VITAMIN B$_6$ ANALOGS

XV.* SYNTHESIS AND PROPERTIES OF 4-ACETYL-3-HYDROXY-5-HYDROXYMETHYL-2-METHYLPYRIDINE 5-PHOSPHATE

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4-Acetyl-3-hydroxy-5-hydroxymethyl-2-methylpyridine 5-phosphate (IV) was synthesized by oxidation of 3-hydroxy-5-hydroxymethyl-4-(α-hydroxyethyl)-2-methylpyridine 5-phosphate (III) with chromic anhydride. Compound III was obtained by phosphorylation of 3-hydroxy-5-hydroxymethyl-4-(α-hydroxyethyl)-2-methylpyridine with polyphosphoric acid (PPA). The UV and PMR spectra of the synthesized compounds were studied.

The synthesis of an analog of pyridoxal phosphate (PLP), modified at the 4'-position, was undertaken according to the following scheme in order to explain the reactivity of the carbonyl group of PLP and the stereochemical peculiarities of reactions catalyzed by PLP-dependent enzymes:
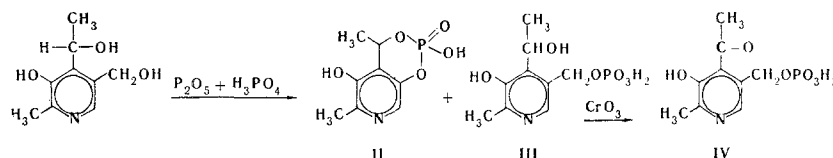
Diol I was obtained by diene condensation of 4-methyl-5-ethoxyoxazole with ethyl β-acetylacrylate with subsequent reduction with lithium aluminum hydride [2]. Two substances, both esters of phosphoric acid, were isolated in the phosphorylation of I with polyphosphoric acid (PPA) with subsequent separation on an ion-exchange resin. The UV spectra of both compounds were similar to the spectra of pyridoxine phosphate (PNP). A 6-H signal at weak field, a 2-CH$_3$ singlet, and a 4'-CH$_3$ doublet at strong field, a double multiplet of the AB part of 5-CH$_2$-O-P- and a multiplet of 4-CH-O-P at 5-6 ppm are observed in the PMR spectrum of the primary reaction product (Fig. 1). These data are in best agreement with a 3-hydroxy-5-hydroxymethyl-4-(α-hydroxyethyl)-2-methylpyridine 4'-O-5'-O-cyclophosphate structure (II). A confirmation of this conclusion is the fact that, under electrophoresis conditions (pH 9), the mobility of the cyclophosphate is less than the mobility of the secondary component by a factor of about two.

A 6-H singlet at weak field at 8.16 ppm and a 2-CH$_3$ singlet and a 4-CH$_3$ doublet (J = 6.6 Hz) at 2.59 and 1.58 ppm, respectively, are observed in the PMR spectrum of the secondary substance. The methine component of the α-hydroxyethyl group is displayed as a quartet at 5.41 ppm. The 5-CH$_2$ signal, which is split into a doublet due to interaction with phosphorus (J = 6.6 Hz), lies at 5.00 ppm. The data obtained make it possible to suppose that this compound has the 3-hydroxy-5-hydroxymethyl-4-(α-hydroxyethyl)-2-methylpyridine 5-phosphate structure (III).

Since seven-membered cyclophosphates are extremely resistant to hydrolysis [3] while the polyphosphates formed in the reaction are phosphorylating reagents, the formation of II during the phosphorylation can be represented in the following way:
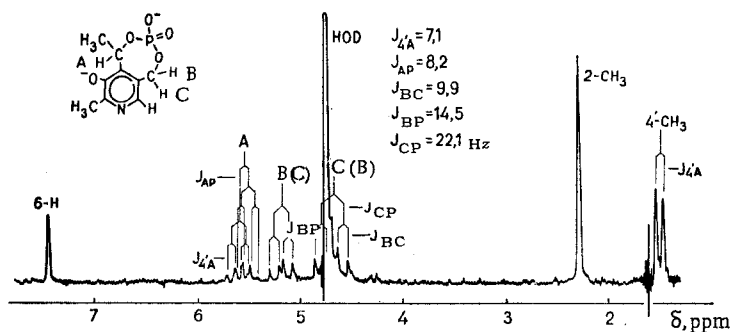
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*See [1] for communication XIV.

Fig. 1. PMR spectrum of 3-hydroxy-5-hydroxymethyl-4-(α-hydroxyethyl)-2-methylpyridine 4'-O-5'-O-cyclophosphate (II) in D_2O.
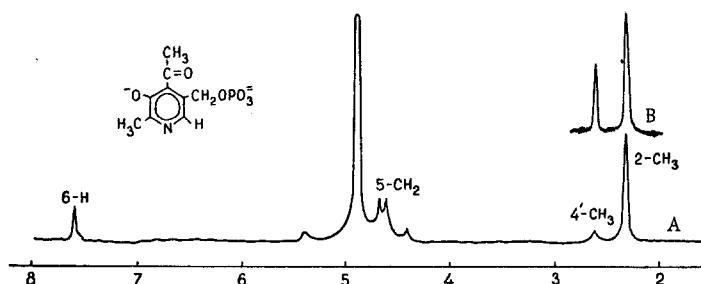

Fig. 2. PMR spectrum of IV: A) in 2 N NaOD; B) on double dilution of a sample with undeuterated water.

TABLE 1. UV Spectra of PLP and Its Analogs*

Ionic form	Medium	λ_{max}, nm ($\varepsilon \cdot 10^{-3}$)			
		PLP†	V ‡	IV	III
Cation, carbonyl form	0,1 N HCl	295 (6,7)	326 (7,4)	295 (8,88)	—
Cation hydrate	0,1 N HCl	338 (1,4)	—	—	291 (9,1)
Dipolar ion, carbonyl form	pH 7	388 (4,9)	382 (7,15)	332 (7,6)	—
Dipolar ion, hydrate	pH 7	330 (2,5)	—	—	320 (7,5)
Anion, carbonyl form	0,1 N KOH	388 (4,9)	380 (7,4)	315 (4,85)	—
Anion, hydrate	0,1 N KOH	305 (1,1)	305 (0,95)	—	305 (7,4)

*In the case of PLP, III, and V, the numbers in parentheses are not the true molecular extinctions, but indicate the absorption of the given ionic form at equilibrium concentrations.
† Data from [8].
‡ Data from [2].
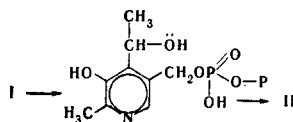
4-Acetyl-3-hydroxy-5-hydroxymethyl-2-methylpyridine 5-phosphate (IV), the structure of which was proved by the IR spectrum ($\nu_{C=O}$ 1720 cm^{-1}, $\nu_{C=\overset{+}{N}} <$ 1650 cm^{-1}) and the PMR spectrum (Fig. 2), was obtained by oxidation of III with chromic anhydride in aqueous acetic acid. The low intensity of the signal from the 4'-CH$_3$ protons is explained by the rapid exchange of these protons with D_2O. When a sample is diluted with water (upper curve in Fig. 2), the intensity of the signal increases in proportion to the water concentration. A similar regularity is observed in the PMR spectra of some 4- and 5-acetylpyridines (Fig. 3).
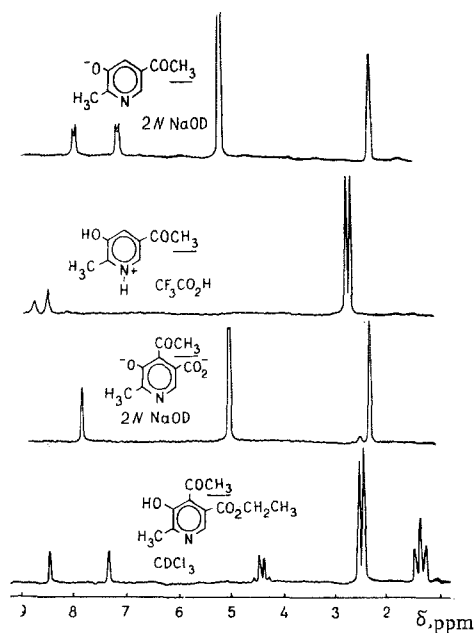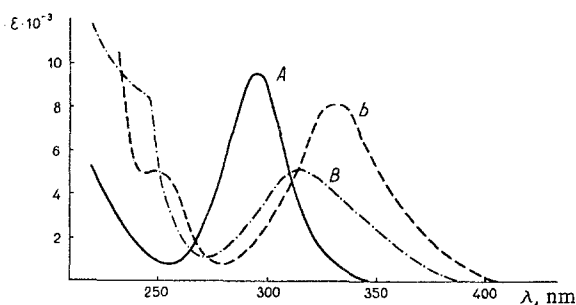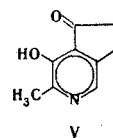
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Fig. 3. PMR spectra of 4- and 5-acetyl-pyridines.

Fig. 4. UV spectra of IV: A) in 0.1 N HCl; B) in 0.1 N phosphate buffer at pH 7; C) in 0.1 N KOH.

The UV spectra of IV was studied (Fig. 4). The absorption maxima must be correlated with an electron transition and an ionic form in order to explain the regularities of the spectra.

The molar extinction value (on the order of 10^4) as well as the fact that a spectral band is observed in acidic medium make it possible to assume that a $\pi \to \pi^*$ transition of the pyridine chromophore is responsible for the long-wave absorption.

We have previously shown [2] that the position of the maximum of the $\pi \to \pi^*$ transition for vitamin B_6 and its analogs depends substantially on the state of the ionogenic groups; in the series of possible ionic forms (neutral form, cation, anion, and dipolar form) the maximum is shifted to long waves. Thus it can be assumed that the absorption maxima at 295, 315, and 332 nm in the case of IV are related to the cation, anion, and dipolar forms, respectively. However, the absorption maximum for the dipolar form of PLP and cyclic ketone V lies at 380 nm.

As shown by Nakamoto and Martell [4], the position of the long-wave maximum in the spectra of 3-hydroxy-4-formylpyridines is explained by the considerable contribution to the excited state of a resonance structure with electron transfer from the nitrogen of the pyridine ring to the oxygen of the carbonyl group. As seen from Table 1, the absorption maximum of IV is shifted to shorter waves by 30-60 nm as compared with PLP and V for the different ionic forms and is extremely close to that for vitamin B_6 derivatives which do not have unsaturated groupings in the 4-position of the pyridine ring.

V

A hypsochromic shift of such magnitude is best explained by disruption of the conjugation of the keto group with the pyridine ring in the IV molecule. This conclusion is confirmed by a study of the UV spectra of the phenylhydrazone of IV, the maximum of which in 0.3 M H_2SO_4 lies at 380 nm (ε = 6433); at the same time, the phenylhydrazone of V under similar conditions absorbs at 410 nm (ε = 18,800). The deviation from coplanarity of conjugated systems is usually estimated from the intensity of the hypsochromic effect, and the average angle of rotation (θ) can be calculated from the Braude equation [5]: $\cos^2 \theta = \varepsilon / \varepsilon_0$. For ε_0 we took the molecular extinction of the phenylhydrazone of V, for which the C $=$ N group is surely fixed in the plane of the pyridine ring. Calculation indicates that the angle of rotation of C $=$ N with respect to the pyridine ring in IV is ~55°.

To determine the relative reactivities we studied the kinetics of the reaction of IV, V, and PLP with phenylhydrazine, during which the second-order rate constants turned out to be $(1.7 \pm 0.8) \cdot 10^{-2}$, $(3.2 \pm 0.7) \cdot 10^{-1}$, and $(3.3 \pm 0.5) \cdot 10^2$ liter-mole^{-1}-min^{-1}, respectively (0.3 M H_2SO_4, 30°). It is apparent that the usual decrease in the rate is observed on passing from the aldehyde to the ketone, but the reaction of IV with phenylhydrazine proceeds at a rate which is lower by a factor of ~20 than that observed with V. As Auld and Bruice [7] pointed out in the case of the reaction of 3-hydroxy-4-formylpyridine and 4-formyl-pyridine with amino acids, the 3-hydroxy group participates in intramolecular general acid catalysis. Thus

the decrease in the rate of reaction of IV with phenylhydrazine is in good agreement with the assumption of the deflection of the carbonyl group of IV from the plane of the pyridine ring, since the catalytic effect of the 3-hydroxy group cannot be manifested in this case.

EXPERIMENTAL

The UV spectra were obtained with a Hitachi ESP-3T spectrophotometer. The IR spectra of mineral oil suspensions were obtained with a UR-10 double beam spectrometer. The PMR spectra were obtained with a JNM-4H-100 spectrometer (100 MHz).

The chemical shifts are indicated in the δ scale, and tert-butanol (1.20 ppm) was used as the internal standard. The electrophoresis was carried out on "Whatmann 3 mm" paper in 0.1% ammonium carbonate (pH 9) at 1500 V (38 V/cm). Chromatography on plates with silica gel was carried out by the method in [6] in n-butanol—ethanol—5% ammonium hydroxide—glacial acetic acid (10 : 10 : 10 : 1). The rates of reaction of PLP and its analogs with phenylhydrazine were determined from the absorption of the phenylhydrazones at 410 nm (ε = 22,400 and 18,800 for PLP and V, respectively) or at 380 nm (ε = 6430 for IV).

Phosphorylation of 3-Hydroxy-5-hydroxymethyl-4-(α-hydroxyethyl)-2-methylpyridine (I). A solution of 450 mg (2 mmole) of the hydrochloride of I in a mixture of 2.7 g of 85% phosphoric acid and 2 g of P_2O_5 was held at room temperature until HCl evolution ceased and was then heated at 40° for 4 h. The mixture was cooled, and 15 ml of alcohol and 70 ml of ether were added to the reaction mass with cooling. The mixture was allowed to stand in a refrigerator for 1 h, the liquid was decanted, and the residue was heated with 25 ml of 1 N HCl for 20 min on a boiling water bath. It was then vacuum evaporated to 5 ml at 45°, and the pH was brought up to 5 by the addition of concentrated ammonium hydroxide. The solution was applied to a 1.6 × 50-cm column filled with Dowex 50 × 4 (H^+ form) and eluted with water at 30 ml/h with recording of the UV absorption at 295 nm and the conductivity of the eluates in the flow. The volume of the empty fraction was 100 ml, while the volume of the fraction containing II was 150 ml, the volume of the intermediate fraction was 200 ml, and, finally, the volume of the fraction containing III was 300 ml. The aqueous solutions of the phosphorylation products were vacuum evaporated at 40° to 30 ml and lyophilized. The yield of II with mp 158° and R_f 0.73 was 44%. UV spectrum in 0.1 N KOH, λ_{max}, nm (ε): 247 (17,500), 308 (8410). Found %: C 40.4; H 5.7; N 5.1; P 11.4. $C_9H_{12}NO_5P \cdot H_2O$. Calculated %: C 41.1; H 5.3; N 5.3; P 11.8.

The yield of III with mp 154° and R_f 0.4 was 24%. Found %: C 38.2; H 5.8; N 4.9; P 11.3. $C_9H_{14}NO_6P \cdot H_2O$. Calculated %: C 38.5; H 5.7; N 5.0; P 11.0.

4-Acetyl-3-hydroxy-5-hydroxymethyl-2-methylpyridine 5-Phosphate (IV). A solution of 300 mg (1.07 mmole) of III in 1 ml of glacial acetic acid and 0.1 ml of a solution of 334 mg of chromic anhydride in 1 ml of water were stirred at room temperature for 3 h and held at 60° for 5-10 min until the chromic anhydride was reduced. The mixture was applied to a 2.5 × 40-cm column filled with Dowex 50 × 4 (H^+ form) and eluted with water at 30 ml/h. The volume of the empty fraction was 150 ml, while the volume of the fraction containing IV was 600 ml. The aqueous solution was vacuum evaporated to 40° to 30 ml and lyophilized. The yield of IV with mp 110° was 50 mg (21%). The phenylhydrazone of IV had mp 160°. Found %: C 38.5; H 5.0; N 4.9; P 11.0. $C_9H_{12}NO_6P \cdot H_2O$. Calculated %: C 38.7; H 5.0; N 5.0; P 11.1.

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