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Pyridoxine Chemistry. IX. (1) Selectively Deuterated Pyridoxols and some Aspects of NMR Spectroscopy of Vitamin B_6 Compounds (2)

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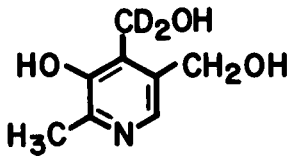
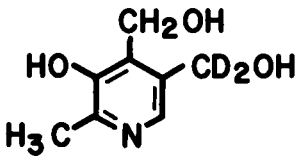
Pyridoxol is converted to the coenzyme pyridoxal phosphate in higher organisms mainly in two steps: first the 5-hydroxymethyl group is phosphorylated, and then the 4-hydroxymethyl group is oxidized to an aldehyde group (3). In these reactions, the α^4-deuterated pyridoxol I is likely to be subject to a primary isotope effect, as conversion of the 4-hydroxymethyl group to aldehyde group must involve a C-D bond breakage. The deuterium isotope effect



I



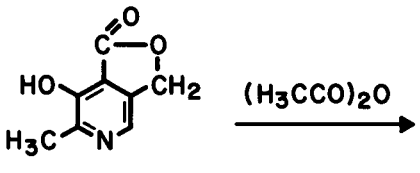
II

has been established to occur in the oxidation of alcohols (4). Since the phosphorylation reaction takes place at the 5-hydroxymethyl group, the α^5-deuterated pyridoxol II is presumably subject to a secondary isotope effect.

The kinetic isotope effect has received some application in pharmacology: marked pharmacological effects have been observed on deuterium substitution in sympathomimetic amines (5); deuteration of the *N*-methyl group of morphine decreases the potency and oxidative *N*-demethylation of the alkaloid (6).

Synthesis of the selectively deuterated pyridoxols I and II has been carried out as part of our studies of the synthesis and biological activity of potential vitamin B_6 antimetabolites. Although the biological studies are still in progress, we would like to describe the synthesis of these compounds and their application to studies of the nuclear magnetic resonance spectra of pyridoxol and its derivatives. Their utility in the elucidation of the fragmentation in mass spectrometry (7) as well as in assignments of infra-red absorption bands will be described later.

Attempts to reduce the readily available 4-pyridoxic acid lactone III with lithium aluminum deuteride



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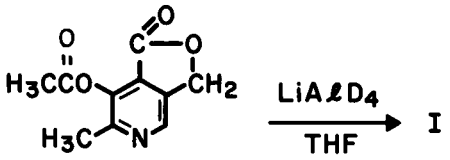
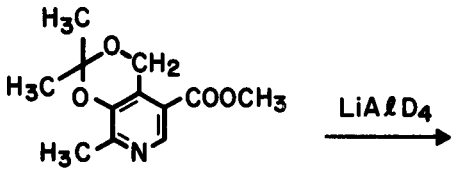


IV

did not provide satisfactory results, as the yield of the desired compound was quite small. Acetylation of the lactone III, followed by reduction of the acetate IV with lithium aluminum deuteride, readily afforded I in 42% yield (8).

The α^5-deuterated pyridoxol II has been obtained by lithium aluminum deuteride reduction of the methyl ester of α^4,3-*O*-isopropylidene-5-pyridoxic acid (V), the synthesis of which has recently been described (9).



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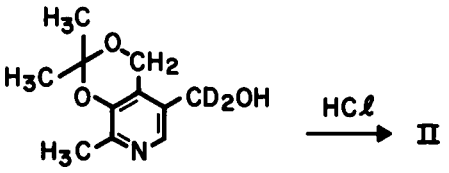


VI

The structures of the compounds I, II, and VI have been confirmed by mass spectrometry (7) and also by NMR spectrometry. Both techniques indicate very high isotopic purity. This is examplified by the NMR spectrum of α^4,3-*O*-isopropylidenepyridoxol-α^5,α^5-d_2 (VI), Fig. 1, in which the 5-methylene peak has virtually disappeared.

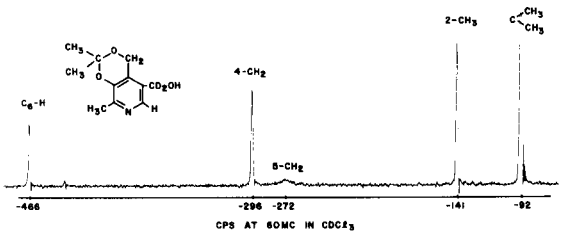


Fig. 1. NMR spectrum of α^4,3-*O*-isopropylidene-pyridoxol-α^5,α^5-d_2 in $CDCl_3$.

A facile base-catalysed deuteration of the 2-methyl-group of quaternized pyridoxol derivatives has been observed earlier (10).

NMR Spectra in Deuterium Oxide and Internal Reference Standards.

The availability of selectively deuterated pyridoxols made possible the unequivocal assignment of methylene peaks whose identification in the spectrum had previously been tentative (10). The original assignment proved to be correct, and the positions of peaks for the several ionic species are shown in Table I. While in the previous study (10) we have used dioxane for an internal standard, we have now applied the more commonly used (11) sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) (12) for the same purpose.

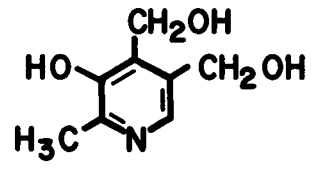
The positions of peaks using the two internal standards differ by significant amounts (for pyridoxol cation 6-7 c.p.s.), and a correlation of the two internal standards appeared to be of some practical importance. Jones *et al.* (13) have estimated the position of 1,4-dioxane from TMS at 6.30 τ (-222 c.p.s. at 60 Mc), and this value has been accepted in our previous study (10). We have now determined the position of the peak for 1,4-dioxane (1% in deuterium oxide) with respect to that of DSS (3% in deuterium oxide) at 0 c.p.s., using a carefully calibrated (14) Varian A-60 instrument. The dioxane peak now appeared at -227.9 $\pm$ 0.1 c.p.s.

When the spectrum of pyridoxol hydrochloride was determined with the dioxane peak as an internal standard set at -228 c.p.s. or 6.20 τ (and not at -222 c.p.s. or 6.30 τ, from TMS, as previously estimated), the positions of the peaks were found to coincide with those obtained with DSS as an internal standard. Thus the two standards can be used interchangeably, with the dioxane peak fixed at -228 c.p.s. The volatility of dioxane permits the sample being recovered without any contamination.

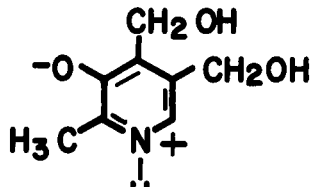
NMR Spectra in Dimethyl Sulfoxide (DMSO).

Many pyridoxol derivatives are not appreciably soluble in water or customary organic solvents for NMR (deuterochloroform or carbon tetrachloride), but are soluble in DMSO. NMR studies, employing this solvent, have been found useful in determining the nature of the hydroxyl function (16) especially in carbohydrates (17) and other natural products (16a,b). Important questions regarding the tautomerism of ribose and deoxyribose nucleosides and the corresponding bases have been answered by NMR spectroscopy employing this solvent (18).

Utilization of DMSO in the NMR spectroscopy of pyridoxol derivatives appeared attractive not only because of the solubility problems sometimes encountered, but also because of the possibility of following existing tautomerism in pyridoxol (VII $\rightleftharpoons$ VIII) and elucidating the nature of the hydroxyl groups in partially substituted pyridoxols.



VII



VIII

The NMR spectra of pyridoxol and of certain of its derivatives in DMSO are summarized in Table II. Here again the methylene peaks were identified by comparison with deuterated compounds. Instead of several peaks due to the two alcoholic protons and the phenolic protons of pyridoxol, a single peak was observed at -388 c.p.s. (Fig. 2, top). The position of this peak is intermediate between that expected for the alcoholic protons (-310 c.p.s.) and that expected for the phenolic proton (-560 c.p.s.), suggesting that the three protons migrate among

the four positions that they can occupy at a rate greater than is necessary for observation of their resonance transition in the several magnetic environments. In pyridoxol hydrochloride, the hydroxyl peak is considerably shifted to the lower field and is very broad.

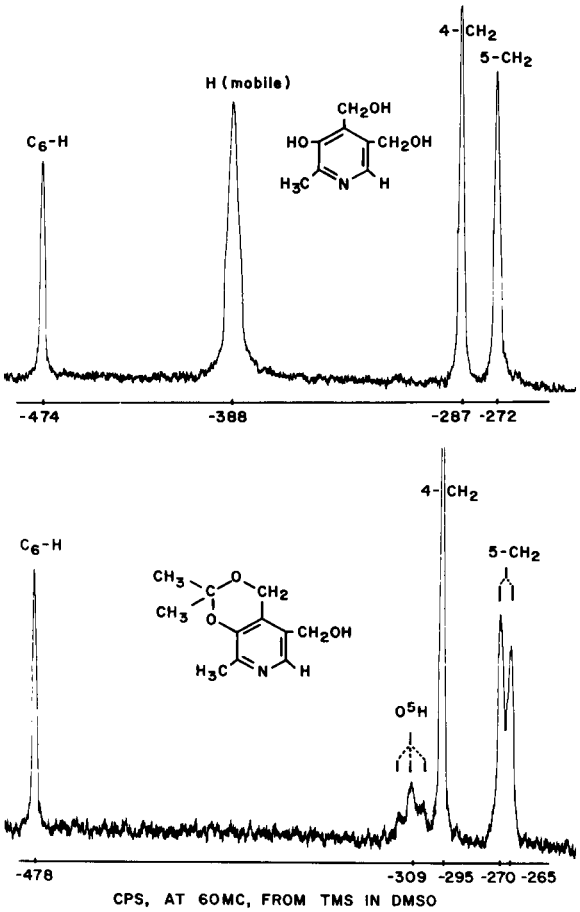
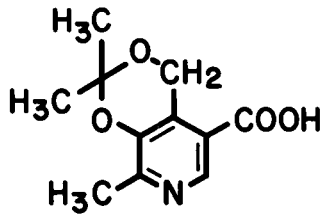


Fig. 2. NMR spectrum of pyridoxol in DMSO (top). The three "mobile" protons give rise to the peak at -388 c.p.s. This is contrasted with the expected splitting pattern of the primary alcoholic group in α^4,3-*O*-isopropylidenepyridoxol (bottom).

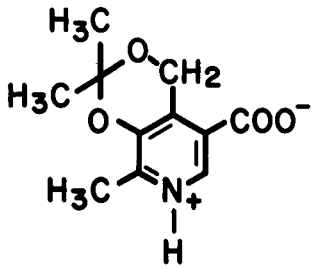
In α^4,3-*O*-isopropylidenepyridoxol, the anticipated hydroxyl splitting takes place; the hydroxyl proton appears as a triplet, and the 5-methylene protons, split by the hydroxyl proton, appears as a doublet (Fig. 2, bottom). Splitting of the hydroxyl groups could also be observed in 3-*O*-methanesulfonylpyridoxol (19). Here the splitting patterns of the two hydroxyl signals overlap to product a quadruplet (Table II). We were able to observe, in a number of other instances, that whenever the phenolic group is blocked, proton exchange is slowed down enough to permit observation of spin-spin splitting caused by hydroxyls. On the other hand, if the phenolic group is free, neither the expected splitting by the hydroxyl protons nor discrete peaks due to the various hydroxyl protons could be observed. Thus the presence of the phenolic group, and possibly the fast equilibrium VII $\rightleftharpoons$ VIII, would appear to result in a fast proton migration. If this explanation holds, it should be possible to estimate the rates of proton exchange by considering the shape of the hydroxyl peaks (20).

A similar phenomenon has been observed by us in some benzene analogs. In *O*-hydroxybenzyl alcohol, no phenolic proton peak could be detected (in DMSO); but in salicylaldehyde, the phenolic proton appeared at the expected position (-647 c.p.s.).

α^4,3-*O*-Isopropylidene-5-pyridoxic acid (9a) and one of its homologs [α^4,3-*O*-isopropylidene-α^5-isopropylideneacetic acid (21)] did not show an NMR signal of the carboxyl proton. Here again the proton has an opportunity to migrate from oxygen to nitrogen, as in IX $\rightleftharpoons$ X (22).



IX



X

The purity of dimethyl sulfoxide is also of importance in observing the hydroxyl protons. While the presence of water in pure DMSO did not seem to interfere with the observation of the hydroxyl splitting pattern in α^4,3-*O*-isopropylidenepyridoxol, no peak corresponding to the hydroxyl proton could be observed in the technical DMSO.

The observations made in the present study indicate the importance of structural factors in determining the nature of exchangeable protons by NMR spectroscopy employing dimethyl sulfoxide as solvent.

TABLE I (a)

	$2CH_3$			$4CH_2OD$			$5CH_2OD$			C_6H		
	Acid	Neutral	Alkaline	Acid	Neutral	Alkaline	Acid	Neutral	Alkaline	Acid	Neutral	Alkaline
Pyridoxol	-162	-148	-143	-305	-292 (b)	-290	-292	-285	-275	-495	-461	-452
Pyridoxol-α^5,α^5-d_2	-162	-148	-143	-305	-292 (b)	-290	-	-	-	-495	-461	-452

(a) NMR spectra were determined as previously described (10), except that sodium 3-(trimethylsilyl)-1-propanesulfonate was used instead of 1,4-dioxane as explained in the text. The shifts are expressed in c.p.s. units at 60 Mc. (b) This peak was obscured by the HDO peak; its value is approximate.

TABLE II

NMR Spectra of Pyridoxol and Some of Its Derivatives in Dimethyl Sulfoxide (a)

	$2CH_3$	$4CH_2OH$	$5CH_2OH$	C_6H	Other
Pyridoxol	-142 (b)	-287	-272	-474	-388 (3 protons)
Pyridoxol-α^5,α^5-d_2	-142 (c)	-289 (c)	-	-477 (c)	
Pyridoxol hydrochloride	-157 (b)	-292	-286	-492	Broad peak extending between -400 and -680 (at *ca.* -550 c.p.s.)
Pyridoxol-α^5,α^5-d_2 hydrochloride	-157 (b)	-290 (c)	-	-491 (c)	
α^4,3-*O*-Isopropylidenepyridoxol	(d)	-295	-265 -270	-478	(5 OH) -309 (triplet)
3-*O*-Methanesulfonylpyridoxol	(d)	-276, -282, -287		-509	(4 OH, 5 OH) -312, -316, -322, -326
α^4,3-*O*-Isopropylidene-5-pyridoxic acid	(d)	-310	-	-515	No peak due to acid proton

(a) Expressed in c.p.s. units at 60 Mc. from tetramethylsilane as internal standard; 10% solutions were used. (b) This value was determined by running the spectrum in deuterated DMSO in a microcell. (c) These values were obtained in a microcell, and are subject to greater error than those obtained in a macrocell. (d) Not determined; sample was dissolved in ordinary DMSO.

EXPERIMENTAL

3-*O*-Acetyl-4-pyridoxic acid lactone (IV).

4-Pyridoxic acid lactone (0.50 g.) was suspended in dry pyridine (5 ml.), cooled, and acetic anhydride (5 ml.) added with shaking. After shaking for 24 hours, the solution was concentrated *in vacuo* to a small volume. The acetate crystallized upon addition of water and cooling. Recrystallization from methyl alcohol gave 0.53 g. (83%) of the acetate, m.p. 169-170° (lit. (8) m.p. 165-176°).

Anal. Calcd. for $C_{10}H_9NO_4$: C, 57.97; H, 4.34; N, 6.76. Found: C, 57.92; H, 4.35; N, 6.57.

Pyridoxol-α^4,α^4-d_2 (I) hydrochloride.

3-*O*-Acetyl-4-pyridoxic acid lactone (0.50 g.) was dissolved in dry tetrahydrofuran (100 ml.). This solution was added drop by drop to a stirred suspension of lithium aluminum deuteride (0.50 g.) in 50 ml. of tetrahydrofuran under nitrogen. The reaction mixture was then refluxed for 8 hours with stirring. After cooling, ethyl acetate (20 ml.) was added, followed by water (5 ml.). The solution was filtered, the residue taken up in methanol, treated with dry ice, and warmed. After filtration, the residue was extracted three or four times with hot methanol. The methanol extracts and the tetrahydrofuran solution were combined, and evaporated to dryness *in vacuo*. After treatment with 1 *N* hydrochloric acid, the residue crystallized from an alcohol-ether mixture (0.21 g., 42%); m.p. 210-211° (dec.), mixture with pyridoxol hydrochloride m.p. 208-209° (dec.).

Anal. Calcd. for $C_8H_{10}ClD_2NO_3$: N, 6.75. Found: N, 6.44.

α^4-3-*O*-Isopropylidenepyridoxol-α^5,α^5-d_2.

Methyl α^4,3-*O*-isopropylidene-5-pyridoxate (0.5 g.) in ether (anhydrous, 25 ml.) was added dropwise over a period of 45 minutes to a stirred suspension of lithium aluminum deuteride (0.16 g.) in ether (100 ml.) under nitrogen. The reaction mixture, after being refluxed for 45 minutes was cooled, and ethyl acetate (10 ml.) added, followed by water (1 ml.). The ether solution was decanted, and the residue extracted with hot ethyl acetate. The combined ether and ethyl acetate extracts were dried, and evaporated under reduced pressure. The residue was crystallized from ether (0.35 g., 78%); m.p. 108-109°, mixture, m.p. with authentic sample (m.p. 115°), 112-113°.

Anal. Calcd. for $C_{11}H_{13}D_2NO_3$: N, 6.63. Found: N, 6.60.

Pyridoxol α^5,α^5-d_2 hydrochloride.

α^4,3-*O*-Isopropylidenepyridoxol-α^5,α^5-d_2 was heated in 1 *N* hydrochloric acid, and the resulting salt was recrystallized from ethyl alcohol; m.p. 209-210° (dec.), m.p. of pyridoxol hydrochloride 206-208°.

Acknowledgment.

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