(b) **From DL-, L-, D-, or *meso*-1,2:3,4-Diepoxybutane.**—To a stirred and cooled mixture of *t*-butyl alcohol (100 ml.) and diethyl ether (100 ml.), methanesulfonic acid (technical grade, 90%, 80 ml.) was added. A solution of the corresponding 1,2:3,4-diepoxybutane[11] (32 ml.) in diethyl ether (100 ml.) was then added dropwise over 2.5 hr. at 15–20°. After 33% of the diepoxybutane had been added the bismethanesulfonate began to crystallize from the reaction mixture. After the addition was completed, stirring was continued for 5 hr. followed by standing in a refrigerator for 16 hr. The crude product was collected by filtration, and washed with a 1:2 mixture of *t*-butyl alcohol–diethyl ether. After several recrystallizations from ethanol, the yield of pure compound was approximately 30% based on diepoxybutane. A higher yield was obtained of the *meso* isomer. The physical properties were identical with those of the corresponding compound obtained by one of the other routes and are listed in Table I.

(c) **From 2,3-*O*-Isopropylidene-DL-, L-, or D-Threitol 1,4-Bismethanesulfonate or 2,3-*O*-Isopropylideneerythritol 1,4-Bismethanesulfonate.**—The corresponding 2,3-*O*-isopropylidene compound (100 g.) was refluxed in 96% ethanol (400 ml.) for 10 hr., after addition of methanesulfonic acid (0.5 ml.). The desired bismethanesulfonate crystallized on cooling and was filtered and washed with ethanol and diethyl ether. After recrystallization from ethanol the yield exceeded 90%, and the physical properties were identical with those of the corresponding compound obtained by one of the other methods; see Table I.

L-1,2:3,4-Diepoxybutane.—A suspension of L-threitol 1,4-bismethanesulfonate (55.8 g.) in diethyl ether (150 ml.) was treated with KOH (25 g.) in water (25 ml.) as described[11] for L-2,3-dibromo-1,4-butanediol resulting in L-1,2:3,4-diepoxybutane (12.8 g.) proved to be identical with authentic material.[11]

General Methods for the Preparation of Cyclic Acetals and Ketals of D-, L-, DL-Threitol- and Erythritol 1,4-Bismethanesulfonates. A.—A mixture of the corresponding bismethanesulfonate (10 g.), concentrated sulfuric acid (20 ml.), and the aldehyde (20% excess: formaldehyde as $(CH_2O)_x$ with 100–200% excess) was heated to 60–70° while stirring for 15–30 min. After cooling, the reaction mixture was poured into ice–water, and the reaction product isolated by filtration or extraction with chloroform or ethyl acetate. The product was then dried and the extraction solvent evaporated. For solvents of recrystallization and physical properties see Table II.

B.—The corresponding bismethanesulfonate (10 g.) was refluxed in excess diethyl acetal for 6–20 hr. after methanesulfonic acid had been added (5 drops). If acetals with a higher boiling point were used, then dilution with chloroform or ethyl acetate (100–200 ml.) was necessary. As for the *meso* isomers, an occasional removal of a solvent–ethanol mixture by distillation was advantageous. After cooling, the reaction product was crystallized by addition of diethyl ether. For solvents of recrystallization and physical properties see Table II.

C.—A mixture of L-threitol 1,4-bismethanesulfonate (12 g.), concentrated sulfuric acid (50 ml.), and α-ethoxy-β-trifluoroethanol (7 g.) was stirred at 70° for 1 hr. and the reaction mixture treated as described in A. The resulting oil was crystallized by treatment with diethyl ether. For the solvent of recrystallization and physical properties see Table II.

D.—To a suspension of the corresponding bismethanesulfonate (10 g.) in a solution of the aldehyde or ketone (50–100% excess) in chloroform or benzene (100 ml.) was added a few drops of methanesulfonic acid, and the mixture was refluxed under a continuous water separator for 3–20 hr. After cooling and dilution with diethyl ether the crude product was isolated by filtration (see Table II).

E.—To a suspension of the corresponding bismethanesulfonate (10 g.) in benzene (100 ml.) was added a few drops of methanesulfonic acid, and the mixture refluxed for 2 hr. while a solution of phenylacetaldehyde dimethylacetal in benzene (200 ml.) was added dropwise. During the reaction a methanol–benzene mixture was removed by distillation resulting in a clear solution (60 ml.). After cooling and dilution with diethyl ether the crude product was isolated by filtration (see Table II).

Vitamin B_6 Analogs. II.[1,2] Synthesis of 4,6-Dimethyl-5-mercapto-3-pyridinemethanol and of 5-Mercapto-6-methyl-3,4-pyridinedimethanol Hydrochlorides

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The 3-mercapto analogs of pyridoxine and 4-desoxypyridoxine have been prepared by the reaction of the appropriate diazonium salt with potassium ethyl xanthate followed by reduction of the xanthate ester with lithium aluminum hydride. These compounds and the requisite intermediates that have been evaluated showed no anti-B_6 activity or antitumor activity. "3-Thiopyridoxine" was capable of replacing B_6 for growth probably by conversion to pyridoxine.

The rationale underlying the synthesis of potential vitamin B_6 antagonists for evaluation as antitumor agents has been discussed.[1] Briefly, the striking inhibition of 4-desoxypyridoxine, alone or in combination with certain other compounds, on the growth of Sarcoma 180 (in Swiss mice maintained on a B_6-deficient diet[3]) encouraged us to search for better B_6 antagonists that might be effective antitumor agents on a complete diet.

One of our early candidates for a possible antagonist of vitamin B_6 was the compound "3-thio-4-deoxypyridoxine" (5-mercapto-4,6-dimethyl-3-pyridinemethanol, VIIa) in which the phenolic group of 4-deoxypyridoxine has been replaced by a mercapto group. The reaction of acetylacetone with 2-cyanoacetamide is known to give 4,6-dimethyl-2-hydroxynicotinonitrile (I)[4] and this served as a starting point for the total synthesis of the target compound. Basic hydrolysis of the nitrile I gave 4,6-dimethyl-2-hydroxynicotinic acid (IIa) which was easily esterified by a modified Fisher procedure to give ethyl 2-hydroxy-4,6-dimethylnicotinate (IIIa). Nitrations of the acid IIa and of the ester

(1) For paper I of this series see J. L. Greene, Jr., and J. A. Montgomery, *J. Med. Chem.*, **6**, 294 (1963).

(2) This work was supported by funds from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740.

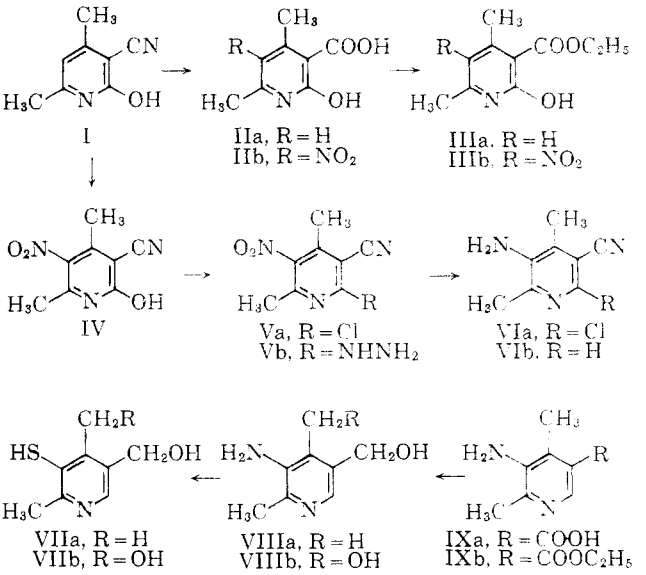
(3) See footnote 3, ref. 1, and H. E. Skipper, J. R. Thomson, and F. M. Schabel, Jr., *Cancer Chemotherapy Rept.*, **29**, 63 (1963).

(4) J. Moir, *J. Chem. Soc.*, **81**, 105 (1902).

IIIa with fuming nitric acid in sulfuric acid gave 4,6-dimethyl-2-hydroxy-5-nitronicotinic acid (IIb) and ethyl 2-hydroxy-4,6-dimethyl-5-nitronicotinate (IIIb), respectively. The prolonged treatment of either IIb or IIIb with the standard chlorinating reagents (phosphorus pentachloride in phosphorus oxychloride) for such compounds failed to replace the 2-hydroxy group of either compound with a chloro group. Nitration of the nitrile I with fuming nitric acid in sulfuric acid gave good yields of 2-hydroxy-4,6-dimethyl-5-nitronicotinonitrile (IV). This hydroxy compound (IV) was readily converted to 2-chloro-4,6-dimethyl-5-nitronicotinonitrile (Va) employing phosphorus pentachloride in phosphorus oxychloride. The 2-chloro compound (Va) reacted readily with hydrazine to give 2-hydrazino-4,6-dimethyl-5-nitronicotinonitrile (Vb). Reduction of Va with stannous chloride in hydrochloric acid as described by Perez-Medina, *et al.*,[5] gave a good yield of 5-amino-2-chloro-4,6-dimethylnicotinonitrile (VIa). We found that refluxing compound VIa in a water suspension of zinc dust for exactly the correct length of time[6] was the most effective way to eliminate chlorine, giving 5-amino-4,6-dimethylnicotinonitrile (VIb).[7] Barium hydroxide hydrolysis of the aminonitrile VIb gave high yields of 5-amino-4,6-dimethylnicotinic acid (IXa) dihydrate. Esterification of the amino acid (IXa) hydrochloride with ethanolic hydrogen chloride produced ethyl 5-amino-4,6-dimethylnicotinate (IXb) in moderate yield. Lithium aluminum hydride reduction of the ester led to very high yields of 5-amino-4,6-dimethyl-3-pyridinemethanol (VIIIa). Diazotization of VIIIa followed by reaction of the diazonium salt with potassium ethyl xanthate and subsequent reduction of the xanthate ester with lithium aluminum hydride[8] resulted in a low yield of 4,6-dimethyl-5-mercapto-3-pyridinemethanol (VIIa) isolated as the hydrochloride.

Diazotization of 3-amino-2-methyl-4,5-pyridinedimethanol (VIIIb), prepared as described by Van der Wal, *et al.*,[9] and by Jones and Kornfeld,[10] followed by reaction of the diazonium salt with potassium ethyl xanthate and reduction of the xanthate ester with lithium aluminum hydride, gave satisfactory yields of "3-thiopyridoxine" (5-mercapto-6-methyl-3,4-pyridinedimethanol, VIIb) isolated as the hydrochloride. This compound was reported by Kreisky[11] but no physical properties were given and the preparation scheme was somewhat vague.

Biological Results.—"3-Thio-4-deoxypyridoxine" (VIIa) and "3-thiopyridoxine" (VIIb) as well as most of the intermediates (I–VI, IXa) leading to these compounds have been evaluated in the three tumor systems of CCNSC (S180, Ca755, and L1210).[12] None of these compounds showed significant activity against these tumors.



The same compounds were evaluated also for their ability to either antagonize or replace the B_6 vitamins in cultures of *Saccharomyces carlsbergensis* by a procedure already described.[13] No B_6 antagonism was observed but "3-thiopyridoxine" (VIIb) did exhibit the ability to replace B_6 for the growth of this bacterium[14]; it showed about one-third the activity of B_6. It could also replace B_6 for the growth of *Neurospora silophila*, showing about one-half the activity of B_6 in this test system. This ability to serve as a source of the B_6 vitamins probably results from the conversion, in these systems, of the mercapto group to hydroxyl thus converting VIIb to pyridoxine itself.

Experimental

All melting points were determined in an open capillary and are corrected.

4,6-Dimethyl-2-hydroxynicotinonitrile (I).—Acetylacetone and 2-cyanoacetamide were condensed under conditions previously described[15] to give I[4] in essentially quantitative yield: m.p. 293–294°.

4,6-Dimethyl-2-hydroxynicotinic Acid (IIa).—A solution of 4,6-dimethyl-2-hydroxynicotinonitrile (62 g.) in aqueous potassium hydroxide (531 g. of 85% reagent KOH in 375 ml. water) was refluxed with stirring for 20 hr. The hot solution was poured into crushed ice (2 l.) and neutralized with concentrated hydrochloric acid (*ca.* 800 ml.). The white solid that precipitated was collected on a filter and thoroughly washed with water before being dried at 80°; it was then recrystallized from glacial acetic acid; yield, 60 g. (85%); m.p. 260–262° dec.

For analysis a sample was recrystallized again from acetic acid, washed with ethanol, and dried at 120°.

Anal. Calcd. for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.52; H, 5.49; N, 8.51.

4,6-Dimethyl-2-hydroxy-5-nitronicotinic Acid (IIb).—The acid IIa was nitrated in a manner quite similar to that described for the ester IIIb to give from 30 g. of starting material 28 g. (75%) of product, which was recrystallized from glacial acetic acid; m.p. 234–235° dec.

Anal. Calcd. for $C_8H_8N_2O_5$: C, 45.28; H, 3.80; N, 13.21. Found: C, 45.11; H, 3.66; N, 13.03.

Ethyl 4,6-Dimethyl-2-hydroxynicotinate (IIIa).—Concentrated sulfuric acid (65 g.) was added to a mixture of 4,6-dimethyl-2-hydroxynicotinic acid (54 g.), sodium chloride (38 g.), absolute ethanol (92 g.), and benzene (400 ml.). This mixture was heated

(5) L. A. Perez-Medina, R. P. Mariella, and S. M. McElvain, *J. Am. Chem. Soc.*, **69**, 2574 (1947).

(6) If reflux of the mixture was continued beyond the time necessary for the reductive elimination of chlorine, reduction of the cyano group to an aminomethyl group commenced.

(7) Another synthesis of this compound has been described by the authors, see ref. 1.

(8) E. Campaigne and S. W. Osborn, *J. Org. Chem.*, **22**, 561 (1957).

(9) B. Van der Wal, T. J. DeBoer, and H. O. Huisman, *Rec. Trav. Chim.*, **80**, 228 (1961).

(10) R. G. Jones and E. C. Kornfeld, *J. Am. Chem. Soc.*, **73**, 107 (1951).

(11) S. Kreisky, *Monatsh.*, **89**, 685 (1958).

(12) *Cancer Chemotherapy Rept.*, **1**, 42 (1959).

(13) E. E. Snell and J. C. Rabinowitz, *Anal. Chem.*, **19**, 277 (1947).

(14) The isomeric "4-pyridoxthiol" was found to be inactive in this system; see U. Schmidt and G. Giesselman, *Angew. Chem.*, **72**, 709 (1960).

(15) S. A. Harris, E. T. Stiller, and K. Folkers, *J. Am. Chem. Soc.*, **61**, 1242 (1939).

at vigorous reflux until no more water collected in the attached water trap (*ca.* 48 hr.). The reaction mixture was diluted with water (800 ml.), made basic with concentrated ammonium hydroxide, and extracted twice with chloroform (400 ml. and 250 ml.). The combined chloroform extracts were dried over Drierite and then evaporated to dryness *in vacuo* to give a white, crystalline residue, which was recrystallized from water (400 ml.). The product was collected on a filter and dried at 80°; yield 51 g. (81%); m.p. 134–135°.

Anal. Calcd. for $C_{10}H_{13}NO_3$: C, 61.52; H, 6.71; N, 7.18. Found: C, 61.42; H, 6.72; N, 7.12.

Ethyl 4,6-Dimethyl-2-hydroxy-5-nitronicotinate (IIIb).—A solution of ethyl 4,6-dimethyl-2-hydroxynicotinate (10 g.) in concentrated sulfuric acid (19 ml.), chilled to 2°, was added all at once to a solution, also at 2°, of fuming nitric acid (16 ml.; *d* 1.5) in concentrated sulfuric acid (38 ml.). The mixture was stirred while the temperature spontaneously rose to 40°. When the temperature of the mixture dropped back to 30°, it was poured with vigorous stirring into crushed ice (300 ml.). The resulting precipitate was collected on the filter, washed with water, and then recrystallized from boiling water (1 l.) to give 9.1 g. (73%) of product; m.p. 194–195°. A sample for analysis was recrystallized from ethanol.

Anal. Calcd. for $C_{10}H_{12}N_2O$: C, 50.00; H, 5.04; N, 11.66. Found: C, 49.96; H, 4.96; N, 11.65.

4,6-Dimethyl-2-hydroxy-5-nitronicotinonitrile (IV).—A solution of 4,6-dimethyl-2-hydroxynicotinonitrile (25 g.) in concentrated sulfuric acid (40 ml.) was added dropwise with external cooling and stirring to a solution of fuming nitric acid (15 ml.; *d* 1.6) at such a rate that the reaction temperature could be maintained at 45 ± 3°. The heat of reaction was allowed to dissipate without cooling, and then the mixture was cooled to 15° and poured with vigorous stirring into crushed ice (500 ml.). The yellow precipitate that formed was collected on a filter, washed thoroughly with water, and then recrystallized from glacial acetic acid (250 ml.) plus water (150 ml.); yield, 24 g. (73%); m.p. 271–272° dec.

Anal. Calcd. for $C_8H_7N_3O_3$: C, 49.94; H, 3.65; N, 21.65. Found: C, 50.07; H, 3.78; N, 21.38.

2-Chloro-4,6-dimethyl-5-nitronicotinonitrile (Va).—A mixture of 4,6-dimethyl-2-hydroxy-5-nitronicotinonitrile (67 g.), phosphorus pentachloride (110 g.), and phosphorus oxychloride (180 ml.) was heated at gentle reflux for 18 hr. The phosphorus oxychloride was removed *in vacuo* leaving a thick, oily residue which was chilled and treated with cold 50% aqueous ethanol (500 ml.). After the reaction had subsided, the mixture was heated briefly on a steam bath and then chilled. The crude solid product thus produced was collected on a filter and washed with water; it was then recrystallized, with charcoal treatment, from methanol (500 ml.) containing a small amount of water. The product crystallized in white, hexagonal plates; yield, 40.5 g. (54%); m.p. 110–112°.

Anal. Calcd. for $C_8H_6ClN_3O_2$: C, 45.40; H, 2.96; Cl, 16.78. Found: C, 45.41; H, 3.22; Cl, 17.06.

4,6-Dimethyl-2-hydrazino-5-nitronicotinonitrile (Vb).—To a solution of 2-chloro-4,6-dimethyl-5-nitronicotinonitrile (6.36 g.) in boiling ethanol (25 ml.) was added, in small increments, a solution of 85% hydrazine hydrate (2.25 g.) and sodium acetate (2.1 g.) in ethanol (30 ml.). The mixture was boiled for 10 min. and then allowed to stand overnight at room temperature. The crystallized product was collected on a filter, washed first with ethanol, and then with water; yield, 4.5 g. (73%); m.p. 285–286° dec. A small sample for analysis was recrystallized from a large volume of ethanol.

Anal. Calcd. for $C_8H_9N_5O_2$: C, 46.37; H, 4.38; N, 33.80. Found: C, 46.24; H, 4.43; N, 33.49.

5-Amino-2-chloro-4,6-dimethylnicotinonitrile (VIa).—To a suspension of 2-chloro-4,6-dimethyl-5-nitronicotinonitrile (3.5 g.) in diethyl ether (15 ml.), was added slowly with stirring a clear solution of stannous chloride (50 g.) in concentrated hydrochloric acid (50 ml.). During the course of the reaction the temperature reached 50° and most of the ether was driven off; ice–water (60 ml.) was added and the mixture chilled in an ice bath for 30 min. before the precipitated crystals were collected and washed first with cold, 5% hydrochloric acid and finally with water. The product was dried at 80°; yield, 2.6 g. (86%); m.p. 143–145°. A sample for analysis was recrystallized from aqueous methanol; m.p. 145–146°.

Anal. Calcd. for $C_8H_8ClN_3$: C, 52.90; H, 4.44; N, 23.15. Found: C, 52.94; H, 4.40; N, 22.98.

5-Amino-4,6-dimethylnicotinonitrile (VIb).—Zinc dust (100 g.) was added with stirring to a solution of 5-amino-2-chloro-4,6-dimethylnicotinonitrile (50 g.) in boiling water (3500 ml.). At the end of 20 hr. more zinc dust (50 g.) was added. Every few hr. during the reaction period a small aliquot was removed from the reaction mixture, filtered hot, and the filtrate allowed to cool. The melting point of the product obtained from each aliquot was used to follow the reaction, which was considered to be complete when the melting point of the aliquot product reached 186–191° (57 hr.). The reaction mixture was filtered and the crude product allowed to crystallize from the chilled filtrate. It was recrystallized from water; yield, 26 g. (64%), m.p. 194–196°.

Anal. Calcd. for $C_8H_9N_3$: C, 65.25; H, 6.16; N, 28.55. Found: C, 65.06; H, 6.44; N, 28.83.

4,6-Dimethyl-5-mercapto-3-pyridinemethanol (VIIa).—A solution of sodium nitrite (1.25 g.) in water (4.0 ml.) was added dropwise to 5-amino-4,6-dimethyl-3-pyridinemethanol (2.6 g.) in 5 *N* hydrochloric acid (8.5 ml.) at 0°. Upon completion of addition of the nitrite solution, the diazonium salt solution was maintained at 0° and added dropwise over 0.5 hr. to a solution of potassium ethyl xanthate (3.2 g.) in water (5 ml.) at 42 ± 2°. The reaction mixture was stirred for an additional 0.5 hr. and then extracted with three 30-ml. portions of ether. The ether extract was dried and then evaporated to give a yellow, oily residue that crystallized. To a solution of these crystals in ether (40 ml.), chilled to 0°, was added with stirring a solution of lithium aluminum hydride (1 g.) in ether (20 ml.). The addition was made in 1-ml. increments over 0.5 hr. The mixture was stirred for an additional hour before the excess hydride was destroyed by the addition of a solution of methanol (5 ml.) in ether (20 ml.) followed by water (4 ml.). The solids were collected on a filter and extracted with three 50-ml. portions of boiling methanol. The combined ether and methanol filtrates were saturated with carbon dioxide before being evaporated to dryness. The residue was taken up in boiling ethanol (100 ml.) and filtered hot. The clear yellow filtrate was saturated with hydrogen chloride, decolorized with carbon, and again evaporated to dryness. The crystalline residue was taken up in absolute ethanol (50 ml.) and reprecipitated by the addition of ether (150 ml.). The solution in ethanol followed by precipitation with ether was repeated once more to give 1.1 g. (32%) of product, m.p. 201–203° dec.

Anal. Calcd. for $C_8H_{11}NOS \cdot HCl$: C, 46.72; H, 5.88; N, 6.81. Found: C, 46.48; H, 5.71; N, 6.58.

5-Mercapto-6-methyl-3,4-pyridinedimethanol (VIIb).[8]—This compound was prepared from 5-amino-6-methyl-3,4-pyridinedimethanol (7.2 g.) in a manner exactly analogous to that described above for the preparation of VIIa; yield, 4.3 g. (44%); m.p. 189–191° dec.

Anal. Calcd. for $C_8H_{11}NO_2S \cdot HCl$: C, 43.34; H, 5.46; Cl, 15.99; N, 6.32; S, 14.46. Found: C, 43.44; H, 5.58; Cl, 16.0; N, 6.34; S, 14.7.

5-Amino-4,6-dimethyl-3-pyridinemethanol (VIIIa).—A solution of lithium aluminum hydride (1.30 g.) in anhydrous ether (25 ml.) was added in 1-ml. increments over 0.5 hr. to a solution of ethyl 5-amino-4,6-dimethylnicotinate (4 g.) in ether (40 ml.) at 0°. This reaction mixture was stirred for 0.5 hr. after completion of the addition. Methanol (5 ml.) in ether (15 ml.) followed by water (3 ml.) was added dropwise to the reaction mixture which was then allowed to stir overnight. The solids were collected on a filter and extracted with three 125-ml. portions of boiling methanol. The methanol and ether filtrates were combined and saturated with carbon dioxide before being evaporated to dryness *in vacuo*. The residue was boiled with ethanol (125 ml.) and filtered hot, the filtrate again being evaporated to dryness *in vacuo*. The residue was taken up in hot absolute ethanol (50 ml.), treated with decolorizing carbon, and evaporated to dryness *in vacuo* to leave a clear oil which quickly crystallized; yield 2.93 g. (93%). A sample for analysis was recrystallized from ethyl acetate containing just enough ethanol to effect solution; m.p. 145–147°.

Anal. Calcd. for $C_8H_{12}N_2O$: C, 63.13; H, 7.95; N, 18.41. Found: C, 63.21; H, 8.00; N, 18.40.

5-Amino-4,6-dimethylnicotinic Acid (IXa).—A mixture of barium hydroxide octahydrate (18.4 g.), water (55 ml.), and 5-amino-4,6-dimethylnicotinonitrile (4 g.) was refluxed until no more ammonia was evolved. A solution of concentrated sulfuric acid (5.88 g.) in water (25 ml.) was added slowly to the hot solution and boiling was continued for 15 min. before the mixture was filtered by suction. The clear filtrate was evaporated to dryness

in vacuo to give a white, crystalline residue, which was suspended in boiling ethanol (100 ml.) and water added dropwise to the first appearance of turbidity. Acetone (100 ml.) was added and the solution allowed to stand for 72 hr. in a refrigerator. The fine, white needles that crystallized were collected, washed with acetone, and dried; yield, 3.6 g. (80%), m.p. 267–269° dec.

Anal. Calcd. for $C_8H_{10}N_2O_2 \cdot 2H_2O$: C, 47.51; H, 6.98; N, 13.86. Found: C, 47.33; H, 6.80; N, 13.74.

The **hydrochloride** salt of the amino acid was formed in ethanolic hydrogen chloride and was recrystallized as described for the free acid, m.p. 282–283° dec.

Anal. Calcd. for $C_8H_{10}N_2O_2 \cdot HCl \cdot 0.5H_2O$: C, 45.18; H, 5.77; N, 13.23; Cl, 16.74. Found: C, 45.08; H, 5.94; N, 13.01; Cl, 16.85.

Ethyl 5-Amino-4,6-dimethylnicotinate (IXb).—A solution of 5-amino-4,6-dimethylnicotinic acid hydrochloride (5 g.) and ethanol saturated with hydrogen chloride (50 ml.) was refluxed for 4 hr. and then allowed to stand overnight. The solvent was removed *in vacuo* before the residue was treated with a sodium bicarbonate solution. The copious, white precipitate thus produced was collected on a filter, washed with water, and dried at 80° before being recrystallized from methanol (25 ml.) and water (50 ml.). Fine, white crystals deposited upon standing: yield, 2.4 g. (59%), m.p. 122–124°.

Anal. Calcd. for $C_{10}H_{14}N_2O_2$: C, 61.85; H, 7.26; N, 14.43. Found: C, 61.65; H, 7.05; N, 14.41.

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Vitamin B_6 Analogs. III. Some 5-Aminomethyl and 5-Thiomethyl Derivatives of Pyridoxine and 4-Desoxypyridoxine[1]

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A number of analogs of pyridoxine and 4-desoxypyridoxine in which the hydroxyl of the 5-hydroxymethyl group is replaced by substituted amino and thio groups have been prepared and evaluated for B_6-antagonism and anticancer activity.

5-Bromomethyl-2,4-dimethyl-3-pyridinol (I) hydrobromide, obtained from 4-desoxypyridoxine (DOP)[2] by the procedure of Sakuragi and Kummerow,[3] was treated with 2-mercaptoethanol in ethanol containing a stoichiometric amount of sodium hydroxide. The resultant 5-[(2-hydroxyethylthio)methyl]-2,4-dimethyl-3-pyridinol (IIa), when treated with thionyl chloride in pyridine, gave no identifiable product. Chlorination in excess thionyl chloride, however, gave moderate yields of 5-[(2-chloroethylthio)methyl]-2,4-dimethyl-3-pyridinol (IIIa) hydrochloride.

2-Mercapto-1-propanol, conveniently prepared by the lithium aluminum hydride reduction of thiolactic acid, and *p*-chlorobenzenethiol reacted in similar fashion to yield 5-[(1-hydroxy-2-propylthio)methyl]-2,4-dimethyl-3-pyridinol (IIb) and 5-[(4-chlorophenylthio)-methyl]-2,4-dimethyl-3-pyridinol (IIc). Chlorination of IIb gave only poor yields of 5-[(1-chloro-2-propylthio)methyl]-2,4-dimethyl-3-pyridinol (IIIb) hydrochloride.

Using the general procedure of Kolesnikov and Mikhailovskaya,[4] 5-bromomethyl-2,4-dimethyl-3-pyridinol (I) hydrobromide was treated with a stoichiometric amount of hexamethylenimine in benzene to yield 5-(hexahydro-1*H*-azepin-1-ylmethyl)-2,4-dimethyl-3-pyridinol (IId).

When ethylenimine was used in place of hexamethylenimine under similar conditions, a product was obtained which is thought to be 5-(1-aziridinylmethyl)-2,4-dimethyl-3-pyridinol (IIe). Some decomposition took place upon removal of the solvent, however, and a test for presence of the aziridinyl ring[5] was negative.

In preparing 5-thiomethyl derivatives of pyridoxine (VI) it was first necessary to block the 4-hydroxymethyl group. After several attempts to duplicate Cohen and Hughes' procedure for the preparation of the cyclic ketal (IV)[6] failed, anhydrous hydrogen chloride was substituted for concentrated sulfuric acid and the time of reaction shortened. This modification[7] produced the desired cyclic ketal (IV) in much higher yield and eliminated the somewhat complex work-up that is necessary in the old procedure.

Isopropylidene pyridoxine (IV) hydrochloride was chlorinated in thionyl chloride to yield 5-chloromethyl-2,2,8-trimethyl-4*H*-*m*-dioxino-[4,5-*c*]pyridine (V) hydrochloride,[8,9] which was subsequently condensed with 2-mercaptoethanol in the manner already described to yield 5-[(2-hydroxyethylthio)methyl]-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-*c*]pyridine (VIa). Chlorination of VIa with thionyl chloride gave 5-[(2-chloroethylthio)-methyl]-2,2,8-trimethyl-4*H*-*m*-dioxino[4,5-*c*]pyridine (VIb) hydrochloride. Mild acid hydrolysis of the hydroxy compound (VIa) gave the expected derivative: 5-[(2-hydroxyethylthio)methyl]-3-hydroxy-2-methyl-4-pyridinemethanol (IXa) hydrochloride, but when the

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