210, 1160, 1120, 1050 cm^{-1}; ^1H nmr: 6.675(s, 2H), 6.0(s, 1H), 3.85(s, 3H), 3.425(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 329 (M^+, 70); meas.: 329.1658, calc. for $C_{19}H_{23}NO_4$: 329.1627; 286(50), 271(68), 270(30), 243(25), 242(43), 240(75), 226(20), 192(100), 160 (28), 85(50), 83(80).

Oxidation of 2-hydroxytaxodine (6) and 2-epihydroxyisotaxodine (8) - The mixture of (6) and (8) (3 mg) from subfraction 2 was oxidised with μ-oxo-bis(triphenyl bismuth dichloride) (10 mg) and 10 mg of potassium carbonate in 1 ml of chloroform for 2 hours. Two oxidation products were obtained, which were separated by multiple development ptlc (5% MeOH/$CHCl_3$). The product with the higher *Rf* value corresponded to the ketone obtained by oxidation of (7) when they were chromatographed under the same conditions, and the product of lower *Rf* corresponded to that from (6).

3.2 *A. selaginoides*

Extraction - Twigs and foliage of King Billy Pine were collected near Zeehan, western Tasmania, in August 1980 and were immersed in 400 liters of methanol as soon as possible after collection. After three days the plant material was removed, drained, and put through a compost shredder. The air-dried material (200 kg) was percolated with methanol until a test sample gave a negative reaction with Mayer's reagent. The combined extracts were concentrated under reduced pressure at a temperature below 40° to a thick gummy dark green syrup, which was dissolved in 10 liters of warm glacial acetic acid and 8 liters of water. The solution was poured in a fine stream into 20 liters of water, which was simultaneously agitated vigorously with a mechanical stirrer. The solution was left to stand overnight, then the precipitate that settled out was filtered off, washed with water until free from alkaloids, and discarded. The extract, combined with the washings, was evaporated to dryness *in vacuo* at a temperature below 35°. The residue was dissolved in 20 liters of water and again evaporated to dryness, and the process of dilution with water and evaporation was repeated once more to get rid of the acetic acid. Finally the residue was basified to pH 8-9 with aqueous ammonia (d 0.88), whereby a heavy precipitate was produced, which was allowed to settle overnight, then filtered off through Hi-Flo Supercel. The dried precipitate and the filtrate were separately extracted with chloroform until no further alkaloid was removed, then the combined chloroform solutions were extracted with 5% aqueous sulfuric acid (40x150 ml) until free from alkaloids as shown by a negative Mayer's test. The aqueous acid solution was basified with aqueous ammonia (d 0.88), extracted with chloroform, again the combined chloroform extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give 14 g of crude alkaloids.

Isolation, purification, and characterisation of the alkaloids - The crude alkaloid mixture (5 g) was separated by short column chromatography on 150 g of silica gel into six fractions. Elution was commenced with chloroform, and was continued with mixtures of chloroform containing gradually increasing amounts of methanol up to 10%. The last two fractions gave no test for alkaloids.

Fraction 1 (0.36 g) contained two components from tlc examination (ethyl acetate/light petroleum : 60/40), which were separated by ptlc with the same solvent system. The less polar component (75 mg) was found to be identical (ms, uv, ir, ^{1}H nmr) with the known alkaloid 3-epischelhammericine (2). The more polar constituent (50 mg) proved to be a new alkaloid, selaginoidine (16), mp 62-63°, $[\alpha]_D^{19}$+166.7 (C=0.25); λ max: 219 (7680), 269 nm(7480); γ max: 2940, 2900, 2850, 1730, 1600, 1550, 1510, 1440, 1300 cm^{-1}; ^{1}H nmr: 6.82(s, 1H), 5.51(s, 1H), 3.85(s, 3H), 3.28(s, 3H) and an unresolved number of protons between 1.5 and 3.5 ppm; when the multiplet at δ 3.5 was irradiated, the singlet at δ 6.82 underwent a pronounced nOe effect; m/z: 317(M^+, 10); meas.: 317.16253, calc. for $C_{18}H_{23}NO_4$: 317.16280; 301(10), 286(18), 259(100), 258(90), 244(10), 217(15), 175(16), 165(5), 146(5); ^{13}C nmr: 159.2 (C=0), 156.8(s, C2;), 141.3(s, C5;), 140.6(s, C4;), 127(s, C6), 122.2(d, C3;), 116.2(d, C1), 74.2(d, C3), 62.2(s, C5), 65.9(q, *Me*OC=0), 51.7(q, MeO), 48.2 and 45.7(t, C8 and C10), 40.0, 31.9, and 28.8(t, C2, C7, and C12), 26.8(t, C14), 18.5(t, C11).

Fraction 2 (0.67 g) contained one component only from tlc with several solvent systems. After purification, this alkaloid proved identical (ms, uv, ir, ^{1}H nmr) with athrocupressine (9).

Fraction 3 (0.43 g) also contained only one compound from tlc, which was found to be identical (ms, ir, uv, ^{1}H nmr) with taxodine (1).

Fraction 4 (0.47 g) contained at least three components from tlc, and it was separated by triple development ptlc (5% MeOH/$CHCl_3$) into

three subfractions, of which that with the highest Rf (37 mg) proved identical (ir, uv, ms, ^{1}H nmr) with homoerythratine (5). The subfraction of lowest Rf (62 mg) was found to be identical (ir, uv, ms, ^{1}H nmr) with 2-hydroxytaxodine (6). The material from the subfraction of intermediate Rf was found by hplc to contain three compounds, two of which could, however, not be completely separated from one another. One component proved to be 2-hydroxyisotaxodine (7) from a comparison of its physical data with those of a sample of (7) isolated in a similar fashion from *A. cupressoides*. The mixture of the other two components, which could not be further separated, was found to correspond in its spectroscopic properties with the inseparable mixture of 2-hydroxytaxodine (6) and 2-epihydroxyisotaxodine (8) which was obtained by hplc under the same conditions from *A. cupressoides*.

LAH reduction of selaginoidine (16) - Selaginoidine (20 mg, 0.063 m mole) was refluxed overnight with suspension of LAH (30 mg in 20 ml of THF) in a current of nitrogen. Excess of LAH was destroyed with water, and the solution was filtered and extracted with chloroform (3x30 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo* to a yellowish oil; purification by ptlc (5% MeOH/$CHCl_3$) yielded the alcohol (17) Rf 0.3 (8 mg, 43%) which could not be crystallised; λ max: 209 (4538), 226 (4538), 285 nm(462); γ max: 3380, 3000, 2900, 2850, 1200, cm^{-1}; ^{1}H nmr: 5.87(s, 1H), 5.45(s, 1H), 5.45(s, 1H), 4.47(s, 2H), 3.33(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 289(M^+, 7); meas.: 289.167, calc. for $C_{17}H_{23}NO_3$: 289.160; 258(10), 232(18), 231(100), 230(50), 214(30), 200(18), 178(16).

3.3 *A. laxifolia*

Extraction - Twigs and foliage were collected near Pine Lake, central Tasmania, in September 1983, from two trees of *A. laxifolia* growing in association with a stand of *A. cupressoides*. The material was immersed as soon as possible after collection in 4 liters of methanol, and after a week it was removed from the solvent, drained, and milled. The dried plant material (1 kg) was percolated with methanol until a test sample gave a negative reaction with Mayer's reagent. The combined extracts were concentrated *in vacuo* at a temperature below 40° to a thick gummy dark green syrup, which was dissolved in 500 ml of glacial acetic acid and 1 liter of water. The solution was left to stand overnight, and the precipitate that settled out was filtered off, washed with water until free from alkaloids, then discarded. The aqueous solution and washings were evaporated to dryness under reduced pressure at a temperature below 35°. The residue was dissolved in 1 liter of water, and the solution was again evaporated to dryness; finally the residue was redissolved in 1 liter of water, and the solution was basified to pH 8-9 with aqueous ammonia (d 0.88). The heavy precipitate that formed was left to settle overnight, then filtered off through Hi-Flo Supercel. The dried precipitate and the solution were separately extracted with chloroform until they gave a negative Mayer's test, and the combined chloroform solutions were extracted with aqueous sulfuric acid (5% w/v, 20x100 ml) until free from alkaloids. The aqueous acid solution was basified with ammonia and again thoroughly extracted with chloroform (30x50 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give 254 mg of crude alkaloids.

Isolation, purification, and characterisation of alkaloids - The crude mixture of alkaloids (254 mg) was separated by ptlc ($CHCl_3$, 7% MeOH)

into six fractions.

Fraction 1 (11.5 mg) contained two bases from tlc (C_6H_6, 30% EtOAc). The mixture was separated by double development ptlc with the same solvent system. The higher *Rf* component was obtained as a yellowish oil, and proved identical with 3-epischelhammericine (2) (ir, uv, ms, ^{1}H nmr). The component of lower *Rf* (4.0 mg), was obtained as a yellowish oil, which was found to be identical (ir, uv, ms, ^{1}H nmr) with selginoidine (16).

Fraction 2 (19 mg) contained two components from tlc ($CHCl_3$, 5% MeOH), which were separated by double development ptlc with the same solvent system. The higher *Rf* component (7.0 mg) proved to be non-alkaloidal. The component of lower *Rf* (8.0 mg) crystallised as colorless needles, mp 150-152^o (Me_2CO), identical (ir, uv, ^{1}H nmr, ms, mixed mp) with taxodine (1).

Fraction 3 (27.5 mg) contained two components from tlc ($CHCl_3$, 5% MeOH), and the same solvent system was used to separate them by double development ptlc. The lower *Rf* component (13.0 mg) was obtained as white needles, mp 178-179^o (Me_2CO), identical (ir, uv, ms, ^{1}H nmr, mixed mp) with homoerythratine (5). The component of higher *Rf* (9.0 mg) proved to be non-alkaloidal.

Fraction 4 (14.0 mg) contained one component only as shown by tlc ($CHCl_3$, 5% MeOH), and was separated into fractions by ptlc using the same solvent system. The component of lower *Rf* (7.0 mg) was obtained as a colorless oil and was found to be identical (ir, uv, ms, ^{1}H nmr) with 2-hydroxy-taxodine (6). The higher *Rf* material (10 mg) could not be further separated by ptlc, but its ^{1}H nmr spectrum showed it to consist predominantly of a mixture of 2-hydroxyisotaxodine (7) and 2-epihydroxyisotaxodine (8) by comparison with the spectra of the corresponding fractions from *A. selaginoides* and *A. cupressoides*.

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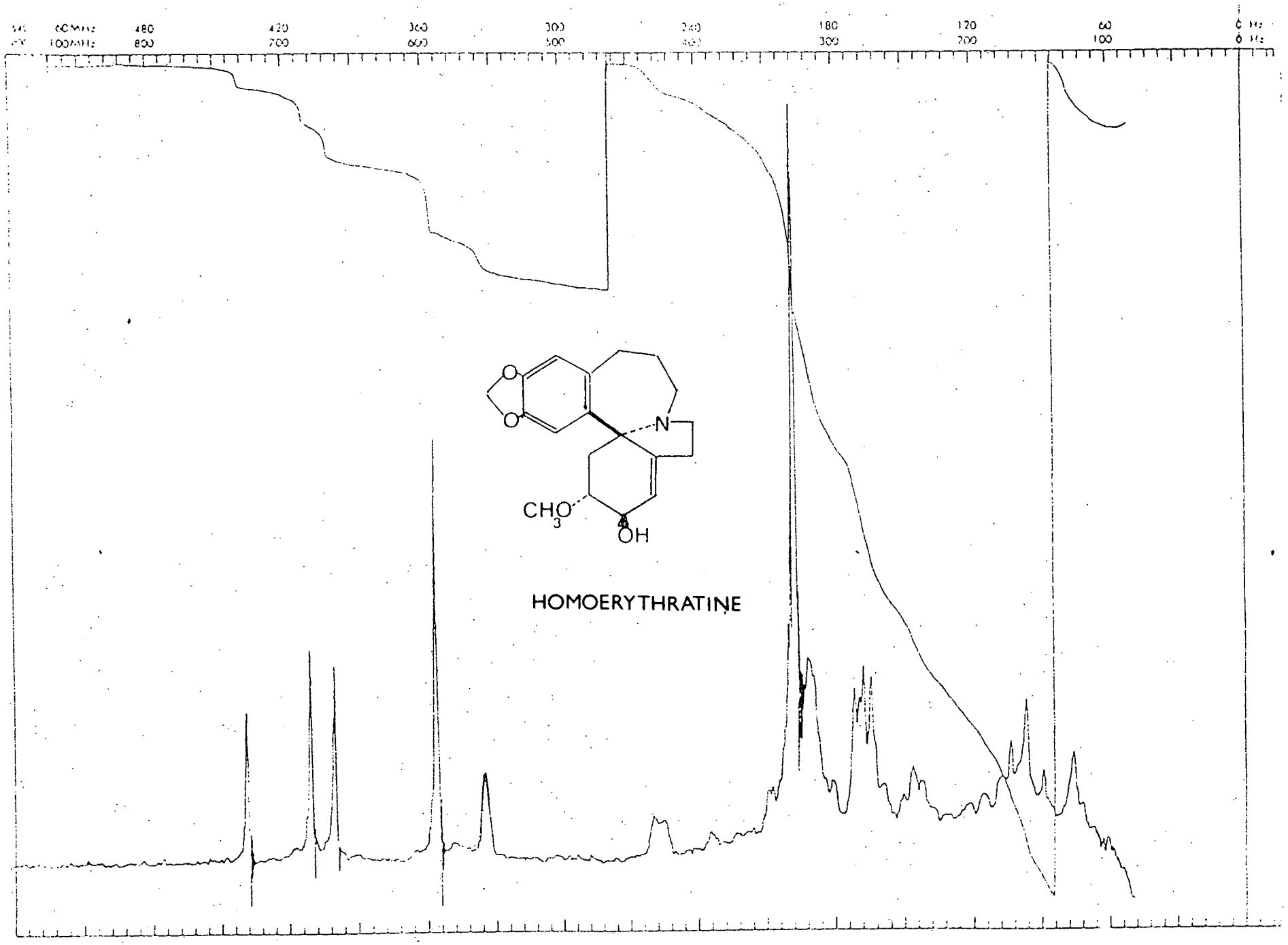
5. Spectrum data of new homoerythrina alkaloids



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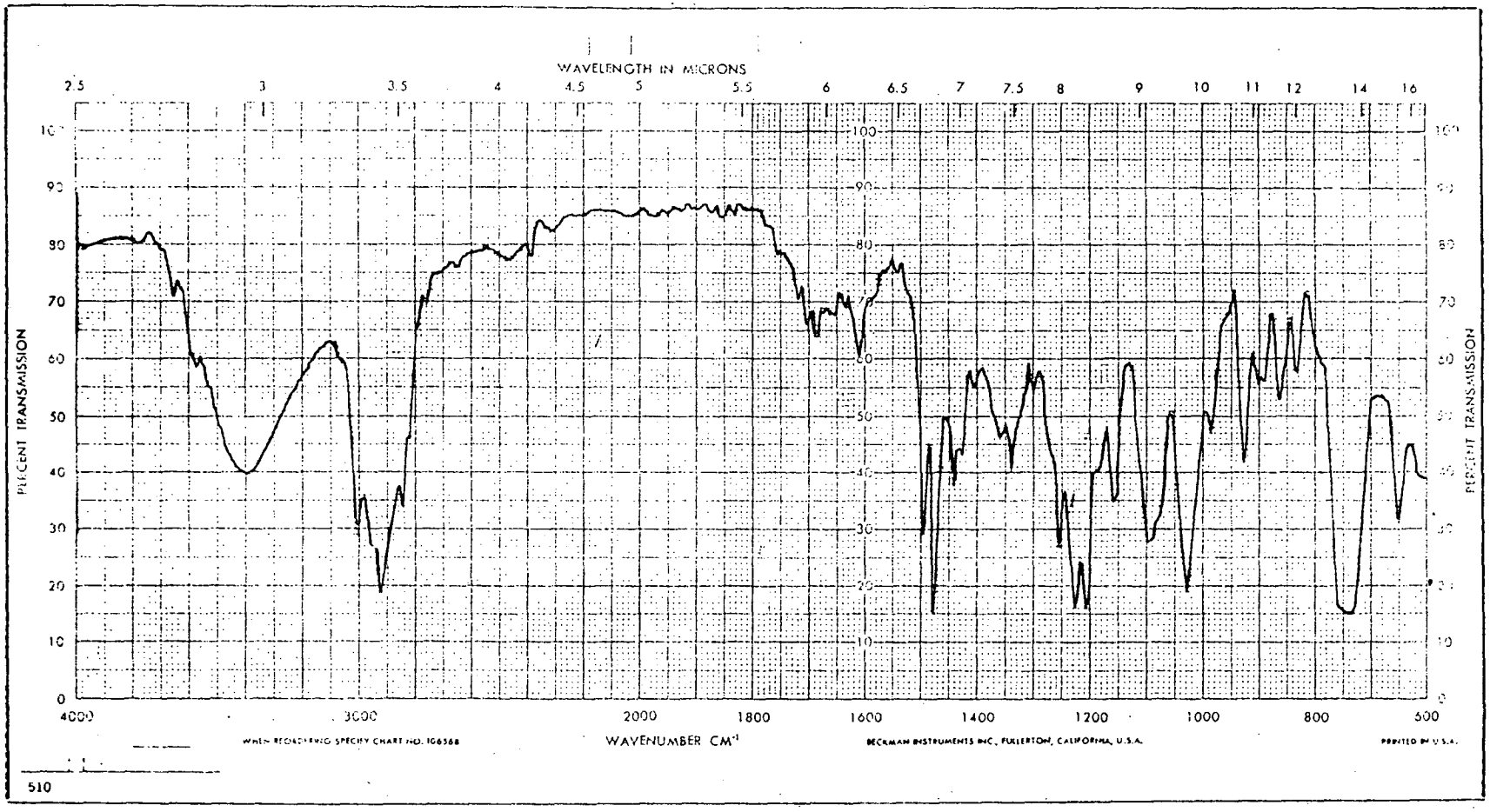

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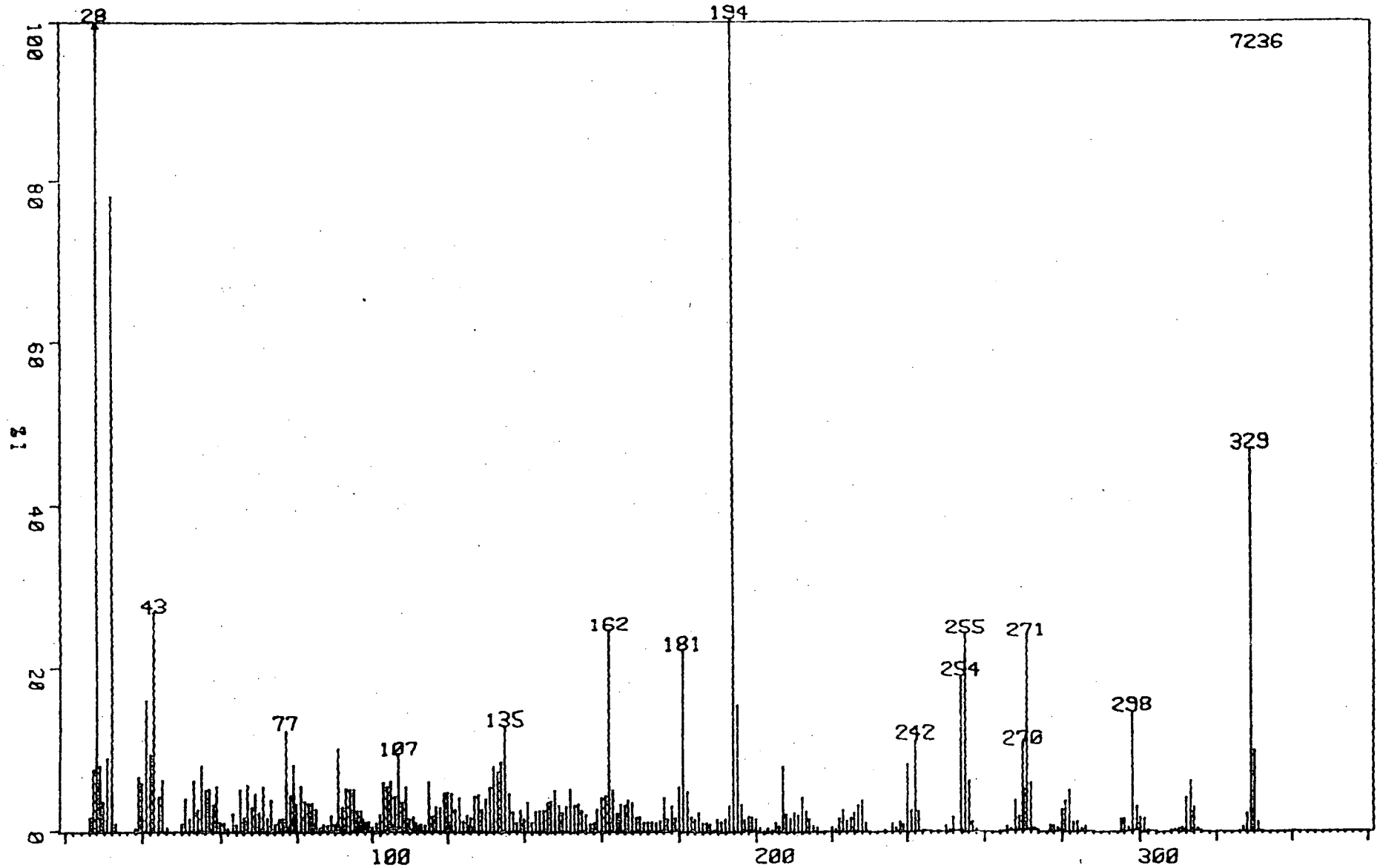

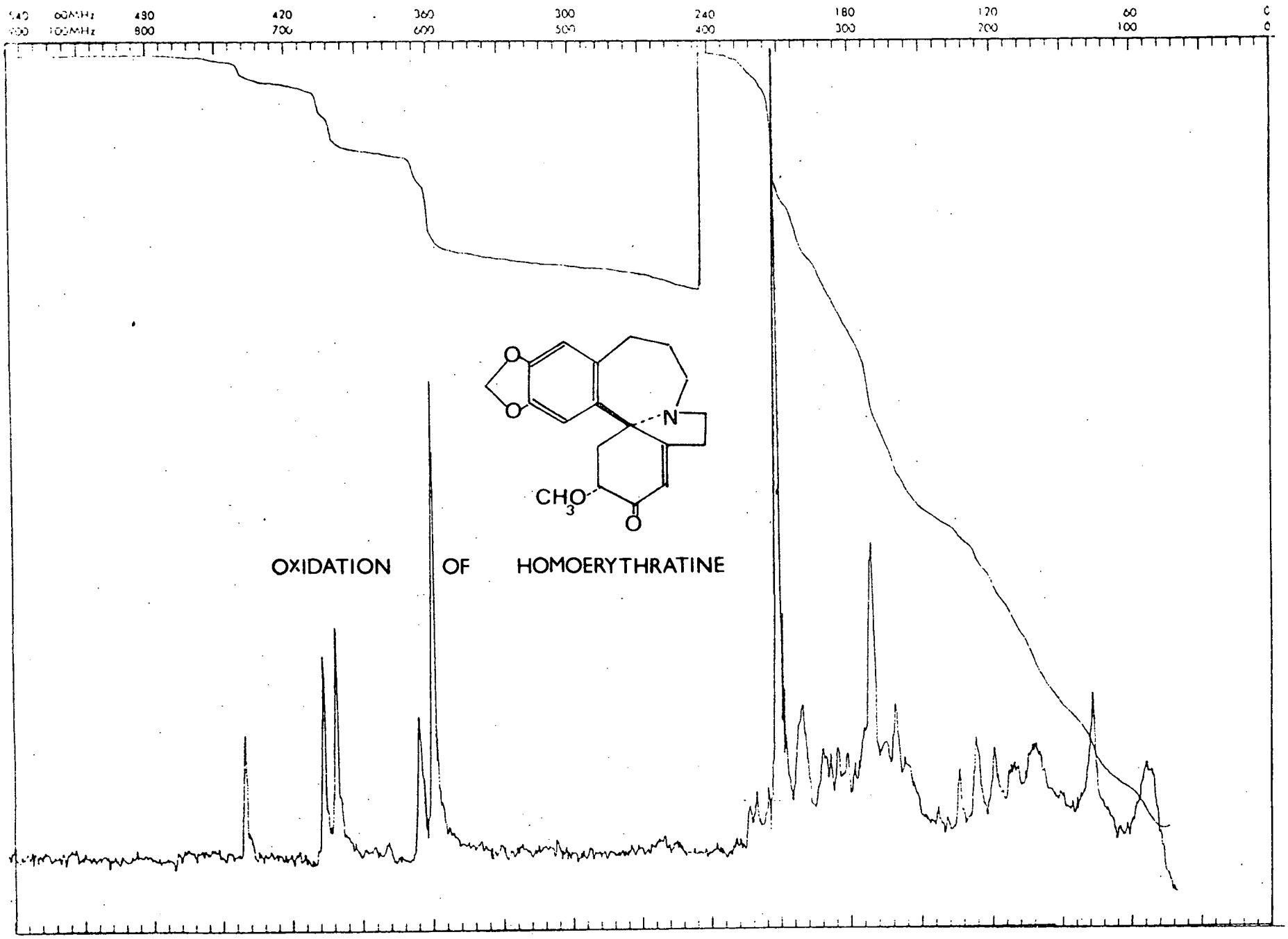
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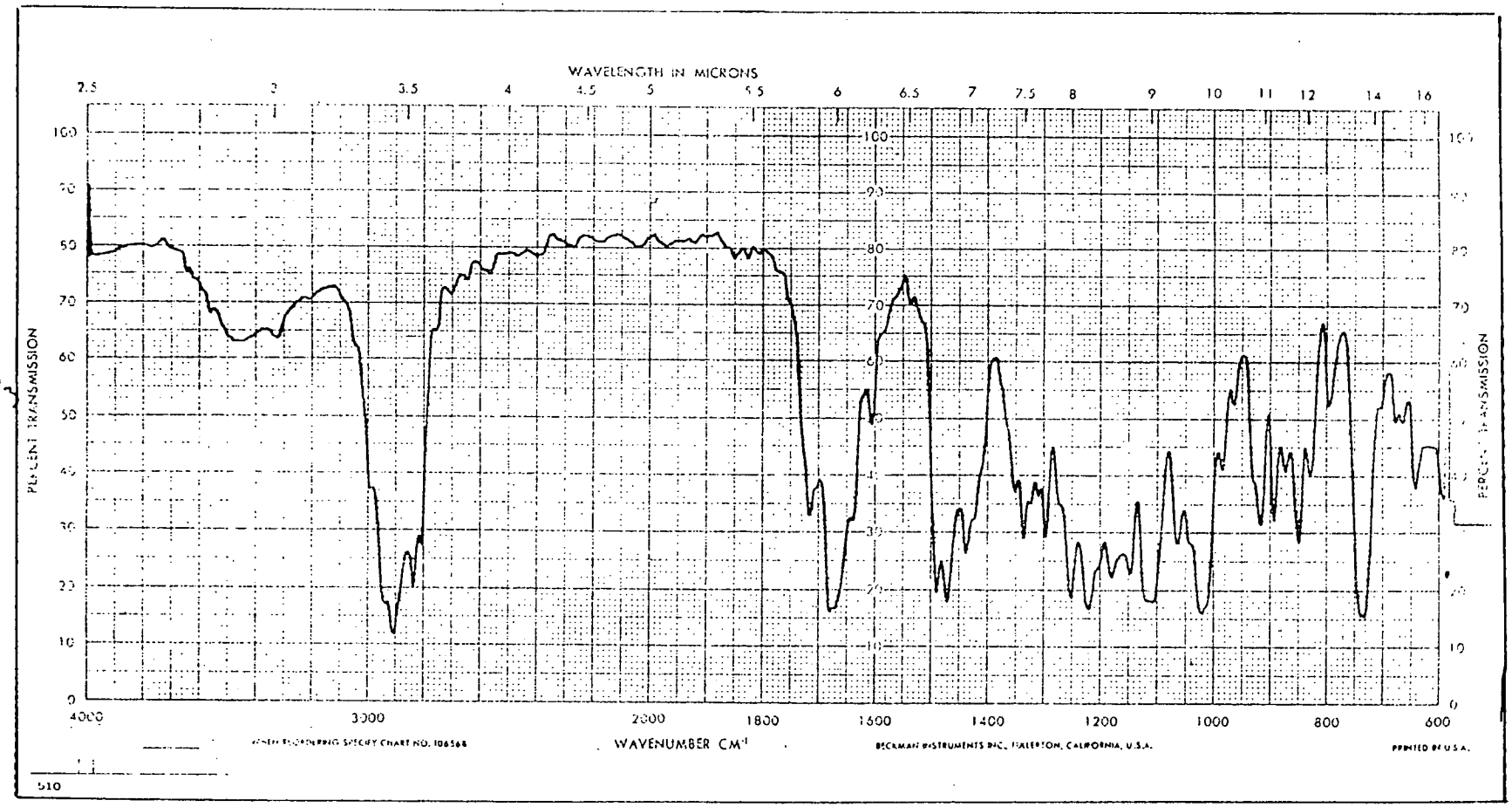

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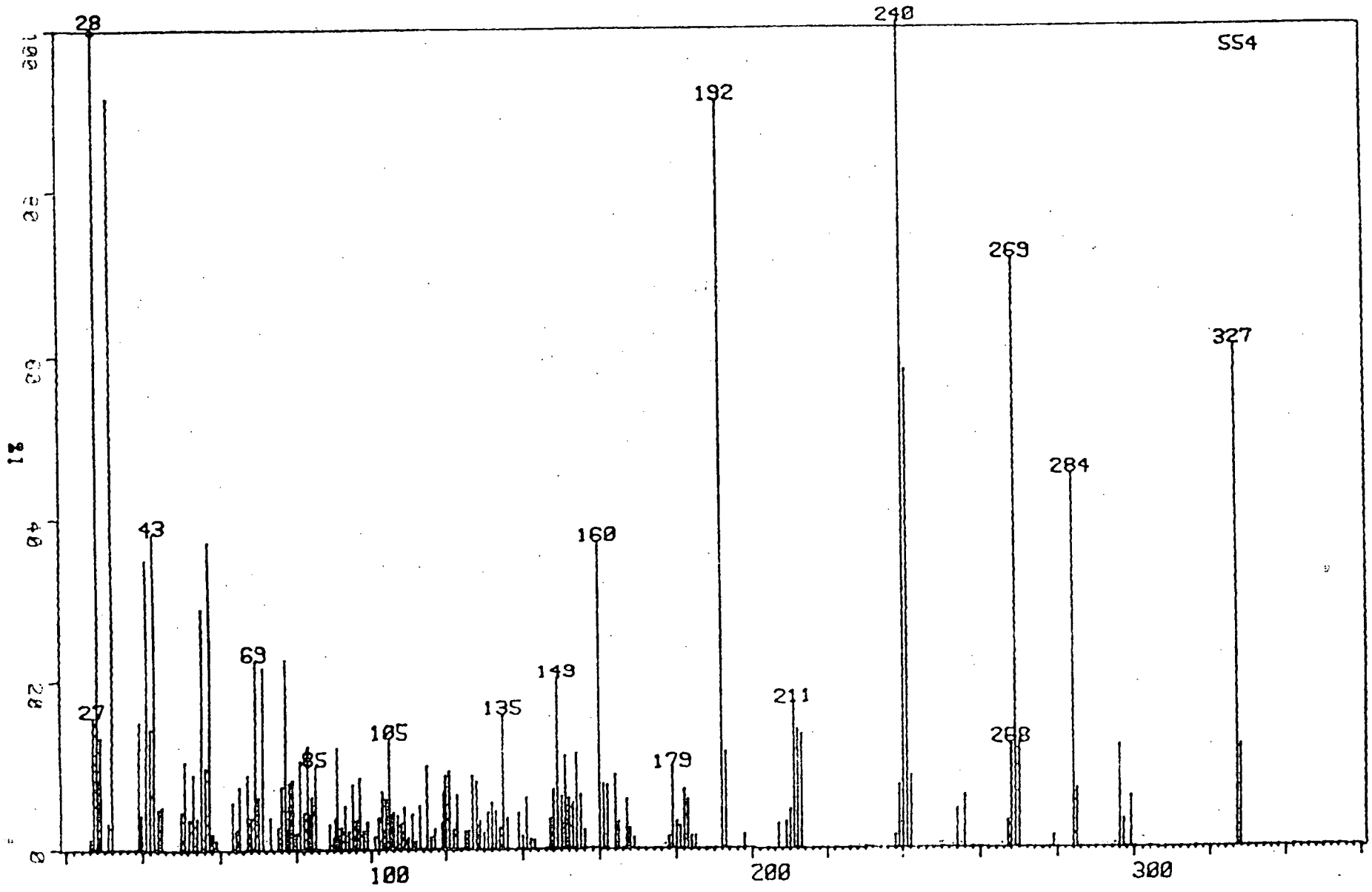

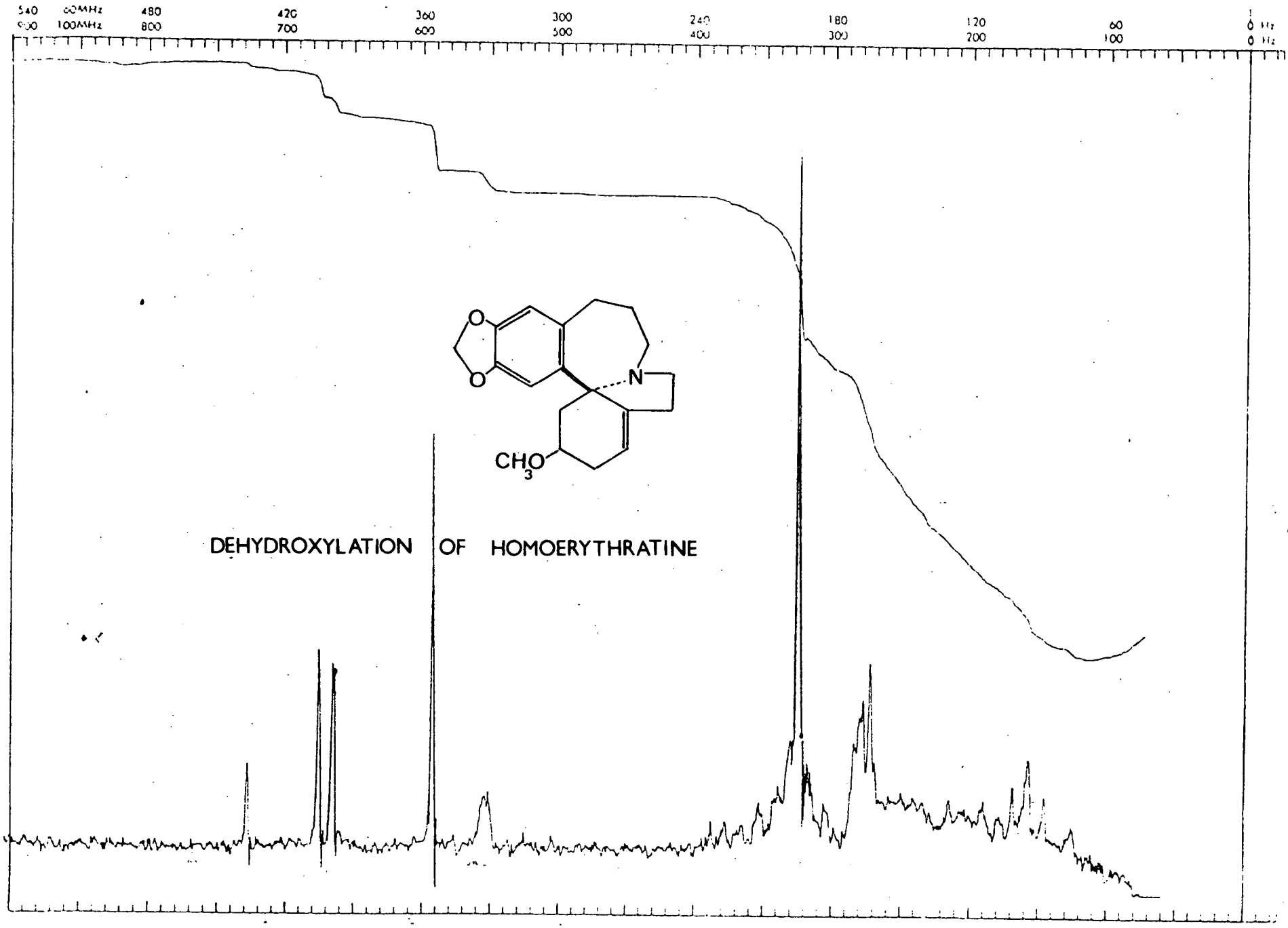
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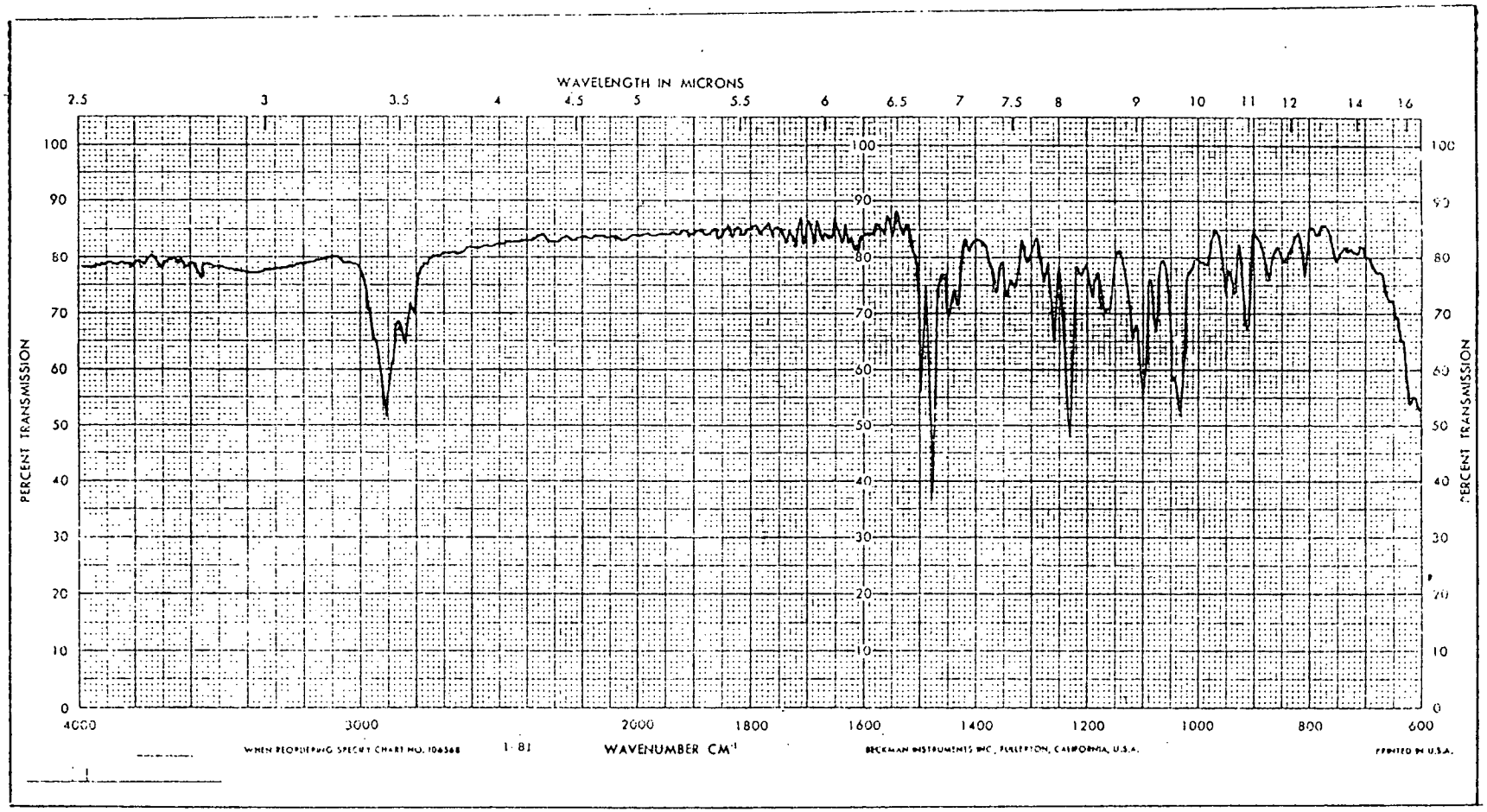

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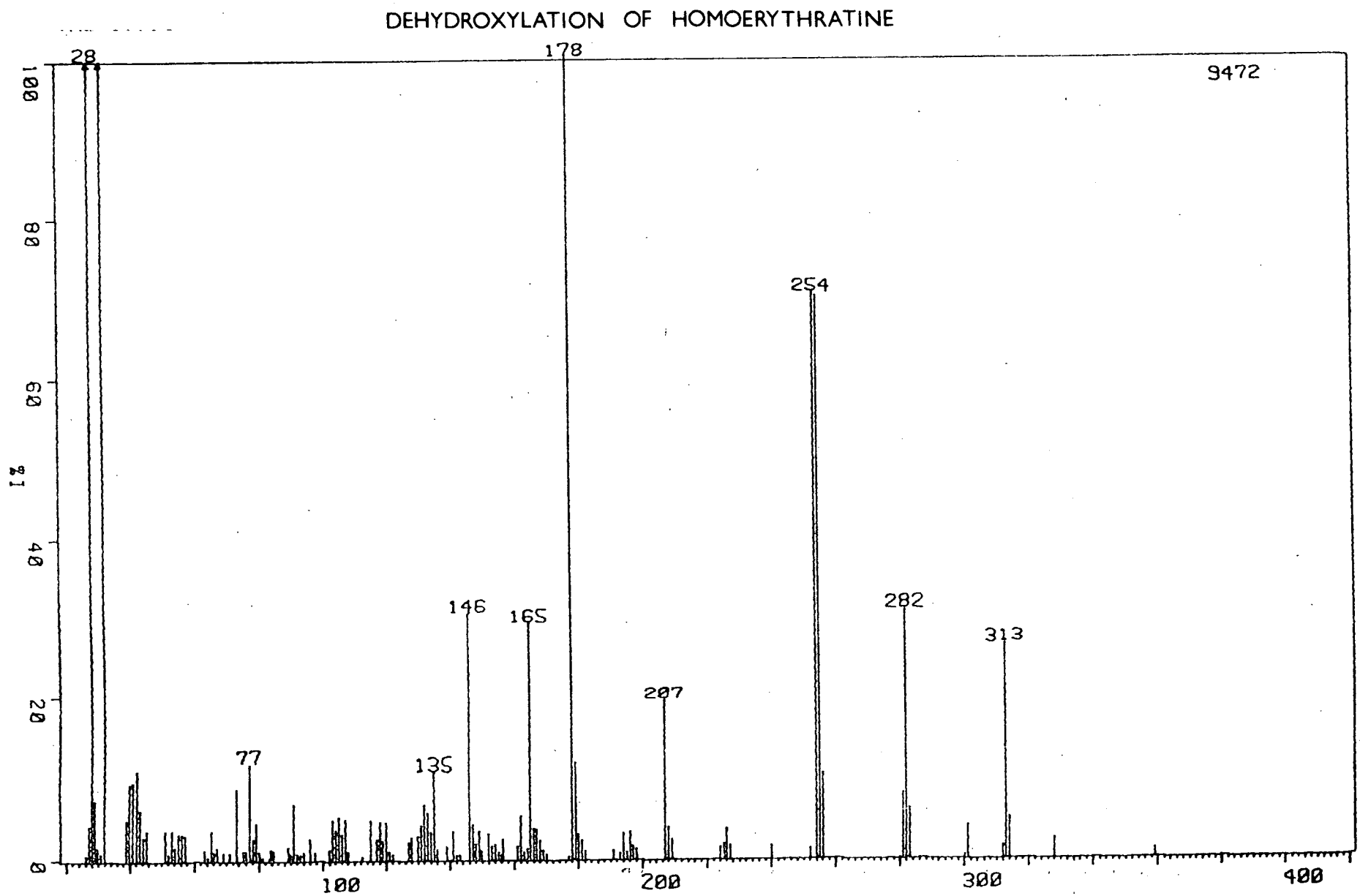
DEHYDROXYLATION OF HOMOERYTHRATINE
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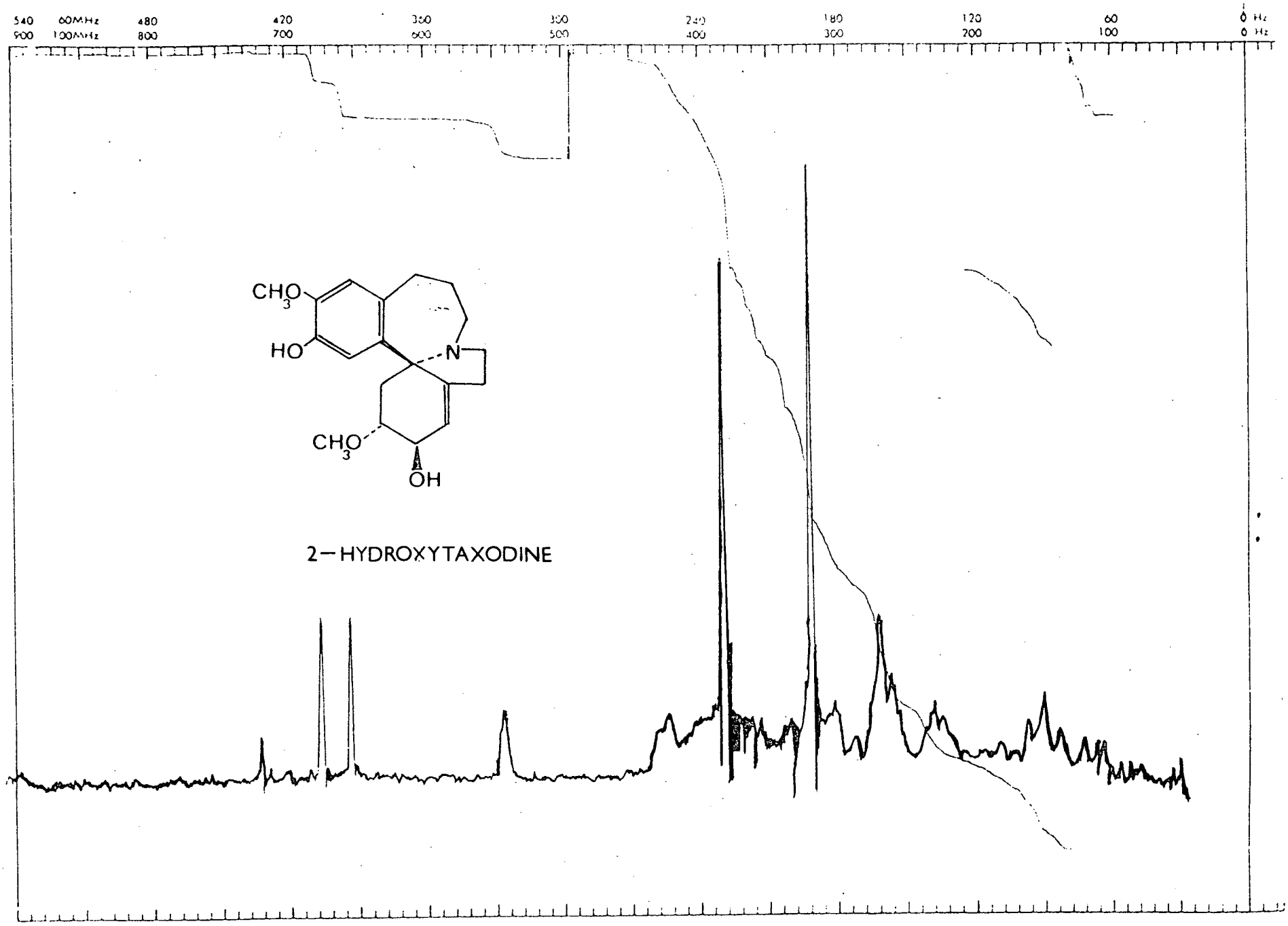
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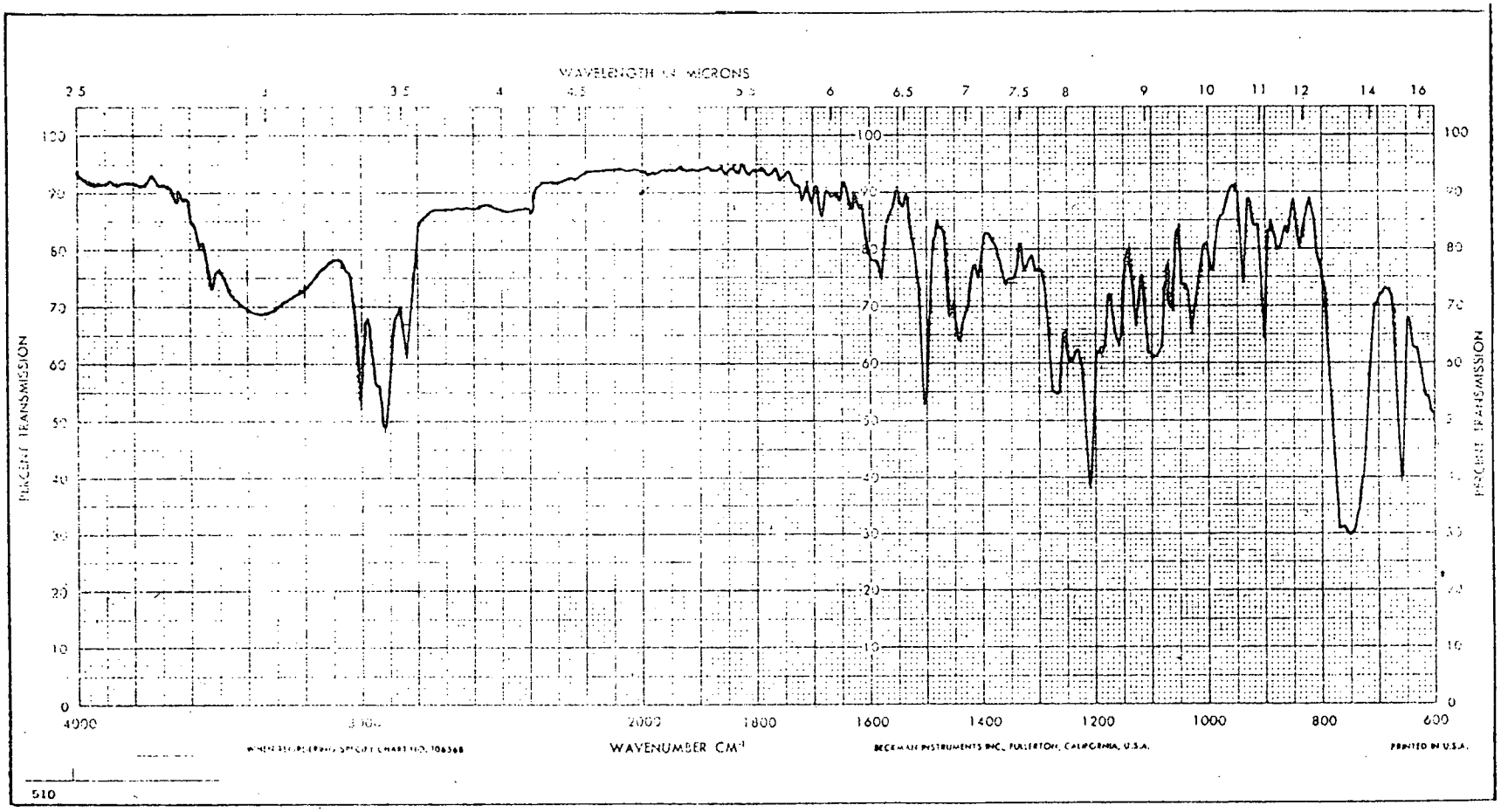

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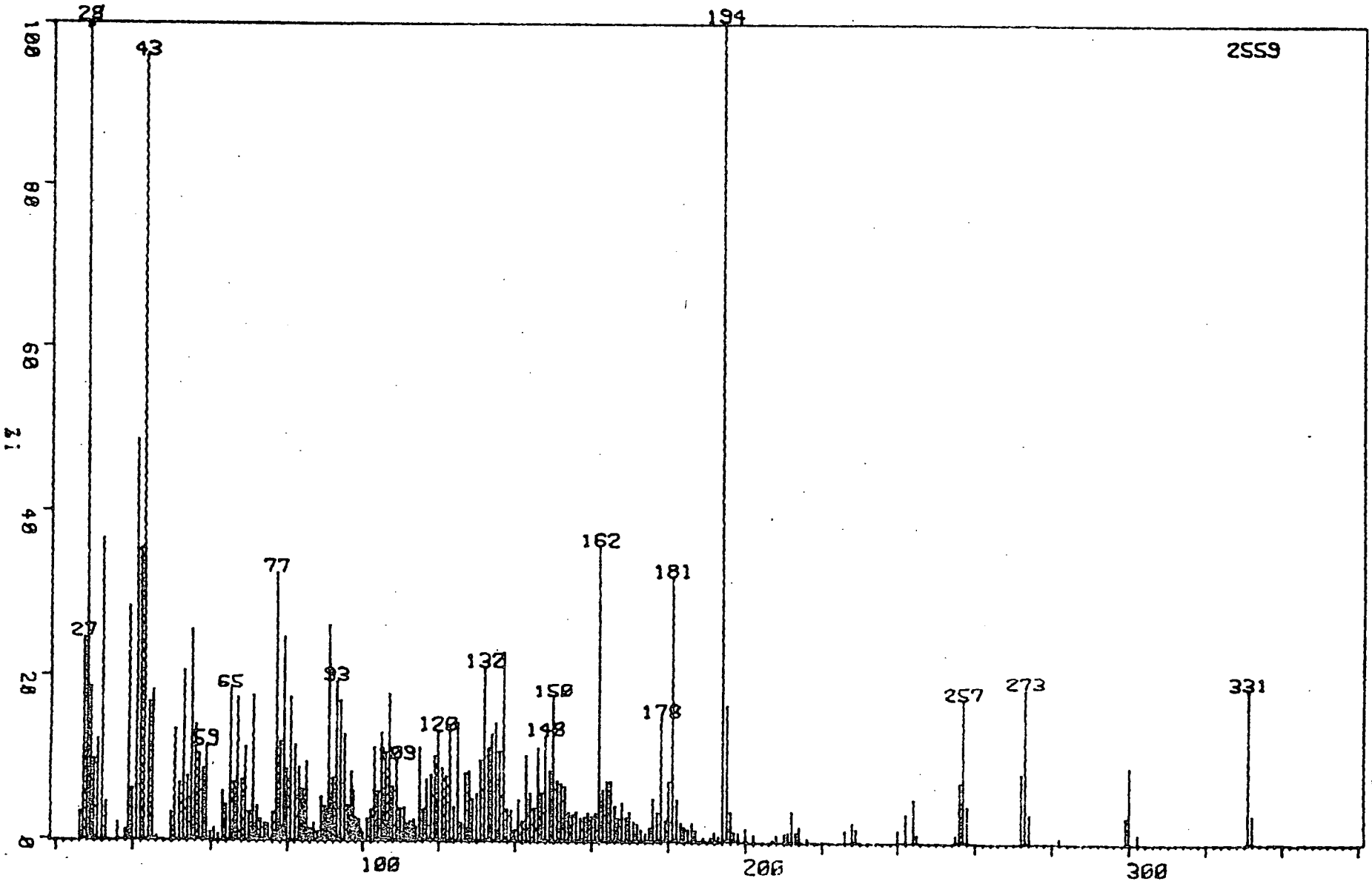

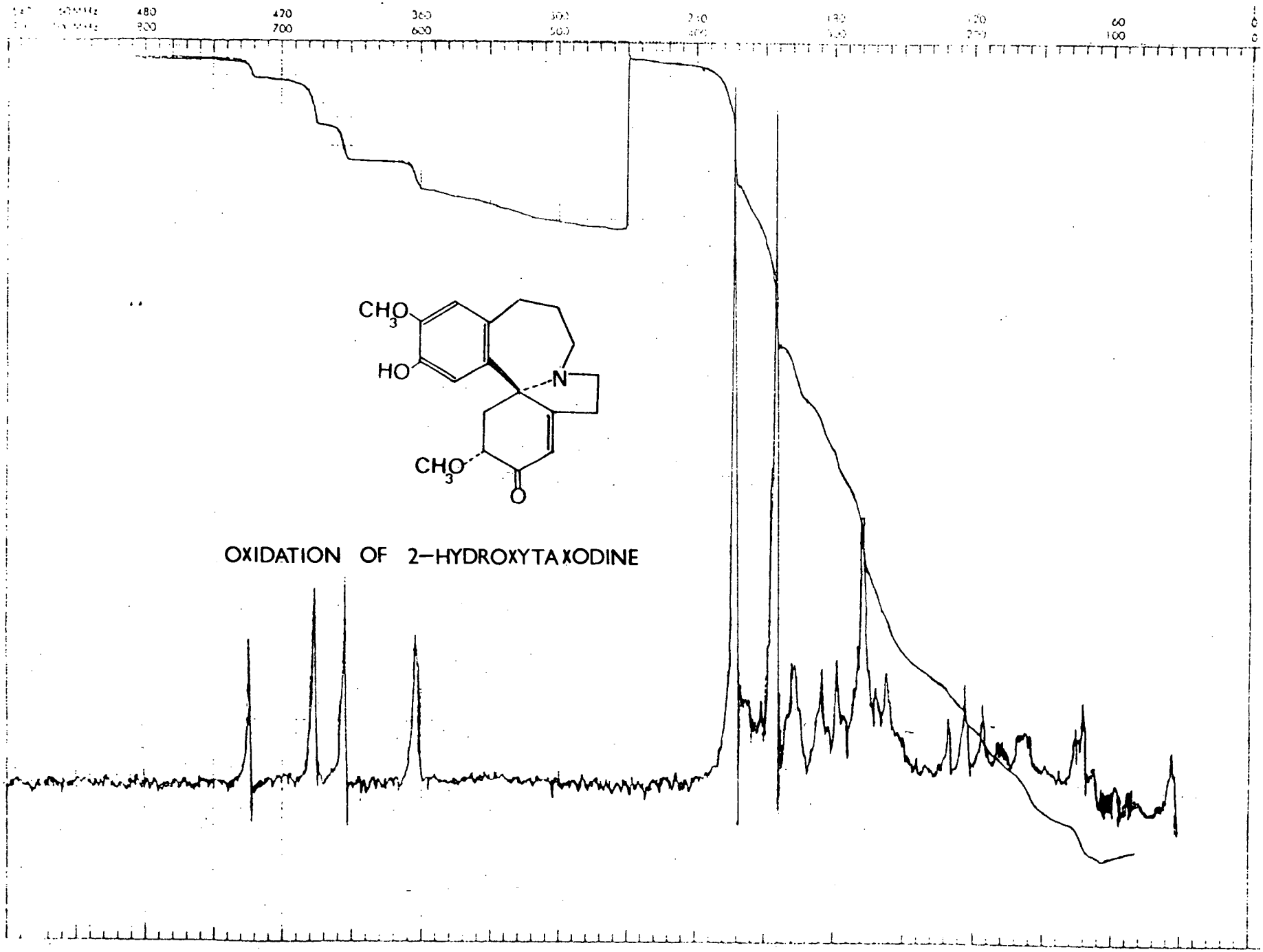

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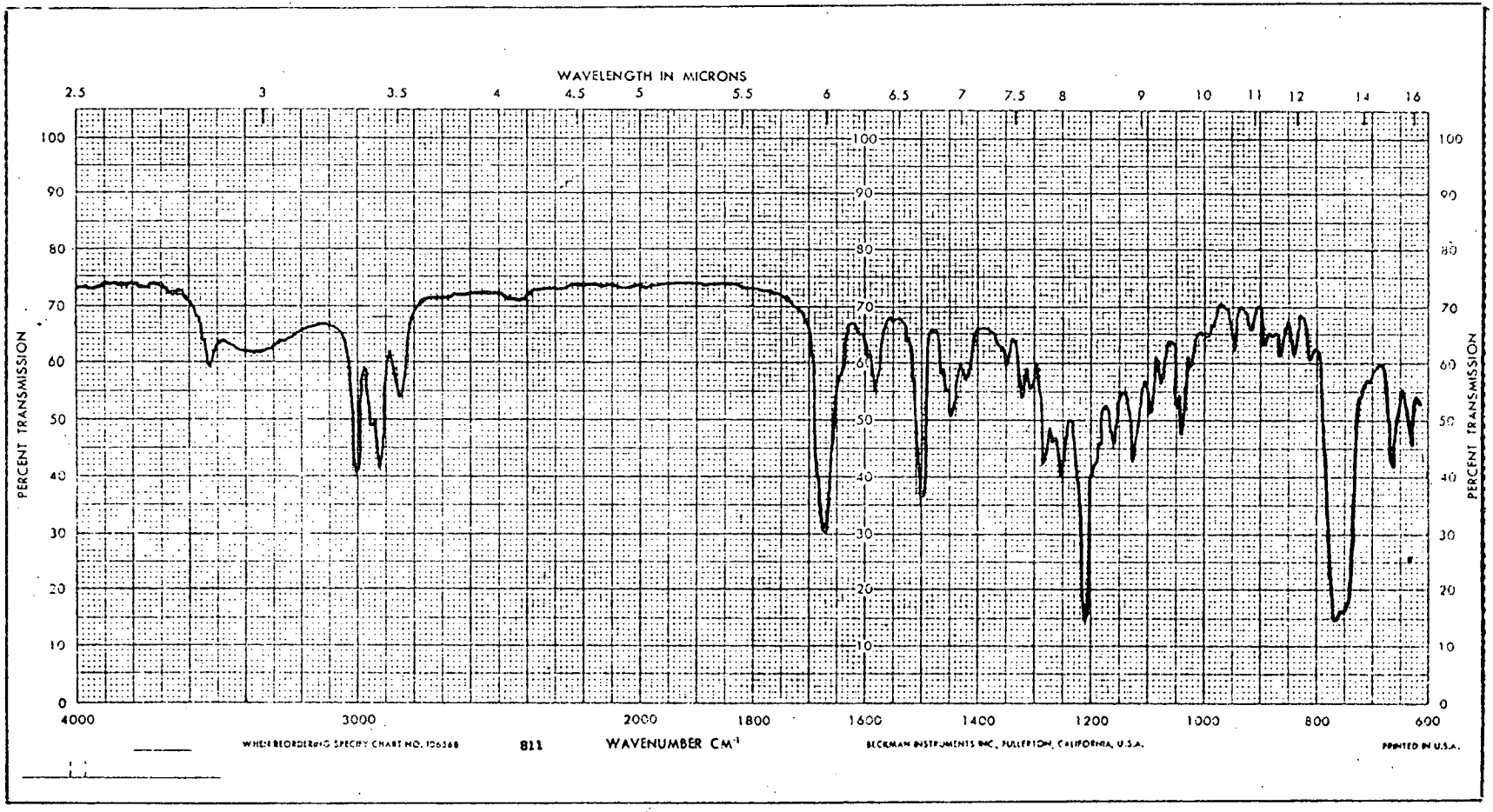

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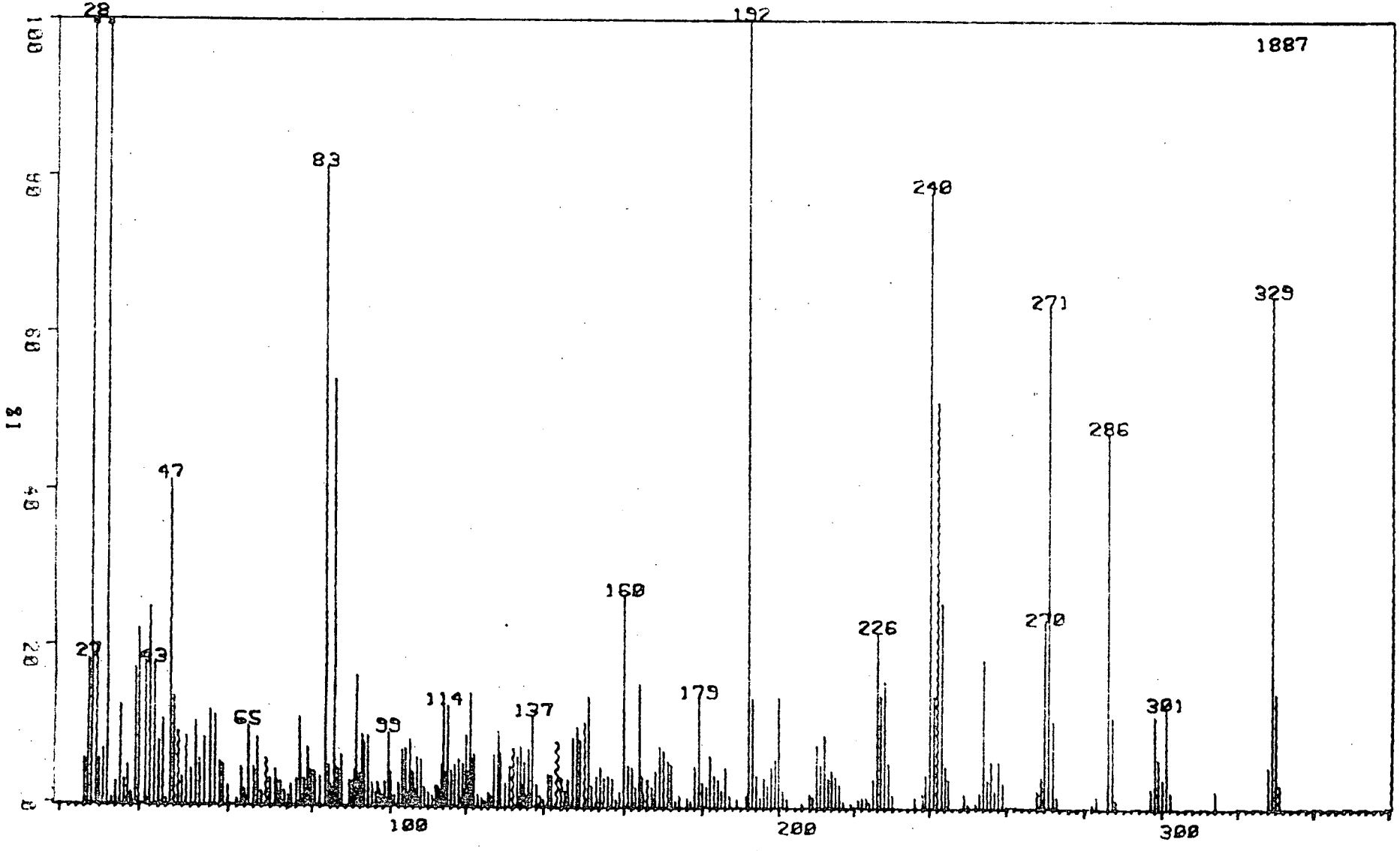

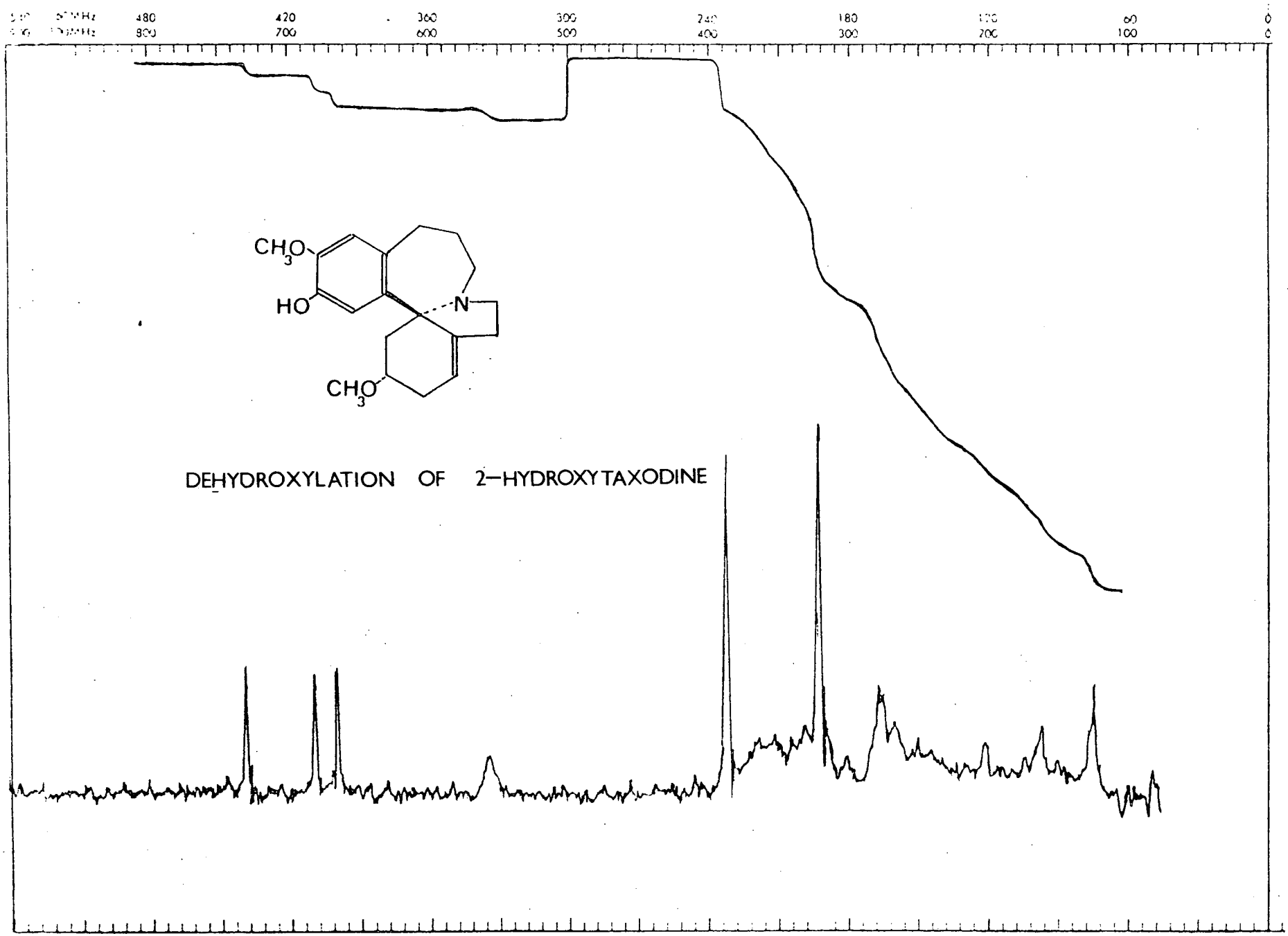
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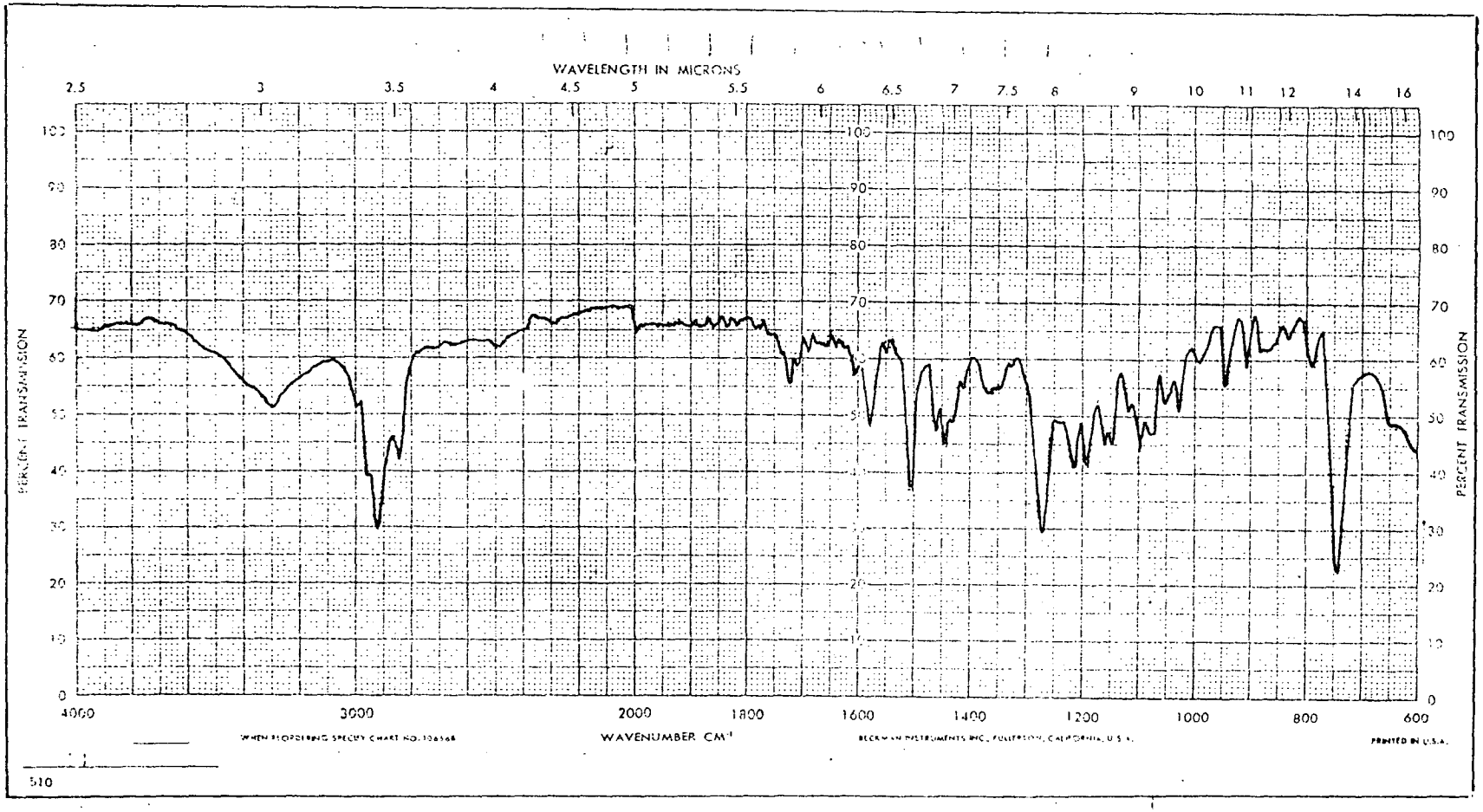

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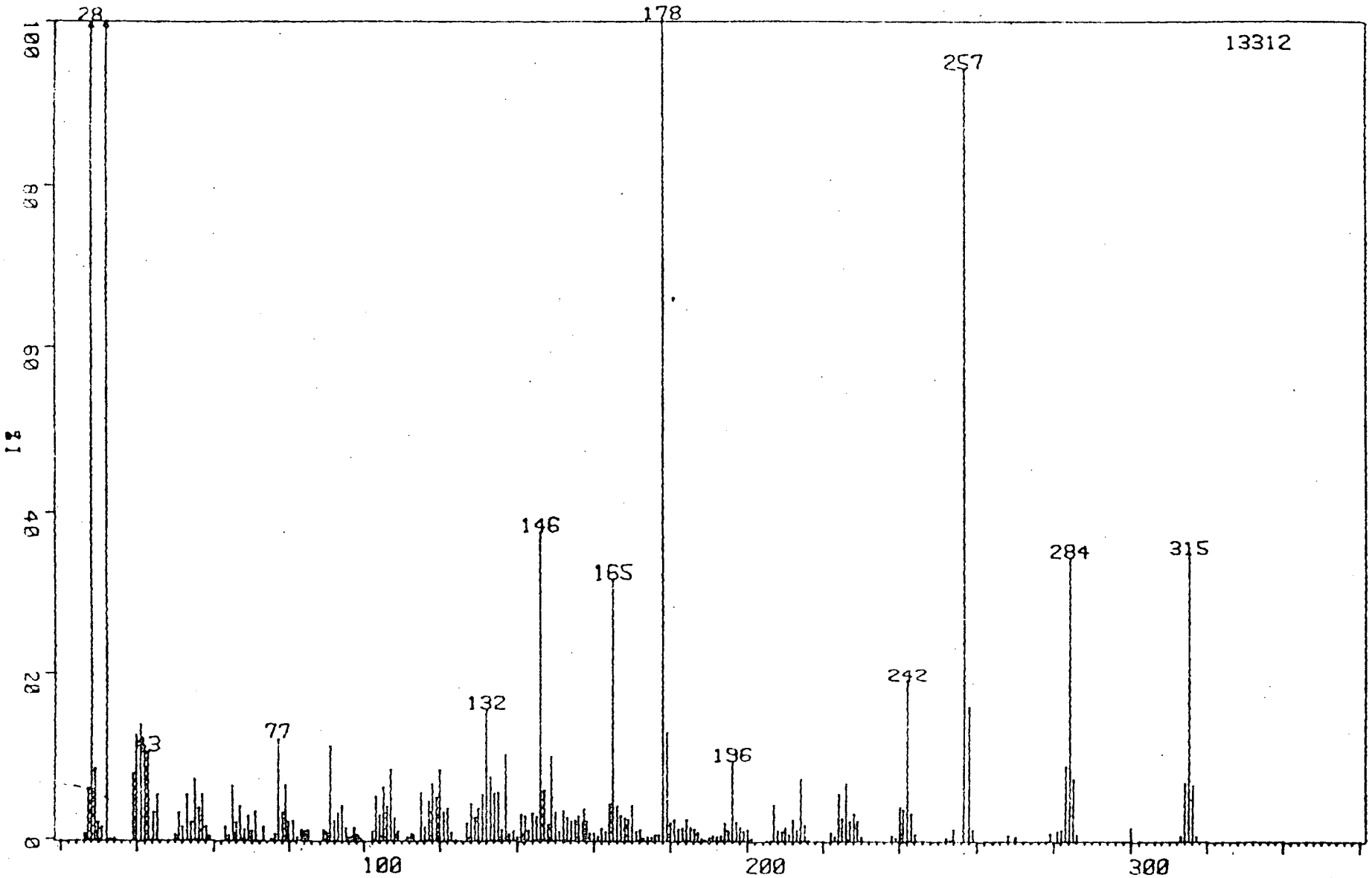

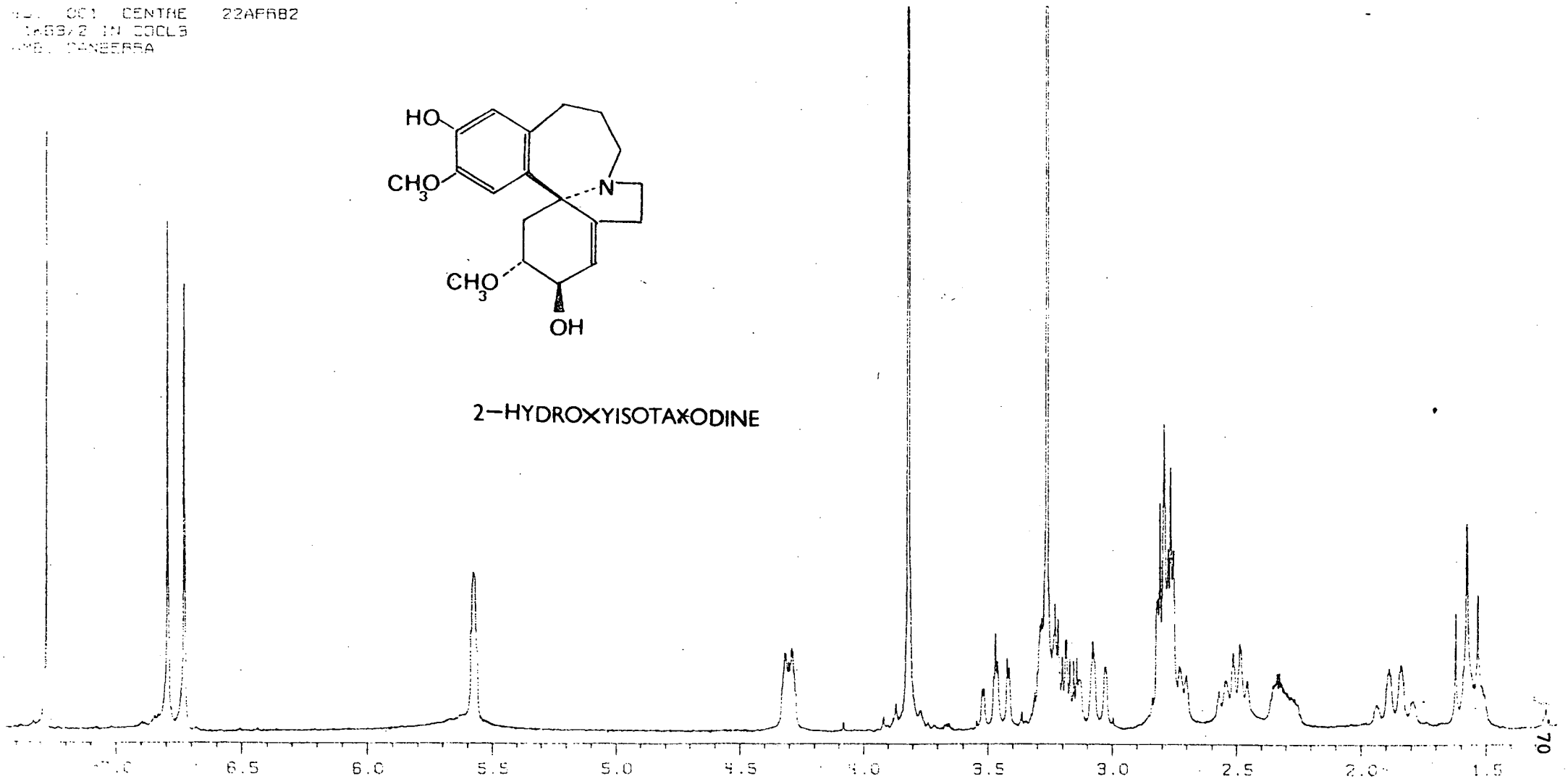

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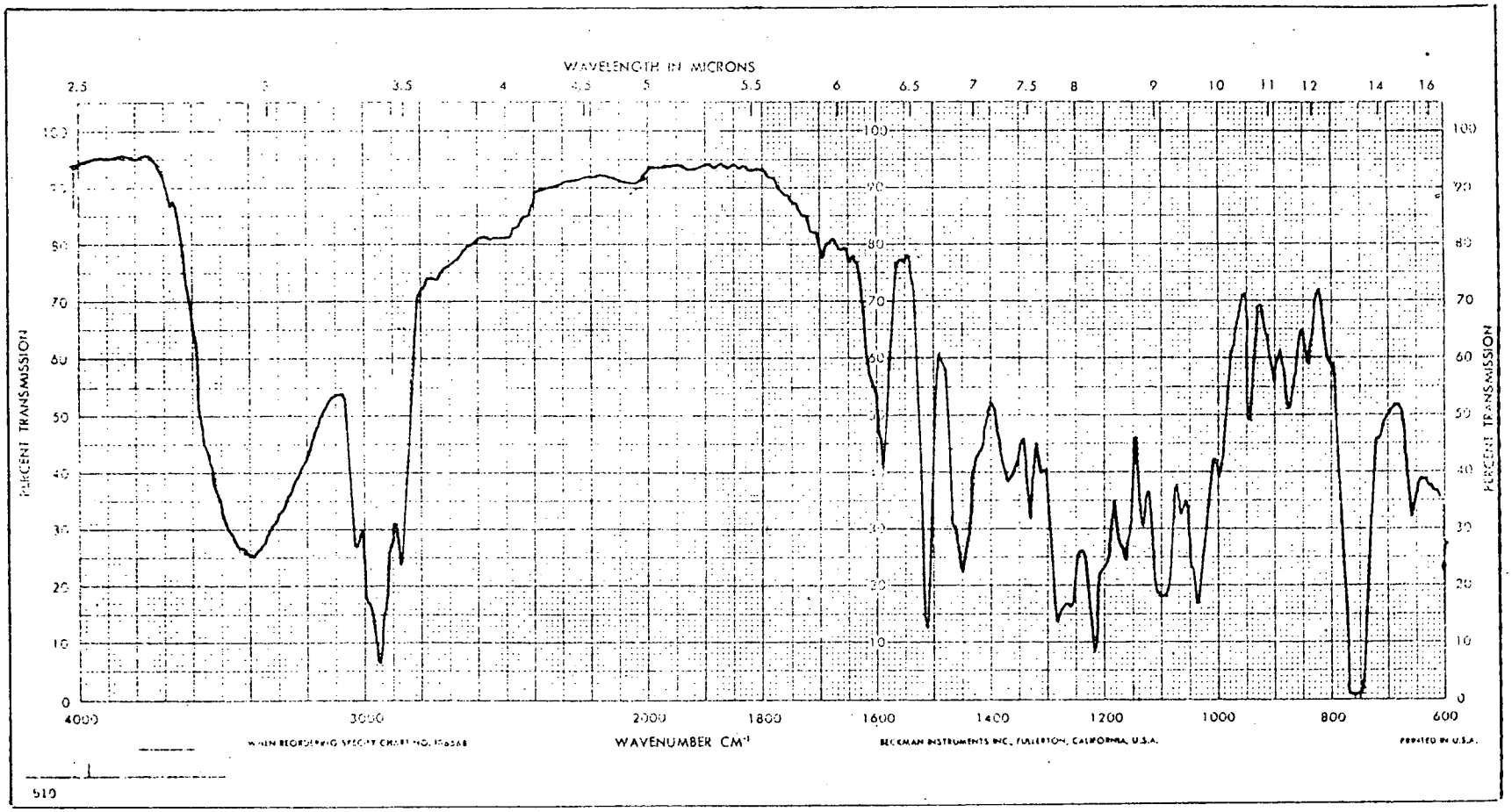

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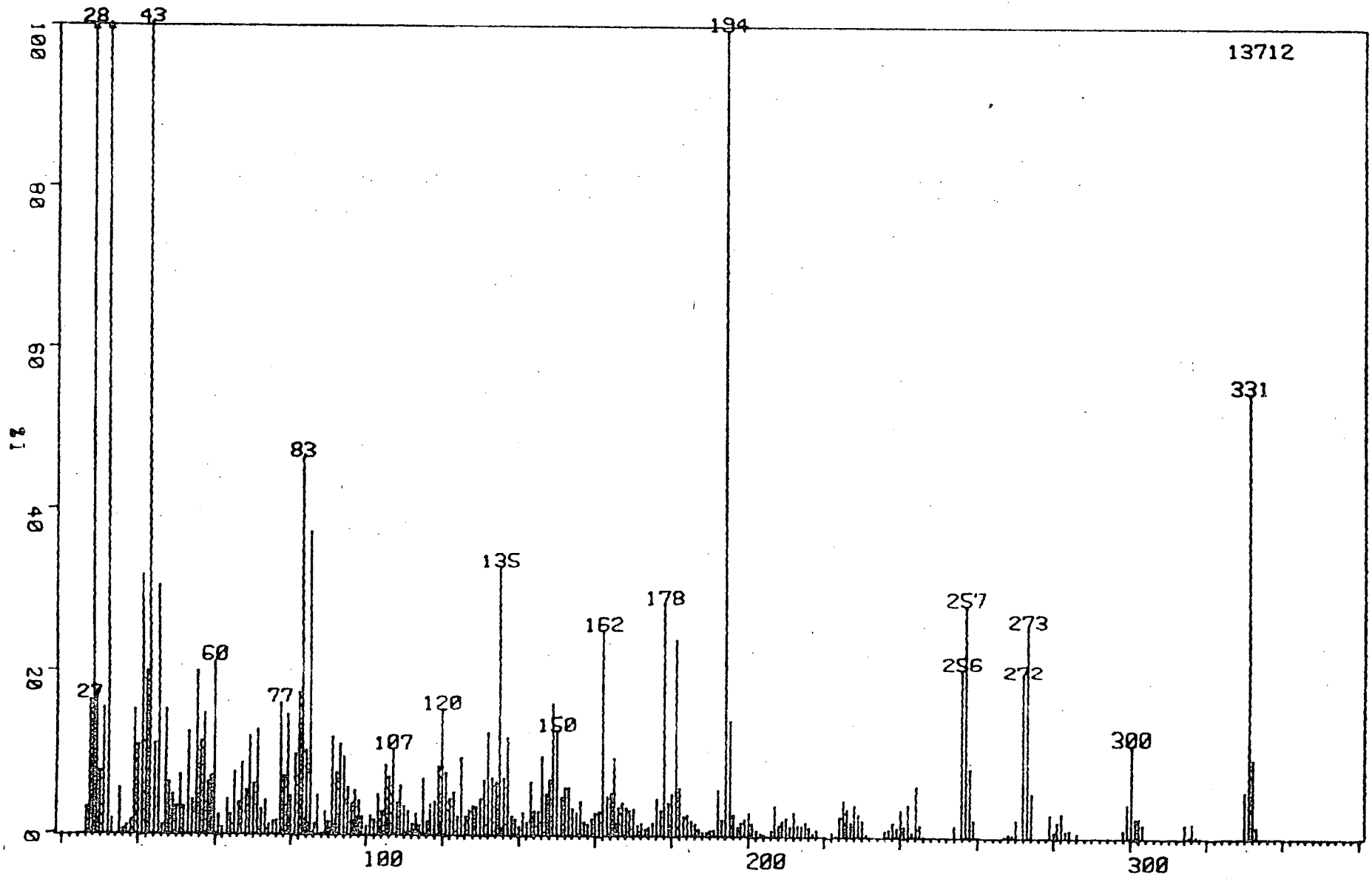

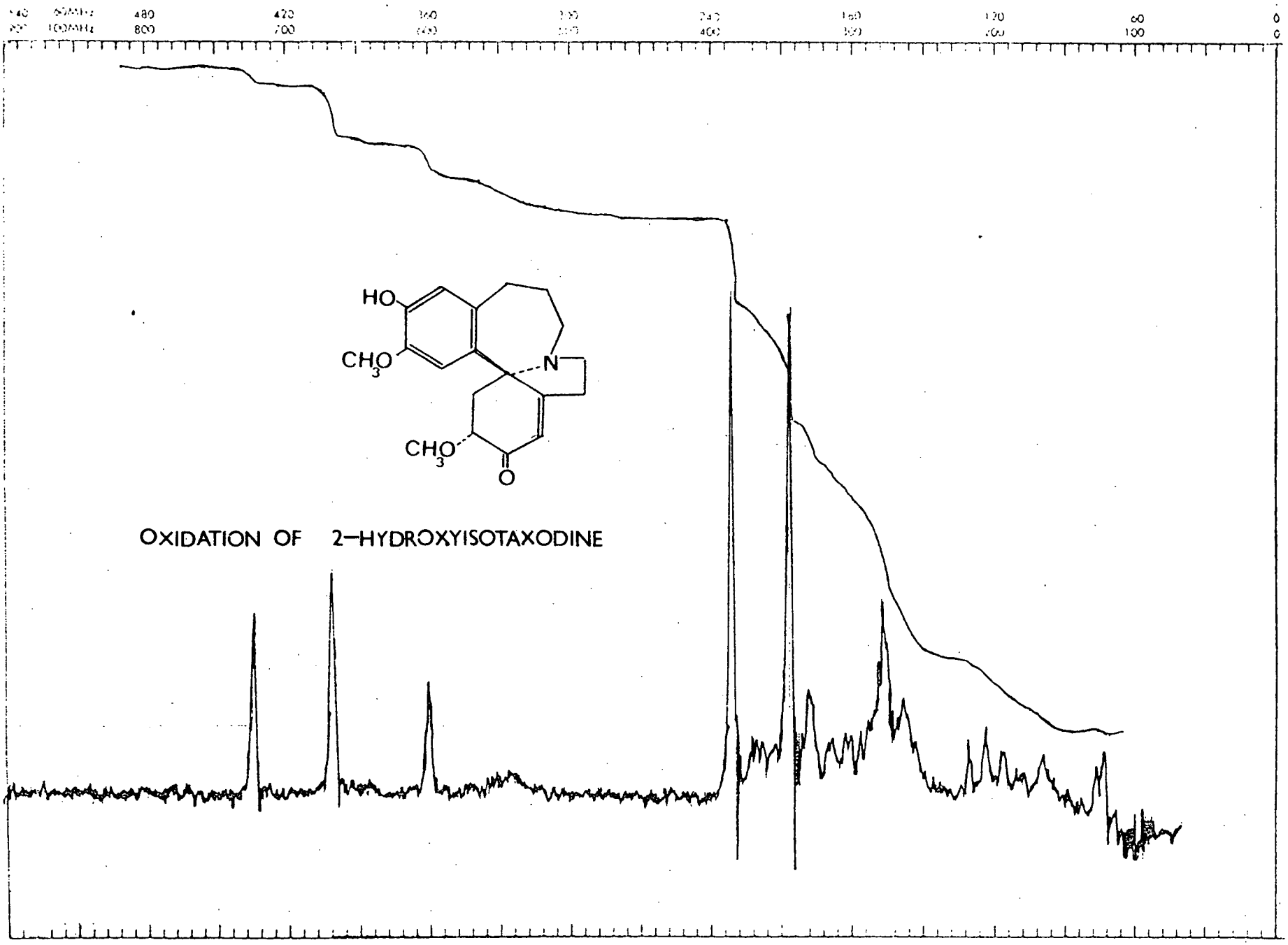
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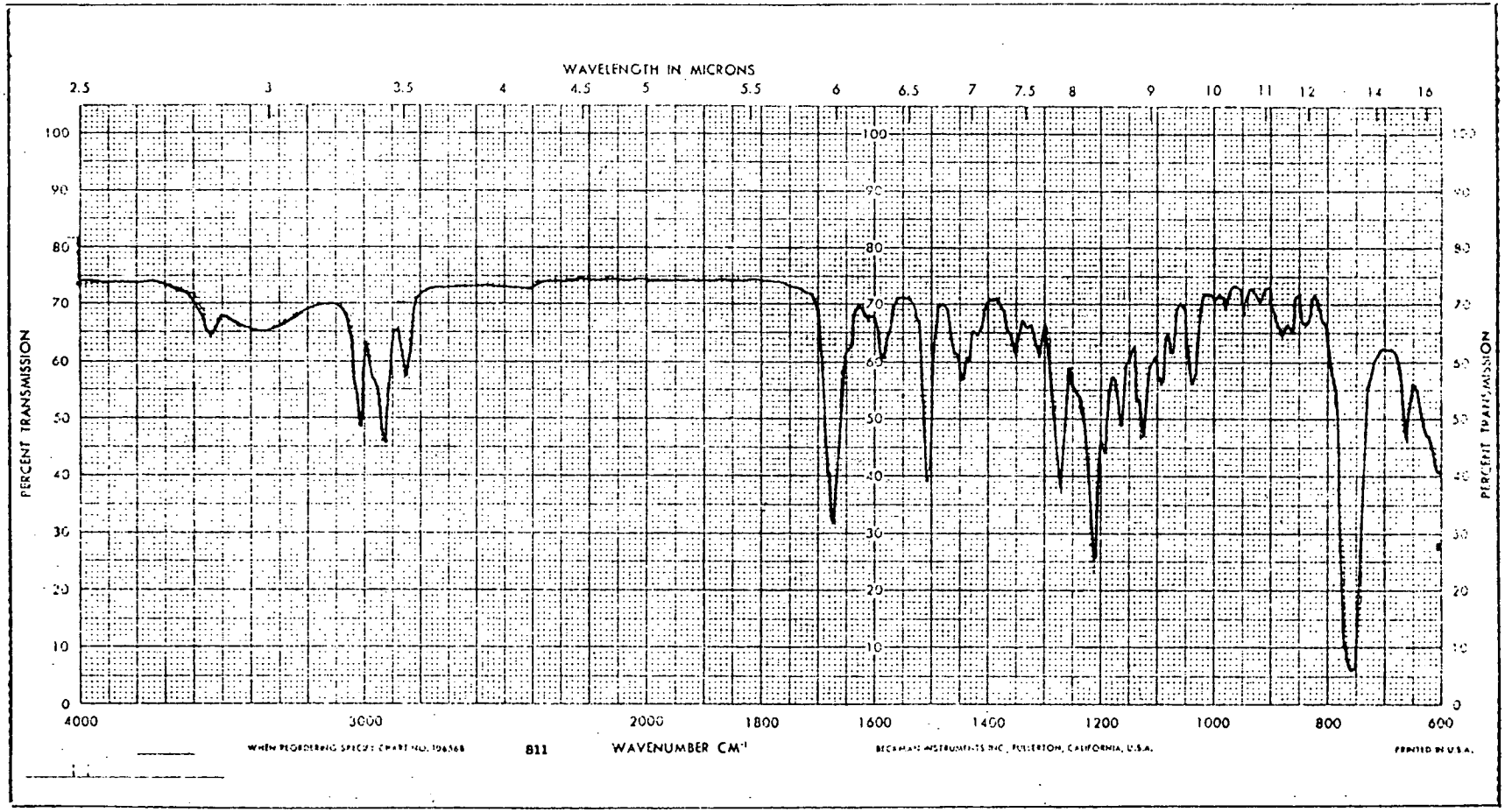

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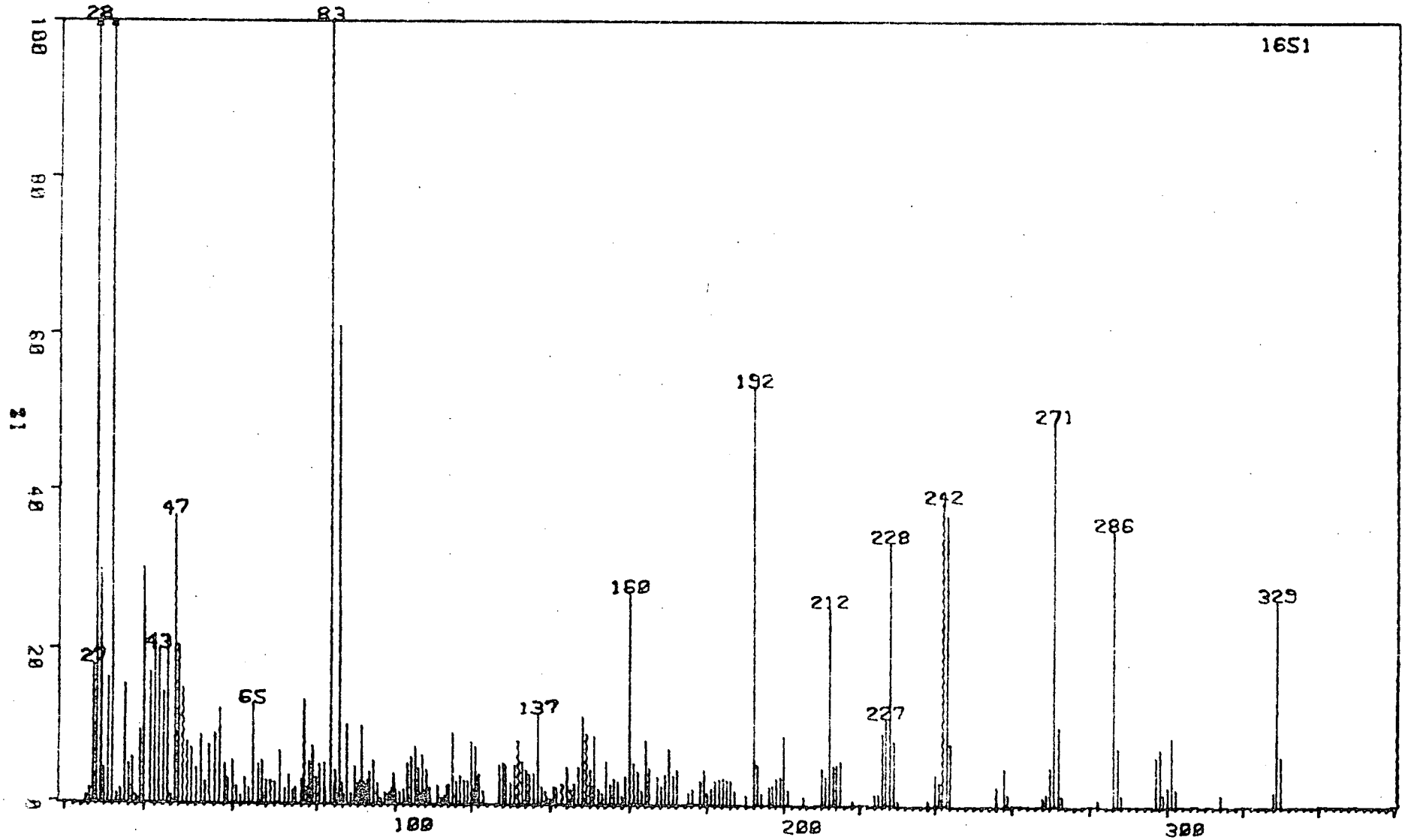
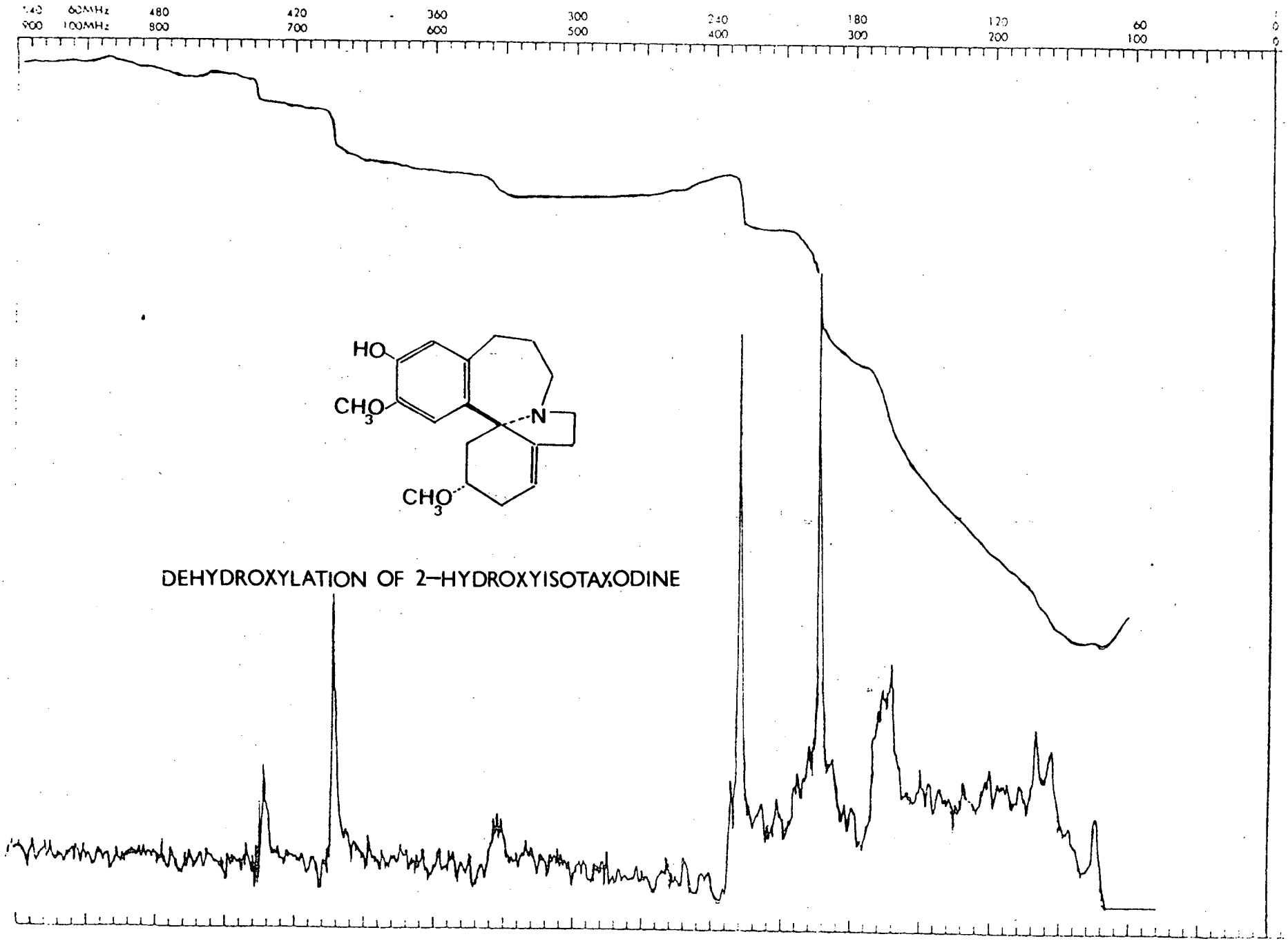
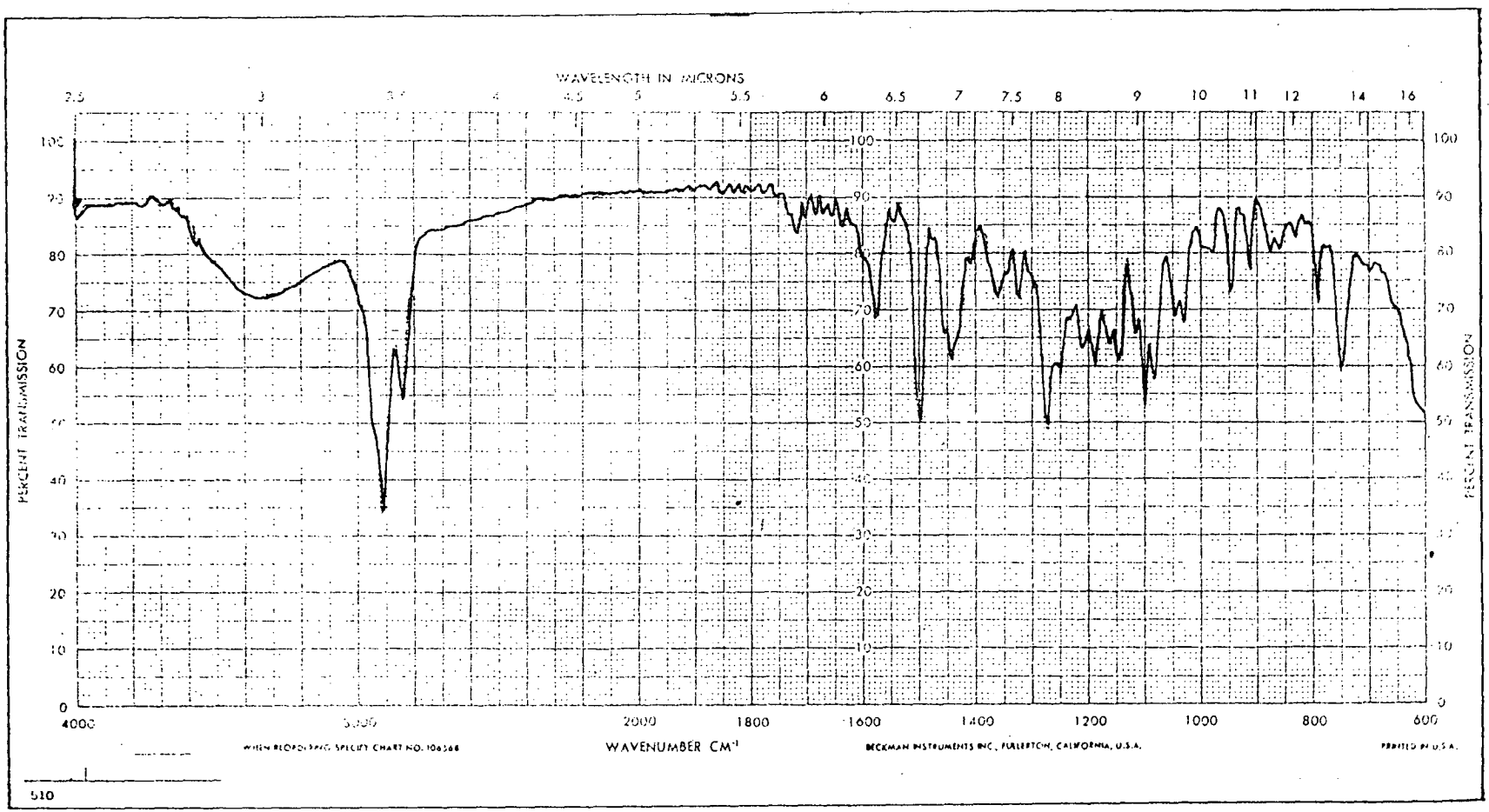
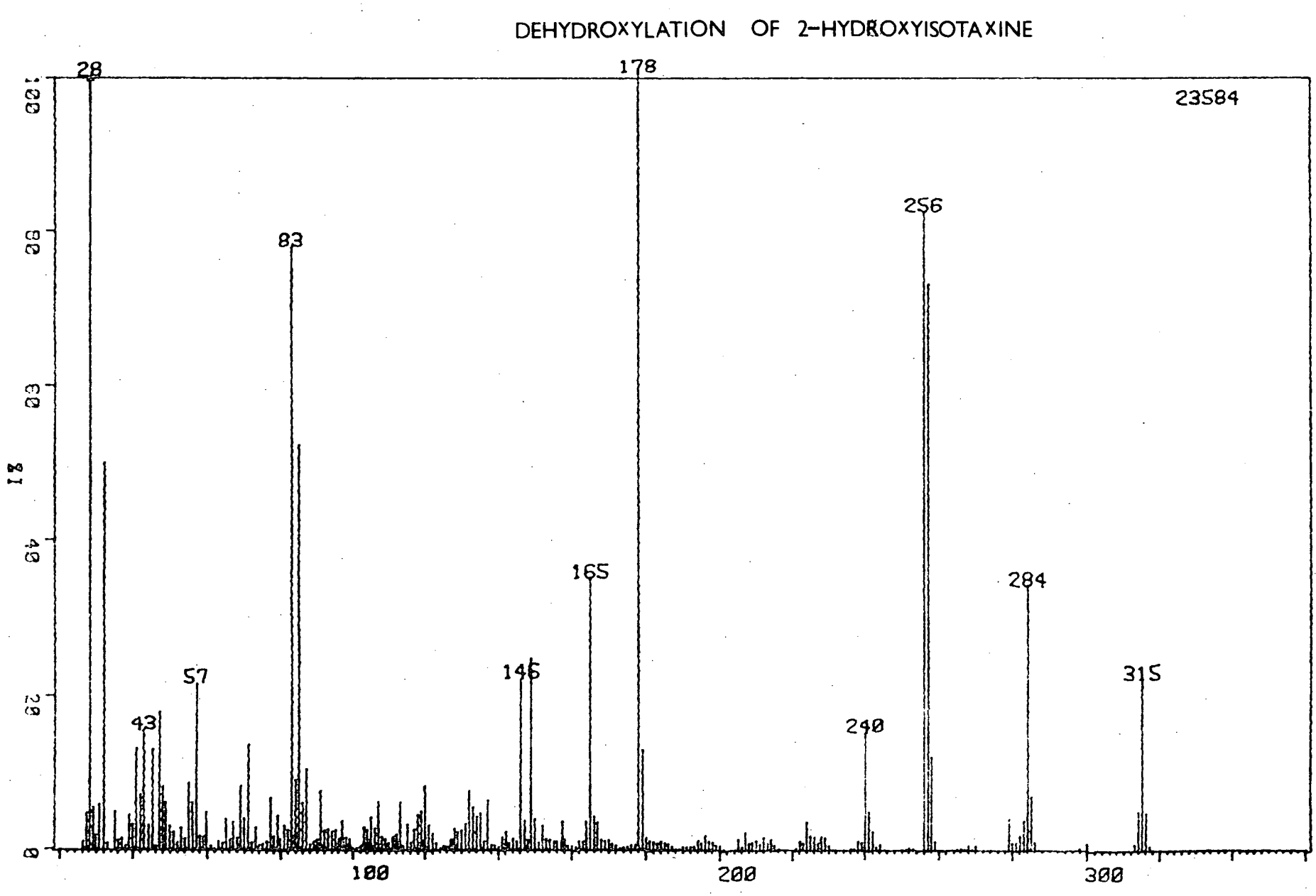


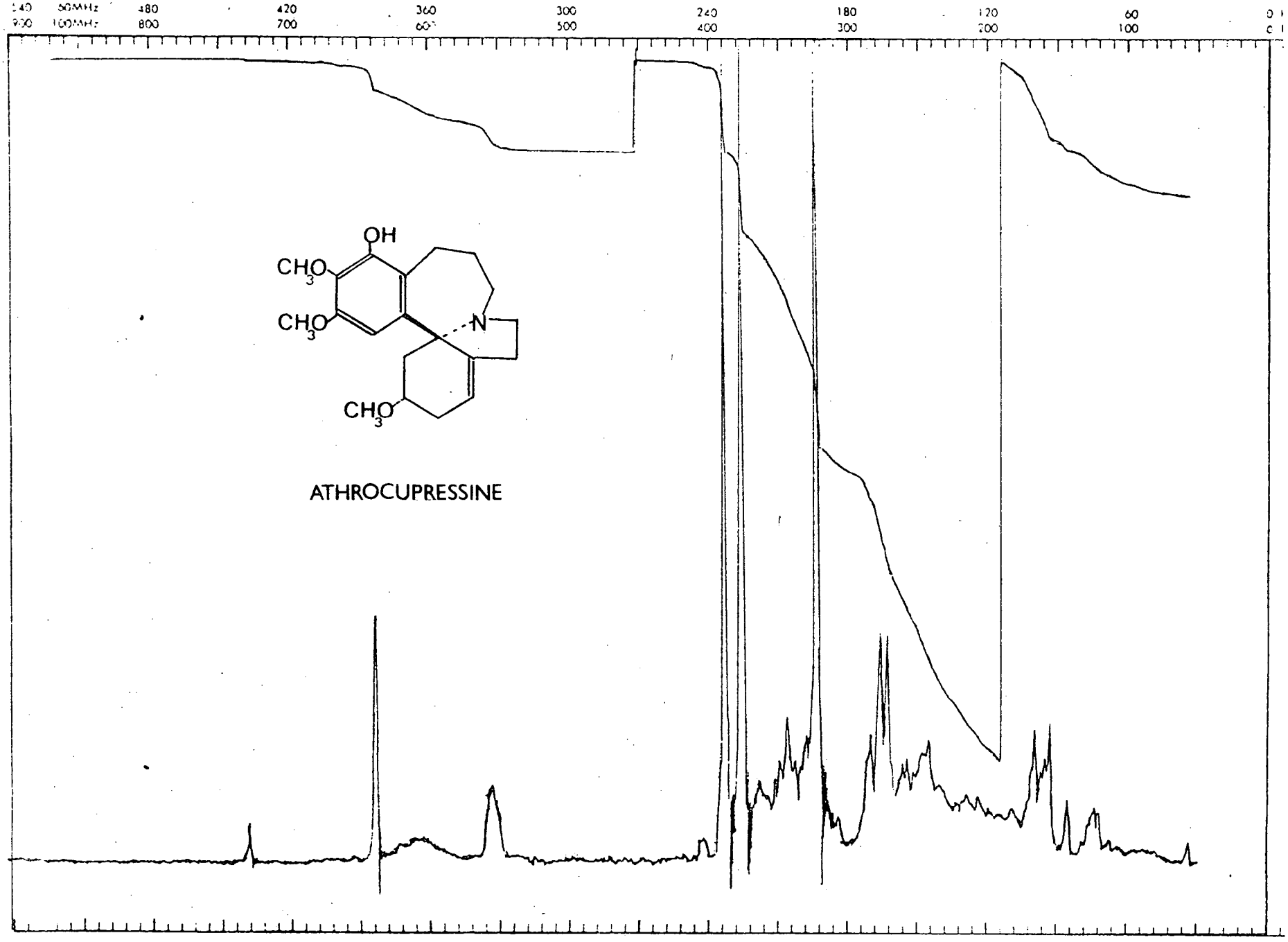


DEHYDROXYLATION OF 2–HYDROXYISOTAXODINE



DEHYDROXYLATION OF 2—HYDROXYISOTAXODINE


DEHYDROXYLATION OF 2-HYDROXYISOTAXINE
28
178
23584
256
83
165
284
57
146
315
43
240
RI
0
20
40
60
80
100
100
200
300



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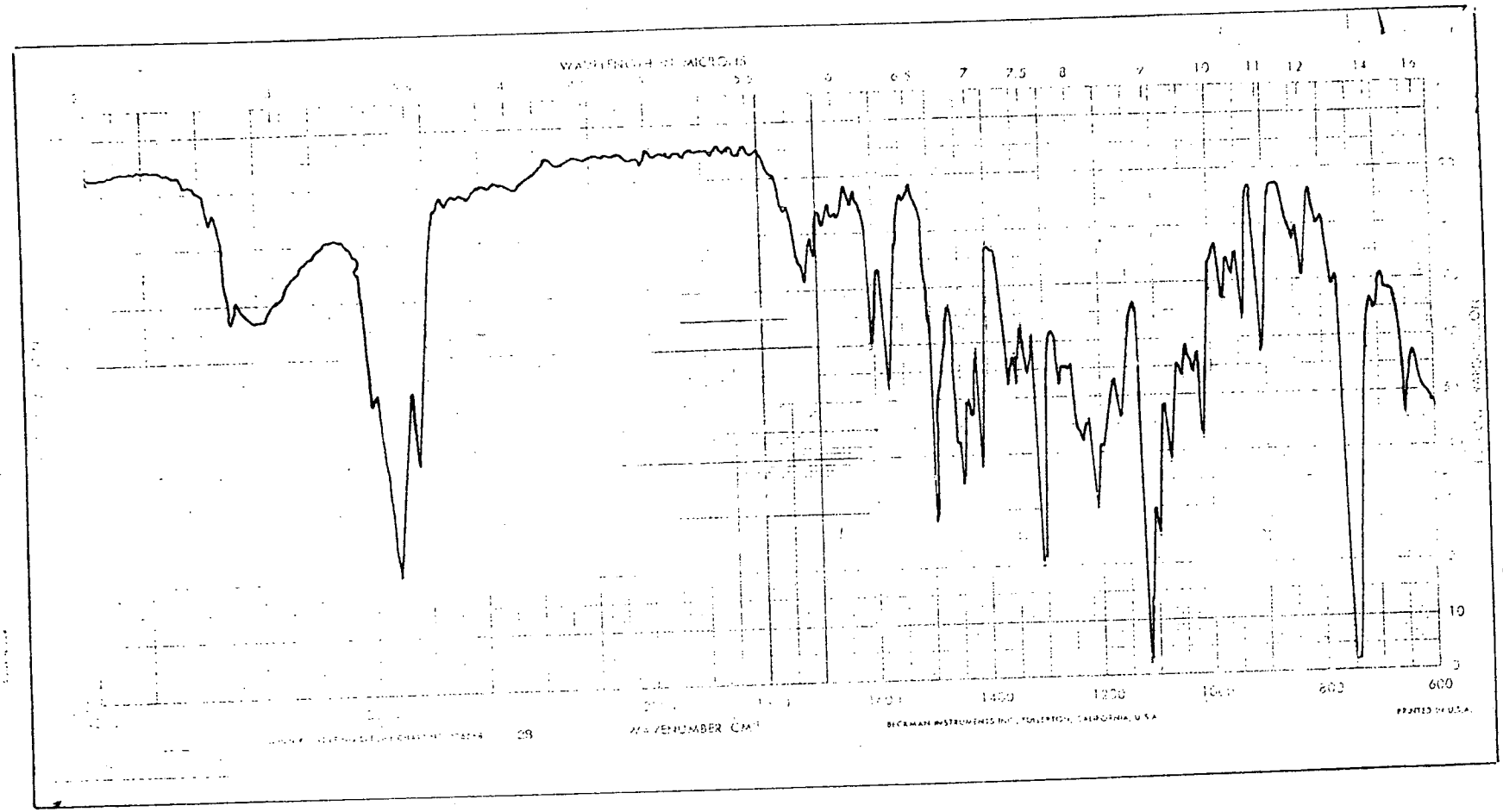

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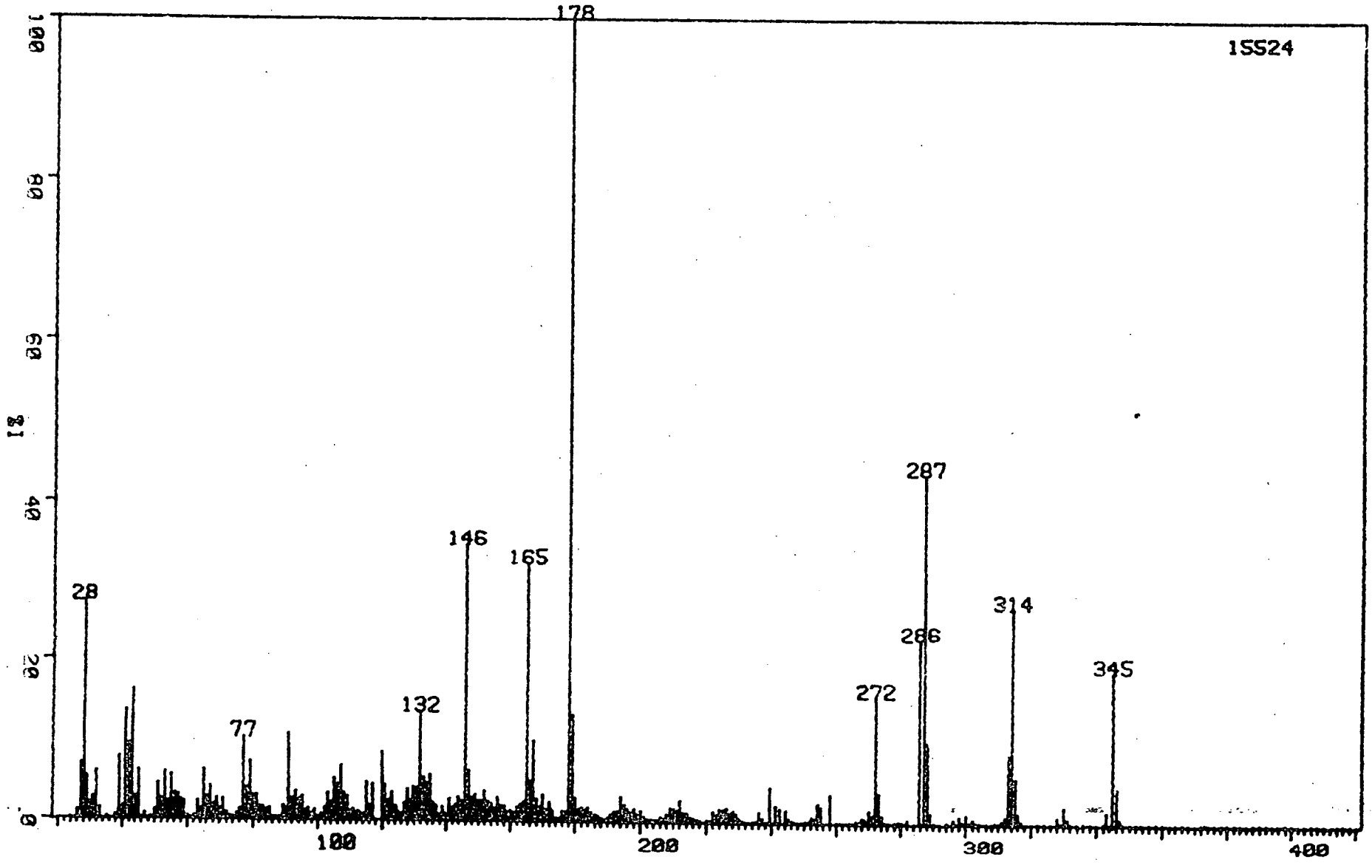
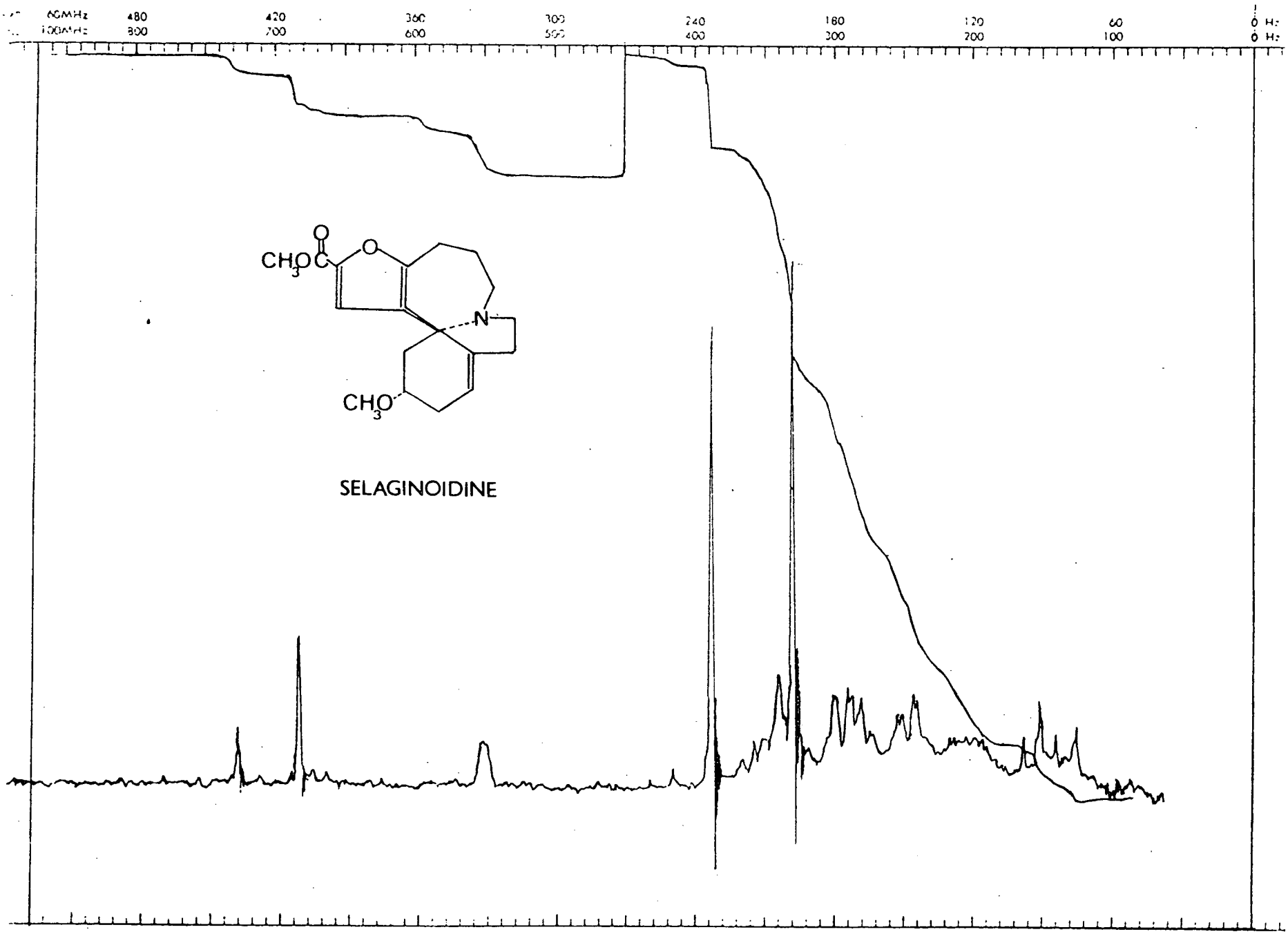
ATHROCUPRESSINE





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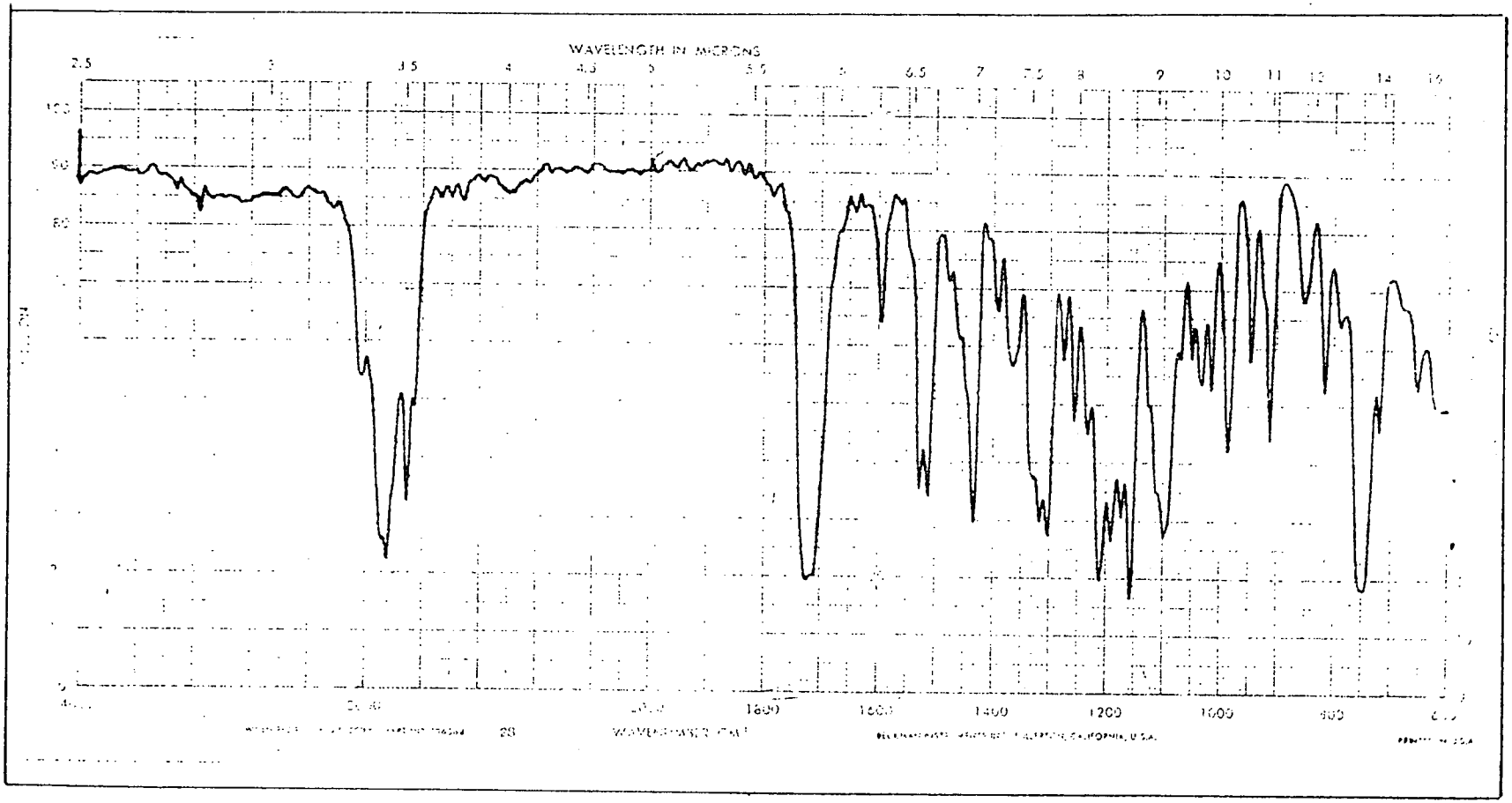
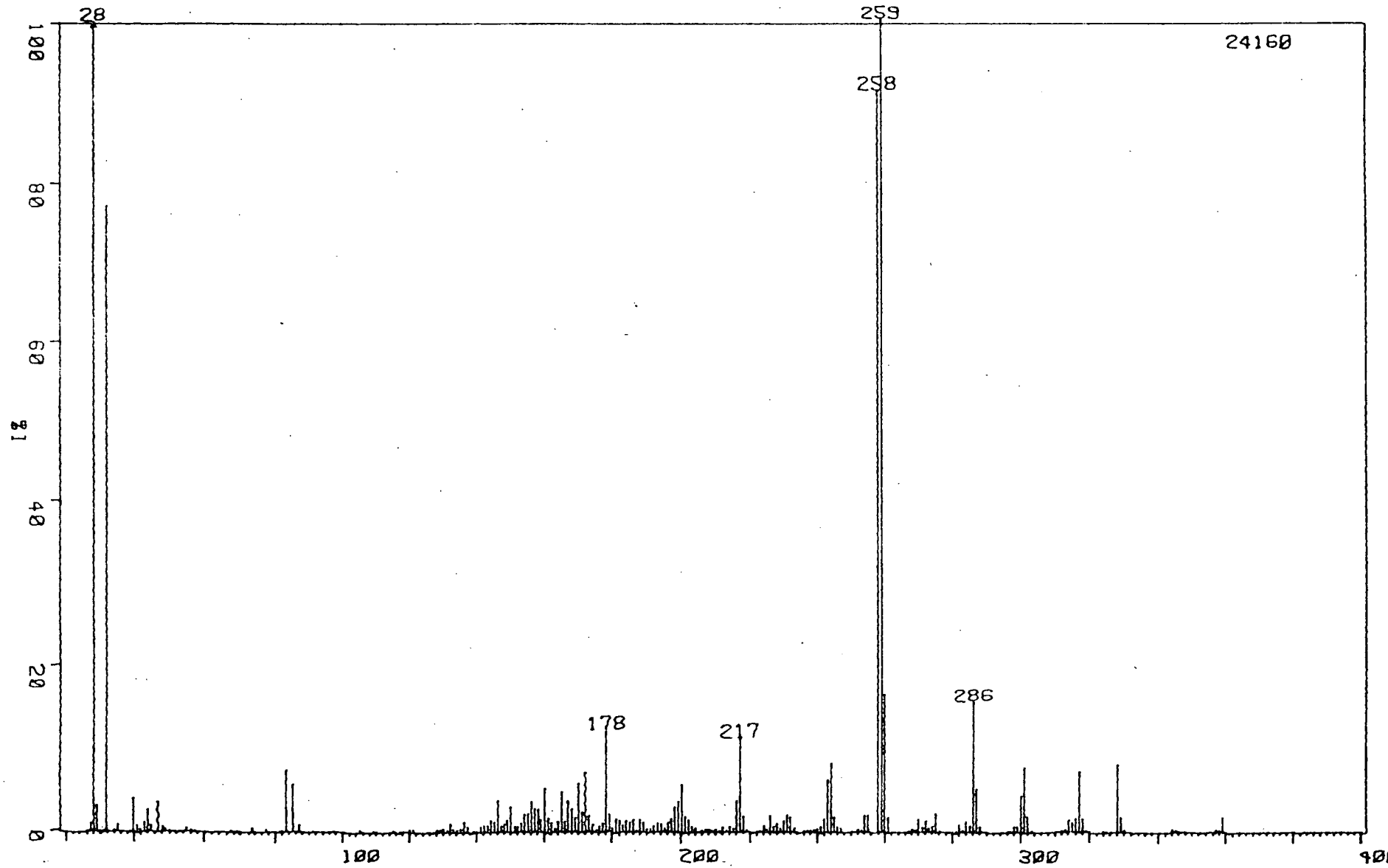
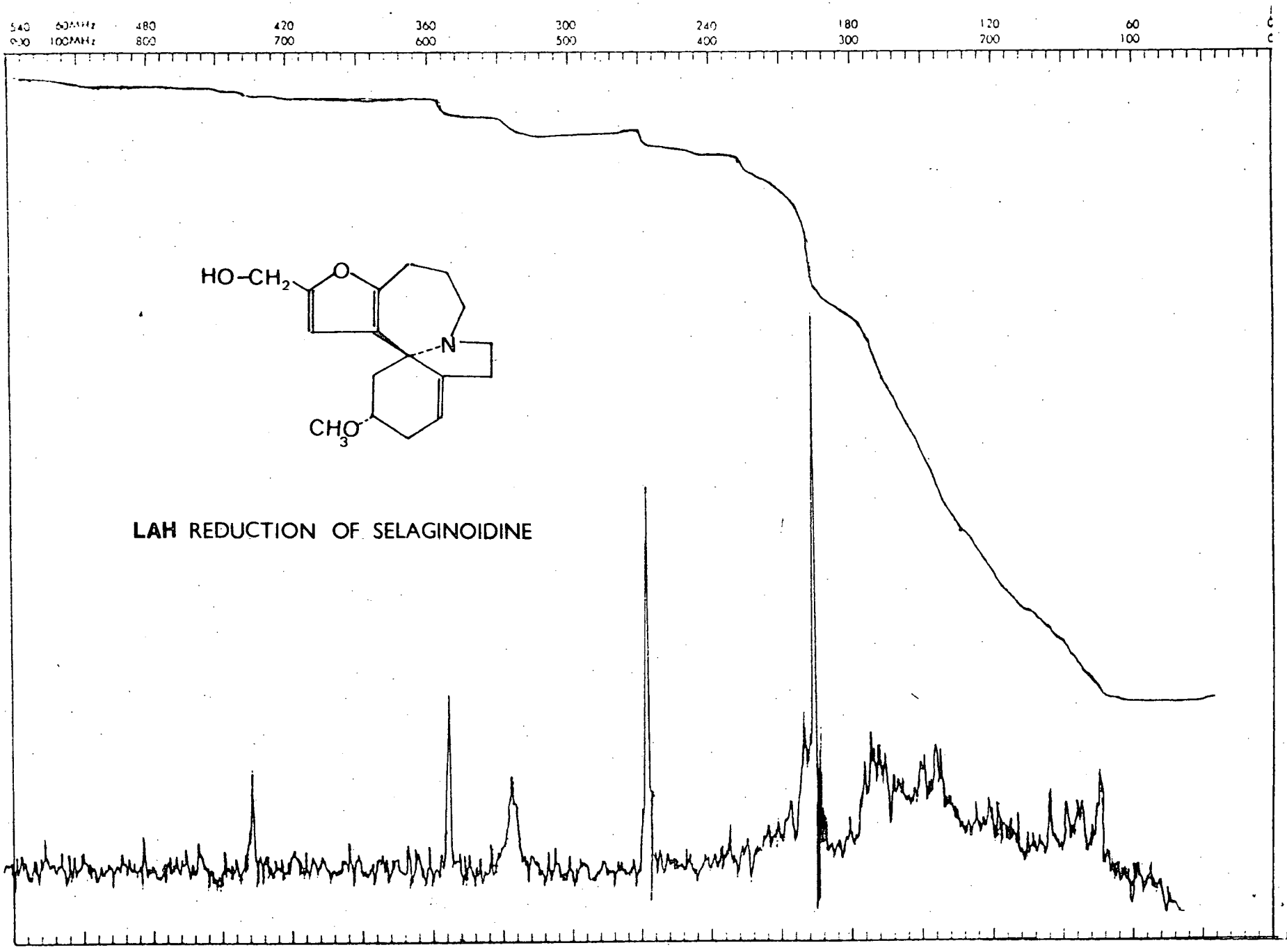


SELAGINOIDINE

SELAGINOIDINE

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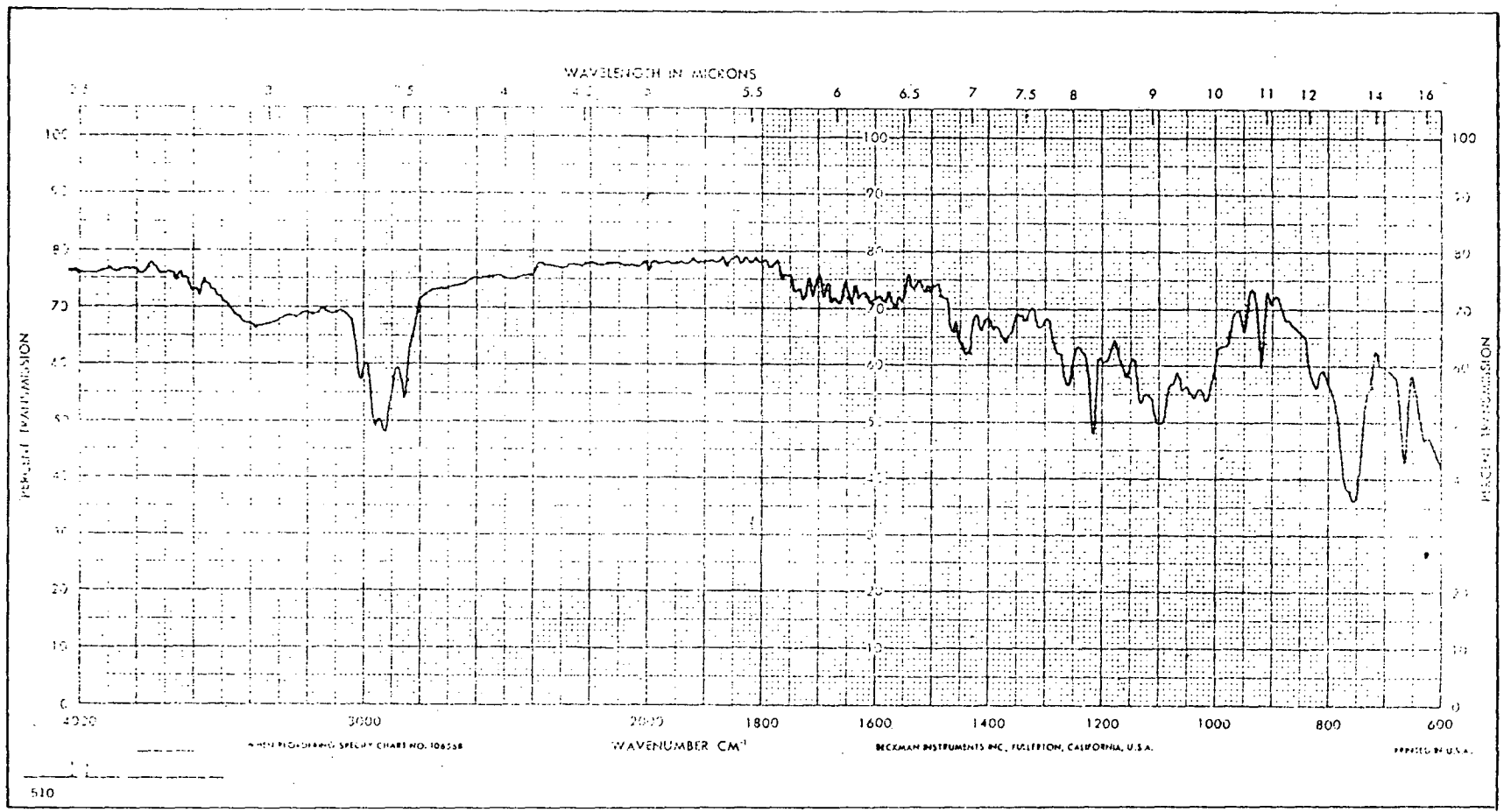
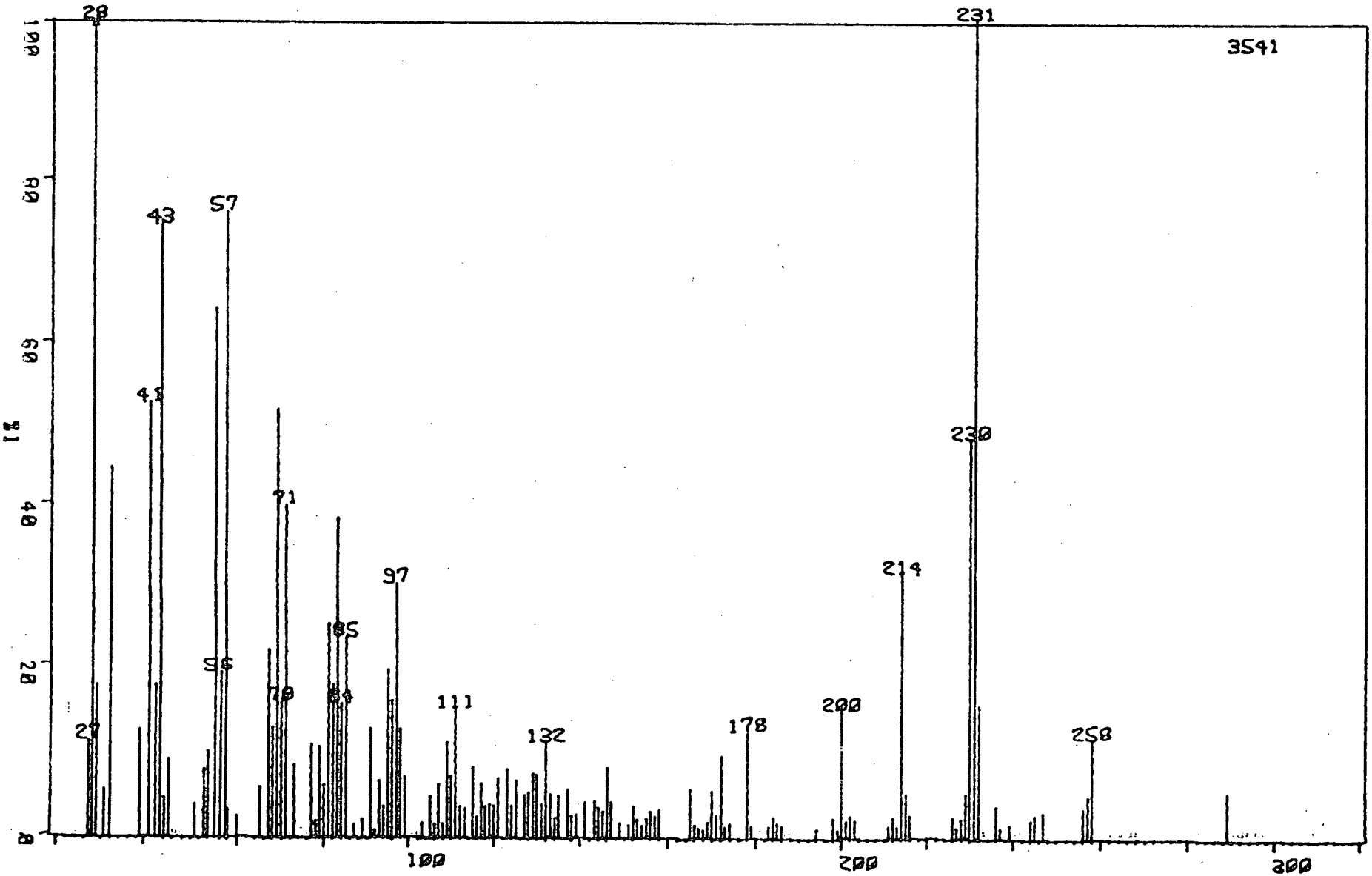
900 100MHz 800 700 600 500 400 300 200 100
HO-CH2
O
N
CH3O
LAH REDUCTION OF SELAGINOIDINE



LAH REDUCTION OF SELGINOIDINE

LAH REDUCTION OF SELAGINOIDINE



CHAPTER III

Attempted Synthesis of Homo*erythrina* Alkaloids

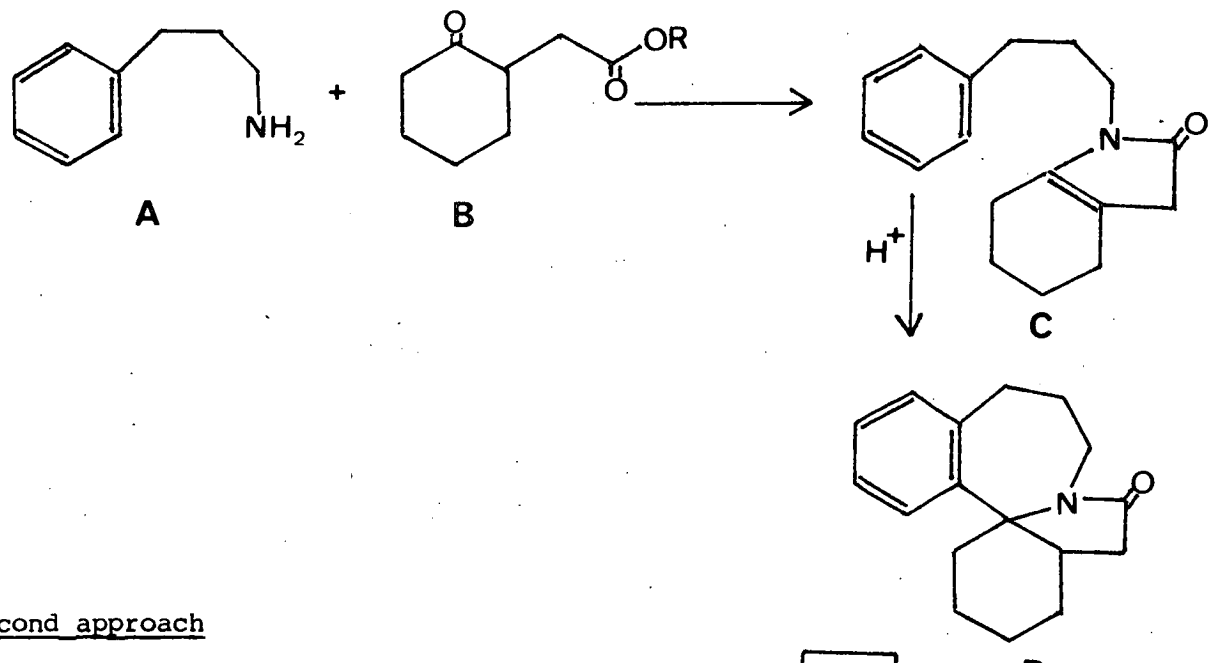
1. Results and Discussion
2. Experimental
3. References

Results and Discussion

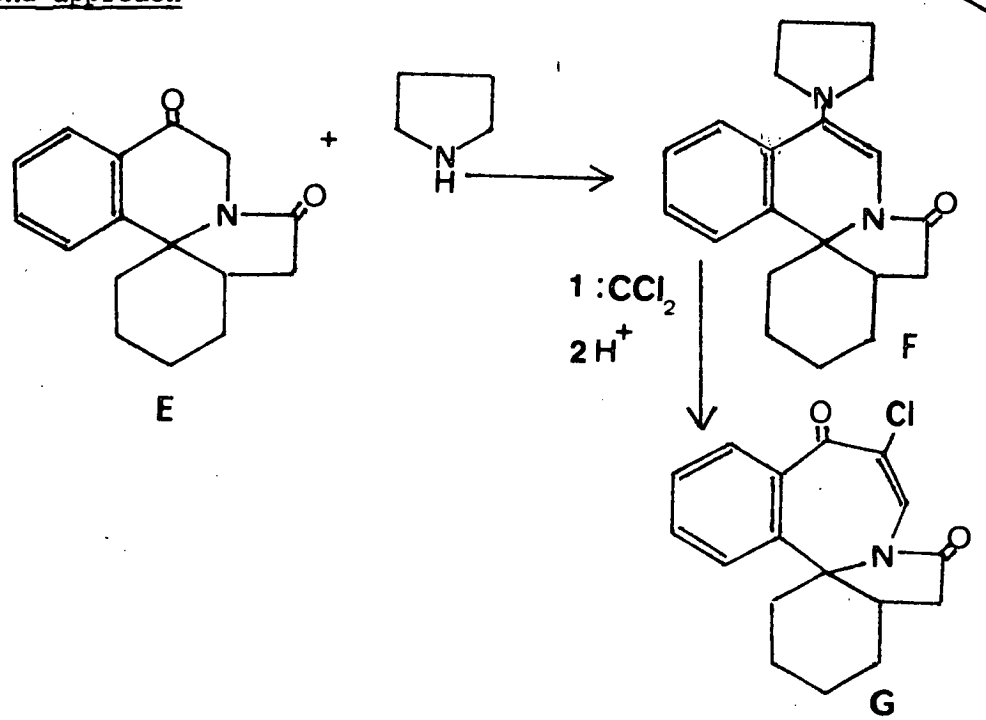
The synthesis of the homo*erythrina* alkaloid skeleton has been attempted by two approaches as shown in scheme I. The acid catalyst cyclisation of condensation product (C) of the amine (A) and the cyclohexanone ester (B) is the key step in the first approach. The ring expansion of the *erythrina* alkaloid nucleus by insertion of dichlorocarbene[7] to give the product (G) comprises the second approach.

Scheme I

First approach

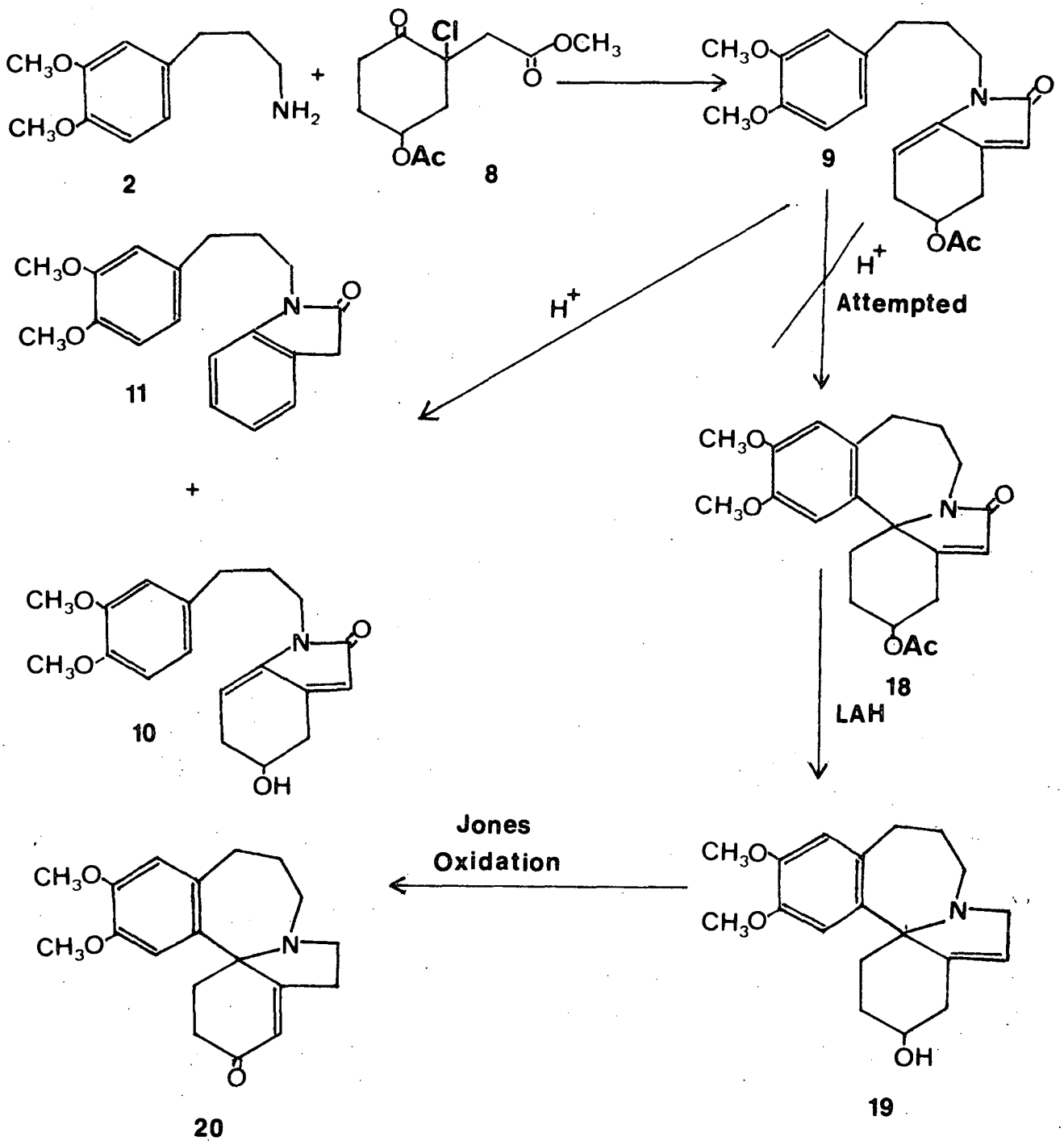


Second approach



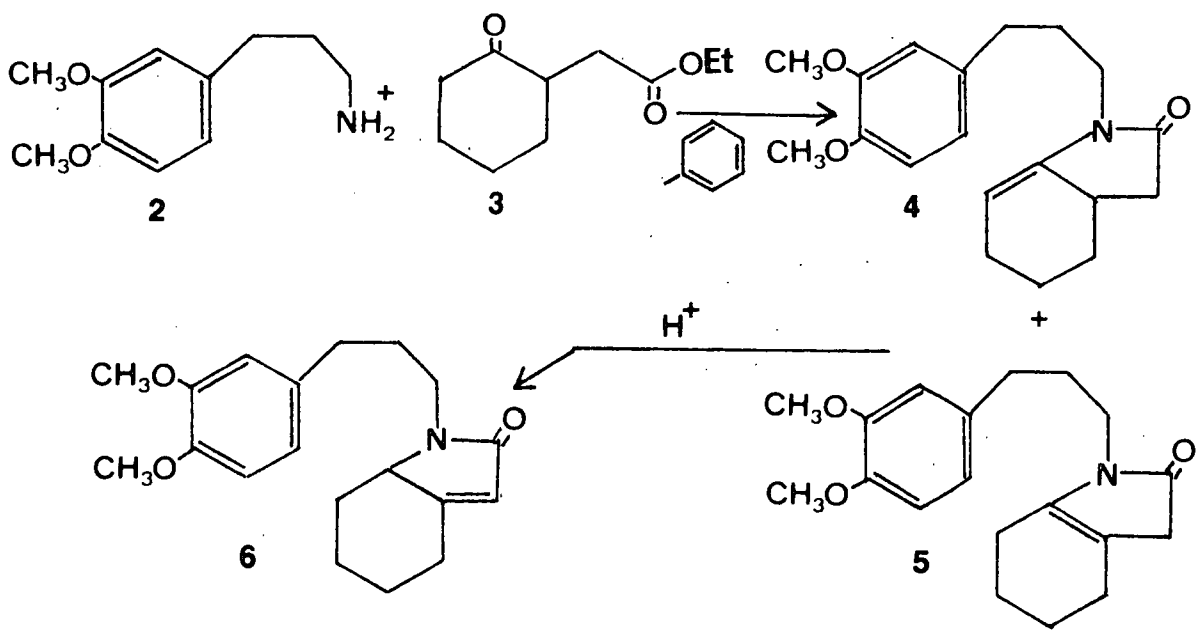
A synthesis of the homo*erythrina* alkaloid skeleton (20) was attempted by following the first approach as shown in scheme II.

Scheme II



The amine (2) was synthesised by hydrogenation of the corresponding phenylacrylonitrile (1) with Raney nickel. The α-chlorocyclohexanone ester (8) was obtained by alkylation[8] of the enamine of 4-acetoxycyclohexanone with methyl bromoacetate, followed by α-chlorination[9] with sulfuryl chloride in dry carbon tetrachloride. When the uncyclised product (9) obtained from the condensation was reacted with 85% phosphoric acid in methanol, the undesired products (10) and (11) of the amide, which were characterised by spectroscopic means, were formed. An attempt was made to cyclise the condensation product (9) by heating it with polyphosphoric acid under a nitrogen atmosphere, but only decomposition products were obtained by this method. Cyclisation with both types of acid catalysis has been successfully used for the *erythrina* alkaloids, and has been employed as a standard method for their synthesis[10,11]. The simplest model synthesis has in consequence been studied by the route shown in scheme III to investigate the reaction conditions.

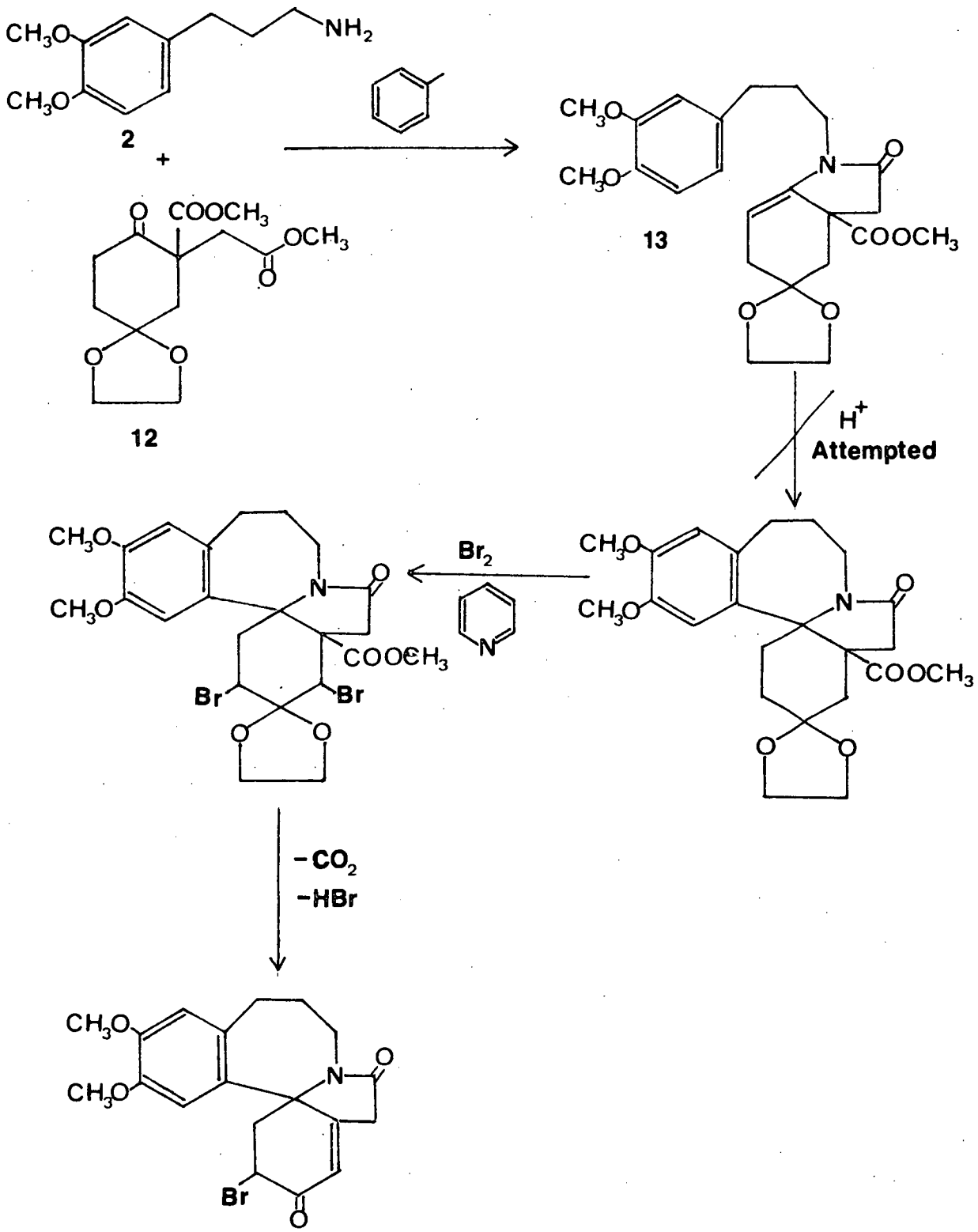
Scheme III



The condensation of the amine (2) and the cyclohexanone ester (3) gave the amide products (4) and (5); both compounds showed the same mass spectroscopic fragmentation, but only the amide (4) revealed a resonance from an olefinic proton (δ 4.2 ppm), which corresponded to the proton of an enamine system[12]. The mixture of amides (4) and (5) was treated successively with p-toluene sulfonic acid, boron trifluoride etherate, 85% phosphoric acid, and polyphosphoric acid. Most of these reagents gave the uncyclised product (6), but under the polyphosphoric acid conditions a decomposition product was obtained. The amide product (6) was characterised by its spectroscopic data: it showed a resonance signal from an olefinic proton at δ 5.62 ppm as a singlet, which distinguished it from the starting material.

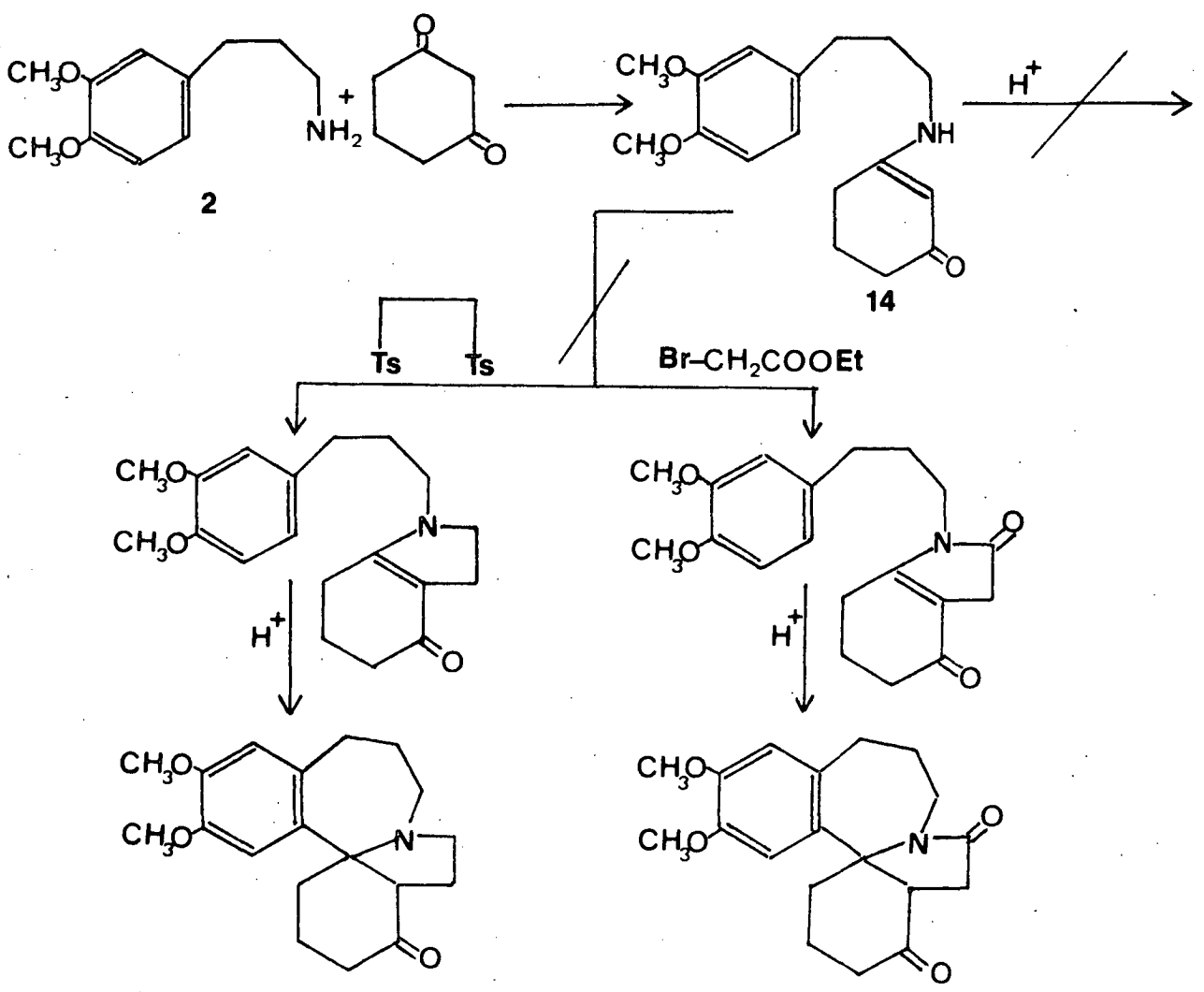
From the undesired products (10), (11), and (6), it could be concluded that the cyclohexanone esters (8) and (3) are not appropriate for this synthetic approach: a suitable cyclohexanone ester should have a blocking group to prevent the migration of the double bond and aromatization; at the same time, the group could be utilized to introduce the double bond in a later step. On the other hand, a double bond introduced at an early stage, as for instance in the amide (9), could increase the strain of the spiro-product (18), and these factors could contribute to the failure of the synthesis. With these points in mind, the cyclohexanone diester (12) was synthesised by alkylation of 2-carbomethoxy-4-ethylene-dioxycyclohexanone with methyl bromoacetate using sodium hydride as a base. This new synthetic approach was attempted using the cyclohexanone diester (12) as shown in scheme IV.

Scheme IV


CH3O
CH3O
NH2
2
+
COOCH3
OCH3
O
O
O
O
12
13
N
COOCH3
H+
Attempted
Br2
N
Br
Br
-CO2
-HBr

The amine (2) and the cyclohexanone diester (12) were refluxed in toluene using p-toluene sulfonic acid as catalyst. The uncyclised amide ester product (13) was obtained and characterised by spectroscopic means. Attempts were made to cyclise the amide ester (13) using boron trifluoride etherate as catalyst, and also by the use of polyphosphoric acid. The product was again decomposed by using polyphosphoric acid, and with boron trifluoride the starting material was recovered. There appeared to be problems involved not only with the strain in the spiro-product, and with the migration of the double bond, but also in the acid-catalysed cyclisation step. A Michael acceptor in the form of the enone amine (14) was in consequence introduced to activate the ene-system for the acid-catalysed cyclisation step, and a further synthetic approach was attempted by the route shown in scheme V.

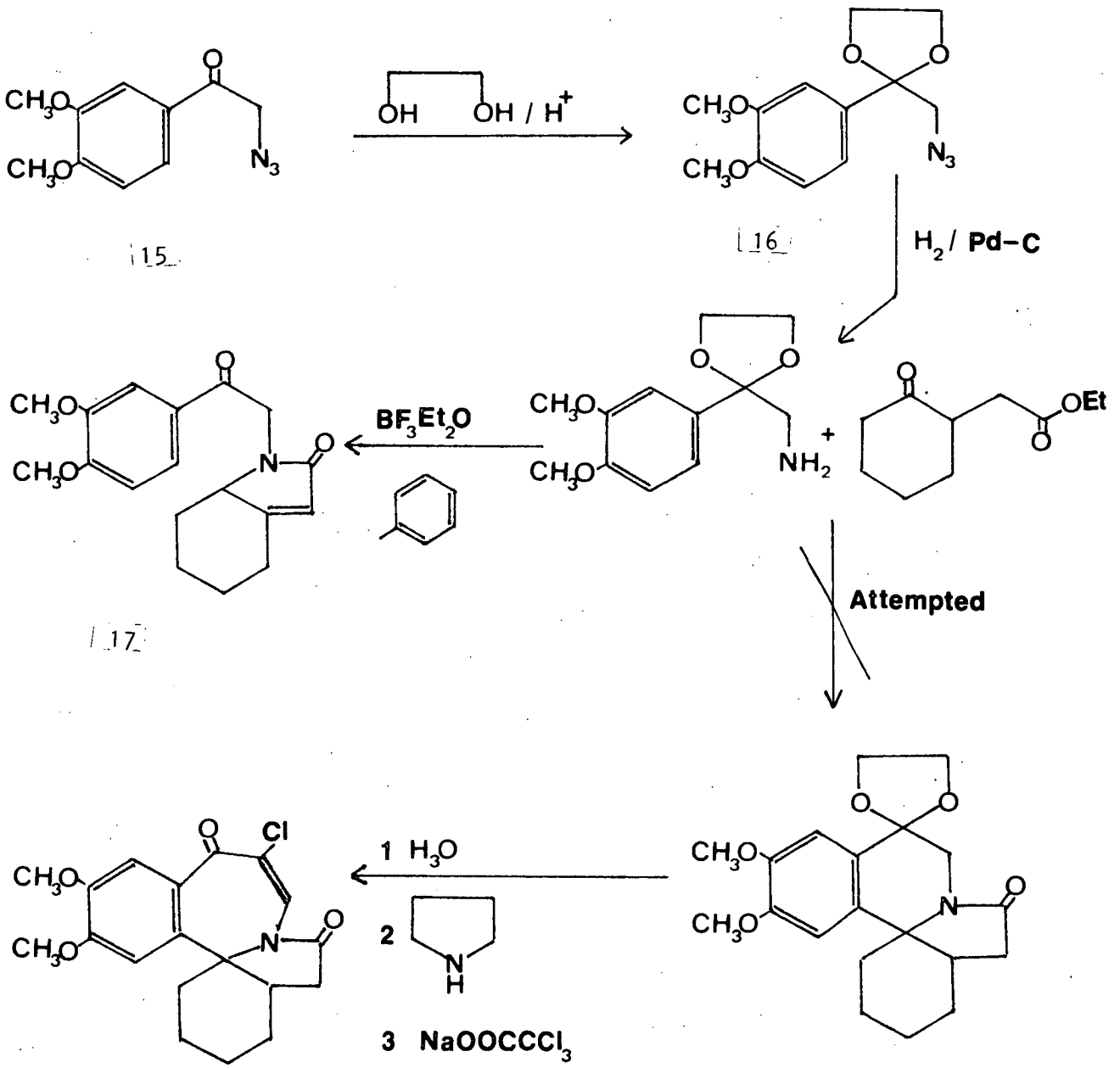
Scheme V



The amine (2) and 1,3-cyclohexadione were refluxed in toluene to yield the keto-enamine (14), which was characterised by spectroscopic means. An attempt was made to alkylate with ethyl bromoacetate using sodium hydride as a base, but only starting material was recovered, both with and without the use of base. The same result was likewise obtained by using ethylene glycol-ditosylate as the alkylating reagent. The keto-enamine (14) is a vinylogous amide and appeared to be very unreactive towards alkylation, so an attempt was made to form the ketal derivative by refluxing with ethylene glycol and p-toluene sulfonic acid in dry benzene, but starting material only was obtained. Finally, an attempt was made to cyclise the keto-enamine (14) using the same acid catalysts and conditions as in the previous approaches. The acid catalysts for the most part yielded starting material, but with poly-phosphoric acid only decomposition products were obtained. It must be concluded that the key step in the first synthetic approach seems an unlikely methodology to obtain the homo*erythrina* alkaloid skeleton.

A second synthetic approach was attempted by the route shown in scheme VI.

Scheme VI



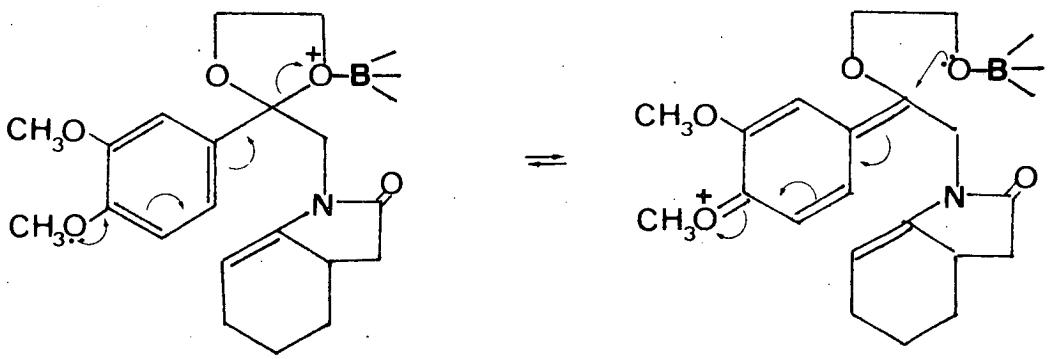
ω-Azido-3',4'-dimethoxyacetophenone, prepared by the method of Bretschneider and Hormann[6], was refluxed with ethylene glycol and p-toluene sulfonic acid in dry benzene to yield the ketal derivative (15). This ketal was hydrogenated over a palladium charcoal catalyst to give the ketalamine (16), which was characterised by spectroscopic means. The ketalamine was condensed with the cyclohexanone ester (3) in boiling toluene under a nitrogen atmosphere, and the crude product, after removal of the solvent, was stirred with boron trifluoride etherate in dry chloroform for 20 hours to yield the keto-amide (17). The ^1H nmr spectrum of this compound showed signals for an olefinic proton at δ 5.72 ppm, and for methylene protons adjacent to a benzoyl group as an AB system at δ 5.1 and 4.3 ppm, with a geminal coupling of 15 Hz. The mass spectrum of this compound showed a molecular ion peak at 315.1466, corresponding to the molecular formula $C_{18}H_{21}NO_4$; its infrared spectrum showed a carbonyl signal at 1670 cm^{-1}. Attempts were made to cyclise the condensation product obtained from the ketalamine (16) and the cyclohexanone ester (3) by the use of 85% phosphoric acid and polyphosphoric acid. Again the keto-amide (17) only was obtained.

From the resulting product (17), migration of the double bond and hydrolysis of the ketal protecting group seem to predominate over the acid catalyst cyclisation; with the use of boron trifluoride etherate as the acid catalyst, the lone pair electrons of the oxygen in the ketal protecting group probably form a complex with the boron atom, so that a carbonium intermediate is formed as shown in scheme VII.

The formation of this complex could be the reason for the deactivation of the aromatic ring, and in consequence the migration of the double bond is favoured. The protonation of the lone pair electrons of the oxygen may also be the reason for the failure when 85% phosphoric acid and polyphosphoric acid were used. The functionalised ketal group

seems to be inappropriate for the intended reaction sequence; on the other hand, the ketone functional group should perhaps be introduced at a later stage. Through lack of time, ring expansion of the *erythrina* alkaloid nucleus has not been further investigated.

Scheme VII



EXPERIMENTAL

Preparation of 3',4'-dimethoxyphenylacrylonitrile (1)

A mixture of verataldehyde (15.4 g, 0.1 mole), cyanoacetic acid (8.7 g, 0.1 mole), 3.0 g of ammonium acetate, 50 ml of toluene, and 50 ml of pyridine was refluxed with a Dean and Stark water trap for two days, then the solvent was removed by evaporation under reduced pressure. The residue was treated with 5% ammonium hydroxide solution (100 ml) and extracted with chloroform (3x250 ml). The combined extracts were dried ($NaSO_4$) and evaporated to obtain the crude product, which crystallised from chloroform petroleum ether (bp 40-60^{o}) as yellow plates (15 g, 79% yield); mp 88-89^{o}, (Lit. 1, mp 88-89^{o}); γ max (nujol): 2960, 2900, 2850, 2220, 1600, 1580, 1520, 1460, 1360, 1270, 1230, 1200, 1160, 1140, 1040, 1020, 960, 800, 750 cm^{-1}; λ max: 215 (6815), 235(7323), 293(8741), 323 nm(10395); ^{1}H nmr: 7.2-6.82(m, AB system, J_{AB}=15 Hz, 2H), 6.9-5.65(m, AB system, J_{AB}=17.5 Hz, 2H), 6.85 (s, 1H), 3.9(s, 6H); m/z: 189(M^{+}, 100), 174(35), 146(25), 118(15), 103(15), 91(15), 76(18).

Preparation of 3-(3',4'-dimethoxyphenyl)propylamine (2)

3',4'-Dimethoxypheylacrylonitrile (1.0 g, 0.005 mole) in methanol (20 ml) and 10 ml ammonium solution (d 0.88) was hydrogenated at room temperature and 60 lb/in^2 over one spatula of Raney nickel until no more hydrogen was absorbed (ca. 10 hours). The reaction mixture was filtered to remove the catalyst, then extracted with chloroform (3x100 ml). The combined extracts were washed with water (100 ml), dried ($NaSO_4$), and evaporated *in vacuo* to obtain the amine (2), (0.9 g, 92.7% yield); γ max (neat); broad peak 3350, 3000, 2920, 2850, 1600, 1580, 1500, 1450, 1420, 1250, 1230, 1150, 1140, 1020, 800, 750 cm^{-1}; λ max: 234(4920), 280 nm (3342); ^{1}H nmr: 6.62(bs, 3H), 3.82(s, 6H), 2.65(m, 4H), 1.75(m, 2H),

1.3(s, 2H, exchangeable with D_2O): m/z: 195(M^+, 60), 178(100), 163(30), 152(60), 151(100), 147(40), 121(60). (Spectroscopic data: Lit. 2).

Reaction of 3-(3',4'-dimethoxyphenyl)propylamine (2) with 2-ethoxycarbonyl-methylcyclohexanone (3)

The amine (2) (0.195 g, 1.0 mole), the cyclohexanone ester (3) (0.184 g, 1.0 m mole), and 3 g of anhydrous sodium sulfate were refluxed in 50 ml of toluene for about 12 hours. The sodium sulfate was filtered off, the solvent was evaporated *in vacuo*, and the residue was separated by ptlc (5% MeOH/$CHCl_3$, double development) to yield two products. The lower *Rf* material proved to be the amide (5) (131.0 mg, 42.8% yield); γ max: 3000, 2920, 2850, 1700, 1680, 1590, 1510, 1460, 1410, 1260, 1150, 1140, 1030, 800 cm^{-1}; λ max: 235(6120), 278 nm(5850); ^1H nmr: 6.60(bs, 3H), 3.75(s, 6H), and an unresolved number of protons between 1 and 3.5 ppm; m/z: 315(M^+, 30), meas.: 315.1833, calc. for $C_{19}H_{25}NO_3$: 315.1833, 313(15), 178(40), 177(35), 151(100), 149(20), 123(20), 122(15).

The higher *Rf* product proved to be the amide (4) (21.6 g, 7% yield); γ max: 3000, 2900, 2850, 1700, 1680, 1590, 1510, 1460, 1410, 1260, 1150, 1140, 1030, 800 cm^{-1}; λ max: 235(6115), 278 nm(5850); ^1H nmr: 6.70(bs, 3H), 4.2(bs, 1H), 3.8(s, 6H), and an unresolved number of protons between 1 and 3.5 ppm; m/z: 315(M^+, 25), meas.: 315.1833, calc. for $C_{19}H_{25}NO_3$: 315.1834, 313(2), 178(20), 151(100), 123(25), 122(20).

Reaction of 3-(3',4'-dimethoxyphenyl)propylamine (2) with 2-ethoxycarbonyl-methylcyclohexanone (3) in the presence of acid catalyst

The amine (2) (0.10 g, 0.5 m mole), the cyclohexanone ester (3) (0.10 g, 0.5 m mole) and 1 g of p-toluene sulfonic acid were refluxed in 50 ml of toluene for about 12 hours. The solvent was evaporated *in vacuo* and the residue was treated with chloroform and water. The organic layer was washed with 5% ammonium hydroxide solution, dried ($NaSO_4$), and

evaporated to yield the crude product (0.10 g), which was purified by ptlc (5% MeOH/$CHCl_3$) to obtain the amide (6), *Rf*=0.5, (0.04 g, 30% yield); γ max: 3000, 2940, 2850, 1670, 1520, 1450, 1410, 1320, 1260, 1150, 1140, 1020, 850 cm^{-1}; λ max: 238(2520), 278 nm(2385); ^1H nmr: 6.60(s, 3H), 5.62(bs, 1H), 3.7(s, 6H), and an unresolved number of protons between 1 and 3.5 ppm; m/z: 315(M^+, 45), meas.: 315.1833, calc. for $C_{19}H_{25}NO_3$: 315.1834, 178(18), 151(100), 150(30), 149(25), 123(30), 122(30).

Reaction of a mixture of the amides (4) and (5) with 85% phosphoric acid

A mixture of amides (4) and (5) (150 mg, 0.5 m mole) from the condensation of the amine (2) and the cyclohexanone ester (3) was dissolved in 100 ml of methanol and 100 ml of 85% phosphoric acid. The mixture was refluxed for 10 hours under a nitrogen atmosphere, poured into ice water, and extracted with chloroform (3x100 ml). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by ptlc (5% MeOH/$CHCl_3$) to yield the amide (6), *Rf*=0.5, (80.0 mg, 53% yield). (The product was identical with the amide (6) as shown by comparison of their physical data).

Reaction of a mixture of amides (4) and (5) with polyphosphoric acid

A mixture of the amides (4) and (5) (150 mg, 0.5 m mole) was dissolved in 50 ml of polyphosphoric acid and heated at 130° in an oil bath under nitrogen for 2 hours. The reaction mixture was cooled to room temperature, then treated with water (100 ml). The solution was extracted with chloroform (3x100 ml), dried ($MgSO_4$), and evapored *in vacuo*. The residue (25 mg), from tlc, contained polar decomposition products only which remained at the base line, and the crude product was in consequence not purified to characterise the structure.

Preparation of quinitol diacetate

Hydroquinone (220 g, 2 mole) in methanol (500 ml) was hydrogenated at 150° and 120 atm over Raney nickel (20 g) until no more hydrogen was absorbed (ca. 40 hours). The reaction mixture was treated with 500 ml of methanol, and the catalyst was filtered off. The filtrate was evaporated under reduced pressure to obtain cyclohexane 1,4 diol (130 g), which was dissolved in acetic anhydride (400 g) and refluxed for 4 hours. The solution was poured into ice water to obtain a precipitate of the product, which was filtered off and recrystallised from acetone to yield white needles (200 g, 50% yield); mp 102-103° (Lit. 3, 102-103°); γ max: 2980, 2900, 2850, 1720, 1520, 1360, 1240, 1050, 950, 900 cm^{-1}; ^1H nmr: 4.8(m, 2H), 2.08(s, 6H), and an unresolved number of protons between 1.5 and 2.0 ppm.

Preparation of quinitol monoacetate

Potassium hydroxide (11 g, pure) in 50% methanol/water (50 ml) was added dropwise during 5 minutes to quinitol diacetate (50 g, 0.25 mole) dissolved in 50% methanol/water (350 ml) at 40-50° with vigorous stirring. The methanol was evaporated *in vacuo*, and the aqueous solution which remained was extracted with chloroform (5x100 ml). The combined extracts were washed with 5% ammonium chloride solution (100 ml), dried ($MgSO_4$), and evaporated. The crude product so obtained was recrystallised from petroleum ether bp 40-60° (25 g, 60% yield), mp 68-72° (Lit. 3, mp 68-72°); γ max:: 3400, 2940, 2850, 1740, 1450, 1370, 1360, 1250, 1070, 1040, 950, 750 cm^{-1}; ^1H nmr: 4.75(m, 1H), 3.75(m, 1H), 2.05(s, 3H), and an unresolved number of protons between 1.3 and 2.0 ppm.

Preparation of 4-acetoxycyclohexanone

Quinitol monoacetate (22 g, 0.14 mole) in glacial acetic acid (50 ml) was oxidised with chromic acid (12 g) in acetic acid (12 ml of

glacial acetic acid and 8 ml of water) at 0°. The solution was allowed to stand overnight, then treated with water (100 ml), and extracted with chloroform (5x100 ml). The combined extracts were washed with a little water, dried (Na_2SO_4), and evaporated under reduced pressure to a yellowish oil, which was distilled to obtain a colorless liquid (15 g, 70% yield), bp 235° (Lit. bp 235°); γ max(neat): 3000, 2950, 2850, 1740, 1430, 1370, 1250, 1120, 1080, 1050, 950, 880 cm^{-1}; ^1H nmr: 5.6(m, 1H), 2.05(s, 3H), and an unresolved number of protons between 1.5 and 2.5 ppm.

Preparation of 2-methoxycarbonylmethy-4-acetoxycyclohexanone (7)

A mixture of 4-acetoxycyclohexanone (15.6 g, 0.1 mole) and pyrrolidine (7.2 g, 0.1 mole) in benzene (100 ml) was refluxed for 6 hours while water was azeotropically removed by a Dean-Stark apparatus. The solvent was evaporated under reduced pressure, and the residue was dissolved in THF (100 ml); the solution was treated with methyl-2-bromoacetate (15.3 g, 0.1 mole) dropwise under a nitrogen atmosphere at room temperature. After addition was completed, the reaction mixture was refluxed for 6 hours, treated with water (100 ml), then again refluxed for 10 hours. The mixture was extracted with chloroform (5x100 ml), and the combined extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. The crude product so obtained was distilled to yield a colorless liquid (13 g, 60% yield); bp 150°, 1.5 mm Hg; γ max(neat): 2960, 2860, 1740, 1440, 1370, 1300, 1120 cm^{-1}; ^1H nmr: 5.17(m, 1H), 3.65(s, 3H), 2.13 and 2.03(s, 3H), and an unresolved number of protons between 1.5 and 3.0 ppm; m/z: 228 (M^+, 1), 209(4), 208(3), 136(100).

Preparation of 2,2-chloro,methoxycarbonylmethyl-4-acetoxycyclohexanone (8)

A mixture of the ketone ester (7) (228 mg, 1.0 m mole) and sulfuryl

chloride (140 mg, 1.1 m mole) in dry carbon tetrachloride (500 ml) was stirred at room temperature under a nitrogen atmosphere for 4 hours, then treated with water and chloroform. The organic layer was washed with 5% ammonium hydroxide (50 ml), dried ($MgSO_4$), and evaporated under reduced pressure to give the product (8) which was used immediately in the following reaction.

Reaction of 3-(3',4' methoxyphenyl)propylamine (1) with 2,2-chloro, methoxycarbonylmethyl-4-acetoxycyclohexanone (8)

A mixture of the amine (2) and the ketone ester (8) was refluxed in toluene (100 ml) under a nitrogen atmosphere for 12 hours. The solvent was evaporated *in vacuo*, and the residue was purified by ptlc (5% MeOH/$CHCl_3$) to give the acetoxyamide (9) as a colorless oil, R*f*=0.6 (250 mg, 67% yield); λ max: 210(17808), 230 nm(17808), 274 nm(19786); γ max(neat): 3000, 2940, 2840, 1740, 1680, 1650, 1600, 1580, 1500, 1450, 1430, 1250, 1150, 1140, 1100, 1030 cm^{-1}; ^1H nmr: 6.68(s, 3H), 5.78(s, 1H), 5.13(m, 1H), 3.86(s, 3H), 2.0(s, 3H), and an unresolved number of protons between 1.5 and 3.8 ppm; m/z: 371(M^+, 60), 369(10), 311(65), 207(10), 178(55), 165(40), 164(20), 147(100), 146(55), 118 (45).

Reaction of the acetoxyamide (9) with 85% phosphoric acid

The acetoxyamide (9) (180 mg, 0.5 m mole) was dissolved in 100 ml of 85% phosphoric acid. The reaction mixture was refluxed for 10 hours under a nitrogen atmosphere, poured into ice water, then extracted with chloroform (3x100 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by ptlc (2% MeOH/$CHCl_3$) to yield two fractions. The product with R*f*=0.5 (100 mg, 32% yield) proved to be the amide (10); λ max: 230(5183), 252(4561), 280 nm(2920); γ max: 3000, 2940, 2840, 1700, 1610, 1510, 1480, 1460, 1360, 1260, 1210, 1150, 1140, 1030 cm^{-1}; ^1H nmr:

7.2-6.9(m, 4H), 6.72(bs, 3H), 3.8(s, 6H), 3.7(t, J=7.5 Hz, 2H), 1.98(m, 2H); m/z: 311(M^+, 80), meas.: 311.1519, calc. for $C_{19}H_{21}NO_3$: 311.1521, 178(35), 165(40), 152(35), 151(40), 147(100), 118(55), 91(35).

The product with Rf=0.2 (45 mg, 13.6% yield) proved to be the amide (11); λ max: 230(11634), 278 nm(8115); γ max: 3400, 3020, 2940, 1680, 1510, 1450, 1420, 1260, 1210, 1150, 1030 cm^{-1}; ^1H nmr: 6.67(s, 3H), 5.75(s, 1H), 5.45(m, 1H), 4.05(m, 1H), 3.8(s, 6H), and an unresolved number of protons between 1.5 and 3.8 ppm; m/z: 329(M^+, 45), meas.: 329.1541, calc. for $C_{19}H_{23}NO_4$: 329.1545, 328(35), 311(10), 285(10), 178(35), 177(40), 165(100), 164(10), 153(40), 136(40), 107(10).

Preparation of dimethyl-γ-ketopimelate

Through a solution of furylacrylic acid (100 g, 0.72 mole) in methanol (400 ml), dry hydrogen chloride gas was passed at a rapid rate with stirring. As soon as the solution reached the boiling point, the passage of gas was diminished to a rate which provided only a small flow from the top of the reflux condenser. Meanwhile the solution was maintained at the boiling point through application of heat when necessary. At the end of 4 hours the solution was concentrated to one-fourth volume *in vacuo*. Benzene (1 liter) was added to the residue, and the distillation was continued at atmospheric pressure until the vapour temperature reached 80°, then the remainder of the benzene was removed *in vacuo*. To the residue was added methanol (500 ml) and 1 ml of 96% sulfuric acid, and the reaction mixture was refluxed for 16 hours. At the end of this time, 300 ml of methanol were distilled off *in vacuo* and the residue was dissolved in benzene (1 liter); the benzene solution was washed with 1 N sodium carbonate solution in 200 ml portions until the aqueous layer remained alkaline, and then with water (200 ml). The benzene solution was dried ($MgSO_4$) and evaporated, and the residue was crystallised to give the required product (75 g, 51% yield), bp 80°,

1.0 mm Hg (Lit. 4, bp 90-93°, 0.1 mm Hg), mp 49-50°, (Lit. 4, mp 49-50°); γ max: 2940, 2900, 2840, 1730, 1700, 1450, 1400, 1370, 1310, 1200, 1100, 960, 850, 800 cm^{-1}; ^1H nmr: 3.63(s, 6H), 2.68(m, 8H).

Preparation of dimethyl-γ-ethylenedioxypimelate

A mixture of dimethyl-γ-ketopimelate (50 g, 0.25 mole), redistilled ethylene glycol (17.0 g, 0.26 mole), and p-toluene sulfonic acid (100 mg) was refluxed in benzene (150 ml) for 13 hours while water was azetropically removed by a Dean and Stark apparatus. Benzene (150 ml) was added to the reaction mixture, and the resulting solution was washed with saturated sodium carbonate (25 ml), then with water (100 ml), and dried ($MgSO_4$). The solvent was evaporated under reduced pressure, and the crude product was purified by distillation (45 g, 73.1% yield), bp 80°, 0.8 mm Hg, (Lit. 4, bp 96-98°, 0.08 mm Hg); γ max(neat): 2950, 1740, 1630, 1500 cm^{-1}; ^1H nmr: 3.85(bs, 4H), 3.6(s, 6H), 2.35(t, J=7.5 Hz, 4H), 1.92(t, J=7.5 Hz, 4H).

Preparation of 2-carbomethoxy-4-ethylenedioxycyclohexanone

Small pieces of sodium metal (0.46 g, 0.02 mole) were suspended in toluene (150 ml) and a solution of dimethyl-γ-ethylenedioxypimelate (4.92 g, 0.02 mole) in toluene (10 ml) was added to the suspension at room temperature under a nitrogen atmosphere. The reaction mixture was refluxed for 5 hours, then glacial acetic acid was until neutral. The resulting solution was extracted with chloroform (3x100 ml) and the combined extracts were washed with saturated sodium carbonate until the aqueous layer remained alkaline. The solution was dried ($MgSO_4$) and evaporated under reduced pressure; the crude product so obtained was purified on a column of silica gel (100 g) prepared in chloroform and eluted with chloroform. The highest Rf fraction contained the desired product (3.0 g, 70% yield); mp 60° (Lit. 4, mp 60-61°);

γ max: 2960, 2880, 1740, 1710, 1650, 1450, 1350, 1300, 1230, 1200, 1150, 1130, 1070, 1000, 950 cm^{-1}; ^1H nmr: 3.95(s, 4H), 3.68(s, 3H), 2.45(m, 3H), 1.82(t, J=7.5 Hz, 4H).

Preparation of 2,2-carbomethoxy,methoxycarbonylmethyl-4-ethylenedioxy-cyclohexanone

2-Carbomethoxy-4-ethylenedioxycyclohexanone (10.7 g, 0.05 mole) was dissolved in THF (20 ml) and added to a suspension of sodium hydride (1.27 g, 0.05 mole) in THF (100 ml) at room temperature under a nitrogen atmosphere. The reaction mixture was stirred overnight, then water was added to destroy the excess of sodium hydride. The resulting solution was extracted with chloroform (5x100 ml), and the combined extracts were washed with water (100 ml), dried ($MgSO_4$), and evaporated under reduced pressure. The crude product so obtained was purified on a column of silica gel (120 g) prepared in chloroform and eluted with chloroform. The highest *Rf* fraction contained the desired product (10 g, 70% yield); γ max: 2950, 2900, 1740, 1430, 1350, 950 cm^{-1}; ^1H nmr: 3.95(bs, 4H), 3.72(s, 3H), 3.62(s, 3H), and an unresolved number of protons between 1.8 and 3.5 ppm; m/z: 286(M^+, 10), meas.: 286.1047, calc. for $C_{13}H_{18}O_7$: 286.1052, 255(10), 229(10), 159(35), 123(10), 100(50), 99(100).

Reaction of 3-(3',4'-dimethoxyphenyl)propylamine (1) with 2,2-carbomethoxy-methoxycarbonylmethyl-4-ethylenedioxycyclohexanone (12) in the presence of p-toluene sulfonic acid

A mixture of the amine (1) (195 mg, 1 m mole), the ethylenedioxy-ketone diester (12) (286 mg, 1.0 m mole), and p-toluene sulfonic acid (100 mg) was refluxed in toluene (100 ml) under a nitrogen atmosphere for 12 hours. The solvent was evaporated off, and the residue was treated with chloroform and water. The organic layer was washed with 5% ammonium hydroxide (100 ml) and water (100 ml), then dried ($MgSO_4$) and evaporated.

The residue was purified by ptlc (5% MeOH/$CHCl_3$) to yield the product (13), Rf=0.5 (200 mg, 46% yield); λ max: 232(4501), 280 nm(1053); γ max: 2950, 2850, 1740, 1680, 1510, 1450, 1300, 1280, 1260, 1230, 1050, 1030, 950, 880, 850, 810 cm^{-1}; ^1H nmr: 6.72(s, 3H), 4.87(m, 1H), 3.9(s, 4H), 3.7(s, 4H), 3.62(s, 3H), and an unresolved number of protons between 1.5 and 3.5 ppm; m/z: 341 (M^+, 60), meas. 431.1935, calc. for $C_{23}H_{29}NO_7$: 431.1944, 387(15), 345(80), 330(50), 313(45), 217(30), 181(40), 178(50), 177(50), 164(30), 151(100), 147(20), 136(30), 121(30).

Reaction of the ethylenedioxyester amide (13) with boron trifluoride etherate

The ethylenedioxyester amide (13) (200 mg, 0.46 m mole) and boron trifluoride etherate (2 ml) were refluxed in dry chloroform (100 ml) under a nitrogen atmosphere for 6 hours, then the solvent was evaporated off under reduced pressure. The residue was dissolved in water (100 ml) and extracted with chloroform (3x100 ml). The combined extracts were washed with 5% ammonium hydroxide (100 ml) and with water (100 ml), then dried ($MgSO_4$), and evaporated under reduced pressure to obtain a product which proved to be the starting material.

Preparation of 1,3-cyclohexanedione

A mixture of sodium hydroxide (24.0 g, 0.6 mole), water (100 ml), and resorcinol (55 g, 0.5 mole) was placed in bomb hydrogenator together with W2 Raney nickel (100 g). The pressure in the bomb was raised to 1000-1500 lb/in^2 with hydrogen, and the temperature was adjusted to 50°. The bomb was shaken and the reaction allowed to proceed for 10 hours, during which time 0.5 mole of hydrogen was absorbed. The apparatus was allowed to cool to room temperature, the pressure was released, and the catalyst was removed by filtration. The filtrate was acidified with concentrated hydrochloric acid, and the solution was cooled to 0° in an ice-salt bath and held at that temperature for 30 minutes before filtration. The crude product

containing sodium chloride was dissolved in 125-150 ml of hot benzene, then the solution was filtered to remove the sodium chloride and allowed to crystallise (53 g, 95% yield), mp 103-104°; (Lit. 5, mp 103-104°).

Reaction of 3-(3',4'-dimethoxyphenyl)propylamine (2) with a mixture of 1,4-cyclohexanedione and ethyl bromoacetate

A mixture of the amine (1) (1.0 g, 5.0 m mole) and 1,3-cyclohexanedione (0.54 g, 5.0 m mole) was stirred at 35-40° in toluene (75 ml) until the solution was clear, then ethyl bromoacetate (0.72 g, 5.0 m mole) was added, and the reaction mixture was refluxed for 12 hours under a nitrogen atmosphere. The solvent was evaporated *in vacuo* to obtain the crude product, which was separated on a column of silica gel (100 g) prepared in chloroform and eluted with chloroform. Two fractions were obtained: the higher *Rf* fraction proved to be ethyl bromoacetate, and the lower *Rf* fraction was evaporated *in vacuo* to obtain the ketone enamine (14) as a colorless oil (0.90 g, 62% yield); λ max: 205(1445), 236(505), 285 nm(2528; γ max: 3250, 3050, 3000, 2950, 1600, 1550, 1360, 1320, 1250, 1200, 1140, 1030 cm^{-1}; ^1H nmr: 6.65(m, 3H), 5.63(bs, 1H exchangeable with D_2O), 5.0(s, 1H), 3.8(s, 6H), 3.05 (m, 2H), 2.6(m, 2H), 2.27(m, 4H), 1.8(m, 4H); m/z: 289(M^+, 50), meas.: 289.1702, calc. for $C_{17}H_{23}NO_3$: 289.1678, 288(15), 274(15), 178(25), 164(15), 163(10), 151(20), 147(15), 125(100), 124(10), 91(10), 78(35).

Reaction of 3-(3',4'-dimethoxyphenyl)propylamine (2) with a mixture of 1,3-cyclohexanedione and ethylene glycol ditosylate

A mixture of the amine (1) (1.0 g, 5.0 m mole) and 1,3-cyclohexanedione (0.54g, 5.0 m mole) was stirred at room temperature in chloroform (100 ml) for one hour, then ethylene glycol ditosylate (1.82 g, 5.0 m mole) was added. The reaction mixture was refluxed

under a nitrogen atmosphere for 3 hours, then ammonia solution (d 0.88) was added. The reaction mixture was extracted with chloroform (3x100 ml) and the combined extracts were washed with water (3x100 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The crude product so obtained was separated on a column of silica gel (100 g) prepared in chloroform and eluted with chloroform. The first fraction (250 ml) was evaporated under reduced pressure to obtain the product (14) (850 mg, 60% yield), identical with the ketone enamine (14) as shown by comparison of their physical data (nmr, ir, uv, ms).

Reaction of the ketone enamine (14) with ethylene glycol ditosylate in the presence of sodium hydride

A mixture of the ketone enamine (14) (289 mg, 1 m mole), and sodium hydride (100 mg, 2 m mole) suspended in THF (30 ml) was stirred at room temperature under a nitrogen atmosphere for 1 hour, then ethylene glycol ditosylate (370 mg, 1 m mole) was added. The reaction mixture was stirred under these conditions overnight, then treated with ethanol to destroy excess of sodium hydride. The resulting solution was extracted with chloroform (3x100 ml), and the combined extracts were washed with water (200 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The crude product so obtained was separated by ptlc (7% MeOH/$CHCl_3$) to give starting material only.

Reaction of the ketone enamine (14) with ethylene glycol in the presence of p-toluene sulfonic acid

A mixture of the ketone enamine (14) (289 mg, 1.0 m mole), ethylene glycol (1 ml), and p-toluene sulfonic acid (50 mg) was refluxed in benzene (50 ml) for 24 hours, then treated with water (100 ml). The reaction mixture was extracted with chloroform (3x100 ml), and the combined extracts were washed with water (200 ml), dried ($MgSO_4$), and

evaporated, leaving a residue of starting material only.

Reaction of the ketone enamine (14) with boron trifluoride etherate

The ketone enamine (14) (289 mg, 1.0 m mole) in dry chloroform (50 ml) and boron trifluoride etherate (3 ml) were stirred at room temperature under a nitrogen atmosphere for 10 hours, then treated with water (100 ml). The reaction mixture was extracted with chloroform (3x100 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure, leaving a residue of starting material only, as shown by a comparison of physical data (nmr, ir, uv, ms).

Reaction of the ketone enamine (14) with 85% phosphoric acid

A mixture of the ketone enamine (14) (289 mg, 1 m mole), 85% phosphoric acid (20 ml) and methanol (30 ml) was refluxed under a nitrogen atmosphere for 6 hours, and then treated with ice water. The solution was basified to pH 8 with concentrated ammonia solution (d 0.88), then extracted with chloroform (3x100 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure, leaving a residue of starting material only, as shown by a comparison of physical data (nmr, ir, uv, ms).

Reaction of the ketone enamine (14) with polyphosphoric acid

A mixture of the ketone enamine (14) (289 mg, 1 m mole) and polyphosphoric acid (30 ml) was heated under a nitrogen atmosphere in an oil bath at 120-150^{o} for 2 hours. The reaction mixture was cooled to room temperature, then treated with water (100 ml). The resulting solution was neutralized with 15% ammonium hydroxide solution and extracted thoroughly with chloroform (3x100 ml). The combined extracts were washed with water (100 ml), dried ($MgSO_4$), and

evaporated under reduced pressure. From tlc (5% MeOH/$CHCl_3$) the residue (38 mg) contained materials which remained at the base line; the crude product was in consequence not purified to characterise the structure.

Preparation of α-chloro-3',4'-dimethoxyacetophenone

A mixture of α-chloro-3',4'-dihydroxyacetophenone (20 g, 0.1 mole), potassium carbonate (26 g, 0.2 mole), and dimethyl sulfate (35 ml, 0.22 mole) was refluxed in acetone (250 ml) and stirred vigorously under a nitrogen atmosphere for 5 hours. The reaction mixture was cooled to room temperature and the salt was filtered off. The filtrate was evaporated under reduced pressure to give a white solid, which was recrystallised from ethanol to obtain white needles, mp 86-87°; γ max: 3000, 2950, 2920, 2840, 1690, 1670, 1590, 1510, 1460, 1410, 1340, 1300, 1270, 1210, 1150, 1020, 870 cm^{-1}; ^1H nmr: 7.74-6.84(AB system, J_{AB}=8 Hz, 2H), 7.45(s, 1H), 4.65(s, 2H), 3.92(s, 6H); m/z: 214(M^+, 10), meas.: 214.0397, calc. for $C_{10}H_{11}ClO_3$: 214.0397, 166(10), 165(100), 137(20), 122(15), 107(10), 92(10), 79(30), 77(25).

Preparation of ω-azido-3',4'-dimethoxyacetophenone

A mixture of α-chloro-3',4'-dimethoxyacetophenone (2.15 g, 10 m mole), and sodium azide (1 g, 15 m mole) was dissolved in 50% acetone/water (150 ml) and refluxed under a nitrogen atmosphere for 1/2 hour. The reaction mixture was poured into ice water (250 ml) and the precipitate was filtered off. The precipitate was recrystallised from methanol to obtain hygroscopic crystals (1.6 g, 72% yield); γ max: 3000, 2950, 2920, 2820, 2100, 1680, 1590, 1510, 1460, 1440, 1340, 1270, 1210, 1160, 1140, 1020, 920, 860, 800 cm^{-1}; ^1H nmr: 7.4(s, 1H), 7.4-6.8(AB system, J_{AB}=8 Hz, 2H), 4.47(s, 2H), 3.8(s, 6H); m/z: 221 (M^+, 1), 165(100), 122(60).

Preparation of ω-azido-3',4'-dimethoxyacetophenone ketal (15)

A mixture of ω-azido-3',4'-dimethoxyacetophenone (2.21 g, 10 m mole), ethylene glycol (5 ml), and dry benzene (100 ml) was refluxed for 3 hours with a Dean and Stark apparatus to remove water. The reaction mixture was treated with water (100 ml), then extracted with chloroform (3x100 ml). The combined extracts were washed with water (50 ml), dried ($MgSO_4$), and evaporated *in vacuo* to obtain the product as a colorless oil (200 mg, 75% yield); γ max: 3000, 2950, 2920, 2880, 2100, 1600, 1510, 1460, 1430, 1410, 1330, 1260, 1210, 1160, 1140, 1020, 940, 920, 860, 840 cm^{-1}; ^1H nmr: 6.98-6.75(AB system, J_{AB}=7.5 Hz, 2H), 6.92(s, 1H), 4.1(m, 2H), 3.9(m, 2H), 3.82(s, 6H), 3.37(s, 2H).

Preparation of α-amino-3',4'-dimethoxyacetophenone ketal (16)

ω-Amino-3',4'-dimethoxyacetophenone ketal (15) (270 mg, 1 m mole) in methanol (30 ml) was hydrogenated at room temperature and 60 lb/in^2 over 10% palladium on charcoal (15 mg) until no more hydrogen was absorbed (ca. 3 hours). The catalyst was filtered off and water was added to the filtrate. The resulting solution was extracted with chloroform (3x50 ml), and the combined extracts were washed with water (50 ml), dried ($MgSO_4$), and evaporated under reduced pressure to obtain the ketalamine product (16) as a colorless oil (240 mg, 98% yield); γ max: 3380, 3300, 2950, 2840, 1600, 1510, 1460, 1410, 1310, 1200, 1180, 1160, 1140, 1030, 940, 850, 810 cm^{-1}; ^1H nmr: 6.95-6.75(AB system, J_{AB}=8 Hz, 2H), 6.9(s, 1H), 4.05(m, 2H), 3.85(m, 2H), 3.87(s, 6H), 2.9(bs, 2H), 1.97(bs, 2H, exchangeable with D_2O).

Reaction of α-amino-3',4'-dimethoxyacetophenone ketal (16) with 2-ethoxycarbonylmethylcyclohexanone (3) and boron trifluoride etherate

A mixture of the ketalamine (16) (245 mg, 1 m mole), the cyclohexanone ester (3) (174 mg, 1 m mole), and sodium sulfate was refluxed

in toluene (50 ml) under a nitrogen atmosphere for about 12 hours, then the sodium sulfate was filtered off. The filtrate was evaporated under reduced pressure to obtain the condensed product, which was dissolved in chloroform (25 ml) and treated with boron trifluoride etherate (3 ml). The reaction mixture was stirred under a nitrogen atmosphere for 20 hours, then 5% ammonium hydroxide (100 ml) was added. The solution was extracted with chloroform (3x50 ml), and the combined extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. The crude product so obtained was purified by ptlc (3% MeOH/$CHCl_3$) to give the keto-amide (17) as needles, R*f*=0.3, mp 101-102°; λ max: 205 (12915), 230(5040), 275 nm(1890); γ max: 2940, 1670, 1500, 1410, 1260, 1140, 1020 cm^{-1}; ^1H nmr: 7.47-6.8(AB system, J_{AB}=8 Hz, 2H), 7.45(s, 1H), 5.72(s, 1H), 5.1-5.3(AB system, J_{AB}=15 Hz, 2H), 3.9(s, 6H), and an unresolved number of protons between 1.3 and 2.8 ppm; m/z: 315(M^+, 2), meas.: 315.1466, calc. for $C_{18}H_{21}NO_4$: 315.1470, 208(10), 209(100), 165(30).

Reaction of the condensation product of the ketalamine (16) and the cyclohexanone ester (3) with polyphosphoric acid

The condensation product from the previous reaction was dissolved in polyphosphoric acid (30 ml). The reaction mixture was stirred vigorously and heated in n oil bath at 130-150° under a nitrogen atmosphere for 2 hours. The mixture was cooled to room temperature, and water (100 ml) was added. The solution was basified to pH 8 with concentrated ammonia solution (d 0.88), then extracted with chloroform (3x50 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. After purification by ptlc (3% MeOH/$CHCl_3$), the product (215 mg, 68% yield) proved to be identical with the keto-amide (17) by comparison of their physical data (mp, nmr, ir, uv, ms).

Reaction of the condensation product of the ketalamine (16) and the cyclohexanone ester (3) with 85% phosphoric acid

The condensation product from the previous reaction was dissolved in methanol (75 ml) and 85% phosphoric acid (75 ml). The reaction mixture was refluxed under a nitrogen atmosphere for 6 hours, then the mixture was poured into ice water (100 ml) and extracted with chloroform (3x50 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated under reduced pressure. After purification by ptlc (3% $MeOH/CHCl_3$), the product (200 mg, 63% yield) proved to be identical with the keto-amide (17) by comparison of their physical data (mp, nmr, ir, uv, ms).

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CHAPTER IV

Alkaloids of *Discaria pubescens* and *D. toumatou*

1. Introduction
2. Results and Discussion
3. Experimental
4. References

1. Introduction

The genus *Discaria* belongs to the family Rhamnaceae and comprises a species of small shrubs growing in South America, New Zealand and Australia. Alkaloids of the cyclopeptide type have been isolated from the South American species *D. longispina*[1] and also *D. pubescens*[3], the only Australian member of the genus. A Chilean species, *D. serratifolia*, however, has been found to contain benzylisoquinoline-type alkaloids[4], and *D. crenata*, another of the South American *Discaria* spp.[2], contains both benzylisoquinoline and cyclopeptide types. The distribution of alkaloids in different species is shown in Table I, and the physical properties of all the cyclopeptide alkaloids isolated so far from *Discaria* spp. is shown in Table II.

Table I.

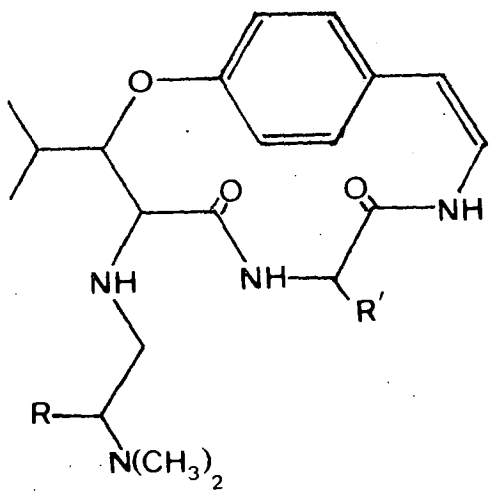
Botanical distribution.

Plant	Alkaloid	Ref.
D. crenata	R(-)-Armepavine	2
	R(-)-N-Methylcoclaurine	2
	Crenatine A	2
D. longispina	Frangulanine	1
	Discarine A	1
	Discarine B	1
	Adouetine Y'	1
	Frangufoline	1
D. pubescens	Pubescine A	3
D. serratifolia	R(-)-O-Methylarmepavine	4
	R(-)-N-Methylcoclaurine	4
	R(-)-N-Methylcolletine	4
	R(-)-Armepavine	4

Table II

Physical properties of *Discaria* cyclopeptide alkaloids

Name	MW	Formula	mp(^{o}C)	$(\alpha)_D$ ($CHCl_3$)
Adouetine Y' (i)	534	$C_{31}H_{42}N_4O_4$	295-297	-390
Crenatine A (II)	568	$C_{34}H_{40}N_4O_4$	223	-292.58
Discarine A (III)	573	$C_{33}H_{43}N_5O_4$	229-231	-282
Discarine B (IV)	573	$C_{33}H_{43}N_5O_4$	235-236	-172
Frangufoline (V)	534	$C_{31}H_{42}N_4O_4$	234-236	-288
Frangulanine (VI)	500	$C_{28}H_{44}N_4O_4$	272-274	-296
Pubescine A (VII)	486	$C_{27}H_{42}N_4O_4$	247-250	-230



Adouetine Y' (I) R = iso-Butyl, R' = Benzyl.

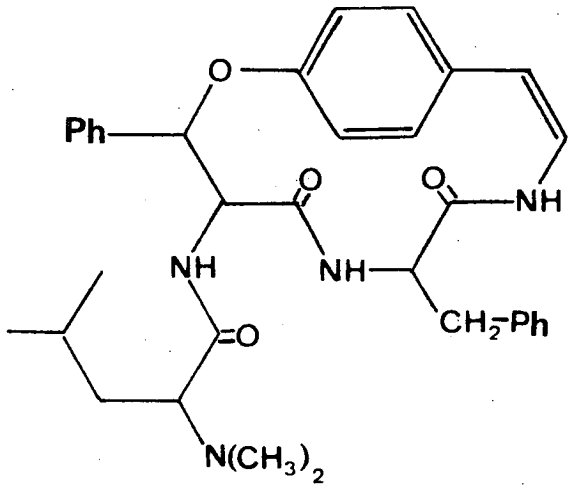
Discarine A (III) R = -Indolylmethyl, R' = sec-Butyl.

Discarine B (IV) R = sec-Butyl, R' = -Indolylmethyl.

Frangufoline (V) R = sec-Butyl, R' = Benzyl.

Frangulanine (VI) R = sec-Butyl, R' = iso-Butyl.

Pubescine A (VII) R = iso-Propyl, R' = iso-Butyl.



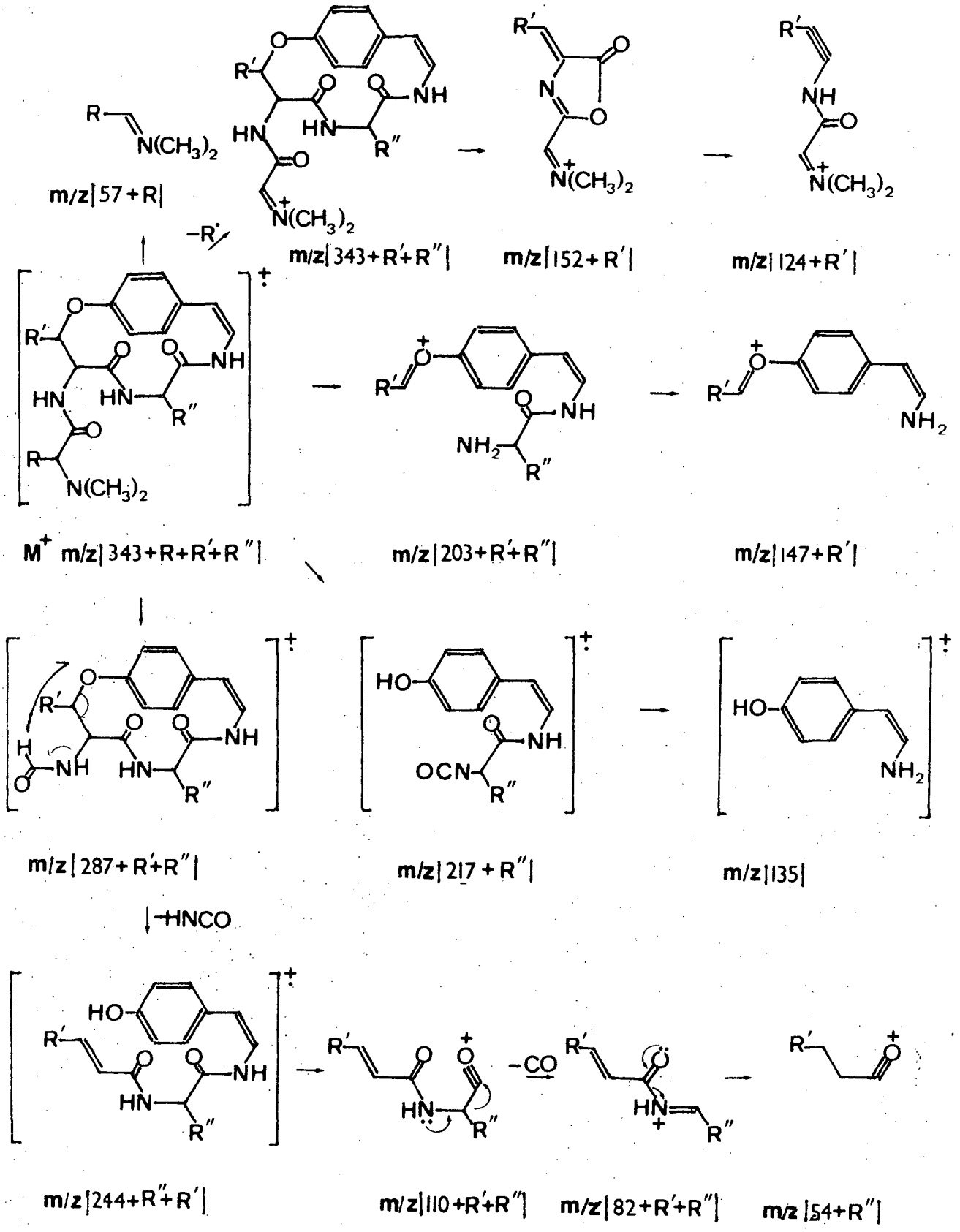
Crenatine A (II)

Structural determination.

Most of the isoquinoline-type bases isolated from *Discaria* spp. are known alkaloids which occur elsewhere. An exception is R(-)-O-methyl-armepavine which was found in *D. serratifolia*[4]; this alkaloid has never been isolated from any other plant, but has previously been obtained by synthesis[5], and also as a chemical degradation product of certain bisbenzylisoquinoline alkaloids[6]. The structures of the cyclopeptide alkaloids have been determined by spectroscopic and chemical means.

Mass spectra of cyclopeptide alkaloids.

The peptide alkaloids of *Discaria* spp. for the most part belong to the frangulanine or the integerrine series. Their structures can be determined largely from their mass spectra[8] (see scheme I); however, the substituents on the aromatic rings cannot be located by this method, nor can leucine be distinguished from isoleucine.



SCHEME I. Mass spectrometric fragmentation of frangulanine- and integerrine-type alkaloids.

NMR Spectra of cyclopeptide alkaloids

In general for the cyclopeptide alkaloids, ^{1}H nmr spectroscopy can be used to study the number and type of NMe and OMe groups, and also to solve the problem posed by isomeric residues such as those of leucine and isoleucine which are not differentiated by mass spectroscopy. A study has been made by the Wenkert group[1] of the spectra of discarine A, discarine B, and frangulanine in which the amide protons have been replaced by deuterium in order to determine the configuration of the α and β methine protons of the hydroxyleucine moiety. The signals for these protons occur at δ 4.40 and 4.77 respectively, the first as a doublet with $J_{\alpha\beta}$ = 8 Hz, and the second as a double doublet with $J_{\alpha\beta}$ = 8 Hz and $J_{\beta\gamma}$ = 2 Hz. An erytho configuration of the α and β methine protons could be assigned from a coupling constant of $J_{\alpha\beta}$= 8 Hz.

^{13}C Nmr spectroscopy of the cyclopeptide alkaloids[8] is a new area, only a few examples of the alkaloids having been studied recently in order to assign chemical shifts.

UV and IR Spectra of cyclopeptide alkaloids

The uv spectra of alkaloids of the frangulanine and integerrine types generally show no characteristic absorption band despite the presence of a double bond and an aromatic nucleus in the styrylamine moiety; the reason[7] for this is to be sought in the strained ring system which prevents the overlapping of p-orbitals in the aryloxy and enamino groups, so that they absorb independently.

The ir spectra[7] show bands at 3285-3400 corresponding to NH oscillations, at ca. 2780-2790 to those of N-methyl groups, at 1230-1240 to phenol ether absorption, and at ca. 1625 cm^{-1} to that of the styryl double bond. Furthermore, the vibrations at 1680-1690 and at 1630-1635 cm^{-1} are diagnostic for the secondary amino group of the peptide linkage.

Chemical degradation of cyclopeptide alkaloids

Hydrogenation of the double bonds of the cyclopeptide alkaloids is followed by complete hydrolysis with 6 N hydrogen chloride in a sealed tube at 120°. The amino acids in the hydrolysate are identified by standard methods, using an amino acid analyzer or paper chromatography. The type and configuration of the amino acids may also be determined even on milligram quantities[9]: the hydrolysate is directly converted into N-trifluoroacetyl or N,N-dimethylamino acid L-methyl esters, which are then subjected to gas chromatography. By comparison with known diastereomers the identity and configuration can be defined.

2. Results and discussion

D. toumatou (Maori name : matagouri) is the sole New Zealand representative of the genus *Discaria*. A previous examination of this plant had indicated the absence of alkaloids[10]; however, the plant showed strong alkaloid tests[11] in preliminary experiments in the field, but the intensity diminished fairly rapidly after collection and was reduced practically to zero after a few days. It was found, however, that if the freshly collected material were immersed as soon as possible in hot methanol, the alkaloid content was stabilized, possibly due to inactivation of plant enzymes which otherwise destroy it. The plant material could then be exhaustively extracted and the alkaloid isolated and purified by standard procedures. R(-)-N-Methyl-coclaurine, one of the bases present in *D. serratifolia*, was isolated[14] from *D. toumatou* and identified by comparison of its physical properties and those of its derivatives with data reported in the literature.

The Australian plant *Discaria pubescens* (Brongn.) Druce (Rhamaceae) was previously found to contain pubescene A. In a re-examination of *D. pubescens* for minor alkaloids, a fresh sample was collected approximately at the same time of year and from the same region as that used in initial study[3], and extracted in a similar fashion. As in the case of *D. toumatou*, the plant material gives a strong test in the field, which diminishes rather rapidly in intensity after collection, but if the fresh material is immersed in methanol as soon as possible, the alkaloid content is stabilized. A careful examination of the methanol extract revealed no trace of cyclopeptide bases, and the only alkaloid to be isolated was R(+)-coclaurine, a widespread benzylisoquinoline base which here, as usual, occurs in a partly racemised form[12]; its identity was confirmed by conversion to its N-methyl and O,N-dimethyl derivatives. This unexpected result may

be due to the drought conditions suffered by the plants during the three years previous to collection, in contrast to the conditions prevailing at the time of the original study. A re-examination of the other *Discaria* spp. under different growth conditions would be of considerable interest.

3. Experimental

3.1 Alkaloid content of *Discaria toumatou*

Leaves and terminal twigs of *D. toumatou* (9 kg), collected around the eastern foot of Porter's Pass, Canberbury, New Zealand, on May 10, 1981, and authenticated in the Division of Botany, DSIR, Lincoln, were immersed as soon as possible in boiling methanol for an hour. The plant material was then removed, air-dried, and milled to a coarse powder (5 kg), which was continuously extracted by cold percolation with methanol until tests showed that the alkaloid content was exhausted. All the methanol extracts were then combined and concentrated almost to dryness under vacuum at a temperature below 40°. The residue was treated with glacial acetic acid (2.5 liters) and water (1 liter), and the whole was warmed to 30° and thoroughly mixed. The homogeneous liquid was then poured into water (3 liters), and the suspension was agitated briskly for 2 hours.

The precipitate formed was filtered off through Hi-Flo Supercell and washed with 25% acetic acid until tests showed no alkaloid remained. The combined filtrates were evaporated to dryness under reduced pressure at 25°. The residue was dissolved in water (3 liters), and the solution was evaporated as before in order to remove as much acetic acid as possible. The residue was again dissolved in water (5 liters), and the solution was basified with conc. ammonia (50 ml) to pH 8-9. The precipitate which appeared was filtered off through Hi-Flo Supercell, and the aqueous solution was thoroughly extracted with chloroform (4 liters). The precipitate was washed with 5% methanol-chloroform until no more alkaloid was removed. The combined chloroform and 5% methanol-chloroform solutions were thoroughly extracted with 0.5% aqueous sulfuric acid (4 liters), and the acid extracts were basified with ammonia to pH 9, then extracted thoroughly with chloroform. The chloroform extracts were

dried (Na_2SO_4) and evaporated under reduced pressure to yield a crude alkaloid fraction (3.1 g, 0.062%).

The crude bases (2 g) were separated on a column of silica gel (Merck G60, 100-200 mesh, 80 g, prepared in 5% methanol-chloroform) which was eluted with methanol-chloroform mixtures ranging from 5% to 15% in concentration. Two hundred 5 ml fractions were collected: fractions 1 to 122 did not contain alkaloid, and subsequent fractions contained only one alkaloid component. The latter fractions, when combined and concentrated *in vacuo*, yielded R(-)-N-methylcoclaurine. Recrystallised from chloroform, the crude base (1.1 g) yielded 800 mg of white needles, mp 181-183^{o} (Lit. 2 181-183^{o}), $[\alpha]_D^{20}$-94.7 (C=11.8, in chloroform) (Lit. 2 $[\alpha]_D^{20}$-92). ^{1}H nmr: 2.48(s, 3H, NCH_3), 2.5-3.8(m), 3.80(s, 3H, OCH_3), 4.6(bs, 2H, OH), 6.0(s, 1H, aromatic), 6.60-6.95(m, A_2B_2, 4H, aromatic), 6.55(s, 1H, aromatic); λ max: 285 (1495), 228 nm (3887); γ max: 3600, 2850 cm^{-1}; m/z: 299(M^+, 1), 298(2.5), 192(100), 178(16), 177(25), 148(22), 107(22). The methiodide and the 0,0-diacetate, prepared by standard methods, had mp 202^{o} and 77-78^{o}, respectively (Lit. 2 202-204^{o} and 77-78^{o}).

3.2 Alkaloid content of *Discaria pubescens*

Leaves and terminal twigs of *D. pubescens* collected around the Shannon River, Hermitage, Tasmania, in April 1983, were immersed as soon as possible in methanol. After three days, the plant material was removed, air-dried, and milled to a coarse powder (10 kg), which was continually extracted by cold percolation with methanol until tests showed that the alkaloid content was exhausted. The methanol extracts were combined and concentrated almost to dryness under vacuum at a temperature below 40^{o}. The residue was treated with glacial acetic acid (4 liters) and water (1 liter), and the whole was warmed to 30^{o} and

thoroughly mixed. The homogeneous liquid was then poured into water (3 liters), and the suspension was agitated briskly for 2 hours. The precipitate formed was filtered off through Hi-Flo Supercell and washed with 25% acetic acid until tests showed no alkaloid remained. The combined filtrates were evaporated to dryness *in vacuo* at 30°, the residue was dissolved in water (3 liters), and the solution was evaporated as before in order to remove as much acetic acid as possible. The solution was again dissolved in water (3 liters), and the solution was basified with conc. ammonia to pH 8-9. The precipitate which appeared was filtered off through Hi-Flo Supercell, and the filtrate was thoroughly extracted with chloroform until no more alkaloid was removed. The combined chloroform and methanol solutions were extracted with 5% sulfuric acid (4 liters), and the aqueous solution was basified with conc. ammonia to pH 8-9, then extracted thoroughly with chloroform. The chloroform extracts were dried ($MgSO_4$) and evaporated under reduced pressure to yield a crude alkaloid fraction (15 g).

A sample of crude bases (2 g) was separated on a column of silica gel (Merck G60, 100-200 mesh, 120 g, prepared in chloroform) which was eluted with chloroform containing gradually increasing amounts of methanol up to 10%. Twenty 50 ml fractions were collected, of which the first 15 were devoid of alkaloids; subsequent fractions contained only one alkaloid component. They were combined and concentrated *in vacuo* to yield R(+)-coclaurine as a foam, $[\alpha]_D^{19}$+19.8 (C=5.9 in methanol) (Lit. 12 $[\alpha]_D$+22 (in ethanol)); λ max: 213 (17100), 228 (14535), 280 nm (4275); after addition of a drop of aqueous NaOH: λ max: 218, 245, 290; γ max (nujol): 3450, 2950, 2920, 2850, 1600, 1500, 1370, 1330, 1270, 1230, 1120 cm^{-1}; ^1H nmr ($CDCl_3$+DMSO-d_6); 6.97-6.65(m, 5H), 6.45(s, 1H), 3.5(bs, 3H, 2OH, NH), 3.77(s, 3H, OCH_3), and an unresolved number of protons between 2.5 and 4.0 ppm; m/z: 285(M^+, 0.3), 284(3), 283(3), 282(6), 179(10), 178(100), 177(6), 163(10).

A sample of the alkaloid was methylated with diazomethane, then with formaldehyde and borohydride in the usual way to yield R(-)-O-methyl-armepavine as a colorless oil, $[\alpha]_D^{19}$-85 (C=2.5 in chloroform); its physical and spectroscopic data corresponded with those of the base isolated from *D. serratifolia*[4]. Its methiodide derivative had mp 136-137° (Lit. 13 mp 135-137°).

Another sample of the alkaloid was N-methylated to give R(-)-N-methylcoclaurine as white needles, mp 182-183° (Lit. 2 mp 182-183°), undepressed on admixture with an authentic sample. Its physical and spectroscopic data corresponded with those of the base isolated from *D. toumatou*.

Voucher specimens of the plant material have been deposited in the collection of dried plants specimens in the Chemistry Dept., University of Tasmania.

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CHAPTER V

Alkaloid of *H. veillardii*

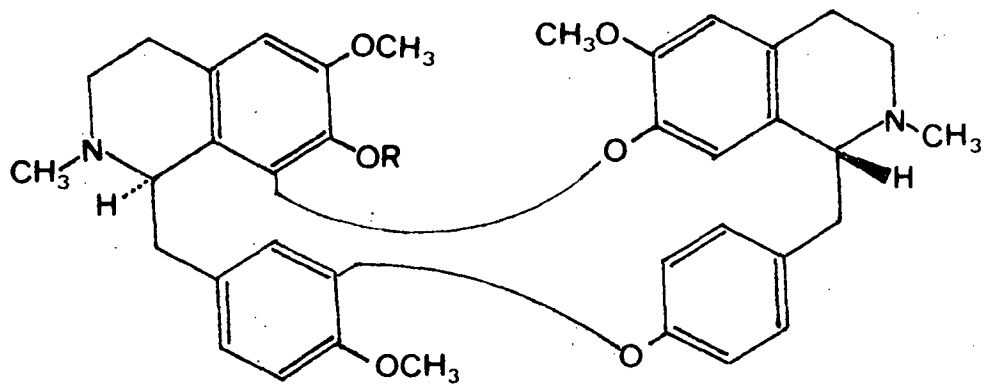
1. Introduction
2. Results and Discussion
3. Experimental
4. References

1. Introduction.

Hypserpa, a member of the family Menispermaceae, comprises a small genus of shrubs which are distributed in New Caledonia, Australia, and China. Phytochemical investigations have been carried out on only two species of this genus: *H. nitida*[4], a species from Hong Kong, has been shown to contain two alkaloids, quaternary alkaloids, and the Australian species *H. laurina*[5], was found to give strong alkaloid tests in a field survey, but no further work on it has been carried out.

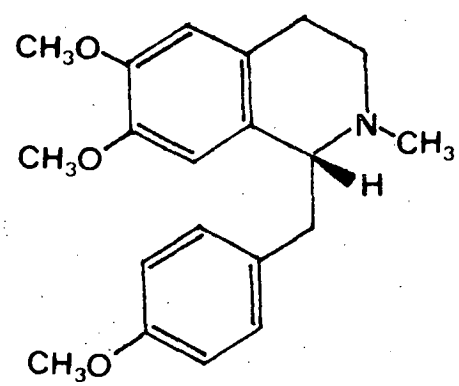
2. Results and discussion.

Plant material from *H. veillardii*, a species from New Caledonia, was collected in 1981. Extraction of the dried root material (6 kg) by standard methods afforded crude bases (7 gm) which were separated on a silica gel column; only one alkaloid could be isolated, the bisbenzyl-isoquinoline base limacine. Its identity has been deduced by comparison with data reported in the literature and confirmed by conversion to its O-methyl derivative followed by Birch reduction.

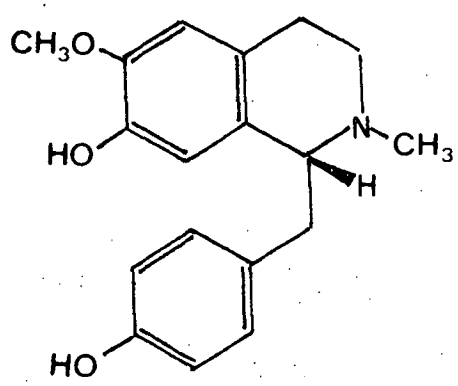


Limacine R=H

Phaeanthine R=CH_3



R(-)-N-Methylarmepavine



R(-)-N-Methylcoclaurine

3. Experimental.

Alkaloids of *H. veillardii*

Finely ground root material (6.0 kg) was continuously extracted by cold percolation with methanol until tests showed that the alkaloid content was exhausted. All the methanol extracts were then combined and concentrated almost to dryness under vacuum at a temperature below 40°C. The residue was dissolved in water (3 liters), and the suspension was agitated briskly for two days. The precipitate formed, which gave a negative test with Mayer's reagent, was filtered off through Hi-Flow supercell and discarded. The filtrate was evaporated to dryness under reduced pressure at 25°, the residue was dissolved in water (3 liters), and the solution was basified with ammonia (d 0.88) to pH 11. The precipitate which appeared was filtered off through Hi-Flow supercell, washed with 10% methanol-chloroform until no more alkaloid was removed, and the aqueous solution was thoroughly extracted with chloroform (5 liters). The combined chloroform and 10% methanol-chloroform solutions were thoroughly extracted with 5% aqueous sulfuric acid (4 liters), and the acid extracts were basified with ammonia (d 0.88) to pH 9. The basified solution was extracted thoroughly with chloroform, then the chloroform extracts were dried ($mgSO_4$) and evaporated under reduced pressure to yield a crude alkaloid fraction (7.0 g).

The crude bases (2 g) were separated on a column of silica gel (Merck G60, 100-200 Mesh) (120 g) prepared in chloroform, which was eluted with methanol-chloroform mixtures ranging from pure chloroform to 10% in concentration. Six fractions (250 ml) were collected: fractions 1 and 2 did not contain alkaloid, and subsequent fractions contained only one alkaloid component. The latter fractions when combined and concentrated *in vacuo*, yield limacine. Recrystallised from acetone-benzene, the crude

base (1.2 g) yielded white needles (700 mg), mp 154-156° (Lit. 1 mp 154-156°); $[\alpha]_D^{19}$-207 (C=0.3 $CHCl_3$) (Lit. 1 $[\alpha]_D$ -212 ($CHCl_3$)); λ max: 235 (5645) 282 nm(2432); after addition of a drop of 2% aqueous sodium hydroxide: 240 and 288 nm; γ max: 3540, 3020, 2950, 2850, 1620, 1590, 1500, 1460, 1450, 1420, 1370, 1320, 1300, 1270, 1220, 1170, 1130, 1060, 1030, 980, 850 cm^{-1}; ^1H nmr 300 MHz: 7.32(dxd, J_1=2.5 Hz, J_2-8.2 Hz, 1H), 7.12(dxd, J_1=2.5 Hz, 1H), 6.86(ABC system, J_{AC}=1.57 Hz, J_{AB}=8.12 Hz, 1H), 6.82(AB system, J_{AB}=8.12 Hz, 1H), 6.79(dxd, J_1=2.51 Hz, J_2=8.48 Hz, 1H), 6.57(d, J=1.58 Hz, 1H), 6.50(s, 1H), 3.91(s, 3H, OCH_3), 3.74(s, 3H, OCH_3), 3.33(s, 3H, OCH_3), 2.59(s, 3H, NCH_3), 2.32(s, 3H, NCH_3), and an unresolved number of protons between 2.5 and 3.5 ppm; m/z: 608(M^+, 45); meas.: 608.2866, calc. for: $C_{37}H_{40}N_2O_6$; 608.2868, 607(35), 382(18), 381(60), 367(30), 192(40), 191(100), 168(20).

O-Methylation of limacine.

Limacine (250 mg) in MeOH/$CHCl_3$ (1:1, 4 ml) was treated with excess ethereal diazomethane, then the mixture was left at room temperature for 3 days. The residue after removal of solvent was separated by ptlc (5% MeOH/$CHCl_3$), and gave phaeanthine as white needles (180 mg), mp 151-152° (acetone) and mp 220-222° (acetone) (Lit. 2 mp 220-222°); $[\alpha]_D^{19}$-305 (C=1.43 $CHCl_3$) (Lit. 2 $[\alpha]_D^{24}$-270 ($CHCl_3$)); λ max 212 (13435), 282 nm(1408); γ max: 2920, 2850, 1600, 1500, 1450, 1430, 1400, 1350, 1300, 1250, 1220, 1150, 1110, 1050, 1020, 960 cm^{-1}; ^1H nmr: 2.275(s, 3H, NCH_3), 2.55(s, 3H, NCH_3), 3.15(s, 3H, OCH_3), 3.35(s, 3H, OCH_3), 5.97(s, 1H), aromatic protons from 7.3 to 6.2, 9H, and an unresolved number of protons between 2.5 and 3.5 ppm; m/z: 622 (M^+, 40); meas.: 622.3042, calc. for: $C_{38}H_{42}N_2O_6$: 622.3042; 621(35), 396(18), 395(59), 381(35), 198(100), 175(40), 174(48).

Birch reduction of phaeanthine.

Phaeanthine (100 mg) was dissolved in benzene-toluene (15 ml of 1:1),

and the solution, cooled to -40°, was treated with liquid ammonia (200 ml). The mixture was stirred vigorously while sodium (2 g), was added in small portions. The temperature was maintained at -40° and stirring was continued for one hour. The ammonia was allowed to evaporate slowly and the mixture was treated with ethanol to destroy excess sodium. The mixture was dilute with water and acidified with 5% (W/V) sulfuric acid; the aqueous acid was basified with ammonia (d 0.88) to pH 8-9 and then extracted with chloroform (5x30 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to obtain a yellow oil which was purified by ptlc (5% MeOH/$CHCl_3$). The higher *Rf* colorless oil (35.0 mg) proved to be identical with R(-)-O-methylarmepavine, $[\alpha]_D^{19}$-86.5 (C=2.33 $CHCl_3$) (Lit. 2 $[\alpha]_D^{23}$-82.3 ($CHCl_3$)); λ max: 232 (2071), 284 nm(1162); γ max: 3000, 2930, 2840, 1600, 1500, 1450, 1340, 1240, 1210, 1170, 1130, 1110, 1090, 1040, 850, 830 cm^{-1}; ^1H nmr: 6.97-6.75(m, A_2B_2, J=8 Hz, 4H), 6.52(s, 1H), 5.9(s, 1H), 3.80(s, 3H, OCH_3), 3.75(s, 3H, OCH_3), 3.5(s, 3H, OCH_3), 2.53(s, 3H, NCH_3), and an unresolved number of protons between 2.5 and 3.5; m/z: 327(M^+, 0.2), 326(1), 310(1), 222(7), 220(5), 207(60), 206(100), 191(20), 190(30), 162(10); methiodide derivative mp 136-137° (Lit. 2 mp 136-137°).

The lower *Rf* white needles (50.0 mg) proved to be identical with R(-)-N-methylcoclaurine, mp 182-184° ($CHCl_3$) (Lit. 3 182-184° ($CHCl_3$)); $[\alpha]_D^{19}$-94.7 (C=11.0 EtOH) (Lit. 3 $[\alpha]_D^{20}$-92.0 (EtOH)). The identity was confirmed by agreement of its physical data with those of an authentic sample (nmr, ir, uv, ms, mmp).

A voucher specimen of the plant material has been deposited in the Herbarium, University of Tasmania.

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