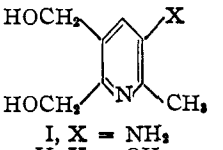
[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Pyridine Syntheses. IV. The Preparation of Some Vitamin B_6 Analogs

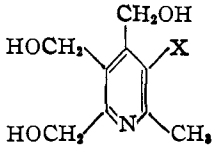
BY REUBEN G. JONES

Five analogs of vitamin B_6 have been synthesized for testing as antagonists of the vitamin. These are 5-amino-2,3-dihydroxymethyl-6-methylpyridine, 2,3-dihydroxymethyl-5-hydroxy-6-methylpyridine, 5-amino-6-methyl-2,3,4-trihydroxymethylpyridine, 5-hydroxy-6-methyl-2,3,4-trihydroxymethylpyridine and 4-amino-2,5-dihydroxymethyl-6-methylpyridine. They were prepared by reduction of the corresponding aminopyridine carboxylic esters with lithium aluminum hydride and conversion of the amino to hydroxy group with nitrous acid. In preliminary tests two of the compounds have shown some biological activity.

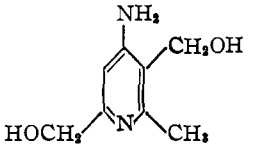
New methods for the synthesis of vitamin B_6 have been reported in recent communications from this Laboratory.[1] Incidental to these studies a number of analogs of vitamin B_6 have been synthesized and tested in the interest of finding antagonists of the vitamin. This paper describes the preparation of five such analogs, I–V.



I, X = NH_2
II, X = OH



III, X = NH_2
IV, X = OH



V

The starting material for the synthesis of I and II was diethyl 5-cyano-6-methyl-2,3-pyridinedicarboxylate.[2] This was treated with concentrated sulfuric acid to convert the cyano group to carbamyl. Reaction of the carbamyl compound with alkaline hypochlorite gave 5-amino-6-methyl-2,3-pyridinedicarboxylic acid. The latter was esterified with alcohol and hydrogen chloride, and the resulting ester was reduced with lithium aluminum hydride to form I. Compound II was obtained by treatment of I with nitrous acid.

For the synthesis of III and IV the starting material was 5-amino-6-methyl-2,3,4-pyridinetricarboxylic acid.[1c] This acid was highly resistant toward esterification with methanol and hydrogen chloride. By heating a suspension of the acid in a mixture of absolute ethanol and benzene containing 2% sulfuric acid it was possible to esterify two of the three carboxyl groups. The resulting compound was believed to be 5-amino-2,3-dicarbethoxy-6-methyl-4-pyridinecarboxylic acid. The reason for assigning the free carboxyl group to position-4 is that the analogous 3-amino-2-methyl-4,5-pyridinedicarboxylic acid is also extremely difficult to esterify with methanol and hydrogen chloride,[1a] whereas its isomer, 5-amino-6-methyl-2,3-pyridinedicarboxylic acid, is esterified with ease under the same conditions (see Experimental).

Compound III was obtained by lithium aluminum hydride reduction of 5-amino-2,3-dicarbethoxy-6-methyl-4-pyridinecarboxylic acid or the corresponding trimethyl ester prepared with diazomethane. Reaction of compound III with nitrous acid led to IV.

The reduction of dimethyl 4-amino-6-methyl-2,5-pyridinedicarboxylate[1c] with lithium aluminum hydride gave compound V.

In an attempt to prepare 5-amino-3-hydroxymethyl-6-methyl-2-trifluoromethylpyridine, methyl 5-amino-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate was reduced with lithium aluminum hydride. The product was isolated but was found to be unstable, and a pure, analytical sample could not be obtained. The instability of this aminotrifluoromethylpyridine compound is perhaps not surprising in view of the relative instability of the analogous *p*-trifluoromethylaniline.[3]

Testing of the various compounds for their ability to antagonize vitamin B_6 was carried out using standard procedures.[4] None of the compounds had any effect at concentrations as high as 1:2000 on the growth of *E. coli 105* or *Strep. viridans*. Compound II inhibited the growth of *Neurospora sitophilia*, and this inhibition was reversed by vitamin B_6. The inhibition ratio was 1 part of B_6 to about 25,000 parts of compound II. Compound V also was a weak antagonist of vitamin B_6, the inhibition ratio being about the same as that for compound II. Compounds I and III had no effect on *Neurospora sitophilia*. The products reported at this time are much less effective antagonists for vitamin B_6 than another compound that we recently described, namely, 3-amino-4,5-dihydroxymethyl-2-methylpyridine.[1a] It had an inhibition ratio of one part of B_6 to about 500 parts of the compound using *Neurospora sitophilia* as the test organism.

In connection with these studies, another related compound, phthalyl alcohol[5] (*o*-dihydroxymethylbenzene), was tested for its effect on the growth of *Neurospora sitophilia*. Surprisingly, it was found not to inhibit, but actually to support the growth of the organism in the absence of vitamin B_6. Its activity was about one five-hundredth that of the vitamin.

Acknowledgment.—The author is grateful to W. L. Brown, H. L. Hunter and W. J. Schenck for the microanalyses and to F. Streightoff for carrying out the microbiological tests.

(1) (a) R. G. Jones and E. C. Kornfeld, THIS JOURNAL, **73**, 107 (1951); (b) R. G. Jones, *ibid.*, **73**, 5244 (1951); (c) R. G. Jones, *ibid.* **73**, 5610 (1951).

(2) E. M. Bottorff, R. G. Jones, E. C. Kornfeld and M. J. Mann, *ibid.*, **73**, 4380 (1951).

(3) R. G. Jones, *ibid.*, **69**, 2346 (1947).

(4) For example see: D. W. Woolley and A. G. C. White, *J. Exptl. Med.*, **78**, 489 (1943).

(5) W. H. Perkin, Jr., *J. Chem. Soc.*, **53**, 6 (1888). The sample used in the present work was obtained by lithium aluminum hydride reduction of diethyl phthalate.

Experimental

Diethyl 5-Carbamyl-6-methyl-2,3-pyridinedicarboxylate.—To 65 g. (0.25 mole) of diethyl 5-cyano-6-methyl-2,3-pyridinedicarboxylate[2] in a 300-ml. round-bottom flask was added 150 ml. of concentrated sulfuric acid. The liquids were mixed, and heat was evolved. The temperature was kept at about 60° by gently warming on the steam-bath for one hour. After cooling to 5° the liquid was poured onto 1 kg. of chipped ice, and the mixture was extracted with four 500-ml. portions of ethyl acetate. The extract was washed with sodium bicarbonate solution, dried and evaporated leaving a white crystalline solid. This was washed by suspension in petroleum ether. The yield was 42 g. (60%); m.p. 140–140.5° (from benzene).

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.76; N, 10.00. Found: C, 55.70; H, 5.88; N, 9.93.

5-Amino-6-methyl-2,3-pyridinedicarboxylic Acid.—To a solution made by absorbing 10.6 g. (0.15 mole) of chlorine in 150 ml. of ice-cold 8 *N* sodium hydroxide solution was added 150 g. of chipped ice followed by 38 g. (0.135 mole) of diethyl 5-carbamyl-6-methyl-2,3-pyridinedicarboxylate. The mixture was stirred, and the solid gradually dissolved. After about one hour at room temperature the solution was heated on the steam-bath for one-half hour, then acidified to about *p*H 2 by carefully adding concentrated hydrochloric acid. The mixture was cooled in an ice-bath, and the precipitate was collected, washed with a little ice-water, acetone and ether, and air-dried. There was obtained 22.5 g. (86% yield) of finely divided, buff-colored, crystalline powder. A sample was recrystallized from water in which it was very sparingly soluble; m.p. 241–243° dec. (uncor.).

Anal. Calcd. for $C_8H_8N_2O_4$: C, 48.98; H, 4.11; N, 14.28. Found: C, 48.85; H, 4.13; N, 14.47.

Dimethyl 5-Amino-6-methyl-2,3-pyridinedicarboxylate.—5-Amino-6-methyl-2,3-pyridinedicarboxylic acid was esterified with methanol and hydrogen chloride in the usual way to give a 77% yield of the dimethyl ester. A sample recrystallized from ethyl acetate–ether–petroleum ether mixture melted at 88–88.5°.

Anal. Calcd. for $C_{10}H_{12}N_2O_4$: C, 53.56; H, 5.40; N, 12.50. Found: C, 54.12; H, 5.53; N, 12.67.

Diethyl 5-Amino-6-methyl-2,3-pyridinedicarboxylate.—This was prepared from the acid by esterification with ethanol and hydrogen chloride. The yield was 65% of fine white powder which melted at 86.5–87° after recrystallization from ether–petroleum ether.

Anal. Calcd. for $C_{12}H_{16}N_2O_4$: N, 11.10. Found: N, 11.06.

5-Hydroxy-6-methyl-2,3-pyridinedicarboxylic Acid.—A suspension of 10 g. of 5-amino-6-methyl-2,3-pyridinedicarboxylic acid in 125 ml. of 5 *N* hydrochloric acid was heated to 80–90° and, with stirring, a solution of 15 g. of sodium nitrite in 20 ml. of water was added dropwise during about 20 minutes. The solid did not dissolve but changed somewhat in appearance. After cooling in an ice-bath the dense buff colored crystalline product was collected on a filter, washed with acetone and ether, and air-dried. The yield was 8.5 g. (85%). It did not melt up to 300°.

Anal. Calcd. for $C_8H_7NO_5$: C, 48.73; H, 3.58; N, 7.11. Found: C, 48.10; H, 3.04; N, 7.60.

Dimethyl 5-Hydroxy-6-methyl-2,3-pyridinedicarboxylate.—This was prepared in 60% yield by esterification of the acid with methanol and hydrogen chloride. A sample was recrystallized from ethyl acetate–ether–petroleum ether mixture; m.p. 157–157.5°.

Anal. Calcd. for $C_{10}H_{11}NO_5$: N, 6.22. Found: N, 6.33.

5-Amino-2,3-dihydroxymethyl-6-methylpyridine (I).—Reduction of dimethyl or diethyl 5-amino-6-methyl-2,3-pyridinedicarboxylate with lithium aluminum hydride by a procedure previously described[1a] gave I in a 70–80% yield after recrystallization from absolute ethaol; m.p. 154–154.5°.

Anal. Calcd. for $C_8H_{12}N_2O_2$: C, 57.12; H, 7.19; N, 16.66. Found: C, 57.36; H, 7.76; N, 16.42.

2,3-Dihydroxymethyl-5-hydroxy-6-methylpyridine (II).—This was prepared from 5-amino-2,3-dihydroxymethyl-6-methylpyridine by treatment with sodium nitrite in hot dilute sulfuric acid solution by a procedure previously described.[1a] The compound was a white crystalline powder readily soluble in water or alcohol, insoluble in petroleum ether or ether, sparingly soluble in ethyl acetate and moderately soluble in acetone. The yield was about 90%. A sample was recrystallized from acetone; m.p. 145–146°.

Anal. Calcd. for $C_8H_{11}NO_3$: N, 8.28. Found: N, 8.71.

The hydrochloride was prepared by saturating a solution in absolute ethanol with dry hydrogen chloride and adding three volumes of dry ether to precipitate the product as fine white crystals; m.p. 169–170°.

Anal. Calcd. for $C_8H_{11}NO_3 \cdot HCl$: Cl, 17.27. Found: Cl, 17.28.

Trimethyl 5-Amino-6-methyl-2,3,4-pyridinetricarboxylate.—A suspension of 12 g. of crude 5-amino-6-methyl-2,3,4-pyridinetricarboxylic acid dihydrate[1c] in 250 ml. of methanol was treated with a solution of about 0.2 mole of diazomethane in 400 ml. of ether. After standing overnight the solution was filtered and evaporated under reduced pressure to a sirup. This was taken up in 400 ml. of dry ether. The solution was filtered and evaporated to 25 ml. and the crystalline solid which had separated was collected on a filter, washed with a little ether and air dried. The yield was 5.7 g. (48%). A sample was recrystallized from a mixture of ether, ethyl acetate and petroleum ether; m.p. 132.5–133.5°.

Anal. Calcd. for $C_{12}H_{14}N_2O_6$: C, 51.06; H, 5.00; N, 9.93. Found: C, 51.00; H, 5.11; N, 10.01.

The triester was not obtained when the acid in methanol solution saturated with hydrogen chloride was heated under reflux for several hours or allowed to stand at room temperature for several days.

5-Amino-2,3-dicarbethoxy-6-methyl-4-pyridinecarboxylic Acid.—A suspension of 9 g. (0.033 mole) of 5-amino-6-methyl-2,3,4-pyridinetricarboxylic acid dihydrate in a mixture of 175 ml. of absolute ethanol, 100 ml. of dry benzene and 5 ml. of concentrated sulfuric acid was heated under reflux. After about one hour the solution became clear, but heating was continued for 20 hours. The solution was evaporated in vacuum to small volume and poured into 100 ml. of water containing 20 g. of sodium bicarbonate. The aqueous solution was washed with 100 ml. of ethyl acetate which was discarded, and then it was carefully acidified with concentrated hydrochloric acid to *p*H 2 after which it was extracted with three 100-ml. portions of ethyl acetate. Evaporation of the dried ethyl acetate extract in vacuum gave 8.5 g. of yellow powder which was very soluble in ethyl acetate, soluble in water and somewhat soluble in ether. A portion of the product, 7.5 g., was stirred with 1500 ml. of dry ether, the solution was filtered from 0.6 g. of insoluble solid and evaporated to dryness leaving 6.8 g. of crystalline solid. A sample was dissolved in anhydrous ether, the solution was decolorized with carbon, evaporated to one-fifth volume, and, after standing, it deposited colorless, stout, needle-like crystals; m.p. 100–102° dec.

Anal. Calcd. for $C_{13}H_{16}N_2O_6 \cdot {}^1/_2H_2O$: C, 51.08; H, 5.62; N, 9.18. Found: C, 51.35, 51.19; H, 5.76, 5.23; N, 9.39.

This experiment was repeated with essentially the same results.

5-Amino-6-methyl-2,3,4-trihydroxymethylpyridine (III).—To a solution of 5 g. of lithium aluminum hydride in 300 ml. of dry ether was added *via* soxhlet extraction during a period of four hours 5.3 g. of trimethyl 5-amino-6-methyl-2,3,-4-pyridinetricarboxylate. The reaction mixture was worked up, in a manner previously described.[1a] The crude product was taken up in 200 ml. of boiling absolute ethanol, and the solution was decolorized with carbon, filtered and diluted with 300 ml. of dry ether. After standing at 0° for several days 1.8 g. of fine white crystalline precipitate was deposited, m.p. 188–190°. The filtrate was partially evaporated and again diluted with ether to give an additional 0.4 g. of product making the total yield 2.2 g. (61%). A sample recrystallized from an absolute ethanol–ether mixture melted at 191–192°.

Anal. Calcd. for $C_9H_{14}N_2O_3$: C, 54.53; H, 7.12; N, 14.14. Found: C, 54.87; H, 7.43; N, 14.34.

5-Amino-2,3-dicarbethoxy-6-methyl-4-pyridinecarboxylic acid was reduced in the same way to give a 47% yield of 5-amino-6-methyl-2,3,4-trihydroxymethylpyridine.

5-Hydroxy-6-methyl-2,3,4-trihydroxymethylpyridine (IV).—A solution of 2 g. of 3-amino-2-methyl-4,5,6-trihydroxymethylpyridine in 40 ml. of 2 *N* sulfuric acid was heated to 70–80° and a solution of 2 g. of sodium nitrite in 10 ml. of

water was added dropwise with stirring during 15 minutes. Heating was continued for 15 minutes after which the solution was brought to *p*H 7 with sodium hydroxide and evaporated on the steam-bath under reduced pressure to dryness. The residue was extracted with 300 ml. of hot acetone, and this extract was evaporated to dryness. Again the residue was taken up in 400 ml. of hot acetone. The solution was filtered, evaporated to 50 ml. and then allowed to evaporate slowly to dryness. A white crystalline residue was left mixed with a little gum. It was triturated with a very little ice-cold, absolute ethanol, collected on a filter and air-dried. The yield was 0.9 g. It was recrystallized from a mixture of acetone, methanol and petroleum ether; m.p. 141–142°.

Anal. Calcd. for $C_9H_{13}NO_4$: N, 7.03. Found: N, 6.92.

4-Amino-6-methyl-2,5-dihydroxymethylpyridine (V).—One gram of dimethyl 4-amino-6-methyl-2,5-pyridinedicarboxylate[1c] was allowed to react with 1 g. of lithium aluminum hydride in 250 ml. of anhydrous ether, and the mixture was worked up in a manner previously described.[1a] There was obtained 0.62 g. (83% yield) of 4-amino-6-methyl-2,5-dihydroxymethylpyridine, m.p. 143–145°. A sample for analysis was recrystallized from absolute ethanol–ethyl acetate–petroleum ether mixture; m.p. 147–148°.

Anal. Calcd. for $C_8H_{12}N_2O_2$: C, 57.12; H, 7.19; N, 16.66. Found: C, 56.69; H, 7.17; N, 16.52.

Ethyl 5-Cyano-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate.—Ethyl ethoxymethylenetrifluoroacetoacetate[6] and β-aminocrotononitrile were allowed to condense, and the mixture was worked up in a manner previously described for similar reactions.[2] The resulting impure ethyl 5-cyano-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate was obtained in 63% yield as a colorless liquid; b.p. 130–140° (4 mm.); 97–102° (0.7 mm.).

Anal. Calcd. for $C_{11}H_9F_3N_2O_2$: C, 51.17; H, 3.51; N, 10.85. Found: C, 51.72; H, 3.82; N, 11.43.

Ethyl 5-Carbamyl-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate.—Ethyl 5-cyano-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate, was treated with concentrated sulfuric acid and the mixture was worked up as described above to give ethyl 5-carbamyl-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate in 60–70% yield. It was a white crystalline solid which melted at 167–168° after recrystallization from ether–petroleum ether.

Anal. Calcd. for $C_{11}H_{11}F_3N_2O_3$: C, 47.83; H, 3.98; N, 10.14. Found: C, 48.01; H, 3.22; N, 10.07.

5-Amino-6-methyl-2-trifluoromethyl-3-pyridinecarboxylic Acid.—This was prepared from ethyl 5-carbamyl-6-methyl-2-trifluoromethyl-3-pyridinecarboxylate by reaction with hypochlorite in the same way as described above for the preparation of 5-amino-6-methyl-2,3-pyridinedicarboxylic acid. The compound was obtained in 88% yield. A sample was recrystallized from water; m.p. 215–216° dec. (uncor.).

Anal. Calcd. for $C_8H_7F_3N_2O_2$: C, 43.64; H, 3.21; N, 12.73. Found: C, 43.87; H, 3.36; N, 12.81.

Attempted Preparation of 3-Amino-5-hydroxymethyl-2-methyl-6-trifluoromethylpyridine.—The above noted 3-amino-2-methyl-6-trifluoromethyl-5-pyridinecarboxylic acid was esterified by heating under reflux for 20 hours in methanol saturated with hydrogen chloride. Isolation of the ester in the usual way gave a viscous sirup which did not crystallize. The crude ester was reduced in ether solution with lithium aluminum hydride and the product isolated as described previously for similar reactions.[1a] It was a white solid, soluble in water, alcohol, or ether, but it appeared to be unstable. All attempts to purify the material resulted in its decomposition, and no analytical sample was obtained.

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(6) R. G. Jones, THIS JOURNAL, **73**, 3684 (1951).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

The Structure of Chondrosine and of Chondroitinsulfuric Acid[1]

BY M. L. WOLFROM, R. K. MADISON AND M. J. CRON

Chondroitinsulfuric acid from cartilage is electrophoretically homogeneous and consumes 1 mole of periodate per disaccharide unit to cleave a glycol in the D-glucuronic acid portion. The component disaccharide, chondrosine (further characterized as the crystalline heptabenzoyl methyl ester derivative), was converted to the crystalline diamide glycitol IV which on periodate oxidation underwent formaldehyde and formic acid scission in the reduced portion to yield crystalline V with the N-acetamido-2-desoxy-D-galactopyranosyl radical intact; further periodate oxidation cleaved a glycol in this hexosamine moiety. Chondrosine is therefore 4-(2-amino-2-desoxy-β(?)-D-galactopyranosyl)-D-glucuronic acid and in the heteropolymer one sulfate acid ester group and the glycosidic attachment of the adjacent D-glucuronic acid group are yet to be partitioned between positions 3, 4 and 6 of each chondrosamine unit. The high yield of chondrosine obtainable by acid hydrolysis characterizes chondroitinsulfuric acid as very probably a linear type polymer.

Chondroitinsulfuric acid is the heteropolysaccharide present in combination with protein in cartilage. It was adequately described by Krukenberg[2] and by Mörner[3] and has been extensively investigated by Levene and co-workers.[4] Its component monosaccharide units[5] are D-glucuronic acid[6,7] and N-acetylchondrosamine[8] (N-acetyl-2-amino-2-desoxy-D-galactose[9]). To these two units is attached one sulfuric acid group as an acid ester. The component disaccharide, designated chondrosine, of this polysaccharide has long been known as its crystalline ethyl ester hydrochloride.[10] It is readily obtainable in high yield by differential acid hydrolysis dependent upon the resistance toward hydrolysis exhibited by the 2-amino-2-desoxyglycosidic linkage. The uronic acid is therefore the reducing component. The high yield of this disaccharide obtained in our work indicates that the polysaccharide is very probably of the linear type with this component as the repeating unit. In his last and posthumously published article,[11] the late Dr. P. A. Levene described a number of crystalline derivatives of this disaccharide but did not succeed in establishing its structure.

Levene reduced the crystalline methyl ester hydrochloride of chondrosine (II, Fig. 1) to the gly-

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(11) P. A. Levene, *J. Biol. Chem.*, **140**, 267 (1941).