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by

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"QUATERNARY ALKALOIDS OF THE STEM AND ROOT BARK OF HUNTERIA EBURNEA PICHON"

September, 1970

To my mother and father

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-.A C K N O W L E D G M E N T S.-

I wish to express my profound gratitude to Prof. R.H. Burnell for the leadership, patience, friendship, and understanding that he has shown throughout the course of this study. I am much obliged to him for providing the financial assistance through National Research Council funds, which made this work possible.

I sincerely thank my wife Nicole for her constant encouragement for the achievement of this work and for typing the first manuscript.

I would like to express my appreciation to my colleagues of the Organic Chemistry Laboratories, particularly M. A. Chapelle, for their cooperation and helpful attitude during the period spent on this investigation.

I sincerely thank M. M. Lavoie for technical help in the running of the columns during summer of 1969, and to Miss D. Thibault for running many of the spectra.

I wish to thank Mme M. Veilleux for accepting the task of typing this thesis and for the excellent performance of it.

-.L I S T O F F I G U R E S.-

The abbreviations used in this thesis are those accepted by TETRAHEDRON, the International Journal of Organic Chemistry.

The following tables are included in this thesis:

-.I N T R O D U C T I O N.-

The study of the Hunteria eburnea Pichon alkaloids has attracted many researchers due to a therapeutical activity attributed to the plant extracts[1-3].

Hunteria eburnea Pichon is a tree varying from 6 to 40 m high abundant in the humid forests of Côte d'Ivoire. A recent approach to the study of the alkaloids occurring in the seeds[4] and leaves[5] of this plant reveals the occurrence of alkaloids specific to the parts of the plant examined but different from those found in the root and stem bark of Hunteria eburnea which constitute the subject of our study.

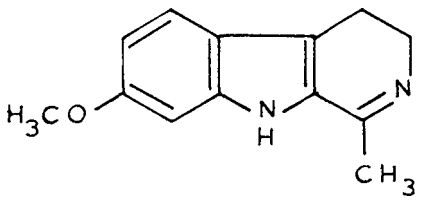
The genus Hunteria is composed of six species from which H. eburnea, H. corymbosa[6] and H. umbellata[7-10] were examined by different research groups. Hunteria eburnea belongs to the Apocynaceae family the richest source of indole alkaloids.

Indole alkaloids fall biogenetically into two broad classes in which a tryptophan residue or its equivalent is either: a) in combination with a ten carbon moiety of terpene derivation: the great majority of the bases fall in this group or b) modified slightly by alkylation and ring closure or by fusion to an anthranilic or mevalonic acid residue[11].

Elucidation of the structures of the first group of alkaloids was slow until the advent of commercial recording ultraviolet and infrared spectrometers allowed the first major break-through. We are presently witnessing a second revolution in the conduct of the art brought about by the availability of protons mappers (nuclear magnetic resonance machines) and mass spectrometers. The application however of the greatest ultimate

promise is structure determination by means of the interpretation of X-ray diffraction data.

Indole alkaloids although amongst the earliest known natural products did not readily lend themselves to structural elucidation and as a result there was an accumulation of physical and experimental data which outpaced understanding. Indole alkaloids have been isolated as early as 1841 when Goebel[12a] obtained harmaline (1) from Peganum harmala L.



1

Occasionally isolation of a particular alkaloid is facilitated by forming a water insoluble salt. Nitric, perchloric, sulphuric and oxalic acids have often been used for this purpose but this does not form part of any systematic procedure except for the removal of water soluble bases from aqueous solution. For quaternary salts picric acid and reinecke salts have been widely used.

In order to follow the course of an alkaloid isolation the classical chemist developed many precipitation and chromogenic methods. They either depended on the water insolubility and colour of complex acid salts or the generation of characteristic colours with oxidizing agents under defined conditions. For indole alkaloids some colour reactions are specific to a particular indolic system and in a very crude mixture such a test is

often more useful than attempting to measure and interpret an ultraviolet absorption spectrum. Useful colours are generated by ceric sulphate, or in sulphuric acid containing a trace of an oxidizing agent, e.g. ferric chloride (Keller), sodium nitrite (Arnold-Vitali), sodium molybdate (Fröhde) and potassium dichromate (Otto).

For precipitations the most well known reagents are Mayer's (potassium mercury iodide), Sonnenschein's (phosphomolybdic acid) and occasionally picric acid. Today these reagents have a new use as detectors in paper and thin layer chromatography.

Once the crude alkaloids have been obtained separation methods are applied which take advantage of previous experience and modern methodology such as column, partition or ion exchange chromatography, countercurrent distribution and the like. The results of the fractionation can be followed by all or as many of the physical methods as need be brought to bear to the problem.

The isolated pure base can be characterized via its melting point, optical rotation (or optical rotatory dispersion curve), ultimate analyses and by the formation of derivatives. All of these measurements were very important up to about 1950 since these were the only means available for comparing compounds. Today many of these pointer readings have diminished in value because more accurate comparisons can be made by a multiplicity of physical methods which sometimes result in a complete proof of structure. The ultraviolet absorption spectrum defines the chromophoric moiety and the infrared spectrum besides "fingerprinting the molecule" by its wealth of bands, detects various functionalities in particular, carbonyl groups and hydrogen attached to nitrogen or oxygen. Nuclear magnetic resonance

spectroscopy yields information from which the proton topography can be deduced. Then there is the mass spectrum, the interpretation of whose line rich spectrum often allows the entire structure of the alkaloid to be deduced.

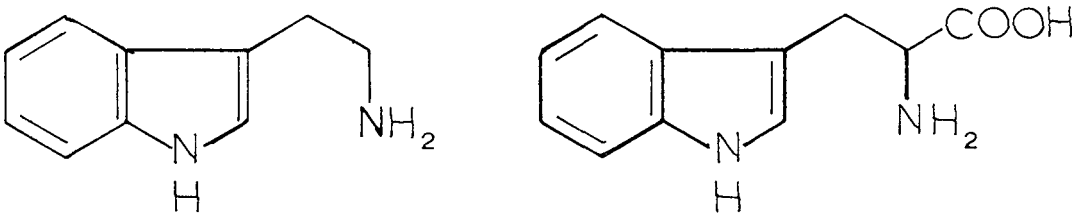
With the advent of computers it has become possible to reduce the interpretation of the X-ray diffraction data of a single crystal containing a heavy atom (e.g. the alkaloid iodide, bromide, brosylate) to a routine. This method is absolute and requires no assumptions as to the structure and it will eventually be used by the chemist as naturally as he has accepted all the other aids which have come his way. It has already enriched our knowledge by solving structural problems for which there was either no chemical penetration available or in some cases simply because the isolated material was insufficient for a chemical examination. This was our case with hunteracine chloride, a new type of quaternary alkaloid we isolated during the investigation of the stem and root bark of Hunteria eburnea Pichon. After a variety of exploratory reactions, we decided to confirm its structure by X-ray analysis[12b] (chapter III).

With the structure of the indole alkaloid largely defined by use of the above methods, the chemist can operate more meaningfully with small amounts of material to complete the structure proof. This can take the form of either partial degradation to or partial synthesis from a known base, which at the same time very often settles the absolute stereochemistry.

With the structure, stereochemistry and conformation now relatively easy to determine and the degradative process eliminated there remains the challenge of synthesis and this must be by a rapid, high yielding stereospecific (or selective) route. There are as yet few landmarks in this area

but it is one to which an increasing amount of attention is being paid.

There can be recognized in the majority of indole alkaloids a 3-β-aminoethylindole moiety (2a) and in agreement with an early hypothesis it has been shown that this is invariably biogenetically derived from tryptophan (2b).



a b

2

It is the source of the remaining portion, a nine or ten carbon fragment, which is presently less certain in spite of a number of sophisticated suggestions and a lot of experimental work. Whatever the progenitor of the ten carbon residue is, it can be seen to be divisible into a linear six carbon plus one carbon residue (6C-1C) linked in one of three ways to a three carbon unit (or 2C if the carboxyl is lost) as shown in Figure 1 where some idea of diversity of indole alkaloids is given. Parenthetically it should be noted that type II and III alkaloid skeleta are theoretically derivable from type I via four-membered ring intermediates, i.e. fission of the C-15, C-16 bond and reclosing of C-17 with either C-14 or C-20, but such rearrangements are presently unknown. I type I precursor is in actual fact being converted into type II and III it is not known whether this happens before or after combination with tryptophan. Some alkaloids are known which lack either the 3C unit or

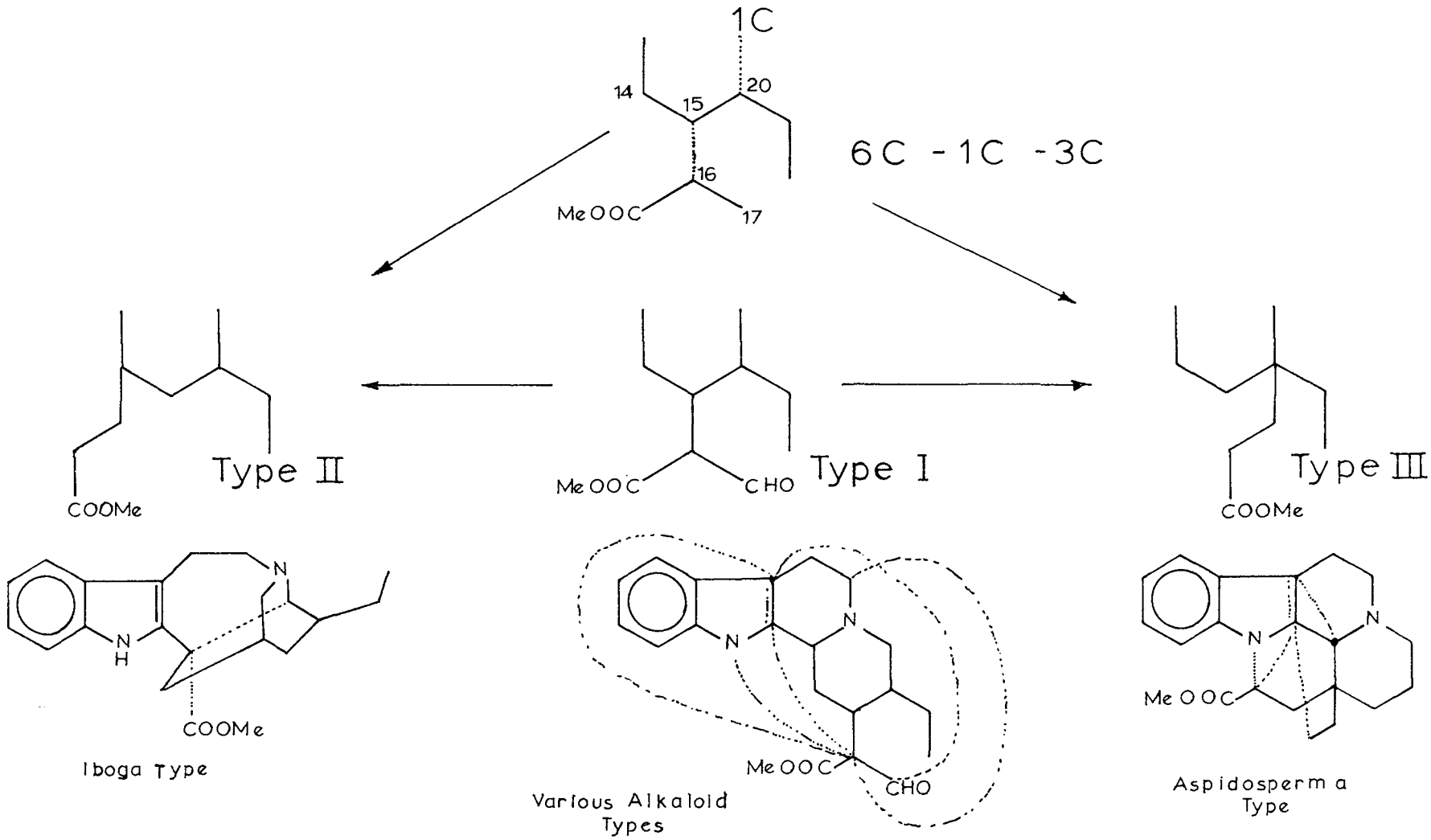
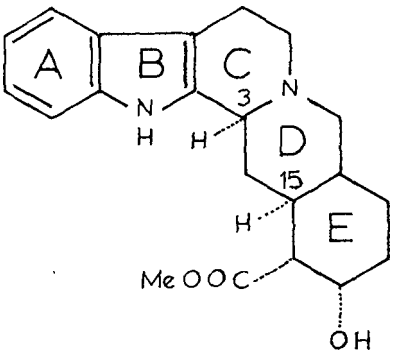


Figure 1.- PRECURSORS OF THE COMPLEX INDOLE ALKALOIDS

the C-1 unit.

The classical view of the origin of the indole alkaloids was predicted almost entirely from the structure of a single alkaloid yohimbine (3) even before the site of its carboxyl was firmly established.



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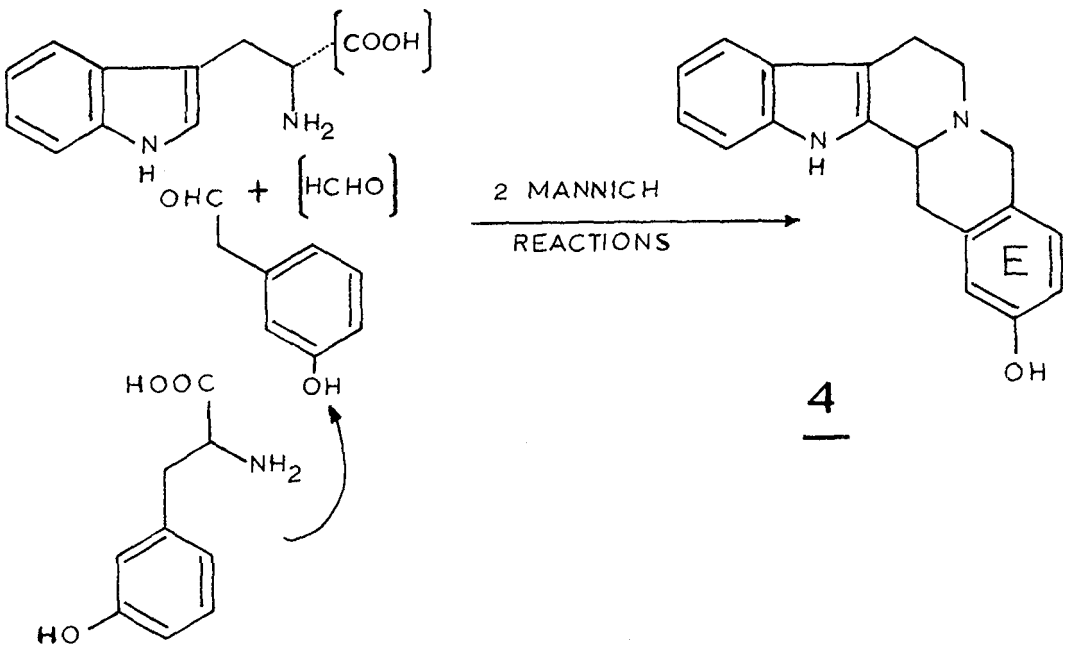
This alkaloid was considered to stem from tryptophan, phenylalanine or its equivalent and formaldehyde via two Mannich condensations.



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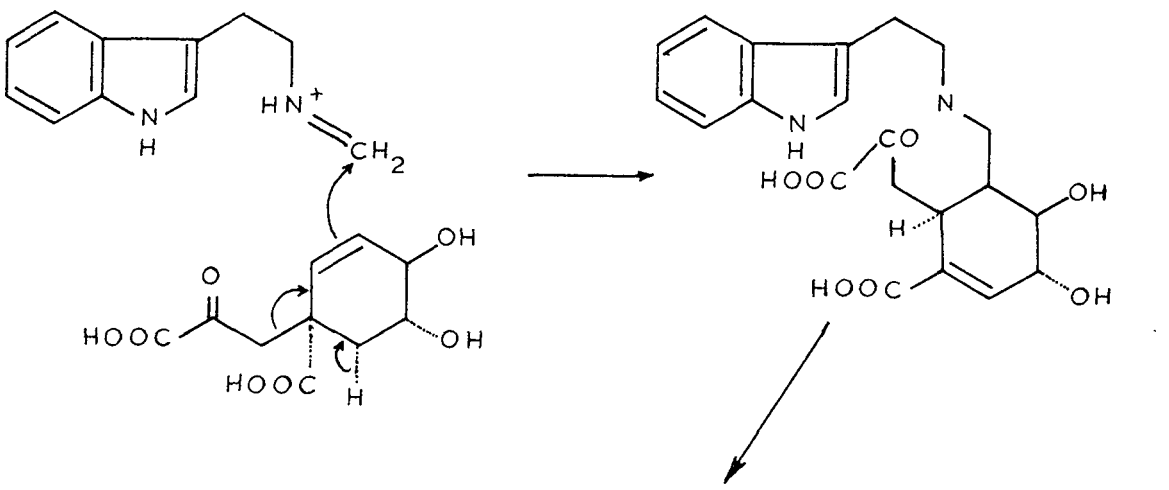
The successful preparation of a model (4) under so-called "physiological conditions" supported this idea but how either the carboxyl group was to be introduced or how reduction of the aromatic ring E was to be accomplished was never seriously discussed. An alternative to the aromatic amino acids hypothesis is one which involves their proximate precursor, viz. a hydrated prephenic acid (5) in which the potential ring E of yohimbine (3) is already in a reduced state and has in addition the necessary carboxylic residue.



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An important conclusion arising out of this theory was the prediction of a uniform stereochemistry in indole alkaloids of type I at the carbon equivalent to C-15 of yohimbine.

In the mid-sixties the idea of prephenic acid was discarded as a precursor to indole biosynthesis. Instead, the C_{9-10} unit of the indole alkaloids was proposed to be of mevalonoid origin. Degradative experiments

were in agreement with head-to-tail combination of the two C_5 units and the logical deduction that geraniol (6) or its equivalent is a precursor of the indole alkaloids was proved to be correct for corynanthe, Aspidosperma and Iboga groups of bases (Figure 2).

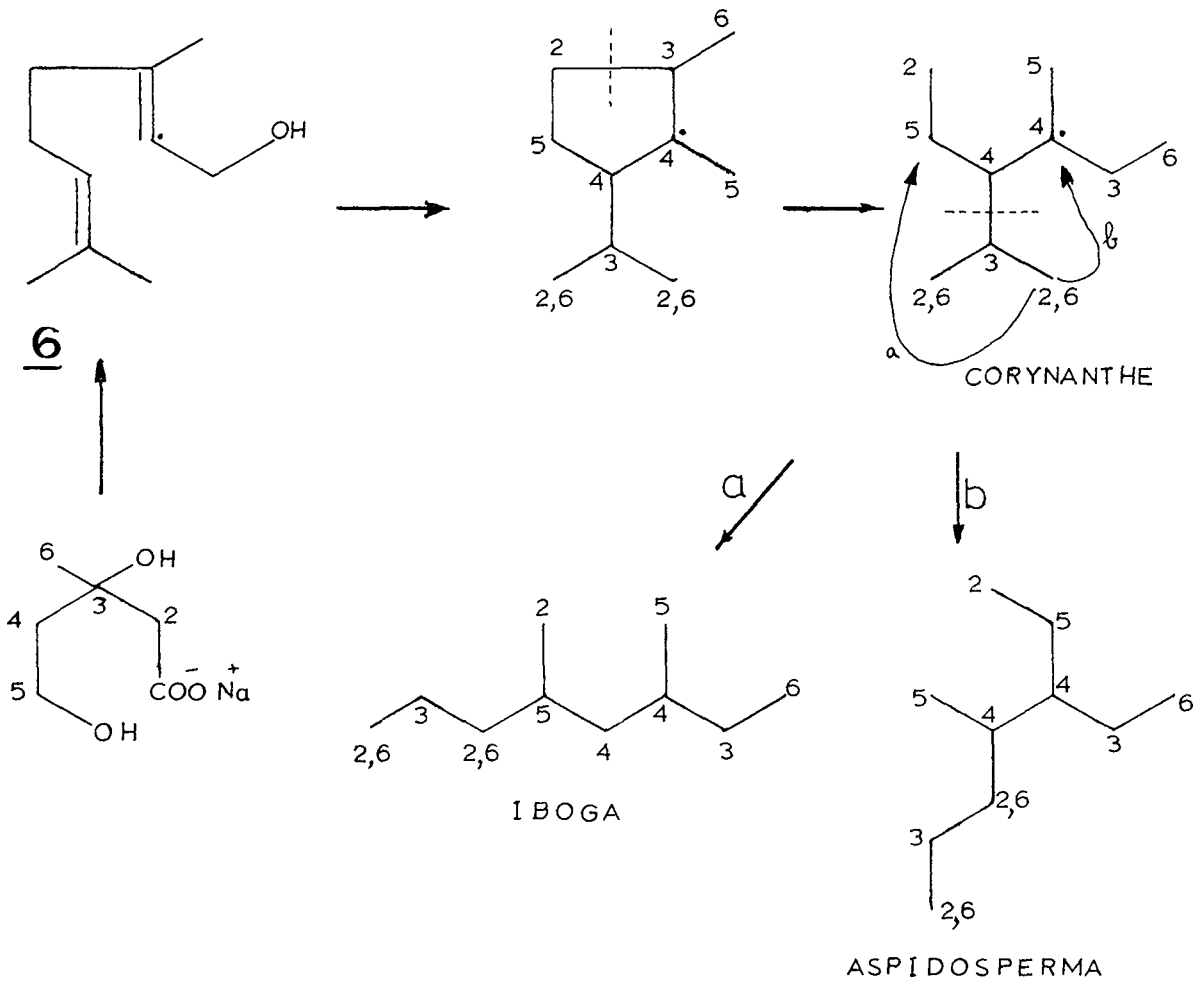
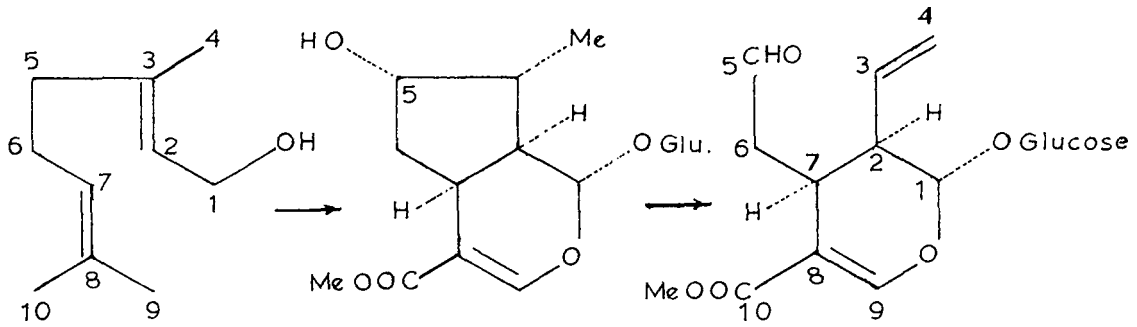


Figure 2.- INDOLE ALKALOIDS BIOSYNTHESIS

More recently the discovery[13] that loganin (7) is a key precursor of the three alkaloidal families opened the problem for detailed study of the later biosynthetic stages. In addition, this finding was in accord with Thomas[14] and Wenkert's[15] suggestion that the indole alkaloids are derived

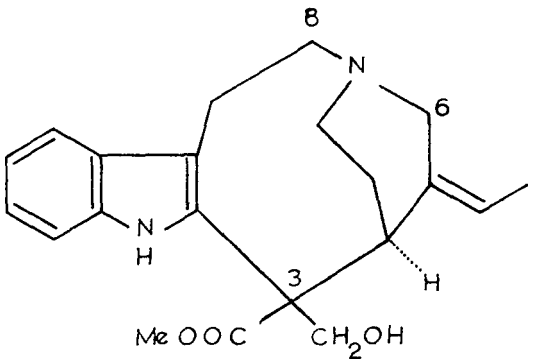
in vivo from a cyclopentane monoterpene by some process involving cleavage of the five-membered ring. Battersby et al.[16] proposed that loganin (7) is cleaved to yield secologanin (8). They reported the successful and specific incorporation of the cyclopentanoid monoterpene loganin (7) into the indole alkaloids elaborated by Vinca rosea. Essentially identical results have been obtained by Arigoni[17], Leete[18], Scott[19] and their respective coworkers.



6 7 8

According to Scott[20], stemmadenine (9) has a crucial biosynthetic position in the rearrangement of the corynanthe skeleton to the Aspidosperma and Iboga alkaloids.

All the plant parts of Hunteria eburnea have been examined by different research groups seeking new alkaloids. The seeds[4] afforded nine alkaloids while eight bases were isolated from the leaves[5]. The stem and root bark are among the richest parts examined. They afforded fourteen tertiary and sixteen quaternary bases. (Table 1).



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In this thesis we report the isolation and elucidation of the structure of five new quaternary alkaloids from the stem and root bark of Hunteria eburnea Pichon.

TABLE 1

Genus	Part of plant studied	Alkaloids separated
H. corymbosa	leaves	Rhazine Corymine
H. umbellata	leaves	Erinine Erinicine Alkaloid PUA-6 Corymine
	seeds	Corymine Acetylcorymine Isocorymine

(Cont. next page)

TABLE 1 (cont.)

H. eburnea	seeds	(+) Eburnamonine (+) Eburnamenine Corymine	
	leaves	Alkaloid I Acetylcorymine Desformocorymine Corymine Erinine Erinicine Geissoschizol Eburnaphylline	
	stem and root bark	tertiary bases	Burnamicine Burnamine Kopsinilam Pleiocarpinilam Pleiocarpinine Pleiocarpine Pleiocarpamine (-) Eburnamine (+) Isoeburnamine (+) Eburnamonine (+) Eburnamenine Hunterine Hunteramine Neburnamine

(Cont. next page)

TABLE 1 (cont.)

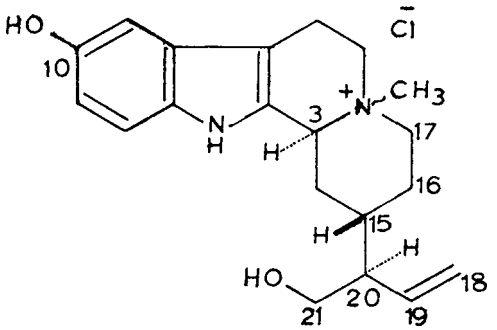
H. eburnea	stem and root bark	quaternary salts	Akuammicine methochloride Antirhine α-methochloride* Dihydrocorynantheol methochloride Hunterburnine α-methochloride Hunterburnine β-methochloride 21-methoxyhunterburnine methochloride* Hunteracine chloride* Hunteriamine Hunteria-alkaloid F =Pleiocarpamine methochloride* Hunteria-alkaloid H Hunteria-alkaloid I Hunteria-alkaloid J =Dihydroantirhine α-methochloride* Hunteria-alkaloid K Hunteria-alkaloid N Huntrabrine methochloride Yohimbol methochloride

* Quaternary alkaloids which constitute the subject of this thesis.

-.C H A P T E R I.-

ANTIRHINES AND HUNTERBURNINES

The structures of an interesting pair of alkaloids were derived by X-ray crystallographic analysis which showed them to be epimeric N_b-methylated quaternary salts of the as yet to be isolated tertiary base hunterburnine[21]. Hunterburnine metho-salts (10a and b) were isolated from the bark of Hunteria eburnea Pichon[21-24] in 1962 and from Pleiocarpa mutica Benth[25] in 1965. Hunterburnine α-methochloride was found without its β-isomer in Ochrosia sandwicensis A. Gray[26].



a : CH_3 = α

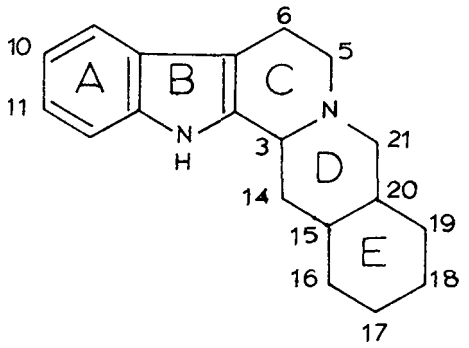
b : CH_3 = β

10

Hypotensive activity is found in the majority of the quaternary bases of this group and some of the tertiary bases are known to show enhanced activity on quaternization[22]. Actually the only pure physiologically useful compound isolated from Hunteria eburnea is hunterburnine α-methochloride[21] (10a).

This was not only the first case of the isolation of this new yohimbinoid variant, but also of such N_b epimers. The natural occurrence of such isomers may turn out to be quite common and it is also possible that the biochemical methylation step, if there is one here, may only be as

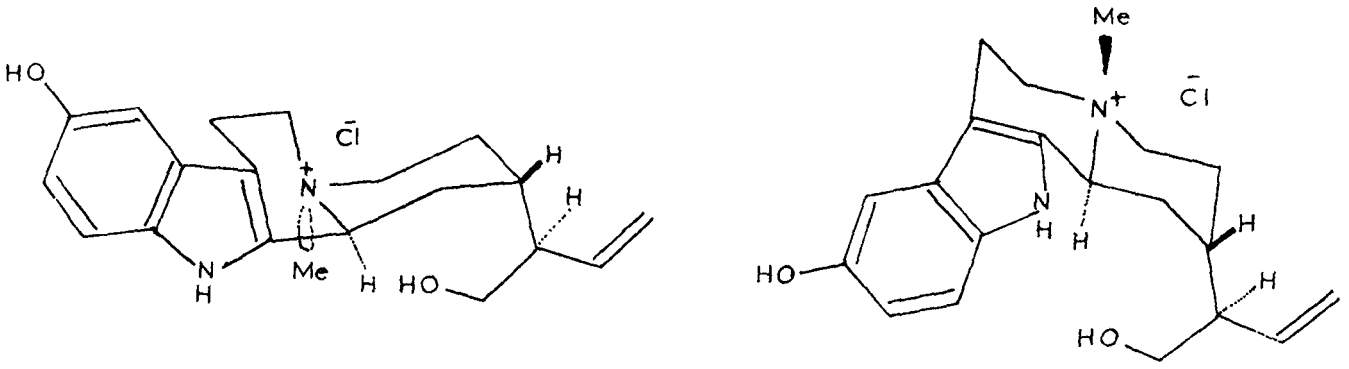
specific as that of the analogous laboratory operation. It is known, for example, that treatment of yohimban (11) with methyl iodide gives both diastereoisomeric methiodides[27].



11

Hunterburnine methochlorides (10) $C_{20}H_{27}O_2N_2Cl$ contain a 5-hydroxy-indole chromophore, an isolated double bond, and an aliphatic hydroxyl group which is readily acetylated, and they occur in the forms of the diastereoisomeric quaternary N_b salts. By the X-ray analysis of their methiodides[21, 23, 24] one of them was shown to have β-oriented N_b-methyl being *trans* to C_{15} substituent, and the other was indicated to have β-oriented N_b-methyl which is *cis* to C_{15} substituent. The former was named as hunterburnine β-methiodide, the latter hunterburnine α-methiodide and they are represented by 12b and 12a respectively.

In the NMR spectra of hunterburnine methochlorides, the β-isomer shows its N-methyl proton signal at 3.31δ and α-isomer at 3.47δ[22] which is in good agreement with the published observations[28, 29] and the conclusion that the former has the *trans* fusion at the C/D ring juncture, while the latter has *cis* fusion at the same position.



a 12 b

The absolute stereochemistry shown in 12a and 12b is only based on biogenetic speculation and the assumption that the rule[30-32] of uniform absolute stereochemistry of the substituent at C_{15} of yohimbine and at the corresponding position of emetine[33, 34] should be valid in this case. It is worthy to note the fascinating proposal by Taylor, et al. on the biogenesis that the skeleton of hunterburnine methosalts (10) could be derived from that of the quaternary corynantheol type of compound (13).

The similar hypothesis was proposed by Djerassi, et al. with vallesiachotamine (14), an alkaloid isolated from Vallesia dichotoma Ruiz et Pav[35]. (Figure 3).

The behaviour of quaternary alkaloids in the mass spectrum has been studied by M. Hesse et al.[36]. According to their work, they suggest that both hunterburnine α- and β-methochlorides undergo either a Hofmann degradation with the loss of HCl or simply the departure of the quaternizing

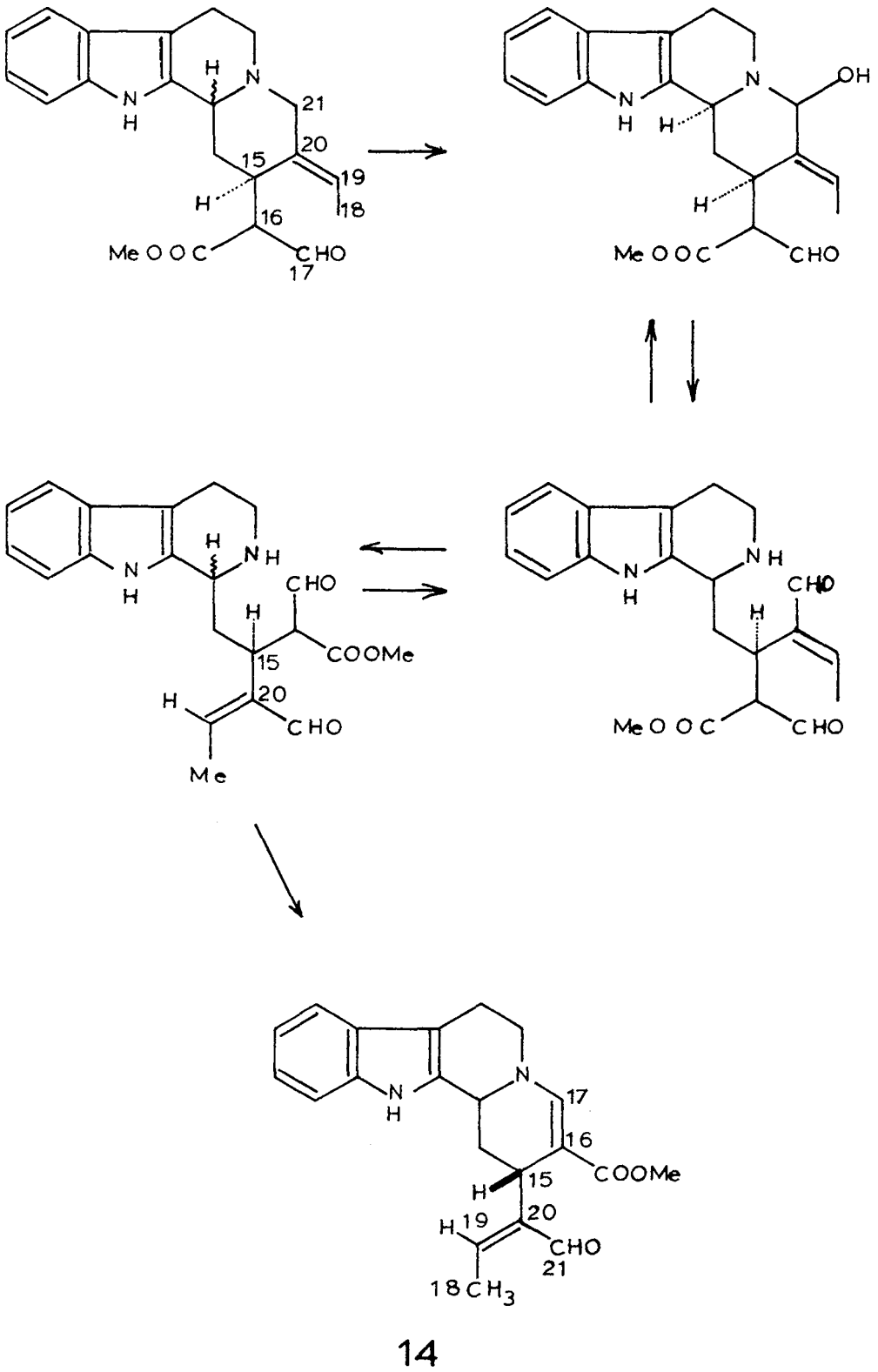


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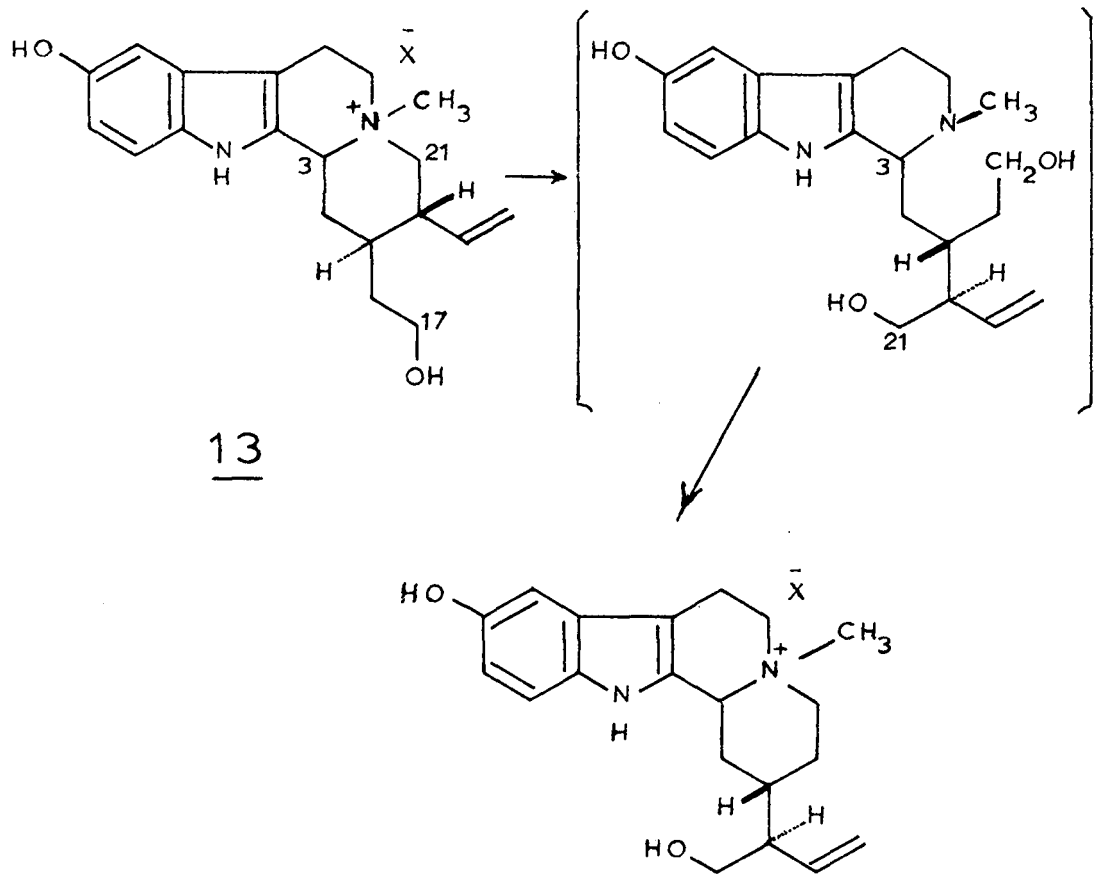
Figure 3.- BIOGENESIS OF VALLESIACHOTAMINE



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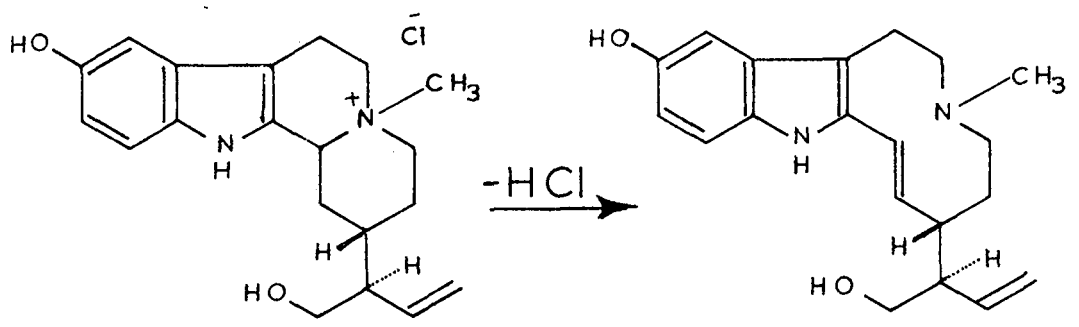
methyl group before undergoing further electronic impact.



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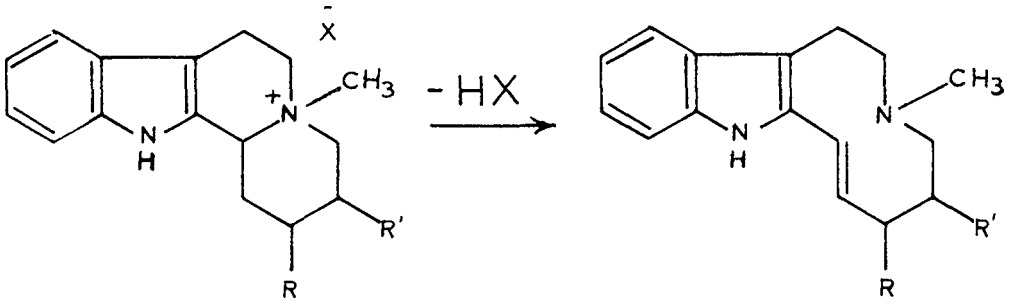
The base (or most intense) peak in the mass spectrum of hunterburnine methochloride is at 255 m/e arising from the loss of the C_{15} substituent in the Hofmann product (15). This behaviour was also noticed in the corynantheine and ajmalicine types of quaternary alkaloids[36, 37] (16) where the opening of the junction between rings C and D gives (17).



16 17

Although the hunterburnines we separated from Hunteria eburnea were easily identified and are in accordance in every way with the published data, we met with a quaternary alkaloid (a fact confirmed by a positive test for chloride ion) having the same UV spectrum as hunterburnine, the same behaviour in alkali, but with slightly different IR, NMR and mass spectra.

While the molecular ion in the mass spectrum of hunterburnine methochloride is at 327 m/e that of the new base is at 341 m/e, probably due to the presence of a methylene residue more than in hunterburnine. For reasons of limited solubility the best NMR spectrum was obtained in CF_3COOH and it confirmed the distribution of the aromatic protons of the indole system, suggested the presence of an olefinic double bond and a quaternary N-methyl group. It showed the absence of C-methyl groups but the three-proton

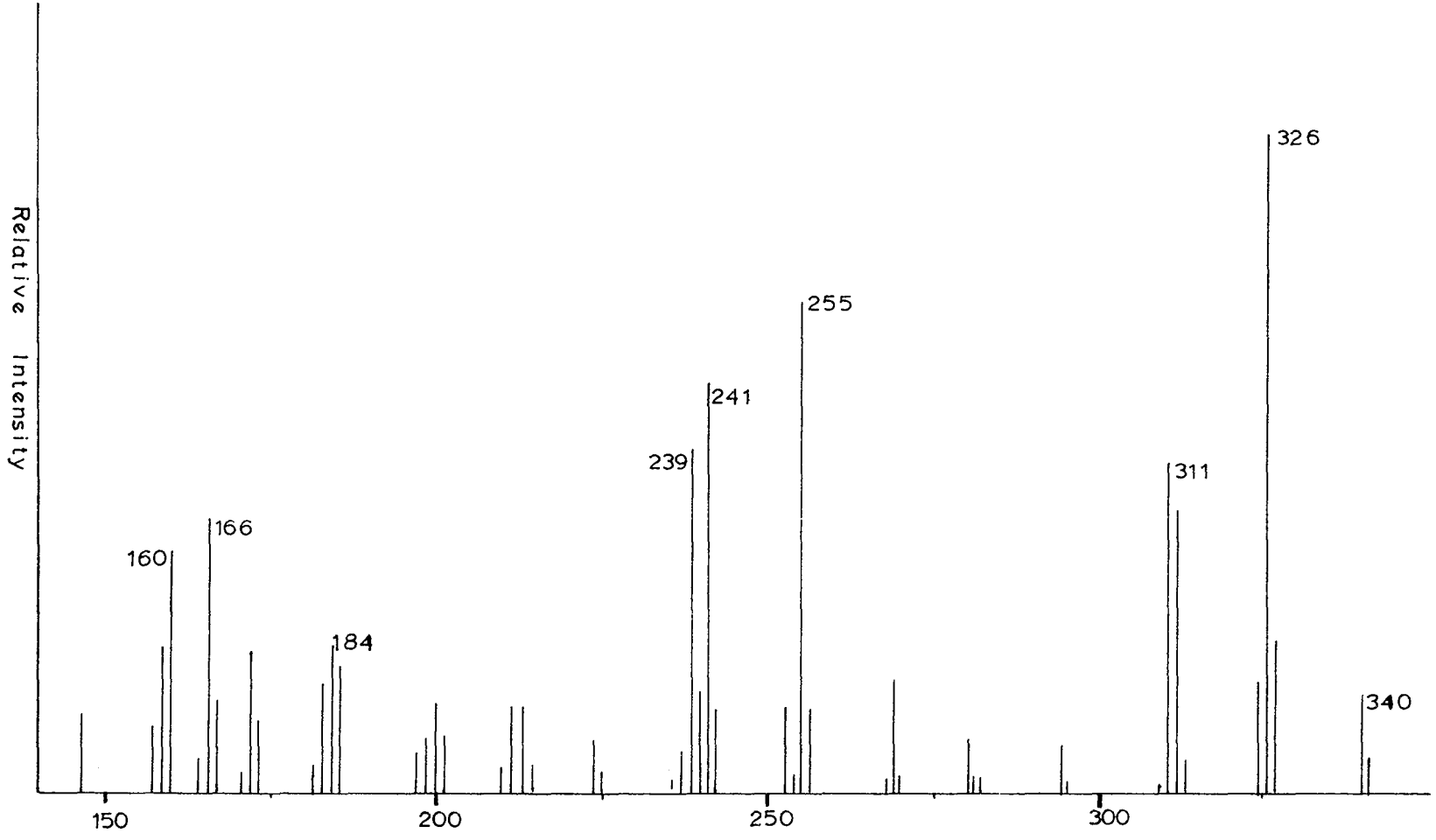
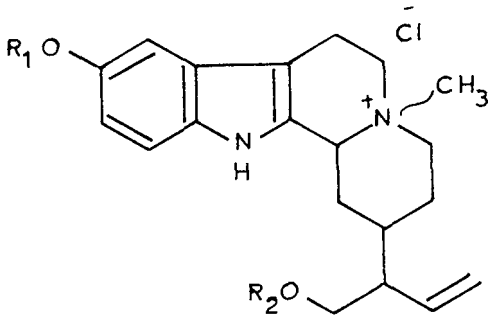


Figure 4_ MASS SPECTRUM OF "21-METHOXYHUNTERBURNINE"* METHOCHLORIDE

* 21-O-METHYL HUNTERBURNINE

singlet at 3.42δ could be attributed to a methoxyl group, the N-methyl group being at 3.25δ.



	R_1	R_2
18	CH_3	H
19	H	CH_3

The possibility to have an aromatic methoxyl group is excluded because its presence could not affect the ultraviolet absorption as this base did specially in the presence of alkali. In addition, the fragmentation pattern in the mass spectrum of this alkaloid is the same as hunterburnine specially in the tetrahydrocarboline and indole portion. Structure (18) could therefore be excluded.

The occurrence of the 255 m/e peak in both hunterburnine and this unknown salt reveals that the extra methylene group is located in the substituent on ring D since the 255 m/e peak is formed by the loss of the C_{15} chain as in hunterburnine (Figure 5). Since the extra methyl group is situated on an oxygen and the only oxygen left is the one of the primary alcohol, we presume that the C_{21} position is the one bearing the methoxyl group as an aliphatic ether. The occurrence of methoxy aliphatic ethers has been reported to occur in indole alkaloids in the case of refractidin[38] (20) and deserpidein[39] (21).

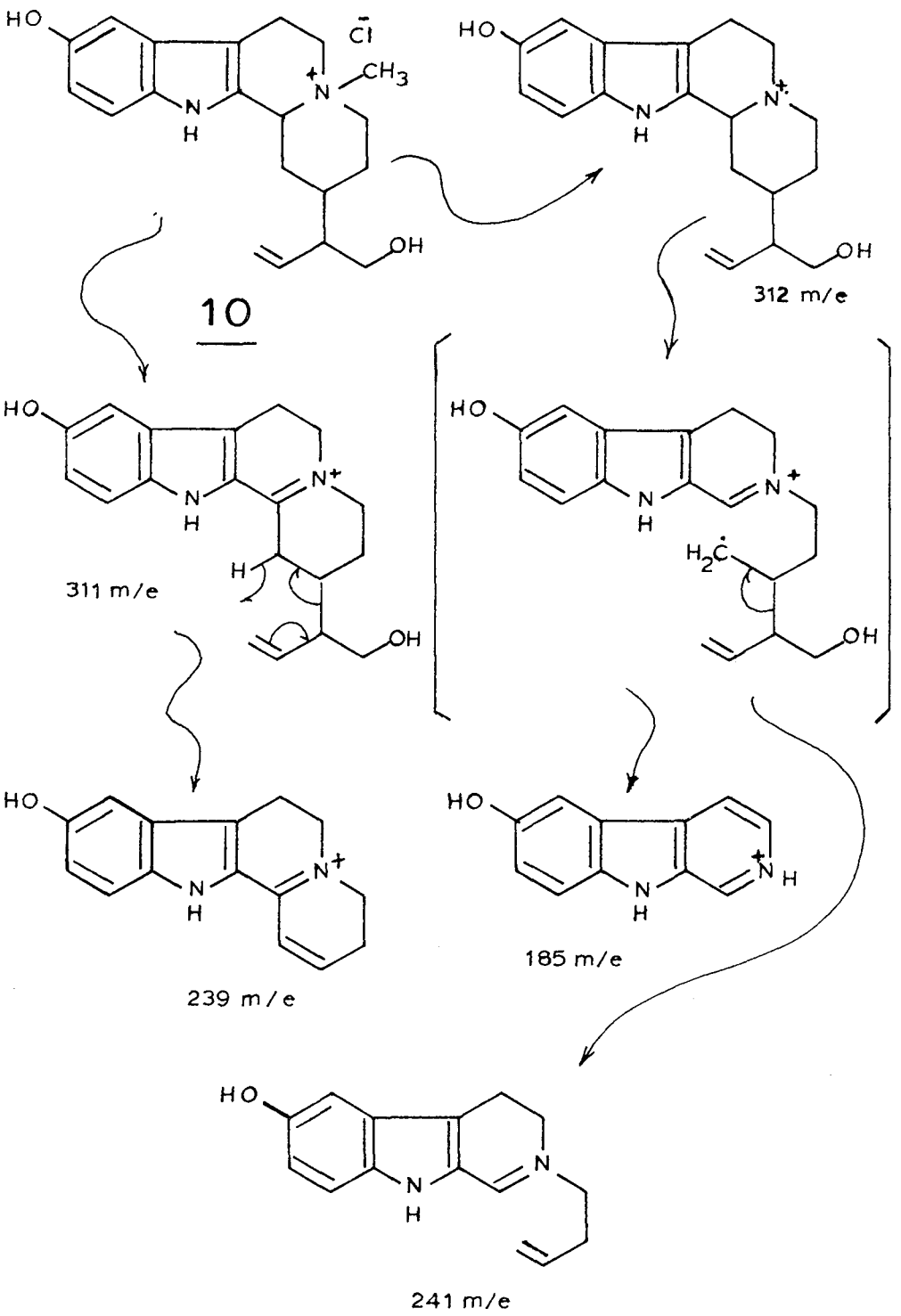
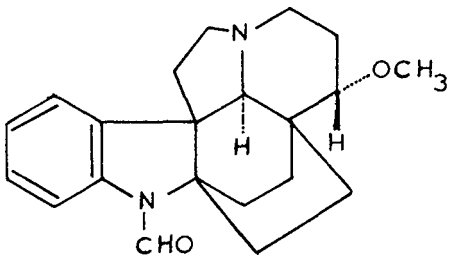


Figure 5._ MASS SPECTROMETRIC FRAGMENTATION OF HUNTERBURNINE METHOCHLORIDE



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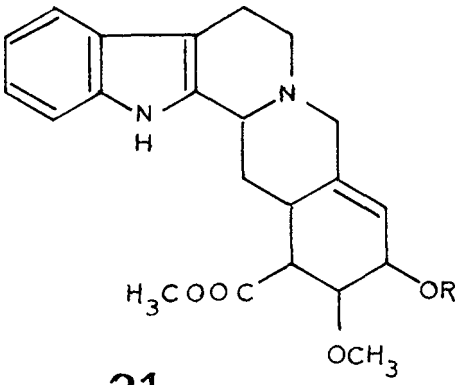

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For the foregoing reasons, we tentatively propose structure (19) for this base of which we isolated unfortunately just sufficient to determine the spectra already discussed. The question whether 21-methoxyhunterburnine methochloride is a natural product or an artifact formed during the extraction or separation of the alkaloids remains without answer.

Fortunately, we isolated a second base from chromatograms B, C and D. Due to its relative abundance, we succeeded to separate and crystallize 1.5 g of pure product, elementary analysis of which revealed its composition to be $C_{20}H_{27}N_2OCl$ with neither $C\text{-}CH_3$ nor $O\text{-}CH_3$ groups. This molecular formula confirms the appearance of a molecular ion at 311 m/e in the mass spectrum and its quaternary nature was confirmed by a positive test for chloride ion. The UV absorption of this base (λ_{max} 222, 272, 289; λ_{min} 240, 286 and λ_{sh} 283 nm) is unaffected by acid or base which suggests an indole chromophore. The infrared spectrum shows peaks typical of an indolic NH (3400 cm^{-1}) and an hydroxyl group (3135 cm^{-1}). The 60 Mc/s NMR spectrum has a broad two-proton signal at δ8.32, arising from protons which are exchangea-

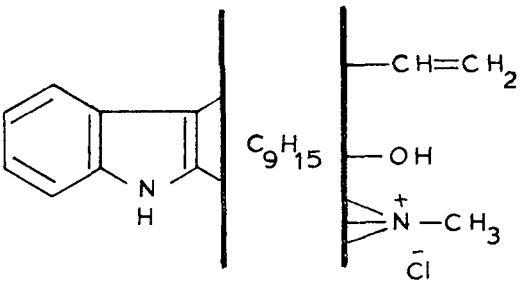
ble in deuterium oxide and can be assigned to hydroxyl and NH protons.

Acetylation of this alkaloid with acetic anhydride in pyridine at 90° affords an O-acetyl derivative (ν_{max} 1740 cm^{-1}) with no N-acetyl group indicating that the hydroxyl group is present as an alcoholic function and that the remaining nitrogen in the molecule is the quaternary one.

A broad four-proton multiplet in the NMR spectrum between 7.1 and 7.7δ shows that the indolic benzenoid ring is unsubstituted and a complex three-proton multiplet at 5.54δ is assigned to a vinyl group.

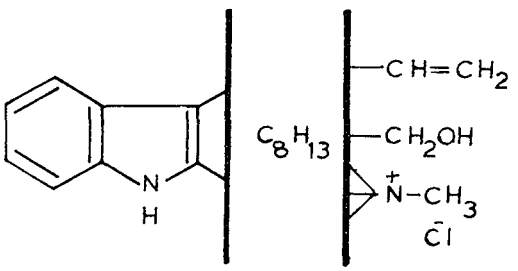
The reduction of this $C_{20}H_{27}N_2OCl$ alkaloid over Adams catalyst gives a dihydro product $C_{20}H_{29}N_2OCl$ with the up-take of only one mole of hydrogen. This dihydro product shows no signal in the double bond region in the NMR spectrum but, unlike the spectrum of the parent alkaloid, shows a broadened three-proton triplet at 0.90δ which can be assigned to a methyl group attached to a methylene group. Formation of this methyl group by reduction confirms the presence of a vinyl group in the parent alkaloid. Hydrogenation did not alter the UV spectrum in various media, thus indicating that the double bond in the starting material was not conjugated with the indole chromophore.

A singlet at 3.47δ which integrates for three protons can be assigned to an $N\text{-}CH_3$ group. Demethylation of the parent alkaloid led to a tertiary base $C_{19}H_{24}N_2O$ which showed no signal in the $N\text{-}CH_3$ region of its NMR spectrum. Since the natural base isolated is quaternary, we could say that the second nitrogen (N_b) is the one bearing the methyl group. Assembling these data permits an approach to the structure as presented by (i).



(i)

The NMR spectrum of the demethylated product shows a two-proton multiplet centered at 3.68 δ which can be assigned to the methylene protons of a hydroxymethylene group because after acetylation the multiplet undergoes a down-field shift due to the anisotropy of the O-acetyl group. This shift provided strong evidence that the formation of the acetate involved the acetylation of a primary alcohol[40]. The partial structure (i) could be extended to (ii).



(ii)

The fragmentation pattern in the mass spectrum of the parent base

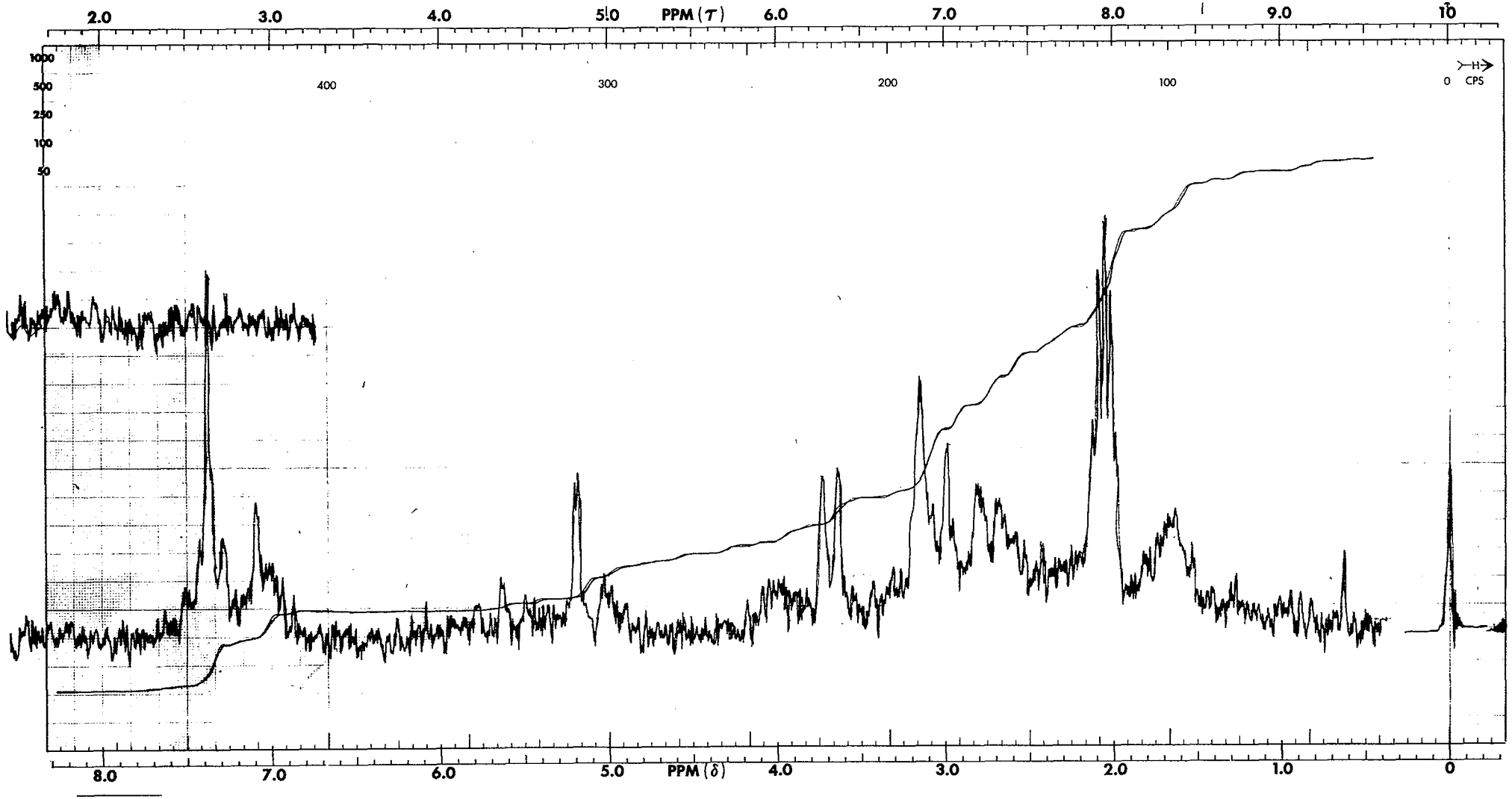
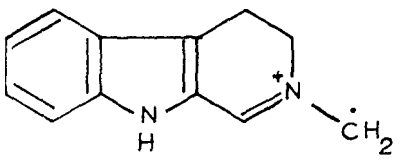


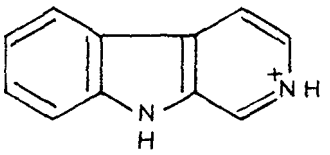
Figure 6.- Nuclear magnetic resonance spectrum of antirhine.

shows the characteristic peaks of tetrahydrocarboline derivatives[41] at 197, 184 (22), 169 (23) and 156 (24) m/e. The unchanged positions of these peaks in the mass spectrum of the acetate show that the hydroxyl group is removed from the β-carboline portion.



184 m/e

22



169 m/e

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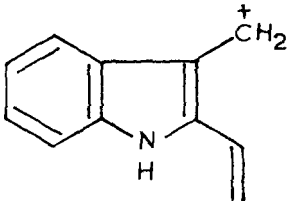
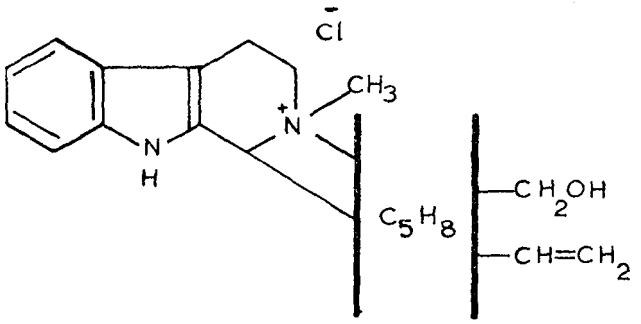


156 m/e

24

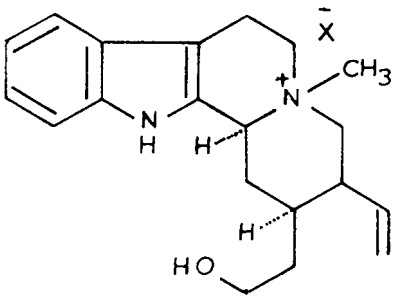
So partial structure (ii) could be extended to (iii).



(iii)

The mass spectrum of the parent base shows the molecular ion at 311 m/e and a strong M-15 peak at 296 m/e while the spectrum of the dihydro product has a molecular ion at 313 m/e and the M-15 at 298 m/e which

confirms the presence of the reducible double bond. A major peak at 225 m/e in the spectrum of the parent base corresponds to an M-86 ion. This same peak appears in the mass spectra of the dihydro, acetylated and demethylated products. This suggests that the 225 m/e peak does not contain the reducible double bond nor the primary alcohol. The unknown parent is therefore isomeric with the known corynane type of alkaloid. The published data on melinonine B (25) isolated from Strychnos melinoniana Baillon[42] and geissoschizol (26) isolated recently from the Hunteria eburnea leaves[5] are different from our parent alkaloid. Comparison of the physical properties (melting points, $[\alpha]_D$ values) of the three alkaloids and their derivatives shows that it is not identical with either.



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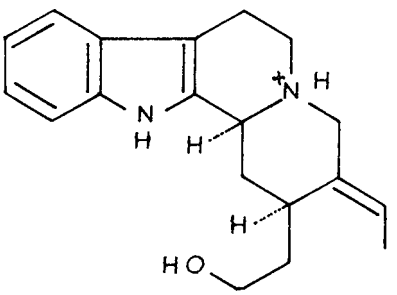
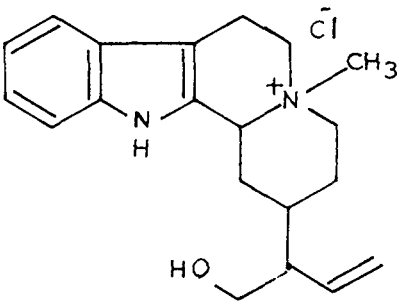


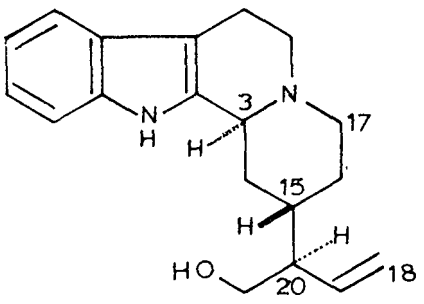
26

On the other hand, comparing the NMR and mass spectra of our unknown base with those of hunterburnine methochloride, they were found to show a strong similarity except in the aromatic region of the NMR spectra. The mass spectrum of hunterburnine methochloride showed most peaks at 16 units higher than the unknown base. The characteristic peaks of hunterburnine[25] at 225, 241 and 239 m/e correspond to peaks in the other spectrum at m/e 239, 225 and 223 respectively. This strong and striking similarity suggests the unknown base to be desoxyhunterburnine methochloride (27).



27

A similar compound has been recently described in the literature by Johns et al.[43] as being antirhine methochloride isolated as the tertiary base antirhine (28) from Antirhea putaminosa (F. Muell.) Bail. Antirhine was easily quaternized with methyl iodide and converted to the chloride form or hydrogenated and then quaternized. We shall call the unknown base antirhine methochloride a proposal yet to be justified. Table 2 shows the differences between the antirhine methochloride and its dihydro product obtained from Hunteria eburnea and that obtained from Antirhea putaminosa.



28

TABLE 2

Plant source	Antirhea putaminosa after quaternization		Hunteria eburnea	
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
Antirhine methochloride (27)	331-334°	-	306°	+75°
Dihydroantirhine methochloride	322-324°	-16.3°	305°	+71.4°

The mass spectra of both antirhine methochlorides* look identical. That the fragmentation observed is identical must be interpreted with caution since the stereochemistry only influences the fragmentation to a negligible extent. Whether our antirhine methochloride is a diastereoisomer of John's antirhine methochloride is a question to be answered.

We first demethylated antirhine methochloride (27) by the sodium thiophenoxide method[44] which, according to M. Shamma, is of the simple S_{N2} type and consists of attack by the thiophenoxide anion in refluxing 2-butanone on the N-methyl group. Kametani et al.[44a] reported recently the application of thiophenol in debenzylation and dealkylation of quaternary ammonium salts. According to their work, the reaction would proceed

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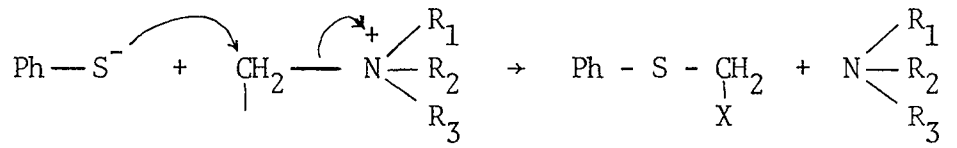
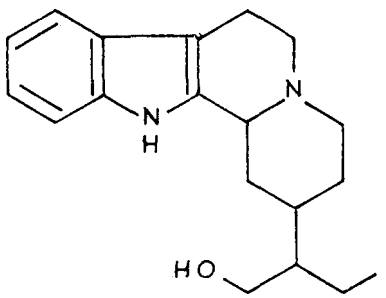
* Sample kindly provided by Dr. S.R. Johns, Csiro Chemical Research Laboratories, Melbourne.

in one stage as follows:



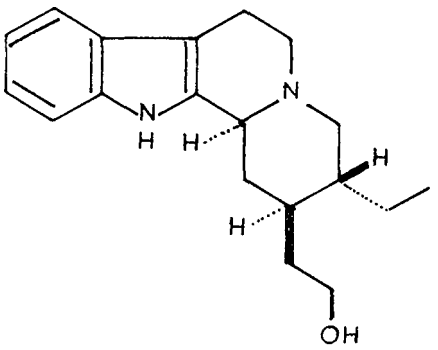
(X=H, Ph, $-CH=CH_2$, $-CH=C(Me)_2$)

Antirhine (28) obtained from this demethylation showed a molecular ion in the mass spectrum at 296 m/e which confirmed the molecular formula $C_{19}H_{24}N_2O$. Reduction of antirhine over Adams' catalyst gives dihydroantirhine (29), the NMR spectrum of which shows no signals in the double bond region but has, unlike the spectrum of antirhine, a broadened three-proton triplet at 0.90δ which can be assigned to a methyl group attached to a methylene group.



29

Formation of this methyl group by the reduction of antirhine confirms the presence of a vinyl group in antirhine. Dihydroantirhine $C_{19}H_{26}N_2O$ (29) is therefore isomeric with the known corynane (17, 18-secoyohimbane) type alkaloids dihydrocorynantheol[42, 45] (30) and corynantheidol[42] (31).



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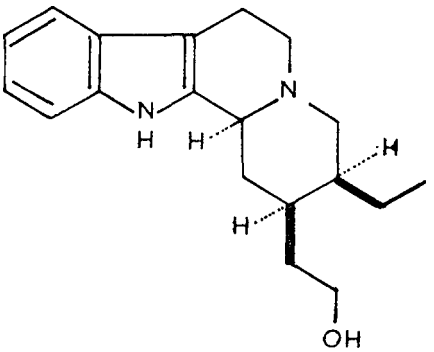


31

The spectral properties of dihydroantirhine suggest close relationship with these two isomeric alkaloids, but comparison of the physical properties of the alkaloids and those of their derivatives show that dihydroantirhine is not identical with either. This conclusion is confirmed by differences in the infrared spectrum of dihydroantirhine (29) and the published spectra of dihydrocorynantheol (30) and corynantheidol (31). Dihydroantirhine on the other hand has been synthesized by two Japanese groups[46, 47] and their published data are in agreement with our values for dihydroantirhine.

A major peak at 225 m/e in the spectrum of antirhine (28) corresponds to a M-71 ion, and the presence of a metastable ion at M^* 171 shows that this ion is derived by a single elimination. The corresponding M-73 ion at 225 m/e in the spectrum of dihydroantirhine (29) proves that the 71 mass units fragment eliminated from antirhine possesses both the reducible double bond and the exchangeable proton of the hydroxyl group. It is proposed that this elimination involves cleavage of the $C_{3,14}$ bond and subsequent elimination of the side chain at C_{15}, as shown in figure 7. A similar

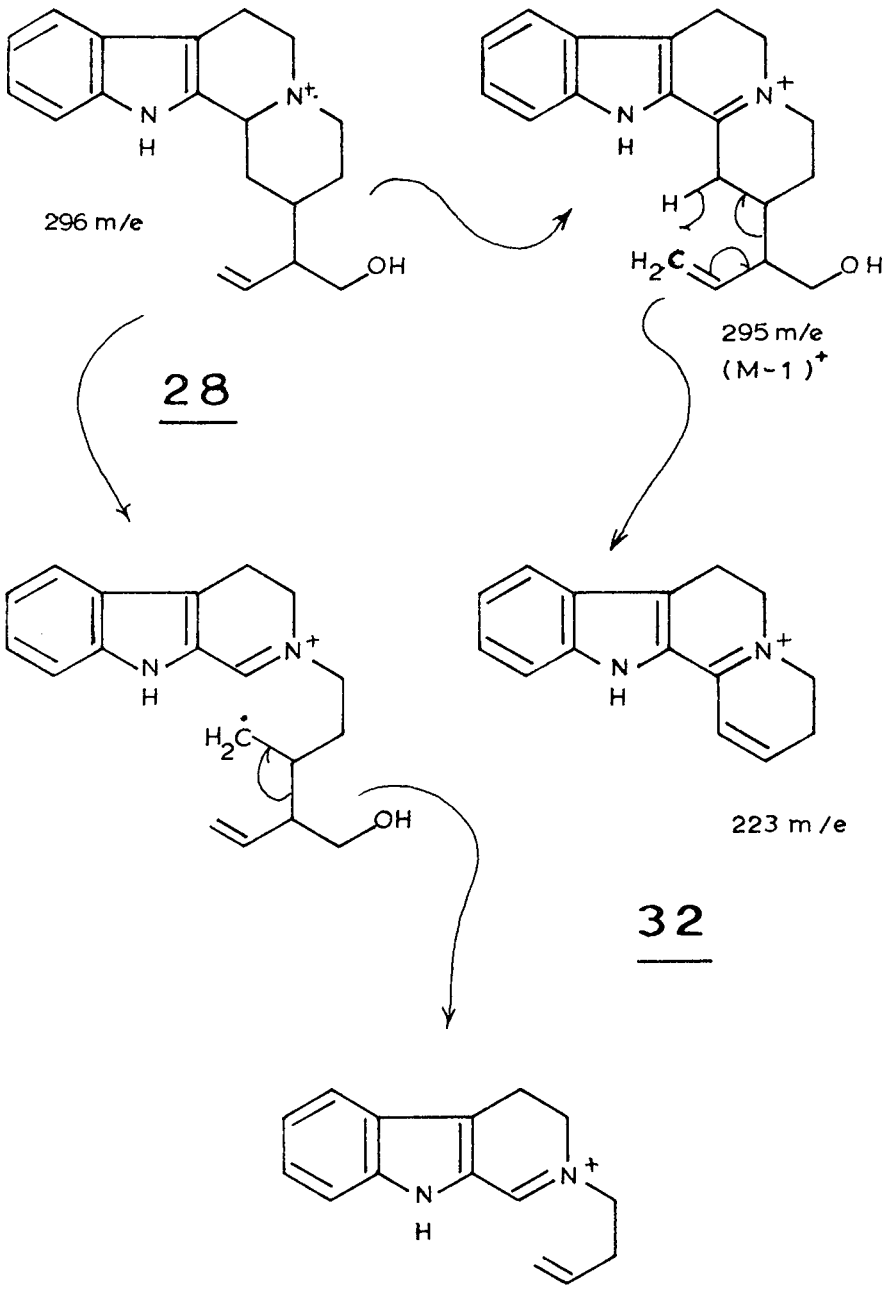
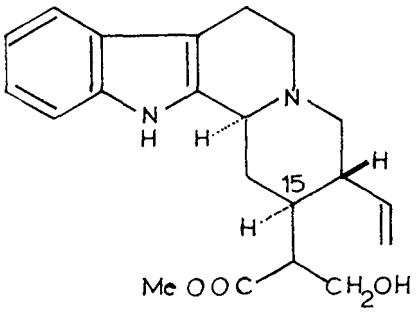


Figure 7.— MASS SPECTROMETRIC FRAGMENTATION OF ANTIRHINE METHOCHLORIDE

elimination of the side-chain at C_{15} has been observed in the spectrum of sitsirikine (33), an alkaloid isolated from Vinca rosea Linn[48].



33

The base peak in the spectrum of antirhine (28) appears at 223 m/e and corresponds to the M-73 ion, and a metastable peak at 168 m/e indicates that this ion is derived from the (M-1) ion. It has been suggested by Johns et al.[44] that this fission involves cleavage of the $C_{15,20}$ bond and McLafferty rearrangement of the C_{14} hydrogen atom as shown in figure 7, to give the ion (32). Since this rearrangement involves the $C_{18,19}$ double bond, no intense peak at 223 m/e is observed in the spectrum of dihydroantirhine (29) and the base peak is the molecular ion, 298 m/e. The remaining peaks in the mass spectra of both antirhine (28) and dihydroantirhine (29) are consistent with the proposed structures. Both possess M-31 ions, which are produced by loss of the hydroxymethylene group, and peaks at 197, 184, 169 and 156 m/e typical of a tetrahydro-β-carboline moiety[41] appear in both spectra.

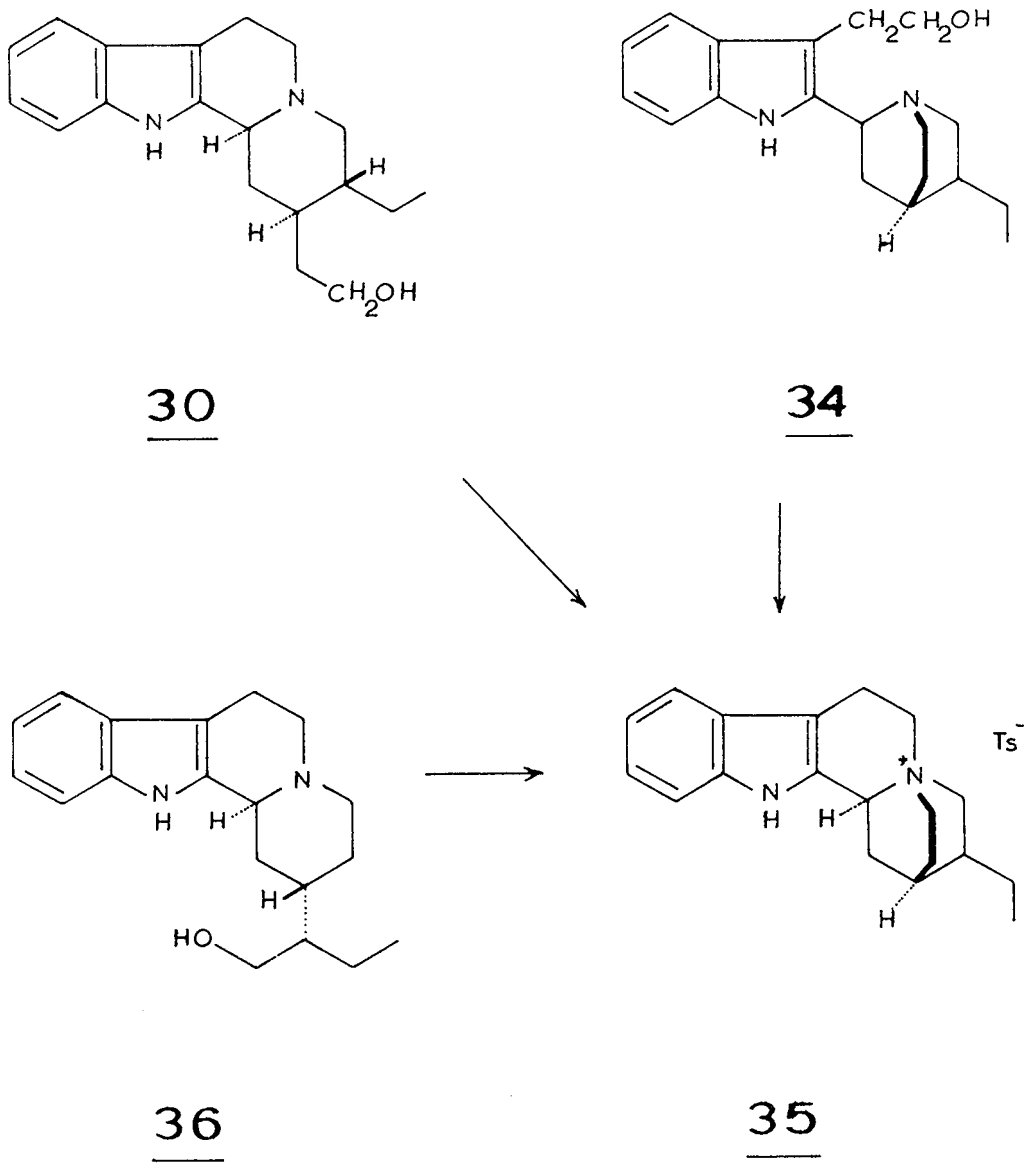
So far we have established the structure of antirhine (28), and dihydroantirhine (29) with no mention of their stereochemistry. When dihydroantirhine (29) is heated with p-toluene-sulphonyl chloride in

pyridine, an O-tosyl derivative is formed, which in refluxing dimethylformamide cyclises to the quaternary tosylate (35). The same tosylate has been obtained from treatment of dihydrocinchonamine (34) and dihydrocorynantheol (30) with p-toluenesulphonyl chloride followed by cyclisation in dimethylformamide[30-32] and comparison of the tosylate (35) prepared from dihydroantirhine (29) with the published physical constants[32] of the tosylate prepared from dihydrocorynantheol showed their identity. The stereochemistry of dihydrocinchonamine and dihydrocorynantheol have been unambiguously shown to be that depicted in formulae (34) and (30) respectively[30-32].

The 15β-configuration in dihydroantirhine (36) established by the formation of the quaternary tosylate (35) is in accord with the biosynthetic hypothesis of Wenkert and Bringi[31, 32], which requires that the C_{15}-H in the normal corynane derivatives have the α-configuration.

Antirhine may be represented as derived from a corynane precursor by cleavage of the C_{21}-N_b bond, rotation about the $C_{14,15}$ bond and subsequent recyclisation linking C_{17} to N_b. Such a formal transposition of bonds as proposed by Taylor[21] and later by Johns[44] requires an inversion of configuration at C_{15}, and consequently a change from the normal 15α-H configuration to a 15β-H configuration.

Antirhine and antirhine methochloride are the parent members of a small group of indole alkaloids which possess a 15β-hydrogen and which were previously represented by the quaternary α- and β-methosalts of hunterburnine (10) and by Vallesiachotamine (14).

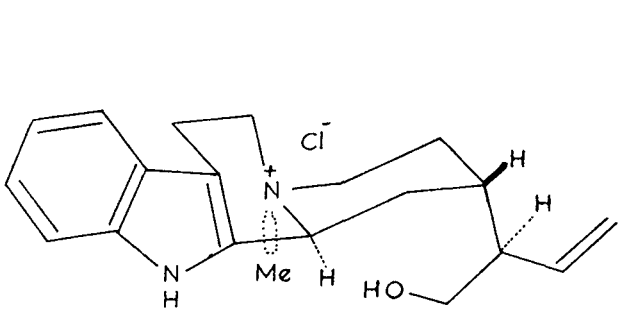


30 34

36 35

A last point to consider is the difference in optical rotation (table 2) between the different antirhine methochlorides. For this we prepared antirhine (28) $[\alpha]_D$ = -2° by demethylation of antirhine methochloride and proved its structure to be identical with antirhine naturally occurring in Antirhea putaminosa[44].

We also prepared antirhine methiodide which was converted to the chloride form on ion exchange resin and we rapidly found that the new antirhine methochloride $[\alpha]_D$ = -17.9° was completely identical with that of Johns (table 2). This means that the change in optical rotation is only attributable to the N-CH_3 linkage since methylation and demethylation affects no other part of the molecule (as assymetric centers are concerned) except the formation or the rupture of the N-CH_3 bond. There are two possible diastereoisomers which could be formed depending on the configuration of the quaternary nitrogen.



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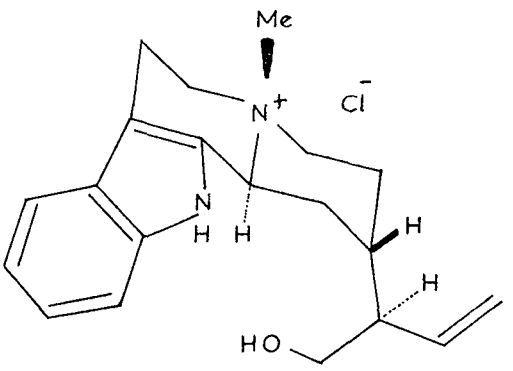


b

37

This type of isomerism has already been reported in the case of the epimeric methiodides of yohimbane[27] (11), and in the naturally occurring hunterburnine α- and β-methosalts[21] (10a and b).

The NMR spectra of naturally occurring antirhine methochloride and that of synthetic origin show a difference in the positions of the N-CH_3 peaks. In agreement with Katritzky's findings[28, 29], the chemical shift

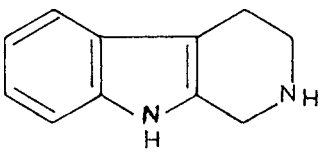
attributed to the quaternary methyl of the cis-quinolizidine (naturally occurring) is found at lower field (δ = 3.47) than in the case of trans (synthetic) (δ = 3.31); both spectra being run in trifluoroacetic acid using tetramethylsilane as reference. We also noticed that on methylation predominantly one isomer is formed as seen from the optical rotation and the singlet in the NMR spectrum integrating for three protons at 3.3δ.

From this we can conclude that naturally occurring antirhine methochloride is the α-isomer (37a) while that formed in the laboratory is mainly the β-isomer (37b). The sample obtained from S.R. Johns is in effect antirhine β-methochloride (37b) and is completely identical (mixed melting point, IR, NMR and mass spectra) with antirhine β-methochloride we prepared from antirhine (28), the latter being the demethylation product of naturally occurring antirhine α-methochloride (37a).

It was noticed that the β-isomer was more soluble in methanol than the α-isomer. According to Jordan and Scheuer[26], a C/D trans ring system renders a product more soluble in methanol than the C/D junction cis. This striking solubility behaviour has been observed with many indole alkaloids having cis or trans C/D ring system. A final confirmation of this point was obtained from the IR spectrum, which clearly showed the diagnostic 3.4 - 3.7μ (2700-2850 cm^{-1}) band, considered characteristic for a C/D trans compound[50, 51].

A third quaternary alkaloid has been isolated from chromatograms C and D. It crystallized easily and its elemental analysis confirmed its molecular formula as $C_{20}H_{29}N_2OCl$. The melting point, infrared and ultraviolet spectra of this base were in close agreement with the salt Taylor and co-workers named Hunteria eburnea alkaloid-J[21].

A typical indole UV spectrum unchanged in different media together with the mass spectrum peaks at 197, 184, 169 and 156 m/e suggest an unsubstituted indole of the tetrahydro-β-carboline[41] type (iv). The absence of O-methyl residues and double bonds and the presence of a terminal methyl group at a saturated carbon, together with the presence of an N-CH_3 group were all suggested from the NMR spectrum.



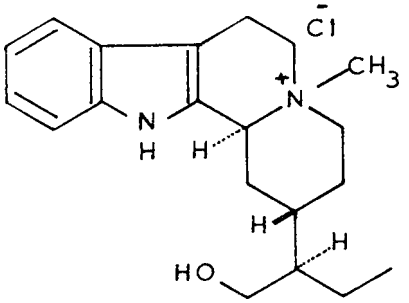
(iv)

Direct comparison of this alkaloid-J with dihydroantirhine α-methochloride (39) prepared by the hydrogenation of naturally occurring antirhine α-methochloride proved them to be identical. Mixed melting point showed no depression, superposable IR, NMR and mass spectra left no doubt that Hunteria alkaloid-J is really dihydroantirhine methochloride (38).

As further proof, alkaloid-J was demethylated employing the sodium thiophenoxide method[45] and the demethylated product obtained was compared with dihydroantirhine obtained from the hydrogenation of antirhine. They were found to be identical in all respects. Proof of the stereochemistry of the dihydroantirhine obtained from alkaloid-J was obtained by the cyclisation reaction with p-toluenesulphonyl chloride in dimethyl formamide[30-32] to the quaternary tosylate (35).

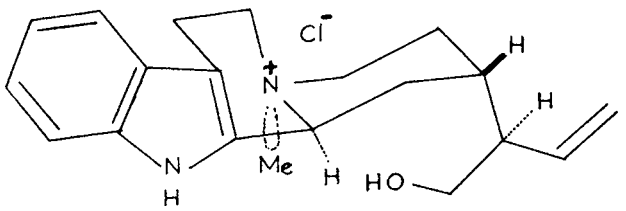
Again this dihydroantirhine reacted with methyl iodide to yield

dihydroantirhine methiodide which was converted on exchange resin to dihydroantirhine methochloride $[\alpha]_D$ = -16°. The NMR spectra of the two dihydroantirhine methochlorides show a different N-CH_3 peak position as was previously noticed in the case of α and β antirhine methochlorides.



38

From these results and in accordance with our previous investigations on antirhine α- and β-methochlorides it is apparent that Hunteria eburnea alkaloid-J is dihydroantirhine α-methochloride (39) and the product synthesized from dihydroantirhine is dihydroantirhine β-methochloride having a C/D trans ring system.



39

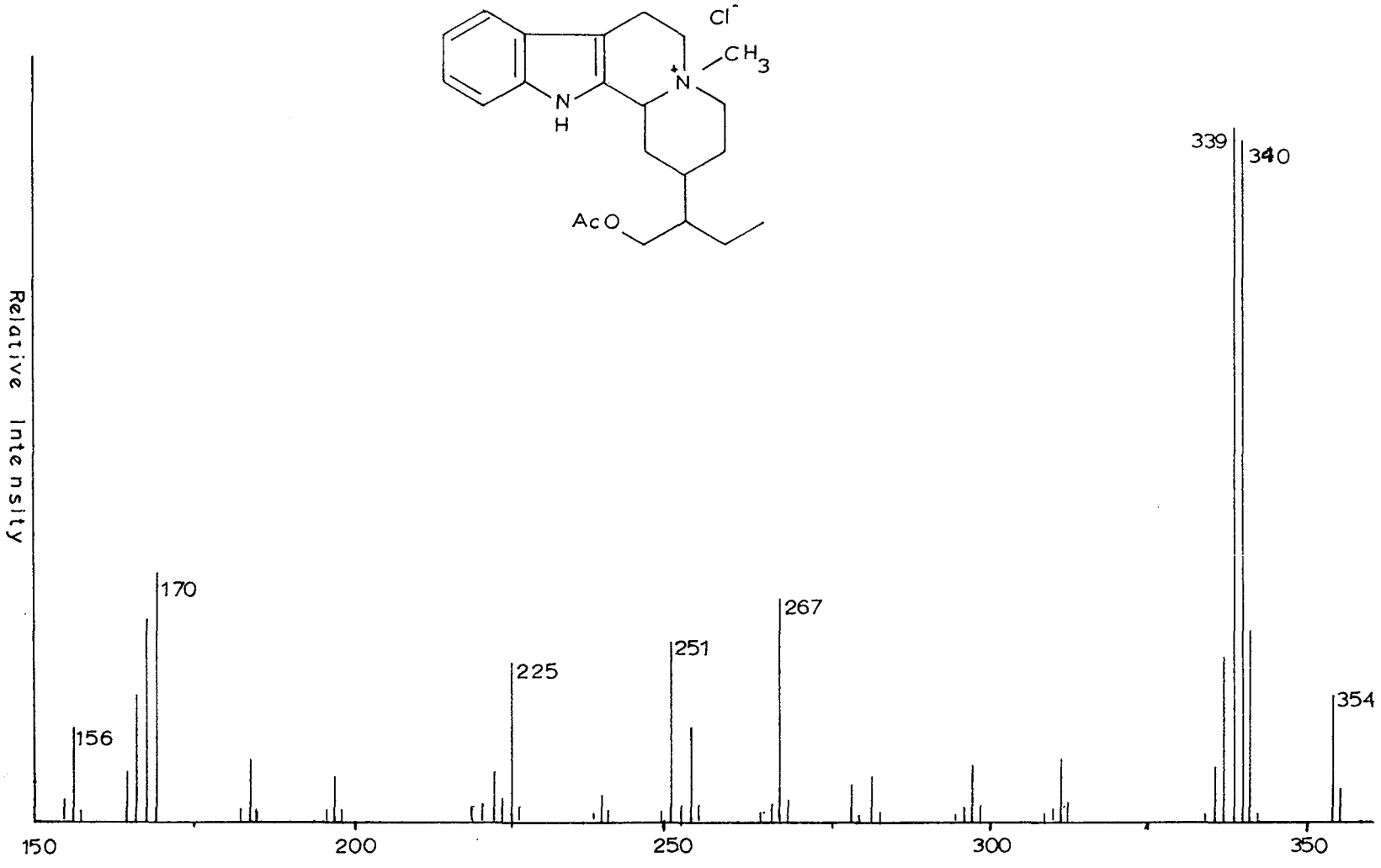


Figure 8._ MASS SPECTRUM OF O-ACETYLDIHYDROANTIRHINE
α-METHOCHLORIDE

Although hunterburnine α- and β-methochlorides (10a, b) do occur together in Hunteria eburnea and Pleiocarpa mutica, only the α-methochlorides of antirhine and dihydroantirhine (37a and 39) occur alone in Hunteria eburnea but we presume if the corresponding β-isomers are present in the same plant, we did not succeed in isolating them. It is worthy of note that N-methylation in vitro yields probably a mixture where the β-isomer is predominant.

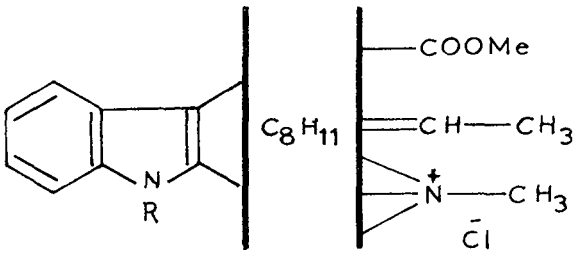
-.CHAPTER II.-

MISCELLANEOUS QUATERNARY ALKALOIDS

An alkaloid isolated from chromatogram B showed a positive test for chloride ion suggesting its quaternary nature. This base gave an unusual royal blue coloration with Vassler's reagent so that it could be easily followed on thin layer chromatography even to the point of deciding the purity of the alkaloid. A faint violet red coloration appears after few seconds with ceric sulphate. Elemental analysis confirmed the molecular formula $C_{21}H_{25}N_2O_2Cl$ with one mole of water of crystallization and the absence of OMe groups. The physical constants of this alkaloid correspond to the published constants of Hunteria eburnea alkaloid-F.

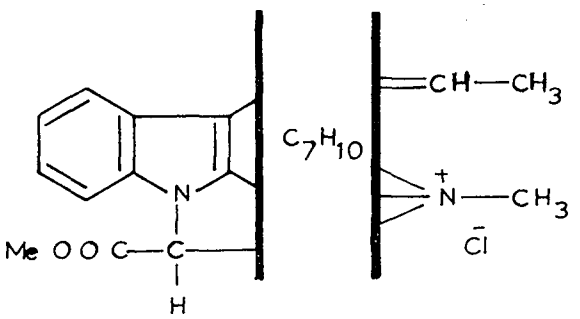
The ultraviolet spectrum of this base shows absorption typical of the indoline moiety[51] where positions 1, 2 and 3 are substituted a fact confirmed by various colour reactions.

The infrared spectrum confirms the presence of the aromatic nucleus and a carbonyl group with absorption at 1737 cm^{-1}. Bands due to indolic NH or hydroxyl groups are absent in the spectrum. The NMR spectrum confirms the presence of four aromatic protons (4H multiplet 6.9 - 8δ) and the lack of NH protons. A peak at 3.70δ integrating for three protons could be assigned to a methyl group on either the indolic or the more basic nitrogen. The N-methyl being at relatively low field it could be assigned to the quaternary rather than the indolic nitrogen. A broad doublet at 5.65δ integrating for one proton accompanied by a doublet for three protons at 1.58δ is clear evidence for an ethylidene side-chain. With this information the partial structure (v) can be written where the C_8 residue could contain up to three rings.



(v)

A very important feature in the nuclear magnetic resonance spectrum is a doublet at 4.99δ integrating for one proton. This proton is only ascribable to a proton adjacent both to an aromatic moiety and a carbon bearing a carbonyl group. This would mean that the C attached to the indolic nitrogen could bear both a single proton and the ester group. Partial structure (v) could be extended to (vi).



(vi)

The mass spectrum of the base was very difficult to interprete. Being sure of the quaternary character of the base, we expected the molecular ion to be at 337 m/e. The presence of peaks at 374, 373 and

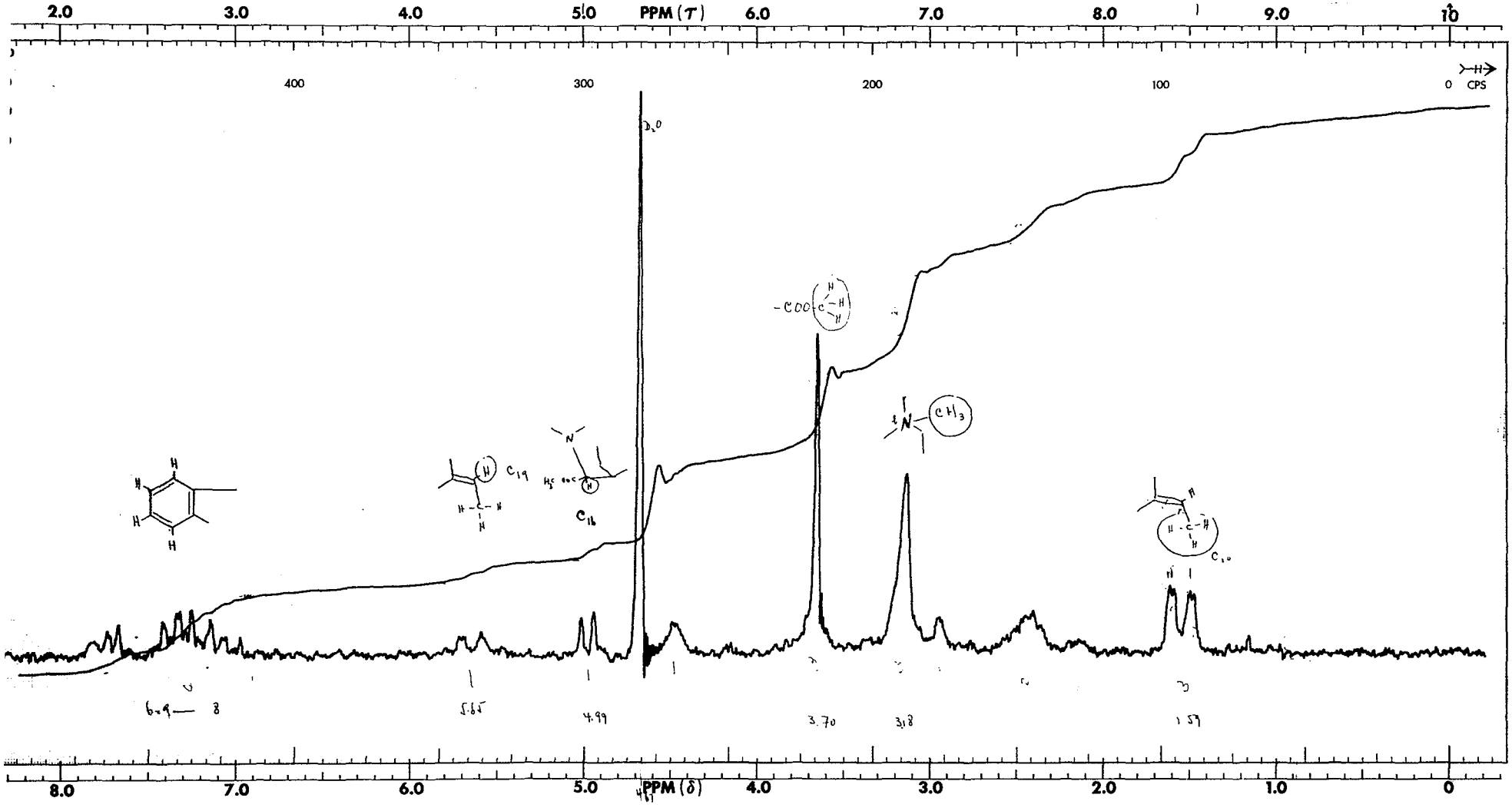
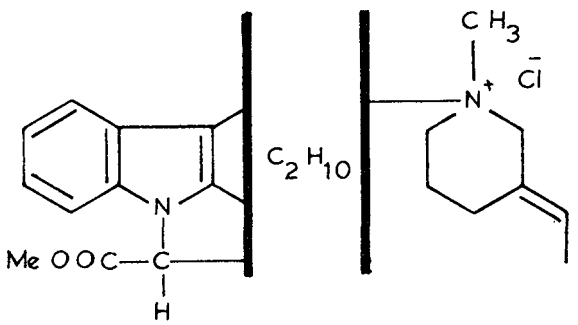


Figure 9.- Nuclear magnetic resonance spectrum of pleiocarpamine methochloride.

372 m/e suggests either a dimeric molecule or the facile incorporation of the chloride anion as a covalently bound chloro substituent. Exchanging the chloride ion by iodide on exchange resin, the mass spectrum of the product showed the highest peak at 464 m/e. This proves that before fragmentation in the mass spectrum, the quaternary base undergoes a certain rearrangement resulting in the incorporation of the halide anion to the molecule.

The presence of the peaks at 122 and 108 m/e in the mass spectrum of the base suggests the formation of a pyridinium ion in the fragmentation[52]. This led to the proposition that the basic nitrogen atom is located in a six-membered ring which also bears the ethylidene side chain. Partial structure (vi) could easily be extended to (vii).

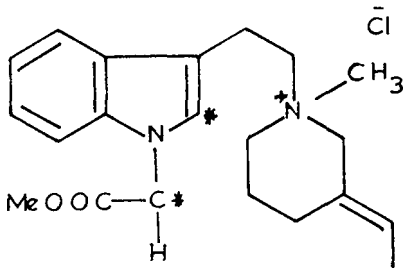


(vii)

Biogenetically most of the indole alkaloids are formed from tryptamine (2a) or tryptophan (2b) and in this case we could suggest the nature of the remaining two-carbon atoms until complete evidence for the structure is available.

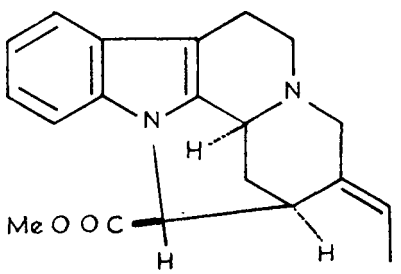
Expanding partial structure (vii) to (viii) would mean that what

remain are the two ring junctions at the starred carbons (viii).



(viii)

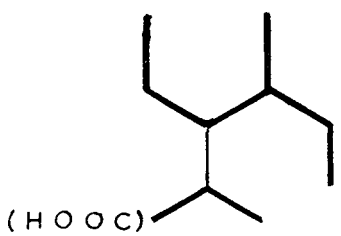
Considering all the possible structures involving these starred carbon atoms and the piperidine ring suggested that this "alkaloid F" might be the methochloride of pleiocarpamine (40), a tertiary base isolated previously from Hunteria eburnea[22]. We demethylated the quaternary salt by the thiophenoxide anion method[44]. Although the yield was poor, probably due to the presence of the ester group, the tertiary alkaloid ($C_{20}H_{22}N_2O_2$) was separated from the rest of the products on a silica gel column. The tertiary base showed a typical indoline alkaloid of the same appearance as the parent base, confirming that the quaternary nitrogen is not the indolic one. The NMR spectrum of the tertiary base showed the absence of the N-methyl group.



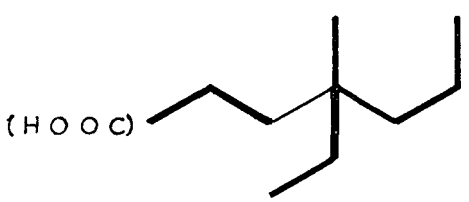
40

Catalytic hydrogenation of the tertiary alkaloid on Adam's Catalyst afforded a dihydro product $C_{20}H_{24}N_2O_2$ when stopping the hydrogenation after the absorption of one mole of hydrogen. The NMR spectrum of the dihydro product still showed the presence of the ethylidene double bond. This may result either of the hydrogenation of another double bond in the molecule or due to ring opening. The UV spectrum of the dihydro product shows a typical dihydroindoline[51] in which positions 1, 2 and 3 are substituted. That the hydrogenation occurs in the indole moiety rather than on the side-chain double bond is a typical case among the indole alkaloid occurring in pleiocarpamine.

According to Taylor[21] none of the quaternary alkaloids of Hunteria eburnea is a quaternary derivative of the co-occurring tertiary bases. Quaternary bases whose structures were determined by Taylor et al.[21, 53] were derived from the yohimbinoid precursor (41), whereas the tertiary bases originated from the aspidospermine precursor (42).



41



42

In fact the tertiary base $C_{20}H_{22}N_2O_2$ was compared with pleiocarpamine*

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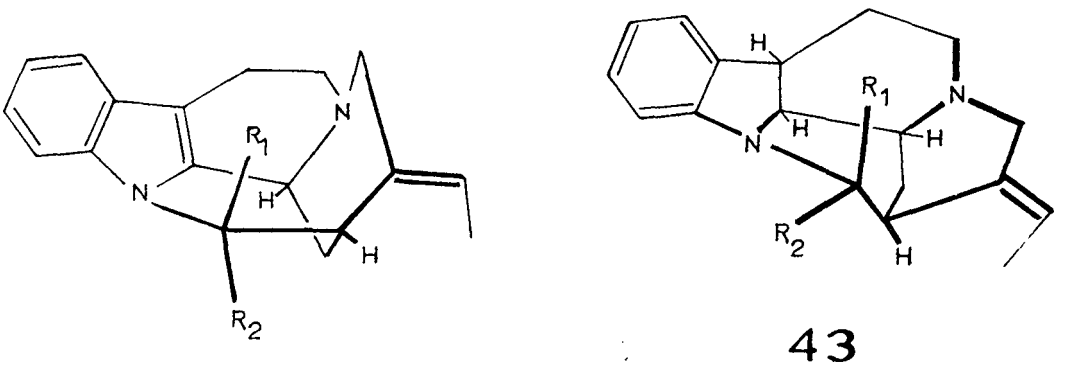
* We thank Dr. M. Hesse (Zürich) for kindly providing us with an authentic sample of pleiocarpamine.

(40) and showed no depression in the m.p. and their IR spectra were superposable. This means that on hydrogenation, pleiocarpamine do give 2,7-dihydropleiocarpamine. Hydrogenation takes place stereospecifically at the 2,7-rather than the 19,20-position[54] to give 2,7-dihydropleiocarpamine (43).

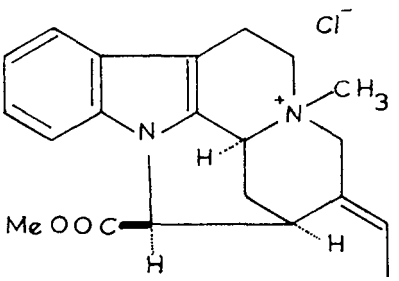
We can therefore say that the quaternary base we isolated and formerly called Hunteria eburnea alkaloid-F[21] is really Pleiocarpamine methochloride (44).



43

R_1 = $COOCH_3$

R_2 = H

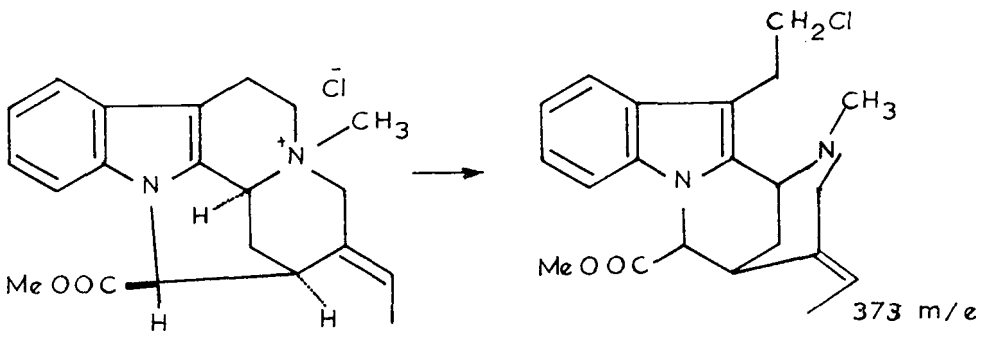


44

Searching in the literature, we find that pleiocarpamine methochloride (44) has been prepared synthetically by quaternizing naturally occurring pleiocarpamine[55] (31) from Pleiocarpa mutica Benth. in 1964[53]. A year later pleiocarpamine methochloride (44) was isolated occurring naturally in the same plant[25].

The behaviour of pleiocarpamine methochloride (44) in the mass spectrum has been studied by M. Hesse et al.[56]. They postulate that mass spectrometric analysis of quaternary nitrogen compounds show that three principal thermal processes occur, namely dealkylation, Hofmann degradation and substitution. (Figure 10).

Since pleiocarpamine methochloride (44) incorporates the halogen in the cation, formation of type (48) occurs (figure 10). This pyrolysis reaction has been confirmed by the synthesis of the supposed pyrolysis product (49).



44 **49**

Both pleiocapamine methochloride (44) and (49) have superposable mass spectra thus confirming the thermal rearrangement.

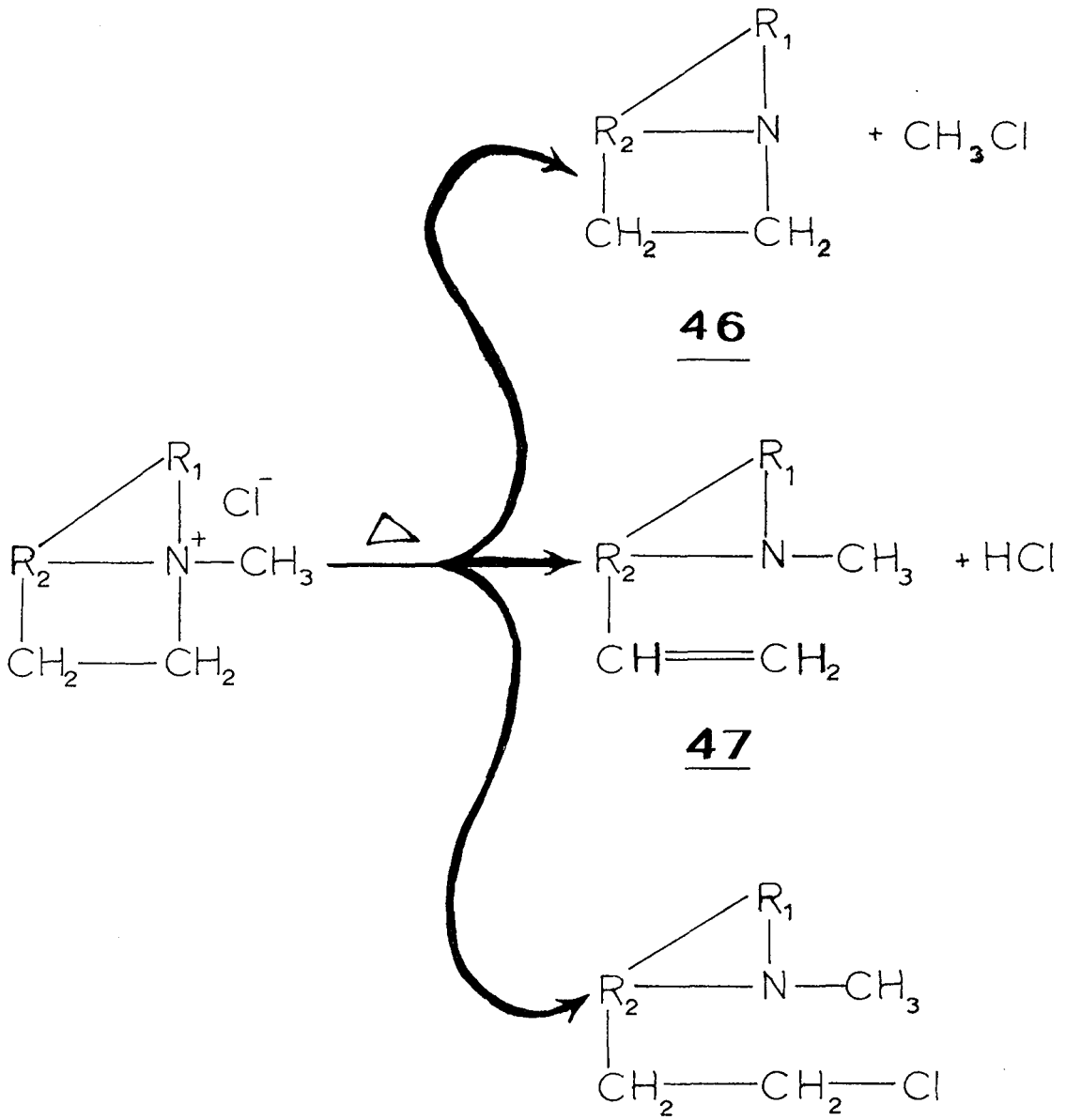


Figure 10.- PYROLYSIS OF QUATERNARY ALKALOIDS IN THE MASS SPECTROMETER

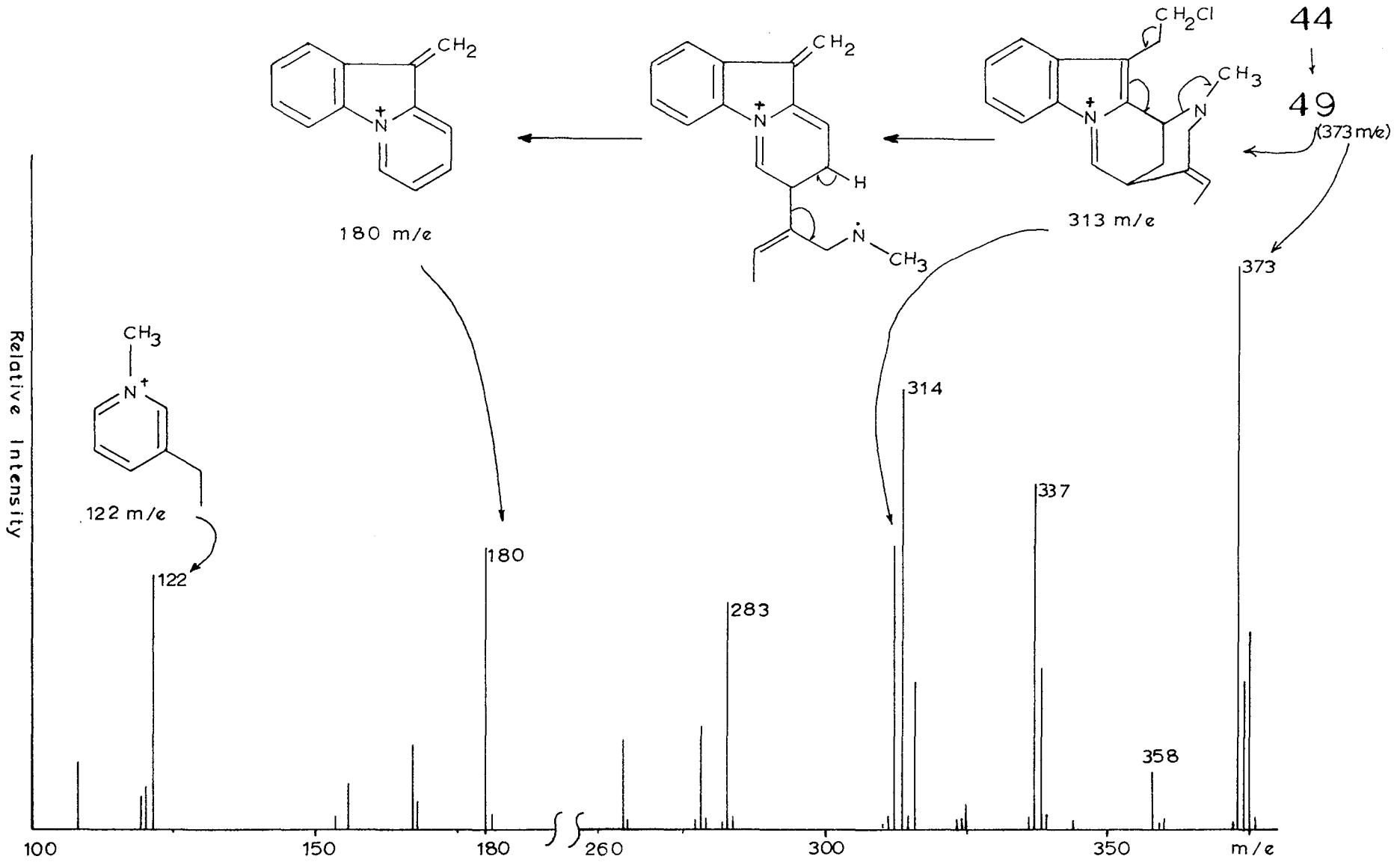


Figure 11 _ MASS SPECTRUM OF PLEIOCARPAMINE METHOCHLORIDE

Another quaternary alkaloid was isolated from chromatograms A, C and D. Its quaternary nature was confirmed by a positive test for chloride ion. The molecular formula $C_{20}H_{27}N_2OCl$ was confirmed by the appearance of a molecular ion at 311 m/e in the mass spectrum. The ultraviolet absorption of this salt (λ_{max} 222, 268, 288; λ_{min} 246, 286 and λ_{sh} 282 nm) is unaffected by acid or base which suggests an indole chromophore. The infrared spectrum shows peaks typical of an indolic NH (3220 cm^{-1}) and a hydroxyl group (3350-3450 cm^{-1}) with the absence of carbonyl bands. The NMR spectrum in CF_3COOH has a broad two-proton signal at δ8.20, arising from protons which are exchangeable in deuterium oxide and can be assigned to hydroxyl and NH protons. The spectrum shows no sign for the presence of $C\text{-}CH_3$, OCH_3 or unsaturation other than the unsubstituted benzenoid ring which appears as a broad four-proton multiplet between 7.1 and 7.6δ.

Acetylation of this alkaloid with acetic anhydride in pyridine at 90° affords an O-acetyl derivative (ν_{max} 1725 cm^{-1}) with no N-acetyl group indicating that the hydroxyl group is present as an alcoholic function. A singlet at 3.3δ which integrates for three protons in the NMR spectra of the isolated base and its O-acetyl derivative can be assigned to an $\overset{+}{N}\text{-}CH_3$ group. The mass spectrum show peaks of tetrahydrocarboline derivatives[41] at 197, 184 (22), 169 (23), and 156 (24) m/e. The unchanged positions of these peaks in the spectrum of the acetate show that the hydroxyl group is not in the β-carboline portion. Assembling this data permits an approach to the structure as presented by (ix).

A multiplet at 5.5δ integrating for one proton in the NMR spectrum of the isolated quaternary salt could be assigned to a proton adjacent to an oxygen. The only oxygen in the molecule being alcoholic, this suggests the hydroxyl group to be secondary. The NMR spectrum of the acetate shows

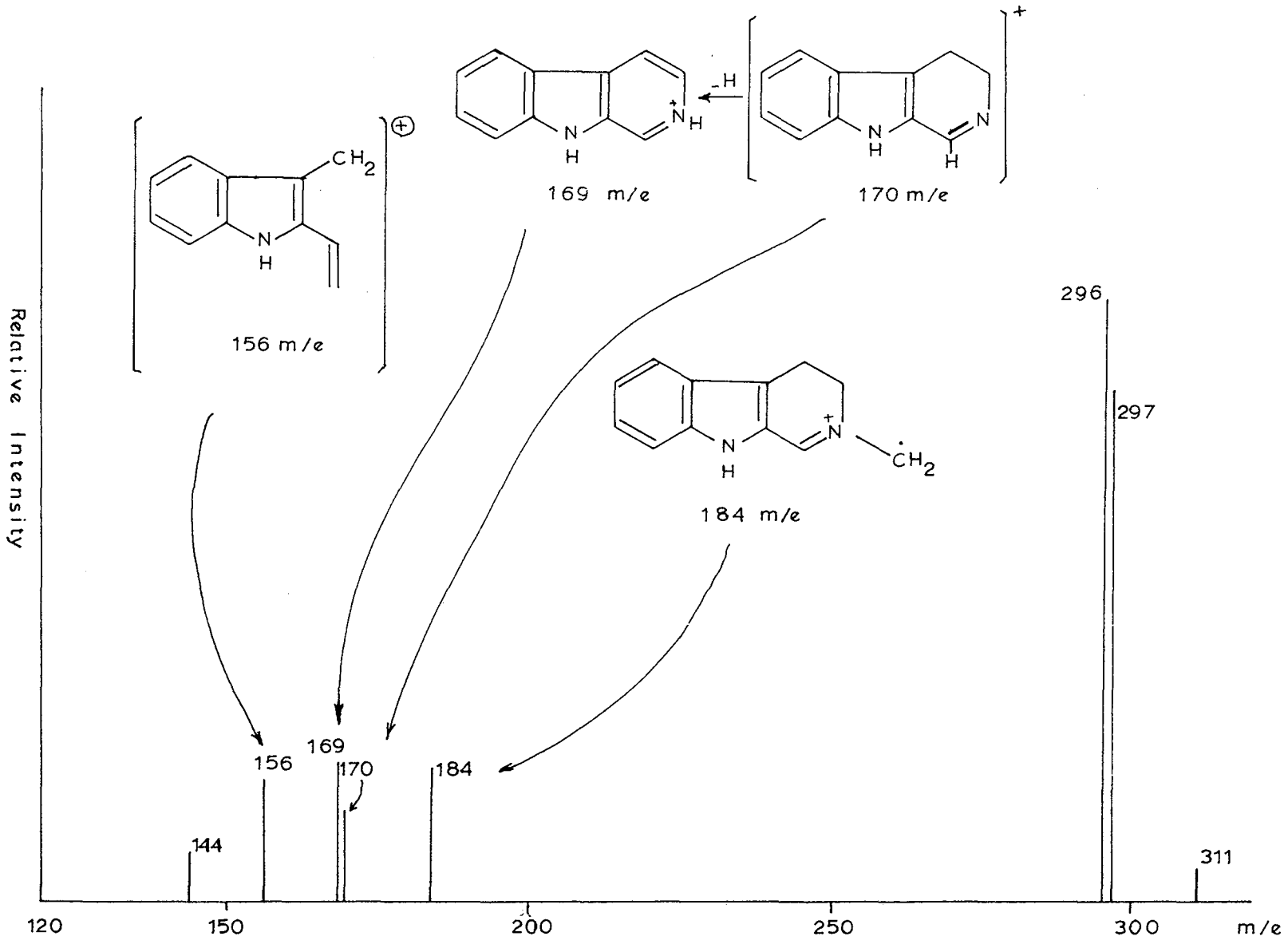
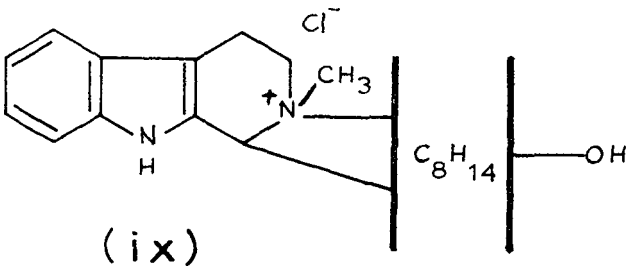


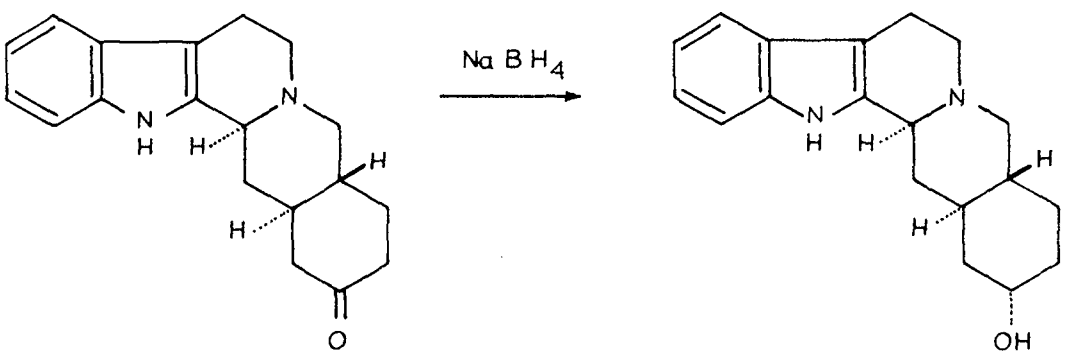
Figure 12._ MASS SPECTRUM OF YOHIMBOL METHOCHLORIDE



(ix)

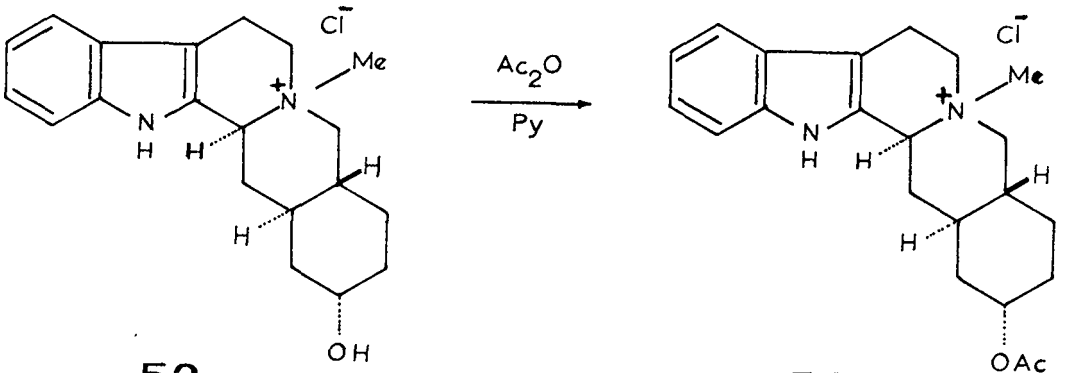
the same multiplet which does not undergo a downfield shift suggesting that the hydroxyl group is not primary[40].

The C_8H_{14} portion in partial structure (ix) could need the formation of up to two rings, one of which should bear the secondary OH group. This led us to think of yohimbol (50), a member of the yohimbine family. Demethylation of the isolated alkaloid affords a tertiary base $C_{19}H_{24}N_2O$, which shows a base peak in the mass spectrum at 296 m/e together with characteristic peaks in the infrared and NMR spectra identical with the published[57, 58] physical constants of yohimbol (50). To confirm the structure, we prepared yohimbol (50) from yohimbone (51) by sodium borohydride reduction.



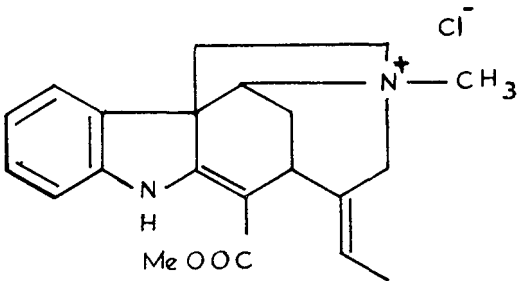
51 50

The obtained yohimbol was quaternized with methyl iodide and then converted to the chloride form on a resin. Yohimbol methochloride (52) prepared by this method was found identical in all respects with the naturally occurring sample.



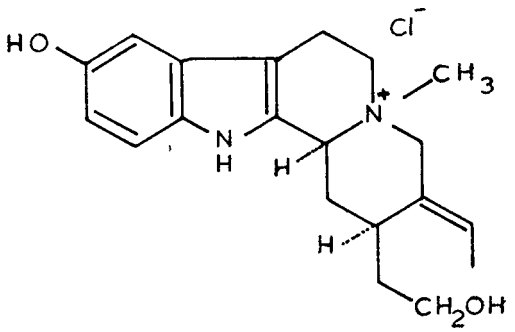
52 53

A third quaternary alkaloid was encountered only once and then from chromatogram B. The ultraviolet absorption of this base (λ_{max} 227, 330, λ_{sh} 300 nm) is unaffected by acid or base which suggests an α-acyldihydroindole chromophore[59]. The NMR spectrum of the salt shows a singlet at 3.57δ integrating for three protons, which could be assigned to an O-CH_3 group while another singlet at 3.04δ integrating for three protons can be attributed to an $\overset{+}{N}$-CH_3. The infrared spectrum shows bands at 1610 and 1662 cm^{-1}, which could be attributed to a double bond and an ester group on a double bond. Unfortunately, lack of material precluded further investigation but from the characteristic UV spectrum and more specifically from the fragmentation in the mass spectrum, we suggest the isolated quaternary alkaloid to be akuammicine methochloride (54). Comparing its physical constants (m.p., IR and UV) with the published data[21], we find they are similar in all respects.



54

A fourth alkaloid in this group was also isolated only once from chromatogram C which sufficed to record the spectra necessary for its identification. Its IR spectrum shows bands for an OH (3413 cm^{-1}) and NH (3120 cm^{-1}). The NMR spectrum shows a multiplet between 6.7 and 7.6δ integrating for (2 + 1) aromatic protons. A broad quartet integrating for one proton together with a doublet at 1.78δ integrating for three protons can be attributed to a vinyl group bearing a terminal methyl. A singlet at 3.05δ can be attributed to an $\overset{+}{N}$-CH_3 group. The characteristic tetrahydrocarboline peaks in the mass spectrum appear at 16 units higher at 200, 185 and 172 m/e. In fact all the physical constants of this quaternary salt agree with the published[21, 25] constants for huntrabrine methochloride (55). Although previous investigators[21] isolated in abundance this salt from the same plant source, we isolated a mere 12 mg. Huntrabrine methochloride (55) has also been isolated from Pleiocarpa mutica Benth[25].



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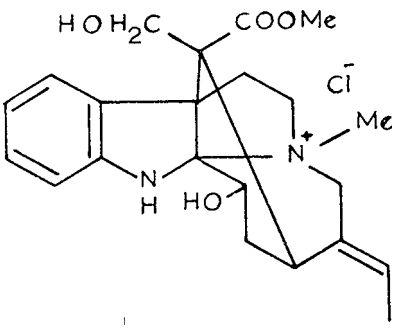
-.CHAPTER III.-

HUNTERACINE CHLORIDE

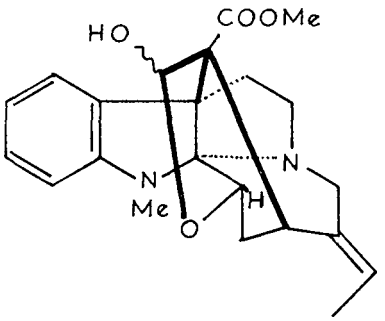
The final alkaloid we isolated from Hunteria eburnea stem and root bark showed a characteristic colour reaction with ceric sulphate, a crimson red which, after some time, turns to a persisting violet rose coloration. Its quaternary character was confirmed by a positive test for chloride ion. Elemental analysis showed the molecular formula to be $C_{18}H_{23}N_2OCl$ and the absence of methoxyl groups. The molecular formula is confirmed by the appearance of a molecular ion at 283 m/e in the mass spectrum. The UV absorption of this base (λ_{max} 234, 289; λ_{min} 216, 254 nm) is unaffected by acid or base which suggests a 2,3-disubstituted indole chromophore[52] similar to echitamine[61] chloride (56).



56

Similar UV spectra have been observed in the case of corymine[62, 63] (57), a tertiary base isolated from Hunteria corymbosa and calycanthine[64] (58). A common feature to these cases is the N-C-N arrangement. The infrared spectrum of the isolated base shows peaks typical of an indolic NH (3150 cm^{-1}) and an hydroxyl group (3440 cm^{-1}) together with a (C=C) double bond[65] (1620 cm^{-1}). The NMR spectrum confirms the distribution of hydrogens on the unsubstituted aromatic ring. It also shows a broad multiplet at 5.20δ integrating for one proton accompanied by a doublet at 1.66δ integrating for

three protons, which is clear evidence for an ethylidene side chain.



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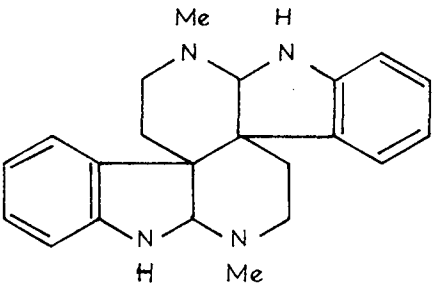
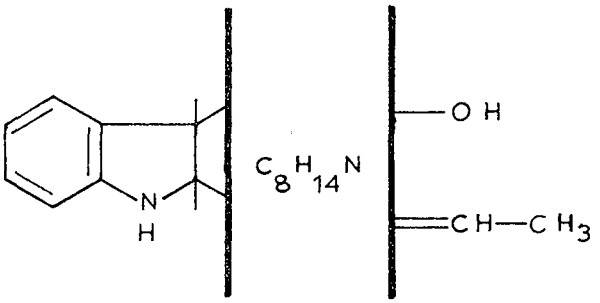


58

Assembling of this data permits an approach to the structure as presented by (x).



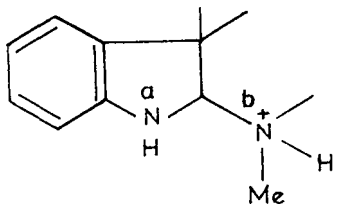
(X)

Hydrogenation of this base over palladium-on-charcoal in ethanol, afforded a dihydro product $C_{18}H_{25}N_2OCl$ confirmed by the appearance in its mass spectrum of a peak at 285 m/e. The NMR spectrum of the dihydro product shows a new three-proton triplet at 0.90δ which could be assigned to a methyl attached to a methylene group. This confirms the presence of an ethylidene side chain in the parent alkaloid.

The absence of an N-alkyl group in the NMR spectra of the isolated

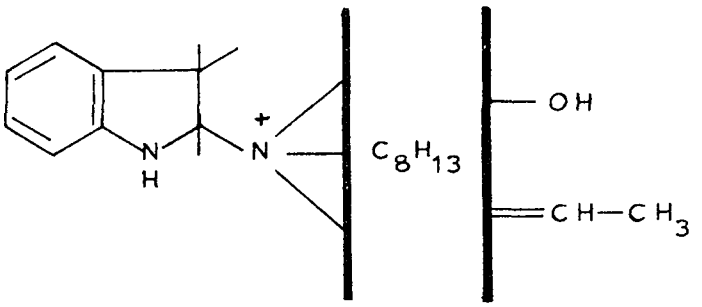
base and its dihydro product means that the quaternary nitrogen is either surrounded by four folded substituents probably forming three rings or the quaternary nitrogen is forming a double bond with one of the neighbouring carbon atoms. On hydrogenation the uptake of hydrogen stopped after one mole forming the dihydro confirmed by NMR and elemental analysis, which is clear evidence of the absence of reducible double bonds other than the ethylidene side chain. This led us to believe that the quaternary nitrogen could be of the same type as in the cyclised product of O-tosyl dihydro-antirhine (35) (Chapter I).

The ultraviolet spectra of echitamine (56), corymine (57) and calycanthine (58) show a hypsochromic effect in acid medium. According to Hodson and Smith[64], calycanthine (58) has an indoline-type spectrum, which is retained in acid solution though with a hypsochromic shift of about 10 nm for both bands in the ultraviolet spectrum. In acid solution the absorbing species is the cation (59), in which the formal positive charge on $N_{(b)}$ has rendered $N_{(a)}$ virtually non-basic: the $N_{(a)}$-electron-pair is thus still available for resonance with the benzene ring, with retention of indoline-type absorption. According to the same authors, the hypsochromic shift must be a result of the closeness of the positive charge on $N_{(b)}$ to the mesomeric system. Since the ultraviolet spectrum of the isolated quaternary salt resembles that of echitamine[21] (56), we assume the closeness of the two nitrogens separated only by one carbon atom forming the system Ar-N-C-N^{+}[66]. Since the $N_{(b)}$ bears the positive charge being quaternary, this explains why the ultraviolet spectrum of the isolated base is unaffected in acid medium.



59

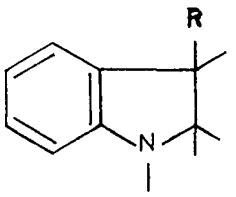
Partial structure (x) could now be extended to (xi).



(XI)

All the physical constants (m.p., $[\alpha]_D$, UV) of this new type of isolated quaternary alkaloid coincide with those published by Taylor et al.[21] for Hunteracine for which they propose partial structure (xii) in which R "could" be a hydroxyl group.

They had suggested (from elemental analyses) the molecular formula $C_{20}H_{27}N_2OCl$. This led us to think that before fragmentation in the mass spectrum, the hunteracine cation could undergo pyrolysis or rearrangement



(XII)

reactions as met in the case of pleiocarpamine methochloride (44) (Chapter II). Our first elemental analysis, although confirmed by the appearance in the mass spectrum of a peak at 283 m/e was believed cautiously since in nature, C_{18} indole alkaloids are of very limited distribution compared to the C_{19} or C_{20} bases. Three different recrystallized samples of hunteracine chloride have been analyzed separately. A sample of the chloride has been exchanged on a resin to the bromide form and also sent for analysis. All the results obtained confirmed the molecular formula $(C_{18}H_{23}N_2O)^+ Cl^-$ or Br^-. The mass spectrum of hunteracine bromide shows the molecular peak at 283 m/e again confirming the elemental analysis and providing proof that the anion is not covalently incorporated in the molecule during the fragmentation in the mass spectrum.

All trials to acetylate or pyrolyse hunteracine chloride failed, the starting material was always recovered unchanged. This suggests that the hydroxyl group present in hunteracine could be tertiary.

Hunteracine chloride or bromide affords a tertiary green-fluorescent product by refluxing for twenty minutes in ethanol in presence of potassium ethoxide. The ultraviolet absorption of this tertiary base (λ_{max} 229, 380;

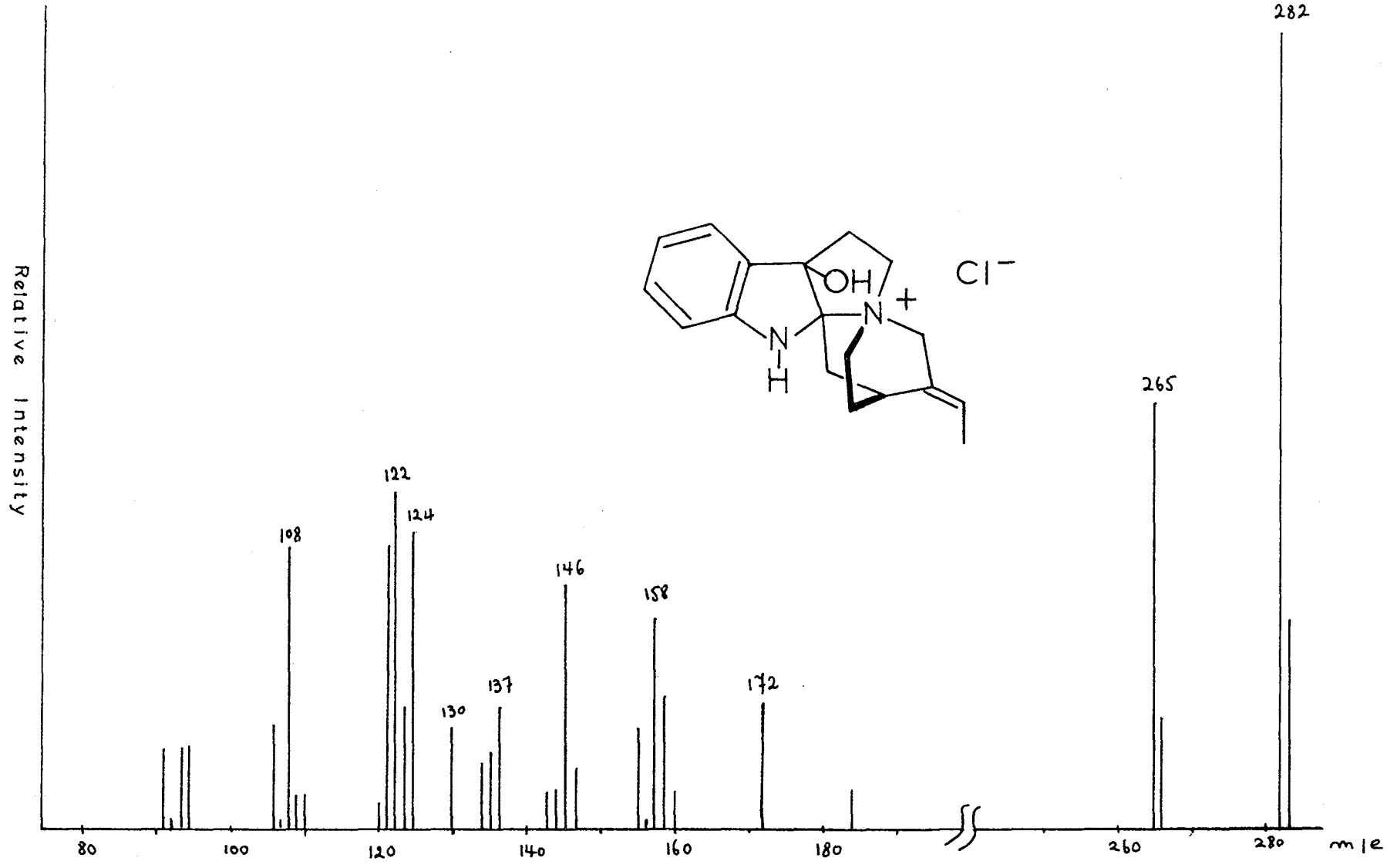
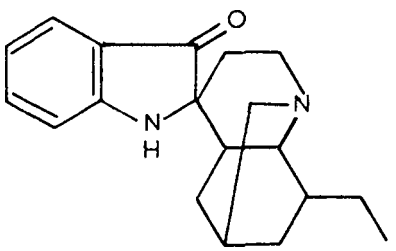


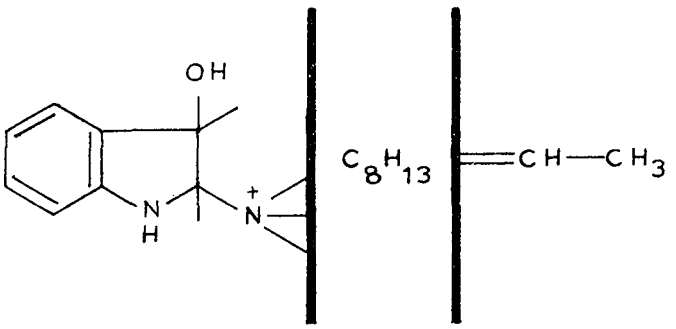
Figure 13.– MASS SPECTRUM OF HUNTERACINE CHLORIDE

λ_{min} 275-290; λ_{sh} 253, 259 and 343 nm) is unaffected in acid or base and suggests a pseudo-indoxyl chromophore[37]. This is confirmed by the appearance in its infrared spectrum of a band at 1692 cm^{-1}. A similar UV spectrum has been observed in the case of desmethoxy-iboluteine[67, 68] (60) where the absorption (230, 250, 256, and 400 nm) is reported to be that of the pseudo-indoxyl.



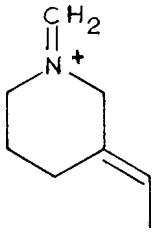
60

Assuming that no deep-seated rearrangement is involved in the formation of the pseudo-indoxyl, the hydroxyl group in hunteracine chloride must be at the β-position (of the indoline system), thus confirming Taylor's original proposal[21]. Partial structure (xi) could be extended to (xiii).



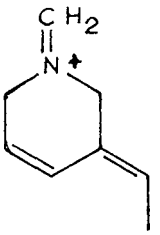
(xiii)

The mass spectrum of hunteracine chloride and bromide show peaks at 124 (61), 122 (62) and 108 (63) m/e. These peaks, which shift to 124 and 110 m/e in the spectrum of dihydrohunteracine, have obvious interpretations as being the progeny of a piperidine ring[52, 69, 74] bearing an exocyclic ethylidene side-chain.



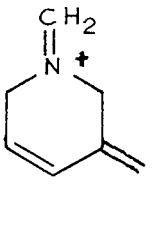
124 m/e

61



122 m/e

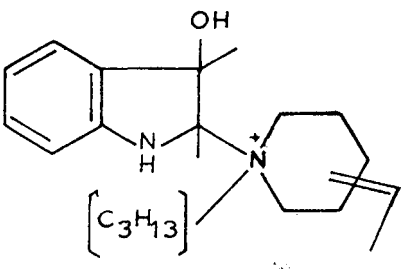
62



108 m/e

63

This would mean that the quaternary nitrogen atom makes part of this piperidine ring. Partial structure (xiii) could be extended cautiously to (xiv).



(XIV)

Hydrogenation of hunteracine chloride over palladium-on-charcoal in presence of traces of acid, affords an "Emde base" with the molecular for-

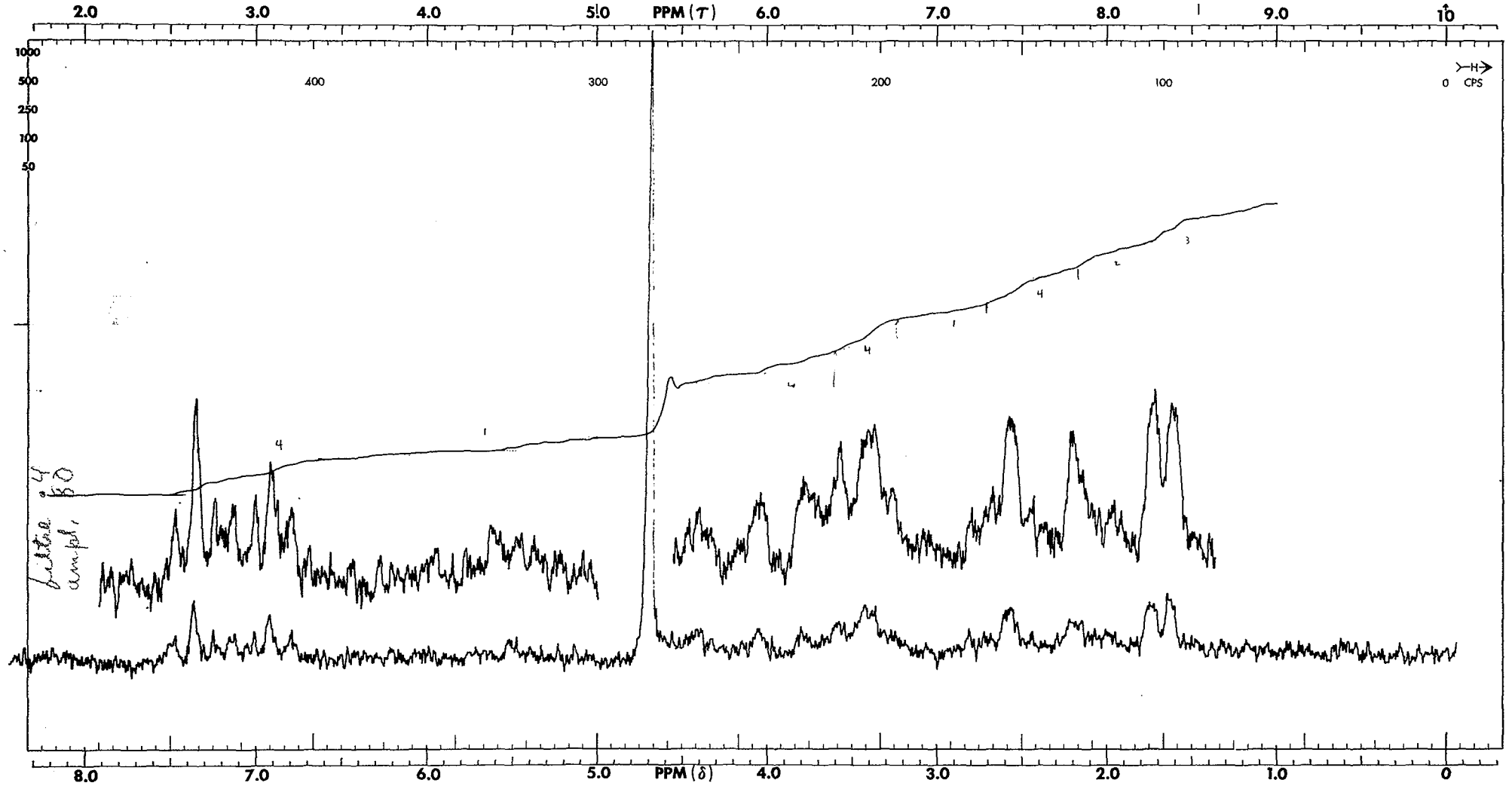
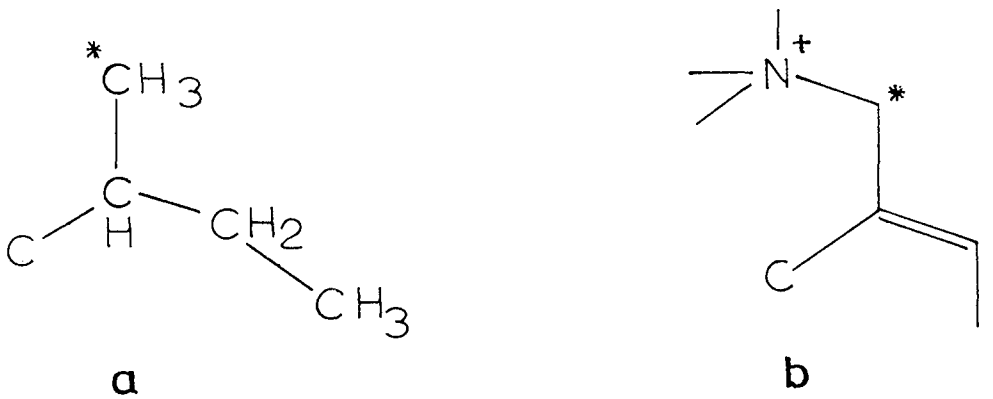


Figure 14.- Nuclear magnetic resonance spectrum of hunteracine chloride.

mula $C_{18}H_{27}N_2O$ again confirmed by the appearance in the mass spectrum of a peak at 287 m/e. The NMR spectrum of this hunteracine "Emde base" shows two triplets centered at 1.07δ (J=7 Hz) and 0.8δ (J=7 Hz), integrating for three protons which is interpretable by assuming the presence of isobutyl residue (64a). Since the Emde reaction results in the rupture of an N-C bond, we can confirm that the starred carbon atom in (64a) is that attached to the quaternary nitrogen and must be vicinal to the carbon atom bearing the ethylidene side-chain (64b).

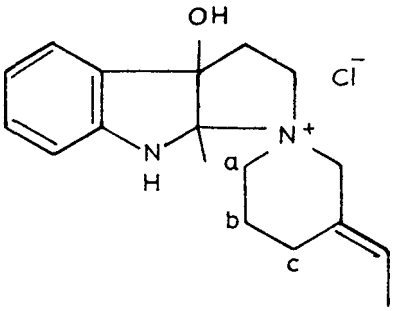


a b

64

Biogenetically, the indole portions of indole alkaloids generate from tryptamine (2a) or tryptophan (2b). We can suggest placing two of the three remaining carbon atoms as being those derived from tryptamine to extend partial structure (xiv) to (xv).

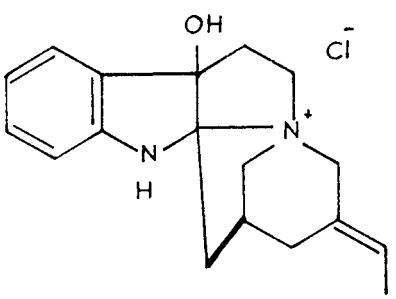
Partial structure (xv) now contains seventeen carbon atoms and there remains to place but one methylene group and to assume the necessary compliment of protons.



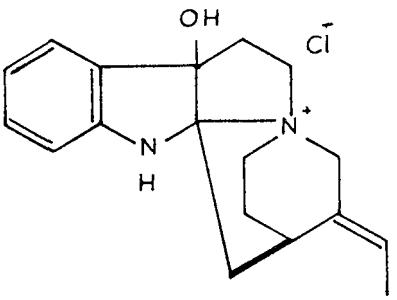
(XV)

Under various reaction conditions, hunteracine chloride does not give rise to the corresponding indole, even in the presence of strong dehydrating agents. This suggests that the carbon atom bridging the two nitrogens does not bear any proton and must be a "spiro" carbon atom. Therefore, the remaining methylene group to place in the molecule must be attached to this spiro carbon atom and is in turn connected to either position a, b or c in the piperidine ring.

Examination of molecular models shows that sterically, position (a) is the less probable and can be excluded and we are left with one of the two possible structures (65) or (66) for hunteracine chloride.



65



66

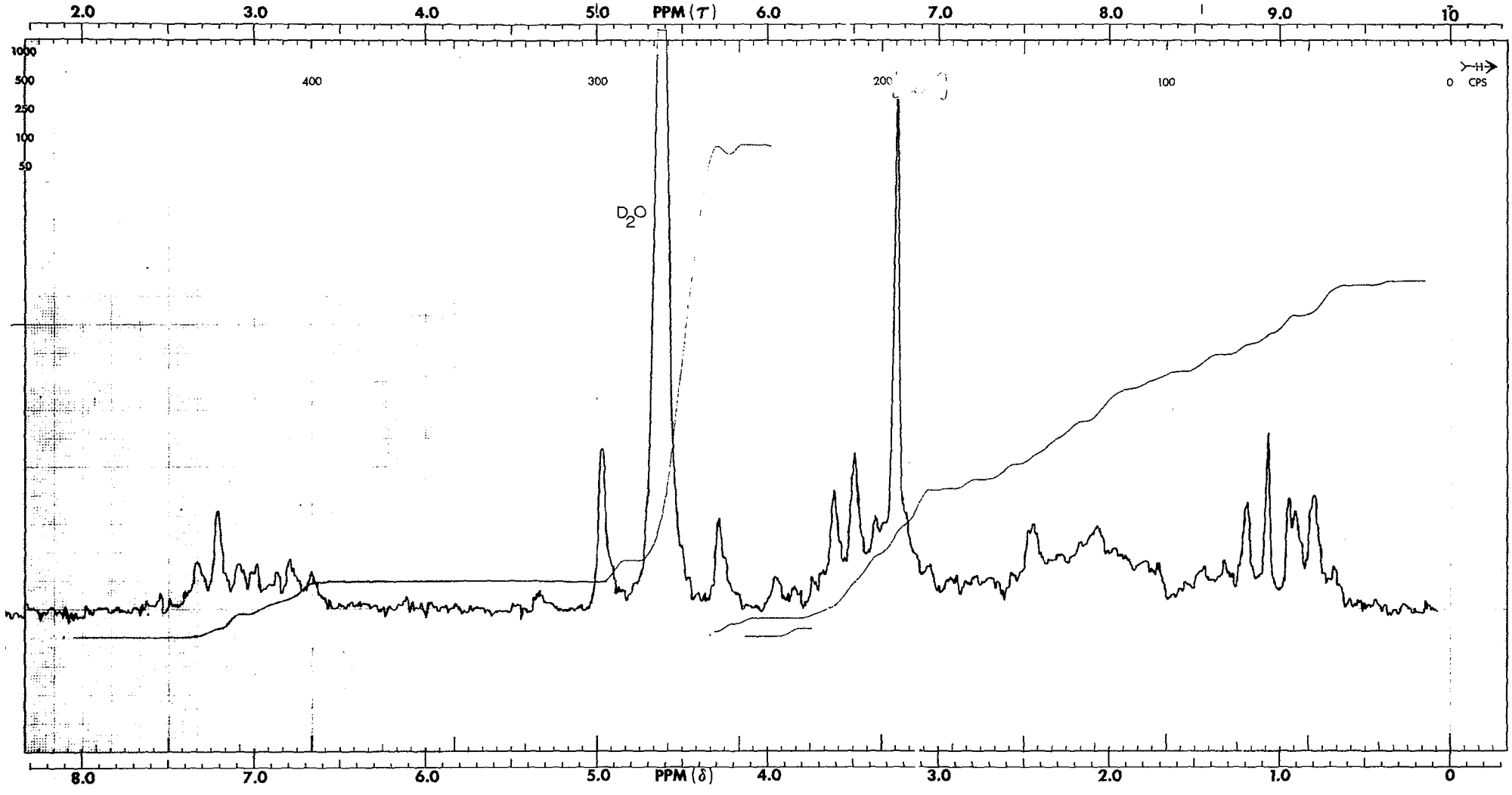
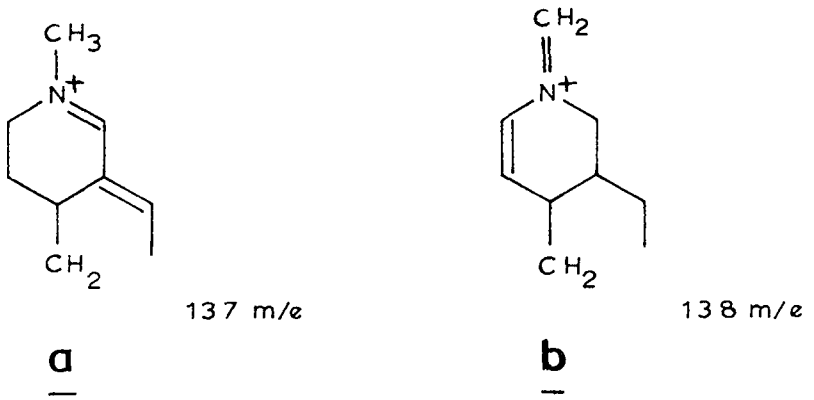


Figure 15.- Nuclear magnetic resonance spectrum of hunteracine Emde base.

The presence in the mass spectrum of hunteracine chloride of a peak at 137 m/e and at 138 m/e in the spectrum of dihydrohunteracine normally attributed to the ions (67a) and (67b) respectively[52, 70, 74] but the 3:5-substitution pattern would satisfy the observations equally as well.

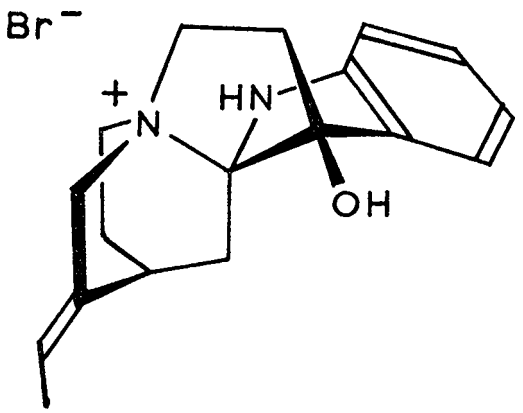


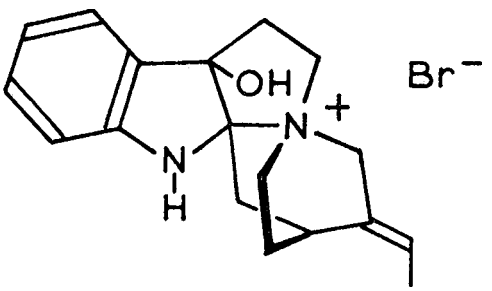
67

However, biogenetically we find that most indole alkaloids with ethylidine side-chains at the 3-position of the piperidine ring are substituted in the 4-position as for example: stemmadenine (9), geissoschizol (26), pleiocarpamine (40) and corymine (57). These considerations led us to favour structure (66) to represent hunteracine chloride rather than (65) which is equally compatible with the evidence on hand.

At this stage of investigation, we felt it necessary to confirm the structure proposed and due to lack of material and lack of an appropriate degradation method, only one route seemed feasible, the determination of the structure of hunteracine by X-ray analysis. For this we chose a crystal of hunteracine bromide from the same sample sent for analysis and on which the mass spectrum had been recorded.

X-ray analysis* confirmed the structure of hunteracine[12b] as proposed where the quaternary nitrogen is shown to participate in three rings, and showed the relative and absolute configuration of the molecule as shown in (68).





68

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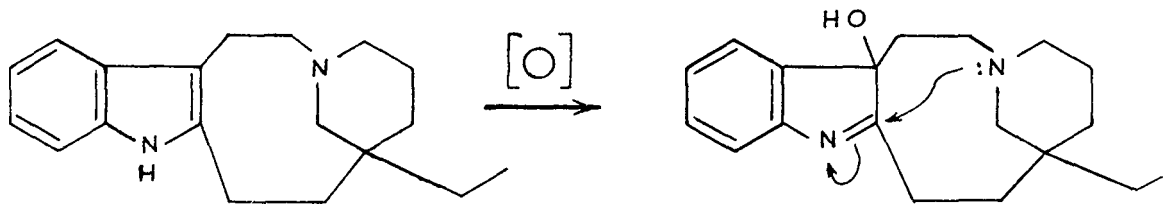
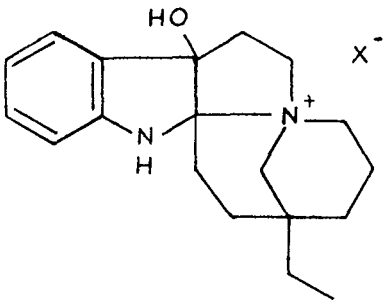
* Results from X-ray analysis will be reported and discussed in detail in M. A. Chapelle's Ph.D. thesis (in preparation) in the laboratories of Prof. R.H. Burnell.

We imagine hunteracine to be derived in the plant from stemmadenine (9) which, according to Scott[20], is biosynthesized from preakuammicine (69). (Figure 16). Stemmadenine can lose the two oxygen bearing carbon atoms and then cyclise as suggested for the formation of rhazidine (70) from rhazidigenine[73] (71) [(-)-quebrachamine].



71 HX 70



72

Rhazidine (70) changes in acid medium to form a quaternary salt rhazidine hydrochloride (72) paralleled by a change in its optical rotation from -612° to -37° returning to approach -612° when re-basified. Thus it becomes practically impossible and perhaps irrelevant to decide if hunteracine exists as such in the plant or has been formed by oxidation during the extractions. However, the apparent lack of decomposition products (oxindoles

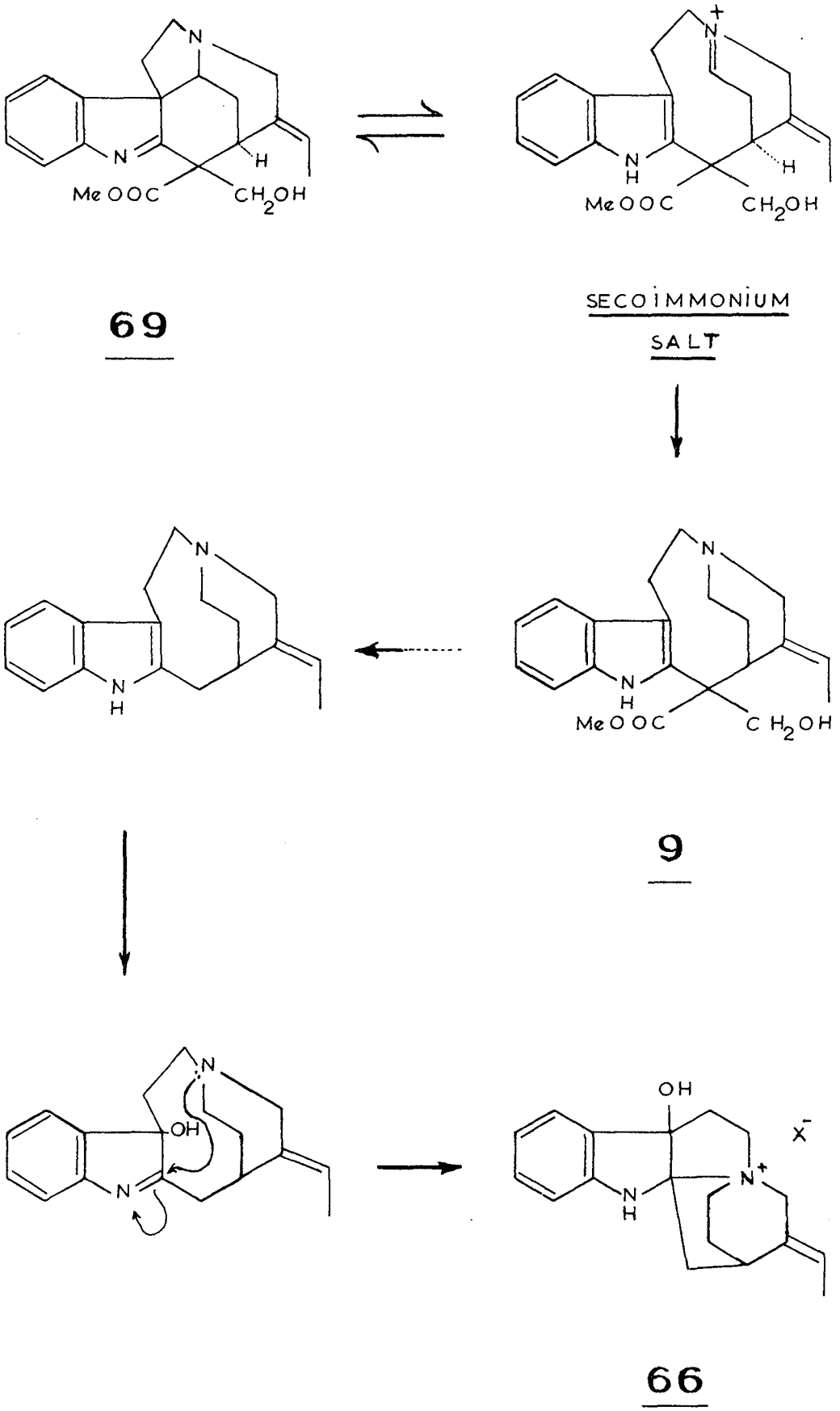
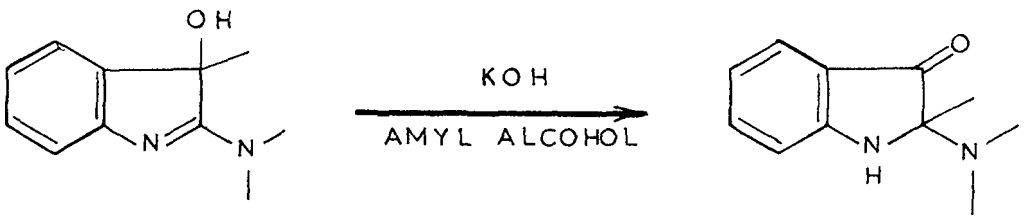


Figure 16 .— PROBABLE BIOGENESIS OF HUNTERACINE CHLORIDE

and indoxyls) which are readily formed during the air oxidation of stemmadenine (9) or its equivalent, seems to preclude this possibility.

Witkop et al.[71, 72] propose a benzylic type transposition by the action of strong base at high temperature for the transformation of β-hydroxyindolenines to their corresponding pseudo-indoxyls (73).



73

Hunteracine chloride being quaternary, we think that as a first step it collapses to a tertiary base followed by the subsequent formation of the indoxyl. The base attracts the indolic proton resulting in the rupture of the C-N_b bond with either the formation of a carbocation at C_2 followed by the migration of the substituent in the β-position of the indole to compensate the charge on C_2 with the subsequent formation of C=O (figure 17), or it might just as well be "concerted".

We propose structure (74) for hunteracine pseudo-indoxyl which is confirmed by the appearance in its mass spectrum of the characteristic peaks of the pyridinium ions[52, 69] (61, 62, 63), suggesting that the piperidine ring with its exocyclic ethylidene chain remained unchanged. The ethylidene double bond does not participate in the pseudo-indoxyl formation since dihydrohunteracine chloride forms a pseudo-indoxyl under the same conditions

with nearly the same yield.

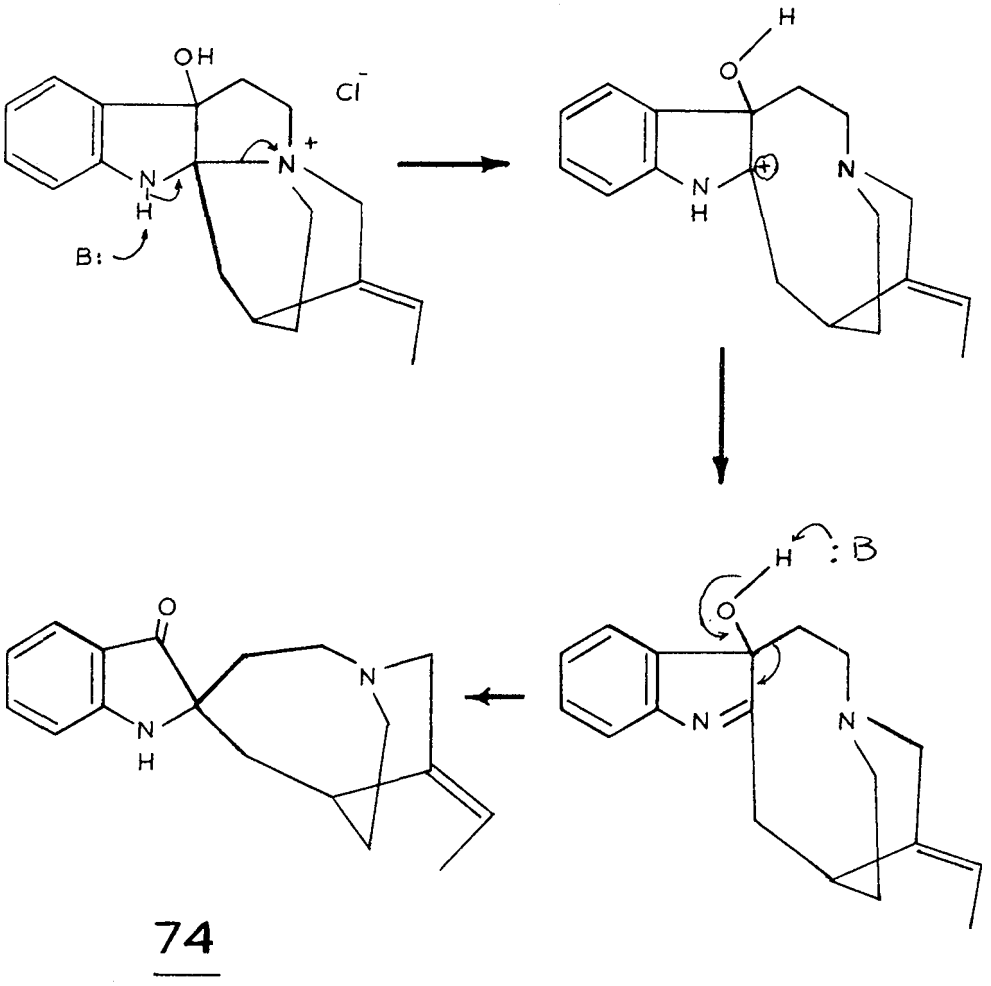
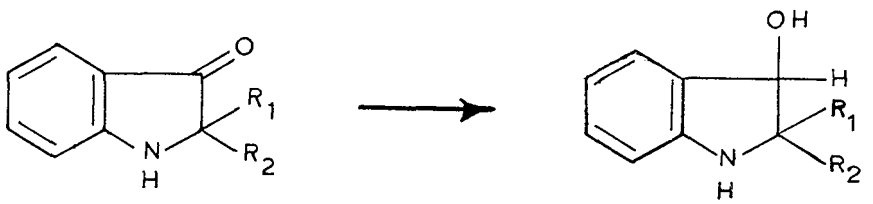


Figure 17._ PROPOSED MECHANISM FOR HUNTERACINE ψ-INDOXYL FORMATION

The ultraviolet spectrum of hunteracine ψ-indoxyl no longer shows the characteristic features of the Ar-N-C-$\overset{+}{N}$ arrangement adding proof that the ruptured bond is that connecting C_2 of the indole moiety to the ex-quaternary nitrogen.

Hofmann degradation of hunteracine chloride in t-butyl alcohol afforded two products in very low yield (15%), the first displaying a molecular ion at 280 m/e in the mass spectrum is unidentified while the second is identical in all respects with hunteracine pseudo-indoxyl (74).

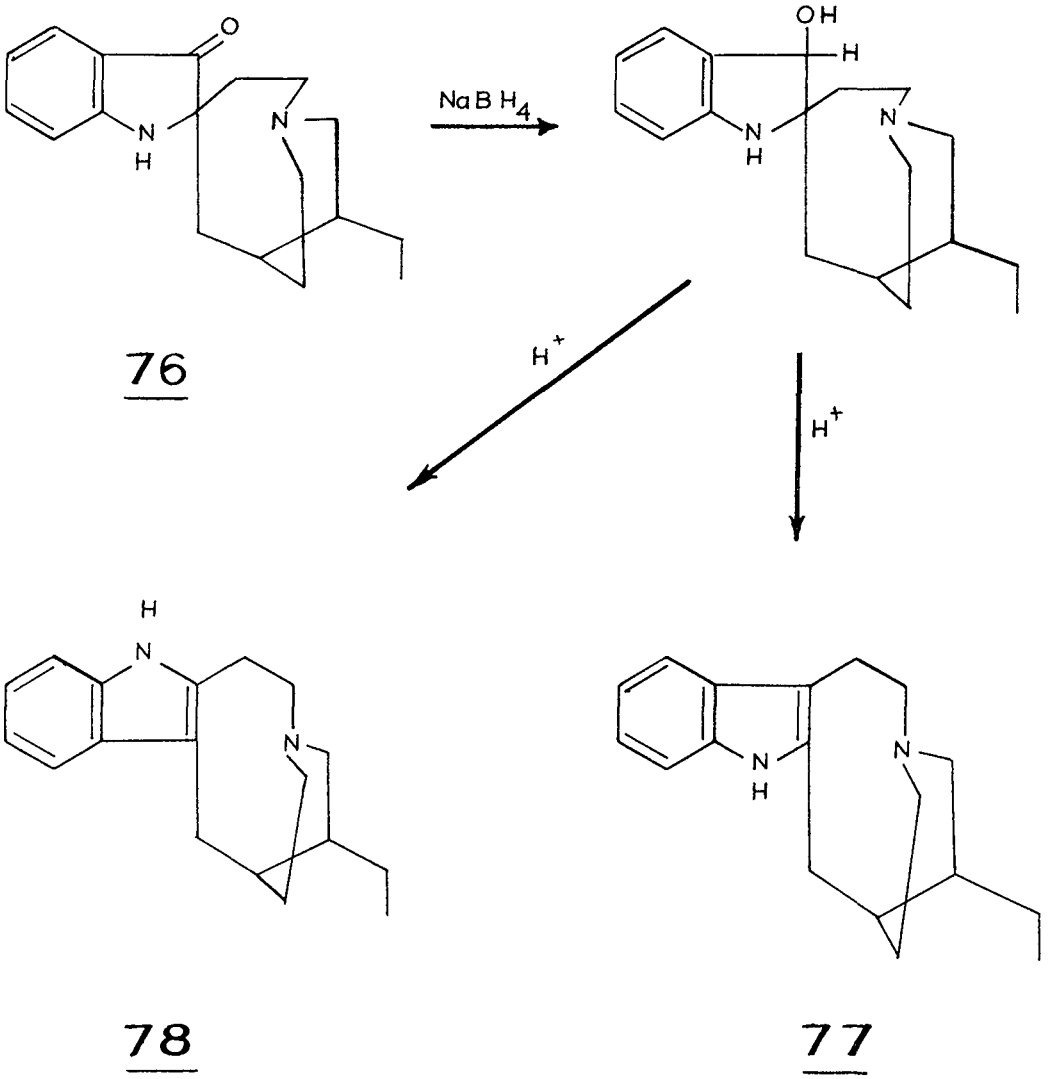
Pseudo-indoxyls can be reduced[72] with $NaBH_4$ or $LiAlH_4$ to the corresponding alcohol. In acid medium rearrangement takes place with the subsequent migration of the more migratory substituent[75, 76] (R_1 or R_2 in 75) to form the corresponding indole.



75

We think that dihydrohunteracine Ψ-indoxyl (76) would give on reduction, the corresponding alcohol which, on rearrangement with acid, would give the corresponding indole (77) and/or the "inverted" structure[75] (78).

The indole (77) has been prepared synthetically by J. Harley-Mason[77,78] and future plans include attempts to synthesize it by the known route[77] described in figure 18.



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77

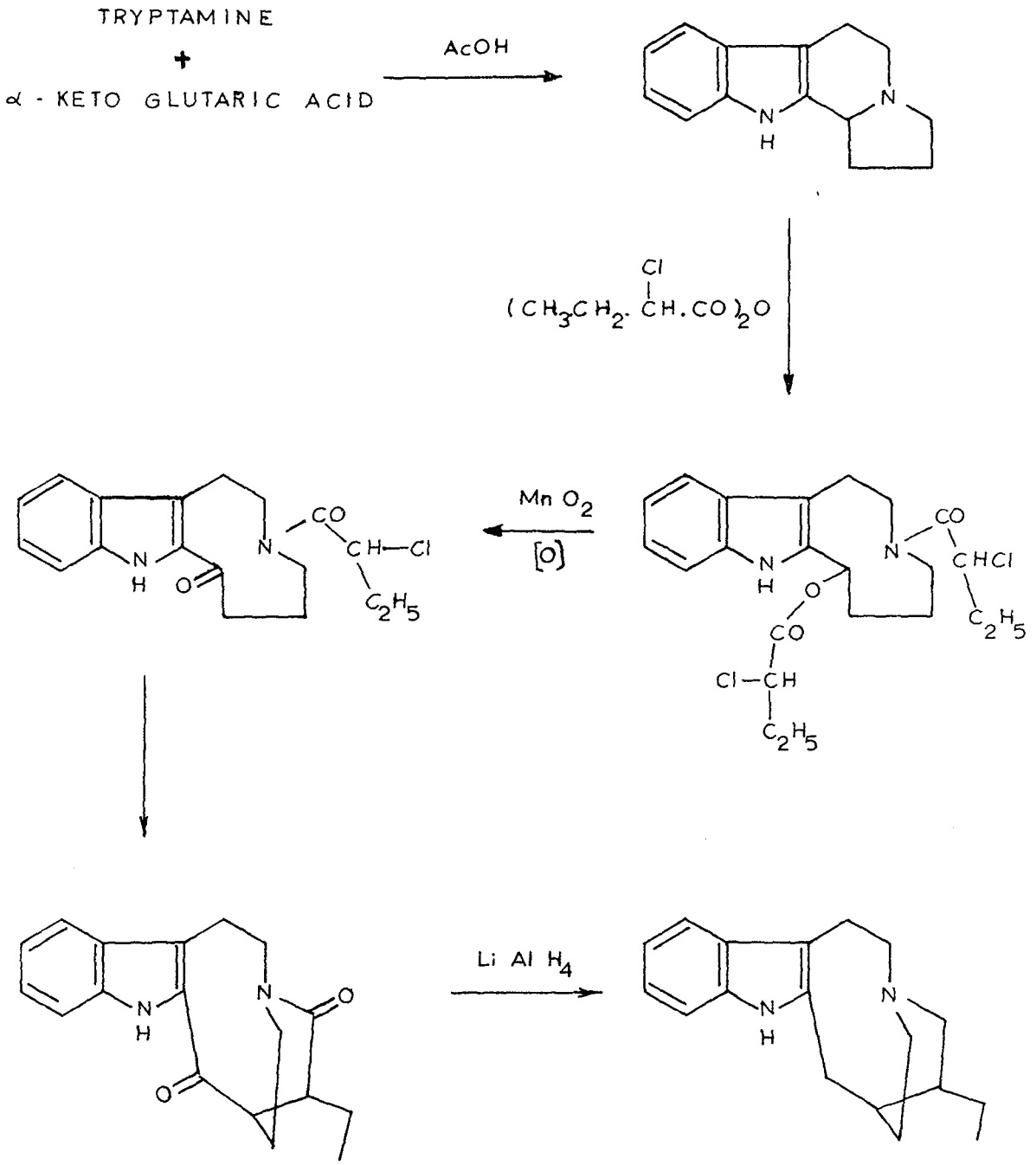


Figure 18.- PROPOSED SYNTHESIS OF DIHYDROHUNTERACINE REARRANGEMENT PRODUCT

-.EXPERIMENTAL.-

-.GENERAL REMARKS.-

Melting points are uncorrected and were registered on an "Electrothermal" apparatus in unsealed capillary tubes. Optical rotations were either registered in a Carl Zeiss 369417 polarimeter with circular scale or in an automatic Carl Zeiss polarimeter at five different wavelengths. Elementary analyses were performed by Dr. Franz Pascher, Bonn, Germany. Analytical samples were routinely dried at 100°C over P_2O_5 in vacuo. Ultraviolet spectra were measured in ethanol (log ε in parentheses) either on a Beckmann spectrophotometer model DK-1A, or on a Jasco model ORD/UV-5. Unless otherwise stated infrared spectra were performed on potassium bromide pellets using a Beckmann model IR-4 or Perkin-Elmer 457 grating infrared spectrophotometer. Nuclear magnetic resonance (NMR) spectra were measured on approximately 5% solutions with Varian Associates spectrometer model A-60. Tetramethylsilane protons taken as 0 p.p.m. Mass spectra were registered using a Varian Associates M-66 spectrometer on precalibrated Varian papers.

ISOLATION OF THE ALKALOIDS.-

Extraction of the bark

The root and stem bark of Hunteria eburnea Pichon was extracted with methylene chloride in order to separate the tertiary bases. The remaining bark was reprocessed with recycling methanol at 40°C to yield 8 kg of extractables from 60 kg of bark.

These 8 kg which represent our starting material were kindly donated to our laboratories by Dr. W.I. Taylor.

The methanolic extract was processed by dissolving a 400 g fraction in 10% acetic acid, filtered, shaken with three portions of methylene chloride which removed some tertiary alkaloids.

The pH of the solution was brought to pH 8-9 with lithium hydroxide, generating a precipitate which was removed by filtration. The filtrate was extracted with methylene chloride, brought to pH 6 with acetic acid, and all traces of methylene chloride were removed by bubbling nitrogen through the solution. This procedure led to a filterable precipitate (450 g) upon addition of lithium picrate solution (300 g of picric acid in 3 ℓ of water with sufficient added lithium hydroxide to give a clear solution). The picrate salts were converted to the chloride salts by stirring with anion exchange resin "Deacidite FF-lp polystyrene resin type SRA-66 (chloride form)" (Koch-light) in acetone-methanol-water (6: 2: 1) for 18 hours yielding the crude chloride salts after lyophilization (chromatogram A).

A sample of the crude aqueous chloride solution was continuously extracted with methylene chloride for 5 days. The methylene chloride extract was evaporated under reduced pressure (chromatogram B).

The aqueous phase of this extraction was heated on a steam bath with Darco decolorizing charcoal, filtered and concentrated in vacuo, and finally freeze dried (chromatogram C).

Material eluted from the charcoal with refluxing methanol in a soxhelet extractor was evaporated under reduced pressure (chromatogram D).

Chromatography

First trials were done to separate the crude quaternary chlorides on different adsorbents. A "Chromax" compressed paper column was used with relatively good separation but was discarded since the maximum quantity to be chromatographed could not exceed 4 g.

The separation on cellulose columns was good and reproducibility maintained. Many solvent mixtures were tried unsuccessfully such as methyl ethyl ketone - methanol - water (12: 4: 1), ethyl acetate - t-butyl alcohol - water (4: 2: 1) and t-butyl alcohol - benzene - water (3: 1: 2). The completely homogeneous system acetone - water which gave poor separations on paper because of excessive streaking proved to be the solvent of choice for the columns.

Preparation of the cellulose columns

Five different columns were prepared having the following dimensions: 10 x 70 cm, 5.5 x 85, 3.5 x 45, 3.3 x 108 and 1.5 x 42 cm. Each column was half-filled with acetone. A flat bed for the cellulose was made of glass wool and Ottawa sand. Cellulose powder CF 11 (Koch-Light Laboratories Ltd.) was placed in a vacuum dessicator, covered with acetone, and the air removed by repeated suction. A portion of this slurry was poured into the column, stirred to break up lumps, allowed to settle and compressed

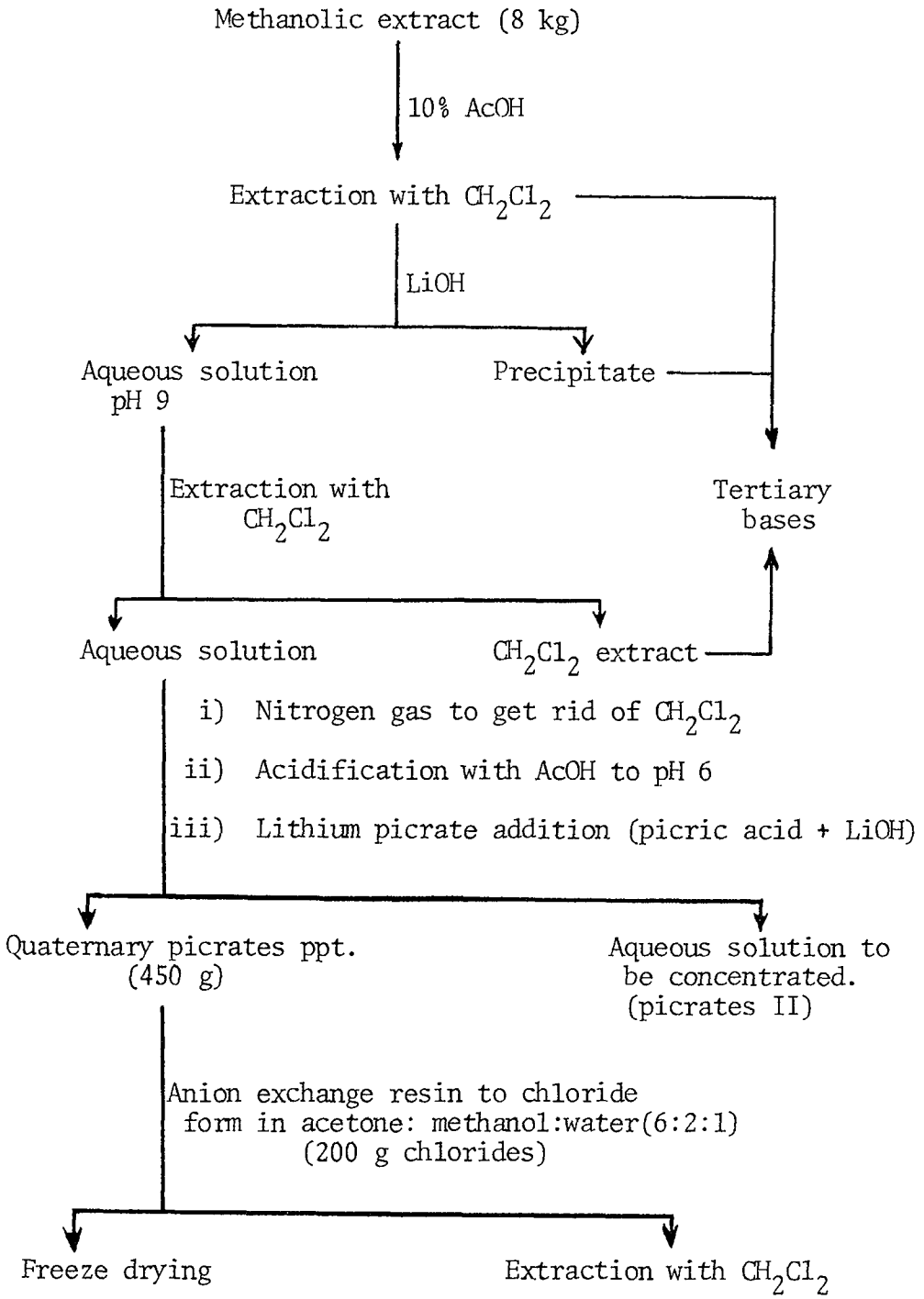
tightly with a tamping rod. Repetition of this procedure resulted in a column which upon washing with increasing percentage of water in acetone caused the cellulose to expand, the top few segments sometimes rising slightly in the column.

Before use the percentage water in the column was reduced by continuous washing while the percentage of water in the acetone was gradually decreased to 1%.

The sample was adsorbed previously on cellulose powder and this mixture poured dry on the top of the column. Elution began with a 3 ℓ portion of 1% water in acetone to rid the chromatogram of traces of unchanged picrate followed by 3 ℓ portions of acetone with steadily increasing water concentration. A rapid increase in water percentage causes considerable damage to the cellulose columns giving rise to channeling.

It takes normally two weeks to prepare a cellulose column. No particular control of the temperature (which could vary from 22 to 24°C) was necessary. Fractions from small and medium columns (10-30 ml) were continuously collected on an LKB fraction collector for a period of two to four weeks. Fractions of 900 ml each were collected from the largest column necessitating from 35 to 40 ℓ solvent for each chromatogram. All the chromatograms have been run at least twice and were readily followed by an ultraviolet lamp together with thin layer chromatography on silica gel; the polarity of the solvants used depended on the nature of the eluates varying from water:acetone (1:9) to methanol:acetone containing a trace of hydrochloric acid.

-.ISOLATION OF HUNTERIA EBURNEA QUATERNARY ALKALOIDS.-



CHROMATOGRAM A

(next page)

CHROMATOGRAM A

Extraction with CH_2Cl_2

1-Hunteracine chloride (400 mg)
2-Yohimbol methochloride (700 mg)
3-Hunterburnine β-methochloride (100 mg)
4-Huntrabrine methochloride (15 mg)
5-Hunterburnine α-methochloride (40 mg)
6-21-Methoxyhunterburnine methochloride (20 mg)

Methylene chloride extract

Aqueous solution

CHROMATOGRAM B

1-Hunteracine chloride (560 mg)
2-Pleiocarpamine methochloride (960 mg)
3-Akuammicine methochloride (20 mg)
4-Antirhine α-methochloride (500 mg)

Darco decolorising charcoal

Aqueous solution freeze dried

Charcoal extracted with methanol

CHROMATOGRAM C

1-Hunteracine chloride (520 mg)
2-Yohimbol methochloride (900 mg)
3-Antirhine α-methochloride (900 mg)
4-Hunterburnine β-methochloride (30 mg)
5-Dihydroantirhine α-methochloride (630 mg)

CHROMATOGRAM D

1-Hunteracine chloride (250 mg)
2-Yohimbol methochloride (400 mg)
3-Antirhine α-methochloride (200 mg)
4-Hunterburnine β-methochloride (40 mg)
5-Hunterburnine α-methochloride (50 mg)
6-Dihydroantirhine α-methochloride (350 mg).

-.C H A P T E R I.-

ANTIRHINES AND HUNTERBURNINES

Hunterburnine α-methochloride (10a)

Hunterburnine α-methochloride was obtained from chromatograms A and D by eluting with 10% water in acetone. It crystallized readily, even in the tubes of the fraction collector and (hunterburnine α-methochloride) was recrystallized from water, m.p. 332°C (decomp.).

UV spectrum	:	λ_{max} 275 (3.99), 303 (3.72);
		λ_{min} 246 (3.37), 299 (3.69);
		λ_{sh} 313 (3.63) nm.
		In 0.1 N NaOH in 95% ethanol:
		λ_{max} 274 (3.98), 325 (3.65);
		λ_{min} 256 (3.84), 295 (3.45) nm.
IR spectrum	:	peaks at 3330 (OH), 3120, 1630, 1600, 1220, 1205, 913, 842 and 824 cm^{-1}.
NMR spectrum (CF_3COOH)	:	6.5 - 7.65δ multiplet aromatic protons.
		5.43δ broad singlet, vinyl protons.
		3.82δ multiplet. 3.43δ singlet ($N-CH_3$).
		1.85δ multiplet.
Mass spectrum	:	326 (65% of base peak), 312 (90), 311 (100%, base peak), 295 (6), 281 (22), 269 (15), 255 (80), 241 (70), 239 (60), 184 (22), 160 (40) and 96 (25) m/e.

Hunterburnine β-methochloride (10b)

Hunterburnine β-methochloride was found in chromatograms A, C and D accompanied by Yohimbol methochloride when eluted with 6% water in acetone. Hunterburnine β-methochloride was recrystallized from methanol, m.p. 303°C (decomp.).

UV spectrum : λ_{max} 222 (4.09), 272 (3.59), 300 (3.24);
λ_{min} 245 (3.05);
λ_{sh} 312 (3.16) nm.
In 0.1 N NaOH in 95% ethanol:
λ_{max} 222 (4.10), 274 (3.57), 294 (3.22);
λ_{min} 256 (3.08) nm.

IR spectrum : peaks at 3330 (OH), 3120, 1630, 1600, 1220, 1205, 913, 842 and 824 cm^{-1}.

NMR spectrum : 6.7 - 7.6δ aromatic protons. 5.55δ singlet vinyl protons. 3.15δ singlet ($N-CH_3$). 2.3δ multiplet.

Mass spectrum : 326 (100%, base peak), 312 (45), 311 (49), 295 (10), 269 (20), 255 (78), 241 (65), 239 (54), 184 (25), 160 (45) and 96 (25), m/e.

21-Methoxyhunterburnine methochloride (19)

This alkaloid was found in very small quantity and the structure is proposed only through spectroscopy. It crystallized from methanol, m.p. 322°C (decomp.).

UV spectrum : λ_{max} 275 (3.99), 303 (3.71);
λ_{min} 246 (3.34), 298 (3.68);
λ_{sh} 313 (3.64) nm.
In 0.1 N NaOH in 95% ethanol:
λ_{max} 274 (3.97), 325 (3.66);
λ_{min} 256 (3.85), 296 (3.43) nm.

IR spectrum : peaks at 3160, 1640, 1590, 1570, 1195, 1030 and 810 cm^{-1}.

NMR spectrum (CF_3COOH) : 6.5 - 7.65δ multiplet aromatic protons. 5.43δ singlet vinyl protons. 3.85δ multiplet. 3.42δ singlet (methoxy). 3.25δ broad singlet ($N-CH_3$). 1.90δ multiplet.

Mass spectrum : 341 (3% of base peak) calculated for $(C_{21}H_{29}N_2O_2)^+$ = 341, 340 (15), 326 (100%, base peak), 312 (43), 311 (50), 295 (7), 281 (8), 269 (17), 255 (75), 253 (13), 241 (62), 239 (52), 224 (8), 213 (13), 212 (13), 200 (13), 184 (22), 172 (21), 166 (41), 160 (37), 146 (13) and 96 (26), m/e.

Antirhine α-methochloride (27)

Antirhine α-methochloride came out from chromatograms B, C and D. It crystallized from ethanol and was recrystallized from the same solvent for analysis, m.p. 306°C (decomp.). $[\alpha]_D^{578}$ = +75°, $[\alpha]_D^{546}$ = +94°, $[\alpha]_D^{436}$ = +175°, $[\alpha]_D^{405}$ = +225°, $[\alpha]_D^{365}$ = +325°. (c = 0.04 in ethanol).

Anal. Found : C, 69.03; H, 7.93; N, 8.07; O, 4.38; Cl, 10.16; OCH_3, 0.0; CCH_3, 0.0%.

$C_{20}H_{27}N_2OCl$ requires: C, 69.24; H, 7.84; N, 8.07; O, 4.61; Cl, 10.22%.

UV spectrum : λ_{max} 222 (4.84), 272 (3.85), 289 (3.80), λ_{min} 240 (3.10), 286 (3.72); λ_{sh} 283 (3.82) nm, with no change in acid or base.

IR spectrum : peaks at 3400, 3135, 1297, 1194, 972, 735 cm^{-1}.

NMR spectrum (D_2O) : 7.1 - 7.7δ multiplet, four aromatic protons. 5.54δ three-proton multiplet (vinyl protons). 3.47δ singlet (N-methyl). 2.78δ singlet. 2.15δ multiplet.

(CF_3COOH) 8.32δ broad two-proton signal.

Mass spectrum : 311 (M^+), 310 (15), 296 (55), 295 (70), 265 (15), 239 (18), 225 (100%), 223 (90), 197 (22), 184 (23), 169 (35), 156 (28), 143 (20) m/e.

Antirhine-methochloride from Antirhea putaminosa*

Mass spectrum : 311 (4% of base peak), 310 (12), 296 (60), 295 (80), 265 (14), 239 (24), 225 (98), 223 (100%, base peak), 197 (15), 184 (19), 169 (21), 156 (17), 143 (10) m/e.

O-Acetylantirhine α-methochloride

Antirhine α-methochloride (130 mg) was acetylated using acetic anhydride (1 ml) in pyridine (2 ml) in a sealed tube for 40 hours at 85°C with occasional shaking. Crystals which appeared in the solution during the reaction were filtered (80 mg), washed with, and then recrystallized from ethanol, m.p. 306°C (decomp.). $[\alpha]_D$ = +74° (c = 0.1 in 27% water in methanol).

Anal. Found : C, 67.72; H, 7.68; N, 7.29; O, 8.05; Cl, 9.19; CCH_3, 6.06%.

$C_{22}H_{29}N_2O_2Cl$ requires: C, 67.93; H, 7.48; N, 7.20; O, 8.22; Cl, 9.11; CCH_3, 6.94%.

UV spectrum : λ_{max} 222 (4.46), 272 (3.87), 289 (3.82); λ_{min} 238 (3.03), 286 (3.74); λ_{sh} 280 (3.87) nm. With no change in acid or base.

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* We thank Dr. S.R. Johns for kindly providing an authentic sample of antirhine methochloride.

IR spectrum : 3130 (NH), 1740 (>C=O), 1370, 1245 (O-acetyl), 1040, 740 cm^{-1}.

NMR spectrum (D_2O) : 6.8 - 7.4δ multiplet four aromatic protons. 5.28δ three-proton multiplet (vinyl protons). 3.75δ singlet due to methylene protons $-CH_2-O-\overset{O}{\overset{\|}{C}}-R$. 3.3δ three-proton multiplet ($N-CH_3$). 1.87δ three-proton singlet (methyl on CO).

Mass spectrum : 352 (13% of base peak), 339 (30), 338 (90), 337 (90), 314 (14), 279 (25), 278 (25), 265 (10), 251 (16), 239 (37), 225 (100%, base peak), 223 (95), 197 (30), 184 (40), 169 (60), 156 (40), 143 (25) m/e.

Dihydroantirhine α-methochloride (38)

Antirhine α-methochloride (100 mg) was dissolved in ethanol and palladium-on-charcoal (10%, 15mg) was added. The mixture was shaken with hydrogen at 50 p.s.i. for 24 hours, filtered and the solvent evaporated under reduced pressure. Only one product was formed as shown by a single spot on thin layer chromatography.

Dihydroantirhine α-methochloride (80 mg) crystallized from ethanol, m.p. 305°C (decomp.), $[\alpha]_D^{578}$ = +71.4°, $[\alpha]_D^{546}$ = +78.9°, $[\alpha]_D^{436}$ = +150.3°, $[\alpha]_D^{405}$ = +187.9°, $[\alpha]_D^{365}$ = +266.9° (c = 0.133 in water).

Anal. Found : C, 67.38; H, 8.51; N, 7.53; O, 6.89; Cl, 9.94; OCH_3, 0.0%.

$C_{20}H_{29}N_2OCl.\frac{1}{2}H_2O$ requires: C, 67.11; H, 8.45; N, 7.82; O, 6.71; Cl, 9.90%.

UV spectrum : λ_{max} 220 (4.59), 272 (3.88), 289 (3.82);
λ_{min} 238 (3.21), 286 (3.75);
λ_{sh} 282 (3.87) nm.

IR spectrum : peaks at 3390 (OH), 3150 (NH), 1300, 1020, 910 and 740 cm^{-1}.

NMR spectrum : 7.25 - 7.8δ aromatic protons. 3.2δ broad singlet. 2.83δ singlet. 1.2δ triplet (J=7 Hz).

Mass spectrum : 312 (5% of base peak), 298 (98), 297 (100%, base peak), 267 (13), 225 (62), 223 (20), 197 (40), 184 (25), 170 (90), 169 (30), 156 (30), 144 (32), 143 (22) m/e.

O-Acetyldihydroantirhine α-methochloride from hydrogenated antirhine α-methochloride

Dihydroantirhine α-methochloride (100 mg) was acetylated in a sealed tube in presence of acetic anhydride (1 ml) and pyridine (2 ml). The tube was heated in a heating block for 48 hours at 90°C with occasional shaking. At the end of the reaction O-acetyldihydroantirhine α-methochloride had partly crystallized in the tube. It was filtered, washed with and recrystallized from methanol, m.p. 303°C (decomp.). $[\alpha]_D$ = +69° (c = 0.03 in 27% water in methanol).

UV spectrum : λ_{max} 218 (4.45), 272 (3.87), 288 (3.76);
λ_{min} 240 (3.10), 285 (3.68);
λ_{sh} 282 (3.78) nm.
with no change in acid or base.

IR spectrum : peaks at 3130 (NH), 1722 (C=O), 1365, 1245 (O-acetyl), 1030, 900, 740 cm^{-1}.

Mass spectrum : 355 (M^+) (6% of base peak), 354 (22), 240 ($M^+ - CH_3$) (95), 339 (100%, base peak), 311 (10), 297 (5), 281 (8), 267 (8), 253 (7), 251 (3), 239 (50), 225 (55), 223 (22), 197 (32), 184 (19), 170 (22), 169 (10), 156 (19), 143 (8) m/e.

Antirhine (28) by demethylation of antirhine α-methochloride

Antirhine α-methochloride (221 mg, 0.64 mmole) was dissolved in 20 ml ethanol and a solution of (235 mg, 1.78 mmoles) of sodium thiophenoxide in 20 ml ethanol was added. After stirring for 20 minutes, the sodium chloride formed was filtered and washed with ethanol. The ethanolic filtrate and washings were combined and the solvent evaporated under reduced pressure. To the residue was added 100 ml of methyl ethyl ketone freshly distilled from zinc dust. The system was then heated to reflux under nitrogen for 36 hours.

Following removal of the solvent 20 ml of water and 50 ml of chloroform were added. The chloroform layer was separated and the aqueous layer extracted three more times with chloroform. Evaporation of the combined chloroform fractions under vacuum was followed by the addition of 10% hydrochloric acid. The aqueous acid solution was then repeatedly extracted with ether.

Neutralization of the aqueous solution with sodium bicarbonate was followed by extraction with chloroform. After drying over anhydrous sodium sulphate and evaporation of the solvent antirhine was recovered and crystallized from chloroform as yellow needles, m.p. 112 - 115°C, $[\alpha]_D = -2°$ (c = 0.1 in chloroform).

UV spectrum : λ_{max} 225 (4.59), 282 (3.77);
λ_{min} 246 (3.35), 287 (3.72);
λ_{sh} 289 (3.73) nm. With no change in acid or base.

IR spectrum (KBr) : peaks at 3420 (NH), 3260 (OH), 2920, 1330, 1280, 1110, 915, 740 cm^{-1}.

NMR spectrum ($CDCl_3$) : 6.8 - 7.4δ aromatic protons. 5.2δ three-proton multiplet vinyl protons. Multiplet at 3.68δ ($\underline{CH_2}OH$). 3.15δ singlet. 2.1δ multiplet.

Mass spectrum : 296 (M) (60% of base peak), 295 (M-1) (80), 265 (10), 225 (100%, base peak), 223 (80), 197 (15), 184 (16), 169 (20), 156 (16), 143 (9) m/e.

Antirhine (28) from Antirhea putaminosa

The published physical constants for antirhine are:

m.p. 112 - 114°C. $[\alpha]_D$ = -2° (c = 0.23 in chloroform).

UV spectrum : λ_{max} 225 (4.41), 282 (3.80);
λ_{sh} 289 (3.68) nm.

IR spectrum ($CHCl_3$) : peaks at 3570 (NH) and 3350 (OH) cm^{-1}.

NMR spectrum : broad four-proton multiplet between 420 and 456 cps (unsubstituted indolic benzenoid), complex three-proton multiplet between 292 and 354 cps (vinyl group), 3.67δ two-proton multiplet ($\underline{CH_2}OH$).

Mass spectrum : 296 (77% of base peak), 295 (75), 265 (12), 225 (94), 223 (100%, base peak), 197 (15), 184 (19), 169 (24), 156 (19), 144 (11) m/e.

Dihydroantirhine (29)

Dihydroantirhine was either obtained by demethylating dihydroantirhine α-methochloride by the thiophenoxide anion method described earlier or simply by hydrogenation of antirhine over Adam's catalyst in ethanol.

Dihydroantirhine in both cases proved difficult to crystallize but showed only one spot on t.l.c. This was also proven by NMR and Mass spectroscopy.

NMR spectrum : 6.8 - 7.5δ multiplet four aromatic protons. 3.4 - 3.8δ multiplet methylene protons. 0.85 broad three-proton singlet terminal methyl group.

Mass spectrum : 298 (M) (95% of base peak), 297 (M-1) (100%, base peak), 267 (4), 253 (10), 225 (50), 223 (8), 197 (28), 184 (14), 170 (18), 169 (17), 156 (12), 144 (8), 143 (7) m/e.

Dihydroantirhine α-methochloride (natural) (39)

Dihydroantirhine α-methochloride was obtained from chromatograms C and D. Two different samples for analysis were crystallized from ethanol.

The physical constants of this quaternary alkaloid correspond to the published constants under the name Hunteria-alkaloid J, m.p. 308 - 310°C (decomp.), $[\alpha]_D$ = +86.7° (c = 0.3 in 27% water in methanol).

Anal. Found : C, 68.64; 68.67; H, 8.14; 8.08; N, 8.25; 7.89; O, 4.69; 4.52; Cl, 10.28; 10.82%.

$C_{20}H_{29}N_2OCl$ requires: C, 68.86; H, 8.32; N, 8.03; O, 4.59; Cl, 10.18%.

UV spectrum : λ_{max} 222 (4.57), 270 (3.98), 289 (3.89);
λ_{min} 242 (3.54), 286 (3.80);
λ_{sh} 280 (3.94) nm. With no change in acid or base.

IR spectrum : peaks at 3440, 3158, 1240, 1050, 905, 750 cm^{-1}.

NMR spectrum (D_2O) : 7.2 - 7.7δ aromatic protons. 3.1δ broad singlet. 2.7δ singlet.broad doublet of doublets centered at 0.9δ.

Mass spectrum : 312 (10% of base peak), 298 (98), 297 (100%, base peak), 267 (18), 225 (24), 223 (7), 197 (6), 184 (10), 170 (34), 169 (27), 156 (18), 144 (12), 143 (9) m/e.

O-Acetyldihydroantirhine α-methochloride from natural dihydroantirhine α-methochloride

Dihydroantirhine α-methochloride (300 mg) was acetylated using acetic anhydride (1 ml) in pyridine (2 ml) in a sealed tube for 48 hours at 90°C with occasional shaking. The O-acetylidihydroantirhine α-methochloride crystallized directly in the tube. It was filtered, and recrystallized from ethanol, m.p. 304-306°C (decomp.), $[\alpha]_D$ = +68° (c = 0.1 in 27% water in methanol).

Anal. Found : C, 64.06; H, 7.53; N, 7.50%.

$C_{22}H_{31}N_2O_2Cl.H_2O$ requires: C, 64.54; H, 8.06; N, 6.85%.

UV spectrum : λ_{max} 218 (4.57), 272 (3.89), 288 (3.82);
λ_{min} 238 (3.07), 285 (3.74);
λ_{sh} 282 (3.87) nm. With no change in acid or base.

IR spectrum : peaks at 3130 (NH), 1730 (C=O), 1235 (O-acetyl), 1035, 900, 740 cm^{-1}.

NMR spectrum (CF_3COOH) : 8.3δ singlet. 7.25 - 7.8δ multiplet, aromatic protons. 3.20δ multiplet. 2.83δ singlet. 2.32δ singlet which appeared after acetylation. 1.1δ broad doublet.

Mass spectrum : 355 (4% of base peak), 354 (20), 341 (30), 340 (98), 339 (100%, base peak), 311 (10), 297 (8), 281 (7), 267 (35), 253 (15), 251 (28), 239 (4), 225 (25), 223 (8), 197 (8), 184 (10), 170 (38), 169 (30), 156 (15), 143 (8) m/e.

Dihydroantirhine (29) from natural dihydroantirhine α-methochloride (39)

Dihydroantirhine α-methochloride (200 mg) was demethylated using the thiophenoxide method described earlier. Dihydroantirhine was found difficult to crystallize. The reaction product (130 mg) was passed through a silica gel column with chloroform containing increasing percentages of ethanol as eluant. Dihydroantirhine although showing one spot on t.l.c. did not crystallize from any solvent except ethanol, m.p. 106 - 107°C, $[\alpha]_D$ = +22° (c = 0.1 in chloroform).

IR spectrum ($CHCl_3$) : 3200-3310, 1620, 1380, 1330, 1300, 1265, 1170, 1105, 1050 cm^{-1}.

NMR spectrum : 6.9 - 7.7δ multiplet four aromatic protons; 3.62δ broad doublet, methylene protons; 2.52δ singlet; 2.13δ singlet; 0.90δ broad triplet terminal methyl group.

Mass spectrum : 298 (M), 297 (M-1) (100%, base peak), 267 (4), 253 (13), 225 (30), 223 (8), 197 (10), 184 (20), 170 (40), 169 (35), 156 (25), 144 (14), 143 (12) m/e.

Dihydroantirhine hydrochloride

Dihydroantirhine which proved difficult to crystallize formed a hydrochloride in 10% hydrochloric acid which crystallized as shining needles from water, m.p. 290 - 292°C (decomp.).

Anal. Found : C, 68.24; H, 7.99; N, 8.27%.

$C_{19}H_{27}N_2OCl$ requires: C, 68.12; H, 8.12; N, 8.36%.

UV spectrum : λ_{max} 223 (4.55), 275 (3.91), 289 (3.78);
λ_{min} 243 (3.13), 286 (3.77);
λ_{sh} 282 (3.88) nm.

IR spectrum : peaks at 3400, 3130, 2560-2700, 1330, 1205, 1040, 1025, 770, 750 cm^{-1}.

Mass spectrum : 299 (M^+), 298 (75% of base peak), 297 (100%, base peak), 253 (13), 225 (30), 197 (10), 184 (20), 170 (40), 169 (35), 156 (25), 144 (14), 143 (12) m/e.

Cyclisation of dihydroantirhine tosylate

Dihydroantirhine (35 mg) and p-toluenesulphonyl chloride (60 mg) were dissolved in pyridine (1 ml) and the solution kept at 0° overnight. The solution was then brought to room temperature and the pyridine evaporated under vacuum. The residue was suspended in water and the tosyl ester extracted into chloroform. The product from evaporation of the chloroform extracts was not purified but was dissolved in dimethylformamide (1 ml) and the solution heated at reflux temperature for 30 min. On cooling the cyclised product (35) separated as colourless needles (15 mg), m.p. 316 - 318°C, $[\alpha]_D$ = -58° (c = 0.1 in ethanol).

UV spectrum : λ_{max} 221 (4.61), 270 (3.82), 289 (3.69);
λ_{min} 239 (3.13), 286 (3.65);
λ_{sh} 282 (3.80) nm. With no change in acid or base.

IR spectrum : peaks at 3140, 3100, 1235, 1170, 1160, 1120, 1030, 1010, 812, 740 cm^{-1}.

Mass spectrum : 370, 369, 354, 340, 314, 312, 300, 286, 271, 265, 258, 240, 238, 237, 214, 212, 185, 183, 171, 157, 137, 129, 126, 123, 121 m/e.

Antirhine β-methiodide from antirhine (28)

Antirhine (60 mg) reacted vigorously with methyl iodide in methanol. Antirhine β-methiodide separated and was recrystallized from methanol, m.p. 288 - 290°C (decomp.), $[\alpha]_D$ = -24.4 (c = 0.1 in ethanol).

UV spectrum : λ_{max} 222 (4.10), 266 (3.48), 288 (3.37);
λ_{min} 236 (3.07), 285 (3.30) nm. With no change in acid or base.

IR spectrum : peaks at 3315, 3210, 2850, 1160, 1000, 950, 750 cm^{-1}.

NMR spectrum (CF_3COOH) : 6.8 - 7.6δ multiplet four aromatic protons. 5.42δ multiplet vinyl protons. 4.6δ multiplet. 3.7δ broad two-proton multiplet (methylene protons). 3.42δ three-proton singlet ($N-CH_3$).

Mass spectrum : 311 (M^+), 310, 296, 295, 265, 239, 225, 223, 197, 184, 169, 156, 144, 143 m/e.

Antirhine β-methochloride (37b)* from antirhine β-methiodide

Antirhine β-methiodide prepared from antirhine was passed through a short column containing Permutit "Isopor SRA-66 (chloride form)" in 50% aqueous acetone.

Evaporation of the solvent under reduced pressure provided the corresponding antirhine β-methochloride which crystallized from aqueous methanol, m.p. 325-328°C (decomp.), $[\alpha]_D$ = -17.9° (c = 0.27 in 27% water in methanol).

UV spectrum	:	λ_{max} 222 (4.21), 268 (3.54), 289 (3.42); λ_{min} 238 (3.16), 286 (3.36) nm. With no change in acid or base.
IR spectrum	:	peaks at 3260, 3130, 2850, 1370, 1240, 1165, 1060, 950, 915, 755 cm^{-1}.
NMR spectrum (CF_3COOH)	:	7.05 - 7.75δ multiplet four aromatic protons; 5.45δ multiplet vinyl protons; 3.42δ three-proton singlet ($N-CH_3$).
Mass spectrum	:	311 (M^+), 310, 296, 295, 265, 239, 225, 223, 197, 184, 169, 156, 143 m/e.

Dihydroantirhine β-methiodide from dihydroantirhine (29)

Dihydroantirhine (50 mg) reacted rapidly with methyl iodide in methanol to yield dihydroantirhine β-methiodide (55 mg) which crystallized from methanol, m.p. 296-298°C. This was readily converted to the chloride form (on ion exchange resin).

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* A mixed melting point with an authentic sample showed no depression and the infrared and mass spectra were superposable with that of antirhine methochloride kindly provided by Dr. S.R. Johns, Chemical Research Laboratories, Melbourne.

Dihydroantirhine β-methochloride (38) from dihydroantirhine (29)

Dihydroantirhine β-methiodide prepared from dihydroantirhine was passed through a short column containing Permutit "Isopor SRA-66 (chloride form)" in 50% aqueous acetone.

Evaporation of the solvent under reduced pressure provided the corresponding dihydroantirhine β-methochloride which crystallized from aqueous ethanol, m.p. 310-312°C, $[\alpha]_D$ = -8° (c = 0.13 in ethanol / water).

UV spectrum	:	λ_{max} 222 (4.69), 270 (3.92), 288 (3.80); λ_{min} 245 (3.54), 286 (3.75); λ_{sh} 282 (3.89) nm. With no change in acid or base.
IR spectrum	:	peaks at 3295 (OH), 3120 (NH), 2840, 1300, 1240, 1165, 1005, 950, 900, 750 cm^{-1}.
NMR spectrum (CF_3COOH)	:	6.9 - 7.8δ multiplet aromatic protons. 3.5δ three-proton singlet (N-CH_3). 0.9δ three-proton broad triplet terminal methyl group.
Mass spectrum	:	312 (4% of base peak), 298 (90), 297 (100%, base peak), 267 (10), 239 (10), 225 (70), 223 (18), 197 (35), 184 (25), 170 (80), 169 (40), 156 (35), 144 (30), 143 (20) m/e.

-.C H A P T E R II.-

MISCELLANEOUS QUATERNARY ALKALOIDS

Pleiocarpamine methochloride (44)

Pleiocarpamine methochloride was encountered only in chromatogram B presumably due to its solubility in methylene chloride. Pleiocarpamine methochloride was crystallized from ethanol / water for analysis.

The physical constants of this alkaloid correspond to the published constants of Hunteria eburnea alkaloid-F, m.p. 242-243°C (decomp.), $[\alpha]_D$ = +165° (c = 0.5 in 27% water in methanol).

Anal. Found	:	C, 64.29; H, 7.03; N, 7.11; O, 12.52; Cl, 9.45%.
$C_{21}H_{25}N_2O_2Cl.H_2O$ requires	:	C, 64.50; H, 7.17; N, 6.96; O, 12.30; Cl, 9.07%.
UV spectrum	:	λ_{max} 223 (4.47), 274 (3.92); λ_{min} 246 (3.52); λ_{sh} 283 (3.87), 292 (3.75) nm. With no change in acid or base.
IR spectrum	:	peaks at 3460, 3410, 1737 (ester), 1211, 1022, 750 cm^{-1}.
NMR spectrum	:	6.9 - 8δ aromatic protons; 5.65δ broad doublet C_{19} proton; 4.99δ doublet C_{16} proton; 3.70δ singlet methyl ester; 3.18δ singlet N-CH_3; 1.58δ doublet C_{20} methyl.
Mass spectrum	:	375 (35% of base peak), 374 (26), 373 (100%, base peak), 358 (10), 338 (28), 337 (62), 316 (26), 314 (78), 313 (50), 283 (40), 278 (18), 264 (16), 180 (50), 167 (15), 156 (8), 122 (45), 108 (12) m/e.

Pleiocarpamine methiodide from pleiocarpamine methochloride

Pleiocarpamine methochloride was converted to the iodide form on passing it over a column containing Permutit "Isopor SRA-66 (iodide form)" in methanol, m.p. 230°C (decomp.).

Mass spectrum : 464 (M^+), 405, 337, 322, 263, 236, 234, 180, 142 (CH_3I^+), 128, 122 m/e.

Pleiocarpamine (40) from pleiocarpamine methochloride (44)

Pleiocarpamine methochloride was demethylated according to the sodium thiophenoxide method described earlier. Although the yield is poor due to the presence of the ester group, the pleiocarpamine was separated from the other reaction products on a silica gel column using chloroform as eluant.

Pleiocarpamine was obtained by eluting with 5% ethanol in chloroform and crystallized from acetone, m.p. 158°C, $[\alpha]_D$ = +134° (c = 0.2 in chloroform).

UV spectrum : λ_{max} 230 (4.42), 284 (3.86) nm.

IR spectrum (KBr) : peaks at 1730 (ester), 1280, 1203, 1150, 1058, 1030, 1012, 995, 855, 750 cm^{-1}.

(CCl_4) : 1738, 1769 cm^{-1}.

NMR spectrum (CD_3COCD_3) : 6.7 - 7.0δ multiplet aromatic protons; 5.17δ broad quartet integrating for one proton (=C<u>H</u>-CH_3); 3.60δ singlet integrating for three protons ($COOCH_3$); 1.45δ multiplet methyl group on a double bond.

A mixed melting point with an authentic sample showed no depression and

the infrared spectrum was superposable with that of pleiocarpamine*.

2,7-Dihydropleiocarpamine (43)

Pleiocarpamine (40 mg) was hydrogenated on pre-reduced platinum oxide catalyst in ethanol at room temperature under atmospheric pressure. The reaction was stopped after the uptake of one mole of hydrogen. After filtration and evaporation of the solvent under reduced pressure, 2,7-dihydropleiocarpamine was recovered and crystallized from acetone / ether, m.p. 144-145°C.

UV spectrum : λ_{max} 254 (4.01), 295 (3.41);
λ_{min} 275 (3.10) nm. With no change in acid or base.

IR spectrum : peaks at 3450, 3410, 2770-2850 (trans bands), 1736, 1211, 750 cm^{-1}.

Yohimbol methochloride (natural) (52)

Yohimbol methochloride was obtained (as was hunteracine chloride) from most of the chromatograms as crystalline needles. It was recrystallized from acetone-water, m.p. 268-270°C, $[\alpha]_D$ = +46° (c = 0.7 in 33% water in ethanol).

UV spectrum : λ_{max} 222 (4.66), 268 (3.98), 288 (3.75);
λ_{min} 246 (3.59), 286 (3.72);
λ_{sh} 282 (3.88) nm. With no change in acid or base.

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* We thank Dr. M. Hesse (Zurich) for kindly providing an authentic sample of pleiocarpamine.

IR spectrum : peaks at 3350-3450 (OH), 3220 (NH), 1250, 1130, 1020, 1000, 945, 820, 750 cm^{-1}.

NMR spectrum (CF_3COOH) : 8.2δ two-proton singlet (hydroxy and indolic imino groups); 7.1 - 7.6δ multiplet four aromatic protons; 5.5δ multiplet C_{17} proton (OH secondary); 3.3δ three H singlet ($N-CH_3$).

Mass spectrum : 311 (M^+) (5% of base peak), 297 (85), 296 (100%, base peak), 184 (22), 170 (15), 169 (23), 156 (20), 144 (8) m/e.

Yohimbol (50) from yohimbone (51)

Yohimbone (100 mg) obtained from yohimbine* was dissolved in methanol and after complete dissolution, sodium borohydride (70 mg) was added and left to reflux for 7 hours. After filtration yohimbol crystallized as fine needles, m.p. 249-251°C.

UV spectrum : λ_{max} 223 (4.63), 270 (3.95), 289 (3.72); λ_{min} 245 (3.55), 287 (3.70); λ_{sh} 282 (3.85) nm. With no change in acid or base.

IR spectrum : peaks at 3200, 1130, 1020, 1000, 945, 820, 750 cm^{-1}.

Mass spectrum : 296 (M, 90% of base peak), 295 (M-1) (100%, base peak), 184 (26), 170 (20), 169 (28), 156 (24), 143 (10) m/e.

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* Yohimbine was extracted from Aspidosperma excelsum Benth. Pierre Benoin's Ph.D. thesis (Laval University).

Yohimbol (50) from yohimbol methochloride (52)

Yohimbol methochloride was demethylated using the thiophenoxide method described earlier.

Yohimbol could also be obtained by pyrolysis of yohimbol methochloride at 300° under high vacuum. Yohimbol crystallized from aqueous methanol, m.p. 250°C.

It was found to be identical in all respects with yohimbol prepared from yohimbone.

Yohimbol methiodide from yohimbol (50)

Yohimbol (40 mg) (from yohimbone reduction) was methylated with methyl iodide in methanol. The reaction was left to proceed overnight. The solvent was evaporated under reduced pressure. Yohimbol methiodide crystallized from ethanol, m.p. 300°C (decomp.).

UV spectrum : λ_{max} 220 (4.52), 267 (3.82), 289 (3.55);
λ_{min} 245 (3.46), 286 (3.46);
λ_{sh} 282 (3.67) nm. With no change in acid or base.

IR spectrum : peaks at 3410, 3220, 1458, 1300, 1060, 1030, 900, 750 cm^{-1}.

Yohimbol methochloride (52) from yohimbol methiodide

Yohimbol methiodide was exchanged on Permutit "Isopor SRA-66 (chloride form)" in 50% aqueous acetone on a short column. The solvent was evaporated

under reduced pressure and yohimbol methochloride was crystallized from ethanol.

It was found that synthetic and natural yohimbol methochlorides have superposable infrared spectra and no depression in their melting points was noticed on admixture.

O-acetyl yohimbol methochloride (53)

Yohimbol methochloride (30 mg) was acetylated with acetic anhydride (2 ml) in presence of pyridine (1 ml) in a sealed tube at 90°C.

After 48 hours with occasional shaking, the reaction products were dried under reduced pressure and O-acetyl yohimbol methochloride (25 mg) crystallized from ethanol, m.p. 265°C (decomp.).

UV spectrum	:	λ_{max} 222 (4.71), 266 (4.05), 287 (3.82); λ_{min} 246 (3.71), 285 (3.79) nm. With no change in acid or base.
IR spectrum	:	peaks at 3600, 3420, 1725 (C=O), 1630, 1375, 1260 (O-acetyl), 1160, 1020, 960, 905, 705 cm^{-1}.
NMR spectrum (CF_3COOH)	:	7.7δ one H singlet indolic imino group. 7.1 - 7.6δ multiplet four aromatic protons; 5.4δ multiplet only one hydrogen adjacent to O-acetyl being secondary; 3.35δ three-proton singlet ($N-CH_3$); 2.3δ singlet.

Akuammicine methochloride (54)

This alkaloid was met with only once from chromatogram B. It crystallized from methanol, m.p. 270°C (decomp.).

UV spectrum : λ_{max} 227 (4.09), 330 (4.20);
λ_{sh} 300 (4.05) nm. With no change in acid or base.

IR spectrum : peaks at 1662, 1610, 1235, 1220, 1105, 756 cm^{-1}.

NMR spectrum (CF_3COOH) : 3.57δ singlet (OCH_3); 3.04δ singlet integrating for three protons ($N-CH_3$); 1.3δ broad multiplet.

Mass spectrum : 322 (M^+-CH_3) (100%, base peak), 292 (8), 280 (25), 278 (75), 265 (27), 264 (90), 263 (23), 249 (18), 233 (37), 222 (18), 208 (24), 194 (20), 181 (32), 180 (34), 172 (64), 167 (25), 158 (85), 157 (45), 156 (28), 144 (34), 143 (18), 130 (10), 122 (62), 121 (70), 115 (18) m/e.

Huntrabrine methochloride (55)

We obtained huntrabrine methochloride only once from chromatogram C. This alkaloid was recognized by its spectra according to published data. It crystallized from methanol / water, m.p. 285-287°C (decomp.).

IR spectrum : peaks at 3413 (OH), 3120, 1629 (C=C), 1220, 1135, 1031, 923, 913, 839, 814 cm^{-1}.

NMR spectrum (D_2O)	:	6.7 - 7.6δ (2 + 1 aromatic protons); 5.95δ broad quartet C_{19} proton; 3.05δ singlet ($N-CH_3$); 1.78δ doublet C_{19}-methyl (J=7 Hz).
Mass spectrum	:	326 (22% of base peak), 312 (100%, base peak), 311 (100), 295 (10), 281 (22), 201 (80), 186 (24), 185 (30), 184 (18), 172 (23), 160 (10), 122 (5) m/e.

Although previous investigators isolated abundant amounts of huntrabrine methochloride, we obtained only 12 mg, which sufficed to record the spectra necessary for its identification.

-.C H A P T E R III.-

HUNTERACINE CHLORIDE

Hunteracine chloride (66)

Hunteracine chloride was obtained from nearly all the chromatograms. This ubiquitous alkaloid, the presence of which could be readily checked by the crimson red coloration it gives with ceric sulphate spray, crystallized from ethanol / water. A sample for analysis was recrystallized from ethanol, m.p. 343°C (decomp.), $[\alpha]_D$ = -90° (c = 0.1 in 27.5% water in methanol).

Anal. Found	:	C, 67.80; H, 7.32; N, 8.73; O, 5.00; Cl, 11.25; OCH_3, 0.00; CCH_3, 4.34%.
$C_{18}H_{23}N_2OCl$ requires	:	C, 67.81; H, 7.27; N, 8.78; O, 5.01; Cl, 11.12; CCH_3, 4.70%.
UV spectrum	:	λ_{max} 234 (3.95), 289 (3.49); λ_{min} 216 (3.91), 254 (2.89) nm. With no shift observed in acid or base. The UV spectrum of hunteracine chloride published by Taylor[21] was: λ_{max} 234 (3.90), 291 (3.34); λ_{min} 218 (3.62), 256 (2.30) nm. With no shift observed in acid or base.
IR spectrum	:	peaks at 3440 (OH), 3150 (NH), 1620 (C=C), 1200, 1140, 1120, 1095, 1060, 1024, 940, 905, 860, 760, 752 cm^{-1}.
NMR spectrum (D_2O)	:	6.65 - 7.5δ multiplet aromatic protons; 5.20δ broad multiplet integrating for one proton; 3.35δ multiplet. 2.58δ broad singlet; 2.2δ doublet; 1.67δ doublet a methyl on a double bond.

Mass spectrum : 283 (M^+) (25% of base peak), 282 (M-1) (100%, base peak), 266 (13), 265 (54), 172 (11), 159 (13), 158 (20), 146 (22), 137 (15), 130 (15), 124 (30), 122 (45), 121 (30), 108 (29) m/e.

Hunteracine bromide (68)

Hunteracine chloride was exchanged on a resin (Permutit, bromide form) in acetone / water.

Hunteracine bromide crystallized from acetone-water. A sample for analysis was recrystallized from ethanol. A crystal of this sample was subjected to X-ray analysis, m.p. 340°C (decomp.).

Anal. Found : C, 59.57; H, 6.35; N, 7.90; O, 4.51; Br, 22.21%.

$C_{18}H_{23}N_2OBr$ requires : C, 59.50; H, 6.33; N, 7.71; O, 4.41; Br, 22.04%.

UV spectrum : λ_{max} 234 (3.92), 291 (3.41); λ_{min} 220 (3.67), 256 (2.51) nm. With no change in acid or base.

Mass spectrum : 283 (M^+) (20% of base peak), 282 (100%, base peak), 266 (10), 265 (50), 172 (10), 159 (12), 158 (22), 146 (22), 137 (20), 130 (15), 124 (30), 122 (50), 121 (28), 108 (23) m/e.

Dihydrohunteracine chloride

Hunteracine chloride (70 mg) in 90% ethanol was hydrogenated in the presence of pre-reduced platinum oxide catalyst with the uptake stopping at

one mole equivalent. After filtration and evaporation of the solvent under reduced pressure, the residue crystallized from methanol / acetone.

A sample for analysis was recrystallized from ethanol, m.p. 313-315°C (decomp.).

Anal. Found	:	C, 67.52; H, 7.95; N, 8.72; O, 5.05; Cl, 11.09; OCH_3, 0.0; CCH_3, 4.43%.
$C_{18}H_{25}N_2OCl$ requires	:	C, 67.50; H, 7.81; N, 8.75; O, 5.00; Cl, 11.09; CCH_3, 4.69%.
IR spectrum	:	peaks at 3140, 1620, 1270, 1135, 1118, 1020, 865, 750 cm^{-1}.
NMR spectrum (D_2O)	:	6.7 - 7.6δ multiplet aromatic protons; 3.45δ singlet; 2.53δ multiplet; 2.2δ multiplet; 2.0δ singlet; triplet centered at 0.90δ (J=6.5 Hz).
Mass spectrum	:	285 (25% of base peak), 284 (90), 268 (25), 267 (100%, base peak), 172 (5), 159 (18), 158 (16), 146 (15), 138 (18), 124 (38), 110 (30) m/e.

Dihydrohunteracine Emde base (67a)

Hunteracine chloride (100 mg) was hydrogenated in presence of 10% palladium-on-charcoal and traces of acetic acid in 90% ethanol at 50 p.s.i. for 24 hours.

After filtration, the dihydrohunteracine Emde base crystallized from methanol. A sample for analysis was recrystallized three times from ethanol, m.p. 292-294°C (decomp.).

Anal. Found	:	C, 67.31; H, 8.57%.
$C_{18}H_{27}N_2OCl$ requires	:	C, 66.92; H, 8.43%.
UV spectrum	:	λ_{max} 282 (3.55), 289 (3.53); λ_{min} 252 (3.29), 286 (3.46); λ_{sh} 223 (4.19), 235 (3.68), 305 (2.68) nm. With no shift in acid or base.
IR spectrum	:	peaks at 3380, 3235, 3140, 1610, 1300, 1240, 1200, 1160, 1045, 970, 910, 740 cm^{-1}.
NMR spectrum (D_2O)	:	6.7 - 7.3δ aromatic four-proton multiplet; triplet centered at 3.50δ (J=7 Hz); broad multiplet at 2.45δ; triplet centered at 1.07δ (J=7 Hz); broad triplet centered at 0.8δ (J=7 Hz).
Mass spectrum	:	287 (25% of base peak), 270 (5), 231 (30), 230 (100%, base peak), 212 (12), 174 (6) m/e.

Hunteracine Pseudo-indoxyl (74)

Hunteracine chloride (220 mg) was refluxed for 30 minutes in ethanol in the presence of potassium hydroxide (250 mg). Aliquots from the reaction medium were tested on t.l.c. until one spot with characteristic green fluorescence reached a maximum intensity and the starting material was no longer present.

At the end of the reaction, the solution was filtered and the residue washed with ethanol. The solvent and the washings were evaporated under reduced pressure affording a brown red residue, which was taken up in water (20 ml) and chloroform (40 ml) was added. The aqueous solution was extracted three more times with chloroform and the combined chloroform layers dried

over anhydrous sodium sulphate and evaporated under reduced pressure.

Hunteracine ψ-indoxyl crystallized from ethanol. A sample for analysis was recrystallized from ethanol, fine yellow needles, m.p. 235°C (decomp.), $[\alpha]_D$ = -355°C (c = 0.1 in ethanol).

Anal. Found	:	C, 60.2; H, 6.79; N, 7.23; O, 7.72;
$C_{18}H_{22}N_2OCl_2C_2H_5OH$ requires	:	C, 60.1; H, 7.00; N, 7.00; O, 8.00;
UV spectrum	:	λ_{max} 229 (4.35), 380 (3.48); λ_{min} 275-290 (2.98); λ_{sh} 253 (3.74), 259 (3.66), 343 (3.26) nm. With no change in acid or base.
IR spectrum	:	peaks at 3200-3400, 1692, 1625, 1490, 1155, 1085, 1045, 900, 860, 752 cm^{-1}.
NMR spectrum ($CDCl_3$)	:	7.4 - 8δ aromatic protons; broad multiplet at 5.8δ integrating for one proton on a vinyl group; broad multiplet centered at 4.6δ; broad multiplet centered at 1.95δ; triplet at 1.4δ (J=7 Hz).
Mass spectrum	:	282 (M), 281 (M-1) (16% of base peak), 266 (9), 238 (24), 237 (28), 236 (24), 224 (12), 210 (25), 201 (44), 196 (20), 185 (90), 183 (100%, base peak), 158 (40), 130 (52), 122 (24), 108 (16) m/e.

Dihydrohunteracine pseudo-indoxyl (76)

Dihydrohunteracine chloride (40 mg) was refluxed in ethanol in presence of alcoholic potassium hydroxide (100 mg). The reaction was

followed by thin layer chromatography using an ultraviolet lamp. The work-up procedure was the same as in the case of hunteracine ψ-indoxyl.

Dihydrohunteracine ψ-indoxyl proved difficult to crystallize from any solvent but showed only one green fluorescent spot on t.l.c. The formation of dihydrohunteracine ψ-indoxyl was confirmed by its behavior in the mass spectrometer.

Mass spectrum : 285, 284, 283, 282, 253, 182, 159, 158, 146, 138, 136, 124, 123, 110, 108, 96, m/e.

Hofmann degradation of hunteracine chloride

Hunteracine chloride (300 mg) was suspended in t-butyl alcohol (15 ml) and a solution of potassium (1 g) in t-butyl alcohol was added slowly. The solution turned to pale brick red. The mixture was refluxed for 24 hours then evaporated to dryness under reduced pressure. The residue (4.6 g) re-dissolved in water and extracted four times with chloroform. The chloroform was dried over anhydrous sodium sulphate and evaporated under reduced pressure.

The chloroform extract showed 2 spots on t.l.c. which were separated on a silica gel column using petroleum ether (boiling range 65-110°C) and chloroform (2:1). The first green fluorescent fraction (15 mg) from the column was eluted with petroleum ether / chloroform (1:1).

IR spectrum ($CHCl_3$) : 1715-1730, 1620, 1475 cm^{-1}.

Mass spectrum : 280, 266, 237, 223, 185, 184, 183, 182, 167, 158, 149, 137, 123, 121, 111, 109 m/e.

The 5% ethanol in chloroform eluates of the above chromatogram gave a residue crystallizing from ethanol / acetone (15 mg), m.p. 230°C (decomp.).

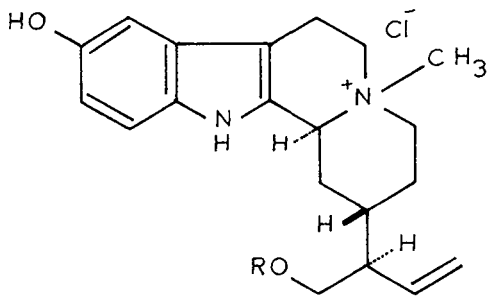
IR spectrum	:	peaks at 3270, 1705, 1620, 1480, 1380, 1300, 1275, 1200, 1160, 980, 955, 900, 755 cm^{-1}.
NMR spectrum	:	6.6 - 7.7δ aromatic protons; 5.32δ broad singlet vinyl proton; broad multiplet centered at 3.4δ; broad triplet at 1.63δ (J=9 Hz).
Mass spectrum	:	282 (M), 281 (M-1), 266, 238, 237, 236, 210, 201, 185, 183 (base peak), 158, 130, 122, 108 m/e.

-.S U M M A R Y.-

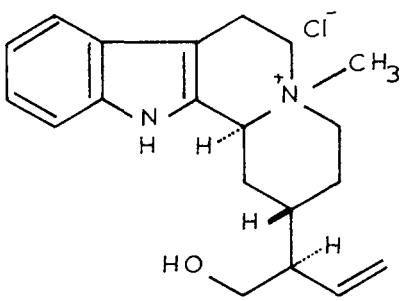
The object of this study was the isolation and elucidation of the structure of unknown quaternary alkaloids occurring in the stem and root bark of Hunteria eburnea Pichon.

We succeeded in isolating ten quaternary bases, the structure of five of which have been elucidated and makes the subject of this thesis. The isolation and separation of the alkaloids were extremely difficult and time consuming especially the chromatography on cellulose columns.

Hunterburnine α- and β-methochlorides have been easily identified according to their published physical constants since they have been reported to occur in different plants. We isolated a quaternary salt and for which we propose the structure as 21-methoxyhunterburnine methochloride.



R = CH_3 21-METHOXY
HUNTERBURNINE
METHOCHLORIDE

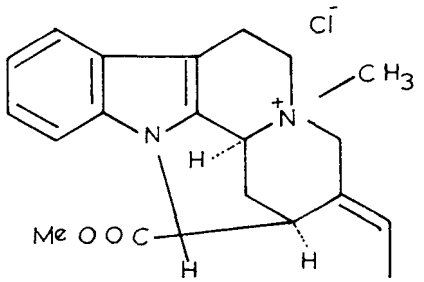


ANTIRHINE
METHOCHLORIDE

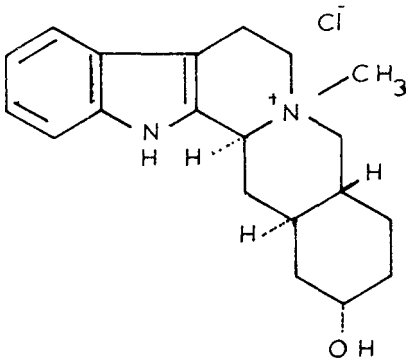
Another pair of quaternary alkaloids belonging to the same skeletal type as hunterburnine has been isolated. One being desoxyhunterburnine α-methochloride which we called antirhine α-methochloride, the second being its dihydro derivative occurring naturally and could be easily prepared from antirhine α-methochloride by hydrogenation. The striking feature in

this pair of bases is the α-configuration of the $\overset{+}{N}-CH_3$ groups. By demethylation and subsequent methylation of the resulting tertiary bases, only the β-isomers were obtained.

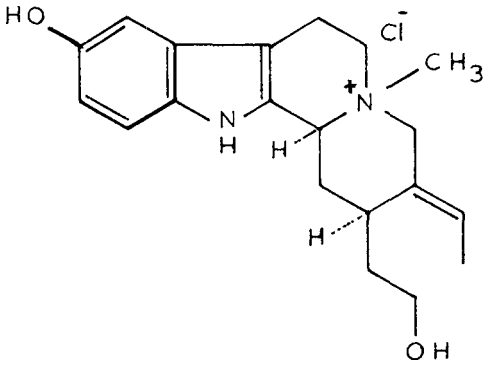
We isolated for the first time from this plant Pleiocarpamine methochloride and this is contrary to the proposal of W.I. Taylor that none of the quaternary alkaloids of Hunteria eburnea originates from the co-occurring tertiary base. Pleiocarpamine has previously been reported to occur in this plant. Yohimbol, Huntrabrine and Akuammicine methochlorides have been easily identified mainly by spectroscopic methods.



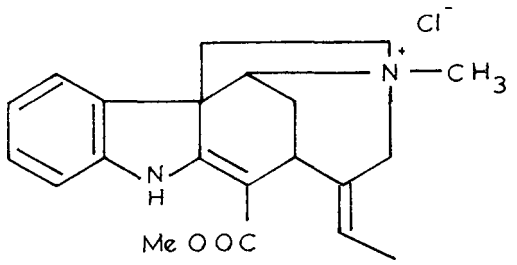
PLEIOCARPAMINE METHOCHLORIDE



YOHIMBOL METHOCHLORIDE

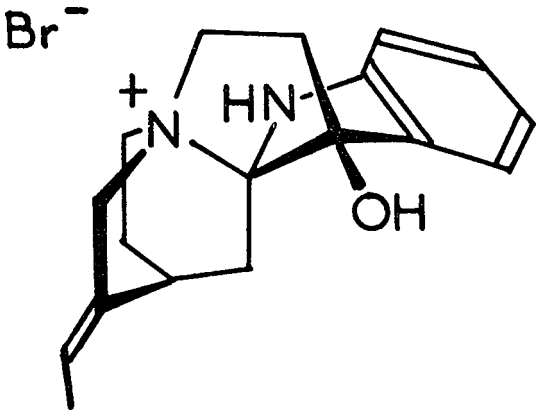


HUNTRABRINE METHOCHLORIDE

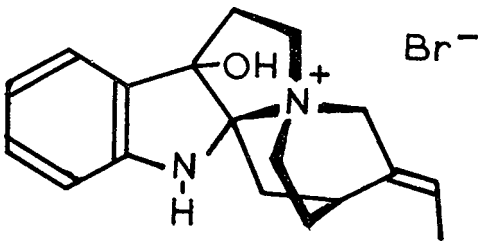


AKUAMMICINE METHOCHLORIDE

The final quaternary alkaloid was difficult to attack by the conventional chemical methods for elucidation of structure. We have proposed a structure which has been confirmed by X-ray analysis.



Hunteracine represents a new structural class where the quaternary nitrogen being at the junction of three rings does not bear an alkyl group.



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