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Alkaloid Studies. XXXIII.[1] Mass Spectrometry in Structural and Stereochemical Problems. VI.[2] Polyneuridine, A New Alkaloid from *Aspidosperma polyneuron* and Some Observations on Mass Spectra of Indole Alkaloids[3]

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From the trunk bark of *Aspidosperma polyneuron* there was isolated normacusine-B (XIVi) and a new alkaloid, polyneuridine, the constitution (XIVa) of which could be established by mass spectrometry, n.m.r. measurements and eventual chemical conversion to normacusine-B (XIVi). Polyneuridine, therefore, is the C-16 epimer of akuammidine (XIVk), which in turn is identical with rhazine. The mass spectra of indole alkaloids related to yohimbine and ajmalicine exhibit certain characteristic peaks to which assignments have been made through the use of deuterated and other substituted indole alkaloids. β-Yohimbine has thus been identified as one of the constituents of *A. eburneum*.

In connection with our continuing search[6] for new *Aspidosperma* alkaloids, we undertook an examination of the leaves and the trunk bark of the Brazilian tree *Aspidosperma polyneuron* Müll. Arg. This plant has already been the subject of several investigations,[7] which resulted in the unambiguous identification of aspidospermine (I),[8] palosine (II)[9] and quebrachamine (III),[10] the principal source[7] being the root bark.

Examination in our laboratory of the leaves of *Aspidosperma polyneuron* provided in very poor yield aspidospermine (I), quebrachamine (III) and a new alkaloid, polyneuridine. Appreciably larger quantities of this new alkaloid, together with a second one (as well as of I and III), were encountered in the trunk bark and these form the subject of the present paper.

Polyneuridine, m.p. 245–247°, $[\alpha]D$ +1° (chloroform), −73° (pyridine), could only be obtained in solvated form, which initially complicated the establishment of its correct empirical formula. This was finally settled in favor of $C_{21}H_{24}N_2O_3$ (352) through its mass spectrum (Fig. 5), which contained a strong molecular ion peak at 352. The ultraviolet spectrum was typical of an indole, while the infrared spectrum exhibited a carbonyl band at 5.81 μ, which could be shown to be due to a carbomethoxy function by saponification to polyneuridinic acid (XIVb) and remethylation to polyneuridine. The remaining oxygen was identified in the form of a hydroxyl group by acetylation to polyneuridine acetate (XIVc). The insolubility of polyneuridine and its acetate precluded n.m.r. measurements, which had to be conducted on a derivative to be described below.

Accompanying polyneuridine was a second alkaloid, double m.p. 246° and 272°, which was shown to possess the empirical formula $C_{19}H_{22}N_2O$ by elementary analysis and mass spectrometric molecular weight determination. Its single oxygen atom was present as a hydroxyl function as demonstrated by the formation of an acetate. The tentative asssumption was made, therefore, that this second alkaloid—subsequently shown to be normacusine-B (XIVi)[11]—was decarbomethoxypolyneuridine. In terms of empirical formula, polyneuridine differs from yohimbine (IVa) by only two hydrogens. As the latter possesses the same functional groups and has also been encountered[12] among *Aspidosperma* species, we suspected initially a close structural relationship between these two alkaloids. Prompted by the recent successful applications[2,10,13,14] of mass spectrometry in the structure elucidation of complex indole and dihydroindole alkaloids, we decided to examine first the mass spectra of yohimbine and some related indole alkaloids. As will be shown below, such mass spectrometric information proved to be of great utility in the structure elucidation of polyneuridine (XIVa) and normacusine-B (XIVi), in spite of the fact that those alkaloids are not based on a yohimbine skeleton.

(1) Paper XXXII, C. Djerassi, J. P. Kutney and M. Shamma, *Tetrahedron*, **18**, 183 (1962).

(2) Paper V, C. Djerassi, T. George, N. Finch, H. F. Lodish, H. Budzikiewicz and B. Gilbert, *J. Am. Chem. Soc.*, **84**, 1499 (1962).

(3) The work at Stanford University was supported by the National Heart Institute (grant No. 2G-682) and the National Institute of Arthritis and Metabolic Diseases (grant No. A-4257) of the National Institutes of Health, U. S. Public Health Service.

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(5) The appointment of B. Gilbert is supported by a grant from the Rockefeller Foundation in connection with a collaborative research program between the Instituto de Quimica Agricola Rio de Janeiro, and Stanford University.

(6) See ref. 2 and earlier references cited therein.

(7) J. Schmutz and H. Lehner, *Helv. Chim. Acta*, **42**, 874 (1959), and earlier references.

(8) For structure see J. F. D. Mills and S. C. Nyburg, *J. Chem. Soc.*, 1458 (1960).

(9) W. I. Taylor, N. Raah, H. Lehner and J. Schmutz, *Helv. Chim. Acta*, **42**, 2750 (1959).

(10) For structure see K. Biemann and G. Spiteller, *Tetrahedron Letters*, 299 (1961).

(11) A. R. Battersby and D. A. Yeowell, *Proc. Chem. Soc.*, 17 (1961).

(12) E. Fourneau and H. Page, *Bull. sci. pharmacol.*, **21**, 7 (1914); J. Schmutz, *Pharm. Acta Helv.*, **36**, 103 (1961).

(13) (a) K. Biemann, *J. Am. Chem. Soc.*, **83**, 4801 (1961); (b) K. Biemann and M. Friedmann-Spiteller, *ibid.*, **83**, 4805 (1961); (c) K. Biemann, M. Friedmann-Spiteller and G. Spiteller, *Tetrahedron Letters*, 485 (1961).

(14) (a) C. Djerassi, S. E. Flores, H. Budzikiewicz, J. M. Wilson, L. J. Durham, J. Le Men, M.-M. Janot, M. Gorman and N. Neuss, *Proc. Natl. Acad. Sci. U. S.*, **48**, 113 (1962); (b) B. Gilbert, J. M. Ferreira, R. J. Owellen, C. E. Swanholm, H. Budzikiewicz, L. J. Durham and C. Djerassi, *Tetrahedron Letters*, 59 (1962); (c) C. Djerassi, H. W. Brewer, H. Budzikiewicz, O. O. Orazi and R. A. Corral, *Experientia*, **18**, 113 (1962); (d) C. Djerassi, H. Budzikiewicz, J. M. Wilson, J. Gosset, J. Le Men and M.-M. Janot, *Tetrahedron Letters*, 235 (1962); (e) M. Plat, J. Le Men, M.-M. Janot, J. M. Wilson, H. Budzikiewicz, L. J. Durham, Y. Nakagawa and C. Djerassi, *ibid.*, 271 (1962).

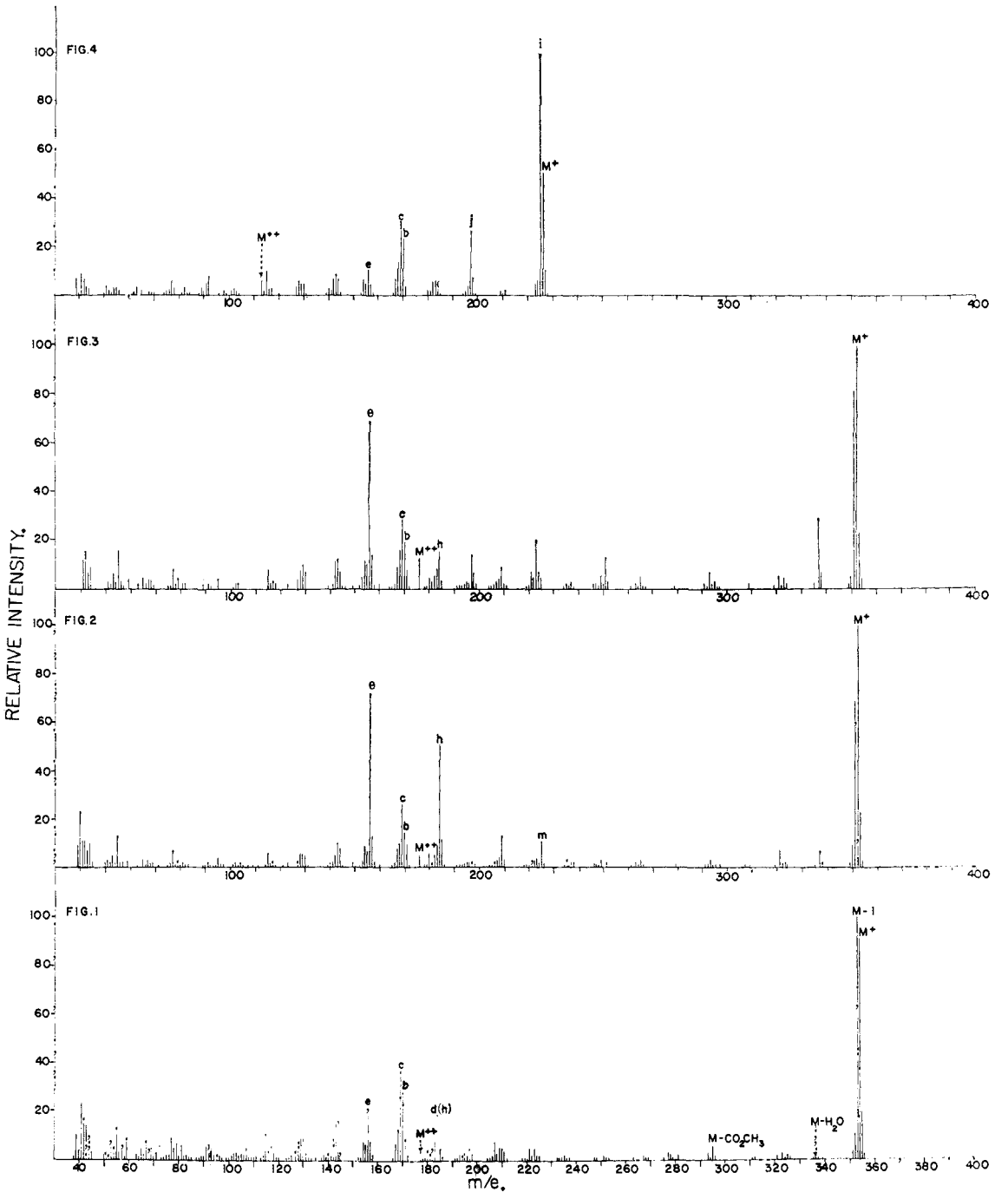


Fig. 1.—Mass spectrum of yohimbine (IVa).
Fig. 2.—Mass spectrum of ajmalicine (IX, 3α, 15α, 20β).
Fig. 3.—Mass spectrum of tetrahydroalstonine (IX, 3α, 15α, 20α).
Fig. 4.—Mass spectrum of tetramethylenetetrahydro-β-carboline (XII).

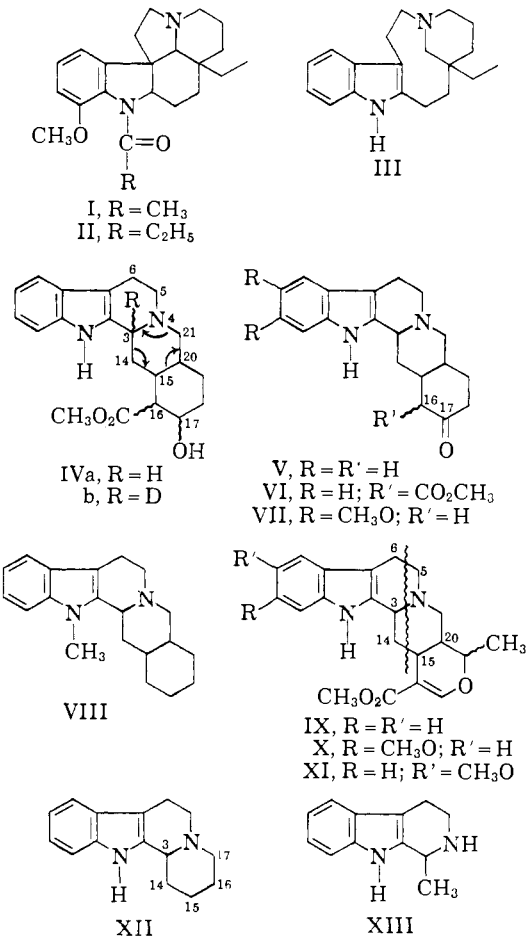
The mass spectrum of yohimbine (IVa) is reproduced in Fig. 1. With the exception of some intensity changes—notably in the M-18 (loss of H_2O) and M-59 (loss of CO_2CH_3) peaks—essentially the same spectrum is also exhibited by the following yohimbine stereoisomers[15]: α-yohimbine, 3-epi-α-yohimbine, β-yohimbine, 3-epi-β-yohimbine, pseudo-yohimbine, allo-yohimbine, corynanthine and 3-epi-corynanthine. Mass spectrometry, therefore, cannot be used as a satisfactory criterion for differentiating among the various yohimbine isomers, but it can be employed very effectively for the recognition of a yohimbine-type skeleton as demonstrated below. Particularly striking is the very strong *m/e* 353 (M-1) peak, which represents the most intense peak in all of these mass spectra. To a considerable extent this is due to loss of the C-3

(15) See M.-M. Janot, R. Goutarel, E. W. Warnhoff and A. Le Hir, *Bull. soc. chim. France*, 637 (1961). We are deeply indebted to Prof. Janot for these specimens.

hydrogen atom yielding species **a**, in which the positive charge is stabilized by conjugation with the indole system as well as by participation of the electron pair on N_b. The correctness of this assumption was established by converting yohimbine (IVa) with mercuric acetate into its 3-dehydro analog[16] and reducing the latter with sodium borodeuteride.[17] The mass spectrum of the resulting 3-deuterioyohimbine (IVb) (*m/e* 355) shows that at least 50% of the hydrogen lost in the yohimbine spectrum (Fig. 1) must come from C-3, because of a strong M-2 peak in the deuterated analog IVb.

The other noteworthy features are the peaks at *m/e* 170 and 169, accompanied by the satellite peaks at 14 mass units higher (*m/e* 184) and lower (*m/e* 156). All these peaks (**b**, **c**, **d**, **e**) must be associated with the indole portion of the molecule since they are also found in 17-deoxy-α-yohimbine (IVa without hydroxy group).[18] the ketones alloyohimbone (V),[19a] 16-ketoyohimbane (V, 16- instead of 17-keto group)[19b] and yohimbinone (VI),[15,20] but are displaced by 60 mass units (*m/e* 216, 229, 230 and 244) in seredone (VII),[21] corresponding to the two extra aromatic methoxyl groups, and by 14 mass units (*m/e* 170, 183, 184 and 198) in N-methylyohimbane (VIII)[22] due to the N_a-methyl substituent. Furthermore, peaks **b**, **c**, **d** and **e** are all shifted by one mass unit in the spectrum of 3-deuterioyohimbine (IVb).

With the above information at our disposal, it becomes a fairly simple matter to attribute likely structures to these four characteristic peaks. Concerted (see arrows in IV) cleavage in the yohimbine ion of the allylically activated 3–14 bond and rupture adjacent to N_b (4–21 bond) would yield the stable dihydro-β-carboline ion **b** corresponding to *m/e* 170. Further loss of one hydrogen leads to the β-carbolinium cation **c** (*m/e* 169), while the *m/e* 184 species simply represents the next higher homolog of **b**. the 20–21 (**d**) or 14–15 (**h**) bond having been cleaved. The last characteristic peak, *m/e* 156, cannot contain the intact β-carboline system, but must, of course, still encompass the indole moiety. We ascribe its genesis to rupture at the two allylic centers C-3 and C-6 of yohimbine (see arrows in **f**), followed by cleavage of the allylically labilized 14–15 bond in **g** to afford the stabilized fragment **e** (*m/e* 156). Strong support for this suggestion is provided in the mass spectra (Figs. 2 and 3) of the



isomeric ring-E heterocyclic bases ajmalicine (IX, 3α, 15α, 20β)[23,24] and tetrahydroalstonine (IX. 3α, 15α, 20α)[24] where the *m/e* 156 peak becomes the strongest of the indole peaks.

This is reasonable in the light of our proposed structure **e** (*m/e* 156), which arises (wavy line in IX) from cleavage (**f** → **g** →**e**) of three allylically activated centers, the 14–15 bond in **g** now being especially labilized because of the additional double bond in ring E. It will also be noted that the *m/e* 184 peak in the ajmalicine (IX, 3α, 15α, 20β) spectrum (Fig. 2) is stronger than the corresponding *m/e* 184 peak of yohimbine (IVa, Fig. 1) and may, therefore, be represented by **h** (cleavage of 14–15 linkage promoted by 16–17 double bond) rather than **d**. There exists a significant difference in the relative intensities of the *m/e* 156, 169 and 184 peaks in the spectrum (Fig. 2) of ajmalicine (IX, 3α, 15α, 20β) as compared to those (Fig. 3) of tetrahydroalstonine (IX, 3α, 15α, 20α) and this can evidently be used as a criterion for a D/E *trans vs. cis* ring juncture. Thus, tetraphylline (X)[25] exhibits exactly the same type of spectrum as ajmalicine (Fig. 2)—except that the *m/e* 156,

(16) F. L. Weisenborn and P. A. Diassi, *J. Am. Chem. Soc.*, **78**, 2022 (1956); E. Wenkert and D. K. Roychaudhuri, *J. Org. Chem.*, **21**, 1315 (1956).

(17) Prepared by the procedures of I. Shapiro, H. G. Weiss, M. Schmich, S. Skolnik and G. B. L. Smith, *J. Am. Chem. Soc.*, **74**, 901 (1952), and H. C. Brown, R. J. Mead and P. A. Tierney, *J. Am. Chem. Soc.*, **79**, 5400 (1957), but substituting lithium aluminum deuteride for lithium aluminum hydride.

(18) P. A. Diassi and R. M. Palmere, *J. Org. Chem.*, **26**, 3577 (1961).

(19) (a) B. Witkop, *Ann.*, **554**, 83 (1943); (b) R. K. Hill and K. Muench, *J. Org. Chem.*, **22**, 1276 (1957).

(20) It is interesting to note that we were unable to detect the molecular ion (352) in this spectrum, the highest peak occurring at *m/e* 294 corresponding to decarbomethoxylation with rearrangement of one hydrogen. The appearance of such a M-58 peak is characteristic of β-keto (methyl) esters; see R. Ryhage and E. Stenhagen, *Arkiv Kemi*, **15**, 545 (1960).

(21) J. Poisson, N. Neuss, R. Goutarel and M.-M. Janot, *Bull. soc. chim. France*, 1195 (1958).

(22) B. Witkop, *J. Am. Chem. Soc.*, **75**, 3361 (1953).

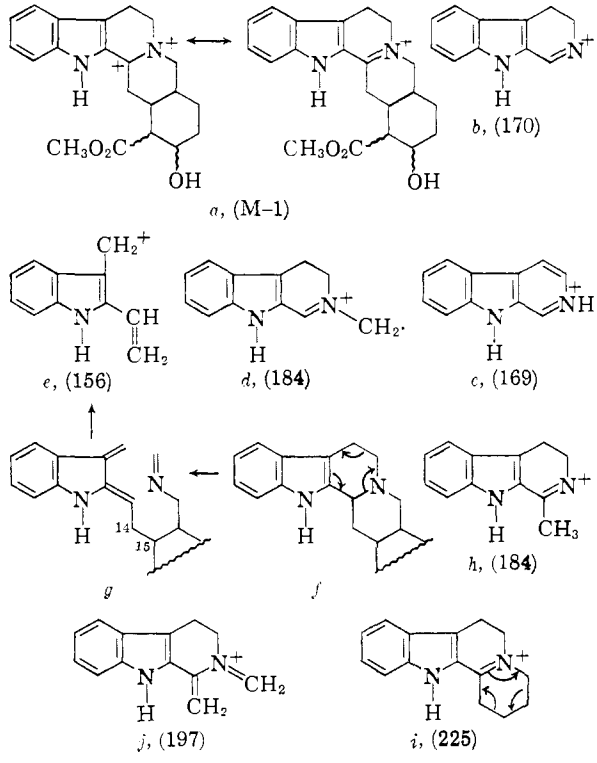
(23) R. Goutarel and A. Le Hir, *Bull. soc. chim. France*, 909 (1951); M. W. Klohs, M. D. Draper, F. Keller, W. Malesh and F. J. Petracek, *J. Am. Chem. Soc.*, **76**, 1332 (1954).

(24) For stereochemistry see E. Wenkert, B. Wickberg and C. L. Leicht, *ibid.*, **83**, 5037 (1961). M. Shamina and J. B. Moss, *ibid.*, **83**, 5038 (1961).

(25) C. Djerassi, J. Fishman, M. Gorman, J. P. Kutney and S. C. Pakrashi, *ibid.*, **79**, 1217 (1957).

169, 170 and 184 peaks are now shifted by 30 mass units (additional aromatic methoxyl group) to *m/e* 186, 199, 200 and 214–while arichine (XI)[26] has a tetrahydroalstonine spectrum (Fig. 3) except for the 30 mass unit shift associated with its extra methoxyl substituent. The spectra of both X and XI contain again the very strong M-1 peak.

We can conclude, therefore, from the mass spectra that tetraphylline (X) has a *trans* (15α, 20β) and aricine (XI) a *cis* (15α, 20α) D/E ring fusion, which is in agreement with n.m.r.[24] and infrared measurements.[27] It is interesting to note that mass spectrometry may be used for stereochemical purposes in the ring E heterocyclic bases (IX–XI), but not in the isomeric yohimbines (IVa), the reason being the presence in the former of a 16–17 double bond which favors cleavage at the C-15 center.



Tetramethylenetetrahydro - β - carboline (XII)[28] represents the simplest indole, where a mass spectral fragmentation pattern of the yohimbine (IVa) and ajmalicine (IX) type may be anticipated. Indeed, as shown in Fig. 4, its spectrum is again characterized by a very strong M-1 peak (i), associated with the removal of the C-3 or C-6 hydrogen atom, and the typical *m/e* 169 and 170 peaks due to **c** and **b**. The two satellite peaks at *m/e* 156 and 184 are smaller, but there appears a rather strong peak at *m/e* 197, which is most likely due to concerted loss (see arrows in i) of ethylene with formation of species **j** (*m/e* 197). Tetrahydroharman (XIII) does not anymore show this typical behavior, since the strongest peak occurs now at M-15 (*m/e* 171) due to the preferential loss of the methyl substituent producing the dihydro-β-carbolinium cation.

For further confirmation of these mass spectrometric assignments, there was synthesized 3,5,6-trideuterioajmalicine by sodium borodeuteride[17] reduction[29] of serpentine hydrochloride.[28,30] The mass spectrum (not reproduced) of this trideuterio derivative, when compared with that (Fig. 2) of ajmalicine (IX), showed several very instructive differences. The molecular ion occurred at *m/e* 355 (as compared to *m/e* 352 for IX) and there was present a M-2 peak of over 50% the intensity of the M-1 peak in the ajmalicine spectrum (Fig. 2). Consequently, at least 50% of the M-1 peak in the latter is due to loss of the C-3 or C-6 hydrogen atoms.

The **e** peak at *m/e* 156 was now observed at *m/e* 158, demonstrating that two of the three deuterium atoms still remain in this fragment. This is in full accord with the proposed structure **e**, where the C-5 deuterium label should be lost. On the other hand, the **h** peak (*m/e* 184 in Fig. 2) was shifted by three mass units to *m/e* 187, thus showing that all three deuterium atoms were still retained. We believe that this is explained most readily by migration of the C-3 deuterium atom to the adjacent methyl group.

The *m/e* 169 peak (**c** in Fig. 2) is shifted in part to *m/e* 171 and in part to *m/e* 172, which again is in agreement with our postulates that its genesis involves the loss of one hydrogen from positions 5 and 6. As these two centers carry each one hydrogen and one deuterium in the labeled trideuterioajmalicine, one would expect shifts of both two and three mass units, because there is no reason why solely a hydrogen or deuterium should be lost exclusively from one of these carbon atoms (C-5 or C-6). The **b** peak (*m/e* 170), on the other hand, is shifted to *m/e* 173 in the anticipated manner as structure **b** does not require the loss of any hydrogen (or deuterium) from positions 3, 5 or 6.

The trideuterioajmalicine also permitted the assignment of still another peak in the ajmalicine spectrum (Fig. 2), namely the one occurring at *m/e* 225. In the trideuterio analog, this peak is moved by three mass units to *m/e* 228, thus requiring that all three deuterium atoms are retained in it. We propose structure **m** for the 225 ion, the formation of which can be visualized as a reverse Diels-Alder reaction (arrows in **k**) followed by rupture of the weakest bond (3–14), which is activated in **l** on either side by a double bond.

This association of characteristic mass spectral peaks with yohimbine-type alkaloids led immediately to the recognition of a recently isolated alkaloid ($C_{21}H_{26}H_2O_3$) from *Aspidosperma eburneum*. F. Allem as a yohimbine stereoisomer, since its mass spectrum exhibited the typical M-1 as well as

(26) A. Stoll, A. Hofmann and R. Brunner, *Helv. Chim. Acta*, **38**, 270 (1955).

(27) N. Neuss and H. Boaz, *J. Org. Chem.*, **22**, 1001 (1957) recognized the correct relationships among these alkaloids, but their actual configurational assignments have to be changed (see ref. 24).

(28) Kindly supplied by Dr. Neuss of Eli Lilly and Co.

(29) E. Wenkert and D. K. Roychaudhuri, *J. Am. Chem. Soc.*, **80**, 1613 (1958), have already shown that sodium borohydride reduction of serpentine nitrate regenerates ajmalicine (IX, 3α, 15α, 20β).

(30) E. Schlittler and H. Schwarz, *Helv. Chim. Acta*, **33**, 1463 (1950).

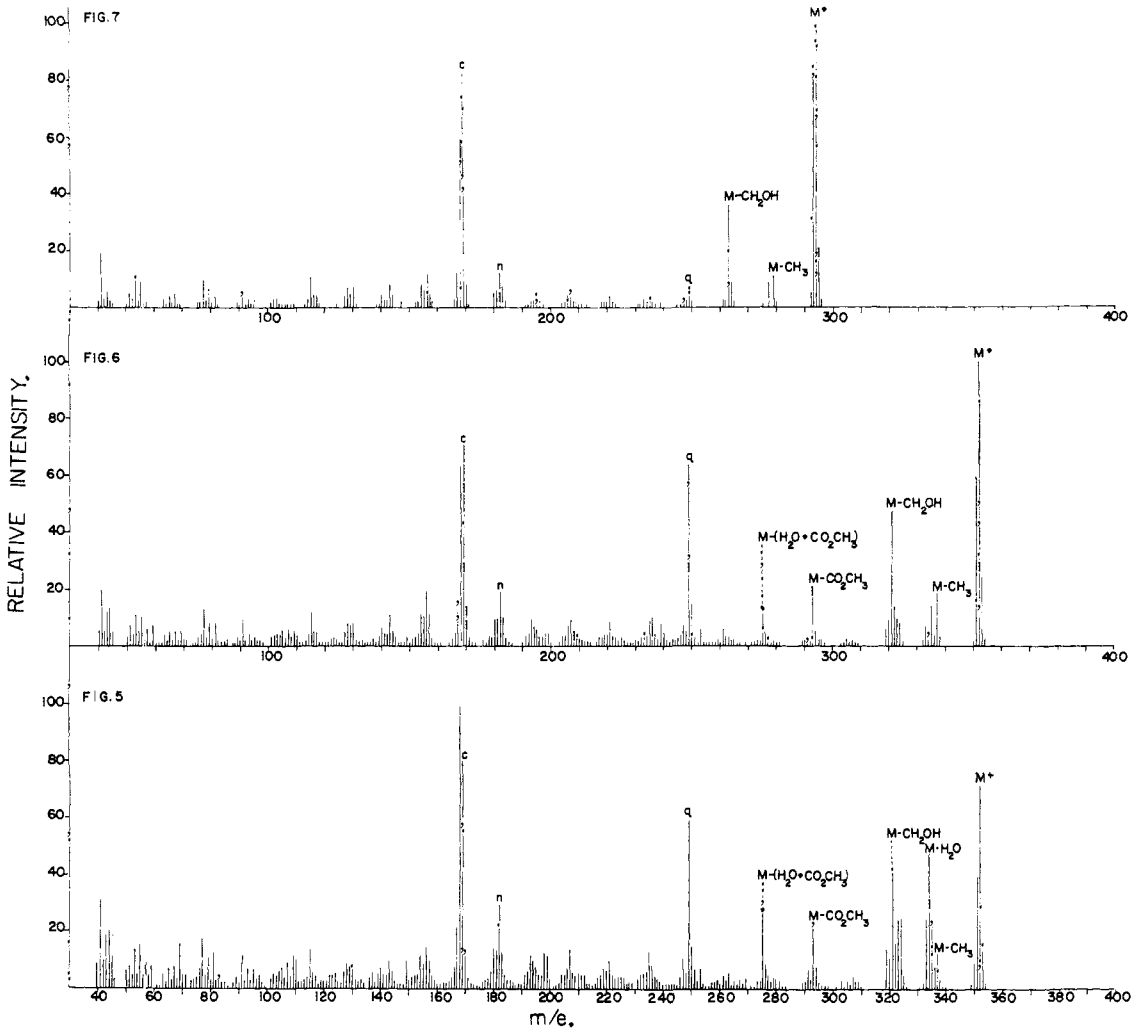
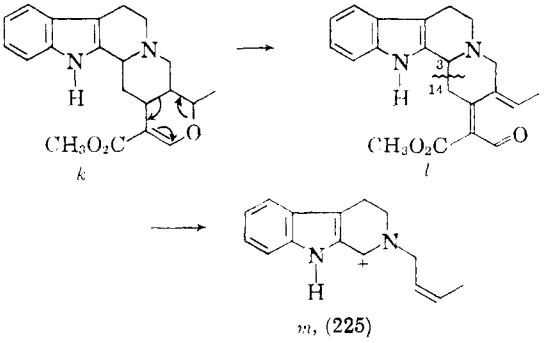


Fig. 5.—Mass spectrum of polyneuridine (XIVa).
Fig. 6.—Mass spectrum of akuammidine (XIVk).
Fig. 7.—Mass spectrum of normacusine-B (XIVi).

b, **c**, **d** and **e** peaks observed with yohimbine (Fig. 1). The physical constants of the alkaloid closely resembled those reported[31] for β-yohimbine (IVa, 3α, 15α, 16α, 17β, 20β) and direct comparison with an authentic sample[15] established its identity. While yohimbine (IVa, 3α, 15α, 16α, 17α, 20β) itself has been isolated earlier from *Aspidosperma* species, this represents the first recorded isolation of β-yohimbine from this genus.



With the above information at our disposal, we can now turn to a consideration of the mass spectrum (Fig. 5) of polyneuridine (XIVa), the new alkaloid from *Aspidosperma polyneuron* Müll. Arg. Aside from a strong molecular ion and a substantial M-1 peak, the most characteristic features of the spectrum are the very intense *m/e* 168 and 169 peaks. These cannot contain the hydroxyl or carbomethoxy groups nor the double bond (*vide infra*) because these same strong peaks are also observed in the mass spectra of all other polyneuridine derivatives (*e.g.*, XIVc, d, f, h) in which these functions are altered. In the light of the above mass spectra of the yohimbine (IVa) and ajmalicine (IX) alkaloids, we feel justified in assuming that the *m/e* 169 peak corresponds to the β-carbolinium cation **c** and the *m/e* peak to the β-carboline ion itself. In order to rationalize this one mass unit difference between these peaks (*m/e* 168, 169) of polyneuridine and the *m/e* 169, 170 peaks of IV and IX, we assumed the presence of an additional

(31) H. Heinemann, *Ber.*, **67**, 15 (1934); A. Le Hir and R. Goutarel, *Bull. Soc. Chim. France*, 1023 (1953).

linkage in ring C of polyneuridine, which has to be broken in the genesis of the β-carboline aromatic system. The identical feature was also observed in the mass spectrum (Fig. 7) of the second alkaloid, subsequently shown to be normacusine-B (XIVi); in addition to the M-1, *m/e* 168 and 169 peaks, both spectra show a strong peak at M-31 (loss of the elements of CH_3O). Since the second alkaloid does not contain a methoxyl group, the M-31 peak was interpreted as the loss of a CH_2OH function, the presence of which could be substantiated readily by n.m.r. measurements.

Examination of the n.m.r. spectra of the parent alkaloids was precluded by their insolubility, but reduction of polyneuridine (XIVa) with lithium aluminum hydride to the diol XIVe followed by acetylation provided polyneuridinol diacetate (XIVf), which was sufficiently soluble in deuteriochloroform, as was the acetate XIVj of the second alkaloid. The spectra of the two acetates were nearly identical in the regions 4.5–8.5 and 0–2.0 p.p.m.[32] which included the four aromatic protons (6.9–7.6 p.p.m.), the indole N_a-hydrogen (8.43 in XIVf and 8.57 in XIVj), a single olefinic proton (quartet between 5.1–5.6 p.p.m.) and most importantly, the three hydrogens of a methyl group (doublet at 1.45–1.70 p.p.m.) located on a double bond. These last two signals establish the presence of an exocyclic ethylidine function, so common among alkaloids of the ajmaline group,[33] and this was confirmed by catalytic hydrogenation to the corresponding dihydroalkaloid (XVI). The signals of the acetate methyl protons occurred at 1.92 and 2.03 (three protons each) in the diacetate XIVf and at 2.0 p.p.m. in the monoacetate XIVj. The primary nature of the alcohol functions, already indicated by the M-31 fragment ion, was demonstrated conclusively by the presence of an n.m.r. signal between 3.9–4.2 p.p.m. corresponding to four protons in the spectrum of VIVf and of a signal at 3.8–4.1 p.p.m. (2 protons) in the monoacetate XIVj. The remaining assignments were only possible after the nuclear structure XIV was established and included hydrogens adjacent to nitrogen (C-3 H: 3.65–3.87; C-21 H_2: 3.4–3.6; C-5 H: 2.8–3.2 p.p.m.), hydrogens adjacent to double bonds (C-6 H_2: 2.8–3.2; C-15 H: 2.55–2.8 p.p.m.) and finally the two protons attached to C-14 at 1.45–1.85 p.p.m.

Chromium trioxide oxidation of the primary hydroxyl group of polyneuridine (XIVa) in acetone solution[34] provided the aldehyde XIVd, which did not undergo exchange with sodium deuterioxide in deuteriomethanol, thus showing that neither the aldehyde nor the ester function contain an adjacent hydrogen atom. This observation afforded the clue to the successful structure elucidation of the two alkaloids:

If we combine the mass spectrometric indication (*m/e* 168 and 169 peaks in Figs. 5 and 7) of a potential β-carboline (with one extra linkage as compared to IV or IX) with the ethylidene function suggestive of the ajmaline group,[33] then we arrive at partial structure XVa, which includes all of the carbon atoms of the molecule. The attachment of ring D is also indicated by the mass spectrum of polyneuridine (Fig. 5), which exhibits a substantial peak at *m/e* 182 (**n**) rather than 184 (**d** or **h**) as observed in the yohimbines (Fig. 1) or ajmalicine (Fig. 2). Such a peak would be accommodated readily in XVa by cleavage at C-15 and C-21, both of which are activated by the allylic double bond coupled with further loss of one hydrogen (as in **o** or **r**). The presumed relationship of the second alkaloid as decarbomethoxypolyneuridine (XIVi) would then lead to partial structure XVb. All that would be necessary for a complete structure is to complete the fifth ring—known to be present from the precise mass spectrometrically determined molecular weight and the hydrogenation experiments—by attaching the carbomethoxy-bearing carbon atom at two locations.

Partial structure XV is so suggestive of the sarpagine skeleton (XIVi with phenolic hydroxyl group attached at C-10)[13a,35] that a literature search was made for structures of type XIVa and XIVi. Indeed, a recent communication by Battersby and Yeowell[11] reported the pyrolysis of the quaternary *Strychnos* alkaloid macusine-B to a substance normacusine-B, for which structure XIVi was established by direct relation to an ajmaline transformation product.[36] The reported[11] double melting point of normacusine-B, was very similar to that of our alkaloid and direct comparison (mixture melting point, thin-layer chromatographic mobility, mass spectrum) with a sample kindly supplied by Dr. A. R. Battersby demonstrated their identity.

In view of the presumed relationship between normacusine-B (XIVi) and polyneuridine, the latter should be assigned structures XIVa or XIVk. One of these two structures has recently been assigned to akuammidine,[37] an alkaloid from *Picralima nitida* Stapf., while the corresponding quaternary methohydroxide, macusine-A, has been encountered[38] in calabash curare and its constitution established by X-ray analysis.

The mass spectrum of akuammidine (XIVk)[39] is reproduced in Fig. 6 and while the qualitative agreement is such as to suggest strongly a very close relationship between akuammidine (XIVk) and polyneuridine (XIVa), there is no question that the intensity differences in Figs. 5 and 6 are so marked as to preclude identity. Indeed, a mixture melting point determination showed a marked depression and the mobility of the two alkaloids in a

(32) For reasons given earlier (C. Djerassi, T. Nakano, A. N. James, L. H. Zalkow, E. J. Eisenbraun and J. N. Shoolery, *J. Org. Chem.*, **26**, 1192 (1961)) all n.m.r. signals are reported in p.p.m. as δ units (δ = c.p.s./60) relative to the internal standard tetramethylsilane.

(33) See for instance N. Neuss "Physical Data of Indole and Dihydroindole Alkaloids," Eli Lilly and Co., Indianapolis, 1960.

(34) K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

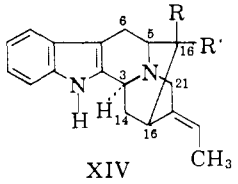
(35) J. Poisson, J. Le Men and M.-M. Janot, *Bull. soc. chim. France*, 610 (1957); D. Stauffacher, A. Hofmann and E. Seebeck, *Helv. Chim. Acta*, **40**, 508 (1957); W. Arnold, W. V. Philipsborn, H. Schmid and P. Karrer, *ibid.*, **40**, 705 (1957).

(36) M. F. Bartlett, E. Schlittler, R. Sklar, W. I. Taylor, R. L. S. Amai and E. Wenkert, *J. Am. Chem. Soc.*, **82**, 3792 (1960).

(37) (a) I. Levy, J. Le Men and M.-M. Janot, *Compt. rend.*, **253**, 131 (1961); (b) NOTE ADDED IN PROOF.—We have been informed by Prof. Janot that recent work in his laboratory (M.-M. Janot, J. Le Men, J. Gosset and J. Levy, in press) has established the absolute configuration implicit in stereoformula XIVk for akuammidine.

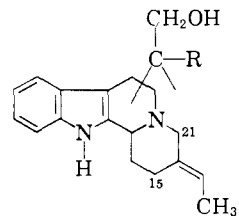
(38) A. T. McPhail, J. M. Robertson, G. A. Sim, A. R. Battersby, H. F. Hodson and D. A. Yeowell, *Proc. Chem. Soc.*, 223 (1961).

(39) We are indebted to Prof. M.-M. Janot for this specimen.

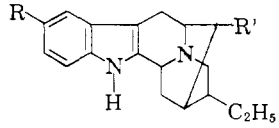


XIV

	R	R'
a	CH_2OH	CO_2CH_3
b	CH_2OH	CO_2H
c	CH_2OAc	CO_2CH_3
d	CHO	CO_2CH_3
e	CH_2OH	CH_2OH
f	CH_2OAc	CH_2OAc
g	CH_2OAc	CD_2OAc
h	$CO_2C(CH_3)_3$	H
i	CH_2OH	H
j	CH_2OAc	H
k	CO_2CH_3	CH_2OH



XVa, R = CO_2CH_3
b, R = H



XVI, R = H; R' = CH_2OH
XVII, R = CH_3O; R' = CH_3

silica gel thin-layer chromatogram was different, polyneuridine (XIVa) moving slightly faster. The French authors[37a] reported that a reverse aldol condensation of akuammidine (XIVk) followed by reduction with lithium aluminum hydride led to normacusine-B (XIVi). When polyneuridine (XIVa) was exposed to potassium *t*-butoxide in refluxing benzene[37a] there occurred retroaldolization as well as ester interchange and epimerization with production of the *t*-butyl ester XIVh (molecular weight established by mass spectrometry), which was reduced with lithium aluminum hydride to normacusine-B. Consequently, polyneuridine (XIVa) and akuammidine (XIVk) are stereoisomers at C-16[40] and this was confirmed further by direct comparison of their respective lithium aluminum hydride reduction products(XIVe)[39] which proved to be identical.

Chatterjee and collaborators[41] reported recently the isolation of a new alkaloid, rhazine ($C_{21}H_{26}N_2O_3$, m.p. 236°), from *Rhazya stricta* Decaisne together with (−)-quebrachamine (III). The physical constants were rather similar to those of XIVa and XIVk and through the courtesy of Mrs. A. Chatterjee a small sample of rhazine was secured. Its mass spectrum proved to be completely identical with that (Fig. 6) of akuammidine ($C_{21}H_{24}N_2O_3$); furthermore, the two alkaloids exhibited the identical mobility in a thin-layer chromatogram and did not give any melting point depression upon admixture. The name rhazine, therefore, should be eliminated from the literature. The Indian workers[41] reported the presence of N-methyl groups in their alkaloid, although structure XIVk does not contain such a grouping (nor does the n.m.r. spectrum of the isomer polyneuridine show such a signal). We have confirmed a positive N-methyl analysis in polyneuridine (XIVa) by the usual Herzig–Meyer method and ascribe it to retroaldolization, followed by eventual transformation of formaldehyde into methyl iodide.

(40) Dr. A. R. Battersby (University of Bristol) has informed us that the quaternary alkaloid, macusine-C, corresponding to polyneuridine (XIVa) has also been encountered in calabash curare (A. R. Battersby and D. A. Yeowell, *J. Chem. Soc.*, in press (1962).

(41) A. Chatterjee, C. R. Ghosal, N. Adityachaudhury and S. Ghosal, *Chemistry & Industry*, 1034 (1961).

The co-occurrence of aspidospermine (I), quebrachamine (III), polyneuridine (XIVa) and normacusine-B (XIVi)[42] in one plant points strongly toward a common biogenetic pathway. The special relationship between aspidospermine and quebrachamine has already been commented upon,[13c,43] while the biosynthesis of all of these alkaloids can be accommodated readily by the terpene–indole schemes proposed recently.[44]

In view of the important role which mass spectrometry is starting to occupy in the structure proof of indole alkaloids,[2,13,14] it seemed to be of interest to comment further upon the mass spectra (Figs. 5–7) of polyneuridine (XIVa), akuammidine (XIVk) and normacusine-B (XIVi). The relatively strong M-1 peak observed in these three spectra, as well as in those[13a] of some related indole alkaloids, is probably due to abstraction of the C-3 or C-6 hydrogen atom, the resulting carbonium ion being stabilized by conjugation with the indole system.[45] Abstraction of a hydrogen atom next to the ethylidene double bond is not involved, since the M-1 peak is equally pronounced in the mass spectrum of dihydronormacusine-B (XVI). The assignment of the β-carboline cation to the *m/e* 168 peak, of **c** to the *m/e* 169 peak and of **n** to the *m/e* 182 peak seems straight forward; these important peaks are found in the mass spectra of XIVa–XIVk as well as in the spectrum of dihydronormacusine-B (XVI) and hence cannot encompass any of these functionalities. Furthermore, these peaks were observed by Biemann[13a] in the sarpagine series (shift of 30 mass units due to the extra aromatic methoxyl group), where they were also ascribed to the β-carboline moiety.

The loss of water (M-18 at *m/e* 334) is particularly pronounced in the spectrum of polyneuridine (Fig. 5) and obviously must involve a rearrangement. We wish to propose the mechanistic path implied by the arrows in **o**, which is completely consistent with the assigned C-16 stereochemistry.[37a] Some support for this suggestion comes from the observation that both the diacetate XIVf and its dideuterio analog XIVg show an M-60 peak corresponding to the loss of acetic acid, which could proceed by the identical mechanism **o**. The peaks at *m/e* 293 (M-59) and *m/e* 275 (M-77) in Fig. 5 are undoubtedly due to the loss of the carbomethoxy function (59 mass units) and to the loss of both carbomethoxy and water. Finally, we ascribe the strong *m/e* 249 peak (**q**) in the spectra of both polyneuridine (Fig. 5) and akuammidine (Fig. 6) to the loss of the one-carbon bridge (C-16, together with the attached carbomethoxy and hydroxymethyl functions) accompanied by rearrangement of one hydrogen. Similarly, a strong *m/e* 249 peak was also observed in the mass spectrum of polyneuri-

(42) Normacusine-B, β-yohimbine together with several other alkaloids have recently been isolated by D. Stauffacher, *Helv. Chim. Acta*, **44**, 2006 (1961), from *Diplorrhynchus condylocarpon* Muell. Arg.

(43) C. Djerassi, A. A. P. G. Archer, T. George, B. Gilbert and L. D. Antonaccio, *Tetrahedron*, **16**, 212 (1961).

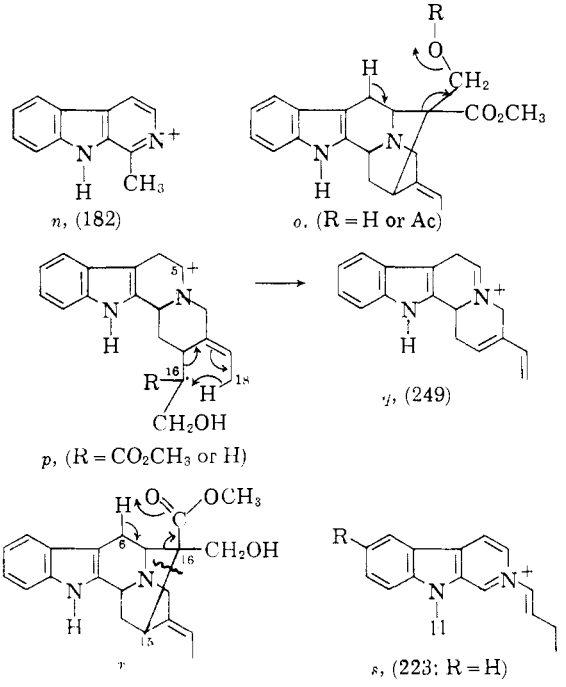
(44) R. Thomas, *Tetrahedron Letters*, 544 (1961); E. Wenkert, *J. Am. Chem. Soc.*, **84**, 98 (1962).

(45) Participation of N_b, as visualized in the M-1 species **a** corresponding to the yohimbines (IV), is unlikely in these alkaloids, because a 2–3 double bond would be sterically very undesirable in view of the additional 5–16–15 ring juncture.

dine acetate (XIVc) and to a lesser extent in that (Fig. 7) of normacusine-B (XIVi).

Two plausible mechanisms can be proposed to rationalize these fragmentation paths. The first implies initial rupture of the 5–16 bond with formation of **p**, this scission being favored by stabilization of the carbonium ion on C-5 through participation of the N_b electrons. Species **p** now undergoes expulsion of C-16 with its attached atoms through the cyclic process indicated by the arrows to yield **q** (*m/e* 249 in Figs. 5–7). Alternatively, the reaction may proceed *via* r with rupture of the 15–16 bond, the principal difference (aside from the location of the extra-nuclear double bond) between the two mechanisms being the origin (C-18 in **p**, C-6 in r) of the hydrogen atom which is transferred to the departing moiety. The greater intensity of the *m/e* 249 (**q**) peak in the polyneuridine (Fig. 5) and akuammidine (Fig. 6) spectra as compared to that (Fig. 7) of normacusine-B may perhaps be ascribed to the lack of participation of mechanism **r** as this requires the presence of the carbomethoxy group.

In the dihydronormacusine-B (XVI) spectrum (not reproduced), the *m/e* 249 peak (**q**) is virtually absent, but there is now found a substantial M-73 peak at *m/e* 223. This *m/e* 223 peak corresponds to the one at *m/e* 253 (thirty mass unit shift due to extra aromatic methoxyl group) noted by Biemann[13a] in O-methyldeoxydihydrosarpagine (XVII) who ascribed structure **s** (R = CH_3O) to it and presented a likely mechanism for its formation.



Experimental[46]

Isolation of Alkaloids from *Aspidosperma polyneuron* Müll. Arg.—The ground and dried stem bark (20 kg.) was extracted in a Soxhlet apparatus with ethanol, the solvent evaporated and the resin extracted with 5 l. of 5% hydrochloric acid. After making basic with ammonia, re-extracting with chloroform and evaporating, there was obtained 31 g. of crude alkaloid mixture. For further separation, it was again taken up in 5% hydrochloric acid and separated into chloroform-soluble (5 g.) and chloroform-insoluble (17.6 g.) hydrochlorides, the former containing principally aspidospermine accompanied by some (−)-quebrachamine (III).

The crude alkaloid derived from the chloroform-insoluble portion was chromatographed on Alcoa basic alumina and the chloroform and chloroform–methanol (1%) eluates were pooled. Crystallization from methanol–benzene provided 3.47 g. (m.p. 248–258°) of alkaloid, which consisted of a mixture of two substances as determined by thin-layer chromatography. Complete separation was effected through the hydrochlorides by dissolving in methanol, acidifying with methanolic hydrochloric acid and evaporating to dryness. Crystallization from water provided 2.47 g. of polyneuridine hydrochloride (m.p. 246–250°), which proved to be homogeneous on thin-layer chromatography.

The water-soluble hydrochloride did not crystallize readily, but regeneration of the base and crystallization from methanol gave beautiful prisms (5 g.) of normacusine-B (XIVi),[11,37] double m.p. 245–246° and 270–272°, $[\alpha]_D$ +28° (*c* 1.02 in pyridine), $[\alpha]$ +40° (*c* 1.14 in methanol); λ_{max}^{EtOH} 225, 280 and 289 mμ, log ε 4.45, 3.80, 3.73; and λ_{min}^{EtOH} 245 and 287 mμ, log ε 3.34, 3.72. Identity with authentic material[11,37] was established by mass spectral comparison (see Fig. 7), mixture melting point determination and thin-layer chromatographic mobility.

Anal. Calcd. for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N, 9.52; O, 5.44; C-methyl, 5.1; mol. wt., 294. Found: C, 78.13; H, 7.56; N, 9.24; O, 5.39; C-methyl, 2.08; mol. wt. (Fig. 7), 294.

Acetylation with acetic anhydride–pyridine at room temperature (48 hr.) provided **normacusine-B acetate (XIVj)**,[37a] which was crystallized from ether; m.p. 212–215°, $[\alpha]_D$ +12° (*c* 1.0 in methanol).

Anal. Calcd. for $C_{21}H_{24}N_2O_2$: C, 74.97; H, 7.19; N, 8.33; mol. wt., 336. Found: C, 74.72; H, 7.07; N, 8.39; mol. wt. (mass spec.), 336.

Hydrogenation with platinum oxide in methanol solution (room temperature, 24 hr.) followed by crystallization from methanol gave **dihydronormacusine-B (XVI)** as small prisms, m.p. 159–160°, the ultraviolet absorption spectrum being identical with that of **normacusine-B**.

Anal. Calcd. for $C_{19}H_{24}N_2O$: C, 76.99; H, 8.16; N, 9.45; mol. wt., 296. Found: C, 76.86; H, 7.98; N, 9.60; mol. wt. (mass spec.), 296.

Extraction of 15 kg. of leaves of the same plant by essentially the same scheme gave 1.0 g. of aspidospermine (I), 0.05 g. of (−)-quebrachamine (III) and 0.1 g. of polyneuridine (XIVa).

Polyneuridine (XIVa).—Regeneration of the base from polyneuridine hydrochloride and crystallization from ethanol provided the ethanol solvate (presence of ethanol proved by n.m.r. spectrum), m.p. 245–247.5°, while from benzene, there was obtained a benzene solvate, m.p. 243–246°, $[\alpha]_D$ −68° (*c* 0.67 of ethanol solvate in pyridine), −73° (*c* 0.82 of benzene solvate in pyridine), $[\alpha]_D$ +1° (*c* 1.03 in chloroform); λ_{max}^{EtOH} 228 and 281 mμ, log ε 4.51, 3.82 and λ_{min}^{EtOH} 249 mμ, log ε 3.33, $\lambda_{max}^{EtOH-HCl}$ 221 and 273 mμ, log ε 4.60, 3.82 and; $\lambda_{min}^{EtOH-HCl}$ 242 mμ, log ε 3.31; λ_{max}^{Nujol} 2.99 and 5.81 μ, pK_a' 6.60 (66% dimethylformamide).[47] The analytical figures of the solvates were not very satisfactory and the correct empirical formula was based on the mass spectrometrically determined molecular weight (Fig. 5).

Anal. Calcd. for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95; mol. wt., 352. $C_{21}H_{24}N_2O_3 \cdot C_2H_5OH$: C, 69.32; H, 7.59; N, 7.03. $C_{21}H_{24}N_2O_3 \cdot 1/3C_6H_6$: C, 72.99; H, 6.93; N, 7.40; O, 12.68; methoxyl, 8.20; C-methyl, 3.97. Found (for ethanol solvate): C, 70.60; H, 7.78; N, 6.72; for benzene solvate: C, 72.81; H, 6.93; N, 7.30; O, 12.51; methoxyl, 7.77; C-methyl, 2.83; mol. wt. (Fig. 5), 352.

(46) Melting points are uncorrected. The microanalyses were performed in part by Mr. E. Meier (Stanford University) and in part by Dr. A. Bernhardt (Mülheim, Germany). Thin-layer chromatograms were performed on silica gel with benzene–ethyl acetate (1:1) containing 10% of 95% ethanol or ethyl acetate–ethanol (1:1) as solvent and using ceric sulfate for detection of the spots.

(47) We are indebted to Dr. H. Boaz of Eli Lilly and Co. for this determination.

The methiodide was formed at room temperature in chloroform solution (24 hr.) and recrystallized from ethanol; m.p. 263–265°; λ_{max}^{EtOH} 271 mμ, log ε 3.76; and λ_{min}^{EtOH} 245 mμ, log ε 3.34.

Anal. Calcd. for $C_{22}H_{27}IN_2O_3$: C, 53.45; H, 5.51; I, 25.67; N, 5.67; O, 9.71. Found: C, 53.15; H, 5.81; I, 25.65; N, 5.36; O, 9.71.

Acetylation with acetic anhydride in pyridine (24 hr., room temperature) and crystallization from methanol yielded **polyneuridine acetate (XIVc)**, m.p. 270–272°, $[\alpha]D$ −92° (*c* 0.71 in pyridine).

Anal. Calcd. for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10; O, 16.23; mol. wt., 394. Found: C, 70.24; H, 6.76; N, 7.02; O, 15.80; mol. wt., 394.

Polyneuridinic acid (XIVb) hydrochloride was obtained by heating under reflux for 5 hr. 100 mg. of polyneuridine (XIVa) with 2 *N* methanolic potassium hydroxide, acidification with hydrochloric acid, evaporation to dryness, trituration with water (hydrochloride insoluble) and recrystallization from ethanol; yield 80 mg., m.p. 255–265°.

Anal. Calcd. for $C_{20}H_{23}ClN_2O_3$: C, 64.08; H, 6.18; Cl, 9.46; N, 7.47; O, 12.80. Found: C, 63.73; H, 6.36; Cl, 9.90; N, 7.29; O, 13.09.

The hydrochloride was dissolved in a large volume of ethanol and left at room temperature for one week with an excess of ethereal diazomethane. Evaporation to dryness, addition of water, filtration and recrystallization from ethanol provided polyneuridine (XIVa) in 40% yield.

Chromium Trioxide Oxidation of Polyneuridine.—Polyneuridine (118 mg.) was dissolved in 5 cc. of acetone and 8 *N* chromium trioxide solution[34] added dropwise at 25°. During the addition of the reagent a dark resin separated, which dissolved only at the end of the reaction after the addition of water. The solution was made alkaline with sodium hydroxide and the product (65 mg.) isolated with ether. Filtration, in chloroform solution, through a short alumina column and recrystallization from ethanol yielded 40 mg. of the **aldehyde XIVd**, m.p. 285–286°.

Anal. Calcd. for $C_{21}H_{22}N_2O_5$: C, 71.98; H, 6.33; N, 8.00; O, 13.70; methoxyl, 8.86; C-methyl, 4.29; mol. wt., 350. Found: C, 72.44; H, 6.48; N, 7.82; O, 13.29; methoxyl, 9.03; C-methyl, 3.43; mol. wt. (mass spec.), 350.

Alkaline saponification of the aldehyde, as described above for polyneuridine, followed by acidification with hydrochloric acid and recrystallization from methanol did not result in decarboxylation, but only loss of the ester function with formation of the acid hydrochloride corresponding to XIVd, m.p. above 320°.

Anal. Calcd. for $C_{20}H_{21}ClN_2O_5$: C, 64.43; H, 5.68; N, 7.51; O, 12.87. Found: C, 64.21; H, 5.87; N, 7.32; O, 12.76.

Attempted deuterium exchange of the aldehyde XIVd by heating under reflux four times with potassium in deuteriomethanol containing some heavy water and mass spectrometric analysis of the product showed that its molecular weight (350) remained unchanged.

Lithium Aluminum Hydride Reduction of Polyneuridine.—A solution of 100 mg. of polyneuridine in 15 cc. of dry tetrahydrofuran was heated under reflux for 2 hr. with excess lithium aluminum hydride and the excess reagent was decomposed with ethyl acetate. A saturated aqueous sodium sulfate solution was added and the **diol XIVe** was isolated with chloroform and recrystallized from the same solvent; yield 95 mg., m.p. 260–265°, $[\alpha]D$ −36° (*c* 1.05 in pyridine), $[\alpha]D$ −14° (*c* 1.1 in methanol); λ_{max}^{EtOH} 228 and 281 mμ, log ε 4.46, 3.75; and λ_{min}^{EtOH} 249 mμ, log ε 3.30, no carbonyl absorption in the infrared. This substance was shown to be identical with akuammidinol (XIVe),[37] the lithium aluminum hydride reduction product of akuammidine (XIVk), by direct comparison (mixture melting point determination, thin-layer chromatographic mobility) with an authentic specimen[39] (m.p. 260–265°).

For further characterization, the diol XIVe was acetylated in the standard manner to the **diacetate XIVf**, which was recrystallized from methanol; m.p. 224–227°. This proved to be the most desirable derivative for n.m.r. measurements as mentioned in the Discussion section.

Anal. Calcd. for $C_{24}H_{28}N_2O_4$: C, 70.58; H, 6.91; N, 6.86; mol. wt., 408. Found: C, 70.65; H, 6.92; N, 6.87; mol. wt. (mass spec.), 408.

The above reaction sequence was repeated with lithium aluminum deuteride to afford the dideuterio derivative XIVg, which exhibited the expected mass spectrometric molecular ion at *m/e* 410.

Conversion of Polyneuridine (XIVa) to Normacusine-B (XIVi).—A solution of 150 mg. of polyneuridine in dry benzene was heated under reflux for 3 hr. in an atmosphere of nitrogen with 100 mg. of twice sublimed (180° and 0.05 mm.) potassium *t*-butoxide and then diluted with water. The crystalline residue (120 mg.) was twice recrystallized from ether; m.p. 246–247°, $[\alpha]D$ +39° (*c* 0.52 in methanol), and proved to be the ***t*-butyl ester XIVh** by mass spectrometry (mol. wt. 364 and very strong peak at M-57 due to the loss of the *t*-butyl fragment). This ester (61 mg.) was reduced with 150 mg. of lithium aluminum hydride in tetrahydrofuran (3 hr. reflux) and the product crystallized from ether to afford 25 mg. of pure **normacusine-B (XIVi)**, the identity of which was confirmed by mixture melting point determination, mass spectral comparison and thin-layer chromatography.

Mass Spectrometric Measurements and Preparation of Deuterated Alkaloids.—All measurements were performed with a Consolidated Electrodynamics Corp. mass spectrometer 21-103C using an all-glass heated inlet system maintained at 200°. The ionizing voltage was 70 ev. and the ionizing current 50 μa.

3-Deuterioyohimbine (IVb) was prepared by adding a suspension of 200 mg. of sodium borodeuteride in tetrahydrofuran to 29 mg. of 3-dehydroyohimbine perchlorate[16] (m.p. 205–206°) in 5 cc. of deuteriomethanol. After the initial vigorous reaction had subsided, the mixture was heated under reflux for 15 min., evaporated under reduced pressure and the residue partitioned between water and chloroform. From the chloroform layer, there was isolated 23 mg. of crude 3-deuterioyohimbine (IVb), which was stirred for 30 min. in methanol (for back-exchange of any deuterium attached to nitrogen or oxygen) and then recrystallized from benzene (m.p. 233°).

3,5,6-Trideuterioajmalicine was synthesized by reduction[30] of 44 mg. of serpentine hydrochloride[29] (m.p. 260°) in 3 cc. of deuteriomethanol with 300 mg. of sodium borodeuteride suspended in 3 cc. of dry tetrahydrofuran. The reaction mixture was processed exactly as described above and after recrystallization from methanol gave fine needles of the 3,5,6-trideuterio analog of ajmalicine (IX), m.p. 254–255° (mol. wt. calcd. for $C_{21}H_{21}D_3N_2O_3$: 355; found mass spectrometrically: 355).

Preparation of Sodium Borodeuteride.[17]—Freshly distilled boron trifluoride etherate (6.5 cc.) in 15 cc. of dry tetrahydrofuran was added over a period of 45 min. in a current of nitrogen to a refluxing and stirred solution of 892 mg. of lithium aluminum deuteride in 40 cc. of dry tetrahydrofuran. The hexadeuterated diborane, thus generated, was passed into a trap containing a solution of 1.52 g. of sodium tetramethoxyborate (prepared by heating under reflux for 5 hr. sodium borohydride with excess absolute methanol and evaporating to dryness *in vacuo*, followed by drying for 4 days at 0.1 mm.) in 15 cc. of tetrahydrofuran. Within 20 min., a precipitate of sodium borodeuteride started to appear and after a reflux time of 1 hr., the tetrahydrofuran in the trap was decanted from the sodium borodeuteride, which was used directly for the above reduction experiments.

Isolation of β-Yohimbine from *Aspidosperma eburneum* F. Allem.—A total of 12.6 kg. of leaves, twigs and bark of *A. eburneum* was extracted exactly as described above for *A. polyneuron*, except that 5% acetic acid was substituted for hydrochloric acid. The crude, chloroform-insoluble alkaloid acetates (10.7 g. of base) were chromatographed on alumina yielding 4.1 g. of alkaloid, m.p. 214–217°, which was transformed into the oxalate and recrystallized from methanol; yield 3.5 g., m.p. 253–254°. The alkaloid was regenerated from the oxalate and after two recrystallizations from methanol led to colorless crystals melting at 235° upon immersion of the capillary at 190°, $[\alpha]D$ −55.4° (*c* 1.0 in pyridine on dried material). The substance exhibited a mass spectrum of the yohimbine type (see Fig. 1) and direct comparison (mixture melting point determination, infrared comparison, thin-layer chromatographic mobility) with an authentic specimen[39] of β-yohimbine (IVa, 3α, 15α, 16α, 17β, 20β)[15,31] established the identity of the alkaloid.

Anal. Calcd. for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90; O, 13.54; methoxyl, 8.76; mol. wt., 354. Found: C, 71.00; H, 7.31; N, 7.86; O, 13.73; methoxyl, 8.72; C or N-methyl, 0; mol. wt. (mass spec.), 354.