Koones for their assistance in the preparation of the vitamin B_6 used in this work.

Summary

1. The methyl ether of vitamin B_6 was oxidized to give a lactone $C_9H_9O_3N$ and a dibasic acid $C_9H_9O_5N$.

2. The acid was shown to be 2-methyl-3-methoxypyridine-4,5-dicarboxylic acid.

3. Vitamin B_6 was shown to be 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine.

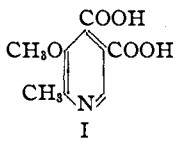
RAHWAY, NEW JERSEY RECEIVED MARCH 15, 1939

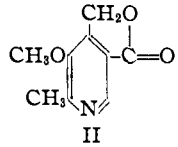
[CONTRIBUTION FROM THE RESEARCH LABORATORY OF MERCK & CO., INC.]

Structure of Vitamin B_6. II

BY STANTON A. HARRIS, ERIC T. STILLER AND KARL FOLKERS

This paper deals with the syntheses of the dibasic acid $C_9H_9O_5N$, I, and the lactone $C_9H_9O_3N$, II, which were obtained by Stiller, Keresztesy and Stevens[1] by the oxidation of the methyl ether of vitamin B_6. The synthetic lactone and acid were


I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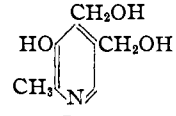
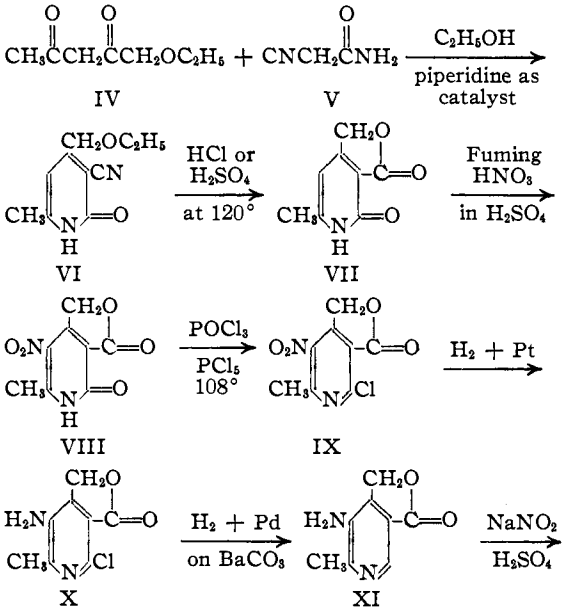
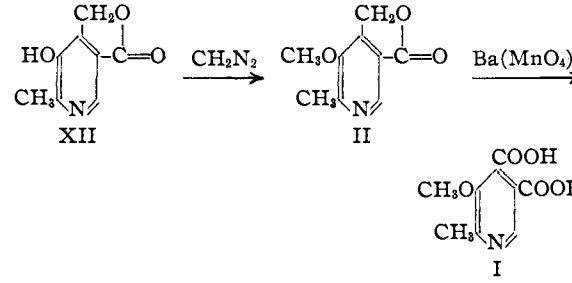

III

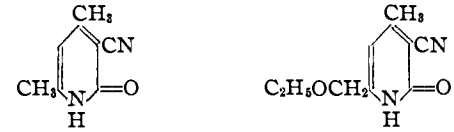
found to be identical with the corresponding compounds obtained from vitamin B_6. Thus, conclusive proof is furnished that vitamin B_6 is 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine, III. The syntheses may be represented graphically in the following manner.


IV V VI VII VIII IX X XI

(1) Stiller, Keresztesy and Stevens, THIS JOURNAL, **61**, 1237 (1939).


XII II I

The synthesis of VI is similar to the synthesis of 3-cyano-4,6-dimethyl-2-pyridone, XIII, which was obtained by the condensation of acetylacetone and cyanacetamide as previously described by Bardhan,[2] and also Simonsen and Nyak.[3]


XIII XIV

The condensation of IV and V might have led to the alternative 3-cyano-4-methyl-6-ethoxymethyl-2-pyridone, XIV. The proof that the condensation product had structure VI, instead of IV, was shown by its conversion on hydrolysis to the lactone VII, which was obtained in 85% yield. It is obvious that the pyridone derivative, XIV, would be incapable of giving a lactone on hydrolysis. The pyridone derivative, VI, was obtained pure in better than 80% yield with no evidence of any other product being formed.

Experimental Part

3-Cyano-4-ethoxymethyl-6-methyl-2-pyridone, VI.—To 65.3 g. of cyanoacetamide,[4] V, dissolved in 500 cc. of hot 95% alcohol, 93.1 g. of ethoxyacetylacetone[5] and ca. 8.5 cc.

(2) Bardhan, *J. Chem. Soc.*, 2223 (1929).

(3) Simonsen and Nyak, *ibid.*, 792 (1915).

(4) Corson, Scott, and Vose, "Org. Syntheses," Coll. Vol. I, John Wiley & Sons Co., Inc., New York, N. Y., 1932, p. 173.

(5) Sommelet, *Bull. soc. chim.*, [4] **1**, 382 (1907).

of piperidine were added with shaking. Since the mixture became warm, it was necessary to cool the solution. Crystals soon appeared. The mixture was allowed to stand overnight, cooled and filtered. The product was washed with 95% alcohol. The yield of white crystals was 92 g. or 81%, m. p. 209–210°, corr. The product was purified by crystallization from boiling 95% alcohol; m. p. 210°.

Anal. Calcd. for $C_{10}H_{12}O_2N_2$: C, 62.50; H, 6.25; N, 14.58. Found: C, 62.38; H, 6.19; N, 14.80.

The Lactone of 3-Carboxy-4-hydroxymethyl-6-methyl-2-pyridone, VII.—Fifteen grams of 3-cyano-4-ethoxymethyl-6-methyl-2-pyridone was mixed with 125 cc. of concentrated hydrochloric acid and heated at 120–125° for four hours. The reaction mixture was then poured into 400 cc. of water and ice mixture, whereupon the lactone of 3-carboxy-4-hydroxymethyl-6-methyl-2-pyridone, VII, crystallized. It was filtered, and washed with water. The dried product weighed 11.1 g. or 87%. The product was recrystallized from water; m. p. above 320°.

Anal. Calcd. for $C_8H_7NO_3$: C, 58.18; H, 4.24; N, 8.48. Found: C, 58.14, 57.90; H, 4.19, 4.19; N, 8.46.

The following method was used later and was found to be preferable. A solution of 93 g. of 3-cyano-4-ethoxymethyl-6-methyl-2-pyridone, VI, in 1120 cc. of 50% sulfuric acid, was refluxed for three hours. The temperature of the liquid was 120°. The reaction mixture was then poured into 3.5–4.0 liters of water and placed in the ice room overnight. On the following day, the crystals were filtered off, washed well with water, alcohol, and ether, and dried at a moderate temperature, 55–60°. The yield of VII was 70.5 g. or 88.2%.

The Lactone of 3-Carboxy-4-hydroxymethyl-5-nitro-6-methyl-2-pyridone, VIII.—A solution of 28 g. of the lactone of 3-carboxy-4-hydroxymethyl-6-methyl-2-pyridone, VII, in 62 cc. of concentrated sulfuric acid was added to an ice-cold mixture of 124 cc. of concentrated sulfuric acid and 52 cc. of fuming nitric acid (sp. gr. 1.5). The mixture warmed spontaneously to a temperature of 35 to 45°. After the temperature had started to fall the mixture was cooled to 15° and poured onto crushed ice. The final volume was about one liter. A yellow solid formed immediately which was filtered and dried at 65°. The yield of the lactone of 3-carboxy-4-hydroxymethyl-5-nitro-6-methyl-2-pyridone was 29.6 g. or 83.5%. On recrystallization from water, it melted at 279–280° with decomposition.

Anal. Calcd. for $C_8H_6N_2O_5$: C, 45.71; H, 2.86; N, 13.33. Found: C, 45.82; H, 2.62; N, 13.21.

The Lactone of 2-Methyl-3-nitro-4-hydroxymethyl-5-carboxy-6-chloropyridine, IX.—About 15.3 g. of the lactone of 3-carboxy-4-hydroxymethyl-5-nitro-6-methyl-2-pyridone, VIII, 50 cc. of phosphorus oxychloride and 75% excess of phosphorus pentachloride were mixed and refluxed until solution occurred (three-quarters of an hour). The phosphorus oxychloride was distilled off under reduced pressure, whereupon a solid separated. This was dissolved in benzene, filtered, and the product precipitated by adding petroleum ether. The total yield of the lactone of 2-methyl-3-nitro-4-hydroxymethyl-5-carboxy-6-chloropyridine, IX, was 12.6 g. or 77%. The product was recrystallized from benzene or ethyl acetate; m. p. 176–178°.

Anal. Calcd. for $C_8H_5N_2O_4Cl$: C, 42.01; H, 2.19; N, 12.25. Found: C, 41.96; H, 2.10; N, 12.44.

The Lactone of 2-Methyl-3-amino-4-hydroxymethyl-5-carboxy-6-chloropyridine, X.—A solution of 11.3 g. of the lactone of 2-methyl-3-nitro-4-hydroxymethyl-5-carboxy-6-chloropyridine, IX, in 250 cc. of glacial acetic acid, with 0.4 g. of platinum oxide catalyst, was shaken with hydrogen at three atmospheres pressure until three moles had been absorbed. The solution was cooled, filtered and the crystalline precipitate well washed with ether. The net yield of the lactone of 2-methyl-3-amino-4-hydroxymethyl-5-carboxy-6-chloropyridine, X, was 8.7 g. or 87.7%. After recrystallization from alcohol, the m. p. was 280–282°.

Anal. Calcd. for $C_8H_7N_2O_2Cl$: C, 48.36; H, 3.53; N, 14.11. Found: C, 48.32; H, 3.46; N, 14.33.

The Lactone of 2-Methyl-3-amino-4-hydroxymethyl-5-carboxypyridine, XI.—A suspension of 5.95 g. of the lactone of 2-methyl-3-amino-4-hydroxymethyl-5-carboxy-6-chloropyridine, X, and 10 g. of 5% Pd–$BaCO_3$ catalyst in 250 cc. of absolute alcohol was shaken with hydrogen at three atmospheres pressure at 60°. The absorption of hydrogen stopped after one mole had been used and, on cooling, spontaneous crystallization of a chlorine-free compound took place. After recrystallization from alcohol, the m. p. was 224–226°. The yield of the lactone of 2-methyl-3-amino-4-hydroxymethyl-5-carboxypyridine, XI, was 4.3 g. or 87.5%.

Anal. Calcd. for $C_8H_8N_2O_2$: C, 58.54; H, 4.88; N, 17.07. Found: C, 58.62; H, 4.76; N, 17.07.

The aminopyridine derivative, XI, was also made directly from the nitrochloropyridine derivative, IX, by dissolving 20.37 g. of the lactone of 2-methyl-3-nitro-4-hydroxymethyl-5-carboxy-6-chloropyridine, IX, in a mixture of 1 liter of ethyl acetate and 1 liter of 95% alcohol, and shaking with hydrogen in the presence of 0.75 g. of platinum oxide catalyst and 20 g. of 5% Pd–$BaCO_3$ catalyst. The first three moles of hydrogen was absorbed in fifteen minutes, whereas it took several hours to absorb the fourth mole. It was filtered from the catalyst, concentrated to dryness and recrystallized from water in the presence of charcoal. The yield of XI was 12.1 g. or 82.5%; m. p. 226°.

The Picrate of the Lactone of 2-Methyl-3-amino-4-hydroxymethyl-5-carboxypyridine.—The above aminopyridine derivative, XI, may be isolated nearly quantitatively as its picrate by adding an alcoholic solution of picric acid to an alcoholic solution of the amine. It was recrystallized from alcohol; m. p. 229–230°.

Anal. Calcd. for $C_{14}H_{11}O_9N_5$: C, 42.75; H, 2.80; N, 17.81. Found: C, 42.86; H, 2.82; N, 17.73.

The Lactone of 2-Methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine, XII.—To 4.85 g. of the lactone of 2-methyl-3-amino-4-hydroxymethyl-5-carboxypyridine, XII, dissolved in 100 cc. of 25% sulfuric acid, and cooled in ice, was added a slight excess of sodium nitrite (2.1 g. in 10 cc. of water). This diazo solution was added slowly to 25 cc. of a boiling 50% sulfuric acid solution. After the addition was complete, the solution was cooled and neutralized

to congo paper with a 30% sodium hydroxide solution. The sodium sulfate was precipitated and well washed with alcohol. The combined filtrates were evaporated to dryness, and residue redissolved in absolute alcohol. After a trace of sulfuric acid was neutralized with sodium bicarbonate, the solution was filtered in the presence of carboraffin and concentrated until crystallization took place. The total yield of the lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine, XII, was 2.1 g. It gave a strong ferric chloride test for a β-hydroxypyridine. After two recrystallizations from alcohol, the final decomposition point was 272–273°.

Anal. Calcd. for $C_8H_7O_3N$: C, 58.18; H, 4.24; N, 8.48. Found: C, 58.24; H, 4.31; N, 8.63.

The Lactone of 2-Methyl-3-methoxy-4-hydroxymethyl-5-carboxypyridine, II.—To a well-cooled solution of 258 mg. of the lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine, XII, in 50 cc. of methyl alcohol, an excess of diazomethane in 70 cc. of dry ether was added. The solution gradually developed a brown color and after standing at room temperature for sixteen hours, the solvents and excess diazomethane were removed by distillation. The residue, a dark brown viscous oil, gave no color with aqueous ferric chloride. The product was sublimed at 100–110° (10^{-4} mm.), and since the crystalline product (241 mg.) had a slight yellow color, it was resublimed at 90–95° (10^{-4} mm.). After recrystallization from water, the lactone of 2-methyl-3-methoxy-4-hydroxymethyl-5-carboxypyridine, II, was obtained as colorless needles; m. p. 108–109°; the mixed m. p. with the lactone, $C_9H_9O_3N$, from vitamin B_6 was 108–109°.

Anal. Calcd. for $C_9H_9O_3N$: C, 60.33; H, 5.03; N, 7.82. Found: C, 60.08; H, 4.91; N, 7.97.

2-Methyl-3-methoxy-4,5-pyridinedicarboxylic Acid, I.—A solution of 177 mg. of the lactone of 2-methyl-3-methoxy-4-hydroxymethyl-5-carboxypyridine, II, in 10 cc. of water was warmed on the water-bath, adding dilute barium hydroxide until the solution was alkaline to phenolphthalein after heating for one hour. After cooling to 25°, a slight excess of 0.1 *M* barium permanganate (3 atoms O) was added in small portions during two hours. The first few additions decolorized rapidly and after the final addition the solution was allowed to stand overnight at room temperature. The small excess of permanganate was destroyed and the manganese dioxide was centrifuged and washed thoroughly with hot water. The combined aqueous liquors were concentrated to 12 cc. and the barium removed quantitatively with 0.1 *N* sulfuric acid. The filtrate and the washings from the barium sulfate were taken to dryness in a stream of dry air at 50°. Since the product still contained some unchanged lactone, it was heated in a subliming apparatus at 90–100° (10^{-4} mm.). By this means, 27 mg. of the methoxy lactone, II, was obtained; m. p. (after recrystallization from water) 107–108°; mixed m. p. 107–108°.

The unsublimed material was taken up in the minimum amount of hot water and a trace of color removed with norite. On cooling the 2-methyl-3-methoxy-4,5-pyridinedicarboxylic acid, I, was obtained as colorless flattened needles; m. p. 207–208° (dec.). The mixed melting point with the dibasic acid, I, from the methyl ether of vitamin B_6, was 207–208° (dec.). For analysis, the acid was dried at 67° in high vacuum for three hours.

Anal. Calcd. for $C_9H_9O_5N$: C, 51.16; H, 4.30; N, 6.63. Found: C, 50.99; H, 4.35; N, 6.55.

Acknowledgment.—A grateful acknowledgment is made to D. F. Hayman and W. Reiss for the microanalyses given in this paper and to M. Sletzinger and A. N. Wilson for technical assistance.

Summary

3-Cyano-4-ethoxymethyl-6-methyl-2-pyridone was made from ethoxyacetylacetone and cyanacetamide. This 2-pyridone derivative was used for the syntheses of the lactone of 2-methyl-3-methoxy-4-hydroxymethyl-5-carboxypyridine and the 2-methyl-3-methoxy-4,5-pyridinedicarboxylic acid. This lactone and this acid were found to be identical with the lactone, $C_9H_9O_3N$, and the dibasic acid, $C_9H_9O_5N$, obtained by the oxidation of the methyl ether of vitamin B_6. Thus, the structure of vitamin B_6 has been proved to be 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine.

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