VITAMIN B_6 ANALOGS

X.* SYNTHESIS AND PROPERTIES OF 3-O-METHYLPYRIDOXAL-5'-PHOSPHATE

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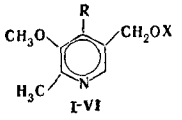
3-O-Methylpyridoxal phosphate and 3-O-methylpyridoxamine phosphate were synthesized and their UV spectra were studied.

Pyridoxal-5'-phosphate (5'-phosphate ester of 2-methyl-3-hydroxy-4-formyl-5-hydroxymethylpyridine) serves as a cofactor of enzymes which bring about transformations of amino acids. One of the structures which manifests catalytic properties in model systems is 3-hydroxy-4-formylpyridine. The hydroxyl group fulfills a number of important functions in the catalytic act, including the accomplishment of intramolecular, general acid catalysis. For a detailed study of the contribution of the 3-hydroxyl group of the coenzyme in enzyme systems it would be desirable to investigate the properties of an enzyme analog which, while preserving the topochemical similarity to the natural cofactor in general features, would not have a free hydroxyl group in the 3-position of the pyridine ring. One such compound is 3-O-methylpyridoxal-5'-phosphate, to whose synthesis this communication is devoted.



I-VI

I R=CH_2OH, X=H; IV R=CH_2NH_2, X=H;
II R=CHO, X=H; V R=CHO, X=PO_3H_2;
III R=CH=NOH, X=H; VI R=CH_2NH_2, X=PO_3H_2

3-O-Methylpyridoxine (I) and 3-O-methylpyridoxal (II) were previously obtained by methylation with diazomethane of pyridoxine [1] and the methylacetal of pyridoxal [2], respectively. We attempted to find a path for the conversion from these compounds to the coenzyme analogs. The application of the method of selective oxidation of the 4-hydroxymethyl group of the pyridoxine analogs with manganese dioxide, which we developed in [3], gives unsatisfactory results in this case. In the isolation of the aldehyde as an oxime the yields do not exceed 20%, as against 70-80% for analogs with a free hydroxyl group. This fact indicates that the hydroxyl group catalyzes the oxidation of the 4-hydroxymethyl group.

An attempt to isolate IV from the reaction mixture in the form of a Schiff base with an aromatic amine was unsuccessful. Since the formation of Schiff bases of pyridoxal analogs with aromatic amines is one of the most convenient methods for obtaining pyridoxal-5'-phosphate analogs, we attempted to obtain 3-O-methylpyridoxylidene-p-anisidine by heating free base II with p-anisidine in absolute tetrahydrofuran. However, a pure Schiff base could not be obtained under these conditions. After removal of the solvent by distillation the mass was esterified directly with polyphosphoric acid. 3-O-Methylpyridoxal-5'-phosphate (V) was, in fact, obtained from this experiment after separation on an ion-exchange resin, but the yield was only 5.2%.

The second possibility for the synthesis of V, which was studied and realized in this investigation, consisted in the use of preparative transamination applied to 3-O-methylpyridoxamine-5'-phosphate (VI). To obtain the latter, aldehyde II was converted to oxime III, the hydrogenation of which results in amine IV.

*See [7] for communication IX.

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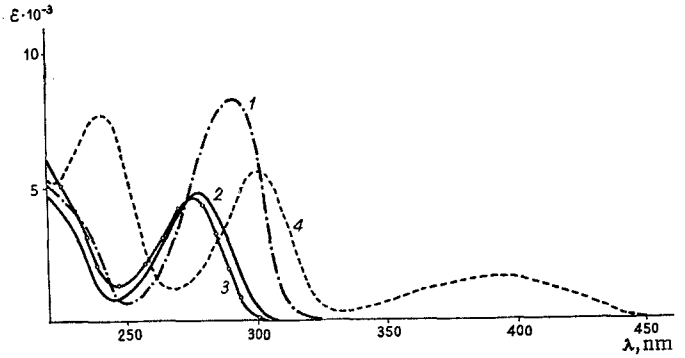


Fig. 1. UV spectra: 3-O-methylpyridoxal (I): 1) in 0.1 N HCl; 2) at pH 7; 3) in 0.1 N KOH; 4) pyridoxal in 0.1 N KOH.

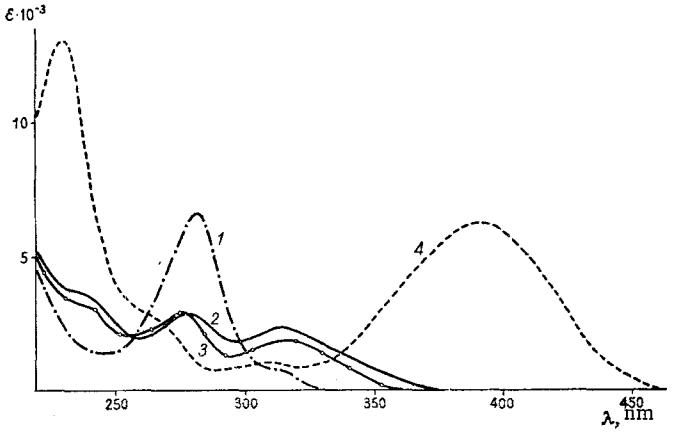
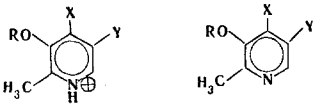


Fig. 2. UV spectra: 3-O-methylpyridoxal-5'-phosphate (V): 1) in 0.1 N HCl; 2) at pH 7; 3) in 0.1 N KOH; 4) pyridoxal-5'-phosphate in 0.1 N KOH.

TABLE 1. UV Spectra of 3-O-Methyl Analogs of Vitamin B_6



Compound	λ_{max}, nm (ε · 10⁻³)	
	R=CH₃	R=H*
VIIa†	261 (8,0)	291 (8,6)
VIIIa	276 (4,57)	286 (5,7)
VIIb, Hemiacetal	292 (8,4)	288 (9,0)
VIIIb, Hemiacetal	276 (4,65)	280 (4,1)
VIIc, (NH_3^+)	285 (7,36)	292 (8,2)
VIIIc	277 (4,02)	287 (3,4)
VIId, Hydrate	282 (6,6)	295 (6,7)
VIId, Aldehyde	315 (0,5) inflection	340 (1,4)
VIId, (PO_3^-), Hydrate	278 (2,9)	—
VIId (PO_3^-), Aldehyde	316 (1,9)	—
VIIe, (NH_3^+)	285 (9,75)	293 (9,0)
VIIIe	276 (5,58)	—

*Data from [5].

† a) X = Y = CH_2OH; b) X = CHO, Y = CH_2OH; c) X = CH_2NH_2, Y = CH_2OH; d) X = CHO, Y = $CH_2OPO_3H_2$; e) X = CH_2NH_2, Y = $CH_2OPO_3H_2$.

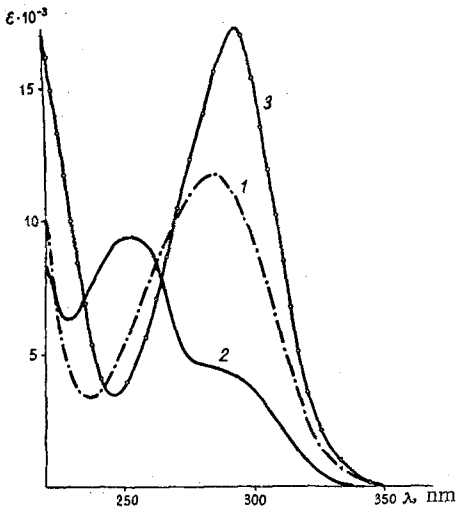


Fig. 3. UV spectra of 3-O-methylpyridoxal oxime (III): 1) in 0.1 N HCl; 2) at pH 7; 3) in 0.1 N KOH.

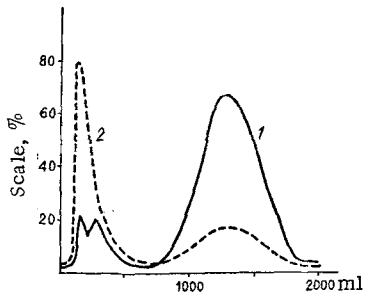


Fig. 4. Chromatography of 3-O-methylpyridoxamine-5'-phosphate (VI) on Amberlite CG-50: 1) absorption at 295 nm; 2) electrical conductivity.

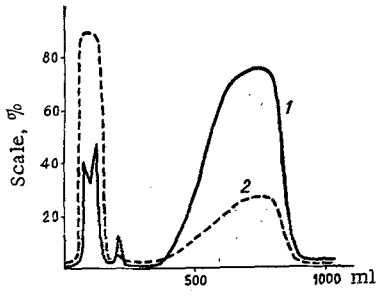


Fig. 5. Chromatography of 3-O-methylpyridoxal-5'-phosphate (V) on Dowex 50 × 4 (the symbols are the same as in Fig. 4).

Compound VI was obtained in 53% yield by phosphorylation of IV according to the method described in [3].

Transamination of VI with glyoxylic acid in the presence of divalent copper gives V in 24% yield. The absence of a free hydroxyl group in this case also leads to a decrease in the yield to 30-40% [3].

The UV spectra of 3-O-methyl analogs of vitamin B_6 have a number of characteristic peculiarities (see Table 1). As would be expected, the bathochromic shift usual for 3-hydroxypyridines is not observed in the spectra on passing to the alkaline region; this is due to ionization of the hydroxyl group.

The absorption maxima of the neutral form (neutral–alkaline pH region) of the 3-O-methyl analogs is shifted by 5-8 nm to the short wave region as compared with the cation. An unusually large shift (16 nm) is observed only for the hemiacetal band of VIIb. An examination of the spectra of VIIb, d and VIId* indicates that equilibrium of the hemiacetal or hydrate–aldehyde is shifted more to the left (Figs. 1 and 2) than for compounds with a free hydroxyl group. In our opinion, this sort of behavior is explained by the absence in neutral and alkaline media of a strongly electron-donating effect of the ionized 3-hydroxyl group. The position of the maximum of the VIIa form (316 nm) is close to that for benzaldehyde (320 nm [4]) but shifted by 20 nm to the short-wave side as compared with the neutral form of pyridoxal [5]. (In [6] it is shown that the ionization of the phosphate group has virtually no effect on the position and intensity of the absorption maxima.)

As seen from the comparative data of Table 1, the maximum hypsochromic shift is observed for aldehyde V.

It is natural to explain this by the fact that, owing to the presence of two ortho substituents, the formyl group of V is somewhat deflected from the plane of the pyridine ring, and this leads to partial disruption of conjugation. This sort of conclusion is confirmed by an examination of the spectra of oxime III (Fig. 3), where a hypsochromic shift is also observed.

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EXPERIMENTAL

The UV spectra were obtained with a JOAN spectrometer (France). 3-O-Methylpyridoxine (I) and 3-O-methylpyridoxal (II) were obtained by methods described in [1] and [2], respectively. The oxidation of I with manganese dioxide and the isolation of II as the oxime and Schiff base with p-phenetidine were carried out according to the methods we developed in [3].

2-Methyl-3-methoxy-5-hydroxymethylpyridine-4-aldehyde Oxime (III). Hydroxylamine hydrochloride (2 g) was added to a solution of 3.8 g (17.5 mmole) of the hydrochloride of II in the minimum amount of water, and the mixture was heated for 10 min at 70°. Sodium acetate dihydrate (9.1 g) was added, and the mixture was heated at 70° for 10 min. The mass was kept at 0-5° for 2 h, filtered, and the precipitate was washed with water and recrystallized from 15% ethanol to give 3.4 g (99%) of III with mp 183-184° (decomp.). Found %: N 14.16. $C_9H_{12}N_2O_3$. Calculated %: N 14.28.

*As in Russian original – Publisher.

2-Methyl-3-methoxy-4-aminomethyl-5-hydroxymethylpyridine Dihydrochloride (IV). A total of 0.5 g of 5% Pd/C was added to a solution of 2.75 g (14 mmole) of III in 130 ml of water and 7 ml of concentrated HCl, and the compound was hydrogenated at room temperature and atmospheric pressure. After 3 h the theoretical amount of hydrogen had been absorbed. The catalyst was removed by filtration and washed with water, and the combined filtrates were evaporated to dryness in vacuo to give 3.1 g (87%) of a compound with mp 162-163° (from alcohol–ether). Found %: C 42.61; H 6.27. $C_9H_{16}Cl_2N_2O_2$. Calculated %: C 42.37; H 6.32.

3-O-Methylpyridoxamine-5'-phosphate (VI). The dihydrochloride of IV [2 g (7.85 mmole)] was added to a mixture of 9 g of 85% H_3PO_4 in 7 g of P_2O_5, and the mixture was held at room temperature until HCl evolution ceased. The mixture was then heated for 2 h at 60°, cooled, 50 ml of ethanol was added, the mixture was stirred thoroughly, and 140 ml of ether was added. The mixture was held at 0-5° for 1 h, the liquid was decanted, and the residue was dissolved in 90 ml of 1 N hydrochloric acid. The mixture was heated for 20 min on a boiling-water bath and evaporated in vacuo to 30 ml. The pH of the solution was brought to 5 with concentrated ammonium hydroxide and applied to a 2.5 × 60-cm column filled with Amberlite CG-50 in the acid form. The column was eluted with water at a rate of 50 ml/h. The separation curve is presented in Fig. 4. Fractions containing VI were evaporated to dryness in vacuo and washed with alcohol–ether to give 1.22 g (52%) of the dihydrate of VI. The compound was electrophoretically homogeneous. Found %: C 35.92; H 6.31. $C_9H_{15}N_2O_5P \cdot 2H_2O$. Calculated %: C 36.24; H 6.42.

3-O-Methylpyridoxal-5'-phosphate (V). A. Sodium glyoxylate (240 mg) was added to a solution of 286 mg (1 mmole) of VI in 7 ml of water and 1 ml of 2 N NaOH under an inert gas, and the mixture (pH 9) was stirred for 10 min at room temperature. The pH of the mixture was brought to 5 with glacial acetic acid, the mixture was stirred for 10 min, and 4 ml of 0.25 M cupric acetate was added slowly. (The pH of the mixture was 6 at the end of the addition.) The mass was stirred for another 45 min and applied to a 1.4 × 40-cm column filled with Dowex 50 × 4 in the acid form. The mixture was eluted with water at a rate of 50 ml/h. The separation curve is presented in Fig. 5. Fractions containing V were evaporated in vacuo to a small volume and lyophilized. The yield was 67 mg (24%). The compound was electrophoretically homogeneous. Found %: C 38.49; H 4.96. $C_9H_{12}NO_6P \cdot H_2O$. Calculated %: C 38.72; H 5.05.

B. A solution of 453 mg (2.5 mmole) of base II and 430 mg (3.5 mmole) of p-anisidine in 10 ml of absolute tetrahydrofuran was refluxed for 6 h, and the solvent was removed in vacuo to dryness. The residue was dissolved in a mixture of 5 g of P_2O_5 and 6.5 g of 85% H_3PO_4, and the mass was heated for 6 h at 50°. After the addition of 10 ml of 0.1 N HCl, the solution was heated for 15 min at 60°, cooled, applied to a 1.6 × 40-cm column filled with Dowex 50 × 4 in the acid form, and chromatographed as in experiment A. The yield of V was 36 mg (5.2%).

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