Synthesis and Physicochemical and Biological Properties of 6-Halogen-Substituted Vitamin B_6 Analogs†

W. Korytnyk* and S. C. Srivastava

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14203. *Received October 24, 1972*

A series of 6-halogen-substituted vitamin B_6 analogs was synthesized, and 6-fluoro analogs were examined most intensively. 6-Fluoropyridoxol was obtained by a modified Schiemann reaction from 6-aminopyridoxol; it was converted to the various vitamer forms, selective oxidation giving 6-fluoropyridoxal, from which 6-fluoropyridoxamine and its 5′-phosphate were obtained. 6-Chloropyridoxol was obtained by two simple methods that supersede the multistep procedure described earlier. 6-Chloropyridoxal was obtained either by selective oxidation of 6-chloropyridoxol or by direct chlorination of pyridoxal ethyl acetal and hydrolysis. The presence of the halogen atom in the 6 position radically changes physicochemical properties of the vitamin, particularly pK_a values, resulting in loss of the zwitterionic forms that are characteristic of the parent vitamers and assumption of a hydrophobic character. All 6-fluoro analogs inhibited cell growth in tissue culture to various degrees, and 6-fluoropyridoxamine phosphate was an inhibitor of pyridoxine phosphate oxidase. Although the 6-chloropyridoxol was not effective as an inhibitor of cell growth, it was found to be a potent convulsant agent.

Analogs of vitamin B_6‡ in which the 6-H has been replaced with a halogen atom (*e.g.*, III, Scheme I) can be expected to interfere with vitamin B_6 metabolism in various ways. The greater electron-withdrawing character of the 6-halogen as compared with the hydrogen, as well as the gradual increase in bulk from fluorine to bromine in such vitamin B_6 analogs, offers an opportunity to correlate these factors with their effects on enzymes, cells, and tissues dependent on vitamin B_6 for their functions. As will be shown, introduction of a halogen can be expected to affect the permeability characteristics of the analogs. Because the size of the fluorine approximates that of hydrogen, 6-fluoro analogs were of particular interest. When we started these studies, only 6-chloropyridoxol had been prepared, and that only by a rather long route.[3] Very recently, some 6-chloro and 6-bromo derivatives of vitamin B_6 have been synthesized.[4] In the present paper, we describe several synthetic approaches for substitution in the 6 position which gave for the first time the 6-fluoro analogs of the various forms of vitamin B_6, as well as some new 6-chloro and 6-bromo analogs.

The most general method that we have developed starts with 6-aminopyridoxol (II, Scheme I). The latter was obtained by hydrogenolysis of 6-phenylazopyridoxol (I), as described by Katritzky, *et al.*,[5] but with an improved yield. A modified Schiemann reaction gave the 6-fluoro compound IIIa, which was then converted to the 4-aldehyde by careful oxidation with MnO_2. The presence of electron-withdrawing atoms in the 6 position (6-F or 6-Cl, IIIa or b) makes the 4-CH_2OH group much more susceptible to oxidation than it is in unsubstituted pyridoxol, and hence the concomitant formation of 6-halopyridoxic acids during oxidation is difficult to avoid. In contrast, oxidation of unsubstituted pyridoxol could be controlled readily.[6] 6-Fluoropyridoxamine (X), obtained by hydrogenation of the oxime IXa, was subsequently phosphorylated with polyphosphoric acid to the cofactor analog XI.§

Although the structures of products of oxidation of 6-fluoropyridoxol could be reasonably formulated as VIII by analogy with the selectivity of oxidation exhibited for other pyridoxol analogs (*e.g.*, 6-chloropyridoxol, as described here), nevertheless it appeared desirable to obtain independent proof of the site of oxidation. This was achieved by preparation of the isomeric 5-oxime XVI obtained from 6-fluoropyridoxol (III, X = F) by the following sequence of reactions: IIIa → XIV → XV → XVI. The two isomeric oximes IX (X = F) and XVI were shown to be different, and hence IX is the 4 isomer.

6-Chloro- and 6-bromopyridoxol (III, X = Cl and Br) were made by the Sandmeyer method from the amine II. We had hoped to improve the yield, particularly that of the 6-fluoro analog, which was obtained in 34% yield from II by starting with the blocked amine 6-amino-3-*O*-benzoyl-α^4,α^5-isopropylidenepyridoxol (VII). This intermediate was obtained from IV by the series of reactions indicated in Scheme I. Nevertheless, the blocking groups were not stable enough to withstand the reaction conditions, even when a limited amount of acid was used. Thus, the blocked intermediate did not offer any advantages in these reactions but may be useful for future applications involving modification of the 6-amino function under neutral conditions.

A more direct method for obtaining 6-chloropyridoxol was achieved by chlorination of α^4,α^5-*O*-isopropylidenepyridoxol (IV) with *tert*-butyl hypochlorite, giving the 6-chloro derivative XIII in excellent yield. This last was hydrolyzed with acid, giving 6-chloropyridoxol (VIIIb). The yield was considerably reduced when unsubstituted pyridoxol was chlorinated. Either of the methods mentioned here is far simpler than the multistep synthesis reported by Blackwood, *et al.*[3]

6-Chloropyridoxal (VIIIb) was also required for biological testing. In analogy with 6-fluoropyridoxal (VIIIa), we obtained it by direct oxidation of 6-chloropyridoxol with MnO_2, and its melting point was 160°. Recently, synthesis of the same compound was reported to have been achieved by direct chlorination of the ethyl acetal of pyridoxal and subsequent hydrolysis; the reported melting point was 124–125°.[4] We have repeated the latter synthesis of VIIIb, and found the product to be identical with ours, with a melting point of 160°.

Attempts to obtain 6-hydroxypyridoxol were only partially successful. The bromine atom in 6-bromopyridoxol could not be replaced with hydroxyl, even when drastic conditions were used (refluxing with 0.5 *N* NaOH at 100° for 24 hr). Similarly, 6-chloro-α^4,α^5-isopropylidenepyridoxol remained

†Chemistry and Biology of Vitamin B_6. 31. For the preceeding paper in this series, see ref 1a. Certain aspects of this study have been presented.[1b]

‡For a review of the synthesis and biological activity of vitamin B_6 analogs, see ref 2.

§The corresponding 6-chloro analog could not be obtained by this route, since the hydrogenation of the oxime IXb causes hydrogenolysis of the C_6-Cl bond, yielding pyridoxamine.

Scheme I

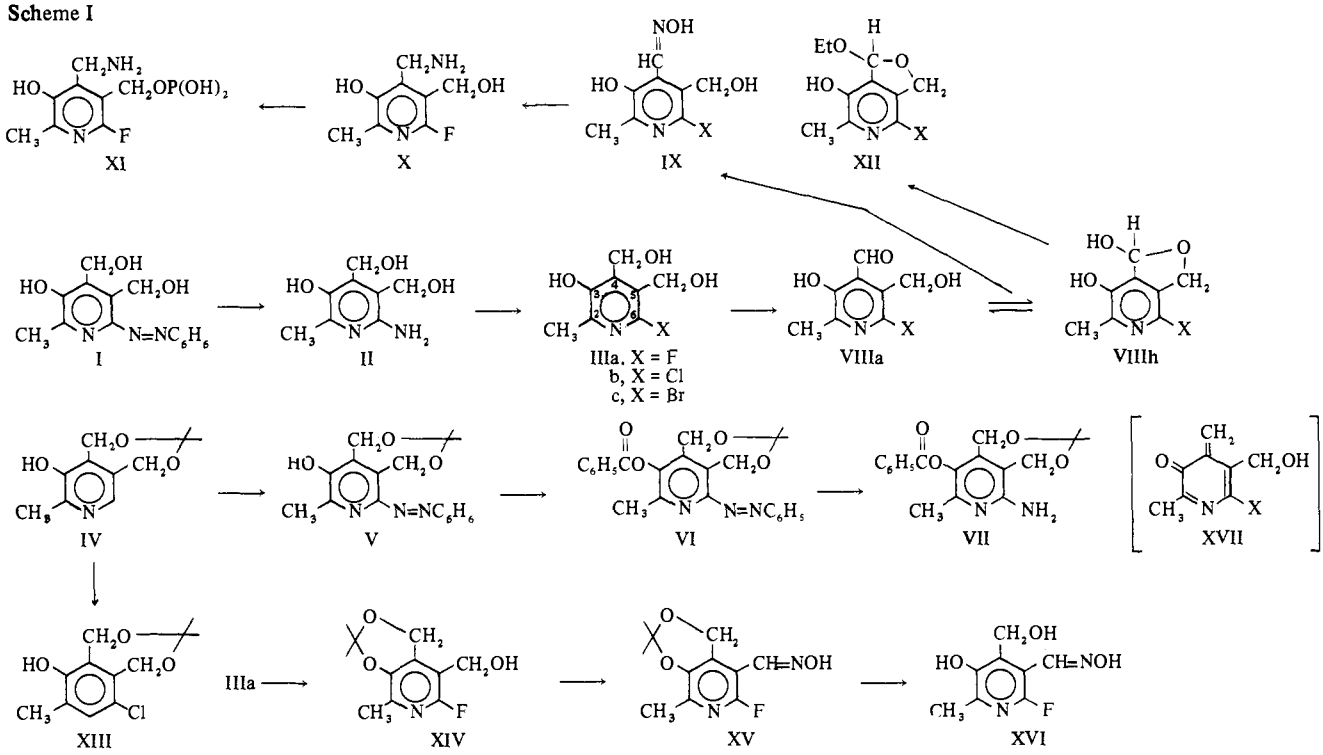


Table I. Uv Spectra and pK_a Values

Compound	Acid medium: 0.1 *N* HCl; λ max, nm (ε × 10⁻³)	Acid medium: 1 *N* HCl; λ max, nm (ε × 10⁻³)	Neutral medium; λ max: pH	Neutral medium; λ max: nm (ε × 10⁻³)	Basic medium, λ max: pH	Basic medium, λ max: nm (ε × 10⁻³)	pK_a (OH)	pK_a (N^+H)[a]
VIIIa [6F-PAL]	288 (4.0)	288 (4.0)	6.65	282 (3.8)	10.1	236 (3.5), 309 (4.2)	7.8 ± 0.1	0.1 ± 0.1
IIIa [6F-PIN]	284 (3.7)	282 (3.8)	6.8	287 (2.5)	10.3	243 (4.4), 317 (4.4)	8.2 ± 0.1	0.2 ± 0.1
VIIIb [6Cl-PAL]	292 (5.7)	302 (8.1)	6.8	287 (4.4)	12.5	247 (11.2), 311 (7.3)	7.2 ± 0.1	0.8 ± 0.1
IIIb [6Cl-PIN]	301 (4.4)	303 (6.0)	6.7	291 (3.7)	9.6	255 (6.1), 319 (9.6)	7.9 ± 0.1	1.1 ± 0.1
IIIc [6Br-PIN]	300 (4.8)	304 (6.3)	6.7	293 (3.8)	10.7	254 (5.2), 319 (7.5)	7.7 ± 0.1	1.0 ± 0.1
Pyridoxol [PIN][b]	291 (8.6)	291 (8.6)	6.8	254 (3.9), 324 (7.2)	1 *N* NaOH	245 (6.3), 310 (6.8)	5.0	9.0
Pyridoxal [PAL][b]	288 (9.0)	288 (9.0)	7.0	317 (8.9)[c] 290 (0.16)[d]	11	240 (8.6), 302 (5.7)	4.2	8.7

[a] Determined as described in ref 8. [b] Data taken from ref 9. [c] Dipolar ion hemiacetal. [d] Dipolar ion aldehyde.

unaffected when heated with 5 *N* NaOH on a steam bath for 74 hr. Fusion with KOH gave an unidentified product, the properties of which indicate degradation of the pyridine ring. This remarkable stability of 6-halopyridoxols is in sharp contrast with the well-documented ease with which the 2-halogen is displaced in 2-chloro- and 2-bromopyridines. The inertness of the 6-halogens in pyridoxol analogs is clearly due to the powerful electron-donating effect of the phenolic hydroxyl, making the C_6–X bond much more resistant to a nucleophilic attack. 6-Hydroxypyridoxol was finally obtained by diazotizing II and subsequent treatment with base. The identity of the compound has been established by mass spectrometry, but an analytically pure sample could not be obtained. In view of the unreactivity of the halogen atom in the 6 position, it was rather surprising to find that the 6-halogen pyridoxols gave a positive Gibbs test.[7]

Ionization Constants and Spectra. The introduction of a 6-halogen greatly affects the ionization constants of the analogs as compared with the parent compounds. The pK_a values and spectra of the halo compounds are presented in Table I and compared with those of the parent vitamers. The negative inductive (–I) effect of the halogen greatly reduces the basicity of the N, so that it can be protonated only in a very strongly acidic solution, as is indicated by the very low pK_a values. The same effect has been observed for 2-halopyridines by Brown and McDaniel,[8] who explained it in terms of the predominance of the –I effect over the opposing resonance effect. The pK_a values for both series of compounds are similar in magnitude, the fluorine analog having the lowest value. The most striking effect of these low pK_a values for the halo vitamin B_6 compounds is that the zwitterionic forms are no longer possible, and thus the substituted compounds are hydrophobic. This property was taken advantage of in the isolation of 6-fluoropyridoxol which was obtained from the acidified reaction mixture by extraction with ether. The pK_a value of the phenolic OH is here slightly basic (pK_a = 7.2–8.2), whereas it is acidic in the unsubstituted vitamers (pK_a = 4–5); thus, under physiologic conditions, this group would be expected to be partly ionized with the undissociated species predominating. The acidity of the phenolic OH increases in the following order: F < Cl < Br. The order is reversed from what would be expected if the –I effect alone were operating, and indicates the overriding effect of resonance, which weakens the acidity of the phenolic hydroxyl.

Conclusions regarding the ionic forms of the halogen analogs are strengthened by study of their uv spectra (Table I).

As would be expected, the uv spectra of pyridoxine and its 6-halogen derivatives in basic media are very similar, since in all cases the anion is obtained by dissociation of the phenolic hydroxyl. (The extinction coefficient for the fluorine analogs, however, was found to be low.) In acid media, on the other hand, pyridoxol and pyridoxal are fully protonated (no change in spectra was observed when the acidity was increased from 0.1 to 1.0 *N*), whereas the chloro and bromo analogs were only partially protonated on nitrogen. The extent of protonation was increased on increasing the acid strength, as can be seen in the spectral shifts, and particularly in the increases in the extinction coefficients. Protonation of the 6-fluoro compounds was not significant, even in 1 *N* HCl. Substantial changes were noted only in 10 *N* HCl solution, and even then the analogs were incompletely protonated. At neutrality, the 324-nm band due to the zwitterionic form of pyridoxine is not present in the halogenated compounds. Instead, there is only one peak which is primarily due to the neutral molecule.

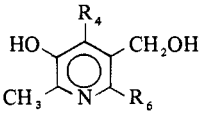
Pyridoxal in a basic solution has an additional small peak at 390 nm due to a small proportion of the free aldehyde form.[9] In contrast to pyridoxal, the halogenated aldehydes (VII, X = F or Cl) lack this peak, indicating that the free aldehyde form is absent even in basic solution. This conclusion is further confirmed by nmr studies. It has been shown previously that pyridoxal loses the characteristic nmr spectrum of the hemiacetal (AB quadruplet due to the 5-methylene protons and splitting of the hemiacetal proton by one of the 5-methylene protons) when dissolved in alkali because of a fast equilibrium between the hemiacetal and the aldehyde forms. Predictably, the hemiacetal proton is shifted downfield by 0.85 ppm at 35° as the amount of the aldehyde form increases.[10] Although there is appreciable sharpening of the hemiacetal and 5-CH_2 peaks for the halogenated aldehydes in alkaline solution, there is very little shift downfield (0.08 ppm for the fluoro compound), indicating that the amount of the aldehyde form is negligible. Ir spectra of the solid aldehydes are also consistent with the hemiacetal structure. Formation of the hemiacetal to the exclusion of the free aldehyde forms is to be expected on the basis of the greater electrophilic character of the aldehyde carbon in the halogenated analogs.

Mass spectra of the most halogenated compounds have been determined and were found to have well-defined molecular ion peaks. Fragmentation patterns could be followed in the chlorinated and brominated compounds by the presence of their characteristic isotope ratios. Fragmentation of the pyridoxol analogs III is initiated by the loss of H_2O to form a quinone methide fragment XVII, as with the unsubstituted analog.[11] Further fragmentation of the latter proceeds by at least four pathways, two of which parallel that for the parent compound,[11] and the other two depend upon the nature of the halogen substituent. Thus, the loss of halogen is of prime importance for the 6-bromo compound (III, X = Br), the *m/e* 150 peak being the base peak of the spectrum, whereas it is only of minor importance for the fluoro derivative. Conversely, the loss of the methyl group, which is the fourth pathway, assumes some importance only in the 6-fluoro compound (III, X = F), which in all other respects behaves like pyridoxol. 6-Fluoropyridoxamine has exactly the same fragmentation pattern as 6-fluoropyridoxol after the quinone methide intermediate XVII resulting from the loss of NH_3 is formed.

The fragmentation of 6-chloro- and 6-fluoropyridoxals differs significantly from that of pyridoxal.[11] Thus, the molecular ion peak is very small relative to the $M^+ - 1$ peak for both compounds, and there is no loss of CHO from M^+, as has been observed for pyridoxal. This may be due to the greater stability of the hemiacetal structure, as has already been discussed. The main difference between the two halogen derivatives is the retention of the fluorine during fragmentation in 6-fluoropyridoxal; in contrast, 6-chloropyridoxal loses chlorine but only after the loss of H_2O from M^+.

Biological Activity. Compounds synthesized in this study were tested as inhibitors of mouse mammary adenocarcinoma (TA-3) cells and Sarcoma 180 (S-180) cells, both grown in tissue culture. Only the 6-fluoro analogs inhibited growth of these cells, as shown in Table II; all were less active than 4-deoxypyridoxine (first compound in Table II). Fluoropyridoxal oxime (IX, X = F) was the most potent antagonist (ID_{50} 4×10^{-6} *M* in the presence of 10^{-7} *M* pyridoxal) but, as was the case with all B_6 antagonists so far tested, was not effective when the standard tissue culture medium containing 10^{-5} *M* pyridoxal was used. Its 5-isomer XVI had an ID_{50} of 2.9×10^{-5} *M* in the presence of 10^{-7} *M* pyridoxal. Fluoropyridoxamine phosphate (XI), in addition to being a cell growth inhibitor, was a very good inhibitor of pyridoxine-*P* oxidase ($K_I = 4.0 \times 10^{-6}$ *M*). This enzyme

Table II. Inhibitory Activity of 6-Halogen-Substituted B_6 Analogs and Related Compounds[a]



No.	R_4	R_6	Additional groups	TA-3 cells,[b] ID_{50}, *M*	PAL added, *M*	S-180 cells,[b] ID_{50}, *M*	
						3 days[c]	4 days[c]
	CH_3	H		6.8×10^{-8}	1×10^{-7}	1.3×10^{-8}	2.3×10^{-8}
VIII, X = F	CHO	F		1.0×10^{-4}	1×10^{-7}	4.4×10^{-6}	2×10^{-5}
III, X = F	CH_2OH	F		10^{-4}	0	2.5×10^{-5}	
X	CH_2NH_2	F		8.0×10^{-5}	1×10^{-7}		
XI	CH_2NH_2	F	5′-PO_4^{2-}	1.2×10^{-4}	1×10^{-7}		
IX, X = F	CH=NOH	F		4.3×10^{-6}	1×10^{-7}		
	CH=NOH	H		7.6×10^{-5}	1×10^{-7}		
III, X = Cl	CH_2OH	Cl		$>10^{-4}$	0		
VIII, X = Cl	CHO	Cl		$>10^{-4}$	10^{-7}		
III, X = Br	CH_2OH	Br		$>10^{-4}$	0		
II	CH_2OH	NH_2		6.0×10^{-5}	1×10^{-7}		

[a] Molar concentration of the compound for half-maximal growth inhibition. [b] It has been established that TA-3 cells required 1×10^{-7} *M* PAL for minimal growth and that S-180 cells did not require any extra PAL. Initially, however, some of the compounds were tested in TA-3 cells without any PAL added, and this has been indicated in the table. [c] Under standard conditions, the duration of testing was 4 days for both cell lines. The results were found to differ when the time was 3 days, as was the case for some compounds with S-180 cells.

catalyzes the formation of pyridoxal phosphate through the oxidation of pyridoxamine phosphate or of pyridoxine phosphate.[12]

The reduced activity of the 6-chloro, -bromo, and -amino compounds as contrasted with 6-fluoro compounds is consistent with the view that bulky substituents in the 6 position preclude analog phosphorylation catalyzed by pyridoxal phosphokinase,[13] most likely a prerequisite for the inhibitory activity of B_6 analogs. This hypothesis is now being tested. The absence of zwitterionic forms in the 6-fluoro and other 6-halo compounds makes them lipophilic, and this property should be expressed on the physiological level by permeability characteristics and tissue specificity which may be different than those of other B_6 analogs.

In vivo toxicity of the 6-halogenated analogs III (X = F and Cl), IX (X = F), and VIII (X = F and Cl) was determined in A/St mice by a single injection (ip) of the drug. The most toxic compound proved to be 6-fluoropyridoxal oxime (IX, X = F). After a single administration of 50 mg/kg of the drug, there were no survivors, and the animals died of acute convulsive toxicity within 1-5 hr. Although the animals survived this acute toxicity with other compounds at this dose level, administration of 6-chloropyridoxol (III, X = Cl) at 100 mg/kg caused convulsions and death to all animals tested, whereas they survived the same dose of 6-fluoropyridoxol (III, X = F). Preliminary results using a single injection of the compounds indicated that they have little or no antitumor activity against TA-3 mouse mammary adenocarcinoma cells.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Tlc (silica gel) was used routinely as described earlier.[14] Ir spectra were determined with a Perkin-Elmer 457 spectrophotometer and nmr spectra with a Varian A-60A instrument, using an 8-15% solution in $CDCl_3$, DMSO, or D_2O; positions of peaks are expressed in cycles per second (cps) from TMS or from dioxane (δ 3.70) as an internal standard. Peaks are assigned on the basis of previous work.[10] Mass spectra were determined with a CEC 21-491 mass spectrometer, direct inlet, ionizing potential 70 eV, ionizing current 18 μA.

6-Aminopyridoxol (II). This analog and intermediate was prepared from 6-phenylazopyridoxol (I) by reduction as described by Katritzky, *et al.*[5] In our hands, catalytic reduction (Pd/C) gave an improved yield of the pure product, but sodium dithionite reduction of the azo compound I gave a product that could not be readily purified from the residual inorganic salt.

To 6-phenylazopyridoxol (4.0 g, 14.6 mmol) in ethanol (400 ml), 10% Pd/C (500 mg) was added, and the compound was hydrogenated in a Parr hydrogenator for 20 hr at 25 lb/in.^{2} The catalyst was filtered, the solvent evaporated, and the residue steam distilled to remove aniline. The aqueous solution was concentrated and was acidified with 12 *N* HCl. The solid was crystallized from cold EtOH, giving a yield of 2.44 g (84%), mp 180–185° dec (lit.[5] mp 180–185° dec).

6-Chloropyridoxol (IIIb). Method A. Compound II (132 mg, 0.60 mmol) was dissolved in concentrated HCl (5 ml). After the solution was cooled to –5°, $NaNO_2$ (50 mg, 0.72 mmol) was added slowly with stirring. The mixture was then brought to 10° and stirred for 2 hr, and the solvent was evaporated. Addition of H_2O and evaporation were repeated three times and finally the residue was dissolved in a small amount of H_2O. The aqueous solution was extracted four times with Et_2O, and the Et_2O solution was dried (Na_2SO_4). Evaporation of Et_2O and crystallization from either MeOH–C_6H_6 or Et_2O yielded faint pink crystals, mp 188° dec. The yield was 70 mg (58%) (lit.[3] mp 192.5–193° dec). The ir spectrum of our material and of that kindly supplied by Dr. R. K. Blackwood is identical.

Method B. To α^4,α^5-*O*-isopropylidenepyridoxol[15] (IV, 500 mg, 2.39 mmol) in *tert*-butyl alcohol (20 ml) was added *tert*-butyl hypochlorite (0.25 ml).[16] The reaction mixture was stirred for 30 min, direct light being excluded. The solvent was distilled off *in vacuo*, and the yellowish residue was shaken with benzene, when some starting material (80 mg) separated out. The C_6H_6 solution was evaporated, and ether and petroleum ether were added to the residue when α^4,α^5-*O*-isopropylidene-6-chloropyridoxol (XIII) separated out; the yield was 400 mg (82%), mp 157–159°. *Anal.* ($C_{11}H_{14}ClNO_3$) C, H.

The product was hydrolyzed by stirring it in 1 *N* HCl (25 ml) overnight. After removal of the solvent, the residue was crystallized from MeOH–Et_2O: yield 350 mg; mp 188°. It was identical with an authentic sample. Chlorination of pyridoxol (free base) with *tert*-butyl hypochlorite under similar conditions gave 6-chloropyridoxol in 24% yield. Tlc of the preparation showed extensive formation of several by-products.

6-Bromopyridoxol (IIIc). Compound II (184 mg, 0.83 mmol) was dissolved in 48% aqueous HBr (5 ml). The solution was cooled to –5°, and solid $NaNO_2$ (70 mg, 1.2 mmol) was added slowly. After stirring for 2 hr at 10°, the solution was neutralized with 1 *N* NaOH to pH 7 and extracted with Et_2O (four times). The extract was dried, evaporated, and crystallized from Et_2O: yield 79 mg (38%); mp 188°. *Anal.* ($C_8H_{10}BrNO_3$) C, H, Br, N.

6-Fluoropyridoxol (IIIa). Compound II (722 mg, 3.49 mmol) was dissolved in 40% HBF_4 (16 ml), and the solution was cooled to –5°; solid $NaNO_2$ (300 mg) was added slowly with stirring, and stirring was continued at 10° for 2 hr. After addition of 5 *N* NaOH at 10° until pH 3–4, the solution was extracted with Et_2O (six times), washed with H_2O, dried (Na_2SO_4), and concentrated. The crystalline material thus obtained was recrystallized from Et_2O or Me_2CO–Et_2O, yielding 225 mg (34%) of IIa, mp 155–156°. *Anal.* ($C_8H_{10}FNO_3$) C, H, F, N.

α^4,α^5-*O*-Isopropylidene-6-phenylazopyridoxol (V) Hydrochloride. To a stirred solution of α^4,α^5-*O*-isopropylidenepyridoxol[15] (IV, 1.04 g, 4.98 mmol) in H_2O (5 ml) and 2.5 *N* NaOH (1 ml) was added diazotized aniline, made from aniline (500 mg, 5.4 mmol), H_2O (5 ml), 12 *N* HCl (5 ml), and $NaNO_2$ (400 mg in 2 ml of H_2O); the pH was maintained at 8 by addition of 2.5 *N* NaOH and the temperature at 0–5°. After being stirred for 1 hr, the solution was acidified with HCl, and the red solid obtained was filtered off and was crystallized from aqueous EtOH: yield 1.1 g (62.5%); mp 90°. *Anal.* ($C_{17}H_{20}N_3O_3Cl$) C, H, N. Mother liquors of V contained 150 mg of the acid cleavage product I.

3-*O*-Benzoyl-α^4,α^5-*O*-isopropylidene-6-phenylazopyridoxol (VI). To the azo compound V (500 mg, 1.21 mmol) in dry pyridine (10 ml) was added benzoyl chloride (0.25 ml in 5 ml of Et_2O) at 10° with stirring. After the reaction mixture was worked up, the product was crystallized from EtOH giving 470 mg (80%) of red crystals, mp 116–118°. *Anal.* ($C_{24}H_{23}N_3O_4$) C, H, N.

6-Amino-α^4,α^5-*O*-isopropylidene-3-*O*-benzoylpyridoxol (VII). To the azo compound VI (370 mg, 0.72 mmol) in EtOH (150 ml), 10% Pd/C (250 mg) was added, and the mixture was hydrogenated at 25 lb/in.^{2} for 54 hr in a Parr hydrogenator. The catalyst was removed by filtration and EtOH was evaporated. After a little EtOH was added, the product was allowed to crystallize. Recrystallization from ether yielded 250 mg (86%) of VII, mp 141°. *Anal.* ($C_{18}H_{21}N_2O_4$) C, H, N.

6-Fluoropyridoxal (VIIIa). To a solution of IIIa (56.7 mg, 0.30 mmol) in H_2O (20 ml), through which N_2 was bubbled to prevent overoxidation, MnO_2 (220 mg) was added, and the mixture was stirred for 2.5 hr. After filtration, the solution was concentrated and worked up by either of the following two methods. A. The solution was streaked on a preparative tlc plate, the streak was developed with EtOAc, and the yellow zone was eluted with a large quantity of Et_2O. On concentration of the Et_2O solution, VIIIa (41.2 mg) crystallized. B. Trituration of the yellow residue with H_2O, filtration, and recrystallization of the residual crystals from warm Et_2O gave 32 mg of VIIIa, mp 140–141°. *Anal.* ($C_8H_8FNO_3$) C, H, N.

Ethyl Acetal of VIIIa (XII, X = F). Compound VIII (10 mg) was refluxed with EtOH (15 ml) for 9 hr, EtOH was evaporated, and the acetal was crystallized from MeOH; mp 133°. *Anal.* ($C_{10}H_{12}FNO_3$) C, H, N.

6-Chloropyridoxal (VIIIb). Method A. To a solution of IIIb (50 mg, 0.25 mmol) in water (10 ml), MnO_2 (200 mg) was added, and oxidation was carried out for 2.5 hr. Preparative tlc (SiO_2, EtOAc) separated compound VIIIb (R_f 0.85) from the corresponding presumed 4-acid (R_f 0.1), which was fluorescent. Elution of the upper yellow streak with EtOAc and recrystallization from Me_2CO–Et_2O gave 37 mg (75%) of VIIIb, mp 160°. *Anal.* ($C_8H_8ClNO_3$) C, H, N.

Method B. Chlorination of the ethyl acetal of pyridoxal was carried out as described by Stambolieva, *et al.*[4] (*Cf.* the chlorination of α^4,α^5-*O*-isopropylidenepyridoxol as described earlier in the present paper.) The melting point of the product (after hydrolysis of the acetal) was 163–165° (lit.[4] mp 124–125°). The sample ob-

tained by method A was identical in ir and nmr spectra with that obtained by method B.

6-Fluoropyridoxal Oxime (IXa). The reaction product from oxidation of IIIa (15 mg) with MnO_2 (50 mg) was heated with $NH_2OH \cdot HCl$ (25 mg) and NaOAc (50 mg), the mixture was stirred for 0.5 hr, and the precipitate was filtered off. After washing with H_2O, the oxime was crystallized from EtOH: yield 11 mg; mp 235–236° dec. *Anal.* ($C_8H_9FN_2O_3$) C, H, N.

6-Fluoropyridoxamine Hydrochloride (X). A solution of the oxime IXa (35 mg, 0.17 mmol) in AcOH (15 ml) was hydrogenated in the presence of 10% Pd/C (38 mg) for 20 hr at atmospheric pressure. The hydrogenation product was converted to the HCl salt by treatment with 6 *N* HCl, repeated evaporation with EtOH, and recrystallization from Et_2O–MeOH, giving 19 mg (48%) of X, mp 190–195° dec. *Anal.* ($C_8H_{12}ClFN_2O_2 \cdot 0.5H_2O$) C, H, N.

6-Fluoropyridoxamine Phosphate (XI). 6-Fluoropyridoxamine hydrochloride (X, 60 mg, 0.27 mmol) and polyphosphoric acid (2 ml, prepared from 5.2 g of 85% H_3PO_4 and 4.0 g P_2O_5) were mixed, and the mixture was heated on a steam bath at 60° for 2 hr. Then 1 *N* HCl (2.5 ml) was added, and the mixture was heated on a steam bath for 15 min. The solution was concentrated *in vacuo*, and concentrated NH_4OH was added to neutralize the acids. The product was chromatographed on an Amberlite CG50 (H^+) column with H_2O. Fractions with a uv absorption of 290 mμ were combined, evaporated, and crystallized from EtOH. The last traces of NH_4Cl were removed by sublimation (at 5×10^{-5} mm), and the compound was crystallized from EtOH–Et_2O, yielding 42 mg (46%) of XI, mp 220–225° dec. *Anal.* ($C_8H_{12}FN_2O_5P \cdot 2H_2O$) C, N; H: calcd, 5.33; found, 4.59.

Acetonation of 6-Fluoropyridoxol. To Me_2CO (40 ml, dried over K_2CO_3) was added 6-fluoropyridoxol (dry, 4.0 mmol, 748 mg), followed by *p*-toluenesulfonic acid monohydrate (1.52 g, 8.0 mmol), and the mixture was shaken for 14 hr. $NaHCO_3$ (5.0 g) in H_2O (25 ml) was added, the mixture was flash-evaporated, and the residue was extracted with EtOAc. After drying, the EtOAc solution was evaporated, and the residue was spotted on tlc. The upper spot (R_f 0.85) was Gibbs positive,[7] indicating that the phenolic hydroxyl was unsubstituted, whereas the lower spot (R_f 0.80) was Gibbs negative, indicating a cyclic acetonide in the form of a six-membered ring. Nmr spectroscopy of the mixture in $CDCl_3$ showed two isopropylidene methyls at δ 1.53 and 1.57, respectively, confirming the presence of cyclic ketals with six- and seven-membered rings. The mixture (50 mg) was separated on a preparative plate provided with a 1 *N* NaOH strip just above the spotting region to retain the phenolic constituent. The plate was developed with EtOAc, and the zone of R_f 0.80 was scraped off and extracted with EtOAc, yielding 30 mg of 6-fluoro-α^4,3-*O*-isopropylidenepyridoxol (XIV) as an oil: nmr ($CDCl_3$) δ 2.37 (2-CH_3), 1.55 [$C(CH_3)_2$ of the acetonide], 4.68, 5.05 (α^4- and α^5-CH_2, respectively).

6-Fluoro-α^4,3-*O*-isopropylideneisopyridoxal (XIV). The crude acetonation product (550 mg obtained from 748 mg of 6-fluoropyridoxol) in $CHCl_3$ (150 ml) was stirred with MnO_2 (2.1 g) for 18 hr at room temperature. Filtration, washing with $CHCl_3$ (200 ml), and evaporation yielded the aldehyde as the major product (R_f 0.91 in EtOAc, positive $PhNHNH_3$ test). A sample (50 mg) of the reaction mixture was purified by preparative tlc, and the aldehyde was obtained as an oil: nmr ($CDCl_3$) δ 1.55 [$C(CH_3)_2$], 2.47 (2-CH_3), 5.23 (4-CH_2), 10.45 (CHO).

6-Fluoro-α^4,3-*O*-isopropylideneisopyridoxal Oxime (XV). The crude oxidation product (220 mg) was dissolved in pyridine (7 ml), $NH_2OH \cdot HCl$ (250 mg) was added, and the mixture was heated on a steam bath for 15 min and then was stirred at room temperature for 2.5 hr. After evaporation *in vacuo*, the residue was dissolved in EtOAc, the solution was washed with H_2O, and the solvent was evaporated. A small amount of crystalline material was obtained on recrystallization from Et_2O–MeOH (25 ml) and the rest (125 mg) on preparative tlc (R_f 0.92 in EtOAc). The compound starts shrinking at 125° and gradually melts until 205°: mass spectrum M^+ 240, $M^+ - 16$, and $M^+ - 18$; nmr ($CDCl_3$) δ 1.58 [$C(CH_3)_2$], 2.42 (2-CH_3), 5.05 (4-CH_2), 8.47 (=NOH). *Anal.* ($C_{11}H_{13}FN_2O_3$) C, H, N.

6-Fluoroisopyridoxal Oxime (XVI). Method A. The isopropylidene oxime (XV, 50 mg) was cleaved by stirring with 50% formic acid (10 ml) overnight at room temperature. The product was filtered, and the acid was evaporated. MeOH was added and evaporated to remove remaining acid, and the process was repeated twice. The residue was recrystallized from H_2O; mp 145–150° (R_f 0.25 in 1:1 C_6H_6–Et_2O). *Anal.* ($C_8H_9FN_2O_3$) C, H, N.

Method B. A small yield of oxime was obtained by hydrolyzing 6-fluoro-α^4,3-*O*-isopropylideneisopyridoxal (XIV, 65 mg) with 0.1 *N* HCl (5 ml) and MeOH (5 ml) at 80° for 1.5 hr, removing the solvent, adding $NH_2OH \cdot HCl$ (25 mg) and NaOAc (50 mg) in aqueous MeOH, heating for 5 min, and subsequent stirring for 2 hr at room temperature. After evaporation and addition of H_2O, 7 mg of the oxime was obtained.

Acknowledgment. This study was supported in part by research grants (CA-08793 and CA-13038) from the National Cancer Institute, U. S. Public Health Service. We are indebted to Dr. M. T. Hakala and Miss A. Mulhern for the tissue-culture data reported and to Dr. G. Grindey for the *in vivo* tests. Mr. N. Angelino determined the inhibitory activity of XI against pyridoxine phosphate oxidase. Pyridoxol hydrochloride was a gift of Pfizer and Co.

References

(1) (a) F. E. Cole, B. Lachmann, and W. Korytnyk, *J. Heterocycl. Chem.*, **9**, 1129 (1972); (b) Abstracts of papers, 5th International Pharmacological Congress, San Francisco, Calif., 1972, p 129.
(2) W. Korytnyk and M. Ikawa, *Methods Enzymol.*, 18A, 524 (1970).
(3) R. K. Blackwood, G. B. Hess, C. E. Larrabee, and F. J. Pilgrim, *J. Amer. Chem. Soc.*, **80**, 6244 (1958).
(4) N. A. Stambolieva, M. Ya. Karpeiskii, and V. L. Florentiev, *Khim. Geterotsikl. Soedin.*, 493 (1971).
(5) A. R. Katritzky, H. Z. Kucharska, M. J. Tucker, and H. M. Wuest, *J. Med. Chem.*, **9**, 620 (1966).
(6) H. Ahrens and W. Korytnyk, *J. Heterocycl. Chem.*, 4, 625 (1967).
(7) H. D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927); J. V. Scudi, *ibid.*, **139**, 707 (1941).
(8) H. C. Brown and D. H. McDaniel, *J. Amer. Chem. Soc.*, 77, 3752 (1955).
(9) D. E. Metzler and E. E. Snell, *ibid.*, 77, 2431 (1955).
(10) W. Korytnyk and H. Ahrens, *Methods Enzymol.*, 18A, 475 (1970).
(11) D. C. DeJongh and W. Korytnyk, *ibid.*, 18A, 483 (1970).
(12) W. Korytnyk, B. Lachmann, and N. Angelino, *Biochemistry*, 11, 722 (1972).
(13) J. Hurwitz, *J. Biol. Chem.*, **217**, 513 (1955).
(14) H. Ahrens and W. Korytnyk, *Methods Enzymol.*, 18A, 489 (1970).
(15) W. Korytnyk, *J. Org. Chem.*, **27**, 3724 (1962).
(16) K. B. Wiberg, *Org. Syn.*, **49**, 9 (1969).