Synthesis and Antagonist Properties of Pyridoxol Analogs Modified in the 5 Position[1]

W. KORYTNYK, B. PAUL, A. BLOCH, AND C. A. NICHOL

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14203

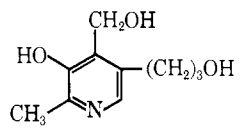
Received December 5, 1966

In order to determine which structural changes in the 5 position of pyridoxol give rise to growth inhibitors, a number of analogs have been synthesized in which the side chain in this position has been modified with respect to length, branching, and nature of the end group. The homologs of pyridoxol obtained by extending the 5-hydroxymethyl group by 1–3 carbon atoms are the most potent antagonists. In a semisynthetic medium these analogs reduced the growth of *Saccharomyces carlsbergensis* by 50% at 5–8 × 10^{-8} *M*. Replacement of the terminal alcoholic group by hydrogen or halogen in the three-carbon homolog did not change the potency significantly, indicating that the *in vivo* phosphorylation of these analogs is not a prerequisite for their activity. Other functional end groups, such as carboxyl or amino, decreased the activity of the analogs markedly, giving 50% inhibition only at 3 × 10^{-4} to 1 × 10^{-5} *M*. Branching of the side chain also decreased the activity. The α^4,3-O-isopropylidene derivatives of these analogs have, in general, less activity than do the respective parent compounds. A stable amino acid analog, 2-(α^5-pyridoxyl)aminoacetic acid, has also been synthesized.

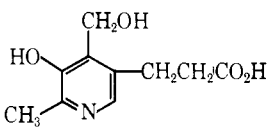
Pyridoxol[2] analogs can interfere with the function of enzymes for which pyridoxal phosphate is a cofactor either by displacing the cofactor or by inhibiting its formation.[3] Other effects of the analogs, such as interference with cellular uptake of the vitamin, may also be involved. Since inhibition of the vitamin B_6 metabolism has been shown to elicit carcinostatic effects, the analogs synthesized may prove of value in cancer chemotherapy.[4]

The present paper is concerned mainly with the chemical methods used for modifying pyridoxol in the 5 position, and with the antagonist activity of the compounds thus obtained, as determined with *Saccharomyces carlsbergensis* (ATTC No. 9080).

Previous papers[5,6] have described the synthesis and the inhibitory properties of 3-hydroxy-4-(hydroxymethyl)-5-(hydroxypropyl)-2-methylpyridine (I), an analog of pyridoxol in which the 5-hydroxymethyl side



I



II

chain is extended by two carbon atoms. Considering the pronounced growth inhibitory activity of this compound it was thought of interest to obtain additional analogs differing in shape and size of the side chain and to compare their activity with that of the three-carbon homolog. Accordingly, the side chain in I was lengthened or shortened by one carbon atom, and some simple branched-chain homologs were prepared.

Since several pyridoxol analogs containing the free 5-hydroxymethyl group are phosphorylated by pyridoxal phosphokinase,[7] the question arose whether phosphorylation of the 5-terminal hydroxy group in I is essential for biological activity. Compounds were therefore prepared wherein the 5-terminal hydroxy group was replaced by hydrogen or halogen.

The observation that the propionic acid II has a considerably lower inhibitory activity than the propyl alcohol I suggested that factors such as polarity may influence the effectiveness of the compounds. Other analogs were therefore prepared in which the polarity was reduced by converting the carboxyl group in II to an amide or an ester.

Because of the nature of the synthetic methods used, most of the analogs described in this paper were available both as the parent compound and as α^4,3-O-isopropylidene derivatives. The biological activities of the two types of analogs are compared.

Chemistry.—An improved method for synthesizing α^4,3-O-isopropylidenepyridoxol[8] and various key intermediates derived from it[9] permitted the systematic chemical modification of pyridoxol in the 5 position. Reactions involving the isopropylidene derivatives were carried out under alkaline or neutral conditions, and the products were subjected to acid hydrolysis to obtain the desired compounds. In most cases, the procedure yielded a single compound of high purity. The synthetic routes used for the preparation of the analogs are shown in Schemes I–VI.

The nitrile XIX and the carboxylic acid XX have also been obtained by Tomita, *et al.*[10] The acid XXIII had no tendency to lactonize, in contrast to the well-known tendency of the 4- and 5-pyridoxic acids toward lactonization.

Condensation of isopropylideneisopyridoxal (XXIV) with nitromethane to give the nitro olefin XXV was carried out in absolute alcohol in the presence of anhydrous sodium carbonate and methylamine hydrochloride. When the reaction was carried out with NaOH instead of sodium carbonate, or in toluene in the pres-

(1) This investigation was supported by U. S. Public Health Service grants (CA-05697 and CA-08793). It represents paper XIII in the series "Pyridoxine Chemistry." Previous paper in this series: W. Korytnyk, G. Fricke, and B. Paul, *Anal. Biochem.*, **17**, 66 (1966). Part of this study was reported at the 148th National Meeting of the American Chemical Society, Chicago, Ill., Aug–Sept 1964, Abstracts, p 12P.

(2) The compound is generally known as pyridoxine. The nomenclature used here and in the previous publications in this series is that recommended by the IUPAC [*J. Am. Chem. Soc.*, **82**, 5545 (1960)], according to which the name pyridoxine has been extended to designate all naturally occurring pyridine derivatives with vitamin B_6 activity. Analogs of pyridoxol are designated as derivatives of the parent structure whenever feasible; otherwise, systematic nomenclature is followed.

(3) E. E. Snell, *Vitamins Hormones*, **16**, 77 (1958).

(4) F. Rosen, E. Mihich, and C. A. Nichol, *ibid.*, **22**, 609 (1964).

(5) W. Korytnyk, *J. Med. Chem.*, **8**, 112 (1965).

(6) 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.

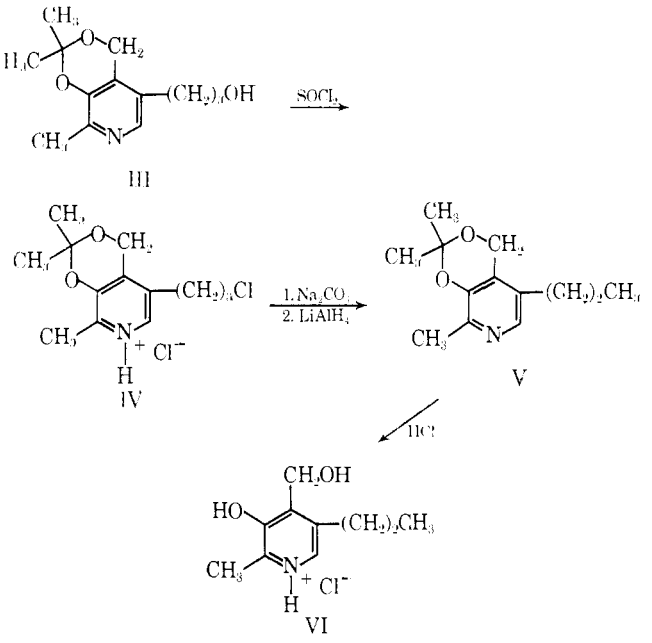
(7) J. Hurwitz, *J. Biol. Chem.*, **217**, 513 (1955).

(8) W. Korytnyk and W. Wiedeman, *J. Chem. Soc.*, 2531 (1962).

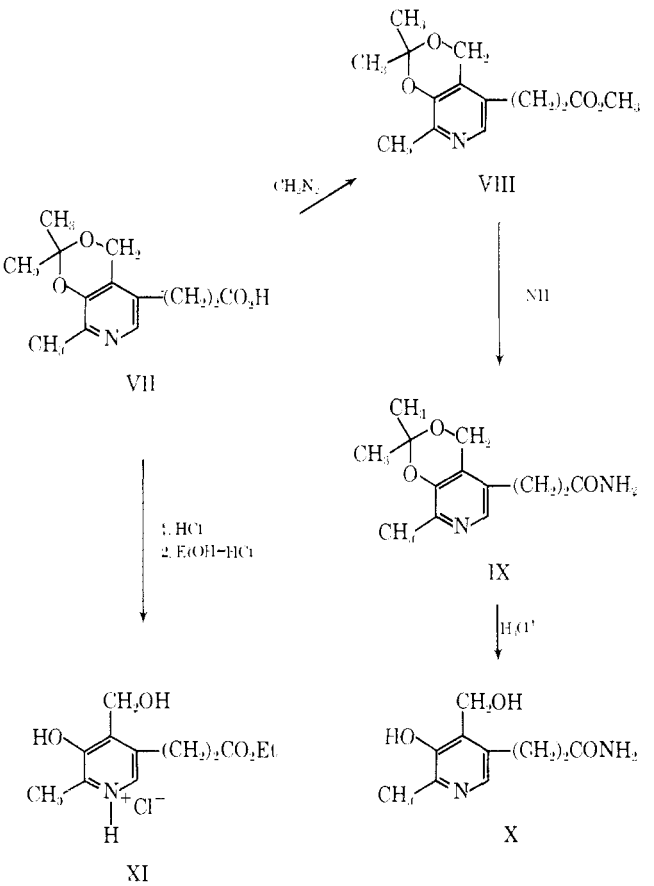
(9) W. Korytnyk, E. J. Kris, and R. P. Singh, *J. Org. Chem.*, **29**, 574 (1964).

(10) I. Tomita, H. G. Brooks, and D. E. Metzler, *J. Heterocyclic Chem.*, **3**, 126 (1966).

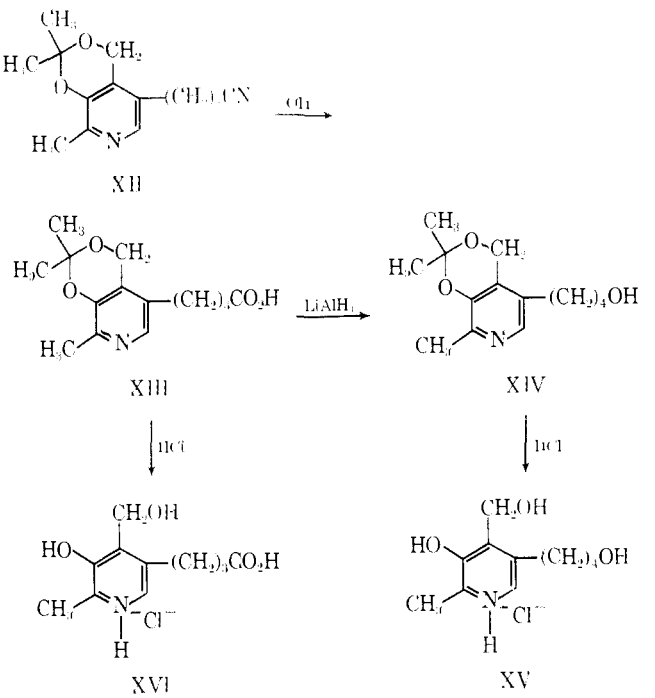
SCHEME I
THREE-CARBON HOMOLOGS (DEOXY DERIVATIVES)



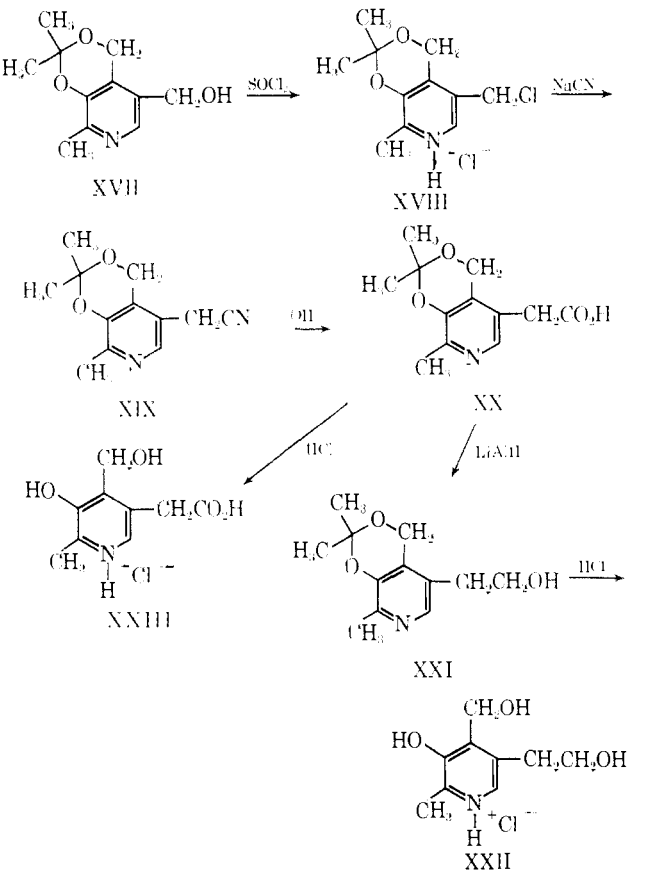
SCHEME II
THREE-CARBON HOMOLOGS (ESTERS AND AMIDES)



SCHEME III
FOUR-CARBON HOMOLOGS AND DERIVATIVES



SCHEME IV
TWO-CARBON HOMOLOGS AND DERIVATIVES

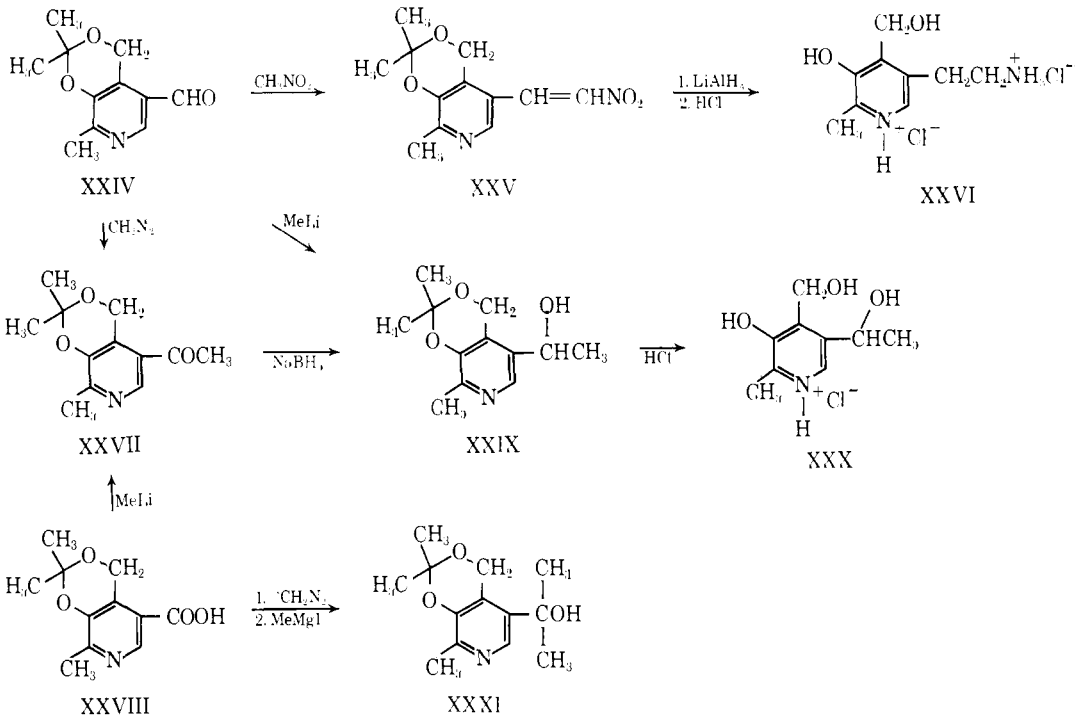


ence of butylamine,[11] a gummy brown product was obtained which did not crystallize. Since the methyl ketone XXVII was obtained only in poor yield by treating the aldehyde XXIV with diazomethane, better preparative methods were investigated. Reaction of the carboxylic acid XXVIII with methyllithium[12] gave XXVII in satisfactory yield. This method was also superior to the Claisen condensation, commonly used for the synthesis of methyl ketones. Thus the Claisen condensation of the methyl ester of XXVIII gave only a poor yield of the methyl ketone isolated as the oxime.

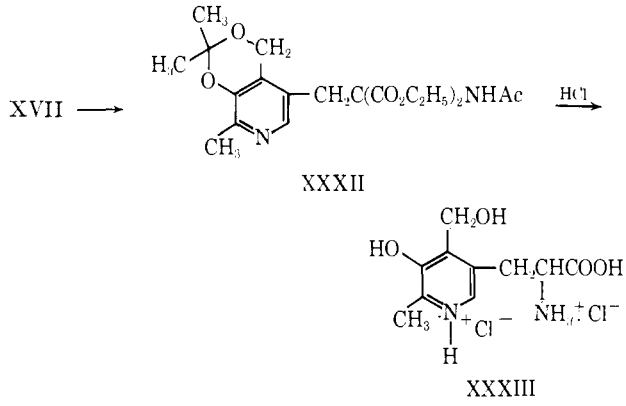
(11) N. Rabjohn, "Organic Syntheses," Coll. Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1965, p 573.

(12) C. Tegner, *Acta Chem. Scand.*, **6**, 782 (1952).

SCHEME V
BRANCHED-CHAIN AND TWO-CARBON HOMOLOGS



SCHEME VI
AMINO ACID ANALOG



When obtained by reaction of the aldehyde XXIV with methyllithium, the secondary alcohol XXIX was apparently contaminated with trace quantities of the starting material, which interfered with microbiological testing. Hydrolysis of the isopropylidene group in XXIX gave the expected product, but hydrolysis of the methyl ketone XXVII under various conditions gave a mixture of at least two products, from which pure material has not been isolated.

Preparation of 5-deoxypyridoxol by hydrogenolysis of the chloro intermediate XVIII and hydrolysis of the isopropylidene group (Scheme IV) is analogous to the method developed by us for synthesizing 4-deoxypyridoxol,[13] and supersedes an earlier method for synthesizing the 5-deoxy isomer.[14]

Nmr spectra have been determined for most compounds and correspond to the assigned structures. In the series represented in Table I, α^4,3-O-isopropylidenepyridoxol (XVII) is taken as the reference compound, and the proton shifts are compared as the substituent in the 5 position is varied. In addition to the electronic effects previously noted,[5,9] steric and anisotropy effects are prominent in this series of compounds, as indicated by α^4- and C_6-proton resonances, especially in the tertiary alcohol XXIX. Further examples of these effects and their extent and nature will be discussed in a separate publication. The hydrogens of the nitro olefin XXV are *trans* to each other, since their splitting constant $J_{\alpha\beta}$ is 14 cps. A number of other nitro olefins previously obtained by similar methods from pyridine aldehydes[15] probably have the same configuration.

The isopropylidene derivatives of these compounds are amenable to gas chromatography and the relationship between the structure and their retention times has been discussed.[16] The mass spectra of certain analogs of pyridoxol described here have been determined, and their fragmentation patterns are being elucidated;[17] the mass spectrum of the methyl ketone XXVII has been presented.[18]

Experimental Section

Syntheses. Three-Carbon Homologs and Derivatives. 5-(3-Chloropropyl)-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine (IV).—To α^4,3-O-isopropylidene-α^5-pyridoxyl-β-ethanol (III, 1.8 g, 7.8 mmoles) in benzene (20 ml), a solution of $SOCl_2$ (1.8 g) in benzene (4 ml) was added dropwise, and then the reaction mixture was cooled. After filtration and washing with ether, a pure product, mp 181–183°, was obtained in almost quantitative yield.

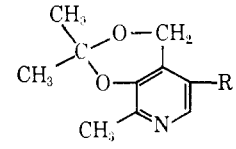
Anal. Calcd for $C_{13}H_{19}Cl_2NO_2$: C, 53.43; H, 6.55; N, 4.79; Cl, 24.27. Found: C, 53.13; H, 6.56; N, 4.87; Cl, 23.95.

5-Propyl-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine (V).—5-(3-Chloropropyl-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine

(13) R. P. Singh and W. Korytnyk, *J. Med. Chem.*, **8**, 116 (1965).
(14) P. Heyl, S. A. Harris, and K. Folkers, *J. Am. Chem. Soc.*, **75**, 653 (1953).
(15) K. W. Merz and H. Stolte, *Arch. Pharm.*, **292**, 496 (1959).
(16) W. Korytnyk, G. Fricke, and B. Paul, *Anal. Biochem.*, **17**, 66 (1966).
(17) Work in progress with Dr. Don C. DeJongh; see also ref 18.
(18) D. C. DeJongh, S. C. Perricone, and W. Korytnyk, *J. Am. Chem. Soc.*, **88**, 1233 (1966).

TABLE I

NMR SPECTRA OF α^4,3-O-ISOPROPYLIDENEPYRIDOXOL DERIVATIVES[a]



No.	R	Ref	Nmr spectra[b] 2-CH_3	4-CH_2	C_5-H	Other protons
XVII	CH_2OH	*b*	−140	−295	−464	$C(CH_3)_2$, −92; 5-CH_2OH, −271
XIX	CH_2CN	*c*	−144	−290	−478	$C(CH_3)_2$, −93; 5-CH_2CN, −212
III	$(CH_2)_3OH$	*d*	−143	−289	−470	$C(CH_3)_2$, −95; 5 side chain, triplet at −220 (α protons), doublet at −110 and −155 (β and α protons).
XXV	$CH_\beta{=}CH_\alpha NO_2$	*c*	−146	−294	−494	$C(CH_3)_2$, −93; H_α, −437, −465 (doublet); H_β, −465, −479 (doublet)
XXVII	$COCH_3$	*c*	−148	−308	−516	$C(CH_3)_2$, −93; $COCH_3$, −156
...	CH_3	*c*	−142	−283	−469	$C(CH_3)_2$, −92; 5-CH_3, −125
XXIX	$CHOHCH_3$	*c*	−138	−295	−468	$C(CH_3)_2$, −91; 5-CH_3, −85 [part of the doublet obscured by $C(CH_3)_2$]; *t*-H, −287 (center of a quadruplet); OH, −240
XXXI	$COH(CH_3)_2$	*c*	−139	−310	−461	$C(CH_3)_2$, −93; $C(CH_3)_2OH$, −90; OH, −220

[a] The spectra were determined on a Varian A-60 instrument in $CDCl_3$ and the positions of peaks are expressed in cycles per second from Me_4Si as an internal standard. The instrument was calibrated by the method of J. L. Jungnickel [*Anal. Chem.*, **35**, 1985 (1963)], and the positions of peaks are accurate within 1 cps. [b] Reference 9. [c] This work. [d] Reference 5.

hydrochloride (IV, 1.00 g) was dissolved in a few milliliters of water, treated with an equivalent amount of Na_2CO_3, and extracted with six 50-ml portions of ether. After evaporation of the ether, the resulting oil was dried by azeotropic distillation with benzene, taken up in tetrahydrofuran (THF) (50 ml), and added to a suspension of $LiAlH_4$ (0.15 g) in THF (25 ml). After refluxing for 2.5 hr and decomposition with ethyl acetate, water was added, and the product was extracted with five 100-ml portions of chloroform. The $CHCl_3$ extract was washed with two 20-ml portions of 6 *N* NaOH and was evaporated *in vacuo*. The oil solidified, yielding 510 mg (67%) of crystalline material, mp 35°. Sublimation at 30° with an aspirator vacuum raised the melting point to 37–38°.

Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.55; H, 8.70; N, 6.33. Found: C, 70.30; H, 8.63; N, 6.60.

4-Hydroxymethyl-2-methyl-5-propyl-3-pyridinol (VI).—5-Propyl-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine (V, 11 mg), in 0.1 *N* HCl (3 ml), was heated on a steam bath for 30 min. Evaporation *in vacuo* gave an oil that could not be made to crystallize. A picrate was prepared by the addition of picric acid (13 mg) dissolved in a minimum amount of water. The melting point of the picrate (17 mg, 78%) was 109°, which did not change on recrystallization from ethanol.

Anal. Calcd for $C_{16}H_{18}N_4O_9 \cdot 0.5H_2O$: C, 45.80; H, 4.56; N, 13.36. Found: C, 45.96; H, 4.27; N, 13.13.

α^4,3-O-Isopropylidene-α^5-pyridoxylacetic Acid Methyl Ester (VIII) Hydrochloride.—α^4,3-O-Isopropylidene-α^5-pyridoxylacetic acid (VII, 1.0 g)[5] was dissolved in methanol (20 ml), to which an excess of diazomethane dissolved in ether was added. Evaporation of the solution gave an oil. Thin layer chromatography (tlc) on silica gel G (MeOH–$CHCl_3$, 1:1) gave a major spot (R_f 0.4) and an impurity at the origin. The oily product was chromatographed on Al_2O_3 (grade II) and the major fraction was eluted with C_6H_6–$CHCl_3$. The product resisted crystallization, and a portion of it was converted to the hydrochloride by passing HCl into an ether solution of the oil, mp 168–170°.

Anal. Calcd for $C_{14}H_{20}ClNO_4$: C, 55.72; H, 6.68. Found: C, 55.48; H, 6.77.

α^4,3-O-Isopropylidene-α^5-pyridoxylacetamide (IX).—α^4,3-O-Isopropylidene-α^5-acetic acid (3.34 g, 1.41 mmoles) was treated with CH_2N_2 in the manner just described. The resulting oil was dissolved in ethanol (150 ml), cooled to 0°, saturated with NH_3 in a pressure tube, and kept at room temperature for 10 days. After evaporation of the ethanol, crystallization began, and some ether was added. The amide X (2.53 g, 73%) was recrystallized from ethyl acetate; mp 164°.

Anal. Calcd for $C_{12}H_{18}N_2O_3$: C, 62.38; H, 7.25; N, 11.19. Found: C, 62.40; H, 7.38; N, 11.39.

α^5-Pyridoxylacetamide (X).—The isopropylidene derivative (IX, 0.40 g, 1.6 mmoles) in 0.1 *N* HCl (20 ml) was heated on a steam bath for 15 min. Water was evaporated *in vacuo* at room temperature, and absolute alcohol was added and likewise evaporated. The crude material contained some starting material, as shown by tlc (silica gel G; MeOH–$CHCl_3$, 1:1). After crystallization from ethanol–ether, 0.22 g of pure amide, mp 150–151°, was obtained.

Anal. Calcd for $C_9H_{13}ClN_2O_3$: C, 48.64; H, 6.13; N, 11.36. Found: C, 48.11; H, 6.31; N, 11.20.

α^5-Pyridoxylacetic Acid Ethyl Ester (XI) Hydrochloride.—A solution of α^4,3-O-isopropylidene-α^5-pyridoxylacetic acid (VII, 1.2 g) in 1 *N* HCl (10 ml) was heated for 1 hr. After evaporation of water *in vacuo*, absolute ethanol (20 ml) and a few drops of concentrated HCl were added, and the mixture was refluxed for 3 hr. After evaporation to a smaller volume, ether was added, and 1.3 g of needles, mp 138–140°, was obtained; recrystallization from an ethanol–ether mixture raised the melting point to 140–142°.

Anal. Calcd for $C_{12}H_{17}ClNO_4$: C, 52.46; H, 6.24; Cl, 12.92. Found: C, 52.62; H, 6.70; Cl, 12.97.

Four-Carbon Homologs and Derivatives. α^4,3-O-Isopropylidene-α^5-pyridoxyl-3-propionic Acid (XIII).—A solution of NaCN (250 mg, 5.1 mmoles) in dimethyl sulfoxide (DMSO) (15 ml, technical) was stirred and heated to 140°. 5-(3-Chloropropyl)-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine (IV, 700 mg, 2.39 mmoles) was added over a period of 25 min. After heating for an additional 30 min, the reaction mixture was cooled with ice, diluted with 50 ml of water, and extracted several times with ether. Evaporation of ether yielded an oily nitrile (XII), which was refluxed for 3 hr in 10% alcoholic KOH (20 ml). The alcohol was evaporated *in vacuo*, and the salt was dissolved in a minimum quantity of water. The solution, kept at 0°, was carefully acidified with concentrated HCl to pH 6. The precipitated acid was filtered, washed with cold water, and dried over P_2O_5; 550 mg (2.16 mmoles, 90%) of the acid, mp 160–161°, was obtained. Crystallization from ethanol did not change the melting point.

Anal. Calcd for $C_{15}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28. Found: C, 63.12; H, 7.33; N, 5.44.

α^5-Pyridoxyl-3-propionic Acid (XVI) Hydrochloride.—A solution of α^4,3-O-isopropylidene-α^5-pyridoxyl-3-propionic acid (XIII, 20 mg, 0.75 mmole) in 0.1 *N* HCl (3 ml) was heated for 30 min on a steam bath. After evaporation of the solvent *in vacuo*, water was added, and evaporation was repeated. The product (17.5 mg, 0.68 mmole) had mp 204°, which was raised to 208–209° by crystallization from ethanol–ether.

Anal. Calcd for $C_{11}H_{16}ClNO_4$: C, 50.48; H, 6.16; N, 5.35. Found: C, 50.91; H, 6.18; N, 5.21.

2,2,8-Trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine-5-butanol (XIV).—A solution of α^4,3-O-isopropylidene-α^5-pyridoxyl-3-propionic acid (XIII, 100 mg, 0.38 mmole) in THF (15 ml) was treated with $LiAlH_4$ (0.3 g). The reaction mixture was stirred for 2 hr at room temperature under N_2, and was then decomposed first with ethyl acetate and then with water. Extraction with $CHCl_3$ and evaporation gave an oil, which crystallized on the addition

of petroleum ether (bp 37–54°), yielding 59 mg (63%) of the alcohol XIV, mp 84°, which was not altered by recrystallization from a mixture of ethyl acetate and petroleum ether.

Anal. Calcd for $C_{14}H_{21}NO_3$: C, 66.90; H, 8.42; N, 5.57. Found: C, 67.22; H, 8.54; N, 5.77.

α^5-Pyridoxyl-3-propanol hydrochloride (XVI) was not isolated, but was obtained in solution on hydrolysis of its isopropylidene derivative (XIV) by heating with 0.1*N* HCl for 30 min on a steam bath.

Two-Carbon Homologs and Derivatives. 2,2,8-Trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine-5-acetonitrile (XIX).—5-(Chloromethyl)-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine hydrochloride (XVIII) was prepared according to the method of Bennett, Burger, and Umbreit[19] from α^4,3-O-isopropylidenepyridoxol obtained by the method of Korytnyk and Wiedeman.[8] The acetonitrile was prepared from the chloromethyl derivative by two methods.

Method A.—To 5-(chloromethyl-2,2,8-trimethyl-4H-*m*-dioxino-[4,5-*c*]pyridine hydrochloride (XVIII, 5.28 g), suspended in 50 ml of acetone, KCN (9.10 g) dissolved in 40 ml of water was added. The two immiscible layers of liquid were heated and stirred for 20 hr while being refluxed. The solvents were removed *in vacuo*, and the mixture solidified. Recrystallization from water and then from petroleum ether provided 3.4 g of XIX, mp 85–89°. A second recrystallization from petroleum ether gave mp 90–91°.

Method B.—To a solution of NaCN (1.67 g, 30 mmoles) in DMSO (250 ml, technical grade), 5-(chloromethyl)-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine hydrochloride (XVIII, 4.1 g, 14 mmoles) was added over a period of 45 min, the temperature being kept at 80°. The temperature was then raised to 140° and maintained for 15 min. After cooling in ice, water was added (500 ml), and the reaction mixture was extracted with seven 150-ml portions of ether. On evaporation of the ether, 3.52 g (91%) of XIX, mp 89–90°, was obtained.

Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.03; H, 6.47; N, 12.84. Found: C, 66.32; H, 6.25; N, 12.57.

2,2,8-Trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine-5-acetic Acid (XX).—2,2,8-Trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine-5-acetonitrile (XIX, 0.7 g) was refluxed in 10% alcoholic KOH (10 ml) for 3 hr, and the alcohol was evaporated *in vacuo*. The residue was dissolved in a small amount of water and was neutralized with diluted HCl to approximately pH 7, when crystallization began. After standing in the cold overnight, the acid was filtered. The yield was 0.35 g, mp 194°. Recrystallization from ethanol did not change the melting point.

Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37. Found: C, 60.50; H, 6.23.

α^4,3-O-Isopropylidene-α^5-pyridoxylmethanol (XXI).—2,2,8-Trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine-5-acetic acid (XX, 0.23 g) was dissolved in 40 ml of THF and slowly added to a stirred suspension of $LiAlH_4$ (0.1 g) in 20 ml of THF. The solution was stirred for 2.5 hr under N_2. It was next decomposed with ethyl acetate, and then water was added. The aqueous solution was extracted three times with chloroform and dried with Drierite. After evaporation to dryness, the oil was taken up in a small amount of ethyl acetate, from which 0.098 g of well-formed needles was obtained; mp 167–169°.

Anal. Calcd for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.71; H, 7.78; N, 6.19.

α^5-Pyridoxylmethanol Hydrochloride (XXII).—α^4,3-O-Isopropylidene-α^5-pyridoxylmethanol (XXI, 70 mg) was dissolved in 5 ml of 0.1 *N* HCl and heated on a steam bath for 2 hr. After evaporation *in vacuo*, the residue was taken up in water and again evaporated. Considerable difficulty was encountered in crystallizing the material. The oil was finally induced to crystallize by boiling it with ethyl acetate, in which the compound is very poorly soluble. The material thus obtained (65 mg) had mp 109–111°.

Anal. Calcd for $C_9H_{14}ClNO_3$: C, 49.21; H, 6.42; N, 6.38. Found: C, 49.22; H, 6.46; N, 6.21.

5-Acetyl-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine (XXVII). Method A.—Diazomethane has been shown to produce methyl ketones from several heterocyclic aldehydes.[20] Diazomethane (0.88 g, 28 mmoles) in 150 ml of ether was added to a solution of α^4,3-O-isopropylideneisopyridoxal (XXIV, 2.0 g, 8.9 mmoles) in methanol (100 ml) at 0°. After standing 30 min at room temperature, the solution was evaporated *in vacuo*, yielding a dark oil. The oil was applied to an Al_2O_3 column (Woelm, No. 1 activity), and was eluted with hexane. The hexane eluate was evaporated, yielding an oil, part of which crystallized. The oily by-product was removed by spreading the mass on a porous plate. The crystalline residue (225 mg, 12%) was sublimed at 80–100° using an aspirator vacuum; mp 107–108°.

Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.09; H, 6.73; N, 6.33.

The acetyl compound was also isolated from the initial dark oil by extraction with ten 10-ml portions of ligroin in the cold. The pure compound was quite unstable and turned dark in a few days. The progress of the reaction was followed by means of tlc (silica gel G; ethyl acetate–chloroform, 80:20). The spots were developed by spraying with 1 *N* HCl, heating at *ca.* 100° for 15 min, and spraying with Gibbs' reagent. After 30 min, no starting material could be detected, and the reaction mixture consisted of the acetyl derivative and an unidentified compound (R_f 0.7 and 0.3, respectively). After 60 min, two additional compounds had formed (R_f 0.6 and 0.2). After 66 hr, another two additional compounds could be detected (R_f 0.4 and 0.5).

Method B.—The methyllithium method described by Tegner[12] for the synthesis of methyl ketones was used. A methyllithium solution in ether (5.0 mmoles) was added all at once to a suspension of α^4,3-O-isopropylidene-α^5-pyridoxic acid (XXVIII, 500 mg) in ice-cooled ether (50 ml). After stirring for 5 min, the initial reddish color of the reaction mixture disappeared, and after stirring for another 10 min in the cold, the mixture was refluxed for 1.5 hr. After cooling in ice, 25 ml of water was added, and the mixture was extracted with ether. Evaporation of ether extracts and recrystallization from petroleum ether yielded 41% of the methyl ketone, identical with that obtained by method A.

The **oxime** of the methyl ketone (XXVII) was obtained by dissolving the ketone (220 mg) in alcohol (15 ml) and adding hydroxylamine hydrochloride (250 mg), followed by 10% aqueous NaOAc (5 ml). The mixture was heated on a steam bath for 2 hr, cooled, and then evaporated to dryness *in vacuo*. The residue was dissolved in water (20 ml) and extracted several times with ethyl acetate. The ethyl acetate extract was evaporated, and the residue was recrystallized from ethyl acetate, yielding 180 mg (76%) of the oxime, mp 194°.

Anal. Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.80; H, 6.82; N, 11.58.

Acid hydrolysis of the isopropylidene group of the methyl ketone and its oxime gave mixtures as determined by tlc. Claisen condensation of the methyl ester of α^4,3-O-isopropylidene-5-pyridoxic acid (prepared according to Korytnyk, *et al.*[9]) with ethyl acetate under standard conditions gave a gummy product, which was converted, in 14% yield, to the oxime of 5-acetyl-3-hydroxy-4-hydroxymethyl-2-methylpyridine, mp 195–196° dec, on crystallization from a mixture of ethyl acetate and ether.

Anal. Calcd for $C_9H_{12}N_2O_3$: C, 55.09; H, 6.17; N, 14.28. Found: C, 54.94; H, 6.24; N, 14.13.

5-(2-Nitrovinyl)-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine (XXV).—Isopropylideneisopyridoxal (XXIV, 1 g) was dissolved in 4 ml of absolute ethanol. Nitromethane (0.7 ml) and methylamine hydrochloride (20 mg) were added, followed by anhydrous Na_2CO_3 (8 mg). The mixture was shaken in a "wrist action" shaker for 6 days, until yellow, needle-shaped crystals separated out. After standing in the cold overnight, the mixture was filtered. The filtrate was concentrated to a small volume under reduced pressure, and additional yellow crystals separated out. The material was filtered again, and the residue was washed with cold ether. The combined residue (0.6 g, 50%) was recrystallized as needles from ether; mp 172–173°.

Anal. Calcd for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.20. Found: C, 57.39; H, 5.69; N, 11.21.

5-(2-Nitrovinyl)-4-hydroxymethyl-2-methyl-3-pyridinol.—5-(2-Nitrovinyl)-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine (XXV, 50 mg) was placed in 10 ml of 20% aqueous acetic acid and heated on a steam bath for 1.5 hr, until the nitro compound dissolved. The solution was evaporated to dryness under reduced pressure, and the residue was crystallized from methanol–ether; mp 197–198° dec.

Anal. Calcd for $C_9H_{10}N_2O_4$: C, 51.42; H, 4.80; N, 13.33. Found: C, 50.86; H, 4.85; N, 12.97.

α^5-Pyridoxylmethylamine Dihydrochloride (XXVI).—5-(2-Nitrovinyl)-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine (XXV, 1.2 g) in 200 ml of anhydrous ether was added drop by drop to a

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(20) L. Capuano and F. Jamaigne, *Ber.*, **96**, 877 (1963).

suspension of $LiAlH_4$ (0.75 g) in 30 ml of anhydrous ether, with stirring, in an atmosphere of dry nitrogen. The reaction mixture was heated and refluxed, with stirring, for 8 hr. It was then cooled in ice, and the unreacted $LiAlH_4$ was decomposed with ethyl acetate (10 ml), followed by water (5 ml) and NaOH solution (5 ml). The ether layer was decanted off, and the residue was extracted several times with ether. The combined ether extract was dried (Na_2CO_3) and evaporated to an oily residue. Addition of HCl gave a hydrochloride (apparently the α^4,3-O-isopropylidene derivative of XXVI) which was very hygroscopic and could not be analyzed. It (0.8 g) was dissolved in 1 *N* HCl (50 ml) and heated on a steam bath for 2 hr. The aqueous layer was evaporated to dryness under reduced pressure, and the residue was crystallized from methanol–ether; mp 204–205.5° dec, yield 0.75 g (61.3%).

Anal. Calcd for $C_9H_{16}Cl_2N_2O_2$: C, 42.35; H, 6.27; N, 10.97. Found: C, 42.59; H, 6.31; N, 10.82.

Branched-Chain Homologs and Derivatives. α^4,3-O-Isopropylidene-α^5-methylpyridoxol (XXIX). Method A.—α^4,3-O-Isopropylideneisopyridoxal (XXIV, 0.570 g, 1.75 mmoles) was dissolved in anhydrous ether (25 ml) and added to methyllithium (2.3 mmoles in 2 ml of ether) during 5 min, the reaction mixture being stirred and cooled in ice. Water was added, and the mixture was extracted with ether. After drying, the solution was evaporated *in vacuo*, yielding an oil, which crystallized. Recrystallization from ethanol gave two crops of crystals: the first, 110 mg, mp 125–128°; and the second, 161 mg, mp 118–120°.

Anal. Calcd for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.66; H, 7.92; N, 6.23.

Method B.—To a solution of XXVII (100 mg) in 75% alcohol (15 ml), $NaBH_4$ (100 mg) was added slowly for 15 min, with stirring. After additional stirring for 30 min, the solvent was evaporated, and the residue was taken up in water (10 ml) and extracted several times with ether. On evaporation, the ether extracts gave a solid residue, which was crystallized from a mixture of ether and petroleum ether, yielding 80% of a product identical with that obtained by method A.

α^5-Methylpyridoxol Hydrochloride (XXX).—α^4,3-O-Isopropylidene-α^5-methylpyridoxol (0.21 g) in 1 *N* HCl (15 ml) was heated for 30 min. Evaporation of the water *in vacuo* and crystallization from ethanol–ether gave 0.21 g of needles, mp 157–158°, which recrystallization raised to 160°.

Anal. Calcd for $C_9H_{14}ClNO_3$: C, 49.21; H, 6.42; N, 6.38. Found: C, 49.07; H, 6.47; N, 6.21.

α^4,3-O-Isopropylidene-α^5,α^5-dimethylpyridoxol (XXXI).—A solution of the methyl ester of α^4,3-O-isopropylidene-α^5-pyridoxic acid[9] (0.5 g) in 10 ml of anhydrous ether was added over a period of 0.5 hr, with stirring, to 0.6 ml of a 1 *M* solution of methylmagnesium iodide in ether. The reaction mixture was refluxed for 1 hr, cooled, and poured into a mixture of ice (*ca.* 25 g) and NH_4Cl (5 g). The resulting solution was extracted several times with ether. The combined ether extract was washed with a sodium thiosulfate solution (10%), followed by water, dried ($MgSO_4$), and evaporated. The residue was crystallized from ether–petroleum ether, yielding 0.332 g (66.4%) of the alcohol, mp 125–126°.

Anal. Calcd for $C_{13}H_{19}NO_3$: C, 65.82; H, 8.01; N, 5.90. Found: C, 66.03; H, 8.13; N, 5.95.

α^5,α^5-Dimethylpyridoxol Hydrochloride.—α^4,3-O-Isopropylidene-α^5,α^5-dimethylpyridoxol (XXXI, 100 mg) was dissolved in 0.1 *N* HCl (10 ml) and was heated on a steam bath for 1 hr. The acid solution was then cooled, filtered, and evaporated *in vacuo*. The residue was crystallized from methanol–ether, yielding 92 mg (92%) of the analog, mp 173–174°.

Anal. Calcd for $C_{10}H_{16}ClNO_3 \cdot 0.25H_2O$: C, 50.42; H, 6.93; N, 5.88. Found: C, 50.77; H, 6.93; N, 5.86.

One-Carbon Analogs. 2,2,5,8-Tetramethyl-4H-*m*-dioxino[4,5-*c*]pyridine.—5-(Chloromethyl)-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine hydrochloride (XVIII, 0.75 g) was dissolved in 10 ml of water and cooled in ice. The solution was made alkaline with $NaHCO_3$, and the resulting free base was extracted with ethyl acetate. The combined ethyl acetate extracts were washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was dissolved in alcohol (40 ml) and hydrogenated with H_2 at 2.1 kg/cm² for 1 hr in the presence of 10% Pd–C (250 mg) in a Parr hydrogenation apparatus. The catalyst was removed by filtration, the filtrate was evaporated to dryness *in vacuo*, and the solute was crystallized from an alcohol–ether mixture. The compound was obtained as the hydrochloride in 91% yield (0.58 g), mp 215–216°. The free base was obtained by treatment with aqueous $NaHCO_3$, and was crystallized from petroleum ether; mp 88–89°.

Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.16; H, 8.00; N, 7.48.

2,5-Dimethyl-3-hydroxy-4-hydroxymethylpyridine (5-Deoxypyridoxol) Hydrochloride.—2,2,5,8-Tetramethyl-4H-*m*-dioxino[4,5-*c*]pyridine hydrochloride (120 mg) was dissolved in 0.1 *N* HCl (15 ml) and was heated on a steam bath for 1 hr. The acid solution was cooled, filtered, and evaporated to dryness under reduced pressure. The residue was crystallized from an alcohol–ether mixture and yielded 91 mg (91%) of the desired compound, mp 143–144°.

Anal. Calcd for $C_8H_{12}ClNO_2$: C, 50.66; H, 6.38; N, 7.39. Found: C, 50.61; H, 6.61; N, 7.45.

Amino Acid Analogs. α^4,3-O-Isopropylidene-α^5-pyridoxylacetamidomalonic Acid Diethyl Ester (XXXII).—Sodium (0.28 g) was dissolved in 15 ml of absolute alcohol, and diethyl acetamidomalonate (2.6 g) was added with stirring and allowed to react for 0.5 hr. Finely powdered 5-(chloromethyl)-2,2,8-trimethyl-4H-*m*-dioxino[4,5-*c*]pyridine hydrochloride (XVIII, 1.5 g) was added, followed by dried KI (0.3 g). The reaction mixture was stirred at room temperature for 48 hr and then evaporated completely under reduced pressure. The residue was taken up in water (10 ml) and extracted several times with ether. The combined ether extract was washed with 10% sodium thiosulfate solution and then with water, dried ($MgSO_4$), and completely evaporated under reduced pressure. The residue was crystallized from a mixture of ether and petroleum ether; mp 117–118° (lit.[10] 122–123°), yield 2.2 g (95.2%).

Anal. Calcd for $C_{20}H_{28}N_2O_7$: C, 58.81; H, 6.91; N, 6.86. Found: C, 59.10; H, 6.92; N, 6.87.

2-(4α^5-Pyridoxyl)aminoacetic Acid (XXXIII). Concentrated HCl (10 ml) was added to diethyl α^4,3-O-isopropylidene-α^5-pyridoxylacetamidomalonate (XXXII, 100 mg) and shaken for 2 hr. The acid solution was diluted with water (10 ml) and heated to reflux for 2 hr. The reaction mixture was then completely evaporated under reduced pressure. The residue was dissolved in water (5 ml), cooled in ice, and neutralized to *ca.* pH 7 with NH_4OH. The neutral solution was then evaporated to dryness under reduced pressure. Alcohol was added to the residue, and the suspension was heated to boiling and filtered. The solid residue was dissolved in water and filtered, and the filtrate was evaporated to a small volume (*ca.* 2 ml). Alcohol (10 ml) was added and evaporated under reduced pressure, when crystalline material separated out. The crystals were filtered, washed with a cold mixture of 2 ml of water and 8 ml of alcohol, and dried. The material was further crystallized from a water–alcohol mixture. The compound showed one spot (R_f 0.55) on tlc with 3:2 EtOH–H_2O and gave positive Gibbs and ninhydrin tests; mp 248–249° dec, yield 34 mg (61.5%). An analytically pure sample could not be obtained because of its extreme solubility in water; its analysis (*Anal.* Calcd for $C_{10}H_{14}N_2O_5$: C, 53.09; H, 6.24; N, 12.38. Found: C, 50.05; H, 6.21; N, 11.93) indicates that it is contaminated with some inorganic salt. It has been further characterized as its methyl ester, as described below.

Methyl 2-(4α^5-Pyridoxyl)aminoacetate.—2-(4α^5-Pyridoxyl)-aminoacetic acid (XXXIII, 100 mg) was taken up in dry methanol (20 ml) and cooled to −10 to −15°. Thionyl chloride (0.1 ml) was added drop by drop, with stirring, over a period of 15 min. Stirring was continued while the reaction mixture was gradually allowed to come to room temperature. Next the methanolic solution was refluxed for 1 hr on a steam bath, cooled, and completely evaporated under reduced pressure. The residue was crystallized from an alcohol–ether mixture, yielding 80 mg (67%) of the ester, mp 194–195° dec.

Anal. Calcd for $C_9H_{18}Cl_2N_2O_5$: C, 42.17; H, 5.75; N, 8.94. Found: C, 41.88; H, 5.45; N, 8.82.

Assay of Antimicrobial Potency and Inhibition Analysis.—The medium of Atkin, *et al.*,[21] modified by the addition of 20 mg of DL-tryptophan and 2.5 mg of nicotinic acid/l. of medium and supplemented with 1 mμg/ml of pyridoxol was used for all growth assays involving *S. carlsbergensis* (ATCC 9080). The assays were carried out by placing 1-ml portions of the medium into 13 × 100 mm Pyrex culture tubes, and adding 1 ml of water or of the solution containing the test compound. Sterilization was carried out by autoclaving for 6 min at 116–121°. The analogs

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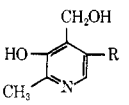
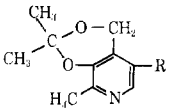
TABLE II

INHIBITION OF GROWTH OF *S. carlsbergensis* BY VITAMIN B_6 ANALOGS[a]

Nature of R		Concn, M, for 50% growth inhib (compd no.)	
Type	Structure		
Two-carbon	CH_2CH_2OH	5×10^{-8} (XXII)	7×10^{-7} (XXI)
	CH_2COOH	Not tested (XXIII)	4×10^{-4} (XX)
	$CH{=}CHNO_2$	1×10^{-4}	5×10^{-6} (XXV)
	$COCH_3$	Not available	1×10^{-3} (XXVII)
	$C(CH_3){=}NOH$	5×10^{-5}	4×10^{-4}
Three-carbon	$(CH_2)_3OH$	5×10^{-8} (I)	1×10^{-7} (III)
	$(CH_2)_3Cl$	Not available	5×10^{-7} (IV)
	$CH_2CH_2CH_3$	1×10^{-7} (VI)	2×10^{-8} (V)
	CH_2CH_2COOH	2×10^{-4} (II)	$>10^{-3}$ (VII)
	$CH_2CH_2CONH_2$	4×10^{-4} (X)	4×10^{-4} (IX)
	$CH_2CH_2COOCH_3$	Not available	5×10^{-4} (VIII)
	$CH_2CH_2COOC_2H_5$	4×10^{-5} (XI)	Not available
Four-carbon	$(CH_2)_4OH$	8×10^{-8} (XV)	1×10^{-6} (XIV)
	$(CH_2)_3COOH$	5×10^{-5} (XVI)	4×10^{-4} (XIII)
Branched	$CH(CH_3)OH$	6×10^{-7} (XXX)	8×10^{-6} (XXIX)
	$C(CH_3)_2OH$	3×10^{-4}	3×10^{-3} (XXXI)

[a] Conditions of testing are described in the text; under the conditions used, 4-deoxypyridoxol inhibited growth of the test organism by 50% at 5×10^{-7} M.

were sterilized by filtration and were then added to the autoclaved medium. The inocula were prepared from cultures of the test organism which were grown in 5 ml of the medium for 20 hr at 30°. Following centrifugation and washing twice with isotonic saline, the cells were resuspended in enough saline to yield an optical density of 0.30 at 470 mμ as measured in a Beckman Model B spectrophotometer. A 1-ml portion of this suspension was diluted tenfold with saline, and 1 drop of this final dilution was placed in each assay tube. Incubation proceeded for 20 hr at 30°. All assays were carried out by shaking the cultures during incubation. The extent of growth was determined by means of a Klett–Summerson photoelectric colorimeter using a red filter (640–700 mμ). To determine their potency as inhibitors of growth, the analogs were added to the medium at concentrations ranging from 10^{-3} to 10^{-10} M.

The inhibitory activity of these compounds is summarized in Table II. To evaluate their antimetabolite nature and to determine the inhibition indices, concentrations of the analogs varying from 10^{-3} to 10^{-6} M were added to the medium containing concentrations of the pyridoxol, pyridoxal, or pyridoxamine ranging from 10^{-4} to 10^{-10} M.

Discussion

Chemical modification of the 5 position of pyridoxol furnished a series of analogs capable of inhibiting the growth of *S. carlsbergensis*, but the extent of inhibition depended upon changes in the length, bulk, and polarity of the side chain (Table II).

The inhibitory activity of most of the parent compounds (Table II, column 3) was compared with that of α^4,3-O-isopropylidene derivatives (Table II, column 4). In most cases the introduction of an isopropylidene group into the molecule decreased the inhibitory potency 2- to 20-fold, but in one case (XXV) a 20-fold increase in potency was noted. Chemical hydrolysis of the isopropylidene group is not expected under the conditions of testing, but the possibility of enzymatic hydrolysis has not been ruled out as yet.

Conversion of the hydroxymethyl group in the 5-position into a straight-chain ω-hydroxyalkyl gave rise to the most potent analogs (I, XV, XXII), and the α^5-(ω-hydroxyalkyl)pyridoxols containing two-, three-, and four-carbon chains all produced 50% growth inhibition at concentrations between 5 and 8×10^{-8} M. Replacement of the terminal hydroxyl group with hydrogen (VI) or chlorine (IV) did not markedly change the biological activity of the three-carbon homolog I, indicating that phosphorylation may not be a prerequisite for the inhibitory effect of this and probably the other alcohols.

The corresponding acids, however, showed a considerable drop in activity. This suggests that either the lipid solubility of this type of compound or their charge or both may be important in determining their activity. Such an implication is strengthened by the fact that esterification of the carboxyl group (XI) restores the activity to some extent. The nature of the side chain may markedly affect the permeability of these analogs and thus their capacity to interfere with the uptake, activation, or cofactor function of the vitamin.[7,22,23]

Branching of the side chain (XXIX, XXXI) also results in decreased activity, the secondary alcohol being inhibitory at 6×10^{-7} M and the tertiary alcohol at 3×10^{-4} M. This decrease indicates that the complexity of the side chain also contributes to the extent of the biological activity of the analogs. Thus, in addition to their lipid solubility, the geometry of the analogs appears to be an important contributing factor in determining their antagonistic potency.

The observation that the analogs containing unbranched alkyl groups demonstrate pronounced biological activity, coupled with the fact that branching of the side chain decreases their effectiveness, suggests that there may exist a hydrophobic region on the receptor site at which the side chain can be bound. Introduction of a carboxyl group or a carbonyl group (such as in compound XXVII) may decrease the bind-

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(23) W. W. Umbreit and J. G. Waddell, *Proc. Soc. Exptl. Biol. Med.*, **70**, 293 (1949).

ing of these analogs to such a region resulting in lower biological activity. Since changes in the α^5 side chain generally are too far removed to influence the electronic configuration of the pyridine ring to any significant extent, electronic effects[5,24] are not expected to be of great importance in this series of compounds. This assumption is supported by the similarity of the positions of the C_6- and α^4-proton resonance peaks in most of the homologs (*e.g.*, Table I: XVII, III, and XXIX). Exceptions are those compounds in which steric and anisotropic effects of the 5 substituent are expected to result in different values (*e.g.*, Table I: XXXI).

The effect of the vitamin on the potency of a number of the unbranched homologs was examined by means of an inhibition analysis.[25] The growth inhibition exerted by these analogs was competitively prevented by the three forms of the vitamin (pyridoxol, pyridoxal, and pyridoxamine), demonstrating the antimetabolite nature of these compounds. The effectiveness of inhibition of the straight-chain homologs and some of their derivatives as measured by their inhibition indices compares well with those of two other potent vitamin B_6 analogs, 4-deoxypyridoxol and ω-methylpyridoxol, previously studied by Rabinowitz and Snell.[26] As determined with pyridoxol, the four-carbon homolog XV has an inhibition index of 2 (ratio of the concentration of the inhibitor to that of the substrate required for 50% growth inhibition), whereas the pyridoxine analog with the *n*-propyl side chain VI has an index of 7. Analysis of the inhibition of various pyridoxol analogs, including those described in this paper, will be discussed in a subsequent publication. The relatively low values of these indices reflect the ability of the unbranched homologs to compete favorably with the vitamin and apparently account for their considerably potency.

Since *S. carlsbergensis* requires only 1 mμg/ml of pyridoxol for approximately half-maximum growth, it proved to be a very sensitive test system for detecting trace contamination of the analogs with the starting materials (*e.g.*, pyridoxol). Such materials were sometimes carried forward through several steps in a synthesis, and their presence could not be detected even by sensitive chemical methods. For example, the interaction of isopropylidenepyridoxal (XXIV) with methyllithium gave the secondary alcohol XXIX, which was pure by all usual chemical standards but sufficiently contaminated with a growth-promoting substance to mask the marked inhibitory activity of the secondary alcohols. When prepared by reducing the methyl ketone XXVII with $NaBH_4$, however, the secondary alcohol XXIX was found to be a good growth inhibitor, free of the growth-promoting contaminant.

The present investigation establishes and extends methods for modifying pyridoxol in the 5 position. The isopropylidene group appears to be ideal for use in such methods because of its stability as a blocking group. Its removal with acid generally yields a pure product. The methyl ketone XXVII, however, is an exception. On hydrolysis it gave a mixture, and this anomaly is under study.

The amino acid analog 2-(α^5-pyridoxyl)aminoacetic acid (XXXIII) is the first analog of its type having the stable C–C linkage between the pyridoxol and the amino acid moieties. Previously, a number of C–N-linked pyridoxyl amino acids have been synthesized[27] which were metabolically activated, presumably through oxidation to a hydrolytically unstable Schiff base.[28]

Modification of the active homologs by conversion of their 4-hydroxymethyl group to the aldehyde form may enhance their biological activity, since such compounds would more closely resemble the cofactor, pyridoxal phosphate. Relatively few analogs of this type have been studied so far. 5-Deoxypyridoxal was found to be much more effective than 5-deoxypyridoxol in inhibiting growth of lactic acid bacteria.[29] Similarly, in Swiss mice, 5-deoxypyridoxal is more toxic than 5-deoxypyridoxol.[3] Tomita and Metzler[29] have converted the 4-hydroxymethyl group of the acid II to the aldehyde form.

With the exception of ω-methylpyridoxol and 4-deoxypyridoxol,[3,26] compounds modified in the 2 and 4 positions were generally found to be weak antagonists. For example, the three-carbon homolog and the secondary alcohol obtained by modification of the 4 position[30] were poor inhibitors of the growth of *S. carlsbergensis*. Modification of the 2 position by replacement of the methyl group with a hydrogen atom resulted in only weak biological activity.[31,32]

The marked potency of the homologs reported in this paper makes it desirable to determine their mode of action. Such a study must take into account the possible interference of the analogs with the uptake of the vitamin, their possible inhibition of pyridoxal kinase activity, and their competition with pyridoxal phosphate for various enzymes for which the vitamin serves as a cofactor. However, the lack of such information does not affect the potential usefulness of the analogs, and their marked potency in the microbial system characterizes them as agents of considerable interest in chemotherapy.

Acknowledgments.—We wish to thank Mr. Robert Mane, Mrs. Barbara Owen, and Mr. George Fricke for their capable technical assistance.

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