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FACULTY OF GRADUATE STUDIES

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FINAL ORAL EXAMINATION

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

of

TADANOBU INABA

B.Eng., The University of Osaka Pref., 1962

M.Sc., The University of British Columbia, 1964

THURSDAY, OCTOBER 26, 1967, AT 3:30 P.M.

IN ROOM 225, CHEMISTRY BUILDING

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STUDIES IN NATURAL PRODUCTS

ABSTRACT

In part I of this thesis is described the structur determination of thamnosin, a minor component obtained from Thamnosma montana Torr. and Frem.

Thamnosin, $C_{30}H_{28}O_6$, was shown by NMR and IR spect to posess a coumarin chromophore and the mass spectrum suggested that this substance was cleaved under electro impact into two equal halves. Catalytic hydrogenation of thamnosin gave its dihydro-derivative and thereby indicated the presence of one easily reduced olefinic linkage. The UV spectrum of the latter suggested that two 6-alkyl-7-methoxycoumarin chromophores were present

The cleavage of thamnosin into lower molecular wei fragments was achieved by osmium tetroxide hydroxylatic of the double bond followed by periodic acid oxidation of the resulting diol. The two aldehydic products ($C_{11}H_8O_4$ and $C_{19}H_{20}O_4$) which were obtained from this reaction were subsequently characterized. The smaller fragment was identified as 7-methoxycoumarin-6-aldehyde by direct comparison with an authentic sample. The oth fragment, $C_{19}H_{20}O_4$, was initially shown by spectroscopi evidence, to possess a 6-substituted 7-methoxycoumarin system. The nature of the substituent at C-6 was subsequently identified as cyclohexene derivative bearing

two methyl groups and a tertiary formyl functions. Summation of the above and other evidence allowed a structural assignment to thamnosin. It is seen that this substance represents a novel system which has not been previously obtained in nature.

A detailed discussion of the mass spectra of thamnosin and its derivatives is presented.

Part II describes a possible synthetic route to the vobasine- and sarpagine-type alkaloids. Three different approaches to the preparation of 5-dehydro salts and to ring closure of corynanthenoid bases via transannular cyclizations were attempted.

Firstly, oxidation of sitsirikine (121) and dihydrocorynantheic acid ethyl ester (126) by mercuric acetate predominantly gave 3-dehydro salts (122) and (127), respectively, while the formation of 5-dehydro salts was not significant to form the bridge between C-16 and C-5 via transannular cyclization.

Secondly, mercuric acetate oxidations of 3-benzylderivatives of corynanthenoid bases followed by transannular cyclizations were attempted. Preparation of the isomeric 3α- and

3β - benzylyohimbines was accomplished by the reaction of benzyl magnesium bromide with 3-dehydroyohimbine perchlorate. The stereochemistry of these compounds was established by NMR and mass spectra. Accordingly, 3α- benzyl-derivatives of dihydrocorynantheine, dihydrocorynantheic acid ethyl and methyl ester and the tetracyclic methyl ketone (157) were prepared. Oxidation of these derivatives by mercuric acetate proceeded but transannular cyclization was not successful.

Thirdly, oxidation of 3,4-seco-corynantheinoid bases by mercuric acetate was attempted. Dihydrocorynantheal ethylene aectal methiodide (173) was treated with sodium in liquid ammonia to give 3,4-seco-N_b-methyldihydrocorynantheal ethylene acetal (174), which could be oxidized by mercuric acetate to the dehydro salt. However subsequent hydrolysis of the ethylene acetal was not successful.

GRADUATE STUDIES

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PUBLICATIONS

J.P. Kutney, A. By, T. Inaba and S.Y. Leong.
A Totally Synthetic Entry into the Veratrum Alkaloid Skeleton, Tetrahedron Letters, 2911 (1965)

J.P. Kutney and T. Inaba - The Structure of Thamnosin
Submitted for Publication

STUDIES IN NATURAL PRODUCTS

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in the Department

of

Chemistry

We accept this thesis as conforming to the

required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September, 1967

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Department of CHEMISTRY

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Acknowledgements

I wish to express my sincere thanks to Dr. James P. Kutney for his constant help and encouragement during the course of my research.

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PART I

STRUCTURE ELUCIDATION OF THAMNOSIN-

A NOVEL COUMARIN SYSTEM

INTRODUCTION

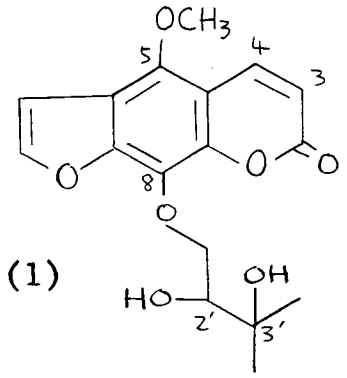
One of the major problems in organic chemistry has always been the structure elucidation of organic molecules which were largely supplied by natural sources. Interestingly the majority of natural products incorporate at least one benzene ring. The plants of the Rutaceae family are well known to contain a large number of benzenoid compounds, coumarins, flavones and some quinoline alkaloids.

The turpentine-broom, Thamnosma montana Torr. and Frem. (Rutaceae), is found in desert mesas and slopes and these shrubby plants were reported to have plant-growth-inhibitor properties[1,2] and to have been used by American Indians in folk medicine[3].

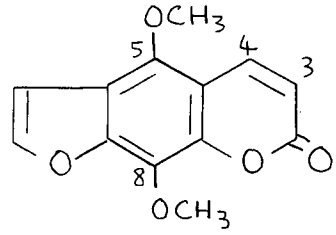
Bennett and Bonner studied the toxicity of aqueous extracts of leaves of eleven desert plant species and found that Thamnosma montana Torr. and Frem. was the most toxic as judged by the response to tomato[1]. The crude material caused death of young tomato plants at concentration of about 1 mg./ml. within seven days.

Three crystalline compounds were isolated from Thamnosma montana and two of them were identified to be byakangelicin (1) and isopimpinellin (2)[1].

The structure of the third compound was elucidated by Dreyer[4] and found to be the diol (3), 5-(3'-methyl-2',3'-dihydroxybutanyl)-8-methoxypsoralen. Dreyer developed a better extraction scheme for isolating not only



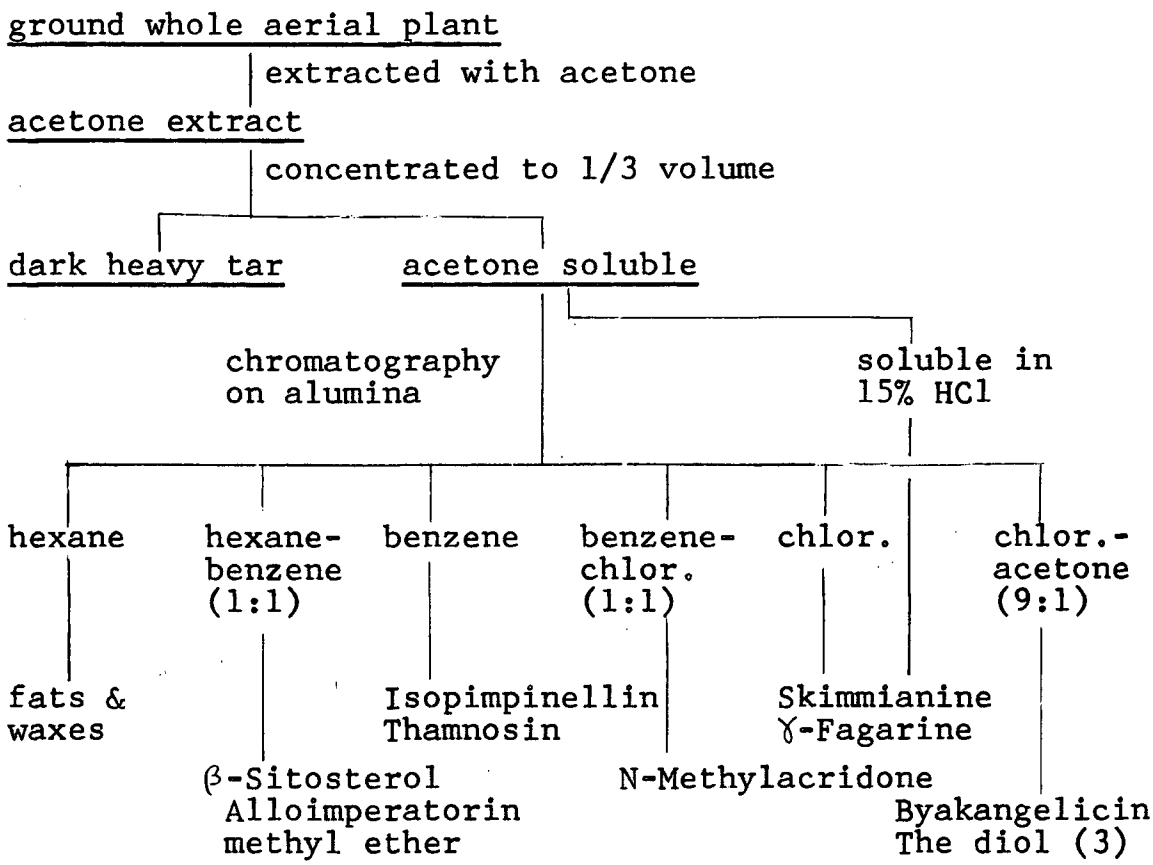
(1)



(2)

the three compounds obtained by Bennett and Bonner but was able to separate six other compounds. This extraction procedure is schematically represented below.

Extraction Scheme



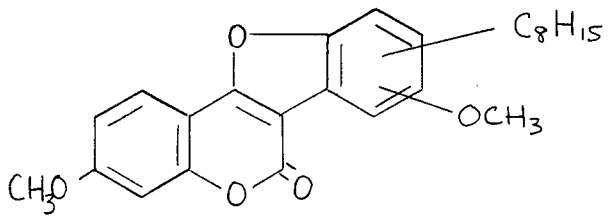
As shown in the extraction scheme and in Table 1, these six compounds were β-sitosterol, three known alkaloids, a known furocoumarin and an unknown compound, thamnosin. This is the first report of N-methylacridone, the parent member of acridone alkaloids[5], occurring as a natural product.

Table 1

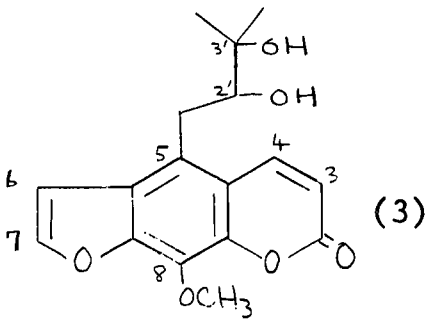
Name	Formula	m.p.	Ref.
β-Sitosterol (4)	$C_{29}H_{50}O$	137-9°	
Alloimperatorin methyl ether (5)	$C_{17}H_{16}O_4$	108-10°	10
Isopimpinellin (2)	$C_{11}H_{10}O_5$	148-9°	1,4
Thamnosin	$C_{25}H_{26}O_5$	244-6°	4
N-Methylacridone (6)	$C_{14}H_{11}ON$	202-3°	4,5
Skimmianine (7)	$C_{14}H_{13}O_4N$	173-5°	7,8,9
γ-Fagarine (8)	$C_{13}H_{11}O_3N$	140-2°	7,8,9
Byakangelicin (1)	$C_{17}H_{18}O_7$	105-7°	1,4
The diol (3)	$C_{17}H_{18}O_6$	174-6°	1,4

Dreyer's proposal for the structure of thamnosin was the following[4]. Thamnosin, m.p. 244-6°, was analyzed for $C_{25}H_{26}O_5$ and its ultraviolet spectrum suggested that thamnosin might be a coumesterol derivative with the long wavelength band shifted slightly to higher energies. The presence of a lactone ring was shown by treatment with 5% sodium hydroxide in aqueous methanol and regeneration

of the starting material upon acidification. Fusion with potassium hydroxide gave resorcinol. The nuclear magnetic resonance spectrum showed the presence of two methyl groups, two methoxyl groups, one olefinic proton and a complex multiplet which was assigned to nine aromatic protons. Dreyer suggested a tentative structure for thamnosin as the following[6].



This section of the thesis will be concerned with the complete elucidation of the structure of thamnosin.



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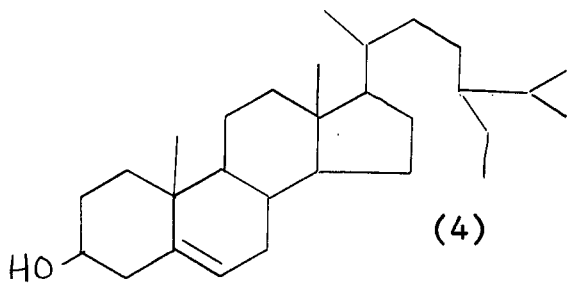

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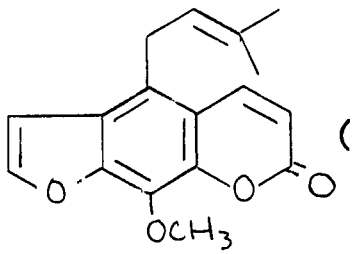

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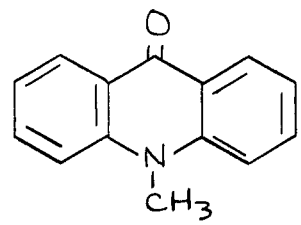

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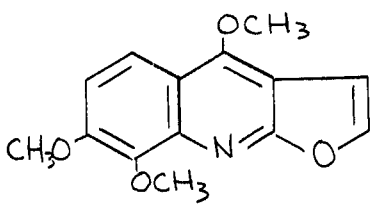

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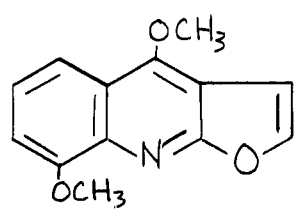

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Biosynthesis of Coumarins

Before discussing the structural elucidation of thamnosin, I felt that it was appropriate to first provide a brief summary of the investigations dealing with the biosynthesis of coumarins.

Before the application of radioactive tracers became possible, attention was focussed on structural regularities within families of compounds and in this way attempts were made to predict plausible biogenetic pathways. Ruzicka's isoprene rule arose from such considerations. In a similar manner, Robinson regarded flavones, chalcones, anthocyanins and related compounds as containing C_6-C_3-C_6 units; cinnamic acids, coumarins and some other groups of natural products as containing C_6-C_3 units[11].

It appears that the large number of benzenoid compounds in nature derive their aromatic rings by one of two routes: (1) via shikimic acid or (2) through polyacetyl chain cyclization[12]. The phenylpropane skeleton (C_6-C_3) is probably the basis of natural coumarins and it has been suggested that the benzene rings arise most likely from shikimic acid in view of the overwhelming preponderance in the C_6-C_3 compounds of the typical shikimic-derived oxidation patterns (Figure 1).

The first proposal for coumarin biosynthesis from cinnamic acids was offered by Haworth[13], who postulated para oxidation of a p-hydroxy-cinnamic acid (9) to yield (11), which could then cyclize by a Michael addition of

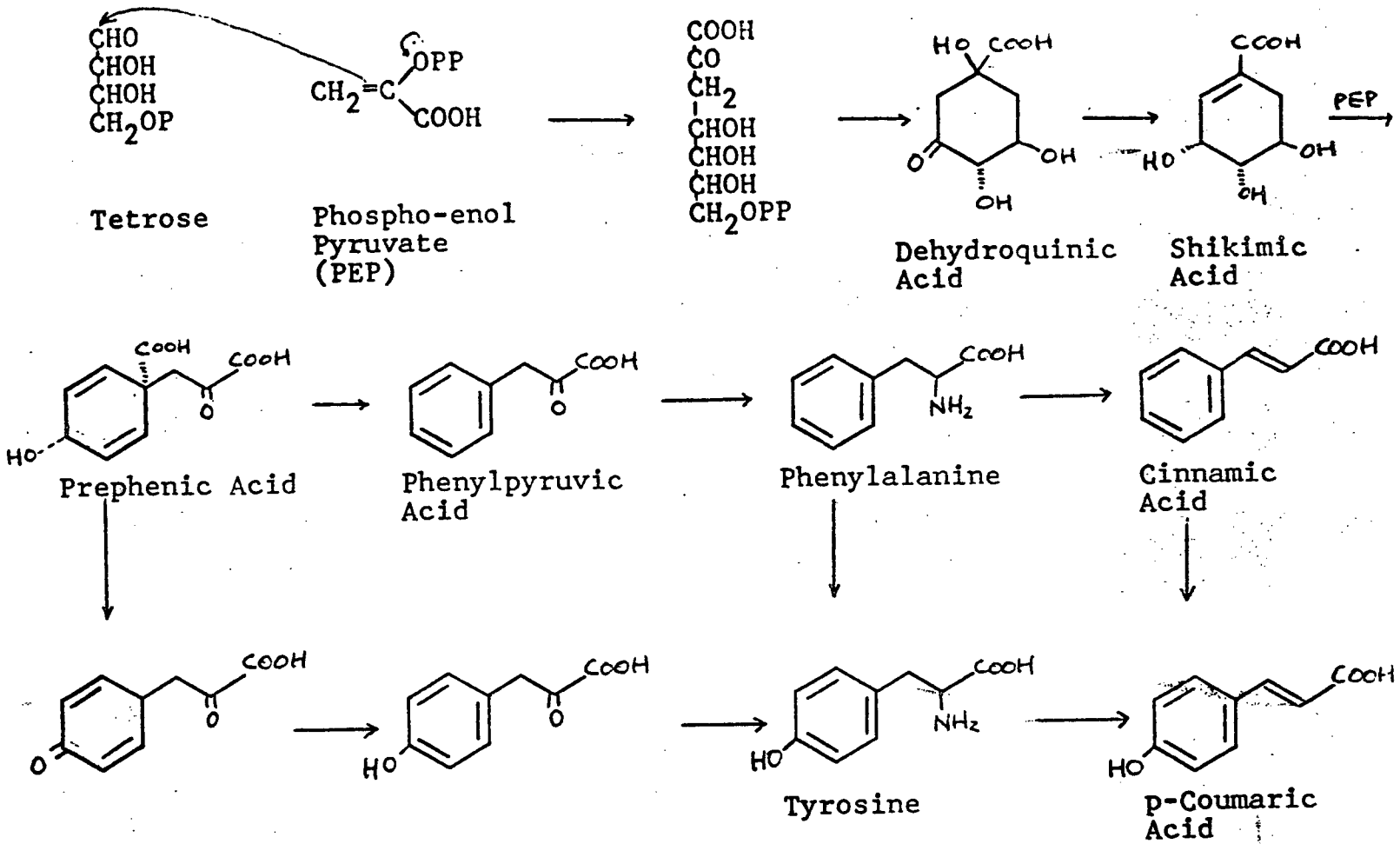


Figure 1 The Shikimic Acid Pathway to Aromatic Compounds

carboxyl, and finally dehydrate to yield a 7-hydroxy-coumarin (15), (Figure 2). It should be noted that most of the known coumarins possess the 7-oxygen function.

Grisebach and Ollis[14] suggested the direct oxidative coupling of the para-position with the cinnamoyl carboxyl, as in (12), whereas Kenner et al[15] have favored initial two-electron oxidation of the carboxyl, as in (10), so as not to have the process dependent on the p-hydroxyl function.

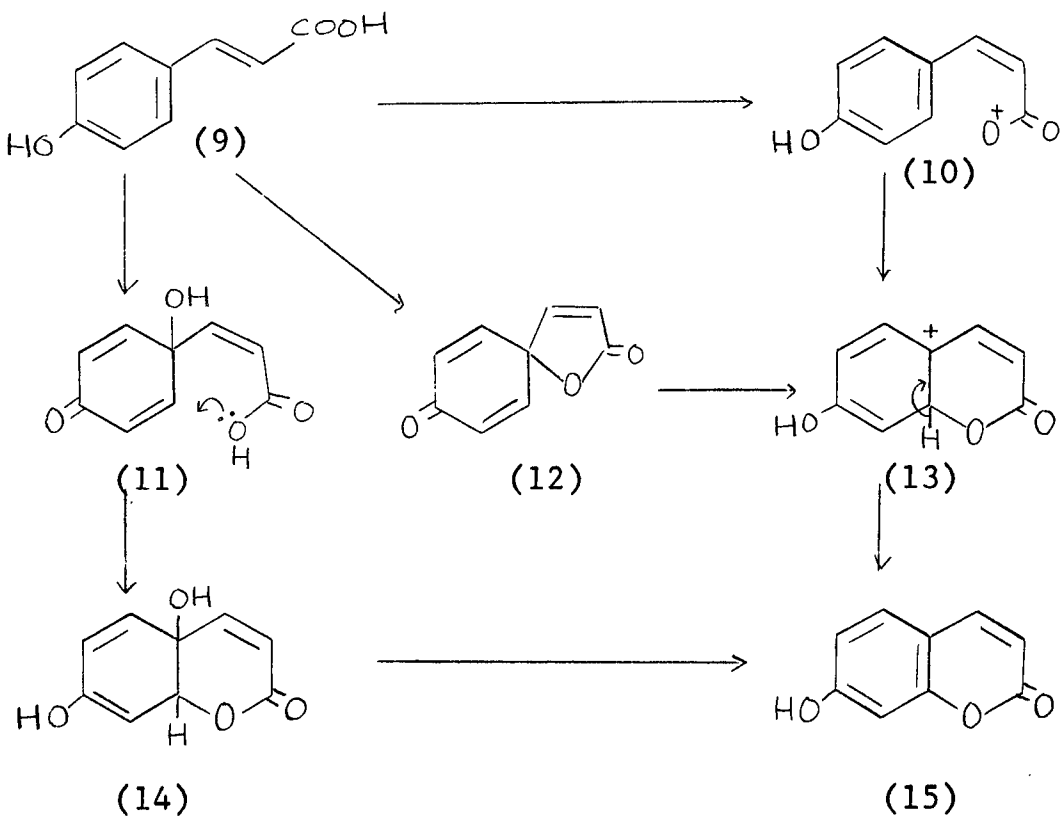
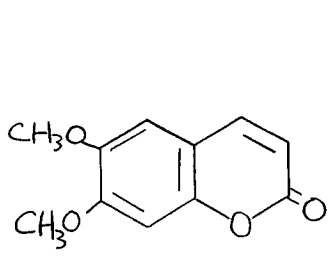


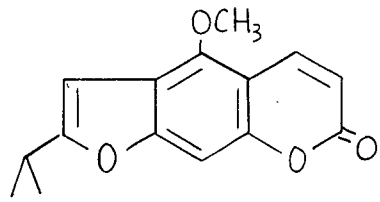
Figure 2

The coumarins display an impressive variation in structure (Figure 3) and therefore provide ample

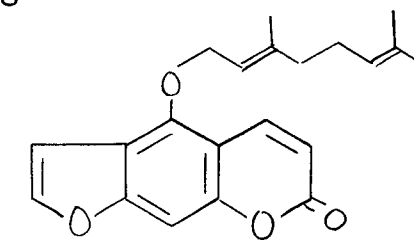
opportunity for biosynthetic investigations.



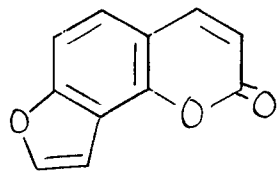
Scopoletin



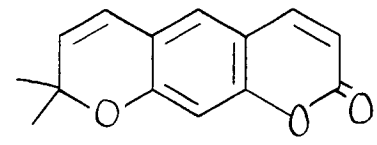
Xanthoxyletin



Bergamotin



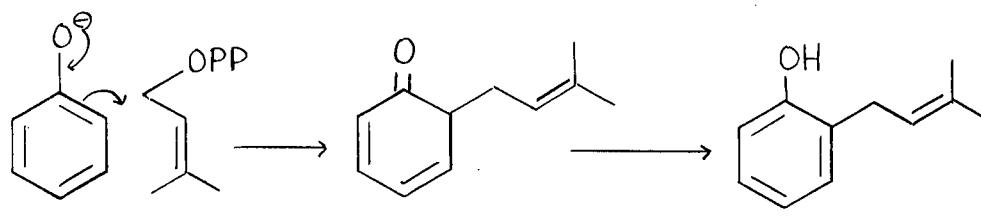
Angelicin



Xanthyletin

Figure 3

The methyl and isopentenyl substituents are always found at aromatic sites conconant with the mechanism of enol alkylation[16].



(pp=pyrophosphate residue)

The ultimate source of these isoprenoid units is mevalonic acid, which eventually gives rise to the active synthetic fragment, isopentenyl pyrophosphate. It seems probable that the course of biosynthesis described above represents the essential nature of these processes but

obviously experiments with labelled intermediates are necessary to establish or exclude them. Some of the recent investigations in this area will be discussed below[34].

Evidence from feeding experiments showed that coumarin (21) was synthesized from shikimic acid-derived phenylpropane precursors (C_6-C_3)[17,18, 19] in preference to acetate condensation.

Brown, Towers and Wright[17] studied coumarin formation with ^{14}C in the perennial grass, Hierochloe odorata, and found that the best precursors were o-coumaric acid (18) and cinnamic acid; phenylalanine (16) and shikimic acid were not as good, acetate and salicylic acid very poor. This work indicated the same results as those of Kosuge's feeding experiment in sweet clover, Melilotus alba[18].

The following results by Kosuge and Conn[20], by Stoker and Bellis[21] and by Gorz and Haskins[33] independently showed that the conversion of trans-cinnamic acid (17) to coumarin in Melilotus alba occurred by way of o-coumaric acid (18), o-coumaric acid β-glucoside (19) and o-coumarinic acid β-glucoside (20). This plant was also shown to contain an trans-cis isomerase enzyme system (19-20)[22] (Figure 4).

Brown[23] has shown that p-coumaric acid was seventy times less effective than cinnamic acid as a precursor of coumarin in Hierochloe odorata, while tyrosine was sixty times less effective than phenylalanine (16). It was clear that a preformed phenolic nucleus posed in fact a decided disadvantage in its use as a precursor. The above

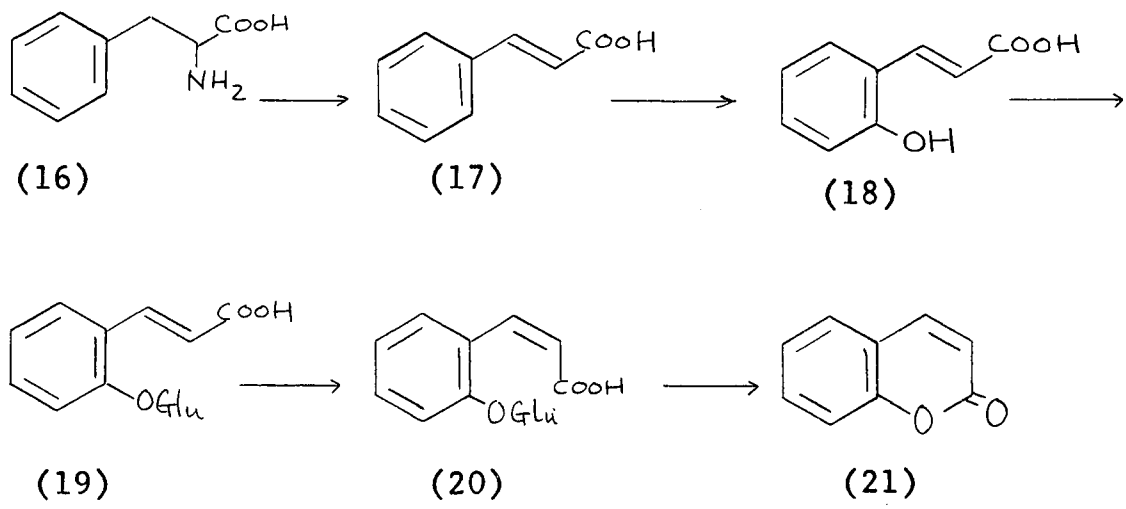
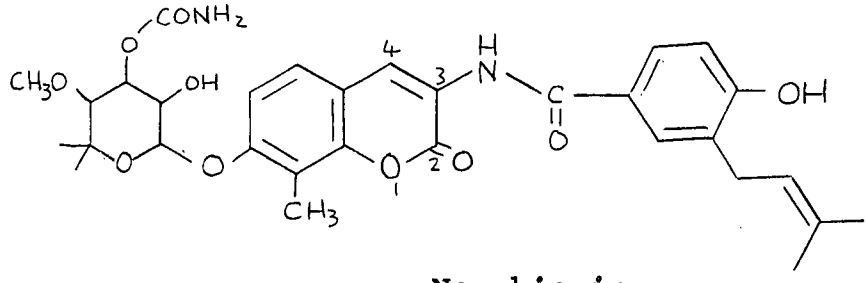


Figure 4 Biosynthesis of Coumarin(21)

results support that the o-hydroxylation mechanism (17→18) is operating in the course of the biosynthesis of coumarin[24].

Kenner et al[15] used tyrosine labelled with ^{18}O in the carboxyl group to prove that the oxygen atom at position 1 in novobiocin originated from carboxyl, i.e. that the simple oxidative cyclization they postulate can also operate.



Novobiocin

The biosynthesis of the 7-oxygenated coumarin, umbelliferone (24), in Hydrangea macrophylla was studied

by Brown, Towers and Chen[25] and by Austin and Meyers[26] and the feeding and trapping experiments with ^{14}C-labelled compounds established the existence of the following pathway. Interestingly radioactive cis-p-hydroxycinnamic acid was incorporated about one-seventh as efficiently as p-coumaric acid (22)[26] (Figure 5).

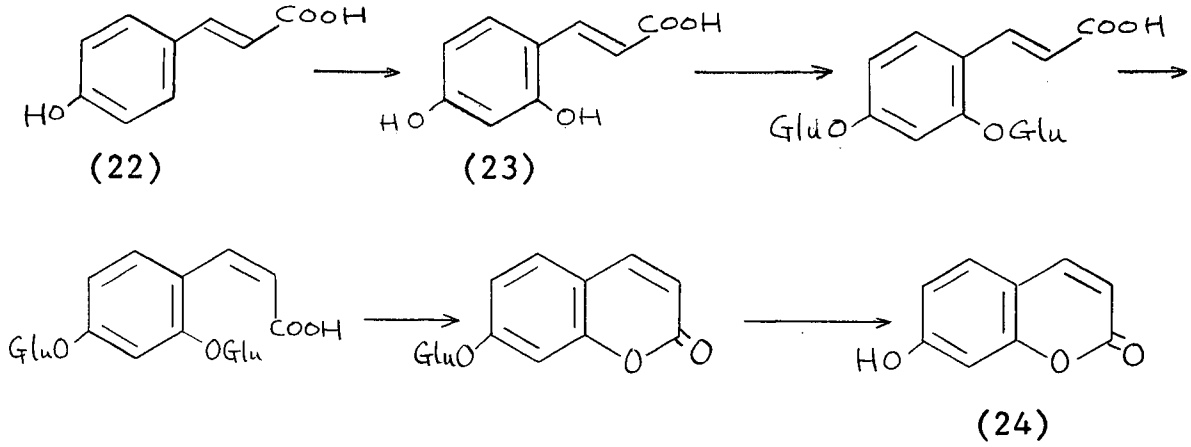


Figure 5 Biosynthesis of Umbelliferone(24)

Analyses of lavender plants by Brown[27,28] with careful precautions to avoid enzymic hydrolysis, have shown that herniarin (27) exists in the plant at least 99% in a bound form, presumably as cis-2-glucosyloxy-4-methoxycinnamic acid (cis-GMC) (26). The use of trapping and feeding techniques enabled Brown to postulate the following pathway for herniarin (27) formation (Figure 6).

Biosynthetic isoprenylation of coumarins is very common, and these compounds often appear in the form of furans with three missing isoprenoid carbon atoms[29,30].

Floss and Mothes studied the incorporation of radio-

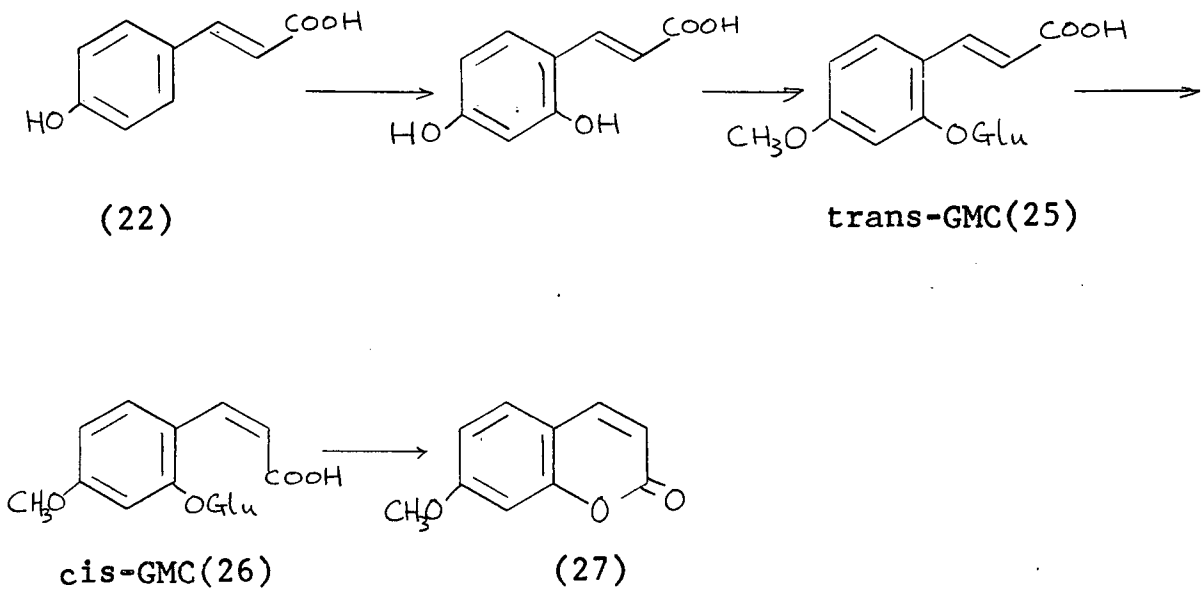


Figure 6 Biosynthesis of Herniarin(27)

active cinnamic acid and umbelliferone into furocoumarins[31]. Evidence was presented that para-hydroxylation of the cinnamic acid precursor preceded ortho-hydroxylation, since umbelliferone was a far better precursor than cinnamic acid, whereas coumarin gave only very poor incorporation.

More recent work by the same authors[32] demonstrated the incorporation of radioactive mevalonic acid into coumarins. Feeding experiments of cinnamic acid-(COOH-^{14}C) and mevalonic acid-(4-^{14}C) into roots of Pimpinella magna and degradation of the labelled furocoumarins, bergapten and pimpinellin, showed that the coumarin part of the furocoumarin skeleton is formed from cinnamic acid, whereas the two extra-carbons of the furan ring originate from C-4 and C-5 of mevalonic acid (Figure 7).

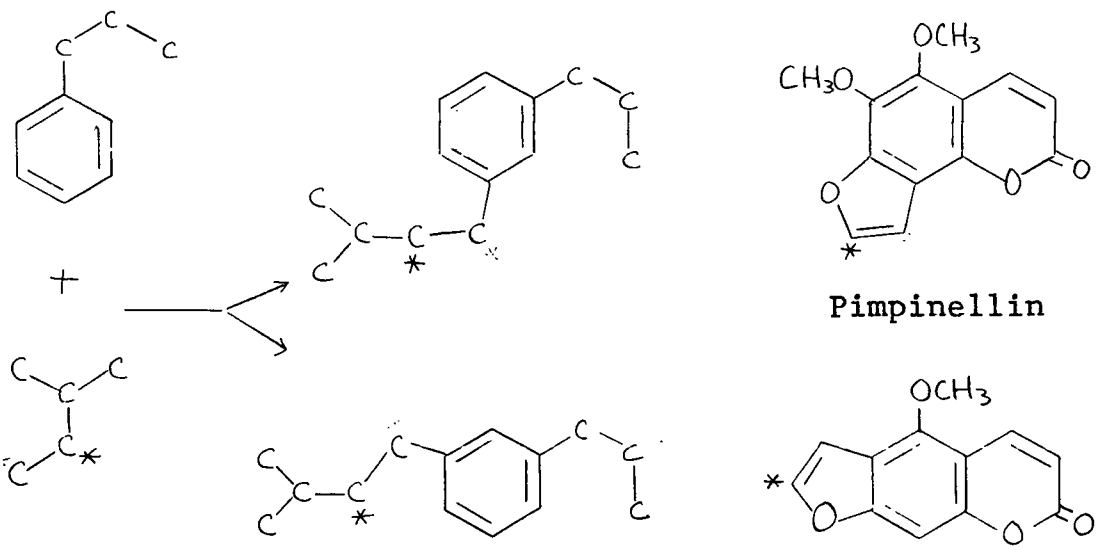
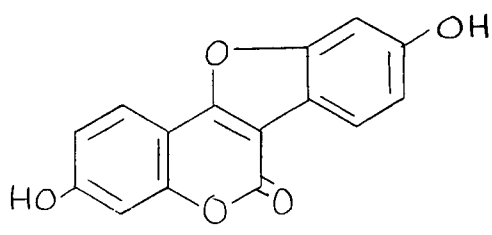


Figure 7

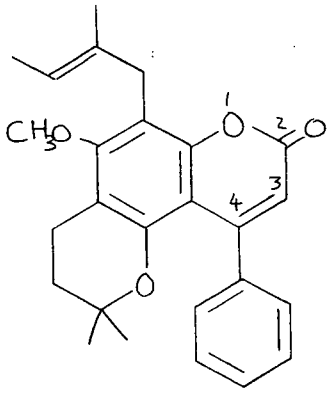
This result reflects the only experiment reported in the literature which casts some evidence on the origin of the two extra carbon atoms of the furan ring.

Grisebach and Barz[35,36] investigated the biosynthesis of coumarano-coumarin, coumestrol, and showed that this compound is an isoflavone, with the benzenoid ring of the coumarin nucleus originating from acetate, and the remaining nine carbons from phenylpropanoid precursors (C_6-C_3).



Coumestrol

Very recent work by Kunesch and Polonsky[37] showed that the specific incorporation of (-)-[3-^{14}C] phenylalanine (a demonstrated precursor of cinnamic acid[14]) into a 4-phenylcoumarin (neoflavanoid), calophyllolide (28), supported the biogenetic scheme suggested by Seshadri[38] and by Ollis[39] (Figure 8).



(28)

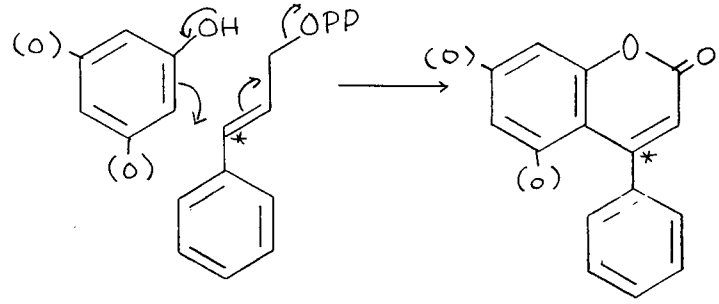


Figure 8

DISCUSSION

I would now like to discuss the experimental results which allow us to postulate a structure for thamnosin, one of the new constituents isolated by Dreyer[4]. As already mentioned in the Introduction (see extraction scheme) thamnosin was obtained as a minor component from Thamnosma montana Torr. and Frem. A sample of this compound was obtained for further structural studies through the kind co-operation of Dr. D. L. Dreyer, Fruit and Vegetable Laboratory, U.S. Department of Agriculture, Pasadena, California. The molecular formula, $C_{25}H_{26}O_6$, had been previously assigned for thamnosin but the reexamination of this substance by high resolution nuclear magnetic resonance (NMR) and mass spectrometry in our laboratory led to the correct molecular formula ($C_{30}H_{28}O_6$, found: M.W. 484.188). A Zeisel determination on thamnosin showed the presence of two methoxyl groups. The strong and complex absorption (λmax 227, 256 and 333 mμ) in the ultraviolet (UV) spectrum and in the appropriate regions of the NMR spectrum suggested the presence of highly conjugated systems. Bands at 1725, 1610 and 1557 cm^{-1} in the infrared (IR) spectrum indicated the presence of α-pyrone or coumarin chromophores[40] and clearly eliminated a γ-pyrone system from consideration. There were also absorption bands indicative of trisubstituted and trans disubstituted double bonds at 820 and 980 cm^{-1}, respectively, but no

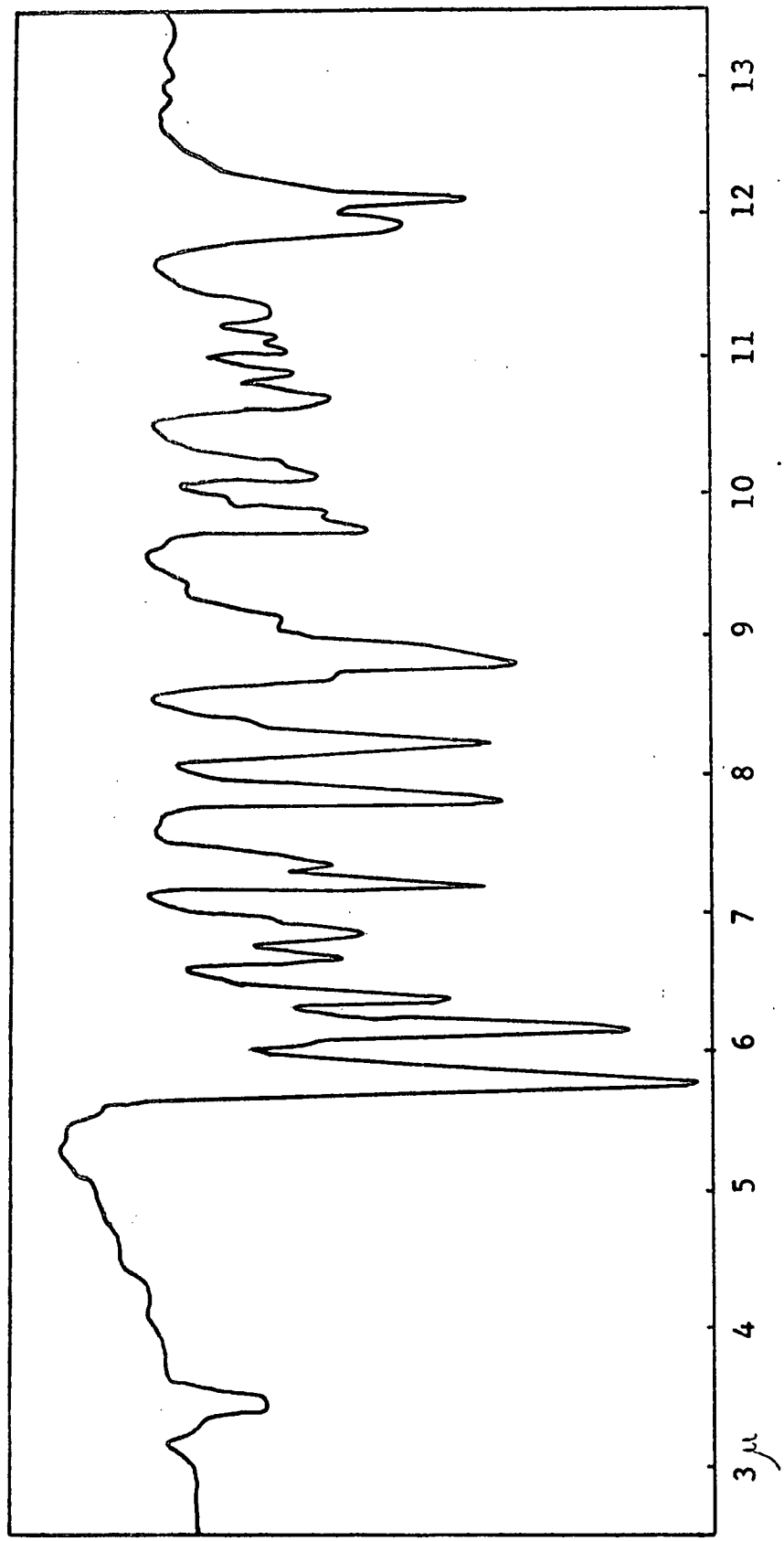


Figure 9 IR spectrum of Thamnosin (KBr)

bands corresponding to hydroxyl absorption (Figure 9).

The mass spectrum of thamnosin was very striking, since there were virtually no peaks between m/e 484 and m/e 243. The fact that the base peak appeared at m/e 242 and that the molecular ion peak was fairly week (8%) suggested that thamnosin was easily cleaved under electron impact into two equal halves. This situation would be consistent with an essentially symmetrical molecule and, as will be seen later, was an important result in the structural elucidation of this compound. The detailed mass spectra of thamnosin and its derivatives will be discussed later in a separate section of this thesis.

The NMR spectrum of thamnosin (Figure 10) indicated the presence of a singlet methyl (probably allylic) at τ8.78, a vinyl methyl at τ 8.20, two methoxyl groups at τ6.29 and τ6.27, a multiplet centered at τ4.75 for an olefinic proton, and a complex multiplet in the region, τ2.4-3.9, which on the basis of its integrated area, could be assigned to ten protons. On the assumption that thamnosin contained two methoxyl groups, the total number of protons in this molecule was twenty eight. The expanded aromatic region of thamnosin, as shown in Figure 12, indicated the presence of an AB system (at τ 3.98 and τ3.82, J_{AB}=16 cps), two sets of doublets at τ3.85 and τ3.83 (J=9.5 cps) which could be tentatively assigned to two protons at the C-3 positions of two coumarin systems and two singlets at τ 3.39 and τ 3.37 which could be assigned

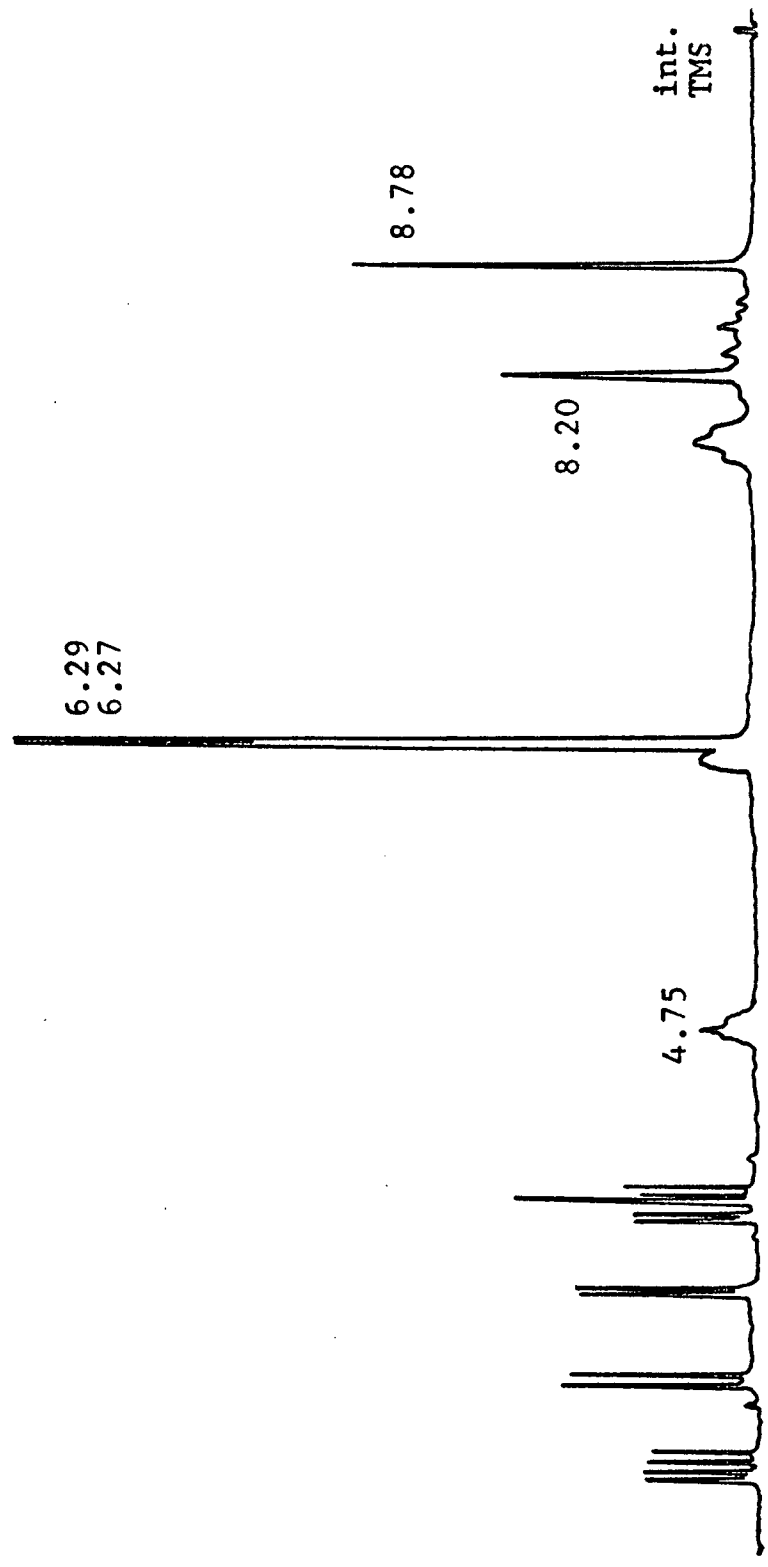


Figure 10 NMR Spectrum of Thamnosin (100 Mc/s)

to protons at the C-8 positions of these systems. In a similar fashion, the two singlets at τ2.94 and τ2.89 and two sets of doublets at τ2.50 and τ2.46 (J=9.5 cps) could be assigned to the C-5 and C-4 protons on the coumarin rings. These assignments are clearly based on the assumption that thamnosin possessed two coumarin chromophores-a postulate which was supported by the UV spectral data (see below).

It was clear from the above molecular formula that seventeen degrees of unsaturation were present in thamnosin and therefore the first selected reaction was catalytic hydrogenation to investigate the nature of any double bonds which may be present.

Thamnosin in the presence of 10% palladium on charcoal in tetrahydrofuran smoothly absorbed one mole of hydrogen. The elemental analysis and the high resolution mass spectrum confirmed that this compound hereafter called dihydro-thamnosin, m.p. 226-228°, had the molecular formula, $C_{30}H_{30}O_6$.

The IR spectrum of dihydro-thamnosin showed very similar bands for the carbonyl and double bond stretching frequencies originally observed in thamnosin but no bands were present at 980 cm^{-1}. The complete disappearance of the AB system in thamnosin (at τ3.98 and τ3.82, J_{AB}=16 cps) as mentioned above was now observed in the NMR spectrum of the dihydro-derivative (Figure 11). The above spectral data confirmed that a <u>trans</u> disubstituted double bond was

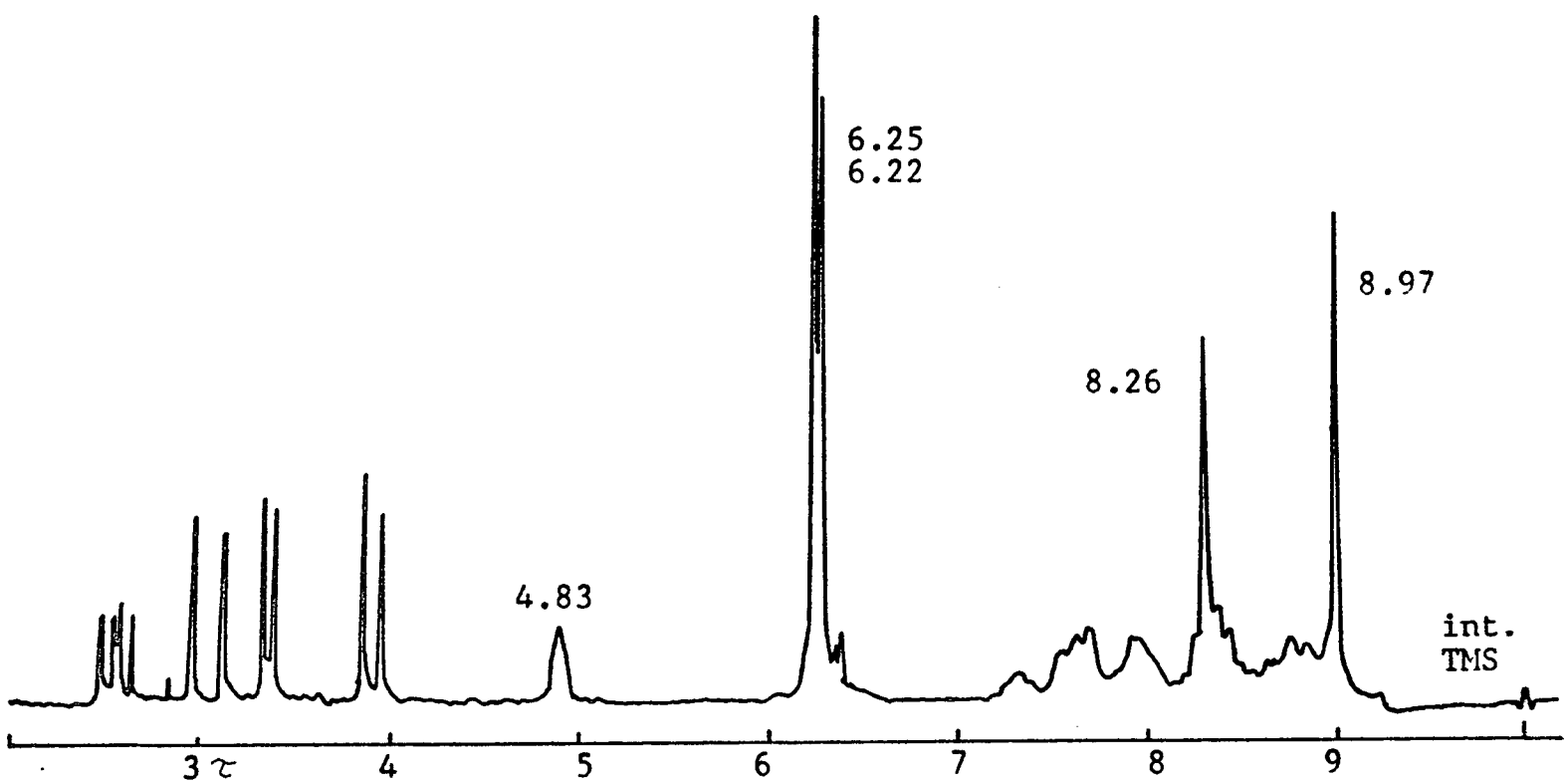


Figure 11 NMR Spectrum of Dihydro-thamnosin (100 Mc/s)

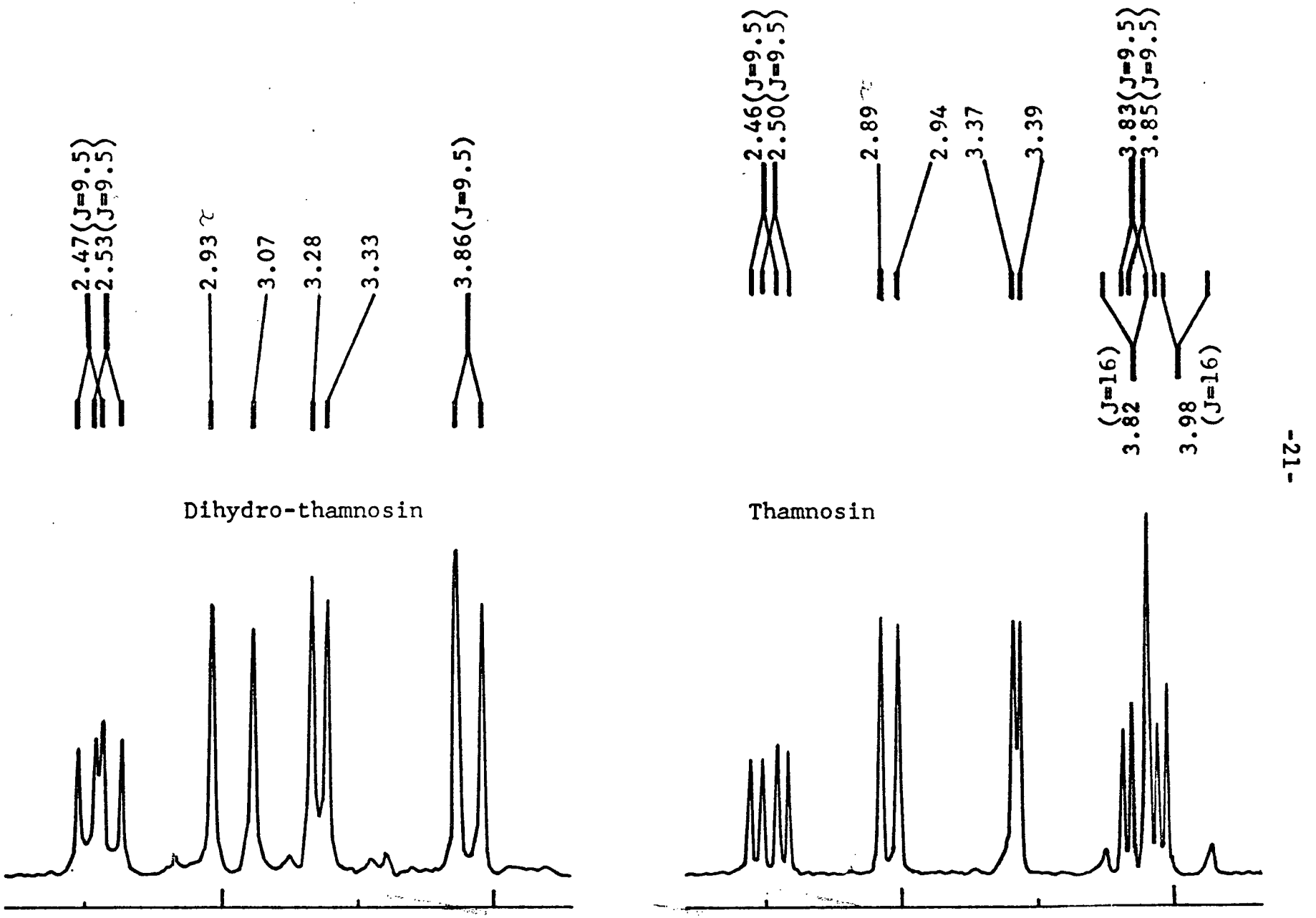
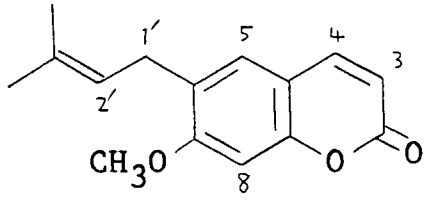


Figure 12 Expanded Aromatic Regions (100Mc/s)

being reduced in the molecule. It could now be further suggested from the chemical shift and the multiplicity pattern of this particular olefinic system that it was linked to an aromatic portion on the one hand and a tertiary carbon atom on the other. The chromophoric change created by the hydrogenation reaction as shown in the UV spectrum confirmed the presence of a double bond conjugated to an aromatic system, the latter most likely being a coumarin chromophore.

As shown in Figure 13, the UV spectrum of dihydro-thamnosin was found to be almost superimposable on that of suberosin[41], 7-methoxy-6-isopent-2'-enylcoumarin. It should be noted that the extinction coefficients in dihydro-thamnosin were almost twice as large as those in suberosin and that suberosin had only four aromatic protons. Therefore the presence of eight aromatic protons in the NMR spectrum of dihydro-thamnosin could suggest that thamnosin consisted of two 7-methoxycoumarin moieties (C_{10} x 2) and a C_{10} alkyl residue linked to the 6-position of these molecules.



Suberosin

The NMR spectrum of dihydro-thamnosin (Figure 11) showed sharp singlets for a methyl group at τ8.97 and a

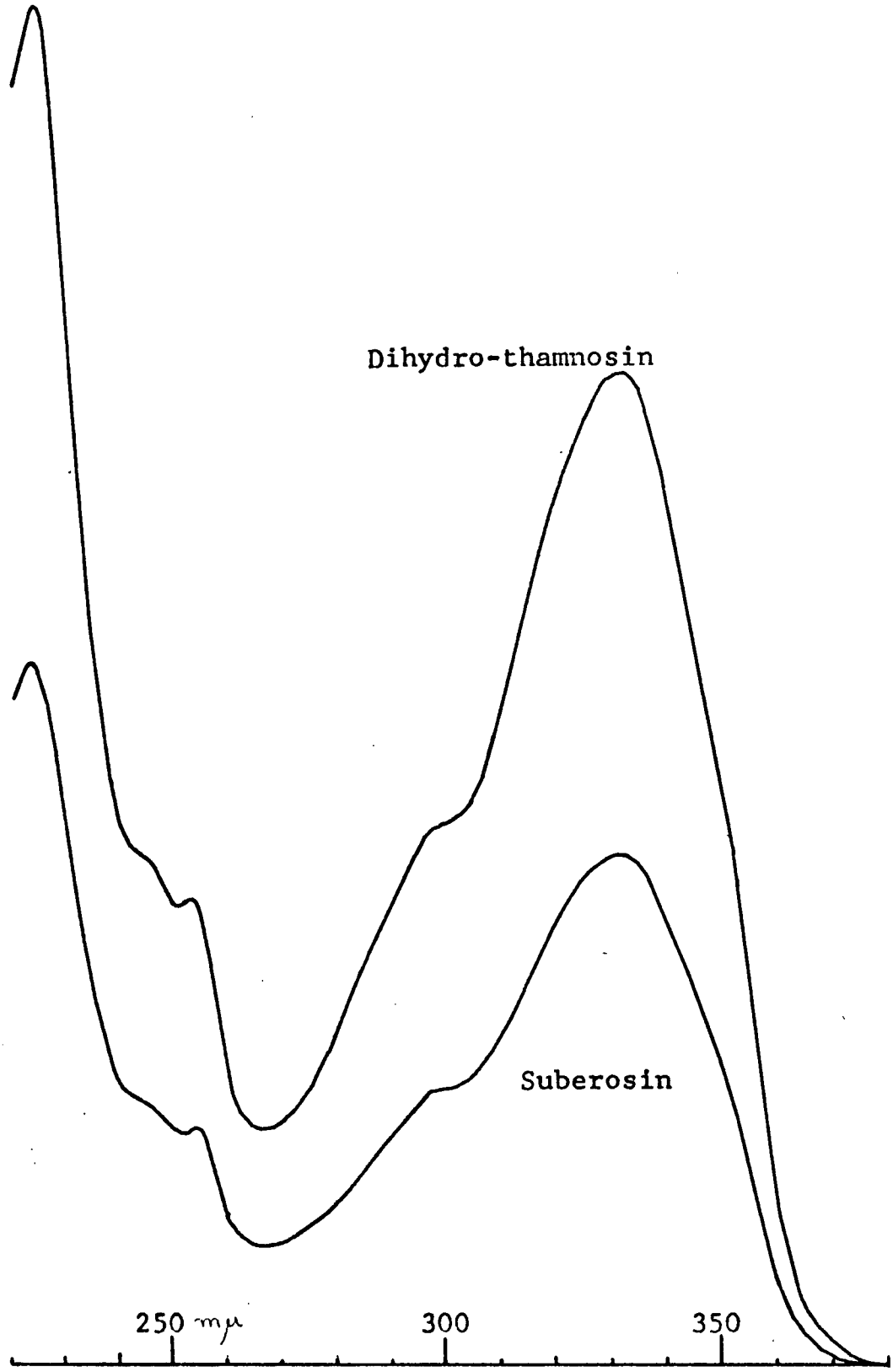
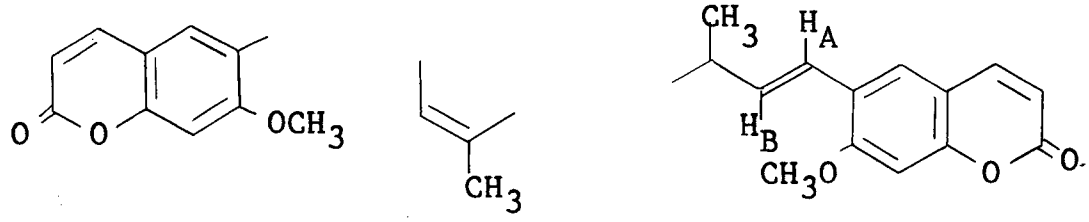


Figure 13 UV Spectra of Dihydro-thamnosin and Suberosin

vinyl methyl at τ8.26, two methoxyl resonances at τ6.25 and τ6.22, an olefinic proton at τ4.83 and a series of signals for eight aromatic protons in the region, τ2.4-3.9. The significant upfield shift of one methyl group signal (8.78→8.97) in converting thamnosin to its dihydro-derivative suggested that the shifted methyl was situated on a carbon atom adjacent to the double bond which had been reduced. Futhermore its appearance as a singlet showed that this methyl was attached to a tertiary carbon atom. On the basis of the above evidence it was possible to postulate, as a working hypothesis, a partial structure (I) for thamnosin.

(I)



The hydrogenation conditions used to obtain dihydro-thamnosin were known to be too mild to attack a coumarin system and this was confirmed when an attempted reduction of the 3,4-double bond in authentic 7-methoxycoumarin met with failure. The presence of a vinyl methyl and an olefinic proton shown by the NMR spectrum and a trisub-stituted double bond indicated by the IR spectrum (a band at 820 cm^{-1}) in dihydro-thamnosin was not surprising since the mild reduction of thamnosin would not be expected to affect the trisubstituted double bond (probably CH_3-$\dot{C}$=CH-).

In order to obtain lower molecular weight fragments which may be more easily compared with compounds of known structure, the cleavage of thamnosin was next considered. The trans disubstituted double bond in thamnosin was thought to be a convenient handle for this purpose and therefore its conversion to a diol was attemped.

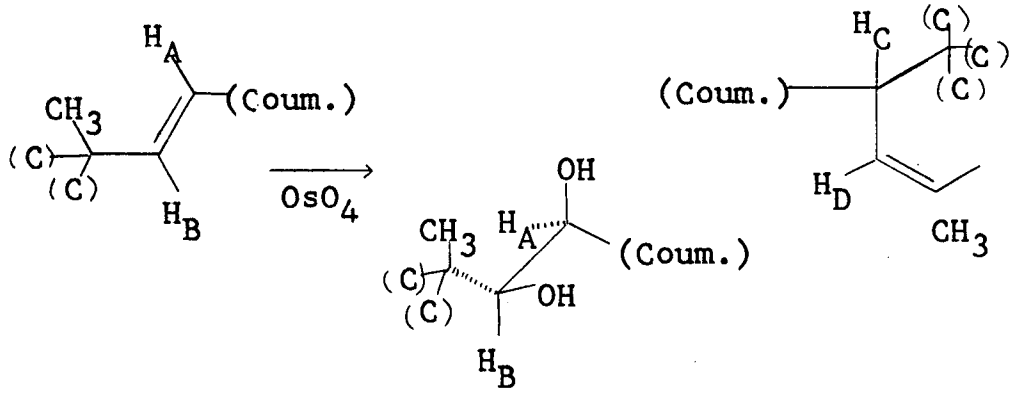
The successful hydroxylation of thamnosin was accomplished by treating it with osmium tetroxide to give thamnosin-diol. Crystallization of the latter compound from ethanol provided two crystalline modifications. One of these (plates), turned out to be the free diol (elemtntal analysis) while the other modification (prisms) was shown by elemental analysis to be the diol bearing one molecule of ethanol of crystallization. The latter could be converted to the former by merely grinding the crystals and heating at 100° for three hours. Interestingly, the crystallization of thamnosin-diol from methanol gave a single product which was analyzed as $C_{30}H_{30}O_8 \cdot CH_3OH$. The above three compounds showed identical behavior on TLC with several solvent systems and the UV spectra were also identical. On this basis it was concluded that thamnosin afforded only one diol in the hydroxylation reaction.

The UV spectrum of thamnosin-diol was almost superimposable on that of dihydro-thamnosin and the IR spectrum showed no absorption at 980 cm^{-1}. As expected, the hydroxylation and the catalytic hydrogenation reactions were both proceeding on the same double bond of thamnosin.

The mass spectrum indicated a very weak molecular ion (1%) but the M-18 peak was sufficiently strong to obtain an accurate mass measurement (found: 500.187).

The IR spectrum showed very intense absorption for hydroxyl groups at 3480 cm^{-1}, the usual bands for coumarin carbonyl functions and double bond absorptions at 1725, 1620 and 1565 cm^{-1} as well as the band at 820 cm^{-1}.

The NMR spectrum of thamnosin-diol (Figure 14 and 15) was very instructive and clearly indicated the presence of all thirty protons. The spectrum showed a three proton singlet at τ8.69, a vinyl methyl at τ8.21, a one proton singlet for H_B at τ7.03, (see below for partial structures), a two proton multiplet at τ6.6, a poorly defined one proton doublet at τ6.28 (J=5 cps, H_C) two methoxyl proton signals at τ6.47 and τ6.16, a broad signal centered at τ4.98 (H_A), an ill-difined one proton doublet at τ4.75 (J=5 cps, H_D) and complex peaks for eight aromatic protons in the region, τ2.3-4.0.



Addition of deuterium oxide to the NMR sample tube containing the diol sharpened the peaks at τ7.03 and τ4.98

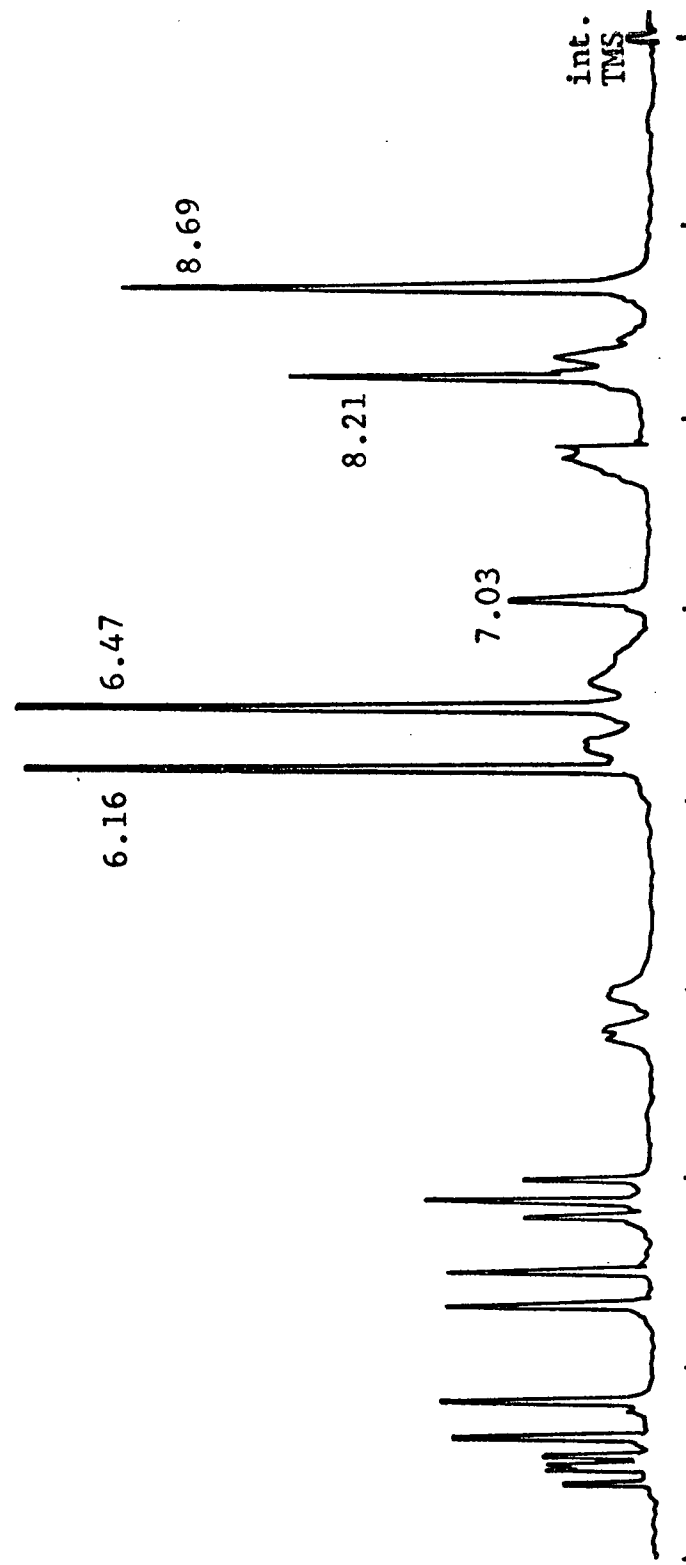


Figure 14 NMR Spectrum of Thamnosin-diol (100 Mc/s)

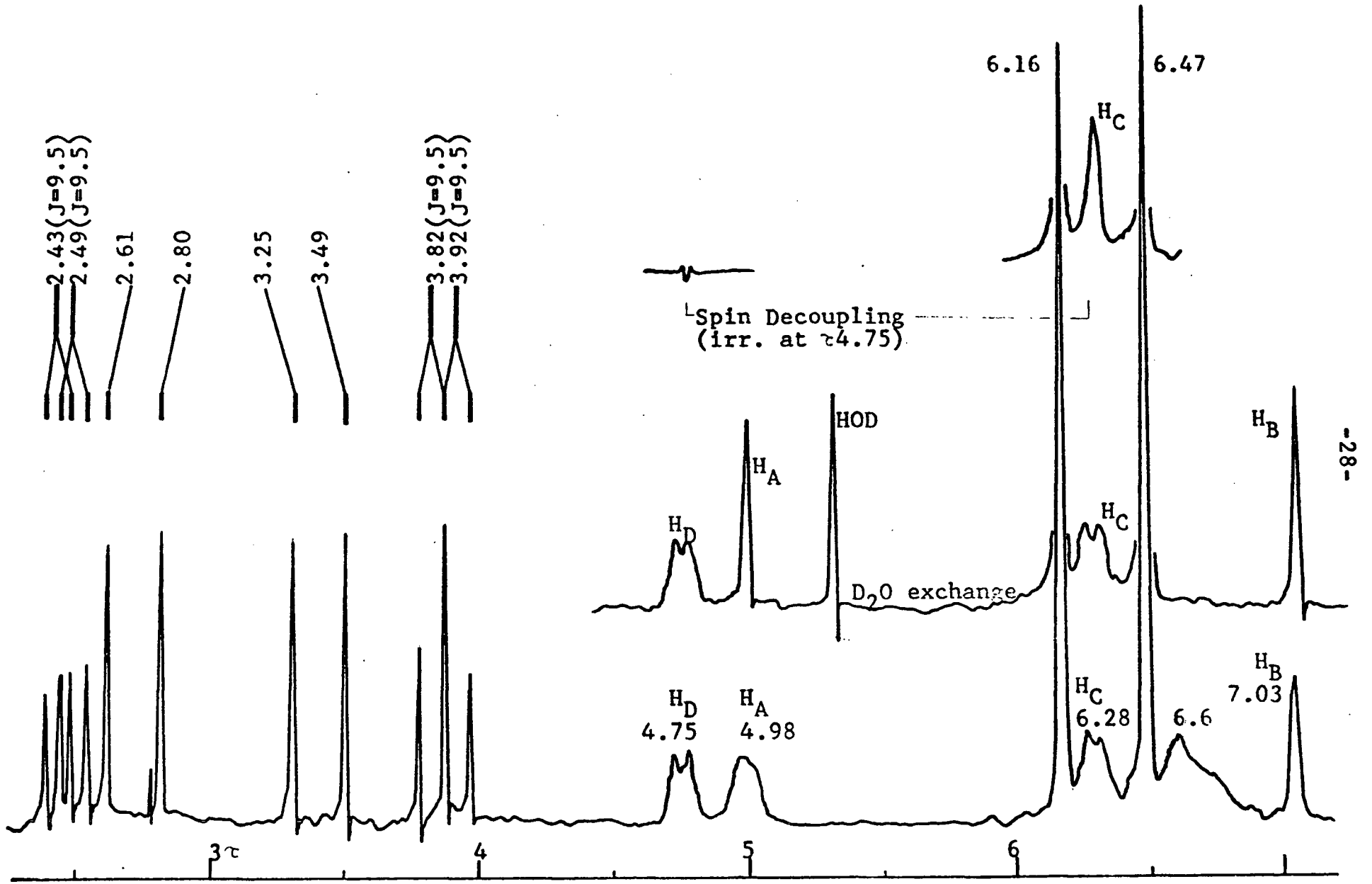


Figure 15 NMR Spectrum of Thamnosin-diol (100 Mc/s)

and caused the two proton multiplet at τ6.6 to disappear. Therefore the multiplet at τ6.6 was assigned to two hydroxylic protons.

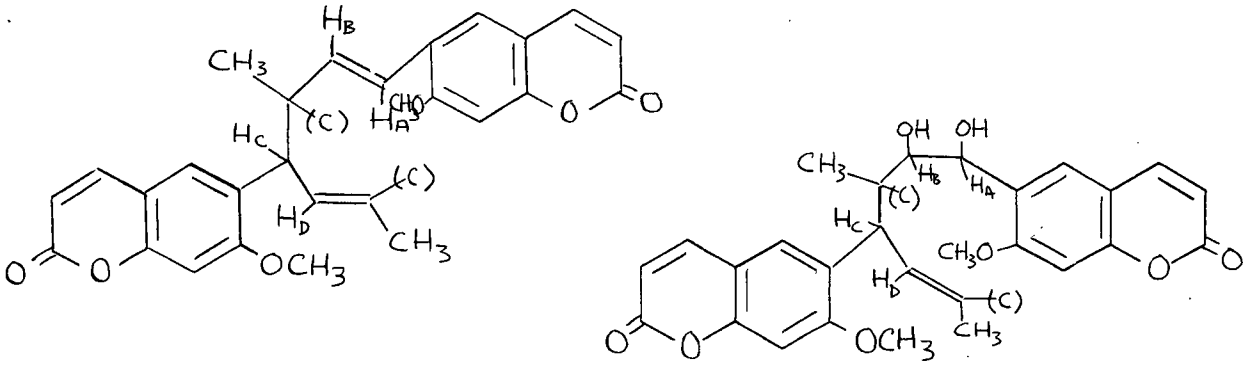
Virtually no couplings between protons H_A and H_B in the diol were observed after deuterium exchange and on this basis it appeared that the dihedral angle between H_A and H_B was close to 90°. A study of molecular models, using the abovementioned proposals, suggested that a cis hydroxylation of the postulated trans double bond would yield a diol with a dihedral angle between H_A and H_B of approximately 60°. According to Karplus[42] this should provide a coupling constant between these protons of approximately 2 cps. But it must be remembered that substituents with high electronegativity are known to reduce the coupling constants of vicinal protons[42]. In this instance both H_A and H_B are attached to carbon atoms bearing hydroxyl groups.

Spin-decoupling experiments (Figure 15) demonstrated that the irradiation at the resonance frequency of the olefinic proton (τ4.75) allowed the doublet at τ6.28 (J=5 cps) to collapse into singlet.

The proton (H_C) at τ6.28 which coupled only with the olefinic proton (H_D) was, on the basis of its chemical shift, situated next to an aromatic system and the signal multiplicity suggested that the adjacent aliphatic carbon atom must be fully substituted.

Consideration of the above spectral evidence allowed us to expand the tentative structure of thamnosin to II

and the diol to III.

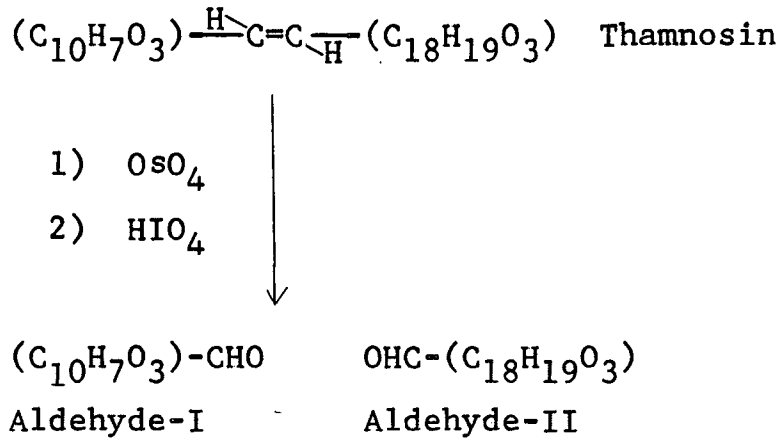


(II) $\xrightarrow{OsO_4}$ (III)

It was now desirable to attempt a cleavage of thamnosin-diol into lower molecular weight fragments. For this purpose, the diol was reacted with periodic acid in aqueous methanol at room temperature. Two aldehydic compounds designated as aldehyde-I and aldehyde-II were obtained from this reaction. Aldehyde-I was more polar than aldehyde-II on a silica gel G thin layer chromatogram (TLC).

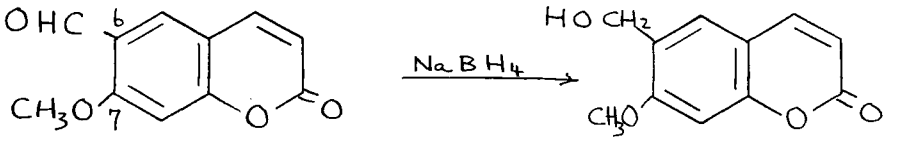
High resolution mass spectra of these aldehydes determined the molecular weights to be 204.042 and 312.138 for aldehyde-I and aldehyde-II, respectively, where $C_{11}H_8O_4$ requires 204.042 and $C_{19}H_{20}O_4$ requires 312.136. Further evidince for the correctness of the above formulae will be presented below. It should be noted that the sum of these two formulae becomes $C_{30}H_{28}O_8$ and indicates conclusively that the periodic acid reaction merely cleaves the molecule

into these two fragments with no loss of any carbon atoms i.e.



Aldehyde-I, m.p. 242, showed an orange spot on a TLC plate after spraying with 2,4-dinitrophenylhydrazine reagent (2,4-DNP) and it was suggested that this compound may be an aromatic aldehyde. The UV spectrum showed a rather complex pattern with λmax at 255, 308 and 329 mμ probably due to an extended conjugation of a coumarin system with the aldehyde group. When sodium borohydride was added to a methanolic solution of aldehyde-I and then the UV spectrum was recorded, the spectrum changed dramatically and was now almost superimposable on the spectra of dihydro-thamnosin and suberosin. The hydroxymethyl group which would be derived from the aldehyde in the hydride reduction would not be expected to contribute significantly to the UV spectrum and it was not surprising that a typical 7-methoxy-coumarin chromophore was now in hand. This result immediately suggested that aldehyde-I could be 6-formyl-herniarin

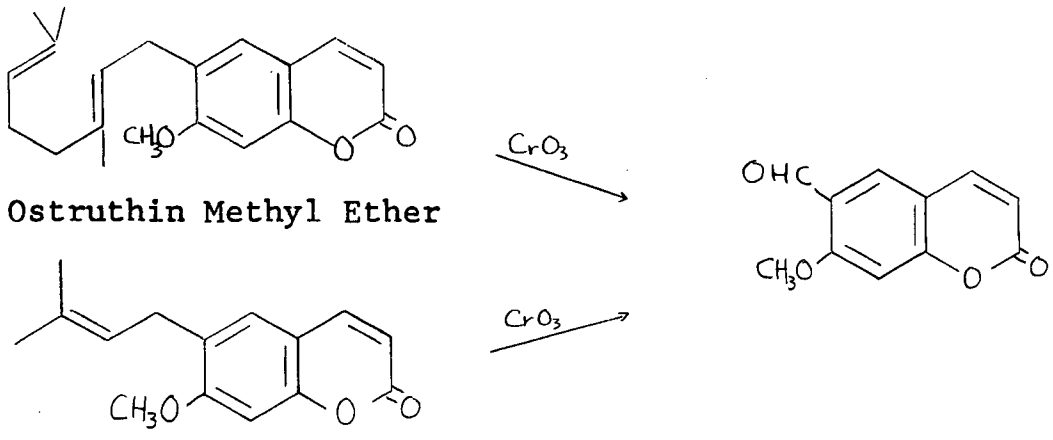
(7-methoxycoumarin-6-aldehyde).



The IR spectrum of aldehyde-I still showed the presence of an α-pyrone system. The NMR spectrum was obtained after some difficulty due to the highly insoluble nature of this compound in the common deuterated solvents and also due to the small amount available. Eventually aldehyde-I in $(CF_2Cl)_2C(OD)_2$ provided an NMR spectrum, from which some evidence of the functional groups could be derived. The presence of one methoxyl group and a sharp one proton singlet at τ -0.23 for an aldehydic proton were suggested from the spectrum.

On the basis of the above spectroscopic evidence the structure of aldehyde-I was proposed to be 7-methoxy-coumarin—6-aldehyde. This latter substance was reported in the literature as a degradation product from ostruthin[43] and from suberosin[41,44].

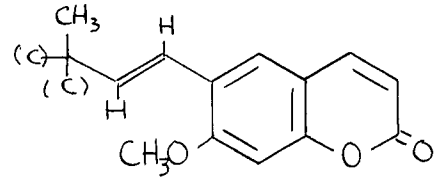
An authentic sample of this compound was kindly supplied by Professor King[41] and a direct comparison with aldehyde-I was made. TLC comparison on silica gel and alumina with several solvent systems, superimposable UV and IR spectra and a mixed melting point determination



Ostruthin Methyl Ether

Suberosin

proved that aldehyde-I was 7-methoxycoumarin-6-aldehyde. On this basis, a portion of the thamnosin structure was now established with certainty.



The less polar aldehyde, aldehyde-II, showed a light yellow spot on a TLC plate when sprayed with 2,4-DNP and resisted crystallization. The UV spectrum of aldehyde-II was essentially superimposable on that of suberosin, which was again indicative of the presence of a 7-methoxycoumarin system bearing a C-6 alkyl side chain. The presence of a coumarin chromophore in aldehyde-II confirmed the previous suggestion that the thamnosin molecule contained two coumarin chromophores most probably linked to a C_{10} alkyl chain.

The NMR spectrum of aldehyde-II (Figure 16) was again

very informative. Two sharp singlets at τ8.82 and τ8.21 confirmed the presence of a saturated and a vinyl methyl group. In addition a methoxyl signal at τ6.20, a one-proton doublet at τ5.84 (J=5 cps), a multiplet at τ4.76 for one olefinic proton, a series of signals for four aromatic protons and a one-proton singlet at τ0.73 for the saturated aldehyde proton were the remaining significant signals.

The chemical shift of one of the methyl signals (τ8.82) and the presence of a singlet for the aldehydic proton gave some evidence that both of these groups may be attached to the same fully substituted carbon atom. This suggestion was made earlier in comparing the NMR spectra of thamnosin and dihydro-thamnosin.

Additional information about the four aromatic protons of aldehyde-II was obtained when the splitting patterns of the aromatic proton signals were examined in the NMR spectra taken at 60 Mc/s and 100 Mc/s. It turned out that this region consisted of two sets of doublets at τ3.84 (J=9.5 cps) and τ2.45 (J=9.5 cps) and two singlets at τ3.31 and τ2.85 for which assignments could be readily made. Table 2 summarizes the published data on several coumarin systems exhibiting similar aromatic regions.

As shown in Table 2, the aromatic regions of ostruthin[46], marmesin[47] and suberosin[48] in particular showed virtually the same patterns and chemical shifts as those of aldehyde-II. This NMR evidence showed without

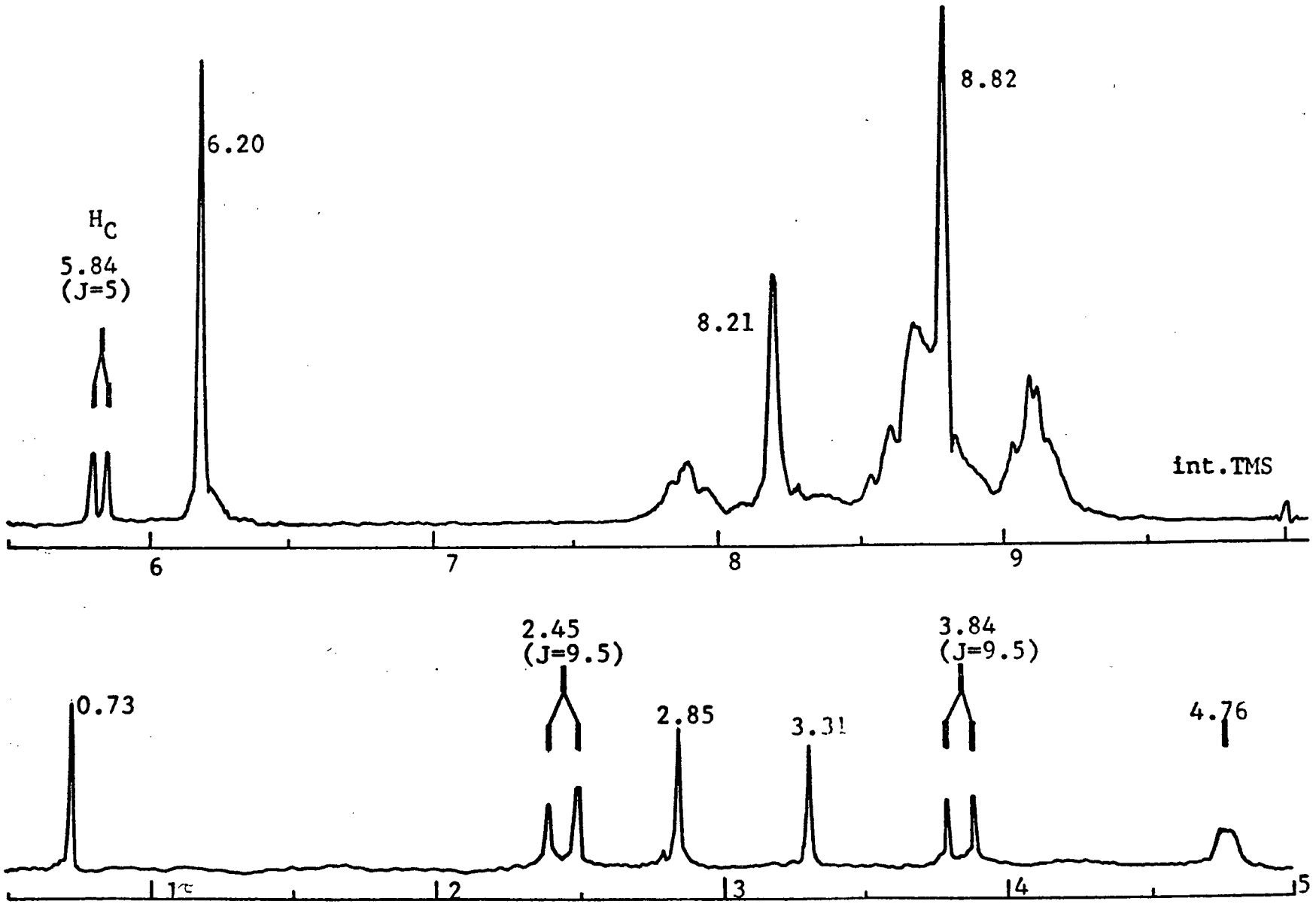
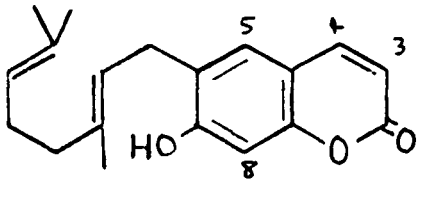


Figure 16 NMR Spectrum of Aldehyde-II (100 Mc/s)

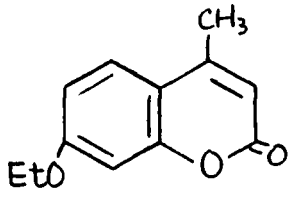
Table 2

Chemical Shifts of Aromatic Protons of Coumarins at 60 Mc/s in Tiers τ scale, J (cps), solvent: $CDCl_3$.

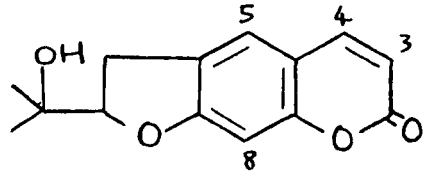
	H_a-C-3	H_b-C-4	H_c-C-5	H_d-C-8	H_e-C-6	Ref.
i)	3.75 d. J_{ab}=10	2.40 d. J_{ab}=10	2.95 s. and 2.80 s.			46
ii)	3.92 s.		2.53 d. J_{ce}=8	3.27 d. J_{de}=2	3.22 q. J=2,8	45
iii)	3.78 d. J_{ab}=10	2.10 d. J_{ab}=10	2.56 s.	3.26 s.		47
iv)	3.77 d. J_{ab}=9.5	2.37 d. J_{ab}=9.5	2.81 s.	3.22 s.		48
v)	3.84 d. J_{ab}=9.5	2.45 d. J_{ab}=9.5	2.85 s.	3.31 s.	(Aldehyde-II)	



i) Ostruthin



ii)



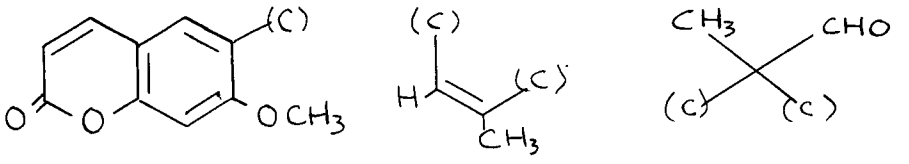
iii) Marmesin

iv) Suberosin

doubt, that aldehyde-II contained a 7-methoxycoumarin system with an alkyl side chain at C-6. Very strong support for this proposal was also available from the UV spectrum of this compound, since the latter spectrum is very sensitive to the relative substituent positions on the coumarin chromophore.

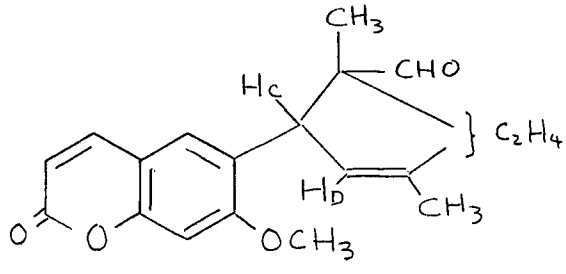
A three proton singlet at τ8.21 and a one proton multiplet at τ4.76 further suggested the presence of a CH_3-C=C-H moiety in aldehyde-II. This suggestion was later substantiated by selective epoxidation of this double bond.

At this point in the discussion the pertinent structural features which are present in aldehyde-II may be summarized as follows:



Further information about the structure of aldehyde-II could now be obtained from comparison of the NMR spectrum of this compound with those of previous compounds already discussed. For example it will be noted from the NMR spectra of thamnosin, dihydrothamnosin and thamnosindiol (Figures 10, 11 and 15) that a broad, one-proton signal always appears beneath or very close to the methoxyl resonances. This particular proton (designated as H_C in partial structure II for thamnosin and on Figures

14 and 15) now appeared clearly as a doublet (J=5 cps see Figure 16) at τ5.84. It was also shown by spin decoupling (see Figure 15) that an olefinic proton (H_D, see partial structure II) was coupled with this particular proton (H_C) in thamnosin-diol (J_{CD}=5 cps). The significant downfield shift of this proton (H_C) in aldehyde-II relative to thamnosin or its other derivatives must be due to its close proximity to the aldehyde group. In order to get some idea about the expected chemical shift of proton H_C, a very approximate calculation was made utilizing the shielding constants for various substituents as given by Shoolery[49] and also mentioned by Silverstein et al[50]. For this purpose, suberosin was selected as a typical system in which the methylene protons are desheilded by a 7-methoxycoumarin ring on the one hand and an olefinic linkage on the other. In this way an approximate value for the shielding constant of the 7-methoxycoumarin system was obtained (see Table 3). A surprisingly good agreement between the calculated and found values was obtained. Therefore if this proton H_C possesses the suggested environment, the partial structure for aldehyde-II may be represented as follows.



Aldehyde-II

Table 3

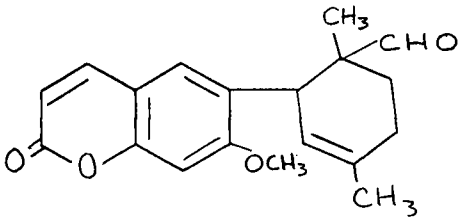
Standard (CH_4)	τ 9.77
Shielding constant C=C[49]	- 1.32
	8.45
$-CH_2-C=C-$	8.45
Obseved for suberosin	- 6.74
	1.71

Approximate shielding constant for 7-methoxycoumarin system is 1.71.

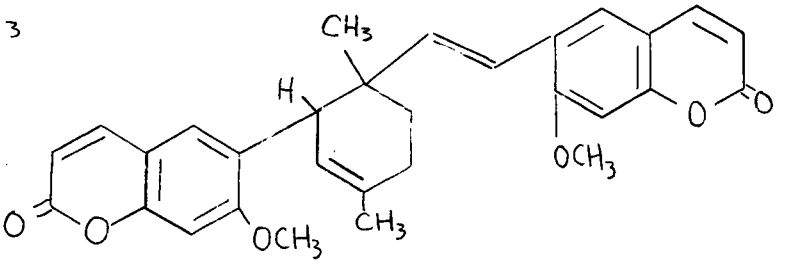
$\underline{CH_3}-\dot{C}-CHO$	8.90
-C=C-	- 1.32
7-methoxycoumarin	- 1.71
Calc. for H_C	τ 5.87
Found for H_C	τ 5.84

If we now consider that the molecular formula established for thamnosin required seventeen degrees of unsaturation, it is noted that we have up to this point in the discussion accounted for sixteen degrees (fourteen (2 x 7) for two coumarin systems and two for olefinic linkages). One degree of unsaturation which still remains unaccounted can now be easily incorporated with the remaining C_2H_4 in aldehyde-II to complete a cyclohexene ring. On this basis, aldehyde-II would have structure III and in turn thamnosin must be assigned structure IV. The remaining portion of the discussion will be devoted to experiments which substantiate these proposals.

In order to provide evidence for the presence of the trisubstituted double bond in a cyclohexene system as



(III)



(IV)

postulated in III and IV, we considered several reactions on dihydro-thamnosin in which the conjugated, disubstituted double bond had been removed.

The epoxidation of dihydro-thamnosin by m-chloroperbenzoic acid in chloroform gave a single product whose high resolution mass spectrum and elementary analyses were in good agreement with the molecular formula of a mono-epoxide, $C_{30}H_{30}O_7$. This epoxide exhibited a UV spectrum which was superimposable on that of dihydro-thamnosin, indicating that the coumarin systems were still intact. The IR spectrum of dihydro-thamnosin-oxide also indicated the three characteristic coumarin bands at 1725, 1612 and 1565 cm^{-1}.

The significant feature of the NMR spectrum (Figure 17) was that one of the two methyl peaks in the starting material had shifted significantly to higher field (8.26→ 8.59) and that no olefinic proton was present in the

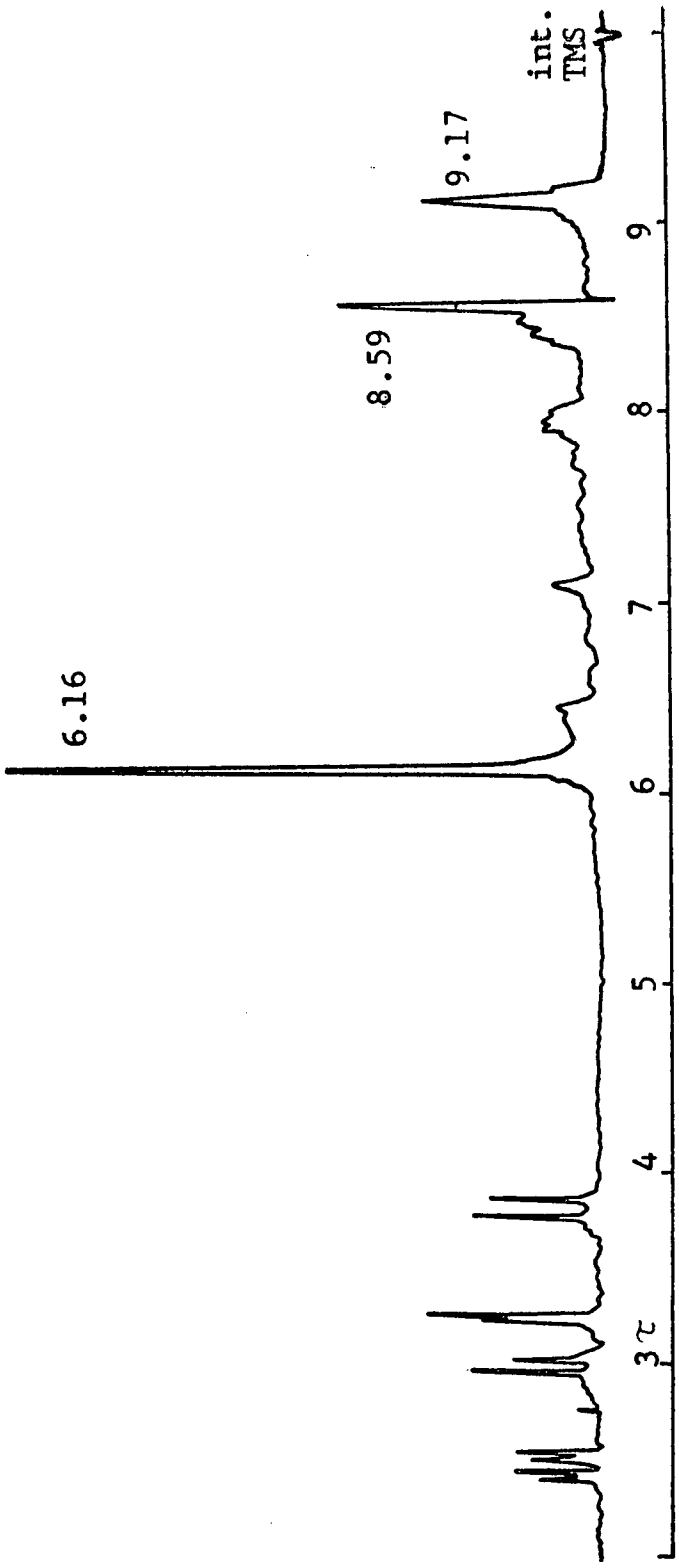
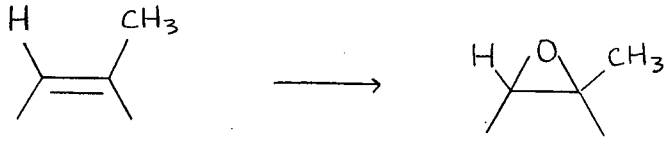


Figure 17 NMR Spectrum of Dihydro-thamnosin-oxide (100 Mc/s)

molecule. This NMR evidence now confirmed the presence of the moiety, CH_3-$\dot{C}$=CH-, in dihydro-thamnosin. The NMR spectrum of dihydro-thamnosin-oxide still showed a sharp three proton singlet at τ9.17, a <u>single</u> peak for two methoxyl groups at τ6.16, and eight protons in the region, τ2.5-3.9. On the basis of the above, it was clear that a straightforward epoxidation of the double bond was occurring, i.e.

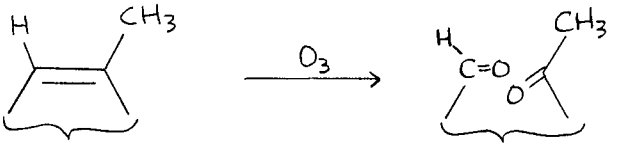


It was hoped that the epoxide could serve as an intermediate for subsequent degradation of the molecule. Unfortunately attempts to cleave the epoxide ring under a variety of conditions always led to a complex mixture of products. Since we were rapidly running short of material, we decided to abandon this approach.

Coumarins are known to be stable towards a very dilute stream of ozone[51,52] and it was felt that this reaction may yield fruitful results. Indeed the controlled ozonolysis of dihydro-thamnosin followed by catalytic reduction of the ozonide gave a single compound, designated as keto-aldehyde-III.

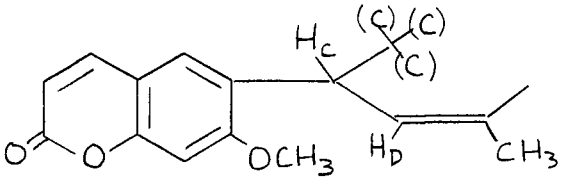
The mass spectrum of this substance showed a very weak molecular ion peak but the M-18 peak, which was more intense, was subjected to an accurate mass measurement. The UV and

IR spectra of this keto-aldehyde-III still indicated the retention of the 6-alkyl-7-methoxycoumarin chromophore.



The NMR spectrum of keto-aldehyde-III (Figure 18) indicated a teriary methyl at τ8.91, two methoxyl groups at τ6.15 and eight aromatic protons for the two coumarin systems. The presence of a sharp, three proton singlet at τ 7.87 (CH_3CO), a one proton doublet at τ 0.02 (-CH_C-CHO, J=2 cps) and a one proton doublet at τ 5.76 (-CH_C-CHO, J=2 cps) confirmed that the ozonolysis had proceeded in the expected fashion.

As suggested above, the aldehydic proton was indeed found to be coupled with a single proton (H_C) when a spin decoupling experiment was carried out (see Figure 18). It was also confirmed that H_C was coupled only with this proton. Therefore the carbon atom bearing H_C could only be connected to an aromatic system, a tetra-substituted carbon atom and a tri-substituted double bond whose olefinic proton was in turn also coupled with H_C. Therefore the immediate environment of H_C must be expressed as follows.



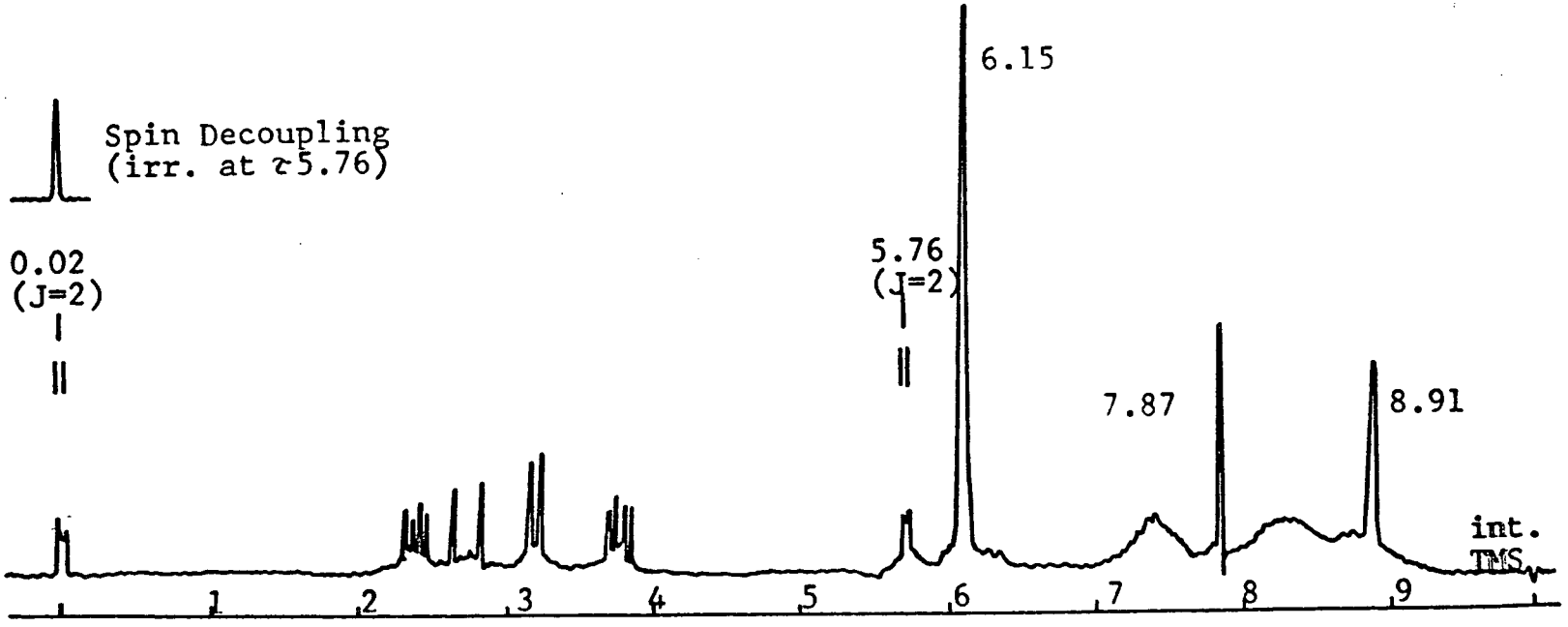
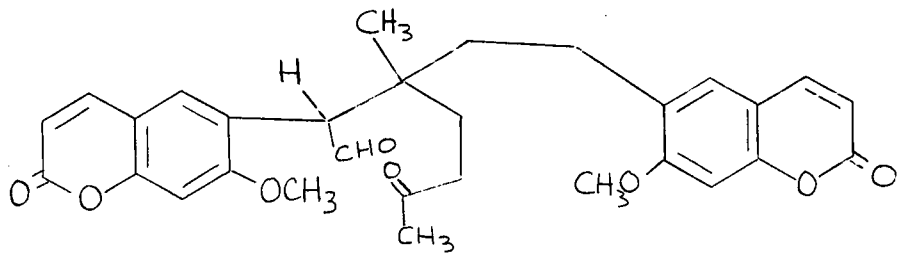


Figure 18 NMR Spectrum of Keto-aldehyde-III (100 Mc/s)

When this evidence is taken in conjunction with the previous results, it is concluded that keto-aldehyde-III had the indicated structure.



The above epoxidation and ozonization products of dihydrothamnosin had now completely identified the nature of the tri-substituted double bond in thamnosin.

Some supporting evidence for keto-aldehyde-III was obtained when the latter was subjected to mild reduction with sodium borohydride. The product, arbitrarily designated as alcohol-IV, had some interesting spectral characteristics, although unfortunately a very small quantity was available and complete characterization was not possible. Consequently the spectral assignments are not entirely definitive. The mass spectrum of this compound showed the presence of M-18 and M-36 peaks along with a very weak molecular ion peak.

The NMR spectrum of alcohol-IV showed a complete dissapearance of the methyl signal at τ7.87, in agreement with the expected reduction of the methyl ketone function.

This discussion now concludes our experimental results on the structural elucidation of thamnosin. The evidence presented allows us to assign structure IV to this natural product. Unfortunately, due to the inavaila-

bility of thamnosin, no further degradation experiments could be carried out and we are unable to make any asignment of stereochemistry at the lone asymmetric centre.

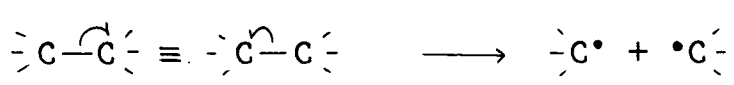
Thamnosin represents a novel system which to the best of my knowledge, has not been previously encountered in any natural source. A biosynthetic study on this molecule, utilizing labelled precursors, could be very interesting and is anticipated.

Mass Spectra of Thamnosin and its Derivatives

Mass spectrometry played an important role in the structure determination of thamnosin and it was felt appropriate to discuss the mass spectral results in a separate section of this thesis. The analysis of these results was aided by previously published investigations as well as by a detailed study on various coumarin systems in our laboratory. A brief discussion of some of these results is presented first before analysis of the thamnosin system is considered.

The mass spectrometric investigations of naturally occurring oxygen heterocycles have been carried out by several groups.[54,55,56,57,58] From these studies it is apparent that the coumarin ring system, under electron impact, fragments with loss of carbon monoxide to provide relatively stable ions. Elimination of a methyl radical from an aromatic methoxyl function and facile cleavage of an alkyl side chain β to the aromatic ring often occurs as well to give rise to conjugated oxonium ions and sometimes, the tropylium ion.

Fragmentations of these molecules will be shown according to the Djerassi convention[53] i.e.



The fragmentations of simple coumarins will be discussed first and then these postulates will be extended

to the thamnosin series. It must be emphasized that the mechanistic interpretations are merely reasonable rationalization to explain the appearance of various fragments in the mass specrometer. In most instances a good deal of additional study is necessary before any definitive comments about these ions can be made.

The loss of CO(M-28) and 2CO(M-56) from the pyrone ring of coumarin (21) and herniarin (27) was observed under electron impact. Herniarin also fragmented with facile loss of a methyl radical as shown in Figure 19[54].

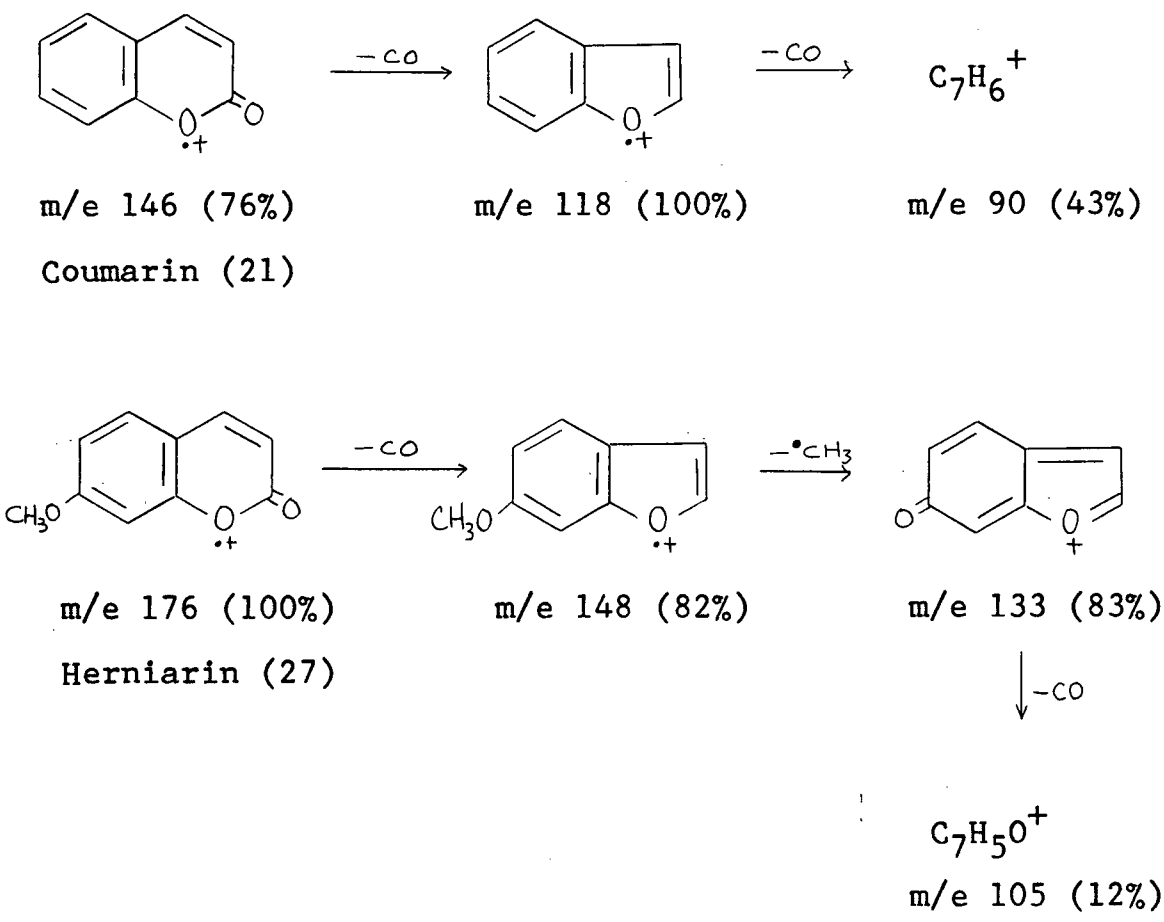


Figure 19

Barnes and Occolowitz[54] reported the interesting breakdown of the prenylated 7-methoxycoumarin, osthol (29) and dihydroosthol (30), as shown in Figures 20 and 21.

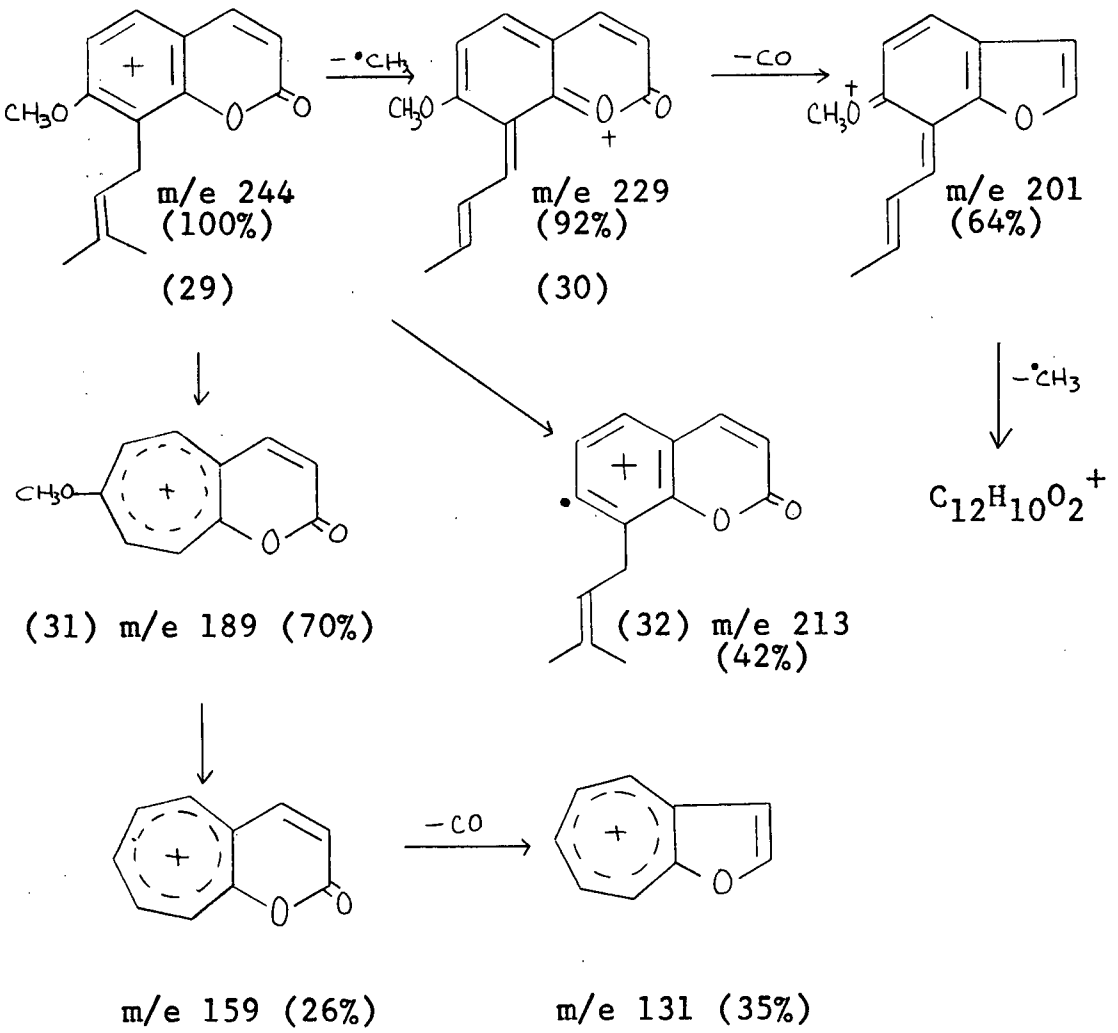


Figure 20

The molecular ion of osthol lost a methyl radical to give an ion at m/e 229 (92%) and it was shown that this methyl radical was fragmented from the prenyl side chain to provide a highly conjugated ion (30). Fission of the side chain

to the ring gave the tropylium-type ion (31) at m/e 189 (70%) and loss of the methoxy group without rearrangement gave the ion (32) at m/e 213 (42%).

As shown in Figure 21, stabilization of the M-15 ion could not occur in dihydroosthol (33) because of the saturated side chain. Loss of a C_4H_7 radical from the molecular ion at m/e 246 (39%) yielded the base peak at m/e 189 which subsequently eliminated a molecule of formaldehyde and carbon monoxide to give the fragment at m/e 131 (36%).

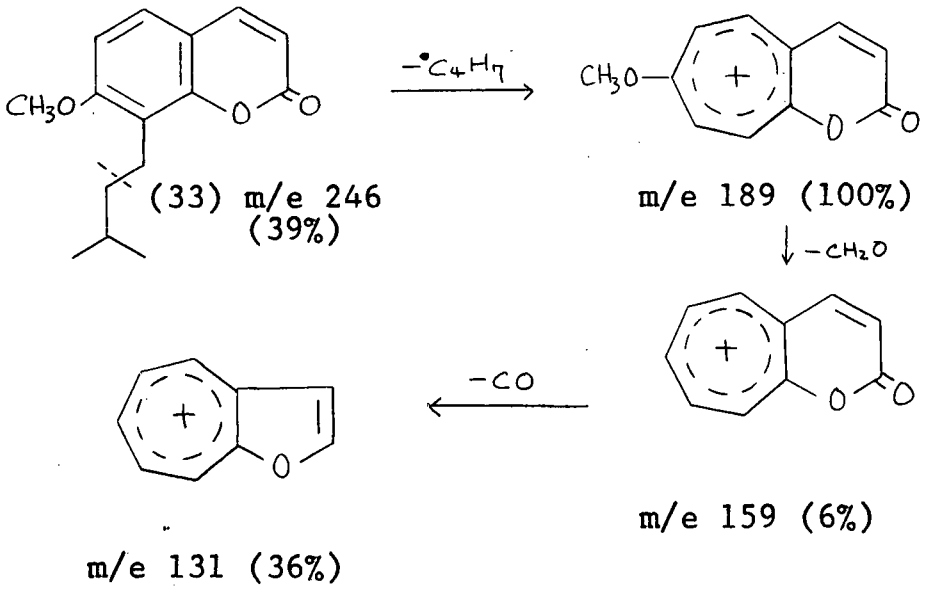
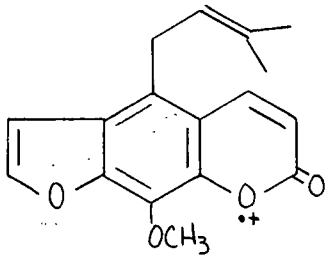


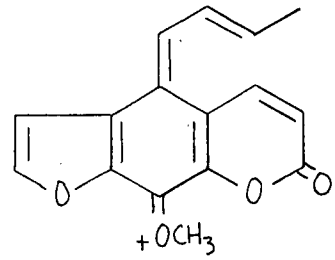
Figure 21

Similar fragmentations of alkyl side chains of furocoumarins were recently observed by Kutney, Eigendorf, Dreyer and Mitsher[57]. A typical example is provided by alloimperatorin methyl ether (34) (Figure 22).

Alloimperatorin methyl ether (34) and its diol (35)



$-\cdot CH_3$

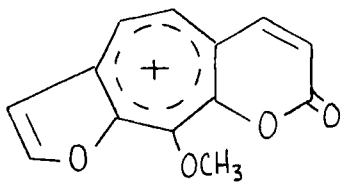


(34) m/e 284 (100%)

m/e 269 (21%)

$-C_4H_7$

$-CO$



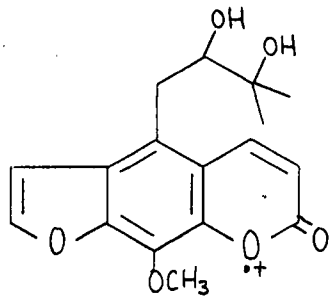
$C_{13}H_9O_3^+$

m/e 201 (6%)

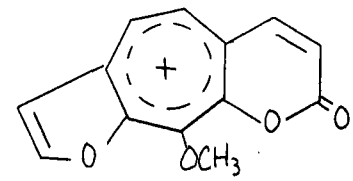
$C_{16}H_{13}O_4$

m/e 241 (12%)

m/e 229 (12%)

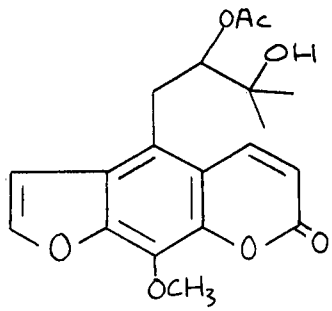


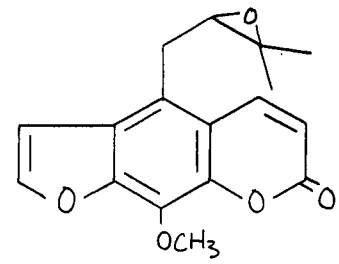
$-\cdot C_4H_9O_2$



(35) m/e 318 (70%)

(36) m/e 229 (97%)





(37)

(38)

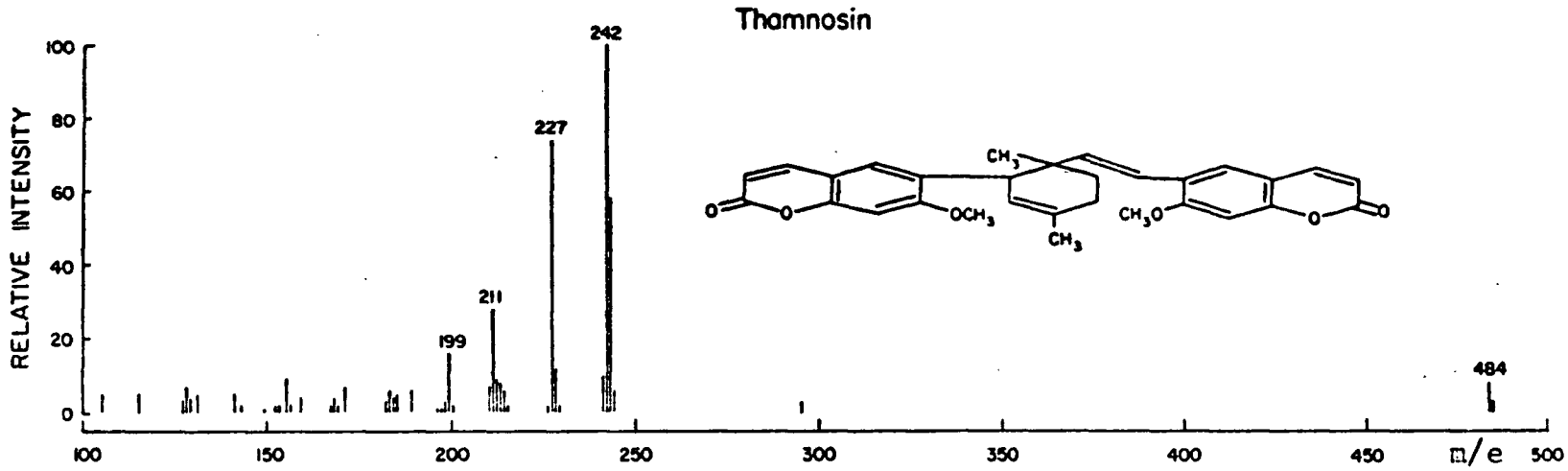
Figure 22

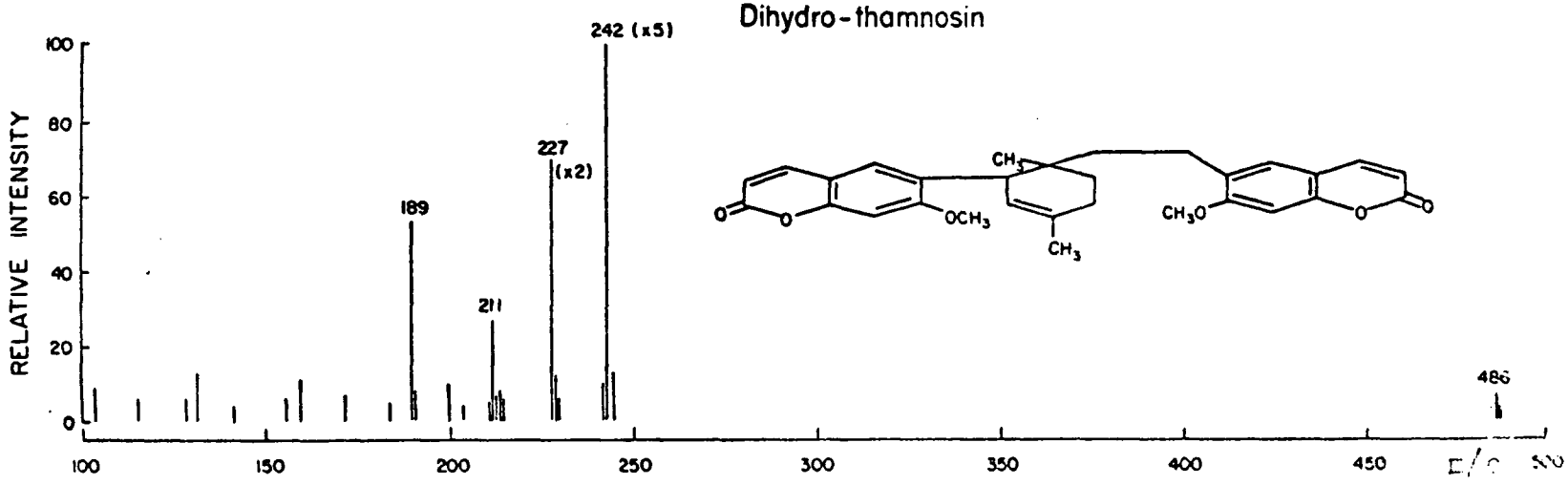
were isolated from Thamnosma montana Torr. and Frem[4]. and their mass spectra were obtained in our laboratory. The difference in the side chain of these two compounds made the relative intensities of peaks in the mass spectra significantly different. The strong ion (36) at m/e 229 (97%) characteristic of furocoumarins was again recorded in the mass spectrum of the diol (35)[57]. This ion (36) was also the base peak in the mass spectra of compounds (37) and (38)[57].

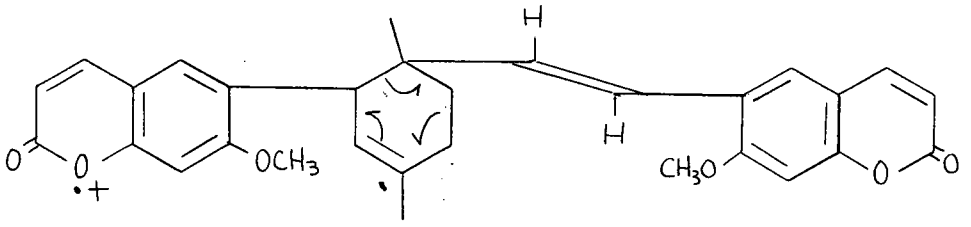
On the basis of the above fragmentations of the coumarin system and other considerations, the mass spectra of thamnosin and its derivatives will be discussed.

It had been already mentioned in the previous section of this thesis that thamnosin and its derivatives showed several interesting features in the mass spectra. Above all, the facile fragmentation of the molecule was noted by the weak molecular ion peak (m/e 484, 8%) and a base peak at m/e 242 (Figure 23). This fission was so efficient that no fragments between these two peaks could be detected. This result immediately suggested that a retro Diels-Alder (D.A.) scission of a cyclohexene ring in thamnosin may be occurring. This type of fragmentation is well known from the mass spectral studies on unsaturated terpenes and steroids in particular.

Furthermore it must be recognized that this process involves cleavage of the molecule into two fragments of equal mass and therfore the cyclohexene ring must be so

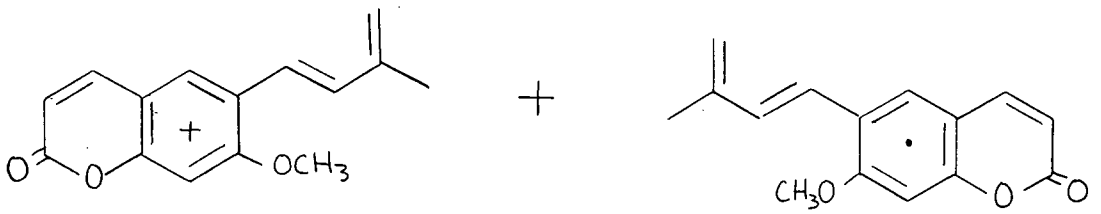

Thamnosin
RELATIVE INTENSITY
100
80
60
40
20
0
242
227
211
199
484
100
150
200
250
300
350
400
450
m/e
500
CH3
OCH3
CH3O
CH3


Dihydro-thamnosin
RELATIVE INTENSITY
100
80
60
40
20
0
242 (x5)
227
(x2)
189
211
486
100
150
200
250
300
350
400
450
m/e
CH3
OCH3
CH3O
CH3

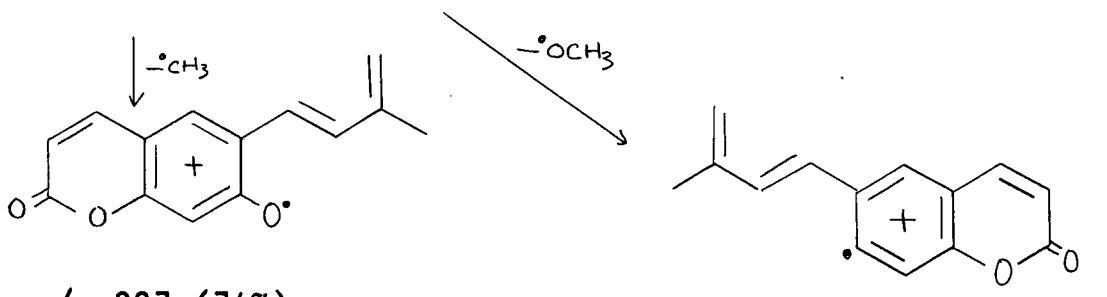


Thamnosin m/e 484 (8%)

retro D.A.

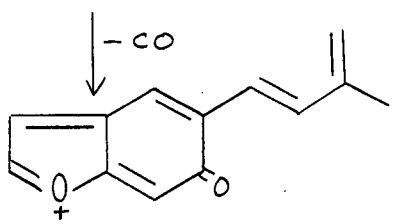


m/e 242 (100%)



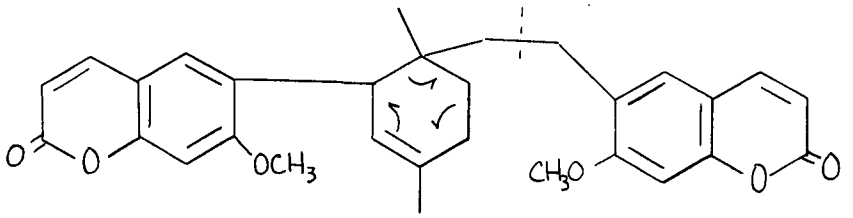
m/e 227 (74%)

m/e 211 (28%)

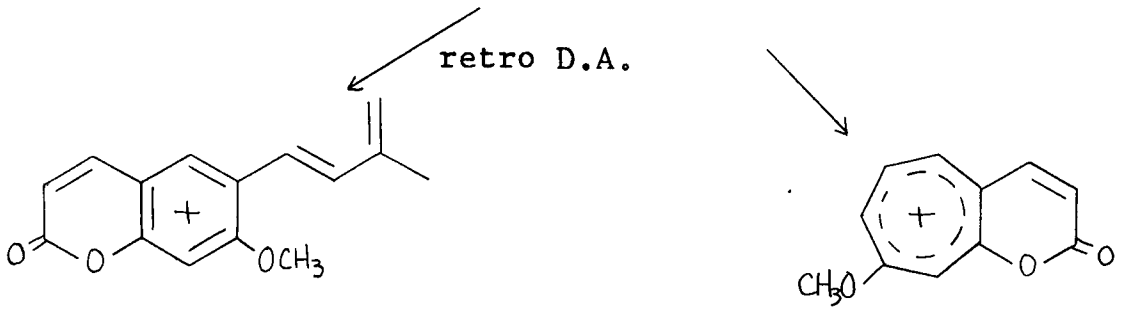


m/e 199 (16%)

Figure 24

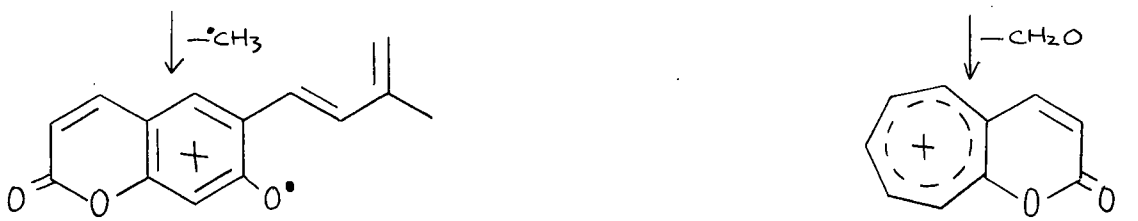


Dihydro-thamnosin m/e 486 (1.4%)



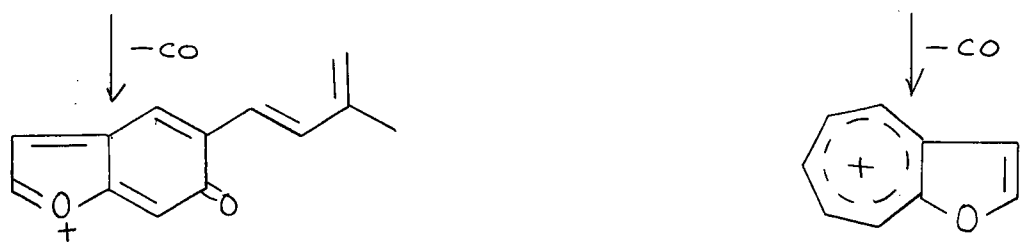
m/e 242 (100%)

m/e 189 (11%)



m/e 227 (28%)

m/e 159 (2%)



m/e 199 (2%)

m/e 131 (3%)

Figure 25

oriented as to accomodate this fact. Indeed this result provided the first suggestion that thamnosin consisted of two coumarin systems linked to a C_{10} unit, with the latter bearing the cyclohexene ring.

Figure 24 illustrates the proposed fragmentation scheme for thamnosin. For the sake of clarity, the correct structure for this substance is utilized. The fundamental retro D.A. fission of the molecule is followed by loss of CO, OCH_3 and CH_3 - the latter fragmentations already noted in the mass spectra of coumarins.

Thamnosin $C_{30}H_{28}O_6$ M.W. 484 ($\geq$ 100)

m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
484	8	227	74	199	16	141	5
244	6	214	6	189	6	131	5
243	58	213	8	185	5	128	7
242	100	212	9	183	6	115	5
241	10	211	28	171	7		
228	12	210	7	155	9		

In dihydro-thamnosin (Figure 25) the retro D.A. reaction also gave the base peak at m/e 242 due to the retention of a cyclohexene in this molecule. The double bond conjugated to a coumarin system is not present in dihydro-thamnosin and so the fission β to the coumarin could now give rise to a species at m/e 189 (11%), postulated as a tropylium-type ion. It should be noted that dihydro-thamnosin shows a weaker molecular ion at m/e 486 (1.4%) than that of thamnosin (Figure 23).

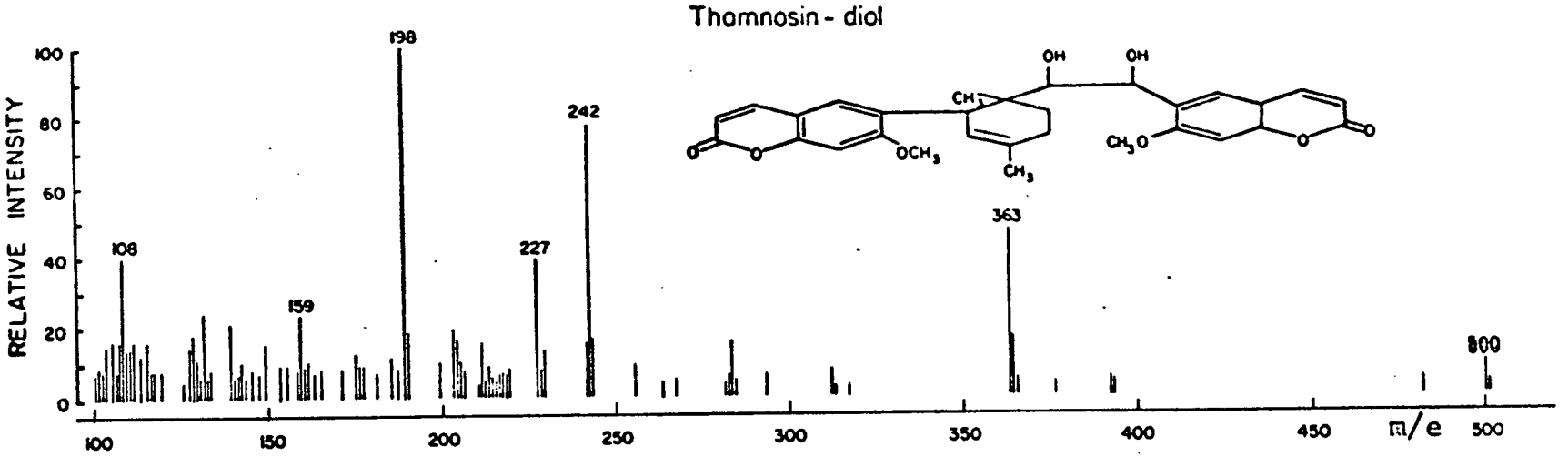
Dihydro-thamnosin $C_{30}H_{30}O_6$ M.W. 486 ($\geqslant$100)

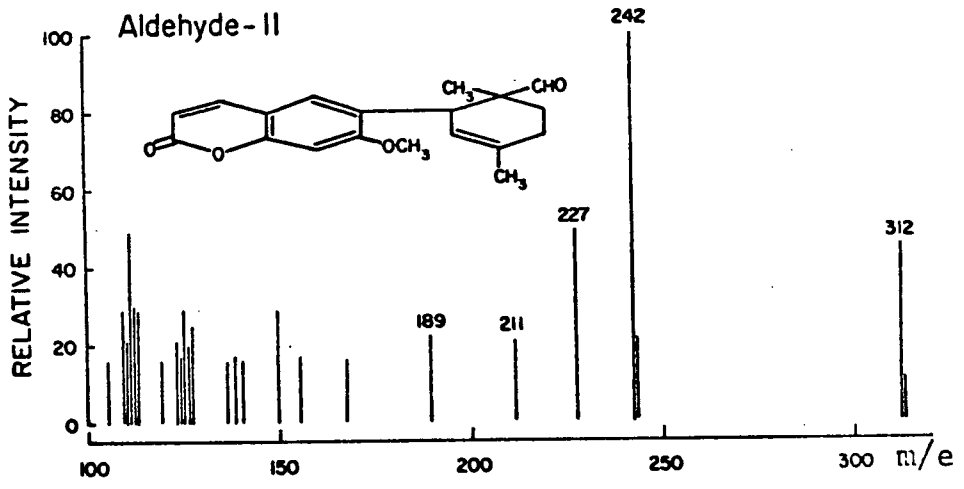
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
486	1.4	227	28.0	199	2.0	155	1.3
244	2.6	214	1.2	190	1.6	141	0.8
242	100.0	213	1.6	189	10.6	131	2.7
241	2.1	212	1.4	183	1.0	128	1.3
229	1.2	210	1.0	171	1.4	115	1.2
228	2.6	203	0.8	159	2.2	103	1.7

Thamnosin-diol, obtained from thamnosin by treatment with osmium tetroxide, has two hydroxyl groups one of which is on a benzylic carbon and it was expected that the molecular ion might be very weak. Indeed immediate loss of H_2O was observed to give a fairly stable ion at m/e 500 (9%) (Figure 26). As shown in Figure 27, further fission α to a carbonyl group and also β to the coumarin system now gave rise to the base peak at m/e 189. The retro D.A. reaction of the cyclohexene ring in this molecule gave a very strog ion at m/e 242 (77%), which was found to be the base peak in the mass spectra of thamnosin and dihydro-thamnosin.

Thamnosin-diol $C_{30}H_{30}O_8$ M.W. 518 ($\geqslant$ 100)

m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
518	0.1	227	39	161	10	113	12
516	0.5	211	15	159	23	111	16
500	9	205	10	149	15	110	14
364	16	204	16	142	10	109	14
363	47	203	19	139	21	108	40
283	15	199	10	131	24	107	16
243	16	190	18	129	11	105	16
242	77	189	100	128	18	103	15
241	15	185	11	127	14		
229	13	175	12	115	16		


Thomnosin - diol
RELATIVE INTENSITY
m/e
198
242
108
227
159
363
800
OH
OH
CH3
OCH3
CH3O
CH3


Aldehyde - II
RELATIVE INTENSITY
m/e
242
227
312
189
211
CH3
CHO
OCH3
CH3

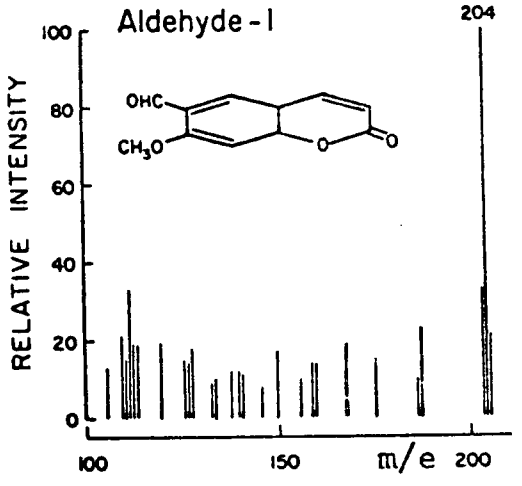
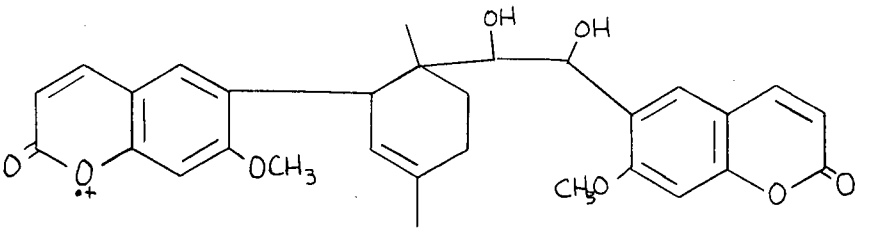
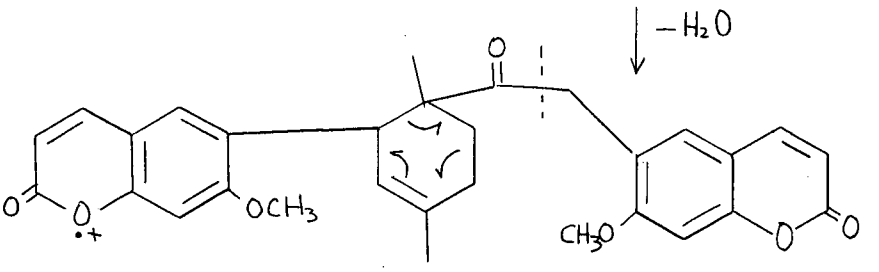

Aldehyde - I
RELATIVE INTENSITY
m/e
204
OHC
CH3O

Figure 26



Thamnosin-diol m/e 518



$(M-18)^+$ m/e 500 (9%)

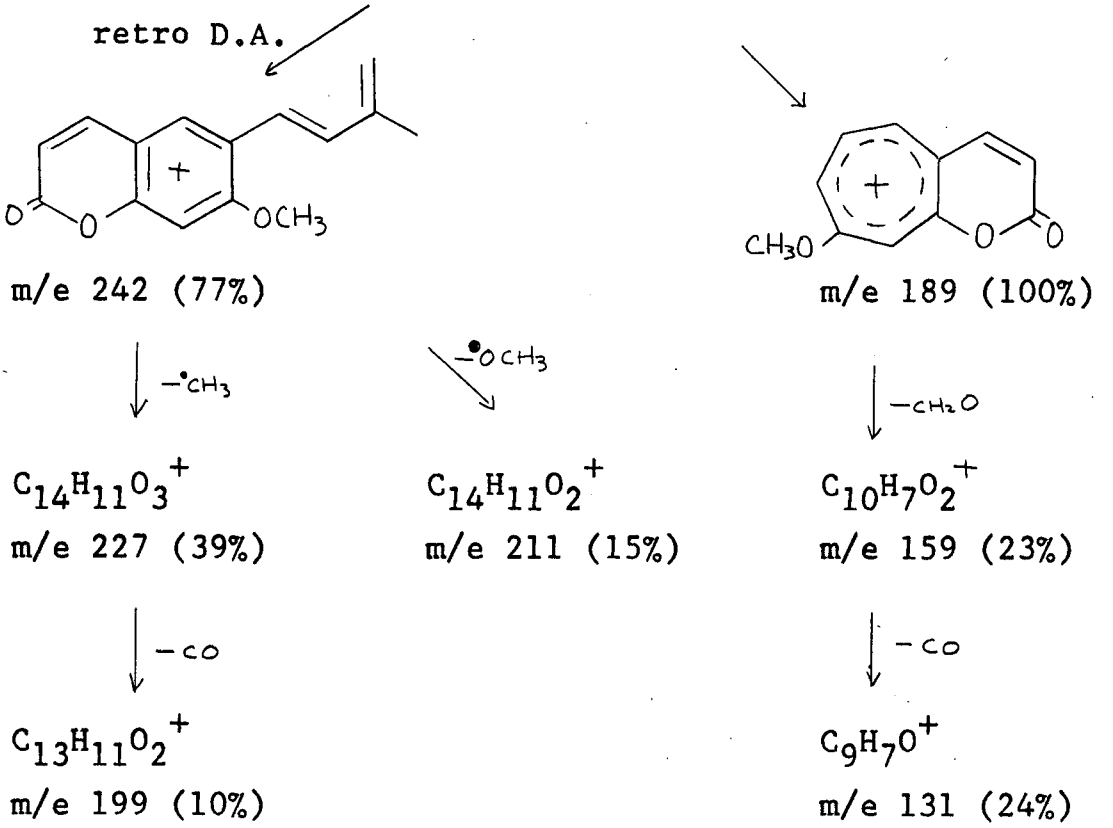


Figure 27

Chemical degradation of thamnosin-diol into two units was accomplished by the action of periodic acid to give two aldehydes. The structure of aldehyde-I was identified to be 7-methoxy-coumarin-6-aldehyde by direct comparison with an authentic sample and this highly conjugated molecule, as anticipated, showed a molecular ion at m/e 204 which also was the base peak. Loss of H and CO gave highly conjugated ions at m/e 203 and m/e 175, successively. (Figure 28)

Aldehyde-I $C_{11}H_8O_4$ M.W. 204 ($\geq$ 100)

m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
205	21	159	14	127	18	113	19
204	100	158	14	126	14	112	19
203	33	149	17	125	21	111	33
187	23	139	12	123	15	110	15
175	15	137	12	119	19	109	21

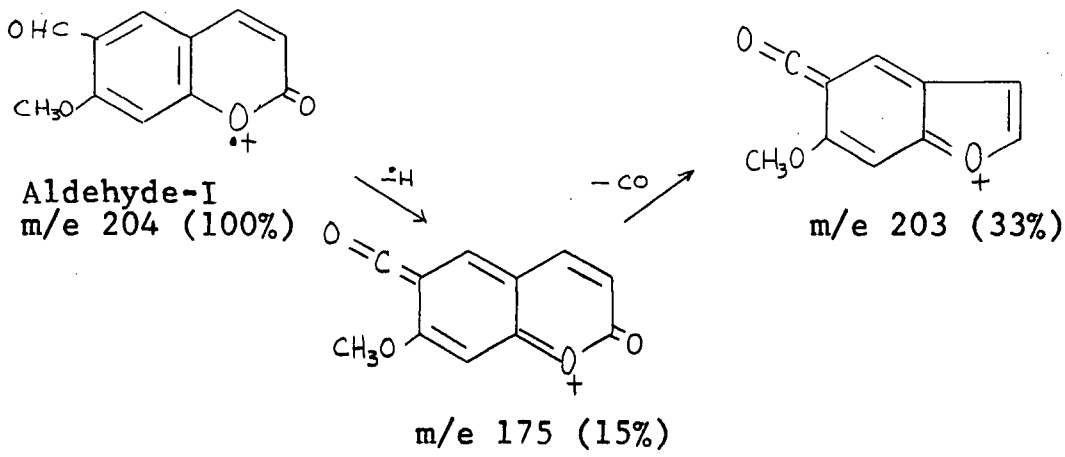
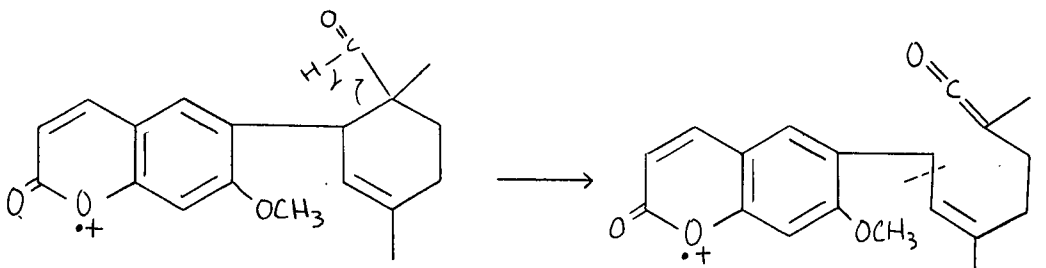


Figure 28

Aldehyde-II $C_{19}H_{20}O_4$ M.W. 312 ($\geqslant$100)

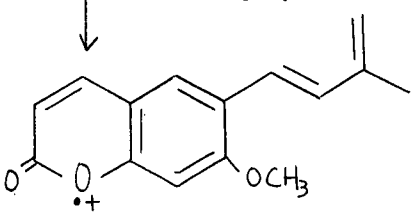
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
312	45	167	16	127	25	113	29
243	21	155	17	126	19	112	30
242	100	149	29	125	29	111	49
227	49	140	16	124	17	110	21
211	21	138	17	123	21	109	29
189	22	136	16	119	16	105	16



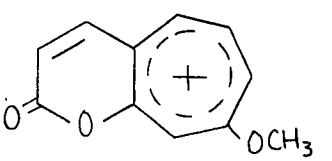
Aldehyde-II

m/e 312 (45%)

retro D.A.



m/e 242 (100%)



m/e 189 (22%)

$\cdot CH_3$

$C_{14}H_{11}O_3^+$

m/e 227 (49%)

$\cdot OCH_3$

$C_{14}H_{11}O_2^+$

m/e 211 (21%)

Figure 29

Another degradation product of thamnosin-diol, aldehyde-II, showed a stable molecular ion at m/e 312 (45%) and the fragmentation pattern in the lower mass region was somewhat similar to that of dihydro-thamnosin (Figure 26). The base peak at m/e 242 must arise from the retro D.A. reaction of the cyclohexene ring. As shown in Figure 9, an aldehydic hydrogen could migrate to form a ketene intermediate and subsequent fission β to the coumarin system could provide an tropylium-type ion at m/e 189 (22%).

In contrast to the characteristic fragmentation patterns of thamnosin and its derivatives discussed above, the dihydro-thamnosin-oxide showed a featureless mass spectrum (Figure 30) except for the fairly stable molecular ion at m/e 502 (23%) and the base peak at m/e 189. The absence of a cyclobexene ring in this molecule and the stability of an epoxide ring under electron impact would be expected to lead to this type of fragmentation.

Dihydro-thamnosin-oxide $C_{30}H_{30}O_6$ M.W. 502 ($\geq$100)

m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
502	23	242	31	185	19	141	22
313	10	229	11	163	12	139	62
282	20	227	10	159	17	131	19
281	13	203	14	158	20	113	13
280	12	190	25	156	55	111	36
245	32	189	100	149	14	103	13

Finally, a mass spectrum of keto-aldehyde-III (Figure 30) was obtained. This compound was obtained by

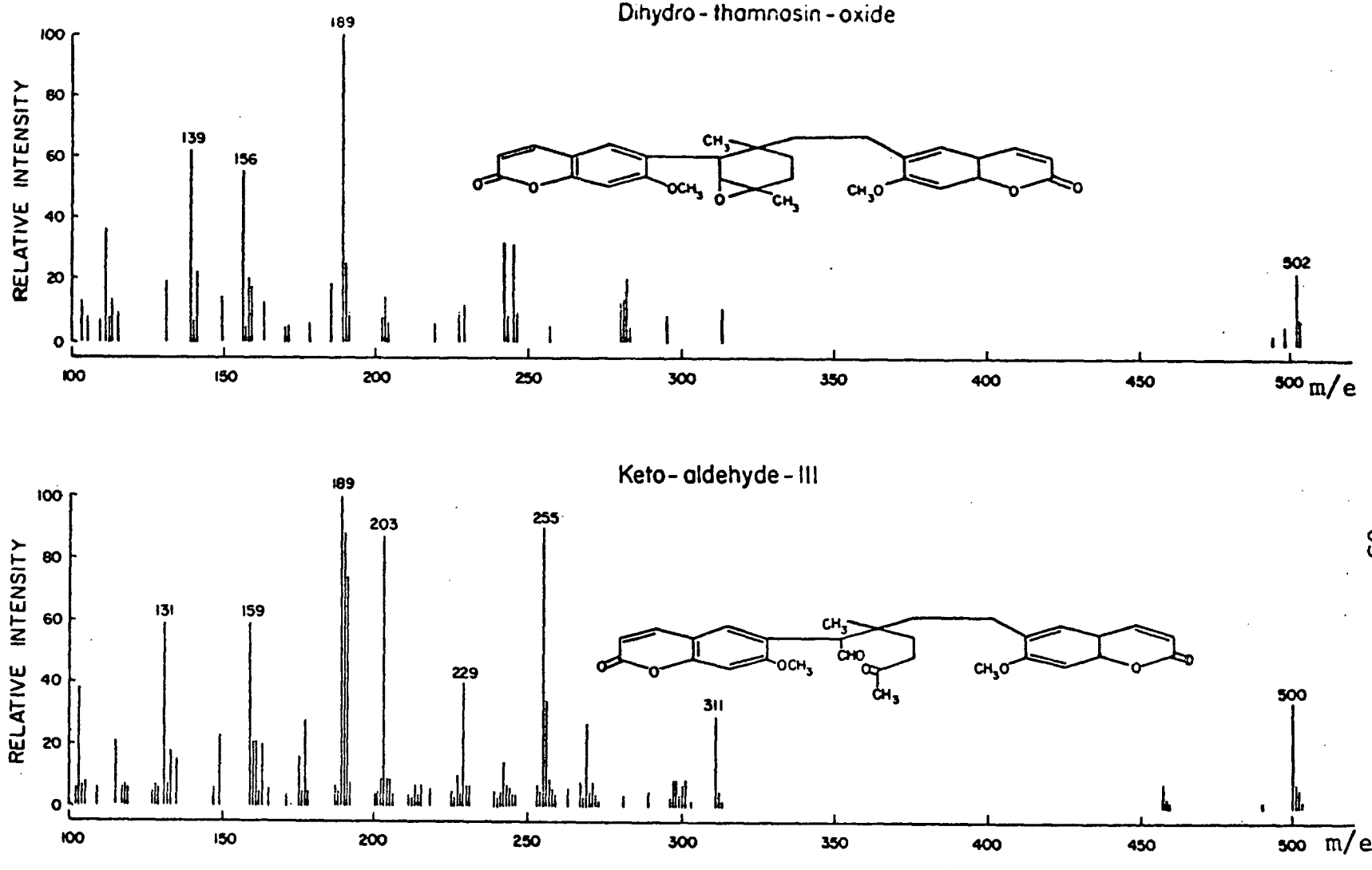
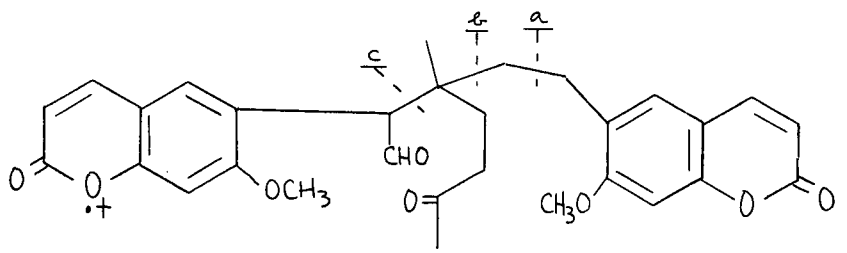

Dihydro - thamnosin - oxide
RELATIVE INTENSITY
100
80
60
40
20
0
189
139
156
502
100
150
200
250
300
350
400
450
500
m/e
Keto - aldehyde - III
RELATIVE INTENSITY
189
203
255
131
159
229
311
500
100
150
200
250
300
350
400
450
500
m/e

Figure 30



Keto-aldehyde-III m/e 518

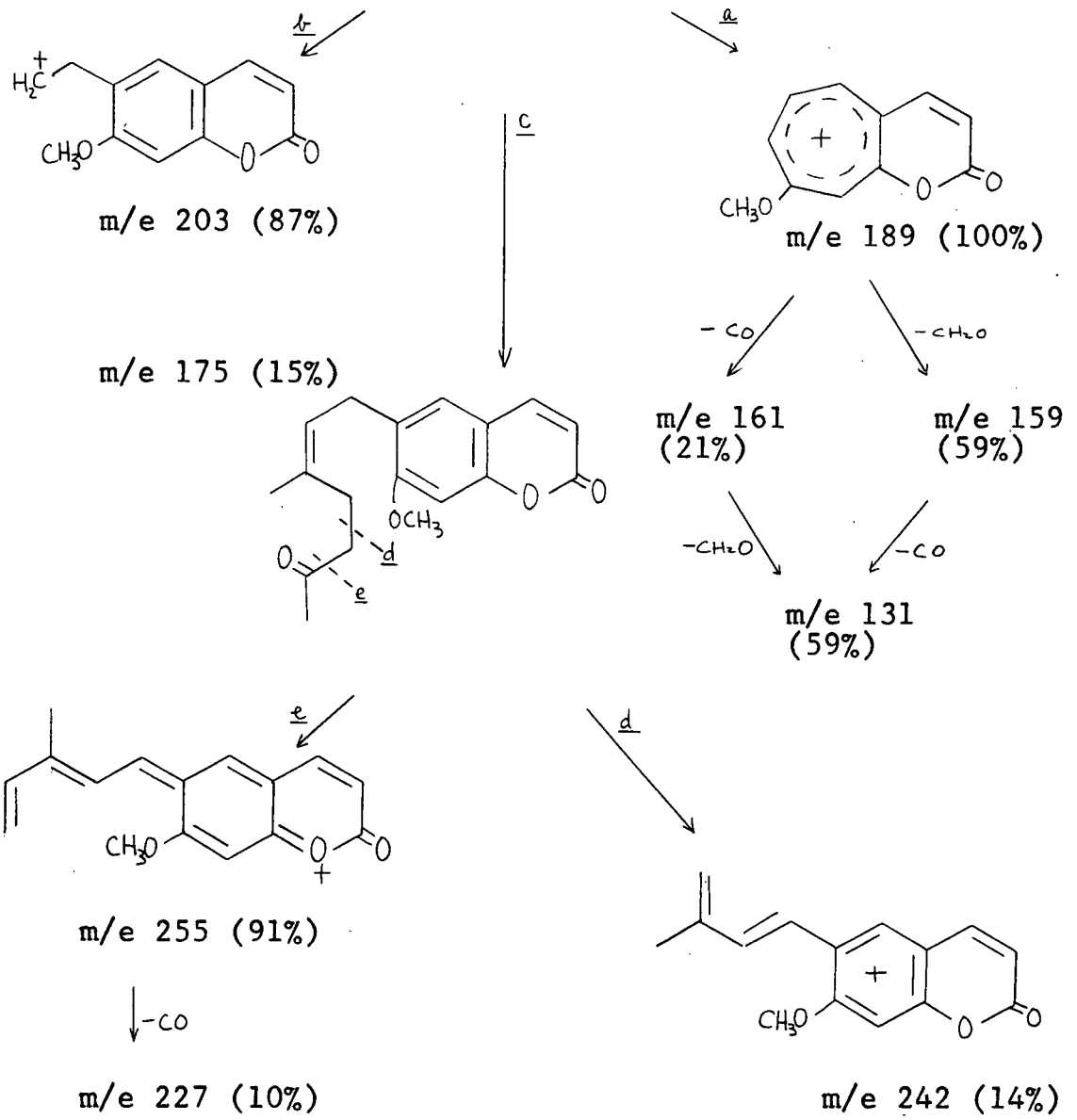


Figure 31

cleavage of a cyclohexene ring in dihydro-thamnosin by treatment with ozone. This molecule, with labile functional groups, was expected to give a characteristic and complex fragmentation under electron impact (Figure 31).

Keto-aldehyde-III $C_{30}H_{30}O_8$ M.W. 518 ($\geqslant$100)

m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
518	0.1	242	14	177	28	149	23
500	34	229	40	175	15	135	15
311	29	203	87	163	20	133	18
269	27	191	74	161	21	131	59
256	34	190	87	160	21	115	21
255	91	189	100	159	59	103	38

As summarized in Figure 31, the strong peaks at m/e 189 (100%), m/e 255 (91%) and m/e 203 (87%) could come from three ways of fission (a, b and c) of Keto-aldehyde-III. Subsequent loss of CO from each fragment could be explained by the fact that each fragment contained a coumarin system.

In conclusion, the mass spectra of thamnosin and its derivatives showed characteristic fragmentation patterns and all the data were in good accord with the proposed structures described in the previous section of this thesis.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. The ultraviolet (UV) spectra were recorded in methanol solution on a Cary 14 spectrophotometer, and the infrared (IR) spectra were taken on Perkin-Elmer Model 21 and Model 137 spectrophotometers. Nuclear magnetic resonance (NMR) spectra were recorded in deuteriochoroform (unless otherwise indicated) at 100 Mc/s on a Varian HA100 instrument. In all instances, spectra were also recorded at 60 Mc/s on a Varian A60 instrument but these are not quoted here. The chemical shifts are given in the Tiers τ scale with reference to tetramethylsilane as the internal standard. Mass spectra were recorded on an Atlas CH-4 mass spectrometer and high resolution determinations were carried out on an AEI MS-9 mass spectrometer. Analyses were performed by Dr. A. Bernhardt, Mulheim (Ruhr), Germany and Mr. P. Borda of the microanalytical laboratory, University of British Columbia. Silica gel G and Woelm neutral alumina containing electronic phosphor were used for thin-layer chromatography (TLC) and Woelm neutral alumina (activity I) was used for column chromatography.

Thamnosin

The crude thamnosin, provided by Dr. D. L. Dreyer, Fruit and Vegetable Laboratory, U.S. Department of Agriculture, Pasadena, California, was recrystallized three times from benzene-dichloromethane to provide the analytical

sample (as prisms), m.p. 244-247°; one bright fluorescent spot on TLC (silica gel G, $CHCl_3$: EtOAc (1 : 1)).

ORD: $[\phi]_D 0°$, $[\phi]_{400}$ 50°, plain positive curve.

IR (KBr): 1725, 1610, 1557 (α-pyrone), 980 (trans disubstituted double bond), 820 (trisubstituted double bond) cm^{-1}.

UV, λmax (ϵ): 227 (30,000), 256 (23,100), 298 (Sh, 14,800) 333 mμ(22,900); λmin (ϵ): 243 (20,600), 282 (12,100).

NMR signals (100 Mc/s): τ 2.46 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.50 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.89 (1H, singlet, H-C_5 coumarin), 2.94 (1H, singlet, H-C_5 coumarin), 3.37 (1H, singlet, H-C_8 coumarin), 3.39 (1H, singlet, H-C_8 coumarin), 3.82 (1H, doublet, J=16 cps, H_B-C=C-), 3.83 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 3.85 (1H, doublet,, J=9.5 cps, H-C_3 coumarin), 3.98 (1H, doublet, J=16 cps, H_A-C=C-), 4.75 (1H, multiplet, H_D-C=C-), 6.18 (1H, multiplet, H_C-C-), 6.27 (3H, singlet, CH_3O-C_7 coumarin), 6.29 (3H, singlet, CH_3O-C_7 coumarin), 8.20 (3H, singlet, CH_3-C=C-), 8.78 (3H, singlet, CH_3-C-C=C-).

Anal. Found: C, 74.26; H, 5.74; O, 20.08; O-Me, 12.87.

Calc. for $C_{30}H_{28}O_6$: C, 74.36; H, 5.82; O, 19.81; (2) O-Me, 12.7.

Molecular wt.: 484.188 (Calc. 484.189).

Attempted Ozonization of Thamnosin

Thamnosin (M.W. 484, 90 mg.) was dissolved in dichloromethane (20 ml.), cooled to -78° and a slow stream of ozone was passed through for 15 minutes. Acetic acid (25 ml.) was added to the mixture and a solution of ferrous

sulfate (1 g.) in water (10 ml.) was added. The mixture was stirred for 30 minutes, heated for 15 minutes and immediately steam distilled into a solution of 2,4-dinitrophenylhydrazine (0.6 g.) in water (40 ml.) and concentrated sulfuric acid (10 ml.). Very slow precipitation was observed after 30 minutes and 250 ml. of distillate was collected. The orange precipitate was separated and chromatographed on acid washed alumina (Shawinigan, pH 3). Elution with benzene-ethyl acetate gave a reddish brown solid (5 mg.), crude m.p. 250° (decomp.). Attempted recrystallization failed. UV, λmax: 224 and 355 mμ (in ether). 2.4-dinitrophenylhydrazine showed λmax at 258 and 342 mμ in ether.

Dihydro-thamnosin

Thamnosin (238 mg.), in absolute tetrahydrofuran (40 ml.), was hydrogenated over 10% palladium on charcoal (220 mg.). The hydrogen uptake ceased after 25 minutes when 1 mol. had been absorbed. After removal of the catalyst and solvent, the product was recrystallized from benzene-petroleum ether to give dihydro-thamnosin (174 mg.), m.p. 226-228°. This compound displayed one dull fluorescent spot on TLC (silica gel, $CHCl_3$: EtOAC (1: 1)) whose R_f value was the same as that of thamnosin.

IR (KBr): 1728, 1618, 1563 (α-pyrone), 820 (trisubstituted double bond) cm^{-1}.

UV, λmax (ϵ): 224 (36,300), 246 (Sh, 13,300), 254 (12,000), 300 (Sh, 14,500), 330 mμ (27,200); λmin (ϵ): 266 (5,900).

NMR singnals (100 Mc/s): 2.47 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.53 (1H, doublet, J=9.5 cps, H-C_4 coumarin) 2.93 (1H, singlet, H-C_5 coumarin), 3.07 (1H, singlet, H-C_5 coumarin), 3.28 (1H, singlet, H-C_8 coumarin), 3.33 (1H, singlet, H-C_8 coumarin), 3.86 (2H, two doublets, J=9.5 cps, H-C_3 coumarin), 4.83 (1H, multiplet, H_D-C=C-), 6.33 (1H, doublet, J=3.5 cps, H_C-C-), 6.22 (3H, singlet, CH_3O-C_7 coumarin), 6.25 (3H, singlet, CH_3O-C_7 coumarin), 8.26 (3H, singlet, CH_3-C=C-), 8.97 (3H, singlet, CH_3-C-).

Anal. Found: C, 73.47; H, 6.43; O, 20.25; O-Me, 12.87.

Calc. for $C_{30}H_{30}O_6$: C, 74.07; H, 6.23; O, 19.73; (2) O-Me, 12.8.

Molecular wt.: 486.204 (Calc. 486.204).

Hydrogenation of 7-Methoxycoumarin

A sample of 7-methoxycoumarin (5 mg.) in absolute ethanol (2 ml.) was hydrogenated over 10% palladium on charcoal (5 mg.). No uptake of hydrogen was detected after stirring for 2 hours. However 7-methoxycoumarin (5 mg.) in absolute tetrahydrofuran (2 ml.) was hydrogenated over 5% rhodium on alumina (5 mg.) in a micro-hydrogenator, and a rapid uptake of 1 mol. was observed. At this point the absorption ceased. The catalyst was filtered off and evaporation of the solvent gave as an oil, 3,4-dihydro-7-methoxycoumarin (5 mg.).

UV, λmax: end absorption (220 mμ), 278, 284 mμ.

IR ($CHCl_3$): 1750 (-CO-O-C=C-), 1626, 1590 (aromatic ring).

Thamnosin-diol

Thamnosin (223 mg.) was dissolved in absolute tetrahydrofuran (40 ml.) and osmium tetroxide (140 mg., 1.2 mol.) was added to the solution. The mixture was allowed to stand for 3 days at room temperature and then methanol (100 ml.) was added. Dry hydrogen sulfide was passed through the mixture for 20 minutes. The sulfide was filtered off to give a pale yellow solution. Evaporation of the solvent gave crystalline thamnosin-diol(150 mg.). Thamnosin-diol crystallized as prisms from methanol, with one molecule of solvent, (a), m.p. 273-276°.

Anal. Found: C, 67.99; H, 6.40. Calc. for $C_{30}H_{30}O_8 \cdot CH_3OH$: C, 67.61; H, 6.23. These prisms were ground and dried in the drying pistol for 3 hours at 100° to afford unsolvated thamnosin-diol (b), m.p. 243-248°, reforming plates, m.p. 267-272°.

Anal. Found: C, 69.01; H, 6.33. Calc. for $C_{30}H_{30}O_8$: C, 69.49; H, 5.79.

Recrystallization from ethanol afforded the unsolvated thamnosin-diol (c) as plates, m.p. 243-248°, reforming plates, m.p. 269-272° mixed melting point with thamnosin-diol (b) showed no depression.

Anal. Found: C, 69.78; H, 5.91; O, 24.51; O-Me, 11.71. Calc. for $C_{30}H_{30}O_8$: C, 69.49; H, 5.83; O, 24.68; (2) O-Me, 12.0.

In addition to the above unsolvated plates (c), thamnosin-diol crystallized, with one molecule of solvent, as prisms

(d), m.p. 267-272°.

Anal. Found: C, 68.01; H, 6.13; O, 25.72. Calc. for $C_{30}H_{30}O_8 \cdot C_2H_5OH$: C, 68.06; H, 6.43; O, 25.51.

These prisms (d) were ground and dried at 100° in the drying pistol for 6 hours to yield unsolvated thamnosin-diol (e), m.p. 243-247°, reforming plates, m.p. 266-272°, whose mixed melting point with thamnosin-diol (c) showed no depression.

The above thamnosin-diol (a, b, c, d and e) showed one spot, respectively, on TLC with the identical R_f valves (alumina and silica gel, benzene-EtOAc, $CHCl_3$, $CHCl_3$-EtOAc, EtOAc).

IR (KBr): 3480 (hydroxyl), 1725, 1620, 1565 (coumarin), 820 (trisubstituted double bond) cm^{-1}.

UV, λmax (ϵ): 223 (36,800), 251 (Sh, 12,900), 300 (Sh, 18,200), 328 (29,700), λmin. (ϵ): 267 (6,400).

NMR signals (100 Mc/s): τ 2.43 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.49 (1H, doublet, J=9.5 cps, H-C_4 coumarin) 2.61 (1H, singlet, H-C_5 coumarin), 2.80 (1H, singlet, H-C_5 coumarin), 3.25 (1H, singlet, H-C_8 coumarin), 3.49 (1H, singlet, H-C_8 coumarin), 3.82 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 3.92 (1H, doublet, J=9.5 cps, H-C_3 coumarin) 4.75 (1H, doublet, J=5 cps, H_D-C=C-), 4.98 (1H, multiplet, H_A-C-O-), 6.16 (3H, singlet, CH_3O-C_7 coumarin), 6.28 (1H, doublet, J=5 cps, H_C-C-), 6.47 (3H, singlet, CH_3O-C_7 coumarin), 6.6 (2H, mutiplet, 2HO-), 7.03 (1H, singlet, H_B-C-O-), 7.8 and 8.3 (4H, two sets of doublets, J=7 cps, -C- CH_2-CH_2-C-), 8.21 (3H, singlet, CH_3-C=C-), 8.69 (3H, singlet, CH_3-C-).

NMR signals ($+D_2O$, 100 Mc/s): 4.98 (1H, sharp singlet, H_A-C-OD), 5.30 (singlet, HOD), no peaks at 6.6 (2DO-), 7.03 (1H, sharp singlet, H_B-C-OD), the rest of the peaks remained the same.
High resolution mass determination, m/e 500 $(C_{30}H_{30}O_8-H_2O)^+$ peak: 500.187 (Calc. 500.184).

Periodate Cleavage of Thamnosin-diol

To a solution of thamnosin-diol (M.W. 518, 200 mg.), in methanol (220 ml.), was added an aqueous solution of periodic acid (1.5 mol.) and the reaction mixture was allowed to stand for 24 hours. The solvent was evaporated in vacuo and the residual material was extracted with dichloromethane. The organic layer was washed with water, aqueous sodium bicarbonate and water and dried over sodium sulfate. Removal of the sovent gave a white solid. The white material was purified by preparative TLC on silica gel ($CHCl_3$: EtOAc (1 : 1)). After the plate was developed, a small portion of the plate was sprayed with 2,4-DNP reagent to give two distinct bands. The more polar compound (aldehyde-I) showed an orange color while the less polar compound (aldehyde-II) was yellow in color. Extraction of these two fractions with methanol/chloroform yielded aldehyde-I (49 mg.) and aldehyde-II (25 mg.). Aldehyde-I was crystallized as prisms from methanol, m.p. 242-246°.
IR (KBr): 1735, 1670, 1610 (α-pyrone, aldehyde).
UV, λmax: 255, 308, 329, 342 (Sh.) mμ, λmin: 237, 278,

318 mμ.

UV (+$NaBH_4$), λmax: 222, 251 (Sh.), 295 (Sh.), 327 mμ, min: 261 mμ.

NMR signals (100 Mc/s in $(CF_2Cl)_2C(OD)_2$): -0.23 (1H, singlet, -C<u>H</u>O), 1.90 (1H, singlet, <u>H</u>-C_5 coumarin), 2.09 (1H, doublet, J=9.5 cps, <u>H</u>-C_4 coumarin), 3.00 (1H, singlet, H-C_8 coumarin), 3.75 (1H, doublet, J=9.5 cps, <u>H</u>-C_3 coumarin) 6.01 (3H, singlet, C<u>H</u>$_3$O-C_7 coumarin).

Molecular wt.: 204.042 (Calc. for $C_{11}H_8O_4$: 204.042).

An authentic sample of 7-methoxycoumarin-6-aldehyde, m.p. 248-251°, was obtained from Dr. F. E. King, Forest Products Laboratories, Ayresburg, Bucks, England, (King et al[41] give m.p. 252-253°).

IR (KBr): 1735, 1670, 1610 cm^{-1}.

UV, λmax: 255, 308, 328, 342 (Sh.) mμ, λmin.: 237, 277, 316 mμ.

Mixed m.p. with aldehyde-I: m.p. 243-246°.

Aldehyde-I was identical with 7-methoxycoumarin-6-aldehyde by all criteria: mixed m.p.; R_f values on TLC (silica gel and alumina, $CHCl_3$, $CHCl_3$-EtOAc, EtOAc, benzene-EtOAc); superimposable UV and IR spectra.

Aldehyde-II resisted crystallization but data was obtained on TLC pure material.

IR ($CHCl_3$): 1720, 1615 cm^{-1} (α-pyrone, saturated aldehyde).

UV, λmax: 229, 254 (Sh.), 296 (Sh.), 328 mμ, λmin: 261 mμ.

NMR signals (100 Mc/s): 2.45 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.85 (1H, singlet, H-C_5 coumarin), 3.31 (1H,

singlet, H-C_8 coumarin), 3.84 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 4.76 (1H, multiplet, H_D-C=C-), 5.84 (1H, doublet, H_C-C-), 6.20 (3H, singlet, CH_3O-C_7 coumarin), 8.21 (3H, singlet, CH_3-C=C-), 8.82 (3H, singlet, CH_3-C-). Molecular wt. : 312.138 (Calc. for $C_{19}H_{20}O_4$: 312.136).

Dihydro-thamnosin-oxide

Dihydro-thamnosin (M.W. 486, 220 mg.), in chloroform (50 ml.), was treated with m-chloroperbenzoic acid (1.5 mol.) and the solution was maintained at room temperature for 36 hours. The solution was then washed with aqueous sodium bicarbonate solution and dried over sodium sulfate. Removal of the sovent gave crystalline material (200 mg.). Recrystallization from diisopropyl ether afforded dihydro-thamnosin-oxide as plates, m.p. 243-246°.

IR (KBr): 1725, 1612, 1565 cm^{-1} (coumarin).

UV, λmax (ϵ): 223 (39,700), 243 (Sh. 12,800), 253 (Sh. 9,700), 298 (Sh. 17,300), 329 mμ (27,400), λmin (ϵ): 263 (3,900).

NMR signals (100 Mc/s): 2.47 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.51 (1H, doublet, J=9.5 cps, H-C_4 coumarin) 3.00 (1H, singlet, H-C_5 coumarin), 3.05 (1H, singlet, H-C_5 coumarin), 3.26 (1H, singlet, H-C_8 coumarin), 3.28 (1H, singlet, H-C_8 coumarin), 3.85 (2H, doublets, J=9.5 cps, 2$\underline{H}$-C_3 coumarin), 6.16 (6H, singlets, 2$\underline{CH_3}O$-C_7 coumarin), 6.4 (1H, multiplet, H_C-C-), 7.11 (1H, broad singlet, H_D-C(=O)-C-), 8.59 (CH_3, singlet, CH_3-C(=O)-C-), 9.17 (3H, singlet, CH_3-C-).

Anal. Found: C, 72,00; H, 5.77; O, 22.29; O-Me, 12.15.
Calc. for $C_{30}H_{30}O_7$: C, 71.69; H, 6.02; O, 22.28; (2) O-Me, 12.3.
Molecular wt. : 502.202 (Calc. 502.199).

Attempted Epoxide Opening on Dihydro-thamnosin-oxide

Dihydro-thamnosin-oxide (M.W. 502, 10 mg.) was added to a boiling 5% aqueous oxalic acid solution (3 ml.) and refluxing continued for 30 minutes. The solution was cooled and extracted with dichloromethane. The extracts were washed with 5% aqueous sodium bicarbonate and dried over sodium sulfate. The solvent was removed to give a white residue (8 mg.). This residue was identified to be the unreacted starting oxide by TLC, UV and IR spectra. Under more forcing conditions (for example refluxing in dioxane for 2 hours) the oxide gave intractable mixtures.

Attempted Hydroxyation of Dihydro-thamnosin

Dihydro-thamnosin (50 mg.), in absolute tetrahydrofuran (2 ml.), was treated with osmium tetroxide (31 mg., 1.2 mol.). The reaction mixture was allowed to stand at room temperature for 5 days, and then was stirred with a solution of sodium bisulfite (100 mg.) in water (5 ml.) and methanol (10 ml.) for 20 hours. The solution was separated, acidified with a few drops of acetic acid, concentrated to a small volume and extracted with chloroform. The organic layer was separated and dried over sodium sulfate.

Evaporation of the solvent afforded a grayish brown solid (23 mg.). TLC on silica gel ($CHCl_3$-EtOAc) indicated that the major component in this mixture was unreacted starting material. Preparative TLC on silica gel (with very poor recovery) showed that the starting material represented 60% while a few more polar compounds represented the remaining 40% of the reaction mixture.

Controlled Ozonization of Osthol

Osthol (8-isopent-2'-enyl-7-methoxycoumarin, 5 mg.) was dissolved in dichloromethane (40 ml.) and cooled to -78°. A slow stream of ozone was passed through the solution for 50 minutes until the excess ozone was detected with aqueous potassium iodide-boric acid at the outlet. The solution was poured into aqueous ferrous sulfate (100 mg.) and the stirring was continuted for 10 minutes. The organic layer was separated and dried over sodium sulfate. Removal of the solvent gave a pale yellow material (4 mg.). This material showed one spot on TLC (silica gel, EtOAc-$CHCl_3$) and its R_f value was smaller than that of osthol. On spraying with 2,4-DNP reagent the ozonization product showed a distinct yellow spot on a TLC plate. Under these conditions, osthol showed no color. The UV spectrum of the ozonization product was the same as that of the starting material. It was apparent that the controlled ozonization of osthol was most likely yielding an aldehydic product.

Controlled Ozonization of Dihydro-thamnosin

Dihydro-thamnosin (100 mg.), in dichloromethane, was ozonized for 1 hour under the same conditions as previously described for osthol. The crude product (90 mg.) was purified by preparative TLC (silica gel, EtOAc-$CHCl_3$) and the aldehydic band was detected by 2,4-DNP as spray reagent. Extraction of the aldehyde by chloroform-methanol afforded an amorphous solid (36 mg.), m.p. 135-140°, designated as keto-aldehyde-III.

IR($CHCl_3$): 1721, 1616 cm^{-1} (coumarin, aldehyde, methyl ketone).

UV, λmax (ϵ): 223 (42,900), 254 (Sh. 12,000), 296 (Sh. 15,500), 329 mμ (29,900), λmin (ϵ): 265 mμ (4,800).

NMR signals (100 Mc/s): 0.02 (1H, doublet, J=2 cps, -CHO), 2.39 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.44 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.68 (1H, singlet, H-C_5 coumarin), 2.86 (1H, singlet, H-C_5 coumarin), 3.18 (1H, singlet, H-C_8 coumarin), 3.27 (1H, singlet, H-C_8 coumarin), 3.77 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 3.81 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 5.76 (1H, doublet, J=2 cps, $\underline{H}_C$-C-CHO), 6.15 (6H, singlets, $2CH_3O$-C_7 coumarin), 7.87 (3H, singlet, CH_3-CO-), 8.91 (3H, singlet, CH_3-C-). High resolution mass determination, m/e 500 $(C_{30}H_{30}O_8-H_2O)^+$ peak: 500.184 (Calc. 500.184).

Sodium Borohydride Reduction of Keto-aldehyde-III

Keto-aldehyde-III (16 mg.), in iso-propanol (2 ml.)

and chloroform (1 ml.), was reduced with sodium borohydride (8 mg.). After the mixture was allowed to stand at room temperature for 55 min., the solvent was removed in vacuo. The resulting residue was extracted with chloroform. Evaporation of the chloroform gave an amorphous solid (8 mg.).

IR ($CHCl_3$): 3436 (hydroxyl), 1718, 1613, 1560 (coumarin) cm^{-1}.

UV, λmax: 225, 254, 298 (Sh.), 329 mμ.

NMR signals (100 Mc/s): 3.43 (2H, doublet, J=9.5 cps, H-C_4 coumarin), 3.66 (1H, singlet, H-C_5 coumarin), 3.84 (1H, singlet, H-C_5 coumarin), 3.29 (2H, singlet, 2H-C_8 coumarin), 3.85 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 3.89 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 6.15 (6H, singlet, 2 CH_3O-C_7), 5.8-6.4 (6H, multiplet), 8.82 (3H, multiplet, CH_3-CH-OH), 9.09 (3H, singlet, CH_3-C-).

Suberosin

Demethylsuberosin (6-isopent-2'-enylumbelliferone) was kindly supplied by Dr. F. E. King, Forest Products Research Laboratories, Ayresburg, Bucks, England. Demethylsuberosin was methylated by refluxing with methyl-iodide and potassium carbonate in acetone for 10 hours to afford suberosin. Recrystallization from diisopropyl ether gave suberosin (7-methoxy-6-isopent-2'-enylcoumarin) as prisms, m.p. 82-87° (King et al[41] give m.p. 87-88°).

IR ($CHCl_3$): 1724, 1621, 1563 (coumarin, α-pyrone) cm^{-1}.

UV, λmax: 223, 253, 297 (Sh.), 330 mμ.

NMR signals (100 Mc/s): 2.47 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.90 (1H, singlet, H-C_5 coumarin), 3.30 (1H, singlet, H-C_8 coumarin), 3.87 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 4.78 (1H, broad triplet, J=7 cps, H-$C_{2'}$), 6.18 (3H, singlet, CH_3O-C_7 coumarin), 6.74 (2H, doublet, J=7 cps, 2H-$C_{1'}$), 8.27 (3H, singlet CH_3-C=C-), 8.33 (3H, singlet, CH_3-C=C-).

Tetrahydro-thamnosin

Dihydro-thamnosin (M.W. 486, 50 mg.), in acetic acid (25 ml.), was hydrogenated over 10% palladium on charcoal (100 mg.). The hydrogenation was interrupted when 1 mol. of hydrogen was absorbed. The catalyst was filtered off and removal of the solvent gave an amorophous solid (40 mg.), tetrahydro-thamnosin.

IR ($CHCl_3$): 1721, 1623, 1560 (coumarin) cm^{-1}.

UV, λmax: end absorption (220 mμ), 254 (Sh.), 300 (Sh.), 332 mμ, λmin: 266 mμ.

NMR signals (100 Mc/s): 2.46 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.50 (1H, doublet, J=9.5 cps, H-C_4 coumarin), 2.92 (1H, singlet, H-C_5 coumarin), 2.97 (1H, singlet, H-C_5 coumarin), 3.26 (2H, singlets, 2H-C_8 coumarin), 3.82 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 3.86 (1H, doublet, J=9.5 cps, H-C_3 coumarin), 6.16 (3H, singlet, CH_3O-C_7 coumarin), 6.18 (3H, singlet, CH_3O-C_7 coumarin), 8.96 (3H, doublet, J=4 cps, CH_3-C-H), 9.16 (3H, singlet, CH_3-C-).

Octahydro-thamnosin

Dihydro-thamnosin (49 mg.), in dichloromethane-methanol (10 ml./10 ml.), was hydrogenated over 10% palladium on charcoal. The hydrogen uptake was ceased after 3 mol. and the catalyst was filtered off. Removal of the solvent gave an amorphous solid (46 mg.), octahydro-thamnosin.

IR ($CHCl_3$): 1761, 1618 cm^{-1} (C=O, aromatic).

UV, λmax: end absorption (220 mμ), 285 mμ, λmin: 253 mμ.

NMR signals (60 Mc/s): 3.25 (2H, broad singlets, 2H-C_5), 3.55 (2H, broad singlets, 2H-C_8), 6.28 (3H, singlet, CH_3O-C_7), 6.32 (3H, singlet, CH_3O-C_7), 9.08 (3H, multiplet, CH_3-CH), 9.23 (3H, singlet, CH_3-C-).

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PART II

STUDIES IN INDOLE ALKALOIDS

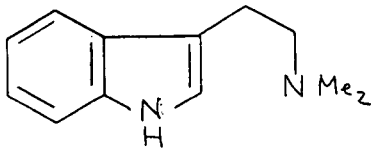
INTRODUCTION

Research on alkaloids interests chemists because of the complex structures, special biological activities and phytochemical importance. Because alkaloids generally are complex molecules, classical structural elucidation methods require large amounts of sample to deduce the original structure from the degradation products and this inconvenience has been the main difficulty in alkaloid chemistry. The problem has been largely overcome by the extensive use of physical methods, especially nuclear magnetic resonance and mass spectroscopy, which require only small quantities of material. In addition to the structural elucidation of alkaloids, a considerable effort has been made towards the synthesis of these substances. In many instances, laboratory construction of complex organic systems has been studied by "biogenetic-type" synthesis.

Studies of the biosynthesis of alkaloids became feasible with the advent of radioactive compounds. For example, incorporation experiments with radioactive tryptophan into gramine (1), ajmaline (2), serpentine (3), vindoline (4)[3], catharanthine (5) and reserpine (6), have now shown conclusively that the indole portion of the indole alkaloids is derived from tryptophan or its biological equivalent[1,2].

In contrast to the "tryptophan" portion of the indole alkaloids, various hypotheses (discussed below) concerned

with the biosynthesis of the "non-tryptophan" or C_{9-10} unit have been put forward.



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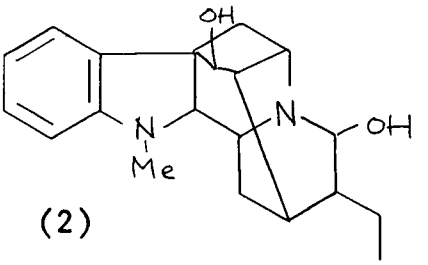

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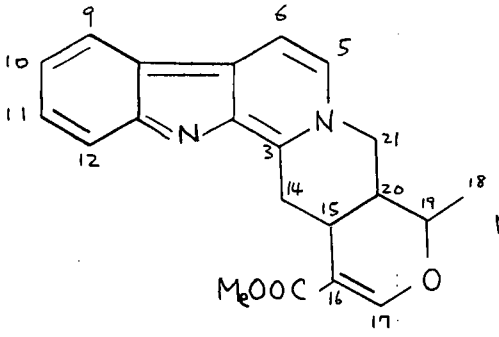

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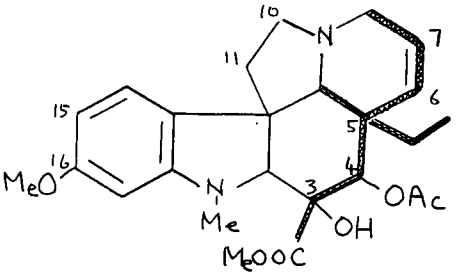

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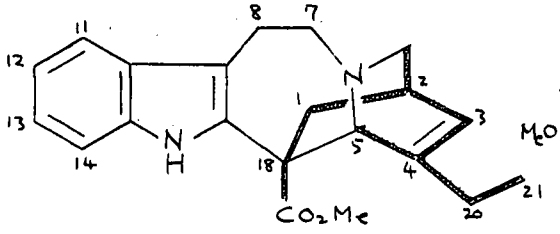

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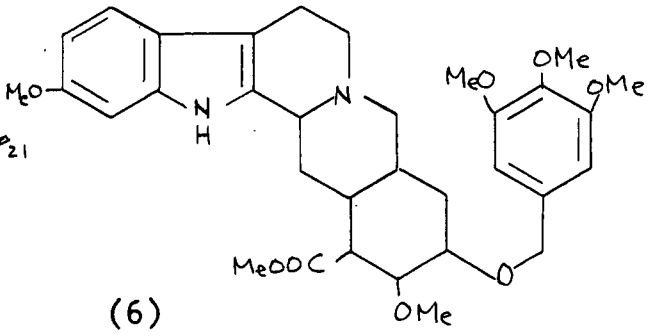

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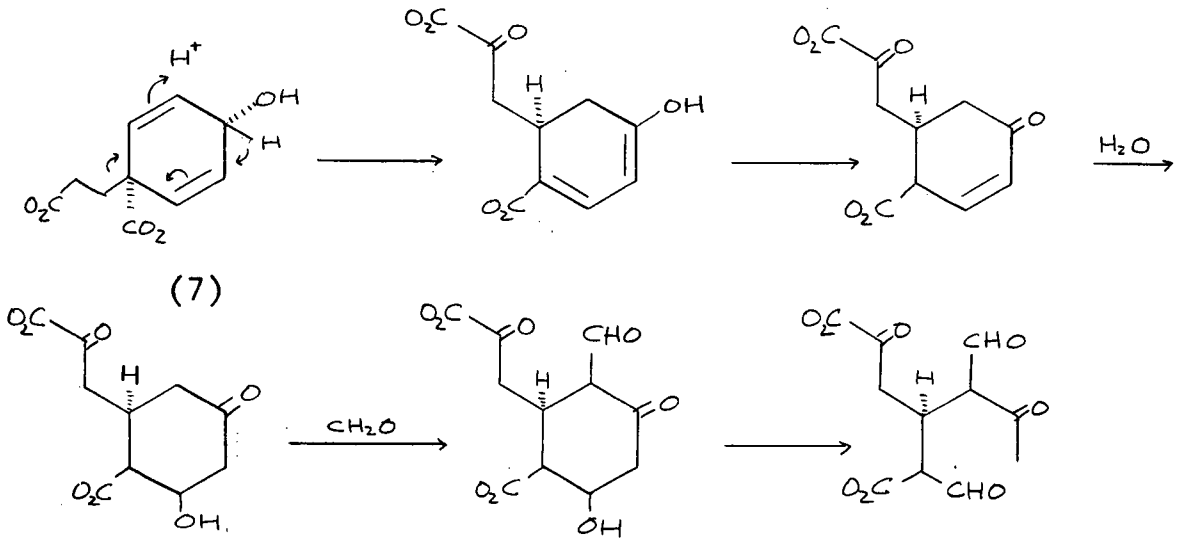
Figure 1

The Shikimic-Prephenic Acid Hypothesis

A biogenetic scheme involving prephenic acid as a precursor was proposed by Wenkert[5] for the biosynthesis



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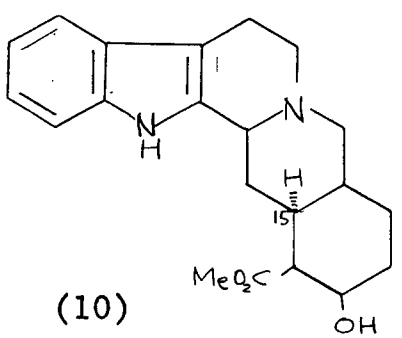
SPF unit(9)



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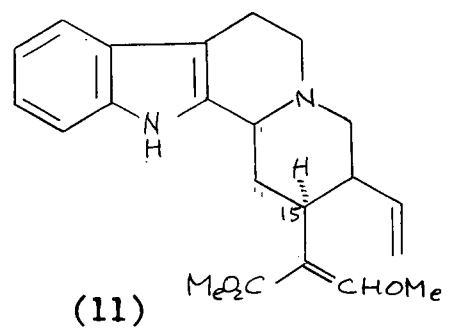

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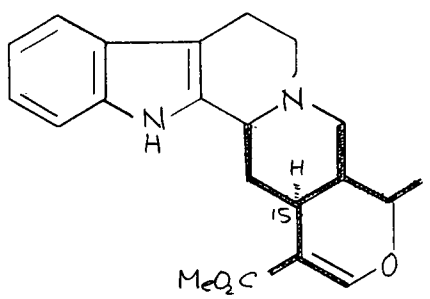

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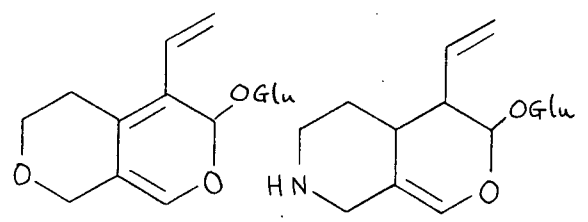

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Figure 2

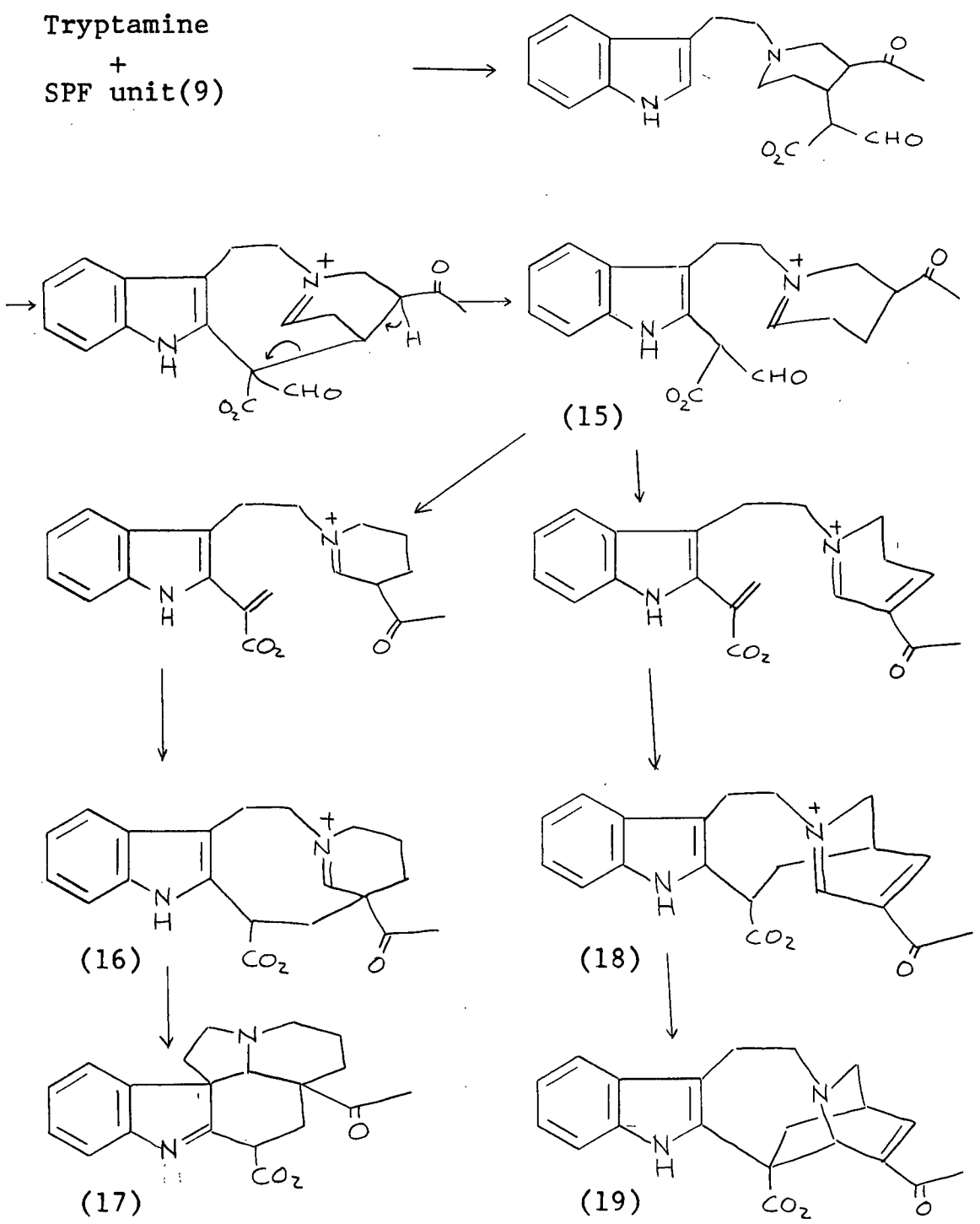


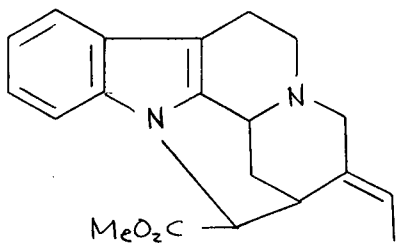
Figure 3

of the "non-tryptophan" portion of indole alkaloids. In this scheme, as shown in Figure 2, prephenic acid (7), known as a precursor of naturally occurring benzenoid compounds, is rearranged and dehydrated to give the intermediate (8), whose role in the biosynthesis of yohimbine (10) could be visualized as follows. Condensation of formaldehyde with the intermediate (8) followed by a retro-aldolization leads to a "seco-prephenate-formaldehyde" (SPF) unit (9) which could be incorporated into corynantheine (11) and ajmalicine (12). The occurrence of neutral plant constituents with a common SPF nucleus (e.g. genitiopicrin (13) and bakankosin (14)) seemed to lend support for the intermediacy of the SPF unit. An importnat feature of the prephenic acid theory was that it accounted for the almost consistantly observed configuration at C-15 in the indole alkalids.

Wenkert's theory also covers the biogenesis of the Aspidosperma and Iboga groups of bases[5]. Condensation of the SPF unit with tryptamine followed by a retro-Michael reaction provides the key product (15). As shown in Figure 3, this cleavage product (15) leads to both the Aspidosperma (17) and Iboga (19) alkaloids. Transannular cyclization reactions similar to the postulated conversions (16→17 and 18→19) have been performed in the laboratory[25, 26,27,28].

The prephenic acid theory has also been extended to cover the biogenesis of Akuamma and Strychnos alkaloids[21].

The new structural patterns found in pleiocarpamine (21)[19] and picraline (22)[20] offer a possible solution to the biogenesis of the Akuamma (24) and of the Strychnos (26) bases, as illustrated in Figure 5.



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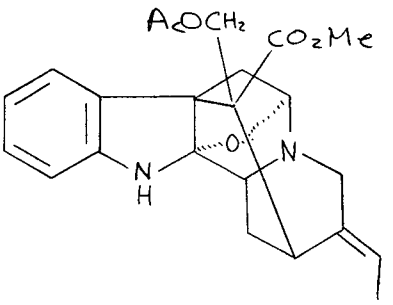

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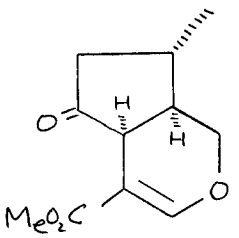

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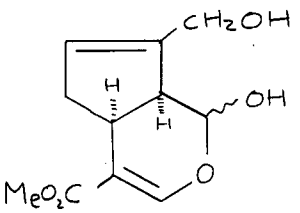


(28)

Figure 4

Although there has been no direct proof of Wenkert's prephenic acid theory, it should be noted that the carbon skeleton of the SPF unit (9) is identical, except for one carbon atom, with that proposed in the monoterpene hypothesis (32) outlined below.

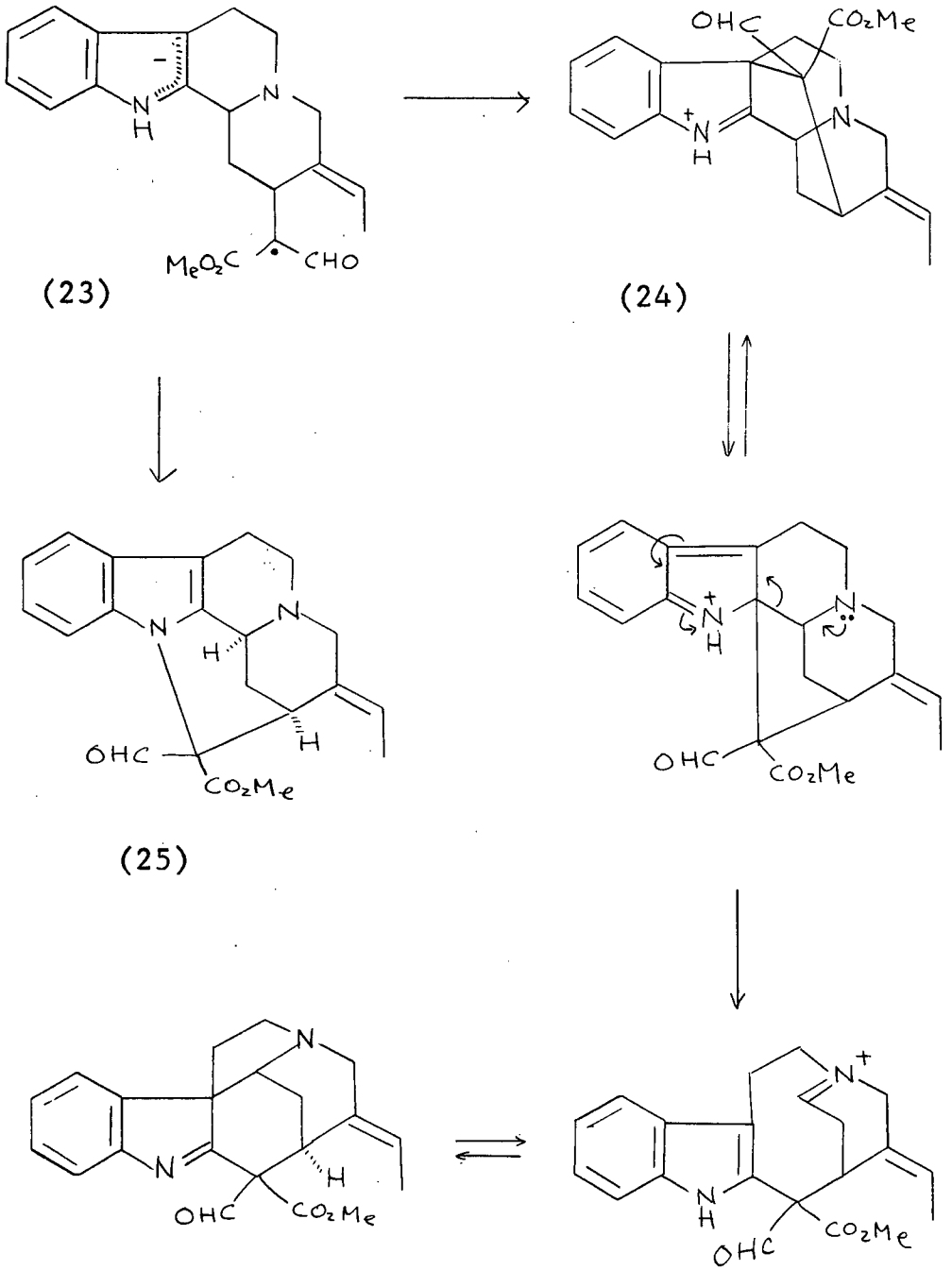


Figure 5

The Monoterpene Hypothesis

Thomas[4] and Wenkert[5] noted the structural similarity between the "non-tryptophan" portion of indole alkaloids and monoterpenic glucosides, e.g. verbenalin (27)[54] and genipin (28)[53] and therefore postulated a monoterpene origin for the "non-tryptophan" portion of indole alkaloids. As shown in Figure 6, the C_{10} monoterpene unit (31) cleaved along the dotted line provides a carbon skeleton very similar to the SPF unit (9). This skeleton (32) and two others obtained by rearrangements (33) and (34) are shown by the thickened bonds in ajmalicine (12), catharathine (5) and vindoline (4), respectively.

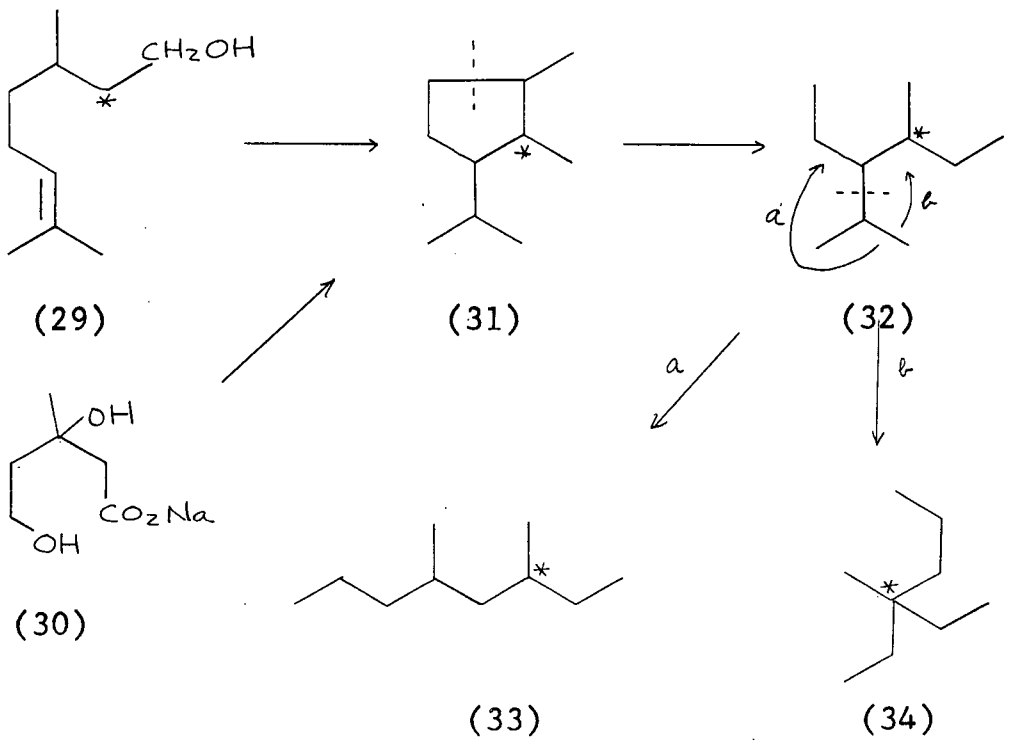


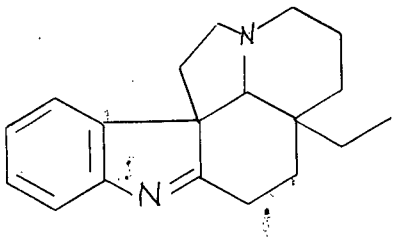
Figure 6

The transformation reactions required to produce these rearranged skeletons and the final alkaloids are similar to those elaborated in the prephenic acid hypothesis. The essential difference between the two theories being in the origin of the initial C_{10} unit, and in explaining the derivation of yohimbine-type alkaloids. In the prephenic acid theory these appear at the very beginning whereas in the monoterpene hypothesis they are at the very end of biogenetic development.

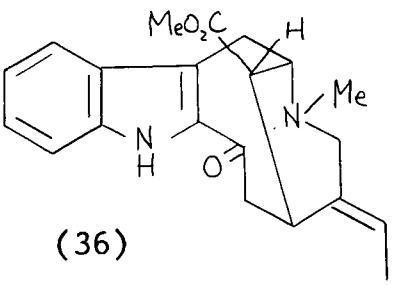
Biosynthesis of the "Non-Tryptophan" Portion of Indole Alkaloids (Yohimbe-, Corynanthe-, Aspidoperma-, Iboga-type)

Recent tracer experiments by several groups have provided very strong evidence for a monoterpene origin. Mevalonate (30), a known precursor of terpenes, has been shown to be incorporated into the "non-tryptophan" portion of vindoline (4)[6,7,8], serpentine (3)[6,10], catharanthine (5)[6], 1,2-dehydroaspidospermidine (35)[6], ajmalicine (12)[6,9] and reserpene (6)[8].

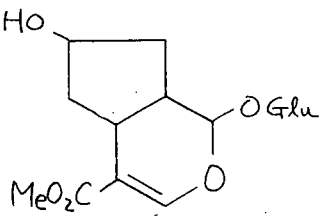
A monoterpene, geraniol (29), was shown to be a precursor of representative examples of the Corynanthe, Iboga and Aspidosperma groups of bases[9,10,11]. Battersby and co-workers[10] reported the incorporation of [2-^{14}C] geraniol into ajmalicine (12), serpentine (3), catharanthine (5), vindoline (4), and radioactive loganin (37)[12] into catharanthine (5), vindoline (4), perivine (36), serpentine (3) and ajmalicine (12) in Vinca rosea plants.



(35)



(36)



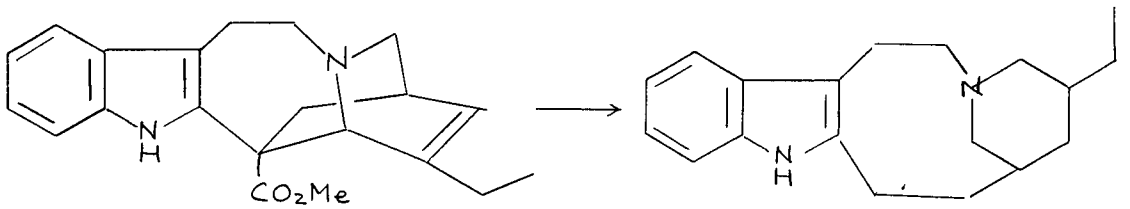
(37)

Figure 7

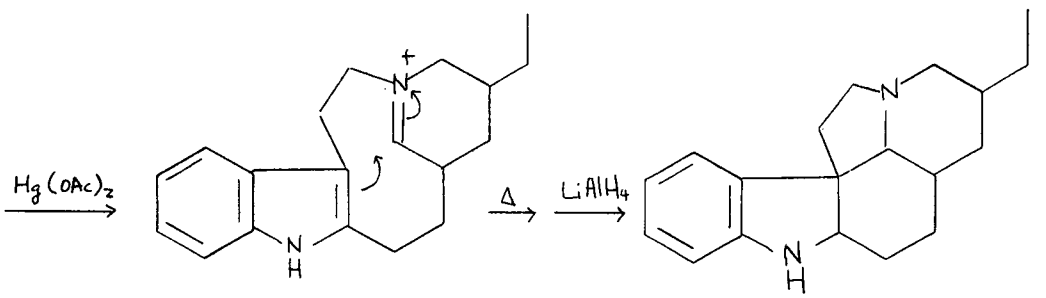
Independently, geraniol (39) was reported to be incorporated into the "non-tryptophan" portion of vindoline (4) by Scott and co-workers[11] and also by Arigoni and co-workers[9] in Vinca rosea plants. Degradation of the radioactive alkaloids fully supported the biogenetic scheme shown in Figure 6.

Biogenetic-type Reactions Leading to Iboga- and Aspidosperma-alkaloids

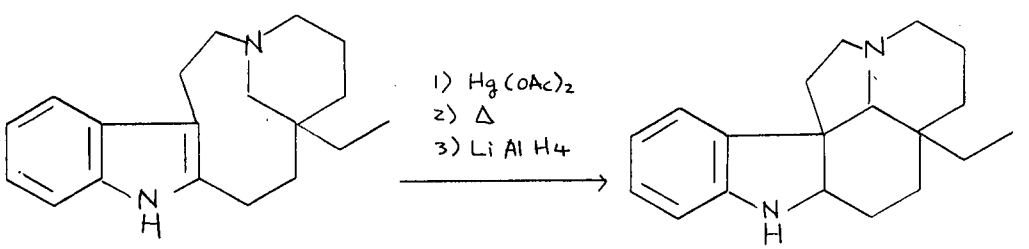
The in vitro conversion of 9-membered ring intermediates to the Iboga and Aspidosperma skeletons (see compounds (16) and (18) in Figure 3) by transannular cyclization reaction was demonstrated by Kutney and co-workers[25,26,27,28]. As Figure 8 illustrates, the Aspidosperma skeleton present in both 7-ethyl-5-desethyl-



(5) (38)



(39)



(40) (41)

Figure 8

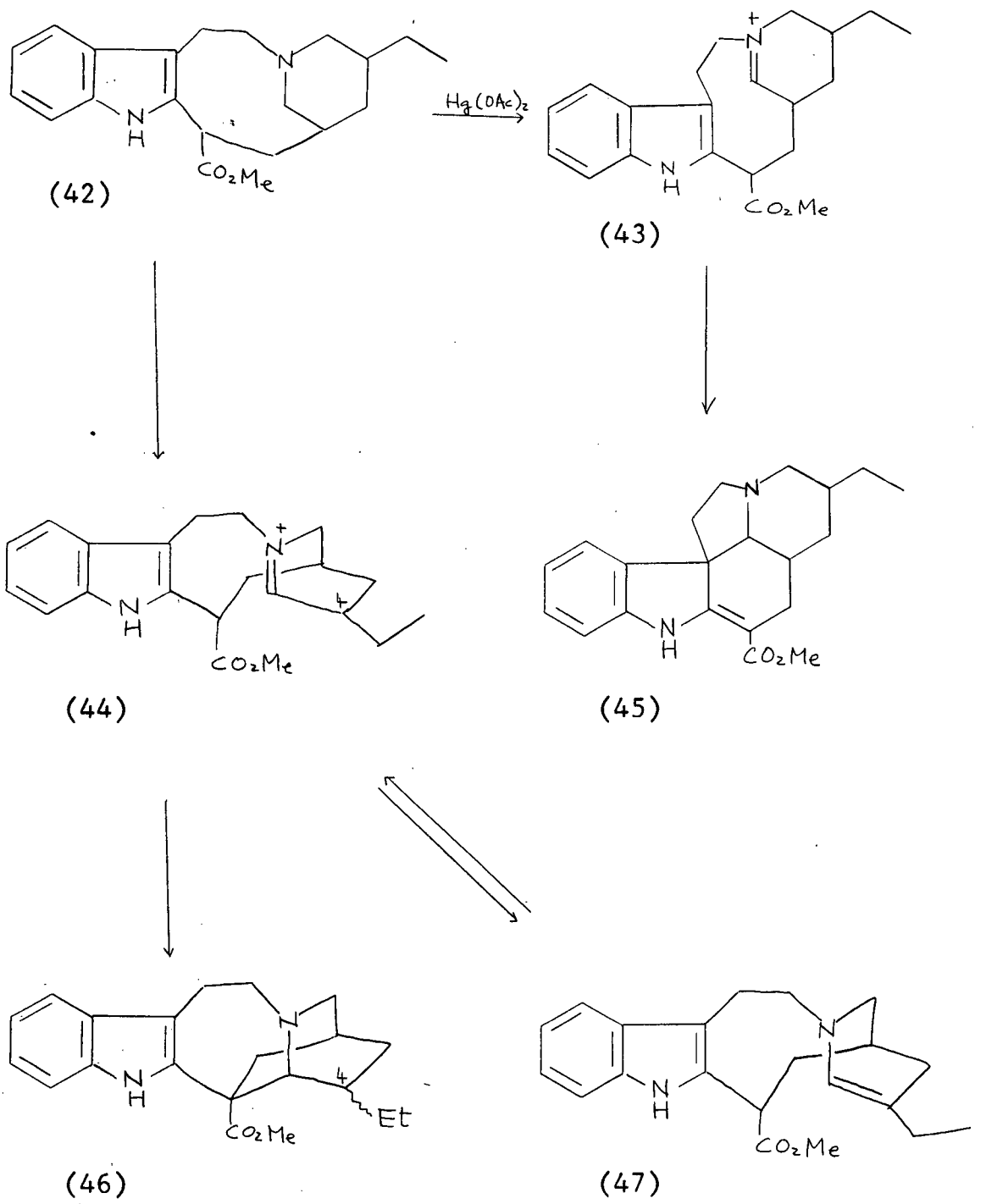
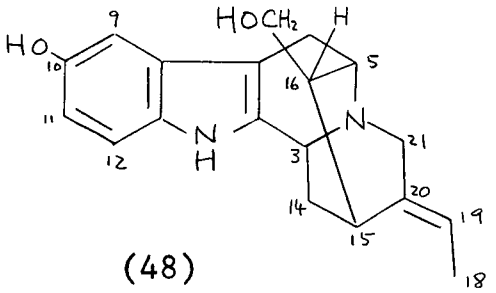


Figure 9

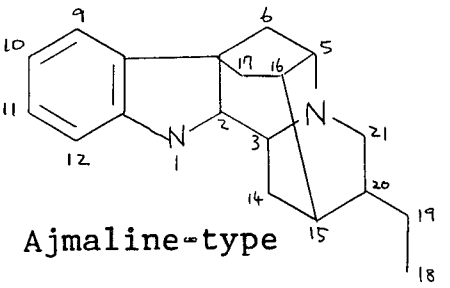
aspidospermidine (39)[25] and (+)-aspidospermidine (41)[26] was constructed from dihydrocleavamine (38) and (-)-quebrachamine (40), respectively. Carbomethoxydihydro cleavamine (42) was also induced to cyclize to yield both the Aspidosperma-[27] and Iboga-type[28] alkaloids (Figure 9). Mercuric acetate oxidation of carbomethoxy-dihydrocleavamine (42) to a mixture of iminium salts (43) and (44), followed by transannular cyclization, provided pseudo-vincadifformine (45) and the Iboga derivatives (46). Both coronaridine (46, β-Et at C-4) and dihydrocatharanthine (46, α-Et at C-4) were isolated since isomerization at C-4 via the enamine (47) occurs during the reaction. This transannular cyclization was thus shown to be a versatile synthetic entry to the Aspidosperma and Iboga alkaloids.

A Biogenetic Theory of Vobasine-, Sarpagine- and Ajmaline-type Bases

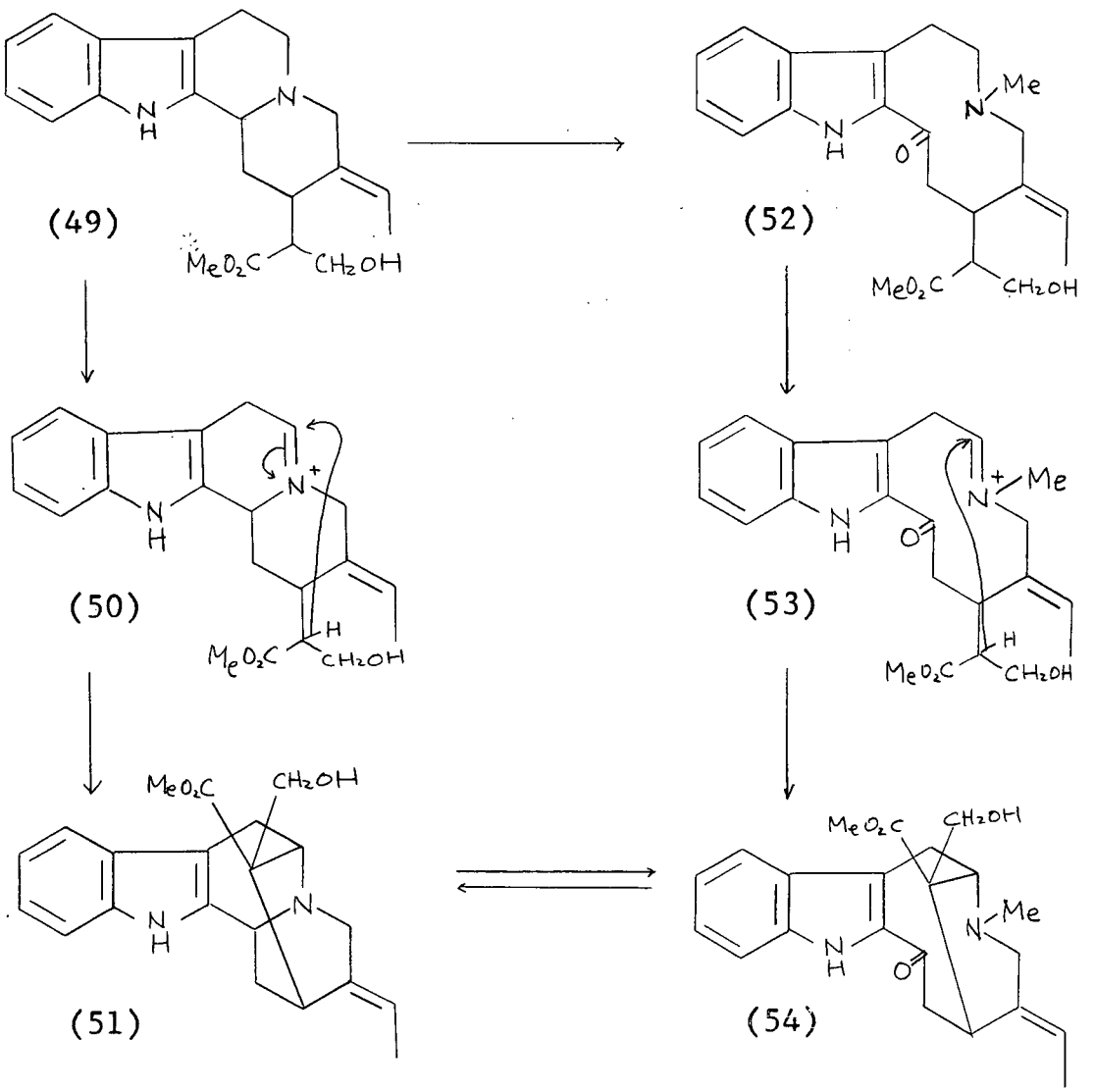
It is quite obvious from structural similarities, that the corynanthenoid skeleton could be related to pentacyclic alkaloids such as sarpagine (48) and then to hexacyclic bases such as ajmaline (2). Interconversion between sarpagine- and ajmaline-like bases seemed to be possible in the plant and this interconversion was simulated in a laboratory as will be discussed later. However it can readily be seen there exist at least two possible routes for the biogenesis of sarpagine-like bases from a corynantheine-type molecule.



(48)



Ajmaline-type

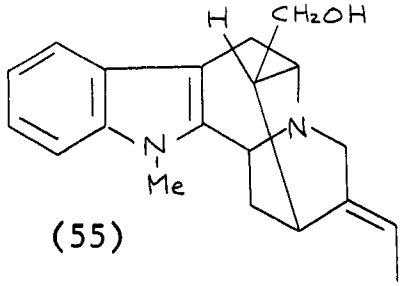


(49) (50) (51) (52) (53) (54)

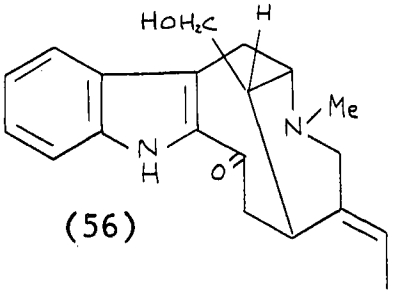
Figure 10

As shown in Figure 10, isositsirikine (49)[17] could be appropriately oxidized to the iminium salt(50) [3] and the subsequent attack of an anion at C-16 via transannular cyclization would lead to akuammidine (51). Quaternization of akuammidine (51)[13], followed by oxidation at C-3 and ring opening could give rise to a 2-acylindole alkaloid, vincadiffine (54)[15].

Alternatively, biogenesis of a 2-acylindole base followed by ring closure to a sarpagine-type base can be visualized. The formation of 3,4-seco base (52) from isositsirikine (49) followed by a transannular cyclization of the iminium salt (53) could yield vincadiffine (54), which, upon ring closure, could furnish a sarpagine-type base (51). The direct biogenetic relationship between these classes of alkaloids draws support from the observed co-occurrence of the sarpagine-type (i.e. affinisine (55)) and of the vobasine-type (i.e. affinine (56)) alkaloids in the same plant, as reported by Cava et al[34].



(55)



(56)

As far as the feeding experiments are concerned, radioactive loganin (37) was reported bo be specificly incorporated into perivine (36) by Battersby and co-workers[12].

Some Reactions of Biogenetic Interest in Sarpagine-, Vobasine- and Ajmaline-type Bases

A considerable amount of work has been done on laboratory synthesis of these types of indole alkaloids. Correlations between sarpagine- and ajmaline-type bases have been tried by Bartlett et al[22], who reported that deoxyajmalal-A(57), upon treatment with strong acid followed by reduction, gave deoxyajmaline (58). This ring closure was also supported by the conversion of tosylated deoxyajmalol-A (60) to 2-hydroxydideoxyajmaline (61) at room temperature (Figure 11).

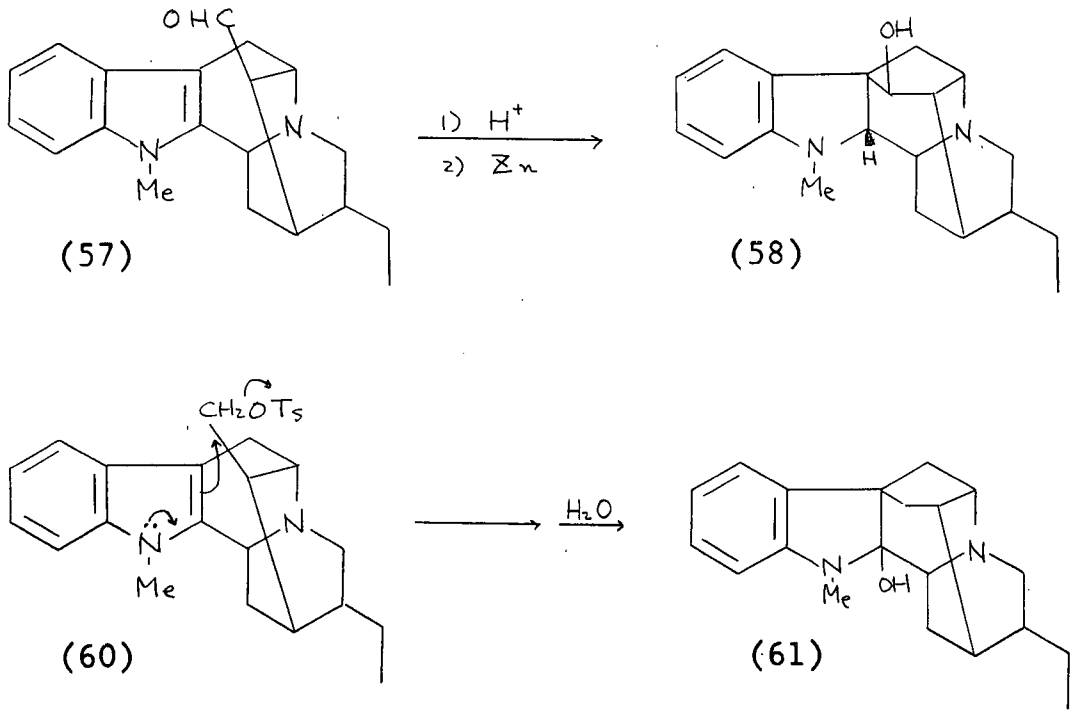
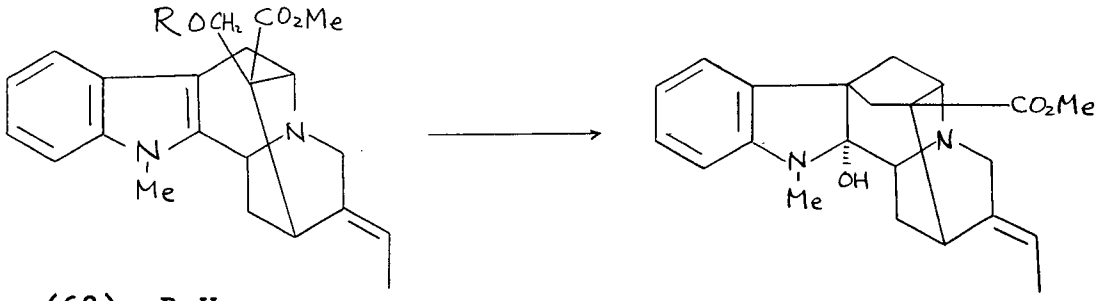


Figure 11

A similar ring closure was reported by Martin et al[23] in the case of voachalotine (62). Methanesulfonyl chloride, p-toluenesulfonyl chloride and thionyl chloride reacted with voachalotine (62) to give, in each case, the same cyclized dihydroindole compound: 2-hydroxy-17-deoxy-vincamajine (63) (Figure 12).



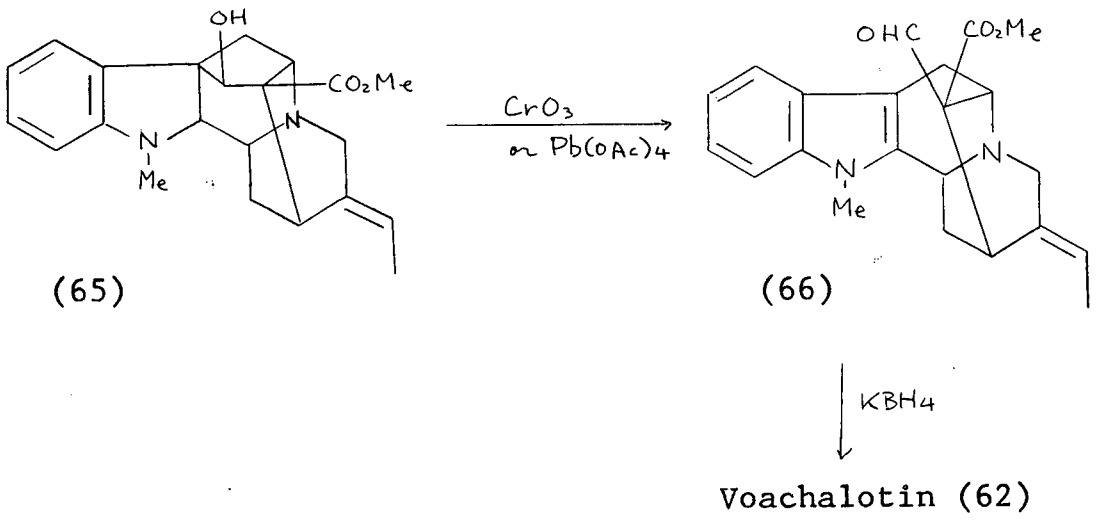
(62), R=H
R=Mes,Ts,ClSO

(63)

Figure 12

The reverse reaction is also possible and has been utilized for the degradation of ajmaline-like alkaloids. As shown in Figure 13, oxidation of vincamajine with chromium trioxide or lead tetraacetate in pyridine gave an indole aldehyde(66), which on reduction furnished the hydroxy ester, voachalotine (62)[24].

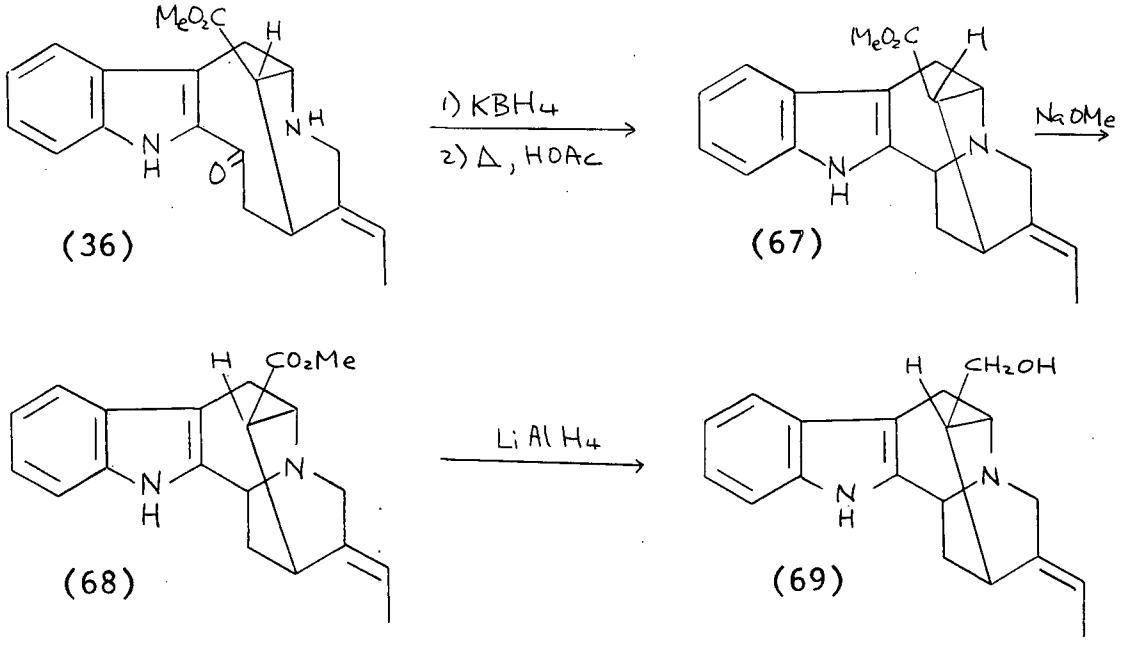
Attempts have also been made to correlate directly between the potentially related pentacyclic sarpagine- and tetracyclic 2-acylindole types. For example, the conversion of perivine (36) into normacusine B (69) was reported by Gorman et al[31] (Figure 14).



(65) (66)

Figure 13

Pericyclivine was isolated during the phytochemical investigations of Gabunia odoratissima[32] and Catharanthus lanceus[33] and found to have the structure (67), a compound previously obtained from perivine (36).

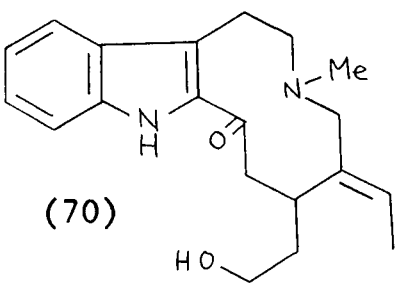


(36) (67)

(68) (69)

Figure 14

A similar ring closure was accomplished in the case of picraphylline, a tetracyclic 2-acyindole compound which was isolated from Picralima nitida. The conversion of this alkaloid to tetrahydroalstonine confirmed the structure and sterochemistry of the former[35,36]. Reduction of picraphylline (71) with potassium borohydride produces an equimolar mixture of epimeric picraphyllinols whose N_b-methochlorides were prepared in the usual manner. Pyrolysis of the methochloride at 280° yielded a single compound identified as tetrahydroalstonine (72).



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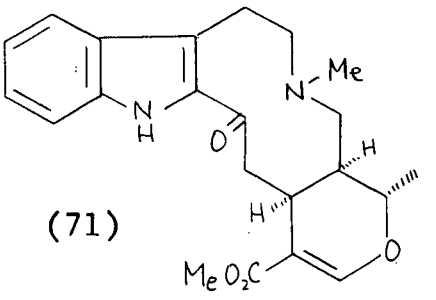
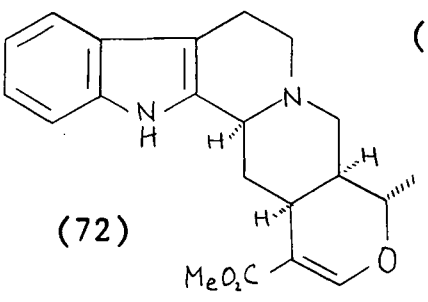


(71)



(72)

It is of interest that N_b-methyltertrahydroalstonine (melinonine A[37]) was also isolated from this plant specimen.

As discussed previously (see Figure 10, 49→52), the reverse reaction (72→71), could also be of significance for the biogenesis of 2-acylindole alkaloids (70)[38] and (71). Dolby and Sakai[39] successfully demonstrated the conversion of dihydrocorynantheine (73) to a tricyclic 2-acylindole,

dihydroburnamicine (77). As shown in Figure 15, dihydrocorynantheine (73) was converted to 7-acetoxy-7H-dihydrocorynantheine by the action of lead tetraacetate. The methiodide (74) of the acetoxyindolenine was treated with boiling acetic acid containing sodium acetate. Extraction from strongly alkaline solution gave 3-keto-3,4-seco-N_b-methyl-dihydrocorynantheine (75), which upon hydrolysis followed by decarboxylation gave the ketoaldehyde(76).

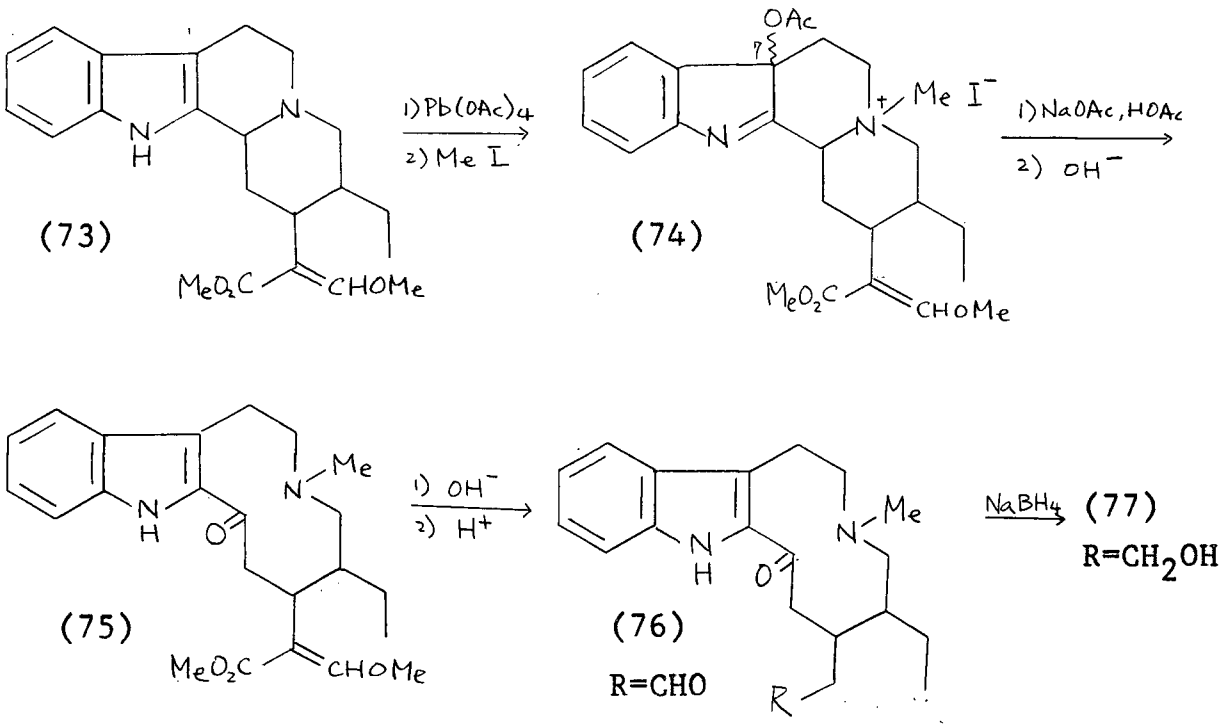


Figure 15

Reduction with sodium borohydride in aqueous methanol yielded dihydroburnamicine (77). This synthesis by Dolby and Sakai was the first reported attempt to obtain the

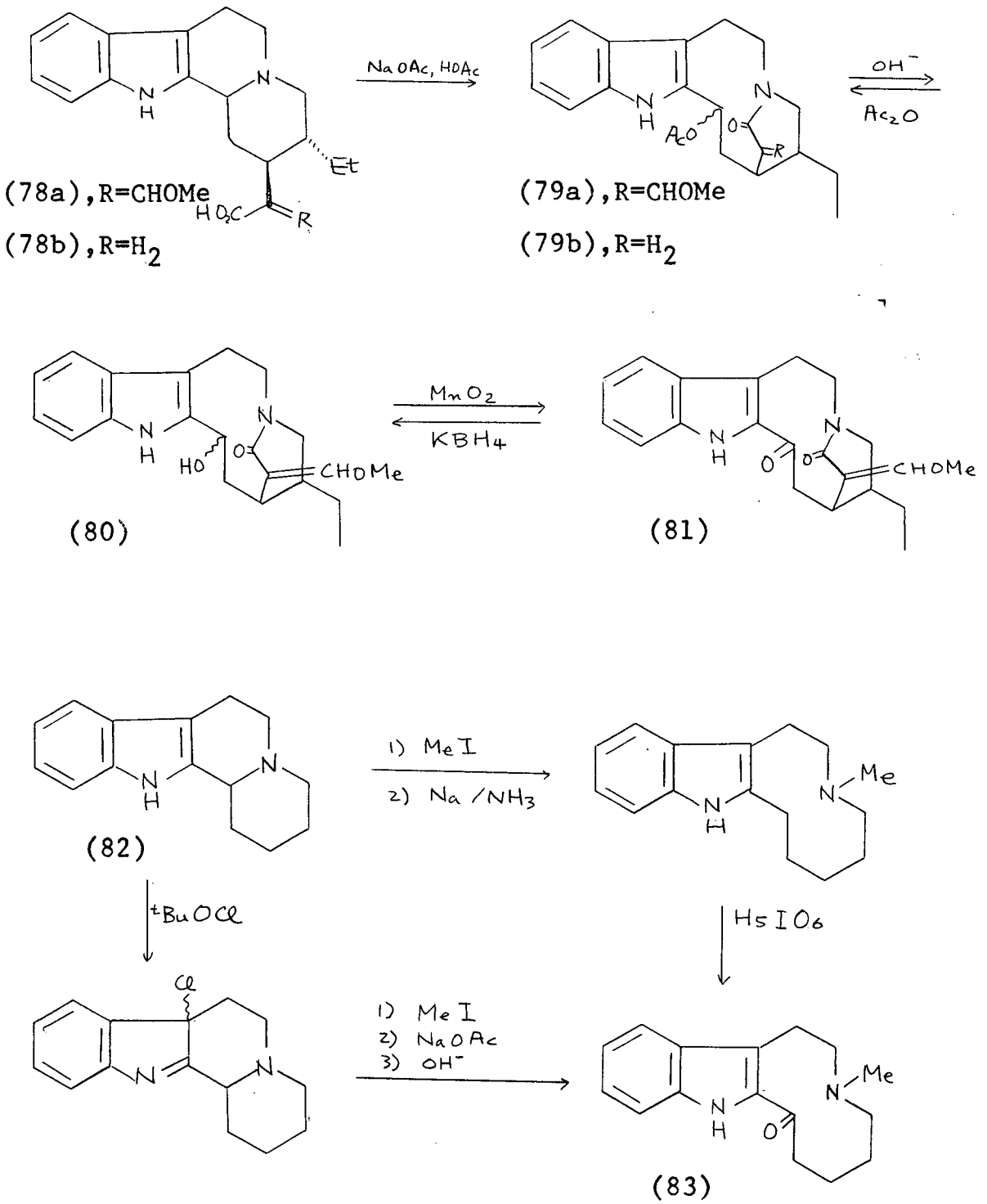

NaOAc, HOAc
OH⁻
Ac₂O
(78a), R=CHOMe
(78b), R=H₂
(79a), R=CHOMe
(79b), R=H₂
MnO₂
KBH₄
(80)
(81)
1) MeI
2) Na/NH₃
(82)
tBuOCl
H₅IO₆
1) MeI
2) NaOAc
3) OH⁻
(83)

Figure 16

2-acyl moiety in indole alkaloids. The same authors[39] also reported the following sequence summarized in Figure 16. Compounds (78a) and (78b), derived from natural dihydro-corynantheine, on treatment with acetic anhydride-sodium acetate yielded the corresponding acetoxylactams (79a) and (79b). Saponification of (79a) yielded the alcohol (80), which reverted to (79a) upon acetylation or was converted to the 2-acylindole (81) on oxidation with manganese dioxide. Attempted preparation of a tricyclic base by hydrolysis of the lactam (81) was not successful.

Very recently, Dolby and Gribble reported other ways of synthesizing 2-acylindoles[52]. They have studied the conversion of the tetracyclic base (82) into the tricyclic ketone (83) by two independent routes. One route is similar to the sequence leading to the synthesis of dihydro-burnamicine (77)[39] and the other includes the direct oxidation of a 10-membered ring (3,4-seco base). The key product of the first sequence is an epimeric mixture of β-chloroindolenines which were alkylated with methyl iodide to form methiodides. The methiodide mixture was treated with sodium acetate in aqueous ethanol and then basified with sodium hydroxide to give the desired tricyclic ketone (83). The second route included Birch reduction on the methiodide of tetracyclic base (82) to furnish 3,4-seco base which was then subjected to periodic acid oxidation to give the tricyclic ketone (83). This oxidation is attractive from the synthetic point of view, since the

introduction of a reactive ketone function can be achieved in the later stage of the synthesis of 2-acylindole alkaloids.

Recently Harley-Mason et al[40] reported briefly a reaction which introduced an acetoxyl at C-3 of the hexahydroindolopyrrocoline (84). As shown in Figure 17, the acetoxyl was converted to a ketone by mild alkaline hydrolysis followed by manganese dioxide oxidation.

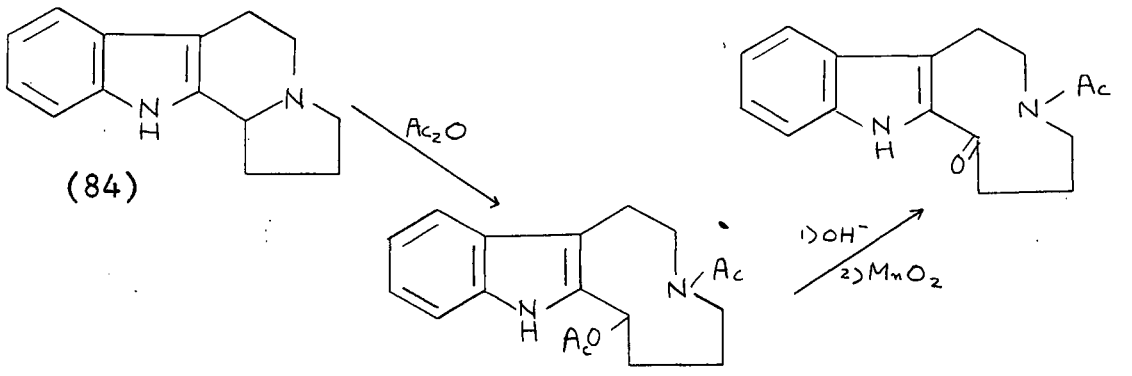


Figure 17

A similar cleavage by acetic anhydride was applied to the preparation of some indolobenzazonines (85, 86) by Freter et al[42], after they observed that treatment of 1-phenyl-2-methyltetrahydro-β-carboline (87) with acetic anhydride gave the 1,2-seco compound (88)[41] (Figure 18).

In addition to these syntheses of 3-keto-3,4-seco bases, preparations of 9- and 10-membered ring compounds by a cleavage of the C/D ring are of particular interest from a synthetic point of view. Hence, the methods now

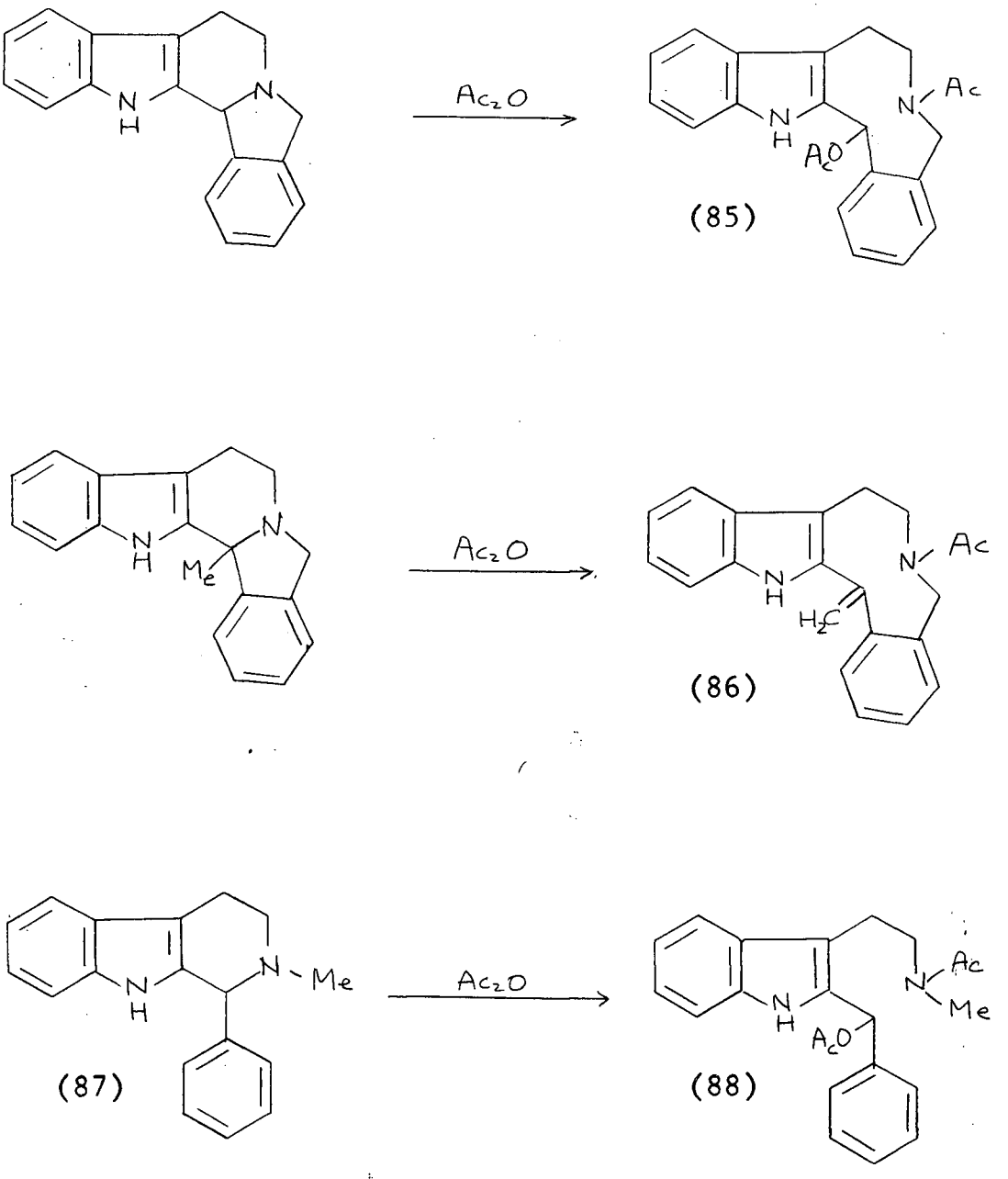
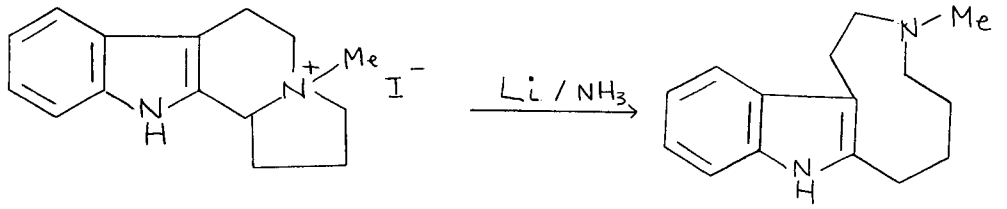


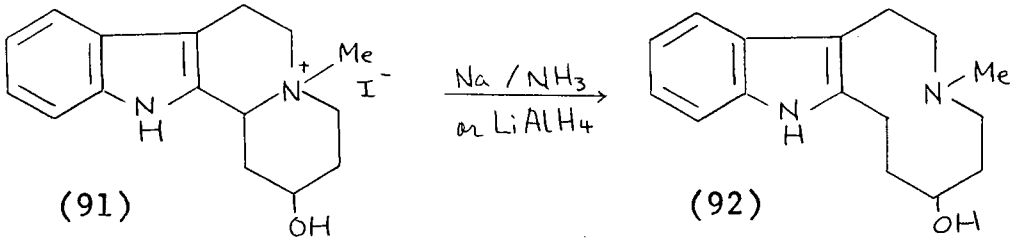
Figure 18

avaiable are reviewed below. Wenkert and co-workers[43] developed a unique synthesis of a quebrachamine model (90) by Birch reduction of the indolopyrrocoline system (89).



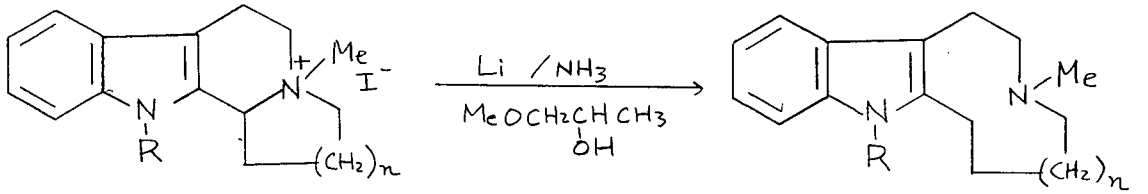
(89) (90)

Dolby and Booth[44] reported the C/D ring cleavage of the octahydro-2-hydroxyindolopyridocoline (91) by lithium aluminum hydride reduction or by Birch reduction to furnish a 10-membered ring compound (92).



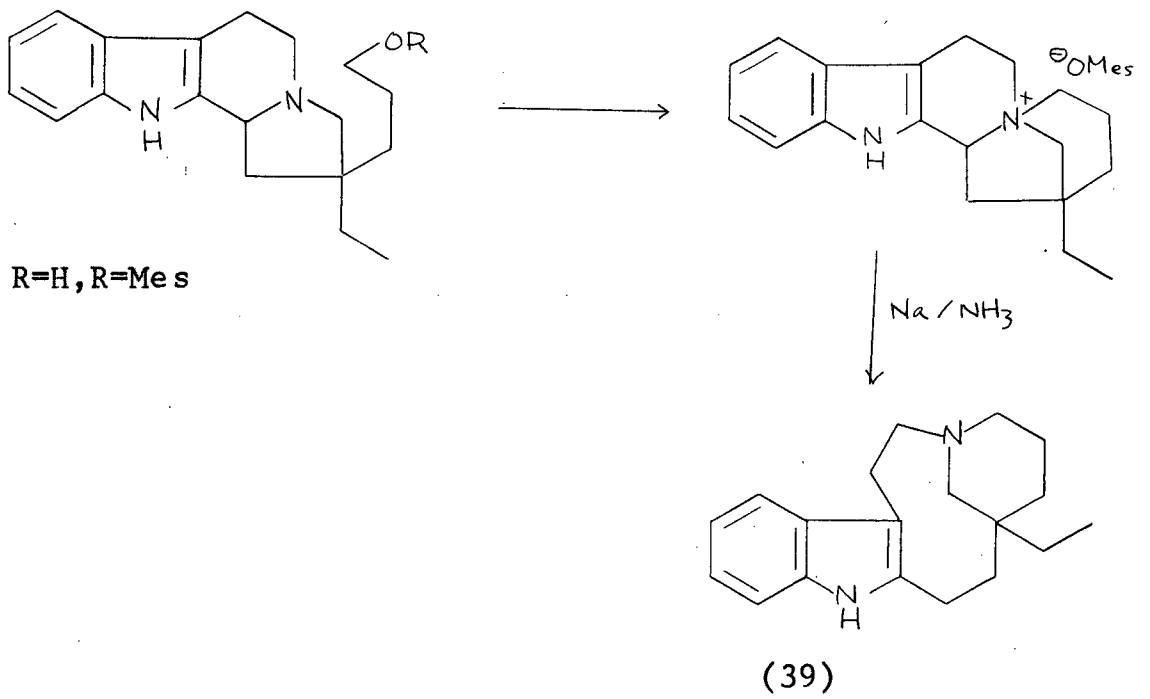
(91) (92)

Herchel Smith and co-workers[45] independently studied the metal-ammonia reduction of hexahydro-4-methylindolo-indolizinium iodide (93) and octahydro-5-methylindolo-quinolizinium iodide (94) in great detail. Optimum yields were obtained by using slightly more than 2 equiv. of lithium and 1 mole of 1-methoxy-2-propanol in liquid ammonia (Figure 19).



(93),n=1 (R=H,Me)
(94),n=2 (R=H,Me)

Figure 19


OR
R=H, R=Mes
⊖OMes
Na / NH3
(39)

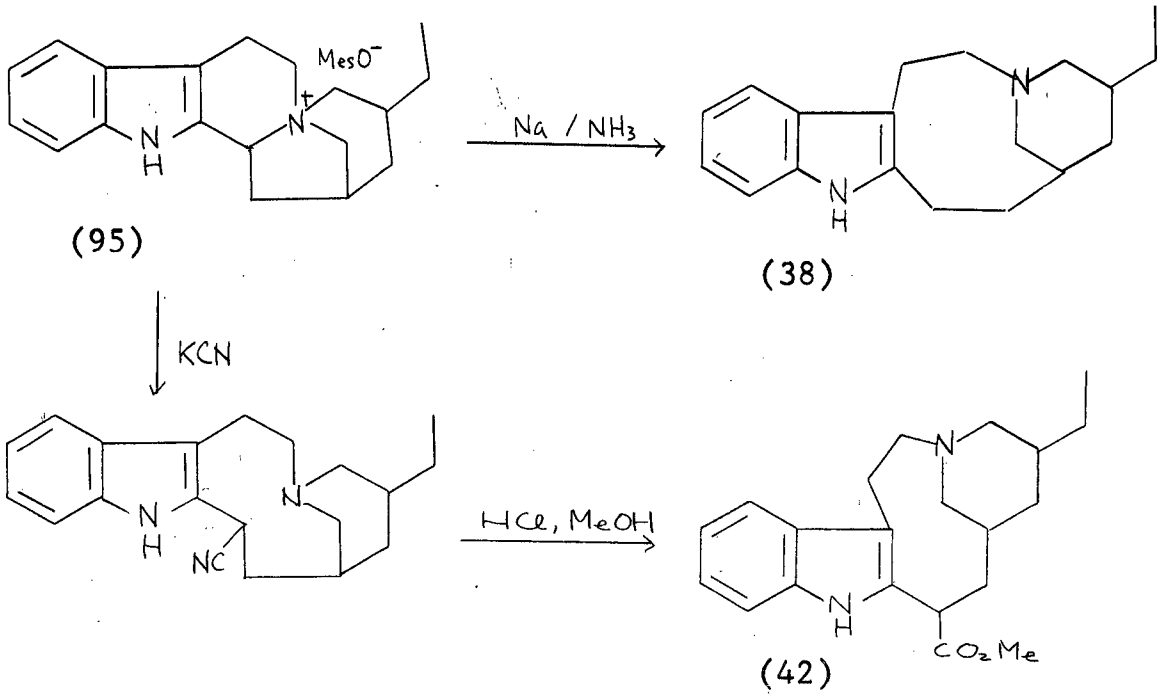

MesO⁻
(95)
Na / NH3
(38)
KCN
NC
HCl, MeOH
CO2Me
(42)

Figure 20

The application of the metal-ammonia reductions mentioned above was the important key to the total syntheses of several alkaloids, quebrachamine[29] (39), dihydrocleavamine [30,46] (38) and carbomethoxydihydrocleavamine[30,47] (42) (Figure 20). Introduction of a carbomethoxy group at C-3 was achieved by treatment of the quaternary mesylate (95) with potassium cyanide followed by hydrolysis of the nitrile with methanolic hydrogen chloride to give (42).

Other possible ways of preparing 3,4-seco- and 3-keto-3,4-seco-corynanthenoid bases could come from applications of carbon-nitrogen hydrogenolysis of allylic quaternary salts reported by Harley-Mason et al[48] or from application of a reaction similar to the ring opening of a 1-azabicyclo-alkane to a 9-membered ring amine as demonstrated by Reinecke et al[50].

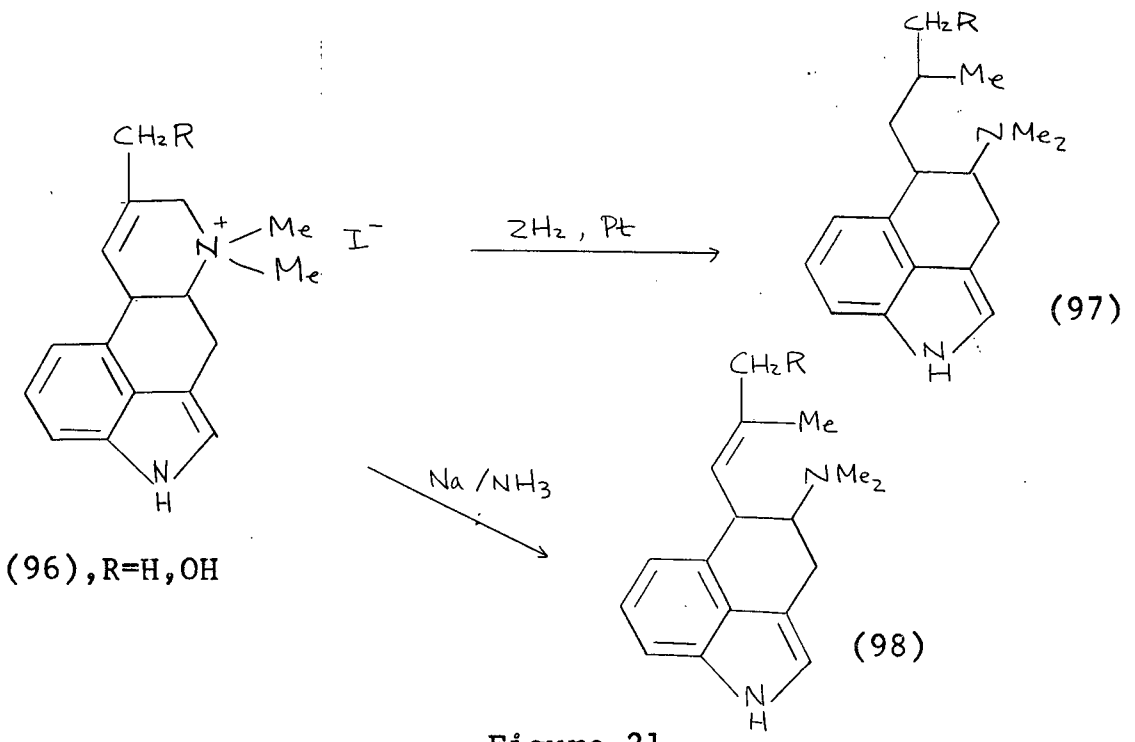
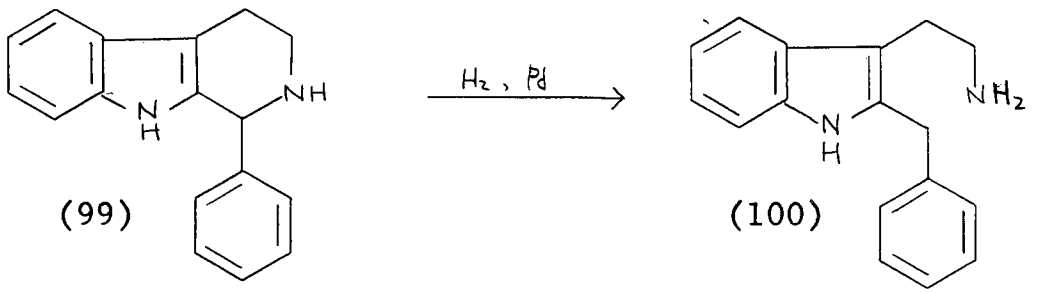


Figure 21

Harley-Mason et al[48] reported that the hydrogenation of agroclavine methiodide (96, R=H) and elymoclavine methiodide (88, R=OH) over Adam's catalyst gave the seco-compound (97, R=H and R=OH, respectively) after two molecules of hydrogen had been smoothly absorbed. As shown in Figure 21, not only the reduction of the double bond but also allylic carbon-nitrogen hydrogenolysis had occurred. However, an Emde reduction of agroclavine and elymoclavine methiodides with sodium and liquid ammonia to furnish (98, R=H and R=OH, respectively) was reported by Birch and co-workers[49].

It should be noted that hydrogenolysis of N-benzyl derivatives to generate the corresponding bases (NH) is well known. The catalytic reduction reported by Freter et al[41] (Figure 22) appears to be the first example where the nitrogen atom and the benzylic bond are in the same 6-membered ring.

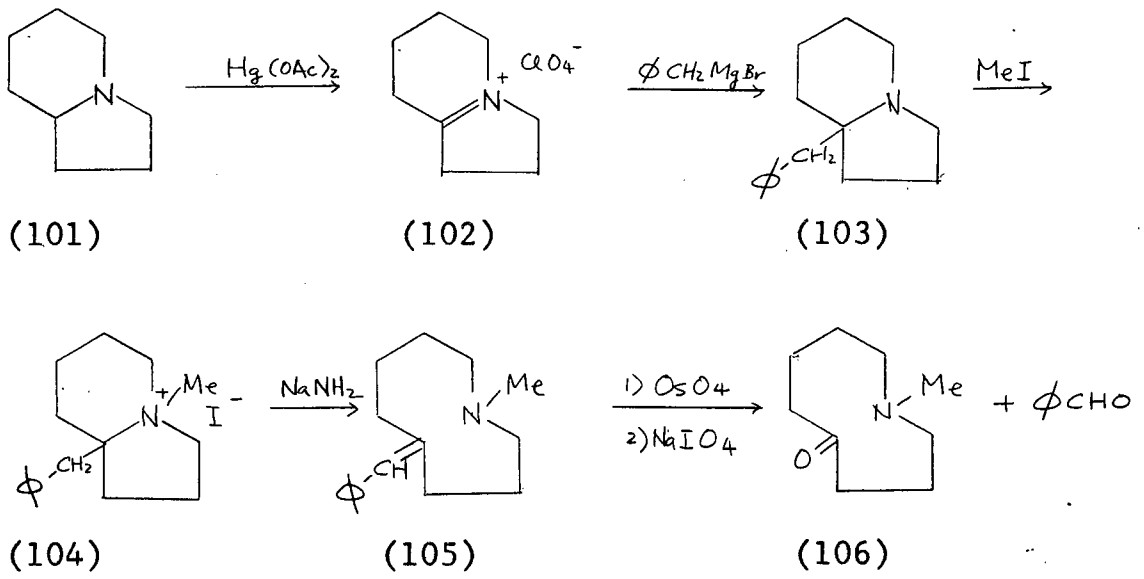


(99) (100)

Figure 22

This hydrogenolysis could be applied to the preparation of 3,4-seco compounds in appropriate indole alkaloids.

Reinecke et al[50] investigated the synthesis of the 9-membered ring aminoketone (106).



(101) (102) (103)

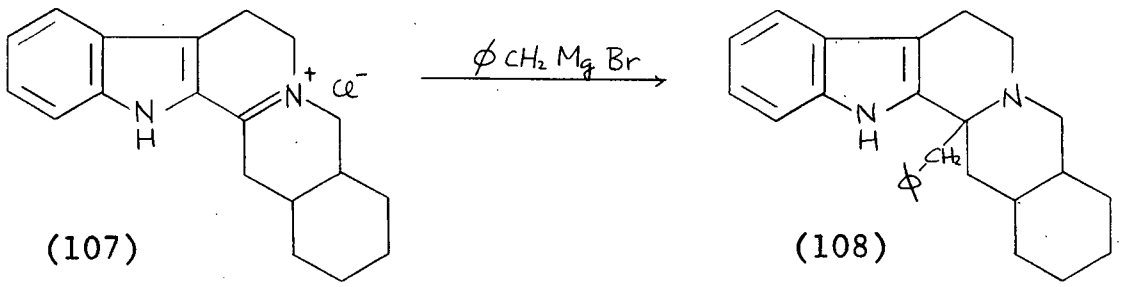
(104) (105) (106)

Figure 23

As shown in Figure 23, reaction of benzyl magnesium bromide with the iminium salt (102), obtained by mercuric acetate oxidation of the 1-azabicycloalkane (101), led to the tertiary amine (103) whose quaternary salt (104) underwent an internal elimination to give the 9-membered ring amine (105). The oxidation (OsO_4-$NaIO_4$) of the amine (105) gave benzaldehyde and an aminoketone (106).

This Grignard reaction has been used by Zinnes et al[51] (Figure 24) to prepare 3-benzylyohimbane (108). Mercuric acetate oxidation of yohimbane yielded the expected 3-dehydroyohimbane salt (107). Reaction of the latter with benzylmagnesium bromide gave 3-benzylyohimbane (108) as the major product. The methiodide of 3-benzylyohimbane

would be expected to give a 10-membered ring upon treatment with base in analogy with Reinecke's sequence and subsequent oxidation (OsO_4-$NaIO_4$) could furnish 3-keto-3,4-seco-yohimbane.



(107) (108)

Figure 24

It can be seen that although several groups of workers have investigated some of the reactions described in Figure 10, no reports concerned with bridge formations between C-15 and C-5 via transannular cyclizations have as yet appeared. Therefore it was of great interest to investigate the possibility of transannular cyclizations on appropriate corynanthenoid-type bases including the 3,4-seco derivatives. These reactions would be of interest not only as biosynthetic models but also as a part of possible synthetic approaches to sarpagine- and vobasine-type alkaloids. This part of the thesis will mainly be concerned with the synthesis of corynantheine-like bases, the generation of iminium double bonds between N_b and C-5 (intermediates (50) and (53) in Figure 10) and with some attempted trasannular cyclizations.

DISCUSSION

The successful transannular cyclizations carried out by Kutney and co-workers[25,26,27,28] providing a general synthetic entry into Iboga and Aspidosperma alkaloids, prompted us to attempt chemical transformations of corynanthenoid-like alkaloids to sarpagine- and vobasine-type bases. This part of the thesis will be concerned with three different approaches to the ring closure of corynanthenoid-like alkaloids and these are summarized briefly below.

a) Oxidation of corynanthenoid bases by mercuric acetate is known to give predominantly the corresponding 3-dehydroiminium salt (109), which might be in equilibrium with the 5-dehydro- (110) and the 21-dehydroiminium salt (111). If a properly generated anion at C-16 would attack the iminium salt to accomplish a ring closure via a transannular cyclization, bond formation between C-16 and C-5 would result, giving the base (112). As shown in Figure 25, other possible bond formations (109 and 111) would yield 4-membered rings, which would be more strained than the 6-membered ring (112).

b) A second approach (Figure 26) to the synthesis of a properly oxidized compound was to block C-3 with a benzyl group in order to prevent the formation of a 3-dehydro

derivative, which is known to be the major product in the case of (a). The benzyl group also might provide a convenient entry into the 2-acyl indole family as described previously (Figure 23).

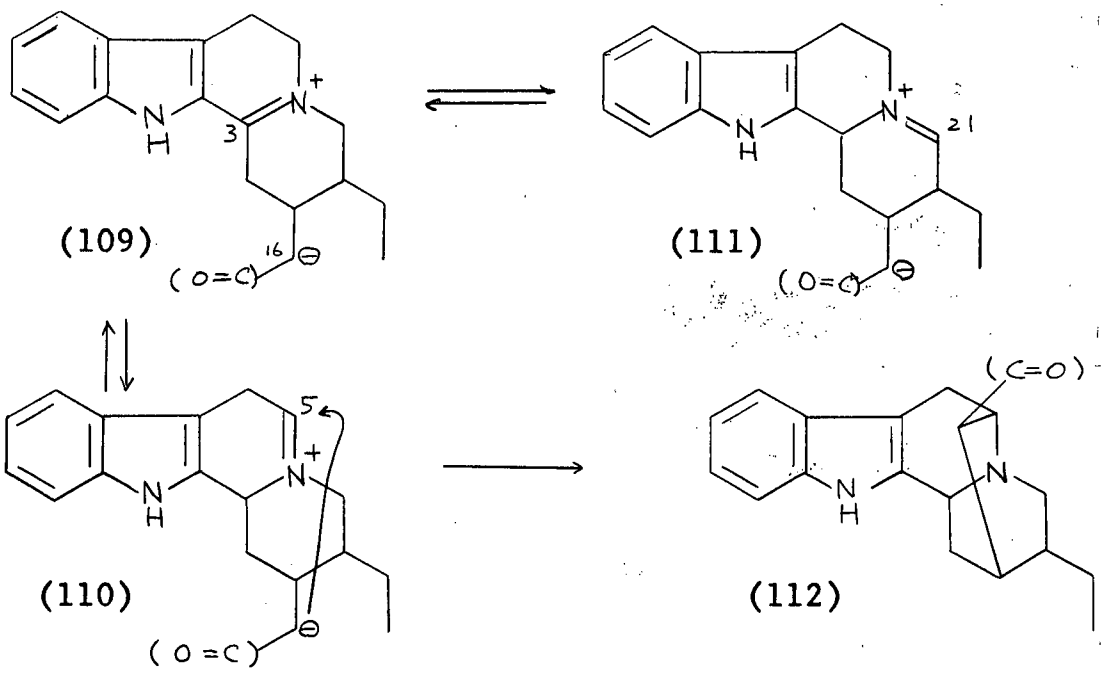


Figure 25

c) The third approach involved the C/D ring cleavage of a corynanthenoid base under Birch reduction conditions to give the corresponding 3,4-seco base, which could be oxidized, by mercuric acetate, to iminium salts (119) and (120). Subsequent attack of an anion at C-16 of the former (119) would give rise to a bridged compound whose skeleton is the same as that of a typical vobasine-type alkaloid (Figure 27).

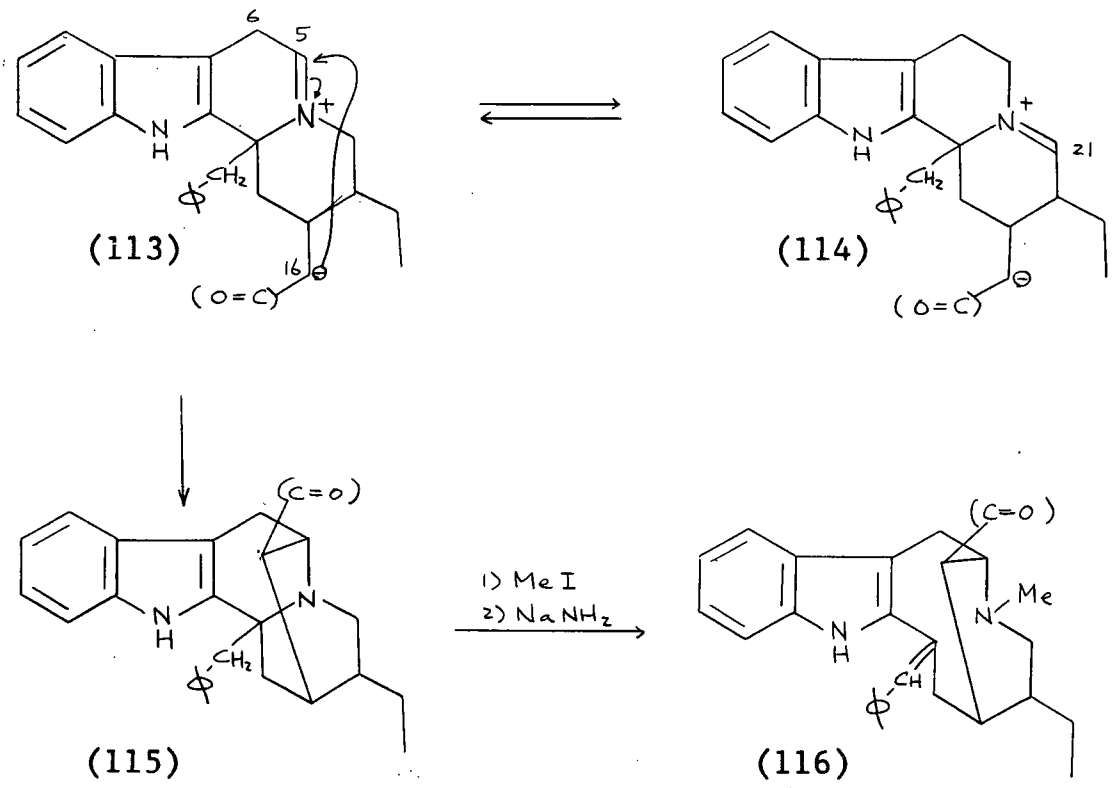


Figure 26

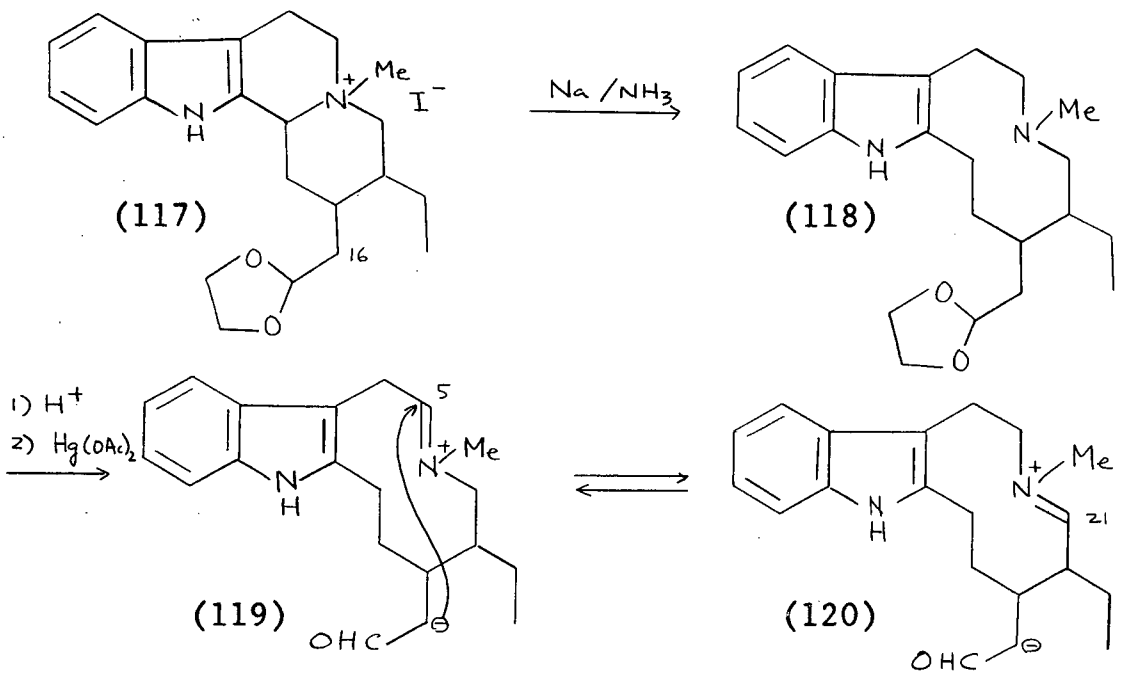


Figure 27

(a) Attempted Transannular Cyclizations of Corynantheine-like Bases

The first attempt of this type was made on sitsirikine (121)[17] by G. Eigendorf[62]. As summarized in Figure 28, mercuric acetate oxidation of sitsirikine (121) in acetic acid gave predominantly a 3-dehydro derivative (122) as shown by the UV spectrum[62].

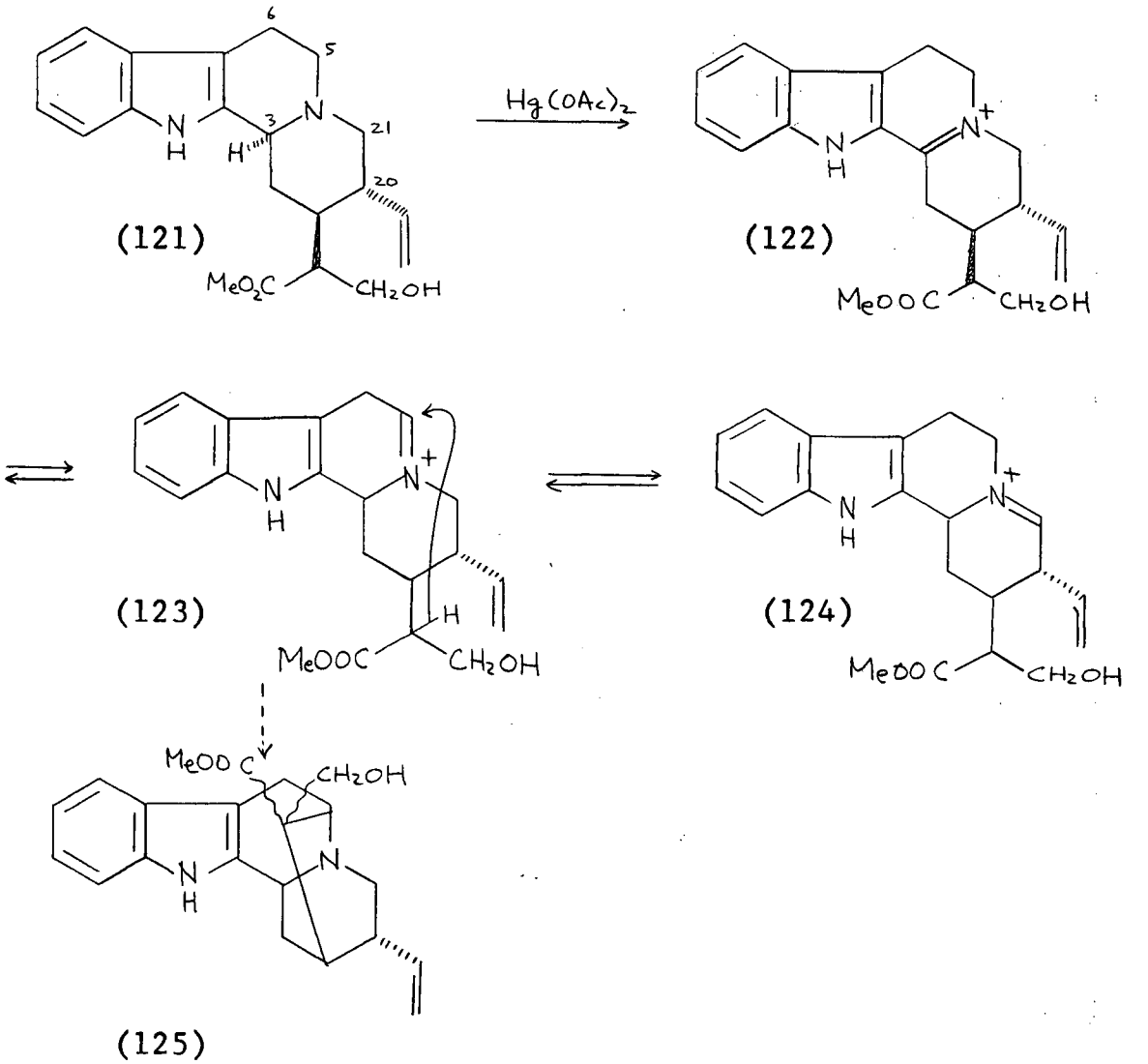
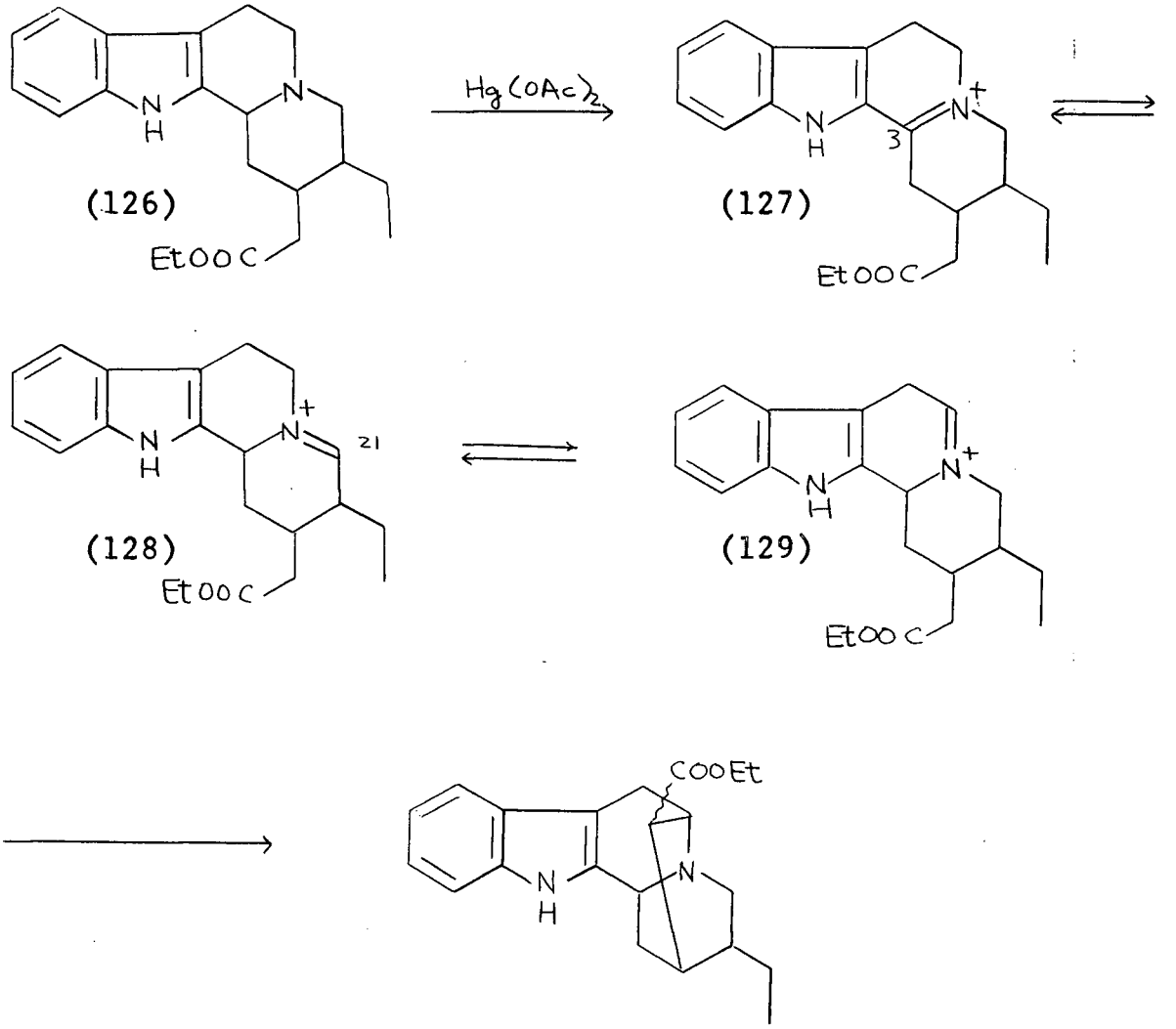


Figure 28

Prolonged stirring of the mixture and subsequent heating for 6 hours gave intractable mixtures (i.e. a tar under forcing conditions). It was concluded that the mercuric acetate oxidation gave mainly the expected iminium salt (122) and that the ring closure beween C-3 and C-16 (resulting in a 4-membered ring) was not favorable. The extent of formation of the iminium salts (123) and (124) either by direct oxidation or by equlibration with the 3-dehydro



derivative (122) was insufficient to detect any of the cyclized base (125). In the case of sitsirikine (121), the tertiary proton on C-16 might not be easily abstracted and this way provide an alternative explanation for the failure of the ring closure reaction.

Dihydrocorynantheic acid ethyl ester (126) was also investigated with regard to the above reaction. When this compound was subjected to mercuric acetate oxidation, the desired ring closure was again not successful. In a typical experiment which involved stirring the reactants at room temperature for 75 hours, a complex mixture of more than 14 compounds (as shown by TLC) was obtained.

(b) Attempted Ring Closures of Corynanthenoid Bases with a Substituent at C-3

As discussed previously, introduction of a benzyl group at C-3 of yohimbane was achieved by Zinnes et al[51], although no NMR or mass spectral data on the 3-benzyl derivative were reported. Since yohimbine was readily available in quantity, this compound was used to investigate in detail, the introduction of a benzyl group into the 3-position. Preparation of 3-dehydroyohimbine perchlorate had been reported previously by Weisenborn and Diassi[58] and this reaction was readily repeated to give a yellow crystalline salt (131) in good yield (Figure 29).

Treatment of 3-dehydroyohimbine perchlorate (131) with a large excess of benzyl magnesium bromide in ether, as

reported by Zinness et al[51], gave essentially a single 3-benzyl derivative in 40% yield after chromatographic separation. The UV spectrum of the Grignard product showed an indole chromophore and the IR spectrum indicated the bands for hydroxyl and methyl ester groups at 3333 and 1709 cm^{-1}, respectively. It was therefore immediately obvious that the introduction of a 3-benzyl group could be accomplished without destroying the methyl ester function at C-16.

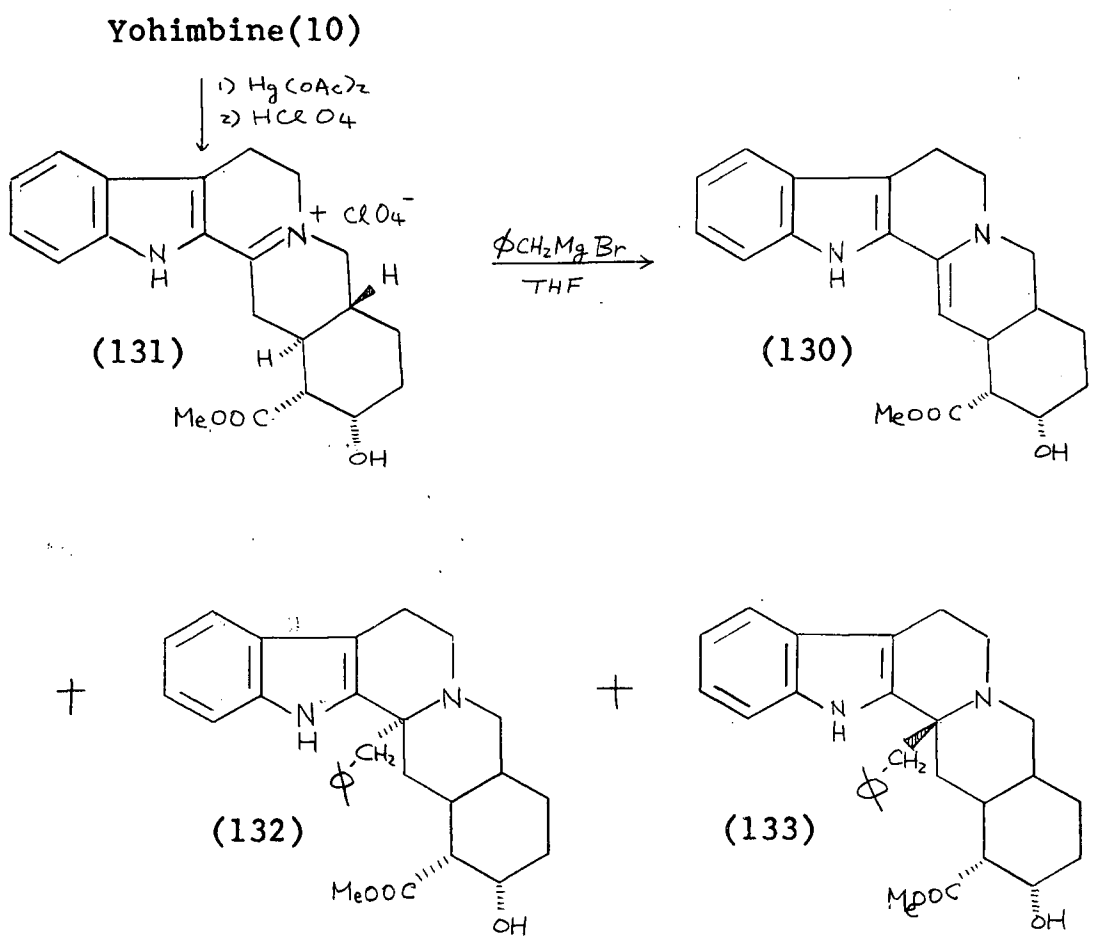


Figure 29

The mass spectrum showed a weak molecular ion at m/e 444 (1.9%) and a base peak at m/e 353, probably due to loss of the benzyl group (M-91). The NMR spectrum (Figure 30) confirmed the presence of a carbomethoxy group (τ6.25), indole NH (τ3.46) and nine aromatic protons in the region, τ2.4-3.2. A deuterium exchange experiment demonstrated a slow exchange of the broad singlet at τ3.46 (30 min. to completion). The chemical shift of the indole NH in this compound when compared to the indole NH resonance in yohimbine (at τ2.21) must be due to the shielding effect of the aromatic ring of the 3-benzyl substituent.

All the physical data supported the successful introduction of a 3-benzyl group into yohimbine, either from the α- or β-side of the molecule. Zinnes et al[51] suggested inconclusively, on the basis of ORD and IR studies, that 3-benzylyohimbane (108) possessed a 3α-benzyl group. In the case of 3-benzylyohimbine, the NMR spectral data suggests that the 3-benzyl group is in the α orientation (132). Molecular models of 3α- and 3β-benzylyohimbine indicated that only the 3α-benzyl group could extensively shield the indole NH as was suggested from its NMR spectrum. It was also seen that the 3α-benzyl group was in a <u>trans diaxial</u> orientation to the lone pair of electrons on the nitrogen (N_b) when the molecule existed in the most favorable conformation.

Apart from the above compound, a slightly more polar spot with a similar color was detected on TLC (3β-benzyl

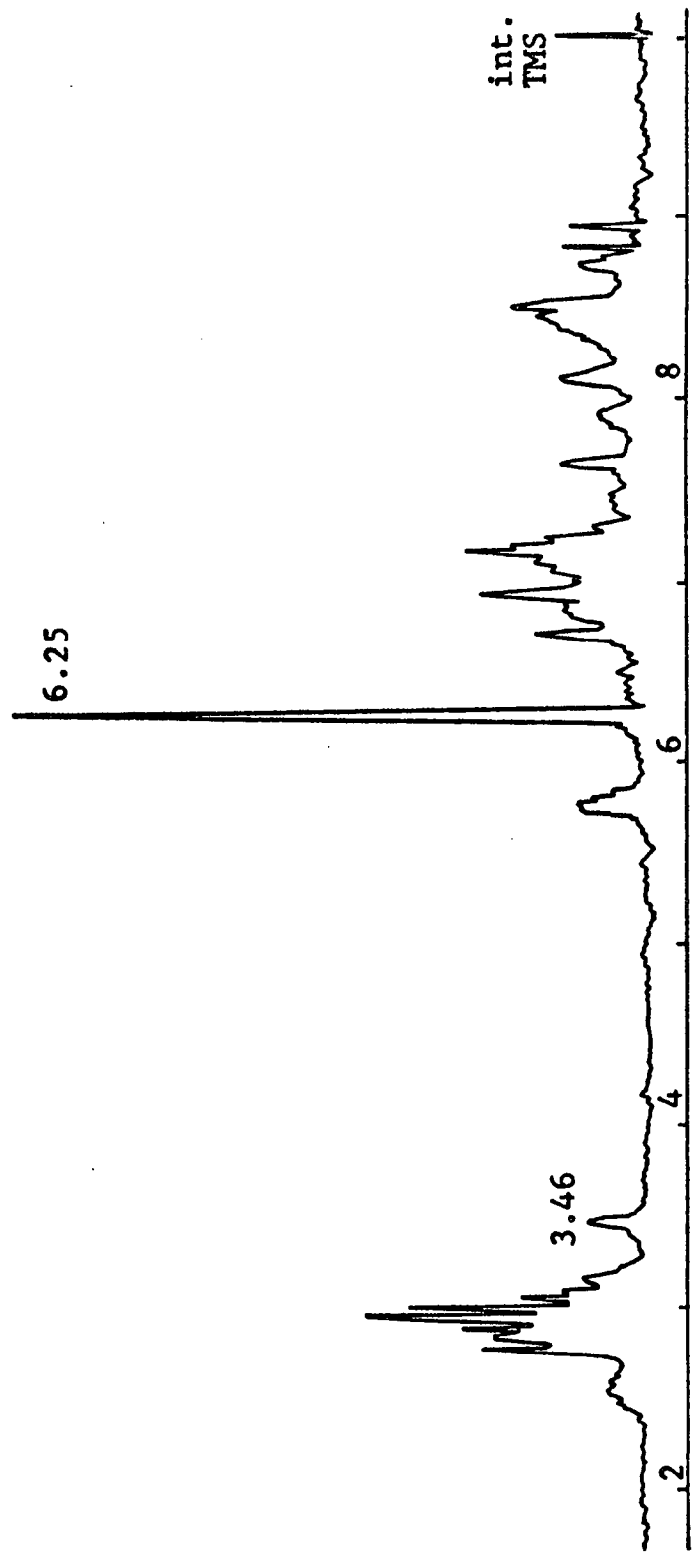


Figure 30 NMR Spectrum of 3α-Benzylyohimbine(132) (60Mc/s)

drivative?), but insufficient quantities were available for complete characterization. This more polar component was obtained as one of the major products when the Grignard reaction was done in tetrahydrofuran, as will be discussed below.

The yield of 3-benzylyohimbine from the Grignard reaction in ether as solvent was low, possibly because the intermediate complex forms an insoluble layer around the yohimbine perchlorate and prevents further reaction. The use of a more polar solvent such as tetrahydrofuran would alleviate this difficulty since it is known that most Grignard intermediate complexes are soluble in this solvent. Indeed when finely powdered 3-dehydroyohimbine perchlorate (131) was added to benzyl magnesium bromide in tetrahydrofuran, solution occurred immediately. Since the perchlorate (131) is not soluble in tetrahydrofuran, it was apparent that reaction must have taken place instantly. The UV spectrum of the crude Grignard products was recorded and indicated the presence of not only an indole chromophore (λmax: 283, 291 mμ) but also a considerable amount of a 3-dehydro base (λmax: 320 mμ). After addition of one drop of concentrated hydrochloric acid to the UV cell, the absorption at 320 mμ was lost and a new peak at 352 mμ appeared. This dramatic bathochromic shift (320→352 mμ)[52] is typical of a 3,14-dehydro base (130) being isomerized to a 3-dehydro iminium salt (131). These UV spectral studies clearly show that the Grignard reagent acts as

a base to pull off a proton at C-14 as well as a nucleophile in attacking the iminium bond at C-3. The extinction coefficients of both the indole chromophore (yohimbine) and the 3-dehydro salt are known and, therefore, the approximate ratio of these components in the mixture could be calculated. In this way, the ratio of saturated indole derivatives to the 3-dehydro salt was found to be 100 : 11.

The crude Grignard product mixture was purified by column chromatography. Elution with benzene-ether gave two products in overall 60% yield. The ratio of the less polar to the more polar material was found, by preparative TLC on silica gel, to be 2 : 1. The less polar component was found to be identical with the 3α-benzylyohimbine obtained previously as the major product by the Grignard reaction in ether.

The more polar material was identical, by TLC, with the minor component obtained in the ether reaction. This material showed an indole chromophore in the UV spectrum and, interestingly, the mass spectrm showed a relatively strong molecular ion peak at m/e 444 (14%) and a base peak at m/e 353 (M-91).

The NMR spectrum of the more polar compound (Figure 31) showed a similar pattern to that of the less polar material except for the chemical shift of the indole NH proton. There was a singlet for the methyl protons of the carbomethoxy group at τ 6.17, and a ten proton multiplet in the region, τ 2.3-3.2. It should be noted that in this

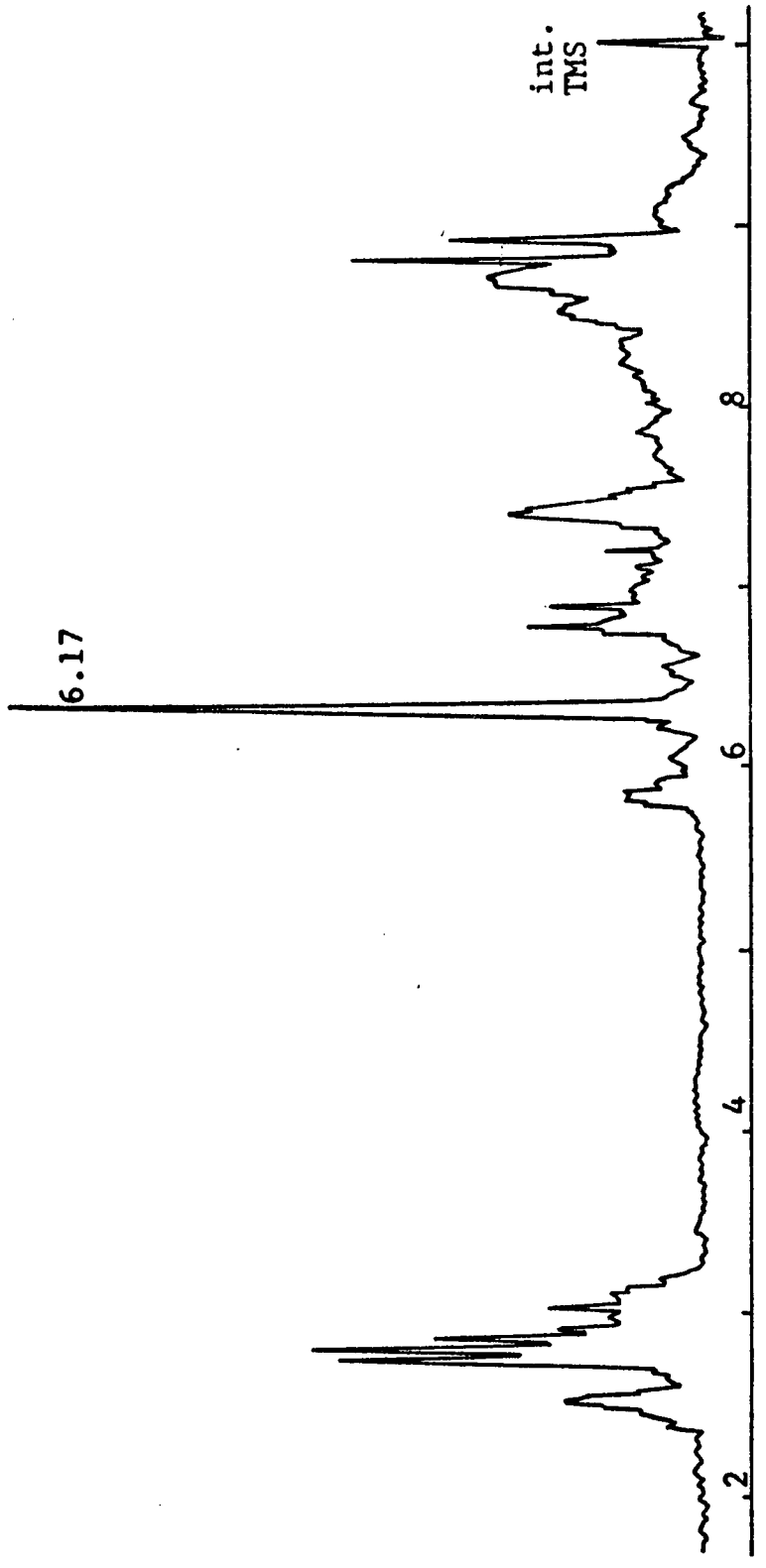
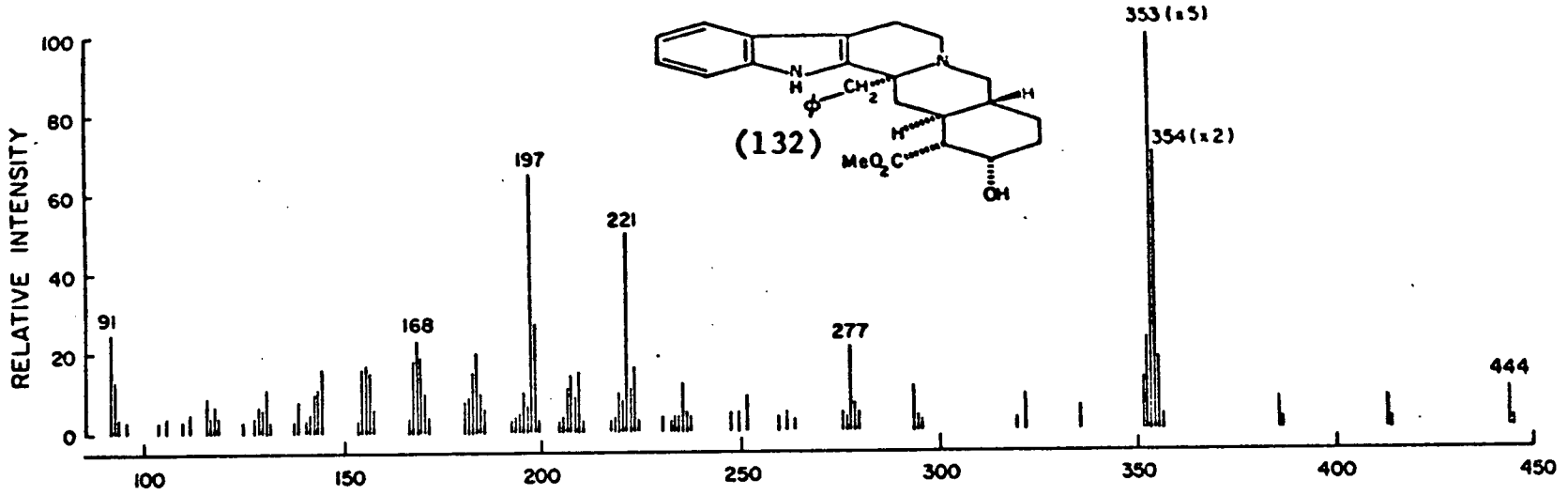


Figure 31 NMR Spectrum of 3β-Benzylyohimbine(133) (60Mc/s)


RELATIVE INTENSITY
100
80
60
40
20
0
100
150
200
250
300
350
400
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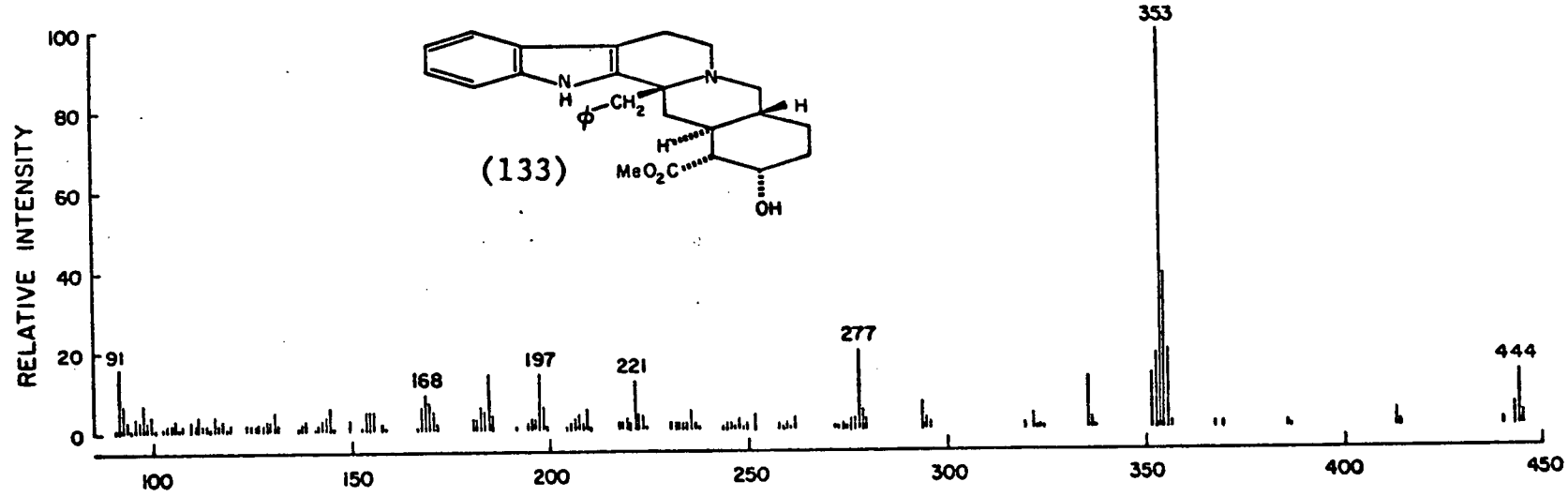
91
168
197
221
277
353 (x5)
354 (x2)
444
(132)
N
H
CH2
H
H
MeO2C
OH


RELATIVE INTENSITY
100
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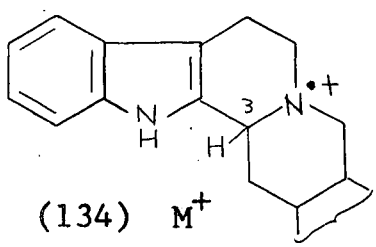
350
400
450
91
168
197
221
277
353
444
(133)
N
H
CH2
H
H
MeO2C
OH

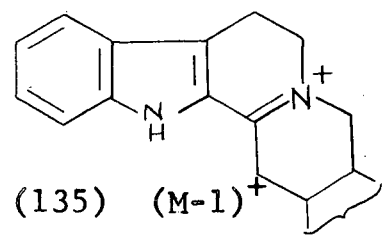
Figure 32

compound the chemical shift of the indole NH became indistinguishable from that of the expected nine aromatic protons. Therefore, it appeared that the benzyl group was not exerting a shielding effect on the indole NH as in the less polar compound. Thus, it could be concluded from the NMR spectra of these two 3-benzyl derivatives that the less polar material bearing the 3α-benzyl group shields the indole NH (τ3.46) whereas the more polar compound with a 3β-benzyl group has little influence. Further evidence to support these proposals could be obtained from the mass spectra of these two isomers.

It is very well known, from mass spectrometric measurements, that yohimbine-, ajmalicine- and corynantheine-type alkaloids (134) give rise to a strong $(M-1)^+$ peak under electron impact and it is speculated that this $(M-1)^+$ peak is due to loss of the proton at C-3[66].

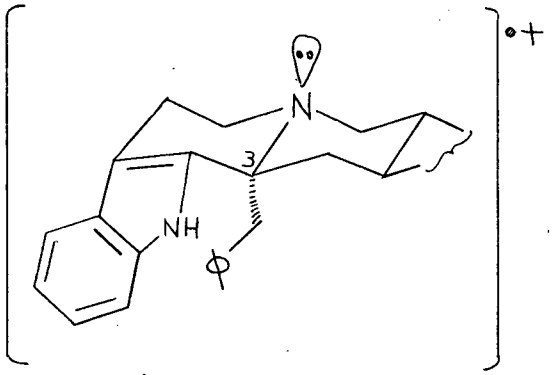


(134) M^+



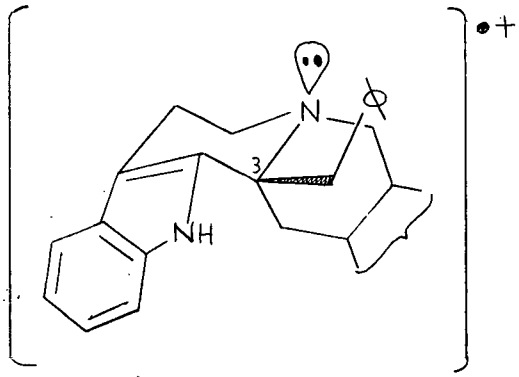
(135) $(M-1)^+$

For example, the mass spectrum of yohimbine (M.W. 354) showed a large $(M-1)^+$ peak at m/e 353[66]. It is not surprising, therfore, that the mass spectra of both 3α- (132) and 3β-benzylyohimbine (133) showed a base peak at m/e 353 undoubtedly due to loss of the benzyl group as shown in Figure 33. The benzyl group and the lone pair of electrons



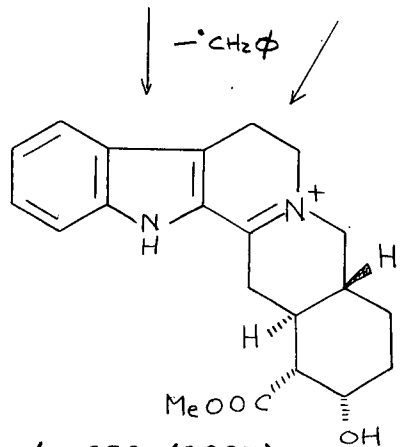
m/e 444 (1.9%)

3α-Benzyl (132)



m/e 444 (14%)

3β-Benzyl (133)



m/e 353 (100%)

Figure 33

on nitrogen (N_b) are considered to be <u>trans diaxial</u> in the case of 3α-benzylyohimbine (132) whereas in the β isomer (133) dihedral angle of approximately 60° exists between them. It would therefore be expected that 3α-benzylyohimbine (132) would lose the benzyl group more easily, under electron impact, Figure 33 bears out this suggestion. The molecular ion peak of 3α-benzylyohimbine (132) was observed at m/e 444 (<u>1.9%</u>) and was found to be weaker than that of 3β-benzyl-

yohimbine (133) (14%). These observations are in good accord with the previous structural assignments.

Further elution of the Grignard product mixture with methanol gave the 3-dehydro base (130) which was characterized as its perchlorate salt.

Although the pentacyclic alkaloid, yohimbine, gave two isomeric 3-benzyl derivatives, it does not necessarily follow that tetracyclic corynanthenoid-type bases would also give two isomeric 3-benzyl compounds. However, the above structural assignments in the yohimbine series, based on NMR and mass spectra, might prove to be useful for similar structural studies on corynanthenoid derivatives.

Another possible blocking group (at C-3) which was considered was the nitrile group, since this function could be easily eliminated by hydrolysis followed by decarboxylation. However, 3-cyanoyohimbine (122) prepared according to the procedure reported by Zinnes et al[51] was found to be very unstable in solution. Loss of hydrogen cyanide occurred even in "spectro grade" methanol while the UV spectrum was being recorded. Purification of 3-cyanoyohimbine was impossible due to the unstable nature of this material in solution and the NMR spectrum was also unobtainable. Although the stereochemistry of 3-cyanoyohimbine was considered to be similar to that of the 3-benzyl derivatives (i.e. predominantly 3α-cyano) no physical data was available to support this assignment. However, treatment of the 3-cyanoyohimbine (137) with benzyl

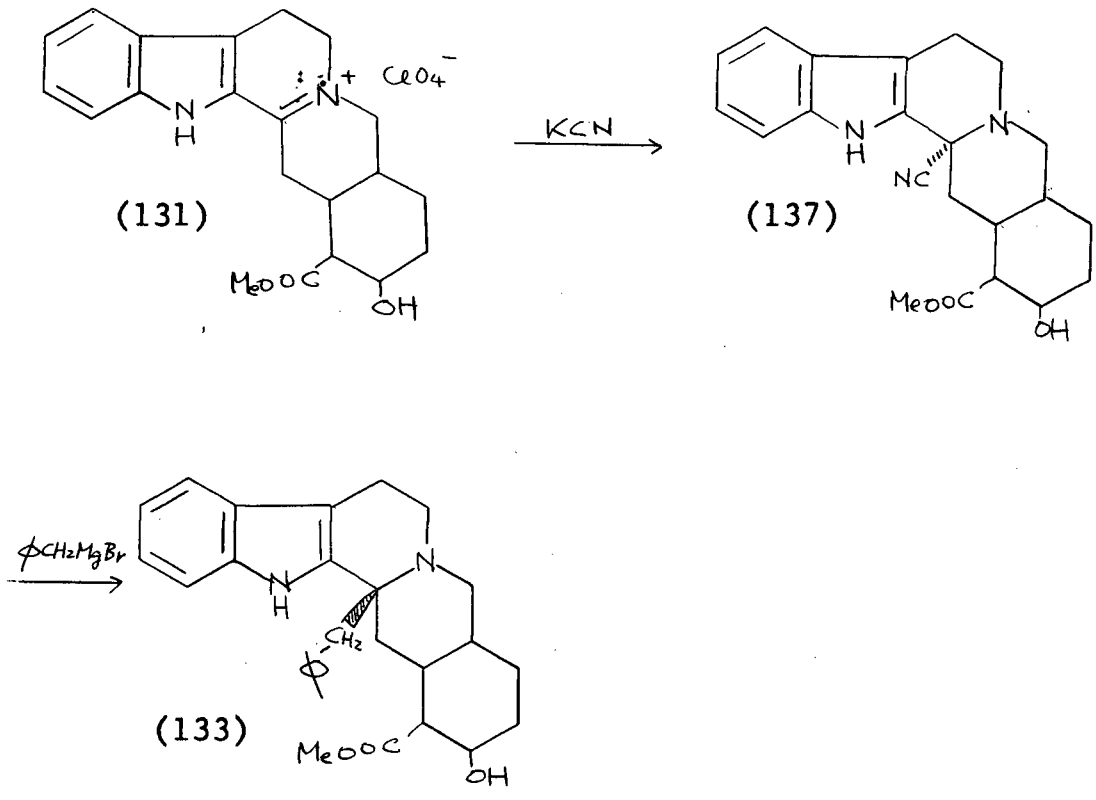


Figure 34

magnesium bromide in tetrahydrofuran furnished predominantly 3β—benzylyohimbine, together with a minor amount of the 3α isomer. This result suggests that the original 3-cyanoyohimbine was predominantly the 3α derivative since the Grignard reaction occurs by Sn_2 displacement of the cyano group.

Before the introduction of the 3-benzyl group into a corynanthenoid-type base was attempted, the reactivity of tetradehydroyohimbine perchlorate (140) with benzyl magnesium bromide was examined (Figure 35). The enamine compound (141) which may be formed in this reaction could

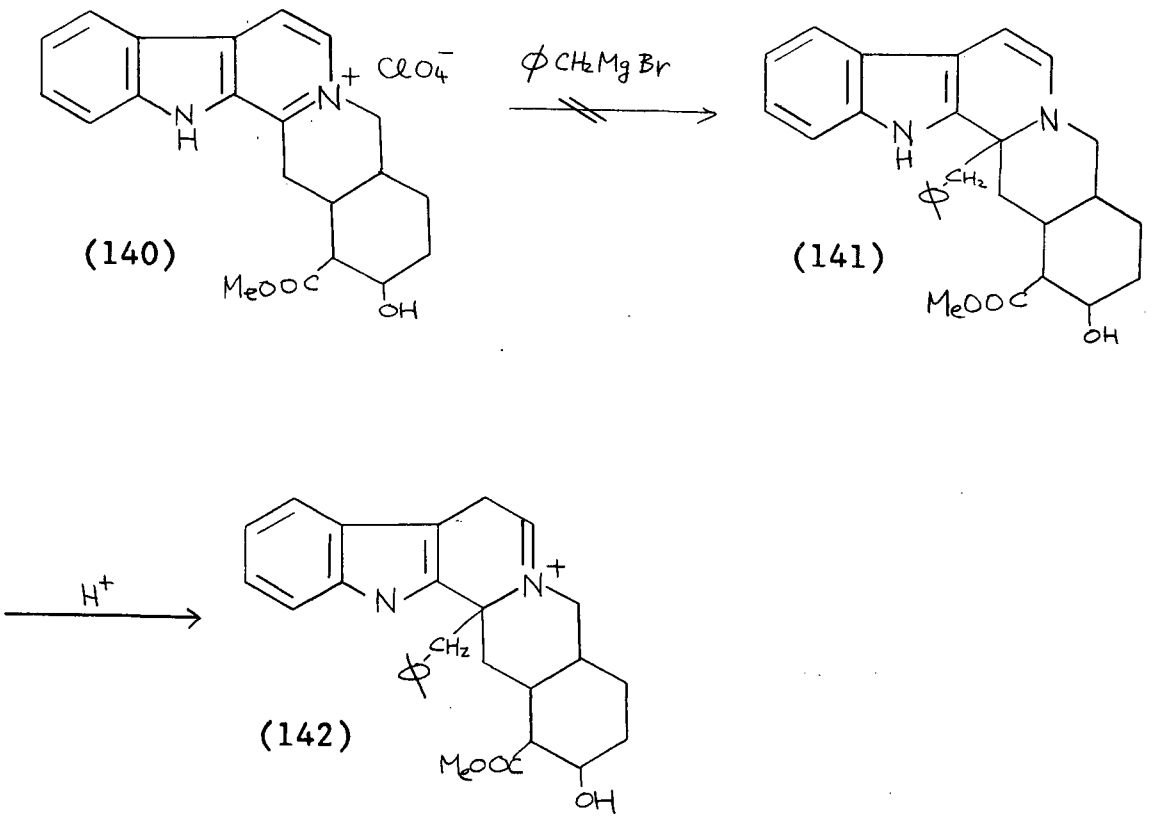


Figure 35

provide, upon treatment with acid, the iminium salt (142), a system which would be very desirable in the corynantheine family. However, tetradehydroyohimbine perchlorate (140)[59] did not react with benzyl magnesium bromide even under refluxing conditions in tetrahydrofuran as solvent.

Since the successful preparation of 3-benzyl derivatives had now been carried out on model compounds, the introduction of a benzyl group at C-3 of dihydrocorynantheine was attempted (Figure 36). The preparation of 3-dehydrodihydrocorynantheine perchlorate (143) was achieved by

mercuric acetate oxidation followed by treatment with perchloric acid. The 3-dehydro salt (143) reacted instantly with benzyl magnesium bromide in tetrahydrofuran and after stirring was continued for 10 minutes, the usual work-up of the reaction mixture gave a single product, as shown by TLC. The UV spectrum of the crude product indicated the presence of a 3-dehydro base (17%).

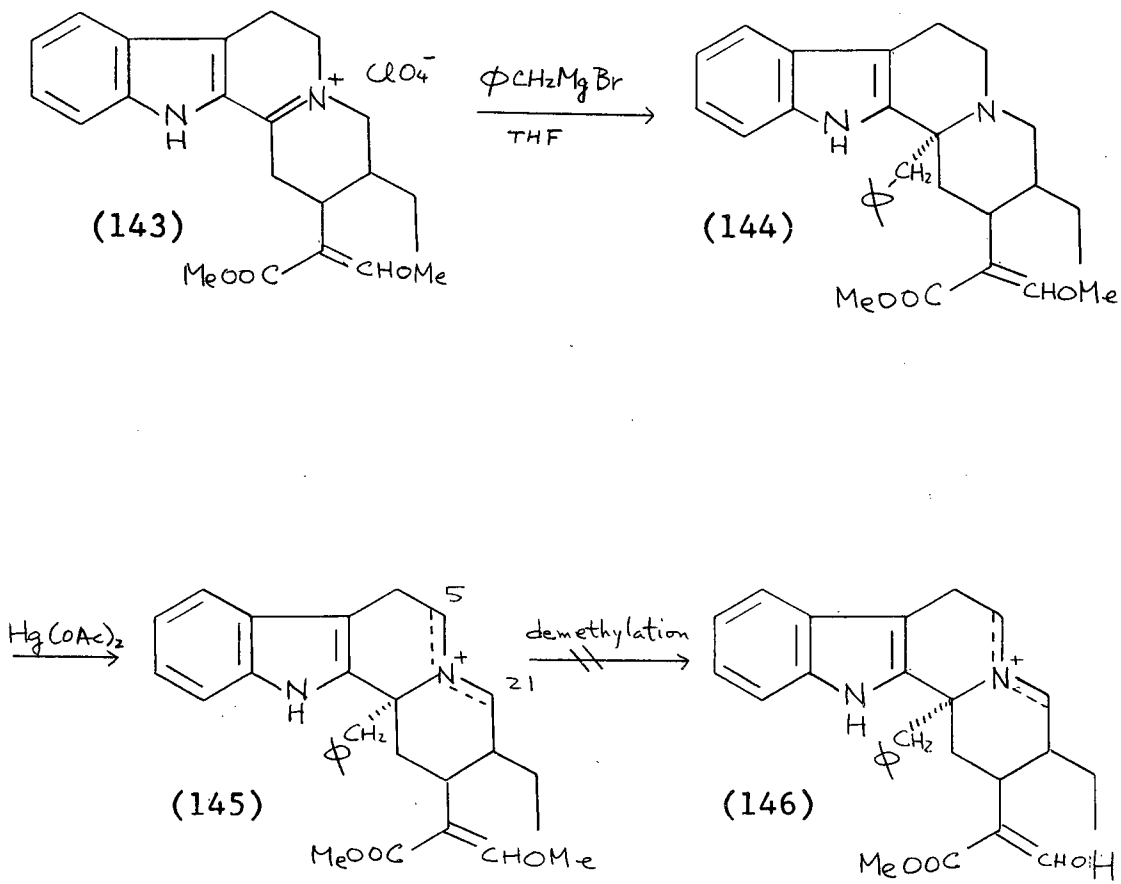


Figure 36

The IR spectrum of the main product (after chromatography) showed the retention of the carbomethoxy (1692 cm^{-1}) and the enol ether (1631 cm^{-1}) groups. The mass spectrum showed a weak molecular ion at m/e 458 (1%) and a base peak at m/e 367 (M-91), thereby confirming the successful introduction of a benzyl group at C-3. The NMR spectrum (Figure 37) showed singlets at τ6.27 and τ6.35 for the methyl protons of the cabomethoxy and methyl ether groups, and a one proton multiplet at τ1.40. Upon addition of deuterium oxide, the multiplet at τ1.40 disappeared immediately and a slow exchange of the singlet at τ 3.40 (30 min. to completion) was observed. The one proton multiplet at τ1.40 could be assigned to the olefinic proton at C-17, being deshielded by the phenyl nucleus of the benzyl group (the olefinic signal of dihydrocorynantheine appears at τ 2.60[63]). The singlet at τ 3.40 could be assigned to the indole NH, and slow exchange of the indole NH may be due to steric hindrance from the benzyl substituent. The chemical shift suggests that the orientation of the benzyl group is α at C-3. The presence of a weak molecular ion (1%) in the mass spectrum also supported this assignment. It was concluded, therefore, that the reaction of benzyl magnesium bromide with 3-dehydrodihydrocorynantheine perchlorate gives exclusively 3α-benzyl- and no 3β-benzyldihydrocorynantheine. It was also clear, from molecular models, that the absence of ring E in dihydrocorynantheine made the molecule less rigid and that the stereospecific attack of

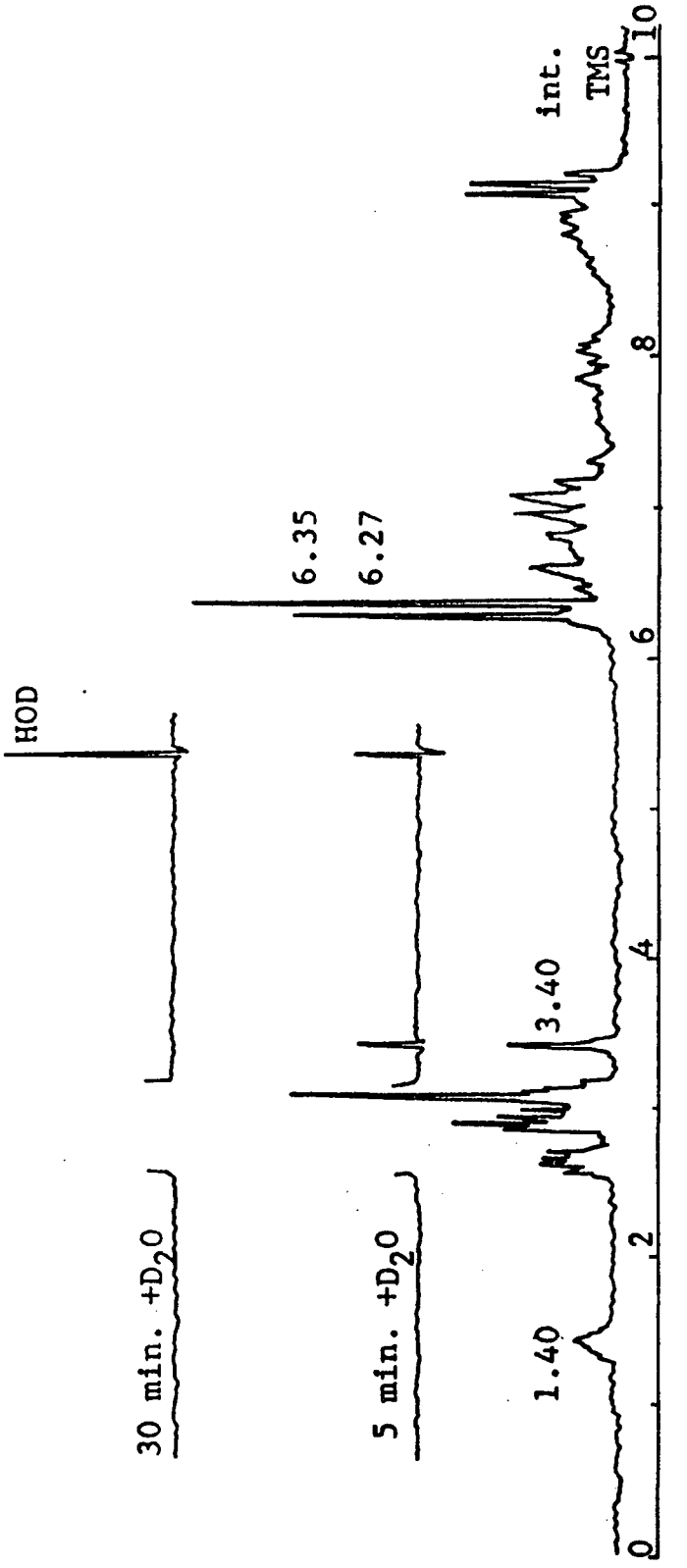


Figure 37 NMR Spectrum of 3α-Benzyldihydrocorynantheine(144) (100Mc/s)

the Grignard reagent from the α-side of the molecule is much more favorable.

In order to study the transannular cyclization of 3α-benzyldihydrocorynantheine (144), it was necessary to prepare an iminium system involving C-5 (146) and to generate a potential anion at C-16.

First, mercuric acetate oxidation of the 3α-benzyl derivative (144) was attempted and the isolation of mercurous acetate (77% of the theoretical amount) and TLC properties supported the formation of an iminium salt involving either C-5 or C-21. However, a subsequent attempt at hydrolysis of the enol ether to the aldehydic function in order to activate C-16, under conditions which were effective in the case of dihydrocorynantheine itself, gave an intractable mixture.

As summarized in Figure 38, an alternative way, involving demethylation of the 3-benzyl derivative (144) and subsequent oxidation to generate the iminium system (147) (Figure 38), was considered and attempted.

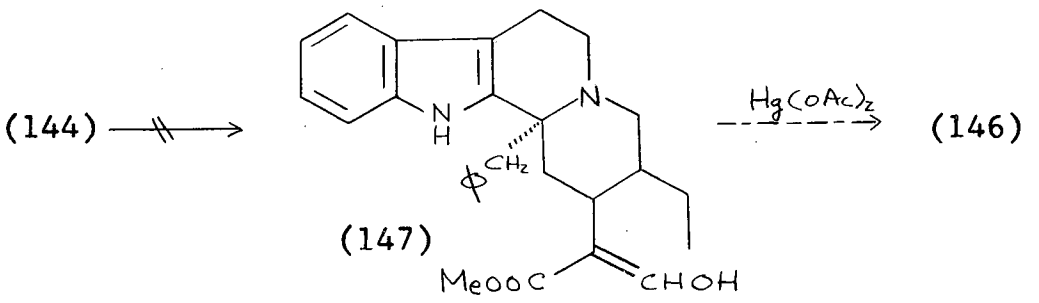


Figure 38

As detailed in the experimental section, six different experiments involving various conditions (hydrogen chloride in acetone, boron trifluoride etherate, hydrogen bromide in methyl acetate, hydrogen bromide in acetone, boron tribromide in dichloromethane, and boron trichloride in dichloromethane) afforded no success in the isolation of the demethyl derivative (147). Therefore, since the enol ether side chain of dihydrocorynantheine evidently caused difficulty in achieving success from this sequence, the preparation of a 3-benzyl derivative with a primary ester at C-15 was studied for possible transannular cyclization.

The synthesis of these 3-benzyl derivatives was carried out under the same conditions as discussed previously. Mercuric acetate oxidation of dl-dihydrocorynantheic acid methyl ester and ethyl ester gave the corresponding 3-dehydro derivatives (149) and (150), respectively. The 3-dehydro methyl ester (149), was treated with benzyl magnesium bromide in tetrahydrofuran and gave a single 3-benzyl compound. The UV spectrum of the crude product showed the presence of a saturated indole chromophore (97%) and 3-dehydro base (3%). The mass spectrum of the main product showed a weak molecular ion at m/e 416 (0.3%) and a base peak at m/e 325 (M-91), indicating that the benzyl group had been successfully introduced at C-3 probably from the α-side. The NMR spectrum showed a singlet for the indole NH at τ 3.35 again indicating the shielding of the phenyl nucleus of the 3α-benzyl group. Additionally, the

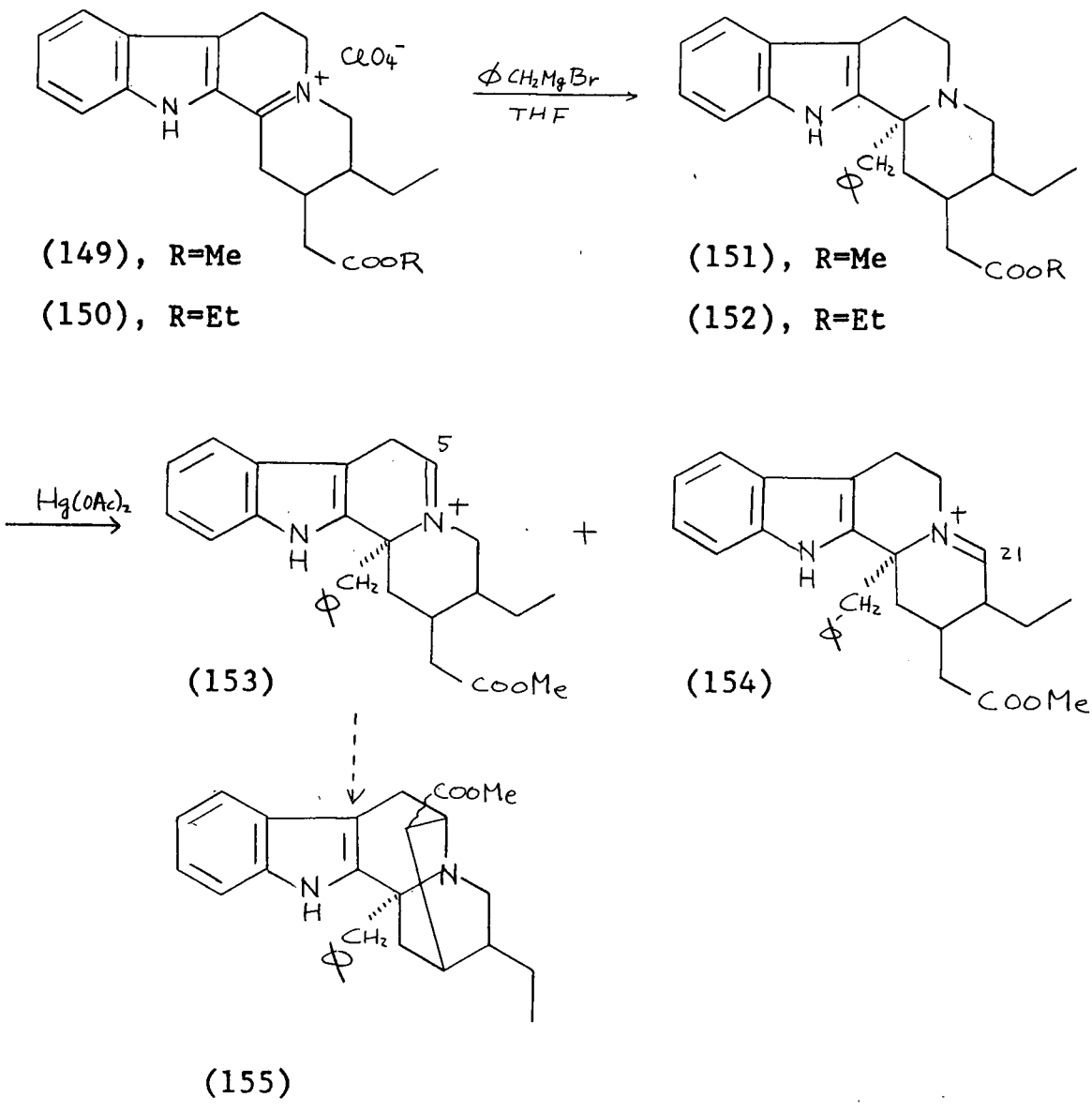


Figure 39

spectrum showed nine aromatic protons in the region τ 2.4-3.2, a singlet for a carbomethoxy group at τ6.29 and a broad singlet for the benzylic protons of the 3-benzyl group at τ 7.18. All the data supported the successful preparation of the 3α-benzyl derivative (151).

Introduction of a 3α-benzyl group was analogously accomplished in the case of the ethyl ester derivative to afford dl-3α-benzyldihydrocorynantheic acid ethyl ester (152).

Mercuric acetate oxidation of the 3α-benzyl methyl ester (151) was attempted and 65% of the theoretical amount of mercurous acetate was isolated. However, treatment of the reaction mixture with sodium borohydride gave back only starting material. Obviously, the conversion (153→ 155) had not occurred, since the cyclized compound (155) could not be attacked by sodium borohydride to give back starting material. Treatment of the 3α-benzyl methyl ester (151) with mercuric acetate under more forcing conditions resulted in the loss of a benzyl group to yield a 3-dehydro derivative. The generation of an anion at C-16 in this molecule might not be very favorable in acidic media. An alternative compound could be the methyl ketone derivative (158) instead of the methyl ester (151). The methyl ketone would be expected to enolize towards the secondary carbon atom rather than the primary carbon atom, to generate a potential anion at C-16.

An efficient way of converting an ester group to a corresponding methyl ketone was recently developed by Corey and Chaykovsky[57]. Although this method has never been applied to an indole containing ester, dl-dihydro-corynantheic acid methyl ester (156) was subjected to this reaction. Thus reaction of methyl sulfinyl carbanion with

the methyl ester (156) gave the corresponding β-ketosulfoxide, which was crystallized from ethyl acetate. Reduction of the ketosulfoxide with aluminum amalgam provided the corresponding methyl ketone (157). The successful conversion was supported by the following physical data. The NMR spectrum showed a singlet for the methyl ketone at τ7.84 and no signals between τ6.7 and τ3.1. The mass spectrum confirmed the presence of the molecular ion peak at m/e 310 (100%).

In a similar manner (Figure 40), dl-3α-benzyldihydrocorynantheic acid methyl ester (151) was converted to the corresponding methyl ketone (158). The success of this transformation was indicated by the following physical data. The NMR spectrum of the product showed a sharp singlet for a methyl ketone at τ7.88, no signals between τ6.5 and τ3.6, a one proton singlet for the indole NH at τ3.58 and nine aromatic protons in the region, τ 2.4-3.2. The chemical shift of the indole NH showed that the configuration at C-3 was retained. The mass spectrum showed a molecular ion at m/e 400 (1.4%) and a base peak at m/e 309 (M-91). The structure of the methyl ketone (158) was thus confirmed.

Mercuric acetate oxidation of the 3α-benzyl methyl ketone (158) was carried out and mercurous acetate (96%) was isolated. After the work-up and chromatography, the product was isolated as the perchlorate salt (32%), m.p. 236-241°. The melting point of this salt was quite

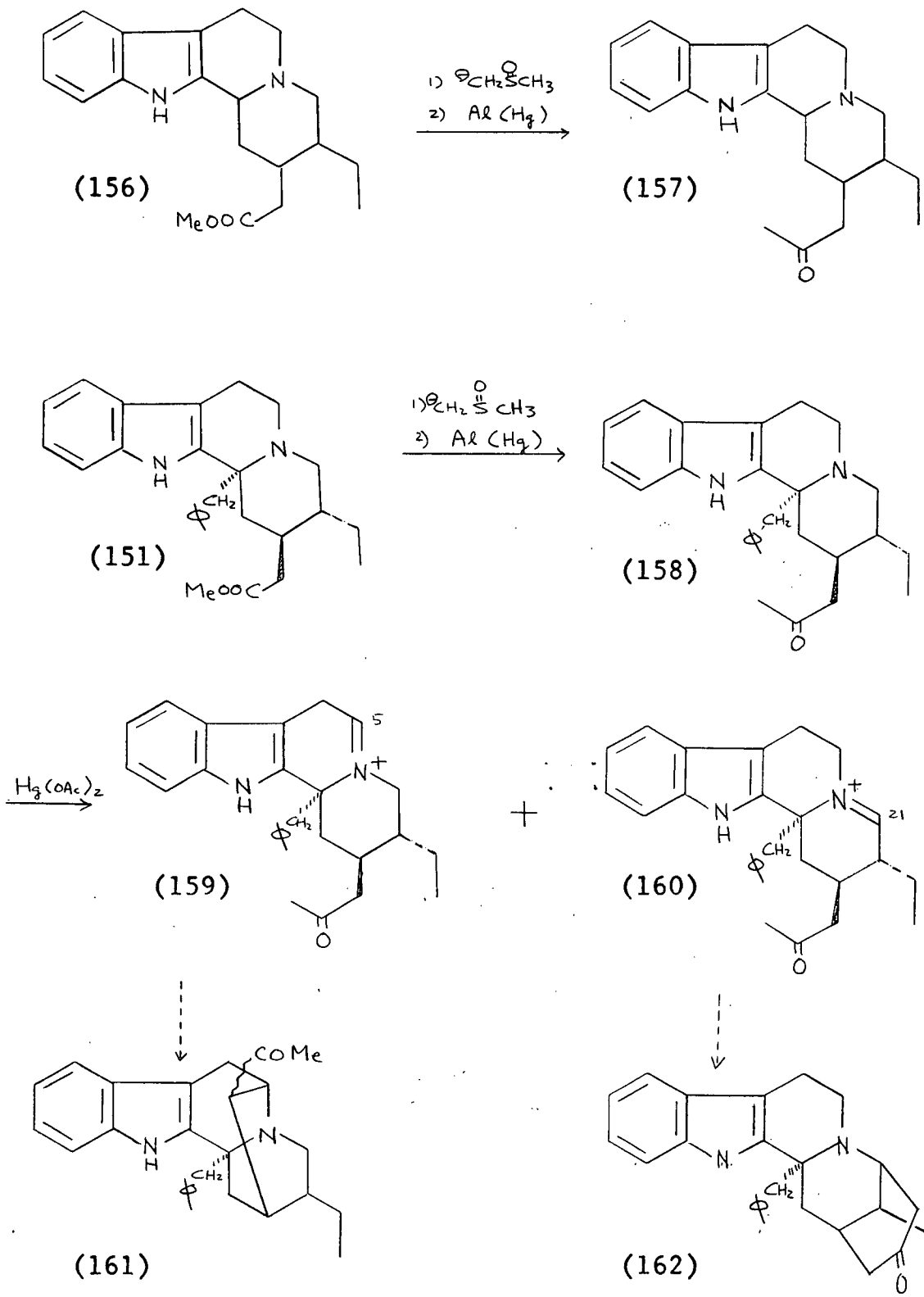

(156)
MeOOC
1) ⊖CH2SCH3
2) Al (Hg)
(157)
(151)
MeOOC
1) ⊖CH2S(=O)CH3
2) Al (Hg)
(158)
Hg(OAc)2
(159)
(160)
(161)
(162)
CO Me

Figure 40

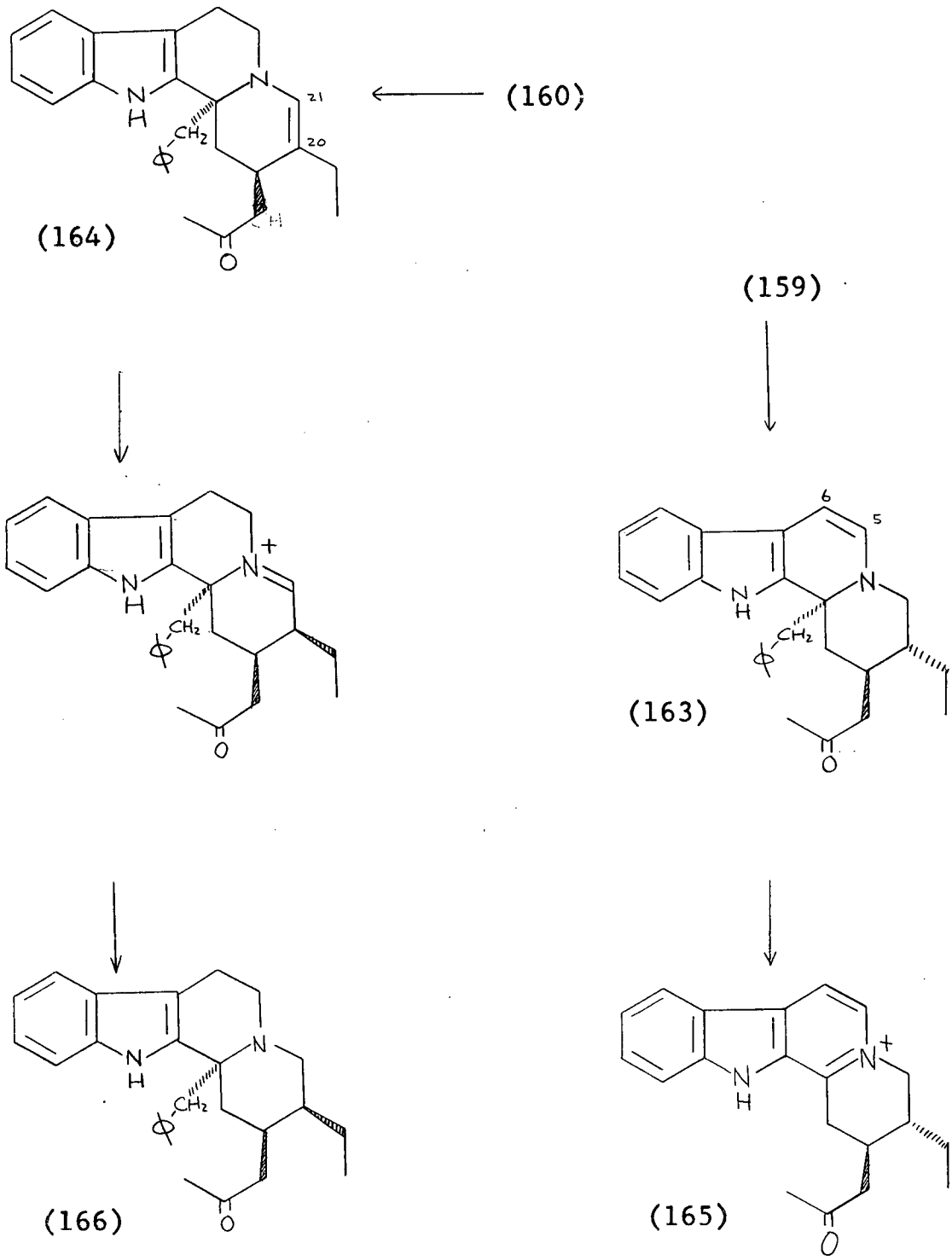


Figure 41

similar to that of the starting material (m.p. 232-237°) but a mixture melting point showed a depression (m.p. 212-215°), indicating that the two compounds were not identical. This oxidation product (as the free base) was shown to contain an indole chromophore in the UV spectrum and an indole NH and a carbonyl absorption at 3289 and 1709 cm^{-1}, respectively, in the IR spectrum. The NMR spectrum still showed nine aromatic protons in the region, τ 2.5-3.3, an indole NH at τ 3.45 and a methyl ketone at τ 7.90. The chemical shifts of the aromatic, indole NH and methyl ketone signals were quite similar to those of the starting methyl ketone (140). The mass spectrum of the product showed a very weak molecular ion at m/e 400 (0.4%, while the starting material had a molecular ion at m/e 400, 1.4%) and a $(M-1)^+$ ion at m/e 399 (1.1%). The ion at m/e 355 (4.7%) appeared in the starting material was no longer present in the mass spectrum of the product. In addition to the base peaks at m/e 309 in the mass spectra of both the starting material and the product, the rest of the fragmentation patterns were almost superimposable.

The cyclized compounds (161) and (162) must have a molecular weight of 398 and the base peaks in the mass spectra could be expected to be at m/e 307. The oxidation product, therefore, cannot have structures (161) or (162).

The enamine compounds (163) and (164) and the iminium salts (159) and (160) for the structure of the oxidation product are also excluded by the mass spectrometry measure-

ments. These physical data show that the product is isomeric with the starting 3α-benzyl methyl ketone (158). One possibility is that this product is the C-20 ethyl epimer (166) formed by a redox reaction[56] as outlined in Figure 41. In the redox reaction, the iminium salt (165) can be reduced to the β-ethyl epimer (166) with the enamine (163) being oxidized to the highly conjugated β-carboline derivative (165).

In conclusion, none of the corynanthenoid-type bases with the 3-benzyl blocking group gave the desired bridged compounds (between C-5 and C-16) under the conditions examined.

It is of interest to summarize the mass spectra of the 3-benzyl derivatives in the corynanthenoid series, since four tetracyclic bases with various substituents at C-15 were available. In all instances, $(M-91)^+$, resulting from easy loss of the 3-benzyl group, was the base peak. It should be noted that all of these compounds (144), (151), (152) and (158) possess carbonyl functions at C-16, either as an ester or a methyl ketone. When a hydrogen γ to a carbonyl group is available to form a 6-membered ring transition state involving the carbonyl bond (see fragment a in Figure 43), it is known that the carbon-carbon bond β to the carbonyl group cleaves under electron impact. Therefore it was expected that an ion at m/e 251 (fragment b) should be common to the spectra of bases (144), (151), (152) and (158). In fact, the mass spectrum of 3-benzyl-

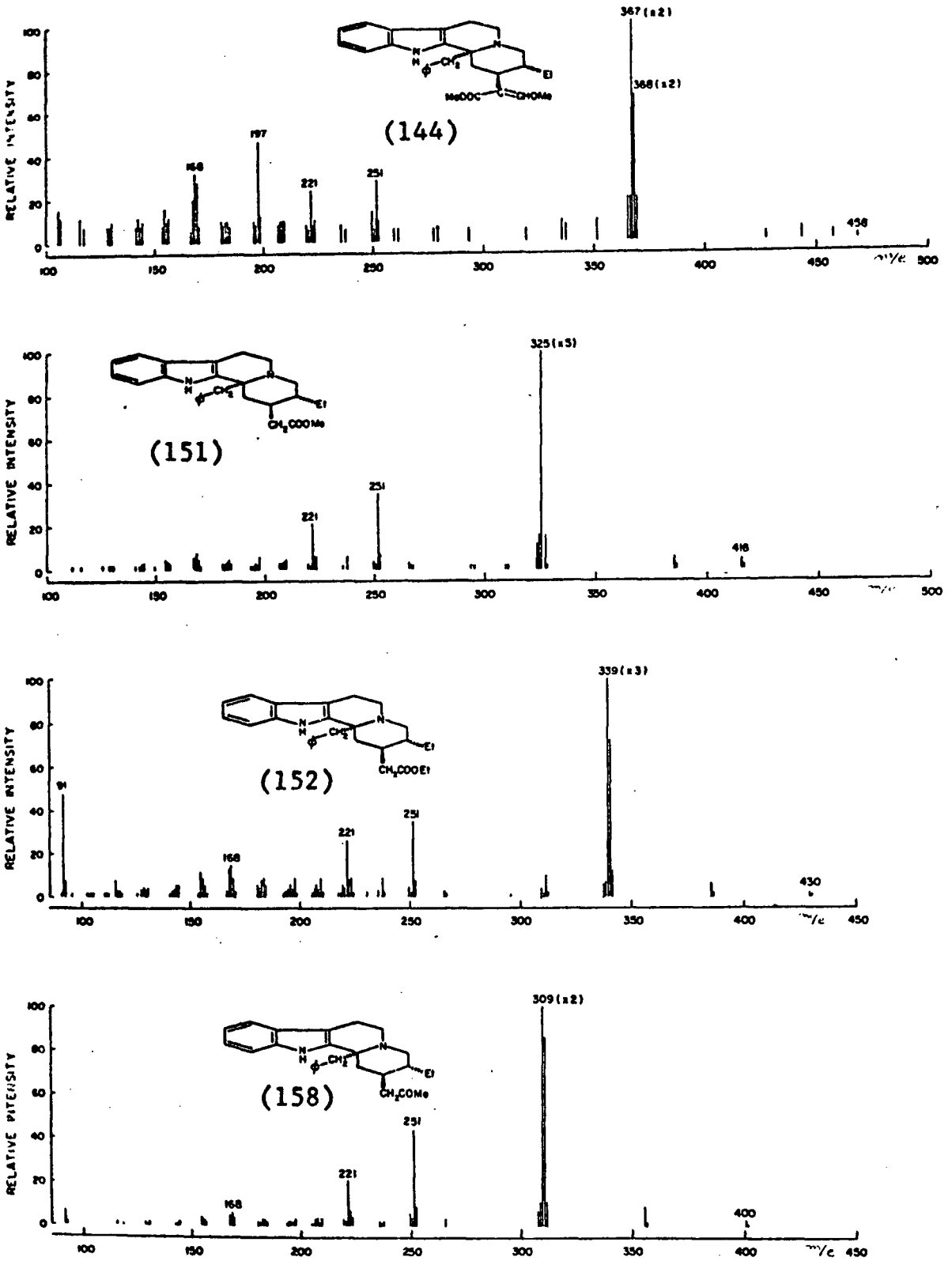
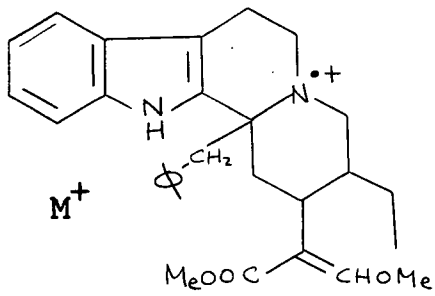
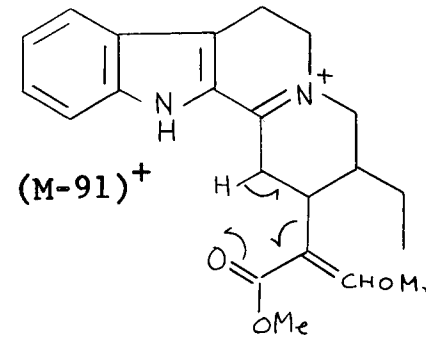

(144)
367(±2)
368(±2)
197
168
221
251
456
RELATIVE INTENSITY
(151)
325(±3)
221
251
416
(152)
339(±3)
168
221
251
430
(158)
309(±2)
221
251
168
400
m/e

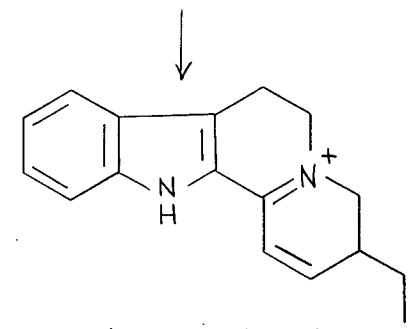
Figure 42



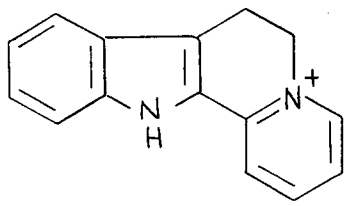
$-\cdot CH_2\Phi$



a m/e 367 (100%)

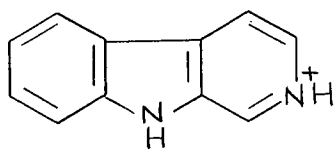


$-C_2H_6$

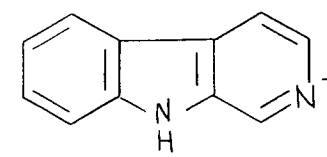


b m/e 251 (14%)

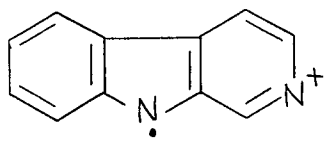
c m/e 221 (12%)



d m/e 169



e m/e 168



f m/e 167

Figure 43

dihydrocorynantheine showed a fairly strong peak at m/e 251 (14%) and also a peak at m/e 221 (c, 12%) probably due to loss of ethane to form the highly conjugated ion (fragment c). As shown in Figure 42, the occurrence of these peaks at m/e 251 and m/e 221 could also be observed in the mass spectra of the 3-benzyl derivatives (151), (152) and (158). There were also common fragments at m/e 169, m/e 168 and m/e 167 in all the four spectra and these ions are known to occur in yohimbine-like bases. It is reasonable to spectulate that ions at m/e 169, m/e 168 and m/e 167 are due to β-carboline fragments d, e and f, respectively[66]. Although these assignments for ion fragments in the mass spectra were not based on direct experimental evidence, the above speculations appear to be very reasonable for the structually similar compounds as discussed.

c) Attemped Transannular Cyclizations of 3,4-seco-Corynanthenoid Alkaloids (Figure 27)

When the preparation of 3,4-seco derivatives was attempted, the only procedure available from the literature was the Birch reduction or lithium aluminum hydride cleavage of the methiodide of the tetracyclic base (91) described by Dolby and Booth[44]. Hence, the methiodides of corynantheic acid ethyl ester (126) were prepared and a sodium in liquid ammonia reduction was attempted. The only products isolated were the recovered starting material and a compound which was probably the 3,4-seco-alcohol (167)

by analogy with the results of Dolby and Booth[44] (Figure 44). The identity of the tricyclic alcohol (167) was established by the following physical data. The NMR spectrum showed a singlet for a N_b-methyl group at τ 7.69, only three protons above τ 9, and the absence of an ester group. The IR spectrum indicated the presence of a hydroxyl and the absence of a carbonyl group.

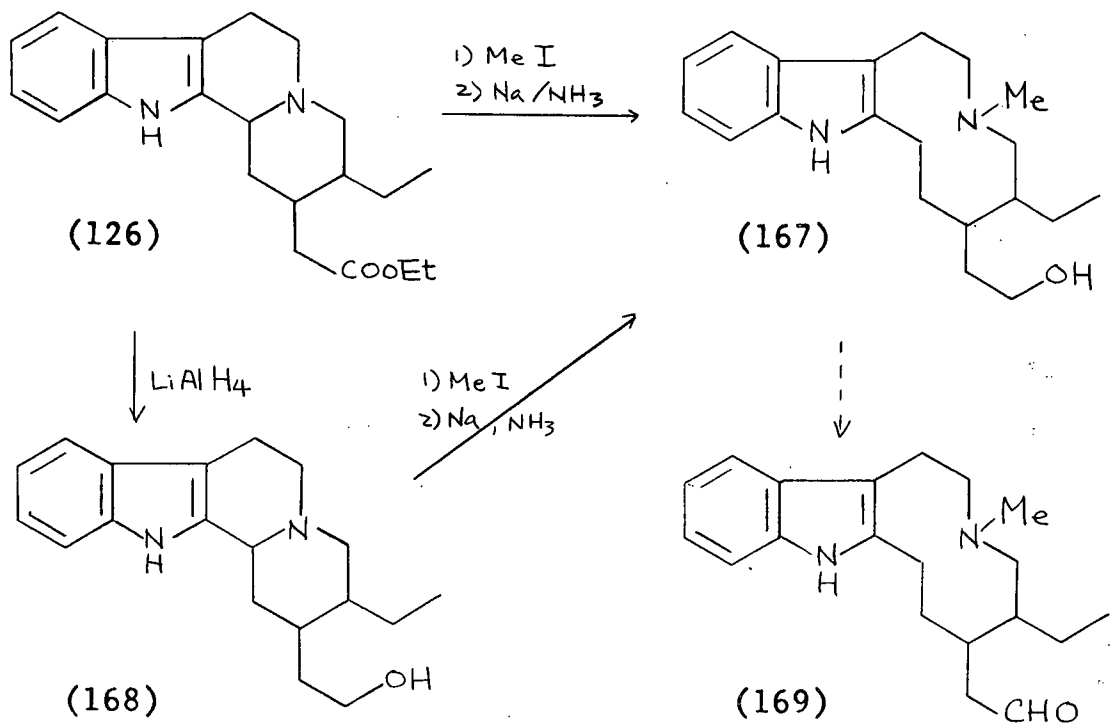


Figure 44

The mass spectrum confirmed the molecular ion at m/e 314 (47%). The poor yield from this reduction was thought to be due to the low solubility of the methiodide of the starting material (126) in liquid ammonia. To overcome this problem the Birch reduction of the methiodide of the

alcohol (168) was attempted, however, the yield of the 3,4-seco-alcohol (167) was not improved.

Since a carbonyl-containing group is necessary to activate a proton at C-16 for the transannular cyclization (Figure 27), attempts were made to oxidize the alcohol (167) to the aldehyde (169). Oxidation of the alcohol (167) with dicyclohexylcarbodiimide in dimethylsulfoxide[65] lead to only a poor conversion to the corresponding aldehyde (169). Since this reaction sequence provides the aldehyde (169) in very poor yield, the alternative sequence (Figure 45) starting from the commercially available dihydrocorynantheine (170) was investigated.

Base catalyzed hydrolysis of dihydrocorynantheine (170) followed by treatment with aqueous acid gave dihydrocorynantheal (171) in good yield[68]. This substance was converted to the acetal (172) and then to the methiodide by the usual methods as outlined in Figure 45. Birch reduction of the methiodide (173) gave some starting material (40%) back and a mixture of 3,4-seco-acetal (174) and dihydrocorynantheal ethylene acetal (172). By recycling the recovered starting material (172 and 173) the 3,4-seco-acetal (174) could be obtained in 70% overall yield. The structure of the 3,4-seco-acetal (174) was fully supported by the mass spectrum which indicated a very stable molecular ion at m/e 356 (100%). The NMR spectrum (Figure 46) was very instructive and showed the presence of N_b-$\underline{CH_3}$ at τ 7.83 and ethylene acetal at τ 6.26 (singlet, 4H) and at τ 5.30 (triplet, J=5

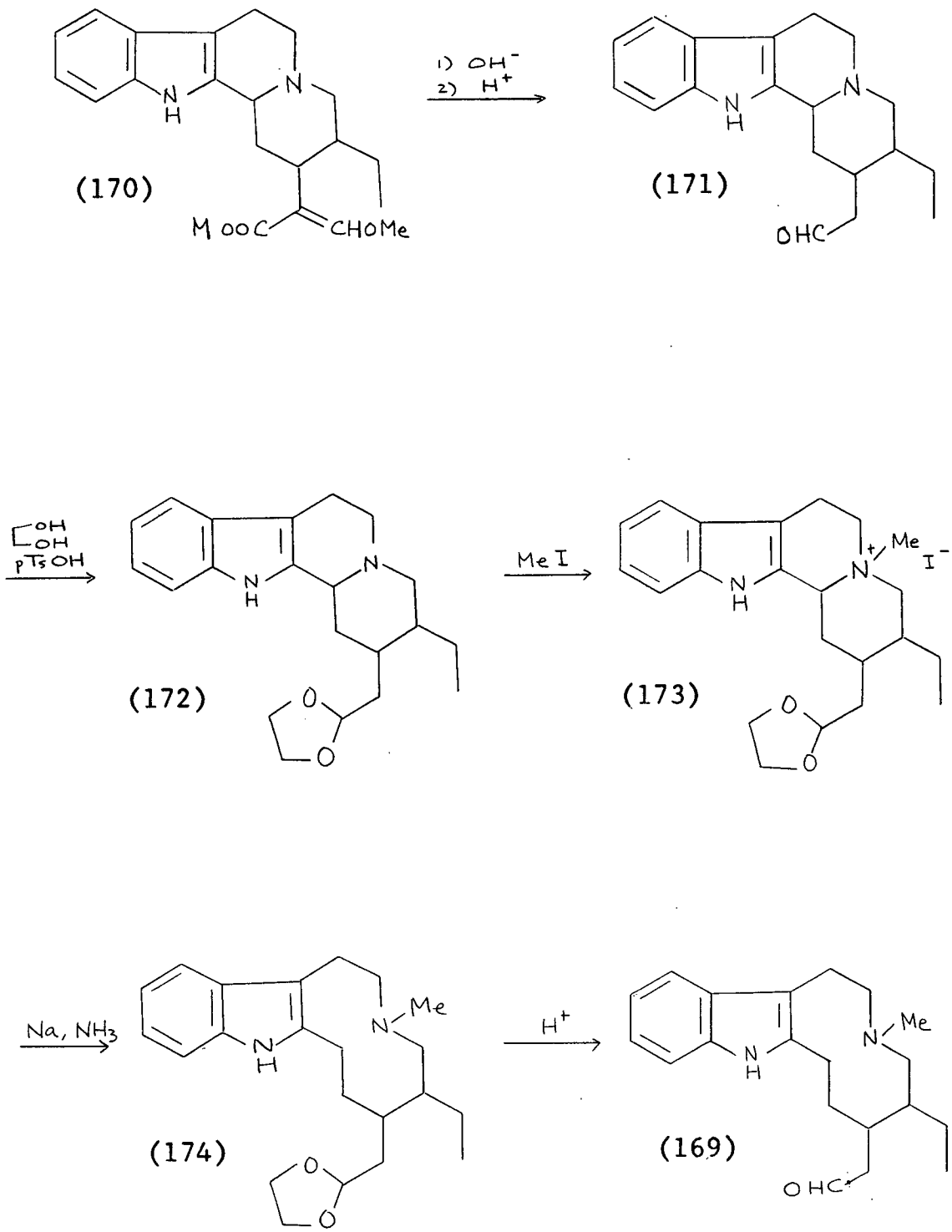

1) OH⁻
2) H⁺
(170)
MeOOC
CHOMe
(171)
OHC
pTsOH
(172)
MeI
(173)
Me
I⁻
Na, NH₃
(174)
H⁺
(169)
OHC

Figure 45

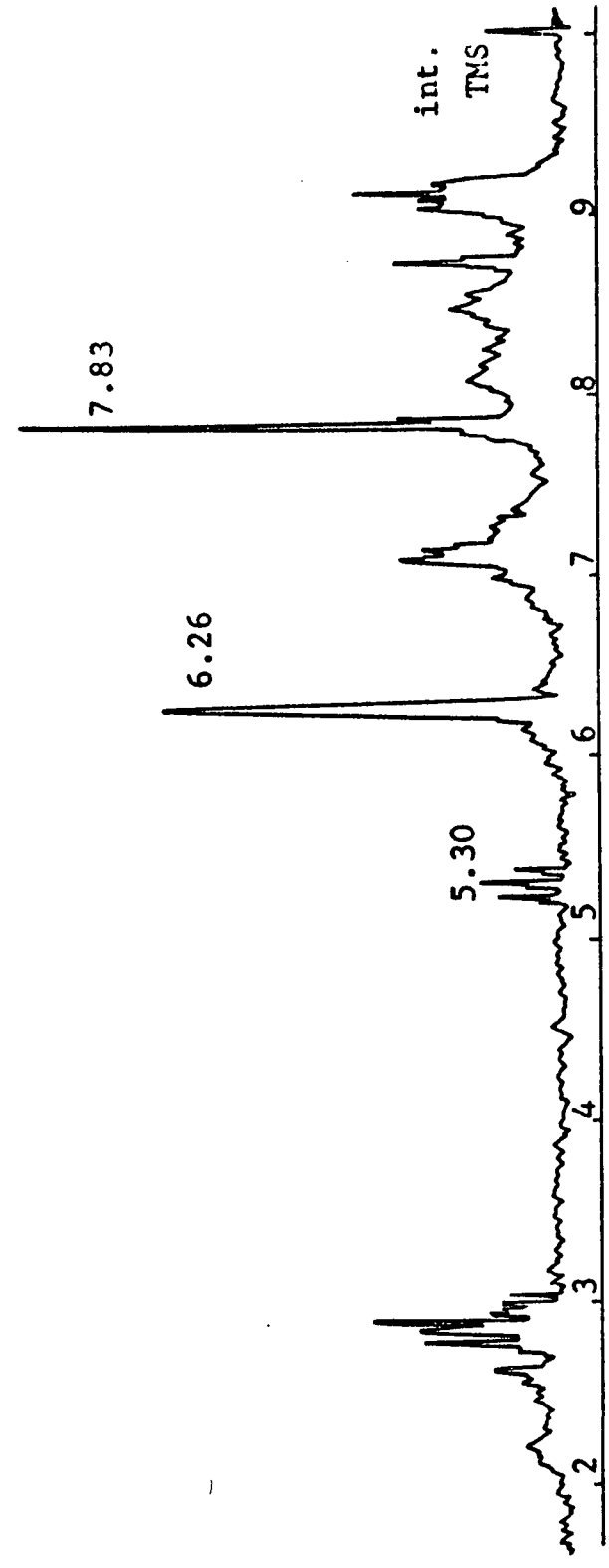


Figure 46 NMR Spectrum of 3,4-seco-Acetal (174) (60Mc/s)

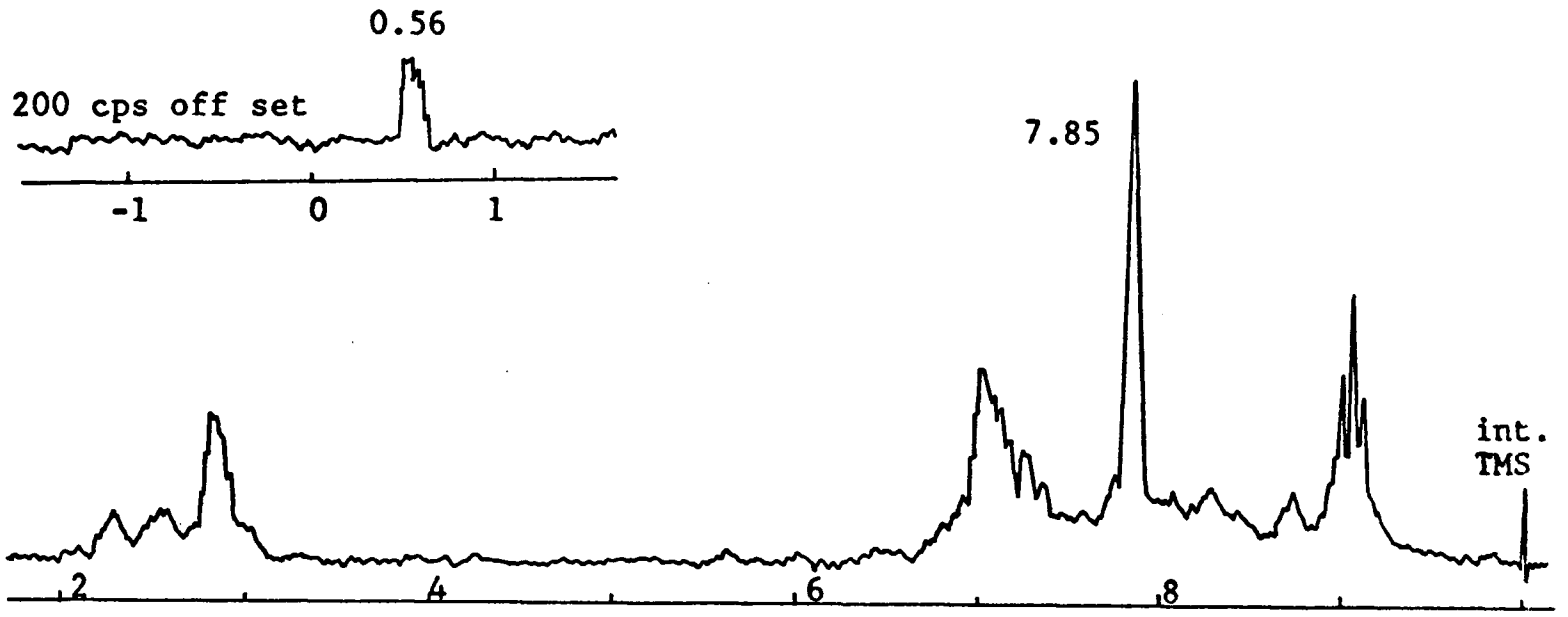


Figure 47 NMR Spectrum of 3,4-seco-Aldehyde(175) (60Mc/s)

cps, $\underline{H}$-C_{17}).

Treatment of the 3,4-seco-acetal (174) with refluxing hydrochloric acid for 5 minutes gave the desired 3,4-seco-aldehyde (175) as shown by the IR spectrum (2835 and 1707 cm^{-1}) and the NMR spectrum (single proton multiplet at τ0.56). This proposal was confirmed by the mass spectrum which showed a molecular ion peak at m/e 312 (91%) compared to that of dihydrocorynantheal at m/e 296 (3%). The tricyclic aldehyde (169) is now a suitable compound for transannular cyclization studies if oxidation to the iminium salt (176) can be achived. Attempted mercuric acetate oxidation of the 3,4-seco-aldehyde (169) under various conditions was not very successful, only 30% of the theoretical amount of mercurous acetate precipitated and prolonged stirring at room temperature did not promote any further oxidation. Thin layer chromatographic examination of the reaction mixture showed only the starting material (169) and very polar material (possibly various iminium salts). No trace of the tetracyclic compound (178), which would not be expected to stay at the base line, could be found.

Since the oxidation of the aldehyde (169) was not very successful, attention was turned to oxidation of the corresponding acetal (174). Mercuric acetate oxidation of the 3,4-seco-acetal (174) proceeded in good yield (85% of the theoretical amount of mercurous acetate was precipitated), however, hydrolysis of the acetal to the desired

aldehyde was a difficult step. Hydrolysis by sodium acetate in acetic acid at room temperature was found to be too mild whereas under reflux only an intractable tar was obtained.

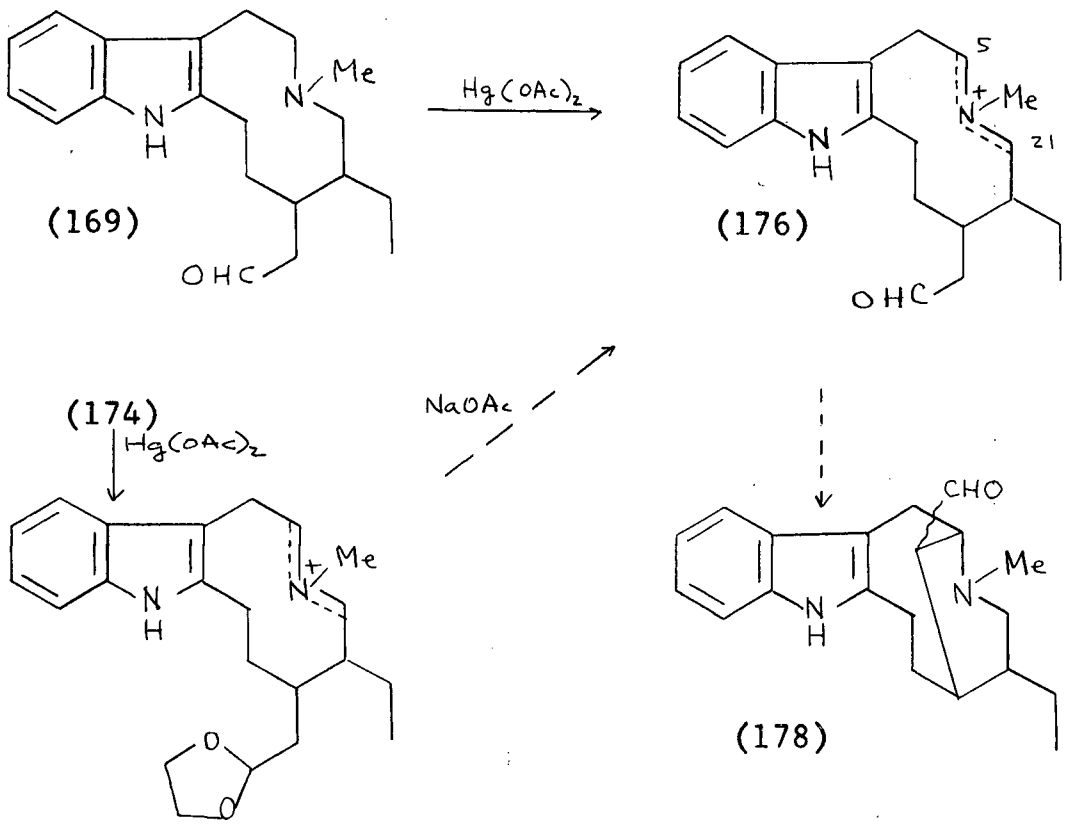


Figure 48

Since most of the difficulties in the proposed synthesis appear to be associated with the aldehydic function, attempts were made to convert this to an ester function (Figure 49). Oxidation of the aldehyde (169) with Jones' reagent and Fisher esterification of the crude oxidation product did not yield any of the desired 3,4-seco methyl ester (179). Oxidation with silver oxide and

esterification with diazomethane again was unsuccessful. Since these initial experiments did not yield the desired ester (179), the more readily available dihydrocorynantheal (171) was used as a model compound to study these reactions.

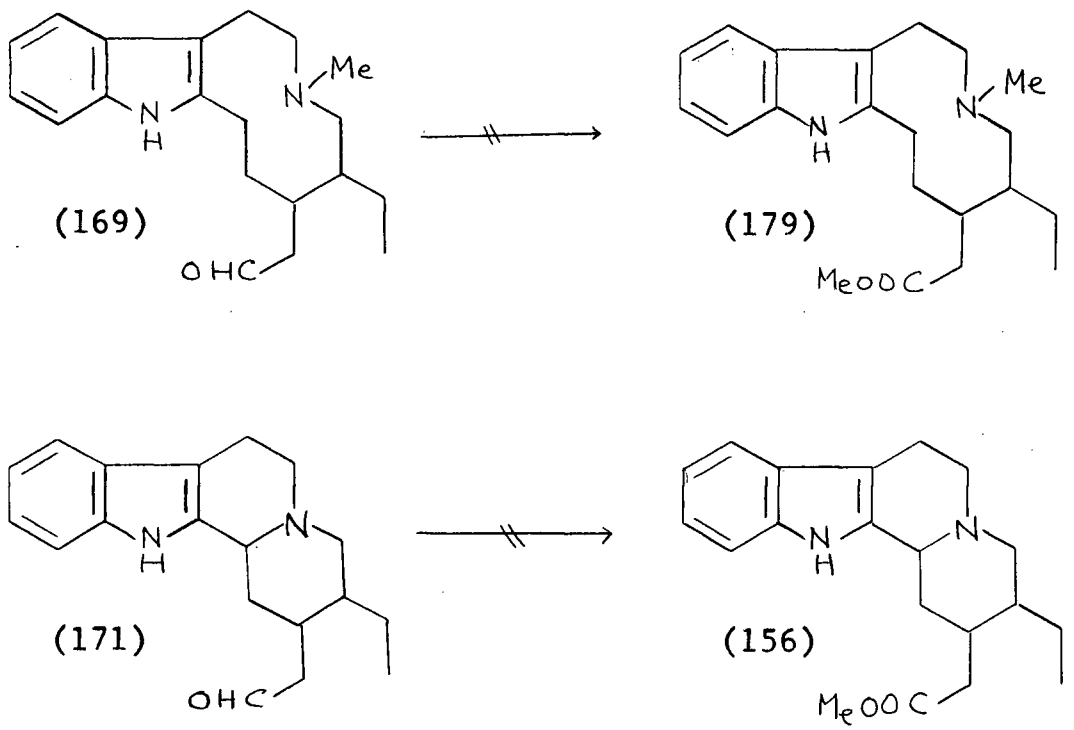


Figure 49

Fourteen different conditions with silver oxide, Jones' or potassium permanganate oxidation and Fisher or diazomethane esterification conditions were tried, but only the starting material or an intractable tar was obtained. Although the desired ester (156) is a known compound and the reaction could be followed by TLC, no trace of the

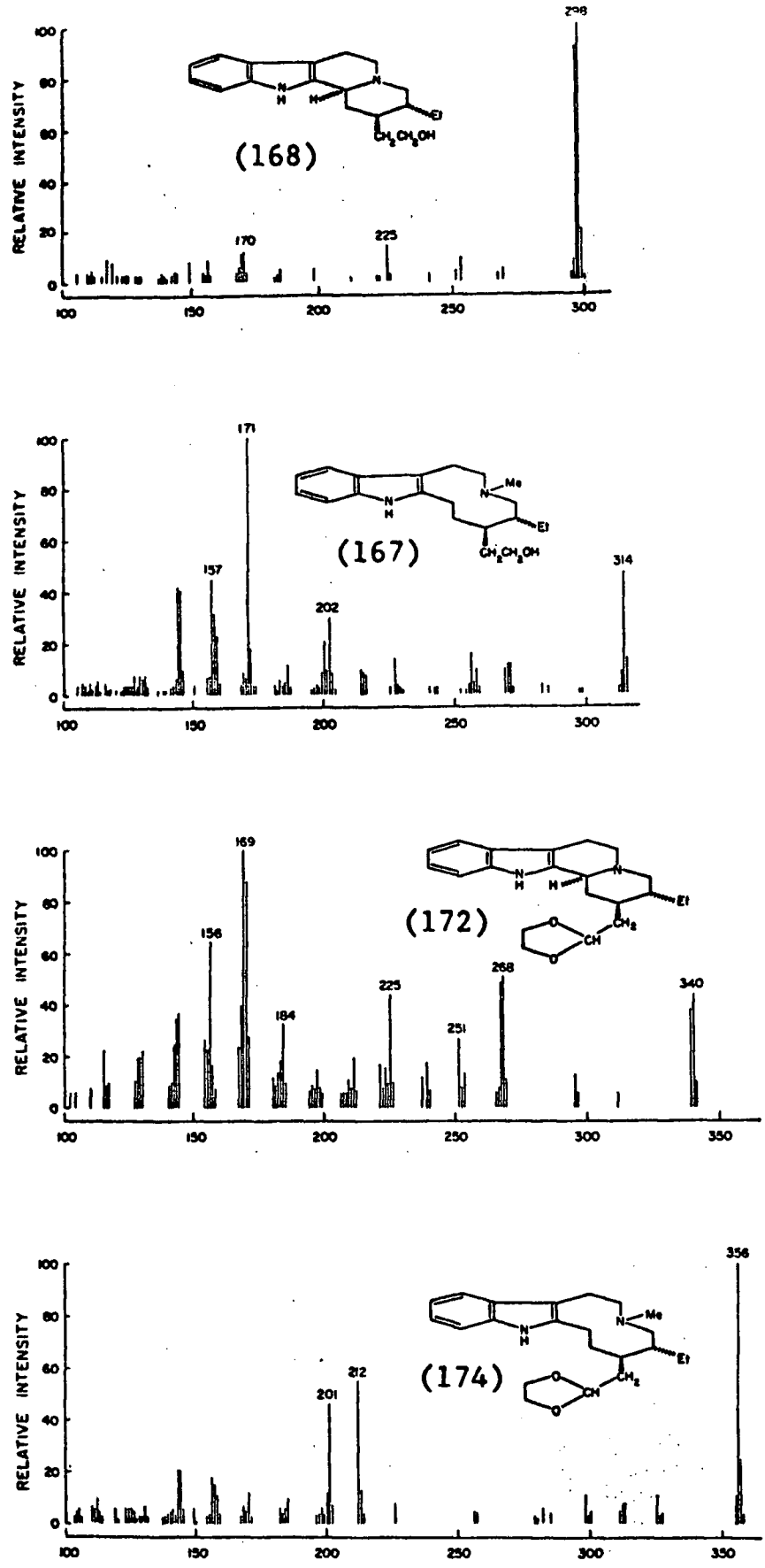
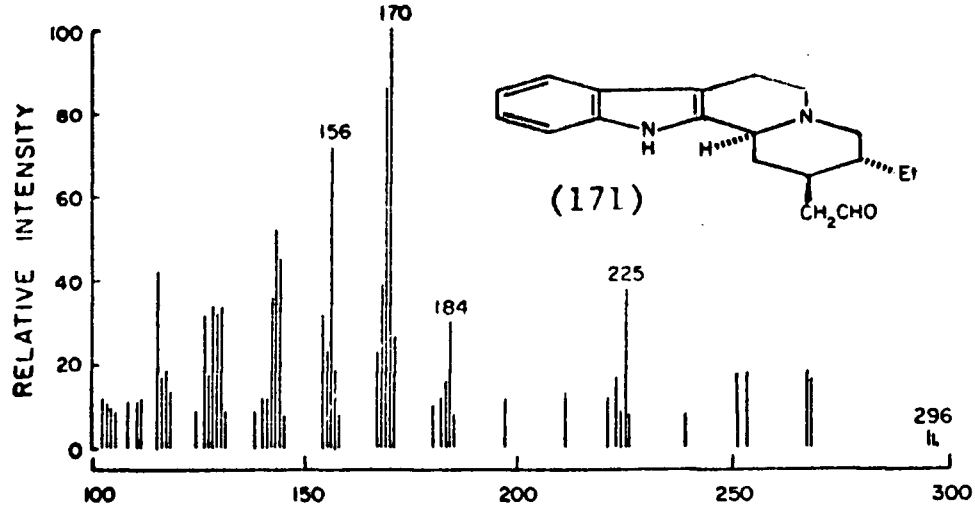

RELATIVE INTENSITY
(168)
170
225
298
(167)
171
157
202
314
(172)
169
156
184
225
251
268
340
(174)
201
212
356

Figure 50


RELATIVE INTENSITY
100
80
60
40
20
0
100
150
200
250
300
170
156
184
225
296
(171)
N
H
H
N
Et
CH2CHO

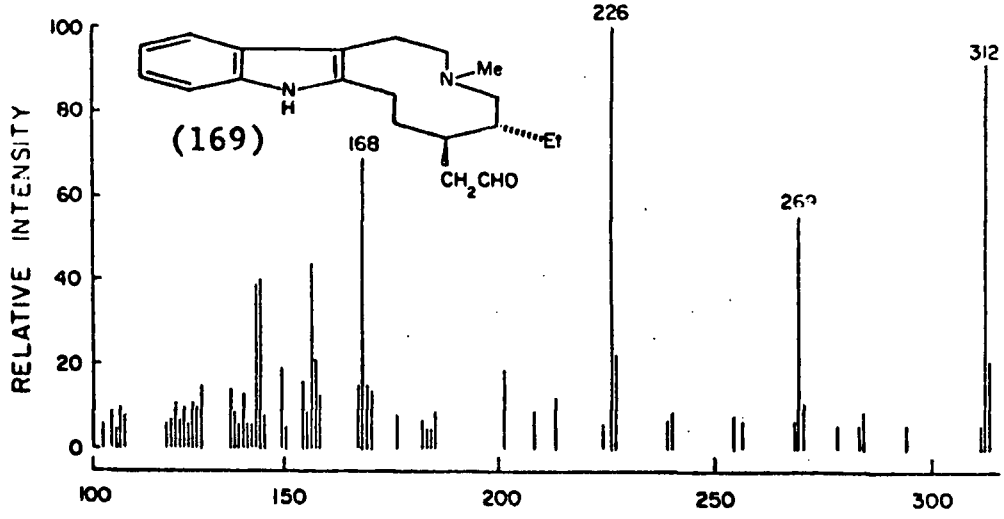
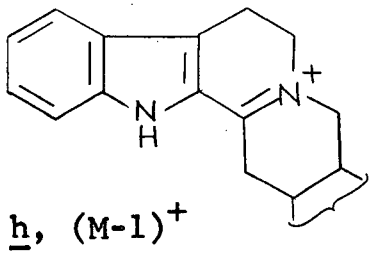

RELATIVE INTENSITY
100
80
60
40
20
0
100
150
200
250
300
226
312
168
(169)
N
H
N
Me
Et
CH2CHO

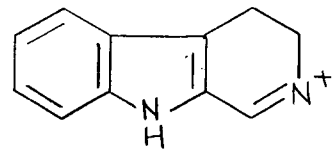
Figure 51

ester (156) could be found in the product mixture from the various reactions. Possibly the indole chromophore had been destroyed by the oxidation mixtures.

The mass spectra of 3,4-seco-corynanthenoid bases have not yet appeared in the literature. Corynanthenoid alkaloids are known to give a strong $(M-1)^+$ peak due to the ion h (see below) and β-carboline-type fragments at m/e 170 (g), m/e 169 and m/e 168 (ions d and e in Figure 43, respectively) under electron impact.



h, $(M-1)^+$



g, m/e 170

The mass spectra of the tricyclic 3,4-seco-bases (Figures 50 and 51) show very weak $(M-1)^+$ peaks as would be expected, since they cannot form a very stable ion of the type h. However the three tricyclic bases prepared did not give very characteristic fragmentation patterns possibly due to the unstable side chains at C-15.

None of the three approaches to study transannular cyclization discussed above were successful, although more detailed investigations were necessary in most cases. Unfortunately, a fire in our laboratory destroyed all the important intermediates necessary for further work at this time. Work is now in progress on a different sequence outlined in Figure 52.

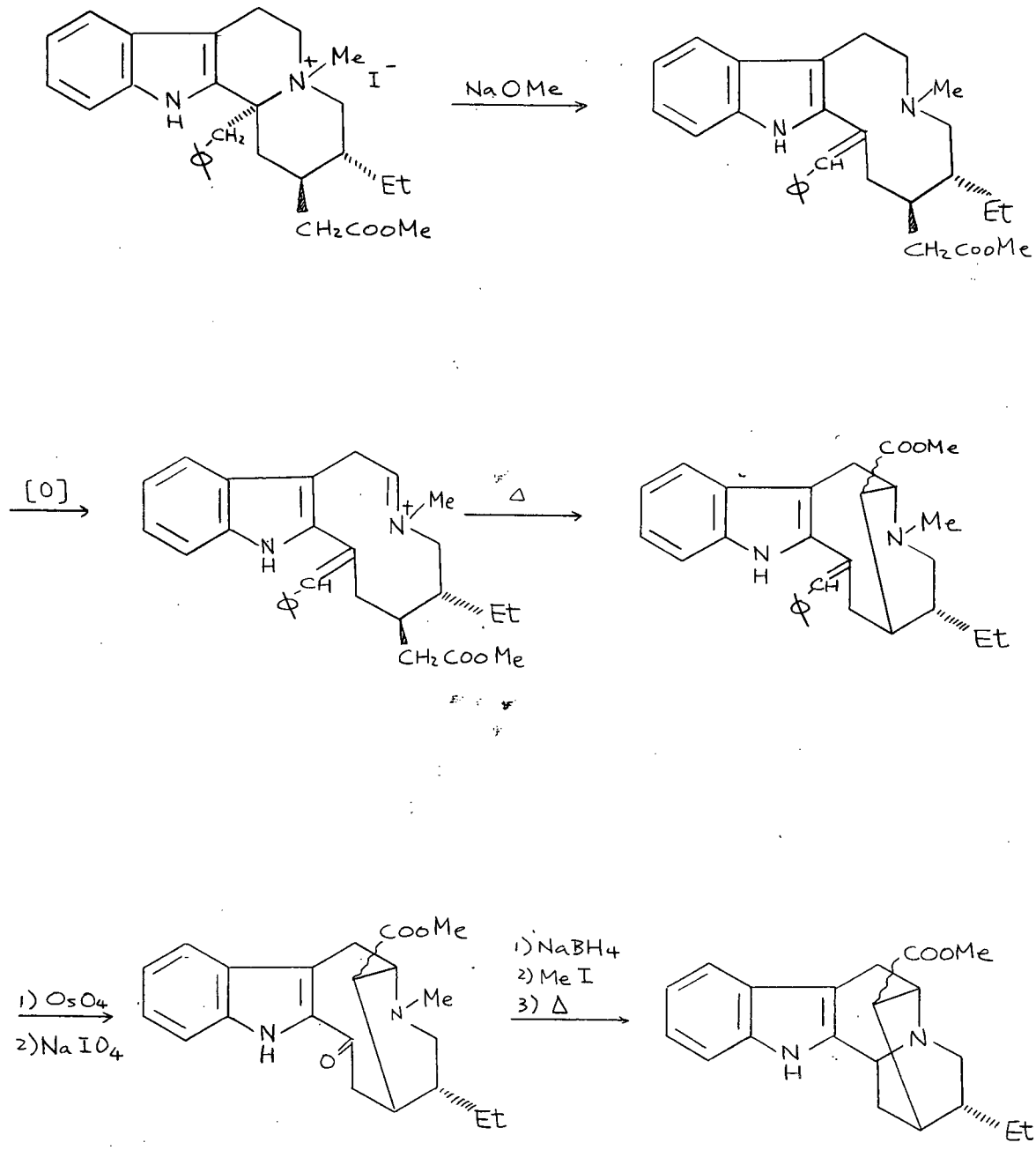


Figure 52 (Proposed Scheme)

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. The ultraviolet (UV) spectra were recorded in methanol solution on a Cary 14 spectrophotometer, and the infrared (IR) spectra were taken on Perkin-Elmer Model 21 and Model 137 spectrophotometers. Nuclear magnetic resonance (NMR) spectra were recorded in deuteriochlroform at 60 Mc/s on a Varian A60 or at 100 Mc/s on a Varian HA100 instrument. The chemical shifts are given in the Tiers τscale with reference to tetramethylsilane as the internal standard. Mass spectra were recorded on an Atlas CH-4 mass spectrometer or on an AEI MS-9 mass spectrometer. Analyses were performed by Dr. A. Bernhardt, Mulheim (Ruhr), Germany and Mr. P. Borda of the microanalytical laboratory, University of British Columbia. Silica gel G and Woelm neutral alumina containing electronic phosphor were used for thin layer chromatography (TLC) and Woelm neutral alumina (activity I) was used for column chromatography.

3-Dehydroyohimbine Perchlorate (131)

The method described by Weisenborn and Diassi[58] was employed.

Yohimbine (7.6 g.) was oxidized by mercuric acetate (7.0 g.) in methanol (50 ml.) at 64° for 30 minutes.

Mercurous acetate was filtered off and hydrogen sulfide was bubbled through the filtrate. The precipitated sulfide was separated by filtration. The filtrate was reduced to a volume of 20 ml. under reduced pressure and was treated with an equimolar amount of perchloric acid to afford crystalline 3-dehydroyohimbine perchlorate (6.0 g.), m.p. 200-205°.

UV, λmax: 246 and 352 mμ. (Lit.[58] m.p. 205-206°, UV, λmax: 246 and 352 mμ)

Reaction of Benzyl Magnesium Bromide with 3-Dehydroyohimbine Perchlorate

a) In Diethyl Ether

A suspension of 3-dehydroyohimbine perchlorate (1 g.) in ether was added to the Grignard reagent (from magnesium (0.8 g.) and benzyl bromide (3.96 ml.)) in ether. The mixture was stirred for 7 hours under reflux. The cooled reaction mixture was poured into aqueous ammonium chloride, the organic layer separated, dried over magnesium sulfate and the solvent evaporated. Chromatography on alumina and elution with benzene gave 1,2-diphenylethane. Elution with ether provided 3α-benzylyohimbine (400 mg.), which crystallized from diisopropyl ether as large prisms, m.p. 155-160°.

IR, νmax (nujol): 3333, 1709 cm^{-1}.

UV, λmax: end absorption (at 230 mμ), 283, 291 mμ.

NMR signals (60 Mc/s): τ 2.4-3.2 (9H, multiplet, aromatic),

3.46 (1H, singlet, indole NH), 6.25 (3H, singlet, CH_3COO-), 7.16 (2H, singlet, $\underline{CH_2}$-C_3).

Mass spectrum: m/e 444 (M^+ Calc. for $C_{28}H_{32}O_3N_2$: 444)

b) In Tetrahydrofuran

3-Dehydroyohimbine perchlorate (500 mg.) was ground to a fine powder and added to the Grignard reagent which was made from magnesium (200 mg.) and benzyl bromide (0.99 ml.) in tetrahydrofuran (100 ml.). After stirring for 2 min. at room temperature, the solution was poured into saturated aqueous ammonium chloride (200 ml.). The organic layer was separated, dried over sodium sulfate and the solvent evaporated leaving white semi-crystalline material, which contained 10% of 3-dehydro base as shown by the UV spectrum. (UV, λmax (neutral): 291, 320 mμ; λ max (acidic): 291 (O.D. 0.47), 353 mμ (O.D. 0.19)) The crystalline material was chromatographed on alumina (30 g.). Elution with benzene gave 1,2-diphenylethane, further elution with benzene-ether (9 : 1) gave a mixture of 3α-and 3β-benzylyohimbine. Further purification by preparative silica gel TLC (ethyl acetata) provided the less polar product (220 mg.), 3α-benzylyohimbine (132), m.p. 155-160° and the more polar product (99 mg.), 3β-benzylyohimbine (133), m.p. 110-115°. The 3α-benzylyohimbine (132) was found to be identical with that from the Grignard reaction in ether, by TLC comparison with several solvent systems. 3β-Benzylyohimbine (133)

showed the typical indole chromophore (λmax: end absorption (at 230 mμ), 283, 291 mμ).

IR, ν max (nujol): 3333, 1709 cm^{-1}.

NMR signals (60 Mc/s): τ 2.3-3.2 (10H, multiplet, aromatic + indole NH), 6.17 (3H, singlet, CH_3COO-), 7.42 (2H, broad singlet, $\phi\underline{CH_2}$-).

Mass spectrum: m/e 444 (M^+ Calc. for $C_{28}H_{32}O_3N_2$: 444).

3-Cyanoyohimbine[51]

The procedure described by Zinnes et al[51] was emplayed. 3-Dehydroyohimbine perchlorate (215 mg.) was dissolved in methanol (20 ml.) and water (15 ml.) and potassium cyanide (93 mg.) added. The solution was stirred for 10 min. at room temperature and the crystalline precipitate of 3-cyanoyohimbine (143 mg.) collected by filtration, m.p. 175° (decomp. softening at 150°).

IR, ν max:2230 (-CN), 1735 (-COOMe) cm^{-1}.

UV, λ max: 233 (O.D. 0.29), 283 (0.43), 292 (0.42), 322 (0.18) mμ. The solution in the UV cell was allowed to stand for 1 hour at room temperature and the UV spectrum was recorded, λmax: 230 (O.D. 0.17), 253 (0.16), 283 (0.22), 292 (0.21), 323 (0.16), 352 (0.26) mμ.

λmax (acidic): 252 (O.D. 0.22), 356 (0.52) mμ.

Zinnes et al[51] reported the following physical constants for 3-cyanoyohimbine (m.p. 175-185°, darkens at 152°, IR (nujol): 3440, 3240, 2300 and 1732 cm^{-1}. UV (95% EtOH), λmax: 228, 257 (Sh), 295 (Sh), 307 and 318 mμ).

Attempted Dehydrogenation of 3-Dehydroyohimbine Perchlorate with 2,3-Dichloro-5,6-dicyanobenzoquinone (DDQ)

A mixture of 3-dehydroyohimbine perchlorate (54 mg.) and DDQ (31 mg., 1.1 mole) in glacial acetic acid (150 ml.) was refluxed for 6 hours. Evaporation of the solvent left a dark brown residue, λmax at 255 (Sh, DDQ), 365 mμ (3-dehydroyohimbine) and there was no indication of aromatization.

Tetradehydroyohimbine Perchlorate (140)

A solution of lead tetraacetate (2.6 g.) in acetic acid (120 ml.) was added dropwise over a 2 hour period to a stirred solution of yohimbine (1 g.) in acetic acid (25 ml.) at 60°. Most of the acetic acid was removed under reduced pressure, chloroform (150 ml.) and water (25 ml.) were added, and the mixture was made alkaline (pH9) by the slow addition of 50% potassium hydroxide. The chloroform solution was dried over sodium sulfate and acidified with methanolic perchloric acid. Upon evaporation the tetradehydroyohimbine perchlorate (0.6 g.) was obtained as needles, m.p. 175-178°. TLC (alumina) showed the presence of a small amount of 3-dehydroyohimbine perchlorate. Purification by column chromatography (alumina) provided the pure tetradehydro salt.

IR, νmax (nujol): 3540, 3190, 1720, 1635, 1570 cm^{-1}.

UV, λmax: 253, 308, 361 mμ.

Anal. Found: C, 54.0; H, 5.6.

Calc. for $C_{21}H_{23}O_3N_2 \cdot ClO_4 \cdot CH_3OH$: C, 54.6; H, 5.6.

Wenkert and Roychaudhri[59] reported the m.p. 200-201° for tetradehydroyohimbine perchlorate. Schlittler et al[60] obtained tetradehydro-α-yohimbine chloride by lead tetraacetate oxidation of 3-epi1-α-yohimbine and the UV spectrum of tetradehydro-α-yohimbine chloride showed λ_{max} (EtOH) at 253, 308 and 361 mμ.

Attempted Grignard Reaction on Tetradehydroyohimbine Perchlorate (140)

Tetradehydroyohimbine perchlorate (50 mg.) was ground to a fine powder and added to the Grignard reagent which was prepared from magnesium (75 mg.) and benzyl bromide (0.4 ml.) in tetrahydrofuran (95 ml.). After stirring for 2 hours under reflux, the insoluble material (30 mg.) was filtered off. This material was found to be the recovered starting material by the UV spectrum. The filtrate was poured into saturated ammonium chloride (100 ml.). The organic layer was separated and dried over sodium sulfate. Evaporation of the solvent gave a brown oil (5 mg.), which was identical with the starting material by TLC and by the UV spectral examination.

3-Dehydrodihydrocorynantheine Perchlorate (143)

A solution of dihydrocorynantheine hydrochloride (2.0 g.), sodium acetate (404 mg.) and mercuric acetate (6.28 g.) in methanol (100 ml.) was stirred for 2 hours

at 40°. The precipitate of mercurous acetate (2.4 g.) was removed by filtration and the filtrate was treated with hydrogen sulfide. The sulfide was filtered off. The filtrate was reduced to a volume of 10 ml. and 60% perchloric acid (1 ml.) was added to give the crystalline perchlorate (1.9 g.). Recrystallization from methanol gave yellow needles, 3-dehydrodihydrocorynantheine perchlorate, m.p. 261-264°.

IR, νmax (KBr): 3260, 1698 (COOMe), 1636 ($>C=N^{+}<$), 1620 ($>C=C-O-$), 1575, 1558 cm^{-1}.

UV, λmax: 240, 352 mμ.

UV (basic), λmax: end absorption (at 250 mμ), 292, 362 mμ.

Anal. Found: C, 56.61; h, 6.00; N, 6.20. Calc. for $C_{22}H_{27}O_3N_2 \cdot ClO_4$: C, 56.6; H, 5.8; N, 6.0.

3α-Benzyldihydrocorynantheine (144)

Benzyl magnesium bromide was prepared by dropwise addition of a solution of benzyl bromide (5.6 ml.) in dry tetrahydrofuran (100 ml.) to a stirred suspension of magnesium (1.2 g.) in tetrahydrofuran (10 ml.) over a period of 30 min. When addition was completed the mixture was stirred at room temperature for 2 hours. To the Grignard reagent was added 3-dehydrodihydrocorynantheine perchlorate (1.9 g.) with vigorous stirring. The reaction mixture was stirred at room temperature for 10 min. and then poured into saturated aqueous ammonium chloride solution (200 ml.). After separation of the layers, the

aqueous layer was extracted with several portions of ether, the combined extracts were dried over sodium sulfate, and were evaporated to dryness. The UV spectrum of the residue indicated the presence of 3-dehydro base (17%) (λmax (acidic): 291 (O.D. 0.25), 352 (O.D. 0.19) mu). The residue was chromatographed on alumina (100 g.). Elution with benzene yielded 1,2-diphenylethane. Elution with benzene-ether (10 : 1) gave the desired 3α-benzyl dihydrocorynantheine (144) as amorphous solid. Crystallization from diisopropyl ether-chloroform gave prisms, m.p. 95-100°.

IR, νmax ($CHCl_3$): 3356 (indole NH), 1692 (ester), 1631 (C=C) cm^{-1}.

UV, λmax (O.D): 226 (1.41), 283 (0.24), 291 (0.21) mμ.

NMR signals (100 Mc/s): τ 1.40 (1H, multiplet, -C=C<u>H</u>OMe), 2,5-3.2 (9H, multiplet, aromatic), 3.40 (1H, broad singlet, indole NH), 6.27 and 6.35 (6H, two singlets, -COO<u>CH</u>$_3$ and CH_3O-C=C).

Mass spectrum: m/e 458 (M^+ Calc. for $C_{29}H_{34}O_3N_2$: 458).

The methiodide was prepared in the usual manner. Recrystallization from diisopropyl ether-methanol gave prisms, m.p. 225-231°. Anal. Found: C, 60.28; H, 6.45; O, 8.20; N, 4.70. Calc. for $C_{30}H_{37}O_3N_2I$: C, 59.99; H, 6.21; O, 7.99; N, 4.66.

Mercuric Acetate Oxidation of 3α-Benzyldihydrocorynantheine (144) followed by attempted Demethylation

3α-Benzyldihydrocorynantheine (84 mg.) was added to a solution of mercuric acetate (238 mg.) in methanol (15 ml.) containing acetic acid (10 drops) and the mixture was stirred at room temperature for 22 hours. Mercurous acetate (74 mg., 77%) was separated. The UV spectrum showed an indole chromophone (λmax, end absorption (at 230 mμ), 285, 292 mμ).

IR, ν max (nujol): 1692 ($COOCH_3$), 1631 (MeO-C=C-) cm^{-1}.

TLC examination of the product showed no starting material (silica gel G). The filtrate was treated with hydrogen sulfide and the sulfide was filtered off. Evaporation of the filtrate gave a light brown residue, which was demethylated with hydrogen bromide in methyl acetate at room temperature for 30 min. and the solvent was evaporated off to yield a brown intractable mixture of more than 9 compounds.

Attempted Demethylation of 3α-Benzyldihydrocorynantheine (144)

a) Hydrochloric Acid in aqueous Methanol

3α-Benzyldihydrocorynantheine (7.5 mg.) in 50% aqueous methanol (2 ml.) was treated with concentrated hydrochloric acid (0.1 ml.). The mixture was allowed to stand at room temperature for 1 hour. TLC (silica gel G) examination of the reaction mixture showed only the starting material.

The mixture was refluxed for 30 min., but no reaction took place as shown by TLC.

b) Hydrogen Chloride in Acetone

3α-Benzyldihydrocorynantheine (50 mg.) in acetone (5 ml.) saturated with hydrogen chloride was allowed to stand for 25 hours at 0°. TLC showed no starting material and none of the spots (streak) on TLC exhibited a positive ferric chloride reaction.

c) Boron Trifluoride Etherate

3α-Benzyl-dihydrocoynantheine (61 mg.) with BF_3. etherate (1 ml.) was heated at 90° for 30 min. and no starting was shown on TLC (silica gel G, EtOAc). The mixture was poured into water and basified with aqueous ammonia. The aqueous solution was extracted with ether and the organic layer was dried over sodium sulfate. Evaporation of the solvent gave an amorphous yellow solid. TLC showed the presence of more than 7 compounds one of which gave a positive ferric chloride test, but further purification by preparative TLC failed due to decomposition of the demethyl derivative.

d) Hydrogen Bromide in Methyl Acetate

3α-Benzyldihydrocorynantheine (50 mg.) was treated with methyl acetate (10 ml.) saturated with hydrogen bromide. The mixture was allowed to stand at room

temperature for 44 hours. TLC (silica gel G) showed a trace of starting material and seven more compounds. Further purification by preparative TLC failed due to decomposition of the products.

e) Hydrogen Bromide in Acetone

3α-Benzyldihydrocorynantheine (50 mg.) was dissolved in acetone (15 ml.) and hydrogen bromide was bubbled through the mixture for 30 min. at 0°. The mixture was allowed to stand overnight at 0°. TLC showed no starting material (positive ferric chloride test), however, purification by column chromatography (alumina) was not successful.

f) Boron Tribromide in Dichloromethane

3α-Benzyldihydrocorynantheine (15 mg.) was dissolved in dichloromethane (7 ml.) and boron tribromide (0.1 ml.) was added. The reddish mixture was stirred at 0° for 2 hours until no starting material was detected. No indole chromophore was present as shown by the UV spectrum (λmax: 229, 304 mu).

g) Boron Trichloride in Dichloromethane

3α-Benzyldihydrocorynantheine (20 mg.) was dissolved in dichloromethane (15 ml.) and boron trichloride was bubbled through the mixture at 0° for 20 min. Only the starting meterial was detected by TLC (silica gel G).

The reaction mixture was allowed to stand at room temperature for further 2 hours, and TLC showed no starting material. The basified mixture (with aqueous ammonia) showed no carbonyl absorptions in the IR spectrum and no indole chromophore in the UV spectrum.

dl-3-Dehydrodihydrocorynantheic Acid Methyl Ester Perchlorate (149)

dl-Dihydrocorynantheic acid methyl ester[61] (1 g.), kindly supplied by Dr. B. Douglas, Smith Kline and French Laboratories, Philadelphia, was dissolved in methanol (50 ml.) and treated with a solution of mercuric acetate (4.1 g., 4 mole) in methanol (100 ml.). The mixture was refluxed for 1 hour, cooled to room temperature and the precipitate of mercurous acetate filtered off. Hydrogen sulfide was passed through the filtrate for 15 min. and the resulting sulfide filtered off. The filtrate was reduced to a volume of 10 ml. and perchloric acid (60%, 0.4 ml.) was added to give a yellow precipitate of 3-dehydro perchlorate (1.5 g.). Recrystllization from methanol afforded an analytical sample of dl-3-dehydrodihydrocorynantheic acid methyl ester perchlorate (149), m.p. 209-212°.

IR, ν max (KBr): 3220, 1727 (C=O), 1643 ($C=N^+$), 1568, 1550 cm^{-1}.

UV, λ max (ϵ): 247 (16,200), 352 (21,300) mμ, λ min: 231 (14,500), 280 (6,000) mμ.

Anal. Found: C, 56.6; H, 6.1. Calc. for $C_{20}H_{25}N_2O_2 \cdot ClO_4$; C, 56.53; H, 5.93.

dl-3-Dehydrodihydrocorynantheic Acid Ethyl Ester Perchlorate (150)

dl-Dihydrocorynantheic acid ethyl ester (1.6 g.) was oxidized by mercuric acetate (6.5 g., 4 mol.) as previously detailed for the methyl ester. The crude yellow perchlorate (1.7 g.) was recrystallized from ethanol to give prisms, m.p. 215-218°.

UV, λmax: 246, 352 mμ.

UV (basic), λmax: end absorption (at 230 mμ), 292, 362 mμ.

Anal. Found: C, 57.62; H, 5.98; N, 6.10.

Calc. for $C_{21}H_{27}O_2N_2 \cdot ClO_4$: C, 57.45; H, 6.20; N, 6.38.

Attempted Synthesis of dl-3-Cyanodihydrocorynantheic Acid Methyl Ester

dl-3-Dehydrodihydrocorynantheic acid methyl ester (25 mg.) in 50% aqueous methanol (30 ml.) was treated with potassium cyanide (11 mg.). No precipitates had formed after 10 min., but the UV spectrum of the reaction mixture (λmax (O.D.): 235 (0.33), 284 (0.58), 292 (0.59), 307 (0.44), 319 (0.42) mμ) did not correspond to that of the starting material (143). The solvent was evaporated off under reduced pressure and further purification by chromatography failed to give any pure compounds. However the peaks at 284 and 292 mμ are probably due to the desired

3-cyano-ester and the peaks at 307 and 319 mμ due to 3-dehydro base after loss of HCN.

dl-3α-Benzyldihydrocorynantheic acid methyl ester (151)

dl-3-Dehydrodihydrocorynantheic acid methyl ester perchlorate (1.15 g.) was added, with vigorous stirring, to the Grignard reagent prepared from magnesium (0.6 g.) and benzyl bromide (3 ml.) in tetrahydrofuran (130 ml.) as previously described. The mixture was stirred for 1 min. at room temperature and then poured into saturated aqueous ammonium chloride (200 ml.). A usual work-up gave a brownish oil. According to the UV spectrum of this oil, approximately 3% of 3-dehydro base was present (λmax (acidic): 291 (O.D. 0.70), 352 (O.D. 0.09) mμ). The oil was chromatographed on alumina and elution with benzene-ether (5 : 1) yielded the desired 3-benzyl derivative. Recrystallization from petroleum ether (30-60°) gave needles (485 mg.), 3α-benzyldihydrocorynantheic acid methyl ester (151), m.p. 141-144°. (perchlorate, m.p. 225-233°)
IR (KBr): 3440 (indole NH), 1730 (COOMe), 1600 (phenyl)cm^{-1}.
UV, λmax (ϵ): 225 (38,500), 275 (Sh, 7,000), 283 (8,300), 291 (6,800) mμ, λmin (ϵ): 252 (2,400), 288 (6,000) mμ.
NMR signals (60 Mc/s): τ 2.4-3.2 (9H, multiplet, aromatic), 3.35 (1H, broad singlet, indole NH), 6.29 (3H, singlet, COOMe), 7.18 (2H, broad singlet, ϕ-$\underline{CH_2}$-C-3).
Anal. Found: C, 77.19; H, 7.65; N, 7.12. Calc. for $C_{27}H_{32}O_2N_2$: C, 77.67; H, 7.74; N, 6.7.

Mass spectrum: m/e 416 (M^+ Calc. for $C_{27}H_{32}O_2N_2$: 416). The methiodide was prepared in a usual manner, m.p. 194-199° (MeOH).

Anal. Found: C,58.90; H, 6.66; N, 4.94. Calc. for $C_{27}H_{32}O_2N_2$.MeI.MeOH: C, 58.5; H, 6.66; N, 4.75.

dl-3α-Benzyldihydrocorynantheic Acid Ethyl Ester

dl-3-Dehydrodihydrocorynantheic acid ethyl ester perchlorate (1.6 g.) was treated with the Grignard reagent prepared from magnesium (1.0 g.) and benzyl bromide (4.8 ml.) in absolute tetrahydrofuran (125 ml.) as previously described. Recrystallization of the crude material from petroleum ether (30-60°) gave 720 mg. of 3α-benzyl-derivative (152) as plates, m.p. 121-125°. (perchlorate, m.p. 216-222°).

UV, λmax: 225, 275 (Sh), 283, 291 mμ.

Anal. Found: C, 77.86; H, 7.77; N, 6.53.

Calc. for $C_{28}H_{34}O_2N_2$: C, 78.09; H, 7.96; N, 6.51.

Mass spectrum: m/e 430 (M^+ Calc. for $C_{28}H_{34}O_2N_2$: 430).

Mercuric Acetate Oxidation of dl-3α-Benzyldihydrocorynantheic Acid Methyl Ester (151)

A mixture of the 3α-benzyl derivative (151) (16.4 mg.) and mercuric acetate (31 mg.) in acetic acid was heated at 90° for 2 hours and the resulting mercurous acetate (13.1 mg., 65%) was filtered off. The reaction mixture was treated with sodium borohydride and worked up as usual.

TLC on silica gel G showed at least six spots and the major spot corresponded to the starting material. Further purification was not attempted.

Preparation of Methylsulfinyl Carbanion

The procedure described by Corey and Chaykovsky[57] was employed. Sodium hydride (3.0 g., 50% mineral oil dispersion; Metal hydrides, Inc.,) was placed in a two-necked 50 ml. flask and washed three times with petroleum ether, by swirling, allowing the hydride to settle, and decanting the liquid portion in order to remove the mineral oil. The flask was immediately fitted with an inlet of nitrogen gas and a rubber cap through which reagents can be introduced via hypodermic syringe. A three-way stopcock, connected to the flask, was connected to a water aspirator and a source of dry netrogen. The system was evacuated until the last traces of petroleum ether were removed from the sodium hydride and was then replaced under nitrogen by evacuating and filling with nitrogen several times. Dimethyl sulfoxide (20 ml., distilled from calcium hydride, b.p. 76° at 3.5 mm Hg) was introduced and the mixture was heated with stirring to 70-75° until the evolution of hydrogen ceased (approximately 60 min.). A somewhat cloudy, pale yellow-grey solution contained 3 moles/liter of sodium salt.

Reaction of dl-Dihydrocorynantheic Acid Methyl Ester (156) with Methylsulfinyl Carbanion and subsequently with Aluminum Amalgam

dl-Dihydrocorynantheic acid methyl ester (62 mg.) was dissolved in dry tetrahydrofuran (5 ml.) with a trace of triphenylmethane. The standard solution of methylsulfinyl carbanion sodium salt as described above (1.1 ml., 16.5 mole) was introduced via hypodermic syringe over a period of 15 min. at 0° and the resulting mixture was stirred for a further 20 min. at room temperature. The reaction mixture was then poured into water and acidified with dilute hydrochloric acid. The acidic solution was brought to pH 8 by aqueous sodium bicarbonate and extracted with chloroform. The extracts were dried over sodium sulfate and evaporated to dryness. The oily residue was crystallized from ethyl acetate to eliminate a small amount of the satrting material (checked by silica gel G TLC plate, 20% EtOAc in benzene).

The purified β-keto sulfoxide, in 10% aqueous tetrahydrofuran (20 ml.), was reacted with aluminum amalgam prepared as follows. Alminum foil (54 mg.) was cut into strips approximately 10 cm. x 1 cm. and immersed, all at once, into a 2% aqueous mercuric chloride for 15 seconds. The strips were rinsed with absolute ethanol and then with ether and cut immediately with scissors, into pieces 1 cm. square, directly into the reaction vessel. The reaction mixture was heated at 65° for 40 min. and the white solid

was filtered off. The filtrate was concentrated to remove most of the tetrahydrofuran, chloroform was added and the chloroform phase was separated from the water, dried over sodium sulfate, and evaporated to leave the oily methyl ketone (50 mg.). Crystallization from petroleum ether (30-60°)-ether yielded the methyl ketone (157) as prisms, m.p. 167-172°.

IR, νmax ($CHCl_3$): 3370 (indole NH), 1695 (C=O) cm^{-1}.

UV, λmax (ϵ): 225 (39,500), 274 (Sh, 8,150), 282 (8,400), 290 (7,100) mμ, λmin (ϵ): 247 (4,200), 288 (6,850) mμ.

NMR signals (60 Mc/s): τ 2.4-3.0 (5H, multiplet, aromatic + indole NH), 7.8 (Sh, singlet, CH_3CO-), 9.03 (3H, triplet, $\underline{CH_3}CH_2$-).

Mass spectrum: m/e 310 (M^+ Calc. for $C_{20}H_{26}ON_2$: 310).

Reaction of dl-3α-Benzyldihydrocorynantheic Acid Methyl Ester (151) with Methylsulfinyl Carbanion and with Aluminum Amalgam

dl-3α-Benzyldihydrocorynantheic acid methyl ester (200 mg.), in tetrahydrofuran (15 ml.), was treated with 3.4 ml. of the standard solution of methylsulfinyl ion sodium salt prepared as previously described (3 moles/liter solution). The resulting β-ketosulfoxide was crystallized from ethyl acetate to yield 143 mg. of white needles. Reduction of the β-ketosulfoxide (124 mg.) with the amalgam prepared from aluminum (75 mg.) gave the desired methylketone (158) (104 mg.). Recrystallization

from diisopropyl ether gave 3α-benzyl methyl ketone (158) as needles, m.p. 115-120°.

IR, νmax (nujol): 3484 (NH), 1718 (C=O), 1592 (phenyl) cm^{-1}.

UV, λmax: 225, 275 (Sh), 283, 291 mμ.

NMR signals (60 Mc/s): τ 2.4-3.2 (9H, multiplet, aromatic), 3.58 (1H, broad singlet, indole NH), 7.20 (2H, broad singlet, ϕ-$\underline{CH_2}$-), 7.88 (3H, singlet, CH_3CO-).

Mass spectrum: m/e 400 (M^+ Calc. for $C_{27}H_{32}ON_2$: 400).

Mercuric Acetate Oxidation of 3α-Benzyl Methyl Ketone (158)

A mixture of 3α-benzyl methyl ketone (140) (63 mg.) and mercuric acetate (210 mg.) in methanol (25 ml.) containing acetic acid (15 drops) was stirred at room temperature for 26 hours. Mercurous acetate precipitated was separated by filtration and weighed (79 mg., 96%). Hydrogen sulfide was bubbled through the filtrate and the sulfide was filtered off. The solvent was evaporated to dryness and the residue was distributed between dichloromethane and aqueous sodium bicarbonate. The organic layer was separated and dried over sodium sulfate. Evaporation of the solvent afforded a light brown residue which was chromatographed on alumina (10 g.). Elution with dichloromethane yielded an oil (containing an indole chromophore checked by the UV spectrum), which was treated with methanolic perchloric acid to afford the crystalline perchlorate (25 mg.), m.p. 236-241°. The starting material gave the perchlorate, m.p. 232-237°, on treatment with methanolic perchloric acid.

A mixed m.p. of the above two perchlorates showed a significant depression, m.p. 212-215°.

IR, νmax (film): 3289 (NH), 1709 (C=O) cm^{-1}.

UV, λmax: 226, 283, 290 mμ.

NMR (100 Mc/s): τ 2.5-3.3 (9H, multiplet, aromatic), 3.45 (1H, singlet, indole NH), 7.90 (3H, singlet, CH_3CO-).

Mass spectrum: m/e 400 (M^+ Calc. for $C_{27}H_{32}ON_2$: 400).

Synthesis of dl-Dihydrocorynantheol (168)[71,72]

dl-Dihydrocorynantheic acid ethyl ester (200 mg.) was reduced with lithium alminum hydride (400 mg.) in tetrahydrofuran (25 ml.) by refluxing for 3 hours. The usual work-up yielded a white solid (168 mg., 96%), which was crystallized twice from chloroform to give an analytical sample, dl-dihydrocorynantheol, m.p. 185-188°.

IR, νmax (CCl_4 film): 3155 cm^{-1} (hydroxyl).

UV, λmax (ϵ): 226 (20,000), 274 (Sh, 6,080), 279 (Sh, 6,330), 290 (5,300), λmin(ϵ); 247 (1,800), 288 mμ (5,100).

NMR signals (100 Mc/s): τ 1.92 (1H, singlet, indole NH), 2.5-3.0 (4H, multiplet, aromatic), 6.32 (2H, triplet, J=6 cps, $-CH_2\underline{CH_2}-OH$).

Anal. Found: C, 76.44; H, 8.70; O, 5.28; N, 9.60.

Calc. for $C_{19}H_{26}ON_2$: C, 76.47; H, 8.78; O, 5.36; N, 9.39.

Mass spectrum: m/e 298 (M^+ Calc. for $C_{19}H_{26}ON_2$: 298).

Sodium Borohydride Reduction of Dihydrocorynantheal (171)

Dihydrocorynantheal (5 mg.), prepared from naturally

occurring corynantheine as described later, was treated with sodium borohydride (5 mg.) in methanol (2 ml.) at room temperature for 10 min. The methanol was evaporated and the white residue was extracted with chloroform. Evaporation of the solvent gave an oil (5 mg.); one spot on TLC (alumina, $CHCl_3$). This oil had identical R_f with dl-dihydrocorynantheol (167) by TLC (silica gel, alumina).

Birch Reduction of dl-dihydrocorynantheol Methiodide

dl-Dihydrocorynantheol (168) (150 mg.) was added to methyl iodide (2 ml.) and methanol (5 ml.), the mixture was kept at room temperature for 12 hours, and the excess reagent was removed under reduced pressure. The residue, in liquid ammonia (50 ml.), was treated with sodium (280 mg.), which was added in small pices with stirring during 30 min., the blue color being allowed to discharge between each addition. On completion of the reaction, ammonium chloride was added and ammonia was evaporated off by passing a stream of nitrogen through. The residue was extracted with chloroform and evaporation of the solvent gave an amorphous solid (155 mg.) which was chromatographed on alumina. Elution with chloroform gave dl-N_b-methyl-3,4-seco-dihydrocorynantheol (167) (53 mg.).

IR, νmax ($CHCl_3$): 3200 (OH) cm^{-1}.

UV, λmax; 226 (O.D. 1.34), 284 (0.31), 291 (0.27).

NMR signals (60 Mc/s): τ 2.4-3.1 (5H, aromatic protons and

indole NH), 7.69 (3H, singlet, CH_3-N_b).
Mass spectrum: m/e 314 (M^+ Calc. for $C_{20}H_3ON_2$: 314).
Further elution with methanol gave the recovered methiodide (89 mg.) identified by spectral and TLC comparisons with an authentic sample.

Birch Reduction of dl-Dihydrocorynantheic Acid Ethyl Ester Methiodide

The methiodide (95 mg.), prepared from dl-dihydrocorynantheic acid ethyl ester (126) in the usual manner, was added to a blue solution of sodium (130 mg.) in liquid ammonia (11 ml.) with t-butanol (1 ml.). The mixture was stirred for 2 min., then ammonium chloride was added to destroy excess sodium. Ammonia was allowed to evaporate and water and chloroform were added. Evaporation of the chloroform layer yielded a white solid (22 mg.), which was identified as dl-N_b-methyl-3,4-seco-dihydrocorynantheol (167) by direct comparison with the Birch reduction product of dl-dihydrocorynantheol methiodide (TLC, UV, IR). The aqueous layer was evaporated to give a solid, which was extracted with chloroform. Evaporation of the chloroform yielded the starting material (55 mg.) (UV, λ max; 220, 272, 282, 289 mμ).

Attempted Conversion of dl-3,4-seco-N_b-Methyldihydrocorynantheol (167) into dl-3,4-seco-N_b-Methyldihydrocorynantheal (169)

The tricyclic alcohol (167) (33 mg.) in dry dimethylsulfoxide (2 ml.) was treated with phosphoric acid (15 mg.) followed by dicyclohexylcarbodiimide (80 mg.).[65] The mixture was allowed to stand at room temperature for 24 hours and poured into aqueous sodium bicarbonate. The mixture was extracted with ether and the ether layer was separated. Evaporation of the solvent gave semi-crystalline material (24 mg.). TLC (alumina) indicated that this material contained mostly the starting material and a very small spot corresponding to the tricyclic aldehyde (169) could be detected.

Preparation of Dihydrocorynantheine (170)

Corynantheine tartrate (1.4 g.), kindly supplied by Professor R. Goutarel of C.N.R.S., Institut de Chimie des Substances Naturalles de Gif-sur-Yvette, France, was treated with aqueous sodium bicarbonate to give a free base corynantheine (995 mg.). Corynantheine (995 mg., M.W. 366), in ethanol (100 ml.), was hydrogenated over 10% palladium on charcoal and 1 mole of hydrogen was absorbed. The catalyst was filtered off and evaporation of the solvent yielded dihydrocorynantheine (990 mg.), which showed one spot on TLC (silica gel G, EtOAc) and was identical with the sample prepared by R. T. Brown[67].

Preparation of Dihydrocorynantheal (171)[68]

A mixture of dihydrocorynantheine (325 mg.), potassium

hydroxide (128 mg.), methanol (5 ml.) and water (1.5 ml.) was refluxed for 7 hours under an atmosphere of nitrogen. The cooled reaction mixture was poured into water (85 ml.) containing concentrated hydrochloric acid (7.3 ml.). The resulting solution was refluxed for 4 hours, then cooled in an ice-bath, made basic by addition of concentrated ammonia water (10 ml.) and extracted thoroughly with chloroform. The combined extracts were washed with a small amount of water and dried over anhydrous magnesium sulfate. Removal of the solvent afforded dihydrocorynantheal (171) as a brownish solid (277 mg.).

IR, ν max ($CHCl_3$): 3390 cm^{-1}(NH), 2703 (-CHO), 1718 (C=O).

UV, λ max: 226, 283, 290 mμ.

Mass spectrum: m/e 296 (M^+ Calc. for $C_{19}H_{24}ON_2$: 296).

Dihydrocorynantheal Ethylene Acetal

A mixture of crude dihydrocorynantheal (700 mg.) and p-toluenesulphonic acid (820 mg.) in ethylene glycol (20 ml.) was heated with stirring in a small vacuum distillation apparatus at 110° and 24 mm Hg pressure for 3 hours. Approximately 3 ml. of distillate was obtained. The cooled reaction mixture was then poured into 100 ml. of ice cold 2N sodium bicarbonate solution and the resulting mixture was extracted with chloroform. The extracts were washed with a small amount of water and then dried over anhydrous sodium sulfate. Removal of the solvent gave a crude brownish material (820 mg.)

which was chromatographed on alumina (40 g.). Elution with benzene/ethyl ether (3 : 1) gave dihydrocorynantheal ethylene acetal (172) (405 mg.), m.p. 219-223° (needles).
IR, ν max ($CHCl_3$): 3380 (NH) cm^{-1}, no C=O absorption.
UV, λ max; 226, 283, 290 mμ.
NMR signals (60 Mc/s): τ 2.1-3.1 (5H, multiplet, aromatic H and indole NH), 5.30 (1H, triplet, J=5 cps, $-\underline{CH}\langle^{O-}_{O-}$), 6.26 (4H, singlet, $-O-CH_2-CH_2-O-$), 7.83 (3H, singlet, CH_3-N_b).
Mass spectrum: m/e 340 (M^+ Calc. for $C_{21}H_{28}O_2N_2$: 340).

Birch Reduction of Dihydrocorynantheal Ethylene Acetal Methiodide (173)

Dihydrocorynantheal ethylene acetal (412 mg.) was added to a solution of methyl iodide (2 ml.) in methanol (50 ml.) and the mixture was allowed to stand at room temperature for 18 hours. Evaporation of the solvent gave the yellow methiodide (583 mg.) which showed no spot corresponding to the starting material on TLC. The methiodide (173), without further purification, was subjected to a Birch reduction.

The methiodide (173) (101 mg.) was suspended in liquid ammonia (25 ml.) and absolute ethanol (0.7 ml.) was added. Sodium (280 mg.) was added in small pieces over a period of 20 min. with continuous stirring. Ammonium chloride was then added until blue color disappeared. Ammonia was allowed to evaporate with a

stream of nitrogen to yield a white residue, which was extracted with ether first and subsequently with chloroform. Evaporation of ether and chloroform yielded a white solid (55 mg.) and a yellow residue (44 mg.), respectively.

The ether extract showed two spots on TLC (alumina, chloroform) and was purified by preparative TLC. The more polar compound (10 mg.), m.p. 220-223°, was identical with dihydrocorynantheal ethylene acetal (172) (TLC, UV, mixed m.p.). The less polar compound (32 mg.) was the desired 3,4-seco-N_b-methyl-dihydrocorynantheal ethylene acetal (174), m.p. 173-176° (plates).

IR, ν max ($CHCl_3$): 3413 cm^{-1} (NH).

UV, λmax; 225, 282, 290 mμ.

NMR signals (60 Mc/s): τ2.29 (1H, singlet, indole NH), 2.4-3.1 (4H, multiplet, aromatic), 5.30 (1H, triplet, J=5 cps, -C<u>H</u>$\langle^{O-}_{O-}$), 6.29 (4H, singlet, -O-CH_2CH_2-O-), 7.86 (3H, singlet, CH_3-N_b), 9.12 (3H, triplet, <u>CH_3</u>-CH_2-).

Anal. Found: C, 74.09; H, 8.87; N, 7.85; O, 8.87.

Calc. for $C_{22}H_{32}O_2N_2$: C, 74.12; H, 9.05; N, 7.86; O, 8.98.

Mass spectrum: m/e 356 (M^+ Calc. for $C_{22}H_{32}O_2N_2$: 356).

<u>3,4-seco-N_b-Methyldihydrocorynantheal (169)</u>

3,4-seco-N_b-Methyldihydrocorynantheal ethylene acetal (174) (40 mg.) in 2N hydrochloric acid (5 ml.) was refluxed for 7 min. The cooled reaction mixture was neutralized with aqueous sodium bicarbonate and extracted with chloroform. The extracts were dried over sodium sulfate and

evaporation of the solvent gave a brownish solid (38 mg.). Crystallization from ether-hexane afforded an analytical sample of the aldehyde (169), m.p. 149.5-152.5.

IR, νmax (KBr): 3420 (NH), 2835 (-CHO), 1707 (C=O) cm^{-1}.

UV, λmax (ϵ): 226 (24,300), 283 (5,570), 291 (5,010), λmin (ϵ): 250 (2,010), 288 (4,860) mμ.

NMR signals (60 Mc/s): τ 0.56 (1H, multiplet, -CHO), 2.1-3.1 (5H, multiplet, aromatic H and indole NH), 7.85 (3H, singlet, N_b-CH_3), 9.08 (3H, triplet, J=3 cps, CH_3CH_2-).

Anal. Found: C, 76.59, H, 9.15; N, 8.84; O, 5.01.

Calc. for $C_{20}H_{28}ON_2$: C, 76.88; H, 9.03; N, 8.97; O, 5.12.

Mass spectrum: m/e 312 (M^+ Calc. for $C_{20}H_{28}ON_2$: 312).

Mercuric Acetate Oxidation of 3,4-seco-N_b-Methyldihydrocorynantheal Ethylene Acetal (174)

A mixture of the tricyclic acetal (174) (16 mg.), mercuric acetate (120 mg., 2.1 mole) and ethanol (17 ml.) was stirred at room temperature for 17 hours and mercurous acetate (80 mg., 85%) was isolated by filtration. A small portion of filtrate spotted on a silica gel TLC plate and developed with ethyl acetate gave no movable spots. The product was treated with sodium acetate (100 mg.) in acetic acid (5 ml.) at 100° for 20 hours. The reaction mixture was poured into saturated sodium bicarbonate solution and extracted with chlorofrom. Evaporation of the solvent gave a black tar.

Mercuric Acetate Oxidation of 3,4-seco-N_b-Methyldihydrocorynantheal (169)

A mixture of 3,4-seco aldehyde (169) (17 mg.), mercuric acetate (34 mg., 2 mole), ethanol (10 ml.) and acetic acid (5 ml.) was stirred at room temperature for 72 hours. The precipitated mercurous acetate was separated by filtration (8 mg., 30%) and the filtrate was evaporated to dryness. The IR spectrum of the brown residue showed no carbonyl bands but a broad band at 1557 cm^{-1}. The UV spectrum indicated the absence of an indole chromophore.

Attempted Conversion of 3,4-seco-N_b-Methydihydrocorynantheal (169) into an Ester

a) Jones' Oxidation and Fisher Esterification

The aldehyde (169) (82 mg.) was dissolved in acetone (15 ml.) and 8N standard Jones' reagent (1.5 mN) was added to the mixture. The reaction mixture was stirred at room temperature for 20 min. and acetone was evaporated under reduced pressure. Anhydrous methanol (15 ml.) was added to the residue and the mixture was refluxed for 1 hour. The whole mixture was poured into aqueous sodium bicarbonate and was extracted with chloroform. The organic layer was dried over sodium sulfate and evaporated to give a dark brown residue (62 mg.). The major product (10 mg.) separated by preparative TLC (alumina, $CHCl_3$) showed an indole chromophore in the UV spectrum but no

carbonyl absorption in the IR spectrum.

b) Silver Oxide Oxidation

A mixture of 3,4-seco-aldehyde (169) (25 mg.), freshly prepared silver oxide (1 mM) and methanol (10 ml.) was stirred at room temperature for 1 hour. The mixture was filtered and the filtrate evaporated to yield a brown residue. The IR spectrum showed a very weak carbonyl absorption and the UV spectrum showed an indole chromophore.

Attempted Conversion of Dihydrocorynantheal (171) into the corresponding Ester (156)

a) Potassium Permanganate Oxidation (1 mole)

Dihydrocorynantheal (30 mg.), in acetone (25 ml.) was treated with 0.1 molar postassium permanganate solution (1 ml.) at 0° and the mixture was stirred for 30 minutes. The resulting brown residue was filtered off and the filtrate was evaporated to give a brown residue (30 mg.). This residue was identified as the starting material by TLC and IR spectra.

b) Potassium Permanganate Oxidation (2.0 mole)

Dihydrocorynantheal (10 mg.), in acetone (10 ml.) was treated with 0.1 molar potassium permanganate solution (0.67 ml.). The mixture was stirred for 30 minutes at 0° and the solvent was evaporated to give a dark brown residue. This residue contained no indole chromophore as

shown by the UV spectrum.

c) Chromium Trioxide in Acetic Acid and Fisher Esterification

Dihydrocorynantheal (15 mg.) was dissolved in 90% acetic acid (1.5 ml.) and chromium trioxide (5 mg.) was added. The reaction mixture was stirred at room temperature for 12 hours. The solvent was evaporated off and the resulting brown oil was dissolved in methanol (3 ml.) with a few drops of concentrated sulfuric acid. The mixture was refluxed for 30 minutes and cooled to 0°. The mixture was treated with saturated sodium bicarbonate and chloroform. The chloroform layer was separated and dried over sodium sulfate. Evaporation of the solvent gave a dark brown residue, which was purified by preparative TLC (alumina). The compound with the same R_f value as that of dihydrocorynantheic acid methyl ester (156) was separated, but the characterization of this compound was not possible due to the poor yield (0.5 mg.).

d) Jones' Oxidation and Esterification with Diazomethane

Dihydrocorynantheal (20 mg.) was treated with Jones' reagent (0.32 mN) in acetone (2 ml.) and the mixture was stirred at room temperature for 20 minutes. Evaporation of the solvent gave a brown residue, which was then dissolved in methanol (2 ml.). A large excess of diazomethane in ether was added to the mixture and the

whole mixture was allowed to stand for 1 hour. The solvent was evaporated and the resulting residue was purified by preparative TLC (alumina). The compound which showed identical behavior with dihydrocorynantheic acid methyl ester (156) was separated (yield: 2 mg.). UV, λ max: 284, 290 mμ (indole). IR, ν max ($CHCl_3$): 1705 cm^{-1} (C=O).

Preparation of N-Formyltryptamine

The procedure described by Szantay et al[64] was employed. Tryptamine formate (20 g., m.p. 167°), prepared from tryptamine and 90% formic acid in methanol, was heated at 180° for 45 minutes. The reaction was monitored by TLC (silica gel, EtOAc). On cooling, crystals formed slowly, m.p. 75-77° (Lit.[64] m.p. 75-76°).
IR, ν max ($CHCl_3$): 3401 (NH), 1689 (Amide I band) cm^{-1}.
The crude N-formyltryptamine was used for the next reaction without further purification.

Preparation of 3,4-Dihydro-β-carboline[64]

The crude N-formyltryptamine (20 g.) prepared above was boiled with anhydrous benzene (400 ml.) until the major part of the substance dissolved. Then the mixture was cooled to 75° and phosphorous oxychloride (60 ml.) was added over a period of 10 minutes. The mixture was refluxed for 30 minutes. The solvent was evaporated under reduced pressure at 40°. The dark brown residue was vigorously shaken with acetic acid (100 ml.). The acidic

solution was separated from black tar and was diluted with water (900 ml.). The resulting brown amorphous solid was separated by filtration and the filtrate was basified at 0° with 50% potassium hydroxide solution to pH 10. The light yellow precipitate of 3,4-dihydro-β-carboline was dried under reduced pressure at 56° for 5 hours(yield 18.6 g).

UV, λmax (neutral): 236, 242, 318 mμ; λmax (+HCl): 246, 359 mμ. (Fleming and Harley-Mason[69] reported λmax (neutral): 236, 242, 318 mμ; λmax (+HCl): 246, 359 mμ).

Preparation of dl-3-Ethyl-1,2,3,4,6,7,12, 12b-octahydro-2-ketoindolo(2,3-a)quinolizine (181)

The procedure of Openshaw and Whittaker[70] was followed. A solution of 3,4-dihydro-β-carboline (18.6 g., 0.11 M) in absolute alcohol (190 ml.) was heated with 3-dimethylamino-methylpentan-2-one methiodide (32.7 g., 0.11 M) under reflux for 2 hours. The cooled mixture was diluted with 5% potassium hydroxide solution (500 ml.) and extracted with chloroform (800 ml.). The extracts were washed with water and dried over sodium sulfate. Evaporation of the solvent gave a dark brown residue. The residue was extracted with hot benzene (700 ml.) and the benzene solution was filtered from amorphous material. Evaporation of the solvent gave a light brown residue, which was crystallized from alcohol to give crude tetracyclic ketone (181) (7.5 g.), m.p. 199-202°. Recrystallization

from alcohol gave prisms, m.p. 205-209°. (Lit.[70] m.p. 205-206°).

IR, νmax ($CHCl_3$): 3401 (indole NH), 1715 (C=O) cm^{-1}.

Wittig Reaction on the Tetracyclic Ketone (181) and subsequent Hydrogenation

A solution of trimethyl phosphonoacetate[55] (12 ml., 60 mM) in dry tetrahydrofuran (100 ml.) was added to solid potassium t-butoxide (prepared from 2.0 g. of potassium and t-butanol). The mixture was cooled to 0°, and a solution of the tetracyclic ketone (181) (5.0 g.) in dry tetrahydrofuran (250 ml.) was added. The solution was allowed to warm to room temperature, stirred for 2 hours and diluted with 2% potassium hydroxide (1000 ml.) and dichloromethane (500 ml.). The organic layer was separated, washed with saturated sodium bisulfite, dried over sodium sulfate and evaporated to dryness to give a yellow resinous product (4.5 g.). TLC (alumina, $CHCl_3$) showed one spot and the product was less polar than the starting material. This α,β-unsaturated ester (182) had the following physical data.

IR, νmax ($CHCl_3$): 1698, 1637 (α,β-unsaturated ester) cm^{-1}.
(Lit.[61] IR, νmax ($CHCl_3$): 1701, 1642 cm^{-1})

UV, λmax: end absorption (250 mμ), 283, 290 mμ.

NMR signals (100 Mc/s): τ 1.59 (1H, singlet, indole NH), 2.5-3.1 (4H, multiplet, aromatic), 4.33 (1H, singlet, vinylic), 6.30 (3H, singlet, COOMe), 9.04 (3H, triplet,

J=7 cps, $C\underline{H}_3CH_2$-) (Lit.[61] τ4.30 (vinylic, singlet) at 60 Mc/s) A small portion of α,β-unsaturated ester was dissolved in methanol and a few drops of concentrated perchloric acid was added. Crystals developed overnight and had m.p. 245-250°.

The Wittig product (182) (4.4 g.), in methanol (250 ml.), was hydrogenated over 10% palladium on charcoal (3 g.) and hydrogen up-take (350 ml.) ceased after 2 hours. The catalyst was filtered off and the filtrate was reduced to a volume of 100 ml. under reduced pressure. The solution was treated with concentrated perchloric acid (2 ml.) and the resulting yellow prisms (3.5 g.) were separated by filtration, m.p. 260-265°. Treatment of the perchlorate with aqueous sodium bicarbonate gave the free base, dl-dihydrocorynantheic acid methyl ester (156), which was crystallized from n-heptane-benzene to give needles, m.p. 142-144°. Direct comparison of this methyl ester (156) with an anthentic specimen[61], kindly supplied by Dr. B. Douglas, Smith Kline and French Laboratories, Philadelphia, Pa., showed the two to be identical (mixed m.p., NMR, TLC, IR).

IR, νmax ($CHCl_3$): 3530 (NH), 1725 (C=O) cm^{-1}.

NMR signals (100 Mc/s): τ1.84 (1H, singlet, indole NH), 2.5-3.1 (4H, multiplet, aromatic), 6.33 (3H, singlet, COOMe), 9.12 (3H, triplet, J=7 cps, $C\underline{H}_3CH_2$-).

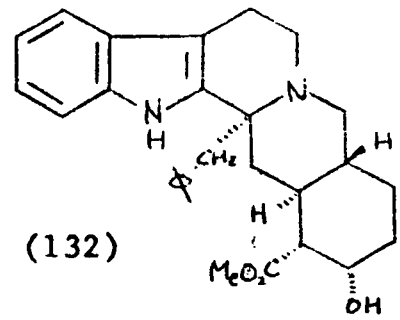
Mass Spectral Data

3α-Benzylyohimbine (132)

$C_{28}H_{32}O_3N_2$

M.W. 444

(≥ 90)



(132)

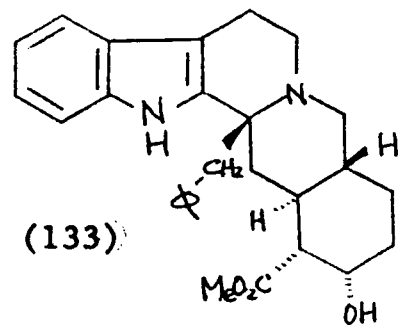
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
444	1.9	223	3.2	195	2.0	154	3.2
355	3.5	222	2.2	184	2.0	144	3.1
354	28.0	221	10.0	183	4.0	143	2.1
353	100.0	219	2.0	182	2.9	142	2.0
352	4.5	209	3.0	169	3.8	130	2.1
351	2.6	207	2.7	168	4.5	92	2.5
293	2.2	206	2.1	167	3.5	91	5.0
277	4.1	198	4.3	156	3.0		
235	2.4	197	13.0	155	3.4		

3β-Benzylyohimbine (133)

$C_{28}H_{32}O_3N_2$

M.W. 444

(≥ 90)



(133)

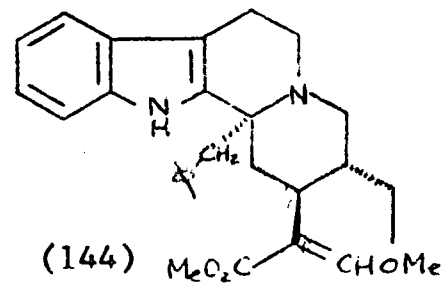
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
444	14	335	13	197	14	155	5
443	6	293	7	184	14	154	5
413	5	278	5	183	5	153	5
355	20	277	20	182	6	144	6
354	39	235	5	170	5	130	5
353	100	221	12	169	7	97	7
352	19	209	5	168	9	92	7
351	14	198	6	167	6	91	16

3α-Benzyldihydro-corynantheine

$C_{29}H_{34}O_3N_2$

M.W. 458

(≈100)



(144)

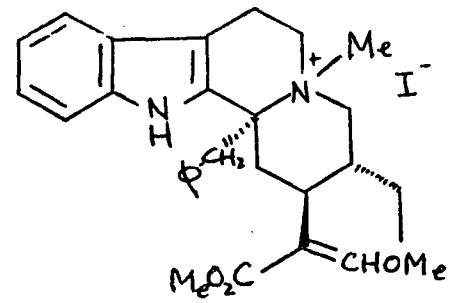
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
458	1	252	5	197	23	155	5
457	2	251	14	195	5	154	8
369	10	249	7	183	5	144	5
368	67	223	5	182	5	142	6
367	100	221	12	180	5	130	5
366	10	209	5	169	14	115	6
365	10	208	5	168	16	106	6
351	5	207	5	167	10	105	8
335	5	198	6	156	6		

3α-Benzyldihydro-corynantheine methiodide

$C_{30}H_{37}O_3N_2I$

M.W. 600

(≈90)



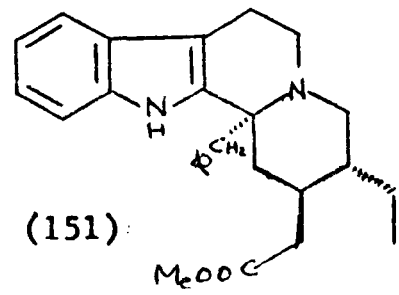
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
472	3	221	6	154	5	128	54
368	23	197	13	143	16	127	93
367	79	196	6	142	100	92	9
295	5	169	6	141	82	91	32
251	10	168	9	140	68		
249	7	167	5	139	72		

3α-Benzyldihydro-corynantheic acid methyl ester

$C_{27}H_{32}O_2N_2$

M.W. 416

(≥ 100)



(151)

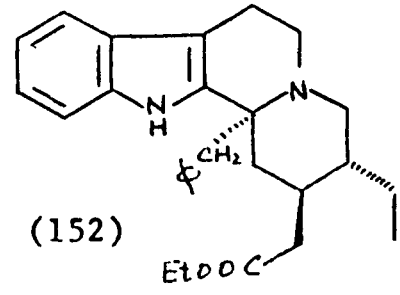
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
416	0.3	323	2.3	222	1.1	169	1.0
415	0.9	252	1.3	221	4.1	168	1.5
385	1.2	251	7.0	209	0.9	167	1.1
327	3.0	249	0.7	197	1.1	155	0.7
325	100.0	237	1.1	183	0.9	154	1.0
324	3.1	223	1.2	182	0.8		

3α-Benzyldihydro-corynantheic acid ethyl ester

$C_{28}H_{34}O_2N_2$

M.W. 430

(≥ 90)



(152)

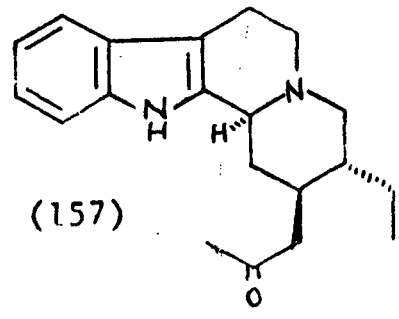
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
430	0.3	252	2.5	197	3.1	156	2.0
429	0.7	251	11.8	195	2.0	155	2.9
341	3.7	237	3.1	184	3.1	154	4.0
340	24.1	223	3.1	183	3.1	144	2.0
339	100.0	222	2.5	182	2.5	143	2.1
338	2.6	221	8.6	169	3.1	115	2.7
337	2.3	219	2.0	168	4.9	92	2.5
311	3.4	209	3.0	167	4.2	91	15.6

Tetracyclic methyl ketone (157)

$C_{20}H_{26}ON_2$

M.W. 310

(≈100)



(157)

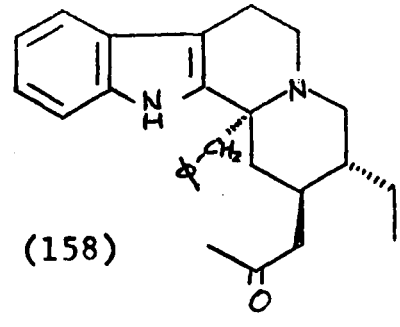
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
311	20	252	9	184	5	149	11
310	100	251	26	170	11	144	4
309	66	225	13	169	11	143	4
308	4	224	4	168	5	111	5
281	9	223	11	156	8	109	5
253	19	221	4	155	5		

3α-Benzyl tetracyclic methyl ketone (158)

$C_{27}H_{32}ON_2$

M.W. 400

(≈90)



(158)

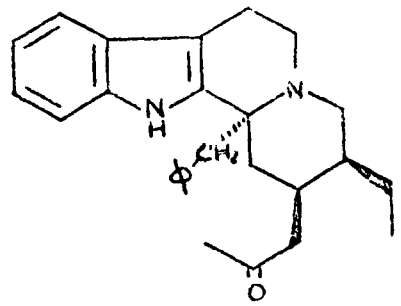
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
400	1.4	265	1.5	221	11.0	182	1.5
355	4.7	252	4.3	219	1.5	169	1.9
311	5.4	251	22.0	209	1.5	168	3.0
310	43.0	250	1.8	207	1.5	167	2.4
309	100.0	249	2.9	206	1.4	155	1.6
308	5.5	223	2.0	197	1.7	154	2.1
307	3.4	222	3.3	183	1.6	91	3.7

$Hg(OAc)_2$ oxidation product from 3d-benzyl tetracyclic methyl ketone (158)

$C_{27}H_{32}ON_2$

M.W. 400

(≈ 90)



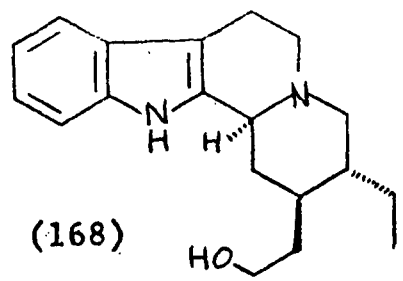
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
400	0.4	251	26.2	209	1.9	168	2.1
399	1.1	250	2.0	207	1.9	167	1.7
311	3.0	249	3.2	206	1.7	155	1.5
310	21.4	223	2.3	197	1.8	154	1.6
309	100.0	222	4.3	195	1.7	91	2.4
308	2.9	221	13.7	183	1.8		
307	2.4	220	1.5	182	1.8		
252	5.3	219	1.9	169	1.7		

dl-Dihydrocorynantheol (168)

$C_{19}H_{26}ON_2$

M.W. 298

(≈100)



(168)

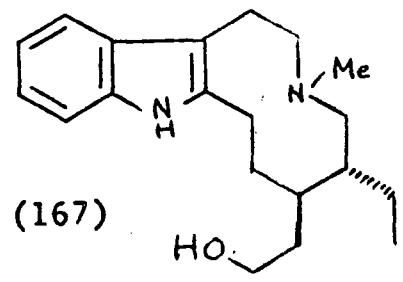
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
299	20.0	225	14.0	156	8.7	117	8.8
298	100.0	197	4.8	154	4.0	111	5.4
297	90.5	184	5.3	149	7.6	110	5.4
296	7.5	170	12.0	144	4.4	109	4.2
269	5.1	169	10.8	143	4.0		
253	9.1	168	6.0	119	7.9		

dl-3,4-seco-N_b-Methyl-dihydrocorynantheol (167)

$C_{20}H_{30}ON_2$

M.W. 314

(≥ 100)



(167)

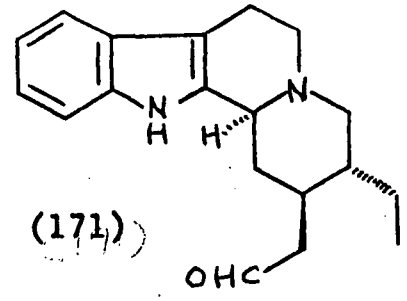
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
315	14	258	10	201	10	159	23
314	47	256	16	200	21	158	32
313	9	227	14	199	9	157	45
271	12	214	10	186	12	146	10
270	12	203	9	172	18	145	14
269	10	202	30	171	100	144	42

Dihydrocorynantheal (171)

$C_{19}H_{24}ON_2$

M.W. 296

(≥ 100)



(171)

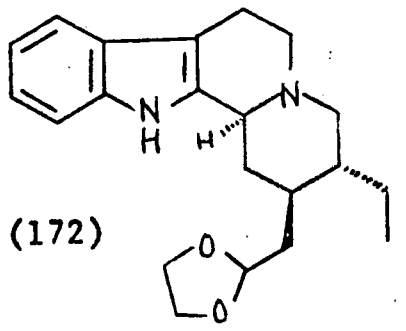
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
296	3.0	223	17	167	23	142	36
295	4.0	184	30	157	19	130	34
268	16	183	16	156	71	129	32
267	18	171	26	155	24	128	34
253	18	170	100	154	32	127	18
251	18	169	86	144	45	126	32
225	38	168	39	143	52	115	42

Dihydrocorynantheal ethylene acetal (172)

$C_{21}H_{28}O_2N_2$

M.W. 340

(≥ 100)



(172)

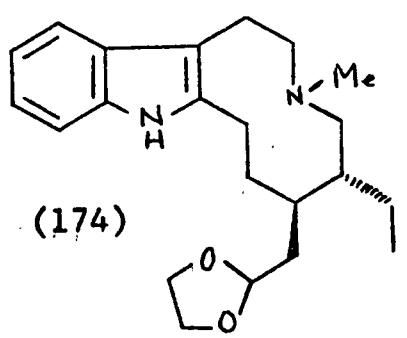
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
340	44	223	16	169	100	143	35
339	38	221	17	168	40	142	25
268	51	211	20	167	24	130	22
267	49	197	15	157	17	129	20
253	14	184	32	156	65	128	20
251	27	183	19	155	23	115	23
239	18	171	28	154	27		
225	44	170	88	144	37		

3,4-seco-N_b-Methyldihydro-corynantheal ethylene acetal

$C_{22}H_{32}O_2N_2$

M.W. 356

(≥ 100)



(174)

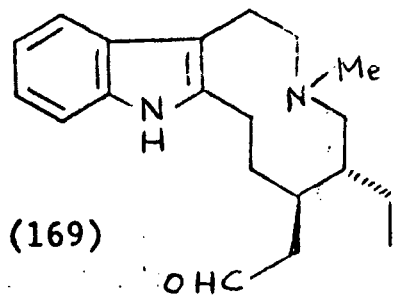
m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
357	25	213	13	170	12	143	21
356	100	212	55	158	11	112	10
355	11	201	46	157	15		
325	11	200	12	156	18		
298	11	185	10	144	21		

3,4-seco-N_b-Methyl-dihydrocorynantheal (169)

$C_{20}H_{28}ON_2$

M.W. 312

(≳ 100)



(169)

m/e	%B.P.	m/e	%B.P.	m/e	%B.P.	m/e	%B.P.
313	21	201	19	158	13	144	40
312	91	170	14	157	21	143	39
269	55	169	15	156	44	140	13
227	22	168	69	154	16	137	14
226	100	167	15	149	19	130	15

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