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.; λ_{max}^{EtOH} 253 (11000), 330 mμ (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°.

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°. The material was sublimed for analysis.

Anal. Calcd. for $C_{11}H_8O_3$ (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°) 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°.

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° 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°. Recrystallization from ether–hexane gave prisms, m.p. 106–107°.

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[1]

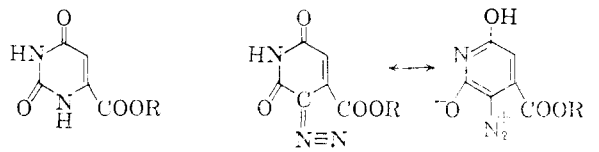
BY ZINON B. PAPANASTASSIOU, ARMAND MCMILLAN, VIRGINIA J. CZEBOTAR AND THOMAS J. BARDOS

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[2] 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.[3] 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"),[4] seems to be related to the structural analogy of the two inhibitors to glutamine; in both azaserine and "DON," the "nitrogen donating"–$CONH_2$ (carboxamide) group of glutamine is replaced by a –$COCHN_2$ (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 –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[5]) is, in 3-diazocitrazinic acid, replaced by a $-COC(N_2)-$ group, thus changing the pyrimidine ring into a pyridine



XI, R = H
XII, R = CH_3
XIII, R = C_2H_5

VIII, R = H
IX, R = CH_3
X, R = C_2H_5

Compound XI is a required growth factor for *Lactobacillus bulgaricus*[6]; as such, it can be replaced by its esters XII and XIII.[7] Compound VIII and its esters IX and X inhibit the growth of this organism; half maximal inhibition is obtained at 250 μg. of VIII, at 100 μg. of IX or at 400 μg. 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

(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.

(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).

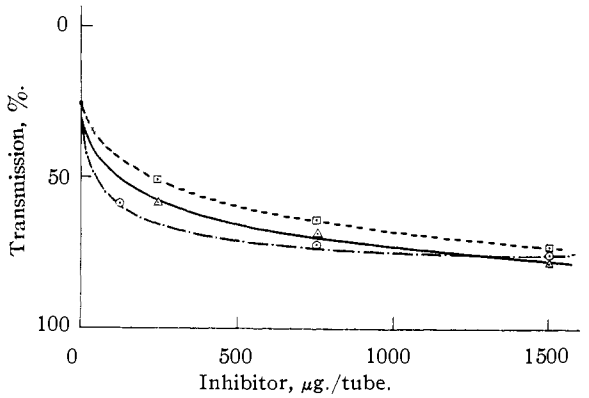


Fig. 1.—Inhibition of growth of *L. bulgaricus* (in the presence of 25 μg./tube orotic acid): X, – – – ⊡ – – –; IX, —·—·⊙·—·—; VIII, ———△———.

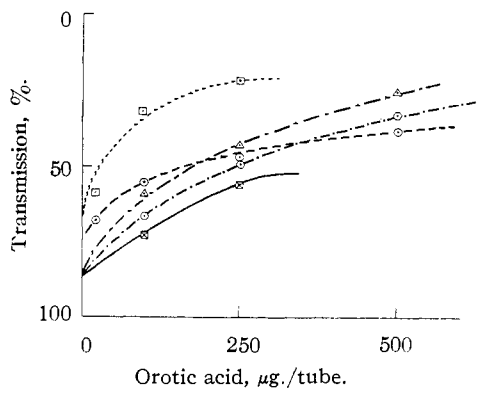
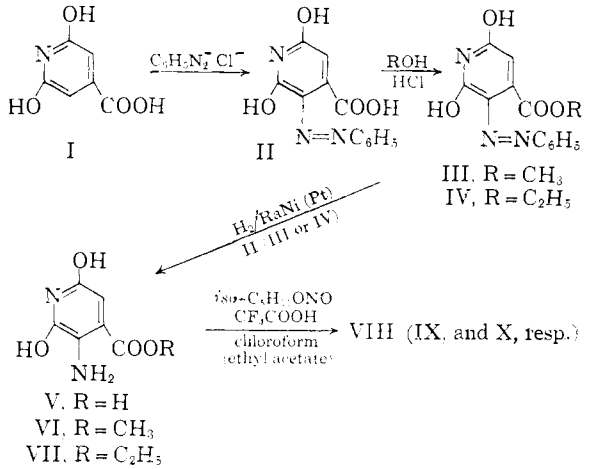


Fig. 2.—Reversal of growth inhibitors of *L. bulgaricus*: – – ⊡ – –, VIII, 1200 μg./tube; – — △ – —, VIII, 2400 μg./tube; – – ⊙ – –, IX, 2500 μg./tube; — · ⊙ · —, VIII, 3600 μg./tube; ———⊠———, IX, 5000 μg./tube.

inhibition indices (*i.e.*, the ratio of inhibitor concentration *vs.* metabolite concentration required for half maximal inhibition) are about 20–25 for all three compounds.

3-Diazocitrazinic acid and its esters were synthesized from commercially available citrazinic acid (I) as the starting material



3-Aminocitrazinic acid (V) and its esters VI and VII, as aminophenols, are very susceptible to oxidation; indeed, they are much more unstable than most aromatic aminophenols. In the presence of traces of water or hydroxy compounds, they immediately turn into colored quinoid compounds which rapidly polymerize. For this reason, as well as because these amino compounds are only very weakly basic, a new method had to be developed for their diazotization. This method involves the use of isoamyl nitrite and trifluoroacetic acid in the presence of chloroform or ethyl acetate, and permits the diazotization of "weak" amines, in the absence of either water or alcohol, in excellent yield. It is believed by the authors that the techniques described in the Experimental part of this paper could be profitably applied to the solution of many, similarly difficult diazotization problems.

Experimental[8]

3-Benzeneazo-2,6-dihydroxy-4-pyridinecarboxylic Acid (3-Benzeneazocitrazinic Acid, II).—Coupling of citrazinic acid (I) (155 g., 1.0 mole) with an equimolecular quantity of benzenediazonium chloride, according to the method of Sell and Easterfield,[9] produced II, in 80% yield, after reprecipitation from dilute (4%) sodium hydroxide solution with dilute hydrochloric acid.

Anal. Calcd. for $C_{12}H_9N_3O_4$: C, 55.6; H, 3.47; N, 16.2. Found: C, 55.4; H, 3.56; N, 16.2.

Methyl 3-Benzeneazo-2,6-dihydroxy-4-pyridinecarboxylate (Methyl 3-Benzene-azocitrazinate, III).—Forty grams (0.155 mole) of very finely powdered II was mixed with 1000 ml. of absolute methanol, and dry hydrogen chloride was passed through the mixture for 4 hours. In order to ensure complete esterification, the product was separated by filtration, the solid was mixed again with 1000 ml. of methanol, and dry hydrogen chloride was again passed through the mixture for 4 hours. The same process was repeated for the third time; then the solid obtained was washed with methanol until free from Cl^- and dried in a vacuum oven; yield 34 g. (80%), m.p. 223–225°.

Anal. Calcd. for $C_{13}H_{11}N_3O_4$: C, 57.14; H, 4.06. Found: C, 57.02; H, 4.22.

Ethyl 3-Benzeneazo-2,6-dihydroxy-4-pyridinecarboxylate (Ethyl 3-Benzene-azocitrazinate, IV).—The ethyl ester was prepared by a procedure similar to the one used for III; yield 80%, m.p. 260°.

Anal. Calcd. for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56. Found: C, 58.30; H, 4.73.

3-Amino-2,6-dihydroxy-4-pyridinecarboxylic Acid (3-Aminocitrazinic Acid, V).—A suspension of 20.7 g. (0.08 mole) of II and three teaspoonfuls of Raney nickel catalyst in 200 ml. of 4% aqueous sodium hydroxide was hydrogenated at 32 p.s.i. initial hydrogen pressure in a Parr low pressure hydrogenation apparatus. After *ca.* 1.5 hours, the dark-red color of the original suspension changed to amber and the theoretical amount of hydrogen was consumed. The mixture was shaken under hydrogen for an additional 0.5 hour, and the catalyst was then removed by filtration and washed with water.[10] The filtrate was acidified under reduced pressure (to avoid oxidation) with 30 ml. of concentrated hydrochloric acid, and the yellow precipitate formed was separated by filtration, washed with liberal amounts of methanol and rinsed with ethyl ether. After being dried under vacuum, the precipitate weighed 12.5 g. (92%).

Anal. Calcd. for $C_6H_6N_2O_4$: C, 42.36; H, 3.55; N, 16.47. Found: C, 42.53; H, 3.87; N, 16.64.

Methyl 3-Amino-2,6-dihydroxy-4-pyridinecarboxylate (Methyl 3-Aminocitrazinate, VI).—A suspension of 3.4 g. (0.0125 mole) of III, 1 g. of PtO_2 catalyst and 220 ml. of

(8) All melting points are uncorrected. Microanalyses were performed by the Midwest Laboratories, Indianapolis, Ind. Infrared spectra were determined on a Perkin-Elmer infrared spectrophotometer by Mr. R. Scott of the Research Division, Armour and Co., using 0.3% of the compounds in KBr pellets.

(9) W. J. Sell and T. H. Easterfield, *J. Chem. Soc.*, **63**, 1035 (1893).

(10) From the moment when the mixture is removed from the hydrogenation apparatus until the time when the final product is completely dry, all operations should be done as fast as possible; otherwise, a blue-colored product may be obtained which cannot be purified.

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[10] 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: λ_{max}^{water} 330 mμ (ε 14.1 × 10³); $\lambda_{max}^{0.1\ N\ HCl}$ 335 mμ (ε 14.6 × 10³); $\lambda_{max}^{0.1\ N\ NaOH}$ 342 mμ (ε 12.3 × 10³); $\lambda_{max}^{(CH_3OCH_3)_2}$ 333 mμ (ε 14.0 × 10³); characteristic infrared absorption band: λ_{max} at 4.7 μ (diazo group).

Anal. Calcd. for $C_6H_3O_4$: 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 isoamyl 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: λ_{max}^{water} 337 mμ (ε 13.4 × 10³); $\lambda_{max}^{0.1\ N\ HCl}$ 335 mμ (ε 9.45 × 10³); $\lambda_{max}^{0.1\ N\ NaOH}$ 343 mμ (ε 13.2 × 10³); $\lambda_{max}^{(CH_3OCH_3)_2}$ 335 mμ (ε 13.0 × 10³); characteristic infrared absorption band: λ_{max} at 4.7 μ (diazo group).

Anal. Calcd. for $C_7H_5N_3O_4$: 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 $C_9H_7N_3O_4$: 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]

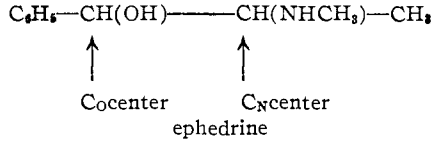
Nuclear Magnetic Resonance Spectra and Configuration. The N.m.r. Spectra of Diastereoisomeric Heterocyclic Derivatives of the Ephedrines

BY J. B. HYNE[1]

RECEIVED MAY 7, 1959

The n.m.r. spectra of derivatives of (−)-ephedrine and (+)-ψ-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 spacial 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 ephedrines 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[2a] by conversion of both (−)-ephedrine and (+)-ψ-ephedrine to (+)-deoxyephedrine that these



ephedrine

diastereoisomers differ in configuration at the Co center and that the other asymmetric center in both forms has the (+)-alanine configuration.[2b] In order to establish a fixed spatial disposition of the groups on the two asymmetric centers, several heterocyclic derivatives of the two ephedrines 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 Co 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 ± 1°. 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).