Vitamin B_6 Antagonists Obtained by Replacing or Modifying the 2-Methyl Group

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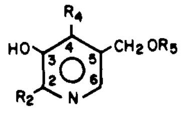
The 2-methyl group of pyridoxol was replaced with various other groups, including 2-amino and 2-methylamino as examples of electron-donating substituents and including 2-carboxyl, 2-carboxamide, and 2-halo as examples of electron-withdrawing substituents. The key intermediate in the synthesis was 3-*O*-benzyl-α^4,α^5-*O*-isopropylidene-α^2-pyridoxol (**15**) or the corresponding 2-aldehyde (**2**). Another approach for modifying the 2 position, but chemically less successful, started with 3-*O*-methylpyridoxol, which was oxidized to the tricarboxylic acid, decarboxylated, esterfied, and reduced with $LiAlH_4$, providing derivatives in which the 2-CH_3 group was replaced with H. The analogues were tested for their growth-inhibitory activity against mouse mammary adenocarcinoma cells in culture. The 2-azine, 2-chloro, and 2-amino analogues were active as inhibitors at $ID_{50} \sim 10^{-5}$ M, whereas the 2-fluoro and 2-carboxylic acid analogues were inactive at 1×10^{-4} M. The results are contrasted with those found earlier for similar modifications in other positions of the vitamin B_6 molecule. Although the 2-chloro analogue was found to inhibit pyridoxal phosphokinase (K_I = 24 μM), the 6-chloro analogue was inactive as an inhibitor at 1 mM.

Pyridoxal phosphate (**1a**), the cofactor form of vitamin B_6, is a constituent of a number of enzymes that catalyze diverse reactions, such as decarboxylation or transamination of amino acids.[1] In an effort to establish the detailed mechanism of this catalysis, it has been proposed that during the enzymatic transamination, pyridoxal phosphate undergoes a rotation around an axis connecting the 2-methyl and 5-CH_2 groups in the molecule.[2]



1 a $R_2 = CH_3$, $R_4 = CHO$, $R_5 = PO_3H_2$

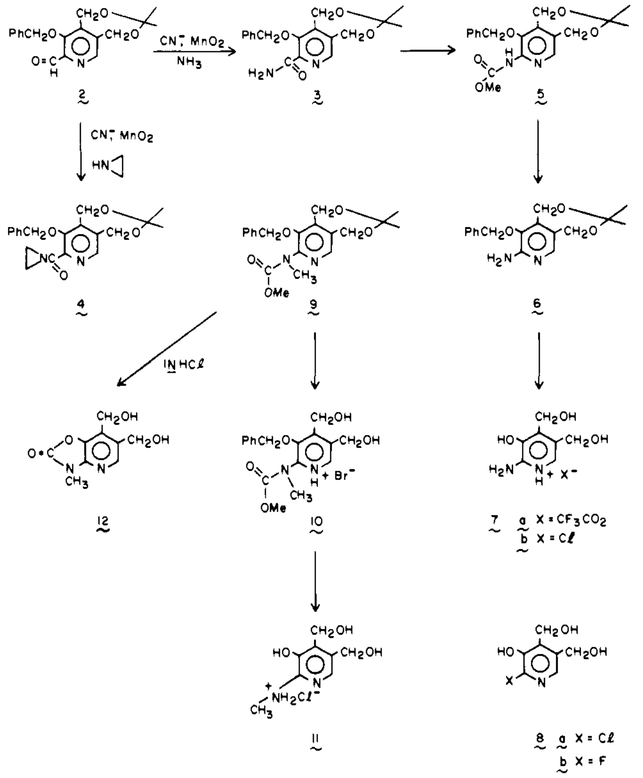
b $R_2 = CH{=}CH_2$, $R_4 = CH_2OH$, $R_5 = H$

To test this postulate, several analogues of pyridoxal phosphate have been synthesized in which the 2-methyl group has been replaced with either a hydrogen atom or a higher alkyl group.[3] In many of the pyridoxal analogues, the cofactor activity was retained,[4] thus indicating some bulk tolerance in the 2 position of the cofactor, which could be exploited in the design of antagonists for this vitamin. Nevertheless, the role of the methyl group in the catalytic activity is probably of lesser importance than was originally postulated.[2]

Earlier, we replaced the 2-methyl group in pyridoxal with vinyl and ethynyl and found that 2-vinyl-2-norpyridoxol (**1b**) was an active inhibitor of mouse mammary adenocarcinoma (TA-3) cells in culture, with an ID_{50} of 9.4 $\times 10^{-6}$ M, and that the inhibition was not reversed by pyridoxal.[5] The availability of a generally useful intermediate for modification of the 2 position, namely the 2-aldehyde (**2**),[5] made it possible to introduce other substituents.

In order to determine the effects of replacing the 2-methyl group of pyridoxol with small electron-withdrawing and -donating substituents on biological activity, we synthesized the 2-amino and 2-(methylamino) analogues (**7** and **11**) as examples of analogues with electron-donating substituents and the 2-carboxylic acid, 2-carboxamide, and 2-halo analogues (**8**) as examples of analogues with electron-withdrawing substituents (Scheme I). Additional considerations led to the preparation of analogues containing a potential alkylating group, *N*,*N*-ethylenecarbamoyl (**4**), or an aminomethyl (**17**) or benzoylthiomethyl (**19**) group (Scheme II). Another approach to modification of the 2 position is illustrated in Scheme III, where the 2-methyl group has been replaced with hydrogen.

Scheme I



As Scheme I shows, the 2-formyl group in **2** was converted to a 2-carbamoyl group as in **3**, by applying Gilman's cyanohydrin procedure.[6] The carboxamide, on Hoffman reaction, gave the 2-methoxycarbonylamido derivative **5**, which on treatment with base gave the 2-amino compound **6**. Treatment of **6** with HCl gave 2-amino-2-norpyridoxol hydrochloride (**7b**), but the yield was unsatisfactory. The yield could be increased on careful treatment of **6** with trifluoroacetic acid. Treatment of **7** with nitrous acid and HCl gave the 2-chloro analogue **8a**, and with nitrous acid and HF the 2-fluoro analogue **8b**. As with the other halogenated derivatives of vitamin B_6, particularly those halogenated in the 6 position, the introduction of halogen abolishes the zwitterionic character of the parent compound, with the result that the analogues are soluble in organic solvents and extractable in ether.[7]

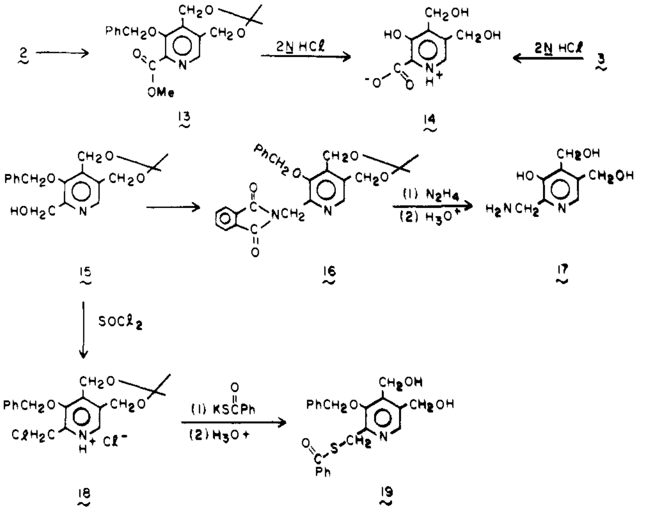
With the introduction of NH_2 and halogen into the 2 position, we have completed the replacement of either H or a functional group of vitamin B_6 with these substituents in all positions. Recent work with such replacements

Table I. Inhibitory Activity of 2-Modified Vitamin B_6 Analogues[a]

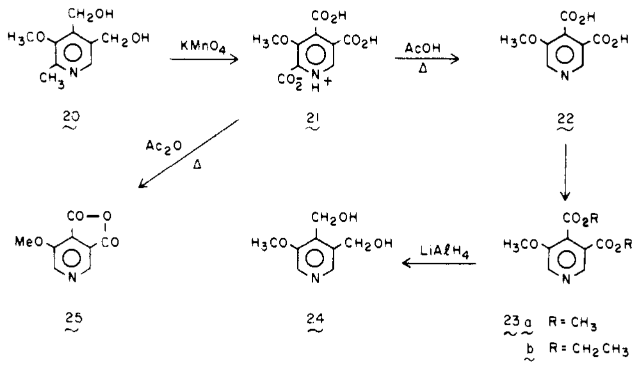
Compd	R_2	Other modifications (positions indicated)	ID_{50}, M[b]	Reversal with PAL (ID_{50}, M[c])
	$CH{=}CH_2$	None	9.4×10^{-6}	9.4×10^{-6}
7	NH_2	None	1.25×10^{-5}	Complete
11	$NHCH_3$	None	1×10^{-4}; 15% inhibn	
10	$NHCH_3CO_2CH_3$	3	1.6×10^{-4}	
8a	Cl	None	4.5×10^{-5}	Complete
8b	F	None	$>1 \times 10^{-4}$	
18	CH_2Cl	3,4, and 5	1.2×10^{-5}	
19	CH_2SBz	3	3×10^{-5}	
17	CH_2NH_2	None	1×10^{-5}; 25% inhibn	
12	NCH_3	3	$>1 \times 10^{-4}$	
2-Aldehyde	CHO	None	1.7×10^{-4}	
14	CO_2H	None	$>1 \times 10^{-4}$	
2-Amide	$CONH_2$	None	1×10^{-4}; 40% inhibn	
2-Hydrazone	$CH{=}NHNH_2$	None	2.3×10^{-4}	
2-Azine	$(CH{=}N)_2$	None	1.6×10^{-5}	1.1×10^{-5}

[a] Inhibition of growth of mouse mammary adenocarcinoma (TA3) cells in culture, as described in ref 13. [b] Pyridoxal at 10^{-7} M to sustain minimal growth. [c] Pyridoxal at 10^{-5} M.

Scheme II



Scheme III



involved the 4 position[8] and the 6 position,[7] and analogues having similar modifications in these and other positions have been reviewed.[9]

In order to explore the bulk tolerance in the 2 position, we have prepared the 2-(methylamino) analogue **11**. To this end the carbamate **5** was methylated to **9**, which, however, could not be crystallized. In an attempt to make the hydrobromide salt, the isopropylidene group was hydrolyzed off, forming **10**. When **9** or **10** was heated in 1 N HCl, a cyclic urethane, **12**, was formed as the only product. An increase in the concentration of the acid to 3 N gave the desired *N*-methyl analogue **11**.

Hydrolysis of either the methyl ester (**13**, Scheme II), which was obtained from **2** by Gilman's cyanohydrin procedure,[6] or the amide **3** gave the desired 2-carboxylic acid analogue **14**, which is most likely a zwitterion.

The methylamino analogue **17** was prepared from the phthalimide **16**, which was obtained in excellent yield in one step from the blocked alcohol **15** by the procedure of Mitsunobu et al.[10] In an attempt to obtain an alkylating function in the 2 position, we replaced the 2-hydroxymethyl group in **15** with a chloromethyl group as in **18**, which was accomplished by treatment of **15** with thionyl chloride. All attempts to deblock **18**, however, resulted in hydrolysis of the chloromethyl group. The chlorine in **18** could be displaced with a thiobenzoate group, and the isopropylidene group could be hydrolyzed, giving **19**. Thus **18** could serve as a useful intermediate for the introduction of various substituents by nucleophilic displacements.

Starting with 2-demethylpyridoxol-2-carboxaldehyde, the synthesis of which was described earlier,[5] we obtained hydrazone, azine, and guanylhydrazone derivatives. These types of derivatives of pyridoxal and of isopyridoxal had been found to be potent pyridoxal phosphokinase inhibitors.[11]

Another synthetic approach to obtaining 2-modified vitamin B_6 analogues has been developed (Scheme III). Kuhn and Wendt[12] have shown that 3-*O*-methylpyridoxol can be oxidized to the tricarboxylic acid **21**, which was not characterized but was converted to the anhydride **25** by treatment with acetic anhydride. We subjected crude 3-*O*-methylpyridoxol (**20**) to vigorous $KMnO_4$ oxidation and purified the tricarboxylic acid **21** by ion-exchange chromatography on a Dowex 1 (OH^- or formate) column. Decarboxylation of **21** was achieved by refluxing with acetic acid, giving **22**, or with acetic anhydride, giving **25**. Esterfication of **25** with ethanolic HCl gave **23b**; similarly, reaction of **22** with diazomethane gave the methyl ester **23a**. This last compound was reduced with $LiAlH_4$ to **24**. Further exploration of this route for the synthesis of 2-modified analogues was not undertaken, because still another approach, one that promises to be more generally applicable, has since been developed.[5] Nevertheless, the intermediates developed in Scheme III should find use for synthesis of certain analogues.

Biological Activity. Compounds synthesized in this study were tested as inhibitors of the growth of mouse mammary adenocarcinoma (TA3) cells grown in cell culture in Eagle's medium with a minimal amount of pyridoxal (1×10^{-7} M) to sustain growth. Inhibition data are summarized in Table I. Some compounds were also tested in the complete medium containing approximately

10^{-5} M pyridoxal (last column in Table I). Under these conditions, the 2-azine derivative (last compound in the table) was not reversed, whereas the 2-amino (**7**) and 2-chloro (**8a**) derivatives were completely reversible. The lack of reversibility for the 2-azine may be due to inhibition of other metabolic pathways besides those involving vitamin B_6. The 2-halogenated derivatives **8a** and **8b** show an interesting contrast with the 6-fluoro and 6-chloro analogues in growth-inhibitory activities. In the 6 series, the fluoro derivative is the only growth inhibitor, since the fluorine atom simulates the hydrogen atom in size, and the higher halogen analogues are inactive.[7] In the 2 series, on the other hand, it is the fluoro derivative that is inactive, and the chloro analogue has some inhibitory activity (Table I), which is to be expected, since the size of the chlorine atom simulates that of the 2-methyl group. Generally, electron-withdrawing substituents, such as carboxyl or carboxamide, are associated with decreased inhibitory activities in the 2 position (Table I) as well as in the 4 position. Also, methylation of the 2-NH_2 group (**11**) has resulted in decreased inhibitory activity as compared with the parent compound **7**, probably due to a steric effect. It should be pointed out that an amino group in the 6 position was associated with a comparable growth-inhibitory activity to that in the 2 position ($ID_{50} = 6.0 \times 10^{-5}$ M),[7] but the 4-amino analogue was devoid of any inhibitory activity at 1×10^{-4} M.[8] Compounds shown in Scheme III were found to be inactive as growth inhibitors of TA3 cells at 1×10^{-4} M.

Recently, growth-inhibitory activity of vitamin B_6 analogues modified in the 4 position has been correlated with their substrate activity for pyridoxal phosphokinase and the inhibition of pyridoxine-P oxidase by the phosphorylated analogues.[13] Although we do not have appropriate data for this series of analogues, nevertheless they have the structural features to become substrates of the pyridoxal kinase, and probably are, in view of permissive structural requirements for this enzyme.[13]

When the 2-chloro analogue **8a** was tested as an inhibitor for the phosphorylation of pyridoxal by pyridoxal phosphokinase, the K_I value was found to be 24 μM, with a competitive type of inhibition. This value is lower than the K_m value of 283 μM for pyridoxal or the K_I value of 105 μM for the 2-C≡CH analogue. The 6-chloro (or 6-bromo)[7] analogue did not inhibit this enzyme at 1 mM, but the 6-fluoro analogue[7] was found to be inhibitory, with a K_I value of 59 μM and competitive kinetics. Enzymatic inhibition studies have been carried out as described earlier.[13]

Experimental Section

Where analyses are indicated only by symbols of elements, analytical results obtained for those 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 Varian A-60A and XL-100A instruments, using 8–15% solutions in $CDCl_3$, Me_2SO, or D_2O; positions of peaks are expressed in parts per million (ppm) units from Me_4Si, or from dioxane (δ 3.70), as an internal standard. Peaks are assigned on the basis of previous work.[15] Solvents were removed in vacuo.

3-*O*-Benzyl-α^4,α^5-*O*-isopropylidene-2-norpyridoxol-2-carboxamide [3,3-Dimethyl-9-(phenylmethoxy)-2,4-dioxepano[5,6-*c*]pyridine-8-carboxamide] (3). Compound **2** (220 mg)[5] was suspended in 16 mL of isopropyl alcohol saturated with ammonia at 0 °C. Powdered NaCN (200 mg) was added, followed by MnO_2 "B" (900 mg). After stirring for 10 min, another portion of MnO_2 (900 mg) was added, and the suspension was stirred for 4 h. After filtration, the solid was washed with chloroform (200 mL), and the chloroform layer was washed with water and dried (Na_2SO_4). The solvent was removed under vacuum, and the residue was crystallized from a mixture of Et_2O and petroleum ether: yield 180 mg (78%); mp 160 °C; IR ν_{max}^{KBr} 3425 (sharp NH stretching), 3200, 3280 (br), 1680 cm^{-1} (C=O). Anal. ($C_{18}H_{20}N_2O_4$) C, H, N.

3-*O*-Benzyl-α^4,α^5-*O*-isopropylidene-2-norpyridoxol-2-(*N*,-*N*-ethylenecarboxamide) [3,3-Dimethyl-9-(phenylmethoxy)-2,4-dioxepano[5,6-*c*]pyridine-8-(*N*,*N*-ethylenecarboxamide)] (4). The same procedure was followed as in the preceding experiment but starting from 1.5 g (4.8 mmol) of **2** and 0.5 mL (9.6 mmol) of ethyleneimine. The product was isolated as an oil (1.25 g, 73.5%), which had the expected NMR spectrum (see below). A fraction of the oil was chromatographed on a silica gel column (eluted with ethyl acetate) and was crystallized from a mixture of Et_2O and petroleum ether. The analytical sample was obtained on recrystallization from Et_2O: mp 83–86.5 °C; NMR 2 × CH_3 1.46, CH_2CH_2 2.43, 2 × CH_2 4.87, CH_2 5.04, Ph 7.42, C_6-H 8.18; IR ν_{max}^{KBr} 2909–3065 (sharp peaks, CH), 1672 cm^{-1} (br, C=O). Anal. ($C_{20}H_{22}N_2O_4$) C, H, N.

3-*O*-Benzyl-α^4,α^5-*O*-isopropylidene-2-(methoxycarbonylamino)-2-norpyridoxol [Methyl [3,3-Dimethyl-9-(phenylmethoxy)-2,4-dioxepano[5,6-*c*]pyrid-8-yl]carbamate] (5). A solution of **3** (80 mg, 2.44 mmol) in a mixture of MeOH (5 mL) and 0.1 N NaOH (2 mL) was added to 0.38 mL (2.68 mmol) of a commercial bleaching solution (5.25% NaOCl) and kept for 2–3 min at 50 °C while being stirred. The mixture was allowed to reach room temperature, and after 30 min, the solvent was evaporated, and the residue was extracted with ethyl acetate, washed with water, and dried (Na_2SO_4). After evaporation, the residue was dissolved in a few drops of Et_2O, and petroleum ether was added until turbidity developed. The product crystallized: yield 66 mg (75%); mp 125–129 °C; IR ν_{max}^{KBr} 3315 (NH), 1745 cm^{-1} (C=O); NMR ($CDCl_3$) 2 × CH_3 1.41, OMe 3.63, 2 × CH_2 4.78, CH_2 4.88, Ph 7.43, C_6-H 7.88, NH 9.33. Anal. ($C_{19}H_{22}N_2O_5$) C, H, N.

2-Amino-3-*O*-benzyl-α^4,α^5-*O*-isopropylidene-2-norpyridoxol [8-Amino-3,3-dimethyl-9-(phenylmethoxy)-2,4-dioxepano-[5,6-*c*]pyridine] (6). A solution of **5** (0.23 g, 0.64 mmol) in MeOH (4 mL) to which 1 N NaOH (4 mL) was added was refluxed for 1.5 h on a steam bath. After the MeOH was evaporated, the compound was extracted with $CHCl_3$, and the chloroform layer was dried (Na_2SO_4). After evaporation of chloroform, anhydrous ether was added, and the compound crystallized: yield 0.177 g (92%). The compound was recrystallized from a mixture of ether and petroleum ether: mp 111.5–114 °C; IR ν_{max}^{KBr} 3480, 3458, 3280 (br), 1630 cm^{-1}; NMR ($CDCl_3$) 2 × CH_3 1.43, 2-NH_2 and 3 × CH_2 4.70, 4.77, 4.82, Ph 7.39, C_6-H, 7.59. Anal. ($C_{17}H_{20}N_2O_3$) C, H, N.

2-Amino-2-norpyridoxol Trifluoroacetate (2-Amino-3-hydroxy-4,5-pyridinedimethanol Trifluoroacetate) (7a). A suspension of **6** (0.165 g, 0.55 mmol) in trifluoroacetic acid (TFA) was stirred for 7.5 h at room temperature and then was heated on a steam bath for 0.5 h. After evaporating the TFA, the residue was dissolved in water, and the solution was extracted with chloroform. The aqueous layer was evaporated, and the residue was taken up in ethyl acetate. On the addition of ether, the compound crystallized. Recrystallization from a mixture of ether and petroleum ether yielded 66 mg (44.8%): mp 122–124 °C. The compound was identical in NMR spectrum and TLC behavior to its hydrochloride, to be described in the following experiment.

2-Amino-2-norpyridoxol (2-Amino-3-hydroxy-4,5-pyridinedimethanol) Hydrochloride (7b). A solution of **6** (79 mg, 0.26 mmol) in 10 N HCl (10 mL) was heated on a steam bath for 10 min and then was left overnight at room temperature, light being excluded. After heating for 15 min on a steam bath, the reaction mixture was evaporated to dryness. The residue was suspended in EtOH, and the suspension was filtered. The filtrate was evaporated, the residue was redissolved in EtOH, and some acetone was added. This precipitated impurities, which were filtered off, and the residue was dissolved in acetone. The acetone solution was separated from acetone insolubles and was evaporated to dryness. Finally, after dissolving in MeOH, filtration, and evaporation to a minimal volume, the compound crystallized: mp 141–142 °C dec; yield 14 mg (26%). The compound is light-sensitive. Anal. ($C_7H_{11}ClN_2O_3$) C, H, N.

2-Chloro-2-norpyridoxol (2-Chloro-3-hydroxy-4,5-pyridinedimethanol) (8a). A solution of **7b** (410 mg, 1.53 mmol) in concentrated HCl (4 mL) was cooled to −10 °C and was stirred, and $NaNO_2$ (130 mg, 1.89 mmol) was added in small portions over a period of 5–10 min, with stirring. Stirring was continued for another 10 min and then for 1 h at 0 °C. The solution was evaporated to dryness, the residue was dissolved in water, and the solution was extracted four times with ether. After evaporation of ether, the residue was dissolved in chloroform, and then some ether was added. On addition of petroleum ether, the compound crystallized. The yield was 72 mg (24.7%), mp 113–118 °C, after recrystallization from a mixture of EtOH, ether, and petroleum ether. Anal. ($C_7H_8ClNO_3$) C, H, Cl.

2-Fluoro-2-norpyridoxol (2-Fluoro-3-hydroxy-4,5-pyridinedimethanol) (8b). To a solution of **7b** (216 mg) in concentrated HF (48%, 3 mL), $NaNO_2$ (73 mg, 1.05 mmol) was gradually added, while the solution was being stirred in a polyethylene centrifuge tube at −10 °C (ice–salt bath) for about 1 h. During this time, the solution was allowed to reach room temperature gradually. After neutralization with 5 N NaOH, the solution was extracted several times with ether. The ether extract was chromatographed on an Amberlite CG-50 column (H^+ form, 200–400 mesh, 1.3 × 15 cm), using water as the eluent. The combined fractions were evaporated and then crystallized from a mixture of ether and petroleum ether: yield 55 mg (35.7%); mp 70–75 °C. Anal. ($C_7H_8FNO_3$) C, H, N.

3-*O*-Benzyl-2-[*N*-(methoxycarbonyl)-*N*-methylamino]-2-norpyridoxol [Methyl [4,5-Bis(hydroxymethyl)-3-(phenylmethoxy)pyrid-2-yl]methylcarbamate] Hydrobromide (10). A solution of **5** (1.42 g, 3.97 mmol) in DMF (20 mL) was added to a suspension of NaH (0.227 g, 5.39 mmol) in DMF (100 mL), which was cooled in an ice bath. After standing at room temperature for 25 min, the mixture was cooled and CH_3I (0.3 mL, 4.5 mmol) added. After stirring for 2 h, a few drops of acetic acid were added. The solution was evaporated to dryness, and the residue was taken up in EtOAc. After washing with H_2O, the organic layer was dried ($MgSO_4$) and evaporated. The oily **9** (1.3 g), the structure of which has been indicated by NMR spectroscopy, could not be made to crystallize, even after chromatography on Bio-Sil A (100–200 mesh, EtOAc as eluent). A portion of the oily compound was dissolved in Et_2O, and ethereal HBr was added. After evaporation of Et_2O, the hydrobromide **10** was crystallized from ethanol–ether: mp 155–156 °C dec; NMR (Me_2SO-d_6), NCH_3 (3.15), $COCH_3$ 3.56, 3 × CH_2 4.63, 4.76, 4.88, Ph 7.43, C_6-H 8.35. Anal. ($C_{17}H_{21}BrN_2O_5$) C, H, N.

2-(*N*-Methylamino)-2-norpyridoxol [3-Hydroxy-2-(*N*-methylamino)-4,5-pyridinedimethanol] Hydrochloride (11). A suspension of **9** (0.30 g of oil) in 3 N HCl (10 mL) was heated for 3 h on a steam bath. The solution was left standing overnight and was then extracted with $CHCl_3$. The aqueous layer was evaporated to an oil, which was crystallized from ethanol–acetone: yield 0.206 g (40%). The compound starts to darken at 140 °C but does not melt. Anal. ($C_8H_{13}ClN_2O_3$) C, H, N.

2-(*N*-Methylamino)-2-norpyridoxol "Cyclic Urethane" [6,7-Bis(hydroxymethyl)-3-methylpyrid[3,2-*b*]oxazol-2-one] Hydrochloride (12). A suspension of **9** (0.25 g, oil) in 1 N HCl (30 mL) was heated on a steam bath for 4 h. The cooled solution was extracted with $CHCl_3$, and the aqueous portion was evaporated to an oil. The oil was taken up in MeOH, and the mixture was filtered. On evaporation of MeOH, the compound crystallized: yield, 0.15 g (51%). Recrystallization from MeOH gave an analytical sample, which softened at 157 °C and decomposed at 165–167 °C. Anal. ($C_9H_{11}ClN_2O_4$) C, H, N.

3-*O*-Benzyl-α^4,α^5-*O*-isopropylidene-2-(methoxycarbonyl)-2-norpyridoxol [Methyl 3,3-Dimethyl-9-(phenylmethoxy)-2,4-dioxepano[5,6-*c*]pyridine-8-carboxylate] (13). A solution of **2** (31.3 mg, 0.1 mmol) in methanol (8 mL), to which NaCN (25 mg) and acetone (10 mL) had been added, was stirred with MnO_2 (350 mg) for 5 h. After filtration and washing with chloroform, the filtrate was evaporated, and the residue was extracted with ether. The ether layer was washed with water and dried (Na_2SO_4). After removal of the solvent, the oil was crystallized from a mixture of ether and petroleum ether: yield 25 mg (73%); mp 57 °C; IR ν_{max}^{KBr} 1735 cm^{-1} (C=O); NMR ($CDCl_3$) $C(CH_3)_2$ 1.65, O=$COCH_3$ 4.02, 2 × CH_2 4.90, CH_2 5.08, C_6H_5 7.47, C_6-H 8.30. Anal. ($C_{19}H_{21}NO_5$) C, H, N.

2-Norpyridoxol-2-carboxylic Acid [3-Hydroxy-4,5-bis(hydroxymethyl)pyridine-2-carboxylic Acid] (14). Compound **13** (0.2 g) was dissolved in 2 N HCl (2 mL), and the solution was heated on a steam bath for 10 h. After evaporating the HCl solution, the solid was taken up in methanol, and the acid crystallized: mp 228 °C dec; yield 0.091 g (76.5%). Anal. ($C_8H_9NO_5$) C, H, N.

Compound **13** has also been formed on similar treatment of the amide **3**.

***N*-(3-*O*-Benzyl-α^4,α^5-*O*-isopropylidene-α^2-pyridoxyl)phthalimide [2-[3,3-Dimethyl-9-(phenylmethoxy)-2,4-dioxepano[5,6-*c*]pyrid-8-ylmethyl]-2*H*-isoindole-1,3-dione] (16).** To a dried mixture of **15** (0.286 g, 0.907 mmol), phthalimide (0.133 g, 0.907 mmol), and triphenylphosphine (0.238 g, 0.907 mmol), THF (40 ml, distilled over $LiAlH_4$) was added. After addition of diethyl azodicarboxylate (0.167 ml, 0.907 mmol), the mixture was stirred for 3 h, with moisture excluded. The solvent was evaporated, and the reaction mixture was chromatographed on a silica gel (100–200 mesh) column. A yellow impurity was eluted with benzene, and the product was eluted with ethyl acetate. The combined fractions were evaporated, and the oil crystallized on the addition of ether and petroleum ether: yield 0.34 g (86%). The compound was recrystallized from hot absolute ethanol, was filtered off, and was washed with a mixture of ether and petroleum ether: mp 133–138 °C; IR ν_{max}^{KBr} 1773, 1715 (C=O); NMR ($CDCl_3$) 2 × CH_2 1.48, 2 × CH_2 5.03, CH_2 4.78, 4.86, C_6H_4 7.76, 7.82, C_6-H 7.96. Anal. ($C_{26}H_{24}N_2O_5$) C, H, N.

α^2-Aminopyridoxol [2-(Aminomethyl)-3-hydroxy-4,5-pyridinedimethanol] (17). To a solution of **16** (0.215 g, 0.48 mmol) in absolute ethanol (10 mL) was added hydrazine (0.2 mL, 95%), and the solution was kept overnight. The precipitated phthalyl hydrazide was filtered off, and the filtrate was evaporated to dryness. The residue was taken up in ether, and the insoluble material was filtered off. The ether solution was washed with water and evaporated, and the residue was stirred with CF_3COOH (5 mL) at room temperature overnight. After cleavage was complete, the CF_3COOH solution was evaporated to dryness. The residue was dissolved in H_2O, and the aqueous solution was chromatographed on Amberlite IR-4B (20–50 mesh, weak anion exchanger), using water as an eluent. Fractions giving a ninhydrin-positive test were combined and concentrated, and ethanol was added. The crystalline compound (42 mg, 47%) was recrystallized from ethanol: mp 167–169 °C dec. Anal. ($C_8H_{12}N_2O_3$) C, H, N.

3-*O*-Benzyl-α^2-chloro-α^4,α^5-*O*-isopropylidenepyridoxol [8-(Chloromethyl)-3,3-dimethyl-9-(phenylmethoxy)-2,4-dioxepano[5,6-c]pyridine] Hydrochloride (18). To a solution of **15** (0.5 g, 1.59 mmol) in benzene (15 mL) was added $SOCl_2$ (0.12 mL, 1.59 mmol), and the solution was heated to reflux. After 1 min, it was cooled to room temperature, and the sovent was evaporated in vacuo. The residue was taken up in warm acetonitrile, and the mixture was filtered. On cooling, the compound crystallized. The crystals were filtered off and were washed with ether and petroleum ether: yield 0.29 g (49%); mp 152–153 °C dec (recrystallized from a mixture of chloroform and petroleum ether); NMR ($CDCl_3$) $C(CH_3)_2$ 1.45, 4 × CH_2 4.85, 4.99, 5.03, 5.16, C_6-H 8.54. Anal. ($C_{18}H_{21}Cl_2NO_3$) C, H, Cl.

3-*O*-Benzyl-α^2-pyridoxyl Thiobenzoate [*S*-[4,5-Bis(hydroxymethyl)-3-(phenylmethoxy)-2-pyridyl]methyl Benzenecarbothioate] (19). A suspension of **18** (0.850 g, 2.3 mmol) in ethanol (20 mL) was flushed with N_2 for 30 min. A solution of potassium thiobenzoate (0.81 g, 4.6 mmol) in water (4 mL) was added, and the solution was stirred for 2 h and then heated on a steam bath for 1.2 h. Next 0.1 N HCl (1 mL) was added, and the heating was continued for another 0.5 h. After evaporating most of the solvent, the reaction mixture was neutralized with $NaHCO_3$ and extracted with EtOAc. After washing (H_2O) and drying (Na_2SO_4), the EtOAc was evaporated to dryness, and the residue was crystallized from ethanol: yield 0.53 g (70%); mp 116–119 °C (from a mixture of chloroform and petroleum ether); IR ν_{max}^{KBr} 1670 cm^{-1}; NMR ($CDCl_3$) 4 × CH_2 4.53, 4.73, 4.78, 5.08, benzyl C_6H_5 7.43 (br s), benzoyl C_6H_5 7.52, 7.90, 7.94, 8.03, 8.07. Anal. ($C_{22}H_{21}NO_4S$) C, H, S.

2-Norpyridoxol-2-carboxaldehyde Hydrazone [3-Hydroxy-4,5-bis(hydroxymethyl)pyridine-2-carboxaldehyde Hydrazone] (8, X = CH=NNH$_2$). A solution of 2-formyl-2-norpyridoxol trifluoroacetate (0.351 g, 1.18 mmol) in ethanol (2.5 mL) was added to hydrazine (1 mL, 95%; ca. 30 mmol), and the mixture was heated on a steam bath for 3 min. After evaporation in vacuo, the compound crystallized from the reaction mixture. It was recrystallized from ethanol: yield 71 mg (31%); mp 142–144 °C. Anal. ($C_8H_{11}N_3O_3$) C, H, N.

2-Norpyridoxol-2-carboxaldehyde Azine [3-Hydroxy-4,5-bis(hydroxymethyl)pyridine-2-carboxaldehyde Azine] (8, X = CH=N–). A suspension of 2-formyl-2-norpyridoxol trifluoroacetate in a mixture of 3.5 M NaOAc (1 mL) and 3.5 M acetic acid (1 mL) was warmed, partially dissolving the solid. Then 95% hydrazine (0.1 mL, 3 mmol) was added, and the mixture was heated on a steam bath for a few minutes. On cooling, the compound crystallized: yield 0.226 g (80.5%). It was recrystallized from DMF. The orange crystals darkened at 215 °C and decomposed at 245 °C. Anal. ($C_{16}H_{18}N_4O_6$) C, H, N.

2-Norpyridoxol-2-carboxaldehyde Guanylhydrazone [3-Hydroxy-4,5-bis(hydroxymethyl)pyridine-2-carboxaldehyde Guanylhydrazone] (8, X = CH=NNHCH=NNH$_2$) Hydrobromide. To a suspension of 2-formyl-2-norpyridoxol trifluoroacetate (0.665 g, 2.24 mmol) in aqueous HBr (16%, 1 mL) was added aminoguanidine sulfate (0.386 g, 3.14 mmol). The mixture was heated on steam bath until a clear solution was obtained. After standing a few minutes, the compound crystallized. The crystals were filtered and washed with some water, with acetone, and finally with ether: yield 0.407 g (45.4%). The compound was recrystallized from warm 16% aqueous HBr, when needles, mp 253–263 °C dec, were obtained. Anal. ($C_9H_{15}Br_2N_5O_3$) C, H, N.

2-Norpyridoxol-2-carboxamide [3-Hydroxy-4,5-bis(hydroxymethyl)pyridine-2-carboxamide] (1, R_2 = CONH$_2$; R_4 = CH$_2$OH; R_5 = H). Compound 3 was deblocked by stirring a trifluoroacetic acid solution of it at room temperature overnight, evaporating the solvent, and passing the residue through an Amberlite CG-50 column, using H_2O as eluent; mp 174–176 °C. Anal. ($C_8H_{10}N_2O_4 \cdot 0.25H_2O$) C, H, N.

3-Methoxypyridine-2,4,5-tricarboxylic Acid (21). To crude 3-*O*-methylpyridoxol (20, 8.7 g, obtained by methylation of pyridoxol with CH_2N_2) in H_2O (100 mL) was added $KMnO_4$ (27.3 g) in H_2O (900 mL). The solution was allowed to stand overnight at room temperature. Again $KMnO_4$ (27.3 g) in H_2O (900 mL) was added, and then 6 N KOH (100 mL) was added. The solution was heated in a steam bath for 8 h. Excess $KMnO_4$ was destroyed with MeOH. The MnO_2 was filtered off and was washed several times with H_2O. The filtrate was passed through a column of Dowex 1-X8 resin in the OH^- form. The column was then washed successively with H_2O and 5, 10, and 20% formic acid. The tricarboxylic acid was eluted with 60% formic acid. The eluent was evaporated to dryness, and the residue was washed with ether and then crystallized from water: yield 8.8 g (77%); turns yellow at 158 °C and darkens at 190–193 °C. The product gave a positive $FeSO_4$ test for a carboxyl group ortho to the ring nitrogen. Anal. ($C_9H_7NO_7 \cdot H_2O$) C, H, N.

3-Methoxypyridine-4,5-dicarboxylic Acid (22). To unrecrystallized 3-methoxypyridine-2,4,5-tricarboxylic acid (12 g) was added acetic acid (450 mL), and the mixture was refluxed for 2 h. All of the solid went into solution before boiling, and CO_2 was given off. After 10 min of refluxing, a solid began to crystallize out. The solid was filtered off and was washed with ether: yield 8.8 g (85%); mp 218–220 °C dec. Anal. ($C_8H_7NO_5 \cdot 0.5H_2O$) C, H, N.

Dimethyl 3-Methoxypyridine-4,5-dicarboxylate (23a). Diazomethane (prepared from 72 g of "diazald"), EtOH (100 mL), KOH (20 g), and H_2O (32 mL) were added to 22 (7.8 g) in methanol (40 mL). The mixture was allowed to stand for 2.5 h at room temperature and was then evaporated to a small volume. After being in a freezer overnight, the dark liquid was filtered, and the solid was dried on a porous plate: yield, 6.5 g. Chromatography showed two or more spots. The solid was dissolved in $CHCl_3$ (25 mL). The solution was heated to boiling and cooled, and the undissolved solid by-product was filtered off: yield, 25 g; mp 233–234 °C. The filtrate was evaporated to an oil under vacuum, EtOH (5 mL) was added, and the product was allowed to crystallize at room temperature: yield, 2.5 g (30%); mp 78–80 °C. Anal. ($C_9H_9NO_5$) C, H, N, methoxyl.

3-*O*-Methyl-2-norpyridoxol (3-Methoxy-4,5-pyridinedimethanol) (24). A solution of 23a (2 g) in ether (110 mL) was added dropwise to a stirring solution of $LiAlH_4$ (0.5 g) in ether (20 mL). The mixture was stirred for 15 min, and then 10% H_2SO_4 (15 mL) was added. The mixture was shaken for 5 min and was then neutralized with 5% NaOH. The water layer was separated off and was continuously extracted for 16 h with ether (200 mL). An oil began to separate out after 3 h and gradually crystallized. The solid was recrystallized from MeOH: yield 0.42 g (30%); mp 133–134 °C. Anal. ($C_8H_{11}NO_3$) C, H, N.

3-Methoxypyridine-4,5-dicarboxylic Anhydride (25). Crude 3-methoxypyridine-2,4,5-tricarboxylic acid (21, 2 g) was heated in acetic anhydride (60 mL) to 110 °C for 30 min, and then to 130 °C for 5 min, by the end of which time all solid material had gone into solution. The acetic anhydride was evaporated off, and the tan solid was sublimed at 80 °C in vacuo (10^{-6} mm). The first sublimate had a low melting point, but the second sublimate had mp 160–161 °C; yield 0.9 g (60%). Anal. ($C_8H_5NO_4$) C, H.

Diethyl 3-Methoxypyridine-4,5-dicarboxylate (23b). To the anhydride 25 (1.1 g) was added EtOH (100 mL) saturated with HCl. After the mixture was refluxed for 2.25 h, the EtOH was evaporated off. EtOH was added again, and the solution was evaporated to an oil. Water (100 mL) was added, and Na_2CO_3 was added until no more bubbles formed, and then a slight excess was added. The mixture was extracted with ether (4 × 75 mL). The ether extract was extracted with water (2 × 50 mL) to remove Na_2CO_3, dried (Drierite), filtered, and evaporated. The residual oil was distilled at 175 °C in vacuo (2 mm). The distillate was slightly yellow: yield, 0.65 g (42%). The sample crystallized on standing. Anal. ($C_{12}H_{15}NO_5$) C, H, N.

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