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ALKALOID STUDIES—L[1]

THE ALKALOIDS OF TWELVE *ASPIDOSPERMA* SPECIES

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Abstract—The results of a survey of Brazilian *Aspidosperma* species are reported and the structures of seven new alkaloids described. Of these, two have the carbon-nitrogen skeleton of aspidospermatidine (LV) and four that of aspidofractinine (LXV). The last is olivacine-N-oxide (XXII). In addition the chemistry of compactinervine, (VI) an alkaloid with the strychane skeleton, is presented.

THE alkaloids of the genus *Aspidosperma* (Apocynaceae) have been extensively studied and an ill-defined but distinct relation between the chemical constituents and botanical classification of the species has been found.[3-5] The examination of minor constituents, however, has revealed the presence of many different types in a single species[6,7] and this would probably prove true of many other members of the genus under more careful scrutiny. Any chemical classification of *Aspidosperma* would therefore have to be made on the basis of the occurrence of a significant quantity of a characteristic type. In pursuance of further data on which to base such a classification we have studied a number of species, whose alkaloids are listed in Table 1.

[1] Part XLIX, A. Walser and C. Djerassi, *Helv. Chim. Acta* **47**, 2072 (1964).

[2] Mr. A. P. Duarte of the Rio de Janeiro Botanical Garden, was responsible for the collection and identification of the majority of the species studied. Botanical data relating to the non-Amazonian species will be published separately, when a general correlation between the chemistry and taxonomy of the genus will be presented.

[3] J. Schmutz, *Pharm. Acta Helv.* **36**, 103 (1961).

[4] N. Dastoor and H. Schmid, *Experientia* **19**, 297 (1963).

[5] J. M. Ferreira, B. Gilbert, R. J. Owellen and C. Djerassi, *Experientia* **19**, 585 (1963).

[6] K. Biemann, M. Spiteller-Friedmann and G. Spiteller, *J. Amer. Chem. Soc.* **85**, 631 (1963); cf. *idem*, *Tetrahedron Letters* 485 (1961).

[7] M. Gorman, A. L. Burlingame and K. Biemann, *Tetrahedron Letters* 39 (1963).

TABLE 1. SPECIES EXAMINED AND ALKALOIDS ISOLATED

Species	Alkaloids (Ref. to previous isolation from *Aspidosperma*)
A. auriculatum Mgf[a]	Dihydrocorynantheol (I)[8,9] Reserpinine
A. oblongum A.DC.[a,b]	19,20-Dehydro-10-methoxydihydro-corynantheol (II)[4,9]
A. species No. RJB 119070[a,c]	10-Methoxydihydrocorynantheol (III)[4,9] Aspidocarpine (IV)[13] N-Acetylaspidospermidine (V)[5] (Demethoxyaspidospermine)
A. compactinervium Kuhlmann[10,d]	Compactinervine (VI)[10] N-Acetyl-11-hydroxyaspidospermatidine (VII)
A. populifolium A.DC.[e]	11-Methoxy-14,19-dihydrocondylocarpine (VIII) 17-Methoxyaspidofractinine (IX) N-Formyl-17-methoxyaspidofractinine (X) 16,17-Dimethoxyaspidofractinine (XI) N-Formyl-16,17-dimethoxyaspidofractinine (XII) Kopsinine (XIII) Aspidofractine (XIV)[14] Refractine (XV)[14] Two alkaloids of unknown structure
A. olivaceum Müll.-Arg.[f]	Uleine (XVI)[15] (−)-Apparicine (XVII)[16] Olivacine (XVIII)[15b,d,e,17a,b 18] Aspidocarpine (IV)[13]

[8] B. Gilbert, L. D. Antonaccio, and C. Djerassi, *J. Org. Chem.* **27**, 4702 (1962).
[9] G. Spiteller and M. Friedmann-Spiteller, *Monatsh. Chem.* **94**, 779 (1963), *ibid.* **93**, 795 (1962).
[10] C. Djerassi, Y. Nakagawa, J. M. Wilson, H. Budzikiewicz, B. Gilbert, and L. D. Antonaccio, *Experientia* **19**, 467 (1963).
[11] R. E. Woodson, *Ann. Missouri Bot. Gard.* **38**, 119 (1951).
[13a] S. McLean, K. Palmer, and L. Marion, *Canad. J. Chem.* **38**, 1547 (1960);
[b] M. Pinar and H. Schmid, *Helv. Chim. Acta* **45**, 1283 (1962);
[c] J. Aguayo Brissolese, C. Djerassi and B. Gilbert, *Chem. & Ind.* 1949 (1962);
[d] C. Ferrari, S. McLean, L. Marion, and K. Palmer, *Canad. J. Chem.* **41**, 1531 (1963).
[e] K. S. Brown Jr. and C. Djerassi, *J. Amer. Chem. Soc.* **86**, 2451 (1964).
[14a] C. Djerassi, A. A. P. G. Archer, T. George, B. Gilbert, and L. D. Antonaccio, *Tetrahedron* **16**, 212 (1961);
[b] C. Djerassi, R. J. Owellen, J. M. Ferreira, and L. D. Antonaccio, *Experientia* **18**, 397 (1962).
[14] C. Djerassi, T. George, N. Finch, H. F. Lodish, H. Budzikiewicz and B. Gilbert, *J. Amer. Chem. Soc.* **84**, 1499 (1962).
[15a] J. Schmutz, F. Hunzicker, and R. Hirt, *Helv. Chim. Acta* **40**, 1198 (1957);
[b] J. Schmutz and F. Hunzicker, *Pharm. Acta Helv.* **33**, 344 (1958);
[c] B. Gilbert, L. D. Antonaccio, A. A. P. G. Archer, and C. Djerassi, *Experientia* **16**, 61 (1960);
[d] M. A. Ondetti and V. Deulofeu, *Tetrahedron Letters* No. 7, 1 (1959);
[e] M. A. Ondetti and V. Deulofeu, *Tetrahedron* **15**, 160 (1961);
[f] M. Ohashi, J. A. Joule, B. Gilbert, and C. Djerassi, *Experientia* **20**, 363 (1964).
[16] J. A. Joule, H. Monteiro, L. J. Durham, B. Gilbert and C. Djerassi, *J. Chem. Soc.* 1965, in press.
[17a] G. B. Marini-Bettolo, *Ann. Chim., Rome* **49**, 869 (1959);
[b] P. Carvalho-Ferreira, G. B. Marini-Bettolo, and J. Schmutz, *Experientia* **15**, 179 (1959);
[c] J. Schmutz and F. Hunzicker, *Helv. Chim. Acta* **41**, 288 (1958).
[18] G. Buchi, D. W. Mayo, and F. A. Hochstein, *Tetrahedron* **15**, 167 (1961).

TABLE 1 (*cont'd*)

A. gomezianum A.DC.[g]	Uleine (XVI) Apparicine (XVII) N-Acetylaspidospermidine (V)[5]
A. eburneum Fr. Allem.[h]	Uleine (XVI) (−)-Apparicine (XVII) Olivacine (XVIII) N-Acetylaspidospermidine (V) Demethylaspidospermine (XIX)[5]
A. subincanum Mart[i].	Uleine (XVI) Olivacine (XVIII)
A. multiflorum A.DC.[j]	Uleine (XVI) (−)-Apparicine (XVII) Dasycarpidone (XX)[15f] Kopsinine (XIII) One Alkaloid of unknown structure
A. nigricans Handro[k]	Olivacine (XVIII) (+)-Guatambuine (XXI)[15d,e,f; 17] Olivacine-N-oxide (XXII)
A. spruceanum Benth.[l]	Aspidolrabine (XXIII)[21,15d] N-Acetyl-N-depropionylaspidoalbine (XXIV)[21,15d] Two alkaloids of unknown structure.

[19] 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).

[20] C. F. P. de Martius, *Flora Brasiliensis* vi.I t 16 (1860).

[21] C. Djerassi, L. D. Antonaccio, H. Budzikiewicz, J. M. Wilson, and B. Gilbert, *Tetrahedron Letters* 1001 (1962).

Footnotes to Table 1

[a] *A. auriculatum* was collected by Mr. Nilo Silva in the reserve of the Instituto Agronômico do Norte, Belém, Pará. This species is a typical member of a large sub-group with deeply indented trunks, often known as "carapanaúba" (mosquito tree, since mosquitoes breed in the water-filled indentations) in the Amazon region where they are commonest. Another member is *A. oblongum*, while two species of similar superficial appearance occurring outside this region are *A. discolor*[4,5] and *A. compactinervium*.[10] All these are classified in the Section *Nitida* by Woodson[11] and the species No. RJB 119070 may be safely assigned to this sub-group which is generally characterized by the presence of alkaloids of the dihydrocorynantheol and tetrahydroalstonine types. *A. auriculatum* is registered in the Rio de Janeiro Botanical Garden under No. 116841.

[b] *A. oblongum* was collected near the Manaus-Itacoatiara highway, km. 31, Amazonas, growing on firm clay soil. Registered under Herbarium numbers: Instituto Nacional de Pesquisas da Amazônia No. 8125 (collector William Rodrigues); Rio de Janeiro Botanical Garden No. 119071.

[c] Collected near the old road to the Ducke Reserve, km. 9, beside Igarapé do Passarinho, growing in firm humid sandy soil, and registered under Herbarium numbers: Instituto Nacional de Pesquisas da Amazônia, Manaus, No. 7614; Rio de Janeiro Botanical Garden, No. 119070.

[d] Collected in the Tijuca forest, Rio de Janerio. This is apparently the species named *A. eburneum* by Woodson.[11] *A. compactinervium* is registered in the Rio de Janeiro Botanical Garden under No. 116926.

[e] *A. populifolium* was collected on the lower slopes of the Serra do Carbral on the eastern side of the Varzea da Palma Valley, Fazenda da Mãe d'Água, Minas Gerais. In this valley were also collected *A. dasycarpon. A. tomentosum, A. cuspa*, and *A. subincanum* while on the top of the serra was encountered in addition to some of these species, *A. macrocarpon*. These species are employed in manufacture of charcoal for iron smelting and we are very grateful to Dr. José Vicente Gonçalves

Pinto and the Belgo-Mineira Iron Company for assistance in their collection. *A. populifolium* was given as synonymous to *A. pyrifolium* by Woodson,[11] but the species are quite distinct in the field although their alkaloids show a definite relation.[18] *A. populifolium* is registered in the Rio de Janeiro Botanical Garden under No. 113341. Similarly *A. dasycarpon* was cited[11] as a synonym of *A. tomentosum*, and preliminary studies on the latter show that its alkaloids are indeed related to those of the former. However the two species often found side by side are distinct in the field.

[f] *A. olivaceum* for this study was collected in its natural habitat in the Serra da Mantiqueira near Hermilo Alves, Minas Gerais, and registered in the Rio de Janeiro Botanical Garden under No. 114452. Material previously studied (Ref. 15c) was collected from trees planted in the Tijuca forest, Rio de Janeiro.

[g] Collected on the dry slopes of the Dois Irmãos Rock, São Conrado, Rio de Janeiro, this species was considered identical to *A. tomentosum* by Woodson.[11] *A. gomezianum* is registered in the Rio de Janeiro Botanical Garden under No. 58974.

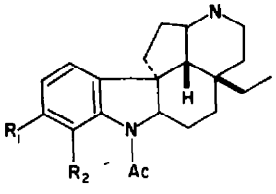
[h] Collected in the Tijuca forest, Rio de Janeiro. As noted in Ref. 8 the species previously investigated by us[10] was incorrectly identified. This species has a smooth trunk and is very distinct from *A. compactinervium*[11] (see note *d* above). *A. eburneum* is registered in the Rio de Janeiro Botanical Garden under No. 19397. Manual labour during the collection of this species was provided by Prof. W. D. Ollis of the University of Sheffield.

[i] Collected on the Fazenda do Chupador (Rio Preto), Unaí, Minas Gerais and later elsewhere in Minas Gerais (note *e*) and botanically identical to the species recorded originally by Martius[20] for the same region. It will be noted that *A. subincanum* collected in Peru also yielded olivacine.[18] *A. subincanum* is registered in the Rio de Janeiro Botanical Garden under No. 113339.

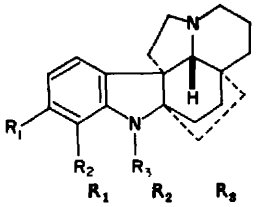
[j] Collected at the Centro de Treinamento of the F.A.O. near the river Curuauna, Santarém, Pará. This species is registered in the Rio de Janeiro Botanical Garden under no. 116848.

[k] *A. nigricans* (popular name, "pereiro amarelo") was collected at km 18 of the Porto Seguro-BR5 road, Bahia. The bark of this species which is vivid yellow, apparently due to the high olivacine content, has local anaesthetic properties. It is registered in the Rio de Janeiro Botanical Garden under No. 119068.

[l] Collected on an island in the Rio Negro above Barcellos, Amazonas. This species is botanically as well as chemically similar to *A. album*, and is registered in the Rio de Janeiro Botanical Garden under No. 116843.

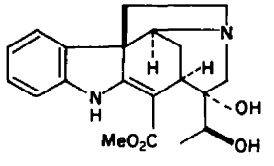


	R_1	R_2
IV	OMe	OH
V *	H	H
XIX	H	OH

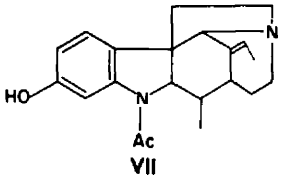


	R_1	R_2	R_3
IX	H	OMe	H
X	H	OMe	CHO
XI	OMe	OMe	H
XII	OMe	OMe	CHO

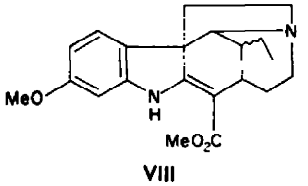
* Stereochemistry unknown.



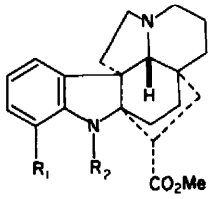
VI



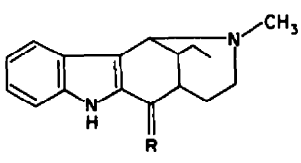
VII



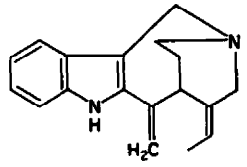
VIII



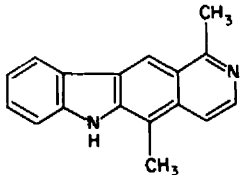
	R_1	R_2
XIII	H	H
XIV	H	CHO
XV	OMe	CHO



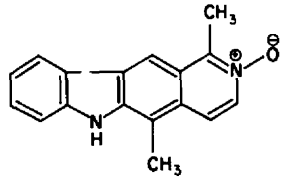
XVI R = CH_2
XX R = O



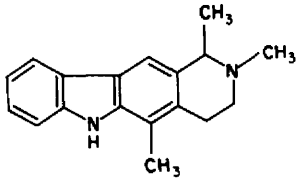
XVII



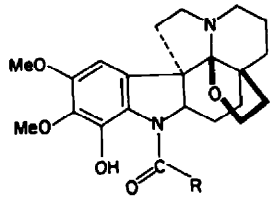
XVIII



XXII

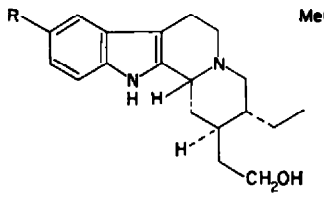


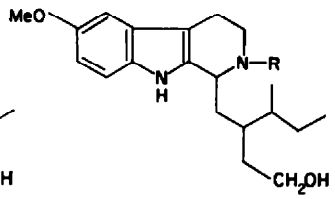
XXI



XXIII R = Et
XXIV R = Me

An as yet preliminary study has been made of three other members of the subgroup *Nitida*, namely the species *A. auriculatum*, *oblongum* and one as yet unidentified (No. 119070) in addition to *A. compactinervium*.[10] In accord with expectation indoles were found to constitute the principal alkaloids. *A. auriculatum*, like the previously studied *A. marcgravianum* Woodson,[8] contains principally dihydrocorynantheol (I) although in this case reserpinine appears in place of aricine. *A. oblongum* has been studied previously[9] and a large number of alkaloids separated and identified by mass spectrometry. Among these were a dehydro-10-methoxydihydrocorynantheol (II) and the corresponding saturated compound 10-methoxydihydrocorynantheol (III), which it was assumed,[9] were probably identical to alkaloids AD-VI and AD-IV respectively of *A. discolor* A.DC.[4] Our reinvestigation of this question has shown that the former, (II), is in fact identical to AD-VI. 19,20-Dehydro-10-methoxydihydrocorynantheol (II) was recognized by its mass[9] and UV spectra[4] as well as by the NMR absorption of its O-acetate. Hydrogenation of II gave a dihydro-derivative different from



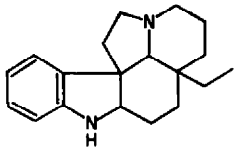


I R = H
II R = OMe; 19,20-dehydro
III R = OMe; stereochemistry uncertain

XXV R = H
XXVI R = CH_3

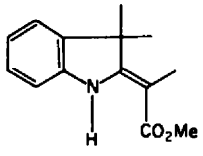
AD-IV (above),[22] as well as a tetrahydro-derivative which is tentatively assigned structure XXV. Hydrogenation of the methochloride of II gave the N-methyl derivative (XXVI) of XXV which was different from the Emde base from huntabrine.[22,23]

The *Aspidosperma* species No. 119070 yielded, in addition to two alkaloids of the aspidospermidine (XXVII) skeleton, an indolic base whose properties were close to those of alkaloid AD-IV of *A. discolor*.[4]



XXVII

The genus *Aspidosperma* has, up to the present, provided only one alkaloid with the strychane skeleton. This compound, compactinervine (VI), from *A. compactinervium* was the subject of a previous communication.[10] The alkaloid, obtainable crystalline only as a solvate of variable composition, was characterized as a number of salts, and its molecular formula $C_{20}H_{24}N_2O_4$ established by mass spectrometry. The high negative rotation, UV and IR spectra (Experimental) were characteristic of the α-methyleneindoline group of alkaloids containing the chromophore XXVIII. The remaining two oxygen atoms were shown to exist as hydroxyl groups by the formation of an O,O-diacetate (XXIX) which on partial hydrolysis with methanolic



XXVIII

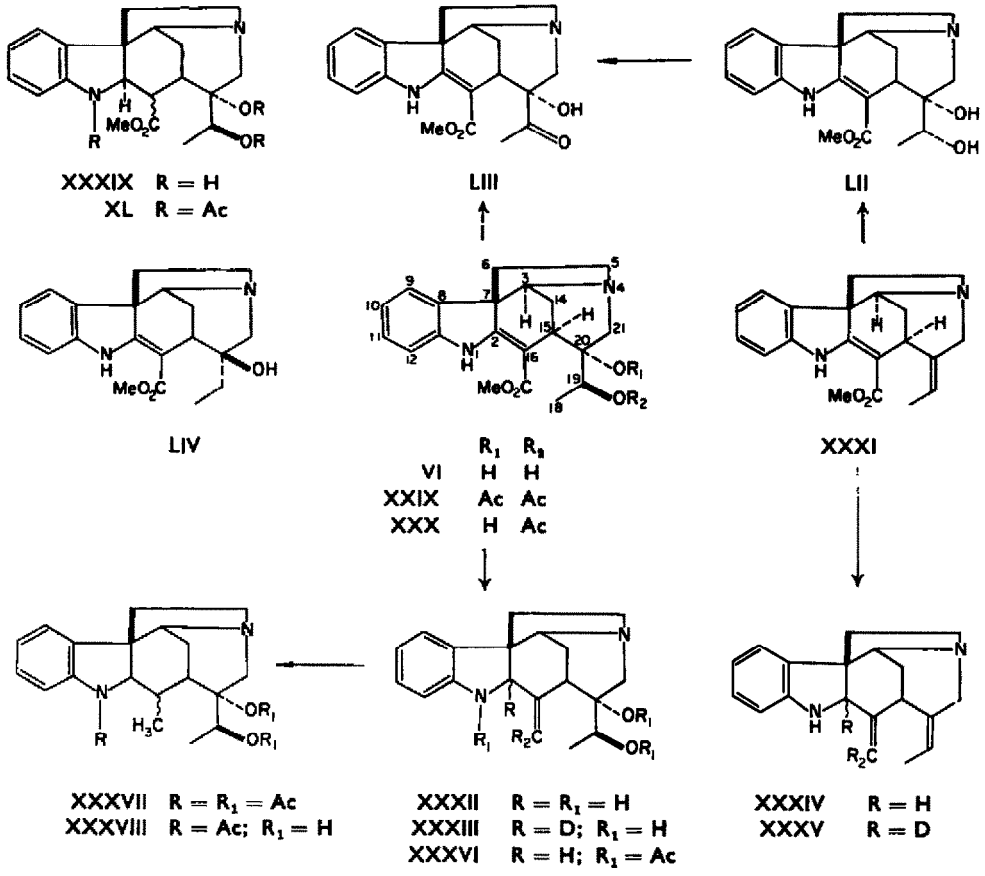
hydrochloric acid gave the monoacetate (XXX). The latter was also available by partial acetylation of compactinervine with acetic anhydride or with ketene. NMR examination of the parent alkaloid and of the mono- and di-acetates confirmed the

[22] The difference between dihydro-AD-VI and AD-IV has been confirmed by Professor H. Schmid (personal communication). The Swiss workers obtained an Emde degradation product (XXVI) probably identical to the Emde base from huntabrine.

[23] M. F. Bartlett, B. Korzun, R. Sklar, A. F. Smith, and W. I. Taylor, *J. Org. Chem.* **28**, 1445 (1963).

presence of the grouping XXVIII unsubstituted in the aromatic nucleus, and showed that the hydroxyl groups were respectively secondary and tertiary since only one proton of the type C*H*-OAc, was visible as a quartet in the spectra of the acetates. This proton was in each case coupled only to a methyl, which appeared as a doublet at 1·30δ in the diacetate and 1·20δ in the monoacetate. The doublet was also present in the spectrum of compactinervine (VI) which therefore contains the grouping $CH_3CH(OH)C$, where the last carbon atom bears no hydrogen atom.

As previously reported[10] compactinervine showed many similarities to akuammicine (XXXI) both in its physical and chemical properties. In particular, the NMR and ORD data pointed to a similar skeleton and absolute configuration. Furthermore, compactinervine showed parallel behaviour on treatment with LAH, zinc and sulphuric

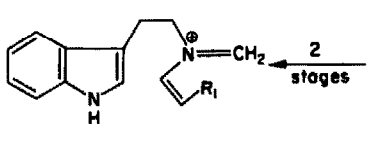


acid, and with hydrochloric acid; reagents which result in characteristic transformations in the akuammicine series (for leading citations see Ref. 24). The LAH product (XXXII) and the corresponding trideuterio-derivative (XXXIII) obtained with LAD exhibited mass spectra parallel to those of the corresponding derivatives (XXXIV) and (XXXV) from akuammicine,[24] but in which fragments containing carbon atoms

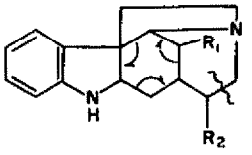
[24] H. Budzikiewicz, J. M. Wilson, C. Djerassi, J. Lévy, J. Le Men and M.-M. Janot, *Tetrahedron* **19**, 1265 (1963).

18, 19 and 20 suffered a shift of 32 units due to the two alcoholic oxygen atoms. The continued presence of these was also established by acetylation of the diol XXXII to the triacetate XXXVI and this could be transformed by successive catalytic reduction and deacetylation to the two saturated 16-methyl-strychane derivatives XXXVII and XXXVIII. Reduction of compactinervine (VI) with zinc and methanolic sulphuric acid also gave the saturated strychane derivative 2,16-dihydrocompactinervine (XXXIX), which yielded an N,O,O-triacetate (XL).

The mass spectra of such saturated derivatives allow a clear distinction between the strychane (tetrahydrodecarbomethoxyakuammicine, XLI) and dihydroaspidospermatidine (XLII) types[6,24] since the former show an *m/e* 199 peak due to fragment *a* which becomes *m/e* 227 due to *b* for alkaloids of the second group having an ethyl



a $R_1 = H$
b $R_1 = CH_2CH_3$ or other side chain



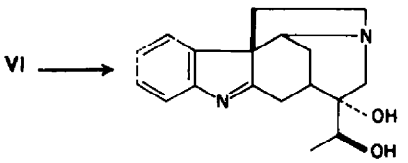
XLI $R_1 = H$; $R_2 = Et$
XLII $R_1 = Et$; $R_2 = H$

side chain (or *m/e* 259 if this chain contains two oxygen atoms). This particular peak was found at *m/e* 199 for XXXVIII and XXXIX, the former showing also a peak at *m/e* 241 (N-acetyl-*a*). This evidence not only indicates the strychane skeleton for compactinervine but also limits the position of the tertiary hydroxyl group to C-20 in such a skeleton, since both oxygen atoms are absent from fragment *a*. This placing requires a *vic*-glycol grouping in the molecule and, in fact, periodic acid oxidation of compactinervine cleaved the side chain to give acetaldehyde in 80% yield.

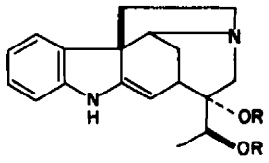
Hydrolysis of compactinervine (VI) with 2N HCl resulted in loss of the CO_2Me group to give decarbomethoxycompactinervine (XLIII). The mass spectrum of this compound paralleled that of its akummicine derived analog. Acetylation of XLIII was accompanied by an isomerization which probably involved a shift of the 1,2-double bond to the 2,16 position to give XLIV. The same isomerization could also be produced by alkaline treatment of XLIII when the product may be represented as XLV. Decarbomethoxycompactinervine (XLIII) underwent the reverse Mannich condensation and reduction on treatment with alkaline KBH_4, to give an indole, a transformation shown by other $\Delta^{1,2}$-strychenes.[25]

While the preceding evidence established the structure of compactinervine as VI, knowledge of the stereochemistry required intertransformation with akuammicine or a derivative. An attempted degradative approach to this failed, for although compactinervine could be carried through the series VI → XLVI → XLVII → XLVIII → XLIX in which the C-19 hydroxyl group has been reduced to hydrogen, dehydration with elimination of the C-20 hydroxyl group and production of a 19,20-double bond as in XXXIV could not be achieved. The tertiary C-20 hydroxyl group of XLIX

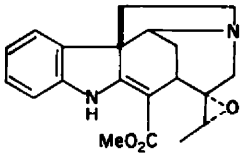
[25a] G. F. Smith and J. T. Wrobel, *J. Chem. Soc.* 792 (1960);
[b] X. Monseur, R. Goutarel, J. Le Men, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Bull. Soc. Chim. Fr.* 1088 (1962).



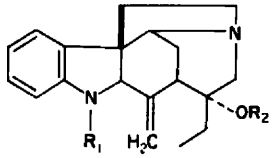
XLIII



XLIV R = Ac
XLV R = H



XLVI



XLVII $R_1 = R_2 = H$
XLVIII $R_1 = R_2 = Ac$
XLIX $R_1 = Ac$; $R_2 = H$
L $R_1 = Ms$; $R_2 = H$
LI $R_1 = Ms$; $R_2 = Ac$

could not be mesylated (for subsequent dehydration or reduction) only the indolic nitrogen suffering attack to give the product L with a free C-20 hydroxyl group which could still be acetylated to the N-mesylate-O-acetate (LI). A synthetic approach however proved successful. *cis*-Hydroxylation of the 19,20-double bond of akuammicine (XXXI) with osmium tetroxide gave the 19-epimer of compactinervine (LII) which on chromic acid oxidation gave the 19-ketone LIII, identical in all respects to the product of similar oxidation of compactinervine. The C-20 hydroxyl group in compactinervine thus has the same configuration as that in LII and its ready acetylation and resistance to dehydration contrast with the similarly placed group in lochneridine (LIV) which cannot be acetylated but suffers ready dehydration.[26] This difference permits the allocation of the α-equatorial configuration to the C-20 hydroxyl groups of compactinervine and LII. Akuammicine is of known absolute configuration and moreover the C-18 and C-15 carbon atoms are *cis*-oriented with respect to the 19,20 double bond,[27] so that *cis*-hydroxylation would result in the C-19 orientation depicted by formula LII. The complete absolute configuration of compactinervine thus follows and is as depicted in formula VI.

A minor alkaloid from *A. compactinervium* proved to be related to compactinervine although in this case the skeleton was of the alternative dihydroaspidospermatidine (XLII) type. The amorphous alkaloid showed hydroxyl and amide absorption in the IR while the UV spectrum (Experimental) was compatible with an N-acyldihydroindolic structure. The mass spectrum with the molecular ion peak at *m/e* 324 suggested the composition $C_{20}H_{24}N_2O_2$, the presence of an M-43 (loss of CH_3CO) peak indicating that the N-acyl group was acetyl. The very intense base peak at *m/e* 136

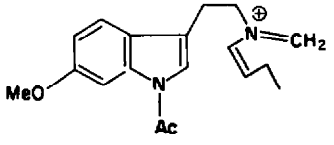
[26] Y. Nakagawa, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Chem. & Ind.* 1986 (1962).

[27a] M.-M. Janot, J. Le Men, A. Le Hir, J. Levy and F. Puisieux, *C. R. Acad. Sci., Paris* **250**, 4383 (1960);

[b] P. N. Edwards and G. F. Smith, *J. Chem. Soc.* 152 (1961);

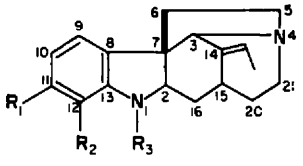
[c] K. Bernauer, F. Berlage, W. von Philipsborn, H. Schmid and P. Karrer, *Helv. Chim. Acta* **41**, 2293 (1958).

pointed to either the aspidospermatidine (LV) or $\Delta^{19,20}$-strychene (dihydrodecarbomethoxyakuammicine, LVI) skeleton[6,24] and the fact that the indolic peaks appeared at *m/e* 146 and 160, 16 units higher than in the spectra of unsubstituted compounds, showed that the hydroxylic oxygen atom lay in the aromatic part of the molecule. Treatment with diazomethane indeed gave an amorphous O-methyl derivative (LVII) whose mass spectrum was almost identical to that of aspidospermatine (LVIII),[6] with molecular ion at *m/e* 338 and indolic peaks now appearing at *m/e* 160 and 174, corresponding to the change OH → OMe. Comparison of the UV spectrum of LVII with those of model methoxyhexahydrocarbazoles[28] demonstrated that the methoxyl group lay in position 11. It remained to confirm the nature of the aliphatic portion of the molecule and distinguish between the two possibilities, VII and LIX, for the parent alkaloid. This was achieved by catalytic reduction of the O-methyl-alkaloid (LVII) to its dihydro derivative (LX) in whose mass spectrum the intensity of the peak at *m/e* 299 (fragment *c*) was approximately double that of the peak at *m/e* 271. The ethyl side chain is thus present in fragment *c* and must be located on C_{14}. The

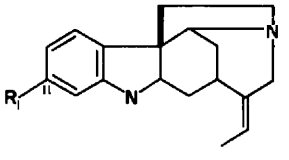


c

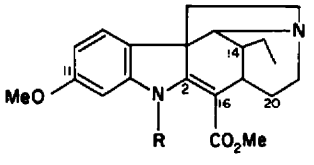
similarity of the breakdown patterns of LVII and aspidospermatine (LVIII) mentioned above provides evidence for the location of the aliphatic double bond at position 14,19 so that the alkaloid itself may be represented as N-acetyl-11-hydroxyaspidospermatidine (VII).



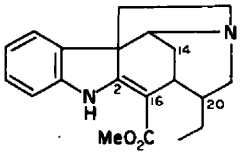
	R_1	R_2	R_3
VII	OH	H	Ac
LV	H	H	H
LVII	OMe	H	Ac
LVIII	H	OMe	Ac
LX	OMe	H	Ac; 14,19-dihydro



LVI $R_1 = R_2 = H$
LIX $R_1 = OH$; $R_2 = Ac$

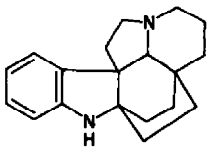


VIII R = H
LXII R = H; 2,16-dihydro
LXIV R = Ac; 2,16-dihydro

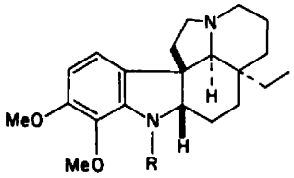


LXI
LXIII; 2,16-dihydro

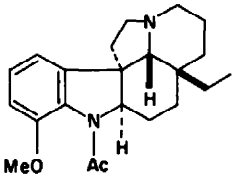
[28] J. R. Chalmers, M. T. Openshaw and G. F. Smith, *J. Chem. Soc.* 1115 (1957).



LXV



LXVI * R = Ac
LXVIII * R = H



LXVII *

* Relative configuration

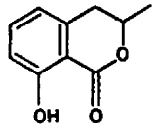
Another base with the aspidospermatidine skeleton was found in the acid benzene extract from *A. populifolium*. The amorphous alkaloid, which exhibits a blue fluorescence in UV light, has IR and UV spectra reminiscent of compactinervine (VI) and thus is indicative of a chromophore related to XXVIII (above). Confirmation came from the NMR spectrum which showed both N*H* (8·80δ) and CO_2CH_3 (3·74δ) in the downfield positions characteristic of this system (for Lit. see Ref. 10). Superimposed on the carbomethoxyl methyl singlet was absorption due to an aromatic methoxyl, in accord with which was the appearance of absorption due to only three aromatic protons. The presence of a poorly resolved 3-proton triplet at 0·70δ and the absence of vinyl absorption indicated the presence of an ethyl rather than an ethylene side chain. A 14,19-dihydromethoxycondylocarpine (e.g. VIII) or a 19,20-dihydromethoxyakuammicine (e.g. LXI) structure was compatible with the mass spectrally determined mol. wt. of 354 ($C_{21}H_{26}N_2O_3$). A distinction between the two possibilities was obtained as in the case of compactinervine (VI) by zinc and sulphuric acid reduction which gave an amorphous dihydroderivative (LXII or LXIII) with a dihydroindole chromophore. The mass spectrum of this compound, identical, apart from a 30 unit shift of indolic peaks to that of tetrahydrocondylocarpine, eliminated the akuammicine-type alternative, LXIII, since the fragment *b* peak appeared at *m/e* 257 showing, as in the case of LX above, that the ethyl group was in position 14. The location of the aromatic methoxyl function follows from comparison of the UV spectra of 11-methoxytetrahydrocondylocarpine (LXII) and its amorphous N-acetyl-derivative (LXIV) with those of known compounds.[28] The alkaloid may therefore be formulated as 14,19-dihydro-11-methoxycondylocarpine (VIII).

All of the other alkaloids (IX–XV) of *A. populifolium* whose structures have so far been determined are of the aspidofractinine (LXV)[29] type. Four, IX-XII, isolated from the chloroform-soluble acetate fraction were of new natural occurrence although the first, 17-methoxyaspidofractinine (IX) proved to be the optical enantiomer of O-methyldeacetylaspidofiline.[13b] It is quite probable that aspidofiline has the "unnatural" configuration since it occurs in *A. pyrifolium* side by side with "unnatural" pyrifolidine (LXVI).[13a] The second base, X, was amorphous and showed amide absorption in the IR. Its N-acyldihydroindole chromophore resembled that of aspidospermine (LXVII) and its mass spectrum suggested[29] that it was the N-formyl derivative of IX. Acid hydrolysis in fact furnished IX and this alkaloid may therefore be formulated as N-formyl-17-methoxyaspidofractinine (X). The two accompanying bases, XI and XII, were interrelated in the same way, the amorphous XII being the

[29] C. Djerassi, H. Budzikiewicz, R. J. Owellen, J. M. Wilson, W. G. Kump, D. J. LeCount, A. R. Battersby and H. Schmid, *Helv. Chim. Acta* **46**, 742 (1963).

N-formyl derivative of the crystalline alkaloid XI. The mass spectra of the two compounds also showed the M-28 and *m/e* 124 and 109 peaks characteristic[29] of the aspidofractinine structure, the positions of the indolic peaks at the same time showing that they were aromatic dimethoxy derivatives of this skeleton. The close similarity of the UV spectra of XI and XII to those of deacetylpyrifolidine (LXVIII) and pyrifolidine (LXVI)[13a] respectively, pointed to a 16,17 orientation for the two methoxyl groups, and this is supported by the appearance in the NMR spectrum of XI of absorption due to two *ortho* aromatic protons and to six aromatic methoxyl protons. The positions of the aromatic proton resonances are compatible with their being in positions 14 (6·86δ) and 15 (6·28δ) so that the two alkaloids may be designated as 16,17-dimethoxyaspidofractinine (XI) and its N-formyl derivative (XII).

As will be seen from the Table the alkaloids so far identified in the species *A. olivaceum* (for earlier work see Ref. 15*b*), *A. gomezianum*, *A. eburneum*, *A. subincanum*, *A. multiflorum* and *A. spruceanum* are known compounds with the exception of (−)-apparicine (XVII) whose structure has been described in a separate paper.[16] In addition to the alkaloids the dihydroisocoumarin mellein[30] was found in *A. spruceanum*.

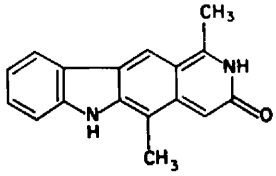


LXIX

Aspidosperma nigricans, a species whose bark contains exceptional amounts of olivacine and (+)-guatambuine, also yielded, in the chloroform fractions from pH4 to pH10, a third alkaloid of intermediate mobility on silica gel. This somewhat unstable compound, m.p. 304–305°, exhibited intense UV absorption not unlike that of olivacine (XVIII). At the same time the NMR spectrum (measured in CF_3CO_2H) was closely similar to that of olivacine although distinct shifts were observed both in the positions of the two aromatic methyl groups present as well as in those of the seven aromatic protons. The mass spectrum showed the molecular ion at *m/e* 262 which is compatible with a mono-oxygenated olivacine with empirical formula $C_{17}H_{14}N_2O$. This oxygen atom however was not involved in a phenolic hydroxyl group (no shift of UV absorption in alkaline solution) nor in a carbonyl group (IR). Up to the base peak at *m/e* 246 (M-16) the mass spectrum resembled closely that of olivacine (Mol. wt. 246) and there was every indication therefore that the molecule lost a single oxygen atom to give the olivacine molecule-ion as the initial step of mass spectral breakdown. This evidence was consistent with the N-oxide structure, XXII, and, in fact, room temperature reduction with zinc and hydrochloric acid in methanol gave olivacine (XVIII). Furthermore, oxidation of olivacine with perbenzoic acid in dimethylformamide gave the N-oxide (XXII) identical in all respects with the natural alkaloid. When the oxidation was conducted with hydrogen peroxide in hot acetic

[30a] T. Yabuta and Y. Sumiki, *J. Agric. Chem. Soc., Japan* **10**, 703 (1934);
[b] E. Nishikawa, *ibid*, **9**, 772, 1059 (1933);
[c] H. S. Burton, *Nature, Lond.* **165**, 274 (1950).

acid[31] this was the initially formed product but rapidly underwent further transformation to a mixture from which could be isolated a red crystalline amide. This substance, isomeric with the N-oxide (XXII), has nevertheless a distinct mass spectral breakdown pattern and is tentatively formulated as LXX.



LXX

EXPERIMENTAL*

Isolation procedure. The dried finely powdered bark was extracted by immersion in cold 95% EtOH for 20 hr followed by distillation of the extract with recycling of the EtOH. Complete extraction required 4 to 8 cycles. The extract partially concentrated by atm. distillation was freed from EtOH under red. press, dispersed in 10% acetic acid (5 to 8 ml per g extract) and allowed to stand at 0° for 24 hr. The aqueous acid filtrate was then exhaustively extracted successively with hexane (or pet. ether), benzene and $CHCl_3$. Three more $CHCl_3$ extractions were then made at pHs 7, 9 and 11, basification being made with NH_4OH (early procedure) or with $NaHCO_3$, Na_2CO_3 and NaOH (later procedure). The residual alkaline aqueous solution was acidified and stored for eventual study of quaternary bases.

Isolation of reserpinine and dihydrocorynantheol. Chromatography of the acid $CHCl_3$ extract (25·4 g from 10·6 kilos bark) of *A. auriculatum*, on active alumina (800 g) gave a fraction (2·8 g) eluted with benzene containing 20–75% ethyl acetate, which on rechromatography on silica gel (50 g) yielded reserpinine (20 mg) eluted with benzene–20% ethyl acetate. The base, m.p. 239·5–241·5°, from MeOH, was identified with authentic reserpinine[32] by IR, UV and mixture m.p. Chromatography of the pH7 $CHCl_3$ extract (24 g) on active alumina gave I (3·2 g) m.p. 185–186°, $[\alpha]_D^{27}$ −18° (*c*, 0·92 in $CHCl_3$), eluted with benzene containing 30–50% ethyl acetate. The alkaloid crystallized from MeOH–water and was identical to dihydrocorynantheol from *A. marcgravianum* Woodson,[8] by IR and mixture m.p.

19,20-*Dehydro*-10-*methoxydihydrocorynantheol* (II)

The EtOH extract (54·5 g) from *Aspidosperma oblongum* yielded a pH 9 $CHCl_3$ fraction weighing 210 mg, of which 70 mg was subjected to thin layer chromatography on silica gel G with MeOH–acetone, 1:1. Elution yielded a solid (33 mg), m.p. 175° which crystallized from EtOH as colourless needles (12 mg), m.p. 181–182·5°, $[\alpha]_D^{26}$ −42° (*c*, 0·76 in pyridine); ν_{max}^{KBr} 3448, 3175 cm^{-1}; λ_{max}^{EtOH} 225 and 280 mμ (log ε 4·44, 3·93); λ_{infl}^{EtOH} 297 and 308 mμ (log ε 3·84, 3·54). (Found: C, 73·68; H, 8·14; N, 8·70; mol. wt. mass spec. 326. Calc. for $C_{20}H_{26}N_2O_3$: C, 73·59; H, 8·03; N, 8·58%; mol. wt. 326·42.) The alkaloid was identical with II (AD-VI) from *A. discolor* A.DC.[4] by IR spectral comparison and mixture m.p.

The mass spectrum (direct introduction into ion-source) showed principal peaks at *m/e*: 326 (M^+, base peak), 325 (M-1, 98%), 311, 295 ($M-CH_3OH$), 281 ($M-CH_2CH_2OH$, 82%), 267, 253, 200, 199 (99%), 186 (76% of base peak), 174.

[31a] E. Ochiai, *J. Org. Chem.* **18**, 548 (1953);
[b] E. Ochiai and M. Ishikawa, *Chem. and Pharm. Bull, Japan* **6**, 208 (1958).

* M.Ps are uncorrected. Microanalyses were performed by Drs. E. Meier and J. Consul. NMR spectra were determined by Dr. Lois J. Durham, mass spectra by Drs. H. Budzikiewicz, J. M. Wilson and M. Ohashi. Many UV and IR spectra and optical rotations were measured by Mrs. D. Aguilar.

[32] A. Hofmann, *Helv. Chim. Acta* **37**, 849 (1954); E. Schlittler, H. Saner and J. M. Müller, *Experientia* **10**, 133 (1954).

O-Acetyl-19,20-dehydro-10-methoxydihydrocorynantheol

The alkaloid (II, 15 mg) was acetylated with acetic anhydride (0·02 ml) and pyridine (1 ml) at room temp for 16 hr with exclusion of light. Water was added, the solution evaporated in vacuum, water and ammonia added and the mixture extracted with $CHCl_3$. Evaporation of the dried extract gave the amorphous acetate, ν_{max}^{chf} 1730 (s), 1631 (m), 1587 (m) cm^{-1}, with UV absorption identical to that of II. The NMR spectrum showed absorption at 1·60 (3 H, doublet, J = 7·5 c/s), 2·00 (3 H, singlet), 3·84 (3 H, singlet), 5·50 (1 H, quartet, J = 7·5 c/s), 6·65–7·0 (2 H, multiplet), 7·22 (1 H, doublet, J = 7·5 c/s), 8·17δ (1 H, singlet). The mass spectrum (ion-source inlet) showed principal peaks at *m/e*: 368 (M^+, 97%), 367 (M-1), 353, 281 (M—CH_2CH_2OAc, 81%) 279 (69%), 200 (81%), 199 (97%), 186 (base peak), 174.

Hydrogenation of 19,20-dehydro-10-methoxydihydrocorynantheol

The alkaloid (II, 50 mg) was hydrogenated over red. PtO_2 at 27° and 1 atm. for 6 hr when uptake of 1 mole H_2 was complete. The crude amorphous product (50 mg) was chromatographed on silica gel in H MeOH–acetone, 1:1, to give two products. The first (25 mg) gave a picrate which crystallized, from EtOH as yellow needles, m.p. 215–217° dec. The free base showed ν_{max}^{KBr} 3420 (s), 2800 (w), 1630 (m), 1428 (m), 1384 (m), 1300 (m), 1212 (m), 1152 (m), 1030 (m) and 800 (m) cm^{-1}; λ_{max}^{EtOH} 228, 280 mμ (log ϵ 4·07, 3·69); λ_{infl}^{EtOH} 295 mμ, (log ϵ 3·64). The mass spectrum (ion source inlet) showed principal peaks at *m/e*: 328 (M^+, 88%), 327 (base peak), 313 (M-CH_3), 299 (M-Et), 297 (M-CH_2OH), 283 (M-CH_2CH_2OH), 281, 255, 214, 200 (63% of base peak), 199, 186, 174, 173. This product was not identical to III (AD-IV) from *A. discolor*,[4] or the possibly isomeric III from *Aspidosperma* species No. RJB-119070 (below) although the mass spectrum is close to that of the latter. The second hydrogenation product (XXV), m.p. 78–81°, λ_{max}^{enf} 2·90, 3·05, 6·00, 6·17 and 6·27 μ, showed UV absorption identical to that of the original alkaloid and had principal mass spectral peaks at *m/e* (ion source inlet): 330 (M^+), 312, 311, 255, 253, 215, 202, 201 (base peak), 186, 158, 100.

Emde degradation of 19,20-dehydro-10-methoxydihydrocorynantheol

To a solution of II (50 mg) in $CHCl_3$ (2 ml) an excess MeI was added and after 24 hr at room temp the solution was evaporated *in vacuo*. The crude methiodide so obtained was vigorously shaken with excess AgCl in water (30 ml), filtered, and evaporated to give the methochloride (which was directly hydrogenated over red. PtO_2 (20 mg) in EtOH (20 ml) at room temp and press. After 6 hr, two moles H_2 had been taken up and the mixture was filtered and evaporated to give a residue (45 mg) which showed one spot on silica gel H, and after 2 crystallizations from ether gave colourless rhombs (7 mg) m.p. 106–108°. This compound was tentatively identified as XXVI by its mass spectrum (heated inlet) which showed principal peaks at *m/e*: 344 (M^+), 310, 216 (55%), 215 (base peak) and resembled but was not quite identical to that of the Emde base from huntabrine.[23]

Isolation of aspidocarpine and N-acetylaspidospermidine

The acidic chloroform extract from *Aspidosperma* species RJB 119070 (Table, footnotes *a*, *c*) gave 5·9 g residue which was chromatographed on neutral alumina (activity III). The benzene eluate gave aspidocarpine which crystallized from MeOH in colourless prisms, (300 mg) m.p. 166–169°, identical chromatographically, by IR and UV spectra and mixture m.p. with an authentic sample.

From the same fractions thin layer chromatography on silica gel using benzene–MeOH, 4:1, yielded V as a clear oil (39 mg), $[\alpha]_D^{25}$ −15° (*c*, 0·5 in $CHCl_3$); ν_{max}^{chf} 2941, 2793, 1642, 1603 cm^{-1}; λ_{max}^{EtOH} 252·2 mμ (log ϵ 3·98); λ_{infl}^{EtOH} 280 and 290 mμ (log ϵ 3·51, 3·45); λ_{min}^{EtOH} 233 mμ (log ϵ 3·73). The mass spectrum showed principal peaks at *m/e*: 324 (M^+), 296 (M-28), 130 and 124 (base peak).

Hydrolysis in conc. HCl (2 ml) at 100° during 2 hr gave, after neutralization and extraction, aspidospermidine as a colourless solid (7 mg) m.p. 90–95°, identical to an authentic sample[8] by mass spectral comparison.

Isolation of 10-methoxydihydrocorynantheol (III)

The pH 7 $CHCl_3$ extract from *Aspidosperma* species No. 119070 gave 1·62 g residue which was chromatographed on neutral alumina (activity IV). The combined benzene and benzene–ethyl acetate eluates (364 mg) were rechromatographed on alumina (11 g) and the semi-crystalline fractions

(108 mg) submitted to thin layer chromatography on silica gel G to give a fraction (19 mg) which crystallized from benzene to give 10-methoxydihydrocorynantheol (7 mg) as colourless crystals, m.p. 153–156°, ν_{max}^{chf} 3401, 3226, 2740, 1616 cm^{-1}; λ_{max}^{EtOH} 227 and 280 mμ (log ϵ 4·38, 3·86); λ_{infl}^{EtOH} 293 mμ (log ϵ 3·82); λ_{min}^{EtOH} 250 mμ (log ϵ 3·50); $\lambda_{max}^{EtOH-HCl}$ 215 and 272 mμ (log ϵ 4·14, 3·86); $\lambda_{infl}^{EtOH-HCl}$ 294 and 306 mμ (log ϵ 3·69, 3·55); $\lambda_{min}^{EtOH-HCl}$ 245 mμ (log ϵ 3·50). The UV spectrum in alkaline solution was identical to that in neutral solution. The mass spectrum showed principal peaks at *m/e*: 328 (M^+, 85%), 327 (M-1, base peak), 299 (M-Et), 297 (M-CH_2OH), 283 (M-CH_2CH_2 OH), 255, 214, 200, 199, 186, 168, 163. This compound may be identical to (AD-IV) from *A. discolor*[4] having a superimposable IR spectrum and identical mobility on thin layer chromatography but differing slightly in mass spectral intensities.

Isolation of compactinervine (VI)

The conc. ethanolic extract (550 g) of the bark of *A. compactinervium* was treated with 10% acetic acid (1:1) and extracted with $CHCl_3$ (500 ml). The aqueous layer was brought to pH 8–9 with NH_4OH (ice cooling) and extracted with $CHCl_3$ (1000 ml). The extract was washed with water (300 ml), dried and evaporated to give a dark resin (20 g). Chromatography on silica gel (200 g) gave *compactinervine* in the $CHCl_3$ and $CHCl_3$–MeOH eluates. Processing of the wood extract (300 g) in the same way yielded 2 g of the alkaloid.

Compactinervine separated from EtOH–water as needles, m.p. 111–120° followed by decomposition at 235–245°, $[\alpha]_D^{25}$ −515° (*c*, 0·55 in EtOH), $[\alpha]_D^{25}$ −680° (*c*, 0·15 in pyridine), ORD negative Cotton effect (*c*, 0·0037 (in MeOH) $[\alpha]_{589}$ −495°, $[\alpha]_{350}$ −12650°, $[\alpha]_{300}$ +21550°, $[\alpha]_{270}$ +8070°, $[\alpha]_{250}$ +1455°; λ_{max}^{EtOH} 237, 297 and 331 mμ (log ϵ 3·97, 3·95, 4·15); λ_{max}^{chf} 2·87, 2·97, 6·04, 6·30 μ; NMR absorption 1·15 (3H, doublet J = 6 c/s, CH_3—CH—), 3·85 (3 H, singlet, CO_2CH_3), 6·6–7·2 (4 H, multiplet, aromatic protons), 8·55δ (1 H, broad singlet, NH). The mass spectrum (introduction into ion-source) showed peaks at *m/e*: 356 (M^+, 39%), 283 (48%), 268, 257, 226 (base peak), 225 (80%), 209, 194, 180, 167 (93%), 154 (40%). (Found: C, 66·24–62·08; H, 6·77–7·80; N, 6·97–8·27. $C_{20}H_{24}N_2O_4$ requires: C, 67·39; H, 6·79; N, 7·86%; mol. wt. 356·428.)

The *picrate* prepared from compactinervine (94 mg) in ether–EtOH solution crystallized from acetone as yellow prisms (80 mg), m.p. 219–220° dec. (Found: C, 53·42; H, 4·81; N, 12·16; 30·42. $C_{26}H_{27}N_5O_{11}$ requires: C, 53·33; H, 4·65; N, 11·96; O, 30·05%.)

The *hydrochloride* prepared from compactinervine (118 mg) in acetone separated from MeOH–acetone (1:4) as colourless prisms (50 mg) m.p. 191—192° dec. (Found: N,6·69; Cl, 9·85. $C_{20}H_{25}ClN_2O_4$ requires: N, 7·13; Cl, 9·05%.)

The *hydrobromide* prepared from compactinervine (111 mg) and conc. HBr aq (0·2 ml) in acetone at 0°, crystallized from MeOH as hygroscopic prisms (50 mg) m.p. 198–200° dec (after drying). (Found: C, 54·28; H, 5·95; N, 6·40; Br, 17·76. $C_{20}H_{25}BrN_2O_4$ requires: C, 54·95; H, 5·77; N, 6·41; Br, 18·28%.)

The *methiodide* was prepared from compactinervine (100 mg) and excess MeI in acetone (30 ml) during one day at room temp. The product crystallized from acetone as colourless prisms (60 mg) m.p. 254–260° dec. (Found: C, 51·44, 50·54, 50·77; H, 5·87, 5·82, 5·80; N, 5·25, 5·17, 4·99. $C_{21}H_{27}IN_2O_4$ requires: C, 50·61; H, 5·46; N, 5·62%).

O,O-*Diacetylcompactinervine* (XXIX)

Compactinervine (150 mg) in pyridine (2 ml) was allowed to react with acetic anhydride (0·5 ml) during 3 days at room temp. The reaction mixture was treated with ice-water, partly concentrated under vacuum, water (10 ml) added and the mixture basified with ammonia. The solution was extracted with $CHCl_3$ and the extract washed (H_2O), dried ($MgSO_4$) and evaporated to give a solid (180 mg), m.p. 150°, which after recrystallization from ethyl acetate gave O,O-*diacetylcompactinervine* as fine needles (95 mg) m.p. 198° (followed by dec with reddening at 208°), $[\alpha]_D^{25}$ −623° (*c*, 0·43 in $CHCl_3$); λ_{max}^{chf} 5·80, 6·00, 6·27 μ; λ_{max}^{EtOH} 230, 294 and 324 mμ (log ϵ 4·14, 4·11, 4·16). NMR absorption occurred at 1·30 (3 H, doublet, J = 6·5 c/s, CH_3CH), 1·98 (3 H, singlet, $OCOCH_3$), 2·15 (3 H, single, $OCOCH_3$), 3·72 (3 H, singlet, CO_2CH_3), 5·45 (1 H, quartet, J = 6·5 c/s, CH_3—CHOAc), 8·95δ (1 H, broad singlet, NH). The mass spectrum (heated inlet system) showed peaks at *m/e*: 440 (M^+), 381, 380, 321, 320. (Found: C, 65·60; H, 6·65; N, 6·29. $C_{24}H_{28}N_2O_6$ requires: C, 65·44; H, 6·51; N, 6·36%, mol. wt. 440·48.)

19-O-*Acetylcompactinervine* (XXX)

(a) *By partial acetylation of compactinervine.* A mixture of compactinervine (100 mg), acetic anhydride (40 mg) and dry pyridine (4 ml) was prepared at −5° and kept at 4° for 14 hr. The mixture was poured onto ice, basified, extracted with $CHCl_3$ and the extract worked up as above to give a mixture (120 mg) of compactinervine (trace), O-acetylcompactinervine (main product) and the diacetyl derivative (trace) by TLC on silica gel in MeOH–acetone). 19-O-*Acetylcompactinervine* (56 mg) was separated by preparative thin layer chromatography and crystallized from MeOH as needles, m.p. 223–225° (dec), λ_{max}^{KBr} 2·79, 3·00, 5·78, 5·99, 6·35 μ; NMR absorption at 1·20 (3 H, doublet, J = 6·5 c/s, CH_3CHOAc), 2·10 (3 H, singlet, $OCOCH_3$), 3·65 (3 H, singlet, CO_2CH_3) 4·60δ (1 H, quartet, J = 6·5 c/s CH_3CHOAc); and principal mass spectral peaks at *m/e*: 398 (M^+), 338 (M-HOAc), 226, 225, 202 (base peak), 200, 194, 180, 167. (Found: C, 66·25; H, 6·73; N, 7·07, $C_{23}H_{36}N_2O_5$ requires: C, 66·31; H, 6·58; N, 7·03%, mol. wt. 398·44.)

A similar result was achieved by acetylation of compactinervine (200 mg) in acetone (20 ml) with ketene at −80° for 1½ hr.

(b) *By partial hydrolysis of* O,O-*diacetylcompactinervine.* O,O-Diacetylcompactinervine (50 mg) was dissolved in 10% methanolic HCl (5 ml) and heated for 7 min under reflux. The mixture was poured into ice-water, basified with ammonia and extracted with $CHCl_3$. The extract was washed (H_2O), dried ($MgSO_4$) and evaporated to give a mixture from which the starting material (25 mg) and 19-O-acetylcompactinervine (6 mg), m.p. 223–225°, were isolated by preparative thin layer chromatography on silica gel in MeOH–acetone.

19β,20α-*Dihydroxy*-16-*methylenestrychane* (XXXII)

LAH (150 mg) was added to a solution of compactinervine (200 mg) in dry tetrahydrofuran (cooling) and the mixture heated under reflux for 16 hr. After cautious decomposition with moist ether followed by sat. Na_2SO_4aq, the mixture was extracted repeatedly with $CHCl_3$. Evaporation of the extracts gave a residue (200 mg) which on recrystallization from MeOH gave 19β,20α-*dihydroxy*-16-*methylenestrychane* (XXXII) as colourless needles, m.p. 255–259° (dec), λ_{max}^{KBr} 2·89, 3·00, 3·30, 5·79 (w), 6·24 μ; λ_{max}^{EtOH} 246 and 300 mμ (log ϵ 3·75, 3·56). The principal mass spectral peaks occurred at *m/e*: 312 (M^+, 50%), 295 (M-OH. 70%), 267, 239 (60%), 226 (59%), 196, 182 (base peak), 144 (74%), 130 (64%), partly shifted by deuterium exchange in MeOD-D_2O to *m/e* 313, 268, 240, 227, 197, 183–4, 145, 131. (Found: C, 72·59; H, 8·33; N, 8·28. $C_{19}H_{34}N_2O_2$ requires: C, 73·04; H, 7·74; N, 8·97%; mol. wt. 312·39.)

A similar reduction of O,O-diacetylcompactinervine (250 mg) gave the same product (100 mg) m.p. 255–259° (dec), which was unaffected by refluxing with 10% ethanolic KOH during 3 hr.

Reduction of compactinervine (100 mg) with LAD (80 mg) gave the corresponding 2,17,17-trideuterio derivative, (XXXIII, 80 mg) m.p. 255–259° (dec) with principal mass spectral peaks at *m/e*: 315 (M^+), 270, 242, 229, 199, 184, 145 and 131.

1-*Acetyl*-19β,20α-*diacetoxy*-16-*methylenestrychane* (XXXVI)

A mixture of the 16-methylene derivative (XXXII, 200 mg) and acetic anhydride (100 mg) in pyridine (3 ml) was kept at room temp for 4 days. Excess acetic anhydride was decomposed with water, the solution reduced *in vacuo* to a small volume, made basic with NH_4OH and extracted with $CHCl_3$ (100 ml). The extract was washed (H_2O) dried ($MgSO_4$) and evaporated to give a residue (200 mg) which crystallized from ethyl acetate in prisms (100 mg), m.p. 187–190°, λ_{max}^{KBr} 2·89–3·30 (br), 5·79 (s), 6·07 (s), 6·27 (w); λ_{max}^{EtOH} 249, 280 and 288 mμ (log ϵ 4·02, 3·47, 3·43). NMR absorption occurred at 1·30 (3 H, doublet, CH_3CHOAc), 2·10 (6 H, singlet, $2OCOCH_3$), 2·35 (3 H, singlet $N\text{-}COCH_3$), 4·9 (2 H, broad singlet, $=CH_2$), 7·15 (multiplet due to 3 aromatic H), 8·0δ (multiplet, 1 aromatic H). The mass spectrum showed principal peaks at *m/e*: (heated inlet) 438 (M^+), 319, 318, 196, 194, 182, 144, 130; (ion source inlet), 439 (M + 1), 379, 378, 320, 319.

1-*Acetyl*-19β,20α-*diacetoxy*-16-*methylstrychane* (XXXVII)

The 16-methylene-derivative (XXXVI, 80 mg) in acetic acid (30 ml) was hydrogenated at atm. press. and 15° in the presence of reduced PtO_2 (30 mg). After 1 hr one mole H_2 had been absorbed. The catalyst was then removed, the solvent concentrated to 1 ml and partitioned between $CHCl_3$ (200 ml) and NH_4OH solution (20 ml). The $CHCl_3$ layer was washed (H_2O), dried ($MgSO_4$) and

evaporated to give a residue (60 mg) which after recrystallization from light petroleum gave 1-*acetyl*-19β,20α-*diacetoxy*-16-*methylstrychane* (XXXVII) as colourless needles (38 mg), m.p. 165–166° (dec), homogeneous by thin layer chromatography. The substance showed λ_{max}^{chf} 5·85 (s), 6·15 (s) μ, and exhibited NMR absorption at 1·16 (3 H, doublet, J = 7 c/s, CH—CH_3), 2·16 (3 H, singlet, OCOCH_3), 2·20 (3 H, singlet, OCOCH_3), 2·39 (3 H, singlet NCOCH_3), 7·1 (3 H, multiplet, 3 aromatic H), 7·3δ (1 H, multiplet, 1 aromatic H). The mass spectrum showed principal peaks at *m/e*: (heated inlet) 320 (M-2AcOH), 291 (M-29-2AcOH), 277 (M-2AcOH-Ac); (ion-source inlet) 440 (M^+), 397 (M-Ac), 380 (M-AcOH), 351 (M-29-AcOH), 339, 321. (Found: C, 68·70; H, 7·29; N, 6·30. $C_{25}H_{32}N_2O_5$ requires: C, 68·16; H, 7·32; N, 6·36%, mol. wt. 440·52.)

1-*Acetyl*-19β,20α-*dihydroxy*-16-*methylstrychane* (XXXVIII)

The above triacetate (20 mg) was dissolved in 20% methanolic KOH solution (2 ml) and heated under reflux for 30 min in N_2. The solution was cooled, diluted to 20 ml with water and extracted with $CHCl_3$. The extract was washed, dried and evaporated to give 1-*acetyl*-19β,20α-*dihydroxy*-16-*methylstrychane* (10 mg) m.p. 198–200°, λ_{max}^{KBr} 6·10 μ, with principal mass spectral peaks (heated inlet) at *m/e*: 356 (M^+), 241, 199, 184, 144 and 130 (Calc. for $C_{21}H_{28}N_2O_3$, mol. wt. 356·45).

2,16-*Dihydrocompactinervine* (XXXIX)

Zn dust (20 g) was added to a solution of compactinervine (200 mg) in 10% absolute methanolic H_2SO_4 (100 ml) and the mixture heated under reflux for 90 min. The mixture was filtered, the residue washed with MeOH and the combined filtrate and washing concentrated to a small volume and distributed between an ice-cold $NaHCO_3$aq and NH_4OH and $CHCl_3$. The $CHCl_3$ extract was washed (H_2O), dried ($MgSO_4$) and evaporated to give a solid (180 mg) m.p. ca. 200°, which crystallized from MeOH as colourless prisms of 2,16-*dihydrocompactinervine* (80 mg), m.p. 265–270° (dec), $[\alpha]_D^{25}$ −44° (*c*, 0·34 in $CHCl_3$). The IR spectrum showed λ_{max}^{chf} 2·90, 3·00, 5·87 and 6·24 μ, and the UV spectrum λ_{max}^{EtOH} 245 and 298 mμ (log ϵ 3·86, 3·55). NMR absorption was observed at 1·20 (3 H, doublet, J = 6·5 c/s, CH—CH_3), 3·90 (3 H, singlet, CO_2CH_3), 4·21 (1 H, broad singlet, N—C*H*—), 6·5–7·2δ (4 H, multiplet, aromatic H), and principal mass spectral peaks (ion source inlet) occurred at *m/e*: 258 (M^+, 6%), 341 (M-OH), 313 (M-CHOHCH$_3$), 270, 228 (93%), 199 (25%), 144 (base peak), 130 (63%). (Found: C, 66·91; H, 7·28; N, 7·78. $C_{20}H_{26}N_2O_4$ requires: C, 67·02; H, 7·31; N, 7·82%; mol. wt. 358·42.)

N,O,O-*Triacetyl*-2,16-*dihydrocompactinervine* (XL)

A solution of dihydrocompactinervine (100 mg) in acetic anhydride (1 ml) and pyridine (2 ml) was kept at room temp (22°) for 48 hr, then poured onto ice and made basic with NH_4OH. The mixture was extracted with $CHCl_3$ and the extract washed thoroughly (H_2O), dried ($MgSO_4$) and evaporated to give a solid which crystallized on trituration with ethyl acetate. Recrystallization from ethyl acetate gave N,O,O-*triacetyl*-2,16-*dihydrocompactinervine* as colourless needles (25 mg), m.p. 173–175° (dec), λ_{max}^{chf} 5·79, 6·10 μ; λ_{max}^{EtOH} 250, 280 and 289 mμ (log ϵ 4·03, 3·47, 3·44). NMR absorption occurred at 1·29 (3 H, doublet, J = 7 c/s; CH—CH_3), 2·11 (3 H, singlet, OCOCH_3), 2·20 (3 H, singlet, OCOCH_3), 2·35 (3 H, singlet, NCOCH_3), 3·45δ (3 H, singlet, CO_2CH_3), and principal mass spectral peaks were observed at *m/e*: (heated inlet) 424 (M-60), 364, 291, 207, 192; (ion source inlet) 441 (M-43), 424, 365 (M-60-59, intense peak). (Found: C, 64·80; H, 6·64; N, 6·15. $C_{26}H_{32}N_2O_7$ requires: C, 64·45; H, 6·66; N, 5·78%; mol. wt. 484·53.)

$\Delta^{1,2}$-*Decarbomethoxycompactinervine* ($\Delta^{1,2}$-19β,20α-*dihydroxystrychene*, XLIII)

A solution of compactinervine (200 mg) in 2N HCl (4 ml) was heated under N_2 at 120° for 5 hr in a sealed tube. The acid solution was extracted with $CHCl_3$ and the extract discarded. The aqueous layer was made basic with conc. NH_4OH and reextracted with $CHCl_3$ and the extract washed, dried and evaporated giving a residue (134 mg) which after 3 recrystallizations from acetone gave $\Delta^{1,2}$-*decarbomethoxycompactinervine* as colourless prisms (27 mg), m.p. 233–235° (dec), $[\alpha]_D^{20}$ −384° (*c*, 0·44 in $CHCl_3$). The IR spectrum showed λ_{max} 2·85–3·20 (br) and 6·25 μ, and the UV, λ_{max}^{EtOH} 215 and 250 mμ (log ϵ 4·30, 3·80); $\lambda_{max}^{EtOH-HCl}$ 220, 260 and 280 mμ (log ϵ 4·25, 3·45, 3·44). The NMR spectrum exhibited absorption at 1·21 (3 H, doublet, J = 6·5 c/s; CH—CH_3) and 7·1 ~ 7·65δ (4 H, multiplet, aromatic H), and principal mass spectral peaks were observed (ion source inlet)

at *m/e*: 298 (M^+), 281 (M-OH), 253, 205, 182 (base peak). (Found: C, 72·11; 72·65; H, 7·36, 7·33; N, 9·48, 9·62, 9·53. $C_{18}H_{22}N_2O_2$ requires: C, 72·45; H, 7·43; N, 9·39%; mol. wt. 298·37.)

$\Delta^{2,16}$-19β,20α-Diacetoxystrychene (XLIV)

A solution of $\Delta^{1,2}$-decarbomethoxycompactinervine (100 mg) in acetic anhydride (1 ml) and pyridine (2 ml) was kept for 3 days at room temp (22°) then diluted with water, concentrated, made basic with NH_4OH, and extracted with $CHCl_3$. The extract was washed (H_2O), dried ($MgSO_4$) and evaporated to give a residue (80 mg), which after recrystallization from ether–MeOH gave *$\Delta^{2,16}$-19β,20α-diacetoxystrychene* as prisms (50 mg), m.p. 262–263°, λ_{max}^{KBr} 5·75 μ; λ_{max}^{EtOH} 225, 284, 290 and 325 mμ (log ϵ 3·76, 3·39, 3·38, 3·55). NMR absorption bands were observed at 1·33 (3 H, doublet, J = 7·0 c/s, ch—C*H*$_3$), 1·68 and 2·20 (each 3 H, singlet, COC*H*$_3$), 5·32 (1 H, quartet, J = 7·0 c/s, C*H*(OAc)·CH$_3$), 6·35 (1 H, broad singlet, NH—C=C*H*—CH), 8·29δ (1 H, broad singlet, N*H*), and the mass spectrum (heated inlet) showed principal peaks at *m/e*: 382 (M^+), 322 (M-AcOH), 304, 295, 278, 262 (M-2AcOH), 158 (base peak), 157, 156, 152, 144, 143, 130. (Found: C, 69·04; H, 6·94; N, 7·31, 7·13. $C_{22}H_{26}N_2O_4$ requires: C, 69·09; H, 6·85; N, 7·33%; mol. wt. 382·44.)

$\Delta^{2,16}$-Decarbomethoxycompactinervine (*$\Delta^{2,16}$-19β,20α-dihydroxystrychene*, XLV)

(a) *By hydrolysis of $\Delta^{2,16}$-19β,20α-diacetoxystrychene.* The diacetyl derivative (XLIV, 100 mg) was heated under reflux with 10% ethanolic KOH (1 ml) in N_2 until hydrolysis was complete (2 hr as shown by thin layer chromatography). The solution was cooled, diluted in ice-water and extracted with $CHCl_3$. The extract was washed (H_2O), dried ($MgSO_4$) and evaporated to give *$\Delta^{2,16}$-decarbomethoxycompactinervine* as colourless needles (10 mg), m.p. 178–179°, λ_{max}^{EtOH} 272 and 312 mμ (log ϵ 4·15, 3·70); λ_{infl}^{EtOH} 225 mμ (log ϵ 3·75). Principal mass spectral peaks (ion source inlet) were observed at *m/e*: 298 (M^+), 280, 265, 254, 253, 225, 194, 180, 168. (Found: C, 72·16; H, 7·45. $C_{18}H_{22}N_2O_2$ requires: C, 72·45; H, 7·43%; mol. wt. 298·37.)

(b) *By alkaline treatment of $\Delta^{1,2}$-decarbomethoxycompactinervine.* $\Delta^{1,2}$-Decarbomethoxycompactinervine (90 mg) was heated under reflux with 10% ethanolic KOH (4 ml) for 3 hr in an N_2. The solution was poured into ice and water and extracted with $CHCl_3$ and the extract washed, dried and evaporated to give a colourless oil (90 mg) which crystallized from ethyl acetate. Recrystallization from the same solvent gave $\Delta^{2,16}$-decarbomethoxycompactinervine as colourless crystals, identical to the product of the preceding reaction by mixture m.p. and thin layer chromatographic behaviour.

Reduction of $\Delta^{1,2}$-decarbomethoxycompactinervine with alkaline potassium borohydride

A solution of $\Delta^{1,2}$-decarbomethoxycompactinervine (100 mg) in 10% methanolic KOH (5 ml) was treated with KBH_4 (200 mg). The mixture was refluxed for 30 min, cooled, diluted with water (20 ml) and extracted with $CHCl_3$ (300 ml). The extract was washed, dried and evaporated to give a crystalline solid (60 mg) which consisted of 4 components. Thin layer chromatography in MeOH–acetone on silica gel enabled separation, and the third component (in decerasing order of mobility) was obtained crystalline (25 mg) and recrystallized from ethyl acetate as colourless cubes (8 mg), m.p. 230–232°. The indolic UV spectrum of this unstable compound showed λ_{max}^{EtOH} 229, 283 and 291 mμ (log ϵ 4·60, 3·92, 3·90) and mass spectral peaks at *m/e*: 300 (M^+), 282 (M-18), 264, 237 (70%), 225 (base peak).

Oxidation of compactinervine with periodic acid

An ice-cold solution of compactinervine (100 mg) in MeOH (1 ml) and pyridine (3 ml) was treated with para-periodic acid (300 mg) in water (1 ml), and allowed to stand at room temp (22°) for 5 min. The solution was then added to dil. NH_4OH (30 ml) and ethyl acetate (100 ml) and the ethyl acetate layer separated, washed (H_2O), dried ($MgSO_4$) and evaporated to give a residue (30 mg). Thin layer chromatography enabled separation (in order of decreasing mobility) of 3 substances. The first (8 mg) crystallized from acetone in needles, m.p. 94–95°, λ_{max}^{KBr} 5·85, 5·97, 6·24 μ; λ_{max}^{EtOH} 229, 296 and 325 mμ (log ϵ 3·96, 3·93, 4·07); λ_{min}^{EtOH} 210, 258 and 304 mμ (log ϵ 3·85, 3·29 and 3·90), with mass spectral molecular ion at *m/e* 312. The second (2 mg) crystallized from $CHCl_3$ in needles, m.p. 245–260° (dec), mass spectral molecular ion at *m/e* 296. The third substance was identified as starting material.

The dil. ammoniacal solution (above) was acidified with 50% H_2SO_4 with cooling while a stream of N_2 was passed first through the solution and secondly through a methanolic solution of 2,4-dinitrophenylhydrazine in 2N-H_2SO_4. The resulting DNP (20 mg) was washed and recrystallized from EtOH to give acetaldehyde 2,4-dinitrophenylhydrazone as yellow needles, m.p. 146–148°, indentical to an authentic sample by IR, and by paper (decalin-DMF, Whatman No. 3 MM) and vapour-phase (acid washed SE30) chromatographic comparison.

A blank experiment under identical conditions gave no acetaldehyde. When the oxidation was conducted in aqueous solution the yield of acetaldehyde-DNP was 80%.

19,20-*Epoxyakuammicine* (XLVI)

A solution of compactinervine (350 mg) in dry pyridine was treated with methanesulphonyl chloride (0·2 ml) and kept at room temp for 3 hr. The solution was then poured into 1N-HCl and ice, extracted with ether to remove non-basic material, then rendered basic with NH_4OH and extracted with ethyl acetate. The ethyl acetate extract was washed (H_2O), dried and evaporated to give a residue (250 mg) which after recrystallization from acetone and ethyl acetate gave 19,20-*epoxyakuammicine* as colourless needles (120 mg) m.p. 200–203° raised by further recrystallization to 206–207°, $[\alpha]_D^{25}$ −664° (*c*, 0·28 in $CHCl_3$). The IR spectrum showed ν_{max}^{KBr} 3330 (sharp), 1660 cm^{-1}, and the UV, λ_{max}^{EtOH} 235, 300 and 330 mμ, (log ϵ 4·00, 3·98, 4·20). NMR absorption was observed at 1·4 (3 H, doublet, CH—CH_3), 3·10 (1 H, quartet, epoxide H), 3·75δ (3 H, singlet, CO_2CH_3), and principal mass spectral peaks (heated inlet) occurred at *m/e*: 338 (M^+), 320 (M-H_2O), 394 (M-C_2H_4O). (Found: C, 71·06; H, 6·76; N, 8·10. $C_{20}H_{22}N_2O_3$ requires: C, 70·98; H, 6·55; N, 8·28%; mol. wt. 338·39.)

Attempted transformation of 19,20-*epoxyakuammicine into* $\Delta^{19,21}$-1-*acetyl*-16-*methylenestrychene*

(a) *Lithium aluminum hydride reduction of the epoxide.* A mixture of 19,20-epoxyakuammicine (50 mg), LAH (40 mg) and tetrahydrofuran (3 ml) was heated under reflux for 16 hr. After decomposition of the excess hydride and its complex with moist ether and sat. Na_2SO_4aq, the mixture was extracted with ethyl acetate (180 ml). Evaporation of the ethyl acetate gave a residue (40 mg) which was separated into 2 principal oily components by thin layer chromatography in dimethylformamide on silica gel. The first (25 mg) which had no carbonyl absorption in the IR and showed λ_{max}^{EtOH} 245 and 295 mμ (log ϵ 3·69, 3·51), with mass spectral molecular ion peak at *m/e* 296 is formulated as 20α-hydroxy-16-methylenestrychane XLVII.

The second component (5 mg) exhibited the same UV spectrum but had the molecular ion peak at *m/e* 314 and may be formulated as 16-hydroxymethylene-20α-hydroxystrychane.

(b) *Acetylation of* 20α-*hydroxy*-16-*methylenestrychane.* A solution of XLVII (40 mg) in acetic anhydride (0·1 ml) and pyridine (2 ml) was kept at room temp for 48 hr and then poured into water (15 ml). The mixture was made basic with NH_4OH and extracted with $CHCl_3$ and the extract washed, dried and evaporated to give a residue (45 mg) from which an amorphous substance formulated as 20α-*acetoxy*-1-*acetyl*-16-*methylenestrychane* (XLVIII) was separated by repeated thin layer chromatography on silica gel in MeOH–acetone. The product showed λ_{max}^{chf} 5·75 and 6·00 μ and had NMR absorption at 0·85 (3 H, triplet, J = 7 c/s, CH_2CH_3), 1·25 (2 H, singlet), 2·10 (3 H, singlet, $OCOCH_3$), 2·18 (3 H, singlet, $NCOCH_3$), 4·93 (1 H, broad singlet, —CH_2), and 5·05δ (1 H, broad singlet, =CH_2). The mass spectral molecular ion was observed (ion-source inlet) at *m/e* 380.

(c) *Alkaline hydrolysis of* 20α-*acetoxy*-1-*acetyl*-16-*methylenestrychane.* The above diacetate (100 mg) was heated under reflux in 5% ethanolic KOH (6 ml) during 30 min, then poured into ice and water and extracted with $CHCl_3$. The extract was washed, dried and evaporated giving a residue (80 mg) from which the principal fraction (10 mg) was isolated by thin layer chromatography MeOH–acetone; silica gel) and recrystallized repeatedly from ethyl acetate to give 1-*acetyl*-20α-*hydroxy*-16-*methylenestrychane* (XLIX); as colourless needles, m.p. 115–116°, λ_{max}^{chf} 6·06 μ. NMR absorption occurred at 0·98 (3 H, triplet, J = 6·5 c/s, CH_2CH_3), 2·30 (3 H, singlet, $NCOCH_3$), 4·86 (1 H, broad singlet, =CH_2) and 4.98δ (1 H, broad singlet, =CH_2) while the mass spectrum exhibited peaks at *m/e*: 338 (M^+), 295 (M-CH_2CO), 281.

Attempted dehydration of this alcohol with $POCl_3$ in pyridine at 10° gave only starting material.

(d) *Mesylation of* 20α-*hydroxy*-16-*methylenestrychene.* A solution of XLVII (58 mg) in dry pyridine (5 ml) was treated with methanesulphonly chloride (0·3 ml) and kept at room temp (22°) for 30 min. The mixture was then poured into ice and water, acidified with HCl aq and extracted

with ethyl acetate. The aqueous layer was rendered basic with NH_4OH and extracted with $CHCl_3$ and the extract washed (H_2O), dried ($MgSO_4$) and evaporated giving a residue (57 mg). Preparative thin-layer chromatography on silica gel in MeOH–acetone enabled separation of 1-*mesyl*-20α-*hydroxy*-16-*methylenestrychane* (L, 5 mg), as an amorphous solid, λ_{max}^{chf} 7·4 (s), 8·65 (s) μ with NMr absorption at 0·99 (3 H, triplet, J = 6·5 c/s, CH_2CH_3), 2·90 (3 H, singlet, SO_2CH_3), 3·52 (2 H, broad singlet), 4·70 (1 H, broad singlet, $-NCHC=$), 5·10 (1 H, singlet, $=CH_2$) and 5·50δ (1 H, singlet, $=CH_2$).

(e) *Acetylation of* 1-*mesyl*-20α-*hydroxy*-16-*methylenestrychane.* A solution of L (13 mg) in dry pyridine (2 ml) and acetic anhydride (0·5 ml) was kept at room temp (22°) for 17 hr. The mixture was diluted with water, made basic with NH_4OH and extracted with $CHCl_3$. The extract was washed, dried and evaporated to give an oily residue (LI, 16 mg), homogeneous on thin layer chromatography and showing λ_{max}^{chf} 5·8 and 7·4 μ, and NMR absorption at 0·85 (3 H, triplet, J = 7·0 c/s, CH_2CH_3), 2·10 (3 H, singlet, $OCOCH_3$), 2·90 (3 H, singlet, SO_2CH_3), 3·52 (1 H, multiplet), 4·59 (1 H, singlet, $-NCHC=$), 5·15 (1 H, singlet, $=CH_2$) and 5·59δ (1 H, singlet, $=CH_2$).

19-*Epicompactinervine from akuammicine*

A mixture of akuammicine (50 mg), dry pyridine (1 ml) and OsO_4 (50 mg) was stirred at room temp under N_2 for 24 hr. $NaHSO_3$ (100 mg) and water (1 ml) were then added and after 1 hr the solution was basified with NH_4OH and extracted with $CHCl_3$. The extract was washed, dried, and evaporated to give a residue (45 mg) which on recrystallization from EtOH gave 19-*epicompactinervine* (LII) as needles (12 mg), m.p. 222–224° (dec), $[\alpha]_D^{25}$ −640° (*c*, 0·15 in pyridine); λ_{max}^{KBr} 3·50, 5·98, 6·31 μ; λ_{max}^{EtOH} 235, 295 and 325 mμ (log ϵ 4·00, 3·95, 4·18).

The mass spectrum exhibited the molecular ion peak at *m/e* 356 and showed close similarity to that of compactinervine. (Found: C, 67·23; H, 6·79; N, 8·01. $C_{20}H_{25}O_4N_2$ requires: C, 67·39; H, 6·79; N, 7·86%; mol. wt. 356·428.)

19-*Dehydrocompactinervine* (LIII)

(a) *By oxidation of compactinervine.* Chromium trioxide (13·35 g) was dissolved in H_2SO_4 (11·5 ml) and water (20 ml) and the solution made up to 50 ml with water. This solution (0·4 ml) was added with stirring and ice cooling to a solution of compactinervine (200 mg) in acetone under N_2. After 12 min the mixture was poured into ice, conc. NH_4OH (1 ml), MeOH (1 ml) and $CHCl_3$ (100 ml). The $CHCl_3$ layer was separated, the aqueous layer reextracted with $CHCl_3$ and the combined $CHCl_3$ extracts washed (H_2O), dried ($MgSO_4$), and evaporated to give a residue (140 mg). Thin layer chromatography (silica gel with MeOH–acetone) enabled separation of the most mobile component (20 mg) which on repeated crystallization from MeOH–acetone gave 19-*dehydrocompactinervine* as fine needles (7 mg), m.p. 220–225° (dec) $[\alpha]_D$ −589° in $CHCl_3$); λ_{max}^{KBr} 5·91, 5·99, 6·06, 6·29 μ. NMR absorption was observed at 2·37 (3 H, singlet, $COCH_3$), 3·70 (3 H, singlet, CO_2CH_3) and 8·91δ (1 H, singlet, N*H*), and principal mass spectral peaks at *m/e*: 354 (M^+), 311 (M-$COCH_3$), 283 (96%), 268, 226 (91%), 209 (76%), 194, 180, 167 (base peak), 154, 140, 129 (88%), 125.

Unchanged compactinervine (80 mg) was recovered from the reaction.

(b) *By oxidation of* 19-*epi-compactinervine* (LII). Chromic acid reagent (see above, 0·02 ml) was added with stirring at 4° to a solution of 19-epicompactinervine (10 mg) in acetone (2 ml) and $CHCl_3$ (0·5 ml) under N_2. After 12 min the mixture was poured into ice and NH_4OH, shaken and extracted with $CHCl_3$. The extract was washed (H_2O), dried ($MgSO_4$), and evaporated giving a residue (7 mg) from which the more mobile of 2 components was separated by thin layer chromatography. The compound (2 mg) recrystallized from MeOH gave 19-dehydrocompactinervine (1 mg) as prisms, m.p. 224–226° (dec; Kofler), 220–225° (dec; capillary) undepressed on admixture with a sample of the oxidation product from campactinervine. The product showed $[\alpha]_D^{25}$ −607° (*c*, in $CHCl_3$), IR, NMR and mass spectrum identical to material prepared by method (a).

N-*Acetyl*-11-*hydroxyaspidospermatidine* (VII)

Direct application of the EtOH-free extract (50 mg) of *A. compactinervium* on a thin layer chromatogram using silica gel G and MeOH enables the separation of amorphous N-*acetyl*-10-*hydroxyaspidospermatidine* (5 mg), with lower R_f than that of compactinervine.[10] The base exhibited ν_{max}^{chf} 3226 and 1639 cm^{-1}; λ_{max}^{EtOH} 218, 252, 294 and 300 mμ (log ϵ 4·23, 3·89, 3·65, 3·65); λ_{infl}^{EtOH} 260 mμ (log ϵ 3·80); λ_{min}^{EtOH} 238 and 272 mμ (log ϵ 3·74, 3·34). The mass spectrum (ion source

inlet) showed principal peaks at *m/e*: 324 (M^+), 309 (M-15), 281 (M-Ac), 160, 146, 136 (base peak), 123.

N-*Acetyl*-11-*methoxyaspidospermatidine* (LVII)

The alkaloid, VII (4 mg), was treated with excess diazomethane in MeOH for 10 hr, more diazomethane added and the solution kept for a further 5 hr. Evaporation gave the chromatographically pure O-methyl ether (LVII) which exhibited principal mass spectral peaks at *m/e*: 338 (M^+), 323 (M-15), 295 (M-Ac), 215 (M-123), 174, 173, 160, 136, 123. The UV spectrum was identical with that of the phenol VII.

N-*Acetyl*-14,19-*dihydro*-11-*methoxyaspidospermatidine* (LX)

The preceding O-methyl ether (LVII, 3 mg) was hydrogenated over reduced PtO_2 (5 mg) in EtOH (10 ml) at 20° to give a colourless amorphous dihydro-derivative (LX, 3 mg). The chromatographically pure product had UV absorption identical to that of the original alkaloid (VII), and showed principal mass spectral peaks at *m/e*: 340 (M^+, 7·6%), 325 (M-15), 312 (M-28), 299 (1·9%), 284 (2·8%), 271 (1·0%), 254, 215, 186, 176, 160, 138 (base peak), 110.

11-*Methoxy*-14,19-*dihydrocondylocarpine* (VIII)

The EtOH-free extract (900 g) of *A. populifolium* yielded an acid benzene extract (7·0 g) which was chromatographed on neutral alumina (activity I). Early fractions gave refractine and some related alkaloids also found in the acid and pH 7 $CHCl_3$ extracts (see below). Middle fractions yielded a new amorphous alkaloid (7 mg), ν_{max}^{chf} 1764, 1672–1661 cm^{-1}; λ_{max}^{EtOH} 253 mμ (log ε 4·00); λ_{infl}^{EtOH} 290 mμ (log ϵ 3·43); λ_{min}^{EtOH} 236 mμ (log ϵ 3·87), with principal mass spectral peaks at 354 (M^+), 341, 324 (M-30), 312, 309, 294 (M-60), 281, 267, 253, 251, 216, 206, 186, 176, 173, 156, 149, 143, 135, 125, 123, 110, 109, 105. Later fractions from the column eluted with $CHCl_3$ and $CHCl_3$–MeOH furnished 3 alkaloids which were separated by repeated thin layer chromatography. The most mobile of these, 11-*methoxy*-14,19-*dihydrocondylocarpine* (VIII, 23 mg) was amorphous exhibiting blue fluorescence in UV light, $[\alpha]_D^{29}$ +213 (*c*, 0·47 in $CHCl_3$), ν_{max}^{chf} 3378, 1672, 1600 cm^{-1}; λ_{max}^{EtOH} 255, 286 and 327 mμ (log ϵ 4·17, 4·04, 4·05); λ_{min}^{EtOH} 275 and 310 mμ (log ϵ 3·98, 4·01). The NMR spectrum showed absorption at 1·70 (3 H, triplet, J = 7 c/s, CH_3CH_2), 3·74 (6 H, singlet, OCH_3 and CO_2CH_3), 6·20 to 6·50 (2 H, multiplet, aromatic H), 6·98 (1 H, doublet, J = 9 c/s, aromatic H), and 8·80δ (1 H, broad singlet, NH). The mass spectrum had principal peaks at 354 (M^+), 339, 323, 311, 297 (M-57), 283, 270, 259 (base peak), 224, 212, 177, 154, 140, 123 and 107.

11-*Methoxy*-2,16,14,19-*tetrahydrocondylocarpine* (LXII)

The alkaloid, VIII (15 mg), was treated portionwise with Zn (ca. 1 g) and H_2SO_4 (0·5 ml) in MeOH (15 ml) during 1·5 hr under reflux. The mixture was heated for a further 1 hr, filtered, evaporated, rendered alkaline with K_2CO_3 and extracted with ether. The dried extract gave 11-*methoxy*-2,16,14,19-*tetrahydrocondylocarpine* (LXII) as a chromatographically homogeneous oil (12 mg), scarlet ceric sulphate reaction, λ_{max}^{EtOH} 246 and 302 mμ (log ϵ 3·73, 3·67); λ_{min}^{EtOH} 270 mμ (log ϵ 3·14). The mass spectrum showed principal peaks at *m/e*: 356 (M^+), 328, 311, 297, 295, 281, 271, 257, 196 (base peak), 185, 174, 173, 168, 160 and 113.

LXII (10 mg) was treated with acetic anhydride (1 ml) and pyridine (1 ml) at room temp. After standing overnight the solution was evaporated in vacuum and the residue partitioned between ether and K_2CO_3aq. The dried ethereal layer gave amorphous N-*acetyl*-11-*methoxy*-2,16,14,19-*tetrahydrocondylocarpine* (LXIV, 9 mg), transient pink ceric sulphate reaction, λ_{max}^{EtOH} 252 and 300 mμ (log ϵ 4·80, 4·61); λ_{infl}^{EtOH} 325 mμ (log ϵ 4·09); λ_{min}^{EtOH} 238 and 272 mμ (log ϵ 4·70 and 4·21). The NMR spectrum exhibited absorption at 0·75 (3 H, ill defined triplet, CH_3CH_2), 2·38 (3 H, singlet $NCOCH_3$), 3·52 (3 H, singlet), 3·80 (3 H, singlet), and 6·00–7·30δ (3 H, multiplet, aromatic H), while principal mass spectral peaks were found at *m/e*: 398, 370, 356, 328, 325, 312, 299, 283, 256, 241, 196, 174, 168, 149, 113.

Isolation of aspidofractinine-type alkaloids from A. populifolium

The acid $CHCl_3$ extract (35 g), from 900 g EtOH–extract, (see above), contains a number of closely similar alkaloids and complete separation by column chromatography on alumina (activites I and IV)

or silica gel, partition chromatography on celite[33] or paper chromatography (Whatman No. 1 with 2% formamide in acetone) was not successful. The benzene and benzene–ether fractions from alumina chromatography yielded however refractine (XV, 7 g) by recrystallization from MeOH. The mother liquors from the above crystallizations were evaporated and a portion of the residue (260 mg) submitted to thin layer chromatography on silica gel G giving, in order of decreasing polarity, refractine (XV, 98 mg), N-*formyl*-17-*methoxyaspidofractinine* (X, 89 mg crude), N-*formyl*-16,17-*dimethoxyaspidofractinine* (XII, 54 mg crude), 17-*methoxyaspidofractinine* (IX, 19 mg crude), and 16-17-*dimethoxyaspidofractinine* (XI, 25 mg crude).

Refractine (XV) had $[\alpha]_D^{22}$ −11° (*c*, 0·9 in $CHCl_3$) and m.p. 185–186°, undepressed on admixture with a sample from *A. refractum*.[14] The IR, UV and NMR spectra and chromatoplate mobility were identical with those of the authentic sample.

N-Formyl-17-methoxyaspidofractinine (X) was purified further by repeated thin layer chromatography to give the amorphous base (15 mg) ceric sulphate reaction purple fading to maroon, ν_{max}^{chf} 1672 cm^{-1}; λ_{max}^{EtOH} 255 mμ (log ε 4·04); λ_{infl}^{EtOH} 288 mμ (log ε 3·42); λ_{min}^{EtOH} 237 mμ (log ε 3·82). The principal mass spectral peaks appeared at *m/e*: 338 (M^+), 310 (M-28), 124 and 109 (base peak).

N-Formyl-16,17-dimethoxyaspidofractinine (XII) was purified by further thin layer chromatography to give the amorphous base (9 mg), ceric sulphate reaction deep purple fading rapidly to fawn, ν_{max}^{chf} 1661 cm^{-1}; λ_{max}^{EtOH} 253 and 287 mμ (log ε 3·86, 3·42); λ_{min}^{EtOH} 245 and 275 mμ (log ε 3·85, 3·38). The principal mass spectral peaks appeared at *m/e*: 368 (M^+), 340 (M-28), 124 and 109 (base peak).

17 Methoxyaspidofractinine (IX) after further chromatographic purification separated from acetone as colourless crystals (10 mg), m.p. 128–130°, $[\alpha]_D^{25}$ +3° (*c*, 0·67 in $CHCl_3$). This alkaloid was identical by IR, UV, and mass spectrum and chromatographically with an authentic sample (O-methyldeacetylaspidofiline) prepared from aspidofiline.[15b] The rotation is opposite in sign (lit.[15b] $[\alpha]_D^{27}$ −7·7°).

The acid benzene extract (see above) also yielded XV, X, IX, XI and kopsinine (XIII). The pH 11 $CHCl_3$ extract (11 g from 900 g EtOH extract of the plant) could not be successfully resolved by column chromatography, although (−)-aspidofractine (0·8 g), m.p. 195–196°, $[\alpha]_D^{24}$ −128° (*c*, 1·3 in $CHCl_3$) was isolated from early fractions and shown to be identical to an authentic specimen[14] by IR, UV, NMR, thin layer chromatography and mixture m.p. Preparative thin layer chromatography on silica gel G gave 16,17-dimethoxyaspidofractine (XI) which crystallized from acetone as colourless crystals, m.p. 140–142°, $[\alpha]_D^{27}$ −141° (*c*, 2·61 in $CHCl_3$). The alkaloid showed no carbonyl absorption in the IR while the UV spectrum showed absorption at λ_{max}^{EtOH} 214 and 298 mμ (log ε 4·50, 3·39); λ_{min}^{EtOH} 267 mμ (log ε 3·00). The NMR spectrum exhibited peaks at the following values: 3·80 (6 H, singlet, 2 OCH_3), 6·28 (1 H, doublet, J = 9 c/s, aromatic H), 6·86δ (1 H, doublet, J = 9 c/s, aromatic H), while principal mass spectral peaks appeared at *m/e*: 340 (M^+), 312 (M-28, 46%), 218, 170, 124, and 109 (base peak).

From the same separation kopsinine (XIII), m.p. 102–103° was isolated and identified with an authentic sample[34,35,14] by IR, UV and mass spectra as well as thin layer chromatography and mixture m.p.

Hydrolysis of the N-*formyl-bases* X *and* XII

N-Formyl-17-methoxyaspidofractine (X, 3 mg) was heated with 2 N HCl for 1·5 hr at 100°. The cooled solution was basified with K_2CO_3 and extracted with ether to give IX (2 mg) m.p. 127–130° alone or admixed with the naturally occurring alkaloid, IX.

N-Formyl-16,17-dimethoxyaspidofractine (5 mg) similarly gave XI which was purified by thin layer chromatography and recrystallized from acetone when it had m.p. 139–142° undepressed on admixture with natural XI. In both cases identity of the deformyl products was confirmed chromatographically.

Isolation of alkaloids from A. olivaceum

The acid benzene (1·1 g) and $CHCl_3$ extracts (12·0 g) (corresponding to 2·5 k of trunk bark)

[33] K. S. Brown and S. M. Kupchan, *J. Chromat.* **9**, 71 (1962).
[34] W. D. Crow and M. Michael, *Austr. J. Chem.* **8**, 129 (1955).
[35] W. G. Kump and H. Schmid, *Helv. Chim. Acta* **44**, 1503 (1961).

were combined and chromatographed on neutral alumina (activity I). Elution with benzene, benzene–ether and pure ether gave an amorphous mixture (6·5 g) which on crystallization from MeOH gave uleine (3·2 g), m.p. 77–98°, $[\alpha]_D^{27}$ +16·9° (*c*, 0·39 in $CHCl_3$), identical to an authentic sample by mixture m.p., chromatographic, IR and UV comparison.

The MeOH filtrate from the above crystallization yielded a solid residue which was crystallized from acetone to give (−)-apparicine (0·41 g) as colourless needles, m.p. 188–191° (phase change at 160°), $[\alpha]_D^{27}$ −179° (*c*, 2·16 in $CHCl_3$), showing identical behaviour on chromatographic, IR, UV, and mass spectral comparison with (+)-apparicine from *A. dasycarpon*.

Later chromatographic fractions yielded further amounts of uleine, apparicine and traces of aspidocarpine (see below).

Thin layer chromatographic separation of the alkaloids of the pH 7 $CHCl_3$ extract enabled isolation of aspidocarpine (20 mg) as colourless crystals, m.p. 164–165°, $[\alpha]_D^{27}$ +139° (*c*, 1·38 in $CHCl_3$), identical to an authentic sample by chromatographic, IR, UV and mass spectral comparison.

Trituration of the pH 11 extract with acetone gave directly olivacine as yellow crystals, m.p. 295–300°, identical to an authentic sample by chromatographic, IR and UV comparison.

Isolation of alkaloids from A. gomezianum

The acid $CHCl_3$ extract from *A. gomezianum* trunk bark (10·3 k) gave on evaporation 6·0 g resin which was dissolved in ether, washed with NH_4OH, dried, and treated with sat. ethereal oxalic acid. The ethereal solution was decanted from the gummy oxalate. A portion of the oxalate (1·5 g) was reconverted to free base with ammonia and ether giving a clear orange amorphous mixture of three principal alkaloids, which was chromatographed on silica gel. Benzene–ether eluted apparicine (20 mg), m.p. 188–191° from ether identical to an authentic sample by chromatographic, IR, UV and mass spectral comparison. Treatment of the mixed free bases (0·5 g) with MeOH resulted in the crystallization of uleine methanolate (0·1 g) m.p. ca. 80°, which after recrystallization from MeOH gave the solvate as colourless prisms, m.p. 80–124°, identical to an authentic sample by IR, UV and chromatographic comparison. The remaining mixed alkaloids were submitted to thin layer chromatographic separation on silica gel G, the fastest moving fraction yielding V (10 g) as a tan-coloured oil, which was identified by IR, UV and mass spectral comparison with an authentic sample (see below and Refs. 5, 6).

Isolation of alkaloids from A. eburneum

The acid $CHCl_3$ extract from *A. eburneum* bark (18 k) was treated in $CHCl_3$ solution with excess oxalic acid to give a crude gummy oxalate (A, 15 g) and a $CHCl_3$ filtrate (B). Solution of A in MeOH resulted in the crystallization of *uleine oxalate* (1·2 g) m.p. 200–205° dec from which the free base uleine was prepared and, after passage over alumina and several recrystallizations from MeOH, obtained as colourless crystals of the methanolate (0·52 g), m.p. 78–80°, identical by IR and chromatographic comparison with an authentic sample. A further amount of uleine oxalate (9·3 g) was isolated from the mother liquors of the above crystallization. Also the pH 7 chloroform extract (8·5 g) gave total ether-soluble alkaloids (5·5 g) from which pure uleine 2.74 g) was isolated as its methanolate.

The $CHCl_3$ filtrate, B (above) was evaporated and the residue partitioned between ether (200 ml) and NH_4OH solution. The aqueous layer was separated, repeatedly extracted with ether, and the combined extracts, washed (H_2O), dried ($MgSO_4$) and treated with a solution of oxalic acid in ether to complete precipitation. The gummy residue (8·0 g) was recovered to free base (7·4 g) and chromatographed on silica gel (140 g). Fractions eluted with ethyl acetate and ethyl acetate–MeOH (100:1) gave an oily mixture (0·79 g), separated by thin layer chromatography into 2 principal components. The more mobile (28 mg) was obtained as a tan oil, $[\alpha]_D^{29}$ +119° (*c*, 0·4 in $CHCl_3$), identical to XIX by IR, UV, NMR and mass spectral comparison. The more polar (9 mg) also obtained as a tan oil was identified as V by its characteristic mass spectral peaks (heated inlet) at *m/e*: 324 (M^+), 296, 281, 152, 144, 130 and 124 (base peak).

Fractions eluted with ethyl acetate–MeOH (20:1) gave a semi crystalline residue (460 mg) which crystallized from MeOH–water to give (−)-apparicine as colourless prisms, m.p. 195–198°, ORD, negative, λ_{max}^{nujol} 3·14, 6·25, 9·0, 11·34, 12·2, 13·6 μ, identical by IR, UV, NMR and mass spectral comparison with a specimen isolated from *A. olivaceum* (above). (Found: C, 81·48; H, 7·50; N, 10·71; C—CH_3, 5·12%; mol. wt. (mass spec.) 264. Calc. for $C_{18}H_{20}N_2$: C, 81·78; H, 7·63; N, 10·60; IC—CH_3, 5·68%; mol. wt., 264·36.)

Fractions eluted with ethyl acetate–MeOH (10:1) gave uleine (3·0 g).

The pH 9 $CHCl_3$ extract was evaporated in the presence of acetic acid (2 ml) and the residue (9·5 g) partitioned between ether (200 ml) and NH_4OH separated, and the aqueous layer extracted with ether (5 × 200 ml). The combined ethereal extracts were washed (H_2O), dried ($MgSO_4$) and evaporated giving an oily base (1·8 g) which on solution in MeOH deposited olivacine (0·5 g) as yellow rhombs, m.p. 318 (dec) identical to an authentic specimen[86] by IR and mixture m.p. comparison.

Isolation of the alkaloids of A. subincanum

Trunk bark and cork (3 k) gave a resinous ethanolic extract (254 g). The acidic $CHCl_3$ extract of this was evaporated and partitioned between ether (300 ml) and dil. NH_4OH (pH 8). The ethereal layer was washed (H_2O) and treated with a sat. solution of oxalic acid in ether to complete precipitation. The precipitated oxalate (22 g) was distributed between NH_4OH and ether, the ether layer yielding mixed free bases (11·4 g), from which uleine methanolate (8·0 g), $[\alpha]_D^{25}$ +11° (*c*, 1·0 in $CHCl_3$) crystallized on solution in MeOH.

A similar isolation procedure enabled the separation of crude oxalate (1·2 g) from the basic $CHCl_3$ extract (3·4 g). Recovery to free base and fractional crystallization from MeOH gave a small amount of uleine and olivacine (28 mg), m.p. 315–318°, identical by IR and mixture m.p. comparison with an authentic sample.[86]

Isolation of the alkaloids of A. multiflorum *A.DC*

The acidic benzene and $CHCl_3$ and pH 7 $CHCl_3$ extracts were identical chromatographically and were combined (15 g, from 300 g EtOH extract) and chromatographed on alkaline alumina (activity I). Benzene, benzene–ether and ether eluted fractions from which uleine was isolated as its methanolate (8 g) by recrystallization from MeOH. Thin layer chromatography of the bases accompanying uleine in the early chromatographic fractions enabled separation of an amorphous and a crystalline base. The amorphous base, $[\alpha]_D^{27}$ +53° (*c*, 2·47 in $CHCl_3$) showed λ_{max}^{chf} 2·90 μ (no carbonyl absorption), and λ_{max}^{EtOH} 217, 283, 292 and 315 mμ (log ϵ 4·57, 3·82, 3·84, 3·70); λ_{min}^{EtOH} 254, 287 and 312 mμ (log ϵ 3·60, 3·81, 3·69); $\lambda_{max}^{EtOH-HCl}$ 211, 282, 289 and 310 mμ (log ϵ 4·67, 3·82, 3·83, 3·57); $\lambda_{min}^{EtOH-HCl}$ 250, 286 and 296 mμ (log ϵ 3·56, 3·75, 3·53); $\lambda_{infl}^{EtOH-HCl}$ 240 and 275 mμ (log ϵ 3·62, 3·77). NMR absorption was observed at 0·99 (3 H, triplet, J = 7 c/s, CH_3CH_2), 2·24 (3 H, singlet, N—CH_3) 4·03 (1 H, multiplet, Ar—C*H*—N), 4·93 and 5·20 (two 1 H singlets, =CH_2), 7·00–7·90 (4 H, multiplet, 4 aromatic H), 8·33–8·58δ (1 H, broad, NH). Principal mass spectral peaks occurred at *m/e*: 266 (M^+), 251, 237, 209, 203, 194, 180, 167. The crystalline base, m.p. 102–103° was identical to Kopsinine (XIII) by mixture m.p., IR UV and chromatographic comparison.

Thin layer chromatographic separation of other alkaloids present in the crude uleine as isolated above gave apparicine, m.p. 188–192°, $[\alpha]_D^{25}$ −126° (*c*, 1·14 in $CHCl_3$) identical to an authentic sample by IR, UV and chromatographic comparison.

A further portion of the combined acidic and neutral $CHCl_3$ extracts (300 g) was filtered through a short alumina column and the most polar material (6 g), eluted with benzene and benzene–ethyl acetate (4:1) distributed between 1N HCl acid and ether. The acid-soluble fraction was recovered to free base (2 g) and separated by thin layer chromatography (1 m plate, silica gel H, benzene–ethyl acetate–EtOH, 2:2:1). One component was obtained homogeneous and identified as the amorphous dasycarpidone[15f] by IR and mass spectral comparison.

Isolation of the alkaloids of A. nigricans

The dried trunk bark (18·4 k) yielded a resinous ethanolic extract (1 k), which was submitted to the standard extraction procedure, with the difference that a semi-solid precipitate (125 g) was collected after neutralization of the aqueous solution to pH 7 ($NaHCO_3$). The acidic $CHCl_3$ extract (17 g) was dissolved in MeOH and a yellow crystalline base (1·2 g) separated which after repeated recrystallization from MeOH and then ethyl acetate gave *olivacine*-N-*oxide* as lemon yellow crystals 125 mg) m.p. 304–305° (dec), ν_{max}^{nujol} 3240 (w), 1605 (w), 1410 (m), 1310 (m), 1248 (m), 1184 (s), 825 (w) 840 (w), 825 (w), 800 (m), 762 (m), 738 (s) cm^{-1}; λ_{max}^{EtOH} 236, 252, 300, 311, 330 and 345 mμ (ϵ 16350, 14280, 51490, 65420, 5420, 5300); $\lambda_{max}^{EtOH-HCl}$ 242, 308 and 354 mμ (ϵ 25060, 75220, 4295). The UV

[86] We are indebted to Dr. Venancio Deulofeu of the University of Buenos Aires for authentic samples of olivacine and (−)-guatambuine.

spectrum showed no shift in alkaline solution. NMR absorption was observed in CF_3CO_2H solution at 2·75 (3 H, singlet, Ar-CH_3), 3·22 (3 H, singlet, Ar—CH_3), and 7·2–8·2δ (7 H, multiplet, aromatic H) and principal mass spectral peaks occurred at *m/e*: 262 (M^+, 50%), 246 (M-16, 100%), 245 (100%), 230, 131, 123, 109. The alkaloid is unstable to moist air but may be stored in vacuum. (Found. C, 72·84; H, 5·88; N, 10·4. $C_{17}H_{16}N_2O \cdot H_2O$ requires: C, 72·84; H, 5·75; N, 9·99%; mol. wt, 262·30.)

The mother liquors were evaporated to give a dark resinous mixture (16·5 g) of which a 3 g portion was chromatographed on alkaline alumina (120 g). Elution with $CHCl_3$ gave successively (+)-guatambuine (1·0 g), olivacine-N-oxide (30 mg) and olivacine (22 mg). Subsequent fractions eluted with ethyl acetate–MeOH (1:1) yielded 4 alkaloids, separable by thin layer chromatography (silica gel, butanol–acetic acid–water, 4:1:5), which are at present under study. (+)-Guatambuine and olivacine were identical to authentic samples by mixture m.p., IR, UV and chromatographic comparison. The semi-solid precipitate (125 g, above) and the neutral, pH 9 and pH 11 $CHCl_3$ extracts yielded (+)-guatambuine, olivacine and small amounts of olivacine-N-oxide as principal components.

Reduction of olivacine-N-oxide. Zn dust (10 mg) was added to a solution of the N-oxide (25 mg) in MeOH (15 ml) and conc. HCl (0·4 ml). After stirring at room temp for 6 hr more Zn dust (10 mg) was added and stirring continued for 7 hr. The mixture was then cooled in ice, basified with 10% NaOHaq and extracted with ethyl acetate (10 × 10 ml). The ethyl acetate extract was evaporated and the residue (20 mg) recrystallized from ethyl acetate–MeOH giving olivacine hydrochloride as yellow crystals (10 mg), m.p. 316–318° (dec) identical to an authentic sample by mixture m.p., chromatographic and IR comparison.

N-Oxidation of olivacine. A solution of perbenzoic acid in $CHCl_3$ was added dropwise to a solution of olivacine (10 mg) in N,N-dimethylformamide (3 ml) stirred at room temp. The concentration of N-oxide present was followed on analytical chromatoplates and reached an optimum value after the addition of ca. 25 mg of perbenzoic acid (0·3 ml solution). The mixture was then diluted with water, extracted with $CHCl_3$ (5 × 10 ml) and the extract washed with 5% NaOHaq, then water, dried ($MgSO_4$) and evaporated. The residue (10 mg) contained 2 components of which one, separated by thin layer chromatography (silical gel, ethyl acetate–MeOH, 7:3), was identified as olivacine-N-oxide (5 mg), m.p. 303–305°. Synthetic and natural samples were identical by mixture m.p. and chromatographic and IR comparison. The second component was unchanged olivacine.

Hydrogen peroxide oxidation of olivacine. H_2O_2 (30 vol%, 0·5 ml) was added to a solution of olivacine (100 mg) in acetic acid (3 ml). After 10 hr at room temp, more H_2O_2 (0·7 ml) was added and after 2 hr the mixture was heated at 70° for 2 hr. The solution was then filtered, the filtrate evaporated to 2 ml and neutralized with sat. Na_2CO_3aq. Extraction with ethyl acetate followed. by evaporation of the extract gave a red crystalline residue, which crystallized from a large volume of ethyl acetate–MeOH to give red crystals (8 mg), m.p. 257–258° (dec), ν_{max}^{KBr} 1658 (s), 1247, 1022, 826, 757, 737, 719 cm^{-1}; λ_{max}^{EtOH} 283 and 395 mμ (ϵ 21200, 6000); λ_{infl}^{EtOH} 325 mμ (ϵ 7300); λ_{min}^{EtOH} 244 mμ (ϵ 9000); $\lambda_{infl}^{EtOH-NaOH}$ 270, 280, 310 and 370 mμ (ϵ 56000, 55000, 46000, 12000); $\lambda_{min}^{EtOH-NaOH}$ 390 mμ (ϵ 2500). The UV spectrum suffered no shift in acidic solution. Principal mass spectral peaks were observed at *m/e*: 262 (M^+, base peak), 207, 203 (84%), 149, 137, 129, 111, 109.

Isolation of the alkaloids of A. spruceanum

Trunk bark (18 k) of *A. spruceanum* gave a resinous EtOH extract (1200 g). The acidic hexane extract from 175 g EtOH extract was chromatographed on a thin layer of silica gel in benzene to give one crystalline fraction (14 mg), m.p. 42°. Further recrystallization gave mellein (LXIX) as fine, colourless needles (6 mg), m.p. 48°, $[\alpha]_D^{25}$ −93° (*c*, 1·14 in $CHCl_3$), λ_{max}^{chf} 3·13, 5·99, 6·16 and 6·29 μ; λ_{max}^{EtOH} 246 and 312 mμ (log ϵ 2·85, 2·60); $\lambda_{max}^{EtOH-NaOH}$ 228, 249 and 343 mμ (log ϵ 3·26, 2·93, 2·74), the UV spectrum showing no shift in acidic medium. NMR absorption was observed at 1·50 (3 H, doublet, J = 6·5 c/s, CH—CH_3), 2·93 (2 H, doublet, J = 8 c/s, Ar—CH_2—CH), 4·72 (1 H, quartet, J = 6·5 c/s, O—$CH(CH_3)CH_2$), 6·73–7·40 (3 H, multiplet, 3 aromatic H) and 11·1δ (1 H, singlet, Ar—O*H*), while principal mass spectral peaks were observed at *m/e*: 178 (M^+), 160, 149 and 134.

As the acidic and neutral $CHCl_3$ extracts were similar in alkaloid content the work up procedure was modified to make a single $CHCl_3$ extraction at pH 8. Evaporation of the $CHCl_3$ yielded a non-crystalline mixture (3·9 g from 560 g EtOH extract). Chromatography on neutral alumina (activity II)

gave a minor alkaloid (85 mg) eluted with benzene, followed by aspidoalbine (XXIII, 640 mg) eluted with benzene and benzene–ethyl acetate (19:1). Later fractions eluted with benzene–ethyl acetate (19:1) and (4:1) gave XXIV (345 mg). Intermediate fractions contained the 2 alkaloids (90 mg). Aspidoalbine, m.p. 168°, and its N-acetyl-analogue, m.p. 175–179°, were identified by IR and mass spectral comparison with authentic samples.[87] A second minor alkaloid was isolated from the aspidoalbine fraction.

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[87] We thank Dr. Léo Marino for authentic samples of aspidoalbine and its N-acetyl analogue (see Ref. 12*d*).