1-Aryl-4,6-diamino-1,2-dihydro-*s*-triazines. Contrasting Effects on Intestinal Helminths, Bacteria, and Dihydrofolic Reductase (1,2)

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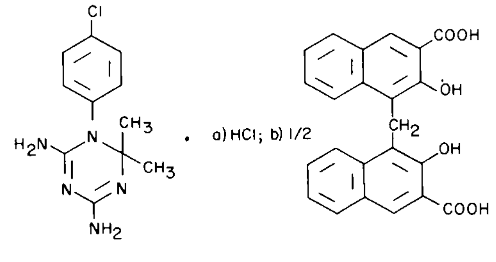
The preparation of a variety of novel 1-aryl-4,6-diamino-1,2-dihydro-*s*-triazines is described. These compounds exhibit an array of contrasting effects on intestinal helminths, bacteria, and dihydrofolic reductase. It is concluded that potent anthelmintic activity among the dihydrotriazines is not dependent solely on the bulky-substituent hypothesis advanced by other investigators, and indeed cannot reliably be predicted on this basis.

Soon after the introduction of chlorguanide, evidence was presented (3,4) indicating that the drug was essentially inactive against malaria parasites *in vitro*, suggesting that a metabolite, or metabolites, are responsible for the antimalarial activity. A search for such substances culminated in a report by Carrington, *et al.*, (5) that 4,6-diamino-1-(*p*-chlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine (cycloguanil) (I) was the active metabolite. However, cycloguanil hydrochloride (Ia) proved to be less active than chlorguanide against *Plasmodium cynomolgi* in monkeys and *P. falciparum* in man, presumably because of rapid excretion. More recently, the synthesis of cycloguanil pamoate (Ib) (6-8) and other dihydrotriazine (7-9) and pyrimethamine (8,10) salts that exhibit remarkable repository antimalarial properties in experimental animals (7-12) was reported. In man, a single intramuscular dose of cycloguanil pamoate has the unusual capacity to protect man for many months against challenges with susceptible strains of *P. vivax*, *P. falciparum*, *P. malariae*, and *P. ovale* (13). Cycloguanil pamoate is also highly effective against cutaneous leishmaniasis in man caused by *Leishmania braziliensis* and *L. mexicanum* (13).



I

During the past two decades, many other 1-aryl-4,6-diamino-1,2-dihydro-*s*-triazines have been synthesized and evaluated for biological activity (1, 14-49). In laboratory studies, many of them exhibited activity against the plasmodia of malaria (1, 14-23, 27, 28), pathogenic (29, 30) and nonpathogenic (16) bacteria, coccidia (23, 31), Toxoplasma (32), intestinal helminths (1, 23, 25), and experimental tumors (33, 34). Many dihydrotriazines are also good enzyme inhibitors in microbiological systems (16,28,35-49). However, none of these has achieved widespread use in human medicine.

In the course of continuing efforts in these laboratories to develop new chemotherapeutic agents, a variety of novel 1-aryl-4,6-diamino-1,2-dihydro-*s*-triazine derivatives (II) was synthesized for biological evaluation. This paper describes the preparation of selected members of this series, and emphasizes their contrasting effects in various biological systems.

CHEMISTRY

1-Aryl-4,6-diamino-1,2-dihydro-*s*-triazine compounds (II) have traditionally been synthesized by three major routes (Chart I): (A) the reaction of a ketone or aldehyde with an arylbiguanide under acidic conditions (5,16,18,19, 21-23); (B) the condensation in one step of an arylamine, dicyandiamide, a ketone or an aldehyde, and one equivalent of acid (14-20, 22-25); and (C) the reaction of dicyandiamide and a protonated Schiff base (18,19,26). In the current work the hydrochloride salts of the 4,6-diamino-

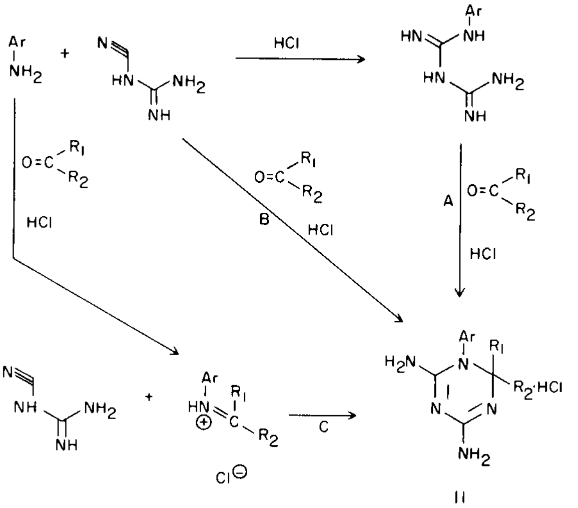
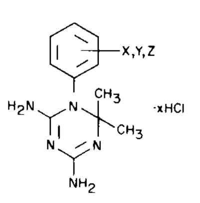


Chart I



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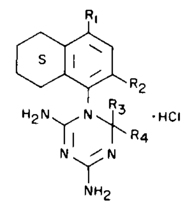

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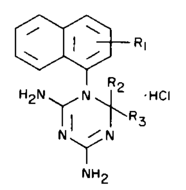

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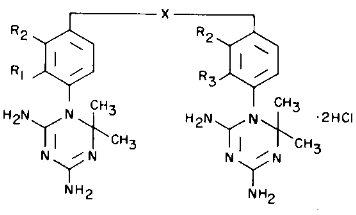

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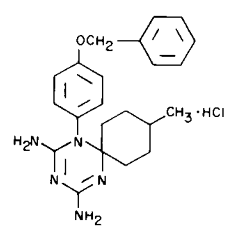
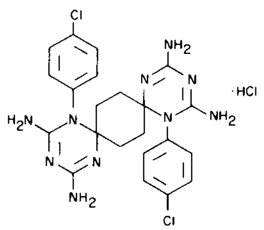
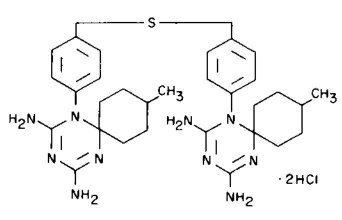


VII



VIII



IX

1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazines (III) (Table I), 4,6-diamino-1,2-dihydro-1-(5,6,7,8-tetrahydro-1-naphthyl)-*s*-triazines (IV) (Table III), 4,6-diamino-1,2-dihydro-1-(1-naphthyl)-*s*-triazines (V) (Table IV), 1,1′-di-*p*-phenylene bis[4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazines] (VI) (Table V), 2,4-diamino-9-methyl-1-[*p*-(benzyloxy)phenyl]-1,3,5-triazaspiro[5.5]undeca-2,4-diene (VII) (**24**, Table II), 2,4,11,13-tetraamino-5,14-bis(*p*-chlorophenyl)-1,3,5,10,12,14-hexaazadispiro[5.2.5.2]-hexadeca-1,3,10,12-tetraene (VIII), and 5,5′-(thiodi-*p*-phenylene)bis[2,4-diamino-9-methyl-1,3,5-triazaspiro[5.5]undeca-1,3-diene] (IX) were prepared by route B. Alternatively, several 4,6-diamino-1,2-dihydro-1-phenyl-*s*-triazines (Table II) were synthesized *via* route A.

In alkali, or upon heating to the melting point, 1-aryl-4,6-diamino-1,2-dihydro-*s*-triazines undergo a characteristic and irreversible rearrangement to their 6-arylamino isomers. This rearrangement is accompanied by characteristic changes in the ultraviolet absorbance (18,20,25). All of the dihydrotriazines described in this work were checked for the appropriate spectral changes upon heating in 0.1*N* sodium hydroxide to confirm that the indicated isomers were obtained.

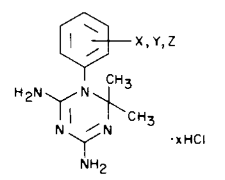
BIOLOGY

The dihydrotriazines were tested against representative pathogens, including *Plasmodium lophurae*, *P. berghei*, *P. cynomolgi*, *Entamoeba histolytica*, *Trichomonas vaginalis*, *Trypanosoma cruzi*, *Syphacia obvelata*, *Aspiculuris tetraptera*, *Hymenolepis nana*, *Schistosoma mansoni*, *Streptococcus pyogenes* (C 203), *Staphylococcus aureus* (UC-76), *Klebsiella pneumoniae* (AD), *Proteus vulgaris* (MGH-1), *Pseudomonas aeruginosa* (No. 28), *Salmonella typimurium* (V-31), *Mycobacterium tuberculosis* ($H_{37}Rv$), herpes simplex, polio, measles, and adenovirus. The compounds were also examined for inhibitory effects on *Streptococcus faecalis*, *Lactobacillus casei*, and dihydrofolic reductase. Many of the dihydrotriazines were highly active in a variety of these biological systems. Detailed structure-activity relationships among compounds studied against *S. obvelata*, *S. faecalis*, *L. casei*, and dihydrofolic reductase are summarized below.

The anthelmintic testing procedure was essentially that described previously (50). Compounds were administered as their hydrochloride salts in single gavage doses. All doses were expressed in terms of free base content. Usually, treatment groups consisted of 7 mice, and control groups of 15 mice. Autopsies and intestinal examinations were performed two days post treatment. When the proportion of uninfected mice in the treatment group was higher than in the control group, the number of infected and uninfected individuals in both groups were checked against fourfold contingency tables (51) to determine whether or not the apparent effect was statistically significant. Numerical entries (other than zero) in Table VI in the "% Cured. . ." column indicate situations in which there was a significant

TABLE I

4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazine Hydrochlorides (a)

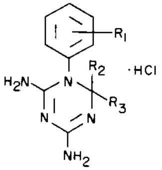


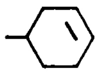
Compd. No.	X, Y, Z	Mp, °C (b)	Yield purified, %	Purification (c) solvent	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1	3-CF_3, 4-Br	239-240	81	A	$C_{12}H_{13}BrF_3N_5\cdot HCl$	35.97	36.15	3.52	3.58	17.48	17.63
2	3-CF_3, 4-Cl	233-234	70	A	$C_{12}H_{13}ClF_3N_5\cdot HCl$	40.46	40.70	3.96	3.90	19.66	19.66
3	3-CF_3, 4-F	233-235	65	A	$C_{12}H_{13}F_4N_5\cdot HCl$	42.42	42.80	4.15	4.25	20.62	20.82
4	4-CF_3	226-227	12	B	$C_{12}H_{14}F_3N_5\cdot HCl$	44.79	44.60	4.70	4.44	21.77	21.41
5	4-C_2H_5	231-233	62	A	$C_{13}H_{19}N_5\cdot HCl$	55.40	55.40	7.15	7.14	24.85	24.94
6	4-COC_2H_5	210-211	22	B	$C_{14}H_{19}N_5O\cdot HCl$	54.27	54.16	6.51	6.26	22.61	22.65
7	2-$OCH(CH_3)_2$	213-217	50	A	$C_{14}H_{21}N_5O\cdot HCl$	53.92	53.96	7.11	6.94	22.46	22.30
8	3,4-$(CH_2)_4$-	235-237	11	B	$C_{15}H_{21}N_5\cdot HCl$	58.52	58.74	7.21	7.34	22.75	22.66
9 (d)	4-$N(CH_2CH_2)_2O$	259-266	16	C	$C_{15}H_{22}N_6O\cdot HCl\cdot H_2O$	50.48	50.37	7.06	7.08	23.55	23.78
10	2-$C(CH_3)_3$	227-229	28	D	$C_{15}H_{23}N_5\cdot HCl$	58.14	57.93	7.81	7.49	22.61	22.74
11	4-$OCH_2CH(CH_3)_2$	213-217	33	E	$C_{15}H_{23}N_5O\cdot HCl$	55.29	54.96	7.42	7.74	21.49	21.62
12	4-$SO_2N(C_2H_5)_2$	218-220	84	A	$C_{15}H_{24}N_6O_2S\cdot HCl\cdot H_2O$ (e)	44.27	44.01	6.69	6.51	20.65	20.56
13 (d)	4-$N(CH_2)_5$	232-236	8	C	$C_{16}H_{24}N_6\cdot HCl\cdot H_2O$	54.16	54.01	7.67	7.59	23.69	23.87
14 (f)	3-$CH_2N(CH_2)_4$, 4-OH	231-233	27	B	$C_{16}H_{24}N_6O\cdot 2HCl\cdot 0.5H_2O$	48.24	47.91	6.83	6.93	21.10	21.03
15	4-$(SC_6H_4NO_2$-*p*)	237-239	84	A	$C_{17}H_{18}N_6O_2S\cdot HCl$	50.17	49.82	4.71	4.54	20.65	20.03
16 (g)	4-OC_6H_5	206-208	36	C	$C_{17}H_{19}N_5O\cdot HCl$	59.04	58.83	5.84	5.56	20.25	20.19
17 (d)	4-$OCH_2CH_2N(C_2H_5)_2$	246-248	36	A	$C_{17}H_{28}N_6O\cdot 2HCl$	50.36	49.79	7.46	7.42	20.73	20.70
18 (h)	4-$CH_2C_6H_5$	217-221	27	C	$C_{18}H_{21}N_5\cdot HCl$	62.87	62.93	6.45	6.51	20.37	20.27
19	4-$OCH_2C_6H_5$	216-219	25	C	$C_{18}H_{21}N_5O\cdot HCl\cdot 1.5H_2O$ (i)	55.88	56.19	6.51	6.50		
20 (f)	3-$CH_2\underset{CH_3}{N}(CH_2)_9CH_3$, 4-OH	227-228	47	B	$C_{23}H_{40}N_6O\cdot 2HCl$	56.43	56.38	8.65	8.49	17.17	17.14

(a) The compounds in this table were prepared by Procedure I, except as noted. (b) With decomposition. (c) A, not recrystallized; B, methanol-ether; C, water; D, ethanol; E, ethanol-ether. (d) Prepared by Procedure I, but with one additional equivalent of concentrated hydrochloric acid in the reaction mixture. (e) Calcd. for H_2O: 4.43. Found: 4.50. (f) Procedure I was followed, but using the dihydrochloride salt of the aniline derivative, and only 0.5 ml. of concentrated hydrochloric acid. (g) Netherlands Patent, 6,500,497, July 19, 1965, reports m.p. 211-212°. (h) B. R. Baker and B.-T. Ho (39) report m.p. 201-203°. (i) Calcd. for H_2O: 6.99. Found: 6.82.

TABLE II

4,6-Diamino-1,2-dihydro-1-phenyl-*s*-triazine Hydrochlorides

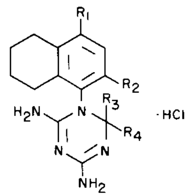


Compd. No.	R_1	R_2	R_3	Mp, °C (a)	Yield purified, %	Procedure	Purification (b) solvent	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
21	4-Cl		H	233-235	21	III	A	$C_{15}H_{18}ClN_5 \cdot HCl$	52.94	53.08	5.63	5.72	20.58	20.56
22	4-Cl	$4\text{-}C_6H_4OCH_2C_6H_5$	H	214-217	43	III	B	$C_{22}H_{20}ClN_5O \cdot HCl$	59.74	59.50	4.79	4.68	15.84	15.97
23	$4\text{-}OCH_2C_6H_5$	C_6H_5	H	222-224	57	III	C	$C_{22}H_{21}N_5O \cdot HCl$	64.78	64.82	5.44	5.37		
24	$4\text{-}OCH_2C_6H_5$	$-CH_2CH_2\underset{\overset{\vert}{CH_3}}{C}HCH_2CH_2-$		209-212	10	IV	D	$C_{22}H_{27}N_5O \cdot HCl$	63.83	63.82	6.82	6.89	16.92	17.10
25	$4\text{-}OCH_2C_6H_5$	$4\text{-}C_6H_4OCH_2C_6H_5$	H	197-200	73	III	E	$C_{29}H_{27}N_5O_2 \cdot HCl$	67.76	67.73	5.49	5.39	13.63	13.68

(a) With decomposition. (b) A, water; B, not recrystallized; C, methanol-ether; D, methanol; E, methanol-2-propanol.

TABLE III

4,6-Diamino-1,2-dihydro-1-(5,6,7,8-tetrahydro-1-naphthyl)-*s*-triazine Hydrochlorides

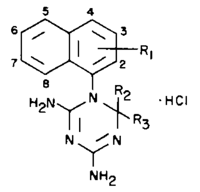


Compd. No.	R_1	R_2	R_3	R_4	Mp. °C (a)	Yield purified, %	Pro-cedure	Purification (b) solvent	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
26	Cl	H	CH_3	CH_3	228-233	14	I	A	$C_{15}H_{20}ClN_5 \cdot HCl$	52.63	52.82	6.19	6.02	20.46	20.67
27	H	H	CH_3	CH_3	225-228	31	I	B	$C_{15}H_{21}N_5 \cdot HCl$	58.52	58.12	7.21	6.94	22.74	22.92
28	Cl	CH_3	CH_3	CH_3	224-226	48	I	B	$C_{16}H_{22}ClN_5 \cdot HCl$	53.93	53.60	6.51	6.53	19.66	19.78
29	H	CH_3	CH_3	CH_3	214-218	29	I	B	$C_{16}H_{23}N_5 \cdot HCl \cdot 2H_2O$ (c)	53.70	53.76	7.88	7.91	19.57	20.08
30	H	H	$-CH_2CH_2\underset{CH_3}{CH}CH_2CH_2-$		236-238	16	IV	B	$C_{19}H_{27}N_5 \cdot HCl$	63.06	62.80	7.80	7.68	19.35	19.50

(a) With decomposition. (b) A, ethanol; B, methanol-ether. (c) Calcd. for H_2O: 10.06. Found: 9.82.

TABLE IV

4,6-Diamino-1,2-dihydro-1-(1-naphthyl)-*s*-triazine Hydrochlorides

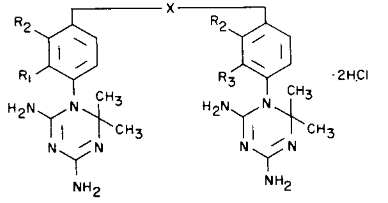


Compd. No.	R_1	R_2	R_3	Mp, °C (a)	Yield purified, %	Pro-cedure	Purifi-cation (b) solvent	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
31	4-Br	CH_3	CH_3	235-237	7	I	A	$C_{15}H_{16}BrN_5 \cdot HCl \cdot 0.17H_2O$ (c)	46.70	46.16	4.53	4.49	18.16	18.51
32	4-Cl	CH_3	CH_3	229-231	25	I	A	$C_{15}H_{16}ClN_5 \cdot HCl$	53.26	52.68	5.07	5.04	20.71	20.85
33 (d)	H	CH_3	CH_3	216-219	23	I	B	$C_{15}H_{17}N_5 \cdot HCl \cdot 0.5H_2O$ (e)	57.60	57.66	6.12	6.08	22.39	22.48
34	2-CH_3	CH_3	CH_3	223-230	31	I	C	$C_{16}H_{19}N_5 \cdot HCl \cdot 0.5H_2O$ (f)	58.79	58.64	6.47	6.39	21.43	21.46
35	4-Cl	$-CH_2CH_2\underset{\mid \atop CH_3}{C}HCH_2CH_2-$		242-243	27	IV	A	$C_{19}H_{22}ClN_5 \cdot HCl$	58.16	57.83	5.91	5.94	17.85	17.79

(a) With decomposition. (b) A, methanol-ether; B, water; C, not recrystallized. (c) Calcd. for H_2O: 0.79. Found: 0.57. (d) P. Mamalis, J. Green, D. J. Outred, and M. Rix, *J. Chem. Soc.*, 3915 (1962) report the anhydrous monohydrochloride, m.p. 226-227°. (e) Calcd. for H_2O: 2.80. Found: 3.00. (f) Calcd. for H_2O: 2.75. Found: 3.29.

TABLE V

1,1′-Di-*p*-phenylenebis[4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine]Dihydrochlorides (a)

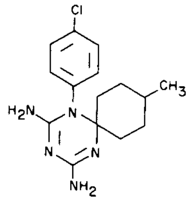
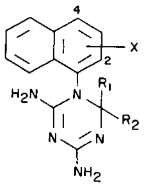
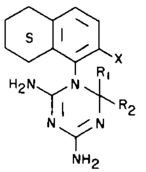
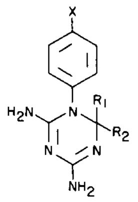
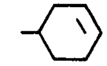


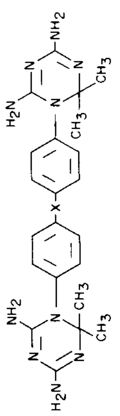
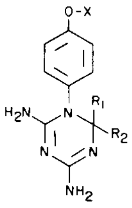
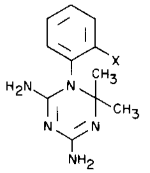
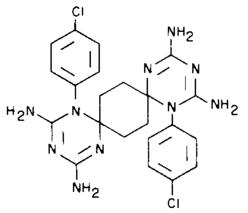
Compd. No.	X	R_1	R_2	R_3	Mp, °C (b)	Yield purified, %	Purification (c) solvent	Formula	Carbon, %		Hydrogen, %		Nitrogen, %	
									Calcd.	Found	Calcd.	Found	Calcd.	Found
36	O	H	H	H	286-288	46	A	$C_{22}H_{28}N_{10}O\cdot 2HCl$	50.67	50.49	5.80	5.93	26.86	27.16
37 (d)	SO_2	H	H	H	266	13	A	$C_{22}H_{28}N_{10}O_2S\cdot 2HCl\cdot 0.67H_2O$ (e)	45.43	45.49	5.43	5.76	24.09	23.57
38	$(SO)_2$	H	H	H	224	16	B	$C_{22}H_{28}N_{10}O_2S_2\cdot 2HCl\cdot 0.33H_2O$ (f)	43.49	43.14	5.09	5.34	23.06	23.00
39	S	H	H	H	276-279	35	A	$C_{22}H_{28}N_{10}S\cdot 2HCl$	49.16	48.81	5.63	5.49	26.06	25.92
40	S_2	H	H	H	246-248	25	A	$C_{22}H_{28}N_{10}S_2\cdot 2HCl\cdot 1.5H_2O$ (g)	44.29	44.19	5.57	5.67	23.48	23.13
41	CH_2	H	NO_2	H	267	36	A	$C_{23}H_{28}N_{12}O_4\cdot 2HCl\cdot 0.33H_2O$ (h)	44.88	44.95	5.02	5.33	27.31	26.97
42	CH_2	H	H	H	290-292	32	C	$C_{23}H_{30}N_{10}\cdot 2HCl$	53.17	52.90	6.21	6.11	26.96	27.00
43	SO_2	CH_3	H	H	244-247	11	A	$C_{23}H_{30}N_{10}O_2S\cdot 2HCl\cdot 0.75H_2O$ (i)	46.27	45.65	5.66	6.14	23.46	23.13
44	CH_2CH_2	H	H	H	274-275	23	A	$C_{24}H_{32}N_{10}\cdot 2HCl$	54.03	53.89	6.42	6.26	26.26	26.45
45	–	CH_3	H	CH_3	278-280	18	B	$C_{24}H_{32}N_{10}\cdot 2HCl\cdot 0.5H_2O$ (j)	53.13	52.77	6.50	6.57	25.82	25.62
46	–	OCH_3	H	OCH_3	280-282	8	A	$C_{24}H_{32}N_{10}O_2\cdot 2HCl\cdot 0.25H_2O$ (k)	50.57	50.00	6.10	6.19	24.58	24.69
47	$C(CH_3)_2$	H	H	H	283-285	38	A	$C_{25}H_{34}N_{10}\cdot 2HCl$	54.84	54.77	6.62	6.52	25.58	25.82
48	$CH_2\underset{COOH}{CH}\text{-}CH_2$	H	H	H	232	9	A	$C_{26}H_{34}N_{10}O_2\cdot 2HCl\cdot 1.5H_2O$	50.48	50.54	6.36	6.63	22.65	22.51
49	$CH\text{-}C_6H_5$	H	H	H	271-273	40	D	$C_{29}H_{34}N_{10}\cdot 2HCl\cdot 0.5H_2O$	57.61	57.69	6.17	6.45	23.17	22.87

(a) The compounds in this table were prepared by Procedure II. (b) With decomposition. (c) A, methanol-ether; B, not recrystallized; C, methanol-water; D, methanol. (d) A. Raychaudhuri, *J. Indian Chem. Soc.*, 35, 75 (1958), reports the anhydrous dihydrochloride, m.p. 266-268°. (e) Calcd. for H_2O: 2.07. Found: 2.33. (f) Calcd. for H_2O: 1.00. Found: 0.90. (g) Calcd. for H_2O: 4.53. Found: 4.63. (h) Calcd. for H_2O: 0.98. Found: 0.73. (i) Calcd. for H_2O: 2.26. Found: 1.85. (j) Calcd. for H_2O: 1.66. Found: 1.62. (i) Calcd. for H_2O: 0.79. Found: 0.62.

TABLE VI

Anthelmintic, Anti-enzymatic, and Antibacterial Activities of 1-Aryl-4,6-diamino-1,2-dihydro-*s*-triazines

Structure	Compd. No.	X	R_1R_2	Anthelmintic Effects in Mice Dose, mg./kg.	 % Cured of *S. obvelata*	Concentrations (μg./ml.) of triazines causing 50% inhibition of: Dihydrofolic Reductase	 *S. faecalis*	 *L. casei*
	XI	–	–	800	83	3.67	2.00	>100
				300	83			
				75	70			
				50	0			
	33 (a)	H	$(CH_3)_2$	100	100	40.0	22.0	>100
				50	Not sig.			
	32	4-Cl	$(CH_3)_2$	1200	100	19.0	11.2	9.0
				150	81			
				75	62 (b)			
	35	4-Cl	$\left.\begin{matrix}CH_2CH_2\\CH_2CH_2\end{matrix}\right>CHCH_3$	600	100	171 (c)	11.5	14.0
				150	57			
	34	2-CH_3	$(CH_3)_2$	600	(Lethal)	390 (c)	>100	> 100
				200	100			
				100	Not sig.			
	27	H	$(CH_3)_2$	600	(Lethal)	53.0	28.5	>100
				100	100			
				50	Not sig.			
	29	CH_3	$(CH_3)_2$	1200	100 (d)	200 (c)	41.5	>100
				100	85			
				50	Not sig.			
	30	H	$\left.\begin{matrix}CH_2CH_2\\CH_2CH_2\end{matrix}\right>CHCH_3$	100	100	200 (c)	43.5	35.0
				50	Not sig.			
	XII (e)	H	$(CH_3)_2$	600	0	0.17 (f)	0.013	1.3
	XIII (e)	H	H, C_6H_5	600	100	7.3	0.38	>100
				75	83			
				50	Not sig.			
	XIV (g)	Cl	H, C_6H_5	600	100	2.3	0.21	>100
				150	Not sig.			
	21	Cl	H, 	600	86	108 (c)	1.7	>100
				150	Not sig.			

	44	CH_2CH_2	–	600	0	0.007	0.014	0.75
	37 (h)	SO_2	–	600	Not sig.	2.2	0.50	>100
	38	$(SO)_2$	–	600	Not sig.	2.5	0.30	12.5
	42	CH_2	–	600 150	86 Not sig.	0.032	0.02	6.25
	47	$C(CH_3)_2$	–	100 50	67 Not sig.	0.048	0.115	33.8
	36	O	–	600 100	90 70 (i)	1.67	0.20	16.0
	39	S	–	300 150	100 Not sig.	0.067	0.05	4.75
	40	SS	–	600 100 50	100 81 Not sig.	13.0	0.60	10.25
	11	$CH_2CH(CH_3)_2$	$(CH_3)_2$	600 150	100 0	1.20	0.005	0.385
	17	$CH_2CH_2N(C_2H_5)_2$	$(CH_3)_2$	600 150	57 0	162 (c)	9.25	>100
	16	C_6H_5	$(CH_3)_2$	600 150	60 0	1.9	0.008	0.775
	19 (j)	$CH_2C_6H_5$	$(CH_3)_2$	100 50	100 Not sig.	0.041	0.005	1.05
	24	$CH_2C_6H_5$	$\begin{matrix}CH_2CH_2\\CH_2CH_2\end{matrix}\!>\!CHCH_3$	150 50	100 Not sig.	3.4	0.67	13.5
	23	$CH_2C_6H_5$	H, C_6H_5	100	Not sig.	5.7	0.03	>100
	10	$C(CH_3)_3$	–	600 200	0(k) Not sig.	0.20	0.06	18.25
	7	$OCH(CH_3)_2$	–	100	0	183 (c)	11.5	>100
	50	–	–	600	0	81	>80	>40

(a) Table IV, footnote d. (b) Statistically significant at 93% confidence level. (c) Run at 50 μg./ml., and extrapolated. (d) Lethal. (e) Ref. 20. Samples of XIII and XIV were kindly supplied by Dr. Modest for testing purposes. (f) B. R. Baker and B.-T. Ho (38) report 50% inhibition of dihydrofolic reductase at 0.11 μM concentration, in a similar assay. (g) Ref. 27. (h) Table V, footnote (d). (i) Statistically significant at 92% confidence level. (j) D. B. Capps, U. S. Patent, 3,150,133, Sept. 22, 1964. (k) Lethal to 5 of 7 mice.

TABLE VII

Correlation of Anthelmintic, Anti-enzymatic, and Antibacterial Properties of 1-Aryl-4,6-diamino-1,2-dihydro-*s*-triazines

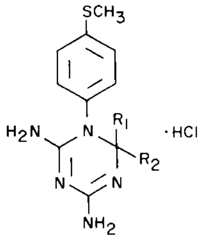
Conc (μg./ml.) causing 50% inhibition of:	Effects Against *S. obvelata* in mice (mg./kg., single oral dose):		
	Active at 200, or lower	Active at 600	Inactive at 600
Dihydrofolic Reductase			
.007 - .099	19, 47	39, 42	44
.100 - .99			XII, 10
1.00 - 9.9	XI, XIII, 24	XIV, 11, 16, 36	37, 38
10.0 - 99	27, 32, 33, 40		50
100 - 400	29, 30, 34, 35	17, 21	
S. faecalis			
.005 - .099	19	11, 16, 39, 42	XII, 10, 44
.100 - .99	XIII, 24, 40, 47	XIV, 36	37,38
1.00 - 9.9	XI	17, 21	
10.0 - 100	27, 29, 30, 32, 33, 35		50
>100	34		
L. casei			
.30 - .99		11, 16	44
1.00 - 9.9	19, 32	39, 42	XII
10.0 - 100	24, 30, 35, 40, 47	36	10, 38, 50
>100	XI, XIII, 27, 29, 33, 34	XIV, 17, 21	37

effect by the drug at a 95%, or greater, level of confidence, except where noted. The figures in this column result from the following calculation: 100X [(fraction of control mice infected with *S. obvelata* at autopsy) - (fraction of treated mice infected with *S. obvelata* at autopsy)] ÷ (fraction of control mice infected with *S. obvelata* at autopsy).

The inhibitory activity of the dihydrotriazines toward dihydrofolic reductase (52) prepared from pigeon liver was measured by the spectrophotometric procedure (53). Details of the antibacterial assay procedures with *S. faecalis* and *L. casei* are given in the Experimental Section.

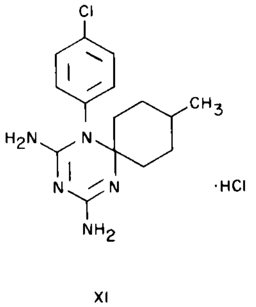
DISCUSSION AND RESULTS

Anthelmintic activity in the dihydrotriazine series was first disclosed by Schalit and Cutler (23) who reported that both 4 6-diamino-1,2-dihydro-2,2-dimethyl-1-[*p*-(methylthio)phenyl]-*s*-triazine hydrochloride (Xa) and 4,6-diamino-1,2-dihydro-1-[*p*-(methylthio)phenyl]-2-propyl-*s*-triazine hydrochloride (Xb) exhibited curative activity against *S. obvelata* and *A. tetraptera* in mice given oral doses of 200 mg./kg./day. In a more extensive series of 1-aryl-4,6-diamino-1,2-dihydro-*s*-triazines studied later by Roth, *et al.*, (25), other dihydrotriazines which lacked hindering substituents were practically devoid of anthelmintic activity, but exhibited potent activity against *L. casei*. Effects against *L. casei* are generally thought to be a measure of anti-folic activity, the presumed basis for the antimalarial activity of cycloguanil. Conversely, compounds with bulky substituents in the *ortho*-position of the benzene ring or at the 2-position of the dihydrotriazine ring, such as B. W. 58-232 (XI), showed high anthelmintic activity and negligible effects against *L. casei* (25). These results led them to the hypothesis that, from a biological point of view, the anthelmintic dihydrotriazines form a different class of compounds from the antimalarial dihydrotriazines, and that these differences can best be explained by increased steric hindrance associated with bulky substitution.



	R_1	R_2
a,	CH_3	CH_3
b,	H	$(CH_2)_2CH_3$

X



XI

This structure-activity hypothesis, surprising in view of the anthelmintic activity of Xa and b, prompted a review of data accumulated in these laboratories as a result of a long interest in dihydrotriazines, and led to the synthesis of other key relatives for biological evaluation. As a result, enough exceptions to the bulky-substituent hypothesis were noted to raise serious questions concerning its general applicability.

The data presented in Table VI allow a comparison of the molecular structure of certain dihydrotriazine derivatives with activity against the pinworm *Syphacia obvelata* (50) in mice. Drug concentrations which inhibit dihydrofolic reductase, *S. faecalis*, and *L. casei*, are also listed, providing three separate indices of anti-folic acid activity for each substance. With many of the compounds listed in Tables I-V, anthelmintic studies were designed to detect only outstanding potencies, and not to locate threshold levels of activity or toxicity. Because these data are not well-suited to the present treatment, they are not included.

The first compound (XI) in Table VI is the outstanding member of the Roth, *et al.*, series which is reported to be active against the human pinworm *Enterobius vermicularis*. In our studies, it was highly active against *S. obvelata* in mice, confirming previous results (25). The bulky substituent in this compound is the methyl-pentamethylene group located in the triazine ring. The next four compounds (**32**, **33**, **34**, and **35**) are derived from α-naphthylamines, and are likewise potent anthelmintics. They have in common the bulky fused ring. It is interesting that the chlorine atom in the 4-position of the aryl group in **32** did not increase the anthelmintic activity over that of **33**, nor did the additional bulk of the methyl-pentamethylene group, in **35**, or the *ortho*-methyl group of **34**. Compounds **27**, **29**, **30**, derived from tetrahydronaphthylamines, showed the same relationships. In sheep, compound **27** caused a high reduction of *Haemonchus* egg counts at 200 mg./kg.

The data on compounds XII and XIII reveal an enhancement of anti-pinworm effect produced by introducing a phenyl group in place of two methyl groups. It is noteworthy that the introduction of a chlorine atom in XIV weakens the anthelmintic activity. Just the opposite effect is found with B.W. -58-232 (XI) and its non-chlorinated analog (**25**). The 2-phenyl group of XIV can be partially hydrogenated (**21**) without much change in effect against *S. obvelata*.

The next eight compounds are members of a series of bis[dihydrotriazines] which vary widely in effects against

S. obvelata, *S. faecalis*, *L. casei*, and dihydrofolic reductase. Compounds **36**, **40**, and **47** are rather potent anthelmintics, yet they have neither *ortho*-substituents in the benzene ring, nor bulky substituents in the 2-positions of the triazine rings. Their activity is thus contrary to expectations based on the bulky-substituent hypothesis.

Six dihydrotriazines with ether substituents appear next in Table VI. With the exception of **24**, their anti-pinworm activity is also at variance with expectations based on the bulky-substituent hypothesis. Of special interest is the remarkable anthelmintic and anti-folic acid activity of **19**. It is noteworthy that the effect against *S. obvelata* is not enhanced by the introduction of bulky groups (*cf*, **23** and **24**), although anti-folic activities decrease as expected in both instances (37).

Compounds **7** and **10** contain bulky groups *ortho* in the benzene ring. The *tert*-butyl derivative (**10**) killed 5 of 7 mice at 600 mg./kg., leaving two alive and still infected. At 200 mg./kg., the effect was not statistically significant. The isopropyl ether (**7**) was not active at 100 mg./kg. It was not tested further against *S. obvelata*, but it was only weakly active against dihydrofolic reductase, *S. faecalis*, and *L. casei*. The last compound (**50**), derived from 1,4-cyclohexanedione, has much of the structure of XI, but lacks the activity. Compounds **7**, **10**, and **50** might have been expected to show strong anthelmintic effects, but were inactive.

Table VII, derived from the data presented in Table VI, depicts the overall relationships between anthelmintic, anti-enzymatic, and antibacterial properties among the dihydrotriazines. The compounds (identified by number) are grouped vertically into three categories based on anthelmintic activity, and horizontally based on relative potency against dihydrofolic reductase, *S. faecalis*, and *L. casei*, respectively.

Recalling that Roth, *et al.*, (25) found an inverse relationship between anthelmintic and antimicrobial activity, as indicated by *L. casei* growth inhibition, one expects the more potent anthelmintics to be the weaker inhibitors of *L. casei*, and the weak anthelmintics to be the strong *L. casei* inhibitors. From Table VII it can be seen that most of the dihydrotriazines with potent activity against *S. obvelata* are, indeed, grouped toward the least active end of the range against *L. casei* as anticipated by Roth, *et al*, (25) but exceptions are found in all but the most-active bracket. The less-active anthelmintics are distributed throughout the range, as are the inactive compounds.

Against *S. faecalis*, another folic acid-requiring organism, the most active group of anthelmintics is not concentrated toward either end of the activity range. There is, however, a correlation in the next most active anthelmintic group, with a direct, not an inverse, relationship. Among the anthelmintically inactive compounds, the inverse relationship is seen. An important enzyme involved in the inhibition, by dihydrotriazines, of *L. casei* and *S. faecalis* growing in a medium containing folic acid is dihydrofolic reductase (52). Data showing the inhibitory activity of these compounds toward this enzyme, prepared from pigeon liver and measured by the spectrophotometric procedure (53), are presented in Tables VI and VII. Here, as in the case of *L. casei*, there seems to be a tendency for the best anthelmintics to be grouped toward the less-active end of the range of dihydrofolic reductase inhibition. This pattern is marred, however, by two striking exceptions (**19** and **47**). Clearer correlations, if they exist, might emerge from a similar treatment of data from a larger series of dihydrotriazines than is presented here (54).

Among key compounds, the potent anthelmintic B.W.-58-232 (XI) lies in the middle of the range against dihydrofolic reductase and *S. faecalis*. It is at the least active end of the range against *L. casei*. By contrast compound **19**, which has comparable anthelmintic potency, is among the most potent compounds against dihydrofolic reductase and *S. faecalis*, and falls within the second most potent category against *L. casei*. A similar pattern is seen with the bis-dihydrotriazine **47**.

The foregoing structure-activity relationships demonstrate that 1-aryl-4,6-diamino-1,2-dihydrotriazines with *ortho*-substituents in the benzene ring, or with bulky groups in the 2-position of the triazine ring may, or may not, exhibit promising anthelmintic activity. Further, certain dihydrotriazines without these special features are, indeed, potent anthelmintics. It is concluded that good anthelmintic activity is not necessarily associated with the presence of bulky groups in these strategic positions, and cannot reliably be predicted on this basis.

EXPERIMENTAL

CHEMICAL

Melting points were taken on a Thomas-Hoover melting point apparatus and are corrected.

Preparation of 1-Aryl-4,6-diamino-1,2-dihydro-*s*-triazine Hydrochlorides (II) (Tables I-V).

1-Aryl-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine Hydrochlorides (Tables I, III, and IV). Procedure I.

One-tenth mole of a substituted aniline was combined with 50-200 ml. of acetone, 10.0 ml. (0.116 mole) of concentrated hydrochloride acid, and 9.0 g. (0.107 mole) of dicyandiamide. The mixture was stirred and heated under reflux for 16-45 hours. If already precipitated, the product was collected and washed with acetone. If the mixture was homogeneous at the end of the heating period, the product was precipitated by cooling, by concentrating the reaction mixture, by adding ether, or by a combination of these. In some cases, methanol (20-350 ml.) was added to dissolve the starting mixture, or to make the suspension more fluid. However, mixtures which were nonhomogeneous at the start of, or throughout the heating period, frequently provided products of good purity directly from the reaction mixture.

Recrystallization solvents, where employed, are indicated in the Tables.

1,1′-Di-*p*-phenylenebis[4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine]Hydrochlorides (VI)(Table V). Procedure II.

This is the same as Procedure I, except that twice the amounts of all reactants but the aniline derivative, which in these cases is difunctional, were employed.

4,6-Diamino-1,2-dihydro-1-phenyl-*s*-triazine Hydrochlorides (Table (II). Procedure III.

A mixture of 13.3 g. (0.063 mole) of 4-(benzyloxy)benzaldehyde, 2.2 ml. (0.025 mole) of concentrated hydrochloric acid, 12.4 g. (0.05 mole) of 1-(*p*-chlorophenyl)biguanide hydrochloride (55), and 100 ml. of methanol was stirred and heated under reflux for 5 hours. The resulting suspension was filtered, and the precipitate washed with methanol. Dried *in vacuo* at 40°, the product, 4,6-diamino-2-[*p*-(benzyloxy)phenyl]-1-(*p*-chlorophenyl)-1,2-dihydro-*s*-triazine, monohydrochloride (**22**), weighed 9.5 g. (43%), m.p. 214-217° dec.

4′,6′-Diamino-1′-arylspiro[cyclohexane-1,2′(1′*H*)-*s*-triazine] Hydrochlorides. (Compounds **24**, **30**, and **35**). Procedure IV.

A mixture of 17.8 g. (0.10 mole) of 1-amino-4-chloronaphthalene 40.0 g. (3.3 moles) of 4-methylcyclohexanone, 10.0 ml. (0.117 mole) of concentrated hydrochloric acid, 9.0 g. (0.107 mole) of dicyandiamide, and 75 ml. of methanol was stirred and heated under reflux for 16 hours. The mixture was cooled and filtered, and the precipitate washed successively with 2-propanol and ether. The 4′,6′-diamino-1′-(4-chloro-1-naphthyl)-4-methylspiro[cyclohexane-1,2′-(1′*H*)-*s*-triazine] monohydrochloride (**35**) thus obtained weighed 22.1 g., m.p. 242-244° dec. Two crystallizations with methanol-ether, gave 10.5 g. (27%), m.p. 242-243° dec.

2,4,11,13-Tetraamino-5,14-bis(*p*-chlorophenyl)-1,3,5,10,12,14-hexaazadispiro[5.2.5.2]hexadeca-1,3,10,12-tetraene Dihydrochloride Dihydrate (VIII).

p-Chlorophenylbiguanide hydrochloride (55) (25.0 g., 0.10 mole), 75 ml. of methanol, 5.0 ml. (0.058 mole) of concentrated hydrochloric acid, and 5.0 g. (0.45 mole) of 1,4-cyclohexanedione were combined and heated under reflux on a steam bath. After 1 hour, a white precipitate was observed, and after 2 hours 75 ml. more methanol was added. After 3 hours, the mixture was cooled and allowed to stand overnight. The precipitate (5.2 g., m.p. > 330°) was recrystallized from water, giving 2.5 g. of VIII, m.p. > 330°, ultraviolet absorption: λ max (MeOH), 241 mμ.

Anal. Calcd. for $C_{22}H_{24}Cl_2N_{10}\cdot 2HCl\cdot 2H_2O$: C, 43.43; H, 4.97; N, 23.03. Found: C, 43.56; H, 5.08; N, 22.75.

5 5′-(Thiodi-*p*-phenylene)bis[2,4-diamino-9-methyl-1,3,5-triazaspiro[5.5]undeca-1,3-diene] Dihydrochloride Hemihydrate (IX).

4,4′-Thiodianiline (21.6 g., 0.10 mole), 100 ml. of methanol, 65 ml. (*ca.* 0.5 mole) of 4-methylcyclohexanone, 20 ml. (0.23 mole) of concentrated hydrochloric acid, and 18.0 g. (0.214 mole) of dicyandiamide were combined, stirred, and heated under reflux for 8 hours. A white solid separated, starting 10 minutes after reflux temperature was reached. The suspension was allowed to stand overnight, and the crude product was recrystallized from methanol-water (4:1) affording 16.2 g. (25%) of (IX), m.p. 290-292° dec.

Anal. Calcd. for $C_{30}H_{40}N_{10}S\cdot 2HCl\cdot 0.5H_2O$: C, 55.03; H, 6.62; N, 21.40. Found: C, 54.71; H, 6.75; N, 21.01.

4-Chloro-5,6,7,8-tetrahydro-2-methyl-1-naphthylamine.

To a stirred solution of 28.0 g. (0.174 mole) of 2-methyl-5,6,7,8-tetrahydro-1-naphthylamine in 150 ml. of glacial acetic acid was added 13.9 g. (0.196 mole) of chlorine over a period of 1.5 hours at 11-33°. The mixture was stirred for 1 hour at 30°, diluted with 600 ml. of water, and filtered. The filtrate, made basic with 500 ml. of 6 *N* sodium hydroxide, was subjected to steam distillation. The first 8 l. of distillate was extracted with ether, and the extracts concentrated to a 13.9 g. residue which, after recrystallizations from isooctane, and aqueous ethanol, yielded 6.5 g. (19%) of 4-chloro-5,6 7,8-tetrahydro-2-methyl-1-naphthylamine, m.p. 56-57.5°, ultraviolet absorption: λ max (Basic MeOH), 242, 293 mμ (ε, 8,850, 2,200).

Anal. Calcd. for $C_{11}H_{14}ClN$: C, 67.51; H, 7.21; N, 7.16. Found: C, 67.55; H, 7.32; N, 7.10.

4-Chloro-5,6,7,8-tetrahydro-1-naphthylamine.

A mixture of 19.7 g. of *N*-(4-chloro-5,6,7,8-tetrahydro-1-naphthyl)acetamide (56), 400 ml. of absolute ethanol, and 30 ml. of concentrated hydrochloric acid was heated under reflux for 5 hours, then concentrated, and chilled, providing 16.1 g. of crude product, m.p. 225-230°. Recrystallization from 2-propanol-water (7:1) provided 8.4 g. of 4-chloro-5,6,7,8-tetrahydro-1-naphthylamine, monohydrochloride, m.p. 259-261° (with preliminary darkening), ultraviolet absorption: λ max (Basic MeOH), 243, 293 mμ (ε, 9,600, 1,830).

1-[*p*-(Benzyloxy)phenyl]biguanide Monohydrochloride.

p-(Benzyloxy)-aniline (25.0 g., 0.13 mole), 11.2 ml. (0.13 mole) of concentrated hydrochloric acid, 100 ml. of water, acid 11.0 g. (0.13 mole) of dicyandiamide were combined and heated under reflux with stirring, for 6 hours. The biguanide salt started to precipitate after 3 hours. After standing overnight, the mixture was filtered, and the precipitate recrystallized from methanol-2-propanol giving 19.7 g. (47%), m.p. 237-240°.

Anal. Calcd. for $C_{15}H_{17}N_5O\cdot HCl$: C, 56.33; H, 5.67; N, 21.90. Found: C, 56.52; H, 5.50; N, 21.74.

ANTIBACTERIAL ASSAYS

Growth antagonism assays with *S. faecalis* ATCC-8043 and *L. casei* ATCC-7469 were carried out in 2.5 ml. cultures in 16 x 150 mm. tubes incubated 24 hours at 37°. Stock cultures were maintained by monthly transfers in a rich medium made by dissolving 105 grams Difco litmus milk, 2 g. tryptose, and 1 g. yeast extract in water and diluting to 1000 ml. Inocula were prepared by transferring cells from the stock culture into a liquid medium consisting of the original riboflavin assay medium of Snell and Strong (57) supplemented with 100 μg. riboflavin per liter. Cells from a 6-hour culture were centrifuged, washed in physiological saline and diluted to give a transmittance reading of 65%. One drop (approximately 2.3 x 10^6 cells) was added to each assay tube.

Assay medium representing minimal requirements for good growth of *L. casei*, and 2.5 times final strength, contained the following per 100 ml.:

Difco casamino acids (treated with 10% Darco G-60 at *p*H 3.5) 1.25 g.; dextrose 1.9 g.; sodium acetate 1.25 g.; dl-tryptophan 0.75 mg.; l-cysteine 25 mg.; asparagine 62.5 mg.; KH_2PO_4 125 mg.; K_2HPO_4 125 mg.; $MgSO_4\cdot 7H_2O$ 50 mg.; $FeSO_4\cdot 7H_2O$ 2.5 mg.; $MnSO_4\cdot H_2O$ 2.25 mg.; NaCl 2.5 mg.; pantothenic acid 6.25 μg.; nicotinic acid 100 μg.; pyridoxal·HCl 7.5 μg.; biotin 31.25 mμg.; folic acid 75 mμg.; riboflavin 6.25 μg.

S. faecalis assay medium was the same except for the following changes: pantothenic acid 5 μg.; nicotinic acid 50 μg.; pyridoxal·HCl 1.25 μg.; biotin 25 mμg.; folic acid 100 mμg.; no riboflavin.

One ml. amounts of concentrated assay medium and calculated amounts of water for final dilutions were dispensed in tubes and autoclaved 10 minutes at 10 psig. The experimental compounds were dissolved in 50% ethanol, then diluted aseptically in water and added in graded amounts to the tubes of sterilized medium to make final volumes of 2.5 ml. Following inoculation the tubes were incubated 24 hours at 37°. The culture turbidities were read in a photoelectric colorimeter and expressed as transmittancies which were plotted against concentrations of inhibitory compounds. Results were expressed either as concentrations required to give 50% inhibition of growth, presented in columns 8 and 9, Table VI, or as inhibition indices. The inhibition index is calculated by dividing the amount of inhibitor per ml. required for half inhibition by the minimum amount of folic acid (or other folate derivative) required to give complete growth.

Reversal studies were sometimes carried out using folic acid, folinic acid, and combinations of the latter with purine and pyrimidine deoxyribosides. It was characteristic of the dihydrotriazines described here that competitive reversal of the inhibition of growth of *S. faecalis* caused by these compounds was produced by folic acid, whereas noncompetitive reversal generally was produced by folinic acid. This indicates that the main inhibitory effect of the dihydrotriazines involves the reduction of folic acid since the inhibitory effect of the compounds could be entirely negated by rather small amounts of a reduced form of folic acid.

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