Pyrido[3,2-*g*]pteridines. 3. 10-Polyhydroxyalkyl Derivatives of 3*H*,10*H*-2,4-Dioxopyrido[3,2-*g*]pteridine (1)

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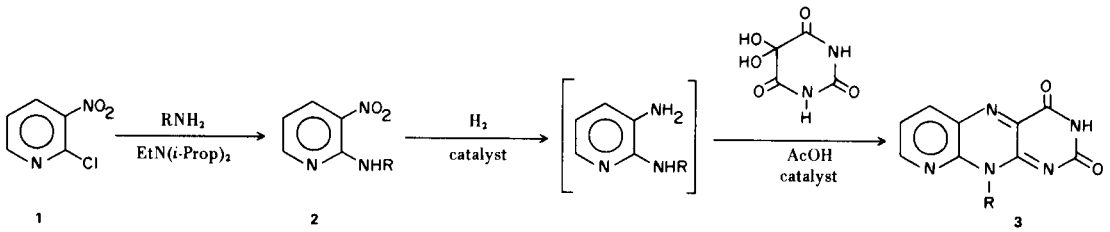
Received September 26, 1972

In the continuing search for new agents of possible value in cancer chemotherapy, we recently turned our attention to the chemistry and biological activity of 10-substituted-3*H*,10*H*-2,4-dioxopyrido[3,2-*g*]pteridines (9-azaisoalloxazines). The tricyclic ring system present in these compounds is structurally related to that found in riboflavin, a vitamin substance essential for normal growth and tissue repair in all animals (2,3). The azaisoalloxazines, however, contain a nitrogen atom in place of the C_9 of riboflavin (4); the location of the additional hetero atom is such that it may be expected to affect the N_1-C_{10a}-C_{4a}-N_5 redox function of the isoalloxazine system and thereby perhaps provide a mechanism for antimetabolic activity for these compounds.

We have already described the synthesis and biological activity of 9-azaisoalloxazines containing, for the most part, terminal hydroxy- or aminoalkyl substituents at the 10 position (5). The impressive growth-inhibitory activity exhibited by many of these agents against serially propagated KB (human epidermoid carcinoma) cells in culture encouraged us to construct 9-azaisoalloxazines more closely related to riboflavin, with sugar-derived side chains at the 10 position. We now wish to report the synthesis of the D-ribityl, L-arabityl, D-galactyl, and D-glucityl derivatives of 3*H*,10*H*-2,4-dioxopyrido[3,2-*g*]pteridine.

The preparation of these compounds is outlined in Scheme I. The synthetic sequence required condensation of 2-chloro-3-nitropyridine (**1**) with glycamines (1-amino-1-deoxyalditols), none of which are commercially available. Accordingly, D-ribose, L-arabinose, D-galactose, and D-glucose were converted to their phenylhydrazones by modification of a procedure originally described by E. Fischer (6). The aldose phenylhydrazones were in turn reduced catalytically to the glycamines according to the method of Wolfrom, Shafizadeh, Wehrmüller, and Armstrong (7), except that Davison sponge nickel catalyst was used in place of Raney nickel. We continue to find that the commercial availability of Davison sponge nickel, its long-term storage under water, and its low pyrophoricity make this material a convenient and worthwhile substitute in many of the applications in which Raney nickel is commonly employed (8,9). The glycamines were

SCHEME I



RNH$_2$ = (a) 1-amino-1-deoxy-D-ribitol; (b) 1-amino-1-deoxy-L-arabinitol; (c) 1-amino-1-deoxy-D-galactitol; (d) 1-amino-1-deoxy-D-glucitol

used directly for reaction with **1** after suitable verification of structure by ir, uv, and nmr, and, with the exception of the ribityl derivative, by melting point.

In our previously described (5) series of azaisoalloxazines, two molar equivalents of the appropriate readily available ω-hydroxy- or aminoalkylamine were heated in ethanol solution with **1** to give the corresponding aminonitropyridine; the purpose of the second equivalent of amine was to serve as the hydrogen chloride acceptor. However, now, to avoid unnecessary waste of glycamine, **1** was treated with a stoichiometric equivalent of the aminodeoxyalditol with ethyl diisopropylamine (10) as the acid acceptor.

The nitro functions of **2a**, **2b**, and **2d** were reduced catalytically in alcohol solution. No attempt was made to isolate or characterize the intermediate diamines. Instead, the alcohol was replaced as quickly as possible by acetic acid and the diamine was caused to react with alloxan monohydrate in the presence of catalyst. Reduction of **2c** was accomplished in 80% acetic acid and, after separation of the catalyst, the reaction solution was used directly for the alloxan condensation. Boric acid, which is generally efficacious in the preparation of alloxazines (11) and azaalloxazines (5,12), served as a suitable catalyst for the formation of the ribityl derivative **3a**. However, boric anhydride appeared to be a better catalyst and led to cleaner products when used in the preparation of **3b**, **3c**, and **3d**.

Boric acid complexes, common with 10-polyhydroxyalkylisoalloxazines (13), were also observed here. These complexes presented a minor inconvenience but no significant difficulty in the purification of the target compounds. The boric acid was removed in each instance as methyl borate by repeated evaporation of a methanolic solution of the crude product until the residue no longer gave a positive reaction for boron in the methyl borate flame test (14).

EXPERIMENTAL (15)

Ir spectra were determined as potassium chloride disks with a Perkin-Elmer Model 137B spectrophotometer; uv spectra were measured with Cary Model 11 and Model 15 spectrophotometers; nmr spectra were obtained by means of a Varian A-60 spectrometer with tetramethylsilane as the internal standard. Melting points were taken by the capillary method on a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) and are uncorrected.

Aldose Phenylhydrazones.

The following is a modification of the procedure described by Fischer (6). The appropriate aldose was dissolved in a small volume of hot water and the aqueous solution was cooled and maintained at 5° while 2 equivalents of phenylhydrazine were added with stirring. The clear slightly yellow reaction mixture was placed in the refrigerator where it solidified within 2-3 days. To remove excess phenylhydrazine, ether was added, the solid yellow mass was ground, and the suspension was stirred vigorously and filtered. The ether extraction process was continued until no yellow color was seen in the ether layer (usually 3 x 800 ml.). The white crystalline aldose phenylhydrazone was washed with ether and dried. Each showed a peak in the uv at λ max (ethanol-water) 277 nm (ε 13,700 ± 10%), in agreement with the observation of O'Donnell and Percival (16). Also, each exhibited in the ir a strong benzene ring aromatic absorption at 6.24 μ and in the nmr (DMSO-d_6 or D_2O solution) a 1 proton doublet (J = 6 Hz) in the region δ 4.66-5.01 ppm for the HC=N and multiple peaks for the 5 proton phenyl group.

D-(-)Ribose phenylhydrazone: 54%; m.p. 126-127° [lit. (17) m.p. 124-127°]; nmr: δ 5.01 and 6.65-7.40 ppm.

L-(-)Arabinose phenylhydrazone: 81%; m.p. 147-148° [lit. (18) m.p. 150-151°]; nmr: δ 4.81 and 6.57-7.35 ppm.

D-(+)Galactose phenylhydrazone: 96%; m.p. 158-159° [lit. (19) m.p. 159-160°]; nmr: δ 4.66 and 6.84-7.49 ppm.

D-(+)Glucose phenylhydrazone: 70%; m.p. 142-143° [lit. (6) m.p. 144-145°]; nmr: δ 4.96 and 6.95-7.55 ppm.

Reduction of Aldose Phenylhydrazones. Preparation of 1-Amino-1-deoxyalditols.

The reduction of the aldose phenylhydrazones in water was accomplished according to a previously described procedure (7), except that Davison sponge nickel (20) was used as the catalyst. After reduction and separation of the catalyst the aqueous solution containing the glycamine was extracted with 4 x 100 ml. portions of 1:1 benzene-ether and concentrated by evaporation on a rotary evaporator or by azeotropic distillation with ethanol to obtain the product. 1-Amino-1-deoxy-D-ribitol was obtained in 64% as a syrup but the other products were solids: 1-amino-1-deoxy-L-arabinitol (96%), m.p. 96-98° [lit. (21) m.p. 98°]; 1-amino-1-deoxy-D-galactitol (75%), m.p. 138-140° [lit. (22) m.p. 143-145°]; 1-amino-1-deoxy-D-glucitol (77%), m.p. 124-126° [lit. (23) m.p. 126-128°]. The products were all transparent in the uv.

2-(D-*Ribo*-2,3,4,5-tetrahydroxypentylamino)-3-nitropyridine (**2a**).

A mixture of 2-chloro-3-nitropyridine (**1**, 2.06 g., 13 mmoles), 1-amino-1-deoxy-D-ribitol (2.0 g., 13 mmoles), and ethyl diisopropylamine (15 ml.) in 50 ml. of a mixed solvent composed of 15 ml. of 1-butanol, 25 ml. of ethanol, and 10 ml. of water was heated at 120-125° for 3 hours with stirring. After evaporation of the reaction mixture to dryness, the yellow residue was treated with 50 ml. of water and the suspension was stirred vigorously and filtered. The solid material was washed with a small volume of water and air dried. Sublimation at 140° (0.005 mm.) gave 1.53 g. (43%) of product, m.p. 158-160°; uv: λ max (ethanol) 267 and 408 nm.

Anal. Calcd. for $C_{10}H_{15}N_3O_6$: C, 43.94; H, 5.54; N, 15.37. Found: C, 43.84; H, 5.54; N, 15.34.

2-(L-*Arabino*-2,3,4,5-tetrahydroxypentylamino)-3-nitropyridine (**2b**).

A mixture of **1** (7.32 g., 46 mmoles), 1-amino-1-deoxy-L-arabinitol (7.0 g., 46 mmoles), and 40 ml. of ethyl diisopropylamine in 200 ml. of 80% ethanol was heated at 90-95° with stirring for 10 hours. The solvent was then evaporated and the residue was treated with 100 ml. of water as in the previous procedure to obtain the crude product. Crystallization from ethanol (charcoal) gave bright yellow needles (10.0 g., 79%), m.p. 163-164°; uv: λmax (ethanol) 267 and 409 nm.

Anal. Calcd. for $C_{10}H_{15}N_3O_6$: C, 43.94; H, 5.54; N, 15.37. Found: C, 43.80; H, 5.43; N, 15.33.

2-(D-*Galacto*-2,3,4,5,6-pentahydroxyhexylamino)-3-nitropyridine (**2c**).

Compound **1** (4.3 g., 27 mmoles), 1-amino-1-deoxy-D-galactitol (5.0 g., 27 mmoles), ethyl diisopropylamine (20 ml.), and 80% ethanol (120 ml.) at 85-90° for 3 hours, followed by the work-up described for **2a**, gave the crude product, which was crystallized from ethanol to give 6.4 g. (78%) of bright yellow crystals, m.p. 181-182°; uv: λ max (ethanol) 223, 266, and 408 nm.

Anal. Calcd. for $C_{11}H_{17}N_3O_7$: C, 43.55; H, 5.66; N, 13.85. Found: C, 43.76; H, 5.66; N, 13.60.

2-(D-*Gluco*-2,3,4,5,6-pentahydroxyhexylamino)-3-nitropyridine (**2d**).

This material was prepared from **1** and 1-amino-1-deoxy-D-glucitol according to the quantities and procedure given for **2c**. After crystallization from ethanol there were obtained 4.5 g. (55%) of bright yellow crystals, m.p. 216-217°; uv: λ max (ethanol) 267 and 408 nm.

Anal. Calcd. for $C_{11}H_{17}N_3O_7$: C, 43.55; H, 5.66; N, 13.85. Found: C, 43.51; H, 5.70; N, 13.59.

3*H*,10*H*-2,4-Dioxo-10-(D-*ribo*-2,3,4,5-tetrahydroxypentyl)pyrido-[3,2-*g*]pteridine (**3a**) Hemihydrate.

A suspension of 1.0 g. (3.6 mmoles) of **2a** and 300 mg. of 5% palladium on charcoal in 150 ml. of methanol was shaken under hydrogen at room temperature on a Parr apparatus for 2 hours or until the yellow color of the aminonitro compound was discharged. After separation of the catalyst, the solvent was quickly evaporated under vacuum and the residue was redissolved in 35 ml. of acetic acid. Water (10 ml.), alloxan monohydrate (0.58 g., 3.6 mmoles), and boric acid (1.55 g., 25 mmoles) were added and the mixture was stirred at room temperature for 4 days. The reaction mixture was taken to dryness, 500 ml. of methanol were added, and the mixture was again evaporated. The residue was crystallized from ethanol (charcoal) to give mustard yellow product, m.p. 270-272° dec., after drying to constant weight; 0.8 g. (66%); uv: λ max (water) 237 (shoulder), 254 (log ε 4.32), 258 (4.32), 297 (3.60), 385 (3.92), and 424 (3.74) nm.

Anal. Calcd. for $C_{14}H_{15}N_5O_6 \cdot \frac{1}{2} H_2O$: C, 46.92; H, 4.50; N, 19.54. Found: C, 47.12; H, 4.49; N, 19.33.

3*H*,10*H*-2,4-Dioxo-10-(L-*arabino*-2,3,4,5-tetrahydroxypentyl)-pyrido[3,2-*g*]pteridine (**3b**).

A mixture of **2b** (8.74 g., 32 mmoles) in 200 ml. of 95% ethanol was shaken under hydrogen on a Parr apparatus for 12 hours in the presence of platinum. The catalyst was removed by filtration under nitrogen. The reaction solution was evaporated quickly to dryness under vacuum and the brownish residue was redissolved in 100 ml. of acetic acid. Alloxan monohydrate (5.12 g., 32 mmoles) and boric anhydride (4.60 g., 32 mmoles) were added and the slurry was warmed with constant agitation at 60° for 45 minutes under nitrogen. The solvent was removed under reduced pressure and the residue was treated with 1500 ml. of methanol. Evaporation of the methanol gave a residue free of boron. The residue was crystallized from water with charcoal to give mustard yellow product, m.p. 285-286° dec.; 6.3 g., (55%); uv: λ max (water) 237.5 (shoulder), 254 (log ε 4.30), 258 (4.30), 300 (3.61), 385 (3.89), and 424 (3.91) nm.

Anal. Calcd. for $C_{14}H_{15}N_5O_6$: C, 48.13; H, 4.34; N, 20.05. Found: C, 47.99; H, 4.73; N, 20.02.

3*H*,10*H*-2,4-Dioxo-10-(D-*galacto*-2,3,4,5,6-pentahydroxyhexyl)-pyrido[3,2-*g*]pteridine (**3c**).

A solution of **2c** (2.0 g., 6.6 mmoles) in 100 ml. of 80% acetic acid was shaken under hydrogen for 2 hours in the presence of platinum catalyst. After removal of the catalyst, alloxan monohydrate (1.05 g., 6.6 mmoles) and boric anhydride (2.80 g., 46 mmoles) were added and the mixture was stirred at room temperature for 4 days. The product was worked-up as described in the previous procedures. Crystallization of the crude material from ethanol (charcoal) gave 1.5 g. (60%) of **3c** as mustard yellow solid, m.p. 225-227° dec.; uv: λ max (water) 237 (shoulder), 255 (shoulder), 260 (log ε 4.32), 304 (3.60), 388 (shoulder), and 425 (3.94) nm.

Anal. Calcd. for $C_{15}H_{17}N_5O_7$: C, 47.48; H, 4.52; N, 18.46. Found: C, 47.56; H, 4.51; N, 18.40.

3*H*,10*H*-2,4-Dioxo-10-(D-*gluco*-2,3,4,5,6-pentahydroxyhexyl)-pyrido[3,2-*g*]pteridine (**3d**).

Compound **2d** (3.0 g., 9.6 mmoles) was reduced in ethanol with platinum catalyst and the reduction product condensed with alloxan monohydrate (1.59 g., 9.7 mmoles) in 50 ml. of acetic acid in the presence of boric anhydride (1.40 g., 20 mmoles) at 60° as described for **3b**. The product was crystallized from water, after treatment with methanol, to give mustard yellow solid, m.p. 264-265° dec.; 2.9 g., (78%); uv: λ max (water) 238 (shoulder), 255 (log ε 4.32), 259 (4.33), 303 (3.63), 389 (3.89), and 423 (3.97) nm.

Anal. Calcd. for $C_{15}H_{17}N_5O_7$: C, 47.48; H, 4.52; N, 18.46. Found: C, 47.32; H, 4.55; N, 18.53.

Acknowledgment.

We thank Miss Karin Tinter for obtaining the quantitative uv spectra.

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