chromatographed on 50 g of silica gel using a C_6H_6–EtOAc solvent system. The chromatography produced 7.3 g (88%) of a light yellow oil. Anal. ($C_{13}H_{13}NO_2$) C, H, N.

3-Cyanomethyl-*N*,2-dimethyl-2-phenylsuccinimide (23). Compound **23** was prepared following the procedure of Seidel and Cook.[6] A suspension of 35 g (0.71 mol) of NaCN in 70 mL of DMF was cooled in an ice bath and 35 mL of concentrated HCl was added dropwise. After the addition had been completed, 6.1 g (0.0028 mol) of **22** in 70 mL of DMF was added to the suspension. After stirring for 18 h, the dark brown suspension was diluted with 200 mL of H_2O and extracted with $CHCl_3$ (4 × 5 mL). The combined $CHCl_3$ extracts were washed with H_2O (2 × 100 mL) and evaporated until only a few mL of solution remained. The dark red solution was poured into 150 mL of an ice–H_2O mixture and a red oil separated. The oil was dissolved in 100 mL of $CHCl_3$, washed with H_2O (2 × 5 mL), dried ($MgSO_4$), and filtered. Removal of the solvent left 5.0 g (74%) of a red oil: IR (film) 4.42 μ (C≡N); NMR ($CDCl_3$) δ 7.50–7.06 (m, 5 H, ArH), 3.50–2.37 (m, 6 H, aliphatic ring CH, CH_2CN, and NCH_3 at 3.17 and 3.12), 1.91 and 1.72 (s, 3 H, CCH_3). The NMR spectrum indicated the product was a mixture of diastereomers and attempts to separate the diastereomers were unsuccessful. The product was not further purified but used directly in the preparation of **24**.

3-(2-Aminoethyl)-*N*,2-dimethyl-2-phenylsuccinimide Hydrochloride (24). Compound **24** was prepared from 5.0 g (0.021 mol) of crude **23**, 8 mL of concentrated HCl, and 0.100 g of PtO_2 in 150 mL of 95% EtOH following a general procedure.[1] The crude hydrochloride was dissolved in absolute EtOH, treated with Darco G-60, and filtered, and Et_2O was added to the point of cloudiness. Upon cooling a white solid crystallized from solution. After drying in vacuo over P_2O_5 the yield was 1.25 g (21%) of a white powder, mp 190–200 °C. A sample for elemental analysis was obtained by recrystallization from an EtOH–Et_2O mixture to yield a powdery solid: mp 185–200 °C; IR (KBr) 3.00–3.20 μ (br NH_3^+); NMR (D_2O) δ 7.40 (s, 5 H, ArH), 3.31–2.92 (m, 6 H, aliphatic ring CH, CH_2, and NCH_3 at 3.07 and 3.01), 2.35–1.53 (m, 5 H, aliphatic CH_2 and CCH_3 at 1.72 and 1.57). Anal. ($C_{14}H_{19}ClN_2O_2$) H, N; C: calcd, 59.46; found, 55.29.

3-(2-Chloroacetamidoethyl)-*N*,2-dimethyl-2-phenylsuccinimide (25). Compound **25** was prepared from 1.0 g (0.0035 mol) of the hydrochloride **24**, 0.599 g (0.0035 mol) of $(ClCH_2CO)_2O$, and 0.354 g (0.0035 mol) of Et_3N in the manner described for the synthesis of **5**. An almost colorless oil was obtained which was purified by column chromatography using 50 g of silica gel and a C_6H_6–EtOAc solvent system. The chromatography yielded 0.200 g (18%) of a white powder: mp 76–79.5 °C. Anal. ($C_{16}H_{19}ClN_2O_3$) C, H, N.

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Inhibitors of Folate Biosynthesis. 1. Inhibition of Dihydroneopterin Aldolase by Pteridine Derivatives

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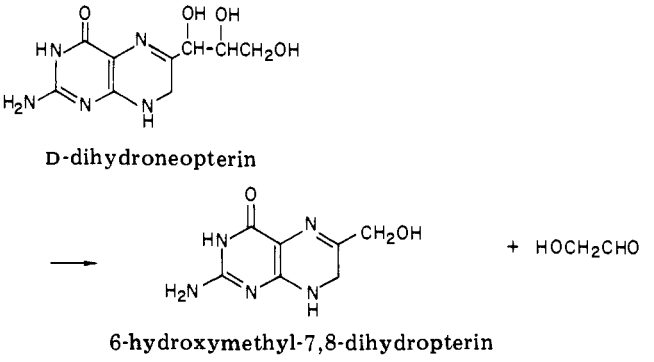
2-Amino-6-carboxamido-7,8-dihydropteridin-4-one and 2-amino-6-hydroxymethyl-7,7-dimethyl-7,8-dihydropteridin-4-one have been shown to be good inhibitors of *Escherichia coli* dihydroneopterin aldolase, an early enzyme of de novo folate biosynthesis.

Many bacteria and some parasites unlike their mammalian hosts have difficulty concentrating certain of the water-soluble vitamins such as folic acid and riboflavin and must, therefore, synthesize them de novo. A favorite approach to the chemotherapy of bacterial and parasitic diseases has been the interference with these biosynthetic sequences. Both of the vitamins cited above are biosynthesized from a guanosine triphosphate precursor[1,2] in separate multistep procedures.[3] The final steps of tetrahydrofolate biosynthesis, those catalyzed by dihydropteroate (or dihydrofolate) synthetase and dihydrofolate reductase, are common chemotherapeutic targets[4] for antibacterial and antiparasitic agents (e.g., methotrexate, pyrimethamine, ethopabate, and sulfas), but inhibitors of the early steps of folate biosynthesis have not been studied in detail.

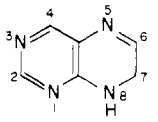
Dihydroneopterin aldolase[5] cleaves a two-carbon segment from D-dihydroneopterin in an early step of pteroate synthesis to form 6-hydroxymethyl-7,8-dihydropterin

Scheme I



(Scheme I). The study of chemical inhibitory requirements for this enzyme was particularly attractive since the substrate is not a phosphorylated derivative obviating the conversion of potential inhibitors to the corresponding

Table I. Inhibition of *E. coli* Dihydroneopterin Aldolase by 7,8-Dihydropteridine Derivatives



Compd (ref)	7,8-Dihydropteridine substituents 2	4	6	7	ID_{50}, μg/mL
1 (6)	NH_2	OH	H	H, H	>50
2 (7)	NH_2	OH	CH_3	H, H	>50
3 (8)	NH_2	OH	CH_2OH	H, H	30
4 (9)	NH_2	OH	CH=NOH	H, H	>50
5 (10)	NH_2	OH	CO_2H	H, H	>50
6 (11)	NH_2	OH	$CONH_2$	H, H	5
7 (12)	NH_2	OH	$CH(OEt)_2$	H, H	>50
8 (13)	NH_2	OH	*n*-C_9H_{19}	H, H	>50
9 (14)	NH_2	OH	$(CH_2)_3Ph$	H, H	>50
10 (15)	NH_2	OH	D-*arabino*-$(CHOH)_3CH_3$	H, H	>50
11 (16)	NH_2	OH	H	CH_2OH, H	>50
12 (17)	NH_2	OH	CH_2OH	CH_3, CH_3	1
13 (18)	NH_2	OH	COOH	OH, H	>50
14 (19)	SH	OH	CH_2OH	H, H	>50
15 (20)	NH_2	NH_2	$CH(CH_3)_2$	$CH(CH_3)_2$, H	>50
16 (8)	NH_2	NH_2	CH_2OH	H, H	>50

phosphoryl or pyrophosphoryl derivatives. Several other enzymes in the folate-synthesizing sequence require phosphorylated substrates (e.g., GTP and 6-hydroxymethylpterin pyrophosphate).

A series of representative pteridine derivatives was therefore examined as inhibitors of dihydroneopterin aldolase.

Results and Discussion

The concentration of a particular dihydropteridine derivative which produced 50% inhibition of the enzyme is shown in Table I.

2-Amino-6-formyl-7,8-dihydropteridin-4-one is known[5] to be a fair inhibitor of dihydroneopterin aldolase (~75% inhibition at 50 μg/mL). Other representative pteridine derivatives are shown which demonstrate the effect of various substituent changes upon inhibition. The enzyme appears to be very sensitive to substituent changes in the 2 and 4 positions of the pteridine ring. 2,4-Diamino-6,7-diisopropylpteridine (**15**), which is reported to be a good inhibitor of dihydrofolate reductase,[21] does not significantly inhibit dihydroneopterin aldolase. Dihydropterins with poly(hydroxyalkyl) side chains at position 6 which do not possess the dihydroneopterin stereochemistry (D-erythro) have been shown to be poor substrates[5] for the enzyme and are also poor inhibitors. In fact, the inhibition data show that the presence of any large or bulky group at C-6 precludes significant inhibitory action (**7–10**). 2-Amino-7,8-dihydropteridin-4-one-6-carboxylic acid (**5**) does not inhibit the aldolase although it has been shown[22] to inhibit the next step in tetrahydrofolate biosynthesis, 7,8-dihydropterin-6-carbinol pyrophosphorylase, and to inhibit the growth of *Escherichia coli*.[23] The anticoccidial and antibacterial agent, 2-amino-6-hydroxymethyl-7,7-dimethyl-7,8-dihydropteridin-4-one (**12**), has also been claimed[24] to inhibit the pyrophosphorylase enzyme, but it is evident from these studies that **12** is an excellent inhibitor of dihydroneopterin aldolase as well.

Experimental Section

***E. coli* Dihydroneopterin Aldolase.** *E. coli* B was grown in a corn steep liquor medium fortified with methionine and glutamic acid. Cells were harvested and broken using a Manton-Gaulin homogenizer. After centrifugation to remove debris, purification through the heat treatment step was carried out as described by Mathis and Brown.[5]

Substrate. Neopterin labeled with ^{14}C was prepared from uniformly labeled arabinose using a procedure to be described elsewhere (specific activity, 4 μCi/μmol; uniformly labeled in the 6 and 7 position of the pteridine and also the trihydroxypropyl side chain). Neopterin was reduced to dihydroneopterin by a modification of the method of Friedkin et al.[25]

Assay Procedure. The assay is based on the production of [^{14}C]glycolaldehyde (see Scheme I). The dihydroneopterin substrate solution was prepared by suspension of unlabeled neopterin (1.6 mg) in 0.05 M potassium phosphate buffer, pH 7.5 (0.25 mL), which was 1.3 M in mercaptoethanol. Then followed the addition of 3.4 μCi of [^{14}C]neopterin in water (0.1 mL) and sodium dithionite (20 mg) and the mixture was warmed until clarified. The total volume was made to 50 mL by addition of 0.55 M ammonium acetate (pH 9.6) which was 0.0125 M in EDTA and 0.18 M in mercaptoethanol.

To the dihydroneopterin solution (0.5 mL) was added H_2O or an aqueous solution of the material to be tested (0.5 mL) and 0.1 mL of enzyme (approximately 0.3 mg of protein). The tubes were capped and incubated in the absence of light at 37 °C for 30 min. Thus, the assay is carried out in the presence of excess reducing agent, ensuring that substrate and inhibitor are in the 7,8-dihydro oxidation state.[21,26] From the fact that neopterin (oxidized form) does not function as a substrate, it is inferred that the pteridines likewise do not function as substrates or inhibitors except in a reduced state. The reaction was terminated by adding 1 mL of a suspension of 100 mg of Norit A/mL of 2 N acetic acid. The tubes were centrifuged and a 1-mL aliquot was removed for counting in the presence of Bray's scintillation counting fluid (10 mL).

Pteridines. Potential pteridine inhibitors were prepared by procedures denoted in Table I (see References and Notes). Treatment of a pteridine derivative (1 mg) with sodium dithionite (13 mg) under conditions as described for the reduction of neopterin vide supra gave a standard solution of the 7,8-dihydropteridine which could be diluted to desired concentrations.

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Pteridines. 41. Synthesis and Dihydrofolate Reductase Inhibitory Activity of Some Cycloalka[*g*]pteridines[1]

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A number of homologous 2,4-diaminocycloalka[*g*]pteridines varying in ring size from 5 to 15 were prepared by (a) condensation of aminomalononitrile tosylate with α-oximinocycloalkanones, deoxygenation of the resulting 2-amino-3-cyanocycloalka[*b*]pyrazine 1-oxides, and guanidine cyclization; (b) guanidine cyclization of the above pyrazine 1-oxides to give 2,4-diaminocycloalka[*g*]pteridine 8-oxides, followed by deoxygenation; or (c) condensation of 2,4,5,6-tetraaminopyrimidine with a cycloalka-1,2-dione (for the cyclohepta- and cycloocta[*g*]pteridines only). These compounds were examined for their activity as dihydrofolate reductase inhibitors against *Lactobacillus casei*, rat liver, L1210, and *Trypanosoma cruzi*. Activity was found to depend upon ring size, with the greatest activity exhibited by the cyclododeca derivative **31**.

Cycloalka[*g*]pteridines are of considerable potential interest, since they represent an unusual class of pteridine derivatives substituted in the pyrazine ring with bulky hydrophobic groups[3] and which are incapable of metabolic oxidation at C-7. Furthermore, the cyclohexa[*g*]pteridines represent intriguing potential intermediates to the biologically interesting benzopteridines.[4] Since initial inhibitory studies against dihydrofolate reductase indicated that 2,4-diaminocyclododeca[*g*]pteridine was some 1000 times more active than 2,4-diaminocyclohexa[*g*]pteridine, we have prepared a number of additional homologues in order to investigate the dependency of dihydrofolate reductase inhibition upon ring size.

Chemistry. The general approach to the synthesis of cyclohexa[*g*]pteridines is illustrated by the synthesis of 2,4-diaminocyclohexa[*g*]pteridine (**7**) outlined in Scheme I. Condensation of aminomalononitrile tosylate with 2-oximinocyclohexanone[5] gave 2-amino-3-cyanocyclohexa[*b*]pyrazine 1-oxide (**1**). Reaction with guanidine then gave 2,4-diaminocyclohexa[*g*]pteridine 10-oxide (**13**) which was deoxygenated to the known 2,4-diaminocyclohexa[*g*]pteridine (**7**).[6] This latter compound could alternatively be prepared by initial deoxygenation of **1** to give 2-amino-3-cyanocyclohexa[*b*]pyrazine (**4**) followed by annelation of the pyrimidine ring by reaction with guanidine. Cyclization of **4** with benzamidine gave 2-phenyl-4-aminocyclohexa[*g*]pteridine (**8**). Similarly, 2-oximinocyclopentanone,[7] 5-methyl-2-oximinocyclohexanone,[8] 4-methyl-2-oximinocyclohexanone, 2-oximinocyclodecanone, 2-oximinocycloundecanone, 2-oximinocyclododecanone, 2-oximinocyclotridecanone, and 2-oximinocyclopentadecanone were all converted by reaction with aminomalononitrile tosylate into the corresponding pyrazine 1-oxides, which were deoxygenated and then cyclized with guanidine to the corresponding 2,4-diaminocycloalka[*g*]pteridines listed in Scheme I and Table I. Because of the ready availability of 1,2-cycloheptanedione and 1,2-cyclooctanedione (by selenium dioxide oxidation of the corresponding monoketones),[9] the cyclohepta-[6] and cycloocta[*g*]pteridines **27** and **28** were prepared from these 1,2-diketones by condensation with 2,4,5,6-tetraaminopyrimidine.

A comparison of the methylcyclohexa[*g*]pteridines **10** and **12** illustrates the value of the unambiguous synthetic route to these compounds involving pyrazine intermediates. The spectral and physical properties of these structural isomers show no significant differences, and it would be extremely difficult to distinguish between them by either chemical or physical means. It is only possible to show that they are different; i.e., although both melt with decomposition at 301 °C, mixtures melt considerably

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