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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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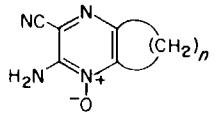
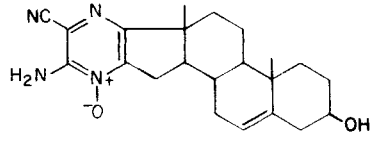
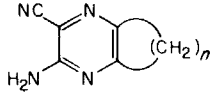
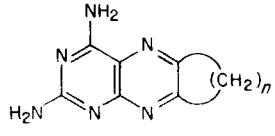
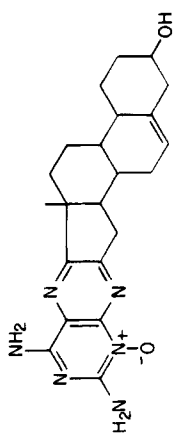
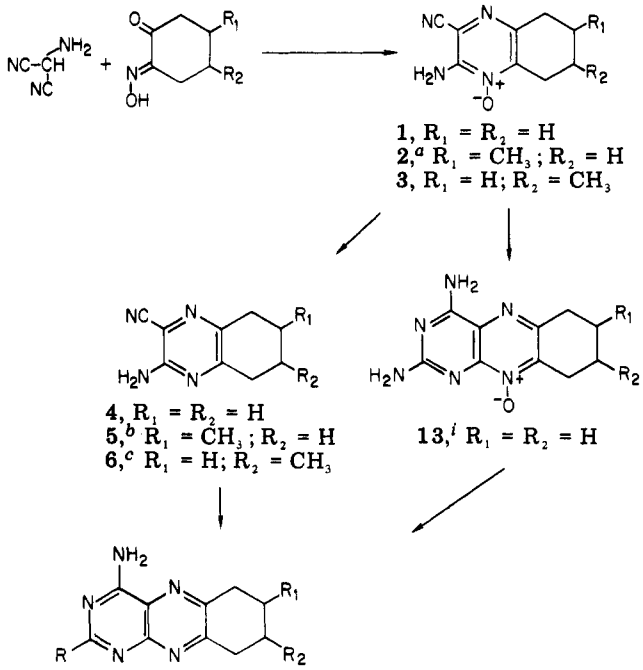
Table I. Cycloalka[*b*]pyrazines and Cycloalka[*g*]pteridines

No.	*n*	Name	Formula	Analyses	Yield, %	Mp, °C
						
14	3	2-Amino-3-cyanocyclopenta[*b*]pyrazine 1-oxide	$C_8H_8N_4O$	C, H, N	43	245-247
1	4	2-Amino-3-cyanocyclohexa[*b*]pyrazine 1-oxide	$C_9H_{10}N_4O$	C, H, N	75	235-238
15	8	2-Amino-3-cyanocyclodeca[*b*]pyrazine 1-oxide	$C_{13}H_{18}N_4O$	C, H, N	40	366-367
16	9	2-Amino-3-cyanocycloundeca[*b*]pyrazine 1-oxide	$C_{14}H_{20}N_4O$	C, H, N	40	333-334
17	10	2-Amino-3-cyanocyclododeca[*b*]pyrazine 1-oxide	$C_{15}H_{22}N_4O$	C, H, N	93.5	227-230
18	11	2-Amino-3-cyanocyclotrideca[*b*]pyrazine 1-oxide	$C_{16}H_{24}N_4O$	C, H, N	34	322-322.5
19	13	2-Amino-3-cyanocyclopentadeca[*b*]pyrazine 1-oxide	$C_{18}H_{28}N_4O$	C, H, N	56	334-338
						
34		2′-Amino-3′-cyano-3β-hydroxyandrost-5-en[17,16-*e*]pyrazine 1-oxide	$C_{22}H_{28}N_4O_2$	C, H, N	72	278-280
						
20	3	2-Amino-3-cyanocyclopenta[*b*]pyrazine	$C_8H_8N_4$	C, H, N	73	224-227
4	4	2-Amino-3-cyanocylohexa[*b*]pyrazine	$C_9H_{10}N_4$	C, H, N	58	203-205
21	8	2-Amino-3-cyanocyclodeca[*b*]pyrazine	$C_{13}H_{18}N_4$	C, H, N	98	306-308
22	9	2-Amino-3-cyanocycloundeca[*b*]pyrazine	$C_{14}H_{20}N_4$	C,[a] H, N[b]	94	274
23	10	2-Amino-3-cyanocyclododeca[*b*]pyrazine	$C_{15}H_{22}N_4$	C, H, N	95.5	215-216
24	11	2-Amino-3-cyanocyclotrideca[*b*]pyrazine	$C_{16}H_{24}N_4$	C, H, N	77	275-276
25	13	2-Amino-3-cyanocyclopentadeca[*b*]pyrazine	$C_{18}H_{28}N_4$	C,[c] H, N	80	288.5-291
						
26	3	2,4-Diaminocyclopenta[*g*]pteridine	$C_9H_{10}N_6$	C, H, N	81	>300
7	4	2,4-Diaminocyclohexa[*g*]pteridine	$C_{10}H_{12}N_6$	C, H, N	72,[d] 99[e]	>360
27	5	2,4-Diaminocyclohepta[*g*]pteridine	$C_{11}H_{14}N_6$	C, H, N	47	>300
28	6	2,4-Diaminocycloocta[*g*]pteridine	$C_{12}H_{16}N_6$	C, H, N	79	338 dec
29	8	2,4-Diaminocyclodeca[*g*]pteridine	$C_{14}H_{20}N_6$	C,[f] H, N	62	>330
30	9	2,4-Diaminocycloundeca[*g*]pteridine	$C_{15}H_{22}N_6$	C, H, N	53	>330
31	10	2,4-Diaminocyclododeca[*g*]pteridine	$C_{16}H_{24}N_6$	C,[g] H, N	80	260-262
32	11	2,4-Diaminocyclotrideca[*g*]pteridine	$C_{17}H_{26}N_6$	C,[h] H, N	47.5	>330
33	13	2,4-Diaminocyclopentadeca[*g*]pteridine	$C_{19}H_{30}N_6$	C,[i] H, N[j]	60	>330

35		2′,4′-Diamino-3β-hydroxyandrost-5-en[17,16-*g*]pteridine 8′-oxide	$C_{23}H_{30}N_6O_2$	C, H, N[k]	99	>300

[a] C: calcd, 68.82; found, 68.22. [b] N: calcd, 22.93; found, 22.01. [c] C: calcd, 71.95; found 71.21. [d] From 4 and guanidine. [e] From dithionite reduction of 13. [f] C: calcd, 61.74; found, 61.06. [g] C: calcd, 63.97; found, 63.27. [h] C: calcd, 64.93; found, 64.35. [i] C: calcd, 66.63; found, 65.88. [j] N: calcd, 24.54; found, 23.95. [k] N: calcd, 18.89; found, 20.01.

Scheme I



1, $R_1 = R_2 = H$
2,[a] $R_1 = CH_3$; $R_2 = H$
3, $R_1 = H$; $R_2 = CH_3$

4, $R_1 = R_2 = H$
5,[b] $R_1 = CH_3$; $R_2 = H$
6,[c] $R_1 = H$; $R_2 = CH_3$

13,[i] $R_1 = R_2 = H$

7, $R = NH_2$; $R_1 = R_2 = H$
8,[d] $R = C_6H_5$; $R_1 = R_2 = H$
9,[e] $R = NH_2$; $R_1 = CH_3$; $R_2 = H$
10,[f] $R = C_6H_5$; $R_1 = CH_3$; $R_2 = H$
11,[g] $R = NH_2$; $R_1 = H$; $R_2 = CH_3$
12,[h] $R = C_6H_5$; $R_1 = H$; $R_2 = CH_3$

[a] 2-Amino-3-cyano-6-methylcyclohexa[*b*]pyrazine 1-oxide: 45%; mp 217–218 °C. Anal. ($C_{10}H_{12}N_4O$) C, H, N. [b] 2-Amino-3-cyano-6-methylcyclohexa[*b*]-pyrazine: 86%; mp 187–191 °C. Anal. ($C_{10}H_{12}N_4$) C, H, N. [c] 2-Amino-3-cyano-7-methylcyclohexa[*b*]-pyrazine: 22%; mp 189–191 °C. Anal. ($C_{10}H_{12}N_4$) C, H, N. [d] 2-Phenyl-4-aminocyclohexa[*g*]pteridine: 34%; mp 293–296 °C. Anal. ($C_{16}H_{15}N_5$) C, H, N. [e] 2,4-Diamino-7-methylcyclohexa[*g*]pteridine: 51%; mp >300 °C. Anal. ($C_{11}H_{14}N_6$) C, H, N. [f] 2-Phenyl-4-amino-7-methylcyclohexa[*g*]pteridine: 37%; mp 301 °C dec. Anal. ($C_{17}H_{17}N_5$) C, H, N. [g] 2,4-Diamino-8-methylcyclohexa[*g*]pteridine: 41%; mp >300 °C. Anal. ($C_{11}H_{14}N_6$) C, H, N. [h] 2-Phenyl-4-amino-8-methylcyclohexa[*g*]-pteridine: 65%; mp 301 °C. Anal. ($C_{17}H_{17}N_5$) C, H, N. [i] 2,4-Diaminocyclohexa[*g*]pteridine 10-oxide: 61%; mp >320 °C. Anal. ($C_{10}H_{12}N_6O$) C, H, N.

lower. The isomer prepared by cyclization of 2-phenyl-4,6-diamino-5-nitrosopyrimidine with 4-methyl-1-morpholinocyclohexene[10] gives an undepressed melting point with **10** but not with **12**. Furthermore, in the case of the 2,4-diaminopteridine derivatives **9** and **11**, it is not even possible to demonstrate readily that the two isomers are different compounds, for neither possesses an observable melting point, and spectral properties are extremely similar. Their structures can, however, be assigned with confidence on the basis of this unambiguous cyclization procedure.

A pteridine with an even larger hydrophobic substituent fused to the 6,7 bond was prepared through the intermediacy of a steroidal α-oximino ketone. Cyclization of aminomalononitrile tosylate with 16-oximino-3-hydroxyandrost-5-en-17-one[11] gave **34**, which was cyclized with guanidine to **35**. However, neither of these *N*-oxides could be deoxygenated, even under strongly reducing conditions (e.g., refluxing triethyl phosphite).

Biological Evaluation. The 2,4-diaminopteridines described in this study were tested against *Lactobacillus casei*, rat liver, L1210, and *Trypanosoma cruzi*. The

Table II. Inhibition of Dihydrofolate Reductases by 2,4-Diaminocycloalka[g]pteridines

No.	ID_{50}			
	Rat liver	L1210	*L. casei*	*T. cruzi*
7	1.9×10^{-4} [a]	2×10^{-4} [b]	6.9×10^{-5} [b]	2.3×10^{-5} [b]
27	3.2×10^{-5} [a]	9×10^{-6} [a]	$>10^{-4}$ [b]	2.1×10^{-5} [b]
28	9×10^{-6} [a]	6.6×10^{-6} [a]	$>10^{-4}$ [b]	2.0×10^{-6} [b]
29	4.6×10^{-7} [a]	3.0×10^{-7} [a]	3.6×10^{-5}	1.0×10^{-6}
30	2.4×10^{-7}	3.5×10^{-7}	1.7×10^{-5}	9.6×10^{-8}
31	2.1×10^{-7} [a]	1.9×10^{-7} [a]	2.7×10^{-5} [b]	1.7×10^{-7} [b]
32	3.7×10^{-7}	3.0×10^{-7}	3.2×10^{-5}	1.5×10^{-7}
33	2.9×10^{-6} [b]	1.5×10^{-5} [b]	3.0×10^{-5} [b]	2.9×10^{-5} [b]

[a] Data from W. E. Richter, Jr., and J. J. McCormack, *J. Med. Chem.*, **17**, 943 (1974). [b] Data from J. J. McCormack in "Chemistry and Biology of Pteridines", W. Pfleiderer, Ed., Walter de Gruyter, Berlin, 1976, p 126.

results are summarized in Table II. It is clear that the activity of these compounds as inhibitors of dihydrofolate reductase does depend upon ring size, with the greatest activity (rat liver and L1210) being exhibited by the cyclododeca derivative **31**.

Experimental Section

2-Amino-3-cyanocyclopenta[*b*]pyrazine 1-Oxide (14). A suspension of 2.26 g (0.02 mol) of 2-oximinocyclopentanone and 5.06 g (0.02 mol) of aminomalononitrile tosylate in 30 mL of 2-propanol was stirred at room temperature for 24 h. Filtration then gave 1.51 g (43%) of a gray microcrystalline solid, mp 229–234 °C, which was homogeneous by TLC. The analytical sample was sublimed at 180 °C (0.5 mm) to give yellow crystals, mp 245–247 °C.

Compounds **1** and **2** were prepared analogously.

2-Amino-3-cyano-7-methylcyclohexa[*b*]pyrazine 1-Oxide (3). A stream of hydrogen chloride gas was bubbled through a stirred solution of 11.2 g (0.10 mol) of 4-methylcyclohexanone in 200 mL of dry ether at −30 °C until the solution was saturated. Passage of hydrogen chloride through the solution was continued while 11.5 g (0.10 mol) of isoamyl nitrite was added dropwise at such a rate that the temperature did not rise above −20 °C. The reaction mixture was then stirred for 30 min at −20 °C, and the solid which had separated was collected by filtration, washed with ether, and air-dried.

A suspension of 0.89 g (5.0 mmol) of crude 4-methyl-2-oximinocyclohexanone hydrochloride and 1.30 g (5.0 mmol) of aminomalononitrile tosylate was stirred in 10 mL of 2-propanol for 48 h. Filtration gave 0.75 g (74%) of a white powder, mp 188–191 °C, which was recrystallized from aqueous acetic acid to give clear colorless crystals of **3**, mp 190–192 °C. Anal. ($C_{10}H_{12}N_4O$) C, H, N.

Compounds **15–19** and **34** were prepared analogously by in situ formation of the appropriate 2-oximinocycloalkanone hydrochloride and subsequent condensation with aminomalononitrile tosylate.

2-Amino-3-cyanocyclopenta[*b*]pyrazine (20). A suspension of 0.53 g (3.0 mmol) of 2-amino-3-cyanocyclopenta[*b*]pyrazine 1-oxide (**14**) in 10 mL of boiling water was treated with 0.75 g (4.3 mmol) of sodium hydrosulfite in small portions. The reaction mixture was stirred for 1.5 h, cooled, and filtered to give 0.35 g (73%) of a dark yellow powder. The analytical sample, mp 224–227 °C, was prepared by sublimation at 180 °C (0.5 mm).

Compounds **4**, **5**, and **6** were prepared analogously.

2-Amino-3-cyanocyclodeca[*b*]pyrazine (21). A solution of 250 mg of 2-amino-3-cyanocyclodeca[*b*]pyrazine 1-oxide in 10 mL of THF was treated with 500 mL of PCl_3 at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and evaporated to a small volume under pressure, and the residue was diluted with ice water. The precipitated solid was collected by filtration, dried by suction, and recrystallized from aqueous ethanol: yield, 230 mg (98%); mp 306–308 °C.

Compounds **22–25** were prepared analogously.

2,4-Diaminocyclopenta[*g*]pteridine (26). To a filtered solution of 1.25 g of guanidine hydrochloride in methanolic sodium methoxide (prepared from 0.35 g of sodium and 25 mL of methanol) was added 0.80 g of 2-amino-3-cyanocyclopenta[*b*]pyrazine. The reaction mixture was heated under reflux for 18 h, cooled, and filtered to give 0.81 g (81%), mp >300 °C. The analytical sample was prepared by sublimation at 205 °C (0.2 mm).

2,4-Diaminocyclohepta[*g*]pteridine (27).[6] A solution of 600 mg of 1,2-cycloheptanedione[9] in 3 mL of methanol was added to a solution of 1.0 g of 2,4,5,6-tetraaminopyrimidine·2.5HCl (neutralized with sodium acetate) in 20 mL of water, and the resulting solution was stirred at room temperature for 30 min. The pale yellow solid which had separated was collected by filtration, washed with water, followed by ethanol and then ether, and dried: yield 470 mg (47%). The analytical sample, mp >300 °C dec, was prepared by recrystallization from DMF.

Compound **28** was prepared analogously.

Compounds **7**, **9**, **11**, **13**, **29–33**, and **35** were prepared as described above from guanidine hydrochloride and the corresponding 2-amino-3-cyanopyrazine intermediate in methanolic sodium methoxide. The use of benzamidine rather than guanidine hydrochloride led to compounds **8**, **10**, and **12**.

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References and Notes

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(2) NSF Predoctoral Fellow, 1967–1971.
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