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AN INVESTIGATION BY MASS SPECTROMETRY OF THE ALKALOIDS OF ASPIDOSPERMA QUEBRACHO-BLANCO

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IN recent years many Aspidosperma species have been investigated chemically and a number of new alkaloids were found and shown to belong to three groups: derivatives of dihydroindoles, indoles, and pyridocarbazoles. The structures of most of these compounds have been elucidated recently.[1] The best known source of aspidosperma alkaloids is the bark of A. quebracho blanco Schlecht.,[2] which contains three alkaloids of known structure; namely, aspidospermine,[3,4] quebrachamine[5] and quebrachine (later found to be yohimbine[6]). In addition to these three alkaloids, Hesse had originally also isolated aspidospermatine, aspidosamine and hypoquebrachine. The existence of the three last-mentioned compounds, not well characterized, was doubted by some of the workers in the field.

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[1] For a review, see J. Schmutz, Pharm. Acta Helv. 36, 103 (1961).

[2] O. Hesse, Liebigs Ann. 211, 249 (1882).

[3] J. F. D. Mills and S. C. Nyburg, Tetrahedron Letters No. 11, 1 (1959).

[4] H. Conroy, P. R. Brook and Y. Amiel, Tetrahedron Letters No. 11, 4 (1959).

[5] K. Biemann and G. Spiteller, Tetrahedron Letters No. 9, 299 (1961).

[6] E. Fourneau and H. Page, Bull. sci. pharmacol. 21, 7 (1914).

Having available in mass spectrometry a very sensitive method which has proven useful in the elucidation of the structure of a number of indole alkaloids,[5,7-9] we undertook a reinvestigation of the alkaloids occurring in the bark of A. quebracho blanco relying mainly on aluminum oxide chromatography and gas chromatography for their separation, and on mass spectrometry and ultraviolet spectroscopy for the elucidation of their structure.

The extract from 300 grams of the powdered, dry bark after removal of neutral and acidic material consisted of 1.95 g , which turned out to be a complex mixture of alkaloids as indicated by a preliminary gas chromatogram and the mass spectra of some of the fractions. It was not possible to achieve a complete separation of the individual components because the isolation of the minor components in amounts sufficient for a good mass spectrum (a fraction of a milligram) necessitated the use of a relatively large amount of liquid phase (6% Apiezon L). Complete separation of all components by chromatography on alumina was also not possible, but further purification of the fractions by gas chromatography permitted the isolation of about twenty compounds in reasonable purity.

Table I lists, in the order of their emergence from the alumina column, fifteen of the compounds investigated in detail. The numbers denoting the components are their molecular weights as deduced from the mass spectra, which also indicated that there are present two groups of compounds designated A and B. Group A was recognized to be related to aspidospermine, the spectrum of which we had determined previously, and was identical with the spectrum of 342A. It has a very strong peak at m/e 124, and this peak

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[7] K. Biemann, Tetrahedron Letters No. 15, 9 (1960).

[8] K. Biemann and M. Friedmann-Spiteller, Tetrahedron Letters No. 2, 68 (1961).

[9] C. Djerassi, B. Gilbert, J. N. Shoolery, L. F. Johnson and K. Biemann, Experientia 17, 162 (1961).

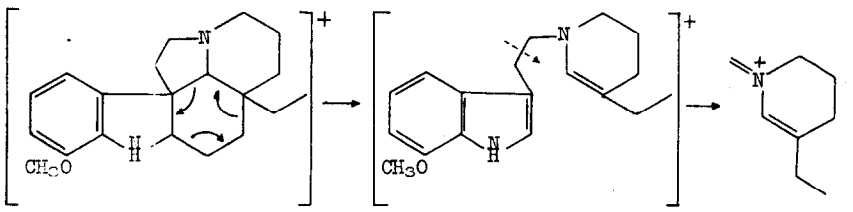
is found in the spectra of all alkaloids of Group A. Group B is characterized by a very intense peak at m/e 136 (138 in 340B). Quebrachamine and yohimbine were identified on the basis of their physical constants.

TABLE I

Compound	Yield[a] %	M. P.	Characteristic Peaks in the Mass Spectrum[b]
Quebrachamine	2.5	146-7	
296A	<.5	c	296; 268; 124
326A	<.5	c	326; 298; 124
280A	2.0	c	280; 251; 210
282A	<1.0	110-2	282; 254; 124
312A	3.0	109-11	312; 284; 124
Aspidospermine (354A)	30.0	208-9	354; 326; 124
384A	1.5	148-50	384; 356; 124
280B	<1.0	c	280; 136; 158[d]; 144[d]
266B	3.0	184-6	266; 136; 144; 130
296B	1.5	c	296; 136; 174; 160
308B	1.0	c	308; 136; 144; 130
338B	3.0	157-9	338; 136; 174; 160
340B	<1.0	c	340; 138; 174; 160
Yohimbine	10.0	223-5	

(a) in % of total bases. (b) for Group A and B only. (c) not crystalline. (d) These two columns are peaks due to the indole part of the molecules. Acyl groups are eliminated during fragmentation.

A discussion of the elucidation of the structure of the remaining twelve alkaloids, three of which turned out to be known derivatives of aspidospermine, follows. The characteristic peaks in the mass spectra of the alkaloids of Group A are summarized in Table I, and their relation to the structure of the molecule is shown below, using deacetylaspidospermine as an example:



312 (MW) 284 (Y) 124 (Z)

The molecular weight indicates the sum of all substituents attached to the basic carbon skeleton (I) and peak Y permits the conclusion that in none of the compounds is there a substituent on the two carbon bridge. The same conclusion can be drawn for the piperidine moiety of the molecules because additional groups there would change the mass of fragment Z.

It follows from the mass spectra that these alkaloids are substituted in the indole moiety of I by methyl, methoxyl, and acetyl groups, respectively. The methyl groups were placed on N_a because of the early emergence of these components from the alumina column. N_a-methyldeacetylaspidospermine[10] was synthesized and found to be identical (mass spectrum and retention time) with compound 326A. The mass spectra of 296A and 326A showed clearly that the two compounds differ only by a methoxyl group. Compound 312A, which must contain a methoxy group, was identified by melting point, mixed melting point, and mass spectrum as deacetylaspidospermine. Compound 384A according to its spectrum is substituted in the indole nucleus by two methoxyl groups and one acetyl group, which suggested it to be pyrifolidine.[9] It was not possible to separate this component from the major alkaloid of the group, aspidospermine, which has only one methoxyl group less. The fractions rich in 384A were hydrolyzed and the deacetyl derivatives were separated by chromatography. The

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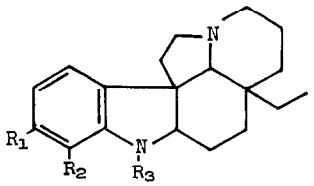
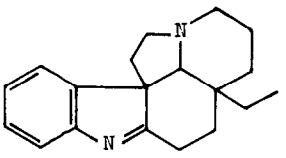
[10] B. Witkop and J. B. Patrik, J. Amer. Chem. Soc. 76, 5603 (1954).

fractions emerging after deacetylaspidospermine had m.p. 144-6° and gave a mass spectrum identical with the one of deacetylpyrifolidine. Upon reacetylation the product had $[\alpha]_D^{28}$ - 93° (chloroform) and is thus (-) pyrifolidine.

Finally, compound 282A does not contain any additional substituents and is, therefore, unsubstituted I. Compound 280A does not exhibit a strong peak at m/e 124 but was recognized to be related to Group A on the basis of its mass spectrum, which was identical with the spectrum of a compound (II) we had isolated earlier on zinc dust distillation of quebrachamine.[5] On reduction 282A was formed.



I



II

282A: I, $R_1 = R_2 = R_3 = H$ 326A: I, $R_1 = H$, $R_2 = CH_3O$, $R_3 = CH_3$

296A: I, $R_1 = R_2 = H$, $R_3 = CH_3$ 354A: I, $R_1 = H$, $R_2 = CH_3O$, $R_3 = CH_3CO$

312A: I, $R_1 = R_3 = H$, $R_2 = CH_3O$ 384A: I, $R_1 = R_2 = CH_3O$, $R_3 = CH_3CO$

The similarity of the mass spectra of Group B indicates that all these compounds contain the same carbon skeleton and differ in the substitution in the dihydroindole moiety, the presence of which is borne out by the UV spectra of 266B and 338B. There is also present a double bond as evidenced by an increase in the molecular weights by two mass units on hydrogenation and the shift of the most intense peak to m/e 138. While the mass spectra of Group B were somewhat reminiscent of the aspidospermine group, they could not have the same carbon skeleton because the characteristic loss of 28 mass units is not observed. Furthermore, the fragment of m/e 136 could not be merely a higher

homolog of the fragment of m/e 124 of Group A because the smallest molecule of Group B is 16 mass units lighter and not heavier than 282A. These findings can best be reconciled with the assumption of a one-carbon bridge in a ring system which on electron impact fragments into a highly stabilized indole system that remains connected to the rest of the molecule only by a single bond, the cleavage of which must give rise to the peak at m/e 136. Structure III would combine all these features, and only the C_2-group and the double bond remain to be placed. Structure IIIa seemed to be an attractive hypothesis, worth testing, but an authentic sample[11] exhibited a mass spectrum very similar but not identical with the spectrum of compound 266B. Since the difference seems to be due to either the location of the double bond or of the C_2-group, both substances were hydrogenated and the mass spectra of the products showed a molecular weight of 268 and a strong peak at m/e 138. The major difference was a peak at m/e 199 in IIIb and a peak at m/e 227 in dihydro-266B. This is best explained by cleavage at b in IV, which suggests structure IIIc for 266B, corroborated by the formation of 3-ethylpyridine on zinc dust distillation of a mixture of 266B and 296B, and by the UV spectrum of 266B (λ_{max} 242, 296 mμ; log ε 3.86, 3.49). The double bond is placed as shown on the basis of deuteration with deuterohydrazine[12] and the absence of a vinyl group according to the infra-red spectrum of 338B.

The major peaks in the mass spectra of the alkaloids of Group B again indicate the presence of methyl, methoxy, and acetyl groups on the indole moiety of 266B, the unsubstituted representative of this class. The structures of these compounds were deduced on the basis of the mass spectra as was done for the aspidospermine group. The position of the methoxyl group follows from the UV spectrum of 338B (λ_{max} 219, 255, 290(sh) mμ; log ε 4.54, 4.10, 3.62).

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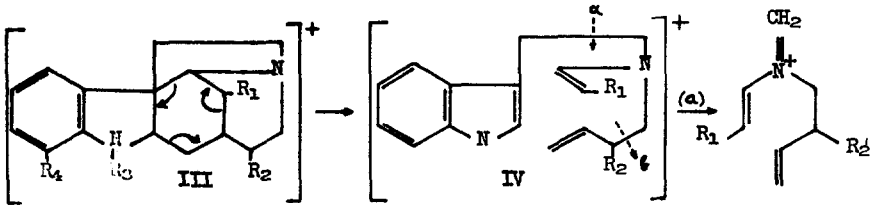
[11] G. F. Smith and J. T. Wrobel, J. Chem. Soc. 1960, 792

[12] Ng. Dinh-Nguyen and R. Ryhage, Arkiv f. Kemi 15, 433 (1960).



IIIa: $R_1 = R_3 = R_4 = H$, $R_2 = CHCH_3$ IIIc: $R_2 = R_3 = R_4 = H$, $R_1 = CHCH_3$

IIIb: $R_1 = R_3 = R_4 = H$, $R_2 = C_2H_5$ IIId: $R_2 = R_3 = R_4 = H$, $R_1 = C_2H_5$

266B: IIIc, $R_3 = R_4 = H$ 308B: IIIc, $R_3 = CH_3CO$, $R_4 = H$

280B: IIIc, $R_3 = CH_3$, $R_4 = H$ 338B: IIIc, $R_3 = CH_3CO$, $R_4 = OCH_3$

296B: IIIc, $R_3 = H$, $R_4 = OCH_3$ 340B: IIId, $R_3 = CH_3CO$, $R_4 = OCH_3$

Additional chemical evidence for these relationships within Group B was obtained by hydrolysis of 308B to 266B and of 338B to 296B, and by hydrogenation of 338B to 340B. While the mass spectrometric molecular weights were sufficient indication of the elemental composition of all the compounds reporte a C, H, N-analysis(correct for $C_{21}H_{26}N_2O_2$) was secured for 338B, the most impor tant representative of Group B. Compound 338B has $[\alpha]_D^{27}$ - 73° (ethanol).

The melting point and the rotation of 338B are very close to the corresponding constants of aspidospermatine (m.p. 163°, $[\alpha]_D^{15}$ - 72°) [6] which Hesse had isolated almost eighty years ago. Although he suggests a formula $C_{22}H_{28}O_2N_2$ for which good C, H, and N values are present, it is possible that he actually had in hand the lower homolog, particularly since the analysis of the chloroplatinate of aspidospermatine definitely agrees better with a C_{21} formula. Since compound 338B is one of the more abundant minor alkaloids of this plant and also has a considerable tendency to crystallize in contrast to many other of the components which we have found, the identity of 338B with aspidospermatine is very likely.

The isolation of so many of the minor alkaloids of Aspidosperma quebracho blanco should have considerable significance for the biogenesis of these

alkaloids. We are inclined to believe that Group A is formed via a quebrachamine-type intermediate followed by ring closure to demethoxydeacetylaspidospermine (282A), which is then substituted in the dihydroindole moiety, particularly since we were unable to find any trace of substituted quebrachamines among these alkaloids. In the structure of the alkaloids of Group B we may have finally found the biogenetic link between the dihydroindole alkaloids of the genus Aspidosperma and ulein,[13] the major alkaloid of Aspidosperma ulei Mgf.

A more detailed account of this work and of the interpretation of the mass spectra will be presented in the full paper.

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[13] G. Büchi and E. W. Warnhoff, J. Amer. Chem. Soc. 81, 4433 (1959).