[JOINT CONTRIBUTION FROM THE RESEARCH DEPARTMENT OF CIBA PHARMACEUTICAL CO., DIVISION OF CIBA CORPORATION, SUMMIT, N. J., AND THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY, STANFORD, CALIF.]

Mass Spectrometry in Structural and Stereochemical Problems. XLVII.[1] Some Observations on Mass Spectra of Pseudoindoxyl Alkaloids[2]

BY NEVILLE FINCH, IVA HSIU-CHU HSU, W. I. TAYLOR, H. BUDZIKIEWICZ, AND CARL DJERASSI

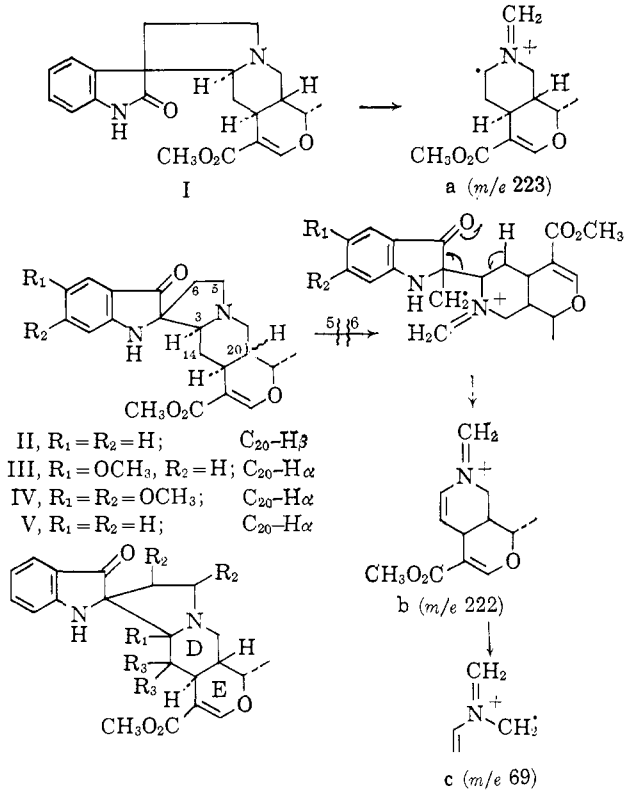
RECEIVED JANUARY 18, 1964

The mass spectra of several pseudoindoxyls derived from tetrahydro-β-carboline alkaloids, by oxidation and rearrangement, have been examined. From deuterated and functional analogs the fragmentation behavior has been shown to be similar to that of the oxindole alkaloids, but possessing a characteristic feature which allows easy recognition of this class of compound. The recent isolation of isoreserpiline pseudoindoxyl from *Aspidosperma discolor* A. DC. indicates that pseudoindoxyls of this type may be of natural occurrence.

The variety of structural types of alkaloids isolated from the genus *Aspidosperma*[3] has been further increased by the isolation of isoreserpiline pseudoindoxyl (IV) from *Aspidosperma discolor* A. DC.[4] The alkaloid has also been encountered in *Rauwolfia vomitoria* Afzel.[5] and *R. ligustrina* Roem. et Shult.[6] Up to this time the only similar alkaloids of the same oxidation state derivable from the tetrahydro-β-carboline bases were the oxindole alkaloids and laboratory methods for realizing this transformation had been developed.[7-9] These reactions have been useful in elucidating the stereochemistry of that class of alkaloid[8] and establishing the structures of new members.[9] It might reasonably have been expected that the indole alkaloids which had naturally occurring oxindole analogs could also have pseudoindoxyl equivalents, derivable by an alternative rearrangement of possibly the same primary oxidation product of tetrahydro-β-carboline moiety. The isolation of isoreserpiline pseudoindoxyl (IV)[4-6] fulfills this expectation as isoreserpiline oxindole, carapanaubine, has already been described.[10]

The mass spectra of oxindole alkaloids[10] show characteristic cleavage products which allow ready recognition of the alicyclic portion and presence of substituents in the aromatic moiety. The mass spectra of pseudoindoxyls prove to be equally definitive, and should help to facilitate the structural elucidation of new members of this class of alkaloid.

The most important fragmentation product of mitraphylline (I) has been shown to arise from cleavage of the spiran ring, and deuterium labeling indicated that no hydrogen transfer accompanied the formation of this fragment a (*m/e* 223). In the case of the isomeric ajmalicine pseudoindoxyl (II)[11] the base peak in the spectrum (Fig. 1) occurs at *m/e* 222. Since it is not shifted by aromatic substitution, as for example in the spectra of aricine (III)[11] and isoreserpiline pseudoindoxyls (IV),[5] it must arise again from the alicyclic portion of the molecule. This conclusion is supported by results obtained from deuterium labeled analogs of II (*viz.*, VIa–VId). A label at C-3 (VIa) causes a shift to *m/e* 223. The 3,5,6-trideuterio derivative VIb has its principal peak at *m/e* 224, indicating that one carbon atom of the tryptamine bridge (C-5) is retained in this fragment. From the shifts noted for the analogs VIc (*m/e* 223) and VId (*m/e* 224), it can be further concluded that one hydrogen atom is lost from C-14 during formation of this main fragment (*m/e* 222) from II and its formulation as b seems to be most reasonable. For its genesis primary cleavage of the tryptamine bridge of the molecular ion occurs between C-5 and C-6 followed by γ-hydrogen transfer *via* a six-membered transition state. (This is typical for the fragmentation of ketones.[12,13])



VIa, R_1 = D, R_2 = R_3 = H
b, R_1 = R_2 = D, R_3 = H
c, R_1 = R_2 = H, R_3 = D
d, R_1 = R_3 = D, R_2 = H

(1) For paper XLVI see G. von Mutzenbecher, Z. Pelah, D. H. Williams, H. Budzikiewicz, and C. Djerassi, *Steroids*, **2**, 475 (1963).

(2) The work at Stanford University was supported by grants No. GM-11309 and AM-04257 from the National Institutes of Health of the U. S. Public Health Service.

(3) H. G. Boit, "Ergebnisse der Alkaloidchemie," bis 1960, unter besonderer Berucksichtigung der Fortschritte seit 1950, Akademie-Verlag, Berlin, 1961, pp. 643–650. For additional references see B. Gilbert, L. D. Antonaccio, and C. Djerassi, *J. Org. Chem.*, **27**, 4702 (1962); C. Djerassi, Y. Nakagawa, J. M. Wilson, H. Budzikiewicz, B. Gilbert, and L. D. Antonaccio, *Experientia*, **19**, 467 (1963).

(4) N. Dastoor and H. Schmid, *ibid.*, **19**, 297 (1963).

(5) N. Finch, W. I. Taylor, and P. R. Ulshafer, *ibid.*, **19**, 296 (1963).

(6) J. M. Mueller, private communication.

(7) J. Shavel and H. Zinnes, *J. Am. Chem. Soc.*, **84**, 1320 (1962).

(8) N. Finch and W. I. Taylor, *ibid.*, **84**, 1318, 3871 (1962).

(9) N. Finch, C. W. Gemenden, I. H.-C. Hsu, and W. I. Taylor, *ibid.*, **85**, 1520 (1963).

(10) B. Gilbert, J. A. Brissolese, N. Finch, W. I. Taylor, H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *ibid.*, **85**, 1523 (1963).

(11) N. Finch, C. W. Gemenden, I. H.-C. Hsu, and W. I. Taylor, in preparation. This paper describes the preparation and properties of this and analogous pseudoindoxyls in detail.

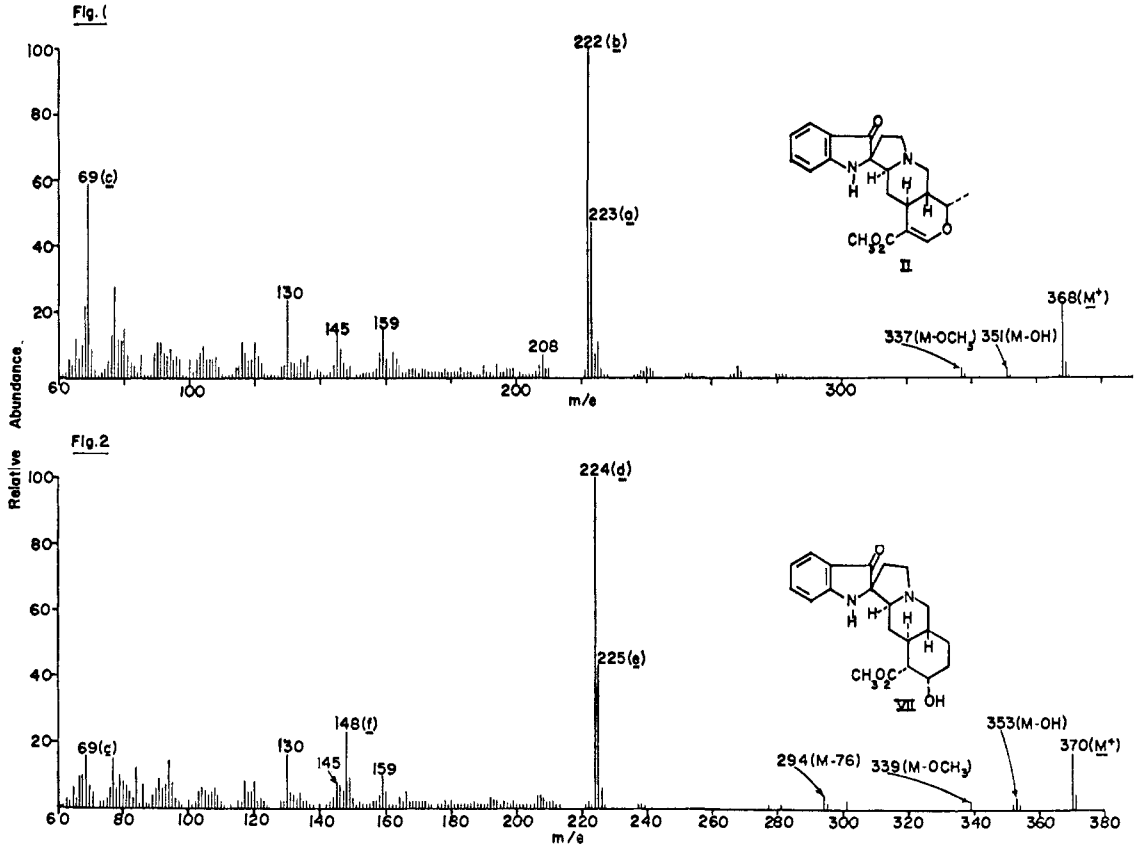


Fig. 1.—Mass spectrum of ajamalicine pseudoindoxyl.
Fig. 2.—Mass spectrum of yohimbine pseudoindoxyl.

Ion b in the spectrum of II (Fig. 1) is accompanied by a fragment, *m/e* 223, of lesser abundance (after subtraction of the isotope contribution of *m/e* 222 (12%), it amounts to 34% of the base peak, *m/e* 222). With the exception of substitution at C-6, none of the deuterium labels in VIa–d are lost and this ion, therefore, is the same as the main cleavage product (a) of mitraphylline (I). The further decomposition products of a, *viz.*, *m/e* 208 (loss of methyl) and *m/e* 69 (c) found in the spectrum of mitraphylline,[10] are also present in Fig. 1.

The fragments containing the indole moiety of II are analogous to those encountered in the spectrum of mitraphylline (I),[10] though in somewhat lesser abundance. The species *m/e* 130 is unaltered in position in the spectrum of VIa, c, and d, but shifted to *m/e* 131–132 in VIb and therefore consists of the indole nucleus, together with an additional carbon atom most probably C-6. The shifts of the indole fragments *m/e* 144–146 and 159, upon deuteration, are difficult to follow because of the low abundance of these ions, but by analogy to the results obtained with the oxindole alkaloids[10] they may be ascribed to species containing the indole nucleus, together with two carbons, or one carbon and one oxygen atom, and the indole nucleus with two carbons and one oxygen atom, respectively.

(12) First proposed by F. W. McLafferty and summarized in F. W. McLafferty, "Determination of Organic Structures by Physical Methods," Vol. 2, F. C. Nachod and W. D. Phillips, Ed., Academic Press, Inc., New York, N. Y., 1962, pp. 129–149.

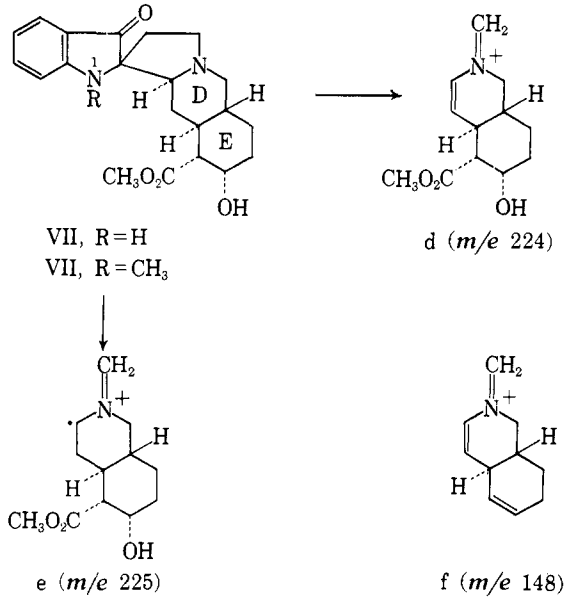
(13) For further references and details of symbolism used in the formulas (arrows = two electron shifts, fishhooks = one electron shift, ⦚ = fission between designated atoms) see H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Interpretation of Mass Spectra of Organic Compounds," Holden-Day, San Francisco, Calif., 1964, pp. xi–xiii.

The second type of pseudoindoxyl base investigated has not yet turned up in nature[14] and is represented by yohimbine pseudoindoxyl (VII)[5] (Fig. 2) and its 1-methyl derivative VIII.[5] The main fragment d, *m/e* 224, is again formed with hydrogen abstraction and comprises the alicyclic moiety. It is accompanied by a species of lesser abundance e, *m/e* 225. The low intensity fragment c (*m/e* 69) present in yohimbine oxindole[10] is also observed. A fragment, *m/e* 148, is unaltered in the spectra of both VII and VIII and must, therefore, be derived from the alicyclic portion. Its origin may be loss of the hydroxyl and carbomethoxyl functions to yield species f. (It should be noted that in the upper mass range of the spectra of VII and VIII a peak at *m/e* 294 due to M − 76 is observed.) Whether f arises from d, or by cleavage of the spiran ring after the loss of the two functional groups in ring E, has not been established. Other indole-containing fragments of rather low abundance occur, as in the case of II, in the region between *m/e* 130 and 159 (Fig. 2).

From the preceding discussion it can be seen that the main fragmentation path of the pseudoindoxyl alkaloids is cleavage of the spiran ring; but in contrast to the oxindole alkaloids, formation of the principal ion from the alicyclic portion is accompanied by hydrogen migration, so that a simple means is available to distinguish between these isomeric types. From the mass of the main fragment the type of alicyclic frame-

(14) This statement may not be correct since methyl isoreserpate pseudoindoxyl has been obtained from *Rauwolfia vomitoria* manufacturing mother liquors, but the process used was such that the compound could have been generated from a primary autoxidation product (P. R. Ulshafer, personal communication).

work can also be deduced. The difference in mass between the main fragment and the molecular ion also allows one to calculate what substituents are present on the indole portion, even if the typical indole fragments are of too low an abundance for their unambiguous identification. This weakness means, however, that no assignment of the stereochemistry of the DE ring junction is possible as was the case with the indole alkaloids[15] on the basis of altered intensity relationships of the key indole fragments. Thus the mass spectra of tetrahydroalstonine pseudoindoxyl (V) and ajmalicine pseudoindoxyl (II) are virtually identical.



Experimental[16]

3,5,6-Trideuterioajmalicine Pseudoindoxyl (VIb).—Serpentine hydrochloride (500 mg.) was dissolved in deuteriomethanol (5 ml.) and cooled in ice. Sodium borodeuteride[15] (340 mg.) was added portionwise during 7–10 min. to the well stirred solution. The progress of the reaction was followed by measuring its ultraviolet spectrum qualitatively. After 30 min. the solution was poured into ice–water and extracted with methylene chloride. The organic phase was dried (Na_2SO_4) and concentrated to yield a foam (323 mg.) whose ultraviolet and infrared spectra were identical with those of ajmalicine as were also its properties upon thin layer chromatography. The foam (320 mg.) was redissolved in methylene chloride (15 ml.) and 16 ml. of 0.07 *M* lead tetraacetate in methylene chloride added. After 10 min. the mixture was shaken with water, the organic phase was dried (Na_2SO_4), and filtered through neutral alumina (Woelm, Activity III). The eluate yielded a residue (150 mg.) which was refluxed for 1 hr. under nitrogen in anhydrous methanol (8 ml.) in which sodium (9.6 mg.) had previously been dissolved. The reaction mixture was poured into ice cold salt solution and extracted with methylene chloride. The resulting isolate (168 mg.) was chromatographed on a plate of silica gel G (Merk, Darmstadt) using ethyl acetate–methanol (19:1) as the developer. The band corresponding to ajmalicine pseudoindoxyl was cut out and extracted to furnish from methanol bright yellow needles of 3,5,6-trideuterioajmalicine pseudoindoxyl (20 mg.), m.p. 215–217°, identical in all respects with the nondeuterated analog; isotopic purity: 3% d_0, 3% d_1, 35% d_2, and 59% d_3.

3-Deuterioajmalicine Pseudoindoxyl (VIa).—Dehydroajmalicine hydrochloride[10] (270 mg.) was suspended in deuteriomethanol (5 ml.), cooled in an ice bath, and treated with sodium borodeuteride (540 mg.) in anhydrous tetrahydrofuran (20 ml.). The reaction mixture was stirred for 90 min. at 0°, diluted with water, and extracted into methylene chloride. Removal of the solvent gave a foam (242 mg.) which was oxidized with lead tetraacetate and methanolized in a manner analogous to the foregoing experiment. The resulting 3-deuterioajmalicine pseudoindoxyl was isolated also by preparative thin layer chromatography; isotopic purity: 14% d_0, 63% d_1, and 23% d_2.

14,14-Dideuterioajmalicine Pseudoindoxyl (VIc).—Dehydroajmalicine hydrochloride[10] (547 mg.) was refluxed for 2 hr. in deuteriomethanol (10 ml.). A portion (5 ml.) was taken to dryness, dissolved in methanol, cooled in an ice bath, and sodium borohydride (300 mg.) was added in small portions until the ultraviolet spectrum became entirely indolic (*ca.* 15 min.). The reaction mixture was poured into ice–water and extracted with methylene chloride. Removal of the solvent gave a foam (228 mg.) which was transformed as for the first experiment into the desired 14,14-dideuterioajmalicine pseudoindoxyl; isotopic purity: 21% d_0, 25% d_1, and 54% d_2.

3,14,14-Trideuterioajmalicine Pseudoindoxyl (VId).—The remainder of the solution (5 ml.), in the above experiment in which dehydroajmalicine hydrochloride was refluxed in deuteriomethanol, was treated with sodium borodeuteride. It was worked up as above to afford 3,14,14-trideuterioajmalicine (258 mg.) which was converted into 3,14,14-trideuterioajmalicine pseudoindoxyl in the same way as described for the other deuterated derivatives; isotopic purity: 14% d_0, 10% d_1, 20% d_2, and 56% d_3.

(15) L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrueggen, H. Budzikiewicz, J. M. Wilson, L. J. Durham, and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 2161 (1962).

(16) The spectra were determined (ionizing energy 70 e.v., ionizing current 50 μa.) with a CEC 21-103C mass spectrometer equipped with a direct inlet system; see J. F. Lynch, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *Experientia*, **19**, 211 (1963). The melting points were determined in a Thomas-Hoover capillary melting point apparatus and are uncorrected.