VITAMIN B_6 ANALOGS

XIII.* 3-DEOXYPYRIDOXAL-5-PHOSPHATE

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Routes for the synthesis of 3-deoxypyridoxal 5-phosphate were studied, and a method for its preparation by the transamination of 3-deoxypyridoxamine 5-phosphate is proposed. Chlorination and reduction of 6-methyl-4-methoxymethyl-3-cyano-2-pyridone converted it to 2-methyl-4-methoxymethyl-5-aminomethylpyridine dihydrochloride which, without isolation, was successively treated with HBr, diazotized, and aminated. The mixture of amines obtained was phosphorylated with polyphosphoric acid, and the phosphate was separated by chromatography. The UV, IR, and PMR spectra of the compounds obtained were investigated.

The data in the literature regarding the effect of a phenolic hydroxyl group on the catalytic properties of pyridoxal 5-phosphate (PP) and its ability to bond with an apoenzyme are based on indirect results and are contradictory. In this connection, we recently synthesized 3-methoxy-PP, and in this communication we describe the synthesis and some physicochemical properties of 3-deoxy-PP and 3-deoxypyridoxamine 5-phosphate.

Preparatively speaking, the most convenient route for the synthesis of PP analogs consists in the selective oxidation of the appropriate pyridoxine analogs with manganese dioxide to the aldehydes, which are isolated from the reaction mixture as Schiff bases with aromatic amines. Phosphorylation of these Schiff bases and subsequent hydrolysis leads to PLP analogs.

However, as pointed out by Snell and co-workers [1], a mixture of the products of oxidation at both hydroxymethyl groups was obtained in a yield of approximately 30% by the oxidation of 3-deoxypyridoxine with manganese dioxide, and the ratio of 3-deoxypyridoxal to 2-methyl-4-hydroxymethyl-5-formylpyridine is 1:6.

Thus this route is practically unsuitable for the preparation of PP analogs modified at the 3-position. We therefore used another approach for the synthesis of 3-deoxy-PP in which the key product is 3-deoxypyridoxamine (see the scheme below).

We initially attempted to obtain this amine from lactone II with the idea of subsequent opening of the lactone ring and production of 2-methyl-4-hydroxymethyl-5-carbethoxypyridine, in which the hydroxyl group could be replaced by a halogen and subsequently by an amino group, while the ester group could be replaced by a hydroxymethyl group. Compound II was obtained by hydrogenation of the lactone of 2-chloro-6-methyl-4-hydroxymethylnicotinic acid (I). The PMR spectrum of the compound obtained contains a signal from the methyl group at 2.47 ppm, a signal from the methylene group at 4.78 ppm, and two singlets from the protons of the pyridine ring at 7.37 ppm (3H) and at 8.44 ppm (6H). The absence of appreciable spin-spin interaction of the latter indicates their para orientation to one another. However, attempts to open the lactone were unsuccessful. Thus, for example, the starting material was recovered on prolonged refluxing of II in alcohol with constant passage of hydrogen chloride. The retention of the characteristic lactone frequencies and the complete absence of a band for an ester grouping in the IR spectra of the isolated compound confirm that the lactone structure has not changed.

*See [12] for communication XII.

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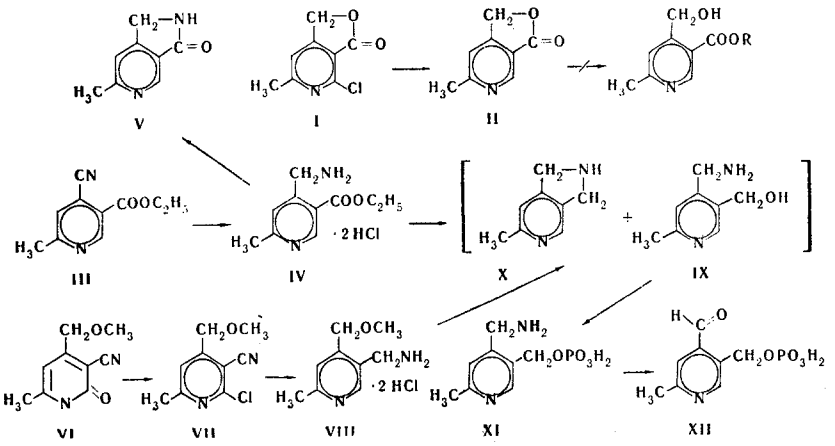


Another possible route to the synthesis of 3-deoxypyridoxamine that we checked consisted in the following: 2-methyl-4-cyano-5-carbethoxypyridine (III) was converted by hydrogenation over palladium on carbon to 2-methyl-4-aminomethyl-5-carbethoxypyridine dihydrochloride (IV), which was then reduced with lithium aluminum hydride. After the usual workup, a reaction product was isolated in a yield of 5-7% and was found to be a mixture of two substances, one of which coincides in its chromatographic behavior with 3-deoxypyridoxamine obtained via the method described below. In connection with the low yield of product, we attempted to isolate base IV and reduce it. However, on treatment of the dihydrochloride of IV with sodium hydroxide in water we obtained the lactam of 6-methyl-4-aminomethylnicotinic acid (V) in 80% yield instead of the expected free base.

A synthetic route based on the following scheme turned out to be suitable for preparative purposes. 6-Methyl-4-methoxymethyl-3-cyano-2-pyridone (VI) was converted to the corresponding chloride (VII), which was converted to 2-methyl-4-methoxymethyl-5-aminomethylpyridine dihydrochloride (VIII) by hydrogenation over 10% palladium on carbon. On treatment with hydrobromic acid the methoxy group is smoothly replaced by bromine to form 2-methyl-4-bromomethyl-5-aminomethylpyridine, which, without isolation, was diazotized and then aminated with ammonium hydroxide. A product was obtained in 80% yield and was a mixture of two substances which are colored by ninhydrin. There are two sets of peaks in the PMR spectrum (Fig. 1) which indicates a mixture of two substances. Two singlets are presented in the region characteristic for the protons of a methyl group (2.76 and 2.78 ppm); peaks which correspond to methylene protons are situated at 4.5-4.7 ppm. At weak field there are two pairs of signals from aromatic protons, of which the two at 8.50 ppm are related to 6H, while the two at 7.61 ppm are related to 3H. The absence of a spin-spin interaction corresponds to a para orientation of these protons. Thus it can be assumed that we have a mixture of 3-deoxypyridoxamine (IX) and 6-methylmerimine (X), but the accurate assignment of the set of peaks to one or another substance is difficult.

The mixture of amines was then treated with polyphosphoric acid under conditions where the 5-hydroxymethyl group of the pyridoxamine is selectively esterified. 3-Deoxypyridoxamine phosphate (XI) can be readily separated from the starting amine by chromatography of the reaction mixture with an ion-exchange resin. The structure of XI was proved by the PMR spectrum (Fig. 2), which contains a signal

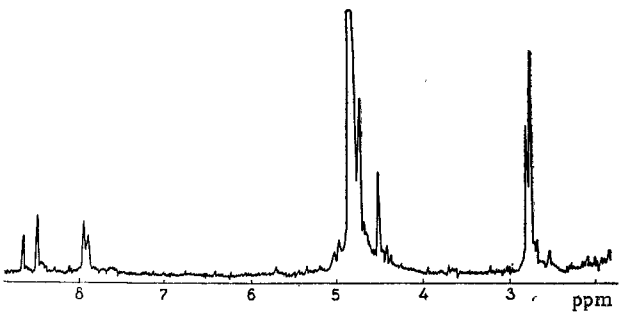


Fig. 1. PMR spectrum of a mixture of amines IX and X (hydrochlorides) in D_2O.

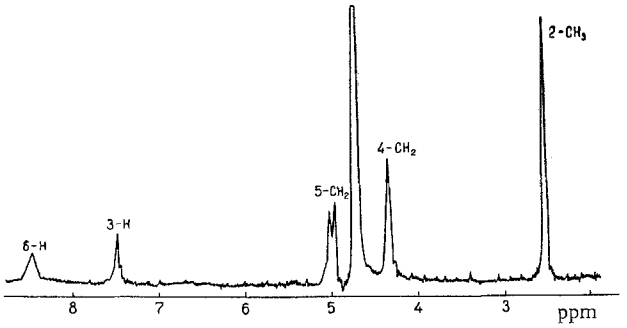
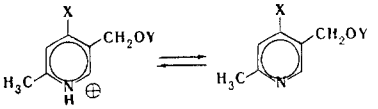


Fig. 2. PMR spectrum of 3-deoxypyridoxamine-5-phosphate in D_2O.

from a methyl group at 2.57 ppm, two methylene peaks at 4.35 ppm ($4CH_2$) and 4.98 ppm ($5CH_2$; $J_{5HP} = 6.1$), and two singlets from the protons of the pyridine ring at 7.47 ppm (3H) and at 8.44 ppm (6' H).

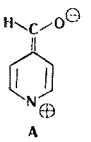
The compound obtained is chromatographically and electrophoretically homogeneous, gives a bright orange coloration with ninhydrin, and its electrophoretic mobility is comparable to that of 3-O-methylpyridoxamine phosphate. 3-Deoxypyridoxal phosphate (XII) was obtained in 20% yield by the reaction of 3-deoxypyridoxamine phosphate (XI) with glyoxylic acid in the presence of divalent copper ions. This decrease in yield, as compared with PP analogs which contain a 3-hydroxy group, and the reduced reactivity of the 4-hydroxymethyl group in 3-deoxypyridoxine are in good agreement with the decrease in the activity of the aldehyde group of 3-O-methylpyridoxal previously noted by us. These results make it possible to assume that the 3-hydroxy group brings about intramolecular general acid catalysis of reactions occurring at the 4-position.

The UV spectra of the compounds obtained have a number of characteristic peculiarities. This is primarily associated with the decrease in the number of ionic forms, since the dipolar and anionic forms are impossible. Thus the equilibrium in solution includes two basic ionic forms (cation and neutral form).



We will not consider the ionization of the ammonium and phosphate groups since, as pointed out in [2], the ionization of these groups does not affect the positions and half widths of the spectral lines. The aldehyde ⇌ hydrate equilibrium must also be taken into account in the case of 3-deoxy-PP.

As seen from Table 1, the usual hypsochromic shift characteristic for $\pi \rightarrow \pi^*$ transitions of pyridine bases [3, 4] is observed on passing from pyridoxamine phosphate to 3-O-methylpyridoxamine phosphate and then to 3-deoxypyridoxamine phosphate. The same regularity is also observed in the spectra of 3-deoxy-PP. This sort of behavior of the long-wave band in the UV spectrum of the aldehyde is somewhat unusual. However, as pointed out by Nakamoto and Martell [5], a maximum corresponding to local excitation of the carbonyl group ($n \rightarrow \pi^*$) is not observed in the spectra of 4-formylpyridines, while the $\pi \rightarrow \pi^*$ transition of the pyridine ring due to charge transfer corresponds to the long-wave absorption. The excited state of this sort of transition can be approximately described by dipolar structure A.

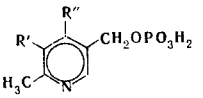


A

In the case of 3-deoxy-PP, the maximum of this band is situated at 305 nm.

In connection with what has been stated above, we feel that it is necessary to note the following. In one of our previous publications devoted to 3-O-methyl-PP we compared the absorption of the aldehyde from (316 nm) with the band at 320 nm of benzaldehyde. However, this comparison is incorrect since the

TABLE 1. UV Spectra of Vitamin B_6 and Its Analogs



Ionic forms	λ_{max}, nm ($\varepsilon \cdot 10^{-3}$)					
	R^1=OH* R^2=CH_2NH_2	R^1=OCH_3 R^2=CH_2NH_2	R^1=H R^2=CH_2NH_2	R^1=OH* R^2=CHO	R^1=OCH_3 R^2=CHO	R^1=H R^2=CHO
Cation	293 (9,0)	285 (9,7)	269 (8,5)	288 (8,7)	282 (6,6)	270 (5,2)
Neutral form	287 (3,4)	276 (5,6)	265 (4,2)	288 (4,1)†	278 (2,9)†	268 (3,0)†
					316 (1,9)‡	305 (1,3)‡
Absorption in acidic phenylhydrazine at 410 nm [9]	—	—	—	22400**	20200	19100

*Data for unphosphorylated compounds from [10].
†Hydrate form.
‡Aldehyde form. In this and the previous cases the numbers in parentheses are not the true molecular extinctions but correspond to the absorption of the given form in equilibrium concentrations.
**Data for pyridoxal-5-phosphate from [11].

$n \rightarrow \pi^*$ transition of the carbonyl group appears at 320 nm. Thus the absorption of pyridoxal analogs must be compared with the first $\pi \rightarrow \pi^*$ transition of benzaldehyde, the band of which lies at 280 nm [6]. It is apparent from the data in Table 1 that the $\pi \rightarrow \pi^*$ band undergoes a considerable red shift when the $-CH-$ link of benzaldehyde is replaced by a nitrogen atom, which is explained by the substantially higher stability of the dipolar excited state in the case of 4-formylpyridines.

EXPERIMENTAL

The UV spectra were obtained with a Hitachi EPS-3T spectrometer. The PMR spectra were obtained with a JNM-4H-100 spectrometer (Japan) and are presented in the δ scale. The IR spectra (mineral oil suspensions) were obtained with a UR-10 spectrometer.

Lactone of 2-Methyl-4-hydroxymethylpyridine-5-carboxylic Acid (II). A total of 1.5 g of 5% Pd/C was added to a solution of 5 g (27 mmole) of I [7] in 120 ml of alcohol and 3 ml of concentrated hydrochloric acid and hydrogenated at room temperature and atmospheric pressure. After absorption of the theoretical amount of H_2 (664 ml), the catalyst was filtered and washed with water, and the combined filtrates were vacuum evaporated to dryness to give 90% of the hydrochloride of the lactone with mp 195-200° (decomp., absolute alcohol). Base II was obtained by the addition of solid $NaHCO_3$ to pH 6 to a solution of the hydrochloride in the minimum volume of water; the yield of product with mp 172-173° (from benzene) was 3.3 g (82%). Found %: C 64.38; H 4.70; N 9.42. $C_8H_7NO_2$. Calculated %: C 64.42; H 4.66; N 9.39. UV spectrum in 0.1 N KOH, λ_{max}, nm ($\varepsilon \cdot 10^{-3}$): 271 (20.4); 321 (9.9). IR spectrum, cm^{-1}: 1770 (C=O); 1146 and 1194 (C−O); 1630 (ring C=C).

2-Methyl-4-aminomethyl-5-carbethoxypyridine Dihydrochloride (IV). A solution of 3.8 g (0.02 mole) of III [8] in 50 ml of alcohol and 25 ml of 2 N HCl was hydrogenated for 4 h at room temperature and atmospheric pressure over 2 g of Pd/C. The catalyst was filtered and washed with water, and the combined filtrates were evaporated to dryness. The resulting oil was stored in a desiccator over alkali until it crystallized to give 4 g (80%) of a product with mp 220-222° (from absolute alcohol). Found %: C 45.01; H 5.99; Cl 26.72. $C_{10}H_{14}N_2O_2 \cdot 2HCl$. Calculated %: C 44.94; H 6.02; Cl 26.55.

6-Methyl-4-aminomethylnicotinic Acid Lactam (V). A solution of 3.6 g of IV in the minimum volume of water was treated with 30% NaOH (with cooling) to pH 12, and the precipitate was filtered and washed with water to give 2 g (90%) of product with mp 222-223° (from acetone). Found %: C 64.30; H 5.42. $C_8H_8N_2O$. Calculated %: C 64.85; H 5.45. UV spectrum in 0.1 N KOH, λ_{max}, nm ($\varepsilon \cdot 10^{-3}$): 263 (3.6), 272 (3.0). PMR spectrum (in 2 N NaOD): $2CH_3$ 2.43 ppm; $4CH_2$ 5.1 ppm; 3H 7.17 ppm; 6H 8.41 ppm.

2-Chloro-3-cyano-4-methoxymethyl-6-methylpyridine (VII). Phosphorus pentachloride [13.7 g (0.066 mole)] was added with cooling and stirring to a suspension of 11.8 g (0.066 mole) of VI in 75 ml of chlorobenzene. The reaction mass was refluxed with stirring for 2.5 h, evaporated, and the residue was treated with 20 ml of absolute alcohol. The solution was filtered and evaporated, and the residue was extracted with petroleum ether to give 11.3 g (87%) of a product with mp 56-58° (from petroleum ether). Found %: C 55.12; H 4.81; N 14.14. $C_9H_9ClN_2O$. Calculated %: C 54.98; H 4.61; N 14.25.

2-Methyl-4-methoxymethyl-5-aminomethylpyridine Dihydrochloride (VIII). Compound VII (4 g) in 150 ml of water and 6 ml of concentrated hydrochloric acid was hydrogenated for 7 h at room temperature and atmospheric pressure over 1 g of 10% Pd/C. The catalyst was filtered and washed with water, and the combined filtrates were evaporated to dryness to give 4.1 g (85%) of the dihydrochloride with mp 153-155° (from alcohol–ether). Found %: C 45.28; H 6.81; Cl 29.77. $C_9H_{14}N_2O \cdot 2HCl$. Calculated %: C 45.19; H 6.74; Cl 29.63.

3-Deoxypyridoxamine Phosphate (XI). A solution of 2.3 g (9.6 mmole) of VIII in 75 ml of 42% HBr was refluxed for 2 h and evaporated. The residue in 65 ml of water and 3.5 ml of concentrated HCl was diazotized at 80° for 3.5 h with a solution of 0.75 g of $NaNO_2$ in 10 ml of water. The reaction mass was evaporated, and the residue was dissolved in 150 ml of 25% ammonium hydroxide and held at room temperature for 48 h. The excess ammonia was removed by refluxing for 2 h, the mixture was evaporated to dryness, and 10 ml of concentrated hydrochloric acid was added to the residue. The excess hydrochloric acid was removed by distillation with water. The mixture of amines was separated on Dowex 50 B × 4 (H^+ form) by elution with 5% ammonium hydroxide to give 1.7 g of a mixture of amines. A total of 1 g of a mixture of the hydrochlorides of the amines was heated at 60-70° for 4 h in polyphosphoric acid (obtained from 5 g of phosphoric acid and 3.8 g of phosphoric anhydride). The mixture was cooled, 25 ml of absolute alcohol was added, the mixture was stirred, 80 ml of dry ether was added, and the mixture was allowed to stand for 30 min in a refrigerator. The solvent was decanted, the residue was heated for 20 min on a boiling-water bath with 45 ml of 1 N HCl, evaporated to about 5 ml, and chromatographed on Amberlite CG-50 (H^+ form with a 2.5 by 60-cm column) by elution with water. The eluate was evaporated to 15 ml at 35-40° and lyophilized to give 0.2 g (18%) of the phosphate. Found %: C 35.77; H 6.28; P 11.39. $C_8H_{13}N_2O_4P \cdot 2H_2O$. Calculated %: C 35.83; H 6.39; P 11.55.

3-Deoxypyridoxal Phosphate (XII). Sodium glyoxylate (192 mg) was added to a solution of 100 mg (0.4 mmole) of XI in 3 ml of water and 0.4 ml of 2 N NaOH, the mixture was stirred at room temperature for 10 min, glacial acetic acid was added to pH 5, and the mixture was stirred for 10 min under an inert gas. After 10 min, 3.2 ml of 0.25 M cupric acetate was added, and the mixture was stirred for 30 min under an inert gas. The green precipitate formed was dissolved by the addition of 2 N HCl, and the mixture was chromatographed on Dowes 50 B × 4 (H^+ form, 1.4 × 30-cm column) by elution with water. The eluate was vacuum evaporated to ~10 ml at 35-40° and lyophilized to obtain 15 mg (20%) of XII. Found %: C 38.48; H 4.83; P 12.47. $C_8H_{10}NO_5P \cdot H_2O$. Calculated %: C 38.56; H 4.85; P 12.43.

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