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Synthesis of Vitamin B_6 Derivatives I. Preparation of some Isopyridoxamine Derivatives from Isopyridoxal (1)

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Improved syntheses of isopyridoxal and isopyridoxamine are described. New 5-*N*-substituted isopyridoxamine derivatives resulting from condensation followed by reduction of the carbonyl group of isopropylideneisopyridoxal with aniline, 2-aminothiazole and benzylamine are also reported.

The 5-hydroxymethyl group of pyridoxal plays no essential part in catalysis of nonenzymic reactions of amino acids (3). However, the phosphate group attached at this position in the coenzyme form of the vitamin is required for the enzymic function of the compound. It probably helps to bind the molecule to the apoenzyme, and it may participate in acid-base catalysis on the enzyme surface. A series of analogs of pyridoxal containing various side chains in place of the 5-hydroxymethyl group would be of interest in the study of both nonenzymic and enzymic catalysis.

We have developed an improved preparation of isopyridoxal, a possible intermediate in a large number of syntheses leading to compounds of this type. Korytnyk, Kris, and Singh have also prepared isopyridoxal (IV) from pyridoxol (I) through conversion to the isopropylidene derivative (II) and oxidation of the latter with chromic anhydride-pyridine complex (4). We have followed a similar procedure (Scheme I) but have employed manganese dioxide as the oxidant. An 87% yield of pure isopropylideneisopyridoxal (III) was obtained. This compound is an intermediate from which a number of analogs of pyridoxal may be prepared. Korytnyk has described several analogs of pyridoxol in which the side-chain has been extended through condensation of compound II with malonic acid and subsequent reactions (5). We have condensed the same compound with amines to produce Schiff bases which have been reduced with sodium borohydride to *N*-substituted isopyridoxamine derivatives (Scheme I). This represents a general method (similar to that employed by Heyl *et al.*, (6) for introduction of groups at the 4-position) by which a variety of 5-substituted pyridoxol derivatives can be prepared.

EXPERIMENTAL

Isopropylideneisopyridoxal (III).

A mixture of 10 g. of manganese dioxide "B" was prepared according to Harnfeist *et al.*, (7) and 5 g. (0.024 mole) of the free base form of isopropylidenepyridoxol (II) (8) in 50 ml. of chloroform was stirred at room temperature (25-30°) for 22 hours. The reaction mixture was diluted with 100 ml. of chloroform and filtered with suction through a one-half inch layer of "Celite". The residue was washed with five 50 ml. portions of boiling chloroform and finally with 50 ml. of boiling methanol. The combined filtrate and washings were evaporated under reduced pressure on a water bath to an oily residue which crystallized upon trituration and cooling in an ice bath to yield 6.85 g. (98%) of crude isopropylideneisopyridoxal. The crude compound was decolorized by placing it on the top of a column of alumina (10 cm. long and 2 cm. in diam.) and eluting either with petroleum ether (Skelly B, b.p. 60-71°) or with a 1:1 mixture of chloroform and petroleum ether (Skelly A, b.p. 26-31°). The eluent was then evaporated to an oil which solidified on standing. The crude solid was twice recrystallized from petroleum ether (Skelly A), wherewith 4.3 g. (87%) of the pure vacuum-dried product was obtained, m.p. 62-63°, (Literature, 61-62°) (4).

Anal. Calcd. for $C_{11}H_{13}NO_3$: C, 63.79; H, 6.32; N, 6.76. Found: C, 63.50; H, 6.21; N, 6.72.

Isopyridoxal (IV).

A solution of 1.04 g. (0.005 mole) of isopropylideneisopyridoxal (III) in 10 ml. of 1*N* hydrochloric acid solution was heated on a steam bath for 15 minutes. The solution was cooled and neutralized carefully to *p*H 6.5 with 10% aqueous sodium hydroxide solution. Upon standing the crystalline product separated. There was obtained 0.81 g. (97%) of the vacuum-dried substance, which melted with decomposition at 178-184° (Literature, 185-186°) (4) after recrystallization from ethyl acetate.

Anal. Calcd. for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.35; H, 5.36; N, 8.07.

Oxime of Isopropylideneisopyridoxal (V).

To a solution of 2.07 g. (0.01 mole) of isopropylideneisopyridoxal (III) in 15 ml. of 95% ethanol, there was added a solution of 0.76 g. (0.011 mole) hydroxylamine hydrochloride and 0.44 g. (0.011 mole) of sodium hydroxide in 5 ml. of water. The crystalline product which separated from solution almost immediately amounted to 2.13 g. (95%) m.p. 205-206° with decomposition.

Anal. Calcd. for $C_{11}H_{14}N_2O_3$: C, 59.45; H, 6.35; N, 12.61. Found: C, 59.75; H, 6.08; N, 12.49.

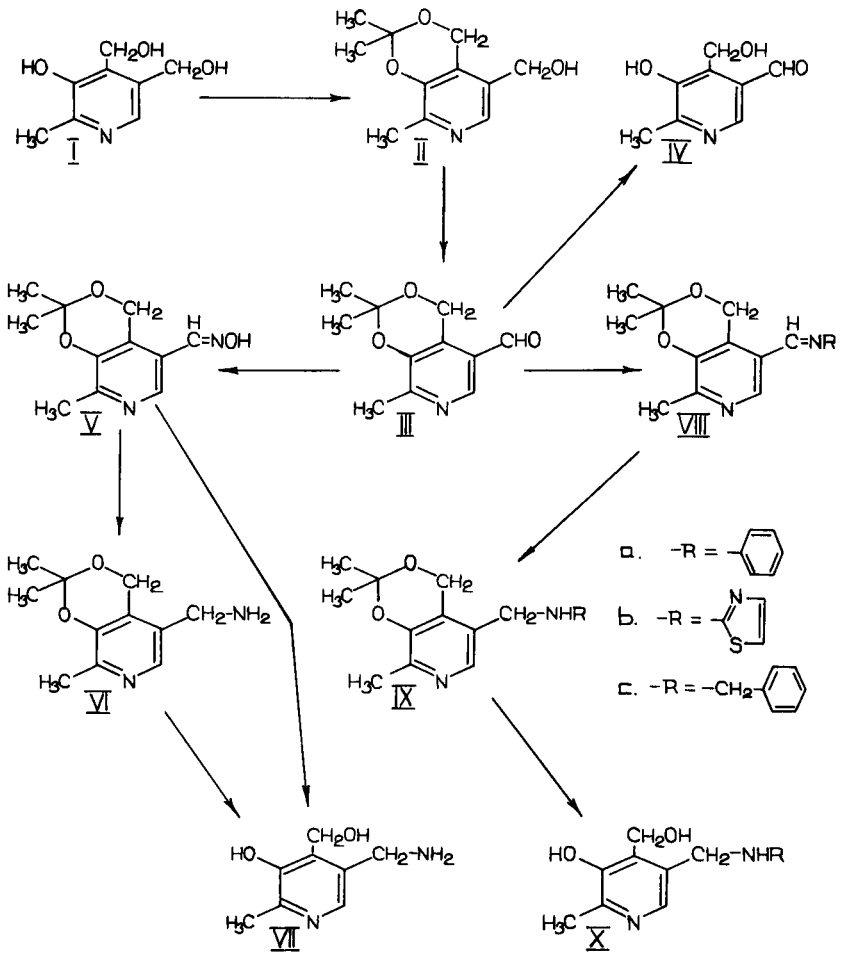
Isopyridoxal Oxime.

A solution of 2.2 g. (0.01 mole) of isopropylideneisopyridoxal oxime in 15 ml. of 1*N* hydrochloric acid solution was heated on a steam bath for 15 minutes, cooled and neutralized to *p*H 6.5 with 10% aqueous sodium hydroxide solution. Upon standing the oxime separated. The yield of the pure product was 1.8 g. (99%), m.p. 191-192° with decomposition.

This substance was also prepared by treating 1.67 g. (0.01 mole) of isopyridoxal (IV) in 10 ml. of hot (90°) water with 0.76 g. (0.011 mole) of solid hydroxylamine hydrochloride. Upon cooling this solution to 40° and neutralizing it to *p*H 6.5 with 10% aqueous sodium hydroxide solution, the product precipitated almost immediately. The yield was 1.8 g. (99%), m.p. 191-192° with decomposition. Mixed m.p. with the substance obtained above showed no depression.

Anal. Calcd. for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.64; H, 5.53; N, 15.14.

SCHEME 1



α^4,3-*O*-Isopropylideneisopyridoxamine (VI).

To a stirred solution of 0.54 g. (0.12 mole) of lithium aluminum hydride in 25 ml. of anhydrous ether maintained at -40° in a "Dry Ice"-acetone bath, there was slowly added a suspension of 2 g. (0.009 mole) of isopropylidene-5-pyridoxal oxime (V) in 100 ml. of anhydrous ether. The cooling bath was removed, and the mixture was allowed to warm up to room temperature, then refluxed for 10 hours. The mixture was again cooled in a "Dry Ice"-acetone bath, and 10 ml. of water was slowly added followed by 4 ml. of 20% aqueous sodium hydroxide. After separating and drying the ether layer over anhydrous sodium sulfate, solvent was removed by evaporation under reduced pressure to leave a semi-solid residue. Recrystallization of this residue from cyclohexane afforded 0.6 g. (32%) of the product; m.p. 89-90°.

Anal. Calcd. for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45. Found: C, 63.51; H, 7.48; N, 13.26.

Isopyridoxamine Dihydrochloride (VII).

Following the procedure of Testa and Fava (9), 3.3 g. (0.018 mole) of isopyridoxal oxime (V) dissolved in 41 ml. of glacial acetic acid and mechanically stirred was treated with 5.5 g. (0.084 g. atom) of zinc dust in three portions. The temperature, which rose spontaneously with the first addition to about 70°, was allowed to fall to 40° before addition of each subsequent portion. The resultant mass was filtered, and the solid residue of zinc dust and salts was washed with 30 ml. of glacial acetic acid. The combined filtrate and washings were evaporated under reduced pressure on a water bath (60°) to an oily residue, which was dissolved in 30 ml. of water. The zinc salts in this solution were removed by treatment with hydrogen sulfide, followed by addition of 2 g. of "Celite" and filtration with suction. The zinc sulfide residue was washed with 20 ml. of water and the combined filtrate and washings were decolorized with "Norit-A", acidified with 10 ml. of concentrated hydrochloric acid and evaporated to dryness under reduced pressure. The crystalline residue was recrystallized from a methanol-ethyl acetate mixture. Yield, 3.7 g. (85%), m.p. 198-199°.

When 0.3 g. (0.0014 mole) of isopropylideneisopyridoxamine (VI) was dissolved in 10 ml. of 1*N* hydrochloric acid solution and heated on a steam bath for 15 minutes, then evaporated to dryness, there was obtained 0.33 g. (97%) of material which was identical with the isopyridoxamine dihydrochloride described in the preceding paragraph, m.p. 198-199°.

Anal. Calcd. for $C_8H_{14}Cl_2N_2O_2$: C, 39.85; H, 5.85; N, 11.62. Found: C, 40.06; H, 5.95; N, 11.96.

Isopropylideneisopyridoxal Anil (VIIIa).

A mixture of 2.07 g. (0.01 mole) of isopropylideneisopyridoxal (III) and 0.93 g. (0.01 mole) of aniline was heated on a steam bath for 15 minutes and cooled. To the resultant oily mass there was added 2 ml. of 95% ethanol and 5 drops of water. Crystallization of the product was induced by trituration of the ethanolic solution while cooling it in an ice bath. After recrystallization from 70% ethanol there was obtained 2.67 g. (89%) of the pure product, m.p. 80-81°.

Anal. Calcd. for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.42; N, 9.92. Found: C, 72.65; H, 6.48; N, 10.04.

α^4,3-*O*-Isopropylidene-5-*N*-Phenylisopyridoxamine (IXa).

To a stirred solution of 3.1 g. (0.011 mole) of isopropylideneisopyridoxal anil (VIIIa) in 35 ml. of warm (50°) absolute methanol, there was slowly added 0.57 g. (0.015 mole) of sodium borohydride. When the evolution of hydrogen had subsided the solution was refluxed for 15 minutes. The reaction mixture then was cooled in an ice bath and treated with 10 ml. of 20% aqueous sodium hydroxide solution and 20 ml. of ice water. Continued cooling with trituration caused the crystalline product to separate, and an additional 50 ml. of ice water then was added. The yield of the crude air-dried product was 3.05 g. (98%), m.p. 110-114°. After two recrystallizations from petroleum ether (Skelly B) 3 g. (96%) of the pure material was obtained, m.p. 114-115°.

Anal. Calcd. for $C_{17}H_{20}N_2O_2$: C, 71.80; H, 7.09; N, 9.85. Found: C, 72.15; H, 6.99; N, 9.92.

5 *N*-Phenylisopyridoxamine (Xa).

A solution of 2.5 g. (0.009 mole) of IXa in 15 ml. of 1*N* hydrochloric acid solution was heated on a steam bath for 15 minutes. The solution was cooled in an ice bath and adjusted to *p*H 8 with 20% aqueous sodium hydroxide solution, wherewith, the crystalline product separated immediately. Subsequent to air-drying and recrystallization from absolute ethanol 2.0 g. (93%) of the desired substance was obtained, m.p. 182-183°.

Anal. Calcd. for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.91; H, 6.67; N, 11.31.

Imine of Isopropylideneisopyridoxal with 2-Aminothiazole (VIIIb).

A solution of 1 g. (0.01 mole) of 2-aminothiazole and 2.07 g. (0.01 mole) of isopropylideneisopyridoxal (III) in 50 ml. of absolute ethanol was refluxed for 8 hours. The alcohol was removed by distillation under reduced pressure, and the viscous oily residue was heated on a steam bath for an additional hour, then cooled and allowed to stand at room temperature (25-30°) for 4 days. After this period of standing in an open flask, the entire mass had solidified. Recrystallization from 40% ethanol afforded 2 g. (69%) of the pure, yellow crystalline product, m.p. 113-114°.

Anal. Calcd. for $C_{14}H_{15}N_3O_2S$: C, 58.11; H, 5.23; N, 14.52. Found: C, 58.19; H, 5.41; N, 14.74.

α^4,3-*O*-Isopropylidene-5-*N*-(2-Thiazolyl)Isopyridoxamine (IXb).

To a solution of 1.77 g. (0.006 mole) of VIIIb in 20 ml. of warm (45°) absolute methanol, there was added slowly with gentle stirring, 0.27 g. (0.007 mole) of sodium borohydride. When the evolution of hydrogen had subsided, the mixture was refluxed for 15 minutes. After cooling in an ice-bath for several minutes the reaction mixture was treated with 20 ml. of ice water, and, after the crystalline product began to separate, an additional 50 ml. of ice water was added dropwise. There was obtained without further recrystallization 1.71 g. (96%) of the desired product, m.p. 118-119°.

Anal. Calcd. for $C_{14}H_{17}N_3O_2S$: C, 57.71; H, 5.88; N, 14.42. Found: C, 57.56; H, 6.17; N, 14.20.

5-*N*-(2-Thiazolyl)-Isopyridoxamine (Xb).

A solution of 1.6 g. (0.0055 mole) of IXb in 10 ml. of 1*N* hydrochloric acid solution was heated on a steam bath for 15 minutes, cooled and adjusted to *p*H 6 with 20% aqueous sodium hydroxide solution, wherewith, the crystalline product separated from solution. Recrystallization from absolute ethanol afforded 1.3 g. (95%) of the desired substance, m.p. 180-182°.

Anal. Calcd. for $C_{11}H_{13}N_3O_2S$: C, 52.57; H, 5.21; N, 16.72. Found: C, 52.80; H, 5.20; N, 16.67.

5-*N*-Benzylisopyridoxamine (Xa).

A mixture of 3.11 g. (0.015 mole) isopropylideneisopyridoxal (III), 1.65 g. (0.015 mole) freshly distilled benzylamine was refluxed with about 0.5 g. sodium carbonate in 30 ml. absolute ethanol for 3 hours. After filtration, the clear solution was evaporated to dryness in vacuum and taken up in 40 ml. of methanol. This methanolic solution was warmed to about 50°, and 0.65 g. (0.17 mole) of sodium borohydride was added gradually in small portions after which the solution was refluxed for 30 minutes. The methanol was evaporated in vacuum, 50 ml. of water was added, and the solution was extracted three times with 100 ml. portions of ether. Evaporation of the ether left a viscous oil which was heated on the steam bath for 20 minutes with 30 ml. of 1*N* hydrochloric acid solution to remove the isopropylidene group. After treatment of the solution with charcoal (Norit) and adjustment of the *p*H to 7.0 with 10% sodium hydroxide, fine needles precipitated.

Recrystallization from hot water or from a mixture of ethanol and acetone yielded white needles, m.p. 119-120° (decomp.), soluble in ethanol but insoluble in ether or benzene.

Anal. Calcd. for $C_{15}H_{18}O_2N_2$: C, 69.80; H, 6.98; N, 10.85. Found: C, 70.25; H, 7.20; N, 10.74.

In one instance the monohydrochloride of the compound, rather than the free base, separated. This was recrystallized from ethanol, m.p. 217° (dec.). The free base could be readily converted into this hydrochloride by dissolving in absolute ethanol, cooling with ice and passing in dry hydrogen chloride, m.p. 217-218°.

Anal. Calcd. for $C_{15}H_{19}O_2N_2Cl$: C, 60.80; H, 6.45; N, 9.51. Found: C, 60.68; H, 6.57; N, 9.48.

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