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SYNTHESIS OF 2-NORPYRIDOXAL-5'-PHOSPHATE

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Pyridoxal-5'-phosphate (PLP) plays an important role as the cofactor of many enzymes of amino acid metabolism.

With the aim of studying the nature of bonds between the coenzyme and apoenzymes, a synthesis of the analog of PLP lacking the methyl group in position "2" of the pyridine cycle, 2-norpyridoxal-5'-phosphate, was developed.

The basic intermediate, 2-norpyridoxine, was prepared by a procedure analogous to the recently reported one for pyridoxine itself (1).

The refluxing of N-formylglycine ethyl ester with 2 equivalents of P_2O_5 in dry chloroform gave 5-ethoxyoxazole (I) in 16% yield, b.p. 74° at 35 mm. I was heated with a twofold excess of dimethyl maleate (II) for 2 hrs at 110°, cooled and dry HCl in absolute methanol was added. The hydrochloride of dimethyl 3-hydroxycinchomerate (hydrochloride III) was isolated in 43,5% yield, m.p. 200-201° (from MeOH); $\lambda_{max.}^{MeOH}$ 302 mμ (ε 4900). [lit. (2): m.p. 195-197°; $\lambda_{max.}$ 302 mμ (ε 3200)].

The free base III was obtained in 95.9% yield by treating hydrochloride III with an equimolar amount of triethylamine.

A solution of III (33 mmole) in tetrahydrofurane was refluxed

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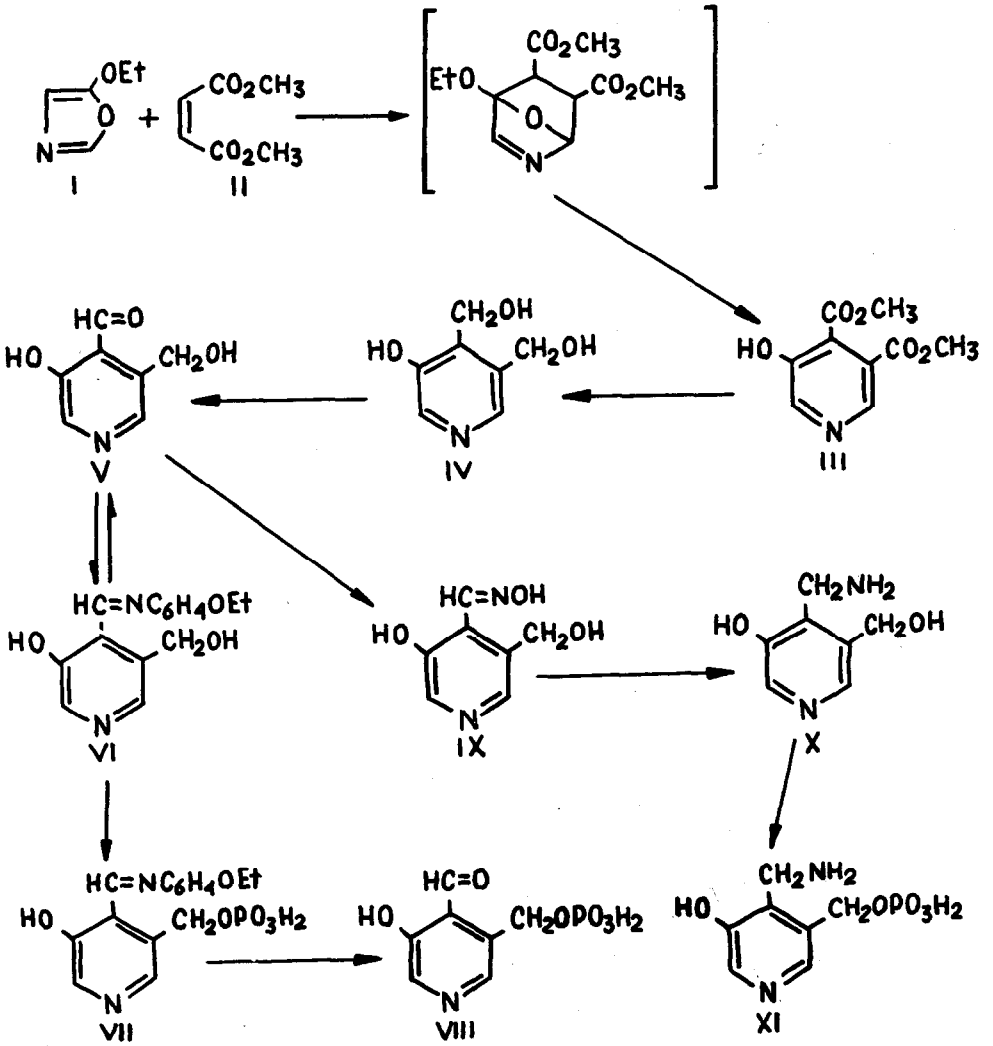
with $LiAlH_4$ (83 mmole) for 6 hrs and kept at room temperature overnight. Excess $LiAlH_4$ was decomposed with water and the hydrochloride of 2-norpyridoxine (hydrochloride IV) was obtained by conventional procedure in 71% yield. M.p. 125-127° (from ether-EtOH-HCl); $\lambda_{max.}^{MeOH}$ 289 mμ (ε 5700). [lit. (2): m.p. 124-126°; $\lambda_{max.}$ 289 mμ (ε 5600)].



The hydrochloride IV was oxidized at the 4-hydroxymethyl side chain with the equivalent amount of MnO_2 "B" (3) in 0.3 M H_2SO_4 at room temperature. 2-Norpyridoxal (V) was isolated from reaction mixture either in the form of its oxime (IX) on addition of hydroxylamine hydrochloride and sodium acetate, or of the Schiff base (VI) on treatment with p-phenetidine and sodium acetate. These derivates were obtained in 75 and 79% yields, respectively; m.p. of VI 192-193° dec. (from EtOH); m.p. of IX 201-203° dec. (from water).

A solution of VI (300 mg) in 5 ml of 1 N HCl was applied to the top of a 40 x 1.4 cm column of Dowex 50W x 4 in acid form, equilibrated with 1 N HCl. It was eluted with 1 N HCl at a rate of 20 ml/hr. Evaporation in vacuo of the fractions containing 2-norpyridoxal (V) (measured by its absorption at 295 mμ), yielded 95.5% of hydrochloride V, m.p. 144-147° dec.

Hydrogenation of the oxime IX in presence of 5% palladium on charcoal gave the dihydrochloride of 2-norpyridoxamine (dihydrochloride X). M.p. 165-169° dec. (from ether-EtOH).

A solution of VI (1.35 mmole) in 7.73 g of a mixture (1.3 : 1) of 85% H_3PO_4 and P_2O_5 was heated at 45° for 6 hrs, 1.4 ml of 0.1 N HCl was added and the resulting syrup was heated again at 60° for 15 minutes. The mixture was brought to pH 3 by addition of 30% NaOH solution. Centrifugation gave 91% of the 5'-phosphoric ester of VI (VII). The latter was dissolved in 2.2 ml of 0.1 N NaOH and p-phenetidine was extracted with ether. To the water layer 2.2 ml of 0.1 N HCl was added. The solution was applied to the top of a 40 x 1.4 cm column of Dowex 50W x 4 in acid form and was eluted with water at the rate of 20 ml/hr. The fractions containing 2-norpyridoxal-5'-phosphate (VIII) were concentrated in vacuo and lyophilized. Yield of VIII, 70%. The substance was homogeneous electrophoretically.

Anal. Calcd. for $C_7H_8NO_6P.H_2O$: C, 33.48; H, 4.02; N, 5.56; P, 12.33, Found: C, 33.22; H, 4.26; N, 5.35; P, 12.60.

Alternatively, the mixture obtained after phosphorilation of VI was directly applied to the column of Dowex 50W x 4 in the acid form and chromatographed as above. Yield of VIII, 73.7%.

The dihydrochloride X was phosphorylated under the conditions for preparation of pyridoxamine-5'-phosphate (4). It was heated with the H_3PO_4-P_2O_5 mixture at 60° for 2 hrs; pyrophosphates were hydrolyzed with 1 N HCl. The solution was neutralized with concentrated ammonia and separated on a column of Amberlite IRC-50. 2-Norpyridoxamine-5'-phosphate (XI) was obtained in 65.5% yield.

Anal. Calcd. for $C_7H_{11}N_2O_5P \cdot 2H_2O$: C, 31.12; H, 5.60; N, 10.36; P, 11.47. Found: C, 30.88; H, 5.69; N, 10.11; P, 11.79.

Ultraviolet spectra of the preparated substances are presented in Table I.

TABLE I

Ultraviolet Spectra of the Derivatives of 2-Norpyridoxal

Substance	$\lambda_{max.}$ mμ (ε)		
	0.1 N HCl	pH 7*	0.1 N KOH
2-Norpyridoxal (V)	283(6600)	249(4600)	241(8600)
		282(2100)	300(5000)
		314(4200)	390(600)
		390(80)	
2-Norpyridoxal-5'-phosphate (VIII)	292(6000)	383(3200)	305(1000)
			386(5600)
2-Norpyridoxamine (X)	292(6800)	251(2600)	243(7500)
		287(3000)	307(5900)
		324(3200)	
2-Norpyridoxamine-5'-phosphate (XI)	292(7100)	248(3700)	243(7300)
		288(3400)	307(5200)
		324(3600)	

* 0.1 N phosphate buffer.

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