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SYNTHESIS AND PROPERTIES OF KETO ANALOGUES OF PYRIDOXAL PHOSPHATE

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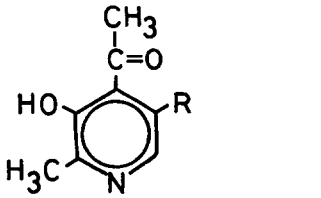
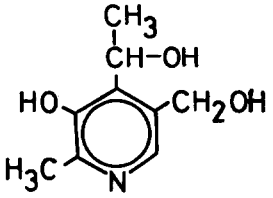
The Schiff bases formation was proved to be an essential step in all transformations of amino acids catalyzed by pyridoxal-5'-phosphate (PLP) or PLP-enzymes[1,2]. Therefore the aldehyde group of the coenzyme molecule is of great importance. Clearly, analogues of PLP containing a keto group in place of the 4-formyl group would be of interest in the study of both nonenzymic and enzymic catalysis. The present paper deals with the synthesis of the PLP keto analogues:



1. R = $CH_2OPO_3H_2$
2. R = CH_3
3. R = H



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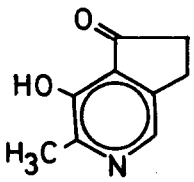

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4'-Methylpyridoxine (4) was obtained according to a previously reported method[3]. It might be expected that polyphosphoric acid would be most suitable agent for selective esterification of the primary 5-hydroxymethyl group of 4. Actually, interaction of 4 with polyphosphoric acid followed by separation on a Dowex-50 column (H^+-form) using water as eluant yielded two compounds[4]. One

was shown to be the 4′,5′-cyclic phosphate of **4** [yield, 44%; m.p., 158–159°; PMR (in NaOD): 1.62δ d. (J=7.1 c/s) Me(4′); 2.37δ s. Me(2); 5.67δ m. (J=7.1, and 8.2 c/s); H(4′); 4.5 to 5.2δ two m. AB pattern of AEX-system (J=9.9, 14.5 and 22.1 c/s) CH_2(5); 7.44δ s. H(6)]. The other was 5′-phosphate of **4** [yield, 24%; m.p., 154–155°; PMR (in NaOD): 1.58δ d. (J=6.6 c/s) Me(4′); 2.59δ s. Me(2); 5.08δ d. (J=7.4 c/s) CH_2(5); 5.41δ q. (J=6.6 c/s) H(4′); 8.16δ s. H(6)].

4′-Methylpyridoxal phosphate (**1**) was prepared by oxidation of the latter compound with equimolecular amount of chromic anhydride in aq. AcOH at room temp for 3 hr. After separation on a Dowex-50 column (H^+-form), 4′-methyl PLP was obtained in 21% yield [m.p., 110–111; m.p. of phenylhydrazone, 160–162°; IR ν_{max}^{Nujol}: 1720 (C=O) and 1650 (C=N±) cm^{-1}; PMR (in NaOD): 2.35δ s. Me(2); 4.26δ d. (J=5 c/s) CH_2(5); 7.57δ s. H(6); the broadened low intensity singlet at 2.58δ is ascribed to Me(4′), the protons of which were recently reported[5] to be exchanged rapidly with D_2O].

2,5-Dimethyl-3-hydroxy-4-acetylpyridine (**2**) and 2-methyl-3-hydroxy-4-acetylpyridine (**3**) were obtained by Diels-Alder condensation of 4-methyl-5-ethoxyoxazole with 3-penten-2-one and methyl vinyl ketone, respectively. These compounds were adequately characterized by IR and PMR analyses.

The UV spectral data of the compounds synthesized are listed in Table. The longer-wavelength absorption maxima were previously shown by Nakamoto and Martell[6] to be attributable to the $\pi \rightarrow \pi^*$ transition. The bands of **1** and **2** in various media show a blue-shift in comparison with PLP, cyclic ketone **5** and **3**. It is reasonable to interpret this shift as resulting from lack of conjugation between the pyridine cycle and the carbonyl group due to steric hindrances. This conclusion is confirmed by consideration of the UV spectra of phenylhydrazones of the compounds obtained (see Table). In the phenylhydrazone of **1** and **2**, two orto-substituent cause a change in both wavelength and absorption intensity. The angle of deflection of the C=N bond from the plane of pyridine cycle (θ) might be calculated approximately by Braude's equation[7]:

$$\varepsilon/\varepsilon_o = \cos^2\theta$$

where ε_o is the molar absorbancy for inhindered compound. The values of the interplanar angle derived in this way are 54 and 60° for the phenylhydrazones of **1** and **2**, respectively.

Table. Same Properties of Keto Analogues of PLP

Compounds	UV spectra: λ_{max} mμ ($\varepsilon \cdot 10^{-3}$)				Interplanar angle θ	Rate constant for interaction with phenylhydrazine, $M^{-1}.sec^{-1}$
	pH 1 (Cation)	pH 7 (Dipolar form)	pH 12 (Anion)	Phenylhydrazones		
PLP[a]	338(1.4)[c] 295(6.7)[d]	388(4.9)[c] 330(2.5)[d]	388(6.4)[c] 305(1.1)[d]	410(21.8)	-	3.78 ± 0.52
4′-Methyl PLP (**1**)	295(6.4)[c]	333(5.9)[c]	315(4.0)[c]	376(6.8)	53°	$(1.45 \pm 0.37) \cdot 10^{-4}$
2,5-Dimethyl-3-hydroxy-4-acetylpyridine (**2**)	292(5.7)[c]	328(5.1)[c]	313(2.9)[c]	360(2.5)	68°	$(1.20 \pm 0.16) \cdot 10^{-4}$
2-Methyl-3-hydroxy-4-acetylpyridine (**3**)	331(4.1)[c]	371(4.1)[c]	345(2.9)[c]	391(16.5)	20°	$(4.4 \pm 0.9) \cdot 10^{-3}$
Cyclic ketone (**5**)[b]	327(7.5)[c]	373(9.2)[c]	375(7.5)[c]	395(18.8)	0°	$(6.3 \pm 1.2) \cdot 10^{-3}$
4-Acetylpyridine	276(5.7)[c]	283(4.0)[e]	283(4.0)[e]	417(26.5)	-	0.021 ± 0.002

a. Data from Metzler and Snell[8].
b. Data from Karpeisky *et al*[9].
c. Carbonyl forms.
d. Hydrate forms.
e. Neutral form.

Spectrophotometric studies of the interaction between phenylhydrazine and keto analogues of PLP in 0.3 M H_2SO_4 at 30° were performed. Secon-order kinetics were obtained with these compounds. As indicated in Table, **1** and **2** react with phenylhydrazine with rate constant approximately 30 fold less, than those for **3** and **5**. Possible explanation for this rate effect might be the absence of intramolecular catalysis by 3-hydroxy group in the case of **1** and **2**, the carbonyl group of which is located out of the plane of pyridine cycle. But if the 3-hydroxyl group catalyzes the reaction, the rate of interaction of 4-acetylpyridine will be less than those of **3** and **5**. However, our results contradict with this postulate. There is little doubt that this observed effect is steric in origin resulting from hindrance for nucleophilic attack in the direction perpendicular to the plane of the carbonyl group.

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