B. From I.—The sodium derivative prepared from 3-phenyloxindole (I) (11 g., 0.05 mole) and sodium hydride (0.06 mole) in dry benzene was refluxed and stirred overnight with 4-(β-chloroethyl)-morpholine (0.06 mole). Isolation of the product in the foregoing manner gave 8.6 g. (48%) of pure XI, m.p. 165–167°, identical (mixed m.p. and infrared spectrum) with the sample obtained from II.

3-(β-Benzylaminoethyl)-3-phenyloxindole Hydrochloride (XII).—3-(β-Chloroethyl)-3-phenyloxindole (II) (5.4 g., 0.02 mole) was heated overnight on the steam-bath with excess benzylamine (25 ml.). The mixture was worked up in the usual way (see preparation of XI) to give 5.2 g. (68%) of pure XII, m.p. 235–237° (from isopropyl alcohol); λ_{max}^{Nujol} 3.11, 5.83μ.

Anal. Calcd. for $C_{23}H_{23}ClN_2O$: C, 72.91; H, 6.12; N, 7.39. Found: C, 72.93; H, 6.33; N, 7.44.

1-Methyl-3-(β-morpholinoethyl)-3-phenyloxindole (XIII) (Base).—A solution of 3-(β-bromoethyl)-1-methyl-3-phenyloxindole (IX) (9.9 g., 0.03 mole) in morpholine (30 ml.) was allowed to stand at room temperature for 48 hr. and then worked up in the usual way (see preparation of XI). In this case, however, the base solidified (5.5 g., m.p. 76–79°, 54%) and was recrystallized from cyclohexane to give pure XIII, m.p. 81–82°, $\lambda_{max}^{CHCl_3}$ 5.85 μ.

Anal. Calcd. for $C_{21}H_{24}N_2O_2$: C, 74.99; H, 7.19; N, 8.33. Found: C, 75.00; H, 7.20; N, 8.54.

3-(β-Cyclopropylaminoethyl)-1-methyl-3-phenyloxindole Hydrochloride (XIV).—A solution of IX (0.03 mole) in cyclopropylamine (30 ml.) and benzene (25 ml.) was refluxed for 48 hr. and worked up as usual to give 7.3 g. (71%) of XIV, m.p. 202–203° (from ethanol); $\lambda_{max}^{CHCl_3}$ 1.63 μ (cyclopropyl-CH_2), 5.86 μ (C=O).

Anal. Calcd. for $C_{20}H_{23}ClN_2O$: C, 70.07; H, 6.77; N, 8.18. Found: C, 70.25; H, 6.68; N, 8.13.

When the bromo compound IX was stirred with liquid ammonia for 24 hr. it was quantitatively recoverable.

Acknowledgment.—We are grateful to Mr. W. H. Washburn and Mr. E. F. Shelberg and their associates, respectively, for the infrared spectra and microanalyses, to Mr. Frank Chadde for the ultraviolet spectra, and to Dr. T. F. Page, Jr., Battelle Memorial Institute, for the determination and interpretation of the n.m.r. spectrum.

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

Application of Mass Spectrometry to Structure Problems. VIII.[1] Quebrachamine[2,3]

BY K. BIEMANN AND G. SPITELLER

RECEIVED JUNE 14, 1962

Quebrachamine was shown to have structure I by comparison of its mass spectrum with the one of Ia, prepared from asipdospermine IIIa. The presence of the same carbon skeleton in both compounds is revealed by these spectra making it unnecessary to remove chemically the methoxyl group of II to prove identity. It was shown that (−)-quebrachamine and (−)-aspidospermine, both occurring in *Aspidosperma quebracho blanco*, have the same absolute configuration at C-5.

The first detailed investigation of the alkaloids of *Aspidosperma quebracho blanco* Schlecht. by Hesse[4] led to the isolation of six alkaloids: aspidospermine, quebrachine (= yohimbine), quebrachamine, aspidospermatine, and two amorphous bases, hypoquebrachine and aspidosamine. Quebrachamine was found in an amount too small to permit its elemental composition to be established, but the alkaloid was later reisolated by Field[5] and shown to have the composition $C_{19}H_{26}N_2$. The presence of one basic tertiary nitrogen was established, and a number of color reactions made it very probable that quebrachamine is an indole derivative. Attempts of oxidative degradation under various conditions failed to yield any actual information.

The problem of the structure of quebrachamine remained dormant for over three decades and only comparatively recently has this alkaloid again received more detailed attention, when Witkop[6] confirmed the presence of an indole moiety on the basis of the ultraviolet spectrum of quebrachamine and reported a number of experiments aimed at the elucidation of the structure of this alkaloid. These consisted of various oxidation and dehydrogenation experiments of which only zinc dust distillation led to products for which definite structures were suggested. When this reaction was carried out fifty times using 50 milligrams of quebrachamine for each experiment, there was obtained from the pooled crude products a basic fraction, a non-basic, steam-volatile fraction, and a non-basic non-volatile fraction. The basic fraction gave a picrate of m.p. 180–181°, identical with the material obtained from aspidospermine on similar treatment[7] and believed to be the picrate of 3,5-diethylpyridine contaminated with some 3-methyl-5-ethylpyridine. The steam-volatile, non-basic fraction was also converted to a mixture of picrates which on repeated recrystallization, raising the melting point from 104° to 132°, gave an analysis indicating a C_3-indole, and was suggested to be a mixture of β-methyl and β-ethylindole picrates contaminated with higher homologs. It is of interest to note that a further recrystallization raised the melting point to 143°, but the amount was insufficient for analysis. Finally, the non-steam-volatile portion was suggested to be a mixture of carbazole and some homolog on the basis of elemental analysis and color reactions.

These findings seemed to establish two points, namely, that quebrachamine may be related to aspidospermine and that in both molecules there is present the carbon skeleton of 3,5-diethylpyridine.

The occurrence of an α,β-substituted indole system in quebrachamine was finally established by its n.m.r. spectrum, which, however, did not offer any additional structural information.[8]

(1) Part VII: K. Biemann, A. L. Burlingame and D. Stauffacher, *Tetrahedron Letters*, **No. 12**, 527 (1962).

(2) For a preliminary communication on this work see K. Biemann and G. Spiteller, *ibid.*, **No. 9**, 299 (1961).

(3) This investigation was supported by a grant (G-5051) from the National Science Foundation.

(4) O. Hesse, *Ann.*, **211**, 249 (1882).

(5) E. Field, *J. Chem. Soc.*, **125**, 1444 (1924).

(6) B. Witkop, *J. Am. Chem. Soc.*, **79**, 3193 (1957).

(7) B. Witkop, *ibid.*, **70**, 3712 (1948).

Obviously the elucidation of the structure of quebrachamine by conventional chemical degradation was hampered by the lack of any functional groups aside from the tertiary nitrogen and the indolic nucleus, and a specific degradation was thus rendered difficult. Even the Hofmann degradation performed on the methiodide led only to regeneration of quebrachamine. It was felt that if more drastic degradation reactions had to be used, a much more detailed investigation of the products of such reactions would be necessary in order to derive any meaningful structural information from them.

Having available in mass spectrometry a very sensitive method thought to be ideally suited for the investigation of the volatile degradation products of alkaloids, we undertook a reinvestigation of the zinc dust distillation of quebrachamine in spite of the fact that we had only a very small amount of this alkaloid at our disposal.

A preliminary experiment had already yielded rather encouraging results. When three milligrams of quebrachamine was heated with zinc dust in a sealed ampoule and the products formed in this reaction were introduced into the mass spectrometer at different temperatures (to achieve fractionation) compounds of mol. wt. 107, 121, 135 were found in the more volatile fraction while the less volatile components had mol. wt. of 131, 145, 159 and 173. The first group corresponds to pyridines with two to four additional carbon atoms while the second group indicates indoles with one to four additional carbon atoms. The spectra also indicated that the C_2-pyridine is present to a much larger extent than the higher homologs, and this observation was in direct contrast to the findings reported previously.[6]

Much more detailed information about the products formed on zinc dust distillation of this alkaloid was obtained when the reaction was repeated with more material (13 milligrams) and the products were first separated by gas chromatography, the individual fractions collected, and their mass spectra determined. The gas chromatogram of the pyridine fraction is shown in Fig. 1, and again it is apparent that one component (A) is formed in a much larger amount than all other ones. According to the mass spectrum it had a molecular weight of 107 and a strong peak at *m/e* 92 corresponding to the loss of an ethyl group. This process is indicative of an alkyl group in the 3-position[9] and suggested the substance to be 3-ethylpyridine, which was confirmed by comparison with the mass spectrum of an authentic sample. The spectra of both fraction B and C showed a molecular weight of 121 and strong peaks at *m/e* 106, which, combined with the absence of peaks at *m/e* 93, suggested the presence of methyl ethylpyridines, or conceivably an isopropylpyridine, with the ethyl or isopropyl group in the 3-position. An *n*-propylpyridine would eliminate ethylene from the side chain and thus give rise to a fragment of *m/e* 93.[10] The two fractions were conclusively identified as 3-ethyl-4-methylpyridine (fraction B) and 3-ethyl-5-methylpyridine (fraction C) by comparison of the spectra with those obtained from authentic samples.

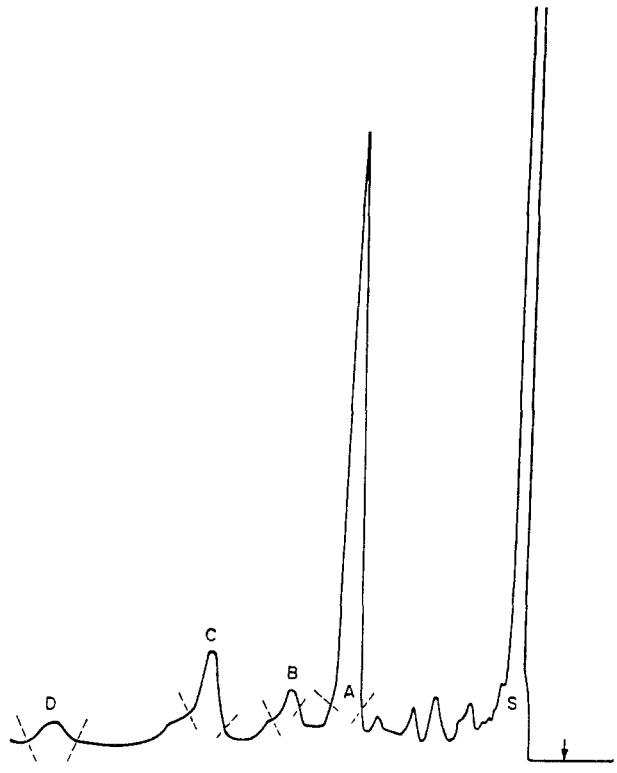


Fig. 1.—Gas chromatogram of the low-boiling fraction of the zinc dust distillation of quebrachamine.

The component emerging last (D) proved to be of molecular weight 135 and had its most intense peak at *m/e* 120, but no appreciable peaks at *m/e* 107 or 93 (elimination of ethylene or propylene, respectively), which indicates the absence of a side chain longer than two carbon atoms. Such a spectrum suggests a diethylpyridine with at least one 3-ethyl group, or conceivably a methyl-isopropylpyridine. The spectrum was not identical with the one of either 2,3-[11] or 3,4-diethylpyridine,[12] but with 3,5-diethylpyridine.[11]

Quantitative evaluation of the gas chromatogram indicates that the 3-ethylpyridine (fraction A) amounts to 75% of all the pyridines formed while fractions B, C and D amount to 5%, 12%, and 5%, respectively. While this experiment confirms Witkop's earlier observation[6] that in this degradation is formed 3,5-diethylpyridine and 3-methyl-5-ethylpyridine, it shows that only the minor products of the reaction had been isolated while the major one escaped detection, presumably owing to the higher solubility of 3-ethylpyridine picrate.

When the zinc dust distillation was repeated and the product separated at higher temperature on a gas chromatographic column coated with Apiezon L, the indole fraction was separated into the individual components, of which mass spectra were

(8) L. A. Cohen, J. W. Daly, H. Kny and B. Witkop, *J. Am. Chem. Soc.*, **82**, 2184 (1960).

(9) For a detailed discussion of the fragmentation of organic molecules and the interpretation of mass spectra see K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962.

(10) K. Biemann and G. Spiteller, to be published.

(11) We are indebted to Dr. G. F. Smith for authentic samples of picrates of these pyridines.

(12) C. T. Kyte, G. H. Jeffery and A. I. Vogel, *J. Chem. Soc.*, 4454 (1960).

taken. The fractions (listed in the order of increasing retention time) were identified by comparison with the mass spectra of authentic samples as (a) indole, (b) a mixture of 2- and 3-methylindole,[13] (c) 3-ethylindole, (d) 2,3-dimethylindole,[13] (e) 3-methyl-2-ethylindole (or the 3-ethyl-2-methyl isomer), (f) 2,3-diethylindole plus non-indolic material. The indole of fraction f could be obtained pure only in another experiment in which a silicon oil column was used for separation. The spectra of isomeric alkylindoles differing only in the point of attachment of the alkyl substituents in the pyrrole ring but not in the structure of these substituents give rather similar mass spectra and can thus be distinguished only if the pure material is available. This was not possible in the experiment just described, and it is for this reason that there is some ambiguity with respect to the exact position of the alkyl substituents at C-2 or C-3. There are, however, definitely absent any alkyl chains longer than two carbon atoms. These would lead to intense peaks at M-29, M-43, etc., which were, however, of only low abundance in the spectra of the degradation products.

A third such experiment aimed at the isolation of products less volatile than alkylindoles was performed, and the crude material separated on a shorter (4′) column. While the separation of this very complex mixture was not complete, it is evident from the mass spectra of the fractions collected that carbazole, methyl, ethyl, methylethyl and, to a small extent, a C_4-carbazole was formed, as judged from the peaks due to the molecular weight and the loss of alkyl groups (only methyl was lost to an appreciably extent). The presence of alkyl chains longer than two carbon atoms is excluded on the basis of the lack of peaks due to elimination of parts of the side chain in the form of an olefin as was discussed in the case of the alkylpyridines. Further separation and characterization of these products was not attempted as the data accumulated at that stage already led to a reasonable working hypothesis for the structure of quebrachamine (see below) which had to be proved by means more subtle than drastic degradations.

The mass spectra of the fractions emerging after the carbazoles indicated the presence of products derivable from quebrachamine by loss or gain of hydrogen or isomerization because the molecular weights of these were 280, 282 and 284. The structures of these compounds and their significance will be discussed later.

The multitude of products isolated and partly identified illustrates how powerful a tool mass spectrometry is for such investigations, particularly if gas chromatography can be used for prior separation of the mixtures. The sensitivity of the method gives, however, rise to a new difficulty owing to the fact that even rearranged products formed only in small quantities are detected. This is best illustrated in our case by the structures of the various pyridines which would lead to the conclusion that there is a trisubstituted piperidine moiety present if none of the products would arise by rearrangement. Nevertheless, as it is possible to detect all the compounds formed and also to establish their quantitative relationship, the major products of such degradation reactions can be clearly recognized and it is these compounds which most probably did not arise by a rearrangement process and therefore are most significant for the determination of the structure of the original alkaloid.

Because of the suspected relationship of quebrachamine with aspidospermine (IIIa), deacetylaspidospermine (IIIb) was heated with zinc dust under the same conditions as quebrachamine. Separation of the pyridine fraction by gas chromatography and identification of the fractions by mass spectrometry showed that also in this case 3-ethylpyridine was formed in largest amount (60%), 3-methyl-5-ethylpyridine and 3,5-diethylpyridine contributed to about 20% each to the total pyridines while only a small amount of 3-ethyl-4-methyl pyridine was found. The absolute yield on pyridines was, however, considerably smaller than in the case of quebrachamine. A possible path for the formation of 3,5-diethylpyridine has been suggested.[14]

Consideration of the pyridines and indoles formed in the zinc dust distillation of quebrachamine make it apparent that there have to be attached to both the piperidine moiety and to the indole moiety at least four carbon atoms each, and these have to be present in C_2-chains. The total number of carbon atoms in this alkaloid indicates that there are only six carbons available which are not incorporated in either the indole or the piperidine moiety, assuming the high yield to be an indication for the fact that the ethyl groups found in the degradation products are not originally a part of these ring systems. We can therefore assume that either a combination of the 2,3-diethylindole and the 3-ethylpyridine, or the 3-ethylindole and the 3,5-diethylpyridine has to be utilized in the construction of the carbon skeleton of quebrachamine.

The first-mentioned possibility is much more likely because of the very large amount of 3-ethylpyridine compared with 3,5-diethylpyridine formed in the zinc dust distillation. The formation of carbazoles is at first difficult to explain. This ring system (*e.g.*, Vc) must be formed during this reaction because the results of the Hofmann degradation,[6] which did not yield any volatile amines nor did it give rise to the loss of a small alkyl group from quebrachamine, demand that the tertiary nitrogen is part of two rings since no $N-CH_3$ grouping is present. These three pieces of evidence lead to structures I and II as possible candidates for quebrachamine. Structure I is particularly attractive because of its clear relationship to aspidospermine, the most abundant alkaloid in quebracho bark. In addition, the location of the ethyl group at a quaternary carbon atom would make the preferred formation of 3-ethylpyridine very likely. On the other hand, formation of 3,5-diethylpyridine would require alkyl migration to a position two carbon atoms away.

Structure II would not require such migration and while it is not related to aspidospermine IIIa, it could be derived from ibogamine IV and it is of in-

(13) Compared with published spectra: J. H. Beynon and A. E. Williams, *Appl. Spectros.*, **13**, 101 (1959).

(14) G. Spiteller, *Monatsh. Chem.*, **93**, 324 (1962).

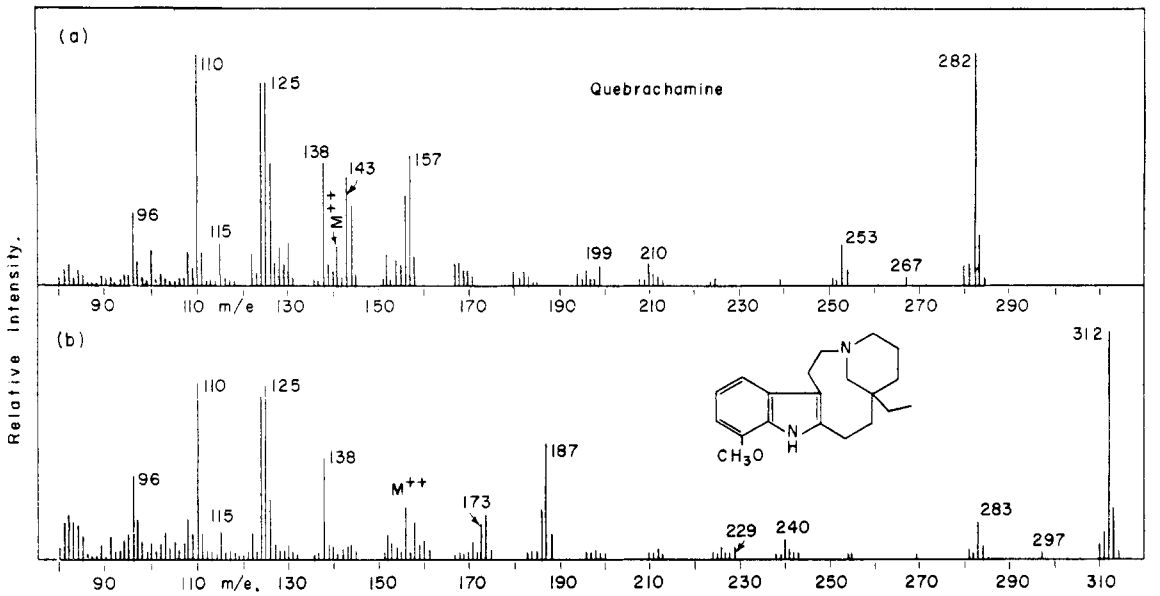
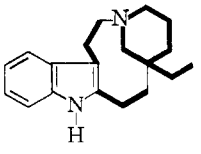
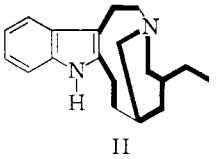


Fig. 2.—Mass spectra of (a) quebrachamine and (b) Ia, derived from aspidospermine.

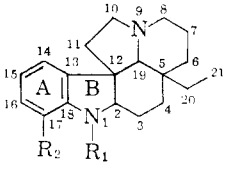
terest to note that (+)-quebrachamine had been isolated[15] from *Stemmadenia donell-smithii* Woods., a plant in which it is accompanied by voacangine, iso-voacangine and tabernanthine, all of which have carbon skeleton IV in common. The iboga alkaloids are, however, known to yield 3-methyl-5-ethylpyridine as the major degradation product on zinc dust distillation, and it would be difficult to see why II should not give the same products, particularly since it would require the cleavage of one bond less. Structure I had, for all these reasons, to be favored over structure II.



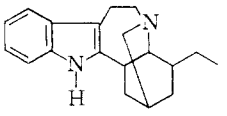
I



II



IIIa, R_1 = CH_3CO, R_2 = CH_3O
b, R_1 = H, R_2 = CH_3O
c, R_1 = R_2 = H

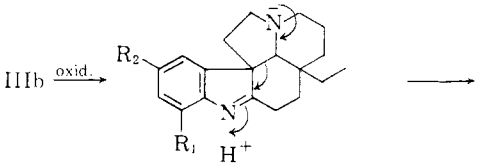


IV

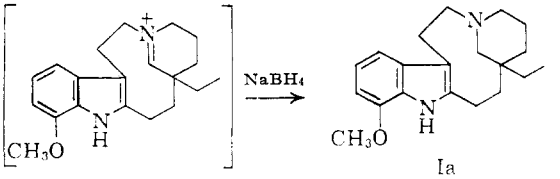
Both Kny and Witkop,[16] and Smith and Wrobel[17] had arrived at the same conclusion based on the previously reported formation of 3,5-diethylpyridine in the zinc dust distillation of both quebrachamine and aspidospermine when the structure of the latter became known.[18,19]

The mass spectrum of quebrachamine (Fig. 2) could not be used at that stage as conclusive proof of the structure of this alkaloid beyond the statement that it showed the presence of an ethyl group (loss of 29 mass units) and that it could be reconciled with both structure I and II. The inavailability of mass spectra of such molecules made any further interpretation difficult and speculative. Previously we had, however, shown that compounds of identical carbon skeleton but differing in additional substituents in the aromatic moiety may be correlated by mass spectrometry.[20] Isomerization of deacetylaspidospermine through opening of the 12–19 bond and aromatization of ring B would lead to such a compound, which would differ from quebrachamine only by a methoxyl group in the benzene ring of quebrachamine, if structure I for this alkaloid were correct.

The most promising approach to this conversion was thought to be the introduction of a 1,2-double bond in deacetylaspidospermine (IIIb) to form the indolenine Va. Compounds of this type had been shown previously to undergo reductive ring opening to indoles on treatment with sodium borohydride.[21]



Va, R_1 = CH_3O, R_2 = H
(Vb, R_1 = R_2 = CH_3O)
(Vc, R_1 = R_2 = H)



Ia

(15) F. Walls, O. Collera and A. Sandoval, *Tetrahedron*, **2**, 173 (1958).

(16) H. Kny and B. Witkop, *J. Org. Chem.*, **25**, 635 (1960).

(17) G. F. Smith and J. T. Wrobel, *J. Chem. Soc.*, 1463 (1960).

(18) J. F. D. Mills and S. C. Nyburg, *Tetrahedron Letters*, No. **11**, 1 (1959).

(19) H. Conroy, P. R. Brook and Y. Amiel, *ibid.*, No. **11**, 4 (1959).

(20) For examples see K. Biemann, *ibid.*, No. **15**, 9 (1960), and K. Biemann and M. Friedmann-Spiteller, *J. Am. Chem. Soc.*, **83**, 4805 (1961). For a detailed discussion of the validity of this approach see Chap. 8 in ref. 9.

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

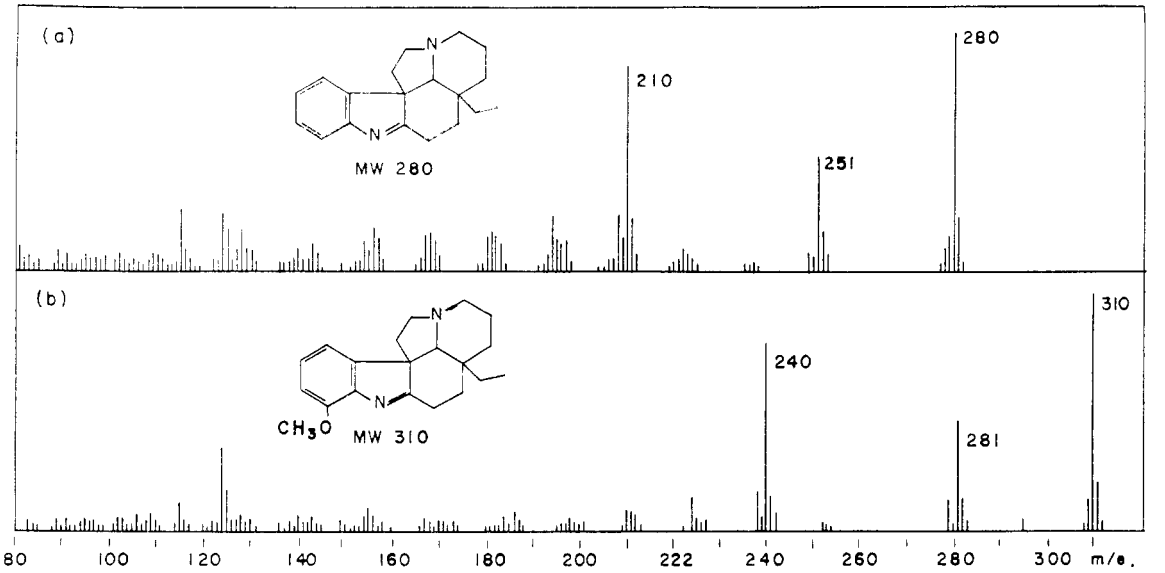
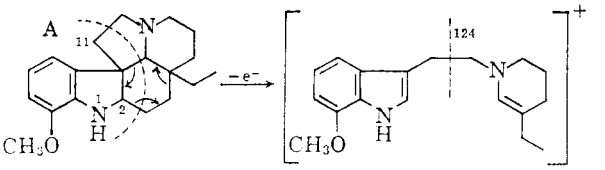


Fig. 3.—Mass spectra of (a) the fraction of mol. wt. 280 obtained from quebrachamine and (b) 1,2-dehydrodeacetylaspidospermine Va.

Oxidation of deacetylaspidospermine with sodium hypoiodite in methanol under very carefully controlled conditions (temperature, time and sequence of addition of reagents) was found to lead to the desired indolenine Va. It was always accompanied by considerable amounts of starting material from which it could not be separated except by gas chromatography, and any attempt to drive the reaction to completion required conditions under which the indolenine Va was destroyed.

The presence of Va in the reaction mixture was established by a number of criteria. First, the mass spectrum of the crude mixture indicated the presence mainly of two compounds, one of molecular weight 312 (unreacted deacetylaspidospermine) and a second one of molecular weight 310. These could be separated by gas chromatography and a mass spectrum of pure Va was obtained in this manner. Second, the ultraviolet spectrum of such a fraction indicated that the material is neither an indole nor a dihydroindole but may well be an indolenine although no authentic methoxyl substituted indolenine is available for comparison. The most convincing proof is, however, its reconversion to deacetylaspidospermine (IIIb) by the action of lithium aluminum deuteride, thus proving that the original carbon skeleton is still present and that the compound does contain a double bond reducible by this reagent. The use of the deuteride rather than the hydride was imperative because of the presence of unreacted starting material in the mixture. The sample of IIIb isolated from this reaction consisted of about two-thirds of normal deacetylaspidospermine and of about one-third of monodeuterio derivative, as indicated by the mass spectrum. It also permits locating the position of the deuterium atom in the molecule on the basis of the peak at *m/e* 124 which did not shift to 125 in the deuterated species, and a doublet at *m/e* 284 and 285 which show that the deuterium atom is retained in this fragment. These fragments are believed to be formed by the process[22]



IIIb *m/e* 284

and the deuterium atom has thus to be either on the dihydroindole moiety or at C-11. Only a 1,2-double bond could be reduced by lithium aluminum deuteride with incorporation of deuterium in part A of (IIIb).

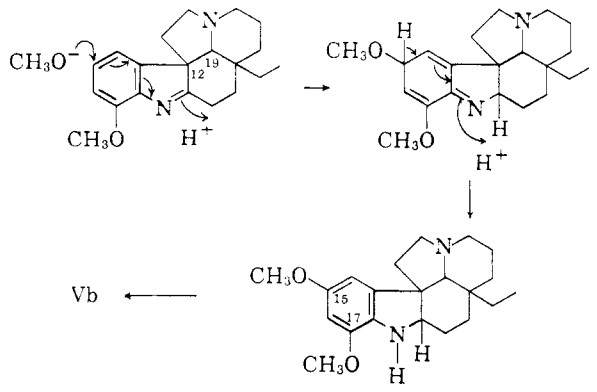
The mass spectrum of Va (Fig. 3b) is very different from the starting material IIIb because the new double bond prevents the fragmentation process pictured above and which is typical for aspidospermine derivatives.[22] This spectrum was, however, surprisingly similar to the one (Fig. 2a) obtained of a fraction (mol. wt. 280) mentioned earlier during the discussion of the zinc dust distillation of quebrachamine. This compound must therefore be Vc and this observation was the first convincing evidence that the carbon skeleton of aspidospermine and of quebrachamine are, in fact, very closely related.[23]

Oxidation of IIIb with sodium hypoiodite in methanol yielded in addition to Va a by-product, the molecular weight of which was 30 mass units higher, *i.e.*, 340. It must be a dimethoxy derivative the mass spectrum is very similar to that of Vc, probably arising by addition of methoxide ion on C_{15} followed by reoxidation of the methoxydeacetylaspidospermine formed during the addition.

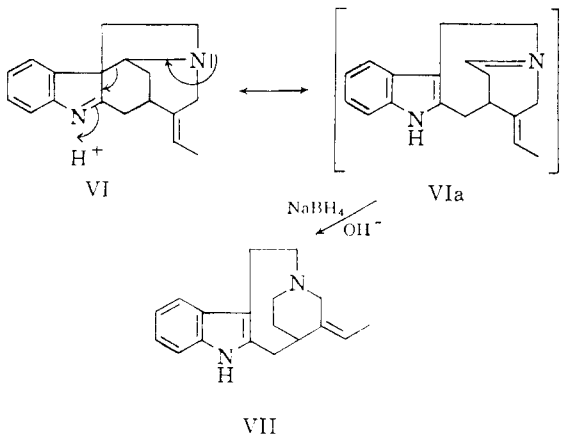
Cleavage of the 12,19-bond of Va was accomplished by treatment of the crude oxidation product

(22) K. Biemann, M. Friedmann-Spiteller and G. Spiteller, *Tetrahedron Letters*, No. 14, 485 (1961).

(23) The absence of both demethoxydeacetylaspidospermine and its 1,2-dehydroderivative from our sample of quebrachamine was ascertained by gas chromatography. The possibility of such a contamination had to be considered when we recently found both IIIc and Vc in the bark of *Aspidosperma quebracho blanco*.[11]



with sodium borohydride in methanol in the presence of sodium hydroxide. Under these conditions VI (a degradation product of akuammicine) is reported[19] to yield VII within 10 min. The efficient conversion of Va to Ia required, however, sixty hours or better of repeated treatment with fresh sodium borohydride over a few twelve-hour periods; the surprising difference in rate for this reaction may be due to the fact that in the case of Va a double bond has to be placed in the intermediate quaternary Schiff base at a one-carbon bridge head of a bicyclic system, the largest ring of which is



nine-membered. A ring of the same size ring is present in VIa, but here the double bond is at a two-carbon bridge and therefore less strained.

The product of the sodium borohydride reaction was chromatographed on alumina which led to the isolation of four compounds, the mass spectra of which indicated that they consisted of two pairs of isomers, mol. wt. 312 and 342. The first fraction was assumed to be the desired Ia while the second one must be its (15?)-methoxy derivative since the mass spectrum was very similar to Fig. 2b with the exception that the peaks in the region above mass 170 were displaced +30 mass units. The third fraction was found to be deacetylaspidospermine (IIIb) and the mass spectrum of the last fraction indicated it to be (15?)-methoxydeacetylaspidospermine as judged from the mass spectrum in which the peaks associated with the aromatic moiety[22] of the molecule were also displaced +30 mass units when compared with IIIb. The spectrum was, in fact, very similar to the one of deacetylpyrifolidine,[22,24] but on the basis of its genesis (see above) we would like to place the methoxyl group at C-15 rather than C-16, a variation which would not appreciably influence the mass spectrum.

The two components eluted from the column first were converted to the corresponding picrates, purified by recrystallization and characterized by the melting points of the picrates (203–205° and 224–228°, respectively).

The free base was regenerated from the picrate of m.p. 203–205° for the determination of mass spectrum, ultraviolet spectrum and optical rotatory dispersion. The mass spectrum is shown in Fig. 2b, and close comparison of it with the spectrum of quebrachamine (Fig. 2a) shows that they do consist of two groups of peaks, one of which formed by the fragment of mass 96, 110, 124, 125, 126 and 138 and present in both spectra—while the second group, which is found at mass 143, 144, 156, 157, 199, 210, 253 and 267, appear 30 mass units higher in the spectrum of compound Ia derived from aspidospermine. These spectra thus provide conclusive evidence for the presence of the same carbon skeleton in both compounds.[20]

The similarity is so close as to eliminate the possibility of a difference in the attachment of the ethyl group (*e.g.*, structure II). If this group would in quebrachamine be attached to a carbon atom other than the quaternary one (C-5 in Ia), one should find an appreciable difference in certain peak intensities, particularly those which for their formation require rupture of a carbon–carbon bond at this point of branching (see discussion below).

As additional support of the reaction sequence which led from deacetylaspidospermine to methoxyquebrachamine *via* the suggested mechanism,[21] the oxidation product was reduced with sodium borodeuteride which led to the formation of deuteriated methoxyquebrachamine, the mass spectrum of which did show that one deuterium atom is incorporated near N-9 because all the fragments containing the piperidine moiety of the molecule but not those retaining only the indole system were displaced for one mass unit. These results are in agreement with a deuterium atom at C_{19} as demanded by the proposed mechanism.

Structure I for quebrachamine is further corroborated by the previously discussed formation of 3-ethylpyridine in such high proportions and by the nature of the products of the zinc dust distillation which showed molecular weight 280 (Vc) and 282. Formation of Vc from quebrachamine requires ring closure, a reversal of the reaction which we had accomplished by chemical means as described above. Furthermore, it turned out that the product of molecular weight 282, which was well separated on gas chromatography from remaining quebrachamine, gave a mass spectrum indicative of a deacetylaspidospermine derivative lacking the methoxy group and which was, in fact, identical with IIIc isolated from quebracho bark.[22]

These findings indicate the closure of a ring under the conditions of the zinc dust distillation which is at first surprising, but a consideration of the model of the quebrachamine molecule shows that C-19

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

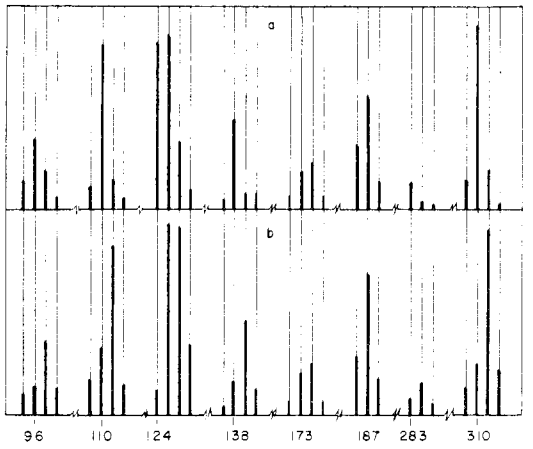
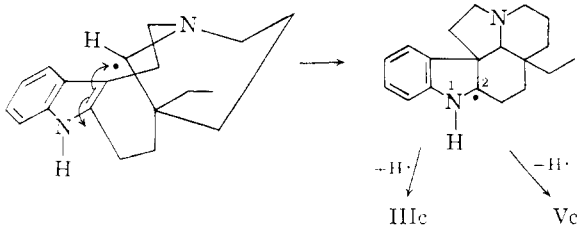


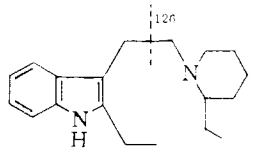
Fig. 4.—Comparison of the major peaks in the spectra of (a) quebrachamine and (b) 19-deuterioquebrachamine.

lies atop the C-2, C-12 double bond, a conformation favoring such a cyclization. It may be depicted starting with the abstraction of a hydrogen atom from C-19 and addition of the resulting radical to the indolic double bond followed either by loss of hydrogen from N-1 to give Vc, or by addition of hydrogen to C-2 resulting in the formation of IIIc. Hydrogen should be available in this system since the reaction is performed in a sealed ampoule.



The ease of cyclization suggests the possibility of the conversion of quebrachamine to the carbon skeleton of aspidospermine by more specific chemical reactions.

It should be mentioned that another fraction obtained in the zinc dust distillation of quebrachamine had a molecular weight of 284 and gave an intense peak at *m/e* 126 suggesting reductive cleavage of the C-4, C-5 bond forming VIII.



VIII

Having shown that the carbon skeleton of aspidospermine (IIIa) contains only one more carbon–carbon bond than quebrachamine (I), it was of interest for biogenetic reasons to determine whether the single remaining asymmetric carbon atom (C-5) in quebrachamine is of the same absolute configuration as the corresponding center in aspidospermine. It had to be the same in Ia since at no time during the reaction was carbon atom 5 involved. The optical rotatory dispersion curves[25] of Ia and quebrachamine[15] are practically superimposable which implies that both belong to the same configurational series, while (+)-quebrachamine of *Stemmadenia donell-smithii*[15] is of the opposite absolute configuration. This result supports the hypothesis that quebrachamine and aspidospermine are biogenetically related. The recent isolation[22] of a number of additional derivatives of aspidospermine from *Aspidosperma quebracho blanco* in which, however, no trace of any other quebrachamine-like alkaloid could be found seemed to indicate that the aspidospermine group is formed *via* quebrachamine or a similar precursor but not *vice versa*.

Too much has already been speculated regarding the biogenesis of aspidospermine and quebrachamine, even before its structure had been established with certainty, and the recent discovery that a polyacetate moiety is the precursor of the alicyclic section of indole alkaloids[26] obviously lends itself to a new variation. We feel, however, that these should first be tested experimentally.

Discussion of the Mass Spectra.—Although the mass spectrometric evidence for the correctness of structure I for quebrachamine did not require a detailed interpretation of the spectra, it should nevertheless be discussed.

Fragmentation of the C-10, C-11 bond in the molecular ion formed on abstraction of an electron is favored because the positive charge is well stabilized by the free electron pair of the nitrogen atom, if retained on C-10.[9] The simultaneous formation of an allyl radical at C-11 further aids this cleavage. The ion IX, still of the same mass as the original molecule, may now suffer fragmentation of the C-3, C-4 bond with rearrangement of a hydrogen (arrows a) to give the ion of mass 138 which may decompose further to mass 110. If, however, the charge distribution upon cleavage of the C-10, C-11 bond is the reverse (a positive change at C-11 would also be stabilized by the aromatic system) then the charge is retained at the aromatic particle leading to a peak at *m/e* 144. Alternatively, the fragment of mass 143 is formed if the hydrogen atom is lost rather than transferred during the cleavage process.

The fragments of mass 125, 124 and 96 seem to arise by cleavage along b followed by the processed indicated in the scheme below. Loss of hydrogen from the radical-ion of mass 125 gives the even better stabilized conjugated ion of mass 124. This suggestion is in agreement with the spectrum of Ia-19-*d* which indicates that this fragment retains the deuterium atom but only to about $^2/_3$ (Fig. 4) (predominant loss of hydrogen due to an isotope effect[27]). Rearrangement of a hydrogen atom from C-19 to C-5 followed by loss of the side chain leads to mass 96. The peaks at mass 261 and 253 are obviously due to loss of methyl and ethyl, respectively.

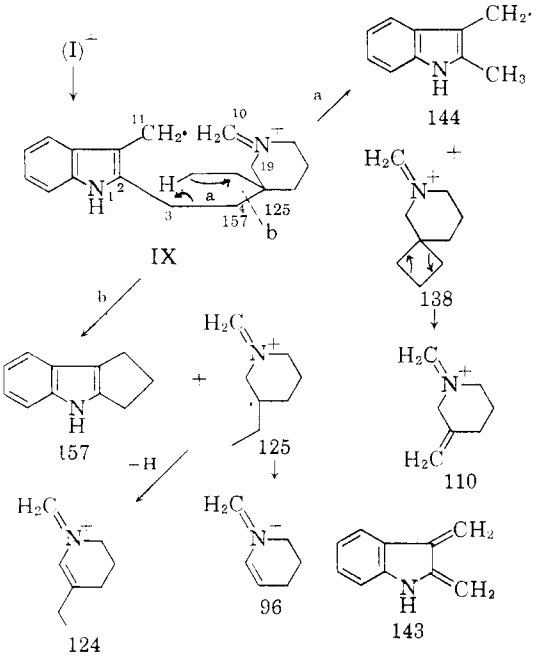
The fragmentation processes pictured above follow the basic assumption that a fragment of high abundance must be formed *via* a path of low energy requirement. This demands the formation of particles with as few as possible free valences and

(25) We are indebted to Prof. Djerassi and his associates for the determination of the O.R.D. curve of Ia.

(26) E. Leete, S. Ghosal and P. N. Edwards, *J. Am. Chem. Soc.*, **84**, 1068 (1962).

(27) The greater ease of loss of H *vs.* D is well known; see, for example, F. L. Mohler and V. H. Dibeler, *Phys. Rev.*, **72**, 158 (1947), and chapter 5 in ref. 9.

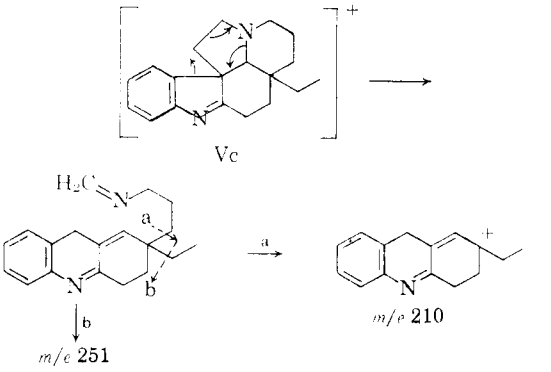
favors the formation of neutral molecules.[9] The processes 138 → 110 and 125 → 96 are corroborated by metastable peaks at *m/e* 88.5 and 74.5, respectively (calcd. 87.7 and 73.8).



In the formation of many of the fragments the ethyl group is involved. If quebrachamine were II rather than I one would expect a considerably different mass spectrum, at least as far as the intensities of the peaks at *m/e* 96, 110 and 138 are concerned.

The mass spectra of Va and Vc contain only very few intense peaks, namely, those for the fragments of mass M-29, M-70 and for the molecular ion (M = 310). It should be noted that these spectra are very different from those of aspidospermine and its close derivatives[22] owing to the presence of the 1,2-double bond which prevents the fragmentation of ring C discussed earlier.

The loss of 70 mass units (310 → 240) must be quite involved and could be due to elimination of either C_5H_{10} or C_4H_8N. The latter is more easily visualized (path b) and was shown to be correct by determination of the accurate mass of the corresponding fragment of Vc. It was found[28] to be 210.129$_6$ in agreement with $C_{15}H_{16}N$ (calcd. 210.1282) rather than $C_{14}H_{14}N_2$ (calcd. 210.1156). Loss of the ethyl group (path b) instead of the nitrogen-containing side chain leads to the fragment of mass 251. In both cases the resulting ion is a highly stabilized one, providing the driving force for the decomposition.



(28) Determined with a double-focusing mass spectrometer (model CEC 21-110), using a photographic plate.

Experimental

Zinc Dust Distillation of Quebrachamine.—To 1 g. of zinc dust, previously dried by heating to 250°, was added 13 mg. of quebrachamine and mixed thoroughly. The mixture was transferred to a glass tube (3 mm. inner diameter, sealed on one end) and covered by an additional gram of dried zinc dust. A plug of lose glass wool was inserted and the tube then narrowed to a capillary, evacuated and sealed off at the end of the capillary. The tube was now inserted at 400–420° into a horizontal oven in such a manner that only the capillary extended from the oven. A yellow distillate began to collect in the cold part after 5 min. and after 1 hr. of heating the tube was cooled and the capillary cut open. The distillate, 9 mg., was injected without solvent into a gas chromatographic column (16% silicon oil 550 on Chromosorb W at 120°, 8 ft., 10 lb. helium) for the separation of the pyridines formed, and the fractions collected in capillary U-tubes held at −70°. Mass spectra were determined of the fractions of this chromatogram shown in Fig. 1.

For the separation of the indolic fraction the zinc dust distillation was repeated with another 13 mg. of quebrachamine and the product (6 mg.) separated by gas chromatography (6% Apiezon L on Chromosorb W at 175°, 8 ft., 16 lb. helium), the fractions collected in glass tubes without external cooling and their mass spectra obtained.

For the least volatile group of products a third zinc dust distillation was performed and the distillate (7 mg.) separated (10% Apiezon on Chromosorb W, at 255°, 4 ft., 12.5 lb. helium). The fractions were collected and mass spectra obtained.

Oxidation of Deacetylaspidospermine (IIIb) with Iodine.—To four portions of 10 ml. of methanol, each containing 250 mg. of iodine, were up to each 5 ml. of 1 *N* NaOH, the mixture swirled once and immediately added to four separate portions of 10 ml. each of a 1% solution of IIIb in methanol. After 3 min. at room temperature all four solutions were combined, and poured into 500 ml. of water. The cloudy solution was extracted three times with 200 ml. of ether, the combined ether solutions were washed with water, dried over potassium carbonate and the solvent evaporated *in vacuo*. The residue was dissolved in 10 ml. of ether, filtered from a trace of insoluble material, and the solvent removed; the remaining brownish oil amounted to 394 mg.

Reduction with Sodium Borohydride.—The product (390 mg.) obtained on oxidation of deacetylaspidospermine was dissolved in 15 ml. of methanol and 30 ml. of 10% solution of sodium methoxide in methanol was added and the mixture heated under reflux. During a period of 10 hr. a solution of 1.5 g. of sodium borohydride in 15 ml. of water was added in portions and the heating continued overnight. The mixture was kept homogeneous by the addition of small portions of methanol. The solution was then concentrated to 15 ml., 100 ml. of water added and acidified with 1 *N* sulfuric acid to decompose the excess of borohydride. The brown clear solution was made alkaline with 2.5 *N* sodium hydroxide and extracted three times with ether. The ethereal solutions were washed with water, dried over potassium carbonate and the solvent distilled. A small amount of the residue was subjected to gas chromatography and found to consist (according to the mass spectra of the fractions) of Ia, IIIb, Va, Vb, a methoxydeacetylaspidospermine and a dimethoxyquebrachamine. The treatment with sodium borohydride was repeated three times to convert all of Va to Ia. The crude product of the last reduction (330 mg.) was dissolved in ether, 1 g. of alumina added and the solvent evaporated. The resulting powder was brought on the top of a chromatographic column consisting of 36 g. of alumina (act. II, neutral) eluted with petroleum ether–benzene 7:3 and collected in 25-ml. fractions. Fractions 1–15 contained Ia (according to the mass spectrum) and were combined (97 mg.) and converted to the picrate (84 mg.). It was recrystallized from methanol and gave 55.6

mg. of deep-orange needles, m.p. 204–206° (capillary, inserted at 175°). From the mother liquor, an additional 12.6 mg., m.p. 203–205° was obtained. The analytical sample was obtained from the first crop.

Anal. Calcd. for $C_{20}H_{28}N_2O$, $C_6H_3N_3O_7$: C, 57.66; H, 5.77; N, 12.93. Found: C, 57.81; H, 5.71; N, 13.08.

The picrate, 35 mg., was converted to the free base by passing its chloroform solution over alumina (5 g., act. I, neutral). The eluate was concentrated and the residue distilled (0.05 mm., 180° air-bath). The colorless glass obtained could not be induced to crystallize. It is characterized by its ultraviolet spectrum in ethanol (λ_{max} 228, 278, 291(sh) mμ; ε 43,600, 8,020, 5,680; λ_{min} 252 mμ, ε 4,300), optical rotatory power ($[\alpha]^{25}D$ −103°, *c* 0.102 in dioxane) and mass spectrum (Fig. 2b), which also permits the determination of the molecular weight of 312.

Fractions 23–30 (eluted with benzene) contained, according to their mass spectra, mainly a compound of molecular weight 342 (a dimethoxyquebrachamine) and were combined (47 mg.) and converted to the picrate. The orange needles obtained on recrystallization from methanol melted under decomposition at 224–228°.

Anal. Calcd. for $C_{21}H_{30}N_2O_2$, $C_6H_3N_3O_7$: C, 56.73; H, 5.82: Found: C, 56.49; H, 5.77.

Fractions 45–47 (22 mg., eluted with benzene–chloroform 10:2) were shown by their mass spectra to consist mainly of deacetylaspidospermine (IIIb). Fractions 48–56 (76 mg., same solvent) contained IIIb and a compound of molecular weight 342, according to its mass spectrum a methoxydeacetylaspidospermine.

Isolation of Va and Vb.—The crude mixture (70 mg.) obtained on oxidation of IIIb was acetylated to facilitate the separation of compound 310 from the unchanged starting material and other by-products. After addition of 3 ml. of acetic anhydride and standing overnight the mixture was poured into 50 ml. of 2.5 *N* NaOH and after standing for 5 min. was extracted three times with ether. The ether phases were washed with water, dried over potassium carbonate and the solvent removed. A sample of the residue, 61 mg. of a brownish oil, was separated by gas chromatography (8% Apiezon L, 260°) permitting the isolation of a pure sample of Va, the mass spectrum of which is shown in Fig. 3b. The ultraviolet spectrum showed λ_{max} 228, 236(sh), 255, 307 mμ; ε 16,500, 13,700, 4,900 and 4,500; λ_{min} 246, 283; ε 4,700, 3,320.

Sodium Borodeuteride Reduction of Crude Va.—The product (94 mg.) of oxidation of 100 mg. of deacetylspidospermine obtained in an experiment similar to the one described above was reduced with 20 mg. of sodium borodeuteride[29] in 2 ml. of water as described earlier. The crude product, 54 mg., was separated by gas chromatography and the methoxyquebrachamine fraction collected. The mass spectrum (Fig. 4) shows the incorporation of one atom of deuterium.

Lithium Aluminum Deuteride Reduction of Crude Va.—The product (32 mg.) of another oxidation of IIIb was dissolved in 5 ml. of tetrahydrofuran and transferred to an ampoule. A large excess of lithium aluminum deuteride was added and the sealed ampoule held at 70° for 1 hr. After cooling, the ampoule was opened, 1 ml. of water and some ether added, shaken vigorously, centrifuged and the ethereal solution decanted. The extraction was repeated three times, the ether phases washed, dried and evaporated. The residue (24 mg.) was separated by gas chromatography and the deacetylaspidospermine fraction collected for a mass spectrum.

(29) The authors are indebted to Prof. R. E. Davis for a gift of sodium borodeuteride.

COMMUNICATIONS TO THE EDITOR

EFFECT OF BOND ENERGY ON REACTIONS OF HOT HYDROGEN ATOMS AT C–H BONDS

Sir:

In a recent letter[1] Root and Rowland have presented a new and significant result on the reaction of hot tritium atoms as produced by nuclear recoil. They investigated hot hydrogen substitution

$$T + RH \longrightarrow RT + H \qquad (1)$$

in mixtures of methane and *n*-butane. It was found that the total probability of this process, per C–H bond, is about the same in the two molecules. On this basis they suggested that steric obstruction is not a significant controlling factor in such reactions. Steric obstruction previously had been suggested[2] as possibly being responsible for observed variations in the yield ratio of substitution (1) relative to abstraction (2)

$$T + RH \longrightarrow HT + R \qquad (2)$$

reactions in various alkanes. Root and Rowland postulate that these variations are caused instead by a sensitive dependence of hot abstraction (but not substitution) on C–H bond energy. This proposal is particularly interesting since in prior work there has been little indication that true hot reactions resemble thermal processes in being extremely sensitive to bond energy.[3] Furthermore, while there must obviously be some effect of bond energy on such hot processes it is most difficult to conceive of a physical basis for as great a dependence as is postulated (see ref. 4).

In this letter we wish to: (1) Present certain experimental data indicating that the effect of the energy of the C–H bond is unlikely to be of sufficient magnitude to be consistent with Root and Rowland's proposal. (2) Point out that Root

(1) J. W. Root and F. S. Rowland, *J. Am. Chem. Soc.*, **84**, 3027 (1962).

(2) D. Urch and R. Wolfgang, *ibid.*, **83**, 2982 (1961).

(3) Certain hot addition reactions result in an intermediate which subsequently de-excites by bond rupture [D. Urch and R. Wolfgang, *ibid.*, **81**, 2025 (1959)]. Such secondary consequences of primary hot reactions will be sensitive to bond energy.

(4) There will, of course, be some effect of a smaller bond energy on reaction probability. The most obvious is that it might extend downward the minimum or threshold energy for reaction. The magnitude of this effect can be estimated using the kinetic theory of hot reactions (P. J. Estrup and R. Wolfgang, *ibid.*, **82**, 2665 (1960)). If reaction occurs with probability $p(E)$ on collision in the energy interval E_0 to ∞ then the total probability of hot reaction is

$$P = 1 - \exp\left(-\frac{f}{\alpha}\int_{E_0}^{\infty}\frac{p(E)}{E}\,dE\right)$$

where f is the probability of collision with a reagent molecule and α the average logarithmic energy loss per collision. If the C–H bond energy is lowered by ΔE, one way of estimating the effect of this would simply be to replace $p(E)$ by $p(E + \Delta E)$ in this equation. However, the shape of the function $p(E)$ is not known. It may be roughly approximated by assuming a constant value between the energy limits E_1 and E_2. In this case the effect of lowering the bond energy might be to drop the lower limit to $E_1 - \Delta E$. Following this procedure, using reasonable values of the parameters involved, and assigning the entire increase in reaction probability to abstraction, it is difficult to see how this effect of bond energy could conceivably reach a magnitude to be consistent with Root and Rowland's proposal.