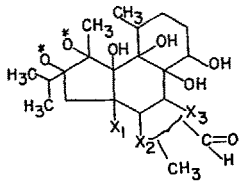
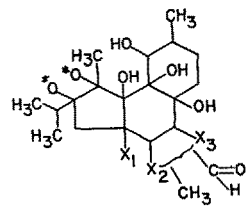
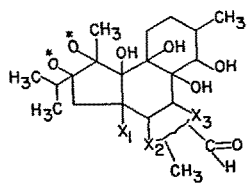


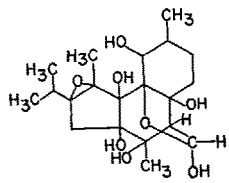
XVII



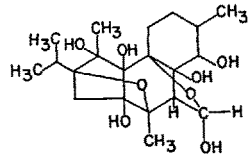
XVIII



XIX



XX



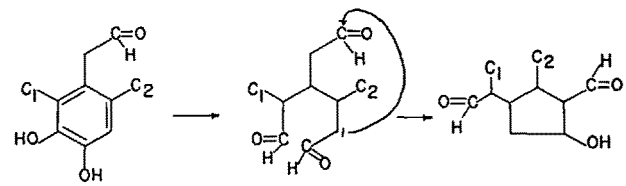
XXI

terpenoid, this argument would make XVIII *a priori* more likely. In our opinion this reasoning is not permissible since the terpenoid origin of the nepetalic acid skeleton is also uncertain.

Recently, THOMAS[5] postulated that the correspondence in structures between the nontryptamine portion of indole alkaloids and the various substances possessing the nepetalic acid skeleton may be explained by discarding the well-known theories of indole alkaloid biogenesis and assuming that the nontryptamine portion of the indole alkaloids is formed by cleavage of a monoterpene with the nepetalic acid skeleton.

We should like to point out that the published data can be explained by an alternative theory which is *a priori* completely equivalent to the THOMAS hypothesis: Compounds with the nepetalic acid skeleton can be biosynthesized by a route involving a Woodward fission of an alkylated phenylacetaldehyde and the resemblance of the product to a terpenoid can be purely coincidental. Such a possible biosynthesis is portrayed in the structures XXII, XXIII, and XXIV.

It is clear that the decision between these two possibilities will have to come from biochemical experiments. In the meantime, however, it does not seem permissible to use the isoprene rule for structural arguments in ryanodine chemistry[6].



XXII XXIII XXIV

Zusammenfassung. Anhydroryanodol besitzt eine den Strukturen I, II oder III entsprechende Laktonformel. Die Struktur des Ryanodols ist in der allgemeinen Formulierung XVII–XIX enthalten. Das Problem der Ryanodolbiogenese wird kurz diskutiert.

Z. VALENTA and K. WIESNER[7], in collaboration with D. R. BABIN, T. BÖGRI, T. P. FORREST, F. FRIED, and H. REINSHAGEN

Organic Chemistry Laboratory, University of New Brunswick (Canada), December 20, 1961.

[5] R. THOMAS, Tetrahedron Letters No. *16*, 544 (1961).

[6] *Acknowledgments.* This work was supported at various stages by the National Research Council, Ottawa, the Research Corporation, New York, the Ciba Company, Summit (New Jersey), the Schering Corporation Ltd., Montreal, and the Hoffmann-La Roche Inc., Nutley (New Jersey). The large amounts of powdered *Ryania speciosa* which were required were donated by S. B. Penick and Company, New York. We wish to thank Professor K. BIEMANN, Massachusetts Institute of Technology, Cambridge, for the mass spectroscopic molecular weight determination of the lactone IV.

[7] The contribution of all our collaborators will be discussed in several papers to be published in the near future.

Mass Spectrometry in Structural and Stereochemical Problems[1] Spegazzinine and Spegazzinidine[2]

A few years ago, we reported[3] the isolation of (−)-quebrachamine[4] and of a new phenolic dihydroindole alkaloid, spegazzinine, from *Aspidosperma chakensis* Spegazzini. With the amount then at our disposal, we were only able to characterize the functional groups and to demonstrate the partial structure I; no firm decision could be reached between the empirical formulae $C_{21}H_{28}N_2O_3$ and $C_{22}H_{30}N_2O_3$, although the former was preferred because of a presumed relationship to aspidospermine (II)[5]. When additional plant material became available, we resumed this investigation, but found that the new extract contained only small quantities of spegazzinine, the principal alkaloid being a new phenolic one, which we have named spegazzinidine. Using mass spectrometry and nuclear magnetic

[1] This paper represents part III. For preceding paper see B. GILBERT, J. FERREIRA, R. J. OWELLEN, C. E. SWANHOLM, H. BUDZIKIEWICZ, L. J. DURHAM, and C. DJERASSI, Tetrahedron Letters, *1962*, 59.

[2] This paper represents Part VI in the La Plata series *Estudios sobre Plantas*; for paper V see T. NAKANO, C. DJERASSI, R. A. CORRAL, and O. O. ORAZI, J. org. Chem. *26*, 1184 (1961).

[3] O. O. ORAZI, R. A. CORRAL, J. S. E. HOLKER, and C. DJERASSI, J. org. Chem. *21*, 979 (1956); Anales Asoc. Quim. Argentina *44*, 177 (1956).

[4] K. BIEMANN and G. SPITELLER, Tetrahedron Letters *1961*, 299.

[5] J. F. D. MILLS and S. C. NYBURG, J. chem. Soc. *1960*, 1458. See also G. F. SMITH and J. T. WROBEL, J. chem. Soc. *1960*, 1463, and H. CONROY, P. R. BROOK, and Y. AMIEL, Tetrahedron Letters No. 11, 4 (1959).

resonance (NMR) spectroscopy, it has now been possible to assign structures to these two alkaloids.

Spegazzinidine (III), m.p. 237–238°, $[\alpha]_D^{24}$ +186° (all rotations in $CHCl_3$), $\lambda_{max}^{CHCl_3}$ 2.85, 6.13 (amide) and 6.33 μ, λ_{max}^{EtOH} 225, 260 mμ (log ε 4.30, 3.89), λ_{min}^{EtOH} 246 mμ (3.78), possesses the empirical formula $C_{21}H_{28}N_2O_4$ (372) as demonstrated by the mass spectrometric molecular weight determination (found: 372) and the elementary analysis (found: C, 67.50, H, 7.64; N, 7.61; O, 17.29; methoxyl, 0.0). Its NMR spectrum[6] was very instructive, the following assigments being compatible with structure III, which is proposed below for spegazzinidine: 11.1 p.p.m. (hydrogen-bonded C-17 phenol), broad peak at 5.83 p.p.m. (C-16 phenol), 2.48 p.p.m. (N-acetyl). The two *ortho* aromatic hydrogens and the ethyl group attached to C-5 were found in the same locations as for pyrifolidine[7] (antipode of II with additional C-16 methoxy group), while the C-2 hydrogen exhibited a doublet at 4.03 p.p.m. (J = 8 c.p.s.) —in contrast to the quartet noted in that region in the spectra[7,8] of aspidospermine (II) or pyrifolidine. It follows that the C-2 hydrogen has only one neighbouring proton and this is confirmed in the NMR spectrum of the ketone V, where the C-2 hydrogen now exhibits a single, sharp signal at 5.11 p.p.m.

Treatment of spegazzinidine (III) with dimethyl sulfate in acetone solution (in the presence of potassium carbonate) provided the dimethyl ether IV, m.p. 167–169°, $[\alpha]_D$ −156°, λ_{max}^{EtOH} 222, 250 and 289 mμ (infl.), log ε 4.48, 3.85, 3.27 ($C_{23}H_{32}N_2O_4$ (400); found: mol. weight, 400 (mass spec.); C, 69.02; H, 8.26; methoxyl, 15.27). Its NMR spectrum was practically identical with that[7] of pyrifolidine, except for the doublet at 4.15 p.p.m. (J = 8 c.p.s.) due to the C-2 hydrogen with its single proton neighbour.

BIEMANN et al.[9] have shown that the mass spectra of aspidospermine (II) and its relatives are characterized by a M-28 peak, corresponding to the fragment *a* due to loss of ethylene (see arrows in II) and by a very intense peak at m/e 124 associated with the unsaturated piperidine fragment *c* arising from further cleavage of the 10–11 bond in *a*. The mass spectra of the spegazzinidine (III, IV) and spegazzinine (VII, VIII) derivatives measured by us exhibited this same intense peak at m/e 124. This suggests strongly that the two alkaloids are based on an aspidospermine (II) skeleton and furthermore proves that the alcoholic hydroxyl group cannot be situated in the portion of the molecule encompassed by fragment *c*. None of the mass spectra of our alkaloids show the loss of 28 mass units (carbon atoms 3 and 4 of II in form of $CH_2{=}CH_2$), but rather of 44 units (spegazzinidine dimethyl ether: m/e 356 in Figure 1, corresponding to *b*; spegazzinine methyl ether: m/e 326 in Figure 2, corresponding to *a*), due to the loss of the oxygenated two carbon bridge as $CH_2{=}CHOH$. When the substance is mixed with D_2O prior to insertion into the mass spectrometer, a M-45 peak is observed (loss of $CH_2{=}CHOD$).

The location of the alcoholic hydroxyl group at C-3—as suggested by the NMR and mass spectra—was proved by JONES oxidation[10] of spegazzinidine dimethyl ether (IV) to the ketone V, m.p. 185–187°, $[\alpha]_D$ −53°, $\lambda_{max}^{CHCl_3}$ 5.84 (six-membered ketone), 6.08 (amide) and 6.2 μ ($C_{23}H_{30}N_2O_4$ (398)); found: mol. weight (mass spec.), 398; C, 69.21; H, 7.52), which exchanged six hydrogen atoms for deuterium upon repeated treatment with NaOD in D_2O-CH_3OD. The extent of deuterium exchange and the location of the deuterium atoms was settled by mass spectrometry. The molecular ion of V at m/e 398 was shifted to m/e 404 in VI and the presence of three deuterium atoms in the N-

[6] Measured on Varian HR-60 or AR-60 spectrometers in $CDCl_3$ solution using tetramethylsilane as internal standard. All signals are reported in p.p.m. as δ units (see C. DJERASSI, T. NAKANO, A. N. JAMES, L. H. ZALKOW, E. J. EISENBRAUN, and J. N. SHOOLERY, J. org. Chem. *26*, 1192 (1961)). We are indebted to Dr. J. N. SHOOLERY (Varian Associates) and Dr. L. J. DURHAM (Stanford University) for these measurements.

[7] C. DJERASSI, B. GILBERT, J. N. SHOOLERY, L. F. JOHNSON, and K. BIEMANN, Exper. *17*, 162 (1961).

[8] C. DJERASSI, A. A. P. G. ARCHER, T. GEORGE, B. GILBERT, J. N. SHOOLERY, and L. F. JOHNSON, Exper. *16*, 532 (1960).

[9] K. BIEMANN, M. FRIEDMANN-SPITELLER, and G. SPITELLER, Tetrahedron Letters *1961*, 485.

[10] K. BOWDEN, I. M. HEILBRON, E. R. H. JONES, and B. C. L. WEEDON, J. chem. Soc. *1946*, 39.

Fig. 2

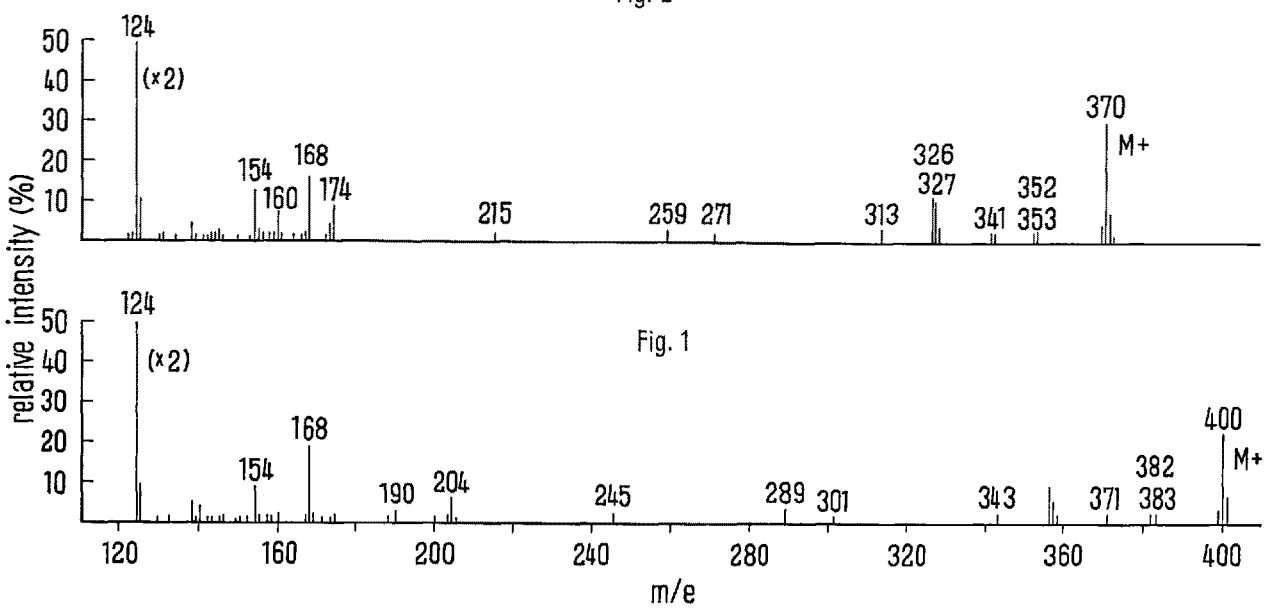
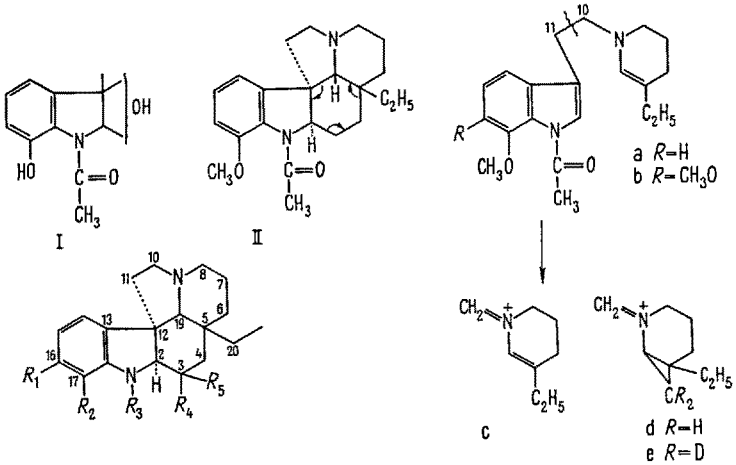


Fig. 1

Fig. 1. Mass spectrum of spegazzinidine dimethyl ether (IV). Fig. 2. Mass spectrum of spegazzinine methyl ether (VIII).

acetyl group was shown by the fact that the m/e 43 peak (CH_3CO^+) was shifted to m/e 46 (CD_3CO^+). The remaining three deuterium atoms must then be present in α-positions to the carbonyl group and such a feature is only possible if the carbonyl function (and hence the hydroxyl substituent in the original alkaloid) is at C-3. Attachment at C-20, though satisfying the deuterium exchange experiment, is excluded by the NMR and mass spectral data.



	R_1	R_2	R_3	R_4	R_5
III	HO	HO	CH_3CO	H	HO
IV	CH_3O	CH_3O	CH_3CO	H	HO
V	CH_3O	CH_3O	CH_3CO	=O	
VI	CH_3O	CH_3O	CD_3CO	=O	(2, 4, 4-d_3)
VII	H	HO	CH_3CO	H	HO
VIII	H	CH_3O	CH_3CO	H	HO
IX	CH_3O	CH_3O	C_2H_5	H	H

The mass spectra of V and VI are extremely interesting, since they demonstrate that a fragmentation process—different from that usually found[9] in aspidospermine (II)-type alkaloids—can occur. The strongest peak in the mass spectrum of the ketone V now occurs at m/e 138 (the 124 peak corresponding to *c* being absent) and is shifted by *two* units to m/e 140 in the deuterated analog VI. The mechanistic implication will be discussed in our detailed paper, but we ascribe the m/e 138 peak to species *d*, and the m/e 140 peak to *e*, both resulting from rupture of the 2–3, 3–4, 10–11, and 12–19 bonds with expulsion of carbon monoxide.

The above mass spectrometric, NMR and chemical data are most compatible with structure III for spegazzinidine[11]. The originally isolated alkaloid spegazzinine[3] can now be assigned structure VII on the following grounds. The NMR spectrum of spegazzinine (VII) closely resembles that of spegazzinidine (III) except for the absence of a signal corresponding to the non-hydrogen-bonded C-16 phenolic group of III and the presence of signals corresponding to *three* aromatic protons. Most importantly, the mass spectrum (Figure 2) of spegazzinine methyl ether (VIII) is virtually identical with that (Figure 1) of spegazzinidine dimethyl ether (IV) except for a 30 mass unit shift (corresponding to the extra methoxyl group of IV) in those peaks (e.g. m/e 400, 383, 382, 371, 356, 343, 301, 289, 245, 204, 190) of IV, in which the aromatic portion of the molecule is still retained. A similar relationship was also observed in the mass spectra of the two parent alkaloids III and VII; as noted earlier[1,4,7,9], this can be considered virtual proof that the two alkaloids possess identical structures and differ only by one substituent in the aromatic ring[12].

Zusammenfassung. Auf Grund von Protonresonanz und massenspektrometrischen Messungen werden die Strukturen III und VII für die Aspidosperma-Alkaloide Spegazzinidin und Spegazzinin vorgeschlagen.

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[11] *Note added in proof.* Chemical verification has now been provided by $LiAlH_4$ reduction of the tosylate of IV which provided the antipode IX ($[\alpha]_D$ −20.6°) of N-ethyldeacetylpyrifolidine ($[\alpha]_D$ + 19.8°), obtained in turn by $LiAlH_4$ reduction of pyrifolidine[7].

[12] *Acknowledgment.* We are indebted to Prof. J. F. Molfino for help with the botanical collection and to Mr. E. Meier and Mr. J. Consul for the microanalyses. The work at Stanford University was supported by the National Heart Institute (Grant No. 2G-682) and the National Institute of Arthritis and Metabolic Diseases (Grant No. A-4257) of the National Institutes of Health, U.S. Public Health Service.

The Reaction Mechanism of Catalase

In his recent review on enzyme models[1], Westheimer has proposed the following scheme for the mechanism of decomposition of hydrogen peroxide by catalase.

$$P\text{–}Fe^{+++} + H_2O_2 \longrightarrow P\text{–}FeO^{+++} + H_2O \quad (1)$$

$$P\text{–}FeO^{+++} + le \longrightarrow P\text{–}FeO^{++} \quad (2)$$

$$P\text{–}FeO^{++} + H_2O_2 \longrightarrow P\text{–}FeO_2^{++} + H_2O \quad (3)$$

$$P\text{–}FeO_2^{++} \longrightarrow P\text{–}Fe^{++} + O_2 \quad (4)$$

$$P\text{–}Fe^{++} + H_2O_2 \longrightarrow P\text{–}FeO^{++} + H_2O \quad (5)$$

[1] F. H. Westheimer, *Enzyme Models. The Enzymes*, 2nd Ed. (Edited by P. D. Boyer, H. Lardy, and K. Myrbäck, Academic Press Inc., New York 1959).