SYNTHESIS OF PYRIDOXAMINE AND ITS 5'-PHOSPHATE ESTER

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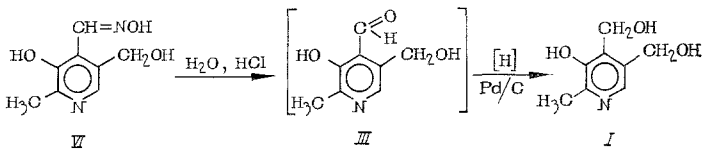
UDC 612.356:577.164.13].012.1

Three compounds that are structurally similar and interconvertible in the organism—pyridoxine (I), pyridoxamine (II), and pyridoxal (III)—constitute the vitamin B_6 group. Pyridoxal (III) is the most important representative of this group. Its phosphate ester—pyridoxal 5'-phosphate (IV)—is the cofactor of several enzymes that catalyze the transformations of amino acids, carbohydrates, and fatty acids [1].

Pyridoxamine 5'-phosphate (V) acts as a coenzyme only in transmission reactions. However, since IV can easily be derived from it, by transamination [2-5] or oxidation [6-8] for example, the development of methods for its synthesis is of considerable interest.

We prepared the starting compound (III) for the synthesis of V by oxidation of I with manganese dioxide in dilute sulfuric acid [9]; subsequent treatment with hydroxylamine gave the oxime of III (VI) in 75.8% yield. This reaction gave a contaminated product when heating was used [10], which necessitated recrystallization to get pure VI. Hydrogenation of VI in aqueous hydrochloric acid over Pd/C catalyst gave II [11]. However, VI was hydrolyzed under these conditions and consequently the resulting II in general was contaminated with I.



We verified the presence of I in the reaction mixture after the reduction of VI by chromatography on paper and Silufol UV-254 plates in various solvent systems and by quantitative chromatographic—spectrophotometric determination of compounds I and II after preparative separation on paper. We found that unpurified II contained 2-5% of I.

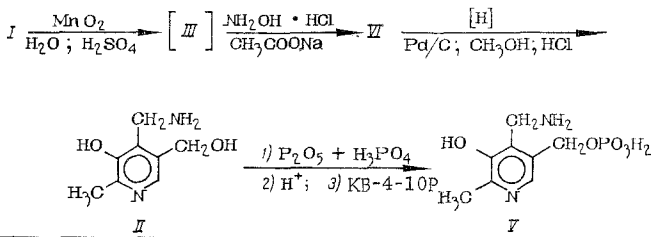
Compound II when isolated after reduction of VI in aqueous hydrochloric acid required further purification to remove the contaminant I.

We had to use an anhydrous solvent to prevent the hydrolysis of VI during its reduction, as in the extant method [12] for the hydrogenation of VI in methanol saturated with hydrogen chloride.

We attempted to simplify the process by carrying out the reduction of VI in methanol in the presence of hydrochloric acid with an equimolar ratio of VI and hydrogen chloride. Chromatography of the reaction mixture on paper and Silufol revealed only a single spot, corresponding in mobility to an authentic sample of II. We isolated II from the reaction mixture in 95% yield.

We carried out the phosphorylation of II with polyphosphoric acid [7]. For the subsequent ion-exchange purification we used the Soviet cation exchanger KB-4-10P, which was a successful substitute for Amberlite IRC-50 and XE-88. Compound V was isolated in 74.7% yield.

Thus on the basis of our work the reaction sequence involved in the synthesis of II and V is



"Vitaminy" Scientific and Industrial Association, Moscow. Translated from Khimiko-farmatsevitcheskii Zhurnal, Vol. 14, No. 6, pp. 79-81, June, 1980. Original article submitted June 12, 1979.

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EXPERIMENTAL

The UV spectra were recorded on an SF-4A instrument. Thin-layer chromatography was carried out on Silufol UV-254 plates in the systems: a) acetone–dioxane–ammonia (9:9:2), b) t-butyl alcohol–85% formic acid–water (7:1.5:1.5), c) ethyl acetate–acetone–ammonia (20:10:1.5), and d) butanol–water (4:5). Paper chromatography was carried out on Whatman No. 1 or 3MM and Filtrak FN-3 (East Germany) in systems b and d. Visualization was in UV light with a 0.5% alcoholic 2,6-dichloroquinone chloroimide and 2% acetone solution of ninhydrin.

2-Methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine Oxime (VI). To a solution of pyridoxine hydrochloride (10 g, 41.4 mmole) in 0.3 M sulfuric acid (165 ml) was added manganese dioxide (4.4 g). The mixture was stirred for 5 h while the solution pH changed from 1.0 to 4.5. Unreacted manganese dioxide was filtered off and washed with water (10 ml). Hydroxylamine hydrochloride (4.27 g) was added to the filtrate. The mixture was heated at 70°C for 10 min, sodium acetate (18.7 ml) was added, and the mixture was again heated at 70°C for 10 min. It was then cooled to 0°C. The precipitated crystals were separated and washed with water until manganese ions were not present in the wash water (test with benzidine). Drying at 70°C under a residual pressure of 15 mm Hg gave (VI) (6.72 g, 75.8%), mp 222-223°C. Literature [10]: mp 225-226°C. The product was chromatographically homogeneous by TLC in systems a, b, c, and d and by paper chromatography in systems b and d.

Pyridoxamine Dihydrochloride (II). A. Compound II was prepared by the method of [11]. Chromatography of a sample of II isolated from the reaction mixture revealed two spots corresponding in mobility to authentic samples of I and II.

The quantitative determination of I and II was carried out by ascending chromatography on Whatman No. 1 paper in system b followed by extraction of the zones containing I and II with 0.1 N hydrochloric acid. The optical density was measured in 1-cm cells at 290 nm for I and 293 nm for II. Each determination was the average result of three measurements. The relative error was 5%.

Preparative chromatography on L 40/100 μ silica gel in system a isolated I and II from the relevant zones, which had UV spectra, mp, and R_f by TLC in systems a, b, c, and d that were identical to those of authentic samples.

B. To a solution of VI (1 g, 5.4 mmole) in methanol (30 ml) and hydrochloric acid (0.5 ml 5.4 mmole) was added Pd/C (0.2 g) containing 5% Pd. The mixture was hydrogenated for 55 min; 230 ml of hydrogen was absorbed. Hydrochloric acid (4.5 ml) was added and the catalyst was separated. The hydrogenate was evaporated to dryness under vacuum. Methanol (1 ml) was added to the dry residue, which was carefully stirred. The crystals were separated and dried over phosphorus pentoxide to give II (1.26 g, 95.2%), mp 225-227°C (decomposition). Literature [11]: mp 225-226°C. The compound was chromatographically homogeneous by TLC in systems a, b, c, and d, and also on paper chromatography in systems b and d.

Pyridoxamine 5'-Phosphate Dihydrate (V). A. To phosphorus pentoxide (2.32 g) with stirring and cooling was added 85% orthophosphoric acid (3.21 g) while the temperature was maintained below 110°C, until the phosphorus pentoxide had completely dissolved. After the solution had cooled to room temperature II (0.5 g, 2.1 mmole) was added. The mixture was stirred for 1 h until the evolution of hydrogen chloride had ceased and then heated at 65°C for 2.5 h. After cooling to 0°C, water (0.25 ml) and anhydrous alcohol (12.5 ml) were added at 10°C. Diethyl ether (37.5 ml) was added to the transparent solution and the mixture was kept at 6°C for 1 h while a white precipitate formed. The precipitate was separated, dried under vacuum, and then hydrolyzed with 1N hydrochloric acid (25 ml) at 100°C for 30 min. The hydrolysate was evaporated under vacuum at 40°C to a volume of 4 ml. It was then neutralized with 25% aqueous ammonia (4.5 ml) to pH 6.0-7.0 and applied to a column (2.77 × 62 cm) of Amberlite XE-88 (H^+ form) cation exchange resin. The column was eluted with water at a rate of 20 ml/h. The neutral fractions with UV absorption maximum at 326 nm that gave an orange coloration with ninhydrin were collected. These fractions were combined (340 ml), evaporated under vacuum at 40°C to a volume of 2 ml, and cooled at 4-6°C for 8 h. The precipitated colorless acicular crystals were separated and washed at 0°C with alcohol (0.2 ml) and ether (0.2 ml). The yield was 0.31 g, mp 231.5-232°C. The filtrate was treated to give a further 0.13 g, mp 231.5-232°C. The total yield of V was 0.44 g (74.7%). Found, %: C 33.76; H 5.85; N 9.9; P 11.0. $C_8H_{13}N_2P \cdot 2H_2O$. Calculated, %: C 33.8; H 6.0; N 9.9; P 10.9. TLC: R_f 0.205 (system b); paper chromatography on Whatman No. 1 R_f 0.2-0.3 (system b).

B. Compound II (0.5 g) was phosphorylated as described in method A. The mixture was applied to a column (2.77 × 62 cm) of the weak cation exchanger KB-4 10P (H form). The column was eluted with water at a rate

of 20 ml/h. After elution of the acidic fractions (100 ml), the neutral fractions with UV absorption at 326 nm that gave an orange coloration with ninhydrin were collected (350 ml). They were evaporated under a vacuum to a volume of 2 ml at 40°C, cooled at 4-6°C for 8 h, and separated as described above. The yield was 0.44 g (74.7%), mp 221.5-232°C. Preparation V had identical chromatographic mobility to V prepared by method A.

LITERATURE CITED

1. A.M. Yurkevich, E. S. Severin, and A. E. Braunshtein, in: A. E. Braunshtein (editor), Enzymes [in Russian], Moscow (1964), p. 147.
2. K. Asano, M. Fusukawa, and S. Shimada, Japanese Patent No. 66-21031 (1966); Chem. Abs., 66, 5401 (1967).
3. T. Kuroda, Japanese Patent No. 63-19739 (1963); Chem. Abstr., 60, 4116a (1964).
4. R. F. Long, British Patent No. 749800 (1956); Chem. Abstr., 51, 1297 (1957).
5. T. Kuroda, J. Vitaminol., 10, 252 (1964).
6. S. Hiroshi, H. Shisuo, and N. Kunio, Japanese Patent No. 70-16949 (1970); Chem. Abstr., 73, 109700m (1970).
7. E. A. Peterson and H. A. Sober, J. Am. Chem. Soc., 76, 169 (1954).
8. Honzo Mikio, Imai Kinchichi, Yoshioka Yoshio, et al., Japanese Patent No. 70-6023 (1970); Ref. Zh. Khim., 2N357P (1971).
9. S. D. L'vova, N. L. Yakovleva, I. S. Cherkasova, et al., Khim.-farm. Zh., No. 11, 23 (1975).
10. D. Neyl, J. Am. Chem. Soc., 70, 3434 (1948).
11. M. V. Balyakina, E. S. Zhdanovich, and N. A. Preobrazhenskii, Zh. Obshch. Khim., 31, 2983 (1961); Inventor's Certificate No. 133885.
12. P. Karrer, H. Viscontini, and O. Foster, Helv, Chim. Acta, 31, 1004 (1948).