is specific. The product formed is destroyed by nitrous acid, and appears to be an amine.

Treatment of pyridoxine with certain oxidizing agents produces a substance with greatly increased growth-promoting power for *S. fecalis* and *L. casei*. This substance is destroyed by incubating with sodium cyanide, or with alkaline acetone or acetaldehyde. It forms a phenylhydrazone, and appears to be an aldehyde.

Adoption of a theory permitting interconversion of pyridoxine, the amine and the aldehyde by a process similar to those known to occur physiologically reduced the possible structures for the latter two compounds to three each. Synthesis of a maximum of four compounds should furnish the active amine and aldehyde. The successful accomplishment of this has been noted elsewhere.[4,12]

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

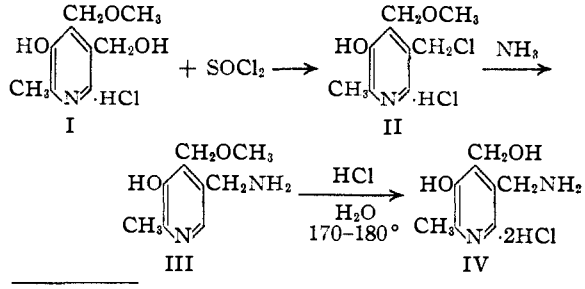
The Vitamin B_6 Group. II. The Structure and Synthesis of Pyridoxamine and Pyridoxal[1]

BY STANTON A. HARRIS, DOROTHEA HEYL AND KARL FOLKERS

In a previous paper,[2] the presence in natural materials of a pyridoxine-like substance which has an extremely high growth promoting power for certain lactic acid bacteria was reported. Snell[3a,3b] showed later that procedures involving amination or oxidation of pyridoxine resulted in substances having this greatly increased activity. Further study indicated that the active product obtained by amination was an amine and the one from oxidation an aldehyde, and that there were three possible structures for each of these compounds.

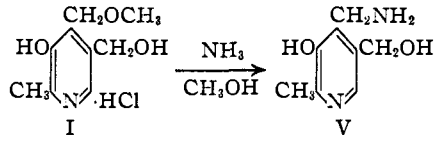
In a recent communication[4] it was stated that as a result of collaboration with Dr. Snell we had succeeded in synthesizing several of his proposed compounds including both an active amine and an active aldehyde, and had proved that these were 2-methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine and 2-methyl-3-hydroxy-4-formyl - 5 - hydroxymethylpyridine, respectively. The former was named pyridoxamine and the latter pyridoxal.[5]

The synthesis of 2-methyl-3-hydroxy-4-hydroxymethyl-5-aminomethylpyridine, IV, was carried out by the following reactions, which leave no doubt concerning the structure of the final product.



The methoxy derivative, I,[6] was converted by means of thionyl chloride to the methoxy chloro compound, II,[7] and this was aminated at room temperature with ammonia to give the methoxy amino derivative, III, which was isolated as the dihydrochloride and then hydrolyzed under pressure to the hydroxy amino derivative, IV. The latter compound, 2-methyl-3-hydroxy-4-hydroxymethyl-5-aminomethylpyridine dihydrochloride, was found to be without significant growth promoting activity[8] on several different test organisms.

This 5-aminomethyl derivative of known structure could then be compared with the amine obtained by the direct amination of pyridoxine or its derivatives. Although the direct amination of pyridoxine diacetate[6] resulted in an active amine,[3b,4] the yields were better when the 4-methoxymethyl derivative, I, was heated with ammonia and methanol in an autoclave at 140°.



The amine, V, differed in chemical properties from the previously obtained amine, IV, and was found to be highly active[5,8] in promoting the growth of *L. casei* and *S. lactis R.* The replacement of the methoxy group by the amino group and the difference between the two amines showed that the active amine was 2-methyl-3-hydroxy - 4 - aminomethyl - 5 - hydroxymethylpyridine. This structure for the active amine was anticipated by us since previous work[6,9] had shown that substitution reactions take place readily on the 4-methylene position of pyridoxine.

The aldehyde resulting from the oxidation of pyridoxine with potassium permanganate was

(1) Presented before the Organic Division of the American Chemical Society at the meeting on September 13, 1944, in New York.

(2) Snell, Guirard, and Williams, *J. Biol. Chem.*, **143**, 519 (1942).

(3) (a) Snell, *Proc. Soc. Exptl. Biol. Med.*, **51**, 356 (1942); (b) Snell, THIS JOURNAL, **66**, 2082 (1944), the first paper of this series.

(4) Harris, Heyl and Folkers, *J. Biol. Chem.*, **154**, 315 (1944).

(5) Snell, *ibid.*, **154**, 313 (1944).

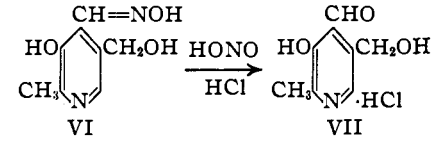
(6) Harris, THIS JOURNAL, **62**, 3203 (1940).

(7) Unpublished work by Dr. R. L. Clark in this Laboratory.

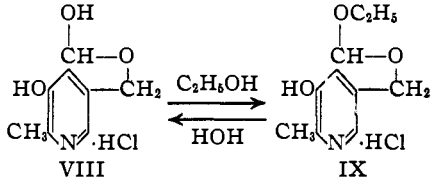
(8) Snell and Rannefeld, in press.

(9) Harris, THIS JOURNAL, **63**, 3363 (1941).

isolated as the oxime and the semicarbazone. The oxime, VI, was converted to the aldehyde, VII, by treatment with sodium nitrite and hydrochloric acid. This aldehyde may also have the structure represented by VIII, and may be readily converted to the cyclic acetal, IX, with alcohol and acid. The reaction is easily reversed in dilute aqueous acid.



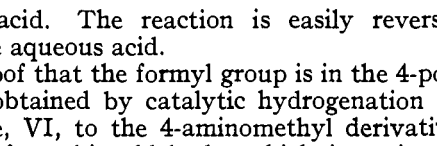
VI VII



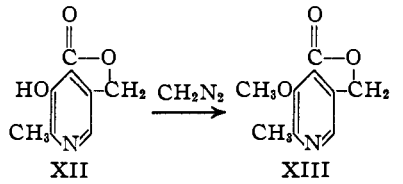
VIII IX

Proof that the formyl group is in the 4-position was obtained by catalytic hydrogenation of the oxime, VI, to the 4-aminomethyl derivative, V. Therefore this aldehyde, which is active, is 2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine.

It was shown previously that 2-methyl-3-methoxy-4,5-bis-(hydroxymethyl)-pyridine, X,[10] was oxidized by potassium permanganate to the lactone of 2-methyl-3-methoxy-4-hydroxymethyl-5-carboxypyridine, XI. Since it has been demonstrated now that pyridoxine is oxidized by permanganate at the 4-hydroxymethyl group to pyridoxal, VII, it is to be expected that the corresponding lactone should be obtainable by the same treatment. A new compound, the lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine, XII, has actually been synthesized from pyridoxine by oxidation with potassium permanganate.[11] It depressed the decomposition point of the isomeric 5-carboxylactone obtained by total synthesis,[12] and the methyl ether, XIII, was found to be different from the methyl ether of the 5-carboxylactone, XI. The oxidation of pyridoxal with ammoniacal silver oxide to this new lactone was direct proof of the structure XII.

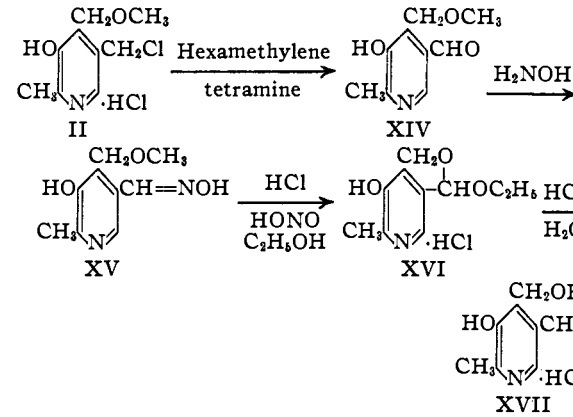


X XI



XII XIII

The isomeric aldehyde, 2-methyl-3-hydroxy-4-hydroxymethyl-5-formylpyridine, XVII, was found by Snell[8] to be inactive for the growth of lactic acid bacteria. It was synthesized by the reactions shown (II → XVII).



II XIV XV XVI XVII

Pyridoxamine and pyridoxal and the isomeric 5-aminomethyl and 5-formyl compounds have been converted to pyridoxine by diazotization and catalytic reduction experiments.

Experimental Part

2-Methyl-3-hydroxy-4-methoxymethyl-5-aminomethylpyridine Dihydrochloride (III).—2-Methyl-3-hydroxy-4-methoxymethyl-5-chloromethylpyridine hydrochloride,[7] II, was prepared by refluxing an ethereal suspension of the methoxy derivative, I, with 2.5 equivalents of thionyl chloride for one and one-half hours. A quantity of 18.3 g. of finely divided chloro derivative, II, (m. p. 137.5–139.5°) was placed in a glass liner in a steel bomb overnight at room temperature with 500 cc. of liquid ammonia. After evaporation of most of the ammonia, the product was dissolved in water and 6.2 g. of sodium hydroxide added. After distillation of water and ammonia under reduced pressure, the dried residue was extracted three times with hot absolute alcohol and treated with excess hydrogen chloride. The 2-methyl-3-hydroxy-4-methoxymethyl-5-aminomethylpyridine dihydrochloride, III, crystallized from alcohol and ether, was obtained in a yield of 7.1 g., m. p. 164–166°. Additional crystals from the filtrate increased the yield to 10.8 g. (55%). The compound was obtained in a pure state after several recrystallizations from alcohol containing a little hydrogen chloride; m. p. 170°, with decomposition.

Anal. Calcd. for $C_9H_{16}O_2N_2Cl_2$: C, 42.36; H, 6.32; N, 10.98. Found: C, 42.18; H, 6.28; N, 10.77.

2-Methyl-3-hydroxy-4-hydroxymethyl-5-aminomethylpyridine Dihydrochloride (IV).—A solution of 6.5 g. of 2-methyl-3-hydroxy-4-methoxymethyl-5-aminomethylpyridine dihydrochloride, III, in 99 cc. of 2.5 *N* hydrochloric acid was sealed in a glass bomb tube and heated for four hours at 170–180°. The acid solution was decolorized with Darco G-60 and evaporated to dryness under reduced pressure. The residue was crystallized from aqueous alcohol; the yield of 2-methyl-3-hydroxy-4-hydroxymethyl-5-aminomethylpyridine dihydrochloride was 5.3 g. (87%), m. p. 197.5–199°, with decomposition. For recrystallization it was necessary to add a little hydrochloric acid to the solvent, as the material tended to lose hydrogen chloride.

Anal. Calcd. for $C_8H_{14}O_2N_2Cl_2$: C, 39.85; H, 5.85; N, 11.62. Found: C, 39.94; H, 5.90; N, 11.64.

(10) Stiller, Keresztesy and Stevens, THIS JOURNAL, **61**, 1237 (1939).

(11) A recent article by Huff and Perlzweig (*Science*, **100**, 15 (1944)) describes a compound melting at 263–265° which was obtained as a metabolite and by oxidation of pyridoxine by permanganate. This compound was characterized as the lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine.

(12) Harris, Stiller and Folkers, THIS JOURNAL, **61**, 1242 (1939).

2-Methyl-3-hydroxy-4-hydroxymethyl-5-aminomethylpyridine.—A little of the dihydrochloride, IV, was treated with two equivalents of sodium bicarbonate in aqueous solution. The resulting solution was evaporated to dryness under reduced pressure; the product was extracted with boiling absolute alcohol and filtered from the cooled solution. After two recrystallizations from alcohol the crystals of 2-methyl-3-hydroxy-4-hydroxymethyl-5-aminomethylpyridine melted at 176–178°.

Anal. Calcd. for $C_8H_{12}O_2N_2$: C, 57.12; H, 7.19; N, 16.66. Found: C, 57.10; H, 7.35; N, 16.91.

Amination of Pyridoxine Diacetate.—Pyridoxine (2 g.) was boiled with 150 cc. of glacial acetic acid for three hours. This procedure has been found to yield the diacetate which had been prepared[6] from the bis-(bromomethyl) derivative with sodium acetate. The excess acid was then removed under reduced pressure and the residue extracted with 150 cc. of ether. After removal of the ether, the remaining oil was dissolved in 50 cc. of saturated alcoholic ammonia and heated in the steel bomb for three hours at 110°. The product, following evaporation of the volatile material, was treated with a saturated alcoholic solution of picric acid, and a crude picrate was obtained. A few milligrams of this was treated with three cc. of 6 *N* hydrochloric acid, and the picric acid extracted with benzene. After concentration of the aqueous solution to dryness, the solid residue was recrystallized from alcohol and ether; m. p. 198–201°. By microbiological tests, Dr. Snell found this material to have the activity of pyridoxamine.

2-Methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine (V).—A mixture of 30 g. of 2-methyl-3-hydroxy-4-methoxymethyl-5-hydroxymethylpyridine hydrochloride, I, 400 cc. of absolute methanol, and 400 cc. of liquid ammonia was heated for fifteen hours in the steel bomb at 140°. After removal of the ammonia and methanol, the residue was dissolved in 100 cc. of water, and a slight excess of 6 *N* sodium hydroxide was added. Most of the liberated ammonia was removed under reduced pressure and the resulting solution cooled in an ice-bath. After filtration the product, which had a yellow color, weighed 16.3 g. (71%). The color was removed from this material by heating it in an aqueous-acid solution with Darco G-60, followed by filtration and reprecipitation of the free base with sodium bicarbonate. After recrystallization from alcohol the melting point of the 2-methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine, V, was 193–193.5°.

Anal. Calcd. for $C_8H_{12}O_2N_2$: C, 57.12; H, 7.19; N, 16.66. Found: C, 57.28; H, 7.33; N, 16.49.

A mixed melting point of a sample of this material with a sample of 2-methyl-3-hydroxy-4-hydroxymethyl-5-aminomethylpyridine was depressed to 166–167°.

2-Methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine Dihydrochloride.—A small amount of the free base, V, was dissolved in alcohol and treated with excess alcoholic hydrochloric acid. After two recrystallizations from alcohol and water containing a little hydrochloric acid, the 2-methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine dihydrochloride melted at 226–227°, with decomposition.

Anal. Calcd. for $C_8H_{14}O_2N_2Cl_2$: C, 39.85; H, 5.85; N, 11.62. Found: C, 39.60; H, 5.70; N, 11.68.

Oxime of 2-Methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine (VI).—Pyridoxine hydrochloride (9.6 g.) was dissolved in 200 cc. of water and neutralized with 3.9 g. of sodium bicarbonate. Two grams of potassium permanganate dissolved in 150 cc. of water was added dropwise during an hour, while the reaction mixture was being stirred mechanically. Hydrochloric acid was then added until the solution had a *p*H of about 3. It was then concentrated to 100 cc. and treated with 20 g. of sodium acetate and 9.7 g. of hydroxylamine hydrochloride. After a few minutes' heating on the steam-bath, the solution was allowed to cool gradually, and the oxime of 2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine was removed by filtration; yield 1.9 g. (22%). After recrystallization from alcohol the melting point was 225–226°, with decomposition.

Anal. Calcd. for $C_8H_{10}O_3N_2$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.61; H, 5.40; N, 15.40.

Semicarbazone of 2-Methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine.—A sample of the solution containing the oxidation product of pyridoxine was evaporated to dryness under reduced pressure and the organic material extracted with absolute alcohol. The latter solution was also concentrated to dryness. The residue, dissolved in water, was treated with semicarbazide hydrochloride and sodium acetate. The solution was heated in a boiling water-bath and allowed to cool slowly, whereupon the semicarbazone of the 4-formyl derivative separated. After recrystallization from water, the semicarbazone melted at 235°, with decomposition.

Anal. Calcd. for $C_9H_{12}O_3N_4$: C, 48.21; H, 5.40; N, 24.99. Found: C, 48.17; H, 5.68; N, 24.66.

Reduction of the Oxime of 2-Methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine (VI).—A solution of 148 mg. of the oxime, VI, in 125 cc. of methyl alcohol was shaken with 0.1 g. of platinum oxide at 24° under a pressure of 2–3 atmospheres of hydrogen for 3.3 hours. The catalyst was removed by filtration, the solution concentrated to dryness and the residue crystallized from alcohol containing hydrogen chloride and ether. After three recrystallizations the hydrochloride was converted to the free base which, after several recrystallizations, consisted of a few milligrams of material; m. p. 189–191°. The mixed melting point with 2-methyl-3-hydroxy-4-aminomethyl-5-hydroxymethylpyridine was 189–192°.

1,3-Dihydro-1-ethoxy-6-methyl-furo[3,4-*c*]-pyridin-7-ol Hydrochloride (IX).—A solution of 0.45 g. of sodium nitrite in 2 cc. of water was added dropwise, with shaking, to a solution of 1.12 g. of the oxime of 2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine, VI, in 18 cc. of 1 *N* hydrochloric acid. The resulting solution was then heated on the steam-bath for about fifty minutes until there was no free nitrous acid present as determined by starch–iodide paper. The solvent was removed under reduced pressure, and the dried residue was treated with 15 cc. of absolute alcohol and allowed to stand overnight. The solution was filtered, concentrated, and the monoethyl acetal, IX, precipitated with ether; yield, 1.1 g. (80%). Recrystallization from alcohol and ether resulted in a product of m. p. 142–143°.

Anal. Calcd. for $C_{10}H_{14}O_3NCl$: C, 51.84; H, 6.09; N, 6.05. Found: C, 51.88; H, 6.33; N, 6.21.

2-Methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine Hydrochloride (VII).—A solution of 144 mg. of 1,3-dihydro-1-ethoxy-6-methyl-furo-[3,4-*c*]-pyridin-7-ol hydrochloride, IX, in 5 cc. of water containing one drop of 6 *N* hydrochloric acid was heated at 50–55° for five minutes and then allowed to stand at room temperature for half an hour. The solution was concentrated to dryness, and the dried residue, after it had crystallized, was washed several times with acetone. The 2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine hydrochloride had no melting point, but decomposed at 173–174°; yield, 121 mg. (96%).

Anal. Calcd. for $C_8H_{10}O_3NCl$: C, 47.18; H, 4.95; N, 6.88. Found: C, 47.01; H, 4.90; N, 6.70.

Lactone of 2-Methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine (XII): Method 1.—To 4.12 g. of pyridoxine hydrochloride dissolved in 150 cc. of water in a three-necked flask equipped with a stirrer and dropping funnel was added during two hours a saturated aqueous solution of 4.27 g. of potassium permanganate. After fifteen minutes of standing the reaction mixture was centrifuged to separate the manganese dioxide. The supernatant solution was concentrated to dryness and the residue extracted with boiling absolute alcohol. The alcoholic solution was acidified with alcoholic hydrochloric acid, and the solution filtered and concentrated to dryness. The residue, dissolved in water, was heated with activated charcoal, filtered, and again concentrated to dryness. This residue, washed once with alcohol, was dissolved in water. A

white precipitate settled out. The filtrate was treated with excess sodium bicarbonate, and more material of the same melting point was obtained. The two fractions were combined and recrystallized from alcohol; yield of the lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine, XII, 0.1 g.; m. p. 272–276°, with decomposition. The mixed melting point with the lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine showed a ten-degree depression.

Anal. Calcd. for $C_8H_7O_3N$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.26; H, 4.28; N, 8.75.

Method 2.—To an aqueous solution of 100 mg. of pyridoxal hydrochloride neutralized with 41 mg. of sodium bicarbonate was added a solution of 336 mg. of silver nitrate, 280 mg. of 25% sodium hydroxide, and 0.7 cc. of ammonium hydroxide in 14 cc. of water. The mixture deposited a silver mirror during the fifteen minutes that it was heated on the steam-bath. Excess hydrochloric acid was added to the reaction mixture. After filtration the solution was evaporated to 6 cc. Sodium bicarbonate was added, but no insoluble material separated out. The solution was made just acid with hydrochloric acid and evaporated to dryness under reduced pressure. The residue was extracted with refluxing alcohol. The material which separated out on concentration was washed with alcohol, dissolved in water, and neutralized with sodium bicarbonate. The lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine, XII, was obtained in a yield of 26 mg. (35%). Recrystallized from alcohol, it melted at 272–273°. There was no depression in the mixed melting point with a specimen of the lactone obtained by direct oxidation of pyridoxine.

Lactone of 2-Methyl-3-methoxy-4-carboxy-5-hydroxymethylpyridine (XIII).—An ethereal solution of diazomethane was added in small portions to 30 mg. of the lactone, XII, obtained from oxidation of pyridoxine, suspended in 4 cc. of absolute methanol cooled in ice. Ten minutes after the evolution of nitrogen had ceased, the solution was concentrated to dryness under reduced pressure, the residue dissolved in ether, boiled with Darco, and crystals of the lactone of 2-methyl-3-methoxy-4-carboxy-5-hydroxymethylpyridine precipitated with petroleum ether; m. p. 116.0–116.5°. The same methyl ether was obtained from the oxidation product of pyridoxal. The mixed melting point with the lactone of 2-methyl-3-methoxy-4-hydroxymethyl-5-carboxypyridine, XI, was depressed thirty degrees.

Anal. Calcd. for $C_9H_9O_3N$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.22; H, 5.08; N, 7.80.

Oxime of 2-Methyl-3-hydroxy-4-methoxymethyl-5-formylpyridine (XV).—2-Methyl-3-hydroxy-4-methoxymethyl-5-chloromethylpyridine hydrochloride, II, (29.7 g.) was dissolved in 480 cc. of 60% alcohol, and the hydrochloride neutralized with 10.5 g. of sodium bicarbonate. Hexamethylenetetramine (17.5 g.) was added, and the solution refluxed in an oil-bath at 100–110° for four hours. When the solution had cooled, 25.0 g. of sodium acetate and 18.7 g. of hydroxylamine hydrochloride were added and the resulting solution heated on the steam-bath for several minutes. After concentration of the solution under reduced pressure to about one-third of its original volume, water was added, and the precipitated oxime filtered and washed with water; yield, 5.9 g. (24%). After two recrystallizations, the melting point of the oxime of 2-methyl-3-hydroxy-4-methoxymethyl-5-formylpyridine was 198–199°, with decomposition.

Anal. Calcd. for $C_9H_{12}O_3N_2$: C, 55.09; H, 6.17; N, 14.27. Found: C, 55.37; H, 6.34; N, 14.54.

1,3-Dihydro-3-ethoxy-6-methyl-furo[3,4-*c*]pyridin-7-ol Hydrochloride (XVI).—The oxime of 2-methyl-3-hydroxy-4-methoxymethyl-5-formylpyridine, XV, 2.0 g., dissolved in 20 cc. of 2 *N* hydrochloric acid at about 50°, was treated with a solution of 0.75 g. of sodium nitrite in 3 cc. of water. The solution was warmed on the steam-bath and shaken until the evolution of nitrogen ceased. The resulting solution contained no excess nitrous acid. This solution, diluted with enough hydrochloric acid so that there was 95 cc. of 2.5 *N* acid, was heated in a sealed tube at 150° for five and one-half hours. The contents of the tube was heated with Darco, filtered, concentrated to dryness under reduced pressure, and the product extracted with absolute ethanol by heating for five minutes on the steam-bath, and then standing for two hours at room temperature. The extract was filtered, and 0.2 g. of 1,3-dihydro-3-ethoxy-6-methyl-furo[3,4-*c*]pyridin-7-ol hydrochloride, XVI, precipitated with ether. By concentration of the mother liquor, the yield was increased to 0.8 g. (34%). Pure material was obtained by two recrystallizations from alcohol and ether. The compound had no melting point.

Anal. Calcd. for $C_{10}H_{14}O_3NCl$: C, 51.84; H, 6.09; N, 6.05. Found: C, 51.86; H, 6.20; N, 6.55.

2-Methyl-3-hydroxy-4-hydroxymethyl-5-formylpyridine Hydrochloride (XVII).—1,3-Dihydro-3-ethoxy-6-methyl-furo[3,4-*c*]pyridin-7-ol hydrochloride, XVI, was dissolved in five cc. of water, heated with Darco, and filtered. One drop of 6 *N* hydrochloric acid was added to the filtrate, which was heated on the steam-bath for a few minutes and then evaporated to dryness under reduced pressure. The residue was crystallized with acetone and ether to give 2-methyl-3-hydroxy-4-hydroxymethyl-5-formylpyridine hydrochloride, XVII, which had no melting point.

Anal. Calcd. for $C_8H_{10}O_3NCl$: C, 47.18; H, 4.95; N, 6.88. Found: C, 47,45; H, 4.84; N, 7.16.

Conversion of Pyridoxamine to Pyridoxine.—To 1.0 g. of pyridoxamine dissolved in 13 cc. of 1 *N* hydrochloric acid was added in small portions a solution of 0.30 g. of sodium nitrite in 2 cc. of water. A vigorous gas evolution took place after each addition. After the reaction mixture had stood with occasional shaking for ten minutes, urea was added until no more free nitrous acid could be detected with starch–iodide paper. The aqueous solution was concentrated to dryness under reduced pressure and the dried residue extracted with absolute alcohol. Crystals melting below 190° were precipitated with ether. More crystals which separated from the mother liquor after it had stood at 0° for five days were recrystallized from alcohol and ether; yield, 4 mg.; m. p. 208–209°; mixed m. p. with pyridoxine hydrochloride, 208–209°.

Conversion of Pyridoxal to Pyridoxine.—Pyridoxal hydrochloride (320 mg.) dissolved in 125 cc. of water and neutralized with 132 mg. of sodium bicarbonate was shaken with about 0.4 g. of Raney nickel catalyst for four hours at 25° under 2–3 atmospheres of hydrogen. After removal of the catalyst and most of the solvent, excess hydrochloric acid was added, and the acid solution was concentrated to dryness under reduced pressure. Pyridoxine hydrochloride was obtained from the alcoholic extract of this residue; yield, 11 mg.; m. p. 207–208°; mixed, m. p. with pyridoxine hydrochloride, 206–208°.

Conversion of 2-Methyl-3-hydroxy-4-hydroxymethyl-5-aminomethylpyridine to Pyridoxine.—The reaction was carried out in the same manner as the conversion of pyridoxamine to pyridoxine, except that the mixture was heated on the steam-bath for eight minutes, and stood at room temperature for fifteen. At the end of this time, there was no free nitrous acid present. The pyridoxine hydrochloride obtained had the m. p. 207–208°, and when mixed with a known sample of pyridoxine hydrochloride it melted at 207°.

Conversion of 2-Methyl-3-hydroxy-4-hydroxymethyl-5-formylpyridine to Pyridoxine.—The conversion was carried out in the same manner as the conversion of pyridoxal to pyridoxine. The temperature, 33°, was slightly higher. The product melted at 209–210° as did a mixture of it and pyridoxine hydrochloride.

Acknowledgment.—The microanalyses were carried out by Messrs. R. N. Boos, L. Rosalsky, E. J. Thornton, W. K. Humphrey, J. H. McGregor, and Mrs. Edith Meiss.

Summary

2-Methyl-3-hydroxy-4-aminomethyl-

5-hydroxymethylpyridine (pyridoxamine) and 2-methyl - 3 - hydroxy - 4 - formyl - 5 - hydroxymethylpyridine (pyridoxal) have been synthesized, and their structures have been proved. These two compounds greatly exceed pyridoxine in growth promoting power for *L. casei* and *S. lactis R.*[5,8] Direct oxidation of pyridoxine yielded, in addition to pyridoxal, the lactone of 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethylpyridine.

Two other closely related compounds, 2-methyl-3-hydroxy-4-hydroxymethyl-5-aminomethylpyridine and 2-methyl-3-hydroxy-4-hydroxymethyl-5-formylpyridine, have been synthesized and found to be inactive toward lactic acid bacteria.

The two amines were converted by nitrous acid to pyridoxine. The two aldehydes were catalytically hydrogenated to yield pyridoxine.

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[CONTRIBUTION FROM THE BAKER LABORATORY OF CHEMISTRY AT CORNELL UNIVERSITY]

Pyridine Derivatives. I. 3-Cyano-4-ethoxymethyl-6-methyl-2-pyridone and Some Related Transformation Products

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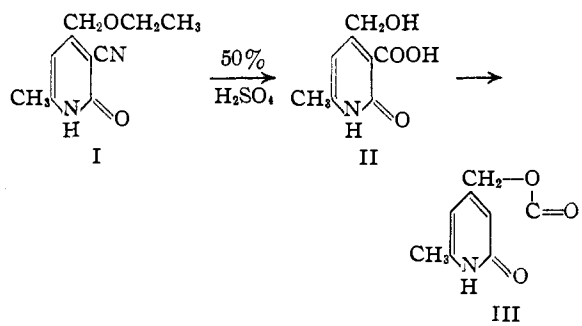
The importance of certain pyridine derivatives in biologically active systems has directed our attention to the synthesis and chemical behavior of some substances containing the pyridine nucleus. Because of our interest in the lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine, as a chick antianemia factor,[1] we have studied the synthesis of this substance reported by Harris, Stiller and Folkers.[2] From this study, certain improvements in the synthesis of the starting materials, some new reactions of the intermediates, involving the behavior of the lactone ring, and a desirable variation in the synthesis have been developed.

The ethoxyacetylacetone required for the ring closure was prepared by Sommelet[3] from ethyl ethoxyacetate and acetone in 42% yield. By a variation of the conditions of reaction and method of isolation, the yield has been increased to 62%. The ethyl ethoxyacetate required for this condensation has been made by the esterification of ethoxyacetic acid in the presence of hydrogen chloride,[4] in 69–72% yield. By using a suitable amount of benzene and sulfuric acid, we have secured a yield of 88–90%.

Hydrolysis of the 3-cyano-4-ethoxymethyl-6-methyl-2-pyridone (I) secured by the method of Harris, Stiller and Folkers gave a product which melted above 320°, in agreement with their description. The initial product, however, was more soluble in water than that which resulted after crystallization from alcohol. The decomposition points, determined by the method of approaching a limit,[5] were 340 and 360°, respectively. Upon analysis, the substance of lower decomposition point had the composition of the hydroxy acid (II), the other product being the lactone (III).



I II III

Treatment of this lactone with ammonia gave a product with the same decomposition point as the lactone, but analysis showed that the product had added a molecule of ammonia. By reaction with ammonia, three other lactones also added one molecule of ammonia. In each case, the change could not be demonstrated by the decomposition point, since loss of ammonia occurred on heating, and the decomposition point observed even in a sealed tube, was that of the starting material. Analysis showed the real nature of the product. Upon reaction with phosphorus pentachloride, the lactone III was readily converted to the α-chloro derivative (IV) and by treatment with bromine to a β-bromo derivative (V). Attempts to reduce the lactone by sodium and alcohol gave only oily products.

The nitration of the α-chloropyridine derivative (IV) gave an 80% yield of the known chloronitrolactone. In our hands, the nitration of the chlorolactone gave consistently better yields than the reverse procedure. This variation, therefore, appears more desirable than the reported procedure for the preparation of the hydroxy lactone.

Reduction of the initial condensation product, I, gave 3-aminomethyl-4-ethoxymethyl-6-methyl-2-pyridone (VI) characterized as the picrate, acetyl and phenylisothiocyanate derivatives. The β-bromo and 4-bromomethyl derivatives of VI were also prepared. By nitration and reduction, I was converted to a diamine.

For comparison with some of the ethoxy de-

(1) Scott, Norris, Heuser, Bruce, Coover, Bellamy and Gunsalus, *J. Biol. Chem.*, **154**, 713 (1944).
(2) Harris, Stiller and Folkers, THIS JOURNAL, **61**, 1242 (1939).
(3) Sommelet, *Bull. soc. chim.*, [4] **1**, 382 (1907).
(4) Fuson and Wojcik, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons Co., Inc., New York, N. Y., 1943, p. 260.
(5) Bruce, *ibid.*, p. 14.