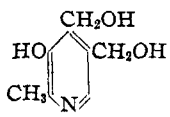
[CONTRIBUTION FROM THE RESEARCH LABORATORY OF MERCK & CO., INC.]

Synthesis of Vitamin B_6

BY STANTON A. HARRIS AND KARL FOLKERS

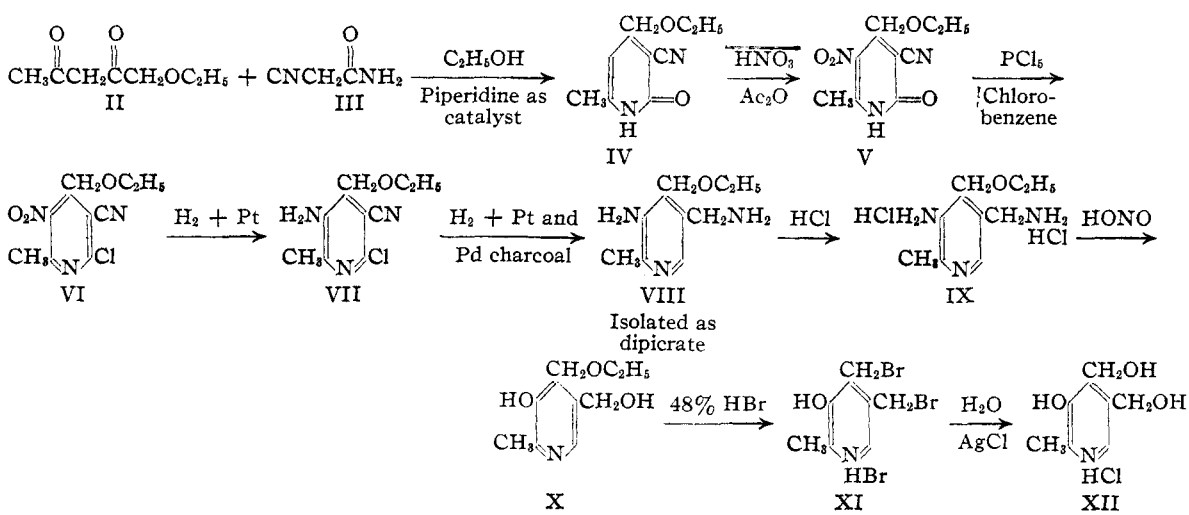
The structure of vitamin B_6 has been fully elucidated as 2-methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine, I, by the researches described in the accompanying two papers[1,2] from this Laboratory, and also by the recent three papers of Kuhn and his co-workers.[3-5]



I

The complete synthesis of the vitamin B_6, I, has been accomplished also, and the authors[6] recently reported that the synthetic vitamin B_6 hydrochloride is chemically identical with the natural vitamin B_6 hydrochloride and that it is biologically active.

This paper describes the details of the reactions used for the synthesis of vitamin B_6, and these may be represented graphically in the following manner



Vitamin B_6 is that factor of the vitamin B complex which prevents or cures an acrodynia-like dermatitis in young rats.[7-9] If factor 2 is added to the usual thiamin and riboflavin supplement, vitamin B_6 not only produces a cure of the dermatitis but also a stimulation of growth.[10] It also has been found that a severe microcytic hypochromic anemia developed in puppies when the rat antidermatitis factor (vitamin B_6) was apparently the only missing component of the diet. This anemia was cured by the addition of this factor to the diet.[11] A biological relationship between vitamin B_6 and unsaturated fatty acids also has been studied, and recently Birch[12] suggested that the physiological function of vitamin B_6 is connected with the utilization of the unsaturated fatty acids.

The biological assay of the synthetic vitamin B_6, which was performed in the Merck Institute of Therapeutic Research by Dr. E. J. Reedman, paralleled the results previously reported by Keresztesy and Stevens[13] for the natural vitamin B_6, a single dose of 100 gamma effecting a complete cure within fourteen days when fed to vitamin B_6 deficient rats. A complete report of these tests and the pharmacological properties of vitamin B_6 will be published elsewhere.

Experimental Part

3 - Cyano - 4 - ethoxymethyl - 5 - nitro - 6 - methyl - 2-pyridone, V.—Five grams of 3-cyano-4-ethoxymethyl-6-

(1) Stiller, Keresztesy and Stevens, THIS JOURNAL, **61**, 1237 (1939).
(2) Harris, Stiller and Folkers, *ibid.*, **61**, 1242 (1939).
(3) Kuhn and Wendt, *Ber.*, **72**, 305 (1939).
(4) Kuhn, Andersag, Westphal and Wendt, *ibid.*, **72**, 309 (1939).
(5) Kuhn, Wendt and Westphal, *ibid.*, **72**, 310 (1939).
(6) Harris and Folkers, *Science*, **89**, 347 (1939).
(7) György, *Nature*, **133**, 498 (1934).
(8) Birch, György and L. J. Harris, *Biochem. J.*, **29**, 741 (1935).
(9) György, THIS JOURNAL, **60**, 983 (1938).
(10) Lepkovsky, *J. Biol. Chem.*, **124**, 125 (1938).
(11) Fouts, Helmer, Lepkovsky and Jukes, *J. Nutrition*, **16**, 197 (1938).
(12) Birch, *J. Biol. Chem.*, **124**, 775 (1938).
(13) Keresztesy and Stevens, *Proc. Soc. Exptl. Biol. Med.*, **38**, 64-65 (1938).

methyl-2-pyridone,[2] IV, in 13 cc. of acetic anhydride was cooled in ice and treated with 2.2 cc. of fuming nitric acid in 2 cc. of acetic anhydride with a little urea. The solid gradually dissolved as the mixture evolved heat. When the temperature had increased to 40–45°, it was cooled to 25° and then allowed to stand until no further heat of reaction was noticeable. When it was poured onto ice, crystallization took place. It was filtered, dissolved in ammonium hydroxide and recrystallized by adding hydrochloric acid. The product was readily soluble in hot water, alcohol, benzene, ethyl acetate, dioxane, and nearly insoluble in ether and petroleum ether. The yield of 2-methyl-3-nitro-4-ethoxymethyl-5-cyano-2-pyridone, V, was about 2 g. (32%). After recrystallization, the melting point was 164–165°.

Anal. Calcd. for $C_{10}H_{11}O_4N_3$: C, 50.64; H, 4.64; N, 17.72. Found: C, 50.93; H, 4.63; N, 17.47.

2 - Methyl - 3 - nitro - 4 - ethoxymethyl - 5 - cyano - 6-chloropyridine, VI.—A mixture of 60 g. of 3-cyano-4-ethoxymethyl-5-nitro-6-methyl-2-pyridone, V, 66 g. of phosphorus pentachloride (25% excess) and 510 cc. of dry chlorobenzene was heated until solution was effected. Heating was continued at such a rate that the phosphorus oxychloride, hydrogen chloride and chlorobenzene distilled off slowly from the solution at atmospheric pressure. After about one-half the solvent had been removed (four to five hours), the evolution of hydrogen chloride had practically stopped. The remaining solvent was removed under reduced pressure (10 mm.) leaving a brown viscous residue. To this cooled residue was added about 100 cc. of water and 20 cc. of ethanol and then the resulting mixture was extracted eight or ten times with petroleum ether. This extract was concentrated on a steam-bath, first at atmospheric pressure and finally at about 1 mm. pressure, in order to remove the last traces of chlorobenzene which interferes with subsequent crystallization. The residue was dissolved in about 50 cc. of 95% ethanol, cooled and a little water was added slowly to reduce the solubility, but not enough to cause a precipitation of the product as an oil. The addition of crystals as seeds was frequently desirable. Originally, this crude chloropyridine derivative was sublimed at 100–125° at 10^{-4} mm. to obtain the pure crystalline substance. The product was recrystallized from alcohol.

The yield of 2-methyl-3-nitro-4-ethoxymethyl-5-cyano-6-chloropyridine, VI, was 20 g. (31%); m. p. 47–48°. About 10% more of the substance can be obtained from the mother liquor.

Anal. Calcd. for $C_{10}H_{10}O_3N_3Cl$: C, 46.96; H, 3.91; N, 16.46. Found: C, 46.79; H, 3.92; N, 16.18.

2 - Methyl - 3 - amino - 4 - ethoxymethyl - 5 - cyano - 6-chloropyridine, VII.—A solution of 25.5 g. of 2-methyl-3-nitro-4-ethoxymethyl-5-cyano-6-chloropyridine, VI, in 300 cc. of 95% alcohol was shaken in the presence of 0.5 g. of Adams platinum catalyst with hydrogen at a pressure of three atmospheres. The hydrogenation was stopped after three moles of hydrogen (one-half hour) had been absorbed and the mixture was allowed to cool. The mother liquor was decanted and the crystalline compound extracted with hot alcohol. Thus, 15 g. of pure 2-methyl-3-amino-4-ethoxymethyl-5-cyano-6-chloropyridine, VII, was obtained. From the mother liquor, an additional 2.2 g. was obtained, making the total yield 76%; m. p. 146–148°.

Anal. Calcd. for $C_{10}H_{12}ON_3Cl$: C, 53.21; H, 5.32; N, 18.63. Found: C, 53.11; H, 5.11; N, 18.48.

Dipicrate of 2-Methyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine, VIII.—A solution of 31 g. of 2-methyl - 3 - amino - 4 - ethoxymethyl - 5 - cyano - 6-chloropyridine, VII, in 1400 cc. of glacial acetic acid with 11.3 g. of sodium acetate, 0.5 g. of Adams platinum catalyst and 30 g. of 5% palladium charcoal catalyst was shaken with hydrogen at a pressure of three atmospheres until three moles had been absorbed. After filtering from the catalyst, the solution was concentrated under diminished pressure and then taken up in alcohol. After separating from sodium chloride, the solution was treated with an alcoholic solution of 70 g. of picric acid. A picrate separated on scratching and standing, and was recrystallized from alcohol. It melted at 186–187° and analyzed for a dipicrate, yield 49 g. (54.5%).

Anal. Calcd. for $C_{22}H_{23}O_{15}N_9$: C, 40.43; H, 3.52; N, 19.30. Found: C, 40.19; H, 3.65; N, 19.55.

The Dihydrochloride of 2-Methyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine, IX.—The dipicrate of 2-methyl - 3 - amino - 4 - ethoxymethyl - 5 - aminomethylpyridine (38.6 g.), VIII, was treated with 100 cc. of hydrochloric acid (1:1) and the liberated picric acid was extracted first with nitrobenzene and finally with ether, until the ether showed no more yellow color. The acid solution was concentrated to a thick sirup under diminished pressure, and an equal volume of alcohol was added. The hydrochloride crystallized after adding acetone and scratching. Further addition of acetone caused complete crystallization of the dihydrochloride; yield 12.9 g. (81.5%). The melting point was 195° after crystallization from absolute alcohol and acetone.

Anal. Calcd. for $C_{10}H_{19}ON_3Cl_2$: C, 44.78; H, 7.09; N, 15.67. Found: C, 45.11; H, 6.99; N, 15.66.

The Hydrochloride of 2-Methyl-3-hydroxy-4-ethoxymethyl-5-hydroxymethylpyridine, X.—The dihydrochloride of 2-methyl-3-amino-4-ethoxymethyl-5-aminomethylpyridine (4 g.), IX, was dissolved in 20 cc. of 2 *N* sulfuric acid and added slowly to a hot (90°) mixture of 2 *N* sulfuric acid (50 cc.) and sodium nitrite (7.5 g.). There was an immediate evolution of nitrogen. The light yellow solution was heated for an additional five minutes, treated with just enough urea to decompose the excess nitrous acid, cooled and neutralized to *p*H 7.2 with sodium hydroxide solution, using brom thymol blue as an outside indicator. This slightly reddish solution was concentrated under diminished pressure until sodium sulfate started to separate. At this point, a dark oily layer was formed which contained most of the desired product. The oily layer was dissolved in alcohol, filtered from separated sodium sulfate, and evaporated to dryness. (In other experiments the entire concentrated mixture was treated with alcohol, etc.) The residue was then dissolved in acetone and filtered from separated sodium chloride. This solution gave a strong ferric chloride test for a β-hydroxypyridine. Dry hydrogen chloride was added to the acetone solution until it was acid to congo paper. A small amount of an oily layer separated and the acetone layer was de-

canted. Addition of a small amount of ether gave a second oily layer which also was separated. On further addition of ether and scratching, crystallization commenced and was allowed to proceed on standing in a cold room at 2–5°. The solution was filtered, yielding 1 g. of a hydrochloride; m. p. 110–120°. This was recrystallized by dissolving in a minimum of absolute alcohol, adding 2–3 volumes of acetone and finally ether until crystallization took place; m. p. 123–125°. This hydrochloride gave a strong positive ferric chloride test similar to that shown by vitamin B_6.

Anal. Calcd. for $C_{10}H_{16}O_3NCl$: C, 51.39; H, 6.85; N, 6.00. Found: C, 51.14; H, 7.12; N, 6.49.

2-Methyl-3-hydroxy-4,5-di-(bromomethyl)-pyridine Hydrobromide, XI.—A solution containing 0.5 g. of the hydrochloride of 2-methyl-3-hydroxy-4-ethoxy-methyl-5-hydroxymethylpyridine, X, in 25 cc. of 48% hydrobromic acid was heated at the boiling point for ten minutes. On cooling in ice water, crystals separated and were filtered, washed with water, acetone and ether; m. p. 223–224°, showing partial decomposition at 219°; yield 0.53 g. (66%).

This compound apparently is identical with the one described by Kuhn and Wendt[14] as having a melting point of 217°. They obtained it from the β-methyl ether of natural vitamin B_6 by the use of 66% hydrobromic acid.

Anal. Calcd. for $C_8H_{10}ONBr_3$: C, 25.53; H, 2.66; N, 3.72. Found: C, 25.95, 25.90; H, 2.89, 2.84; N, 3.73.

Vitamin B_6 Hydrochloride or 2-Methyl-3-hydroxy-4,5-di-(hydroxymethyl)-pyridine Hydrochloride, XII.—The 2-methyl-3-hydroxy-4,5-di-(bromomethyl)-pyridine hydrobromide (1.78 g.), XI, was converted to vitamin B_6 hydrochloride by boiling in 150 cc. of water for twenty minutes and removing the bromide ions with freshly prepared silver chloride. The filtrate was evaporated to dryness, dissolved in 1 cc. of water and 5 cc. of alcohol, filtered with charcoal and crystallized by adding acetone; m. p. 206–208°, mixed m. p. with natural vitamin B_6 hydrochloride, 206–208°. The yield was 0.42 g. of crystals plus 0.30 g. of crystalline residue making the total yield about 75%.

Anal. Calcd. for $C_8H_{12}NO_3Cl$: C, 46.72; H, 5.84; N, 6.81. Found: C, 46.64; H, 5.69; N, 6.75.

Acknowledgments.—The authors wish to thank Drs. Major, Engels, Stevens and Kereszstesy for helpful advice and encouragement, Messrs. Hayman and Reiss for the microanalyses, and Messrs. Sletzinger and Wilson for technical assistance.

Summary

A complete synthesis of vitamin B_6 starting with ethoxyacetylacetone and cyanoacetamide has been accomplished. The synthetic vitamin B_6 hydrochloride is identical with the natural vitamin B_6 hydrochloride. A single dose of 100 gamma of synthetic vitamin B_6 hydrochloride gave a curative effect which paralleled that of the natural vitamin B_6.

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(14) Kuhn and Wendt, *Ber.*, **72**, 311 (1939).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MICHIGAN COLLEGE OF MINING AND TECHNOLOGY]

Chelate Compounds as Flotation Reagents. I

BY C. C. DE WITT AND FREDERICK VON BATCHELDER

The purpose of this paper is to present quantitative data obtained with a new series of flotation reagents.[1] The present report deals with a compound of known chelate structure, salicylaldoxime, and its isomers, meta and para hydroxybenzaldoxime. It is perhaps significant that the ortho derivative when used as a flotation reagent recovers in commercial yields not only the copper sulfides, but also the copper carbonates and cuprous oxide from a siliceous gangue. The only existing report on the use of oximes as flotation reagents of which the authors are aware is that of Holman,[2] who used dimethylglyoxime as a flotation reagent for the recovery of oxidized nickel ores.

(1) Previous work by the senior author in this Laboratory had shown the effectiveness of certain chelate compounds including salicylaldoxime in the separation of heavy metal minerals from siliceous gangue.

(2) B. W. Holman, *Bull. Inst. Min. and Met. Nr.*, 314 (1930).

Preparation of Oximes.—Salicylaldoxime and the *m*- and *p*-hydroxybenzaldoximes were prepared by the method of Brady and Dunn.[3] The crude salicylaldehyde[4] was purified by bisulfite precipitation, washing this precipitate with alcohol to remove phenol, etc., followed by recrystallization from water, acidification and steam distillation, drying, and finally distillation at atmospheric pressure. The *m*- and *p*-hydroxybenzaldehydes were Eastman best grade; these were carefully recrystallized from alcohol.

Preparation of Synthetic Copper Ores.—The copper minerals used were authentic, massive samples of relatively pure chalcocite, covellite, azurite, malachite and cuprite procured from a reputable source. These samples were examined mineralogically by Professor W. A. Seaman and analyzed for copper content in this Laboratory. These minerals were crushed and sized. Those portions which passed through a 40-mesh sieve and were retained on a 60-mesh sieve were reserved for flotation tests. The

(3) Brady and Dunn, *J. Chem. Soc.*, **105**, 821 (1914).

(4) Acknowledgment is made to the Dow Chemical Company for generous amounts of crude salicylaldehyde supplied for this work.