reaction with ethylene glycol as described above for the preparation of Ie, also was first obtained as crystals melting at 176°. After recrystallization the form melting at 208–209° was again obtained.

**Alkaline Hydrolysis.**—By refluxing Ih in 5% methanolic KOH under nitrogen for an hour, the 3-glycol ketal of testosterone (Ig), m.p. 180–181°, was restored.

**Acidic Hydrolysis.**—When 50 mg. of Ih was heated for 5 minutes in 5 cc. of acetone containing a drop of dilute hydrochloric acid, 38 mg. of **testosterone propionate**, m.p. 121–122°, was recovered.

**Cyanohydrin of Androstane-3β-ol-17-one (IIIb).**—Androstane-3β-ol-17-one (2.25 g.) was dissolved by gentle warming in 3 cc. of freshly prepared undistilled[8] acetone cyanohydrin. The cyanohydrin of the steroid ketone precipitated immediately. The product was collected after 2 hours and washed with petroleum ether, 2.370 g. (96% yield), m.p. 170° dec.[14] The analytical sample was recrystallized from ethanol (m.p. unchanged).

*Anal.* Calcd. for $C_{20}H_{31}O_2N$: C, 75.67; H, 9.84; N, 4.41. Found: C, 75.21; H, 9.59; N, 4.65.

**17-Cyanohydrin of Androstane-3,17-dione (IIIc).**—To a suspension of 2.37 g. of the above cyanohydrin (IIIb) in a mixture of 20 cc. of ethylene dichloride and 50 cc. of acetic acid, a solution of 725 mg. of chromic anhydride in 15 cc. of 90% acetic acid was added at room temperature. After 24 hr. all the product went into solution. Ether and water were added, the ether layer was separated, washed with water, dried and evaporated. The crystalline solid (2.1 g.) when recrystallized from ether afforded 1.980 g. (88%) of a product melting at 207° dec.

*Anal.* Calcd. for $C_{20}H_{29}O_2N$: C, 76.15; H, 9.27; N, 4.44. Found: C, 76.45; H, 9.40; N, 4.58.

Hydrolysis of 200 mg. of the product with pyridine-containing ethanol gave 180 mg. of androstane-3,17-dione (IIIa), m.p. and mixed m.p. 130–131°.

**Androstane-17β-ol-3-one (IIIg) through the 3-Diethyl Ketal of Androstane-3,17-dione-17-cyanohydrin (IIId).**—The reaction of the cyanohydrin IIIc with ethyl orthoformate and the purification was carried out as described for the preparation of IIa. The 3-diethyl ketal of androstane-3,17-dione-17-cyanohydrin (IIId) was obtained as an oily residue, which when heated with ethanol and a little pyridine yielded the known 3-diethyl ketal of androstane-3,17-dione (IIIe), m.p. 121°, undepressed on admixture with an authentic specimen.[2]

By repetition of the above procedure starting with 1.6 g. of IIIc, 1.7 g. of oily IIId was obtained which, after reduction with 1.7 g. of sodium in 45 cc. of 1-propanol and the usual processing, yielded the 3-diethyl ketal of androstane-17β-ol-3-one (IIIf) as a crude product melting at 120–130°. Attempts to recrystallize gave oily products.[15] All the fractions were recombined and heated for 15 min. on the steam-bath with 50 cc. of ethanol containing 1 cc. of 4 *N* hydrochloric acid. Dilution with water yielded 1.326 g. (91%) of androstane-17β-ol-3-one (IIIg), m.p. 176–178°, undepressed on admixture with an authentic specimen.

**17β-Acetoxyandrostane-3-one (IIIk) through the 3-Diethyl Ketal of 17β-Acetoxyandrostane-3-one (IIIh).**—By repetition of the above procedure starting with 2.2 g. of androstanedione-17-cyanohydrin (IIIc), 2.1 g. of crude 3-diethyl ketal of androstane-17β-ol-3-one (IIIf), m.p. 120–130°, was obtained. This substance was dissolved in 20 cc. of pyridine and acetylated at room temperature overnight with 10 cc. of acetic anhydride. The water-diluted reaction mixture was extracted with ether. The alkali-washed and dried ether layers yielded after evaporation 2.2 g. of an oily residue of 3-diethyl ketal of 17β-acetoxyandrostane-3-one (IIIh), which was dissolved in 10 cc. of acetone and refluxed for 10 min. with 0.2 cc. of 36% hydrochloric acid. The crystalline product furnished after recrystallization from petroleum ether 1.850 g. (80% from IIIc) of dihydrotestosterone acetate (IIIk), m.p. 153–156°, undepressed on admixture with an authentic specimen.

**Acknowledgment.**—The authors wish to express their gratitude to Louis F. Fieser and Mary Fieser for revising the manuscript.

(14) Literature[6] 210°.

(15) A. Serini and H. Köster[2] give no description of IIIf.

CASATENOVO (COMO), ITALY

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

# Chemistry of Vitamin $B_6$. IX. Derivatives of 5-Desoxypyridoxine

BY DOROTHEA HEYL, STANTON A. HARRIS AND KARL FOLKERS

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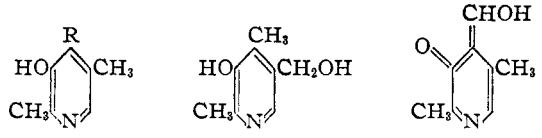
5-Desoxy derivatives of pyridoxine, pyridoxal and pyridoxamine have been prepared and characterized. These compounds are of biological interest because they can participate normally in biochemical reactions involving the substituent at the 4-position, but they cannot be phosphorylated like pyridoxine, pyridoxal and pyridoxamine. As might be expected, the 5-desoxy compounds not only have no vitamin $B_6$ activity, but are effective antimetabolites. Codecarboxylase has been catalytically hydrogenated to produce 5-desoxypyridoxine; under the same conditions, pyridoxal and pyridoxine each produce a mixture of 4-desoxypyridoxine and 5-desoxypyridoxine.

5-Desoxy derivatives of pyridoxine, pyridoxal and pyridoxamine have been prepared. These compounds are of interest, both biologically and chemically, because of their relationship to phosphates of the vitamin $B_6$ group. With the 5-hydroxymethyl group missing, these 5-desoxy derivatives cannot be converted to 5-phosphates, although the functional group in the 4-position is presumably still capable of normal participation in biochemical reactions. For these reasons, the 5-desoxy compounds would be expected to have no vitamin $B_6$ activity and might be antimetabolites. Actually, 5-desoxypyridoxal and 5-desoxypyridoxamine have been found to be potent vitamin $B_6$ inhibitors.[1] The inhibition is of the same high order of activity as that caused by 4-desoxypyridoxine (IV),[2] in which the functional group in position 4 is blocked.



I, R = $CH_2OH$
II, R = CHO
III, R = $CH_2NH_2$

IV

V

Because of the absence of the 5-hydroxymethyl group, the hemiacetal form of pyridoxal[3] is impossible in 5-desoxypyridoxal (II). This same restric-

(1) J. C. Rabinowitz and E. E. Snell, *Arch. Biochem. Biophys.*, in press.

(2) W. H. Ott, *Proc. Soc. Exptl. Biol. Med.*, **61**, 125 (1946); W. W. Cravens and E. E. Snell, *ibid.*, **71**, 73 (1949).

(3) D. Heyl, E. Luz, S. A. Harris and K. Folkers, THIS JOURNAL, **73**, 3431 (1951).

tion occurs also in codecarboxylase (pyridoxal-5-phosphate),[3] and a striking similarity can be seen by comparison of the absorption spectra of 5-desoxypyridoxal (Fig. 1) and pure codecarboxylase.[4] These curves are almost identical at both acidic and alkaline *p*H's, whereas those for pyridoxal[3] are considerably different, especially in alkaline solution. 5-Desoxypyridoxal, like codecarboxylase, forms a deep yellow color in alkaline solution. This yellow color and other absorption characteristics may be ascribed, as in codecarboxylase, to a quinoid structure (V).

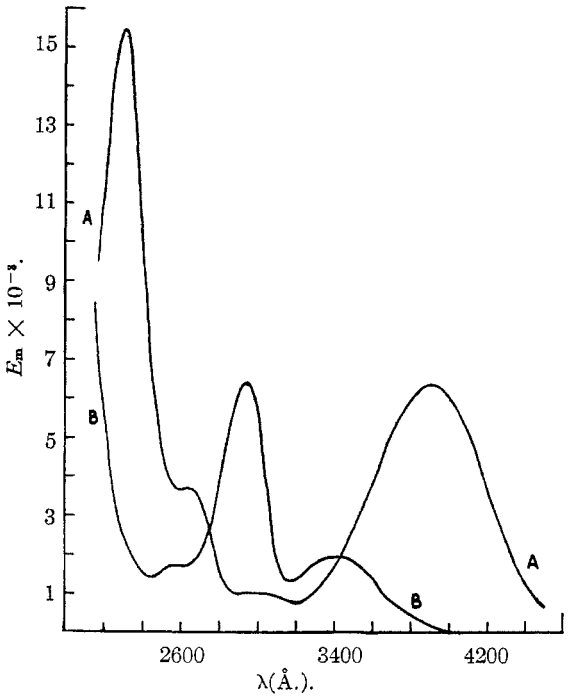
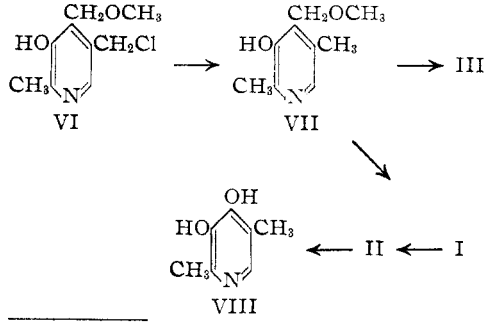



Fig. 1.—Absorption spectrum of 5-desoxypyridoxal (II) at *p*H 11.0 (A) and *p*H 1.9 (B).

The 5-desoxy compounds were prepared by reactions already described for analogs and indicated by the accompanying formulas. 2-Methyl-3-hydroxy-4-methoxymethyl-5-chloromethylpyridine hydrochloride (VI)[5] was reduced catalytically to 2,5-dimethyl-3-hydroxy-4-methoxymethylpyridine hydrochloride (VII) which was converted to 5-desoxypyridoxamine (III) by ammoniation in a bomb with methanol and liquid ammonia. 5-Desoxypyridoxamine was characterized also by preparation of diacetyl and di-*p*-toluenesulfonyl derivatives. The 4-methoxymethyl intermediate VII was hydrolyzed to 5-desoxypyridoxine (I) with hydrochloric acid in a sealed tube at 180–190°, and 5-desoxypyridoxine was oxidized to 5-desoxypyridoxal (II) with manganese dioxide and sulfuric acid in the manner described for the preparation of pyridoxal.[6] 5-Desoxypyridoxal was characterized as the oxime, and was also converted to 2,5-dimethyl-3,4-dihydroxypyridine (VIII) with hydrogen peroxide in alkaline solution, as described for the oxidation of pyridoxal and codecarboxylase.[7]




5-Desoxypyridoxine (I) was found to be the main product of the hydrogenation of codecarboxylase in aqueous solution with a palladium catalyst. No 4-desoxypyridoxine was produced, although hydrogenation of both pyridoxal and pyridoxine[8] produces a mixture of the 4- and 5-desoxypyridoxines. Consequently, the catalytic reaction of hydrogen with codecarboxylase is a direct hydrogenolysis and not catalytic hydrolysis of the phosphate group followed by hydrogenation of the product. 5-Desoxypyridoxine was found to be insensitive to further hydrogenation.

## Experimental[9]

**2,5-Dimethyl-3-hydroxy-4-methoxymethylpyridine Hydrochloride (VII).**—A solution of 2.38 g. of 2-methyl-3-hydroxy-4-methoxymethyl-5-chloromethylpyridine hydrochloride (VI)[5] in 125 ml. of methyl alcohol was shaken with hydrogen in the presence of 2 g. of 5% palladium-on-Darco. The hydrogenation required 10 minutes. After removal of the catalyst by filtration and concentration of the filtrate to 20 ml., crystals of 2,5-dimethyl-3-hydroxy-4-methoxymethylpyridine hydrochloride were precipitated by addition of ether; yield 1.5 g. (75%). After one recrystallization from alcohol–ether, the material melted at 152–153°.

*Anal.* Calcd. for $C_8H_{14}NO_2Cl$: C, 53.07; H, 6.93; N, 6.88. Found: C, 53.09; H, 6.89; N, 6.89.

Larger amounts of material can be reduced in this manner only if the solution is kept dilute. A sample of 23.7 g. of 2-methyl-3-hydroxy-4-methoxymethyl-5-chloromethylpyridine hydrochloride was reduced in two equal portions, each one in 600 ml. of methyl alcohol and with 5 g. of the palladium catalyst. The total yield of 2,5-dimethyl-3-hydroxy-4-methoxymethylpyridine hydrochloride was 19.0 g. (94%).

**5-Desoxypyridoxine Hydrochloride (2,5-Dimethyl-3-hydroxy-4-hydroxymethylpyridine Hydrochloride (I)).**—A solution of 1.47 g. of 2,5-dimethyl-3-hydroxy-4-methoxymethylpyridine hydrochloride in 50 ml. of 4 *N* hydrochloric acid was sealed in a bomb-tube and heated at 180–190° for 3 hours. After filtration of the colorless solution, the filtrate was concentrated to dryness and the water removed by distillation with alcohol and benzene. The residue crystallized from alcohol–ether in two fractions. The total yield was 0.96 g. (70%) of 5-desoxypyridoxine hydrochloride. After one recrystallization from alcohol–ether, the material melted at 143.0–143.5°.

*Anal.* Calcd. for $C_8H_{12}NO_2Cl$: C, 50.66; H, 6.38; N, 7.39. Found: C, 50.98; H, 6.56; N, 7.41.

**5-Desoxypyridoxine.**—An aqueous solution of 5-desoxypyridoxine hydrochloride was treated with an excess of sodium bicarbonate. After cooling, the white crystals of the base were collected on a filter and recrystallized from alcohol. The product melted at 181–182°.

*Anal.* Calcd. for $C_8H_{11}NO_2$: C, 62.72; H, 7.24; N, 9.15. Found: C, 62.85; H, 7.13; N, 9.09.

(4) M. Viscontini, C. Ebnöther and P. Karrer, *Helv. Chim. Acta*, **34**, 1837 (1951).

(5) S. A. Harris, D. Heyl and K. Folkers, THIS JOURNAL, **66**, 2088 (1944).

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

(7) D. Heyl, E. Luz and S. A. Harris, *ibid.*, **73**, 3437 (1951).

(8) S. A. Harris, *ibid.*, **62**, 3203 (1940).

(9) We are indebted to Dr. Charles Rosenblum and Mr. Edward H. Smith for the ultraviolet absorption measurements, and to Mr. Richard Boos and his associates for the microanalyses.

**5-Desoxypyridoxamine (2,5-Dimethyl-3-hydroxy-4-aminomethylpyridine (III)).**—2,5-Dimethyl-3-hydroxy-4-methoxymethylpyridine was recovered from the hydrochloride by neutralization with sodium bicarbonate in aqueous solution, concentration to dryness under reduced pressure, extraction of the dried residue with ether and removal of the ether. A mixture of 3.1 g. of this material, 50 ml. of methyl alcohol and 50 ml. of liquid ammonia was heated in a bomb at 130° for 18 hours. The solution was distilled to dryness under reduced pressure, and methyl alcohol added and removed twice by distillation to remove ammonia. The residue was taken up in ether and the solid material filtered and washed with ether; yield of 5-desoxypyridoxamine, 1.68 g. (60%). After one recrystallization from methyl alcohol, the product melted at 160–161°.

*Anal.* Calcd. for $C_8H_{12}N_2O$: C, 63.13; H, 7.95; N, 18.41. Found: C, 63.48; H, 8.00; N, 18.74.

A small sample was converted to 2,5-dimethyl-3-*p*-toluenesulfonoxy-4-*p*-toluenesulfonylaminopyridine hydrochloride, which after one recrystallization from alcohol melted at 194–195°.

*Anal.* Calcd. for $C_{22}H_{25}N_2O_5ClS_2$: C, 53.15; H, 5.07; N, 5.64. Found: C, 52.71; H, 5.01; N, 5.71.

**2,5-Dimethyl-3-acetoxy-4-acetylaminomethylpyridine.**—A small sample of 5-desoxypyridoxamine was heated on a steam-bath for 20 minutes with acetic anhydride. The solution was concentrated to dryness and the excess of acetic anhydride removed by treatment with alcohol and distillation to dryness. A dilute hydrochloric acid solution of the residue, after decolorization with Darco, was neutralized with sodium bicarbonate. After thorough chilling, the crystals of 2,5-dimethyl-3-acetoxy-4-acetylaminopyridine were collected and recrystallized from benzene containing a few drops of alcohol. The acetate melted at 174–175°.

*Anal.* Calcd. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 61.07; H, 6.66; N, 11.88.

**5-Desoxypyridoxal (2,5-Dimethyl-3-hydroxy-4-formylpyridine, (II)).**—A mixture of 5.7 g. of 5-desoxypyridoxine hydrochloride (I), 2.8 g. of manganese dioxide, 1.5 ml. of sulfuric acid and 75 ml. of water was stirred at 60–70° for 2 hours. The *p*H of the final solution was 4–5, and the manganese dioxide had disappeared. After removal of a little insoluble material by filtration, the filtrate was concentrated to a sirup under reduced pressure. The sirup was taken up in 15 ml. of water, and an excess of solid sodium acetate was added. The thick, crystalline precipitate which appeared was cooled in ice and then collected on a filter and washed with ice-water. It consisted of 1.30 g. (29%) of 5-desoxypyridoxal which melted at 108–109° after one recrystallization from petroleum ether.

*Anal.* Calcd. for $C_8H_9NO_3$: C, 63.55; H, 6.00; N, 9.27. Found: C, 63.43; H, 6.15; N, 9.55.

When the aqueous filtrate, after separation of the aldehyde, was treated with 2 g. of hydroxylamine hydrochloride, 0.9 g. (18%) of the oxime of 5-desoxypyridoxal was obtained.

**Oxime of 5-Desoxypyridoxal.**—The oxidation in the previous experiment was repeated. After clarification of the reaction mixture by filtration, 12 g. of sodium acetate and 4.5 g. of hydroxylamine hydrochloride were added. The mixture was heated on a steam-bath for 10 minutes, then cooled in an ice-bath. The crystals of the oxime of 5-desoxypyridoxal, 2.43 g. (49%), were collected on a filter and washed with water, alcohol and ether. A hot solution of the material in hydrochloric acid was decolorized with Darco. The oxime was reprecipitated by addition of an excess of sodium bicarbonate to the cooled solution. After recrystallization from alcohol, the oxime melted at 239–240° dec.

*Anal.* Calcd. for $C_8H_{10}N_2O_2$: C, 57.82; H, 6.07; N, 16.86. Found: C, 58.10; H, 5.77; N, 17.10.

**5-Desoxypyridoxal Hydrochloride.**—A chloroform solution of 5-desoxypyridoxal was treated with an excess of alcoholic hydrogen chloride. The solution was evaporated to dryness under reduced pressure. A little water was added and removed under reduced pressure. Chloroform was added to the residue, and the crystals of 5-desoxypyridoxal hydrochloride, m.p. 191–193° dec., were collected on a filter.

*Anal.* Calcd. for $C_8H_{10}NO_3Cl$: C, 51.21; H, 5.37; N, 7.47. Found: C, 50.92; H, 5.33; N, 7.81.

**2,5-Dimethyl-3,4-dihydroxypyridine (VIII).**—A suspension of 90 mg. of 5-desoxypyridoxal (II) in 1 ml. of water was cooled in ice and adjusted to *p*H 11 with 6 *N* sodium hydroxide. Four drops of 30% hydrogen peroxide was added, and in a few seconds the deep amber solution suddenly became colorless, with the appearance of white crystals. The mixture was adjusted to *p*H 3 with hydrochloric acid and was cooled further in ice. The 2,5-dimethyl-3,4-dihydroxypyridine, 70 mg. (85%), was collected on a filter and washed with water, alcohol and ether. The material decomposed over a range, 262–270°.

*Anal.* Calcd. for $C_7H_9NO_2$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.43; H, 6.30; N, 10.30.

**Hydrogenation of Codecarboxylase (2-Methyl-3-hydroxy-4-formyl-5-pyridylmethylphosphoric Acid).**—A sample of crude calcium codecarboxylase (contaminated with inorganic material) weighing 0.5 g. was suspended in water and treated with 0.7 ml. of 6 *N* hydrochloric acid. A white insoluble substance was removed by filtration. The filtrate was diluted to 50 ml. and shaken with hydrogen at atmospheric pressure in the presence of 0.5 g. of 10% palladium-on-charcoal. After 2.25 hours the material had practically stopped absorbing hydrogen. The catalyst was removed by filtering and the solution concentrated to dryness under reduced pressure. The residue was dissolved in about 3 ml. of water, and solid sodium bicarbonate was added in excess. The insoluble, inorganic material was separated by filtration and was washed well with water.

The combined filtrate and washings was concentrated under reduced pressure to 5 ml. and was extracted continuously with chloroform for 21 hours. Removal of the chloroform left 0.05 g. of residue which was converted to the hydrochloride by addition of alcoholic hydrogen chloride. No crystalline insoluble 4-desoxypyridoxine hydrochloride was obtained. Addition of ether precipitated 0.07 g. of crystals, m.p. 140–141°. When this product was mixed with a sample of 5-desoxypyridoxine hydrochloride, there was no depression in melting point.

The aqueous solution remaining from the chloroform extraction was concentrated to dryness and the water removed by distillation several times with a mixture of absolute alcohol and benzene. An alcoholic extract of the residue was found to yield only 0.02 g. of yellow oil.

**Hydrogenation of Pyridoxal.**—A procedure as close as possible to the one for the hydrogenation of codecarboxylase was carried out. The original solution contained 0.35 g. of pyridoxal hydrochloride, and 0.10 g. of calcium oxide and 0.17 g. of phosphoric acid was added. After hydrogenation the chloroform extract yielded 0.16 g. of residue, which was dissolved in alcohol and made acid with alcoholic hydrogen chloride. Crystals of 4-desoxypyridoxine hydrochloride separated at once; yield 0.08 g. (24%). On recrystallization they melted at 264–265° dec. and were identified by "mixed melting point." By addition of ether to the alcoholic filtrate, 0.11 g. (33%) of 5-desoxypyridoxine hydrochloride, m.p. 142–143°, was obtained. From the aqueous filtrate left from the chloroform extraction, and after concentration to dryness, extraction with alcohol, and acidification of the alcoholic solution with alcoholic hydrogen chloride, 0.11 g. (30%) of crystalline material was obtained which consisted mostly of pyridoxine hydrochloride.

**Hydrogenation of Pyridoxine.**—A mixture of 0.40 g. of pyridoxine hydrochloride, 0.3 ml. of 6 *N* hydrochloric acid and 50 ml. of water was hydrogenated as described for codecarboxylase. The hydrogenation lasted for 4–5 hours. The chloroform extract, treated as described for pyridoxal, yielded 0.16 g. (42%) of 4-desoxypyridoxine hydrochloride and 0.09 g. (24%) of 5-desoxypyridoxine hydrochloride.

**Hydrogenation of 5-Desoxypyridoxine.**—This material when shaken with hydrogen under the conditions described for codecarboxylase absorbed no hydrogen. The starting material was recovered quantitatively.

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