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Pyridoxal Hydrazine Derivatives for Cancer Chemotherapy

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A series of pyridoxal and pyridoxal phosphate derivatives have been prepared for cancer chemotherapy evaluation. The dimethylhydrazone and methylhydrazone are relatively toxic and the latter has a borderline tumor growth retardation activity against Sarcoma 180. Catalytic reduction cleaves the hydrazone linkage to form pyridoxamine.

Interest in the tumor growth retardation characteristics of a series of methyl and dimethylhydrazones[1,2] has prompted the study of similar derivatives of pyridoxal. These derivatives were prepared from pyridoxal hydrochloride as such or in solution obtained on manganese dioxide[3] oxidation of pyridoxine. The pyridoxal methylhydrazone could not be isolated from the crude pyridoxine oxidation mixture and had to be made from the isolated pyridoxal hydrochloride. Pyridoxal phosphate was obtained by manganese dioxide oxidation of pyridoxamine phosphate[4] and was converted to derivatives without isolation. Catalytic reduction of pyridoxal dimethylhydrazone in methanol, ethanol, dioxane, or acetic acid over platinum oxide or in ethanol with added hydrochloric acid gives pyridoxamine.

(1) R. H. Wiley and G. Irick, *J. Org. Chem.*, **24**, 1925 (1959).
(2) R. H. Wiley, S. C. Slaymaker, and H. Kraus, *ibid.* **22**, 204 (1957).
(3) E. E. Snell, *J. Am. Chem. Soc.*, **66**, 2082 (1944).
(4) E. A. Peterson and H. A. Sober, *ibid.*, **76**, 169 (1954).

Reduction of pyridoxal dimethylhydrazone diacetate in methanol over palladium on carbon gives an unidentified compound $C_{10}H_{16}N_2O_2$.

Preliminary screening data[5] have shown these results: pyridoxal methylhydrazone −,±(9); T(30,125); and pyridoxal dimethylhydrazone −(2,4); T(8,30,125) in Sarcoma 180 retardation studies and pyridoxal isonicotinoylhydrazone (±) against four organisms in anaerobic bacterial inhibition. The significance of these ratings has been discussed elsewhere.[5] None of the other compounds described herein showed significant activity against Sarcoma 180. The results of other testing will be reported elsewhere.

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Experimental[6]

The manganese dioxide used in the experiments was prepared by addition of saturated aqueous potassium permanganate to a hot (90°) solution of 15.0 g. of manganese sulfate monohydrate in 300 ml. of water until the purple color persisted. The brown precipitate of manganese dioxide was collected and dried at 110°.

Pyridoxal Dimethylhydrazone.—A mixture of 20.5 g. of pyridoxine hydrochloride and 8.64 g. of manganese dioxide in 9.8 g. of concd. sulfuric acid and 50 ml. of water was stirred and heated at 60–70° for 1 hr. The solution was concentrated to 150 ml. by distillation under vacuum and neutralized by addition of 21 g. of potassium carbonate in 50 ml. of water. Addition of 6.9 g. of dimethylhydrazine resulted in the precipitation of the crude product after 10 hr. at room temperature and 2 hr. at 0°. The crude product was recrystallized from methanol–water to give 6.9 g. (32.8%) of the product, m.p. 119–122°.

Anal. Calcd. for $C_{10}H_{15}N_3O_2$: N, 20.08. Found: N, 19.97.

The **hydrochloride** was prepared by passing dry hydrogen chloride into a dry butanol (10 ml.) solution of the dimethylhydrazone (100 mg.) at 0°. Recrystallization of the precipitated product from isopropyl alcohol–petroleum ether (b.p. 60–90°) gave the analytical sample, m.p. 233° dec.

Anal. Calcd. for $C_{10}H_{16}N_3O_2Cl$: N, 17.11; neut. equiv., 245.5. Found: N, 16.94; neut. equiv., 239.

The **diacetate** was prepared by refluxing the dimethylhydrazone (1.05 g.) with 10 ml. of acetic anhydride for 15 min. The volatile fraction was removed by heat-

(5) The authors are indebted to Drs. C. C. Stock, R. K. Barclay and D. A. Clarke, Sloan Kettering Institute, for conducting these tests. The rating scales and procedures for the Sarcoma 180 test are given in *Cancer Research*, Suppl. No. 1, 91 (1953), Suppl. No. 2, 179 (1955), and *Cancer Research*, **18**, No. 8, 49 (1958).

(6) Analyses by Micro-Tech Laboratories. M.ps. are uncorrected. The pyridoxal hydrochloride, pyridoxine hydrochloride, and pyridoxamine dihydrochloride were used as supplied by Nutritional Biochemicals Co.

ing on the steam bath at 30 mm. vacuum. The residue was treated with 15 ml. of ethanol, again evacuated, and the residue taken up in 25 ml. of ether. The ether solution was washed with 5% aqueous bicarbonate solution and with water, dried, and evaporated to give an oil from which crystals of the crude product separated. Recrystallization from ethyl acetate–petroleum ether (b.p. 60–90°) at −20° gave 0.65 g. (44.2%) of the pure product as colorless needles, m.p. 68–70°. In other experiments in which the crude oil from the ethanol treatment was recrystallized directly at −20° yields of 50% of product were obtained.

Anal. Calcd. for $C_{14}H_{19}N_3O_4$: N, 14.33. Found: N, 14.56.

The hydrochloride of the pyridoxal dimethylhydrazone acetate was prepared in butanol solution by the procedure given above. Recrystallization of the crude solid from isopropyl alcohol–petroleum ether gave the pure hydrochloride, m.p. 173–176°.

Anal. Calcd. for $C_{14}H_{20}Cl\,N_3O_4$: N, 12.73. Found: N, 12.84.

Hydrazones prepared from pyridoxal and phenylhydrazine, diethylhydrazine, *p*-nitrophenylhydrazine, and 2,4-dinitrophenylhydrazine were prepared but were not obtained analytically pure. The last was recrystallized from acetic acid and analyzed as a complex containing one mole of acetic acid.

Anal. Calcd. for $C_{14}H_{13}N_5O_6 \cdot CH_3CO_2H$: N, 17.2. Found: N, 17.19.

Pyridoxal Methylhydrazone.—Two grams of pyridoxal hydrochloride (Nutritional Biochemicals Company) was dissolved in 25 ml. of water. To this solution was added 1.2 g. of methylhydrazine. The solution became warm and the crude product precipitated within a few minutes. Water (15 ml.) was added and the solution cooled to 0° to complete the precipitation. The solid was collected and dried to give 1.70 g. (89.2%) of product, m.p. 167–170°. Two recrystallizations from benzene–petroleum ether (b.p. 60–90°) gave the analytically pure product, m.p. 168–170°.

Anal. Calcd. for $C_9H_{13}N_3O_2$: N, 21.53. Found: N, 21.35.

Pyridoxal Semicarbazone.—Addition of a solution of 0.25 g. of semicarbazide hydrochloride and 0.7 g. of sodium acetate in 7 ml. of water to 0.51 g. of pyridoxal hydrochloride in 7 ml. of water gave a precipitate of 0.52 g. (92.8%) of the product, m.p. 230° dec. Recrystallization from ethanol gave the analytical sample.

Anal. Calcd. for $C_9H_{12}N_4O_3$: N, 24.99. Found: N, 24.75.

Pyridoxal Thiosemicarbazone.—Addition of 0.1 g. of thiosemicarbazide in 5 ml. of hot water to 0.2 g. of pyridoxal hydrochloride in 5 ml. of hot water gave a precipitate of 0.15 g. (62.5%) of the crude product on standing. Recrystallization from ethanol gave the analytically pure sample., m.p. 232° dec.

Anal. Calcd. for $C_9H_{12}N_4O_2S$: N, 23.32. Found: N, 23.51.

Pyridoxal Carboxymethyloxime.—A solution of 0.7 g. of aminoxyacetic acid hemihydrochloride in 15 ml. of 75% ethanol, 1.02 g. of pyridoxal hydrochoride in 10 ml. of 95% ethanol, and 0.65 g. of sodium acetate in 3 ml. of water was refluxed 15 min. and cooled to precipitate 0.92 g. of the crude product, m.p. 201–203° dec. Recrystallization from dimethylformamide gave the analytically pure sample, m.p. 208° dec.

Anal. Calcd. for $C_{10}H_{12}N_2O_5$: N, 11.66. Found: N, 11.76.

Pyridoxylideneaminomorpholine.—An aqueous solution containing approxi-

mately 0.113 mole of 4-aminomorpholine prepared as previously described[7] was concentrated to 30 ml. under vacuum, diluted with methanol and filtered to remove sodium chloride. One-half of this solution was added to 1.02 g. of pyridoxal hydrochloride in 15 ml. of water. The solution was refluxed for 15 min. and cooled to precipitate 0.36 g. (29%) of the crude product. Recrystallization from methanol gave the analytically pure sample, m.p. 203° dec.

Anal. Calcd. for $C_{12}H_{17}N_3O_3$: N, 16.72. Found: N, 16.77.

Pyridoxal Methylphenylhydrazone.—This product was prepared from oxidized pyridoxine hydrochloride and methylphenylhydrazine in 28% yield; m.p. 196–198° (from chloroform).[8]

Anal. Calcd. for $C_{15}H_{17}N_3O_2$: C, 66.42; H, 6.32. Found: C, 66.16; H, 6.42.

Pyridoxal Isopropylhydrazone.—This product was prepared from pyridoxal hydrochloride and isopropylhydrazine hydrochloride in 65% yield[8]; m.p. 139–140° (from ethanol–water).

Anal. Calcd. for $C_{11}H_{17}N_3O_2$: C, 59.16; H, 7.68; N, 18.82. Found: C, 59.37; H, 7.69; N, 18.59.

The **hydrochloride**,[9] m.p. 224° dec., crystallized from ethanol–ethyl acetate.

Anal. Calcd. for $C_{11}H_{18}ClN_3O_2$: C, 50.8; H, 6.95; N, 16.4. Found: C, 50.83; H, 7.22; N, 16.20.

The pyridoxal phosphate solution used in the experiments to be described was prepared as follows.[4] Two grams of pyridoxamine dihydrochloride was carefully mixed with 10 ml. of anhydrous phosphoric acid prepared by adding 1 part of phosphorus pentoxide to 1.3 parts of 85% phosphoric acid. The mixture was heated at 50–70° for 2 hr., cooled, poured onto 150 ml. of ice and water, and stirred to give a solution. The pH was adjusted to 6.0 with 30% aqueous sodium hydroxide and 0.8 g. of manganese dioxide was added. After stirring 20 min. at 60° the solution was cooled, filtered, and used in the following preparations.

Pyridoxal Phosphate Isonicotinoylhydrazone.—The aqueous solution of pyridoxal phosphate was warmed to 95° with 0.82 g. of isonicotinoylhydrazine in 20 ml. of water. The precipitate was collected and dried to give 2.2 g. (99%) of the crude product, m.p. 239° dec. Reprecipitation from solution in 3 *N* hydrochloric acid by addition of (a) sodium bicarbonate until the solution was but slightly acidic and (b) excess saturated sodium acetate gave the product, m.p. 255° dec. (sealed tube). The water-insoluble product was thoroughly washed with water. The product gave the analysis of a monosodium salt.

Anal. Calcd. for $C_{14}H_{14}N_4O_6NaP$: N, 14.40. Found: N, 14.02.

Pyridoxal Phosphate Thiosemicarbazone.—This was prepared by the procedure given in the preceding example. The product decomposes over a wide temperature range over 200° with no appearance of melting.

Anal. Calcd. for $C_9H_{13}N_4O_6PS$: N, 17.50. Found: N, 17.32.

Pyridoxal Phosphate Dimethylhydrazone.—The aqueous solution of pyridoxal phosphate was acidified to pH 5 with hydrochloric acid and treated with 0.60 g. of dimethylhydrazine. After heating at 50° and cooling to 0°, 1.05 g. (44%) of the

(7) R. H. Wiley, H. K. White, and G. Irick, *J. Org. Chem.*, **24**, 1784 (1959).

(8) The authors are indebted to R. L. Clevinger for the preparation of this compound.

(9) The authors are indebted to J. S. Ridgway for the preparation of this compound.

yellow, crystalline product separated. The analytical sample was recrystallized from water, m.p. 243° dec. (sealed tube).

Anal. Calcd. for $C_{10}H_{16}N_3O_5P$: C, 41.53; H, 5.58; N, 14.53. Found: C, 41.41; H, 5.71; N, 14.25.

Hydrogenation.—Hydrogenation of 2.1 g. of pyridoxal dimethylhydrazone over 0.1 g. of platinum oxide catalyst in 200 ml. of methanol at 2 atm. hydrogen pressure for 16 hr. gave 1.2 g. of a solid which was recrystallized from water as the dihydrate of pyridoxamine, m.p. 194°.

Anal. Calcd. for $C_8H_{16}N_2O_4$: C, 47.05; H, 7.90; N, 13.72; neut. equiv., 204. Found: C, 47.52; H, 8.01; N, 13.64; neut. equiv., 204.

Hydrogenation under similar conditions but in acetic acid gave 0.4 g. of pyridoxamine, m.p. 178° dec., from 1.2 g. of hydrazone. The infrared spectrum of a recrystallized portion of this sample, m.p. 181–183°, was identical in all respects with that of an authetic sample of pyridoxamine. Hydrogenation in ethanol, dioxane, and ethanol with an equivalent amount of hydrogen chloride all gave pyridoxamine.

In several (but not all) of the hydrogenations in methanol a product, m.p. 239°, separated on evaporation of the filtered reaction mixture. Recrystallization from methanol–ether gave colorless, crystalline, non-basic solid, m.p. 247°; soluble in water, positive ferric chloride test. This apparently is pyridoxamine hydrochloride.

Anal. Calcd. for $C_8H_{13}ClN_2O_2$: C, 46.90; H, 6.36; N, 13.7. Found: C, 46.94; H, 6.50; N, 13.37.

The hydrogenation of pyridoxal diacetate dimethylhydrazone (1.0 g.) in methanol over 5% palladium on carbon catalyst at 2 atm. for 23 hr. gave, on evaporation of the filtered reaction mixture, 0.4 g. of a colorless solid. This was washed with chloroform, dissolved in methanol, and reprecipitated by adding ether and cooling to −20° as colorless crystals, m.p. 235°; picrate, m.p. 163°; positive ferric chloride test. The analytical and characterization data are in agreement with the structure of a desoxypyridoxamine monoacetate: 2,5-dimethyl-3-hydroxy-4-acetamidomethylpyridine.

Anal. Calcd. for $C_{10}H_{14}N_2O_2$: C, 61.8; H, 7.27; N, 14.4; neut. equiv., 194. Found: C, 61.91; H, 7.34; N, 14.33; neut. equiv., 195 (weak).