1-Trifluoroacetylamino-3-hydroxy-4-methyl-5-phenylpyridinium N-Betaine.—A suspension of 100 mg. of IXa in 2 ml. of benzene was treated, at 5–12°, with 0.3 ml. of trifluoroacetic anhydride. The base immediately dissolved, and after 20 sec. the solution was evaporated to a colorless oil. The oil crystallized on treatment with water followed by a drop of methanol. The colorless plates of the trifluoroacetate were filtered, washed with water and dried; m.p. 178–179°, 107 mg. Recrystallization from methanol–water gave glistening prisms, m.p. 179–180°, pK_A' 6.0 (50% methanol); $\lambda_{max}^{H_2O \text{ or } 0.1\ N\ NaOH}$ 229 (ϵ 23,400), 322 mμ (7,300); $\lambda_{max}^{0.1\ N\ HCl}$ 228 (20,000), 292 mμ (7,100).

Anal. Calcd. for $C_{14}H_{11}N_2O_2F_3$ (296.25): C, 56.76; H, 3.74; N, 9.46. Found: C, 56.88; H, 3.95; N, 9.52.

This compound was virtually insoluble in water or acid, but was freely soluble in carbonate solution; it was significantly more soluble in organic solvents such as benzene or ether than was the acetate.

A sample (20 mg.) of this derivative was hydrolyzed by warming in 0.5 cc. of 10% potassium hydroxide solution for 20 min. The solution, which contained a trace of oil, was cooled and treated with one ml. of water and a few drops of methanol; white needles separated. This material was filtered and dried; wt. 11 mg., m.p. and m.m.p. with the original base IXa, 195–196° dec.

1-Trifluoroacetylamino-3-methoxy-4-methyl-5-phenylpyridinium N-Betaine (XVIIb).—A solution of 340 mg. of the trifluoroacetylbetaine in 6 ml. of methanol was treated with excess ethereal diazomethane. When the vigorous gas evolution had ceased, the solution was evaporated *in vacuo*. The oil was dissolved in ether and the solution crystallized upon the addition of petroleum ether. The product was recrystallized twice from methanol–water, furnishing 150 mg. of XVIIb, white prisms, m.p. 134–135°, no pK_A' between *p*H 2 and 12; λ_{max}^{EtOH} 233 (ϵ 15,000), 295 mμ (9,600); the spectra in acidic and alkaline solutions were unchanged.

Anal. Calcd. for $C_{15}H_{13}N_2O_2F_3$ (310.27); C, 58.06; H, 4.22; N, 9.03. Found: C, 58.10; H, 4.17; N, 9.71, 9.85.

A 60-mg. sample of this product was heated for 20 min. on the steam-bath in 1 ml. of 5% methanolic potassium hydroxide solution. Evaporation of the solution gave a dark brown water-insoluble oil which was dissolved in methanol and treated with a methanol solution of 50 mg. of picric acid. Dark gold plates of the picrate separated immediately; recrystallization from ethanol gave 50 mg. of derivative, m.p. 171–172°, no depression with picrate (m.p. 174–175°) prepared by hydrolysis of XVIIa.

1-(N-Methylacetylamino)-3-hydroxy-4-methyl-5-phenylpyridinium Betaine (XXIII).—A 60-mg. sample of IXb was dissolved in 0.3 ml. of acetic anhydride; the solution became warm and developed an orange color. After standing for 30 sec. at 60°, 0.5 ml. of water was added and the solution was evaporated to a sirup. This compound was exceedingly difficult to crystallize. Treatment with chloroform and ether gave an amorphous solid which slowly crystallized from a mixture of methanol, acetone and ether to give 38 mg. of of slightly colored prisms, m.p. 194–198°. After treatment with carbon, recrystallization from the same solvent mixture gave short white prisms, m.p. 198–200°.

For analysis the compound was converted to the picrate which was twice recrystallized from ethanol, m.p. 179–180°, pK_A 6.0.

Anal. Calcd. for $C_{21}H_{19}O_9N_5$ (485.4): C, 51.96; H, 3.94; N, 14.43. Found: C, 51.87; H, 4.00; N, 14.36.

A solution of 16 mg. of the acetylation product in 0.1 ml. of methanol was allowed to stand for 2.5 hours with excess ethereal diazomethane. Evaporation of the solution gave a crystalline residue, m.p. and m.m.p. with IXb, 150–152°.

Acknowledgment.—We are indebted to Dr. John Vandenbelt and Mrs. Carola Henrich Spurlock for determination of ultraviolet spectra and pK measurements.

NEWARK, DEL.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF DELAWARE]

Heterocyclic Studies. VII. The Preparation and Reactions of 2-Amino-5-hydroxypyridines; the Formation of an Azaquinone[1]

BY JAMES A. MOORE AND FRANK J. MARASCIA[2]

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2-Amino-5-hydroxypyridine has been prepared by the diazotization of 5-amino-2-benzamidopyridine and by reduction of 5-hydroxy-2-*p*-nitrobenzeneazopyridine; the diazo coupling of 3-hydroxypyridines has been shown to be a generally useful method for the preparation of 2-amino-5-hydroxypyridines. Treatment of 2-amino-5-hydroxypyridine and 2-amino-5-hydroxy-4-methyl-3-phenylpyridine with excess nitrous acid furnished nitrosodihydroxypyridines. Hydrolysis of 2-nitroso-3,6-dihydroxy-4-methyl-3-phenylpyridine led to an azaquinone. Several reactions of the latter compound are described.

In connection with other work in this series, samples of 2- and 6-amino-3-hydroxy-4-methyl-5-phenylpyridine and information on some reactions of 2-amino-5-hydroxypyridines were required. Although several generally applicable synthetic paths for the preparation of 2-amino-3-hydroxypyridines are available, no satisfactory synthetic methods leading to the 2-amino-5-hydroxypyridine system have been described. The studies reported in this paper were undertaken to develop preparative methods for 2-amino-5-hydroxypyridines and to obtain precise information on the properties and reactions of this type of substituted pyridine. Parallel investigations were carried out with the 4-methyl-5-phenyl- and the 4,5-unsubstituted pyridine series, the latter serving as model compounds.

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(2) Predoctoral Research Fellow of the National Cancer Institute, Public Health Service.

2-Amino-5-hydroxypyridine has had a checkered past, and appears never to have been adequately characterized. The compound was reported by Koenigs, Gerdes and Sirot[3] to arise by reduction of the nitration product of 3-hydroxypyridine, but it was subsequently established by Schickh, *et al.*,[4] and by Plazek[5] that the product from this sequence of reactions is the 2-amino-3-hydroxy isomer. Renewed interest in 2-amino-5-hydroxypyridine stemmed from a study of the metabolic product of sulfapyridine in the rabbit and dog.[6–8] Hydrolysis of this metabolite furnished an aminohydroxypyridine,[6,8] characterized as the picrate and hydrochlo-

(3) E. Koenigs, H. C. Gerdes and A. Sirot, *Ber.*, **61**, 1022 (1928).

(4) O. V. Schickh, A. Binz and A. Schulze, *ibid.*, **60**, 2600 (1936).

(5) E. Plazek and Z. Rodewald, *Rocz. Chem.*, **16**, 502 (1936).

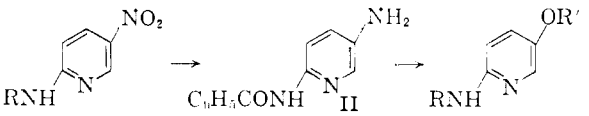
(6) C. V. Weber, J. J. Lalich and R. H. Major, *Proc. Soc. Expt. Biol. Med.*, **53**, 190 (1943).

(7) J. V. Scudi, *ibid.*, **55**, 197 (1944).

(8) H. G. Bray, F. C. Neale and W. V. Thorpe, *Biochem. J.*, **40**, 406 (1946).

ride, which was tentatively assigned the 2-amino-5-hydroxy structure.[8,9] Attempts to synthesize the compound by several methods were rather inconclusive. 2-Amino-5-ethoxypyridine has been prepared by a circuitous but unambiguous route[10]; the parent hydroxy compound was not described.

The first objective of the present work was an unequivocal synthesis of 2-amino-5-hydroxypyridine; this was accomplished by a very simple sequence of reactions beginning with the readily available 2-amino-5-nitropyridine. The 2-amino group was protected by benzoylation and the 2-benzamide Ib was then reduced catalytically to the amine II which was converted in 56% yield to the hydroxybenzamide IIIb by diazotization. Vigorous acid hydrolysis of IIIb led to 2-amino-5-hydroxypyridine which was very sensitive to air oxidation. Correlation of this synthesis with that of 2-amino-5-ethoxypyridine[10] was achieved by conversion of IIIb to the ethyl ether IIIc and hydrolysis to IIId. The melting points of the hydrochloride and picrate of IIIa corresponded to those reported[6] for the aminohydroxypyridine obtained from the urinary sulfapyridine metabolite and, although a direct comparison could not be made, there seems little doubt that the latter compound is correctly assigned the 2-amino-5-hydroxy structure.



Ia, R = H
b, R = C_6H_5CO

IIIa, R = R′ = H
b, R = C_6H_5CO; R′ = H
c, R = C_6H_5CO; R′ = Et
d, R = H; R′ = Et
e, R = CH_3CO; R′ = Et

Although this synthesis was structurally unambiguous and proceeded in a satisfactory yield, a method based on 3-hydroxypyridine as a starting point now was desired for application to the substituted series, in which the only readily accessible starting material was the 3-hydroxy-4-methyl-5-phenylpyridine (VIII).[11] Since 3-hydroxy- and -alkoxypyridines give predominantly exclusively 2-substituted products in typical aromatic substitution reactions such as nitration or halogenation, previous efforts in this direction have led to indirect syntheses entailing the removal of an unwanted 2-substituent.[10] Moreover, the conditions usually employed for the nitration of 3-hydroxypyridine would be expected to lead to substitution in the phenyl substituent of VIII.

On the other hand, the behavior of 3-hydroxypyridine parallels that of phenol in several typical color tests including the Gibbs indophenol reaction, which has been used as a diagnostic test for the presence of an unsubstituted 6-position in pyridoxine and other 3-hydroxypyridines.[12] It would, therefore, appear that similar phenol reactions such as nitrosation or diazo coupling of 3-hydroxypyridine might lead to 6-substituted products. The nitrosation of 3-hydroxypyridine has been attempted without success,[13] and we have also found that the compound is unaffected under the usual nitrosation conditions. The coupling reaction of 3-hydroxypyridine was originally reported by Mills and Widdows,[14] who obtained an azo product of unspecified structure on treatment with benzenediazonium chloride. Quite recently, the coupling reaction with *p*-nitrobenzenediazonium chloride has been reported to furnish an azo compound which on reduction gave 2-amino-3-hydroxypyridine.[13]

In the light of the Polish report, our attention was directed to the coupling reaction as a source of the 2-amino-3-hydroxy-4-methyl-5-phenylpyridine. When the substituted 3-hydroxypyridine (VIII) was coupled with *p*-nitrobenzenediazonium chloride in slightly alkaline solution, two azo compounds, separated by extraction with benzene, were obtained in approximately equal amounts. As described below, these two dyes on reduction gave two different aminohydroxypyridines.

This result prompted a reinvestigation of the coupling reaction of 3-hydroxypyridine under the same conditions. Crystallization of the crude coupling product, obtained in 91% yield, furnished three differently colored substances, all of which were converted on reduction, however, to a single aminohydroxypyridine which was shown by direct comparison of several derivatives to be the 2-amino-5-hydroxy isomer IV obtained in the synthesis described above. When the coupling reaction was carried out in weakly acidic solution as described by Bojarska-Dahlig and Urbanski, and the crude azo product was then chromatographed on alumina, two zones were resolved which were eluted separately and reduced. The major (slower-moving) band furnished IV and the minor, more readily eluted band which comprised 13% of the total azo product, gave 2-amino-3-hydroxypyridine, identical with the product obtained *via* nitration of 3-ethoxypyridine.

The diazo coupling reaction of 3-hydroxypyridine thus furnishes *mainly the p-azo product*, paralleling the behavior of phenol and of 2-pyridone[14]; it seems likely that *para* coupling of 3-hydroxypyridines is a generally useful synthetic procedure. The formation of larger amounts of the 2,3-isomer in the coupling of the 5-phenyl substituted compound VIII must be ascribed to the effect of the latter substituent.

The most satisfactory method for the reductive cleavage of the azopyridines was found to be catalytic hydrogenation with palladium in acetic acid solution. Since both reduction fragments were extremely sensitive to air oxidation in alkaline solution, advantage could not be taken of the acidic properties of the aminohydroxypyridines for separation from *p*-phenylenediamine, but the latter

(9) H. G. Bray, F. C. Neale and W. V. Thorpe, *Biochim. J.*, **46**, 506 (1950).

(10) H. J. den Hertog, C. Jouwersma, A. A. van der Wal and E. C. C. Willebrands-Schogt, *Rec. trav. chim.*, **68**, 275 (1949); H. J. den Hertog, J. P. Wibaut, F. R. Schepman and A. A. van der Wal, *ibid.*, **69**, 700 (1950).

(11) J. A. Moore and H. H. Püschner, THIS JOURNAL, **81**, 6041 (1959), paper V.

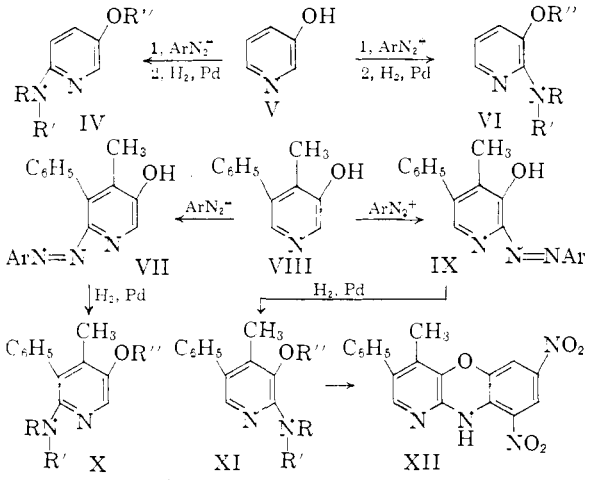
(12) E. T. Stiller, J. C. Keresztesy and J. R. Stevens, *ibid.*, **61**, 1237 (1939).

(13) H. Bogjarska-Dahlig and T. Urbanski, *Prace Placowek Nauk-Badawcz. Min. Przemyslu Chem.*, 1 (1952); *C. A.*, **48**, 1338 (1954).

(14) W. H. Mills and S. T. Widdows, *J. Chem. Soc.*, 1372 (1908).

was conveniently removed by precipitation of the hydrobromide which is very sparingly soluble in acetic acid. The aminohydroxypyridines were then isolated as benzoyl derivatives.

Reduction of the benzene-soluble azo product obtained from the coupling of VIII furnished a compound which was isolated as a tribenzoyl derivative. On vigorous acid hydrolysis an aminohydroxypyridine was obtained which was identified as the 2-amino-3-hydroxy isomer XIa by formation of the phenoxazine XII on treatment with picryl chloride.[15] From the benzene-insoluble azo compound was obtained in a similar way an aminohydroxypyridine isolated as a dibenzoyl derivative, which was assigned the 2-amino-5-hydroxy-4-methyl-3-phenyl structure X.



In VII and IX, Ar = *p*-nitrophenyl
In IV, VI, X and XI: a, R = R′ = R″ = H; b. R = C_6H_5CO, R′ = R″ = H; c, R = R″ = C_6H_5CO, R′ = H; d, R = R′ = R″ = C_6H_5CO

Comparison of the properties and reactions of the two 2,3-isomers VI and XI and the 2,5-isomers IV and X provided an additional basis for the assignment of structures to the amines obtained from the coupling reaction in the 4-methyl-5-phenyl series, neither of which was available by an alternative synthesis. No distinction between the 2,3- and 2,5-series was possible from the pK_A values, which were very similar for all four compounds, but a characterization of the two types was readily made by means of the Gibbs reaction. All of the 2-amino or -benzamido-3-hydroxy compounds gave the characteristic deep blue indophenol color, whereas none of the compounds assigned 2-amino- or -benzamido-5-hydroxy structures gave a positive reaction. This test provides a very convenient method for the detection of a small amount of the 2,3-isomer in preparations of the 2,5-isomer obtained by the coupling reaction.

Another contrast between the 2,3- and 2,5-aminohydroxy compounds was encountered in the benzoylation of the several substances. Treatment of either of the 2-amino-3-hydroxypyridines VIa or XIa with excess benzoyl chloride in pyridine led in good yield to a tribenzoyl derivative, formulated as the N,N-dibenzamide esters VId and XId. Under the same conditions, the O,N-dibenzoylamide esters IVc and Xc were obtained from the 2-amino-5-hydroxypyridines; the tribenzoyl derivative IVd was formed under Schotten–Baumann conditions. The facile formation of N,N-diacyl derivatives of 2-aminopyridines has been previously noted on several occasions.[16] All of the dibenzoyl and tribenzoyl derivatives were very readily hydrolyzed with mild alkali to the 2-benzamidopyridines IVb, VIb, Xb and XIb.

The most significant distinction between the 2-amino-3-hydroxy and 2-amino-5-hydroxy series was found in the reaction with nitrous acid. The conversion of 2-aminopyridines to 2-pyridones by diazotization is a widely used reaction[17] and generally proceeds in high yield. Although Plazek and Rodewald[5] reported the transformation of 2-amino-3-hydroxypyridine to the 2,3-diol,[18] Koenigs, *et al.*,[3] failed to isolate a product. We were similarly unable to isolate products from the diazotization of either VIa or XIa, although intensive efforts were not made in the case of VIa. Only amorphous highly colored mixtures were obtained on treatment of XIa with nitrous acid under a variety of conditions.

The diazotization of IVa and Xa presented a quite different pattern. On reaction of IVa with one equivalent of nitrous acid, 2,5-dihydroxypyridine (XIV) was obtained in good yield; this compound had previously been prepared in very low yield by diazotization of 5-amino-2-methoxypyridine followed by cleavage of the ether linkage.[19] In a completely analogous manner, a compound assigned the 2,5-dihydroxy-4-methyl-3-phenylpyridine structure XIII was obtained from Xa.

When on one occasion excess nitrous acid was used in the diazotization of Xa, a mixture of products was obtained, one of which had the composition $C_{12}H_{10}O_3N_2$, corresponding to the nitrosodihydroxypyridine XVI. When the unsubstituted 2,5-isomer IVa was then subjected to the same conditions, a product with strikingly similar spectral properties was obtained which is assigned the 2,5-dihydroxy-6-nitroso structure XVII. The same compound, which was obtained in two differently colored crystalline modifications, was also obtained from the 2,5-diol XIV on treatment with nitrous acid. As mentioned above, 3-hydroxypyridine does not undergo nitrosation, but 2,6-diaminopyridine[20] and 2-amino-6-hydroxypyridine[21] are reported to furnish 3-nitroso derivatives on treatment with nitrous acid.

In addition to the nitroso diol XVI obtained from the reaction of Xa with nitrous acid, two other products were characterized. One of these,

(15) This reaction has previously been used[5] as a basis for the structural assignment of a 2-amino-3-hydroxypyridine.

(16) (a) O. Seide, *Ber.*, **57**, 791, 1804 (1924); (b) E. H. Huntress and H. C. Walker, *J. Org. Chem.*, **13**, 735 (1948); (c) S. T. Lur'e, *Zhur. Obshchei Khim.*, **20**, 195 (1950); *C. A.*, **44**, 5880 (1950).

(17) H. S. Mosher, The Chemistry of the Pyridines, in "Heterocyclic Compounds," edited by R. C. Elderfield, Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1950.

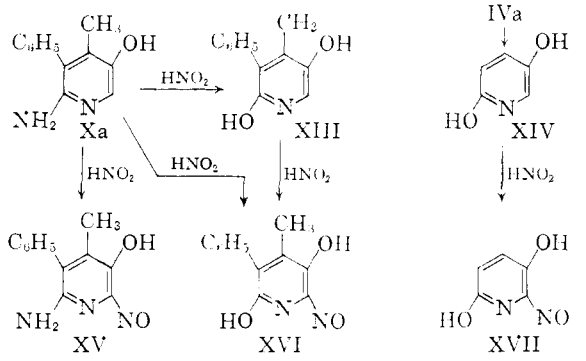
(18) *Cf.* footnote 13, paper V.

(19) R. Adams and T. R. Govindachari, THIS JOURNAL, **69**, 1806 (1947). An improved synthesis of 2,5-dihydroxypyridine has been described by E. J. Behrman and B. M. Pitt, *ibid.*, **80**, 3717 (1958).

(20) A. E. Chichibabin, *J. Russ. Phys. Chem. Soc.*, **50**, 522 (1920); *C. A.*, **18**, 1486 (1924).

(21) A. I. Titov, *J. Gen. Chem. U.S.S.R.*, **8**, 1983 (1938); *C. A.*, **33**, 4248 (1939).

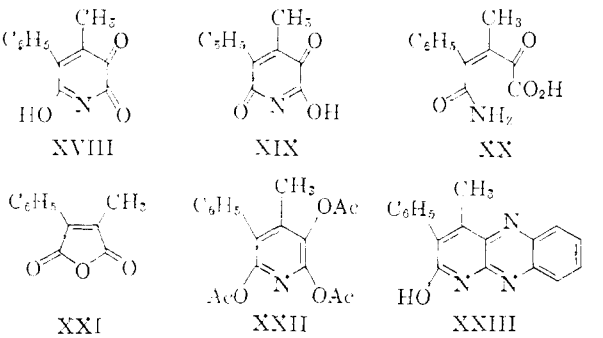
a yellow basic compound, is evidently the 6-amino-3-hydroxy-2-nitroso derivative XV, nitrosation having occurred without diazotization, as previously observed.[20,21]



The second companion substance obtained in the diazotization of Xa with excess nitrous acid proved to be of considerable interest. It was a very weakly acidic, pale yellow compound which sublimed readily and could be recrystallized from ether or water. Analysis indicated the composition $C_{12}H_9O_3N$. This empirical formula, corresponding to addition of water to the nitroso compound and loss of the elements of hydroxylamine, suggested that the compound was a hydrolysis product of XVI, and this was confirmed by hydrolysis of XVI with sulfuric acid, which furnished the $C_{12}H_9O_3N$ compound in 40% yield together with a small amount of a steam-volatile product, $C_{11}H_8O_3$. The latter substance was in turn obtained directly by hydrolysis of the $C_{12}H_9O_3N$ compound. On the reasonable premise that the phenyl and methyl substituents were retained intact during these reactions, the nitrogen-free compound could only be methylphenylmaleic anhydride (XXI). This conclusion was corroborated by direct comparison with an authentic sample of XXI which had been prepared in the course of other work by the N-bromosuccinimide dehydrogenation[22] of α-methyl-α'-phenylsuccinic anhydride.

The formation of the $C_{12}H_9O_3N$ compound from XVI by hydrolytic treatment and its subsequent hydrolysis to XXI can be rationalized only by an azaquinone structure. The preparation of quinones by hydrolysis of *p*-nitrosophenols or their quinone monoxime tautomers has been described by Karrer and Hoffmann,[23] but the reaction has not been widely used, and the application to nitrosohydroxypyridines has not been recorded.[24] Information on azaquinones[25] is rather sparse. The literature has recently been reviewed by Boyer and Kruger,[26] who obtained highly colored, sparingly soluble quinhydrones from the oxidation of 5-amino-2-pyridones with potassium bromate. The insoluble, infusible product obtained by oxidation of 2,3-dihydroxypyridine is clearly not the monomeric quinone as originally formulated.[27] 4-Aza-3-hydroxy-1,2-benzoquinone, obtained[28] by oxidation of 2,3,4-trihydroxypyridine, and bicyclic 4-aza-1,2-naphthoquinones derived from hydroxyquinolines have been reported[29,30] whose properties are consistent with quinone formulations, however, and it appears that with substitution in the heterocyclic ring, azaquinones can be prepared which exhibit the expected behavior.

The compound obtained from hydrolysis of the nitrosodiol XVI was characterized as a quinone by reductive acetylation to the triacetoxymethylphenylpyridine XXII and by the reversible oxidation reduction potential of 0.40 v. The quinone reacted with *o*-phenylenediamine to give a quinoxaline XXIII. The data available are insufficient to distinguish between the 1,2- (XVIII) and 1,4-quinone (XIX) structures, although the latter would be expected to be the more stable tautomer. The hydrolytic cleavage of the pyridine ring, leading to the anhydride XXI, presumably involves formation of the α-ketoglutaconamic acid (XX) followed by decarbonylation.



Attempts to obtain an analogous quinone in the 4,5-unsubstituted series by hydrolysis of the 2-nitroso-3,6-dihydroxypyridine (XVII) have so far been unsuccessful. An alternative approach to the unsubstituted quinone *via* 2-amino-3,6-dihydroxypyridine was briefly explored. The amine was obtained by stannous chloride reduction of the nitroso compound XVII and characterized as the dibenzoyl derivative. When an acid solution of the amine was neutralized with bicarbonate an intensely colored precipitate was immediately obtained. This extremely insoluble purple solid dissolved in base to give a pure blue color which became red on acidification; treatment with sodium hydrosulfite regenerated the aminohydroxypyridine. This product appears to be very similar in nature to the quinhydrone described by Boyer and Kruger.[26] It is hoped to extend these observations on azaquinones and related compounds.

Acknowledgments.—Infrared and ultraviolet spectra were obtained through the courtesy of Dr. Harold Beachell of these laboratories and Dr. John Vandenbelt, Parke, Davis and Co., respec-

(22) L. E. Miller, H. B. Staley and D. J. Mann, THIS JOURNAL, **71**, 374 (1949).

(23) P. Karrer and O. Hoffmann, *Helv. Chim. Acta*, **22**, 654 (1939).

(24) Dr. J. H. Boyer has informed us that studies on the hydrolysis of pyridine dioximes are in progress in his laboratories.

(25) In this discussion the term azaquinone is restricted, as defined in ref. 26, to compounds in which the heteroatom is a member of the quinonoid ring.

(26) J. H. Boyer and S. Kruger, THIS JOURNAL, **79**, 3552 (1957).

(27) R. Kudernatsch, *Monatsh.*, **18**, 624 (1897).

(28) H. Ost, *J. prakt. Chem.*, [2] **27**, 260 (1890); A. Peratoner, *Gazz. chim. ital.*, **41**, 619 (1912).

(29) M. Passerini, T. Bonciani and N. Di Gioia, *ibid.*, **61**, 959 (1931).

(30) O. Kruber and O. Rappen, *Chem. Ber.*, **81**, 483 (1948).

tively. We wish to thank Dr. Robert Wood for advice on the measurement of the E_o value of the quinone, and the Dow Chemical Co. for a generous supply of 3-hydroxypyridine.

Experimental[31]

4,5-Unsubstituted Series

5-Amino-2-benzamidopyridine (II).—A solution of 10 g. of 2-amino-5-nitropyridine[32] and 22 g. of benzoyl chloride in 100 ml. of pyridine was warmed for 45 min., poured into carbonate solution and steam distilled to remove pyridine. The resulting nitrobenzamide Ib was filtered and recrystallized from ethanol, giving 17 g. (98%) of pale tan needles, m.p. 167°. A solution of 10 g. of this amide in 180 ml. of glacial acetic acid was hydrogenated with 500 mg. of 10% palladium–charcoal catalyst; uptake of hydrogen was complete in 15 min.; concd. hydrochloric acid, 7.2 ml., then was added and the mixture of amine hydrochloride and catalyst was filtered and extracted with alcohol. Dilution of the colorless alcohol solution (75 ml.) with ten volumes of ether furnished 7.5 g. (67%) of colorless solid, m.p. 220–230°, which was the **dihydrochloride**. (*Anal.* Calcd. for $C_{12}H_{13}ON_3Cl_2$: Cl, 24.7. Found: Cl, 24.3.) The base obtained by neutralization with bicarbonate was recrystallized from water containing a trace of sodium hydrosulfite, m.p. 141–142°. The analytical sample was purified by sublimation.

Anal. Calcd. for $C_{12}H_{11}ON_3$ (213.3): C, 67.59; H, 5.21; N, 19.71. Found: C, 67.59; H, 5.22; N, 19.73.

2-Benzamido-5-hydroxypyridine (IIIb) from **II**.—To a solution of 1.0 g. of 2-benzamido-5-aminopyridine dihydrochloride in 5 ml. of 20% sulfuric acid at 0° was added 0.28 g. of sodium nitrite in 3 ml. of water. After standing at 0° for 15 minutes, urea was added to the solution to remove unreacted nitrous acid and the solution was added during 30 min. to 50 ml. of 20% sulfuric acid at 95°. The reaction mixture was then made alkaline with carbonate and the dark red aqueous solution was treated with 3 ml. of benzoyl chloride. The resulting amorphous precipitate was then hydrolyzed with methanolic potassium hydroxide and the product was taken up in water and treated with charcoal in both alkaline and acid solution. The colorless hydrochloride, m.p. 215–220°, which separated from 5 *N* hydrochloric acid solution was converted to the free base with bicarbonate. Ether extraction furnished 425 mg. (54%) of short white needles, m.p. 181–182°; recrystallization from methanol did not change the m.p.; pK'a 2.6, 8.8 (50% methanol); λ_{max}^{EtOH} 300 (11,300), 273 (11,400), 226 mμ (12,800); $\lambda_{max}^{EtOH+HCl}$ 325 (13,300), 261 mμ (15,400); $\lambda_{max}^{EtOH+NaOH}$ 322.5 mμ (12,800); λ_{max}^{KBr} 3.10, 3.25–3.35, 6.11 (C=O amide) 6.24 μ.

Anal. Calcd. for $C_{12}H_{10}O_2N_2$ (214.22): C, 67.28; H, 4.71; N, 13.08. Found: C, 67.21; H, 4.76; N, 13.18.

The 3-benzoate ester IVc was prepared by shaking a solution of 100 mg. of the above product in 5 ml. of potassium carbonate solution with three drops of benzoyl chloride. The resulting solid was recrystallized from methanol to give 130 mg. of silky needles, m.p. 148–149°; λ^{KBr} 3.1, 3.3, 5.8, 6.0 μ.

Anal. Calcd. for $C_{19}H_{14}O_3N_2$ (318.32): C, 71.69; H, 4.43; N, 8.80. Found: C, 71.33; H, 4.42; N, 8.70.

Hydrolysis of IVc with methanolic potassium hydroxide furnished the 2-benzamido-5-hydroxy compound IVb (same as IIIb) in 85% yield, m.p. and m.m.p. 180–182°.

2-Amino-5-ethoxypyridine (IIId).—To a solution of 3.0 g. (0.014 mole) of IIIb in 10 ml. of ethanol containing 0.015 mole of sodium ethoxide was added 0.93 ml. of ethyl bromide. After refluxing for 2.5 hours, the solution was evaporated to dryness *in vacuo*. The solid residue was dissolved in aqueous potassium carbonate solution and extracted with ether. Evaporation of the ether gave 900 mg. of tan needles of the 2-benzamido-5-ethoxy derivative IIIc; neutralization of the aqueous solution furnished 1.75 g. of unreacted IIIb. The crude 5-ethoxy amide was then refluxed for 2 hours in concd. hydrochloric acid solution and after removing benzoic acid by extraction with ether the aqueous solution was evaporated, giving 350 mg. of dark brown solid. Crystallization (charcoal) from ethanol–ether furnished 150 mg. of short white needles of the **hydrochloride**, m.p. 143–145°. **The free base** was prepared by neutralization of the hydrochloride with sodium bicarbonate in the presence of a trace of sodium hydrosulfite. Extraction with ether gave a brown oil which was distilled in a sublimation apparatus. After scratching the resulting colorless oil in the presence of hexane, colorless crystals of IIId, m.p. 47–48°,[33] were obtained. **The picrate** was obtained as yellow needles from ethanol, m.p. 240–241° (s. 230°). **The 2-acetylamino-5-ethoxypyridine** (IIIe) was prepared by refluxing a solution of 10 mg. of the base with acetic anhydride. After removal of the anhydride *in vacuo*, crystallization from ethanol furnished 8 mg. of white needles, m.p. 108–109°.

5-Hydroxy-2-(*p*-nitrobenzenazo)-pyridine.—To a cooled, well-stirred solution of 19 g. of 3-hydroxypyridine and 11.2 g. of potassium hydroxide in 500 ml. of water were added simultaneously solutions of *p*-nitrobenzenediazonium chloride (0.2 mole) and 58 g. of potassium hydroxide in 1 l. of water. The solution was maintained at a neutral to slightly basic *p*H during the addition, which required 20 min. After stirring for an additional hour, 50 ml. of glacial acetic acid was added and the bright red precipitate was filtered and dried, giving 44.6 g. (91%) of the crude coupling product. Crystallization from ethanol furnished three differently colored fractions: violet needles, m.p. 213–230°; red needles, m.p. 231–232° and long orange needles, m.p. 214–226°. The spectra (ultraviolet and infrared) of all of these fractions were very similar. The orange form was further crystallized ethanol to give orange-red needles, m.p. 231–232°.

Anal. Calcd. for $C_{11}H_8O_3N_4$ (244.2): N, 22.94. Found: N, 23.12.

2-Benzamido-5-hydroxypyridine from Reduction of Azo-coupling Product. a. Hydrogenation.—A solution of 25 g. of 5-hydroxy-2-(*p*-nitrobenzenazo)-pyridine (recrystallized from methanol) in 150 ml. of glacial acetic acid was shaken with 0.3 g. of 10% palladium–charcoal catalyst at 45 lb. hydrogen pressure. After the absorption of the theoretical quantity of hydrogen (30 min.) the hydrogen atmosphere was replaced with nitrogen and 48 ml. of 48% hydrobromic acid was added. The resulting precipitate of *p*-phenylenediamine dihydrobromide and catalyst was filtered and washed with four 25-ml. portions of acetic acid. The acetic acid solution was then evaporated *in vacuo* and the residue was dissolved in water, made alkaline with sodium carbonate, and shaken with 34 g. of benzoyl chloride in the presence of a trace of stannous chloride. The crude benzoylation product was filtered and hydrolyzed with methanolic potassium hydroxide. After evaporation to small volume the alkaline solution was acidified with hydrochloric acid and the precipitate of benzoic acid and the hydrochloride of 2-benzamido-5-hydroxypyridine was filtered and washed with ether to remove benzoic acid. The crude hydrochloride, m.p. 215–220°, 20.5 g. (76%), was converted to the base with aqueous bicarbonate, furnishing white needles, m.p. and m.m.p. 180–181° after recrystallization from ethanol.

b. **Stannous Chloride Reduction. Isolation of 2-Dibenzamido-5-benzoyloxy-pyridine Benzoate** (IVd).—To a warm solution of 2.5 g. of 5-hydroxy-2-(*p*-nitrobenzeneazo)-pyridine in 250 ml. of concentrated hydrochloric acid and 150 ml. of methanol was added 25 g. of stannous chloride in small portions. After the addition was complete, the reaction mixture was warmed on a steam-bath until a test drop showed no color change when made strongly alkaline with 50% potassium hydroxide solution. The solvent was evaporated and the residue was redissolved in 25 ml. of ice-water. Sufficient 50% potassium hydroxide solution was added to dissolve the precipitated tin salts and the solution was shaken with 10 ml. of benzoyl chloride at 0–5°. After decanting the aqueous phase and washing with water, the oily semi-solid residue was allowed to stand overnight with 10% sodium carbonate solution. The resulting solid was filtered, dried, and continuously extracted with methanol; the methanol-insoluble dibenzoyl derivative of *p*-phenylenediamine, m.p. >250° was discarded. Concentration of the methanol solution furnished 2.30 g. (47%) of short white needles, m.p. 179–183°. Recrystallization from methanol gave pure IVd as needles, m.p. 182–183°, m.m.p. with 5-hydroxy-2-benzamidopyridine <170°; λ_{max}^{EtOH} 240, 273 mμ; λ^{KBr} 5.74 (ester), 5.81–5.85 μ (bis-amide).

(31) Infrared spectra of all compounds were obtained in KBr disks. Only the most significant bands of the more important compounds are recorded.

(32) W. T. Caldwell and E. C. Kornfeld, THIS JOURNAL, **64**, 1696, (1942).

(33) Reported[10] for IIId: base, m.p. 50–50.5°; acetyl, m.p. 113°; picrate, m.p. 239–240°.

Anal. Calcd. for $C_{26}H_{18}O_4N_2$ (422.2): C, 73.92; H, 4.30; N, 6.63. Found: C, 74.13; H, 4.25; N, 6.70.

3-Hydroxy-2-(*p*-nitrobenzeneazo)-pyridine.[13]—To a solution of *p*-nitrobenzenediazonium chloride prepared from 1.38 g. of *p*-nitroaniline was added sodium acetate until the solution was no longer acid to congo red, and this solution was then added at 25° to 0.85 g. of 3-hydroxypyridine dissolved in 50 ml. of water. After standing overnight the red precipitate was filtered and dried; 1.78 g. A 31-mg. sample of this product was dissolved in 2 ml. of ethanol and absorbed on a column of 3 g. of alumina. The chromatogram was developed with chloroform–ethanol (4:1), and a bright red band remained at the top of the column. Elution with 150 ml. of solvent removed a smaller brownish-red band from the column; evaporation of this eluate gave 5.5 mg. (17%) of red powder, m.p. 234–235°. Recrystallization from methanol gave red needles of the 3-hydroxy-2-azopyridine, m.p. 234–235°.

Anal. Calcd. for $C_{11}H_8O_3N_4$ (244.2): N, 22.94. Found: N, 23.16.

From a similar chromatogram of a sample of the crude azo-coupling product prepared in *alkaline* solution, a 13.4% yield of the 3-hydroxy-2-azo isomer was eluted; prolonged elution then furnished the 5-hydroxy-2-azo isomer.

The 3-hydroxy-2-azo structure for the more readily eluted dye was established by catalytic reduction as described for the 5-hydroxy isomer. Hydrogenation of a 40-mg. sample of the azo compound obtained by chromatography of the neutral coupling product gave a crude amino-hydroxypyridine which was benzoylated in bicarbonate solution to yield the **N,N,O-tribenzoyl derivative VId,** identical with a sample prepared from authentic 2-amino-3-hydroxypyridine (VIa).

For the preparation of comparison samples of VId and other benzoylation products, a sample of VIa was prepared by cleavage of 2-amino-3-ethoxypyridine with hydrobromic acid.[34] The tribenzoyl derivative VId was obtained by treatment of 500 mg. of VIa hydrobromide in sodium bicarbonate solution with 1 ml. of benzoyl chloride; after shaking for several minutes, the white solid which separated was collected, washed and dried; 900 mg., m.p. 168–170°. Recrystallization from methanol furnished white needles, m.p. 169–170°.

Anal. Calcd. for $C_{26}H_{18}O_4N_2$ (422.4): C, 73.92; H, 4.30; N, 6.63. Found: C, 74.17; H, 4.24; N, 6.74.

The monobenzoyl derivative VIb was obtained by hydrolysis of VId with methanolic potassium hydroxide, analogous to the preparation of IVb. Crystallization from aqueous methanol gave white needles, m.p. 95–96°. For analysis this material was converted to the **picrate** which crystallized in stout yellow needles from ethanol, m.p. 237–238°.

Anal. Calcd. for $C_{18}H_{13}O_9N_5$ (443.3): N, 15.80. Found: N, 15.77.

2-Amino-5-hydroxypyridine (IIIa).—A solution of 7.0 g. of the benzamide IIIb in 20 ml. of 48% hydrobromic acid was refluxed for 3 hours and then concentrated *in vacuo*. The hydrobromide of IIIa was isolated by dissolving the sirupy residue in a small volume of ethanol and diluting with ether; the salt precipitated as a brown powder; 5.7 g., m.p. 120–125°. For preparation of the base, a sample of the hydrobromide was dissolved in bicarbonate solution containing a few mg. of sodium hydrosulfite. After continuous extraction of the neutral solution with ether the solvent was removed and IIIa was obtained as a gray powder which rapidly darkened in air. Recrystallization from methanol–benzene gave nearly colorless needles, m.p. 116–117°. Satisfactory analytical results could not be obtained for the base.

The picrate was prepared in ethanol solution and crystallized as tiny yellow needles, m.p. 225–227° dec.[35]

Anal. Calcd. for $C_{11}H_9O_8N_5$ (339.2): C, 38.95; H, 2.76; N, 20.65. Found: C, 39.18; H, 2.70; N, 20.78.

The hydrochloride of IIIa was prepared by prolonged hydrolysis of IIIb with concd. hydrochloric acid. The salt was isolated as described for the hydrobromide; recrystallization from ethanol–ether furnished stout white needles, m.p. 125–126°.[35]

3,6-Dihydroxy-2-nitrosopyridine (XVII).—To a cooled, stirred solution of 2-amino-5-hydroxypyridine hydrobromide in 20 ml. of 20% sulfuric acid was added a solution of 4.5 g. of sodium nitrite in 5 ml. of water. After the addition was complete the dark red precipitate was collected, washed with water and dried; 1.75 g. Two recrystallizations from water gave bright red needles, dec. 210°, pK_a 8.4 (H_2O); λ_{max}^{EtOH} 221 (6000), 247 (5100), 350 mμ (3000); $\lambda_{max}^{EtOH + base}$ 240 (12,000), 461 mμ (6600).

Anal. Calcd. for $C_5H_4O_3N_2$ (140.1): C, 42.86; H, 2.87; N, 20.00. Found: C, 43.15; H, 2.67; N, 19.91.

2,5-Dihydroxypyridine Benzoate.—A 500-mg. sample of the hydrobromide of IIIa was added to 3 ml. of cold concd. sulfuric acid. After the evolution of hydrogen bromide had ceased, 170 mg. of sodium nitrite was added and the cold solution was stirred for 10 min. and then heated until nitric oxide was no longer evolved. The solution was then cooled, poured onto ice, neutralized with solid sodium bicarbonate and finally treated with 1 ml. of benzoyl chloride. The resulting solid was extracted with ether and the ether solution was dried and evaporated to give 550 mg. of white powder which was sublimed and then recrystallized from benzene as short white needles; 350 mg., m.p. 187–189°.

Anal. Calcd. for $C_{12}H_9O_3N$ (215.3): C, 66.95; H, 4.21; N, 6.54. Found: C, 66.98; H, 4.52; N, 6.38.

For conversion to **2,5-dihydroxypyridine,** a solution of 45 mg. of the benzoate in 1 ml. of 48% hydrobromic acid was refluxed for 30 min. and then cooled, diluted with water, and extracted with ether to remove benzoic acid. The aqueous solution was then neutralized with bicarbonate and extracted with benzene–ethanol (1:1). The dark oil obtained after removing the solvent was distilled to give a pale yellow glass which crystallized from ethanol to give 15 mg. of short white needles, m.p. 245–247° (s. 225°).

For nitrosation, a 100-mg. sample of the benzoate was hydrolyzed with acid as described above and the acid solution was evaporated to dryness. The crude hydrobromide obtained by evaporating the solution to dryness was then dissolved in 20% sulfuric acid and treated at 15–20° with 75 mg. of sodium nitrite. The resulting red precipitate was filtered and recrystallized to give pale red needles of XVII, identical with the product obtained by nitrosation of IIIa.

2-Amino-3,6-dihydroxypyridine.—To a solution of 200 mg. of XVII in 4 ml. of concd. hydrochloric acid and 4 ml. of ethanol was added 500 mg. of stannous chloride. The solution was warmed on the water-bath, and the color changed progressively from red to green and finally dark yellow. After evaporation to about one-half volume, 190 mg. of pale yellow needles separated from the solution. The crystals, which were presumably the hydrochloride of the aminodihydroxypyridine, darkened rapidly in air, and an aqueous solution was stable only in the presence of sodium hydrosulfite or stannous chloride. On adding a sample of the yellow needles to bicarbonate solution, an immediate brilliant indigo precipitate was obtained, with a deep blue color imparted to the solution. Acidification of the solution caused a color change to red, and addition of hydrosulfite regenerated the yellow crystals.

A solution of 15 mg. of the hydrochloride in 2 ml. of pyridine was treated with three drops of benzoyl chloride, and after brief warming the solution was poured into iced hydrochloric acid. The solid which separated was collected and recrystallized from chloroform–ethanol to give 20 mg. of fluffy white needles of a dibenzoyl derivative, presumably the **2-benzamido-3-benzoyloxy-6-pyridone,** m.p. 243–244°; λ^{KBr} 5.79, 6.12 μ.

Anal. Calcd. for $C_{19}H_{14}O_4N_2$ (334.3): C, 68.25; H, 4.22; N, 8.38. Found: C, 68.68; H, 3.93; N, 8.20.

4-Methyl-5-phenyl Series

Azo Coupling of 3-Hydroxy-4-methyl-5-phenylpyridine (VIII).—To a solution prepared from 3.2 g. (0.017 mole) of VIII[11] dissolved in 100 ml. of water containing one equivalent of sodium hydroxide was added a solution of 0.017 mole of *p*-nitrobenzenediazonium chloride; sodium hydroxide was added concurrently at a rate to maintain a neutral reaction to indicator paper. After the addition was complete, stirring was continued for one hour and the solution was then made acid and the heavy red precipitate was filtered and dried; 6.83 g. The crude coupling product was then extracted with benzene in a Soxhlet apparatus until the returning solvent was only pale yellow. The benzene-

(34) J. Bernstein, B. Stearns, M. Shaw and W. A. Lott, THIS JOURNAL, 69, 1158 (1947).

(35) Reported[6] for the hydrolysis product of the urinary metabolite of sulfapyridine: picrate, m.p. 212°; hydrochloride, m.p. 126°.

insoluble residue, 3.9 g. (57% of crude product), was recrystallized from ethanol to give stout red needles of the **5-hydroxy-2-azo isomer VII**, m.p. 261–269° dec.

Anal. Calcd. for $C_{18}H_{14}O_3N_4$ (334.3): C, 64.66; H, 4.22: N, 16.76. Found: C, 64.54; H, 4.19; N, 16.72.

Recrystallization of the benzene-soluble fraction from benzene furnished golden-red laths of the **3-hydroxy-2-azo isomer IX**, m.p. 230–235°.

Anal. Found: C, 64.94; H, 4.34; N, 16.93.

2-Amino-3-hydroxy-4-methyl-5-phenylpyridine (XI) and Benzoyl Derivatives.—A solution of 1.77 g. of the benzene-soluble coupling product IX in 150 ml. of acetic acid and 0.25 g. of 10% palladium–charcoal catalyst was shaken with hydrogen until the theoretical amount of hydrogen was absorbed. The *p*-phenylenediamine was removed as described previously by precipitation with hydrobromic acid and filtration of the mixture of hydrobromide and catalyst; extraction of the precipitate with water and benzoylation under Schotten–Baumann conditions gave 1.83 g. of dibenzoyl-*p*-phenylene diamine. The acetic acid solution of the aminopyridine was evaporated, dissolved in aqueous potassium hydroxide and shaken with benzoyl chloride. The resulting oily precipitate was extracted with ether and the ether solution was extracted with sodium bicarbonate, washed with water, dried and evaporated to give 2.80 g. of colorless crystalline residue. Repeated recrystallization from ethanol gave white needles of the **N,N,O-tribenzoyl derivative XId**, m.p. 182°, no pK_A between 2 and 12; λ^{KBr} 5.75, 5.85–5.92 μ.

Anal. Calcd. for $C_{33}H_{24}O_4N_2$ (517.5): C, 77.30; H, 4.72; N, 5.47. Found: C, 77.05; H, 4.84; N, 5.37.

For hydrolysis to the **monobenzoyl derivative XIb**, a solution of 250 mg. of XId and 500 mg. of potassium hydroxide in 6 ml. of 80% methanol was refluxed for 0.5 hour. After evaporation of the methanol, addition of water resulted in the precipitation of a sodium salt which was then acidified with dilute acid and finally neutralized with bicarbonate. The poorly crystalline base XIb which separated was converted to the **picrate** for characterization; yellow filaments from ethanol, m.p. 213°.

Anal. Calcd. for $C_{25}H_{19}O_9N_5$ (533.4): C, 56.30; H, 3.59; N, 13.13. Found: C, 56.54; H, 3.61; N, 13.09.

The free aminopyridine XIa was obtained by acid hydrolysis of the tribenzoyl derivative XId; a solution of 280 mg. of XId in 5 ml. of 48% hydrobromic acid was refluxed for 1 hour and then chilled. The precipitate of benzoic acid and amine hydrobromide was extracted with ether and the base was obtained by neutralization with bicarbonate; 125 mg. of cream-colored solid. Recrystallization from ethyl acetate gave 30 mg. of colorless needles of XIa, m.p. 210° dec.; pK_A 6.05, 9.9 (50% methanol); λ_{max}^{EtOH} 256 (6900), 307 mμ (9000); $\lambda_{max}^{EtOH\ +\ acid}$ 312 mμ (10,700), $\lambda_{max}^{EtOH\ +\ base}$ 319 mμ (12,000). Since analytical data for the base were unsatisfactory, the **picrate** was prepared; golden needles from ethanol, m.p. 260° dec.

Anal. Calcd. for $C_{18}H_{15}O_8N_5$ (429.3): C, 50.35; H, 3.52; N, 16.31. Found: C, 50.39; H, 3.54; N, 16.41.

Acylation of XIa with benzoyl chloride in pyridine solution gave the **2-benzamido-5-benzoate ester XIc**, as white needles from ethanol, m.p. 195–196°; λ^{KBr} 3.05, 5.78, 6.02 μ.

Anal. Calcd. for $C_{26}H_{20}O_3N_2$ (408.4): C, 76.45; H, 4.94; N, 6.86. Found: C, 76.42; H, 4.92; N, 6.84.

7,9-Dinitro-4-methyl-3-phenyl-10-pyrido[3.2-b][1.4]benzoxazine (XII).—To a solution of 15 mg. of sodium in 3 ml. of ethanol was added 25 mg. of XIa and 50 mg. of recrystallized picryl chloride. After heating for 10 min. on the water-bath the dark solution was cooled and poured into water. The resulting dark oily solid was recrystallized twice from methanol to give 20 mg. of long red prisms, m.p. 196°.

Anal. Calcd. for $C_{18}H_{12}O_5N_4$ (364.3): C, 59.34; H, 3.32; N, 15.38. Found: C, 59.38; H, 3.31; N, 15.43.

2-Amino-5-hydroxy-4-methyl-3-phenylpyridine (X) and Benzoyl Derivatives.—A 1.94-g. sample of the benzene-insoluble azo coupling product VII was hydrogenated exactly as described for IX; 1.42 g. of the dibenzoyl derivative of *p*-phenylenediamine was isolated. Benzoylation of the crude aminohydroxypyridine furnished a non-crystalline polybenzoyl derivative which was treated directly with methanolic potassium hydroxide. Crystallization of the **benzamide Xb** from aqueous methanol furnished beautiful prisms, m.p. 124–130°, which contained methanol of crystallization. Recrystallization from chloroform–ether gave white needles, m.p. 216–217°.

Anal. Calcd. for $C_{19}H_{16}O_2N_2$ (304.3): C, 74.98; H, 5.30; N, 9.21. Found: C, 75.06; H, 5.32; N, 9.21.

For hydrolysis to the **free aminopyridine**, a solution of 325 mg. of Xb in 3 ml. of concd. sulfuric acid was warmed for 10 min. and then poured onto ice. After extraction with ether, which furnished 120 mg. of benzoic acid, neutralization of the acid solution with sodium bicarbonate solution gave 205 mg. of a light yellow solid. This material was twice recrystallized from ethyl acetate to give 60 mg. of colorless rods of Xa, m.p. 190–195° dec.; pK_A 6.05, 10.25 (50% methanol); λ_{max}^{EtOH} 210 (21,000), 319 mμ (5600); $\lambda_{max}^{EtOH\ acid}$ acid 331 mμ (7500); $\lambda_{max}^{EtOH\ base}$ 336 mμ (5500).

Anal. Calcd. for $C_{12}H_{12}ON_2$ (200.2): C, 71.98; H, 6.04; N, 13.99. Found: C, 71.93; H, 6.03; N, 13.96.

The 2-benzamido-5-benzoate ester Xc was prepared by treatment of Xb in ether solution with pyridine and benzoyl chloride. After evaporation of the ether, the oily residue was triturated with bicarbonate solution and the resulting solid was twice recrystallized from ethyl acetate to give stout white needles, m.p. 199–200°, λ^{KBr} 3.10, 5.74, 6.08 μ.

Anal. Calcd. for $C_{26}H_{20}O_3N_2$ (408.4): C, 76.45; H, 4.94; N, 6.86. Found: C, 76.01; H, 4.89; N, 6.73.

The same derivative was obtained by benzoylation of Xa; hydrolysis of this ester with methanolic alkali furnished Xb. Further derivatives and characterization of Xa will be described in a subsequent communication.

2,5-Dihydroxy-4-methyl-3-phenylpyridine (XIII).—To a solution of 100 mg. of Xa in 2.5 ml. of 60% sulfuric acid at −5° was added 35 mg. of sodium nitrite. After stirring at 0° until gas evolution had ceased the solution was warmed to 50° and then chilled again and neutralized by addition of potassium carbonate. A white precipitate separated when filtered, washed thoroughly with water and bicarbonate solution. Recrystallization from methanol gave 60 mg. of colorless needles, m.p. 250–260°.

Anal. Calcd. for $C_{12}H_{11}O_2N$ (201.2): C, 71.62; H, 5.51; N, 6.96. Found: C, 71.57; H, 5.55; N, 6.98.

2,5-Dihydroxy-6-nitroso-4-methyl-3-phenylpyridine (XVI).—A solution of 850 mg. of Xa in 20 ml. of 20% sulfuric acid was treated at room temperature with a solution of 1.20 g. of sodium nitrite in 7 ml. of water. After stirring for 10 min., the reddish-brown precipitate was filtered, washed with water and dried *in vacuo;* 650 mg. (63%). Recrystallization from ethanol gave golden-red prisms, m.p. 250–253°, pK_A below 2 and 8.65 (50% methanol); λ_{max}^{EtOH} 263 (5100), 319 mμ (8000); $\lambda_{max}^{EtOH\ acid}$ 326 mμ (6000); $\lambda_{max}^{EtOH\ base}$ 417 mμ (8400).

Anal. Calcd. for $C_{12}H_{10}O_3N_2$ (230.2): C, 62.60; H, 4.38; N, 12.17. Found: C, 62.81; H, 4.50; N, 12.16.

The strongly acid mother liquor from the crude nitroso derivative was extracted several times with ether. Evaporation of the dried ether solution gave 70 mg. of light yellow solid which was recrystallized from water to give cream-colored needles of the quinone (XVIII–XIX). This material was identical with a sample prepared by hydrolysis of XVI as described below.

Neutralization of the ether-extracted aqueous acid solution with solid sodium bicarbonate then furnished 110 mg. of a crystalline yellow precipitate which was very sparingly soluble in ether or water. Recrystallization from acetone gave 58 mg. of stout yellow needles of the **2-amino-6-nitroso derivative XV**, m.p. >280°.

Anal. Calcd. for $C_{12}H_{11}O_3N_2$ (229.2): C, 62.87; H, 4.84; N, 18.33. Found: C, 62.69; H, 5.05; N, 17.94.

2-Hydroxy-4-methyl-5-phenyl-1-azaquinone (XVIII-XIX).—A suspension of 150 mg. of XVI in 2.5 ml. of 40% sulfuric acid was heated on the steam-bath until a clear solution was obtained. After cooling, the solution was neutralized with solid sodium carbonate and then extracted with ether. Evaporation of the dried ether solution gave a crystalline orange residue which was recrystallized from water to give 60 mg. of pale yellow needles, m.p. 160–161°; the melting point was unchanged by sublimation at 140° (0.1 mm.). The reversible oxidation-reduction potential for the reaction $QH_2 \rightleftarrows Q + 2H^+ + 2e^-$ was determined by measurement of the potential of the half-cell formed from the

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]

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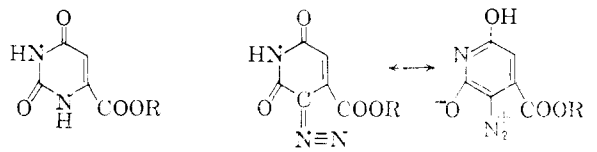
A new variation of "structural analogy" was devised and biologically tested. Replacement of a nitrogen atom in the pyrimidine ring of orotic acid by the carbon atom of a diazomethine radical leads to the structure of 3-diazocitrazinic acid. This compound, and its esters, were synthesized, and they were 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).