yield 6.71 g. (35%); m.p. 54–55°, which did not change on recrystallization.

Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.52; H, 6.71; N, 7.18. Found: C, 61.77; H, 6.51; N, 7.24.

3-Pyridoxal Methyl Ether (VII).—A solution of 4.68 g. of the monomethyl acetal of 3-pyridoxal methyl ether in water was adjusted to *p*H 2 with hydrochloric acid. After brief warming and subsequent standing at room temperature for three-quarters of an hour, the solution was concentrated almost to dryness, and acetone and ether were added. Crystals of the hydrochloride of the monomethyl acetal of 3-pyridoxal methyl ether crystallized in a yield of 3.72 g. (67%); m.p. 152–153°.

Anal. Calcd. for $C_{10}H_{14}NO_3Cl$: C, 51.84; H, 6.09; N, 6.05. Found: C, 52.03; H, 6.00; N, 6.04.

Under the conditions of this experiment, pyridoxal monoethyl acetal is completely hydrolyzed to pyridoxal.[4]

On neutralization of an aqueous solution of the hydrochloride with sodium bicarbonate, subsequent distillation to dryness, and extraction of the residue with petroleum ether, crystals of the monomethyl acetal of 3-pyridoxal methyl ether melting at 53–54° were obtained. The melting point of a mixed sample of these crystals and the material from which the hydrochloride was made, was the same.

The hydrolysis was repeated under more vigorous conditions. A solution of 1.0 g. of the monomethyl acetal of 3-pyridoxal methyl ether hydrochloride in 15 ml. of water was treated with 3.5 ml. of 6 *N* hydrochloric acid. After 30 minutes on the steam-bath and two days at room temperature, the solution was distilled almost to dryness under reduced pressure. The crystalline 3-pyridoxal methyl ether hydrochloride was suspended in acetone and then filtered; yield 0.81 g. (86%); dec. 179–180°. This decomposition point was not changed on recrystallization from water–acetone.

Anal. Calcd. for $C_9H_{12}NO_3Cl$: C, 49.66; H, 5.56; N, 6.44. Found: C, 50.14; H, 5.88; N, 6.64.

Monoethyl Acetal of 3-Pyridoxal Acetate Hydrochloride (VIII).—Five milliliters of acetic anhydride containing 0.23 g. of the monoethyl acetal of pyridoxal hydrochloride and two drops of alcoholic hydrogen chloride was stirred for two hours at approximately 50°. The resulting homogeneous solution was "freeze-dried." After a little alcohol had been added to the residue and the volatile material removed under reduced pressure, the residue was crystallized from alcohol–ether; yield 0.22 g. (82%). After recrystallization from alcohol–ether, the monoethyl acetal of 3-pyridoxal acetate hydrochloride melted at 161–162°. It gave no color reaction with ferric chloride solution.

Anal. Calcd. for $C_{12}H_{16}NO_4Cl$: C, 52.65; H, 5.89; N, 5.12. Found: C, 52.84; H, 5.79; N, 5.58.

RAHWAY, N. J. RECEIVED JANUARY 12, 1951

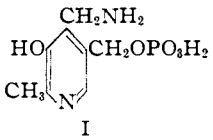
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Phosphates of the Vitamin B_6 Group. III. Pyridoxamine Phosphate

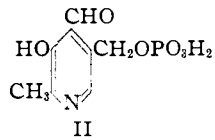
BY DOROTHEA HEYL, EILEEN LUZ, STANTON A. HARRIS AND KARL FOLKERS

Pyridoxamine phosphate (2-methyl-3-hydroxy-4-aminomethyl-5-pyridylmethylphosphoric acid) has been synthesized, isolated as an amorphous calcium salt, and characterized as a crystalline di-*p*-toluenesulfonyl derivative. Attempts to obtain pyridoxamine phosphate by catalytic hydrogenation of codecarboxylase oxime have resulted in removal of the phosphate group.

Pyridoxamine phosphate (I) has been synthesized and characterized as a crystalline di-*p*-toluenesulfonyl derivative. This phosphate (I) is the pyridoxamine analog of codecarboxylase (II).[1]



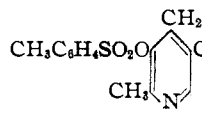
I



II

It has been shown that pyridoxamine phosphate is a growth factor for lactic acid bacteria.[2,3] Since pyridoxamine phosphate was prepared[2] "in solution" (not isolated) by autoclaving codecarboxylase with glutamic acid, the phosphoric acid group must be in the same position in pyridoxamine phosphate as in codecarboxylase.

Pyridoxamine phosphate has now been prepared by direct phosphorylation of pyridoxamine in aqueous solution with phosphorus oxychloride, and has been isolated as a crude calcium salt. Since this material[2] has the same activity as that[2] prepared by amination of the aldehyde, the free acid has structure I. On reaction with *p*-toluenesulfonyl chloride in the presence of sodium hydroxide, and subsequent acidification, 2-methyl-3-*p*-toluenesulfonoxy - 4 - *p* - toluenesulfonylaminomethyl - 5-pyridylmethylphosphoric acid (III) was obtained in crystalline form and was satisfactorily characterized. This compound no longer gives color tests with ferric chloride or diazotized aniline, as do pyridoxamine and pyridoxamine phosphate. Therefore, one *p*-toluenesulfonyl group is on the 3-hydroxy group; the second is on the primary amino group.



III, R = PO_3H_2
IV, R = H

A similar di-*p*-toluenesulfonyl (IV) derivative was prepared from pyridoxamine by the same method.

The hydrogenation of pyridoxal oxime to pyridoxamine has been described previously.[4] Attempts to convert codecarboxylase oxime to pyridoxamine phosphate by hydrogenation over palladium or nickel catalysts have resulted in removal of the phosphoric acid group.

Experimental[5]

Calcium Pyridoxamine Phosphate (Calcium Salt of 2-Methyl-3-hydroxy-4-aminomethyl-5-pyridylmethylphosphoric Acid (I)).—A solution of 5 g. of pyridoxamine dihydrochloride in 35 ml. of water was surrounded by a cold water-bath. The solution was stirred mechanically while 25 ml. of phosphorus oxychloride was added at such a rate that the temperature did not exceed 50°; the addition, which required 75 minutes, was followed by 30 minutes of stirring. After as much hydrogen chloride as possible had been removed *in vacuo*, the solution was surrounded by an

(1) Heyl, Luz, Harris and Folkers, THIS JOURNAL, **73**, 3434 (1951).
(2) McNutt and Snell, *J. Biol. Chem.*, **182**, 557 (1950).
(3) Hendlin, Caswell, Peters and Wood, *ibid.*, **186**, 647 (1950).
(4) Harris, Heyl and Folkers, THIS JOURNAL, **66**, 2088 (1944).
(5) We are indebted to Mr. Richard Boos and his associates for the microanalyses.

ice-water-bath, and a suspension of 150 g. of calcium carbonate in 110 ml. of water was added with occasional stirring. The resulting reaction mixture had a *p*H of 5–6. After an hour of chilling, the precipitated salts were collected on a filter and washed with 60 ml. of ice-water. The combined filtrate and washings were cleared by filtration through a fine-grained sintered glass disc to give 100 ml. of a light greenish-yellow solution. After this filtrate had been diluted with 300 ml. of ethyl alcohol and chilled, the white gelatinous material was separated by centrifuging and was washed twice with alcohol and once with ether. The dried material consisted of 0.4 g. of crude calcium pyridoxamine phosphate. Addition of ammonium hydroxide to the supernatant solution from the first centrifuging (the washings were discarded) until the solution had a *p*H of 8, and chilling overnight, caused the separation of 0.42 g. of crude calcium pyridoxamine phosphate. It was washed twice with alcohol and once with ether. Both fractions were dried in a vacuum desiccator.

2-Methyl-3-*p*-toluenesulfonoxy-4-*p*-toluenesulfonylaminomethyl-5-pyridylmethylphosphoric Acid (III).—A suspension of 0.82 g. of crude calcium pyridoxamine phosphate in 15 ml. of 1 *N* sodium hydroxide was shaken with a solution of 1.5 g. of *p*-toluenesulfonyl chloride in 10 ml. of ether for four hours. The resulting mixture was centrifuged and the ether layer decanted. The aqueous layer was extracted twice with ether, and the extract separated each time by centrifuging and decanting. After the last centrifuging, the water layer was removed. The white solid remaining was treated with an excess of dilute hydrochloric acid. The resulting solid material was collected by centrifuging and was washed twice with water, and once each with alcohol and ether. The yield of 2-methyl-3-*p*-toluenesulfonoxy-4-*p*-toluenesulfonylaminomethyl-5-pyridylmethylphosphoric acid was 190 mg. The derivative was recrystallized by solution in aqueous sodium bicarbonate and reprecipitation with hydrochloric acid. After collection by centrifuging, the material was washed with water until the washings were free of chloride ions, then with alcohol, and finally with ether; m.p. 189–190° (dec.).

Anal. Calcd. for $C_{22}H_{25}N_2O_9PS_2$: C, 47.47; H, 4.53; N, 5.03; P, 5.57. Found: C, 47.41; H, 4.58; N, 5.15; P, 5.8.

2-Methyl-3-*p*-toluenesulfonoxy-4-*p*-toluenesulfonylaminomethyl-5-hydroxymethylpyridine Hydrochloride (IV).—A solution of 1 g. of pyridoxamine dihydrochloride and 0.93 g. of potassium hydroxide in 15 ml. of water was shaken with a solution of 1.59 g. of *p*-toluenesulfonyl chloride in 10 ml. of ether for four hours. The resulting mixture was centrifuged, and the clear ether and water layers were removed from the dark oil at the bottom by decanting. After the oil had been washed with sodium bicarbonate solution, it was dissolved in alcohol and acidified with alcoholic hydrogen chloride. Addition of ether and cooling in an ice-bath caused crystallization of 0.98 g. (46%) of 2-methyl-3-*p*-toluenesulfonoxy-4-*p*-toluenesulfonylaminomethyl-5-hydroxymethylpyridine hydrochloride. After recrystallization from alcohol, it melted at 187–189°.

Anal. Calcd. for $C_{22}H_{25}N_2O_6ClS_2$: C, 51.50; H, 4.91; N, 5.46. Found: C, 51.72; H, 4.97; N, 5.76.

RAHWAY, N. J. RECEIVED JANUARY 12, 1951

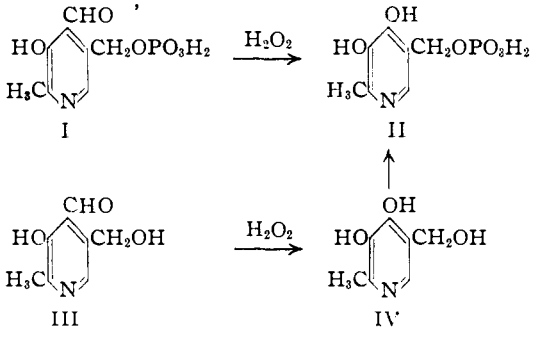
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK AND CO., INC.]

Phosphates of the Vitamin B_6 Group. IV. An Oxidation Product of Codecarboxylase

BY DOROTHEA HEYL, EILEEN LUZ AND STANTON A. HARRIS

Oxidations of codecarboxylase and pyridoxal with hydrogen peroxide in alkaline solutions have yielded 2-methyl-3,4-dihydroxy-5-pyridylmethylphosphoric acid and 2-methyl-3,4-dihydroxy-5-hydroxymethylpyridine, respectively. The latter has been converted into two methyl and two *p*-toluenesulfonyl derivatives. This oxidation of codecarboxylase provides further evidence that the phosphoric acid group is on the 5-hydroxymethyl group of pyridoxal.

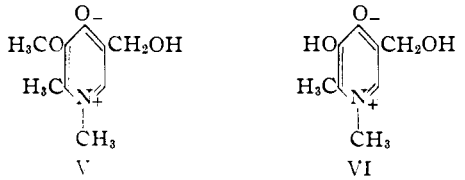
Codecarboxylase (I),[1] when oxidized with hydrogen peroxide in alkaline solution, yields 2-methyl-3,4-dihydroxy-5-pyridylmethylphosphoric acid (II). Pyridoxal (III) also undergoes this same oxidation, yielding 2-methyl-3,4-dihydroxy-5-hydroxymethylpyridine (IV), which can be phosphorylated in aqueous solution with phosphorus oxychloride to yield compound II.



The reaction of hydrogen peroxide with ortho-hydroxyaldehydes to give ortho-dihydroxy derivatives was described by Dakin.[2] Its application to codecarboxylase provides further evidence that the phosphoric acid group is on the 5-hydroxymethyl group of the pyridoxal nucleus.[1,3,4]

Compounds II and IV do not show the yellow color characteristic of codecarboxylase[1] and pyridoxal in alkaline solutions. Their ultraviolet absorptions at *p*H 11 in the range of 2200 to 3400 Å. (Fig. 1)[5] also differ from those of codecarboxylase and pyridoxal.[1]

Two methyl derivatives of compound IV were prepared by the action of dimethyl sulfate. In solutions close to neutral, the betaine of 1,2-dimethyl-3-methoxy-4-hydroxy-5-hydroxymethylpyridine (V) was formed. The use of strongly alkaline solutions produced the betaine of 1,2-dimethyl-3,4-dihydroxy-5-hydroxymethylpyridine (VI). The former gave no color with ferric chloride; the latter showed the characteristic deep



(1) Paper I of this series, Heyl, Luz, Harris and Folkers, THIS JOURNAL, **73**, 3430 (1951).

(2) Dakin, *Proc. Chem. Soc.* (*London*), **25**, 194 (1909).

(3) Heyl and Harris, THIS JOURNAL, **73**, 3434 (1951).

(4) Heyl, Luz, Harris and Folkers, *ibid.*, **73**, 3436 (1951).

(5) We are indebted to Dr. Charles Rosenblum and his associates for the ultraviolet absorption measurements.