Pyridoxine Chemistry. VI. Homologs of Pyridoxol and of 5-Pyridoxic Acid[1,2]

W. KORYTNYK

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo 3, New York

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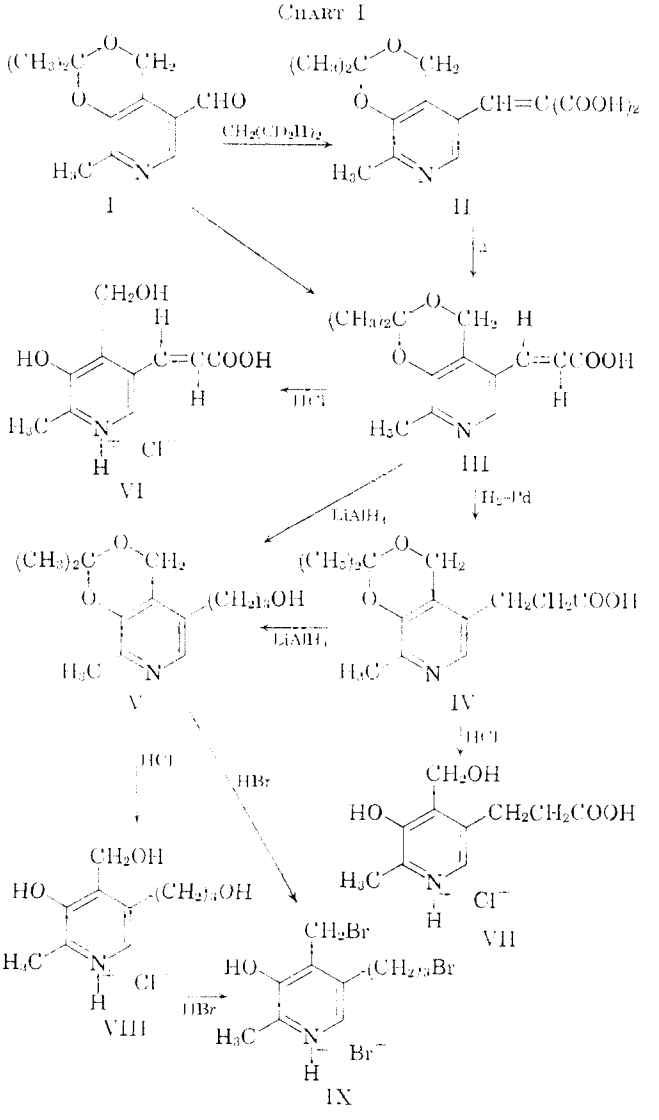
The 5-side chain in pyridoxol has been extended by two carbon atoms to yield homologs of 5-pyridoxic acid and pyridoxol. Derivatives of these homologs have been prepared. Some of these compounds are very potent antagonists of pyridoxine. Their n.m.r. spectra are discussed in relation to their biological properties.

Relatively little attention has been given to designing antagonists of vitamin B_6, the dimensions of which would approach those of its cofactor form, namely pyridoxal phosphate. Generally, emphasis has been placed on small modifications of the pyridoxol molecule itself, which alter its shape and size to only a small extent. It was therefore of considerable interest to obtain compounds in which the side chain in the 5-position is extended, as in α^5-pyridoxylacetic acid (VII) or in the pyridoxol homolog VIII. It can be readily demonstrated with molecular models that the 5-side chain in these compounds is almost isosteric with the 5-methylphosphoric acid group of pyridoxal phosphate. Along similar lines, Bennett, Burger, and Umbreit[3] previously described the synthesis and some biological properties of 5-deoxypyridoxine-5-phosphonic acid and its derivatives.[4] It should be pointed out that the space occupied by the 5-methyl phosphoric acid group on the apoenzyme surface is considerably greater than that occupied by the substituents in other positions of the molecule. It becomes obvious, therefore, to modify the 5-position of the pyridoxol molecule in such a way that the resulting derivatives can be "fitted" into the space otherwise occupied by the 5-methylphosphoric acid group of pyridoxal phosphate. Such compounds could displace the cofactor directly, without the necessity of being phosphorylated first. It has been postulated that 4-deoxypyridoxine has to be phosphorylated first in order to displace the cofactor.[5] The synthesis of the homologs and their derivatives is portrayed in Chart I.

Reduction of either α^4,3-O-isopropylidene-α^5-pyridoxylideneacetic acid[6] (III) or α,3-O-isopropylidene-α^5-pyridoxylacetic acid (IV) with lithium aluminum hydride in tetrahydrofuran gave α^4,3-O-isopropylidene-α^5-pyridoxylethanol (V). In the case of the unsaturated acid III, it was impossible to reduce the carboxyl group selectively without reducing the double bond at the same time, even when mild conditions were used.[7]

CHART I



Nevertheless it was found convenient to reduce the double bond catalytically before undertaking reduction with lithium aluminum hydride. The product thus obtained was purer as shown by bioassay with *Saccharomyces carlsbergensis* ATCC 9080.

In contrast to 5-pyridoxic acid, the homologous α^5-pyridoxylacetic acid hydrochloride VII[8] had no tendency to lactonize.

Recently McCasland, *et al.*,[9] have halogenated the 4-

(1) Preceding paper in this series: W. Korytnyk, E. J. Kris, and R. P. Singh, *J. Org. Chem.*, **29**, 574 (1964).

(2) Presented in part at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept. 1963.

(3) R. Bennett, A. Burger, and W. W. Umbreit, *J. Med. Pharm. Chem.*, **1**, 213 (1959).

(4) In this connection, it should be mentioned that M. Matsuda and M. Makino [*Biochim. Biophys. Acta*, **48**, 194 (1961)] found that the activation of glutamic acid decarboxylase by pyridoxal 5-phosphate is inhibited competitively by pyridoxal 5-sulfate.

(5) J. Harwitz, *J. Biol Chem.*, **217**, 513 (1955); W. W. Umbreit and J. G. Waddell, *Proc. Soc. Exptl. Biol. Med.*, **70**, 293 (1949).

(6) It was convenient to name these and other compounds referred to in this paper as pyridoxol derivatives according to nomenclature consistent with that used in previous papers of this series.[1]

(7) R. F. Nystrom and W. G. Brown [*J. Am. Chem. Soc.*, **69**, 2548 (1947)] have made a similar observation in the case of the closely analogous cinnamic acid.

(8) Dr. David E. Metzler (private communication) and his co-workers have obtained this acid by a different method. The identity of the products of the two methods has been demonstrated by a mixture melting point determination.

(9) G. E. McCasland, L. K. Gottwald, and A. Furst, *J. Org. Chem.*, **26**, 3511 (1961).

and 5-side chains of pyridoxol and 3-amino-3-deoxypyridoxol in the hope of obtaining alkylating agents having pyridoxol-like structures. Similarly, we have treated the analog VIII and its isopropylidene derivative V each with hydrobromic acid and have obtained the corresponding dibromide hydrobromide IX.

The 5-hydroxymethyl side chain in isopropylidenepyridoxol was found to behave in an anomalous fashion, and a number of derivatives could not be obtained.[10] Thus, the compound did not yield a *p*-toluenesulfonate by interaction with *p*-toluenesulfonyl chloride in pyridine.[10,11] The isopropylidenepyridoxol homolog V appears to react in the normal way, giving the *p*-toluenesulfonate ester under normal reaction conditions.

The inability to form normal sulfonates from α^4,-3-O-isopropylidenepyridoxol is probably due to the formation of a quaternary salt. The primary hydroxy group in α^4,3-O-isopropylidenepyridoxol probably reacts first with *p*-toluenesulfonyl chloride in a normal manner to give a very reactive *p*-toluenesulfonate, which immediately quaternizes to yield the pyridinium *p*-toluenesulfonate salt. Such quaternizations are known to occur in *p*-toluenesulfonates of some carbohydrates.[12]

Although there are three one-carbon side chains in the pyridoxol molecule, only the side chain in the 2-position has been extended previously to provide higher homologs. This has been accomplished readily either by adaptations[13] of the Harris–Folkers pyridoxol synthesis or by adaptations[14] of the method developed by Cohen, *et al.*[15] Although the higher homologs of pyridoxol exhibit little or no antimetabolite activity, the ethyl homolog ("ω-methylpyridoxine") is one of the most interesting antimetabolites, and has been studied extensively in various biological systems.[16]

The homologs described in this paper are also of some biological interest. 2-(α^5-Pyridoxyl)-1-ethanol (VIII) was found to be a more potent inhibitor of the growth of *Saccharomyces carlsbergensis* (ATCC 9080) than 4-deoxypyridoxine. In an assay medium containing 1 mγ/ml. of pyridoxal, 2-(α^5-pyridoxyl)-1-ethanol inhibited growth of the test organism by 50% at the concentration of 5×10^{-8} *M*, whereas 4-deoxypyridoxine was only one-tenth as active. Inhibition by the homolog VIII was prevented in a competitive manner by pyridoxol, pyridoxal, and pyridoxamine. The dibromide IX and the *p*-toluenesulfonate of α^4,3-O-isopropylidene-α^5-pyridoxyl-1-ethanol were about as active as 2-(α^5-pyridoxyl)-1-ethanol, presumably because they were hydrolyzed to the parent compound under the conditions of testing. On the other hand, α^5-pyridoxylacetic acid hydrochloride (VII) was found to be less active in the same biological system. The metabolism of these compounds is being studied, expecially in microbial systems, and a preliminary report on this work has appeared.[17]

Nuclear Magnetic Resonance Spectra.—The recent finding[18] that the trifluoro analog of 4-deoxypyridoxine (5-hydroxy-6-methyl-4-trifluoromethyl-3-pyridinemethanol) was completely inactive as an antagonist of pyridoxine clearly indicates that the electronic effects of substituents play an important role in determining the biological activity of pyridoxine analogs. N.m.r. spectroscopy has now emerged as an excellent tool for assessment of electron densities; satisfactory correlations have been made in biologically important molecules between proton shifts and electron densities as calculated by the molecular orbital method.[19]

A similar relationship should apply also to pyridoxine analogs, and it has been shown in a number of instances that proton shifts could be correlated with the expected changes in electron densities.[1,20] Thus, the positions of the C-6 proton, the 2-CH_3 protons, and 5-hydroxymethyl protons, respectively, are almost identically the same in the spectra of the most potent antimetabolites of pyridoxine, 4-deoxypyridoxol, and 4-methoxypyridoxol, as in that of pyridoxol itself. Thus it appears that these molecules must have very similar electron distributions. On the other hand, the positions of the peaks in the spectrum of 3-deoxypyridoxol, a much weaker antimetabolite, are appreciably different.[20]

Comparisons (Table I) of the n.m.r. spectra of the pyridoxylethanol anion (XIII) and the pyridoxol anion (XIV), the corresponding cations (XV and XVI), and the isopropylidene derivatives (XVII and XVIII) indicate that the positions of corresponding peaks are similar, and hence that the molecules must have similar electronic properties. Thus, in addition to the steric consideration mentioned at the beginning of this paper, electronic properties should be studied and related to biological activities.

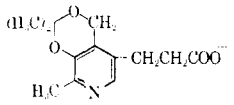
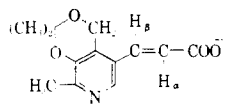
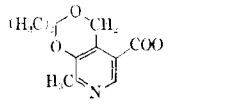
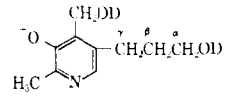
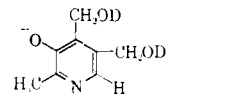
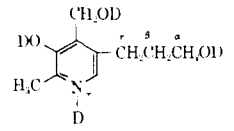
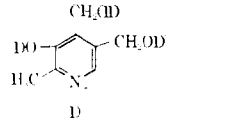
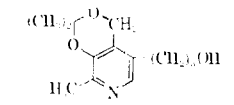
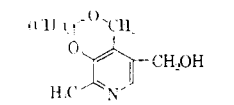
A comparison of the n.m.r. spectra of the carboxylic acids X–XII demonstrates the effect of the carboxylate anion on the shielding of the C-6 proton. The electron-withdrawing effect of the carboxylate anion is much in evidence in α^4,3-O-isopropylidene-5-pyridoxic acid (XII), in which the C-6 proton is considerably deshielded with respect to the saturated two-carbon acid X. As expected, the electron-withdrawing effect of the carboxylate anion is not transmitted through a saturated system in X, but the C-6 proton is deshielded in the unsaturated acid XI, indicating that its electron-withdrawing effect is transmitted through a conjugated system. The magnitude of the splitting constant of H_α,H_β in the unsaturated acid XI is 16 c.p.s., and hence it must be *trans*. The same configurations should apply to analogous β-pyridylacrylic acids[21] described in the literature.

Infrared Spectra.—Pyridinecarboxylic acids in which the carboxyl group is attached directly to the pyridine nucleus were found to have anomalous infrared

(10) J. Baddiley and A. P. Mathias, *J. Chem. Soc.*, 2583 (1952).
(11) W. Korytnyk and W. Wiedeman, *ibid.*, 2531 (1962).
(12) R. S. Tipson, *Advan. Carbohydrate Chem.*, **8**, 107 (1953).
(13) (a) S. A. Harris and A. N. Wilson, *J. Am. Chem. Soc.*, **63**, 2526 (1941); (b) D. Heyl, E. Luz, S. A. Harris, and K. Folkers, *ibid.*, **75**, 4079 (1953).
(14) (a) A. Cohen and J. A. Silk, *J. Chem. Soc.*, 4386 (1952); (b) H. Daroll and F. B. Kipping, *ibid.*, 1395 (1953).
(15) A. Cohen, J. W. Haworth, and E. C. Hughes, *ibid.*, 4374 (1952).
(16) E. E. Snell in "Vitamins and Hormones," Vol. XVI, Academic Press Inc., New York, N. Y., 1958, p. 77.
(17) C. A. Nichol, A. Bloch, W. Korytnyk, E. Mihich, and F. Rosen Abstracts, Sixth International Congress of Biochemistry, New York, N. Y., 1964, p. 433.
(18) J. L. Greene, Jr., and J. A. Montgomery, *J. Med. Chem.*, **6**, 294 (1963).
(19) A. Veillard and B. Pullman, *Compt. rend.*, **253**, 2418 (1961).
(20) W. Korytnyk and R. P. Singh, *J. Am. Chem. Soc.*, **85**, 2813 (1963).
(21) L. Panizzon, *Helv. Chim. Acta*, **24**, 24E (1941); R. N. Castle and A. Burger, *J. Am. Pharm. Assoc., Sci. Ed.* **43**, 163 (1954); C. S. Marvel, L. E. Coleman, and G. P. Scott, *J. Org. Chem.*, **20**, 1785 (1955).

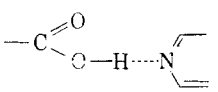
TABLE I

NUCLEAR MAGNETIC RESONANCE SPECTRA[a]

No.	Compd.	Solvent	2-CH	3-OH	C=CH	$C(CH_3)_2$	Others
X		1 *N* NaOD	−134	−292	−466	−89	Multiplet at −253
XI		1 *N* NaOD	−132	−289	−478	−87	H_α −370, −386 (doublet) H_β −467, −483 (doublet)
XII		1 *N* NaOD	−138	−308	−498	−89	
XIII		1 *N* NaOD	−136	−284	−447	...	Multiplets at −105 and −147 (β- and γ-protons) Triplet at −214 (α-protons)
XIV		1 *N* NaOD	−138	−286	−449	...	
XV		D_2O	−154	−289	−483	...	Multiplets at −108 and −164 (β- and γ-protons) Triplet at −216 (α-protons)
XVI		D_2O	−156	−299	−488	...	
XVII		$CDCl_3$	−143	−290	−467	−92	Multiplet at −250
XVIII		$CDCl_3$	−140	−296	−466	−91	OH, −265

[a] Expressed in c.p.s. units at 60 Mc. In D_2O solution, 1,4-dioxane was used as an internal standard, as described in ref. 1. Spectra in chloroform-*d* were determined from tetramethylsilane as an internal standard.

spectra.[22] Instead of having absorption bands characteristic of dimeric carboxyl groups at 7.04 (1420 cm.$^{-1}$) and 10.77 μ (920 cm.$^{-1}$), they have broad bands at 4.08 (2450 cm.$^{-1}$) and at 5.26 μ (1900 cm.$^{-1}$). This has been interpreted as indicating intermolecular hydrogen bonding of the following type.



It was of interest to observe that the pyridinecarboxylic acids III and IV, which have the carboxyl groups on the extended side chains, also show similar broad bands at 4.10 (2439 cm.$^{-1}$), 4.27 (2342 cm.$^{-1}$), and 5.40 μ (1852 cm.$^{-1}$), but no bands which could be assigned to dimeric structures. The corresponding pyridine acids with free phenolic and 4-hydroxymethyl groups (VI and VII) do not have absorption bands which are either consistent with the dimeric structure or the intermolecular hydrogen bond as discussed above. Here opportunities exist for intermolecular hydrogen bonding of a different type, involving the phenolic oxygen function. The corresponding bands are probably so broadened that they could not be recognized in the spectrum.

In contrast to pyridoxol hydrochloride, which exhibits only one OH stretching band at 3.07 μ (3257 cm.$^{-1}$), the homolog VIII exhibits two well-resolved bands at 2.97 (3367 cm.$^{-1}$) and 3.15 μ (3175 cm.$^{-1}$), respectively.

(22) S. Yoshida and M. Asai, *Chem. Pharm. Bull.* (Tokyo), **7**, 162 (1959); *Chem. Abstr.*, **54**, 22008*i* (1960).

Experimental

α^4,3-O-Isopropylidene-α^5-pyridoxylidenemalonic Acid (II). — Isopropylideneisopyridoxal[1] (5.0 g., 24.1 mmoles) and malonic acid (2.51 g., 24.1 mmoles) were dissolved in 95% aqueous ethanol containing 0.5 ml. of pyridine. After gentle refluxing for 45 min., pyridoxylidenemalonic acid precipitated out (4.9 g., 56%); m.p. 200–201° dec. after a single crystallization from ethanol.

Anal. Calcd. for $C_{14}H_{15}NO_6$: C, 57.33; H, 5.16; N, 4.78. Found: C, 56.98; H, 5.26; N, 4.75.

α^4,3-O-Isopropylidene-α^5-pyridoxylideneacetic Acid (III). A.—Isopropylideneisopyridoxal (19.72 g., 95.2 mmoles) and malonic acid (9.92 g., 95.2 mmoles) were dissolved in anhydrous pyridine (25 ml.) to which piperidine (2 ml.) had been added. The reaction mixture was heated for 2 hr. on a steam bath, and was then kept at 5° overnight. Filtration, followed by washing with ether, yielded 19.52 g. (82%) of the product, m.p. 220–221°. An analytical sample was recrystallized from ethanol; $\lambda_{max}^{0.1\ N\ HCl}$ 237.2 mμ (ε 19,900), 307.5 mμ (ε 9800); λ_{max}^{Nujol} 4.10, 4.27, 5.40 (broad), 5.89 (C=O), 7.86, 8.13, 8.72, 9.39, 10.14, 10.43, 10.69, 10.99, 11.35, 11.48, 12.43, 13.73 μ.

Anal. Calcd. for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.58; H, 6.01; N, 5.76.

B.—The same product was obtained in only 19% yield under conditions similar to those described for the preparation of α^4,3-O-isopropylidene-α^5-pyridoxylidenemalonic acid, except that refluxing was continued for another 7 hr. The product (m.p. 220–221°) crystallized from the reaction mixture when kept at 5° overnight.

α^5-Pyridoxylideneacetic Acid Hydrochloride (VI).—α^4,3-O-Isopropylidene-α^5-pyridoxylideneacetic acid (0.413 g., 2.0 mmoles) was treated with 200 ml. of ether containing 10 ml. of 0.1 *N* aqueous HCl. After standing 16 hr. at room temperature, the resulting precipitate was collected and recrystallized from aqueous ethanol; yield, 0.35 g. (72%); m.p. 255–260° dec.; $\lambda_{max}^{0.1\ N\ HCl}$ 233 mμ (ε 23,700), 305 mμ (ε 11,600); λ_{max}^{Nujol} 3.12 (OH), 5.83 (C=O), 6.47, 6.61, 8.04, 8.52, 9.70, 11.40, 12.18, 13.0 μ.

Anal. Calcd. for $C_{10}H_{12}ClNO_4$: C, 48.89; H, 4.92; N, 5.70. Found: C, 48.87; H, 4.95; N, 5.93.

α^4,3-O-Isopropylidene-α^5-pyridoxylacetic Acid (IV).—α^4,3-O-Isopropylidene-α^5-pyridoxylideneacetic acid (10.77 g, 43 mmoles) was suspended in ethyl alcohol (150 ml.) and was hydrogenated with H_2 at 2.81 kg./cm.^{2} (40 p.s.i.) for 4 hr. in the presence of 5% palladium on charcoal in a Parr hydrogenation apparatus. After filtration and evaporation to 25 ml., 10.52 g. (97.5%) of the product, m.p. 188–190°, crystallized on standing at 5° overnight; $\lambda_{max}^{0.1\ N\ HCl}$ 290 mμ (ε 8900), shoulder at 225 mμ (ε 3300); λ_{max}^{Nujol} 4.12, 4.28, 5.20 (broad), 5.90 (C=O), 7.76, 7.93, 8.20, 8.79, 9.41, 10.42, 10.99, 10.25, 11.50, 12.45, 15.00 μ.

α^5-Pyridoxylacetic Acid Hydrochloride (VII).—A solution of α^4,3-O-isopropylidene-α^5-pyridoxylacetic acid (1.0 g., 4.2 mmoles) in 50 ml. of 0.1 *N* aqueous HCl was heated on a steam bath for 1 hr. Evaporation *in vacuo* on a flash evaporator gave a solid product, which was recrystallized from ethanol, m.p. 214–215° dec.; λ_{max}^{Nujol} 3.11 (OH), 5.86 (C=O), 6.52, 7.92, 8.09, 8.47, 9.68, 10.25, 11.44, 11.94, 12.60, 14.38 μ.

Anal. Calcd. for $C_{10}H_{14}ClNO_4$: C, 48.51; H, 5.70; Cl, 14.32; N, 5.66. Found: C, 48.79; H, 5.74; Cl, 14.22; N, 5.56.

α^4,3-O-Isopropylidene-α^5-pyridoxyl-1-ethanol (V).—A solution of α^4,3-O-isopropylidene-α^5-pyridoxylacetic acid (7.5 g., 29.9 mmoles) in 250 ml. of tetrahydrofuran was added to a well-stirred suspension of $LiAlH_4$ (3.0 g.) in tetrahydrofuran (50 ml.) over a period of 10–15 min. Stirring was continued for 1 hr. Unreacted $LiAlH_4$ was destroyed by the addition of ethyl acetate, water (500 ml.) was added, and the solution was extracted three times with ethyl acetate. The ethyl acetate extract was dried (Drierite), evaporated to 10–15 ml., and allowed to crystallize at 5°, yielding 5.0 g. (71%), m.p. 112–113°. Recrystallization from ethyl acetate raised the m.p. to 117–118°; $\lambda_{max}^{0.1\ N\ HCl}$ 290 mμ (ε 8800), 232 mμ (shoulder) (ε 2700); λ_{max}^{EtOH} 281 mμ (ε 5700); $\lambda_{max}^{0.1\ N\ NaOH}$ 281 mμ (ε 6400); λ_{max}^{Nujol} 3.12 (OH), 6.22, 6.48, 7.82, 8.02, 8.13, 8.32, 8.77, 9.18, 9.42, 10.50, 10.82, 11.58, 12.63, 13.22, 13.82, 14.34, 15.02 μ.

Anal. Calcd. for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.59; H, 8.15, N, 6.03.

Treatment of this compound with an excess of *p*-toluenesulfonyl chloride in collidine for 24 hr. gave the *p*-toluenesulfonate, m.p. 88–90° (from ethanol).

Anal. Calcd. for $C_{20}H_{25}NO_5S$: C, 61.51; H, 6.44; S, 8.19. Found: C, 61.66; H, 6.67; S, 8.19.

2-(α^5-Pyridoxyl)-1-ethanol Hydrochloride [3-Hydroxy-4-hydroxymethyl-5-(3-hydroxypropyl)-2-methylpyridine Hydrochloride] (VIII).—α^4,3-O-isopropylidene-α^5-pyridoxyl-1-ethanol (0.12 g., 0.51 mmole) in 20 ml. of 1 *N* aqueous HCl was heated on a steam bath for 30 min. The water was evaporated *in vacuo*, and the yield was 0.11 g. (92%), m.p. 142–143°. The sample was recrystallized from aqueous ethanol; $\lambda_{max}^{0.1\ N\ HCl}$ 292 mμ (ε 9200); $\lambda_{max}^{ethanol}$ 293 mμ (ε 6500); $\lambda_{max}^{0.1\ N\ NaOH}$ 244 mμ (ε 6100), 307 mμ (ε 7100); λ_{max}^{Nujol} 2.97 (OH), 3.15 (OH), 6.53, 7.72, 8.10, 8.22, 9.47, 9.58, 9.70, 9.82, 10.40, 10.78, 11.23, 11.84, 13.17, 13.83 μ.

Anal. Calcd. for $C_{10}H_{16}ClNO_3$: C, 51.41; H, 6.90; Cl, 15.18; N, 6.00. Found: C, 51.61; H, 6.97; Cl, 15.40; N, 6.11.

3-Hydroxy-4-bromomethyl-5-(3-bromopropyl)-2-methylpyridine Hydrobromide (IX).—3-Hydroxy-4-hydroxymethyl-5-(3-hydroxypropyl)-2-methylpyridine hydrochloride (0.24 g.) was heated for 30 min. with 41% aqueous HBr, and then was refluxed for 10 min. Keeping the mixture in a refrigerator for several hours provided bunches of needles. These were filtered and washed with acetone. The yield was 0.110 g., m.p. 130–132°.

Anal. Calcd. for $C_{10}H_{14}Br_3NO$: C, 29.73; H, 3.49; Br, 59.35. Found: C, 30.32; H, 3.73; Br, 59.04.

The same dibromide hydrobromide (IX) was obtained when α^4,3-O-isopropylidene-α^5-pyridoxyl-1-ethanol (V) was treated with HBr under similar reaction conditions.

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