BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 41 161—165 (1968)

A New Synthesis of Pyridoxal-5-phosphate

Masaru IWANAMI, Toshiko NUMATA and Masuo MURAKAMI

Central Research Laboratory, Yamanouchi Pharmaceutical Co., Ltd., Azusawa-cho, Itabashi-ku, Tokyo

(Received June 5, 1967)

By the hydrolysis of pyridoxal-5-phosphate Schiff bases, pyridoxal-5-phosphate was prepared in a crystalline form without any further purification. Pyridoxal-5-phosphate Schiff bases were prepared in good yields by the phosphorylation of pyridoxal Schiff bases and were easily purified by alkali-acid reprecipitation. The pyridoxal-5-phosphate thus obtained was demonstrated to be identical with an authentic sample by a study of its UV, IR, and NMR spectra.

It is generally accepted that the biological activity of vitamin B_6 is due to the participation of a phosphorylated derivative, pyridoxal-5-phosphate[1,2] (I). The synthesis of I has been reported by many investigators. Peterson and Sober first obtained I in a crystalline form by the oxidation of pyridoxamine phosphate and by purification through chromatography on cation-exchange resin.[3] The Hoffman La Rosche Co. improved this method and obtained I by the transamination of pyridoxamine phosphate with pyruvic acid in the presence of a metal ion and by purification

1) W. W. Umbreit and I. C. Gunsalus, *J. Biol. Chem.*, **179**, 279 (1949).
2) D. Heyl, E. Jus, S. A. Harris and K. Folkers, *J. Am. Chem. Soc.*, **73**, 3430 (1951).
3) E. A. Peterson and H. A. Sober, *ibid.*, **76**, 169 (1949).

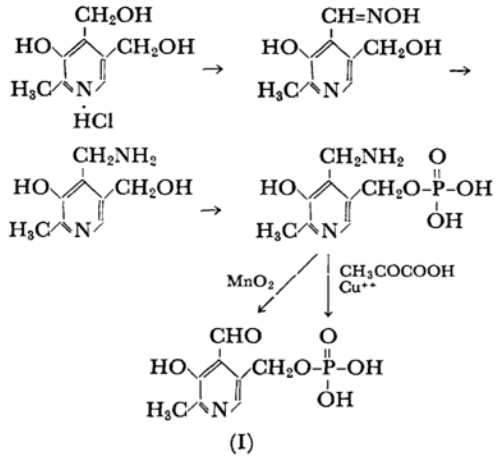


Fig. 1. Synthesis of pyridoxal-5-phosphate from pyridoxamine.

through chromatography on cation-exchange resin,[4] as shown in Fig. 1. In the present study it was established that I can be successfully prepared by the phosphorylation of pyridoxal Schiff bases followed by the hydrolysis of the phosphorylated products.[5]

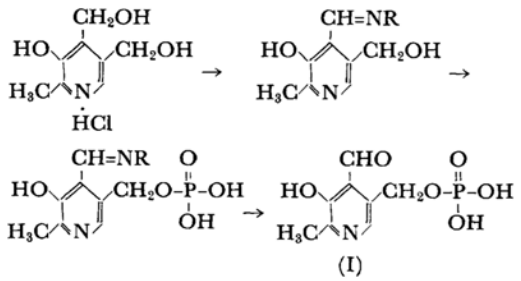


Fig. 2. A new synthesis of pyridoxal-5-phosphate from pyridoxal Schiff bases.

There have been few investigations concerning the synthesis of pyridoxal Schiff bases,[6] which were prepared in this paper from the three different substances described below. Pyridoxal (II) was used at first as the starting substance.[6] Pyridoxal Schiff bases were prepared from II by reaction with amines in methanol. Secondly, a pyridoxal sodium bisulfite addition compound (III), which could be obtained more easily than II from pyridoxine hydrochloride (IV), was used. Various pyridoxal Schiff bases were synthesized from III in yields varying from 30 to 95%, as Fig. 3 shows. Further, a more convenient method for the preparation of pyridoxal Schiff bases was established by the manganese dioxide oxidation of IV, followed by subsequent condensation with amines.

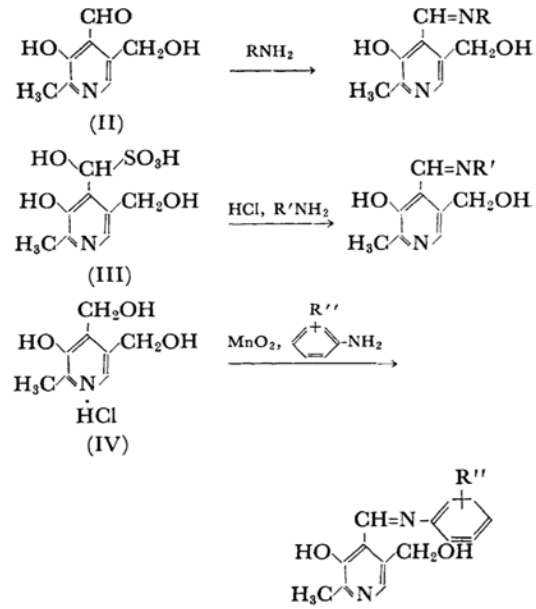


Fig. 3. Synthesis of pyridoxal Schiff bases.

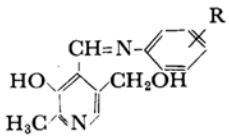
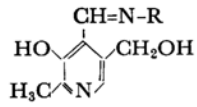
This procedure is best suited to the synthesis of aromatic Schiff bases, since they are insoluble in water and therefore can be isolated as precipitates from the reaction mixture. For example, pyridoxal *p*-toluidine and pyridoxal *o*-phenetidine Schiff bases were obtained in good yields (80—85%) by this procedure.

Because of their instability, especially under acidic conditions, the Schiff bases have generally been considered to be unsuitable for the protection of the aldehyde group. Aromatic pyridoxal Schiff bases are unstable compared with pyridoxamine, which has been used for the starting material of I in a previous method.[3,4] On the other hand, it was found that pyridoxal Schiff bases were stable in a nonaqueous solution, and a mixture of 85% phosphoric acid and phosphorus pentoxide (1.1 : 1) was used for the phosphorylation of pyridoxal Schiff bases. In the case of pyridoxamine, the phosphorylation could be carried out at 60°C without decomposition, but aromatic pyridoxal Schiff bases decomposed almost completely at the same temperature and the yields of I were very low. However, aromatic pyridoxal Schiff bases gave I in good yields when the temperature for phosphorylation was fixed at 40—50°C, while the phosphorylation of aliphatic pyridoxal Schiff bases was effected at higher temperatures, such as at 60°C for 18 hr. The yields of I from various pyridoxal Schiff bases are shown in Table 1.

It can be noticed that aromatic pyridoxal Schiff bases react more rapidly than aliphatic Schiff bases with the phosphorylating agent, and that the presence of such an electron-repulsive substituent as a methoxy, ethoxy, or methyl group in the benzene rings of aromatic pyridoxal Schiff bases

4) R. F. Long, U. S. Pat. 2755284 (1956).
5) M. Murakami, M. Iwanami and T. Numata, Belgium Pat. 651001.
6) D. H. Ellen Luz and S. A. Harris, *J. Am. Chem. Soc.*, **70**, 3669 (1948).

TABLE 1. THE YIELD OF PLP* FROM PL** SCHIFF BASES (ISOLATION THROUGH CATION-EXCHANGE RESIN)

PL aromatic Schiff bases[a] 		PL aliphatic Schiff bases[b] 	
R	Yield of PLP(%)	R	Yield of PLP(%)
p-Cl	40.3	CH_3	68.2
H	60.5	*n*-C_4H_9	67.0
o-CH_3	75.0	*t*-C_4H_9	61.5
p-CH_3	90.0	*n*-C_6H_{13}	65.6
m-CH_3	85.3	$-CH_2-C_6H_5$	72.5
o-OCH_3	87.9		
p-OCH_3	87.9	$-CH_2-CH_2-C_6H_5$	43.4
o-OC_2H_5	85.0		
p-OC_2H_5	92.0		

* Pyridoxal-5-phosphate ** Pyridoxal
a) Reaction condition: 45°C, 6 hr
b) Reaction condition: 60°C, 18 hr

increases the yields of the phosphorylated products.

Pyridoxal-5-phosphate was isolated in a crystalline form from pyridoxal phosphate Schiff bases obtained by the above-mentioned procedure by a simple treatment. Pyridoxal phosphate Schiff bases were precipitated from the reaction mixture by adjusting the pH to 3—4 and were purified by alkali-acid reprecipitation. Purified pyridoxal phosphate Schiff bases were converted to pure I and amines simply by making the solution strongly alkaline by the addition of sodium hydroxide at room temperature. After the amines had been extracted with organic solvents, I remained as its sodium salt in the water layer, I was isolated in a crystalline form by the adjusting pH to 3—4 by the addition of hydrochloric acid. All of the earlier methods for preparation of I required chromatography on cation-exchange resin and the condensation of the dilute eluate up to the crystalline form, the present method is unique in that it does not involve such a complicated procedure. In the case of *p*-toluidine and *p*-phenetidine Schiff bases, phosphorylated products were obtained in more than 90% yields.

Experimental

Pyridoxal Methylamine Schiff Base. To an aqueous solution of 1.5 g of II, 3 m*l* of 30% methylamine were added. The reaction mixture was then neutralized to pH 7 with a sodium carbonate solution, and extracted twice with 30 m*l* portions of chloroform. The evaporation of the solvent gave a crude pyridoxal methylamine Schiff base, which was then recrystallized from *n*-hexane, mp 148—150°C. Pyridoxal *n*-hexylamine and *n*-butylamine Schiff base were prepared similarly. (Table 2).

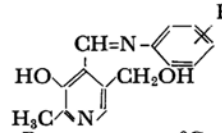
Pyridoxal *t*-Butylamine Schiff Base. To a mixture of 1.0 g of II and 0.36 g of *t*-butylamine in 3 m*l* of absolute ethanol, alcoholic potassium hydroxide was added and the solution was heated to reflux for 2 hr. After cooling, the evaporation of the solvent gave an oily residue which was taken up in ether. The evaporation of the ether gave a crude *t*-butylamine Schiff base which was recrystallized from benzene, mp 56—57°C.

Pyridoxal Schiff Base from III (General Procedure). A solution of 3.0 g of III in 10 m*l* of 25% hydrochloric acid was heated at 60°C for 10 min under a nitrogen atmosphere. After cooling, amine was added to the solution. The pyridoxal Schiff base was precipitated from the solution by neutralization with a potassium carbonate solution.

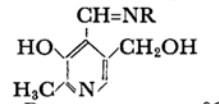
Pyridoxal' *p*-Toluidine Schiff Base from Pyridoxine Hydrochloride. To an aqueous solution of 10.0 g of pyridoxine hydrochloride, manganese dioxide which had been prepared from 8.0 g of potassium permanganate, 9.5 g of sodium bisulfite, and 10.0 g of 50% sulfuric acid were added. After the solution had then been stirred for 4—5 hr, 7.8 g of *p*-toluidine were added. The pyridoxal *p*-toluidine Schiff base was precipitated from the solution, mp 274—275°C. Yield 10.2 g (82%)

The pyridoxal *o*-phenetidine Schiff base, the pyridoxal

TABLE 2. PYRIDOXAL SCHIFF BASES

PL* aromatic Schiff bases 

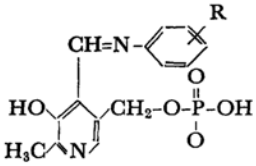
R	mp, °C	Analysis N% Found	Calcd
p-Cl	231	10.20	10.12
o-CH_3	191—192	10.60	10.90
p-CH_3	191—192	11.20	10.90
m-CH_3	188—189	10.69	10.90
H**	178—179	11.47	11.58
o-OCH_3	194—195	10.25	10.30
p-OCH_3	215—216	10.49	10.30
o-OC_2H_5	209—210	10.00	9.78
p-OC_2H_5	200—201	9.48	9.78

PL* aliphatic Schiff bases 

R	mp, °C	Analysis N% Found	Calcd
n-C_4H_9	72— 74	12.77	12.60
t-C_4H_9	56— 57	12.31	12.60
n-C_6H_{13}	83— 84	11.29	11.18
$-CH_2-C_6H_5$ **	113—114	10.85	10.94
$-C_2H_4-C_6H_5$ **	103—104	10.70	10.36

* Pyridoxal.
** Lit. *J. Am. Chem. Soc.*, **70**, 3669 (1948).

TABLE 3. PYRIDOXAL PHOSPHATE SCHIFF BASES



R	Formula	Analysis, % Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	Yield %
p-Cl	$C_{14}H_{14}N_2O_4P\,Cl$	47.10	3.96	7.85	46.91	4.01	7.65	35.0
H	$C_{14}H_{15}N_2O_4P$	52.17	4.65	8.70	51.90	4.82	8.43	68.0
p-CH_3	$C_{15}H_{17}N_2O_4P$	53.57	5.06	8.34	53.23	5.29	8.19	82.0
o-OCH_3	$C_{15}H_{17}N_2O_5P$	51.20	4.86	7.94	50.17	4.94	7.62	78.5
p-OC_2H_5	$C_{16}H_{19}N_2O_5P$	52.46	5.19	7.65	52.02	5.19	7.38	92.0
o-OC_2H_5	$C_{16}H_{19}N_2O_5P$	52.46	5.19	7.65	52.30	5.29	7.75	77.0

p-anisidine Schiff base, *etc.*, were prepared similarly (Table 2).

Pyridoxal Phosphate Schiff Base (General Procedure). To a mixture of 5.0 g of phosphorus pentoxide and 6.5 g of 85% phosphoric acid, 1.0 g of the pyridoxal Schiff base was added. The reaction mixture was then heated at 45°C for 6 hr. After cooling, 5 m*l* of 0.1 N hydrochloric acid was added and the solution was heated at 60°C for 10 min. The reaction mixture was then neutralized with a 50% sodium hydroxide solution. The pyridoxal phosphate Schiff base was precipitated from the solution. The pyridoxal phosphate Schiff bases thus obtained are summarized in Table 3.

Isolation of Pyridoxal-5-phosphate through Chromatography on Cation-exchange Resin (General Procedure) in the Case of Aliphatic Pyridoxal Schiff Bases. To a mixture of 5.0 g of phosphorus pentoxide and 6.5 g of 85% phosphoric acid, 1.0 g of pyridoxal Schiff base was dissolved. The mixture was then heated at 60°C for 18 hr. The resulting solution, after the addition of 5 m*l* of 0.1 N hydrochloric acid, was again heated at 60°C for 10 min and then carefully applied to a 70 m*l* Amberlite IR-120 (acid form) column and eluted with water. The eluate was collected in 10—20 m*l* fractions. As the ultraviolet absorption spectra of pyridoxal-5-phosphate have a maximum at 390 mμ in a neutral or alkaline solution, the absorption spectra of each fraction were measured at 320 and 390 mμ, and those in which $E390\,m\mu/E320\,m\mu = 2.4 \pm 0.1$ were mixed. The first fraction, containing inorganic acid, was subjected to the column again. All the fractions containing pure pyridoxal-5-phosphate were combined and evaporated under reduced pressure below 40°C. A representative elution curve is presented in Fig. 4.

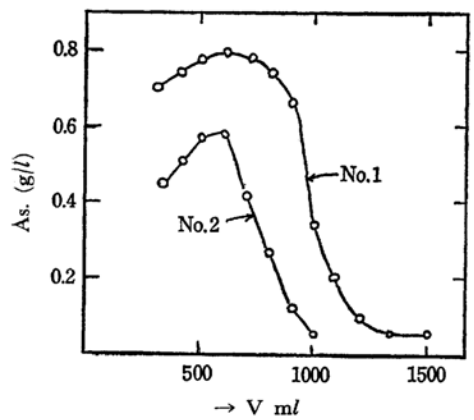


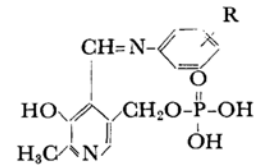
Fig. 4. A elution curve of pyridoxal-5-phosphate.

The curve No. 1 shows the elution pattern of pyridoxal-5-phosphate, while the curve No. 2 shows the elution pattern recovered from the acid part. The yields of pyridoxal-5-phosphate have been summarized in Table 1.

In the Case of Aromatic Pyridoxal Schiff Bases. In a mixture of 5.0 g of phosphorus pentoxide and 6.5 g of 85% phosphoric acid, 1.0 g of the pyridoxal Schiff base was dissolved. The solution was heated at 45°C for 4 hr, and to this 0.1 N hydrochloric acid was added after cooling. The resulting solution was heated again at 60°C for 10 min, carefully subjected to the column after cooling, and eluted with water. Pyridoxal-5-phosphate was obtained from the eluate by the method described above. The yields of pyridoxal-5-phosphate have been summarized in Table 1.

Isolation of Pyridoxal-5-phosphate from Pyridoxal Phosphate Schiff Base (General Procedure). To a suspension of 2.0 g of the pyridoxal phosphate

TABLE 4. THE YIELD OF PLP* FROM PLP* SCHIFF BASES



R	Yield of PLP %
p-Cl	63.0
H	70.2
o-CH_3	61.0
p-CH_3	74.7
m-CH_3	61.0
o-OC_2H_5	76.0
p-OC_2H_5	71.0

* Pyridoxal-5-phosphate

TABLE 5. UV SPECTRA DATA OF PLP

Solvent	Max. mμ	E_M	E_M (Lit.)
0.1 N HCl	295	6750	6700
pH 6.9 Buffer soln.	330 388	2500 4920	2500 4900
0.1 N NaOH	305 388	1100 6700	1100 6600

Schiff base in 5 m*l* of water, 10 m*l* of a 2 N sodium hydroxide solution was added. The reaction mixture was extracted twice with 20 m*l* portions of ether. To the water layer, 10 m*l* of 2 N hydrochloric acid was added. The solution was then cooled at 0—5°C overnight. Pure pyridoxal-5-phosphate was thus obtained in a crystalline form from the solution.

The yields of pyridoxal-5-phosphate are summarized in Table 4.

The pyridoxal-5-phosphate thus obtained was demonstrated to be identical with an authentic sample by a study of its UV, IR, and NMR spectra. The ultraviolet spectra data are summarized in Table 5.

The authors wish to express their gratitude to the staff of the analysis laboratory for their elementary analysis and infrared and ultraviolet spectra measurements