was made to 0.5 ml with H_2O. The mixt was incubated for 5 min at 37°. Controls were included to correct for nonenzymatic decarboxylation.

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5-Homopyridoxals, 5-Thiopyridoxal, and Related Compounds. Synthesis, Tautomerism, and Biological Properties[1]

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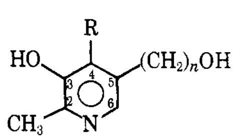
Homopyridoxals with 1 or 2 additional CH_2 groups in the 5 position have been obtained by controlled oxidn of the corresponding homopyridoxols with MnO_2. More vigorous oxidn yielded the corresponding 4-homopyridoxic acids. 4-Deoxyhomopyridoxols have also been prepd from homopyridoxols by hydrogenolysis with hydrazine. Reaction of hydrazine-d_4 with pyridoxol gave 4-deoxypyridoxol in which the α^2 and α^4 Me groups and the 6 position were deuterated. These deuterations as well as the formation of 4-deoxypyridoxol have been rationalized assuming the formation of quinone methide intermediates. 5-Thiopyridoxal was prepd and was found to be a hemiacetal in the narrow pH range in which it was stable. Likewise, the two homopyridoxals exist in a cyclic (hemiacetal) form in acid and neutral soln, whereas in an alkaline medium a marked tendency to revert to the aldehyde form, particularly in the 2 C homolog, has been observed. Derivatives of both the aldehyde and hemiacetal forms of these pyridoxal analogs have been obtained. Pyridoxal was found to undergo a Cannizzaro reaction when treated with alkali. The oximes of homopyridoxals and the 4-deoxyhomopyridoxols are inhibitors of pyridoxal phosphokinase. The effect of some of these compds on *Saccharomyces carlsbergensis*, tissue culture cells, and certain enzymes *in vivo* has also been determined.

In efforts to develop more selective antagonists of vitamin B_6 that might be active as anticancer agents[2] we previously synthesized a series of homologs of pyridoxol (I, R = CH_2OH) by extension and branching of the 4- and 5-hydroxymethyl side chains.[3,4] Compds obtained by extension of the 5 position (I, R = CH_2-OH; n = 2–4) were found to be inhibitors of *Saccharomyces carlsbergensis*[4] but were ineffective in inhibiting mammalian systems.[5]



I

It was hoped that by modifying the 4-hydroxymethyl to a formyl (I, R = CHO; n = 2,3) or Me (I, R = CH_3; n = 2,3), improved inhibitors could be obtained, since they would more closely resemble the biologically more active form of vitamin B_6 or the well-known antimetabolite 4-deoxypyridoxol (I, R = CH_3; n = 1), resp. (It has also been found that when 5-deoxypyridoxol was converted to the corresponding 4-aldehyde, toxicity was increased markedly.[6])

(1) (a) Pyridoxine Chemistry. 26. Preceding paper in this series: W. Korytnyk and B. Lachmann, *J. Med. Chem.*, **14**, 641 (1971). (b) A brief report of this study has appeared: Abstracts of the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, MEDI 48.

(2) For a review of the syn and biol activity of vitamin B_6 analogs see W. Korytnyk and M. Ikawa, *Methods Enzymol.*, **18A**, 524 (1970).

(3) W. Korytnyk and B. Paul, *J. Med. Chem.*, **13**, 187 (1970).

(4) W. Korytnyk, B. Paul, A. Bloch, and C. A. Nichol, *ibid.*, **10**, 345 (1967).

(5) Compd IX was tested against S-180 in Swiss mice fed complete or vitamin B_6 deficient diets. It was found to be inactive at doses up to 400 mg/kg per day x 7 ip or 0.025% in diets (Dr. E. Mihich, personal communication).

(6) F. Rosen, E. Mihich, and C. A. Nichol, *Vitam. Horm. (New York)*, **22**, 609 (1964).

In addn to compds of the types already mentioned, we have synthesized 5-thiopyridoxal (XVIII) and two 5-homopyridoxic acids (VIII and XIII). Chem properties of these compds, particularly ring–chain tautomerism, have been studied, and have been compared with those of pyridoxal. Some of these compds have been evaluated for their biol and enzymatic activity in several systems.

Chemistry. Synthesis.—Scheme I depicts the synthesis of the homopyridoxals (III and X), homopyridoxic acid (VIII and XIII), their derivs, and 4-deoxyhomopyridoxols (VI and XII); and Scheme II that of 5-thiopyridoxal (XVIII) and its ethyl acetal deriv.

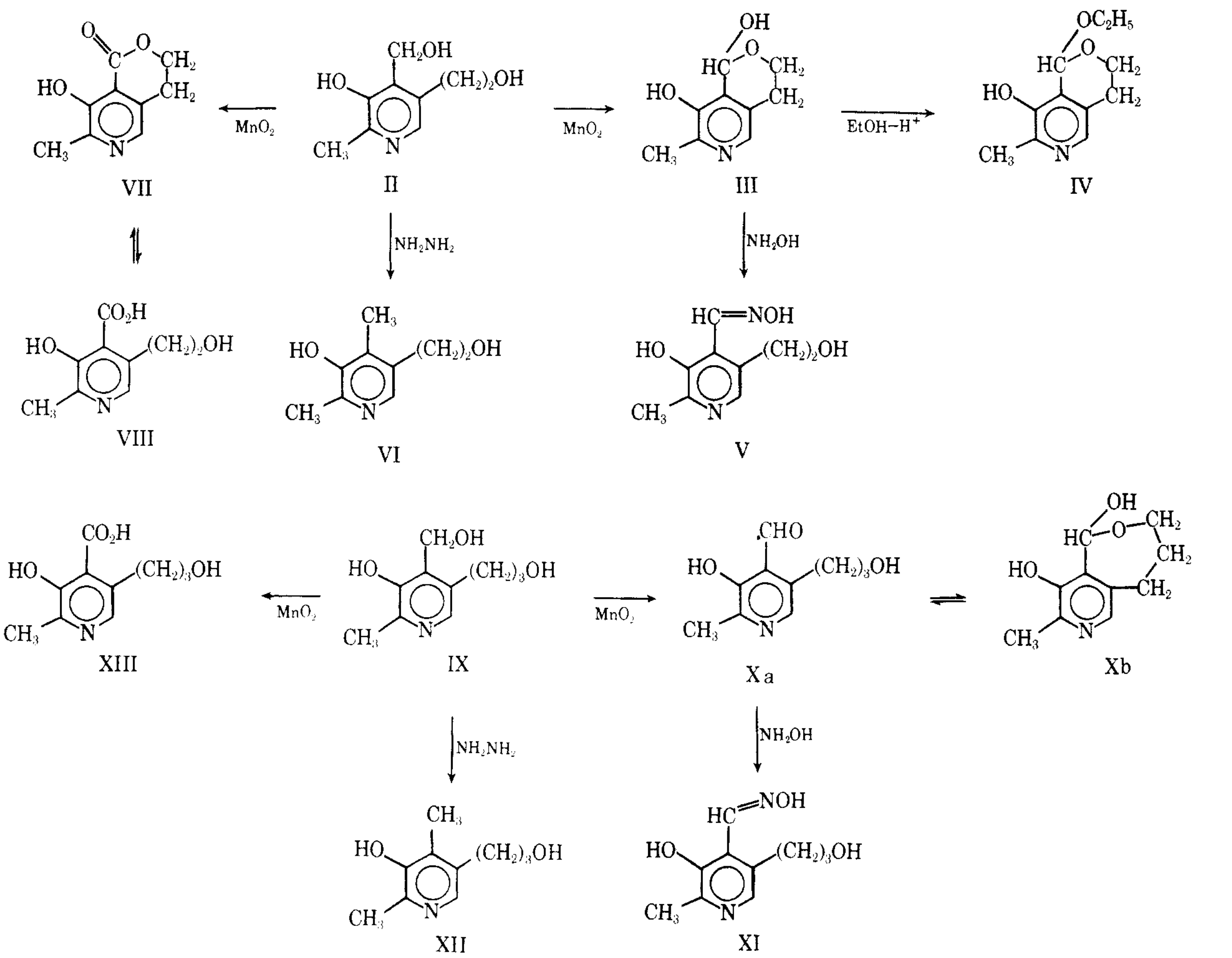
Oxidn of the 4-CH_2OH group to the CHO and COOH groups has been carried out with MnO_2 as shown in Scheme I. Conditions for this oxidn had to be varied for each compd. Probably because of the ring–chain tautomerism of these compds (see below), the length of the side chain had a profound effect on the oxidizability of the 4-CH_2OH group. Thus conditions[7] that had been worked out earlier for the oxidn of pyridoxol to pyridoxal and 4-pyridoxic acid could not be applied.

In the synthesis of 5-thiopyridoxal (XVIII, Scheme II), it was necessary to block the SH group of 5-thiopyridoxol by benzoylation, as in XVI. This was accomplished in a more direct manner and in better yield (from the blocked chloro derivative XIV) than has been reported previously.[8] The most crucial step in this synthesis was the deblocking step to yield 5-thiopyridoxal from XVII. Both acid and alkaline hydrolysis of the thiobenzoate XVII gave a mixt of products, but a base-catalyzed transesterification with

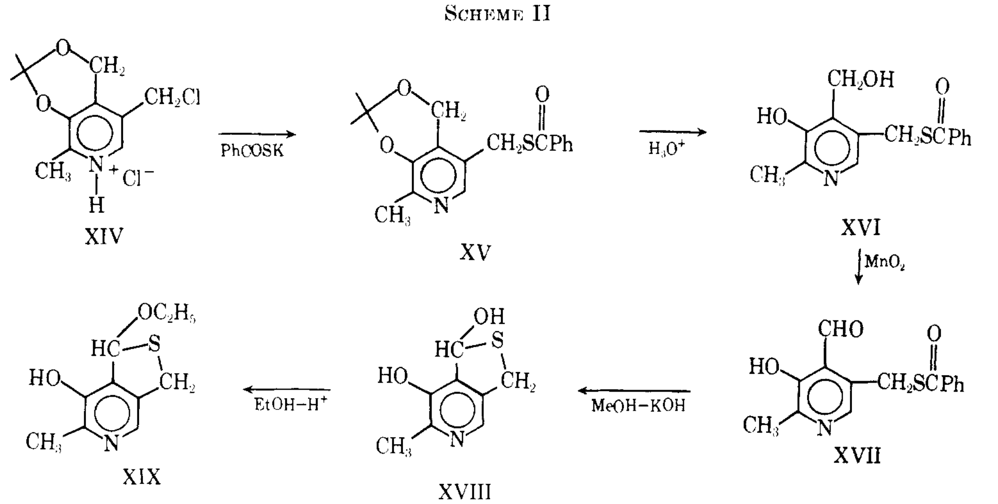
(7) H. Ahrens and W. Korytnyk, *J. Heterocycl. Chem.*, **4**, 625 (1967).

(8) B. Paul and W. Korytnyk, *Tetrahedron*, **25**, 1071 (1969).

SCHEME I



SCHEME II



MeOH proved to be most satisfactory. The thiopyridoxal thus obtained was stable in a rather narrow pH range. Elemental anal. of this compd were not satisfactory, but the structure was indicated beyond any doubt by nmr spectroscopy, and by conversion to the ethyl acetal, which gave satisfactory anal. data.

Ring–Chain Tautomerism.— Ring–chain tautomerism of these compds has been studied by ir and nmr spectroscopy and by the prepn of appropriate derivs. In this regard, we have reexamined the ring–chain tautomerism of pyridoxal in the light of more recent reports.[9-11]

Pyridoxal has been found to exist in the hemiacetal form in both neutral and acid soln, as indicated by the

(9) W. Korytnyk and H. Ahrens, *Methods Enzymol.*, **18A**, 475 (1970).

(10) K. F. Turchin, V. F. Bystrov, M. Ya. Karpeisky, A. S. Olkhovoy, V. L. Florentiev, and Yu. N. Sheinker, "Pyridoxal Catalysis: Enzymes and Model Systems," E. E. Snell, A. E. Braunstein, E. S. Severin, and Yu. M. Torchinsky, Ed., Wiley, New York, N. Y., 1968, p 67.

(11) O. A. Gransow and R. H. Holm, *Tetrahedron*, **24**, 4477 (1968); *J. Amer. Chem. Soc.*, **91**, 5984 (1969).

presence of an AB quadruplet due to α^5-CH_2 protons, one of which was found to be coupled to the α^4 (hemiacetal) proton.[9] In alkaline soln the AB quadruplet collapsed to a singlet and the α^4 (hemiacetal) proton was shifted downfield. This has been explained by a fast equil between the aldehyde and hemiacetal forms by one group of investigators[10] or by an equil between the hemiacetal and the hydrated form by another group.[11] The evidence obtained in the present study does not support the possibility of a hydrate $\rightleftharpoons$ hemiacetal equil, but provides further arguments for the aldehyde $\rightleftharpoons$ hemiacetal equil in addn to those adduced by us earlier.[9] Thus pyridoxal was found to undergo a Cannizzaro reaction when heated with $Ba(OH)_2$, giving a mixt of pyridoxol (50%) and 4-pyridoxic acid, the latter isolated as the lactone (31%); this result is expected from the free aldehyde form. Addnl evidence of the existence of an aldehyde $\rightleftharpoons$ hemiacetal equil was indicated by studies of the ring–chain tautomerism of the 2 homologs of pyridoxal, as about to be described.

5-Homopyridoxal (III) has also been shown to exist in the hemiacetal form in the solid state. (No C=O has been observed in its ir spectrum.) Its nmr spectrum is also consistent with the hemiacetal structure. In contrast to pyridoxal, homopyridoxal did not exhibit a shift of the hemiacetal proton in alkaline soln and hence the equil is entirely on the hemiacetal side.[12] This indication of the greater stability of the 6-membered hemiacetal ring in III as compared with the 5-membered ring in pyridoxal can be expected on the basis of Brown's I-strain theory.[13] Homopyridoxal readily forms an ethyl acetal (IV); but a strong nucleophile, like NH_2OH, opens up the ring to give an oxime (V). Homopyridoxic acid (VIII) readily lactonizes in acid solution to VII, a behavior that is quite analogous to that of 4-pyridoxic acid.[7]

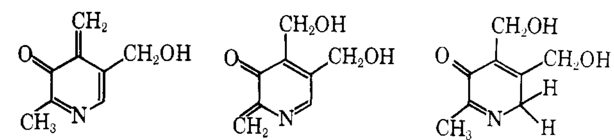
The next higher homolog of pyridoxal (X) forms a hemiacetal containing a 7-membered ring in the solid state. In D_2O, at acid and neutral pH, it also exists in the hemiacetal form; but in alkaline soln, it is converted entirely to the aldehyde form, as is shown by the aldehyde peak at -623 cps in its nmr spectrum. Here the equil between the aldehyde and hemiacetal forms as a function of pH is clearly indicated and cannot be misinterpreted as in the case of pyridoxal. Likewise, the stability of the hemiacetal rings with 5, 6, and 7 members is reflected by the equil in alkaline soln, and is in the expected order of $6 > 5 > 7$.

The lactonization of homopyridoxic acids has also been compared. No tendency to form the 7-membered lactone ring in XIII could be observed, which is in sharp contrast to the 2 lower homologs (4-pyridoxic acid and VIII), which lactonize readily.

5-Thiopyridoxal was found to exist in the hemiacetal form as a solid, and in soln in the narrow pH range where it was stable. As was expected, it forms an ethyl acetal with EtOH.

4-Deoxyhomopyridoxols and Deuterations with Hydrazine-d_4.—Homopyridoxols (II and IX, resp) have also been converted to 4-deoxyhomopyridoxols (VI and XII, resp) by refluxing with anhyd N_2H_4 (Scheme I). This method was introduced by Taborsky[14] for the prepn of 4-deoxypyridoxol, and it seems to be general for this type of system.

In an effort to learn more about the nature of this reaction, we treated pyridoxol with hydrazine-d_4. Unexpectedly, we found that the resulting 4-deoxypyridoxol was almost fully deuterated in the 2- and 4-Me groups and in the 6 position, with only the 5-CH_2 protons left intact. Under the same conditions, 4-deoxypyridoxol was found to be deuterated in precisely the same manner. Thus deuteration is not introduced during hydrogenolysis of the 4-CH_2OH group. No exchange reactions were observed when γ-collidine was treated with hydrazine-d_4. Thus the phenolic OH ortho to the CH_3 and 4-CH_2OH groups activates those groups, presumably *via* quinone methide intermediates (XXa,b). Similarly, a quinonoid type of intermediate (XXc) may be invoked in explaining the proton exchange in the 6 position.[15] The existence of these hypothetical quinone methide forms would also explain the selectivity of the hydrogenolysis of pyridoxol and its 5-homologs by hydrazine: refluxing with hydrazine produces an equilibrium mixture of XXa–XXc, of which only XXa is reduced to 4-deoxypyridoxol



XXa XXb XXc

(the diimide mechanism for reduction with hydrazine may be invoked)[16] whereas the other two intermediates could readily revert to the starting pyridoxol. The process is continued until no starting material is left.

Deuteration of pyridoxol and its 4-deoxy analog provides for the first time experimental evidence of the relative lability of protons in these and related molecules. Earlier we observed facile deuteration of the 2-CH_3 group of pyridoxol *N*-methiodide in 1 *N* NaOD soln, but the 2-CH_3 group in the parent (nonquaternized) compd was found to be inert.[17,18]

Quinone methide intermediates of the type XXb have been postulated in photophosphorylation reactions of pyridoxal Schiff bases.[19] Quinone methides derived from pyridoxol, such as XXa, may explain the reactivity of its 4 position toward alcohols and in self-condensation reactions.[20] The same type of quinone methide (XXa) is most likely formed in the first fragmentation step from pyridoxol and pyridoxamine during mass spectrometry.[21] Indeed, when pyridoxamine was re-

(12) The corresponding 5-amino analog of III has been prepared [T. L. Fisher and D. E. Metzler, *J. Amer. Chem. Soc.*, **91**, 5323 (1969)] and was also found to exist exclusively as the cyclic aldimine.

(13) H. C. Brown, R. S. Fletcher, and R. B. Johannesen, *J. Amer. Chem. Soc.*, **73**, 212 (1951).

(14) R. Taborsky, *J. Org. Chem.*, **26**, 596 (1961).

(15) S. F. Contractor and B. Shane, *Biochemical Pharmacol.*, **19**, 1669 (1970), have studied the "metabolic" stability of ^3H in labeled pyridoxol. From their experiments and ours, it would appear that the α^5 position would be the most stable for ^3H labeling.

(16) (a) E. J. Corey, W. L. Mock, and D. J. Pasto, *Tetrahedron Lett.*, 347 (1961); (b) S. Hunig, H. R. Muller, and W. Thier, *ibid.*, 353 (1961).

(17) W. Korytnyk and R. P. Singh, *J. Amer. Chem. Soc.*, **85**, 2813 (1963).

(18) The reported deuteration of the 2-CH_3 of pyridoxol in D_2O [R. Hüttenrauch and K. Mattey, *Z. Chem.*, **6**, 421 (1966)] is in error.

(19) (a) K. Makino, Y. Murakami, and Y. Kobayashi, International Symposium on Pyridoxal Enzymes, Nagoya, 1967, Maruzen Co., Ltd., Tokyo, 1968, p 129; (b) Y. Kobayashi and K. Makino, *Jikeikai Med. J.*, **15**, 249 (1968).

(20) S. A. Harris, *J. Amer. Chem. Soc.*, **63**, 3363 (1941).

(21) D. C. DeJongh and W. Korytnyk, *Methods Enzymol.*, **18A**, 483 (1970), and ref cited therein.

fluxed with hydrazine, some 4-deoxypyridoxol was formed.

B. Biological Activity.—Selected compds described in this study were evaluated in various biol systems under the supervision of Drs. A. Bloch, M. Hakala, and F. Rosen of our department.

Antagonist Activity against *S. carlsbergensis*.[22]—Although α^5-homopyridoxol (II) was found[4] to inhibit the growth of *S. carlsbergensis* by 50% at 5×10^{-8} *M*, the corresponding value for the 4-aldehyde (hemiacetal III) was 5×10^{-5} *M*; the corresponding ethyl acetal (IV) had a similar activity. Inhibitory activity was lost when this compd was converted to the carboxylic acid (VIII), which does not inhibit at 10^{-3} *M*. The lactone (VII) and oxime of the 3-C homolog XI also did not show any inhibitory activity at 10^{-3} *M*. By conversion of 5-thiobenzoylpyridoxol (XVI), which was found to be inactive at 10^{-3} *M*, to the corresponding 4-aldehyde XVII, the compd became active at 5×10^{-6} *M*. Thus, depending on the substituent in the 5 position, conversion of the 4-CH_2OH group to a related group increases or decreases the inhibitory activity of the resulting compd in relation to the parent compd.

Tissue-Culture Studies.[23]—Compds VIII, XVII, and XVIII were ineffective as inhibitors of the mouse mammary adenocarcinoma cells grown in suspension in Eagle's vitamin B_6 free medium. The inactivity of the thio analog XVIII may be due to *in vivo* desulfuration, which was observed previously with 3-thiopyridoxol by Green and Montgomery.[24]

***In Vivo* Systems.**[25]—No significant depression of liver L-tyrosine aminotransferase activity was seen in rats fed a vitamin B_6 deficient diet containing 20 mg% of the 4-DOP homolog XII for 5 days. Physical signs of vitamin B_6 deficiency were not aggravated, and the weight gain of the animals fed the diet containing the analog was slightly greater than that of those receiving the vitamin B_6 deficient diet only. There was no evidence of leukopenia, although 3 out of 4 animals showed a definite depression in neutrophil count. In the same experiment, homopyridoxol (II) was found to be without significant activity on lymphoid tissues, and had no effect on hepatic L-serine hydroxymethylase and L-serine dehydratase, but did depress L-tyrosine aminotransferase activity by 23%.

Inhibition of Pyridoxal Phosphokinase.—Some compds in this series were shown to be inhibitors of pyridoxal phosphokinase from rat liver.[26] With increasing length of the 5 side chain in 4-deoxyhomopyridoxols their binding to this enzyme progressively decreased as indicated by the following K_I values: 4-deoxypyridoxol, 3.6×10^{-4} *M*; VI, 5.2×10^{-4} *M*, whereas XII did not bind. A conversion of the 4-CH_3 group to a 4-CH_2OH group also decreased the binding somewhat, as is shown by comparison of the K_I value for homopyridoxol (II) (8.5×10^{-4} *M*) with that of the corresponding 4-deoxy compd, VI (5.2×10^{-4} *M*).

Oximes of homopyridoxals were shown to be more potent inhibitors of pyridoxal phosphokinase. The oxime of pyridoxal at 9.6×10^{-6} *M* inhibited the enzyme by 27%, the oxime of homopyridoxal (V) at 8.0×10^{-5} *M* by 31% (and at 8.0×10^{-4} *M* by 72%), and the oxime of the next higher homolog (XI) at 4.0×10^{-5} *M* by only 4% (and at 4×10^{-4} *M* by 65%). Thus a decrease of inhibitory activity with the lengthening of the 5 side chain is also indicated in the oxime series.

Experimental Section

Where analyses are indicated only by symbols of the elements, anal. results obtained for these elements were within +0.4% of the theoretical values.

Tlc was used routinely as described earlier.[27] Ir spectra were determined with a Perkin-Elmer 457 spectrometer, and nmr spectra with a Varian A 60A instrument, as 8-15% solutions in $CDCl_3$ or D_2O; positions of peaks are expressed in cps from TMS, or from sodium 3-(trimethylsilyl)-1-propanesulfonic acid as internal standards. Peaks were assigned on the basis of previous work.[17,28]

α^5-Homopyridoxal (III, 7-Methyl-3,4-dihydro-1*H*-pyrano-[4,3-*c*]pyridine-1,8-diol).—α^5-Pyridoxylmethanol·HCl (II, 60 mg)[4] was dissolved in H_2O (20 ml), to which concd H_2SO_4 (0.02 ml) was added. After addn of MnO_2 (500 mg),[29] the reaction mixt was shaken vigorously for 70 sec, then the MnO_2 was filtered off and washed with H_2O (25 ml). (The time for the reaction and washing should not exceed 6 min.) The filtrate was extd with $CHCl_3$ (3 × 50 ml), which removed α^5-homo-4-pyridoxic acid lactone (VII), 9.8 mg (20%), mp 112-114°.

The aq layer was evapd *in vacuo* at 30°, dissolved in H_2O (1 ml), and carefully neutralized by the addn of solid $NaHCO_3$, when crystn occurred. The yield was 36.5 mg (74%) of material melting around 135°, which was recrystd from H_2O-Me_2CO: tlc, 1:1 MeOH-$CHCl_3$, R_f 0.77, retarded by boric acid[27] to R_f 0.46; nmr (in 1 *N* NaOD), (2-CH_3) −137.5, (α^5-CH_2) −163.5 (tr J = 6 cps), (β^5-CH_2) −192 (tr J = 6 cps), (α^4-H) −381 (broad), (C_6-H) −422; at a neutral pH peaks appear broadened, and α^4-H is at −364 cps; uv, $\lambda_{max}^{0.1\ N\ NaOH}$ 218.5 (ϵ 6700); 247 (ϵ 5800), 310 mμ (ϵ 5750); $\lambda_{max}^{0.1\ N\ HCl}$ 230 (ϵ 2500) shoulder, 292 mμ (ϵ 6750); $\lambda_{max}^{pH\ 7}$ 255 (ϵ 4400), 312 (ϵ 7450); ir no C=O group (KBr pellet). *Anal.* ($C_9H_{11}NO_3$) C, H, N.

Ethyl Acetal of α^5-Homopyridoxal (IV, 1-Ethoxy-7-methyl-3,4-dihydro-1*H*-pyrano[4,3-*c*]pyridin-8-ol).—α^5-Pyridoxylmethanol·HCl[4] (II, 750 mg) in aq H_2SO_4 (30 ml of H_2O and 0.27 ml of concd H_2SO_4) was shaken with MnO_2[16] (5.0 g) for 90 sec at room temp. The MnO_2 was filtered and washed with 40 ml of H_2O in small portions. The combined aq solns were extd with $CHCl_3$ (4 × 20 ml). The $CHCl_3$ exts yielded 133 mg of α^5-homo-4-pyridoxic acid lactone, mp 114-116°.

The aq layer was evapd to dryness, dried at 0.1 mm, and shaken with EtOH (abs, 50 ml) for 24 hr. After filtration, the soln was evapd, and the oily material was purified on a silica gel column. The pure ethyl acetal was eluted with 1:1 $CHCl_3$-EtOH, giving 176 mg (25%) of the product, mp 135°. Further purification was accomplished by sublimation (0.15 Torr, 80-90°): mp 139-140°; nmr (in $CDCl_3$), (2-CH_3) −147, (α^4-H) −337, (α^5-CH_2) −166 (tr, J = 7.5 cps), (β^5-CH_2) −233 (multiplet), (C_6-H) −472, (OEt) −80 (tr, J = 7.0 cps), −233 (multiplet); uv: $\lambda_{max}^{0.1\ N\ NaOH}$ 218.5 (ϵ 6600), 248 (ϵ 6400), 311 (ϵ 6650); $\lambda_{max}^{0.1\ N\ HCl}$ 293 (ϵ 7500), 230 (ϵ 3000 shoulder); $\lambda_{max}^{pH\ 7.0}$ 254.5 (ϵ 5200), 323 (ϵ 8650). *Anal.* ($C_{11}H_{15}NO_3$) C, H, N.

The ethyl acetal (IV, 20.1 mg) was hydrolyzed by dissolving in H_2O (3 ml) and heating to 55° for 96 hr. An almost quant yield (17.3 mg) of pure hemiacetal (III) was obtained.

Oxime of 3-Hydroxy-5-(2-hydroxyethyl)-2-methylpyridine-4-carboxaldehyde (V).—α^5-Pyridoxylmethanol·HCl (II, 210 mg) in dil acid (30 ml of H_2O plus 0.05 ml of concd H_2SO_4) was shaken with 2.0 g of MnO_2[29] for 90 sec at room temp. After filtration and washing of the ppt with H_2O (30 ml, in small portions), the filtrate was extd with $CHCl_3$ (3 × 25 ml) to remove

(22) Dr. A. Bloch, personal communication; testing procedure described in ref 4.

(23) Dr. M. Hakala, personal communication.

(24) J. L. Green and J. A. Montgomery, *J. Med. Chem.*, **7**, 17 (1964).

(25) Dr. F. Rosen, personal communication.

(26) Pyridoxal phosphokinase was obtd from rat liver as described by D. B. McCormick, M. E. Gregory, and E. E. Snell, *J. Biol. Chem.*, **236**, 2076 (1961).

(27) H. Ahrens and W. Korytnyk, *Anal. Biochem.*, **30**, 413 (1969).

(28) W. Korytnyk and B. Paul, *J. Heterocycl. Chem.*, **2**, 481 (1965).

(29) O. Mancera, G. Rosenkranz, and F. Sondheimer, *J. Chem. Soc.*, 2189 (1953).

VII. The H_2O phase was evapd to 3.5 ml, and NaOAc (1.1 g) and $NH_2OH \cdot HCl$ (150 mg) were added. After heating on a steam bath for 10 min, V crystd; it was kept at room temp overnight before filtration. The yield was 125 mg (67%), mp 197–198° dec. Recrystn from EtOH and from pyridine–H_2O raised the mp to 200–201° dec. *Anal.* ($C_9H_{12}N_2O_2$) C, H, N.

α^4-Deoxy-α^5-pyridoxylmethanol·HCl (VI).—α^5-Pyridoxylmethanol·HCl (II, 257 mg) was refluxed with anhyd N_2H_4 (2.0 ml, purified according to the method of Taborsky[14]) for 18 hr, moisture being excluded. Excess N_2H_4 was distd off at 90° and 0.1 Torr. The cryst residue was taken up in EtOH (5 ml), and $N_2H_4 \cdot HCl$ was filtered off. Addn of methanolic HCl and some Et_2O pptd additional amts of $N_2H_4 \cdot HCl$. The filtrate was evapd to approx 1.5 ml, and was treated with drops of Et_2O until turbid. On standing in a refrigerator for 12 hr, 122.5 mg of IV, mp 154–156°, was obtd. A further 14.1 mg was obtd from the mother liquors on addn of Et_2O, raising the yield to 137 mg (58%). Recrystn from EtOH and from MeCN raised the mp to 156–157°: nmr, (2-CH_3 and 4-CH_3) −149.5 and −160, (α^5-CH_2) −184 (tr J = 7.0 cps), (β^5-CH_2) −234.5 (tr J = 7.0 cps), (C_6-H) − 482; uv, $\lambda_{max}^{0.1\ N\ HCl}$ 228 mμ (ϵ 2100), 286 (ϵ 7800); $\lambda_{max}^{1\ N\ NaOH}$ 245 (ϵ 6100), 302 (ϵ 6700); λ_{max}^{EtOH} 285 (ϵ 6950), 225 (shoulder). *Anal.* ($C_9H_{14}ClNO_2$) C, H, N.

3-Hydroxy-5-(2-hydroxyethyl)-2-methylpyridine-4-carboxylic Acid (4 → 5) Lactone (VII).—A soln of II (200 mg) in H_2O (5 ml) was added to a mixt of MnO_2 (1.65 g, prepd according to the method of Mancera, *et al.*[29]), H_2O (15 ml), and concd H_2SO_4 (0.1 ml) and was stirred for 1 hr at room temp. MnO_2 was filtered off and washed with H_2O (30 ml). The filtrate was shaken with $CHCl_3$ (3 × 30 ml). The $CHCl_3$ ext was washed with H_2O (25 ml), dried ($CaSO_4$), and evapd, giving 125 mg (76%) of lactone VII, mp 119°. Recrystn from Et_2O–petr ether gave the anal. sample: mp 120°; nmr ($CDCl_3$); (2-CH_3) −151, (α^5-CH_2) −183 (tr J = 6.0 cps, broad), (β^5-CH_2) −279 (tr J = 6.0 cps, sharp), (C_6-H) −479 (broad), (3-OH) −634 (disappears on addn of D_2O); uv, $\lambda_{max}^{0.1\ N\ HCl}$ 247 (ϵ 2200, sh) 329 (ϵ 6550); ir, λ_{max}^{Nujol} 1700 cm^{-1} (C=O). *Anal.* ($C_9H_9NO_3$) C, H, N.

3-Hydroxy-5-(2-hydroxyethyl)-2-methylpyridine-4-carboxylic Acid (VIII).—3-Hydroxy-5-(2-hydroxyethyl)-2-methylpyridine-4-carboxylic acid (4 → 5) lactone (VII, 25.0 mg) was dissd by gentle heating in 1 N NaOH (3 ml). After adding 0.1 N HCl till pH 8, the soln was evapd to 1.0 ml. More 0.1 N HCl was added, until, at pH 6, the acid crystd. After cooling in ice, the acid was filtered, yielding 16.5 mg (60%), mp 226°. Recrystn from EtOH did not raise the mp: nmr (in 1 N NaOD); (2-CH_3) −136, (α^5-CH_2) −160 (tr J = 7.0 cps), (β^5-CH_2) (tr J = 7.0 cps), (C_6-H) −440; $\lambda_{max}^{0.1\ N\ NaOH}$ 220 mμ (ϵ 10,500), 347 (ϵ 5500), 308 (ϵ 6400). *Anal.* ($C_9H_{11}NO_4$) C, H, N.

3-Hydroxy-5-(3-hydroxypropyl)-2-methylpyridine-4-carboxaldehyde (X).—(4-Hydroxymethyl)-5-(3-hydroxypropyl)-2-methyl-3-pyridinol·HCl (IX, 200 mg)[30] was dissolved in H_2O (25 ml). On addn of 1 N NaOH (4 ml) and MnO_2[29] (4.0 g), the mixt was stirred for 8 hr at room temp. The MnO_2 was filtered off and washed with H_2O (30 ml). The filtrates were evapd, giving a yellow oil. A very small amt of H_2O was added. The mixt was neutralized with 6 N HCl to pH 5.9, when crystals formed, which were filtered off and washed with H_2O. The yield was 89.1 mg, mp 124–126°. From the mother liquors, another 39.6 mg, mp 125–126°, was isolated. The total yield was 128.7 mg (76%). The first fraction of this material was converted to the hydrochloride by dissolving in dry Me_2CO (10 ml) and adding Et_2O (0.5 ml) satd with HCl gas. More Et_2O was added till cloudiness, and the product was allowed to cryst. The hydrochloride was then recrystd from Me_2CO: mp 132–135°; nmr (D_2O), pH 1–3.73 (2-CH_3) −160, (α^4-H) −390 (C_6-H) −485; pH 8.45–11.0, (2-CH_3) −139, (C_6-H) −432 (very broad), (CHO) −623; the compd was not adequately sol in D_2O around pH 7 for an nmr study; uv $\lambda_{max}^{0.1\ N\ HCl}$ 295 mμ (ϵ 8300); $\lambda_{max}^{0.1\ N\ NaOH}$ 307 (ϵ 1850), 394 (ϵ 6500), shoulder at 265 mμ; $\lambda_{max}^{pH\ 7}$ 323 (ϵ 5200), 384 (ϵ 2750), shoulders at 249 and 266 mμ; ir, no C=O (KBr pellet). *Anal.* ($C_{10}H_{14}ClNO_3$) C, H, N, Cl.

Attempts to convert the aldehyde X to an ethyl acetal (ethanol and HCl) gave mixts of products, which were not further investigated.

Oxime of 3-Hydroxy-5-(3-hydroxypropyl)-2-methylpyridine-4-carboxaldehyde (XI).—To a soln of X·HCl (56.5 mg) in H_2O (5 ml), 1 N NaOH (1 ml) and MnO_2 (0.5 g, prepd according to the method of Mancera, *et al.*[29]) were added, and the mixt was stirred for 10 hr. Tlc (1:1 $CHCl_3$–MeOH) indicated the presence of an aldehyde (R_f 0.77) and a trace of fluorescent material (R_f 0.59). Excess MnO_2 was filtered off. NaOAc (0.2 g) and $NH_2OH \cdot HCl$ (50 mg) were added, and the soln was heated on a steam bath for 10 min, when the oxime pptd. After standing for 6 hr, the oxime was filtered. After drying, the yield was 32.9 mg (65%), mp 200–203°. Recrystn from pyridine raised the mp to 206–208°. *Anal.* ($C_{10}H_{14}N_2O_3$) C, H, N.

5-(3-Hydroxypropyl)-2,4-dimethyl-3-pyridinol (XII).—4-(Hydroxymethyl)-5-(3-hydroxypropyl)-2-methyl-3-pyridinol·HCl (IX, 250 mg) and N_2H_4 (95%+, 3 ml) were refluxed for 18 hr. Excess N_2H_4 was removed by distn under reduced pressure. The residue was extd with hot MeOH. The methanolic ext was concd to a small vol, when $N_2H_4 \cdot HCl$ separated out; the latter was removed by filtration. The filtrate (MeOH ext) was evapd completely *in vacuo*, and the residue was extd several times with hot EtOAc. Combined EtOAc exts were evapd *in vacuo* to a small vol, when white cryst material sepd out. The material was cooled, filtered, washed with anhyd Et_2O, and dried; yield, 155 mg (80%). The compd was crystd from EtOAc after treatment with charcoal, mp 135–136°. *Anal.* ($C_{10}H_{15}NO_2$) C, H, N.

3-Hydroxy-5-(3-hydroxypropyl)-2-methylpyridine-4-carboxylic Acid (XIII).—To 4-(hydroxymethyl)-5-(3-hydroxypropyl)-2-methyl-3-pyridinol (IX, 1.0 g) in 0.03 N HCl (70 ml), MnO_2[31] (20 g) was added, and the reaction mixt was heated to 70°, with vigorous stirring, for 1.5 hr. The mixt was filtered (Celite Filter Aid), and the solid material was washed with H_2O and EtOH. After evapn to a small vol, the soln was applied to a Bio-Rad AG 1 × 8 anion-exchange column in the formate form (1.6 × 45 cm), and was eluted with 0.25 N HCO_2H. Fractions contg fluorescent material were combined, evapd to dryness, and crystd from EtOH. The yield was 146 mg plus 44 mg from the mother liquors (21% total); the compd decompd above 240°. *Anal.* ($C_{10}H_{13}NO_4$) C, H, N.

α^5-*S*-Benzoylpyridoxthiol (XV).—Potassium thiobenzoate[32] (5.0 g) in H_2O (30 ml) was added to XIV[4] (7.92 g) in EtOH (180 ml) under N_2 for 30 min and was stirred for 1.5 hr. H_2O (150 ml) was added, and the soln was kept at 100° for 2 hr to hydrolyze the isopropylidene group. The reaction mixt was concd to 100 ml *in vacuo*, and was neutralized ($NaHCO_3$). The resulting ppt was collected and was washed with H_2O: yield 8.09 g (100%); mp 160–162°. The compd was identical with α^5-*S*-benzoylpyridoxthiol prepared by a multistep procedure.[8]

α^5-Thiobenzoylpyridoxal (XVII).—Compd XV (1.0 g) was dissolved in dry $CHCl_3$ (100 ml) and stirred for 2 hr with MnO_2[29] (5.0 g), moisture being excluded. The MnO_2 was filtered off and washed with $CHCl_3$ (100 ml), and the combined filtrates were evapd to about 10 ml, yielding 0.685 g of cryst material, plus 0.135 g from the mother liquors (81%). The anal. sample was obtained by filtration over a silica gel column (Woelm, activity grade I; EtOAc was used for elution): mp 116° dec; ir λ_{max}^{KBr} 1660 cm^{-1} (C=O); nmr ($CDCl_3$), (2-CH_3) −151, (5-CH_2) −275.5, (Ph) −463 (multiplet), (C_6-H) −492.5, (α^4-H) (aldehyde) −629, (phenolic OH) −693 cps; uv λ_{max}^{EtOH} 271 (ϵ 9850), 354 mμ (ϵ 2460). *Anal.* ($C_{15}H_{13}NO_3S$) C, H, N.

α^5-Thiopyridoxal (XVIII).—α^5-Thiobenzoylpyridoxal (XVII, 200 mg) was added to 10 ml of dry MeOH containing 1% KOH, and the mixt was stirred for 2 hr. After thorough removal of MeOH *in vacuo*, the yellow residue was shaken with dry $CHCl_3$ (20 ml) to remove $PhCO_2Me$ formed in the transesterification reaction. The yellow residue was dissolved in 2.3 ml of H_2O, and the pH was carefully adjusted to 9.0 with 6 N HCl (a pH meter was used), when crystn of the product began. At this point, more HCl was added, without any change in the pH, and the addn was interrupted once the pH started falling. The soln was chilled briefly in a freezer (*ca.* 10 min.), filtered, washed with ice-H_2O, and dried (P_2O_5, oil pump). The yield was 80 mg (63%). The compd decompd gradually between 150 and 170° (R_f 0.25 in EtOAc). The product was recrystd once from Me_2CO: ir spectrum, no C=O; nmr (DMSO-d_6) (2-CH_3) −143 (5-CH_2) −244, −264 (AB quadruplet, J = 15 cps), (α^4-H, hemiacetal) −401.5, (C_6-H) −480 cps; uv, $\lambda_{max}^{pH\ 7.0}$ 256 (ϵ 5850), 324 (ϵ 8950), $\lambda_{max}^{0.1\ N\ NaOH}$ 247.5 (ϵ 7400), 308 mμ (ϵ 7350). *Anal.* ($C_8H_9NO_2S$) C, H, S: calcd, 17.50; found, 15.29.

Various attempts to improve the anal. by using different sol-

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vents were without success. The compd is quite unstable, and readily forms a black polymer. It was further characterized as the ethyl acetal (XIX).

α^5-Thiopyridoxal Ethyl Acetal (XIX).—α^5-Thiopyridoxal (XVIII, 50 mg) was suspended in EtOH (5 ml, dry), and 5 drops of Et_2O satd with HCl gas were added. The mixt was allowed to stand at room temp. The reaction was followed by tlc (EtOAc, R_f 0.25 starting material, 0.45 ethyl acetal). After standing for 7 days, only traces of starting material were left. The mixt was evapd to 1 ml *in vacuo*, and the product was sepd by preparative tlc. The material was eluted with EtOAc and evapd to a small vol, and petr ether was added. Crystn yielded 6.8 mg of the ethyl acetal, hygroscopic crystals: mp 120–122° dec, nmr ($CDCl_3$) (CH_3CH_2) −77 (tr), (2-CH_3) −151, (CH_3CH_2) −218 (m), (5-CH_2) −257 (broad), (α^4-H, hemiacetal) −405 (split singlet), (C_6-H) −487. *Anal.* ($C_{10}H_{13}NSO_2$) C, H, S.

Cannizzaro Reaction of Pyridoxal.—Pyridoxal·HCl (50 mmoles, 102 mg), dissolved in 10 ml of satd $Ba(OH)_2$ soln, was refluxed for 24 hr. Tlc of the reaction mixt indicated only spots due to pyridoxol and pyridoxic acid. (The identities of the products were confirmed by retardation by boric acid and a positive Gibbs test, as described earlier.[27]) The mixt was then evapd to dryness, and was thoroughly dried *in vacuo*. The dry white powder was acetylated for gas chromatog (2.5 ml of pyridine and 2.5 ml of Ac_2O for 4 hr). Samples of this mixt were injected into a gas chromatograph operating under standard conditions.[33] Two peaks were observed, with retention times of 9.1 min (pyridoxol acetate) and 2.25 min (4-pyridoxic acid lactone acetate). Comparison of the areas under the curves with each other and with standards run separately showed that the total amts of the acetates were 24.9 mmoles for pyridoxol and 15.4 mmoles for 4-pyridoxic acid. The apparent loss of 4-pyridoxic acid during the reaction may be due to decarboxylation and degradation.

A similar mixt was obtained when pyridoxal·HCl (51 mg, 25 mmoles) was dissolved in strong NaOH soln (1 g of NaOH in 2.5 ml of water) and was heated to 110° for 24 hr.

Hydrazine-d_4 Experiments. (a) **4-Deoxypyridoxol-α^2-d_3,α^4-d_3,α^6-d from Pyridoxol.**—Pyridoxol·HCl (251 mg) and hydrazine-d_4 (2 ml, anhyd, supplied by Volk Radiochemical Co.) were refluxed for 16 hr, moisture being excluded. After evapn of excess hydrazine [80° (0.1 mm)], the residue was extd with boiling EtOH (5 ml) for 10 min and cooled, and the hydrazine·2HCl that crystd was removed by filtration. To the filtrate, 1.5 ml of methanolic HCl (11.2% HCl) was added. On chilling, cryst 4-deoxypyridoxol·HCl pptd. The yield was 178 mg (73%), mp 254° dec. On addn of Et_2O to the mother liquors, a further 40 mg of 4-deoxypyridoxol could be obtd; but it was contaminated. Recrystn of the main crop from boiling EtOH gave the pure product, mp 271° (lit.[14] mp 273°), migrating as one spot on tlc (50:50 $CHCl_3$–MeOH; R_f 0.75, not retarded by boric acid). The nmr spectrum in 1 *N* D_2SO_4 shows only an α^4-H_2 peak at −321 cps; α^2-H_3, α^4-H_3, and C_6-H appear as small bumps, indicating virtually complete deuteration.

(b) **4-Deoxypyridoxol-α^2-d_3,α^4-d_3,α^6-d from 4-Deoxypyridoxol.**—4-Deoxypyridoxol·HCl (251 mg) and hydrazine-d_4 (2.5 ml) were refluxed for 110 hr. The reaction mixt was worked up as in the preceding expt, yielding 139 mg (60%) of deuterated 4-deoxypyridoxol·HCl. By using an internal standard and integration, it could be established that α^5 protons were not exchanged, but that α^2-H_3, α^4-H_3, and α^6-H protons were exchanged to the extent of 94–95%.

(c).—Collidine (0.40 ml, pure by nmr spectroscopy) was heated with hydrazine-d_4 (2.0 ml) at 120° for 120 hr. On cooling, the reaction mixt sepd into 2 layers, the upper one containing mostly γ-collidine. The nmr spectrum of this layer was exactly the same as that of the starting γ-collidine, indicating no D exchange.

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Antiestrogenic and Antifertility Compounds. 4. 1,1,2-Triarylalkan-1-ols and 1,1,2-Triarylalk-1-enes Containing Basic Ether Groups[1]

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In an attempt to relate structure to antiestrogenic and antifertility activity, several 1,1,2-triarylalkan-1-ols and 1,1,2-trialkylalk-1-enes containing a basic ether group have been synthesized, and their biological activities examined. Assignments of geometric isomerism in the triarylalkenes are made on the basis of nmr data.

The discoveries that the compds **3a**,[2,3] **1**,[4,5] and **2**[6] are orally active antifertility agents, and that by sc administration they inhibit simultaneously applied estradiol,[2,7–9] prompted us to undertake the synthesis of **3b** and **3c**, which are previously unknown positional isomers of **3a**, and of compds **4**, which are open chain analogs of **1** and **2**. After we began this work, patents[10,11] appeared describing some compds of the general type **4**, but these included only 2 of those described in this paper.

Chemistry.—Most of the compds were prepared by standard procedures described in the Experimental Section. Attempts to prepare 1-{*p*-[2-(*N*,*N*-diethyl-

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