VITAMIN B$_6$ ANALOGS

XVI.* SYNTHESIS AND PROPERTIES OF 2'- AND 5'-MODIFIED ANALOGS OF PYRIDOXAL 5-PHOSPHATE

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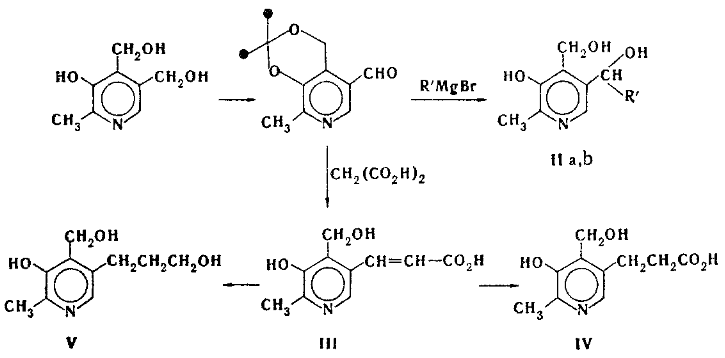
The synthesis of 2'- and 5'-modified analogs of pyridoxine from which analogs of pyridoxal and pyridoxal 5-phosphate were obtained is described. The transition to analogs of pyridoxamine and pyridoxamine 5-phosphate is realized by hydrogenation of the oximes of these aldehydes.

The synthesis of analogs of pyridoxal 5-phosphate and pyridoxamine 5-phosphate that are modified in the 2 and 5 positions of the pyridine ring was undertaken.

The synthesis of the 2-modified analogs (Ia, b) was carried out by a previously developed scheme [2].†

The analogs modified in the 5 position were obtained from pyridoxine:



IIa R′=CH$_3$; b R′=C$_6$H$_5$

According to the PMR spectral data, the product of the condensation of 3,4'-O-isopropylideneisopyridoxal with malonic acid–unsaturated acid V–has the trans configuration in relation to the double bond (the spin–spin coupling constant of the ethylene protons is 16 Hz).

The oxidation of the 4-hydroxymethyl group of the pyridoxine analogs to a formyl group was accomplished with manganese dioxide in sulfuric acid or [in the oxidation of III and IV to carboxy analogs of pyridoxal 5-phosphate (VI and VII)] with active manganese dioxide in a nonpolar solvent. The corresponding aldehydes were isolated as the oximes or Schiff bases with p-anisidine. Compounds VI and VII were isolated in satisfactory yields as the aldehydes, and retention of the trans configuration of starting unsaturated acid III during oxidation was confirmed for VII by means of PMR spectral data.

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*See [1] for communication XV.

†In the final stages of our research, a paper was published by Takuichi and Taisuke [3] in which the synthesis of these two pyridoxine analogs was described.

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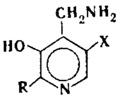
TABLE 1. Assignment of the Absorption Maxima to Ionic Forms in the UV Spectra of Vitamin B_6 Analogs

Compounds	λ_{max}, nm (ε)		
	cation (0.1 N HCl)	Dipolar form (pH 7)	anion (0.1 N NaOH)
Pyridoxine analogs:			
Ia	292 (9300)	253 (4700); 321 (7100)	248 (6500); 307 (7800)
Ib	294 (10400)	256 (4900); 323 (8400)	248 (6800); 309 (7900)
IIb	290 (11200)	256 (4900); 318 (9300)	248 (7000); 304 (7400)
Pyridoxamine analogs:			
VIIIa	299 (9800)	254 (4800); 330 (6700)	248 (6700); 311 (7900)
VIIIb	302 (10700)	252 (4700); 328 (7600)	249 (6100); 314 (9300)
VIIIc	296 (9600);	254 (5100); 322 (7400)	248 (6400); 312 (8200)
VIIId	296 (11500)	254 (4900); 326 (10400)	250 (5800); 310 (9000)
VIIIe	304 (8300)	255 (3100); 332 (6400)	249 (4700); 318 (6900)
VIIIf	295 (9500)	250 (4500); 325 (7500)	245 (6100); 306 (8000)
Pyridoramine 5-phosphate analogs:			
IXa	299 (10900)	254 (4900); 322 (11100)	248 (5700); 313 (7200)
IXb	298 (10500)	252 (4300); 331 (8200)	248 (6900); 314 (9500)
IXc	298 (10200)	254 (4500); 323 (9200)	248 (5800); 313 (7400)
IXd	295 (7400)	252 (2900); 324 (7200)	244 (4100); 308 (5600)
Pyridoxal 5-phosphate analogs:			
Xa	299 (9200) a 340 (1150) b	324 (3100) a 399 (2600) b	306 (2200) a 399 (7600) b
Xb	301 (7000) a 305 (1000) b	328 (1800) a 387 (1300) b	305 (1700) a 396 (4300) b
Xc	295 (8300) a 337 (1400) b	323 (3500) a 391 (4300) b	304 (2100) a 395 (5400) b
Xd	296 (7400) a 344 (2700) b	320 (4200) a 384 (3800) b	306 (1300) a 389 (7500) b
VII	294 (6400) a 352 (4600) b	321 (2700) a 395 (5800) b	303 (1100) a 393 (7200) b

[a] Hydrated form. [b] Aldehyde form. The numbers in parentheses are not the true molecular extinctions of the given forms but show the absorption at the equilibrium concentration.

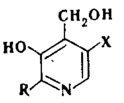


VIII

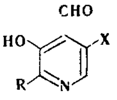
R=sec-C_4H_9, X=CH_2OH; b R=$CH_2C_6H_5$, X=CH_2OH; c R=CH_3, X=CH(OH)CH_3; d R=CH_3, X=CH(CH)C_6H_5; e R=CH_3, X=CH_2CH_2COOH; f R=CH_3, X=$CH_2CH_2CH_2OH$

Hydrogenation of the oximes leads to pyridoxamine analogs (VIIIa-d, f). However, the hydrogenation of 5'-desoxy-5'-carboxymethylenepyridoxal oxime is accompanied by reduction of the double bond and gives, as in the case of 5'-desoxy-5'-carboxymethylpyridoxal, amine VIIIe.

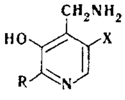
Phosphorylation of the Schiff bases and the pyridoxamine analogs with polyphosphoric acid (PPA) under the conditions that we described in [2] makes it possible to obtain analogs of pyridoxal phosphate and pyridoxamine phosphate:



Ia, b



VI, VII, X a-e



VIII a-f IX a-e

R= sec-C_4H_9; b R=$CH_2C_6H_5$; c—f R=CH_3; I X=CH_2OH; VI R=CH_3, X=CH_2CH_2OH; VII R=CH_3, X=$CHCHCO_2H$; VIIIa X=CH_2OH, b X=CH_2OH, c X=CH(OH)CH_3; d X=CH(OH)C_6H_5; e X=$CH_2CH_2CO_2H$, f X=$CH_2CH_2CH_2OH$; IX, Xa, X=$CH_2OPO_3H_2$, b X=CH_2OPO_3H, c X=CH(CH_3)OPO_3H_2, d X=$CH_2CH_2CH_2OPO_3H_2$

In the phosphorylation of 5'-phenylpyridoxamine (VIIId) and the Schiff base of 5'-phenylpyridoxal with polyphosphoric acid we were unable to isolate identifiable products; this, in our opinion, is associated with the high reactivity and lability of the pyridylphenylcarbinol grouping involved in this reaction.

TABLE 2. Derivatives of Pyridoxal Analogs

Compound	mp, °C	UV spectrum, λ_{max}, nm (ε)	Yield, %
2'-Isopropylpyridoxal oxime	203—206 (dec.)	278 (6020) and 329 (5400)^a	77
2'-Phenylpyridoxal oxime	175—176 (dec.)	280 (9160) and 330 (8710)^a	58
5'-Methylpyridoxal oxime	202—203 (dec.)	279 (6300) and 330 (5600)^a	64
5'-Phenylpyridoxal oxime	214—215 (dec.)	281 (9400) and 332 (9050)^a	23
5'-Desoxy-5'-β-hydroxyethylpyridoxal oxime	187—189 (dec.)	278 (6200) and 326 (4400)^a	68
Schiff bases with p-anisidine:			
2'-Isopropylpyridoxal	140—141	352 (13300) and 365 (13600)^b	72
2'-Phenylpyridoxal	152—153	356 (13800) and 366 (14300)^b	63
5'-Methylpyridoxal	173—174 (dec.)	354 (13400) and 366 (13600)^b	68
5'-Phenylpyridoxal	180—181 (dec.)	358 (14000) and 368 (14800)^b	31
5'-Desoxy-5'-β-hydroxyethylpyridoxal	149—151 (dec.)	230 (10500) and 300 (4500) ^{b} 351 (8000) and	74

^{a}In 0.1 N HCl. ^{b}In ethanol.

TABLE 3. Pyridoxamine Analogs

Dihydrochloride	mp, °C	Empirical formula	Found, %		Calculated, %	
			C	H	C	H
VIII a	228—230 (dec.)	$C_{11}H_{18}N_2O_2 \cdot 2HCl$	46,3	7,4	46,6	7,1
VIII b	193—195 (dec.)	$C_{14}H_{16}N_2O_2 \cdot 2HCl$	52,8	5,6	53,0	5,7
VIII c	184—186 (dec.)	$C_9H_{14}N_2O_2 \cdot 2HCl$	42,3	6,4	42,4	6,3
VIII d	204—206 (dec.)	$C_{14}H_{16}N_2O_2 \cdot 2HCl$	53,4	5,5	53,0	5,7
VIII e	195—197 (dec.)					
VIII f	191—193 (dec.)	$C_{10}H_{16}N_2O_2 \cdot 2HCl$	44,8	6,6	44,6	6,7

The assignment of the absorption maxima in the UV spectra of the compounds to ionic forms is presented in Table 1.

EXPERIMENTAL

The UV spectra were recorded with an ESP-3T spectrophotometer (Japan). The PMR spectra were recorded with an R-20 spectrometer (60 MHz, Japan) with hexamethyldisiloxane (HMDS) or tert-butyl alcohol (1.20 ppm) as the internal standard. Thin-layer chromatography was carried out as described in [4].

5-Ethoxyoxazoles. These compounds were obtained by cyclization of ethyl esters of N-formylamino acids under the conditions described in [2]. 4-sec-Butyl-5-ethoxyoxazole, with bp 62-64° (4 mm), was obtained in 59% yield from N-formylleucine ethyl ester. Found %: C 63.6; H 9.1. $C_9H_{15}O_2$. Calculated %: C 63.9; H 8.9.

4-Benzyl-5-ethoxyisoxazole, with bp 111-115° (3 mm), was obtained in 35% yield from N-formylphenylalanine ethyl ester. Found %: C 70.9; H 6.6. $C_{12}H_{13}O_2$. Calculated %: C 70.9; H 6.4.

Dimethyl Esters of 5-Hydroxycinchomeronic Acids. A mixture of 0.1 mole of 5-ethoxyoxazole and 0.2 mole of dimethyl maleate was heated at 110° for 6 h, after which it was cooled, and 20 ml of a 25% solution of dry HCl in methanol and 200 ml of ether were added to it successively. The mixture was stirred thoroughly and allowed to stand overnight in a refrigerator. The crystals were removed by filtration, washed with ether, and recrystallized from acetone.

6-sec-Butyl-5-hydroxycinchomeronic Acid Dimethyl Ester Hydrochloride. This compound, with mp 152-153°, was obtained in 51% yield. Found %: C 51.4; H 5.9. $C_{13}H_{17}NO_5 \cdot HCl$. Calculated %: C 51.4; H 6.0. PMR spectrum, δ, ppm (CF_3COOH*): 4- and 5-CO_2CH_3, 3.74 and 3.76 s; 6-H 8.20 s; 2-CH_2 2.77 d (J = 7.3 Hz); 2'-CH 1.98 m; 2"-CH_3, 0.66 d (J = 6.6 Hz).

Dimethyl 6-Benzyl-5-hydroxycinchomeronate Hydrochloride. This compound with mp 146-147° was obtained in 79% yield. Found %: C 57.2; H 4.7. $C_{16}H_{15}NO_5 \cdot HCl$. Calculated %: C 56.9; H 4.8. PMR spectrum, δ, ppm (CF_3COOH): 4- and 5-CO_2CH_3, 3.75 and 3.82 s; 6-H, 8.21 s; 2-CH_2, 4.29 s; 2'-C_6H_5, 7.12 s. The hydrochlorides were converted to the free bases by the action of sodium bicarbonate.

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*Here and subsequently, s is singlet, d is doublet, and m is multiplet.

TABLE 4. Pyridoxamine 5-Phosphate Analogs[a]

Dihydrate	Empirical formula	Found, %		Calculated, %		Yield, %
		C	H	C	H	
IXa	$C_{11}H_{19}N_2O_5P \cdot 2H_2O$	40,2	7,3	40,5	7,1	57
IXb	$C_{14}H_{17}N_2O_5P \cdot 2H_2O$	46,2	5,6	46,7	5,9	39
IXc	$C_9H_{15}N_2O_5P \cdot 2H_2O$	35.9	6,5	36,2	6,4	35
IXd	$C_{10}H_{17}N_2O_5P \cdot 2H_2O$	38,4	6,9	38,4	6,8	43

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[a] All of the compounds were chromatographically and electrophoretically homogeneous. [b] PMR spectrum, δ, ppm (2 N NaOD): 2-C_6H_5, 7.2 s; 2-CH_2, 4.12 s; 4-CH_2, 3.75 s; 5-CH_2, 4.05 d (J = 5.5 Hz); 6-H, 7.50 s.

TABLE 5. Pyridoxal 5-Phosphate Analogs[a]

Monohydrate	Phosphorylation formula	Empirical formula	Found, %		Calculated, %		Yield, %
			C	H	C	H	
Xa	60°, 4 h	$C_{11}H_{16}NO_6P \cdot H_2O$	42,6	5,7	43,0	5,9	72
Xb	60°, 6 h	$C_{14}H_{14}NO_6P \cdot H_2O$	48,9	4,5	49,3	4,7	38
Xc	45°, 6 h	$C_9H_{12}NO_6P \cdot H_2O$	38,3	5,2	38,7	5,0	47
Xd	45°, 6 h	$C_{10}H_{14}NO_6P \cdot H_2O$	40,7	5,6	41,0	5,5	65

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[a] All of the compounds obtained were chromatographically and electrophoretically homogeneous. [b] PMR spectrum, δ, ppm (2 N NaOD): 2-C_6H_5, 7.25 s; 2-CH_2, 4.32 s; 5-CH_2, 4.03 d (J = 5.5 Hz); 6-H, 7.59 s.

Pyridoxine Analogs. 2'-Isopropylpyridoxine (Ia) and 2'-phenylpyridoxine (Ib) were obtained by reduction of the free bases of diesters of 5-hydroxycinchomeronic acids via the method in [2]. The hydrochloride of Ia, with mp 211-213° (dec., from alcohol–ether), was obtained in 74% yield. Found %: C 53.5; H 7.5. $C_{11}H_{17}NO_3 \cdot HCl$. Calculated %: C 53.4; H 7.3. The hydrochloride of Ib, with mp 198-200° (dec., from alcohol–ether), was isolated in 71% yield. Found %: C 59.7; H 5.5. $C_{14}H_{15}NO_3 \cdot HCl$. Calculated %: C 59.7; H 5.7.

5'-Methylpyridoxine (IIa). This compound was obtained by the method in [2].

5'-Phenylpyridoxine (IIb). A solution of 4 g (19.3 mmole) of 4',3-O-isopropylideneisopyridoxal [5] in 20 ml of absolute ether was added slowly with cooling and stirring to a solution of a Grignard reagent prepared from 0.5 g of magnesium and 3.3 g of bromobenzene in 20 ml of absolute ether. At the end of the addition, the mixture was refluxed for 2 h, cooled, and decomposed by the addition of 10 g of ice. The ether layer was separated, and the aqueous layer was extracted with three 10-ml portions of ether. The combined ether extracts were dried and evaporated to dryness. The residue was dissolved in 100 ml of 10% hydrochloric acid, and the solution was refluxed for 30 min and vacuum evaporated to dryness. The residue was triturated thoroughly with dry acetone, and the mixture was filtered. The yield of the hydrochloride was 1.9 g (35%). Found %: C 59.0; H 5.5. $C_{14}H_{15}NO_3 \cdot HCl$. Calculated %: C 59.7; H 5.7.

5'-Desoxy-5'-carboxymethylpyridoxine (IV) and 5'-Desoxy-5'-β-hydroxyethylpyridoxine (V). These compounds were obtained by the method of Korytnik and co-workers [5].

5'-Desoxy-5'-carboxymethylenepyridoxine (III). A 0.5-g (2 mmole) sample of 3,4'-O-isopropylidene-5'-desoxy-5'-carboxymethylenepyridoxine [5] was dissolved in 25 ml of 0.1 N HCl, and the solution was heated at 80° for 2 h. It was then evaporated to dryness in vacuo, and the residue was triturated with dry acetone. The resulting crystals were removed by filtration, washed with acetone and ether, and dried. The yield of the hydrochloride of III with mp 293-297° (dec.) was 0.4 g (81.5%). PMR spectrum, δ, ppm (2 N NaOD): 2-CH_3, 2.32 s; 4-CH_2, 4.82 s; 5-CH, 7.56 d (J = 16 Hz); 5'-CH, 6.34 d; 6-H, 7.71 s. According to [5], the hydrochloride melts at 295-300° (dec.). The hydrochloride was converted to the free base by the action of sodium acetate.

Oxidation of Pyridoxine Analogs. A) 2'-Isopropyl-, 2'-phenyl-, and 5'-desoxy-5'-β-hydroxyethylpyridoxines were oxidized with manganese dioxide in sulfuric acid by the method in [2]. The pyridoxal analogs were isolated from the reaction mixtures as either the oximes or the Schiff bases with p-anisidine. The compounds obtained are presented in Table 2.

B) 5'-Desoxy-5'-carboxymethyl- (VI) and 5'-Desoxyl-5'-carboxymethylenepyridoxals (VII). A 4-g sample of "B" manganese dioxide [6] was added to a suspension of 4 mmole of free base III or IV in a mixture of 20 ml of chloroform and 40 ml of dioxane, after which the mixture was refluxed for 5 h. It was then filtered, and the solid was washed with dioxane. The combined filtrates were evaporated to dryness, and the residue was extracted with five 50-ml portions of boiling chloroform. The chloroform extracts were filtered, and the filtrate was evaporated to a small volume. The precipitated base (VI or VII) was removed by filtration and dried. The yield of VI with mp 165-166° (mp 167-168° [7]) was 44%. The yield of VII was 32%. Found %: C 57.8; H 4.3. $C_{10}H_9NO_4$. Calculated %: C 57.9; H 4.4. PMR spectrum, δ, ppm (2 N NaOD): 2-CH_3, 2.29 s; 4-H, 10.15 s; 5-CH, 7.5 d (J = 16 Hz); 5'-CH, 6.21 d; 6-H, 7.44 s.

Pyridoxamine Analogs. The pyridoxamine analogs presented in Table 3 were obtained by hydrogenation of the oximes over Pd/C. Chromatographically pure substances in yields that were close to quantitative were obtained in all cases.

Pyridoxamine Phosphate Analogs. These compounds were obtained by phosphorylation of the pyridoxamine with polyphosphoric acid under the conditions described in [2]. The compounds obtained are presented in Table 4.

Pyridoxal 5-Phosphate Analogs. These compounds were obtained by phosphorylation of the Schiff bases of the pyridoxal analogs with polyphosphoric acid and subsequent separation on Dowex-50 (H^+ form) via the method we described in [2]. The compounds obtained are presented in Table 5.

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