um compound was then decomposed with H_2O (100 ml). The Et_2O layer was separated, washed with H_2O, and concentrated. The solid was collected by filtration to give 3.3 g (40% yield) of the desired product, mp 217–220°. Recrystallization from acetone–cyclohexane yielded analytically pure **5f** as white crystals, mp 218–220°. *Anal.* ($C_{22}H_{13}F_6NO$) C, H, N.

3,6-Bis(trifluoromethyl)-9-phenanthryl 4-Pyridyl Ketone (5g). A solution of 2.1 g (0.005 mol) of **5f** in 100 ml of AcOH was treated with a saturated solution of CrO_3 (2.0 g, 0.02 mol) in H_2O. The reaction mixture was allowed to stand overnight at room temperature, and excess oxidizing agent was then destroyed by the addition of MeOH. The resulting mixture was made basic with aqueous NaOH. The product was isolated by Et_2O extraction (frequent filtration was required to break up the emulsion). On evaporation of the Et_2O solution there was obtained 1.26 g (60% yield) of crude **5g**, mp 157–159°. An analytical sample was obtained as white needles upon recrystallization from MeOH, mp 162–164°. *Anal.* ($C_{22}H_{11}F_6NO$) C, H, N.

3,6-Bis(trifluoromethyl)-α-(4-piperidyl)-9-phenanthrene-methanol Hydrochloride (4). A solution of 1.8 g of **5f** in 250 ml of EtOH and 1 ml of HCl was hydrogenated in the presence of Adams catalyst at 5 kg/cm² for 4 hr. The reaction mixture was then heated to dissolve the precipitated solid product and filtered to remove the catalyst. The filtrate was evaporated to dryness *in vacuo* and the residue recrystallized from MeOH to give **4** as white crystals, mp 323–325° dec. The combined yield of pure **4** from two similar runs was 2.35 g (60%). This compound could also be prepared by hydrogenation of 3,6-bis(trifluoromethyl)-9-phenanthryl 4-pyridyl ketone (**5g**) under the same reaction conditions. *Anal.* ($C_{22}H_{19}F_6NO\cdot HCl$) C, H, N.

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A General Method for Modifying the 2-Methyl Group of Pyridoxol. Synthesis and Biological Activity of 2-Vinyl- and 2-Ethynylpyridoxols and Related Compounds†

W. Korytnyk,* S. C. Srivastava, N. Angelino, P. G. G. Potti, and B. Paul

Department of Experimental Therapeutics, Roswell Park Memorial Institute (New York State Department of Health), Buffalo, New York 14203. *Received April 9, 1973*

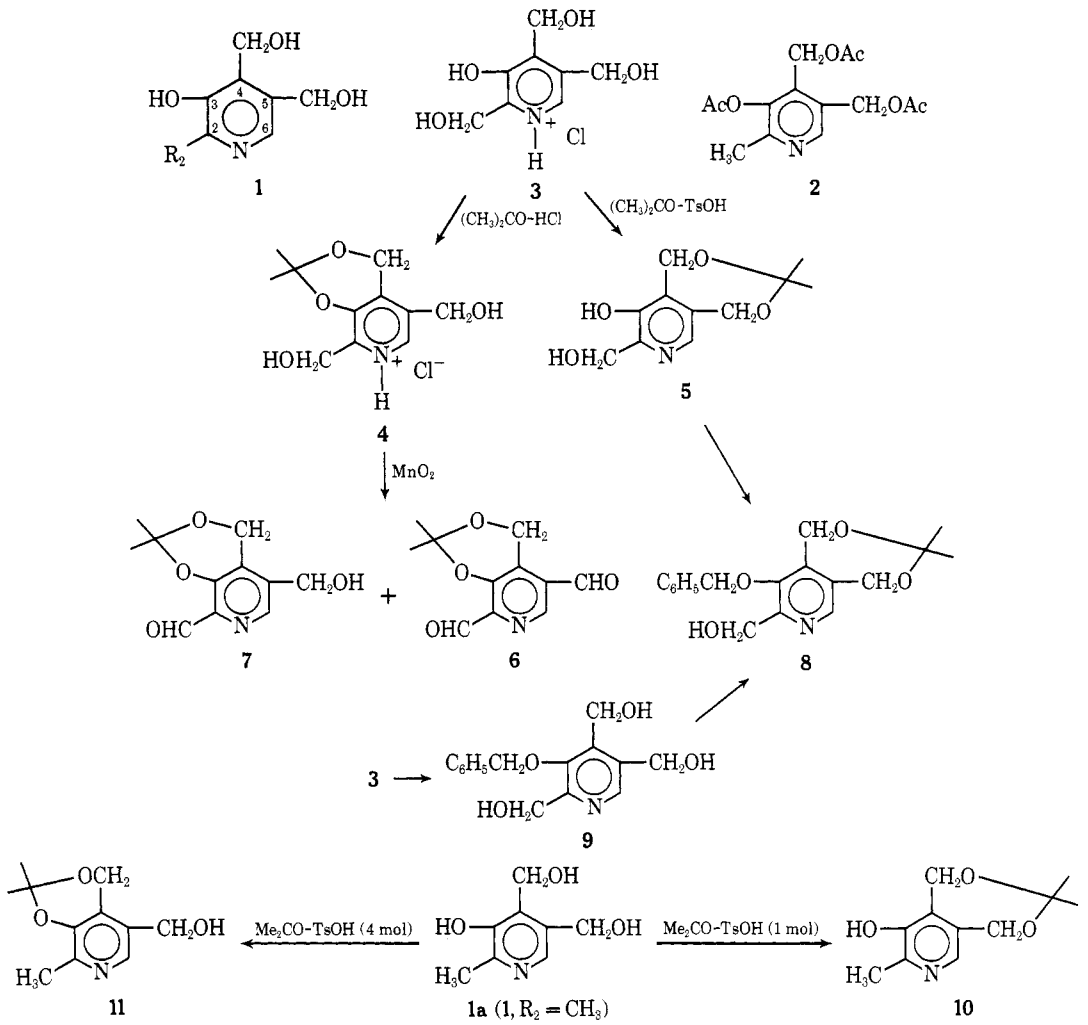
In order to explore the bulk tolerance indicated in the 2 position of vitamin B_6 for enzymes both dependent on the vitamin and metabolizing it, we have developed a general method for modifying the 2 position. To this end, formation of cyclic ketals from acetone and pyridoxol or α^2-hydroxypyridoxol has been studied, using various amounts of catalyst, principally *p*-toluenesulfonic acid. With a limited amount of catalyst, ketal formation occurs across the α^4- and α^5-OH groups, without involving the phenolic OH. When the ratio of acid catalyst to substrate is increased (*e.g.*, to 4:1), acetone condenses with the 3- and α^4-OH groups in these compounds but not with the 3- and α^2-OH groups in α^2-hydroxypyridoxol. By blocking the 3-OH group in α^2-hydroxypyridoxol with benzyl, and the α^4- and α^5-OH groups with acetone, a generally useful intermediate for modifying the 2 position was obtained. An alternative, more efficient method for synthesizing the compound was developed, starting from 3-*O*-benzylpyridoxol, converting it to the α^4,α^5 cyclic ketal, N-oxidizing that, rearranging the *N*-oxide with trifluoroacetic anhydride, and hydrolyzing the 2-(trifluoroacetyl) group. The 2-CH_2OH group of this intermediate was oxidized to 2-CHO, which on mild hydrolysis gave 2-formyl-2-norpyridoxol. The 2-CHO groups of the blocked intermediate were converted by Wittig reactions to 2-CH=CH_2 and 2-CH=CHCl groups; dehydrochlorination of the latter gave the 2-C≡CH derivative. The blocking groups in all of these compounds were readily hydrolyzed with acid, and the resulting analogs were tested as inhibitors of the growth of mouse mammary adenocarcinoma (TA-3) cells *in vitro*. 2-Vinyl-2-norpyridoxol, with an ID_{50} of 9×10^{-6} *M*, was the best inhibitor in this series. In contrast to the inhibition caused by the 4-vinyl analog of pyridoxal, readily reversed by pyridoxal, that caused by the 2-vinyl analog of pyridoxol could not be reversed by the vitamin.

Analogs of vitamin B_6[2] modified in the 2 position, such as α^2-methylpyridoxol (**1**, R_2 = Et), have been of considerable biochemical interest in the development of ideas in regard to pyridoxal catalysis[3] and the mode of binding of the cofactor analogs,[4] and in studies of the active sites of enzymes metabolizing vitamin B_6.[5] Generally, the analogs that were studied had the 2-methyl group replaced either with 2-CH_2OH (**1**, R_2 = CH_2OH) or with various other alkyl groups. The results of these studies indicated that there is a certain bulk tolerance in the 2 position with respect to several enzymes requiring pyridoxal phosphate and also with respect to enzymes involved in metabolic interconversions of different forms of vitamin B_6. In order to exploit this bulk tolerance in the design of potential antagonists of vitamin B_6, we had to develop a method for modifying the 2-methyl group of pyridoxol in such a way that the method would also be suitable for the introduction of reactive groups. The present paper describes the synthesis of a blocked intermediate that satisfies these requirements. The utility of this intermediate parallels that of α^4,3-*O*-isopropylidenepyridoxol for modifying the 5-CH_2OH group[6] and that of 3,α^5-*O*-dibenzylpyridoxol for modifying the 4-CH_2OH group.[1a,7]

Initially, we investigated the acetonation of α^2-hydroxypyridoxol (Scheme I). The latter compound was prepared from tri-*O*-acetylpyridoxol by a simplified version of the

†Chemistry and Biology of Vitamin B_6. 33. Previous paper in this series, ref 1a. A brief report of part of the present study has appeared.[1b] Part of the present study was submitted by P. G. G. Potti in partial fulfillment of requirements for the Ph.D. degree.

Scheme I



procedure described by Bedford, *et al.*,[8] in which the intermediates were not isolated. Using HCl as the catalyst, we did not obtain the expected (from our previous experience[9]) cyclic ketal **5**, but its isomer **4**, irrespective of the reaction temperature or the concentration of HCl. The structure of **4** was indicated partly by its spectral properties and partly by oxidation experiments. The uv spectrum of **4** at pH 7.0 has a maximum at 283 nm, which is not shifted in basic (pH 9) solution; a red shift would be expected if the phenolic OH were unsubstituted.[7] The nmr spectrum of **4** in DMSO-d_6 shows a splitting of the methylene protons by the hydroxyl protons. It was demonstrated earlier that whenever the phenolic OH is free, such splitting is not observable, and all of the exchangeable protons appear as one single peak ("mobile" protons).[10]

These data, however, were not sufficient to distinguish **4** from its isomer α^2,3-*O*-isopropylidene-α^2-hydroxypyridoxol. Hence, we decided to conduct oxidations of the remaining two hydroxymethyl groups. Previous experience indicated that if these groups occupy positions ortho to each other, as in pyridoxol, the resulting product of oxidation will be a hemiacetal or, if further oxidized, an acid or a lactone.[11] Oxidation of **4** with MnO_2 gave rise to two compounds. The nmr spectrum of one compound showed clearly two aldehyde peaks at δ 10.38 and 10.58, and hence that compound was assigned the structure **6**. The other compound had a single aldehyde peak at δ 10.50 and hence was assigned the structure **7**. The position of the aldehyde group in **7** has been established unequivocally by removing the isopropylidene and demonstrating the identity of the resulting compound with the product of the hydrolysis of **15**.

The preference for the formation of **4** *vs.* the other isomer with a six-membered ring, the one that would be formed by joining the 2 and 3 positions with isopropylidene, may be related to the bulk of the anion (Cl^- or *p*-toluenesulfonate), which would hinder the approach of acetone to form a hemiacetal with the α^2-OH group, the reaction considered to be the first step in the formation of cyclic ketals.[12,‡] The cyclic ketal **5**, with a seven-membered ring, was obtained by acetonation of **3** with *p*-toluenesulfonic acid in an equimolar ratio to substrate. The presence of the free phenolic OH group in this compound is indicated by its uv spectrum (maxima at 253 and 315 nm at pH 6.8, which shifted to 244 and 305 nm in 0.1 *N* NaOH). The desired intermediate **8** was obtained either by direct benzylation of **5** with dimethylphenylbenzylammonium chloride,[7] or by first benzylating α^2-hydroxypyridoxol to give **9**, and then subjecting the latter to acetonation.

Our experience with the acetonation of **3** with *p*-toluenesulfonic acid and dimethoxypropane led us to reinvestigate the acetonation of pyridoxol with these reagents. When HCl was used as the catalyst as originally described,[9] the yield of **10** was variable, since it was not al-

‡The following experiments support this idea. 3-Hydroxy-2-hydroxymethylpyridine does not form an acetonide under any of various conditions (HCl and *p*-toluenesulfonic acid were used as catalysts); similarly, a second isopropylidene group could not be introduced into **5**. An alternative mechanism preventing the formation of the acetonide across α^2-OH and 3-OH groups would be intramolecular hydrogen bond formation between the α^2-OH and N^+-H of the pyridine ring.

ways possible to reproduce the original reaction conditions for the synthesis of this key intermediate. When *p*-toluenesulfonic acid was used as the catalyst, however, either **10** or **11** or both would be formed, depending on the molar ratio of catalyst to pyridoxol hydrochloride. If that ratio was equimolar, **10** was obtained exclusively, in excellent yield. If the proportion of the catalyst was increased, mixtures of **10** and **11** were obtained, the yield of **11** increasing with the catalyst concentration. Finally, at a 4:1 ratio of catalyst to substrate, **11** was the only product. For practical purposes, the *p*-toluenesulfonic acid method is superior for the synthesis of **10**, but the HCl method[13] is still the method of choice for the synthesis of **11**, since it gives a better yield and a cleaner product.

The possibility was considered that **10** was the initial product even when a large proportion of the catalyst was used and that the formation of **10** is followed by isomerization to **11**. Apparent isomerization of **10** to **11** has been reported to occur in acetone with HCl.[9] To test this hypothesis, we prepared α^4,α^5-*O*-isopropylidene-d_6-pyridoxol by reacting **1a** (**1**, R_2 = CH_3) with acetone-d_6. We subjected this labeled material to the "isomerization procedure," using *p*-toluenesulfonic acid in acetone, and we isolated **11** lacking the label. This finding indicates that the "isomerization procedure" first causes the isopropylidene group in **10** to be completely hydrolyzed off, and then the cyclic ketal **11**, with a six-membered ring, is formed directly. It also makes it likely that ketal formation is under kinetic control; *i.e.*, the outcome of the reaction is not dependent on the relative stabilities of the cyclic ketals formed but on the relative rates of their formation.§ Thus, acid media increase the rate of formation of ketals involving the phenolic OH group relative to those involving only the alcoholic OH groups.

After the preceding investigation was completed, we eventually developed a new method for the synthesis of **8** (Scheme II). In this new method, the least satisfactory step is the benzylation of pyridoxol to **12**, which is obtained in only 53% yield. Other steps gave almost quantitative yields, and it was not necessary to isolate the product in every case. The crucial step in the synthesis is the rearrangement of the *N*-oxide **14** with trifluoroacetic anhydride and the subsequent hydrolysis of the α^2-*O*-trifluoroacetyl group. These steps were carried out under very mild conditions, without any concomitant hydrolysis of the acid-sensitive blocking groups. The 2-aldehyde **15** was the key intermediate in this method and was obtained by MnO_2 oxidation of **8**. The blocking groups could be removed by mild acid hydrolysis (typically, heating with 1 *N* HCl on a steam bath for 1 hr). Such treatment of **15** gave 2-formyl-2-norpyridoxol [**1b** (**1**, R_2 = CHO)], which was identical with the product of the hydrolysis of **7**, thus indicating the structure of **7**.

With the ready availability of **15**, it is now possible to introduce various groups into the 2 position of pyridoxol. Initially we have chosen to introduce 2-vinyl, 2-ethynyl, and related groups, since pyridoxol analogs with these groups in the 4 position have been shown to be very potent reversible inhibitors of vitamin B_6[1a,15] or irreversible inhibitors directed to cofactor sites of apoenzymes dependent on pyridoxal phosphate.[16]

The aldehyde group in **15** was converted into vinyl, as in **11**, by means of a Wittig reaction in which the required ylide was generated from triphenylphosphonium bromide, using both *n*-butyllithium and potassium *tert*-butoxide as the base, as has been described earlier for the synthesis of the 4-vinyl analog.[1a] Mild acid hydrolysis gave 2-vinyl-2-norpyridoxol [**1c** (**1**, R_2 = $CH{=}CH_2$)], which on hydrogenation (Pd/C) gave the known α^2-methylpyridoxol.[5,17] Further hydrogenolysis occurred very readily, giving 2-ethyl-3-hydroxy-4,5-dimethylpyridine. The blocked β-chlorovinyl analog **17** was similarly prepared from **15** by a Wittig reaction (chloromethyltriphenylphosphonium chloride was used), giving **17** as a mixture of cis and trans isomers. The two isomers were not separated but were subjected to hydrolysis, giving **1c** (**1**, R_2 = CH=CHCl), and to dehydrochlorination ($NaNH_2$ in liquid ammonia), giving in good yield the blocked ethynyl compound **18**, which was similarly hydrolyzed to **1d** (**1**, R_2 = C≡CH).

The ethynyl proton in **18** is shifted in **1d** (**1**, R_2 = C=CH) by 1.07 ppm downfield, indicating an intramolecular H bonding between the OH and ethynyl groups. An analogous intramolecular H bonding has been postulated for *o*-hydroxyphenylacetylene.[18] Like the 4-vinyl analog of pyridoxol, the 2-vinyl and 2-ethynyl analogs are less reactive than the corresponding unsubstituted pyridine compounds, since the phenolic OH ortho to these groups has a deactivating influence. The relative ease with which the blocked intermediate **15** can now be synthesized and the ease of removal of the blocking groups make it feasible to introduce other, more reactive groups into the 2 position. The present method extends and supplements the procedures applied earlier to modification of the 2-methyl group in vitamin B_6. Such procedures were based on methods for synthesizing the vitamin itself,[17] and their scope is limited.

Biological Activity. Compounds synthesized in this study were tested as inhibitors of the growth of mouse mammary adenocarcinoma (TA-3) cells grown in tissue culture in Eagle's medium containing a minimal amount of pyridoxal (1×10^{-7} *M*) to sustain optimal growth.[15] 2-Vinyl-2-norpyridoxol was the most active inhibitor in the series, with an ID_{50} of 9.4×10^{-6} *M*. Although it was less active than the 4-vinyl analog of pyridoxal (ID_{50} 1×10^{-7} *M* under the same conditions), it was not reversed by pyridoxal at approximately 10^{-5} *M*. As in the case of the 4-vinyl analog, saturation of the double bond almost abolished its inhibitory activity; the ID_{50} for α^2-methylpyridoxol was approximately 3×10^{-4} *M*. 2-(β-Chloro)vinyl, 2-ethynyl, 2-formyl, and 2-(hydroxymethyl) analogs inhibited the half-maximal growth of these cells at 1×10^{-4}, 1.7×10^{-4}, 1.7×10^{-4}, and 1×10^{-4} *M*, respectively. The substantial activity of the 2-vinyl analog and the apparently irreversible nature of the inhibition make it attractive to design other vitamin B_6 analogs incorporating the 2-vinyl group.

Experimental Section

Where analyses are indicated only by symbols of elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Tlc (silica gel) was used routinely as described earlier.[19] Ir spectra were determined with a Perkin-Elmer 457 spectrophotometer, and nmr spectra with a Varian A-60A instrument, using 8–15% solutions in $CDCl_3$, DMSO, or D_2O; positions of peaks are expressed in parts per million from TMS, or from dioxane (δ 3.70), as an internal standard. Peaks are assigned on the basis of previous work.[20]

Tri-*O*-acetylpyridoxol (2). To a suspension of pyridoxol hydrochloride (7.5 g, 36 mmol) in pyridine (dry, 50 ml), Ac_2O (30 ml) was added, and the mixture was heated on a steam bath for 1 hr.

§This was also supported by the following experiment. 5-Deoxypyridoxol was acetonated in the presence of a 1 *M* proportion of *p*-toluenesulfonic acid. Thus it is not necessary to have a larger proportion of the catalyst for the formation of a cyclic ketal with a six-membered ring in the absence of vicinal hydroxymethyl groups competing for the formation of a cyclic ketal with a seven-membered ring. Since the proportions of *p*-toluenesulfonic acid used are large, a reviewer has suggested describing it as a "reaction carrier" instead of a "catalyst."

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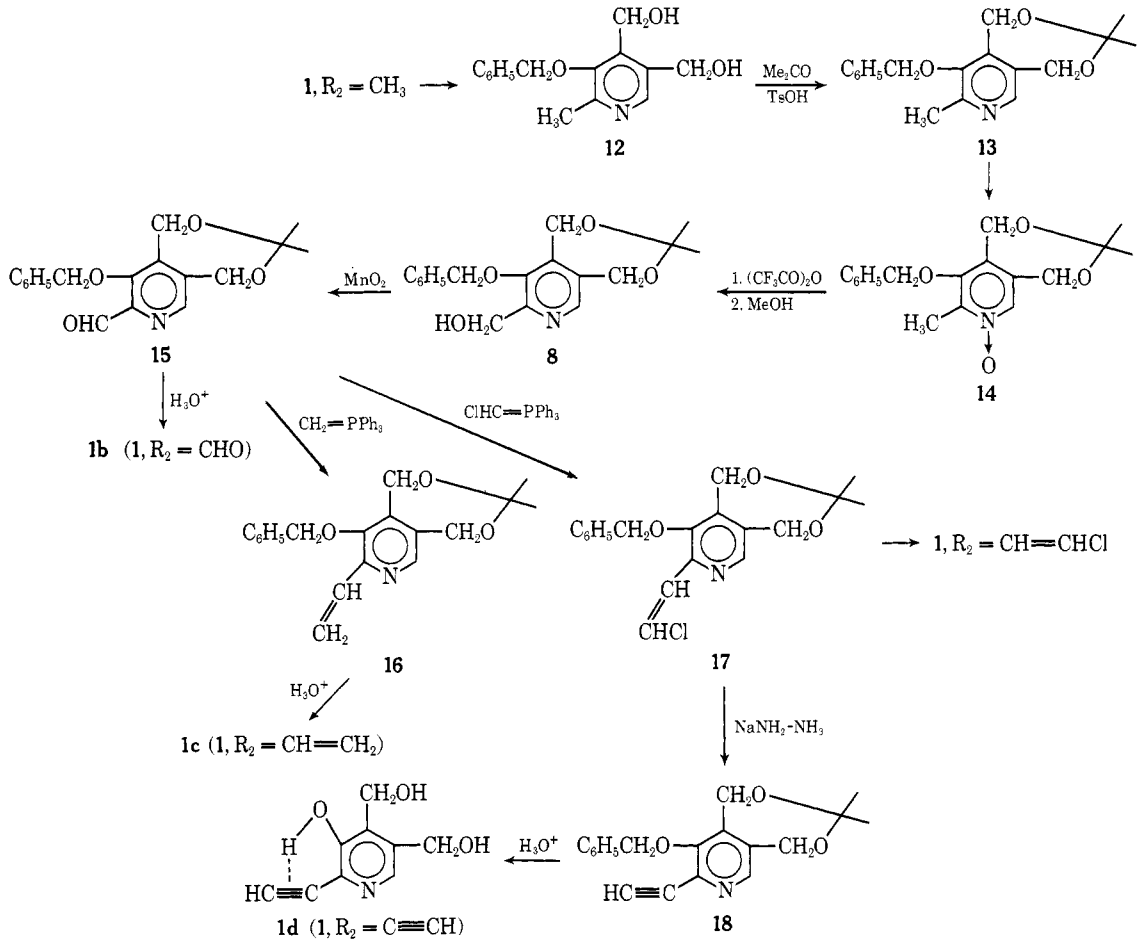
A preliminary report has been published by Sartorelli, *et al.*,[14] on the synthesis of the thiosemicarbazone of 2-formyl-2-norpyridoxol, as well as that of ketal **5**.

Scheme II



The clear solution thus obtained was stirred at room temperature for 15 hr, and the solvent was evaporated. Next, EtOH was added to the residue and was then evaporated. The residue was taken up in EtOAc, and the solution was washed with H_2O (three times) and dried (Na_2SO_4). The solvent was completely removed, yielding 9.5 g (85%) of oily product.

α^2-Hydroxypyridoxol (3, 3-Hydroxy-2,4,5-tris(hydroxymethyl)pyridine Hydrochloride). Tri-*O*-acetylpyridoxol (**2**, 10.0 g, 32.5 mmol) in $CHCl_3$ (200 ml) was treated with *m*-chloroperbenzoic acid (12.0 g, 69.5 mmol) for 12 hr in the dark. The mixture was washed with $NaHSO_3$ solution (10% in H_2O), followed by $NaHCO_3$ solution and H_2O. Evaporation of $CHCl_3$ gave an oil (10 g), which was heated with Ac_2O (100 ml) at 100° for 1 hr. The residue from evaporation of the reagent was dissolved in EtOH, and the solution was again evaporated. The residue was heated on a steam bath with 2.5 *N* HCl (100 ml) and was then stirred at room temperature for 12 hr. At this stage, benzoic acid crystallized. It was filtered off and was washed with a little 2.5 *N* HCl. The combined filtrates were concentrated, when the desired compound crystallized. It was suspended in Et_2O, filtered, and washed with Et_2O. Recrystallization from MeOH-Et_2O gave 4.0 g (64%) of the product, mp 171–173°.

Acetonation of α^2-Hydroxypyridoxol Hydrochloride. α^4,3-*O*-Isopropylidene-α^2-hydroxypyridoxol Hydrochloride (4) by HCl Catalysis. α^2-Hydroxypyridoxol (200 mg, 0.90 mmol) was suspended in acetone (10 ml, freshly distilled over K_2CO_3). The suspension was cooled to −10° (ice–salt bath) and saturated with HCl. The flask, well stoppered, was stirred for 30 min at room temperature. The product was poured into Et_2O (400 ml, anhydrous), kept at −15° overnight, filtered, and washed with Et_2O: yield 162 mg (69%); mp 166–168.5° dec from EtOH. *Anal.* ($C_{11}H_{16}ClNO_4$) C, H, N. The free base was obtained by neutralization of the hydrochloride with 10% K_2CO_3 and extraction with EtOAc: mp 110° (from MeOH-Et_2O).

α^4,α^5-*O*-Isopropylidene-α^2-hydroxypyridoxol (5). α^2-Hydroxypyridoxol hydrochloride (**3**, 960 mg, 4.33 mmol) was suspended in acetone (dried, 15 ml) to which *p*-toluenesulfonic acid (823 mg, 4.33 mmol) and 2,2-dimethoxypropane (10 ml) had been added, and the reaction mixture was stirred for 3–5 days. The precipitate was filtered, dissolved in 5% $NaHCO_3$ (*ca.* 50 ml, final pH about 8), and extracted continuously with EtOAc. After drying (Na_2SO_4), the EtOAc solution was evaporated, and the residue was crystallized from EtOH, yielding 630 mg (65%) of product, mp 149–151°. *Anal.* ($C_{11}H_{15}NO_4$) C, H, N.

Acetonation of α^2-Hydroxypyridoxol Hydrochloride, Catalyzed by Increasing Proportions of *p*-Toluenesulfonic Acid. The preceding experiment was repeated, using *p*-toluenesulfonic acid and α^2-hydroxypyridoxol hydrochloride in a molar ratio of 6:1. The experimental procedures were the same, except that after neutralization (10% Na_2CO_3), the product was extracted with petroleum ether to remove the acetone condensation products. The cyclic ketal with a six-membered ring predominated over the one with a seven-membered ring, in a ratio of 6:1, as estimated by the ratio of the isopropylidene peaks in the nmr spectrum of the mixture. The former was isolated from the reaction mixture and amounted to 205 mg (21%) of pure product.

When the ratio of *p*-toluenesulfonic acid to α^2-hydroxypyridoxol hydrochloride was 4:1, the ratio of the cyclic ketal with a six-membered ring to the one with a seven-membered ring was 5:3.

Oxidation of α^4,3-*O*-Isopropylidene-α^2-hydroxypyridoxol. Experiment A. Compound **4** (free base, 50 mg, 0.22 mmol) was dissolved in a mixture of $CHCl_3$ (5 ml) and EtOH (2 ml), and the solution was stirred with MnO_2 (500 mg) for 70 hr. The major spot had an R_f of 0.78 (EtOAc, red spot with phenylhydrazine). The reaction mixture was heated for 5 min on a steam bath with a solution of NaOAc (200 mg) and NH_2OH·HCl (100 ml) in H_2O (5 ml), when the dioxime of **6** separated out in a yield of 40 mg (72%), mp 230° dec (shrinks at 210°, from MeOH). *Anal.* ($C_{11}H_{13}N_3O_4$) C, H, N. The parent dialdehyde **6** was obtained as an oil by preparative tlc (eluted with EtOAc): nmr ($CDCl_3$) δ 1.67 (isopropylidene CH_3's), 5.38 (4-CH_2), 10.38, 10.58 (2-, 5-CHO), 8.87 (C_6-H).

Experiment B. Compound **4** (free base, 225 mg, 1 mmol) was dissolved in H_2O (15 ml). MnO_2 (650 mg) was added, and the suspension was stirred for 3 hr. After the MnO_2 was filtered and was washed with H_2O (200 ml), the combined filtrate and washings were concentrated *in vacuo*. Tlc (EtOAc) of the reaction mixture showed two aldehyde spots: R_f 0.78 for the dialdehyde **6** (see ex-

periment A), and R_f 0.48 for the monoaldehyde **7**. The latter was isolated by preparative tlc (the upper spot yielded 20 mg of oily dialdehyde). The yield of monoaldehyde **7** was 60 mg; mp 65° (from Me_2CO-Et_2O); uv λ_{max} (0.1 *N* NaOH) 250 nm (ϵ 8400), 285 (10,800), 320 (6800); ir λ_{max} (KBr) 1695 (C=O), 3400 cm^{-1} (broad, probably due to water of hydration) (OH); nmr ($CDCl_3$) δ 1.65 (isopropylidene CH_3's), 4.80 and 5.08 (4- and 5-CH_2's), 8.40 (C_6-H), 10.50 (2-CHO). *Anal.* ($C_{11}H_{13}NO_4$) C, H.

3-*O*-Benzyl-α^2-hydroxypyridoxol (9). To a solution of 1.67 g (72 mmol) of Na in absolute EtOH (100 ml), **3** (8.05 g, 36 mmol) was added. The solution was stirred for 1 hr and cooled to 0°, and to it was added dropwise a solution of benzyldimethylphenylammonium chloride (8.95 g, 37 mmol) in 100 ml of absolute EtOH. The dark-colored mixture was stirred at room temperature for 2 hr, the solvent was removed *in vacuo*, and the residue was twice flash-evaporated with toluene and dried. Xylene (dry, 100 ml) was then added, and the mixture was refluxed for 2.5 hr. After evaporation of the xylene, the syrupy residue was dissolved in EtOAc (2 l.), and the EtOAc solution was washed with H_2O and dried (Na_2SO_4). After removal of the EtOAc, the residue was dissolved in H_2O and was subjected to steam distillation to remove dimethylaniline. The residue was extracted with EtOAc and dried (Na_2SO_4), the solvent was removed, and the residue was crystallized from EtOAc-Et_2O, yielding 4.5 g (45%), mp 84°. *Anal.* ($C_{15}H_{17}NO_4$) C, H, N.

3-*O*-Benzyl-α^4,α^5-*O*-isopropylidene-α^2-hydroxypyridoxol (8). To a solution of **9** (4.0 g, 15 mmol) in Me_2CO (dry, 150 ml), *p*-toluenesulfonic acid (8.0 g, 42 mmol) was added, and the solution was stirred for 16 hr at room temperature. The reaction mixture was neutralized with a 25% aqueous solution of $NaHCO_3$, concentrated, and extracted with Et_2O. After washing with H_2O and drying (Na_2SO_4), the solvent was removed, and the residue was crystallized from MeOH, yielding 3.5 g (76%): mp 144°; ir λ_{max} (KBr) 3160 cm^{-1} (OH). *Anal.* ($C_{18}H_{21}NO_4$) C, H, N. Benzylation of **5** with benzyldimethylphenylammonium chloride gave **8** in 76% yield.

3-*O*-Benzyl-2-formyl-α^4,α^5-isopropylidene-2-norpyridoxol (15). To a solution of **8** (630 mg, 2 mmol) in $CHCl_3$ (60 ml), MnO_2 (2.25 g) was added, and the suspension was stirred for 24 hr at room temperature. The reaction mixture was worked up in the usual way. The oily residue was crystallized from a mixture of Et_2O and petroleum ether, yielding 500 mg (80%): mp 52°; ir λ_{max} (KBr) 1705 cm^{-1} (C=O); nmr δ ($CDCl_3$) 5.08 (CH_2), 4.94 (CH_2), 4.86 (CH_2), 10.30 (CHO), 7.50 (C_6H_5), 8.40 (C_6-H). *Anal.* ($C_{18}H_{19}NO_4$) C, H, N.

The oxime of **15** was obtained by heating **15** in aqueous EtOH solution with NH_2OH·HCl and NaOAc on a steam bath for 1 hr: mp 114–115° (from Et_2O-petroleum ether). *Anal.* ($C_{18}H_{20}N_2O_4$) C, H, N.

α^4,α^5-*O*-Isopropylidenepyridoxol (10). To pyridoxol (free base, 100 mg, 0.59 mmol) in Me_2CO (2 ml, dried over $CaSO_4$), 0.5 ml of 2,2-dimethoxypropane and finally 112 mg (0.59 mmol) of *p*-toluenesulfonic acid were added, and the solution was stirred for 20 hr. The resulting precipitate was filtered, washed with a little Me_2CO, and poured into a cold saturated $NaHCO_3$ solution while it was being stirred. The resulting solution was extracted with EtOAc continuously for 6 hr, and the EtOAc was dried (Na_2SO_4) and evaporated. Tlc gave a single product: mp 182° (lit.[9] mp 185°); yield 120 mg (94%). The compound was identical with an authentic sample in ir and nmr spectra.

α^4,α^5-*O*-Isopropylidene-d_6-pyridoxol was prepared exactly like the nondeuterated compound, except that acetone-d_6 (99.5%) was used. The melting point was identical with that of the nondeuterated compound, and the degree of isotopic purity was 88%.

α^4,3-*O*-Isopropylidenepyridoxol (11). To a suspension of pyridoxol hydrochloride (1.0 g, 4.86 mmol) in Me_2CO (25 ml, dried over $CaSO_4$) and 2,2-dimethoxypropane (15 ml), *p*-toluenesulfonic acid (3.7 g, 19.4 mmol) was added, and the mixture was stirred for 16 hr. The resulting dark-brown solution was neutralized with K_2CO_3 and then concentrated *in vacuo*. After extraction with $CHCl_3$, washing with H_2O, and drying (Na_2SO_4), the $CHCl_3$ was removed, and the residue was crystallized from EtOH-Et_2O: yield 0.64 g (63%). The compound was identical with authentic material in ir and nmr spectra.[13] The preparation has been scaled up, starting with 41 g (0.2 mol) of pyridoxol hydrochloride, and yielding 31.8 g (76%) of **11**.

"Isomerization" of α^4,α^5-*O*-Isopropylidene-d_6-pyridoxol. To a solution of **11**-d_6 (50 mg, 0.23 mmol) in acetone (dried over $CaSO_4$, 2.5 ml), 0.177 g (0.93 mmol) of *p*-toluenesulfonic acid was added, and the mixture was stirred for 2 days. The mixture was then neutralized (Na_2CO_3) and was extracted with $CHCl_3$. Evaporation yielded 30 mg (62%) of α^4,3-*O*-isopropylidenepyridoxol without the deuterium label, as shown by ir and nmr spectroscopy.

3-*O*-Benzylpyridoxol (12). Pyridoxol hydrochloride (2.0 g, 0.97 mmol) was added to a solution of NaOEt [from Na (0.5 g) in 25 ml of absolute EtOH], and the resulting solution was stirred at room temperature for 1 hr. The latter solution was cooled in ice, and a solution of dimethylphenylbenzylammonium chloride (3 g) in EtOH (25 ml) was added slowly, with stirring. The mixture was stirred at room temperature for 2 hr and was then evaporated *in vacuo*. Twice, dry C_6H_6 was added to the residue and evaporated to remove traces of EtOH. Dry benzene (25 ml) was added to the residue, and the reaction mixture was gently refluxed for 0.5 hr and was then evaporated completely *in vacuo*. Water (25 ml) was added, and the mixture was extracted several times with $CHCl_3$. The combined $CHCl_3$ extracts were dried over $CaSO_4$ and evaporated *in vacuo*, when crystalline material separated out. It was cooled, filtered, washed with a little Et_2O, and dried: yield 1.43 g (57%); mp 113–114° (lit.[21] mp 118–120°). *Anal.* ($C_{15}H_{17}NO_3$) C, H, N. The hydrochloride had a melting point of 198–199° (lit.[21] mp 178°).

3-*O*-Benzyl-α^4,α^5-*O*-isopropylidenepyridoxol (13). To a solution of **12** (9.2 g, 35.5 mmol) in Me_2CO (dried over $CaSO_4$, 100 ml), 2,2-dimethoxypropane (15 ml) and *p*-toluenesulfonic acid (6.98 g, 36.8 mmol) were added, and the mixture was stirred at room temperature for 4.5 days, until only a trace of the starting material could be detected by tlc. The reaction mixture was poured into ice-cold 10% aqueous Na_2CO_3 with rapid stirring, and the alkaline solution was extracted with $CHCl_3$, washed with H_2O, dried (Na_2SO_4), and evaporated. The oily product did not crystallize and was used for preparing the *N*-oxide.

3-*O*-Benzyl-α^4,α^5-*O*-isopropylidenepyridoxol *N*-Oxide (14). The oily product **13** from the previous experiment was dissolved in $CHCl_3$ (100 ml, dried over $CaSO_4$), and the solution was cooled to 0°. To this solution, rapidly stirred, was added *m*-chloroperbenzoic acid (7.22 g, 0.0355 mol on the basis of 85% purity of the reagent). After being stirred at room temperature for 2 hr, until no starting material was detected by tlc, the reaction mixture was extracted with Na_2SO_3 (10%, 2 × 100 ml), $NaHCO_3$ (5%, 2 × 100 ml), and H_2O. After drying (Na_2SO_4), $CHCl_3$ was evaporated, and the residual oil was used in the next experiment. Part of the oil was crystallized (Et_2O-petroleum ether) and recrystallized (EtOH-Et_2O): mp 109–112°. *Anal.* ($C_{18}H_{21}NO_4$) C, H, N.

3-*O*-Benzyl-α^4,α^5-isopropylidene-α^2-hydroxypyridoxol (8). A solution of **14** (the total amount of the oily material from the preceding experiment) in CH_2Cl_2 (dried over $CaSO_4$, 75 ml) was cooled (ice bath). Trifluoroacetic anhydride (2.0 ml) was added while the solution was being stirred, and more (5.0 ml) was added after 5 min. The reaction mixture was stirred for 8 hr at room temperature, until no starting material could be detected by tlc. Then it was cooled to 0°, and MeOH (30 ml) was added while stirring was continued. The solvents were then evaporated. The oil was taken up in $CHCl_3$ and was washed with Na_2CO_3 (20%), followed by H_2O until neutral. It was then crystallized from EtOH-$CHCl_3$, yielding 8.24 g (74%, based on amount of **12**). The material was identical with **8** obtained by the other two methods already described.

2-Formyl-2-norpyridoxol (1, R_2 = CHO). A solution of 74 mg (0.24 mmol) of **15** in trifluoroacetic acid (10 ml) was stirred at room temperature for 3 hr, and then the acid was removed *in vacuo*. The residue was dissolved in H_2O (1 ml), applied to an Amberlite CG-50 (200–400 mesh, H^+ form, 1.1 × 15 cm) column, and eluted with H_2O. The acid fractions emerged first and were followed by neutral fractions containing the aldehyde. The neutral fractions were combined, and the aldehyde was crystallized from H_2O-Me_2CO: yield 15 mg (35%); mp 123–125°; uv λ_{max} (pH 6.8) 248, 327, 386 nm; uv λ_{max} (0.1 *N* NaOH) 243, 384 nm. *Anal.* ($C_8H_9NO_4$) C, H, N.

3-*O*-Benzyl-α^4,α^5-*O*-isopropylidene-2-vinyl-2-norpyridoxol (16). Triphenylmethylphosphonium bromide (900 mg, 3.43 mmol) was added under N_2 to a stirred and cooled (0°) mixture of a 1.6 *M* solution of *n*-butyllithium (1.5 ml) and a solution of 0.5 g of a 1:1 mixture of potassium *tert*-butoxide and *tert*-butyl alcohol in 8 ml of Et_2O. The resulting mixture was stirred at room temperature for 2 hr. Then **15** (450 mg, 1.43 mmol) in Et_2O (3 ml) was added within 15 min, and stirring was continued for another 12 hr. The reaction mixture was filtered, and the filtrate was diluted with Et_2O. The dilute solution was treated with saturated $NaHSO_3$ and NH_4Cl solutions, and finally with H_2O. After drying ($MgSO_4$), the solvent was evaporated to an oil, and the vinyl compound was separated from $(C_6H_5)_3PO$ by chromatogra-

phy on silica gel, using EtOAc as the eluent: yield 358 mg (80%); nmr ($CDCl_3$) δ 1.47 [$(CH_3)_2C$], 4.89 (3 × CH_2), 5.43–6.67 (2-CH=CH_2), 7.00–7.73 (2-CH=CH_2), 7.50 (C_6H_5), 8.23 (C_6-H); ir λ_{max} (neat) 1605 cm^{-1} (C=C). *Anal.* ($C_{19}H_{21}NO_3$) C, H.

2-Vinyl-2-norpyridoxol [1c (1, R_2 = CH=CH_2)] Hydrochloride. A solution of **16** (300 mg) in 1 *N* HCl (10 ml) was heated on a steam bath for 1 hr. After evaporation *in vacuo*, the residue was evaporated several times with H_2O to remove excess HCl and benzyl alcohol: mp 201–203° dec from EtOH; yield 170 mg (77%); nmr (D_2O) δ 4.78 (5-CH_2), 5.02 (4-CH_2), 5.90–6.50 (2-CH=CH_2), 6.83–7.35 (2-CH=CH_2), 8.27 (C_6-H); ir λ_{max} (KBr) 3340, 3200, 3100 (OH), 1612 cm^{-1} (C=C); uv λ_{max} (0.1 *N* HCl) 324 nm (ϵ 6485); uv λ_{max} (0.1 *N* NaOH) 353 nm (ϵ 6360); uv λ_{max} (pH 7) 367 nm (ϵ 6800). *Anal.* ($C_9H_{12}ClNO_3$) C, H, Cl.

α^2-Methylpyridoxol Hydrochloride. To a solution of **18** in EtOH (5 ml), 5% Pd/C (12 mg) was added, and the mixture was hydrogenated for 30 min. After filtration and evaporation of the solvent, the product was crystallized from EtOH–Et_2O: mp 192–195° (lit.[17a] mp 192°); yield 32 mg (79%).

2-Ethyl-3-hydroxy-4,5-dimethylpyridine Hydrochloride. To a solution of 2-vinyl-2-norpyridoxol hydrochloride (50 mg) in EtOH (3 ml) 10% Pd/C (30 mg) was added, and the solution was stirred for 24 hr in an atmosphere of H_2. After filtration and evaporation the compound was crystallized from EtOH–petroleum ether: yield 32 mg (74%); mp 154–156°. *Anal.* ($C_9H_{14}ClNO$) C, H, N. This compound has also been obtained from α^2-methylpyridoxol hydrochloride on hydrogenolysis under the conditions described here.

3-*O*-Benzyl-α^4,α^5-isopropylidene-2-(β-chlorovinyl)-2-norpyridoxol (17). The reaction conditions were similar to those described for **16**, except that the ylide was generated from (chloromethyl)triphenylphosphonium chloride (1.9 g, 5.47 mmol) and was allowed to react with **15** (1.0 g, 2.9 mmol). Chromatography (silica gel, EtOAc as eluent) gave an oil (782 mg, 67%); nmr ($CDCl_3$) δ 1.45 [$(CH_3)_2C$], 4.87 (3 × CH_2), 5.83–7.33 (CH=CHCl), 7.50 (C_6H_5), 8.17 (C_6-H); ir λ_{max} (neat) 1610 cm^{-1} (C=C). The compound was also characterized by the two hydrolysis products obtained as described in the next two paragraphs.

3-*O*-Benzyl-2-(β-chlorovinyl)-2-norpyridoxol Hydrochloride. A solution of **17** in Et_2O was treated with ethereal HCl. The precipitated hydrochloride was crystallized from hot EtOH, which resulted in cleavage of the isopropylidene group: mp 166–168° dec; nmr δ ($CDCl_3$) 4.82, 4.70, 5.02 (3 × CH_2), 6.67–7.67 (CH=CHCl), 7.48 (C_6H_5), 8.25 (C_6-H); ir λ_{max} (KBr) 3380, 3310 (-OH), 1615 cm^{-1} (C=C). *Anal.* ($C_{16}H_{17}Cl_2NO_3$) C, H, Cl.

2-(β-Chlorovinyl)-2-norpyridoxol (1, R_2 = CH=CHCl) Hydrochloride. The blocking groups in **19** were hydrolyzed as described for **16**: yield 73%; mp 177–179° dec; nmr (D_2O) δ 4.87 and 5.02 (4- and 5-CH_2), 6.70–7.83 (CH=CHCl), 8.47 (C_6-H); ir λ_{max} (KBr) 1612 cm^{-1}. *Anal.* ($C_9H_{11}Cl_2NO_3$) C, H, N.

3-*O*-Benzyl-α^4,α^5-*O*-isopropylidene-2-ethynyl-2-norpyridoxol (18). Fresh $NaNH_2$ was prepared according to the method of Vaughn, *et al.*,[22] from Na (69 mg), liquid NH_3 (10 ml), and $Fe(NO_3)_2$ (0.6 mg). A solution of **17** (346 mg, 1 mmol) in Et_2O (anhydrous, 10 ml) was added dropwise to the $NaNH_2$ solution during 1 hr. The reaction mixture was left at room temperature for 15 hr. The sodium acetylide formed was hydrolyzed by careful addition of 10% NH_4Cl solution, and the products were extracted with Et_2O, washed with H_2O, and dried ($MgSO_4$). The oily mixture obtained on evaporation of Et_2O was separated on a silica gel column (eluted with 1:1 C_6H_6–Et_2O), yielding 96 mg (30%) of **18** as an oil: nmr ($CDCl_3$) δ 1.42 [$(CH_3)_2C$], 4.77 and 4.93 (4- and 5-CH_2), 5.20 (C$H_2$$C_6H_5$), 3.45 (C≡C$H$), 7.47 ($C_6H_5$), 8.07 ($C_6$-H); ir λ_{max} (neat) 3290 (C≡CH stretching), 2210 cm^{-1} (C≡C). *Anal.* ($C_{19}H_{19}NO_3$) C, H.

2-Ethynyl-2-norpyridoxol [1d (1, R_2 = C≡CH)] Hydrochloride. The blocking groups in **18** were hydrolyzed with 1 *N* HCl as described for **16**. The yield was only 32%; mp 155–157° dec, from EtOH–Et_2O, with some difficulty; nmr (D_2O) δ 4.52 (C≡CH), 4.84 and 4.88 (4- and 5-CH_2), 8.37 (C_6-H); ir λ_{max} (KBr) 3260 (C≡CH stretching), 2120 cm^{-1} (C≡C). *Anal.* ($C_9H_{10}ClNO_3$) C, H.

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