

hydroquinone, prepared by reduction of the quinone in a Jones reductor, and the quinone at two different  $QH_2/Q$  ratios. The  $E_0$  value for the reaction, measured against a calomel electrode, was  $-0.40v.$ ;  $\lambda_{max}^{EtOH}$  253 (11000), 330  $m\mu$  (3000);  $pK_A$  9.2 (50% methanol).

*Anal.* Calcd. for  $C_{12}H_9O_3N$  (215.2): C, 66.97; H, 4.22; N, 6.51. Found: C, 66.96; H, 4.26; N, 6.56.

The azaquinone was also obtained by nitrosation of the dihydroxypyridine XIII. A 10-mg. sample of XIII dissolved in 0.5 ml. of 20% sulfuric acid was treated with 20 mg. of sodium nitrite and the solution was allowed to stand for 2 days at room temperature, during which large tan prisms separated. This material, 5.5 mg., had m.p. and mixed m.p. with the above preparation  $159-160^\circ$ .

**3-Methyl-4-phenylmaleic Anhydride (XXI).**—A solution of 250 mg. of the nitroso derivative XVI in 7 ml. of 40% sulfuric acid was refluxed for one hour, during which long colorless crystals began to form in the condenser. This material was then isolated by steam distillation, water being added until no further solid distilled. The product was extracted from the distillate with ether, and the ether solution after drying and evaporation furnished 75 mg. of colorless needles, m.p.  $95-96^\circ$ . The material was sublimed for analysis.

*Anal.* Calcd. for  $C_{11}H_9O_3$  (188.2): C, 70.21; H, 4.29. Found: C, 69.94; H, 4.35; N, trace ( $<0.2\%$ ).

The infrared spectrum of this material was superimposable on that of a sample (m.p.  $95^\circ$ ) prepared previously in 20%

yield by the reaction of 3-methyl-4-phenyl succinic anhydride with N-bromosuccinimide followed by attempted distillation of the bromination product. The latter preparation was also found to steam distil without hydrolysis.

Hydrolysis of the quinone XVIII-XIX with 40% sulfuric acid for one hour followed by steam distillation furnished the anhydride XXI in 65% yield.

**2-Hydroxy-4-methyl-3-phenylpyrido[2,3-b]quinoxaline (XXIII).**—A solution of 21 mg. of the quinone XVIII-XIX and 10.2 mg. of *o*-phenylenediamine in 2 ml. of acetic acid was warmed on the water-bath for one hour. After removal of the acetic acid *in vacuo*, addition of ethanol furnished 22 mg. of yellow prisms. Recrystallization from acetic acid gave pale yellow needles, m.p.  $275^\circ$ .

*Anal.* Calcd. for  $C_{18}H_{13}ON_3$  (287.3): C, 75.24; H, 4.56; N, 14.63. Found: C, 75.12; H, 4.73; N, 14.32.

**2,3,6-Trihydroxy-4-methyl-5-phenylpyridine Triacetate (XXII).**—A solution of 50 mg. of the quinone XVIII-XIX in 3 ml. of acetic anhydride was heated with 500 mg. of zinc dust at  $75^\circ$  for one hour. After filtration of the zinc and evaporation *in vacuo*, the residue was dissolved in ether, and after filtration, the ether solution deposited 55 mg. of colorless prisms, m.p.  $105-107^\circ$ . Recrystallization from ether-hexane gave prisms, m.p.  $106-107^\circ$ .

*Anal.* Calcd. for  $C_{18}H_{17}O_6N$  (343.2): C, 62.97; H, 4.99; N, 4.08. Found: C, 63.50; H, 5.41; N, 4.06.

NEWARK, DEL.

[CONTRIBUTION FROM THE RESEARCH DIVISION, ARMOUR AND COMPANY]

### 3-Diazocitrazinic Acid, A New Antimetabolite of Orotic Acid<sup>1</sup>

BY ZINON B. PAPANASTASSIOU, ARMAND McMILLAN, VIRGINIA J. CZEBOTAR AND THOMAS J. BARDOS

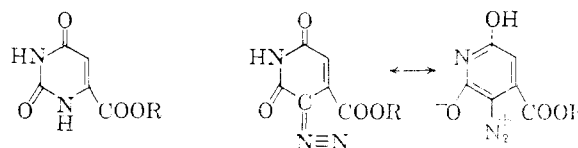
RECEIVED MAY 25, 1959

A new variation of "structural analogy" was devised and biologically tested. Replacement of a nitrogen atom in the pyrimidine ring of orotic acid by the carbon atom of a diazomethine radical leads to the structure of 3-diazocitrazinic acid. This compound, and its esters, were synthesized, and they were found biologically active as competitive antagonists of orotic acid. In the course of this work, a new diazotization technique was developed which permits the diazotization of particularly unstable (as well as only weakly basic) amino compounds in satisfactory yields.

The anti-tumor action of azaserine<sup>2</sup> has been linked to its ability to inhibit a specific step of nucleic acid biosynthesis which is dependent on glutamine as the donor of two purine-ring nitrogens.<sup>3</sup> The metabolite-antimetabolite relationship between glutamine and azaserine, as well as the similar but even more potent inhibitory activity of 6-diazo-5-oxo-L-norleucine ("DON"),<sup>4</sup> seems to be related to the structural analogy of the two inhibitors to glutamine; in both azaserine and "DON," the "nitrogen donating"  $-\text{CONH}_2$  (carboxamide) group of glutamine is replaced by a  $-\text{COCHN}_2$  (diazoacetyl) group.

A similar structural relationship exists between orotic acid (XI), a precursor of nucleic acid pyrimidines, and 3-diazocitrazinic acid (VIII), one of the new compounds described in this paper. Here, the "reactive" carboxamide  $-\text{CON(H)}-$  portion of the

orotic acid ring structure (*i.e.*, the nitrogen which would participate in the enzymatic reaction with 5-phosphoribosyl pyrophosphate to form the nucleotide<sup>5</sup>) is, in 3-diazocitrazinic acid, replaced by a  $-\text{COC(N}_2)-$  group, thus changing the pyrimidine ring into a pyridine



XI, R = H  
XII, R =  $\text{CH}_3$   
XIII, R =  $\text{C}_2\text{H}_5$

VIII, R = H  
IX, R =  $\text{CH}_3$   
X, R =  $\text{C}_2\text{H}_5$

Compound XI is a required growth factor for *Lactobacillus bulgaricus*<sup>6</sup>; as such, it can be replaced by its esters XII and XIII.<sup>7</sup> Compound VIII and its esters IX and X inhibit the growth of this organism; half maximal inhibition is obtained at 250  $\mu\text{g.}$  of VIII, at 100  $\mu\text{g.}$  of IX or at 400  $\mu\text{g.}$  of X, per 5-ml. assay tube (Fig. 1).

The action of these inhibitors can be reversed "competitively" with XI, as shown in Fig. 2. The

(5) I. Crawford, A. Kornberg and E. S. Simms, *J. Biol. Chem.*, **226**, 1093 (1957).

(6) L. D. Wright, J. W. Huft, H. R. Skeggs, K. A. Valentik and D. K. Bosshardt, *THIS JOURNAL*, **72**, 2312 (1950).

(7) J. W. Laakso, *Diss. Absts.*, **17**, 965 (1957).

(1) The work described in this paper was presented before the Division of Medicinal Chemistry, at the 135th Meeting of the American Chemical Society, Boston, Mass., April, 1959.

(2) C. C. Stock, H. C. Reilly, S. M. Buckley, D. A. Clarke and C. P. Rhoads, *Nature*, **173**, 71 (1954).

(3) B. Levenberg and J. M. Buchanan, *THIS JOURNAL*, **78**, 504 (1956).

(4) D. A. Clarke, H. C. Reilly and C. C. Stock, Abstracts of Papers presented at 129th Meeting of the American Chemical Society, Dallas, Texas, April, 1956, p. 12M.; J. M. Buchanan, J. G. Flaks, S. C. Hartman, B. Levenberg, L. N. Lukens and L. Waren, in "Ciba Foundation Symposium on the Chemistry and Biology of Purines," J. and A. Churchill, Ltd., London, 1957, p. 250.



ethyl acetate was hydrogenated at 30 p.s.i. initial hydrogen pressure. The color of the suspension changed from red to yellow when the theoretical amount of hydrogen was consumed. The catalyst was removed by filtration<sup>10</sup> and the filtrate washed with (a) three 20-ml. portions of 0.5 *N* hydrochloric acid, (b) three portions of water and (c) two portions of saturated sodium chloride solution, and then dried over Drierite. The solution was concentrated under reduced pressure and the solid residue recrystallized from 80 ml. of ethyl acetate. A yellow crystalline powder was obtained weighing 1.3 g. (57%) and melting at 192–193°.

*Anal.* Calcd. for  $C_7H_8N_2O_4$ : C, 45.66; H, 4.38; N, 15.21. Found: C, 45.88; H, 4.88; N, 15.41.

**Ethyl 3-Amino-2,6-dihydroxy-4-pyridinecarboxylate (Ethyl 3-Aminocitrazinate, VII).**—The ethyl ester VII was prepared from IV by the same method described for VI; yield ca. 45%, m.p. 206–208°.

*Anal.* Calcd. for  $C_8H_{10}N_2O_4$ : C, 48.48; H, 5.09; N, 14.14. Found: C, 48.73; H, 5.19; N, 14.15.

**3-Diazo-2-oxy-6-hydroxy-4-pyridinecarboxylic Acid (3-Diazocitrazinic Acid, VIII).**—A solution of 25 ml. of iso-amyl nitrite in 35 ml. of chloroform was added during ca. 1 hour to a well-stirred suspension of V (7.18 g., 0.042 mole), in 30 ml. of trifluoroacetic acid and 100 ml. of chloroform, cooled at 0°. After the addition was complete, the mixture was stirred in the cold for an additional 2 hours; then it was concentrated under reduced pressure to about half its original volume, and 100 ml. of ethyl ether was added. The yellow powder was separated by filtration, washed with ethyl ether, and dried under vacuum; yield 7.25 g. (92%), decomposes without melting at 250°; absorption in the ultraviolet:  $\lambda_{max}^{water}$  330  $m\mu$  ( $\epsilon$  14.1  $\times 10^3$ );  $\lambda_{max}^{0.1N HCl}$  335  $m\mu$  ( $\epsilon$  14.6  $\times 10^3$ );  $\lambda_{max}^{0.1N NaOH}$  342  $m\mu$  ( $\epsilon$  12.3  $\times 10^3$ );  $\lambda_{max}^{CH_3COCH_3}$  333  $m\mu$

( $\epsilon$  14.0  $\times 10^3$ ); characteristic infrared absorption band:  $\lambda_{max}$  at 4.7  $\mu$  (diazo group).

*Anal.* Calcd. for  $C_6H_5O_4$ : C, 39.78; H, 1.67; N, 23.20. Found: C, 39.40; H, 1.97; N, 23.08.

**Methyl 3-Diazo-2-oxy-6-hydroxy-4-pyridinecarboxylate (Methyl 3-Diazocitrazinate, IX).**—A solution of 35 ml. of iso-amyl nitrite in 100 ml. of ethyl acetate was added during ca. 90 minutes to a well-stirred solution of VI (5.5 g., 0.0295 mole), in 22 ml. of trifluoroacetic acid and 550 ml. of ethyl acetate, at 0°. The reaction mixture was stirred for an additional 30 minutes in the cold, and was then concentrated to ca. 75 ml. under vacuum. The precipitate formed was separated by filtration and washed with liberal amounts of ethyl ether. After being dried under high vacuum, the yellow crystalline precipitate weighed 4.2 g. (74%) and melted at 172–174° dec. An additional quantity of product, ca. 1.0 g., m.p. 170° dec., can be obtained by diluting the filtrate with 100 ml. of ethyl ether and 100 ml. of petroleum ether, with cooling; absorption in the ultraviolet:  $\lambda_{max}^{water}$  337  $m\mu$  ( $\epsilon$  13.4  $\times 10^3$ );  $\lambda_{max}^{0.1N HCl}$  335  $m\mu$  ( $\epsilon$  9.45  $\times 10^3$ );  $\lambda_{max}^{0.1N NaOH}$  343  $m\mu$  ( $\epsilon$  13.2  $\times 10^3$ );  $\lambda_{max}^{CH_3COCH_3}$  335  $m\mu$  ( $\epsilon$  13.0  $\times 10^3$ ); characteristic infrared absorption band:  $\lambda_{max}$  at 4.7  $\mu$  (diazo group).

*Anal.* Calcd. for  $C_7H_8N_2O_4$ : C, 43.08; H, 2.58; N, 21.53. Found: C, 43.45; H, 2.88; N, 21.92.

**Ethyl 3-Diazo-2-oxy-6-hydroxy-4-pyridinecarboxylate (Ethyl 3-Diazocitrazinate, X).**—The ethyl ester X, was prepared from VII by the general method used for IX; yield 85%, m.p. 162–164° dec. The ultraviolet and infrared spectra were similar to those of IX.

*Anal.* Calcd. for  $C_8H_{10}N_2O_4$ : C, 45.94; H, 3.37; N, 20.09. Found: C, 45.99; H, 3.62; N, 20.69.

CHICAGO 9, ILL.

[CONTRIBUTION NO. 1551 FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

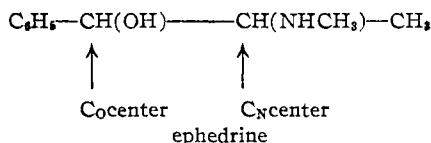
## Nuclear Magnetic Resonance Spectra and Configuration. The N.m.r. Spectra of Diastereoisomeric Heterocyclic Derivatives of the Ephedrine

By J. B. HYNE<sup>1</sup>

RECEIVED MAY 7, 1959

The n.m.r. spectra of derivatives of (–)-ephedrine and (+)- $\psi$ -ephedrine synthesized by ring closure across the hydroxyl and amino functions of the amino-alcohols are analyzed in terms of the differing configurations in the molecules. The influence of the magnetic anisotropy of the phenyl group in the derivatives studied is the key factor which enables detailed interpretation of the spectra in terms of the relative spatial distribution of the various groups in the molecules.

**Introduction.**—The primary purpose of this work was to investigate the effect of configurational differences on the n.m.r. spectra of diastereoisomeric compounds. The ephedrine were chosen for this study since the configuration of the two asymmetric centers in the various isomers has been well established by previous work. It has been shown<sup>2a</sup> by conversion of both (–)-ephedrine and (+)- $\psi$ -ephedrine to (+)-deoxyephedrine that these



diastereoisomers differ in configuration at the  $C_{\alpha}$  center and that the other asymmetric center in both forms has the (+)-alanine configuration.<sup>2b</sup> In order to establish a fixed spatial disposition of the groups on the two asymmetric centers, several het-

erocyclic derivatives of the two ephedrine were prepared by bridging across the hydroxyl and amino groups. This bridging effectively restricts rotation about the asymmetric carbon linkage, and the relative spatial distribution of the various groups on both centers is known. Projection formula, systematic names and trivial names by which the structures will hereafter be identified are shown in Fig. 1. The configuration about the  $C_{\alpha}$  center is drawn in anticipation of the n.m.r. spectral evidence.

**N.m.r. Spectra.**—All n.m.r. spectra were taken on a Varian V4300, 40 mc. machine with 5 mm. spinning sample tube at  $20 \pm 1^\circ$ . Chemical shifts were measured relative to benzene (external standard) by the side band technique. Peaks were identified with specific types of hydrogens by a combination of peak area, spin-coupling splitting and previously known chemical shifts for similar groups. Schematic presentations of the spectra for the six heterocyclic derivatives are shown in pairs in Fig. 2.

As different solvents were used for different pairs of diastereoisomers, discussion of the spectra will be

(1) Department of Chemistry, Dartmouth College, Hanover, N. H.  
(2) (a) H. Emde, *Helv. Chim. Acta*, **12**, 365 (1929); (b) K. Freudenberg and F. Nikolai, *Ann.*, **510**, 223 (1934).