STUDIES ON TERPENOIDS FROM *TECOMA STANS*

AND

EPERUA FALCATA

by

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PART I SUMMARY

The chemistry and biosynthesis of cyclopentanoid monoterpene alkaloids is reviewed. The isolation of pyrindane and perhydropyrindane alkaloids from several samples of *Tecoma stans* (Juss) and their separation by column and preparative vapour-phase chromatography is described. Four new alkaloids are characterised, two known alkaloids (tecomanine, boschniakine) are found and tentative structures are suggested for two more new alkaloids.

A new attempted synthesis of the pyrindane ring system (1), which is unsuccessful, is described. This involves a Diels-Alder reaction between an oxazole and a cyclopentanoid dienophile, cyclopentene-3:5-dione and both chemical and photochemical methods are used.

PART II SUMMARY

Part II of the thesis describes work carried out on products isolated from the exudate and wood of the Wallaba tree, *Eperua falcata*.

The acid component of the exudate is shown to be eperuic acid. The neutral component is separated by chromatography on an alumina column and the benzene fraction is shown to contain a mixture of involatile esters, the main component of which is thought to be eperuyl eperuate.

Separation of the methyl esters of the mixed acids from the branchwood is attempted by both preparative-layer and column chromatography but is unsuccessful.

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Part I

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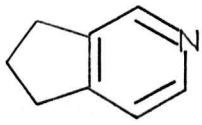
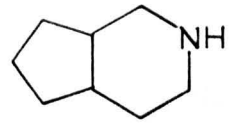
Part II

THE CHEMISTRY OF CYCLOPENTANOID MONOTERPENE ALKALOIDS

These alkaloids have structures which are based either on the pyrindane (1) or perhydropyrindane (2) ring system. These contain the cyclopentanoid monoterpene skeleton (3).[1]



(1)



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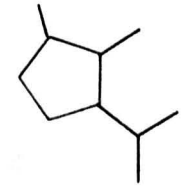
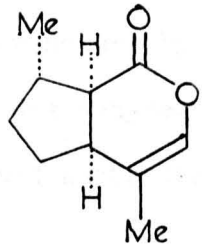
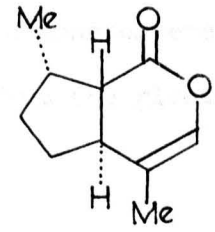


(3)

The structural unit (3) was first encountered in the nepetalactones (4) and (5) extracted from oil of catnip, a constituent of the plant Nepeta cataria.[2,3]



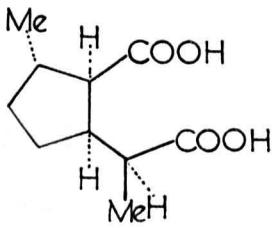
cis-trans nepetalactone (4)



trans-cis nepetalactone (5)

In the designation of configuration in these compounds, the

stereochemistry of the ring junction is given first and the relation of the methyl groups on the five-membered ring to the adjacent ring fusion is given second. The nepetalactones have been converted into four nepetalinic acids (6) - (9), which have been used as reference compounds in the correlation of the structure and stereochemistry of the cyclopentanoid monoterpene alkaloids.[20]



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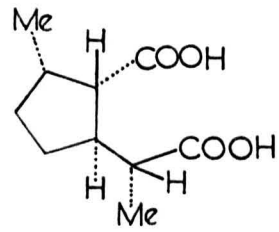

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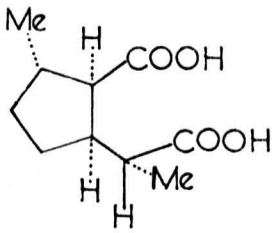

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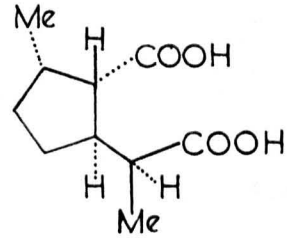
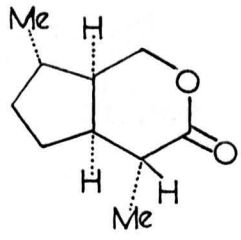


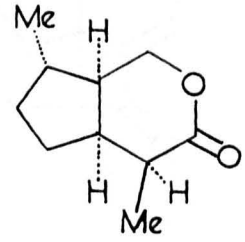
(9)

Other naturally occurring cyclopentanoid monoterpenes are the lactones and dialdehydes (10) - (13) extracted from the glands of Argentinian and Australian ants.



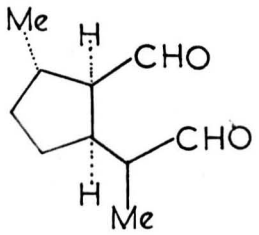
(10)

Iridomyrmecin[1,4,5]



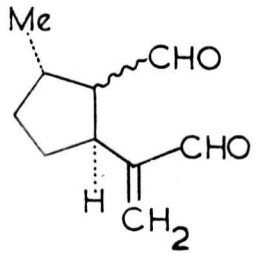
(11)

Isoiridomyrmecin[1,4,6]



(12)

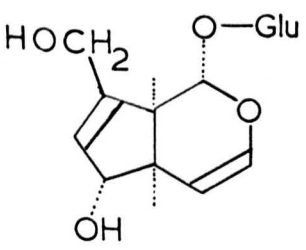
Iridodial[1]



(13)

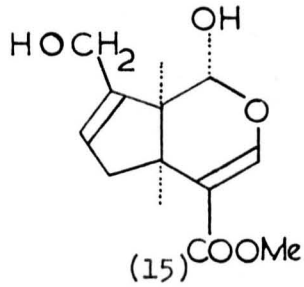
Dolichodial[1,7,8]

Also there are several plant glycosides and related natural products possessing the cyclopentanoid monoterpene structure, for example (14) - (18).[9]



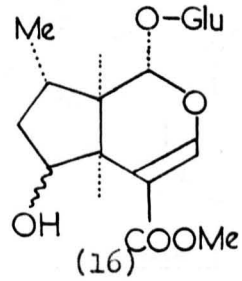
(14)

Aucubin[11]



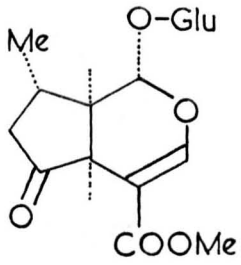
(15)

Genepin[10]



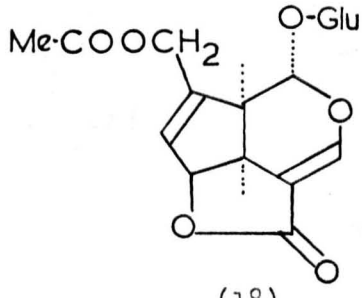
(16)

Loganin[12]



(17)

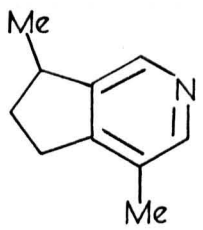
Verbenalin[13]



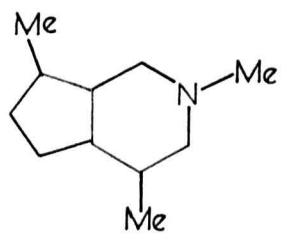
(18)

Asperuloside[14]

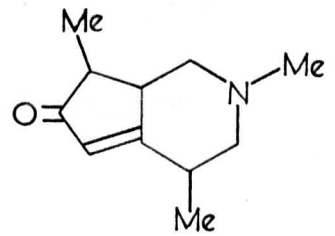
Up to the present time the structure of about a dozen cyclopentanoid monoterpene alkaloids has been reported in the literature. These are shown in figure 1. Their names derive mainly from the name of the plant from which they were isolated. It is intended to briefly review the structure determination and properties of these alkaloids and then deal in general terms with their biosynthesis. They have all been isolated from plants of the Apocynaceae and Bignoniaceae families.



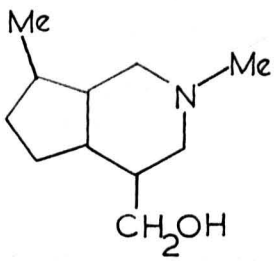
Actinidine



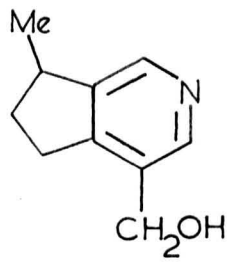
Skytanthine



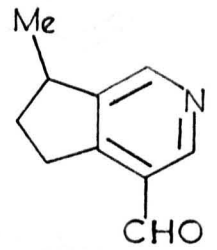
Tecomanine



Tecostanine

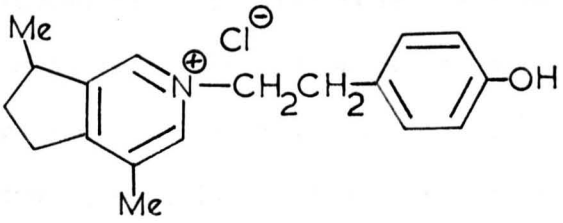


Tecostidine

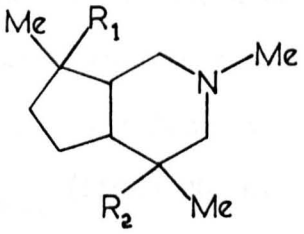


Boschniakine

Fig. I



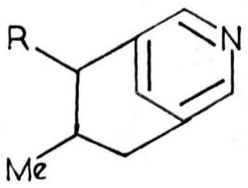
Principal Alkaloid from
Valeriana officinalis



HSI, R_1 = H, R_2 = OH

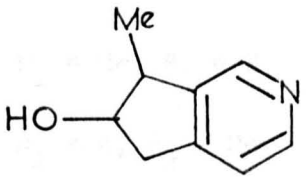
HSII, R_1 = OH, R_2 = H

Hydroxyskytanthine



R = CO_2H, Plantagonine

R = CHO, Indicaine

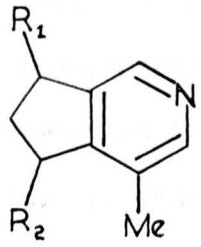


Alkaloid RW47

or

Venoterpene

Actinidine (19) was the first cyclopentanoid monoterpene alkaloid to be discovered.[15] It was isolated, together with a second compound, matatabilactone, from the leaves and gall of *Actinidia polygama* Miq. (Dilleniaceae), a Japanese plant known to be attractive to members of the Felidae (cat) animal family. Actinidine is a liquid, b.p. 100 - 103°/9 mm., $[\alpha]_D^{11}$ - 7.2° (C, 17.54, $CHCl_3$) and forms a picrate, m.p. 143°. Its ultraviolet spectrum, $\lambda_{max.}$ (EtOH) 262 mμ (ε 2400), and its infrared absorption at 1587 cm^{-1} (C=N) and the violet colour produced with 2:4-dinitrochlorobenzene and alkali suggested it was a pyridine derivative. By comparison of the permanganate oxidation product of actinidine with known pyridine carboxylic acids, two possible structures (19) and (20) were deduced for the alkaloid.

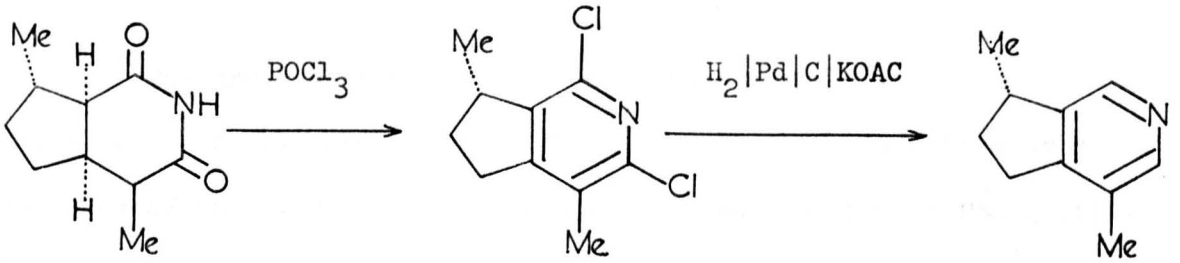


(19) R_1 = Me, R_2 = H

(20) R_1 = H, R_2 = Me

Structure (19) was considered to be the more likely as it has the same skeleton as nepetalactone. Also, matatabilactone, isolated with actinidine, was shown to be a mixture of iridomyrmecin (10) and

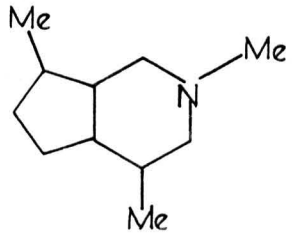
isoiridomyrmecin (11), and both of these structures support structure (19) as being the correct one for actinidine. Confirmation was obtained by synthesis of actinidine from nepetalinic acid imide (21) as shown below.



(21)

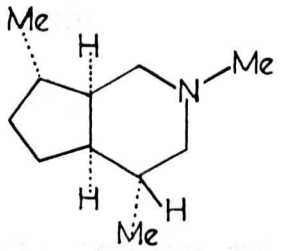
The product was identical with natural actinidine. Two further syntheses of actinidine have been published.[16,17]

A second cyclopentanoid monoterpene alkaloid, skytanthine, was isolated from the stems and leaves of *Skytanthus acutus* (Meyen), a Chilean member of the Apocynaceae. Its structure was determined by two groups of workers, Djerassi et al. in America[18] and Casinovi et al. in Italy.[19] The crude alkaloid is a liquid, b.p. 54°/1.5 mm., $[\alpha]_D$ + 42° ($CHCl_3$) with empirical formula $C_{11}H_{21}N$. Its structure (22) was determined on the basis of Hofmann degradation and nuclear magnetic resonance studies on the parent compound and its dehydrogenation product. The latter was shown to be identical to racemic actinidine by a comparison of the melting-points and infrared spectra of the respective picrates.



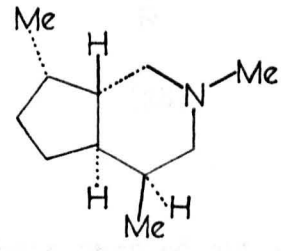
(22)

The crude alkaloid was shown to be a mixture of three diastereoisomers by vapour phase chromatography (VPC) and these were separated by thin-layer chromatography (TLC) and column chromatography. Their stereochemical structures have been correlated with the known nepetalinic acids (6) - (9).[20] The acids were converted to their diols by lithium aluminium hydride reduction and the bis-tosylates of the diols heated under pressure with methylamine gave the skytanthines. The configurations of the four skytanthines produced are shown below (23) - (26).



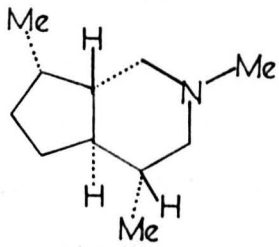
(23)

α-Skytanthine
Picrate m.p. 120°



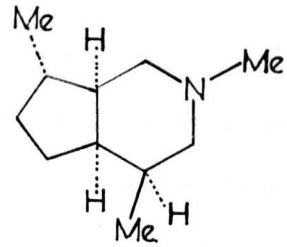
(24)

β-Skytanthine
Picrate m.p. 135°



(25)

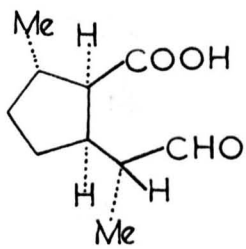
γ-Skytanthine
Picrate m.p. 162°



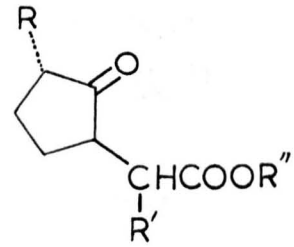
(26)

δ-Skytanthine
Picrate m.p. 139°

α-Skytanthine (23) has also been prepared from a nepetalic acid (27) and δ-skytanthine (26) has been prepared from iridomyrmecin (10) and from a nepetalic acid.[21]



(27)

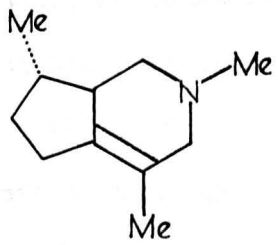


(28)

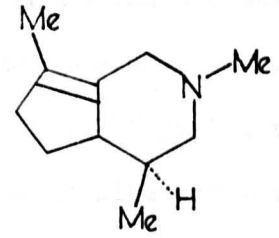
Recently, Volodina and co-workers[22] have carried out stereospecific syntheses of several perhydropyridines, including skytanthine, starting from cyclopentane oxo-esters (28). The product corresponding to skytanthine was found to be a mixture of two diastereoisomers which were identical with

β- and γ-skytanthines.

It was found by Casinovi et al.[23] that crude skytanthine contained approximately 60 - 65% of β-skytanthine with only trace amounts of α- and δ-skytanthines. The remainder of the volatile basic fraction was a mixture of two compounds only one of which formed a picrate, m.p. 127° (ca. 20% of total crude material). The free base was shown to be unsaturated and n.m.r. studies and VPC comparison of its reduction products with known skytanthines suggested it was derived from δ-skytanthine (26) implying it had structure (29) or (30), i.e. that of a dehydroskytanthine.[24]



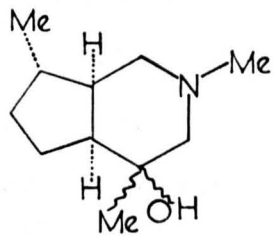
(29)



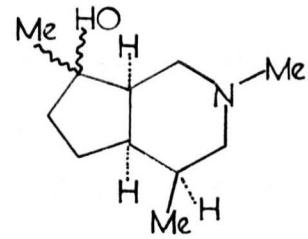
(30)

The non-volatile fraction of the basic extract from *Skytanthus acutus* yielded a crystalline alkaloid, m.p. 93°. This was shown to be an alcohol which on dehydration with thionyl chloride gave a dehydroskytanthine identical to that described above. This suggests two possible structures

(31) or (32) for the alkaloid.[24]



(31)



(32)

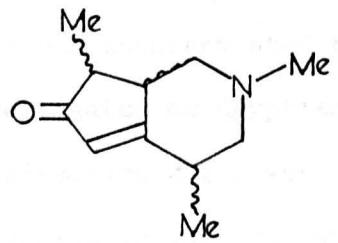
Later, Adolphen et al.[25] succeeded in isolating and separating both of these alcohols from a sample of *Skytanthus acutus*. Hydroxyskytanthine I (HSI) (31), m.p. 94-5°, obtained in 0.005% yield, was assumed to be the same as that above and also the same as an alkaloid D obtained by Appel and Müller from the same plant.[26] The isomeric hydroxyskytanthine II (HSII) (32), m.p. 119 - 120°, was obtained in 0.001% yield. The alkaloids were isolated by chloroform extraction after the greater part of the skytanthines had been removed by steam distillation and were separated by chromatography on alumina. Their structure and stereochemistry was determined largely by a detailed study of their n.m.r. spectra and also from mass spectral evidence.

A rich source of cyclopentanoid monoterpene alkaloids is the *Tecoma stans* (Juss) plant, a member of the Bignoniaceae family. The Tecoma species have for a long time been a subject of botanical, chemical

and pharmacological interest.[27-33] The leaves of *Tecoma mollis* (Juss) have been used by Mexican natives as an anti-diabetic drug and it is claimed that they contain alkaloidal material.[34] However, there is some doubt as to the validity of this claim.[35] *Tecoma stans* (Juss) is a shrub up to 4 ft. in height and it occurs in South and Central America, the Southern United States and in Egypt.

Boorsma first reported the presence of alkaloids in *Tecoma stans* (Juss) in 1899[36] and in 1959 Hammouda and Motawi isolated two alkaloids from the plant.[37] One of these, which they named Tecomine, was a liquid base having the following properties: it formed a methiodide, m.p. 265°, $[\alpha]_D^{23}$ - 20 ± 2, corresponding to the molecular formula $C_{12}H_{20}ONI$. It showed carbonyl stretching in its infrared spectrum at 1670 cm^{-1} (nujol) and formed a 2:4-dinitrophenylhydrazone, m.p. 260° and a picrate, m.p. 154°. The second alkaloid was a crystalline solid, m.p. 275°, $[\alpha]_D^{23}$ + 50 ± 2.

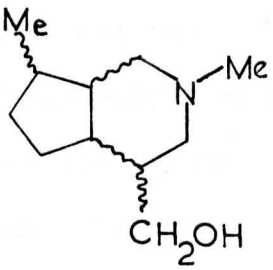
The *Tecoma stans* (Juss) plant was re-examined in 1961 by Jones, Fales and Wildman.[38] They obtained an alkaloid which had similar properties to tecomine, described above. They showed it to have structure (33) and called it tecomanine.



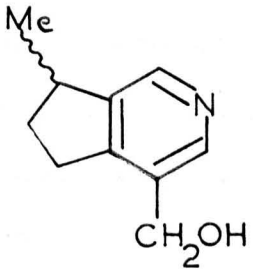
(33)

Tecomanine is a colourless unstable liquid, b.p. 125°/0.1 mm., $[\alpha]_D^{24}$ - 175° (C, 1.17, $CHCl_3$), $\lambda_{max.}$ (EtOH) 226 mμ (log ε 4.10), $\lambda_{max.}$ (acidic EtOH) 223 mμ (log ε 4.13). Its infrared spectrum shows carbonyl stretching at 1700 cm^{-1} and double-bond stretching at 1620 cm^{-1}, typical of an α,β-unsaturated cyclopentenone. Its structure was deduced on the basis of n.m.r. studies and reduction to a dihydrotecomanine. Further evidence was obtained by reduction of the alkaloid with platinum oxide/acetic acid to a mixture of saturated ketones followed by Huang-Minlon reduction of the mixture and dehydrogenation. A pyridine was isolated and identified as dl-actinidine (19)[15,18] by comparison of its picrate, m.p. 138 - 140° with the literature value, 139 - 140°.[15] The product was also shown to be identical in U.V. and i.r. spectra as well as VPC retention time with an authentic sample of dl-actinidine. Huang-Minlon reduction of dihydrotecomanine gave a skytanthine whose picrate, m.p. 152-3°, was different from the picrates of the four isomers of skytanthine synthesised by Eisenbraun.[20] Therefore, this work did not allow the complete stereochemistry of Tecomanine to be elucidated.

Up to the present time the isolation of two more alkaloids from *Tecoma stans* (Juss) has been reported. A short while after its publication, the structure of tecomanine was substantiated by the findings of Hammouda and Le Men.[39,40] They investigated an Egyptian sample of *Tecoma stans* (Juss) and obtained three alkaloids which were separated by TLC. One of these was identical with tecomanine and the other two were shown to have the structures (34) and (35) and named tecostanine[39a] and tecostidine,[40a] respectively.



(34)



(35)

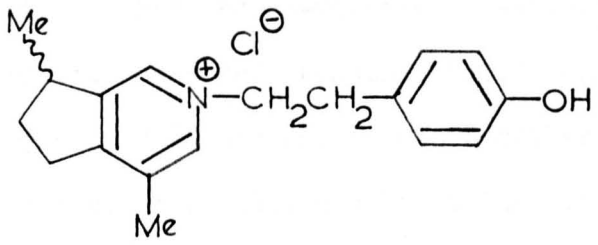
Tecostanine is a solid, m.p. 82°, $[\alpha]_D^{20}$ 0 ± 2° (MeOH). It has i.r. absorption at 3180 cm^{-1} and forms an acetate. Its U.V. spectrum is transparent above 215 mμ and its n.m.r. spectrum indicated the presence of -N-Me, -HC-CH_3, OH and -CH-CH_2OH groups. The tosylate was reduced with lithium aluminium hydride to a skytanthine which formed a picrate, m.p. 145°. This indicates it is not one of the known isomers of skytanthine. Further evidence for the structure was provided by the mass spectra of tecostanine and its degradation products. Investigations by Hammouda and Amer[39b] have shown that tecomanine citrate and tecostanine hydrochloride can be used effectively as anti-diabetic drugs.

Tecostidine is optically active, $[\alpha]_D^{22}$ - 4° (C, 1.221, $CHCl_3$) and forms a picrate, m.p. 152-3°. Its U.V. spectrum corresponds to a pyridine derivative, $\lambda_{max.}$ (EtOH) 262 mμ ($\log_{10}$ ε 3.27), 270 mμ ($\log_{10}$ ε 3.21) and shows a hypsochromic shift and an increase in intensity on acidification. The main bands in the i.r. spectrum are at 3400 and 3200 cm^{-1}, indicative of a hydroxyl group. This evidence, supported by its n.m.r. spectrum and that of its deuterated derivative, suggested (35) as

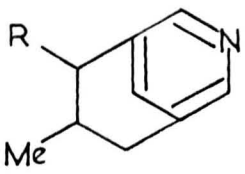
the structure of tecostidine.

Cavill and Zeitlin[40] have synthesised D(+)-tecostidine and shown natural tecostidine to be an enantiomer of this, i.e. D(-)-hydroxyactinidine, thus defining the absolute configuration of the natural product. Attempts to synthesise tecostanine (or its enantiomer) from tecostidine by reduction of the pyridine ring lead to the formation of a mixture of stereoisomers. Also, attempts to reduce 8-hydroxyactinidine to hydroxyskytanthine II (31) were unsuccessful.

Torssel and Wahlberg have reported the isolation[41] of an alkaloid having structure (36) from the dried roots of *Valeriana officinalis*. They isolated it as its chloride. It forms a picrate, m.p. 152° and a trifluoroacetate, m.p. 203°. Structure determination was based on exhaustive spectral data. When pyrolysed it gave a mixture from which it was possible to isolate actinidine (19).



(36)

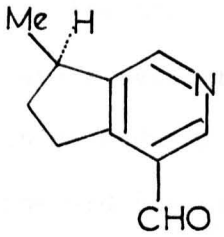


(37) R = $-CO_2H$

(38) R = -CHO

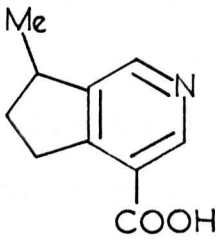
The rather unlikely structures (37) and (38) have been assigned to two alkaloids, named plantagonine (37) and indicaine (38) isolated from *Pedicularis olgae*.[42] They were first isolated in 1952 from *Plantago indica*[43] and again in 1956 from *Plantago ramosa*[44] but no structures were put forward on these occasions.

An alkaloid having the structure (39) was isolated by Sakan and Murai in 1963.[45] No details of isolation or structure determination were given at this time.

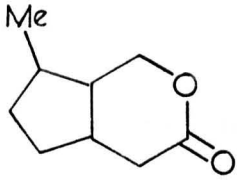


(39)

Later, Sakan and co-workers[46] re-isolated this alkaloid from a Japanese plant, *Boshniakia rossica* (Hult) found on the slopes of Mount Fuji and they called it boschniakine. Together with boschniakine they isolated boschnialactone (41) and boschniakinic acid (40) from the same source.



(40)



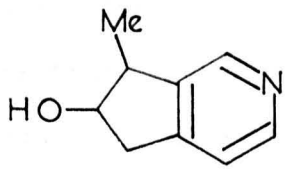
(41)

Boschniakine (39) is a fragant liquid, b.p. 80 - 90°/3 mm., $[\alpha]_D$ + 21.02°, corresponding to the molecular formula $C_{10}H_{11}NO$. It forms a picrate, m.p. 126.5 - 128° and a semicarbazone, m.p. 227 - 228° (dec.). The base obtained by Huang-Minlon reduction and its picrate had the same infrared spectrum as actinidine and its picrate. The U.V. spectrum, $\lambda_{max.}$ (EtOH) 239, 268, 282 mμ and i.r. absorption maxima (3050, 2725, 1700 and 1580 cm^{-1}) both suggest the presence of an aldehyde group conjugated with a pyridine ring. This indicated that boschniakine probably had structure (39) and this was confirmed by synthesis.

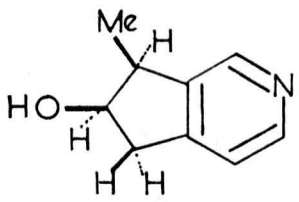
It is interesting that the pyrindane and perhydropyrindane bases reported previous to boschniakine have the C-8 methyl group taking an S-configuration while in boschniakine it has the reverse configuration.

Boschniakinic acid (40), a solid, m.p. 215 - 220° (dec.) was obtained from the high-boiling fraction of the basic extract by the distillation of boschniakine. Its structure was deduced from its n.m.r. spectrum and confirmed by the oxidation of boschniakine with silver oxide. It seems to be an auto-oxidation product of boschniakine. Both boschniakine and boschnialactone have a marked physiological action on the cat, similar to the constituents of *Actinidia polygama*.

Three independent sources have reported the isolation of the cyclopentanoid monoterpene alkaloid having the structure (42). It has been isolated from the mother liquors remaining from the isolation of echitamine and called scholarine.[47]



(42)



(43)

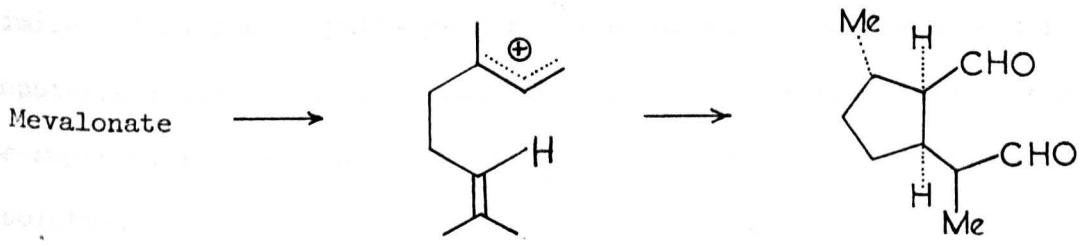
An examination of the alkaloids of *Rauwolfia verticillata* (Lour) of Hong Kong led to the identification of the known alkaloids yohimbine, δ-yohimbine, reserpine, serpentine and ajmaline, and to the isolation of three alkaloids of unknown constitution. Arthur and co-workers[48] have shown that one of the latter alkaloids obtainable only from the wood of the plant is monoterpene and has the structure (42). This alkaloid, which they designated RW47 has, m.p. 130 - 132°, $[\alpha]_D$ + 27 ($CHCl_3$) and a molecular formula $C_9H_{11}NO$. On the basis of further evidence provided by n.m.r. spectral studies this structure was confirmed and the alkaloid was shown to have the stereochemical structure (43) in which the C-8 methyl group has the same configuration as it does in boschniakine (39). It is noteworthy that *Rauwolfia* like *Skytanthus acutus* (Meyen), the source of skytanthine, belongs to the family Apocynaceae.

More recently, workers in India have isolated a similar alkaloid to RW47 from the mature fruits of *Alstonia venenata* R.Br. which they have named venoterpine,[49] and to which they have assigned structure (42). Venoterpine, m.p. 128 - 130° has a molecular formula $C_9H_{11}NO$, $\lambda_{max.}$ (EtOH) 259 mμ (log ε 3.50). Like RW47 it is sensitive to light and air and turns purple on keeping. The structure (42) was deduced from its n.m.r. spectrum. It is pointed out by the authors that although many of the properties of venoterpine are quite similar to those of alkaloid RW47, the n.m.r. spectrum of their alkaloid differs from that of RW47 in the $-\overset{|}{C}HOH$ region. This leads them to the conclusion that venoterpine is possibly a stereoisomer of RW47.

BIOSYNTHESIS OF CYCLOPENTANOID MONOTERPENE ALKALOIDS

Most of the early schemes put forward for the biosynthesis of the cyclopentanoid monoterpenes involved iridodial (12) or similar compounds as an intermediate.[50,51] It seems reasonable to assume that iridodial is derived from the cyclisation of two isoprene units with oxidation-reduction occurring prior to or after the cyclisation (fig. II).

Figure II



Iridodial is thought also to be the precursor of the nepetalactones and related compounds such as the plant glycosides.[2,52,53] The cyclopentanoid monoterpene alkaloids could arise from the reaction of a precursor such as iridodial with ammonia or its equivalent. For example, skytanthine can be derived from an 'iridodial' type precursor through reaction with the biological equivalent of methylamine.[54] They might also be produced at a later stage in the biosynthesis of cyclopentanoid

monoterpenes possibly from lactonic or glycosidic compounds.

The only perhydropyrindane alkaloid which has had its biosynthesis closely studied is skytanthine (22). Studies with labelled precursors have shown that the biosynthesis of the alkaloid occurs by the terpene route.[55] *Skytanthus acutus* seeds were incubated with $^{14}CH_3.CO_2.Na$, $^{14}Ph.CH_2.CH(NH_2).CO_2H$ and mevalonic-2-^{14}C acid for 14 days. The latter compound showed a much higher incorporation (80 times as great) into the alkaloid fraction of the plantlets than the other two compounds.

Skytanthus acutus has been shown to also contain indole alkaloids[56] and this led to the speculation that both types of alkaloid derive from a common precursor. Some more circumstantial evidence[49] for similar biosynthetic pathways for indole alkaloids and cyclopentanoid monoterpene alkaloids is provided by their co-existence in *Alstonia venenata* R.Br, the plant from which venoterpene, described above, was isolated.

Evidence that the indole alkaloids[57] also derive from isoprenoid precursors has been obtained by two groups of workers[58,59] who have shown that 2-^{14}C-mevalonate is incorporated with the indole alkaloids, Vindoline and Reserpinine.

Recent work by Battersby and co-workers[60] has confirmed their earlier experiments[61,62] on the biosynthesis of indole alkaloids. They have rigorously established that loganin (16) is a key intermediate in the biosynthesis of some indole alkaloids. This work therefore supports the theory that glycosides such as loganin and also other lactonic compounds

such as (14) - (18) may be intermediates in the biosynthesis of cyclopentanoid monoterpene alkaloids since the latter often occur in the same plant as the indole alkaloids.

The same workers[63] have also established the biological derivation of loganin from a terpene precursor, geraniol, by feeding (1 - ^{3}H) geraniol to the *Menyanthes trifoliate* plant and isolating radioactive loganin.

Thus, from these experiments, the complete biosynthetic pathway from mevalonate through to the indole alkaloids has been established and it seems reasonable to assume that a similar pathway exists for the cyclopentanoid monoterpene alkaloids. Confirmation of this will require extensive studies of feeding experiments with the plants from which these latter alkaloids have been isolated.

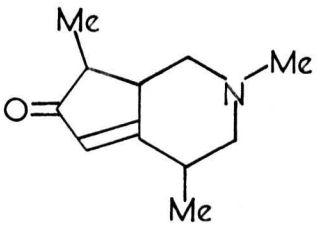
DISCUSSION

Investigation of the Alkaloid Content of *Tecoma stans* (Juss).

During the course of this work samples of *Tecoma stans* (Juss) from three countries, Cuba, Florida (twice) and Mexico, were examined. It was of interest to compare the alkaloids extracted from each sample and, with the Mexico sample, to compare the alkaloid content of the root with that of the leaves of the plant. The results of this investigation showed that there was a considerable variation in alkaloid content with the place of origin of the plant and, in the case of the Florida samples, with the time of the year at which the plant was collected. Only cyclopentanoid monoterpene alkaloids were isolated from the available samples and Table 2 at the end of this section (p. 59) shows the distribution of alkaloids which was found.

Examination of the Cuban Sample of *Tecoma stans* (Juss).

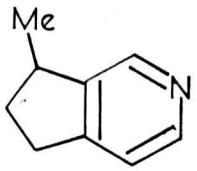
The extraction of the whole plant was performed as described in the experimental section, using ethanol as the extraction solvent. VPC analysis of the crude alkaloid mixture showed that there were three components present. Most of the main component (called alkaloid B) was removed as its picrate formed by addition of ethanolic picric acid to the mixture. Alkaloid B has been investigated by Jones, Fales and Wildman[38] who called it tecomanine and gave it structure (44) (see introduction, p. 12).



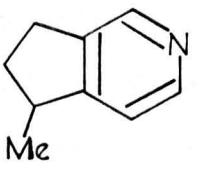
(44)

The residue, after removal of tecomanine picrate, was separated by column chromatography on Florisil, using benzene and ethyl acetate as eluting solvents. Two quite pure alkaloids were obtained from the column (called alkaloids A and C).

Alkaloid A, the first to be eluted (by benzene) was a liquid with a pungent odour and it was purified through picrate formation. The picrate, m.p. 116 - 117°, analysed correctly for a molecular formula of $C_9H_{11}N$ for the free base. The mass spectrum of the alkaloid gave a molecular weight of 133 which was in agreement with this formula. The U.V. spectrum of alkaloid A had absorptions at 259.5 and 267 mμ (with a doubling of intensity on acidification) which were close to those of a 3:4-dialkyl pyridine.[64] Support for this was provided by the n.m.r. spectrum of alkaloid A picrate. This showed evidence of pyridine protons with signals at τ2.02 (doublet, β-pyridine proton), 1.25 (singlet, α-pyridine proton) and 1.20 (doublet, α-pyridine proton). This pattern also suggested a 3:4-disubstituted pyridine. The other significant signal in the spectrum was a methyl doublet at τ8.4 assignable to a CH_3-$\overset{|}{C}H$- group and also there were groups of multiplets at τ6.8 - 6.2 (three protons), 7.5 - 7.0 (one proton) and 8.0 - 7.6 (one proton). This data indicated one of two possible structures (45) or (46) for alkaloid A.



(45)



(46)

Structure (45) was preferred because of its relation to cyclopentanoid monoterpene alkaloids previously reported, such as actinidine (19). Hence, alkaloid A is a 4-noractinidine. The structure was confirmed when a mixed melting-point of alkaloid A picrate with a sample of 8-epi-4-noractinidine picrate from asperuloside supplied by Professor L.H. Briggs showed no depression.

Alkaloid A (45) completes an interesting oxidation sequence in the cyclopentanoid monoterpene group of alkaloids starting from actinidine, through tecostidine, boschniakine, boschniakinic acid to alkaloid A, (figure III).

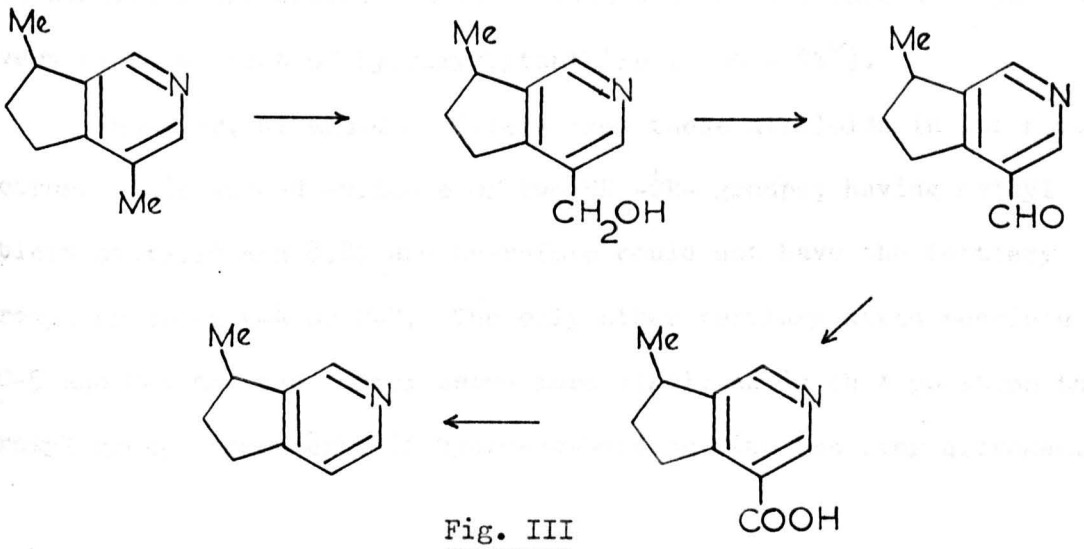


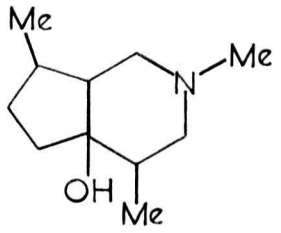
Fig. III

This suggests that the alkaloids may arise in the plant by such oxidation steps starting from actinidine which is itself produced from skytanthine (22) or N-norskytanthine (48) by dehydrogenation, i.e., oxidation. The alkaloids present in any one sample of the plant would then depend on the oxidative processes occurring within the plant.

The third alkaloid eluted from the Florisil column, alkaloid C, also formed a crystalline picrate, m.p. 168 - 170°, which gave analytical figures corresponding to a molecular formula of $C_{11}H_{21}NO$ for the free base. This formula was confirmed by the mass spectrum of alkaloid C which had a molecular ion, $M^+ = 183$. The free base, regenerated from its picrate was a solid which was purified by sublimation to give white crystals, m.p. 91 - 92°. Its ultraviolet spectrum was transparent above 220 mμ and the main feature of the infrared spectrum was a tertiary alcohol hydroxyl stretching at 3609 cm^{-1}. From its molecular formula it can be seen that alkaloid C is isomeric with tecostanine from *Tecoma stans*[39a] and the hydroxyskytanthines from *Skytanthus acutus*.[25] Like alkaloid C these three alkaloids are solids and the melting-point of alkaloid C (91 - 92°) is very close to that of hydroxyskytanthine I (94 - 95°).

However, alkaloid C differs from these alkaloids in its n.m.r. spectrum. This showed evidence of two CH_3-$\overset{|}{C}H$- groups, having methyl doublets at τ9.10 and 8.85 and therefore could not have the tertiary hydroxyl group on C-4 or C-8. The only other tertiary sites possible are at C-5 and C-9 and the former seems more likely as in this position the hydroxyl group cannot exhibit hydrogen-bonding with the ring nitrogen.

This is in agreement with the infrared spectrum which shows no evidence of hydrogen-bonding. An axial hydroxyl group on C-9 could readily hydrogen-bond with the piperidine nitrogen as in 3-piperidinols.[65] Thus, the structure (47) is suggested for alkaloid C.



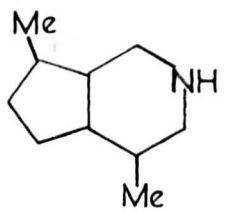
(47)

The structure of alkaloid C, including its stereochemistry is discussed further on page 35.

Examination of the Florida (1) Sample of *Tecoma stans* (Juss).

VPC analysis of the crude alkaloid extract showed it to contain three main components. By comparison of the retention times of these peaks with that of authentic tecomanine it was shown that this alkaloid was present in the mixture. Addition of ethanolic picric acid to the mixture leads to the formation of a picrate, called A_1 picrate. This was removed, the solution diluted and more picric acid added, when a further picrate was precipitated. This was shown to be tecomanine picrate.

Crude alkaloid A_1 picrate was purified by recrystallisation from methanol to give yellow crystals, m.p. 179 - 180° and analytical figures which did not differentiate rigorously between the formulae $C_{10}H_{19}N$ and $C_{10}H_{17}N$ for the free base. The mass spectrum gave a molecular weight of 153 for the free base, corresponding to the formula $C_{10}H_{19}N$. The infrared spectrum of alkaloid A_1 showed NH stretching in the region 3600 - 3150 cm^{-1} and the presence in its n.m.r. spectrum of two methyl doublets at τ9.15 and 9.03 suggested two CH_3-$\overset{|}{C}$H- groups in the molecule. Also significant was the absence of a signal corresponding to an -$\overset{|}{N}$-CH_3 group. On the basis of this spectral evidence, a possible structure for alkaloid A_1 is (48), i.e., it is an N-norskytanthine.



(48)

Alkaloid A_1 was treated with benzoyl chloride/pyridine and formed a liquid N-benzoyl derivative. The n.m.r. spectrum of the product showed that the benzoylation had taken place on nitrogen and therefore supported the assignment of an N-H group in the free base. This was confirmed by the absence of NH stretching absorption and the presence of carbonyl stretching absorption ($\nu_{max.}$: 1615 cm^{-1}) in the infrared spectrum of the N-benzoyl compound. An attempt was made to purify the crude product by distillation from a bulb-tube in the hope of obtaining an analytical sample but the distillate failed to analyse correctly for $C_{17}H_{23}NO$.

To obtain further evidence for the structure proposed for alkaloid A_1 and also to investigate its stereochemistry it was methylated by the method of Clarke, Gillespie and Weisshaus,[66] using formaldehyde and formic acid (figure IV). It was expected that the product, a skytanthine, could be converted to its picrate and this, by comparison with the known skytanthine picrates, would provide information on its stereochemistry and, therefore, also on the stereochemistry of alkaloid A_1.

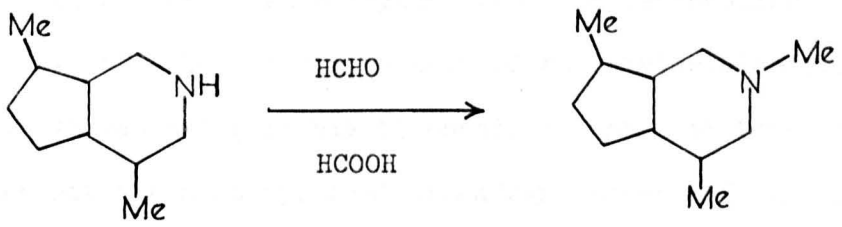


Fig. IV

The n.m.r. spectrum of the product of this reaction had a methyl singlet at τ7.73, assignable to the $-\overset{|}{N}-CH_3$ group in skytanthine, showing that the methylation had taken place. The product formed a picrate, m.p. 139 - 142°, after recrystallisation from methanol, which analysed correctly for skytanthine picrate. The free skytanthine, recovered from its purified picrate, was distilled from a bulb-tube and the colourless distillate gave analytical figures that were in agreement with the formula $C_{11}H_{21}N$.

Of the four skytanthine picrates of known stereochemistry (23) - (26) synthesised by Eisenbraun,[20] only one, δ-skytanthine picrate, has a melting-point (139°) close to that of the skytanthine picrate derived from alkaloid A_1. Since the stereochemistry of δ-skytanthine has been established by synthesis[20] identification of the present skytanthine with δ-skytanthine would have established the stereochemistry of the former. However, admixture of the picrate of the skytanthine from alkaloid A_1 with an authentic sample of δ-skytanthine picrate supplied by Dr. Casinovi produced a depression in melting-point of the latter showing that the present skytanthine is not the δ-isomer.

Le Men has obtained a skytanthine from Tecostanine[39a] which forms a picrate, m.p. 142 - 143° close to that of the skytanthine picrate from alkaloid A_1. It was not possible to obtain a sample of this for a mixed melting-point but the mass spectral breakdown pattern of our derivative was very similar to that of the skytanthine derived from tecostanine.

Unfortunately this does not give us any information on the stereochemistry of alkaloid A_1 since the stereochemistry of tecostanine is not established.

Further confirmation of the proposed structure for alkaloid A_1 was provided by its behaviour on dehydrogenation with palladium/charcoal[18b] (figure V). The product was, as would be expected, actinidine (19). This was contaminated by unchanged alkaloid A_1 which was removed by acetylation of the mixture when alkaloid A_1 formed a neutral N-acetyl derivative from which actinidine was separated by acid extraction.

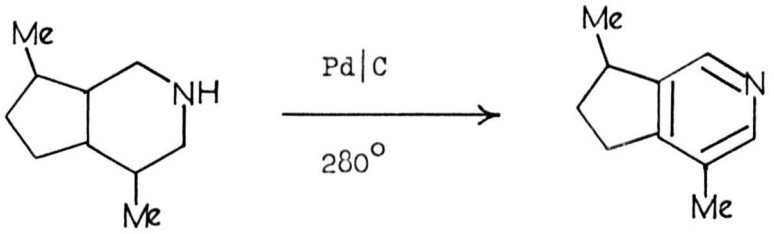


Fig. V

The n.m.r. spectrum of the product (of purity 95%) was almost identical to that of racemic actinidine (picrate m.p. 139 - 140°) obtained by Djerassi et al. by dehydrogenation of skytanthine[18b] and also that of synthetic D(+)-actinidine prepared by Cavill and Zeitlin.[40b] It had a methyl singlet at τ7.78 and a methyl doublet at τ8.65 assignable to the C-4 methyl group on the pyridine ring and the C-8 methyl group

respectively. The quartet at τ6.7 could be attributed to the methine proton on C-8 coupling with the adjacent methyl group and the broad peak centred at τ1.75 could be interpreted as two overlapping singlets due to the α and α' protons on the pyridine ring. The two reference spectra mentioned differed only in that the two pyridine proton signals occurred as distinct singlets at τ1.81 and 1.74.

The actinidine from alkaloid A_1 formed a picrate, m.p. 144 - 146°, which correctly analysed for the formula: $C_{10}H_{13}N.C_6H_3N_3O_7.H_2O$. Casinovi obtained an optically inactive actinidine by dehydrogenation of skytanthine which gave a picrate, m.p. 143 - 148° and Sakan subjected boschniakine to Huang-Minlon reduction to produce an actinidine with a picrate, m.p. 143 - 144°.

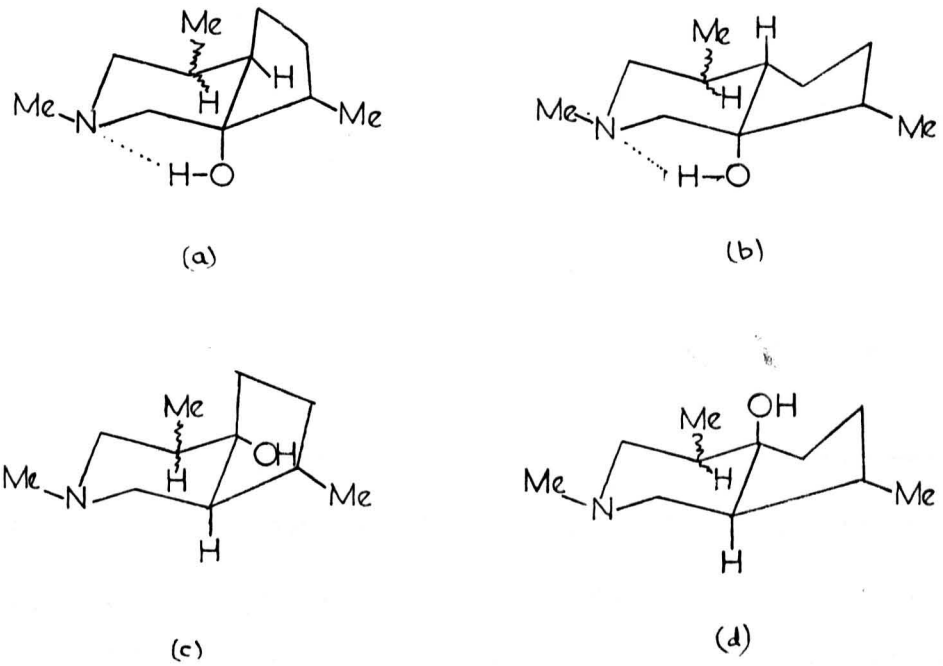
The basic residue, after removal of most of the tecomanine and alkaloid A_1 as picrates, was shown by VPC to contain predominantly one component although small amounts of tecomanine and alkaloid A_1 were still present. A small sample of the residue was treated with benzoyl chloride/sodium hydroxide/acetone to form the benzoyl derivative of alkaloid A_1, so that the last traces of this alkaloid could be removed from the mixture by acid extraction. However, the benzoylation was incomplete and the recovery of the other alkaloids was rather poor. To try and improve the reaction a modified procedure was tried on a larger sample of the basic residue. It was treated with benzoyl chloride/sodium hydroxide/dimethoxyethane and this time all of alkaloid A_1 underwent benzoylation and was removed from the mixture. The mixture was then chromatographed on an alumina column and eluted with benzene-ethyl acetate mixtures. VPC analysis of the fractions collected indicated that the main component of the mixture had been eluted by 20 - 50% ethyl acetate in benzene and was of quite high purity. By comparison of its retention time with that of an authentic sample it appeared that this alkaloid was alkaloid C (47) (isolated previously from the Cuban sample of the plant). This was confirmed by its n.m.r. and infrared spectra which were identical to those obtained for alkaloid C. Also, this alkaloid, like alkaloid C, turned purple on exposure to light and air, even in solution. However, unlike the previous sample which was a solid, m.p. 91 - 92°, this alkaloid was a liquid. This may have been due to the fact that the present sample of alkaloid C was only 90% pure. Later fractions from the

column contained an ester or ketone along with alkaloid C (C=O, 1735 cm^{-1}) which was not removed by attempted D.N.P. formation. The crude alkaloid C from the column was converted into its picrate, m.p. 170 - 172° but there was insufficient of this after recrystallisation to give satisfactory analytical figures.

A larger sample of alkaloid C was required for enquiry into its stereochemistry. Dehydration, followed by reduction of alkaloid C, should give a skytanthine which could then be compared with the skytanthines of known stereochemistry.[20] Accordingly, the remainder of the basic mixture was benzoylated to remove alkaloid A_1 using the same conditions as were used for the smaller sample. Unfortunately, on this occasion the conditions were too drastic. The benzoylated mixture was chromatographed on Florisil and it was found that most of the benzene fractions from the column contained a compound which showed carbonyl stretching at 1715 cm^{-1} and no O-H stretching in its infrared spectrum which suggested that alkaloid C had benzoylated on oxygen to form alkaloid C benzoate. The n.m.r. spectrum confirmed this, being very similar to that of alkaloid C except for the signals due to the benzoyl phenyl group at τ2.6 - 2.2 (3H multiplet) and τ1.9 - 1.6 (2H multiplet). The benzoate had a low retention time on VPC analysis and this uggested that it eliminated benzoic acid in the injection heater (temperature = 220°) to give an unsaturated skytanthine which would have a relatively low retention time.

Four possible stereochemical structures can be written for alkaloid C (figure VI, (a) - (d)), two cis-fused, (a) and (c), and two trans-fused, (b) and (d).

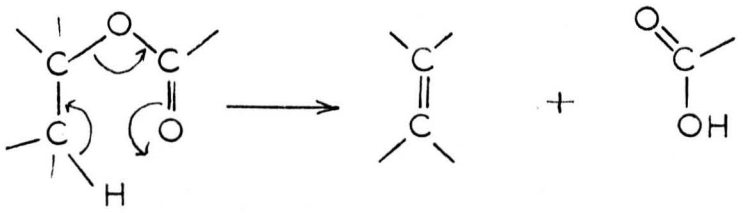
Fig. VI



Structures (a) and (b) can be eliminated since in these the hydroxyl group is near enough to the ring nitrogen to hydrogen-bond with it and this is contrary to the evidence of the infrared spectrum of alkaloid C which shows no evidence of intramolecular hydrogen-bonding. Thus the hydroxyl group is situated on C-8 and this agrees with the

conclusion reached in earlier discussion of alkaloid C (p.27).

Of the two structures (c) and (d), (c) is the more likely since in the benzoate derived from this arrangement of alkaloid C, there is more chance of the benzoate group being co-planar with an adjacent proton, allowing elimination of benzoic acid by a cyclic mechanism such as:



Infrared spectra of the later fracitons from the column showed that they contained mixtures of alkaloid C and its benzoate and several attempts were made to separate these mixtures.

Efforts to sublime alkaloid C from the mixture were unsuccessful and extensive experiments with preparative layer chromatography on Kieselgel also failed to give either component in a purified state.

Some of the benzene fractions contained pure alkaloid C benzoate (from VPC). This was a grey amorphous powder which did not melt up to 320° and could not be induced to crystallise in a variety of solvents. Since there was a relatively large amount of the benzoate available,

experiments were carried out with the aim of discovering suitable conditions for its hydrolysis to alkaloid C. The following hydrolysis conditions were tried on small samples of the benzoate.

a) Heating under reflux with 2% methanolic potassium hydroxide.

b) Heating under reflux with 5% ethanolic potassium hydroxide.

c) Heating on a steam-bath with 5% aqueous potassium hydroxide with ethylene glycol present to produce homogeneity.

Conditions a) and b) failed to hydrolyse the benzoate while condition c) caused partial hydrolysis. The mixture obtained from c) was chromatographed on Florisil but no separation was achieved. Further attempts at separation were not pursued.

Examination of the Mexican Sample of *Tecoma stans* (Juss).

The alkaloid content of the leaves and roots of this sample were examined separately. 10 Kg. of the dried leaves were extracted as described in the experimental section and the basic extract, which amounted to 40 g., was distilled to give the alkaloid fraction boiling at 80 - 135°/0.2 mm. Ethanolic picric acid was added to this and produced a precipitate of tecomanine picrate which was identified by a mixed melting-point with an authentic sample.

VPC analysis of the residue showed the presence of at least eight components. In view of the fact that previous samples of the plant have yielded only three or four alkaloids it was suspected that some neutral components, carried over in the acid extraction, were present in the 'alkaloid' extract. This suspicion was confirmed by treatment of a sample of the extract with 4N hydrochloric acid, followed by ether extraction of the basified acid extract, when VPC analysis showed 5 peaks only. These five components had retention times: 2.8, 3.3, 5.4, 8.0, 9.4 min. After extraction with ether the basic extract was also extracted with chloroform to yield a further quantity of the alkaloid of retention time 5.4 min. in about 90% purity. From their retention times, two of the alkaloids in the mixture were tentatively identified as tecomanine (9.4 min.) and alkaloid A_1 (3.3 min.).

The bulk of the 'alkaloid' extract was now treated with acid in

a similar manner in order to remove neutral components and a basic residue of 2.0 g. was obtained. VPC showed that this consisted predominantly of two components (retention times: 2.8, 8.0 min.) in the ratio 1:4 with a trace of another alkaloid with retention time 5.4 min. This meant that no alkaloid A_1 (i.e. the alkaloid of retention time 3.3 min.) had been extracted by 4N hydrochloric acid from the bulk of the basic extract. Although the separated neutral extract was re-extracted with 5N hydrochloric acid no alkaloid A_1 was obtained. After ether extraction, the basic extract was extracted with chloroform and removal of solvent left 500 mg. of the alkaloid of retention time 5.4 min.

The 2 g. of mixed alkaloids in the ether extract was successfully separated by preparative vapour phase chromatography (for the conditions used, see experimental section). A preliminary analytical run on the preparative machine showed the presence of two peaks in the approximate ratio 4:1 with respective retention times, 19.9 min. (with a small shoulder, probably due to tecomanine) and 11.2 min. This was considered a sufficiently good resolution to allow separation of the two main components on a preparative scale. The alkaloid mixture was dissolved in acetone and the solution injected manually from a 5 ml. syringe. The fractions collected after the separation were examined by analytical VPC.

Alkaloids Separated by Preparative VPC.

Alkaloid R_1

The first alkaloid collected was named alkaloid R_1 and it had a retention time of 2.8 min. It was a colourless liquid with a pungent odour and appeared from VPC to be completely pure. Its ultra-violet spectrum had absorptions at 258 and 266 mμ, showing an increase in intensity on acidification, suggesting a pyridine derivative. This was confirmed by its n.m.r. spectrum which had signals due to pyridine protons at τ1.45 (doublet, C-3 proton), 1.4 (singlet, C-1 proton) and 2.7 (doublet, J = 7 c/s, C-4 proton). The other significant signal in the spectrum was a methyl doublet at τ8.65 indicative of a CH_3-$\overset{|}{C}H$- group. These spectral characteristics corresponded exactly with those of 8-epi-4-noractinidine (45) isolated previously from *Tecoma stans* (Cuban origin). Alkaloid R_1 formed a picrate, m.p. 135 - 137° which analysed correctly for $C_9H_{11}N.C_6H_3N_3O_7$. The n.m.r. spectrum of this picrate was also identical to that of alkaloid A picrate from the Cuban sample. An interesting variation in the properties of the picrates of these two samples of 8-epi-4-noractinidine was in their melting-points. Alkaloid A picrate, prepared in ether and recrystallised from methanol, had m.p. 116 - 117°, while alkaloid R_1 picrate also prepared in ether and recrystallised from methanol had m.p. 135 - 137°. It was noticed during the melting-point determination of R_1 picrate that the crystals appeared to undergo a change

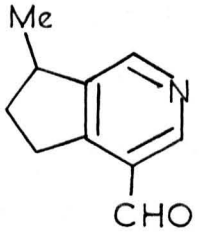
in shape at about 110 - 115°. Briggs et al.,[67] who prepared 8-epi-4-noractinidine from asperuloside, reported that its picrate, prepared in ethanol and recrystallised from chloroform-carbon tetrachloride, had m.p. 115 - 116°, while a crystal modification (large plates) obtained by recrystallisation of the picrate from methylene chloride-ethanol had m.p. 129 - 130°. The conclusion drawn from this data is that alkaloid R_1 picrate is a crystal modification of alkaloid A picrate and it would appear that the transition from one crystalline form to another occurs at 115 - 120°.

Alkaloid R_3

The other main component of the basic mixture separated by preparative VPC had a retention time of 8.0 min. and was a liquid alkaloid of ca. 90% purity. VPC analysis of the fraction showed that the impurities were tecomanine (10%) and 8-epi-4-noractinidine (2%). Its infrared spectrum showed evidence of an aldehyde group (C-H stretching at 2750 cm^{-1}, C=O stretching at 1700 cm^{-1}) and the ultraviolet spectrum had $\lambda_{max.}$: 235, 265, 271.5 mμ and $\lambda_{max.}$ (acidic ethanol) 257.5 mμ, suggesting an aldehyde group conjugated with a pyridine ring. Nicotinaldehyde, i.e., pyridine-3-aldehyde, with $\lambda_{max.}$ 225, 265, 330 mμ and $\lambda_{max.}$ (acidic ethanol) 258 mμ, has absorptions very similar to those of alkaloid R_3 implying that the aldehyde group in the latter is in a β-position with respect to the pyridine nitrogen.

The n.m.r. spectrum of alkaloid R_3 showed the presence of a CH_3-CH- group (methyl doublet at τ8.62) and two α-pyridine protons (two singlets at τ1.23, 1.01) and also a one proton singlet at very low field, (- 0.45). The latter signal could be due to an **acid** proton, a highly de-shielded aldehyde proton or a phenolic hydroxyl proton. The signal does not disappear from the spectrum on shaking with deuterium oxide and therefore cannot be due to a phenol hydroxyl group. The other spectral evidence suggests the presence of an aldehyde group rather than a carboxylic acid. Most aldehyde protons appear in the region τ0.00 - 1.00 in the n.m.r. spectrum. The negative value for the boschniakine aldehyde proton can probably be explained as being due to a combination of steric repulsion[68a] and the inductive effect[68b,68c] of the pyridine nitrogen.

The above spectral information suggests (49) as the structure for alkaloid R_3.



(49)

Sakan et al. have isolated an alkaloid of this structure from *Boschnia rossica* and they have called it boschniakine.[46] The spectral data for alkaloid R_3 corresponds very closely with that reported for

boschniakine. The structure of boschniakine has been confirmed by synthesis.[46]

Alkaloid R_3 forms a picrate, m.p. 134 - 136° and a semicarbazone, m.p. 217 - 220° (dec.). The corresponding values for the boschniakine derivatives are 126.5 - 128° and 227 - 228° (dec.). Further supporting evidence for structure (49) for alkaloid R_3 was obtained when R_3 semicarbazone showed no depression in melting-point on admixture with an authentic sample of boschniakine semicarbazone supplied by Dr. Sakan. Also the infrared spectrum of R_3 semicarbazone ($\nu_{max.}$: 3510, 3405, 3325, 1690, 1610, 1560 cm^{-1}) was quite close to that of boschniakine semicarbazone ($\nu_{max.}$: 3410, 3210, 3145, 1690, 1605, 1575 cm^{-1}) and the ultraviolet spectrum of boschniakine ($\lambda_{max.}$: 239, 268, 282 mμ) is virtually identical to that of alkaloid R_3. It was not possible to obtain satisfactory elemental analyses for either alkaloid R_3 picrate or semicarbazone. This was probably due to traces of tecomanine and 8-epi-4-noractinidine, which could not be completely removed by recrystallisation. This fact might also account for the slight differences in the infrared spectra of the semicarbazones of alkaloid R_3 and boschniakine.

An attempt was made to purify crude alkaloid R_3 via its semicarbazone. It was found that alkaloid R_3 (an aldehyde) forms a semicarbazone more readily than tecomanine(a ketone). Tecomanine was found to be more soluble in dilute acid than the semicarbazone of alkaloid R_3 so acid extraction of the mixture removed tecomanine and alkaloid R_3 was recovered by heating the semicarbazone with acid. VPC analysis showed

that most of the tecomanine was removed by this procedure. Unfortunately, there was insufficient crude alkaloid R_3 left to carry out the purification on a reasonable scale.

Alkaloid R_2

The basic material obtained by chloroform extraction of the basified acid extract after separation into basic and neutral components weighed 500 mg. and VPC showed that it contained predominantly one alkaloid with retention time 5.4 min. The other alkaloids present in small quantities were: tecomanine, 7%; boschniakine, 2%; nor-actinidine, 1%. The alkaloid, which was a liquid, was designated alkaloid R_2. The n.m.r. spectrum of crude alkaloid R_2 contained a peak at τ6.3 which vanished on exchange with deuterium oxide. This indicated that the peak could be due to either an -OH or -$\dot{N}$H group. To discover which of these groups was present a small sample of the crude alkaloid was acetylated with acetic anhydride. If the compound contained an -$\dot{N}$H group the acetyl derivative would be an amide and (assuming no more basic nitrogen in the compound) would be neutral. Alternatively, if a hydroxyl group was present, the acetyl derivative would be an acetate and the acetylated compound would still be basic. The result of the experiment was that the product was basic, supporting a conclusion that alkaloid R_2 contained a hydroxyl group. VPC of the product showed a peak at retention time 10.8 min., and peaks representing the same impurities as were present in crude alkaloid R_2,

showing that acetylation had taken place. It might have been expected that the acetate of alkaloid R_2 could have been separated from the impurities by distillation and hence a pure sample of alkaloid R_2 obtained by hydrolysis of the acetate. However, on bulb-tube distillation of the acetate, VPC analysis indicated that there was no improvement in purity. Alkaloid R_2 failed to form a picrate and could not therefore be purified via this derivative.

It was decided to attempt to purify crude alkaloid R_2 by column chromatography on Florisil. The column was eluted with benzene-ethyl acetate mixtures. Several of the 50:50 benzene-ethyl acetate fractions were found on VPC analysis to contain almost pure alkaloid R_2. A liquid film infrared spectrum of the pure alkaloid provided evidence for hydrogen-bonded OH (3600 - 3100 cm^{-1}) and the solution infrared spectrum ($CHCl_3$) in addition showed free hydroxyl absorption at 3578 cm^{-1}. Dilution had little effect on these absorptions suggesting that the bonding was intra-molecular. The only other significant band in the solution spectrum was at 1120 cm^{-1}, assignable to C-O stretching of either a secondary or tertiary alcohol. Nakanishi[68d] gives the values for C-O stretching frequencies in alcohols as: secondary OH, 1120 - 1090 cm^{-1}, tertiary OH, 1160 - 1140 cm^{-1}; if the hydroxyl group is on a ring, subtract 50 cm^{-1}. This gives a range of 1070 - 1040 cm^{-1} for a secondary alcohol and 1110 - 1090 for tertiary OH. This data suggests that alkaloid R_2 contains a tertiary hydroxyl group.

Examination of the n.m.r. spectrum of alkaloid R_2 (figure VII) showed that there was a CH_3-$\overset{|}{C}H$- group (methyl doublet at τ9.05) and an -$\overset{|}{N}$-CH_3 group (methyl singlet at τ7.70) present in the alkaloid. Since there was evidence for only one CH_3-$\overset{|}{C}H$- group and since the hydroxyl group present was probably tertiary it seemed likely that the latter was situated on C-4 or C-8 along with a methyl group in the perhydropyrindane skeleton. Support for this was provided by the singlet at τ8.71 which could be assigned to this methyl group. This would imply that alkaloid R_2 is one of the hydroxyskytanthines (31) and (32) isolated by Hammouda et al.[24] and isolated and characterised by Aldolphen et al.[25] or an isomer. A comparison of the n.m.r. spectra of hydroxyskytanthine I (figure VIII) (with the hydroxyl group on C-4) and that of hydroxyskytanthine II (figure IX) (with the hydroxyl group on C-8), the alkaloids isolated from *Skytanthus acutus* by Aldolphen et al., with that of alkaloid R_2 (figure VII) suggests that the latter has the hydroxyl group on C-4. The spectrum of HSI closely resembles that of alkaloid R_2 in peak positions but not in their relative intensities. It can be seen from figure VII that the integration of the spectrum does not agree with the structure (31) (i.e. hydroxyskytanthine I) for alkaloid R_2. The peak at τ8.71 integrates to more than 3 protons (taking the -$\overset{|}{N}$-CH_3 and -$\overset{|}{C}H$-CH_3 peak integrations as equivalent to 3 protons), implying that there is some extra proton absorption beneath it.

The stereochemical structure put forward for hydroxyskytanthine I,[25] on the basis of a detailed study of its n.m.r. spectrum and also because

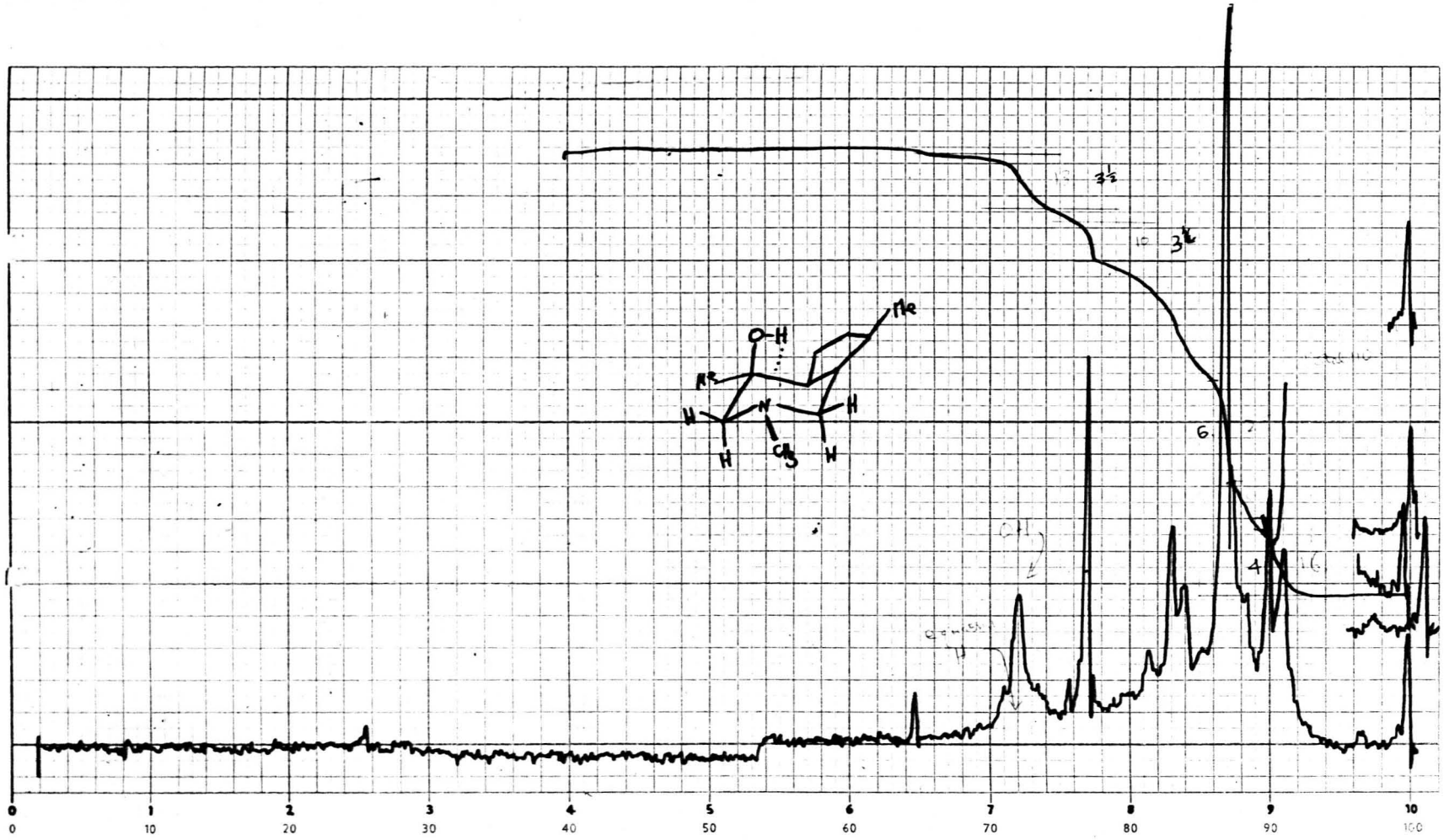


Fig. VII

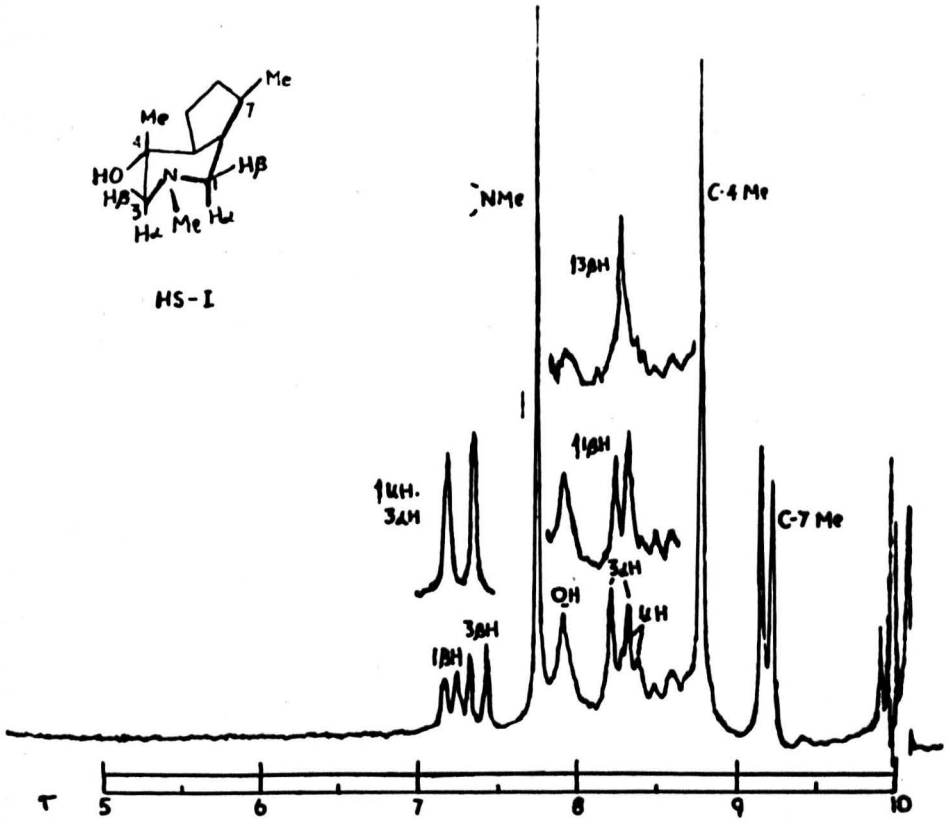


Fig. VIII

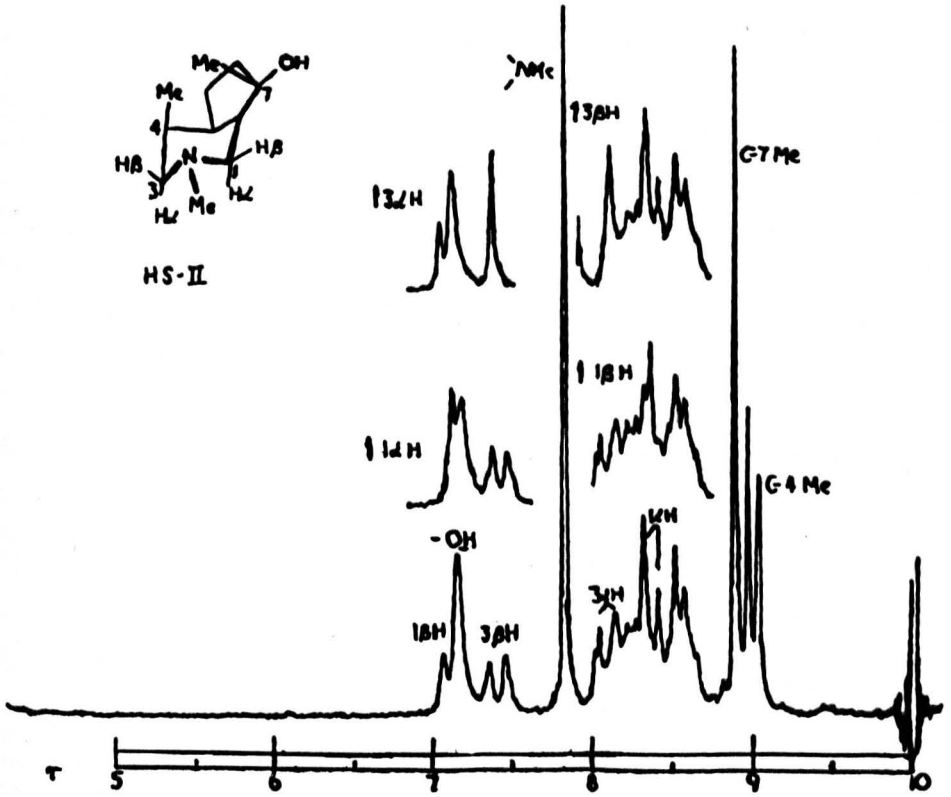
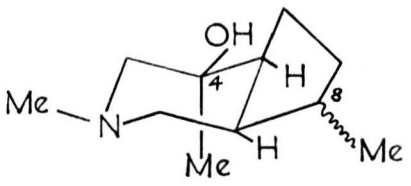


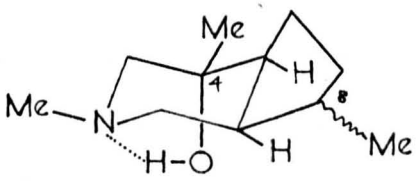
Fig. IX

of the absence of evidence for intramolecularly hydrogen-bonded OH in its infrared spectrum is shown below (50).



(50)

The hydroxyl group on C-4 was assigned an equatorial conformation because it cannot then intramolecularly hydrogen-bond with the ring nitrogen. 3-Hydroxypiperidine which approximates to hydroxyskytanthine I with an axial OH shows considerable hydrogen-bonding in its infrared spectrum.[68e] Since the infrared spectrum of alkaloid R_2 shows evidence of hydrogen-bonding it was felt that it might have an axial OH group on C-4 which could readily hydrogen-bond with the piperidine nitrogen. (51)



(51)

This may explain the small differences in peak positions in the n.m.r. spectrum of alkaloid R_2 as compared with that of hydroxyskytanthine I. However, it does not explain the failure of the integral for the former spectrum to agree with structure (51). Both this n.m.r. spectrum and the fact that alkaloid R_2 was a liquid (while hydroxyskytanthine I was a solid) suggested that the alkaloid was impure. VPC analysis had given a chromatogram containing only one smooth peak for alkaloid R_2 at high column temperatures. In order to investigate the possibility of it being a mixture, VPC analysis of the alkaloid was carried out at lower column temperatures. Again, only one smooth peak was obtained for alkaloid R_2.

Aldolphen et al.[25] had separated HSI and HSII by thin-layer chromatography on Kieselgel G prepared with 0.1 N sodium hydroxide, using methanol-chloroform (1:1). Alkaloid R_2 was chromatographed on silica-gel plates using the same solvent mixture and two spots were observed of R_f values: 0.7 - 0.8, 0.04 - 0.08. However, when the separation was repeated on a preparative scale and the bands eluted with ether, alkaloid R_2 was found to be contaminated with a carbonyl compound (C=O, 1740 cm^{-1}). There was insufficient of the alkaloid to permit further attempts at purification.

The assignment of structure (51) to alkaloid R_2 can only be considered tentative since its n.m.r. spectrum and thin-layer chromatography suggest it is a mixture, although it is possible that the compound of this structure is the principal component of the mixture.

Examination of the Root Extract of the Mexican Sample of *Tecoma stans* (Juss).

The ground root was extracted in an identical manner to the leaves and yielded 3.9 g. of alkaloids on distillation of the basic extract. Two fractions were collected, b.p. 80 - 115°, 115 - 145°/0.05 mm. and VPC analysis of these showed that both were predominantly tecomanine (80, 90%, respectively) with only trace amounts of the other alkaloids found in the leaves of the sample. No further work was done on the root extract.

Examination of the Florida (2) Sample of *Tecoma stans* (Juss).

The main interest in this particular sample was to compare its alkaloid content with that of the previous sample of the plant from Florida. The basic extract was distilled and yielded three fractions, the first two containing 4 components (VPC) and the last one 3 components (mainly tecomanine). Only the first two fractions were examined. Most of the tecomanine was removed from these by picrate formation and the alkaloid mixture left was successfully separated by preparative vapour-phase chromatography.

The first fraction, after removal of tecomanine, was shown by VPC analysis to contain 2 principal components and 1 minor component. (Retention

times: 5.6, 7.6, 3.6 min., respectively). This mixture was separated and the three alkaloids obtained in quite high purity by preparative VPC. (100, 98, 75% purity from analytical VPC). About a dozen samples of the alkaloid mixture, each of 0.5 g. were separated in this way with varying degrees of success. Table 3 in the experimental section gives a summary of the conditions used and the quantities involved in all these separations. The main drawback of this technique of separation for these alkaloids was the low recovery from the preparative column. (Average yield, 25%, maximum yield, 40%). The principal cause of this was the instability of the alkaloids to the relatively high column and injection heater temperatures (160 - 200°) which were used during the separations. It would have been rather impracticable to use much lower temperatures (say, <150°) since these would have made the retention times of the alkaloids on the column prohibitively long. Experiments were conducted to try and improve the yields by lowering the injection heater temperature and on a few occasions considerable success was achieved, the recovery from the column being as much as 40%. Unfortunately, it was not possible to reproduce the conditions exactly so most of the yields were about 25%.

Most of the separations gave small quantities (<50 mg.) of the two main components; a solid alkaloid of retention time 5.6 min., called alkaloid X, of purity >99% (according to VPC), and a liquid alkaloid of retention time 7.6 min., called alkaloid Y and shown by VPC to be contaminated with 2 - 5% of tecomanine. Only 5 of the separations gave

any of the alkaloid of retention time 3.6 min. and VPC showed that most of the samples were contaminated by some material which appeared as a substantial shoulder on the 3.6 min. peak. One of the separations allowed a small quantity (5 mg.) of this contaminant to be isolated (retention time, 2.1 min.) and an infrared spectrum was obtained. However, VPC indicated that this material was only 60% pure.

Alkaloids Separated by Preparative VPC.

Alkaloid X

This alkaloid, with retention time 5.6 min., was a white solid, m.p. 87 - 94°, which turned purple on exposure to light and air. Some of the fractions collected appeared (from VPC) to have a purity >99%. The infrared spectrum of alkaloid X showed evidence of intramolecular hydrogen-bonding (3500 - 3000 cm^{-1}, no change on dilution) and C-O stretching at 1092 cm^{-1} (tertiary alcohol). It formed a methiodide, m.p. 310 - 312° (dec.) which gave an elemental analysis in agreement with the formula $C_{11}H_{21}NO$ for the free alkaloid. The mass spectrum of a sample of the alkaloid taken directly from the preparative separation and done on a combined VPC-mass spectrometer showed a molecular ion of m/e 183, confirming the above molecular formula. It can be seen that alkaloid X is isomeric with the hydroxyskytanthines I (31) and II (32)[25] tecostanine (34)[39a] and alkaloid C (47). It has similar physical properties to hydroxyskytanthine I (solid, m.p. 94 - 95°), alkaloid C (solid, m.p. 91 - 92°) and also the alkaloid D

obtained by Appel and Müller from *Skytanthus acutus* (solid, m.p. 93°).[26] Alkaloid X also behaves similarly to hydroxyskytanthine I in not forming a picrate and like alkaloid D forms a methiodide with m.p. >300°.

The n.m.r. spectrum of one of the 99% pure samples of alkaloid X is shown in figure X. This spectrum differs appreciably from that of the isomeric hydroxyskytanthines (figures VIII and IX) and alkaloid R_2 (figure VII) but shows close similarities to that of alkaloid C (see experimental, p. 82). TLC of this sample of alkaloid X on Kieselgel G plates made with 0.1N sodium hydroxide solution, using 1:1 methanol-chloroform as eluent, showed it contained two trace impurities at Rf values: 0.77 - 0.73, 0.67 - 0.63 with the principal component having Rf, 0.6 - 0.54. The n.m.r. spectrum of a pure sample of alkaloid X (according to TLC) was identical to that of the above sample, showing that the trace impurities had no effect on the spectrum. Therefore figure X is the n.m.r. spectrum of a pure compound of molecular formula $C_{11}H_{21}NO$. This formula implies that alkaloid X is a skytanthine with a tertiary hydroxyl group attached at some point in the ring system. Since the spectrum shows no methyl singlet in the region τ8.6 - 8.9 (present in the spectra of hydroxyskytanthines I and II) it can be assumed that the tertiary hydroxyl group is not on C-4 or C-8. The only other free tertiary sites are the points of ring-fusion, C-5 and C-9. Alkaloid C has the hydroxyl group on C-5 and in such a position that it cannot intramolecularly hydrogen-bond with the ring nitrogen. The infrared spectrum of alkaloid X shows evidence of intramolecular hydrogen-bonding and this suggests that the hydroxyl group

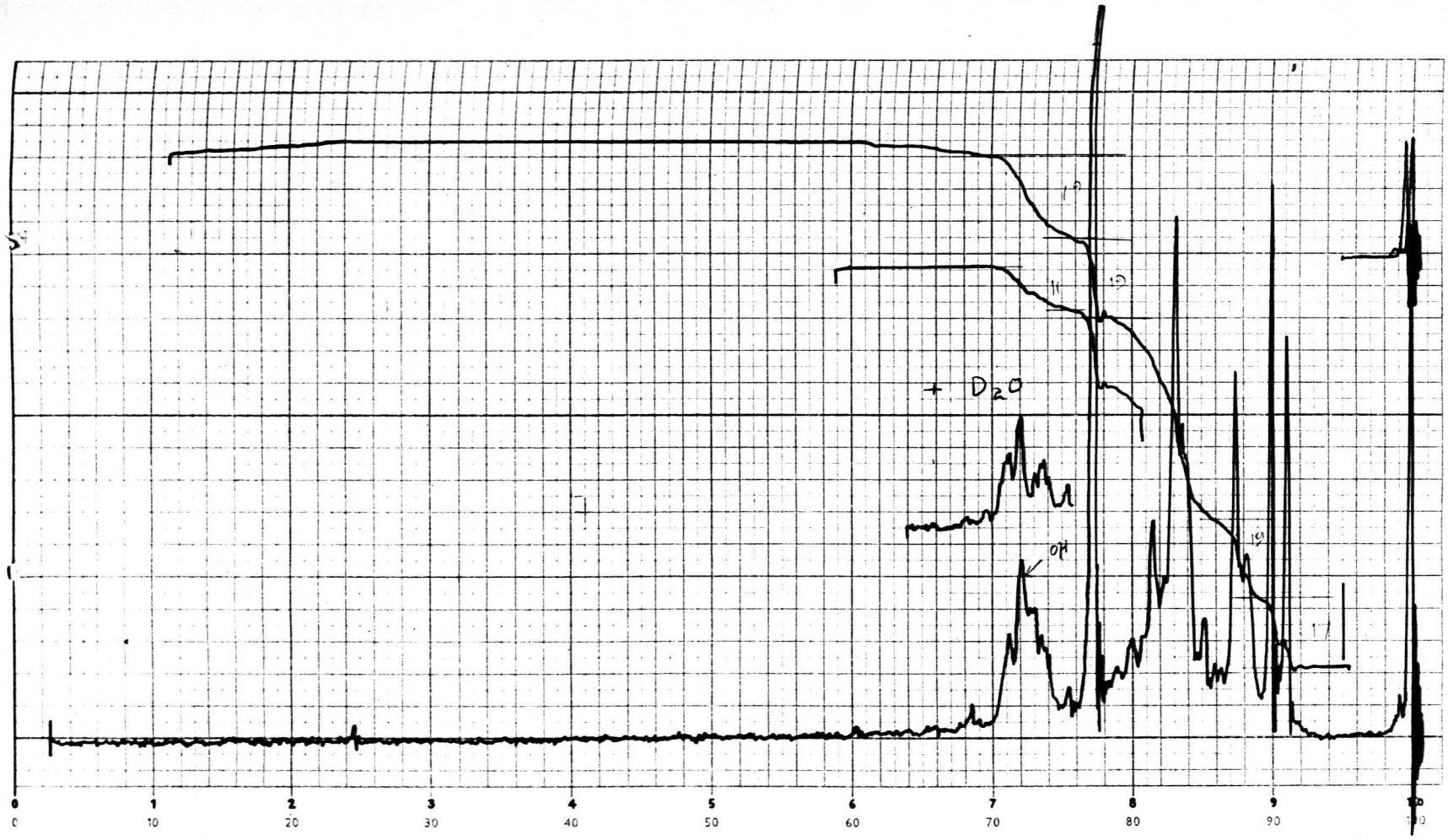
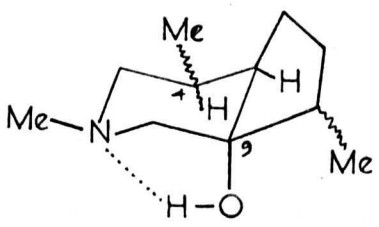


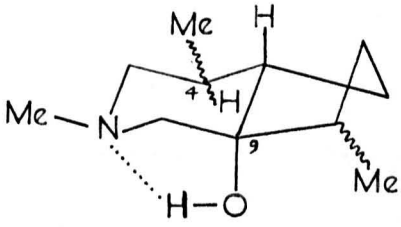
Fig. X

is situated on C-9 giving two possible structures (52a) and (52b) for alkaloid X.



(52a)

cis-fused



(52b)

trans-fused

The n.m.r. spectrum of alkaloid X (figure X) can be correlated with either of these structures as follows: the doublet at 9.05τ, integrating as three protons can be assigned to the C-4 methyl group coupled to the methine proton also on C-4. The three-proton singlet at τ7.72 is due to the $-\overset{|}{N}-CH_3$ group. The methyl group on C-8 which should appear as a doublet, being split by the C-8 proton, would be expected to appear down-field from the C-4 methyl signal since it is in a β-position to the hydroxyl group. Therefore, the distorted doublet at τ8.80 can be assigned to the C-8 methyl group. The n.m.r. spectrum of alkaloid C has

a similar doublet at τ8.85 which is due to the C-4 methyl group (which also has a β-hydroxyl group) in this alkaloid. The shift in position of a methyl signal due to a β-hydroxyl group is given as τ0.3 - 0.15 in the literature.[68f,68g] The spectrum of alkaloid X agrees with this, the shift in this case being τ0.25. The multiplet at τ8.0 - 8.5 in the spectrum of alkaloid X is also present in the spectrum of alkaloid C and can be assigned to the α-protons (above the plane of the ring) on the ring system. The multiplet at τ7.0 - 7.4 is due to the β-protons (below the plane of the ring) next to the piperidine nitrogen and the hydroxyl proton. The sharp peak at τ7.2 is due to the latter since it disappears on exchange with deuterium. If the integral of the $-\overset{|}{N}-CH_3$ or C-4 methyl signal corresponds to 3 protons, the total spectrum integral is equivalent to 21 protons, which is in agreement with the formula $C_{11}H_{21}NO$. From this n.m.r. spectrum it is not possible to decide between the cis- and trans-fused forms for the structure of alkaloid X.

In the mass spectrum of alkaloid X the fragment of m/e 166 (40% of base peak) can be attributed to the fragment left by loss of the hydroxyl group from the alkaloid (loss of m/e 17). No other fragments could be identified.

Alkaloid Y

This alkaloid was a liquid with a retention time of 7.6 min., which, like alkaloid X, turned purple on exposure to light and air. It was not possible to obtain a pure sample; all the samples from the column were contaminated with tecomanine (2 - 5%) and alkaloid X (1%). Alkaloid Y

would not form a picrate and attempts to prepare a methiodide led to break-down of the ring system. Therefore, the alkaloid could not be purified sufficiently for elemental analysis or mass spectroscopy. The structure deduced for this alkaloid (56) is based solely on its n.m.r. and infrared spectra and because of this must be considered rather tentative.

The infrared spectrum of alkaloid Y had absorption at 3500 - 3000 cm^{-1}, suggesting intramolecular hydrogen-bonding, and evidence of C-O stretching at 1080, 1125 cm^{-1}. Its n.m.r. spectrum (figure XI) was very different to those of the alkaloids which have been discussed previously. It showed the presence of an $-\overset{|}{N}-CH_3$ group (singlet at τ7.68), an ethoxy group (quartet, centred at τ6.35 and a triplet, centred at τ8.63) and an olefinic proton (poorly resolved doublet at τ4.43). The broad peak at τ5.80, equivalent to one proton could be due to a shielded olefinic proton or a highly de-shielded methine proton such as that in the grouping $-\overset{|}{C}H-O-$. The spectrum shows no observable change on shaking the alkaloid in $CDCl_3$ with deuterium oxide. This would suggest that there is no hydroxyl proton in the alkaloid which conflicts with the infrared evidence. The same effect was observed in the n.m.r. spectrum of alkaloid C. In both of these spectra the hydroxyl proton signal could be concealed beneath another peak and its disappearance go unobserved (although the integration of the spectrum should change). The infrared spectrum suggests the presence of a hydroxyl group intramolecularly-bound to the piperidine nitrogen and it is possible that the alkaloid could contain a hydroxyl group (on C-4, C-8 or C-9 for hydrogen-bonding to nitrogen) and also an ethoxyl group.

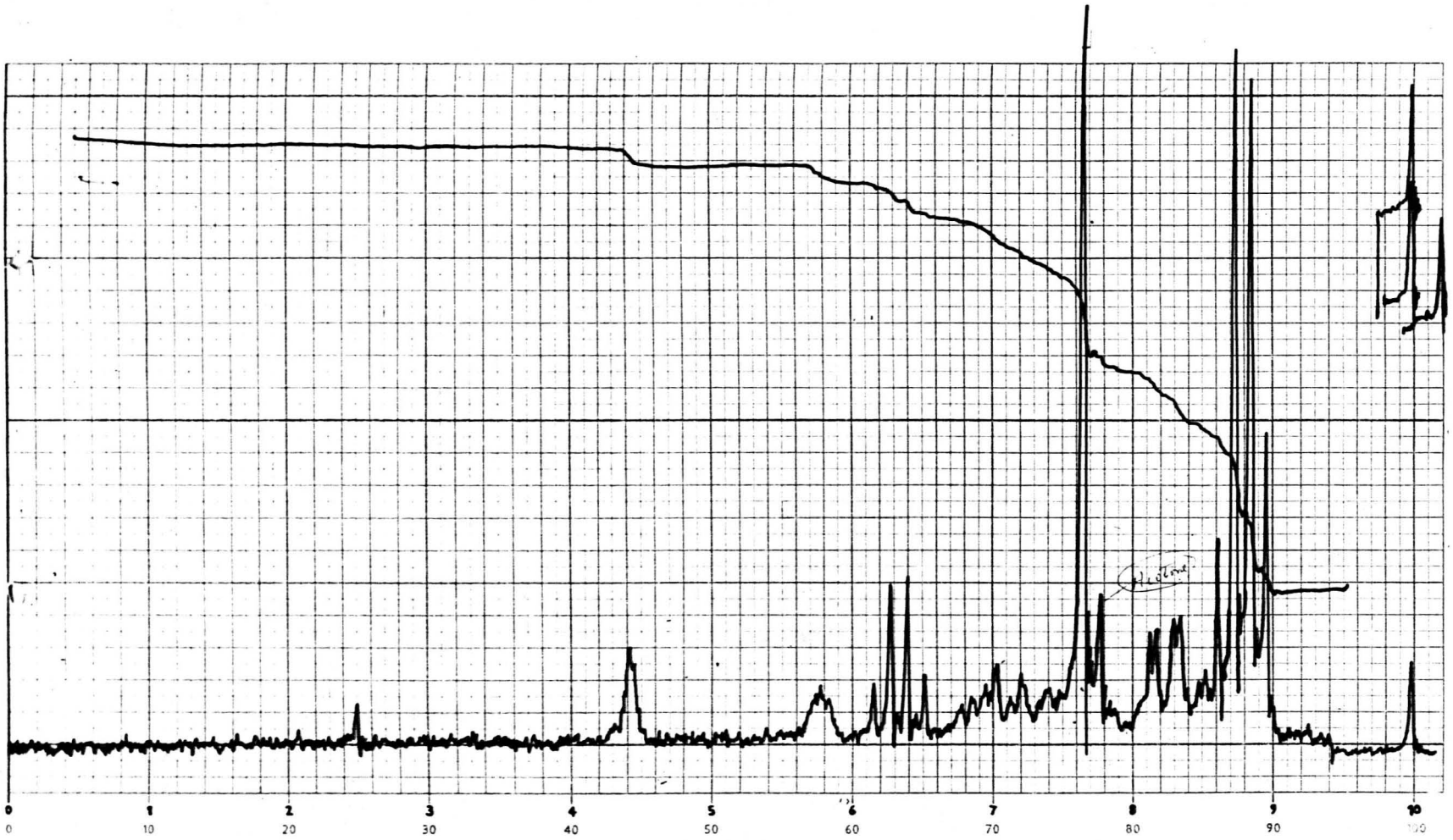
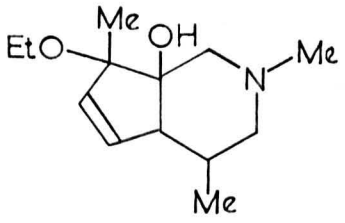
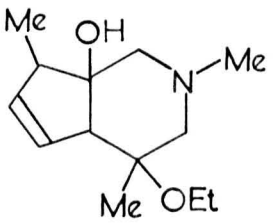


Fig. XI

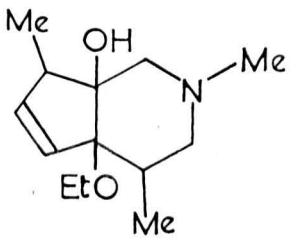
Evidence for the latter in the infrared spectrum is the strong, broad C-O absorption at 950 - 1150 cm^{-1}. On the basis of the n.m.r. spectrum four possible structures for alkaloid Y are (53), (54), (55) and (56):



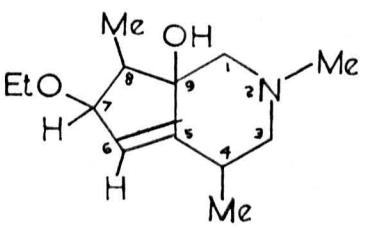
(53)



(54)



(55)



(56)

and also the six corresponding structures with the hydroxyl group on C-4, C-8 and C-4 or C-8 and again, C-4 or C-8, respectively.

A close examination of the multiplet at τ8.6 - 9.0, equivalent from the integration to 9 protons (probably 3 methyl groups), indicates that it is made up of a triplet over two overlapping doublets all with J = 6.5 — 7 c/s. This eliminates all the above structures except for (55) and (56).

Another possible structure for the alkaloid is the hemi-acetal of tecomanine, i.e., tecomanine (44) with a hydroxyl group and an ethoxyl group both on C-7. This sort of compound is quite common among natural products. However, this structure does not contain a proton which could be assigned to the broad signal at τ5.80 in the n.m.r. spectrum of the alkaloid. Assuming the integral of the olefinic proton signal at τ4.43 is equivalent to one proton, the total integral of the spectrum is equivalent to ca. 25 protons. Structures (55) and (56) contain 23 protons. This discrepancy could be explained by the impurities in the alkaloid. Of these two structures (56) seems the more sterically favourable. In structure (55) there would be considerable steric repulsion between the ethoxy group and the olefinic proton on C-6. Therefore, taking structure (56) as a rather speculative suggestion for alkaloid Y, the following principal proton assignments can be made: $-\overset{|}{N}-CH_3$ (singlet at τ7.68), C-4, C-8, methyls, (doublets at τ8.93, 8.82 or vice versa), ethoxy group (quartet at τ6.35 and triplet at τ8.63), methine proton on C-7 (broad peak at τ5.80, coupled with C-6 olefinic proton and proton on C-8), olefinic proton on C-6 (doublet at τ4.43, split by neighbouring proton on C-7). It must be emphasised that this structure is purely speculative and more

substantial evidence, e.g., molecular formula from elemental analysis of a pure sample and the mass spectrum of the pure alkaloid is required to confirm or negate the assignment.

Two further alkaloids of retention times 3.6 and 2.1 min., of respective purities, 92, 60%, were isolated in low yield by preparative VPC. The second one was too impure to investigate. The alkaloid of retention time 3.6 min. showed stretching in the carbonyl region of its infrared spectrum at 1708 cm^{-1}, along with evidence of intramolecular hydrogen-bonding. An n.m.r. spectrum was run on 14 mg. of the alkaloid using a computer of average transients (C.A.T) to multiply the spectrum 4 times. The spectrum was not very informative. It showed the presence of an $-\overset{|}{N}-CH_3$ group (singlet at 7.83) and possibly two $CH_3-\overset{|}{C}H-$ groups (overlapping doublets, badly resolved at 9.02) and the absence of any olefinic or aromatic protons. However, there was not enough information for a structural assignment.

Table 2, below, shows the distribution of the alkaloids found in *Tecoma stans* (Juss.) based on the present work and the work by Hammouda et al. on an Egyptian sample of the plant.[39,40] The most striking features are the occurrence of tecomanine in all the samples and the fact that, for the Mexican sample, the roots contain a much higher proportion of tecomanine than do the leaves. It is also interesting that the one plant should produce such a wide diversity of alkaloids and that only a limited number appear in any one sample.

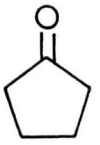
Distribution of Cyclopentanoid Monoterpene Alkaloids in *Tecoma stans* (Juss.)

Table 2

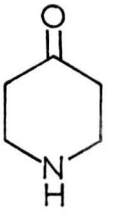
Alkaloid	Plant Source					
	Cuba	Florida (1)	Florida (2)	Mexico Leaves	Mexico Roots	Egypt
N-norskytanthine (48) (Alkaloid A_1)		+		+		
5-hydroxyskytanthine (47) (Alkaloid C)	+	+				
4-hydroxyskytanthine (51) (Alkaloid R_2)				+		
9-hydroxyskytanthine (52) (Alkaloid X)			+			
9-hydroxy-7-ethoxy-5:6-dihydroskytanthine (56) (Alkaloid Y)			+			
Tecomanine (44)	+	+	+	+	+	+
Boschniakine (49)				+	+	
8-epi-4-noractinidine (45) (Alkaloid **A**, R_1)	+			+	+	
Tecostanine (34)						+
Tecostidine (35)						+

Attempted Synthesis of the Pyrindane Ring System

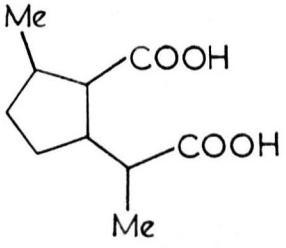
Previous syntheses of the pyrindane ring system have used three main precursors: cyclopentanone (57), 4-piperidone (58) and nepetalinic acid derivatives (59).



(57)

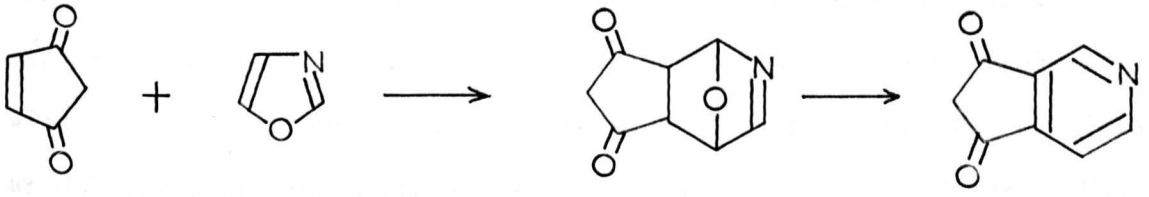


(58)



(59)

The present attempt to synthesise the system was an entirely new approach, involving a Diels-Alder reaction between an oxazole and a five-membered dienophile, cyclopentene-3:5-dione (60). The theoretical reaction is shown in figure XII.



(60)

Fig. XII

Two oxazoles were synthesised for use in the reaction; 2:5-dimethyl oxazole (61) and 5-methyl-2-carbethoxy oxazole (62, R = Et). These were expected to react with cyclopentene-3:5-dione according to the equations shown below (figure XIII).

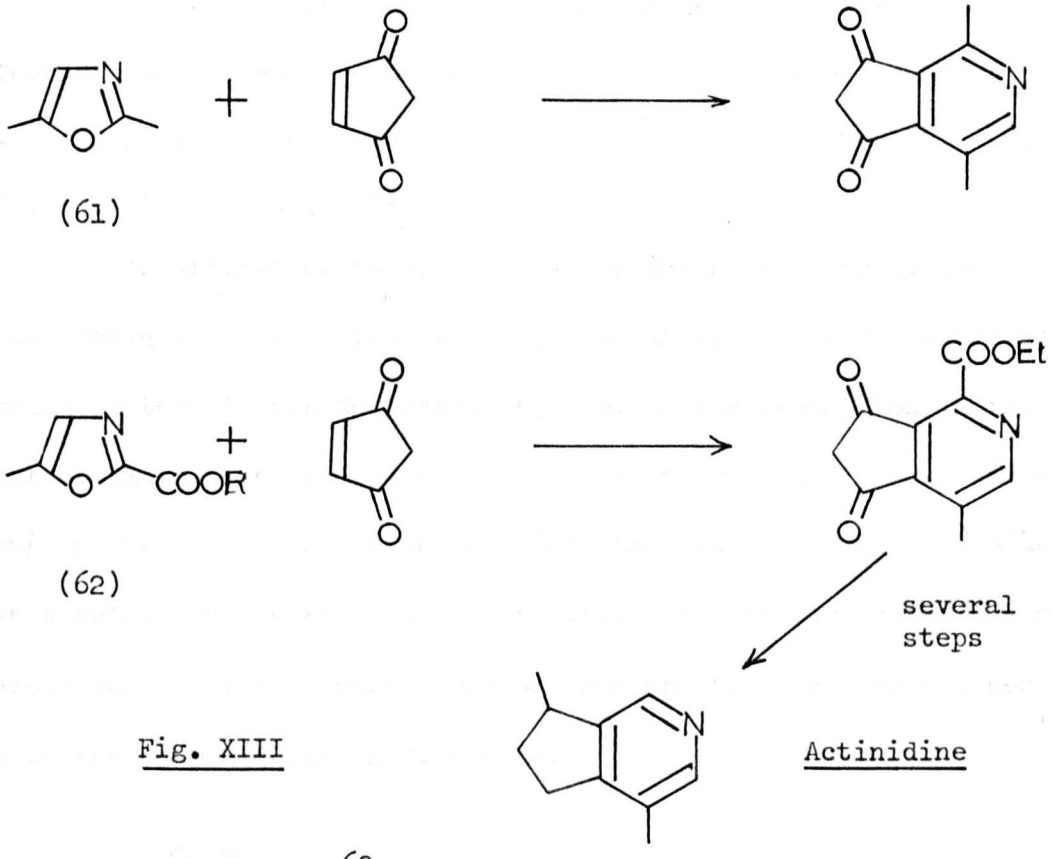


Fig. XIII

Previous work[69] has shown that oxazoles can act as dienes in the Diels-Alder reaction with such reactive dienophiles as maleic anhydride and maleimide. For example, heating 4:5-dimethyl oxazole with maleimide in benzene in the presence of hydroquinone for 2 hr. gave an 85% yield of 5:6-dimethyl pyridine-3:4-dicarboximide (figure XIV).

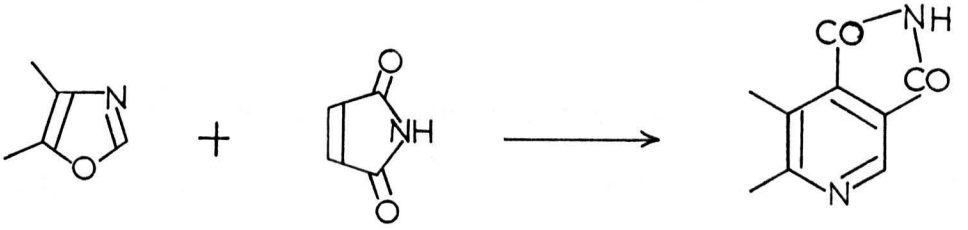
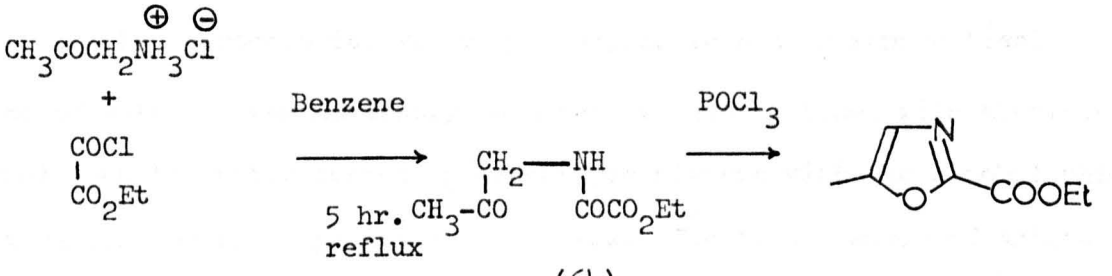


Fig. XIV

The same workers[70] established an order of reactivity of the effect of substituent groups on the oxazole in the reaction with maleic anhydride. This was, in order of decreasing activity: RO, R, 4-Ph, Acetyl, - CO_2Et, 2:5-diphenyl-.

In attempting to synthesise the pyrindane ring system it would be desirable to have a group which is easily removable at C-1 in the product of the Diels-Alder reaction, i.e. in the 2-position in the starting oxazole, such as a carbethoxy group. Also, if there was a methyl group in the 5-position in the original oxazole the expected product would have a methyl on C-4 and would be a partial actinidine system. A suitable oxazole was 5-methyl-2-carbethoxy oxazole and this was synthesised according to the scheme shown in figure XV.

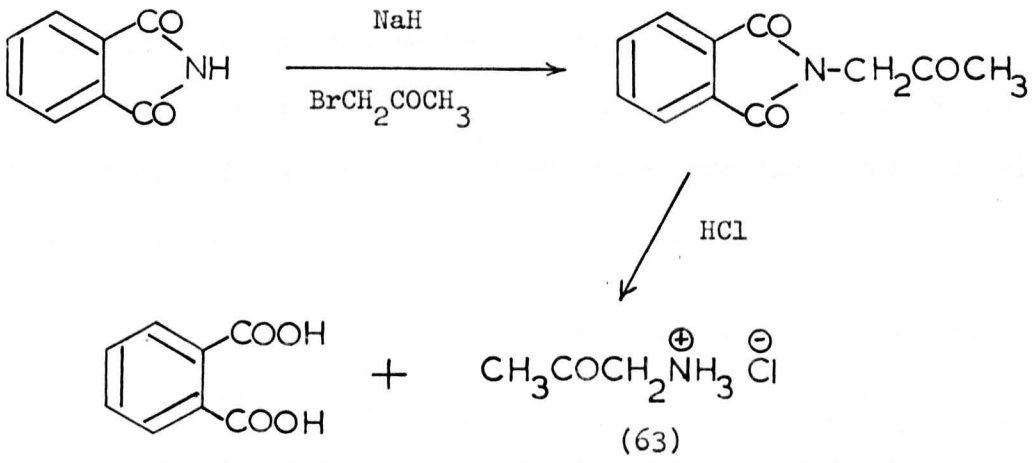


(64)

Fig. XV

Japanese workers[71] have prepared the corresponding phenyl compound, 5-phenyl-2-carbethoxy oxazole, by a parallel route, except for the final stage of the reaction where they used phosphorus oxychloride in benzene at 0^{o} as the cyclising conditions.

Aminoacetone hydrochloride (63) was prepared by a Gabriel synthesis from bromoacetone,[72] phthalimide and sodium hydride, followed by acid hydrolysis of the resulting phthalimidoacetone.[73] (figure XVI)



(63)

Fig. XVI

The hydrochloride was very hygroscopic and to remove final traces of water it was necessary to treat it several times with absolute alcohol when the water formed an azeotropic mixture with the alcohol which could be removed by evaporation to dryness. The first sample of amino-acetone hydrochloride prepared was contaminated with quantities of ethanol which were not removed at water-pump pressure on a steam-bath and when it

was reacted with ethoxalyl chloride the product, ethoxalylaminoacetone (64), contained a high proportion of diethyl oxalate formed by reaction of ethanol with ethoxalyl chloride. This could not be removed by fractional distillation. This difficulty was avoided in later preparations by heating the hydrochloride at 40° at 0.05 mm. pressure for several hours when all trace of ethanol was removed. However, the hydrochloride still failed to crystallise; it existed as a very viscous liquid. Ethoxalyl chloride was made by the action of phosphorus pentachloride on diethyl oxalate.[74] It was then heated under reflux in benzene with aminoacetone hydrochloride to give ethoxalylaminoacetone.

The cyclisation of ethoxalylaminoacetone to the oxazole was the most difficult part of the reaction scheme to accomplish. The following conditions were tried:

a) Heating with concentrated sulphuric acid on a steam-bath.[75]

b) Treatment with phosphorus oxychloride in benzene at 0°.[71]

c) Heating with phosphorus pentachloride on a steam-bath.[76]

These conditions had proved successful in oxazole syntheses involving cyclisation of a similar intermediate to ethoxalylaminoacetone.

The following conditions were also tried:

d) Heating with poly-phosphoric acid at 150°.

e) Heating under reflux with phosphorus pentachloride in benzene.

Conditions b) and c) failed to give any reaction. The n.m.r.

spectrum of the product of the reaction done under conditions a) showed some evidence of cyclisation. It had a methyl singlet at τ7.5 assignable to a 5-methyl group on an oxazole, while the starting material, ethoxalylaminoacetone, shows a methyl singlet in its n.m.r. spectrum at τ7.7 (methyl next to carbonyl). The former spectrum also contained an aromatic proton (oxazole proton on C-4) at τ3.02 and an acidic proton at -1.35τ (not effected by shaking with D_2O, therefore not phenolic). This indicated that cyclisation of ethoxalylaminoacetone had occurred with concomitant hydrolysis to 5-methyl oxazole-2-carboxylic acid (62, R = H). There were still signals in the n.m.r. spectrum attributable to an ethoxyl group which may have been due to incomplete hydrolysis of the oxazole ester or to diethyl oxalate present as an impurity in ethoxalylaminoacetone. Unfortunately, all attempts to reproduce this reaction in concentrated sulphuric acid were unsuccessful.

Method d) gave a small yield of the cyclised product (ca. 20%). This was insufficient for this to be a suitable method for cyclising the intermediate.

Method e), using phosphorus pentachloride in benzene gave a low yield (5%) of a white, crystalline solid, insoluble in chloroform, which from its n.m.r. spectrum appeared to be 5-methyl oxazole-2-carboxylic acid. There was no trace of signals due to ethoxyl protons. The spectrum in trifluoracetic acid had only two signals; a methyl singlet at τ7.25 attributable to the 5-methyl group on the oxazole ring and a singlet at τ2.2 due to the C-4 oxazole proton. After removal of this solid acid the residue was examined to see if any oxazole ester was present. Removal of

chloroform left only 1.8 g. of material and the n.m.r. spectrum showed that this was ethoxalylaminoacetone. However, this left nearly 3 g. of starting material unaccounted for. It was considered possible that the rest of the ethoxalylaminoacetone had cyclised and hydrolysed and the resulting solid acid had undergone immediate decarboxylation to give the free oxazole, 5-methyl oxazole. Since this would be basic it was expected that it would remain in the acid solution after chloroform extraction. The acid solution was therefore neutralised, saturated with sodium chloride (since simple methyl oxazoles are water-soluble), and extracted with chloroform. However, a negligible amount of residue was obtained on evaporation of the solvent. Another possibility was that the simple oxazole, being quite volatile (the boiling-points of most of the alkyl oxazoles are $<100^{\circ}$) had distilled over on boiling off the benzene from the reaction mixture. To test this the benzene distillate was extracted with strong hydrochloric acid and the basified aqueous extract (after saturation with sodium chloride) was extracted with ether. Removal of solvent at $<40^{\circ}$ left no residue.

Several different solvents and solvent mixtures were used in attempts to recrystallise 5-methyl oxazole-2-carboxylic acid, but none were found to be suitable. The main reason for this appeared to be the ease with which the acid underwent decarboxylation to 5-methyl oxazole. Presumably the carboxyl group is very labile when positioned on carbon between two heterocyclic atoms. The solid acid did seem to dissolve in

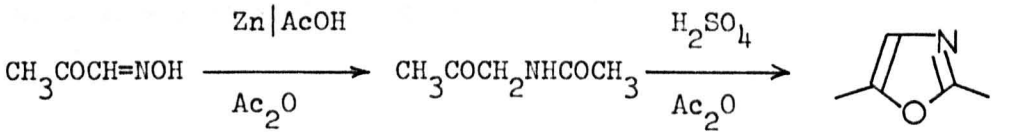
chloroform on prolonged boiling but no crystals could be obtained on cooling the solution and there was no residue on evaporation of the solvent. This behaviour suggested decarboxylation of the acid at the boiling-point of chloroform, followed by loss of the volatile oxazole.

Cyclisation of ethoxalylaminoacetone was finally accomplished by heating it under reflux with a large excess of phosphorus oxychloride. The reaction product was shown to be a mixture of the required oxazole (65%) and starting material (35%) by its n.m.r. spectrum which had methyl singlets at τ7.5 (5-methyl of oxazole) and τ7.7 (methyl next to carbonyl in ethoxalylaminoacetone) in the ratio 2:1. Heating the reaction mixture for up to 4 hr. did not improve the yield. Attempts to purify the crude product by column chromatography on alumina were unsuccessful. The fractions collected from the column appeared from their n.m.r. spectra to be more complex than the original mixture, containing some aliphatic compound absorbing the region τ8.5 - 9.0, and the recovery from the column was very low (<50%) in spite of extracting the alumina with glacial acetic acid. It was found that the crude product could be separated by fractional distillation. It was possible to obtain a satisfactory yield of 5-methyl-2-carbethoxy oxazole by repeatedly treating the residue after distillation with more phosphorus oxychloride and, in turn, fractionally distilling the product. A small sample of the oxazole ester was distilled from a bulb-tube but the distillate failed to analyse correctly for $C_7H_9NO_3$.

The mercuric chloride complex of 5-methyl-2-carbethoxy oxazole was prepared in aqueous solution and recrystallised several times from ethanol to yield white needles, m.p. 110 - 113°, which analysed correctly

for the formula $C_7H_9NO_3.2HgCl_2.H_2O$.

Reference to the reactivity order of oxazoles with given substituents (p. 62) shows that a carbethoxy group tends to deactivate the oxazole towards reaction in the Diels-Alder reaction. Therefore, in anticipation of the possibility of 5-methyl-2-carbethoxy oxazole not reacting with cyclopentene-3:5-dione it was decided to prepare 2:5-dimethyl oxazole (56) which would be expected to be more reactive. It was prepared by a method due to Treibs and Sutter.[77] This involved reductive acylation of isonitrosoacetone followed by cyclisation of the intermediate acetylaminoacetone with concentrated sulphuric acid, (figure XVII).

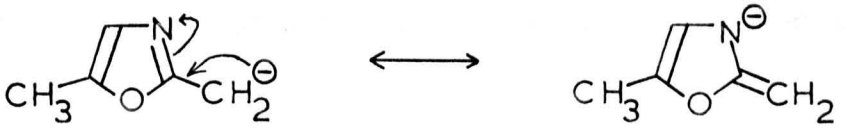


(56)

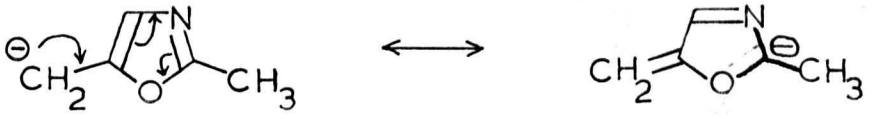
Figure XVII

Isonitrosoacetone was prepared in good yield (ca. 70%) by the reaction of nitrous acid and potassium hydroxide with ethyl acetoacetate.[78] It was then reduced with zinc and acetic acid in the presence of acetic anhydride to give acetylaminoacetone which cyclised to the required oxazole under the influence of sulphuric acid. The cyclisation stage did not go to completion and the crude product was purified by distillation from potassium hydroxide.

It would be desirable to have 5-methyl oxazole available to try in the Diels-Alder reaction with the dione and a possible way of obtaining it was by selective oxidation of the 2-methyl group in 2:5-dimethyl oxazole followed by decarboxylation of the resultant acid. The reagent used for oxidation was selenium dioxide. This has been used in selective oxidation of dimethyl pyridines.[79] It would be expected that the 2-methyl group in 2:5-dimethyl oxazole would be more reactive than the 5-methyl group since the anion formed by loss of a proton (probably the first step in oxidation of the methyl group) from the 2-methyl group can be stabilised by the resonance structure (65) with the negative charge on nitrogen, while the corresponding anion from loss of a proton from the 5-methyl group is stabilised by a form (66) with the negative charge on carbon, a less stable entity.



(65)



(66)

However, the oxidation failed to work with 2:5-dimethyloxazole and no recognisable product was isolated.

Another approach to obtaining 5-methyl oxazole was from 5-methyl-2-carbethoxy oxazole by hydrolysis and decarboxylation. The ester was heated under reflux with 10% aqueous potassium hydroxide and this led to rupture of the heterocyclic ring with the production of a compound containing aliphatic protons in its n.m.r. spectrum. Less drastic conditions have been used by Cornforth[80] to successfully hydrolyse 2-methyl-4-carbethoxy oxazole to the corresponding acid, (aqueous potassium hydroxide at steam-bath temperatures for 30 min.). However, it was found that these conditions broke down the oxazole ring of 5-methyl-2-carbethoxy oxazole. In fact, even milder conditions such as stirring the oxazole ester at room temperature for 20 hr. with 2% aqueous potassium hydroxide led to ring breakdown. Further attempts at hydrolysis were not pursued.

The dienophile to be used in the Diels-Alder reaction, cyclopentene-3:5-dione, was prepared by the reaction sequence shown in figure XVIII.

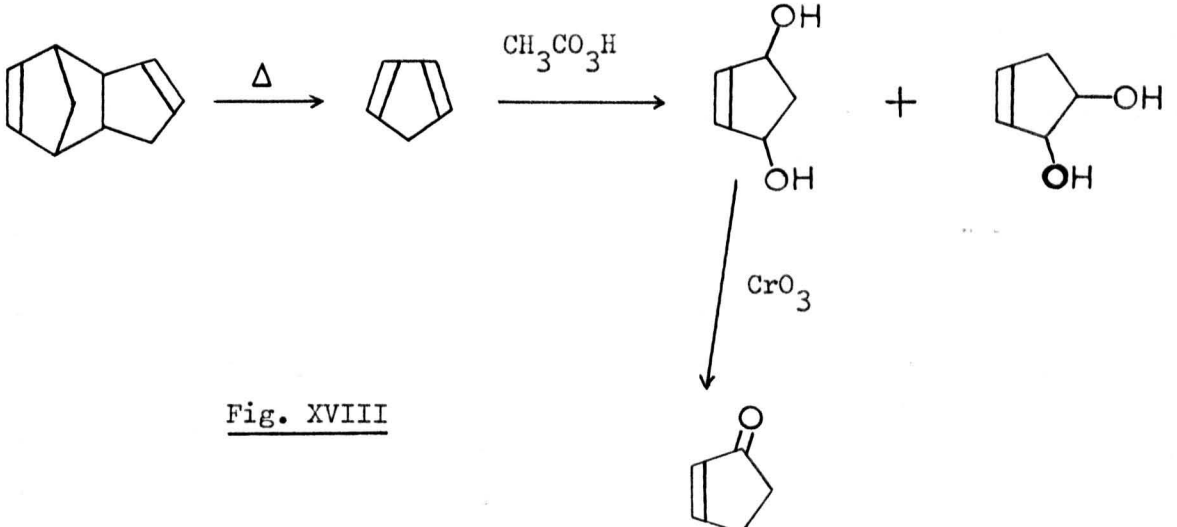


Fig. XVIII

The main difficulty encountered in obtaining a good yield of the dione was the preparation of a peracetic acid solution of sufficient strength to bring about the oxidation of cyclopentadiene to the mixed diols. Several methods of preparation were tried[81,82] but the peracetic acid solutions produced gave negligible yields of diols. Eventually, a commercial sample of 40% peracetic acid was obtained and using this a satisfactory conversion of cyclopentadiene to the mixed diols was achieved.

Cyclopentadiene was obtained from technical dicyclopentadiene by pyrolysis[83] and then oxidized with peracetic acid to an epoxide which was hydrolysed to a mixture of 3:4- and 3:5-cyclopentenediols.[84] The final stage in the synthesis was oxidation of the 3:5-diol only to the required cyclopentene-3:5-dione.[85] The product is known to be a very reactive dienophile in the Diels-Alder reaction being less reactive than maleic anhydride but more reactive than acrylonitrile.

Attempted Diels-Alder Reaction to Produce the Pyrindane Ring System.

The reaction was first tried with cyclopentene-3:5-dione and 5-methyl-2-carbethoxy oxazole, heating the reactants under reflux in benzene with a trace of hydroquinone. These were the conditions used successfully by Kondrat'eva et al.[69] with various oxazoles and maleic anhydride or maleimide. However, on this occasion no reaction took place. The reaction was repeated using a more reactive dienophile, maleic anhydride, but the only products isolated were maleic acid and unchanged oxazole ester.

As mentioned previously, 2:5-dimethyl oxazole would be expected to be more reactive as a diene in this reaction than 5-methyl-2-carbethoxy oxazole since the latter contains a deactivating carbethoxy group. The reaction was performed with the dimethyl oxazole and cyclopentene-3:5-dione but none of the expected pyridine derivative was obtained, only starting material being isolated. The reaction was repeated in toluene, with similar results.

Finally, the reactants were mixed and heated in the absence of any solvent up to 100° and maintained at this temperature for 30 min. A sample of the mixture was removed and its n.m.r. spectrum showed that no reaction had occurred. The mixture was then heated under reflux (reflux temperature was about 115°) for 1½ hr. but the residue in the flask was an amorphous black powder which was insoluble in any solvent

and did not melt up to 320°. This was not investigated further.

Kondrat'eva et al. had carried out the reaction successfully with 2:5-dimethyl oxazole and maleic anhydride obtaining a good yield of the expected product (figure XIX). We repeated this reaction using the same reaction conditions but obtained only a 5% yield of 2:5-dimethyl pyridine-3:4-dicarboxylic acid.

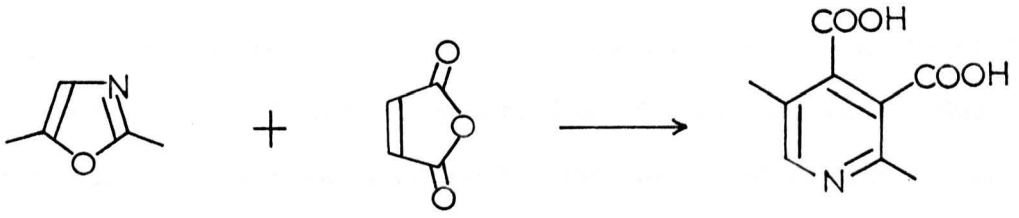


Fig. XIX

Because of the lack of success of these chemical methods it was decided to try and bring about the reaction by photochemical means. It is known that the Diels-Alder reaction can proceed by a radical mechanism and it was hoped that irradiation with light of a suitable wavelength would promote the reaction.

The most common solvents used in photochemical reactions are saturated hydrocarbons such as n-hexane. This is because of their low chemical reactivity and non-absorption of U.V. light. Unfortunately, cyclopentene-3:5-dione was only partially soluble in n-hexane although 2:5-dimethyl oxazole dissolved completely. The dione did dissolve to a greater extent in ether but there was a risk in using this solvent of the

possible formation of explosive peroxides if oxygen was not rigorously excluded from the reaction mixture. Aromatic solvents such as benzene would not have been suitable since they absorb U.V. light in the same region of the spectrum as oxazoles and might compete in the reaction.

It was decided to use n-hexane as solvent with the expectation that the dione would go into solution as it reacted. The solvent was purified by running it through an alumina column (to remove unsaturated hydrocarbons) and de-gassed by bubbling nitrogen through it for 30 min. This was to remove oxygen, which is a radical scavenger, and might interfere with the reaction. Equimolar quantities of reactants were added to n-hexane and nitrogen was bubbled through the solution for 20 min. The solution was irradiated for 18 hr. with a medium pressure mercury arc lamp. After 3 hr. irradiation a sample of the solution was removed, solvent evaporated, and the n.m.r. spectrum of the residue examined. Both this and VPC analysis of the sample of the solution showed that no reaction had occurred after 3 hr. After 18 hr. irradiation hexane was removed but only a small quantity of residue remained and this was found to be unchanged dione. The main product was a red-brown amorphous powder, insoluble in hexane, which did not melt up to 320°. This was thought to be a product of the polymerisation of the dione.

The same reactants were also irradiated in the absence of solvent by a super-pressure mercury arc lamp emitting a wavelength continuum. The irradiation was performed both at room temperature and at 40°. No reaction occurred at room temperature, but at 40° the mixture

solidified to a dark-red mass which was shown to be the same as the amorphous powder obtained previously. Further photochemical methods were not attempted.

EXPERIMENTAL

Preliminary Notes

Melting-points were determined on a Kofler block and are uncorrected.

Infrared absorption spectra were measured on Perkin Elmer 257 and Unicam SP200G grating spectrometers. The spectra were determined as liquid films (film) or in solution (e.g. $CHCl_3$). Ultraviolet spectra were determined on a Unicam SP700 instrument.

Nuclear magnetic resonance (n.m.r.) spectra were recorded on a Perkin Elmer 60 mc. instrument and are quoted as 'tau' (τ) values from an internal tetramethyl silane standard (10.00τ).

Microanalyses were carried out on an F & M carbon/hydrogen/nitrogen analyser by Mr. John Boulton of Keele University.

Analytical vapour phase chromatography (VPC) was performed on a Pye series 104 instrument using a 4' x $\frac{1}{8}$" spiral glass column packed with chromosorb G (treated with dimethyldichlorosilane) coated with 5% S.E. 30 silicone grease. The mobile phase was nitrogen with a flow rate of 50 $ml.min^{-1}$.

Preparative VPC was carried out on a Pye series 105 instrument using a 15' x $\frac{1}{4}$" spiral glass column packed with 60/72 mesh diatomaceous earth (DMCS-treated) impregnated with 25% S.E. 30 silicone grease. The mobile phase was nitrogen with a flow rate of about 200 $ml.min^{-1}$.

Optical rotations were determined in chloroform unless otherwise indicated.

Mass spectra were determined on an MS9 instrument and on a LKB 9000 combined gas chromatograph - mass spectrometer.

Separation of the Volatile Neutral and Basic Components of *Tecoma stans* (Juss.)

General Extraction Procedure

The ground plant material was exhaustively extracted with hot ethanol or methanol (ca. 15 l.), the extract concentrated in vacuo and absorbed on Celite. The Celite was stirred with successive portions of water (once) and N hydrochloric acid until the extracts gave no further precipitate with Meyer's reagent. The extracts were bulked and basified (with dilute ammonia or sodium carbonate) and extracted with chloroform. The chloroform solution was evaporated in vacuo and the residue distilled. The fraction, b.p. 80 - 120°/1 mm., was dissolved in absolute alcohol (150 ml.) and treated with alcoholic picric acid to give a picrate, called A_1 picrate. The filtrate was diluted to 500 - 600 ml. and treated with a further quantity of alcoholic picric acid to give tecomanine picrate. The filtrate, after removal of both picrates, was evaporated, basified (LiOH) and extracted with chloroform. The chloroform was distilled to yield a dark residue which was chromatographed on Florisil or separated by preparative vapour-phase chromatography.

This procedure indicates the general approach adopted in the extraction of the *Tecoma stans* plant. The conditions that were used for

each particular sample of the plant and the quantities of plant and extract involved are summarised in table 2.

Table 2

Plant Source	Ground Plant Weight	Extraction Solvent	Alkaloid Fraction	Boiling Range	A_1 Picrate	Tecomanine Picrate
Cuba	12 kg.	EtOH	8.5 g.	80-134°/ 0.25 mm.	-	6.1 g.
Florida (1)	20 kg.	EtOH	20.1 g.	80-120°/ 0.1 mm.	6.2 g.	8.5 g.
Florida (2)	20 kg.	EtOH	22.5 g.	85-130°/ 0.05 mm.	-	7.9 g.
Mexico (Leaves)	10 kg.	MeOH	3.1 g.	80-135°/ 0.2 mm.	-	2.0 g.
(Roots)	9 kg.	MeOH	3.9 g.	80-145°/ 0.05 mm.	-	5.7 g.

Cuban Sample of *Tecoma stans* (Juss)

Column chromatography of fraction: b.p. 80 - 100°/0.25 mm.

The basic extract (8.5 g.) was chromatographed on a column of Florisil (300 g.) and the column was eluted with benzene and ethyl acetate. The following fractions were collected:

Solvent Volume	Solvent	Fraction Weight	Contents
600 ml.	Benzene	-	non-basic
1400 ml.	Benzene	2.1 g.	Base A
1600 ml.	10% benzene-EtAc.	1.8 g.	Tecomanine (B)
1000 ml.	25% benzene-EtAc.	-	Mixture (some tec.)
1000 ml.	50% benzene-EtAc.	-	Mixture (some tec.)
1400 ml.	Pure EtAc.	1.1 g.	Base C

Examination of the alkaloids isolated by column chromatography.

Base A. (45)

The picrate was prepared in ether and purified by recrystallisation several times from methanol, m.p. 116 - 117°. Attempts to dry at 80° led to decomposition. An analysis sample was dried at

25^{o}/0.2 mm.

Found: C, 49.3, 49.6; H, 4.26, 4.34; N, 14.88, 15.06%

$C_9H_{11}N.C_6H_3N_3O_7$ requires: C, 49.7; H, 3.9; N, 15.5%

$2\ C_9H_{11}N.C_6H_3N_3O_7\ .CH_3OH$ requires: C, 49.2; H, 4.26; N, 14.81%

Treatment of pure A picrate with aqueous lithium hydroxide followed by extraction with chloroform gave a liquid with $\lambda_{max.}$ (EtOH): 259.5, 267 mμ ($\log_{10}$ ε 3.05). Double intensity on acidification. $[\alpha]_D^{24}$ + 3°, $[\alpha]_{36}^{24}$ + 21°, $[\alpha]_{380}^{24}$ + 49° (C, 2.34). For A picrate, n.m.r. ($CDCl_3$): τ 8.44 (3H doublet, J = 6.5 c/s), 2.02 (1H doublet, J = 7 c/s), 1.25 (1H singlet), 1.2 (1H doublet).

Base C. (47)

The picrate was prepared in ether and recrystallised several times from water, m.p. 170 - 170.5°. The free base was regenerated from the picrate and sublimed at 110°/0.25 mm. to give crystals, m.p. 91 - 92°. For base C picrate,

Found: C, 49.43; H, 5.85; N, 13.52%

$C_{17}H_{24}N_4O_8$ requires: C, 49.5; H, 5.87; N, 13.6%

For base C methiodide (prepared in ethanol, needles, m.p. 293-5° (dec)).

Found: C, 44.5; H, 7.48; N, 4.15; I, 38.58%

$C_{12}H_{24}NOI$ requires: C, 44.3; H, 7.44; N, 4.31; I, 39.00%

The molecular weight of the free base, from its mass spectrum was 183.

n.m.r. ($CDCl_3$): τ 9.10, 8.85 (3H doublets, J = 6 c/s), 7.68 (3H singlet)

U.V. spectrum was transparent above 220 mμ.

$\nu_{max.}$ ($CHCl_3$): 3609 cm^{-1} (tertiary OH).

Investigation of the Florida (1) Sample of *Tecoma stans* (Juss)

Alkaloid A_1

The picrate was prepared in ether and recrystallised from methanol, m.p. 179 - 180°.

Found: C, 50.5; H, 5.48; N, 14.5%

$C_{16}H_{22}N_4O_7$ requires: C, 50.26; H, 5.8; N, 14.65%

$C_{16}H_{20}N_4O_7$ requires: C, 50.5; H, 5.3; N, 14.73%

From the mass spectrum of the picrate, molecular weight of the free base = 153 (agrees with $C_{16}H_{22}N_4O_7$ for A_1 picrate).

The free base was recovered from the crude picrate (m.p. 170 - 174°, 500 mg.) by treatment with aqueous lithium hydroxide followed by extraction with chloroform. The yellow oil (180 mg.) obtained was distilled from a bulb-tube at 125 - 130°/13 mm.

$\nu_{max.}$ ($CHCl_3$): 3600 - 3150 (NH), $[\alpha]_D^{22}$ + 35° (Rather inaccurate because of dark-coloured solution)

n.m.r. (CCl_4): τ 9.15 (3H doublet, J = 6 c/s), 9.03 (3H doublet, J = 6.5 c/s), 6.10 (1H broad peak, disappears on shaking with D_2O).

Methylation of alkaloid A_1 to a skytanthine

Alkaloid A_1 (500 mg.) was heated under reflux for 13 hr. with a mixture of formaldehyde (2 ml., 40%) and formic acid (3.1 ml., 98%). Water

was added and the solution was extracted with ether. The aqueous layer was basified with sodium carbonate and extracted with ether. The ether extract was concentrated to 5 ml. and to this was added concentrated ethereal picric acid. The skytanthine picrate (200 mg.) separated on cooling and was recrystallised twice from ethanol, m.p. 139 - 142°. An analysis sample was dried at 80°/0.03 mm.

Found: C, 51.2; H, 5.96; N, 13.8%

$C_{17}H_{24}N_4O_7$ requires: C, 51.5; H, 6.1; N, 14.1%

The concentration of the mother liquors gave another crop of crystals (450 mg.), m.p. 132 - 142°. Recrystallised from ethanol, m.p. 140 - 144° (365 mg.).

A mixed melting-point of the first crop of skytanthine picrate (m.p. 139 - 142°) with an authentic sample of δ-skytanthine picrate supplied by Dr. Casinovi (m.p. 145 - 147°) gave a value, 131 - 142°.

The skytanthine (120 mg.) was regenerated from its picrate (500 mg.) by treatment with aqueous lithium hydroxide followed by extraction with chloroform. It was distilled from a bulb-tube at 105°/15 mm. to give a colourless distillate. $[\alpha]_D^{22}$ + 5.5° (C, 3.3; $CHCl_3$). The $[\alpha]_D$ values for the four synthetic skytanthines are: α, + 79°; β, + 16°; γ, + 59°; δ, + 9°.

Found: C, 78.6; H, 12.93; N, 8.7%

$C_{11}H_{21}N$ requires: C, 78.9; H, 12.6; N, 8.4%

n.m.r. ($CDCl_3$): τ 9.02, 9.15 (two 3H doublets, J = 6 c/s), 7.73 (3H singlet, -N-Me).

Benzoylation of alkaloid A_1

A sample of alkaloid A_1 (110 mg.) was heated with redistilled benzoyl chloride (130 mg.) and dry pyridine (5 ml., distilled over KOH) on a steam-bath for 1 hr. The pyridine solution was cooled and poured into dilute hydrochloric acid (40 ml.) and the acid solution extracted with chloroform. The chloroform extract was washed with sodium carbonate solution and dried over sodium sulphate and after distillation gave an oily residue (108 mg.). The oil failed to crystallise on trituration with light petroleum and leaving several days at 0°.

$\nu_{max.}$ ($CHCl_3$): 1615 (C=O, aromatic amide), 1580 cm^{-1} (C=C).
n.m.r. ($CDCl_3$): τ 9.05, (6H, broad peak), 7.1 (2H triplet, J = 13 c/s), 2.45 (5H singlet, benzoyl group).

A sample of the benzoyl derivative of A_1 (96 mg.) was distilled from a bulb-tube at 155°/0.01 mm. Elemental analysis of the distillate was not quite correct.

Found: C, 80.5; H, 9.18; N, 5.3%
$C_{17}H_{23}NO$ requires: C, 79.33; H, 9.01; N, 5.44%

Dehydrogenation of Alkaloid A_1

Alkaloid A_1 (240 mg.) was heated with Pd/C (100 mg. 10%) at 280° for 30 min. The mixture was extracted with ether to give, on evaporation, an oil (137 mg.). Extraction with hot chloroform yielded a further quantity (75 mg.). VPC analysis of the product indicated that there was still some A_1 present (25%). Most of the excess A_1 (except for 5%) was removed by heating the residue with acetic anhydride (0.5 ml.) on a steam-bath for 5 min., diluting with water and extracting with ether. The aqueous extract was then basified and ether extracted to yield, on evaporation of the ether, a yellow oil (76 mg.). VPC indicated that this was 95% pure.

$\lambda_{max.}$ (EtOH): 263 mμ (ε 2200)

n.m.r. (CCl_4): τ 8.65 (3H doublet, J = 6.5 c/s), 7.78 (3H singlet), 6.7 (1H quartet, J = 6.5 c/s), 1.75 (2H broad peak).

The picrate of the actinidine (120 mg.) was prepared in ethanol and purified by heating twice with animal charcoal in methanol, m.p. 140-145°. Four recrystallisations from methanol gave crystals, m.p. 144 - 146°. An analysis sample was dried at 60°/0.2 mm.

Found: C, 48.2; H, 3.98; N, 14.2%

$C_{10}H_{13}N.C_6H_3.N_3O_7.H_2O$ requires: C, 48.7; H, 4.5; N, 14.2%

Benzoylation of basic extract after removal of tecomanine and alkaloid A_1 as picrates.

A sample of the residue (1.16 g.) was shaken at room temperature for 40 hr. with 10% aqueous sodium hydroxide solution (10 ml.), benzoyl chloride (1 ml.) and dry dimethoxyethane (5 ml.). The solution was diluted with water (75 ml.) and extracted six times with ether. The ethereal layer was washed three times with dilute hydrochloric acid. Evaporation of the dried ether extract gave the neutral material (228 mg.)

$\nu_{max.}$ (film): 3600 - 3100 (N-H), 1715, 1620 cm^{-1} (two kinds of C=0). n.m.r. ($CDCl_3$): τ 9.05 (6H broad peak), 7.15 (triplet, J = 12 c/s), 2.5 (5H singlet, benzoyl group). VPC analysis indicated that there were three components in the neutral fraction.

Basification of the acid extract with sodium carbonate, followed by ether extraction, yielded a basic residue (660 mg.) VPC indicated there were 5 components with one predominating (ca. 60%).

Column chromatography of the basic residue after benzoylation

The basic extract (660 mg.) was dissolved in benzene and run onto a column of Florisil (20 g.) and eluted successively with benzene and benzene-ethyl acetate mixtures. The following fractions were collected:

fraction	solvent	weight	contents
1 - 8	Benzene	0.2 g.	mixture
9 - 16	10, 20% benzene-EtAc	0.21 g.	mostly base C
17 - 28	25, 50% benzene-EtAc	0.12 g.	mixture
25 - 35	pure EtAc	0.08 g.	Base C + ketone

Fraction 15 contained pure **base** C (VPC showed one peak)

$\nu_{max.}$ (CCl_4): 3609 (tertiary OH), 1100 cm^{-1} (C-O).

n.m.r. ($CDCl_3/CCl_4$): τ 9.08, 8.80 (two 3H doublets, J = 6 c/s), 7.75 (3H singlet, N-Me) Signal due to hydroxyl proton not visible. There was no apparent change in the spectrum on shaking with D_2O.

Base C picrate was made in ether, recrystallised from water, m.p. 170 - 172°.

Benzoylation of the bulk of basic residue

The bulk of the basic extract (10.2 g.) was benzoylated as above by shaking at room temperature with 10% sodium hydroxide solution (100 ml.), benzoyl chloride (10 ml.) and dimethoxyethane (50 ml.) for 28 hr. The solution was diluted with water, extracted with ether, the ether extract in turn extracted with acid and the acid layer basified with sodium carbonate. Extraction of the basified extract with ether gave a basic residue (6.3 g.). The neutral fraction (1.7 g.) was obtained on evaporation of the initial ether extract.

Column chromatography of the bulk of the basic extract after benzoylation

The basic extract (6.35 g.) was chromatographed on Florisil (250 g.) and eluted successively with benzene and benzene-ethyl acetate mixtures. The following fractions (100 ml.) were collected.

fraction	solvent	weight	contents
1 - 8	Benzene	nil	-
9 - 43	Benzene	2.00 g.	Base C + benzoate
44 - 59	10% EtAc-benzene	2.1 g.	Base C + mixture
60 - 75	25% EtAc-benzene	1.3 g.	Base C + mixture
76 - 87	50% EtAc-benzene	0.38 g.	Base C + mixture
88 - 101	Pure EtAc	0.88 g.	Base C + mixture

Base C benzoate (amorphous solid)

$\nu_{max.}$ (CCl_4): 1715 cm^{-1} (C = O, ester)

n.m.r. ($CDCl_3$): τ 9.1, 9.0 (two 3H doublets, J = 6 c/s), 7.7 (3H singlet, $-\overset{|}{N}-Me$), 2.6 - 2.2 (3H multiplet), 1.9 - 1.6 (2H multiplet).

Investigation of Mexican sample of *Tecoma stans* (Juss)

Basic extract obtained from the leaves.

Vapour-phase chromatography (VPC) indicated that the basic fraction (8.7 g.) contained about 8 compounds. Most of these were shown to be neutral compounds carried over in the acid extraction by treating the basic extract with 4N hydrochloric acid, basifying and extracting with ether. The basic residue obtained this time (2.01 g.) showed two main and two minor peaks on VPC analysis. The predominant peaks had retention times: 2.8, 8.0 min. The compounds were called R_1, R_2 respectively. (Retention times were measured at an oven temperature of 144°, a nitrogen flow-rate of 50 ml.min^{-1}, measured at the operating temperature, with 1.5 µl of solution injected each time). With constant use, the permeability of the column packing increased slightly and effected the retention times of samples. To counteract this, before each analytical run, the conditions were adjusted so that a reference sample of the extract gave known peaks having retention times the same as those recorded at the start of the work. In this way it was possible to identify peaks in an unknown chromatogram with reasonable certainty by comparison of their retention times with those of the reference sample.

Preparative vapour-phase chromatography of the basic residue.

An analytical run on the preparative instrument of the basic extract (after removal of neutral material) at 200° showed the presence of two main components. The peak separation on the chromatogram (9 min.) was considered sufficient to allow a good separation on a preparative scale. The preparative separation was carried out isothermally at 200° with a nitrogen flow-rate of about 200 ml.min^{-1} and a sample volume, injected manually, of 500 mg. (neat alkaloid mixture). The fractions were collected in U-tube traps, cooled in a dry ice-acetone bath, and examined by analytical VPC. In this trial preparative run the fraction weights were not recorded. VPC analysis indicated that two of the fractions were pure. The remainder of the basic residue (1.4 g.) was chromatographed under the same conditions and the following fractions collected:

Fraction	Weight (mg.)	Alkaloids present (R_1,.... R_4) Retention time in brackets (min.)
1	20	pure R_1 (2.8)
2	5	R_2 (5.4) + 10% impurity
3	50	R_3 (8.0) + 10% R_4 (9.4)
4	3	not examined
5	26	pure R_1 (2.8)
6	10	R_2 (5.4) + 10% impurity
7	76	R_3 (8.0) + 10% R_4 (9.4)
8	10	R_3 (8.0) + 10% impurity
9	33	pure R_1 (2.8)
10	10	R_2 (5.4) + 10% R_1 (2.8)
11	90	R_3 (8.0) + 10% R_4 (9.4)
Total	335	

The recovery from the column, ca. 25%, was rather low and could be accounted for by the following losses:

1) Unavoidable loss of 25% due to the inefficiency of the collection procedure.

2) High loss due to some decomposition of the alkaloids at the high column temperature (200°).

3) Loss in injection and in transfer from traps.

Examination of the pure fractions from preparative VPC

Base R_1 (Fractions 1, 5, 9)

This was shown to be 100% pure from VPC, with a retention time of 2.8 min. The following spectral characteristics indicated that R_1 was 4-nor-actinidine. (45)

$\lambda_{max.}$ (EtOH) 258, 266 mμ, increase in intensity in acidic ethanol.

$\nu_{max.}$ ($CHCl_3$): 1600 cm^{-1} (C=C), otherwise rather featureless.

n.m.r. ($CDCl_3$): τ 8.65 (3H doublet, J = 8 c/s), 2.7 (1H doublet, J = 6 c/s), 1.4 (1H singlet), 1.45 (1H doublet J = ?)

R_1 picrate was prepared in ether, m.p. 135 - 137° and recrystallised from methanol. An analysis sample was dried at room temperature/0.5 mm., m.p. 135 - 137°.

Found: C, 49.4; H, 3.9; N, 15.2%

$C_{15}H_{14}N_4O_7$ requires: C, 49.7; H, 3.9; N, 15.5%

n.m.r. ($CDCl_3$): τ 8.5 (3H doublet, J = 7 c/s), 2.1 (1H doublet, J = 5 c/s), 1.25 (1H singlet), 1.2 (1H doublet, J = ?).

Base R_3 (Fractions 3, 7, 11)

VPC analysis indicated that this was 85% pure with a retention time of 8.0 min. Spectral data showed that the base was boschniakine (39).

$\lambda_{max.}$ (EtOH) 235, 265, 271.5 mμ, $\lambda_{max.}$ (acid EtOH) 257.5 mμ.
$\nu_{max.}$ ($CHCl_3$): 2750 (C-H, aldehyde), 1700 (C=O), 1585 cm^{-1} (C=C).
n.m.r. ($CDCl_3$): τ 8.62 (3H doublet, J = 7 c/s), 1.23 (1H singlet), 1.01 (1H singlet), - 0.45 (1H singlet).

R_3 picrate was prepared in ether, m.p. 120 - 128° and recrystallised from methanol, m.p. 134 - 136°.

Preparation of boschniakine semicarbazone.

Semicarbazide hydrochloride (90 mg.) and crystalline sodium acetate (140 mg.), in a small amount of water, was added to the crude alkaloid (90 mg.) and the mixture heated on a steam-bath for 2 min. On cooling, a white precipitate (56 mg.) separated out. It was recrystallised from ethanol, m.p. 217 - 220° (dec.).

$\lambda_{max.}$ ($CHCl_3$): 3510, 3405, 3325 (NH), 1690 (C=O), 1610, 1560 cm^{-1} (C=C).

An authentic sample of boschniakine semicarbazone was obtained

from Dr. Sakan, m.p. 217 - 221° (dec.). A mixed melting-point with R_3 semicarbazone (m.p. 217 - 220° dec.) had the value 216 - 219° (dec.).

Base R_2 (51)

This base (500 mg.) was obtained by chloroform extraction of the initial basic extract after it had been treated to remove neutral material and ether extracted. VPC analysis showed it was ca. 90% pure and had a retention time of 5.4 min.

$\nu_{max.}$ ($CHCl_3$): 3600 cm^{-1} (O-H)
n.m.r. ($CDCl_3$): τ 9.05 (doublet), 8.85 (doublet), 8.40, 8.30 (singlets), 7.70 (singlet), 6.30 (broad peak, disappears on shaking with D_2O).

Acetylation of R_2.

Base R_2 (15 mg.) was dissolved in acetic anhydride (0.3 ml.), the mixture heated on a steam-bath for 5 min., diluted with ice-water, basified with sodium carbonate and extracted with chloroform. Evaporation of solvent left a residue (12 mg.). VPC showed disappearance of most of the peak at 5.4 min., appearance of peak at 10.8 min. The acetylated product was extracted with 4N hydrochloric acid and then with chloroform. There was no residue in the chloroform extract. Basification of the acid extract, followed by chloroform extraction gave the acetylated product (12 mg.).

$\nu_{max.}$ ($CHCl_3$): 1715 cm^{-1} (O-C=O)

n.m.r. ($CDCl_3$): Appearance of a new peak at τ 7.97, disappearance of the broad peak at τ 6.30.

VPC analysis showed the acetylated product to be 70% pure. A sample (60 mg.) was distilled from a bulb-tube at 110 - 120°/0.1 mm. but the distillate was identical to the starting-material.

Column chromatography of crude base R_2.

VPC analysis of the crude extract indicated that it contained the following alkaloids: 4-nor-actinidine, R_1, 2.8 min., (1%), base R_2, 5.4 min.,(90%), boschniakine, R_3, 8.0 min., (2%), Tecomanine, R_4, 9.4 min., (7%). The relative proportion of each alkaloid was determined by comparing peak areas.

The crude extract (280 mg.) was chromatographed on Florisil (15 g.) and the column eluted successively with benzene, ethyl acetate, acetone and methanol.

fraction	solvent	weight	content
1 - 8	Benzene	nil	-
9 - 12	5% EtAc-Benzene	9 mg.	mostly tecomanine
13 - 19	10% EtAc-Benzene	41 mg.	tecomanine, boschniakine
20 - 28	50%, 100% EtAc-Benzene	80 mg.	Base R_2
29 - 46	EtAc/Acetone mixtures, acetone, methanol.	120 mg.	mixtures

The fractions 30 - 46 contained a light brown amorphous powder which did not melt up to 320° and was not investigated further.

Base R_2 (51)

VPC analysis indicated that it was ca. 99% pure (impurity was 4-nor-actinidine).

$\nu_{max.}$ (film): 3600 - 3150 (OH, bonded), 1115 cm^{-1} (C-O).

$\nu_{max.}$ ($CHCl_3$): 3650, 3578 (OH, non-bonded), 3500 - 3100 (OH, bonded, no change on dilution).

n.m.r. ($CDCl_3$): τ 9.05 (doublet, J = 6 c/s), 8.7 (singlet), 7.7 (singlet), 7.2 (broad peak, disappears on shaking with D_2O), (figure VII).

Attempted sublimation of Base R_2.

A sample of R_2 from the column (35 mg.) was heated slowly up to 100°/0.2 mm. Some white crystals appeared on the cold-finger condenser (2 mg., m.p. 60 - 85°). They were contaminated with an oil which had condensed with them. This method of purification was not pursued further.

Mexico Root Extract.

The dried, ground root (9 kg.) was extracted in a similar manner to the leaves and yielded 3.9 g. of alkaloids on distillation. Two fractions were collected, b.p. 80 - 115°, 115 - 145°/0.05 mm. VPC analysis of the fractions indicated that they were predominantly tecomanine.

Fraction 1: tecomanine (9.4 min.), 80%, unknown base (7.2), 2%, base R_2 (5.4), 4%, 4-nor-actinidine (2.8), 10%.

Fraction 2: tecomanine (9.4 min.), 90%, unknown base (7.2), 6%.

Addition of ethanolic picric acid to each fraction produced a precipitate of tecomanine picrate, (4.1, 1.62 g., respectively).

Investigation of whole plant extract from Florida (2).

The basic extract from the second sample of *Tecoma stans* (Juss) from Florida was distilled at 0.05 mm. to yield three fractions: up to 85° (3.37 g.), 85 - 95° (7.2 g.), 95 - 130° (12.04 g.). VPC analysis of these gave the following results:

Fraction 1: 4 main peaks, retention times (min.) 3.6 (with a pronounced shoulder), 5.6, 7.6, 9.4 (tecomanine) in the approximate ratio; 1:7:3:8.

After removal of most of the tecomanine as its picrate, ratio was 1:6:3:1.

Fraction 2: 4 main peaks, retention times (min.), 3.6 (with shoulder), 5.6, 7.6, 9.4 (tecomanine) in the approximate ratio, 1:6:2:9.

After removal of most of the tecomanine as its picrate, the ratio was 1:6:2:1.

Fraction 3: 3 main peaks, retention times, 5.6, 7.6, 9.4 in the ratio 3:1:10, i.e. mainly tecomanine. Not treated with ethanolic picric acid.

Preparative vapour phase chromatography of the basic extract from the Florida (2) sample of *Tecoma stans* (Juss)

Suitable conditions for separation of the alkaloids by VPC were

determined by an analytical run on the preparative instrument. The preparative separation was carried out by injecting, manually, a sample of the basic residue after removal of most of the tecomanine as its picrate (400 mg.), dissolved in acetone (1.0 ml.) at a column temperature of 192° and a nitrogen flow-rate of 200 ml.min^{-1} (measured with a bubble flowmeter). The total retention time of the mixture was similar on both analytical and preparative instruments. The fractions collected were examined by analytical VPC. Two quite pure compounds were obtained of retention times 5.6, 7.6 min.

A second preparative run was carried out under identical conditions and the weight of all the fractions collected in the two runs amounted to only 100 mg., giving a recovery from the column of approximately 12%. A dozen more preparative runs were carried out and attempts were made to improve the recovery from the column. For example, several of the runs were performed with the injection heater half-on and with a lower column temperature in the hope of preventing or at least decreasing the decomposition of the alkaloids at the head of the column. In two of the runs the system began to leak and most of the sample was lost. Table 3 summarises the conditions employed and the weights of material collected in all the preparative separations.

Table 3.

Run	Column Temp.	Injection heater setting	Nitrogen flow-rate ($ml.min^{-1}$)	Fractions collected (weight in mg.) (purity)					Recovery from column	Comments
				2.1 min.	3.6 min.	5.6 min.	7.6 min.	9.4 min. (tecomanine)		
1	192°	max.^{m}	200	-	6 75%	-	10 98%	not weighed	12%	
2	192°	max.^{m}	200	-	-	20 98%	8 98%	"	12%	
3	190°	max.^{m}	200	-	4 75%	20 >99%	12 98%	"	12%	
4	164°	max.^{m}	220	-	14	87	35	"	30%	Collected both runs in same traps.
5	166°	max.^{m}	220	-	92%	>99%	98%	"		
6	170°	Half-on (4)	200	5 60%	18 60%	81 98%	32 95%	20 92%	40%	
7	175°	(4)	220	-	11 50%	50 >99%	23 90%	17 95%	40%	Drastic cutting of fractions to improve purity.
8	175°	(4)	220	-	-	26 95%	-	-	-	Started to leak half-way through the run.
9	175°	(4)	220	-	-	60	24	not	25%	
10	170°	(4)	220	-	-	95%	75%	weighed		
11	170°	(4)	220	-	-	-	-	-	-	Immediate leak, no collection.
12	172°	(8)	200	-	-	30 99%	10 93%	not weighed	25%	
13	170°	(5)	200	-	-	16 >99%	7 90%	"	25%	Drastic cutting of fractions for mass-spectrum samples.

Examination of alkaloids separated by preparative VPC.

Alkaloid X (Retention time: 5.6 min., VPC showed purity >99%)

This compound was a white crystalline solid which went purple on exposure to air and light, m.p. 87 - 94°.

$\nu_{max.}$ ($CHCl_3$): 3650, 3580 (OH, non-bonded), 3500 - 3000, (OH, bonded, no change on dilution), 1092 cm^{-1} (C-O).

n.m.r. ($CDCl_3$): τ 9.05 (3H doublet, J = 6.5 c/s), 8.75 (singlet over a multiplet), 8.32 (singlet over a multiplet), 7.73 (3H singlet), 7.2 (1H broad peak, disappears on shaking with D_2O), (figure X).

The methiodide was made in ethanol by adding methyl iodide and heating the mixture for 5 min. on a steam-bath, then cooling, when white crystals appeared, m.p. 325 - 330° (dec.). Recrystallised from ethanol, m.p. 310 - 312° (dec.), darkened at 287°.

Found:	C, 44.4;	H, 7.25;	N, 4.3%	
$C_{12}H_{24}NOI$ requires:	C, 44.3;	H, 7.4;	N, 4.3%	

The mass spectrum of the free alkaloid showed a strong molecular ion peak at m/e 183 (100% base peak) and the other main peaks were: 166 (40), 150 (70), 122 (37), 107 (45), 98 (38), 85 (40), 83 (58), 74 (90), 72 (54), 70 (63), 55 (90), 45 (32), 39 (54), 29 (72), 27 (58). No metastable ions.

Alkaloid Y (Retention time: 7.6 min.)

VPC showed purity was about 97%.

$\nu_{max.}$ ($CHCl_3$): 3660 (OH, non-bonded), 3600 - 3000 (OH, bonded, no change on dilution), 1125, 1090 cm^{-1} (C-O).

n.m.r. ($CDCl_3$): τ 8.94 (3H doublet, J = 6 c/s), 8.81 (3H triplet (?), J = 7 c/s), 8.34, 8.17 (two 1H doublets, J = 3 c/s), 7.68 (3H singlet), 6.35 (2H quartet, J = 7 c/s), 5.78 (1H broad peak), 4.44 (1H singlet with slight splitting, J = 2 c/s), (fig. XI).

Unable to prepare picrate in ether or methiodide in ethanol. Heating the base with methyl iodide in ethanol on a steam-bath for $1\frac{1}{2}$ hr. led to its decomposition.

Base, retention time 3.6 min. (liquid)

VPC indicated that it was 92% pure.

$\nu_{max.}$ ($CDCl_3$): 3600, 3400 (OH, non-bonded), 3600 - 3450 (OH, bonded, no change on dilution), 1708 (C=O), 1355 cm^{-1} (?).

n.m.r. ($CDCl_3$): τ 9.02 (triplet, badly resolved), 7.83 (singlet, N-Me), 7.17 (singlet).

Base, retention time 2.1 min. (liquid, 60% pure)

$\nu_{max.}$ ($CHCl_3$): 1728, 1712 cm^{-1} (two C=O ?)

Attempted Synthesis of the Pyrindane Ring System.

Preparation of the components for the attempted Diels-Alder reaction.

Preparation of Bromoacetone.

Bromine (177 ml.) was added with stirring over 1-2 hr. to a mixture of water (800 ml.), acetone (250 ml.) and glacial acetic acid (186 ml.) maintained at ca. 65°. The mixture was stirred for a further 20 min. and then diluted with water (400 ml.), cooled to 10°, made neutral to Congo Red with solid anhydrous sodium carbonate (500 g.) and the oil which separated was collected and dried over anhydrous calcium chloride. The oil was distilled and the fraction boiling at 38 - 48°/ 13 mm. was collected. (Yield 230 g., 50%).

Aminoacetone hydrochloride. (59)

Phthalimide (77 g.) and sodium hydride (24 g. of 50% dispersion in oil) were boiled and stirred, under nitrogen, in dimethoxyethane (500 ml.) for 2 hr. Bromoacetone (70 g.) was added with stirring over 2-3 hr. and the mixture boiled overnight. It was concentrated and the almost pure phthalimido-acetone (203 g.) filtered off. This was heated under reflux with 7N hydrochloric acid (1250 ml.) for 2 hr. The mixture was allowed to cool and re-heated for a further 2 hr. The mixture was cooled, phthalic acid removed and the filtrate evaporated to complete dryness at reduced pressure at 40 - 50°. The residue was heated with absolute alcohol (200 ml.) and 3N alcoholic hydrogen chloride (20 ml.) and ammonium chloride filtered

off and washed with boiling alcohol (50 ml.) into the filtrate which was evaporated to dryness. The treatment was repeated, yielding a very viscous brown oil which was dried over concentrated sulphuric acid in vacuo. It was then heated at 40 - 50°/0.5 mm. for 1 hr. to remove final traces of alcohol. It failed to crystallise. (Yield: 47 g., 50%).

Ethoxalyl chloride.

Phosphorus pentachloride (200 g.) was powdered and mixed with excess diethyl oxalate (180 g.) and heated under reflux with stirring on a steam-bath for 2 hr., with the exclusion of moisture. Phosphorus oxychloride was distilled using a Fenske column (27°/18 mm.) and the residue heated with Pd/C (0.5 g., 10%) at atmospheric pressure to 95 - 98° until no more ethyl chloride was evolved. Fractional distillation gave ethoxalyl chloride, b.p. 129° (110 g.). This was contaminated with diethyl oxalate (186°). The ethoxalyl chloride was purified by redistillation (80 g.).

Ethoxalylaminoacetone. (58)

Aminoacetone hydrochloride (63 g.) was suspended in dry benzene (200 ml.). Ethoxalyl chloride (80 g.) was added with continuous stirring and the mixture was heated under reflux for 5 hr. The benzene was removed in vacuo and water (150 ml.) added to the dark green residue. The mixture was extracted with chloroform, the extract dried over sodium sulphate and,

on evaporation, yielded ethoxalylaminoacetone as a dark brown oil (75 g.). This was purified by distillation at 124 - 134°/0.3 mm.

$\nu_{max.}$ ($CHCl_3$): 1740 - 1690 cm^{-1} (C=O)

n.m.r. ($CDCl_3$): τ 8.6 (3H triplet, J = 7 c/s), 7.7 (3H singlet), 5.65 (2H doublet), 5.55 (2H quartet, J = 7 c/s), 2.05 (1H broad multiplet).

Attempted cyclisation of ethoxalylaminoacetone to 5-methyl-2-carbethoxy oxazole (57, R = Et).

The following reagents and reaction conditions were tried:

i) Ethoxalylaminoacetone (1.3 g.) was heated for 15 min. with concentrated sulphuric acid (7 ml.) on a steam-bath, the mixture cooled, poured onto ice (100 g.), basified with sodium carbonate and extracted with chloroform. Evaporation of the solvent left starting material (0.2 g.). The aqueous extract was then made neutral with acid and re-extracted to give an oil (100 mg.).

n.m.r. ($CDCl_3$): τ 8.6 (triplet, J = 6.5 c/s), 7.5 (singlet), 5.65 (distorted quartet, J = 6.5 c/s), 2.95 (broad peak), 2.4 - 1.3 (several multiplets), - 1.35 (broad peak).

ii) Ethoxalylaminoacetone (1.9 g.) was heated at 140 - 150° for 15 min. with polyphosphoric acid (30 g.). The mixture became homogeneous.

It was poured onto crushed ice (150 g.) and extracted with chloroform. The residue (0.8 g.) after removal of solvent was distilled from a bulb-tube at 110 - 130°/0.05 mm.

n.m.r. ($CDCl_3$): τ 8.55 (distorted triplet), 7.6 (3H singlet), 5.6 (distorted quartet, <2H), 2.95 (broad peak, <1H). Small signals due to unchanged starting material at τ 7.7, 2.1.

iii) Ethoxalylaminoacetone (5.1 g.) in dry benzene (10 ml.) was cooled to 0° and stirred magnetically. Phosphorus pentachloride (14 g.) was added over 10 min., with stirring, and the mixture heated under reflux for 30 min. The solution became homogeneous after 15 min. The benzene was distilled, the residue added to crushed ice (150 g.) and the aqueous solution was extracted with chloroform. A white solid (200 mg.) was filtered off, m.p. 119 - 122° (dec.). This had:

n.m.r. (T.F.A.): τ 7.27 (3H singlet), 2.23 (1H singlet)

$\lambda_{max.}$ (EtOH) 248 mμ (ε 11,000), $\lambda_{max.}$ (acid EtOH) 253 mμ, $\lambda_{max.}$ (alkaline EtOH) 238 mμ.

The chloroform extract yielded a mixture (1.83 g.) containing mostly starting material.

iv) Ethoxalylaminoacetone (0.7 g.) and phosphorus pentachloride (1 g.) were heated on a steam-bath for 30 min., then at 150° for 10 min. Water

(50 ml.) was added and the mixture heated for 5 min. on the steam-bath. More water (100 ml.) was added and the solution extracted with chloroform to yield, on evaporation, a residue (0.23 g.), n.m.r. spectrum indicated very little cyclisation had occurred.

v) Ethoxalylaminoacetone (0.6 g.) was dissolved in phosphorus oxychloride (10 ml.) and the mixture heated under reflux for 1¼ hr. The phosphorus oxychloride was removed by distillation under reduced pressure, water added to the residue, and the aqueous solution extracted with chloroform. The residue after removal of solvent was found to be a mixture of starting material and the cyclised product.

n.m.r. ($CDCl_3/CCl_4$): τ 7.5 (singlet, methyl of oxazole), 7.7 (singlet, methyl of starting material) in the ratio 4:1. 8.7 - 8.4 (two overlapping triplets), 5.65 - 5.25 (two overlapping quartets), 2.92 (slightly split singlet), 2.14 - 1.96 (multiplet).

The reaction product from v) was purified by distillation from a bulb-tube at 120 - 145°/10 mm. to give the required 5-methyl-2-carbethoxy oxazole (60 mg.) as a yellow oil, m.p. ca. 10° (57, R = Et). A larger sample of ethoxalylaminoacetone was treated with phosphorus oxychloride and the reaction product (4.8 g.) was distilled at 15 mm. to give the following fractions:

1. 128 - 137°, almost pure oxazole (1.05 g.)
2. 137 - 160°, 95% oxazole, 5% ethoxalylaminoacetone (0.97 g.)
3. 165 - 185°, 40% oxazole, 60% ethoxalylaminoacetone (0.86 g.)

Fraction 1, almost pure 5-methyl-2-carbethoxy oxazole,

$\nu_{max.}$ (CCl_4): 1742 cm^{-1} (C=O),

$\lambda_{max.}$ (EtOH) 255 mμ (ε 2200), $\lambda_{max.}$ (acid EtOH) 257 mμ (ε 1700)

n.m.r. (CCl_4): τ 8.55 (3H triplet, J = 7 c/s), 7.53 (3H singlet),

5.55 (2H quartet, J = 7 c/s), 3.00 (1H singlet).

A larger sample of 5-methyl-2-carbethoxy oxazole (9.74 g.) was prepared by heating the remaining ethoxalylaminoacetone under reflux with phosphorus oxychloride, distilling the product, collecting the required fractions and treating the remaining impure fractions again with phosphorus oxychloride.

Mercuric chloride complex of 5-methyl-2-carbethoxy oxazole.

A sample of the oxazole was dissolved in a small quantity of 95% ethanol and the solution added to cold, aqueous mercuric chloride solution to produce an immediate white precipitate of the mercuric chloride complex, m.p. 110 - 113° after recrystallisation twice from ethanol.

Found: C, 11.4; H, 1.21; N, 1.97%

$C_7H_9NO_3.2HgCl_2$ requires: C, 11.9; H, 1.3; N, 2.0%

$C_7H_9NO_3.2HgCl_2.H_2O$ requires: C, 11.7; H, 1.53; N, 1.95%

Preparation of isonitrosoacetone.

A mixture of ethyl acetoacetate (100 g.), potassium hydroxide (50 g.) and water (1800 ml.) was stirred at room temperature for 24 hr. Sodium nitrite (62 g.) in water (200 ml.) was added, the mixture cooled to 5-6° and 20% sulphuric acid (430 g.) added. The solution was neutralised with 30% sodium hydroxide solution and crushed ice, extracted with ether, the aqueous extract acidified at 5° by 20% sulphuric acid and ether extracted. The product, white crystals, had m.p. 62-8°, (43 g., 70%).

2:5-dimethyl oxazole. (56)

To isonitrosoacetone (43 g.) in a mixture of acetic anhydride (150 ml.) and glacial acetic acid (150 ml.) was added sodium acetate (3.5 g.) and a few crystals of mercuric chloride, then zinc dust (100 g.) in small portions, with cooling. The mixture was boiled for 30 min., cooled, zinc acetate filtered and the precipitate washed with acetic acid. The solution was evaporated on a steam-bath at water-pump pressure to yield the crude acetylamino ketone (67 g.). This was dissolved in acetic anhydride (67 g.) and a mixture of concentrated sulphuric acid (37 ml.) and acetic anhydride (67 g.) was added, with cooling. The solution was warmed to 80 - 90° on a water-bath for 30 min. and solvent distilled. The residue was treated with ice-water (200 ml.) and the solution neutralised with 40% aqueous sodium hydroxide (ice-cold) and then steam-distilled to

yield, on saturation of the distillate with sodium carbonate, the oxazole as an oil. This was distilled from solid potassium hydroxide at 25 - 26°/8 mm. (8.7 g.).

n.m.r. ($CDCl_3$): τ 7.68, 7.55 (two 3H singlets), 3.28 (1H slightly-split singlet).

Cyclopentadiene.

Technical dicyclopentadiene (110 g.) was distilled through a short, well-lagged vigreux column and the fraction boiling over 38 - 48° collected in a receiver cooled by an ice-salt mixture. Distillation was continued until about two-thirds of the dicyclopentadiene had been pyrolysed. The cyclopentadiene collected (65 g.) was stored at dry-ice temperatures.

3:4- and 3:5-cyclopentenediols.

Peracetic acid (76 g., 40%) was added, with stirring over 30 min. at 20°, to a mixture of cyclopentadiene (56 g.), anhydrous sodium carbonate (106 g.) and methylene chloride (500 ml.). The mixture was stirred for a further hour at room temperature. Solid was removed by suction filtration and the filter-cake washed with methylene chloride. The combined filtrate and washings were added, with rapid stirring over 1 hr., to cold water (500 ml. at 5 - 10°). The stirring was continued for 1 hr. at room temperature. The methylene chloride layer was extracted twice with water (25 ml.) and the aqueous extracts were combined and the water removed on

a rotary evaporator at 40°/30 mm. The residue (30 g.) was distilled at 94 - 114°/1 mm. to give the mixed diols as a pale yellow oil (22.6 g.).

Cyclopentene-3:5-dione. (55)

A solution of chromium trioxide (50 g.) in concentrated sulphuric acid (80 ml.) and water (225 ml.) was added, with stirring, to a mixture of 3:4 and 3:5 cyclopentenediols (22.6 g.), water (100 ml.) and methylene chloride (150 ml.) which had been cooled to between -5 and 0° by dry ice/MeOH/H_2O/Acetone. The temperature was maintained at this value during the addition and also while the mixture was stirred for a further hour. Then more methylene chloride (100 ml.) was added and the resulting mixture stirred for 10 min., the organic layer separated, dried over magnesium sulphate and concentrated under reduced pressure at room temperature. The product, yellow plates, had m.p. 31 - 35° (10.3 g.) n.m.r. ($CDCl_3$): τ 7.05, 2.52 (two 2H singlets).

Attempted Diels-Alder Reaction to Produce the Pyrindane Ring System

The following reactants and conditions were tried:

a) Cyclopentene-3:5-dione (0.5 g.), 2:5-dimethyl oxazole (0.5 g.) and hydroquinone (0.1 g.) were dissolved in dry benzene (10 ml.) and the solution heated under reflux for 5 hr. A small amount of brown solid was filtered (ca. 10 mg.) and the benzene removed on a steam-bath. An n.m.r. spectrum of the residue showed that no reaction had occurred.

b) Reaction a) was repeated in dry toluene. The solution was extracted with dilute hydrochloric acid to remove any excess oxazole. The toluene was distilled on a steam-bath at reduced pressure. A residue (80 mg.) was shown to be the starting dione. The acidic extract was basified with sodium carbonate and extracted with chloroform. Evaporation of solvent left a small amount of material (40 mg.) shown to be unchanged oxazole. No further material was obtained on saturating the aqueous extract with sodium carbonate and re-extracting with chloroform.

c) Cyclopentene-3:5-dione (0.5 g.) was dissolved in 2:5 dimethyl oxazole (0.5 g.) and the mixture heated to 100° and maintained there for 30 min. A small sample was removed and its n.m.r. spectrum showed no reaction had occurred. The mixture was heated under reflux (ca. 115°) for 1½ hr. It formed a black amorphous powder which did not melt up to 320°.

d) Maleic anhydride (0.5 g.), 2:5 dimethyl oxazole (0.5 g.) and hydroquinone (0.1 g.) were dissolved in dry benzene (10 ml.) and the mixture heated under reflux for 5 hr. The benzene was removed by steam-distillation. On cooling the residue, white crystals appeared (45 mg.), m.p. 262° (dec.).

n.m.r. (T.F.A.): τ 7.25, 6.81 (two 3H singlets), 1.00 (1H singlet).

No more solid was obtained on evaporating the residue to dryness. Water was added and the solution extracted with chloroform. Boiling down the chloroform extract yielded a mixture of solid and liquid, mostly maleic acid.

e) Maleic anhydride (0.6 g.) and 5-methyl-2-carbethoxy oxazole (0.6 g.) were dissolved in dry benzene (10 ml.) and the mixture heated under reflux for 5 hr. The solvent was steam-distilled and yellow crystals appeared in the residue (0.5 g.), m.p. 134 - 138°. These were shown to be maleic acid, m.p. 131°. Extraction of the filtered aqueous solution with chloroform produced a small residue (40 mg.) which was shown to be unchanged oxazole. The benzene distillate was separated from the aqueous layer and extracted with 5N hydrochloric acid. The acid extract was neutralised with sodium carbonate, saturated with sodium chloride and ether extracted to yield, on removal of solvent, a brown oil (160 mg.), shown to be unchanged oxazole ester.

Photochemical Methods

a) Cyclopentene-3:5-dione (0.6 g.) and 2:5-dimethyl oxazole (0.6 g.) were dissolved in n-hexane (120 ml.) which had been purified by passage through an alumina column and de-gassed by bubbling nitrogen through it for 30 min. The dione was only partially soluble in the n-hexane. The solution was irradiated for 18 hr. under nitrogen with a medium pressure mercury arc tube which emitted predominantly radiation of wavelengths 254, 265, 297, 313 and 366 mμ. A sample of the solution was removed after 3½ hr. An n.m.r. spectrum showed it to contain only starting materials. Distillation of the hexane at the end of the irradiation gave a small residue (90 mg., unchanged dione). The predominant product was a dark-brown amorphous powder which failed to melt up to 320°.

b) Cyclopentene-3:5-dione (1.5 g.) was dissolved in 2:5-dimethyl oxazole (1.5 g.) and nitrogen bubbled through the solution for 30 min. in a quartz cell. The mixture was irradiated for 2 hr. under nitrogen with a super-pressure mercury arc lamp, emitting a wavelength continuum. An n.m.r. spectrum of a sample taken after 2 hr. indicated no change. Irradiation for a further 3 hr. failed to produce any reaction.

The procedure was repeated at a temperature of 40°. After 2 hr. the solution solidified to a dark-red mass which was shown to be the same material as the brown amorphous powder formed in a). Photochemical methods were not further pursued.

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INTRODUCTION

The Chemistry and Stereochemistry of Eperuic Acid and Related Diterpenoids

INTRODUCTION

The wallaba tree, *Eperua falcata*, with other members of the *Eperua* genus, occurs extensively in British Guiana. Although used locally as a timber for constructional purposes and also as a fuel, uses in other fields have been investigated because of its relatively common occurrence, e.g., for paper-making[1] and as a tanning agent.[2] Attempts to find other uses for it have been largely unsuccessful.[3]

Treatment of the heartwood with boiling alcohol yields considerable amounts (ca. 25%) of a reddish phenolic resin, but no chemically homogeneous material has been isolated from this. The resin has found use in the production of high-grade metallurgical coke by destructive distillation.

Making incisions in the living tree produces a pale oleoresin which may be collected as it exudes from the tree. This is quite distinct from the phenolic material mentioned above. King and Jones[4] have shown that this consists largely (ca. 85%) of a monocarboxylic diterpene, eperuic acid. Other work[40] has shown that the remaining 15% of the exudate is neutral material containing a C_{20} monocyclic alcohol very readily cyclised to a bicyclic alcohol and a smaller quantity of another compound which is probably an acyclic secondary alcohol.

Extraction of the wood from various parts of the tree with light petrol yielded a pale, viscous resin similar to the exudate which

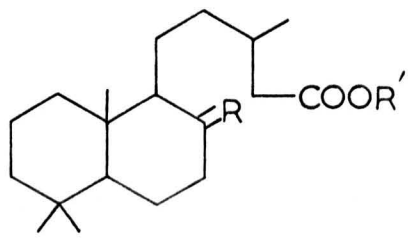
has been shown to contain eperuic acid.[4] The proportion of non-acidic material obtained from the wood was approximately the same as from the exudate (10-15%) but none of the C_{20} monocyclic alcohol was found. The neutral fraction was shown[5] by chromatography on alumina to consist of four major components; one of these was established as oleyl alcohol and another as a mixture of high-boiling esters formed from oleyl alcohol and diterpene acids. A second alcohol and a hydrocarbon were also isolated, but were not further examined.

The occurrence of oleyl alcohol and its esters in combination with terpenoids in vegetable matter is unprecedented. The higher aliphatic alcohols are found in nature mainly in some marine animal oils. Oleyl alcohol is the most important of this group and occurs as the major constituent of the higher alcohols present in the head and blubber oils of the sperm whale and the porpoise. It has also been reported as existing in the heart of whale and the sea-anemone. Although oleyl alcohol has not been reported previously in vegetable matter, lower unsaturated straight-chain alcohols (e.g., $C_9H_{17}OH$) have been detected in the growing leaves of a number of plants and higher saturated alcohols (C_{24}- C_{26}) are found in plant cuticle waxes.

Although petroleum extracts from heartwood, sapwood and branch-wood from the same sample of wallaba appear to contain the same acidic constituents, different samples of wood afford different acid mixtures. It seems likely that the variation in acid content arose because the samples of wood come from trees cut at different times of the year or of

a slightly different variety. The constitution of the neutral fractions, unlike the acid fraction, appears to be independent of the sample of wood.

The structure of eperuic acid, which has been isolated from both the exudate and petrol extracts of the wood, was established by King and Jones[4] as (1, R = CH_2, R' = H).

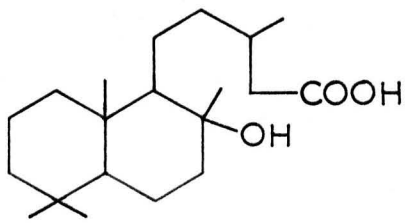


(1)

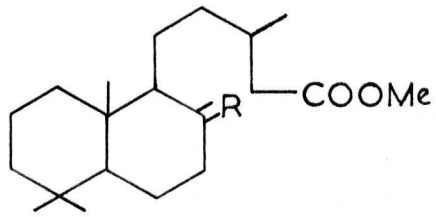
Selenium dehydrogenation of its methyl ester yields naphthalene derivatives, including 1:2:5-trimethyl naphthalene. The ozonolysis products of methyl eperuate are formaldehyde, formic acid, and a keto-ester $C_{20}H_{34}O_3$, and, when the latter is dehydrogenated, pimanthrene (1:7-dimethylphenanthrene) is obtained. These indications of a relation to the manool-agathic acid group of terpenes were substantiated by stepwise oxidation of dihydroeperuic acid, through an acid $C_{19}H_{34}O_2$ and methyl ketone $C_{18}H_{32}O$, to a C_{17} acid which yielded 1-ethyl-2:5 dimethyl naphthalene when aromatised and decarboxylated with selenium. These reactions show that eperuic acid has structure (1).

The acid was conveniently purified through its methyl ester (1, R = CH_2, R' = Me) and proved to be stereochemically homogeneous.

Cocker and Halsall[6] have isolated from gum labdanum a diterpenoid hydroxy acid, labdanolic acid, and have shown that its structure (2) is closely related to that of eperuic acid.[7] Dehydration of methyl labdanolate yielded a homogeneous product (3, R = CH_2) which proved to be identical with methyl eperuate except in its stereochemistry.



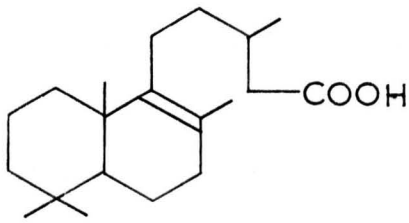
(2)



(3)

They also showed that on treatment of both eperuic acid and dehydrolabdanolic acid or their esters with acid, the same double-bond isomer (4), ignoring the stereochemistry, was formed.

Further work on gum labdanum by Halsall and Moyle[8] led to the isolation of a second diterpene carboxylic acid shown to be 6-oxocativic acid (5).



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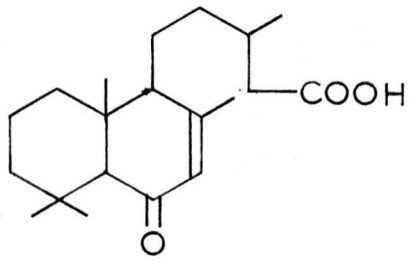
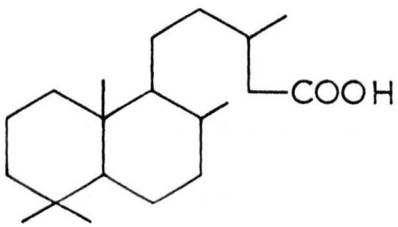
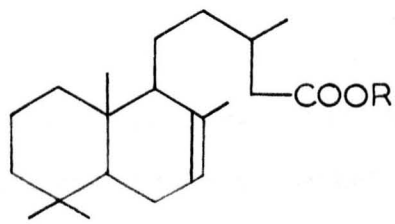


(5)

This was reduced to dihydrocativic acid (6) by a Wolff-Kishner reduction under vigorous conditions. This proves that the carbon skeleton of the oxo-acid is the same as that of both labdanolic and eperuic acids.



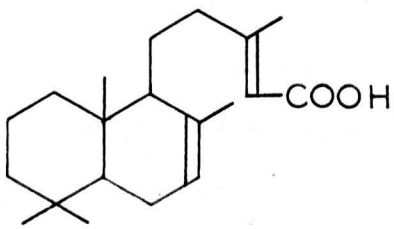
(6)



(7)

The structure of cativic acid itself, (7, R = H) was established by Zeiss and Grant[9] using similar methods to those already described and they also showed that it was isomeric with eperuic acid but has the same configuration for rings A and B as labdanolic acid. Unlike eperuic acid, it could be obtained in a crystalline form, m.p., 80 - 82°.

Nakano and Djerassi[10] have obtained from the resin of Brazil Copal a mixture of diterpene acids which they have called copalic acid. They showed that it is a mixture consisting of the acid of structure (8) and its double-bond isomers. (Vapour phase chromatography showed three major components with relative proportions 5:3:2, respectively).



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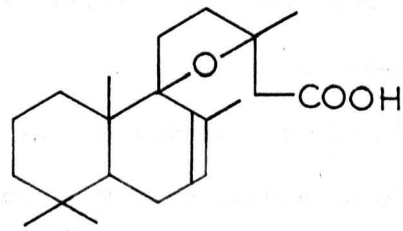


(9)

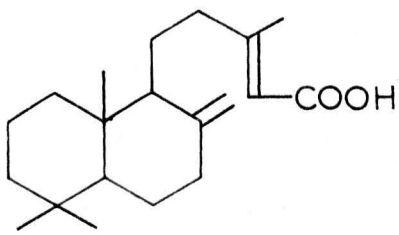
The double-bond in the side-chain conjugated with the carbonyl group, was selectively reduced in the methyl ester to give methyl dihydrocopalate which on ozonolysis yielded a mixture of keto-esters from which were obtained two oximes. One of these was identified with the oxime obtained from the keto-acid (1, R = O, R' = H) showing that copalic acid has the same carbon skeleton as all the related diterpene acids discussed above.

Panizzi, Mangoni and Belardini[11] have reported the isolation of a new diterpenoid acid related to the above acids, grindelic acid, obtained from the resin of *Grindelia robusta* which belongs to the *Compositae* family.

They have shown it to have the structure (9).

As the acid is readily esterified (unlike related acids with the ester grouping on the C-4 carbon atom) and selenium dehydrogenation gives, as it does with eperuic acid, 1:2:5-trimethylnaphthalene, it was assumed to belong to the class of bicyclic diterpenoid acids discussed here.

More recently Sandemann, Bruns and Reichelm[12] have extracted the wood of *Oxystigma oxyphyllium* with ether-benzene mixtures and obtained a mixture of diterpene acids which they have shown consists of eperuic acid (1, R = CH_2, R' = H), an acid related to eperuic acid containing two double-bonds (10) and another acid which they were unable to obtain pure, which they believe may be a double-bond isomer of (10) possibly with the same structure as the main constituent of copalic acid (8).

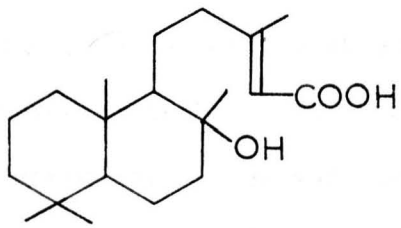


(10)

They separated the methyl esters of the acids by chromatography on silver nitrate-impregnated silica and elucidation of the structure of the esters was dependent mainly on n.m.r. and i.r. spectral evidence and also on their mass spectra.

The acid (10) has also been isolated by Hugel et. al.[12a] from

the trunk oleoresin of *Trachylobium verrucosum*, a large tropical tree native to East Africa and Madagascar. The resin was also found to contain labdanolic acid (2) and a diterpene acid related to labdanolic acid but containing a double-bond in the side-chain. This was shown to have the structure (10a).



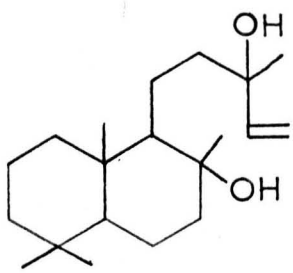
(10a)

Related Diterpenoids

There are a number of diterpenes which are related quite closely to the family of resin acids dealt with above. The chemistry of some of these can be found in standard texts[13,14]. A comprehensive review by Tsutsu[15] covers a large number of diterpenoids and selected topics have been dealt with in more detail by Barltrop and Rogers.[16]

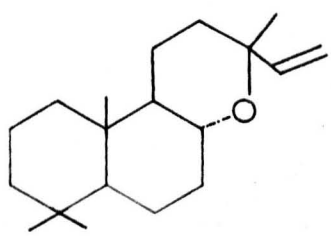
One related group of diterpenoids which has been known for much longer than the eperuic-labdanolic acid group includes sclareol, manool, manoyl oxide and ketomanoyl oxide and these were isolated and assigned structures between 1928 and 1936.

Sclareol[13] was first isolated from the leaves of *Salvia sclorea L.* by Volmar and Jermstad and later shown to have the structure (11). Both manoyl oxide (12) and ketomanoyl oxide (13) were obtained from the wood of silver pine (*Dacrydium Colensoi*) by Hosking and Brandt,[13] who later established their structures. These workers also isolated manool[13] (14) from the wood oil of the yellow pine (*Dacrydium biforme*) and from this same plant Carmen and Grant[17] obtained biformene (15), another related diterpenoid.



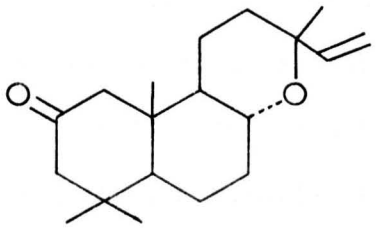
(11)

Sclareol



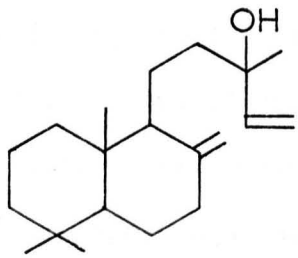
(12)

Manoyl oxide



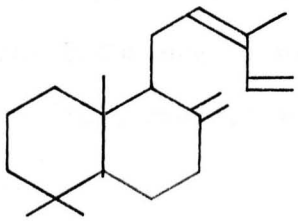
(13)

Keto-manoyl oxide



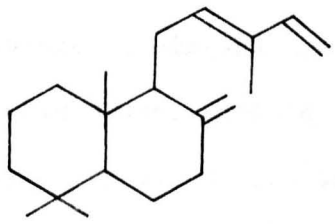
(14)

Manool



(15)

Biformene



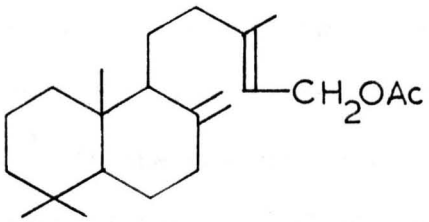
(16)

Cis-biformene

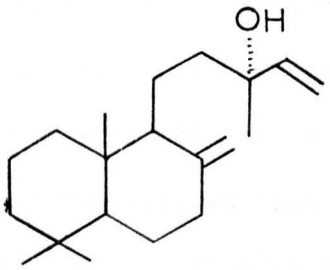
The same general methods have been used in determining the structures of all these compounds. In all cases catalytic hydrogenation and selenium dehydrogenation were used. In addition, the formation of solid hydrohalides was employed to relate one terpene to another. Another useful technique was degradative oxidation with ozone or permanganate, especially before the use of i.r. spectra, to determine the kinds of double-bond present. The position of the keto group in ketomanoyl oxide was confirmed in 1960 by Grant and Hodges[18] by deuteration of the α-carbon atoms. Hodges[19] has compared the rotatory dispersion curves of 2-ketodihydromanoyl oxide and its 1- and 3-keto isomers.

As mentioned above, both manool and biformene occur in the same plant (*D. biformene*) and from biogenetic considerations the naturally occurring biformene is expected to be a mixture of cis-and trans-isomers, (15) and (16), respectively, and the synthetic product should contain both isomers. Carmen and Dennis[20] have recently synthesised both the cis and trans forms, obtaining the pure isomers by chromatography over alumina impregnated with silver nitrate. They also showed that Canada Balsam contains cis-biformene but no detectable amounts of the trans-isomer.

Another source of diterpenes related to the labdanolic-eperuic acid family is Siberian larch resin. Russian workers[21] have chromatographed the diterpene content of this tree on alumina and obtained fractions containing labda-8(20)-13-diene-15-ol acetate (17a), impure biformene (15) and 13-epimanool (17b). They were able to purify the biformene fraction by chromatography on silver nitrate-impregnated alumina.



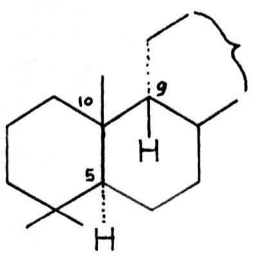
(17a)



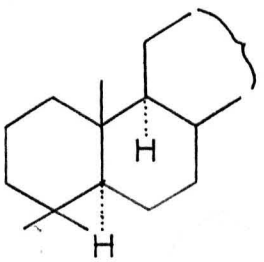
(17b)

Stereochemistry

Although the biogenetic isoprene rule[22] has been used with some success in correlating stereochemical aspects of triterpene chemistry it was not until 1963 that similar success was attained in the diterpene family.[23] In the past there was a reluctance to exercise rigorous application of the rule of antiparallel cationic 1,2-addition to the appropriate C_{20} precursors and this was occasioned by a small but important group of diterpenoids with an apparently[24] exceptional common stereochemical feature. Thus, the proposed configuration at C-5, C-9 and C-10 in each member of this irregular set results in the trans-syn (18) rather than the trans-anti (19) arrangement.



(18)



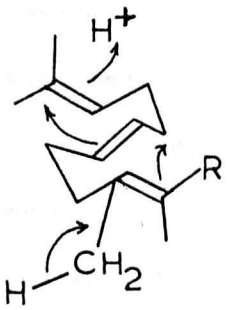
(19)

This group of diterpenes with apparently irregular stereochemistry includes members of the bicyclic, tricyclic and tetracyclic diterpenoids. Eperuic acid is one of the bicyclic diterpenes within this group, but it has now been shown, by work described below, to conform to the biogenetic isoprene

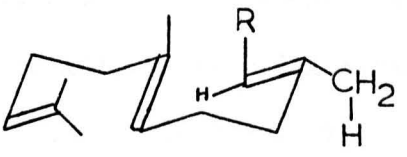
rule in its stereochemistry.

In accordance with this rule rings A, B and C in polycyclic diterpenoids are expected to exhibit trans-anti stereochemistry, resulting from concerted trans-antiplanar cyclisation of a suitably oriented geranyl-geraniol precursor (20).

Now, for this to occur, a pseudo two-chair conformation (21) is required in the C_{20} precursor. Thus, cyclisation of a potential ring B-boat precursor (22) would lead to configuration (23) with a C_5 side-chain (R) in the more encumbered axial position while the product of cyclisation of the two-chair conformation would have a configuration (24).



(20)



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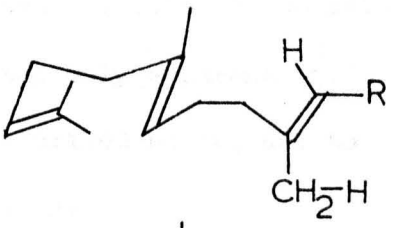
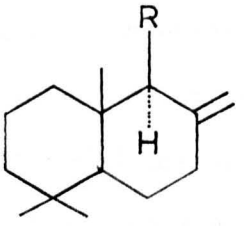


(22)



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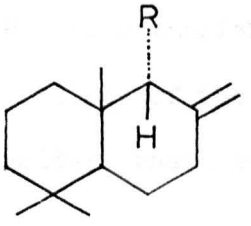


(23)

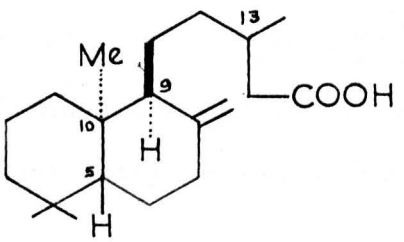
Although apparent analogy for this event might be advocated when one considers the cyclisation of squalene, it must be pointed out that ring B in the triterpenoid-steroid series eventually realises a chair

conformation by rearrangement and so many examples of stereochemistry (24) as a result of cyclisation of (21) are known to render the intervention of (23) an unlikely event. In recent years, several workers[23,30] have corrected the previously assigned stereochemistry of these anomalous compounds and, at the present time, no authentic member of this irregular group exists in the diterpenoid family.

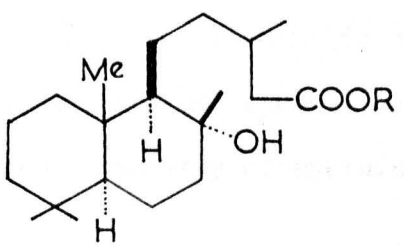
For example, of the diterpenes thought to disobey the biogenetic isoprene rule, cafestol and kahweol,[25] gibberellic acid[26] and rosololactone and its cogeners[27] have been shown to follow the orthodox pattern, largely by the X-ray-crystallographic studies of Scott, Sim and their colleagues. Similarly Isopimaric acid[28] and rimuene[29] have been acquitted as normal.

However, in 1964 there still remained the only apparent exception to the rule, eperuic acid. The work described below, due to Graham and Overton,[30] has since removed this anomaly.

Eperuic acid (originally proposed[31] and for a time accepted stereochemistry (25)[31,32]) had been shown by Djerassi and Marshall[31] to be essentially antipodal to labdanolic acid (26, R = H), of established stereochemistry,[7] by a comparison of the rotatory dispersions of the keto-esters (1, R = O, R' = Me) derived from the two acids; these were found to be near but not complete mirror images.



(25)



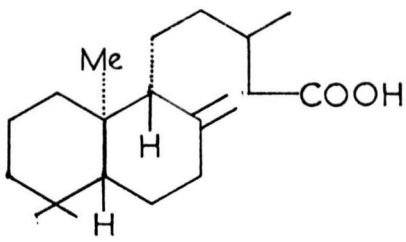
(26)

This minor discrepancy and the more significant difference in the melting-points (223° vs. 190°) of the oximes from the corresponding keto-acids (1, R = O, R' = H) was attributed[31] to a possible difference in configuration at C-9.

This would mean that the side-chain in the keto-ester from eperuic acid, and so presumably in eperuic acid itself, is axially attached, thus resulting in an anomalous trans-syn backbone. Consideration of the conditions employed[4,7] to hydrolyse the two keto-esters prior to oximation (methanolic 2N. potassium hydroxide solution under reflux for 1 hr. and 2 hr. respectively) convinced Graham and Overton that the side-chain in both oximes must have the stable equatorial configuration and that the stereochemical difference in these derivatives must therefore reside at C-13, the only other relevant asymmetric centre. A comparison[7] of the physical constants of the products arising from stepwise degradation of the side-chains in the labdanolic and eperuic series already suggests that the two become completely antipodal as soon as the asymmetry at C-13 is removed.

Graham and Overton confirmed this by a comparison of the oximes from the keto-acids derived from methyl labdanolate, methyl 13-epi-labdanolate and methyl eperuate. The last two gave oximes which were undistinguishable on melting-point, and i.r. spectra and exactly opposite in optical rotation but differed from the oxime from labdanolic acid. This indicated that the keto-acids from labdanolic and eperuic acids are antipodal except at C-13. The revised stereochemistry of eperuic acid

is therefore (27) compared with the original assignment (25).

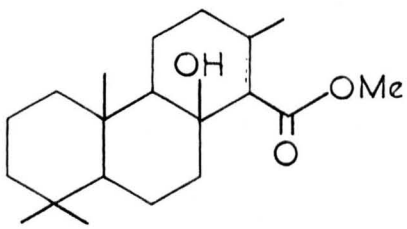


(27)

As mentioned above, the absolute configuration at C-13 is the same in both eperuic and labdanolic acids. Two groups of workers[33,36] have provided evidence in favour of a 13(R)-configuration in labdanolic acid. More recently, Overton and Renfrew[37] have questioned the arguments put forward by these workers in support of their conclusions. Initially, Bory and Lederer[33] had used Stallberg-Stenhagen's observation[38] that in a diastereomeric pair of β-methyl carboxylic acids the more laevorotatory stereoisomer has the (R)-configuration at the β-carbon atom. Bigley, Rogers and Barltrop[36] rightly questioned the validity of such a configurational assignment when based on molecular rotations in situations (such as the present) where intramolecular hydrogen-bonding may be involved. However, they then accepted Bory and Lederer's assignment in the mistaken belief that methyl labdanolate and methyl 13-epi-labdanolate do not exhibit

intramolecular hydrogen-bonding. It is clear from their own observations and from subsequent infrared studies[39] with methyl labdanolate that extensive intramolecular hydrogen-bonding does occur.

On this basis the C-13 configurations in both labdanolic and eperuic acids must be regarded as undefined. Overton and Renfrew[37] were able to produce more convincing evidence in favour of a 13 (R)-configuration in both acids, depending largely on n.m.r. spectral studies of the tricyclic hydroxy esters (28). They also obtained supporting evidence from the antipodal nature of the circular dichroism curves of the bicyclic diketones (29, 30) derived from the two acids and by X-ray analysis of p-bromophenacyl labdanolate.



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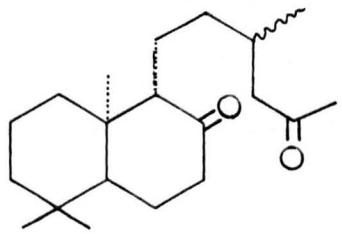

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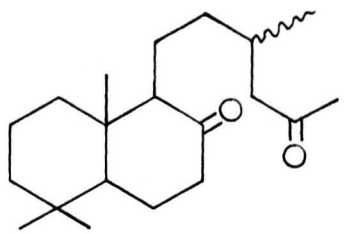


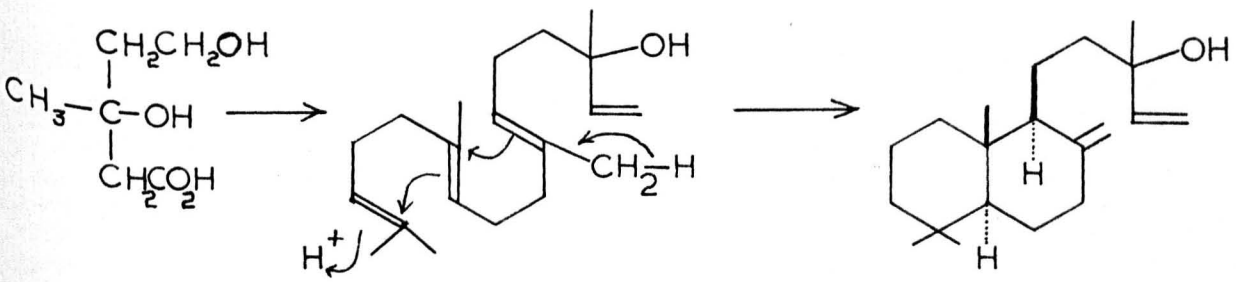
(30)

Biosynthesis

It has been shown by experiments with acetates labelled with ^{13}C or ^{14}C that acetic acid is a carbon source for the biosynthesis of steroids and terpenoids. Subsequent work on the degradation of labelled terpenoids derived from isotopically labelled acetic acid has shown that the two carbon atoms of acetic acid to a very large extent preserve their identity and appear at specific points in the terpenoid skeletons.

The acetic acid is converted via acetate in the form of its coenzyme-A into mevalonic acid (MVA, 30a). Decarboxylation of this molecule leads to a C_5 isoprenoid unit which can condense with itself to form polyisoprenoids, i.e., terpenoids.

According to the biogentic isoprenoid rule, it is geranyl-geraniol (20) or a related system such as geranyllinalool (30b) which is the precursor of diterpenes and from which the latter substances are derived by cyclisation by a cationoid entity, usually a proton, followed where appropriate by skeletal rearrangement, oxidation, etc. Thus, the labdane skeleton (30c) is formed from (30b) by two cyclisations:



(30a) (30b) (30c)

The Acidic Fraction from the Exudate of Eperua Falcata

The acidic terpene fraction was isolated from the pet. ether extract of the exudate by treatment with 0.5N aqueous sodium carbonate and subsequent acidification with concentrated hydrochloric acid to yield, on ether extraction, the acid fraction as a viscous, yellow syrup. The acids were converted to their methyl esters by treatment with ethereal diazomethane. Vapour phase chromatography (VPC) of the ester mixture showed it to consist essentially of one product. From previous work,[4] this was expected to be methyl eperuate (1, R = CH_2, R' = Me).

The ester, isolated from the solvent as a pale yellow oil, was distilled at 0.2 mm. to give three fractions all boiling within the range 149 - 166°, together with a very small quantity of a white, crystalline solid.

VPC analysis of the three fractions showed them to be substantially pure and comparison of infrared (i.r.) and nuclear magnetic resonance (n.m.r.) spectra of each fraction indicated that they were identical. Their optical rotations in carbon tetrachloride were similar (-26.6°, -22.0°, -25.5°) and in reasonable agreement with the known value[4] for methyl eperuate (α_D = -28.2° C, 3.98). Re-determination of the rotation of one of the fractions in chloroform gave a value very close to the literature value. Hence, the above discrepancies are probably attributable to solvent effects.

Fraction A was hydrolysed by 2N. methanolic potassium hydroxide and the cyclohexylamine salt of the resulting acid was prepared. This

was recrystallised to constant rotation and gave a final value (α_D, -29.5°) in close agreement with the known value (α_D, -30.2°)[41] for the cyclohexylamine salt of eperuic acid.

A sample of the acid was esterified using the Fischer method (1% sulphuric acid-methanol). This was done to investigate the likelihood of acid-catalysed isomerisation of eperuic acid to a mixture of its double-bond isomers. The mixed esters from the wood of the tree examined in the course of this work had been produced[40] from their respective acid fractions by the above method of esterification and the resultant mixture of esters may have been due to isomerisation caused by the acidic conditions used. However, the acid fraction from the exudate underwent no double-bond migration and the only product of the esterification was methyl eperuate.

This evidence allows us to make the assumption that the acid fractions from the present samples of the wood of the wallaba tree all contain mixtures of acids and not a single acid as with the exudate.

Attempted Separation of the Branchwood Esters

of

Eperua Falcata

Eperuic acid (α_D, -27°), which was first obtained by King and Jones[4] from the exudate of *Eperua falcata*, has also been extracted by them from samples of the wood. However, from samples of the sapwood they obtained a dextrarotatory acid (α_D, ca. +2°).[41] Samples of sapwood, heartwood and branchwood extracted subsequently have yielded a weakly dextrarotatory acid (α_D, ca. -8°). This section deals with the esters obtained by Fischer esterification of the acid fraction from the branchwood.

Several workers have used chromatography of the methyl esters to purify acids. Cocker, Halsall and Bowers[6] successfully separated both labdanolic and 6-oxo-cativic acids in this way and Panizzi, Mangoni and Belardini[11] used this method to separate grindelic acid. However, Nakona and Djerassi[10] were unable to separate the methyl esters of the acids in the mixture of double-bond isomers obtained from Brazil Copal. VPC of methyl copalate showed the presence of at least three components (ca. 50%, 30% and 20% of the whole). Similarly, Hugel, Oehlschlager and Ourisson[12a] were unable to separate double-bond isomeric esters from *Trachylobium verrucosum* by repeated chromatography on silica gel impregnated with silver nitrate. In the present work the same difficulties were experienced in attempts to separate mixtures of diterpenoid esters.

The mixture of esters from branchwood was shown by VPC analysis to consist of three components in the relative proportions 4:3:1, the major component being methyl eperuate. A sample of the esters was

chromatographed on a column of active alumina. The first 12 fractions collected, eluted by pet. ether, contained only a small amount of material which was shown by VPC to be identical to the original ester mixture. Nothing else came off the column although it was eluted successively with petrol-benzene mixtures, benzene, chloroform and ethanol. The conclusion drawn from this was that the esters had undergone hydrolysis to their parent acids on the column. This method of separation was abandoned.

Our next attempt at separation of the mixed esters was by means of preparative layer chromatography (PLC). Preliminary tests to determine suitable conditions were carried out on thin-layer plates. These were eluted with benzene and then with benzene-ethyl acetate mixtures. Although some slight separation was achieved (about four spots could be observed) it was considered insufficient to warrant repeating the procedure on a preparative scale.

Work being done simultaneously by A.D. Healey on the sapwood esters had indicated that the main constituents of these esters were methyl eperuate and possibly methyl cativate (7, R = Me) in the ratio 4:5. If the principal component was methyl cativate then it was likely (by comparison of VPC retention times) that there was a significant amount of this ester in the branchwood esters. Another approach to the problem of separating the mixed esters was based on the fact that silver nitrate forms a loose π-complex with compounds containing olefinic double-bonds. The esters in the mixtures, being double-bond isomers, might be expected

to form complexes of varying strength, depending on the substitution at the double-bond. It was hoped that this effect could be made use of by chromatographing samples of the esters on PLC plates impregnated with silver nitrate when the differences in strength of the complexes with the silver ions in the thick-layer would allow separation.

Again, preliminary tests were performed on TLC plates. Various eluting solvents were tried but no separation was achieved. This may have been due to the inability to prepare good silver nitrate-impregnated layers at this time.

At a later date it was possible to prepare more satisfactory TLC and PLC plates impregnated with silver nitrate (see experimental section). Therefore, further efforts to separate the mixed esters by this method were made. The branchwood ester mixture was subjected to both TLC and PLC using a wide selection of solvents and solvent mixtures as eluent. The results of this work are discussed below.

To find a suitable eluting solvent samples of the mixed esters were dissolved in ether and spots of the solution applied to TLC plates. The spots were eluted radially with various solvent mixtures including pet. ether-benzene, propyl alcohol-glacial acetic acid, pet. ether-ether, benzene-ethanol and hexane-ether. Two of the solvent mixtures, pet. ether-ether (8:2) and benzene-ethanol (95:5) appeared to give a suitable separation on TLC plates.

Branchwood ester samples were developed on TLC plates using these two solvent mixtures. Pet. ether-ether gave the better separation.

Benzene-ethanol (95:5) appeared to be too polar. On changing the solvent ratio to (100:1) an excellent separation was achieved with the latter solvent pair.

Now that a suitable eluting solvent had been found the separation was attempted on PLC plates of 1 mm. layer thickness. The absorbent used in the slurry contained fluorescent material and this allowed the progress of the bands of sample up the plate to be followed by observing them under u.v. light when they showed up as a brown band on a green background.

A sample (300 mg.) was applied to the plates and movement of the band was achieved by elution with benzene-ethanol (100:1). The upper band contained 200 mg. of material which was seen from VPC analysis to consist of at least three components. A lower band contained only 10 mg., shown to consist of four components. It was decided that this development was too rapid to allow separation and a less polar solvent mixture would have to be used.

Development five times with ether-pet. ether (5:95) lead to an apparently good separation. However, recovery of material from the plate was again rather poor, there being only 23 mg. in the lower band (VPC:two components) and 28 mg. in the upper band. VPC analysis of the upper band did show it to be reasonable pure so the procedure was repeated on two more PLC plates to obtain a larger sample of pure material.

Spectra of Material Eluted from Upper and Lower Bands.

Lower band.

The infrared spectrum was very similar to that of methyl eperuate, having carbonyl stretching absorption at 1745 cm^{-1} and C-H out of plane deformation of $=CH_2$ at 890 cm^{-1} (very strong).

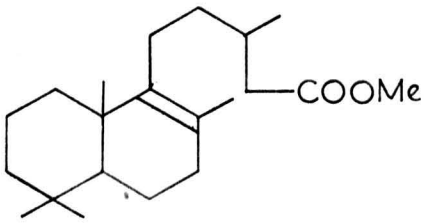
Similarly, a comparison of its n.m.r. spectrum with that of methyl eperuate (from ref. 12) indicated it was mostly this ester. (VPC had shown about 20% impurity).

Upper band.

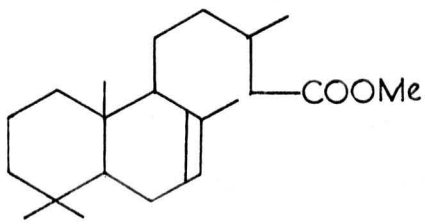
The material eluted from the upper band, which was shown by VPC analysis to contain up to 15% impurity, had a similar infrared spectrum to methyl eperuate except for the absence of bands at 3080 and 890 cm^{-1} (characteristic of the exomethylene group). However, there was a strong absorption band at 860 cm^{-1} which may be due to a tri-substituted double-bond. The absorption pattern in the C-O stretching region (1250 - 1050 cm^{-1}) was slightly different to that of methyl eperuate.

The significant difference in the n.m.r. spectrum when compared with that of methyl eperuate was a 3H-singlet at 8.4τ not present in the spectrum of methyl eperuate. This could be due to a methyl group on a double-bond. Another significant feature of the spectrum was the absence of any signal due to an exomethylene group (5.1, 5.4τ).

From this spectral data two possible structures can be considered. (A, B)



(A)



(B)

(A) is methyl dehydro-labdanolate and (B) is methyl cativate. As noted above, the branchwood esters have been shown by VPC analysis to consist of three components in the ratio 4:3:1, the major component being methyl eperuate. Since PLC has allowed separation of the methyl eperuate the material in the upper band should be a mixture of esters in the ratio ca. 3:1. Therefore, the content of this upper band could be a mixture of (A) and (B) in the ratio 3:1 or 1:3. VPC analysis of the material in the upper band showed a mixture of two components in the approximate ratio 4:1.

In structure (A) the double-bond is adjacent to the bridge-head methyl group on C-10 and it would be expected to de-shield the methyl group so that its signal would appear at lower field in the spectrum than it does in methyl eperuate (9.3τ). The spectrum of the upper band material has no signal at 9.3τ but does have a 3H-singlet at 9.15τ (as well as at 9.05, 9.1τ). The double-bond in structure (B) would have a similar de-shielding effect to that in methyl eperuate. (A significant bulge on the side of the 3H-singlet at 9.15τ could be due to the bridge-head methyl in methyl cativate (B) if this ester was the minor component

of this mixture).

Again, comparing the spectrum with that of methyl eperuate, it has a 3-H singlet at 8.4τ which can be assigned to the methyl group on the double-bond in either (A) and (B). The fact that this peak is sharp would point to structure (A) since in (B) it would be expected to be split slightly by allylic coupling with the olefinic proton. The smaller singlet at 8.7τ could then be due to the methyl on the double-bond in (B) (although this peak is not split either). If structure (B) were correct, we would expect absorption at ca. 4.6τ due to the olefinic proton. There is a small, broad signal at 4.3τ and this integrates as $^1/_3$ proton. This would support the theory that (B) was the minor component of the mixture.

The evidence provided by the n.m.r. spectrum is not very conclusive since the material under investigation is a mixture. However, it may be suggested, with reservation, that the components of the branchwood esters are methyl eperuate, methyl dehydro-labdanolate and methyl cativate in the proportions ca. 4:3:1. Conclusive proof would require separation of the esters into pure components and examination of each of these.

Attempted Separation of the Heartwood Esters by a Chemical Method.

VPC analysis showed that the heartwood esters consisted of approximate equal quantities of methyl eperuate and another ester. It was decided to subject the mixture to mild ozonolysis in the hope of forming the keto-ester of methyl eperuate only. If the ozonolysis was mild enough the isomeric ester might not react (since this would involve rupture of one of the carbocyclic rings) and the keto-ester of methyl eperuate could be removed as a suitable derivative. The reagent used to form the derivative of the ketone was Girard's 'T' reagent, since the derivative formed is water-soluble and the non-ketonic material is readily removed by ether extraction.

The ozonolysis was carried out in methylene chloride at -10°. At 2 min. intervals samples of the reaction mixture were removed and subjected to VPC analysis to follow the progress of the reaction. It was found that methyl eperuate was attacked by ozone before the other ester was, but it was difficult to determine at what point all the eperuate had reacted. The fractions removed at 2 and 4 min. showed gradual disappearance of methyl eperuate and formation of its keto-ester; fractions after 4 min. were complex mixtures, indicating that ozone had attacked the other ester.

The ozonolysis was repeated and terminated after 4 min. The reaction mixture yielded 1 g. of residue. This was treated with Girard's 'T' reagent to remove any ketone that had been formed. However, both the

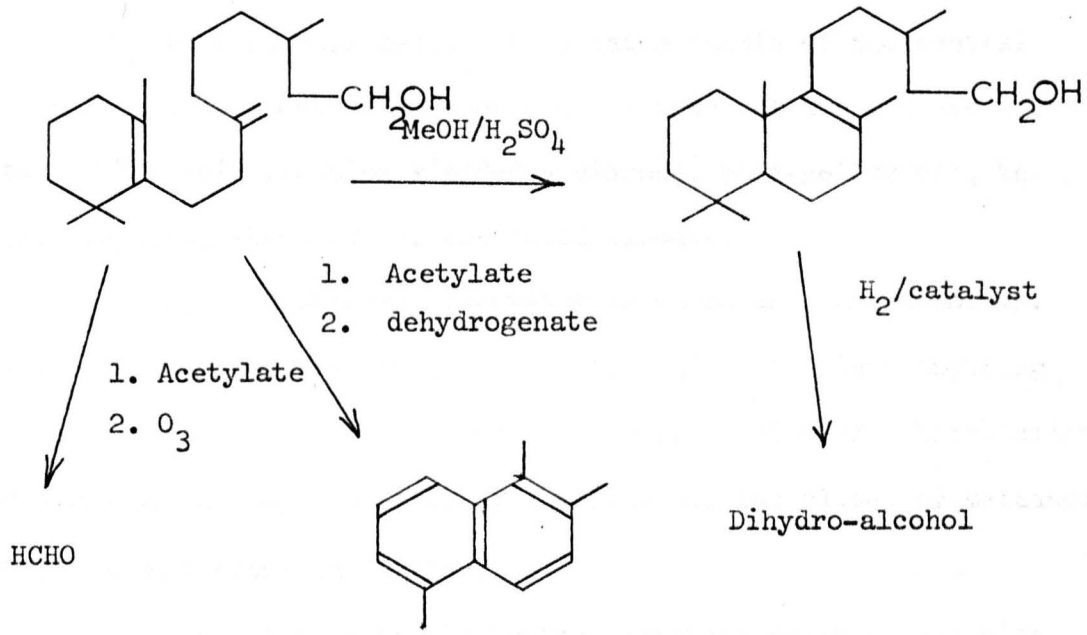
Girard's 'T' extract after decomposition of the complex and the non-ketonic material extracted with ether were found on VPC analysis to be complex mixtures.

A sample of the sapwood esters which had been partially purified by PLC was treated in a similar manner. The ozone was bubbled through the solution for only 1 min. but, again, complicated mixtures were obtained. This method was not pursued any further.

The Neutral Fraction from the Exudate of Eperua Falcata

Investigation of the Neutral Fraction from the Exudate.

From a previous sample of the neutral fraction of the wallaba exudate Dr. G. Jones[40] has isolated a primary alcohol, $C_{20}H_{36}O$ with two double-bonds, one of them exomethylene. Treatment with acid readily converted it into a compound having only one double-bond. Dehydrogenation of the acetylated alcohol yielded 1:2:5-trimethylnaphthalene. He suggests the following explanation:



A smaller quantity of another alcohol, showing infrared absorption at 1100 cm^{-1} (C-O stretching, acyclic secondary alcohol) was also obtained.

Work by Blake and Jones[5] has shown that the neutral fraction obtained from extraction of wood samples of *Eperua falcata* contains four

main constituents:-

1) A hydrocarbon (ca. 4%)

2) A mixture of oleyl esters of acids in the acid fraction (ca. 60%)

3) Oleyl alcohol (ca. 2%)

4) An unidentified alcohol (ca. 20%)

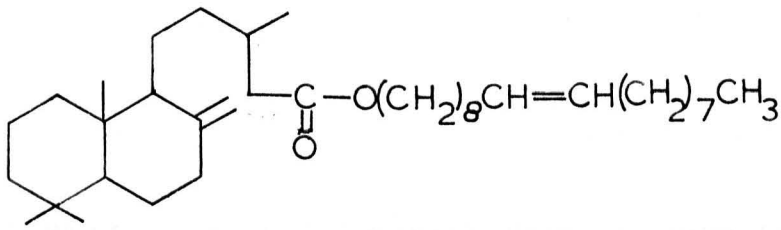
Mixture 2) was found to be inseparable by column chromatography.

The present work deals with a later sample of the neutral fraction from the exudate. The petroleum extract of the exudate after removal of the acid fraction yielded a viscous, pale-yellow oil, the neutral fraction, about 10% of the total exudate.

A sample of this was chromatographed on an alumina column. The first eluting solvent used, pet. ether, failed to elute anything from the column, but benzene eluted a large proportion of a high-boiling ester mixture. A small quantity of a yellow oil was eluted by methanol and this was not examined further.

The present work on the neutral fraction deals solely with the high-boiling ester mixture eluted with benzene. The low volatility of the esters prevented the use of VPC to determine the number of components in the mixture. A comparison of the infrared spectrum of the mixed esters with that of the ester mixture isolated by Blake and Jones, i.e. mainly oleyl eperuate (31), suggested that although the acid

component in the present ester mixture was the same (i.e. eperuic acid), they differed in alcohol component. The infrared spectrum of the ester mixture showed no absorption at 720 cm^{-1}. Absorption at this wavelength is characteristic of a chain of four or more methylenes and is present in the spectrum of oleyl eperuate, since this compound contains such a grouping.

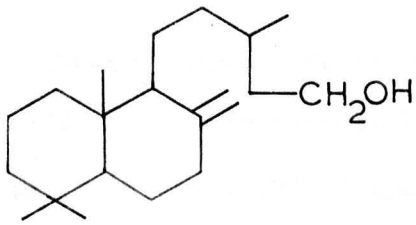


(31)

No n.m.r. spectrum of oleyl eperuate was available for comparison so the n.m.r. spectrum of the involatile esters was compared with one of a synthetic sample of oleyl alcohol. The proton absorption in the olefinic region of the spectrum (ca. 4 - 6τ) were totally dissimilar, the mixed esters showing no signals due to cis hydrogens on a double-bond which appear at 4.6τ in the spectrum of oleyl alcohol. This evidence strongly suggested there were no oleyl esters in the high-boiling ester mixture.

A larger sample of the ester mixture was obtained by chromatography of the neutral fraction. The esters were hydrolysed by 10% ethanolic potassium hydroxide and examination of the i.r. and n.m.r.

spectra of the products indicated that the acid component was eperuic acid and the alcohol component (shown by VPC analysis to be a mixture) may contain eperuyl alcohol (32).

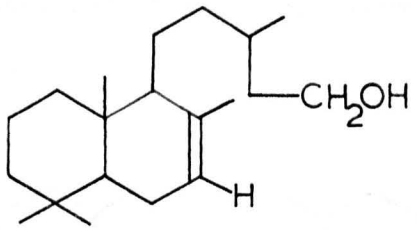


(32)

The i.r. spectrum of the alcohol component showed a strong band at 890 cm^{-1}, suggesting the presence of an exo-methylene group (CH_2=). The rest of the spectrum below 3000 cm^{-1} was very similar to that of eperuic acid and so it was reasonable to suggest that eperuyl alcohol was present in the mixture. This was supported by its n.m.r. spectrum which also showed the alcohol component was a mixture. The high-field signals were very similar to those of eperuic acid and methyl eperuate and there was a triplet at 6.4τ which could be attributed to the methylene group next to oxygen in the side-chain. Signals at 5.2 and 5.5τ could be assigned to the exo-methylene group, the coupling constant (J = 18 c/s) being of the magnitude expected for gem. hydrogens on a carbon-carbon double-bond. The alcoholic hydroxyl group signal does not appear to stand out from the high-field absorption. The ratio of high-field to low-field protons is 8:1. This agrees with the 32:4 ratio expected for the 36 protons of eperuyl alcohol. However, the

only peaks expected for eperuyl alcohol in the middle-field region are those due to the exo-methylene group and the methylene next to the hydroxyl group. The n.m.r. spectrum of the alcohol component of the ester has these signals, 5.2, 5.5 and 6.4τ, respectively but, in addition there is broad absorption at 4.6 - 4.9τ. Integration of the spectrum shows that the signals at 6.4, 5.2, 5.5 and 4.6 - 4.9τ represent about $1^1/_3$, $^5/_6$, $^5/_6$ and $1^1/_3$ protons respectively (making a total of 4 protons) which suggests there is about 70% of eperuyl alcohol present in the alcohol mixture.

The broad signal at 4.6 - 4.9τ could be tentatively assigned to the olefinic proton in cativyl alcohol (32a), one of the double-bond isomers of eperuyl alcohol. This suggestion finds support from the spectrum of cativic acid which has a broad peak centred at 4.6τ.



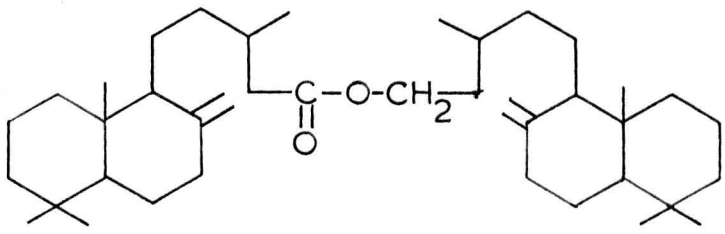
(32a)

The conclusion drawn from this spectral evidence is that the principal alcohol component in the involatile ester mixture from the neutral fraction of the exudate is eperuyl alcohol; there is the possibility that one of the other components is cativyl alcohol, although

the evidence for this is not very conclusive.

The infrared spectrum of the acid component from the ester was identical to that of the crude acid fraction from the exudate (main absorptions:- 3080, 1720, 1650, 1390, 890 cm^{-1}) which has been shown to be eperuic acid.

No n.m.r. spectrum of eperuic acid was available for comparison, but the acid had a very similar spectrum to that of methyl eperuate, having the two signals at 5.2 and 5.5τ due to the exo-methylene group and showing a general similarity in the high-field region (signals at 9.3, 9.2, 9.1τ). Absorption between 6.2 - 6.6τ in the spectrum of the acid suggested the presence of double-bond isomers of eperuic acid as a slight impurity. Thus, hydrolysis of the involatile ester mixture and examination of the products has shown that the principal ester in the neutral fraction of the present sample of exudate is eperuyl eperuate (33) with, possibly, a significant amount of cativyl eperuate present as well.



(33)

This is the first time that eperuyl eperuate has been found in the neutral fraction of either the exudate or wood extracts of *Eperua falcata*.

The only information published concerning the neutral fractions of similar resins is that gum labdanum contains a saturated hydrocarbon (ca. C_{30}) and that cativo gum contains an involatile ester assumed to be cativyl cativate.

An attempt was made to purify the alcohol component of the ester mixture from *Eperua falcata* by chromatography on an alumina column using the usual range of solvents. However, no separation was achieved and the investigation was terminated at this point.

E X P E R I M E N T A L

Melting points were determined on a Kofler block and were uncorrected. Infrared absorption spectra were determined on a Unicam S.P. 200G spectrophotometer and n.m.r. spectra on a Perkin-Elmer 60 mc/s R-10 n.m.r. spectrometer.

Analytical V.P.C. analysis was carried out on a 10 ft. spiral glass column packed with Gaschrom P coated with 1% S.E.30 silicone grease.

Unless otherwise stated rotations refer to solutions in chloroform at room temperature.

All temperatures are measured in degrees centigrade. Unless otherwise indicated petroleum ether refers to 40 - 60° petroleum ether.

In n.m.r. spectra tetramethyl silane (T.M.S.) was used as an internal standard at 10τ.

Extraction of the Exudate from *Eperua Falcata* and Separation of the Extract into Acid and Neutral Fractions

The exudate, a highly-viscous dark resin (75 g.), containing wood chippings, was extracted with petroleum ether (ca. 2 l.) and the wood was removed by filtration (2 g.).

The acid fraction was separated from the neutral by shaking the extract with 0.5 N aqueous sodium carbonate. A thick, persistent emulsion formed which was removed by allowing the solutions to stand several hours in large conical flasks. As the top petrol layer was continually decanted off it was shaken with fresh sodium carbonate solution until no further emulsion formed. The emulsion was washed repeatedly with petrol to remove the neutral component and the washings were in turn treated with sodium carbonate solution. The petrol layers and washings were bulked, dried over sodium sulphate, and the petrol distilled to yield the crude neutral fraction as a pale yellow oil (3.15 g.).

The emulsion, containing the sodium salts of the acids, was acidified with concentrated hydrochloric acid and the petrol layer which formed was separated, washed with water, dried over sodium sulphate, and the solvent distilled. (Crude acid fraction, 68 g.)

Esterification of the acid fraction.

A sample of the crude acids (10 g.) was dissolved in ether (100 ml.), cooled in ice, and treated with a solution of diazomethane in ether at 5° (the diazomethane was prepared by the addition of N-nitroso-methylurea (20 g.) to a mixture of ether (200 ml.) and 40% potassium hydroxide (60 ml.)). Addition of diazomethane was continued dropwise, with swirling, until its yellow colour persisted. The mixture was allowed to stand for 15 min., after which the excess diazomethane was neutralised by a few drops of glacial acetic acid. The ethereal solution was washed with sodium carbonate solution and dried over sodium sulphate. Removal of the ether left a pale yellow oil (8.2 g.).

Vapour phase chromatography (VPC) of the product showed it to be almost pure (ca. 5% impurity) and spectral evidence indicated that it was methyl eperuate.

Distillation of the ester from the acid fraction.

The ester (7.5 g.) was distilled at 0.2 mm. pressure and the following fractions collected.

		Weight	b.p.
A:	Colourless oil containing some colourless solid.	1.23 g.	149 - 154°
B:	Colourless oil containing trace of solid.	3.06 g.	149 - 154°
C:	Colourless oil.	0.79 g.	154 - 156°

VPC analysis of the fractions showed them all to be completely pure and identical to one another. Both infrared (main absorptions: 1735, 1640, 980 cm^{-1}) and n.m.r. spectra (peaks at 9.15, 9.2, 9.35, 6.4, 5.2 and 5.5τ) of A, B and C were substantially the same and indicative of methyl eperuate.

The optical rotation of each fraction was determined in carbon tetrachloride with the following results:-

$$_{A}\alpha_{D}^{24} \quad -26.6^{\circ}, \qquad _{B}\alpha_{D}^{24} \quad -22.0^{\circ}, \qquad _{C}\alpha_{D}^{24} \quad -25.5^{\circ}$$

The differences in the values can probably be attributed to experimental error in measurement and the effect of the solid impurity. The literature value[5] for the rotation of methyl eperuate in chloroform at 18°C is -28.2° (C. 3.98).

The rotation of fraction C was re-determined in chloroform at a later date and the value obtained ($_{C}\alpha_{D}^{22^{\circ}}$, -29.0°) was much closer to the literature value. This difference could be due to the change in solvent. Unfortunately, there was none of fractions A or B left to re-determine their value in chloroform.

Examination of the solid material in fractions A and B.

The white crystalline solid was found to be insoluble in petroleum ether and was separated from the oil by shaking the mixture with this solvent. However, the total weight of solid obtained was small (ca. 20 mg.) in comparison with the oil. A rough melting-point gave a value of 110 -120°. Attempts to identify it were abandoned

because of its negligible amount compared with the liquid ester. It was thought that it might be some sort of crystalline polymer.

Hydrolysis of the ester from the acid fraction.

Fraction B (3.06 g.) was hydrolysed by 2N-methanolic potassium hydroxide (50 ml.) by heating under reflux for 1 hr. The methanol was distilled until the solution went cloudy, water was added, and the mixture extracted with ether. The aqueous layer was acidified with concentrated hydrochloric acid, extracted with chloroform, (chloroform was used because the semi-solid white material obtained on acidification was insoluble in ether) and, after drying the chloroform extract over sodium sulphate, the solvent was removed, to give the free acid (2 g.).

The cyclohexylamine salt of the acid was prepared by direct treatment with cyclohexylamine (cyclohexylamine (2 ml.) was added to the acid (2 g.) in acetone (40 ml.)). The salt was recrystallised to constant rotation from ethyl acetate and gave a final value, α_D^{22}, -29.5°. The literature value[41] for the cyclohexylamine salt of eperuic acid is -30.2°.

Esterification of the crude acid fraction from the exudate by the Fischer method.

The crude acid fraction (4.96 g.) was treated with a 1% sulphuric acid (0.22 ml.) - methanol (25 ml.) mixture by heating under reflux for 1 hr. The excess methanol was distilled and the solution

shaken with water and then extracted with ether. Unchanged eperuic acid was removed from the ether layer by shaking with sodium carbonate solution. This caused an emulsion which was removed by addition of sodium chloride. A small quantity of ester was obtained (ca. 1 g.) and VPC analysis showed only one main peak, corresponding to methyl eperuate, indicating that the acid conditions had not caused any isomerisation of the acid fraction.

Attempted Separation by Column Chromatography of the Methyl Esters from Branchwood

The crude ester mixture (5.2 g., b.p. 142 - 150°/0.5 mm.) was dissolved in dry petroleum ether and run onto a column of activated alumina (Woelm, grade I, 260 g.). The column was eluted successively with pet. ether, benzene, chloroform and methanol, with no abrupt changes in solvent. 100 ml. fractions were collected, solvent removed, and any involatile material left was weighed and subjected to VPC analysis. Any fractions containing less than 50 mg. of material were discarded.

The following fractions were collected:-

Fraction	Solvent	Weight in grams
1 - 17	pet. ether	0.323
18 - 22	5% benzene-pet. ether	nil
23 - 36	10%, 25%, 50%, 100% benzene	nil
37 - 39	10% chloroform-benzene	nil
40 - 43	50%, 100% chloroform	nil
44 - 46	10% methanol-chloroform	nil
47 - 50	50%, 100% methanol	nil

VPC analysis of fractions 1 - 17 indicated that no separation of the mixture had occurred. Since only 0.323 g. of the ester mixture was

recovered from the column it was assumed that the esters had undergone hydrolysis to their parent acids on the column. This method of separation was therefore abandoned.

Thin-layer chromatography of the branchwood esters.

Preparation of the thin-layer plates.[42]

Silica gel G (25 g.) was shaken vigorously with distilled water (50 ml.) for 2 min. in a mechanical shaker. The resultant slurry was applied to 20 x 5 cm. glass plates, which had been previously cleaned with scouring powder, distilled water, and methanol, by means of a special applicator and the wet plates were left exposed to the air for 10 min. The silica was activated by heating the plates at 120 - 140° for 45 min.

The mixed esters were dissolved in benzene and the solution spotted onto the plates with a capillary tube. Development was achieved by standing the plates vertically in a gas-jar containing developing solvent to a depth of about ½ in. The atmosphere in the gas-jar was kept saturated with solvent vapour by a strip of filter paper dipping into the liquid. When the solvent front had advanced about three-quarters of the way up the plate, the solvent was dried off and the spots located by iodine vapour.

Preparation of thin-layer plates impregnated with silver nitrate.

The following methods were used:-

1) Spraying the normal plates with saturated aqueous silver nitrate solution. This was a failure because the silica gel layer flaked and cracked during the spraying.

2) A slurry was made up from silica gel G (60 g.) and saturated aqueous silver nitrate solution (60 ml.) and applied to the plates in the usual way. The plates were left in air overnight, but the silver nitrate crystallised out and rendered the layers useless.

3) The slurry was made up as in 2) and the plates were exposed to the air for 10 mins. They were activated at 110° for 1½ hr. Most of the plates seemed satisfactory and these were stored in a light-proof box.

The ester mixture was applied to the plates in benzene solution and petroleum ether and petroleum ether-benzene mixtures were used as eluting solvents. The spots were located with 0.05% aqueous fluorescein solution, followed by treatment with bromine vapour. Using benzene as eluent, the plates flaked badly.

Preparation of TLC plates using 10% impregnation of silver nitrate.

The slurry was made from Kieselgel PF (fluorescent) (25 g.), water (40 ml.) and silver nitrate (2.5 g.). This gave a 10% impregnation

of silver nitrate with respect to weight of absorbent. The slurry was spread on 20 x 5 cm. glass plates and the layers activated at 105 - 110° for 40 min. The plates were stored in a light-proof box.

Attempted separation of the branchwood esters by preparative-layer chromatography (PLC).

Preparation of PLC plates.

The slurry was made up from Kieselgel PF_{254} (150 g.), water (320 ml.) and silver nitrate (15 g.). The mixture was shaken for 10 min. in a mechanical shaker and applied to clean, 20 x 40 cm. glass plates by means of a PLC applicator set to produce a layer of thickness 1 mm. The plates were left exposed to the air in darkness for 2 hr. and activated at 110 - 120° for 3 hr.

The sample of mixed esters was dissolved in pet. ether and the solution sprayed on the plates in a band $^1/_4$inch broad along the length of the plates about 1 inch from the edge. Development was performed in a large glass chromatography tank. The plates were eluted by multiple runs in the same solvent; up to ten such runs was often needed to move the bands the width of the plate. After each run the solvent was allowed to evaporate from the plate in a fume-cupboard and the final traces were removed by exposing the layers to a warm air source.

The bands were located using a u.v. lamp emitting light of wavelength 254 mμ. After marking the position of the band, the absorbent was removed and extracted with ether.

Spectral Data of the Material Extracted from the Plates

The content of the lower band after PLC of the branchwood esters was shown from spectral evidence to be methyl eperuate. It had the following infrared absorptions:-

3090 cm^{-1}	-C-H stretching	(= CH_2)
1745 cm^{-1}	C=O stretching	(ester)
1640 cm^{-1}	C=C stretching	(unconjugated)
890 cm^{-1}	C-H out of plane deformation	(= CH_2)

It had the following main peaks in its n.m.r. spectrum:-

9.3τ, 3 proton singlet (bridge-head Me group at C-10)

9.1, 9.15τ, two 3 proton singlets (gem.dimethyl group on C-4)

6.3τ, 3 proton singlet (ester methyl group)

5.1, 5.4τ, both broadened 1 proton singlets (exomethylene protons, J = 18 c/s).

The material in the upper band from PLC of the branchwood esters had infrared absorptions at:-

1735 cm^{-1}	C=O stretching	(ester)
1645 cm^{-1}	C=C stretching	(non-conjugated)

860 cm^{-1} ? (may be out of plane deformation of hydrogen on a trisubstituted double-bond).

Note the significant absence of absorption at 3080 cm^{-1} and 890 cm^{-1} due to the $=CH_2$ group present in methyl eperuate.

Main peaks in n.m.r. spectrum:-

9.05τ, 9.1τ, 9.15τ, three 3-proton singlets (bridge-head Me at C-10 and gem dimethyl at C-4)

8.4τ, 3 proton singlet (methyl on a double-bond)

6.3τ, 3 proton singlet (ester methyl group)

4.4 - 4.7 , broad absorption (< 1 proton) (may be olefine proton in methyl cativate).

There was also a sharp peak at 8.7τ.

Attempted separation of heartwood esters by ozonolysis.

The heartwood esters had been shown from VPC analysis to consist of methyl eperuate and another ester in the ratio 1:1.

The mixed esters (1 g.) were dissolved in methylene chloride (35 ml.) and the solution cooled in a salt-ice mixture to -10°.

Pure oxygen was passed through the ozoniser at a rate of 1 l./min. and the resultant ozone was bubbled through the cooled methylene chloride solution and then through a normal solution of potassium iodide in dilute sulphuric acid (20 ml.). The latter was to give some indication

when the reaction was complete, when excess ozone would liberate iodine from the potassium iodide. At 2 min. intervals samples of the reaction mixture were removed, warmed for several minutes with an equal volume of water (to decompose the ozonide) and the layers separated. The methylene chloride layer was subjected to VPC analysis.

The procedure was repeated and the ozonolysis discontinued after 4 min. After treatment of the reaction mixture with water (10 ml.), the methylene chloride layer was boiled down to yield 1.0 g. residue. To remove any ketonic material, the residue was treated with Girards' 'T' reagent ($(CH_3)_3\overset{+}{N}CH_2CO\cdot NHNH_2.\overset{-}{Cl}$) (0.25 g.) in alcohol (2.25 ml.) and glacial acetic acid (0.25 ml.) by heating under reflux for 30 min. on a steam-bath. The cooled solution was added to water (10 ml.) containing some ice and sodium bicarbonate (0.16 g.). A yellow cloudiness appeared. The solution was cooled and extracted with ether. There was 0.51 g. of non-ketonic material in the ether extract. The aqueous extract was treated with 0.5 N hydrochloric acid and left at room temperature for 1 hr. The liberated ketonic compound was isolated by ether extraction (50 mg.).

Examination of the Neutral Fraction of the Exudate

Column chromatography of the neutral fraction.

A sample of the crude neutral fraction (1.05 g.) was dissolved in pet. ether (60 - 80°) and run onto a column of active alumina (Woelm, grade II, 75 g.). The column was eluted successively with pet. ether (60 - 80°), benzene, ether and ethanol, no abrupt changes being made between any pair of solvents.

The following fractions (75 ml.) were obtained:-

Fraction	Solvent	Weight in grams.
1 - 8	pet. ether (60 - 80°)	nil
9 - 17	5%, 10%, 50% benzene-pet. ether	nil
18 - 21	100% benzene	0.597
22 - 23	10% ether - benzene	nil
24 - 26	50% ether - benzene	0.079
27 - 28	50% ethanol - ether	0.137
	Total	0.813

recovery = ca. 80%

Benzene eluted the fraction containing the largest amount of material and subsequent work was done on this. From previous work[5] done on the

neutral fraction from the wood of the tree it was expected that the benzene fraction would contain a mixture of high-boiling esters.

Distillation of esters from the column.

A sample of the esters (0.28 g.) was distilled from a bulb-tube at 0.002 mm. (mercury diffusion pump) but decomposition occurred at around 200°. A very dark brown distillate was collected at 205 - 210°. There was insufficient of this to run its n.m.r. spectrum.

Spectral data for the mixed esters.

Infrared absorption bands at:-

1730 cm^{-1}	C=O stretching (ester)
1640 cm^{-1}	C=C stretching (non-conjugated)
1120, 1150 cm^{-1}	C-O stretching (ester)
890 cm^{-1}	C-H out of plane deformation ($=CH_2$)

Main peaks in the olefinic region of the n.m.r. spectrum:-

5.1, 5.4τ, two broadened 1 proton singlets (exomethylene protons, $CH_2=C$, J=18 c/s)

5.9, 4.7τ, broad absorption

Main peaks in olefinic region of n.m.r. spectrum of oleyl alcohol:-

4.65τ	2 proton triplet, J=5 c/s (cis protons on double-bond)
6.4τ	3 proton multiplet (methylene next to oxygen, and hydroxyl proton)

Hydrolysis of the Involatile Ester Mixture

The residue from the bulb-distillation of the ester (ca. 0.1 g.) was hydrolysed by potassium hydroxide (1 g.) in methanol (10 ml.) by heating under reflux for two hours. Most of the methanol was removed by distillation and the mixture was diluted with water and extracted with ether. The aqueous layer was separated, treated with concentrated hydrochloric acid and the organic acid extracted with ether.

The first ether extract, containing the alcohols, was dried over sodium sulphate and boiled down to yield the alcoholic component of the esters (40 mg.).

The organic acid was shown to be pure eperuic acid by comparison of its spectra with those of an authentic sample. The alcohol component was found to contain no oleyl alcohol by comparison of its i.r. and n.m.r. spectra with those of a synthetic sample of this alcohol.

A further sample of the involatile ester mixture was obtained by column chromatography of the remainder of the neutral fraction (2 g.). The ester was eluted immediately with pure benzene. The optical rotation of the crude ester fraction was determined in chloroform, giving a value $\alpha_D^{24^\circ}$ -33.7°.

Hydrolysis of the second sample of involatile esters.

The involatile esters (1.63 g.) were hydrolysed by heating under

reflux for 1 hr. with potassium hydroxide (5 g.) in methanol (50 ml.). On acidifying the aqueous layer after ether extraction, a white crystalline precipitate (sodium chloride) was obtained. This was redissolved by adding more water. The aqueous layer was extracted with ether but no residue was obtained on removing the ether.

The first ether extract yielded a residue of 1.62 g. i.e. similar to the weight of the starting ester mixture. An infrared spectrum of this had no absorption in the carbonyl region but did show strong alcoholic O-H absorption. This indicated that the hydrolysis had taken place and gone to completion. The only explanation for the absence of the acid component of the ester was that the sodium salt of the acid had gone into the ether layer as an emulsion. This assumption was supported by the i.r. spectrum of the residue which had strong bands in the 1550 - 1650 cm^{-1} region, a characteristic of the carboxylate anion, $-COO^{-}$.

A small sample of the residue (20 mg.) was treated with pet. ether and a white precipitate was formed. This was filtered, dissolved in water and on treatment with concentrated hydrochloric acid and extraction with ether, yielded the acid (identified from its i.r. spectrum).

The alcohol was isolated from the filtered pet. ether solution but an i.r. spectrum showed that it was contaminated with organic acid (C=O at 1720 cm^{-1}). This contamination was probably due to the small amounts of material involved, leading to difficulty in separating the layers.

The rest of the residue from the hydrolysis was treated in a

similar manner with pet. ether and the precipitated sodium salt extracted twice with water. This caused a great deal of foaming which was removed by addition of sodium chloride. The petrol extract was washed with sodium carbonate solution (to remove any acid), then by water, and the washings were added to the aqueous extract. The aqueous layer was acidified with concentrated hydrochloric acid and extracted twice with ether to give the acid as a dark-coloured viscous oil (0.78 g.). Boiling down the pet. ether extract gave the alcoholic component (ca. 0.5 g.).

Spectra of the hydrolysis products.

Alcohol component.

Infrared absorption bands at:-

3600-3150 cm^{-1}	O-H stretching (intermolecularly hydrogen-bonded)
1650 cm^{-1}	C=C stretching (non-conjugated)
1385 cm^{-1}	$C(CH_3)_2$ bending
1050 cm^{-1}	C-O stretching (primary alcohol)
890 cm^{-1}	C-H out of plane deformation ($=CH_2$)

Main peaks in the n.m.r. spectrum:-

9.3, 9.15, 9.1τ, three 3 proton singlets (bridge-head methyl on C-10 and gem. dimethyl on C-4)

6.4τ, 2 proton triplet, J = 6 c/s., ($-CH_2-O$).

5.9 - 6.0τ, broad peak, O-H

5.2, 5.5τ, 2 broadened 1 proton singlets J = 18 c/s. ($=CH_2$)

4.6 - 4.9τ, broad absorption

Acid component.

Infrared absorption bands at:-

3000 - 2450 cm^{-1}	O-H stretching (bonded, carboxylic acid)
3085 cm^{-1}	C-H stretching ($=CH_2$)
1720 cm^{-1}	C=O stretching (carboxylic acid)
1650 cm^{-1}	C=C stretching (non-conjugated)
1390 cm^{-1}	$>C(CH_3)_2$ bending
890 cm^{-1}	C-H out of plane deformation ($=CH_2$)

Principal peaks in the n.m.r. spectrum:-

9.1, 9.2τ, two 3 proton singlets (gem.dimethyl on C-4)

9.3τ, 3 proton singlet (bridge-head methyl on C-10)

6.2 - 6.6τ, broad absorption

5.2, 5.5τ, two broadened singlets J = 18 c/s., ($CH_2=C$)

0.05τ, 1 proton, broad absorption (O-H, carboxylic acid, 10% carbon tetrachloride solution)

Attempted separation of the alcohol mixture by column chromatography.

VPC analysis showed that the alcohol component was a mixture of at least 3 components.

A sample of the alcohol mixture (0.31 g.) was dissolved in pet. ether and run onto a column of active alumina (Woelm, grade IV, 75 g.). The column was eluted successively with pet. ether and benzene.

The following fractions (35 ml.) were collected:-

Fraction	Solvent	Weight in grams.
1 - 16	pet. ether	0.036
17 - 24	10%, 25% benzene - pet. ether	0.076
25 - 30	50%, 100% benzene	0.155

VPC analysis showed that all the fractions were mixtures. No further investigation was pursued.

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