Papierchromatographie-Beitrag zur Bestimmung der C_3-Konfiguration bei Reserpoiden

Beim Studium von 105 Substanzen aus den verschiedensten Phasen der Reserpin- und Reserpoid-Synthese[1] erwiesen sich als geeignete Lösungsmittel für die Trennung und Charakterisierung dieser Substanzen am besten die Formamidsysteme. Da Endprodukte der Reserpoid-Synthesen schon von basischem Charakter vorliegen, verursacht Zugabe von Ammoniumformiat zu Formamid eine Abnahme der Rf-Werte sowie Fleckenabrundung. Diese Erscheinung konnte zu einer interessanten und eindeutigen Bestimmung ausgewertet werden, je nachdem es sich um ein Derivat handelte, dem die C_3-Konfiguration des Reserpins zuzuschreiben ist, oder ob es sich um ein Isoderivat handelte. Dies galt selbst dann, wenn nicht beide Isomere nebeneinander vorlagen und wir nicht über die entsprechenden Standardsubstanzen verfügten. Wurde das Alkaloid nebeneinander auf mit Formamid imprägniertem Papier und auf demselben Papier mit 5% Ammoniumformiat-Zusatz entwickelt, so enthält man bei der Verbindung der normalen Reihe auf dem Streifen mit Ammoniumformiat eine Abnahme des R_M-Wertes um 0,71–0,86 ($R_M = \log (1/Rf - 1)$)[2], während die Abnahme bei der Iso-Reihe lediglich 0,38–0,48 beträgt (Tab.)[3].

Im übrigen ist der direkte Unterschied zwischen den Derivaten der normalen Reihe und den Isoderivaten ziemlich beträchtlich: Auf mit Formamid imprägniertem Papier beträgt R_M ungefähr 0,80, auf Papier mit Ammoniumformiat sogar 1,15.

Summary. A method was devised for the estimation of the configuration on C_3 of reserpoides, based on the differences between Rf-values on paper impregnated with formamide and on paper impregnated with formamide containing ammonium formiate.

	Benzol-Cyclohexan 1:1		ΔR_M
	Formamid	Formamid mit Ammoniumformiat	
Reserpin	0,61	0,20	0,80
3-Isoreserpin	0,91	0,79	0,38
Deserpidin	0,75	0,34	0,77
3-Isodeserpidin	0,95	0,88	0,41
10-Fluordeserpidin	0,74	0,36	0,71
Methyl-O-(3,5-diäthoxy-4-methoxybenzoyl)-reserpat	0,88	0,57	0,74
Methyl-O-(m-methoxy-phenoxy-acetyl)-reserpat	0,50	0,12	0,86
10-Methyl-3-isodeserpidin	0,97	0,92	0,45
Methyl-O-acetyl-10-methoxy-3-isodeserpidat	0,69	0,42	0,48

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Forschungsinstitut für Pharmazie und Biochemie, Prag (Tschechoslowakei), 3. August 1960.

[1] K. MACEK und S. VANĚČEK, Coll. Czech. Chem. Comm., im Druck.
[2] E. C. BATE-SMITH und R. G. WESTALL, Biochim. biophys. Acta *4*, 427 (1950). – I. M. HAIS und K. MACEK (Herausg.), *Handbuch der Papierchromatographie* (G. Fischer Verlag, Jena 1958), Bd. I, p. 64.
[3] Die in der Tabelle angeführten Substanzen wurden von den Arbeitsgruppen von Dr. PROTIVA und Dr. WEICHET in unserem Institut dargestellt.

Alkaloid Studies XXVI.[1] The Constitution of Pyrifolidine

In two recent papers from our laboratories[1,2] there has been recorded the isolation from Brazilian *Aspidosperma* species of five new alkaloids—refractine, pyrifoline, pyrifolidine, cylindrocarpine, and cylindrocarpidine—as well as the structure proof of the last two. In the present article, we propose a constitutional formula for pyrifolidine (I), based almost completely upon deductions from two physical tools, nuclear magnetic resonance and mass spectrometry, which have been introduced only very recently into indole alkaloid chemistry.

The elementary analyses[2] of pyrifolidine did not differentiate between $C_{22}H_{30}N_2O_3$ and $C_{23}H_{32}N_2O_3$, but the integrated NMR proton count (found: 32.0 ± 0.7) requires the presence of 32 hydrogen atoms, thus settling the empirical formula. The analytical and infrared spectral properties[2] of the alkaloid showed that the three oxygen atoms are present in one N-acetyl and two methoxyl functions. The NMR spectrum (Fig. 2) of pyrifolidine exhibits the typical aspidospermine (II) fingerprint (Fig. 1) in the region of $\delta = 2.90$–3.31[3], including the characteristic peaks ($\delta = 0.67$) of the C-ethyl group. Indeed, the only significant differences between the NMR spectra (Fig. 1 and 2) of aspidospermine (II) and pyrifolidine (I) lie in the aromatic and methoxyl regions, the latter alkaloid possessing *only two aromatic hydrogens* and both methoxyl groups ($\delta = 3.77$, 3.84) being aromatic. Since we had already indicated earlier[1] that such similarities in NMR spectra are of great diagnostic value, and as the large spin-coupling (J = 8.2 cps) of the benzenoid hydrogens requires that they be adjacent, the NMR evidence is compatible only with structures I, VI, or VII for pyrifolidine. Of these expressions I or VI appear to be more plausible, since all alkaloids of the aspidospermine (II) class which have been encountered so far in nature possess a 7-methoxy-N-acyldihydroindole moiety.

Furthermore, moving a methoxyl group to the top of the ring (C-1′) should change the NMR fingerprint appreciably, while adding a second methoxyl (at C-3′) would be expected to have little effect. On these grounds, we prefer structure I for the alkaloid. The ultraviolet absorption spectrum[2] of pyrifolidine (I) is somewhat shifted as compared to that[4] of aspidospermine (II), but

[1] Paper XXV, C. DJERASSI, A. A. P. G. ARCHER, T. GEORGE, B. GILBERT, J. N. SHOOLERY, and L. F. JOHNSON, Exper. *16*, 532 (1960).
[2] B. GILBERT, L. D. ANTONACCIO, A. A. P. G. ARCHER, and C. DJERASSI, Exper. *16*, 61 (1960).
[3] For definition of δ, see [1], as well as C. DJERASSI, T. NAKANO, A. N. JAMES, L. H. ZALKOW, E. J. EISENBRAUN, and J. N. SHOOLERY, J. org. Chem., in press.
[4] See J. R. CHALMERS, H. T. OPENSHAW, and G. F. SMITH, J. chem. Soc. *1957*, 115.

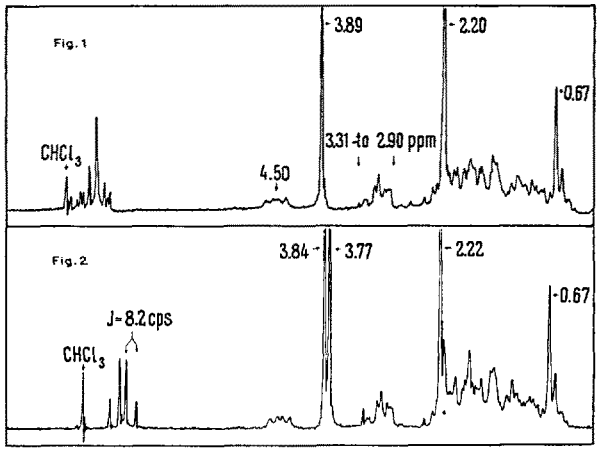
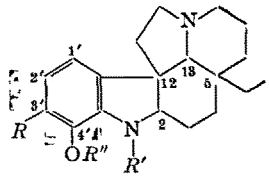
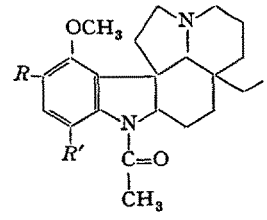


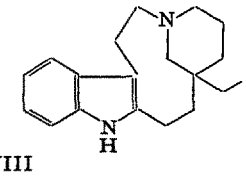
Fig. 1. NMR spectrum of aspidospermine (II). Fig. 2. NMR spectrum of pyrifolidine (I).



I: $R = OCH_3$; $R' = CH_3CO$; $R'' = CH_3$
II: $R = H$; $R' = CH_3CO$; $R'' = CH_3$
III: $R = OCH_3$; $R' = H$; $R'' = CH_3$
IV: $R = R' = H$; $R'' = CH_3$
V: $R = OCH_3$; $R' = CH_3CO$; $R'' = H$



VI: $R = H$; $R' = OCH_3$
VII: $R = OCH_3$; $R' = H$



VIII

we have been unable to find in the literature any suitable models with this chromophore in either the dihydroindole or hexahydrocarbazole series[4]. Indeed, this type of oxygenation pattern in the aromatic ring appears to be unique among the naturally occurring indole alkaloids.

This uniqueness and consequent difficulty in relating pyrifolidine (I) chemically to a known indole alkaloid prompted us to seek confirmation from another physical method, namely, mass spectrometry, which has been employed recently to resolve similar problems regarding the structures of sarpagine[5] and iboxygaine[6].

The mass spectra of deacetylpyrifolidine (III), (m.p. 147–149°, $[\alpha]_D + 7°$ ($CHCl_3$); λ_{max}^{EtOH} 215 and 293 mμ, log ε 4.40, 3.50; *Anal.* calc. for $C_{21}H_{30}N_2O_2$: C, 73.64; H, 8.83; N, 8.18; O, 9.34; methoxyl, 18.13; C-methyl, 4.39; found: C, 73.39; H, 9.08; N, 8.31; O, 9.36; methoxyl, 18.19; C-methyl, 3.49), and deacetylaspidospermine (IV) both exhibit a very strong peak at m/e 124 ($C_8H_{14}N$) and a group of smaller ones at m/e 138 and 152, all arising from the piperidine portion of the molecule. Another group of peaks (190, 203, 204, 314, and 341 in III, *vs.* 160, 173, 174, 284, and 311 in IV) is due to fragments still containing the indole nucleus, the difference in one methoxyl group accounting for the shift of 30 mass units. This difference is also expressed in the molecular ion peaks at m/e 342 and 312 (doubly charged species at m/e 171 and 156).

After completion of our work, there appeared a paper by McLEAN, PALMER, and MARION[7], in which they proposed structure V for the new *Aspidosperma* alkaloid aspidocarpine. Consequently, pyrifolidine (I) should be identical with O-methylaspidocarpine[7] and deacetylpyrifolidine (III) with deacetyl-O-methylaspidocarpine[7]. Indeed, the recorded (ref. [2,7] and the present paper) physical constants of these two pairs are in reasonable agreement, *except for the specific rotations which are opposite in sign*. Through the kind cooperation of Dr. L. MARION, it was possible to show that the infrared spectra (chloroform) of pyrifolidine (I) and O-methylaspidocarpine[7] were identical, and this also applied to the pair deacetylpyrifolidine (III) and deacetyl-O-methylaspidocarpine[7]; furthermore, the appropriate pairs exhibited identical mobility in chromatoplates on silica with the solvent system acetone-ethyl acetate (1:1). Most importantly, the optical rotatory dispersion curves of pyrifolidine and O-methylaspidocarpine[7] were of mirror-image type throughout the spectral range (700–300 mμ), thus establishing their enantiomeric relationship[8]. Since aspidocarpine has been related to aspidospermine (II), *pyrifolidine* (I) *must possess the antipodal absolute configuration at all four asymmetric centers* (C-2, -5, -12, and -13). We believe that this observation, coupled with the fact that both antipodes of the *Aspidosperma* alkaloid quebrachamine (VIII)[9] occur in nature[10], is of utmost significance in any biogenetic scheme[11] and we intend to comment on this point in our full paper.

Zusammenfassung. Die Struktur des *Aspidosperma*-Alkaloids Pyrifolidin (I) wird durch Protonresonanz und durch Vergleich mit Aspidospermin und Aspidocarpin bewiesen. Dieses Alkaloid ist in seiner absoluten Konfiguration ein Antipode des Aspidospermins.

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[5] K. BIEMANN, Tetrahedron Letters No. 15, 9 (1960).
[6] K. BIEMANN and M. FRIEDMANN-SPITTELER, Tetrahedron Letters, in press.
[7] S. MCLEAN, K. PALMER, and L. MARION, Can. J. Chem. *38*, 1547 (1960).
[8] See C. DJERASSI, *Optical Rotatory Dispersion* (McGraw-Hill Book Co., New York 1960).
[9] This biogenetically attractive structure has been suggested by G. F. SMITH and J. T. WROBEL, J. chem. Soc. *1960*, 1463. — H. KNY and B. WITKOP, J. org. Chem. *25*, 635 (1960).
[10] F. WALLS, O. COLLERA, and A. SANDOVAL, Tetrahedron *2*, 173 (1958).
[11] See also, P. N. EDWARDS and G. F. SMITH, Proc. chem. Soc. *1960*, 215.
[12] *Acknowledgement.* Financial support has been provided by grant H-5048 of the National Heart Institute, National Institutes of Health (U.S. Public Health Service), and by the Conselho Nacional de Pesquisas (Brazil). The postdoctorate research fellowship of B. GILBERT in Rio de Janeiro was financed from a grant of the Rockefeller Foundation in support of a collaborative research program between Stanford University and the Instituto de Quimica Agricola, Rio de Janeiro (Brazil).