Tetrahedron, 1966, Vol. 22, pp. 321 to 336. Pergamon Press Ltd. Printed in Northern Ireland

THE STRUCTURAL ELUCIDATION OF SITSIRIKINE, DIHYDROSITSIRIKINE AND ISOSITSIRIKINE

THREE NEW ALKALOIDS FROM VINCA ROSEA LINN[1]

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(*Received* 31 *May* 1965; *in revised form* 6 *July* 1965)

Abstract—The chemical structures of three new alkaloids, sitsirikine, dihydrositsirikine and isositsirikine have been elucidated. These alkaloids are members of the corynantheine class.

Vinca rosea Linn (*Catharanthus roseus* G. Don or *Lochnera* Reichb.) is an Apocynaceous plant which is widely grown in gardens all over the world for ornamental purposes. It has attracted a great deal of interest in recent years largely because of the biological properties shown by some of its alkaloidal components and as a result, a great number of alkaloids have been isolated from this plant.[2]

The detailed investigations of the Lilly group in this area led to the isolation of an alkaloid, sitsirikine, as a crystalline sulfate ($C_{21}H_{26}O_3N_2 \cdot \frac{1}{2}H_2SO_4$)[2,3] and on the basis of preliminary results it was suggested that sitsirikine may represent a new yohimbine isomer. Through the kind cooperation of Dr. Marvin Gorman, we obtained a generous supply of the crude alkaloid (as its sulfate) from which on further purification in our laboratory it was shown that three new alkaloids are in fact present. We have termed these alkaloids as sitsirikine, dihydrositsirikine and isositsirikine. A preliminary communication on our investigations regarding the first two alkaloids has appeared[4] and we wish now to present a more detailed discussion of this work as well as provide new evidence for the structural elucidation of the third alkaloid, isositsirikine. For the sake of clarity these results are discussed separately below.

A. *Sitsirikine and dihydrositsirikine*

The crude sulfate provided by Dr. Gorman was made basic and the resulting amorphous alkaloid which was obtained from the ether extract showed three distinct spots on thin-layer chromatography (TLC). Several different purification techniques were applied without success—chromatography on alumina and silica gel, sublimation, fractional recrystallization of the salts. Finally it was found that after several recrystallizations of the base from acetone–pet. ether, a material melting sharply at 181° was obtained. This now showed only two spots on TLC and analyzed for the acetone solvate, $C_{21}H_{26}O_3N_2 \cdot CH_3COCH_3$. Attempts to separate the two components were of no avail, and indeed the mixture behaved as a homogeneous compound in all criteria except on TLC. The unsolvated alkaloid, for which we retain the name sitsirikine,

[1] Financial support for this work was provided by the National Cancer Institute of Canada and Eli Lilly and Company. One of us (R. T. B.) is also indebted to the Canadian Commonwealth Scholarship and Fellowship Committee for a scholarship. We are very grateful to Dr. M. Gorman, Eli Lilly and Company, for the generous gift of the crude alkaloids and for numerous discussions during the course of this work.

[2] G. H. Svoboda, I. S. Johnson, M. Gorman and N. Neuss, *J. Pharm. Sci.* **51**, 707 (1962).

[3] G. H. Svoboda, M. Gorman, N. Neuss and A. J. Barnes, *J. Pharm. Sci.* **50**, 409 (1961).

[4] J. P. Kutney and R. T. Brown, *Tetrahedron Letters* 1815 (1963).

could be obtained in purest form from aqueous methanol, m.p. 206–208°, $[\alpha]_D^{26}$ —58° (MeOH) and analyzed well for $C_{21}H_{26}O_3N_2$. This molecular formula was supported by elemental analyses on the picrate, m.p. 226–228° (dec) and finally substantiated by a mass spectral mol. wt. determination (354).[5] It must be emphasized that the purest sample which we could obtain always indicated a trace impurity on thin layer chromatoplates and this was subsequently shown to be due to dihydrositsirikine.

The UV spectrum of sitsirikine, with maxima at 226, 282 and 290 mμ indicated an unsubstituted indole chromophore. This was confirmed by signals in the NMR spectrum at 0·06τ (indolic NH) and in the aromatic region (four proton multiplet centered at 2·8τ). A strong band in the IR spectrum at 5·86 μ was readily attributed to a carbonyl group and an absorption at 2·98 μ was compatible with the presence of NH and/or hydroxyl groups. In addition to a spike at 6·38τ (OCH_3), the NMR spectrum of sitsirikine displayed a two-proton multiplet centered at 6·1τ that was possibly due to the methylene protons of a primary alcoholic function. The presence of a hydroxyl group was confirmed by the formation of a monoacetate, $C_{23}H_{28}O_4N_2$, m.p. 198°, whose NMR spectrum was particularly instructive. Apart from the expected signal at 8·02τ due to the acetyl group, the multiplet present at 6·11τ in the spectrum of the alcohol had shifted downfield and now appeared at 5·6τ. This shift of 0·5τ upon acetylation is characteristic of *primary* alcohols, whereas the corresponding shift for *secondary* alcohols is about 1τ unit.[6]

Besides the above-mentioned signals the NMR spectra of sitsirikine and its acetate displayed a multiplet centered at 4·7τ due to olefinic protons, which integrated for rather less than two hydrogen atoms. A series of microhydrogenations was run and it was found that only 0·6–0·7 moles of hydrogen was taken up. Moreover the reduction product gave only *one* spot on TLC whose R_f value corresponded to the smaller of the two spots exhibited by the original alkaloid. This evidence suggested that the two components of the mixture differed from each other by the presence of an olefinic bond in one of the alkaloids. This conclusion was fully borne out by subsequent work. The unsaturated alkaloid was named sitsirikine, whereas the corresponding dihydro derivative will be referred to as dihydrositsirikine.

Catalytic hydrogenation of the alkaloid mixture on a larger scale and recrystallization from acetone afforded solvated dihydrositsirikine, m.p. 180°, which analyzed for $C_{21}H_{28}O_3N \cdot {}_2CH_3COCH_3$. Further recrystallizations from aqueous methanol gave the unsolvated alkaloid, $C_{21}H_{28}O_3N_2$, m.p. 215°, $[\alpha]_D^{26}$ —55° (MeOH). The NMR spectrum of dihydrositsirikine showed a complete disappearance of the olefinic proton absorption. A strong band at 5·85 μ in the IR spectrum of the reduced material excluded any conjugation between the carbonyl group and the double bond in sitsirikine, and the UV spectrum was unchanged. Elemental analyses on the crystalline picrate, m.p. 228–230° (dec), acetate, m.p. 187°, and *p*-bromobenzoate, m.p. 174°, supported the formula assigned to dihydrositsirikine, and final confirmation was obtained from a mass spectral mol. wt. determination (356).

Evidence that the olefinic linkage in sitsirikine was in fact a terminal double bond

[5] We are very grateful to Professor Carl Djerassi, Stanford University, for all the mass spectral results mentioned in this paper.

[6] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, p. 55. Pergamon Press, Oxford (1959).

was provided by the appearance of a new C-methyl signal at 9·07τ in the NMR spectrum of the reduction product. This was corroborated when ozonolysis of the original alkaloid gave formaldehyde, identified by paper chromatography of its 2,4-dinitrophenylhydrazone.[7] A conventional Kuhn-Roth determination on dihydrositsirikine indicated 0·93 moles C-methyl, while a modified procedure[8] yielded propionic acid and thus showed that the new C-methyl function was in fact part of a C-ethyl group. These experiments established the presence of a vinyl group in sitsirikine.

However it was still necessary to explain why the olefinic proton absorption in the NMR spectrum of sitsirikine integrated for two rather than the three hydrogen atoms expected for a vinyl group. The TLC and microhydrogenation results had suggested that the impurity present in the original alkaloid was dihydrositsirikine, and a close scrutiny of the NMR spectrum of the original, impure sitsirikine revealed a slight absorption at 9·02τ integrating for about one hydrogen atom, whereas the mass spectrum on this material indicated a small peak at m/e 356 in addition to the mol. ion peak at m/e 354. A Kuhn-Roth determination on impure sitsirikine showed 0·38 moles C-methyl and the modified method afforded propionic acid. From these results it was deduced that the original alkaloid was a mixture of sitsirikine and dihydrositsirikine in an approximate ratio of 2:1.

Since dihydrositsirikine was the only component which was easily obtained in pure form, it was used as the starting material in all of our subsequent studies.

Besides providing the information discussed above, the NMR spectrum of dihydrositsirikine was very useful in establishing the nature of the oxygen functions present in the molecule. In the region of 6·1τ there was a two proton absorption attributable to hydrogen atoms attached to an oxygen-bearing carbon atom, which upon acetylation moved downfield to 5·6τ. This paralleled the behaviour of the original sitsirikine and confirmed the presence of a primary alcohol. The presence of a methoxyl group at 6·42τ was confirmed by a Zeisel determination on dihydrositsirikine.

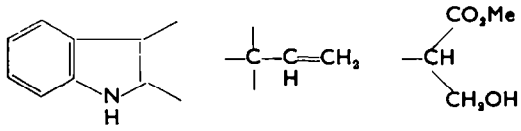
Since the UV and NMR spectra exlcuded the possibility that the methoxyl was attached to the indole system, it was deduced that it must be part of a carbomethoxy group, even though the position of the carbonyl absorption in the IR spectrum was more suggestive of a ketone.

Reduction of dihydrositsirikine with LAH yielded a crystalline diol, m.p. 203°, which analyzed for $C_{20}H_{28}O_2N_2$. The IR spectrum of the diol showed no carbonyl absorption, and the NMR spectrum indicated a complete absence of the methoxyl signal. The presence of a carbomethoxy group in dihydrositsirikine was thus confirmed.

Treatment of the diol with acetone containing *p*-toluenesulfonic acid afforded an acetonide, m.p. 105–109°, as shown by a six-proton NMR signal at 8·62τ (*gem*-dimethyl). Since both alcoholic groups were primary the formation of this derivative meant that the hydroxyl groups were in a 1,3-relationship, and hence dihydrositsirikine itself must contain a β-hydroxy-ester grouping. The NMR spectrum of the acetonide was even more instructive in that the signal at 6·25τ, due to four methylene protons on the two oxygen-bearing carbon atoms, was split into a *doublet*, thereby indicating that there must be one proton on the carbon atom linking the hydroxymethyl and carbomethoxy groups in dihydrositsirikine.

[7] D. F. Meigh, *Nature, Lond.* **170**, 579 (1962).
[8] H. Bickel, H. Schmid and P. Karrer, *Helv. Chim. Acta* **38**, 649 (1955).

At this point it had been established that sitsirikine possessed a tetracyclic skeleton and the following features:



During the course of the chemical investigations, mass spectrometric analyses of sitsirikine, dihydrositsirikine and various derivatives were undertaken. The mass spectrum of dihydrositsirikine (Fig. 1), run by the direct inlet procedure,[9] was most helpful and is discussed in some detail.

The mol. ion peak at m/e 356 established the molecular formula assigned to dihydrositsirikine, and fragments at m/e 338 (M—H_2O) and 325 (M—CH_2OH) were consistent with the presence of a primary alcohol. A strong peak at m/e 253 was considered to arise from loss of the entire oxygen-containing portion of the molecule, i.e. M—CH$<^{CH_2OH}_{CO_2Me}$. More important, however, were the ions at m/e 184, 170, 169 and 156. It was immediately apparent from these four peaks that rings A, B and C of the dihydrositsirikine skeleton were of the type encountered in the yohimbine and related alkaloid classes[10,11] where these ions were also attributed to the fragments shown in Fig. 1. The occurrence of significant peaks at m/e 169 and 170 and *not* at m/e 168 and 169 excluded the type of pentacyclic ring system found in polyneuridine.[10]

Further information regarding the ring skeleton was obtained from semimicro dehydrogenation experiments. Treatment of dihydrositsirikine with lead tetraacetate afforded a product which exhibited UV spectra (λ_{max} 253, 308 and 365 mμ in neutral or acid solution) in good agreement with those of tetradehydroyohimbine and similar compounds (I).[12] Dehydrogenation of dihydrositsirikine with 10% Pd-C at 250° gave a mixture of products which were separated by TLC. The main product (compound A) displayed UV spectra very similar to harman (II). In neutral and alkaline media the spectra were the same, with maxima at 234, 250, 282, 288, 337 and 349 mμ, whereas in acid solution there was a bathochromic shift to 254, 303 and 372 mμ. These results were sufficient to confirm the tetrahydro-β-carboline structure indicated by the mass spectrum.

Dihydrositsirikine hydrobromide was then subjected to Pd–dehydrogenation at 280°, and the resulting mixture separated by TLC. The UV absorption of one major fraction (compound B) was in close correspondence with that of 5,6-dihydroflavocoryline hydrochloride[13] with maxima at 221, 312 and 385 mμ. This result provided the first piece of evidence for the entire ring system in sitsirikine. Final confirmation was obtained when the dehydrogenation product was oxidized further with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone to another substance (compound C) which now possessed a completely aromatized ring system. The UV spectrum with

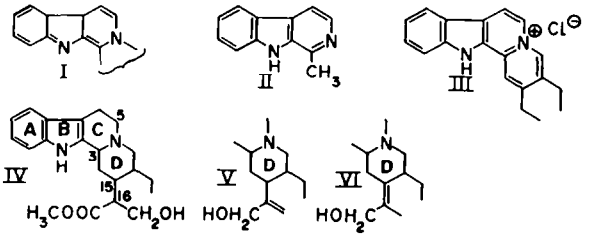
[9] J. F. Lynch, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Experientia* **19**, 211 (1963).

[10] L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J. M. Wilson, L. J. Durham and C. Djerassi, *J. Amer. Chem. Soc.* **84**, 2161 (1962).

[11] H. Budzikiewicz, C. Djerassi and D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectrometry* Vol. 1: *Alkaloids* p. 77. Holden-Day (1964).

[12] F. E. Bader, D. F. Dickel, C. F. Huebner, R. A. Lucas and E. Schlittler, *J. Amer. Chem. Soc.* **77**, 3547 (1955).

maxima at 237, 291, 345 and 385 mμ was in good agreement with that reported for flavocoryline hydrochloride (III).[13] An authentic sample of flavocoryline hydrochloride was subsequently obtained[14] and the UV spectrum was found to be superimposable on that of the compound derived from dihydrositsirikine. Furthermore, the two materials had the same R_f value on paper chromatograms run in several different solvent systems. Although the minute amounts of dehydrogenation products available prevented complete characterization, the UV spectral data established the ring structure, and also suggested that sitsirikine was a relative of the corynantheine class of alkaloids. A provisional structure such as IV could thus be considered for dihydrositsirikine.



The orientation of the hydrogen atom at C-3 in IV was indicated as α by the presence of Bohlmann bands[15,16] at 3·56 and 3·62 μ in the IR spectrum of dihydrositsirikine. Furthermore, the hydrogen atom at C-15 could be assumed to have the α-configuration since this orientation has been found to be constant at the corresponding position in the related alkaloids.[17]

During their investigations on corynantheine, Karrer *et al.*[18] had prepared two isomeric alcohols: desmethoxydihydrocorynantheine alcohol and isodesmethoxydihydrocorynantheine alcohol (V and VI respectively, rings A, B, C as in IV). Since the configurations at C-3 and C-15 in dihydrocorynantheine were the same as those projected for the corresponding positions in dihydrositsirikine, it seemed feasible to attempt a correlation between dihydrositsirikine and V or VI.

Accordingly, dihydrositsirikine was treated with methoxide in dry methanol to afford an α,β-unsaturated ester, which recrystallized from aqueous methanol as needles, m.p. 84–89 and analyzed for the methanol solvate, $C_{21}H_{26}O_2N_2 \cdot CH_3OH$. The presence of a terminal olefin was shown by a band in the IR spectrum at 6·17 μ and NMR signals at 3·73 and 4·41 τ, each of which integrated for one proton. This olefinic ester was then reduced with LAH, but the product contained relatively little terminal olefin as determined by the NMR spectrum. By a combination of chromatographic and recrystallization techniques the major component was obtained, as needles, m.p. 204°, $[\alpha]_D^{26}$ −24° (MeOH), which analyzed for $C_{20}H_{26}ON_2$. The m.p. and specific rotation were in excellent agreement with the corresponding values quoted[18] for VI. This result was good evidence for the structure proposed above for

[13] K. B. Prasad and G. A. Swan, *J. Chem. Soc.* 2024 (1958).

[14] We are very grateful to Dr. G. A. Swan, King's College, University of Durham, for providing us with this sample.

[15] F. Bohlmann, *Chem. Ber.* **91**, 2157 (1958); *Angew. Chem.* **69**, 641 (1957).

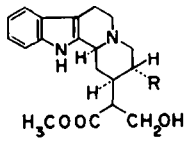
[16] W. E. Rosen, *Tetrahedron Letters* No. 14, 481 (1961).

[17] E. Wenkert and N. V. Bringi, *J. Amer. Chem. Soc.* **81**, 1474 (1959).

[18] P. Karrer, R. Schwyzer and A. Flem, *Helv. Chim. Acta* **35**, 851 (1952).

dihydrositsirikine but was not entirely conclusive, since unfortunately no sample of the iso-alcohol (VI) could be obtained for direct comparison.

However the correlation between dihydrocorynantheine and dihydrositsirikine was achieved by the following sequence of reactions. Mild acid hydrolysis converted dihydrocorynantheine to desmethyldihydrocorynantheine,[19] which on reduction with $NaBH_4$ yielded a product (VII) identical in every respect with dihydrositsirikine. Having thus established the structure of dihydrositsirikine, we could immediately assign the structure and stereochemistry to sitsirikine as depicted in VIII.



VII, R = $-CH_2CH_3$
VIII, R = $-CH{=}CH_2$

B. *Isositsirikine*

From the amorphous post-perivine fractions of the chromatography of fraction B, the Lilly group isolated[2] another alkaloid which they also referred to as sitsirikine but to which we have given the name, isositsirikine, since we have now shown that it is a new alkaloid. Although the base, $[\alpha]_D^{26}$ $-20°$ ($CHCl_3$), was an amorphous powder, it was homogeneous by TLC and gave a sharp melting crystalline sulfate, m.p. 263·5°, and picrate, m.p. 216°. Analyses on isositsirikine and its salts indicated a formula of $C_{21}H_{26}O_3N_2$ for the base. This formula was subsequently confirmed by a mass spectral mol. wt. determination which showed a value of 354. Standard Kuhn-Roth and Zeisel determinations showed the presence of one C-methyl and one O-methyl group respectively. Maxima at 224, 283 and 291 mμ in the UV spectrum were characteristic of an unsubstituted indole chromophore, and an absorption band at 5·81 μ gave evidence for a carbonyl group. The NMR spectrum of isositsirikine confirmed that the indole system was unsubstituted and a sharp, three-proton singlet at 6·28τ was readily attributed to the methoxyl group found in the Zeisel determination.

The presence of one olefinic hydrogen atom was shown by a quartet centered at 4·53τ, whereas a doublet at 8·40τ indicated that the methyl group was attached to an olefinic carbon atom. Since the coupling constants were the same (7 c/s) in both cases, these signals almost certainly denoted an ethylidene group containing a tri-substituted double bond. Catalytic hydrogenation resulted in the uptake of one mole of hydrogen to afford an amorphous mixture of two compounds as shown by TLC. The major component (which was *not* the same as dihydrositsirikine) was separated as an amorphous powder by column chromatography and characterized as dihydro-isositsirikine, $C_{21}H_{28}O_3N_2$. In the NMR spectrum of the dihydro compound the signals due to the ethylidene group had disappeared, and a new methyl absorption at 9·03τ became evident. Final confirmation of the ethylidene group was obtained when ozonolysis of isositsirikine yielded acetaldehyde, identified by paper chromatography of its 2,4-dinitrophenylhydrazone derivative.[7]

Carbonyl and methoxyl functions accounted for two of the oxygen atoms in isositsirikine. The nature of the third oxygen atom was revealed when acetylation

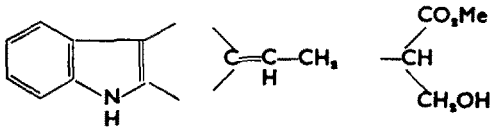
[19] A. Chatterjee and P. Karrer, *Helv. Chim. Acta* 33, 802 (1950).

gave an amorphous acetate, $C_{23}H_{28}O_4N_2$, which displayed the appropriate bands in the IR region at 5·78 and 8·1 μ. The NMR spectrum of this acetate was very helpful in that apart from the expected new sharp signal at 8·15τ due to the acetyl group, a two-proton doublet now appeared at 6·05τ, whereas the two-proton absorption present at 6·6τ in the spectrum of the original alkaloid had disappeared. The most obvious interpretation was that the doublet was due to the methylene protons of a *primary* alcohol which had undergone a paramagnetic shift of 0·5τ on acetylation.

When isositsirikine was reduced with LAH a diol was obtained, which showed neither a carbonyl absorption in the IR spectrum nor a methoxyl signal in the NMR spectrum. The presence of a carbomethoxy group in isositsirikine was thereby established.

Treatment of the reduction product with acetone containing *p*-toluenesulfonic acid gave a crystalline acetonide and the NMR spectrum of the latter quickly established a *gem*-dimethyl group with a pair of sharp signals at 8·63 and 8·68τ. Since the diol had two primary alcohol functions, the formation of the above derivative established a 1,3 relationship between these hydroxyl groups. Hence isositsirikine itself must possess a β-hydroxy ester grouping similar to that found in sitsirikine.

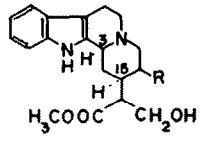
At this point the following features of the alkaloid structure had been established:



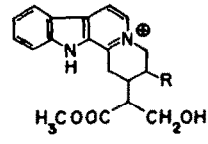
Since it was now apparent that a close relationship may exist between sitsirikine and isositsirikine and since it had been possible to degrade dihydrositsirikine to flavocoryline by a combination of Pd and quinone dehydrogenation reactions, a similar procedure was followed with dihydroisositsirikine.

Dihydroisositsirikine was converted to the amorphous hydrochloride and the salt, without further purification, was then heated with Pd-black at 280°. The resulting residue from this reaction was then taken up in acetic acid and treated with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone. From the reaction mixture was isolated a crystalline hydrochloride, m.p. 280–282°, which was identical in every respect (mixed m.p., superimposable IR and UV spectra, identical R_f values on TLC) with authentic flavocoryline hydrochloride (III).

From these results the gross structure IX (no stereochemistry implied at this point) could be assigned with some certainty to isositsirikine since the alternative structure with the positions of the ethylidene and β-hydroxy-ester functions interchanged was considered very unlikely on biogenetic grounds.



IX, R = =CHCH$_3$
X, R = CH$_2$CH$_3$



XI, R = CH$_2$CH$_3$

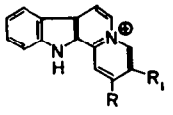
The stereochemistry of this molecule still remained for consideration. At first, the C-3 hydrogen atom was thought to have a β-orientation, since the IR spectrum of

isositsirikine did not display any Bohlmann bands in the C—H region.[15] However, dihydroisositsirikine exhibited strong absorptions at 3·56 and 3·62 μ, and thus presumably had the α-configuration at C-3.[16] This suggestion was substantiated when lead tetraacetate oxidized dihydroisositsirikine (X) to the tetradehydro compound, XI, which on subsequent reduction with $NaBH_4$ regenerated the starting material. Since this sequence is known to give the isomer with the C-3 hydrogen atom in the α-orientation,[20] it followed that dihydroisositsirikine, and hence isositsirikine, must have this configuration at C-3.

The α-orientation of the hydrogen atom at C-15 could be assumed on the basis of the constant stereochemistry which always prevails at this position[17] and therefore the structure postulated for isositsirikine was IX.

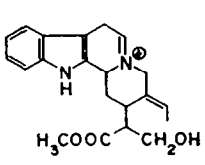
After the evidence indicated above for isositsirikine was already on hand, a mass spectrum (Fig. 2) of this alkaloid was obtained. In general, the mass spectrum provided valuable evidence about the structure and thus supplemented the chemical investigations.

The peaks at m/e 336 (M—H_2O) and 335 ((M-1)—H_2O) were considerably stronger than the parent ions at m/e 354 (M^+) and 353 (M-1)—a facile dehydration compatible with the presence of a labile proton at C-16. A series of ions at m/e 184, 170, 169 and 156 corresponded to a similar sequence displayed by dihydrositsirikine, but on the whole, the spectrum of isositsirikine differed markedly from the spectrum of dihydrositsirikine. In particular, a series of strong signals were obtained at m/e 275, 261, 247, 232 and 219 in the spectrum of the former but not in the instance of the latter alkaloid. These peaks could be plausibly attributed to various ions (XII) in which the entire tetracyclic structure of isositsirikine has been aromatized. For example, the ion in which $R = R_1 = CH_2CH_3$ possesses the mass 275, whereas the ion possessing R = H, $R_1 = CH_2CH_3$ would have mass 247 etc. It must be emphasized that this is merely speculation and we do not have any more direct evidence for

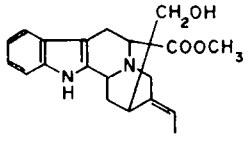


XII

the existence of these species. Since these ions are not produced in the fragmentation of dihydrositsirikine (or sitsirikine) it must be assumed that the presence of a double bond exo to the D-ring leads to its ready aromatization, and subsequently to that of the C-ring.



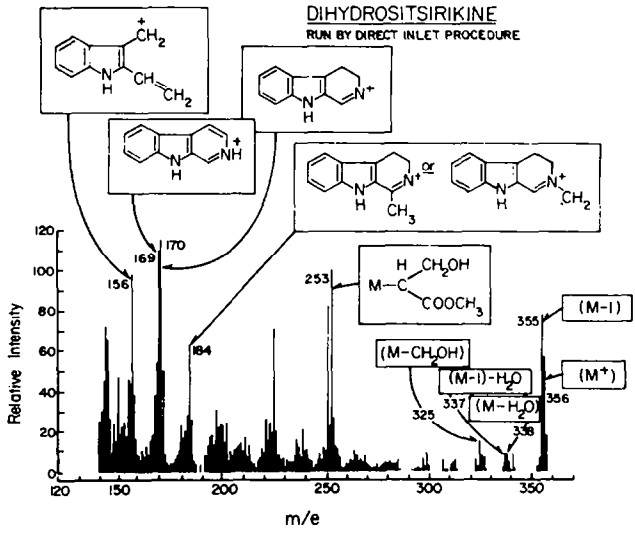
XIII

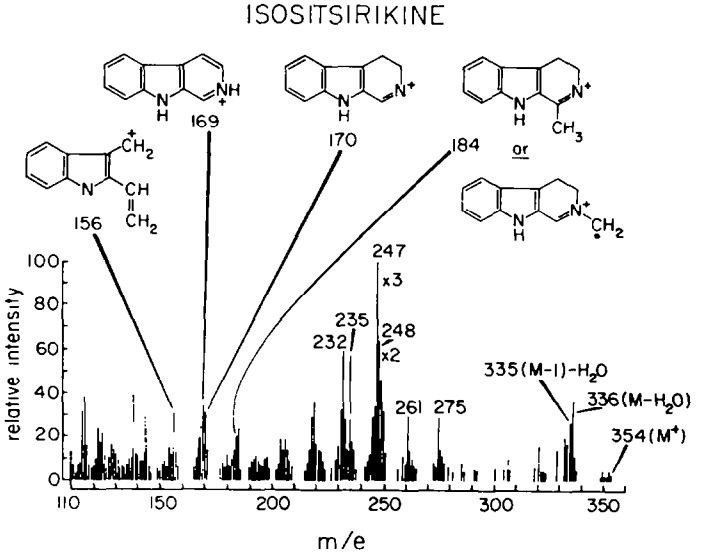


XIV

[20] F. L. Weisenborn and P. A. Diassi, *J. Amer. Chem. Soc.* **78**, 2022 (1956); F. E. Bader, D. F. Dickel, C. F. Huebner, R. A. Lucas and E. Schlittler, *Experientia* **10**, 298 (1954); E. Wenkert and D. P. Roychaudhuri, *J. Org. Chem.* **21**, 1315 (1956).

The above results conclude our evidence for the structures of sitsirikine and its two relatives. It is of interest to note that these alkaloids constitute a variation of the corynantheine series which may lie on one possible biogenetic pathway to pentacyclic alkaloids such as polyneuridine.[10] For example, the iminium derivative, XIII, easily





derivable from isositsirikine, can proceed via a transannular cyclization process to polyneuridine (XIV) or its C-16 epimer, akuammidine. The significance of such a reaction in Nature still remains an open question.

We wish to point out that the isolation of sitsirikine derivatives has now been reported by two other research groups. Spiteller and Spiteller-Friedman[21] have isolated several related alkaloids from *Aspidosperma oblongum* A.DC. These alkaloids

[21] G. Spiteller and M. Spiteller-Friedman, *Monatsh.* **94**, 779 (1963).

isolated in trace amounts by means of TLC, were assigned structures on the basis of mass spectral cracking patterns. On the basis of their results, these authors suggested structures corresponding to sitsirikine and/or isositsirikine for an alkaloid of mol. wt. 354. They actually preferred the isositsirikine structure for their alkaloid but unfortunately a comparison of the mass spectra of our alkaloid and its dihydro derivative with those obtained from Spiteller's compounds revealed distinct differences. A more detailed comparison could not be made since no further supply of alkaloid 354 was available from their investigations.

In an investigation on alkaloids from *Pausinystalia yohimbe* Pierre, some workers[22] have recently described the isolation of an alkaloid $C_{21}H_{28}O_3N_2$, for which these authors have independently derived a structure which corresponded to dihydrositsirikine. Indeed the IR spectrum of their alkaloid was superimposable on that of our compound and there is no question that they are identical.

EXPERIMENTAL

M.ps were determined on a Kofler block and are uncorrected. UV absorption curves were measured in MeOH solution on a Cary 14 Spectrometer, and IR spectra were taken on a Perkin-Elmer Model 21 Spectrophotometer. NMR spectra were recorded at 60 megacycles/sec. on a Varian A60 instrument; the line positions or centres of multiplets are given in the Tiers τ scale with reference to tetramethylsilane as the internal standard; the multiplicity, and integrated area and type of protons are indicated in parentheses. Silica gel G plates were used for TLC and were developed by ethyl acetate, ethyl acetate–$CHCl_3$ or ethyl acetate–EtOH mixtures as given below. The alumina used for column chromatography was Shawinigan reagent grade, deactivated with 3% of 10% aqueous acetic acid, unless otherwise stated. Analyses were performed by Dr. A. Bernhardt and his associates, Mulheim (Ruhr), Germany and by the Microanalytical Laboratory, University of British Columbia. Every mol. wt. quoted was determined mass spectrometrically.

Isolation of sitsirikine. The crude sulphate (1 g) provided by Dr. M. Gorman, Eli Lilly Research Laboratories, was dissolved in MeOH (20 ml) and water (250 ml), the solution cooled in ice and made basic with NH_4OH aq. The precipitate was taken up in ether (200 ml) the layers separated, and the aqueous portion further extracted with ether (3 × 100 ml). After drying over $MgSO_4$, the combined ethereal extracts were evaporated to give a powder, which showed three spots on TLC (EtOAc).

Several recrystallizations from acetone–pet. ether (b.p. 60–80°) afforded needles (320 mg) m.p. 178–179°, which now displayed only two spots on TLC. Repeated recrystallizations failed to resolve the mixture, which behaved as a pure compound by all criteria except TLC, and hence this was called sitsirikine. The purest samples of the alkaloid and its derivatives are described below.

Sitsirikine crystallized from acetone with one molecule of solvent as needles, m.p. 181°, $[\alpha]_D^{26}$ −52° (MeOH). (Found: C, 69·52; H, 7·84. Calc. for $C_{21}H_{26}O_3N_2.Me_2CO$: C, 69·88; H, 7·82%.)

The unsolvated material was obtained from aqueous MeOH as stout needles, m.p. 206–208°; $[\alpha]_D^{26}$ −58° (MeOH); λ_{max} (log ε): 226 (4·56), 282 (3·90), 290 (3·84) mμ; $\tilde{\nu}_{max}$ (Nujol): 2·98 (NH and/or OH), 5·86 (C=O)μ; NMR signals (CD_3COCD_3): 2·8 (multiplet, 4H, aromatic), 4·7 (multiplet, 1·8 H, olefinic), 6·1 (multiplet, 2 H, CH_2O), 6·38 (singlet, 3H, CH_3O), 9·02 (1H)τ. (Found: C, 71·15, 71·43; H, 7·51, 7·66; O, 14·00; N, 7·89, 7·77; C–Me, 1·61; mol. wt. 354. Calc. for $C_{21}H_{26}O_3N_2$: C, 71·16; H, 7·39; O, 13·54; N, 7·90; (1) C–Me, 4·24%; mol. wt. 354.

Sitsirikine picrate. A saturated alcoholic solution of picric acid (2 ml) was added to sitsirikine (50 mg) in EtOH (1 ml) and the mixture heated to boiling. The precipitate was recrystallized from MeOH to afford yellow hexagonal prisms (45 mg), m.p. 226–228° (dec). (Found: C, 55·55, 55·70; H, 5·46, 5·26; N, 12·10. Calc. for $C_{27}H_{29}O_{10}N_5$: C, 55·57; H, 5·01; N, 12·00%.)

Sitsirikine acetate. Sitsirikine (110 mg) was dissolved in pyridine–acetic anhydride (1:1; 2 ml) and left overnight. The solution was poured into ice-water (10 ml), basified with ammonia, and the precipitate taken up in ether. After washing several times with water, the ethereal solution was dried over $MgSO_4$ and evaporated. Recrystallization from aqueous MeOH afforded the acetate as needles

[22] Th H. van der Meulen and G. J. M. van der Kerk, *Rec. Trav. Chim.* **83**, 148, 154 (1964).

100 mg), m.p. 198°; $[\alpha]_D^{26}$ −26° (MeOH); two spots on TLC (EtOAc–$CHCl_3$, 1:1); $\tilde{\nu}_{max}$ (Nujol): 2·99 (NH), 5·76, 5·88 (C=O), 8·06 (OAc)μ; NMR signals (CD_3COCD_3): 2·7 (multiplet, 4H, aromatic), 4·7 (multiplet, 1·8H, olefinic), 5·6 (multiplet, 2H, CH_2OAc), 6·37 (singlet, 3H, CH_3O), 8·02 (singlet, 3H, CH_3C=O), 9·02 (1H)τ. (Found: C, 69·65; H, 7·32; N, 7·01. Calc. for $C_{22}H_{28}O_4N_2$: C, 69·67; H, 7·12; N, 7·07%.)

Dihydrositsirikine (VII). Sitsirikine (450 mg), in MeOH (10 ml), was hydrogenated over Pd–C (24 mg). The H_2 uptake ceased after 30 min when 0·65 mole had been absorbed. After removal of the catalyst and solvent, the product was recrystallized twice from acetone–pet. ether (b.p. 60–80°) to give dihydrositsirikine (405 mg), m.p. 177–179°. This compound displayed only *one* spot on TLC (EtOAc) which corresponded to one of the two spots shown by sitsirikine. Therefore the impurity which could not be removed from sitsirikine was in fact the dihydro compound. Dihydrositsirikine crystallized from acetone, with one molecule of solvent, as needles, m.p. 180°. (Found: C, 69·42; H, 7·91; N, 6·97. Calc. for $C_{21}H_{28}O_3N_2 \cdot Me_2CO$: C, 69·53; H, 8·27; N, 6·76%.)

Recrystallization from aqueous MeOH afforded the unsolvated alkaloid as prisms, m.p. 215°; $[\alpha]_D^{26}$ −55° (MeOH); λ_{max} (log ε): 226 (4·61), 282 (3·95), 290 (3·87) mμ; λ_{min} (log ε): 247 (3·40), 287·5 (3·85) mμ; $\tilde{\nu}_{max}$ ($CHCl_3$): 2·87 (NH and OH), 3·56 and 3·62 (Bohlmann bands)[16], 5·85 (C=O)μ; $\tilde{\nu}_{max}$ (Nujol): 2·94 (NH), 3·12 (OH), 5·85 (C=O)μ; NMR signals (CD_3COCD_3): 2·8 (multiplet, 4H, aromatic) 6·1 (multiplet, 2H, CH_2O), 6·42 (singlet, 3H, CH_3O), 9·07 (broad singlet, 3H, CH_3C)τ. (Found: C, 70·80; H, 7·78; O, 13·49; N, 7·77; O–Me, 9·01; C–Me, 3·95; mol. wt. 356. Calc. for $C_{21}H_{26}O_3N_2$: C, 70·76; H, 7·92; O, 13·47, N, 7·86; (1) O–Me, 8·72; (1) C–Me, 4·22%; mol. wt. 356.)

Dihydrositsirikine picrate. Dihydrositsirikine (60 mg) and a solution of picric acid were reacted in the manner described above and the derivative recrystallized from MeOH to yield amber prisms (55 mg), m.p. 228–230° (dec). (Found: C, 55·30, 55·46; H, 5·55, 5·71; N, 11·95. Calc. for $C_{27}H_{31}O_{10}N_5$: C, 55·38; H, 5·34; N, 11·96%.)

Dihydrositsirikine acetate. The acetate was prepared by treatment of dihydrositsirikine (200 mg) with acetic anhydride in pyridine as above. The product was recrystallized twice from acetone–pet. ether (b.p. 60–80°) to afford needles (155 mg), m.p. 187°; one spot on TLC (EtOAc–$CHCl_3$, 1:1); $[\alpha]_D^{26}$ −31° (MeOH); $\tilde{\nu}_{max}$ (Nujol): 2·95 (NH), 5·75, 5·86 (C=O), 8·0 (OAc)μ; NMR signals ($CDCl_3$): 1·34 (singlet, 1H, NH), 2·8 (multiplet, 4H, aromatic), 5·6 (multiplet, 2H, CH_2OAc), 6·38 (singlet, 3H, CH_3O), 7·96 (singlet, 3H, CH_3C=O), 9·02 (broad singlet, 3H, CH_3C)τ. (Found: C, 69·39, 68·98; H, 7·75, 7·59; O, 16·46; N, 6·85. Calc. for $C_{23}H_{30}O_4N_2$: C, 69·32; H, 7·69; O, 16·06; N, 7·03%.)

Dihydrositsirikine p-*bromobenzoate.* *p*-Bromobenzoyl chloride (130 mg) was added to dihydrositsirikine (65 mg) in dry pyridine (3 ml). After standing overnight the solution was poured into ice-water, made basic with aqueous ammonia and stirred for 10 min. The precipitate was taken up in ether, the ethereal solution washed twice with water and dried over $MgSO_4$. Removal of the solvent gave a gum which was dissolved in benzene and filtered through alumina (5 g). The benzene eluate was concentrated and pet. ether (b.p. 60–80°) added dropwise to the boiling solution until a permanent turbidity was obtained. The product was recrystallized from acetone–pet. ether (b.p. 60–80°) to yield the *p*-bromobenzoate as slender needles (60 mg), m.p. 174°; one spot on TLC; $\tilde{\nu}_{max}$ (Nujol): 2·97 (NH), 5·80, 5·86 (C=O)μ; NMR signals ($CDCl_3$): 2·5 (multiplet, 8H, aromatic), 5·4 (multiplet, 3H, CH_2O), 6·37 (singlet, 3H, CH_3O), 9·10 (broad singlet, 3H, CH_3C)τ. (Found: C, 62·34; H, 5·70; N, 5·06. Calc. for $C_{28}H_{31}O_4N_2Br$: C, 62·33; H, 5·79; N, 5·19%.)

Saponification of dihydrositsirikine. Dihydrositsirikine (200 mg) was heated under reflux with 2N methanolic NaOH (20 ml) for 2 hr. After removal of the solvent, the residue was taken up in water and extracted with CH_2Cl_2 to remove unsaponified material. The aqueous solution was acidified with HCl and evaporated to dryness. The residue was leached with absolute EtOH and the solution filtered from NaCl. Evaporation and recrystallization from aqueous alcohol gave an $\alpha\beta$-unsaturated acid hydrochloride, m.p. 260–263°; $\tilde{\nu}_{max}$ (Nujol): 5·90 (C=O), 6·17 (C=C)μ; NMR signals (CF_3CO_2H): 3·2 (multiplet, 4H, aromatic), 3·77 (singlet, 1H, olefinic) and 4·20 (singlet, 1H, olefinic)τ.

Dihydrositsirikine diol. A solution of dihydrositsirikine (250 mg) in tetrahydrofuran (10 ml) was run slowly into a stirred suspension of LAH (200 mg) in tetrahydrofuran (10 ml) and heated under reflux for 3 hr. After the mixture had stood overnight, the excess hydride was decomposed with saturated Na_2SO_4aq (10 ml), followed by water (20 ml). The aqueous suspension was then extracted with CH_2Cl_2 (4 × 25 ml), and the combined extracts dried over Na_2SO_4. Removal of the solvent and recrystallization from aqueous acetone gave the diol as needles (180 mg), m.p. 203°; one spot

on TLC (EtOAc–EtOH, 1:1); $[\alpha]_D^{25}$ −3° (MeOH); λ_{max} (log ε): 226 (4·53), 282 (3·85), 290 (3·78)mμ; $\bar{\nu}_{max}$ (Nujol): 3·11 (NH and OH)μ; NMR signals (CD_3COCD_3): 2·8 (multiplet, 4H, aromatic), 6·4 (multiplet, 4H, 2 CH_2O), 9·02 (broad singlet, 3H, CH_3C)τ. (Found: C, 73·30; H, 8·46; N, 8·41. Calc. for $C_{20}H_{28}O_2N_2$: C, 73·13; H, 8·59; N, 8·53%.)

Acetonide of dihydrositsirikine diol. The above diol (150 mg) was dissolved in dry acetone (20 ml) and *p*-toluenesulphonic acid (110 mg) added. After standing at room temp for 48 hr, the solution was neutralized with aqueous ammonia and the acetone removed under vacuum. The product was isolated with ether, the ethereal solution dried over $MgSO_4$, and evaporated to leave a gum. On trituration with a little anhydrous ether crystals formed, which were filtered off and found to be unreacted diol (95 mg).

The filtrate was evaporated, the residue taken up in benzene and passed through a column of alumina (3 g). Removal of the solvent gave the acetonide as an amorphous powder (30 mg) which crystallized from MeOH with one molecule of solvent, m.p. 105–109°; one spot on TLC (EtOAc–$CHCl_3$, 1:1); $\bar{\nu}_{max}$ (Nujol): 3·12 (NH)μ; NMR signals ($CDCl_3$): 1·61 (singlet, 1H, NH), 2·8 (multiplet, 4H, aromatic), 6·25 (doublet, 4H, 2 CH_2O), 8·62 (singlet, 6H, $(CH_3)_2CO_2$), 9·06 (broad singlet, 3H, CH_3C)τ. (Found (powder): C, 74·56; H, 9·03; N, 7·31. Calc. for $C_{23}H_{32}O_2N_2$: C, 74·96; H, 8·75; N, 7·60%. Found (solvate): C, 72·21; H, 8·71. Calc. for $C_{23}H_{32}O_2N_2$·MeOH: C, 71·96; H, 9·06%.)

Modified Kuhn-Roth oxidation[6] *of dihydrositsirikine.* Dihydrositsirikine (5 mg) and 10% chromic acid (2 ml) were put in a distillation apparatus, double-distilled water (2 ml) added, and distillation begun immediately. It was continued with periodic addition of water until 30 ml of idstillate had been collected. This was neutralized with 2N KOH (pH meter), and evaporated to dryness. The residue was taken up in pure water (0·3 ml) and put on a small column of Dowex 50 acid resin (2 × 0·5 cm); the flask was washed with water (2 × 0·5 ml) and this also added to the column. To the filtrate was added a few drops of 70% aqueous ethylamine, and it was then concentrated under vacuum at 30–40° down to 1–2 drops. This was spotted on Whatman No. 1 paper together with standard solutions of the ethylamine salts of acetic, propionic and butyric acids.

The paper was developed by descending chromatography, using a 0·025M ethylamine solution in water-saturated n-butanol as the stationary phase, and water-saturated n-butanol as the mobile phase.[8] After 24 hr the paper was sprayed with alcoholic bromocresol green solution, when the acids became visible as blue spots on a yellow background.

Dihydrositsirikine gave acetic and propionic acids, and a blank oxidation showed only the barest trace of acetic acid.

Sitsirikine under these conditions also gave acetic and propionic acids, while isositsirikine gave acetic acid only.

Ozonization of sitsirikine and isositsirikine. Sitsirikine (5 mg) in glacial acetic acid (1 ml) was ozonized for 5 min, then transferred to a distillation apparatus containing 5% $FeSO_4$aq (15 ml). After 30 min the mixture was steam distilled into an aqueous solution of 2,4-dinitrophenylhydrazine sulphate until about 10 ml of water had passed over. The solution was extracted several times with benzene, the combined extracts dried with $MgSO_4$ and flushed through a column of Fisher acid-washed alumina (10 g). After concentration of the benzene solution to about 0·5 ml, a few drops were spotted on Whatman No. 1 paper, together with standard solutions (5 mg/ml) of the 2,4-dinitrophenylhydrazones of formaldehyde, acetaldehyde and acetone.

The paper was developed by descending chromatography,[7] using MeOH–heptane as the stationary phase and heptane as the mobile phase. After 6 hr the paper was sprayed with 10% NaOHaq. The 2,4-dinitrophenylhydrazone of formaldehyde (red-brown spot, R_f 0·10) was clearly indicated, and a trace of acetone (dark-brown spot, R_f 0·30) was also present. Blank experiments gave no spots corresponding for formaldehyde or acetaldehyde, but always showed a trace of acetone.

Repitition of the same procedure for isositsirikine (5 mg) showed formation of acetaldehyde 2,4-dinitrophenylhydrazone (R_f 0·19).

Lead tetracetate dehydrogenation of dihydrositsirikine. Lead tetracetate (400 mg) was added in small portions over a period of 10 min to a solution of dihydrositsirikine (100 mg) in glacial acetic acid (10 ml). The mixture was kept at 50–60° for a further 20 min, then poured into ice-cold 50% NaOHaq and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with a little water, dried over Na_2SO_4, and acidified to Congo red with 8N ethanolic HCl. Evaporation of the solvent afforded

tetradehydrodihydrositsirikine hydrochloride as a gum (70 mg), which could not be induced to crystallize; λ_{max} (acid and neutral solution): 253, 308, 365 mμ; λ_{max} (alkaline solution): 284, 328 mμ; $\bar{\nu}_{max}$ ($CHCl_3$): 5·88 (C=O), 6·13 (aromatic)μ.

Palladium-charcoal dehydrogenation of dihydrositsirikine. Dihydrositsirikine (50 mg) was well mixed with 10% Pd–C (250 mg) and heated under N_2 at 250° for 15 min. The residue was extracted with hot MeOH and the UV spectrum run; λ_{max}: 230, 290, 310, 385 mμ.

After removal of the MeOH, the product was taken up in ether–water, the ether layer separated and dried over Na_2SO_4. Removal of the solvent gave a gum (30 mg); λ_{max}: 230, 288, 317 mμ.

The aqueous portion was made strongly alkaline (pH > 10) and extracted with $CHCl_3$. After drying, the $CHCl_3$ solution was evaporated to afford a gum (5 mg); λ_{max}: 295, 313, 348, 389 mμ.

The neutral extract was dissolved in a few drops of MeOH and spotted on a preparative TLC plate (silica gel, 0·5 mm thick). The plate was developed in $CHCl_3$–ethyl acetate (3:1) for 45 min, dried, and then developed two more times. Under UV light four bands could be seen, and each was cut out and extracted with MeOH in a Soxhlet apparatus for several hr. The extract from the main band (compound A) displayed UV spectra similar to those of harman; λ_{max} (neutral and alkaline solution): 234, 250, 282, 288, 337, 349 mμ; λ_{max} (acid solution): 254, 303, 372 mμ. Evaporation of the MeOH yielded a gum (10 mg); $\bar{\nu}_{max}$ ($CHCl_3$): 5·85 (C=O), 6·15 (C=C)μ.

Palladium-charcoal dehydrogenation of dihydrositsirikine hydrobromide. The hydrobromide (50 mg) was well mixed with 10% Pd–C (200 mg) and heated under N_2 at 280° for 15 min. The residue was extracted with hot MeOH, filtered, and the UV spectra run; λ_{max} neutral and acidic solution): 221, 250, 308, 366, 380 mμ; λ_{max} (alkaline solution): 282, 310, 382 mμ.

The solvent was removed and the residue dissolved in water, the solution made basic (pH 8) with ammonia and shaken with ether. After separation and drying the ether was removed to leave a gum (5 mg); λ_{max}: 290, 355 mμ.

50% NaOHaq was added to the aqueous portion until it was strongly alkaline (pH > 10), and the solution was then extracted with $CHCl_3$. The red $CHCl_3$–extract was washed with water, dried and acidified with 8N ethanolic HCl (yellow solution). Evaporation of the solvent afforded a gum (22 mg); λ_{max} (acid and neutral solution): 222, 308, 383 mμ; λ_{max} (alkaline solution): 283, 315, 380 mμ.

This material was dissolved in a little MeOH and spotted on a preparative TLC plate (silica gel, 0·5 mm thick). This plate was run for 20 min in ethyl acetate, and then twice in EtOH–ethyl acetate (1:1) for 40 min.

Under UV light a separation into two main bands was observed. These were cut out and extracted with MeOH in a Soxhlet apparatus for several hr.

One fraction gave a UV spectrum analogous to that of tetradehydrodihydrositsirikine hydrochloride; λ_{max}: 251, 307, 365 mμ. The other fraction (compound B) gave a UV spectrum similar to that of 5,6-dihydroflavocoryline hydrochloride;[13] λ_{max}: 221, 312, 386 mμ; λ_{min}: 215, 277, 338 mμ.

Quinone dehydrogenation of compound B. The methanolic solution of compound B was evaporated to give a gum (2 mg), which was dissolved in glacial acetic acid (0·5 ml). 2,3-Dichloro-5,6-dicyano-*p*-benzoquinone (10 mg) was added, and the mixture heated at 80–90° for 6 hr. The solution was then diluted with water and extracted several times with ether. After making strongly alkaline with 50% NaOHaq, the aqueous solution was shaken with $CHCl_3$, the organic layer separated, washed with a little water and dried over Na_2SO_4. Acidification with 8N ethanolic HCl and removal of the solvent afforded a yellow gum (0·8 mg). This (compound C) displayed a UV spectrum very similar to that of flavocoryline hydrochloride;[13] λ_{max}: 237, 291, 345, 385 mμ; λ_{min}: 274, 304, 373 mμ.

The compound displayed the same R_f value (0·43) as an authentic sample of flavocoryline on paper chromatography using an ethyl acetate–pyridine–water (8:2:1) system.

Dihydrositsirikine olefinic ester. Dihydrositsirikine acetate (1·3 g) was heated under reflux with 0·1N NaOMe in dry MeOH (60 ml) for 45 min. Solid CO_2 was then added, the MeOH removed under vacuum, and the residue taken up in ether–water. The ethereal solution was dried and the solvent removed. Chromatography of the product on alumina (50 g) afforded the desired material (410 mg) on elution with benzene–ether (19:1). Ether eluted dihydrositsirikine (605 mg).

Recrystallization from MeOH afforded the solvated olefinic ester as needles (290 mg), m.p. 84–89°; one spot on TLC (EtOAc–$CHCl_3$, 1:1); $[\alpha]_D^{26}$ +2° (MeOH); $\bar{\nu}_{max}$ (Nujol): 3·16 (NH), 5·85 (C=O), 6·17 (C=C)μ; NMR signals ($CDCl_3$): 1·60 (singlet, 1H, NH), 2·8 (multiplet, 4H, aromatic), 3·73 (singlet, 1H, olefinic), 4·41 (singlet, 1H, olefinic), 6·23 (singlet, 3H, CH_3O), 9·06 (broad singlet,

3H, CH_3C)τ. (Found: C, 71·41; H, 8·08; O, 12·71; N, 7·12. Calc. for $C_{21}H_{26}O_3N_2$·MeOH: C, 71·32; H, 8·16; O, 12·96; N, 7·56%.)

Lithium aluminum hydride reduction of dihydrositsirikine olefinic ester. The olefinic ester (150 mg) and LAH (100 mg) in ether–tetrahydrofuran (1:1, 30 ml) were heated under reflux for 2 hr. Excess hydride was decomposed with Na_2SO_4aq (20 ml), the organic layer separated, and the aqueous suspension extracted with CH_2Cl_2 (3 × 10 ml). The combined organic extracts were dried over $MgSO_4$ and evaporated to leave a crystalline solid (140 mg). This material had no carbonyl absorption in the IR region, but showed three spots on TLC. The NMR spectrum indicated that the mixture contained only 25% of the expected terminal olefinic alcohol.

The product was taken up in benzene and chromatographed on alumina (5 g, deactivated with 0·3% of glacial acetic acid). Ethyl acetate eluted the major fraction (70 mg), which was recrystallized twice from acetone–pet. ether (b.p. 60–80°) and once from aqueous MeOH to afford light brown needles (23 mg), m.p. 204°; one spot on TLC (EtOAc); $[\alpha]_D^{26}$ −24·3° (MeOH); $\bar{\nu}_{max}$ (Nujol): 3·12μ (NH and OH); NMR signals ($CDCl_3$): 1·25 (singlet, 1H, NH), 2·8 (multiplet, 4H, aromatic)τ, no olefinic protons. (Found: C, 77·39; H, 8·49; N, 8·79. Calc. for $C_{20}H_{24}ON_2$: C, 77·38; H, 8·44; N, 9·03%.)

The constants quoted in the literature[18] for isodesmethoxydihydrocorynantheine alcohol are m.p. 204°, $[\alpha]_D^{17}$ −24·0° (MeOH).

Desmethyldihydrocorynantheine. A solution of dihydrocorynantheine (500 mg) in acetone (50 ml) was cooled in ice and dry HCl passed in for 15 min. After standing at 5° for 15 hr, the solution was evaporated under vacuum to small bulk, diluted with water (100 ml) and extracted with $CHCl_3$ (10 × 50 ml). The water layer was then made basic with aqueous ammonia, extracted with ether (3 × 50 ml) and the combined ether extracts dried over $MgSO_4$. Removal of the ether afforded desmethyldihydrocorynantheine as an amorphous powder (240 mg), which gave a positive $FeCl_3$ test (purple); $\bar{\nu}_{max}$ ($CHCl_3$): 2·87 (NH) 5·83 (C=O), 6·03 (HO—C=C—C=O), 6·22 (C=C)μ; NMR signals (CD_3COCD_3): 1·01 (singlet, 1H, NH), 2·8 (multiplet, 4H, aromatic), 6·28 and 6·52 (2 singlets, 3H, CH_3O), 9·1 (broad singlet, 3H, CH_3C)τ. (Found: C, 71·22; H, 7·91; N, 8·21. Calc. for $C_{21}H_{26}O_3N_2$: C, 71·16, H, 7·39; N, 7·90%.)

Synthesis of dihydrositsirikine. Desmethyldihydrocorynantheine (200 mg), in MeOH (10 ml), was reduced with $NaBH_4$ (500 mg). After 1 hr the MeOH was removed under vacuum, the residue treated with water and then extracted with ether. The ethereal layer was separated, dried over $MgSO_4$ and the solvent evaporated. The resulting material was dissolved in benzene and chromatographed on alumina (10 g). Elution with ether yielded a solid which was recrystallized twice from pet. ether (b.p. 60–80°) to afford stout needles (65 mg), m.p. 215°; one spot on TLC (EtOAc); $[\alpha]_D^{26}$ −56° (MeOH); $\bar{\nu}_{max}$ (Nujol): 2·94 (NH), 3·12 (OH) 5·85 (C=O)μ. (Found: C, 70·81; H, 7·94; N, 7·96. Calc. for $C_{21}H_{28}O_3N_2$: C, 70·76; H, 7·92; N, 7·86%.)

This compound was identical with dihydrositsirikine by all criteria: m.p. and mixed m.p.; optical rotation; R_f value on TLC; superimposable IR spectra.

Isositsirikine (IX). Isositsirikine was obtained from Dr. M. Gorman, Eli Lilly Laboratories as the crystalline sulphate, m.p. 263·5°. (Found: C, 62·70, H, 6·69; O, 20·14; N, 6·93; S, 3·97; C–Me, 2·95; O–Me, 7·65. Calc. for $C_{21}H_{26}O_3N_2 \cdot \frac{1}{2}H_2SO_4$: C, 62·52; H, 6·74; O, 19·84; N, 7·00; S, 3·97; (1) C–Me, 3·72; (1) O–Me, 7·69%.)

The free base was an amorphous powder, $[\alpha]_D^{25}$ −20° ($CHCl_3$); one spot on TLC (EtOAc); λ_{max} (log ε): 224 (4·55), 283 (3·92), 291 (3·84) mμ; $\bar{\nu}_{max}$ ($CHCl_3$): 2·94 (NH and OH), 5·80 (C=O)μ; no Bohlmann bands[15]; $\bar{\nu}_{max}$ (Nujol): 3·03 (NH and OH), 5·81 (C=O)μ; NMR signals ($CDCl_3$): 1·33 (singlet, 1H, NH), 2·7 (multiplet, 4H, aromatic), 4·53 (quartet, J = 7 c/s, 1H, C=CH—CH_3), 6·28 (singlet, 3H, CH_3O), 8·40 (doublet, J = 7c/s, 3H, CH_3—CH=C)τ. (Found: C, 70·65; H, 7·75; O, 14·15; N, 7·53; C–Me, 3·28; O–Me, 9·17; mol. wt. 354. Calc. for $C_{21}H_{26}O_3N_2$: C, 71·16; H, 7·39; O, 13·54; N, 7·90; (1) C–Me, 4·24; (1) O–Me, 8·76%; mol. wt. 354.)

Isositsirikine picrate was prepared in the manner described for sitsirikine and recrystallized from MeOH as yellow needles, m.p. 216°. (Found: C, 55·60, 55·69; H, 5·20, 5·38; O, 27·29; N, 12·17, 11·92. Calc. for $C_{27}H_{29}O_{10}N_5$: C, 55·57; H, 5·01; O, 27·42; N, 12·00%.)

Acetylation of isositsirikine with acetic anhydride in pyridine gave an amorphous monoacetate which was homogeneous by TLC (EtOAc–$CHCl_3$, 1:1); $\bar{\nu}_{max}$ (CCl_4): 2·96 (NH), 5·78 (C=O), 8·10 (OAc)μ; NMR signals ($CDCl_3$): 1·40 (singlet, 1H, NH), 2·7 (multiplet, 4H, aromatic), 4·39 (quartet, 1H, C=CH—CH_3), 6·05 (doublet, 2H, CH—CH_2OAc) 6·27 (singlet, 3H, CH_3O), 8·15 (singlet, 3H,

$CH_3C{=}O$), 8·36 (doublet, 3H, $\underline{CH_3}{-}CH{=}C)\tau$. (Found: C, 69·84; H, 7·78; N, 7·44. Calc. for $C_{22}H_{28}O_4N_2$; C, 69·67, H, 7·12; N, 7·07%.)

Acetonide of isositsirikine diol A solution of isositsirikine (300 mg) in tetrahydrofuran (10 ml) was run into a suspension of LAH (300 mg) in ether (5 ml), and the mixture heated under reflux for 3h Sat. Na_2SO_4aq (10 ml) was added with stirring and the organic layer separated. After dilution with water (20 ml) the aqueous layer was extracted with CH_2Cl_2 (3 × 20 ml), and the combined organic extracts were dried over Na_2SO_4. Evaporation of the solution afforded a gum (240 mg); $\bar{\nu}_{max}$: 3·12 (OH and NH); no carbonyl absorption; NMR signals (CD_3COCD_3): 2·8 (multiplet, 4H, aromatic), 4·53 (quartet, 1H, $C{=}\underline{CH}{-}CH_3$), 8·37 (doublet, 3H, $\underline{CH_3}{-}CH{=}C)\tau$, no methoxyl absorption.

Since it could not be induced to crystallize, the gum was taken up in dry acetone (10 ml), *p*-toluenesulphonic acid (150 mg) added, and the mixture heated under reflux for 30 min. After standing overnight the solution was made basic with aqueous ammonia, and the acetone removed under vacuum. The residue was extracted with ether, the ethereal solution dried over Na_2SO_4 and evaporated to leave a gum. This was taken up in benzene and filtered through alumina (5 g). Removal of the benzene and recrystallization from MeOH afforded isositsirikine acetonide as needles (42 mg), m.p. 105–109°; one spot on TLC (EtOAc); $[\alpha]_D^{26}$ −53° ($CHCl_3$); $\bar{\nu}_{max}$ (Nujol): 3·12 (NH)μ; NMR signals ($CDCl_3$): 1·87 (singlet, 1H, NH), 2·8 (multiplet, 4H, aromatic), 4·40 (quartet, 1H, $C{=}\underline{CH}{-}CH_3$), 8·30 (doublet, 3H, $CH_3{-}CH{=}C$), 8·63 (singlet, 3H, $(CH_3)_2CO_2$), 8·68 (singlet, 3H, $(\underline{CH_3})_2CO_2)\tau$. (Found: C, 72·10, 72·19; H, 8·39; 8·46; N, 7·20. Calc. for $C_{23}H_{30}O_3N_2{\cdot}MeOH$: C, 72·33, H, 8·60; N, 7·03%.)

Dihydroisositsirikine (X). Isositsirikine base (200 mg), in MeOH (5 ml), was hydrogenated over Pd–C (20 mg). Uptake of hydrogen ceased after 5 hr, when 1·02 mole had been absorbed. Removal of the catalyst and the solvent yielded an amorphous product, which showed two spots on TLC (EtOAc). The major component (120 mg) was isolated from the ether–benzene (1:3) eluate during chromatography on alumina (10 g).

Dihydroisositsirikine was an amorphous powder which could not be induced to crystallize, but was homogeneous on TLC; λ_{max} (log ε): 226, (4·57), 284 (3·92), 291 (3·84) mμ; $\bar{\nu}_{max}$ ($CHCl_3$): 2·87 (NH and OH), 3·56 and 3·62 (Bohlmann bands)[16] 5·81 ($C{=}O)\mu$; NMR signals ($CDCl_3$): 2·01 (singlet, 1H, NH), 2·8 (multiplet, 4H, aromatic), 6·20 (singlet, 3H, CH_3O), 9·03 (broad singlet, 3H, $CH_3C)\tau$. (Found: C, 70·30; H, 7·53; N, 8·13. Calc. for $C_{21}H_{28}O_3N_2$: C, 70·76; H, 7·92; N, 7·86%.)

Dihydroisositsirikine picrate was formed in the usual manner and recrystallized from aqueous MeOH as yellow platelets, m.p. 187°. (Found: C, 54·65, 54·30; H, 5·68, 4·84; O, 27·57; N, 11·89. Calc. for $C_{27}H_{31}O_{10}N_5{\cdot}\frac{1}{2}H_2O$: C, 54·56; H, 5·43; O, 28·23; N, 11·79%.)

Lead tetracetate oxidation of dihydroisositsirikine. The dihydro compound (100 mg) was dissolved in acetic acid (10 ml), lead tetracetate (400 mg) added in small portions, and the mixture heated at ca. 60° for 2 hr. After removal of the solvent the residue was taken up in water (20 ml), made strongly alkaline with 50% KOHaq and extracted with $CHCl_3$ (3 × 20 ml).

The combined extracts were dried over Na_2SO_4, acidified with 8N ethanolic HCl and evaporated to dryness. Tetradehydrodihydroisositsirikine (XI) hydrochloride was thus obtained as a red glass (70 mg); λ_{max}: 253, 308, 360 mμ; $\bar{\nu}_{max}$ ($CHCl_3$, free base): 5·78 ($C{=}O$), 6·19 μ: TLC (EtOAc) showed no starting material.

Sodium borohydride reduction of tetradehydrodihydroisositsirikine. The above hydrochloride (60 mg), in MeOH (5 ml), was treated with $NaBH_4$ (200 mg), and heated under reflux for 1 hr. The solvent was removed under vacuum, the residue taken up in water (10 ml), and extracted with ether (4 × 10 ml). After drying, the ethereal solution was evaporated, the product taken up in benzene, and chromatographed on alumina (3 g). Benzene–ether (3:1) eluted a compound (21 mg) which was found to be identical to dihydroisositsirikine (UV and IR spectra, TLC).

Palladium dehydrogenation of dihydroisositsirikine hydrochloride. Dihydroisositsirikine (200 mg) was converted to the amorphous hydrochloride salt, which was then intimately mixed with Pd–C (200 mg) and heated under a N_2 atmosphere at ca. 280° for 10 min. The residue was taken up in hot glacial acetic acid (5 ml), the solution filtered and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (200 mg) added. The mixture was then heated at 90–95° for 5 hr. After removal of the solvent under vacuum, dilute aqueous ammonia (20 ml) was added (pH 8), and the solution extracted several times with ether to remove weak bases. The aqueous solution was then made strongly alkaline (pH > 10)

with 50% KOHaq and extracted with $CHCl_3$ (3 × 20 ml). When the $CHCl_3$-extract had been dried over K_2CO_3, it was acidified with 8N ethanolic HCl and evaporated to yield a yellow gum (40 mg), which displayed a flavocoryline-type UV spectrum. Paper chromatography (as above) indicated that it was a mixture of two compounds—the major constituent having the same R_f value as flavocoryline. Three recrystallizations from $CHCl_3$ afforded the major component as yellow needles (6 mg), m.p. 280–282° (block preheated to 150°); λ_{max} (log ε): 238 (4·54), 247 (4·50), 290 (4·13), 345 (4·27), 384 (4·23) mμ; λ_{min} (log ε): 210 (4·26), 244 (4·49), 273 (4·04), 303 (4·01), 373 (4·15) mμ; $\tilde{\nu}_{max}$ (Nujol): 2·98 (NH), 6·06, 6·13, 6·60 μ; one spot on paper chromatography.

The compound was found to be identical with authentic flavocoryline hydrochloride in all respects: m.p. and mixed m.p.; superimposable IR and UV spectra; same R_f values on paper chromatography.