Tetrahedron Letters No. 2, pp. 59-67, 1962. Pergamon Press Ltd. Printed in Great Britain.

MASS SPECTROMETRY IN STRUCTURAL AND STEREOCHEMICAL PROBLEMS[1]

PYRIFOLINE AND REFRACTIDINE

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(Received 6 December 1961; in revised form 22 December 1961)

FROM the Brazilian tree Aspidosperma pyrifolium Mart., there have been isolated[3] two alkaloids, pyrifolidine (A)[4] and pyrifoline, the latter having been characterized[3] as an N-acetyl-7-methoxydihydroindole with an additional aliphatic methoxyl function and possessing the probable empirical formula $C_{23}H_{30}N_2O_3$. By utilizing largely the mass spectrometric fragmentation patterns of suitably labelled derivatives without resorting to classical chemical degradations, it has now been possible to narrow down the constitution of the alkaloid to a few hexacyclic types. Indeed, if biogenetic

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[1] For paper I, see H. Budzikiewicz and C. Djerassi, J. Amer. Chem. Soc. 84, in press (1962).

[2] Recipient of fellowship from the U.S. National Academy of Sciences, National Research Council while on leave from the Instituto de Quimica Agricola, Rio de Janeiro.

[3] B. Gilbert, L.D. Antonaccio, A.A.P.G. Archer and C. Djerassi, Experientia 16, 61 (1960).

[4] 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 and B. Gilbert, Tetrahedron in press.

likeliness is taken into account, then only two expressions (IB or IC) need to be considered seriously. Furthermore, we have been able to demonstrate that a newly discovered alkaloid, refractidine (VII), is based on the identical skeleton.

The NMR spectrum[5] of pyrifoline closely resembles that[6] of aspidospermine (antipode of A without C-16 methoxyl group) in the aromatic region as well as in the location of the aromatic methoxyl (3.81 p.p.m.) and N-acetyl (2.12 p.p.m.) signals. These results, taken in conjunction with the ultraviolet spectroscopic data,[3] settle the substitution pattern in the dihydroindole moiety. The three most striking features in the NMR spectrum of pyrifoline (I), when contrasted with that[6] of aspidospermine, are the absence of any signals between 3.81-6.5 p.p.m. (thus demonstrating the absence of a hydrogen atom at C-2 with its characteristic quartet[6] near 4.5 p.p.m. as well as of any olefinic protons), the absence of any C-methyl groups and the presence of an aliphatic methoxyl function (3.33 p.p.m.). Barring the presence of a non-reducible, tetrasubstituted double bond pyrifoline must be hexacyclic.

Aside from the molecular ion (m/e 382, thus establishing the $C_{22}H_{30}N_2O_3$ rather than $C_{23}H_{30}N_2O_3$[3] empirical formula), there are six important groups of peaks (identified as a - f) in the mass spectrum (Fig. 1) of pyrifoline, which can be assigned with such certainty so as to lead to only very few structural possibilities for the alkaloid. For the sake of simplicity, the results will be discussed below in terms of the skeleton B. The mass spectra of all seven pyrifoline derivatives (I-VI and 7,7-dideuterio VI) show an important peak at M-28 (peak f) due to the loss of ethylene.

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[5] Measured with Varian HR-60 or AR-60 spectrometers in deuteriochloroform with tetramethylsilane as internal standard; all signals are reported in p.p.m. as δ units (δ = c.p.s./60).

[6] C. Djerassi, A.A.P.G. Archer, T. George, J.N. Shoolery and L.F. Johnson, Experientia 16, 532 (1960).

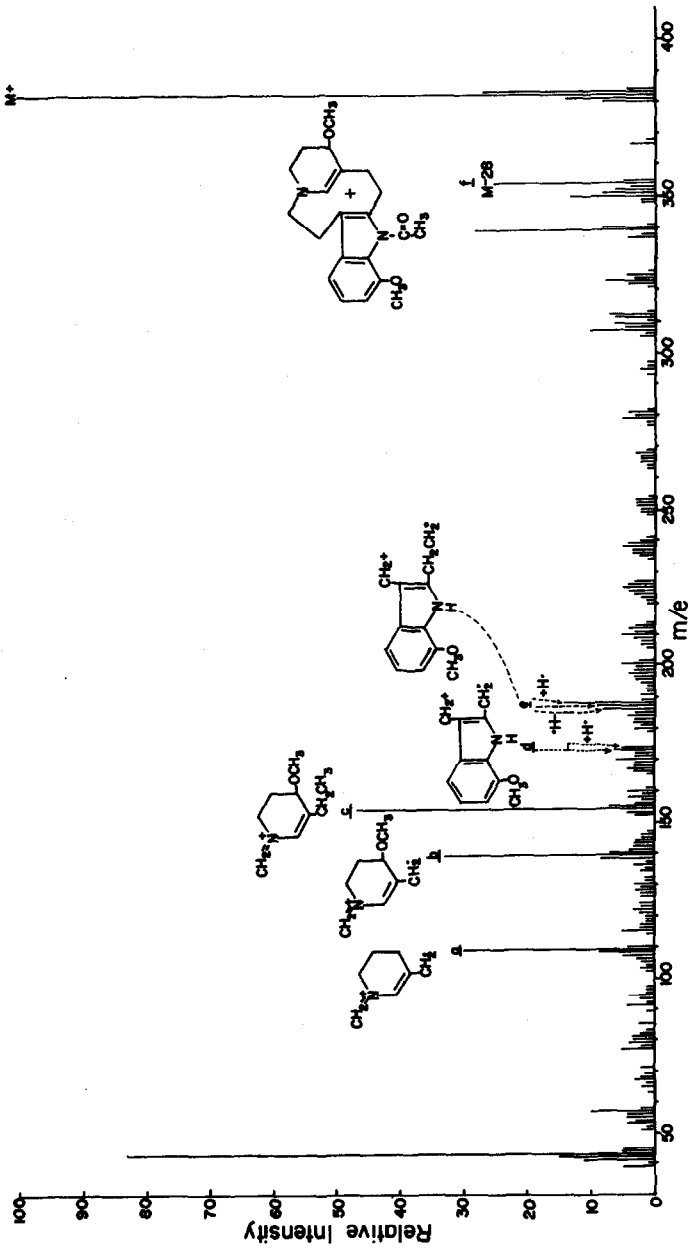

FIG. 1.

Mass spectrum of pyrifoline (IB).

Such a characteristic peak has been recognized first by Biemann et al.[7] in the mass spectra of aspidospermine derivatives and can be attributed to the presence of an unsubstituted ethylene bridge (carbon atoms 3 and 4), the driving force for its expulsion (see arrows in B) being the relief of steric strain in the highly fused system B, the aromatization of the dihydroindole and the formation of a double bond adjacent to nitrogen. The peaks at m/e 339 (loss of CH_3CO), 323 (loss of $CH_2 = CH_2$ and methoxyl) and 307 (loss of acetyl and methanol) are trivial and are not observed in those derivatives which lack the N-acetyl or C-6 methoxyl substituents.

Species d (m/e 173, 174) and e (m/e 186, 187, 188) are present in the spectra (Table 1) of all of the derivatives (I-VI) and are considerably more intense in the deacetyl series (II-IV, VI). They must, therefore, represent the indole moiety with two (d) respectively three (e) carbon atoms attached. This is proved rigorously below in the spectra of various refractidine analogs (XIII-XVI). The assignment to e and d is based on what appear to be energetically the most favored fragmentations from IB or f, but alternatives such as e' are, of course, not excluded and do not affect the overall conclusions regarding the structure of pyrifoline.

Peaks a (m/e 109), b (m/e 139) and c (m/e 154) must be derived from the non-aromatic region of the alkaloid, which bears the aliphatic methoxyl substituent, since alterations in that portion of the molecule produce characteristic shifts (Table 1). Cleavage of pyrifoline with dilute acid yielded deacetylpyrifoline (II), while more drastic acid treatment provided deacetyl-demethylpyrifoline (III), in which the aromatic methoxyl group was retained (NMR spectrum and unchanged mass spectral peaks d and e). Peak a (m/e 109) was observed only in the mass spectra of those pyrifoline and

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[7] K. Biemann, M. Friedmann-Spiteller and G. Spiteller, Tetrahedron Letters No. 14, 485 (1961).

refractidine derivatives, which possess the aliphatic methoxyl group. We attribute its genesis to a rearrangement from b with loss of the elements of CH_2O.

O,N-Diacetylation (pyridine-acetic anhydride) of III gave demethyl-pyrifoline O-acetate (V), which represented an important substance, because it now permitted the recognition of the C-6 hydrogen atom in its NMR spectrum due to the strong downfield shift resulting in a quartet between 4.45 and 4.8 p.p.m. This unambiguous NMR assignment indicates that C-6 has only two, non-equivalent neighboring protons. Oppenauer oxidation of III led to the ketone (unchanged U.V. spectrum) deacetyl-demethyl-6-dehydropyrifoline (VI), the infrared spectrum of which exhibited a band at 5.89μ (Nujol) typical of a six-membered ketone. Its pK_a' (33% DMF) occurred at 5.90 in contrast to 7.25 for its precursor III, a shift which has also been observed in ajmalidine,[8] the keto group of which bears the same relationship to its basic nitrogen as is proposed for VI. Exchange with NaOD in boiling CH_3OD resulted in the introduction of two deuterium atoms (partial exchange of the hydrogen at N_a was also observed by an additional one unit shift of the molecular ion and of the d and e peaks) adjacent to the carbonyl group (7,7-d_2 analog of VI). The deuterium exchange experiment confirmed the conclusion from the NMR analysis of the acetate V that there are present only two hydrogen atoms adjacent to C-6, thus excluding C-7 as a possible point of attachment of the original methoxyl group.

The above experiments prove conclusively that peaks b and c contain the piperidine moiety (six-membered size indicated by I.R. spectrum of VI), the aliphatic methoxyl substituent (with its adjacent CH_2 group) and two (b), respectively three (c) additional carbon atoms. If we assume cleavage of

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[8] S.C. Pakrashi, C. Djerassi, R. Wasicki and N. Neuss, J. Amer. Chem. Soc. 77, 6687 (1955); M. Gorman, N. Neuss, C. Djerassi, J.P. Kutney and P.J. Scheuer, Tetrahedron 1, 328 (1957).

the 10-11 bonc, as has been done in the case of aspidospermine[7] (stabilization of resulting carbonium ion and/or radical by the electrons on nitrogen or the indole double bond), then structures b and c (capture of one hydrogen) are the only plausible ones for these fragments. The mass

TABLE 1

Substance	m.p.	$[\alpha]_D^{CHCl_3}$	Principal mass spectrometric peaks				
			b	c	d	e	f
I	142-144°	+102°	139	154	173,174	186-188	354
II	glass	-14°	139	154	173,174	186-188	312
III	202-203°	-20°	125	140	173,174	186-188	298
IV	-	-	126	141	173,174	186-188	299
V	197-199°	+170°	167	182	173,174	186-188	382
VI	158-160°	+24°	123	138	173,174	186-188	296
1,7,7-d_3-VI	-	-	125	140	174,175	187-189	299
VII	158-160°	-140°	139	154	143,144	156-158	310
VIII	glass	-53°	139	154	143,144	156-158	282
IX	163-164°	-24°	125	140	143,144	156-158	268
X	193-194°	+44°	167	182	143,144	156-158	352
XI	218-219°	+39°	125	140	143,144	156-158	310
XII	132-136°	-	123	138	143,144	156-158	266
XIII	104-106°	-86°	139	154	157,158	170-172	296
XIV	-	-	139	154	159,160	172-174	298
XV	160-161°	-44°	125	140	157,158	170-172	282
XVI	153-157°	-	125	140	159,160	172-174	284

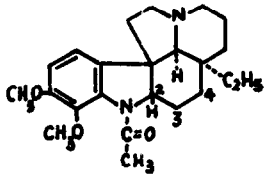
spectral data, therefore, are only consistent with the expressions f or f' for the species M-28, since either one will lead to fragments a-e (Fig. 1). Only a CH_2CH_2 bridge needs to be inserted with simultaneous generation of two more rings to complete the structure of pyrifoline. This can be done in six ways, yielding structures B-G, of which B-D (see arrows) lead to f, and E-G (see arrows) to f'. From a biogenetic standpoint, only B and

C[9] appear likely and in the absence of further information, we prefer B because of its closer relationship to the aspidospermine class of alkaloids and the co-occurrence in the same plant of pyrifoline with pyrifolidine (A).[4]

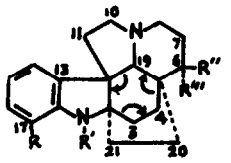
In our earlier paper,[3] we reported on the isolation of a new alkaloid, refractine, from Aspidosperma refractum Mart. Subsequently, we encountered a second alkaloid, refractidine, which now is shown to be closely related to pyrifoline (IB). Refractidine (VII), m.p. 158-160°, $[\alpha]_D$-140° ($CHCl_3$), pK_a' 6.5, possesses the empirical formula $C_{21}H_{26}N_2O_2$ as demonstrated by elementary analysis (one methoxyl, no C-methyl) and mass spectrometric molecular weight determination. Its ultraviolet absorption spectrum (λ_{max}^{EtOH} 208, 253, 278, 288 mμ, log ε 4.36, 4.16, 3.72, 3.67) is typical of an unsubstituted N-acyl dihydroindole and this was confirmed by the recognition of four aromatic protons in its NMR spectrum. The latter did not contain any signals corresponding to olefinic protons, aromatic methoxyl, C-methyl or the C-2 hydrogen (e.g. A), but did show the presence of an aliphatic methoxyl (3.33 p.p.m.) and an N-formyl group. This latter feature was confirmed by acid cleavage to deformylrefractidine (VIII) and reformylation to refractidine (VII). Heating with concentrated hydrochloric acid provided deformyldemethylrefractidine (IX), which was acetylated to the O,N-diacetate X, the NMR spectrum of which showed a quartet due to the C-6 hydrogen exactly as discussed above for the corresponding pyrifoline derivative V. Saponification removed the O-acetate function to yield XI, while Oppenauer oxidation of IX produced the ketone XII (λ_{max}^{Nujol} 5.90μ).

Except for the absence of the aromatic methoxyl group and the substitution of an N-formyl for an N-acetyl grouping, the above reactions indicated

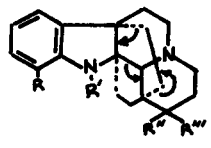
[9] Structure C bears a very strong resemblance to the various Hunteria alkaloids (e.g. H), described by M.F. Bartlett and W.I. Taylor, J. Amer. Chem. Soc. 82, 5941 (1960), and a simple biogenetic connection between C and eburnamonine (H) could be visualized.



A



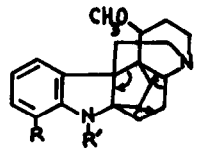
B



C

	R	R'	R''	R'''
I	CH_3O	Ac	CH_3O	H
II	CH_3O	H	CH_3O	H
III	CH_3O	H	HO	H
IV	CH_3O	H	HO	D
V	CH_3O	Ac	AcO	H
VI	CH_3O	H	=O	
VII	H	CHO	CH_3O	H
VIII	H	H	CH_3O	H

	R	R'	R''	R'''
IX	H	H	HO	H
X	H	Ac	AcO	H
XI	H	Ac	HO	H
XII	H	H	=O	
XIII	H	CH_3	CH_3O	H
XIV	H	CD_2H	CH_3O	H
XV	H	CH_3	HO	H
XVI	H	CD_2H	HO	H



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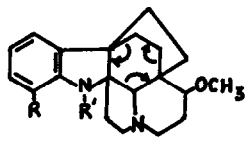

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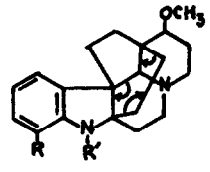
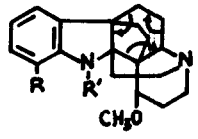
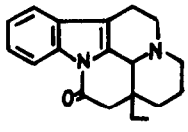


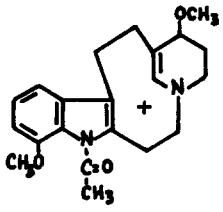
F



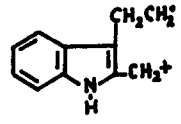
G



H



f'



e'

a striking resemblance between refractidine and pyrifoline. That these were indeed the only structural differences could be demonstrated mass spectrometrically by a technique first employed by Biemann et al.[7,10] Thus comparison of the mass spectra of the pairs II vs. VIII, III vs. IX, V vs. X and VI vs. XII demonstrated identical a, b and c peaks for each pair, but 30 mass unit differences (due to the extra aromatic methoxyl in pyrifoline) in the d, e and M-28 peaks. This coincidence or 30 mass unit shift in peaks also applied to the smaller peaks (unassigned in Fig. 1), whereupon it follows that both alkaloids are based on the identical skeleton and differ only in the nature of the C-17 and N-acyl substituents. The presence of the indole nucleus in the d and e peaks was confirmed by "mass spectrometric labelling" through the N_a-CH_3 and N_a-CD_2H ($LiAlD_4$ reduction of N_a-CHO) refractidine derivatives XIII-XVI (Table 1).

The present results with pyrifoline and refractidine, just as in the earlier case of aspidospermatine,[7] represent further examples of how the constitutions of unknown alkaloids based on new skeletal structures can be elucidated by mass spectrometry (coupled with NMR measurements) of simple derivatives, which afford "labels" of different portions of the molecule. It is instructive to note that in the present structure assignment to pyrifoline and refractidine, none of the carbon atoms (with the exception of the N_a-acyl substituent) of these complicated alkaloids was identified in the sense of classical, degradative chemistry.

We are indebted to Drs. Dardano da Andrade Lima and Alberto Sarmento as well as the Rio Sao Francisco Valley Commission for the plant collections, to the Rockefeller Foundation for supporting the collaborative research program between Stanford University and the Instituto de Quimica Agricola, to the National Institutes of Health for financial aid (grants No. 2G-682 and A-4257) and to Prof. K. Biemann for a stimulating discussion.

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[10] K. Biemann, Tetrahedron Letters No. 15, 9 (1960); K. Biemann and G. Spiteller, Ibid. No. 9, 299 (1961).