STUDIES IN ALKALOID CHEMISTRY

A thesis submitted to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

by

Matthew Ira Suffness

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January 19<u>70</u>

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To Professors: Hart

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This thesis having been approved in respect to form and mechanical execution is referred to you for judgment upon its substantial merit.

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Approved as satisfying in substance the doctoral thesis requirement of the University of Wisconsin.

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Date of Examination, Dec. 16 1969

STUDIES IN ALKALOID CHEMISTRY

by Matthew I. Suffness

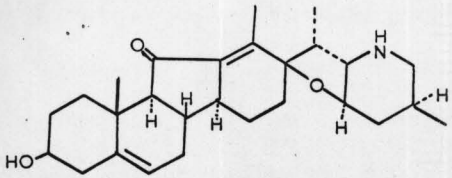
(Under the supervision of Professor S. Morris Kupchan)

An active-principle-directed fractionation of an ethanolic extract of Senecio triangularis Hook led to the isolation and characterization of the alkaloids senecionine and senecionine-N-oxide, which were shown to possess reproducible tumor-inhibitory activity against the Walker 256 intramuscular carcinosarcoma in the rat.

The C-nor-D-homosteroidal alkaloid jervine (1) was interrelated with veratrobasine, whose structure had been elucidated by X-ray crystallographic analysis. The interrelation made possible unequivocal assignments of the relative and absolute configurations at all centers in jervine, 11-deoxojervine, verarine, veratramine, and veratrobasine.

Investigation of the alkaloids of Stephania abyssinica Walp. resulted in isolation of the new alkaloids oxoxylopine (2) and stephavanine (3). The structure of oxoxylopine, an oxoaporphine alkaloid, was proven by interrelation with the aporphine alkaloid xylopine. The structure of stephavanine, a hasubanan alkaloid, was elucidated by chemical degradation and extensive nmr and mass spectral studies of the parent compound and derivatives.

Investigation of the alkaloids of Stephania hernandifolia Walp. led to the isolation and characterization of the new alkaloids stephisoferuline (4), oxoepistephanine (5), 4-demethylnorhasubanonine (6) and 4-demethylhasubanonine (7). The structures of stephisoferuline (4) and 4-demethylnorhasubanonine (6)



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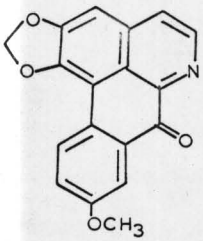

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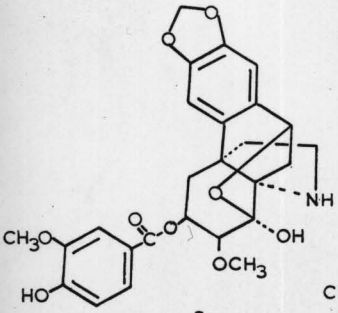

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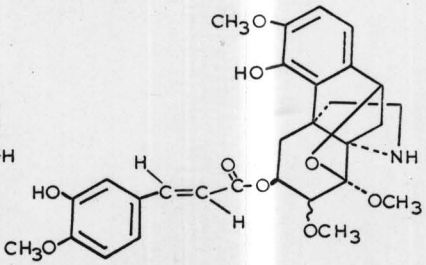

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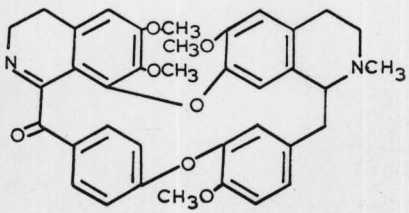

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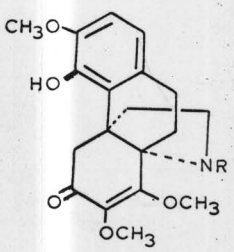

6, R=H

7, R=CH_3

were proven by interrelation with 4-demethylhasubanonine (7), the molecular structure and relative and absolute configurations of which were established by X-ray crystallography. The structure of oxoepistephanine was proposed on the basis of spectral studies and analogy to epistephanine, a known alkaloid also isolated in this investigation. New correlations have been noted between mass spectral fragmentations and substitution patterns among hasubanan alkaloids.

APPROVED S. Morris Kupchan

DATE December 2, 1969

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A thesis submitted in partial fulfillment of the

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at the

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1969

In memory of my father.

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The excellent advice and experienced supervision of Professor S. Morris Kupchan was instrumental in the successful completion of the studies herein.

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OF THE

TUMOR INHIBITORY PRINCIPLES OF *SENECIO*

TRIANGULARIS

INTRODUCTION

The Senecio (pyrrolizidine) alkaloids have a long and varied pharmacological history. They have been implicated in numerous livestock diseases, the common feature of which is extensive hepatic necrosis.[1] The most common of these diseases occur in horses and cattle and have unequivocally been traced to the ingestion of plants containing pyrrolizidine alkaloids. In independent studies the isolated alkaloids have been shown to have the same toxic effects. The most common of these livestock diseases are Pictou disease (Nova Scotia), Winton disease (New Zealand), Molteno disease (South Africa), zdar disease (Czechoslovakia), and "walking disease" (Nebraska).

These diseases are all characterized by profound liver lesions and all are eventually fatal. Poisoning by pyrrolizidine alkaloids is not generally acute since Senecio and related genera are not especially palatable to stock but ingestion of small amounts over extended periods of time will cause chronic liver damage. There is a significant latent period between ingestion and toxicity ranging from less than one month to over five months making this type of poisoning very insidious since by the time symptoms of pyrrolizidine poisoning occur severe and perhaps fatal liver damage has already been done. In this manner whole herds of animals may be affected. The economic significance of pyrrolizidine poisoning cannot be overemphasized as thousands of

cattle and horses are lost each year in each of the affected areas and indeed the toll is probably much higher than is generally recognized because of the latent period (when symptoms appear the animals may not be grazing in an area containing Senecio or related genera and the disorder is thus not associated with pyrrolizidine poisoning).

Pyrrolizidine poisoning in livestock is characterized by weakness, uneasiness, signs of abdominal pain, and emaciation. The animals become very restless and wander aimlessly, often not avoiding objects in their path, hence the name "walking disease." There have also been a number of cases of Senecio poisoning reported in man. In Africa poisoning is generally due to bread prepared from flour which has been contaminated with seeds or fragments of Senecio. In the West Indies natives brew a "medicinal tea" from a number of indigenous plants one of which is frequently Senecio. The cases of human poisoning all appear to be associated with liver malfunction although the documentation is by no means thorough.

In all cases of poisoning in animals there are found rather severe lesions of the liver, characterized by the presence of a large number of megalocytes. There is apparently derangement of the normal mitotic processes which is implicated in formation of malignancies of the liver.[2] It is noteworthy that the present work and earlier studies in these laboratories[3] and elsewhere[4] have

shown that pyrrolizidine alkaloids have significant tumor-inhibitory properties. The earlier studies showed inhibition of adenocarcinoma 755 carried in the rat, and the present work has shown that certain pyrrolizidine alkaloids are effective tumor inhibitors against the Walker muscular 256 carcinosarcoma carried in the rat.

The mechanism of the hepatotoxic action of the pyrrolizidine alkaloids has been and continues to be a subject for much work and speculation. Structure-activity relationships were first advanced by Schoental, [5] who noted the necessity of having a Δ^1 unsaturation in the alkamine portion of the alkaloid in order for it to process hepatotoxic activity. The first real mechanistic proposal for the hepatotoxic action of these alkaloids was made by Culvenor et al. [6] who showed that the allylic ester present in those molecules having a Δ^1 unsaturation, as proposed by Schoental, could be displaced by reactive nucleophiles such as benzyl mercaptan anion and that alkylation was the probable mode of action of pyrrolizidine alkaloids in the liver. Alkylation as a mechanism of action was supported by the observation that the toxicity of pyrrolizidine alkaloids was significantly reduced in the presence of cysteine. However it was also noted in the same paper that the allylic displacement reaction only proceeded in the presence of rather reactive nucleophiles and was not demonstrable with weaker nucleophiles analogous to those present in biological systems. The work on anti-tumor activity quoted earlier seems to support alkylation as a mechanism of action

since the tumor systems found to be responsive are known to be especially sensitive to alkylating agents.

Recently, metabolic studies by Mattocks[7] have shown that there is conversion of Δ^1 pyrrolizidine alkaloids to reactive pyrrole type compounds in the liver and that these compounds possess the same order of hepatotoxic activity as do the parent alkaloids. Furthermore, these pyrroles gave positive results as alkylating agents with standard substrates such as 4(p-nitrobenzyl)-pyridine and 4-picoline in contrast to the results obtained earlier by Culvenor in his work on the parent alkaloids.[6] In addition there was a strong correlation between the amounts of pyrrole bodies formed and toxicities of the alkaloids; those alkaloids which do not contain a double bond in the 1 position of the pyrrolizidine molecule and are not hepatotoxic are also observed to produce relatively minor amounts of "metabolic pyrroles". At the present time therefore, all the available data point toward these alkaloids acting as alkylating agents after being first converted to a more reactive form (e.g. V) in the liver.

The chemistry and structure elucidation of the pyrrolizidine alkaloids has been extensively reviewed[8-12] and will not be presented here. Work on this series of alkaloids has been rather extensive (at present there are nearly 100 alkaloids of the pyrrolizidine type known), for several reasons: Senecio is one of the largest genera of the family Compositae and species have a worldwide distribution,

the alkaloids are generally fairly abundant and are not especially difficult to work with, they have a great economic importance as livestock poisons, and most recently, they are of interest as a result of their potential anti-tumor acitvity.

A valuable book reviewing the chemistry, toxicity, and biological properties of the pyrrolizidine alkaloids has been recently written by Bull, Culvenor and Dick.[17]

DISCUSSION

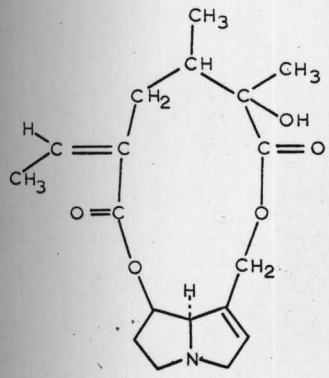
Our work was concerned with the isolation and structure elucidation of the tumor-inhibitory components of Senecio triangularis, the ethanolic extracts of which showed reproducible tumor-inhibitory activity against the Walker 256 intramuscular carcinosarcoma.

The activity was shown to be concentrated into the alkaloidal fraction on solvent partitioning, and this fraction was shown to consist of two major alkaloids, senecionine and senecionine N-oxide, which were responsible for the activity noted. Senecionine (I) is a well-characterized compound which has been previously isolated from a number of plants of the genus Senecio and related genera. Senecionine N-oxide (II) had not been previously characterized. This work represents the first phytochemical investigation of S. triangularis.

Characterization of senecionine N-oxide was achieved by reduction to senecionine and conversely senecionine was converted to senecionine N-oxide by peroxide oxidation. Senecionine was also hydrolyzed to give the alkamine retronecine (III) and senecic acid (IV) the physical constants of which were in good agreement with those reported.

One interesting observation was made concerning the nmr spectra of senecionine and senecionine N-oxide. The signals for the protons on carbon adjacent to nitrogen are shifted downfield to a very significant extent in the case of the N-oxide. The spectra

of these two compounds are presented in Figure 1 and the resonance assignments are found in Table 2. The shifts observed vary from 0.7 to 0.9 τ units for the protons at C-3, C-5, and C-8 which are the protons on carbon bearing nitrogen, whereas, the largest shift observed for any of the other protons is 0.4 τ units. Provided that these shifts are observed consistently in other cases they could be useful in the structure elucidation of alkaloids. By oxidation of the free base to the N-oxide and comparison of chemical shifts observed, the number of methylene and methine protons on carbon adjacent to nitrogen could be readily determined. This could also give information about protons further away by virtue of the coupling of these protons to the protons which are shifted. Thus this technique may serve as a spectral probe to determine the structure and perhaps stereochemistry of that part of an alkaloidal molecule which is in the vicinity of the nitrogen atom.



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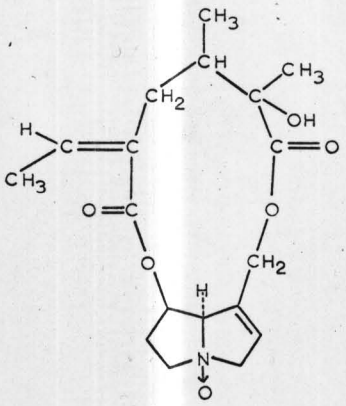

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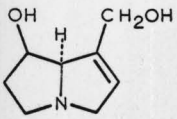

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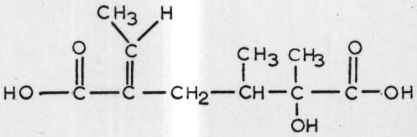

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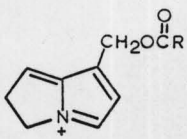

V

FIGURE 1. SENECIONINE AND DERIVATIVES.

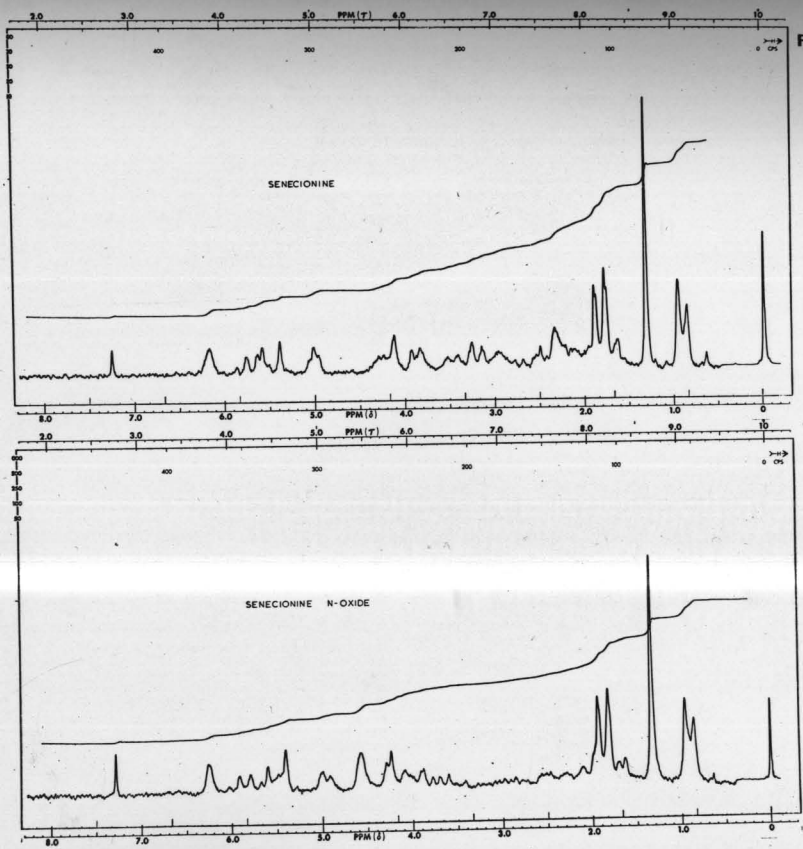

FIG. 2

TABLE 1. NMR Spectral Comparison of Senecionine and Senecionine N-Oxide.

	H2	H3	H5	H6	H7	H8
Senecionine	3.82	6.33	7.05	7.65	4.97	5.73
Senecionine N-oxide	3.75	5.43	6.30	7.50	4.53	5.03
Downfield Shift	.07	.90	.75	0.15	.44	0.70

Chemical shifts are in τ units for $CDCl_3$ solutions relative to tetramethylsilane.

EXPERIMENTAL*

Isolation of Alkaloids. Continuous extraction of plant material (1.5 kg) with ethanol gave on evaporation of solvent a gummy residue (290 g). A portion of this material was partitioned between chloroform and 3% citric acid solution (2 l. each) and the aqueous phase was washed with chloroform (3 l.). The aqueous phase was basified with sodium carbonate and extracted with chloroform (4 x 1 l.). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give the crude alkaloidal fraction (13 g). This material was crystallized from ethanol to give the major alkaloid, senecionine (I, 3.65 g), which was recrystallized twice from ethanol to give colorless crystals (2.44 g): mp 235-236° (d, in a sealed tube); $[\alpha]_D^{28}$ -51° (c 2.18, $CHCl_3$) (lit. -56°)[8]; λ_{max}^{Nujol} 5.72, 5.81, 6.00 μ; picrate, mp 190-191° (d, lit. 191°[13]): nitrate, mp 214-215° (d, lit. 214°[14]): methiodide, mp 240-243° (d, lit. 249°[14]).

* Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected for stem exposure. Infrared spectra were determined on Beckman models IR-5A and/or IR-9 recording spectrophotometers. Ultraviolet spectra were determined on a Beckman model DK2A recording spectrophotometer. Optical rotations were measured on a Zeiss-Winkel polarimeter and are approximated to the nearest degree. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan. Thin layer chromatography (tlc) was performed on plates prepared from aluminum oxide G which were visualized with Dragendorff's reagent. These chromatograms were developed in methanol-chloroform solvent systems.

The mother liquors from the first crystallization of senecionine showed the presence of a second, more polar alkaloid. A portion of these mother liquors (2 g) was chromatographed over acid-washed alumina (100 g) in chloroform. Elution with chloroform gave senecionine (1 g) and subsequent elution with methanol-chloroform (1:50) gave senecionine N-oxide (800 mg) (II). This material was crystallized twice from chloroform-petroleum ether to give colorless needles (259 mg): mp 141-142° (d); λ_{max}^{Nujol} 5.73, 5.81, 6.04 μ; $[\alpha]_D^{28}$ -22° (c 1.32, $CHCl_3$).

Anal. Calcd for $C_{18}H_{25}NO_6 \cdot 2/3\ CHCl_3$: C, 52.02; H, 6.00; N, 3.25; Cl, 16.45. Found: C, 51.52; H, 6.24; N, 3.55; Cl, 17.64.

Hydrolysis of Senecionine (I) to Retronecine (III) and Senecic Acid (IV). - Senecionine (500 mg) and barium hydroxide octahydrate (1 g) were added to water (5 ml) and heated under reflux for one hour. After cooling the reaction was saturated with carbon dioxide, filtered, acidified with HCl, and continuously extracted with ether for 48 hours. The ethereal solution was dried (Na_2SO_4) and evaporated to give crude senecic acid (IV, 210 mg), which on crystallization from ether-petroleum ether gave colorless needles: mp 143-144° (lit. 146°[15]); λ_{max}^{Nujol} 2.92, 5.78, 5.92, 6.12 μ; $[\alpha]_D^{29}$ +14° (c 2.65, EtOH).

Anal. Calcd for $C_{10}H_{16}O_5$: C, 55.54; H, 7.46. Found: C, 55.62; H, 7.39.

The remaining aqueous solution was evaporated to dryness and the residue was taken up in hot ethanol and filtered. The filtrate was evaporated and redissolved in cold ethanol and again filtered. The filtrate was concentrated to a syrup which crystallized on addition of ethyl acetate. The crystals were washed with benzene, ethyl acetate, and chloroform to give retronecine (III) hydrochloride as colorless needles: mp 163-165° (lit. 164°[16]); λ_{max}^{Nujol} 3.01, 3.15, 3.45, 3.92 μ.

Oxidation of Senecionine (I) to Senecionine N-oxide (II). Treatment of senecionine (I, 157 mg) in a mixture of chloroform (2 ml) and ethanol (5 ml) with 30% hydrogen peroxide solution (0.35 ml) gave after heating at 60° for 24 hours a homogeneous product on tlc corresponding to II. Evaporation of the solvent and filtration through an acid-washed alumina column gave senecionine N-oxide (II, 177 mg) which was crystallized four times from chloroform-petroleum ether to give colorless needles identical in mp, mixture mp, tlc, mixture tlc and infrared spectrum to II.

Reduction of Senecionine N-oxide (II to Senecionine (I). Several drops of $CuSO_4$ solution and zinc dust (0.4 g) were added to senecionine N-oxide (II, 50 mg) in 2 N sulfuric acid (10 ml) and the reaction was allowed to proceed for two hours at ambient temperature. The acidic solution was filtered, made strongly basic with excess ammonium hydroxide and extracted with chloroform. The chloroform extract was evaporated and the residue taken up in hot ethanol and filtered. The filtrate was concentrated and crystallized to give pure I as colorless prisms (43 mg) which showed mp,

mixture mp, tlc, mixture tlc and ir spectrum identical to senecionine.

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PART II

THE STEREOCHEMISTRY OF JERVINE AND RELATED ALKALOIDS

INTRODUCTION

Jervine, $C_{27}H_{39}NO_3$, is an alkaloid obtained from several species of Veratrum, most noteably V. viride., V. album and V. grandifolium (fam. Liliaceae).

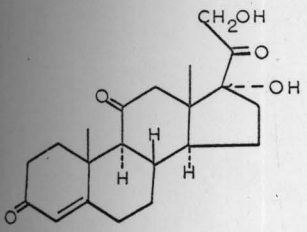
The alkaloid became the focus of a number of structural studies shortly after the discovery of cortisone (I) by Kendall in the 1930's. The extremely small amounts of cortisone available from adrenal glands and the difficulty of processing these glands made the cost of cortisone prohibitive, and indeed supplies were so short that at times the drug could not be had at any price. One of the key structural requirements for cortisone-like activity is an oxygen function at C-11, and this presented the greatest barrier to synthesis since no methods for functionalization of the steroidal C ring and been developed up to that time. Early structural studies on jervine revealed the presence of an abnormally unreactive ketone function which should logically be placed at either C-11 or C-12. These positions are sterically hindered, and such ketones are known to show the same order of reactivity as the ketone function present in jervine. Hence jervine, which is available fairly readily from Veratrum species, became important as a potential precursor of cortisone. In 1949 Jacobs and Sato proposed structure II for jervine indicating a close relationship to cortisone. However, additional studies pointed out several discrepancies in the proposed structure and, in 1951, a revised structure (III) was presented by Fried, Wintersteiner, Moore, Iselin, and Klingsberg.[2] This structure was quite

novel as it was the first of the C-norD-homo steroids. All known reactions of jervine can be accounted for in terms of this structure and so only the stereochemistry remained to be established.

Mitsuhashi and Shimizu[3] interrelated jervine (III) and hecogenin (IV), which is of known relative and absolute configuration at all centers, through V, thereby establishing the stereochemistry at C-3, C-8, C-9, C-10, and C-14. Since C-9 is adjacent to the C-11 ketone the possibility of epimerization at C-9 was considered. To prove unequivocally that the configuration of C-9 was α, jervine was interrelated with veratramine (VI), which had been previously interrelated with hecogenin through an intermediate lacking the ketone at C-11 and hence incapable of undergoing epimerization at C-9.[4,5]

The stereochemistry of ring E in veratramine was investigated by Sicher and Tichý. Comparison with model pipecolinol derivatives showed that the substituents at C-22 and C-23 were trans. These workers were also able to assign the equatorial configuration to the hydroxyl group at C-23.[6] Since it had been shown previously that the configurations at C-22 and C-23 were the same in veratramine and jervine, this defined the stereochemistry of jervine at these centers also. It was shown by synthesis from citronellal (of established absolute configuration) that the methyl group at C-25 had the (S) configuration in jervine.[8]

The question of the relative configurations of C-22 and C-25 was approached by Augustine[9] who presented arguments leading to assignment of the C-22 and C-25 substituents as cis. His argument was based on the assumption that the bulky C-22 substituent was conformation determining



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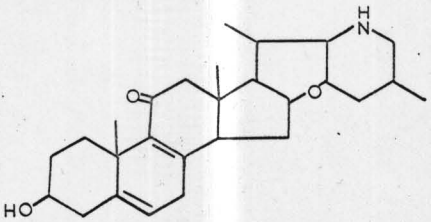

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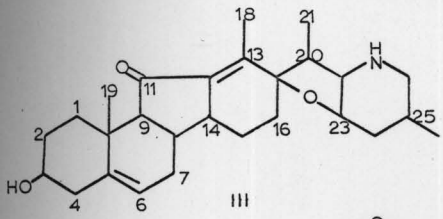

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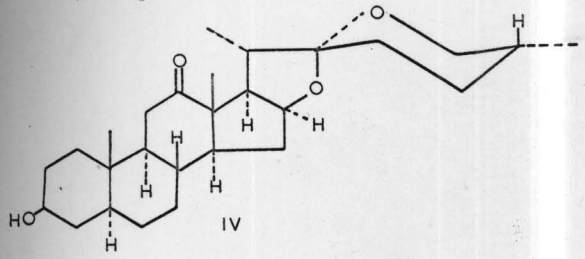
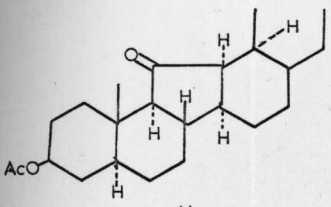
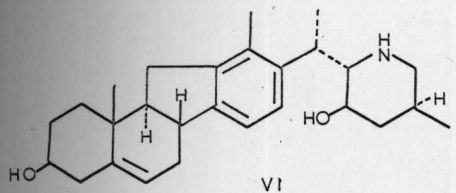


IV

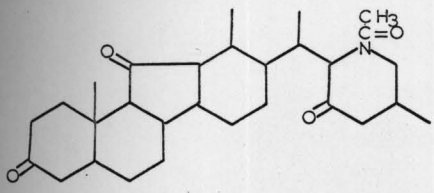


V

FIGURE 3. JERVINE AND RELATED COMPOUNDS.

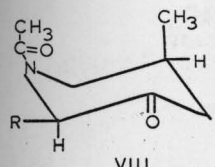


VI

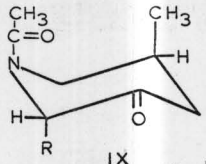


NaOMe / MeOH → C - 22 EPIMER

VII

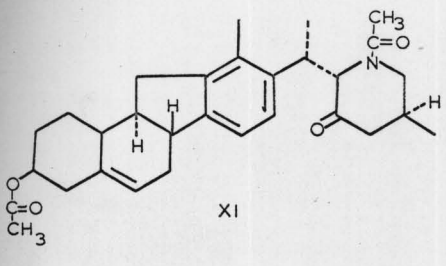


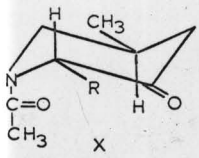
NaOMe / MeOH



VIII IX

ring flip





XI X

FIGURE 3. - CONTINUED.

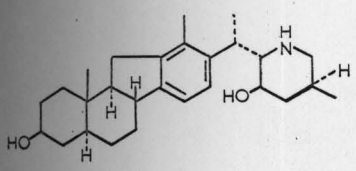
and equatorial in compound VII. Since VII underwent epimerization to a C-22 epimer, C-25 must be axial, for on epimerization and flipping of the ring this would result in both substituents being equatorial. Since the compound did epimerize to the extent of 75% at C-22, this seemed to be a valid conclusion. This series of experiments as interpreted by Augustine is shown diagrammatically in structures VIII - X, where R is the bulky side chain consisting of rings A through D. A few years later, in a series of somewhat more rigorous experiments, Masamune and co-workers derived the same results from experiments on 23-dehydro-3,N-diacetylveratramine (XI).[10]

However in 1967 this work was re-examined by Johnson and co-workers[11] who prepared 5α,6-dihydroveratramine (XII) and its C-23 epimer (XIII). By examination of the 100 MHz nmr spectra of both compounds Johnson was able to show that the methyl group at C-25 was axial and in fact the C-22 and C-25 substituents were trans, as opposed to to the earlier conclusions cited above. The results of Augustine and Masamune were explained by additional studies by Johnson's group which showed that there was a very strong steric factor induced by the presence of the amide. To relieve steric crowding both the substituents at C-22 and C-25 are axial and on equilibration would be expected to give C-22 epimers which can exist with the substituents at C-22 and C-25 axial and equatorial respectively. Thus these experiments can be represented diagrammatically by structures XIV-XVI rather than VIII-X.

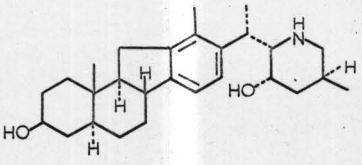
At this point the stereochemistry was known at all centers with the exceptions of C-17 and C-20, the configurations of which were assigned solely on the basis of biogenetic analogy.[7] Masamune and co-workers reported a formal total synthesis of jervine[12] (III) which did not, however, allow proof of the configuration of C-20. Further, the analogy used for the assignment of stereochemistry at C-17 did not appear to be firmly grounded. Simultaneously, Johnson et al. completed a total synthesis of veratramine (VI)[13] but also assigned C-20 only on the basis of biogenetic analogy (there is no asymmetric center at C-17 in veratramine).

Thus although the formal total syntheses of both veratramine and jervine had been recorded, the configuration at C-20 (and in the case of jervine C-17) were not unequivocally defined. In 1968 Reeke et al. elucidated the structure of veratrobasine* (XVII) by X-ray diffraction determination and noted its close relationship to jervine (11 β-alcohol at C-11 vs. 11 ketone in jervine). However, they also noted that; "it remains to be seen whether the stereochemistry of the two molecules is the same at corresponding asymmetric centers."[14] The absolute configuration of veratrobasine could not be established in this work.

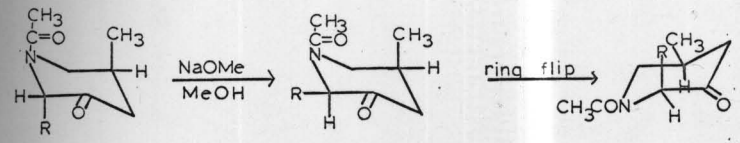
* Veratrobasine is a minor alkaloid from Veratrum album, the structure of which had been previously unknown as was its relationship to other C-nor-D-homosteroidal alkaloids.



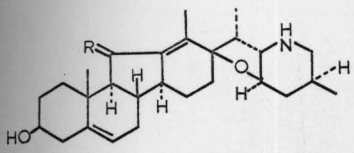
XII



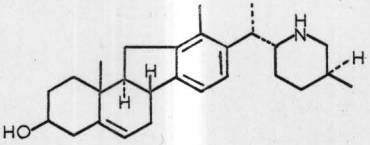
XIII



XIV XV XVI



XVII , R = β-OH , H
XVIII , R = O
XIX , R = H_2



XX

FIGURE 4. STEREOCHEMISTRY OF JERVINE AND RELATED COMPOUNDS.

DISCUSSION

In view of the question of the assignments of stereochemistry at C-17 and C-20 in jervine it was decided to attempt the interrelation of jervine and veratrobasine. A survey of the literature revealed that a compound tentatively designated as jervine 11β-ol, reported by Iselin, Moore, and Wintersteiner in 1956, [15] possessed nearly the same physical constants as veratrobasine. [16] Following a slightly modified procedure from that recorded, we were able to prepare jervin-11β-ol in moderate yield. Direct comparison of this product with a reference sample of veratrobasine [17] showed them to be identical (by mixture melting point, mixture tlc, optical rotation, high-resolution ir, 100 MHz nmr, and mass spectral comparisons).

This correlation established the following: (1) The β-orientation of the 17-oxide in jervine (XVIII) (and hence also in 11-deoxojervine (XIX) [18]); it should be noted that this is a revision of the configuration proposed in the report on the total synthesis of jervine [12]; (2) The α-orientation of the methyl substituent at C-20 in jervine (and hence in 11-deoxojervine (XIX) [18], veratramine (VI) [19] and verarine (XX), [10] as had been suggested earlier by biogenetic analogy [7]; (3) The revised C-22α and C-23β configurations for the substituents at the respective positions in jervine and related alkaloids as shown by Johnson and co-workers; and (4) The absolute configuration of veratrobasine (XVII) as that which occurs in normal steroids since jervine (XVIII) had previously been interrelated with hecogenin (IV) of established absolute configuration. [3,20]

In conclusion, this work conclusively established the relative and absolute configurations of all asymmetric centers in jervine (XVIII), veratrobasine (XVII), 11-deoxojervine (XIX), veratramine (VI), and verarine (XX).

EXPERIMENTAL *

Reduction of Jervine (XVIII) to Veratrobasine (XVII). - To a solution of jervine (1.5 g) in tetrahydrofuran (45 ml) under nitrogen lithium aluminum hydride (400 mg) in tetrahydrofuran (5 ml) was gradually added. The reaction was stirred at room temperature for one hour and gradually assumed a gelatinous consistency. Excess lithium aluminum hydride was destroyed by cautious addition of water and when evolution of hydrogen had ceased an additional 100 ml of water was added. This solution was extracted with chloroform (3 x 100 ml) and the combined chloroform extracts were washed successively with dilute sodium hydroxide solution (100 ml) and water (100 ml). The organic phase was dried (Na_2SO_4) and evaporated to give 1.21 g of amorphous material. This material was chromatographed over silica gel (0.05-.2 mm, Brinkmann, 120 g) in chloroform gradually increasing the polarity of the eluant with increasing amounts of methanol. Elution with 5% methanol-chloroform gave 2 compounds, 12,13-dihydrojervine (692 mg) and subsequently veratrobasine (190 mg). The 12,13-

* Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected for stem exposure. High resolution ir spectra were determined on a Beckman model IR-9 recording spectrophotometer. Optical rotations were measured in a Zeiss-Winkel polarimeter and are approximated to the nearest degree. NMR spectra were measured on a Varian HA-100 spectrometer equipped with a time-average computer in tetradeuterioacetic acid solution. Mass spectra were determined on a Hitachi RMU-6A spectrometer. TLC determinations were made on silica gel F_{254} plates (Brinkmann) in methanol-chloroform solvent systems and visualization was achieved by spraying with ceric sulfate (3%) in sulfuric acid (3 N) solution and heating.

dihydrojervine gave needles from acetone: mp 248-250° (lit. 240-242°[15]), and showed an unconjugated carbonyl band in the ir. The veratrobasine was crystallized twice from methanol-acetone to give colorless needles (21 mg): mp 283-288° (d) (lit. 278-283°[15], 285-288 (d)[16b]); $[\alpha]_D^{27}$ - 111° (c 0.72 pyr.) (lit. -126°[16b]); λ_{max}^{KBr} 2.91, 2.97, 3.04, 3.39, 6.85, 9.47 μ; m/e 427 (M^+).

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PART III

THE ISOLATION AND STRUCTURE ELUCIDATION OF ALKALOIDS FROM *STEPHANIA ABYSSINICA*

INTRODUCTION

Stephania abyssinica (Walp) is a creeping plant indigenous to Eastern and Southern Africa which is reputed to possess medicinal properties.[1] In Tanganyika the plant is pounded in water and the extract so obtained is used as a mild purgative in children. The juice of the stem is used as an emetic for relief of pains in the chest. In tropical Africa the leaf is used as a purgative and the root is used for treatment of roundworm and menorrhagia. The Zulus use a decoction of the root in the treatment of boils.

The only chemical investigations of S. abyssinica were conducted by de Waal et al.,[2] who isolated from the plant the well characterized flavonoid rutin, veratric acid and an alkaloid designated as neostephanine which they later showed to be identical to metaphanine,[3] a hasubanan alkaloid previously isolated from Stephania japonica and characterized by Tomita et al.[4]

Our work on Stephania abyssinica was initiated as a part of the screening program for tumor-inhibitory natural products sponsored by the National Cancer Institute in conjunction with the Cancer Chemotherapy National Service Center (CCNSC) and the United States Department of Agriculture (USDA). In this program, extracts of Stephania abyssinica were shown to have reproducible tumor-inhibitory activity against the Lewis Lung tumor carried in the mouse.

Thus far we have been unable to isolate the principal tumor-inhibitory

principle(s) of this plant, but we have isolated and characterized two new alkaloids, oxoxylopine and stephavanine. The latter alkaloid is quite toxic and has shown somewhat erratic tumor-inhibitory activity against the test system cited. Oxoxylopine is a member of the oxoaporphine class of alkaloids and stephavanine is a member of the rather rare series of hasubanan alkaloids which thus far appear to be limited in occurrence to species of the genus Stephania.

DISCUSSION

Occurrence of Oxoaporphine Alkaloids. The oxoaporphines all have the basic ring system I and are presumed to arise in the plant by oxidation of the corresponding aporphine nucleus (II). [5] This is supported by the observation that oxoaporphines are generally present in small amounts relative to the quantities of co-occurring aporphine alkaloids. The oxoaporphines are highly colored, generally yellow, orange, or red, as is expected from their highly conjugated ring system. The oxoaporphines are interconvertible with the corresponding aporphines by reduction of the oxoaporphine under modified Clemmensen conditions or by oxidation of the aporphine using chromium trioxide-pyridine. [6,7] The number of known oxoaporphines is quite limited, but they are of considerable economic importance since their colors are intense enough, even at very low concentrations, to impart a significant degree of color to derived plant products. One of the first investigations leading to the discovery of this class of alkaloids was made by Buchanan and Dickey on Liriodendron tulipfera, the yellow poplar, in order to isolate and characterize unknown compounds which were coloring the wood of this tree and thereby making it unsuitable for use as a source of paper pulp. [8] The major constituent which they isolated was liriodenine (III) which was characterized shortly thereafter as the first oxoaporphine alkaloid. [9]

The structures, chemistry, and some physical properties of a number of oxoaporphine bases have been reviewed [5] and recently data on

the mass spectra of these alkaloids have been published. [10]

Isolation and Structure Elucidation of Oxoxylopine (V). An ethanolic extract of S. abyssinica rhizomes [34] was prepared and worked up for alkaloids. The strongly acidic HCl-soluble fraction was partially basified to pH 5 and extracted with chloroform to give a fraction designated as weak bases, from which oxoxylopine was separated by column chromatography on silica and alumina. Crystallization of a fraction from the alumina chromatography from chloroform gave bright orange crystals of oxoxylopine (V), mp 319-320°(d).

The alkaloid had the molecular formula $C_{18}H_{11}NO_4$ and showed a corresponding molecular ion in the mass spectrum at m/e 305. An absorption band at 6.02 μ in the infrared spectrum showed the presence of a conjugated ketone. A series of sharp aromatic absorption bands were also present. There were no absorption bands assignable to -OH or -NH. The color of the compound, its high melting point, and its uv spectrum strongly supported a very highly conjugated aromatic structure, as did the carbon to hydrogen ratio from the elemental analysis. The ir and uv spectra very closely resembled those of liriodenine [8] (III), strongly indicating substitution at positions 1 and 2 and confirming the initial classification of this compound as an oxoaporphine alkaloid. The nmr spectrum showed no aliphatic protons, one methoxyl group, one methylenedioxy group and 6 aromatic protons. Since substitution of C-1 and C-2 was indicated by ir and uv measurements, it was logical to place the methylenedioxy group there. One of the aromatic protons was a sharp singlet, indicating the absence of substitution at C-3.

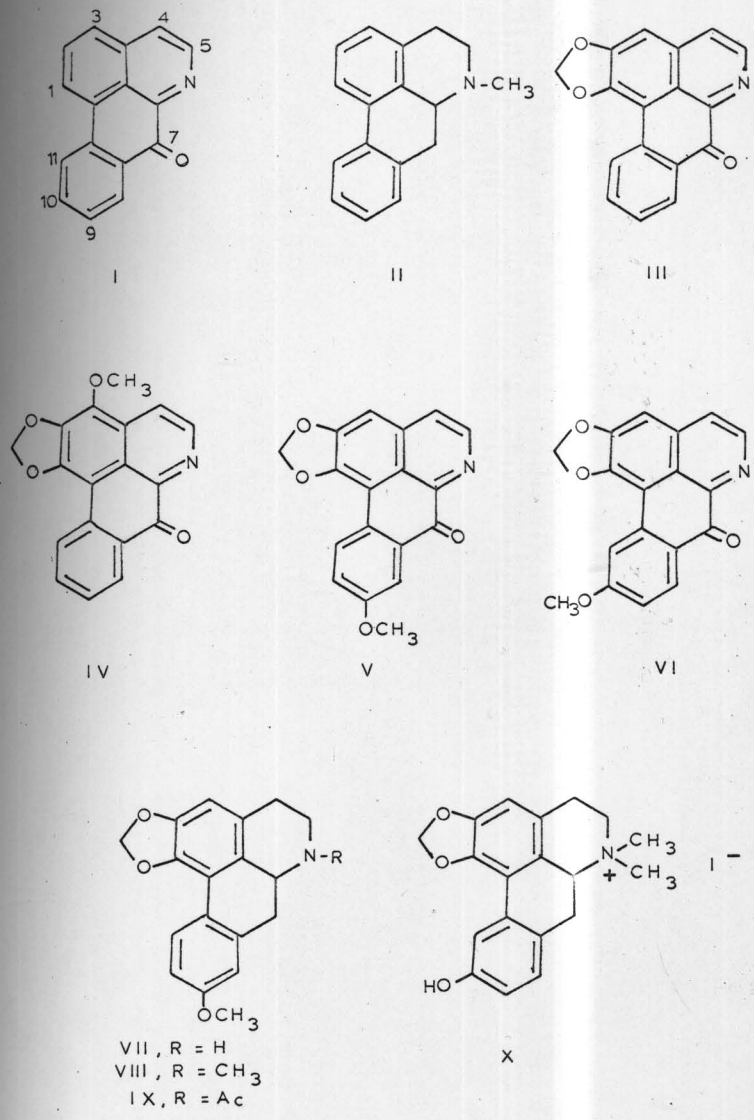


FIGURE 5. OXOXYLOPINE AND RELATED COMPOUNDS.

TABLE 2

Nmr Assignments of Oxoxylopine (V)*

H-3	H-4	H-5	H-8	H-10	H-11	$-O\diagdown\!\diagup CH_2$	$-OCH_3$
2.47	1.22	1.55	1.93	2.33	1.22	3.35	5.88
s	d(6)	d(6)	d(3)	dd(3, 9)	d(9)	s	s

* Chemical shifts are recorded in τ units in CF_3COOH solution at 60 MH_z relative to tetramethylsilane.

In addition, the compound so substituted is known (atherospermidine, IV) and has different physical constants. The possibility of substitution at C-4 or C-5 could be excluded on the basis of the nmr spectrum, which showed signals for a pair of isolated adjacent protons (AB quartet, J=6Hz), consistent only with the C-4 and C-5 hydrogens. This limited placement of the methoxyl group to the D ring. Examination of the nmr spectrum of V showed a clear 1, 2, 4 pattern for the protons assigned to the D ring, which is readily distinguishable from the 1, 2, 3 proton pattern that would be present if the methoxyl group were placed at either C-8 or C-11. The nmr assignments for oxoxylopine are given in Table 2.

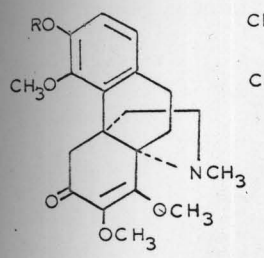
As a result of the arguments advanced thus far, the methoxyl group could be placed only in position 9 or 10. Since aporphines having either of these substitution patterns are known, reduction of oxoxylopine would be expected to give a compound which could be readily correlated. The 1, 2-methylenedioxy-9-methoxy substitution pattern is exemplified by either xylopine (VII) or isolaureline (VIII), whereas the 1, 2-methylenedioxy-10-methoxy substitution pattern is typified by michepressine iodide (X). The uv spectra of aporphines are very sensitive to the positions of oxygen substituents and there is a significant difference in the recorded uv spectra of these position isomers. [5]

Reduction of oxoxylopine (V) with zinc and hydrochloric acid in acetic acid [7] gave a compound whose uv spectrum was identical with that recorded for xylopine (VIII). [13] The product was acetylated to give (±)-N-acetylxylopine (IX) which, after purification, was compared with

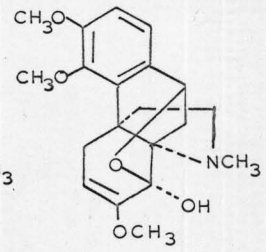
authentic (-)-N-acetylxylopine[14] by uv spectrum, high resolution ir spectrum and tlc properties. The comparisons proved the structure of oxoxylopine to be V.

Recently, a report concerning the structure elucidation of a new oxoaporphine alkaloid, lanuginosine, from Michelia lanuginosa Wall (Magnoliaceae) has appeared.[15] Lanuginosine, which has physical constants clearly different from oxoxylopine, has also been assigned structure V. However, lanuginosine has not been interrelated with a known compound and the spectral data are insufficiently unique to warrant the assignment made. Lanuginosine shows a 1, 2, 4-proton pattern in the nmr spectrum corresponding to the ring D aromatic protons. As discussed above, this could apply to either a 1, 2-methylenedioxy-9-methoxy substitution pattern or to a 1, 2-methylenedioxy-10-methoxy substitution pattern. Oxoxylopine has been interrelated with xylopine, which has been correlated[13] with isolaureline. The latter alkaloid has been prepared by unequivocal total synthesis[16] so that the assignment of structure V to oxoxylopine rests on firm ground. Therefore, lanuginosine is probably the 1, 2-methylenedioxy-10-methoxy isomer of oxoxylopine and should be represented by structure VI. It is noteworthy that michepressine iodide (X), having the same oxygenation pattern as VI, has been isolated from Michelia species.[17]

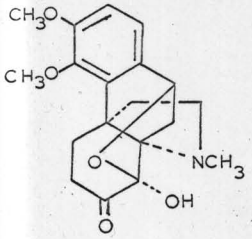
Significance and Occurrence of Hasubanan Alkaloids. There are only eight naturally occurring members of the class of hasubanan alkaloids recorded in the literature. These are hasubanonine (XI),[18] homostephanoline



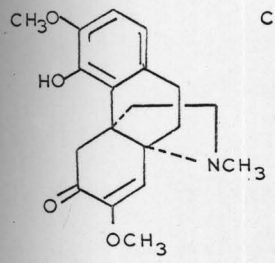
XI , R = CH_3
XII, R = H



XIII



XIV



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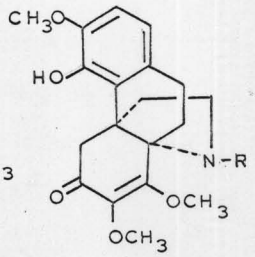
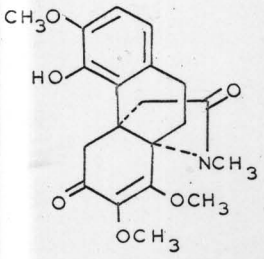
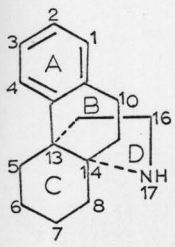


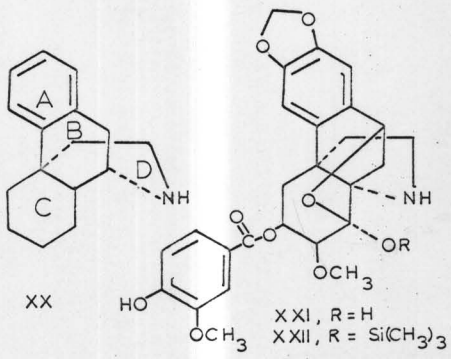
XVI , R = CH_3
XVII, R = H



XVIII



XIX



XX

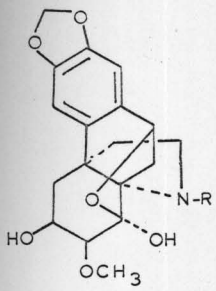
XXI, R = H
XXII, R = $Si(CH_3)_3$

FIGURE 6. HASUBANAN ALKALOIDS .

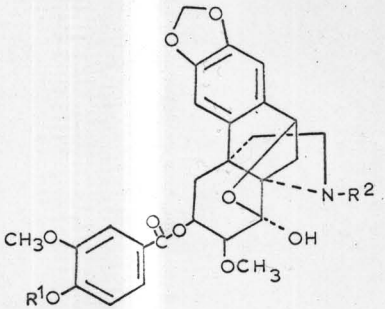
(XII),[21] prometaphanine (XIII),[20] metaphanine (XIV),[19] cepharamine (XV),[22] 4-demethylhasubanonine (XVI),[23] 4-demethylnorhasubanonine (XVII),[23] and aknadilactam (XVIII).[24] All have been isolated from plants of the genus Stephania (family Menispermaceae). The very close structural relationship of hasubanan (XIX) to morphinan (XX) makes the hasubanan series of alkaloids interesting from a biogenetic viewpoint. This close relationship also begs the question of whether pharmacological activity of the morphine type might possibly be found in analogous members of the hasubanan series and, if so, whether the hasubanan alkaloids would be more or less efficacious than the morphine alkaloids as medicinal agents.

The biogenesis of hasubanan alkaloids has not as yet been examined experimentally but Battersby has postulated a logical biogenetic sequence based on a phenol coupling reaction induced by an unusual methylation pattern in the benzylisoquinoline precursor.[25] Most recently there have been three synthetic entries into the hasubanan series, two via total syntheses[26,27] and one via conversion from the morphine series.[28]

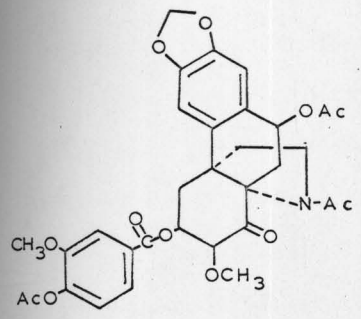
Isolation and Preliminary Characterization of Stephavanine (XXI). The acid-soluble fraction of an ethanolic extract of rhizomes of Stephania abyssinica[34] was partially neutralized to pH 5 and extracted with chloroform to give a fraction designated as the weak base fraction. The sparingly soluble stephavanine hydrochloride could be readily isolated from this fraction by crystallization and was converted to the free base, stephavanine (XXI), $C_{26}H_{27}NO_9$, mp 229-230°(d). The compound was characterized by the following salts: oxalate, mp 178-179°(d); formate, mp 192-193°(d);



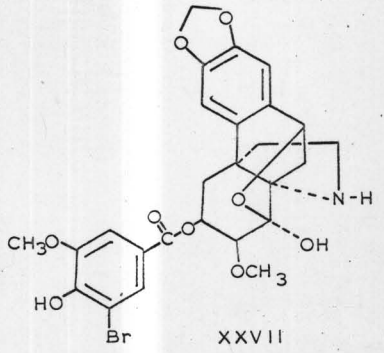
XXIII, R= H
XXVIII, R= CH_3



XXI, $R^1 = R^2 = H$
XXV, $R^1 = R^2 = CH_3$
XXVI, $R^1 = CH_3$, $R^2 = H$



XXIV



XXVII

FIGURE 7. STEPHAVANINE AND DERIVATIVES.

malonate, mp 174-175°(d); hydrochloride, mp 217-218°(d); and hydrobromide, mp 191-192°(d). Stephavanine showed an infrared absorption band at 5.88 μ, attributable to an unsaturated ester and also showed absorption bands assigned to -OH (2), -NH (1), and aromatic systems. The uv spectrum indicated the presence of aromatic nuclei (λ_{max} 264, 288 $m\mu$) and the nmr spectrum of the trimethylsilyl ether (XXII), $C_{29}H_{35}NO_9Si$, showed resonances assigned to two methoxyl groups (τ 6.10 (3H, s) and τ 6.43 (3H, s), 5 aromatic protons (τ 2.67 to τ 3.52), an aromatic methylenedioxy linkage (τ 4.28 (1H, d.) and τ 4.78 (1H, d.), and 3 aliphatic protons on carbon bearing oxygen (τ 4.83 (1H, m.), τ 5.12 (1H, d.), and τ 5.67 (1H, d.). The mass spectrum of the alkaloid showed only one major fragmentation which gave rise to an ion at m/e 214, the relative intensity of which was characteristic of a hasubanan type alkaloid.[29]

Hydrolysis of stephavanine gave the alkamine stephine (XXIII), $C_{18}H_{21}NO_6$, and vanillic acid, which was identified by comparison with an authentic sample. Stephine showed no carbonyl absorption in the infrared but did show absorptions for 2 OH and 1 NH groups. Acetylation of stephavanine gave a triacetyl derivative (XXIV) which showed a carbonyl absorption in the infrared attributable to a ketone, indicating the presence in the alkaloid of a masked ketone function in the form of a hemiketal. Thus the nine oxygens in the molecule can be accounted for as methoxyl (2), methylenedioxy (2), unsaturated ester (2), hemiketal (2), and phenol (1, from vanillate ester) while the nitrogen is present as a secondary amine.

A major portion of the structure elucidation of stephavanine was accomplished through mass spectral and nmr measurements of the parent compound and derivatives. The results and conclusions obtained from each of these techniques are discussed separately in subsequent sections.

The Mass Spectra of Stephavanine and Derivatives. In the mass spectrum of stephavanine, $C_{26}H_{27}NO_9$, the molecular ion appears at m/e 497 and the base peak appears at m/e 214. The mass spectrum of the derived alkamine (stephine, XXIII) also showed the base peak at m/e 214 with a strong peak at m/e 215 (85%). This was very striking since the only other peak in the spectrum having a relative abundance greater than 20% was the molecular ion at m/e 347. High resolution spectrometry showed the base peak to have the elemental composition $C_{13}H_{12}NO_2$.

It has been noted earlier by Tomita and co-workers that the mass spectral fragmentation pattern for hasubanan alkaloids is very characteristic,[29] usually involving loss of the C-ring to give rise to the base peak in the spectrum. This is a very facile cleavage due to the fact that both of the carbon atoms at the B-C-D ring juncture are quaternary. This provides a ready differentiation from the morphine series, in which only one of the elements of the D ring is attached to the B-C ring juncture and loss of ring C cannot effectively occur (compare structure XIX with structure XX). The mass spectra of stephavanine and derivatives are presented in Table 3 and it is noteworthy that in each of the compounds examined the base peak arises from cleavage at the B-C-D ring juncture. These results are

TABLE 3

Mass Spectra of Stephavanine and Derivatives

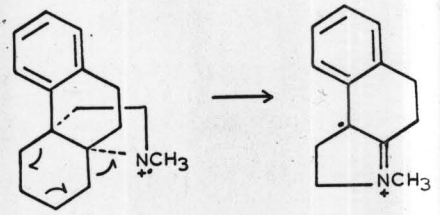
Compound	Formula	M^+ (%)	Base Peak m/e	Other m/e (%)
Stephavanine (XXI)	$C_{26}H_{27}NO_9$	497 (23)	214	215 (43), 213 (95), 151 (26).
Stephavanine trimethylsilyl ether (XXII)	$C_{29}H_{35}NO_9Si$	569 (30)	213, 214	215 (80), 151 (81), 73 (58).
Stephavanine bromide (XXVII)	$C_{26}H_{26}NO_9Br$	576 (3)	213, 214	578 (3), 248 (65), 246 (66),
Stephavanine bromide trimethylsilyl ether (XXVIIa)	$C_{29}H_{34}NO_9BrSi$	647 (4)	213	649 (3), 215 (30), 214 (82).
Stephine (XXIII)	$C_{18}H_{21}NO_6$	347 (19)	214	288 (6), 215 (85).
Bis-(trimethylsilyl) stephine (XXIIIa)	$C_{24}H_{37}NO_6Si_2$	491 (10)	214	215 (60), 213 (93), 73 (25).
Stephavanine methyl ether (XXVI)	$C_{27}H_{29}NO_9$	511 (18)	213, 214	215 (37), 182 (13), 165 (25).
N-Methyl stephavanine methyl ether (XXV)	$C_{29}H_{31}NO_9$	525 (5)	227	229 (80), 228 (93), 182 (23), 165 (18).

TABLE 3 (Cont'd)

Mass Spectra of Stephavanine and Derivatives

Compound	Formula	M^+ (%)	Base Peak m/e	Other m/e (%)
O,O,N-Triacetylstephavanine (XXIV)	$C_{32}H_{33}NO_{12}$	623 (.2)	255	381 (8), 213 (40), 168 (40), 153 (12), 151 (6).
6-Oxostephine (XXIX)	$C_{18}H_{19}NO_6$	345 (8)	214	286 (18), 244 (11), 213 (83).
8-Oxostephine (XXX)	$C_{18}H_{19}NO_6$	345 (33)	301	327 (17), 269 (67), 241 (36), 214 (41).
N-Methyl stephine (XXVIII)	$C_{19}H_{23}NO_6$	361 (10)	229	228 (66), 227 (18).

extremely significant and provide strong evidence for a hasubanan skeleton in stephavanine. The general fragmentation mechanism for a typical hasubanan alkaloid is shown below, using hasubanan as an example.



m/e 241 m/e 185

The major ion thus exhibits seven degrees of unsaturation (rings plus double bonds) which is expected to be the case for the typical hasubanan alkaloid. The fragment $C_{13}H_{12}NO_2$, m/e 214, which is the base peak in stephavanine and stephine, exhibits eight degrees of unsaturation. This is readily explicable by the fact that an aromatic methylenedioxy group is present in these compounds. Furthermore, the fragmentation makes possible the assignment of the methylenedioxy group to ring A. Mass shifts are observed in the base peak in compounds in which the functionality of the nitrogen is altered. Thus in N-methylstephine (XXVIII) vs. stephine (XXIII) and in N-methylstephavanine methyl ether (XXV) vs. stephavanine methyl ether (XXVI), there is an increase of 14 mass units in the base peak. Likewise in O, O, N-triacetylstephavanine (XXIV) there is a shift of 42

mass units in the base peak, attributable to the acetamide function generated in ring D. These data strongly support the presence of a secondary nitrogen atom in the hasubanan skeleton.

The position of the hemiketal in the molecule can be proposed in the light of the structures of metaphanine (XIV) and prometaphanine (XIII), in both of which the hydroxyl portion of the hemiketal is located at C-10. This location is in accord with biogenetic considerations and the chemical shift of the proton on the carbon bearing the hemiketal hydroxyl is indicative that this is a benzylic position. In the mass spectrum of metaphanine this oxygen function is lost concurrently with ring C[29] and in all of the derivatives of stephavanine examined, this is also the case. A mechanistic view of the loss of this oxygen atom with ring C is presented in Figure 8. There are apparently two mechanistic possibilities after the first cleavage to give ion a; there can be either a simple cleavage of the 5-13 bond to give ion b or there can be a hydrogen rearrangement to C-10 (or C-13) concomitant with cleavage of the 5-13 bond to give ion c (or c'). Thus for stephine (XXIII), these processes would be expected to lead to ions at m/e 214 and 215, both of which are observed at high relative abundances (100% and 85% respectively). The 347 to 215 transition is supported strongly by the presence of the appropriate metastable peak in the mass spectrum of stephine.

The presence of a phenolic nucleus in the side chain of stephavanine is confirmed by mass shifts of 14 units in the diazomethane methylation product (stephavanine methyl ether, XXVI) and a shift of 78 and 80 mass

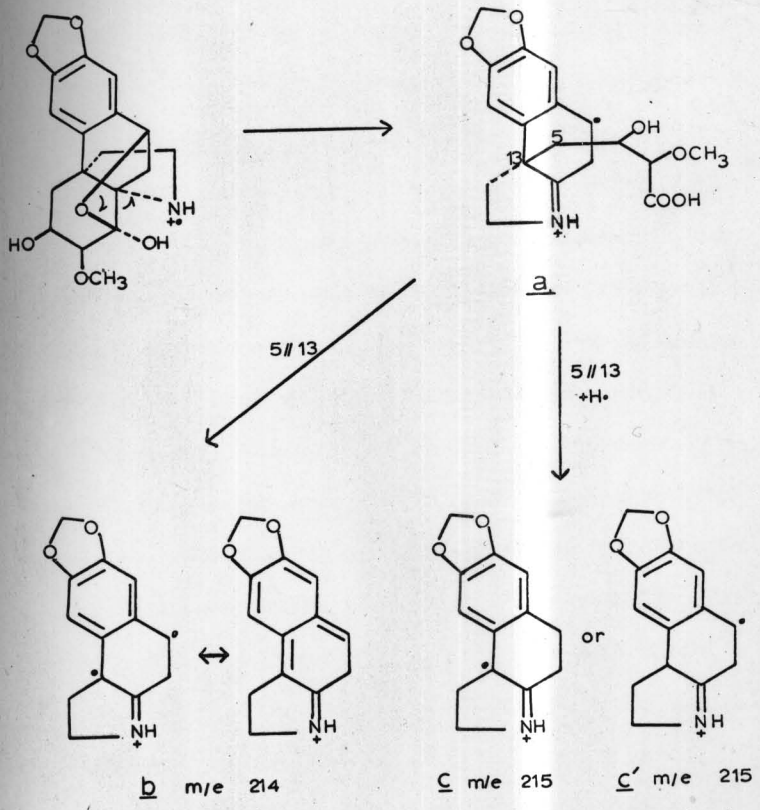


FIGURE 8. MASS SPECTRAL FRAGMENTATION OF STEPHINE.

units in stephavanine bromide (XXVII) in both the m/e 168 ion, $C_8H_8O_4$ (vanillic acid), and the m/e 151 ion, $C_7H_8O_3$ (corresponding acylonium ion).

Oxidation of stephine (XXIII) using Jones' reagent in acetone-acetic acid gave a single product, 6-oxostephine (XXIX), when the reaction was worked up with sodium bicarbonate. However, if sodium hydroxide was used in the workup a second product of lower R_f was also detected. This product was isomeric with 6-oxostephine and could be prepared by mild acid or base treatment of 6-oxostephine. The latter observation supported formulation as a hemiketal isomer in which the C-10 alcohol was bound to the newly formed C-6 ketone, rather than the C-8 ketone, resulting in formation of a 7 membered oxygen ring rather than the 5 membered one present in 6-oxostephine. In this isomer, the free ketone is at C-8 rather than at C-6, so this compound has been designated as 8-oxostephine (XXX). The change from the 5 membered to the 7 membered oxygen bridge is supported by the nmr spectra of the two compounds. In the nmr spectrum of 6-oxostephine (XXIX), the proton on carbon bearing the hemiketal hydroxyl at C-10 appears as a doublet (the dihedral angle with one of the two C-9 hydrogens is $\backsim 90^{\circ}$, giving rise to a coupling constant of zero Hz) but in 8-oxostephine (XXX) rings C and B must alter conformation in order to form the 7 membered oxygen ring and the two C-9 protons become equivalent with respect to the C-10 proton, which now appears as a triplet. The marked difference in mass spectral fragmentation patterns of 6-oxostephine (XXIX) and 8-oxostephine (XXX) is noteworthy. In XXIX, the mass spectrum shows the typical pattern discussed earlier for stephine,

the base peak at m/e 214 in the spectrum of XXIX arising by loss of the C ring and the benzylic oxygen of the hemiketal from C-10.

As can be seen from Figure 9, the loss of the benzylic oxygen atom in 6-oxostephine is concerted with formation of a double bond to nitrogen, which is the key step in directing the fragmentation. In the case of 8-oxostephine the relative positions of the hemiketal terminus and the nitrogen are altered and it is no longer possible to transfer electrons in a concerted manner. Not surprisingly, the base peak arises by a completely different type of fragmentation in this compound and, in fact, corresponds to the loss of ring D rather than ring C, to give an ion at m/e 301. The cleavage patterns for 6-oxostephine and 8-oxostephine are found in Figure 9, together with possible mechanisms for their formation. The cleavage of 8-oxostephine is regarded to be initiated by homolysis of the 14-17 bond and subsequent hydrogen transfer to nitrogen followed by homolysis of the 13-15 bond and the loss of ring D.

Since both the C ring and the D ring are bound to quaternary carbon at the ring juncture with ring B in hasubanan alkaloids, it would appear logical that the loss of either ring C or ring D could occur readily. The observed preference for the loss of ring C is anticipated in most cases since this provides for the stabilization of the radical ion on nitrogen. The observation in the case of 8-oxostephine of loss of the D ring is extremely important since this also serves to indicate that both the C ring and the D ring are quaternary, giving further support to the assignment of the hasubanan skeleton to stephavanine. This cleavage of the D ring

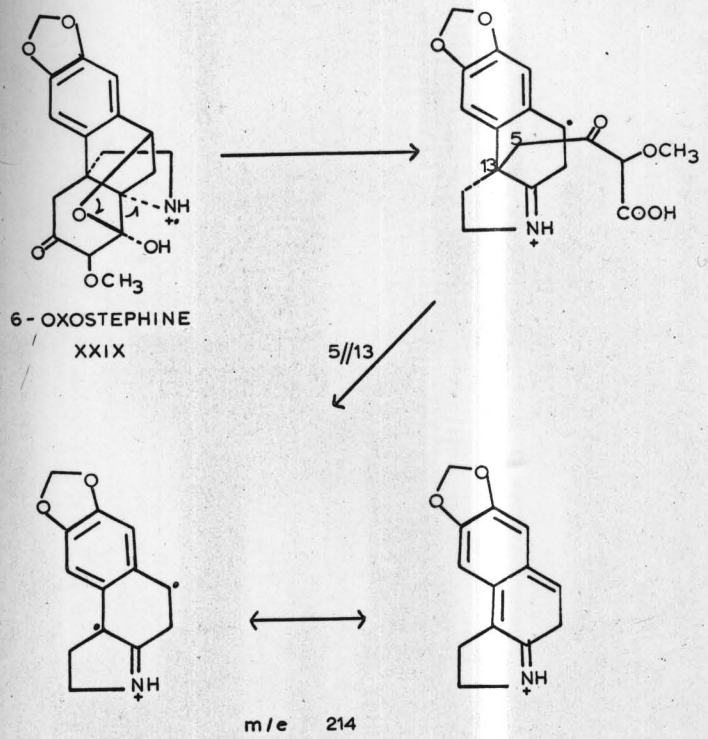
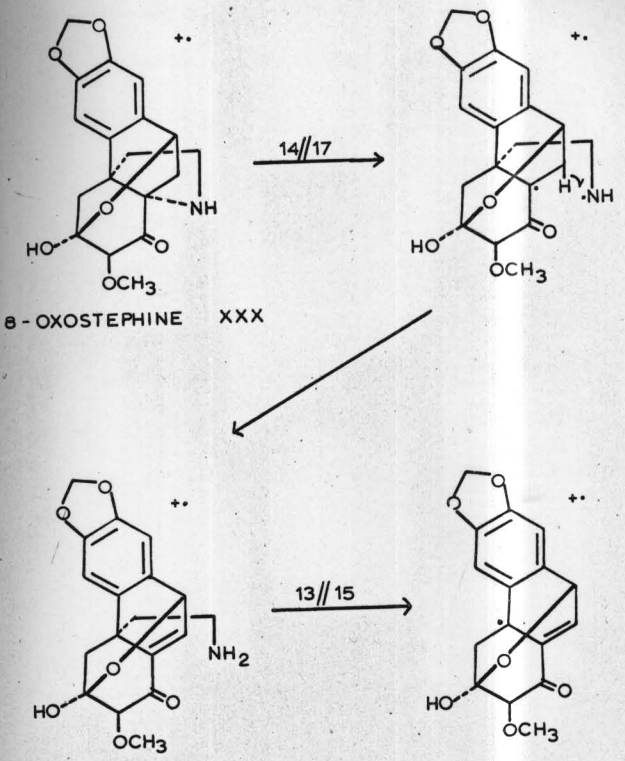


FIGURE 9. MASS SPECTRAL FRAGMENTATIONS OF 6-OXOSTEPHINE AND 8-OXOSTEPHINE.



8-OXOSTEPHINE XXX

FIGURE 9. - CONTINUED.

observed in the case of 8-oxostephine has been observed earlier in our studies of 4-demethylhasubanonine (XVI) and some of its derivatives which are discussed in Part IV of this thesis.

Thus it would appear that the observation of Tomita et al.[29] that the loss of ring C is characteristic of and diagnostic for hasubanan alkaloids should be extended to include the alternative loss of the D ring, since either one of these modes of cleavage can occur readily and give rise to the major fragment ion in the spectrum. While the preference generally seems to be for loss of ring C, the loss of ring D does occur in a significant number of cases, depending on the specific substitution pattern of the hasubanan alkaloid and should be considered as being equally diagnostic for members of this series.

Furthermore, the occurrence of both of these cleavages in different derivatives of the same parent compound, as is observed in 6-oxostephine and 8-oxostephine, gives independent evidence of both a quaternary C ring and a quaternary D ring. Such data should be recognized as the strongest type of spectroscopic evidence for the hasubanan nucleus.

NMR Spectra of Stephavanine (XXI) and Derivatives. The data available from the mass spectral fragmentation of stephavanine and derivatives thereof have shown that it is a hasubanan type alkaloid having a methylenedioxy group in Ring A, a benzylic oxygen function as part of a hemiketal in Ring B and a secondary nitrogen atom in Ring D. Stephavanine is not sufficiently soluble in most of the common nmr solvents to

obtain useful spectral data. It is sufficiently soluble in D_6-DMSO or D_4-acetic acid but the resolution obtained in these solvents was inadequate. Therefore stephavanine was converted to its trimethylsilyl ether, the nmr spectrum of which is presented in Figure 10. The initial silation of stephavanine forms a bis-(trimethylsilyl) ether which, upon silica gel chromatography, is converted to the more stable monotrimethylsilyl ether. This derivative contains two deuterium exchangeable protons (one OH and the NH functionality) and was used for the nmr measurements.

The three downfield signals in the nmr spectrum of stephavanine trimethylsilyl ether represent a classic 1, 2, 4 aromatic proton pattern and are assigned to the aromatic protons of the vanillate ester. The other two signals at τ 3.48 and τ 3.52, are singlets assigned to the A ring of the alkamine portion of the molecule. Since these signals appear as singlets in all the nmr spectra of stephavanine and derivatives they can be securely assigned to protons para to each other and thus, the methylenedioxy group in ring A must be in the 2 and 3 positions. The nmr signals of the methylenedioxy group are rather unusual because of the extremely high field resonance of one of the protons and the large difference in chemical shift between the resonances assigned to the protons involved. An examination of reference spectra[30] showed that for 14 aromatic methylenedioxy groups listed in 13 different compounds of a variety of chemical types, the chemical shifts were distributed over a relatively narrow range, varying from τ 3.77 to τ 4.16. A chemical shift of approximately 4.0 τ units is considered as typical for an aromatic methylenedioxy group.[31] In those cases where

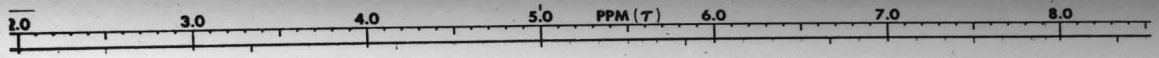
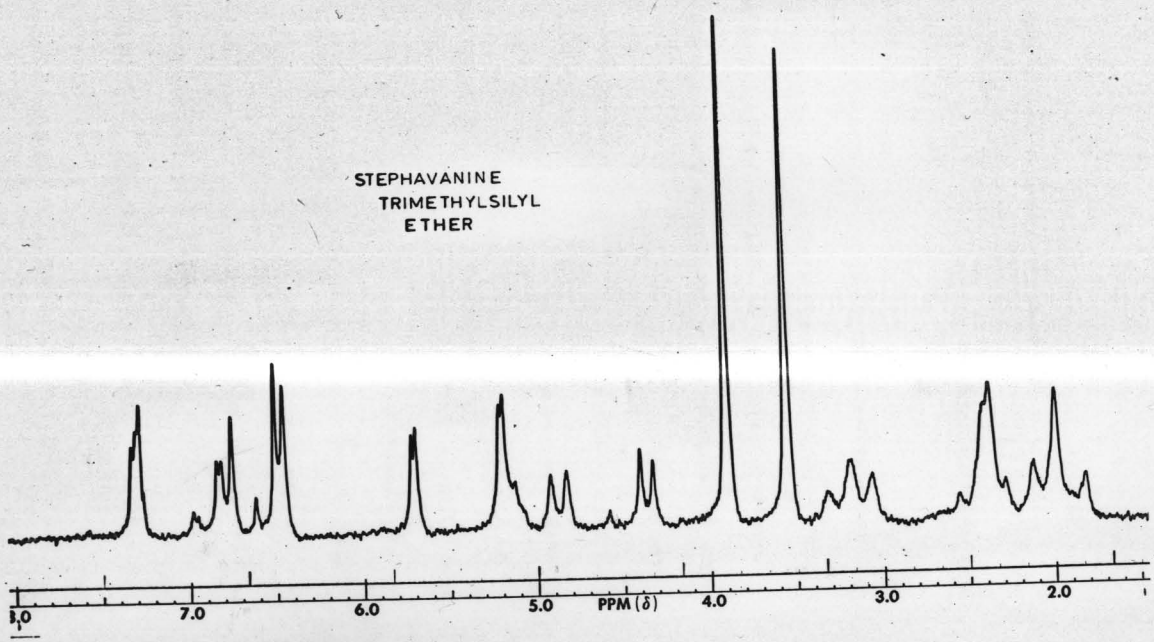

2.0
3.0
4.0
5.0
PPM (τ)
6.0
7.0
8.0
400
300
200
100

FIG. 10

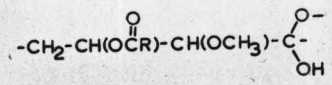

STEPHAVANINE
TRIMETHYLSILYL
ETHER
8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0

non-equivalence of the two protons was observed, there was not more than 0.15 τ units difference in the chemical shift of the two protons. The non-equivalent situation is frequently observed in aporphine alkaloids, due to the presence of a twisted biphenyl system[31,32] wherein the adjacent non-coplanar aromatic ring exhibits a selective deshielding towards one of the two protons.

In stephavanine trimethylsilyl ether (XXII) the high field proton resonates at τ 4.78 and the low field proton resonates at τ 4.28. Both of these signals are upfield of the normal range, the high field proton being more than 0.6 τ units upfield from any of those compounds examined in the spectral compilation. This observation is extremely significant in view of the normally narrow range within which these types of protons are found. The line separation between the upfield and downfield protons is also extremely large (0.5 τ) in comparison with the examples examined. The profound shielding of the upfield proton supports assignment of the axial configuration for the ester side chain, in which the aromatic ring of the vanillate ester is in close proximity to the methylenedioxy group. The protons of the latter group lie above the plane of the aromatic ring and are consequently exposed to a large shielding effect due to the induced diamagnetic circulation of the π-electrons around the aromatic ring.[33] This shielding mechanism is supported by the fact that this phenomenon is only noted in those cases in which the ester is present. In the alkamine and all of its derivatives, the signal for the methylenedioxy group appears as a 2-proton singlet in the normal range. Chemical evidence for the axial configuration of the C-6 ester is presented in a later section.

ester is presented in a later section.

The nmr signals of stephavanine and derivatives are found in Table 4. Proof that the substituents in ring C are arranged in the order indicated is shown by the following series of experiments. Acetylation of stephavanine (XXI) gives O, O, N-triacetylstephavanine (XXIV) in which the hemiketal is converted to a ketone assigned to C-8. There is only one proton which is shifted downfield by virtue of being adjacent to a ketone and this is a doublet at τ 5.67 which is shifted to τ 4.73 in XXIV with no change in coupling constant. This signal is assigned to the C-7 proton. This proton is shown to be coupled to the proton on carbon bearing hydroxyl in bis-(trimethylsilyl)-stephine (XXIIIa) by decoupling experiments. Oxidation of stephine (XXIII) to 6-oxostephine (XXIX) changes the signal assigned to the C-7 proton from a doublet with a coupling constant of 4Hz to a singlet and also results in a downfield shift of this signal, since the C-7 proton is now adjacent to a ketone. The C-7 proton signal is shifted from τ 6.00 (d, J=4) in XXIIIa to a singlet at τ 5.38 in (XXIX). These transformations are shown in Figure 11. The fact that the proton assigned to C-6 gives rise to a complex multiplet in the nmr spectra of all of the compounds in which it is present indicates that it must be adjacent to a methylene group. This would indicate the following partial structure for ring C of stephavanine:



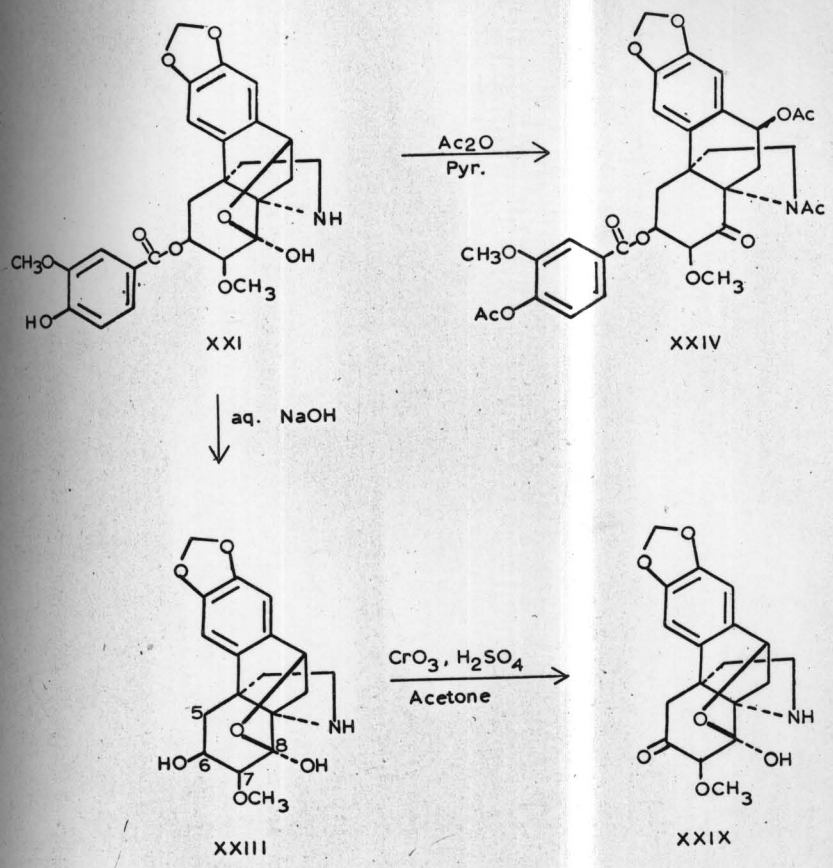


FIGURE 11. TRANSFORMATIONS OF STEPHAVANINE (XXI).

TABLE 4

Nmr Signals of Stephavanine and Derivatives[a]

	C-1, C-4	C-2, C-3	C-6	C-7	C-10	OCH_3	Vanillate ester	Other Signals
Stephavanine trimethylsilyl ether (XXII)	3.48 s 3.52 s	4.28 d(1.5) 4.78 d(1.5)	4.83 m	5.67 d(4)	5.12 d(5.5)	6.10 s 6.43 s	2.67 d (1.5) 3.12 d,d (1.5, 8) 3.28 d (8)	$Si(CH_3)_3$ 9.88 s
N-Methyl stephavanine methyl ether (XXV)	3.50 s 3.57 s	4.32 d(1.5) 4.90 d(1.5)	4.82 m	5.80 d(5)[b]	5.11 d(6)	6.07 s (6H) 6.38 s	2.72 d (1.5) 3.02 d,d (1.5,8.5) 3.32 d (8.5)	$N\text{-}CH_3$ 7.42 s
Stephavanine methyl ether (XXVI)	3.52 s (2H)	4.32 d(1.5) 4.90 d(1.5)	4.83 m	5.80 d(5)	5.17 d(5.5)	6.07 s (6H) 6.37 s	2.70 d (2) 2.98 d,d (2,8) 3.30 d (8)	
O,O,N-Triacetyl-stephavanine (XXIV)	3.40 s 3.47 s	4.22 d(1) 4.55 d(1)	4.62 m	4.73 d(4)	5.03 d(6)	6.15 s 6.50 s	2.57 s 3.12 s (2H)	Acetate 7.68 s 7.80 s 7.95 s
Stephine bis-(trimethylsilyl) ether (XXIIa)	3.37 s 3.45 s	4.10 s (2H)	6.13 m	6.00 d(4)	5.20 d(5.5)	6.53 s	-	$Si(CH_3)_3$ 9.83 s 10.17 s

TABLE 4 (Cont'd)

Nmr Signals of Stephavanine and Derivatives[a]

	C-1, C-4	C-2, C-3	C-6	C-7	C-10	OCH_3	Vanillate ester	Other Signals
N-Methyl stephine (XXVIII)	3.34 s 3.50 s	4.15 s (2H)	6.17 m	6.17 m	5.28 d(6)	6.48 s		$N-CH_3$ 7.53 s
6-Oxostephine (XXIX)	3.32 s 3.48 s	4.07 s (2H)	-	5.38 s	5.28 d(6)	6.40 s		
8-Oxostephine (XXX)	3.10 s 3.18 s	4.03 s (2H)		c	5.18 t(3)	6.20 s		

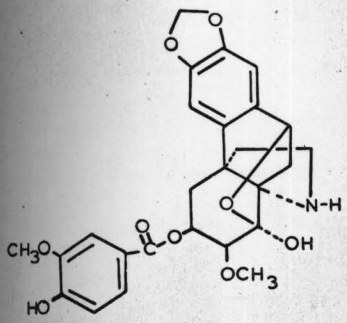
a τ values (60 MH_z) in $CDCl_3$ (TMS).

b after deuteration.

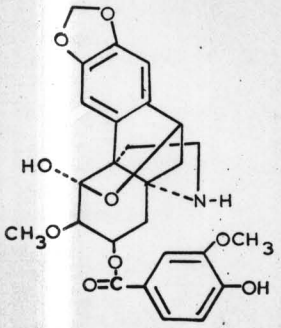
c unresolved

Considering the hasubanan nucleus and the mass spectral data in conjunction with the above nmr data there are only two probable formulations for stephavanine, structure XXI, the one assigned, and structure XXXI, which differs in the orientation of the partial structure of ring C. These structures are shown in Figure 12. Structure XXXI can be eliminated on the following grounds: (1) The hemiketal from C-10 to C-5 is appreciably more strained and it is, in fact, impossible to construct a model of it. (2) The shielding of the methylenedioxy protons discussed earlier can only be achieved by an axial ester at C-6, whereas there is no opportunity for interaction between these protons and a C-7 ester in either configuration regardless of conformation of ring C. (3) Concerted loss of the benzylic oxygen of the hemiketal, and of ring C, in the mass spectrum is not feasible (see Figure 8) and the mass spectral data obtained for stephavanine are not consistent with structure XXXI. (4) Biogenetic analogy shows that C-5 is not oxygenated in any of the naturally occurring hasubanan alkaloids whereas C-8 is oxygenated in every case but one. Furthermore all of the compounds in this series which have oxygen bridges between the B and C rings are bridged from C-10 to C-8.

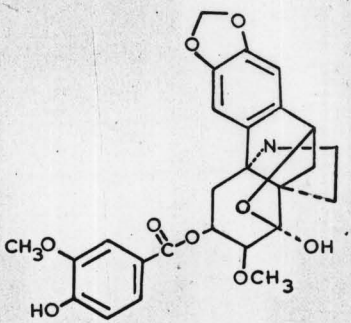
Thus, if stephavanine indeed has a hasubanan skeleton, as shown by the mass spectral data, it must be formulated as XXI. There is but one other remote possibility for the structure of stephavanine, which is structure XXXII. This is not a hasubanan structure and is not a member of any known series of alkaloids but it might be expected to show similar nmr spectral properties to those observed and the chemical reactions



XXI



XXXI



XXXII

FIGURE 12. POSSIBLE STRUCTURES OF STEPHAVANINE.

undergone by stephavanine would not be inconsistent with such a structure. However, due to the different relative positions of the nitrogen and the benzylic hemiketal oxygen it appears that the type of mass spectral fragmentation undergone by hasubanan alkaloids having an oxide bridge would no longer be applicable (see Figure 8). The loss of the benzylic oxygen together with the C ring in structure XXXII is very difficult to rationalize mechanistically and the high relative abundance of the ion derived from this process militates strongly against structure XXXII.

In view of the known occurrence of hasubanan alkaloids exclusively in Stephania species[18-24] and in S. abyssinica in particular[3], and the biogenetic suggestions advanced for the formation of the hasubanan nucleus[25], structure XXXII is improbable on biogenetic grounds as well as on the basis of mass spectral fragmentation patterns. The possibility of such a structure for stephavanine must therefore be considered as highly unlikely.

Stereochemistry of Stephavanine. The absolute configuration of all of the previously-known naturally occurring hasubanan alkaloids is the same[18-24] and has been established by chemical interrelation with the indolecodeine series of alkaloids which are of known absolute configuration[18] and confirmed by X-ray crystallography[23]. It has been assumed for purposes of this discussion that the absolute configuration of stephavanine is the same as that of the other naturally occurring hasubanan alkaloids and this configuration is the one which has been used in the structural drawings, wherein the nitrogen ring is projected towards the alpha face of the molecule.

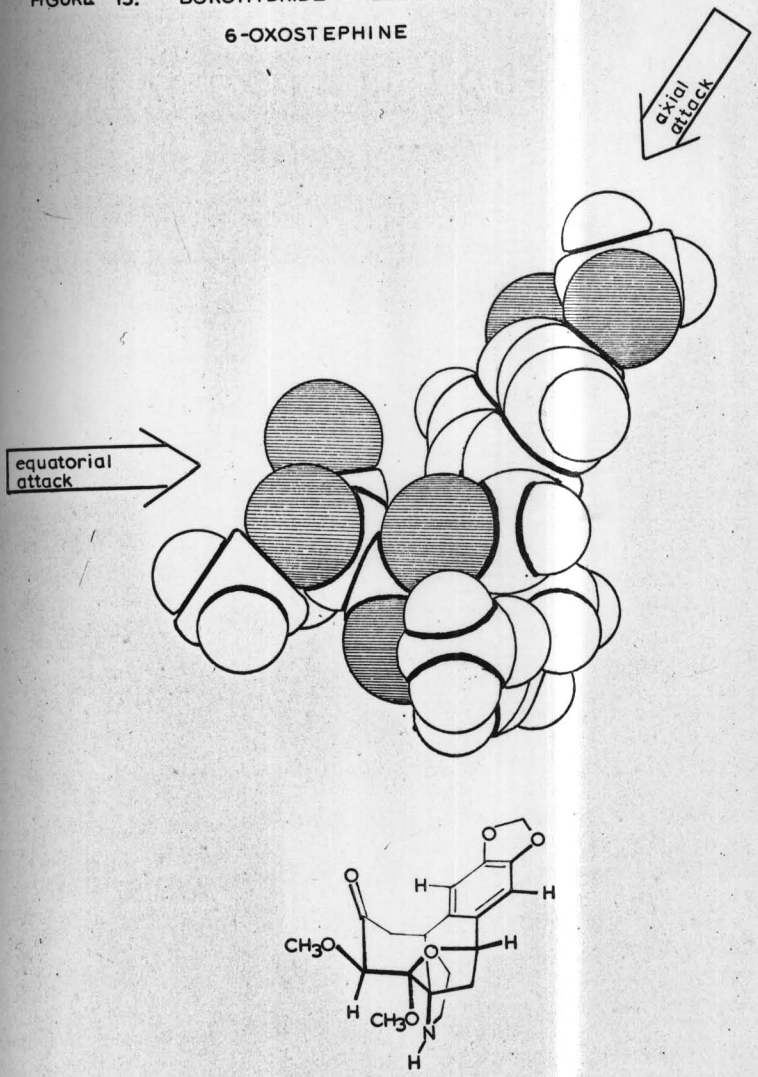
There are six asymmetric centers in stephavanine, located at C-6, C-7, C-8, C-10, C-13 and C-14. Since carbons 13 and 14 are common to three rings at the B-C-D ring junction they form the pivot carbons of a propellerane type of system in which it is readily shown that the ring fusions must all be cis. The stereochemistry at C-8 and C-10 is also readily defined, since the alpha projection of the nitrogen ring constrains the bonds to the oxygen bridge to the beta configuration. Thus the oxygen ring of the hemiketal is beta while the hemiketal hydroxyl at C-8 and the C-10 hydrogen are of the alpha configuration.

An argument for the axial nature of the C-6 ester in stephavanine has already been advanced on the basis of the unusually large shielding of the ring A methylenedioxy protons in the nmr spectrum, and this assignment has been confirmed by an independent experiment. Reduction of 6-oxostephine XXIX with sodium borohydride in methanol gave a single alcohol shown to be pure by tlc and nmr spectroscopy. The product was shown to be identical to stephine, from which the 6-oxostephine had originally been prepared. There exists here a rather unusual case of completely stereospecific reduction of a ketone by sodium borohydride at room temperature. Since this reduction is generally considered to be a relatively fast reaction at room temperature, the steric hindrance to approach from one direction must be very large to obtain the specificity observed. Examination of a Dreiding model of 6-oxostephine shows that there is only one possible chair conformation for ring C because of the cis ring fusion at the B-C-D ring juncture and the hemiketal oxide bridge which prevents inversion of

ring C to an alternate chair. It can be seen in Figure 13 that the proximity of the ketone to the aromatic ring sterically hinders axial delivery of hydride, since the top side of the molecule is very effectively caged by the A ring and the oxide bridge of the hemiketal. Therefore delivery of hydride must be exclusively from the equatorial direction to give the axial alcohol. Since this product is identical to the natural alcohol it follows that the C-6 alcohol in stephine and hence the corresponding ester in stephavanine must be axial and beta.

The methoxyl group at C-7, the only remaining asymmetric center, has been shown to be in its most stable configuration in stephavanine since on vigorous treatment of stephavanine with sodium methoxide in methanol at reflux, it is recovered unchanged. In basic media an equilibrium should be established between the hemiketal and the ketoalcohol such that there is a finite amount of C-8 ketone present. This equilibrium should provide opportunity for epimerization at C-7. Since stephavanine is recovered unchanged, the C-7 methoxyl group must already be in its most stable configuration. Further evidence on this point is available from the hydrolysis of stephavanine to stephine in 2.5 N sodium hydroxide solution, to afford a single alcohol in high yield. The coupling constant of the C-7 hydrogen is the same in the nmr spectra of both stephavanine and stephine, indicating that no epimerization has taken place and that the C-7 methoxyl is in its most stable configuration. It has been assumed that the most stable configuration of the C-7 methoxyl group is equatorial, since in the axial configuration the methoxyl group should be destabilized by two 1,3 diaxial

FIGURE 13. BOROHYDRIDE REDUCTION OF 6-OXOSTEPHINE



interactions; however this assignment cannot be regarded as unequivocal. An attempt has been made to prepare the C-6 epimeric alcohol of stephine in order to study the coupling constants of the C-7 hydrogen to alcohols of both configurations at C-6, to provide additional data on the stereochemistry of the C-7 methoxyl group. However, sodium and alcohol reduction of 6-oxostephine gave only very polar products, apparently due to reduction of the masked C-8 ketone as well as the C-6 ketone. Lithium aluminum hydride reduction showed the same specificity as sodium borohydride, giving stephine in quantitative yield.

The problem of a definitive determination of the absolute configuration of stephavanine is complicated by the fact that interrelation with any of the other hasubanan alkaloids is virtually precluded, due to the difference in oxygenation patterns in ring A. All of the other hasubanan alkaloids bear oxygen substituents at C-3 and C-4 while in stephavanine the methylenedioxy group is located at C-2 and C-3. Comparison of stephavanine and stephine with other hasubanan derivatives was attempted by the use of optical rotatory dispersion but the results were unclear, presumably as a result of the differences in the aromatic chromophore. Therefore in order to establish the absolute configuration of stephavanine and to provide unequivocal evidence for the stereochemistry at C-7 a single crystal X-ray diffraction study of stephavanine hydrobromide has been undertaken by Professor George Sim, at the University of Sussex, England.

EXPERIMENTAL*

Isolation of Oxoxylopine (V) and Stephavanine (XXI). In a typical experiment 7 kg of dried, ground plant material was extracted continuously with ethanol until the extract returning to the pot was nearly colorless. Evaporation of the ethanolic extract gave a mobile semi-solid residue (901 g) which was triturated three times with 5% (W/V) HCl solution (31.). Filtration gave a gummy residue (143 g). The filtrate was partially basified with conc. NH_4OH to pH 5 and was then extracted with chloroform (4-1 l. portions) to give after evaporation the "weak base" fraction (28.7 g). The remaining aqueous solution was separated from insoluble residue (70 g) and was basified to pH 8 with concentrated ammonium hydroxide. Extraction with chloroform (4-1 l. portions) gave on evaporation the "strong base"

* Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected for stem exposure. Optical rotations were measured on a Zeiss-Winkel polarimeter and are approximated to the nearest degree. Infrared spectra were determined on Beckman model IR-5A or IR-9 recording spectrophotometers. Ultraviolet spectra were determined on a Beckman model DK-2A recording spectrophotometer. NMR spectra were determined on a Varian A-60A spectrometer in deuteriochloroform solution with tetramethylsilane as the internal standard except where indicated. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Michigan. Mass spectra were obtained from the Purdue Mass Spectrometry Center Hitachi RMU-6A spectrometer. Petroleum ether refers to the 60-68° boiling fraction. Thin layer chromatography was carried out on silica gel F_{254} plates (Brinkmann) and spots were developed by heating plates sprayed with a 3% (W/V) solution of ceric sulfate in 3N sulfuric acid. All evaporations were carried out below 40° at reduced pressure (water pump).

Rhizomes of Stephania abyssinica Walp. were collected in Ethiopia in April and June, 1965. We acknowledge with thanks receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, Beltsville, Maryland in accordance with the program developed with the U. S. D. A. by the Cancer Chemotherapy National Service Center.

fraction (10.4 g). The "weak base" fraction was suspended in a minimal volume of either acetone or chloroform and shaken to dissolve most of the material. A crystalline insoluble residue was removed by filtration through a sintered glass funnel (medium porosity) to give 1.4 g of material. Two recrystallizations of the solid from methanol gave needles of stephavanine (XI) hydrochloride (1.02 g): mp 217-218° (d); $[\alpha]_D^{32}$ + 16° (c 0.73, MeOH); λ_{max}^{KBr} 2.82, 2.95, 3.39, 4.05, 5.87, 6.25, 7.82, 9.63 μ; λ_{max}^{MeOH} 265 mμ (ϵ 12,240), 290 mμ (ϵ 9,280); m/e 497 (M^+).

Anal. Calcd for $C_{26}H_{28}ClNO_9$: C, 58.46; H, 5.29; Cl, 6.62; N, 2.62. Found: C, 58.54, 58.50; H, 5.33, 5.27; Cl, 6.49, 6.53; N, 2.49, 2.56.

Additional stephavanine (XXI) could be isolated upon chromatography of the weak base fraction or of the strong base fraction, the free base being eluted from acid-washed alumina columns with 5% methanol-chloroform or from silica gel or silicic acid columns with from 2% to 5% methanol-chloroform. The free base, stephavanine (XXI), upon recrystallization from methanol-chloroform gave colorless needles: mp 229-230°(d); $[\alpha]_D^{32}$ +30° (c 0.90, pyr.); λ_{max}^{KBr} 2.80, 2.98, 3.38, 5.88, 6.24, 7.82μ; λ_{max}^{MeOH} 264 mμ (ϵ 15,830), 288 mμ (ϵ 11,900); m/e 497 (M^+).

Anal. Calcd for $C_{26}H_{27}NO_9$: C, 62.77; H, 5.47; N, 2.82. Found: C, 62.71, 62.84; H, 5.38, 5.26; N, 2.84.

The weak base fraction, after removal of stephavanine hydrochloride, was chromatographed on silicic acid (900 g) eluting with chloroform and subsequently methanol-chloroform mixtures. The fraction eluted with 2 1/2% methanol-chloroform (6 g) was rechromatographed over acid-washed alumina using benzene-chloroform mixtures as eluant. A fraction (150 mg) eluted

with 2:1 benzene-chloroform crystallized on standing to give orange prisms (108 mg). Recrystallization from chloroform gave yellow-orange prisms of oxoxylopine (V): mp 319-321°; λ_{max}^{KBr} 3.88, 6.02, 6.24, 6.86 μ; $\lambda_{max}^{CHCl_3}$ 246.5 mμ (ϵ 28,650), 271 mμ (ϵ 21,800), 314 mμ (ϵ 5,960), 381 mμ (ϵ 3,010), 430 mμ (ϵ 4,750); $\lambda_{max}^{.5\underline{N}HCl}$ 257 mμ (ϵ 20,570), 284 mμ (ϵ 15,500), 356 mμ (ϵ 3,780), 395 mμ (ϵ 3,780), 505 mμ (ϵ 2,215); m/e 305 (M^+).

Anal. Calcd for $C_{18}H_{11}NO_4$: C, 70.81; H, 3.63; N, 4.59.

Found: C, 70.88; H, 3.76; N, 4.63.

Conversion of Oxoxylopine (V) to (+)-N-Acetylxylopine (IX).

Oxoxylopine (V, 22 mg) in HOAc-H_2O (2:1, 2 ml) was treated with powdered zinc (3 g) and conc. HCl (6 ml). The reaction mixture was heated with stirring at 100° for 18 hours, at which time all of the zinc was consumed and the reaction mixture turned red indicating that the reaction was incomplete (oxoxylopine gives a cherry red solution in HCl). Additional zinc powder (1 g) and conc. HCl (3 ml) was added and the reaction was again heated with stirring at 100° for an additional 24 hours, at which time all of the zinc had been consumed and the reaction mixture was colorless. The acidic solution was made strongly basic with a large excess of conc. NH_4OH and was extracted with chloroform (5-150 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give crude xylopine (18 mg), which was then acetylated without further purification. The crude xylopine was dissolved in pyridine (1 ml), acetic anhydride (1 ml) was added and the resulting clear solution was heated at 70° for 1/2 hour. The

reaction mixture was allowed to cool and was kept at room temperature for three hours. The solution was evaporated to dryness, and residual pyridine was azeotroped with dry benzene until the odor of pyridine was no longer discernable. The residue was dissolved in ether-chloroform (3:1, 50 ml) and washed successively with 50 ml portions of 0.5 N HCl, 1 N NaOH, and water. The organic phase was dried (Na_2SO_4) and evaporated to leave a solid residue. Two crystallizations from acetone-ether gave colorless needles (9 mg). The crystalline material was chromatographed on a silicic acid column (5 g) in chloroform. Elution with chloroform gave the desired material in the third, fourth, and fifth 10 ml fractions which showed uv λ_{max}^{EtOH} 216.5 mμ (ε 32,200), 283 mμ (ε 16,700); ir $\lambda_{max}^{CHCl_3}$ 3.32, 3.41, 3.45, 3.52, 6.12, 6.33μ. The spectra were indistinguishable from those of an authentic sample.

Hydrolysis of Stephavanine (XXI) to Stephine (XXIII) and Vanillic Acid.

Stephavanine (232 mg) was dissolved with stirring in 2.5 N NaOH (15 ml) at room temperature and stirring was continued for four hours to give a copious white precipitate. The reaction mixture was adjusted to pH 1 with 0.5 N hydrochloric acid and extracted with chloroform (5-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give the non-nitrogenous fraction (58 mg) as crystalline material. Three recrystallizations from acetone-chloroform gave colorless needles (mp 209-210°) showing identical high resolution ir, uv, and nmr spectra to authentic vanillic acid, (Aldrich). A mixture melting point was not depressed.

The acidic solution was basified to pH 8 with saturated $NaHCO_3$ solution and was extracted with chloroform (4-50 ml portions) to give crude stephine (165 mg). Two crystallizations from chloroform-ether gave colorless prisms: mp 224-226° (d); $[\alpha]_D^{26}$ +99° (c 0.84, AcOH); λ_{max}^{KBr} 2.84, 3.03, 3.14, 3.39, 6.71 μ; $\lambda_{max}^{.5NHCl}$ 243 mμ (ϵ 1880), 290 mμ (ϵ 2425); m/e 347 (M+).

Anal. Calcd for $C_{18}H_{21}NO_6$: C, 62.24; H, 6.10; N, 4.03. Found: C, 62.10; H, 6.16; N, 4.03.

Conversion of Stephavanine (XXI) to Stephavanine Bromide (XXVII). Stephavanine (200 mg) was dissolved in dimethyl sulfoxide (5 ml) and bromine (10% in CCl_4) was added dropwise at ambient temperature until a distinct reddish-brown color persisted. After one hour the excess bromine was discharged by the addition of 5% sodium thiosulfate solution. The reaction mixture was diluted with water (50 ml) and extracted with methylene chloride (5-50 ml portions). The combined extracts were evaporated to dryness and chromatographed on acid-washed alumina (15 g) in chloroform. Elution with chloroform removed demethylsulfoxide and subsequent elution with methanol-chloroform (1:20) gave stephavanine bromide (XXVII, 60 mg) which was crystallized three times from methanol-chloroform to give colorless prisms: mp 218-219° (d); λ_{max}^{Nujol} 2.86, 3.15, 5.90 μ; λ_{max}^{EtOH} 272 mμ (ϵ 10,270); m/e 576, 578, (M+).

Anal. Calcd for $C_{26}H_{26}NO_9Br$: C, 54.18; H, 4.55; N, 2.43; Br, 13.87. Found: C, 54.38; H, 4.57; N, 2.72; Br, 15.72.

Conversion of Stephavanine Bromide (XXVII) to Stephavanine Bromide Trimethylsilyl Ether (XXVIIa). To stephavanine bromide (350 mg) in pyridine (10 ml) was added hexamethyldisilazane (7 ml) and trimethylchlorosilane (3.5 ml). After five minutes the reaction mixture was poured into water (50 ml) and extracted with chloroform (5-25 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated and azeotroped with dry benzene until the odor of pyridine was no longer discernable. Chromatography of the residue on silica gel (100 g) with methanol-chloroform (1:100) as the eluant gave 250 mg of material. Three crystallizations from chloroform ether gave stephavanine bromide trimethylsilyl ether (XXVIIa): mp 197-198° (d); λ_{max}^{KBr} 2.97, 3.38, 3.46, 5.88 μ; λ_{max}^{MeOH} 268 mμ (ϵ 12,150); m/e 647, 649, (M+).

Anal. Calcd for $C_{29}H_{34}NO_9BrSi$: C, 53.70; H, 5.29; N, 2.16. Found: C, 53.72, 53.85; H, 5.30, 5.36; N, 2.18, 2.21.

Conversion of Stephavanine (XXI) to Stephavanine Trimethylsilyl Ether (XXII). Stephavanine hydrochloride (85 mg) in anhydrous pyridine (12 ml) was treated with hexamethyldisilazane (1.6 ml) and trimethylchlorosilane (0.8 ml) for five minutes. The reaction mixture was poured into water (50 ml) and extracted with chloroform (3-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated, and azeotroped with dry benzene until the odor of pyridine was no longer discernable. The residue so obtained (120 mg) was chromatographed on a silicic acid column and eluted with methanol-chloroform (1:20). Three crystallizations from chloroform gave colorless needles: mp 201-202° (d);

λ_{max}^{KBr} 2.96, 3.38, 3.46, 5.89 μ ; λ_{max}^{MeOH} 262 mμ (ϵ 11,650), 291 mμ (ϵ 9,690); m/e 569 (M^+).

Anal. Calcd for $C_{29}H_{35}NO_9Si$: C, 61.00; H, 6.14; N, 2.46. Found: C, 60.97; H, 6.13; N, 2.52.

Conversion of Stephine (XXIII) to Bis-(trimethylsilyl) Stephine (XXIIIa). Stephine (100 mg) in pyridine (5 ml) was treated with hexamethyldisilazane (1 ml) and trimethylchlorosilane (0.5 ml). After five minutes the reaction was poured into water (50 ml) and extracted with chloroform (3-50 ml portions) and the combined, dried (Na_2SO_4) chloroform extracts were evaporated, and azeotroped with benzene to remove residual pyridine to give one-spot material (110 mg) which upon crystallization from carbon tetrachloride gave colorless prisms: mp 197-199° (sinters); λ_{max}^{KBr} 2.90, 3.38, 7.98, 11.79 μ; λ_{max}^{MeOH} 243 mμ (ϵ 3,720), 294 mμ (ϵ 4,160); m/e 491 (M^+).

Anal. Calcd for $C_{24}H_{37}NO_6Si_2 \cdot 1/3\ CCl_4$: C, 53.82; H, 6.87; N, 2.58. Found: C, 53.72; H, 7.02; N, 2.49.

Conversion of Stephavanine (XXI) to Stephavanine Hydrobromide. Stephavanine (50 mg) was suspended in methanol (10 ml) and excess solution of saturated HBr in methanol was added to pH 1. The solution so obtained was evaporated to dryness to give a colorless residue which was crystallized twice from methanol-ethyl acetate to give colorless needles: mp 191-192°(d); λ_{max}^{KBr} 2.94, 3.38, 4.06, 5.86, 7.78 μ ; λ_{max}^{MeOH} 259 mμ (ϵ 11,950), 291 mμ (ϵ 9,450).

Anal. Calcd for $C_{26}H_{28}NO_9Br$: C, 53.79; H, 4.88; N, 2.42; Br, 13.82. Found: C, 53.76; H, 4.91; N, 2.30; Br, 13.90.

Conversion of Stephavanine (XXI) to Stephavanine Oxalate.

Stephavanine (50 mg) and oxalic acid dihydrate (13 mg) were dissolved in methanol (5 ml), heated to boiling on the steam bath and ether was added to faint turbidity. The mixture was allowed to stand overnight and gave colorless needles (35 mg) of stephavanine oxalate: mp 178-179°(d); λ_{max}^{KBr} 2.90, 3.38, 5.87, 7.78μ ; λ_{max}^{MeOH} 262 mμ (ϵ 11,100), 291 mμ (ϵ 9,240).

Anal. Calcd for $C_{28}H_{29}NO_{13}$: C, 57.23; H, 4.98; N, 2.38. Found: C, 57.18, 57.14; H, 5.00, 5.03; N, 2.34, 2.37.

Conversion of Stephavanine (XXI) to Stephavanine Formate. To stephavanine (50 mg) in methanol (5 ml) excess formic acid (88%) was added dropwise with shaking until the alkaloid had dissolved and the solution was distinctly acidic. The solution so obtained was evaporated, azeotroping excess formic acid with benzene. The residue was crystallized twice from methanol-ether to give colorless needles: mp 192-193°(d); λ_{max}^{KBr} 2.88 - 3.03 (br), 3.36, 4.03, 5.89, 7.75μ ; λ_{max}^{MeOH} 262 mμ (ϵ 11,200), 291 mμ (ϵ 9,380).

Anal. Calcd for $C_{27}H_{29}NO_{11}$: C, 59.67; H, 5.38; N, 2.58. Found: C, 59.87, 59.76; H, 5.38, 5.37; N, 2.50, 2.61.

Conversion of Stephavanine (XXI) to Stephavanine Malonate.

Stephavanine (40 mg) and malonic acid (20 mg) were mixed in methanol (10 ml) and evaporated. The residue was crystallized from methanol-ethyl acetate to give 40 mg of colorless rosettes: mp 174-175° (d) (softens 145°);

λ_{max}^{KBr} 2.86, 3.08, 3.39, 4.00, 5.79, 5.88, 7.81μ; λ_{max}^{MeOH} 260 mμ (ϵ 13,700), 291 mμ (ϵ 10,610).

Anal. Calcd for $C_{29}H_{31}NO_{13} \cdot CH_3OH$: C, 56.88; H, 5.57; N, 2.21. Found: C, 56.93, 56.87; H, 5.54, 5.59; N, 2.06, 2.08.

Conversion of Stephavanine (XXI) to O, O, N-Triacetylstephavanine (XXIV). Stephavanine hydrochloride (290 mg) was dissolved in acetic anhydride (4 ml)-anhydrous pyridine (4 ml dried over KOH) and heated with stirring at 90° for three hours. The reaction mixture was poured into ice water (100 ml), neutralized with saturated aqueous $NaHCO_3$ (200 ml) and extracted with chloroform (4-100 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated, and residual pyridine was azeotroped with dry benzene to give 380 mg of a faintly yellow foam. Crystallization from chloroform-ether gave colorless needles: mp 189-190°; $[\alpha]_D^{28}$ + 73° (c 2.47, $CHCl_3$); λ_{max}^{KBr} 3.38, 5.64, 5.72, 5.85, 5.94, 7.74μ; λ_{max}^{MeOH} 235 mμ (Sh) (ϵ 13,850), 291 mμ (ϵ 7,500); m/e 623 (M^+).

Anal. Calcd for $C_{32}H_{33}NO_{12}$: C, 61.63; H, 5.33; N, 2.25. Found: C, 61.53; H, 5.37; N, 2.24.

Methylation of Stephavanine (XXI) to Stephavanine Methyl Ether (XXVI) and N-Methyl Stephavanine Methyl Ether (XXV). Stephavanine hydrochloride (300 mg) was suspended in methanol (2 ml) and ether (20 ml) and treated with a large excess of distilled ethereal solution of diazomethane and allowed to stand overnight. Examination of the reaction mixture by tlc showed considerable starting material so the material was treated with a

second portion of diazomethane for an additional 24 hours. The solution was then evaporated and the residue (300 mg) was chromatographed on a silicic acid column (30 g) packed in acetone-methylene chloride (1:2). The fractions eluted with acetone-methylene chloride (1:2) gave N-methyl stephavanine methyl ether (140 mg) which was crystallized from ether to give colorless needles: mp 175-176° (d); $[\alpha]_D^{24}$ -18° (c 0.97, $CHCl_3$); λ_{max}^{KBr} 2.83, 3.39, 5.87, 7.86 μ; λ_{max}^{MeOH} 260 mμ (ϵ 10,200), 292 mμ (ϵ 7,970); m/e 525 (M^+), 227, 228.

Anal. Calcd for $C_{28}H_{31}NO_9$: C, 63.99; H, 5.95; N, 2.67. Found: C, 63.89; H, 5.98; N, 2.61.

Subsequent elution with acetone gave fractions containing stephavanine methyl ether (XXVI, 150 mg). Crystallization from methanol-ether gave needles: mp 142-143° (sealed tube); $[\alpha]_D^{27}$ -14° (c 0.90, $CHCl_3$); λ_{max}^{KBr} 2.89, 3.38, 5.85, 7.88 μ; λ_{max}^{MeOH} 253 mμ (ϵ 12,550), 291 mμ (ϵ 9,410); m/e 511 (M^+), 213, 214, 182, 165.

Anal. Calcd for $C_{27}H_{29}NO_9 \cdot 1/2\ CH_3OH$: C, 62.61; H, 5.92; N, 2.66. Found: C, 62.11, 62.14; H, 5.99, 5.96; N, 2.64, 2.66.

Methylation of Stephine (XXIII) to N-Methyl Stephine (XXVIII). Stephine (120 mg) dissolved in methanol (20 ml) and dioxane (10 ml) was treated with 37% formaldehyde solution (4 ml) and allowed to stand for fifteen minutes at room temperature to give a clear solution. The solution was cooled to 0° and stirred and sodium borohydride (1.0 g) was gradually added over fifteen minutes. The reaction was then stirred at 0° for three hours, poured into water (50 ml) and extracted with chloroform (4-50 ml

portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give the colorless crude product (120 mg), contaminated with a small amount of starting material. Chromatography on a preparative silica gel F_{254} plate in 6% methanol-chloroform and subsequent elution of the major band with methanol-chloroform (1:1) gave crystalline N-methyl stephine (85 mg), mp 128-30°, which was recrystallized twice from ether-petroleum ether to give colorless needles: mp 141-143°; $[\alpha]_D^{25}$ +182° (c 0.96, MeOH); λ_{max}^{MeOH} 242 mμ (ε 3,540), 294 mμ (ε 4,680); $\lambda_{max}^{CHCl_3}$ 2.86, 3.42, 6.75, 7.95μ; m/e 361 (M^+), 229, 228.

Anal. Calcd for $C_{19}H_{23}NO_6$: C, 63.14; H, 6.42; N, 3.88. Found: C, 63.24, 63.02; H, 6.43, 6.58; N, 3.89, 4.10.

Oxidation of Stephine (XXIII) to 6-Oxostephine (XXIX) and 8-Oxostephine (XXX). To a solution of stephine (XXIII, 150 mg), in a mixture of glacial acetic acid (5 ml) and acetone (10 ml), a slight excess of 8N chromium trioxide in sulfuric acid-water (Jones' reagent) was added. After one minute the reaction was poured into excess dilute sodium hydroxide solution and partially neutralized to pH 8 with dilute hydrochloric acid. The aqueous solution was extracted with chloroform (4-100 ml portions) and the combined, dried (Na_2SO_4) chloroform extracts were evaporated to give two spot material (135 mg). Preparative layer chromatography on silica gel F_{254} in 8% methanol-chloroform separated the two materials. Subsequent elution of the higher R_f band with methanol-chloroform (1:1)

gave 6-oxostephine (XXIX, 86 mg), which was crystallized three times from chloroform-ether to give colorless prisms: mp 191-192° (d); $[\alpha]_D^{26}$ + 58° (c 1.1, AcOH); λ_{max}^{KBr} 3.03, 3.37, 5.75, 6.72 μ; λ_{max}^{MeOH} 289 mμ (ϵ 5,590); m/e 345 (M^+), 286, 214, 213.

Anal. Calcd for $C_{18}H_{19}NO_6$: C, 62.60; H, 5.55; N, 4.06. Found: C, 61.84; H, 6.03; N, 4.11.

Elution of the lower Rf band with methanol-chloroform (1:1) gave 8-oxostephine (XXX, 30 mg) which after 2 crystallizations from chloroform showed: mp 128-30°; $[\alpha]_D^{29}$ -191° (c 1.13, MeOH); λ_{max}^{KBr} 2.89, 2.96, 3.38, 5.93 μ; λ_{max}^{MeOH} 233 mμ (ϵ 14,180), 257 mμ (ϵ 12,180); m/e 345 (M^+), 327, 301, 269.

Anal. Calcd for $C_{18}H_{19}NO_6$: C, 62.60; H, 5.55; N, 4.06. Found: C, 61.43; H, 5.50; N, 4.09.

Attempted Epimerization of Stephavanine (XXI). Stephavanine (67 mg) in methanol (10 ml) and chloroform (5 ml) was treated with sodium methoxide (12 mg) at vigorous reflux for 22 hours. The reaction mixture was cooled, and evaporated to dryness. Tlc of the product showed it to be a single spot identical in Rf and color with ceric sulfate reagent to stephavanine. This product in pyridine (5 ml) was treated with hexamethyldisilazane (1 ml) and trimethylchlorosilane (0.5 ml) for five minutes, followed by dilution with water (30 ml) and extraction with chloroform (4-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to dryness and residual pyridine was azeotroped with dry benzene to give a light brown foam (105 mg). The nmr spectrum of this material was indistinguishable

from that of stephavanine bis-(trimethylsilyl) ether.

Attempted Epimerization of Stephine (XXIII). Stephine (85 mg) in methanol (15 ml) and sodium methoxide (20 mg) was held at reflux for 14 hours. After cooling, the reaction mixture was poured into water (50 ml) and extracted with chloroform (4-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to dryness to yield 85 mg of product which showed one spot on tlc identical in R_f and color with ceric sulfate reagent to stephine. Silation afforded a product whose nmr spectrum was indistinguishable from that of stephine bis-(trimethylsilyl) ether.

Reduction of 6-Oxostephine (XXIX) to Stephine (XXIII). 6-Oxostephine (100 mg) in 2-propanol (10 ml) and methylene chloride (5 ml) was treated with sodium borohydride (100 mg, 99%, Metal Hydrides, Inc.) and stirred at room temperature for thirty minutes. Tlc at this point indicated that the reaction was complete. The reaction mixture was poured into excess saturated solution of sodium bicarbonate and extracted with chloroform (3-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to dryness to yield 100 mg of product which, on tlc examination, had the same R_f and color with ceric sulfate reagent as stephine (XXIII). This product was dissolved in pyridine (5 ml) and treated with hexamethyldisilazane (1 ml) and trimethylchlorosilane (0.5 ml) for five minutes. The reaction mixture was then poured into water (30 ml) and extracted with chloroform (3-50 ml portions). The combined, dried (Na_2SO_4)

chloroform extracts were evaporated to dryness. The nmr spectrum of this material was indistinguishable from that of stephine bis-(trimethylsilyl) ether.

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PART IV

ALKALOIDS OF *STEPHANIA HERNANDIFOLIA*

INTRODUCTION

Stephania hernandifolia Walp, is a menispermaceous slender twining shrub found in India on the west and east coasts, Cachar, Sikkim, East Bengal, and Assam.[1] The roots are said to be useful in the treatment of fever, diarrhea, dyspepsia, and urinary diseases.[2] The presence of l-quercitol and a mixture of alkaloids was reported from S. hernandifolia gathered on the east coast of Austrailia.[3] In 1969 Tomita and Ueda reported the isolation of isotrilobine, a new tertiary phenolic base, and β-sitosterol.[4] Moza et al. have reported that the plant contained alkaloids, steroids, and fats.[5] They have isolated four alkaloids,[6,7] of which two are regarded as being identical with the known alkaloids epistephanine[8] and steponine.[7] The other two have been assigned the names aknadinine and aknadicine, of elemental formulas $C_{20}H_{25}NO_5$ and $C_{19}H_{23}NO_5$,[6] respectively. Russian workers have isolated an alkaloid from S. hernandifolia of elemental formula $C_{20}H_{25}NO_5$, which they have named hernandoline[9] and which may well be identical to the aknadinine isolated by Moza.

In previous work in these laboratories the isolation from S. hernandifolia of Indian origin of the alkaloids dl-tetrandrine, fangchinoline, d-tetrandrine, and d-isochondrodendrine was reported.[10] Subsequent biological studies determined that all four of these alkaloids showed significant cytotoxicity against human carcinoma of the nasopharynx carried in tissue culture (KB), and that dl-tetrandrine and d-tetrandrine also showed significant inhibitory activity in vivo against the Walker 256 intramuscular carcinosarcoma in the rat. Our present work deals with the investigation

of a new sample of roots of S. hernandifolia collected in India in January, 1965.[12] This sample, however, contained none of the alkaloids isolated earlier in these laboratories. The alkaloids 4-demethylhasubanonine, 4-demethylnorhasubanonine, epistephanine, oxoepistephanine and stephisoferuline, all of which are new alkaloids except epistephanine have been isolated and characterized. After our report on the structure elucidation of 4-demethylhasubanonine and 4-demethylnorhasubanonine,[13] a preliminary note on the structure of aknadinine appeared.[14] This was followed by a more detailed report on the structure elucidation of both aknadinine and aknadicine, which are identical to 4-demethylhasubanonine and 4-demethylnorhasubanonine, respectively.[15]

The alkaloids which we have isolated are members of two distinct classes, the bisbenzylisoquinoline alkaloids and the hasubanan alkaloids. The former are significant insofar as several members of this group have shown reproducible tumor inhibitory activity against the Walker 256 intramuscular carcinosarcoma (e.g. tetrandrine). The hasubanan alkaloids are of interest because of their apparent close biogenetic relationship to the morphine alkaloids and their novel skeleton. Hasubanan alkaloids have thus far been found only in species of the genus Stephania.

DISCUSSION

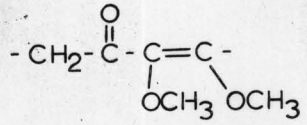
Isolation and Structure Elucidation of 4-Demethylhasubanonine (I).

A concentrated methanolic extract of the defatted roots of S. hernandifolia Walp. was triturated with 6% hydrochloric acid and the acid solution was basified with ammonium hydroxide and extracted with chloroform to yield the crude non-quaternary alkaloids.[16] The crude alkaloids were fractionated by continuous ether extraction. The ether soluble alkaloids were chromatographed on silica to yield a fraction rich in the new alkaloid. Treatment of this fraction with oxalic acid in methanol and repeated recrystallizations of the oxalate salt from methanol-ether yielded 4-demethylhasubanonine (I) oxalate, $C_{20}H_{25}NO_5 \cdot C_2H_2O_4$, mp 198-199°, $[\alpha]_D^{32}$ -123°, m/e 359 (M^+), λ_{max}^{EtOH} 266 mμ (ε 8,400). The infrared spectrum of the free base (KBr) showed absorption at 5.95 μ, indicative of an α,β -unsaturated ketone. The alkaloid was also characterized as the perchlorate salt, mp 229-230°, $[\alpha]_D^{32}$ -145°, and the brosylate ester (II), mp 209-211°, $[\alpha]_D^{29}$ -149°, m/e 576 (M^+).

The nmr spectrum of I showed signals at τ 3.32 and 3.45 (2H, q, J=9Hz, 2 ortho aromatic H), 3.88 (1H, OH), 5.92, 6.18, and 6.32 (9H, 3 OCH_3), 7.48 (3H, NCH_3).

The 5 oxygen atoms in the molecule are present as a ketone, 3 methoxyl groups and one hydroxyl group. The hydroxyl group was shown to be phenolic (positive ferric chloride test) and para to an unsubstituted position (positive reaction towards Gibbs reagent). The uv, ir and nmr

spectra indicated the presence of the following partial structure:



The nmr data were particularly important since the respective shielding and deshielding of methoxyl groups on the alpha and beta carbons of α, β -unsaturated ketones gives rise to characteristic chemical shifts for these groups, (see Table 5). It was suspected that this alkaloid was a member of the hasubanan series and further that the compound was a demethyl hasubanonine. Methylation of I with diazomethane gave a mixture of starting material and a product of higher R_f which was separated by column chromatography on acid-washed alumina. The product of the reaction was purified through its oxalate salt and the free base was characterized as hasubanonine (III) by comparison of the physical constants of the alkaloid and its methiodide with those in the literature.[17,18]

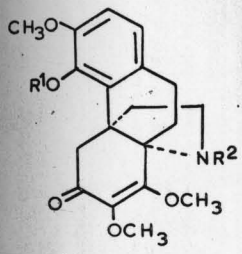
A comparison of the nmr methoxyl resonances of known hasubanan alkaloids with those of the new compound showed that the methoxyl groups which were present corresponded to those located at C-7, C-8 and either C-4 or C-3. These data are found in Table 5. The C-3 phenolic isomer, homostephanoline (V), is a well-characterized compound whose physical constants are clearly different from those of our compound; hence the 4-demethylhasubanonine structure (I) was deemed the most plausible. This is in agreement with the fact that the phenol should have an unsubstituted

TABLE 5

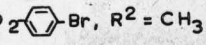
NMR Methoxyl Resonances in the Hasubanan Series *

Compound		C-3	C-4	C-7	C-8
4-Demethylhasubanonine (I)		6.18		6.32	5.92
Hasubanonine (III)	17,18	6.19	6.09	6.36	5.92
Cepharamine (VII)	19	6.15		6.35	
Homostephanoline (V)	17		6.14	6.40	5.92
O-Ethylhomostephanoline (VI)	20		6.05	6.35	5.93
4-Demethylnorhasubanonine (IV)		6.15		6.30	5.87

* τ values for $CDCl_3$ solutions measured at 60 MHz relative to tetramethylsilane.

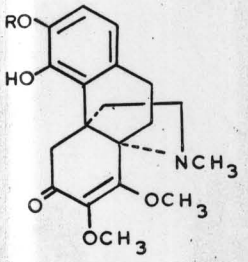


I ; $R^1 = H$, $R^2 = CH_3$

II ; $R^1 =$  $R^2 = CH_3$

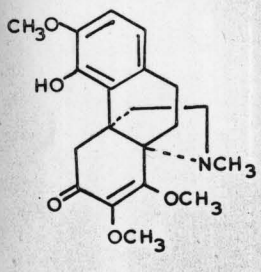
III ; $R^1 = R^2 = CH_3$

IV ; $R^1 = R^2 = H$

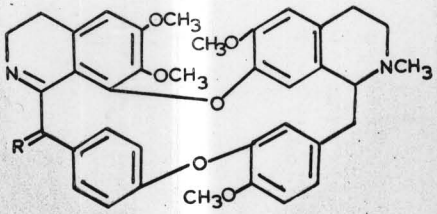


V , R = H

VI , $R = C_2H_5$



VII



VIII , $R = H_2$

IX , R = O

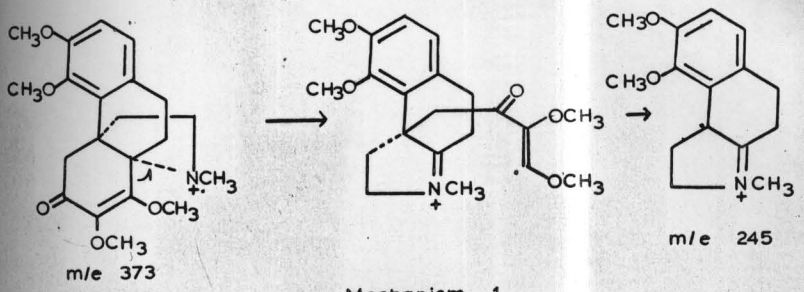
FIGURE 14. STEPHANIA HERNANDIFOLIA ALKALOIDS AND RELATED COMPOUNDS.

para position as indicated by the positive Gibbs test.

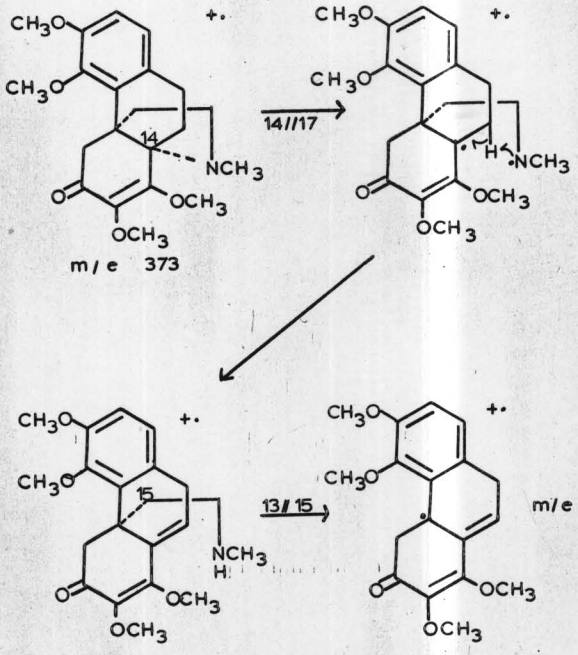
Unequivocal proof of structure I was achieved by X-ray crystallographic analysis of the brosylate (II).[13]

The mass spectrum of I was relatively simple, the major fragmentation being loss of 58 mass units to give an ion at m/e 301. In the mass spectrum of II m/e 300 is the base peak, arising through loss of 58 mass units and cleavage of the brosylate ester. The methyl ether of I, hasubanonine (III), also showed the base peak to arise by loss of 58 mass units, but showed a mass shift of 14 units and was located at m/e 315. The mass spectral fragmentation of hasubanan alkaloids has been examined by Tomita et al.,[21] who have noted a very characteristic pattern of C ring cleavage as mechanism I at the top of Figure 15 illustrates for the case of hasubanonine. For the same compound we have noted a cleavage of ring D for which we propose mechanism 2 in the same figure. Thus the mode of cleavage seems to depend in large part on the specific conditions under which the spectra are determined.

The designation of the M-58 fragment as having lost C_3H_8N is supported by exact mass measurement, the fragment at m/e 315 corresponding to $C_{18}H_{19}O_5$ (calculated: 315.12325 a. m.u.; Found 315.12333 a. m.u.; error 0.08 m.m.u). Furthermore in the case of 4-demethylnorhasubanonine (IV) there is a loss of 44 mass units rather than 58 in the analagous fragment ion, showing a mass shift of 14 units which is exactly as anticipated for the difference between the secondary amine and the corresponding N-methyl tertiary amine, establishing beyond doubt the loss of the nitrogen atom from



Mechanism 1



Mechanism 2

FIGURE 15. MASS SPECTRAL CLEAVAGE OF HASUBANONINE.

ring D. The mechanism which is proposed herein fits the data well. It cannot, however, be considered as proven since no deuterium labelling studies have been conducted to determine if the source of the hydrogen atom which is abstracted by the nitrogen is intramolecular. The significance of both C ring and D ring cleavage in structural assignments of hasubanan alkaloids has been discussed in Part III of this dissertation (q.v.).

Isolation and Structure Elucidation of 4-Demethylnorhasubanonine (IV). Fractions of slightly lower R_f than those which yielded 4-demethylhasubanonine from the above-mentioned silica column were combined and rechromatographed on a silica gel column to give material homogeneous on tlc which was crystallized from methanol-ether as the oxalate salt, mp 192-193° (d); $[\alpha]_D^{25}$ -159°. Liberation of the free base with bicarbonate followed by crystallization from chloroform-ether gave pure 4-demethylnorhasubanonine, mp 116-119°; $[\alpha]_D^{25}$ -219°; λ_{max}^{MeOH} 266 mμ (ϵ 11,750); λ_{max}^{KBr} 3.02, 6.02 μ; m/e 345 (M^+); nmr τ3.32, 3.45 (2H, aromatic H, q., J=9 Hz), 5.87, 6.15, 6.30 (9H, 3 x OCH_3). The spectral data indicated that this compound was an alkaloid of the hasubanonine type but having one less methoxyl group and lacking the N-methyl moiety, making it the first alkaloid in this series found to be a secondary amine. The positions of the methoxyl groups are in agreement with those anticipated for a compound lacking the C-4 methoxyl. This was supported by the phenolic nature of the alkaloid (ferric chloride test) and its positive reaction toward Gibbs reagent. Thus it was presumed that this compound was the N-demethyl

analog of I. Methylation of IV with methyl iodide under neutral conditions gave a mixture of the N-methyl derivative, starting material and a trace of very polar material presumed to be the methiodide. It is interesting that neither I nor IV forms a methiodide salt except under forcing conditions, whereas the corresponding fully-methylated compound, hasubanonine (III)[7], does so readily. This is perhaps due to the partially zwitterionic nature of the phenolic compounds which results in a decreased nucleophilicity of the nitrogen atom.

Chromatography of the above reaction mixture on silica gel separated the less polar product from starting material and this less polar product was shown to be identical to I by ir spectra, nmr spectra, mixture tlc and a mixture melting point of the respective oxalate salts.

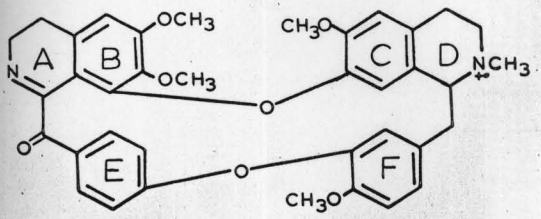
Isolation and Structure Elucidation of Epistephanine VIII. After elution of 4-demethylnorhasubanonine from the aforementioned silica column the polarity was increased and subsequent fractions rich in another alkaloid were eluted. These fractions were combined and rechromatographed on silicic acid and then on neutral alumina to give homogeneous material which was crystallized from benzene to give colorless crystals of epistephanine (VIII): mp 134-135° (lit 133-134°[8]); $[\alpha]_D^{27}$ +204° (c 0.83, $CHCl_3$) (lit +196°[22], +218°[8]); λ_{max}^{MeOH} 229 mμ (sh, ε 44,930), 281 mμ (ε 11,960); m/e 606 (M^+); λ_{max}^{KBr} 3.40, 3.52, 3.60, 6.25, 6.62μ.

The methiodide of VIII showed mp 243-245° (d); (lit 245°[22]). The isolation of epistephanine from S. hernandifolia has been reported by Moza et al.[8]

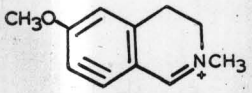
Isolation and Structure Elucidation of Oxoepistephanine(IX).

Fractions less polar than those containing epistephanine were rich in a new alkaloid which was isolated by silica gel chromatography followed by alumina chromatography. Crystallization of the new alkaloid from methanol-ether gave colorless needles, mp 224-226° (d). The ir spectrum showed a conjugated carbonyl absorption at 5.97 μ and several aromatic C-H bands. The nmr spectrum showed the compound to be of the bisbenzylisoquinoline type and was extremely similar to that of epistephanine, except that one of the aromatic protons was shifted to lower field as would be expected for a benzylic ketone. The positions of the four methoxyl groups and the N-methyl group were nearly identical to those observed in epistephanine. Elemental analysis showed the compound to possess the molecular formula $C_{37}H_{36}N_2O_7$, compared with $C_{37}H_{38}N_2O_6$ for epistephanine, in agreement with the replacement of a methylene group by a ketone.

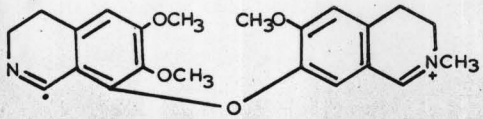
The mass spectrum of oxoepistephanine shows a strong molecular ion at m/e 620 (base peak), in agreement with the assigned molecular formula. There are fairly intense peaks at m/e 381, 380, and 379 which correspond to double benzylic fission of the molecular ion to give an ion such as a m/e 380, the 379 and 381 ions being formed by hydrogen transfer (see Fig. 16). The double benzylic cleavage of unsymmetrical bisbenzylisoquinoline alkaloids to give the type of ion shown in the figure is well established and has been studied extensively in several laboratories[23-25]. There is also an ion at m/e 174 which can be formulated as ion b resulting from the cleavage of the C and D rings from the remainder of the molecule.[23]



IX, m/e 620



b m/e 174



a m/e 380

FIGURE 16. MASS SPECTRAL FRAGMENTATION OF OXOEPISTEPHANINE.

This would establish the methylation pattern of ring C and the location of the N-methyl group as being the same as in epistephanine (VIII). The presence of the m/e 380 ion as a result of double benzylic cleavage requires that the ketone be in the lower part of the molecule and therefore occupy one of two positions, either benzylic to ring E or to ring F. The absence of a fragment ion corresponding to the lower portion of the molecule is anticipated since the charge should preferentially be located on nitrogen. The ion resulting from the double benzylic cleavage which would compliment the m/e 380 ion is expelled as a neutral fragment.

Thus oxoepistephanine should be a modified epistephanine with a ketone moiety benzylic to either ring E or ring F. The latter seems unlikely since (a) the N-methyl group in oxoepistephanine is not appreciably shifted from its location in epistephanine as would be anticipated due to carbonyl shielding effects if the ketone were benzylic to ring F; (b) oxoepistephanine is stable towards treatment with base, suggesting the absence of an asymmetric center adjacent to the ketone; and (c) biogenetically, the methylene group benzylic to ring E in epistephanine is activated by the presence of the imine double bond, making it a more likely site for oxidation. The last reason is based on the assumption that oxoepistephanine is derived from epistephanine by oxidation in the plant which seems extremely reasonable since these compounds do occur together and all evidence available on the biosynthesis of bisbenzylisoquinolines suggests that they are formed in the plant by direct coupling of two benzylisoquinoline units. Therefore epistephanine should be the precursor

of oxoepistephanine and not the reverse.

On the basis of these arguments the isomer in which the ketone is benzylic to ring E is favored and oxoepistephanine should possess structure IX. It was desired to prove this assignment by partial synthesis of oxoepistephanine from epistephanine. It has been reported that in the corresponding monomeric series 3, 4-dihydro-1-benzylisoquinolines are converted to the corresponding 3, 4-dihydro-1-benzoylisoquinolines quite readily by either alumina chromatography or treatment with sodium hydroxide solution[26-28]. Since the 1, 2, 3, 4-tetrahydro-1-benzylisoquinolines are stable under these conditions the imine group is clearly a key activating factor in these benzylic oxidations. It therefore seemed likely that oxidation of epistephanine should be selective and should lead to the isomer in which the benzylic ketone is conjugated to the imine. Several attempts to oxidize epistephanine by either sodium hydroxide or alumina led to quantitative recovery of starting material, as did air oxidation and treatment of a basic solution of epistephanine with molecular oxygen. The failure of these reactions must be attributed to steric factors resulting from the conformation of the macrocyclic ring in the bisbenzylisoquinoline nucleus of epistephanine. When oxidations were attempted under more vigorous conditions with either manganese dioxide in benzene or chromium trioxide in acetone or chromium trioxide in acetic acid,[29] a complex mixture of products was obtained in which the presence of oxoepistephanine could not be detected.

An attempt was made to convert oxoepistephanine (IX) to epistephanine (VIII) by Raney nickel desulfurization. However, the required

thioketal could not be formed even with the use of boron triflouride etherate as catalyst. The failure of this reaction can again be attributed to steric difficulties induced by the 18 membered macrocyclic ring.

Another potential method of interrelating VIII and IX was through reduction of IX to one of the two epimeric dihydroepistephanines by zinc and acid treatment (the imine group of epistephanine is reduced to yield the epimeric dihydroepistephanines under these conditions[30]). However, when oxoepistephanine was treated with zinc and sulfuric acid a complex mixture resulted which contained neither of the dihydroepistephanines.

Isolation and Characterization of Stephisoferuline (X). Fractions intermediate in polarity between oxoepistephanine (IX) and epistephanine (VIII) on silica were rich in a new alkaloid, stephisoferuline (X).

Stephisoferuline was purified by rechromatography on silica gel and crystallization from chloroform-ether gave a chloroform solvate, mp 133-135°. The alkaloid showed uv maxima at 229, 287, and 325 mμ and elemental analysis supported the formula $C_{29}H_{33}NO_9$. The infrared spectrum showed broad hydroxyl absorption and a carbonyl absorption at 5.89μ assigned to an unsaturated ester. The compound was phenolic (ferric chloride test) and gave a positive Gibbs test indicating the presence of at least one phenolic hydroxyl with an unsubstituted para position. The nmr spectrum showed signals for 5 aromatic protons, 2 trans olefinic protons, a proton on carbon bearing an ester, two other protons on carbon bearing oxygen, one of which appeared to be benzylic, and 4 methoxyl groups

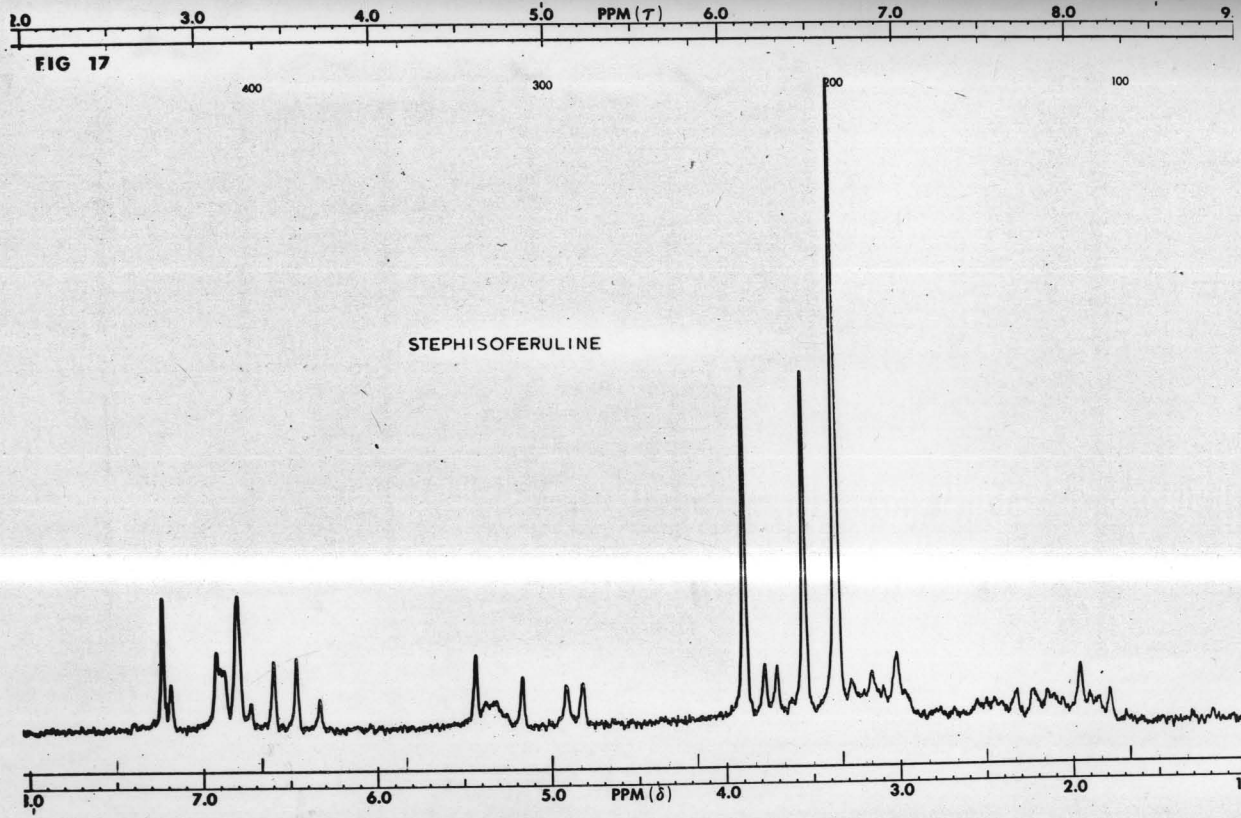
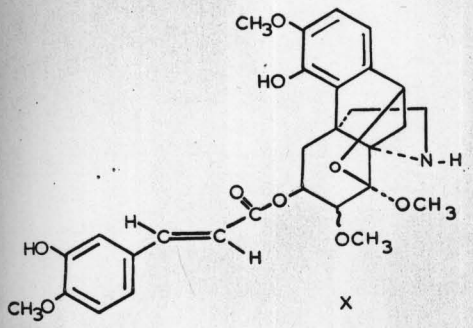
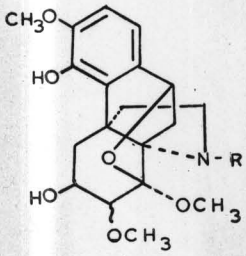

2.0
3.0
4.0
5.0
PPM (τ)
6.0
7.0
8.0
9
400
300
200
100
STEPHISOFERULINE
1.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.

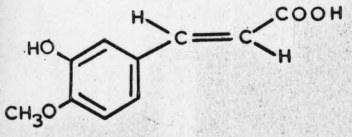
FIG 17



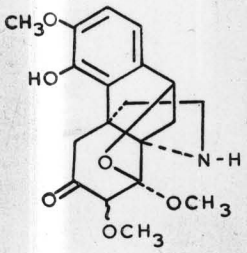
X



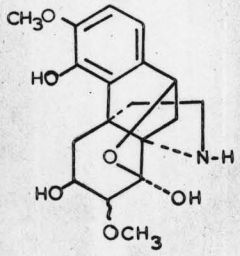
XI, R = H
XIII, R = CH_3



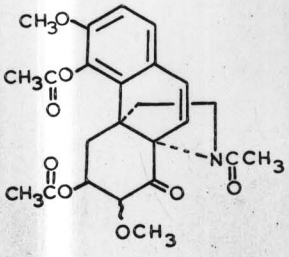
XII



XIV



XV



XVI

FIGURE 18. STEPHISOFERULINE AND DERIVATIVES.

TABLE 6

NMR Signals of Stephisoferuline and Derivatives[a]

	C-1, C-2H	C-6H	C-7H	C-10H	OCH_3	Other Signals
Stephisoferuline (X)	3.32, 1H d(8.5) 3.58, 1H d(8.5)	4.63 m	6.25 d(4)	5.12 d(5.5)	6.08, s 6.43, s 6.60, s (6H)	isoferulate ester 3.07-3.18 (3H, aromatic H) 2.43 (1H, d (16), olefinic H) 4.68 (1H, d (16), olefinic H)
Stephuline (XI)	3.43, 2H s	5.88 m	6.33 d(4)	5.17 d(6)	6.23, s 6.42, s 6.53, s	
6-Oxostephuline (XIV)	3.42, 2H s	-	5.72 s	5.21 d(5.5)	6.15, s 6.42, s 6.50, s	
N-Methylstephuline (XIII)	3.41, 2H s	5.87 m	6.32 d(4)	5.12 d(6)	6.22, s 6.43, s 6.53, s	N-methyl 7.43 (3H, s)
8-Demethylstephuline (XV)	3.42, 2H s	5.80 m	6.33 d(4)	5.15 d(6)	6.22, s 6.48, s	
Triacetyl-10-desoxy-9, 10-dehydrostephuline (XVI)	2.97, 1H d(8) 3.17, 1H d(8)	4.45 m	5.80 d(4)	3.40 d(10)	6.20, s 6.53, s	C-9 Acetate 4.18 7.67(3H, s) d(10) 7.87(3H, s) 8.30(3H, s)
Triacetyl-10-desoxy-stephuline (XVII)	3.00, 1H d(9) 3.17, 1H d(9)	4.68 m	5.85 d(3.5)	c	6.22, s 6.50, s	Acetate 7.65 (3H, s) 7.90 (3H, s) 8.67 (3H, s)

TABLE 6 (Cont'd)

NMR Signals of Stephisoferuline and Derivatives[a]

	C-1, C-2H	C-6H	C-7H	C-10H	OCH_3	Other Signals
Triacetyl-3, 8-dimethoxy-4, 6-dihydroxy-norhasubanan-7-one (XVIII)	3.08, 1H d(9) 3.20, 1H d(9)	4.53 m	-	c	6.25, s 6.43, s	Acetate 7.70 (3H, s) 8.03 (3H, s) 8.82 (3H, s)
4-Demethylnorhasubanonine-6αol (XX)[b]	3.15, 1H d(8) 3.28, 1H d(8)	5.38 t(6)	-	c	6.05, s 6.23, s 6.27, s	
4-Demethylnorhasubanonine-β-ol (XIX)[b]	3.18, 1H d(8) 3.33, 1H d(8)	5.12 t(5)	-	c	5.95, s 6.18, s 6.30, s	

[a] All values in τ for $CDCl_3$ solutions at 60 MHz relative to tetramethylsilane unless otherwise specified

[b] D_5 - Pyridine solution

[c] Signal not distinguishable

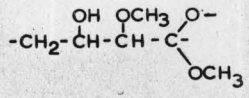
in addition to a number of unassignable complex aliphatic protons. The nmr spectrum of stephisoferuline is presented in Figure 17. The nmr data of stephisoferuline and derivatives are summarized in Table 6.

Hydrolysis of stephisoferuline (X) gave an acid, $C_{10}H_{10}O_4$, and the alkamine stephuline (XI), $C_{19}H_{25}NO_6$. The spectral data of the acid were consistent with a trans-cinnamic acid having one hydroxyl and one methoxyl group and a survey of the literature showed that the physical constants of the acid were in accord with those recorded for isoferulic acid (XII). Direct comparison with an authentic sample of isoferulic acid (by ir, uv, and nmr spectra, and mixture melting point) showed the two compounds to be identical. Both isoferulic acid and stephuline (XI) gave positive Gibbs tests, indicating the presence of at least two phenolic hydroxyl groups in the parent compound. The isoferulate ester could not have been bound to the phenolic hydroxyl of the alkamine since the signal for hydrogen on carbon bearing ester in the parent compound appeared at much higher field in the nmr spectrum of stephuline.

The infrared spectrum of stephuline showed both OH and NH absorption but no carbonyl absorption, indicating that the ester was the only carbonyl function present in the molecule. Proof that stephuline is a secondary amine was achieved by methylation with formaldehyde and sodium borohydride to give N-methylstephuline (XIII), which showed a three-proton signal in the nmr spectrum at τ 7.43. Jones oxidation of stephuline gave a monoketone, 6-oxostephuline (XIV), indicating the presence of only one secondary alcohol in the molecule and making the point of attachment of the

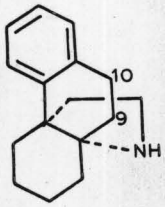
isoferulate ester unambiguous. In the nmr spectrum of XIV, the signal for one of the protons in XI on carbon bearing oxygen which was coupled to the proton on carbon bearing the secondary alcohol as a doublet (J=4Hz) now appeared as a singlet at lower field as a consequence of being adjacent to the newly formed ketone.

Five of the six oxygen atoms in stephuline could be accounted for as three methoxyl groups (indicated by signals for three three-proton singlets in the nmr spectrum), a phenolic group, and a secondary alcohol. Treatment of stephuline with dilute acid led to facile demethylation to give 8-demethylstephuline (XV), showing signals for only two methoxyl groups in its nmr spectrum. Thus the sixth oxygen atom must be part of a ketal grouping. Acetylation of stephuline followed by dilute acid treatment gave triacetyl-9-desoxy-9,10-dehydrostephuline (XVI) which shows a carbonyl absorption in the ir attributable to a ketone function, lending further substance to the assumption that a ketal is present in stephuline. The nmr spectrum of XVI showed a downfield shift of the signal for the proton on carbon bearing oxygen which was coupled to the proton on carbon bearing the secondary alcohol. This indicates that the former proton is adjacent to the ketal in stephuline. Since the proton on carbon bearing the secondary alcohol is a complex multiplet, it may be assumed that it is adjacent to a methylene group. Therefore, partial structure A must be present in stephuline

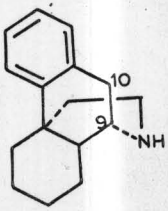


A

The nmr of XVI also shows the presence of a styrene system in which the two olefinic protons are coupled only to each other, indicating that the beta carbon is bound to a quaternary center. This strongly suggests the presence of a hasubanan nucleus in stephisoferuline and effectively eliminates the possibility of a morphine skeleton since C-9 in morphine bears a nitrogen substituent and thus cannot also bear an olefenic proton.



HASUBANAN



MORPHINAN

The presence of a styrene system also suggests very strongly the presence of a benzylic oxygen function in the starting material which is part of the ketal function and accounts for the remaining oxygen atom in the molecule.

The mass spectra of stephisoferuline and derivatives strongly support the presence of a hasubanan nucleus. A characteristic fragmentation pattern of hasubanan alkaloids occurs through loss of ring C,[21] and this mode of cleavage gives rise to relatively intense ions in the spectra of all of the derivatives examined. The mass spectra of stephisoferuline and derivatives are presented in Table 7 and the fragments arising from cleavage of ring C are underlined for ready recognition.

TABLE 7

Mass Spectra of Stephisoferuline and Derivatives

Compound	Formula	M^+ (%)	Base Peak m/e	Other m/e (%)
Stephisoferuline (X)	$C_{29}H_{33}NO_9$	539 (.1)	194	270 (12), 248 (10), 215 (21), 179 (53), 133 (40), 83 (50).
Stephuline (XI)	$C_{19}H_{25}NO_6$	363 (15)	217	216 (77), 215 (67), 202 (29), 184 (60), 182 (35), 154 (36).
N-Methylstephuline (XIII)	$C_{20}H_{27}NO_6$	377 (4)	231	230 (22)
6-Oxostephuline (XIV)	$C_{19}H_{23}NO_6$	361 (6)	214	360 (12), 257 (15), 183 (32), 181 (30), 153 (17).
8-Demethylstephuline (XV)	$C_{18}H_{23}NO_6$	349 (4)	217	348 (19), 216 (65), 215 (32), 184 (30).
Triacetyl-10-desoxy-9, 10-dehydrostephuline (XVI)	$C_{24}H_{27}NO_8$	457 (1)	257	299 (44), 215 (23), 182 (19), 154 (16), 71 (87), 43 (23).
Triacetyl-10-desoxystephuline (XVII)	$C_{24}H_{29}NO_8$	459 (14)	71	431 (24), 399 (21), 372 (17), 313 (19), 301 (44), 271 (32), 259 (76), 243 (26), 217 (44), 43 (95)
4-Demethylnorhasubanomine-6α-ol (XX)	$C_{19}H_{25}NO_5$	347 (36)	285	329 (24), 253 (78), 217 (26), 55 (24), 45 (66).
4-Demethylnorhasubanonine	$C_{19}H_{23}NO_5$	345 (45)	301	300 (31), 269 (38), 237 (19).

TABLE 7 (Cont'd)

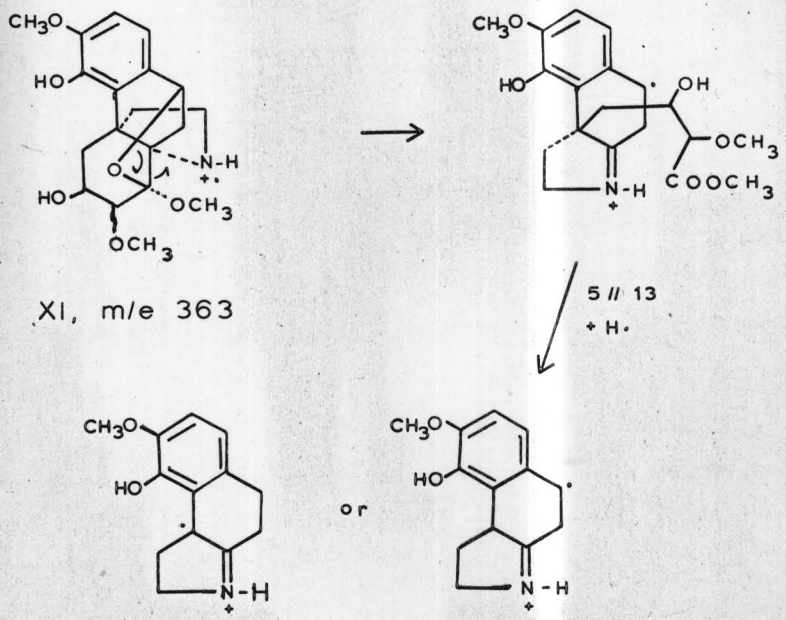
Mass Spectra of Stephisoferuline and Derivatives

Compound	Formula	M^+ (%)	Base Peak m/e	Other m/e (%)
Triacetyl-3, 7-dimethoxy-4, 6-dihydroxy-hasubanan-7-one (XVIII)	$C_{24}H_{29}NO_8$	459 (-)	259	399 (86), 313 (71), 301 (66), 285 (64), 271 (95), 243 (73), 217 (61), 216 (58), 87 (41), 43 (73).
4-Demethylnorhasubanonine-6β-ol (XIX)	$C_{19}H_{25}NO_5$	347 (93)	285	303 (97), 302 (73), 286 (47), 253 (53), 243 (71), 217 (39)

In the mass spectrum of stephuline (XI), the alkamine of stephisoferuline, the base peak occurs at m/e 217 and corresponds to $C_{13}H_{15}NO_2$ by exact mass measurement (calc: 217.11028 a.m.u.; found: 217.10707 a.m.u.; error 3.21 millimass units). This ion arises by cleavage of the C ring and the benzylic oxygen atom and its formation is shown diagrammatically in Figure 19. The mechanism is supported by the appropriate metastable peak for the M^+ to 217 transition. A discussion of the diagnostic significance of C-ring cleavage as an indicator of the presence of a hasubanan skeleton has been presented in Part III, in connection with the discussion of the mass spectra of stephavanine and derivatives (q.v.).

The use of mass shift technique confirmed the presence of a secondary amine in stephuline since a shift of 14 mass units was observed in both the molecular ion and the fragment corresponding to loss of ring C in the N-methyl derivative XIII. In the triacetyl derivative XVI there are mass shifts of 42 units for both an N-acetyl group and a phenolic acetate in the ion formed by loss of ring C, showing that both a secondary amine and a phenol are present in rings A, B, and D.

To this point, it appeared likely that stephuline (and hence stephisoferuline) has one methoxyl and one phenolic hydroxyl in ring A, a benzylic ketal oxygen in ring B, a secondary nitrogen atom in ring D, and partial structure "A" in ring C. Assignment of the positions of the phenol and the methoxyl group in ring A was made in the following way. The nmr signals of the two aromatic protons of stephuline show them to be ortho coupled. Since stephuline is a Gibbs-positive phenol the position para to the phenol



$C_{13}H_{15}NO_2$

m/e 217

FIGURE 19. MASS SPECTRAL FRAGMENTATION OF STEPHULINE (XI).

must be unsubstituted, limiting its placement to C-1 or C-4. The methoxyl group must be at C-2 (if the phenol is at C-1) or at C-3 (if the phenol is a C-4), in order to provide two ortho aromatic hydrogens. The hydroxyl and methoxyl cannot be located at C-1 and C-4 with the 2 aromatic hydrogens at C-2 and C-3, since there would then be no free para position for phenolic coupling.

The isomer having the methoxyl at C-3 and the phenol at C-4 appeared to be most likely since the other two hasubanan alkaloids isolated from Stephania hernandifolia, 4-demethylhasubanonine (I), and 4-demethylnorhasubanonine (IV) both have this pattern. It was therefore proposed that stephuline should possess structure XI and stephisoferuline would then be represented by structure X.

Interrelation of 4-Demethylnorhasubanonine (IV) and Stephuline (XI). In order to establish the molecular structure of stephuline, it has been interrelated with 4-demethylnorhasubanonine through the series of reactions shown in Figure 20. 4-Demethylnorhasubanonine is of known relative and absolute configuration at all centers, since it has been converted to 4-demethylhasubanonine, the stereochemistry of which has been unequivocally determined by X-ray crystallography.[13]

Triacetyl derivative XVI was hydrogenated over platinum oxide in ethanol to give XVII, which showed the expected spectral features and lacked the olefinic protons present in XVI. Treatment of XVII with acetone dimethyl acetal and p-toluenesulfonic acid in methanol gave a mixture of starting material and an isomeric product identified as the keto-triacetyl derivative XVIII.

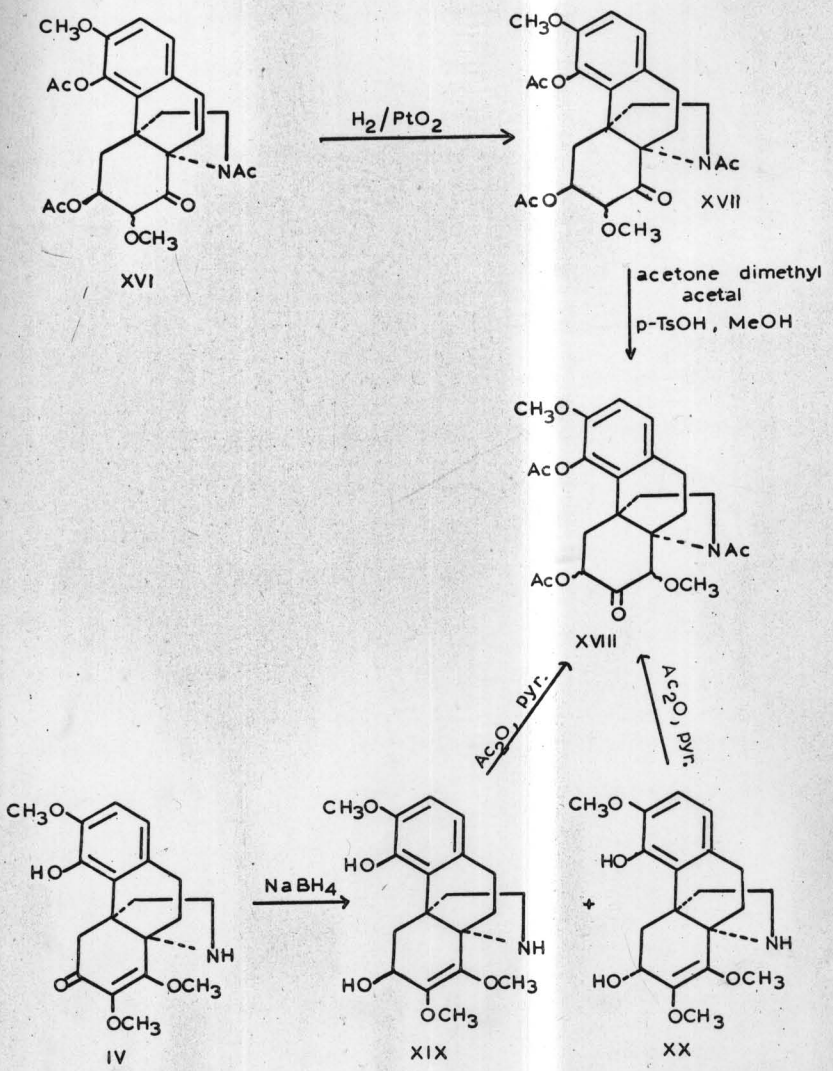


FIGURE 20. INTERRELATION OF 4-DEMETHYLNORHASUBANONINE AND STEPHULINE.

Sodium borohydride reduction of 4-demethylnorhasubanonine (IV) gave a mixture of the C-6 epimeric alcohols XIX and XX which were separated on silica gel. The less polar alcohol, which also showed the signal for the proton on carbon bearing hydroxyl at lower field in the nmr, was assigned as the quasi-axial alcohol (XIX), while the more polar alcohol in which the proton on carbon bearing hydroxyl resonated at higher field was assigned as the quasi-equatorial alcohol (XX).[17] Acetylation of each of these alcohols gave unstable triacetyl derivatives which on standing in chloroform gave a single compound shown to be identical to keto-triacetyl derivative XVIII (by high resolution ir, uv, mass spectra, mixture tlc and sign of optical rotation).

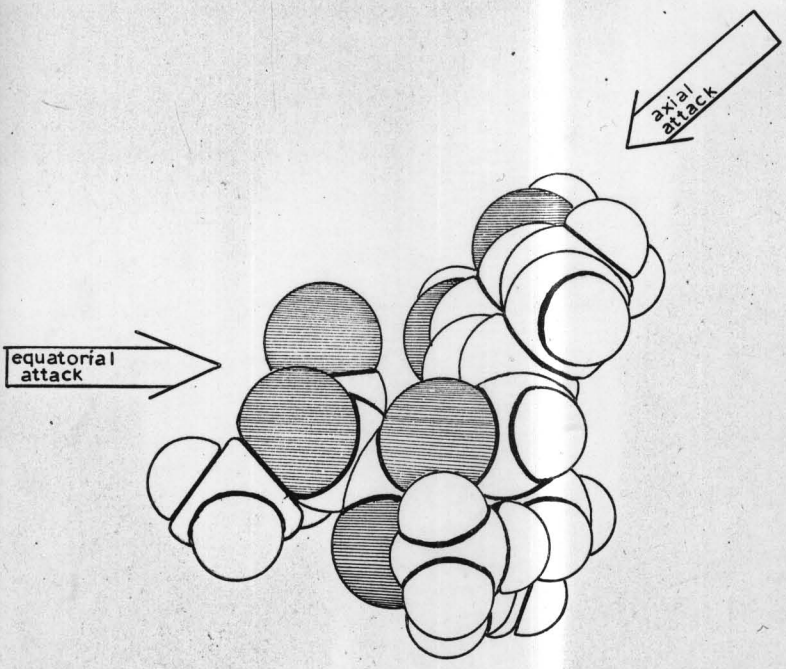
This correlation conclusively established the oxygenation pattern of stephuline and hence that of the parent compound stephisoferuline. It also established the relative stereochemistry of four of the six asymmetric centers in stephuline (C-8, C-10, C-13, and C-14) and the absolute configuration of the molecule. The fact that the nitrogen ring has the alpha configuration at C-13 and C-14 constrains the oxide bridge of the ketal to be beta at C-8 and C-10.

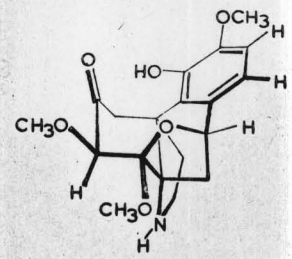
The stereochemistry at C-6 was investigated by means of sodium borohydride reduction of 6-oxostephuline (XIV), which gave a single alcohol, shown to be identical to stephuline (XI). Since reduction of 4-demethylnorhasubanonine (IV) or hasubanonine (III) with sodium borohydride gives approximately equimolar quantities of both C-6 alcohols, it is apparent that there is a very great steric factor introduced by the presence of the

oxide bridge of the ketal. This oxide bridge prevents the C-ring from flipping to an alternate chair conformation so that there is only one low energy conformation for the C-6 ketone. The Dreiding model of 6-oxostephuline (XIV) supports the view that attack of hydride from the axial direction is extremely hindered whereas there is no significant steric hindrance to the equatorial delivery of hydride. A diagrammatic representation of this argument is offered in Figure 21. Since this reduction is stereospecific, the sole product must be derived from equatorial delivery of hydride and must hence be the axial alcohol. Since this alcohol obtained is identical to the natural product, stephuline (XI), the axial configuration is favored for the alcohol at C-6.

The investigation of the stereochemistry of the methoxyl group at C-7 is more difficult to attack. While the C-7 methoxyl group could perhaps be epimerized through the ketone in 6-oxostephuline, and the C-6 ketone then stereospecifically reduced with sodium borohydride, the products might not be helpful in assignment of configuration. Since the C-6 hydrogen would be axial, it would give rise to axial-equatorial and equatorial-equatorial couplings of the C-6 hydrogen to the C-7 epimers. The dihedral angles involved are very similar (from examination of models) and the ring strain introduced by the oxide bridge to ring C would be expected to cause bond angle deformations of unknown magnitude, making any differentiation between these two isomers extremely tenuous. The approach chosen was to attempt preparation of the C-6 alcohol of unnatural configuration. If this compound could be prepared, it would have an axial hydrogen at C-6

FIGURE 21. BOROHYDRIDE REDUCTION OF 6-OXOSTEPHULINE





and the C-6 to C-7 coupling constant could be easily differentiated as being either axial-equatorial or axial-axial allowing ready assignment of configuration at C-7. Since both sodium borohydride and lithium aluminum hydride reduction of 6-oxostephuline gave only the axial alcohol, other methods of reduction of ketones were attempted. An attempt at Meerwein-Pondorff-Verley reduction of 6-oxostephuline led to recovery of starting material. This was not unexpected since cyclic ketones do not readily undergo this reaction, especially if highly substituted.[31] Sodium in alcohol reduction led to a low recovery of starting material and no indication (by tlc) of more polar products. Model studies on hydrogenation using stephine gave a complex mixture of products, presumably formed by hydrogenolysis of the benzylic ketal oxygen and further transformations therefrom.

The close similarity between stephavanine (see Part III) and stephisoferuline suggests that they are formed biogenetically by analogous processes. The configuration of the esters at C-6 in both cases is axial and the nmr coupling constants for the C-6 to C-7 hydrogens is 4Hz in both cases suggesting that the configurations at C-7 should be the same. Evidence for the equatorial configuration of the C-7 methoxyl group in stephavanine has been presented, and therefore this configuration also seems most likely for stephisoferuline. Derivatives are now being prepared for X-ray crystallographic studies aimed at establishing the configuration at C-7 of stephisoferuline.

EXPERIMENTAL *

Extraction and Preliminary Fractionation.[15] The dried roots (41.4 kg) of S. hernandifolia were extracted twice with hexane (4 l./kg) and the hexane extracts discarded. The defatted roots were extracted three times with methanol (3 l./kg). The combined methanolic extracts were concentrated in vacuo to a volume of 8 l. This material was triturated five times with 6% HCl (total of 200 l.) to give an acid solution and 1.3 kg of residual gums. The acid solution was extracted with ether (58 l.) to give 16.5 g of ether-extractable solids. The remaining acid layer was basified to pH 9.1 with NH_4OH and extracted twice with 80 l. of chloroform. The chloroform soluble material yielded on evaporation the non-quaternary alkaloids (486 g). The aqueous solution was acidified to pH 1.8 with HCl and treated with ammonium Reineckate to precipitate the quaternary alkaloids (658 g, as Reineckates). The remaining aqueous solution was discarded. Continuous ether extraction of a portion of the non-quaternary alkaloids (172 g) for 21 days gave 96 g of ether soluble alkaloids and left a residue of 75.5 g of ether-insoluble alkaloids.

The ether soluble alkaloids (96 g) were chromatographed on 3.4 kg of SilicAR CC-7 (100-200 mesh, Mallinckrodt) eluted with chloroform and then methanol-chloroform mixtures, and fractions varying from 1 to 3 l., depending on the amount of material being eluted, were collected. A total of 64 fractions were collected and they were combined on the

* For conditions of spectral measurements and special procedures see the beginning of the Experimental section in Part III.

TABLE 8

Chromatographic Fractions of Ether Soluble Alkaloids of S. Hernandifolia

Aggregate Fraction	Fractions	Eluant	Volume	Weight	Constituents
A	1-5	chloroform	8 l.	2.87 g	nonalkaloidal material.
B	6-15	1% methanol-chloroform	13 l.	27.25 g	4-demethylhasubanonine (I)
C	16-20	"	6.5 l.	4.56 g	I + unidentified minor alkaloid
D	21-24	"	5.5 l.	5.78 g	4-demethylnorhasubanonine (II) + I
E	25-32	"	8.5 l.	14.53 g	II + unidentified mixture of minor components
F	33-43	2.5% methanol-chloroform	15.5 l.	12.12 g	oxoepistephanine (IX) + stephisoferuline (X)
G	44-49	"	11 l.	13.35 g	stephisoferuline + epistephanine (VIII)
H	50-55	5% methanol-chloroform	15 l.	9.56 g	VIII + unidentified minor alkaloids + non-alkaloidal material
I	56-63	15% methanol-chloroform	15.5 l.	6.48 g	unidentified complex alkaloidal mixture + much non-alkaloidal material
J	64	methanol	4 l.	8.01 g	mainly non-alkaloidal material

basis of tlc into the aggregate fractions indicated by letters A through J in Table 8.

Isolation of 4-Demethylhasubanonine. A portion of fraction B (18.96 g) was dissolved in methanol (30 ml) and treated with oxalic acid dihydrate (6.7 g) in methanol (30 ml). The resulting solution was heated to boiling on the steam bath, anhydrous ether was added to turbidity, and the mixture was allowed to crystallize. Two subsequent recrystallizations from methanol-ether gave pure 4-demethylhasubanonine (I) oxalate (12.32 g): mp 198-199°; $[\alpha]_D^{32}$ -123° (c 3.46, MeOH); λ_{max}^{KBr} 2.90, 3.39, 4.02, 5.85, 5.96 μ; λ_{max}^{EtOH} 266 mμ (ϵ 8,400); m/e 359 (M^+), 344, 328, 301, 300, 245.

Anal. Calcd for $C_{20}H_{25}NO_5 \cdot C_2H_2O_4$: C, 58.79; H, 6.06; N, 3.12. Found: C, 58.91; H, 6.02; N, 3.58.

4-Demethylnorhasubanonine (IV). Fraction D (5.78 g) from the large scale chromatography of the ether-soluble alkaloids was filtered through a column of neutral alumina (Woelhm, activity 1) eluting with chloroform and 10% methanol-chloroform to give 4.98 g of material. A portion of this (2.67 g) was chromatographed over silica gel (0.05 - .2 mm Brinkmann, 300 g) in chloroform, first collecting a forerun of 2 l. and then eluting with 1% methanol-chloroform and collecting 15 ml fractions. Combination of similar fractions by tlc gave three major fractions, the first being a mixture of IV and higher R_f material (832 mg), the second fraction being one spot IV (840 mg) and the third fraction consisting of a

mixture of IV and more polar material (634 mg). The second fraction was crystallized from acetone-petroleum ether or from chloroform-ether to give crystals of IV: mp 116-119° (softens 110°); $[\alpha]_D^{25}$ -219° (c 1.30, MeOH); λ_{max}^{KBr} 3.02, 3.40, 6.02, 6.24μ; λ_{max}^{MeOH} 266 mμ(ϵ 11,750); m/e 345 (M^+), 302, 301, 299, 269.

Anal. Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71, N, 4.06. Found: C, 66.14; H, 6.79; N, 3.98.

The remainder of the material isolated from the alumina filtration was chromatographed over SilicAR CC-7 (100-200 mesh, Mallinckrodt, 300 g) in 10% acetone-chloroform. Fractions were collected and combined on the basis of similarity in tlc properties. This gave two main fractions, the first consisting of a mixture of IV and less polar material (1.5 g) and the second being one-spot 4-demethylnorhasubanonine (IV, 630 mg). The latter fraction was treated with oxalic acid dihydrate (230 mg) in methanol (15 ml) and crystallized twice from methanol-ether to give colorless prisms: mp 192-193° (d); $[\alpha]_D^{25}$ -159° (c 1.34, MeOH); λ_{max}^{KBr} 2.89, 3.38, 5.85, 5.98, 6.13μ; λ_{max}^{MeOH} 232 mμ (sh) (ϵ 11,185), 264 mμ(ϵ 9,320).

Anal. Calcd for $C_{19}H_{23}NO_5 \cdot C_2H_2O_4$: C, 57.92; H, 5.79; N, 3.22. Found: C, 57.97; H, 5.92; N, 3.15.

Conversion of 4-Demethylnorhasubanonine (IV) to 4-Demethylhasubanonine (I). 4-Demethylnorhasubanonine (IV, 45 mg) was dissolved in chloroform (2 ml) and treated with methyliodide (1 ml) at 70° under reflux for one hour. The reaction mixture was evaporated to dryness and tlc showed it to be a mixture of less polar product, a small amount of starting

material and a trace of very polar material. Chromatography on silica gel (0.05-.2 mm Brinkmann, 15 g) using chloroform as the eluant achieved separation, giving the less polar material (22 mg) and starting material (15 mg) in pure form. The product was shown to be identical to 4-demethylhasubanonine (I) by nmr and ir spectra, mixture tlc and mixture melting point of the oxalate salts.

Isolation of Epistephanine (VIII). Fraction G (13.35 g) was chromatographed over SilicAR CC-7 (100-200 mesh, Mallinckrodt, 300 g) eluting with 0.5% methanol-chloroform to give a fraction (3.94 g) rich in epistephanine. Rechromatography of this fraction on neutral alumina (Woelhm, activity 1, 300 g) in 50% benzene-chloroform gave one spot material (2.8 g) which was crystallized three times from benzene to give epistephanine (524 mg): mp 134-135°, $[\alpha]_D^{27}$ +204° (c 0.83, $CHCl_3$); λ_{max}^{KBr} 3.40, 3.52, 3.60, 6.25, 6.62 μ; λ_{max}^{MeOH} 229 mμ (sh) (ϵ 44,930), 281 mμ (ϵ 11,960); m/e 606 (M^+).

Anal. Calcd for $C_{37}H_{38}N_2O_6 \cdot C_6H_6$: C, 75.41; H, 6.48; N, 4.09. Found: C, 75.27, 75.26; H, 6.58, 6.58; N, 4.12, 4.17.

Isolation of Stephisoferuline (X) and Oxoepistephanine (IX). Fraction F (12.12 g) was chromatographed over silica gel (0.05 - 0.2 mm, Brinkmann, 700 g in chloroform) eluting with chloroform (2 l.), 2% methanol-chloroform (2 l.), and 4% methanol-chloroform, collecting 100 ml fractions. Fractions 6 through 10 gave a mixture of stephisoferuline and oxoepistephanine (8 g) and fractions 11 through 24 gave one spot stephisoferuline (4 g) which was

crystallized from chloroform-ether to give 1.13 g of material. Two recrystallizations from the same solvents gave colorless needles: mp 133-135° (softens 105°, evacuated capillary); $[\alpha]_D^{32}$ +48° (c 0.82, MeOH); λ_{max}^{KBr} 2.84, 2.98, 3.38, 5.89μ; λ_{max}^{EtOH} 229 mμ(sh) (ε 19,500), 287 mμ (ε 15,100), 325 mμ(ε 15,100); m/e 539 (M^+).

Anal. Calcd for $C_{29}H_{33}NO_9 \cdot 2/3\ CHCl_3$: C, 57.55; H, 5.48; N, 2.26; Cl, 11.45. Found: C, 57.30, 57.44; H, 5.44, 5.52; N, 2.21, 2.16; Cl, 10.54, 10.59.

Fractions 6 through 10 of the above chromatography were rechromatographed on acid-washed alumina (Merck, 300 g) in chloroform and 15 ml fractions were collected. Fractions 3 through 15 gave oxoepistephanine (IX, 1.12 g) which was crystallized twice from methanol-ether to give colorless needles: mp 224-226° (d); $[\alpha]_D^{27}$ +272° (c 1.38, $CHCl_3$); λ_{max}^{KBr} 3.40, 3.52, 3.58, 5.97, 6.25, 6.62μ; λ_{max}^{MeOH} 235 mμ (ε 42,760), 278 mμ (ε 17,100); m/e 620 (M^+), 605, 590, 575, 559, 381, 380, 379; nmr τ 1.95 - 3.67 (10 H, aromatic H), 6.10 (9H, 3 OCH_3), 6.65 (3H, OCH_3), 7.45 (3H, NCH_3).

Anal. Calcd for $C_{37}H_{36}N_2O_7$: C, 71.60; H, 5.85; N, 4.51. Found: C, 71.29, 71.41; H, 6.01, 6.02; N, 4.71, 4.54.

Stephuline (XI). To a solution of stephisoferuline (X, 100 mg) in methanol (3 ml) 2.5 N NaOH (5 ml) was added and the reaction mixture was refluxed for 18 hours. The reaction mixture was cooled and partially neutralized to pH8.3 The solution was diluted to 50 ml and extracted with

chloroform (3-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give crude stephuline (XI, 75 mg) which was crystallized twice from chloroform-ether to give colorless needles: mp 223-225°; $[\alpha]_D^{32}$ +93° (c 0.55, MeOH); λ_{max}^{KBr} 2.81, 3.03, 3.38, 3.50, 6.19, 6.73, 7.86 μ; λ_{max}^{EtOH} 226 mμ (ϵ 8,200), 283 mμ (ϵ 1,850); m/e 363 (M^+), 217, 216, 215, 202, 184, 154.

Anal. Calcd for $C_{19}H_{25}NO_6$: C, 62.79; H, 6.93; N, 3.85. Found: C, 62.59; H, 7.05; N, 3.84.

The above aqueous solution was acidified to pH 3 with 0.5 N HCl and extracted with chloroform (3-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give crude isoferulic acid (XII, 32 mg) which was crystallized twice from methanol-chloroform to give colorless needles: mp 232-235° (lit 230°); λ_{max}^{KBr} 2.94, 3.41, 5.92, 6.00 μ; λ_{max}^{EtOH} 232 mμ (ϵ 9,200), 241 mμ (ϵ 9,540), 294 mμ (ϵ 12,100), 324 mμ (ϵ 13,050); nmr (D_6DMSO), τ 2.52 (1 H, d (J=16 Hz) olefinic H), τ 2.82-3.15 (3 H aromatic H), τ 3.75 (1H, d (J=16 Hz) olefinic H), τ 6.17 (3H, OCH_3); m/e 194 (M^+).

6-Oxostephuline (XIV). To a solution of stephuline (XI, 57 mg) dissolved in acetone (15 ml) at 0° Jones reagent was added dropwise until a brown color just persisted. The reaction was kept at 0° for 10 minutes and then poured into ice cold 5% sodium bicarbonate solution (200 ml) and extracted with chloroform (4-100 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give a green residue (65 mg)

which was chromatographed on a preparative silica gel plate (Silica gel F_{254}, 20 x 20 x .2 cm, Brinkmann) in 10% methanol-chloroform. The major band was scraped off, powdered and eluted with 50% methanol-chloroform to give 6-oxostephuline (XIV, 50 mg). Two crystallizations from acetone-petroleum ether gave needles: mp 225-228° (d); $[\alpha]_D^{27}$ + 75° (c 0.27, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.82, 3.39, 3.52, 5.76, 6.72, 7.80 μ; λ_{max}^{MeOH} 280 mμ (ϵ 4,230); m/e 361 (M^+), 360, 215, 214, 183, 181.

Anal. Calcd for $C_{19}H_{23}NO_6$: C, 63.14; H, 6.42; N, 3.88. Found: C, 63.24; H, 6.84; N, 3.64.

Triacetyl-10-desoxy-9,10-dehydrostephuline (XVI). Stephuline (XI, 50 mg) in anhydrous pyridine (2 ml, dried over KOH) was treated with acetic anhydride (3 ml) and heated at 60° for 16 hours. The solution was allowed to cool and was poured into saturated sodium bicarbonate solution (200 ml). After evolution of CO_2 had ceased, the mixture was extracted with chloroform (3-150 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give a gummy residue which was taken up in chloroform (20 ml). The resultant solution was saturated with gaseous hydrogen chloride and allowed to stand for 30 minutes. This solution was poured into saturated sodium bicarbonate solution (200 ml) and was extracted with chloroform (3-100 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give a gummy residue which was chromatographed on a preparative silica gel plate (Silica gel F_{254}, 20 x 20 x .2 cm, Brinkmann) in 8% methanol-chloroform. The

major band was scraped off, powdered, and eluted with 50% methanol-chloroform to give triacetyl derivative XVI (70 mg). Crystallization from methanol-ether gave colorless needles: mp 181-183°; $[\alpha]_D^{27}$ -92° (c 0.29, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 3.32, 3.40, 3.46, 3.52, 5.70, 5.75, 6.08 μ; λ_{max}^{MeOH} 268 mμ (ϵ 14,300); m/e 457 (M^+), 299, 257, 215, 182, 154, 71, 43.

Anal. Calcd for $C_{24}H_{27}NO_8 \cdot CH_3OH$: C, 61.34; H, 6.38; N, 2.86. Found: C, 61.04; H, 5.79; N, 2.83.

N-Methylstephuline (XIII). Stephuline (XI, 50 mg) was dissolved in dioxane-methanol (4 ml each) and 37% formalin solution (2 ml) was added. After stirring for 30 minutes sodium borohydride (1.20 g) was added and the reaction was stirred for 1.5 hours. The reaction mixture was poured into 5% potassium carbonate solution (50 ml) and extracted with chloroform (5-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to give N-methylstephuline (XIII, 74 mg), which was crystallized from ether to give colorless needles: mp 126-128°; $[\alpha]_D^{27}$ +92° (c 0.54, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.83, 3.32, 3.40, 6.73, 8.46 μ; λ_{max}^{MeOH} 254 mμ (ϵ 4,700), 279 mμ, (ϵ 5,340); m/e 377 (M^+), 231, 230.

Anal. Calcd for $C_{20}H_{27}NO_6$: C, 63.64; H, 7.21; N, 3.71. Found: C, 63.66; H, 7.23; N, 3.75.

8-Demethylstephuline (XV). Stephuline (XI, 49 mg) was dissolved in 0.5 N HCl (5 ml) and heated at 60° for four hours. The reaction mixture was poured into saturated sodium bicarbonate solution (30 ml) and extracted with chloroform (5-40 ml portions). The combined, dried (Na_2SO_4) chloro-

form extracts were evaporated to give 8-demethylstephuline (XV, 49 mg) which gave colorless crystals from ethyl acetate:ether: mp 178-180°; $[\alpha]_D^{26}$ +107° (c 0.47, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.83, 3.32, 3.40, 6.73, 8.46, 9.27 μ; λ_{max}^{MeOH} 254 mμ, (ϵ 3,490), 278 mμ, (ϵ 4,530); m/e 349 (M^+), 348, 217, 216, 215, 184.

Anal. Calcd for $C_{18}H_{23}NO_6$: C, 61.88; H, 6.64; N, 4.01. Found: C, 61.86; H, 6.77; N, 4.08.

Triacetyl-10-desoxystephuline (XVII). Triacetyl compound XVI (33 mg) in ethanol was hydrogenated over platinum oxide in a microhydrogenation apparatus until uptake of hydrogen had ceased (30 minutes). The catalyst was removed by filtration and washed with several portions of ethanol. The combined filtrate and washings were evaporated to give crystalline triacetyl compound XVII (29 mg), which was crystallized from acetone to give colorless needles: mp 199-201°; $[\alpha]_D^{26}$ +16° (c0.67, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 3.21, 3.41, 3.50, 5.67, 5.75, 6.06, 6.72 μ; λ_{max}^{MeOH} 254 mμ (ϵ 5,420), 279 mμ, (ϵ 5,280); m/e 459 (M^+), 431, 301, 259.

Anal. Calcd for $C_{24}H_{29}NO_8$: C, 62.73; H, 6.36; N, 3.05. Found: C, 62.68; H, 6.47; N, 2.92.

4-Demethylnorhasubanonine-6α-ol (XX) and 4-Demethylnorhasubanonine-6β-ol (XIX). 4-Demethylnorhasubanonine (IV, 245 mg) in methanol (15 ml) was cooled to 0°, and stirred with sodium borohydride (500 mg) in an ice-salt bath for three hours. The reaction mixture was poured into 5% sodium bicarbonate solution (200 ml) and extracted with

chloroform (5-75 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to dryness to give three spot material (by tlc) consisting of a small amount of starting material and two more polar compounds. This material was chromatographed on a preparative silica gel plate (Silica gel F_{254} Brinkmann, 20 x 20 x .2 cm) in 30% methanol-chloroform. The three bands visible under uv light were scraped off, powdered, and eluted with 50% methanol-chloroform. The highest R_f band gave starting material (16 mg). The band of middle R_f gave the axial alcohol (XIX, 161 mg) and the lower R_f band gave the equatorial alcohol (XX, 72 mg).

Alcohol XIX was crystallized four times from chloroform-ether to give colorless prisms: mp 181-182°; $[\alpha]_D^{32}$ -114° (c 0.85, MeOH); λ_{max}^{KBr} 2.80, 2.94, 3.39, 7.78, 9.30μ; λ_{max}^{MeOH} 282 mμ (ϵ 2,420); m/e 347 (M^+).

Anal. Calcd for $C_{19}H_{25}NO_5 \cdot 2/3\ CHCl_3$: C, 55.31; H, 6.06; N, 3.28; Cl, 16.63. Found: C, 55.15, 55.02; H, 6.03, 6.13; N, 3.41; Cl, 15.17, 15.12.

Alcohol XX was crystallized twice from acetone to give colorless microcrystalline material: mp 214-215° (d); $[\alpha]_D^{32}$ -100° (c 0.97, MeOH); λ_{max}^{KBr} 2.90, 3.06, 3.39, 7.78, 9.18, 9.51μ; λ_{max}^{MeOH} 282 mμ (ϵ 2,420); m/e 347 (M^+).

Anal. Calcd for $C_{19}H_{25}NO_5$: C, 65.69; H, 7.25; N, 4.03. Found: C, 65.25, 65.14; H, 7.09, 7.03; N, 3.91, 3.89.

Triacetyl-3,7-dimethoxy-4,6-dihydroxyhasubanan-7-one (XVIII). To triacetyl derivative XVII (39 mg) was added p-toluenesulfonic acid (3 mg), methanol (2 ml) and acetone dimethyl acetal (10 ml). The mixture was allowed

to stand for ten minutes and was then distilled to half volume on a rotary evaporator. Excess sodium methylate (10 mg) in methanol (1 ml) was added, to quench the reaction mixture, which was then evaporated to dryness to give a gummy residue. This residue was partitioned between water and chloroform (30 ml each) and after removal of the organic phase, the aqueous phase was extracted with additional chloroform (4-30 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to dryness. Tlc of the residue indicated about 20% conversion to a higher R_f spot. The residue was then treated under the some reaction conditions as above, except that methylene chloride (10 ml) was added as a co-solvent. Workup in the manner outlined gave a residue which showed about 40% conversion to higher R_f product (estimated by tlc). The reaction was repeated two more times under identical conditions and after the last workup, conversion of the starting material to higher R_f product was estimated at about 70% (by tlc). The residue from the last workup was chromatographed on a preparative tlc plate (20 x 20 x .2 cm, Brinkmann) in 7% methanol-chloroform. The plate was developed, dried, and redeveloped to effect separation. The main band of higher R_f was cut out, powdered, and eluted with 50% methanol-chloroform to yield triacetyl derivative XVIII (25 mg). This material was purified by precipitation with petroleum ether from chloroform solution to give an amorphous solid: $[\alpha]_D^{26}$ -71° (c 0.57, $CHCl_3$); $\lambda_{max}^{CHCl_3}$ 3.30, 3.40, 5.65, 5.88, 6.06, 6.71 7.10, 7.80, 8.14 μ; λ_{max}^{MeOH} 270 mμ (ϵ 5,550); m/e 399 (M^+-60), 313, 301, 285, 271, 259.

Anal. Calcd for $C_{24}H_{29}NO_8$: C, 62.73; H, 6.36; N, 3.05.

Found: C, 62.58; H, 6.25; N, 2.98.

Alcohol XIX (55 mg) in dry pyridine (2 ml, dried over potassium hydroxide) and acetic anhydride (2 ml) was stirred at room temperature for 18 hours. The reaction mixture was poured into saturated sodium bicarbonate solution (50 ml) and extracted with chloroform (4-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to dryness, residual pyridine being azeotroped with benzene, to give 55 mg of material. Tlc showed this material to be different from XVIII immediately after preparation, but after standing in chloroform solution the R_f was changed and was then identical to that of XVIII. The infrared, ultraviolet, and mass spectra as well as optical rotation and mixture tlc showed this compound to be identical to XVIII.

Alcohol XX (53 mg) in dry pyridine (2 ml, dried over potassium hydroxide) and acetic anhydride (3 ml) was stirred for 18 hours at room temperature. The reaction mixture was poured into saturated sodium bicarbonate solution (50 ml) and extracted with chloroform (4-50 ml portions). The combined, dried (Na_2SO_4) chloroform extracts were evaporated to dryness, residual pyridine being azeotroped with benzene, to give 55 mg of material. After standing in chloroform this material was indistinguishable from XVIII (by infrared, ultraviolet and mass spectra, and sign of rotation and mixture tlc).

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