by a Wood's metal bath. In the decompositions of II, IV, and VI, the bath temperature was slowly raised until the product distilled. In the decompositions of I, III, and VII, the bath temperature was maintained at about 300° for 1.5, 5.5, and 5 hr., respectively, and the product was isolated by dissolving the residue in ethanol–water or ethanol–water–tetrahydrofuran, treating with charcoal, cooling, and filtering. The amines from the decomposition of I, VI, and VII were recrystallized from ethanol–water; the amine from III was recrystallized from ethanol–water–dioxane.

Pyridoxine Chemistry. V. Synthesis of Isopyridoxal, 5-Pyridoxic Acid Lactone, and Their Derivatives[1,2]

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The present paper describes convenient methods of synthesis for isopyridoxal, 5-pyridoxic acid lactone, and certain of their derivatives. The physical and chemical properties and some biological properties of these various compounds have been compared with the corresponding properties of some of their isomeric derivatives in the pyridoxal–pyridoxic acid series. The amide and hydrazide of 5-pyridoxic acid were found to be hydrolyzed with extreme ease, indicating neighboring-group participation of the 4-hydroxymethyl group during hydrolysis. Infrared and proton magnetic resonance spectra of these compounds have been studied. It has been shown that isopyridoxal exists in the hemiacetal form both in the solid state and in aqueous solution.

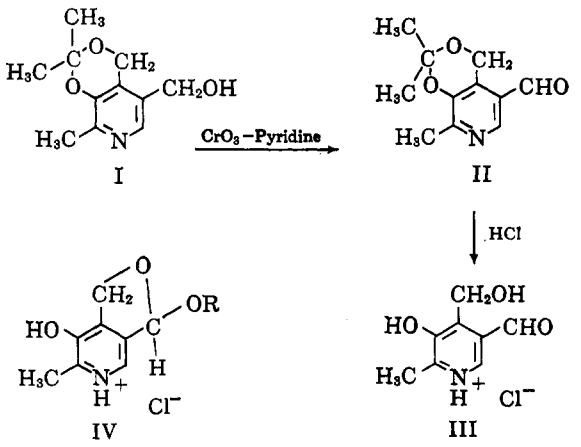
Pyridoxal is metabolized in man and rodents to pyridoxal phosphate and 4-pyridoxic acid or its lactone (β-pyracin). Although pyridoxal is closely related to the cofactor, 4-pyridoxic acid is excreted in the urine, following ingestion of either form of the vitamin. Chemically, both metabolites are formed from pyridoxal with relative ease, since the 4-hydroxymethyl side chain of pyridoxal is preferentially attacked by oxidizing agents.[3]

The ready availability of the starting compounds made it possible to obtain a number of derivatives selectively modified at the 4-position, many of which were found to have a pronounced biological activity. Thus, several hydrazones of pyridoxal were found to inhibit pyridoxal phosphokinase *in vitro*, and pyridoxal methylhydrazone and isonicotinoylhydrazone had weak inhibitory activity on the growth of Sarcoma 180 in mice and also inhibited the growth of some microorganisms.[4] Several thiosemicarbazones show promise as antitubercular drugs,[5] and 4-pyridoxhydrazone was found to inhibit the growth of malignant cells in tissue culture.[6] Derivatives of 4-pyridoxic acid, such as the hydrazone, also have been shown to be active as bacteriostatic agents.[7]

In our studies concerning the synthesis of potential antimetabolites for pyridoxine, it was of interest to determine whether similar modifications in the 5-hydroxymethyl side chain of pyridoxol would yield compounds capable of inhibiting certain enzymes essential in the metabolism of amino acids, and whether such compounds would be of some potential chemotherapeutic value. Isopyridoxal, 5-pyridoxic acid, and the lactone of the latter recently have been found to be metabolites of pyridoxol in certain microorganisms (*Pseudomonas sp.*).[8]

Methods for the synthesis of isopyridoxal[9] and 5-pyridoxic acid lactone (α-pyracin)[10] have been described, but gave poor yields and were impractical. In a preliminary communication,[11] we described a simple and unequivocal method for the synthesis of isopyridoxal, starting from α^4,3-O-isopropylidenepyridoxol (I).[12] (Another name for I is O^3O^4-isopropylidenepyridoxine. The nomenclature of this type of compound presents problems.[13]) Later we developed a convenient method for the preparation of the starting material (I).[14]

Oxidation of I with the chromic anhydride–pyridine complex at room temperature for 2 days gave only a 30% yield of the aldehyde (II). When the reaction mixture was refluxed for 90 min., however, the yield increased to 83%. In the relatively few cases in which the reagent has been used for the synthesis of aldehydes, an increased yield was obtained at moderately elevated temperatures. Very recently, in a systematic study of



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(3)(a) S. A. Harris, D. Heyl, and K. Folkers, *J. Am. Chem. Soc.*, **66**, 2088 (1944); (b) J. W. Huff and W. A. Perlzweig, *J. Biol. Chem.*, **155**, 345 (1944); (c) D. Heyl, *J. Am. Chem. Soc.*, **70**, 3434 (1948).

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(9) S. A. Harris, D. Heyl, and K. Folkers, *J. Am. Chem. Soc.*, **66**, 2088 (1944).

(10) S. A. Harris, E. T. Stiller, and K. Folkers, *ibid.*, **61**, 1242 (1939); J. Baddiley and A. P. Mathias, *J. Chem. Soc.*, 2583 (1952).

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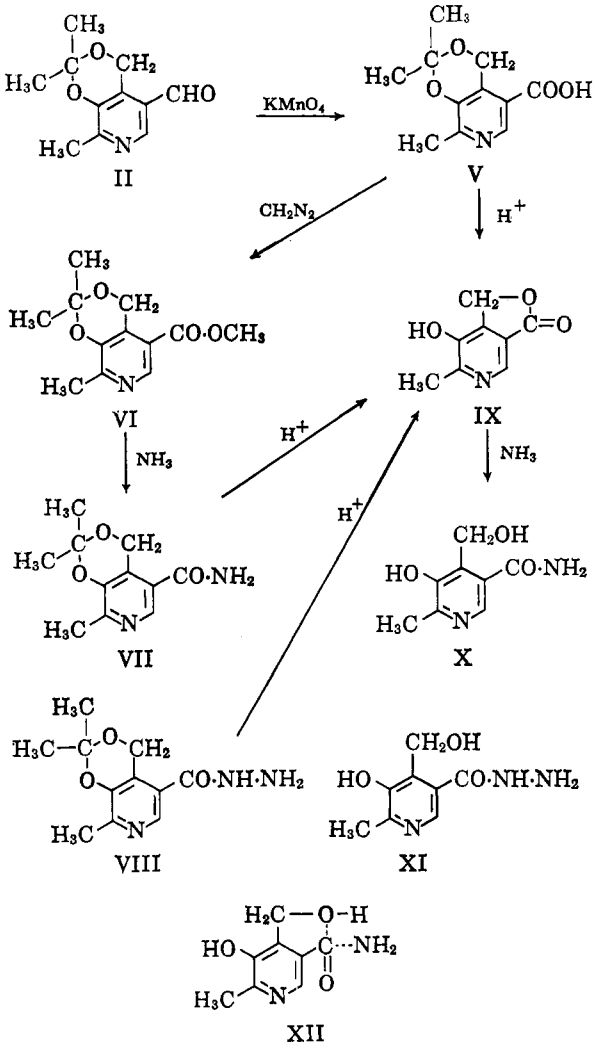
(13) W. Korytnyk, *J. Org. Chem.*, **27**, 3724 (1962).

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

SCHEME I



this reagent, citronellol was converted by the chromium trioxide–pyridine complex to citronellal in only 25% yield when the reaction was carried out at room temperature for 21 hr., but the yield increased to 47% when the reaction was carried out at 40° for 4.5 hr.[15] Our present results indicate that the reflux temperature would probably increase yield and shorten reaction time.

The isopropylidene group in II was hydrolyzed with weak hydrochloric acid to give isopyridoxal hydrochloride, from which the free base was liberated. The melting point, mixture melting point, and ultraviolet and infrared spectra of the synthetic material were found to be identical with those of the natural product isolated from a culture medium of *Pseudomonas sp.*[8,16]

The aldehyde function of pyridoxal and pyridoxal phosphate plays an important part in the catalysis of various biochemical reactions, and considerable attention has been focused on its nature.[17] It has been shown by ultraviolet spectroscopy,[18] and more recently by proton magnetic resonance spectroscopy,[1] that pyridoxal exists in the hemiacetal form. The infrared spectrum of solid pyridoxal[19] lacks a carbonyl band, and a hemiacetal structure has been postulated.[19b]

We have compared the infrared spectra of isopyridoxal with those of pyridoxal in Nujol mull. Both compounds show an absence of carbonyl bands which is, however, evident in isopropylideneisopyridoxal (II) at 5.89 μ (1698 cm.$^{-1}$). This indicates that, in the solid form, both pyridoxal and isopyridoxal exist in the hemiacetal form. Another feature of the spectra is the presence of well-defined peaks for both phenolic and alcoholic hydroxyls (at 3.79 and 3.00 μ, respectively) in isopyridoxal in contrast to that of pyridoxal. This may be related to the possibility of intramolecular hydrogen bonding in the latter.

In solution, isopropylideneisopyridoxal (II) readily forms a dimedone derivative, whereas isopyridoxal does not. Pyridoxine derivatives which have both the phenolic and the 4-hydroxymethyl groups unsubstituted form borate complexes. Their formation can be demonstrated by shifts in the ultraviolet spectrum.[14,20] Isopyridoxal did not give this shift in neutral solution, and this again supports the hemiacetal structure.

Proton magnetic resonance spectra of these compounds are also consistent with the hemiacetal structure and will be discussed below.

Careful oxidation of α^4,3-O-isopropylideneisopyridoxal (II) with alkaline potassium permanganate gave α^4,3-O-isopropylidene-5-pyridoxic acid (V) (Scheme I) in 82% yield. The yield was considerably lower (44%) when isopropylidenepyridoxol (I) was oxidized under similar reaction conditions and decreased when more vigorous conditions were used. This loss presumably occurs through oxidation of the protecting ketal group under the more vigorous conditions.

The acid V on heating with dilute acetic acid gave the lactone IX (α-pyracin) in excellent yield.

Methylation of the acid with diazomethane gave the methyl ester (VI), which was readily converted to the amide VII and the hydrazide VIII. We have now attempted to hydrolyze the protecting isopropylidene group selectively so as to obtain the unsubstituted amide IX and hydrazide X. A selective hydrolysis of the isopropylidene group has been readily accomplished with several esters of isopropylidenepyridoxol without appreciably affecting the ester link.[14] In the case of the amide and hydrazide, however, even mild hydrolysis gave the lactone IX and not the desired unprotected derivatives. Under similar or even more drastic conditions of hydrolysis, nicotinamide has been recovered unchanged. It is very probable that the situation in the case of acid hydrolysis is due to the participation of the 4-hydroxymethyl group in the hydrolysis step, as shown in XII. A somewhat analogous case of intramolecular participation of a neighboring group during hydrolysis has been studied by Bender, *et al.*[21] The hydrolysis rate of phthalamic acid was found to be enhanced by the presence of a neighboring carboxylic acid group.

The amide (X) and hydrazide (XI) of 5-pyridoxic acid were finally obtained by the interaction of liquid ammonia and hydrazine, respectively, with the lactone

(15) J. R. Holum, *J. Org. Chem.*, **26**, 4814 (1961).

(16) We are greatly indebted to Dr. E. E. Snell for making the natural metabolite available to us.

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TABLE I

PROTON MAGNETIC RESONANCE SPECTRA OF ISOPYRIDOXAL, PYRIDOXAL, AND THE CORRESPONDING LACTONES[a]

Compound	2-CH_3			Hemiacetal H			CH_2OR			C_6–H		
	Acid	Neutral	Alkaline	Acid	Neutral	Alkaline	Acid	Neutral	Alkaline	Acid	Neutral	Alkaline
Isopyridoxal (IV, R = H)	−157	−154	−140	−401	−397	−404	−317 −319	−307 −314	−300	−497	−487	−455
Isopyridoxal methyl acetal (IV, R = CH_3)	−156	−144	−139	−384	−379	−374	−316	−307	−303 −305	−492	−465	−455
Isopyridoxal ethyl acetal (IV, R = C_2H_5)	−158	[b]	[b]	−390	[b]	[b]	−318	[b]	[b]	−498	[b]	[b]
Pyridoxal	−159	−145	−139	−402 −404	−392	−425	−315	−304 −307	−289	−492	−457	−442
Pyridoxal ethyl acetal	−159	−145	−140	−404 −405	−378	−376 −378	−314	−304	−301 −305	−492	−455	−448
5-Pyridoxic acid lactone (IX)	−163	[c]	−138	...	...	...	−349	[c]	−314	−526	[c]	−475
4-Pyridoxic acid lactone	−164	[c]	−140	...	...	...	−333	[c]	−304	−496	[c]	−437

[a] Spectra were obtained at 60 Mc., using a Varian A-60 instrument and 1,4-dioxane as internal standard as described in ref. 1. [b] Not determined. [c] Too insoluble in neutral solution.

TABLE II

PROTON MAGNETIC RESONANCE SPECTRA OF PYRIDOXINE DERIVATIVES IN CARBON TETRACHLORIDE OR DEUTERIOCHLOROFORM[a]

Compound	2-CH_3	4-CH_2OR	5-CH_2OR	C_6–H	Others
α^4,3-O-Isopropylidenepyridoxol (I)	−140	−296	−272	−466	$-C(CH_3)_2$, −91; −OH, −265
5-O-Benzoyl-α^4,3-O-isopropylidenepyridoxol[b]	−142	−293	−312	[c]	$-C(CH_3)_2$, −91
α^4,3-O-Isopropylidene isopyridoxal (II)	−148	−306	...	−506	$-C(CH_3)_2$, −93; ald. H, −604
α^4,3-O-Isopropylidene 5-pyridoxamide (VII)	−147	−309	...	−499	$-C(CH_3)_2$, −94; $-CONH_2$, −384
α^4,3-O-Isopropylidene 5-pyridoxic acid methyl ester (VI)	−148	−312	...	−521	$-C(CH_3)_2$, −96; $-OCH_3$, −235
3-O-Benzoyl-α^4,α^5-O-isopropylidenepyridoxol (XIII)[d]	−142	−283	−287	[c]	$-C(CH_3)_2$, −84
3-O-Methanesulfonyl-α^4,α^5-O-isopropylidenepyridoxol (XIV)[d]	−150	−293	−287	−484	$-C(CH_3)_2$, −86; $-SO_2CH_3$, −183
3-O-Methylpyridoxol	−146	−281 or −286	−286 or −281	−482	−OH, −328; $-OCH_3$, −226
Tri-O-acetylpyridoxol		−307 or −315	−315 or −307	−503	$-CH_3$, −118, −122, −139, −142

[a] Expressed in c.p.s. units at 60 Mc. from tetramethylsilane as internal standard. [b] See ref. 14. [c] Peak was obscured by benzene proton resonance lines. [d] See ref. 13.

IX. The corresponding derivatives of 4-pyridoxic acid have been synthesized previously.[3c]

Proton Magnetic Resonance Spectra.—Finally, as an extension of our studies in the application of the proton magnetic resonance spectroscopy in the pyridoxine series,[1] we have compared the p.m.r. spectra of isopyridoxal and its acetals with those of pyridoxal and ethyl acetal (Table I). There is a remarkably similarity between the spectra of the parent compound and its cyclic acetal derivative, and between the spectra of pyridoxal and isopyridoxal themselves. In pyridoxal as well as in isopyridoxal (Fig. 1), the free aldehyde proton peak which was found for isopropylidene isopyridoxal (II) at −604 c.p.s. in CCl_4 (Table II) is missing. The C_6–H is somewhat less shielded in isopyridoxal than in pyridoxal. This is probably because the potential aldehyde group in pyridoxal, being *meta to* the C_6–H, has less influence on the shielding of this proton. This effect is much more pronounced for the corresponding isomeric lactones (Table I).

The close resemblance of the p.m.r. spectra of pyridoxal and isopyridoxal to those of their hemiacetal derivatives, together with the other supporting evidence discussed above, strongly indicates that these compounds exist in the hemiacetal form. The situation closely resembles that found in the case of sugars, which usually exist in the hemiacetal form in the solid state and in solution, and yet are capable of undergoing most of the reactions that are typical of aldehydes.

A doubling or broadening of peaks of protons in the side chains which are involved in the hemiacetal formation can often be observed for isomeric pyridoxals and their methyl acetals (Table I). This effect could be due to the puckering of the five-membered ring.[1] A doubling of the corresponding peaks in isomeric pyridoxic acid lactones is absent, indicating a planar structure.

Many substituted derivatives of pyridoxol are not sufficiently soluble in water, and their p.m.r. spectra were taken either in deuterated chloroform or carbon tetrachloride.[22]

The spectra (Table II) were usually found to be simple and, in most cases permitted unequivocal assignments of 2-CH_3 and C_6–H peaks. The two 2-proton peaks of the hydroxymethyl side chains of pyridoxol and its derivatives are usually close together, and

(22) Both solvents can be used interchangeably, as p.m.r. spectra determined in both carbon tetrachloride and deuterated chloroform were found to be identical. The solubility of 3-O-methylpyridoxol permitted its spectrum to be determined in both $CDCl_3$ (Table II) in this study, and in D_2O in the previous paper.[1] Although the similarity is closer than could be anticipated, the results indicate that spectra taken in an organic solvent and in D_2O can be compared only qualitatively.

in some cases it was not possible to distinguish them. In other cases, when there were good structural analogies, it was possible to tell the two peaks apart.

Thus, in α^4,3-O-isopropylidenepyridoxol (I), the two 2-proton peaks appear at −272 and −296 c.p.s., respectively. Benzoylation of I is expected to decrease the shielding of methylene protons on the 5-hydroxymethyl group, leaving the shielding on the 4-side chain much less affected. The relevant peaks in the benzoate are found at −293 and −312 c.p.s., respectively, and, therefore, the −296 c.p.s. peak in I and the −293 c.p.s. peak in its benzoate must be due to the 4-methylene protons. It should be noted that the 2-CH_3 peaks in the parent compound I and its benzoate appear at a similar position.

A much more substantial change in the shielding of adjacent protons occurs when the 5-hydroxymethyl group is converted to the aldehyde group as in II, the carbomethoxy group as in VI, or the amide group as in VII. Here a comparison of the effects of substituents, the electron-withdrawing property of which increases from hydroxymethyl to carbomethoxy, can be made in regard to complex derivatives of pyridine. A previous study of the p.m.r. spectra of various simple 4-substituted pyridine derivatives has been found useful in indicating electron densities at the 2- and 3-positions.[23]

It can be seen (Fig. 2) that the 6-proton peak for the *gem*-dimethyl group in the isopropylidene group does not change greatly. The greatest change occurs in the peaks for the groups adjacent to the 5-side chain. The shielding of C_6–H proton, being *ortho* to the modified group and directly attached to the ring, is most sensitive to changes in the electronic nature of the substituent. Nevertheless, the shielding of every proton in the molecule decreases in a regular fashion as the electron-withdrawing power of the 5-side chain increases.

Spectra of two derivatives of the seven-membered cyclic ketal of pyridoxol[13] (XIII and XIV) also have been determined (Table II). These spectra, besides confirming the structure of the seven-membered cyclic ketal, also offer an opportunity to study the electronic effects of substituent groups. Protons of the *gem*-dimethyl group are more shielded here than in the isomeric ketal, probably because the group is more removed from the electron-withdrawing influence of the ring. The methanesulfonyl group exerts a stronger electron pull than the benzoyl group does, as is evident from the changes in the shielding of the 2-CH_3 protons (Fig. 3). The assignment of the two 2-protons peaks of the side chains is based upon the assumption that the shielding of the protons in the 4-side chain is decreased in XIV by virtue of the electron-withdrawing properties of the adjacent methylsulfonyl group, whereas it remains unchanged in the 5-side chain.

In connection with p.m.r. spectral studies and some other synthetic work, we needed some tri-O-acetylpyridoxol, which has been obtained previously, but not adequately described.[24]

The triacetate has been obtained in high yield as the hydrobromide (see Experimental).

Biological Properties.—Pyridoxal has been condensed with a number of carbonyl reagents, such as

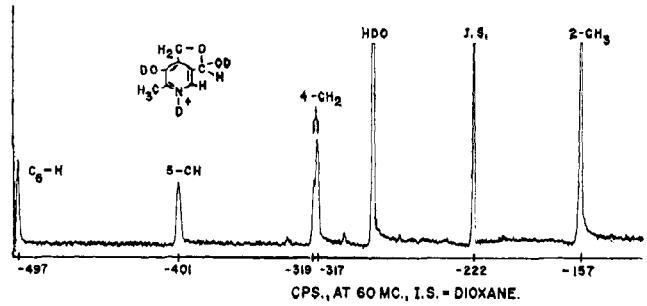

Fig. 1.—The p.m.r. spectrum of isopyridoxal cation in deuterium oxide.

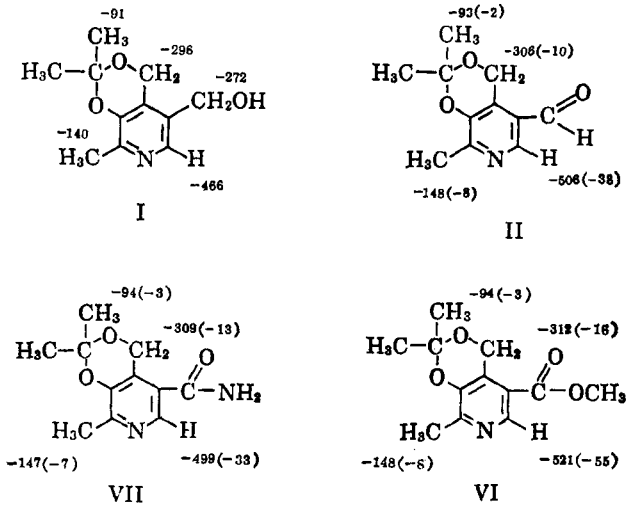

Fig. 2.—Comparison of the p.m.r. spectra of some α^4,3-O-isopropylidene derivatives of pyridoxine in deuteriochloroform. Positions of peaks are indicated in c.p.s. units. Numbers in parentheses represent shifts with respect to α^4,3-O-isopropylidenepyridoxol (I).

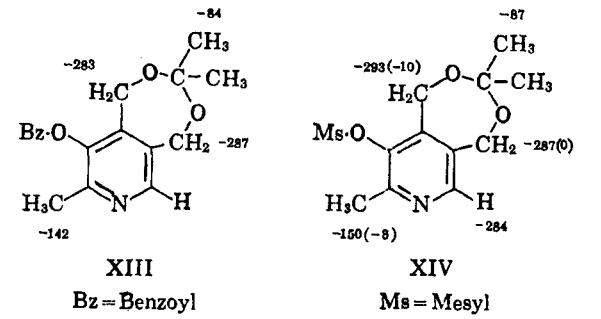

Fig. 3.—Comparison of the p.m.r. spectra of two derivatives of α^4,α^5-O-isopropylidenepyridoxol. Positions of peaks are indicated in c.p.s. units. Numbers in parentheses represent shifts with respect to 3-O-benzoyl-α^4,α^5-isopropylidenepyridoxol (XIII).

hydrazines and hydroxylamine, and some of these were found to be inhibitors of pyridoxal kinase *in vitro*.[25] We have prepared a number of similar derivatives from isopyridoxal for testing in various biological systems. The azine, hydrazone, phenylhydrazone, dimethylhydrazone, and phenylsemicarbazone of isopyridoxal were found to inhibit pyridoxal phosphokinase, but isopyridoxal and its oxime and thiosemicarbazone were ineffective.[26] The activities found in this system for the compounds in the isopyridoxal series closely parallel those found by McCormick and Snell[25] for the corre-

(23) A. R. Katritzky and J. M. Lagowski, *J. Chem. Soc.*, 43 (1961).
(24) R. Kuhn and G. Wendt, *Ber.*, **71**, 780 (1938); S. A. Harris, *J. Am. Chem. Soc.*, **62**, 3203 (1940).
(25) D. B. McCormick and E. E. Snell, *J. Biol. Chem.*, **236**, 2083 (1961).
(26) Dr. D. B. McCormick, private communication.

sponding derivatives in the pyridoxal series. On the other hand, in contrast to the thiosemicarbazone of pyridoxal,[5] the thiosemicarbazone of isopyridoxal was found to be ineffective as an inhibitor of Mycobacterium tuberculosis *in vitro*.[27]

The amide of 5-pyridoxic acid (X) shares structural features with both nicotinamide and pyridoxine and hence might be able to interfere with the metabolism or uptake of both vitamins (dual antagonist[28]). The amide of pyridoxic acid was found to be inactive for *Saccharomyces carlsbergensis*, but inhibits the growth of *S. faecalis*.[29] Additional biological properties of these compounds are under investigation and will be reported elsewhere.

Experimental

α^4,3-O-Isopropylideneisopyridoxal (II).—Various precautionary measures have been adopted in the preparation of the chromium trioxide–pyridine complex. Initially we prepared the complex by the gradual addition (*ca.* 1 hr.) of 17.0 g. of CrO_3 to 250 ml. of pyridine (A. R., dried over KOH), the solution being vigorously stirred with a powerful stirrer and covered with a blanket of dry nitrogen. Occasional cooling in ice–water kept the temperature at approximately 20°. Addition of the first portions of CrO_3 invariably caused flames to appear, even though the addition of subsequent portions went smoothly. When the pyridine had been distilled over $KMnO_4$, however, no such difficulty was encountered, and CrO_3 could be added to the pyridine in a much shorter time (10–15 min.). Isopropylidenepyridoxol (25.0 g. dried) in pyridine (100 ml.) was added at once to the complex. The reaction mixture was brought to reflux temperature in 2 hr. (oil bath) and was refluxed for 90 min. After cooling, water (500 ml.) was added, and the mixture was continuously extracted with ether. After evaporation of solvents *in vacuo*, 20.5 g. (83%) of the aldehyde, m.p. 59–60°, was obtained. Sublimation at water-pump vacuum at 80° gave the raised m.p. 64–65°; λ_{max}^{EtOH} 282 mμ (ε 5.5 × 10³); $\lambda_{max}^{0.1\ N\ HCl}$ 292.5 mμ (ε 7.4 × 10³); after heating for 15 min. at 85°, λ_{max} 284 mμ (ε 9.2 × 10³).

Anal. Calcd. for $C_{11}H_{13}O_3N$: C, 63.75; H, 6.32; N, 6.76. Found: C, 63.57; H, 6.26; N, 6.95.

The **dimedone** derivative (from 10% aqueous ethanol) had m.p. 210° dec.

Anal. Calcd. for $C_{27}H_{35}O_6N$: C, 69.05; H, 7.51. Found: C, 68.89; H, 7.68.

The **hydrochloride** was obtained by passing dry hydrogen chloride gas into an ether solution of the aldehyde (II).

Anal. Calcd. for $C_{11}H_{13}O_3N$: C, 54.21; H, 5.79; N, 5.75; Cl, 14.55. Found: C, 54.10; H, 6.08; N, 5.54; Cl, 14.55.

Hydrazone of α^4,3-O-Isopropylideneisopyridoxal.—The aldehyde (3.0 g., 14.5 mmoles) was refluxed with hydrazine (15.0 ml.) in ethanol (15.0 ml.) for 1.5 hr. After keeping overnight at 5° and filtering, 2.73 g. (85%) of the hydrazone, m.p. 175–176°, was obtained. Crystallization from ethanol gave the raised m.p. 176–177°; $\lambda_{max}^{0.1\ N\ HCl}$ 310 mμ (ε 8.6 × 10³; rapid change on standing at room temperature).

Anal. Calcd. for $C_{11}H_{15}O_2N_3$: C, 59.71; H, 6.83; N, 18.99. Found: C, 59.51; H, 7.07; N, 18.63.

Isopyridoxal Hydrochloride (IV, R = H).—Isopropylideneisopyridoxal (0.20 g.) was heated in 0.1 *N* hydrochloric acid (100 ml.) at 85° (steam bath) for 40 min. Pure isopyridoxal hydrochloride was obtained on evaporating the water at room temperature (flash evaporator) and drying; λ_{max} 257 and 302 mμ (in phosphate buffer, pH 6.8; did not change on saturation with boric acid and adjustment to the original pH).

Anal. Calcd. for $C_9H_{10}ClO_3N$: C, 47.18; H, 4.95; Cl, 17.41; N, 6.88. Found: C, 47.30; H, 5.03; Cl, 17.86; N, 7.17.

Isopyridoxal.—The hydrochloride (0.23 g.) was dissolved in a few drops of water, and 1 *N* NaOH (1.0 ml.) was added. The precipitated isopyridoxal was recrystallized from water, giving fine needles, m.p. 185–186° dec., which was not depressed on addition of natural isopyridoxal.

Anal. Calcd. for $C_8H_9O_3N$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.25; H, 5.62; N, 8.58.

Isopyridoxal Methyl Acetal Hydrochloride (IV, R = CH_3).—Isopyridoxal hydrochloride (1.0 g., 4.92 mmoles) was dissolved in absolute methanol (50 ml.) and allowed to stand for 16 hr. Part of the methanol was evaporated. Ether was added to the point of turbidity, and the solution was allowed to stand in a refrigerator, giving 0.91 g. (85%) of the methyl acetal, m.p. 155–157° dec. Recrystallization from methanol–ether raised the decomposition point to 156–158°.

Anal. Calcd. for $C_9H_{12}ClO_3N$: C, 49.63; H, 5.56; OCH_3, 14.26. Found: C, 49.78; H, 5.61; OCH_3, 14.48.

Isopyridoxal Oxime.—Isopyridoxal hydrochloride (0.105 g.) was added to 2 ml. of 10% aqueous sodium acetate and heated with hydroxylamine hydrochloride (0.1 g.) for 1 hr. After crystallization from ethanol, the oxime had m.p. 198–199° dec. (lit.[8] m.p. 192–193° dec.).

Anal. Calcd. for $C_8H_{10}O_3N_2$: C, 52.74; H, 5.53. Found: C, 52.75; H, 5.76.

Isopyridoxal thiosemicarbazone was obtained similarly. Crystallization from wet dimethylformamide gave a product with m.p. 239° dec.

Anal. Calcd. for $C_9H_{13}O_2N_4S$: C, 44.79; H, 5.42; S, 13.29. Found: C, 45.04; H, 5.26; S, 13.25.

Isopyridoxal phenylhydrazone was obtained from phenylhydrazine and isopyridoxal in acetic acid–sodium acetate buffer. After crystallization from ethanol, the phenylhydrazone had m.p. 219–220° dec.

Anal. Calcd. for $C_{14}H_{15}O_2N_3$: C, 65.35; H, 5.88; N, 16.33. Found: C, 65.07; H, 6.12; N, 16.25.

Isopyridoxal 4-Phenylsemicarbazone Hydrochloride.—The phenylsemicarbazone was obtained by heating isopyridoxal hydrochloride and 4-phenylsemicarbazide hydrochloride in acetic acid–sodium acetate buffer for 1 hr. The free base (m.p. 210–220° dec.) was dissolved in ether and the hydrochloride, precipitated with dry hydrogen chloride gas, had m.p. 219–220° dec.

Anal. Calcd. for $C_{15}H_{17}ClO_3N_4$: C, 53.50; H, 5.09; N, 16.63. Found: C, 53.78; H, 5.30; N, 16.46.

Isopyridoxal Azine.—To a solution of isopyridoxal hydrochloride (3.04 g., 12.5 mmoles) in 20 ml. of sodium acetate buffer, pH 4.5, was added an equimolar amount of hydrazine. After boiling for 2–3 min., the reaction mixture was filtered, and the precipitate was washed with water. The azine was obtained in 82% yield and was crystallized from dimethylformamide.

Anal. Calcd. for $C_{16}H_8O_4N_4$: C, 58.17; H, 5.49; N, 16.96. Found: C, 58.01; H, 5.29; N, 16.80.

Isopyridoxal Hydrazone.—Isopyridoxal hydrochloride (2.04 g., 8.4 mmoles) was dissolved in sodium acetate–acetic acid buffer (18 ml.), and the pH was adjusted to 8.5 by the addition of hydrazine. The reaction mixture was heated at 95–100° for 2–3 min., kept in a refrigerator overnight, and then filtered. The hydrazone (0.83 g.), after crystallization from ethanol, had m.p. 175–176° dec.

Anal. Calcd. for $C_8H_{11}O_2N_3$: C, 53.03; H, 6.12; N, 23.19. Found: C, 53.26; H, 5.95; N, 23.23.

Isopyridoxal Dimethylhydrazone.—Isopyridoxal hydrochloride (2.0 g., 9.8 mmoles) was dissolved in 4 ml. of water and treated with 2.0 g. of dimethylhydrazine at room temperature. After standing at 0° for 16 hr., the reaction mixture was filtered. The dimethylhydrazone (1.65 g., 80%), m.p. 176–177° dec., was crystallized from ethanol.

Anal. Calcd. for $C_{10}H_{15}O_2N_3$: C, 57.40; H, 7.23; N, 20.08. Found: C, 57.50; H, 7.47; N, 19.86.

α^4,3-O-Isopropylidene-5-pyridoxic Acid (V). A. From Isopropylideneisopyridoxal.—To a vigorously stirred suspension of the aldehyde (5.0 g., 24 mmoles) was added a saturated aqueous solution of potassium permanganate (5.5 g., 35 mmoles) over a period of 2–5 min., the mixture being heated on a steam bath. Towards the end, several drops of 1.0 *N* NaOH solution was added. Manganese dioxide was filtered off with a filter aid, and the solution was evaporated *in vacuo* to approximately 10 ml. Careful acidification with concentrated HCl to pH 5 and cooling gave the main crop of the acid. Further acidification of the mother liquors gave a further portion. The combined yield was 4.4 g. (82%), m.p. 220–221° dec. The analytical sample, m.p. 221–222° dec., was obtained by crystallization from ethanol; λ_{max}^{EtOH} 294 mμ (ε 6.4 × 10⁴); $\lambda_{max}^{0.1\ N\ HCl}$ 299.5 mμ (ε 8.5 × 10³).

Anal. Calcd. for $C_{11}H_{13}O_4N$: C, 59.18; H, 5.87; N, 6.28. Found: C, 59.38; H, 6.09; N, 5.98.

(27) Dr. P. Mantegazza, private communication.

(28) T. J. Bardos, D. B. Olsen, and T. Enkoji, *J. Am. Chem. Soc.*, **49**, 4704 (1957).

(29) Dr. C. A. Nichol, preliminary observations.

B. From Isopropylidenepyridoxol.—Isopropylidenepyridoxol (2.85 g.), oxidized with potassium permanganate (3.0 g.) under reaction conditions similar to those in A, gave 1.35 g. (44%) of the acid, m.p. 220–221° dec.

α^4,3-O-Isopropylidene-5-pyridoxic Acid Methyl Ester (VI).—Isopropylidene-5-pyridoxic acid (5.0 g., 2.24 mmoles) in tetrahydrofuran (500 ml.) was treated with an excess of diazomethane in ether. After standing for 4 hr., the solvent was evaporated *in vacuo*, and the residue was extracted three times with ether. The combined ether extracts were evaporated and yielded 4.63 g. (87%) of the methyl ester, m.p. 85–87°. The analytical sample was crystallized from ethanol and had m.p. 86.5–87°; λ_{max}^{EtOH} 296 mμ (ε 6.3 × 10³). $\lambda_{max}^{0.1\ N\ HCl}$ 301 mμ (ε 8.5 × 10³); after heating for 3 hr. on steam bath (85°), λ_{max} 291 mμ (ε 8.0 × 10³) and 252 (4.4 × 10³).

Anal. Calcd. for $C_{12}H_{15}O_4N$: C, 60.75; H, 6.37; N, 5.90; OCH_3, 13.0. Found: C, 61.06; H, 6.57; N, 5.89; OCH_3, 12.96.

α^4,3-O-Isopropylidene-5-pyridoxamide (VII).—Isopropylidene-5-pyridoxic acid methyl ester (1.0 g., 4.2 mmoles) was suspended in aqueous ammonia (10 ml.), which was saturated at 0° with additional NH_3 and kept in a stoppered tube at room temperature for 7 days. After evaporation *in vacuo* and crystallization from ethanol, 0.85 g. (92%) of the amide, m.p. 174–175°, was obtained; λ_{max}^{EtOH} 290 mμ (ε 5.1 × 10³); $\lambda_{max}^{0.1\ N\ NaOH}$ 288 mμ (ε 5.2 × 10³). $\lambda_{max}^{0.1\ N\ HCl}$ 295 mμ (ε 7.4 × 10³); after heating for 2 hr. on the water bath, the spectrum was identical to that of 5-pyridoxic acid lactone.

Anal. Calcd. for $C_{11}H_{14}O_3N_2$: C, 59.45; H, 6.35; N, 12.60. Found: C, 59.83; H, 6.65; N, 12.39.

α^4,3-*O*-Isopropylidene-5-pyridoxic Acid Hydrazide (VIII).—The methyl ester (1.15 g., 4.88 mmoles) was dissolved in 10 ml. of 50% aqueous methanol, hydrazine (10 g.) was added, and the reaction mixture was heated on a steam bath for 2 hr. Evaporation of the volatiles *in vacuo* yielded a solid, which was crystallized from ethanol and yielded 0.87 g. (75%) of the hydrazide, m.p. 175° dec.; λ_{max}^{EtOH} 289 mμ (ε 6.6 × 10³); $\lambda_{max}^{0.1\ N\ NaOH}$ 298 mμ (ε 8.6 × 10³).

Anal. Calcd. for $C_{11}H_{15}O_3N_3$: C, 55.68; H, 6.37; N, 17.71. Found: C, 55.81; H, 6.74; N, 17.21.

Lactone of 5-Pyridoxic Acid (IX).—Isopropylidene-5-pyridoxic acid (1.01 g., 4.52 mmoles) was dissolved in 15% aqueous acetic acid (150 ml.) and heated on a steam bath for 3 hr. After evaporation and crystallization from ethanol, 0.68 g. (91%) of the lactone, m.p. 265–270° dec., was obtained. After recrystallization from ethanol, m.p. 275–278° dec. (lit.[10] m.p. 272–273°, 268–269° dec.).

Anal. Calcd. for $C_8H_7O_3N$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.36; H, 4.53; N, 8.30.

5-Pyridoxamide (X).—The lactone (0.57 g., 3.45 mmoles) was treated with a large excess of liquid ammonia at room temperature for 30 hr. The excess ammonia was allowed to evaporate, and the solid residue was crystallized from methanol, yielding 0.46 g. (73%) of the amide, m.p. 273–275° dec.; λ_{max}^{EtOH} 290 mμ (ε 5.8 × 10³); $\lambda_{max}^{0.1\ N\ NaOH}$ 314 mμ (ε 6.6 × 10³) and 250 (sh) (5.1 × 10³); $\lambda_{max}^{0.1\ N\ HCl}$ 293 mμ (ε 9.2 × 10³).

Anal. Calcd. for $C_8H_{10}O_3N_2$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.97; H, 5.67; N, 15.32.

5-Pyridoxic Acid Hydrazide (XI).—5-Pyridoxic acid lactone (0.254 g., 1.54 mmoles) was suspended in water (20 ml.), and hydrazine (1.0 g.) was added. The resulting clear solution was kept on a steam bath for 1 hr. and was evaporated to dryness. The solid residue was redissolved in an ethanol–ether–benzene mixture and was repeatedly evaporated to remove traces of hydrazine. Finally, the solid was suspended in ethanol, filtered, and washed with ether. The yield was 0.231 g. (76%) of the hydrazide, m.p. 252–255° dec. An analytical sample was obtained by crystallization from a dimethylformamide–ethanol mixture.

Anal. Calcd. for $C_8H_{11}O_3N_3$: C, 48.72; H, 5.62; N, 21.31. Found: C, 48.84; H, 5.73; N, 21.39.

Tri-O-acetylpyridoxol Hydrobromide.—To a solution of pyridoxol hydrochloride (40 g.) in dry pyridine (150 ml.) 70 ml. of acetic anhydride was added, and the mixture was shaken until a clear solution was obtained. After standing overnight at room temperature, excess solvent was evaporated under reduced pressure. Water was added and the mixture was allowed to stand at 0° for 4 hr. to hydrolyze acetic anhydride. The solution was now flash evaporated; the process was repeated several times to remove most of the acetic acid. The residue was made alkaline with sodium carbonate and extracted three times with ether. Removal of the solvent gave a viscous residue which was dissolved in methanol and to which aqueous hydrogen bromide was added, yielding the hydrobromide (79.0 g., 90%) in colorless needles, m.p. 155°.

Anal. Calcd. for $C_{14}H_{15}NO_6 \cdot HBr$: C, 44.93; H, 4.31; N, 3.74; Br, 21.40. Found: C, 44.72; H, 4.52; Br, 21.61.

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Quinoxaline Studies. XII. Stereodirective Syntheses of *cis*- and *trans*-Decahydroquinoxalines and *cis*- and *trans*-Decahydroquinoxalones-2[1,2]

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5,6,7,8-Tetrahydroquinoxalone-2 (I) was prepared in 64% yield by condensing 1,2-cyclohexanedione with glycinamide in aqueous methanol–sodium hydroxide solution. I was catalytically reduced with platinum to give 100% yield of *cis*-decahydroquinoxalone-2, chemically reduced with sodium–amyl alcohol to give 58% yield of only *trans*-decahydroquinoxaline, and electrolytically reduced in aqueous sulfuric acid at a lead cathode to give 8% yield of *trans*-decahydroquinoxaline plus 13% yield of *trans*-decahydroquinoxalone-2. *cis*-Decahydroquinoxaline was prepared in 12% yield by the catalytic reduction over platinum of an aqueous solution of 1,2-cyclohexanedione and ethylenediamine, as was *trans*-decahydroquinoxaline in 36% yield by a similar reduction of an ethanol solution of *trans*-1,2-cyclohexanediamine and glyoxal. Also reported is a study of the ratios of *cis*- and *trans*-decahydroquinoxalones-2 and *cis*- and *trans*-decahydroquinoxalines obtained in certain syntheses.

Relatively large quantities of the two geometrical isomers of decahydroquinoxaline were needed for complexation studies. The unequivocal syntheses of *cis*- and *trans*-decahydroquinoxalines, the corresponding decahydroquinoxalones-2, and decahydroquinoxaline-diones-2,3 were described in the preceding paper.[2] The purpose of this paper is to report syntheses of *cis*- and *trans*-decahydroquinoxalines, *via* other synthetic routes more efficacious for the preparation of large amounts of materials.

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(2) Paper XI of this series: E. Brill and H. P. Schultz, *J. Org. Chem.*, **28**, 1135 (1963).