STRUCTURAL AND SYNTHETIC STUDIES OF ALKALOIDS OF THE PROTEACEAE

by

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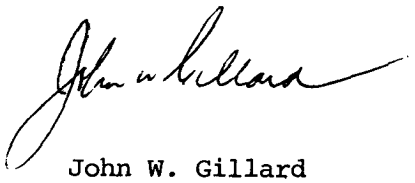
Except as herein stated, this thesis contains no material or paraphrase of material presented or accepted for the award of any degree or diploma in any university. To the best of my knowledge, this thesis contains no copy or paraphrase of material previously published, except where due reference is made in the text.



John W. Gillard

Contents

ABSTRACT

Following the discovery of the presence of alkaloids in an endemic Tasmanian Proteaceous species, Bellendena montana R.BR., a detailed phytochemical examination for alkaloids has been undertaken. In addition, three other species of the Proteaceae, Darlingia darlingiana (F. MUELL) L.A.S. JOHNSON, Darlingia ferruginea, J.F. BAILEY, and Agastachys odorata R.BR., also found to contain alkaloids, have been subjected to a similar examination.

This study has resulted in the isolation of fifteen alkaloids, of which twelve have been structurally identified by spectroscopy and synthesis. The structures of the bases have been shown to be derivatives of the tropane class; eight alkaloids with novel structures have been classified in two new tropane categories, those of γ-pyronotropanes and 2-benzoyl tropanes. Use has been made of proton magnetic resonance and carbon magnetic resonance spectroscopy in determining relative stereochemistries of the bases, and a discussion of the ^{13}C N.M.R. of γ-pyrones is given. Two stereochemical series of tropane diol esters have been prepared and the spectroscopic data relating to these bases are presented.

A general synthetic procedure has been developed to apply to the γ-pyronotropanes; each of the naturally occurring alkaloids has been synthesized by a condensation between tropan-3-one and an enol ether derivative of a β-keto acid chloride.

The probable biogenetic relationships between the alkaloids of the species under study and those recently described, of Knightia deplanchei VIEILL. ex BROGN et GRIS (Proteaceae) are described in terms of a biosynthesis from ornithine and a polyketide chain terminated with a non-acetate moiety.

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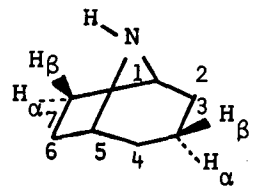
CHAPTER 1

1.1. Introduction

The therapeutic application of extracts of a number of species of the Solanaceae dates from mankind's early history. The use of Mandragora officinarum L. (Mandrake) as a soporific and analgesic is recorded in the Ancient Egyptian Ebers Papyrus of 1500 B.C.[1,2]; Hyoscyamus niger (Henbane), Atropa belladonna L. (Belladonna) and Datura stramonium L. (Thorn Apple) are each recorded as contributing to the pharmacopoeia of cultures as early and diverse as those of the Aztec Indians[3] and the Babylonians[4].

This long-standing recognition of the powerful physiological action of these extracts has contributed to the zeal with which chemists and pharmacologists have studied the alkaloids they contain, as it is these which are responsible for the physiological activity associated with the plant.

The basic skeleton of this group of alkaloids, also found in the Convolvulaceae, Erythroxylaceae, Euphorbiaceae, Rhizophoraceae, and Proteaceae is 8-azabicyclo[3.2.1]octane. (1.I).

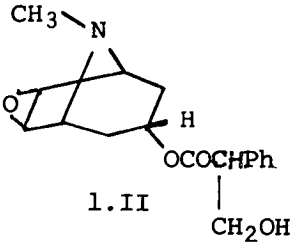


1.I

Five distinct classes of naturally occurring alkaloids are derived from substitution of this ring system; the first three have provided the basis for much of the structural and synthetic knowledge of the tropanes, and the literature pertaining to these classes has received excellent coverage in reviews by Fodor[5(a)(b)(c)] and others[6]. The

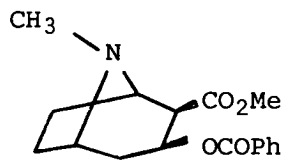
majority of bases are N-methylated and are hydroxylated at position C-3. The simplest class of tropanes, bearing only these substituents, are termed tropanols, and their esters, tropeines. Variety in this class results from the range of organic acids, which esterify the secondary hydroxyl group, the most common being 2-methylbutyric, (isovaleric) 2-methylbut-2-enoic, (tiglic) and 3-hydroxy-2-phenyl propanoic acid (tropic). In addition, unusual acids such as 2-hydroxy-5-phenyl propanoic[112] and butane-1,2-dithiolane-3-carboxylate[6] have been noted. Two series occur within this class depending on the stereochemistry of the hydroxylation of C-3. There is general acceptance of the Fodor proposal[7] that the steroid nomenclature convention should apply to substituted tropanes. Hence, substituents lying above the plane of the carbocyclic ring in Figure 1.I are termed β and those below the plane are termed α. The 3α nomenclature (or endo) corresponds to the tropine series and the 3β nomenclature (or exo) to the pseudotropine series of the early literature.

Oxidation of the ethano bridge carbons C-6 and/or C-7 in addition to C-3, produces a second distinct class of tropane alkaloids. Esterification of the resultant alcohol generates the tropane diol and triol ester series, the same convention being used to describe the stereochemistry. All naturally occurring diol and triol esters have the 6β, 7β configuration. In addition, epoxidation may occur across C-6 and C-7 to produce the scopine series: 6β, 7β-oxy tropan-3-ols. Esterification of the C-3 hydroxyl with tropic acid produces the medicinally useful scopolamine (1.II).

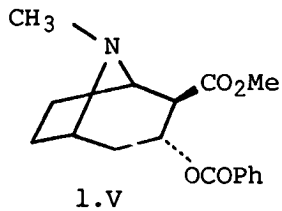


1.II

Those alkaloids which are carboxylated at position C-2 and hydroxylated at C-3 are known as the ecgonines. The carboxyl group in this class is esterified with either methanol or ethanol and a variety of acids esterify the C-3 hydroxy group; thus, cocaine constitutionally is the benzoate of 2-carbomethoxy tropan-3-ol. The epimeric centres C-2 and C-3, however, generate four configurational forms whose chemical interrelationships[8,9] and proton magnetic resonance (P.M.R.) spectroscopic parameters[10] are known. Cocaine (1.IV) is the benzoate derived from 2β-carbomethoxy tropan-3β-ol, allococaine (1.V) from 2β-carbomethoxy tropan-3α-ol, pseudococaine (1.VI) from 2α-carbomethoxy tropan-3β-ol, and allopseudococaine (1.VII) from 2α-carbomethoxy tropan-3α-ol.



1.IV



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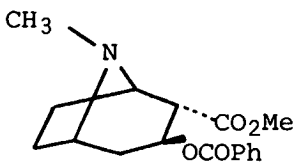
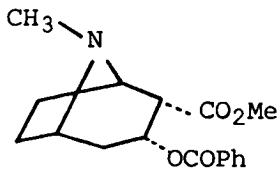


1.VI



1.VII

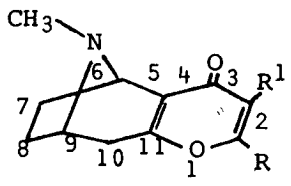
Hydrolysis of the cocaines followed by dehydration and methylation produces a compound identical with the naturally occurring base methyl anhydroecgonine[11].

The three classes described above have, until recently, constituted the entire tropane field. The remaining two classes have been

discovered recently in the family Proteaceae; a detailed examination of these novel alkaloids is the subject of this thesis.

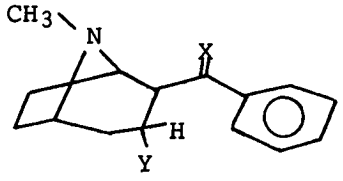
The incorporation of the 2- and 3-positions of the tropane nucleus* into a γ-pyrone ring is the structurally characteristic aspect of the tropane alkaloids isolated from the Bellendena and Darlingia species.

These constitute a new class of tropane bases which have been called pyronotropanes[12].



R = H, R^1 = CH_3	Bellendine (1.VIII)	
R = CH_3, R^1 = H	Isobellendine (1.IX)	
R = CH_3, R^1 = CH_3	Darlingine (1.X)	
R = CH_3, R^1 = H 5,11 cis dihydro	Dihydroisobellendine (1.XI)	

The Proteaceous species Darlingia ferruginea BAILEY and the related species Knightia deplanchei[13] VIEILL. ex BROGN. et GRIS also elaborate closely related alkaloids of the type 1.XII constituting the fifth class of tropane alkaloids derived from 2-acyl tropane.



1.XII

X = O,	Y = H	Ferrugine	
X = 2H,	Y = OCOR	Knightia bases	
= H,OH		Minor ferruginea bases	

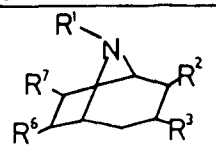
A summary of the known tropane bases is shown in Table 1-1.

*No indication of absolute stereochemistry is intended by specifically noting substituents at C-6, C-7, C-2 or C-3 in this table.

In later chapters, where only relative stereochemistries are known, the lowest numbering sequence is employed and one projection is arbitrarily employed, there being no intention of implying a knowledge of absolute configuration.

TABLE 1-1

TROPANE ALKALOIDS OF KNOWN CONSTITUTION AND CONFIGURATION



Name	Molecular Formula	Molecular Weight	Melting Point	Substituents on Tropane Ring					Plant Source	Ref.
				R^1	R^2	R^3	R^6	R^7		
Tropine	$C_8H_{15}ON$	141.22	64	CH_3	H	α-OH	H	H	Atropa belladonna	5,6
Pseudotropine	$C_8H_{15}ON$	141.22	108	CH_3	H	β-OH	H	H	Datura innoxia (Fastuosa)	"
Atropine	$C_{17}H_{23}O_3N$	289.37	116-17	CH_3	H	α-(±)-O-Tropoyl	H	H	Atropa belladonna	"
(-)-Hyoscyamine	$C_{17}H_{23}O_3N$	289.37	108-11	CH_3	H	α-(-)-O-Tropoyl	H	H	Atropa acuminata	"
Tropacocaine	$C_{15}H_{19}O_2N$	245.33	49	CH_3	H	β-O-Benzoyl	H	H	Erythroxylon coca	"
Tigloidine	$C_{13}H_{21}O_2N$	223.32	Liquid	CH_3	H	β-O-Tigloyl	H	H	Datura innoxia (Fastuosa)	"
Convolamine	$C_{17}H_{23}O_4N$	305.37	114-15	CH_3	H	α-O-Veratroyl	H	H	Convolvulus pseudocantabrica	"
Convolvine	$C_{16}H_{21}O_4N$	291.35	115	H	H	α-O-Veratroyl	H	H	Convolvulus pseudocantabrica	"
Phyllalbine	$C_{16}H_{21}O_4N$	291.35	209-10	CH_3	H	α-O-Vanilloyl	H	H	Phyllantus discöides Muell, arg.	69
Poroidine	$C_{12}H_{21}O_2N$	211.31	Liquid	H	H	α-O-3-Methylbutyryl	H	H	Duboisia myoporoides	5,6
Isoporoidine	$C_{12}H_{21}O_2N$	211.31	Liquid	H	H	α-O-(+)-2-Methylbutyryl	H	H	Duboisia myoporoides	5,6
Valerine	$C_8H_{15}O_2N$	157.22	212	CH_3	H	α-OH	β-OH	H	Erythroxylon coca	5,6
Valeroidine	$C_{13}H_{23}O_3N$	221.32	85	CH_3	H	α-O-3-Methylbutyryl	β-OH	H	Datura sanguinea, Duboisa myoporoides	107
6-Tigloyl-3,6-dihydroxy-tropane	$C_{13}H_{21}O_3N$	239.32	Amorph	CH_3	H	α-OH	H	β-Tigloyloxy		108
Ditigloyl-3,6-dihydroxy-tropane	$C_{18}H_{27}O_5N$	337.42	Amorph	CH_3	H	α-O-Tigloyl	H	β-Tigloyloxy	Datura cornigera	5,6

TABLE 1-1 (continued)

Teloidine	$C_8H_{15}O_3N$	173.33	168-9	CH_3	H	α-OH	β-OH	β-OH	Datura ferox, etc.	5,6
Meteloidine	$C_{13}H_{21}O_4N$	255.32	141-2	CH_3	H	α-O-Tigloyl	β-OH	β-OH	Datura ferox, (Fastuosa) etc.	"
Tigloylmetelo-idine	$C_{18}H_{27}O_5N$	337.42	Amorph	CH_3	H	α-O-Tigloyl	OH	β-Tigloyloxy	Datura ferox, etc.	108
Scopine	$C_8H_{13}O_2N$	155.21	76	CH_3	H	α-OH		β-Oxide		5,6
Scopolamine	$C_{17}H_{21}O_4N$	303.36	82-3	CH_3	H	α-O-(±)-Tropoyl		β-Oxide	A. belladonna	"
Hyoscine	$C_{17}H_{21}O_4N$	303.36	59	CH_3	H	α-O-(-)-Tropoyl		β-Oxide	A. acuminata, A. belladonna, etc.	"
Oscine	$C_8H_{13}O_2N$	155.21	110	CH_3	H	α-oxide				
(-)-Ecgonine	$C_9H_{15}O_3N$	185.23	205	CH_3	β-COOH	β-OH	H	H	Erythroxylon coca	"
(-)-Cocaine	$C_{17}H_{21}O_4N$	303.36	98	CH_3	β-$COOCH_3$	β-O-Benzoyl	H	H	" "	"
(+)-Pseudo-ecgonine	$C_9H_{15}O_3N$	185.23	254-7	CH_3	α-COOH	β-OH		H	" "	"
(+)-Pseudo-cocaine	$C_{17}H_{21}O_4N$	303.36	46-47	CH_3	α-$COOCH_3$	β-O-Benzoyl	H	H	" "	"
α-Truxilline	$C_{38}H_{46}O_8N_2$	658.80	Amorph	CH_3	β-$COOCH_3$	β-O-α-Trixilloyl	H	H	" "	"
β-Truxilline	$C_{38}H_{46}O_8N_2$	658.80	Amorph	CH_3	β-$COOCH_3$	β-O-β-Trixilloyl	H	H	" "	"
Cinnamylcocaine	$C_{19}H_{23}O_4N$	329.40	121	CH_3	β-$COOCH_3$	β-O-Cinnamoyl	H	H	" "	"
Tropanone	$C_8H_{13}NO$	139.20	42	CH_3	H	Oxo	H	H	Nicandra spp.	111
6β-Acetoxy-3α-tigloyloxy-tropane	$C_{15}H_{23}NO_4$	281.36	liq	CH_3	H	α-Tigloyloxy	H	β-Acetoxy	Datura sanguinea	108
									Agastachys odorata	Thesis
3α-Acetoxy-tropane	$C_{10}H_{17}NO_2$	183.25	liq	CH_3	H	α-Acetoxy	H	H	Datura sanguinea	108
Valtropine	$C_{13}H_{23}NO_2$	225.34	liq	CH_3	H	α-O-3-Methylbutoxy	H	H	Duboisia spp	5,6

TABLE 1-1 (continued)

3α-Tigloyloxy-6β-hydroxy tropane	$C_{13}H_{21}NO_3$	239.32	liq	CH_3	H	α-tigloyloxy	H	6-hydroxy	Datura sanguinea	108
Butropine	$C_{12}H_{21}NO_2$	211.31	salt	CH_3	H	α-isobutoxy	H	β-OH	Duboisia spp.	5
Littorine	$C_{17}H_{23}NO_4$	275.35	96	CH_3	H	α-(2-hydroxy-5-phenyl)propoxy	H	H	Anthocercis littorea Datura sanguinea.	112 110
Bellendine	$C_{12}H_{15}NO_2$	205.26	162	CH_3		γ-pyrone-3-methyl			Bellendena montana	Thesis
Isobellendine	$C_{12}H_{15}NO_2$	205.26	114	CH_3		γ-pyrone-2-methyl			" "	"
Dihydroiso-bellendine	$C_{12}H_{17}NO_2$	207.27	liq	CH_3		5,11-dihydro-γ-pyrone-2-methyl			" "	"
6β-Acetoxy-3α-isobutoxy tropane	$C_{14}H_{23}NO_4$	269.33	liq	CH_3	H	α-isobutoxy	H	β-acetoxy	" "	"
6β-Isobutoxy-3α-acetoxy	$C_{14}H_{23}NO_4$	269.33	liq	CH_3	H	α-acetoxy	H	β-isobutoxy	" "	"
Darlingine	$C_{13}H_{17}NO_2$	219.38	103	CH_3		γ-pyrone-2,3-dimethyl	H	H	Darlingia darlingiana, D. ferruginea	"
2β-Benzoyl-tropane	$C_{15}H_{19}NO$	229.31	liq	CH_3	H	β-Benzoyl	H	H	Darlingia ferruginea	"
2-Acetyl-trop-2-ene	$C_{10}H_{15}NO$	165.22	liq	CH_3	$COCH_3$	$\Delta^{2,3}$			" "	"
2β-Benzyl-3α-acetoxy tropane	$C_{17}H_{23}NO_2$	273.36	liq	CH_3	β-Benzyl	α-acetoxy	H	H	Knightia deplanchei	13
2β-Benzyl-3α-benzoyloxy tropane	$C_{22}H_{25}NO_2$	335.42	liq	CH_3	β-Benzyl	α-benzoyloxy	H	H	" "	"
2β-Benzyl-3α-hydroxy 6β-benzoyloxy-tropane	$C_{22}H_{25}NO_3$	351.42	168	CH_3	β-Benzyl	α-hydroxy	H	β-benzoyloxy	" "	"

TABLE 1-1 (continued)

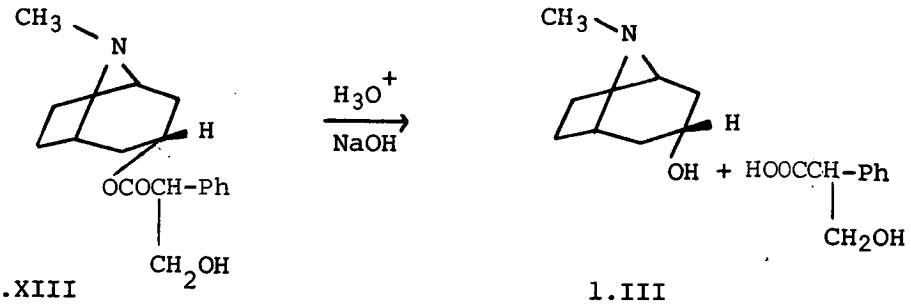
2β-Benzyl-3α-cinnamoyloxy-6β-hydroxy-tropane	$C_{24}H_{27}NO_3$	377.45	174	CH_3	β-Benzyl	α-cinnamoyloxy	H	β-hydroxy	Knightia deplanchei	13
Methyl ecgonidine	$C_{10}H_{15}NO_2$	181.24	liq	CH_3	COOMe	$\Delta^{2,3}$	H	H	Erythroxylon coca	5,6
Methyl ecgonine	$C_{10}H_{17}NO_3$	199.25	liq	CH_3	COOMe	β-hydroxy	H	H	" "	"
Benzoyl ecgonine	$C_{16}H_{19}NO_4$	289.34	195	CH_3	COOH	β-benzoyloxy	H	H	" "	"
6β-Isovaleroxy-7β-hydroxy-3α-tigloyloxy-tropane	$C_{18}H_{29}NO_5$	339.41	liq	CH_3	H	α-tigloyloxy	β-OH	β-isovaleroxy	Datura sanguinea	110
3α-Tigloyloxy-tropane	$C_{13}H_{21}NO_2$	223.31	liq	CH_3	H	α-tigloyloxy	H	H	" "	"
6β-(2-Methyl-butanoyloxy)-3α-hydroxy tropane	$C_{13}H_{23}NO_2$	225.33	liq	CH_3	H	α-hydroxy	H	β-methylbutoxy	Datura ceratocaula	109
2-(hydroxy-benzyl)-3α-cinnamoyloxy-tropane	$C_{24}H_{27}NO_3$	377.45	liq	CH_3	hydroxy-benzyl	α-cinnamoyloxy	H	H	Knightia deplanchei	38
2-(hydroxy-benzyl)-3α-hydroxy-6β-benzoyloxy-tropane	$C_{22}H_{25}NO_4$	367.42	liq	CH_3	hydroxy-benzyl	α-hydroxy	H	β-benzoyloxy	" "	"
Brugine	$C_{12}H_{19}NO_2S_2$	273.41	decomp.	CH_3	H	α-1,2-dithiolane-3-carboxylate	H	H	Bruguiera sexangula	5,6
2-(hydroxy-benzyl)-3α-benzoyloxy-tropane	$C_{22}H_{25}NO_3$	351.42	138	CH_3	hydroxy-benzyl	α-benzoyloxy	H	H	Darlingia ferruginea	Thesis

1.2. Structural Determination of the Tropane Bases

1.2.1. Tropan-3-ol Esters

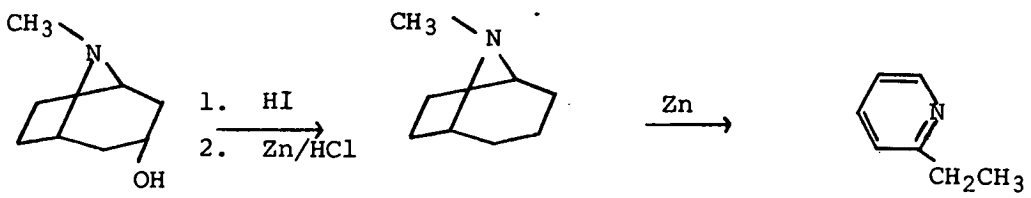
Under hydrolytic conditions, cleavage of the tropane esters takes place [13]. The amino-alcohol so formed may be mono-, di-, or trihydroxylated depending on the degree of oxidation of the parent base. Selective procedures are available for specific hydrolysis of ester functions at C-6 or C-7[14], enabling differentiation of the esterifying acids at these positions vs C-3.

As a representative C-3 tropine ester, atropine (1.XIII) yields tropan-3α-ol (tropine) and racemic tropic acid. Hyoscyamine yields (-) tropic acid and tropan-3α-ol. Atropine, which is formed from hyoscyamine by the action of heat or alkali, is thus the racemate of hyoscyamine[15].



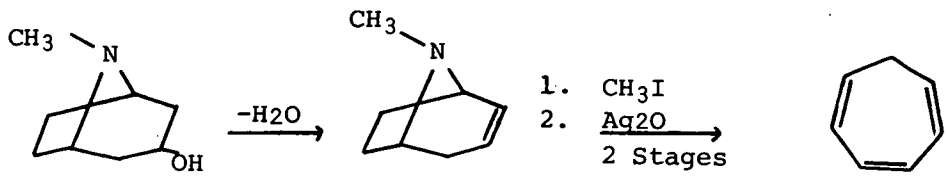
1.XIII 1.III

The ring structure of tropan-3-ol (1.III) was deduced by the reductive elimination of the C-3 hydroxy with hydriodic acid, followed by zinc and acid to give tropane (1.I)[16]. Degradation of tropane with zinc dust gives 2-ethyl pyridine, indicating the presence of a 6-membered ring containing nitrogen [16].



1.I

Hofmann degradation of trop-2-ene (1.XIV), the dehydrated product of tropan-3-ol, leads to the carbocyclic compound, cycloheptatriene[17].



1.XIV

Thus, a reconstruction of these data indicates that tropane has the bicyclic structure (1.I).

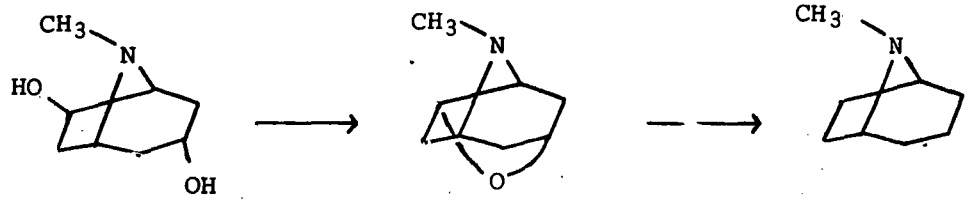
The location of the hydroxyl substituent in tropan-3-ol was achieved by oxidation to tropanone followed by nitrosation, leading to the 2,4-dioximino tropanone. This confirmed the presence of two free methylenes adjacent to the carbonyl function, unambiguously locating this group at position C-3[18].

The reduction of tropan-3-one yields the epimeric alcohols tropan-3α-ol (tropine) and tropan-3β-ol (pseudotropine). Chemical methods of reduction such as sodium in alcohol, or lithium aluminium hydride, favour the β alcohol series, whereas catalytic hydrogenation produces the α alcohol exclusively. House[19] has interpreted these results on the basis of steric approach control in chemical reductions and the geometry of complexation to the catalytic surface in catalytic hydrogenation. Such reductive methods confirmed the C-3 epimerism of the tropanols, both isomers having arisen from the hydrolysis of naturally occurring tropanol esters.

1.2.2. C-6, C-7 Hydroxylated or Epoxidized Tropan-3-ols

(i) Tropan-3,6-diol Esters

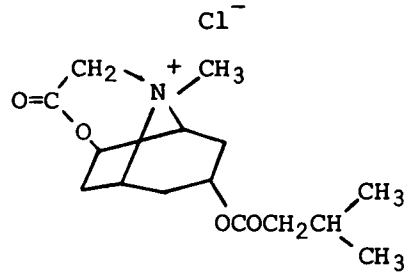
Hydrolysis of valeroidine, a representative of the above class of ester, yields 3-methylbutyric acid and the corresponding amino alcohol, tropan-3,6-diol (1.XV), which was found to be identical to the naturally occurring base from Erythroxylon coca[20]. This base, which undergoes dehydration[21] with p-toluenesulphonic anhydride to yield an internal ether (1.XVI) has therefore two neighbouring hydroxy groups. The dehydrated product may be totally reduced with hydroiodic acid and phosphorus to tropane (1.I).



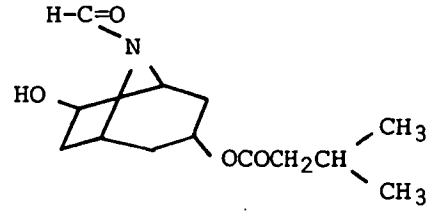
1.XV (3S,6S) 1.XVI 1.I

The quaternized parent base undergoes lactone salt formation - a method utilized by Fodor et al[22] to examine the configuration of C-6 and/or C-7 hydroxy groups relative to the bridging nitrogen in tropanes. Lactonization (1.XVII) implied the presence of a C-6 or C-7 hydroxy function and furthermore, indicated a β configuration. However, such evidence was not unambiguous, given lactonization through a 3β-OH group could possibly occur. Such a configuration was not excluded by the ether experiment, provided the 6-hydroxy had the α configuration. An

elegant series of stereochemical experiments confirmed the initial assignment. The cyclic urethane claimed to be formed on treating valeroidine with $KMnO_4$ was subsequently shown by P.M.R. spectroscopy to be N-formyl nor-valeroidine. (1.XVIII)[23].



1.XVII



1.XVIII

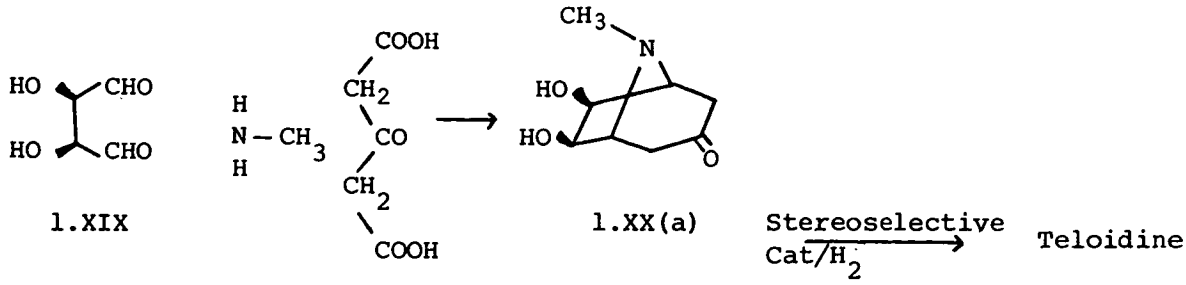
The assignment of the hydrolysis product as tropan-3α,6β-diol was confirmed by a synthesis of the parent base from

(a) the thermal decomposition product of valeroidine phenylurethane[24].

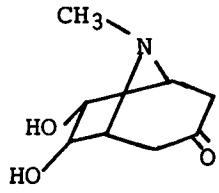
(b) selective deacylation of 3,6-diisovaleroxy tropane[24].

(ii) Tropan-3,6,7-triols and Esters

The simplest member of this series of compounds is meteloidine, which is optically inactive and not resolvable. A diacetyl compound was formed on acetylation of the parent base, which also yielded on hydrolysis one mole of 2-methylbut-2-enoic acid and an amino triol, assumed to be tropan-3,6,7-triol (teloidine)[25]. This assignment was proved by a Robinson-Schöpf synthesis from meso-tartaric dialdehyde (1.XIX).

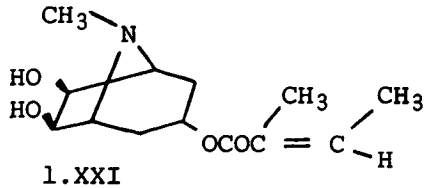


1.XIX 1.XX(a) Stereoselective Cat/H_2 Teloidine



1.XX(b)

The meso-aldehyde must undergo cyclization with the hydroxy groups cis to each other, hence products 1.XX(a) and 1.XX(b) were expected: catalytic hydrogenation of the product produced teloidine and a small amount of pseudoteloidine, its C-3 isomer, indicating the stereoselectivity of incorporation of the dialdehyde[26]. Spectroscopic evidence has subsequently shown that meteloidine is 6β,7β-dihydroxy-3α-tigloyloxytropane[27] (1.XXI).

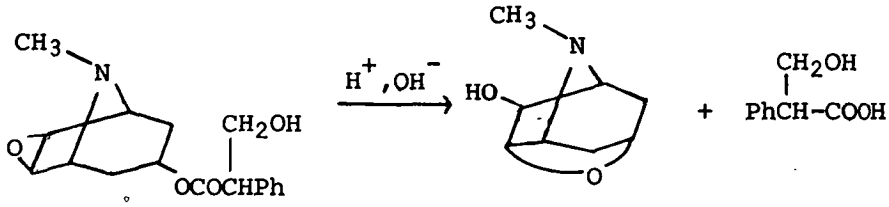


1.XXI

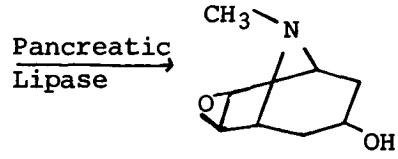
(iii) 3-hydroxy-6β,7β-epoxy tropanes

The hydrolysis of hyoscine (1.II) or scopolamine, its racemate, under acidic or basic conditions leads to racemic oscine (1.XXII) and (-) or (±) tropic acid (1.XXIII), respectively[28]. However, selective enzymatic hydrolysis using pancreatic lipase produces the

optically inactive, nonresolvable scopine (1.XXIV) and (-) or (±) tropic acid respectively.

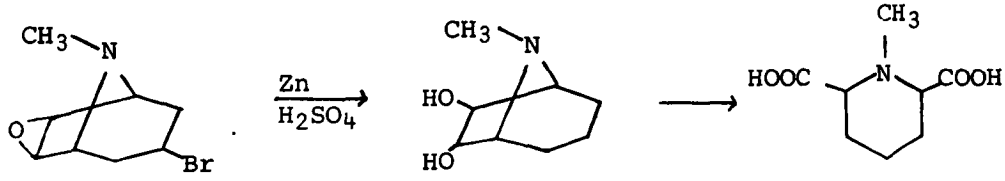


1.II 1.XXII 1.XXIII



1.XXIV

The fact that the optical activity resides in the tropic acid moiety was proven by dehydration of both hyoscine and scopolamine to give an inactive and unresolvable product. Hence, oscine was seen to be a rearrangement product and scopine was a primary hydrolysis product[29]. The bridging ether of oscine (1.XXII) was opened by the action of hydrogen bromide to give "hydroscopoline bromide" (1.XXV) which on debromination yielded hydroscopoline[30] (1.XXVI).

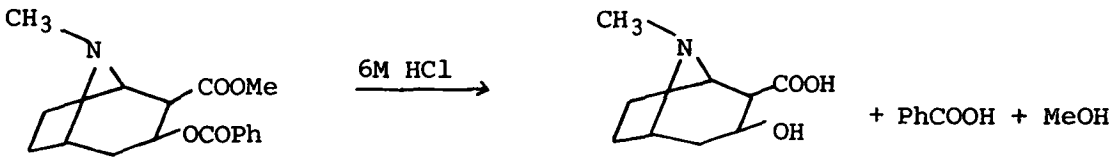


1.XXV 1.XXVI 1.XXVII

The oxidation of 1.XXVI with chromic acid to 2,6-dicarboxy-1-methyl-piperidine (1.XXVII) confirmed the 6,7-dihydroxy tropane structure[31].

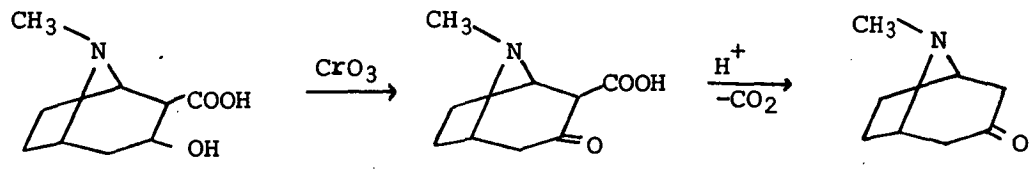
1.2.3. Ecgonines

Acid hydrolysis of the natural base cocaine (1.IV) leads to methanol, benzoic acid and an amino-carboxylic acid (-) ecgonine (1.XXVIII)[32].



1.IV 1.XXVIII

Ecgonine is oxidized in chromic acid to ecgoninone (1.XXIX), a β-keto carboxylic acid, which readily decarboxylates to tropan-3-one (1.XXX)[33].



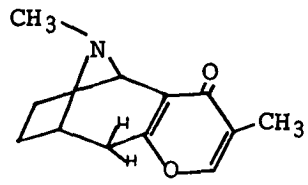
1.XXVIII 1.XXIX 1.XXX

In 1901 Willstaetter[34] achieved the synthesis of ecgoninone from tropan-3-one sodium enolate and carbon dioxide. The carboxylate was then reduced to ecgonine and pseudoecgonine, which were interconvertable with base, but were not enantiomers (their optical rotations differing in absolute terms and their melting points being different). Based on analogy with the tropan-3-ol series, Willstaetter assumed that isomerism in the cocaines was the result of C-3 epimerism. Fifty years later elegant stereochemical experiments by Fodor et al[55,56] and Findlay[57,59,61] related the stereochemistries of all four cocaines. It was proved that cocaine-pseudococaine isomerism was a consequence of C-2 epimerism, whereas cocaine-allococaine isomerism was a consequence of C-3 epimerism.

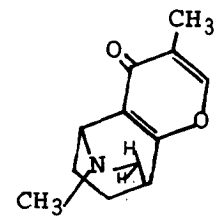
Benzoylation[34] of Willstaetter's reduced products and a difficult resolution of the bases, through the d-α-bromocamphorsulphonic acid salts, afforded the natural bases pseudococaine and cocaine.

1.2.4. Pyronotropanes

The structural elucidation of the bases from the Bellendena and Darlingia genera has been based on spectroscopic, diffraction and synthetic evidence. Interpretation of the ultraviolet, (U.V.), infrared, (I.R.), nuclear magnetic resonance, (N.M.R.) and mass spectra, (M.S.) data (Chapter 2) indicated that bellendine had structure 1.VIII or 1.XXXI.



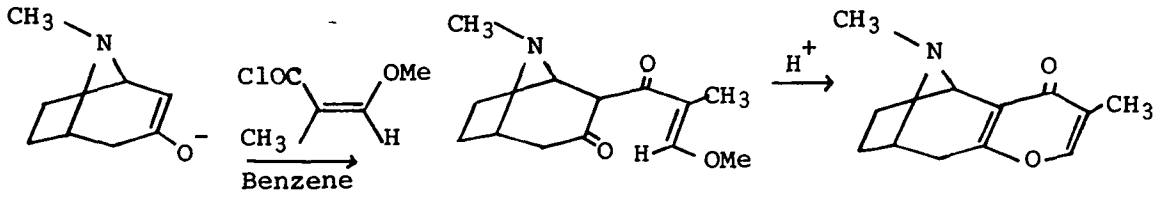
1.VIII



1.XXXI

The tropane structure (1.VIII) was favoured on biosynthetic grounds; however, the isoquinuclidine structure (1.XXXI) could not be excluded because of the observed "splitting" of the proton magnetic resonances associated with the geminal methylene protons when the spectrum was run in trifluoro-acetic acid. This resulted in the methylene signal being assigned a position adjacent to nitrogen. However, the large apparent coupling constant (15 Hz) was significantly in excess of values observed for systems involving the coupling of α methylene protons to protonated tertiary nitrogen. This fact cast doubt over structure 1.XXXI.

The tropane structure (1.VIII) was subsequently shown to be correct through a direct method X-ray crystal structure determination[35], and through a synthesis of the compound[36] from the sodium enolate of tropan-3-one and 3-methoxy-2-methyl prop-2-enoyl chloride (Scheme 1-1).



1.XIII

Scheme 1-1

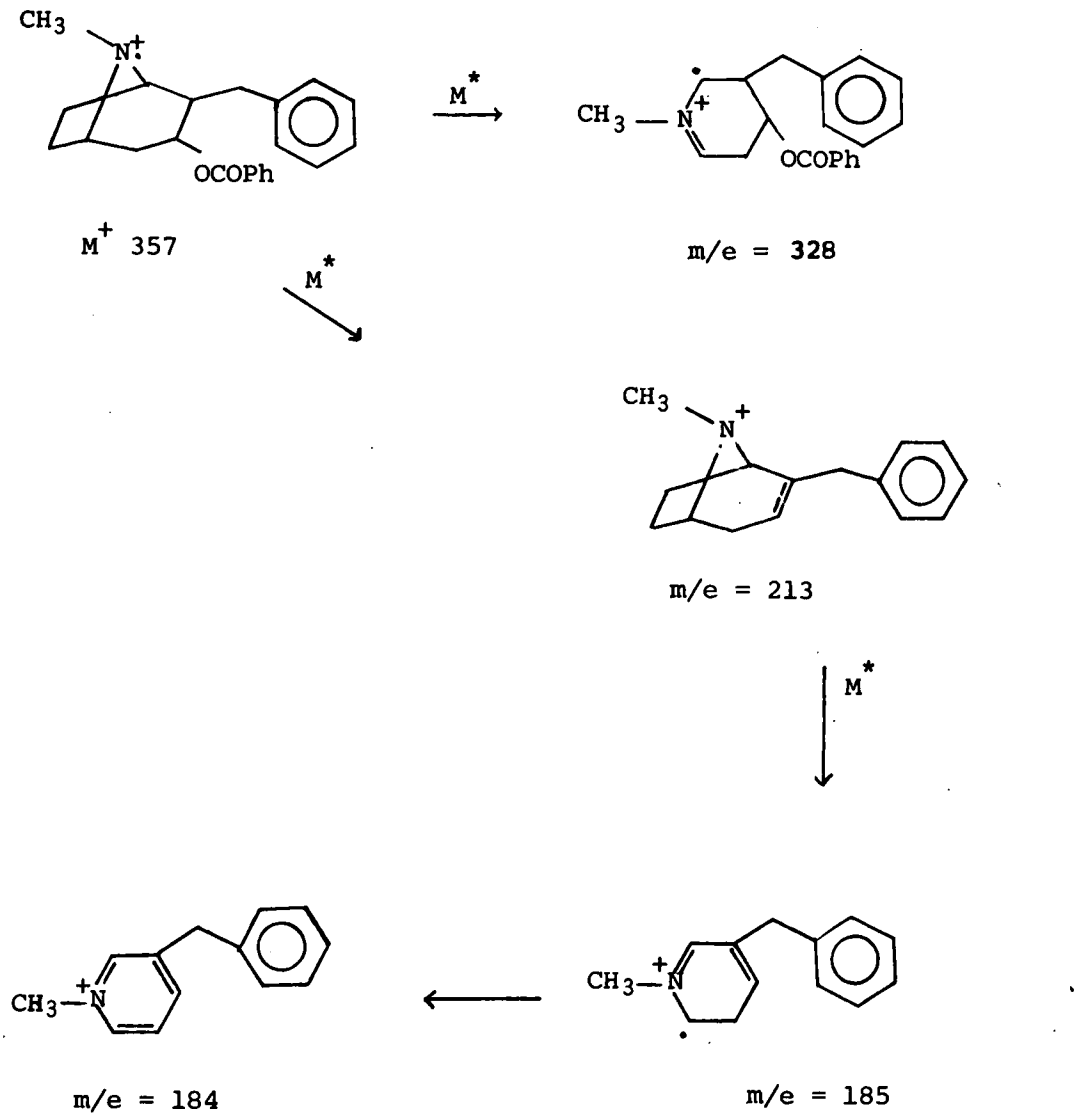
The structures of isobellendine (1.IX) and darlingine (1.X) were determined by analysis of their spectroscopic data as compared to bellendine and by analogous syntheses to the bellendine case. The structure of dihydroisobellendine (1.XI) was determined from a study of its spectroscopic data, in particular through P.M.R. decoupling experiments and the use of shift reagents to unambiguously assign proton resonances. From a knowledge of H-2 and H-3 coupling constants in the cocaine series[10] it was possible to assign the stereochemistry of the dihydropyrono-tropane ring junction as being cis endo. The structure (1.XI) was confirmed by synthesis from ecgonidinoyl chloride and sodium acetone enolate. Details of the structural assignment and synthesis appear in Chapters 2 and 4, respectively.

1.2.5. 2-Benzyl and 2-acyl tropanes

The relative structures of the 2-benzyl tropane alkaloids from Knightia deplanchei[13] have been determined through interpretation of spectroscopic data, and in two cases confirmation has been made through synthesis[37]. The mass spectra of these alkaloids and their hydrolysed products provided evidence for a substituted tropane ring system, bearing a benzyl group in position 2. The fragmentation patterns were

analogous to the known tropan-3-ol ester and tropan-3,6-diol ester series; however, those peaks arising from fragments in which the piperidine ring remained intact, were displaced by 90 mass units when compared to the parent tropane. This was due to the benzyl substituent at position 2.

Fragmentation of K. deplanchei Product A[13]

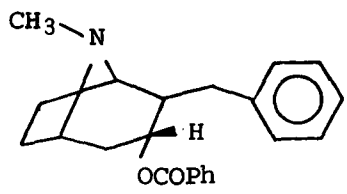


Scheme 1-2

The P.M.R. spectra were consistent with this structural assignment, and further, showed in all cases the characteristic triplet (J = 5 Hz) at τ = 4.8-5.1 for the C-3 proton in products A,B and D, indicating C-3α substitution of the tropane nucleus by an acyloxy substituent. In the case of product C the triplet appeared at higher field, indicating a C-3α-OH substituent.

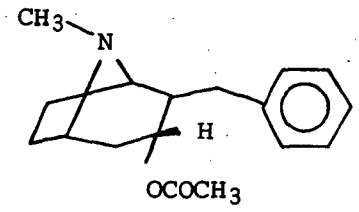
In products C and D additional resonances were apparent at 4.15τ in C and 5.20τ in D, appearing as a doublet of doublets (J = 10 Hz, 4 Hz), consistent with a C-6 or C-7 acyloxy substituent in the β configuration in C, and a C-6 or C-7 hydroxy substituent in the β configuration in D. The benzyl group was apparent as a 5-proton aromatic multiplet at 2.78τ. The benzylic protons appeared as a singlet in product D. Hindered rotation in A,B and C resulted in the appearance of a geminal AB system J_{AB} = 18 Hz, $\Delta\nu_{AB}$ = 8 Hz for the benzylic protons.

Structures of the Major K. deplanchei Alkaloids



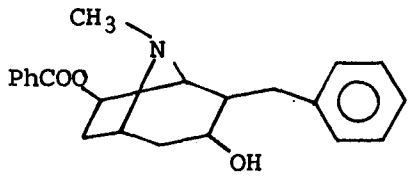
Product A

1.XXXII



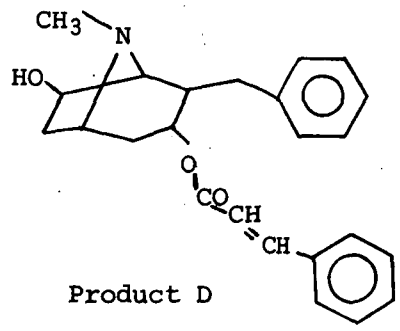
Product B

1.XXXIII



Product C

1.XXXIV

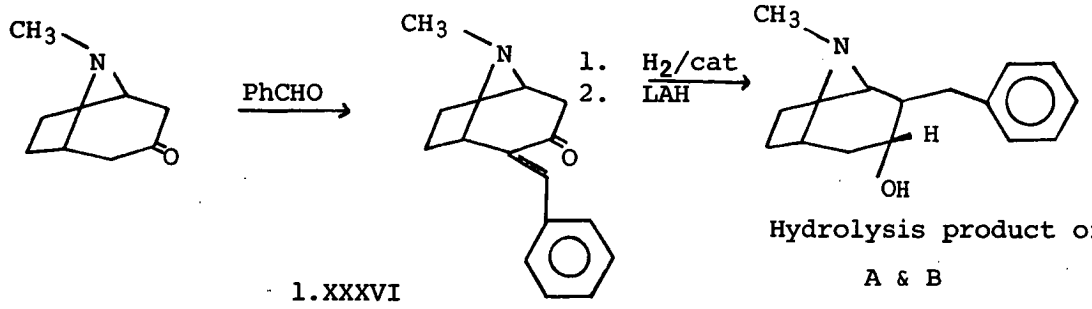


Product D

1.XXXV

The stereochemistry at C-2 has been tentatively assigned as β in the case of products A and B[37]. The stereochemistry of products C and D is not currently known and the relationship between the benzyl group and ethano bridge oxy-function must await P.M.R. spin decoupling on a high frequency instrument.

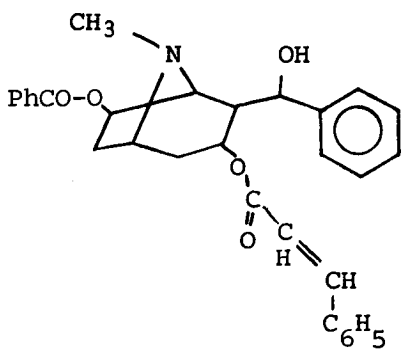
The synthesis of products A and B has been achieved by the formation of trans-2-benzylidine tropan-3-one (1.XXXVI), which on catalytic hydrogenation of the C-C double bond, followed by lithium aluminium hydride reduction, led to a mixture of four isomers. One of the reduced products was identical to the hydrolysis product of the bases (1.XXXII) and (1.XXXIII).



Scheme 1-2

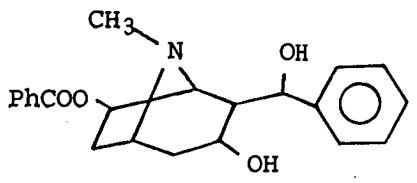
This was assumed to be the 2β-benzyl-3α-hydroxy tropane isomer although no decoupling data have been presented.

Lounasmaa[38] has recently published the structures of two minor alkaloids of Knightia deplanchei, products E and F, (1.XXXVII) and (1.XXXVIII) respectively.



Product E

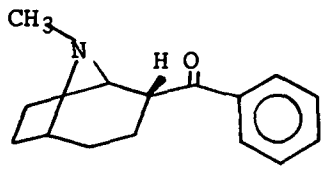
XXXVII



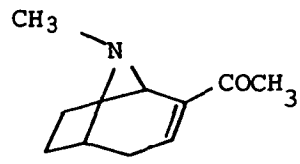
Product F

XXXVIII

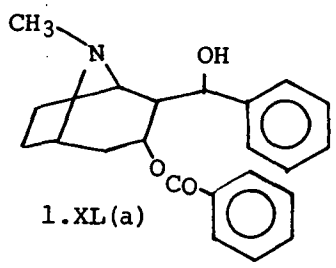
The isolation of these bases, whose structures were determined spectroscopically by analogy with the previously isolated bases, serves to support a hypothesis proposing a similar biogenesis for the Knightia alkaloids and the minor alkaloids of the related Proteaceous species Darlingia ferruginea. The minor alkaloids of the Darlingia species ferruginea have been shown to be 2α-benzoyltropane (1.XXXIX)[39], 2-acetyl-trop-2-ene (1.XL) and 2-(hydroxybenzyl)-3α-benzoyloxy tropane. (1.XL(a)) (Chapter 3).



1.XXXIX

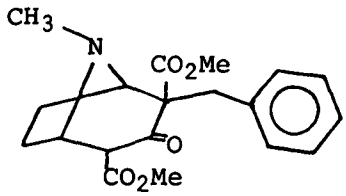


1.XL

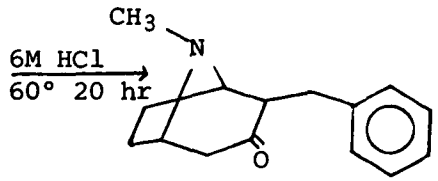


1.XL(a)

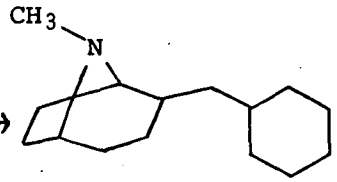
These structures have been deduced from an analysis of spectroscopic data and by synthesis. In particular, use was made of the characteristic M.S. fragmentation patterns of substituted or dehydrotropanes[40]. 2,4-Dicarbomethoxy-2-benzyltropan-3-one (1.XLI) was hydrolysed and decarboxylated; the resultant 2-benzyltropanone (1.XLII) was totally reduced to 2-(methyl cyclohexyl)tropane 1.XLIII which proved to be identical to the reduced product of the alkaloid 1.XXIX.



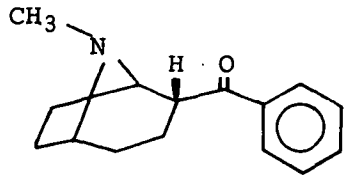
1.XLI



1.XLII

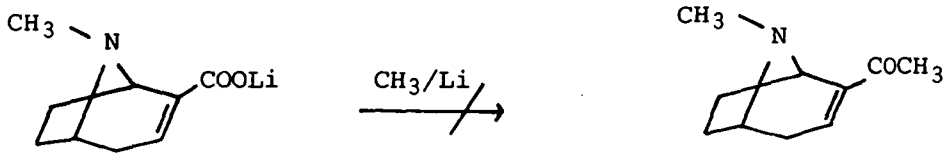


1.XLIII



1.XXIX

The P.M.R. spectroscopic properties of anhydroecgonine methyl ester were analogous to 2-acetyl trop-2-ene, although a synthesis from the lithium salt of anhydroecgonine and methyl lithium was not successful.



1.XLIV 1.XL

1.3. Configurational Assignments and Conformation Studies

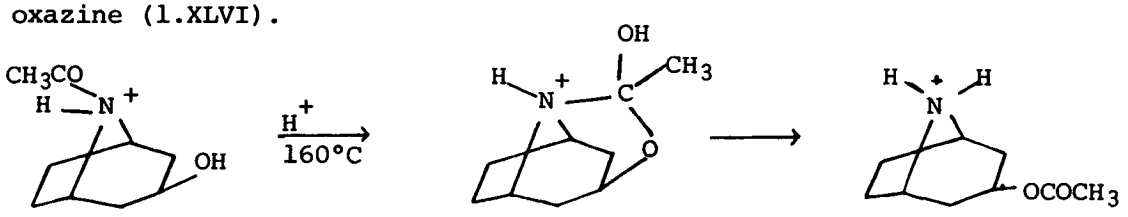
1.3.1. Chemical Methods

(i) Tropanol Esters

The simplest tropanol epimers, tropine (tropan-3α-ol) and pseudotropine, (tropan-3β-ol), were shown to be related to each other by Willstaetter[41] even before the constitution of the bases was known. Reduction of the chromic acid oxidation product, tropan-3-one, of either alcohol led to a mixture of the epimeric alcohols. Furthermore, tropine could be transformed into pseudotropine by the action of sodium amyloxide. Despite this early evidence, when the constitution of the tropanes became known it was argued[42] that the tropine/pseudotropine pair were actually dl and meso forms of tropanol. With the ultimate failure to resolve tropine, Willstaetter's structure and stereochemical rationalization of the lack of optical activity was accepted. Both the isomers must be meso forms as it is impossible to construct the aza bridge in the trans configuration.

The ultimate assignment of configuration was based on the proof of the steric proximity of the C-3 hydroxy group in pseudotropine to the ring nitrogen. This was achieved by both Fodor[43] and Fieser[44] simultaneously through O → N acyl migration experiments. Fieser

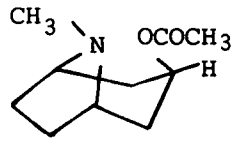
demethylated pseudotropine acetate using the von Braun cyanogen bromide method. The treatment of N-acetylnorpseudotropine (1.XLV) with acid at 160°C produced quantitatively the O- acetyl derivative (1.XLVII). The reaction involves a transition through the cyclic oxazine (1.XLVI).



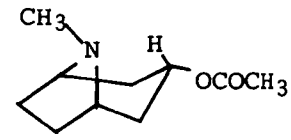
1.XLV 1.XLVI 1.XLVII

When pseudotropine assumes the boat conformation, the steric proximity of the hydroxy group and the quaternary nitrogen enables the oxazine to form. However, in the case of tropine, such a close relationship is not possible and acyl migration via the oxazine intermediate does not occur. This differential reactivity enabled Fieser and Fodor to unambiguously assign the α and β hydroxy configurations to tropine and pseudotropine respectively.

Further evidence has supported this assignment. Pseudotropine is the thermodynamically more stable isomer, in keeping with an equatorial configuration of the hydroxy group. Furthermore, esters of pseudotropine hydrolyse more rapidly than corresponding esters of tropine[45], as expected for equatorial esters. However, in order for the hydrolysis data to stand as independent evidence of configuration, the conformation of the piperidine portion of the tropane ring system must be known. If the molecule were to assume the boat conformation predominantly, then the opposite stereochemical interpretation must be placed on the relative rates of hydrolysis, that is, the faster rate of hydrolysis must be associated with 1.XLIX due to the greater steric inhibition in the case of 1.XLVIII.



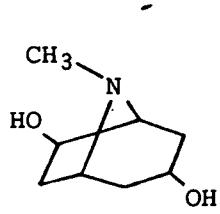
1.XLVIII



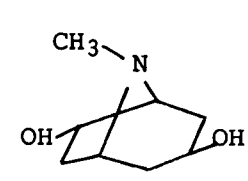
1.XLIX

Such were the proposals of the contemporary workers in this field at the time of the N → O acyl migration experiments. The unambiguous assignment of configuration based on this experiment meant that as a corollary, the ground state conformation of pseudotropine must be the chair form. Measurements of molecular polarizability[46], and N.M.R. parameters[47], and X-ray crystal structure determinations[48,49], have subsequently supported this conclusion. At the high temperature of the N → O acyl migration experiment a greater degree of conformational mobility permits cyclization to occur <u>via</u> the oxazine.

In Section 1.2.2. reference was made to the techniques of forming cyclic ethers, urethanes and lactone salts (1.LVI) in order to assign the steric relationship of the hydroxy groups of the tropan-3,6-diols and tropan-3,6,7-triols. Such techniques do not unambiguously discriminate between forms such as 1.XV or 1.L, both of which are capable of undergoing the abovementioned cyclizations.



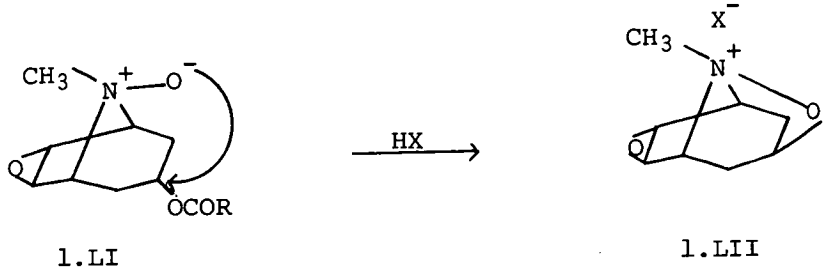
1.XV



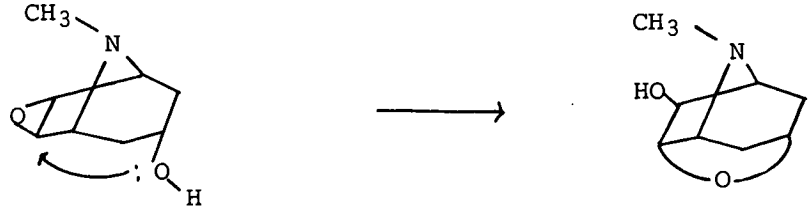
1.L

The solution to this problem was achieved through an elegant application of stereochemical principles and some simple experimentation. As a consequence, the configuration of the epoxy bridge across carbons 6 and 7 in scopolamine was also determined.

The ready attack of the N-oxide oxygen in scopolamine N-oxide[50] (1.LI) to form scopinium bromide (1.LII) via displacement of tropic acid provided evidence for the assignment of the stereochemistry of scopolamine as C-3α-OCOR[51]. This assignment was consistent with the observed formation of oscine (1.XXII) from scopine (1.LIII), which can occur via a concerted nucleophilic attack only if the epoxide ring in scopine has the 6β, 7β configuration[51].

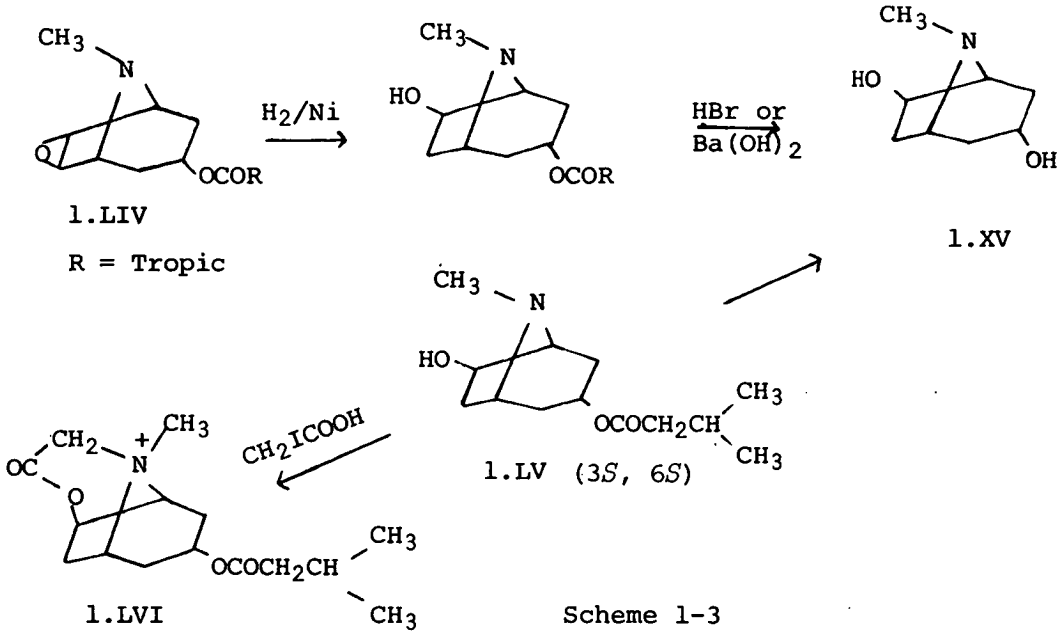


1.LI 1.LII



1.XXII 1.LIII

When scopolamine (1.LIV) was catalytically reduced and hydrolysed, it formed a compound, 1.XV (±) 3α, 6β-dihydroxytropane[52] structurally similar to that produced by the hydrolysis of the natural base valeroidine (1.LV). As the epoxide reduction proceeded with retention of the C-6-OH configuration, the stereochemistry of valeroidine could be assigned as 6β-hydroxy-3α-isovaleroxytropane (1.LV). At the same time, Stoll, Becker and Jucker[53] reported the synthesis of this compound.



1.LIV

R = Tropic

1.XV

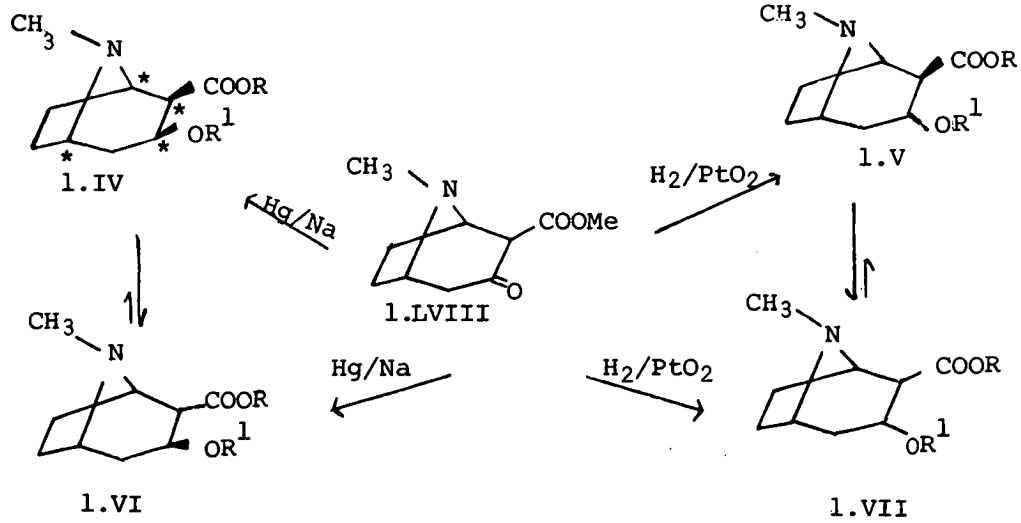
1.LV (3*S*, 6*S*)

1.LVI

Scheme 1-3

(ii) Configuration of the four Cocaine epimers

The cocaine structure (1.IV) shows the presence of four asymmetric centres (shown by *). The linkage of two such centres through aza bridging reduces the number of possible stereochemical forms to four racemic pairs (1.IV), (1.V), (1.VI), (1.VII). Absolute configurations are shown.



1.IV

1.V

1.LVIII

1.VI

1.VII

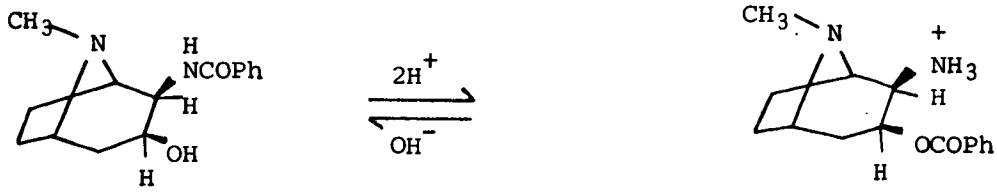
1.IV	Cocaine	R = Me, R^1 = COPh	1.V	Allococaine	R = Me, R^1 = COPh
	Ecgonine	R = H, R^1 = H		Alloecgonine	R = Me, R^1 = H
1.VI	Pseudo-cocaine	R = Me, R^1 = COPh	1.VII	Allopseudo-cocaine	R = Me, R^1 = COPh
	Pseudo-ecgonine	R = H, R^1 = H		Allopseudo-ecgonine	R = H, R^1 = H.

The investigation of the stereochemical interrelationship of these forms dates from Einhorn's[54] conversion of the hydrolysis product of (-) cocaine (IV) i.e. (-) ecgonine to "(+) ecgonine" by the action of base. The concluding chapter was the definitive synthetic and P.M.R. study of Beyerman *et al*[10].

Willstaetter recognized that the product obtained from (-) ecgonine on treatment with base was not the enantiomer, (+) ecgonine, as proposed by Einhorn, but the diastereomer. On the basis of the "analogous" tropine/pseudotropine conversion, he named the isomer pseudoecgonine. No evidence was given to support C-3 epimerism other than the qualitative similarity of the reaction conditions in both cases. The subsequent synthesis of ($\bar{+}$) pseudoecgonine and ($\bar{+}$) ecgonine was realized through the reduction of 2-carbomethoxy tropan-3-one (1.LVII). These two racemates were the major products, although a minor product, "das drittes racemisches Ekgonin" was also isolated. The assumption that as the two major racemates were the products of reduction of a common 3-keto compound they were C-3 epimers, was subsequently proven incorrect.

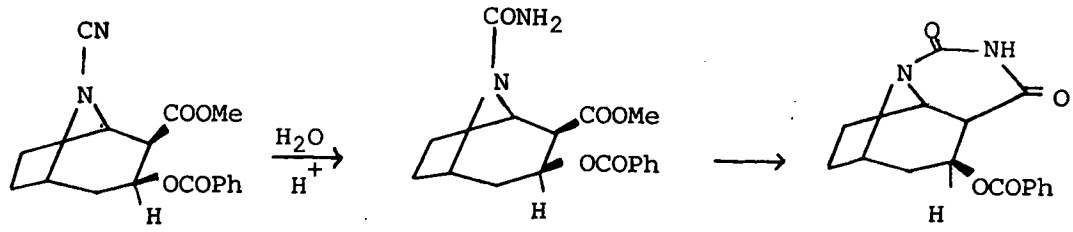
Fodor[55] succeeded in effecting the reversible N → O acyl migration for N-acetylnorpseudoecgonine ethyl ester. This confirmed the C-3β-OH configuration in pseudoecgonine. The same experiment failed for ecgonine. This was interpreted as being in accordance with the Willstaetter view of C-3 epimerism for the compounds. Findlay[57] was able to show this to be invalid when he achieved the facile O → N acyl migration of O-benzoylnorecgonine, proving the C-3β-OH configuration for ecgonine. Hence ecgonine and cocaine were shown to differ from pseudoecgonine and pseudococaine at the C-2 position. Facile interconversion has now been shown for these isomers[57], consistent with the ready epimerization of axial carbomethoxy groups to equatorial positions.

The assignment of the C-2 stereochemistry of ecgonine and pseudoecgonine was made by Fodor[55,56] on the basis of further acyl migration studies. Ecgonine and pseudoecgonine were subjected to Curtius degradation, which was assumed to take place with retention of configuration. The N-benzoyl derivatives were then examined for migration from N → O.



1.LVIII 1.LIX

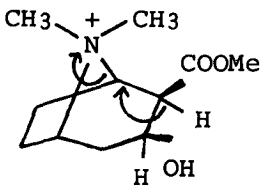
The ecgonine derivative underwent amide-ester interchange to 1.LIX, but no migration was observed for the pseudoecgonine derivative. This was taken as evidence of the cis nature of the relationship between the C-3β-OH and the C-2 carboxyl function in ecgonine and a trans relationship in pseudoecgonine. The configuration of the carbomethoxy group was shown to be β in cocaine and α in pseudococaine. This assignment received confirmation when the von Braun degradation product of cocaine, N-cyanonorcocaine (1.LX) was converted via the carbamate (1.LXI) to the lactam[58] (1.LXII).



1.LX 1.LXI 1.LXII

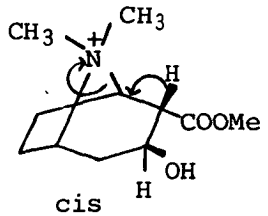
The knowledge that cocaine and pseudococaine were C-2 epimers with the C-3β-OH configuration meant that the other two cocaines were C-2 epimers based on a C-3α-OH configuration. Findlay[59] was the first to unambiguously synthesize these compounds, although his nomenclature is the reverse of that proposed by Preobrazhenskii[60] and accepted by most workers.

The assignment of the stereochemistry of these epimers was based on an observation related to the relative rates of Hofmann degradation of their methiodides. The facile Hofmann degradation of ecgonine methiodide was interpreted as being due to the trans relationship between the C-2 hydrogen and the departing dimethylammonium group. This E2 elimination reaction occurs so readily that preparations of the methiodide of ecgonine were always contaminated by the hydroiodide of the degradation products[61,9]. No such contamination was observed with pseudoecgonine, due to the higher energy requirement for cis elimination.



trans

Fig. 1-1



cis

Similarly the compound "allococaine" (allopseudococaine (1.IV)) formed a pure methiodide in contrast to "allopseudococaine" (allococaine (1.VII)) which formed a mixture of products.

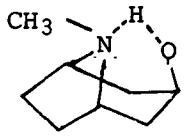
Various other experimenters[55,62] have made configurational assignments to allococaine and allopseudococaine. At best, such interpretations have been tentative; in all cases, the possibility of epimerization due to the labile Hα to the carbomethoxy group, or conformational mobility favouring a greater degree of boat form, cannot be excluded. The use of spectroscopic methods provides an alternative

and sometimes superior approach to the problem of structural, configurational and conformational analysis.

1.3.2. Spectroscopic Methods

(i) Infrared Spectroscopy

The infrared spectra of tropine and pseudotropine in CS_2 solution show the presence of a sharp band at 3620 cm^{-1} and a fairly strong broad band at $\tilde{\nu}$ 3300 cm^{-1}. Zenitz[63] et al attributed the former to a free hydroxyl and the latter to a hydroxy with hydrogen bonding. A dilution experiment by these workers was designed to show the presence of intermolecular hydrogen bonding in tropine and intramolecular bonding in pseudotropine. The ratio of the integrated areas under the "free" OH band and the "bonded" OH band were compared for each of the epimers at progressively increasing dilution. It was found that pseudotropine showed a greater ratio over all concentrations down to the minimum measured (2×10^{-2}M). The conclusion was drawn that pseudotropine was the cis (3β) isomer and existed in a conformation permitting intramolecular hydrogen bonding. (1.LXIII).



1.LXIII

House[64] has subsequently shown in a detailed I.R. study of cyclic amino alcohols that neither pseudotropine nor tropine exhibit intramolecular hydrogen bonding. It was asserted that the concentrations used in the preceding experiment were too great to exclude intermolecular bonding. At concentrations of 0.5×10^{-3}M no intramolecular bonding was

observed although the presence of a free OH band was clearly seen. Furthermore, it has been shown that the energy gained by intramolecular hydrogen bonding is not sufficient to force a piperidine ring out of the chair conformation into the boat conformation unless appreciable repulsions exist for the chair form[65]. Thus, House concluded that both tropine and pseudotropine exist as the chair conformers, and that the configurational assignment based on the hydrogen bonding studies was invalid.

Preobrazhenskii[60] has used infrared spectroscopy to determine the relative configurations of the four cocaine epimers. The approach was based on hydrogen bonding studies and the correlation of observed ν_{C-O} values for tropine and pseudotropine with values of known axial and equatorial hydroxy cycloalkanes.

The data supporting the hydrogen bonding arguments are tenuous. It was claimed that in ecgonine, allopseudoecgonine, and to a lesser extent pseudoecgonine, an intramolecular hydrogen bonding interaction was possible between the C-3-OH and the carbonyl at the C-2-COOMe, these groups being <u>cis</u> (a,e; e,a) in the former pair and <u>trans</u> (e,e) in the latter. The I.R. spectrum (CS_2 solution) showed a large broad band at $\tilde{\nu}$ 3400-3500 cm^{-1} in all cases, attributed to intermolecular hydrogen bonding. This band was appreciably reduced in intensity upon dilution, a result which is consistent with the assignment. The bands appearing at 3536 cm^{-1}, 3530 cm^{-1} and 3630 cm^{-1} respectively, for the above isomers were attributed to intramolecularly hydrogen bonded OH because they remained unchanged or slightly increased on dilution. One can criticize this approach on the grounds that the bands may not be correctly assigned. Firstly, such bands appear in all four ecgonines; secondly, the sharpness of the bands, the close wavenumber relationship to "free OH" (ν_{OH} = 3630 cm^{-1}) in tropine, and the increase in intensity

on dilution, are facts more consistent with a "free O-H" assignment. The use of hydrogen bonding studies, upon which many of the arguments were based, has been criticized by House[64]. The results presented by Preobrazhenskii were consistent with the stereochemical assignment proposed earlier by Findlay.

The identification of the ν_{C-O} absorption frequencies for axial and equatorial substituents was made through a solvent shift technique for the tropanol epimers. An analysis of the ν_{C-O} values for cyclohexane alcohols showed that ν_{C-O} for axial hydroxy groups occurs at lower wavenumber than the corresponding equatorial group. This relationship was found to occur in tropine and pseudotropine, namely, ν_{C-O} = 1049 cm^{-1} and 1063 cm^{-1} respectively.

Extension of this approach to cocaine and pseudococaine c.f. allococaine and allopseudococaine enabled the assignment of the C-3β-OH configuration to the cocaine, pseudococaine epimeric pair, ν_{C-O} = 1075 cm^{-1}, and the C-3α-OH configuration to the allococaine, allopseudococaine epimeric pair, ν_{C-O} = 1049 cm^{-1}.

(ii) Proton Magnetic Resonance (P.M.R.)

The utility of P.M.R. methods in conformational and configurational studies of the tropane alkaloids results from application of the Karplus equation[66] or Williamson and Johnson's modification of it[67] to a study of the multiplet associated with the C-3 proton[68]. Two factors are responsible for the concentration of attention on this resonance. Firstly, the difference between the chemical shift of the C-3 proton and the vicinal protons to which it is coupled, $\Delta\delta^{2,3}$, is significantly larger than the vicinal coupling constants, $J_{3,2ax2eq}$.

For atropine $\frac{\Delta\delta^{3,2ax}}{J_{3,2ax}} = 5.5$; $\frac{\Delta\delta^{3,2eq}}{J_{3,2eq}} = 16$

For tropacocaine $\frac{\Delta\delta^{3,2ax}}{J_{3,2ax}} = 30$; $\frac{\Delta\delta^{3,2eq}}{J_{3,2eq}} = 44$

A "first order" interpretation of the spectrum is thus possible. The spin system associated with the tropan-3-ol series C-3 proton is best described as AA'BB'X. Furthermore, the AB coupling is large ($\tilde{\nu}$ 14 Hz); under these circumstances the system can be regarded as two almost independent A_2 ($H_{2,4ax}$) and B_2 ($H_{2,4eq}$) systems[68]. The X part of the system (H_3) therefore consists of a triplet of triplets, the splittings being simply J_{AX}, J_{BX}.

Secondly, since the molecule is predominantly in the chair conformation, that is, not undergoing chair → boat → chair conversions,* these couplings represent the H_{3ax}-$H_{2,4ax}$ and H_{3ax}-$H_{2,4eq}$ vicinal interactions for pseudotropine and the H_{3eq}-$H_{2,4ax}$ and H_{3eq}-$H_{2,4eq}$ vicinal interactions for tropine. From these coupling constants and the modified Karplus equation for constrained cyclohexanes viz

$$J_{H,H'} = 10 \cos^2\phi \qquad 0° < \phi < 90°$$

$$J_{H,H'} = 16 \cos^2\phi \qquad 90° < \phi < 180°$$

the dihedral angle may be calculated.

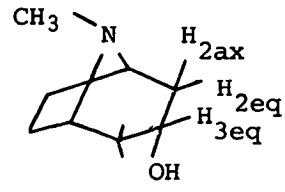
The analysis has been performed for a number of tropan-3-ols and their esters. Some examples of the present work are included for comparison. It is generally considered that the modified Karplus

---

*The fusion of the piperidine and pyrrolidine ring systems prevents chair-boat-chair interconversion; moreover complimentary evidence of dipole moments[49], molecular polarizability[46], and X-ray data[47,48] suggest that the molecule is predominantly in the chair conformation.

equation gives more self-consistent results for dihedral angles in fused ring systems[69], although the Karplus equation has been shown to give dihedral angles for pseudotropine which are close to those found by X-ray crystallography[48]. The latter analysis uses values for coupling constants which are at variance with those of other laboratories. Note Table 1-2.

Tropan-3α-ol and Esters



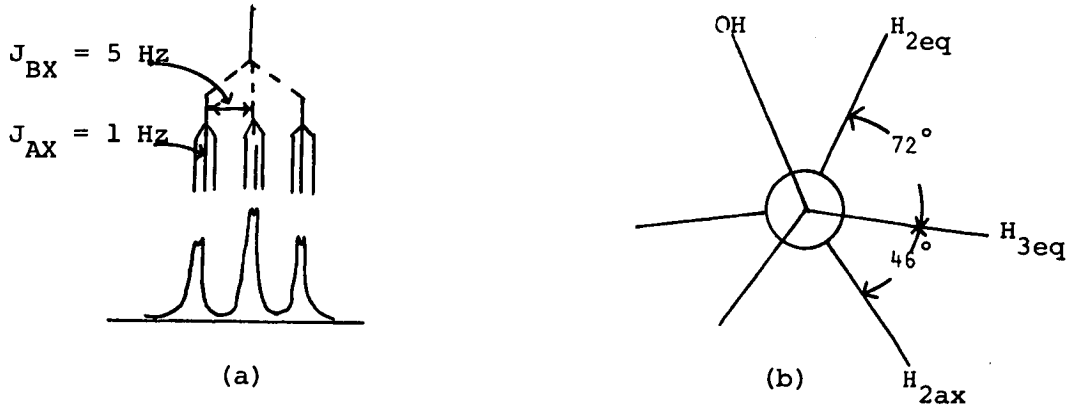


Fig. 1-2. (a) Diagram showing observed splittings in H_{3eq} in tropine.
(b) View along C-3,2 indicating calculated dihedral angles.

TABLE 1-2

Comparison of Coupling Constants and

Calculated Dihedral Angles, α alcohol Series

Compound	J_{BX}, J_{AX}		Angles Calculated[a] (Approx.)		Ref.
Tropan-3α-ol	4.8	1-2	47°	72°	69
Tropan-3α-ol[b]	4.8	1-2	40°	80°	49,48
Phyllalbine	5.2	1-2	40°	80°	69
Atropine	5.0	1-2	43°	75°	69
Methylphyllalbine	5.2	1-2	40°	80°	69
6β-Acetoxy-3α-Isobutoxy tropane	5.0 ± 0.2	1-2	43°	75°	Thesis
6β-Isobutoxy-3α-acetoxy tropane	4.8 ± 0.2	1-2	47°	72°	"
6β-Acetoxy-3α-Isovaleroxy tropane	5.2 ± 0.2	1-2	40°	80°	"
2-benzyl-3α-benzoyl tropane	5.2 ± 0.5	<1	40°	80°	13[c]
2-benzyl-3α-[d] acetoxy tropane	4 ± 0.5	<1	50°	70°	13[c]
Scopine	5	1	43°	75°	68(b)

(a) Dihedral angles calculated from Williamson and Johnson equation.

(b) Dihedral angles calculated from Karplus equation.

(c) From spectra kindly supplied by Dr. M. Lounasmaa.

(d) The substitution of the piperidine ring by a benzyl group may alter the coupling constant by mechanisms other than dihedral angle change.

It is seen from the table that in all cases of 3α substituted tropanes, the coupling constants are consistent with a distorted chair conformation with deviations from normal dihedral angles observed in cyclohexane of $\tilde{\sim}$ 15°. Furthermore, it is seen that as the steric bulk of the esterifying acid increases, the distortion from an ideal chair conformation becomes greater. However the experimental error is too large in these determinations to support such an hypothesis quantitatively.

The distortion of the piperidine ring from an ideal chair conformation in tropine is rationalized by consideration of the interaction between the ethano bridge endo (C-6,7α) protons and the bulky esterifying group. Steric repulsions are reduced by the molecule assuming a more flattened conformation. This distortion, which is apparent from a similar analysis of the pseudotropine series, but to a lesser extent, does not prevent discrimination between the two series. J_{AX} in the tropine series is sufficiently small to enable the C-3H multiplet to be qualitatively described as a "triplet" (Fig. 1-2). In the pseudotropine series, the multiplet has the appearance of a "quintuplet" (Fig. 1-3).

Tropan-3β-ol and Esters

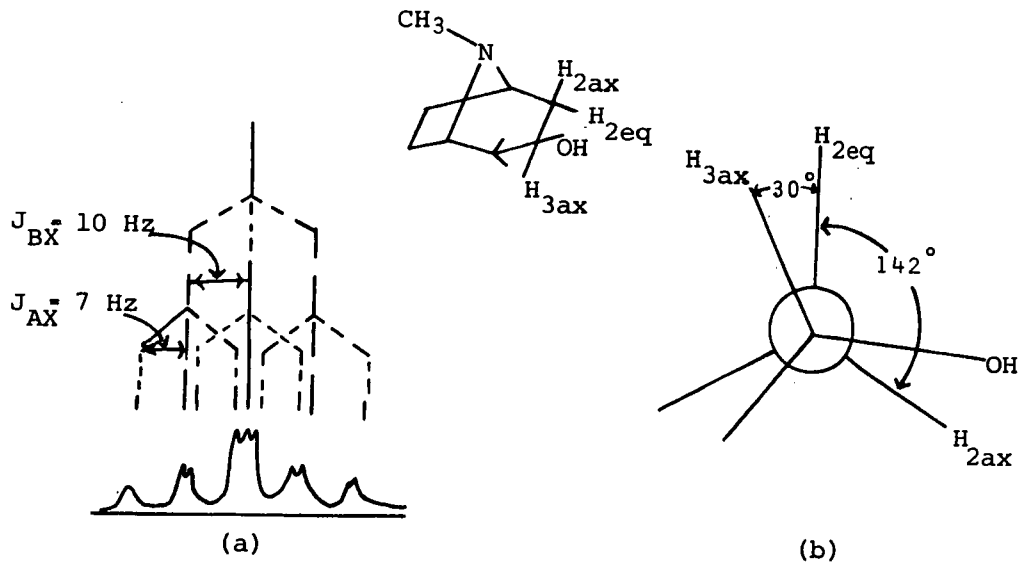


Fig. 1-3. (a) Diagram showing observed splittings in H_{3ax} in pseudotropine.

(b) View along C-3,2, indicating calculated dihedral angles.

TABLE 1-3

Comparison of Coupling Constants and

Calculated Dihedral Angles, β Alcohol Series

Compound	J_{BX}, J_{AX}	Angles Calculated[a] (Approx.)		Ref.
Tropan-3β-ol	9.9 7.5	142	30	69
Tropan-3β-ol[b]	10.2 6.6	159	40	49,48
Tropacocaine	9.9 7.5	142	30	69
6β-Acetoxy-3β-hydroxy tropane	9 ± 0.5 7 ± 0.5	147	35	Thesis
6β-Acetoxy-3β-Isovaleroxy tropane	9 ± 0.5 7 ± 0.5	147	35	"

(a) Dihedral angles calculated from Williamson and Johnson equation.

(b) Dihedral angles calculated from Karplus equation.

It is noted that in cases of 3β substituted tropanes, there is slightly less distortion from ideal chair conformation than in the case of the 3α series. Esterification of the equatorial hydroxyl in the pseudotropine series has no effect on the piperidine ring conformation - within the tolerances permitted by experimental error.

Ecgonine and Cocaine Series

Sinnema, Beyerman et al[10] have used the modified Karplus equation to compare and umbiguously assign the stereochemistries of all four cocaine and ecgonine stereoisomers. The chemical shift differences of each of the relevant signals was sufficiently large to enable first order analysis of the multiplets. Despite the large coupling constant between H_{4ax}-H_{4eq}, there was no virtual coupling observed in H_2 which

enabled more ready extraction of coupling constants from the multiplets. From a consideration of the calculated angles in Table 1-4 it is seen that the substituent configuration for the cocaine series can be readily determined, and that the results are in accordance with the previously assigned stereochemistries. The newly isolated base, dihydroisobellendine, is also seen to have a stereochemistry closely related to allopseudococaine.

Lambert[70] has described a method for calculating the degree of ring flattening by comparing the ratios of the coupling constants $J_{3,4ax}$ to $J_{3,4eq}$ for similarly substituted cyclohexanes or heteroderivatives. A ratio of $\tilde{\sim}$ 2:1 indicates that the molecule is close to an ideal chair conformation, whereas values less than this correspond to flattening of the chair. It is seen that the chair form in cocaine is less flattened than pseudococaine. Such a result is contrary to expectations; the diequatorial configuration of Pseudococaine would be expected to cause less significant distortion from the ideal chair conformation than cocaine.

(iii) Carbon-13 Magnetic Resonance (C.M.R.)

The application of C.M.R. spectroscopy to structural and stereochemical problems provides valuable complementary information to P.M.R. spectroscopy. The widely used techniques of noise-off-resonance decoupling (N.O.R.D.)[71] and single-frequency-off-resonance decoupling (S.F.O.R.D.) permit shift assignments to be made with greater certainty. Two structurally diagnostic effects, in addition to the standard chemical shift theory for ^{13}C nuclei[72], are used in C.M.R. spectroscopy of natural products. Substitution of (5 or 6-membered) alicyclic or heterocyclic systems leads to significant shifts at the substitution site (α effect), the neighbouring carbon (β effect) and the carbon one further removed (γ effect). For example, in piperidines[73,74] the presence of an equatorial methyl group causes deshielding α,β and γ

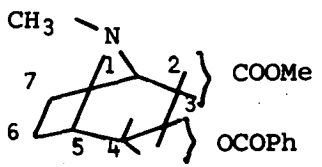


TABLE 1-4

Comparison of Coupling Constants and Stereochemistries in the Cocaine Series[10]

Compound	Vicinal Coupling Constants				Calculated Angles °				Configuration	
	$J_{2,3}$	$J_{3,4ax}$	$J_{3,4eq}$	$J_{1,2}$	H_2/H_3	H_3/H_{4ax}	H_3/H_{4eq}	H_1/H_2	2	3
Cocaine	6.0	11.6	6.0	3	39	148	39	60	β	β
Pesueodocaine	10.4	10.4	6.8	3	145	145	35	60	α	β
Allococaine	1-2	5	1-2	3	63	45	63	60	β	α
Allopseudococaine	5	5	1-2	-	45	45	63	-	α	α
Dihydro-isobellendine(a)	5	5	1-2	3	45	45	63	60	α	α

(a) Current work.

effects of 5.6, 8.9 and 0 p.p.m. respectively whereas axial methyl groups cause α and β deshielding effects of 1.1 and 5.2 p.p.m. respectively, but a γ shielding effect of 5.4 p.p.m.

Secondly, an effect known as endocyclic homoallylic shielding[75] has been noted in which a double bond within a 6 or 7-membered ring causes a shielding of $\tilde{\sim}$ 5.0 p.p.m. in the chemical shift of the homoallylic carbon. Use has been made of this effect in assigning the ethano bridge carbons in tropidine (1.XIV)[71].

^{13}C Data are available for the tropane alkaloids which unequivocably assign the orientation of the N-methyl as equatorial; the assignment is based on the weak shielding of C-2 and C-4 (negligible γ effect) and from the strong deshielding of the ethano bridge carbons C-6, C-7. Quaternized tropanes show distinctly different ^{13}C chemical shifts for axial and equatorial N-methyl groups[71].

Wenkert's assignment of tropane alkaloid ^{13}C resonances was based on a combination of knowledge of 2-methyl-N-methylpyrrolidine chemical shifts, and those for substituted N-methyl piperidines. Linkage of these two data sets through the standard chemical shift theory enables an unambiguous assignment to be made to all resonances. The data for tropane alkaloids presented by Maciel[76] are clearly misassigned. Furthermore in the spectrum of scopolamine, a spurious signal at δ^{TMS} 25.2 is assigned to C-2,4. Such an assignment supports the overall pattern presented in the paper but is not comparable with Wenkert's data, or those found for C-2,4 in darlingine/bellendine or 2α-benzoyltropane in the current work.

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footnote: In a paper received after this manuscript was prepared[76(a)] a good example of an accentuated β effect resulting from ring flattening is seen. The chemical shifts of C-2, C-4 for 3α-phenyl-3β-carbethoxy tropane and 3α-phenyl-3β-benzoyl tropane 41.3δ and 44.7δ, respectively, are more deshielded than the shifts for 3β-phenyl-3α-carbethoxy tropane and 3β-phenyl-3α-benzoyl tropane at 38.1δ and 38.6δ, respectively, expressing an increase in steric compression at the β carbon through a flattening of the piperidine ring.

TABLE 1-5

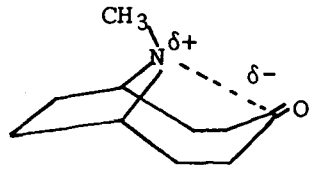
Comparison of ^{13}C Chemical Shifts for Tropane Alkaloids

Compound	$C_{1,5}$	$C_{2,4}$	C_3	$C_{6,7}$	C_8	Ref.
Nortropane	54.7	32.9	17.2	29	-	71
Tropane	61.2	29.9	15.9	25.6	40.4	71
Tropan-3β-ol	60.1	38.3	62.7	26.7	39.2	71
Tropan-3α-ol	59.8	39.1	63.6	25.7	40.0	71
Tropan-3α-ol	64.3	25.6	60.0	38.0	39.2	76
Scopolamine	58.2	38.7	66.6	55.9	40.4	71
Scopolamine (H^+)	53.8	25.2	64.4	58.2	53.4	76
Tropan-3-one	60.2	47.1	207.8	27.3	37.8	71
Trop-2-ene	58.9 57.8	130.8 29.9	122.9	33.9 31.8	36.6	71
Bellendine	58.4 56.2	124.0 29.8	161.3	34.0	37.6	Thesis
Darlingine	58.4 56.2	125.2 29.8	161.7	34.1	37.3	Thesis
2α-benzoyl tropane	63.9 61.6	47.3 29.6	18.6	25.9 22.7	40.0	Thesis

The small difference observed between tropine (tropan-3α-ol) and pseudotropine (tropan-3β-ol) was interpreted by Wenkert as being in accordance with a flattening of the piperidine ring. The flattening results in a lessening of the 1,3 diaxial interaction in tropine which is normally responsible for the γ shielding effect of axial substituents in cyclohexanes or piperidines. (See footnote preceding page).

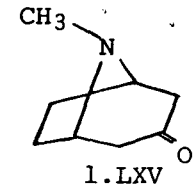
The chemical shift of the carbonyl carbon in tropan-3-one (1.LXV) is δ^{TMS} 207.8. This may be compared with that obtained for carbonyls in a number of heterocyclanones[77]. The very slight shift for tropan-3-one

(1.LXV) vs Bicyclo[3,2,1]octan-2-one, $\Delta\delta^{TMS}_{C=O}$ = 3.6 p.p.m., as compared with 1.LXIV, $\Delta\delta^{TMS}_{C=O}$ = 81.6 p.p.m., indicates a negligible transannular interaction in the former case, which is in accordance with tropan-3-one being predominantly in the chair conformation.



1.LXIV

$\delta^{TMS}_{C=O}$ 129.8



1.LXV

$\delta^{TMS}_{C=O}$ 207.8

(iv) Shift Reagents

In most cases of configurational and conformational studies of tropane alkaloids or piperidines, the coupling constants have been determined by an analysis of the C-3 proton only. The P.M.R. spectra of tropine, pseudotropine and tropan-3-one have been examined under the influence of the paramagnetic shift-reagent, tris (dipivalomethanato)-europiumIII[78]. This reagent enables differentiation of the axial and equatorial proton signals associated with C-2, and C-4, permitting the ready evaluation of the appropriate coupling constants. It was shown that

(a) No conformational change was induced by the shift reagent, as indicated by failure of protons attached to sites close to the complex bonding sites to broaden on addition of the shift-reagent.

(b) Complexing in tertiary amine alcohols occurred via the alcohol whilst tertiary amine ketones complexed via the amine function.

The results of the experiment indicated that the shifts for pseudotropine occurred in the order $OH>>H_3>>H_{2ax}>H_{2eq}>>H_1>N\text{-}CH_3$. The coupling

constant, $J_{2eq,3}$ was found to be 6 Hz, which from the Karplus relationship gives a dihedral angle of $\tilde{\sim}$ 40°. This was shown[78] to be in accordance with the previously assigned dihedral angle for pseudotropine[49]. In the case of tropine, the shift order was found to be $H_3 > H_{2eq} > H_{6\alpha} >> H_{2ax} > H_1 > H_{6\beta} > N\text{-}CH_3$. The observed coupling constant $J_{2eq,3}$ indicated a dihedral angle of $\tilde{\sim}$ 80° from the Karplus equation which was consistent with a flattened chair conformation.

The evidence from tropan-3-one was not conclusive as both functional groups appeared to complex with the shift-reagent. However, the data available suggested dihedral angles for H_1/H_{2ax} of 40° and for H_1/H_{2eq} of 80° which further supported the semiplanar nature of the piperidone ring in tropan-3-one.

In addition to the lanthanide shift-reagents, use has been made of nickel and cobalt diacetylacetonates in obtaining qualitative information about conformation in tropane alkaloids[79]. The analysis was based on two assumptions. Firstly, that paramagnetic shifts for $Co(acac)_2$ complex systems are due, as for the lanthanide systems, to the sum of a constant contact shift and an anisotropic pseudocontact shift which is proportional to $(3Cos^2\theta-1)/r^3$, where r is the distance between the resonating proton and the Co atom, and θ is the angle between the r vector and the Co-N axis (Fig. 1-4). Secondly, it was assumed that complexing occurred on nitrogen only for the $Co(acac)_2$ complex.

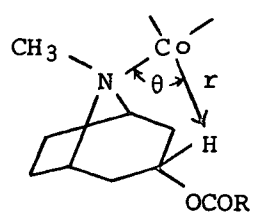


Fig. 1-4

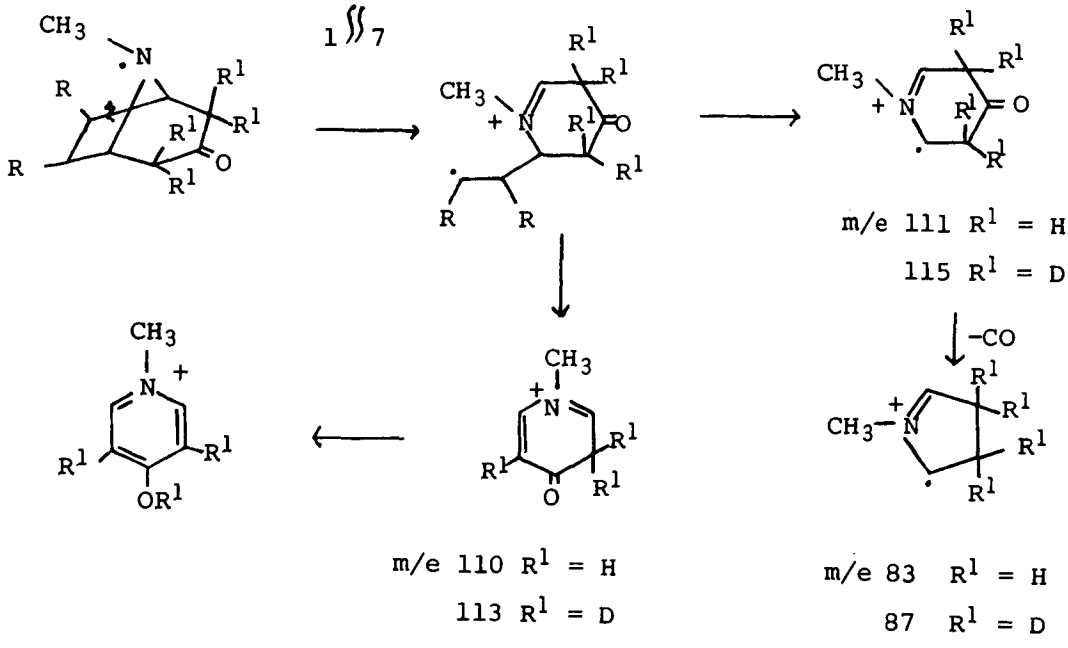
Shifts were calculated for θ and r values based on a nearly semiplanar form for tropine, and these were in close accord with the values observed.

1.4. Fragmentation of Tropane Alkaloids under Electron Impact

1.4.1. α-Cleavage Mechanism

Mass spectral fragmentation patterns for tropane alkaloids[40,69] were interpreted on the basis of the well-known cleavage which occurs α to tertiary nitrogens. The effect of such fragmentation is to stabilize the radical cation in the form of an immonium ion. (Scheme 1-4).

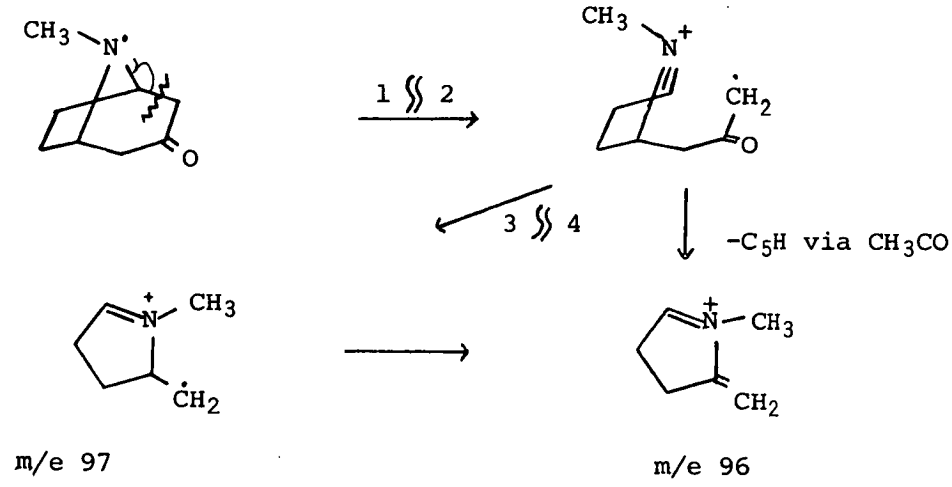
If such cleavage occurred via the 1-7 bonds, then fragments as shown in Scheme 1-4 are seen for tropan-3-one and its deuterated analogues.



Scheme 1-4

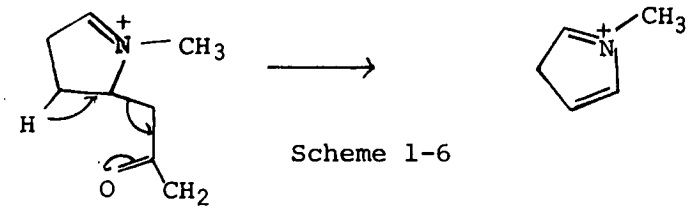
That the fragment m/e 83 resulted from the piperidone moiety and not the pyrrolidine ring was shown by deuterium labelling and the presence of the appropriate metastable peaks.

The α cleavage mechanism may also cause a 1-2 bond to break, in which case the fragmentation pattern is represented by Scheme 1-5.

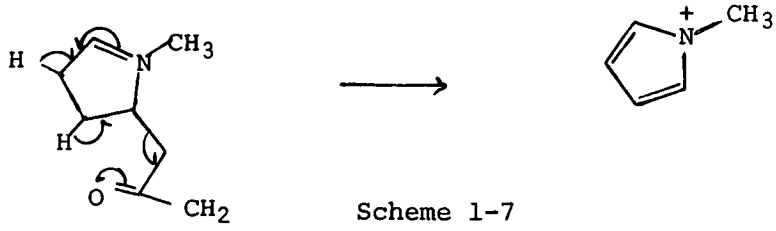


Scheme 1-5

The base peak in tropan-3-one, tropine, and pseudotropine occurs at m/e 82. Deuterium labelling supports the fragmentation pattern outlined in Scheme 1-6, in which m/e 82 is seen to result from a secondary fragmentation involving hydrogen transfer from C-6. The accompanying weak peak at m/e 81 was attributed to N-methylpyrrole, which was assumed to arise through a double hydrogen transfer. (Scheme 1-7).

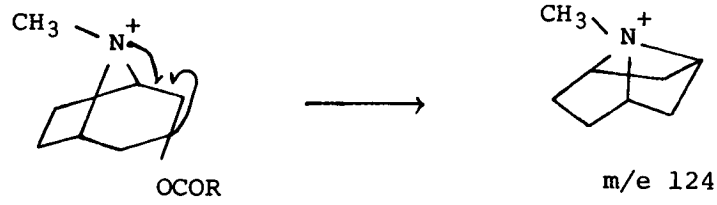


Scheme 1-6



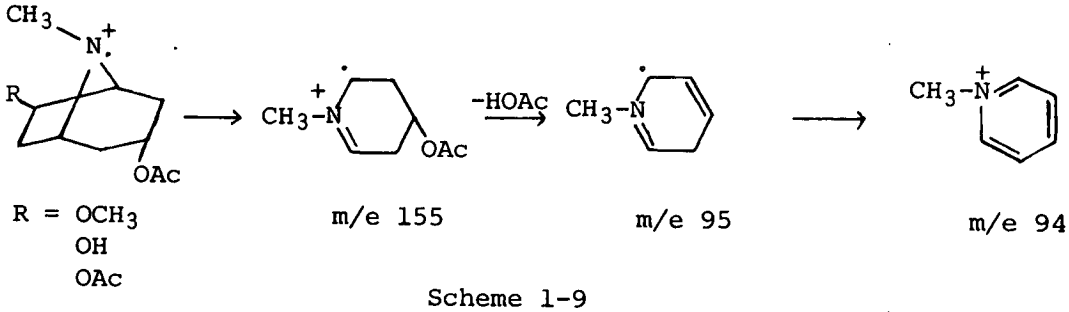
Scheme 1-7

Tropine esters, and to a lesser extent pseudotropine esters, show an important peak at m/e 124. This has been interpreted[69] as being due to the bridged species shown in Scheme 1-8. in which nitrogen behaves as a quaternary ammonium cation.



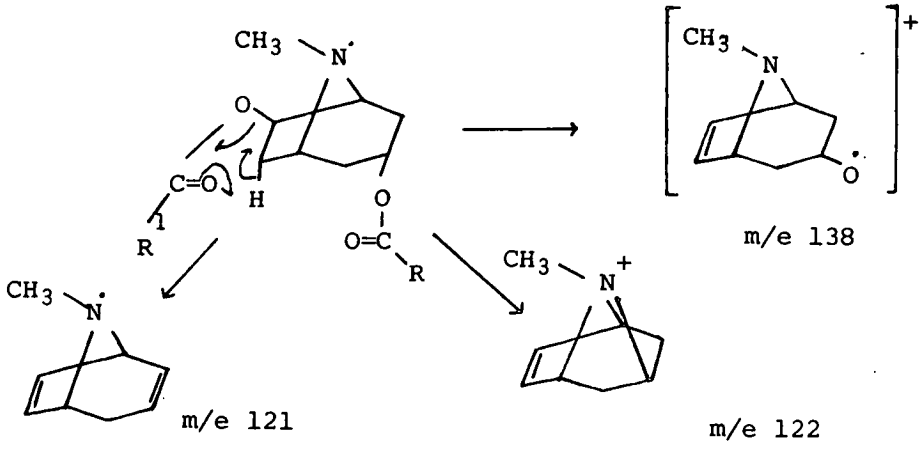
Scheme 1-8

Substitution or dehydrogenation of the C-6, C-7 ethano bridge in C-3 hydroxylated tropanes facilitates cleavage of the bridge through retrocyclization. The base peak in such cases is seen at m/e 94 (Scheme 1-9).



Scheme 1-9

Other important peaks observed in C-6 or C-7 substituted tropane alkaloids occur at m/e 138, m/e 122 and m/e 121. These result from a McLafferty rearrangement as shown in Scheme 1-10.



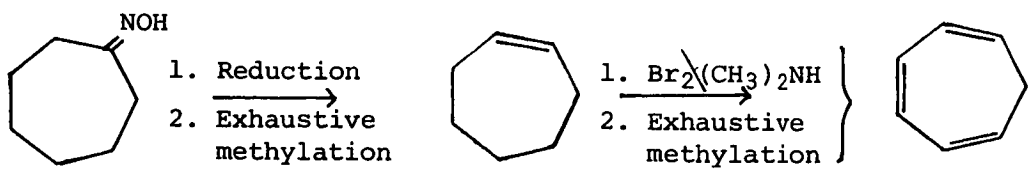
Scheme 1-10

1.5. Synthesis of Tropane Alkaloids

Synthetic routes to tropanes have involved, as the key step, the preparation of tropan-3-one. Reduction, either by chemical means or by catalytic hydrogenation has afforded entry to the pseudotropine or tropine series, while carboxylation of tropan-3-one has led to the ecgonines[34]. Techniques currently available enable the facile elaboration of this ring system to permit synthesis of all known types of tropane alkaloids[5,6].

1.5.1. Willstaetter's Tropan-3-one Synthesis

The initial synthesis of tropan-3-one was long and labourious, involving fifteen steps[34]. The synthesis can be considered in two phases. The first involved the preparation of cyclohepta-1,3,5-triene from cycloheptanone oxime (1.LXVI) (Scheme 1-11).

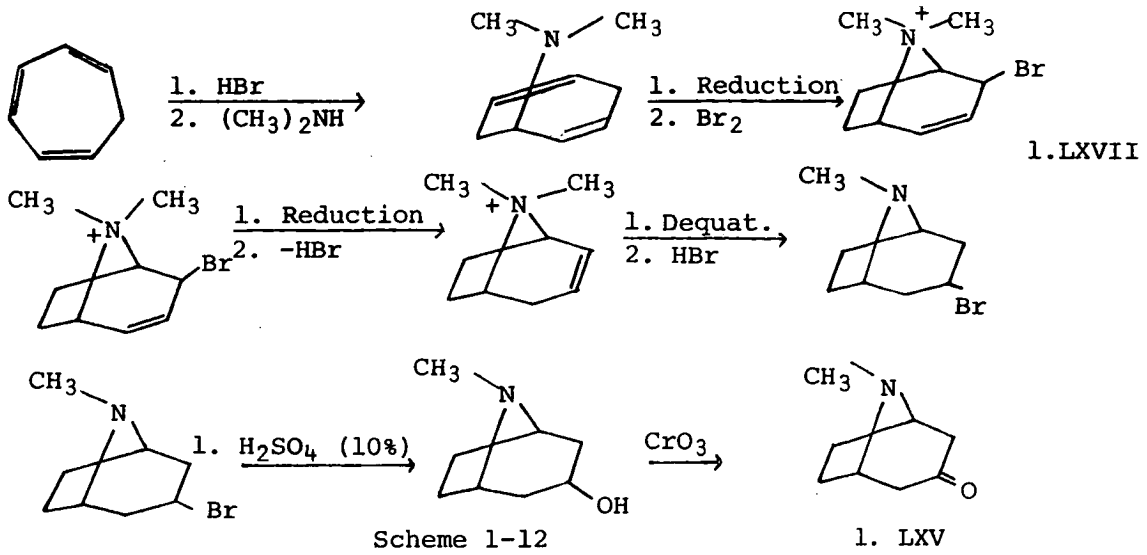


1.LXVI

Scheme 1-11

The second phase involved cyclization with an aza bridge, reduction, dehydrobromination to trop-2-ene methobromide, (1.LXVII), dequaternization, hydrobromination, hydroxy displacement to tropine,

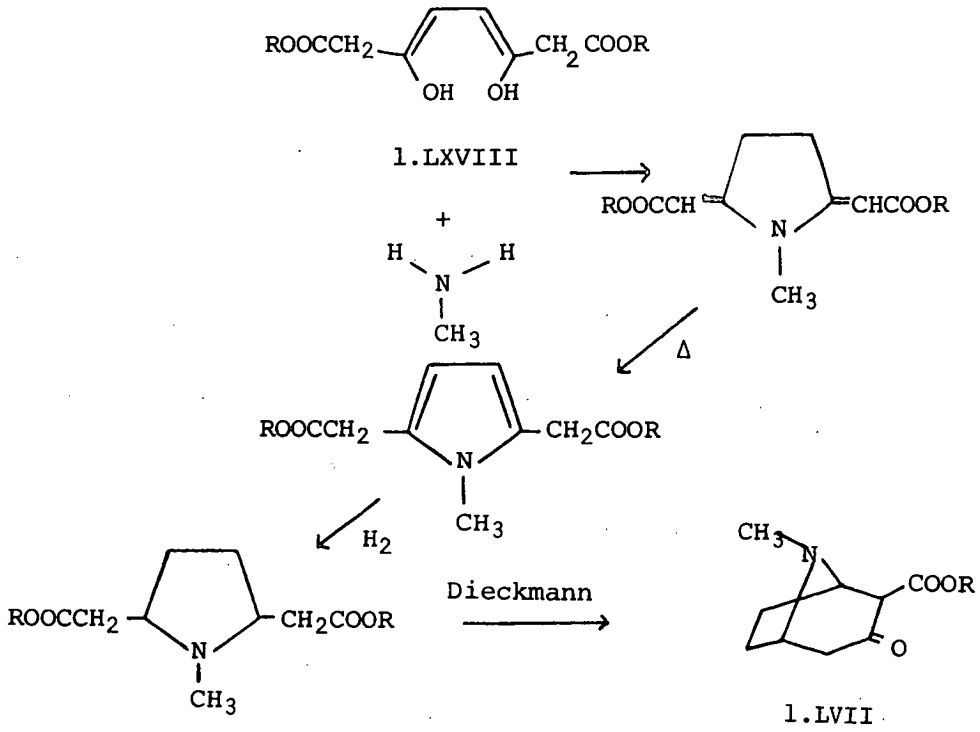
and finally oxidation with chromic acid to tropan-3-one. (Scheme 1-12).



Scheme 1-12

1. LXV

A recent reinvestigation[80] has been made of another of the Willstaetter tropan-3-one syntheses[81]. This method utilizes a reaction between hexa-1,3-diyne dicarboxylic ester with methylamine, in contrast to the 1,5-diyne employed by Parker et al[82]. The two methods are analogous, both presumably acting through the common dienediol (1.LXVIII) (Scheme 1-13).



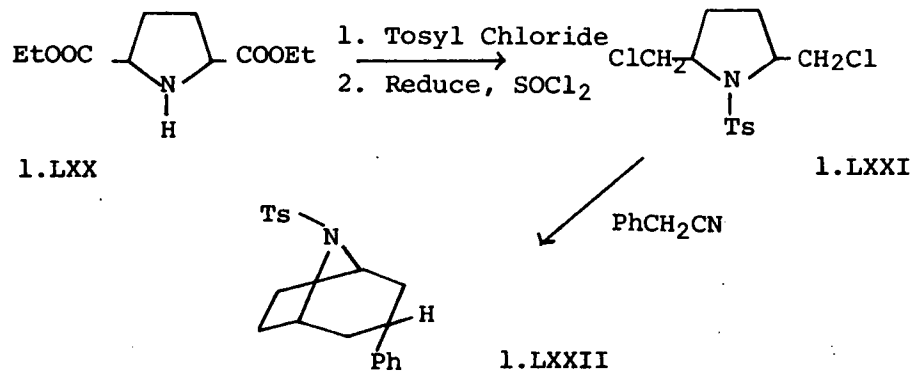
1. LXIX

1.LVII

Scheme 1-13

N-methylpyrrolidine-2,5-diacetic ester (1.LXIX), produced by hydrogenation of the corresponding pyrrole, undergoes the Dieckmann condensation to produce 2-carbomethoxy tropan-3-one (1.LVII).

In another recent approach[83], 2,5-dicarboethoxy pyrrolidine (1.LXX) was converted via its reduced product to a 2,5-dichloromethyl pyrrolidine (1.LXXI), which was condensed with benzyl cyanide to give N-tosyl 3α-phenyltropane (1.LXXII). (Scheme 1-14). (See also reference 76(a)).



1.LXX 1.LXXI 1.LXXII

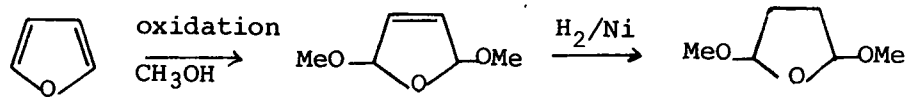
Scheme 1-14

1.5.2. Robinson-Schöpf Method

In contrast to the Willstaetter approach, Robinson's synthesis[84] of tropan-3-one is elegantly simple. It involves a double Mannich condensation of succindialdehyde, methylamine and acetone. The reaction occurs at room temperature, with moderate yields; when doubly activated acetone dicarboxylic acid is used, the yields are very high. (>85%)[85].

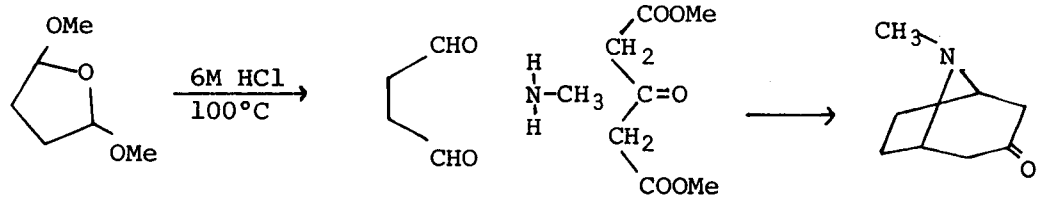
The reaction has been investigated in greater detail by Schöpf and Lehman[85], who found that a dilute buffered succindialdehyde, methylamine hydrochloride, and acetone dicarboxylic acid mixture gave tropan-3-one in 83% yield. Succindialdehyde is unstable and was prepared by the hydrolysis of pyrrole in hydroxylamine to form succindialdoxime which hydrolysed in situ to the reactive dialdehyde.

Clauson-Kaas[86(a)] has subsequently shown that 2,5-dimethoxy-2,5-dihydrofuran (1.LXXIII) formed by the anodic oxidation of furan in the presence of methanol and ammonium bromide, can be hydrogenated to the tetrahydro derivative, 1.LXXIV the cyclic methyl acetal of succindialdehyde.



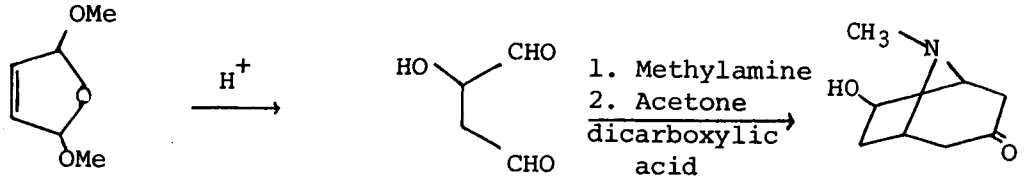
1.LXXIII 1.LXXIV

2,5-Dimethoxy tetrahydrofuran is readily hydrolysed in 6M HCl; the resultant dialdehyde remains relatively stable under acidic conditions, and after neutralization is used in the Robinson-Schöpf reaction to produce tropan-3-one in yields of 93%. (Scheme 1-15).



Scheme 1-15 1.LXV

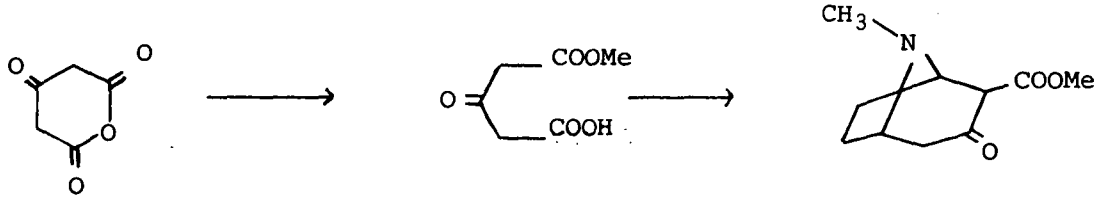
This route also provides access to the tropanes which are hydroxylated or esterified at positions C-6 or C-7. Under acidic hydrolysis conditions, the dihydrofuran derivative (1.LXXIII) is hydrated to yield 2-hydroxysuccindialdehyde which undergoes analogous condensation to 6β-hydroxy tropan-3-one (1.LXXV).



1.LXXIII 1.LXXV

This compound has been used as the starting point in synthesis of valeroidine[53], teloidinone[86], and through 6,7 dehydrotropan-3α-ol, to hyoscine, scopine, and scopolamine[87]. A discussion of the steric course of the reaction is given in Chapter 4.

Findlay[88] has shown that the cocaine intermediate, 2-carbomethoxy-tropan-3-one, may be conveniently synthesized through the use of monomethyl-β-ketoglutaric ester (1.LXXV) in the Robinson-Schöpf synthesis. The ester was available through the methanolysis of β-ketoglutaric anhydride (1.LXXVI).



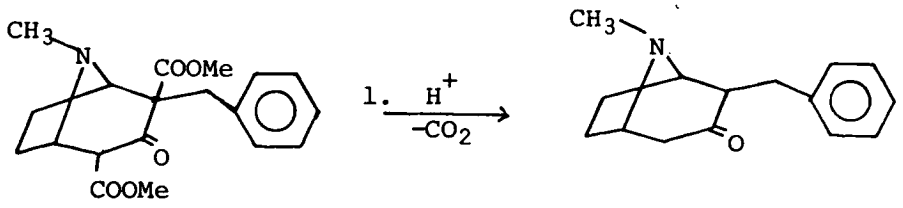
1.LXXVI 1.LXXV 1.LVIII

In addition to the synthesis of naturally occurring tropane alkaloids, the Robinson-Schöpf method has been used to prepare a large range of analogues used for pharmacological testing[89]. Through substituted dialkoxy tetrahydrofurans, substituents have been introduced to the tropan-3-one ring at positions 6 and/or 7[90].

Beyerman[91] has investigated the use of ammonia in place of methylamine in a thorough re-examination of the conditions of the Robinson-Schöpf reaction. The resultant nortropanes have been converted to a variety of tertiary and quaternary amines which have also been utilized in pharmacological testing. Use of amino-acids as amines has enabled the synthesis of a range of N-substituted tropanes[92].

It has also been shown (Chapter 3) that the Robinson-Schöpf reaction occurs in high yields when the acetone dicarboxylic acid moiety is substituted with a benzylic group. Hydrolysis and

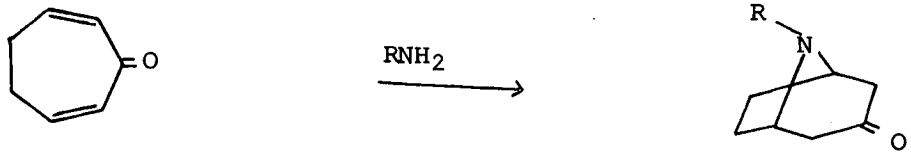
decarboxylation of the condensation product (1.XLI) leads to 2-benzyl tropan-3-one (1.XLII).



1.XLI 1.XLII

1.5.3. Michael Addition Method

Kashman and Cherkez (b)[93] have extended the early work of Bottini and Gál (a)[94] by synthesizing a wide range of nortropan-3-one derivatives (1.LXXXIX) from cycloheptadienone (1.LXXVII). This Michael addition method was shown by the original workers to effect the synthesis of N-substituted nortropanes in high yield.



1.LXXVIII 1.LXIX

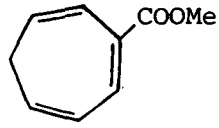
(a) R = CH_3 -
C_2H_5 -
$PhCH_2$ -

(b) R = $R^1_1 \diagup N-$, $R^1CONHNH-$,
$R_1 \diagdown$
R^1-O-NH-, HO-NH-

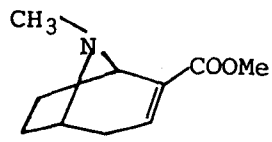
Kashman and Cherkez have shown that semicarbazones, oximes, and hydrazines are each capable of undergoing the addition reaction.

Anhydroecgonine methiodide undergoes the Hofmann degradation with great ease, generating in one step, cycloheptatriene carboxylic acid (1.LXXX). Grundmann and Ottmann[95] have synthesized 1.LXXXI by the

retro-Hofmann addition of methylamine to cycloheptatriene carboxylic acid.



NH_2Me →

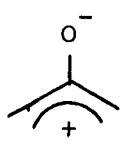


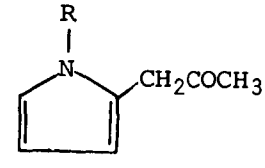
1.LXXX 1.LXXXI

1.5.4. $4_\pi + 2_\pi$ Cyclo-addition Reactions

(i) 1,3-dienes and oxyallyl zwitterions

Pyrrole and its N-alkylated derivatives undergo Diels-Alder type reactions only with difficulty. However, the oxyallyl species (1.LXXXII) is a strong dienophile and capable of undergoing cyclo-addition reactions with N-carbomethoxy pyrrole. This reaction gives tropan-3-one derivatives, although a strongly competing reaction is the electrophilic substitution reaction to produce 1.LXXXIII.

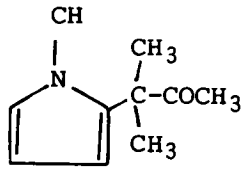




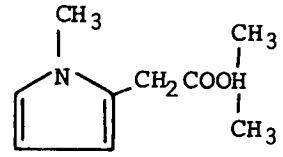
1.LXXXII 1.LXXXIII

Turro[96] found that the reaction of 2,2-dimethyl cyclopropanone with N-methyl pyrrole produced 1.LXXXIV and 1.LXXXV on vapour phase chromatography of the products. Two mechanisms were proposed for the production of the electrophilic substitution products. The first involved a cyclopropane and pyrrole cyclo-addition to produce 2,2-dimethyl trop-6-en-3-one (1.LXXXVI); subsequent V.P.C. caused decomposition to the observed products. The second mechanism involved the oxyallyl species acting as an electrophile directly on the pyrrole.

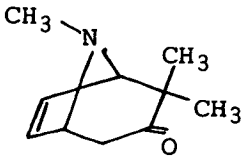
The second mechanism was discarded when 1.LXXXVI was isolated from the reaction mixture and found to decompose under the chromatography conditions to the observed products.



1.LXXXIV

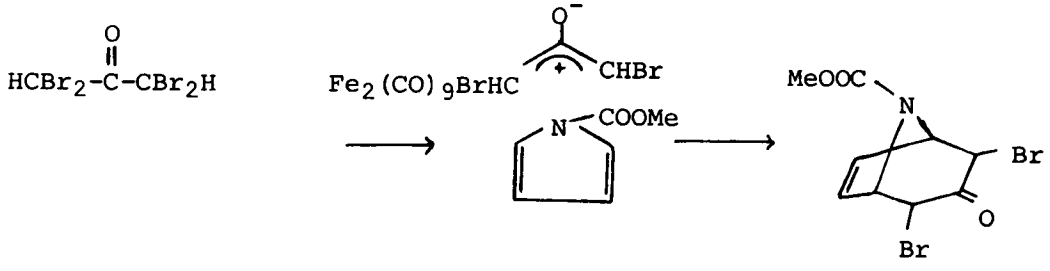


1.LXXXV



1.LXXXVI

However, it has been found subsequently that the oxyallyl cation generated from tetrabromoacetone (1.LXXXVII) and $Fe_2(CO)_9$ reacts with N-carbomethoxy pyrrole in high yields to produce (cis and trans) carbomethoxy 2,4-dibromo trop-6-en-3-one[106] (1.LXXXVIII) Electrophilic substitution was reduced by involving the pyrrole-nitrogen lone-pair electrons in tautomeric bonding to the carbomethoxy group.

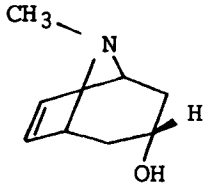


1.LXXXVII

1.LXXXVIII

Debromination was effected quantitatively by a zinc/copper couple in methanol. Reduction of the resultant N-carbomethoxy trop-6-en-3-one with diisobutylaluminium hydride gave the key intermediate in tropane alkaloid synthesis, trop-6-en-3α-ol (1.LXXXIX), in high yield (92%).

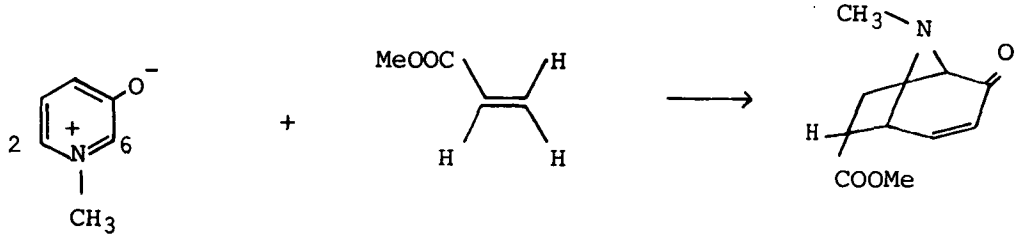
The availability of methods for converting 1.LXXXIX to the tropanes scopolamine, teloidine, and hyoscine, means that this approach is the most valuable alternative currently available to the Robinson-Schöpf method.



1.LXXXIX

(ii) 1,3 Dipoles and Dipolarophiles

The betaine, 1-methyl-3-oxidopyridinium, (1.XC) reacts as a 1,3 dipole across the 2 and 6 positions with a number of dipolarophiles[97] such as methyl methacrylate, methyl acrylate or acrylonitrile.

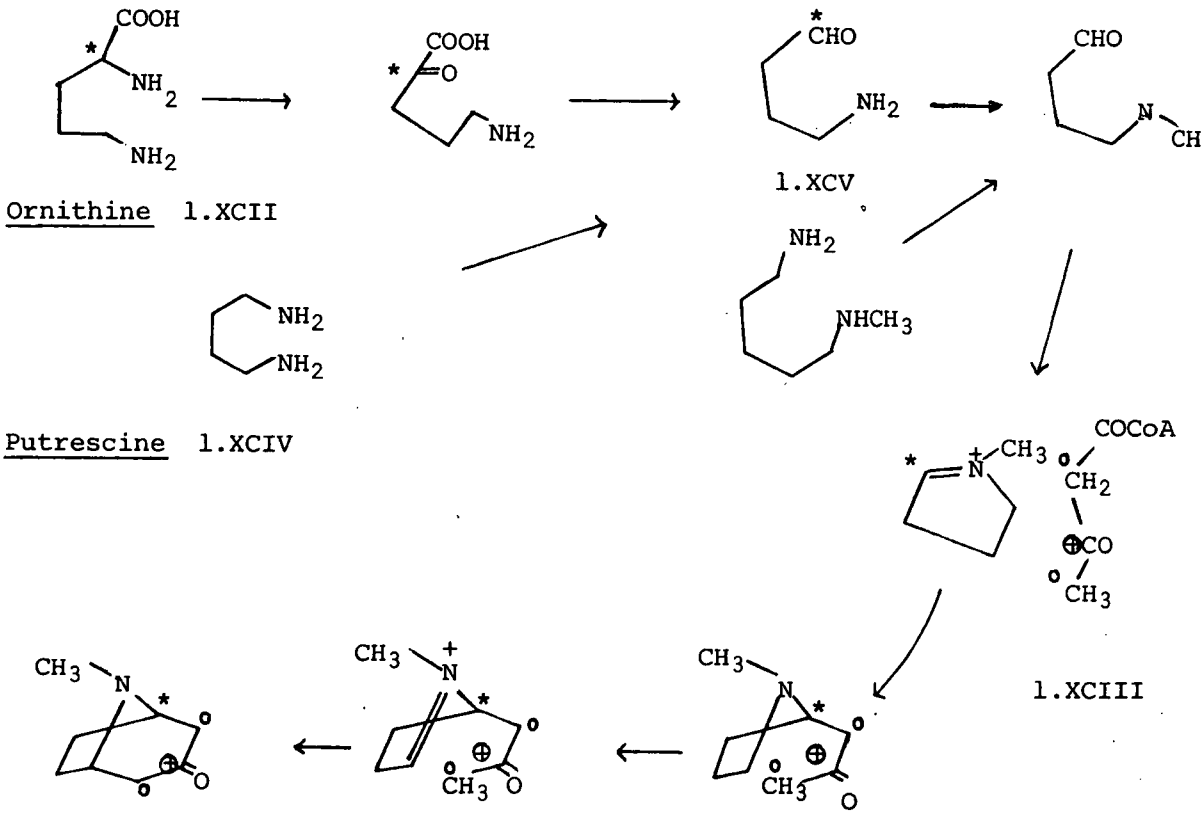


1.XC 1.XCI

The resultant compound from 1.XC and methyl acrylate was a 6-substituted trop-3-en-2-one (1.XCI), whose structure was deduced by P.M.R. spectroscopy. It was concluded that the C-1 proton peak appeared as a doublet because of coupling with the C-7β proton whereas the C-5 proton peak was a doublet of quartets due to coupling with the C-4 proton and the C-6β proton.

1.6. Biosynthesis of Tropane Alkaloids

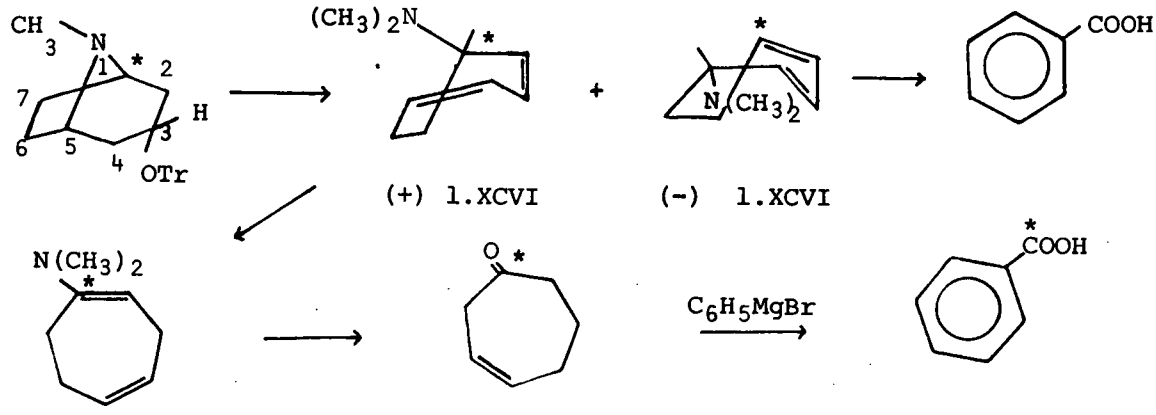
The biosynthesis of the tropane ring system has received considerable attention in recent years. The extensive work of Leete[98,99,100] and others[101] has shown that the carbons 1,5,6,7 of the tropine moiety of hyoscyamine are derived from ornithine (1.XCII). The administration of ornithine 2- ^{14}C to Datura stramonium plants afforded tropine which was labelled exclusively and stereospecifically at C-1 or C-5. Liebisch[102] has shown that only the δ-amino nitrogen of ornithine is incorporated into tropine. Carbons 2,3 and 4 of tropine are derived from acetone, or its biosynthetic equivalent acetoacetyl co-enzyme A[101,103] (1.XCII). A generalized scheme for the in vivo synthesis of tropan-3-one is outlined in Scheme 1-16 (after Leete[99]).



Scheme 1-16

Putrescine 2- ^{14}C (1.XCIV) and N-methyl putrescine have also been incorporated into tropine[100]. It was claimed that the incorporation resulted from oxidation of putrescine to 4-amino butanal (1.XCV), the common precursor in both routes prior to cyclization. Furthermore, ornithine is not decarboxylated to putrescine prior to cyclization, as the symmetry of this compound would have caused the ^{14}C label to be evenly distributed between C-1 and C-5.

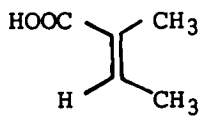
The determination of the stereospecificity of incorporation of labelled ornithine at C-1 or C-5 was based on Willstaetter's degradation[4] (Manske, Vol. 1, p. 278). Scheme 1-17.



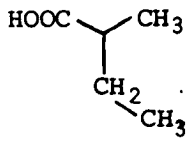
Racemic 5-(dimethylamino)-cyclohepta-1,3-diene (1.XCVI) was resolved and each of the enantiomers subjected to the degradation shown above, leading to benzoic acid. Only the dextrorotatory isomer ((+) 1.XCVI) gave a radioactive product, proving stereoselective incorporation of ornithine to hyoscyamine.

6,7-Dihydroxy tropine (teloidine), and thus meteloidine (1.XXI), was claimed to arise in the plant from erythrose[104]; it has been shown subsequently that in common with hyoscyamine, hyoscine, and 6,7 dihydroxy hyoscyamine, teloidine is biosynthesized from ornithine[105].

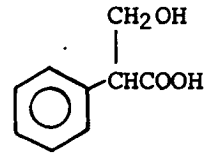
The origin of the esterifying acids in the tropane alkaloids, tiglic acid (1.XCVII), 2-methylbutanoic acid (1.XCVIII), and tropic acid (1.XCIX) has been studied. Tiglic acid was shown to be derived from L-isoleucine (1.C) via 2-methylbutanoic acid[106] (1.XCVII).



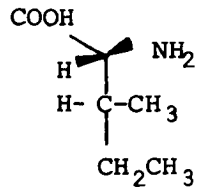
1.XCVII



1.XCVIII

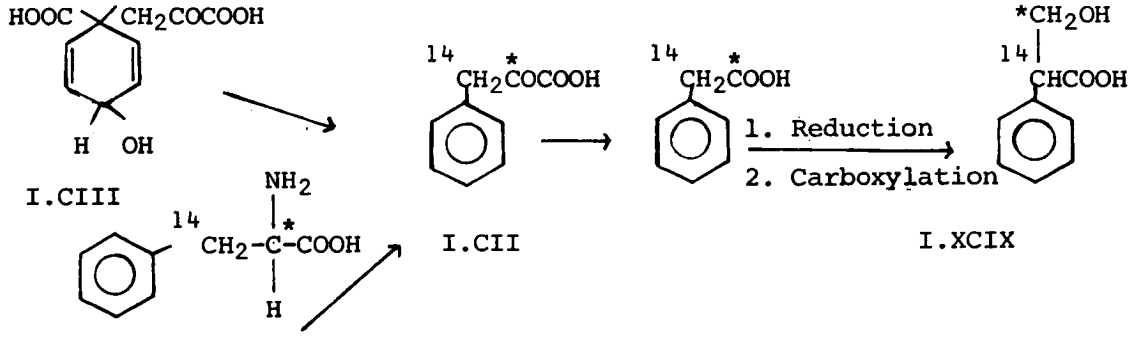


1.XCIX



I.C

Tropic acid has been shown through labelling experiments to arise from phenylalanine[107] (1.CI). This may not represent the actual course of biosynthesis however, as phenyl pyruvic acid (1.CII) is an intermediate, which may originate also from prephenic acid (1.CIII) and this may be the original source of the tropic acid moiety. (Scheme 1-18).



I.CIII

I.CI

I.CII

I.XCIX

Scheme 1-18

It was shown[108] (Scheme 1-18) that 3- ^{14}C phenylalanine (1.CI) led to 2- ^{14}C tropic acid, also that a 2- ^{14}C phenylalanine precursor, when fed to Datura stramonium[109] produced 3- ^{14}C tropic acid[109]. These data indicate that carboxylation takes place in addition to terminal reduction of one carbonyl group to a hydroxy methyl substituent (Scheme 1-18).

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CHAPTER 2

Isolation and Structural Elucidation of Alkaloids from the Proteaceous Species Bellendena montana R.BR. and Agastachys odorata R.BR.

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2.1. Introduction

The Proteaceae comprise some 1,400 species which are distributed widely in the Southern Hemisphere[1]. A schematic representation of the phylogeny as described by Johnson and Briggs[2] is shown in Fig. 2-1. An investigation of the alkaloidal constituents of certain species of the Proteaceae arose from the discovery[3] in 1969 that two monotypic species endemic in Tasmania, Bellendena montana R.BR. and Agastachys odorata R.BR., gave positive Mayers tests for alkaloids. At that time there were no reports of alkaloids having been isolated from the Proteaceae, although Webb[4] had obtained positive tests from two Queensland species. An alkaloid testing survey to supplement that of Webb was undertaken to determine whether other species of this family contained alkaloids. The results of this survey are shown in Table 2-1.

The two Queensland species of the Darlingia genus gave positive tests and an Argentinian plant, Embothrium coccineum, was also positive. Concurrently, a private communication[5] indicated that the New Caledonian species Knightia deplanchei VIELL. ex BROGN. et GRIS, had shown the presence of alkaloids.

A preliminary investigation[6] of the Bellendena species indicated the presence of at least three bases, and the principal base, bellendine, was characterized spectroscopically. A detailed structural examination of the Bellendena bases is described in Section 2.2 of this chapter. Agastachys odorata has been investigated and the structural data relating

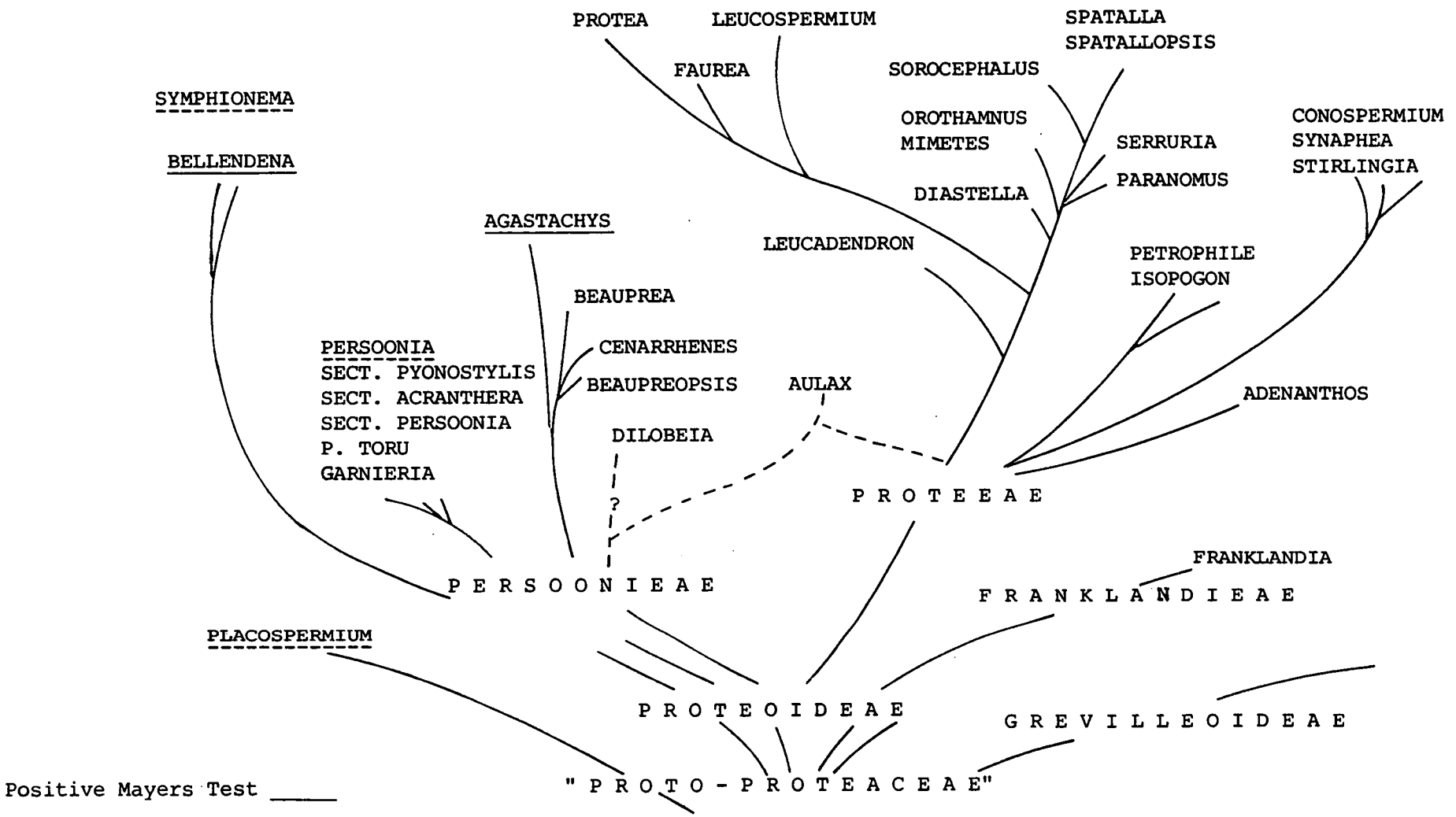

PROTEA
LEUCOSPERMIUM
SPATALLA
SPATALLOPSIS
FAUREA
SOROCEPHALUS
SYMPHIONEMA
OROTHAMNUS
MIMETES
SERRURIA
CONOSPERMIUM
SYNAPHEA
STIRLINGIA
BELLENDENA
PARANOMUS
DIASTELLA
AGASTACHYS
LEUCADENDRON
PETROPHILE
ISOPOGON
BEAUPREA
PERSOONIA
SECT. PYONOSTYLIS
SECT. ACRANTHERA
SECT. PERSOONIA
P. TORU
GARNIERIA
CENARRHENES
BEAUPREOPSIS
AULAX
ADENANTHOS
DILOBEIA
?
P R O T E E A E
FRANKLANDIA
P E R S O O N I E A E
F R A N K L A N D I E A E
PLACOSPERMIUM
P R O T E O I D E A E
G R E V I L L E O I D E A E
" P R O T O - P R O T E A C E A E"
Positive Mayers Test _____
Negative Mayers Test -----

Fig. 2-1

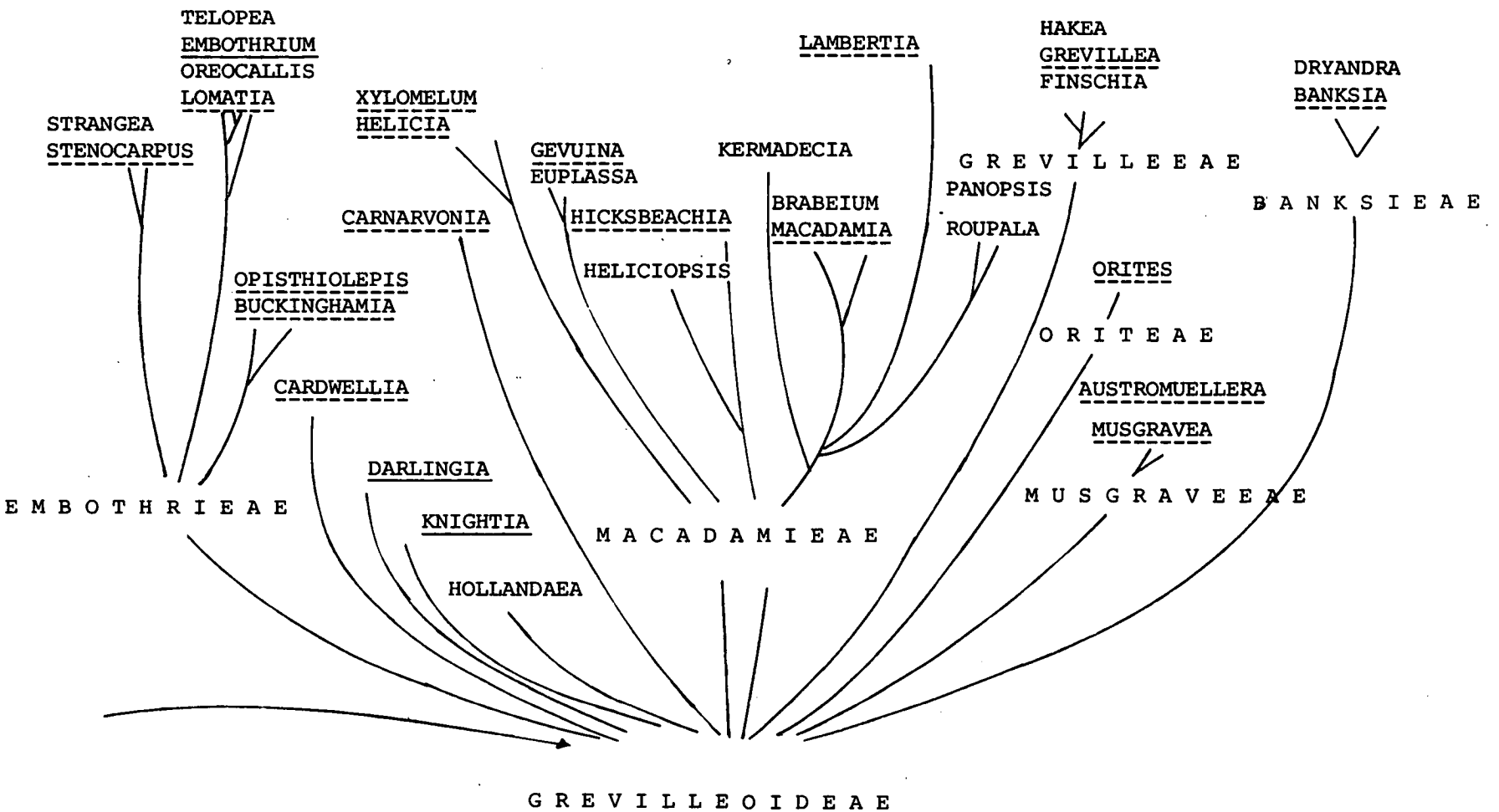


Fig. 2-1 (continued)

TABLE 2-1

Alkaloid Testing of the Proteaceae

Species	Location	Test (Mayers) [a]	Ref.
Bellendena montana	Tasmania	+++	(b)
Agastachys odorata	Tasmania	+	(b)
Darlingia darlingiana	Atherton Q'ld.	++++	(b)
Darlingia ferruginea	Davies Creek Q'ld.	++++	(b)
Knightia deplanchei	New Caledonia	+ve	5
Embothrium coccineum	Argentina	+	(b)
Embothrium wickhamii	N.S.W.	-ve	(b)
Persoonia tenuifolia	Q'ld.	+ve -ve	4 (b)
Symphionema montanum	N.S.W.	-ve	(b)
Symphionema paludosum	N.S.W.	-ve	(b)
Musgravea stenostachya	N.S.W.	-ve	(b)
Opisthiolepsis heterophylla	Q'ld.	-ve	(b)
Placospermum coriaceum	Q'ld.	-ve	(b)
Gevuinea bleadsdalii	Q'ld.	-ve	(b)
Austromuelleria trinervia	Q'ld.	-ve	(b)
Persoonia falcata	Q'ld.	-ve	4
Persoonia cornifolia	Q'ld.	-ve	4
Persoonia mitchellii	Q'ld.	-ve	4
Buckinghamia celsissima	Q'ld.	-ve	4
Carnarvonia aralifolia	Q'ld.	-ve	4
Banksia integrifolia	N.S.W.	-ve	4
Hicksbeachia pinnatifolia	N.S.W.	-ve	4
Grevillea floribunda	Q'ld.	-ve	4

TABLE 2-1 (continued)

Orites excelsa	Q'ld.	-ve	4
Hakea microcarpa	N.S.W.	-ve	4
Lomatia longifolia	N.S.W.	-ve	4
Lambertia formosa	N.S.W.	-ve	4
Stenocarpus sinuatus	Q'ld.	-ve	4

(a) According to the method of Fitzgerald and Culvenor (Section 2.11.1.).

(b) Current work.

to the alkaloids of this species are reported in Section 2.9. The Darlingia bases are considered in Chapter 3.

2.2. Isolation of the Bellendena bases

Bellendena montana is prevalent in two locations in Tasmania. The species appears only on mountain plateaux above 1000m in southern Tasmania, where it grows to a height of ca. 0.5m; whereas in a lower region, near Guildford, western Tasmania, the species grows to a height of ca. 1m. In those plants collected from the Hartz Mountains, the alkaloids were found only in the flowering heads, while plants collected from the Guildford area had a more even distribution, the greatest concentration appearing in the flowers and root-bark. The preliminary investigation was carried out only on flowering heads of plants from the Hartz Mountains. Plants from the Guildford area were collected for the present study because of their higher alkaloid content; however, in view of the observed differences in distribution, the flowering heads were examined separately from the roots and stems.

2.2.1. Alkaloids of the Flowering Heads

The extraction procedure involved cold percolation of the flowers with Prollius solution[7] until the extract no longer gave a positive Mayers test. The extract was concentrated in vacuo and dissolved in a minimum quantity of glacial acetic acid. Precipitation of non-alkaloidal material was achieved by pouring the acetic acid solution in a thin stream into rapidly stirred water. Basification of the aqueous solution with ammonia released the free bases, which were extracted into chloroform.

The extract was examined by two methods. Analytical thin-layer chromatography (T.L.C.) revealed seven alkaloids. Preparative thin-layer chromatography (P.T.L.C.) was carried out on a third of the extract. It was possible to cut five bands from P.T.L.C.; however, in view of the similar R_f values for the constituents of these bands in a number of solvent systems, none was sufficiently pure to enable characterization. Repeated P.T.L.C. separated small quantities of material sufficiently pure to crystallize in two cases. Use of seed crystals from these separations enabled the fractional crystallization of two bases, B2, and bellendine, from the original P.T.L.C. extracts.

TABLE 2-2

Summary of P.T.L.C. Bands isolated from the Flowering Heads

(Silica gel 12% MeOH, $CHCl_3$, NH_4OH 2 drops)

Code	R_f	Constituents	Comments
B1	1.0	12 mg Impure	Not further examined
B2	0.89	127 mg 2 components	Crystallized B2, P.T.L.C. mother liquod B2'
B3	0.78	17 mg 1 component	Apparently pure
B4	0.66	400 mg 2 components	Bellendine and 1 other
B5, B6	0.43-0.30	200 mg 2 components	Impure, poorly resolved in all solvent systems

In view of the unsatisfactory separation achieved by thin-layer chromatography and the attendant loss of material on using repeated chromatography, the balance of the flower extract was subjected to Craig distribution between chloroform as the stationary phase and 0.5×10^{-3}M H_2SO_4 as the mobile phase. The fractions cut from this distribution were complementary to those separated by P.T.L.C. In particular, it was possible to discriminate between fractions containing bellendine and those containing its isomer, isobellendine, due to the very slight difference in the colour reaction of the pure compound with respect to potassium iodoplatinate spray reagent (Schlittlers Reagent)[8] on T.L.C.

TABLE 2-3

Summary of Craig Distribution Fractions

($CHCl_3$ stationary phase, 0.5×10^{-3}M H_2SO_4 mobile phase)

Craig No.	P.T.L.C. Code	Comments and Identification
1-8	B5,6	Impure, poorly resolved low R_f
9-16	B5	Pure by T.L.C. B5
17-24	B4	Bellendine
25-28	B4	Isobellendine
29-35	-	New base, bright blue Schlittler test
36-40	B2'	Small quantity B2'
41-60	-	Impure, poorly resolved high R_f

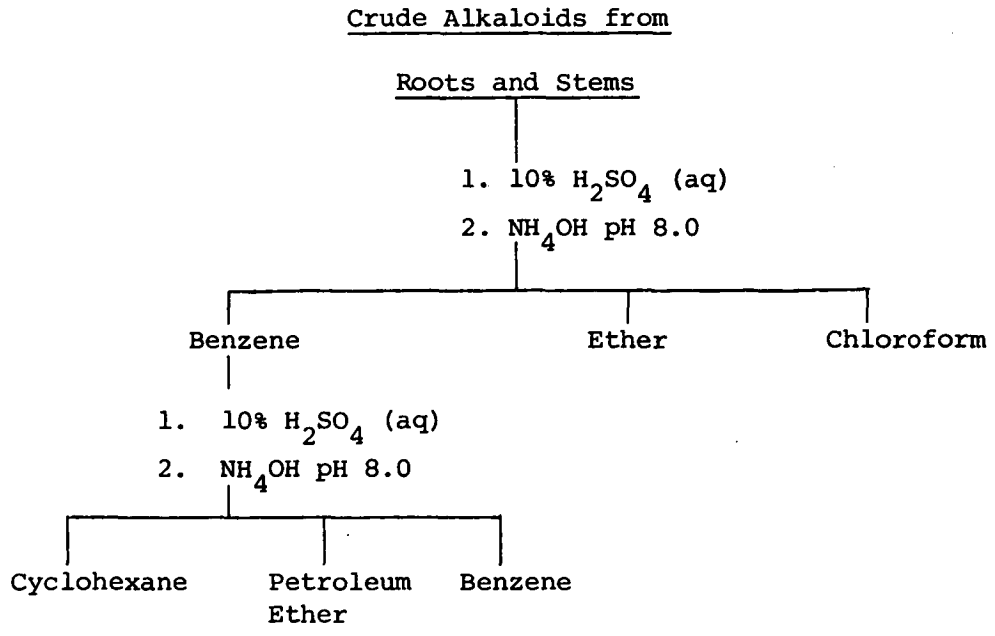
Craig fractions identical with bands isolated from P.T.L.C. were combined with those bands. The impurity in P.T.L.C. band B4 was recognized as isobellendine; bellendine, the major component, was fractionally crystallized from this and the mother liquor was combined

with the appropriate Craig fraction. The mother liquors from the crystallization of alkaloid B2 from band B2 were likewise combined with fractions 36-40, which contained principally B2'.

2.2.2. Alkaloids of the Roots and Stems

A similar extraction procedure to that used for the flowers was utilized for the ground and dried root and stem material. The extract, examined by T.L.C., revealed qualitative similarities to the flower extract. However, B2' was absent from the B2 band, B5 was present in significantly greater quantities, and it was subsequently found that the major band, B4, consisted of isobellendine instead of bellendine.

In order to achieve a further fractionation or selective concentration, a solvent extraction separation technique was utilized for the crude extracts as outlined in Scheme 2-1.



Scheme 2-1

By this technique it was possible to separate and concentrate certain fractions, which after further purification by P.T.L.C. could be bulked or combined with the appropriate fractions isolated from the flowers by the methods described in 2.2.1. and 2.2.2.

TABLE 2-4

Summary of Alkaloids isolated from

Bellendena montana

(8 kg wet material)

Code	Name	Quantity
B1	B1	10 mg
B2	B2	18 mg
B2'	2-acyl-trop-2-ene	30 mg
B3	6β-acetoxy-3α-isobutoxy tropane	7 mg
B3'	6β-isobutoxy-3α-acetoxy tropane	8 mg
B4	Bellendine	400 mg
B4'	Isobellendine	400 mg
B5	Dihydroisobellendine	120 mg
B6	6β-OH-3α-acetoxy tropane	40 mg

2.3. Structural Elucidation of Bellendine

Bellendine; $C_{12}H_{15}NO_2$, m.p. 162° (sublimation), $[\alpha]_D^{19}$ + 168°, ($CHCl_3$), has been analysed by P.M.R., C.M.R., I.R., U.V., and mass spectroscopy (M.S.).

2.3.1. P.M.R. Spectroscopy

Three diagnostic features of the P.M.R. spectrum of bellendine in $CDCl_3$ (Fig. 2-1), are the signals at 7.68τ (3H, singlet), 8.11τ

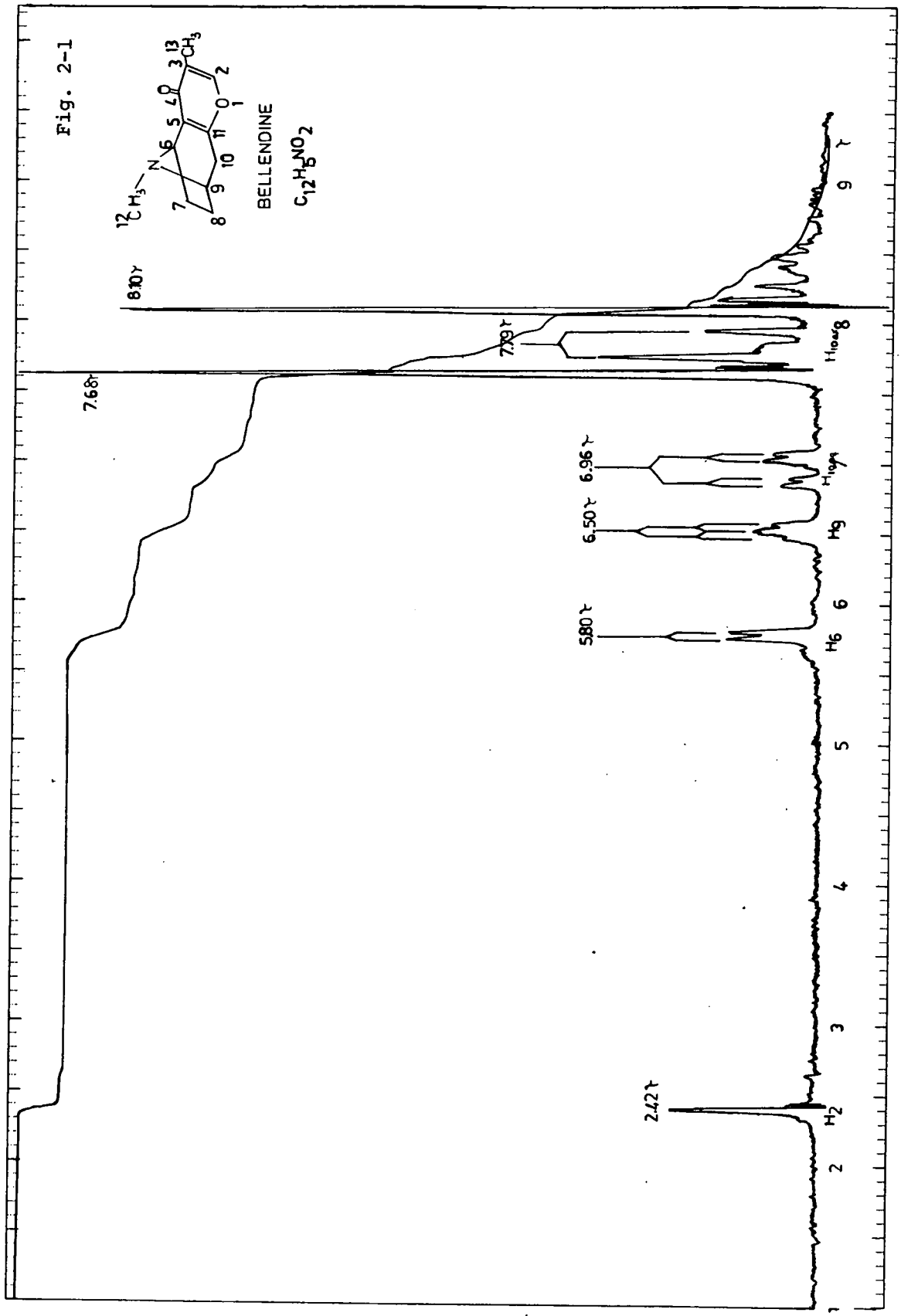


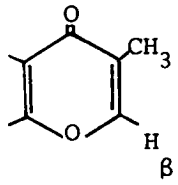
Fig. 2-1
BELLENDINE
$C_{12}H_{15}NO_2$
8.10τ
7.68τ
7.79τ
6.96τ
6.50τ
5.80τ
2.42τ
H2
H6
H9
H10a9
H10a8
9τ
1
2
3
4
5
6

(3H, doublet, J = 1.2 Hz) and 2.42τ (1H, quartet, J = 1.2 Hz). The signal at 7.68τ falls in the range for N-methyl groups[9,10] (7.5-8.0τ). Use of trifluoroacetic acid (T.F.A.) as solvent protonates nitrogen, which results in the N-methyl proton signal being split to a doublet, J = 7.8 Hz. At the same time the N-methyl signal shifts downfield by 0.92τ. Double irradiation indicated that the splitting resulted from coupling and was not due to an equal distribution of species[9] with equatorially or axially protonated nitrogen. The signal at 8.11τ corresponds to a C-methyl which is significantly deshielded partly due to the anisotropy of an adjacent carbon-carbon double bond. Values observed[11] for methyl protons in a vinyl system are in the range 8.4τ-8.6τ.

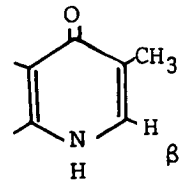
The allylic coupling, J = 1.2 Hz, observed in the methyl signal at 8.11τ further confirmed the existence of an adjacent olefinic system. A combination of carbonyl deshielding and olefinic anisotropy results in a lowering of the methyl group resonance to the value observed, 8.11τ. Williams[10] compilation, listing the C-methyl resonances of α,β unsaturated carbonyls correlates the 8.11τ resonance with an α methyl group. On scale expansion, the coupling of 1.2 Hz which splits this C-methyl signal into a doublet, was observed in a one proton quartet centred at 2.42τ, indicative of an allylic system with 1,3 coupling. A spin decoupling experiment in which the signal at 2.42τ was irradiated, caused the collapse of the signal at 8.11τ to a three proton singlet. Irradiation at 8.11τ caused the quartet centred at 2.42τ to collapse to a singlet, providing proof of the allylic system.

The quartet at 2.42τ is at extremely low field for an olefinic proton, and furthermore is coupled only through the allylic system to the methyl group, which suggests that the β position of the α,β unsaturated carbonyl chromophore bears an electronegative substituent

with no protons capable of coupling with the olefinic proton. From the molecular analysis, $C_{12}H_{15}NO_2$, the only two systems compatible with these data are 2.I or 2.II.



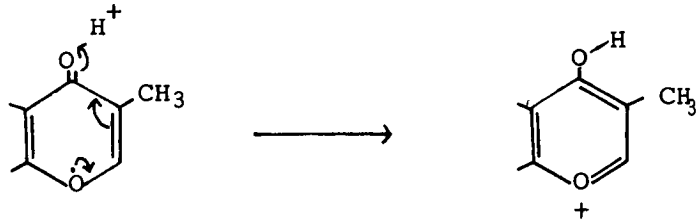
2.I



2.II

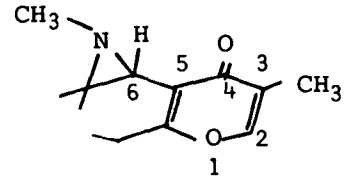
Chemical shift values for H_β are reported[12] for systems such as 2.I to lie between 1.9 and 4.0τ, and for 2.II, between 2.0 and 4.3τ. The basicity of the compound, pK_b = 6.7, and the position of the N-methyl resonance suggest that the compound is not a vinylogous amide (2.II): pK_b values for vinylogous amides are in the range 13.0-12.0. This suggestion is further supported by the absence of a significant hypsochromic shift[13] in the ultraviolet absorption spectrum on addition of acid.

The pyrone residue (2.I) was tentatively assigned as the structure responsible for the resonances centred at 8.11τ and 2.42τ. This assignment is supported by correlations in the infrared and ultraviolet spectra, discussed in a later section. Further evidence for this structure was given by the P.M.R. spectrum of the alkaloid in T.F.A., in which the allylic coupling was lost. This may arise by protonation and electron delocalization, resulting in an aromatic system in which no coupling is observed between the allylic proton and methyl group (2.III).



2.III

The doublet at 5.80τ (J = 6 Hz) integrates for one proton and is coupled to a multiplet at 7.70τ. The position of this resonance corresponds to a proton in a deshielded environment arising from an adjacent electronegative substituent. The presence of oxygen in an ether linkage would result in deshielding of this order; however, this is not possible if both oxygens are present in a γ-pyrone ring. An adjacent nitrogen would result in a shift of smaller magnitude unless factors such as carbonyl anisotropy resulted in further deshielding. If the γ-pyrone moiety is present in the molecule, then the proton responsible for the 5.80τ resonance is likely to be H_6 in 2.IV.



2.IV

An unresolved resonance occurs at 6.50τ (1H) in $CDCl_3$; this peak is resolved in benzene solution and appears as a doublet of doublets, partially superimposed. One of the doublets is due to coupling with the multiplet at 7.70τ. The second coupling is to one proton of a geminal quartet centred at 6.96τ. A broadened, downfield resonance of this type suggests that the signal at 6.50τ results from a proton adjacent to nitrogen.

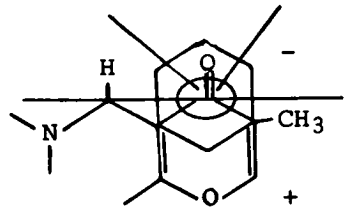
The signal at 6.96τ (1H, doublet of doublets, J = 17 Hz, 6Hz) arises from part of a geminal methylene group; decoupling of this signal results in the appearance of a singlet at 7.79τ from the other proton of the geminal pair. The difference in chemical shifts of the individual protons in the geminal pair (τ = 6.96, τ = 7.79) suggests large differential shielding due to the presence of an adjacent heteroatom lone pair.

The resonance at 6.96τ appears to be split further by interaction with the proton on nitrogen when the spectrum is run in T.F.A. as solvent. The large coupling constant, J = 17 Hz, is indicative of a very strong interaction for which no parallel could be found in the literature: the accepted values of coupling constants for methylene protons coupled to protonated nitrogen are J = 6-8 Hz[8]. Furthermore it was not possible to decouple the multiplet by irradiation. This suggests that although the geminal pair may be adjacent to nitrogen, the "splitting" may arise from an equal distribution of two different protonated species, for example on nitrogen and on the oxygen of the pyrone carbonyl. This may cause the methylene signals associated with each form to shift so that two peaks are coincident and the spectrum has the appearance of a triplet in this region.

Two other signals are apparent in the P.M.R. spectrum: an unresolved multiplet centred at 7.70τ which integrates for two protons, either or both of which are coupled to the protons which resonate at 5.80τ and 6.50τ, and a two-proton multiplet at 8.35τ. This latter multiplet corresponds to part of a geminal methylene group in a saturated environment, J_{gem} = 10 Hz.

The P.M.R. spectrum was determined in deutero-benzene solvent to investigate shifts induced by solvent effects. It has been shown[14-17] that protons lying in regions of high electron density are deshielded in benzene relative to chloroform, and that protons in regions of low electron density are shielded. These predictions are valid if steric factors do not prevent the approach of the π cloud of the benzene to electron deficient areas during solvation. The reference plane rules for solvent-induced shifts of cyclic ketones[18] postulate that protons lying in regions above a reference plane through the carbon and perpendicular to the carbonyl bond will be shielded relative to chloroform

when the spectrum is observed in benzene as solvent. Protons in regions below the reference plane are deshielded. (2.V).



2.V

Protons lying in the plane, or close to it, are not appreciably shifted. The methyl resonance at 8.11τ was shifted upfield by +0.14 p.p.m. in benzene; such a shift is consistent with an α-C-methyl substituent as previously assigned. The greatest observed downfield shift, a deshielding of -0.20 p.p.m., was observed in the signal assigned to the methine proton adjacent to nitrogen and under the deshielding influence of the carbonyl. The benzene solvent experiment therefore supports the assignment of this methine resonance.

2.3.2. Infrared Spectroscopy

The infrared spectrum of bellendine (Fig. 2-2) was determined as a nujol mull, and in $CHCl_3$ solution. A strong carbonyl band at 1650 cm^{-1} has been assigned to a conjugated carbonyl group. Conjugation between a carbonyl and an olefinic system results in the infrared stretching frequencies of both chromophores falling to lower wavenumber due to a charge resonance form of the type $\overset{+}{C}H-C=C-\overset{-}{O}$, diminishing the double bond character of the carbonyl. However, the value observed, 1650 cm^{-1}, falls below the common absorption value of 1685 cm^{-1} for acyclic or hexacyclic α,β unsaturated ketones. Release of electrons from a β-substituent with +M characteristics will further induce the

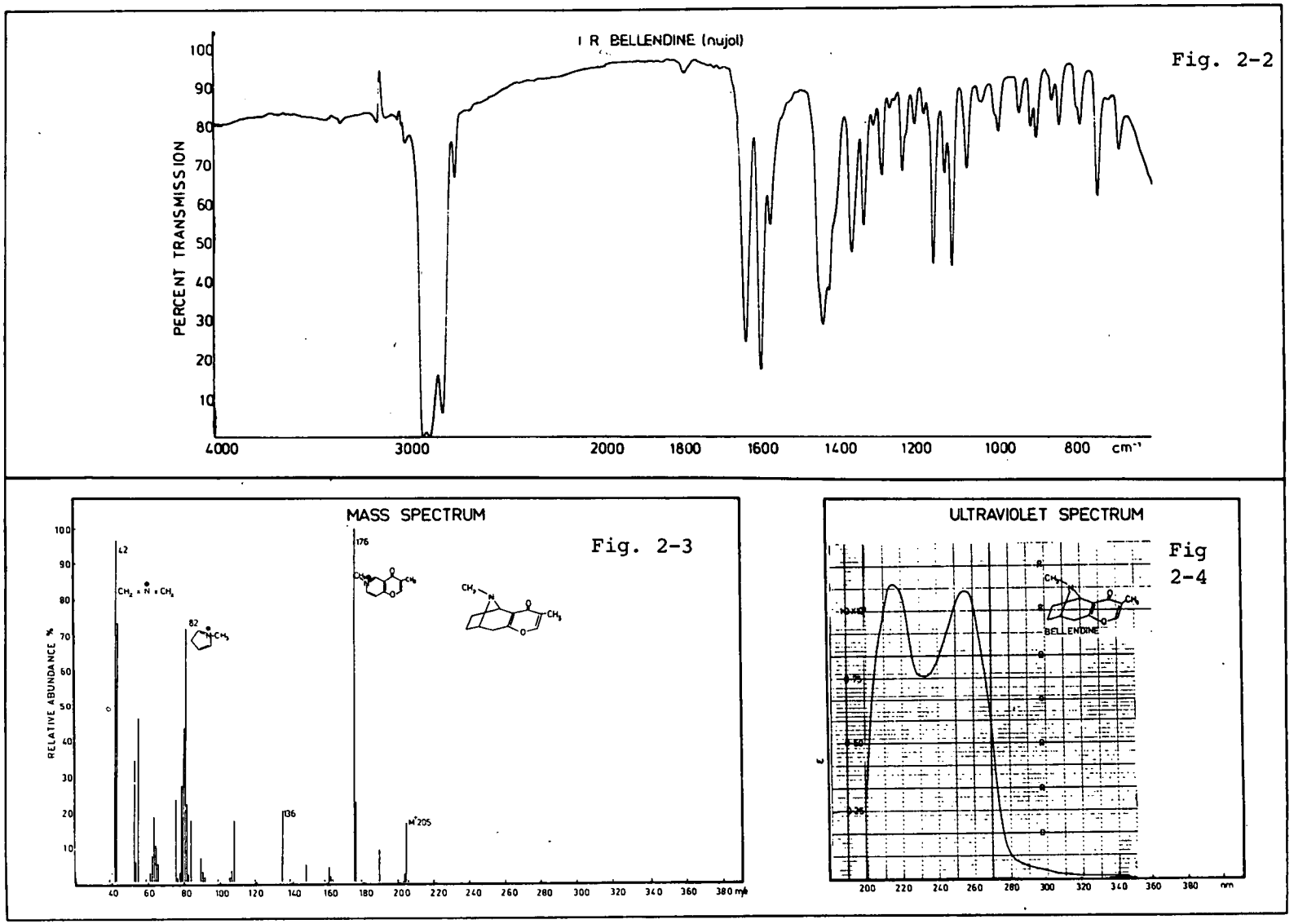


Fig. 2-2

Fig. 2-3

Fig 2-4

mesomeric form above and result in greater lowering of the carbonyl absorption frequency. Cyclization to form rings of less than six members introduces a ring strain of the order of +40 cm^{-1} in the carbonyl absorption frequency; hence, in bellendine, this chromophore must be present in a hexacyclic ring. Another feature of the infrared spectrum is the intensity of the olefinic absorption. Such an intense olefinic absorbance is normally associated with a heterosubstituent or a β-substituted α,β unsaturated carbonyl. γ-Pyrones have infrared absorption characteristics identical to those observed in this region of the infrared spectrum.

2.3.3. Ultraviolet Spectroscopy

The ultraviolet spectrum of bellendine (Fig. 2-4) was examined in water and MeOH. The spectrum had two absorption maxima: 209 nm and 257 nm for which the extinction coefficients were 9,800 and 10,600, respectively. The 209 nm band cannot be assigned to any structurally significant feature, but the 257 band suggests a chromophore containing an α,β unsaturated carbonyl (the presence of a weak $n \rightarrow \pi^*$ transition at 287 nm indicates the presence of a carbonyl).

The empirical rules of Woodward and Fieser[19] for the calculation of absorption maxima of α,β unsaturated carbonyls give 256 nm for an α,β unsaturated, hexacyclic ketone with an oxy function in the β position.

A small hypsochromic shift was apparent when acid was added to the solvent; a shift of this order is likely in such a chromophore because partial protonation of the ether oxygen lone-pair electrons prevents electron delocalization via the α,β unsaturated system. Vinylogous amides undergo hypsochromic shifts of 12-14 nm in acid solution[13]. An examination of the spectral properties of model γ-pyrones[20,21] showed

that the U.V. absorption maxima occur at 260 nm, ε_{max} 10,500.

2.3.4. Mass Spectral Fragmentation

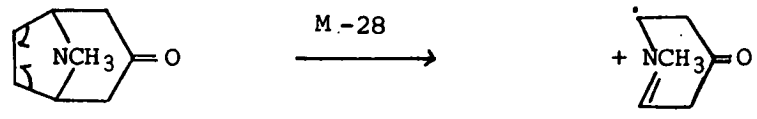
Initial mass spectral analysis at 70eV (ambient), revealed a molecular ion at 205 mass units. High-resolution of this peak indicated a molecular formula of $C_{12}H_{15}NO_2$, in agreement with elemental analysis.

The base peaks in the spectrum (Fig. 2-3) occurred at 176 and 42 mass units. High-resolution of the 176 peak showed that it corresponded to a loss of C_2H_5; a peak of moderate intensity (relative abundance 41%) at 177 arises from the loss of C_2H_4.

The intensity of the 176 peak suggested that an ion of high stability was being formed through C_2H_5 elimination. Stability of this nature is generally conferred on a molecule by a conjugated or aromatic structure. From the P.M.R. analysis, no free ethyl group occurs in the molecule so that an ethyl elimination must arise from an ethylene radical generation and ready hydrogen transfer from the resulting ion radical.

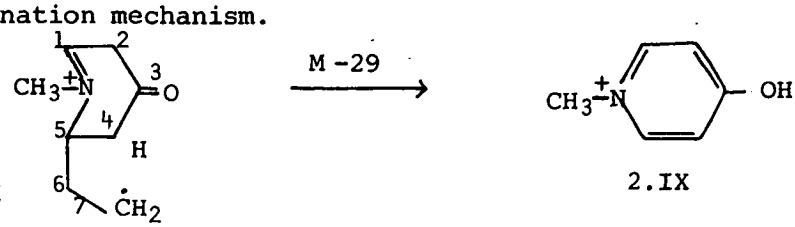
Extensive studies on the tropane alkaloids[22-24] (Section 1.4) using deuterium labelling, have shown that the ethylene bridge may be eliminated by a retro-Diels-Alder type of fragmentation, leaving the stabilized ion radical (2.VII); for example in tropan-3-one (2.VI):

Ethylene elimination mechanism.



2.VI 2.VII

Ethyl elimination mechanism.



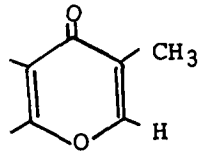
2.VIII 2.IX

Hydrogen transfer from position 4 results in ethyl elimination.

The mass spectrum of bellendine shows metastables corresponding to the loss of C_2H_4 and C_2H_5; furthermore the simplicity of the spectrum suggests that the characteristic α-cleavage of cyclic amines has resulted in generating an immonium ion that has aromatic stability. Such evidence suggests an ethylene bridge structure similar to the tropane ring system.

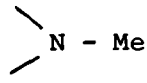
2.3.5. Structural postulates from spectral evidence

On the basis of consistent P.M.R., I.R., and U.V. spectral evidence the γ-pyrone chromophore is strongly favoured. (2.X).



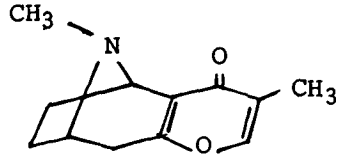
2.X

Furthermore, there can be little doubt of the presence of an N-methyl group. (τ = 7.68) (2.XI).



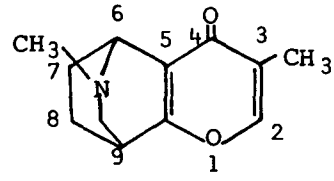
2.XI

The signals at 5.80τ and 6.55τ have been attributed to protons adjacent to nitrogen, that at 5.80τ being within the carbonyl deshielding zone. The proton producing the 6.55τ signal is coupled to the equatorial proton of the geminal system at 6.96τ ; the axial proton of this system, at 7.96τ, shows no coupling other than J_{gem} = 17 Hz. On the basis of these assignments, structure 2.XII is proposed for bellendine.



2.XII

However, if the nitrogen protonation evidence is accepted as indicating a methylene adjacent to nitrogen for the geminal system seen at 6.96τ and 8.02τ, then the isoquinuclidine structure 2.XIII must also be considered. Such a structure is less favoured than 2.XII due to the necessity of assigning the 6.55τ resonance to H_9.



2.XIII

Both structures are capable of undergoing the fragmentation observed in the mass spectrum via facile cleavage α to the nitrogen, leading to the elimination of ethylene, or by hydrogen transfer, to the elimination of an ethyl radical.

2.3.6. X-Ray Diffraction Study

Bellendine was sublimed in vacuo to deposit fine white needles suitable for X-ray crystallography. The development of a direct-method crystallography package by the Cambridge group, Motherwell and Isaacs[25], enabled these workers to show the structure of bellendine to be 2.XII. Bellendine was shown to crystallize in space group $p2_12_12_1$, with orthorhombic crystals, for which the unit cell dimensions were a = 9.733 ∓ 0.008, b = 12.624 ∓ 0.008 and c = 8.507 ± 0.007 Å. The density was found to be 1.305 cm^{-1}.

2.3.7. ^{13}C N.M.R. Spectroscopy

The ^{13}C spectrum was determined in $CDCl_3$ in the off-resonance decoupled mode, and the assignments of the δ^{TMS} scale are shown in Fig. 2-5. A detailed discussion of ^{13}C spectroscopy of γ-pyrono tropanes is made in Chapter 3.

Fig. 2-5

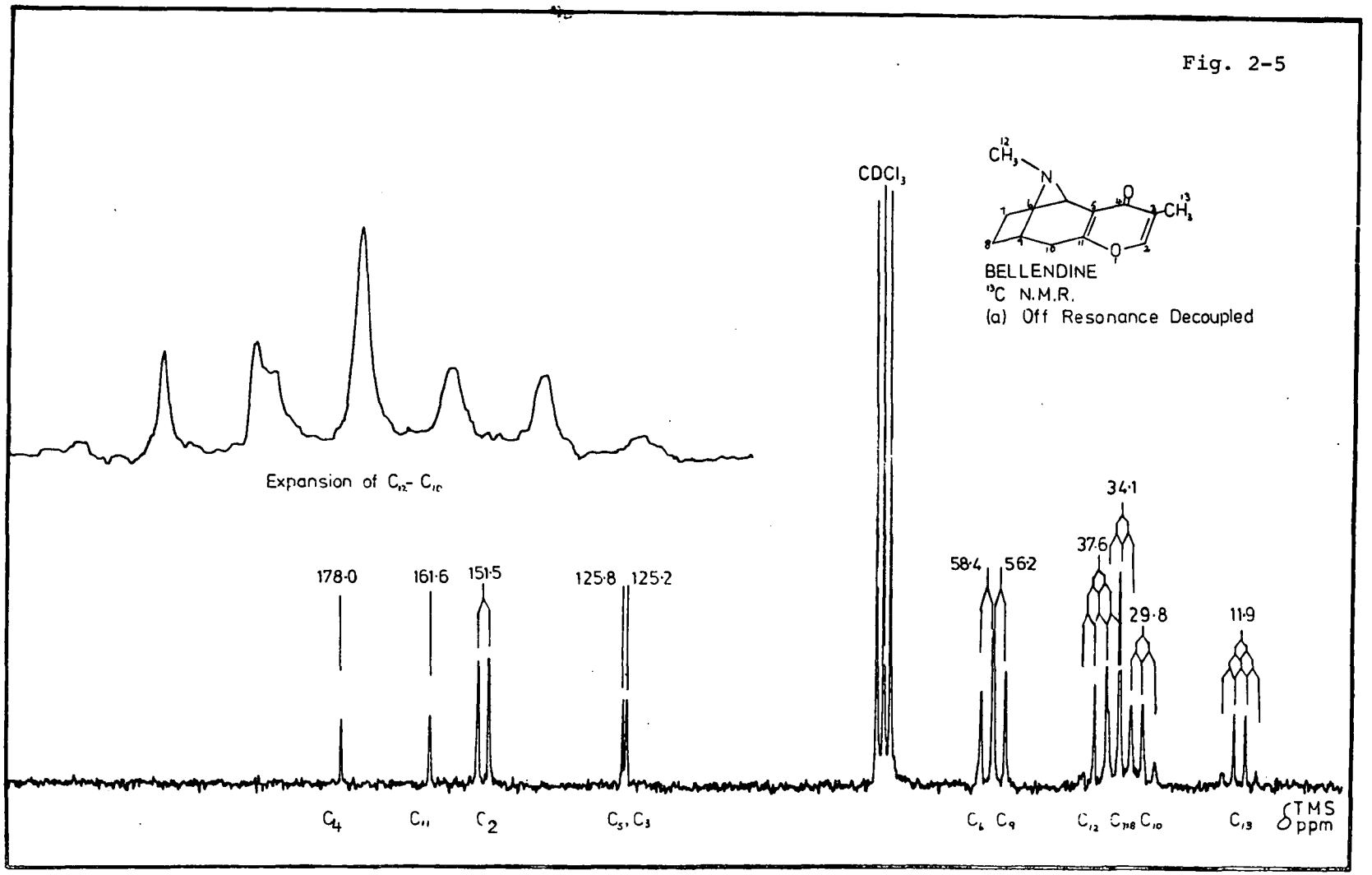

CDCl3
BELLENDINE
13C N.M.R.
(a) Off Resonance Decoupled
Expansion of C12- C10
178·0
161·6
151·5
125·8
125·2
58·4
56·2
37·6
34·1
29·8
11·9
C4
C11
C2
C5, C3
C6 C9
C12 C7,8 C10
C13
δTMS ppm

TABLE 2-5

Assignment of P.M.R. Signals and Couplings in Bellendine

τ	H	Assignment	Coupling Constants	
			$J_{x,y}$	Hz
2.42	1	H_2	$J_{2,13}$	1.2
5.80	1	H_6	$J_{6,7\ \beta}$	4.0
6.55	1	H_9	$J_{9,10\beta}$	6.0
			$J_{9,8\beta}$	5.0
6.96	1	H_{10}	$J_{10\beta,9}$	6.0
			$J_{10\beta,10\alpha}$	17.0
7.79	1	H_{10}	$J_{10\alpha,10\beta}$	17.0
7.68	3	$N\text{-}CH_3$		
7.70	2	$H_{7\beta,8\beta}$	$J_{7\beta,6}$	6.0
			$J_{7\beta,7\alpha}$	10.0
8.85	2	$H_{7\alpha,8\alpha}$	$J_{7\alpha,7\beta}$	10.0
8.10	3	$C\text{-}CH_3$		

2.3.8. Bellendine synthesis

The successful synthesis of bellendine is discussed in Chapter 4.

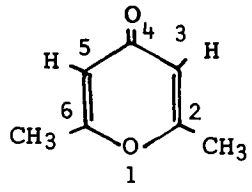
2.4. Structural Elucidation of Isobellendine

The second main alkaloid isolated from Bellendena montana, isobellendine, was crystallized from ether, and sublimed (80°, 1.0×10^{-4} mm Hg) to yield white needles m.p. 114° $[\alpha]_D$ = +143°. High-resolution mass spectroscopy indicated a molecular formula of $C_{12}H_{15}NO_2$, in exact agreement with the elemental analysis and

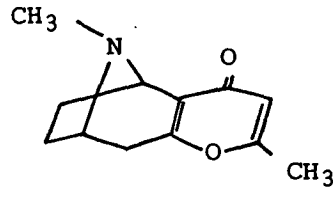
isomeric with bellendine. The fragmentation pattern was identical to bellendine.

2.4.1. P.M.R. Spectroscopy

The P.M.R. spectrum (Fig. 2-6) bore similarities to the bellendine spectrum, but differed in the following respects: the resonance at 2.42τ associated with the olefinic proton in bellendine was no longer present, but a new olefinic singlet appeared at 3.97τ. The signal assigned to the C-3-CH_3 in bellendine at 8.11τ was absent and a new three-proton singlet appeared at 7.78τ. The chemical shift of the olefinic proton resonance and the methyl resonance in isobellendine were characteristic of the H_3 and 2-methyl resonances in 2,6-dimethyl pyrone[26] (2.XIV).



2.XIV



2.XV

From these data and the mass spectral fragmentation pattern, structure 2.XV was proposed for isobellendine.

2.4.2. Infrared Spectroscopy

The infrared spectrum (Fig. 2-7) shows the characteristic γ-pyrone absorptions $\nu_{C=O}$ 1655 cm^{-1}, $\nu_{C=C}$ 1600 cm^{-1}. There are small skeletal vibrational differences between bellendine and isobellendine in the fingerprint region at 1020 cm^{-1} and 1170 cm^{-1}.

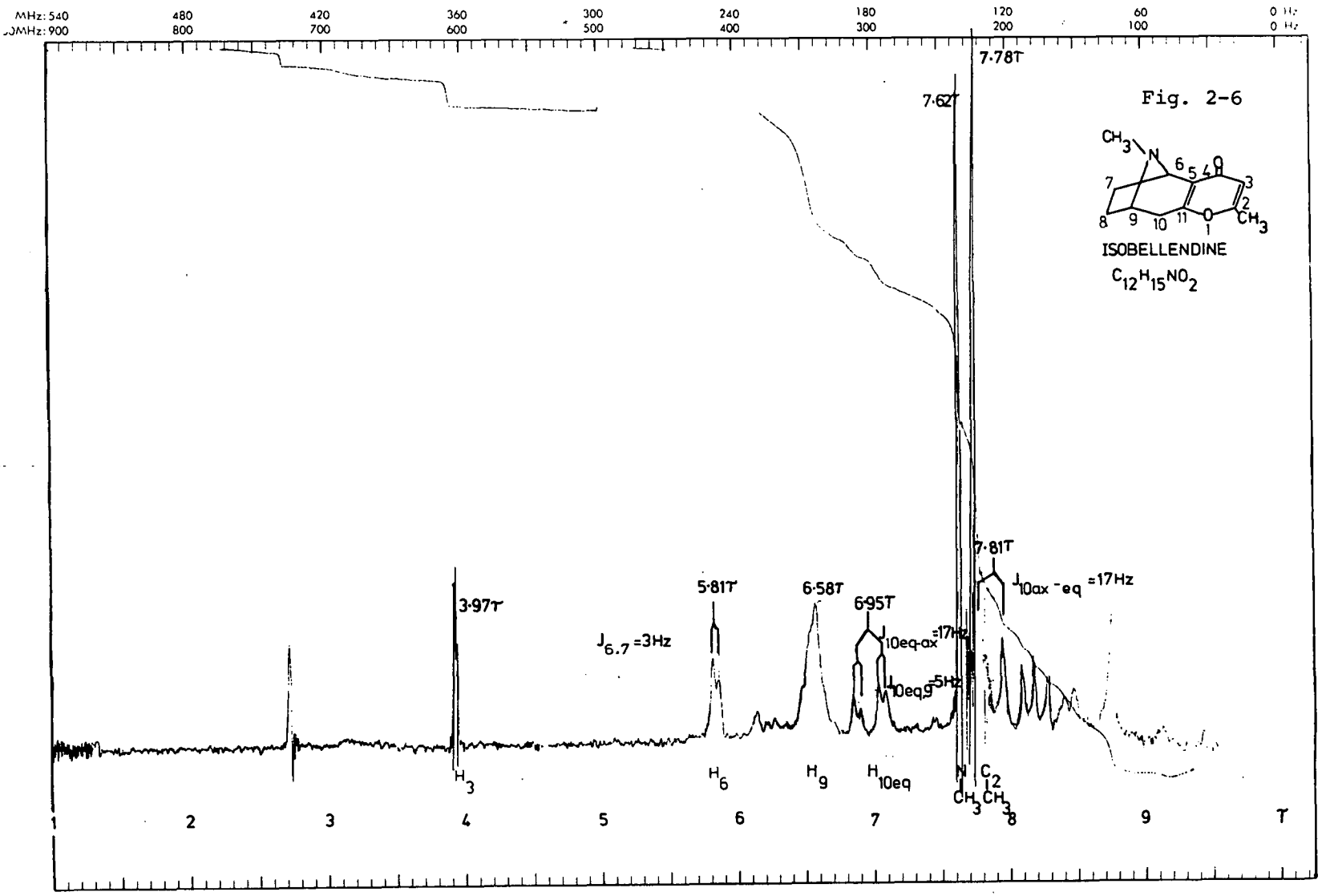


Fig. 2-6

ISOBELLENDINE

$C_{12}H_{15}NO_2$

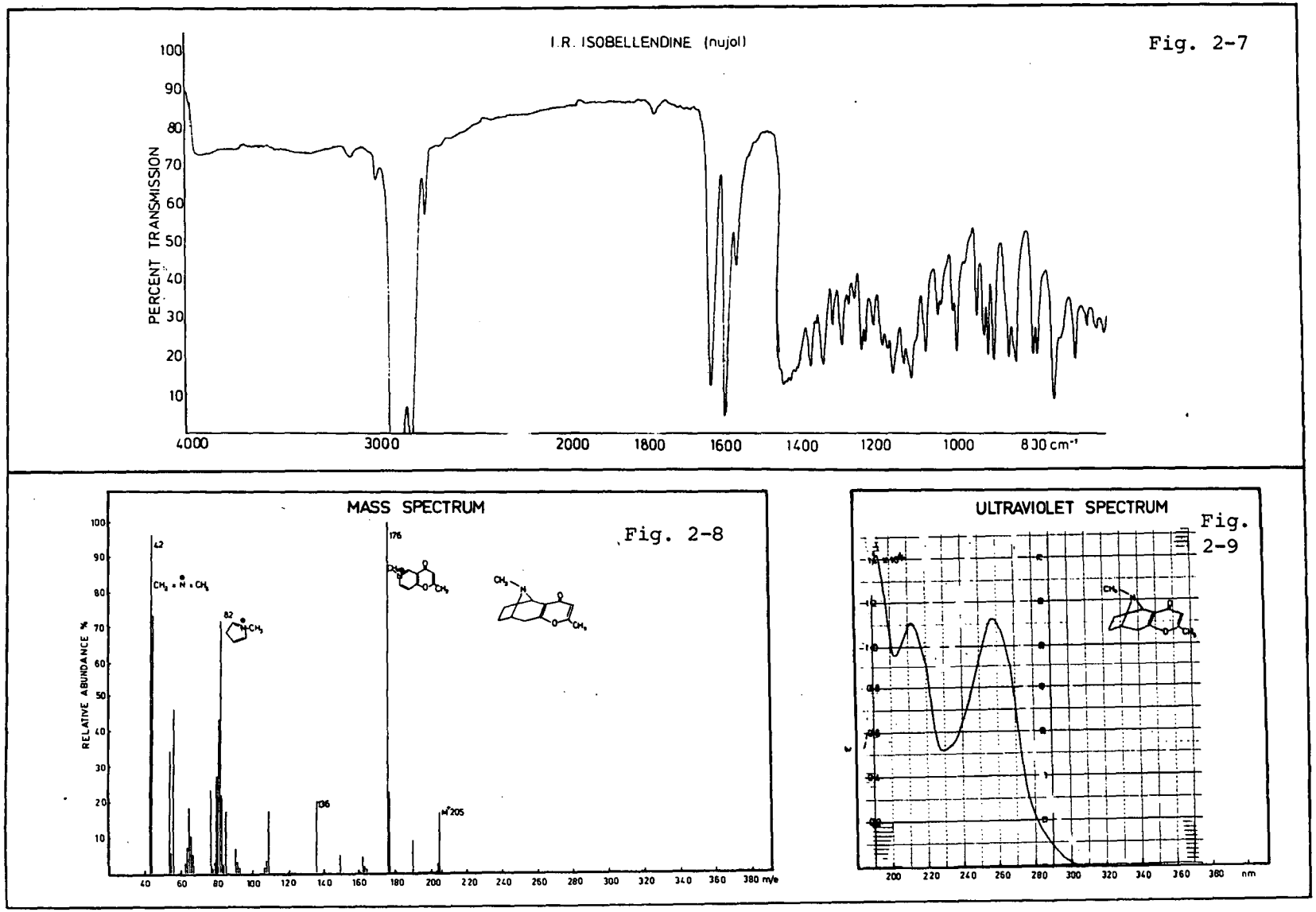


Fig. 2-7

Fig. 2-8

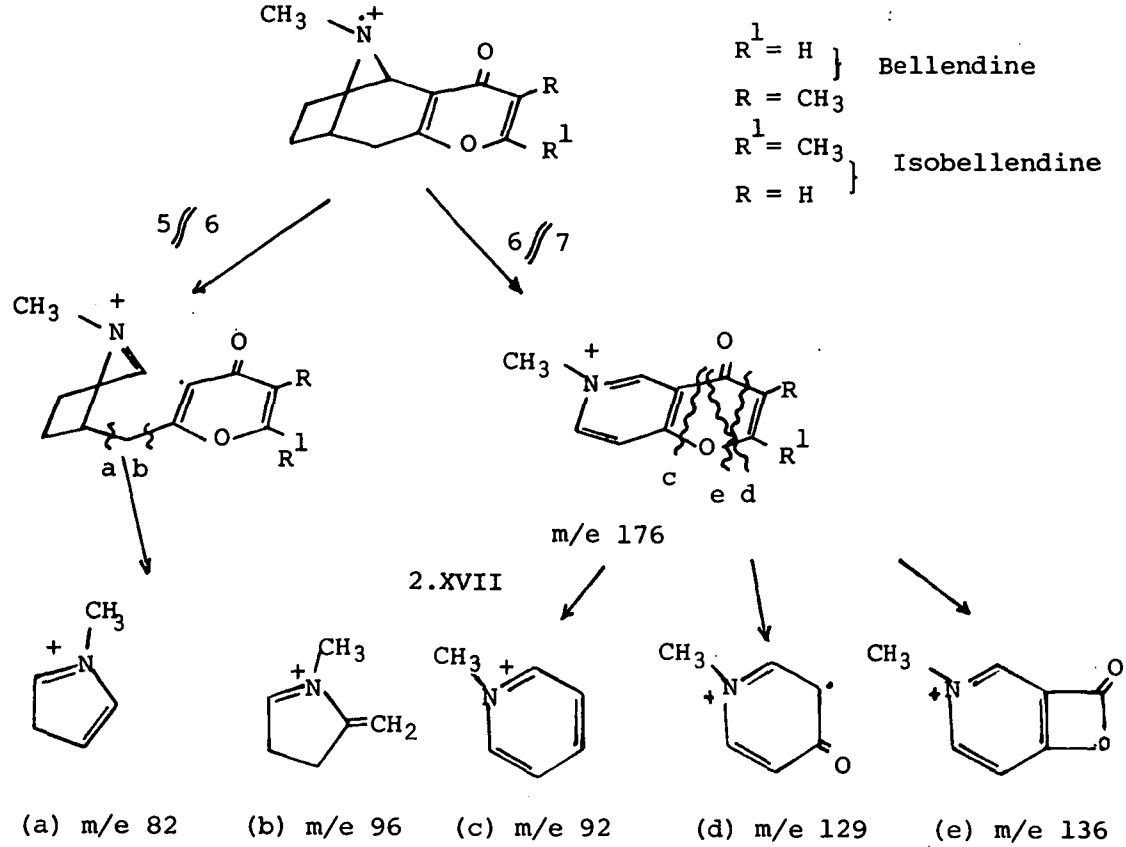
Fig. 2-9

2.4.3. Ultraviolet Spectroscopy

The ultraviolet spectrum (Fig. 2-9) λ_{max} = 257, ε_{max} = 10,600 indicates the presence of a γ-pyrone chromophore. The small difference between the absorption maxima of bellendine and isobellendine, +2 nm, is consistent with the empirical rules of Woodward and Fieser[19] which assign substituent effects of +10 nm to alkyl substituents attached to α positions in α,β unsaturated ketones and +12 nm to alkyl substituents in β positions.

2.4.4. Mass Spectroscopy

Under electron impact, isobellendine fragmented identically to bellendine (Fig. 2-8). The base peak, resulting from the tertiary amino α-cleavage mechanism and loss of the ethano bridge, appears at m/e 176. This leads to the stable pyrono-pyridinium cation 2.XVII as the base peak. A representation of the fragmentation pattern of the two Bellendena pyrono-tropanes is shown in Scheme 2-2.



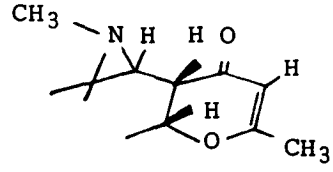
Scheme 2-2

2.5. Structural Elucidation of Dihydroisobellendine

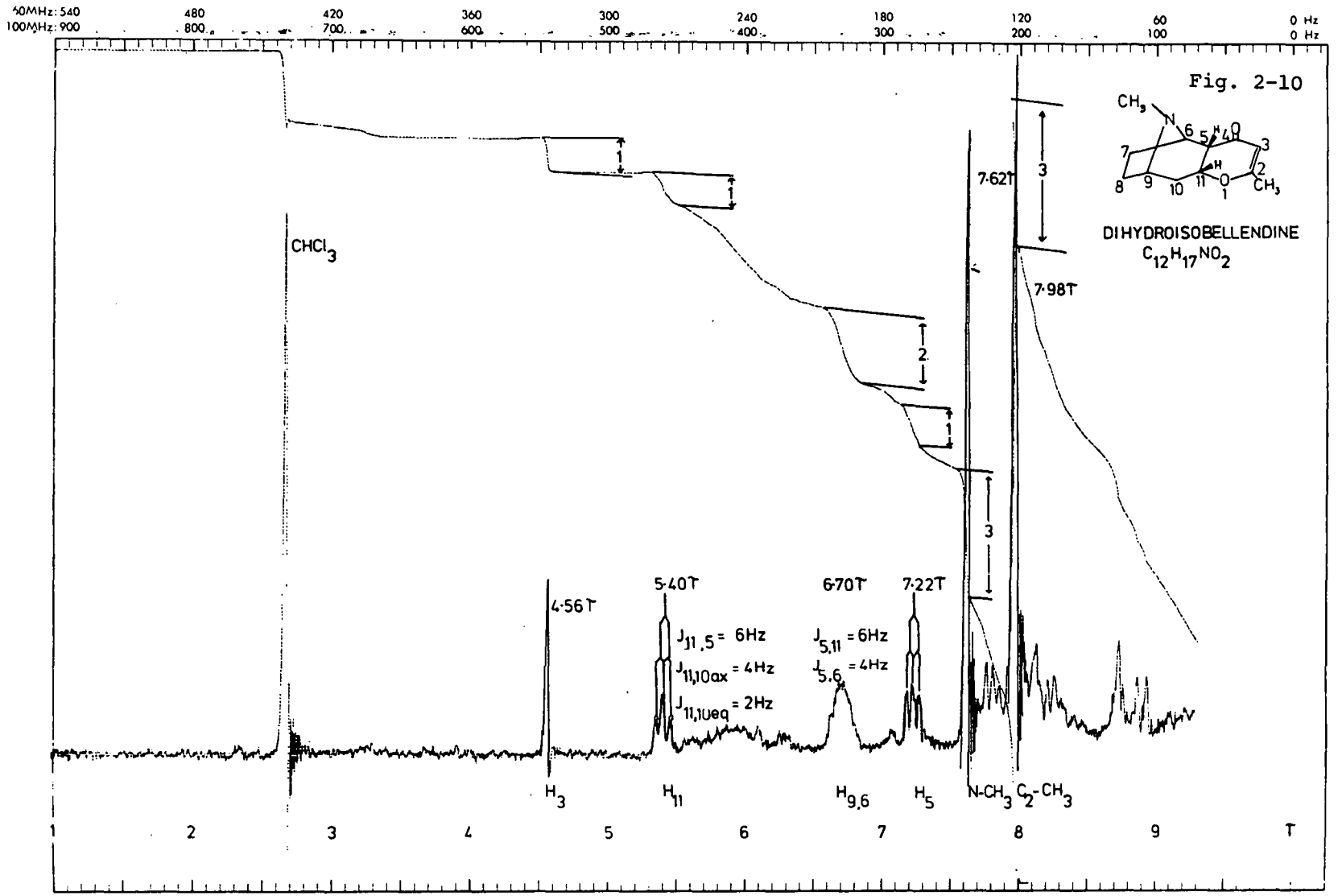
Preparative thin-layer chromatography of B5 enabled the separation of a third major alkaloid from Bellendena montana as a viscous oil, $[\alpha]_D = -53°$. The compound could not be induced to crystallize nor form crystalline hydrobromide or picrate derivatives. On attempted vacuum sublimation, the compound decomposed. Its structural characteristics have been determined through a spectroscopic study and confirmed by synthesis. The molecular formula was found by high-resolution mass spectroscopy to be $C_{12}H_{17}NO_2$.

2.5.1. P.M.R. Spectroscopy

The P.M.R. spectrum (Fig. 2-10) revealed two methyl resonances, one at 7.63τ consistent with a N-methyl substituent, and the other at 7.98τ, which correlated with C-methyl values of enolic diketo systems[27]. An olefinic resonance was present, in a more shielded environment than that of isobellendine. No exchangeable protons were apparent on shaking the solution with D_2O, hence one oxygen of the enolic system was assumed to be present in a γ-pyrone ring. Two of the lowerfield resonances at 5.42τ and 7.25τ were found to be coupled to each other (J = 6 Hz). The downfield signal was assigned to a methine proton adjacent to a pyrone oxygen. The upfield resonance at 7.25τ was attributed to the position α to the carbonyl of the γ-pyrone. This signal was also coupled (J = 4 Hz) to one of the remaining two broadened lowerfield resonances at 6.75τ (M, 2H) which were assigned to methine protons adjacent to nitrogen. These data suggested the partial structure 2.XVI for dihydroisobellendine.



2.XVI

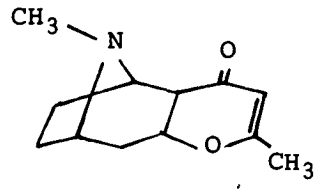

60MHz: 540
100MHz: 900
480
800
420
700
360
600
300
500
240
400
180
300
120
200
60
100
0 Hz
0 Hz
Fig. 2-10
CHCl3
4·56T
5·40T
6·70T
7·22T
7·62T
7·98T
J11,5 = 6Hz
J11,10ax = 4Hz
J11,10eq = 2Hz
J5,11 = 6Hz
J5,6 = 4Hz
H3
H11
H9,6
H5
N-CH3
C2-CH3
DIHYDROISOBELLENDINE
C12H17NO2
1
2
3
4
5
6
7
8
9
T

2.5.2. I.R. and U.V. Spectroscopy

The infrared spectrum (Fig. 2-11) indicates the presence of a conjugated carbonyl $\nu_{C=O}$ 1660 cm^{-1} and a strong olefinic stretch at $\nu_{C=C}$ 1610 cm^{-1}. The spectrum in this region showed qualitative similarity to that of isobellendine which was assigned to the γ-pyrone chromophore. The ultraviolet spectrum (Fig. 2-13) indicates a major diagnostic band at λ_{max} 266 nm. The wavelength of this band was consistent with a γ-pyrone structure; however, the ε value, ε_{max} = 6,700, was indicative of a dihydropyrone chromophore (λ_{max} = 262, ε_{max} = 6,500)[28]. In both the above cases, the evidence supports the original dihydro-γ-pyrone assignment.

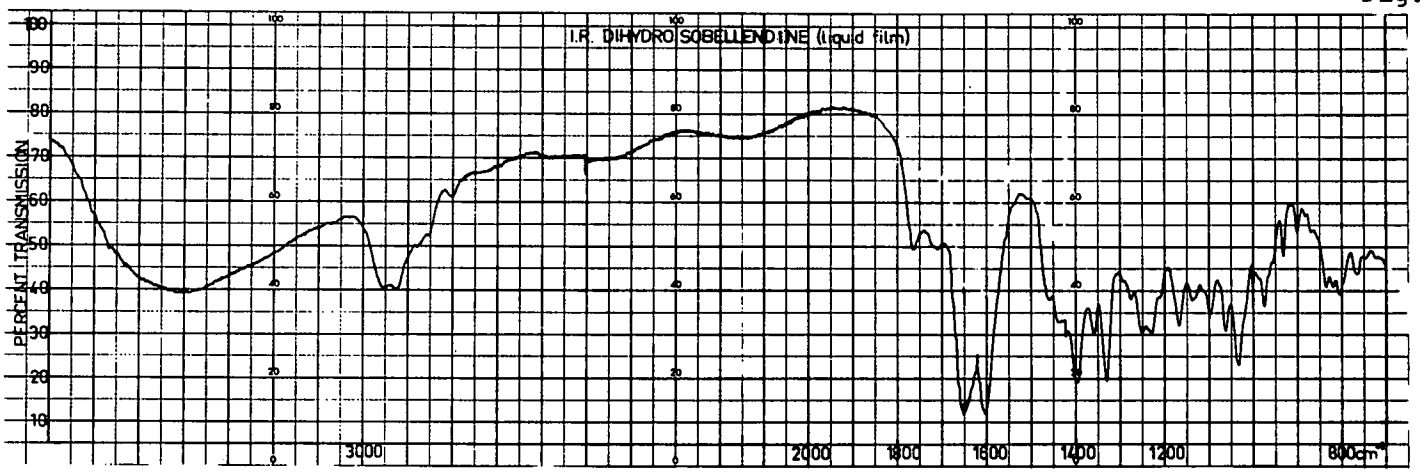
2.5.3. Mass Spectroscopy

The compound was subjected to mass spectral fragmentation and the principal fragments were highly resolved. (Fig. 2-12). The peaks at 97, 96 and 82 confirmed the presence of a tropane moiety in the molecule. The observed fragmentation pattern has been interpreted on the basis of a ring-opening hydrogen-transfer mechanism from a structure such as 2.XVII to give the enol form of the diketone. (Scheme 2-3). Subsequent cleavages α to the carbonyls produce the important peaks at m/e 164, 150 and 122.



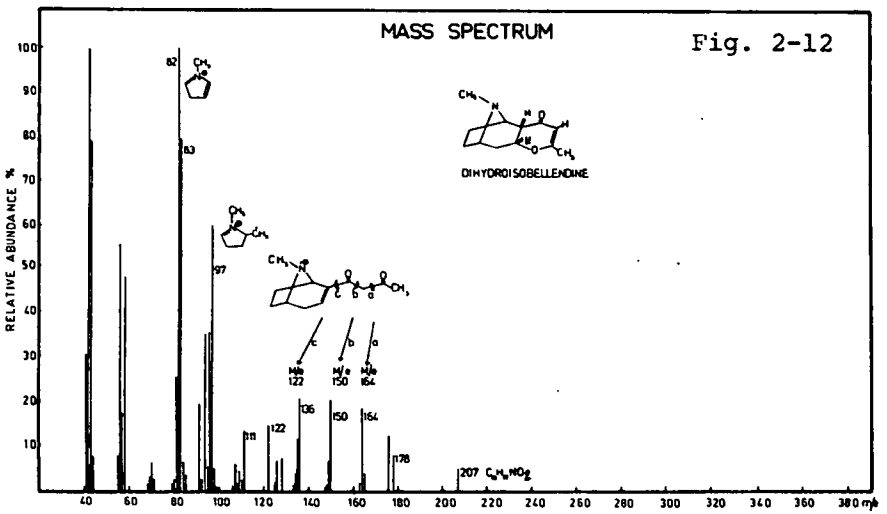
2.XVII

Fig. 2-11



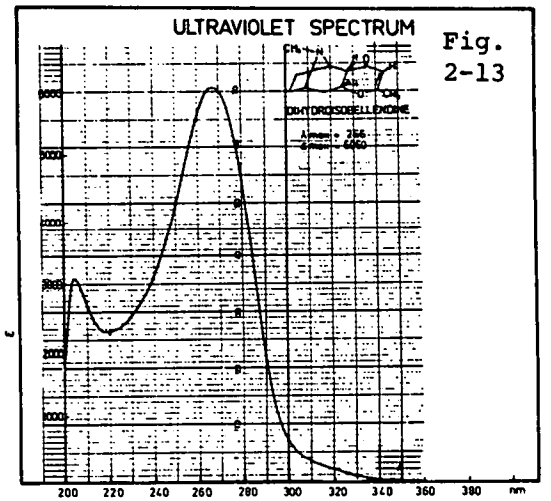
MASS SPECTRUM

Fig. 2-12

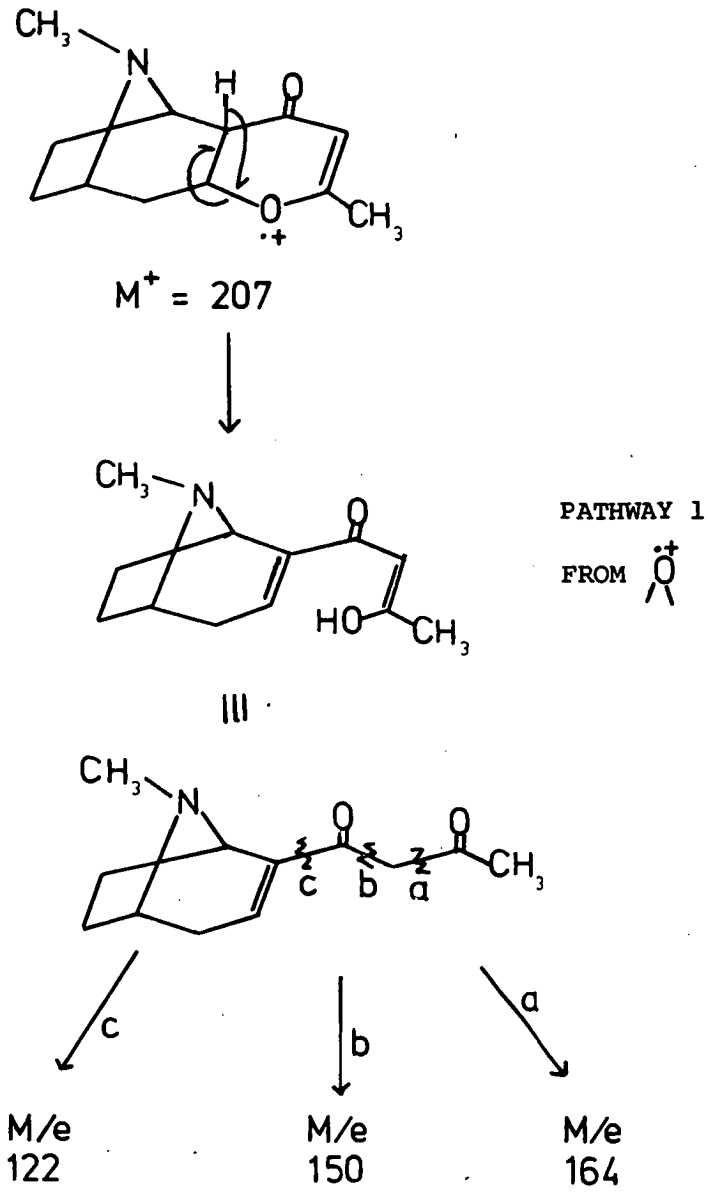


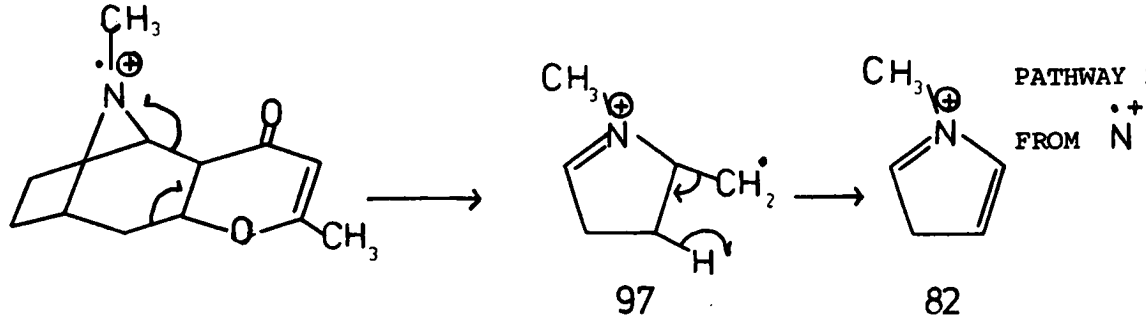
ULTRAVIOLET SPECTRUM

Fig. 2-13



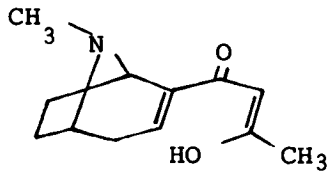
Mass Spectral Fragmentation Pattern, Dihydroisobellendine





Scheme 2-3

An identical pattern was observed in the mass spectrum of the synthetic intermediate of dihydroisobellendine, 2.XVIII. (Fig. 4-28).



2.XVIII

The characteristic tropane fragments in the spectrum are due to cleavages based on a nitrogen radical cation; the resultant α-cleavages lead to the fragments at m/e 97, 96, and 82. Only a small peak at m/e 178 arises from the retro-Diels-Alder loss of the ethano bridge. This is in contrast to the bellendine/isobellendine case in which such a loss conveys aromatic stability on the system as a pyrono-pyridinium cation.

2.5.4. Determination of the Stereochemistry of Dihydroisobellendine

(i) Coupling constants and Calculated Dihedral Angles

The well-defined P.M.R. signals associated with the protons of the pyronotropane ring junction enabled an analysis of the coupling constants to be undertaken. The significant chemical shift difference between each of the interacting spins permitted a first order interpretation of the multiplets. The signal associated with H_{11}, 5.40τ, was a triplet, $J_{11,5}$ = 5 Hz, $J_{11,10eq}$ =1.5 Hz, $J_{11,10ax}$ = 5 Hz. Dihedral angles calculated from the modified Karplus equation for each of these vicinal interactions were found to be H_{11}/H_5 = 45°, H_{11}/H_{10eq} = 63°, H_{11}/H_{10ax} = 45°. The multiplet associated with H_5 also proved to be a triplet, $J_{5,11}$ = 5 Hz, $J_{5,6}$ = 4 Hz, which gave the dihedral angles H_5/H_{11} = 45°, H_5/H_6 = 60°. Dreiding stereomodels were constructed for each of the four possible configurations of the ring junction. In all cases except the cis endo configuration, at least one trans diaxial interaction was present.

Such an interaction would be reflected in a large coupling constant of the order of 10-11 Hz. No such coupling was observed, and, furthermore, the dihedral angles calculated from the coupling constants fitted exactly those of models of the cis endo (2α, 3α) configuration when the molecule assumed the chair conformation.

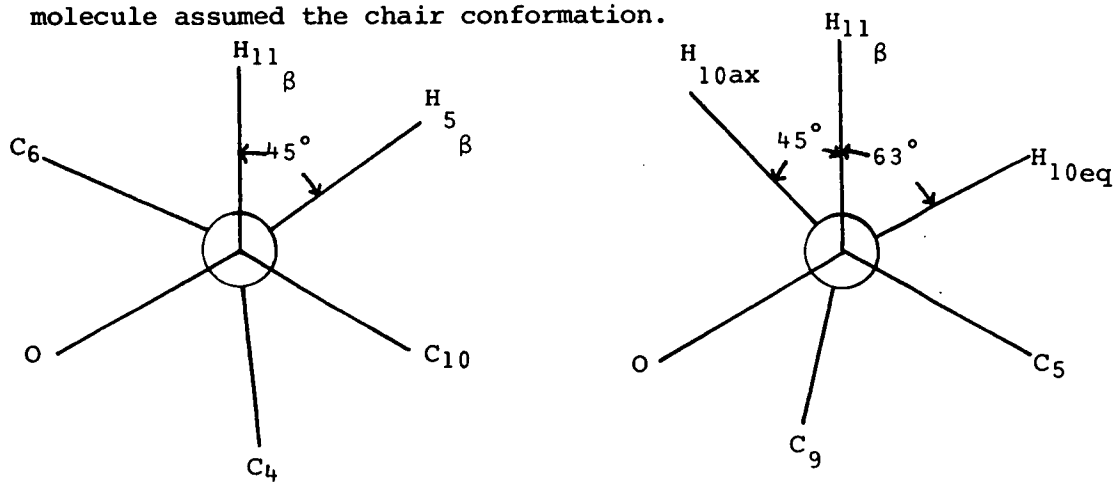


Fig. 2-14

(a) View along C_{11}-C_5 showing dihedral angle calculated for H_{11}/H_5.

(b) View along C_{11}-C_{10} showing dihedral angles calculated for H_{11}/H_{10ax} and H_{11}/H_{10eq}.

(ii) Shift Reagents

In view of the stereochemical deductions above, it was considered that the addition of a lanthanide shift reagent, $Eu(DPM)_3$, would cause substantial deshielding of the C-5 and C-11 proton resonances (also H_{10ax}). Such an hypothesis was based on the assumption that complexing of the shift reagent would occur via the nitrogen lone-pair electrons. The graph of observed shifts (Fig. 2-15) partially substantiates such an hypothesis, the greatest gradient being observed for the shift of the H_5 resonance. It is also noted that the H_3 resonance is subjected to a substantial shift; such an observation may result from there being a second site for complexation - the carbonyl

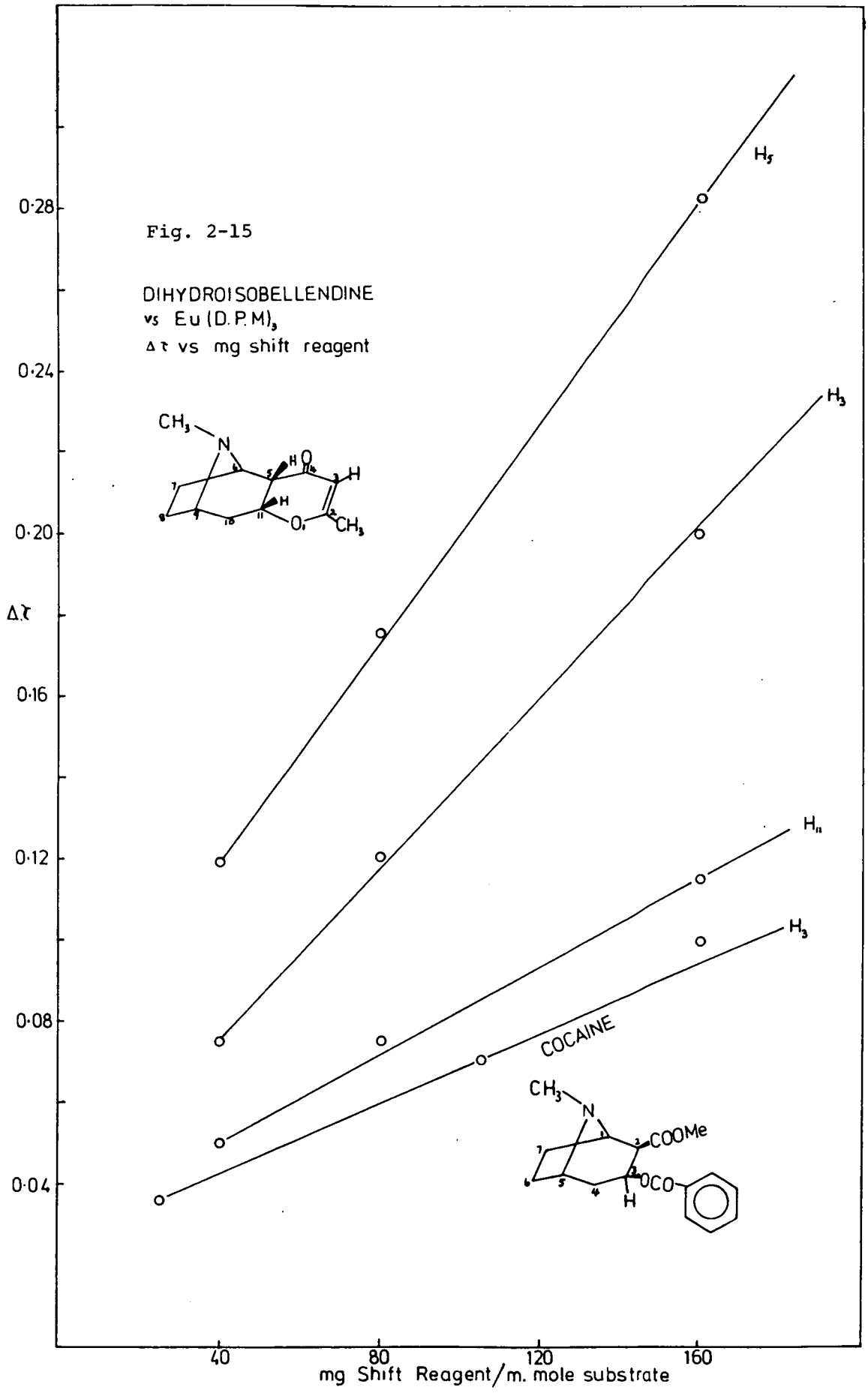
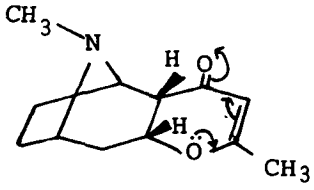


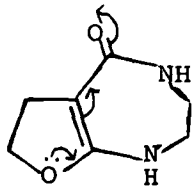
Fig. 2-15
DIHYDROISOBELLENDINE
vs Eu(D.P.M)3
Δτ vs mg shift reagent
Hs
H3
Hn
H3
COCAINE
0·28
0·24
0·20
0·16
0·12
0·08
0·04
Δτ
40
80
120
160
mg Shift Reagent/m. mole substrate

oxygen. I.R. and U.V. data indicate that delocalization of the lone-pair electrons from the pyrone oxygen causes a considerably greater electron density at the carbonyl oxygen of pyrones than for normal ketones (2.XIX).

In the lactam, 2.XX, complexing has been shown to occur *via* the lactam carbonyl rather than the secondary amino function[29].



2.XIX



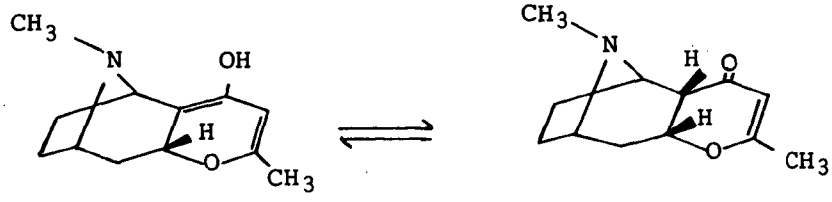
2.XX

The equilibrium between forms complexed at either site will be apparent as an average effect in the observed lanthanide-induced chemical shift[30]. The shift gradient observed for the C-11 proton is seen to be substantially greater than that for the C-3α-H in cocaine, which has the opposite stereochemistry to that assigned to 2.XIX. This can be taken as a reflection of the differing stereochemical relationships between dihydroisobellendine and cocaine with regard to the complexing sites and the proton under consideration. Data from other experiments indicate that polyfunctional compounds containing tertiary amino groups and ester functions, such as cocaine, complex with lanthanide shift reagents *via* the nitrogen[31]. Thus for cocaine, the shift gradient for H_{4ax} was found to be substantially in excess of that observed for the C-3α proton. In view of the dual complexing sites in 2.XIX, it is not possible to deduce from a comparison of these data unambiguous configurational information about H_{11} in dihydroisobellendine.

(iii) Synthetic Studies

Dihydroisobellendine was synthesized from anhydroecgoninoyl

chloride and sodium acetone enolate. Whereas the condensation was rapidly effected, the cyclization process was only achieved after three months under mild acid conditions. In view of the cyclization from the enol to the product of natural configuration, it is apparent that the Michael addition of the enol oxygen to the C_{11} occurs from the least-hindered α face, and the resultant enol epimerizes to the thermodynamically more stable equatorial isomer 5,11-cis, endo-dihydroisobellendine (2.XIX).



2.XXI 2.XIX

2.6. Structural Elucidation of B3 (6β-Acetoxy-3α-isobutoxy tropane and 6β-isobutoxy-3α-acetoxy tropane)

Alkaloid band B3 was revealed on P.T.L.C. at R_f = 0.68 (10% MeOH in $CHCl_3$) by Schlittler's reagent. The band caused no quenching of the adsorbent fluorescence. A small quantity (17 mg) was isolated by extraction of this band, and analytical T.L.C. in several solvent systems indicated a substantially pure product. Nevertheless, it could not be induced to form a crystalline picrate, hence the molecular formula, $C_{14}H_{23}NO_4$, was determined by high-resolution mass spectroscopy.

2.6.1. Mass Spectral Fragmentation

The molecule fragmented according to the characteristic pattern for tropan-3,6-diol esters (Section 1.4.1) (Fig. 2-17). The presence of a substituent on the ethano bridge resulted in facile retro-Diels-Alder cleavage of this fragment, and the loss of a C-3 ester function via a

McLafferty rearrangement produced the base peak in the spectrum at m/e 94. The other characteristic tropane fragments, m/e 83, 82 and 42, were also observed.

2.6.2. Spectroscopic Studies

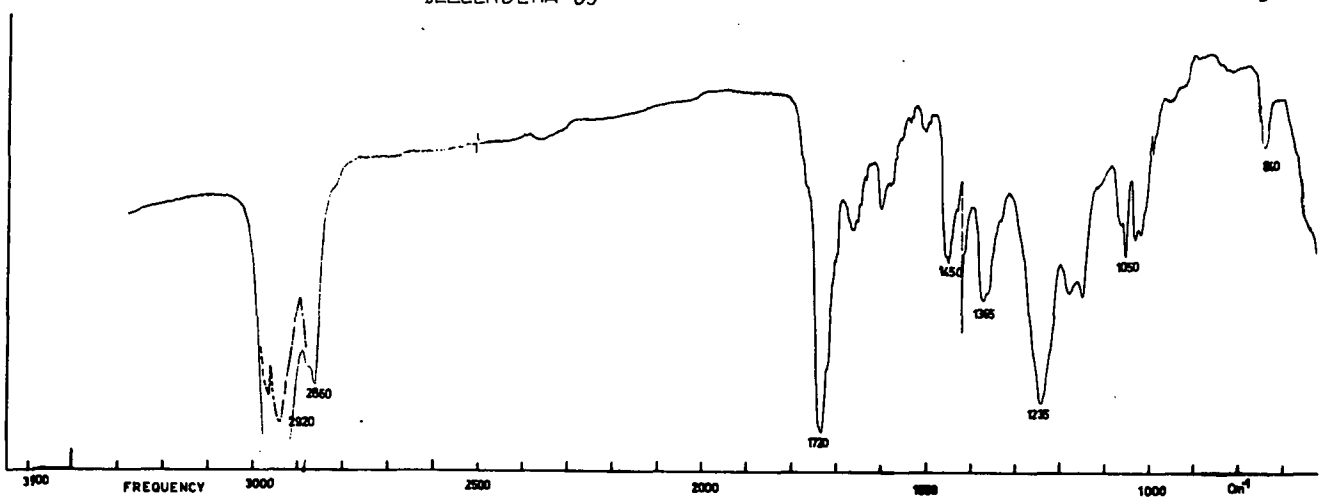
The P.M.R. spectrum (Fig. 2-19) shows the presence in the molecule of an N-methyl group at 7.49τ (s, 3H), an acetoxy C-methyl group at 7.95τ (s, 3H), and an apparent isopropyl group split into two doublets at 8.80τ (d. of d., 6H, J = 7 Hz, J = 3 Hz). The downfield resonances at 4.50τ (q, 1H, J = 7.5 Hz, J = 2 Hz), and 4.90τ, (t, 1H, J = 5 Hz), were assigned to protons adjacent to acylated oxygen. Two other broadened downfield multiplets are apparent at 6.49τ, (1H), and 6.61τ, (1H), which were assigned to protons adjacent to nitrogen. The presence of these four downfield resonances was strongly indicative of a tropan-3,6-diol ester; moreover, the resonance at 4.90τ, an apparent triplet, is an indication of C-3α-acyloxy stereochemistry of the tropane, whereas the quartet at 4.50τ, coupled (J = 2 Hz, J = 7.5 Hz) to the resonances at 7.60τ and 6.49τ respectively, is considered to show C-6α-acyloxy substitution of the tropane ring (see Section 1.3.2). This assignment was supported by the simultaneous collapse of the signal at 6.61τ (H_{11}) on irradiation at 7.6τ ($H_{7\beta}$).

The infrared spectrum (Fig. 2-16) indicated the presence of an ester carbonyl, $\nu_{C=O}$ = 1730 cm^{-1} and ν_{C-O} = 1235. No ultraviolet chromophore was observed.

It was apparent from the spectroscopic data that B3 had either structure 2.XXII or 2.XXIII.

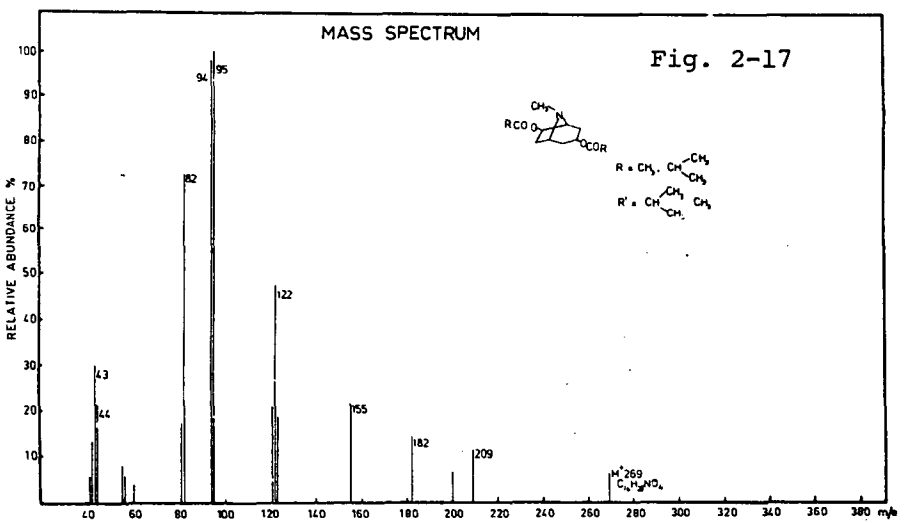
BELLENDENA B3

Fig. 2-16



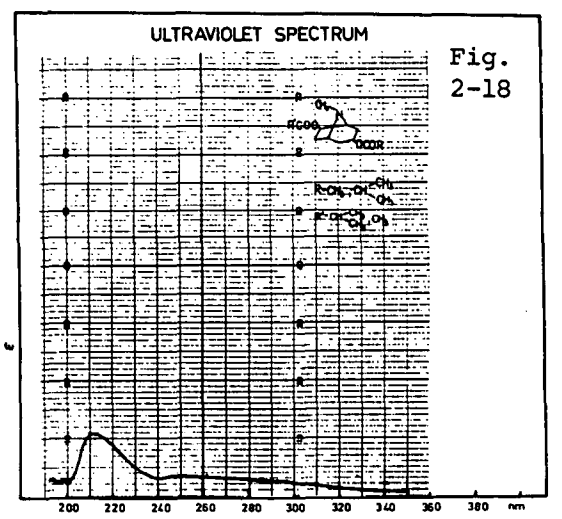
MASS SPECTRUM

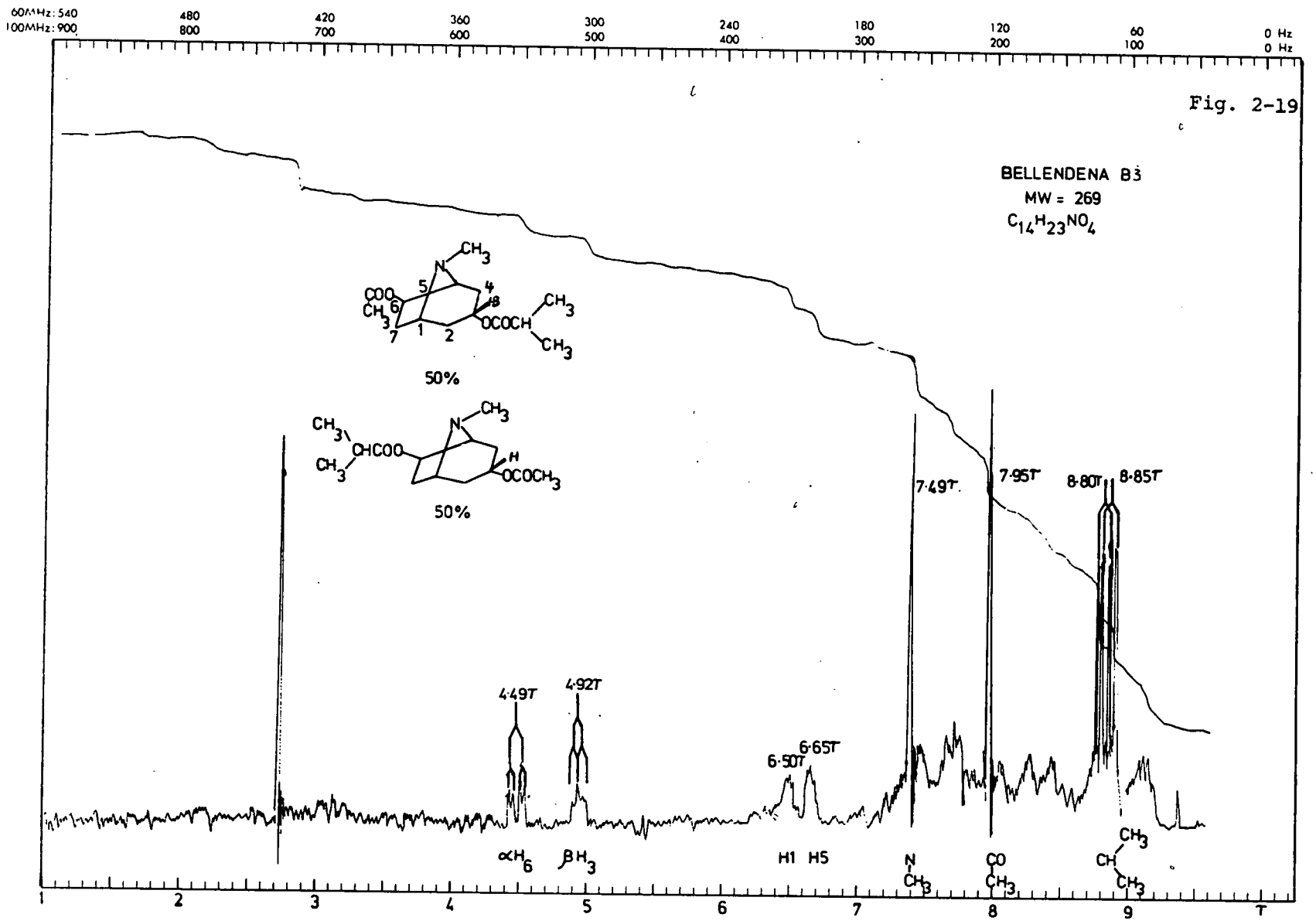
Fig. 2-17

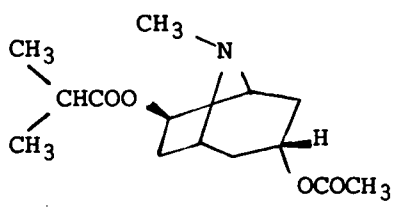


ULTRAVIOLET SPECTRUM

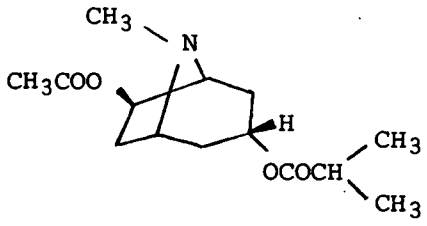
Fig. 2-18




60MHz: 540
100MHz: 900
480 800
420 700
360 600
300 500
240 400
180 300
120 200
60 100
0 Hz
0 Hz
Fig. 2-19
BELLENDENA B3
MW = 269
$C_{14}H_{23}NO_4$
50%
50%
4·49T
4·92T
6·50T
6·65T
7·49T
7·95T
8·80T
8·85T
αH6
βH3
H1 H5
N CH3
CO CH3
CH CH3 CH3
1 2 3 4 5 6 7 8 9 T



2.XXII



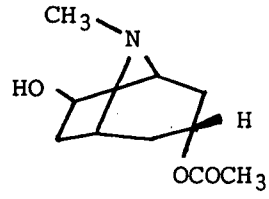
2.XXIII

Due to the small amount of compound available, (14 mg), a selective Kuntz hydrolysis[32] to remove the C-6 esterifying acid was not initially attempted. Instead, a synthesis was undertaken (details Section 2.11.2) of 2.XXII and 2.XXIII to compare spectroscopic properties with those of the natural product. The P.M.R. spectrum of each compound proved identical in all respects to that of the natural compound, except for the isopropyl resonances, which for 2.XXII and 2.XXIII were doublets at 8.78τ and 8.82τ respectively, (J = 7 Hz), instead of the doublet of doublets noted for B3, (J = 7 Hz, Δτ 2.5 Hz). In the belief that such splitting in B3 may have been the consequence of a conformational effect in which a boat conformation for the piperidine ring caused undue electronic or steric effects on a C-3β substituent, this series of compounds was synthesized for comparative purposes. The C-3β series (pseudotropines) showed distinct differences in the chemical shifts and coupling constants of all the diagnostic signals when compared to the C-3α series, (tropines). The C-6α proton was found at 4.95τ, (doublet of doublets, J = 5 Hz, J = 3 Hz), whereas the C-3α proton was a quintuplet 5.18τ, (J = 5 Hz), in accordance with the previously discussed theory (Section 1.3.2.). In both 6β-isobutoxy-3β-acetoxy tropane (2.XXIV) and 6β-acetoxy-3β-isobutoxy tropane (2.XXV), the isopropyl resonances appeared as doublets. A closer examination of

exact chemical shifts of the isopropyl resonances of the C-3α series, 2.XXII and 2.XXIII, showed that these matched exactly the upper and lower field components of the isopropyl resonance in B3. When the spectrum of a 50:50 mixture of 2.XXII and 2.XXIII was recorded (Fig. 2-19 (a)), it was superimposible on that of the natural product B3. Likewise, a comparison of the infrared spectra of the mixture and B3 showed complete accord. Attention was given to separation of the components. Repeated T.L.C. showed only partial separation of the parent bases. A Kuntz hydrolysis was undertaken on the mixture in order to identify the products by mass spectroscopy. P.T.L.C. on the reaction mixture indicated two products of differing R_fs which were isolated and found to be tropan-3,6-diol and 3α-isobutoxy tropan-6β-ol. The latter compound was isolated in sufficient quantities for P.M.R. and mass spectral confirmation of structure. The former, being very water soluble, was isolated only in trace quantities sufficient for mass spectral examination. Under the conditions of the Kuntz hydrolysis, one component of B3, 6β-isobutoxy-3α-acetoxy tropane, was totally hydrolysed to the diol. From these data, and a comparison of the synthetic and natural product, it was concluded that B3 was a mixture of 6β-isobutoxy-3α-acetoxy tropane and 6β-acetoxy-3α-isobutoxy tropane.

2.7. Structural Elucidation of B6

The lowest R_f alkaloid (R_f = 0.26 12% MeOH $CHCl_3$ silica gel) was isolated in small quantities (12 mg) and formed a picrate, m.p. 182-184°, which analysed for $C_{10}H_{17}NO_3.C_6H_3N_3O_7$, M^+ 199. Mass spectroscopy indicated that the compound was a tropane diol ester with structure 2.XXVI.



2.XXVI

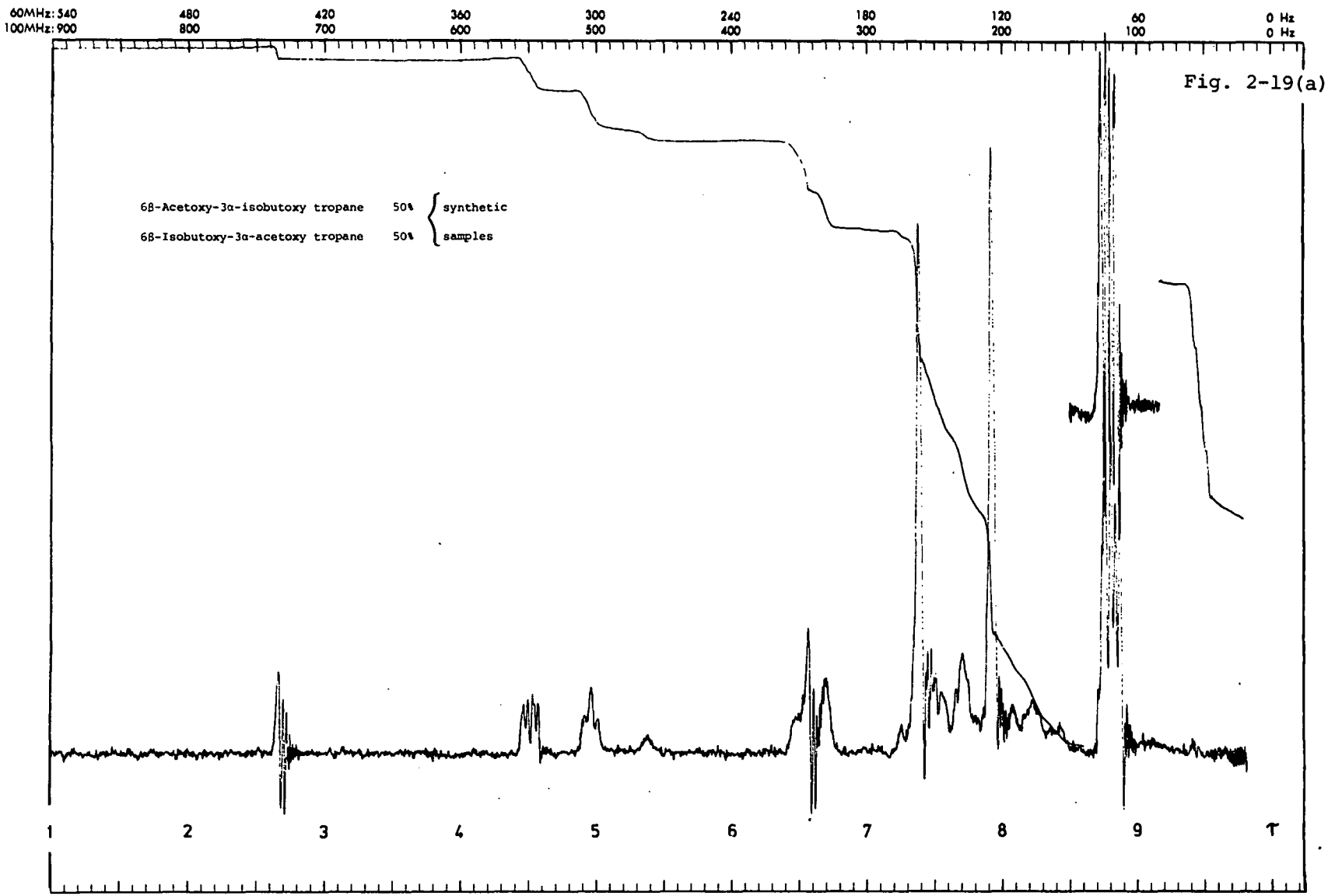

60MHz: 540
100MHz: 900
480
800
420
700
360
600
300
500
240
400
180
300
120
200
60
100
0 Hz
0 Hz
6β-Acetoxy-3α-isobutoxy tropane 50% synthetic
6β-Isobutoxy-3α-acetoxy tropane 50% samples
1
2
3
4
5
6
7
8
9
τ

Fig. 2-19(a)

The compound was synthesized from the Kuntz hydrolysis of 6β-acetoxy-3α-acetoxy tropane. (Section 2.11.2.). The melting point and mixed melting point of the two compounds were identical, likewise their infrared spectra (Figs. 2-20, 2-21). From these data, it was concluded that B6 was (±) 6β-hydroxy-3α-acetoxy tropane.

2.8. Structural Elucidation of B2

Band 2 from P.T.L.C. was found to be a mixture of two alkaloids, the major component of which crystallized as white needles m.p. 186-187° (ethanol) with a molecular formula of $C_{15}H_{21}NO_6$ as determined by high-resolution mass spectrometry.

This base is not a tropane alkaloid, and its structure is not currently known. The very small quantity of compound isolated (12 mg) has not permitted a complete study, although it has been examined spectroscopically and several structural possibilities are presented. From the infrared spectrum, (Fig. 2-23) at least one carbonyl was present as a γ-lactone, $\nu_{C=O}$ 1750 cm^{-1}, and two strong ether bands appeared at 1010 cm^{-1} and 1170 cm^{-1}. Support for the lactone assignment was provided by the ^{13}C N.M.R. spectrum which showed two carbonyl resonances at δ^{TMS} 178.1 and 178.8 p.p.m.[33].

2.8.1. N.M.R. Studies on B2

(i) P.M.R. Spectroscopy

The P.M.R. spectrum (Fig. 2-22) shows three groups of signals: six protons in a group centred at 5.75τ, consisting of two two-proton multiplets (triplets of doublets), and a two-proton singlet; three protons in an apparent triplet centred at 6.90τ; and twelve protons in an envelope between 7.20τ and 8.10τ. The triplets in the lowest field

Fig. 2-20

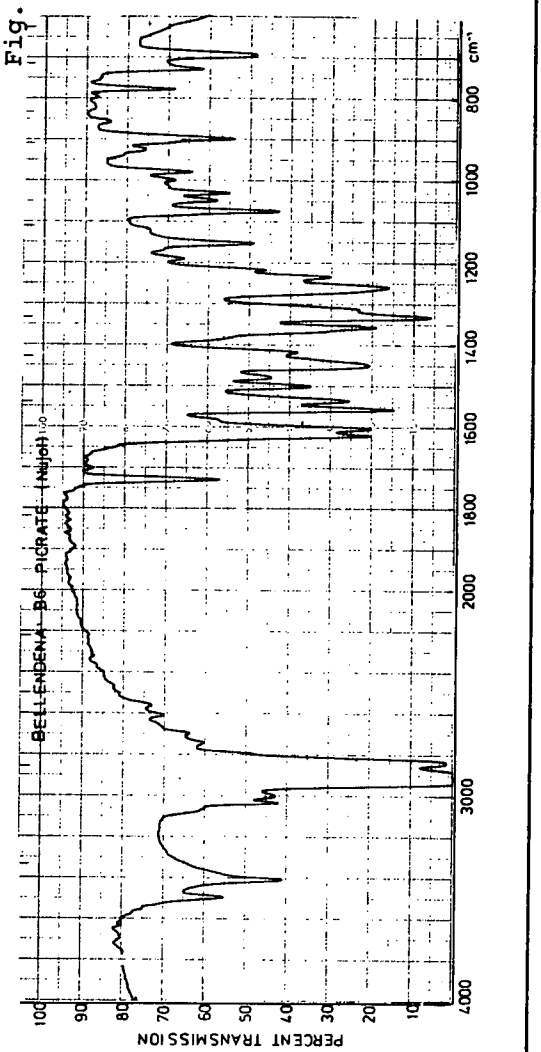
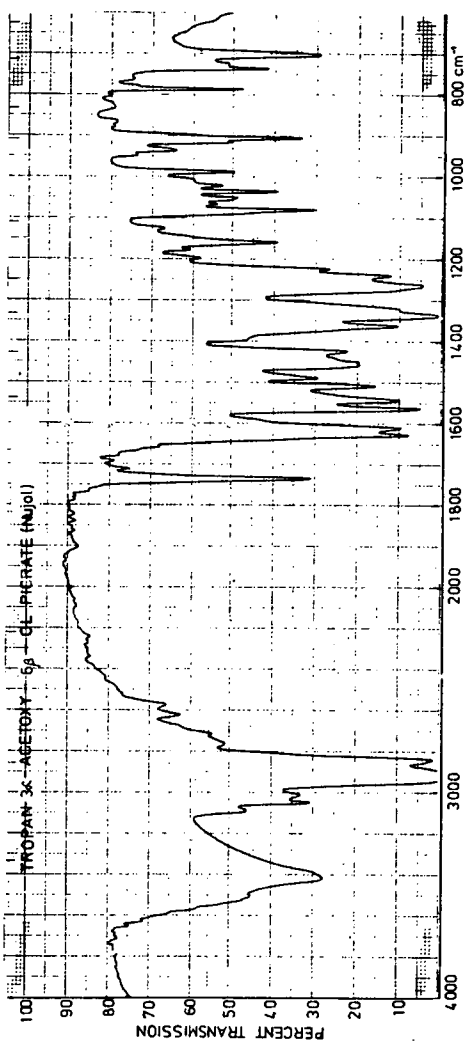


Fig. 2-21



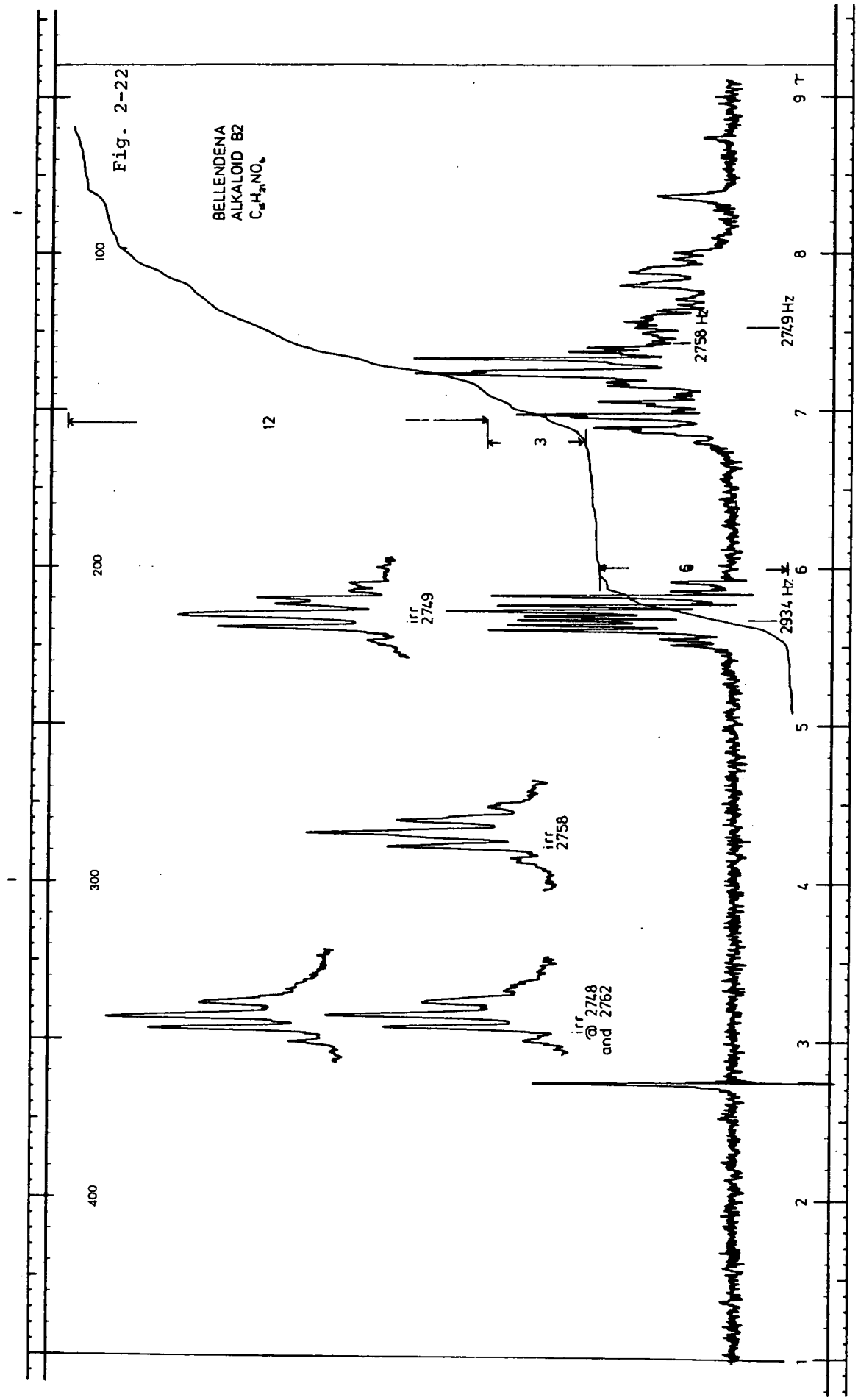


Fig. 2-22
BELLENDENA
ALKALOID B2
$C_{18}H_{21}NO_4$
irr
2749
irr
2758
irr
@ 2748
and 2762
2749 Hz
2758 Hz
2934 Hz
12
3
6
6
100
200
300
400
1
2
3
4
5
6
7
8
9 τ

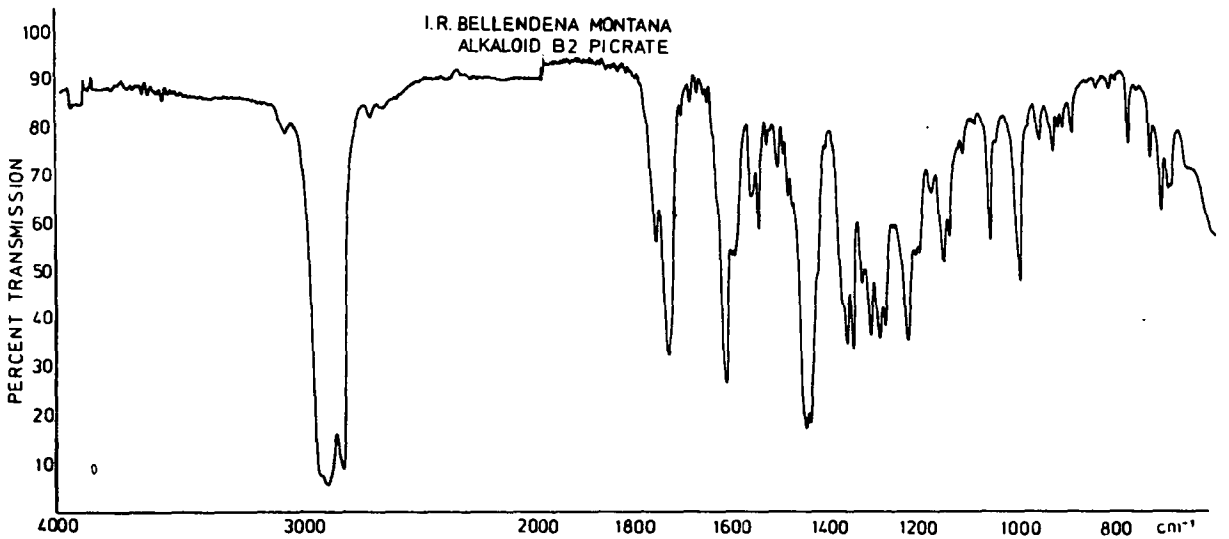


Fig. 2-23

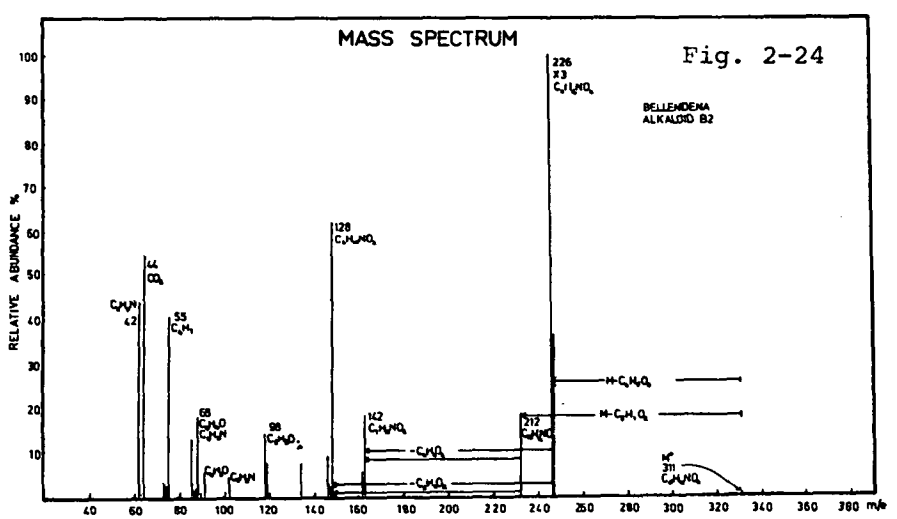


Fig. 2-24

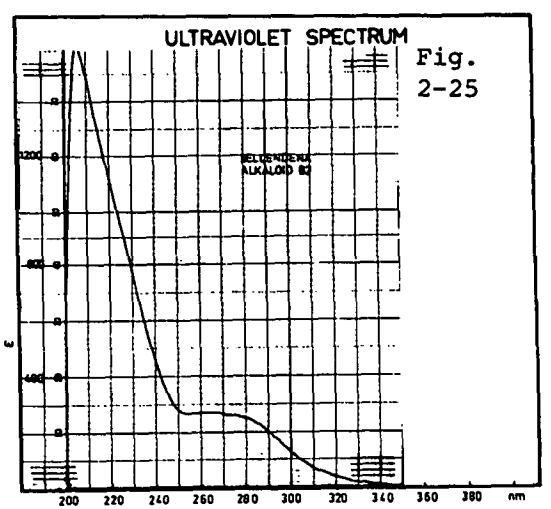


Fig. 2-25

group may result from a combination of geminal coupling and vicinal interaction with a methine proton to form part of an ABX system. Thus, for the resonance centred at 5.89τ, J_{gem} = 10.5 Hz, J_{vic} = 6.00 Hz, and $\Delta\tau$ = 10.50 Hz. For the resonance centred at 5.62τ, J_{gem} = 8.8 Hz, J_{vic} = 4.5 Hz and $\Delta\tau$ = 8.00 Hz. In order to discriminate couplings from signal overlap, the spectrum was recorded in a variety of solvent systems. In d6 acetone, the "triplet" centred at 6.90τ separated into three one-proton triplets, indicating that it was a composite resonance made up of three methine protons in nearly equivalent environments. The chemical shift and coupling of these resonances suggested that the environments were adjacent to nitrogen and methylene groups. The remaining twelve protons were distributed in a complex set of well-resolved multiplets.

(ii) C.M.R. Spectroscopy

The ^{13}C spectrum (Fig. 2-26) was recorded only in the noise-off-resonance decoupled mode (noise 2.5 KHz, power = 37 watts, scans = 18,500, rep. = 3.5 sec) due to the small quantity of compound available. From this spectrum only five distinct resonances could be detected. It is seen from the spectrum that two resonances show further splitting, and the line-widths of the remaining resonances are too broad to be accounted for in terms of single carbon resonances. In view of the discrimination normally achieved in the chemical shifts of carbon nuclei, such coincident resonances suggest that the molecule has considerable symmetry. The recorded ^{13}C chemical shifts of morpholine and γ-butyrolactone[33] have been used to assign some of the resonances in the ^{13}C spectrum of B2. (Fig. 2-26).

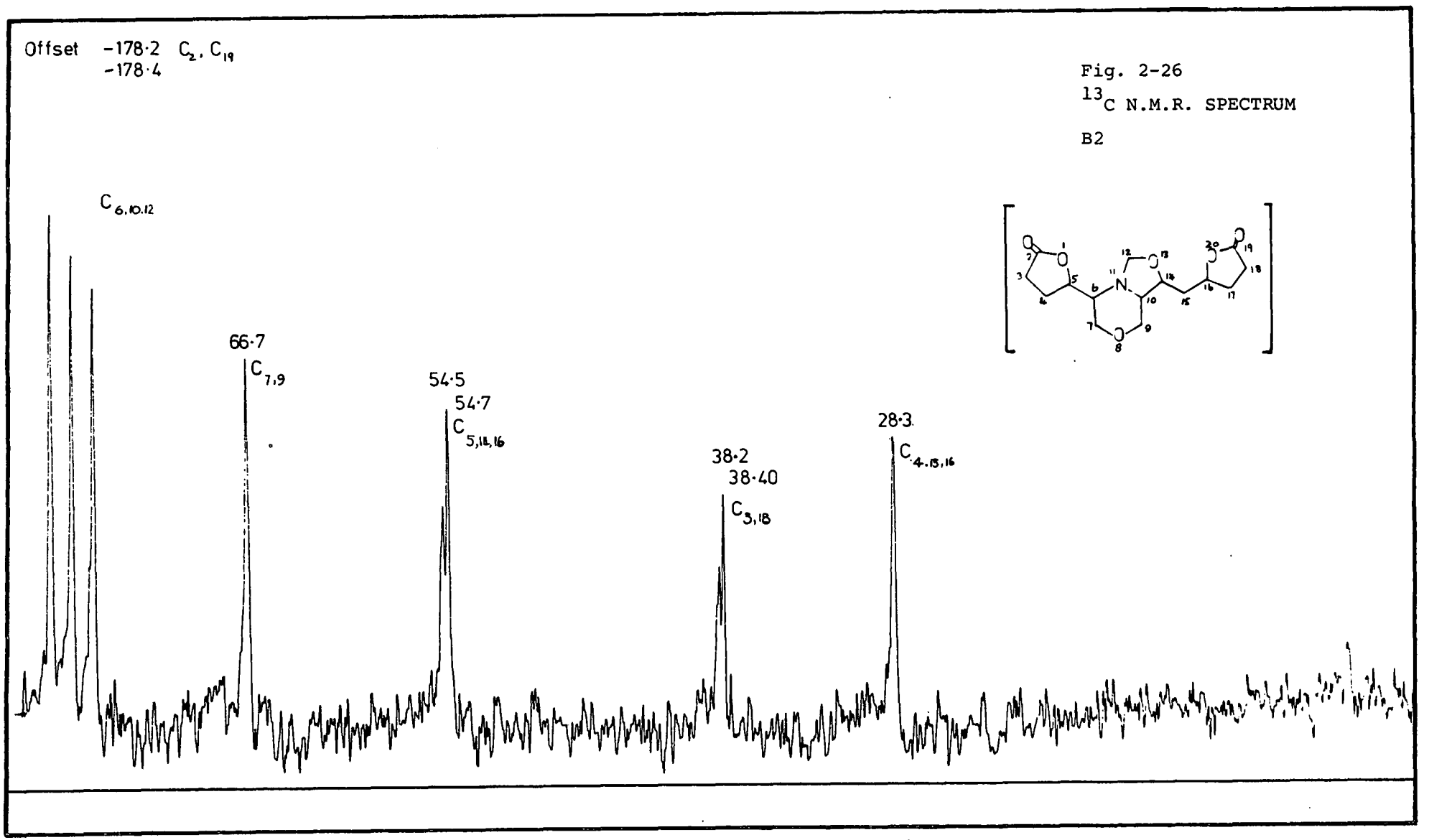
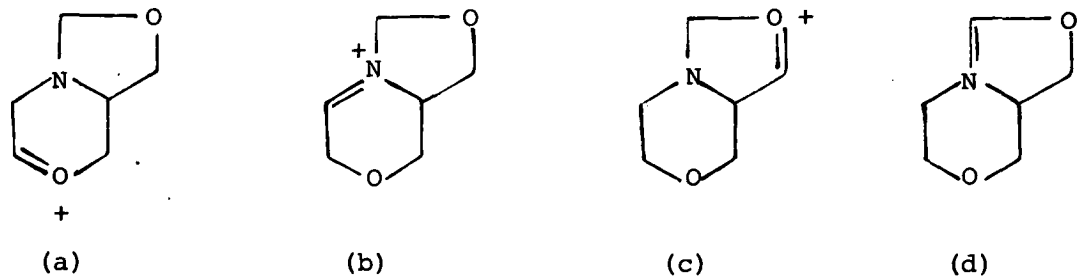


Fig. 2-26
^{13}C N.M.R. SPECTRUM
B2

2.8.2. Mass Spectral Fragmentation and Spectral Interpretation

The fragmentation pattern of this molecule is consistent with the consecutive loss of two lactone moieties from the exceedingly weak parent ion. The subsequent loss of the second lactone takes place with hydrogen transfer to produce the second most prominent peak in the spectrum, m/e 128, $(C_6H_{10}NO_2)^+$. It is noteworthy that the homologue of this peak, m/e 142, $(C_7H_{12}NO_2)^+$ is also present, indicating that a methylene group may intervene between the lactone and heterocyclic nucleus. The P.M.R. spectrum indicated that the nitrogen was tertiary and was bridging two rings, there being no exchangeable signal on addition of D_2O, and no terminal aliphatic groups. C.M.R. evidence indicated the presence of two lactone carbonyls and no other low field signal was observed. In view of the degree of unsaturation of the $C_6H_{10}NO_2$ fragment, and the high possibility of an immonium or oxonium ion to stabilize the positive charge, the oxygens of this fragment are therefore likely to be present as cyclic ethers. On the basis of these deductions, structures 2.XXVII (a), (b), (c), or (d) are considered likely for the fragment $C_6H_{10}NO_2$.

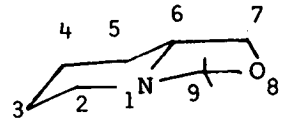


(a) (b) (c) (d)

2.XXVII

The proton distribution in the lowest field region of the P.M.R. spectrum is comprised of two triplets and a singlet, totalling six protons. The resonances of the C-2 and C-6 protons of morpholine[26] fall in the range

5.6τ-6.3τ, consistent with the observed pattern. Such a constitution may account for four of the six low field protons. The remaining two protons may be part of an oxazolidine N-CH_2-O system in which the two methylene protons resonate at between 5.65τ and 5.90τ[35]. The degree of coupling observed, J_{gem}, in trans fused 8-oxa-1-azabicyclo[4,3,0]nonane (2.XXVIII) for the H_9 protons was only 0.8 Hz. In the case of the cis fused system, J_{gem} = 5 Hz. However, in both of these cases there was a separation of the axial and equatorial resonances. The resonance assigned to the proton in question in B2 appears as a singlet.



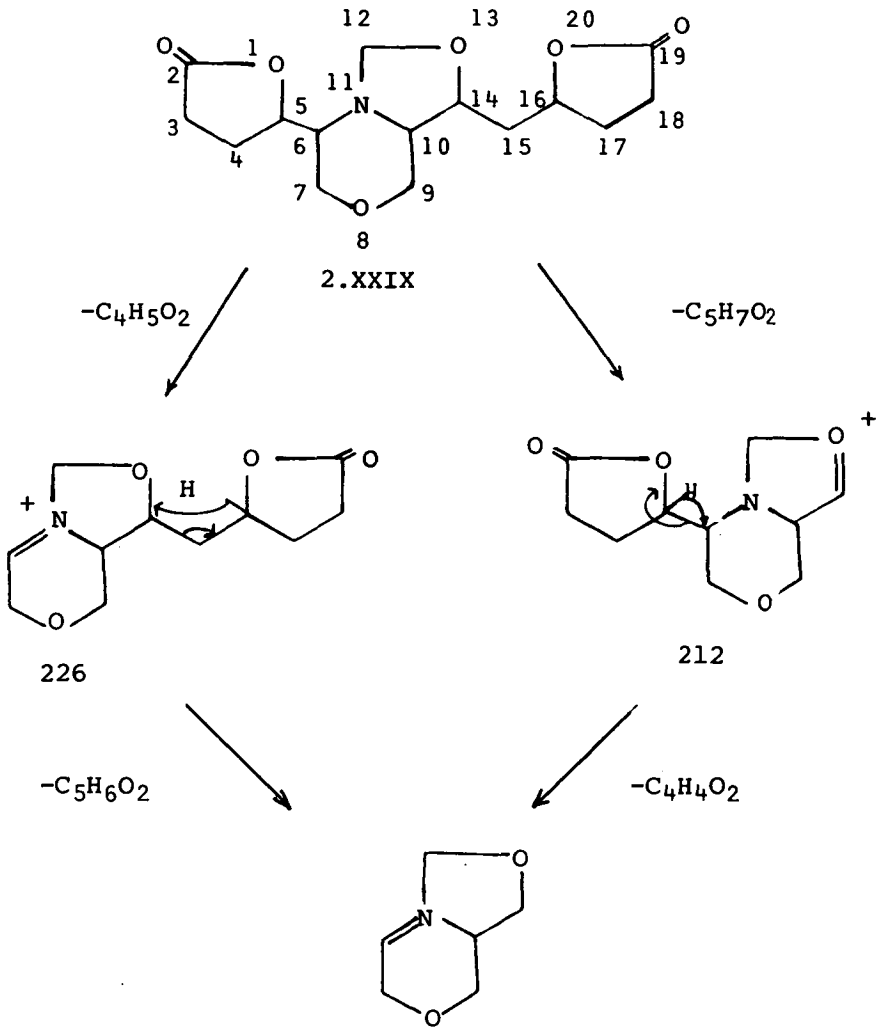
2.XXVIII

H_7 protons for 2.XXVIII absorb in the range 6.5-6.8τ. In B2 a triplet is centred at 6.92τ, which consists of three separate protons superimposed upon each other. One of these may be due to the H_{14} proton in 2.XXIX, whereas the other two protons in this multiplet may be the lactone protons H_5 and H_{16}. The mass spectral fragmentation of B2 is consistent with that of a molecule such as 2.XXIX (Scheme 2-4).

2.9. Isolation of the Agastachys odorata Bases

Agastachys odorata is a Tasmanian endemic, prevalent in all areas of the western and north-western highland regions where it grows to a height of $\tilde{\sim}$ 3 metres. Field testing for alkaloids gave a weak positive response which was subsequently supported by the very low yield of alkaloids recovered from the extraction (0.002%). This was achieved by an initial stripping of the waxy coating from the plant material with petroleum ether, followed by milling and exhaustive cold

Mass Spectral Fragmentation Pattern, B2



Scheme 2-4

extraction with Prollius solution. The extract was concentrated *in vacuo* and the alkaloidal component separated as described in Section 2.2.1.

2.9.1. Separation of the Agastachys bases

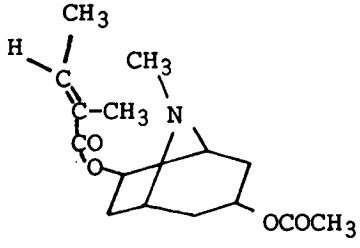
Analytical T.L.C. (12% MeOH in $CHCl_3$, silica gel) indicated three Schlittler-positive spots of R_f = 0.82, 0.26, 0.10, and one other non-alkaloidal phenolic component. The extract was then subjected to preparative thin-layer chromatography, and each of the alkaloidal bands separated and extracted. The lowest band, R_f 0.10, yielded only 6 mg of impure material which was not further investigated. The principal alkaloidal band, A1, R_f = 0.82, was isolated in 30 mg yield and the band at R_f = 0.26, A2, yielded 12 mg. The phenolic component P1 was isolated in 100 mg yield. Each was subjected to spectroscopic examination.

2.9.2. Structural Elucidation of A1

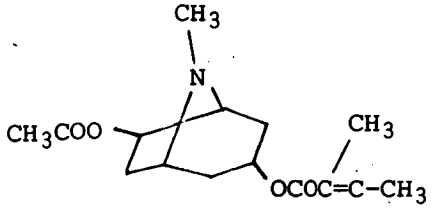
High-resolution mass spectroscopy indicated that the molecular formula of A1, (oil) $[\alpha]_D$ = -14° was $C_{15}H_{23}NO_4$. The principal peaks in the mass spectrum (Fig. 2-29) were highly resolved; those occurring at m/e 82 (C_5H_8N), m/e 94 (C_6H_8N), m/e 95 (C_6H_9N) were indicative of a tropane structure. The appearance of strong peaks at m/e 122, 138, and m/e 222 gave further support for this assignment, and indicated that the compound was a tropane diol ester. The infrared spectrum (Fig. 2-28) showed the presence of two ester carbonyls at 1735 cm^{-1} and 1710 cm^{-1}. The lower frequency band was assigned to an α,β unsaturated carbonyl for which the complementary olefinic absorption appeared with moderate intensity at 1645 cm^{-1}. Two other strong bands were present at ν_{C-O} 1285, 1245 cm^{-1}. The ultraviolet spectrum (Fig. 2-30) confirmed the

presence of an α,β unsaturated carbonyl chromophore, λ°_{max} = 217 and ε_{max} = 16,000. Values calculated from the Woodward-Fieser rules[19] for tiglic acid esters were in accordance with that observed.

The P.M.R. spectrum (Fig. 2-27) showed the presence of an N-methyl, 7.50τ, an acetoxy C-methyl, 7.92τ, and two other C-methyl resonances each split into a doublet, J = 0.8 Hz, characteristic of a tigloyl moiety for which the olefinic resonance appears as a multiplet at 3.12τ. The molecule was characterized as a tropan-6,3-diol ester by the appearance of a one-proton quartet (J = 7 Hz, J = 5 Hz) at 4.52τ, and an apparent triplet (J = 5 Hz) at 4.90τ. Those protons adjacent to nitrogen appear at 6.70τ and 6.80τ. From the spectroscopic data, it was concluded that A1 had structure 2.XXX or 2.XXXI.



2.XXX



2.XXXI

Compound 2.XXXI has recently been isolated by Evans from Datura sanguinea[36] and its picrate prepared. The picrate of A1 was prepared and the melting point, 180-182° compared with that recorded for the Datura base, 182-186°. In order to confirm the structure, racemic 6β-acetoxy-3α-tigloyloxy tropane was prepared from 6β-acetoxy-3α-tropanone by catalytic hydrogenation, and esterification of the resultant 6β-acetoxy tropan-3α-ol with tigloyl chloride was undertaken. The picrate of the racemate m.p. 214° (Lit. 213°) had an identical infrared

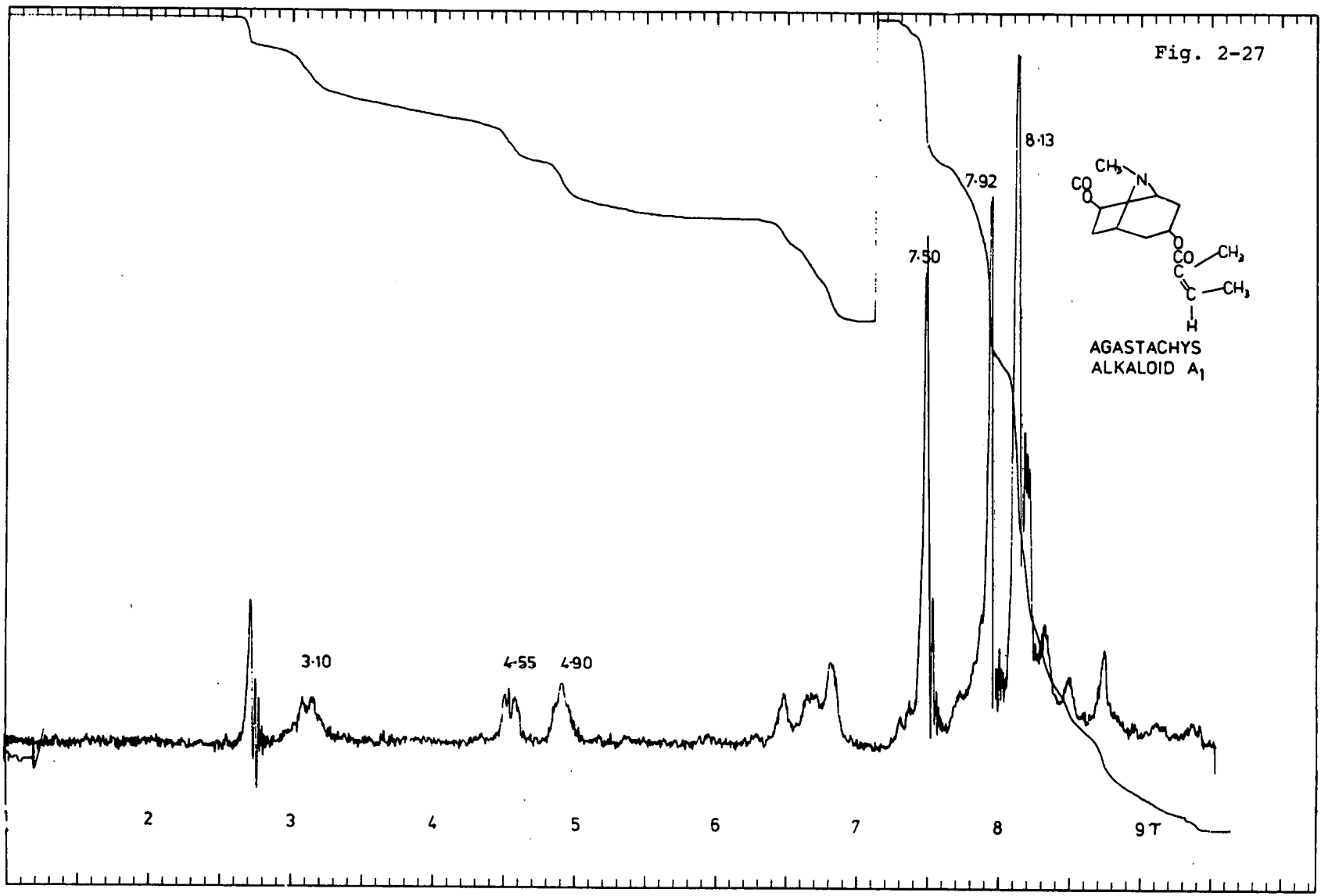


Fig. 2-27
8·13
7·92
7·50
3·10
4·55
4·90
2
3
4
5
6
7
8
9 T
AGASTACHYS
ALKALOID A1

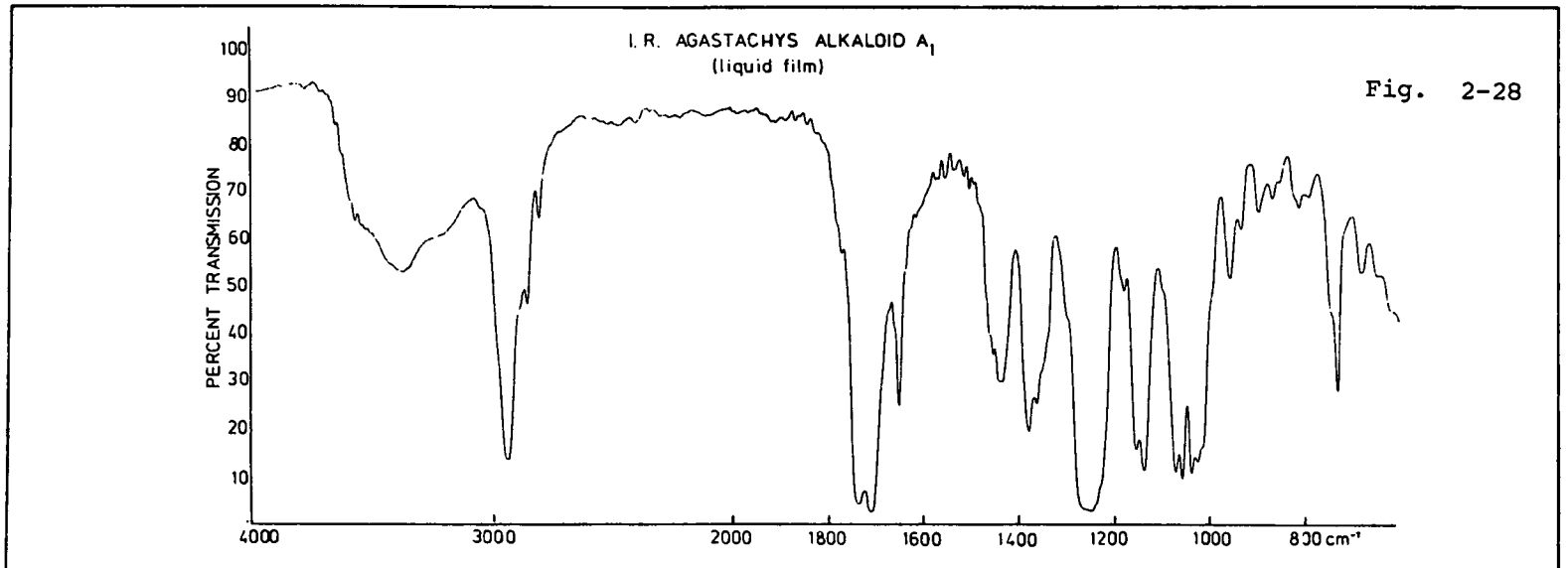


Fig. 2-28

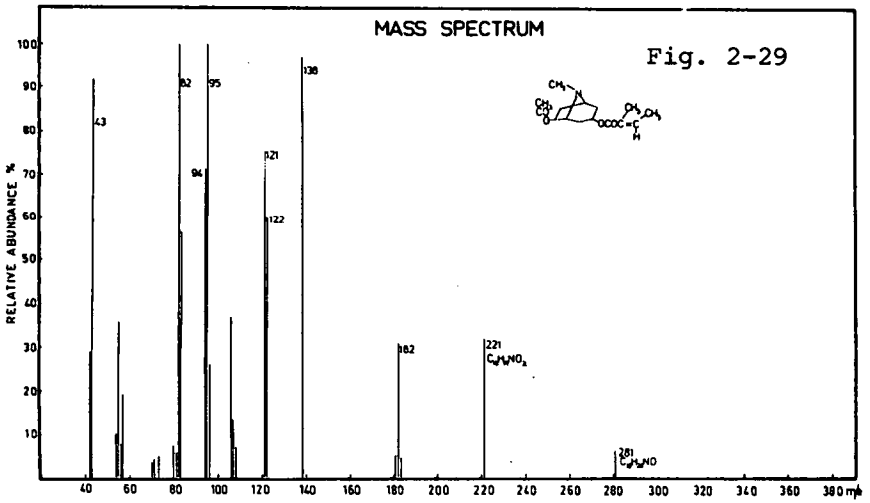


Fig. 2-29

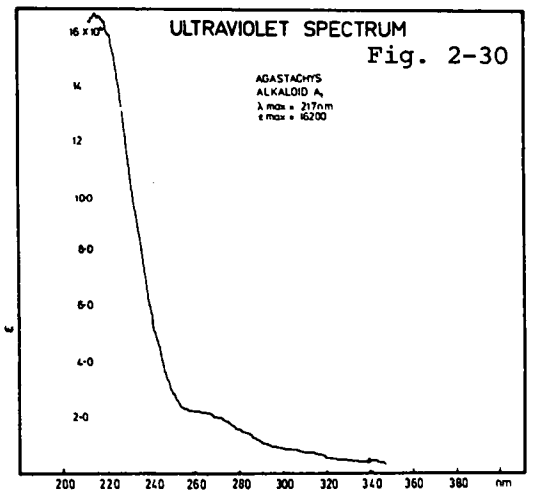


Fig. 2-30

spectrum to that of the natural base (Fig. 2-31). The mass spectral fragmentation pattern shown in Fig. 2-30 is consistent with the assigned structure.

2.10. Structural Elucidation of A2

The second base isolated from Agastachys odorata by P.T.L.C. in trace quantities (12 mg) as a brown oil, R_f = 0.26 (12% MeOH in $CHCl_3$, silica gel), was further purified by vacuum sublimation (90°, 1 × 10^{-5} mm Hg). The oil which distilled with no apparent decomposition, was found by high-resolution mass spectroscopy to have a molecular formula of $C_{15}H_{17}NO_3$, and to show characteristic tropane fragments at m/e 82 and m/e 95 (Fig. 2-33). The infrared spectrum (Fig. 2-32) showed two strong hydroxy bands at 2700 cm^{-1} and 3400 cm^{-1}, a carbonyl band at 1710 cm^{-1} and strong aromatic bands at 1610 cm^{-1} and 1580 cm^{-1}. The ultraviolet spectrum (Fig. 2-34) has characteristics which are consistent with a p-hydroxy benzoyl residue, which from the carbonyl absorption frequency in the infrared spectrum was assumed to be an ester. The small quantity of compound available did not enable a well-resolved P.M.R. spectrum to be determined. The p-hydroxy benzoyl chromophore was seen to be present by the appearance of an AA'BB' aromatic pattern at 2.10τ (2H, doublet, J = 8.0 Hz) and 3.16τ (2H, doublet, J = 8.0 Hz). An ill-resolved triplet at 4.59τ (1H, J = 5 Hz) was indicative of a tropan-3α-ol ester. An unusually high-field N-methyl signal at 7.84τ and an olefinic resonance at 3.78τ, (2H, doublet, J = 2 Hz) were consistent with values observed for N-methyl nortrop-6-en-3α-ol[37].

Based on these data, and the mass spectral fragmentation pattern (Scheme 2-5) in which the principal peaks were highly resolved,

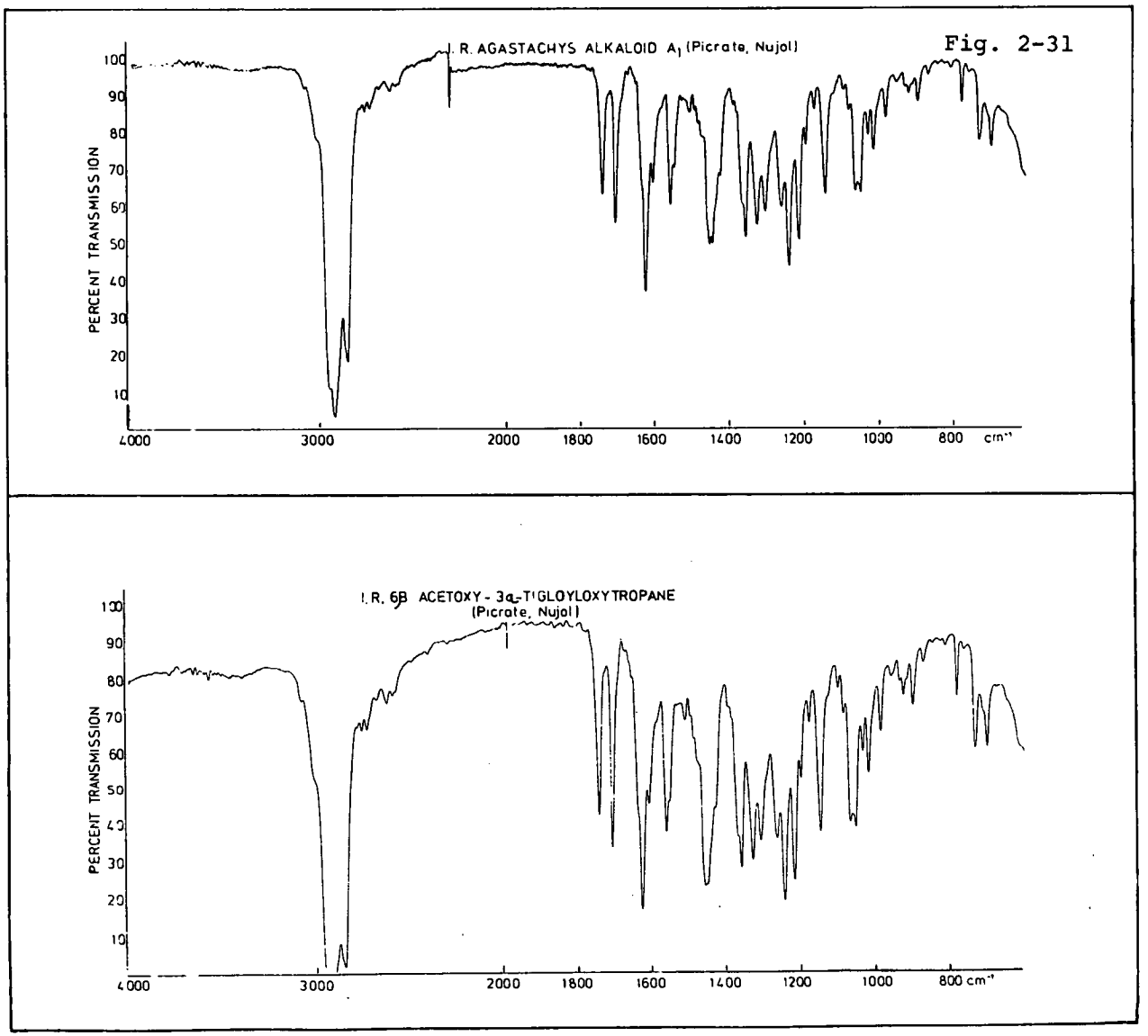

I. R. AGASTACHYS ALKALOID A_1 (Picrate, Nujol)
PERCENT TRANSMISSION
100
90
80
70
60
50
40
30
20
10
4000
3000
2000
1800
1600
1400
1200
1000
800
cm⁻¹
I. R. 6β ACETOXY - 3α - TIGLOYLOXYTROPANE
(Picrate, Nujol)
PERCENT TRANSMISSION
100
90
80
70
60
50
40
30
20
10
4000
3000
2000
1800
1600
1400
1200
1000
800 cm⁻¹

Fig. 2-31

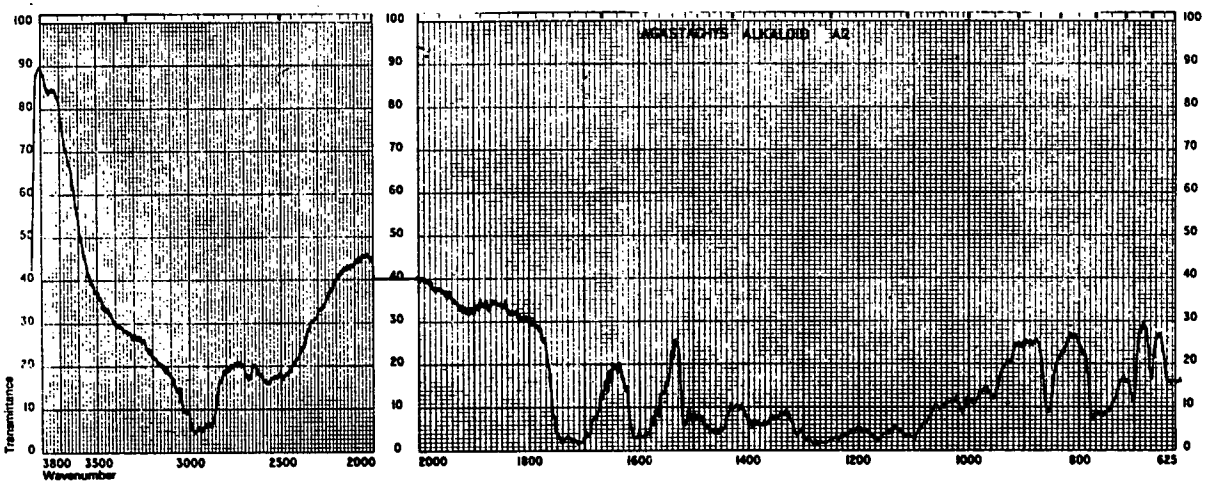


Fig. 2-32

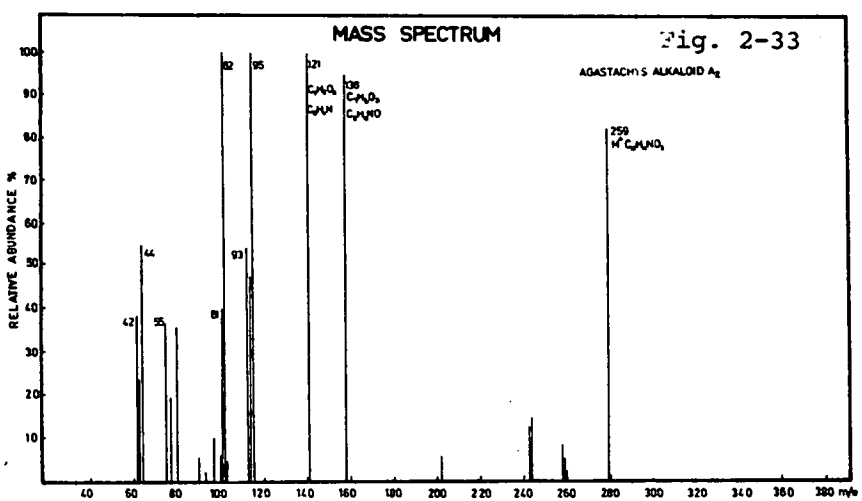


Fig. 2-33

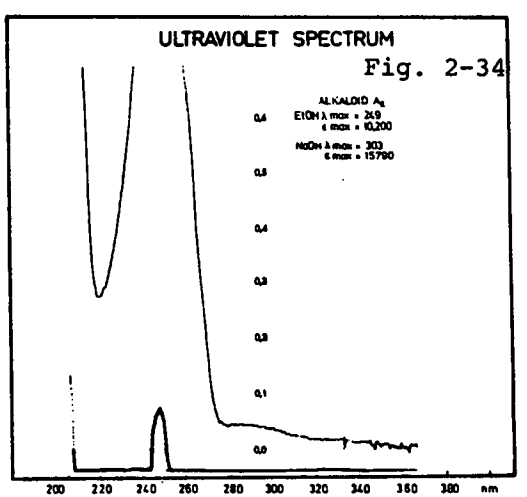
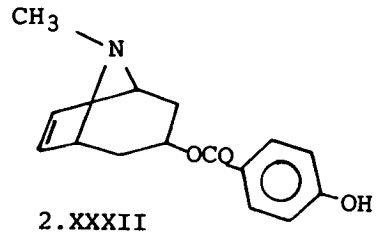


Fig. 2-34

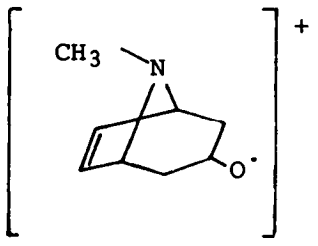
structure 2.XXXII is proposed for A2.



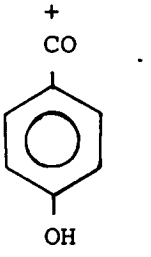
2.XXXII

a b

(c)

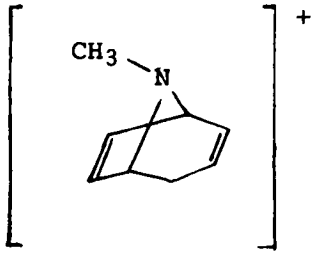


m/e 138, $C_8H_{12}NO$

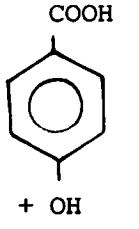


m/e 121 $C_7H_5O_2$

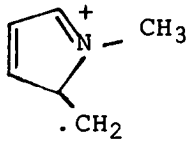
(b)



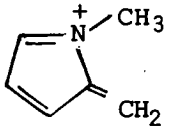
m/e 121 $C_8H_{11}N$



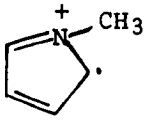
m/e 138 $C_7H_6O_3$



m/e 95



m/e 94



m/e 81

Scheme 2-5

The peaks at m/e 95 and m/e 94 are in accordance with this structural assignment, indicating the additional element of unsaturation over normal tropanes, for which these fragments occur at m/e 97 and m/e 96.

In view of the importance given to compounds bearing a C-6 - C-7 double bond in the biosynthetic routes to epoxidized tropanes[38], and the fact that such bases have never been isolated, the dried plant was re-extracted on a large scale (36 kg) in the hope of isolating this constituent in greater quantities and confirming its identity by hydrogenation and hydrolysis. The base has considerable water solubility, but despite exhaustive extraction of the aqueous phase, no trace of any alkaloid of this R_f could be found. The plant was subsequently collected during the flowering season and extracted rapidly, prior to drying, but no indication of the previously isolated A2 was observed.

The possibility that the compound is an artefact which was produced during unusual drying or extraction conditions on the first lot of material thus cannot be excluded. Evidence for the lack of decomposition during sublimation of this compound was a T.L.C. comparison of the original and sublimed materials; both had similar R_f values, but the poor resolution of the bases on silica gel did not completely exclude the possibility that decomposition had occurred.

Finally, in the absence of any chemical evidence, the structure proposed should be considered to be tentative.

MEMORANDUM

In the ensuing experimental sections, the following common procedures were adhered to. All evaporations were carried out in vacuo, analytical samples were dried over P_2O_5 at 5×10^{-4} mm Hg for 24 hrs. Melting points were determined on a hot stage device and are uncorrected. Optical Rotations were determined at 19°C in chloroform solutions. P.M.R. spectra were recorded on a JOEL JNM 100 100 MHz instrument, C.M.R. spectra were recorded on a Varian CFT-20 instrument in deuterochloroform. Infrared spectra were recorded on a Beckman IR-33 instrument in the media as represented on the spectra. Ultraviolet spectra were recorded on a Perkin-Elmer UV-124 instrument.

2.11. Experimental

2.11.1. Field Testing procedure (According to Fitzgerald[37(a)])

2.11.2. Extraction of the Flowering Heads of Bellendena montana

8 kilograms of dried upper leaves, flowers and flowering stems from plants collected at Guildford, western Tasmania, were ground to a fine mesh and extracted by cold percolation with Prollius solution until a test sample gave a negative Mayers reaction[(a)]. The extract was concentrated in vacuo, < 50°C, to yield 600 g of tar which was dissolved in 800 ml of glacial acetic acid. The solution was poured in a fine stream, with rapid agitation, into 4.5 ℓ of water. The non-alkaloidal material which precipitated was filtered off through celite and the aqueous filtrate was chilled and basified (pH 8.5) with 0.880 NH_4OH and extracted with chloroform (10 × 500 ml).

The chloroform solution was concentrated to 500 ml in vacuo and extracted with 10% H_2SO_4 (5 × 50 ml) until free from alkaloid (Mayers test). The acid solution was chilled, basified, and extracted exhaustively with chloroform (10 × 25 ml). The dried ($MgSO_4$) chloroform extract was evaporated in vacuo to yield 4.6 g crude alkaloids. Part of the extract (1.6 g) was subjected to preparative thin-layer chromatography on five 1 M plates coated with Camag DSF 5 silica gel. (10% MeOH in $CHCl_3$). Five bands were identified by edge spraying with Schlittler's reagent. The appropriate bands were combined and extracted with $CHCl_3$:MeOH (50:50) and five drops NH_4OH (0.880). Each extract was dried, ($MgSO_4$) evaporated and rechromatographed to give fractions as shown in Table 2-6.

(a) Handbook of Chemistry and Physics, Chemical Rubber Co. Cleveland, 28th Edition, page 1288.

TABLE 2-6

Chromatography Fractions, Flowers

Code	Chromatography Condition	R_f	Schlittler Colour	Mass
B1	$CHCl_3$ 4% MeOH	0.82	Red-brown	12 mg
B2	$CHCl_3$ 4% MeOH	0.62	Purple	127 mg
B3	$CHCl_3$ 4% MeOH	0.56	Red-purple	17 mg
B3'	$CHCl_3$ 8% MeOH	0.50	Bright blue	22 mg
B4	$CHCl_3$ 10% MeOH	0.55	Blue/grey → white	380 mg
B5	$CHCl_3$ 10% MeOH	0.45	Grey	120 mg
B6	$CHCl_3$ 12% MeOH	0.40	White-grey	80 mg

The balance of the alkaloid extract (3 g) was subjected to a Craig distribution between chloroform and 0.5×10^{-3} M H_2SO_4. From every tenth fraction a sample was withdrawn, basified, (NH_4OH), and extracted with $CHCl_3$. The concentrated extracts were then chromatographed (T.L.C.) against standards isolated from P.T.L.C. Similar fractions were bulked, the volume of the aqueous acid reduced in vacuo and the solution in each case basified (NH_4OH) and extracted with chloroform. Those fractions which were similar to the P.T.L.C. fractions or crystalline products were combined with them and further chromatographed or crystallized as appropriate. A summary of the combinations is shown in Table 2-3.

The B2 fraction was found to consist of two components of similar R_f (0.62, 0.60 4% MeOH in $CHCl_3$, silica gel) which were separated from each other by fractionally crystallizing the latter component from a hot ethanolic solution. B2, (17 mg), had m.p. 185-187° and formula $C_{12}H_{21}NO_6$ (H.R.M.S.).

The residue B2' was combined with that isolated from the Craig distribution.

B4 was found to be a mixture of two very similar components, one of which, bellendine, was crystallized from ether/petroleum ether, m.p. 162-163°, and the mother liquors combined with the appropriate Craig function and chromatographed. The semi-crystalline product recovered from P.T.L.C. was sublimed in vacuo (5×10^{-5} mm, 65-70°C), to deposit fine white needles of isobellendine, m.p. 115-116°C, $[\alpha]_D^{19} = +143°$.($CHCl_3$). $C_{12}H_{15}NO_2$ requires C, 70.24%; H, 7.32%; N, 6.83%. Found C, 70.31%; H, 7.28%; N, 6.71%.

All attempts to crystallize B5, or its picrate, hydrobromide or hydrochloride, were unsuccessful. In an attempt to sublime the compound, extensive decomposition occurred at $\tilde{\sim}$ 90°C (5×10^{-4} mm Hg). A small amount of B5 was retained for comparison with the subsequent Craig distribution fractions, from which sufficient was isolated for elucidation of the structure as dihydroisobellendine.

2.11.3. Extraction of the Roots and Stems of Bellendena montana

27 kg of roots and lower stems of Guildford plants were ground to a fine mesh and extracted by cold percolation with Prollius solution. The extract was concentrated in vacuo to 1.4 kg which was dissolved in warm glacial acetic acid and the non-alkaloidal material precipitated by pouring this solution into 8 ℓ of rapidly stirred water. The non-alkaloidal material was filtered through celite and the cooled aqueous filtrate was neutralized, filtered, and basified (NH_4OH) to pH 8.5. 1 kg of salt was added and the solution was extracted with chloroform until no further Mayers test was given (6 × 1 ℓ). The dried chloroform layer was concentrated in vacuo to 1 ℓ and the crude alkaloid solution was extracted into 5% H_2SO_4 (5 × 100 ml). The acid was then basified and extracted initially with benzene (5 × 50 ml), followed by diethyl ether (5 × 50 ml), and chloroform (10 × 50 ml). Each of the extracts

was examined by T.L.C. to monitor the fractionation achieved. The benzene solution which proved to contain most of the alkaloidal constituents of the original extract, was further fractionated by extraction into 5% H_2SO_4 (5 × 30 ml), basification (NH_4OH), and successive extraction with cyclohexane, petroleum ether (60-80°) and benzene. The effect of this was to separate and concentrate B2 in the benzene extract and a new alkaloid giving a bright blue Schlittler test in the cyclohexane extract. The concentrates from each of the solvent extractions were chromatographed on preparative thin-layer plates and a total of twenty-five alkaloid bands cut and extracted. These were examined by analytical T.L.C. against the bases isolated from the flowers and stems; by the use of Schlittler's reagent, R_f, and U.V. characteristics they were combined into nine groups of moderate purity. Each group was then further chromatographed on thin-layer plates to separate the principal component.

TABLE 2-7

Chromatography Fractions, Roots and Stems

12% MeOH in $CHCl_3$

Code	Fraction	R_f	Schlittler Colour	Composition
B1	1,2,4	1.0	Red-brown	Impure, not investigated
B2	3	0.89	Purple	B2
	5	0.87	Purple-red	B2, B2'
B3	6,7	0.78	Red-purple	6β-acetoxy-3α-isobutoxy-tropane 6β-isobutoxy-3α-acetoxy-tropane
B_{blue}	8,9,10	0.68	Bright-blue	B_{blue}
B4	13,14,18,20	0.66	Blue grey-white	Bellendine, Isobellendine
B5	15,19	0.46	Grey	Dihydroisobellendine
B6	17,22	0.26	White	6β-OH-3α-acetoxy tropane
B7	23,25	0.21	Mauve	Minor alkaloid, impure not investigated

Alkaloid B2

B2 was crystallized from hot ethanol (95%) in needles, m.p. 185-187°, $[\alpha]_D$ = +80°, R_f = 0.89 (12% MeOH in $CHCl_3$ silica gel). High-resolution mass spectroscopy established the molecular formula of $C_{15}H_{21}NO_6$. (Rotation error $\pm$ 20°).

Attempted Formation of Hydrobromide of B2

7 mg of B2 was dissolved in 1 ml dimethoxy ethane containing absolute ethanol (3 drops). 1 drop 48% HBr was added and the solution placed in a desiccator at room temperature. After 24 hr. no crystallization had occurred, but a brown gum had formed which was taken up in ethanol and left stand in a desiccator at 0°. No crystallization occurred; the solution was basified and extracted with $CHCl_3$. Analytical T.L.C. showed 5 alkaloidal spots indicating extensive decomposition.

Alkaloid B2'

From the mother liquors of B2, a component was isolated by P.T.L.C. (10% MeOH in $CHCl_3$, silica gel), R_f = 0.78, named B2'. The compound could not be crystallized. Spectral data suggest that the compound may be an acyl trop-2-ene derivative, bearing a γ-lactone group.

M.S. 249 (22%), 234 (13%), 220 (47%), 206 (28%), 180 (12%), 168 (44%), 164 (100%), 122 (51%), 100 (78), 94 (56%), 83 (74%), 81 (72%), 80 (70%), 55 (98%), 43 (100%).

P.M.R. 3.23τ (d of d, 1H, J = 2.5 Hz olefinic), 3.75, 4.23τ (1H, enolic). 600 (d, 1H, J = 7 Hz, CH-N-Me), 7.72τ (S, 3H, N-Me).

I.R. $\nu_{C=O}$ 1780 cm^{-1} γ-lactone; $\nu_{C=O}$ 1680 cm^{-1} α,β unsaturated carbonyl; $\nu_{C=C}$ 1610 cm^{-1}.

U.V. λ_{max} = 250 nm, ε_{max} = 8,000; λ_{max} = 218 nm, ε_{max} = 12,500.

Alkaloid B3

A clear viscous oil (17 mg) was extracted from band B3, R_f = 0.78 (12% MeOH in $CHCl_3$, silica gel), H.R.M.S. indicated a molecular formula of $C_{14}H_{23}NO_4$. A picrate derivative could not be crystallized. The composition of B3 was found from a P.M.R. study to be an equal mixture of 6β-acetoxy-3α-isobutoxy tropane and 6β-isobutoxy-3α-acetoxy tropane. In connection with this study a number of model compounds were prepared. N.M.R. and I.R. data are shown in Appendix 2.1.

Synthesis of (-) 6β-acetoxy-3α-isovaleroxy tropane (acetyl valeroidine)

25 mg (-) valeroidine oxalate (from a sample kindly supplied by Dr. W.C. Evans) were dissolved in water (5 ml), basified (NH_4OH) and extracted with chloroform (5 × 5 ml). The extract was dried (Na_2SO_4) reduced in volume in vacuo and 3 ml acetic anhydride added. The solution was refluxed for 1.5 hr, extracted with 20% K_2CO_3 solution, dried, and concentrated in vacuo. P.T.L.C. on silica gel (10% MeOH in $CHCl_3$) enabled the separation of 14 mg pure (-) acetyl valeroidine, R_f = 0.74. Picrate m.p. 269-271° (d), M^+ 283. Found: C, 49.37%; H, 5.55%. Calculated for $C_{15}H_{25}NO_4.C_6H_3N_3O_7$: C, 49.27%; H, 5.48%. (N.M.R. Fig. 2-35).

Synthesis of (±) 6β-hydroxy tropan-3-one

Prepared according to P. Nedenskov and N. Clauson-Kaas[39] and crystallized from dimethoxy ethane. m.p. = 121-122° (lit. 122°). Picrate m.p. 199° (d) (lit. 199°).

Synthesis of (±) 6β-isovaleroxy-3α-acetoxy tropane

150 mg 6β-OH-tropan-3-one were dissolved in 10 ml $CHCl_3$ and 1 g freshly distilled isovaleroyl chloride was added. The mixture was heated under gentle reflux for 0.5 hr. At the end of this time, the solvent was removed in vacuo and the brown oil chromatographed on P.T.L.C. The desired hydrochloride of 6β-isovaleroxy-tropan-3-one was separated, R_f = 0.68 (12½% MeOH in $CHCl_3$, silica gel). The hydrochloride was transferred in ethanol to a Parr bomb and hydrogenated over Adams catalyst (60 p.s.i.) for 3 hr. The reduced product, freed from catalyst by centrifugation, was concentrated in vacuo and chromatographed R_f = 0.31 (P.T.L.C. 12% MeOH in $CHCl_3$, silica gel). The required 6β-isovaleroxy-3α-hydroxy tropane was acylated with freshly distilled acetic anhydride by refluxing for 1 hr. Water was carefully added to the chilled solution, which was then basified (Na_2CO_3), and extracted with chloroform (6 × 10 ml). The dried chloroform extracts were combined and chromatographed; 60 mg of an oil, R_f = 0.72, (10% MeOH in $CHCl_3$, silica gel) were recovered which had spectral characteristics very similar to those of (-) acetyl valeroidine. Picrate m.p. 133-134°.
Found: C, 49.31%; H, 5.56%. $C_{15}H_{25}NO_4.C_6H_3N_3O_7$ requires: C, 49.27%; H, 5.48%.

Synthesis of (±) 6β-acetoxy-3α-isovaleroxy tropane

500 mg of 6β-OH-tropan-3-one in 5 ml acetic anhydride was left to stand for 10 hr, then refluxed for 0.5 hr. The solution was cautiously neutralized with (aq.) $NaHCO_3$, basified, and extracted with chloroform (3 × 10 ml). T.L.C. indicated a substantially pure compound, R_f = 0.78 (10% MeOH in $CHCl_3$, silica gel) and whose spectral characteristics were consistent with 6β-acetoxy tropan-3-one (N.M.R. Fig. 2-36) which formed a

picrate m.p. 178-179° M^+ = 197. The oil from this acylation (0.52 g) was dissolved in 6 ml isopropyl alcohol and 100 mg $NaBH_4$ was added. The mixture was left to stand for 24 hr, then water was added (8 ml) and the solution was exhaustively extracted with chloroform (6 × 15 ml). The combined chloroform extracts were dried ($MgSO_4$) and concentrated in vacuo to give 285 mg of an oil. P.T.L.C. enabled the separation of the major band, 125 mg, R_f = 0.30 (14% MeOH in $CHCl_3$, silica gel), whose spectral characteristics were consistent with 6β-acetoxy-3β-hydroxy tropane (N.M.R. Fig. 2-37). 65 mg of the 3β-alcohol was dissolved in chloroform (4 ml) and 100 mg isovaleroyl chloride added. The mixture was left stand overnight, refluxed for 1.5 hr, then extracted with saturated $NaHCO_3$ solution. The residue was dried, concentrated and chromatographed on T.L.C. (12% MeOH in $CHCl_3$, silica gel). The major band, R_f = 0.68, was found to contain the desired 6β-acetoxy-3β-isovaleroxy tropane. The base formed a picrate from dry ethanol; m.p. = 155°C. Found: C, 49.38%; H, 5.66%. Calculated for $C_{15}H_{25}NO_4.C_6H_3NO_7$: C, 49.27%; H, 5.48%.

Synthesis of (±) 6β-acetoxy-3α-isobutoxy tropane

6β-Acetoxytropan-3-one was prepared as above. 700 mg was hydrogenated in ethanol over freshly prepared Adams catalyst (0.01 g) at 60 p.s.i. for 4 hr after which no further H_2 uptake occurred. The catalyst was removed by centrifugation and the solvent removed in vacuo. Portion of the light oil remaining formed a picrate, m.p. 182-184°, of 6β-acetoxy-3α-hydroxy tropane, (lit. 182-184°)[36]. 200 mg of the reduction product was dissolved in dry chloroform and 1 g isobutyroyl chloride was added. The solution was refluxed for 2 hr, washed with 20% K_2CO_3 solution, dried, and evaporated in vacuo to yield 200 mg of the hydrochloride of (±) 6β-acetoxy-3α-isobutoxy tropane, M^+ 269. (N.M.R. Fig. 2-38). A picrate crystallized from absolute ethanol had m.p. = 144-146°, M^+ 269.

Found: C, 48.00%; H, 5.14%; N, 10.49%. Calculated for $C_{14}H_{23}NO_4.C_6H_3N_3O_7.\frac{1}{2}C_2H_5OH$: C, 48.00%, H, 5.19%, N, 11.00%. (I.R. Fig. 2-39).

Synthesis of (±) 6β-acetoxy-3β-isobutoxy tropane

6β-Acetoxy-3β-hydroxy tropane was prepared as above (60 mg) and acylated with isobutroyl chloride (200 mg) in chloroform by refluxing for 1.5 hr. The solution was washed with saturated $NaHCO_3$ solution, dried (Na_2SO_4), concentrated in vacuo and the residue chromatographed on P.T.L.C. (12% MeOH in $CHCl_3$, silica gel). An extract of the principal band (R_f = 0.92) had spectral characteristics in accord with the desired product (N.M.R. Fig. 2-41). The picrate crystallized from ethanol had m.p. = 149-150°, M^+ 269. Found: C, 48.12%; H, 5.17%. Calculated for $C_{14}H_{23}NO_4.C_6H_3N_3O_7$: C, 48.10%; H, 5.19%. (I.R. Fig. 2-43).

Synthesis of (±) 6β-isobutoxy-3α-acetoxy tropane

6β-Isobutoxy tropan-3-one was prepared by acylation of 400 mg 6β-hydroxy tropan-3-one with 1 g isobutyric anhydride in the same manner as 6β-acetoxy tropan-3-one. A picrate crystallized from aqueous ethanol had m.p. 157-158°, M^+ 225. The acylated base (410 mg) was hydrogenated over Adams catalyst at 60 p.s.i. for 4 hr to yield 400 mg of 6β-isobutoxy-3α-hydroxy tropane. The reduced product was acylated by refluxing with 1 g acetyl chloride in chloroform for 1.5 hr, and the cooled solution was extracted with 5% H_2SO_4 (5 × 10 ml). The combined acid extracts were basified and extracted with chloroform (5 × 10 ml). The combined chloroform extracts were dried, and evaporated in vacuo to yield 500 mg of an oil which was chromatographed on P.T.L.C. (8% MeOH in $CHCl_3$, silica gel). The major band (R_f = 0.50) was extracted to yield 370 mg of the desired

(±) 6β-isobutoxy-3α-acetoxy tropane (N.M.R. Fig. 2-42). The base formed a picrate m.p. 113°; M^+ 269. Found: C, 47.86%; H, 5.13%, Calculated for $C_{13}H_{23}NO_4.C_6H_3N_3O_7$: C, 48.10%; H, 5.18%, (I.R. Fig. 2-40).

Kuntz Hydrolysis of B3 (mixture)

A solution of 12 mg of B3 in 0.5 ml acetone and 1 ml 0.1 M NaOH was maintained at 40° for 1 hr, then neutralized with 2 M HCl and evaporated in vacuo to dryness. 1 ml H_2O was added and the solution was basified with K_2CO_3 and extracted with chloroform (5 × 5 ml). T.L.C. indicated only slight hydrolysis had taken place. The procedure was repeated at 55° for 2 hr, and the reaction products, worked up as before, yielded two products on P.T.L.C.: 2 mg of an oil R_f = 0.12, (10% MeOH in $CHCl_3$, silica gel) M^+ 157, fragmentation indicative of tropan-3,6-diol, and 4 mg of an oil R_f = 0.46 (10% MeOH in $CHCl_3$, silica gel) M^+ 227, whose fragmentation pattern and P.M.R. were consistent with 6β-hydroxy-3α-isobutoxy tropane.

Alkaloid B_{blue}

The band at R_f = 0.68 (12% MeOH in $CHCl_3$, silica gel) was extracted to yield 24 mg of a pungent-smelling oil which was characterized spectroscopically. M^+ 239 (2%), 238 (18%), 224 (32%), 178 (45%), 176 (33%), 154 (22%), 136 (36%), 96 (84%), 85 (98%), 83 (90%), 82 (80%), 81 (90%), 71 (100%), 57 (100%), 43 (100%).

P.M.R. 5.10τ (1H, m), 6.18τ (1H, d, J = 10 Hz), 6.75τ (1H, m), 7.48τ (3H, s, N-Me), 8.78τ (6H, d of d, J = 6 Hz), 8.85τ (3H, s).

I.R. $\nu_{C=O}$ 1730 cm^{-1}; $\nu_{C=O}$ 1660 cm^{-1}, $\nu_{C=C}$ 1615 cm^{-1} pyrone.

U.V. λ_{max} = 260 nm, ε_{max} = 10,300, λ_{max} = 219 nm, ε_{max} = 10,700.

Alkaloid B4 (Bellendine and Isobellendine)

The two components of B4, bellendine and isobellendine, were not easily separable by P.T.L.C. Success was achieved by High-Pressure Liquid Chromatography (H.P.L.C.) using a Chromatronix pump and column system at 180 p.s.i. and a Pye hot-wire F.I.D. detector. The column (glass, 60 cm, I.D. 1 cm) was packed with Merck silica gel for H.P.L.C. (Art. 7729) and a gradient elution program set to increase methanol concentration in chloroform from 0% to 10%, linearly, over 1 hr. The two components emerged at 32 min and 38 min as broad bands, partially separated. Fractions were cut over this range enabling the separation of 65 mg isobellendine and 40 mg bellendine. Bellendine was purified by crystallization from ether/petroleum ether, m.p. 162-163°, $[\alpha]_D$ = +168°, pK_b = 6.7, $C_{12}H_{15}NO_2$ (H.R.M.S. and analysis, $C_{12}H_{15}NO_2$ requires C, 70.24%; H, 7.32%; N, 6.83%. Found C, 69.98%; H, 7.63%; N, 6.77%).

Alkaloid B5 (Dihydroisobellendine)

B5 was chromatographed on P.T.L.C. (12% MeOH in $CHCl_3$, silica gel). The principal band of R_f = 0.46 was extracted to yield 70 mg of a clear oil, $[\alpha]_D$ = -53°. The oil was triturated with ether, petroleum ether, ethanol and chloroform, but did not crystallize. 15 mg were placed in a sublimation device, the pressure reduced to 2×10^{-5} mm Hg, and the temperature increased to 90°. No sublimation took place. T.L.C. of the residue indicated considerable decomposition. A picrate derivative was attempted from 7 mg of dihydroisobellendine in 1 ml ethanol to which was added 1 ml of a saturated ethanolic solution of picric acid. No

crystallization took place.

High-resolution mass spectroscopy on the oil gave a molecular formula of $C_{12}H_{17}NO_2$.

Alkaloid B6 (6β-hydroxy-3α-acetoxy tropane)

Band 6 was chromatographed on P.T.L.C. (12% MeOH in $CHCl_3$, silica gel). The oil, (R_f = 0.26), 17 mg, was converted to a crystalline picrate, m.p. 182-184°, M^+ 199. Found: C, 44.80%; H, 4.48%. Calculated for $C_{10}H_{17}NO_3.C_6H_3N_3O_7$: C, 44.85%; H, 4.68%.

6β-Hydroxy-3α-acetoxy tropane

6β-Isobutoxy-3α-acetoxy tropane (25 mg) was dissolved in 1 ml acetone and 1.5 ml 0.1 M NaOH. The solution was heated to 45° for 1 hr. Water was added (3 ml) and the solution extracted with chloroform (3 × 5 ml). The chloroform was dried and removed in vacuo and the oil which remained (16 mg) was dissolved in ethanol (1 ml) and a saturated ethanolic solution of picric acid added (1 ml). The picrate which separated as yellow rosettes had m.p. 182-183°, mixed m.p. with B6, 182-183°.

2.11.4. Extraction of the Leaves, Flowers and Stems of Agastachys odorata

18 kg of ground, dried leaves and stems of plants collected near Scotts Peak, S.W. Tasmania were continuously extracted at room temperature with Prollius solution. The extract was concentrated in vacuo to a very dark viscous mass (3 kg) which was dissolved with difficulty in 3.2 ℓ glacial acetic acid. The solution was poured into 25 ℓ of water with vigorous agitation. Non-alkaloidal material, which precipitated and polymerized to a viscous black tar, was removed by filtration through a thick bed of celite. Neutralization with NH_4OH produced a further precipitate which was similarly removed. The solution was just basified and extracted with chloroform (7 × 5 ℓ) until no further positive test was given by the extract. The chloroform solution was dried (Na_2SO_4), concentrated in vacuo to 2 ℓ, washed with saturated brine, then extracted with 10% H_2SO_4 (5 × 100 ml). The acid extract was basified and continuously extracted with chloroform.

The dried chloroform extract yielded 3.5 g of crude material which was chromatographed on seven 1 m P.T.L.C. Plates (10% MeOH in $CHCl_3$, silica gel). The plates were run twice in this solvent and the bands A1 and A2 which gave positive Schlittler tests were combined according to R_f and colour. The major band isolated from thin-layer chromatography was non-alkaloidal, P1 (1.2 g).

Alkaloid A1 (-) 6β-acetoxy-3α-tigloyloxy tropane)

Band A1 was rechromatographed on preparative thin-layer plates (12% MeOH in $CHCl_3$, silica gel). The band giving a purple-red colouration with Schlittler's reagent, R_f = 0.82, was extracted to yield 23 mg of a light coloured oil $[\alpha]_D$ = -14°. $C_{15}H_{23}NO_4$ (H.R.M.S.).

Picrate of A1 (6β-acetoxy-3α-tigloyloxy tropane)

Band A1 (10 mg) was dissolved in 10 ml ethanol (95%) and a saturated ethanolic solution of picric acid (3 ml) was added. The solution slowly deposited rosettes of yellow needles, m.p. 180-182° (lit. 182-186°).

Preparation of (±) 6β-acetoxy-3α-tigloyloxy tropane

6β-Acetoxy-3α-hydroxy tropane was prepared as previously described from 6β-acetoxy tropan-3-one. The hydrochloride of the reduced product (200 mg) was dissolved in 10 ml $CHCl_3$ and 1 g freshly prepared tigloyl chloride was added. The solution was refluxed for two hours, washed with 20% K_2CO_3 solution and then with water, dried (Na_2SO_4), and concentrated in vacuo. The product was chromatographed on P.T.L.C. (12% MeOH in $CHCl_3$, silica gel) and 120 mg of the desired product, 6β-acetoxy-3α-tigloyloxy tropane, was extracted, A picrate was prepared from 15 mg of the above and a saturated ethanolic picric acid solution, m.p. 214°, (lit. 213°)[36]. Found: C, 48.66%; H, 5.12%. Calculated for $C_{15}H_{23}NO_4.C_6H_3N_3O_7$: C, 48.48%; H, 5.00%.

Alkaloid A2

Band A2 was rechromatographed on P.T.L.C. (12% MeOH in $CHCl_3$, silica gel). The band at R_f = 0.26 with a purple-red Schlittler test was extracted to yield 12 mg of an oil which was sublimed at 90° (1×10^{-5} mm Hg). $C_{15}H_{17}NO_3$ (H.R.M.S.).

Appendix - 2.1

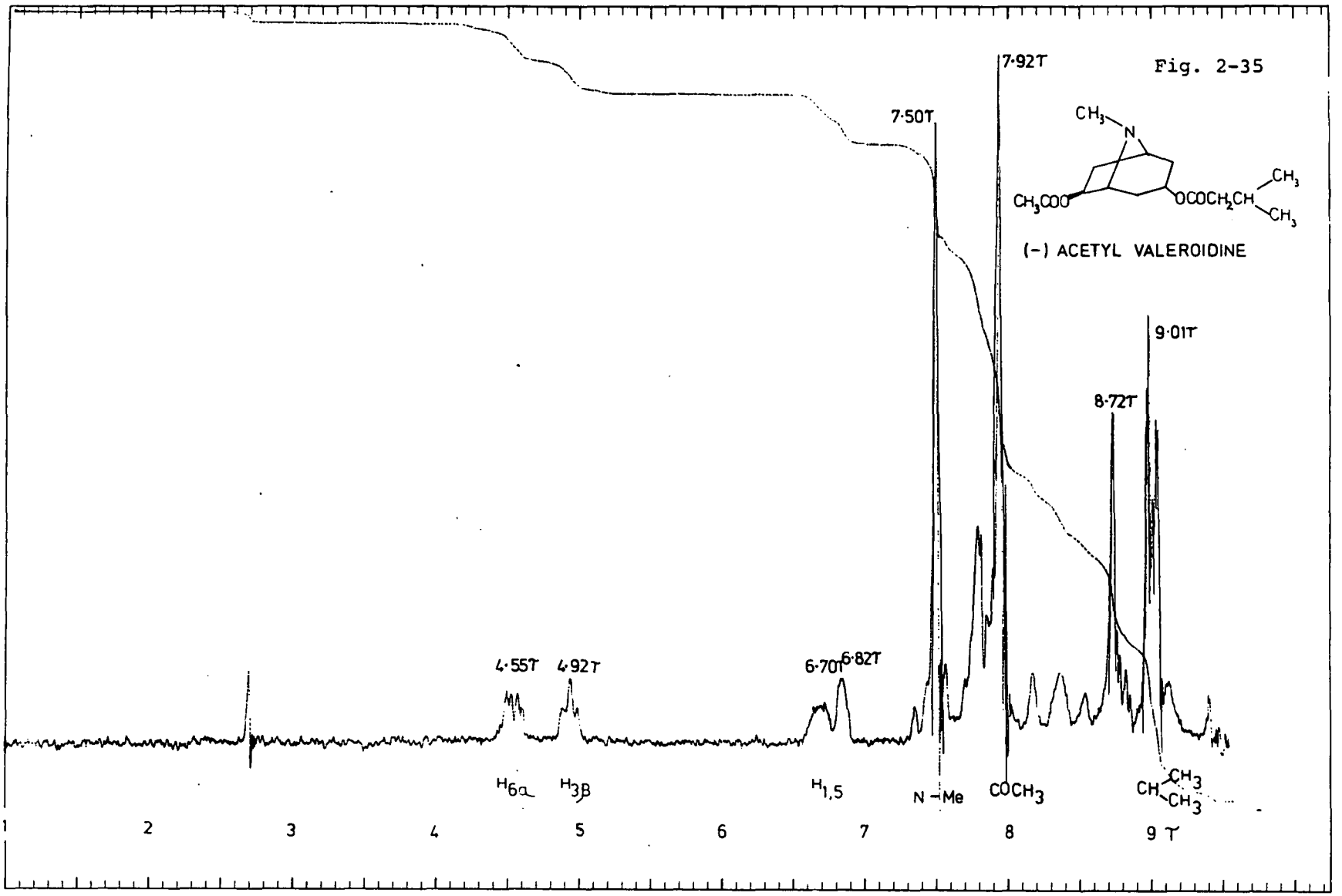


Fig. 2-35
7·92τ
7·50τ
9·01τ
8·72τ
4·55τ
4·92τ
6·70τ
6·82τ
H_{6a}
$H_{3\beta}$
$H_{1,5}$
N – Me
$COCH_3$
$CH(CH_3)_2$
1
2
3
4
5
6
7
8
9 τ
CH_3
N
CH_3COO
$OCOCH_2CH(CH_3)_2$
(–) ACETYL VALEROIDINE

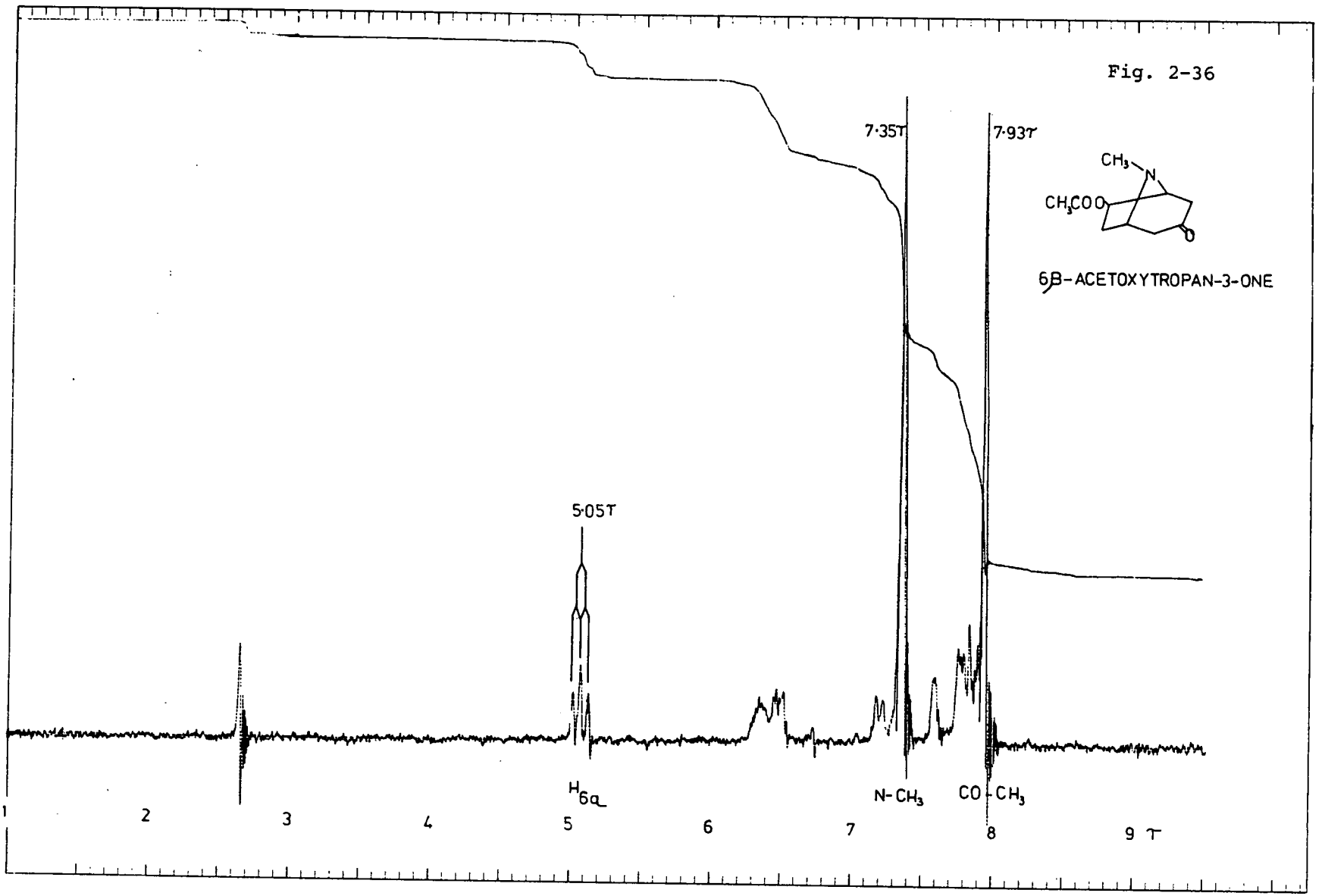


Fig. 2-36
7·35τ
7·93τ
CH3
N
CH3COO
O
6β-ACETOXYTROPAN-3-ONE
5·05τ
H6α
N-CH3
CO-CH3
1
2
3
4
5
6
7
8
9 τ

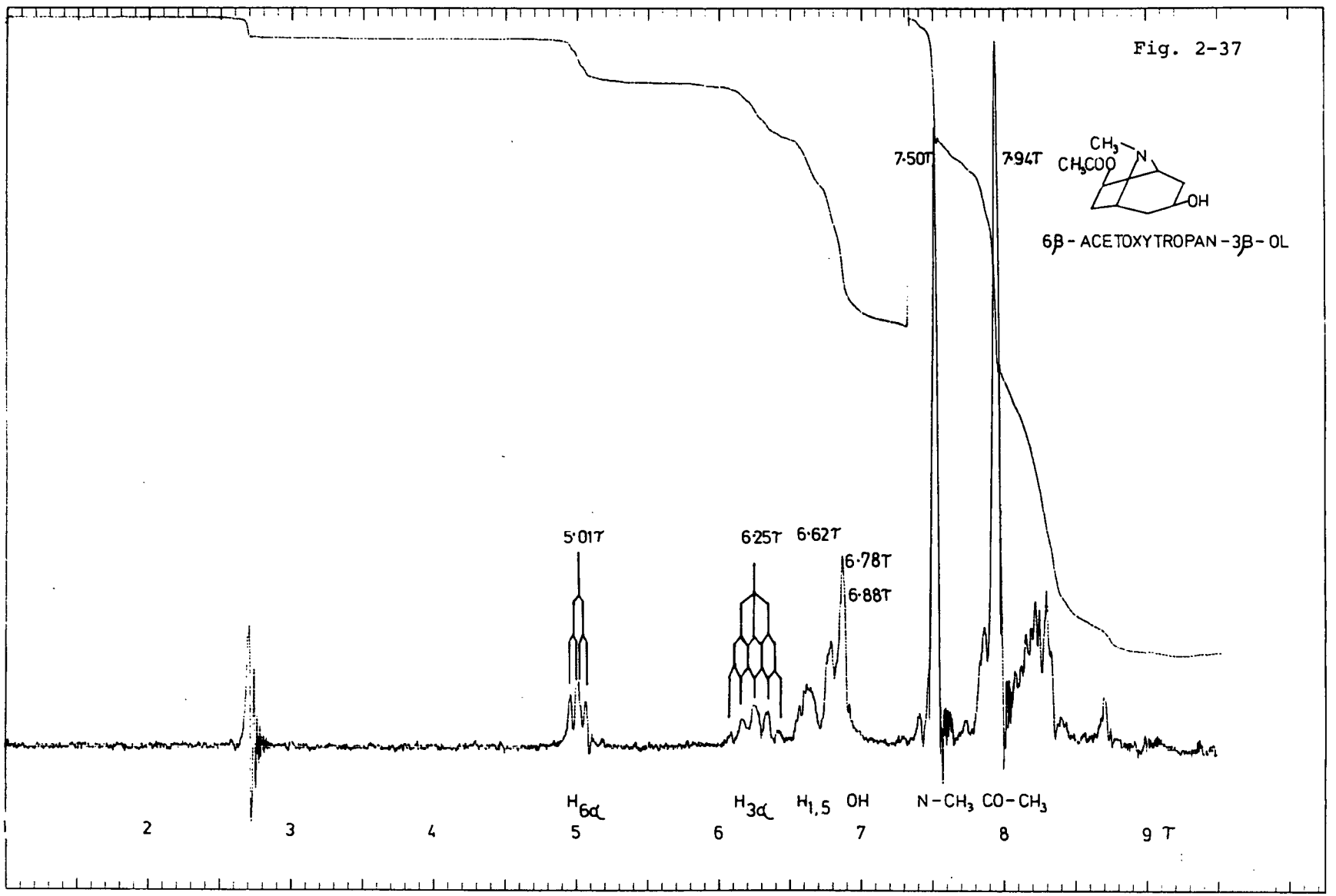


Fig. 2-37
7·50τ
7·94τ
CH3
CH3COO
N
OH
6β - ACETOXYTROPAN - 3β - OL
5·01τ
6·25τ
6·62τ
6·78τ
6·88τ
H6α
H3α
H1,5
OH
N-CH3
CO-CH3
2
3
4
5
6
7
8
9 τ

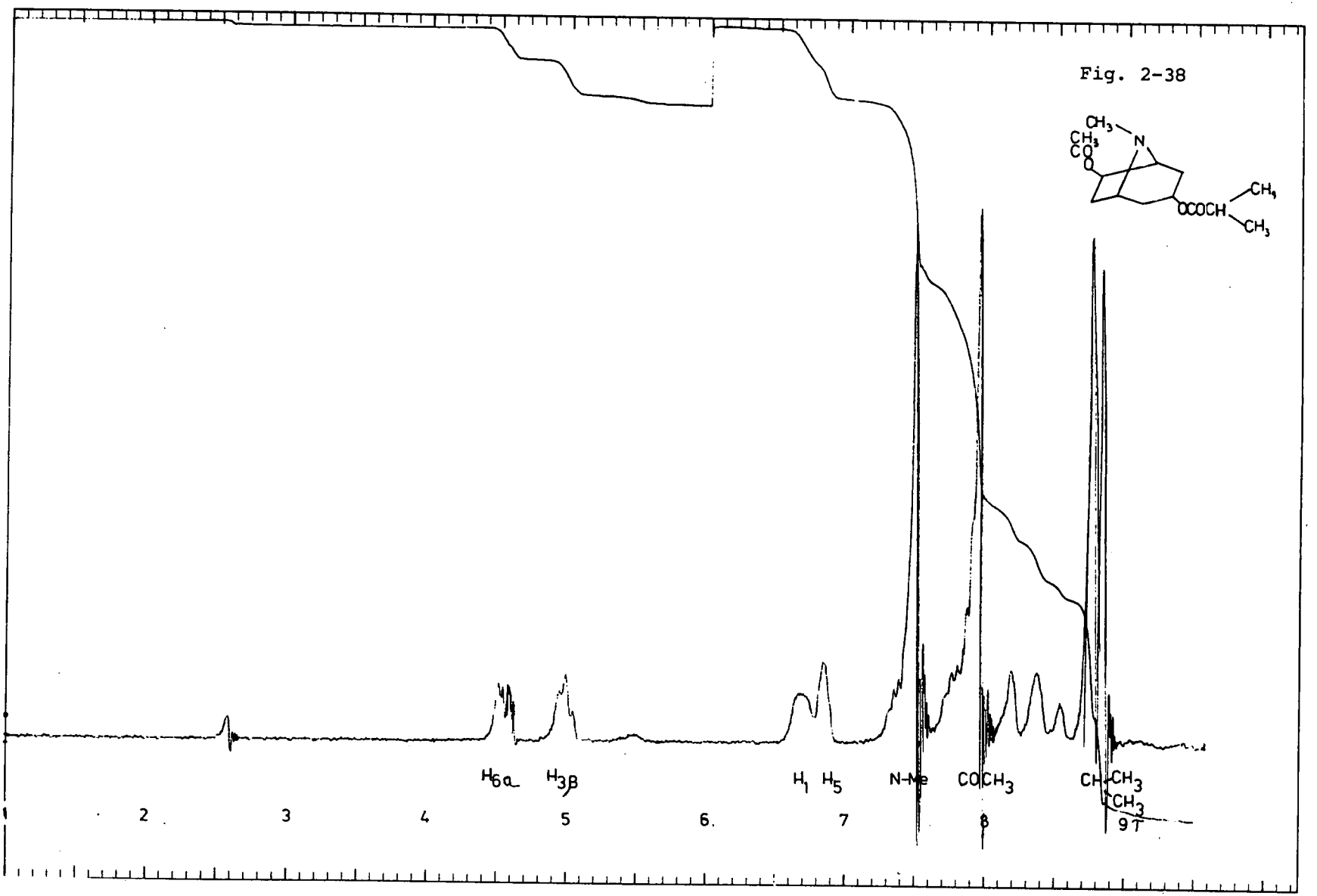


Fig. 2-38
H6α
H3β
H1 H5
N-Me
COCH3
CH-CH3
CH3
1
2
3
4
5
6.
7
8
9τ

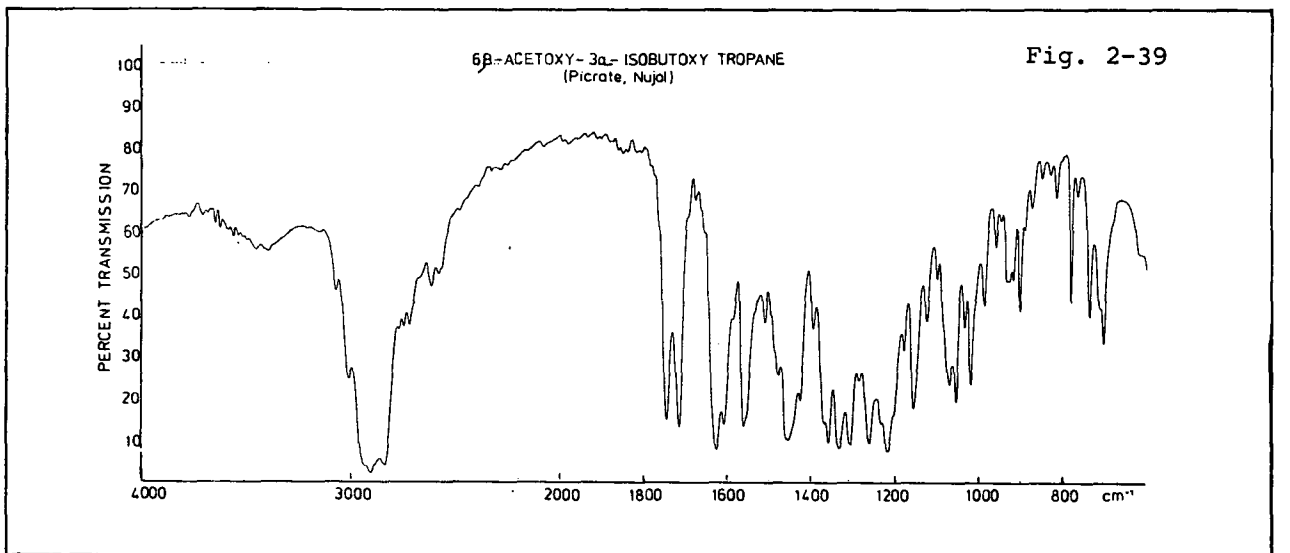


Fig. 2-39

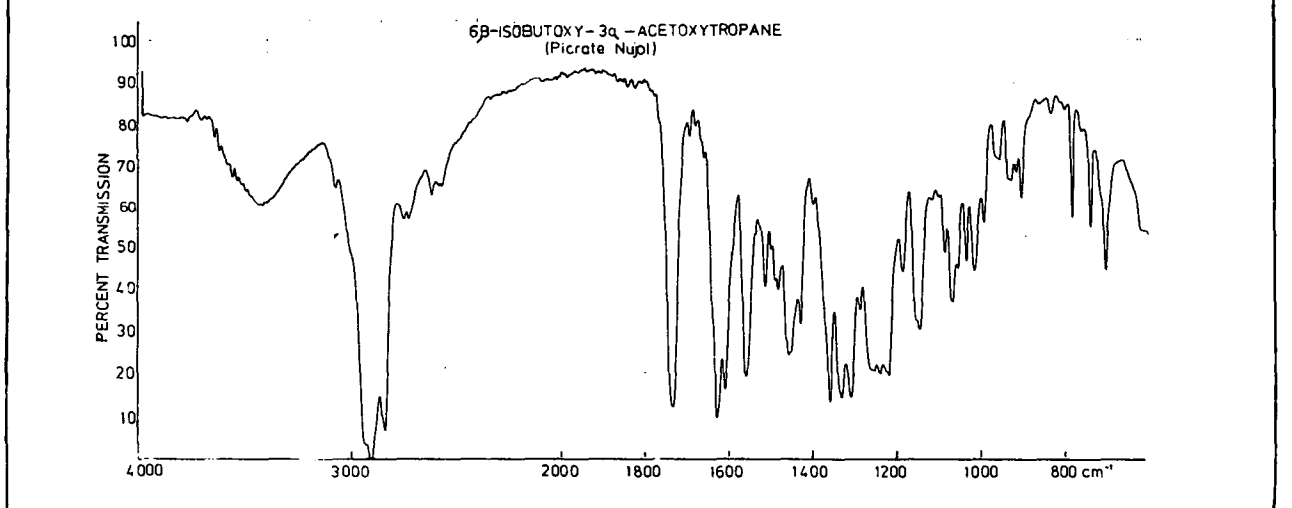


Fig. 2-40

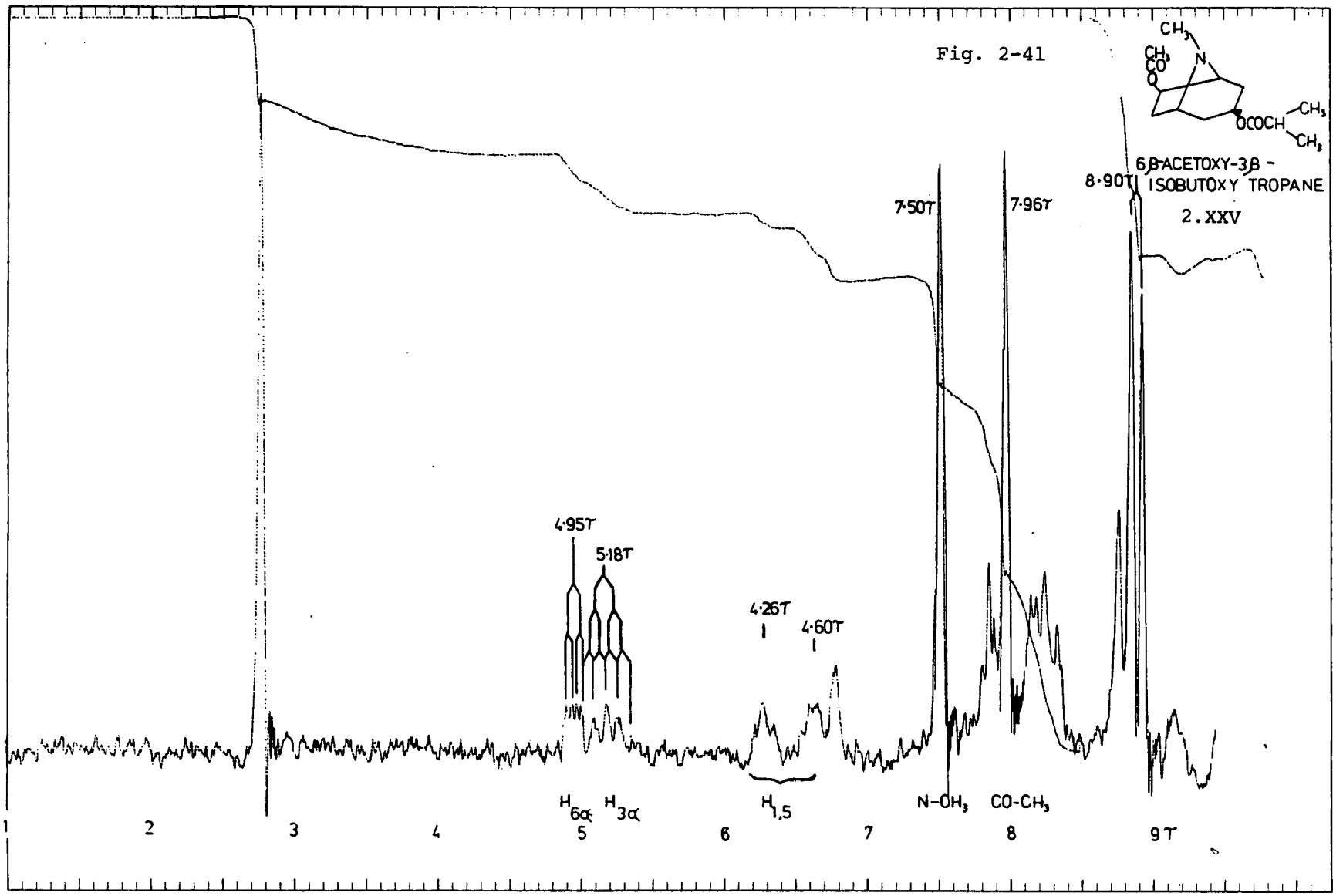


Fig. 2-41
6β-ACETOXY-3β - ISOBUTOXY TROPANE
2.XXV
8·90τ
7·50τ
7·96τ
4·95τ
5·18τ
4·26τ
4·60τ
H6α H3α
H1,5
N-CH3
CO-CH3
1
2
3
4
5
6
7
8
9τ

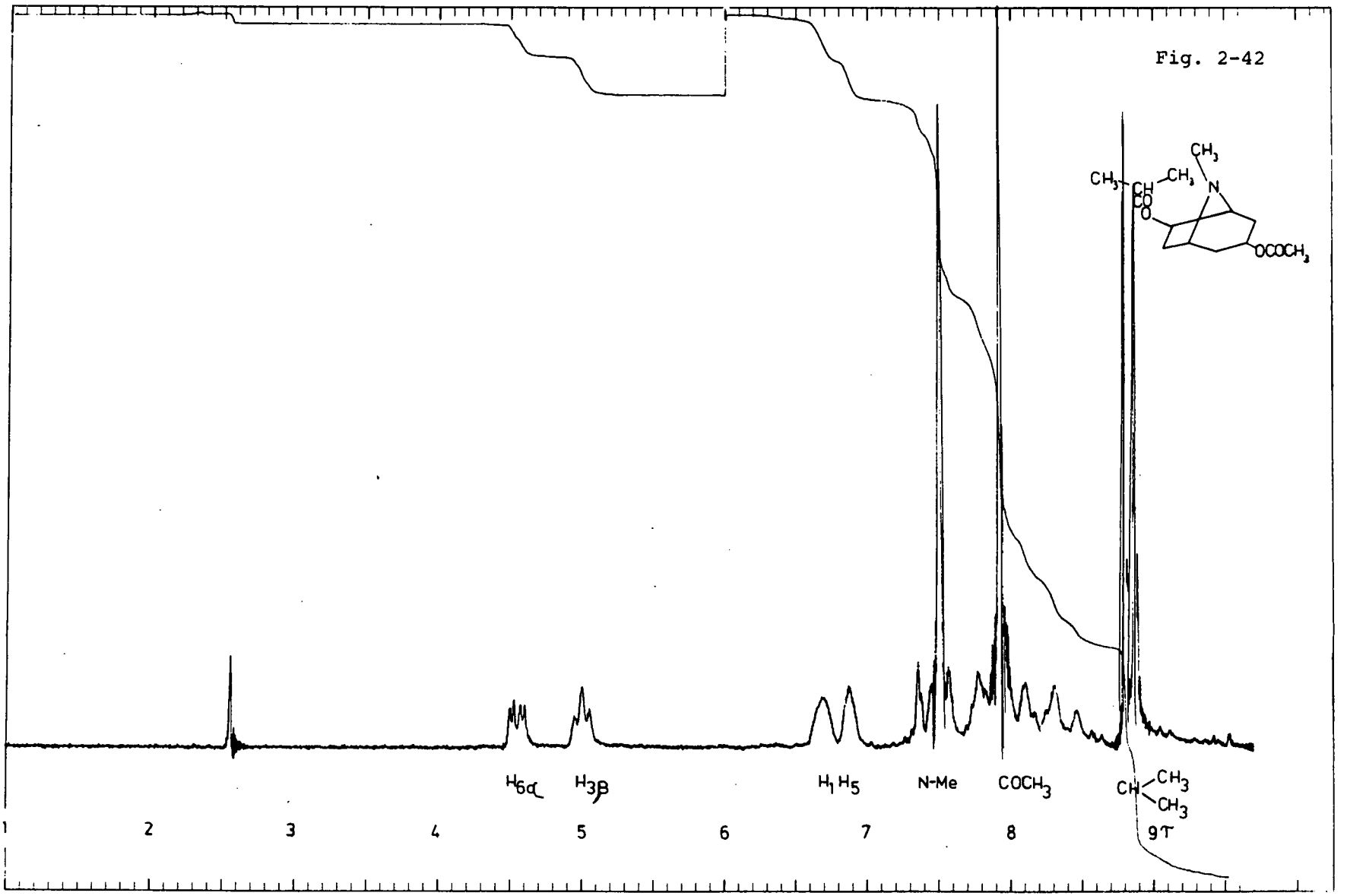
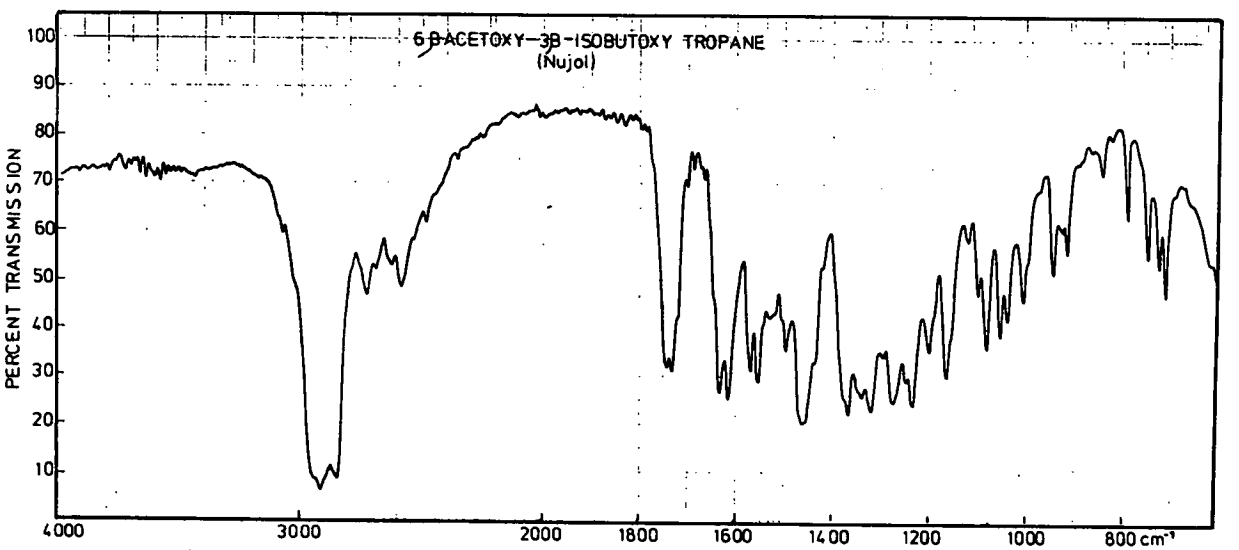


Fig. 2-42
H6α
H3β
H1 H5
N-Me
COCH3
CH
CH3
CH3
1
2
3
4
5
6
7
8
9τ

Fig. 2-43


6β-ACETOXY-3β-ISOBUTOXY TROPANE
(Nujol)
PERCENT TRANSMISSION
100
90
80
70
60
50
40
30
20
10
4000
3000
2000
1800
1600
1400
1200
1000
800 cm⁻¹

CHAPTER 3

Isolation and Structural Elucidation of Alkaloids from the Proteaceous species Darlingia darlingiana (F. MUELL), L.A.S. JOHNSON and Darlingia ferruginea J.F. BAILEY

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Isolation and Structural Elucidation of Alkaloids from the Proteaceous species Darlingia darlingiana (F. MUELL), L.A.S. JOHNSON and Darlingia ferruginea J.F. BAILEY

3.1. Introduction

Two other Australian species of the Proteaceae, Darlingia darlingiana and D. ferruginea, were found to give positive alkaloid tests. These two Darlingias are grouped with the Knightia species in an unclassified assemblage of Proteaceous genera which also includes Hollandaea and Cardwellia. The basis for this grouping is the retention by all four genera of the primitive unreduced tetraploid chromosome number n = 14. Certain morphological features, especially the common peduncle of the flower pairs, are associated with the Darlingia and Knightia genera. The use of chromataxonomic principles based on alkaloids in relating species and genera has achieved some notable success in the Monimiaceae[40]. In the Proteaceae it may provide a useful adjunct to other criteria. It has been found that the major alkaloid of both Darlingia species is darlingine, a substituted γ pyronotropane[41]. The minor alkaloids were distinctly different in both species, but those of D. ferruginea bore a very close relationship to the major alkaloids of K. deplanchei. The Cardwellia and Hollandaea species examined were found not to contain alkaloids.

3.2. Isolation of the bases of Darlingia darlingiana

The leaves and stems of D. darlingiana collected from Atherton, North Queensland, were dried, ground, and extracted by cold percolation with Prollius solution. The alkaloidal fraction was isolated in the

manner described in Section 2.2.1.

Analytical T.L.C. revealed four Schlittler-positive spots at R_f = 0.48 (darlingine), 0.42 (D2), 0.30 (D3), and 0.22 (D4) (10% MeOH in $CHCl_3$, silica gel). The major band, R_f = 0.48, (darlingine), was well separated from the lower R_f alkaloids and the corresponding base was readily isolated by P.T.L.C. The remaining bases were substantially more polar than darlingine and were not readily separable by P.T.L.C. due to extensive tailing and overlapping.

Use was made of Craig distribution to achieve separation of the bases by partitioning between chloroform as the stationary phase and 0.5×10^{-3} M sulphuric acid as the mobile phase. This technique was successful in enabling the isolation of purified D3, which after further P.T.L.C. was crystallized from petroleum ether. All fractions containing darlingine were accompanied by D4, which was easily separated from the major constituent by P.T.L.C. D2 was not isolated in sufficient quantity to permit characterization.

TABLE 3-1

Summary of Craig fraction constituents

Fractions	P.T.L.C.	Comments and Identification
0-30	-	No constituents
30-185	D1, D4	Fraction, darlingine 95%, D4 5%
186-240	D3	Darlingiana D3
241-245	D2, D1	Darlingiana D2 (very impure)
246-300	D5	Low R_f impure bands

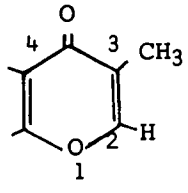
3.3. Structural elucidation of Darlingine

3.3.1. Spectroscopic studies

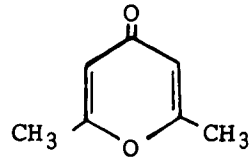
Darlingine, separated by P.T.L.C. from Craig fractions 30-185, was crystallized from ether/petroleum ether, and had m.p. 162-163°C, $[\alpha]_D^{19}$ +104°. High-resolution mass spectroscopy indicated a molecular weight of 219.1268 corresponding to a molecular formula of $C_{13}H_{17}NO_2$, which was confirmed by elemental analysis.

The P.M.R. spectrum (Fig. 3-1) at 100 MHz, which bore considerable similarity to the bellendine spectrum, revealed however three singlets at τ = 7.65, 7.75, 8.08, each of which integrated for three protons. The signal at 7.65τ was shifted downfield and split to a doublet on protonation by trifluoroacetic acid as solvent, confirming the assignment of $\diagdown N\text{-}CH_3$ to this resonance.

The remaining two singlets were assigned to C-methyl groups. The resonance at 8.08τ, which in bellendine appeared as a doublet (J = 1.2 Hz) resulting from allylic coupling of the C-3 methyl group to the C-2 proton, was a singlet at identical chemical shift. The



3.I



3.II

absence in the darlingine spectrum of the olefinic proton resonance at 2.42τ, in addition to the lack of allylic coupling, suggested that the C-2 position was substituted; furthermore, the additional three proton resonance observed in darlingine at 7.75τ indicated a C-2 methyl substituent, the chemical shift bearing close correlation to that observed for the methyl resonances of 2,6-dimethyl-γ-pyrone (3.II) and

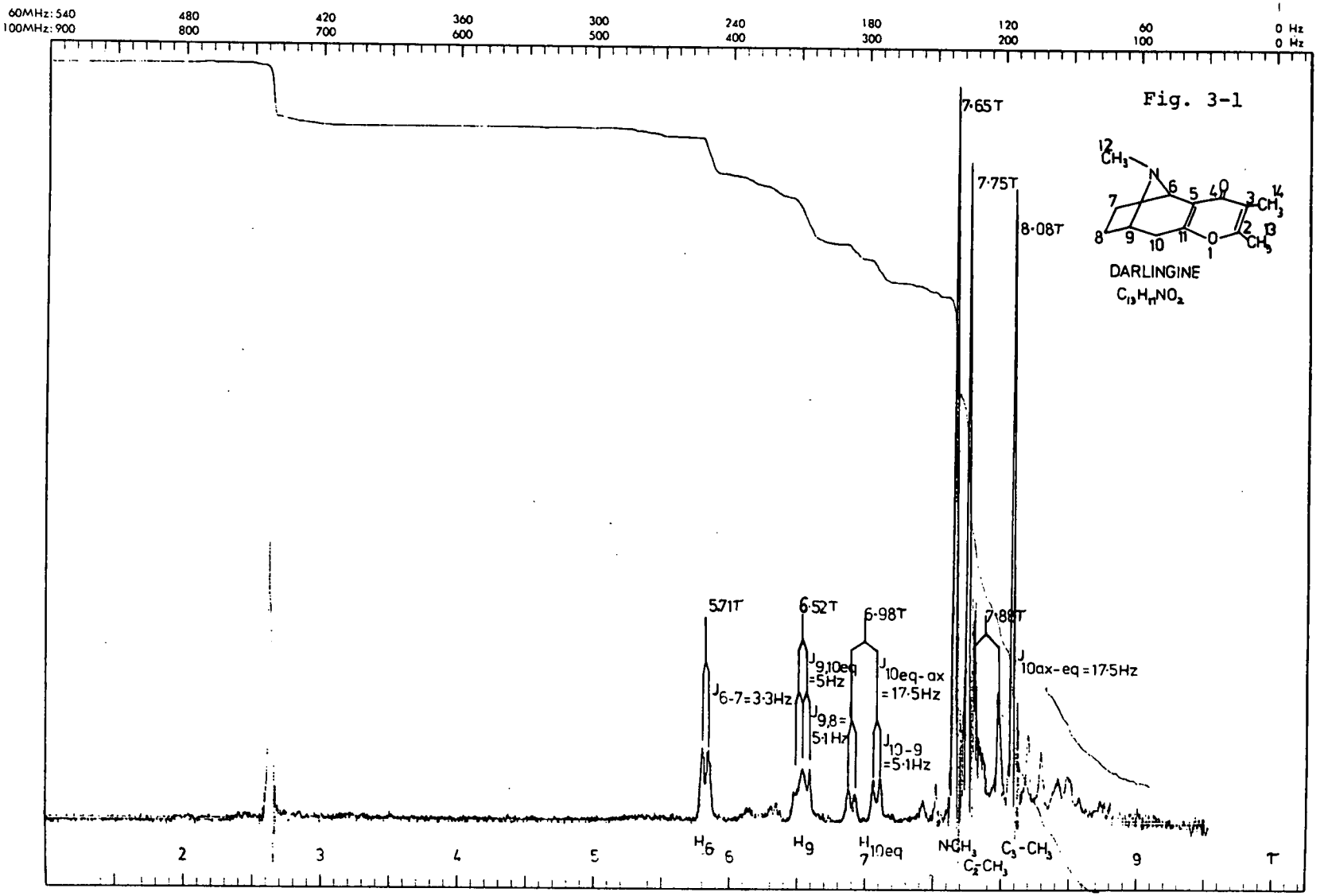

60MHz: 540
100MHz: 900
480
800
420
700
360
600
300
500
240
400
180
300
120
200
60
100
0 Hz
0 Hz
7·65T
7·75T
8·08T
5·71T
6·52T
6·98T
7·88T
$J_{6-7}=3·3Hz$
$J_{9,10eq}=5Hz$
$J_{9,8}=5·1Hz$
$J_{10eq-ax}=17·5Hz$
$J_{10-9}=5·1Hz$
$J_{10ax-eq}=17·5Hz$
H_6
H_9
H_{10eq}
$N\text{-}CH_3$
$C_2\text{-}CH_3$
$C_3\text{-}CH_3$
2
3
4
5
6
7
9
T
DARLINGINE
$C_{13}H_{17}NO_2$

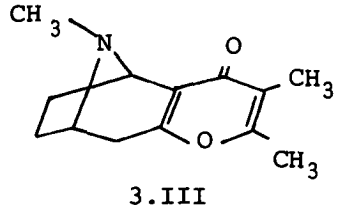
Fig. 3-1

the position of the C-2 methyl resonance in isobellendine (7.78τ).

The infrared spectrum of darlingine (Fig. 3-2) is closely similar to that of bellendine. The characteristic bands, interpreted earlier as being due to a γ-pyrone chromophore, are present, shifted fractionally in wavenumber: $\nu_{C=O}$ 1660 cm^{-1}, $\nu_{C=C}$ 1610 cm^{-1}. Some minor skeletal vibrational changes were apparent, and the symmetrical and asymmetrical ν_{C-O} stretch at 1150 cm^{-1} and 1170 cm^{-1} have reversed intensity.

The ultraviolet spectrum (Fig. 3-4) is nearly identical to that of bellendine. The γ-pyrone chromophore associated with such a spectrum has been discussed earlier. The empirical rules of Woodward and Fieser for α,β unsaturated esters and acids are less satisfactory in predicting the ultraviolet absorption for darlingine than for bellendine/isobellendine. $\lambda_{max,obs}$ = 256 nm, $\lambda_{max,cal}$ = 261 nm.

The molecular formula and the above spectroscopic data indicate that darlingine is a homologue of bellendine (3.III).



3.III

The mass spectral fragmentation pattern (Fig. 3-3) is shifted by fourteen mass units for all fragments in which the pyrone ring is intact. The characteristic tropane fragments are apparent in the darlingine spectrum as in bellendine, resulting from the facile α cleavage of the C-5-C-6 bond, which stabilizes the radical cation as an immonium ion. Subsequent cleavage of this ion results in the formation of either the stable pyrrolinium ion at m/e 82 or the conjugated pyrroline adduct at m/e 96. Both of these cleavages were supported by metastable peaks. The base peak in the mass spectrum occurs at m/e 190; high-resolution

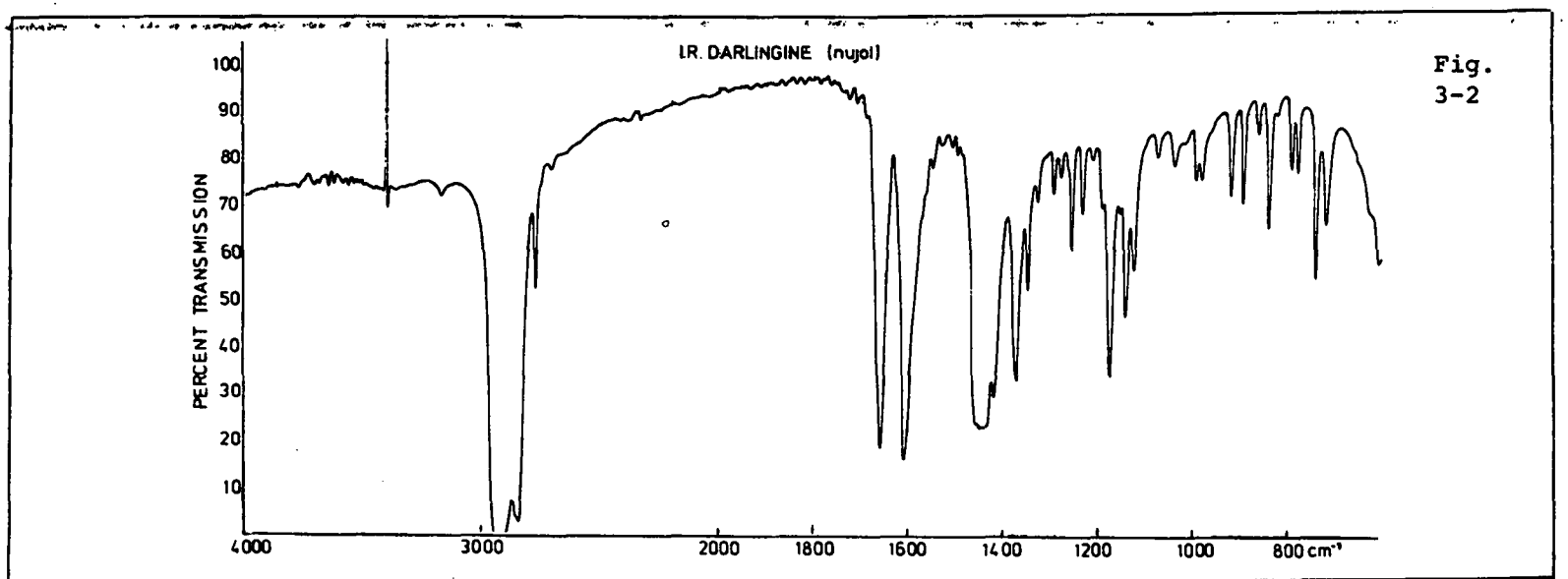


Fig. 3-2

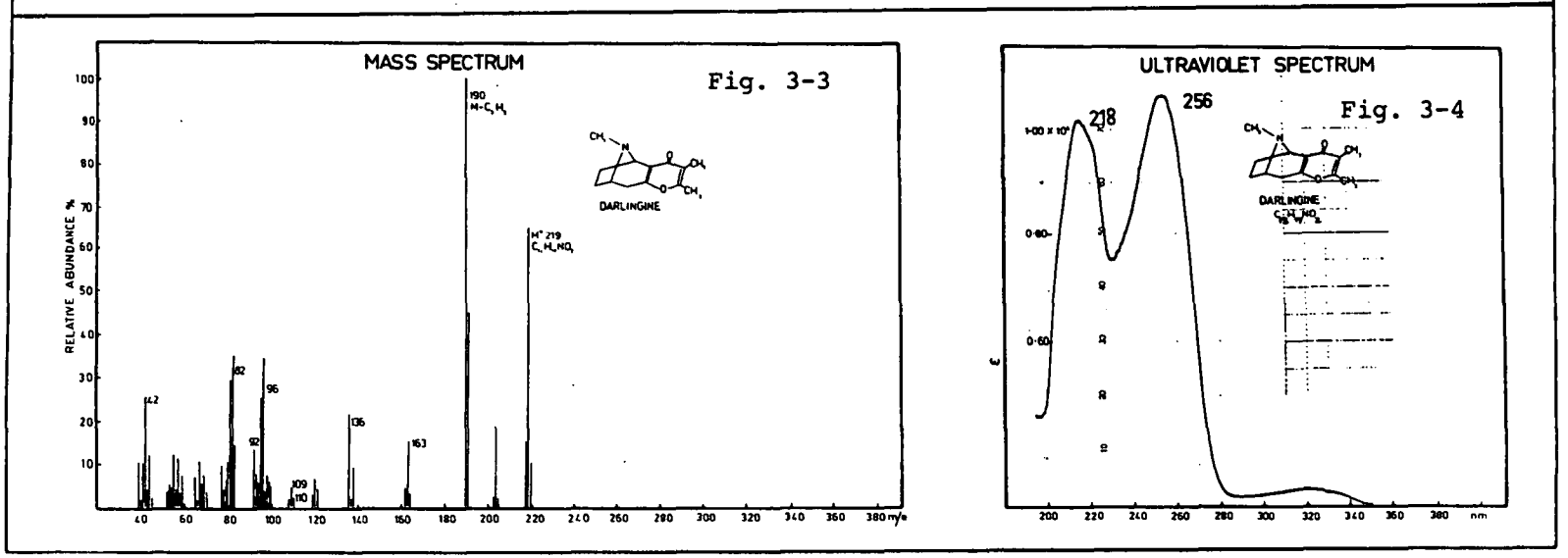
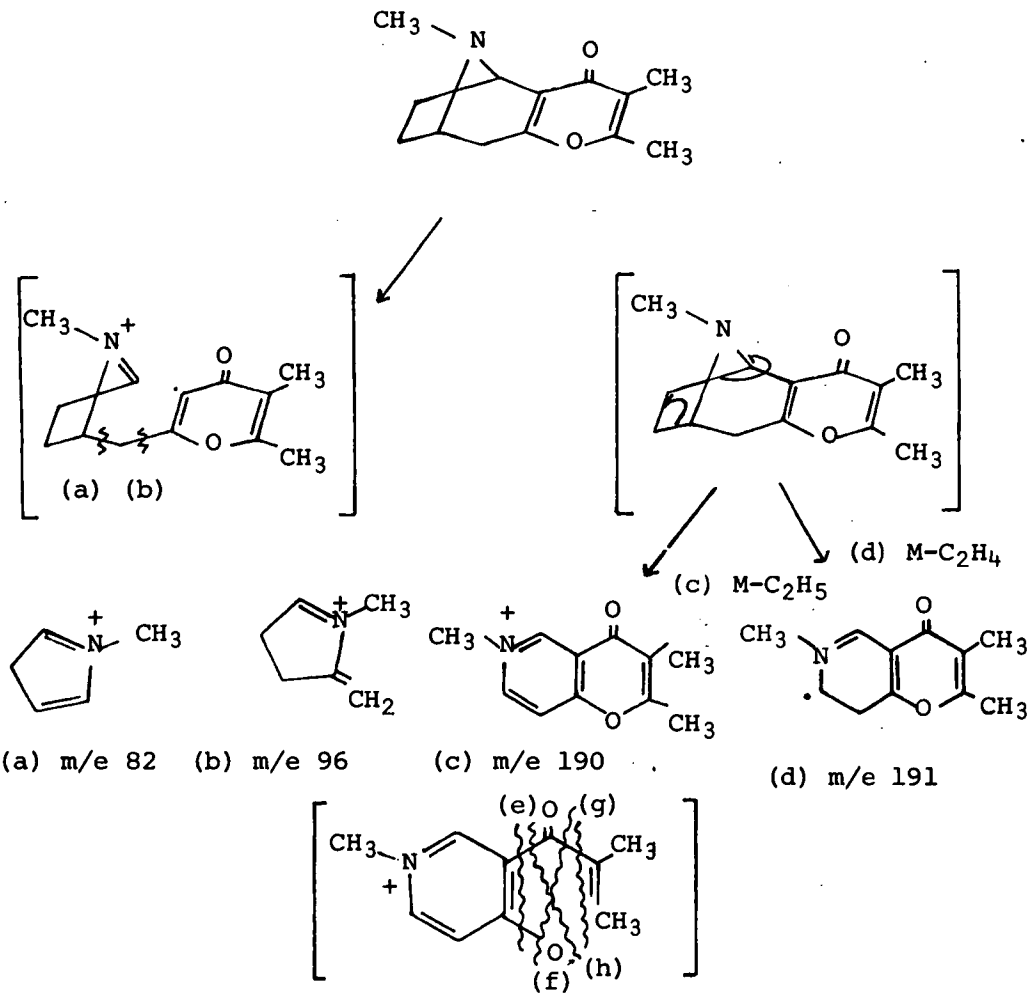


Fig. 3-3

Fig. 3-4

revealed a molecular formula of $C_{11}H_{12}NO_2$ for this ion. The loss of an ethyl group was analogous to the bellendine fragmentation, the stable aromatic base-peak bearing in this case an additional methyl group. Subsequent fragmentation leads to the common fragments m/e 92, 109, 120 and 136.

Mass spectral Fragmentation Pattern

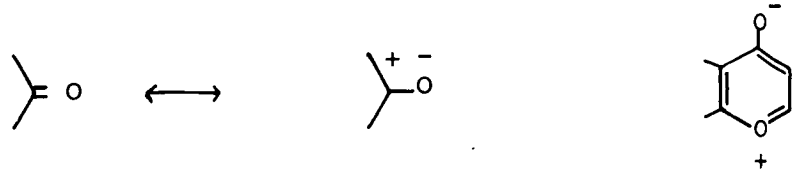


Scheme 3-1

3.3.2. ^{13}C Nuclear Magnetic Resonance of Darlingine and Bellendine

The ^{13}C N.M.R. spectrum of darlingine was determined in the off-resonance decoupled and noise-decoupled modes on a JEOL Fourier transform instrument. The noise-decoupled spectrum is presented in Fig. 3-5; in this spectrum, the nuclear Overhauser enhancement factor and the signal enhancement resulting from multiplet collapse, enabled the exact chemical shifts to be found with a reduced number of scans. A ten second repeat time was selected to enable complete relaxation of quaternary carbons.

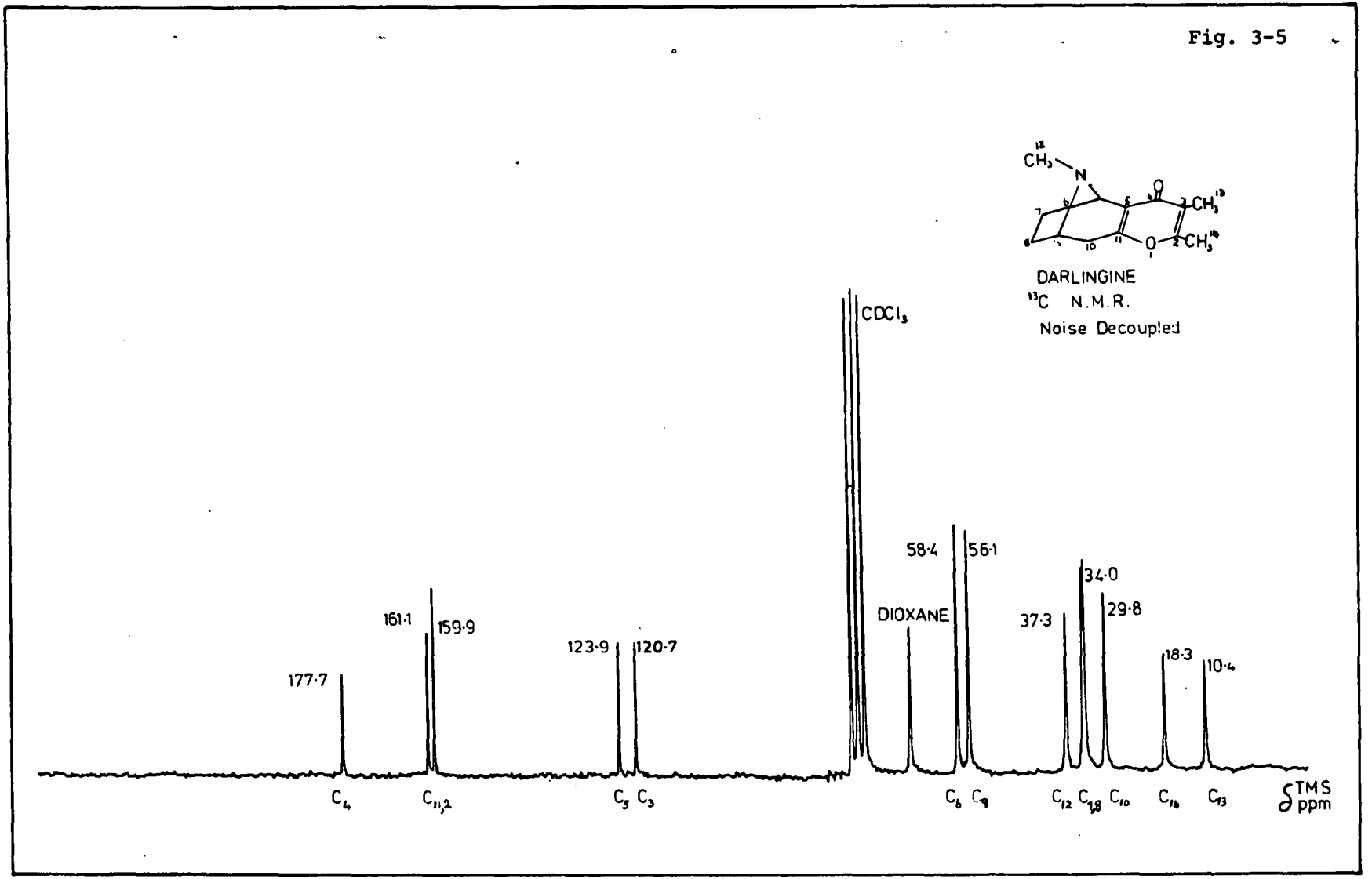
The spectrum has been interpreted on the following basis: the resonance at 177.7δ, which is appreciably upfield of normal carbonyls, was assigned to the pyrone carbonyl carbon. This shift results from a decrease in the carbonyl bond polarity, shown in canonical form (3.V) for normal carbonyls. Lone pair electron release from the conjugated



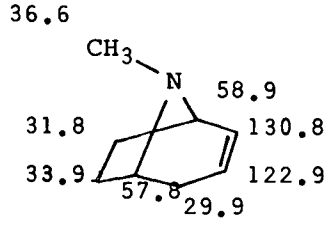
3.IV 3.V 3.VI

oxygen of the pyrone ring results in forms such as 3.VI in which the carbonyl carbon electron density is increased, with attendant upfield shifts being observed in the ^{13}C resonance[42]. Four other quaternary carbon resonances are apparent in two groups of two at 161.1δ, 159.9δ and 123.9δ, 120.7δ. The chemical shifts of these resonances are indicative of sp^2 hybridization. A small degree of differential shielding exists for the lower field resonances, which are assigned to C_2, and C_{11} adjacent to oxygen in the γ-pyrone ring. The higher field resonance is assigned to C_{11} as a consequence of the mild γ-effect which is experienced from the piperidine ring nitrogen.

Fig. 3-5


DARLINGINE
^{13}C N.M.R.
Noise Decoupled
$CDCl_3$
DIOXANE
177·7
161·1
159·9
123·9
120·7
58·4
56·1
37·3
34·0
29·8
18·3
10·4
C_4
$C_{11,2}$
C_5 C_3
C_6 C_9
C_{12} $C_{1,8}$ C_{10}
C_{14}
C_{13}
δ^{TMS} ppm

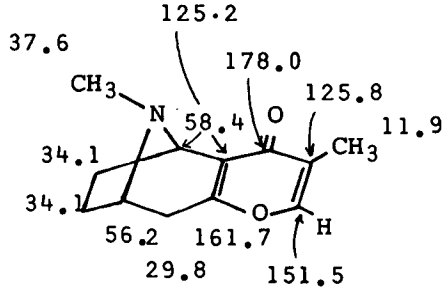
The fact that these two resonances are at appreciably lower field than the remaining pair is indicative of contributors such as 3.VI to the resonance forms of the pyrone ring. The remaining olefinic resonances at 123.9δ and 120.77δ are due to C_3 and C_5, both carbons being α to the carbonyl. The characteristic shift for carbons β to an electron withdrawing group such as nitrogen[43] is the reason for the differentiation of the signals and permits the assignment of the resonance at 123.9δ to C_5 and that at 120.7δ to the C_3 carbon. The off-resonance decoupled spectrum showed the two signals at 58.4δ and 56.1δ in Fig. 3-5 to be doublets; furthermore the chemical shifts are consistent with methine carbons adjacent to nitrogen, which enables the assignment of these resonances to C_6 and C_9. The differentiation of these two resonances may be due to two factors: as in tropidine (3.VII), a homo-allylic effect[44] may cause the C_9 resonance to be shielded; however, the very small difference in chemical shift observed for the C_7 and C_8 resonances suggests that endocyclic homoallylic shielding is less significant where there is delocalized bonding as in the pyrone ring. The shielding of the C_9 resonance may be due to diamagnetic anisotropy resulting from the carbonyl oxygen.



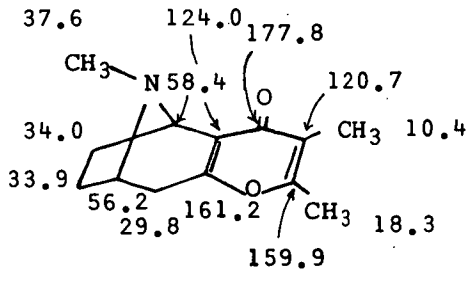
3.VIII

The primary carbons were easily recognized as quartets in the off-resonance spectrum. The N-methyl signal appearing at 37.3δ is consistent with a higher degree of electron density in the $\Delta^{2,3}$ piperidine ring, e.g. tropidine (36.6δ). The saturated tropanes and 3α-O-acyl tropanes have N-methyl values of $\tilde{\sim}$ 40δ. The remaining two primary carbon resonances at 18.3δ and 10.4δ are due to the methyl substituents on the pyrone ring. The C.M.R. spectrum of bellendine shows only one resonance in this region at 11.9δ due to the C-3 methyl group, hence the assignment of the 18.3δ resonance in darlingine to the C-2 methyl group.

A comparison of the darlingine and bellendine spectra indicates that fairly close accord exists between observed values for chemical shifts of the pyrone ring carbons and those predicted by chemical shift substituent effects on olefinic systems.



Bellendine 3.VIII



Darlingine 3.III

Substituents	δ C_2	δ C_3
H	151.5	125.8
CH_3	159.9	120.7
α Δδ	= + 8.4 p.p.m.	β Δδ = -5.1 p.p.m.

Thus it can be seen that the C-2 methyl group exercises an α effect of +8.4 p.p.m. and a β effect of -5.1 p.p.m. In the series of hexacyclic alkenes considered by Stothers[45], alkyl substituents on olefinic systems caused very similar shifts; an α effect of +7.0 p.p.m. was observed and a β effect of -4.9 p.p.m.

3.4. Structural elucidation of Darlingiana D3

The minor alkaloid of D. darlingiana, D3, was isolated by preparative thin-layer chromatography from Craig fractions 186-200. It was crystallized from petroleum ether (60-80°), to yield yellow plates, m.p. 93-94°, $[\alpha]_D$ = +62°. High-resolution mass spectroscopy indicated a molecular formula of $C_{17}H_{23}NO$.

3.4.1. Spectroscopic Studies

The P.M.R. spectrum (Fig. 3-6) indicated the presence of an N-methyl group at 7.60τ (s, 3H), and an aromatic multiplet at 2.71τ (m, 5H). Two prominent complex broadened downfield resonances at 6.84τ and 7.30τ were assigned to protons adjacent to nitrogen, and a third downfield resonance at 5.40τ was attributed to a proton adjacent to oxygen. An exchangeable proton appears at 3.80τ. A complex set of olefinic resonances involving four protons is centred at 3.42τ, and the remaining eight protons are distributed in a broad band between 7.80τ and 8.50τ.

The mass spectrum (Fig. 3-8) was recorded in the high-resolution mode and the molecular ion, $C_{17}H_{23}NO$ so determined, indicated seven centres at unsaturation. No carbonyl absorption was present in the infrared spectrum (Fig. 3-7), but a band at 3,200 cm^{-1}, and the exchangeable P.M.R. signal indicated the presence of an hydroxy group. The intense base peak in the mass spectrum at m/e 84 ($C_5H_{10}N$) contained

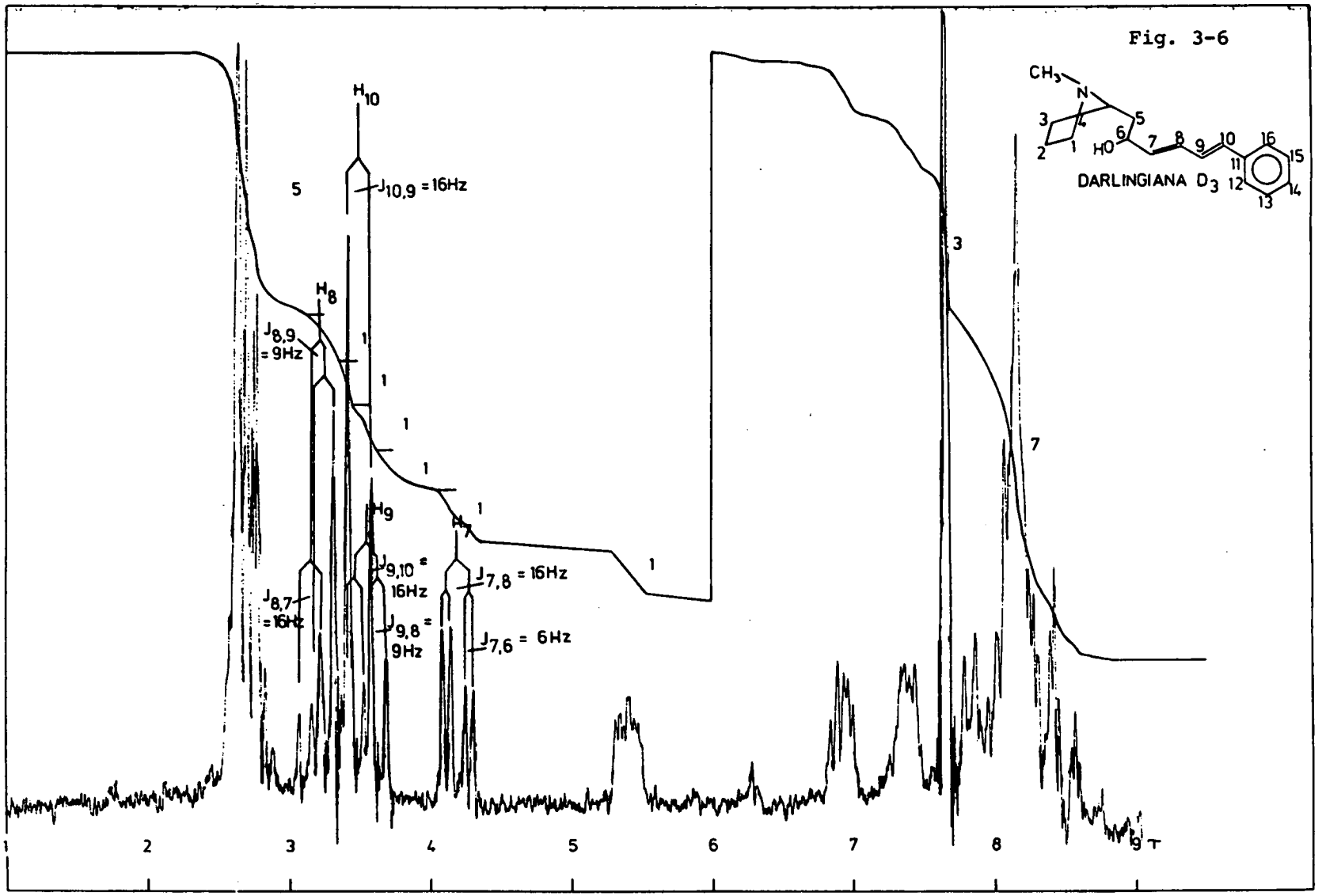


Fig. 3-6
DARLINGIANA D3
H10
J10,9 = 16Hz
H8
J8,9 = 9Hz
H9
J9,10 = 16Hz
J9,8 = 9Hz
J8,7 = 16Hz
H7
J7,8 = 16Hz
J7,6 = 6Hz
5
1
3
7
1 2 3 4 5 6 7 8 9 τ

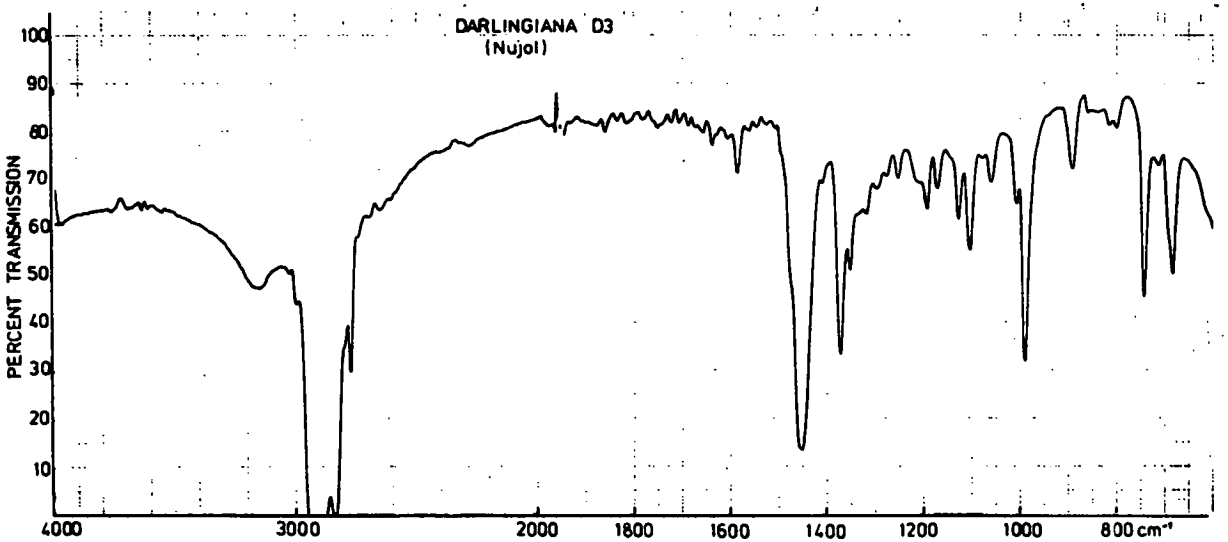


Fig. 3-7

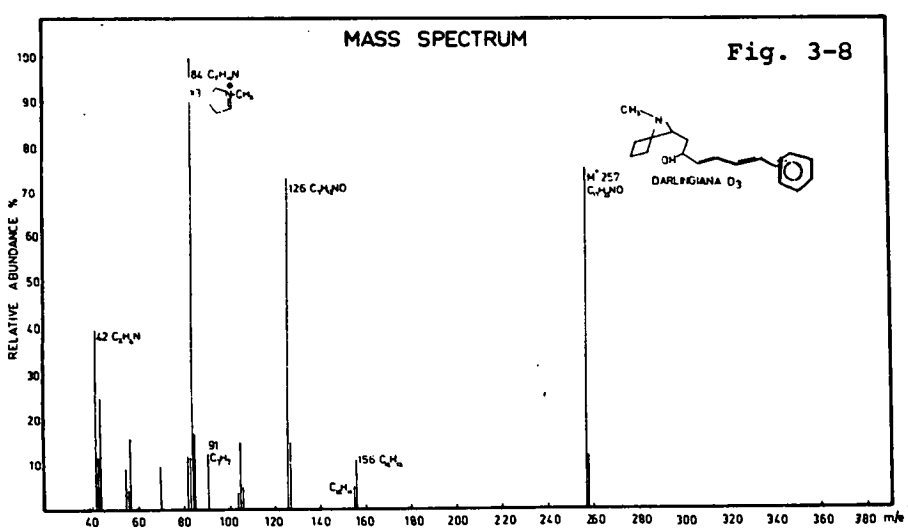


Fig. 3-8

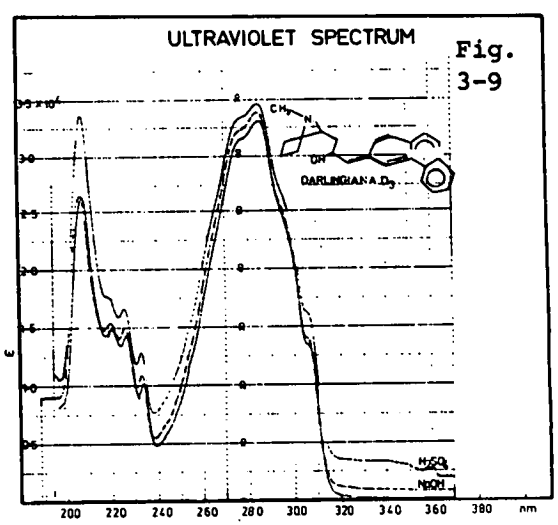
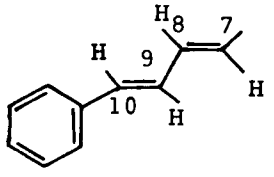


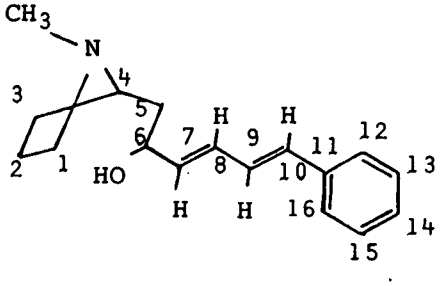
Fig. 3-9

one less element of saturation than tropane bases; this was taken as an indication that the compound was a pyrrolidine base. The ultraviolet spectrum (Fig. 3-9) was indicative of a conjugated aromatic compound. The intense band at 290 nm, ε_{max} = 32,000, was taken as being due to a 4-phenyl buta-1,3-diene system. A first order analysis of the olefinic resonances in the 100 MHz P.M.R. spectrum of D3 supports a buta-1,3-diene moiety. Two sets of <u>trans</u> interactions are indicated, from the measurement of coupling constants (J = 16 Hz) in the multiplets centred at 4.2τ, 3.1τ and 3.6τ, 3.5τ, respectively, which are assigned to H_7-H_8 and H_9-H_{10}. An intermediate value for the H_8-H_9 interaction, J = 9 Hz, is seen in the multiplets centred at 3.1τ and 3.6τ. Hence, the partial structure 3.IX is suggested from these data.



3.IX

The additional vicinal coupling between H_7 and the allylic protons on C_6 was detected by irradiation of the signal at 5.80τ. The chemical shift of this proton is consistent with a location close to both oxygen and an allylic system. The reconstruction of these data suggested a structure like 3.X for Darlingiana D3.



3.X

H_{10}: 3.50τ, Doublet, $J_{10,9}$ <u>trans</u> = 16 Hz

H_9 : 3.60τ, Doublet of doublets $J_{9,10}$ <u>trans</u> = 16 Hz; $J_{9,8}$ = 9 Hz

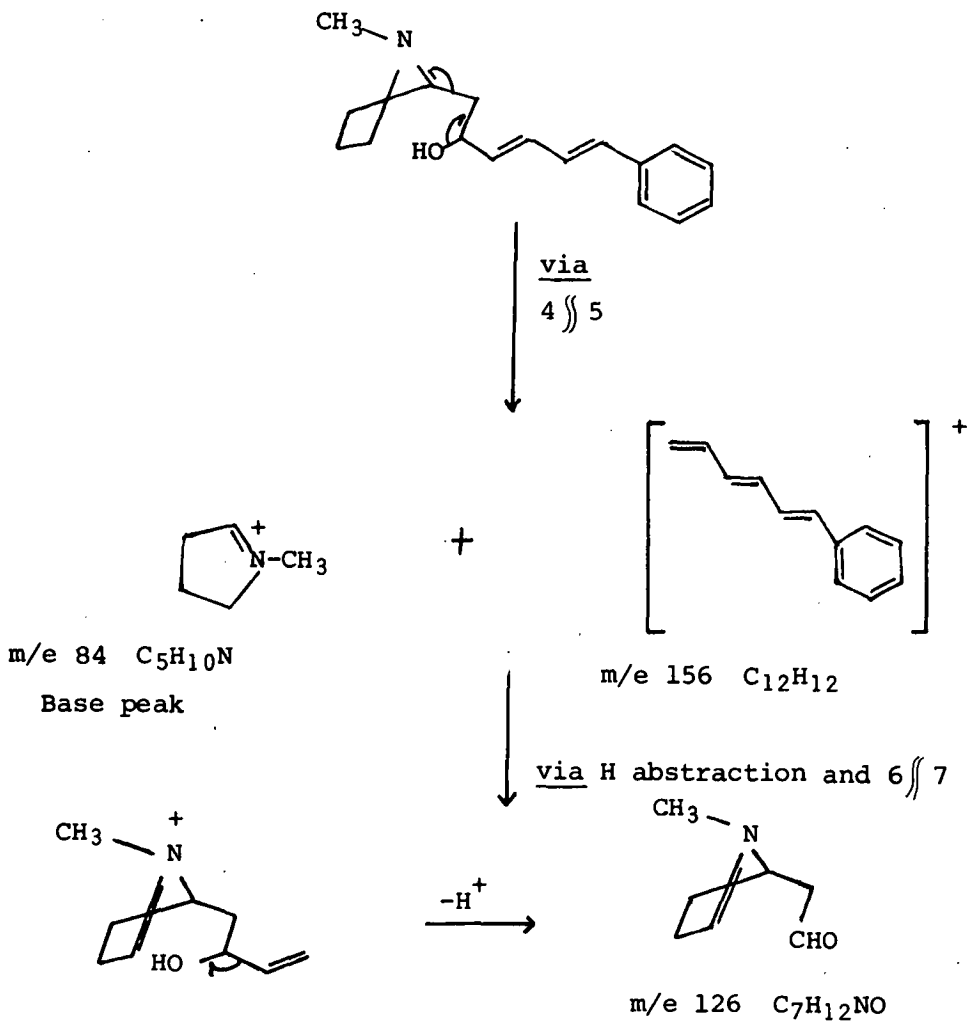
H_8 : 3.12τ, Doublet of doublets $J_{8,7}$ <u>trans</u> = 16 Hz; $J_{8,9}$ = 9 Hz

H_7 : 4.23τ, Doublet of doublets $J_{7,8}$ <u>trans</u> = 16 Hz; $J_{7,6}$ = 6 Hz

This structure necessitates the assignment of the multiplet at 7.82τ, to the remaining methylene proton adjacent to nitrogen in the pyrrolidine ring. Such a structure is consistent with biosynthetic theories for pyrrolidine bases. A condensation is indicated between N-methyl-Δ^1-pyrroline and a polyketide chain, terminated by a cinnamoyl residue. Subsequent reduction and elimination may introduce the additional conjugation to the olefinic system.

The mass spectral fragmentation pattern (Scheme 3-2) is consistent with a structure such as 3.X for Darlingiana D3.

Mass Spectral Fragmentation Pattern, D3



Scheme 3-2

The base peak, m/e 84 ($C_5H_{10}N$), arises from the characteristic α-cleavage mechanism of tertiary amines and the complementary hydrocarbon fragment may arise from simultaneous loss of a hydroxyl radical to produce the unsaturated hexatriene which is subsequently ionized.

3.5. Isolation of the bases of Darlingia ferruginea

Darlingia ferruginea JOHNSON is an arborial species closely related to D. darlingiana, and like the latter, it contains alkaloids in significant quantities. An extraction of the dried stems and leaves was carried out using an identical procedure to that for the Darlingiana species. Analytical thin-layer chromatography indicated the presence of at least four alkaloids. The major base, F1, was found to be darlingine (R_f = 0.48, m.p. 162-163°, $[\alpha]_D$ = 102°), which was separated from the remaining minor alkaloids. The resolution of these latter on silica gel was poor: F2 (ferrugine, R_f = 0.37) was separated from F3, (ferruginine, R_f = 0.22) by P.T.L.C. using multiple development (10% MeOH in $CHCl_3$). The intermediate P.T.L.C. zone, R_f = 0.29, contained another base named F2'. Each base was further purified by P.T.L.C. using multiple development, and their structures have been found by spectroscopic and chemical methods to be novel tropane derivatives.

3.6. Structural Elucidation of Alkaloid F2 (2α-Benzoyltropane)[46]

The P.T.L.C. band F2, R_f = 0.37 (10% MeOH in $CHCl_3$, silica gel), was rechromatographed three times using progressively decreasing solvent polarity (10%, 8% and 7% MeOH in $CHCl_3$, silica gel). This enabled the isolation of a pure clear oil (120 mg, $[\alpha]_D$ = +55°, for which a molecular formula of $C_{15}H_{19}NO$ was indicated by high-resolution mass spectroscopy. Preparative gas-liquid chromatography (G.L.C.) proved effective in

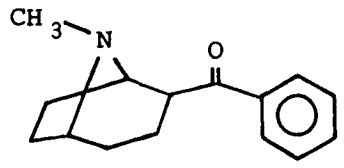
separating F2 from F3.

3.6.1. Spectroscopic studies

The presence of prominent peaks in the mass spectrum (Fig. 3-12) at m/e 82 (C_5H_8N) and m/e 96 ($C_6H_{10}N$) was indicative of a tropane system. The P.M.R. spectrum (Fig. 3-10) suggests the presence of a benzoyl moiety: two distinct low-field resonances at 2.05τ and 2.45τ, integrating for two and three protons respectively, arise from the carbonyl-deshielded ortho protons and the remaining aromatic protons. Three one-proton multiplets at 6.20τ, 6.55τ and 6.75τ result from the two protons adjacent to nitrogen, and a methine proton adjacent to a carbonyl. An N-methyl signal appears at 7.60τ, and the remaining eight protons attached to the tropane ring resonate in the range 7.70-8.60τ.

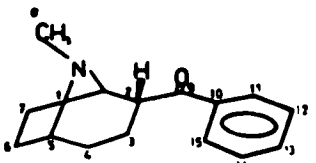
The infrared spectrum (Fig. 3-11) supports the presence of a benzoyl group: the carbonyl absorption, $\nu_{C=O}$ 1675 cm^{-1}, aromatic skeletal vibrations $\nu_{C=C}$ 1580 cm^{-1}, 1600 cm^{-1} and mono-substituted aromatic out-of-plane C-H bands ν_{CH} 725 cm^{-1} and 780 cm^{-1} are all consistent with this assignment. The ultraviolet spectrum (Fig. 3-13) also shows accord: λ_{max} = 243 nm, ε_{max} = 12,800; c.f. acetophenone: λ_{max} = 242 nm, ε_{max} = 12,700.

The combination of the above data indicates a plane structure 3.XI for alkaloid F2.



3.XI

Fig. 3-10



DARLINGIA FERRUGINEA
ALKALOID F_3

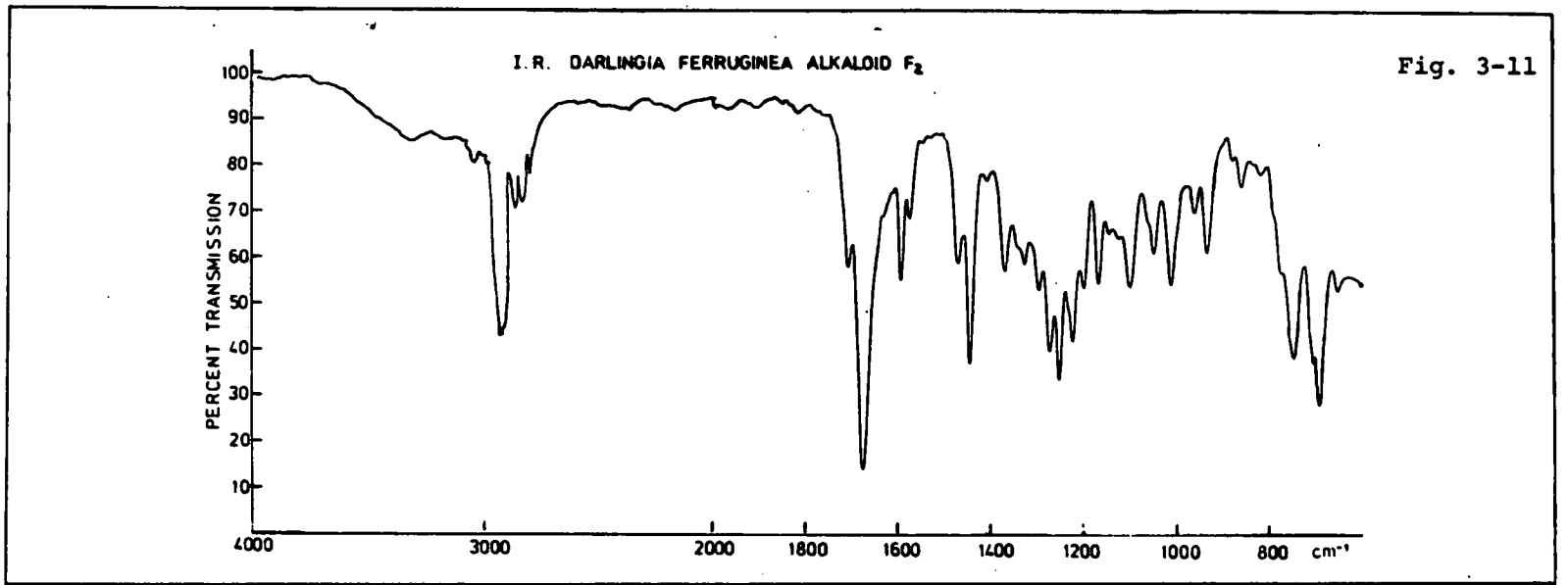


Fig. 3-11

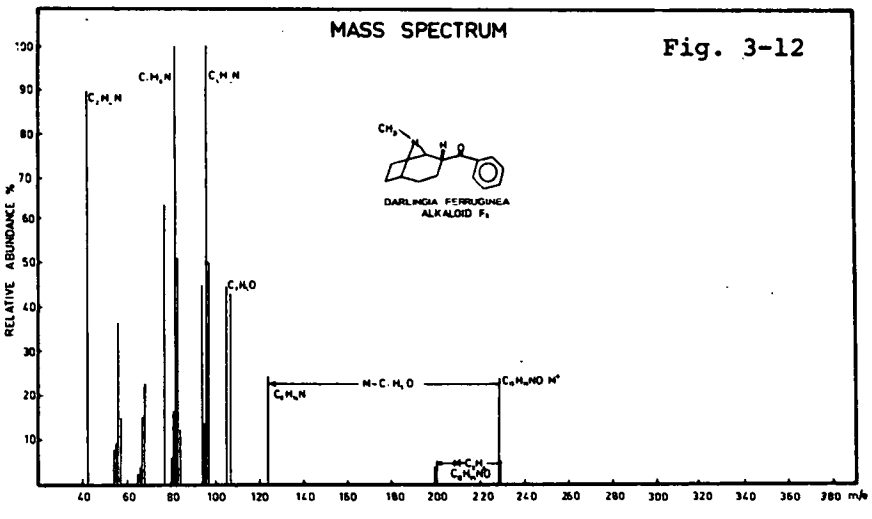


Fig. 3-12

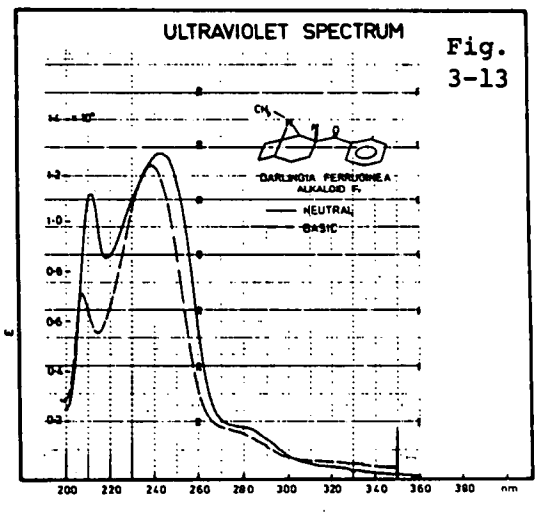
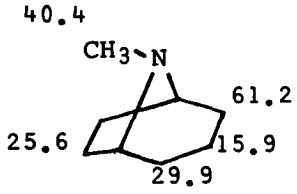
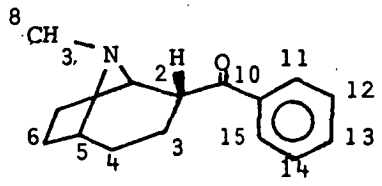


Fig. 3-13

The unresolved multiplet associated with the C-2 proton in the P.M.R. spectrum at 6.2τ did not enable the evaluation of the H_{leq}-H_2 coupling constant. Stereochemical assignment based on P.M.R. parameters was not therefore possible. The powerful shift technique of C.M.R. spectroscopy was utilized to achieve this assignment[47]. The ^{13}C chemical shifts of tropane[48] 3.XII and the assigned shifts of the new base, shown in Fig. 3-14, are seen to correlate closely, strongly supporting the structural assignment 3.XIII.



3.XII



3.XIII

Use of standard chemical theory and model acetophenone compounds[49] enabled assignment of the aromatic resonances, and the C-2α stereochemistry was determined from the fact that the C_4 resonance in F2, δ^{TMS} = 29.6 p.p.m., is only slightly shifted from the value for unsubstituted tropane, $\Delta\delta C_2$ = 0.3 p.p.m. Such a small shift is in accordance with the negligible γ effect exerted by equatorial substituents on conformationally fixed six-membered rings; axial substituents, acting through a trans 1,3-diaxial mechanism, exercise a shielding effect of $\tilde{\sim}$ 5.0 p.p.m. on the chemical shift of the γ carbon[50]. From these data, the structure of F2 (ferrugine) was determined as 2α-benzoyl tropane (3.XIII). The mass spectral fragmentation pattern for ferrugine is shown in Scheme 3-3.

3.6.2. Total Reduction of F2 to 2-(methyl cyclohexyl)tropane

In order to confirm the structure 3.XIII assigned to F2, a total reduction was undertaken to convert the base to 2-benzyl tropane 3.XIV. However, catalytic hydrogenation, using Adams catalyst in acidified ethanol, produced the totally reduced product 3.XIV(a), 2-(methyl

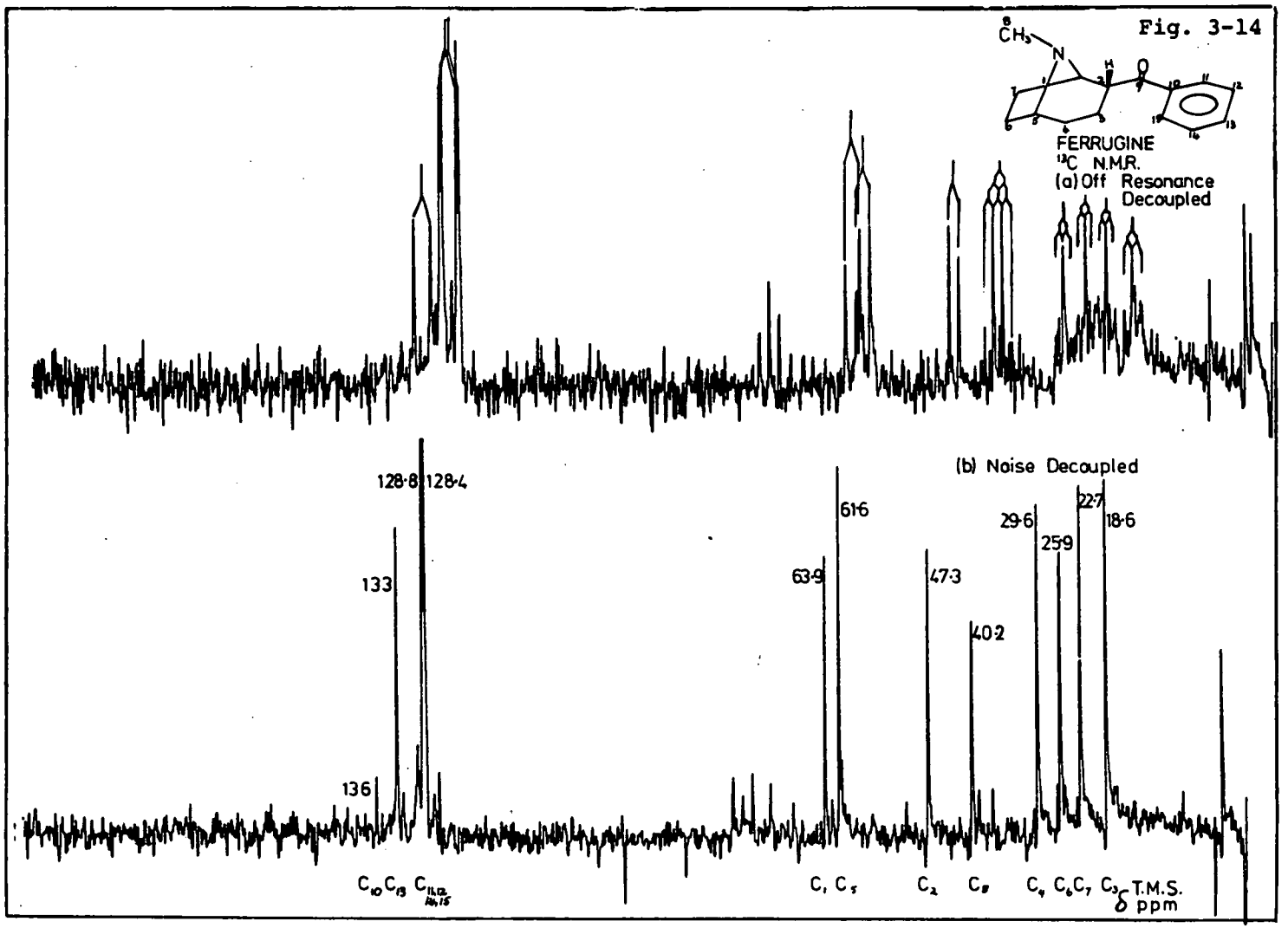
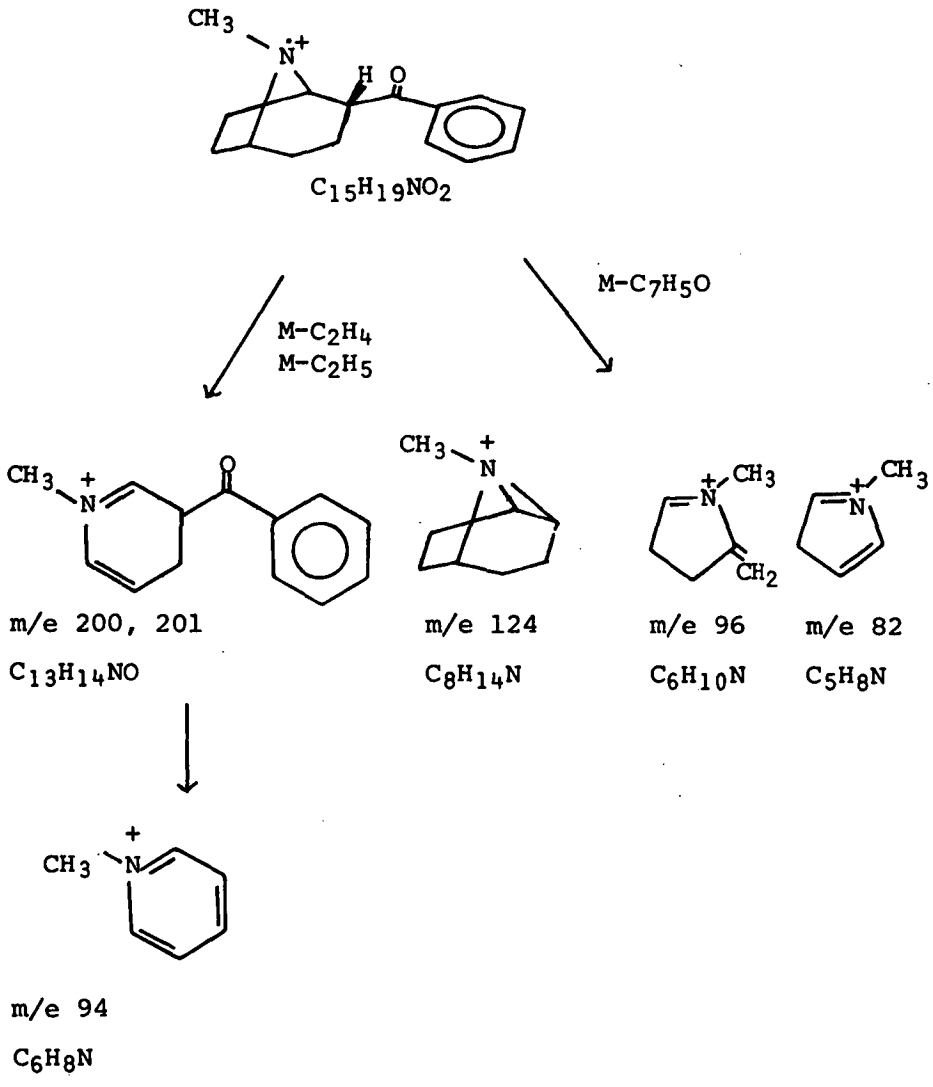


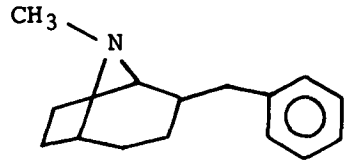
Fig. 3-14
FERRUGINE
^{13}C N.M.R.
(a) Off Resonance Decoupled
(b) Noise Decoupled
128·8
128·4
133
136
63·9
61·6
47·3
40·2
29·6
25·9
22·7
18·6
$C_{10}C_{13}$ $C_{11,12,14,15}$
C_1 C_5
C_2
C_8
C_4 C_6C_7 C_3
δ T.M.S. ppm

cyclohexyl) tropane. This was compared with 2-(methyl cyclohexyl)-tropane synthesized from 2-benzyl tropan-3-one (3.XV).

Mass Spectral Fragmentation of F2 (Ferrugine)

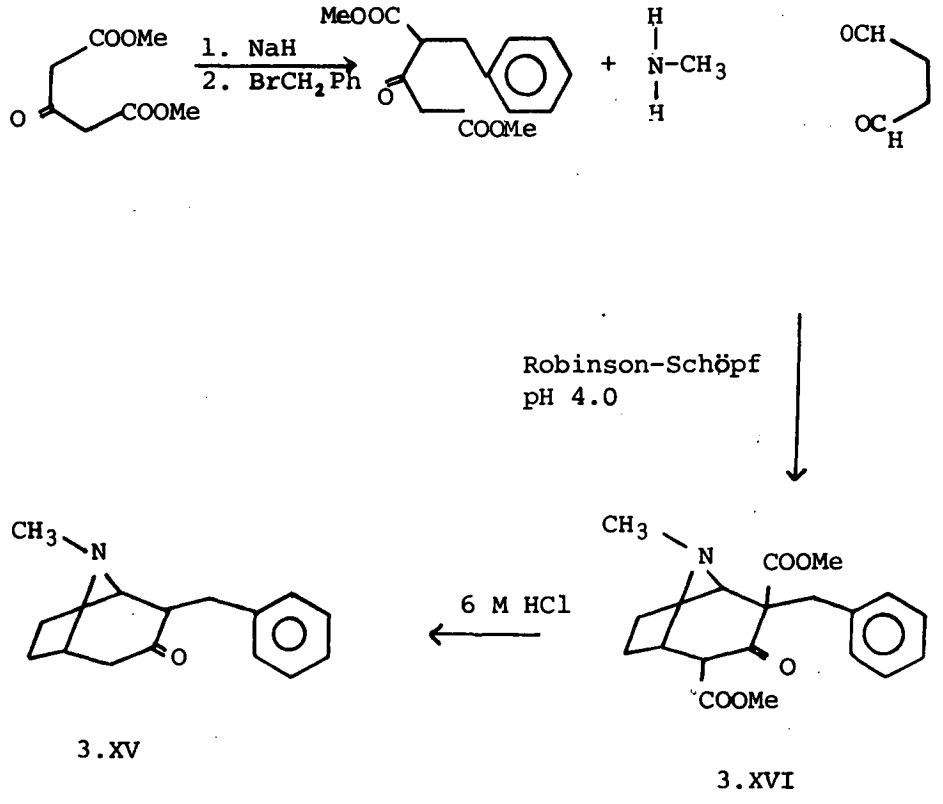


Scheme 3-3



3.XIV

The route undertaken to 2-benzyl tropan-3-one is outlined in Scheme 3-4. It involved a Robinson-Schöpf reaction of dimethyl 2-benzyl-3-oxo-glutarate leading to 2,4-dicarbomethoxy-2-benzyl tropan-3-one (3.XVI). P.M.R., I.R., M.S., and U.V. data are shown in Figs. 3-15 - 3-18. Hydrolysis and decarboxylation was achieved by heating 3.XVI with 6 M HCl to produce 2-benzyl tropan-3-one (3.XV). Spectral data relating to 3.XV are shown in Figs. 3-19 - 3-22. (Figs. 3-15 - 3-22 appear in Appendix 3.1).

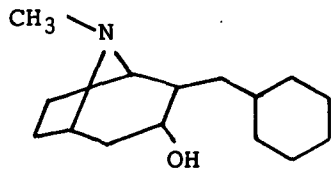


3.XV

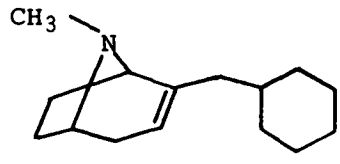
3.XVI

Scheme 3-4

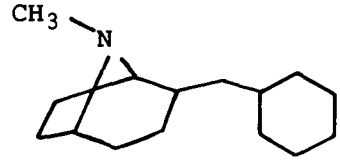
A number of methods were tried, without success, to achieve total reduction of 3.XV. Catalytic hydrogenation of tropan-3-one in 1 M HCl over Adams catalyst has been shown to effect the efficient reduction of this ketone to tropane[51]. Under the same conditions, the 2-benzyl derivative was not reduced. Use of longer reaction times caused reduction of the aromatic ring to produce 2-(methyl cyclohexyl)-3α-hydroxy tropane. (3.XVII). Clemmensen reduction conditions gave 2-benzyl-3β-hydroxy tropane as the major product.



3.XVII



3.XVIII



3.XIV(a)

Total reduction was eventually achieved by partial hydrogenation to the alcohol, dehydration to 3.XVIII, and hydrogenation to 2-(methyl cyclohexyl) tropane 3.XIV(a). Spectral data is shown in Figs. 3-14(a) and 3-14(b).

3.7. Structural Elucidation of Alkaloid F2'

The second base separated from the repeated chromatography of fraction F2 had R_f = 0.29 (12% MeOH in $CHCl_3$, silica gel) and crystallized in needles from petroleum ether, with m.p. 136-138° $[\alpha]_D$ = +28°. High-resolution mass spectroscopy indicated a molecular formula $C_{22}H_{25}NO_3$.

3.7.1. Spectroscopic Studies

The P.M.R. spectrum (Fig. 3-23) indicated a complex aromatic pattern consisting of a multiplet at 2.18τ, (2H), and an ill-defined multiplet at 2.65τ (3H), assigned to the aromatic protons of a benzoyloxy

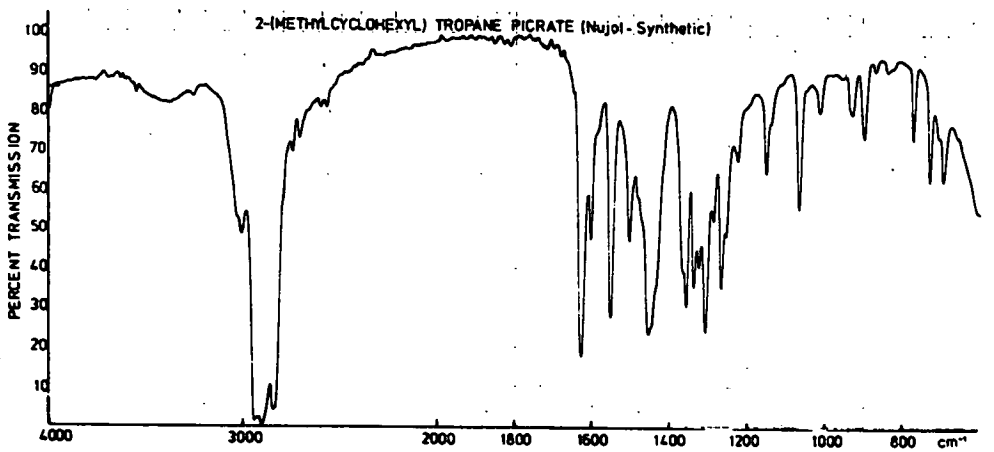


Fig. 3-14(a)

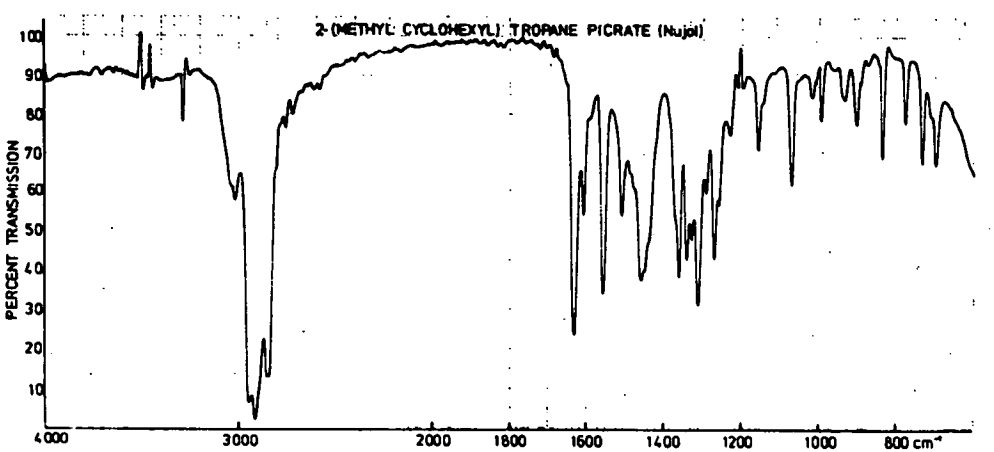


Fig. 3-14(b)

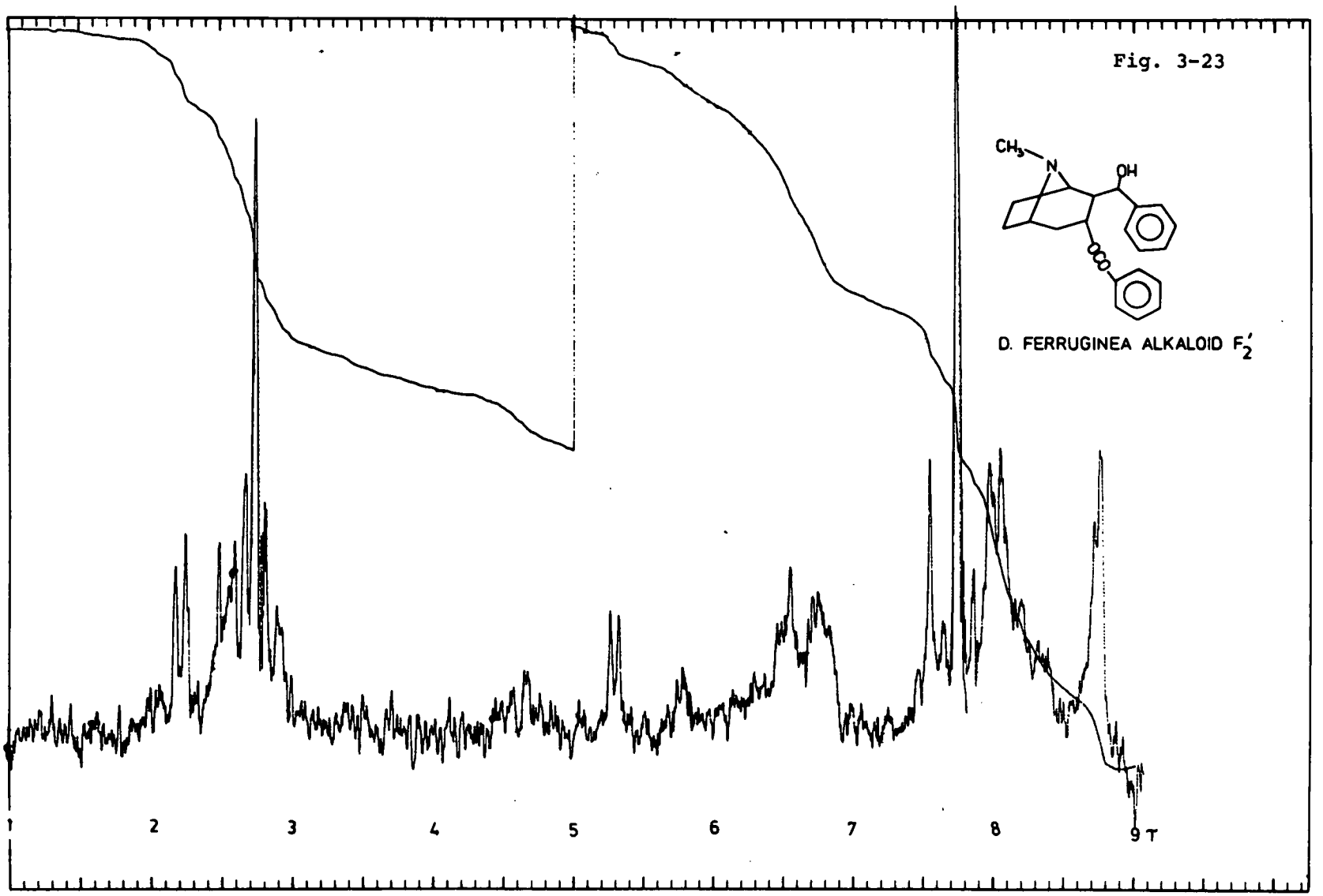
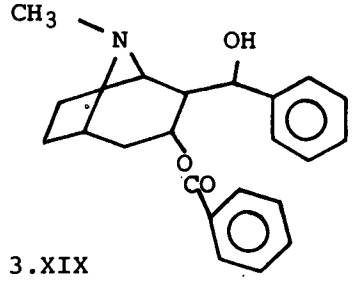


Fig. 3-23
CH3
N
OH
D. FERRUGINEA ALKALOID F2'
1
2
3
4
5
6
7
8
9 T

group. A complex multiplet of five protons over the range 2.45-2.80τ corresponding to benzylic protons. A doublet at 5.28τ, J = 8 Hz, was assigned to a methine proton on a hydroxy benzylic carbon, and a broad multiplet at 5.72τ to a methine adjacent to an acyloxy substituent. Two one-proton multiplets present at 6.65τ and 6.80τ were assigned to methine protons adjacent to nitrogen. A singlet at 7.75τ (3H) was attributed to an N-methyl group. The remaining seven protons contributed to the complex envelope of signals in the range 7.90τ-8.50τ.

The infrared spectrum (Fig. 3-24) showed the presence of an hydroxy group at 3400 cm^{-1}, and an aromatic ester carbonyl at $\nu_{C=O}$ 1710 cm^{-1}, supported by aromatic bands at 1580 cm^{-1} and 1600 cm^{-1}. The ultraviolet spectrum (Fig. 3-26) confirmed the aromatic ester assignment: λ_{max} = 280 nm, ε_{max} 1,000, λ_{max} = 233 nm, ε_{max} = 10,200, λ_{max} = 210 nm, ε_{max} = 10,480. These data for F2' were interpreted in terms of structure 3.XIX.



3.XIX

The mass spectrum is in good accord with this structure. The base peaks occurred at m/e 96 and 82, consistent with the tropane moiety, and peaks at m/e 244 and m/e 230, 229 corresponded to a loss of a hydroxy benzyl group and a benzoic acid moiety respectively. The peaks observed at m/e 122, 105 and 77 were characteristic of fragments derived from the benzoate ester group. The mass spectral fragmentation is outlined in Scheme 3-5.

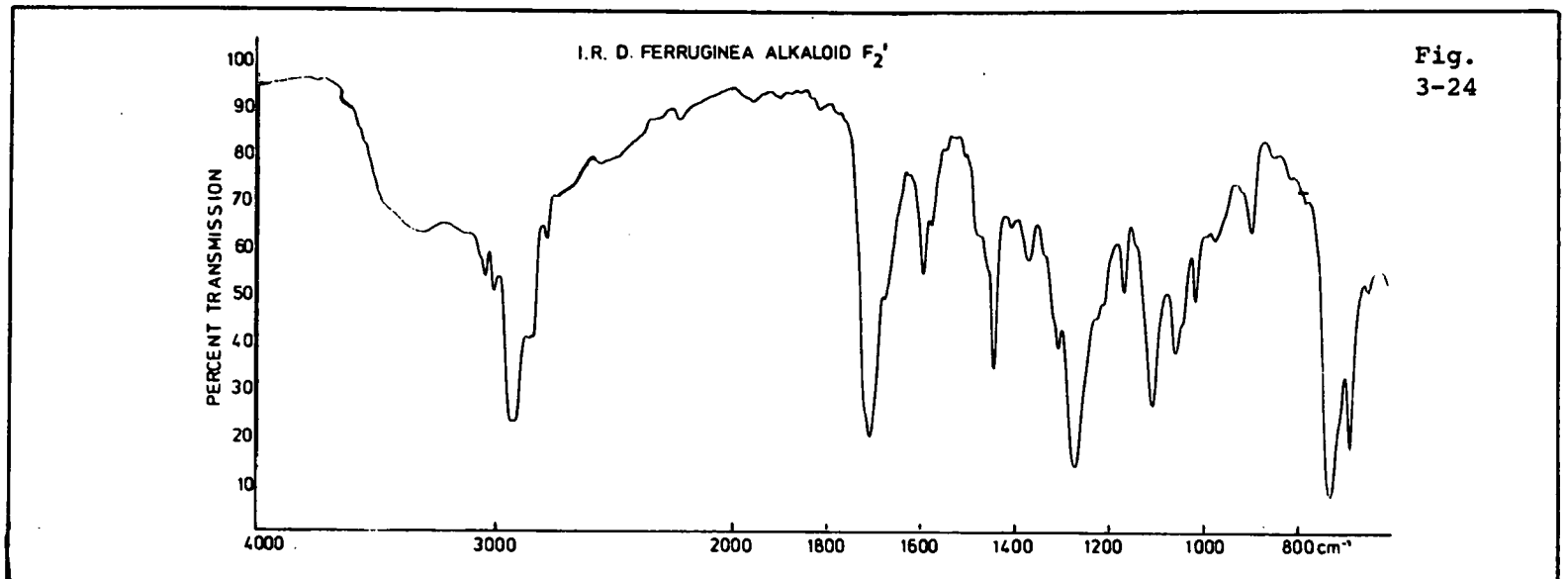


Fig. 3-24

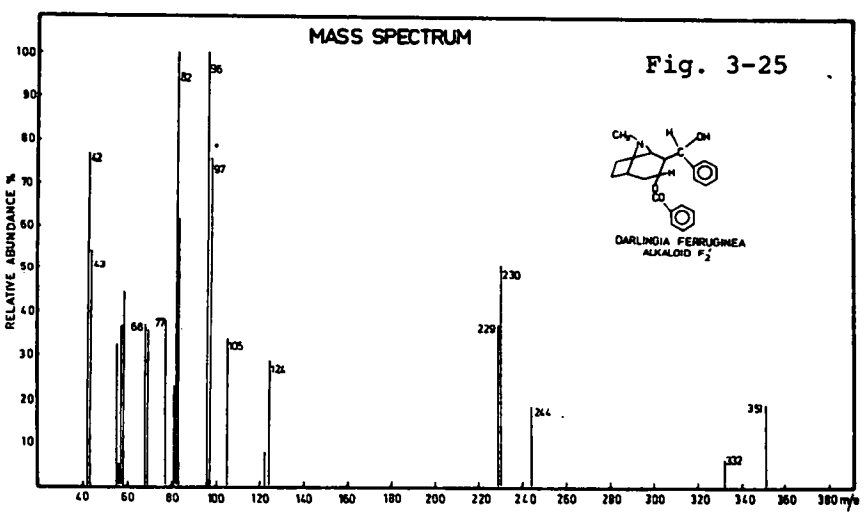


Fig. 3-25

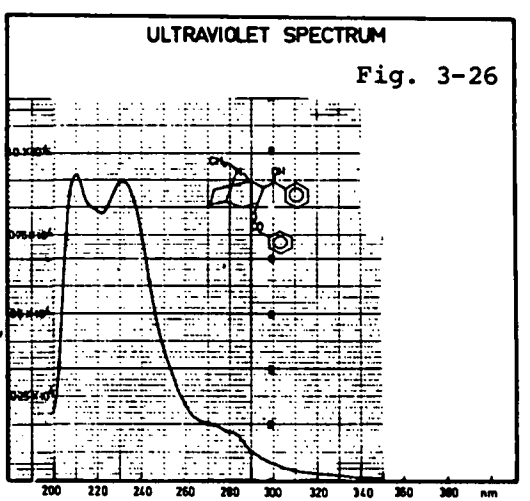
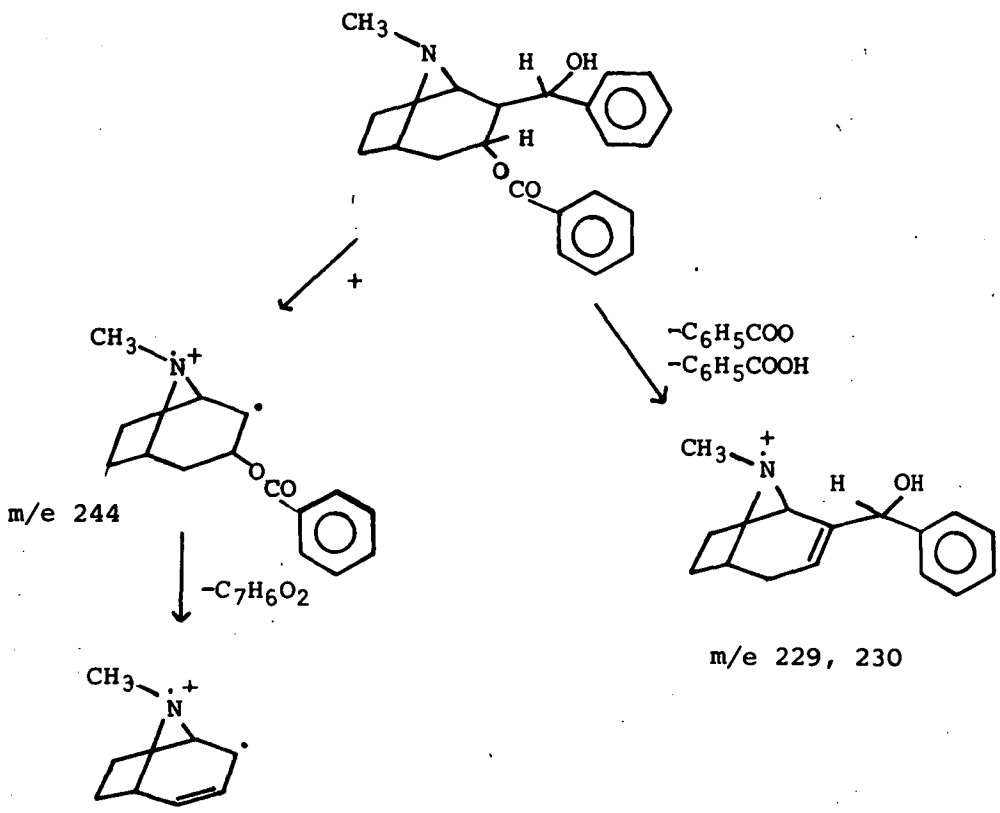
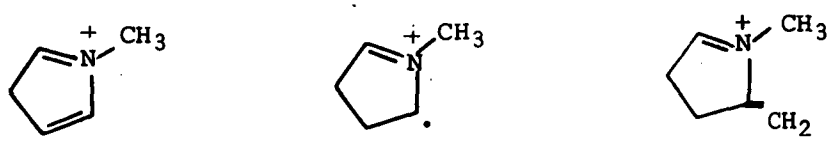


Fig. 3-26

Mass Spectral Fragmentation Pattern, Alkaloid F2'





m/e 82 m/e 83 m/e 96

Scheme 3-5

3.7.2. Relationship of D. ferruginea and K. deplanchei bases

It is likely that the minor unidentified base, m/e 351, reported by Lounasmaa to occur in the related plant Knightia deplanchei has the same structure as alkaloid F2'. F2' is seen to bear a close relationship to the minor bases of K. deplanchei and indicates the likelihood of a similar biogenesis involving reduction of a 2-benzoyl tropan-3-one In D. ferruginea, total reduction of the tropane-carbonyl

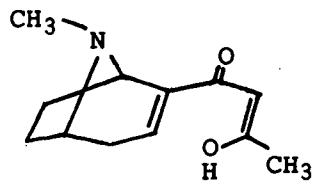
produces Ferrugine (3.XIII). In K. deplanchei total reduction of the benzoyl-carbonyl produces products A,B,C, and D. (Section 1.2.5). Partial reduction of the benzoyl-carbonyl produces F2' in D. ferruginea, and products E and F in K. deplanchei.

3.8. Structural Elucidation of Alkaloid F3 (Ferruginine)

The base which was isolated from the low R_f band 0.22 of D. ferruginea was obtained as a mobile oil, $[\alpha]_D^{19} = +37°$*, with a molecular formula of $C_{10}H_{15}NO$ as shown by high-resolution mass spectroscopy coupled to a gas chromatograph.

3.8.1. Spectroscopic Studies

The P.M.R. spectrum (Fig. 3-28) shows an olefinic triplet at 3.30τ (1H, J = 5 Hz), ascribed to the X part of an ABX system; a doublet at 6.08τ (1H, J = 7 Hz), assigned to a methine proton adjacent to nitrogen and in the deshielding zone of a carbonyl group (analogous to the H_6 signal in the γ-pyronotropane series); a broadened multiplet at 6.72τ (1H) attributed to a methine proton adjacent to nitrogen; and the A part of an ABX system at 7.30τ, coupled geminally (J_{gem} = 17 Hz) to the B proton at 7.90τ. Two three-proton singlets at 7.70τ and 7.75τ were assigned to an N-methyl group and a deshielded acetyl-C-methyl group, respectively. The spectrum bore considerable similarity to that of the 2-acyl-trop-2-ene intermediate 3.XX in the dihydroisobellendine synthesis.



3.XX

*The purity of this non-crystalline compound may be estimated only from the NMR spectrum (Fig. 3.28).

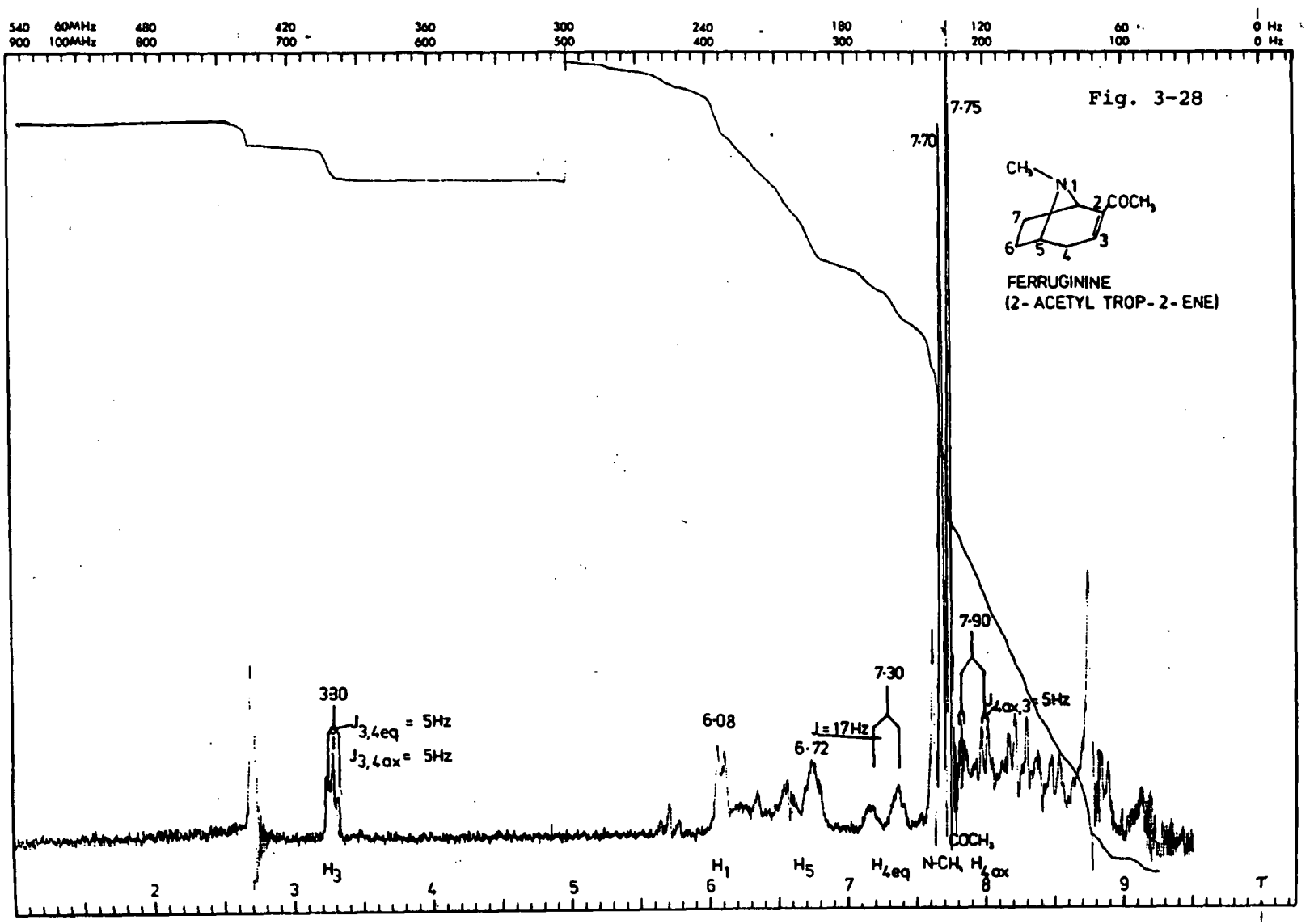
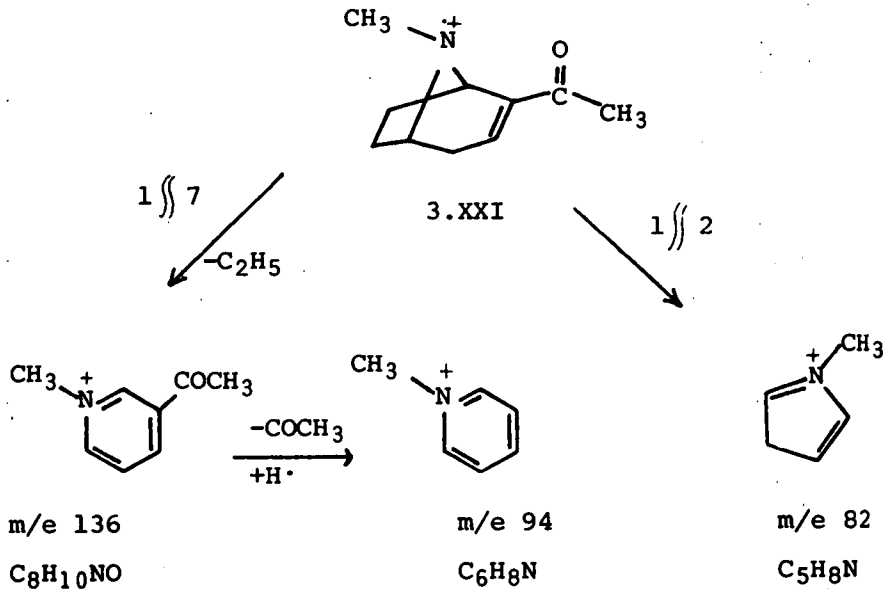


Fig. 3-28
FERRUGININE
(2- ACETYL TROP- 2- ENE)
7·75
7·70
7·90
7·30
6·72
6·08
3·30
J = 17Hz
J3,4eq = 5Hz
J3,4ax = 5Hz
J4ax,3 = 5Hz
H3
H1
H5
H4eq
N-CH3
H4ax
COCH3
τ

Two bands at $\nu_{C=O}$ 1680 cm^{-1} and $\nu_{C=C}$ 1600 cm^{-1} in the infrared spectrum (Fig. 3-29) of 3.XX indicated the presence of an α,β unsaturated carbonyl group, an assignment which was supported by the ultraviolet spectrum (Fig. 3-31) in which the chromophore absorbed at λ_{max} = 233 nm, ε_{max} = 10,070. The Woodward-Fieser rules for α,β unsaturated carbonyls give a value of 235 nm for this system. The mass spectrum (Fig. 3-30) supported structure 3.XXI, 2-acetyl trop-2-ene, for alkaloid F3. The principal fragments, which were highly resolved, may arise from a fragmentation process outlined in Scheme 3-6.

Mass Spectral Fragmentation Pattern, Alkaloid F3 (Ferruginine)



Scheme 3-6

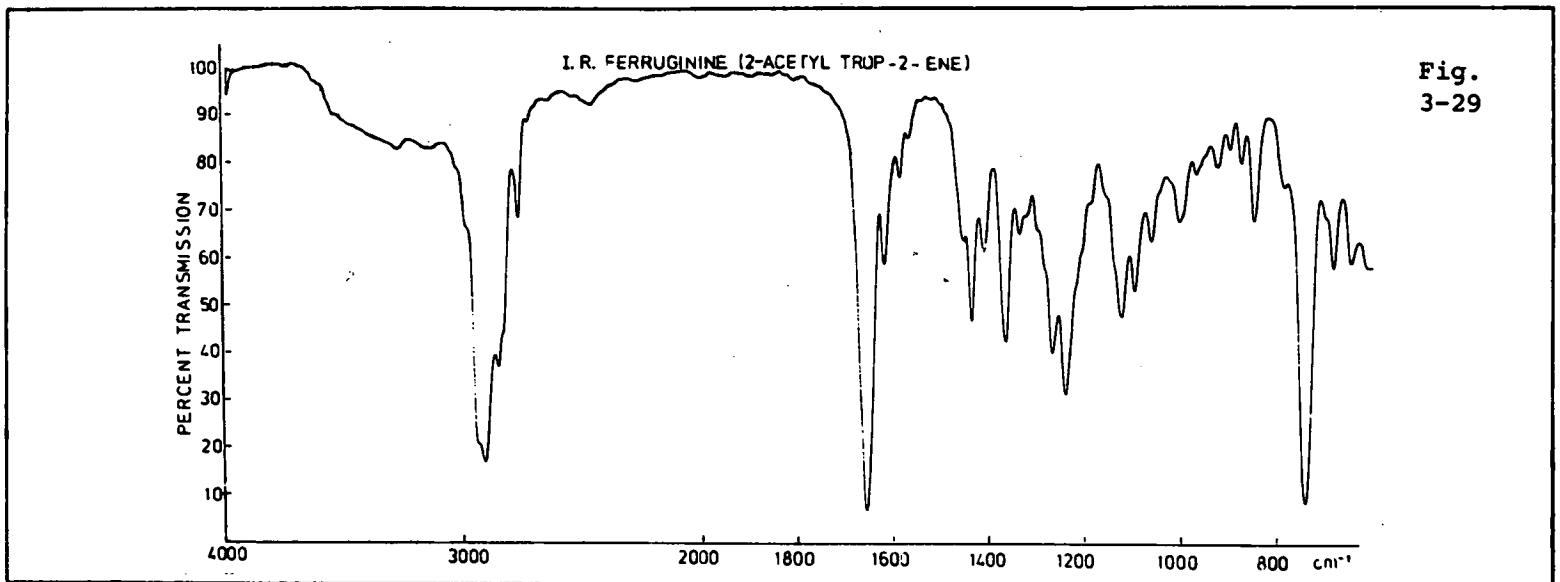


Fig. 3-29

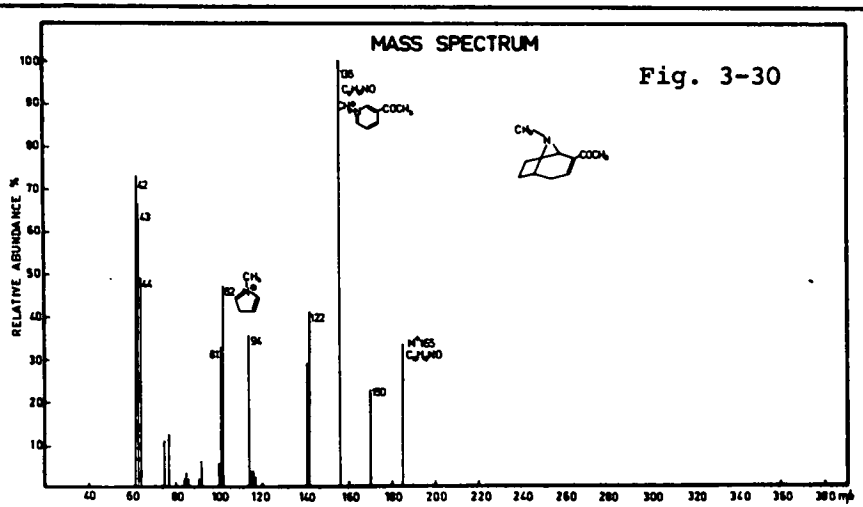


Fig. 3-30

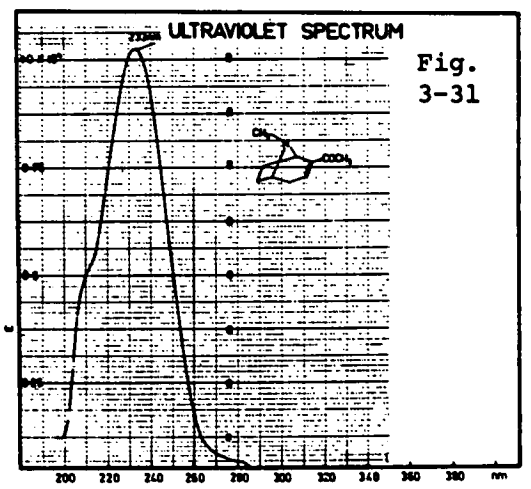
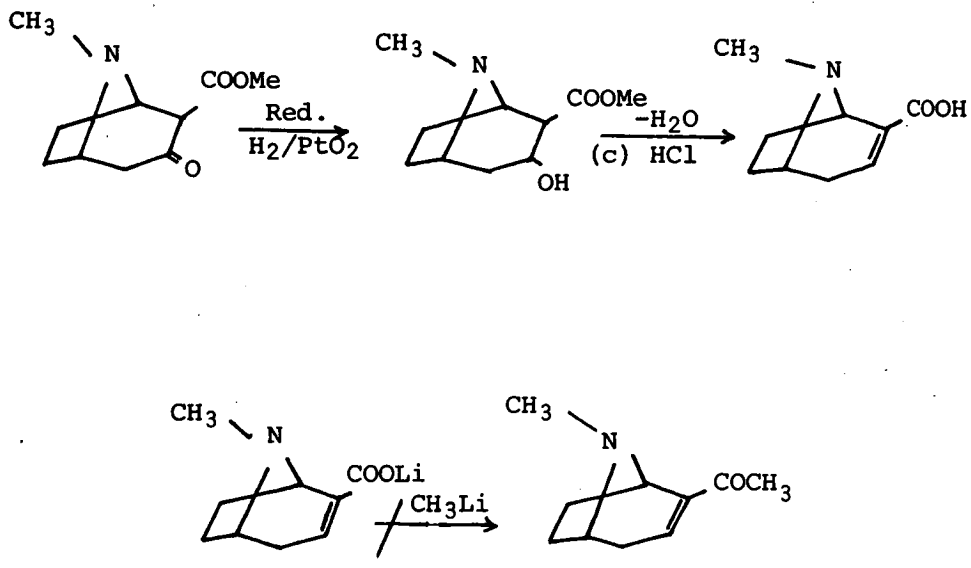


Fig. 3-31

3.8.2. Attempted Synthesis of Alkaloid F3

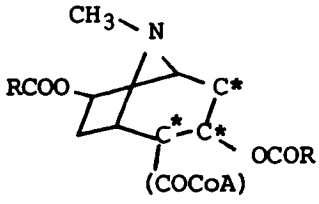
An attempt to synthesize Alkaloid F3 by a condensation of the lithium salt of anhydroecgonine and methyl lithium[52], as outlined in Scheme 3-7, resulted in recovery of the salt of anhydroecgonine at the final stage. The failure to react may have been due to the partial solubility of the lithium salt in the solvent, dimethoxyethane. To compensate for this, a long reaction time was utilized, however after periods of four and eight hours, no appreciable reaction had taken place.



Scheme 3-7

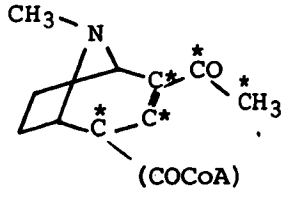
3.9. Conclusion

The species of the Proteaceae under study have been shown to produce alkaloids which offer diversity to the well-described tropane class. Such diversity can be rationalized, biogenetically, as being due to an extension of the di-acetate chain which contributes carbons C-2, C-3, and C-4 to the nucleus of the classical tropane alkaloids. The Proteaceae produce tropanes containing two, three and four acetate units, leading to the structures shown in Scheme 3-8.



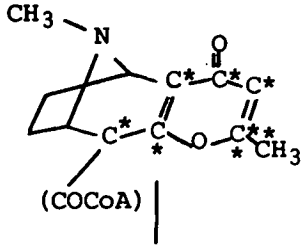
2 Acetate Units

Alkaloids B3, B6



3 Acetate Units

Alkaloid F3



4 Acetate Units

Isobellendine

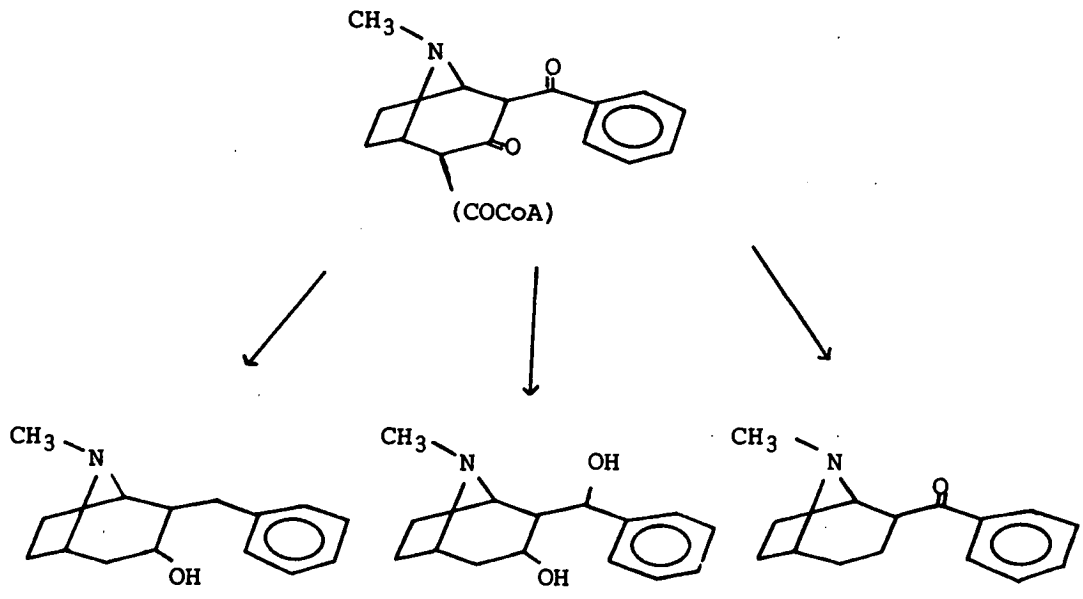
Reduction → Dihydroisobellendine

1,2 Methyl shift → Bellendine

Methylation → Darlingine

Scheme 3-8

In addition to bases which are derived from an extended acetate chain, there appear two types of Proteaceous alkaloids which arise from an α polyketide bearing a non-acetate terminal moiety. The F2, F2' and Knightia bases may arise from a condensation between ornithine and a polyacetate unit terminated by a benzoyl group. Subsequent partial or total reduction of the carbonyl groups leads to the bases shown in Scheme 3-9.



Knightia deplanchei | Knightia deplanchei, Darlingia ferruginea | Darlingia ferruginea

Scheme 3-9

The pyrrolidine base, Darlingiana D3, (3.X) may arise from a polyacetate chain terminated by a cinnamoyl group. Reduction and elimination to the conjugated diene may prevent the cyclization leading to the tropane nucleus.

As phylogenetic variables, the alkaloid structures provide useful additional information in discriminating the two Darlingia species from each other, and from the Knightia species, whilst linking each of these species through a probable common biogenetic pathway. The limitation of alkaloid chemotaxonomy is clearly seen however, when reference is made to the phylogeny of the Proteaceae in Fig. 2-1. The presence of γ-pyronotropane alkaloids in species arising from such diverse tribes as the Persoonieae and the Grevilleoideae indicates the need for caution when proposing phylogenetic schemes which rely exclusively on chemical evidence.

3.10. Experimental

3.10.1. Extraction of D. darlingiana

Finely ground upper stems and leaves of Darlingia darlingiana (F. MUELL.) L.A.S. JOHNSON from Atherton, North Queensland (13 kg) were exhaustively extracted with Prollius solution. The extract was concentrated at reduced pressure to a resinous tar (500 g) and the concentrate dissolved in warm glacial acetic acid (1 ℓ). This solution was poured in a fine stream into 7 ℓ of water whilst rapidly agitating the solution. After 2 hr, a black resinous precipitate had settled out and this was removed by filtration through a bed of celite. The filtrate was neutralized with $NaHCO_3$, a second precipitate was removed by filtration, and the solution basified to pH 8 with ammonia (d = 0.880) before extraction with chloroform (10 × 500 ml). The combined chloroform extracts were dried (Na_2SO_4) and freed of solvent in vacuo to yield 18 g of a viscous brown oil.

Part of the crude alkaloid mixture (10 g) was dissolved in chloroform and subjected to Craig distribution using chloroform as the stationary phase and water as the mobile phase. No alkaloid was eluted in the first 100 transfers; the mobile phase was changed to 0.5×10^{-3} M H_2SO_4 and every tenth fraction was monitored by T.L.C. after concentration, basification and extraction of the aqueous eluant. The bulking summary is shown in Table 3-2. Bulked fractions from the Craig distribution were further purified by P.T.L.C.

3.10.2. Characteristics of the D. darlingiana bases

TABLE 3-2

Summary of Craig Distribution Fractions

(From 7 Kg dry plant material)

Craig No.	P.T.L.C. Code	Comments and Identification
0-130	-	No constituents
131-285	D1, D4	Darlingine 1 and D4 (4 g)
286-340	D3, D4	D3 major component, trace D4 (700 mg)
341-345	D4	Trace quantity
346-400	D2	Trace quantity

Darlingine, the major alkaloid, was extracted from the major band of fractions 131-258, and recrystallized from ether/petroleum ether, m.p. 162-163° $[\alpha]_D^{19}$ = 102°. Found: C, 70.94%; H, 7.70%; N, 6.23%. Calculated for $C_{13}H_{17}NO_2$: C, 71.20%; H, 7.74%; N, 6.38%. High-resolution mass spectroscopy indicated the molecular formula to be $C_{13}H_{17}NO_2$.

Synthesis of Darlingine

The successful synthesis of darlingine is discussed in Chapter 4.

Alkaloid D3

Craig fractions 286-340 were combined and chromatographed on P.T.L.C. (12% MeOH in $CHCl_3$, silica gel). Two bands of nearly identical R_f were separated from each other by multiple development using

the same solvent system. Material from the top (major) band was purified by crystallization from petroleum ether to give D3, m.p. 93.5°, $[\alpha]_D$ = +62°, $C_{17}H_{23}NO$ (H.R.M.S.). An attempt to form a hydrobromide of the base resulted in decomposition. The bottom band from P.T.L.C. was not further investigated. , $C_{17}H_{23}NO$ requires C, 79.38%; H, 8.95%; N, 5.45%. Found C, 79.19%; H, 8.86%; N, 5.33%.

Reduction of D3

A 25 mg sample of D3 was dissolved in ethanol (3 ml), Adams catalyst (10 mg) was added and the base hydrogenated at 60 p.s.i. for 4 hr. The catalyst was removed by centrifugation, the solvent distilled *in vacuo* and the oil (25 mg) was chromatographed on P.T.L.C. (12% MeOH in $CHCl_3$, silica gel). Only one product was formed, R_f = 0.60, for which spectroscopic evidence suggested the structure 1-methyl-2-(2'-hydroxy-6'-cyclohexyl-hexyl)pyrrolidine, M^+ 267, base peak 84.

Preparation of D3 acetate

D3 (25 mg) was dissolved in 1 ml chloroform and 2 ml acetyl chloride was added. The solution was heated on a water bath at 70° for 0.75 hr, chloroform was added (10 ml) and the solution was extracted with 20% aq. Na_2CO_3 (5 × 2 ml). After drying, the chloroform solution was concentrated and chromatographed on P.T.L.C. (10% MeOH in $CHCl_3$, silica gel). The band at R_f = 0.65 was extracted to yield 9 mg of an oil which had spectral characteristics consistent with an acetate of a secondary alcohol.

3.10.3. Extraction of D. ferruginea

The dried roots and stems of Darlingia ferruginea J.F. BAILEY (18 kg) collected from Davies Creek, North Queensland, were finely ground

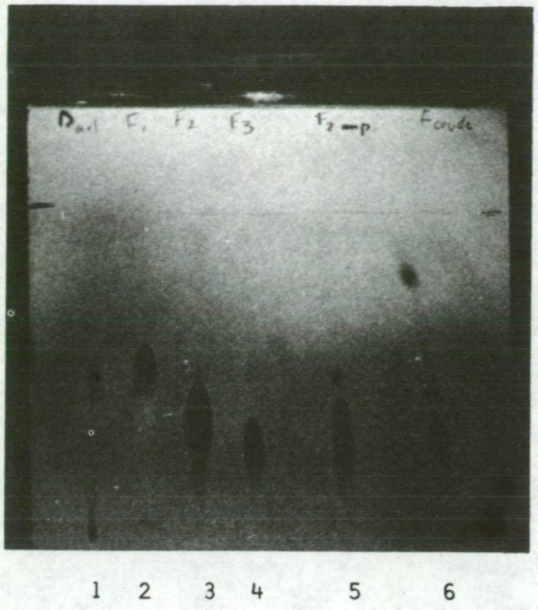
and continuously extracted at room temperature with Prollius solution. The extract was concentrated in vacuo (< 35°C) to yield a tarry mass (250 g). The concentrate was dissolved in warm glacial acetic acid. (2.5 ℓ) and the solution poured in a thin stream into rapidly agitated water (16 ℓ). The non-alkaloidal precipitate was removed by filtration through a bed of celite. A further non-alkaloidal precipitate which formed upon neutralization was similarly removed. The filtrate was basified (NH_4OH 0.880), whereupon a further precipitation occurred which partly occluded some alkaloid. The precipitate was removed by filtration, dried and dissolved in 1 ℓ of 50:50 chloroform methanol. Addition of an equal quantity of 1% H_2SO_4 caused the separation of an aqueous alkaloid-positive phase which was removed, washed with chloroform (3 × 500 ml), basified and extracted with chloroform (3 × 500 ml). The second extract was reserved and combined with the chloroform extract of the initial basified aqueous phase (5 × 3 ℓ).

Concentration of the dried chloroform extract in vacuo yielded a crude alkaloid fraction (18 g). This was further purified by redissolving in chloroform (300 ml) and extraction with 5% H_2SO_4 (5 × 50 ml). The aqueous extract was basified and re-extracted with chloroform (4 × 50 ml) to yield 14 g of crude alkaloids.

3.10.4. Isolation and characteristics of the D. ferruginea bases

The crude fraction was chromatographed on 1 m silica gel thin-layer plates (12% MeOH in $CHCl_3$, silica gel). The major base, darlingine, was isolated by extraction of the grey, Schlittler-positive band at R_f = 0.50. The thin-layer chromatogram of the minor bases is shown in Fig. 3-32 to be extensively overlapped. Preparative thin-layer chromatography on plates coated with silica gel prepared with 0.5 N KOH, enabled a better resolution of the bases: upper and lower cuts of the

T.L.C. Comparison of D. ferruginea alkaloids



1. Darlingine standard
2. Darlingine
3. 2α-Benzoyl tropane (F_2)
4. 2-acetyl trop-2-ene (F_3)
5. mid-band PTLC impure (F_2')
6. D. ferruginea crude alkaloids.

Fig. 3-32

G.L.C. Eluant Curve

X = Solvent, Attn 50 × 10^4

Y = Attn 50 × 10^2

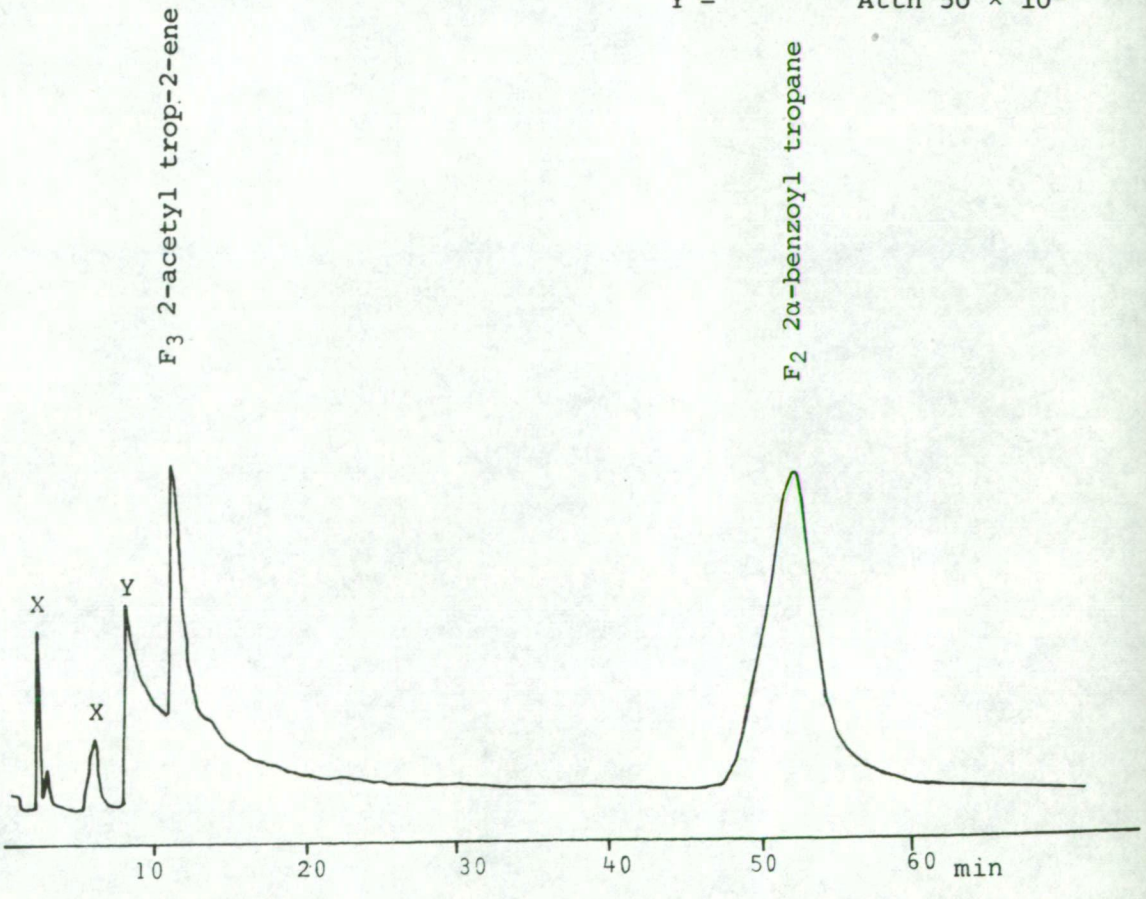


Fig. 3-33

band at R_f = 0.37 separated F2 and F3 which were purified by further P.T.L.C. The mid-band was rechromatographed to yield 25 mg of F2'.

G.L.C. on a mixture of the minor bases separated F2 from F3; F2 was retained on the column. Column: glass; 6" × 0.25"; column packing: Oviol. Temperature; column, isothermal, 190°C; injector, 250°C; detector, 250°C. Carrier gas: He 40 ml/min. The eluant curve is shown in Fig. 3-33.

TABLE 3-3

Summary of D. ferruginea minor bases

(Silica gel, 12% MeOH in $CHCl_3$)

Code	R_f	Composition
F2	0.37	2α-benzoyl tropane 110 mg
F2'	0.29	2-(hydroxybenzyl)-3α-benzoyloxy tropane
F3	0.22	2-acetyltrop-2-ene

Alkaloid F2

High-resolution mass spectroscopy indicated a molecular formula of $C_{15}H_{19}NO$. The picrate, crystallized from ethanol, had m.p. 192-194°. Found: C, 53.68%; H, 4.71%. Calculated for $C_{15}H_{19}NO.C_6H_3N_3O_7$: C, 53.56%; H, 4.60%.

3.10.5. Synthesis of 2-(methyl cyclohexyl) tropane

(i) Synthesis of dimethyl-2-benzyl-3-oxoglutarate

Dimethyl-3-oxoglutarate (17.4 g) prepared according to the method for the ethyl ester in Organic Synthesis Coll. Vol. 1, p. 237 was dissolved in dry benzene (250 ml) and 4 g NaH (60% suspension in oil) were added in small quantities with stirring and refluxing. After 2 hr, the soluble sodium enolate was treated with benzyl bromide (17.1 g) and the solution refluxed for 6 hr. The precipitated NaBr was removed by filtration and the solution washed with sat. NH_4Cl solution. The dried (Na_2SO_4) solution was then concentrated in vacuo and the final product vacuum distilled (180-182°, 5×10^{-2} mm) to give a product whose spectral characteristics were consistent with dimethyl-2-benzyl-3-oxoglutarate.

(ii) Synthesis of 2-benzyl-2,4-dicarbomethoxy tropanone

2,5-Diethoxytetrahydrofuran was prepared from 2,5-diethoxy-dihydrofuran by high pressure hydrogenation (2000 p.s.i.) over Raney nickel according to the method of N. Elming[39]. 2,5-Diethoxy tetrahydrofuran (5.4 g) was dissolved in 3.5 ml of 1 M HCl and refluxed for 0.5 hr. After neutralization, it was added to a solution of 8.0 g of dimethyl-2-benzyl-3-oxoglutarate and 2.5 g methylamine hydrochloride in 230 ml of methanol. The reaction mixture (pH 4.3) was left to stand at room temperature for 36 hr and 0° for 48 hr. After this time, the pH had dropped to 3, and approximately half the solvent was removed in vacuo; water was added (50 ml), followed immediately by the appearance of crystals which were removed by filtration (4 g). The reaction mixture was then basified ($NaHCO_3$) and extracted with $CHCl_3$ (5 × 100 ml). The oil (6 g) which remained after removal of the solvent was crystallized from aqueous dimethoxy ethane, m.p. 130-130.5°. Found: C, 66.06%;

H, 6.76%; N, 3.89%. Calculated for $C_{19}H_{23}NO_5$ C, 66.10%; H, 6.70%; N, 4.03%.

(iii) Hydrolysis and decarboxylation of 2-benzyl-2,4-dicarbomethoxy tropanone

A number of reaction conditions were examined in order to effect hydrolysis and decarboxylation of the diester. A table of reaction conditions summarizes the results of these experiments.

TABLE 3-4

Table of Hydrolysis Conditions

Reaction No.	Reaction Conditions	Product and Comment
1	2 M NaOH (aq) MeOH R.T. 24 hr	Starting material 40% mono ester 8% 2-Benzyl tropan-3-one 6%
2	2 M NaOH (aq) MeOH R.T. 24 hr, H^+ (c)	Starting material 40% mono ester 10% 2-Benzyl tropan-3-one 15%
3	Dowex, X-2, H^+	Starting material 85% mono ester 10%
4	6 M H_2SO_4 (aq) Reflux 1 hr	Unchanged starting material
5	6 M HCl (aq) Reflux 20 hr (N_2)	Starting material 20% mono ester 10% 2-Benzyl tropan-3-one 30%
6	2 M NaOH (aq) MeOH Reflux 0.5 hr 80°	2-Benzyl tropan-3-one 15% Extensive resinification

The use of aqueous base and methanol at 80° (Reaction 6) was found to effect total hydrolysis, but very extensive resinification occurred. Reaction 5 was found to be the most satisfactory hydrolysis method: the acidic medium caused spontaneous decarboxylation of the β-keto acid to 2-benzyl tropan-3-one. 2-Benzyl-2,4-dicarbomethoxy tropan-3-one (1 g) was dissolved in 50 ml 6 M HCl and the solution refluxed for 20 hr under N_2. A black precipitate which formed was removed by filtration through celite, the filtrate was basified with ammonia and extracted with chloroform (5 × 50 ml). The dried chloroform solution was concentrated in vacuo and chromatographed P.T.L.C., (10% MeOH in $CHCl_3$, silica gel), to yield 300 mg of 2-benzyl tropan-3-one. The spectral data for this compound are shown in Appendix 3.1.

The base formed a picrate m.p. 202-203°, M^+ 229. Found: C, 53.62%; H, 4.49%. Calculated for $C_{15}H_{19}NO.C_6H_3N_5O$; C, 53.56%; H, 4.60%.

Reduction of 2-benzyl tropan-3-one

(i) Hydrogenation

2-Benzyl tropan-3-one (80 mg) was dissolved in 6 ml 1 M HCl and Adams catalyst (10 mg) was added. The base was hydrogenated at 60 p.s.i. and monitored by T.L.C. at 1.5 hr, 3.0 hr and 12 hr; only a small degree of change occurred in the two initial examinations. The product isolated after 12 hr was found to be 2-(methylcyclohexyl)tropan-3α-ol m.p. 131-133°, M^+ 237 after P.T.L.C. For spectral data, see Appendix 3.1.

(ii) Clemmensen Reduction

Amalgamated zinc was prepared by dissolving 60 mg $HgCl_2$ in 1 ml of 0.33 M HCl. To this solution, zinc dust, (600 mg) was added and the suspension stirred for 5 min. The supernatant liquor was decanted and 2 ml of 7 M HCl containing 320 mg of 2-benzyl tropan-3-one was added.

The solution was refluxed for 24 hr under N_2, 0.5 ml (conc) HCl being added over this time. The remaining zinc was filtered off and the solution basified and the precipitate $Zn(OH)_2$ which formed was filtered off, dried, and extracted with $CHCl_3$. The aqueous phase was then extracted until no further Mayers test was given by the extract. The dried chloroform solution was reduced in volume in vacuo and chromatographed on P.T.L.C. (10% MeOH in $CHCl_3$, silica gel). The major band which was extracted (R_f = 0.50) had spectral characteristics consistent with 2-benzyl-3β-hydroxy tropane as discussed in Chapter 2.

(iii) Dehydration

2-(Methyl cyclohexyl)tropane (78 mg) from (i) above was dissolved in a solution of 1 ml glacial acetic acid and 2 ml (conc) sulphuric acid. The solution was slowly heated on a water bath to 100° for 20 min. At the end of this time water was cautiously added, (10 ml) and the solution basified with ammonia. The solution was then extracted with chloroform (5 × 10 ml) and the combined chloroform extracts dried and chromatographed on P.T.L.C. (6% MeOH in $CHCl_3$, silica gel). The major band, R_f = 0.63, was extracted to yield 48 mg of a light coloured oil which was used directly in the hydrogenation below.

(iv) Hydrogenation

2-(Methyl cyclohexyl)trop-2-ene (48 mg) was dissolved in 5 ml ethanol and 2 drops (conc) HCl was added. The solution was hydrogenated over Adams catalyst (20 mg) for 3 hr. The catalyst was removed by centrifugation and the solution concentrated in vacuo. A saturated solution of ethanolic picric acid was added and a picrate, m.p. 239-243°, slowly deposited. M^+ 221. $C_{15}H_{27}N.C_6H_3N_3O_7$ requires C, 54.5%; H, 6.66%. Found C, 54.72%; H, 6.78%.

Total Reduction of Alkaloid F2

F2 (18 mg) was dissolved in ethanol (6 ml) and 3 drops (conc) HCl were added. The solution was hydrogenated over Adams catalyst at 65 p.s.i. for 10 hr. The catalyst was removed by centrifugation and the solvent evaporated *in vacuo*. The product had spectral characteristics of 2-(methyl cyclohexyl)tropane hydrochloride. A picrate from ethanol had m.p. = 238-240°. $C_{15}H_{27}N.C_6H_3N_3O_7$ requires C, 54.5%; H, 6.66%. Found C, 56.11%; H, 6.92%.

Reduction of Alkaloid F2

F2 (82 mg) was dissolved in an amalgamated zinc suspension prepared in an identical manner to (2) above. The solution was refluxed for 24 hr and extracted in the same manner as (2). P.T.L.C. was carried out on the reaction products; the major band was found to be 2-(hydroxy benzyl)tropane, M^+ 231.

3.10.6. Synthesis of Knightia deplanchei Alkaloid B

2-Benzyltropan-3-one (45 mg) was dissolved in 95% ethanol (15 ml) and 10 mg Adams catalyst was added. The solution was hydrogenated for 4 hr at 60 p.s.i., the catalyst removed by centrifugation and the solvent distilled *in vacuo*. The oil (45 mg) which remained was dried over P_2O_5 (5×10^{-2} mm Hg) for 24 hr, after which it was dissolved in 10 ml chloroform and acetyl chloride (3 ml) was added. The solution was refluxed for 0.5 hr and left to stand for 24 hr. $NaHCO_3$ (1 g) was added and 1 ml of water, then the solution was extracted to yield 40 mg of an oil which was purified by P.T.L.C. and shown by spectroscopy to be identical to 2-benzyl-3α-acetoxy tropane (*Knightia deplanchei* Product B).

3.10.7. Attempted Synthesis of Alkaloid F3 (2-acetyltrop-2-ene)

(i) Synthesis of 2-carbomethoxy tropan-3-one

2-Carbomethoxy tropan-3-one was prepared according to the method of Findlay using a Robinson-Schöpf reaction involving monomethyl-3-oxo-glutarate as the diketone. The product was extracted and chromatographed on P.T.L.C. (10% MeOH in $CHCl_3$, silica gel) R_f = 0.42. The extracted material from the plates, on sublimation *in vacuo* (95°, 5 × 10^{-5} mm Hg) deposited fine white needles, m.p. 100-105° (lit., anhydrous form, 101-104°). P.M.R. analysis indicated an equal mixture of the 2α and 2β forms.

(ii) Synthesis of Anhydroecgonine Methyl Ester

Reduction of 2-carbomethoxy tropan-3-one to the 3β alcohol was carried out by dissolving 200 mg of the starting ketone in 15 ml methanol and adding 3 g $NaBH_4$. The reaction was left to stand for 12 hr and 15 ml water was added. The basic aqueous phase was extracted with chloroform (5 × 30 ml) and the chloroform solution dried (Na_2SO_4) and evaporated *in vacuo* to yield 170 mg of an oil which had spectral properties characteristic of an 80:20 mixture of 2-carbomethoxy tropan-3β-ol and 2-carbomethoxy tropan-3α-ol. The oil was dissolved in 10 ml of 10 M HCl and refluxed under nitrogen for 1.5 hr. 3 ml of the acid solution was withdrawn and added to 10 ml of methanol and this solution was refluxed for 5 hr. The methanol was removed *in vacuo*, water was added (2 ml), and the aqueous phase was basified and extracted with chloroform (5 × 20 ml). The oil was chromatographed on P.T.L.C. (10% MeOH in $CHCl_3$, silica gel) and the major band (R_f = 0.48) was extracted and found to have spectral characteristics identical to those of anhydroecgonine methyl ester; the principal peaks in the P.M.R.

spectrum were analogous to those of alkaloid F3.

P.M.R. 3.28τ (t, 1H), H_3; 6.28τ (s, 3H), COOMe; 6.20τ (d 1H), H_1; 6.78τ (t, 1H), H_5; 7.29τ, 8.00τ (d of d 2H), $H_{4ax, 4eq}$; 7.67τ (s, 3H), N-Me.

M^+ 181. I.R. $\nu_{C=O}$ 1700 cm^{-1}; $\nu_{C=C}$ 1610 cm^{-1}.

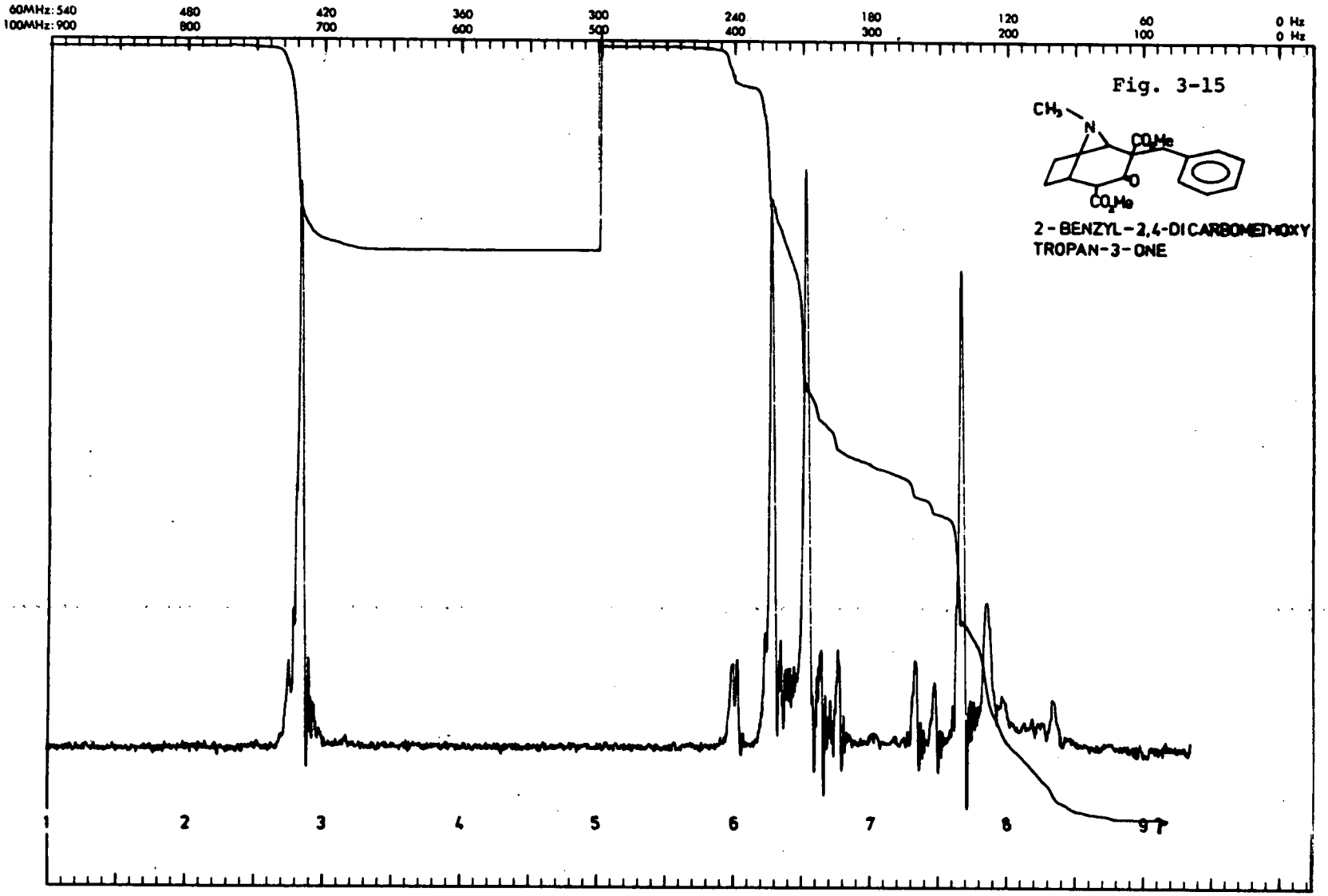
U.V. λ_{max} = 220 nm, ε_{max} = 8.600.

(iii) Attempted Methylation of Lithium Anhydroecgonine

The remainder (7 ml) of the above dehydration and hydrolysis reaction was evaporated in vacuo to dryness and the crystalline hydrochloride (110 mg, 0.05 millimoles) which deposited was dissolved in dry methanol (10 ml). Two molar equivalents of lithium methoxide (1.0 millimole) in methanol (3 ml) were added. The solution was evaporated to dryness, dissolved in dimethoxy ethane (6 ml) and 1.0 millimole of methyl lithium in dimethoxy ethane was added. The solution was refluxed for 4 hr, cooled and water was added (6 ml). The solution was then extracted with ether (10 × 10 ml) and the dried ether extract examined by T.L.C. No basic product was found in the ether extract. The aqueous solution was neutralized (2 M HCl) and evaporated to dryness. A partially crystalline product was converted to the methyl ester by refluxing in MeOH saturated with HCl. The product, recovered from P.T.L.C. was anhydroecgonine methyl ester.

The above reaction was repeated using a longer reaction time (8 hr). No basic material was extracted in the ether phase.

Appendix - 3.1


60MHz: 540
100MHz: 900
480
800
420
700
360
600
300
500
240
400
180
300
120
200
60
100
0 Hz
0 Hz
Fig. 3-15
CH3
N
CO2Me
O
CO2Me
2-BENZYL-2,4-DICARBOMETHOXY
TROPAN-3-ONE
1
2
3
4
5
6
7
8
9 τ

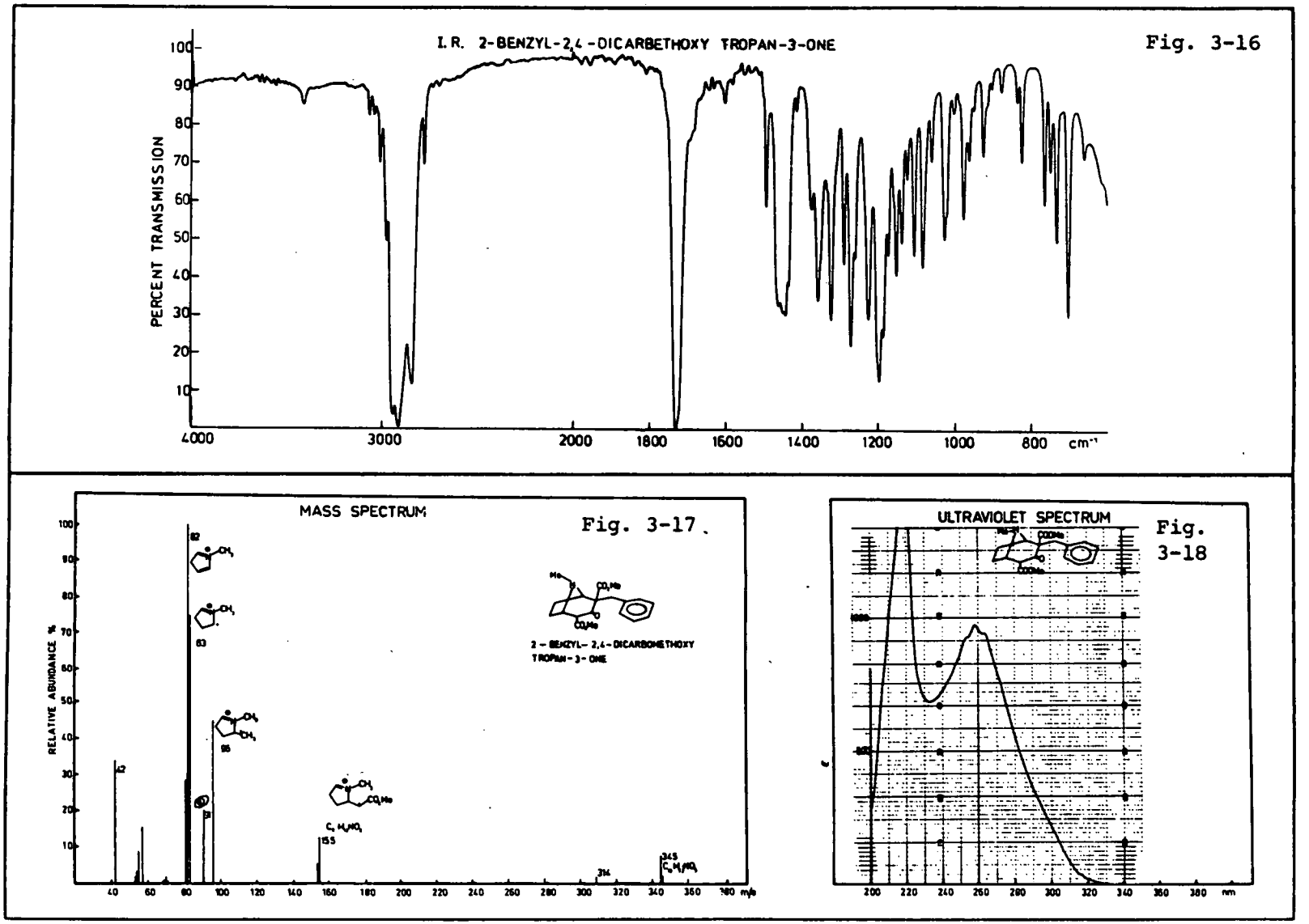
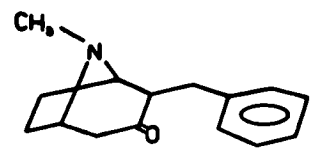


Fig. 3-16

Fig. 3-17

Fig. 3-18

Fig. 3-19



2-BENZYLTROPAN-3-ONE

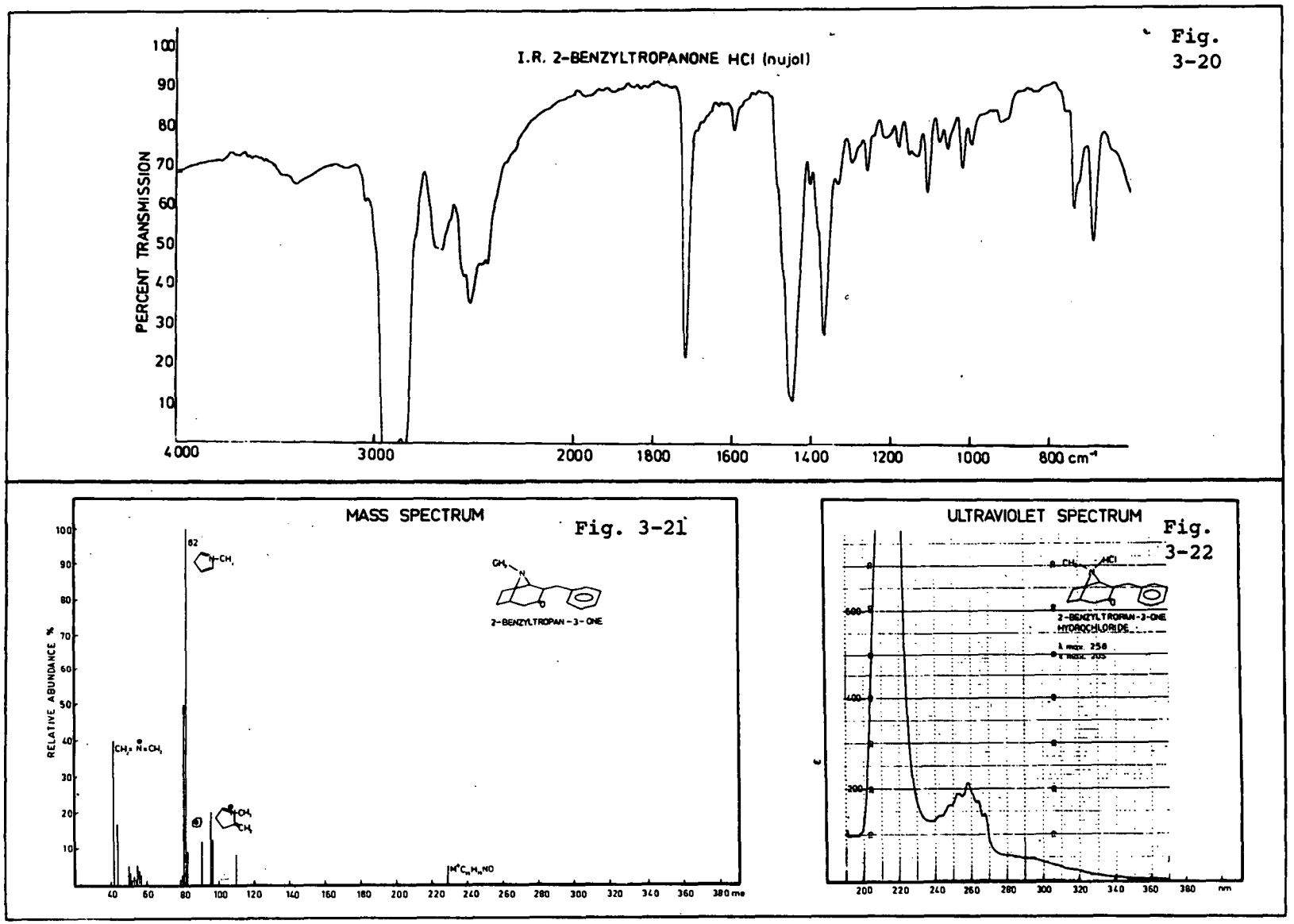
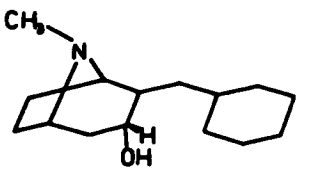


Fig. 3-20

Fig. 3-21

Fig. 3-22

Fig. 3-27



2-(METHYLCYCLOHEXYL)
TROPAN-3α-OL

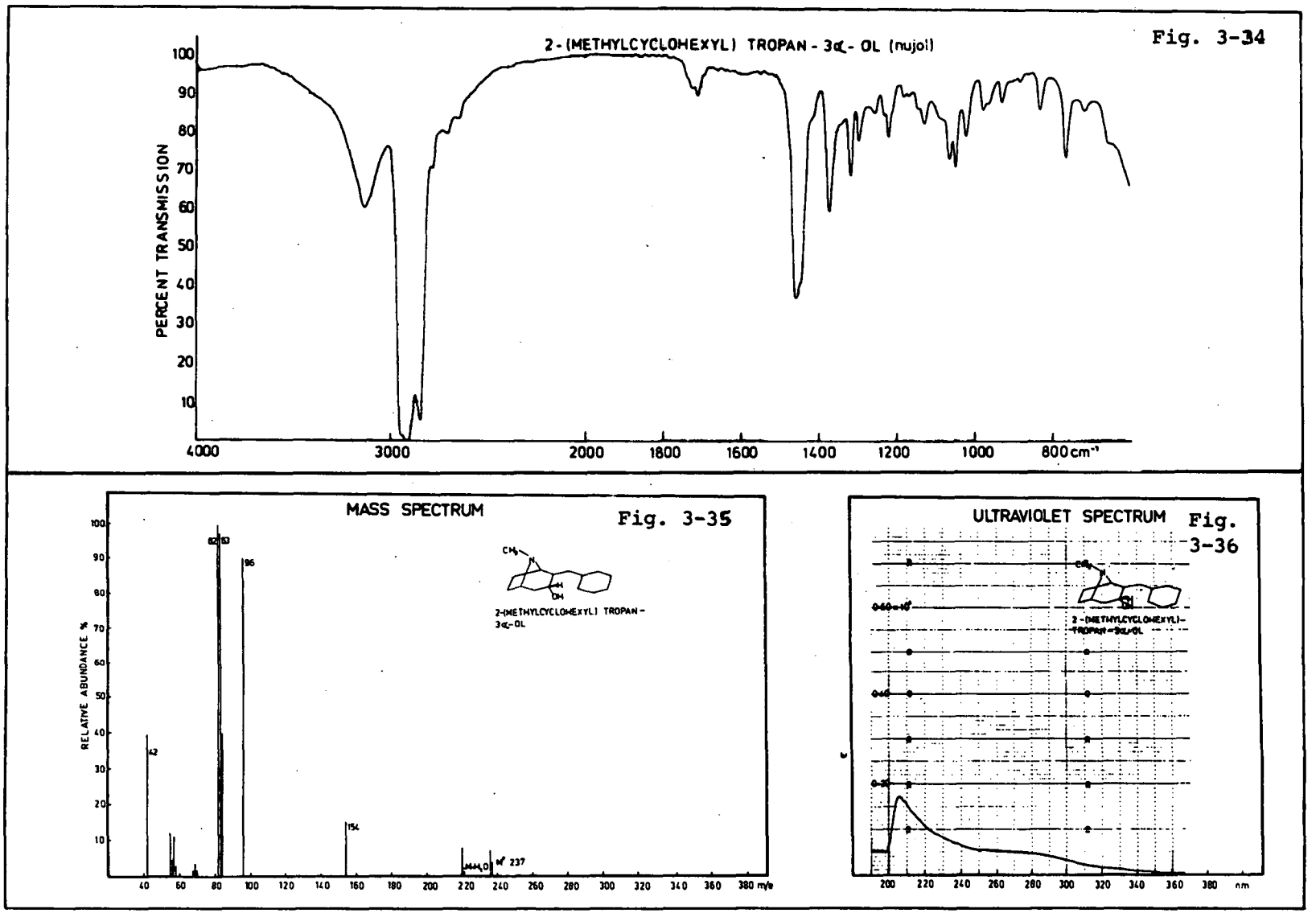


Fig. 3-34

Fig. 3-35

Fig. 3-36

CHAPTER 4

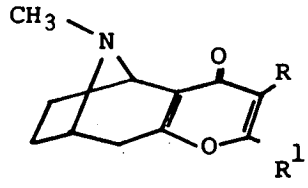
Synthesis of Pyronotropanes

CHAPTER 4

Synthesis of Pyronotropanes

4.1. Strategy

The development of a strategy for the synthesis of pyronotropanes (4.I) was influenced by two factors which offer potential simplification of the problem.



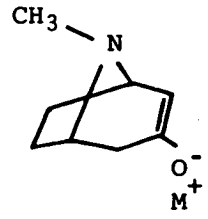
$R = CH_3$, $R^1 = H$	Bellendine	4.I
$R = H$, $R^1 = CH_3$	Isobellendine	4.II
$R = CH_3$, $R^1 = CH_3$	Darlingine	4.III

The first factor is the facility with which Robinson-Schöpf reaction furnishes the basic tricyclic substrate, tropan-3-one. The second is the well-proven acid catalysed cyclization of β-triketones to γ-pyrones[53]. The incorporation of these two reaction sequences in one approach necessitated the acylation of tropan-3-one at the 2-position with a species bearing a potential oxo-function β to the attacking carbonyl (4.IV).

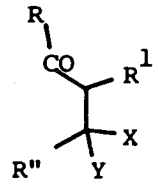
The symmetrical acylation sites in tropan-3-one, C_2 and C_4, are active methylenes, and there are a large number of methods available for base catalysed condensations[53,54] involving combinations of solvent, base and acylating agent which offer control over enolate solubility and regio-selectivity, O vs C, of acylation.

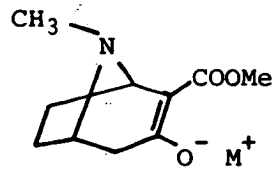
A minor modification of the Robinson-Schöpf method (Chapter 1) enables the introduction of a carbomethoxy group to the 2-position, which influences another variable in acylation: the acidity of the methylene

protons (4.V). The ease of enolate anion formation is an important factor in the success of chelating bases such as TlOEt or EtMgBr offering regio-specificity in acylation.



4.IV





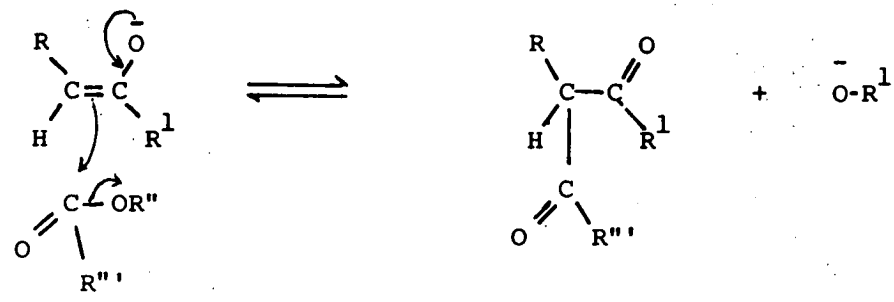
4.V

The choice of conditions for the reaction was also influenced by the intention to generalise the approach to include the three known pyronotropanes (4.I , 4.II and 4.III) and other synthetic analogues.

4.1.1. Selection of Acylating Agent

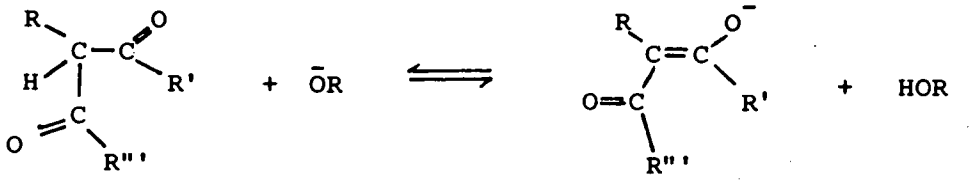
Carboxylic acid derivatives, particularly esters, are the commonly employed acylating agents in Claisen condensations. The reactivity of these reagents increases in the order $RCONR'_2$ < RCOOH < RCOOR < RCOOPh < RCOOCOR < RCOCl, RCH=C=O. Acid chlorides, ketenes and anhydrides offer the advantage over esters of irreversibility of the acylation step, which is due to the lack of nucleophilicity of the chloride or carboxylate anions (4.VI). [A particular use of the reaction of diketene with enamines in synthesizing γ-pyrones is discussed in Section 4.3.3].

Reversible ester acylation



4.VI

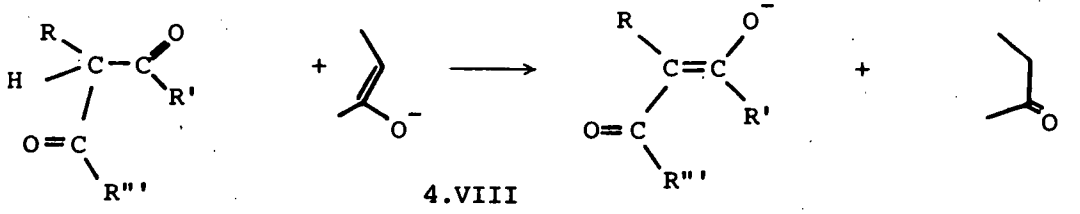
In order to improve the yields of ester acylation reactions, the second stage of the sequence, the equilibrium formation of a stable 1,3-dicarbonyl enolate (4.VII), may be forced to completion by continuous removal of the alcohol so formed.



4.VII

Alternatively, use of a strong base such as NaH or $NaNH_2$ consumes the alcohol, generating the 1,3-dicarbonyl enolate anion and liberating the alkoxide to react further with the mono-keto substrate.

Use of an acid chloride as acylating agent necessitates the provision of two molar equivalents of enolate anion, due to the consumption of one equivalent in converting the acylated product to its enolate anion (4.VIII), this is achieved by the alkoxide ion in ester acylations.

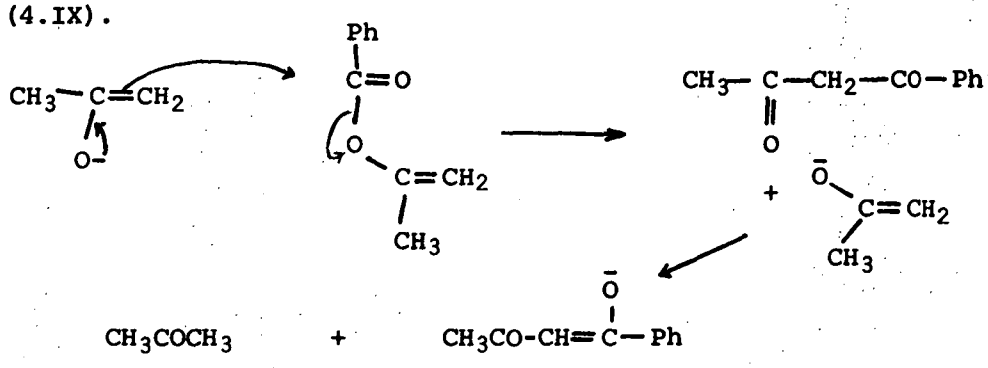


4.VIII

An important factor in acid chloride systems is the increased tendency for O-acylation to occur over C-acylation. This observation has been interpreted as being due to the relative importance of S_N1 reaction mechanisms vs S_N2 mechanisms in the acylation process[55]. The two sites, C and O, on the enolate system are discriminated from each other on the basis of the greater nucleophilicity of carbon, compared with the greater electron density of oxygen. Thus, a highly ionic transition state results in the attack of an 'acylium' ion on the electron rich oxygen of the enolate anion via an S_N1 mechanism with

resultant O-acylation. With less reactive acylating agents, the transition state is S_N2, and due to the greater nucleophilicity of the carbon atom in the enolate system, C-acylation results. It should be noted that such discrimination represents the two extremes, which are not likely to be observed exclusively. Furthermore, kinetic data supporting the above assertions are not unequivocal. The reaction of acyl halides with nucleophiles has been shown[55] to be bimolecular in almost every instance, implying an S_N2 mechanism. Despite this, acetyl chloride reacts with nucleophiles 10^3 times faster than does ethyl-chloroformate, implying a transition state for the acid chloride/ambident anion which has considerable ionic character, thereby favouring O-acylation. This latter hypothesis is supported by evidence which shows that on increasing the solvent polarity, and changing the leaving group from halide to perchlorate, a large increase in the proportion of O-acyl product is observed.

By employing two or three molar equivalents of enolate anion per equivalent of acyl halide, use is made of the kinetically controlled O-acylation reaction. Such an approach utilizes the capacity of the O-acylate to undergo further reaction with another equivalent of enolate to produce the thermodynamically more stable 1,3-diketo enolate anion[56] (4.IX).



4.IX

In the bellendine synthesis, use was made of both esters and acid chlorides; the successful synthesis used an acid chloride under conditions favouring C-acylation.

4.1.2. Selection of Base

Studies with 1,3-diketo enolate systems[57] have shown that those bases whose cations are capable of chelating, or forming tightly bound ion pairs to the enolate anion, favour C-acylation. The rationale for these observations is that chelation occurs *via* the oxygen of the enolate system, thereby preventing the attack of an acylium ion directly on oxygen. Use has been made of R-MgBr, R-MgOEt and TlOEt[58] in regio-specific acylations of 1,3-diketo systems; however, the pK_a values of monoketones (> 20) are usually too large to enable the formation of enolate anions by bases derived from chelating cations. Sodium hydride is a strong base of considerable utility in effecting enolate formation from monoketones. Na^+ is of intermediate-low chelating ability, however, as is reflected by the proportions of O *vs* C-acylation products in the acylation of 2-carbomethoxy cyclohexanone with acetyl chloride. NaH was compared with EtMgBr, both bases in tetrahydrofuran as solvent[57]. For NaH, the proportion of O-acylation was 99% from a total yield of 61%. For EtMgBr, the proportion of C-acylation was 100% from a total yield of 79%. It is likely that steric factors, which are considered to be of great significance in the regio-specific acylation of hexacyclic ketones[59], had a greater influence on the weakly associated sodium enolate in influencing O-acylation than the rigidly chelated magnesium bromide enolate. Cycloheptanones and cyclopentanones are more favoured to acylate on carbon than oxygen, due to the decreased steric interference to the attacking acylating agent by axial hydrogens α to the enolate carbon.

The simplicity of use of sodium hydride in forming enolate acids, its non-nucleophilicity, and concomitant lack of acyloin formation, has led to its widespread use in base-catalysed condensations. In order to influence the orientation of acylation towards the synthetically valuable C-acylate, variations of solvent and reactant ratios have been utilized.

4.1.3. Selection of Solvent

The selection of an acid chloride as an acylating agent restricts the range of media for acylation to aprotic solvents. In view of the earlier discussion relating to the enhancement of S_N1 mechanisms by stabilization of ionic reaction intermediates and assisting the ionization of the acylating agent, non-polar solvents are favoured over polar solvents in increasing the yields of C-acylated products. However, solubility considerations and the slow rate of enolate formation, with resultant aldol condensation between unionized ketone and enolate anion, may restrict the use of non-polar solvents such as benzene. Two polar solvents which are widely used in acylation reactions, dimethoxy ethane and tetrahydrofuran, offer the advantage of solubilizing the enolate system. Both polar solvents ($NH_{3(liq)}$, dimethoxy ethane), and non-polar solvents (benzene, xylene) were tried in the acylation reactions of tropan-3-one. The successful reaction utilized benzene.

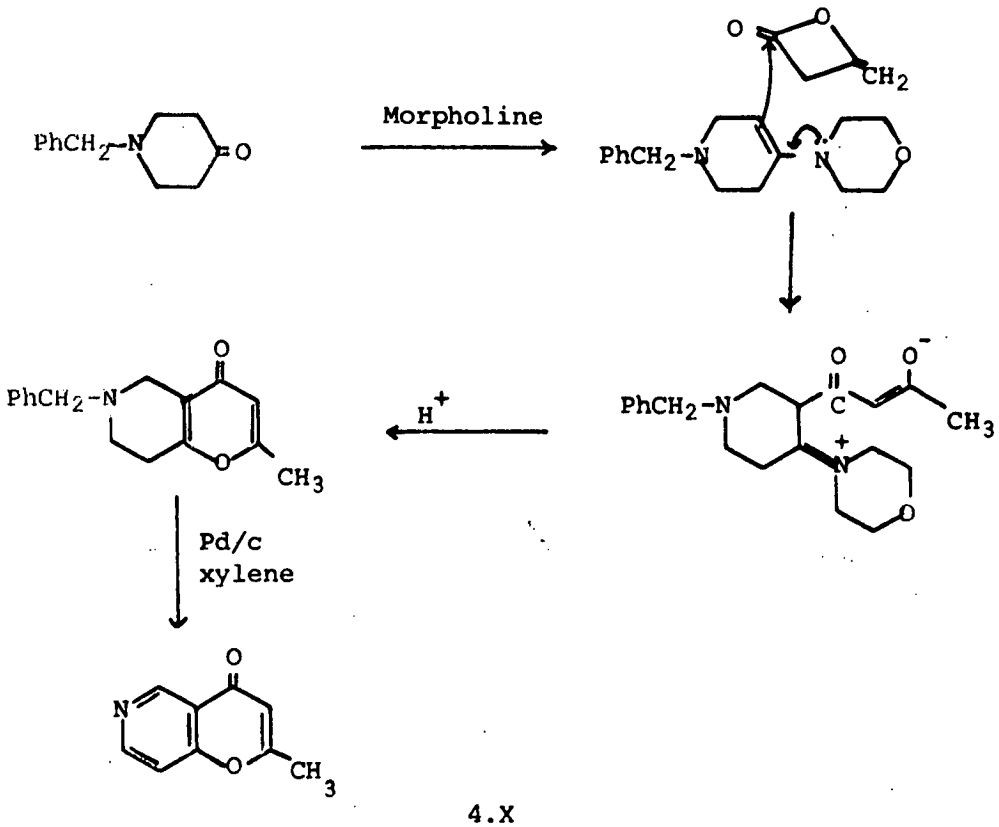
4.1.4. Selection of carbonyl protecting groups

The choice of a 1,3-diketo acylating system using an acid chloride necessitated a protecting group for the β-carbonyl. It was also considered necessary to deactivate the α-protons of the acylating system to avoid proton abstraction from the acid chloride by the stronger monoketo enolate base and subsequent self-condensation. Furthermore,

the protecting groups had to be base stable and acid labile to enable removal and simultaneous cyclization to the γ-pyrone. Acetals, ketals and enol ethers[60] fulfil these requirements and each protecting group was tried in attempting to synthesize the pyronotropanes. The successful method utilized an enol ether system as a protecting group.

4.1.5. Acylation of enamines

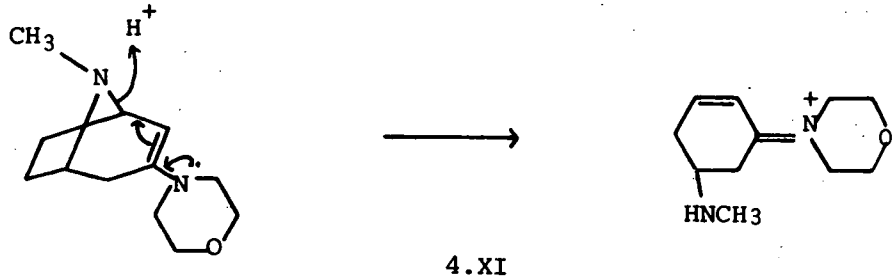
Fused γ-pyrones have been synthesized by the reaction of diketene and enamines of cyclic ketones[61]; in particular, the heterocyclic aza-chromone (4.X) was readily synthesized by the reaction shown in Scheme 4-1.



4.X

Scheme 4-1

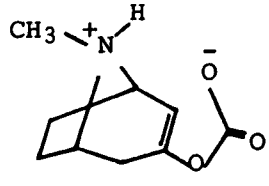
The analogous pyrone elaboration was considered for the synthesis of isobellendine (4.II). However, attempts to form the pyrrolidine or morpholine enamines of tropan-3-one were not successful. At low temperatures there was no reaction; at high temperature, using p-tosic acid as catalyst in refluxing xylene, extensive decomposition occurred which may have been due to a degradation as shown in 4.XI. This approach was not proceeded with for these reasons.



4.XI

4.2. Tropan-3-one Acylation Reactions

The literature pertaining to base-catalyzed acylations of tropan-3-one is restricted to three examples. Willstaetter[62] succeeded in carboxylating tropan-3-one sodium enolate in his synthesis of cocaine. Evidence at this stage suggested the simultaneous formation of a betaine from the O-acylation product. (4.XII).



4.XII

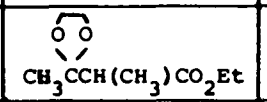
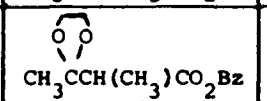
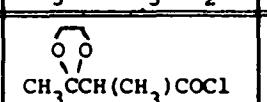
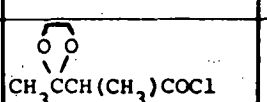
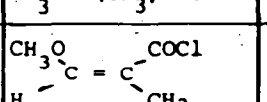
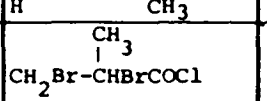
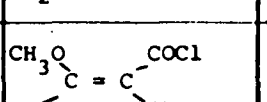
Findlay[63] described the low yield carbethoxylation of the sodium enolate of tropan-3-one with ethyl chloroformate. The reaction produced "a multitude of side products" and only a very low yield of the desired 2-carbethoxy tropan-3-one. Findlay also repeated the work of Preobrazhenskii[64] who claimed a 75-80% yield of 2-carbethoxy tropan-3-one

from the sodium enolate of tropan-3-one and diethyl carbonate in refluxing xylene. He found that the quoted yields could not be repeated. Beyerman[65] repeated Willstaetter's benzoylation of 2-carbomethoxy tropan-3-one in pyridine to produce the O-benzoyl product, the structure of which was proved spectroscopically.

A summary of the reaction conditions attempted in the bellendine synthesis is shown in Table 4-1. Based on a consideration of the above acylation experiments and the literature pertaining to acylation reactions, the initial attempts at bellendine synthesis utilized a non-polar solvent and an ester with an acetal protecting group for the β-aldehyde, as acylating reagent. This experiment, Reaction No. 1 in Table 4-1, repeated the conditions of Willstaetter: tropan-3-one was refluxed with sodium wire for several hours, and the acylating ester added slowly over a period of an hour. An aqueous work-up followed by chromatography of the products produced tropan-3-one as the major product. A considerable amount of highly polar basic material which did not chromatograph was not isolated. The facility with which the O-acyl product was hydrolysed under aqueous acid conditions was not initially recognized. It was considered that the failure to react may have been due to incomplete enolate generation; in order to improve this, sodamide in liquid ammonia was used as a base/solvent system. Aqueous work-up and chromatography again produced tropan-3-one as the major high R_f product. Under more forcing conditions, molten sodium in refluxing xylene was used as a base/solvent system and the darlingine-precurser ketal-ester shown in Reactions Nos. 3 and 4 was employed as acylating agent. This reaction produced a multitude of poorly resolved products; the major product was again tropan-3-one. The experiment was repeated using a shorter reaction time and a more carefully controlled basification procedure (low temperature, $NaHCO_3$) in

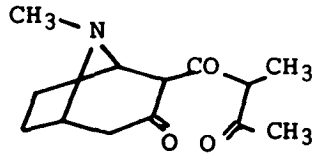
TABLE 4-1

SUMMARY OF TROPAN-3-ONE ACYLATION REACTIONS

Rn. No.	BASE	SOLVENT	ACYLATING AGENT	WORK-UP	RESULTS
1	Na	benzene	$(EtO_2C)_2CH(CH_3)CO_2Et$	Aqueous H^+; OH^- (pH 9) Extract $CHCl_3$	Tropan-3-one 100%
2	$NaNH_2$	liq. NH_3	"	Aqueous H^+; OH^- (pH 9) Extract $CHCl_3$	"
3	Na	xylene		"	Tropan-3-one 80% resinification extensive
4	Na	xylene		Aqueous H^+ - cold Basify $NaHCO_3$. Extract $CHCl_3$	7 Bands on P.T.L.C. isolated 8 mg consistent NMR
5*	NaH	dimethoxyethane		Evaporate to dryness PTLC HCl Salt	P.T.L.C. 3 Bands Tropan-3-one 60% Major band 35% minor 5%
6	NaH	dimethoxyethane	D_2O	DCl in D_2O Na_2CO_3, extract $CHCl_3$	Mass-spectrum and NMR indicate α D substitution
7	NaH	dimethoxyethane		Evaporate to dryness PTLC HCl salt	P.T.L.C. 3 Bands Tropan-3-one 60% Major band 35% + 1 other
8	NaH	dimethoxyethane		"	P.T.L.C. 3 Bands Tropan-3-one 45% Major band 45% + 1 other
9	NaH	benzene		"	P.T.L.C. 3 Bands Tropan-3-one 30% Major band 40% minor 30%
10	NaH	benzene		"	P.T.L.C. 3 Bands Tropan-3-one 50% Major band 40% minor 10%

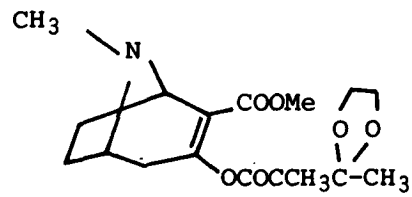
* Reaction 5 Used 2-carbomethoxy tropan-3-one

order to reduce the possibility of basic cleavage of the β-triketone. The intensely coloured extract of this reaction was chromatographed, and a trace quantity of one of the products had P.M.R. spectral characteristics consisistent with 4.XIII (Fig. 4-1). An attempt to cyclize the triketone using acetic acid and concentrated hydrochloric acid was too severe and resulted in destruction of the compound.



4.XIII

It was apparent from the dark colouration of the enolate/base systems in the above attempts, that extensive decomposition was occurring prior to acylation. In order to improve enolate generation the strong non-nucleophilic base, sodium hydride, was applied to the doubly activated methylene system, 2-carbomethoxy tropan-3-one in the polar solvent, dimethoxyethane. The rapidly formed sodium enolate was readily soluble in this solvent, and an equivalent of the acid chloride shown in Reaction No. 5 was added. The major product from this reaction, from a non-aqueous work-up, was 2-carbomethoxy tropan-3-one, but a minor product was isolated in substantial quantities. This product had spectral characteristics, particularly a high frequency carbonyl absorption in the I.R. spectrum (Fig. 4-3), consistent with an O-acyl product (4.XIV).



4.XIV

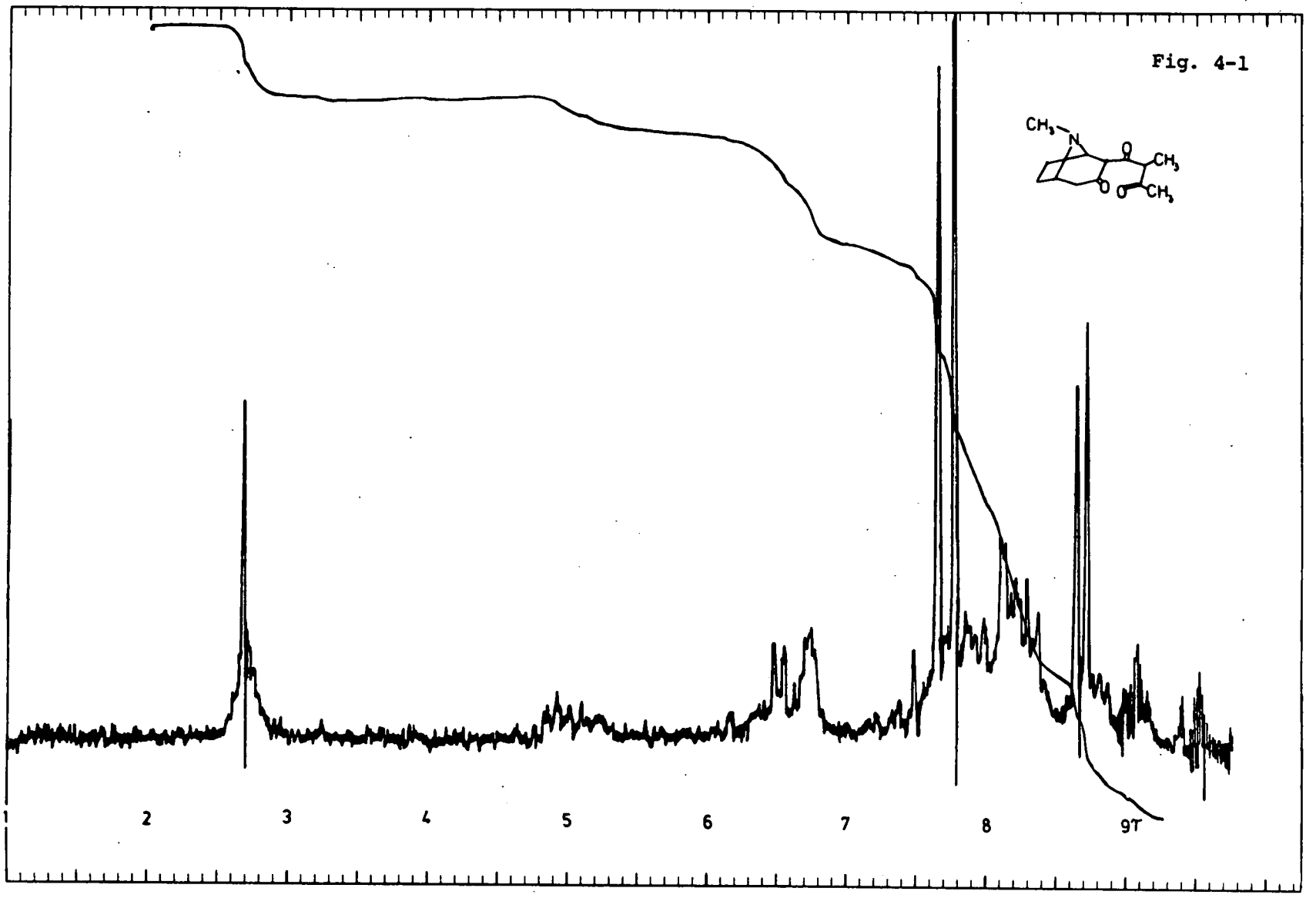


Fig. 4-1
CH3
N
O
CH3
O
O
CH3
1
2
3
4
5
6
7
8
9τ

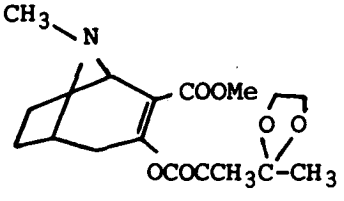
The ultraviolet spectrum (Fig. 4-5) also supported this assignment. The P.M.R. spectrum (Fig. 4-2) provided evidence for acylation, but was not unequivocal in indicating whether C- or O-acylation had occurred. One interpretation of the split N-methyl signal at 7.50τ was that C-acylation had taken place, producing the axially and equatorially substituted forms on C-2 which influenced the N-methyl signal. Chemical evidence confirmed the O-acyl assignment: mild aqueous acid conditions totally hydrolysed the product to 2-carbomethoxy tropan-3-one.

Reaction No. 5

TABLE 4-2

Acylation of 2-Carbomethoxy Tropan-3-one

Hydrolysis reactions



4.XIV

Major product.

Reaction No. 5

Hydrolysis Conditions	Product
Dilute HCl	2-carbomethoxy tropan-3-one
Dilute H_2SO_4	"
Acetone & H_2SO_4	"
$HClO_4$/THF	"
NH_4OH/NH_4Cl	"
NaOH 1 M	"
Zeocarb 225	Starting material

A minor product of the reaction, which had spectroscopic properties in accord with the C-acylated product, was isolated by P.T.L.C. However, attempts to hydrolyze the tertiary carbomethoxy group in acid were unsuccessful; likewise under milder conditions using ion-exchange resins. Under basic conditions, retro-Claisen cleavage occurred to

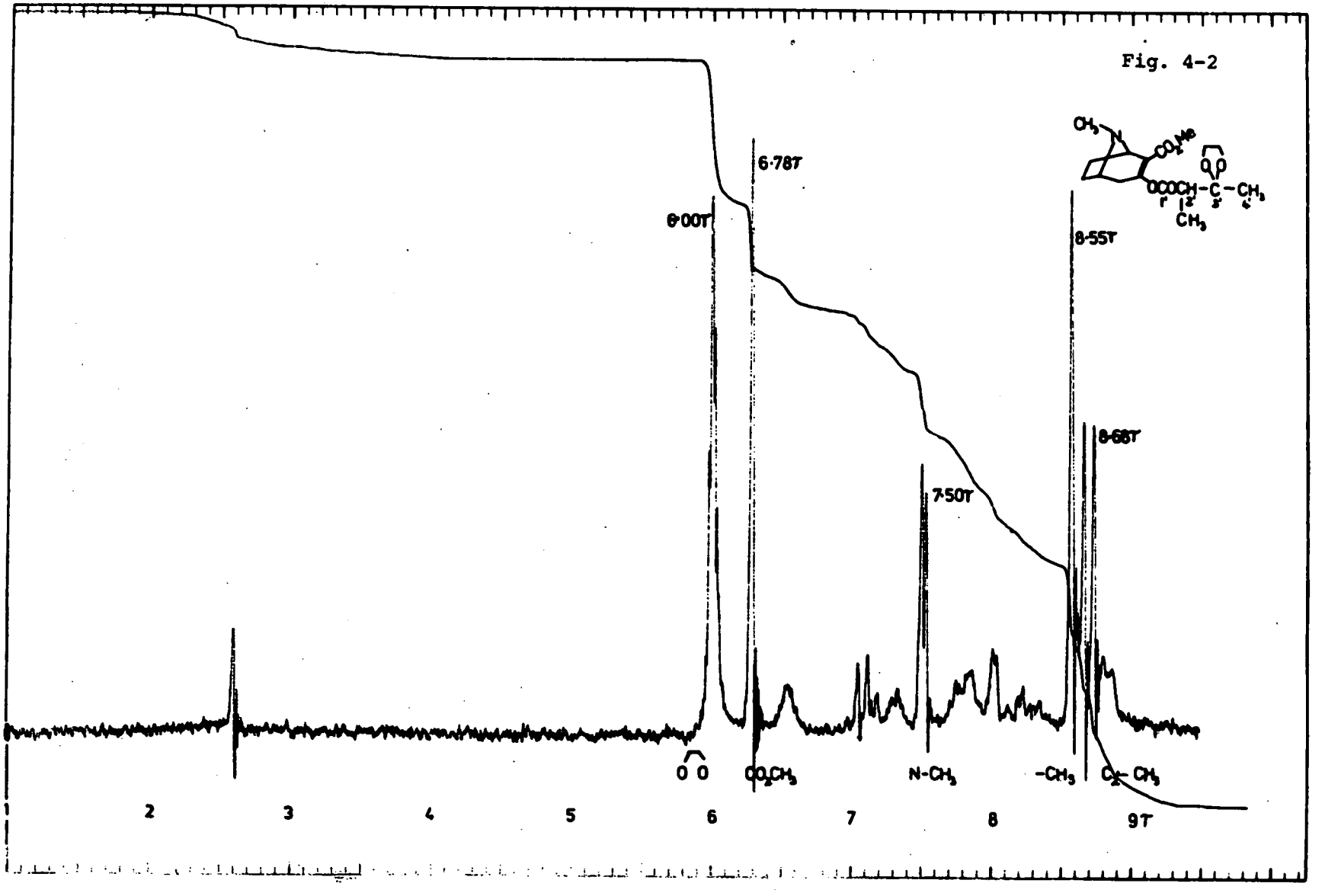
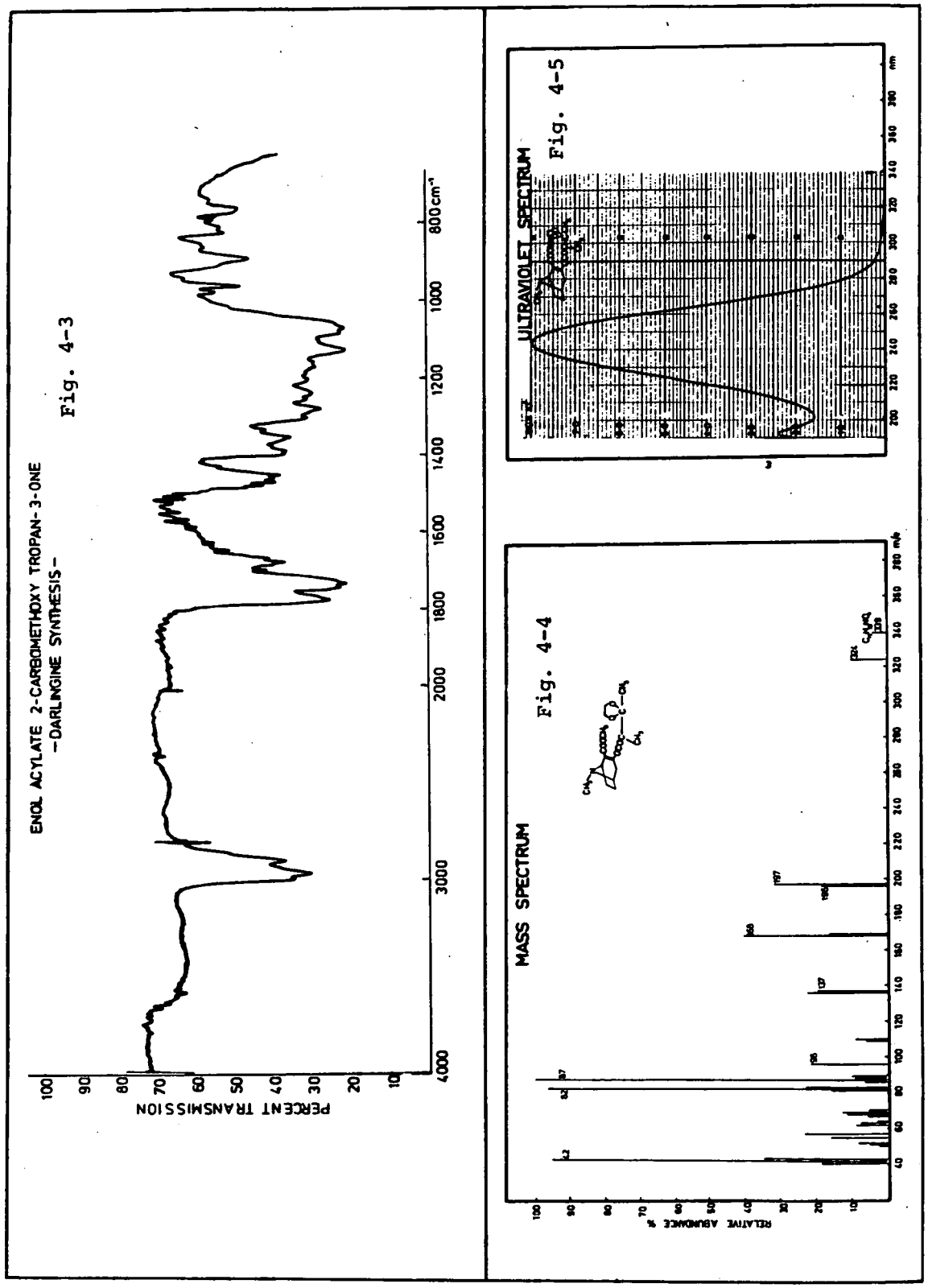


Fig. 4-2
6·78T
6·00T
8·55T
8·68T
7·50T
O O
CO_2CH_3
N-CH_3
-CH_3
C_2-CH_3
2
3
4
5
6
7
8
9T


ENOL ACYLATE 2-CARBOMETHOXY TROPAN-3-ONE
-DARLINGINE SYNTHESIS-
Fig. 4-3
PERCENT TRANSMISSION
4000
3000
2000
1800
1600
1400
1200
1000
800 cm⁻¹
ULTRAVIOLET SPECTRUM
Fig. 4-5
MASS SPECTRUM
Fig. 4-4
RELATIVE ABUNDANCE %

produce the starting ester.

The conditions of Reaction No. 5 were repeated for tropan-3-one after a D_2O exchange reaction had shown that α-deuteration readily occurred for the base under these conditions. The major acylation product of this reaction was again the O-acylate, for which spectral characteristics are shown in Figs. 4-6 - 4.9. The P.M.R. spectrum shows the olefinic C-2 proton at 4.46τ as a doublet, J = 8 Hz, coupled to the C-1 proton at 6.20τ. The minor acylation product, which was examined by mass spectroscopy, had a fragmentation pattern consistent with the C-acylated product (Scheme 4-2). However, only a small quantity was available, and a series of hydrolysis conditions monitored by T.L.C. indicated failure to effect the conversion to darlingine.

The high proportion of O-acylation and the stability of the ketal to hydrolysis caused the above approach to be dropped in favour of an attempt with an enol-ether acid chloride, 3-methoxy-2-methyl prop-2-enoyl chloride, shown in Reaction No. 8. The major product of this reaction was again the O-acylate, which was readily hydrolysed to the starting ketone under mild aqueous acid conditions. Spectral properties are shown for this compound in Figs. 4-10 - 4-13.

In an unsuccessful γ-pyrone approach involving a base-induced cyclization *via* an S_N2 attack of the enolate oxygen on a bromine-substituted β-carbon, benzene was used as the acylation solvent. This approach resulted in a considerable increase in the proportion of C-acylated product (4.XVI), consistent with the previously discussed theory concerning non-polar solvents. It is considered that the product of the displacement reaction in dimethoxy ethane was the Favorski product (4.XVII), arising from the attack of the more

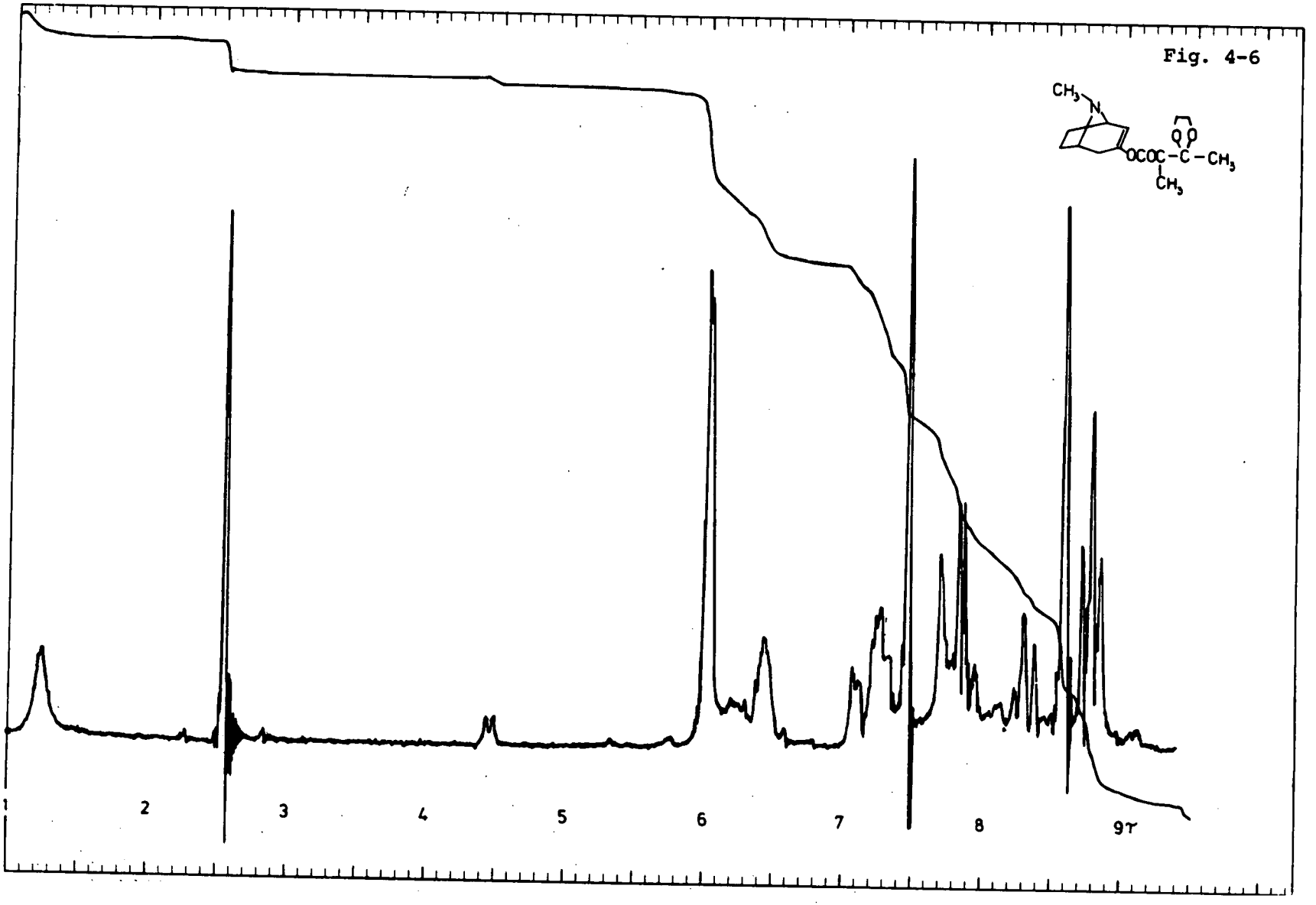
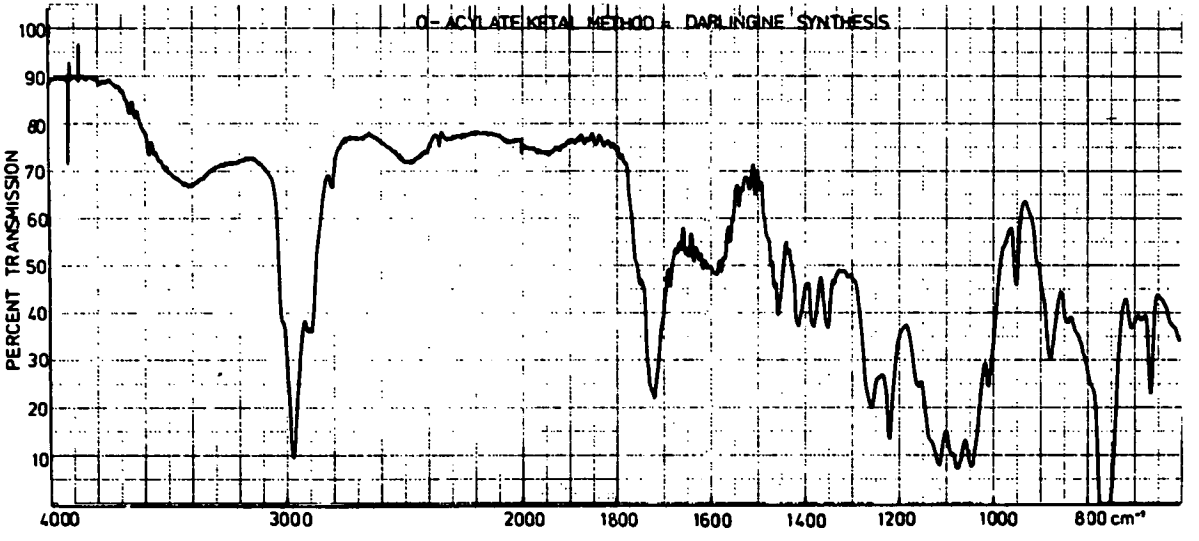


Fig. 4-6
CH3
N
OCOC-C-CH3
CH3
2
3
4
5
6
7
8
9τ

Fig. 4-7



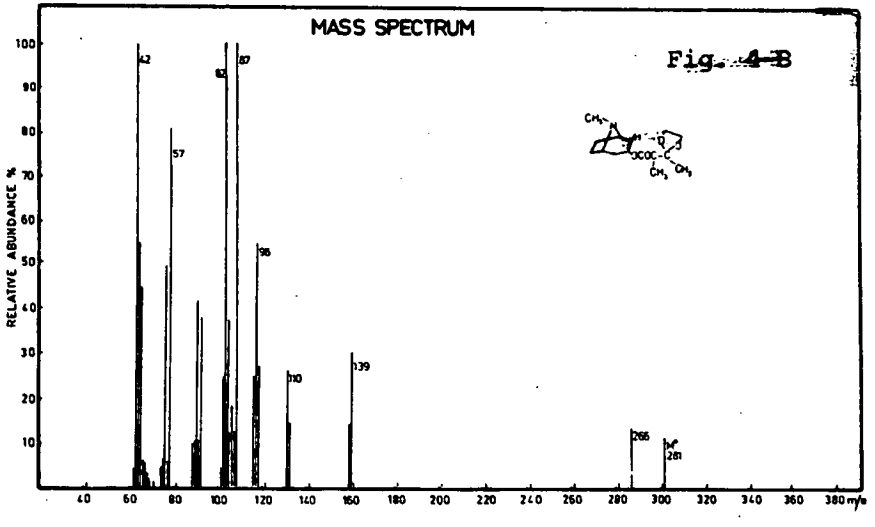


Fig. 4-8

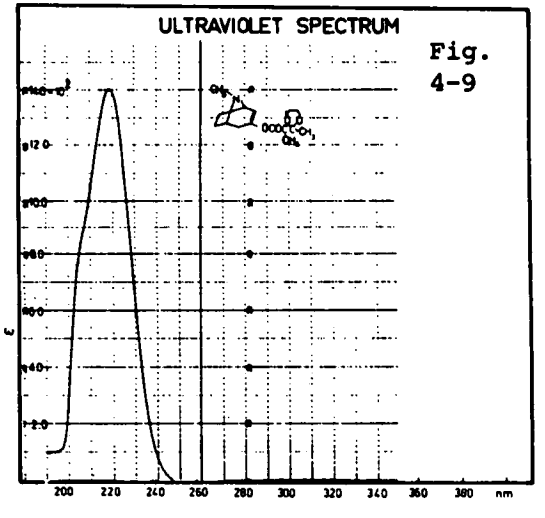
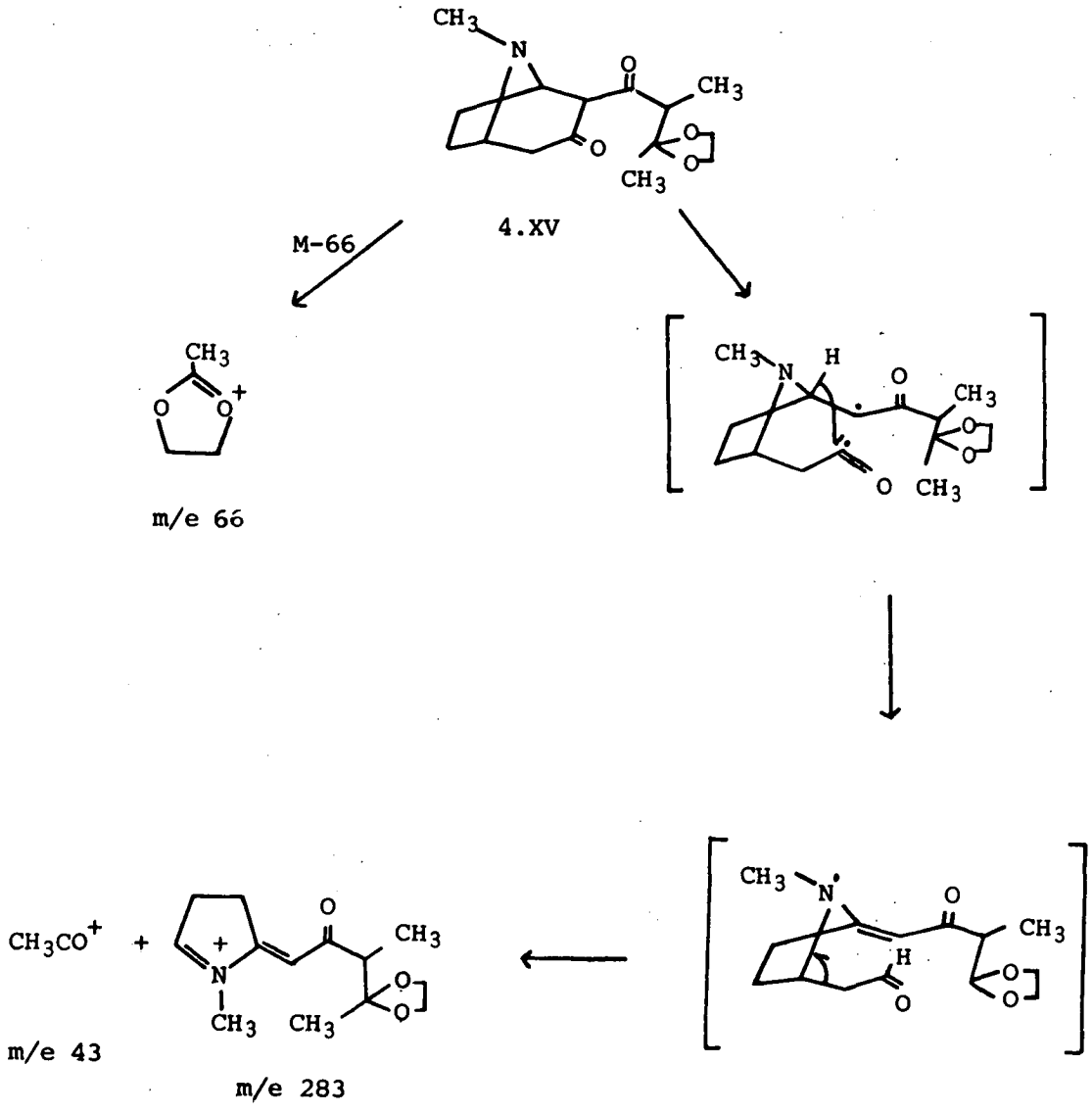


Fig. 4-9

Fragmentation of Ketal Acylation Product



Scheme 4-2

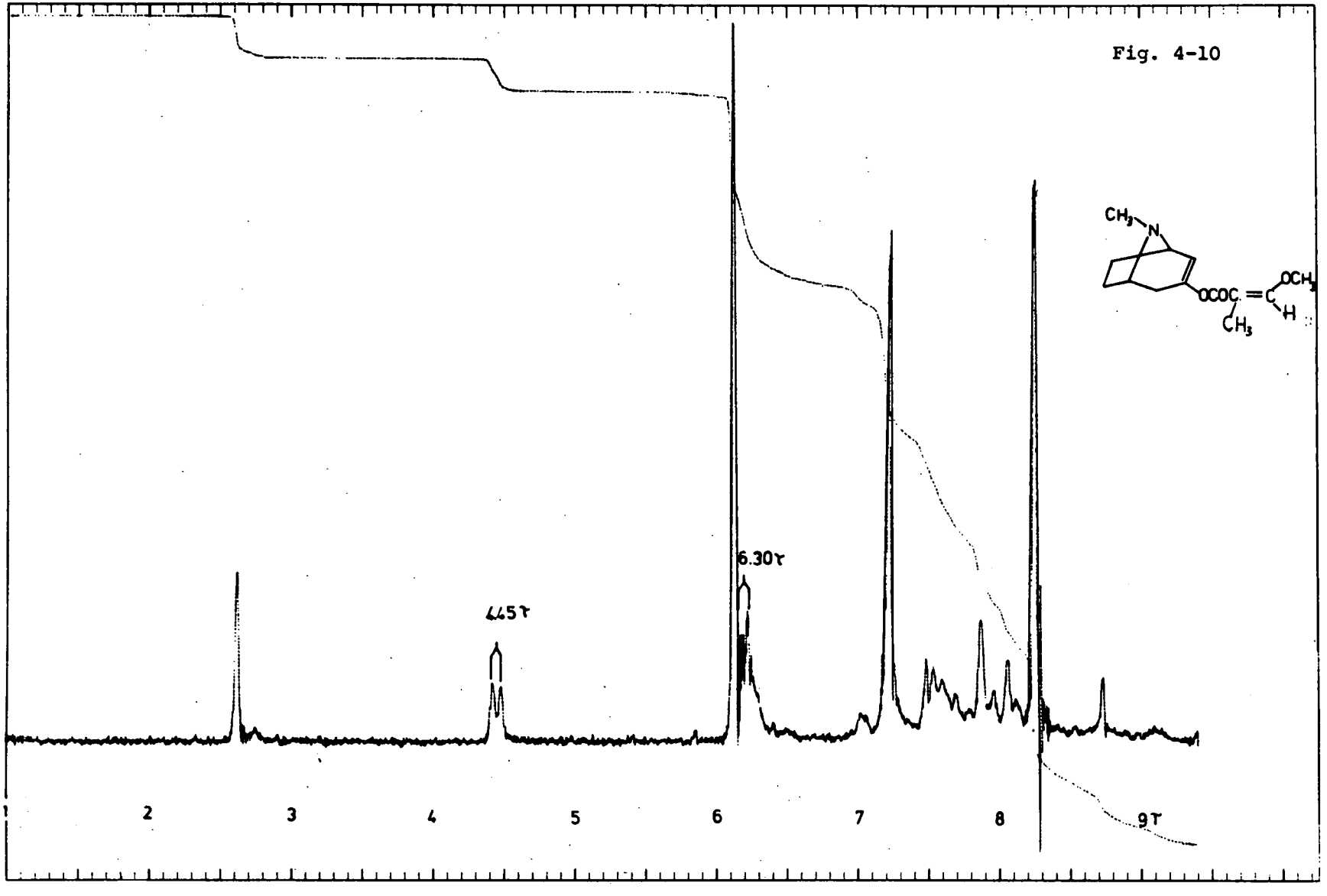


Fig. 4-10
CH3
N
OCOC
OCH3
H
CH3
6.30τ
4.45τ
2
3
4
5
6
7
8
9τ

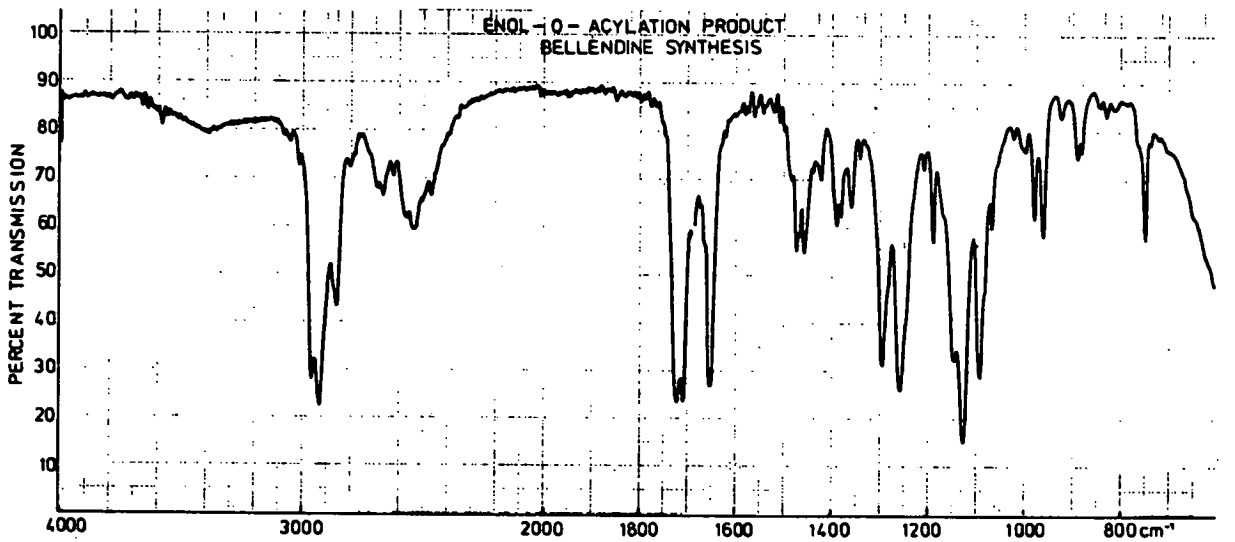


Fig. 4-11

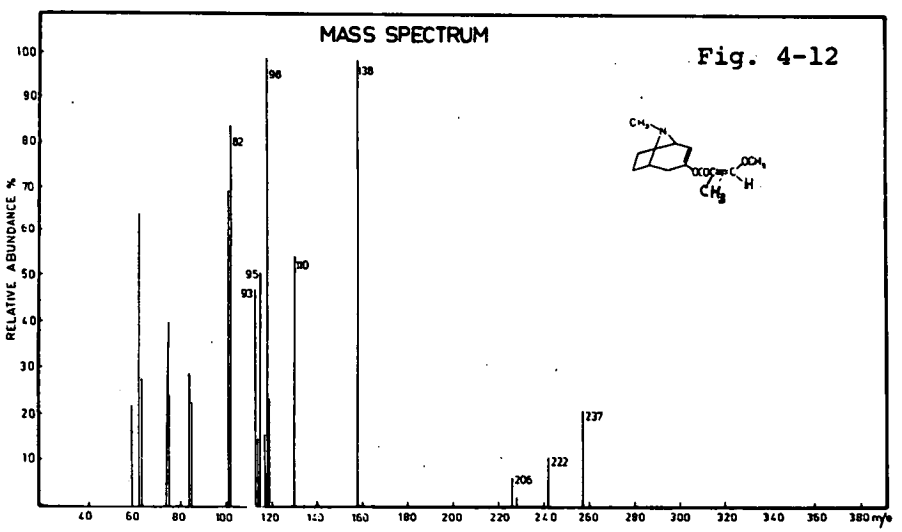


Fig. 4-12

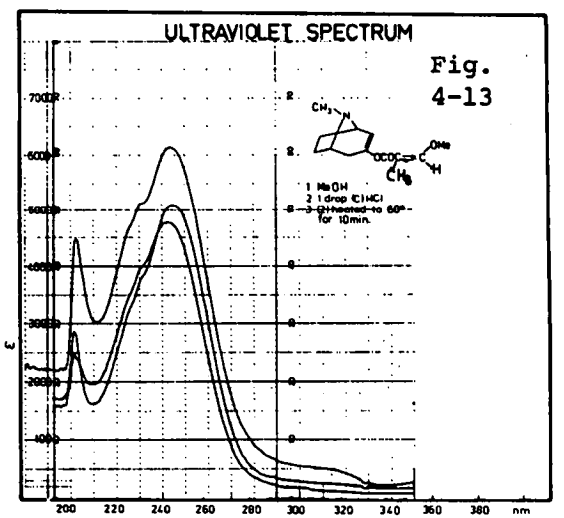
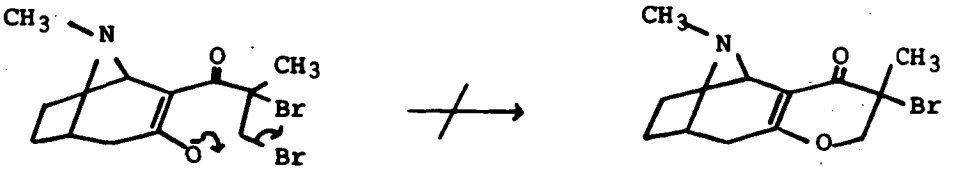
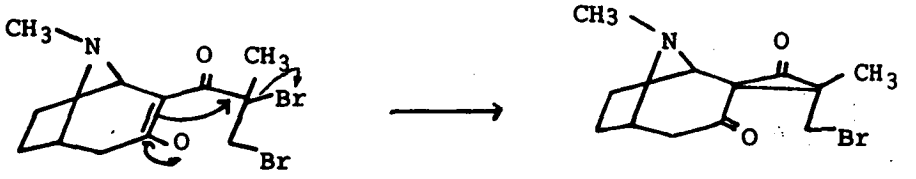


Fig. 4-13

nucleophilic carbon centre on the highly reactive α-bromo centre.



4.XVI



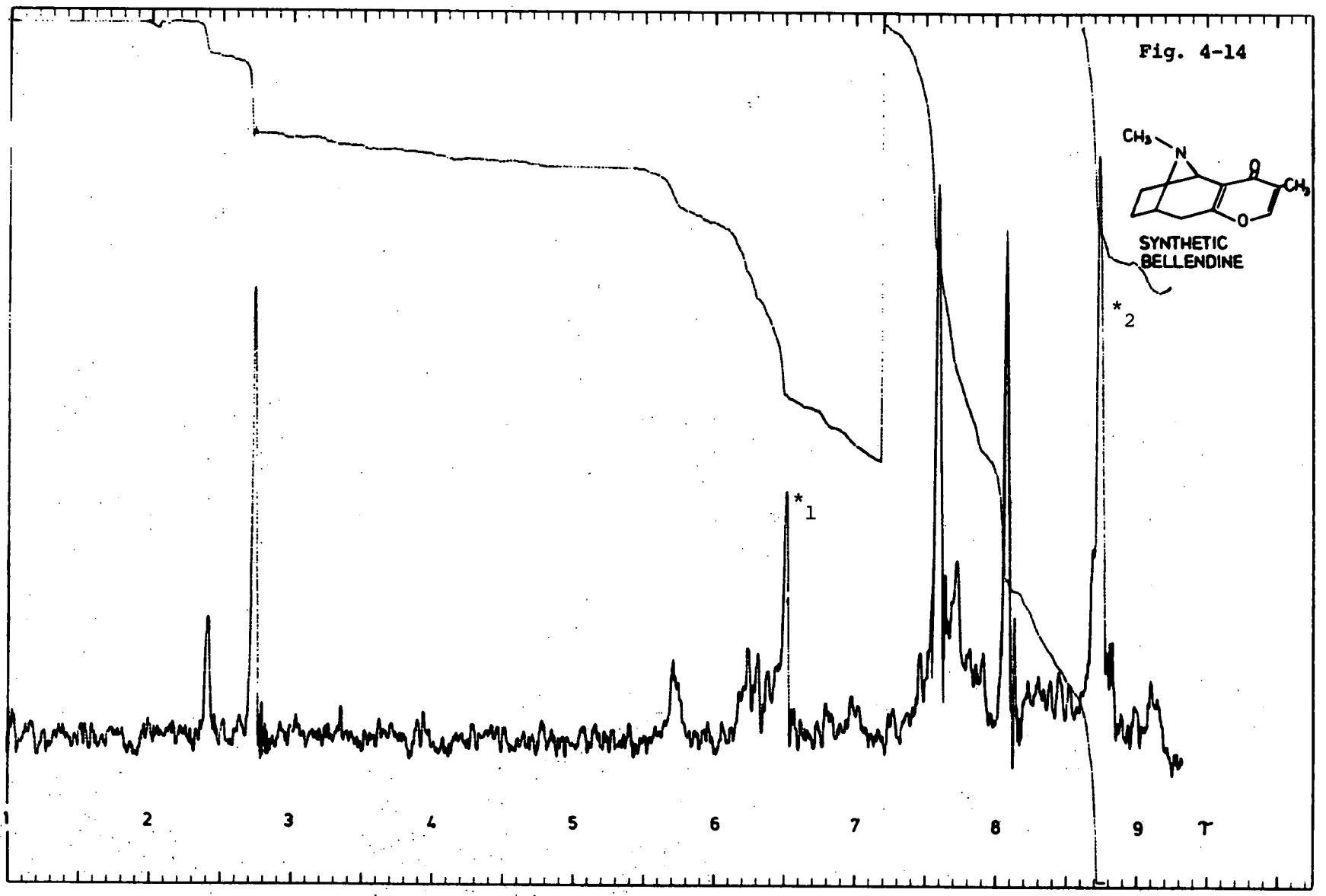
4.XVII

The conditions of Reaction No. 9 were repeated in Reaction No. 10, using the enol-ether acid chloride, 3-methoxy-2-methyl prop-2-enoyl chloride which resulted in a substantial increase in the proportion of C-acylated product. In dilute aqueous acid, this product was quantitatively converted to the γ-pyrone, giving bellendine[66] (Figs. 4-14 - 4-17).

This method has been employed for the synthesis of isobellendine (4.II) and darlingine (4.III), using 3-ethoxy-but-2-enoyl chloride and 3-ethoxy-2-methyl-but-2-enoyl chloride respectively. (Scheme 4-3). (Figs. 4-18 - 4-25).

4.3. Synthesis of Dihydroisobellendine

Dihydropyrones have been synthesized by two routes which involve, as the final step, the cyclization of a β-keto enol oxygen to the β-carbon of an unsaturated system (4.XVIII)[67,68].



*1 H_2O Impurity *2 Impurity from P.T.L.C.

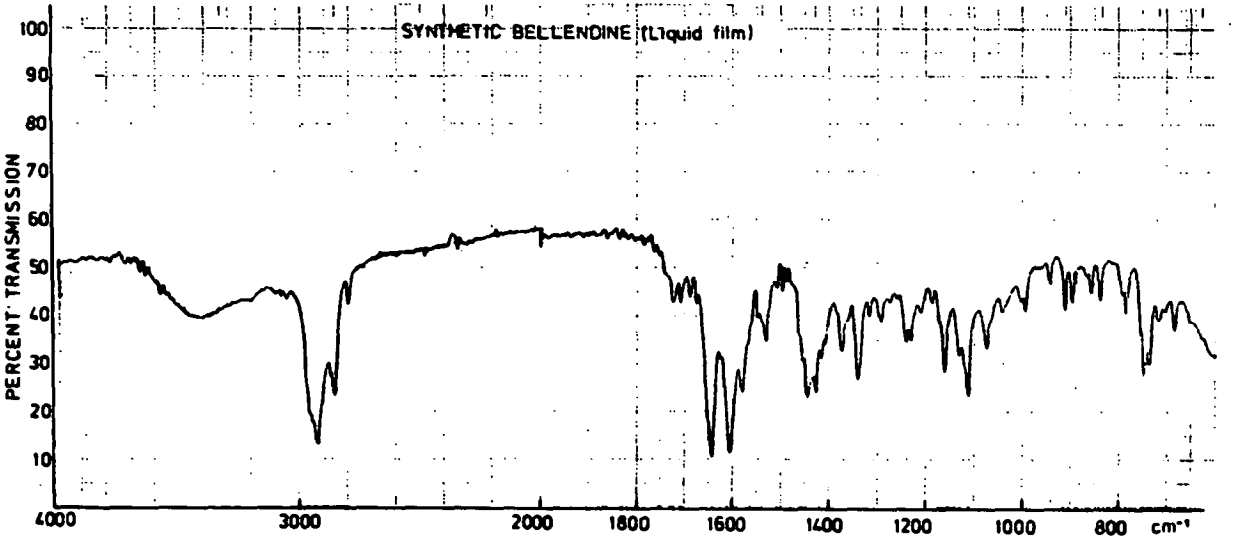


Fig. 4-15

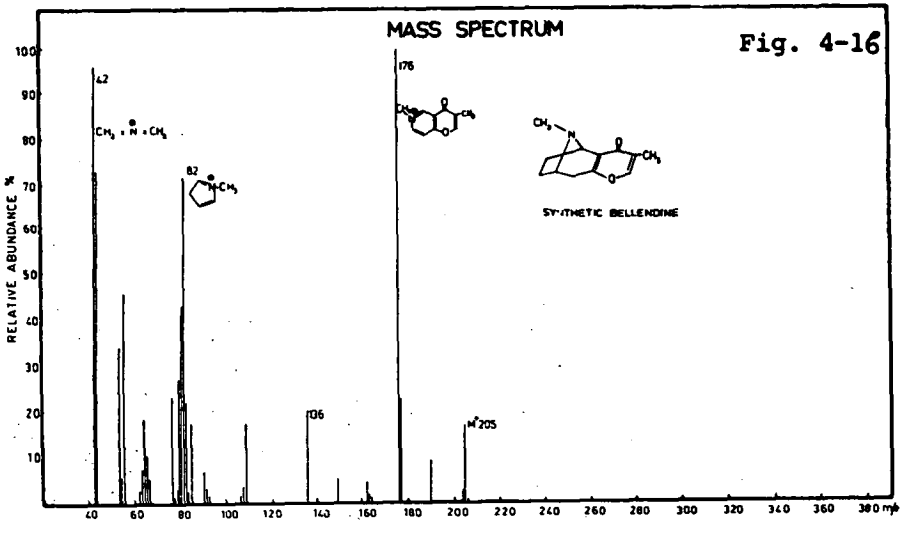


Fig. 4-16

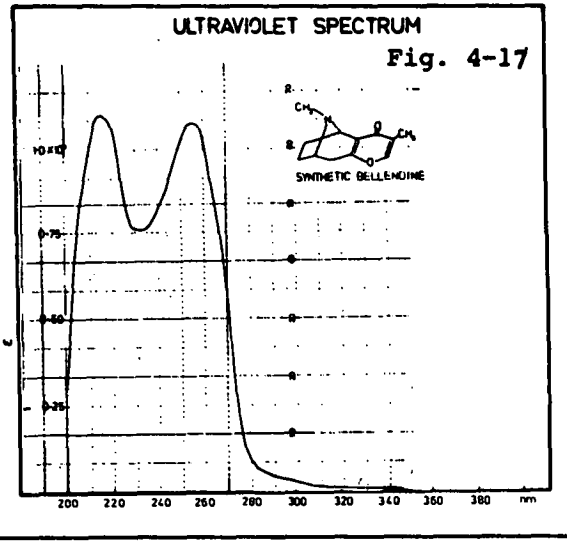
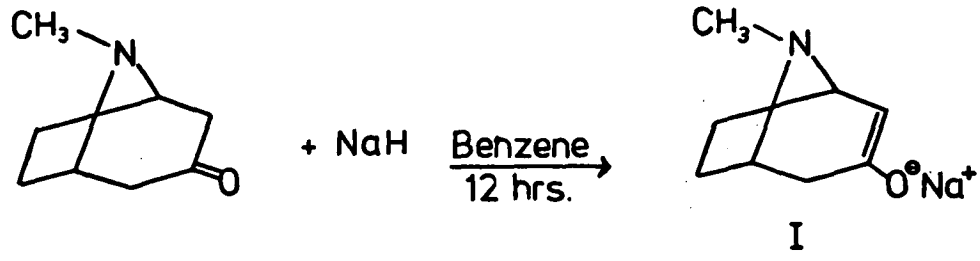


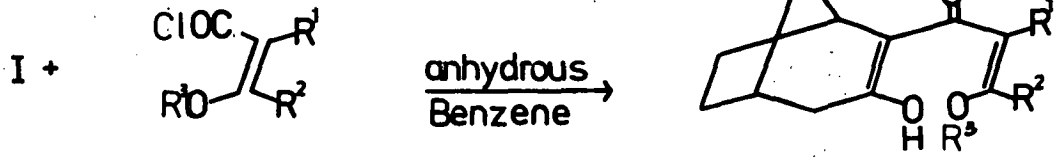
Fig. 4-17

SYNTHETIC SEQUENCE FOR

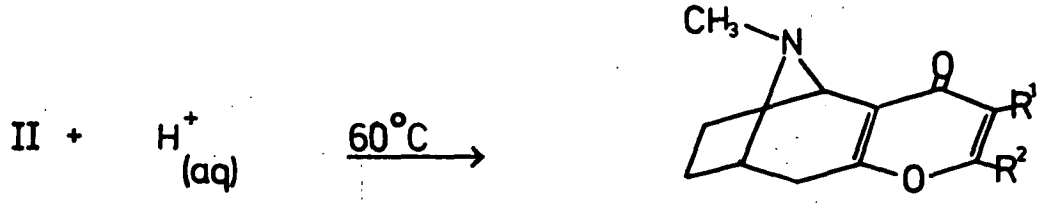
BELLENDINE, ISOBELLENDINE, DARLINGINE



I



II



$R^1 = CH_3$ $R^2 = H$ $R^3 = CH_3$ } Bellendine	$R^1 = H$ $R^2 = CH_3$ $R^3 = C_2H_5$ } Isobellendine	$R^1 = CH_3$ $R^2 = CH_3$ $R^3 = C_2H_5$ } Darlingine
4.I	4.II	4.III

Scheme 4-3

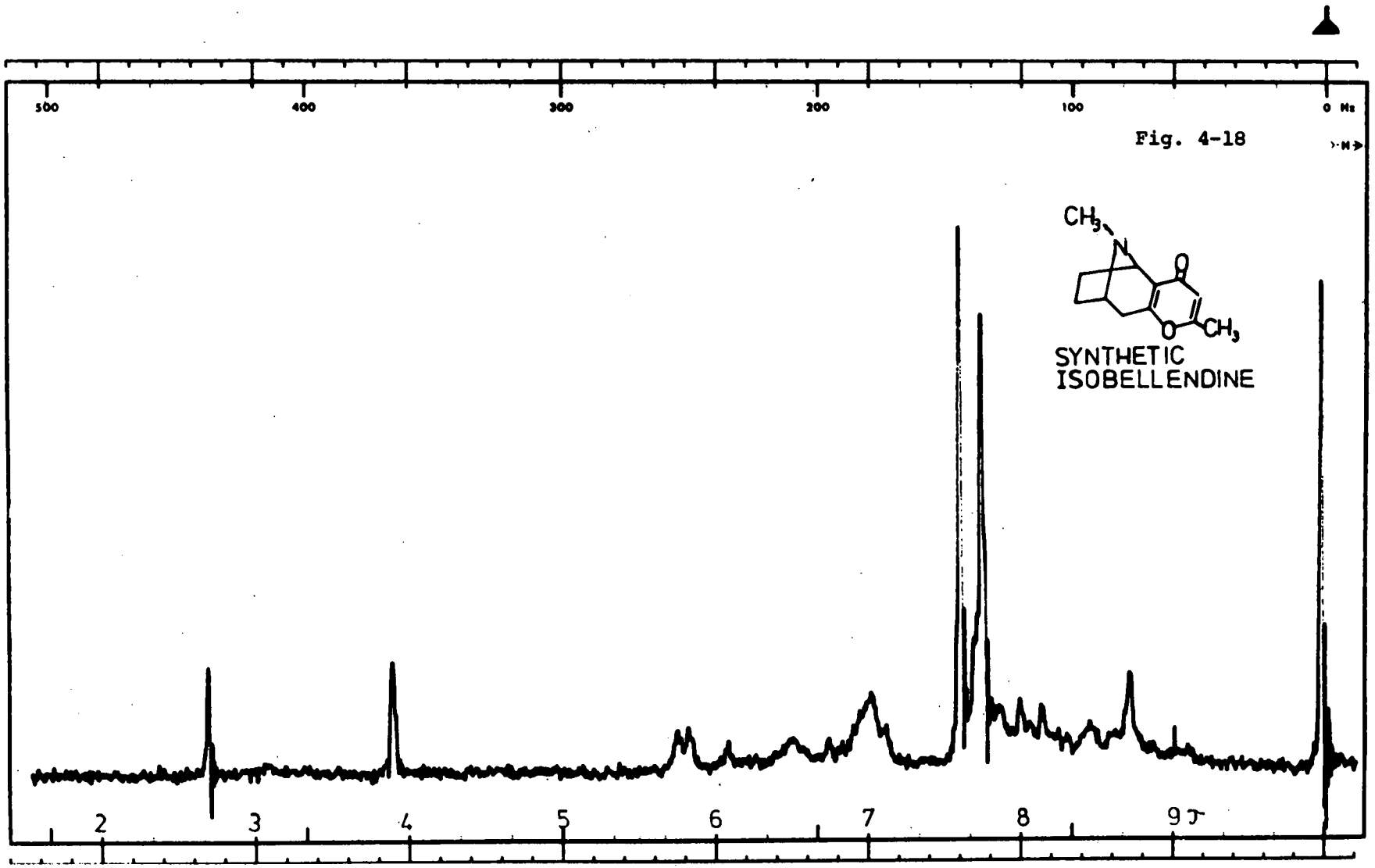

500
400
300
200
100
0 Hz
CH3
N
O
O
CH3
SYNTHETIC
ISOBELLENDINE
2
3
4
5
6
7
8
9 τ

Fig. 4-18

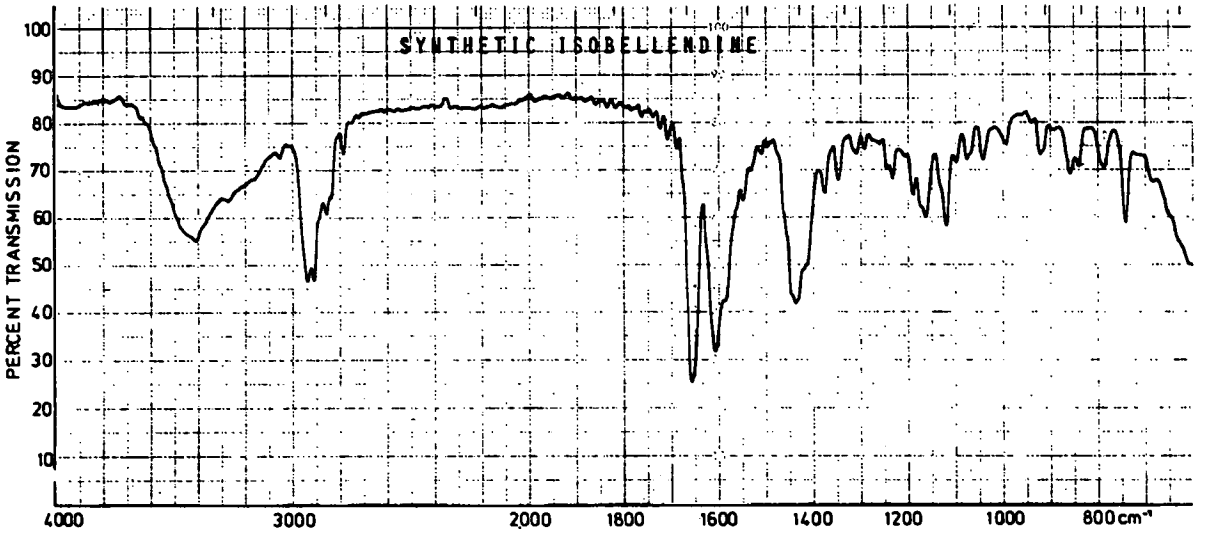


Fig. 4-19

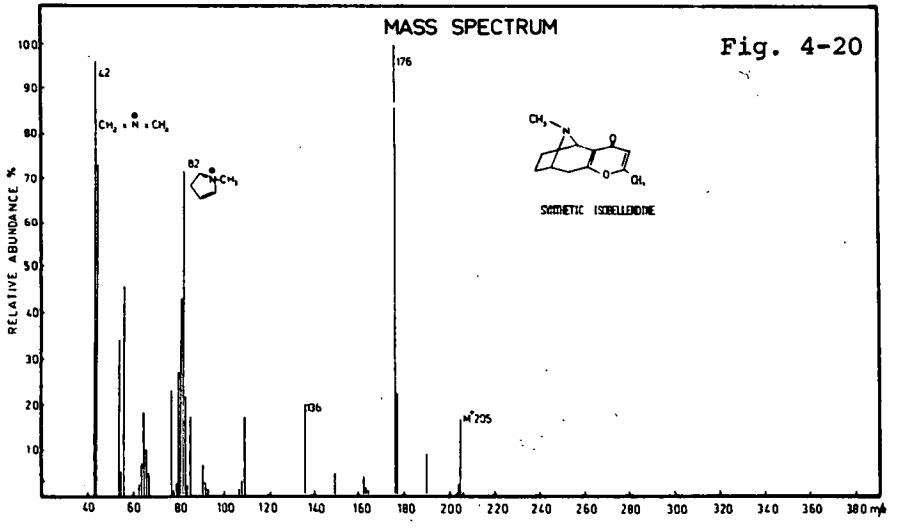


Fig. 4-20

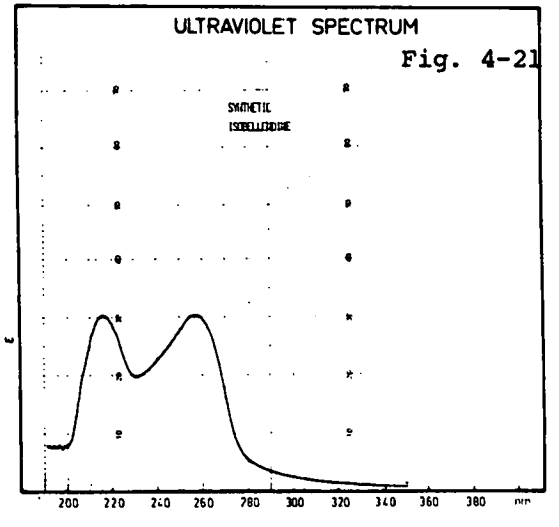


Fig. 4-21

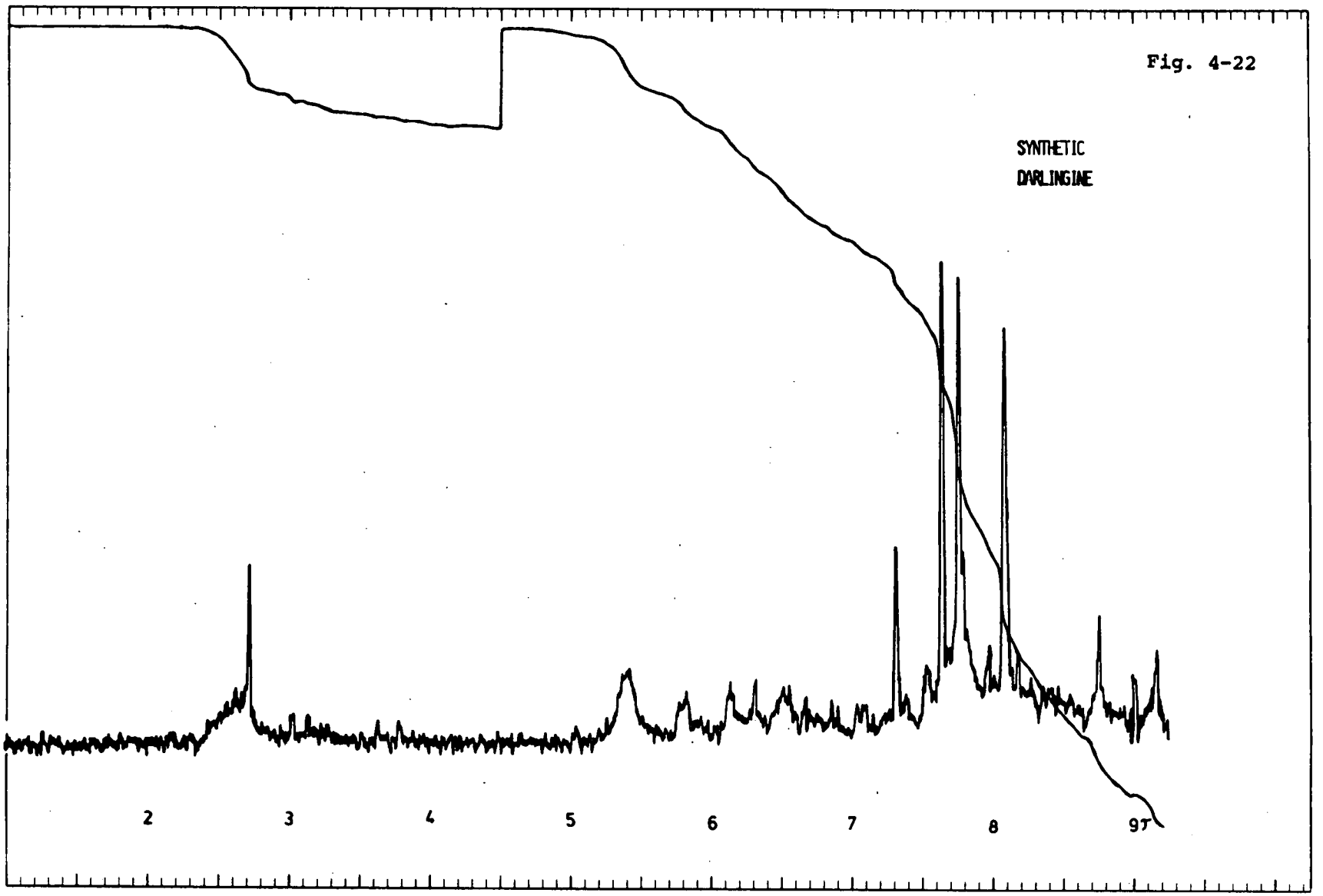


Fig. 4-22

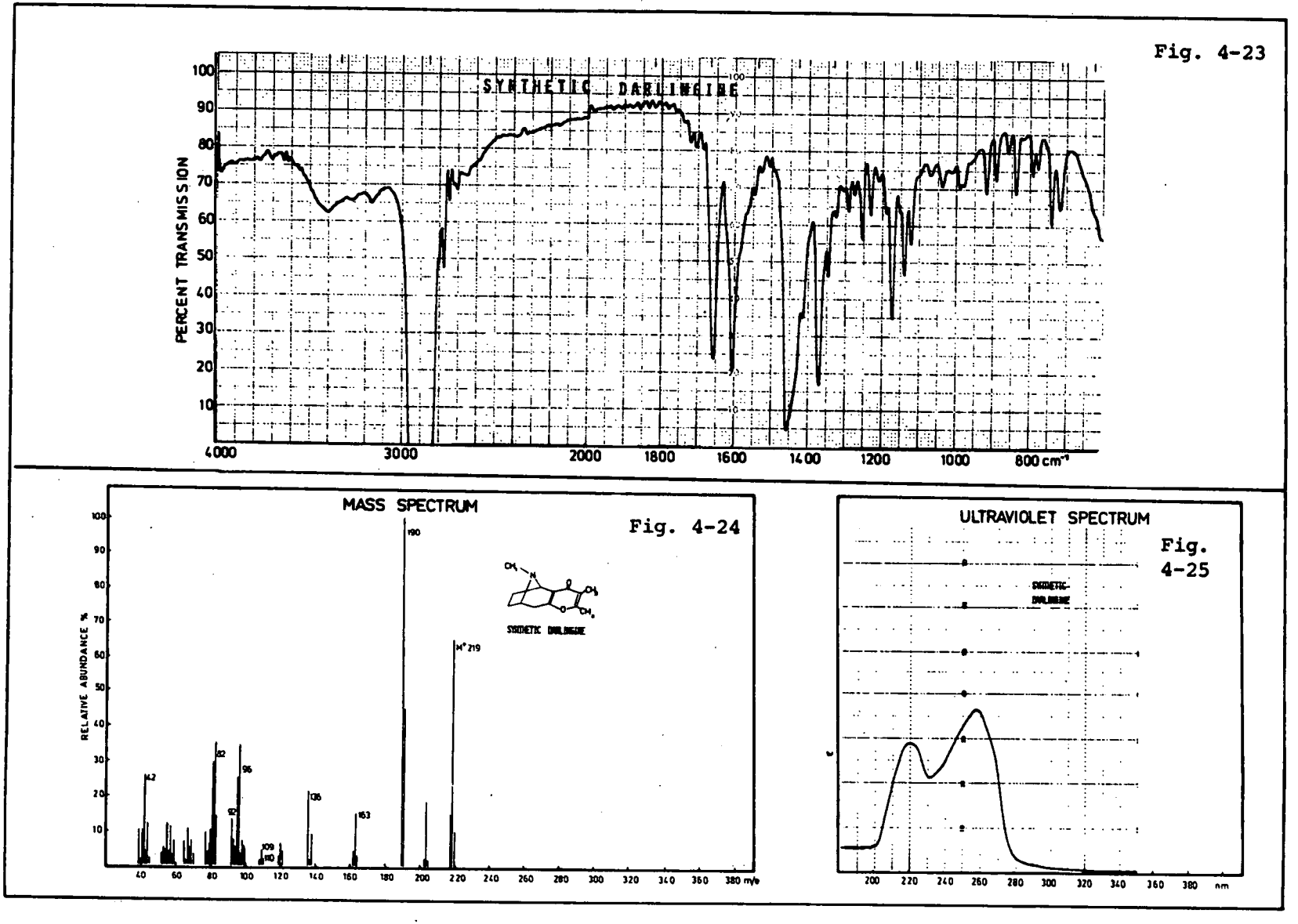


Fig. 4-23

Fig. 4-24

Fig. 4-25

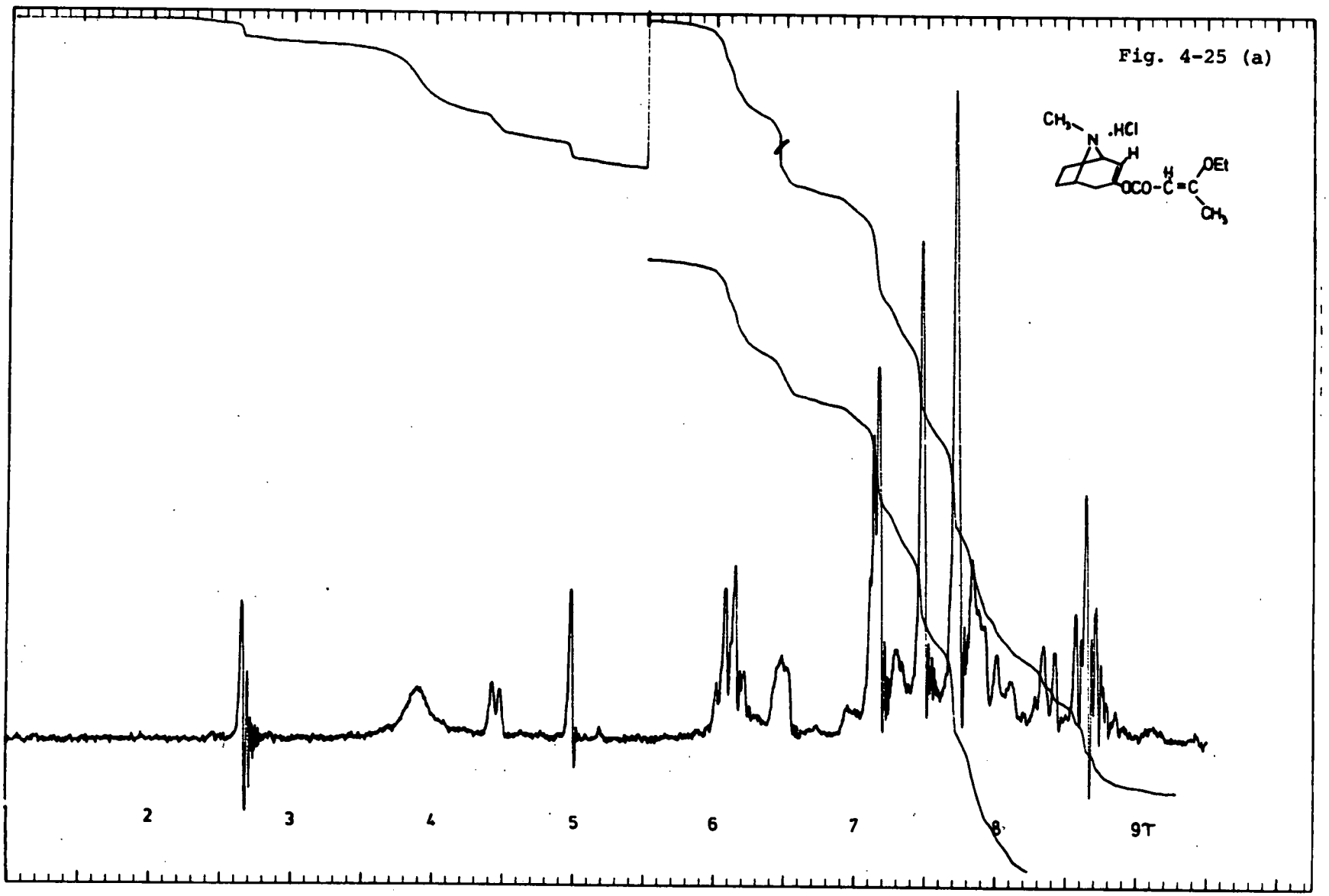
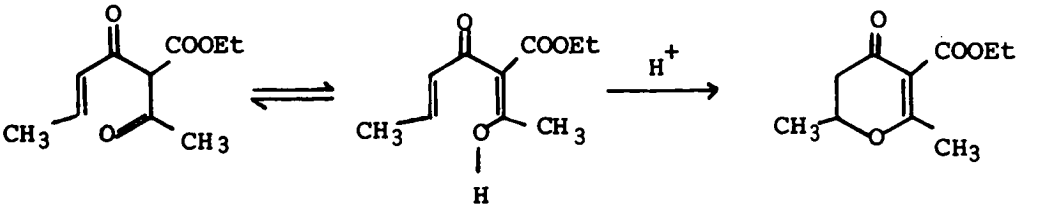


Fig. 4-25 (a)
CH3
N
.HCl
H
OCO-C=C
H
OEt
CH3
2
3
4
5
6
7
8
9T



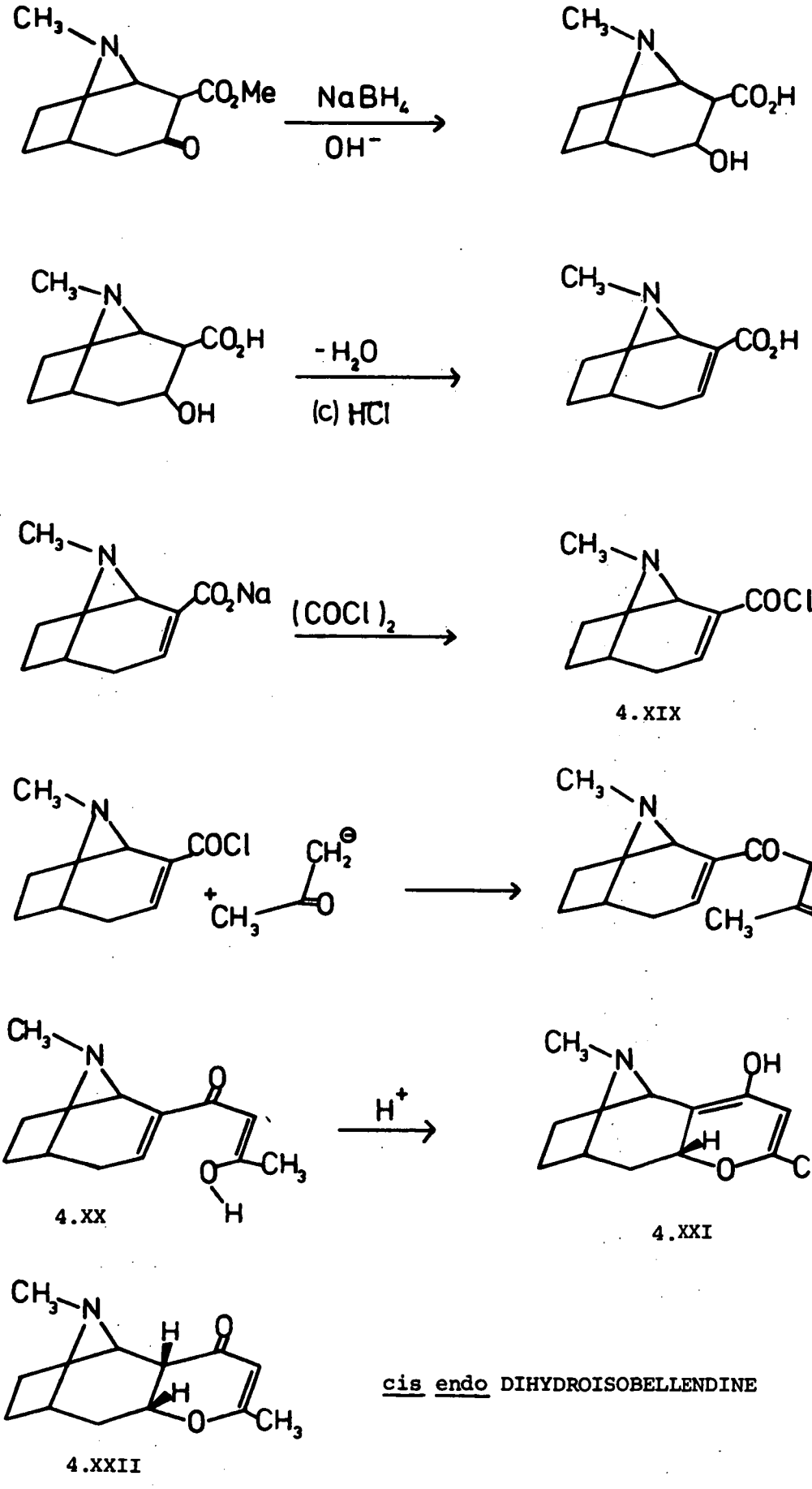
4.XVIII

The application of this approach to the dihydroisobellendine case necessitated the synthesis of anhydroecgoninoyl chloride (4.XIX) and condensation of this acid chloride with sodium acetone enolate. (Scheme 4-4). The stereochemistry of 5,11-dihydroisobellendine has been shown to be cis endo (i.e. 5α,11α). An examination of Dreiding stereomodels of the condensation product indicated that the most probable attack of the enol oxygen to effect ring closure should occur from the least hindered α face. Furthermore, the enol so produced was considered likely to equilibrate to favour the more thermodynamically stable equatorial (5α) derivative. No axial (5β) derivative was found in the plant extract, which had been subjected to strong acid conditions, favouring enolization and epimerization, in the work-up procedure. The acylation was effected by using a four-fold excess of the sodium enolate of acetone in benzene, produced from sodium wire and acetone, and slow addition to the benzene solution of the acid chloride in benzene/dimethoxy ethane.

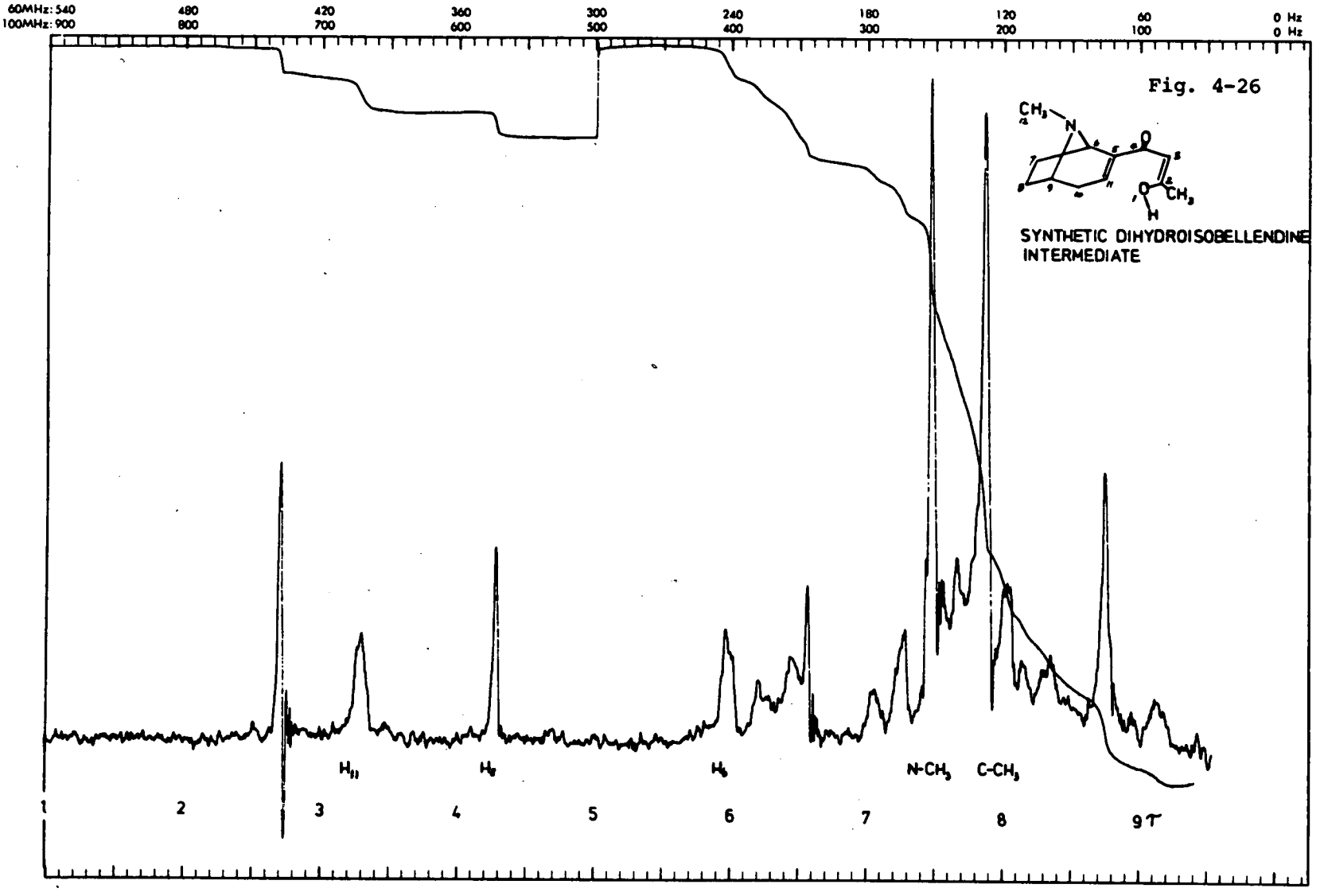
The major product isolated from this reaction by P.T.L.C. was the C-acylation product (4.XX). Spectral characteristics for this compound are shown in Figs. 4-26 - 4-29. Cyclization to the dihydro-γ-pyrone was achieved after three months in buffered (pH 4) aqueous conditions at $\tilde{\sim}$ 4°C.

SYNTHETIC SEQUENCE

cis endo DIHYDROISOBELLENDINE



Scheme 4-4


60MHz: 540
100MHz: 900
480
800
420
700
360
600
300
500
240
400
180
300
120
200
60
100
0 Hz
0 Hz
Fig. 4-26
SYNTHETIC DIHYDROISOBELLENDINE
INTERMEDIATE
H11
H9
H6
N-CH3
C-CH3
1
2
3
4
5
6
7
8
9τ

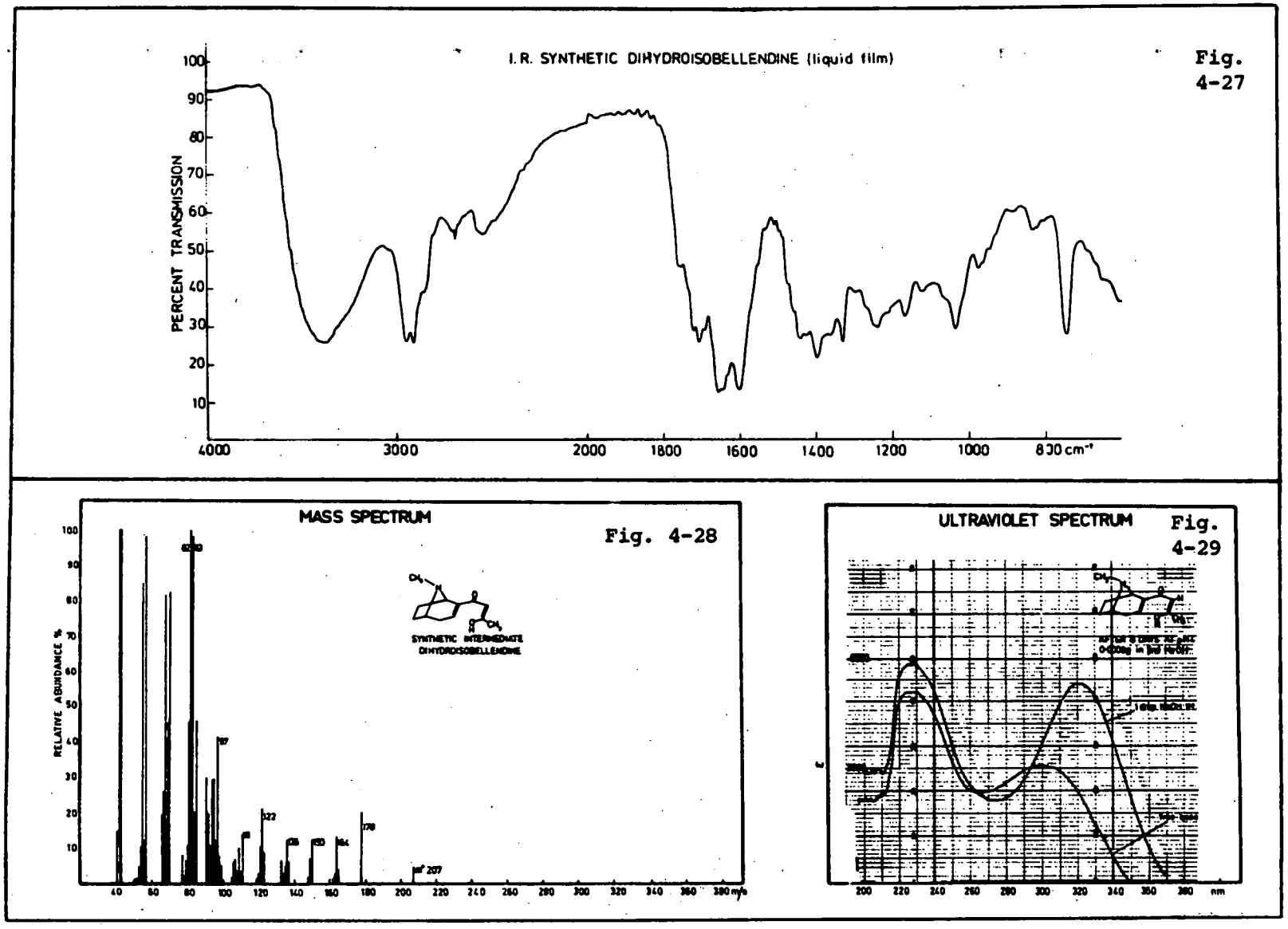


Fig. 4-27

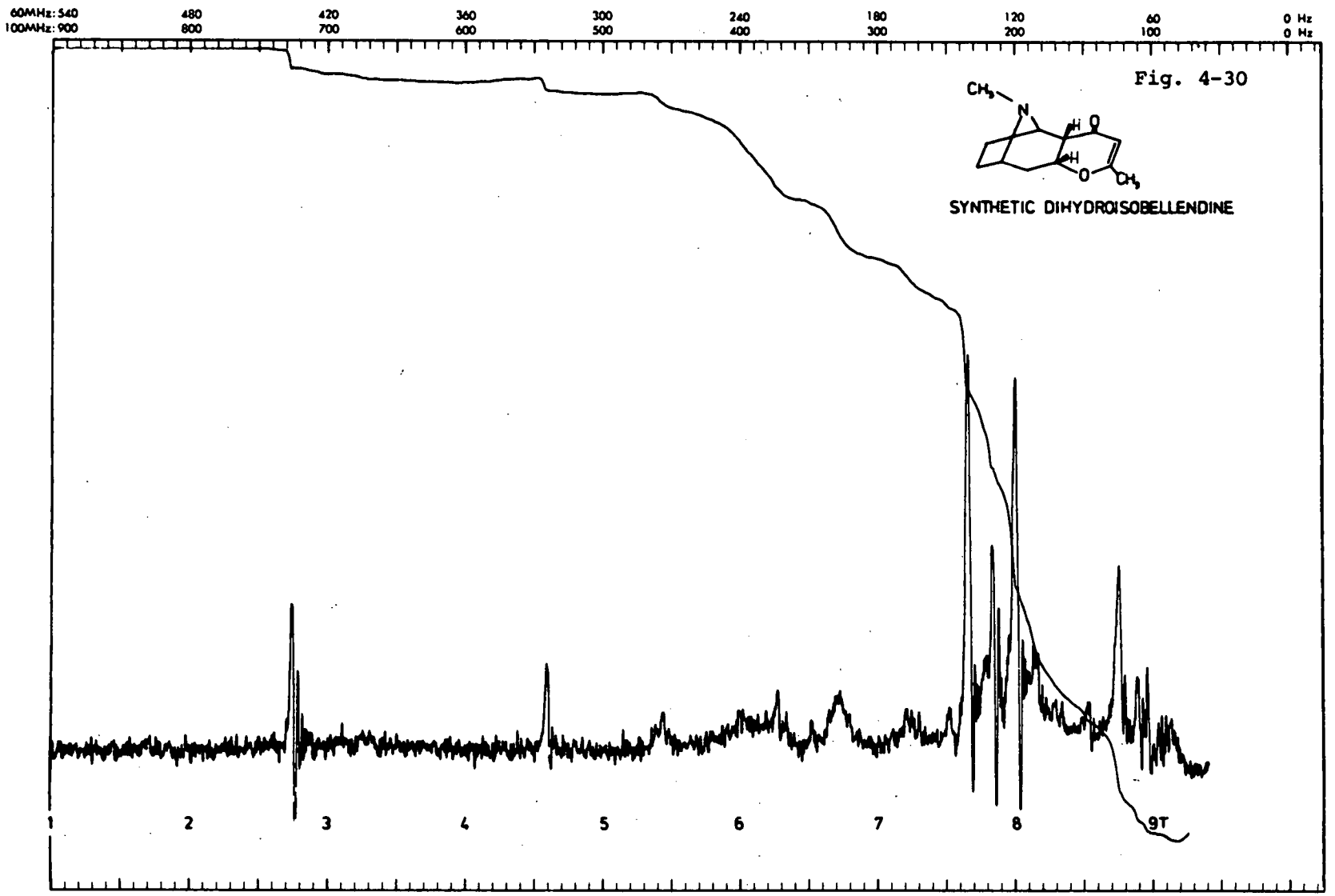
Fig. 4-28

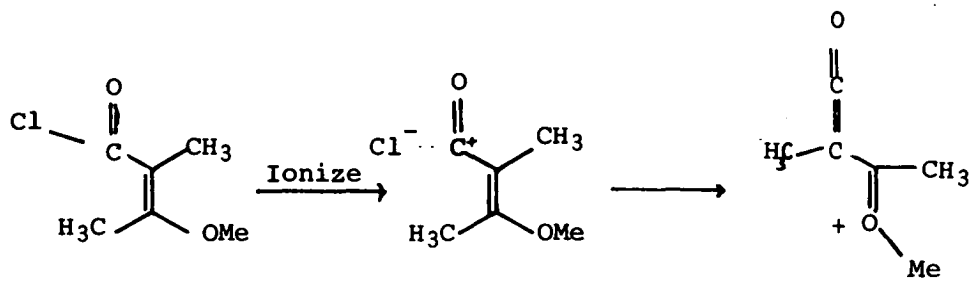
Fig. 4-29

However, the fact that incomplete cyclization occurred prevented an entirely unequivocal interpretation of the P.M.R. spectrum (Fig. 4-30) concerning C-11 stereochemistry. Although the multiplet associated with the C-5 proton at 7.21τ was not readily interpretable in favour of either stereochemistry, the line-width of the H_{11} resonance at 5.40τ suggested that the signal was a triplet, reflecting the C-11α-stereochemistry. Furthermore, only the exact correlation of the synthetic N-methyl and C-methyl resonances with those of the natural product support the assignment of the natural configuration to the synthetic product, which is not sufficient to be vigorous proof. The synthetic product was seriously impure, but present in such small amounts as to inhibit further purification. The purity may be estimated from the P.M.R. spectrum.

4.4. Conclusion

It has been found that the tropan-3-one acylation reactions reflect closely the theoretical and empirical considerations concerning acylation reactions. The favouring of O-acylation over C-acylation is clearly seen in all cases, suggesting steric influences in the approach of the acylating agent to the tricyclic enolate system in tropan-3-one. Under conditions which favour C-acylation: excess of enolate anion, non-polar solvents, and long reaction times, a very low yield of C-acylated product was isolated. Furthermore, the higher yield of C-acylated products in the case of enol-ether acylating agents suggests that electronic factors may be responsible for a decrease in the charge density on the carbonyl carbon of the acylating agent. This may thus reduce the tendency to O-acylate in the case of enol-ether acylating agents (4. XXIII).


60MHz: 540
100MHz: 900
480
800
420
700
360
600
300
500
240
400
180
300
120
200
60
100
0 Hz
0 Hz
Fig. 4-30
SYNTHETIC DIHYDROISOBELLENDINE
1
2
3
4
5
6
7
8
9T



4.XXIII

The dihydroisobellendine synthesis was achieved with little complication from O-acylation due to the lack of steric restrictions, the use of a non-polar reaction solvent, and a large excess of enolate anion. These facts suggest that this method, employing a protected β-keto acid chloride derived from 2-carbomethoxy tropan-3-one, acting on acetone enolate, may give the pyronotropanes in higher yields than the methods utilized in this study.

4.5. Experimental

Synthesis of Tropan-3-one

Prepared according to the method of Elming[39], m.p. 42° (lit. 42°).

Synthesis of 2-Carbomethoxy tropan-3-one

Prepared according to the method of Findlay[63], m.p. 101-105° subl. (lit. 101-104° anhyd.).

Synthesis of Methyl-2-methyl-3,3-diethoxy propanoate

Prepared according to the method of Kupiecki and Coon[69], b.p. 78° at 14 mm Hg (lit. 90° at 16 mm Hg).

4.5.1. Tropan-3-one Acylation Reactions

Reaction No. 1

Dry, freshly sublimed tropan-3-one (400 mg) was dissolved in 5 ml benzene and treated with methyl-2-methyl-3,3-diethoxy propanoate (800 mg) in 8 ml benzene. Sodium wire (70 mg) was added, the solution heated to near incipient fusion of the wire and refluxed for 10 min. During this time the solution turned dark red, after which it was left stand for 3 hr, treated with methanol (3 ml), and extracted with 10% H_2SO_4. The acid solution was heated for 1 hr at 45°, basified, extracted with chloroform (5 × 10 ml) and chromatographed to yield tropan-3-one (290 mg); no other Schlittler-positive bands on P.T.L.C. were observed.

Reaction No. 2

$NaNH_2$ (100 mg) was prepared from 57 mg sodium in 100 ml liquid ammonia. To this solution, tropan-3-one (350 mg) was added directly and methyl-2-methyl-3,3-diethoxy propanoate (500 mg) in 20 ml ether was added over a period of 1 hr, during which time the liquid ammonia was allowed to evaporate and the temperature to rise to 18°. The ether was extracted with 10% H_2SO_4, (3 × 5 ml), the acid layer warmed on a water bath for 15 min (60°), basified (NH_4OH) and extracted with chloroform (5 × 5 ml). P.T.L.C. (10% MeOH in $CHCl_3$, silica gel) isolated tropan-3-one as the only product (270 mg).

Reaction No. 3

Preparation of Ethyl-2-methyl acetoacetate-3,5-ethylene ketal

Ethyl-2-methyl acetoacetate was prepared according to the method of Folkers and Adkins[70], and used in the ketalization procedure of Burkhalter and Brown[71] to produce ethyl-2-methyl acetoacetate-3,3-ethylene ketal, b.p. 122-124° (0.40 mm Hg).

Acylation Reaction

Tropan-3-one (1 g) was dissolved in xylene and sodium wire (200 mg) was added. The solution was heated to fusion of the sodium and refluxed for 5 min, then the ketal ester (1.4 g) in xylene (20 ml) was added dropwise. On completion of addition, the solution was refluxed for 0.5 hr. An acid-extraction work-up and hydrolysis procedure identical to that described in Reaction No. 1 was employed and the products chromatographed (P.T.L.C., 10% MeOH in $CHCl_3$, silica gel). The major product isolated was tropan-3-one (780 mg).

Reaction No. 5

Preparation of 2-methyl acetoacetyl chloride-3,3-ethylene ketal

The ethyl ester of Reaction No. 3 was hydrolyzed by dissolving 10 g in 100 ml aqueous alcohol containing 2.3 g NaOH. The solution was refluxed for 4 hr and concentrated in vacuo. The white solid sodium salt which deposited was washed with ethanolic ether and dried over P_2O_5 (5×10^{-2} mm Hg). The sodium salt (5 g) was added in small portions to 6.7 g oxalyl chloride in 18 ml dry benzene over 1 hr.

After the addition, the suspension was refluxed for 2 hr, cooled, filtered to remove NaCl, and the solvent removed *in vacuo*. The residue was fractionally distilled *in vacuo*, b.p. 46° (0.05 mm Hg), to produce 2.8 g of acid chloride.

P.M.R. 8.65τ (s, 3H) C-4 methyl; 8.68τ (d, 3H, J = 8 Hz) C-2 methyl; 6.80τ (q, 1H, J = 8 Hz) C-2 H; 6.05τ (m, 4H) ethylene ketal protons.

I.R. $\nu_{C=O}$ 1800 cm^{-1}.

Acylation Reaction

To 2-carbomethoxy tropan-3-one (197 mg) in 10 ml dry dimethoxy ethane, was added sodium hydride (from 40 mg of 60% oil dispersion). The reaction was kept at 50° for 0.5 hr (under N_2), during which time it effervesced freely. The acid chloride from above (210 mg) in dimethoxy-ethane (8 ml) was added dropwise over a period of 0.5 hr. The solution was gently refluxed and stirred magnetically for 10 min. A fine grey precipitate of NaCl was filtered off and the solvent removed *in vacuo*. The product was chromatographed on P.T.L.C. (10% MeOH in $CHCl_3$, silica gel). In addition to 2-carbomethoxy tropan-3-one, the major product was found to be the O-acylate, which was readily hydrolysed to the starting β-keto-ester: 2 mg samples were heated with aqueous acids at 60° for 10 mins in conditions as outlined in Table 4-2.

Reaction No. 6

α-Deuteration of Tropan-3-one

Tropan-3-one (47 mg) was dissolved in 10 ml dimethoxyethane and added to a NaH suspension in 5 ml dimethoxyethane (from 14 mg NaH, 60%

oil dispersion). The solution was refluxed for 5 hr, quenched with 0.2 M DCl (2 ml) and evaporated to near dryness. The solution was basified (Na_2CO_3) and extracted with chloroform (3 × 5 ml). The oil from the concentrated chloroform extracts was sublimed in vacuo (40°, 5×10^{-2} mm Hg) to deposit 35 mg of fine white needles, m.p. 42°. P.M.R. spectroscopy indicated mono deuteration with approximately 70% axial replacement at C-2.

Mass spectroscopy confirmed the mono deuteration assignment with principal peaks observed at m/e 140, m/e 111 and m/e 83.

Reaction No. 7

The conditions of Reaction No. 5 were repeated for Reaction No. 7, using tropan-3-one (140 mg) in place of 2-carbomethoxy tropanone, and employing a longer reaction time (1.75 hr). The acid chloride (210 mg) was dissolved in dimethoxy ethane (10 ml), and added over a period of 1 hr to the cooled enolate solution, which then was refluxed for 0.5 hr and left stand for 8 hr at room temperature.

The solution was partly concentrated in vacuo, ether (10 ml) was added and the solution filtered and washed with water (10 ml). The concentrated solution was chromatographed and three bands recovered. The principal band was found to be tropan-3-one, constituting 60% of the recovered products. The second band, R_f = 0.48, (10% MeOH in $CHCl_3$, silica gel) proved to be the O-acylate (35 mg), and was subjected to a series of acidic hydrolysis reactions which resulted in the hydrolytic cleavage of the enol ester. (Table 4-3).

The minor product (7 mg) was examined by mass spectroscopy, and was found to be isomeric with the O-acylate; however, conditions were not found which readily hydrolysed the ketal to produce the triketone.

Reaction No. 8

Methyl-3-methoxy-2-methyl prop-2-enoate was prepared according to the method of Shaw and Warrener[72], b.p. 66-70°, 10 mm Hg (lit. 66-67°, 10 mm Hg).

Preparation of 3-methoxy-2-methyl prop-2-enoyl chloride

The above ester (39 g) was heated with 2 M NaOH (250 ml) at 90° on a water bath with stirring until a clear solution resulted (3 hr). The solution was cooled and acidified with 2 M HCl. The acid layer which separated was removed, and the aqueous remainder extracted with chloroform (5 × 50 ml). The combined chloroform extracts were dried and evaporated, and the remaining oil combined with the initial acid separation (30 g). The sodium salt, prepared by the addition of 13.5 g NaOH in aqueous ethanol and evaporation of the solvent, was dried over P_2O_5 at 100°C and 5×10^{-2} mm Hg for 8 hr.

Sodium 3-methoxy-2-methyl propenoate (9 g) was added to a solution of oxalyl chloride (10 g) in refluxing dry benzene (50 ml) over 0.5 hr. The suspension was refluxed for 2 hr, then filtered and fractionally distilled *in vacuo*. The acid chloride distilled at 40-42° (0.40 mm Hg).

Acylation Reaction

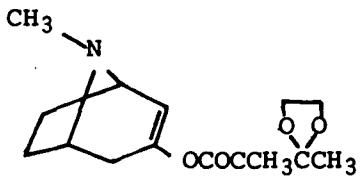
The acylation procedure was carried out as described in Reaction No. 7, utilizing 140 mg (1.0 millimole) of tropan-3-one and 135 mg of acid chloride (1.0 millimole). The major acylation product was isolated by P.T.L.C. and found spectroscopically to be the O-acyl product. This compound was subjected to the same set of hydrolysis conditions as outlined in Table 4-3.

Reaction Nos. 7 & 8

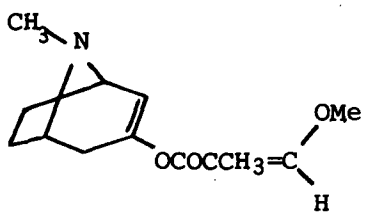
TABLE 4-3

Acylation of Tropan-3-one

Hydrolysis reactions



Reaction No. 7



Reaction No. 8

Hydrolysis Conditions [1]	
5% HCl, Neutralize	Tropan-3-one
H_2SO_4 [2] "	"
H_2SO_4 [4] 0°C 5 min	"
D_2O, DCl NMR tube [2]	"
$HClO_4$ in THF	"
$MgSO_4$ in wet benzene	Starting material
H_2SO_4 [2] in acetone	Tropan-3-one
HCl [5] in MeOH	"
Dowex 2 - X8 .	Starting material

(1) 3 mg acylated product dissolved in 2 ml of each solvent and left at 20° for 12 hr except where indicated.

(2) 15 mg examined at variable temperatures, reaction worked up at disappearance of ketal peak.

(3) Concentrated.

(4) Dilute.

(5) Gaseous.

Reaction No. 9

Preparation of 2,3-dibromo-2-methylpropanoyl chloride

Methyl methacrylate (200 g) in 1 ℓ CCl_4 was maintained at 6° and Br_2 (320 g) in CCl_4 (500 ml), was added dropwise. The reaction was left stand for 10 hr, and excess bromine removed by washing with 10% $NaHSO_3$ (1 ℓ) and water (3 × 1). The dried CCl_4 solution was fractionally distilled in vacuo, the brominated product distilling at 67-70° (5 mm Hg).

The above brominated methyl ester (40 g) was hydrolysed at 35° with 10% NaOH (500 ml) for 3 hr. On acidification, an oil precipitated, this was separated, and the aqueous phase extracted with chloroform (5 × 50 ml). The chloroform extracts were combined and dried, the solvent removed in vacuo and the oil fractionated at 88-90° (0.4 mm Hg) to give 2,3-dibromopropanoic acid. The acid (35 g) was dissolved in 125 ml benzene, and thionyl chloride (19 g) containing 3 drops of dimethyl formamide, was added dropwise. The solution was refluxed for 3 hr, and excess thionyl chloride and solvent removed in vacuo. The product was fractionally distilled at 45-48° (0.5 mm Hg).

Acylation Reaction

Tropan-3-one (1.39 g, 0.01 mole) in 25 ml dry benzene was added to NaH (from 400 mg, 0.01 mole of a 60% oil dispersion) in 60 ml benzene containing 1 drop absolute alcohol. The mixture was refluxed for 8 hr, cooled to $\tilde{\sim}$ 4°, and the acid chloride from above (2.66 g, 0.01 mole) in benzene (10 ml) added dropwise. The reaction was left for 1 hr at $\tilde{\sim}$ 0° and allowed to come to room temperature. The solution was filtered, concentrated in vacuo, and chromatographed to yield two major acylation

products. The major product, R_f = 0.40 (10% MeOH in $CHCl_3$, silica gel) had spectroscopic properties characteristic of the O-acyl product, and was readily hydrolysed in water to tropan-3-one.

The minor product, which was present in significantly greater quantities than in previous reactions, was found to be the C-acyl derivative, $\nu_{C=O}$ 1740 cm^{-1} (α halo ketone), $\nu_{C=O}$ 1630 cm^{-1} (enol carbonyl), ν_{OH} 3400 cm^{-1} (enolic hydroxy).

Attempted base-catalysed cyclization of Reaction No. 9 Product

The dibromo C-acyl product from the above reaction (30 mg) was dissolved in dry dimethoxyethane (10 ml), and added to 4 mg of NaH suspended in dimethoxyethane (10 ml). The solution was refluxed for 3 hr, cooled, and a saturated solution of ammonium chloride added (3 ml). Water (10 ml) containing NH_4OH was then added to the solution, which was extracted with chloroform. The chloroform solution was concentrated in vacuo and chromatographed. The major product, which rapidly turned red whilst extracting and evaporating, had $\nu_{C=O}$ 1785 cm^{-1}, $\nu_{C=O}$ 1720 cm^{-1}, M^+ 286, indicating the formation of a Favorski cyclopropanone product.

Reaction No. 10

Synthesis of Bellendine

Tropan-3-one (700 mg) (0.05 mole) was dissolved in 35 ml dry benzene and added to 0.05 mole NaH (from 200 mg of a 60% oil dispersion in 25 ml dry benzene. The suspension was refluxed for 20 hr after which 3-methoxy-2-methyl prop-2-enoyl chloride (370 mg, 0.05 mole) in 15 ml of dry benzene was added dropwise at 10°C. The solution was then refluxed for 0.5 hr, during which time it turned light purple. A saturated

solution of NH_4Cl (3 ml) was added to the cold solution, which extracted a considerable amount of basic material. The aqueous washing was basified and extracted with benzene and the combined benzene extracts were dried and chromatographed. The T.L.C. pattern is shown in Fig. 4-31. Three bands were separated by P.T.L.C. (10% MeOH in $CHCl_3$, silica gel). The major band (R_f = 0.47, Band 3, 120 mg) was found to be the O-acylate, the second band (R_f = 0.59, Band 2, 130 mg) was recovered tropan-3-one, and the minor band (R_f = 0.65, Band 1, 17 mg) was the C-acylated product.

Hydrolysis and cyclization of 2-(3'methoxy-2'-methyl propanoyl)tropan-3-one

Two samples of the C-acylated product (3 mg) were added to 0.5 ml 2 M H_2SO_4 and each heated at 75° on a water bath. The first sample was removed after 10 min, the second after 20 min. The cooled aqueous solutions were basified and extracted with chloroform. Thin-layer chromatography indicated quantitative hydrolysis and cyclization to bellendine in both cases. (Fig. 4-32, Fractions 5,6).

The balance of the C-acylated product (11 mg) was hydrolysed for 10 min under the same conditions, and similarly worked up. The oil was purified by P.T.L.C. followed by sublimation (85°, 1.5×10^{-4} mm Hg) to yield 7 mg of (±) bellendine, m.p. 128.5°, mixed m.p. with authentic (+) bellendine 153°, M^+ 205, $C_{12}H_{15}NO_2$ (H.R.M.S.). Spectral data is shown in Figs. 4-14 - 4-17.

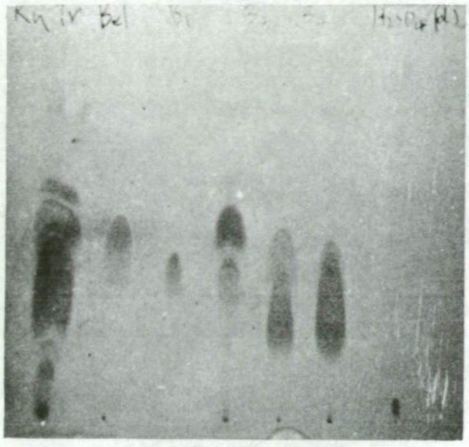
Synthesis of Isobellendine

Preparation of ethyl-3-methoxybut-2-enoate

Ethyl acetoacetate (60 g) and ethyl orthoformate (60 g) were mixed with 20 ml HCl saturated absolute alcohol. The solution was left stand

T.L.C. Summary of Reaction Products No. 10

(10% MeOH in $CHCl_3$)



Band 1
Band 2
Band 3
Band 4

1 2 3 4 5 6

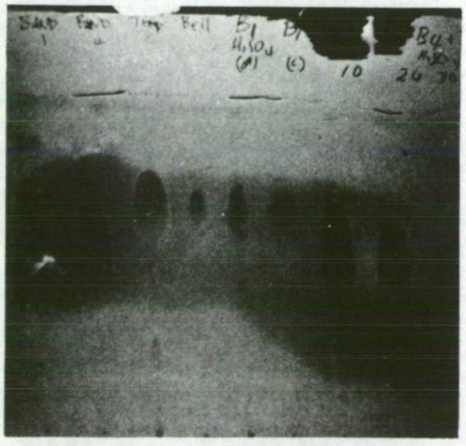
1. Reaction Mixture No. 10.
2. Tropan-3-one Standard.
3. Bellendine Standard.
4. Band 1 C-Acylate
5. Band 3 } O-Acylate
6. Band 4 }

Fig. 4-31.

T.L.C. Summary of Hydrolysis and Cyclization Products

Reaction No. 10

(10% MeOH in $CHCl_3$)



1 2 3 4 5 6 7 8 9

1. Band 1.
2. Band 4.
3. Tropan-3-one Standard
4. Bellendine Standard
5. Band 1 (10 min 75°, 4 M H^+)
6. Band 1 (20 min 75°, 4 M H^+)
7. Band 4 (10 min 75°, 4 M H^+)
8. " " (20 min 75°, 4 M H^+)
9. " " (30 min 75°, 4 M H^+)

Fig. 4-32.

for 24 hr, then fractionally distilled and the enol ether fractionated at 58-62° (6 mm Hg). G.L.C. analysis showed the distillate to be 98% pure.

To the enol ether (60 g) was added 16.6 g NaOH in 200 ml of 95% ethanol. The solution was refluxed for 2 hr and evaporated to dryness. The sodium salt was dried (P_2O_5), washed with ether and used to prepare the acid chloride in an identical manner to that described for 3-methoxy-2-methyl prop-2-enoyl chloride (Reaction No. 8). 2-Methoxy but-2-enoyl chloride distilled at 44-46° (0.5 mm Hg).

Acylation of tropan-3-one

The acylation and work-up procedures were identical to those described for Reaction No. 10. Chromatography of the reaction products afforded a smaller proportion (10 mg) of the C-acyl product than the corresponding product in the bellendine synthesis, which was hydrolysed and cyclized using the bellendine procedure. The product was purified by thin-layer chromatography to give a semicrystalline oil (5 mg), which had close spectroscopic properties to those of naturally occurring isobellendine. (Figs. 4-18 - 4-21). M^+ 205. A sublimed sample (2 mg) was analyzed. $C_{12}H_{15}NO_2$ requires C, 70.24%; H, 7.32%; N, 6.83%. Found C, 70.42%; H, 7.18%; N, 6.46%.

Synthesis of darlingine

Preparation of ethyl-2-methyl acetoacetate

Prepared according to the method of Fokers and Adkins[70]. The product was distilled in vacuo at 75-76° (15 mm Hg).

Preparation of ethyl-2-methyl-3-methoxy but-2-enoate (cis and trans)

Equal quantities of the starting ester, ethyl-2-methyl acetoacetate (60 g) and methyl orthoformate (60 g), were combined with 20 ml HCl-saturated absolute methanol for 20 hr. The product was redistilled in vacuo, b.p. 90-94° (4.5 mm Hg).

N.M.R. 6.2τ (q) 2H; 6.79 (s) 1.5H; 6.72 (s) 1.5H, cis and trans OMe; 9.0-9.4τ multiplet, 9H, 2(CH_3C=C) and CH_3-. 50:50 cis and trans mixture 40 g, 78%.

Preparation of 2-methyl-3-methoxy but-2-enoyl chloride

The enol ether from above (50 g) was hydrolysed in the same manner as described for 2-methoxy-but-2-enoyl chloride, and the sodium salt from the hydrolysis converted to the acid chloride with oxalyl chloride in the same manner. The product was fractionally distilled in vacuo, b.p. 64-66° (0.5 mm Hg). N.M.R. 8.40τ, (s), 3H, α-CH_3; 7.80τ, (s), 5H, β-CH_3; 6.30τ, (s), 1.5H; 6.38τ, (s), 1.5H; cis and trans OMe.

Acylation of tropan-3-one

The identical acylation and work-up procedure to that described in Reaction No. 10 using the above acid chloride was employed and the products of the acylation were chromatographed to yield 14 mg of the C-acylated product. Hydrolysis and cyclization using identical conditions to the bellendine synthesis produced (±) darlingine, m.p. 100°, mixed m.p. 88-92° M^+ 219. Spectral properties for synthetic darlingine are shown in Figs. 4-22 - 4-25, indicating close similarity to the natural product.

4.5.2. Synthesis of Dihydroisobellendine

Anhydroecgonic acid was prepared according to the method described in Section 3.9.8 (ii).

The acid chloride of this acid was prepared by suspending 200 mg of anhydroecgonic acid in dry benzene (5 ml) and adding 12 ml redistilled thionyl chloride. After refluxing for 4 hr the solution was evaporated to dryness to form a red crystalline hydrochloride of anhydroecgoninoyl chloride.

The acid chloride (60 mg) was dissolved in dry dimethoxy ethane (10 ml) and added to a solution of acetone (1 g) in benzene (15 ml), and sodium wire (80 mg) was added. After a very vigorous initial reaction, the solution was refluxed for 5 min. After cooling, 5 ml benzene was added to the solution, which was poured into cold saturated NH_4Cl solution (20 ml). The solution was then acidified (5% H_2SO_4), shaken, and the aqueous phase removed. This was then basified (NH_4OH, pH 8) and extracted with chloroform (5 × 10 ml). The combined chloroform extracts were dried, concentrated in vacuo and chromatographed on P.T.L.C. (10% MeOH in $CHCl_3$, silica gel). The major band, R_f = 0.24 (25 mg), proved to be 2-acetoacetyl trop-2-ene by spectroscopy.

The product formed a picrate, yellow needles from ethanol, m.p. 197-198° M^+ 207. Found: C, 49.73%; H, 4.69%. Calculated for $C_{12}H_{17}NO_2.C_6H_3N_3O_7$: C, 49.51%; H, 4.58%.

The product proved difficult to cyclize; conditions are set out in Table 4-4 for the attempts made to cyclize the adduct to dihydroisobellendine. The synthetic product recovered from the acid cyclization by basification and extraction with chloroform (5 × 10 ml) was chromatographed, (P.T.L.C. 12% MeOH in $CHCl_3$, silica gel) and the major band, R_f = 0.48, isolated and extracted. The spectral properties of this compound indicated that substantial cyclization had occurred to produce dihydroisobellendine. Spectral characteristics are shown in Figs. 4-26 - 4-30.

TABLE 4-4

Cyclization Conditions, Dihydroisobellendine

Conditions	Temperature	Time	Result
10% H_2SO_4	50°	1 hr	Starting material
25% H_2SO_4	60°	1.5 hr	" "
pH 2	20°	4 days	" "
3	"	" "	" "
4	"	" "	" "
5	"	" "	" "
3-4	0-5°	3 months	Dihydroisobellendine

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Ferrugine, a Novel Tropane Alkaloid

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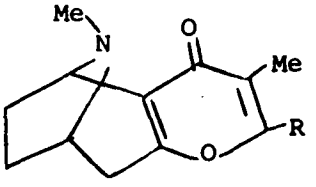
Since the discovery of the first alkaloid, bellendine[1] (I) from a Tasmanian plant of the family Proteaceae, a second species[2] from New Caledonia has been reported to yield alkaloids; the bases so far described all have a tropane nucleus, but have structural features distinct from other tropane alkaloids. We now report the isolation of two alkaloids from a further species, from Queensland, Darlingia ferruginea. By standard extraction procedures of leaf and stem material, followed by ptlc on silica gel, we obtained the major alkaloid, m.p. 162.5°, $[\alpha]_D^{19}$ 104°, whose structure was established as 2-methyl bellendine (II) by microanalysis and by correlation of its spectroscopic data with those of bellendine (I); we have also isolated (II) from the related species D. darlingiana[3]. A minor constituent of D. ferruginea was isolated as a basic oil, $[\alpha]_D^{19}$ 55°; its formula $C_{15}H_{19}NO$ was determined by hrms, which also indicated the loss of a benzoyl group from the molecular ion. Subsequent cleavage of the ion so formed led to the characteristic tropane fragments[4] C_5H_8N and C_6H_9N; however, the base peak at $C_6H_{10}N$ indicated that the piperidine ring was monosubstituted and underwent cleavage not involving the usual β-hydrogen abstraction.

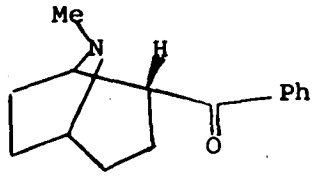
The presence of a benzoyl group was supported by the uv and ir spectra, and by the appropriate multiplets in the aromatic region of the pmr spectrum. The remainder of the latter was consistent with a 2-substituted tropane structure, the C-2 proton at 3.25δ being an

unresolved multiplet. The cmr spectrum was in complete accord with structure III; standard chemical shift theory enabled each resonance to be correlated precisely with a tropane[5] bearing a 2-benzoyl group, and the spectrum in addition gave stereochemical information: the C-4 resonance at 29.6 ppm relative to tms was depressed by only 0.3 ppm from the standard value for tropane[5] by substitution at the C-2 position, and this negligible γ effect indicated that the benzoyl group was an equatorial substituent. Ferrugine thus has the 2α-benzoyl tropane structure (III), and appears to have a close biosynthetic relationship with 2-benzyl-3β-acetoxytropane (IV) and the accompanying alkaloids isolated from the New Caledonian Knightia deplanchei[2].

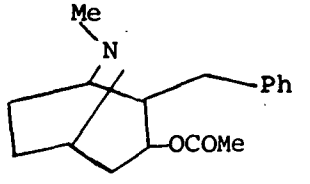


(I) R = H

(II) R = Me



(III)



(IV)

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W.D.S. Motherwell, N.W. Isaacs, O. Kennard, I.R.C. Bick, J.B. Bremner, and J. Gillard, Chem. Commun., 1971, 133.

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Bellendine, the First Proteaceous Alkaloid, a γ-Pyronotropane: *X*-Ray Structure Determination by Direct Methods

By W. D. S. Motherwell,* N. W. Isaacs, and Olga Kennard†
(*University Chemical Laboratory, Lensfield Road, Cambridge* CB2 1EW)
and I. R. C. Bick, J. B. Bremner, and J. Gillard
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Bellendine, the First Proteaceous Alkaloid, a γ-Pyronotropane: *X*-Ray Structure Determination by Direct Methods

By W. D. S. MOTHERWELL,* N. W. ISAACS, and OLGA KENNARD†
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and I. R. C. BICK, J. B. BREMNER, and J. GILLARD
(*Chemistry Department, University of Tasmania, Australia*)

Summary Bellendine, the first alkaloid isolated from a proteaceous plant, has been shown to have a γ-pyronotropane structure by direct methods of *X*-ray determination.

BELLENDINE[1] (I) occurs in the flowers of the monotypic shrub *Bellendena montana*, endemic on Tasmanian mountain plateaux, and constitutes the first alkaloid from the *Proteaceae*, a large family especially well-represented in the southern hemisphere. Extraction of the fresh flowers with Prollius solution[2] followed by standard methods of fractionation gave a benzene concentrate which was chromatographed on silica gel. The major alkaloid, bellendine, was obtained in 0·0013% yield together with at least two other bases whose structures have not yet been studied in detail.

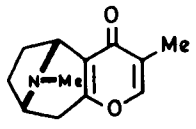
Bellendine forms colourless crystals, $[\alpha]_D^{20}$ + 168·5° ($CHCl_3$), m.p. 162—163°, from ether–petroleum, or by sublimation. Mass spectrometry established the formula $C_{12}H_{15}NO_2$, and the n.m.r. spectrum showed the presence of an *N*-methyl group, and a *C*-methyl attached to an olefinic carbon. Both the i.r. and the u.v. spectra suggested the presence of a conjugated carbonyl [ν_{max} 1650 cm^{-1}; λ_{max} (EtOH) 258, 213 nm (ϵ_{max} 10,680, 10,290)], and the chemical shift of the *C*-methyl group (τ 8·11) pointed to its being substituted α to the carbonyl group. The *C*-methyl is coupled allylically to the only olefinic proton present, the chemical shift of which (τ 2·42) indicated that it was attached to a carbon bearing an oxygen or a nitrogen atom; however, the basicity of bellendine (pK_a 7·3) precluded the possibility of it being a vinologous amide. The evidence is thus in accord with a γ-pyrone structure with a 3-methyl group, and is further supported by the fact that in CF_3CO_2H solution, the allylic coupling in the n.m.r. spectrum is lost due to the increased aromatic character of the ring.

An intense $M - 29$ peak in the mass spectrum was shown by high resolution to be due to loss of C_2H_5; however, the n.m.r. spectrum showed the absence of an ethyl group as such. The formation of this strong ion suggested the presence of a 6-membered nitrogen-containing ring which on electron impact aromatises with loss of an ethylene bridge and hydrogen transfer to the neutral fragment. These

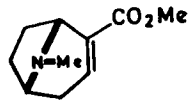
† External staff, Medical Research Council.

observations are in complete accord with a tropane system.

The absolute stereochemistry has not yet been determined, and is written arbitrarily as (I) to correspond with the structurally analogous naturally-occurring *Coca* alkaloid methyl ecgonidine (II).



(I)



(II)

The structure of bellendine was established by an *X*-ray diffraction analysis of the crystals using direct mathematical methods[3] with no chemical assumptions.

Crystal data: Bellendine $C_{12}H_{15}NO_2$, *M* 205, orthorhombic, $a = 9{\cdot}733 \pm 0{\cdot}008$, $b = 12{\cdot}624 \pm 0{\cdot}008$, $c = 8{\cdot}507 \pm 0{\cdot}007$ Å, $D_c = 1{\cdot}305$ g cm^{-3}, $Z = 4$, space group $P2_12_12_1$, $F(000) = 440$.

The intensities of 1010 reflexions were measured in about 10 days with an automatic diffractometer operating in the θ—2θ scan mode. The corrected intensities were converted into $|E|$ values and the highest 153 were input to a computer program[4] which selects reflexions to be given symbolic phases in the tangent formula method of solution. Three symbolic reflexions and one origin selecting reflexion were chosen by the program. These were input to an iterative tangent formula calculation[5,6] which generated 32 sets of phases for 153 of the highest $|E| > 1{\cdot}396$. A Karle map computed with the phased *E* of the set with lowest *R* (Karle) (19·4%) revealed the complete molecule

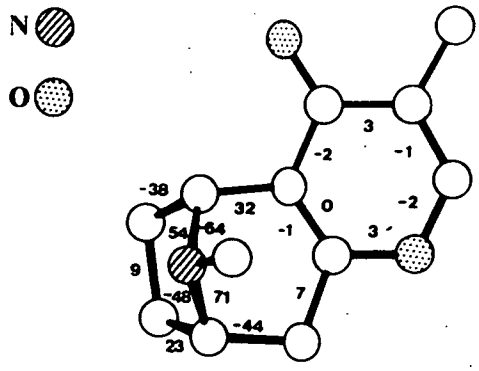


FIGURE. *A perspective view of the bellendine molecule with intra-annular torsion angles. Numbers refer to the relevant torsion angles in degrees.*

as (I). The atomic positions, taken from the Karle map, were refined through two cycles of isotropic and two of anisotropic full-matrix least-squares calculations. Hydrogen positions were calculated where possible by using the heavier atom co-ordinates. The current reliability factor is 7·3%. The solution and refinement of the structure was completed in approximately 2·5 h on an IBM 360/44 computer.

(*Received, October 30th*, 1970; *Com.* 1886.)

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PERGAMON PRESS
OXFORD · NEW YORK

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THE SYNTHESIS OF BELLENDINE

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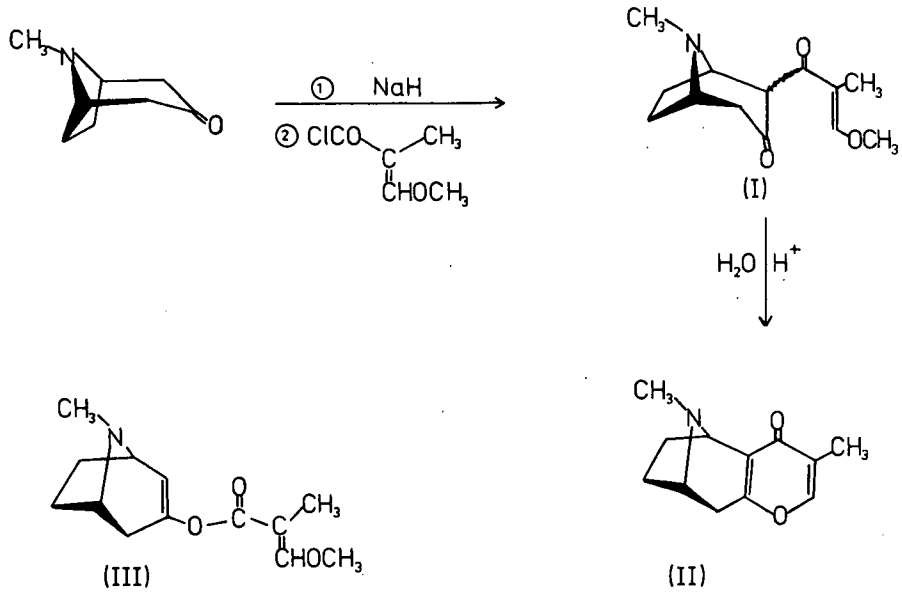
(Received in UK 30 October 1973; accepted for publication 8 November 1973)

Bellendine was the first alkaloid to be isolated[1] from the Proteaceae, a plant family widely distributed in the southern hemisphere, and its structure was shown to be (II) by direct methods of X-ray crystallography[2]. The plant from which it was obtained, Bellendena montana, contains other alkaloids of analogous structure, and several other tropane or pyranotropane alkaloids related to it have subsequently been isolated from New Caledonian[3], Queensland[4], or Tasmanian[4] endemic species.

We have synthesised racemic bellendine by the following process:



Tropinone in dry benzene was refluxed with sodium hydride for 20 hours, then 3-methoxy-methacryloyl chloride[5] in dry benzene was added at 10°C. The mixture was refluxed for 15

minutes, cooled, and shaken with saturated aqueous ammonium chloride, then the aqueous solution was extracted with benzene, the combined extracts were evaporated, and the residue was purified by PTLC on silica gel in 10% methanol-chloroform. Material from the high R_f band was removed and hydrolysed by warming briefly on a water bath with dilute sulphuric acid. The base was recovered in low overall yield from the neutralised solution and purified by PTLC. The crystalline product, further purified by sublimation, had UV, IR, NMR and mass spectra identical with those of bellendine. It melted at 128.5° alone and at 153° when mixed with (+) bellendine (m.p. 162°).

In addition to (I) and some unchanged tropinone, the acylation produced a considerable amount of crystalline product which, from its spectroscopic properties and ready hydrolysis to tropinone, corresponded to the O-acyl derivative (III) of the latter [$\nu_{C=O}$ 1715, $\nu_{C=C}$ 1650 cm^{-1}; λ_{max} 243 nm, ε_{max} 2,750; τ 8.27 (s, 3H), 7.50 (s, 3H), 6.15 (s, 3H), 4.41 (d, H), 6.50 (m, 2H); M_f^+ 237].

The method of synthesis is being extended to the preparation of other pyranotropane-type alkaloids[4] from proteaceous plants.

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3 C. Kan-Fan and M. Lounasmaa, Acta. Chem. Scand., 1973, 27, 1039.

4 I.R.C. Bick, J.B. Bremner, and J.W. Gillard, unpublished results.

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Aust. J. Chem., 1974, 27, 2515–8

Alkaloids of *Anthocercis tasmanica* (Solanaceae)

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Abstract

The major alkaloids of *Anthocercis tasmanica* are shown to be hyoscine and nicotine.

Anthocercis tasmanica is endemic in Tasmania and is one of some twenty species of *Anthocercis*, all indigenous to Australia.[1] Three Western Australian species have been found[2,3] to contain a variety of tropane alkaloids, most of which also occur in other solanaceous plants. *A. tasmanica* is now quite rare, and only a few bushes near the east coast of Tasmania are known to exist.

Extraction of a sample of air-dried leaves and stems gave 0·064% of crude base, which from t.l.c. contained two main alkaloids and several minor ones. The major alkaloid, separated by preparative t.l.c., gave an n.m.r. spectrum corresponding closely to that of hyoscine; a second oily base was considerably more volatile, and had a pronounced pyridine-like smell. On t.l.c. it had an R_F corresponding to that of nicotine, and the spot gave the same characteristic colour as nicotine when sprayed with iodoplatinate reagent.[4] A sample isolated by preparative t.l.c. gave an n.m.r. spectrum which agreed with that of nicotine.

The crude alkaloids extracted from a further quantity of plant material were separated into steam-volatile and involatile fractions. The first fraction formed a picrate identical with (−)-nicotine picrate, and the second a hydrobromide identical with that of (−)-hyoscine. T.l.c. showed the presence of other alkaloids, but in quantities insufficient for further examination; some of them are evidently steam-volatile from g.l.c.–m.s. of the crude nicotine fraction, but no indication of the presence of nornicotine could be observed.

Hyoscine has been found amongst the alkaloids of *A. littorea*,[3] and has been identified by t.l.c. as occurring in *A. viscosa*.[3] These species contain other tropane alkaloids, but no nicotine-type bases have been recorded in them; *A. tasmanica* is one

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[3] Evans, W. C., and Treagust, P. G., *Phytochemistry*, 1973, **12**, 2505.
[4] Smith, I., 'Chromatographic and Electrophoretic Techniques' 2nd Edn, Vol. I, p. 98 (Heinemann Medical Books: London 1960).

of the few solanaceous plants in which both these alkaloid types have been found to occur together.[5]

Experimental

Melting points are uncorrected, and were measured on a Gallenkamp apparatus. Mass spectra were measured by direct insertion on an EAI Quad mass spectrometer. N.m.r. spectra were measured in deuterochloroform on a Jeol JNM-4H-100 instrument with $SiMe_4$ as internal standard. Evaporations were carried out under reduced pressure and, particularly during the workup of the fresh material and isolation of nicotine, the temperature was maintained below 40°. T.l.c. was performed on Merck silica gel GF_{254}.

The plant material was collected at 'Kelvedon', near Swansea, in February 1973 and February 1974. Voucher specimen No. TCD-2 has been deposited in the University of Tasmania herbarium.

Extraction of Dried Plant Material

Dried milled leaves and twigs (730 g) were exhaustively extracted by cold percolation with methanol, and the extract was concentrated in a vacuum. The syrupy residue was dissolved in glacial acetic acid (200 ml) and the solution well shaken with water (600 ml). The precipitated material was redissolved in glacial acetic acid and again diluted as before. The insoluble material gave a negative Mayer's test and was discarded; the aqueous extracts were basified with ammonia and exhaustively extracted with chloroform. The chloroform solution was then repeatedly extracted with aqueous sulphuric acid (1%), and the acid extracts were united, rebasified and extracted with successive quantities of chloroform until a Mayer's test showed virtually complete removal of alkaloid. The combined chloroform extracts were dried (Na_2SO_4) and evaporated to yield a brown oil (452 mg) with a strong amine-type odour.

Separation of Major Alkaloids

The crude alkaloids were applied to two 40-cm preparative t.l.c. silica plates which were developed with chloroform–methanol (7%). Spraying of portion of the plates with iodoplatinate reagent[4] revealed at least four bands, with the two major ones somewhat overlapped. These were removed, the bases recovered by extraction with methanol, and the preparative t.l.c. repeated on each separate component. That of higher R_F, run as before, afforded an oil (29 mg), with a pronounced pyridine-like odour; on a t.l.c. plate with iodoplatinate reagent it gave a mauve-coloured spot which rapidly faded to a pale purplish white, as for nicotine. The n.m.r. spectrum of the oil corresponded closely with that of authentic nicotine.

For the component of lower R_F, chloroform–diethylamine (5%) was used for development. The alkaloid (45 mg) recovered from the main band gave a permanent purple spot with iodoplatinate reagent, and its n.m.r. spectrum was practically identical with that of authentic hyoscine.

Extraction of Fresh Material

Freshly collected leaves and stems (946 g) were cut up and exhaustively extracted in large Soxhlets with methanol. The extract was evaporated to dryness under reduced pressure, and the residue was dissolved in glacial acetic acid (150 ml) with gentle warming. The solution was diluted with water to 1·5 l. with vigorous agitation, filtered, and the residue again dissolved in glacial acetic acid and the solution diluted as before. The combined filtrates were basified with ammonia and thoroughly extracted with chloroform, the aqueous solution being reserved for later treatment to recover nicotine. The chloroform extracts were carefully evaporated and the residue steam-distilled until no further Mayer's test could be observed on the distillate, which was also reserved for the recovery of nicotine.

Isolation of Hyoscine

The residual liquid in the still after steam distillation was acidified with glacial acetic acid, filtered, washed with chloroform to remove impurities, then basified with ammonia and exhaustively extracted

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with chloroform. The extract was dried (Na_2SO_4) and evaporated, leaving a residue of crude non-volatile alkaloids (360 mg), which were applied to a 1-m preparative t.l.c. silica plate and eluted with chloroform–methanol (10%). The major band, free from contamination with a band due to nicotine, was removed and extracted as before. The base obtained was dissolved in methanol (*c.* 1 ml) to which a few drops of hydrobromic acid (40%) were added. The crystalline hydrobromide which slowly separated, after recrystallization from methanol, melted at 96°, resolidified, and remelted at 197°. An authentic sample of (−)-hyoscine hydrobromide, recrystallized in the same way, behaved similarly on heating, and its m.p. (lit.[6] 195°) was not depressed when mixed with the sample of plant origin. The picrate of the plant base, recrystallized from aqueous ethanol and dried in vacuum, formed yellow needles which melted in a sealed tube at 187–188°, undepressed on admixture with authentic (−)-hyoscine picrate (lit.[7] 187–188°).

Isolation of Nicotine

The aqueous ammoniacal solution remaining after chloroform extraction was further basified with sodium hydroxide and extracted with ether in an apparatus in which the two liquids were boiled in separate flasks with one reflux condenser common to both; the condensates separated into two phases which were continually returned to their respective flasks. From time to time the ether extract was removed and replaced with fresh ether; the extract was dried (Na_2SO_4) and evaporated, and the process was repeated until virually no residue was left on evaporation of the last extract.

The steam distillate obtained previously was extracted with ether in a continuous extractor until it gave no further Mayer's test. The ether extract was dried (Na_2SO_4) and evaporated, and the residue, together with those from the ether extracts above, was dissolved in 5% aqueous sulphuric acid (10 ml). The solution was thoroughly washed with ether to remove volatile impurities, then the aqueous solution was basified (NaOH) and extracted with successive quantities of ether (20× 10 ml). The combined ether extracts were dried (Na_2SO_4) and evaporated to an oil (142 mg) with a strong nicotine-like smell. When a portion was treated with saturated aqueous picric acid which had been slightly acidified with hydrochloric acid, crystals of nicotine picrate slowly separated, m.p. 217°, undepressed on admixture with authentic (−)-nicotine picrate (lit.[8] 218°). The mass spectra of the two samples of nicotine picrate were identical.

Minor Alkaloids

Fractions from preparative t.l.c. of the original extract were rechromatographed, chloroform–methanol (10%) being used for development on an analytical silica plate, against authentic specimens of some of the alkaloids found previously in *Anthocercis* spp. These (with their R_F values) included nicotine (0·53), hyoscine (0·33), littorine (0·20) and hyoscyamine (0·14).

Spots with the following R_F values could be detected in the alkaloid fractions: 0·52 (nicotine), 0·43, 0·34 (hyoscine), 0·21 (littorine?). The latter spot was ill resolved and could contain several components of lower R_F.

The crude steam-volatile bases gave a mass spectrum which, in addition to molecular and fragment ions corresponding to nicotine, showed several other prominent peaks including ones at *m/e* 223, 167 and 149, but no appreciable signal which could be ascribed to the molecular ion of nornicotine. The same material, applied to a g.l.c. column similar to that described by Massingill and Hodgkins,[9] gave prominent peaks with retention times of 2·20 and 3·46 min, the first of which matched that of an authentic nicotine sample.

Acknowledgments

We are grateful to Messrs A. and S. R. Himson for their invaluable assistance in locating and collecting the plant material, and to Mrs A. T. Cotton, from whose

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property the material was obtained. We thank also Dr J. R. Cannon and Mr L. J. McLeod for gifts of alkaloid samples, and the Australian Research Grants Committee for a grant to assist this work. The award of a Commonwealth Postgraduate Scholarship to one of us (J.W.G.) is gratefully acknowledged.

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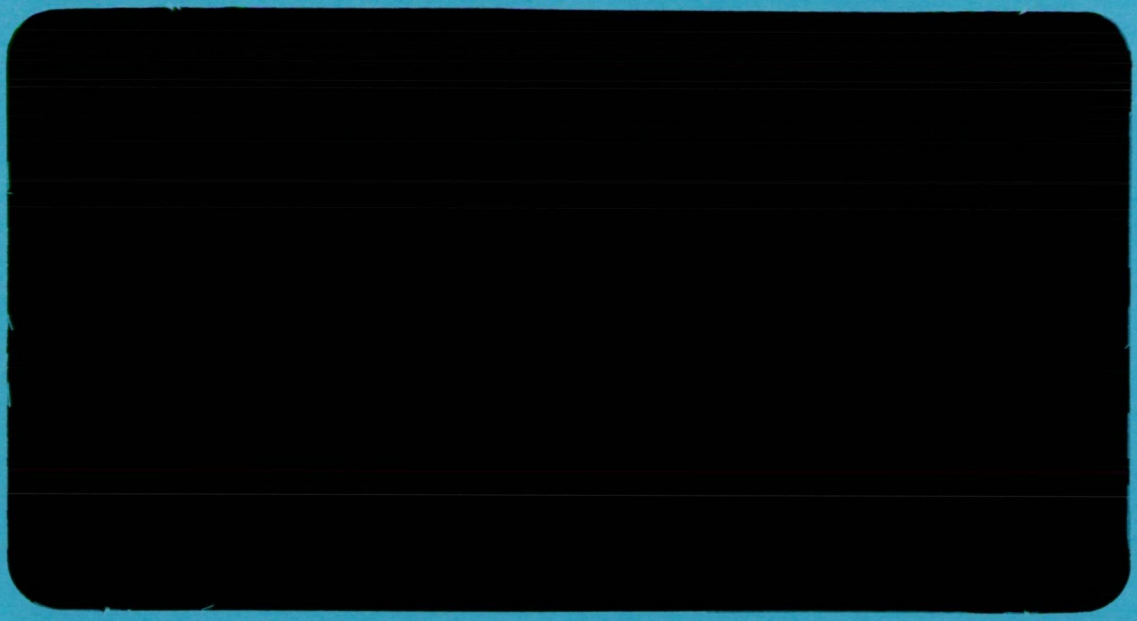

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Dionysia are so far very uniform and do not as yet give any indication of correlations with phylogeny in the genus.

In the previous paper on the flavonoids of the Primulaceae,[4] the provisional identification of the rare farina constituent, primetin (5,8-dihydroxyflavone) in *P. chionantha* was mentioned. This identification has now been fully confirmed (see Experimental) by means of direct comparison with natural material from *P. modesta*[5] and with a synthetic sample.[6]

EXPERIMENTAL

Plant material. Plant material was provided by Professor P. Wendelbo or Mr. J. C. Archibald (as indicated in Table 1) and identified by them. It was supplied as fresh material, except for *D. microphylla*, which was dried leaves and flowers, collected by P. W. in Afghanistan from plants growing at Maimana, Darrah Abdullah near Belcheragh. Voucher specimens are held by Professor Wendelbo.

Flavonoid identifications. Flavonoids were identified in leaf, flower or farina by methods outlined earlier.[4] Hirsutin was identified in flowers of *D. microphylla* by direct chromatographic and spectral comparison with an authentic sample from *P. capitata* flowers.[4] It was further identified by acid hydrolysis to give hirsutidin. Due to shortage of material it could only be identified in flowers of the other three *Dionysia* species by chromatographic comparison and by its colour properties.

Primetin (5,8-dihydroxyflavone) was identified in the farina of *P. chionantha* (cf. Ref. 4) by direct comparison with both a natural specimen supplied by Professor Hattori and a synthetic specimen supplied by Professor W. Baker. Material from all three sources had the following properties: λ_{max} in EtOH 282, 366, in EtOH–$AlCl_3$, 296, 360, and in EtOH–NaOEt, 290, 350 nm; R_f 0·76 on SiO_2 in 10% HOAc in $CHCl_3$, 0·68 on SiO_2 in 45% EtOAc in C_6H_6, 0·91 on paper in *n*-BuOH–HOAc–H_2O (4:1:5) and 0·24 in 15% HOAc; yellow in visible light, dull brown in u.v. light, immediate blue with Folin Ciocalteu reagent. Primetin differed in R_f, colour and/or spectral maxima from a number of other simple flavones examined at the same time, including 5-, 6-, and 7-monohydroxyflavone, 5,6-, 7,8- and 3,4′-dihydroxyflavone and 5-hydroxy-8-methoxyflavone.

Acknowledgements—The author thanks Professor P. Wendelbo for his generous interest and advice and, with Mr. Archibald, for the provision of the plant material. He also thanks Professor W. Baker for kindly supplying specimens of natural and synthetic flavones.

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PROTEACEAE

METHYL (*p*-HYDROXYBENZOYL) ACETATE AND AN ALKALOID, BELLENDINE, FROM *BELLENDENA MONTANA*

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(*Received* 5 *June* 1970)

Abstract—The previously unreported methyl (*p*-hydroxybenzoyl) acetate, and the first alkaloids from the Proteaceae, have been isolated from the flowers of *Bellendena montana*. The major alkaloidal constituent, bellendine, was obtained crystalline and characterized spectroscopically.

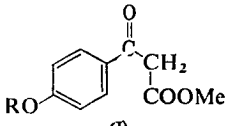
INTRODUCTION

THE MONOTYPIC *Bellendena montana*, an endemic Tasmanian proteaceous shrub abundant on mountain plateaux,[1] first attracted our attention in a survey of the local flora for the presence of alkaloids. The flowers of this plant gave a relatively strong Mayer's test for alkaloids and in view of the complete lack of reports of alkaloids in the Proteaceae,*[,3,4] detailed phytochemical work was initiated. We now wish to report the preliminary results of this work.

RESULTS AND DISCUSSION

Extraction of the fresh flowering heads with Prollius solution[4] and subsequent fractionation by standard methods, gave a crude basic fraction which contained both non-alkaloidal and alkaloidal material.

From the non-alkaloidal material a colourless, crystalline compound, m.p. 83–84°, was isolated. The high-resolution mass spectrum of this compound indicated the molecular formula $C_{10}H_{10}O_4$, a base peak from loss of $C_3H_5O_2$, and other prominent peaks due to loss of $C_3H_4O_2$ and CH_3O. The i.r. spectrum showed two of the oxygens to be in an ester group (ν_{max} 1740 cm^{-1}), and the other two in hydroxy and carbonyl groups (3300 and 1670 cm^{-1}). A *para*-disubstituted benzene ring was suggested by absorption bands at 1600, 1570, 1507, 1430 and 825 cm^{-1}. The latter deduction was supported by the aromatic proton absorptions (τ 2·62, q, 4H, J 9 Hz) in the NMR spectrum which also showed that the compound was a methyl ester (τ 6·28, s, 3H), and suggested a β-keto ester structure for it, with an unsubstituted α-methylene group (τ 6·02, s, 2H). An exchangeable proton singlet at τ 8·4 was assigned to a phenolic hydroxyl group. The u.v. spectrum in water showed absorption maxima at 209 and 272 nm (ϵ_{max} 8878 and 10,940) and on addition of alkali, the latter band shifted to 328 nm. A bathochromic shift of this order suggested a *p*-hydroxyphenone chromophore on the basis of Scott's Rules,[5] the calculated values being 271 nm and 324 nm respectively. The structure which is uniquely consistent with the above data is methyl (*p*-hydroxybenzoyl) acetate (I, R=H). While (I, R=H) has not been recorded previously, it has been suggested[6] as a precursor in the biosynthesis of coumarins and chromones. Methylation of (I, R=H) gave the known[7] methyl ether (I, R=CH_3).



(I)

TLC analysis (silica gel, $CHCl_3$–10% methanol) of the alkaloidal material from the original basic fraction indicated the presence of at least three alkaloids of R_f 0·62, 0·48

* Four other proteaceous plants were reported to give positive tests, but no alkaloids have been isolated.[2,3]

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[5] A. I. SCOTT, *Interpretation of the Ultraviolet Spectra of Natural Products*, p. 109, Pergamon Press, Oxford (1964).
[6] T. A. GEISSMAN, in *Biogenesis of Natural Compounds*, (edited by P. BERNFELD) 2nd edn., p. 790, Pergamon Press, Oxford (1967).
[7] *Beilsteins Handbuch der Organischen Chemie*, Suppl. I, Vol. X, p. 462. Verlag von Julius Springer, Berlin (1932).

and 0·46. The major alkaloid (R_f 0·48) which we have named bellendine, was isolated as colourless needles, m.p. 162–163°, in 0·0013 per cent yield (fr. wt.), while the other two alkaloids were obtained as oils. A high-resolution mass spectrum of bellendine revealed the formula $C_{12}H_{15}NO_2$, and indicated the ready loss of an ethyl group (base peak). The characteristic features of the NMR spectrum included an *N*-methyl group at τ 7·68, and a methyl group (τ 8·11, s, 3H, J 1·2 Hz) with allylic coupling to a single proton (τ 2·42, q, J 1·2 Hz). Protonation of the nitrogen in trifluoroacetic acid resulted in a downfield shift of the methyl resonance of 0·97 τ, and a splitting of the signal into a doublet (J 7·8 Hz) which was confirmed by spin decoupling. Both the i.r. and u.v spectra pointed to the presence of a conjugated carbonyl group possibly containing a β-substituent with +M characteristics (ν_{max} 1650 cm^{-1}; λ_{max} (EtOH) 257, 212 nm; ϵ_{max} 10,680, 10,290). No complete structural assignment for bellendine could be made on the basis of these data, and further work on the structural elucidation of this and other constituents of the plant is proceeding.

EXPERIMENTAL

M.ps are uncorrected. NMR spectral data (100 Mc/s) refer to solutions in $CDCl_3$; chemical shifts are quoted as values relative to tetramethylsilane.

Source of Plant Material

The flowers of *B. montana* were collected in January from the Hartz Mountain area of Southern Tasmania.

Extraction and Isolation Procedure

Fresh flowers together with their stalks (7 kg) were extracted with Prollius solution (MeOH–$CHCl_3$–0·88 NH_4OH 30:10:2) (4 × 6 l.) until the extract gave only a weak Mayer's test. The plant material was allowed to dry in the shade and the dried, ground material (1 kg) then re-extracted with Prollius solution. The combined Prollius extract was concentrated by evaporation of the solvents *in vacuo*, and $CHCl_3$ (300 ml) added to the residue. The $CHCl_3$ solution was then exhaustively extracted with 1N H_2SO_4, the acidic extract basified (0·88 NH_4 OH) and extracted successively with benzene and $CHCl_3$. Evaporation of the dried ($MgSO_4$) extracts *in vacuo* and combination of the residues afforded a crude basic fraction (9 g) which was adsorbed in silica gel (200 g; 200 mesh) from a warm benzene solution. The eluate was passed through two other silica gel columns (100 g and 60 g) and then collected automatically in 10 ml fractions (104 l. × 10 ml). Elution with benzene, benzene–$CHCl_3$ (5, 10, 15, 20, 25–99% $CHCl_3$), $CHCl_3$, and $CHCl_3$–MeOH (2, 2½, 3, 3½ 5, 10 and 15% MeOH) afforded the following:

(a) Crude *Methyl* (p-*hydroxybenzoyl*)*acetate* (I, R=H) (1·7 g, 0·024%) purified by crystallization from benzene–petroleum ether; m.p. 83–84°. Methyl ether (I, R=CH_3), m.p. 24° (lit.[8] 26–27°) NMR, 2·62τ 4H, q, aromatic protons), 7·4τ (3H, s, aromatic methoxyl), 6·35τ (3H, s, methyl ester) and 6·22τ (2H, s, methylene protons).

(b) Alkaloid A_1, (13 mg, 0·002%) as an oil, after purification by preparative TLC (silica gel; $CHCl_3$–10% MeOH).

(c) Bellendine (65 mg) after recrystallization from ether–petroleum ether, m.p. 162–163°; $[\alpha]_D^{20}$ + 168·5° (c, = 0·64, $CHCl_3$); pK_a 7·3. A further quantity of bellendine (28 mg) was obtained as colourless needles, m.p. 162°, after separation from alkaloid A_2 (18 mg; 0·003%) by preparative TLC and sublimation (85°/1·5 × 10^{-4} mm).

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ROSACEAE

AROMATIC HYDROCARBONS: EXAMINATION OF PEACH FRUIT AND FOLIAGE VOLATILES

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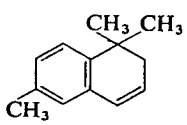
Abstract—Peach fruit volatiles obtained by steam distillation contained 1,2-dihydro-1,1,6-trimethylnaphthalene while foliage volatiles also contained this compound in addition to 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene (ionene) and two $C_{14}H_{22}$ hydrocarbons. Other compounds isolated from foliage were hexanal, *trans*-2-hexenal, *trans*-3-hexen-1-ol, benzaldehyde, nonanal, methyl salicylate and eugenol.

INTRODUCTION

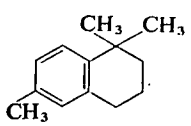
SEVERAL recent papers[1–3] on the composition of food volatiles have revealed the presence of a number of aromatic hydrocarbons including methylnaphthalenes and dimethylnaphthalenes. Pippen *et al.*[4] have suggested that these hydrocarbons are produced from carotenoids during the cooking process and result in a characteristic "naphthalene" aroma. In a previous paper[5] we reported a new naphthalene derivative, 1,2-dihydro-1,1,6-trimethylnaphthalene, as a component of strawberry volatiles. As part of an investigation of the occurrence of hydrocarbons in cooked foods we have examined peach fruit and foliage volatiles.

RESULTS AND DISCUSSION

We now wish to report 1,2-dihydro-1,1,6-trimethylnaphthalene (I) and a similar hydrocarbon 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene (ionene, II) in peach foliage volatiles. The identification of ionene was confirmed by comparison of the mass spectra, i.r. and GLC retention data of ionene synthesized in our laboratory and that isolated from peach foliage. In the case of peach fruit volatiles 1,2-dihydro-1,1,6-trimethylnaphthalene was shown to be present by mass spectral, u.v. and GLC data; however, the presence of ionene was not established although a very small peak with the correct retention time for ionene



(I)



(II)

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