

appears to be a combination of indole and 2-acylindole chromophores: λ_{\max} ($m\mu$) 291 (ϵ 7230), 282 (8400), 274 (8120), and 220 (39,100), λ_{sh} 310 $m\mu$ (ϵ 2020). The infrared spectrum (chloroform solution) shows carbonyl absorption at 1640 cm^{-1} and the n.m.r. spectrum exhibits a sharp signal at τ 8.06 ascribed to the N-methyl group. Dihydroburnamicine has a pK_a of 9.02 (50% methanol-water) which is very close to that reported for burnamicine.²

The mass spectrum of dihydroburnamicine⁸ provides further confirmation for the proposed structure. The parent mass peak at $m/e = 328$ was also the most intense line in the spectrum. The anticipated fragmentations along lines a, b, and c are those observed for burnamicine.² The expected fission along a gave medium intensity peaks at $m/e = 128$, 129, and 130 from the indole fragment. A medium intensity peak at $m/e = 170$ is considered to arise by cleavage along b and by loss of carbon monoxide (mass 28) from the fragment of mass 198 formed from fission along a. The peak at $m/e = 170$ thus corresponds to the strong peak at $m/e = 168$ in the mass spectrum of burnamicine. The second strongest peak in the spectrum, $m/e = 143$, and a peak at $m/e = 144$ correspond to the intense peaks at the same m/e in the mass spectrum of burnamicine and could have arisen as previously suggested for burnamicine. The mass spectrum of dihydroburnamicine also shows a peak at $m/e = 310$ which corresponds to the peak at $m/e = 308$ in the mass spectrum of burnamicine resulting from the loss of the elements of water in both cases.² The synthesis of other indole alkaloids from compound II is under investigation.⁹

(8) We are indebted to Dr. Taylor for the mass spectrum and his helpful interpretation.

(9) Satisfactory analytical data were obtained for all new compounds described in this communication.

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RECEIVED SEPTEMBER 14, 1964

Viomycin. I. The Structure of Viomycin

Sir:

On complete acid hydrolysis, the tuberculostatic *streptomyces* antibiotic viomycin¹ yields carbon dioxide, ammonia, urea L-serine, L- α,β -diaminopropionic acid,^{1,2} L- β -lysine,^{3,4} and a basic amino acid^{1,2} which we have named viomycinine (I). Ion-exchange separation of viomycin hydrolysate followed by charcoal chromatography of the fraction containing strongly basic amino acids gave I as a crystalline hydrochloride, m.p. 200–208° dec., $[\alpha]^{30D} -78^\circ$ (c 1.78, water). *Anal.* Calcd. for $C_6H_{10}O_2N_4 \cdot HCl$: C, 35.00; H, 5.36; O, 15.48; N, 27.65; Cl, 17.18. Found: C, 35.07; H, 5.31; O, 14.20; N, 27.49; Cl, 16.97. C-Methyl, O-methyl, N-methyl, and primary amino groups were shown to be absent. Viomycinine had pK_a values of 2.8, 5.87, and 13.4 (in 66% dimethylform-

amide) and 1.3 (estimated), 5.50, and 12.6 (in water)⁵; it gave positive Sakaguchi, Weber, and ninhydrin tests and negative Tollens, Benedict, and Benedict-Behre tests. Viomycinine hydrochloride showed strong infrared absorption at (μ) 2.98, 3.18, 3.50, 5.91, 6.06, 6.87, and 7.10, among others; it displayed only end absorption in the ultraviolet region. The n.m.r. spectrum of I-hydrochloride in deuterium oxide solution showed five nonexchangeable protons present. Absorptions were present at τ 4.37 (1H, triplet, $J = 2.4$ c.p.s.), 5.38 (2H, multiplet), and 7.43 (2H, triplet, $J = 1.9$ c.p.s.).

Guanidine was detected as a product of nitric acid oxidation of I; the strongly basic group present in I was assigned to a monosubstituted guanidine function because of the strongly positive Sakaguchi reaction. The nonguanidine nitrogen of I was characterized as an imine due to (i) its weakly basic nature, (ii) its differential ultraviolet spectrum (λ_{\max} 212 $m\mu$ (ϵ 2530), pH 9.80 vs. 3.82),⁶ characteristic of tertiary amines,⁶ and (iii) a positive *o*-aminobenzaldehyde test.^{7,8} I consumed 1 mole of hydrogen (platinum-acetic acid). Thus viomycinine contains a strongly acidic carboxyl group, a monosubstituted guanidine function, one reducible double bond, and, therefore, one ring. Because I is stable to vigorous acid hydrolysis, three- and four-membered rings containing nitrogen are excluded and I must contain a Δ^1 -pyrroline ring.

Hydrolysis of I by hot barium hydroxide solution furnished nearly 3 moles of ammonia¹⁰; no other volatile base was formed. From the hydrolysate pyrrole-2-carboxylic acid¹¹ was isolated in 21% yield. Sodium hydroxide fusion of I gave 2-aminopyrimidine,^{11,12} pyrrole-2-carboxylic acid,¹¹ and glycine^{11,12}; no volatile base other than ammonia was formed. The products of alkaline hydrolysis of viomycinine place the carboxyl group at an α -position of the Δ^1 -pyrroline nucleus.

The n.m.r. spectra of viomycinine and related compounds¹³ indicate the absence of an olefinic-type proton in I.

(5) We are grateful to Dr. Harold Boaz, Eli Lilly and Co., for obtaining these data.

(6) G. W. Stevenson and D. Williamson, *J. Am. Chem. Soc.*, **80**, 5943 (1958).

(7) W. B. Jakoby and J. Fredericks, *J. Biol. Chem.*, **234**, 2145 (1959).

(8) In this test, I showed λ_{\max} 301 $m\mu$. Δ^1 -Pyrroline showed λ_{\max} 288 $m\mu$; 2-methyl- Δ^1 -pyrroline-5-carboxylic acid⁹ showed λ_{\max} 296 $m\mu$. Viomycinine and all imines and Δ^1 -pyrrolines tested showed broad shoulders of low extinction in the region 420–480 $m\mu$, responsible for the yellow color of the positive test.

(9) We are grateful to Dr. H. Gershon, Pfister Chemical Works, Inc., for a sample of this compound.

(10) Under these conditions arginine gives only 2 moles of ammonia.

(11) Identified by comparison with an authentic sample, melting point, mixture melting point, infrared, ultraviolet, and n.m.r. spectra, color reactions, and paper chromatographic behavior. The transformation of I into this substance and the structural inferences derived therefrom will be fully developed in the complete paper.

(12) The formation of 2-aminopyrimidine and glycine on base fusion is considered to proceed through a derivative of β -guanidopropionaldehyde, derivable from I by isomerization, ring opening, and cyclization.

(13) The n.m.r. spectrum of I in trifluoroacetic acid showed absorptions at τ 4.07 (1H), 5.05 (2H), and 7.23 (2H); guanidinium absorptions were present from τ 1.5 to 3.0. The n.m.r. spectrum of Δ^1 -pyrroline in trifluoroacetic acid solution showed absorptions at τ 1.20 (position 2, 1H), 6.75 (position 3, 2H), 7.58 (position 4, 2H, quintet, $J = 4.0$ c.p.s.), and 5.73 (position 5, 2H); no absorption was present that might be assigned to the protonated imine. The olefinic proton of isobutylideneethylamine absorbs at τ 2.47 (neat liquid) and at 1.66 (trifluoroacetic acid solution); the olefinic proton of isobutylideneazirine absorbs at τ 2.32 (neat liquid); the olefinic proton of 5,5-dimethyl- Δ^1 -pyrroline absorbs at τ 3.1 (neat liquid).¹⁴

(14) R. Bonnett and D. E. McGreer, *Can. J. Chem.*, **40**, 177 (1962).

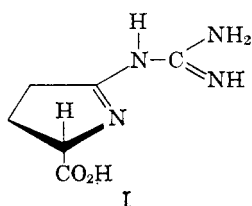
(1) T. H. Haskell, S. A. Fusari, R. P. Frohardt, and Q. R. Bartz, *J. Am. Chem. Soc.*, **74**, 599 (1952).

(2) L. H. Mason, Ph.D. Thesis, University of Illinois, 1953.

(3) H. E. Carter, W. R. Hearn, E. M. Lansford, Jr., A. C. Page, Jr., N. P. Salzman, D. Shapiro, and W. R. Taylor, *J. Am. Chem. Soc.*, **74**, 3704 (1952).

(4) E. E. van Tamelen and E. E. Smissman, *ibid.*, **74**, 3713 (1952).

Viomycin was most conveniently characterized by a highly crystalline N-2,4-dinitrophenyl derivative, m.p. 171.5–172.5°. *Anal.* Calcd. for $C_{12}H_{12}O_6N_8 \cdot 2H_2O$: C, 38.71; H, 4.33; N, 22.58. Found: C, 38.71; H, 4.57; N, 23.03. On acetylation using aqueous acetic anhydride, I was converted into a crystalline N-acetyl derivative, m.p. 256–257°, $[\alpha]^{25}_D$ 41.5° (*c* 2.4, water). *Anal.* Calcd. for $C_8H_{12}O_3N_4$: C, 45.28; H, 5.70; N, 26.43. Found: C, 45.41; H, 5.91; N, 26.59. The derivative gave positive Weber and Sakaguchi tests but negative ninhydrin and *o*-aminobenzaldehyde tests; it showed only end absorption in the ultraviolet region and had pK_a values of 4.86 and 13.0 (in 66% dimethylformamide).⁵ Acid hydrolysis of the acetyl derivative converted it into viomycin as the only observable product. These data suggest an N-acetyl- Δ^2 -pyrroline structure¹⁵ for acetylviomycin. The positions of the double bond and the guanidine group of viomycin were determined by a study of the ozonolysis products of acetylviomycin. When acetylviomycin was subjected to ozonolysis, oxidative work-up, and acid hydrolysis, guanidine and aspartic acid¹⁶ were produced in good yield and as the only observable products. Thus acetylviomycin is 1-acetyl-2-guanido- Δ^2 -pyrroline-5-carboxylic acid and viomycin (I) is 2-guanido- Δ^1 -pyrroline-5-carboxylic acid.^{18–20} Because viomycin is more dextrorotatory in acid ($M_D -10.3^\circ$) than in



water ($M_D -37.6^\circ$), application of the Clough-Lutz-Jirgensons rule²¹ suggests the L (or *R*) configuration for the asymmetric center present.

Acknowledgment.—This work was supported by Research Grant E-2007 from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service. We are grateful to Dr. L. M. Long, Parke, Davis and Co., for supplies of viomycin.

(15) Δ^1 -Pyrrolines give, on acetylation, either N-acetyl- Δ^2 -pyrrolines, ring-opened N-acyl carbonyl compounds, or a mixture of the two derivatives. See, for example, F. C. Uhle and F. Sallman, *J. Am. Chem. Soc.*, **82**, 1190 (1960), and P. J. A. Demoen, P. A. J. Janssen, and J. L. M. Loomans, *ibid.*, **81**, 6286 (1959).

(16) The procedure used was similar to that of Zbiral,¹⁷ who in this way obtained aspartic acid from Δ^1 -pyrroline-5-carboxylic acid. The aspartic acid isolated was racemic, racemization apparently having occurred during treatment of I with hot pyridine-acetic anhydride.

(17) E. Zbiral, *Monatsh. Chem.*, **94**, 639 (1963).

(18) We thank Dr. E. F. Ullman for suggesting that structures similar to I would be stable to hydrolysis, and Dr. Jack Hine for valuable discussions.

(19) To our knowledge, viomycin is the first stable compound containing an α,β -unsaturated guanidine unit. This formulation is also suggested by the differential ultraviolet spectrum shown by the guanidine group of viomycin (pH 9.0 vs. pH 13.1, λ_{max} 222 $m\mu$ (ϵ 1690)).⁵ Saturated alkyl-guanidines do not display differential ultraviolet spectra.

(20) The n.m.r. absorptions of the protons of viomycin in deuterium oxide solution are assigned as follows: C-3(2H), τ 5.38; C-4(2H) 7.43, C-5(1H) 4.37.

(21) J. P. Greenstein, *Advan. Protein Chem.*, **9**, 121 (1954).

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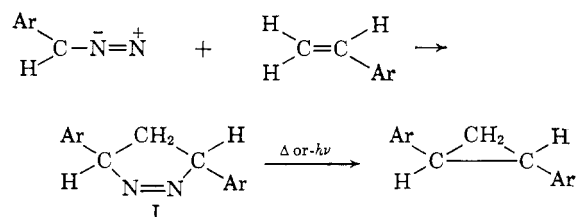
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RECEIVED OCTOBER 16, 1964

cis-3,5-Bis(*p*-Methoxyphenyl)-1-pyrazoline. A *cis-trans* Isomer Pair of Cyclic Azo Compounds

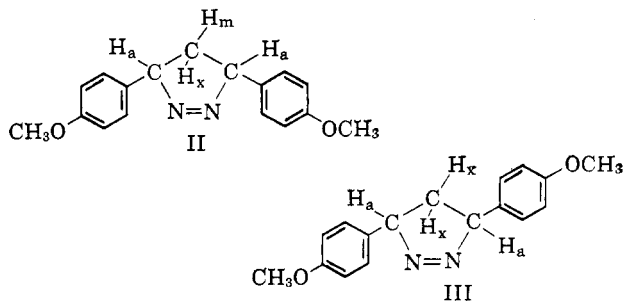
Sir:

Previous communications in this series^{1–3} had described the synthesis of *trans*-3,5-diaryl-1-pyrazolines (I) via the presumed stereospecific 1,3-dipolar addition⁴ of aryl diazoalkanes to the corresponding styrenes. Evidence for their *trans*-configuration² and their stereospecific thermal decomposition^{1,3} to the corresponding *trans*-1,2-diarylcyclopropanes was also presented.



However, since the corresponding *cis*-1-pyrazolines were not available, no comparative study of their chemical behavior with that of the *trans* isomers could be made. We would like to report, for the first time, the isolation of a *cis*-3,5-diaryl-1-pyrazoline of type I.

As an extension of the 1-pyrazoline synthesis from aryl diazoalkanes and styrenes,³ the reaction of *p*-methoxyphenyldiazomethane with *p*-methoxystyrene was investigated. A 36% yield of what proved to be a mixture of the *cis* and *trans* isomers of 3,5-bis(*p*-methoxyphenyl)-1-pyrazoline was obtained. The *cis-trans* ratio was estimated to be 55:45 by n.m.r. spectral analysis. By careful fractional crystallization, each isomer was separated in 95% minimum purity.



The expected *trans*-3,5-bis(*p*-methoxyphenyl)-1-pyrazoline (III) crystallized as off-white plates (from methanol), m.p. 129° dec., λ_{max}^{EtOH} 332 $m\mu$ (ϵ_{max} 533); the $-N=N-$ bond appeared as a weak absorption at 1555 cm^{-1} . The n.m.r. spectrum consisted of a quartet at τ 2.98 (aromatic protons), a triplet at 4.25 (benzylic protons), a singlet at 6.26 (methoxy protons), and a triplet at 7.95 (methylene protons). This perfect agreement with the spectral data of the other *trans*-3,5-diaryl-1-pyrazolines² leaves no doubt as to the *trans* configuration of the 3,5-substituents of this isomer.

cis-3,5-Bis(*p*-methoxyphenyl)-1-pyrazoline (II) was isolated as silvery plates (from methanol), m.p. 114° dec., λ_{max}^{EtOH} 329 $m\mu$ (ϵ_{max} 329); its infrared spectrum had a weak band at 1545 cm^{-1} assigned to the azo linkage. The n.m.r. spectrum was more complicated

(1) C. G. Overberger and J.-P. Anselme, *J. Am. Chem. Soc.*, **84**, 869 (1962).

(2) C. G. Overberger, J.-P. Anselme, and J. R. Hall, *ibid.*, **85**, 2752 (1963).

(3) C. G. Overberger and J.-P. Anselme, *ibid.*, **86**, 658 (1964).

(4) R. Huisgen, *Angew. Chem.*, **75**, 604, 741 (1963).