chloroisogriseofulvin where the atypical 4-methoxy and 6′-methyl resonances hint at some minor, but nonetheless significant, environmental change.

In assessing the general character of the data, it is apparent that the clear delineation of all proton types together with the sensitivity of the chemical shifts toward structural change enables n.m.r. to play a very important role in investigating the griseofulvin species. Changes in functionality influence the shielding values

TABLE III

Proton type	τ^a (Griseofulvins)	τ^a (Isogriseofulvins)	$\Delta\tau$
4-CH_3O	5.97	6.10	+0.13
5-H	3.86	3.94	+ .08
3′-H	4.48	4.60	+ .12
6′-CH_3	9.02	8.96	− .06

[a] Average deviation for all values ±0.02 or less.

of nearby protons in a characteristic fashion. Furthermore, when more than one functional group is introduced, the individual effects appear to be additive. These two features are of obvious utility and allow the setting up of a list of incremental shift values reminiscent of the work in steroids.[5,6]

Although most of the spectra could be interpreted satisfactorily in terms of the foregoing simple rules, there were several atypical effects. The cases of 3′-chloroisogriseofulvin and the 6-hydroxy-4′-isopropoxy analog of isogriseofulvin have already been mentioned. Other anomalies were: (1) the selective downfield shifts of the 6′-methyl and 3′-olefinic proton in griseolactone (VI), (2) the collapse of the long range C_3'–C_5' proton coupling in 6′-desmethyldehydrogriseofulvin and (3) the perturbation of the $-CH_2CH-$ pattern by 5-chlorination.

The last example serves as a reminder that a remote functionality is not always without influence. It is reasonable to suppose that the effect is an indirect one, arising from a change in ring or possibly 3-keto anisotropy induced by the 5-chloro group.

The proton magnetic resonance data were obtained with a 60 megacycle Varian Associates Model 4300B spectrometer. Unless otherwise stipulated, spectra were run as dilute solutions (5% or less) in $CDCl_3$. The resonance positions were determined relative to an external benzene reference and scaled by the usual side band method.[7] The shielding numbers were calculated from the equation[8] $T = \Delta\gamma/\gamma_0 + 3.60$, where $\Delta\gamma$ is the observed displacement from benzene in cycles per second, and γ_0 is the spectrometer frequency in megacycles. For acetone, 2.85 was used as the constant in place of 3.60. The precision of the chemical shifts and coupling constants is approximately ±1 cycle.

(5) J. Shoolery and M. T. Rogers, *J. Am. Chem. Soc.*, **80**, 5121 (1958).
(6) N. R. Trenner, B. H. Arison, D. Taub and N. L. Wendler, *Proc. Chem. Soc.*, 214 (1961).
(7) J. T. Arnold and M. E. Packard, *J. Chem. Phys.*, **19**, 1608 (1951).
(8) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY, CAMBRIDGE 39, MASS.]

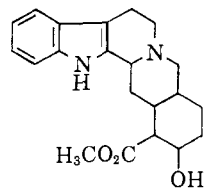
Application of Mass Spectrometry to Structure Problems. X.[1] Alkaloids of the Bark of *Aspidosperma quebracho blanco*[2]

BY K. BIEMANN, MARGOT SPITELLER-FRIEDMANN AND G. SPITELLER

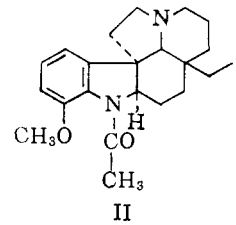
RECEIVED AUGUST 10, 1962

A detailed investigation, with the aid of gas chromatography and mass spectrometry, of the crude alkaloid mixture obtained from the bark of *Aspidosperma quebracho blanco* led to the detection of over twenty compounds. Sixteen of these were isolated in quantities sufficient for the determination of their structure. Three were those known to occur in this plant yohimbine (I), aspidospermine (II) and quebrachamine (III), while the remaining ones turned out to be related to II or to belong to a new group of which aspidospermatine (338B) is the most abundant derivative. The interpretation of the mass spectra which made it possible to arrive at the structures of these alkaloids is discussion in detail.

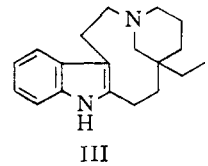
Investigations of the alkaloids of the bark of *Aspidosperma quebracho blanco* Schlecht. during the last century led to the isolation[3] of six alkaloids, aspidospermine, quebrachamine, quebrachine, aspidospermatine, hypobrachine and aspidosamine. Of these, quebrachine was later found[4] to be identical with yohimbine (I) and only recently the structures of two others, aspidospermine (II)[5] and quebrachamine (III),[6] were established. The remaining three alkaloids were not well characterized and had not attracted any serious attention since their isolation.[3] In spite of the great interest which the Apocynaceae family in general and the genus Aspidosperma in particular received during the last decade,[7] *A. quebracho blanco* has not been reinvestigated by more modern techniques although the plant material is readily available. An exception is a recent short note reporting the isolation of a new glucoalkaloid, quebrachacidin.[8]



I



II



III

Recently we have shown that mass spectrometry can be used advantageously for the determination of the structure of alkaloids.[9a,b] This technique was also used for the determination of the structure of quebrachamine (III)[6] which necessitated the determi-

(1) Part IX, H. K. Schnoes, A. L. Burlingame and K. Biemann, *Tetrahedron Letters*, **No. 22**, 993 (1962).
(2) For a preliminary report on this subject see K. Biemann, M. Friedmann-Spiteller and G. Spiteller, *ibid.*, **No. 14**, 485 (1961).
(3) O. Hesse, *Ann.*, **211**, 249 (1882).
(4) E. Fourneau and H. Page, *Bull. sci. pharmacol.*, **21**, 7 (1914).
(5) J. F. D. Mills and S. C. Nyburg, *Tetrahedron Letters*, **No. 11**, 1 (1959); H. Conroy, P. R. Brook and Y. Amiel, *ibid.*, **No. 11**, 4 (1959).
(6) K. Biemann and G. Spiteller, *ibid.*, **No. 9**, 299 (1961); *J. Am. Chem. Soc.*, **84**, 4578 (1962).
(7) For a review see J. Schmutz, *Pharm. Acta Helv.*, **36**, 103 (1961).
(8) P. Tunmann and J. Rachor, *Naturwiss.*, **47**, 471 (1960).
(9) (a) K. Biemann, *Tetrahedron Letters*, **No. 15**, 9 (1960); (b) For a detailed discussion of this subject see K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Chapter 8.

TABLE I

ALKALOIDS OF *A. quebracho blanco* LISTED IN THE ORDER OF ELUTION FROM AN ALUMINA COLUMN[d]

Compound	Eluent	Abundance, %	M.p., °C.	$[\alpha]_D$	Ultraviolet maxima, mμ (log ε)[a]
III	Petr. ether–C_6H_6 1:1	2.5	146–147		
296A	1:2	0.5	[b]		
326A	1:5	0.5	[b]		
280A	1:9	2	[b]		222 (4.39), 228 (4.26), 253 (3.78)
278[c]	1:9	0.5	[b]		
282A	C_6H_6–$CHCl_3$ 10:0.5	1	110–112		
312A	10:1	3	109–111		247 (3.85), 291 (3.41)
342A	10:1	0.5	144–147		215 (4.38), 293 (3.50)
354A(= II)	10:1	30	208–209		218 (4.53), 255 (4.07), 290 (3.45)
384A	10:1.5	1.5	148–150	−93°	223 (4.45), 252 (4.0), 286 (3.35)
280B	10:1.5	1	[b]		
266B	10:2	3	184–186		242 (3.83), 296 (3.49)
296B	10:2	1.5	[b]		
308B	10:3	1	[b]		
338B	10:5	3	157–159	−73°	219 (4.54), 255 (4.10), 290 (3.62)
340B	*10:5*	*1*	[b]		
I	$CHCl_3$	10	223–225	+104°	

[a] In ethanol. [b] Not obtained crystalline. [c] Eburnamenine (see ref. 1). [d] 1.95 g. total alkaloids from 300 g. of powdered bark.

nation and detailed interpretation of the mass spectrum of this alkaloid and some of its derivatives. It thus was of interest to use this very sensitive technique for the detection of alkaloids related to III in the plant in which it is most abundant, namely *A. quebracho blanco*. A reinvestigation of the alkaloids occurring in the bark of this South American tree did not reveal any trace (limit of detection is estimated to be less than 0.1% of crude alkaloid mixture) of other compounds with the carbon skeleton of III but led to the detection of about twenty new alkaloids. The structures of fifteen of these, among them Hesse's aspidospermatine, were reported in a preliminary communication[2] and the present paper presents the details of this work.

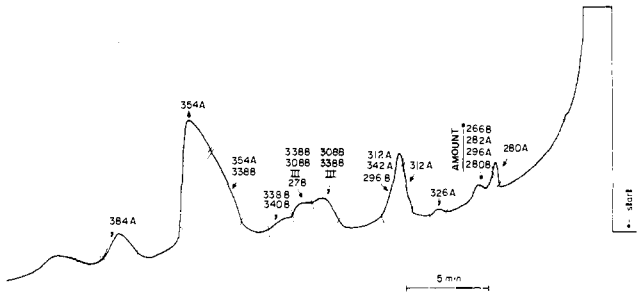


Fig. 1.—Gas chromatogram of alkaloid mixture isolated from *A. quebracho blanco* (8 ft. column, 265°, 6% Apiezon L on Chromosorb W, 10 p.s.i. helium).

When the crude extract of dry powdered bark was separated in neutral, acidic and basic fractions and the basic one put on a gas chromatographic column operated at 265°, a record was obtained (Fig. 1) which showed at least eight distinct peaks indicating the presence of a larger number of compounds than previously reported.[7] The mass spectra of the fractions collected between the slanted lines in the figure revealed that most of the peaks contain, in fact, more than one component and it was eventually possible to detect more than 20 alkaloids in this mixture.

From the mass spectra it was possible to glean a preliminary relationship among some of these compounds; namely, one group exhibited an intense peak at *m/e* 124, a fragment characteristic for aspidospermine (II), the spectrum of which we had determined before, while the other group gave spectra which were dominated by an intense peak at mass 136. These characteristics combined with the molecular weight of the compound which could be determined even in mixtures from spectra determined with an electron energy of about 10 e.v., led to a preliminary nomenclature for all these compounds which will also be used throughout this paper instead of Roman numerals. The molecular weight followed by A designates all those alkaloids exhibiting an intense peak at mass 124 and thus probably belonging to the aspidospermine family while B indicates those substances showing an intense peak at mass 136 (or 138 in the case of the dihydro derivatives) for the group of compounds which belong to a new type of indole alkaloids as will be demonstrated below. Aspidospermine (II) will thus be henceforth called 354A. Because of the complexity of this mixture in which many components were present only in a rather small amount, overloading of the column was necessary which led to a somewhat low degree of resolution. Rather than collecting and rechromatographing the unresolved peaks, the entire mixture was preseparated on an alumina column and about 185 fractions were collected. The chromatogram was followed by gas chromatography and mass spectrometry which revealed the fact that quebrachamine (III) was eluted first, followed by group A, then by group B and finally by yohimbine (I). The even more polar components could be eluted with chloroform–methanol mixtures but were not further investigated in the course of this work. On the basis of these data suitable groups were combined and the major components purified by crystallization while the remaining compounds were obtained pure by gas chromatographic separation and collection of the individual components. This permitted the determination of the mass spectra of all fractions and, where necessary, also their ultraviolet and infrared spectra.

The results of this separation on alumina are summarized in Table I, listing the compounds in the order of their elution from the column. The figure in the second column indicates the amount to which these alkaloids are present in the crude extract as judged from the weights of the fractions and their relative composition, determined from the gas chromatogram. It should not imply, however, the actual yield in which these compounds were obtained. Many but not all of the fractions could be induced to crystallize. The small amounts of material we had at hand in some cases precluded further efforts to achieve crystallization. In addition to the compounds listed in Table I and discussed in the present paper there were found traces of other compounds, namely 294A, 294B,

308A, 320B, 322B, 324B, 368A and 398A. The classification as A and B is for some of these still tentative. The structures of these compounds are presently being investigated.

The Aspidospermine Group.—The mass spectra of the fractions designated III, 312A, and 354A, indicated that they represent quebrachamine, deacetylaspidospermine, and aspidospermine, the spectra of which we had determined previously in connection with the elucidation of the structure of quebrachamine.[6] In addition, compound 280A was found to give a spectrum identical with one[6] obtained from a dehydrogenation product of quebrachamine, namely, 1,2-dehydroaspidospermidine (for nomenclature see below). This was confirmed by reduction with lithium aluminum deuteride to a compound of molecular weight 283, the spectrum of which was analogous to the one of 282A (Fig. 3a) except that the peaks at *m/e* 130, 143, 144, 254 and 282 are now shifted to *m/e* 131, 144, 145, 255 and 283. As will be shown below, 282A represents the unsubstituted carbon skeleton of aspidospermine, for which we have chosen the name aspidospermidine in an attempt to simplify the nomenclature of all these alkaloids and to avoid the necessity of giving a trivial name to each one. All the derivatives discussed henceforth will be named either as substituted aspidospermidine or aspidospermine, whichever is shorter.

Based on the "mass spectrometric shift" technique outlined previously,[9a,10] it can be shown that the alkaloids belonging to group A are derivatives of aspidospermidine substituted with methyl, methoxyl and acetyl groups since certain peaks in the spectra are displaced 14, 30 and 42 mass units, or a combination thereof (Fig. 3).[11] These substituents were shown to be present in the dihydroindole moiety on the basis of the following detailed analysis of the mass spectrum of deacetylaspidospermine (312A, Fig. 2).

The most characteristic peaks in this spectrum are found at *m/e* 124 and 284. The latter arises by a fragmentation of ring C (see Scheme I) accompanied by expulsion C-3 and C-4 as ethylene. This fragmentation is somewhat analogous to the one exhibited by six-membered rings containing a double bond, in which fragmentation of one of the allylic bonds leads to a retro-Diels–Alder type decomposition.[12] In the present

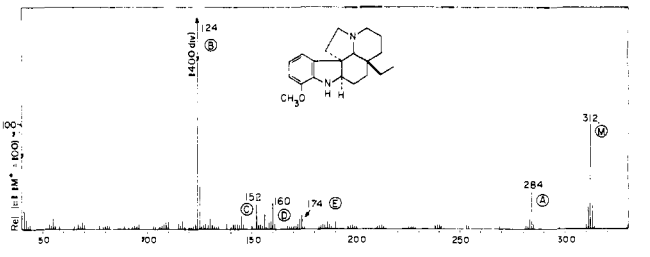
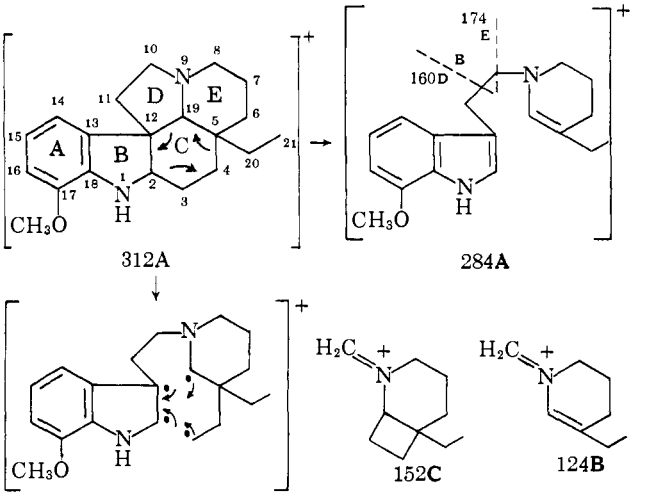


Fig. 2.—Mass spectrum of deacetylaspidospermine (312A).[11]

case the fragmentation is initiated by cleavage of the C-12, C-19 bond, which is most prone to break in this



Scheme I.

molecule as it connects one benzylic carbon atom with one attached to nitrogen. This process is energetically favorable because although three bonds have to be broken, much energy is regained by the formation of a highly conjugated system, an indole derivative, a trisubstituted double bond and a neutral molecule, ethylene. In addition, the somewhat strained polycyclic ring system is relieved of any strain.

The resulting charged particle **A** now contains a bond linking two carbon atoms, one of which (C-11) is attached to the aromatic system and the other one (C-10) to nitrogen. Cleavage of this bond is favored because the resulting radical (at C-11) is well stabilized by the aromatic moiety while the positive charge on C-10 is stabilized by the free electron pair on N-9, resulting in the ion **B** of mass 124. The opposite charge distribution is much less favorable although a positive charge at C-11 would also be stabilized by the ring system, but the radical remaining at C-10 would not receive any particular stabilization. It is for this reason that the fragment **B** (*m/e* 124) is in all these spectra much more intense than fragment **D** (*m/e* 160 in 312A, for example).

Metastable peaks corroborate this two-step process and its sequence, namely, M → **A** → **B**. For example, in the spectrum of 282A such peaks are found with maxima at *m* = *m/e* 229.7 and 60.8 (calcd. 228.8 for 282 → 254; 60.5 for 254 → 124). It is worth noting that the abundance of the fragment **A** (M − 28; molecular weight minus 28 mass units) is strongly dependent on the substitution of the indole moiety. The addition of methoxyl groups decreases the intensity of the peak **A** from 70% of M^+ in 282A to 35% of M^+ in 312A and further to 24% of M^+ in 342A. Similarly, a methyl group at N-1 decreases this peak from 70% to 29% of M^+ (282A *vs.* 296A) and from 35% to 10% of

(10) (a) Both in our original communication[9a] describing this technique and on later occasions (see ref. 9b and references cited therein) we have pointed out that such correlations may be considered conclusive if these additional substituents are attached to the aromatic moiety and if the various spectra all exhibit the same peaks, either shifted for the appropriate mass difference or unchanged, and if the intensity relationships are quite similar.

It is implicit in the reasoning on which this technique is based that the mere occurrence of some fragments of the same or analogous mass in the spectra of two compounds should never be construed as proof for the presence of an identical carbon skeleton in the two compounds (a good example is the pairs 266B and VI, both exhibiting intense peaks at *m/e* 136, or 268B and VII with peaks at *m/e* 138). This applies also to strychnanone,[10b] whose mass spectrum exhibits peaks at *m/e* 124, 130, 144 and M − 28. The absence of a peak at *m/e* 152 and the quite different intensity ratios (the M^+-peak is relatively small) should immediately have cast doubt on any assumption that this compound was of the aspidospermine type. Comparison of the spectrum of strychnanone[10b] and the actual aspidospermine analog of the corresponding molecular weight (aspidospermidine, 282A, Fig. 3a) illustrates this point quite clearly.

A detailed evaluation of the influence of functional groups in the alicyclic part of the skeleton is important for the interpretation of the mass spectra of compounds substituted in that manner. Such a discussion, which would go far beyond the framework of this paper, can be found elsewhere (see ref. 9b).

(b) C. Djerassi, H. W. Brewer, A. Budzikiewicz, O. O. Orazi and R. A. Corral, *J. Am. Chem. Soc.*, **84**, 3480 (1962).

(11) All peaks ≥ 2% of the abundance of the molecular ion M^+, and falling in the mass range shown in the particular figure, are shown in the figures. Peaks due to multiply charged fragments of odd mass and/or to metastable ions are omitted even if their intensity would exceed the above limit. In some figures the lower mass range, which does not contain significant peaks (unless mentioned in the text), as borne out by Fig. 2 for example, is omitted for the sake of better readability of the graphs. Note that the most intense peak in all these spectra is too high to be shown in the correct proportion. Its correct intensity (in terms of M^+ = 100 div.) is indicated in each case.

(12) (a) K. Biemann, *Angew. Chem.*, **74**, 102 (1962); *Intern. Ed.*, **1**, 98 (1962). (b) For a more detailed discussion of the interpretation of the mass spectra of complex organic molecules, see ref. 9b, especially Chapter 3.

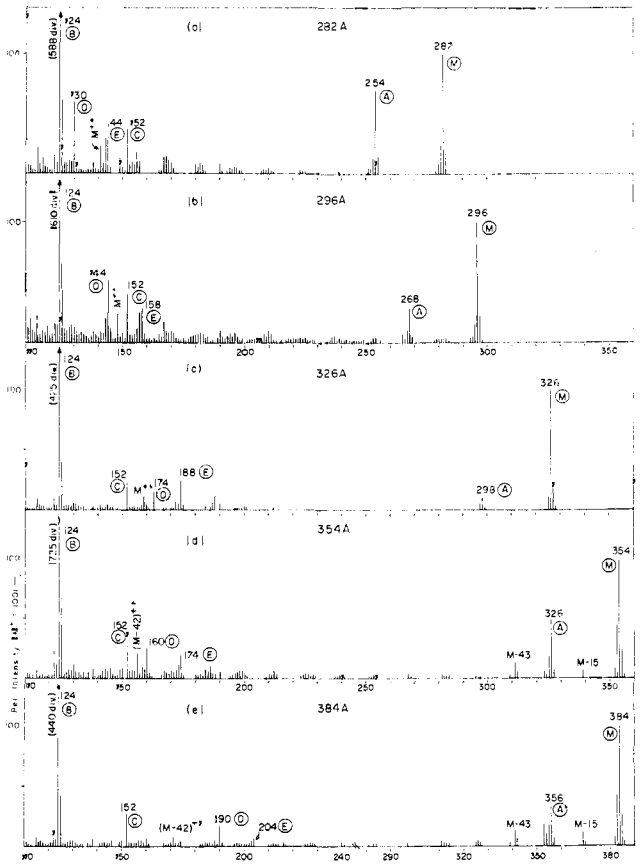
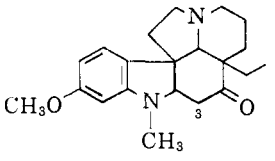


Fig. 3.—Mass spectra of the aspidospermine group.[11]

M^+ (312A *vs.* 326A). Conversely, acetylation increases the abundance of this fragment from 35% (in 312A) to 49% (in 354A).

Fragmentation of the N-9, C-10 bond gives rise to fragment **E**. Simple cleavage of a C–N bond generally[12b] leads to retention of the positive charge at carbon because a sextet at nitrogen is energetically unfavorable. This ion contains C-10 and C-11 as shown rather than C-3 and C-11 as deduced from the mass spectrum of IV, a derivative of vindoline.[13] It exhibited a peak at 188, unchanged upon incorporation of deuterium at C-3.



IV

Retention of C-3 and C-4 on the piperidine ring leads to the ion **C** of mass 152. Its appearance would seem to indicate that the cleavage of ring C is not completely concerted but is initiated by the splitting of the C-12, C-19 bond followed by the C-2, C-3 bond. The resulting diradical (see Scheme 1) can either form a new C-3, C-19 bond or, more frequently, eliminate ethylene by further rupture of the C-4, C-5 bond, giving rise to fragment **A** (M − 28). Cleavage of the C-10, C-11 bond leads then to the fragments of mass 152 and 124, respectively. The spectrum of the above-mentioned ketone IV deuterated at C-3 supports the assignment of these fragments. It exhibited peaks at *m/e* 124 (fragment **B**) and at *m/e* 168 (166 in undeuterated compound) for fragment **C**.

The mass spectrum (Fig. 3d) of aspidospermine (354A) illustrates the influence of an acetyl group at N-1 upon the mass spectra. Aside from an increase of the molecular weight for 42 mass units, peaks are found which correspond to the loss of 15 (CH_3) and 43 (CH_3CO). In addition, a peak at mass 43 is also found (not shown in Fig. 3d). On the other hand, elimination of the acetyl group in the form of ketene (42 mass units) from fragments seems to be a favorable process because the indole peaks **D** and **E** are of the same mass as the corresponding fragments in the non-acetylated derivative (312A, Fig. 2). The driving force for this elimination might be the better stabilization of the indole system, if the electron pair at N-1 is fully available.

It is also of interest to note that in contrast to the unacetylated compounds the M^{++} peak is absent while an intense peak is found for the doubly charged species arising by loss of ketene $(M - 42)^{++}$. The stability of a molecular species lacking two electrons is probably higher in the absence of an electron-withdrawing substituent such as acetyl at the indolic nitrogen.

The lack of an appreciable peak in all these spectra at mass M − 29 is at first surprising in view of the presence of an ethyl group attached to a quaternary carbon atom. Loss of this group would, however, lead to a bridgehead carbonium ion and is thus very unfavorable compared with the cleavage of another bond (such as C-4, C-5) at this center.

On the basis of the fragmentation processes outlined above for deacetylaspidospermine, it is a simple matter to assign structures to the alkaloids not previously reported in *A. quebracho blanco*, namely 282A, 296A, 326A, 342A and 384A, the spectra of most of which are shown in Fig. 3. All of them have to be based on the carbon skeleton of aspidospermine as they all exhibit the characteristic peaks at *m/e* 124, 152 and M − 28. The first one, 282A, shows the fragments **D** and **E** at *m/e* 130 and 144, *i.e.*, 30 mass units (H *vs.* CH_3O) lower than in deacetylaspidospermine (312A). It therefore corresponds to the demethoxy derivative and represents thus the basic carbon skeleton of aspidospermine and is named aspidospermidine for reasons outlined above.

Alkaloid 296A (Fig. 3b) shows the fragments **A**, **D**, **E**, and the molecular weight increased for 14 mass units compared with 282A and is thus a higher homolog with a methyl group in the indole moiety. This group seems to be attached to the nitrogen rather than to the benzene ring because the compound was eluted from the alumina column earlier than 282A, an assumption supported by the structure of 326A. Its mass spectrum (Fig. 3c) has the peaks **A**, **D**, **E** and M displaced to higher mass by 14 units *vs.* deacetylaspidospermine (312A) and by 30 mass units *vs.* 296A. Compound 326A is thus the higher homolog of 312A and was thought to be the N-1 methyl derivative for reasons discussed above. This was proved to be correct on comparison with synthetic N-methyldeacetylaspidospermine, prepared by a procedure described previously,[11] which showed identical mass and infrared spectra and had the same retention time on gas chromatography. Like the natural material, it is reported[14] as noncrystalline. Because of the considerable differences in polarity between compounds possessing a secondary amino group and those lacking hydrogen on nitrogen, we consider the similarity in the chromatographic behavior of 296A and 326A (both are eluted from the alumina column much earlier than the demethyl analogs) sufficient proof for the placement of the methyl group at N-1 also in 296A.

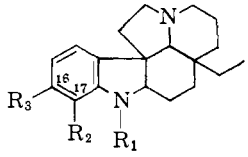
Inspection of Fig. 3e leads to the conclusion that compound 384A contains an N-acetyl group (peaks at

(13) M. Gorman, N. Neuss and K. Biemann, *J. Am. Chem. Soc.*, **84**, 1058 (1962).

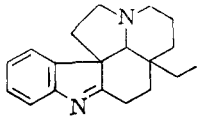
(14) B. Witkop and J. B. Patrik, *ibid.*, **76**, 5603 (1954).

m/e 43, M − 43 and M − 15) in addition to two methoxyl groups in the indole moiety (indole peaks at *m/e* 190 and 204). On hydrolysis (see below) it was converted to 342A, the mass spectrum of which was in turn identical with the one[15] of deacetyl-(+)-pyrifolidine, determined earlier in our laboratory. The identity of ultraviolet spectrum and melting point proved the 16,17-position of the two methoxyl groups in 342A, and thus also in 384A.

The structures of the alkaloids of group A can thus be summarized as



282A (R_1 = R_2 = R_3 = H): aspidospermidine
296A (R_1 = CH_3, R_2 = R_3 = H): 1-methylaspidospermidine
312A (R_2 = OCH_3, R_1 = R_3 = H): deacetylaspidospermine
326A (R_1 = CH_3, R_2 = OCH_3, R_3 = H): 1-methyldeacetylaspidospermine
342A (R_1 = H, R_2 = R_3 = OCH_3): deacetylpyrifolidine
354A (= II; R_1 = CH_3CO, R_2 = OCH_3, R_3 = H): aspidospermine
384A (R_1 = CH_3CO, R_2 = R_3 = OCH_3): (−)-pyrifolidine



280A: 1,2-Dehydroaspidospermidine

Because of the occurrence in nature of both antipodal forms of 16,17-oxygenated derivatives of aspidospermine, namely aspidocarpine (1-acetyl-16-methoxy-17-hydroxyaspidospermidine)[16] which has the same absolute configuration as aspidospermine, while (+)-pyrifolidine had the opposite one,[15] it became of importance to determine the optical rotation of our dimethoxy derivative (384A). Isolation of sufficient amounts in pure form required hydrolysis of those chromatographic fractions which contained 384A in addition to 354A because the deacetyl derivatives could be separated more efficiently on alumina. Reacetylation of 342A, whose rotation is too low ($[\alpha]_D$ +7°)[15] to permit accurate measurement with the small amounts available, yielded (−)-pyrifolidine (384A), $[\alpha]^{28}_D$ −93°. As was expected the absolute configuration of 384A, isolated from *A. quebracho blanco*, is thus the same as of the major alkaloid of that plant, aspidospermine, but opposite to the one of pyrifolidine found in *A. pyrifolium*.[15]

The presence of unacetylated derivatives (*e.g.*, 312A and 342A) in the alkaloid mixture made it necessary to ascertain that these are not artifacts formed during isolation from the more abundant acetyl derivatives (354A and 384A). This possibility is eliminated because the deacetyl derivatives listed in Table I could be detected in the corresponding gas chromatographic fractions of a total extract that had not been treated with acid or base. Furthermore, when pure aspidospermine (354A) was subjected to conditions identical with those for the extraction of the powdered bark and the isolation of the alkaloid fraction, no deacetylaspidospermine (312A) could be detected by gas chromatography and mass spectrometry.

The Aspidospermatine Group.—The determination of the structure of the alkaloids of group B presented a more formidable problem because their mass spectra

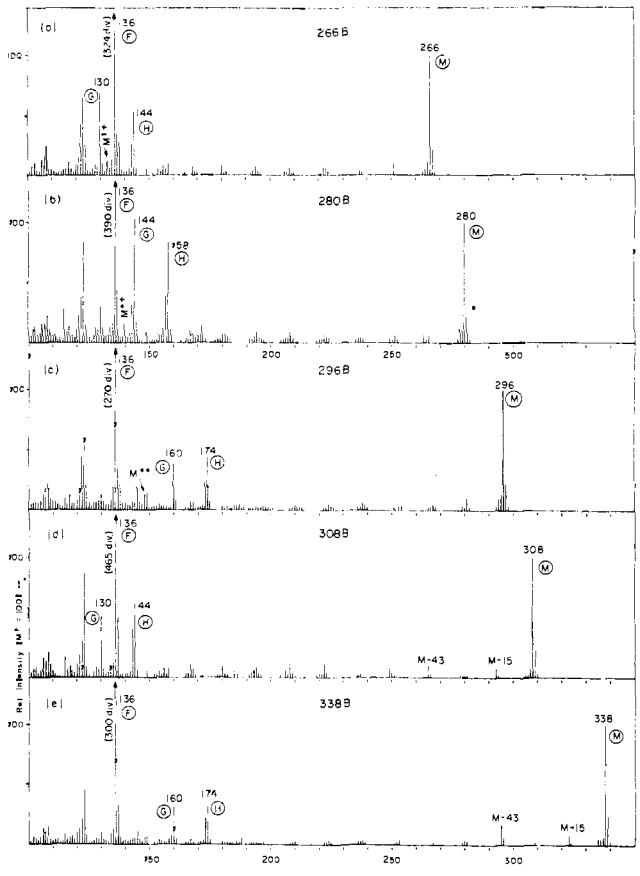


Fig. 4.—Mass spectra of the aspidospermatine group.[11]

clearly indicated that they do not belong to the aspidospermine group (lack of peaks at *m/e* 124 and M − 28) and also did not resemble any other mass spectral type we had encountered at that time.

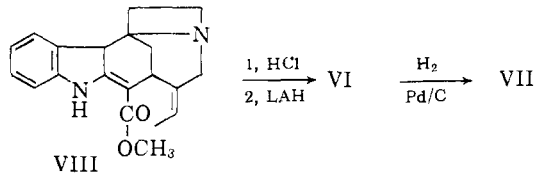
The peaks at *m/e* 130 and 144 in the spectrum (Fig. 4a) of 266B as well as its ultraviolet spectrum (Table I) indicated the presence of a dihydroindole moiety similar to group A. The intense peak[11] at *m/e* 136 present in all representatives of group B (except *m/e* 238 in 340B) could, however, not be an unsaturated higher homolog of the fragment of mass 124 characteristic of group A because the smallest representative of the latter had a molecular weight (282) higher and not lower than the smallest compound of group B (266B). Furthermore, no C_2-fragment is eliminated, indicating the absence of a two-carbon bridge of the type present in aspidospermine.

These facts and the assumption that the high abundance of the ion of *m/e* 136 arises by a mechanism reminiscent of the formation of the fragment of mass 124, discussed above, are best reconciled by the proposal of a one-carbon bridge linking the dihydroindole and piperidine moieties in a tryptamine-derived structure. A carbon skeleton (V) fulfilling these requirements is present in all alkaloids related to the Wieland–Gumlich aldehyde.

Cyclic decomposition (see Scheme II) of ring C initiated by cleavage of the C-3, C-7 bond (the equivalent of the C-12, C-19 bond in group A) leads in this case without elimination of a neutral fragment to an "open" structure in which the two major parts are connected by the tryptamine bridge (C-5, C-6). Cleavage of this bond and retention of the positive charge at C-5 leads to ion **F** of *m/e* 136 while the ions of *m/e* 130 and 144 (**G** and **H**) are formed in the fashion discussed for ions D and E from the aspidospermine type. A compound of such a structure (VI) has been described

(15) C. Djerassi, B. Gilbert, J. N. Shoolery, L. F. Johnson and K. Biemann, *Experientia*, **17**, 162 (1961); C. Djerassi, A. A. P. G. Archer, T. George, B. Gilbert and L. D. Antonaccio, *Tetrahedron*, **16**, 212 (1961).

(16) S. McLean, K. Palmer and L. Marion, *Can. J. Chem.*, **38**, 1547 (1960).



as a degradation product of akuammicine (VIII).[17] Comparison of its mass spectrum (Fig. 5a) with the one of 266B (Fig. 4a) indicated the general correctness of this hypothesis. In spite of their great similarity there are differences (for example, the group of peaks in the

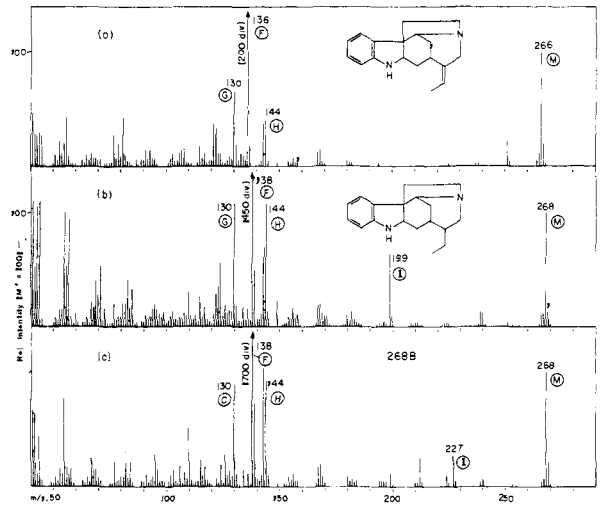
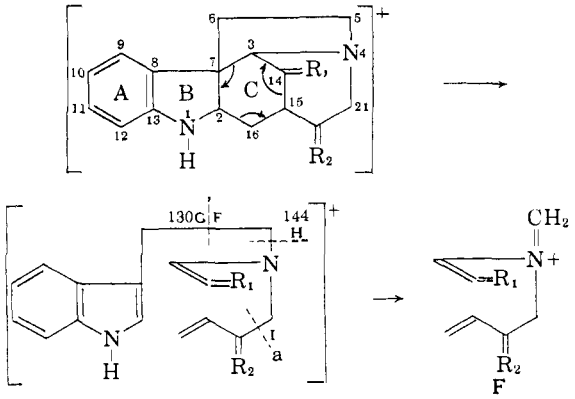


Fig. 5.—Mass spectra of compounds VI (a), VII (b) and 268B (c).[11]

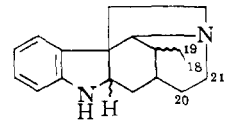
region of *m/e* 120 to 125) which precluded the identity of the two substances. The spectral differences then could be explained by variation of the position of the double bond, of the attachment of the C_2 side chain, or both.



V, $R_1 + R_2 = H_2 + =CHCH_3$
VI, $R_1 = H_2$, $R_2 = =CHCH_3$
VII, $R_1 = H_2$, $R_2 = H$, C_2H_5
136 for $R_1 + R_2 = H_2 + =C_2H_4$

Scheme II

Saturation of the double bond was thought to yield information regarding this point. The mass spectrum of the reduced products VII and 268B (Fig. 5b, and 5c, respectively) differed even more than the unsaturated compounds, thus excluding the possibility of double bond isomerism. The most striking differences are at *m/e* 227 (in 268B) *vs.* *m/e* 199 (in VII). The latter indicates loss of 69 mass units by cleavage of the C-20, C-21 bond (a in Scheme II) while 268B loses only 41 mass units, showing the absence of a substituent at C-5 and, of course, also at C-3 and C-4. This leaves C-21, C-5, C-3 and C-14, of which all but the last one are excluded by the identification of 3-ethylpyridine as the major pyridine fraction formed on zinc dust distillation of these alkaloids requiring attachment of the C_2-chain to a potential 3-position with respect to N-4. The structure of compound 268B is thus

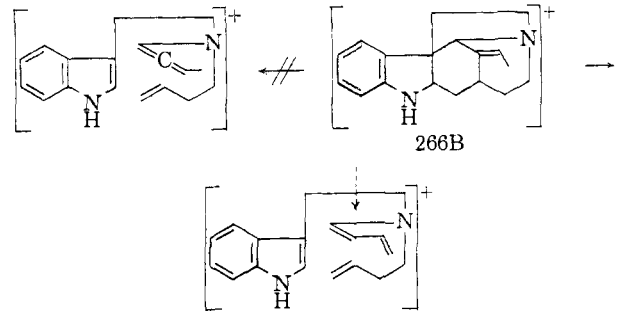


268B

Two problems remain to be solved: (1) the position of the double bond and (2) the type and position of the additional substituents in the other alkaloids of group B.

For the double bond, which must be present in the moiety giving rise to fragment **F** because its mass increases for 2 mass units upon hydrogenation, only the positions 18,19; 20,21; and 14,19 do not violate Bredt's rule. Of these all but the last possibility are eliminated on the following grounds: (a) The n.m.r. spectrum of 338B (the most abundant alkaloid of this group) exhibited a doublet at 8.45 and 8.34 τ (allylic methyl split by one neighboring hydrogen) in addition to two singlets at 7.80 τ ($-COCH_3$) and 6.14 τ ($-OCH_3$); (b) the infrared spectrum indicated the absence of a vinyl group as it exhibited no intense bands at 910 and 990 cm.$^{-1}$; and (c) saturation of the double bond (in 296B) with deuterium (using N_2D_4) led to dideuterio-298B of molecular weight 300 which lost only one of the two deuterium atoms with the C_2-chain.

A 14,19-double bond would seem to require the formation of an allene moiety during the electron-impact induced decomposition of ring C. Double bond migration in molecular (and fragment) ions is, however, frequently observed.[12b] Migration to 19,20 before or during this fragmentation would lead to a highly conjugated ion



266B

Having settled the detailed structure of the simple representative of the alkaloids of group B, the structure of all the other ones listed in Table I can now be deduced from the corresponding mass spectra in the same fashion, as outlined for group A with the exception of the exact position of the aromatic methoxy group which was deduced from the ultraviolet spectrum.[18]

Compound 280B (Fig. 4b) is a higher homolog of 266B with the additional substituent in the indole moiety (fragments **G** and **H** at *m/e* 144 and 158). This methyl group is placed at N-1 because of its behavior on alumina (see discussion of 296A and 326A).

Compound 296B (Fig. 4c) contains an aromatic methoxyl group (molecular weight 296 and fragments **G** and **H** at *m/e* 160 and 174). It was shown to be deacetylaspidospermatine as it is obtained on hydrolysis of 338B.

(17) G. F. Smith and J. T. Wrobel, *J. Chem. Soc.*, 792 (1960).

(18) For the ultraviolet spectra of the various methoxyhexahydrocarbazoles and their N-acetyl derivatives, see J. R. Chalmers, H. T. Openshaw and G. F. Smith, *ibid.*, 1115 (1957).

TABLE II

COMPARISON OF PHYSICAL CONSTANTS OF HESSE'S ASPIDOSPERMATINE[3] AND ALKALOID 338B

	M.p., °C.	$[\alpha]D$ (ethanol)	C	H	N	Pt	H_2O
Aspidospermatine (Hesse)	162	−72.3°	75.07	7.95	7.91	16.65 (16.70)	6.28
338B (present invest.)	157–159	−73°	74.59	7.73	8.28	...	..
Calcd. for: $C_{21}H_{26}N_2O_2$			74.52	7.74	8.27	...	..
$C_{22}H_{28}N_2O_2$			75.00	7.95	7.95	...	..
$(C_{21}H_{26}N_2O_2)_2H_2PtCl_6 \cdot 4H_2O$			...	..	..	16.83	6.22
$(C_{22}H_{28}N_2O_2)_2H_2PtCl_6 \cdot 4H_2O$			...	..	..	16.44	6.04

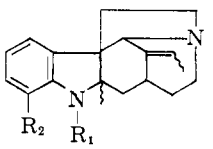
Compound 308B is 42 mass units heavier than 266B but exhibits peaks **F**, **G** and **H** at the same mass (138, 130 and 144) indicating an N-acetyl derivative (see above). This is confirmed by the peaks at *m/e* 43, M − 43 and M − 15 (the last two are less characteristic in this system) as well as hydrolysis to 266B.

The mass spectra of 338B and 296B exhibit the same relationship as 308B and 266B (see Fig. 4). The ultraviolet spectrum of 338B (Table I) corresponds to the one of aspidospermine[18] and the presence of the N-acetyl group is corroborated by a strong band at 1633 cm.$^{-1}$ in the infrared spectrum. This major alkaloid of group B is thus 1-acetyl-12-methoxy-266B.

The relatively high abundance of this substance compared with the other newly discovered alkaloids, its relative ease of crystallization, the melting point, and optical rotation made it highly probable that our alkaloid 338B is, in fact, a substance isolated by Hesse in 1882 and named aspidospermatine.[3] It had been assigned a molecular formula $C_{22}H_{28}N_2O_2$, but the values reported in the earlier work (Table II, where necessary recalculated for better comparison with the theoretical values based on presently accepted atomic weights) agree with the exception of the value for carbon, as well, if not better (*e.g.*, the chloroplatinate), with the C_{21}-formula. Compound 338B is, therefore, named aspidospermatine and for reasons outlined earlier the name aspidospermatidine is adopted for the simple derivative 266B.

Compound 340B is the only one in this group to show a peak at *m/e* 138 (instead of 136) characteristic for analogs of 268B. It was shown to be 14,19-dihydroaspidospermatine on the basis of the identity of its mass spectrum with the one of the compound obtained on hydrogenation of 338B.

The alkaloids of group B are thus best represented by the structures



266B (R_1 = R_2 = H): aspidospermatidine
280B (R_1 = CH_3, R_2 = H): 1-methylaspidospermatidine
296B (R_1 = H, R_2 = OCH_3): deacetylaspidospermatine
308B (R_1 = CH_3CO, R_2 = H): 1-acetylaspidospermatidine
338B (R_1 = CH_3CO, R_2 = OCH_3): aspidospermatine
340B: 14,19-dihydroaspidospermatine

Conclusions.—The determination of the structure of so many new alkaloids reported in the present investigation underscores again the usefulness of mass spectrometry in this field, a potentiality which had been pointed out in the first investigation of this type.[9] The complementary nature of ultraviolet and mass spectra, both requiring less than a milligram of substance, makes it possible to detect alkaloids present to only a very small amount in the plant and to derive their structure. It should be recalled that for none of the structural assignments reported in the present work was an elemental analysis necessary, which would have required more substance than we had available of many of the compounds. The possibility of deducing the main features, or even the complete structure, of an alkaloid isolated by gas chromatography opens the way to much more thorough and complete investigation of the complex alkaloid mixtures produced by plants. The significance of such detailed studies for a better understanding of the biogenetic relationship has been pointed out on previous occasions.[2,6]

The fruitfulness and broad applicability of the mass spectrometric approach and of the detailed interpretation of the fragmentation processes of the carbon skeletons of aspidospermine, aspidospermatine and akuammicine is illustrated by the fact that it has not only led to the elucidation of the indole alkaloids reported in this paper. In the short time since the appearance of our preliminary communication outlining the interpretation of these spectra,[2] it has in addition been used successfully both by us[19] and by the Stanford–Paris group[20] for the determination of a considerable number of other related indole alkaloids.

The purity of those compounds which are not crystalline (see Table I) may be doubted, but the emergence as a single gas chromatographic peak (after preseparation on alumina), coupled with the appearance of the mass spectrum (ref. 9b, Sec. 4–2), indicate that they were reasonably pure.[21]

The lack of elemental analyses (except for 338B) is balanced by the presentation of mass spectrometric molecular weights (and the entire spectrum), which are very reliable for this type of compound (ref. 9b, Sec. 3–1A) and leave as little ambiguity (albeit of a different kind) regarding the true empirical formula as does a conventional elemental analysis. Furthermore, we feel that the mass spectrum is in general at least as good a method for the characterization of organic compounds as other spectra at present accepted for this purpose.

The apparent failure to characterize some of the minor alkaloids "in the presently accepted ways" is, in our opinion, more than offset by the fact that it was possible to put forward structures for these compounds using amounts so small as to preclude such conventional characterization. The complete stereochemistry of the new compounds could not be deduced on the basis of the data at hand. However, the presence of aspidospermine, deacetylaspidospermine, (−)-pyrifolidine, and (−)-quebrachamine (which was shown[6] to be stereochemically related to aspidospermine) makes it very likely that the other compounds (280A, 282A, 296A and 326A) belong to the same stereochemical series. For group B the relative stereochemistry of

(19) (a) Vindoline: see ref. 13; (b) condylocarpine: K. Biemann, A. L. Burlingame and D. Stauffacher, *Tetrahedron Letters*, **No. 12**, 527 (1962).

(20) (a) Pyrifoline and refractidine: B. Gilbert, *et al.*, *ibid.*, **No. 2**, 59 (1962); (b) spegazzinine and spegazzinidine: C. Djerassi, *et al.*, *Experientia*, **18**, 113 (1962); vindolinine: C. Djerassi, *et al.*, *Proc. Natl. Acad. Sci. U.S.*, **48**, 113 (1962); refractine and aspidofractine: C. Djerassi, *et al.*, *J. Am. Chem. Soc.*, **84**, 1499 (1962); vincadifformine: C. Djerassi, *et al.*, *Tetrahedron Letters*, **No. 6**, 235 (1962); tabersonine: M. Plat, *et al.*, *ibid.*, **No. 7**, 271 (1962); mossambine: X. Monseur, *Bull. soc. chim. France*, 1088 (1962); echitamidine: C. Djerassi, *et al.*, *Tetrahedron Letters*, **No. 15**, 653 (1962).

(21) This and the following three paragraphs have been added on suggestion of the Editor, to indicate clearly the extent of our departure from past methods.

only two centers (C-2 and C-19) remains ambiguous because all others are fixed in this bridged system.

Experimental[22]

Isolation of Alkaloids from the Tree Bark of *Aspidosperma quebracho blanco*.—The powdered bark (100 g.) was refluxed with 3 portions of 500 ml. of ethanol for 2 hr. each. After-filtering, the combined ethanolic solutions were evaporated under reduced pressure and the brown residue was extracted three times with 100-ml. portions of hot chloroform. The chloroform solution was extracted first with 1 *N* sodium hydroxide and then with dilute sulfuric acid. The aqueous acidic solution was made slightly alkaline and immediately extracted with several portions of chloroform. After drying and evaporation of the solvent, 0.70 g. of crude alkaloids was obtained. The gas chromatogram shown in Fig. 1 was obtained with a small amount of this material.

For the isolation of the individual components of this mixture, three such portions were combined (total 1.959 g.) and chromatographed on 185 g. of alumina (act. II). Elution was started with petroleum ether–benzene 5:3 and changed gradually to pure chloroform which eluted yohimbine (see Table I). About 60% of the starting material had been recovered at that point. The more polar substances remaining on the column were not further investigated.

Most of these fractions (185 in all) still represented mixtures of a few compounds which could be obtained pure on further separation by gas chromatography (80-cm. column, 10% Apiezon L on Chromosorb W, 250°, 14 lb. helium). Frequently, it was necessary to combine adjacent fractions of the alumina chromatogram before gas chromatography. The individual fractions were collected simply by inserting into the exit tube of the gas chromatograph an unsealed melting point capillary on the cold parts of which the substance condensed immediately. The capillary containing the sample was transferred directly into the inlet system of the mass spectrometer to obtain the mass spectrum of the fraction or, alternatively, rinsed with ethanol to obtain a solution for ultraviolet spectroscopy. Melting points were also obtained in some instances from the gas chromatographic fractions, except for the major alkaloids which could be obtained crystalline from the alumina chromatogram (for physical data of these fractions see Table I).

Isolation of (−)-Pyrifolidine (384A).—The fractions emerging from the alumina column immediately after the bulk of 354A were found by mass spectrometry to consist of a difficult to-separate mixture of 354A and 384A. To facilitate separation, the mixture was hydrolyzed with boiling hydrochloric acid (10%, 4 hr.). The reaction mixture was then made alkaline and extracted with ether. After drying and evaporation of the solvent, the product (187 mg.) was chromatographed on alumina. Benzene eluted 312A (96.8 mg., m.p. 109–110°). Benzene–chloroform 19:1 eluted 342A (10.6 mg., m.p. 144–147°) which was dissolved in 0.5 ml. of pyridine and 0.5 ml. of acetic anhydride. After 15 minutes reflux, the mixture was evaporated, digested with dilute sodium hydroxide, and extracted with ether. The ether phase was dried and evaporated to yield 9.0 mg. of 384A, m.p. 148–150°, $[\alpha]^{28}D$ −93° ($CHCl_3$, *c* 0.90) (pyrifolidine from *A. pyrifolium*[15]: m.p. 147.5–150°, $[\alpha]D$ +90°).

Reduction of 1,2-Dehydroaspidospermidine (280A) with Lithium Aluminum Deuteride.—The purest fractions of 280A obtained from the alumina chromatogram were combined (12 mg.) and treated with 20 mg. of $LiAlD_4$ in 1 ml. of tetrahydrofuran in a sealed tube. On work-up, 9 mg. of crude material was obtained which was purified by gas chromatography. The mass spectrum of the collected material (282A-2-*d*) exhibited the major peaks at *m/e* 131, 144, 145, 152, 254 and 283.

Catalytic Reduction of Aspidospermatidine (266B) and 12-Methoxyaspidospermatidine (206B).—Fractions containing both 266B and 296B were combined (48 mg.; ratio 1:1 as determined by gas chromatography), dissolved in 2 ml. of methanol and hydrogenated on 20% Pd-on-charcoal for 2 hr. The filtrate, on evaporation, yielded 44 mg. of an oil which was separated by gas chromatography into two components. The one emerging first showed a molecular weight of 268 (Fig. 5c), the second 298 (characteristic peaks in the mass spectrum at *m/e* 138, 160, 174, 242, 257, 298).

Hydrolysis of Aspidospermatine (338B).—Aspidospermatine, 3 mg., was heated to reflux with 2 ml. of 10% hydrochloric acid for 4 hr. After cooling, the solution was made alkaline with bicarbonate and extracted with ether. After drying and evaporation of the solvent, the residue was distilled at 0.05 mm. (180–200° bath). The mass spectrum of the oil so obtained was identical with that of deacetylaspidospermatine (296B) shown in Fig. 4c.

Catalytic Reduction of Aspidospermatine (338B).—Aspidospermatine (4 mg.) was hydrogenated in 2 ml. of methanol with 10 mg. of 20% Pd-on-charcoal. After 40 min. the catalyst was filtered off, the solvent evaporated, and the residue purified by gas chromatography (one peak). The mass spectrum (major peaks at *m/e* 138, 160, 174, 299 and 340) of the collected material was identical with that of dihydroaspidospermatine (340B) isolated from the alkaloid mixture (see Table I).

Zinc Dust Distillation of the Aspidospermatine Group.—The mixture (25 mg.) of 268B and 298B obtained on catalytic hydrogenation of 266B and 296B (see above) was thoroughly mixed with 1 g. of zinc dust, transferred into a glass ampoule and covered with 1 g. of zinc dust and glass wool. The ampoule was evacuated, sealed and heated for 1 hr. to 400–410° in a horizontal furnace. The volatile degradation products condensed in the part of the tube extending from the furnace. After cooling and opening of the tube, the more volatile components were separated on a gas chromatographic column (2.5 m., 16% silicon oil 550 on Chromosorb W, 110°, 10 lb. helium), and the individual fractions collected. The lower boiling ones were found on the basis of their mass spectra to consist mainly of 3-ethylpyridine and small amounts of 3-methyl-5-ethylpyridine and 3,5-diethylpyridine. The higher boiling fractions seemed to consist of indoles and carbazoles.

Reduction with Perdeuteriohydrazine.—A sample (19 mg.) of deacetylaspidospermatine (296B) still containing some aspidospermatidine (266B) was heated to 90–100° with perdeuteriohydrazine in dioxane–D_2O. The progress of the reduction was followed by mass spectrometry (withdrawing a small sample in 1-day intervals). After 4 days the reduction was essentially complete. The reaction mixture was then concentrated under reduced pressure, extracted with ether, the solvent removed, and the residue purified by gas chromatography which permitted the isolation of a pure specimen of deuteriated dihydrodeacetylaspidospermatine (298B). Its mass spectrum showed a molecular weight of 300 and peaks at *m/e* 259, 270, 271, and the most intense at *m/e* 140.

Acknowledgments.—We are indebted to Dr. G. F. Smith for a sample of decarbomethoxydihydroakuammicine (VI); to S. B. Penick and Company for a supply of powdered quebracho bark; to the Petroleum Research Fund of the American Chemical Society and, in part, to the Upjohn Company, for financial support.

(22) Mass spectra were determined with a CEC 21-103C mass spectrometer, equipped with a heated inlet system operated at 140°. Ionizing potential 70 e.v.

COMMUNICATIONS TO THE EDITOR

CYANOETHYLATIONS AND MICHAEL ADDITIONS. I. THE SYNTHESIS OF ALLYLIC CYCLOHEXENOLS BY γ-CYANOETHYLATION OF AN α,β-UNSATURATED ALDEHYDE[1,2]

Sir:

Cyanoethylations of α,β-unsaturated carbonyl compounds are reported to occur, similarly to mechanistically related alkylations,[3] in position α to the carbonyl function, on the originally unsaturated carbon atom,[4a–d] even in the absence of a hydrogen substituent in this position.[4b,c] We now wish to record the *first*

(1) Paper XXII in the series on Steroids and Related Products; for Paper XXI, see S. Rakhit, R. Deghenghi and Ch. R. Engel, *Can. J. Chem.*, **41** (1963) (in press).

(2) Abbreviated from the doctoral thesis of J. Lessard to be submitted to the School of Graduate Studies, Laval University, Quebec; presented, in part, at the 2nd International Symposium on the Chemistry of Natural Products, Prague, August–September, 1962.

(3) *Cf., e.g.*, (a) J.-M. Conia, *Bull. Soc. Chim. France*, 690 (1954); J.-M. Conia and A. Le Craz, *ibid.*, 1327 (1960); (b) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives and R. B. Kelly, *J. Am. Chem. Soc.*, **76**, 2852 (1954); (c) H. J. Ringold and S. K. Malhotra, *ibid.*, **84**, 3402 (1962).

(4) *Cf., e.g.*, (a) H. A. Bruson and T. W. Riener, *ibid.*, **65**, 18 (1943); (b) H. A. Bruson and T. W. Riener, *ibid.*, **66**, 56 (1944); (c) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, *ibid.*, **74**, 4223 (1952); (d) S. Julia, *Bull. Soc. Chim. France*, 780 (1954).