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

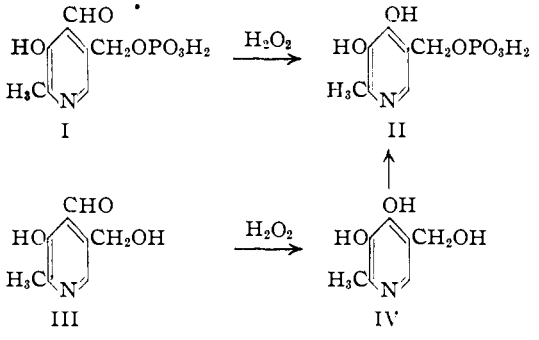
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK AND CO., INC.]

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.

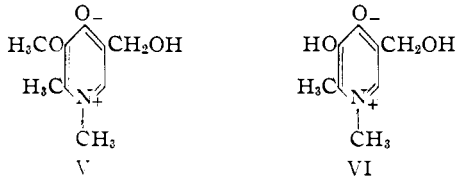
Codecarboxylase (I),[1] when oxidized with hydrogen peroxide in alkaline solution, yields 2-methyl-3,4-dihydroxy-5-pyridylmethylphosphoric acid (II). Pyridoxal (III) also undergoes this same oxidation, yielding 2-methyl-3,4-dihydroxy-5-hydroxymethylpyridine (IV), which can be phosphorylated in aqueous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

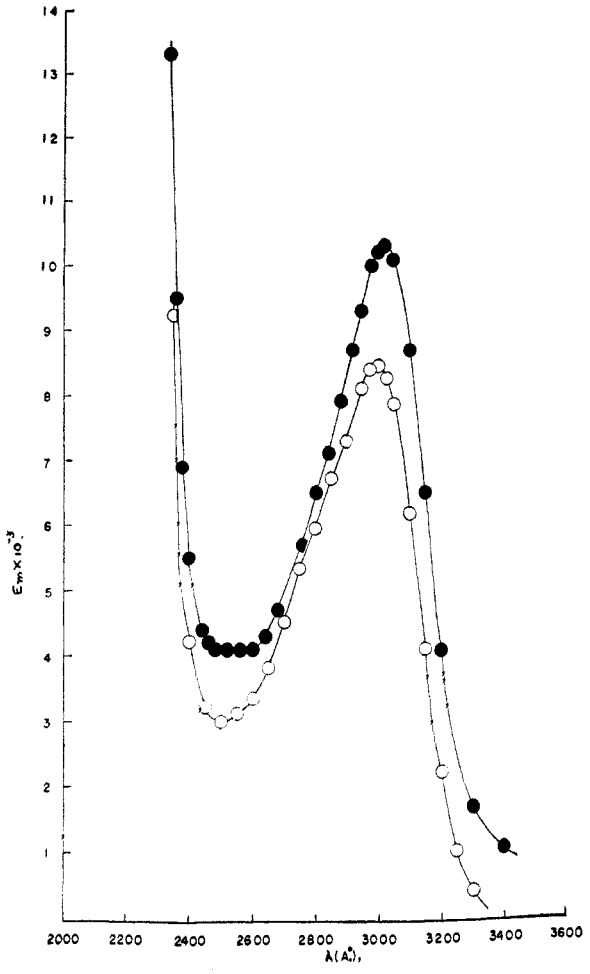
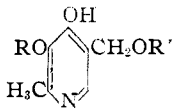


Fig. 1.—Absorption spectra at *p*H 11: O, 2-methyl-3,4-dihydroxy-5-pyridylmethylphosphoric acid (II); ●, 2-methyl-3,4-dihydroxy-5-hydroxymethylpyridine (IV).

red color given by 3-hydroxypyridines. Compounds II and IV showed with ferric chloride a purple color, which is known to be produced by 3,4-dihydroxypyridine compounds.[6]

Compounds VII and VIII, two derivatives of compound IV, were prepared by the action of *p*-toluenesulfonyl chloride. Neither one gave any color with ferric chloride solution.



VII, R = $SO_2C_6H_4CH_3$, R′ = H
VIII, R and R′ = $SO_2C_6H_4CH_3$

Experimental[7]

2-Methyl-3,4-dihydroxy-5-pyridylmethylphosphoric Acid (II).—A saturated oxalic acid solution was added dropwise with thorough stirring to 100 mg. of calcium codecarboxylase (estimated by biological assay to be about 80% pure) until the yellow solid had all disappeared. The white precipitate of calcium oxalate was removed by centrifuging and washed twice with water. The combined solution and washings were chilled in an ice-bath and made alkaline (*p*H 10) by the dropwise addition of sodium hydroxide solution. Several drops of 30% hydrogen peroxide were added, also dropwise, and the solution warmed to room temperature. The color change from bright yellow to a very pale yellow required about ten minutes. After removal of some calcium hydroxide by centrifuging, the solution, chilled in ice, was acidified (*p*H 2) with 6 *N* hydrochloric acid. The 2-methyl-3,4-dihydroxy-5-pyridylmethylphosphoric acid crystallized promptly. It was washed successively with ice-water, alcohol and ether; yield 18.0 mg., m.p. 229–230° (dec.).

Anal. Calcd. for $C_7H_{10}NO_6P$: C, 35.75; H, 4.29; N, 5.96; P, 13.19. Found: C, 35.55; H, 4.63; N, 5.73; P, 13.00.

2-Methyl-3,4-dihydroxy-5-hydroxymethylpyridine (IV).—A solution of 5.0 g. of pyridoxal hydrochloride dissolved in 15 ml. of water was cooled in an ice-bath and made alkaline to *p*H 10 with 6 *N* sodium hydroxide. Hydrogen peroxide (30%) was added, several drops at a time, to the bright yellow mixture, which had been removed from the ice-bath. After about 5 ml. had been added, the mixture became almost colorless. It was cooled in ice and acidified (*p*H 5) with hydrochloric acid. After further cooling, the crystals of 2-methyl-3,4-dihydroxy-5-hydroxymethylpyridine were collected on a filter and washed successively with water, alcohol and ether; yield 2.91 g. (76%); m.p. 226–227° (dec.). Two recrystallizations from water did not change the melting point.

Anal. Calcd. for $C_7H_9NO_3$: C, 54.16; H, 5.85; N, 9.03. Found: C, 54.40; H, 5.94; N, 8.97.

Calcium Salt of 2-Methyl-3,4-dihydroxy-5-pyridylmethylphosphoric Acid (II).—Twenty-five ml. of phosphorus oxychloride was added dropwise with mechanical stirring to 4.95 g. of 2-methyl-3,4-dihydroxy-5-hydroxymethylpyridine (IV) suspended in 35 ml. of water. The rate of addition was regulated so that the temperature did not rise above 50°. The addition required almost two hours. After an additional half hour of stirring and removal of hydrogen chloride under reduced pressure, the mixture was surrounded by a water-bath at 5°, and a suspension of calcium carbonate in water was added until the color turned pink and the solution showed a *p*H of 5. After one hour of chilling, the precipitate was removed by filtering and washed with ice water. The aqueous filtrate and washings, totaling 120 ml., was diluted with three volumes of alcohol. After two hours of cooling, the pink precipitate was centrifuged and washed twice with alcohol and once with ether; yield 0.33 g. After two days further chilling, an additional 0.55 g. was obtained.

2-Methyl-3,4-dihydroxy-5-pyridylmethylphosphoric Acid (II).—The calcium salt described above was suspended in water, cooled in ice, and 2.5 *N* hydrochloric acid was added until the *p*H of the solution was 2. The crystals of free 2-methyl-3,4-dihydroxy-5-pyridylmethylphosphoric acid were filtered and washed successively with water, alcohol and ether; m.p. 233–234° (dec.), after extraction with hot water, filtration and washing as before. The melting point was not depressed when the material was mixed with a sample prepared by oxidation of codecarboxylase.

Anal. Calcd. for $C_7H_{10}NO_6P$: C, 35.75; H, 4.29; N, 5.96; P, 13.19. Found: C, 35.12; H, 3.97; N, 5.84; P, 13.00.

Betaine of 1,2-Dimethyl-3-methoxy-4-hydroxy-5-hydroxymethylpyridine (V).—A solution of 1.2 g. of 2-methyl-3,4-dihydroxy-5-hydroxymethylpyridine (IV) in 1 ml. of 6 *N* sodium hydroxide was treated with 0.5-ml. portions of dimethyl sulfate until a total of 2 ml. had been added. The mixture was shaken between each addition until the dimethyl sulfate had reacted, and sodium hydroxide was added to bring the resulting acidic solutions back to alkalinity. The final solution was cooled in an ice-bath and adjusted to *p*H 5 with 6 *N* sulfuric acid. It was extracted continuously with chloroform for ten hours. The chloroform extract was distilled to dryness and the residue crystallized from chloroform–ether and then alcohol–ether. The 140 mg. of the betaine of the 1,2-dimethyl-3-methoxy-4-hydroxy-5-hydroxymethylpyridine was again recrystallized from alcohol–ether; m.p. 163–164°. Additional material recovered from the filtrates increased the yield to 270 mg. (19%).

Anal. Calcd. for $C_9H_{13}NO_3$: C, 59,00; H, 7.15; N, 7.65; OCH_3, 16.92; NCH_3, 8.19. Found: C, 59.09; H, 7.27; N, 7.43; OCH_3, 17.00; NCH_3, 7.55.

(6) Maier-Bode and Altpeter, "Das Pyridin und seine Derivate," Halle, 1934, p. 144.

(7) We are indebted to Mr. Richard Boos and his associates for the microanalyses.

Betaine of 1,2-Dimethyl-3,4-dihydroxy-5-hydroxymethylpyridine (VI).—A solution of 1.32 g. of 2-methyl-3,4-dihydroxy-5-hydroxymethylpyridine (IV) in 10 ml. of 6 *N* sodium hydroxide was cooled in ice and then shaken with 1.8 ml. of redistilled dimethyl sulfate until the solution was clear. About 15 minutes were required. The solution, which had become warm spontaneously, was cooled in ice and acidified (to *p*H 5) with 6 *N* sulfuric acid. The solution was extracted continuously with chloroform for five hours. The betaine of 1,2-dimethyl-3,4-dihydroxy-5-hydroxymethylpyridine (0.47 g., 33%) suspended in the chloroform was collected on a filter and recrystallized from alcohol; m.p. 228–229°. Further extraction of the aqueous acid solution with chloroform did not increase the yield.

Anal. Calcd. for $C_8H_{11}NO_3$: C, 56.79; H, 6.56; N, 8.28. Found: C, 56.65; H, 6.59; N, 8.56.

Treatment of 2-Methyl-3,4-dihydroxy-5-hydroxymethylpyridine (IV) with *p*-Toluenesulfonyl Chloride.—To a suspension of 300 mg. of 2-methyl-3,4-dihydroxy-5-hydroxymethylpyridine in 2.5 ml. of pyridine cooled in an ice-bath, 924 mg. of *p*-toluenesulfonyl chloride was added in several portions. The solution became clear. After five minutes standing at room temperature, the mixture was added to an excess of ice-water. An oil appeared, which crystallized after ether had been added to the mixture. The mixture was cooled, and the solid material collected on a filter and washed well successively with water, alcohol and ether. The crude 2-methyl-3-*p*-toluenesulfonoxy-4-hydroxy-5-hydroxymethylpyridine (VII) weighing 160 mg. (27%) was recrystallized three times from alcohol, the final product melting at 228–229° (dec.).

Anal. Calcd. for $C_{14}H_{15}NO_5S$: C, 54.36; H, 4.89; N, 4.53. Found: C, 54.66; H, 5.09; N, 4.70.

The ether was distilled from the filtrate from which the crude material in the above preparation had been separated, and the aqueous solution was diluted with water and cooled in an ice-bath. The resulting crystals (530 mg., 59%) of 2-methyl-3-*p*-toluenesulfonoxy-4-hydroxy-5-*p*-toluenesulfonoxymethylpyridine (VIII) were filtered and washed successively with water, alcohol and ether. After two recrystallizations from alcohol, the melting point was constant at 140–141°.

Anal. Calcd. for $C_{21}H_{21}NO_7S_2$: C, 54.41; H, 4.57; N, 3.02; S, 13.83. Found: C, 54.53; H, 4.27; N, 3.25; S, 13.71.

RAHWAY, N. J. RECEIVED JANUARY 12, 1951

[CONTRIBUTION FROM THE MORLEY CHEMICAL LABORATORY, WESTERN RESERVE UNIVERSITY]

The Synthesis of 6-, 7- and 8-Methyl-1,2-benzfluorenes

BY MALCOLM E. GROSS[1] AND HERMAN P. LANKELMA

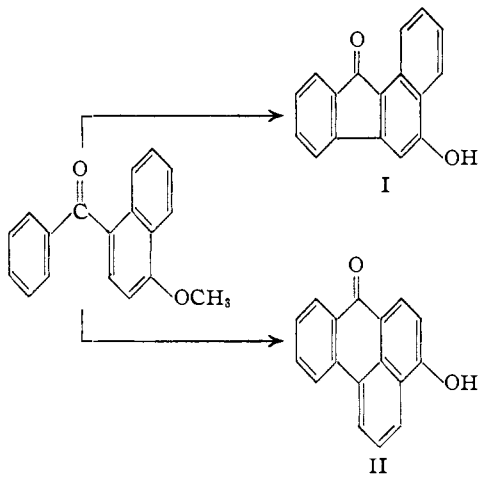
6-, 7- and 8-methyl-1,2-benzfluorenes have been synthesized from 4-methoxy-1-naphthyl *p*-, *m*- and *o*-tolyl ketones, respectively, by ring closure through the Scholl reaction to the 6, 7, and 8-methyl-3-hydroxy-1,2-benzfluorenones followed by stepwise reduction to the 3-hydroxy-1,2-benzfluorenols, the 3-hydroxy-1,2-benzfluorenes and the 1,2-benzfluorenes. The parent hydrocarbon, 1,2-benzfluorene, was prepared for comparison purposes by the same procedure. The fact that the three isomeric ketones gave three isomeric methyl-3-hydroxy-1,2-benzfluorenones is of interest since several cases have been reported where ring closure by the Scholl reaction gave, as a result of migration of the methyl group from the ortho to the meta position, only two products from three isomeric toluyl ketones.[1a] The three methyl-1,2-benzfluorenones have been further characterized by their ultraviolet absorption spectra. Ring closure of the 4-methoxy-1-naphthyl *m*-tolyl ketone could give 5-methyl-3-hydroxy-1,2-benzfluorenone by condensing ortho to the methyl group, although steric hindrance would make this improbable. An attempt was made to synthesize the 7-methyl-1,2-benzfluorene by a method which would leave no doubt as to its structure, but was unsuccessful. A method for the reduction of a phenol to an aromatic hydrocarbon with zinc dust which gives greatly improved yields, is described.

Only one of the eleven possible mono-methyl-1,2-benzfluorenes, the 9-methyl derivative,[2] has been reported. The present work describes the synthesis of three additional methyl-1,2-benzfluorenes, namely, the 6-, 7- and 8-methyl derivatives.

Scholl[3] in 1912 and Fierz-David[4] in 1928 reported that 4-hydroxy-1-benzoylnaphthalene, or its ethers would undergo a cyclodehydrogenation in the presence of aluminum chloride to give principally 3-hydroxy-1,2-benzfluorenone (I) with minor amounts of 2-hydroxy-1,9-benz-10-anthrone (II).

In the present work this reaction was employed with the three isomeric 4-methoxy-1-(*p*-, *m*- and *o*-toluyl)-naphthalenes to give the desired 6-, 7- and 8-methyl-3-hydroxy-1,2-benzfluorenones which were reduced stepwise to the 6-, 7- and 8-methyl-1,2-benzfluorenes. This may be illustrated by the synthesis of 6-methyl-1,2-benzfluorene from 4-methoxy-1-(*p*-toluyl)-naphthalene (III to VII).

After numerous trials it was found that the best method for preparing the 1-naphthyl tolyl ketones



(III) was the addition of slightly more than one mole of aluminum chloride to a solution of the toluyl chloride and methyl 1-naphthyl ether in a mixture of nitrobenzene and tetrachloroethane at 0–5°. The products were very clean and easy to purify and were consistently obtained in yields of about 95%.

The ring closure of the ketones to the 3-hydroxy-1,2-benzfluorenone was done by the Scholl reaction using a mixture of sodium chloride–aluminum

(1) B. F. Goodrich Co. Research Center, Brecksville, Ohio.

(1a) L. F. Fieser and E. B. Hershberg, THIS JOURNAL, **60**, 1658 (1938); F. Mayer, E. Fleckenstein and H. Gunther, *Ber.*, **63**, 1464 (1930); ref. 5.

(2) G. M. Badger, *J. Chem. Soc.*, 535 (1941).

(3) R. Scholl and C. Seer, *Ann.*, **394**, 111 (1912).

(4) H. E. Fierz-David and G. Jaccond, *Helv. Chim. Acta*, **11**, 1042 (1928).