

tween the thiazolidinecarboxylic acid on the one side, and cysteine and aldehyde on the other. Thus irreversible removal of one product of the equilibrium will eventually result in complete dissociation of the compound. It appears likely that the observed activity of this compound for the various assay organisms may result solely from this process, which would be accelerated by utilization of the pyridoxal for growth and perhaps by other ingredients of the medium. Active utilization of the compound *per se* by the various organisms is, of course, also a possibility.

In contrast to the activity of these products, the condensation product formed between histidine and pyridoxal (4-(2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridyl)-1-imidazo(c)tetrahydropyridine-6-carboxylic acid) was essentially inactive for all organisms. Pyridoxyl- $\beta$ -alanine was also inactive.

### Summary

A series of pyridoxylamino acids and some related structures were tested for vitamin and anti-vitamin activities against four microorganisms of diverse types. For *L. casei*, *S. faecalis* and *S. carlsbergensis*, these compounds were essentially

inactive. Although growth-promoting activity was shown at high concentration levels, the magnitude of this action was such that it could well be attributed to trace impurities, or to purely chemical breakdown during incubation in the medium. For *N. sitophila*, the compounds had somewhat greater activity which, however, was still of a low order. No significant "antivitamin" activity was demonstrated with these organisms.

When autoclaved in extremely dilute solutions containing dissolved air and subsequently tested, the compounds show high activity, approaching that of equimolar quantities of pyridoxal for all test organisms. This is ascribed to their oxidation to the corresponding Schiff bases, with subsequent hydrolysis to yield pyridoxal or pyridoxamine. The presence of ascorbic acid, cysteine or other antioxidants prevents this change.

True Schiff bases of pyridoxal and the thiazolidinecarboxylic acid formed by condensation of pyridoxal with cysteine exhibit high growth-promoting activity for all organisms. In both cases, such activity probably results from spontaneous dissociation, which proceeds to completion as the pyridoxal formed is utilized for growth.

MADISON, WISCONSIN

RECEIVED MAY 25, 1948

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

## The Chemistry of Vitamin B<sub>6</sub>. V.<sup>1</sup> Conversion of Pyridoxine to the Lactone of 4-Pyridoxic Acid<sup>2</sup>

BY DOROTHEA HEYL

Several syntheses of the lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine have been described.<sup>3,4,5</sup> Direct oxidation of pyridoxine produces the lactone<sup>3,4</sup> in poor yield and is not convenient for the preparation of the lactone in quantity. Stepwise conversion of pyridoxine to the lactone of 4-pyridoxic acid has now been found to be more successful.

Oxidation of pyridoxine to pyridoxal is accomplished in better yield with manganese dioxide and sulfuric acid than with potassium permanganate.<sup>3</sup> Pyridoxal was isolated as the oxime (II), which was then acetylated and dehydrated to the diacetyl nitrile IV. By variation in the length of reaction time, it was found that the acetylation and dehydration could be accomplished either sepa-

rately, with the isolation of the intermediate triacetyl oxime III, or in one operation. The diacetyl nitrile IV was hydrolyzed in alkaline solution to 4-pyridoxic acid (V), which was isolated by acidification. Lactonization occurred when the acid was refluxed with alcoholic hydrogen chloride.

The lactone VI was converted to the hydrochloride by treatment with saturated alcoholic hydrogen chloride. Pure 4-pyridoxic acid was prepared from the lactone by saponification.

Removal of the acetyl group from the phenolic hydroxyl group of the diacetyl nitrile IV by treatment with alcoholic sodium ethoxide resulted in the production of 2-methyl-3-hydroxy-4-cyano-5-acetoxymethylpyridine (VII).

Thionyl chloride was also used to dehydrate pyridoxal oxime (II). The resulting 2-methyl-3-hydroxy-4-cyano-5-chloromethylpyridine (VIII) was hydrolyzed by water to 2-methyl-3-hydroxy-4-carbamyl-5-hydroxymethylpyridine (IX).

### Experimental

**Pyridoxal Oxime (II).**—In 1.5 l. of water in a 3-l. round-bottomed flask equipped with a mechanical stirrer, 102.8 g. of pyridoxine hydrochloride (I) was dissolved. In this

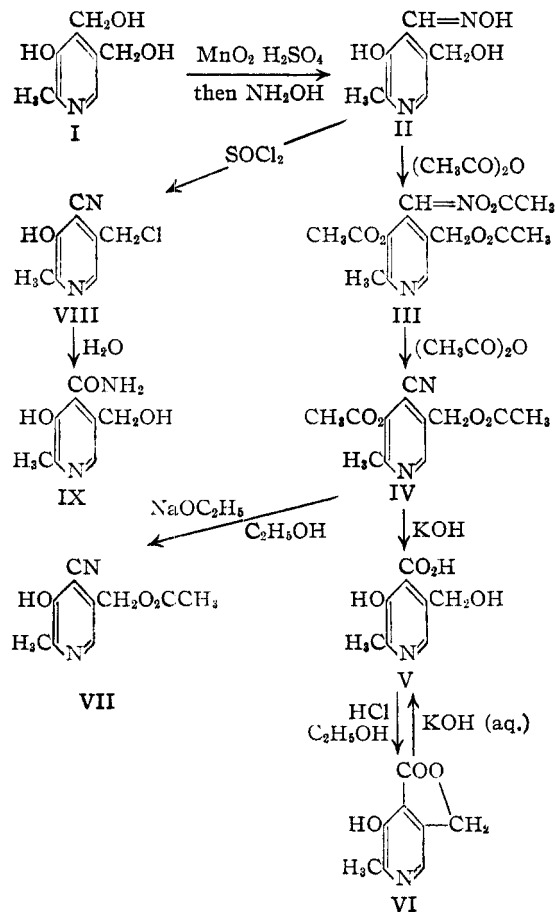
(1) For the preceding paper of this series, see Harris, *THIS JOURNAL*, **63**, 3363 (1941).

(2) 4-Pyridoxic acid is the name designated for 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine by Huff and Perlzweig (ref. 4). The name " $\beta$ -pyracin" given to the lactone by Scott, Norris, Heuser and Bruce (*J. Biol. Chem.*, **158**, 291 (1945)) was later transferred to the acid by Daniel, Scott, Norris and Heuser (*ibid.*, **160**, 265 (1945)).

(3) Harris, Heyl and Folkers, *THIS JOURNAL*, **66**, 2088 (1944).

(4) Huff and Perlzweig, *J. Biol. Chem.*, **155**, 345 (1944).

(5) Scott, Norris, Heuser and Bruce, *THIS JOURNAL*, **67**, 157 (1945).



solution 51.2 g. of 85% manganese dioxide was suspended, and 49.1 g. of concentrated sulfuric acid was added slowly from a dropping funnel. When all the sulfuric acid had been added, the mixture was heated in an oil-bath at 60–70° until the pH of the solution was about 6 and the manganese dioxide had all disappeared (approximately two hours were required). The oxime precipitated immediately on the addition of 123.0 g. of sodium acetate and 52.1 g. of hydroxylamine hydrochloride. After the mixture had been heated on a steam-bath for about ten minutes, it was cooled in ice; the pyridoxal oxime was collected on a filter, washed with water, and dried; yield, 53.8 g. (59%). This oxime was dissolved in hot ethyl alcohol, decolorized with Darco, and filtered. The crystals which separated from the cooled solution melted at 225–226° dec. A mixture of this material and pyridoxal oxime obtained through the permanganate oxidation of pyridoxine<sup>3</sup> also melted at 225–226° dec.

Pyridoxal oxime was obtained in slightly higher yields (65–70%) when pyridoxine or pyridoxine sulfate was used as starting material, instead of the hydrochloride.

**Acetoxime of 2-Methyl-3-acetoxy-4-formyl-5-acetoxymethylpyridine (III).**—One gram of pyridoxal oxime (II) was refluxed for twenty minutes with 10 cc. of acetic anhydride. After removal of the unchanged acetic anhydride by distillation under reduced pressure, ethyl alcohol was added and also removed by distillation. The residue was dissolved in 30 cc. of ethyl alcohol, and after sixteen hours the volatile material was again removed. An ethereal solution of the residue was washed twice with dilute sodium bicarbonate solution and three times with water. After decolorization with Darco, the solution was filtered and the solvent removed; the residue was dried further by distillation with benzene. The acetoxime of 2-methyl-3-acetoxy-4-formyl-5-acetoxymethylpyridine

(III) was crystallized from dilute ethyl alcohol; m. p. 114.5–115°.

*Anal.* Calcd. for  $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_6$ : C, 54.54; H, 5.23; N, 9.09. Found: C, 54.97; H, 5.01; N, 9.06.

**2-Methyl-3-acetoxy-4-cyano-5-acetoxymethylpyridine (IV).**—Fifty grams of pyridoxal oxime (II) was refluxed with 500 cc. of acetic anhydride for two and one-half hours. After removal of the unchanged acetic anhydride under reduced pressure, the residue was boiled with ethyl alcohol and the volatile material again removed under reduced pressure. An ethereal solution of the residue, after four washings with saturated sodium bicarbonate solution and three washings with water, was boiled with Darco and filtered. The ether was evaporated and the residue dried by distillation with benzene. After crystallization from ether-petroleum ether (b. p. 30–60°), the 2-methyl-3-acetoxy-4-cyano-5-acetoxymethylpyridine (IV), m. p. 63–64°, weighed 52.9 g. Further crystals, m. p. 62–63°, from the filtrate increased the yield to 59.5 g. (88%).

*Anal.* Calcd. for  $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_4$ : C, 58.06; H, 4.87; N, 11.29. Found: C, 58.40; H, 4.67; N, 11.42.

**4-Pyridoxic Acid (V).**—Twenty-four grams of 2-methyl-3-acetoxy-4-cyano-5-acetoxymethylpyridine (IV) was refluxed with 500 cc. of 3 N potassium hydroxide for seven hours. After filtration the solution was made acid to congo red with concentrated hydrochloric acid and the resulting material cooled in ice and filtered. The solid was washed with dilute hydrochloric acid, repeatedly with water, then with ethyl alcohol, and finally with ether. After drying, the impure 4-pyridoxic acid (V), m. p. 253–254° dec., weighed 16.9 g. (96%).

**Lactone of 4-Pyridoxic Acid (VI).**—A suspension of 3.7 g. of 4-pyridoxic acid (V) was refluxed for two and one-half hours with 100 cc. of absolute ethyl alcohol to which had been added 20 cc. of concentrated alcoholic hydrogen chloride. The resulting mixture was cooled in an ice-bath. The product was collected on a filter, washed with ethyl alcohol, dissolved in water, and treated with an excess of sodium bicarbonate. The crystalline lactone of 4-pyridoxic acid, after collection on a filter, was washed successively with water, ethyl alcohol and ether. It was recrystallized from ethyl alcohol; m. p. 273–273.5° dec.; yield, 3.0 g. (91%).

*Anal.* Calcd. for  $\text{C}_8\text{H}_7\text{NO}_3$ : C, 58.18; H, 4.27; N, 8.48. Found: C, 58.28; H, 4.27; N, 8.63.

**Hydrochloride of the Lactone of 4-Pyridoxic Acid.**—Two-hundred milligrams of the lactone of 4-pyridoxic acid (VI) was suspended for sixteen hours in 20 cc. of ethyl alcohol saturated with hydrogen chloride. The resulting material, after collection by filtration, thorough washing with ethyl alcohol and drying, decomposed at 252–253°, with previous darkening.

*Anal.* Calcd. for  $\text{C}_8\text{H}_8\text{NO}_3\text{Cl}$ : C, 47.66; H, 4.00; N, 6.95. Found: C, 47.88; H, 3.98; N, 7.25.

**4-Pyridoxic Acid (V).**—Two and one-half grams of the lactone of 4-pyridoxic acid (VI) dissolved in 100 cc. of 0.1 N potassium hydroxide was heated on the steam-bath for two hours. The resulting solution was filtered, cooled in an ice-bath, and acidified with concentrated hydrochloric acid. After collection on a filter, the product was washed successively with water, ethyl alcohol, and ether; m. p. 258–258.5° dec.

*Anal.* Calcd. for  $\text{C}_8\text{H}_7\text{NO}_3$ : C, 52.46; H, 4.95; N, 7.65. Found: C, 52.74; H, 4.98; N, 7.70.

**2-Methyl-3-hydroxy-4-cyano-5-acetoxymethylpyridine (VII).**—Seven grams of 2-methyl-3-acetoxy-4-cyano-5-acetoxymethylpyridine (IV) was refluxed for two hours with 200 cc. of anhydrous ethyl alcohol in which 200 mg. of sodium had been dissolved. The solution was cooled and poured into an excess of ice-cold, dilute hydrochloric acid. The mixture was concentrated to 50 cc. under reduced pressure, cooled, and the precipitated material collected on a filter. The 2-methyl-3-hydroxy-4-cyano-5-acetoxymethylpyridine (yield 5.2 g., 90%) was purified

by two recrystallizations from ethyl alcohol; m. p. 209–210°. The deep red color produced in ferric chloride solution indicated that the phenolic group was unsubstituted.

*Anal.* Calcd. for  $C_{10}H_{10}N_2O_3$ : C, 58.24; H, 4.89; N, 13.59. Found: C, 58.37; H, 4.99; N, 13.49.

**2-Methyl-3-hydroxy-4-cyano-5-chloromethylpyridine (VIII).**—Three grams of pyridoxal oxime (II) was treated very cautiously with 15 cc. of thionyl chloride. After the vigorous exothermic reaction had subsided, the mixture stood at room temperature for ten minutes; it was then diluted with ether and filtered. After the solid material had been decolorized by treatment with Darco in hot water, the solution was filtered and cooled. The resulting crystals of 2-methyl-3-hydroxy-4-cyano-5-chloromethylpyridine were collected on a filter and washed with water; yield, 1.4 g. (47%); m. p. 167–168° dec. This material gave a precipitate of silver chloride when heated with silver nitrate solution.

*Anal.* Calcd. for  $C_8H_7N_2OCl$ : C, 52.62; H, 3.86; N, 15.34. Found: C, 52.83; H, 3.86; N, 15.65.

**2-Methyl-3-hydroxy-4-carbamyl-5-hydroxymethylpyridine Hydrochloride (IX).**—Thirteen cubic centimeters of water containing 0.2 g. of 2-methyl-3-hydroxy-4-cyano-5-chloromethylpyridine hydrochloride (VIII) was refluxed for forty minutes. After decolorization with Darco, the solution was concentrated to dryness under

reduced pressure, and the residue was crystallized from ethyl alcohol. The crystals of 2-methyl-3-hydroxy-4-carbamyl-5-hydroxymethylpyridine hydrochloride weighed 0.1 g. and melted at 210–211° dec.

*Anal.* Calcd. for  $C_8H_{11}N_2O_3Cl$ : C, 43.94; H, 5.07; N, 12.82. Found: C, 44.30; H, 5.10; N, 12.72.

**Acknowledgment.**—The microanalyses were carried out by Messrs. W. K. Humphrey, J. McGregor, L. Rosalsky, E. Thornton, Mrs. Edith Meiss and Mrs. Dorothy Sellers.

### Summary

Pyridoxal, isolated as the oxime, has been prepared from pyridoxine by means of manganese dioxide and sulfuric acid. Pyridoxal oxime has been dehydrated with acetic anhydride to form 2-methyl-3-acetoxy-4-cyano-5-acetoxy-methylpyridine. This has been converted to 4-pyridoxic acid, which has then been lactonized.

Dehydration of pyridoxal oxime with thionyl chloride has yielded 2-methyl-3-hydroxy-4-cyano-5-chloromethylpyridine.

RAHWAY, N. J.

RECEIVED JUNE 3, 1948

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF PARKE, DAVIS & Co.]

## The Synthesis of 2,3-Disubstituted-4-thiazolidones<sup>1</sup>

BY H. D. TROUTMAN AND LOREN M. LONG

During the course of our investigation concerning the synthesis of 2,3-disubstituted-4-thiazolidones, there appeared papers by Erlenmeyer and Oberlin<sup>2</sup> and by Surrey<sup>3</sup> reporting the preparation of derivatives of the same ring system. Because none of the derivatives which we have prepared corresponds to those reported by the aforesaid authors<sup>2,3</sup> and since some variation in preparative technique was employed, we wish to report the results of our work at this time. The compounds reported in the earlier papers<sup>2,3</sup> are, for the most part, 2,3-diaryl derivatives, whereas the compounds herein reported are 2-aryl-3-alkyl or 2-hetero-3-alkyl derivatives. In addition, we have oxidized a number of the derivatives listed in

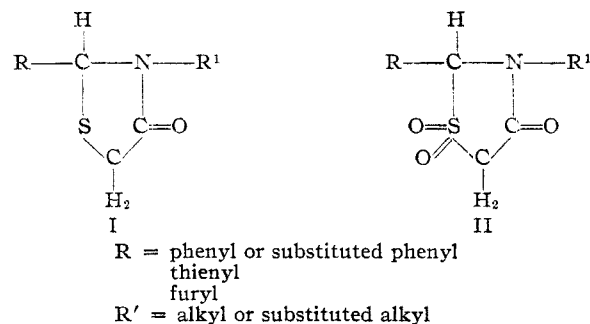
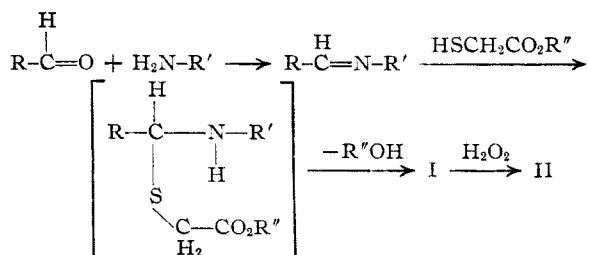


Table I to the corresponding 2,3-disubstituted-4-thiazolidone-1-dioxides (Formula II).

It was thought that the synthesis of 4-thiazolidones as represented by I and the corresponding dioxides as represented by II might possibly lead to compounds possessing anticonvulsant activity. The fact that various amides, sulfides and sulfones exhibit such activity has been indicated in numerous publications by various authors, and the results of pharmacological testing summarized by Merritt and Putnam.<sup>4</sup>

The following scheme represents the method which was used in the preparation of all of the 4-thiazolidones listed in Table I. It will be noted



that the method of choice recommended by Surrey<sup>3</sup> involves the use of thioglycolic acid instead of an ester of thioglycolic acid as shown above. Surrey reports that in an attempt to prepare 2,3-diphenyl-4-thiazolidone from the corresponding Schiff base and ethyl thioglycolate only an 8%

(1) Presented before the Division of Medicinal Chemistry, Chicago, Ill., April 21, 1948.

(2) Erlenmeyer and Oberlin, *Helv. Chim. Acta*, **30**, 1329 (1948).

(3) Surrey, *This Journal*, **69**, 2911 (1947).

(4) Merritt and Putnam, *Epilepsia*, **3**, 51 (1945).