Selective Modification of the 2-Position of Pyridoxol

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Pyridoxol, protected by acetylation of the hydroxyl groups, has been converted to its *N*-oxide which upon reaction with perfluoroacetic anhydride yields a 2-nor-2-hydroxymethylpyridoxol derivative as an intermediate. This compound undergoes acyl migration from the 3-position. Protection of the pyridoxol hydroxyls by benzylation followed by the same treatment yields the unrearranged α^2-hydroxy derivative. This compound has been converted to a series of α^2-substituted pyridoxols (X = -Cl, -Br, $-OCOCH_3$, $-OCH_3$, $-OC_2H_5$).

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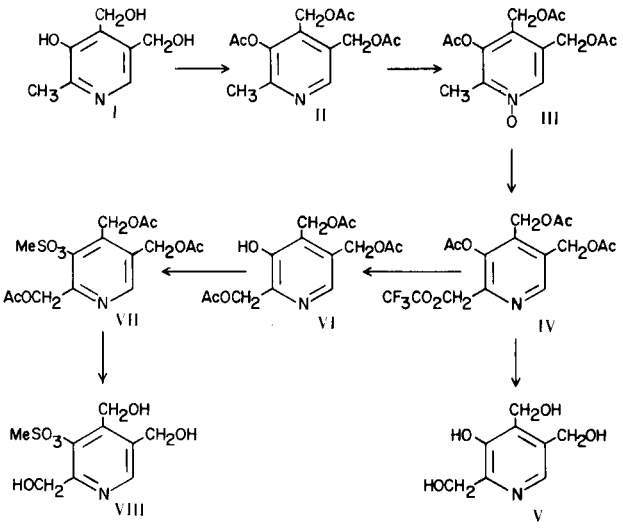
The B_6 group of vitamins is known to be responsible for many non-oxidative enzymic amino acid transformations and catalyzes such reactions as decarboxylation, transamination, racemization and α- and β-eliminations in amino acid metabolism (2). One of the active forms of this vitamin is pyridoxol, I, which because of its biological importance has been the subject of numerous studies directed at chemical modification of the basic molecule. Such modifications have been made principally at the 3-, 4- and 5- positions which present functionalities convenient for chemical reaction.

However, because these positions are intimately involved at the active site of the enzyme, such modifications must be expected to have rather drastic effects on the enzyme activity of the analogs. For this reason we have initiated an extensive study of the effects of minor modifications at more distant parts of the coenzyme and in this note we report our initial studies directed towards modification of the 2-methyl group. Few such studies have been made prior to this work (3,4).

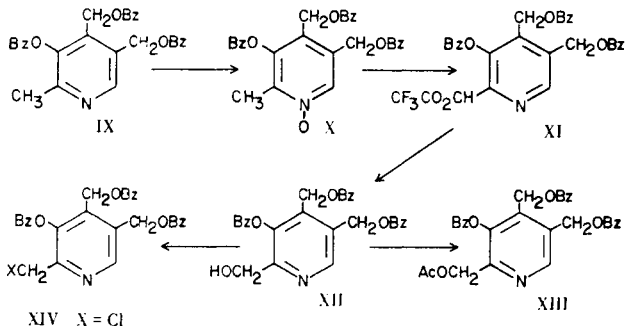
A most attractive approach appears to involve making use of the proximity of the pyridine nitrogen and the activation of the α-methyl hydrogens by the *N*-oxide function (Scheme 1). With this goal in mind, triacetyl pyridoxol, II, was prepared by the previously described procedure (5). This was readily converted to the *N*-oxide, III, by reaction with *meta*-chloroperbenzoic acid. The reaction of picoline *N*-oxides with anhydrides has been shown to yield acyloxymethylpyridines, and the reaction most suited to our purpose is found to be favored with electron deficient anhydrides (6). Thus, treatment of III

Scheme 1



with perfluoroacetic anhydride resulted in the quantitative formation of the trifluoroacetate IV. This compound was not isolated, but could be hydrolyzed to the known hydrochloride V (5). Under milder conditions (methanol, 65°) the trifluoroacetate solvolyzed to yield a triacetate assigned the structure VI. This assignment was made on the basis of consistent analytical and spectral data as well as positive ferric chloride and Gibbs tests (phenol unsubstituted in the *para*-position) (7). Such acyl migrations have been thoroughly characterized in related pyridoxine derivatives wherein an acetyl group migrated from the

Scheme II



XIV X = Cl
XV X = Br
XVI X = OCH_3
XVII X = OC_2H_5

α^3 -O- position to the 4-O-position in the monoacetylpyridoxol (8). The structure of VI was substantiated by converting it to its methane sulfonate VII followed by acid catalyzed hydrolysis to a compound which gave a negative Gibbs test and was assigned the structure VIII on the basis of its spectral properties.

In view of this undesirable acyl migration, an alternative route to the substituted pyridoxols was utilized (Scheme 2). Pyridoxol was converted to the tribenzyl derivative, IX. The free base, IX, reacted cleanly with *meta*-chloroperbenzoic acid to produce the *N*-oxide, X which was most readily purified as its hydrochloride, Xa. Treatment of X with perfluoroacetic anhydride again gave excellent yields of the trifluoroacetate, XI which hydrolyzed readily in the air or in methanol to give XII.

This compound provides a useful starting point for the synthesis of a variety of α^2-substituted pyridoxols. Thus, treatment of XII with acetyl chloride produced the acetate XIII while reaction with thionyl chloride resulted in the corresponding chloride. This chloride XIV was surprisingly stable and showed no tendency to dimerize or undergo hydrolysis. On the other hand, the bromide XIV produced upon reaction of XII with phosphorus tribromide could not be isolated as such. Attempts to isolate the hydrochloride of this compound under the usual conditions lead only to the chloride XIV. The bromide however does provide a convenient method for obtaining other α^2-substituents. Thus, work-up of the bromination reaction in methanol or ethanol yields the corresponding ethers under extremely mild conditions.

EXPERIMENTAL

All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. The nuclear magnetic resonance spectra were recorded on a Varian A-60, Perkin Elmer R-20B or R-24 spectrometers using 10% solutions with internal TMS. Infrared spectra were run on a Perkin Elmer 337 Grating Infrared Spectrophotometer. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Inc., Woodside, New York.

3,α^4,α^5-Tri-*O*-acetylpyridoxol 1-Oxide (III).

The following is a modification of the procedure of Katritzky and coworkers (5). Tri-O-acetylpyridoxol (1.95 g., 6.6 mmoles) was dissolved in chloroform (25 ml.) and to the solution was added 3.7 g. (18 mmoles) of *m*-chloroperbenzoic acid (85%). The mixture was stirred at room temperature for 72 hours after which time the produced *m*-chlorobenzoic acid was removed by filtration. The chloroform solution was washed successively with saturated sodium bicarbonate solution (3 x 10 ml.) and water (10 ml.) and then dried (magnesium sulfate). After removal of the drying agent, the solvent was removed *in vacuo* to give a yellow solid. The product crystallized from benzene to give white needles (0.88 g., 43%) m.p. 114-118° (lit. (5) 114-115°); nmr (deuteriochloroform): δ 2.05, 2.15, 2.43 (S, 3H each, CH_3CO_2-); 2.37 (S, 3H, CH_3-); 5.18, 5.33 (S, 2H each, -CH_2O-); 8.40 (S, 1H, pyridine ring H).

Attempted Synthesis of α^2-Hydroxy-3,α^4,α^5-tri-*O*-acetylpyridoxol.

A solution of tri-*O*-acetylpyridoxol 1-oxide (2.13 g., 6.8 mmoles) in methylene chloride (7 ml.) was cooled to 0°, and to the stirred solution was added 2 ml. (14 mmoles) of trifluoroacetic anhydride over a period of 15 minutes. The solution became warm and the evolution of a gas was observed as the solution turned pale pink. Stirring was continued for a further 1 hour whereupon the solvent and excess of reagent were removed under vacuum to yield a pink gummy residue; nmr (deuteriochloroform): δ 2.05, 2.13, 2.43 (S, 3H each, CH_3CO_2-); 5.27, 5.45, 5.55 (S, 2H each, -CH_2O-); 8.80 (S, 1H, pyridine ring H). The residue was dissolved in anhydrous methanol (10 ml.) and stirred at 25° overnight. The solution was evaporated under vacuum to give a clear gummy residue which gave a strong positive Gibbs test and ferric chloride test; nmr (deuteriochloroform): δ 2.08, 2.12 (S, 3H each, CH_3CO_2-); 5.27, 5.30, 5.35 (S, 2H each, -CH_2O-); 8.30 (S, 1H, aromatic H). Attempts to isolate this compound as its hydrochloride were unsuccessful, but it was readily converted to α^2-hydroxypyridoxol hydrochloride m.p. 170-171° (lit. (5) 172-173° dec.) by treatment with hot methanolic hydrochloric acid. Alternatively the gummy residue (2.73 g., 8.7 mmoles) was dissolved in pyridine (17 ml.) and cooled to 0°. To this solution was added methanesulfonylchloride (1.5 ml., 20 mmoles) over a period of 10 minutes. The mixture was stirred overnight and evaporated under vacuum without heating. The residue was dissolved in water (20 ml.) and extracted with ether (5 x 20 ml.). The combined ether extracts were dried (magnesium sulfate) and evaporated to dryness under reduced pressure to yield a residue which gave negative ferric chloride and negative Gibbs tests; nmr (deuteriochloroform): δ 2.04, 2.10, 2.13 (S, 3H each, CH_3CO_2-); 3.53 (S, 3H, CH_3SO_2-); 5.37, 5.40, 5.45 (S, 2H each, -CH_2O-); 8.85 (S, 1H, aromatic H). The residue (2.5 g., 6.4 mmoles) was dissolved in anhydrous methanol (20 ml.) and saturated with anhydrous hydrogen chloride gas. The white crystals which precipitated were filtered from the solution and recrystallized from methanol to give 1.8 g. (81% yield) of white needles of 3-*O*-methanesulfonyl-α^2-hydroxypyridoxol (VIII) hydrochloride m.p. 158-162° dec.; nmr (deuterium oxide): δ 3.71 (S, 3H, CH_3SO_2-); 4.94, 5.00, 5.02 (S, 2H each, -CH_2O-); 8.27 (S, 1H, aromatic H).

Anal. Calcd. for $C_9H_{14}ClNO_6S$: C, 36.06; H, 4.67; N, 4.67. Found: C, 36.31; H, 4.84; N, 4.99.

3,α^4,α^5-Tri-*O*-benzylpyridoxol (IX).

Pyridoxol hydrochloride (24.7 g., 120 mmoles) was added in

small portions over 1 hour with cooling and stirring to a suspension of sodium hydride (20.0 g., 720 mmoles) in dry dimethylformamide (400 ml.). The reaction mixture was stirred for an additional 45 minutes with external cooling (ice-water). Benzyl chloride (60.6 g., 480 mmoles) was added dropwise with cooling over a period of 1 hour. The reaction mixture was gradually warmed to room temperature and stirring was continued for 48 hours during which time the mixture turned from yellow-green to brown. The mixture was cooled to 0° and the excess of sodium hydride was decomposed with 250 ml. of 95% ethanol followed by 300 ml. of water. The mixture was extracted with ether (5 x 150 ml.) and the combined extracts were washed with water (100 ml.) and saturated aqueous sodium chloride (100 ml.) and then dried over Drierite. The dried ether solution was then saturated with dry hydrogen chloride gas whereupon a white sticky precipitate formed. This crude product was purified by trituration with benzene (5 ml.) to yield white crystals which were filtered and washed with ether. The benzene and ether washings were cooled to yield an additional small amount of product. These combined crystals were recrystallized from methanol-ether (3:1) to yield white crystals (28.3 g., 53.2%) m.p. 179-181° (somewhat dependent on rate of heating); nmr (deuteriochloroform): δ 2.79 (S, 3H, CH_3-); 4.51, 4.57, 4.62, 4.66, 4.98 (S, 2H each, -CH_2O-); 7.30-7.40 (broad, 15H, phenyl H); 8.59 (S, 1H, pyridine ring H).

Anal. Calcd. for $C_{29}H_{30}ClNO_3$: C, 73.26; H, 7.31; N, 2.94; Cl, 7.45. Found: C, 73.40; H, 6.32; N, 2.95; Cl, 7.40.

The free base was prepared by suspending its hydrochloride (21.6 g., 45 mmoles) in 50 ml. of water, adding 100 ml. of a 10% aqueous sodium hydroxide solution and 100 ml. of ether and stirring for 30 minutes. The ether layer was separated, and the aqueous layer was washed with ether (3 x 25 ml.). The combined ether extracts were washed with water (25 ml.), saturated aqueous sodium chloride (25 ml.) and were then dried over Drierite. The ether solution was filtered to remove the drying agent and the solvent was removed on a rotary evaporator leaving a colorless viscous residue (18.1 g., 91%); nmr (carbon tetrachloride): δ 2.55 (S, 3H, CH_3-); 4.50, 4.55, 4.65, 4.90 (S, 2H each, -CH_2O-); 7.36, 7.40, 7.42 (3 apparent S, total of 15H, phenyl H); 8.30 (S, 1H, pyridine ring H).

Anal. Calcd. for $C_{29}H_{29}NO_3$: C, 79.25; H, 6.64; N, 3.18. Found: C, 79.23; H, 6.48; N, 3.01.

3,α^4,α^5-Tri-*O*-benzylpyridoxol 1-Oxide (X).

The following is a modification of the procedure of Katritzky and coworkers (5). Tri-*O*-benzylpyridoxol (18.1 g., 41 mmoles) was dissolved in methylene chloride (400 ml.) and *m*-chloroperbenzoic acid (85%, 25.1 g., 123 mmoles) was added to the solution. The reaction mixture was stirred for 48 hours during which time a white precipitate formed. The precipitate was filtered and was washed with cold methylene chloride (25 ml.). The solution was washed with 10% aqueous sodium hydroxide solution (3 x 50 ml.), water (50 ml.), saturated aqueous sodium chloride (50 ml.) and was then dried over Drierite. The solution was filtered free of drying agent and the solvent was removed under vacuum leaving a pale yellow viscous residue (16.8 g., 90.5%). The *N*-oxide could be purified by dissolving this residue in anhydrous ether (50 ml.) and saturating the solution with dry hydrogen chloride gas. The resultant precipitate was recrystallized from chloroform-ether (2:1) to yield white crystals (17.2 g., 95%), m.p. 170-171°; nmr (deuteriochloroform): δ 2.35 (S, 3H, CH_3-); 4.35, 4.45, 4.78 (S, 4H, 4H, 2H, -CH_2O-); 7.19, 7.21, 7.25 (3 apparent S, 5H each, phenyl H); 8.20 (S, 1H, pyridine ring H).

Anal. Calcd. for $C_{29}H_{30}ClO_4N$: C, 70.81; H, 6.10; N, 2.85, Cl, 7.21. Found: C, 69.46; H, 6.69; N, 3.08; Cl, 7.53.

The free base was liberated by suspending the hydrochloride (17.2 g., 35 mmoles) in 50 ml. of water, adding 100 ml. of a 10% aqueous sodium hydroxide solution and 100 ml. of ether. The mixture was stirred for 30 minutes, the ether layer was separated and the aqueous layer was washed with ether (3 x 25 ml.). The combined ether extracts were washed with water (25 ml.), saturated aqueous sodium chloride solution (25 ml.) and were then dried over Drierite. The drying agent was removed by filtration and the solvent was evaporated under vacuum leaving white crystals (15.6 g., 97%) which upon recrystallization from carbon tetrachloride had a m.p. 74-76°.

α^2-Hydroxy-tri-*O*-benzylpyridoxol (XII).

The following is a modification of the procedure of Koenig (6). Tri-*O*-benzylpyridoxol 1-oxide (4.8 g., 10.6 mmoles) was dissolved in methylene chloride (50 ml.) and to the stirred solution was slowly added freshly distilled trifluoroacetic anhydride (22.0 g., 10.6 mmoles). Vigorous bubbling commenced immediately and continued until *ca.* 5 minutes after the addition was complete. The reaction mixture was heated under reflux for 15 minutes and was stirred for an additional 45 minutes at room temperature. The solvent and the excess of trifluoroacetic anhydride were removed under vacuum. The residue was dissolved in 50 ml. of ether and the solution was washed with a saturated aqueous sodium bicarbonate solution (3 x 25 ml.), water (25 ml.) and a saturated aqueous sodium chloride solution (25 ml.). The solution was then dried over Drierite and the solvent was removed under reduced pressure. The residue was dissolved in methanol (10 ml.) and heated under reflux for 1 hour. The solvent was then removed under vacuum leaving a viscous residue which slowly solidified. Recrystallization from carbon tetrachloride gave white crystals m.p. 64-65° (2.30 g., 51%); nmr (carbon tetrachloride): δ 4.37, 4.45, 4.49, 4.57, 4.65, 4.80 (S, 2H each, -CH_2O-); 5.32 (S, 1H, -OH); 7.18, 7.23 (15H, phenyl H); 8.32 (S, 1H, pyridine ring H); ir (carbon tetrachloride) 3420, 3045, 1550, 1451, 1400, 1225, 1040 cm^{-1}.

Anal. Calcd. for $C_{29}H_{29}O_4N$: C, 76.49; H, 6.32; N, 3.08. Found: C, 76.30; H, 6.33; N, 3.08.

The hydrochloride was prepared by dissolving the base in anhydrous ether (50 ml.) and saturating the solution with dry hydrogen chloride gas. The solvent was decanted from the white crystals which were recrystallized from chloroform-ether (1:1) to give 2.46 g. (100%) of crystalline product m.p. 162-163°.

α^2-Acetoxy-3,α^4,α^5-tri-*O*-benzylpyridoxol (XIII).

Acetylchloride (395 mg., 5 mmoles) was added to the hydroxy compound XII (455 mg., 1 mmole) in the absence of solvent. The reaction mixture immediately became warm and the solution rapidly developed a white precipitate. The reaction mixture was stirred (80°) for 2.5 hours. After 1 hour the precipitate had dissolved completely to yield a clear yellow solution. The excess of acetyl chloride was removed under vacuum to leave a brown oily residue; ir (carbon tetrachloride): 1752 cm^{-1}. Ether (10 ml.) was added to this residue and a small amount of white crystals formed. Dry hydrogen chloride gas was passed through the solution causing more crystals to form. The white solid was filtered, washed with dry ether and recrystallized from methanol-ether (1:4). The product had a m.p. 112-113.5°; nmr (DMSO-d_6): δ 2.01 (S, 3H, CH_3CO_2-); 4.42, 4.57, 4.86, 4.99 (4H, 4H, 2H, 2H, -CH_2O-); 7.18, 7.23 (15H, phenyl H); 8.50 (S, 1H, pyridine ring H).

Anal. Calcd. for $C_{31}H_{32}ClO_5N$: C, 69.73; H, 6.00; N, 2.62;

Cl, 6.65. Found: C, 69.59; H, 6.12; N, 2.80; Cl, 6.40.

α^2-Chloro-3,α^4,α^5-tri-*O*-benzylpyridoxol (XIV).

To 994 mg. (2.2 mmoles) of the free base XII was added thionyl chloride (2.62 g., 22 mmoles) dropwise with stirring. The reaction mixture warmed immediately. Stirring and gentle heating (40°) was continued for 2 hours. The excess of thionyl chloride was removed under vacuum and ether was added to the residue to give a cloudy solution which upon cooling (-70°) yielded a precipitate of crude yellow material. This product was removed by filtration and recrystallized from chloroform-ether (1:3) to give a white crystalline material m.p. 105-107°; nmr (carbon tetrachloride): δ 4.37, 4.44, 4.48, 4.64, 4.61 (S, 2H each, $-CH_2O-$); 4.95 (S, 2H, $-CH_2Cl$); 7.15, 7.20, 7.25 (15 H, phenyl H); 8.35 (S, 1H, pyridine ring H).

Anal. Calcd. for $C_{29}H_{29}Cl_2NO_3$: C, 68.24; H, 5.69; Cl, 13.92; N, 2.75. Found: C, 68.90; H, 5.64; total Cl, 13.40; ionic Cl, 7.66; N, 2.92.

Bromination of α^2-Hydroxy-3,α^4,α^5-tribenzylpyridoxol.

The free base XII (455 mg., 1 mmole) was dissolved in dry cold benzene (10 ml.) and to this solution was added phosphorus tribromide (144 mg., 0.42 mmole). The reaction mixture was slowly warmed to room temperature and was stirred overnight. The mixture was then poured on to finely crushed ice (50 g.) when a sticky yellow solid immediately formed. Solid sodium bicarbonate (2 g.) was added and the mixture was stirred until the ice had melted. The mixture was extracted with chloroform (3 x 25 ml.), the combined chloroform extracts were washed successively with water (25 ml.) and saturated aqueous sodium chloride solution (25 ml.), and then dried (magnesium sulfate). The drying agent was removed and the solvent was evaporated under vacuum leaving a pale pink viscous residue (392 mg.). The limited attempts to prepare the hydrochloride of this material resulted only in the isolation of a compound which proved to be identical with chloride XIV. The substance at this stage however, proved to be quite satisfactory for further reaction and had not been hydrolyzed during the above work-up procedure. Thus, when the pink viscous residue was dissolved in methanol (10 ml.) and added dropwise under nitrogen to a solution of sodium methoxide in methanol and allowed to stir overnight at room temperature, the methoxy derivative XVI could be isolated. This however is not the method of choice for the synthesis of the methyl ether.

α^2-Methoxy-3,α^4,α^5-tribenzylpyridoxol (XVI).

α^2-Hydroxyl-3,α^4,α^5-tribenzylpyridoxol (228 mg., 0.5 mmole) was dissolved in cold dry benzene (5 ml.) and phosphorus tribromide (57 mg., 0.21 mmole) was added from a syringe. The mixture was warmed to room temperature and was stirred overnight. To this mixture was added dropwise with cooling, a solution of sodium methoxide, prepared by dissolving 230 mg. (0.01 g.-atom) of sodium in methanol (5 ml.). An immediate cloudiness developed and the solution changed color from pale pink to deep orange. The mixture was allowed to warm to room temperature and was stirred for 4 hours under an atmosphere of nitrogen. The reaction mixture was poured into cold water (25 ml.) and extracted with chloroform (3 x 25 ml.). The chloroform extracts were combined, washed successively with water (20 ml.) and with saturated aqueous sodium chloride (2 x 25 ml.) and then dried (magnesium sulfate). After removal of the drying agent, the solvent was removed under vacuum leaving an oily residue which was dissolved in ether (10 ml.) and saturated with dry hydrogen chloride gas, whereupon the crude hydrochloride precipitated as a yellow sticky solid. The crude product was separated from the solvent by decantation, dissolved in methanol and recrystallized from methanol-ether (1:4) to yield fine white needles m.p. 71-73° (157 mg., 64%); nmr (deuteriochloroform): δ 3.50 (S, 3H, $-OCH_3$); 4.49, 4.59, 4.61, 4.70, 4.83, 5.05 (S, 2H each, $-CH_2O-$); 7.29, 7.33 (15H, phenyl H); 8.73, (S, 1H, pyridine ring H).

Anal. Calcd. for $C_{30}H_{32}ClNO_4$: C, 71.21; H, 6.33; Cl, 7.02; N, 2.76. Found: C, 71.18; H, 6.31; Cl, 7.05; N, 2.64.

α^2-Ethoxy-3,α^4,α^5-tri-*O*-benzylpyridoxol (XVII).

The ethyl ether was prepared in the same manner as the methyl ether using ethanol instead of methanol as the reactant. The hydrochloride was recrystallized from methanol-ether (1:4) to give long white needles m.p. 54-55° (32% yield); nmr (deuteriochloroform): δ 1.21 (T, J = 7Hz, 3H, $-CH_3$); 3.62 (Q, J = 7Hz, 2H, $-OCH_2CH_3$); 4.44, 4.49, 4.58, 4.62, 4.64, 4.98 (S, 2H each, $-CH_2O-$); 7.25, 7.29, 7.33 (15H total, phenyl H); 8.52 (S, 1H, pyridine ring H).

Anal. Calcd. for $C_{31}H_{34}ClNO_4$: C, 71.60; H, 6.54; Cl, 6.83; N, 2.69. Found: C, 71.35; H, 6.51; Cl, 5.22; N, 2.68.

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