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A Novel Synthesis of Pyridoxal-5′-phosphate

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Pyridoxal-5′-phosphate was synthesized in excellent yield by phosphorylation of 1-secondaryamino-1,3-dihydro-7-hydroxy-6-methyl-furo(3,4-c)pyridine which was readily obtained by a condensation reaction between pyridoxal and a secondary amine.

Pyridoxal-5′-phosphate (**1**) has been known as a coenzyme of decarboxylase and many other enzymes.[1] Since the first success of synthesis of **1** by Gunsalus *et al.*[2] in 1944, many studies on the synthesis have been reported and these were reviewed by Harris.[3] The present paper deals with a new and simple synthesis of **1** as shown in Fig. 1 and Table I. Pyridoxal (**2**) condensed with a given secondary amine to yield the intermediate (**3**), of which **1** was obtained by phosphorylation in a high yield.

The intermediates **3**a-e were generally obtained as follows. Pyridoxal was dissolved in a secondary amine with or without a solvent. After removal of the solvent, the excess of amine and water resulting from condensation, **3** was obtained as a slightly yellowish crystal and it was further purified by recrystallization from benzene-hexane mixture.

The structure of **3** was determined by IR, PMR, MS spectra and elementary analysis. The IR spectrum showed a broad absorption band from 2400 to 2900 cm^{-1} by internal hydrogen bond between the phenolic proton and 1-substituted nitrogen, and no absorption was observed for carbonyl groups. The PMR spectrum showed only one D_2O exchangeable proton. A long range coupling was reported for a related compound of **3** without making any references to the position of coupling.[4] A spin-spin coupling between methine (C–1 position) and methylene (C–3) protons was demonstrated for **3** by spin decoupling procedure in which each proton signal was sharpened by irradiating the other.

The reaction between **2** and a secondary amine was markedly infuenced by basicity of the amine but not by steric factors. An aliphatic or alicyclic amine (even diisopropylamine) condensed readily with **2** to form **3** in benzene or dioxane solution. Whereas, weakly basic N-methylaniline which could yield **3**e in N,N-dimethylformamide, did not yield **3**e in either solution. The very weakly basic diphenylamine, however, did not react with **2** in any solution. Since **3** was hydrolyzed by water alone, the condensation reaction was reversible. Amine exchange reaction between **3** and another secondary amine took place without any catalyst.

The transformation of **3** to **1** by phosphorylation was described bellow. Any of **3** was stirred with excess of polyphosphoric acid under the condition shown in Table I. The reaction mixture was hydrolyzed by the addition of water and by keeping at 55°C for half an hour with stirring. After cooling it to r.t., the solution was neutralized with aq. sodium hydroxide and filled up to a volume with distilled water. The quantitative determination of **1** in the solution was made by the known method.[5] The results were summarized in Table I.

About 72% of total amount of **1** was recovered as a crystalline form from the solution by a usual procedure (absorption on activated charcoal[6] and purification by ion-exchange

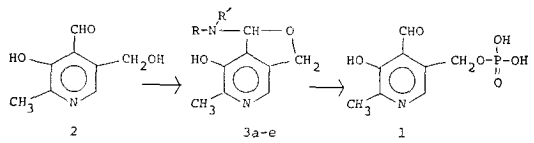

FIG. 1.

TABLE I. RESULTS OF SYNTHESIS OF **1**

Secondary amine	Yield of **3**	Condition of phosphorylation	Yield of **1**
Morpholine (3a)	88%	95°C 1 hr	97%
Piperidine (3b)	86%	95°C 1 hr	99%
Diethylamine (3c)	94%	70°C 3 hr	99%
Diisopropylamine (3d)	70%	70°C 3 hr	32%
N-Methylaniline (3e)	64%	70°C 3 hr	75%

column chromatography).[7]

By the present procedure, **1** was easily synthesized in a excellent yield.

EXPERIMENTAL

All mp are uncorrected. IR spectra refer to Nujol mull. PMR spectra are recorded at 60 MHz with TMS as an internal standard.

1,3-Dihydro-7-hydroxy-6-methyl-1-morpholino-furo (3,4-c) pyridine (**3a**)

After 1.0 g of **2** was dissolved in 5 ml of morpholine by warming in a hot water bath, the excess of morpholine and water formed was evapolated under reduced pressure. The slightly yellowish crystalline residue was recrystallized from benzene-hexane to obtain **3a** as a colorless needle. mp 166°C. IR ν_{max} cm^{-1}: 2500, 1581, 1212. MS *m/e*: 236 (M^+), 150, 122. PMR δ^{CDCl_3}: 2.57 (s, 3H) 2.75 (m, 4H) 3.75 (t, 4H, J=5.0 Hz) 5.12 (d, 2H, J=2.1 Hz) 5.97 (t, 1H, J=2.1 Hz) 7.0 (broad s, 1H) 8.05 (s, 1H). *Anal.* Found: C, 61.12; H, 6.82; N, 11.83. Calcd. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.82; N, 11.86%.

1,3-Dihydro-7-hydroxy-6-methyl-1-piperidino-furo (3,4-c) pyridine (**3b**)

The same procedure for **3a** was applied for **3b** preparation. mp 142°C. IR ν_{max} cm^{-1}: 2470, 1587, 1308. PMR δ^{CDCl_3}: 1.55 (m, 6H) 2.58 (s, 3H) 2.72 (m, 4H) 5.20 (d, 2H, J=2.1 Hz) 6.01 (t, 1H, J=2.1 Hz) 6.7 (broad s, 1H) 8.01 (s, 1H). *Anal.* Found: C, 66.64; H, 7.74; N, 11.96. Calcd. for $C_{13}H_{16}N_2O_2$: C, 66.90; H, 7.73; N, 11.95%.

1-Diethylamino-1, 3-dihydro-7-hydroxy-6-methyl-furo (3, 4-c) pyridine (**3c**)

To the mixture of 2 ml of diethylamine and 20 ml of benzene were added 1.0 g of **2** and 0.5 g of CaH_2. The reaction mixture was then stirred at r.t. for 18 hr. After removal of CaH_2 by filtration, the same procedure for **3a** was applied. mp 85°C. IR ν_{max} cm^{-1}: 2600, 1301, 1280. PMR δ^{CDCl_3}: 1.16 (t, 6H, J=7.0 Hz) 2.80 (q, 4H, J=7.0 Hz) 2.59 (s, 3H) 5.10 (d, 2H, J=2.1 Hz) 6.21 (t, 1H, J=2.1 Hz) 7.0 (broad s, 1H) 8.01 (s, 1H). *Anal.* Found: C, 64.84; H, 8.16; N, 12.60. Calcd. for $C_{12}H_{18}N_2O_2$: C, 64.80; H, 8.12; N, 12.55%.

1-Diisopropylamino-1,3-dihydro-7-hydroxy-6-methyl-furo (3,4-c) piridine (**3d**)

This was obtained by the same procedure for **3c** except 1.0 g of anhydrous K_2CO_3 was used instead of CaH_2. mp 128°C. IR ν_{max} cm^{-1}: 2600, 1300, 1280. PMR δ^{CDCl_3}: 1.16 (d, 6H, J=7.0 Hz) 2.46 (s, 3H) 3.13 (septet, 1H, J=7.0 Hz) 4.95 (d, 2H, J=2.1 Hz) 6.20 (t, 1H, J=2.1 Hz) 7.89 (s, 1H) 8.0 (broad s, 1H). *Anal.* Found: C, 67.17; H, 8.86; N, 11.19. Calcd. for $C_{14}H_{22}N_2O_2$: C, 67.07; H, 8.79; N, 11.22%.

1,3-Dihydro-7-hydroxy-6-methyl-1-(N-methylanilino)-furo(3,4-c)pyridine (**3e**)

After 1.0 g of **2** was dissolved in the mixture of 2 ml of N-methylaniline and 5 ml of DMF, the same procedure for **3a** was applicable to obtain **3e**. mp 181°C. IR ν_{max} cm^{-1}: 2560, 1600, 1306. PMR δ^{CDCl_3}: 2.54 (s, 3H) 2.69 (s, 3H) 5.07 (d, 2H, J=2.1 Hz) 5.5 (broad s, 1H) 6.60 (t, 1H, J=2.1 Hz) 7.23 (s, 5H) 8.00 (s, 1H). *Anal.* Found: C, 69.66; H, 6.99; N, 10.74. Calcd. for $C_{15}H_{16}N_2O_2$: C, 69.74; H, 7.02; N, 10.85%.

3a *by transamination*

To 5 ml of morpholine was added 1.11 g of **3c**. The reaction mixture was stirred at 50°C for 2 hr, and then concentrated *in vacuo*. The residue was crystallized from benzene-hexane to yield 1.06 g of pure **3a**.

General phosphorylation procedure for all of **3**

Ten mmole of **3** and 24 g of polyphosphoric acid (Wako Pure Chem., P_2O_5/85%H_3PO_4=about 1.4) were stirred together according to the condition in Table I. To the reaction mixture was added 24 ml of water and the stirring was continued for another 30 min at 55°C. The solution was adjusted to pH 7 with aq. NaOH and diluted accurately to 50 ml with distilled water. The quantitative determination of **1** for the diluted solution was achieved by essentially the same procedure of Oike *et al.*[5]

Isolation of **1**

To the unneutrallized solution prepared from 4.5 g of **3a** as described above was added 30 g of activated charcoal (Shirasagi). The solution was stirred for 2 hr at r.t. The charcoal was collected by suction and washed with water until pH of the washing became about 4. It was then washed with 0.5 M NaOH (300 ml)

and again with water. The eluent was concentrated to a small volume under the reduced pressure at a low temperature, and subjected to ion-exchanging column chromatography (Amberlyst IR 120 [H^+], water). The eluent was concentrated to about 25 ml and refrigerated overnight to obtain **1** as yellowish crystal (3.64 g, 72%). UV λ_{max} (0.1 N NaOH) nm(ε): 305 (955), 388 (6750). *Anal.* Found: C, 36.09; H, 4.60; N, 5.31. Calcd. for $C_8H_{10}NO_6P \cdot H_2O$: C, 36.00; H, 4.52; N, 5.28%.

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