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ANALOGUES OF VITAMIN B_6

SYNTHESIS AND PROPERTIES OF 3-DEOXYPYRIDOXAL PHOSPHATE AND 3-O-METHYLPYRIDOXAL PHOSPHATE

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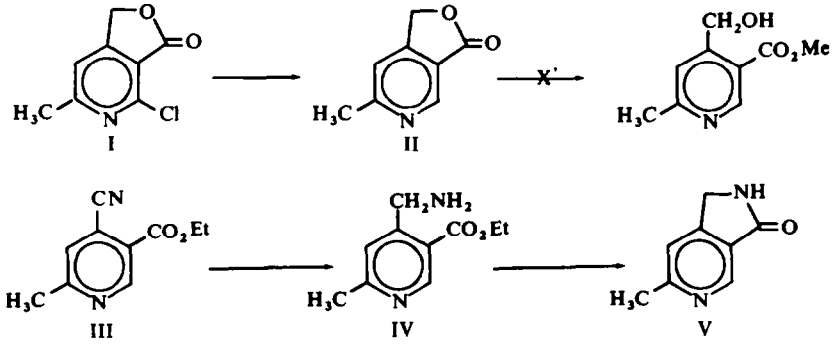
Abstract—3-Deoxypyridoxal phosphate and 3-O-methylpyridoxal phosphate have been synthesized by transamination of the corresponding pyridoxamine phosphate analogues. The amine phosphates were obtained by reduction of oximes of pyridoxal analogues followed by phosphorylation with polyphosphoric acid. PMR, UV spectra and TLC have been studied.

COMPARATIVE studies of the chemical and enzymological properties of analogues of pyridoxal phosphate (PLP) in which the hydroxy group at position 3 is replaced by hydrogen or methoxy group are of importantce for closer assessment of relations between structure and function of the coenzyme.

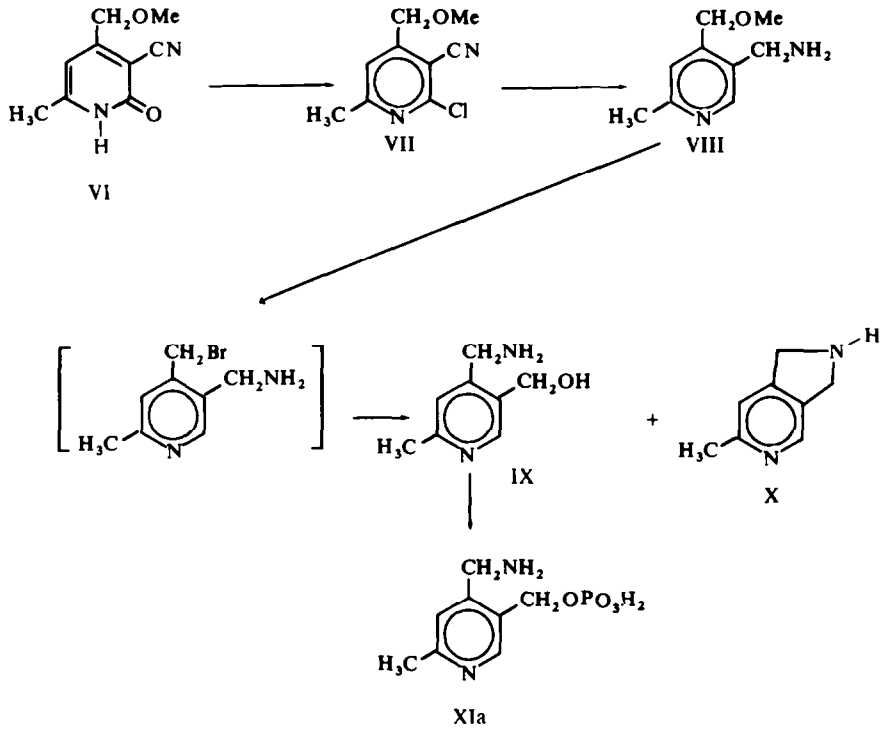
An appropriate route for preparation of PLP analogues is phosphorylation of Schiff bases of pyridoxal analogues, obtained by selective oxidation of the pyridoxine analogues.[1] However, as reported by Snell *et al.*[2] the oxidation of 3-deoxypyridoxine results in formation of a mixture of 3-deoxypyridoxal and 3-deoxyisopyridoxal in a ratio of 1:6; this mixture was obtained in 30% yield. Therefore, this route does not appear suitable, currently, for the synthesis of PLP analogues modified at position 3 of the pyridine ring. To obtain such compounds in sufficient amounts, their synthesis *via* the corresponding pyridoxamine phosphate analogues was attempted.

At the beginning, we were faced with difficulties. Lactone (II), which was prepared by hydrogenation of the 2-chloro-lactone (I), failed to undergo conversion to methyl 6-methyl-4-hydroxymethylnicotinate. Hydrogenation of ethyl 6-methyl-4-cyano-nicrotinate (III) followed by reduction of the dihydrochloride of the aminomethyl



derivative (IV) prepared with LAH, yielded only trace amounts of 3-deoxypyridoxamine. An attempt to obtain the free base of IV was unsuccessful; the lactam of 6-methyl-4-aminomethyl nicotinic acid (V) was isolated in 80% yield.

The following route proved convenient for achieving the preparative aims:

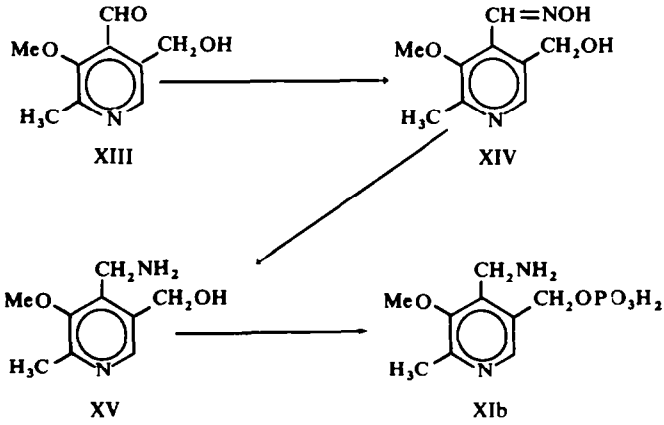


6-Methyl-4-methoxymethyl-3-cyano-2-pyridone (VI) was converted to the corresponding chloride (VII), which, upon hydrogenation on Pd-C catalyst, yielded the dihydrochloride of VIII. Interaction of VIII with hydrobromic acid gave the 4-bromomethyl derivative. Without isolation, the latter compound was deaminated with nitrous acid and then aminated with aqueous ammonia. The mixture of two amines was obtained in 80% overall yield. The PMR spectrum of this mixture shows two sets of resonance signals. A pair of singlets at higher field are ascribed to the Me groups, four singlets at lower field are assigned to α- and γ-protons of the pyridine ring. Absence of coupling between these protons indicated its para-position. Signals of 5- and 4-CH_2 are observed in the range of 4–5δ.

On this basis it is reasonable to assume that this mixture consists of 3-deoxypyridoxamine and 6-methylmerimine. This conclusion is confirmed by the data of mass-spectrometric analysis. The hydrochloride of vitamin B_6 was recently shown to dissociate thermally in the inlet system into free base and HCl, and to give a spectrum of free base with the spectrum of HCl superimposed.[3] The mass spectrum of the mixture has two molecular ion peaks at m/e 153 and m/e 135 which are assigned to IX and X, respectively, and peaks at m/e 36 and 38 attributable to HCl^+.

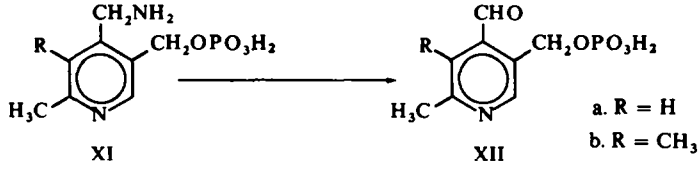
The mixture of amines thus prepared was phosphorylated with polyphosphoric acid (PPA) under conditions appropriate for selective esterification of the 5-hydroxymethyl group. 3-Deoxypyridoxamine phosphate (XIa) obtained in this way was separated from the starting amines by chromatography on a weak cation-exchange resin, employing water as the eluant. The structure of XIa was proved by its PMR spectrum. The singlet at 2·57 δ is ascribed to the 2-Me group, the singlet at 4·35 δ and doublet at 4·98 δ (J = 6·1 c/s) are assigned to 4- and 5-CH_2 groups, respectively. In addition, a pair of singlets at 7·78 δ and 8·44 δ are ascribed to 3-H and 6-H.

3-O-Methylpyridoxamine phosphate (XIb) was synthesized as follows:



3-O-Methylpyridoxal (XIII) was converted to the oxime (XIV), which was reduced to 3-O-methylpyridoxamine (XV). The phosphate (XIb) was prepared by phosphorylation of XV with PPA in 53% yield.

3-Deoxy PLP (XIIa) and 3-O-methyl PLP (XIIb) were obtained by preparative transamination of amines (XI) with glyoxylic acid as chromatographically pure



compounds in yields ranging from 25%. The molar absorbances of the phenylhydrazones of XIIa and XIIb were 19,100 and 20,200, respectively, while in the case of PLP ε was 22,400.

UV spectra of the compounds prepared have a number of characteristic features. Notably, the equilibrium in aqueous solutions involves, as distinct from PLP, only two principal ionic forms: a cation and a neutral form. Ionization of the 5'-phosphate group and 4'-amino group was recently shown[4] not to affect the absorption spectrum. In addition, equilibrium between aldehyde and hydrate must be taken into account in the case of PLP analogues.

As indicated in Table 1, the $\pi \rightarrow \pi_1^*$ bands of the amine phosphates show the usual blue-shift[5,6] in the sequence: pyridoxamine phosphate → XIb → XIa. The behaviour

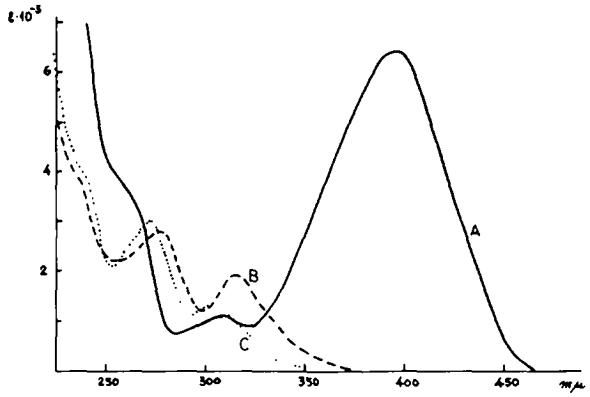


FIG 1. UV spectra in 0·1 N KOH. A: pyridoxal phosphate; B: 3-O-methylpyridoxal phosphate; C: 3-deoxypyridoxal phosphate

TABLE 1. UV SPECTRA OF VITAMIN B_6 ANALOGUES MODIFIED AT POSITION 3 OF THE PYRIDINE RING

(data for $\pi \rightarrow \pi_1^*$ transition)

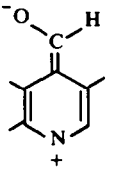
Compounds	λ_{max} mμ (ε.10⁻³)			
	Cation	Δλ mμ	Neutral form	Δλ mμ
3-Deoxypyridoxamine phosphate	269 (8·5)	23	265 (5·1)	22
Pyridoxamine[a]	292 (8·2)		287 (3·4)[c]	
3-O-Methylpyridoxamine	285 (7·4)	7	277 (4·0)	10
3-O-Methylpyridoxamine, phosphate	285 (8·7)		277 (5·6)	
Pyridoxal, hemiacetal[a]	288 (9·0)	−4	280 (4·1)[d]	4
3-O-Methylpyridoxal, hemiacetal	292 (8·4)		276 (4·7)	
Pyridoxal phosphate, aldehyde	340 (1·4)[e]		—	
o-Methoxybenzaldehyde[b]	—		310 ($\pi \rightarrow \pi^*$)	6
3-O-Methylpyridoxal phosphate, aldehyde	—		316 (1·9)[e]	11
3-Deoxypyridoxal phosphate, aldehyde	—		305 (1·3)[e]	
Benzaldehyde[b] (in EtOH)	—		280 ($\pi \rightarrow \pi^*$) 328 ($n \rightarrow \pi^*$)	
Pyridoxal phosphate,[a] hydrate	295 (6·7)[e]	7	—	
3-O-Methylpyridoxal phosphate, hydrate	282 (6·6)	12	278 (2·9)[e]	10
3-Deoxypyridoxal phosphate, hydrate	270 (5·2)		268 (3·0)[e]	

[a] Data from Metzler and Snell.[8] [b] Data from Nakamoto and Martell.[7] [c] In 98% aqueous dioxane. [d] In 60% aqueous dioxane. [e] In these cases, the figures in brackets are not true molar absorbances; they show the absorbance of the given ionic form at equilibrium concentration, i.e. when the total concentration of all forms is 1 M.

TABLE 2. SYNTHESIS OF PYRIDOXAL PHOSPHATE ANALOGUES MODIFIED AT POSITION 3 OF THE PYRIDINE RING

Monohydrate of	Purification		Yield %	Formula	Analysis			
	Volume of void fractions, (ml)	Volume of fractions containing XII (ml)			Calc		Found	
					C	H	C	H
3-Deoxypyridoxal phosphate	700	400	20	$C_8H_{10}NO_5P \cdot H_2O$	38·56	4·85	38·48	4·83
3-O-Methylpyridoxal phosphate	500	400	24	$C_9H_{12}NO_5P \cdot H_2O$	38·72	5·05	38·49	4·96

of longer wavelength absorption maxima of PLP, XIIb and XIIa (Fig 1) is in agreement with the rule mentioned above, since this band was recently reported by Nakamoto and Martell[7] to be attributable not to the $n \rightarrow \pi^*$ transition, but to the $\pi \rightarrow \pi_1^*$ transition. The excited state may be described approximatively by polar resonance structures such as shown below:



The stabilizing effect of the pyridine N-atom on such excited states accounts for the red-shift of this band in comparison to carbocyclic analogues (see Table 1).

Examination of UV spectra shows that the degree of hydration of the aldehyde group is increased in compounds without a free OH group at position 3 of the pyridine ring. Assignment of UV absorption maxima to the ionic forms of the compounds prepared is presented in Table 1.

EXPERIMENTAL

UV spectra were taken on a ESP-3T "HITACHI" spectrophotometer. Absorbancy in acid phenylhydrazine was determined according to Wada and Snell.[9] IR spectra were obtained on an UR-10 spectrophotometer (suspension in Nujol). PMR spectra were determined on an 100 Mc/s "JEOL" instrument. The chemical shifts are reported in δ values in ppm with the t-BuOH signal (1·20 ppm) as internal standard. Mass spectra were taken on a MS 13-02 spectrometer.

1 *Lactone of 6-methyl-4-hydroxymethylnicotinic acid* (II). To a soln of 5 g (27 mmoles) of lactone (I)[10] in 120 ml of EtOH and 3 ml of conc HCl was added 1·5 g of 5% Pd-C catalyst. Hydrogenation was carried out at room temp and atm pressure until about the theoretical amount of H_2 (664 ml) had been absorbed. The catalyst was removed by filtration, using a hot water wash, and the filtrate was evaporated to dryness *in vacuo*. Yield of lactone (II) hydrochloride, 4·5 g (90%); m.p. 195–200° (dec, from abs EtOH).

The free base of II was obtained by addition of solid $NaHCO_2$ to a soln of the hydrochloride of II in a minimum volume of water (pH 6). Yield, 3·3 g (82%), m.p. 172–173° (from benzene); UV $\lambda_{max}^{0.1\,N\,KOH}$ (ε): 271 (20,400) and 321 (9,900) mμ; IR ν_{max}^{Nujol}: 1770 (C=O), 1194 and 1146 (C—O), 1630 cm^{-1} (C=C, pyridine). (Found: C, 64·38; H, 4·70; N, 9·42. Calc for $C_8H_7NO_2$: C, 64·42; H, 4·64; N, 9·39%).

2 *Dihydrochloride of ethyl 6-methyl-4-aminomethylnicotinate* (IV). Hydrogenation of a soln of 3·8 g (20 mmoles) of ethyl 6-methyl-4-cyanonicotinate (III)[11] in 50 ml of EtOH and 25 ml conc HCl on 2 g of Pd-C catalyst was continued at room temp and atm pressure for 4 h. The catalyst was filtered off, washed with water, and evaporated *in vacuo* to dryness. The residue was dried in vacuum desiccator over KOH. The yield of IV was 4 g (80%); m.p., 220–222° (from abs EtOH). (Found: C, 45·01; H, 5·99. Calc for $C_{10}H_{16}Cl_2N_2O_2$: C, 44·92; H, 6·02%).

3 *Lactam of ethyl 6-methyl-4-aminomethyl nicotinate* (V). To a soln of 3·6 g of IV in a minimum volume of water, 30% KOH aq was added to pH 12 with cooling. The ppt was filtered, washed with water and dried. Yield of V, 2 g (90%); m.p., 222–223° (from Me_2CO). UV $\lambda_{max}^{0.1\,N\,KOH}$ (ε): 263 (3,600) and 272 mμ (3,000); PMR δ ppm (in 2 N NaOD): 2-Me at 2·43; 4-CH_2 at 5·1; 3-H at 7·17 and 6-H at 8·41 (all peaks are singlets). (Found: C, 64·30; H, 5·42. Calc for $C_8H_8N_2O$: C, 64·85; H, 5·45%).

4 *2-Chloro-3-cyano-4-methoxymethyl-6-methylpyridine* (VII). To a suspension of 11·8 g (66 mmoles) of VI in 75 ml of chlorobenzene, 13·7 g (66 mmoles) of PCl_5 was added with stirring and cooling. The mixture was refluxed for 2·5 h and evaporated *in vacuo*; the residue was shaken with 20 ml of abs EtOH. After evaporation, the residue was extracted with boiling hexane. The solvent was removed and VII was obtained in 87% (11·3 g) yield. M.p., 56–58° (from hexane). (Found: C, 55·12; H, 4·81. Calc for $C_9H_9ClN_2O$: C, 54·98; H, 4·61%).

5 *Dihydrochloride of 2-methyl-4-methoxymethyl-5-aminomethylpyridine* (VIII). Hydrogenation of VII

(4 g) in 150 ml of water and 6 ml of conc HCl under the conditions for preparation of II yielded 4·1 g (85%) of VIII, m.p. 153–155° (from ether—EtOH). (Found: C, 45·28; H, 6·81. Calc for $C_9H_{16}Cl_2N_2O$: C, 45·19; H, 6·79%).

6 *3-Deoxypyridoxamine phosphate* (XIa). A soln of 2·3 g (9·6 mmoles) of VIII in 75 ml 42% HBr aq was refluxed for 2 h and evaporated *in vacuo* to dryness. The residue was dissolved in 65 ml water and 3·5 ml conc HCl and heated to 80°. To this mixture, a soln of 0·75 g $NaNO_2$ in 10 ml of water was added dropwise and stirring. Heating at 80° was continued for 3·5 h. The mixture was evaporated *in vacuo* to dryness. To the cooled reaction mixture, 150 ml 25% aq NH_3 was added, and the resulting soln was allowed to stand at room temp for 48 h. This mixture was refluxed for 2 h and then evaporated *in vacuo* to dryness. To the residue was added 10 ml conc HCl, the excess of which was removed by repeated evaporation with water. The solid was dissolved in 10 ml water and applied to the top of a 1·4 × 30 cm column of Dowex 50W × 4 in the acid form. The column was eluted with water and then with 5% aq NH_3. Evaporation of the ammonia effluent followed by repeated evaporation with 2N HCl yielded 1·7 g of IX and X dihydrochlorides. A soln of 1 g of this mixture in PPA (prepared from 3·8 g of P_2O_5 and 5 g of 85% H_3PO_4) was kept until evolution of HCl was complete. The resulting syrup was heated at 60° for 4 h, cooled and mixed thoroughly with 25 ml EtOH followed by 80 ml ether. After refrigeration for 1 h, the liquid was decanted, and residue was dissolved in 45 ml N HCl. This soln was heated on a steam bath for 20 min and evaporated *in vacuo* at 35–45° to a volume of 5 ml; the mixture was brought to pH 5 with 25% aq NH_3 and the resulting soln was applied to the top of a 2·5 × 60 cm column of Amberlite CG-50 in the acid form. This column was eluted with water at a rate of 30 ml/h. The effluent containing the amine phosphate* was evaporated to a small volume *in vacuo* at 35–45° and lyophilized. Yield of XIa, 0·2 g. (Found: C, 35·77; H, 6·28; P, 11·39. Calc for $C_8H_{13}N_2O_4P{\cdot}2H_2O$: C, 35·83; H, 6·39; P, 11·55%).

7 *Oxime of 2-methyl-3-methoxy-4-formyl-5-hydroxymethylpyridine* (XIV). To a soln of hydrochloride of 3-O-methylpyridoxal[12] (3·8 g, 17·5 mmoles) in a minimum volume of water was added 2 g of $NH_2OH{\cdot}HCl$, and the mixture was heated at 70° for 10 min. Then 9·1 g of $NaOAc{\cdot}3H_2O$ was added and the soln was heated again at 70° for 10 min and allowed to stand in the refrigerator for 2 h. The ppt was filtered off, washed with cold water, dried and recrystallized from 15% aq EtOH. Yield of XIV, 3·4 g (99%), m.p., 183–184° (dec). (Found: N, 14·16. Calc for $C_9H_{12}N_2O_3$: N, 14·28%).

8 *Dihydrochloride of 2-methyl-3-methoxy-4-aminomethyl-5-hydroxymethylpyridine* (XV). To a soln of XIV (2·75 g, 14 mmoles) in 130 ml of water and 7 ml conc HCl was added 0·5 g of 5% Pd-C catalyst. Hydrogenation was carried out at room temp and atm pressure until about a 10% excess over the theoretical amount of H_2 had been absorbed. The catalyst was removed by filtration, using a hot water wash; the filtrate was evaporated to dryness *in vacuo* at a temp below 45°. Recrystallization from ether–ethanol mixture yielded 3·1 g (87%) of XV, m.p., 162–163°. (Found: C, 42·61; H, 6·27. Calc for $C_9H_{16}Cl_2N_2O_2$: C, 42·37; H, 6·32%).

9 *3-O-Methylpyridoxamine phosphate* (XIb). Phosphorylation of XV (2 g, 7·85 mmoles) with a mixture of 9 g 85% H_3PO_4 and 7 g P_2O_5 by the method described above for preparation of XIa yielded 1·22 g (52%) of XIb dihydrate. (Found: C, 35·92; H, 6·31. Calc for $C_9H_{15}N_2O_5P{\cdot}2H_2O$: 36·24; H, 6·42%).

10 *Pyridoxal phosphate analogues* (XIIa) *and* (XIIb). To solns prepared from 0·4 mmole each of the pyridoxamine phosphate analogues, 3 ml water and 0·4 ml 2N NaOH, 192 mg of sodium glyoxylate was added. The mixture was stirred at room temp for 10 min, then the soln was brought to pH 5 with glacial AcOH and stirred for another 10 min. To this was added, dropwise and with stirring, 3·2 ml 0·25 M soln of cupric acetate under a slow stream of N_2 for 30 min; the resulting mixture was then applied to the top of a 1·4 × 30 cm column of Dowex 50W × 4 in the acid form and eluted with O_2-free water at a rate of 50 ml/h. The effluent containing the aldehyde phosphate was concentrated *in vacuo* to a volume of 20 ml at a temp not exceeding 30–35° and lyophilized. The compounds obtained are listed in Table 2.

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* The effluent was controlled by flow measurement of absorption at 295 mμ and of conductivity.

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