Synthesis and Antimicrobial Testing of 2-Amino-6-hydroxymethyl-4-(3*H*)pyrido[3,2-*d*]pyrimidinone

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Synthesis of 2-amino-6-hydroxymethyl-4-(3*H*)pyrido[3,2-*d*]pyrimidinone (**5**) from 2-amino-6-methyl-4-(3*H*)-pyrido[3,2-*d*]pyrimidinone (**2**) was accomplished by selenium dioxide oxidation of **2** to the aldehyde **4** followed by sodium borohydride reduction. Compound **2** was available in four steps from 5-aminouracil or in two steps from 5-nitroisocytosine (**3a**). Catalytic reduction of **4** or **5** gave a mixture of 2-amino-6-methyl-5,6,7,8-tetrahydro-4-(3*H*)pyrido[3,2-*d*]pyrimidinone (**6a**) and the 6-hydroxymethyl compound **6b**. These compounds showed only weak inhibitory activity in the coupled reactions catalyzed by 7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase and 7,8-dihydropteroate synthetase from *E. Coli*. No significant antibacterial activity was observed.

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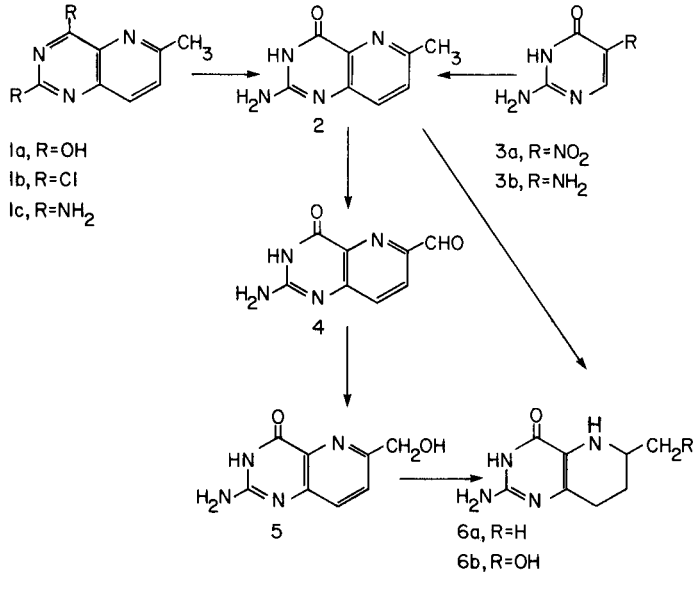
These laboratories have maintained a long standing interest in the preparation of pyridopyrimidines as potential chemotherapeutic agents (1-6). A large series of 2,4-diaminopyrido[2,3-*d*]pyrimidines were found to be inhibitors of dihydrofolate reductase with species specificity (6). The antibacterial and antiprotozoal activities exhibited by this series were markedly potentiated when used in combination with sub-inhibitory levels of sulfadiazine (6). Because the biosynthetic steps inhibited by sulfonamides such as sulfadiazine (7), and by 2,4-diaminopyrido[2,3-*d*]-pyrimidines are in sequence on the same biochemical pathway considerable synergistic effects result (8,9). Inhibition of a third enzyme in this biochemical sequence, 7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase, has resulted in a synergistic effect with the triple combination of 2-amino-4-hydroxy-6-hydroxymethyl-7,7-dimethyl-7,8-dihydropteridine, a sulfonamide, and a dihydrofolate reductase inhibitor (10,11). The 8-deazapterin, 2-amino-6-hydroxymethyl-4-(3*H*)pyrido[3,2-*d*]pyrimidinone (**5**), or a di- or tetrahydro- derivative, was considered attractive as a candidate inhibitor of the pyrophosphokinase. If **5**, or its reduced analog **6b**, inhibited the kinase it would have potential as a synergistic agent in combination with a sulfonamide and/or a dihydrofolate reductase inhibitor. It has been reported that 8-deazafolates and 8-deazapteroates have antimicrobial activity (12). Two laboratories have recently reported new synthetic routes to pyrido[3,2-*d*]pyrimidines of potential biological interest (13,14). We now report the preparation and antimicrobial activity of 8-deaza-6-hydoxymethylpterin **5** and some of its derivatives.

The synthesis of **5** was accomplished as outlined in Scheme I. Starting with 5-aminouracil, the 2,4-diaminopyridopyrimidine **1c** was prepared in three steps via the literature method (15,16). Alkaline hydrolysis of **1c**, in analogy with earlier work (12), gave the 8-deaza-6-methylpterin **2** in excellent yield. Compound **2** was also prepared from 5-nitroisocytosine (**3a**) (17). Reduction of **3a** by catalytic hydrogenation gave **3b** which was condensed with crotonaldehyde as for preparation of **1a** (15) to give **2**. The pyridine ring of **2** was easiy reduced by catalytic hydrogenation to give **6a**. Bromination of **2** resulted in a complex mixture of products. Oxidation of the methyl in **2** with selenium dioxide gave the aldehyde **4** contaminated with unreacted **2**, a highly polar material of unknown structure, and selenium. Due to the low solubility of **4** purification was not feasible. Catalytic reduction of crude **4** resulted in a mixture of **6a** and **6b** in a ratio of one to three. Crude **4** was then reduced with sodium borohydride to give **5**, which was purified by low-pressure, reverse-phase, column chromatography. The pyridine ring of **5** was reduced by catalytic hydrogenation in 1*N* hydrochloric acid but produced a mixture of **6a** and **6b**. This mixture was assayed for antimicrobial activity without isolation.



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The 2-amino-4-oxopyridopyrimidines **2**, **5**, **6a**, and **6b** were tested at $10^{-4}M$ for inhibition of the coupled reactions catalyzed by 7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase and 7,8-dihydropteroate synthetase from *Escherichia coli* (11). Only **5** gave significant inhibition with an $I_{50} = 1.67 \pm 0.11 \times 10^{-4}M$. No activity was observed when these compounds were tested for *in vitro* antibacterial activity against twenty-four bacteria at 100 μg./ml. (18). When they were tested in combination with trimethoprim or sulfamethoxazole only slight potentiation was observed for **5** at 50 μg./ml. (18).

EXPERIMENTAL

The melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian XL-100-15-FT, a Varian T-60, or a Hatachi Perkin-Elmer R-24 spectrometer using tetramethylsilane as an internal standard. Ultraviolet absorption spectra were obtained on a Cary 118 spectrophotometer. Hplc analysis was conducted with a Waters hplc system using two model 6000A pumps, a 660 solvent programmer, a U6K injector, a 3.9 mm. x 30 cm. μBondapak C_{18} column and a Schoffel SF 770 variable wavelength ultraviolet detector. Preparative chromatography was performed on a 0.5 x 23 inch Laboratory Data Control glass column with a Fluid Metering Inc. Model RP-SY pump. The column was packed with EM Reagents LiChroprep RP-18 absorbant, particle size 25-40 μm. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on tlc (Analtech, Inc. Uniplate Silica Gel GF) with either benzene:ethanol 1:1 or chloroform:ethanol 1:1. The analytical samples gave combustion values for C, H, N within 0.4% of theoretical.

2-Amino-6-methyl-4-(3*H*)pyrido[3,2-*d*]pyrimidinone Hydrochloride (**2**).

Method A.

A solution of 13.2 g. (75 mmoles) of **1c** (16), 300 ml. of 1*N* sodium hydroxide, and 60 ml. of 2-methoxyethanol was heated on a steam bath for 18 hours. The solution was filtered and acidified with 25 ml. of acetic acid with rapid stirring. The resulting white solid was collected and washed with water to yield 12.2 g. (92%) of **2**, m.p. > 300° dec. The analytical sample was obtained in 79% yield by recrystallization from dilute hydrochloric acid/ethanol and then from water/ethanol, m.p. > 300° dec.; uv (0.1*N* hydrochloric acid): λ max 318 nm (sh) (ε 4,700), 311 nm (ε 5,000), 286 nm (sh) (ε 3,200), 246 nm (ε 12,100); (*p*H 7.0): λ max 325 nm (ε 4,700), 315 nm (ε 4,800), 262 nm (ε 10,800); (0.1*N* sodium hydroxide): λ max 333 nm (ε 5,200), 267 nm (ε 9,100), 233 nm (ε 23,300); nmr (deuteriotrifluoroacetic acid): δ 8.61 (q, 2H, ArH_2), 3.17 (s, 3H, CH_3).

Anal. Calcd. for $C_8H_8N_4O$·HCl: C, 45.2; H, 4.27; N, 26.3. Found: C, 45.5; H, 4.28; N, 26.3.

Method B.

A solution of 10.16 g. (65 mmoles) of **3a** (17), 600 mg. of 10% palladium on carbon, and 200 ml. of 1*N* hydrochloric acid was shaken in the presence of hydrogen at 2-3 atmospheres for 1.5 hours. The reaction was filtered and spin evaporated *in vacuo*. The residual solid was collected and washed with acetone to yield 13.0 g. (99%) of crude **3b** dihydrochloride. A solution of 11.2 g. of this solid, 120 ml. of 20% aqueous hydrochloric acid, and 4.60 g. (65.6 mmoles) of crotonaldehyde was refluxed with stirring for 2 hours. The reaction was filtered and spin evaporated *in vacuo*. The residue was neutralized with ammonium hydroxide and evaporated. The resulting solid was collected and washed with ethanol to yield 3.50 g. (36%) of **2**, m.p. > 300° dec. which was identical to that obtained from **1c**.

2-Amino-6-methyl-5,6,7,8-tetrahydro-4-(3*H*)pyrido[3,2-*d*]pyrimidinone Hydrochloride (**6a**).

A mixture of 2.50 g. (11.5 mmoles) of **2**, 50 mg. of platinum oxide, 150 ml. of acetic acid, and 50 ml. of 2-methoxyethanol was shaken in the presence of hydrogen at 2-3 atmospheres for 2 hours. The reaction was filtered and spin evaporated *in vacuo*. The residue was dissolved in dilute hydrochloric acid and reevaporated with addition of water in three portions. The white solid was collected and washed with acetone to yield 2.20 g. (88%) of **6a**, m.p. > 250° dec. Recrystallization of a sample from water/acetone gave the analytical sample of unchanged m.p.; uv (0.1*N* hydrochloric acid): λ max 259 nm (ε 4,400); (*p*H 7.0): λ max 267 nm (ε 3,900), 285 nm (sh) (3,500); (0.1*N* sodium hydroxide): λ max 245 nm (ε 5,000), 298 nm (ε 3,600); nmr (deuterium oxide): δ 3.43 (m, 1H, C-6 H), 2.70 (m, 2H, C-8 H_2), 2.00 (m, 2H, C-7 H_2), 1.35 (d, 3H, CH_3).

Anal. Calcd. for $C_8H_{12}N_4O$·HCl: C, 44.3; H, 6.05; N, 25.9. Found: C, 44.3; H, 5.86; N, 25.5.

2-Amino-6-hydroxymethyl-4-(3*H*)pyrido[3,2-*d*]pyrimidinone (**5**).

A solution of 177 mg. (1.0 mmoles) of **2** which had been converted to the free base with 5% aqueous sodium bicarbonate, 255 mg. (2.0 mmoles) of selenium dioxide and 10 ml. of glacial acetic acid was refluxed with stirring for 5 hours. The cooled mixture was stirred with 20 ml. of 0.1*N* hydrochloric acid and filtered through a pad of Celite which was washed with 100 ml. of 0.1*N* hydrochloric acid. The filtrate and wash were spin evaporated *in vacuo*. The residue was triturated with acetone to yield 155 mg. (68%) of **4**, m.p. > 300° dec. which was shown by hplc to be contaminated with **2** and a highly polar material of unknown structure. This mixture was used without further purification; nmr (DMSO-d_6): δ 9.90 (d, 1H, J = 0.4 Hz, CHO).

To a stirred solution of 2.70 g. (60 mmoles) of crude **4** from several selenium dioxide reactions in 540 ml. of 0.1*N* sodium hydroxide was added 8.10 g. (210 mmoles) of sodium borohydride in several portions over 0.5 hour. After 2 hours the reaction was acidified with acetic acid to *p*H 5-6 and spin evaporated *in vacuo*. The residue was dissolved in water and neutralized with 5% aqueous sodium bicarbonate. Hplc analysis (μBondapak C_{18} column–30% methanol/water eluate) showed that **5** was contaminated with **2** and a highly polar material. This crude **5** was purified by preparative low-pressure, reverse phase, column chromatography with the fractions monitored by hplc. The mixture was pumped into the column in 90 ml. of water and the column was washed with water until the eluate was clean. Compound **5** was eluted with 5% methanol in water and **2** was removed with methanol. Fractions containing a mixture of **5** and **2** were combined, concentrated and rechromatographed. Fractions containing pure **5** were combined and spin evaporated *in vacuo*. Recrystallization from methanol gave 0.275 g. (10%) of **5**, m.p. > 320° dec.; uv (0.1*N* hydrochloric acid): λ max 315 nm (sh) (ε 4,700), 307.5 nm (ε 5,200), 248.5 nm (ε 12,300); (*p*H 7.0): λ max 322 nm (sh) (ε 4,300), 314 nm (ε 4,400), 265 nm (ε 10,000); (0.1*N* sodium hydroxide): λ max 333 nm (ε 4,900), 271 nm (ε 8,800), 235.5 nm (ε 21,800); nmr (DMSO-d_6): δ 11.13 (br s, 1H, NH), 7.61 (q, 2H, ArH_2), 6.46 (br s, 2H, NH_2), 5.41 (t, 1H, J = 0.6 Hz, OH), 4.56 (d, 2H, J = 0.6 Hz, CH_2O).

Anal. Calcd. for $C_8H_8N_4O_2$: C, 50.0; H, 4.20; N, 29.2. Found: C, 49.9; H, 4.24; N, 29.1.

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