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Studies on Antitumor Substances. VIII.[1] Syntheses of 4-Amino-6-substituted Amino-2-substituted *sym*-Triazine Derivatives

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In order to synthesize a variety of 4-amino-6-substituted amino-2-substituted *sym*-triazine, 1-substituted biguanide was allowed to react with ethyl chloroacetate to give 4-amino-6-substituted amino-2-chloromethyl-*sym*-triazine, from which the purpose compounds were derived. The reactions of 4-amino-6-substituted amino-2-chloromethyl-*sym*-triazine with amines and alkylthiols were successfully carried out to give 4-amino-6-substituted amino-2-substituted aminomethyl-*sym*-triazine and 4-amino-6-substituted amino-2-alkylthiomethyl-*sym*-triazine, respectively. However, in the reaction with sodium alkanethiosulfonate, it was found to result in the formation of the corresponding sulfone afforded by the partial desulfurization in the intermediately formed 4-amino-6-substituted amino-2-methoxythiosulfonyl-*sym*-triazine. This sulfone was confirmed to be identical with that obtained by oxidation of the corresponding sulfide. The mercaptylation of 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine with thiourea was resulted in the formation of the corresponding disulfide rather than the thiol compound anticipated.

Biological activities have been known in some *sym*-triazine derivatives[3] and we also have found that N-amidino-4-amino-6-morpholino-*sym*-triazine-2-carboxamide[4] exerted a selective inhibitory effect on the multiplication of polio virus. In the basis of these findings, we have attempted to synthesize other derivatives of *sym*-triazine, which are properly substituted with an alkylating group, in order to find carcinostatic compounds. This paper deals with the reaction of N,N-pentamethylenebiguanide and 1-(3-oxapentamethylene)-biguanide, which have been known to possess biological activities,[5a,b] with esters of some halogenofatty acids, followed by the conversion to the purpose triazine derivatives.

Syntheses of 4-Amino-6-substituted Amino-2-substituted Aminomethyl-*sym*-triazine

N,N-Pentamethylenebiguanide and 1-(3-oxapentamethylene)biguanide were respectively submitted to react with ethyl chloroacetate or ethyl β-bromopropionate. In the reaction with ethyl chloroacetate, 4-amino-6-piperidino- or 4-amino-6-morpholino-2-chloromethyl-*sym*-triazine (I)[6a,b] anticipated was obtained in the presence of an equimolar amount of sodium methoxide at −40°. In the reaction with ethyl β-bromopropionate, however, corresponding 2-bromoethyl derivatives (II) could not be obtained under the similar reaction condition, but 4-amino-6-piperidino- or 4-amino-6-morpholino-2-(β-methoxyethyl)-*sym*-triazine

1) Part VII: S. Hayashi, M. Furukawa, J. Yamamoto and Y. Nishizima, *Chem. Pharm. Bull.* (Tokyo), **16**, 474 (1968).
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3) F.L. Rose, J.A. Hendry and A.L. Walpole, *Nature*, **165**, 993 (1950); F.S. Philips and J.B. Thiersch, *J. Pharmacol. Exptl. Therap.*, **100**, 398 (1950); M.R. Lewis and M.L. Crossley, *Arch. Biochem.*, **26**, 319 (1950); H. Iensch, *Angew. Chem.*, **50**, 891 (1937); E.J. Modest, E. Foley, M.M. Pechet and S. Farber, *J. Am. Chem. Soc.*, **74**, 855 (1952).
4) T. Ueda, S. Toyoshima, M. Furukawa and Y. Seto, *Chemotherapy*, **12**, 148 (1964).
5) *a*) K.S. Pilcher and K.F. Soikes, *Antibiotics & Chemotherapy*, **11**, 881 (1961); *b*) B. Melander, *Antibiotics & Chemotherapy*, **10**, 34 (1960); *Toxicology & Applied Pharmacol.*, **2**, 474 (1960).
6) *a*) S.L. Shapiro and C.G. Overberger, *J. Am. Chem. Soc.*, **76**, 98 (1954); *b*) S.L. Shapiro, E.S. Issac, V.A. Parrino and L. Freedman, *J. Org. Chem.*, **26**, 68 (1961).

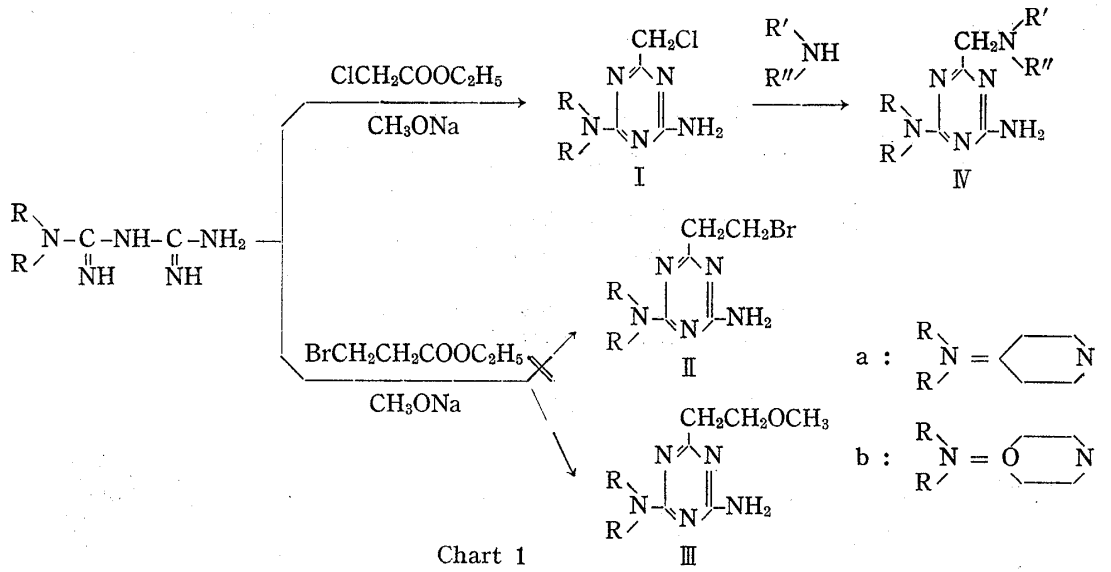
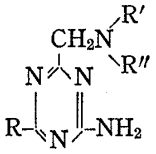


Chart 1

(III) was given in 23% and 3% yields, respectively, according to Chart 1. When the same reactions were carried out at room temperature, the yields were increased to 55% and 48%, respectively. 2-Chloromethyl derivatives (I) easily reacted with various aliphatic amines, such as piperidine, morpholine, pyrrolidine and diethanolamine, to afford the corresponding 2-substituted aminomethyl derivatives (IV). The reactions were successfully carried out by heating in ethanol for two to three hours in comperatively good yields, using one mole of 2-chloromethyl compound (I) and two moles of these amines. These products synthesized were listed in Table I. Contrary to our expectation, the reaction of 2-chloromethyl

Table I. 4-Amino-6-substituted Amino-2-substituted Aminomethyl-*sym*-triazine



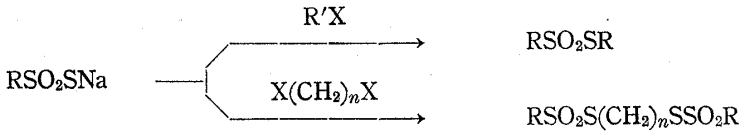
R	R′, R″	Appearance	mp (°C)	Yield (%)	Formula	Anal. (%) Calcd. C	H	N	Found C	H	N
N	N	prisms	167.5	60	$C_{14}H_{24}N_6$	60.84	8.75	30.41	61.16	8.91	30.41
N	O N	plates	153—154	75	$C_{13}H_{22}ON_6$	56.09	7.97	30.20	56.40	8.17	29.77
N	N	prisms	159	77	$C_{13}H_{22}N_6$	59.51	8.45	32.05	59.64	8.57	32.10
N	$HOCH_2CH_2$ $HOCH_2CH_2$ >N	prisms	108	93	$C_{13}H_{24}O_2N_6$	52.68	8.16	28.39	52.63	8.24	28.40
O N	N	prisms	145.5	75	$C_{13}H_{22}ON_6$	41.83	5.28	30.49	42.00	5.18	30.71
O N	O N	prisms	154	54	$C_{12}H_{20}O_2N_6$	51.41	7.19	30.00	51.41	7.40	29.52
O N	N	needles	155—156	62	$C_{12}H_{20}ON_6$	54.53	7.63	31.80	54.70	7.56	31.93
O N	$HOCH_2CH_2$ $HOCH_2CH_2$ >N	prisms	107	89	$C_{12}H_{22}O_3N_6$	48.30	7.43	28.17	48.26	7.45	28.28

derivatives (I) with ethylene imine failed to recover any product. Chlorination of 4-amino-6-substituted amino-2-diethanolamino-*sym*-triazine, prepared by treating 2-chloromethyl derivatives (I) with diethanolamine, with thionyl chloride also were unsuccessful, resulting in polymerization. Moreover, 4-amino-6-morpholino-2-chloromethyl-*sym*-triazine (Ib) only

reacted with morpholine to yield directly the hydrochloride of 4-amino-6-morpholino-2-morpholinomethyl-*sym*-triazine, even though a large excess of morpholine was used. This hydrochloride was readily converted into the free base by treating with sodium ethoxide in ethanol.

Syntheses of 4-Amino-6-substituted Amino-2-alkylmercaptomethyl-*sym*-triazine and the Related Compounds

By treating sodium alkanethiosulfonate with alkyl halide, Hayashi, *et al.*[7] have prepared the several kinds of alkyl alkanethiosulfonate having antitumor activity, according to the following scheme.



By means of the similar method, attempts were made to synthesize the corresponding-*sym*-triazinylmethyl alkanethiosulfonate from 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine (Ia) and sodium alkanethiosulfonate. Sodium alkanethiosulfonates employed were as follows: sodium methanethiosulfonate, sodium phenylmethanethiosulfonate, sodium benzenethiosulfonate and sodium *p*-toluenethiosulfonate. These reactions were satisfactorily carried out by refluxing 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine (Ia) with these sodium alkanethiosulfonate in ethanol or in acetone containing a small amount of water on account of increasing the solubility of sodium alkanethiosulfonate. The infrared spectra of the resulting products showed two absorption bands assigned to sulfonyl group at near 1300 cm^{-1} and 1100 cm^{-1}, as shown in Table II. The elemental analysis showed no corres-

Table II. Infrared Absorptions

R	$\nu_{SO_2}^{KBr}$ (cm^{-1})		R	$\nu_{SO_2}^{KBr}$ (cm^{-1})	
CH_3	1311	1128	$C_6H_4CH_3$ (*p*)	1290	1126
Ph	1304	1157	$PhCH_2$	1295	1119

pondence to 4-amino-6-piperidino-2-alkoxythiosulfonylmethyl-*s*-triazine (V), but good agreement with 4-amino-6-piperidino-2-alkylsulfonylmethyl-*sym*-triazine (VI), probably formed by the subsequent partial desulfurization in the substituent of the intermediary product (V), as shown in the Chart 2.

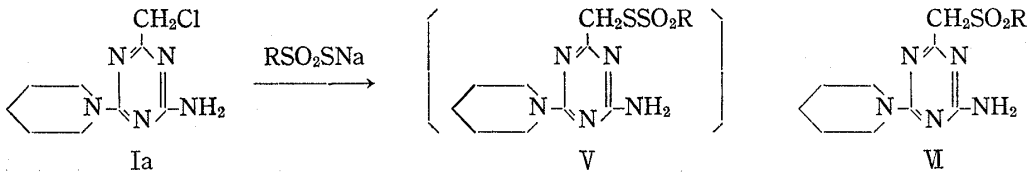


Chart 2

In order to confirm the structures of these compounds, 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine (Ia) was allowed to react with sodium phenylmercaptide, followed by the oxidation to the corresponding sulfone compound (VIII), as shown in the Chart 3. The oxidation[8] of 4-amino-6-piperidino-2-phenylmercaptomethyl-*sym*-triazine (VII) readily suc-

7) S. Hayashi, H. Ueki, S. Harano, J. Komiya, S. Iyama, K. Harano, K. Miyata, K. Niigata and Y. Yonemura, *Chem. Pharm. Bull.* (Tokyo), **12**, 1271 (1964).
8) T. Wood, *J. Am. Chem. Soc.*, **50**, 1226 (1928).

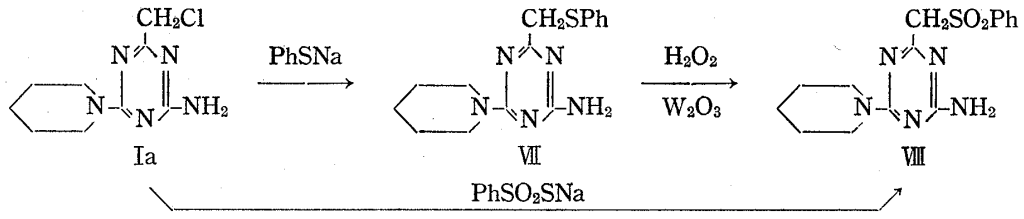
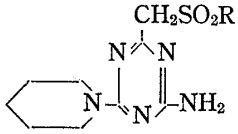


Chart 3

ceeded with excess hydrogen peroxide in the presence of the catalytic amount of tangustic acid at the temperature between 60° and 65° in ethanol. Resulting 4-amino-6-piperidino-2-phenylsulfonylmethyl-*sym*-triazine (VIII) proved to be identical with the product obtained by the reaction of 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine (Ia) with sodium benzenethiosulfonate, by mixed melting point test and infrared spectra comparison. 2-Alkylsulfonylmethyl compounds synthesized according to the Chart 2 were listed in Table III.

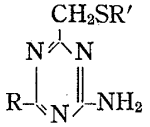
Table III. 4-Amino-6-piperidino-2-alkanesulfonylmethyl-*sym*-triazine



R	Appearance	mp (°C)	Yield (%)	Formula	Anal. (%) Calcd. C	H	N	Found C	H	N
CH_3	needles	197—198	51	$C_{10}H_{17}O_2N_5S$	44.26	6.32	25.81	44.29	6.36	26.11
Ph	needles	224—225	43	$C_{15}H_{19}O_2N_5S$	54.03	5.74	21.01	54.16	5.76	20.98
$C_6H_4CH_3(p)$	plates	222—223	33	$C_{16}H_{21}O_2N_5S$	55.31	6.09	20.16	55.51	6.19	20.36
$PhCH_2$	plates	224	64	$C_{16}H_{21}O_2N_5S$	55.31	6.09	20.16	55.52	6.16	20.22

4-Amino-6-substituted amino-2-chloromethyl-*sym*-triazine (I) readily reacted with not only sodium phenylmercaptide but also other sodium mercaptides to yield the corres-

Table IV. 4-Amino-6-substituted Amino-2-alkylthiomethyl-*sym*-triazine



R	R'	Appearance	mp (°C)	Yield (%)	Formula	Anal. (%) Calcd. C	H	N	Found C	H	N
	C_2H_5	prisms	122—123	80	$C_{11}H_{19}N_5S$	52.15	7.95	27.65	52.42	7.72	27.86
	n-C_4H_9	needles	83.5	83	$C_{13}H_{23}N_5S$	55.48	8.82	24.88	55.73	8.32	25.11
	Ph	needles	132—133	78	$C_{15}H_{19}N_5S$	59.77	6.35	23.24	60.03	6.36	23.27
	$PhCH_2$	needles	119	91	$C_{16}H_{21}N_5S$	60.92	6.71	22.20	61.11	6.67	22.25
	C_2H_5	prisms	91	86	$C_{10}H_{17}ON_5S$	47.03	6.71	27.43	47.03	6.76	27.54
	n-C_4H_9	needles	71	69	$C_{12}H_{21}ON_5S$	50.86	7.47	24.71	50.93	7.49	24.99
	Ph	needles	134	92	$C_{14}H_{17}ON_5S$	55.42	5.65	23.09	55.22	5.67	23.14
	$PhCH_2$	needles	135—136	94	$C_{15}H_{19}ON_5S$	56.75	6.03	22.06	56.76	6.03	22.10

ponding 2-alkylthiomethyl compounds under the similar condition. These compounds synthesized were summarized in Table IV.

Furthermore, in order to synthesize 4-amino-6-piperidino-2-mercatomethyl-*sym*-triazine (XI), the reaction between 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine (Ia) and thiourea was attempted, according to the Chart 4. Thus, the mixture of equimolar amount of

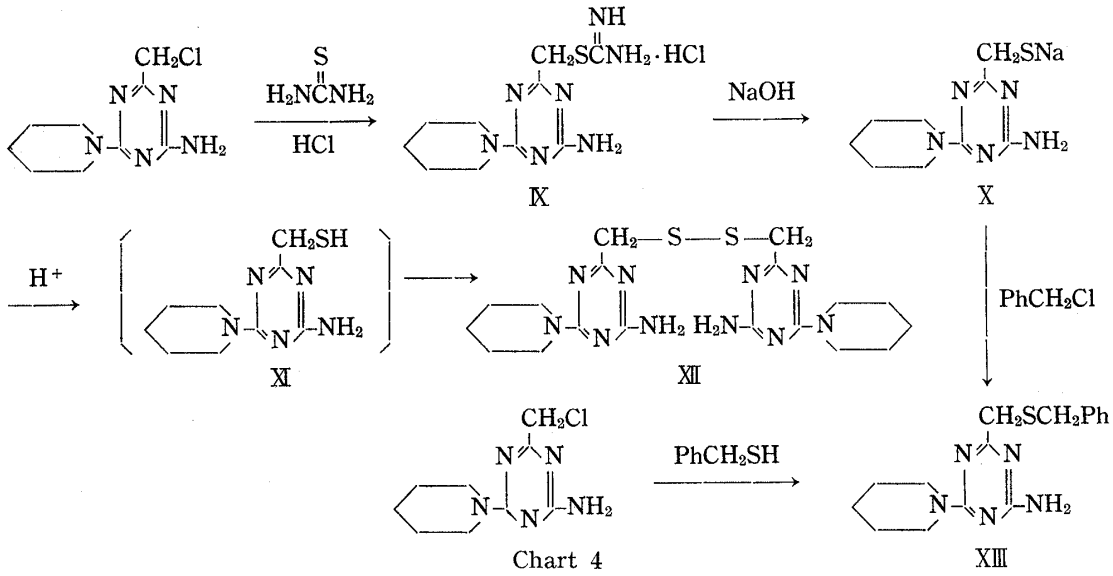


Chart 4

4-amino-6-piperidino-2-chloromethyl-*sym*-triazine (Ia) and thiourea was heated at 100° in dioxane to yield 2-(4-amino-6-piperidino-*sym*-triazinyl)methyl isothiuronium chloride (IX) in excellent yield, followed by decomposition with an aqueous solution of sodium hydroxide under heating. A small amount of the resulted precipitates was filtered off, and then the filtrate was carefully neutralized with acetic acid. The infrared spectrum of the product thus obtained showed no absorption assignable to a thiol group. Considering from the experimental elemental analysis, it seemed to be the corresponding disulfide (XII), probably afforded by the immediate oxidation of the intermediately formed thiol compound (X) in the course of neutralization. The same compound was obtained by the treatment of 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine (Ia) with sodium hydrosulfide in ethanol. In order to give evidence that the thiol compound (XI) was intermediately formed, the decomposition product of 2-(4-amino-6-piperidino-*sym*-triazinyl)methyl isothiuronium chloride (IX) with alkali was immediately submitted to react with benzyl chloride. The resulted product was confirmed to be identical with 4-amino-6-piperidino-2-benzylthiomethyl-*sym*-triazine (XIII) prepared from 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine (Ia) and benzylthiol. From these results, it might be assumed that thiol compound (XI) was immediately oxidized by air to convert into the corresponding disulfide (XII).

The pharmacological activities of the compounds synthesized will be reported in the other paper.

Experimental

4-Amino-6-morpholino-2-chloromethyl-*sym*-triazine (Ib)——It was prepared from 1-(3-oxapentamethylene)biguanide and ethyl chloroacetate by the method of Shapiro.[6] The yield was 67%. mp 178°. *Anal.* Calcd. for $C_8H_{12}ON_5Cl$: C, 41.83; H, 5.28; N, 30.49. Found: C, 42.00; H, 5.18; N, 30.71.

General Procedure for Synthesis of 4-Amino-6-substituted amino-2-(β-methoxyethyl)-*sym*-triazine (III)——1) To a solution of 18.1 g (0.1 mole) of ethyl β-bromopropionate in 80 ml of abs. MeOH containing MeONa prepared from 2.3 g (0.1 atom) of Na, 0.1 mole of 1-substituted biguanide powdered finely was added slowly at −40°. After completion of the addition, the reaction mixture was stirred for 2 hr at this temperature, and then poured onto 200 g of crushed ice. The resulted precipitates were collected by filtration and recrystallized from EtOH. By this procedure, 4-amino-6-piperidino-2-(β-methoxyethyl)-*sym*-triazine was obtained in 23% yield, which melted at 130—131°. *Anal.* Calcd. for $C_{11}H_{19}ON$: C, 55.67; H,

8.07; N, 29.52. Found: C, 55.23; H, 8.21; N, 29.15. 4-Amino-6-morpholino-2-(β-methoxyethyl)-*sym*-triazine was obtained in 3% yield, which melted at 105—106°. *Anal.* Calcd. for $C_{10}H_{17}O_2N_5$: C, 50.19; H, 7.16; N, 29.27. Found: C, 50.29; H, 7.26; N, 28.90.

2) To a solution of 0.02 mole of 1-substituted biguanide in 100 ml of abs. MeOH containing MeONa prepared from 0.46 g (0.02 atom) of Na, 0.02 mole of ethyl β-bromopropionate was added dropwise with stirring at room temperature. After completion of the addition, the reaction mixture was stirred for 3 hr at room temperature. After a half volume of MeOH was removed by evaporation, NaBr separated was filtered off. The filtrate was cooled and allowed to stand. Thus, 4-amino-6-piperidino-2-(β-methoxyethyl)-*sym*-triazine and 4-amino-6-morpholino-2-(β-methoxyethyl)-*sym*-triazine were obtained in 55% and 48% yield, respectively.

General Procedure for Synthesis of 4-Amino-6-substituted Amino-2-substituted Aminomethyl-*sym*-triazine (IV)——A mixture of 0.02 mole of 4-amino-6-substituted amino-2-chloromethyl-*sym*-triazine and 0.04 mole of amine in 100 ml of EtOH was heated to reflux for 2—3 hr. After completion of the reaction, the mixture was concentrated, and the crystals separated on cooling were collected by filtration. The product was recrystallized from EtOH or MeOH.

4-Amino-6-morpholino-2-morpholinomethyl-*sym*-triazine——A mixture of 4.6 g (0.02 mole) of 4-amino-6-morpholino-2-chloromethyl-*sym*-triazine and 3.5 g (0.04 mole) of morpholine in 100 ml of EtOH was refluxed for 3 hr. On cooling, the crystals were separated to give 4.1 g of 4-amino-6-morpholino-2-morpholinomethyl-*sym*-triazine hydrochloride melting at 292°. *Anal.* Calcd. for $C_{12}H_{20}O_2N_6 \cdot HCl$: C, 45.49; H, 6.78; N, 26.53. Found: C, 45.28; H, 6.71; N, 26.58.

General Procedure for Synthesis of 4-Amino-6-piperidino-2-alkylsulfonylmethyl-*sym*-triazine (VI)——1) A mixture of 0.02 mole of 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine and 0.02 mole of sodium alkanethiosulfonate in 150 ml of abs. EtOH was refluxed for 10 hr. The mixture was then allowed to stand overnight. The resulted precipitates were collected by filtration, washed with H_2O and recrystallized from EtOH. Analytical data were summarized in Table III.

2) To a solution of 0.02 mole of 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine in 80 ml of acetone, a solution of 0.02 mole of sodium alkanethiosulfonate in 20 ml of H_2O was added dropwise with stirring and heating. Stirring and heating were continued for additional 5—6 hr and the reaction solution was then allowed to stand overnight. The precipitates deposited were recrystallized from EtOH. Yields were as follows; 4-amino-6-piperidino-2-methanesulfonylmethyl-*sym*-triazine, 49%. 4-Amino-6-piperidino-2-benzenesulfonylmethyl-*sym*-triazine, 41%. 4-Amino-6-piperidino-2-*p*-toluenesulfonylmethyl-*sym*-triazine, 24%. 4-Amino-6-piperidino-2-phenylmethanesulfonylmethyl-*sym*-triazine, 68%.

General Procedure for Synthesis of 4-Amino-6-substituted Amino-2-alkylthiomethyl-*sym*-triazine——1) To a solution of EtONa prepared from 0.46 g (0.02 atom) of Na and 100 ml of abs. EtOH, 0.02 mole of thiol and then 0.02 mole of 4-amino-6-substituted amino-2-chloromethyl-*sym*-triazine were added. After the mixture was refluxed for 2—3 hr, the mixture was filtered to remove NaCl deposited and concentrated. The resulted precipitates were recrystallized from EtOH.

2) Alkylthiol was dissolved in a solution of an equimolar amount of NaOH in H_2O. To this solution, an equimolar amount of 4-amino-6-substituted amino-2-chloromethyl-*sym*-triazine and a suitable amount of EtOH were added and the mixture was refluxed for 2—3 hr. Then, EtOH was removed by evaporation and the residue was washed with H_2O and recrystallized from MeOH.

Oxidation of 4-Amino-6-piperidino-2-phenylthiomethyl-*sym*-triazine (VII) to 4-Amino-6-piperidino-2-benzenesulfonylmethyl-*sym*-triazine (VIII)——To a solution of 1.5 g (0.005 mole) of 4-amino-6-piperidino-2-phenylthiomethyl-*sym*-triazine in 60 ml of EtOH containing a trace of tungstic acid, 30% aqueous solution of 0.02 mole of H_2O_2 was added dropwise with stirring at 60—65°. Stirring was continued for additional 3 hr at this temperature and the reaction mixture was filtered. The precipitates deposited on cooling were recrystallized from EtOH to show mp 224—225°. Yield was 71%. It showed no depression of melting point on admixture with an authentic sample of 4-amino-6-piperidino-2-benzenesulfonylmethyl-*sym*-triazine.

2-(4-Amino-6-piperidino-*sym*-triazinyl)methyl Isothiuronium Chloride (IX)——A mixture of 4.6 g (0.02 mole) of 4-amino-6-piperidino-2-chloromethyl-*sym*-triazine and 1.6 g (0.021 mole) of thiourea in 50 ml of dioxane was heated with stirring at 100°. The white precipitates were gradually deposited during the heating. After cooling, the precipitates were collected by filtration and recrystallized from EtOH to give needles melting at 196° (decomp.). The yield was almost theoretical. *Anal.* Calcd. for $C_{10}H_{17}N_7S \cdot HCl$: C, 39.53; H, 5.94; N, 32.27. Found: C, 39.37; H, 5.88; N, 32.44.

Bis(4-amino-6-piperidino-*sym*-triazinylmethyl) disulfide (XII)——To an aqueous solution of 30 ml of 2N NaOH was added 5.8 g (0.02 mole) of 2-(4-amino-6-piperidino-*sym*-triazinyl)methyl isothiuronium chloride. The mixture was heated with stirring for 2 hr on a water bath. A small amount of the precipitates deposited were filtered off and the filtrate was carefully neutralized with 10% AcOH. The resulted crystals were recrystallized from EtOH to give prisms melting at 194—195°. Yield was 31%. *Anal.* Calcd. for $C_{18}H_{28}N_{10}S_2$: C, 48.19; H, 6.71; N, 31.23. Found: C, 48.79; H, 6.46; N, 30.69.

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