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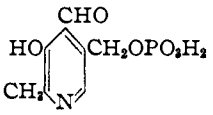
Phosphates of the Vitamin B_6 Group. V.[1] A Synthesis of Codecarboxylase[2]

BY ANDREW N. WILSON AND STANTON A. HARRIS

Codecarboxylase (2-methyl-3-hydroxy-4-formyl-5-pyridylmethylphosphoric acid) has been prepared from pyridoxamine hydrochloride by phosphorylation and oxidation. It was purified by adsorption on charcoal, followed by elution with ammonium hydroxide.

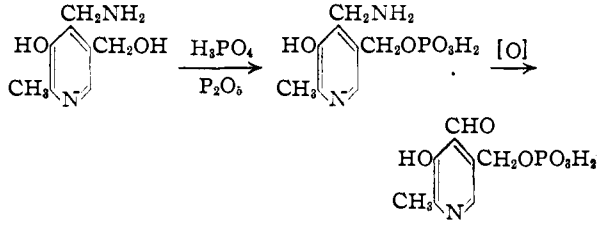
It has been shown previously[3] that codecarboxylase or pyridoxal phosphate, has the structure



A method for the preparation of codecarboxylase by phosphorylation of pyridoxal hydrochloride in aqueous solution with phosphorus oxychloride has been described.[4] The yield of product obtained by this method, however, is very low.

In seeking a method which would give a better yield, anhydrous phosphoric acid was prepared[5] and utilized in an attempt to phosphorylate pyridoxal hydrochloride. A considerable amount of decomposition occurred, however, and so the procedure was altered to allow the use of the corresponding pyridoxamine dihydrochloride, with subsequent oxidation of the phosphorylated amine to codecarboxylase.

This series of reactions is illustrated by the equation



The most convenient method of isolating the phosphorylated amine from the solution of inorganic acids was by adsorption of the organic material on activated charcoal: Darco G-60 was used. The adsorption as determined by ultraviolet absorption spectral data was virtually quantitative. It was found, however, that when the charcoal was washed with dilute ammonia water, the eluted material was a solution of the ammonium salt of pyridoxal phosphate and not of pyridoxamine phosphate. The oxygen adsorbed on the surface of the charcoal had spontaneously oxidized the amino group to the corresponding aldehyde. In one case, however, a particular sample of charcoal gave only partial oxidation, and a mixture of the pyridoxamine and pyridoxal phosphoric acid esters was obtained. This result was due apparently to the fact that the charcoal held less surface-held oxygen than was customarily so. The phosphorylated amine and aldehyde can be readily distinguished, however, by means of their ultraviolet absorption spectra.

To eliminate the differences in samples of charcoal, therefore, it was found expedient to oxidize the phosphorylated amine with manganese dioxide[6] before its adsorption on the charcoal. As the oxidized solution is deep yellow-brown in color the amount of adsorption can be followed visually. Samples of pyridoxal phosphate obtained by either method are indistinguishable.

The ammonium salt of codecarboxylase prepared by these methods has given the oxime[7] in a 50% yield. Bioassay, however, indicates that this sample has a codecarboxylase activity of 80%.

Experimental[8]

Ammonium Salt of 2-Methyl-3-hydroxy-4-formyl-5-pyridylmethylphosphoric Acid.—Twenty-five grams of phosphorus pentoxide was dissolved in 33 g. of 85% orthophosphoric acid to give anhydrous phosphoric acid, according to the method described by Ferrel, Olcott and Fraenkel-Conrat.[5] To 20 g. of this anhydrous mixture, cooled to room temperature, was added 2 g. of pyridoxamine dihydrochloride. As the solid dissolved there was a vigorous evolution of hydrogen chloride, which was dissipated by vigorous stirring. When most of the gas had been evolved, the clear, viscous solution was allowed to stand in a desiccator over phosphorus pentoxide for 3–6 days.

The reaction mixture was poured into about 300 ml. of a mixture of crushed ice and water. To the resulting colorless solution was added 35–40 g. of acid-washed charcoal (Darco G-60), which adsorbed the phosphorylated amine. The charcoal adsorbent was filtered by suction and washed well with water to remove the bulk of occluded inorganic salts. It was then suspended in water, mixed with one or two volumes of Polycel or some similar cellulose material to facilitate washing, and placed in a column where it was further washed with 1–2% hydrochloric acid to remove the remainder of the phosphoric acid. It was then washed with water to remove the hydrochloric acid. Both of these washings should be carried out thoroughly, as none of the phosphorylated material is removed by them.

The charcoal adsorbent was then washed with 1–2% ammonia in order to elute the phosphorylated product. The eluate was vivid yellow in color due to the presence of the codecarboxylase ammonium salt. When about 700 ml. of eluate had been collected, most of the product was removed. This liquor was concentrated to about 50–100 ml. volume under reduced pressure and at a low temperature. It was then freeze-dried to give 1.5 g. (63%) of an amorphous, brown, hygroscopic powder; $\lambda_{max.}^{H_2O}$ (*p*H 10.5) 2300 Å., *E*% 300; shoulder 2625–2715 Å., *E*% 95–83; plateau 3050–3200 Å., *E*% 40; $\lambda_{max.}$ 3900 Å., *E*% 194.

Anal. Calcd. for $C_8H_{16}N_3O_6P$: C, 34.17; H, 5.74; N, 14.94; P, 11.02. Found: C, 34.27; H, 5.71; N, 13.06; P, 13.25, 13.26.

(1) Paper IV, Heyl, Luz and Harris, THIS JOURNAL, **73**, 3437 1951).

(2) Material included in this paper was presented to the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., September 11, 1951.

(3) (a) Heyl, Luz, Harris and Folkers, THIS JOURNAL, **73**, 3430 (1951); (b) Heyl and Harris, *ibid.*, **73**, 3434 (1951).

(4) Gunsalus, Umbreit, Bellamy and Foust, *J. Biol. Chem.*, **161**, 743 (1945).

(5) Ferrel, Olcott and Fraenkel-Conrat, THIS JOURNAL, **70**, 210 (1948).

(6) D. Heyl, *ibid.*, **70**, 3434 (1948).

(7) Heyl, Harris and Folkers, *ibid.*, **73**, 3430 (1951).

(8) We wish to thank Mr. R. N. Boos and his associates for the analytical results reported in this paper.

Ammonium Salt of 2-Methyl-3-hydroxy-4-formyl-5-pyridylmethylphosphoric Acid Using Manganese Dioxide:—Pyridoxamine dihydrochloride (2.5 g.) was dissolved in 25 g. of anhydrous phosphoric acid as described above. After standing for several days at room temperature under anhydrous conditions, the mixture was poured into about 200 ml. of a mixture of ice and water. A solution of 30% sodium hydroxide was added carefully until the reaction mixture was about *p*H 6.

One gram of manganese dioxide was added and the mixture was heated at 60° for 20 minutes with frequent shaking. The manganese dioxide was replaced by a light-colored inorganic solid, and the colorless solution became yellow-brown due to the presence of the aldehyde phosphate. The solution was cooled, filtered from the inorganic salt, diluted with an equal volume of water, and re-acidified to congo red with phosphoric acid. The organic material was adsorbed on Darco G-60 as described above, and isolated in an identical manner. The yield of freeze-dried residue was 2.6 g. (88%) $\lambda_{max.}^{H_2O}$ (*p*H 11) 2300 Å., *E*% 300; shoulder 2650–2750 Å., *E*% 90–70; plateau 2850–3100 Å., *E*% 38; $\lambda_{max.}$ 3900 Å., *E*% 180.

Isolation of Codecarboxylase as the Calcium Salt.—Twenty-five grams of pyridoxamine dihydrochloride was dissolved in 250 g. of anhydrous phosphoric acid as described above, and was converted to the aldehyde phosphate by means of manganese dioxide oxidation. Instead of concentrating the ammonia eluate to dryness, however, it was concentrated under reduced pressure to a volume of about 1 liter. The concentrate was acidified to about *p*H 4 with dilute acetic acid, and to it was added a solution of 18.5 g. of calcium acetate in water. Additional acetic acid may be added if necessary to maintain solution. A small amount of amorphous insoluble material was removed by filtration through Super-cel, and the clear filtrate was diluted with three volumes of ethyl alcohol. The mixture was allowed to stand overnight in the refrigerator. The precipitate was centrifuged, washed with a mixture of alcohol and water, with a mixture of alcohol and ether, finally with ether, and dried in a vacuum oven at 40–45°. The yield of bright yellow calcium salt of codecarboxylase was 29 g. (97%); $\lambda_{max.}^{H_2O}$ (*p*H 11) 2280 Å., *E*% 375; shoulder 2650–2750 Å., *E*% 90–60; $\lambda_{max.}$ 3075 Å., *E*% 37; $\lambda_{max.}$ 3900 Å., *E*% 164.

Codecarboxylase Oxime.—Five grams of pyridoxamine dihydrochloride was phosphorylated in 50 g. of the anhydrous phosphoric acid as described above, and was oxidized to the aldehyde with 2 g. of manganese dioxide. This oxidized solution was filtered from the inorganic salts and made just acid to congo red paper with hydrochloric acid. The volume of the resulting solution was about 400 ml. To this solution was added 4 g. of hydroxylamine hydrochloride dissolved in a little water. On scratching, crystallization of the oxime took place. After standing at room temperature for several hours, the crystals were collected, washed and dried. The yield of codecarboxylase oxime was 2.6 g. (50%); m.p. 218° dec. A melting point of a mixture of this sample with a known sample of codecarboxylase oxime showed no depression.

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[CONTRIBUTION FROM THE COATES CHEMICAL LABORATORIES, LOUISIANA STATE UNIVERSITY]

The Synthesis of Some Simple and Mixed Ethers as Contact Insecticides[1]

BY TIEN-CHIH CHEN[2a] AND W. T. SUMERFORD[2b]

The phenomenal activity of DDT (2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane) as a contact insecticide has been attributed to its neurotoxic bis-(*p*-chlorophenyl)-methylene group and its lipophilic trichlorinated methyl radical.[3]

Attempts to combine these insecticidally-active groups in other molecules to obtain additional, and possibly improved, insecticides has met with varying degrees of success, and in some instances with complete failure. In projecting this general effort to obtain substitutes for DDT and to prepare compounds which might be toxic to DDT-resistant insects, especially house flies,[4] it seemed worthwhile to synthesize a series of benzyl aryl ethers and allyl aryl ethers having one or more halogen atoms in either or both of the aliphatic and aromatic hydrocarbon radicals. The benzyl aryl series of compounds would possess a neurotoxic haloaryl group, an ether linkage having lipophilic properties in the Overton–Meyer concept of narcosis, as well as a benzyl radical, of which the alcohol and other derivatives are known to be neurotropic in character. In addition to the haloaryl group and the ether linkage, the allyl aryl series would depend for activity upon a 2- or a 3-chloroallyl grouping, both of which might be expected to have a fumigant action against insects.[5] Added support for the possible insecticidal activity of these series of compounds is found in the potent contact insecticides: rotenone, 2-butoxy-2′-thiocyanodiethyl ether[6] and 2,2-bis-(*p*-methoxyphenyl)-1,1,1-trichloroethane,[7] all of which have one or more ether linkages in their structures.

A series of benzyl and halobenzyl ethers with phenol, halophenols and nitrophenols has been credited in the patent literature with having general insecticidal activity[8]; and *p*-chlorobenzyl *p*-chlorophenyl ether has been shown to be active against the citrus red mite.[9] The 2-chloroallyl ethers of phenol, certain alkyl-, aryl-, chloro- and nitro-substituted phenols have been patented as fly sprays[10] as have the related compounds: 2-chloroallyl 2-(*p*-chlorophenyl)-ethyl ether, 2-chloroallyl 2-(2-chloro-4-*t*-butylphenyl)-ethyl ether and 2-(3-chloro-2-methylallyloxy)-2′-(2-phenoxy)-diethyl ether.[11]

The nine benzyl aryl ethers and the seven chloroallyl aryl ethers prepared in this study, which are

(1) This paper has been constructed from the Doctorate dissertation presented by Tien-Chih Chen to the Louisiana State University in August, 1949.

(2) (a) Chemistry Department, National Nankai University, Tientsin, China. (b) Technical Development Services, Communicable Disease Center, U. S. Public Health Service, Savannah, Georgia.

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(4) Frank H. Babers, "Development of Insect Resistance to Insecticides," Publication E-776, Bur. Entomol. and Plant Quarantine, U. S. Dept. of Agri., Washington, D. C., 1949, 31 pp.

(5) O. I. Snapp, "Publication E-558, Bur. Entomol., and Plant Quarantine," U. S. Dept. of Agri., Washington, D. C., 1942, 4 pp.

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(8) W. F. Hester, U. S. Patent 2,243,479 (1941).

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(10) G. H. Coleman, *et al.*, U. S. Patent 2,207,721 (1940).

(11) C. L. Moyle and G. H. Coleman, U. S. Patent 2,244,309 (1941).