VITAMIN B_6 ANALOGS

XIV.* 6-HALO DERIVATIVES OF PYRIDOXAL 5-PHOSPHATE

N. A. Stambolieva, M. Ya. Karpeiskii, and V. L. Florent'ev

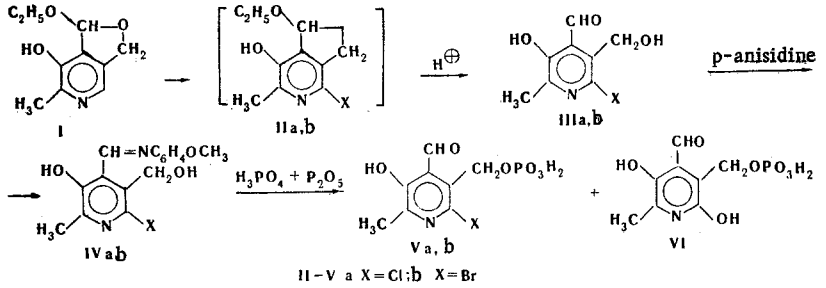
UDC 547.824.07:543.422.6:541.67

6-Chloropyridoxal and 6-bromopyridoxal were synthesized by halogenation of pyridoxal ethylacetal with tert-butyl hypochloriate and dioxane dibromide, respectively. 6-Halo analogs of pyridoxal-5-phosphate were obtained by phosphorylation of the Schiff bases. The UV and PMR spectra were studied.

6-Chloro and 6-bromo analogs of pyridoxal-5-phosphate were synthesized to study some aspects of the mechanism of the action of pyridoxal phosphate-dependent enzymes. The synthesis was accomplished via the following scheme.

6-Bromopyridoxal (IIIb) was obtained in 80-83% yield by the bromination of pyridoxal ethylacetal (I) with dioxane dibromide with subsequent hydrolysis. The halogenation was carried out in the presence of triethylamine to eliminate the deactivating effect of the protonated nitrogen of the pyridine ring.

The reaction of I with tert-butyl hypochlorite in tert-butanol makes it possible to obtain 6-chloropyridoxal (IIIa) in good yields. The structures of the compounds obtained were proved by the UV and PMR spectra (Figs. 1 and 2).



An attempt to synthesize 6-halopyridoxamines by catalytic hydrogenation of 6-halopyridoxal oximes was unsuccessful, since the rate of hydrogenolysis of halogen is comparable to the rate of hydrogenation of the oxime group in the case of the 6-chloro derivative and appreciably exceeds that for the 6-bromo derivative.

A mixture of two compounds was obtained by phosphorylation of Schiff base IVb with polyphosphoric acid with subsequent separation on an ion-exchange resin. The PMR spectrum of the mixture (Fig. 3) contains 2-CH_3, 5-CH_2, and 4'-H signals but does not contain signals from the ring protons. All of the spectral lines are broad. The UV spectrum (Fig. 4) makes it possible to obtain important information. Characteristic for the mixture is the presence of two long-wave absorption maxima at 396 and 474 nm (Fig. 4, curve A). However, after separation by means of electrophoresis on paper, each of the two components has only

*See [6] for communication XIII.

Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 4, pp. 493-498, April, 1971. Original article submitted December 2, 1969.

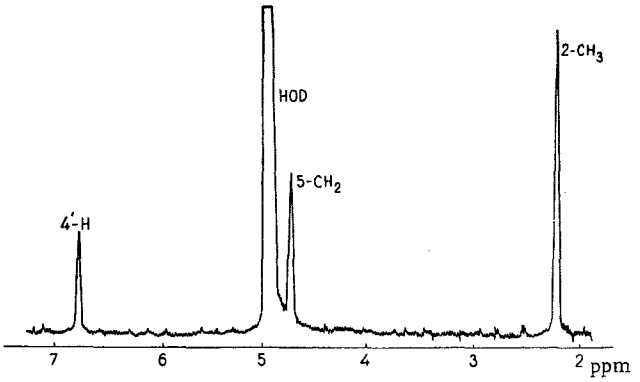


Fig. 1. PMR spectrum of 6-bromopyridoxal (IIIb) in 2 N NaOD.

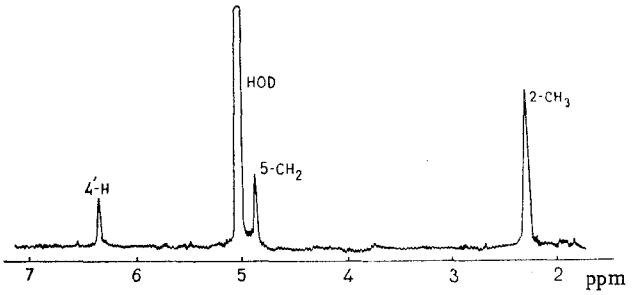


Fig. 2. PMR spectrum of 6-chloropyridoxal (IIIa) in 2 N NaOD.

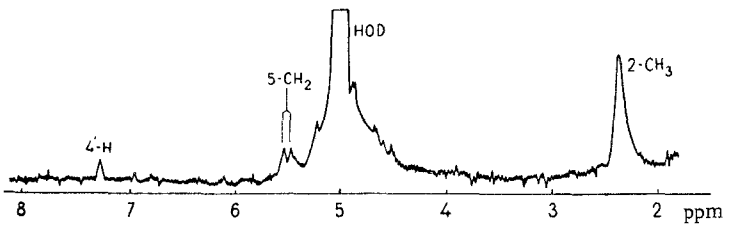


Fig. 3. PMR spectrum of the mixture obtained by phosphorylation of 6-bromopyridoxylidene-p-anisidine in 2 N NaOD.

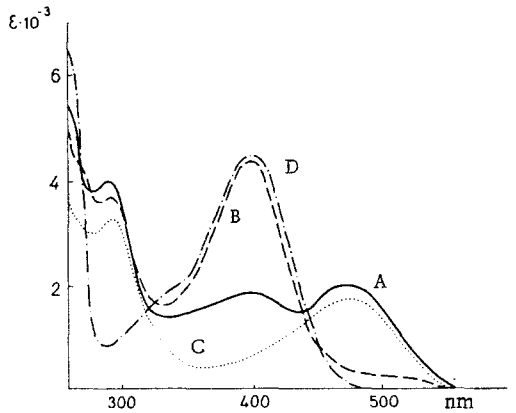


Fig. 4. UV spectra at pH 7: A) mixture obtained by phosphorylation of IVb; B) 6-bromopyridoxal phosphate (Vb); C) 6-hydroxypyridoxal phosphate (VI); D) 2-methyl-3-hydroxyl-4-formyl-5-methoxymethyl-6-bromopyridine (VII).

TABLE 1. Correlation of the Ionic Forms in the UV Spectra of 6-Halo Analogs of Pyridoxal*

Medium	Ionic forms	λ_{max}, nm ($\varepsilon \cdot 10^{-3}$)		
		pyridoxal†	6-chloro-pyridoxal	6-bromo-pyridoxal
0,1 *N* HCl	Cation, hemiacetal	288 (9,0)	294 (5,8)	297 (7,2)
pH 7	Dipolar ion, hemiacetal	317 (8,9)	—	—
	Dipolar ion, aldehyde	390 (0,16)	—	—
	Neutral form, hemiacetal	—	293 (4,1)	295 (5,1)
	Anion, hemiacetal	—	312 (3,4)	312 (4,8)
0,1 *N* KOH	Anion, hemiacetal	302 (5,7)	311 (6,8)	312 (8,6)
	Anion, aldehyde	390 (1,7)	—	—

*The maxima of the $\pi \rightarrow \pi^*_1$ transitions are presented.
†Data from [2].

TABLE 2. Correlation of the Ionic Forms in the UV Spectra of 6-Halo Analogs of Pyridoxal Phosphate*

Medium	Ionic forms	λ_{max}, nm ($\varepsilon \cdot 10^{-3}$)				
		pyridoxal phosphate†	6-chloro-pyridoxal phosphate	6-bromo-pyridoxal phosphate	5-O-methyl-6-bromo-phosphate	6-hydroxy-pyridoxal phosphate
0,1 *N* HCl	Cation, hydrate	295 (6,7)	300 (3,5)	305 (3,4)	305 (2,0)	300 (3,0)
	Cation, aldehyde	338 (1,4)	340 (2,4)	346 (2,8)	358 (3,3)	330 (1,5)
pH 7	Neutral form, hydrate	—	295 (3,7)	297 (3,6)	—	297 (3,5)
	Dipolar form, hydrate	330 (2,5)	—	—	—	—
	Dipolar form, aldehyde	388 (4,9)	—	—	—	—
	Anion, aldehyde	—	390 (3,2)	398 (4,5)	400 (4,4)	475 (1,9)
0,1 *N* KOH	Anion, hydrate	303 (1,1)	322‡	323‡	318 (1,3)	323 (3,6)
	Anion, aldehyde	388 (6,6)	390‡	398‡	400 (5,0)	470 (3,2)

*The maxima of the $\pi \rightarrow \pi_1^*$ transitions are presented.
†Data from [2].
‡Accurate values of the molecular extinction cannot be obtained since the indicated compounds decompose rapidly in alkaline media.

one of the indicated absorption maxima (Fig. 4, curves B and C). A study of the UV spectrum of 2-methyl-3-hydroxy-4-formyl-5-methoxymethyl-6-bromopyridine (VII) (Fig. 4, curve D), which models 6-bromo-pyridoxal phosphate, makes it possible to conclude that the component with an absorption maximum at 396 nm is Vb. A similar pattern is observed in the phosphorylation of IVa.

The following fact is important for establishing the structure of the second component. As indicated by electrophoresis, chromatography, and UV spectroscopy, the preparation of Va, like that of Vb, is accompanied by the formation of the same side product. These data, like the PMR spectrum, make it possible to assume with a high degree of confidence the 6-hydroxypyridoxal phosphate structure (VI) for the second component. This conclusion is also confirmed by a study of the stabilities of Va and Vb as a function of the pH of the medium. Both compounds are relatively stable at pH 1-9, but are rapidly converted to VI at higher pH values. The unphosphorylated aldehydes are stable up to pH 12, and the explanation for this interesting phenomenon apparently consists in the action of the dianion of the phosphate group via the following mechanism:

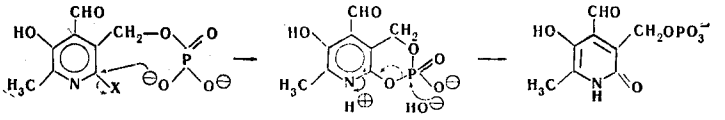


TABLE 3. Oximes and Schiff Bases of 6-Halo Analogs of Pyridoxal

Compound	mp, °C	R_f	UV spectrum, λ_{max}, nm ($\varepsilon \cdot 10^{-3}$)	Empirical formula	Found, %				Calc., %				Yield, %
					C	H	X	N	C	H	X	N	
6-Chloropyridoxal oxime	235—237 (dec.)	0,636	253 (16,2)*; 356 (8,0)	$C_8H_9ClN_2O_3$	44,61	4,30	16,24	13,11	44,35	4,18	16,37	12,93	86
6-Bromopyridoxal oxime	230—232 (dec.)	0,636	255 (17,0)*; 355 (8,5)	$C_8H_9BrN_2O_3$	37,43	4,68	30,09		36,89	3,48	30,62		92
6-Chloropyridoxylidene-p-anisidine	187—192 (dec.)	—	233 (17,8)†; 296 (6,4); 356 (12,0); 374 (10,8)	$C_{15}H_{15}ClN_2O_3$	57,99	4,98	11,51	9,19	59,05	4,92	11,55	9,13	42
6-Bromopyridoxylidene-p-anisidine	188—190 (dec.)	—	233 (20,6)†; 296 (8,0); 396 (12,5); 374 (11,9)	$C_{15}H_{15}BrN_2O_3$	52,21	4,65	20,59	7,61	51,29	4,30	22,49	7,82	48

* In 0.1 N KOH.
† In ethanol.

A comparison of the UV spectra of the 6-halo analogs with the spectra of pyridoxal and pyridoxal phosphate (Tables 1 and 2) indicates that the introduction of a halogen into the 6-position of the pyridine ring has little effect on the position of the absorption maxima.

The data in Tables 1 and 2 make it possible to draw the following conclusions:

1) The basicity of the nitrogen of the pyridine ring is sharply reduced in the same way as the acidity of a phenolic hydroxyl group. This is reflected in the fact that a neutral form $\rightleftharpoons$ anion equilibrium rather than a neutral form $\rightleftharpoons$ dipolar form equilibrium, as in the case of vitamin B_6, is characteristic in the neutral pH region for the 6-halo analog;

2) In accordance with the previously noted regularity [1], the introduction of a substituent in the 6-position of the pyridine ring results in a shift in the aldehyde $\rightleftharpoons$ hemiacetal or aldehyde $\rightleftharpoons$ hydrate equilibria to the right.

The authors sincerely thank Academician A. E. Braunshtein for his constant interest in this work, and K. F. Turchin and V. F. Bystrov for obtaining the PMR spectra.

EXPERIMENTAL

The UV spectra were obtained with an EPS-3T (Hitachi) spectrometer. The PMR spectra were obtained with a "Jeol" JNM-4H-100 spectrometer. The chemical shifts are presented in the δ scale with tert-butanol (1.2 ppm) as the internal standard. The IR spectra of mineral oil emulsions were obtained with a UR-10 spectrometer. Thin-layer chromatography was carried out according to the method described in [3] in an ethyl acetate–acetone–25% ammonium hydroxide system (20:10:15). Electrophoresis on Whatmann 3 mm paper required 1.5 h at 1500 V in an ethyl aminoacetate buffer with pH 6.5.

6-Bromopyridoxal (IIIb). Dioxane dibromide [22.2 g (92.25 mmole)] was added with stirring in the course of 1.5 h to a solution of 12 g (61.5 mmole) of the free base of pyridoxal ethylacetal [4] and a mixture of 450 ml of dry dioxane and 12.5 ml (92.25 mmole) of triethylamine, and the mixture was stirred for another 4 h at room temperature. The triethylamine hydrobromide was filtered and washed with dry ethyl acetate. The filtrates were vacuum evaporated (30°) to dryness, the residue was dissolved in 20 ml of 2 N HCl, 20 ml of water was added, and the mixture was heated at 60° for 30 min. The solution was cooled, and 160 ml of water was added to it. The mixture was allowed to stand for 2 h in a refrigerator to give 10.8 g of the monohydrate of IIIb. Neutralization of the filtrate with sodium bicarbonate gave another 2 g of product. The overall yield of the monohydrate of IIIb with mp 160-162° (decomp., from alcohol) and R_f 0.185 was 12.8 g (83%). Found %: C 36.90; H 4.12. $C_8H_8BrNO_3 \cdot H_2O$. Calculated %: C 36.42; H 3.82.

2-Methyl-3-hydroxy-4-formyl-5-methoxymethyl-6-bromopyridine (VII). A total of 0.32 g (45%) of VII with mp 96-97° (from petroleum ether) and R_f 0.952 was obtained by the method of the previous experiment from 0.5 g (2.8 mmole) of the free base of 5-O-methylpyridoxal [5]. Found %: C 41.84; H 4.13. $C_9H_{10}BrNO_3$. Calculated %: C 41.56; H 3.99. PMR spectrum in 2 N NaOD (ppm): 2-CH_3 2.28; 5-OCH_3 3.35; 5-CH_2 4.53.

6-Chloropyridoxal (IIIa). A total of 9.6 ml (80 mmole) of tert-butyl hypochlorite was added in the course of 10 min to a solution of 12 g of I in 400 ml of tert-butanol. The mixture was stirred at room temperature for 30 min and vacuum evaporated to dryness at 30°. The resulting oil was washed with 30 ml of benzene and 30 ml of ether. The residue was treated with HCl, as in the case of IIIb, to give 10 g (80.6%) of IIIa with mp 124-125° (from petroleum ether) and R_f 0.185. Found %: C 48.56; H 4.30; Cl 17.20. $C_8H_8NO_3Cl$. Calculated %: C 47.65; H 4.11; Cl 17.50.

6-Halopyridoxal Oximes. Hydroxylamine hydrochloride (10 mmole) was added to a solution of 5 mmole of III in 10 ml of 0.5 N HCl and 10 ml of alcohol, and the mixture was heated at 70° for 10 min. The alcohol was removed in vacuo, and the aqueous solution was neutralized with solid sodium acetate and stored in a refrigerator for 2 h. The oxime was filtered, washed with water, and recrystallized from alcohol–water. The properties of the compounds obtained are presented in Table 3.

Schiff Bases of 6-Halopyridoxal (IV). Compound III (4 mmole) was dissolved in 10 ml of 2 N HCl, and 12 ml of 0.5 N p-anisidine hydrochloride was added to the solution. The mixture was stirred for 15 min at room temperature and neutralized with 2 N sodium acetate. After standing at room temperature for 5 h, the Schiff base was filtered, washed with water, and recrystallized from methanol. The properties of the compounds obtained are presented in Table 3.

Hydrogenation of the Oximes. A total of 150 mg of 5% Pd/C was added to a solution of 1 mmole of the 6-halopyridoxal oxime in 20 ml of alcohol and 0.5 ml of concentrated HCl and hydrogenated at room temperature and atmospheric pressure. Pyridoxal oxime was obtained in 90% yield by the hydrogenation of 6-bromopyridoxal oxime. A mixture of pyridoxamine and the starting oxime in a ratio of 3 : 2 was obtained in the case of 6-chloropyridoxal oxime.

Analogs of Pyridoxal Phosphate (Va and Vb). A solution of 6.5 mmole of IV in a mixture of 22.9 g of 85% H_3PO_4 and 18.6 g of P_2O_5 was heated at 55-60° for 24 h. After cooling, 19.5 ml of 0.1 N HCl was added to the mass, and it was again heated at 60° for 15 min. The resulting solution was applied to a 3 × 94-cm column filled with Dowex 50B × 4 in the acid form. It was eluted with water at 30 ml/h. The fractions containing V were vacuum evaporated at 30° to 30 ml and lyophilized to give 11-13% of a mixture of VI and V. The subsequent purification was carried out according to one of the following methods.

A) Repeated chromatography with a 3 × 94-cm column (Dowex 50B × 4, H^+ form) under the previously described conditions made it possible to obtain 15 mg of pure Va. The remaining material (first four to five fractions) was still a mixture of Va and VI.

B) Preparative electrophoresis on paper made it possible to isolate both components of the mixture in pure form. The product obtained by the phosphorylation of IVb was treated in this way. Zones containing Vb and VI were eluted with water, desalinized with a column filled with Dowex 50B × 4 (1.4 × 40 cm, H^+ form), and lyophilized. A total of 8 mg each of Vb and VI was obtained from 50 mg of the mixture.

LITERATURE CITED

1. N. A. Doktorova, L. V. Ionova, M. Ya. Karpeiskii, N. Sh. Padyukova, K. F. Turchin, and V. L. Florent'ev, Tetrahedron, 25, 3527 (1969).
2. D. E. Metzler and E. E. Snell, J. Am. Chem. Soc., 77, 2431 (1955).
3. E. N. Dement'eva, N. A. Drobinskaya, L. V. Ionova, M. Ya. Karpeiskii, and V. L. Florent'ev, Biokhimiya, 33, 350 (1968).
4. D. Heyl and S. A. Harris, J. Am. Chem. Soc., 73, 3434 (1951).
5. N. A. Doktorova, M. Ya. Karpeiskii, N. Sh. Padyukova, K. F. Turchin, and V. L. Florent'ev, Khim. Geterotsikl. Soedin., 365 (1971).
6. N. A. Stambolieva, M. Ya. Karpeiskii, A. M. Kritsyn, and V. L. Florent'ev, Khim. Geterotsikl. Soedin., 487 (1971).