Pyridoxine Chemistry. VII.[1a] Some Modifications in the 4-Position of Pyridoxol[1b]

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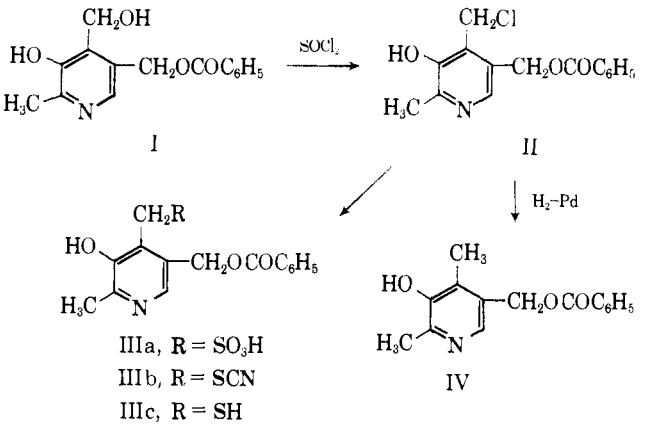
Among many antagonists of vitamin B_6 which have been tested, 4-deoxypyridoxine appears to be one of the most active *in vivo* against sarcoma 180 tumor, as well as in causing lymphoid depression in rats.[2] In spite of its high potency in animals fed with vitamin B_6 deficient diet, 4-deoxypyridoxine is only slightly active under normal dietary conditions. This suggests that the antagonist is readily displaced by the vitamin contained in the diet. Furthermore, the effects of 4-deoxypyridoxine are different from those of dietary depletion alone in the sense that the drug causes marked side effects, such as a central nervous system toxicity, in addition to the desired effect.[2] Thus, it was of interest to explore the possibility of other modifications of the 4-position in pyridoxol in an effect to obtain more selective and more firmly bound antimetabolites than 4-deoxypyridoxine.

Recently[3] the synthesis of α^5-O-benzoylpyridoxol[4] (I) has been reported from this laboratory. Interaction of this derivative with thionyl chloride gave 4-(chloromethyl)-5-hydroxy-6-methyl-3-pyridinemethanol benzoate hydrochloride (II). While this work was in progress, Schmidt and Giesselmann[5] reported the preparation of this intermediate.

Hydrogenolysis of the 4-chloromethyl intermediate II in the presence of palladium-on-charcoal catalyst gave a quantitative yield of 4-deoxypyridoxine 5-benzoate (IV). Alkaline hydrolysis of the benzoate yielded 4-deoxypyridoxine. Although other methods for the preparation of 4-deoxypyridoxine have been described,[6] this method is more general and yields a product of greater purity as shown by bioassay with *Saccharomyces carlsbergensis* ATCC 9080.[7]



The chlorine atom in II proved to be a convenient leaving group for several substitution reactions. Thus the chlorine was replaced by sulfonate to yield IIIa, by thiocyano (IIIb) and by sulfhydryl group (IIIc) under mild conditions.

The compounds have been evaluated as potential inhibitors of *S. carlsbergensis*,[7] and also their effect on the depression of peripheral lymphocytes in rats has been studied.[8] 4-Deoxypyridoxine 5-O-benzoate was inactive as an inhibitor of *S. carlsbergensis*, but was as active as 4-deoxypyridoxine in lowering the number of peripheral lymphocytes[9] in rats fed a diet deficient in pyridoxine. These results suggest that the benzoate group can be removed by an esterase in the mammalian, but not in the microbial cells. 5-Hydroxy-6-methyl-4-(sulfomethyl)-3-pyridinemethanol benzoate (IIIa) was found to be about one-half as active as 4-deoxypyridoxine in suppressing lymphocyte count in rats fed on vitamin B_6 deficient diet. Other compounds described in this paper were found to be devoid of biological activity in these two systems.

Experimental[10]

α^4,3-O-Isopropylidene-α^5-O-benzoylpyridoxol has been obtained as described previously.[3] The picrate formed yellow needles from ethanol, m.p. 209–210° dec.

Anal. Calcd. for $C_{18}H_{19}NO_4 \cdot C_6H_3N_3O_7$: C, 53.14; H, 4.10; N, 10.33. Found: C, 53.27; H, 4.23; N, 10.33.

The hydrochloride formed needles, m.p. 177–178° dec.

Anal. Calcd. for $C_{18}H_{18}NO_4 \cdot HCl$: C, 61.80; H, 5.76; Cl, 10.14; N, 4.00. Found: C, 61.73; H, 5.96; Cl, 10.40; N, 3.88.

α^5-O-Benzoylpyridoxol (I) has been obtained by the hydrolysis of the isopropylidene group by dilute formic acid as described previously,[3] and formed a picrate which crystallized from ethanol in bright yellow needles, m.p. 200–201° dec.

Anal. Calcd. for $C_{15}H_{15}NO_4 \cdot C_6H_3N_3O_7$: C, 52.10; H, 3.81; N, 11.63. Found: C, 51.85; H, 3.73; N, 11.52.

α^5-O-Benzoylpyridoxol Hydrochloride.—α^4,3-O-Isopropylidene-α^5-O-benzoylpyridoxol (10 g.) was dissolved in 50 ml. of *N* HCl and the solution was heated over a steam bath for 1 hr. The excess of the solvent was evaporated *in vacuo* and crystallization of the residue from ethanol–ether gave the hydrochloride (7.96 g., 95%) as colorless needles, m.p. 180–181° dec.; ν_{max}^{Nujol} 1720 (C = O), 3350 (alcoholic OH), 2650 s (bonded N⁺–H), 715 cm.$^{-1}$ (benzoate CH).

Anal. Calcd. for $C_{15}H_{15}NO_4 \cdot HCl$: C, 54.88; H, 4.61; Cl, 21.63; N, 4.27. Found: C, 54.78; H, 4.79; Cl, 21.38; N, 4.19.

4-(Chloromethyl)-5-hydroxy-6-methyl-3-pyridinemethanol Benzoate (II) Hydrochloride.—Thionyl chloride (50 ml.) and benzoate I were refluxed for 4 hr. under nitrogen. Excess thionyl chloride was evaporated *in vacuo* at room temperature. Absolute ethanol (100 ml., cooled at −6°) was added, stirred for 15 min., and kept at −6° for 4 hr. Filtration and washing with petroleum ether yielded 6.1 g. of crude product, which was recrystallized from methanol–ether: m.p. 178–179° dec., lit.[5] m.p.

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190.5° dec.; ν_{max}^{Nujol} 2350, 2400 (bonded N⁺–H or O–H), 1700 (C=O), 765 (C–Cl), 712 cm.⁻¹ (benzoyl CH).

Anal. Calcd. for $C_{15}H_{14}ClNO_3 \cdot HCl$: C, 54.88; H, 4.61; Cl, 21.63; N, 4.27. Found: C, 54.78; H, 4.79; Cl, 21.38; N, 4.19.

Attempted Preparation of Free Base of II.—II (1.1 g.) was added to 20% aqueous sodium bicarbonate solution, the suspension was stirred for 2 hr., and the solid was filtered, washed with water, and dried. The material (0.5 g.) was insoluble in most organic solvents, NaOH, or HCl. The melting point of the compound was above 350°. A sample was dried at 100° (0.5 mm.) over P_2O_5 and was analyzed without further purification (Found: C, 61.75; H, 5.17; N, 4.66.). Its n.m.r. spectrum in dimethyl sulfoxide-d_6 gave indistinct peaks indicating a polymer. An analogous polymerization has been observed for 2-methyl-3-hydroxy-4,5-dibromomethylpyridine hydrobromide.[6]

4-Deoxy-5-benzoyloxypyridoxine Hydrochloride (IV).—II (1.1 g.) in 20 ml. of methanol was hydrogenated with H_2 at 2.81 kg./cm.² (40 p.s.i.) for 6 hr. in the presence of 5% palladium on charcoal. Filtration, evaporation under reduced pressure, and crystallization from ethanol–water gave 4-deoxy-5-benzoyloxypyridoxine hydrochloride (0.84 g., 85%) as colorless needles, m.p. 225–226° dec.

Anal. Calcd. for $C_{15}H_{15}NO_3 \cdot HCl$: C, 61.33; H, 5.49; Cl, 12.07; N, 4.77. Found: C, 61.03; H, 5.66; Cl, 12.20; N, 4.61.

The free base precipitated from aqueous solution on addition of sodium carbonate. It was crystallized from aqueous ethanol in needles, m.p. 140°.

Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.05; H, 6.13; N, 5.44.

The picrate formed yellow needles from ethanol, m.p. 210–211° dec.

Anal. Calcd. for $C_{15}H_{15}NO_3 \cdot C_6H_3N_3O_7$: C, 52.10; H, 3.81; N, 11.63. Found: C, 52.10; H, 3.73; N, 11.52.

4-Deoxypyridoxine.—4-Deoxy-5-benzoyloxypyridoxine (IV, 2.57 g.) was refluxed for 2.5 hr. with 2 *N* aqueous KOH. The solution was neutralized, 4-deoxypyridoxine was filtered off and converted into its hydrochloride by the ethanolic HCl. The material (1.42 g., 95%) was recrystallized from ethanol–ether and melted at 235°, which was not depressed by admixture of an authentic sample.

Anal. Calcd. for $C_8H_{11}NO_2 \cdot HCl$: Cl, 50.66; H, 6.38: Cl, 18.69; N, 7.39. Found: C, 50.40; H, 6.43; Cl, 18.94; N, 7.55.

5-Hydroxy-6-methyl-4-(sulfomethyl)-3-pyridinemethanol Benzoate (IIIa, R = SO_3H).—To 1.09 g. of II in ethanol (15 ml.) a solution of sodium bisulfite (0.71 g.) in water (5 ml.) was added, and the mixture was stirred at room temperature for 20 hr. The resulting solid was collected; from the mother liquor another crop was obtained on acidification and concentration. Recrystallization from a large volume of aqueous ethanol (charcoal) yielded the sulfonic acid (0.56 g., 50%) in needles, which did not have a melting point; ν_{max}^{Nujol} 1315, 1160 (—SO_3H), 1710 (C=O), 720 cm.⁻¹ (benzoyl CH).

Anal. Calcd. for $C_{15}H_{15}NO_6S$: C, 53.41; H, 4.48; N, 4.13; S, 9.48. Found: C, 53.11; H, 4.75; N, 3.92; S, 9.41.

5-Hydroxy-6-methyl-4-(thiocyanomethyl)-3-pyridinemethanol Benzoate (IIIb, R = SCN).—To a solution of II in anhydrous ethanol (15 ml.) potassium thiocyanate (0.68 g.) was added and refluxed for 30 min. The contents were cooled to 0° and KCl was removed by filtration. The filtrate, after clarification with charcoal, was evaporated and recrystallized from aqueous methanol to yield 0.57 g. of needles, m.p. 184° dec.; ν_{max}^{Nujol} 1428, 1316 (—SCN), 1710 (C=O), 710 cm.⁻¹ (benzoyl CH).

Anal. Calcd. for $C_{16}H_{14}N_2SO_3$: C, 61.14; H, 4.49; S, 10.18. Found, C, 60.88; H, 4.38; S, 10.15.

5-Hydroxy-4-(mercaptomethyl)-6-methyl-3-pyridinemethanol Benzoate Hydrochloride (IIIc, R = SH).—To a stirred solution of 0.82 g. of II in 10 ml. of ethanol was added a solution of 0.5 g. of sodium sulfhydrate in 2.0 ml. of water over a period of 5 min. The mixture was stirred at room temperature for 8 hr. Excess solvent was evaporated and the residue was dissolved in absolute ethanol and passed through a column of Dowex 50 in the H^+ form in order to remove Na ions. Evaporation of the solvent followed by the crystallization from aqueous ethanol afforded 0.36 g. (50% based on the amount of II not recovered) in prisms, m.p. 117–119° dec.; ν_{max}^{Nujol} 2270 (–SH), 1705 (C=O), 710 cm.⁻¹ (benzoyl CH).

Anal. Calcd. for $C_{15}H_{15}NSO_3$: C, 62.28; H, 5.23; N, 4.84; S, 11.08. Found: C, 62.09; H, 5.03; N, 4.86; S, 11.35.

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Substitution in the Hydantoin Ring. I. N-3-Aminomethyl Derivatives

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A series of N-3-aryl (and alkyl) aminomethyl hydantoins have been prepared (Tables I–III) from 5,5-disubstituted hydantoins and spirohydantoins by condensation with formaldehyde and the appropriate amine.[1-3] The hydantoins used in this study were prepared from the corresponding ketones by a modification of the Bucherer–Berg reaction as described by Goodson and co-workers.[4]

In a basic solution the aminomethyl group is cleaved from the N-3 position, and the parent hydantoin is regenerated. Thus in one experiment N-3-anilinomethyl-5-ethyl-5-phenylhydantoin was converted quantitatively into 5-ethyl-5-phenylhydantoin upon standing in an alkaline solution at room temperature.

In addition to their preparation by the general procedure as described in the Experimental section, N-3-morpholinomethyl-5,5-dimethylhydantoin and N-3-anilinomethyl-5,5-dimethylhydantoin were also prepared by the reaction of hydroxymethyl-5,5-dimethylhydantoin with morpholine and aniline, respectively.

N-1,N-3-Bis(morpholinomethyl)-5,5-dimethylhydantoin was prepared by the general procedure and by allowing hydroxymethyl-5,5-dimethylhydantoin to react with formaldehyde and 2 equiv. of morpholine. Attempts at preparing N-1,N-3-bis(anilinomethyl)-5,5-dimethylhydantoin from either hydroxymethyl-5,5-dimethylhydantoin, formaldehyde, and 2 equiv. of aniline, or from 5,5-dimethylhydantoin and 2 equiv. each of formaldehyde and aniline resulted only in the formation of N-3-anilinomethyl-5,5-dimethylhydantoin.

N,N′-Bis(5,5-disubstituted 3-hydantoinylmethyl)-piperazine derivatives of 5,5-dimethylhydantoin and 5,5-diphenylhydantoin have been prepared by permitting 2 equiv. each of the hydantoin and formaldehyde to react with 1 equiv. of piperazine.

Infrared spectrograms of a number of the compounds reported here appear in the Sadtler Standard Spectra Catalog, No. 21157–21197, Sadtler Research Laboratories, Philadelphia, Pa.

Pharmacology.—Various chemotherapeutic and pharmacologic tests on representative members of this group of hydantoins were conducted by Merck Sharp and Dohme Research Laboratories, Division of Merck

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