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Pyridoxine Chemistry. VIII. (1) Synthesis of 5-Norpyridoxol (2).

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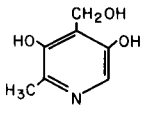
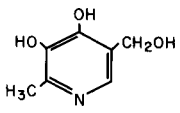
5-Norpyridoxol (3,5-dihydroxy-4-hydroxymethyl-6-methylpyridine, I) has been synthesized. In contrast to the other two isomeric norpyridoxols (II and III), it proved to be inhibitory to *Saccharomyces carlsbergensis*. Improved methods of synthesis for some intermediates previously described have been developed.

Potent antagonists of vitamin B_6 can be obtained by extension of the 5-hydroxymethyl side-chain in pyridoxol (3). Therefore, it was of interest to explore the biological properties of 3,5-dihydroxy-4-hydroxymethyl-6-methylpyridine (I), in which the same side-chain of pyridoxol has been replaced by a hydroxyl group. For convenience, this compound can be called "5-norpyridoxol", since it lacks the



I



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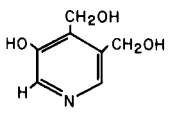
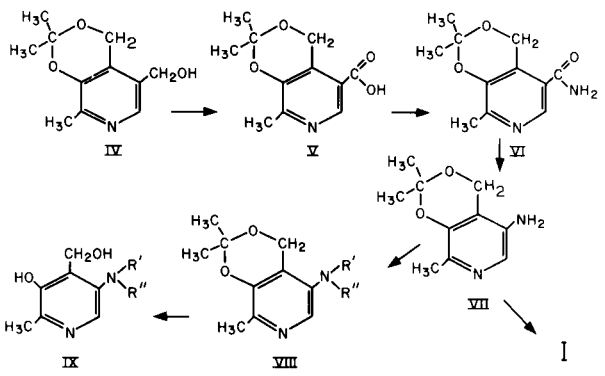


III

methylene group which is present in the 5-position of pyridoxol. Synthesis of the other two lower homologs of pyridoxol, 4-norpyridoxol (3,4-dihydroxy-5-hydroxymethyl-2-methylpyridine, II) (4) and 2-norpyridoxol ["nor-vitamin B_6", 3-hydroxy-4,5-bis(hydroxymethyl)pyridine, III] (5), has already been reported.

The synthesis of the new norpyridoxol (I) is outlined in Scheme I. The synthesis of some of the

SCHEME I



intermediates in this scheme has been described earlier (6). Nevertheless, we have introduced some improvements that should be of general applicability in their preparation. Thus, the yield of the acid V has been improved from 44% to 66% by the oxidation of isopropylidenepyridoxol (IV) with potassium permanganate in acetone instead of in water (6). The naturally-occurring 5-pyridoxic acid lactone can be prepared from the carboxylic acid V in one step by hydrolysis with dilute acetic or hydrochloric acid (6), as previously described (7). It should be noted that permanganate oxidation of the alcohol IV also yielded, as a by-product, isopropylideneisopyridoxal in 19.6% yield, together with some starting material (IV). The amide VI has now been obtained directly from the acid by the mixed anhydride method (8). This amide was converted to the amine VII by treatment with sodium hypochlorite (9).

It was found that either the monoacyl (R' = aryl, R'' = H) or diacyl (R' = R'' = acyl) derivatives of the amine VIII were obtained under the same conditions if the amine was allowed to react in pyridine with either the same or double the same molecular quantity, respectively, of methanesulfonyl chloride or benzoyl chloride. An excess of acetic anhydride in pyridine, however, gave only a monoacetyl derivative (R = acetyl, R'' = H). This finding may be related to the ease with which hydrolysis of the diacetyl derivatives takes place in water (10); hydrolysis could have occurred during the process of working up the material.

The ease with which diacyl derivatives of the amine VII can be formed is surprising, since the resulting molecules are very crowded. Although a number of 3-aminopyridines have been studied in some detail, diacyl derivatives of these compounds have not been described (11). (Diacylation of 2-aminopyridines gave rise to anomalous products (11).) The isopropylidene group was hydrolyzed by dilute acid to yield compounds of the general structure IX, but the acyl groups in the diacyl and monoacyl derivatives were unaffected under the same conditions.

Hydrolysis of the parent amine VII with 1 *N* hydrochloric acid and heating, or treatment with concentrated hydrochloric acid at room temperature, gave 5-amino-3-hydroxy-4-hydroxymethyl-2-methylpyridine (IX, R' = R'' = H) as a monohydrochloride, and not as the expected dihydrochloride. This is surprising since 3-aminopyridines are known to

TABLE I

Ultraviolet Absorption Spectra of Norpyridoxols and Related Compounds

	0.1 *N* HCl		Alcohol		0.1 *N* NaOH	
	λ max	ε max	λ max	ε max	λ max	ε max
5-Norpyridoxol (I)	299	8.35×10^3	296.5	7.2×10^3	315	6.8×10^3
	250	2.0×10^3	258	1.8×10^3		
4-Norpyridoxol (II)	271	6.0×10^3	281	13.1×10^3	303	9.2×10^3
	244	3.9×10^3			223	18.4×10^3
5-Amino-3-hydroxy-4-hydroxymethyl-2-methylpyridine (IX, R' = R'' = H)	315.5	6.1×10^3	306	5.1×10^3	315.5	7.1×10^3
	272.5	5.3×10^3	243	6.8×10^3	212	18.8×10^3
	226.5	15.8×10^3	217.5	17.4×10^3		
Pyridoxol (a)	291	8.6×10^3	286	5.7×10^3	310	6.8×10^3
	232 (s)	2.1×10^3			245	6.3×10^3

(a) These data were taken from D. E. Metzler and E. E. Snell, *J. Am. Chem. Soc.*, 77, 2431 (1955).

combine with two molecules of mineral acid (12). It is possible that the opposite charges on the phenolic and amino groups in IX (R' = R'' = H) cause the groups to enter into intermolecular association, resulting in the formation of a quasi-dimer or -polymer. This would leave only the heterocyclic nitrogen available for salt formation.

3,5-Dihydroxy-4-hydroxymethyl-6-methylpyridine (I) was obtained in good yield from the amine VII by diazotization with sodium nitrite in 1 *N* hydrochloric or 1 *N* sulfuric acid, followed by hydrolysis of the diazotized compound with boiling water.

Biological Properties

Preliminary testing with *Saccharomyces carlsbergensis* (ATCC 9080) has indicated that 5-norpyridoxol (I) as well as the amines VII and IX (R' = R'' = H) were growth inhibitors producing half maximal inhibitors at concentrations in the range of 10^{-5} to 10^{-6} M in the presence of 1 mμ/ml. of pyridoxal in the assay medium. Acyl substituents on the amino group decreased the activity of the parent compound ten to hundredfold (13).

This activity sharply contrasts with the inactivity of the isomers II (14) and III (5a, 15).

NMR Spectra

The structures of the compounds reported have been confirmed by NMR spectroscopy. Determinations of spectra in D_2O were performed as previously described (16), but instead of dioxane as an internal standard, the recently introduced sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used, with the result that peaks appear somewhat shifted from previous values (2). The availability of the two isomeric hydroxy analogs I and II and the amino analog IX (R' = R'' = H) provided an opportunity to study the effect of replacing the 4- or 5-hydroxymethyl group with the much more electron-donating amino or phenolic hydroxy group.

All signals were more shielded for the analogs XI, XII, and XIII than for the pyridoxol cation X (Fig. 1); and the methyl and C_6 protons were more shielded for the 5-hydroxy (XII) and 5-amino (XIII) analogs than for the 4-hydroxy analog XI. This result can be easily rationalized: substitution of an electron-donating hydroxyl group for the hydroxymethyl group in the 5-position will affect the shielding of the methyl protons in the *para* position, and especially the proton *ortho* to it and directly attached to the aromatic system, to a greater extent than a similar change in the 4-position of the molecule, in which case the C_6 and 2-methyl protons are *meta* to that of the 4-hydroxyl. The shielding of the relevant protons in the methanesulfonyl derivative XIV approaches that of the pyridoxol cation X. Here the electron-donating effect of the 5-amino group, as evidenced in XIII, is counteracted by the electron-withdrawing effect of the methanesulfonyl group, resulting in virtual cancellation of the two opposing effects.

A similar relationship is found by comparing the anions of the analogs XVI, XVII, and XVIII with the pyridoxol anion XV (Fig. 2). Here the additional dissociated phenolic group in XVI and XVII has a marked effect on the shielding of protons, especially those *ortho* and *para* to it. The amino group has a smaller effect on the shielding of protons in anions, as is shown in XVIII (Fig. 2), than in cations (Fig. 1).

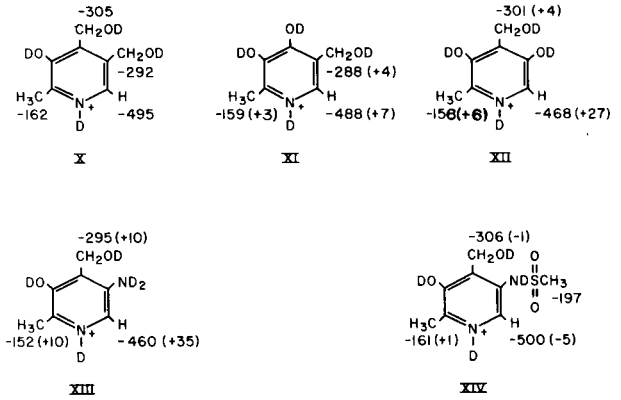


Fig. 1. Comparison of NMR spectra of pyridoxol analog cations in D_2O. Positions of peaks are indicated in c.p.s. units (60 Mc). Numbers in parentheses represent displacement of the peaks in c.p.s. units with respect to the pyridoxol cation X.

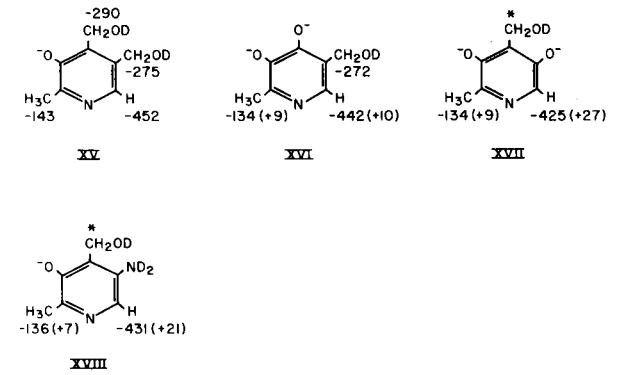


Fig. 2. Comparison of NMR spectra of pyridoxol analog anions in D_2O (1 *N* NaOD). Positions of peaks are indicated in c.p.s. units (60 Mc). Numbers in parentheses represent displacement of the peaks in c.p.s. units with respect to the pyridoxol anion XV. Starred protons have been obscured by the HDO peak of the solvent.

Ultraviolet Spectra

The ultraviolet spectra of 3,5- and 3,4-dihydroxypyridine have been compared in water and 0.1 *N* hydrochloric acid solution by Den Hertog *et al.* (17). The main band was shifted markedly towards the red for the 3,5-derivative relative to the 3,4-derivative, whereas the band at the lower wavelength was shifted in the opposite direction. A similar relationship was observed for 5- and 4-norpyridoxol (Table I). The high extinction coefficient of 4-norpyridoxol in alcohol is remarkable and quite comparable with that found for 4-pyridone (18). This indicates that a 4-pyridone structure should be considered for 4-norpyridoxol. Ultraviolet spectra have also been determined for the 5-amino analog IX (R' = R'' = H) and compared with that of pyridoxol (Table I).

EXPERIMENTAL

α^4,3-*O*-Isopropylidenepyridoxol (IV).

This compound was synthesized by the method of Korytnyk and Wiedeman (19).

3,4-Dihydroxy-5-hydroxymethyl-2-methylpyridine (4-norpyridoxol, II).

This compound was synthesized by the method of Heyl *et al.* (4).

α^4,3-*O*-Isopropylidene-5-pyridoxic acid (V).

To a solution of isopropylidenepyridoxol (25 g.) in acetone (400 ml.) containing 10% aqueous sodium hydroxide solution (0.5 ml.), finely powdered potassium permanganate (35 g.) was added in small portions for one hour, while the reaction mixture was vigorously stirred and refluxed. After an additional hour of refluxing, the temperature was lowered to 40-45°, and was kept there for 36 hours. The mixture was filtered and washed with acetone, and the residue was extracted with boiling water (6 x 100 ml.). Concentration of the aqueous extracts to 40 ml. *in vacuo*, acidification with concentrated hydrochloric acid to *p*H 6, filtration, and washing gave 17.7 g. (66.3%) of acid, identical with α^4,3-*O*-isopropylidenepyridoxic acid (6) (m.p. 220-221°, dec., not depressed by the addition of an authentic sample). The acetone filtrate was evaporated. Water (25 ml.) was added, and it was continuously extracted with petroleum ether (b.p. 36-52°). On concentration and cooling, the extract yielded 4.9 g. (19.6%) of isopropylideneisopyridoxal, m.p. 60-61°, which was identical (mixed m.p., I.R., and TLC) with the aldehyde reported earlier (6). From the aqueous layer, 2.56 g. of starting material (isopropylidenepyridoxol), m.p. 111-112°, was isolated.

α^4,3-*O*-Isopropylidene-5-pyridoxamide (VI).

A suspension of finely powdered isopropylidene-5-pyridoxic acid (10 g.) in dry tetrahydrofuran (200 ml.) was immersed for 5-10 minutes in a cooling bath of dry ice-acetone. Triethylamine (7 ml.) was added dropwise for 10 minutes and was allowed to react for 5 minutes. Then ethyl chloroformate (7 ml.) was gradually added, while stirring and cooling were continued for an additional 30 minutes. A strong stream of ammonia gas was passed through the mixture for 5 minutes and the stirring continued at room temperature for 12 hours. After evaporation *in vacuo*, the residue was extracted with ethyl acetate (5 x 100 ml.). Evaporation of the extract *in vacuo* yielded 7.4 g. of the amide, m.p. 174-175°, undepressed by addition of an authentic sample (6).

5-Amino-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine (VII).

A freshly prepared 0.5 *N* sodium hypochlorite solution (20 ml.) was warmed to 50°. While the warmed solution was stirred vigorously, finely powdered α^4,3-*O*-isopropylidene-5-pyridoxamide (1 g.) was added. After 5 minutes the solution became clear, and turned slightly yellow. The temperature of the bath was raised to 85°, and stirring was continued for an additional 45 minutes. After cooling in ice, potassium hydroxide (5 g.) was added, and the mixture was extracted with hot benzene (5 x 100 ml.). The benzene extract was dried over potassium carbonate, filtered, and evaporated *in vacuo*. Recrystallization from a mixture of ether and petroleum ether yielded 0.464 g. (53%) of the amine, m.p. 159-160° (lit. (9) m.p. 160-161°).

Anal. Calcd. for $C_{10}H_{14}N_2O_2$: C, 61.83; H, 7.27; N, 14.42. Found: C, 62.11; H, 7.44; N, 14.82.

5-(Acetylamino)-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine (VIII, R' = $COCH_3$, R'' = H).

To a solution of the amine VII (0.5 g.) in pyridine (5 ml.), acetic anhydride (0.7 ml.) was added. After being shaken for 12 hours, the reaction mixture was evaporated *in vacuo*, and pyridine was removed by repeated evaporation with water. The residual oil crystallized on cooling yielding 0.58 g. of crystals which showed one spot in TLC. Sublimation *in vacuo* gave the analytical sample m.p. 130-131°.

Anal. Calcd. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 61.03; H, 6.84; N, 11.66.

5-(Benzoylamino)-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine (VIII, R' = COPh, R'' = H).

This compound was obtained from equimolecular quantities of the amine VII and benzoyl chloride in pyridine, and was crystallized from alcohol; m.p. 170-171°.

Anal. Calcd. for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.24; H, 6.15; N, 9.43.

5-(Dibenzoylamino)-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine (VIII, R' = R'' = COPh).

This compound was obtained by allowing the amine VII to react with twice the same molecular quantity of benzoyl chloride in pyridine; m.p. 174-175° from alcohol.

Anal. Calcd. for $C_{24}H_{22}N_2O_4$: C, 71.62; H, 5.51; N, 6.96. Found: C, 71.63; H, 5.64; N, 7.04.

5-(Methanesulfonylamino)-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine (VIII, R' = SO_2CH_3, R" = H).

This compound was obtained from equimolecular quantities of the amine VII and methanesulfonyl chloride, and was isolated as the hydrochloride, which was recrystallized from alcohol; m.p. 217-218° (dec.).

Anal. Calcd. for $C_{11}H_{16}N_2O_4S\cdot HCl$: C, 42.78; H, 5.55; N, 9.07. Found: C, 42.92; H, 5.42; N, 8.79.

5-Bis(methanesulfonyl)amino-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]-pyridine (VIII, R' = R" = SO_2CH_3).

This compound was obtained by allowing a solution of the amine VII (0.2 g.) in pyridine (5 ml.) to react with methanesulfonyl chloride (0.3 ml.); m.p. 188-189°, after recrystallization from alcohol.

Anal. Calcd. for $C_{12}H_{18}N_2S_2O_6$: C, 41.14; H, 5.18; N, 8.00. Found: C, 41.52; H, 5.43; N, 7.74.

5-Amino-3-hydroxy-4-hydroxymethyl-2-methylpyridine hydrochloride (IX, R' = R" = H).

A solution of 5-amino-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine (VII) (0.1 g.) in 1 *N* hydrochloric acid (20 ml.) was heated at 80° for 45 minutes and evaporated *in vacuo*. The residue was crystallized from alcohol-ether, yielding 89 mg. (90.7%) 5-amino-3-hydroxy-4-hydroxymethyl-2-methylpyridine monohydrochloride, m.p. 189-190° (dec.).

Anal. Calcd. for $C_7H_{10}N_2O_2\cdot HCl$: C, 44.10; H, 5.81; N, 14.70; Cl, 18.59. Found: C, 44.23; H, 5.86; N, 14.89; Cl, 18.56.

The same monohydrochloride was also obtained by treating the same amine with a large excess of concentrated hydrochloric acid at room temperature for two hours, and evaporating the acid at 40° *in vacuo*.

5-(Acetylamino)-3-hydroxy-4-hydroxymethyl-2-methylpyridine (IX, R' = $COCH_3$, R" = H).

5-(Acetylamino)-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine (VIII, R' = $COCH_3$, R" = H) (70 mg.) was heated with 20% acetic acid (10 ml.) for 1 hour. Evaporation and crystallization from water yielded the acetate, m.p. 163-164° (dec.), in almost quantitative yield.

Anal. Calcd. for $C_9H_{12}N_2O_3\cdot{}^1/_2H_2O$: C, 52.67; H, 6.38; N, 13.64. Found: C, 52.72; H, 6.58; N, 13.63.

5-(Benzoylamino)-3-hydroxy-4-hydroxymethyl-2-methyl pyridine hydrochloride (IX, R' = COPh, R" = H).

5-(Benzoylamino)-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine (VIII, R' = COPh, R" = H) was heated with 0.2 *N* hydrochloric acid on a steam bath for 2 hours. Evaporation and crystallization from alcohol-ether yielded the benzoate, m.p. 210-211° (dec.) in almost quantitative yield.

Anal. Calcd. for $C_{14}H_{15}N_2O_3Cl\cdot{}^1/_2H_2O$: C, 55.36; H, 5.30; N, 9.22. Found: C, 55.80; H, 5.10; N, 9.25.

5-(Dibenzoylamino)-3-hydroxy-4-hydroxymethyl-2-methylpyridine hydrochloride (IX, R' = R" = COPh).

5-(Dibenzoylamino)-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine (VIII, R' = R" = COPh) was heated in 0.2 *N* hydrochloric acid on a steam bath for 2 hours. Evaporation and crystallization from alcohol-ether provided, in almost quantitative yield, the dibenzoate, m.p. 218-219° (dec.).

Anal. Calcd. for $C_{21}H_{18}N_2O_4\cdot HCl$: C, 63.24; H, 4.80; N, 7.02. Found: C, 62.99; H, 4.86; N, 6.87.

5-(Methanesulfonylamino)-3-hydroxy-4-hydroxymethyl-2-methylpyridine hydrochloride (IX, R = SO_2CH_3, R" = H).

This compound was obtained in quantitative yield by heating 5-(methanesulfonylamino)-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-c]pyridine hydrochloride (VIII, R' = SO_2CH_3, R" = H) in 0.2 *N* hydrochloric acid on a steam bath for 2 hours. The solution was evaporated, and the residue was crystallized from alcohol-ether; m.p. 187-188° (dec.).

Anal. Calcd. for $C_8H_{12}N_2O_4S\cdot HCl$: C, 35.75; H, 4.87; N, 10.43. Found: C, 35.45; H, 4.85; N, 10.05.

5-(Bismethanesulfonylamino)-3-hydroxy-4-hydroxymethyl-2-methylpyridine (IX, R' = R" = SO_2CH_3).

Hydrolysis of the 5-bismethanesulfonylamino compound (VIII, R' = R" = SO_2CH_3) with 1 *N* hydrochloric acid gave a hygroscopic hydrochloride. The latter was converted to the free base, which was then crystallized from alcohol; m.p. 195-196° (dec.).

Anal. Calcd. for $C_9H_{14}N_2O_6S_2$: C, 34.84; H, 4.55; N, 9.03. Found: C, 34.62; H, 4.72; N, 9.24.

3,5-Dihydroxy-4-hydroxymethyl-6-methylpyridine hydrochloride (I).

To a solution of the amine VII (0.2 g.) in 1 *N* hydrochloric acid (20 ml.), a sodium nitrite solution (0.095 g. in 4 ml. of water) was added at 3-4°, with stirring for 10 minutes. The diazotized solution was then added slowly (15 minutes) to boiling water (50 ml.), and was refluxed for another 45 minutes. After evaporation *in vacuo*, the residue was taken up in absolute alcohol, and was filtered from the sodium chloride. The filtrate was concentrated *in vacuo*, ether was added until turbidity was produced, and the material crystallized in the cold. Recrystallization from absolute alcohol yielded 0.145 g. (73.5%) of the hydroxy compound, m.p. 204-205° (dec.).

Anal. Calcd. for $C_7H_{10}NO_3Cl$: C, 43.87; H, 5.26; N, 7.31. Found: C, 44.14; H, 5.33; N, 7.33.

The same reaction could also be carried out in 1 *N* sulfuric acid instead of hydrochloric acid. The free base has been obtained from an aqueous solution of the sulfate by making the solution alkaline with sodium bicarbonate and extracting with ethyl acetate; m.p. 192° (dec.). It decomposed slowly at room temperature.

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