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The Synthesis of a Metabolite of Pyridoxamine[1,2]

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A metabolite of pyridoxamine, 2-methyl-3-hydroxypyridine-5-carboxylic acid, was synthesized. The key step in this synthesis involved the hydrolysis, with 10% aqueous sodium hydroxide, of 2-methyl-3-amino-5-cyano-6-chloropyridine to 2-methyl-3-amino-6-chloropyridine-5-carboxylic acid. Hydrolysis of the same compound with concentrated hydrochloric acid gave 3-carboxy-5-amino-6-methyl-2(1)pyridone. A mechanism is proposed for this hydrolysis.

In connection with studies[3-7] in this laboratory on antagonists and analogs of vitamin B_6, 2-methyl-3-hydroxypyridine-5-carboxylic acid (VI) (Fig. 1) was synthesized. This compound has recently[8] been isolated as a metabolite of pyridoxamine in *Pseudomonas sp.* MA. In this paper we report the synthesis of the metabolite.

The simplest route for the synthesis of the desired compound VI seemed to involve the sequence I → II → IV → V → VI, as compound II has been reported to be synthesized.[9] However, we found that the compound reported as II was actually compound VIII, the same compound obtained by acid hydrolysis of compound VII. The identity of the compounds obtained by hydrolysis of I and VII was proven by melting point, mixed melting point, infrared, and ultraviolet spectra. It is obvious that during refluxing with concentrated hydrochloric acid (which represented drastic conditions of hydrolysis) both the cyano and the chloro groups were hydrolyzed. The activating effect on chlorine of the two electron withdrawing groups is apparent. Hydrolysis of the chlorine of the α-chloropyridines, with concentrated hydrochloric acid has been observed in similar compounds.[10-12]

It was hoped that the replacement of the nitro group by an amino group might prevent the hydrolysis of the chlorine; therefore the route I → III → IV → V → VI was undertaken. However, when compound III was refluxed with concentrated hydrochloric acid, compound IX was formed, indicating that the chlorine was still hydrolyzable. Under milder conditions, that is, dilute hydrochloric acid (1:1) at 60° for twenty-four hours, no hydrolysis occurred. Compound IX was also obtained by catalytic hydrogenation of VIII and acidic hydrolysis of IV. The hydrolysis of III to IX with concentrated hydrochloric acid is explained as follows: Compound III is protonated in the strong acidic solution to form the carbonium ion (A) (Fig. 2) which on attack by water gives the oxonium ion

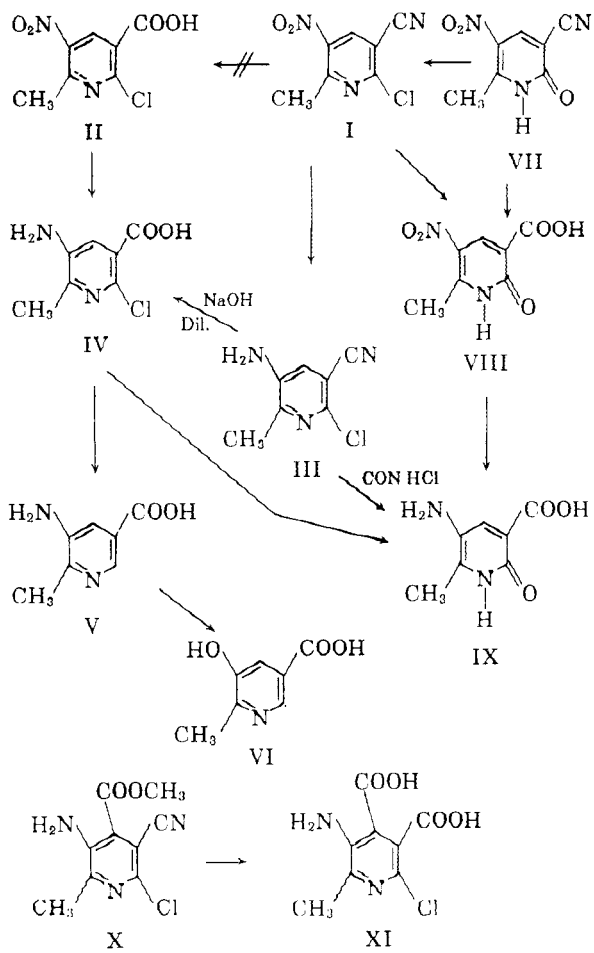

Figure 1

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(2) Portion of a thesis to be presented by C. J. Argoudelis as partial fulfillment of the requirement for the degree of Doctor of Philosophy in Food Technology.

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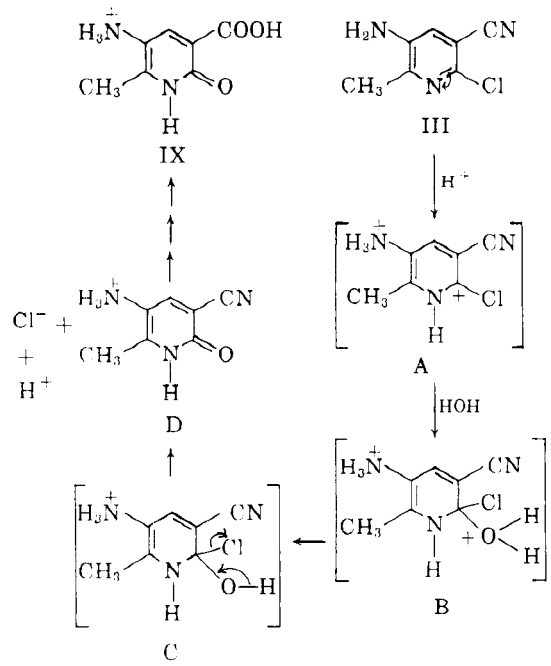

Figure 2

(B) and subsequently, IX. The proposed mechanism for the formation of IX from III is in agreement with the findings of Banks[13] that the protonation of a heteronitrogen should increase its electron-withdrawing power.

Although we failed to prepare IV by acidic hydrolysis, we were able to obtain it by alkaline hydrolysis. When compound III was hydrolyzed with 10% aqueous sodium hydroxide, compound IV was obtained. Obviously, under basic conditions the electron withdrawing power of the heteronitrogen is depressed. Furthermore, electrons are supplied to the pyridine nucleus from the electron-rich amino group so that attack of an hydroxyl anion is prevented and thus no nucleophilic displacement of chlorine can occur.

To verify our results of this specific alkaline hydrolysis, we synthesized compound X. Blackwood *et al.*[10] were unable to hydrolyze this compound to XI. However, when compound X was hydrolyzed with 10% aqueous sodium hydroxide, compound XI was obtained.

With the key intermediate (IV) available, the synthesis of compounds V and VI was a straightforward matter. Catalytic hydrogenolysis with palladium on barium carbonate removed the chlorine; compound V was isolated as the sulfate salt, which was consequently diazotized to give VI.

EXPERIMENTAL[14]

Compounds I, III, and VII were synthesized according to the procedure of McElvain *et al.*[15]

Hydrolysis of 3-cyano-6-methyl-5-nitro-2(1)pyridone. This compound was hydrolyzed according to the procedure of Mariella *et al.*[9] However, when the isolated crystals were decolorized with active carbon, they gave white needles of m.p. 271–272° dec.; reported[9] 268°. This compound (VIII) had an ultraviolet spectrum in acid-methanol with, λ_{max} 219 mμ, log ε 4.15. In base-methanol the peaks were λ_{max} 369 mμ, log ε 4.16; λ_{max} 311 mμ, log ε 3.90 and a shoulder at 225 mμ, log ε 4.07.

Hydrolysis of 2-chloro-3-cyano-6-methyl-5-nitropyridine. This compound was also hydrolyzed according to the procedure of Mariella *et al.*[9] Recrystallization from boiling water of the isolated crystals gave white needles of m.p. 271–272° dec.; reported[9] 261–262°. This compound had a negative sodium fusion test for chlorine. It did not depress the melting point of compound VIII, and both had identical ultraviolet and infrared spectra.

Anal. Calcd. for $C_7H_6N_2O_5$: C, 42.43; H, 3.05; N, 14.14. Found: C, 42.30; H, 3.05; N, 14.19.

3-Carboxy-5-amino-6-methyl-2(1)pyridone (IX) *A. From* III. A solution of 2 g. of III in 20 ml. of concd. hydrochloric acid was heated in an oil bath (temp. *ca.* 140°) for 6 hr., 20 ml. of water added, and the solution evaporated to dryness. The residue was dissolved in 50 ml. of water and the solution was again evaporated to dryness. The addition of water and evaporation to dryness was repeated three times. After this treatment addition of water did not dissolve the residue. The compound was filtered; yield 1.22 g. (61%). The sample prepared for analysis was recrystallized from 50% acetic acid to give yellow crystals of m.p. 319–320° dec. and gave a negative sodium fusion test for chlorine. Compound IX had an ultraviolet spectrum in acid-methanol with λ_{max} 337 mμ, log ε 3.93 and λ_{max} 234 mμ, log ε 3.89. In base-methanol the peaks were λ_{max} 335 mμ, log ε 3.78 and λ_{max} 239 mμ, log ε 3.84.

Anal. Calcd. for $C_7H_8N_2O_3$: C, 50.00; H, 4.80; N, 16.66. Found: C, 50.03; H, 5.00; N, 16.37.

B. From IV. A solution of 0.5 g. of IV in 5 ml. of concd. hydrochloric acid was heated in an oil bath (temp. *ca.* 130°) for 6 hr. The solution was diluted with 10 ml. of water and evaporated to dryness. Ten milliliters of water was added to the residue and the suspension was heated on a steam bath. After cooling it was filtered and the filter cake washed with water and with 95% ethanol; yield 0.38 g. (81%). A sample recrystallized from 50% acetic acid had m.p. 317–318° dec., had a negative sodium fusion test for chlorine, and did not depress the melting point of the compound obtained from III. The ultraviolet and infrared spectra were the same as those of the compound obtained from III.

C. From VIII. A solution of 1 g. of VIII in 50 ml. of 0.1*N* sodium hydroxide was hydrogenated for 6 hr. over 1 g. of palladium on carbon at room temperature and slightly above atmospheric pressure. The catalyst was removed by filtration, the filtrate concentrated to 10 ml. and 10 ml. of 1*N* hydrochloric acid added when yellow crystals precipitated. The precipitate was filtered, washed with water and with 95% ethanol; yield 0.67 g. (79%). A sample recrystallized from 50% acetic acid had a m.p. of 319–320° dec. and did not depress the melting point of the compound obtained from III. The ultraviolet and infrared spectra were the same as for the compound obtained from III.

2-Methyl-3-amino-6-chloropyridine-5-carbolylic acid (IV). Ten grams of III and 200 ml. of 10% aqueous sodium hydroxide were mixed in a 500 ml. round-bottom flask and refluxed on a steam bath, with shaking from time to time.

(13) C. K. Banks, *J. Am. Chem. Soc.*, 66, 1127 (1944).

(14) All melting points are uncorrected. Microanalyses were carried out by Clark Microanalytical Laboratories, Urbana, Ill. Ultraviolet absorption spectra were determined on a model 11M Cary recording spectrophotometer with the test compounds at *ca.* 0.002% in methanol solution, either 0.01*N* in sodium hydroxide (base) or 0.01*N* in hydrochloric acid (acid). Infrared spectra were determined with the aid of a Beckmann model IR-7 recording spectrophotometer, Nujol was used as solvent.

(15) L. A. Perez-Medina, R. P. Mariella, and S. M. McElvain, *J. Am. Chem. Soc.*, 69, 2574 (1947).

Solution was attained within 2 hr. After 12 hr. of heating, the solution was cooled and filtered to remove a very small amount of undissolved material. The solution was brought to *p*H 2–3 by adding hydrochloric acid (1:1). After cooling 9.3 g. (84%) of 2-methyl-3-amino-6-chloropyridine-5-carboxylic acid was recovered by filtration, m.p. 226–227°. This compound gave a positive sodium fusion test for chlorine. The sample prepared for analysis was decolorized with active carbon and recrystallized from 95% ethanol, m.p. 227–228°. The ultraviolet spectrum in acid-methanol had λ_{max} 347 mμ, log ε 3.47; λ_{max} 275 mμ, log ε 3.81 and a shoulder at 223 mμ, log ε 3.98. The ultraviolet spectrum in base-methanol had λ_{max} 312 mμ, log ε 3.72 and λ_{max} 258 mμ, log ε 4.00.

Anal. Calcd. for $C_7H_7ClN_2$: C, 45.06; H, 3.78; N, 15.01; Cl, 19.00; neut. equiv., 186.6. Found: C, 45.16; H, 4.04; N, 15.30; Cl, 18.95; neut. equiv., 188.3.

2-Methyl-3-aminorpyridine-5-carboxylic acid half sulfate (V). Four grams of IV was dissolved in a suspension of 2.5 g. of barium carbonate in 100 ml. of water. Then 3 g. of 5% palladium on barium carbonate was added and the mixture shaken in a Parr low-pressure hydrogenation apparatus at room temperature and 30 lbs. pressure. The hydrogenation was carried out for *ca.* 3 hr. by which time the pressure had dropped to a constant value. The catalyst was removed by filtration and the barium ions were precipitated with 6*N* sulfuric acid as barium sulfate which was removed by filtration. The filtrate was concentrated to a small volume, 95% ethanol added to bring the volume to 200 ml. and stored in the freezer overnight. Three grams of 2-methyl-3-aminopyridine-5-carboxylic acid half sulfate was isolated by filtration, m.p. 262–263° dec. On concentration of the mother liquor, another 0.5 g. was isolated; total yield 3.5 g. (81%). An analytical sample was prepared by recrystallization from 70% ethanol, m.p. 262–263° dec. The ultraviolet spectrum in acid-methanol had λ_{max} 342 mμ, log ε 3.75; λ_{max} 274 mμ, log ε 3.90; and λ_{max} 224 mμ, log ε 4.14. In base-methanol the peaks were λ_{max} 309 mμ, log ε 3.77, and λ_{max} 249 mμ, log ε 3.93.

Anal. Calcd. for $C_7H_8N_2O_2.1/2\ H_2SO_4$: C, 41.79; H, 4.51; N, 13.92; S, 7.97; neut. equiv. 100.6. Found: C, 42.02; H, 4.69; N, 13.96; S, 8.17; neut. equiv. 101.4.

2-Methyl-3-hydroxypyridine-5-carboxylic acid (VI). To a solution of 3.40 g. of the half-sulfate salt of (V) in 50 ml. of 2*N* hydrochloric acid was added at room temperature and in *small portions a solution of* 2.33 g. of sodium nitrite in 10 ml. of water. The resulting faint yellow solution was slowly added to 50 ml. of 2*N* hydrochloric acid at the boiling point. The solution was kept boiling until the evolution of gas ceased. It was then concentrated and brought to *p*H 3 with 10% aqueous sodium hydroxide, at which time crystals precipitated. After cooling 1.67 g. of 2-methyl-3-hydroxypyridine-5-carboxylic acid was collected by filtration. On concentration of the mother liquor another 0.34 g. was obtained. Total yield 2.01 g. (77%). An analytical sample was prepared by recrystallization from water. This sample darkened at 319° and sublimed at *ca.* 325°. Reported[8] m.p. 320° and 325° respectively. The ultraviolet spectrum in acid-methanol had λ_{max} 300 mμ, log ε 3.94, and λ_{max} 242 mμ, log ε 3.62. In base-methanol the peaks were λ_{max} 316 mμ, log ε 3.79, and λ_{max} 254 mμ, log ε 3.83. In 0.1*N* hydrochloric acid the peaks were λ_{max} 298 mμ, log ε 3.95, and λ_{max} 240 mμ, log ε 3.61. Reported[8] λ_{max} 298 mμ, log ε 3.93, and λ_{max} 241 mμ, log ε 3.57. In 0.1 *N* sodium hydroxide the peaks were λ_{max} 316 mμ, log ε 3.79, and λ_{max} 254 mμ, log ε 3.78. Reported λ_{max} 317 mμ, log ε 3.80, and λ_{max} 256 mμ, log ε 3.79.

Anal. Calcd. for $C_7H_7NO_3$: C, 54.90; H, 4.61; N, 9.15; neut. equiv., 153. Found: C, 55.06; H, 4.43; N, 8.79; neut. equiv., 151.5.

The *methyl ester of VI* was prepared as follows. A solution of 0.5 g. of VI in 25 ml. of absolute methanol saturated with hydrochloric acid was refluxed for 12 hr. The solvent was evaporated *in vacuo*. The residue was dissolved in 25 ml. of water and sodium bicarbonate was added until no more carbon dioxide evolved. The precipitated compound was filtered and washed with water. After recrystallization from ethyl acetate 0.35 g. of methyl 2-methyl-3-hydroxy-5-pyridinecarboxylate was collected, m.p. 243–244°; reported[8] 239–240°.

Hydrolysis of 2-methyl-3-amino-4-carbomethoxy-5-cyano-6-chloropyridine (X). This compound was synthesized according to the procedure of Blackwood, *et al.*[10] A suspension of 0.25 g. of X in 5 ml. of 10% aqueous sodium hydroxide was heated on a steam bath; solution was attained within 5 min After 19 hr. of heating the solution was filtered, acidified to *p*H 2 with hydrochloric acid (1:1), and heated on a steam bath. After cooling overnight 0.15 g. of 2-methyl-3-amino-6-chloropyridine-4,5-dicarboxylic acid was collected by filtration, m.p. 218–219° dec.; reported[10] 217.8–218.2° dec. This compound had an ultraviolet spectrum in acid-methanol with λ_{max} 360 mμ, log ε 3.77; λ_{max} 253 mμ, log ε 3.96; and λ_{max} 227 mμ, log ε 4.07. In base-methanol the peaks were λ_{max} 332 mμ, log ε 3.69, and λ_{max} 227 mμ, log ε 4.20. Reported[10] values for the ultraviolet spectra were: In acid-methanol λ_{max} 359 mμ, log ε 3.78; λ_{max} 253 mμ, log ε 3.96; and λ_{max} 227 mμ, log ε 4.06. In base-methanol λ_{max} 332 mμ, log ε 3.67, and λ_{max} 228 mμ, log ε 4.13.

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