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Analogs of Tetrahydrofolic Acid. XXIII. 1-(ω-Phenylalkyl)-4,6-diamino-1,2-dihydro-*s*-triazines as Inhibitors of Dihydrofolic Reductase (1,2)

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A series of 1-(ω-phenylalkyl)-4,6-diamino-1,2-dihydro-*s*-triazines was synthesized by acid catalyzed condensation of 1-(ω-phenylalkyl)biguanides with acetone, aromatic aldehydes and ω-phenylalkyl aldehydes; the intermediate biguanides were prepared by fusion of ω-phenylalkylamines with cyanoguanidine at 150°. 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-(4-phenylbutyl)- and 1-(3-phenylpropyl)-*s*-triazines (Xe and Xd) were potent inhibitors of dihydrofolic reductase, being complexed with the enzyme about two hundred times better than the substrate, dihydrofolic acid; further Xd and Xe were about 15-fold better inhibitors of dihydrofolic reductase than the earlier investigated 1-(*p*-chlorophenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine, the antimalarial drug.

When the 2,2-dimethyl group was replaced by *p*-acetamidophenyl (IX) or phenyl (VIII), in inhibitors such as Xd and Xe, activity was reduced only 5-30 fold; the corresponding change in 1-(*m*-chlorophenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine (V) gave a 19,000-fold reduction in activity with the 2-(*p*-acetamidophenyl) analog (VII) and a 550-fold decrease in activity with the 2-phenyl analog. These decreases in activity are attributed to a combination of intramolecular steric effects, intermolecular (enzyme-inhibitor) steric inhibition of binding, and inductive effects. By evaluation of suitable candidate compounds, these effects were partially separated for individual study.

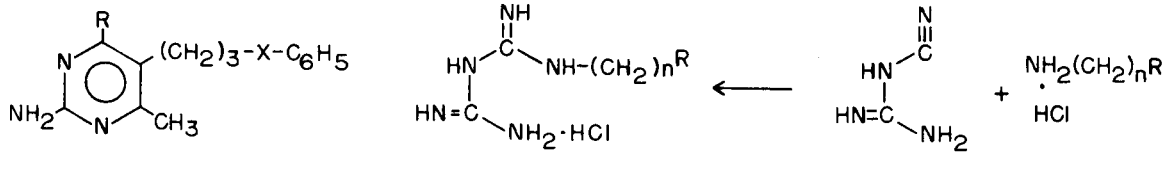
That 2,4-diamino-5-arylpyrimidines and 1-aryl-4,6-diamino-1,2-dihydro-*s*-triazines (such as V) are potent inhibitors of folic reductase *in vivo* and *in vitro* has been known for some years (3). The coupling of these observations with the observed strong inhibition of dihydrofolic reductase by 5-(3-anilinopropyl)-2,4-diamino-6-methylpyrimidine (I) (4) suggested that 4,6-diamino-1,2-dihydro-*s*-triazines bearing an ω-anilinoalkyl or ω-phenylalkyl (X) side chains at the 1-position be synthesized and evaluated as dihydrofolic reductase inhibitors; since the 5-(4-phenylbutyl) side-chain on 2-amino-4-hydroxy-6-methylpyrimidine (II) gave even better binding to dihydrofolic reductase than the corresponding pyrimidine with a 5-(3-anilinopropyl) side chain (III) (5), the synthesis and enzymic evaluation of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(4-phenylbutyl)-*s*-triazine (Xe) was undertaken first.

The phenylbutyl triazine (Xe) was indeed an excellent inhibitor of dihydrofolic reductase (Table I), being about three-fold more effective than the corresponding 1-phenyl triazine, Xa (3b).

In order to determine if the number of methylene groups between the phenyl and triazine moieties could influence the inhibitory properties, the remaining three derivitives with 1, 2 or 3 methylene groups were synthesized. The phenylpropyl triazine (Xd) was just as potent as the phenylbutyl triazine (Xe), but the 1-phenylethyl (Xc) and 1-benzyl (Xb) triazines were remarkably poor inhibitors in comparison, being 20-100 times less effective on the dihydrofolic reductase from pigeon liver. That the 1-benzyl triazine (Xb) would be expected to be a poorer inhibitor of dihydrofolic reductase than the 1-phenyl triazine (Xa) might have been anticipated from the results previously obtained with 5-(*p*-chlorophenoxy)- and 5-*p*-chlorophenyl-6-methyl-2,4-diaminopyrimidines as inhibitors of the dihydrofolic reductase from this source (3b). Whether longer side chains than phenylbutyl would increase or decrease the potency remains to be determined; similarly, whether halogen substitution on the benzene ring will increase potency as seen by conversion of Xa to V (3b) (Table I), also remains to be determined.

The anilinopropyl group of I and III presumably binds to a locus on the enzyme where the *p*-aminobenzoyl moiety of dihydrofolic reductase is bound (4,6), whereas the aryl group of V and 5-aryl-2,4-diaminopyrimidines presumably has a different locus of binding on the enzyme (4b). It was therefore considered possible that a *m*-phenylethylphenyl side-chain (XIc) or a *m*-benzylphenyl side-chain (XIb) on a triazine might give even greater potency by binding at both loci. Unfortunately, no such increment in binding was observed, that is, XIb and XIc had about the same potency as the phenylbutyl triazine (Xe).

Since there was no increment in binding with XI compared to Xe, it appeared that only one of the

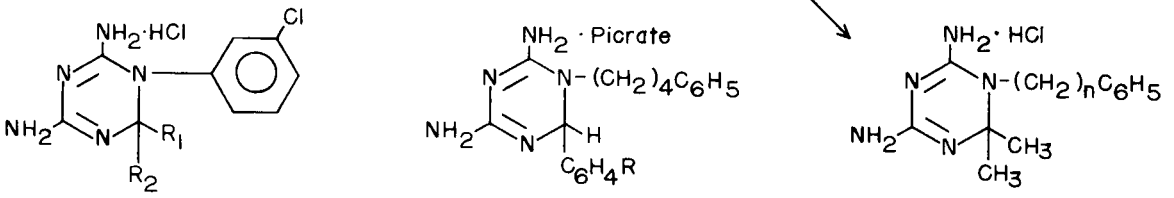


I, X = NH, R = NH_2

II, X = CH_2, R = OH

III, X = NH, R = OH

IV



V, R_1 = R_2 = CH_3

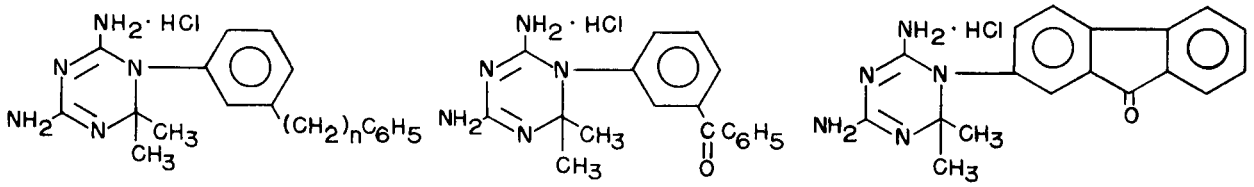
VI, R_1 = H, R_2 = $C_6H_5(CH_2)_n$-

VII, R_1 = H, R_2 = 4-$AcNHC_6H_4$-

VIII, R = H

IX, R = 4-AcNH

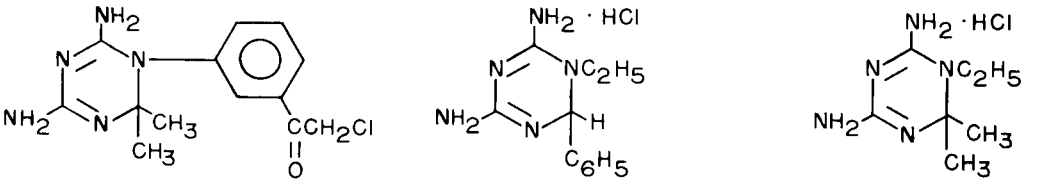
X



XI

XII

XIII



XIV

XV

XVI

a series, n = 0

b series, n = 1

c series, n = 2

d series, n = 3

e series, n = 4

two phenyl groups of XIb or XIc was binding to the enzyme. It is difficult to establish which one of two benzene rings of XIb and XIc was binding to the enzyme. However, a notable 50-fold decrease in binding was observed when the methylene group of XIb was converted to a ketone; the same order of decrease in binding was noted with a 1-phenyl triazine bearing an aliphatic ketone group (XIV) (7); these data would indicate that at least in the case of the benzoyl ketone (XII) all of the phenyl binding could be accounted for by the 1-phenyl group of the triazine and not the benzoyl, although to establish such a point with more certainty is again difficult. With the conformationally fixed fluorenonyl group (XIII) inhibition is decreased another 80-fold, showing that there is less tolerance on the enzyme surface for such a large, flat group.

It has been previously proposed (4b) that the 1-aryl group of V or the corresponding 5-aryl group on a 2,4-diaminopyrimidine might be complexed to an enzymic locus - presumably an imidazole ring - that acts as a proton donor during the TPNH reduction of (dihydro)folic acid. Molecular models confirm what is readily apparent to the eye, that is, the phenyl of a phenylbutyl group (Xe) cannot fold back to occupy the same position as the phenyl group of Xa due to the attachment of the alkyl group to the 1-position of the triazine. However, it is still possible for the phenylbutyl group to fold back in order to complex the phenyl with the "imidazole" locus, but approach it from a different angle than the 1-phenyl group of Xa. The fact that the 2-phenyl group in a 1-phenyltriazine (VIa) causes a 550-fold loss in binding compared to the 2,2-dimethyl triazine (V) (3b) suggested a way in which additional light could be shed on this problem.

There are at least three ways in which the 2-phenyl group of VIa could cause a reduction in binding

TABLE I

Inhibition of Dihydrofolic Reductase by 1-R_1-2-R_2-2-R_3-4,6-Diamino-1,2-dihydro-*s*-triazine Hydrochlorides

Compound number	R_1	R_2	R_3	μM Conc. for 50% Inhibition	Inhibitor: Substrate (a)
V	3-ClC_6H_5-	CH_3	CH_3	0.012 (b)	0.0020
A	4-ClC_6H_4-	CH_3	CH_3	0.44 (b)	0.072
Xa	C_6H_5-	CH_3	CH_3	0.11	0.018
Xb	$C_6H_5CH_2$-	CH_3	CH_3	3.3	0.55
Xc	$C_6H_5(CH_2)_2$-	CH_3	CH_3	0.71	0.12
Xd	$C_6H_5(CH_2)_3$-	CH_3	CH_3	0.028	0.0047
Xe	$C_6H_5(CH_2)_4$-	CH_3	CH_3	0.041	0.0068
XIb	3-$C_6H_5CH_2C_6H_4$-	CH_3	CH_3	0.019	0.0032
XIc	3-$C_6H_5(CH_2)_2C_6H_4$-	CH_3	CH_3	0.024 (c)	0.0040
XII	3-$C_6H_5COC_6H_4$	CH_3	CH_3	1.1	0.18
XIV (d)	3-$ClCH_2COC_6H_4$-	CH_3	CH_3	1.7	0.26
XIII	2-fluorenonyl	CH_3	CH_3	85.	14.
VIa	3-ClC_6H_4-	H	C_6H_5	5.5 (b)	0.19
VII	3-ClC_6H_4-	H	4-$AcNHC_6H_4$-	190.	32.
XVI	C_2H_5-	CH_3	CH_3	220.	37.
XV	C_2H_5-	H	C_6H_5	15,000.	2500.
VIb	3-ClC_6H_4-	H	$C_6H_5CH_2$-	1.1	0.18
VIc	3-ClC_6H_4-	H	$C_6H_5(CH_2)_2$-	0.070	0.012
VIII (e)	$C_6H_5(CH_2)_4$-	H	C_6H_5	1.2 (e)	0.20
IX (e)	$C_6H_5(CH_2)_4$-	H	4-$AcNHC_6H_4$-	0.62 (e)	0.10

Dihydrofolic reductase was a 45-90% ammonium sulfate fraction isolated from pigeon liver acetone powder and assayed with 6 μM dihydrofolate and 12 μM TPNH in 0.05 M Tris buffer (*p*H 7.4) as previously described (4a). (a) Ratio of concentration of inhibitior to 6 μM dihydrofolic acid giving 50% inhibition. (b) Previously reported in reference 3b. (c) The same concentration for 50% inhibition was also required for inhibition at twice, one-half, or one-quarter the usual concentration of enzyme thus showing that the inhibition was independent of the enzyme concentration (11), that the inhibitor was not "titrating" the enzyme (11b) in a stoichiometric manner (11c), that the observed inhibition was therefore due to competition between substrate and inhibitor, and the inhibition was not due to pseudo-irreversible inhibition (11b) of the enzyme as in the case of amethopterin at *p*H 5.9 (11c, 11e). (d) B. R. Baker and B.-T. Ho, unpublished. (e) Picrate salt; a separate experiment demonstrated that picric acid at this concentration showed no inhibition of the enzyme.

TABLE II

Physical Constants of 1-R_1-2-R_2-2-R_3-4,6-Diamino-1,2-dihydro-*s*-triazine Hydrochlorides

Compound Number	R_1	R_2	R_3	Method; (Reaction time, hrs.)	% Yield	m.p. °C	Analyses Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
VIb (a)	3-ClC_6H_4-	$C_6H_5CH_2$-	H	B (190)	36	212-215 (b)	54.9	4.89	20.0	54.8	5.00	19.9
VIc	3-ClC_6H_4-	$C_6H_5(CH_2)_2$-	H	B (48)	48	206-211 (c)	56.1	5.26	19.2	56.1	5.23	19.1
VII	3-ClC_6H_4-	4-$AcNHC_6H_4$-	H	B (120)	65	184-186 (b)	51.9	4.61	21.4	51.7	4.73	21.1
VIII (d)	$C_6H_5(CH_2)_4$-	C_6H_5-	H	E (17)	28	187-189 (e)	54.5 (d)	4.76	20.4	54.9	4.76	20.1
IX (d)	$C_6H_5(CH_2)_4$-	4-$AcNHC_6H_4$-	H	E (24)	15	177-178 (e)	53.4 (d)	4.81	20.8	53.1	4.58	21.0
Xb	$C_6H_5CH_2$-	CH_3	CH_3	D (48)	65	187-188 (b)	53.8	6.77	26.2	53.5	7.00	25.9
Xc	$C_6H_5(CH_2)_2$-	CH_3	CH_3	D (20)	30	199-203 (f)						
Xd	$C_6H_5(CH_2)_3$-	CH_3	CH_3	D (20)	22	203-206 (b)	56.8	7.50	23.7	56.8	7.56	23.5
Xe	$C_6H_5(CH_2)_4$-	CH_3	CH_3	C (24)	26	206-207 (c)	58.1	7.81	22.6	58.4	7.83	22.4
XIb	3-$C_6H_5CH_2C_6H_4$-	CH_3	CH_3	A (17)	84	180-181 (b)	62.9	6.45	20.4	62.6	6.43	20.5
XIc (g)	3-$C_6H_5(CH_2)_2C_6H_4$	CH_3	CH_3	A (17)	75	185-186 (b)	63.8	6.76	19.6	63.7	6.90	19.3
XII	3-$C_6H_5\overset{O}{\overset{\|}{C}}C_6H_4$-	CH_3	CH_3	A (17)	87	200-202 (b)	60.4	5.63	19.6	60.2	5.72	19.3
XIII	2-fluorenonyl	CH_3	CH_3	B (17)	73	230-233 (b)	60.8	5.10	19.7	60.7	5.16	19.5
XV	C_2H_5	C_6H_5	H	E (20)	53	248-249 (c)	52.1	6.36	27.6	52.0	6.17	27.4
XVI	C_2H_5	CH_3	CH_3	D (20)	16 (h)	206-207 (c)	40.9	7.84	34.0	41.0	7.77	33.8
Xa	C_6H_5	CH_3	CH_3	A (17)	74	200-202 (b, i)						

(a) A picrate salt could be prepared in 95% ethanol and recrystallized from 50% ethanol to give yellow crystals, m.p. 210-212°. *Anal.* Calcd. for $C_{16}H_{16}N_5Cl \cdot C_6H_5N_3O_7$: C, 48.7; H, 3.53; N, 20.6. Found: C, 48.7; H, 3.60; N, 20.4. (b) Recrystallized from absolute alcohol-ether. (c) Recrystallized from absolute alcohol-petroleum ether. (d) Picrate salt. (e) Recrystallized from 50% ethanol. (f) Lombardino (19) has reported m.p. 195-198°. (g) The *m*-amino-1,2-diphenylethane needed for this triazine was prepared by catalytic reduction of *m*-nitrostilbene (20a) in the presence of 5% palladium-charcoal (20b). (h) The acetone-insoluble crude crystals were obtained in 78% yield, m.p. 168-171°; no attempt was made to recover additional material from the recrystallization filtrate. (i) Modest (21) has reported m.p. 200-203° and yield of 63%.

TABLE III

Physical Constants of 1-R-Biguanide Hydrochlorides

R	% Yield	m.p. °C	Analyses Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
C_2H_5	47	186-187 (a)	29.0	7.30	42.3	29.3	7.42	42.5
$C_6H_5CH_2$- (b)	33 (c)	197-199 (c)						
$C_6H_5(CH_2)_3$-	46	143-145 (a)	51.7	7.09	27.4	51.8	7.20	27.3
$C_6H_5(CH_2)_4$-	62	162-164 (a)	53.4	7.47	26.0	53.1	7.69	25.7
2-fluorenonyl (d)	75	238-240 (e)	57.0	4.47	22.2	57.0	4.61	22.4
3-ClC_6H_4- (d)	48	199-200 (f)						

(a) Recrystallization from absolute alcohol-ether. (b) It was necessary to run the fusion at 170° for 1 hour to get melting. Shapiro *et al.* (19a) record a m.p. of 196-197°, but do not record the yield. (c) Recrystallized from absolute alcohol. (d) Prepared by the general method of Curd and Rose (14). (e) Recrystallized from methanol-ether. (f) Curd and Rose (14) record a yield of 30% and m.p. 208°.

compared to the 2-methyl group of V:

(a) The 2-phenyl group could force the 1-phenyl group somewhat out of coplanarity with the triazine ring, a phenomenon known to reduce activity (3b, 8).

(b) The 2-phenyl group, being at a 54° angle with the plane of the triazine ring, may sterically interact intermolecularly with the enzyme, thus making it difficult for both the triazine and 1-phenyl group to approach their proper loci on the enzyme surface.

(c) The 2-phenyl group, through an inductive effect on the triazine ring, may cause a decrease in binding of the 4,6-diaminotriazine moiety.

In order to study effects (b) and (c) without effect (a), 4,6-diamino-1,2-dihydro-1-ethyl-2-phenyl-*s*-triazine (XV) and the corresponding 1-ethyl-2,2-dimethyl-*s*-triazine (XVI) were synthesized and evaluated as inhibitors of dihydrofolic reductase. The binding of XVI to the enzyme should give a base-line for the amount of binding of the 4,6-diamino-*s*-triazine system without the contribution of the 1-phenyl. Fifty percent inhibition of dihydrofolic reductase by XVI was observed at 0.22 millimolar concentration under the assay conditions, whereas the 2-phenyl analog (XV) required 15 millimolar; this 70-fold reduction in binding by the 2-phenyl group of XV cannot be due to factor (a). However, since the 2-phenyl group on the 1-phenyl-*s*-triazine (VIa) caused a 550-fold reduction in binding compared to V and since the 2-phenyl group on the 1-ethyl-*s*-triazine (XV) caused only a 70-fold reduction in binding compared to XVI, the remaining 8-fold difference can most probably be attributed to the intramolecular steric factor (a).

That the intermolecular steric factor (b) could be an important reason for reduced activity was supported by two lines of evidence. Introduction of a 2-(*p*-acetamidophenyl) group (VII) caused a still further reduction in activity, being 19,000-fold less effective than the parent 2,2-dimethyl derivative (V). This further reduction in binding of VII compared to V cannot be due to factor (a) since the *p*-acetamido group does not come in contact with the 1-phenyl group. An increased inductive factor (c) is not likely since the acetamido group has a sigma-value of near zero (9). Therefore the intermolecular steric interaction with the enzyme is the most likely explanation.

The second line of evidence in favor of the steric factor (b) was obtained by synthesizing and evaluating the 2-benzyl (VIb) and 2-phenylethyl (VIc) analogs, which are sufficiently removed from the triazine to reduce the inductive effect (c) and the intramolecular steric effect to negligable quantities. The 2-benzyl analog (VIb) was only a 5-fold better inhibitor than the 2-phenyl analog (VIa), whereas the 2-phenylethyl analog (VIc) was an 80-fold better inhibitor; in fact, VIc was only 6-fold less effective than the parent 2,2-dimethyl analog (V). Since molecular models show that the 2-benzyl group of VIb does not interact with the 1-phenyl group (factor (a)) and since inductive effects are negligable (factor (c)), then the intermolecular steric interaction of the benzyl group with the enzyme (factor (b)) is the most logical explanation. Thus, if the 2-substituent is sterically interacting with the enzyme, this effect decreases in the order of *p*-acetamidophenyl (VII), phenyl (VIa), benzyl (VIb), phenylethyl (VIc), methyl (V).

Since these dihydrotriazines containing a 2-aryl group have an asymmetric center at C-2, it would be worth investigating the effect of resolution of VIa, VIII or XV on activity. The inductive effect (c) and the intramolecular steric effect (a) will remain the same in the *d*- and *l*-isomers, but the intermolecular steric effect (b) should be considerably different due to the 2-phenyl group being 54° above the plane of the triazine ring when one isomer complexes with the enzyme, and 54° below the plane with the other isomer.

The effect of a 2-aryl substituent on the inhibition of dihydrofolic reductase by the 4,6-diamino-1-

phenylbutyl-*s*-triazine system was then investigated. Replacement of the 2,2-dimethyl group of Xe by 2-phenyl (VIII) gave a 30-fold reduction in binding to the enzyme, about the same order (70-fold) as observed with 1-ethyl series (XV and XVI), but clearly a magnitude different (550-fold) than in the 1-(3-chlorophenyl) series (V and VIa). Thus, the intramolecular steric effect (a) is clearly not influencing binding of the phenyl of the phenylbutyl group, but the effects (b) and (c) remain characteristic of phenyl substitution in the ethyl series (XV and XVI). It is therefore not possible to answer the question posed earlier: does the phenyl of the phenylbutyl triazine (Xe) bind to the locus where the *p*-aminobenzoyl moiety of folic acid is bound or does the phenylbutyl group fold back to the "imidazole" locus where the 1-phenyl group of V is presumably bound. It is clear that the binding of the 1-phenyl group of Xe to the enzyme is not hindered by introduction of either a 2-phenyl (VIII) or 2-(*p*-acetamidophenyl) groups (IX) regardless of which locus the 1-phenylbutyl group is bound. Further studies to resolve this question are continuing with bicyclic pyrimidine derivatives where the phenyl group cannot fold back to the "imidazole" locus.

Regardless of the mode of binding of the 1-phenylbutyl group, two utilities arise from the study of the 2-aryl derivatives which were the underlying goal of all this work. A structure such as 2-(*p*-acetamidophenyl)-4,6-diamino-1,2-dihydro-1-phenylbutyl-*s*-triazine (IX) is still an excellent inhibitor of dihydrofolic reductase, being complexed with the enzyme about 10-fold better than the substrate, dihydrofolate; therefore structures related to IX where the acetamido group has been replaced by a group capable of forming a covalent linkage with the enzyme are good candidates for active-site-directed irreversible inhibitors (10) of dihydrofolic reductase. Similarly, a covalent forming group could be placed on the 2-phenyl group of 1-(*m*-chlorophenyl)-4,6-diamino-1,2-dihydro-2-phenylethyl-*s*-triazine (VIc), which binds to dihydrofolic reductase about 80-times better than substrate. In contrast, 1-aryl-2-(*p*-acetamidophenyl)triazines such as VII are poor candidates because of the 300-fold loss in reversible binding compared to IX, attributed to steric effects (a) and (b) which might become further magnified as the acetamido group is modified or its position on the benzene ring is changed in VII.

CHEMICAL METHODS

Synthesis.

Thorough studies of the synthesis of 1-aryl-4,6-diamino-1,2-dihydro-*s*-triazines such as V-VII has been reported by Modest (12) and Carrington *et al.* (13). 1-Arylbiguanide hydrochlorides (IVa, R = aryl) can be synthesized by reaction of cyanoguanidine with the arylamine hydrochloride in water (14). Ring closure of IVa (R = aryl) with aldehydes or methyl ketones afford dihydrotriazines of type V-VII (12b, 13); Modest (12b) has called this the "two-component" method. Since the conditions for formation of the 1-arylbiguanide hydrochloride and the reaction with a ketone are similar, direct interaction of cyanoguanidine, aryl amine hydrochloride and ketone can be performed to give the dihydro-*s*-triazine directly - the so-called "three-component" method (12a). Modest (12a) has reported that the dihydro-*s*-triazines are best distinguished from the aryl biguanides with ammoniacal cupric sulfate; this sensitive color test for biguanides is negative with the dihydro-*s*-triazines and in this work is used to check the absence of biguanide in our dihydro-*s*-triazine preparations and to follow completeness of reaction; the ultraviolet spectra of these two classes of compounds can be used for differentiation of the pure compounds, but the cuprammonium test is obviously better for a mixture.

Five of the compounds in Table II were prepared by the "three component" method (12a) with some variants. The 1-(3-chlorophenyl)-2-phenyl-*s*-triazine (VIa) (3b) was prepared by condensation of benzaldehyde with *m*-chloraniline and cyanoguanidine in ethanol containing one mole-equivalent of 12 N aqueous hydrochloric acid; compounds XIc and XV were prepared similarly. In the case of XIf, the hydrochloride salt of *m*-aminodiphenylmethane was prepared separately, then condensed with cyanoguanidine in dry acetone.

Since the previously described method for the preparation of the amines required for synthesis of XI and XII was improved, a few comments are in order. *m*-Nitrobenzophenone (15) was selectively reduced catalytically in benzene in the presence of 5% palladium-on-charcoal, which afforded a 95% yield of *m*-aminobenzophenone; this catalytic method was considered simpler than the reported chemical reductions by iron and hydrochloric acid (16a) or stannous chloride in hydrochloric acid (16d). Oelschläger (17) has catalytically reduced both the nitro and oxo groups by adding dropwise a solution of *m*-nitrobenzophenone in glacial acetic acid to a suspension of the catalyst in glacial acetic acid - sulfuric acid at 70°; the high temperature and dropwise addition is presumably needed to keep the sulfate salt from coating and inactivating the catalyst and the mineral acid is necessary to promote hydrogenolysis of the intermediate carbinol. This cumbersome procedure has now been considerably simplified by using methanesulfonic acid as the promoter; the methanesulfonate salts of product and intermediates are soluble in acetic acid and the reduction proceeded smoothly at room temperature in standard fashion in 86% yield.

Four of the 1-aryl-*s*-triazines (XIII, VII, VIb, VIc) were made by the standard "two-component" method (12b). Reaction of 1-(3-chlorophenyl)biguanide hydrochloride (14) with hydrocinnamaldehyde in absolute alcohol to give VIc proceeded considerably more rapidly in the presence of an extra 0.5 mole-equivalent of hydrochloric acid (12b), however, the yield was twice as great without additional hydrogen chloride even though the reaction time was considerably longer. Therefore, compounds VIb and VII

were prepared without additional hydrochloric acid, the reactions requiring 5-8 days reflux for completion.

The alkyl biguanides (IV) with 1-ethyl or 1-phenylalkyl groups used as intermediates for the preparation of the 1-alkyltriazines, VIII-X, XV and XVI were prepared by fusion of cyanoguanidine with the appropriate amine hydrochloride at 155° according to the method of Shapiro *et al.* (18). Modest (12) was unable to synthesize 1-alkyl-1,2-dihydrotriazines by his methods. However, by continous removal of water using anhydrous calcium sulfate in the thimble of a Soxhlet apparatus, Lombardino (19) was able to prepare triazines of this type by the "two-component" method when a 0.2 mole-equivalent of excess hydrogen chloride was present; with aliphatic aldehydes or acetone he obtained 7-20% yields and with aromatic aldehydes he obtained 20-50% yields. The 2-aryl-*s*-triazines, VIII, IX, and XV, have now been prepared in 28, 15, and 53% yields respectively by direct addition of anhydrous calcium sulfate to the alcoholic reaction medium; no excess hydrochloric acid was used in the case of the 2-(*p*-acetamidophenyl)-*s*-triazine (IX) in order to avoid excessive alcoholysis of the acetamido function. Since two of the compounds (VIII, IX) did not give crystalline hydrochlorides, they were isolated as their crystalline picrates.

The preparation of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-phenylethyl-*s*-triazine hydrochloride (Xc) in 20% yield has been described by Lombardino (19). Since his procedure is inconvenient for acetone condensation and since 2,2-dimethoxypropane is an excellent scavenger for water, Xc has been prepared in 30% yield by use of 2,2-dimethoxypropane in place of calcium sulfate. Similarly, Xb, Xd, and Xe were prepared in 22-30% yield. The 2,2-dimethoxypropane obviously cannot be used as a water scavenger for preparation of the 2-aryl-*s*-triazines such as VIII or XV, since the generated acetone would also form a 2,2-dimethyl-*s*-triazines such as X or XVI.

EXPERIMENTAL

Methods.

Melting points were taken on a Fischer-Johns apparatus and those below 230° are corrected. Infrared spectra were taken in KBr pellet with a Perkin-Elmer 137B spectrophotometer and ultraviolet spectra with a Perkin-Elmer 202 spectrophotometer; all of the compounds reported in this paper gave spectra in agreement with their assigned structures.

m-Aminoacetophenone.

m-Nitroacetophenone was prepared in 95% yield, m.p. 89-92°; from *m*-nitrobenzoyl chloride, benzene and aluminium chloride as previously described (15). For hydrogenation purposes the material was further purified by recrystallization from ethanol with 78% recovery, m.p. 93-94°.

A solution of 2.27 g. (10 mmoles) of *m*-nitrobenzophenone in 100 ml. of reagent grade benzene was stirred with 227 mg. of Norit for about 30 minutes, then filtered; the carbon pad was washed with 100 ml. of benzene. To the benzene solution was added 200 mg. of 5% palladium-charcoal and the mixture was shaken with hydrogen at 2-3 atm. until uptake was complete (about 1 hour). The filtered solution was spin-evaporated *in vacuo* and the residue was triturated with 5 ml. of water; yield, 1.88 g. (95%) of yellow crystals, m.p. 78-81°. One recrystallization from water gave 1.73 g. (88%) of product, m.p. 83-84°. Novák and Protiva (16a) have recorded a m.p. of 82° and a yield of 79% for this compound prepared by iron-hydrochloric acid reduction.

m-Aminodiphenylmethane.

A solution of 2.27 g. (10 mmoles) of *m*-nitrobenzophenone in 100 ml. glacial acetic acid was stirred with 227 mg. of Norit for 30 minutes, then filtered and the filter cake washed with 50 ml. of glacial acetic acid. After addition of 300 mg. of 5% palladium-charcoal and 1.92 g. (20 mmoles) of methanesulfonic acid, the mixture was shaken with hydrogen at 2-3 atm. for 24 hours when hydrogen uptake had ceased (5 mole-equivalents). The filtered solution was spin-evaporated *in vacuo* leaving a crystalline residue of the methanesulfonate salt. This salt was dissolved in 40 ml. of water; after clarification by filtration, the solution was adjusted to about *p*H 9 with 10% aqueous sodium hydroxide. The product was collected on a filter and washed with water; yield, 1.57 g. (86%), m.p. 46°, which was unchanged when recrystallized from petroleum ether. When 0.61 g. in 20 ml. of ether was treated with hydrogen chloride, a 97% yield of the salt, m.p. 163-165° was obtained. Oeschläger (17) has recorded a m.p. of 53°.

1-(*m*-Benzylphenyl)-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine hydrochloride (XIb).

A mixture of 0.659 g. (3 mmoles) of *m*-aminodiphenylmethane hydrochloride, 0.269 g. (3.2 mmoles) of cyanoguanidine and 2 ml. of acetone was refluxed with magnetic stirring for 17 hours, during which time the product separated. After 3 hours at about -10°, the product was collected on a filter and washed with acetone; yield, 0.865 g. (84%) m.p. 178-179°. Two recrystallizations from absolute alcohol-ether gave white crystals, m.p. 180-181°; ν max 3350, 3150 (NH); 1650 ($C=NH^+$); 1625, 1585, 1550-1540, 1500 (C=N, C=C, NH); 755, 712, 696 cm^{-1} (phenyl). See Table II for analytical data. This "three-component" method is listed as method A in Table II with the following slight modifications:

For synthesis of VIa, Xa, XIc, and XII, the free base such as *m*-amino-1,2-diphenylethane (20), and 1 mole-equivalent of 12 N aqueous hydrochloric acid were employed.

1-(*m*-Chlorophenyl)-4,6-diamino-1,2-dihydro-2-phenylethyl-*s*-triazine hydrochloride (VIc).

A mixture of 124 mg. (0.5 mmole) of 1-(*m*-chlorophenyl)biguanide hydrochloride (14), 100 mg. (0.745 mmole) of hydrocinnamaldehyde and 1 ml. of absolute ethanol was refluxed for 48 hours, then spin-evaporated *in vacuo*. Trituration of the residue with acetone gave 88 mg. (48%) of white crystals, m.p. 202-208°. Two recrystallizations from absolute ethanol-petroleum ether gave the analytical sample, m.p. 205-209°; ν max 3350, 3250, 3150 (NH); 1675 ($C=NH^+$); 1650, 1600, 1590, 1550, 1530, 1500 (C=N, C=C, NH); 750, 695 cm^{-1} (C_6H_5-). See Table II for analytical data. This procedure is listed as method B in Table II.

When an additional 0.5 mole-equivalent of 12 N aqueous hydrochloric acid was added to the reaction mixture at the start, crystallization occurred rapidly and after 16 hours of reflux only a 22% yield of VIc was obtained; in both cases a negative cuprammonium test was obtained at the end of the specified time, showing that no arylbiguanidine (IVa) remained.

Although Modest (12) reports that methyl ethyl ketone can also be used, we have found that methyl phenylethyl ketone fails to undergo condensation with 1-(*m*-chlorophenyl)biguanide under these conditions.

1-(4-Phenylbutyl)biguanide hydrochloride (IVe, R = C_6H_5).

Commercial 4-phenylbutylamine was converted to its hydrochloride, m.p. 167-168°, with hydrogen chloride in ether in 98% yield. A mixture of 1.86 g. of this hydrochloride (10 mmoles) and 0.84 g. (10 mmoles) of cyanoguanidine was gradually heated in an oil bath. The mixture began to liquify at 120° and was completely liquid at 130°. After being heated at 150° for 1 hour, the cooled transparent mass was triturated with acetone; yield, 1.66 g. (62%), m.p. 155-158°. Recrystallization from absolute ethanol-ether gave white crystals, m.p. 162-164°; ν max 3500, 3350, 3200 (NH); 750, 700 cm^{-1} (C_6H_5); λ max (H_2O), 233 mμ. See Table III for analytical data and for similar compounds prepared by this method.

4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-(4-phenylbutyl)-*s*-triazine hydrochloride (Xe).

A mixture of 675 mg. (2.5 mmoles) of IVe (R = C_6H_5), 6 ml. of acetone, 1 ml. of 2,2-dimethoxypropane, and 0.040 ml. (0.48 mmole) of 12 N aqueous hydrochloric acid was refluxed with magnetic stirring

for 24 hours; at no time was solution complete. After an additional 12 hours at 5°, the white solid was collected on a filter and washed with acetone; yield, 200 mg. (26%), m.p. 206-209°. Two recrystallizations from absolute alcohol-petroleum ether gave 99 mg. of white crystals, m.p. 204-206°; ν max 3350, 3150 (NH); 1675 (C=NH$^+$); 1630, 1590, 1570, 1490 (NH, C=N, C=C); 742, 698 cm^{-1} (C_6H_5); λ max (H_2O) 245 mμ. See Table II for analytical data; additional compounds prepared by this method are listed in Table II under method C.

4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-phenylethyl-*s*-triazine hydrochloride (Xc).

A solution of 1.21 g. (5 mmoles) of 1-phenylethylbiguanide hydrochloride (IVc, R = C_6H_5), 10 ml. of absolute ethanol, 2 ml. of acetone, 2 ml. of 2,2-dimethoxypropane and 0.090 ml. (1 mmole) of 12 N aqueous hydrochloric acid was refluxed for 20 hours, then spin-evaporated *in vacuo*. Trituration of the residue with acetone gave 0.425 g. (30%) of white crystals, m.p. 197-200°. Recrystallization from absolute ethanol-ether raised the m.p. to 199-203°; λ max (H_2O) 245 mμ; ν max 3350, 3150 (NH); 1675 (C=NH$^+$); 1620, 1590, 1575 (NH, C N, C-C); 750, 700 cm^{-1} (C_6H_5). Other compounds prepared in this way are listed in Table II under method D.

Lombardino (19) has recorded a m.p. of 195-198° and a yield of 20% for Xc by continous removal of water with calcium sulfate in the thimble of a Soxhlet apparatus.

4,6-Diamino-1,2-dihydro-2-phenyl-1-(4-phenylbutyl)-*s*-triazine picrate (VIII).

To a solution of 540 mg. (2 mmoles) of 1-(4-phenylbutyl)biguanide hydrochloride (IVe, R = C_6H_5), 4 ml. of absolute ethanol, 0.22 ml. (2.2 mmoles) of benzaldehyde, and 0.020 ml. (0.24 mmole) of 12 N aqueous hydrochloric acid was added 200 mg. of anhydrous powdered calcium sulfate. After being refluxed with magnetic stirring for 17 hours, the mixture was filtered and the filtrate was spin-evaporated *in vacuo*. This resultant hydrochloride of VIII could not be crystallized. To a hot solution of the residue in 3 ml. of 95% ethanol was added a hot solution of 458 mg. (2 mmoles) of picric acid in 2 ml. of 95% ethanol. After 3 days at room temperature, the yellow crystalline picrate was collected on a filter and washed with ethanol; yield, 200 mg. (28%), m.p. 187-189°. Two recrystallizations from 50% aqueous ethanol gave 159 mg. of yellow crystals of unchanged m.p. See Table II for analytical data; additional compounds prepared by this method are listed in Table II under method E.

REFERENCES

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(2) For the previous paper of this series see B. R. Baker and J. K. Coward, *J. Pharm. Sci.*, 54, in press (1965).

(3a) For a review of leading references see T. H. Jukes and H. P. Broquist, "Metabolic Inhibitors," edited by R. M. Hochster and J. H. Quastel, Academic Press, Inc., New York, N. Y., 1963, pp. 481-534; (b) B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, 53, 1137 (1964), paper XVI of this series.

(4a) B. R. Baker, B.-T. Ho, and T. Neilson, *J. Heterocyclic Chem.*, 1, 79 (1964), paper XII of this series; (b) B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, 53, 1457 (1964); paper XV of this series.

(5) B. R. Baker, B.-T. Ho, and G. B. Chheda, *J. Heterocyclic Chem.*, 1, 88 (1964); paper XIII of this series.

(6) B. R. Baker, D. V. Santi, P. I. Almaula, and W. C. Werkheiser, *J. Med. Chem.*, 7, 24 (1964); paper X of this series.

(7) B. R. Baker and B.-T. Ho, unpublished.

(8a) E. J. Modest, S. Farber, and G. E. Foley, *Proc. Am. Assoc. Cancer Research*, 1, 33 (1954); (b) E. J. Modest, *J. Org. Chem.* 21, 1 (1956); (c) B. R. Baker, W. W. Lee, W. A. Skinner, A. P. Martinez and E. Tong, *J. Med. Pharm. Chem.*, 2, 633 (1960).

(9) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill, Inc., New York, N. Y., 1940, p. 188.

(10) B. R. Baker, *J. Pharm. Sci.*, 53, 347 (1964).

(11a) O. H. Strauss and A. Goldstein, *J. Gen. Physiol.*, 26, 559 (1943); (b) W. W. Ackermann and V. R. Potter, *Proc. Soc. Exptl. Biol. Med.*, 72, 1 (1949); (c) W. C. Werkheiser, *J. Biol. Chem.*, 236, 888 (1961); (d) J. L. Webb, "Enzyme and Metabolic Inhibitors," Academic Press, Inc., New York, N. Y., 1963, Chap. III; (e) J. R. Bertino, B. A. Booth, A. L. Bieber, A. Cashmore and A. C. Sartorelli, *J. Biol. Chem.*, 239, 479 (1964).

(12a) E. J. Modest, *J. Org. Chem.*, 21, 1 (1956); (b) E. J. Modest and P. Levine, *ibid.*, 21, 14 (1956).

(13) H. C. Carrington, A. F. Crowther, and G. J. Stacey, *J. Chem. Soc.*, 1017 (1954).

(14) F. H. S. Curd and F. L. Rose, *J. Chem. Soc.*, 362 (1946).

(15) B. B. Stewart and H. A. Smith, *J. Am. Chem. Soc.*, 79, 5457 (1957).

(16a) L. Novák and M. Protiva, *Coll. Czechoslov. Chem. Comm.*, 24, 3966 (1959); (b) R. Geigy and W. Koenigs, *Ber.*, 18, 2401 (1885).

(17) H. Oelschläger, *ibid.*, 89, 2025 (1956).

(18a) S. L. Shapiro, V. A. Parrino and L. Freedman, *J. Am. Chem. Soc.*, 81, 3728 (1959); (b) *ibid.*, 81, 2221 (1959).

(19) J. G. Lombardino, *J. Med. Chem.*, 6, 213 (1963).

(20a) J. I. G. Cadogan, E. G. Duell and P. W. Inward, *J. Chem. Soc.*, 4164 (1962); (b) F. Bergmann and D. Schapiro, *J. Org. Chem.*, 12, 57 (1947).

(21) E. J. Modest, U. S. 2,900, 385; *Chem. Abstr.*, 54, 1573 (1960).

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