[Chem. Pharm. Bull. 12 (5) 607 ~ 614] UDC 547.495.9 : 547.461.4

87. Kaname Takagi and Takeo Ueda : Reaction of 1-Substituted Biguanide with Diethyl Formylsuccinate and Diethyl Succinate.*1

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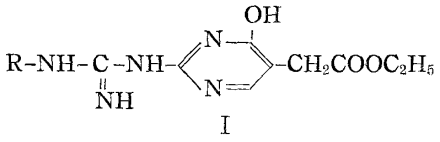
Recently, Furukawa,[1] one member of our group synthesized several compounds of 4,6-diamino-*s*-triazine-2-carboxamide having a biguanido moiety in structure by the condensation of 1-substituted biguanide with diethyl oxalate, and then Ueda, *et al.*[2] showed that some of these compounds especially 4-(N-morpholino)-6-amino-*s*-triazine-2-carboxyguanidide exerted a significant effect on viruses of polio, measles and common cold in tissue culture and PR-8 strain of influenza A virus in mice. In these studies, it was assumed that the effect of this compound on viruses of polio, measles and common cold might be due to the existence of guanidino group, while that on the influenza virus might be ascribed to other partial structure, probably morpholino group and/or triazine ring.

These findings led the authors to conceive an idea to synthesize heterocyclic compounds having biguanido moiety and carboxamido rest in their structures.

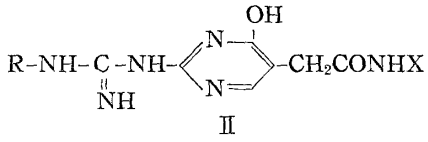
For the purpose of finding new antiviral agents, attempts were made to synthesize ethyl 2-(substituted guanidino)-4-hydroxy-5-pyrimidineacetate, ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propionate, their acid hydrazides and acid guanidides.

Synthesis of Ethyl 2-(Substituted guanidino)-4-hydroxy-5-pyrimidineacetate

At first, the synthesis of ethyl 2-(substituted guanidino)-4-hydroxy-5-pyrimidineacetate (I) was considered as the intermediate to the objective 5-acetamido derivatives (II). No compound of this series has ever been reported. However, the compounds related to this series were reported by some researchers.[3~5]



I



II

(X stands for $-NH_2$ or $-C(=NH)-NH_2$)

Todd, *et al.*[3] and Andersag, *et al.*[4] obtained several derivatives of ethyl 4-hydroxy-5-pyrimidineacetate by reacting amidines with diethyl formylsuccinate, as shown in Chart 1.

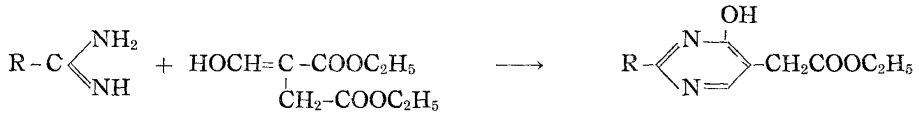


Chart 1.

*1 Papers were read at the 83rd Annual Meeting of the Pharmaceutical Society of Japan (1963).

*2 Shinano-machi Shinjuku-ku, Tokyo (高木 要, 上田武雄).

1) M. Furukawa, T. Ueda : This Bulletin, **11**, 596 (1963).
2) T. Ueda, S. Toyoshima, M. Furukawa, T. Seto : The 82nd Annual Meeting of the Pharmaceutical Society of Japan (1962).
3) A. R. Todd, F. Bergel, H. L. Fraenkel-Conrat, A. Jacob : J. Chem. Soc., **1936**, 1601.
4) H. Andersag, K. Westphal : Ber., **70**, 2035 (1937).
5) E. Peters, H. J. Minnemeyer, A. W. Spears, H. Tieckelmann : J. Org. Chem., **25**, 2137 (1960).

These facts suggested that I might be produced by the condensation of 1-substituted biguanide with diethyl formylsuccinate. This assumption was supported by the finding[6] that 2-arylguanidino-6-methyl-4-pyrimidinol was obtained by the reaction of 1-arylbiguanide with ethyl acetoacetate.

Thus, attempts were made to condense some compounds of 1-substituted biguanide with diethyl formylsuccinate in anhydrous ethanol, and several compounds of type (I) were exclusively obtained by the reaction between the both reactants, as shown in Chart 2.

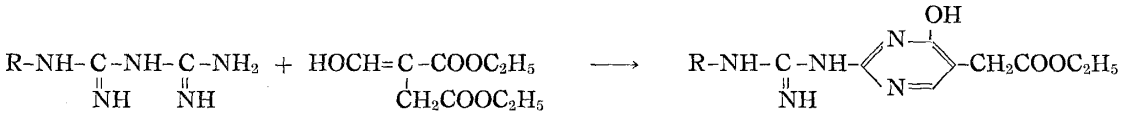
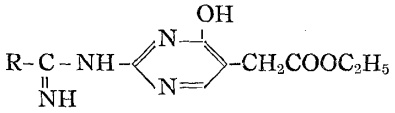
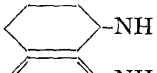
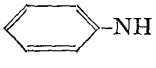


Chart 2.

The structures of these products were confirmed by examining their infrared absorption spectra and their analytical data.

The infrared spectra of the products showed the band assignable to C=O in the pyrimidone form of hydroxypyrimidine near 1650 cm^{-1}, and the band of ester carbonyl group at 1730 cm^{-1}. The compounds synthesized are listed in Table I.

Table I. 

R	m.p. (°C)	Yield (%)	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
	167	40	$C_{11}H_{17}O_3N_5$	49.43	6.41	26.20	49.31	6.22	26.39
	171~172	28	$C_{14}H_{21}O_3N_5$	54.71	6.89	22.79	54.54	6.73	23.04
	177	60	$C_{13}H_{19}O_4N_5$	50.48	6.19	22.65	50.27	5.95	22.81
	189~190	24	$C_{15}H_{23}O_3N_5$	56.06	7.21	21.79	56.25	7.08	21.92
	211	75	$C_{15}H_{17}O_3N_5$	57.13	5.43	22.21	56.92	5.29	22.28

Next, the authors made an attempt to synthesize the 5-pyrimidineacetate derivatives through another synthetic process, as shown in Chart 3.

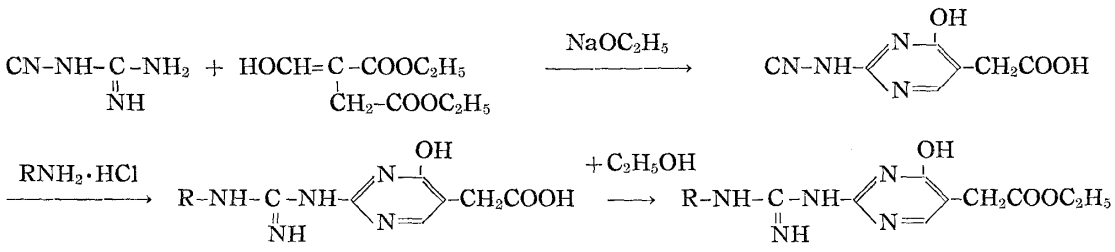


Chart 3.

6) F. H. S. Curd, F. L. Rose : J. Chem. Soc., **1946**, 362.

Pohl[7] reported that the reaction of dicyanodiamide with β-keto-ester such as ethyl acetoacetate and ethyl α-formylpropionate afforded 2-cyanaminopyrimidine derivatives. His finding suggested that this reaction would be held true of that between dicyanodiamide and diethyl formylsuccinate.

In fact, 2-cyanamino-4-hydroxy-5-pyrimidineacetic acid was obtained in a good yield by condensing dicyanodiamide with diethyl formylsuccinate in anhydrous ethanol in the presence of sodium ethoxide, as shown in Chart 3. In this reaction, the employment of an alkali catalyst was proved indispensable for the formation of the condensation product.

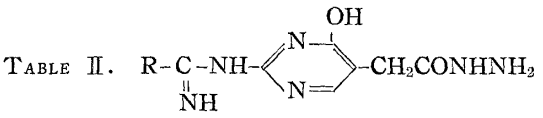
The infrared spectrum of the product showed the strong bands at 2220 cm^{-1} and 1730 cm^{-1} assigned to C≡N and to C=O in carboxyl group. Also, the analytical data of the product was found favorable to the formation of 2-cyanamino-4-hydroxy-5-pyrimi dineacetic acid.

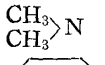
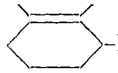
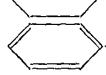
The reaction of 2-cyanamino-4-hydroxy-5-pyrimidineacetic acid with several amine hydrochlorides were succeedingly examined. Anilinium chloride was found to produce, in a good yield, hydrochlorides of 2-phenylguanidino-4-hydroxy-5-pyrimidineacetic acid by heating with 2-cyanamino-4-hydroxy-5-pyrimidineacetic acid in water. Hydrochlorides of aliphatic amines such as dimethylamine, piperidine, morpholine and cyclohexylamine did not give the corresponding guanidino derivatives by the reaction in water, but produced the objective guanidino derivatives by the reaction in pyridine. The products thus obtained were easily converted into corresponding ethyl esters by the heating in 5 per cent ethanolic hydrochloric acid.

These esters were identified by the mixed melting point method and the comparison of their infrared spectra with those of the products obained by the condensation of biguanide with diethyl formylsuccinate.

Reaction of Ethyl 2-(Substituted guanidino)-4-hydroxy-5-pyrimidineacetate with Hydrazine and Guanidine

The compounds (I) described above were converted, in success, to the corresponding acid hydrazides by the reaction with hydrazine hydrate, to obtain pharmacologically 1-substituted active agents. The hydrazides synthesized are listed in Table II.

TABLE II. 

R	m.p. (decomp.) (°C)	Reaction solvent	Yield (%)	Formula	N (%) Calcd.	N (%) Found
	230~231	ethanol	60	$C_9H_{15}O_2N_7$	38.72	38.78
	212~213	〃	51	$C_{12}H_{19}O_2N_7$	33.45	33.26
	226~227	〃	62	$C_{11}H_{17}O_3N_7$	33.21	33.39
	245~247	pyridine	57	$C_{13}H_{21}O_2N_7$	31.92	31.73
	266~267	〃	30	$C_{13}H_{15}O_2N_7$	32.56	32.77

Analogously, attempts were made to react I with guanidine to prepare the corresponding 5-pyrimidineacetoguanidides. The reaction product by this process, however, did not crystallize and any of the objective compound could not be obtained.

7) F. Pohl: J. Prak. Chem., (2) **77**, 542 (1908).

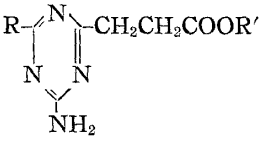
Synthesis of Ethyl 4-(Substituted amino)-6-amino-*s*-triazine-2-propionate

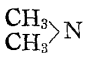
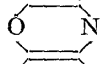
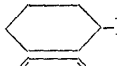
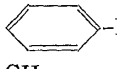
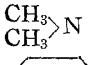
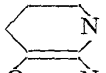
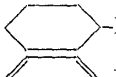
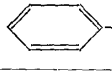
Attempts were made to synthesize ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propionate as the intermediate to 4-(substituted amino)-6-amino-*s*-triazine-2-propioguanidide of pharmacological interest.

Sokolovskaya, *et al.*[8] already reported that the reaction of 1-phenylbiguanide with diethyl succinate gave 4-phenylamino-6-amino-*s*-triazine-2-propionic acid, its ethyl ester and 2,2′-ethylenebis(4-phenylamino-6-amino-*s*-triazine), in the presence of sodium ethoxide. According to the method of Sokolovskaya, a mixture of 1-substituted biguanide and diethyl succinate was heated on a water bath in the presence of sodium ethoxide. The reaction resulted in the formation of thick precipitates.

The reaction mixture was divided into the ethanol soluble filtrate and insoluble precipitates by the filtration. The latter was further divided into the water soluble and water insoluble part by the treatment with water.

From the ethanolic filtrate, ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propionate was obtained after evaporation of the solvent. The water soluble part, when it was made acidic with acetic acid, deposited 4-(substituted amino)-6-amino-*s*-triazine-2-propionic acid. This product was found convertible in a good yield to the corresponding ethyl ester by heating with 5 per cent ethanolic hydrochloric acid. The water insoluble part was identical with 2,2′-ethylenebis[4-(substituted amino)-6-amino-*s*-triazine] by the comparison with the authentic sample which had been prepared by the reaction of ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propionate with 1-substituted biguanide having the same substituent group to that of the former. The synthetic processes are shown in Chart 4 and the compounds obtained are listed in Table Ⅲ and Ⅳ.

TABLE Ⅲ. 

R	R′	m.p. (°C)	Recryst. solvent	Formula	N (%) Calcd.	N (%) Found
	H	239～240	ethanol-water	$C_8H_{13}O_2N_5$	33.16	33.28
	〃	207～208	〃	$C_{11}H_{17}O_2N_5$	27.87	27.73
	〃	227	〃	$C_{10}H_{15}O_3N_5$	27.66	27.48
	〃	195～196	〃	$C_{12}H_{19}O_2N_5$	26.40	26.49
	〃	225～226	ethanol	$C_{12}H_{13}O_2N_5$	27.02	27.05
	C_2H_5	83～84	petr. benzin	$C_{10}H_{17}O_2N_5$	29.27	29.11
	〃	79～81	〃	$C_{13}H_{21}O_2N_5$	25.07	24.96
	〃	125	petr. benzin-ethanol	$C_{12}H_{19}O_3N_5$	24.91	24.73
	〃	92～93	petr. benzin	$C_{14}H_{23}O_2N_5$	23.87	23.72
	〃	129	ethanol	$C_{14}H_{17}O_2N_5$	24.38	24.91

8) S. V. Sokolovskaya, V. N. Sokolova, O. Yu Magidson : Zhur. Obshchei Khim., **27**, 1968 (1957) (C. A., **52**, 5426 (1958)).

Table V. R-(2,4-diamino-s-triazin-6-yl)-CH_2CH_2-(2,4-diamino-s-triazin-6-yl)-R

R	m.p. (°C)	Formula	N (%) Calcd.	N (%) Found
$(CH_3)_2N$	267~268	$C_{12}H_{20}N_{10}$	46.02	45.79
piperidino	241~243	$C_{18}H_{28}N_{10}$	36.43	36.31
morpholino	277~277.5	$C_{16}H_{24}O_2N_{10}$	36.06	35.89
anilino	211~212	$C_{20}H_{32}N_{10}$	33.94	34.07
o-toluidino	261~262	$C_{20}H_{20}N_{10}$	34.86	34.75

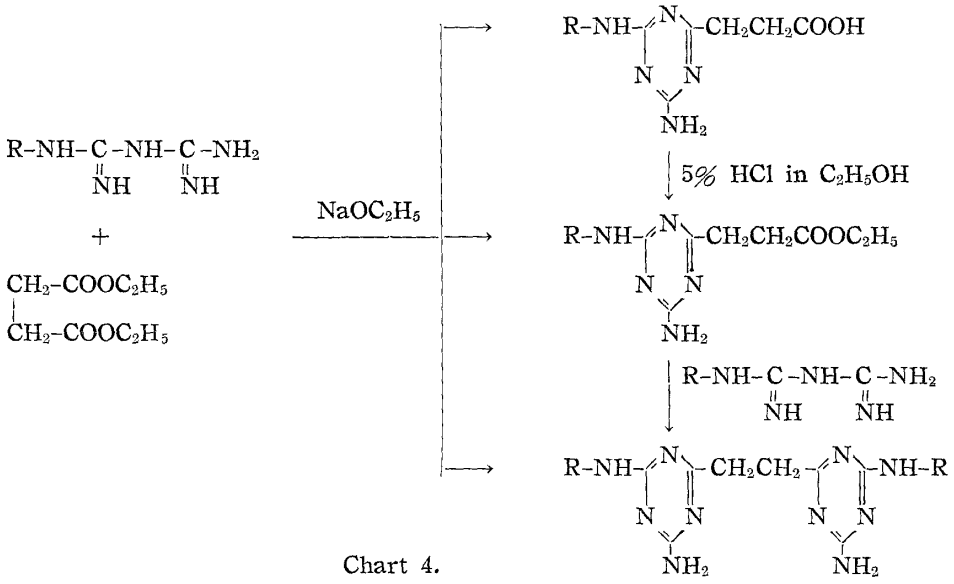


Chart 4.

Reaction of Ethyl 4-(Substituted amino)-6-amino-*s*-triazine-2-propionate with Hydrazine and Guanidine

The reaction of ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propionate with hydrazine hydrate was tried to obtain the objective acid hydrazide. In this reaction, 4-(substituted amino)-6-amino-*s*-triazine-2-propionic acid hydrazide was readily produced without any solvent, as shown in Chart 5. The hydrazides synthesized are listed in Table V.

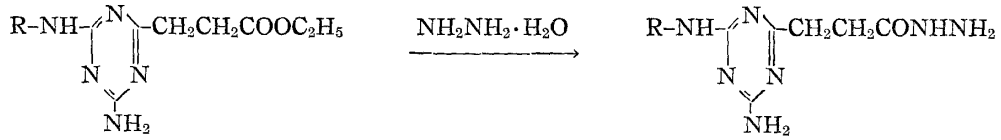
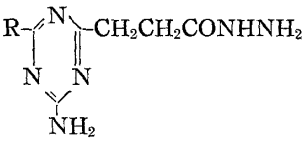
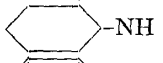
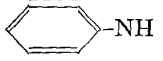


Chart 5.

The reaction of the above ester with guanidine was successively undertaken to prepare the objective propioguanidide.

Furukawa[1] showed that 4-(N-morpholino)-6-amino-*s*-triazine-2-carboxyguanidide was obtained by the reaction of ethyl 4-(N-morpholino)-6-amino-*s*-triazine-2-carboxylate

TABLE V. 

R	m.p. (°C)	Recryst. solvent	Formula	N (%) Calcd.	N (%) Found
	186	ethanol	$C_8H_{15}ON_7$	43.52	43.55
	181～182	〃	$C_{11}H_{19}ON_7$	36.96	37.02
	178	〃	$C_{10}H_{17}O_2N_7$	36.69	36.89
	142～143	〃	$C_{12}H_{21}ON_7$	35.11	34.96
	190～191	ethanol-water	$C_{12}H_{15}ON_7$	35.88	35.84

with guanidine. This finding suggested that this reaction would be held true of that between triazine-2-propionic acid ester and guanidine. Thus, attempts were made to condense ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propinate with guanidine in anhydrous ethanol. This reaction, however, did not afford any objective acid guanidide, but give guanidinium salt of 4-(substituted amino)-6-amino-*s*-triazine-2-propionic acid, as shown in Chart 6. The guanidinium salts obtained are listed in Table Ⅵ.

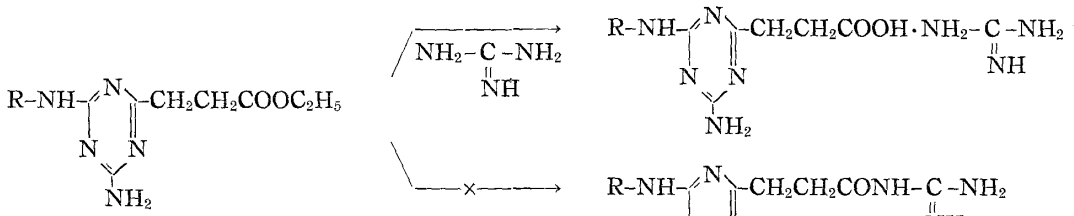
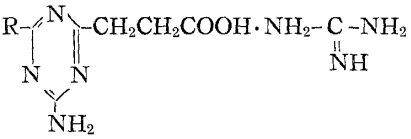
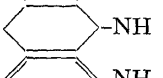
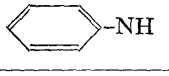


Chart 6.

TABLE Ⅵ. 

R	m.p. (decomp.) (°C)	Formula	N (%) Calcd.	N (%) Found
	205～207	$C_9H_{18}O_2N_8$	41.47	41.29
	127～129	$C_{12}H_{22}O_2N_8$	36.11	35.88
	223～225	$C_{11}H_{20}O_3N_8$	35.89	35.83
	198～201	$C_{13}H_{24}O_2N_8$	34.56	34.58
	236～237	$C_{13}H_{18}O_2N_8$	35.22	35.03

These guanidinium salts were identified by the mixed melting point method and the comparison of their infrared spectra with the authentic samples prepared by the reaction of 4-(substituted amino)-6-amino-*s*-triazine-2-propionic acid with guanidine.

In this reaction, it is inferred that the hydrolysis of the ester might take place before the formation of propioguanidide, owing to the strong basicity of guanidine.

As described above, the authors synthesized new derivatives of 2-guanidino-5-pyrimidineacetic acid and 4,6-diamino-*s*-triazine-2-propionic acid, having biguanido moiety and carboxamido rests. These compounds are of interest as antiviral and pharmacologically active agents.

The pharmacological studies with these agents will be published in a medical journal in the near future.

Experimental

Synthesis of Ethyl 2-Phenylguanidino-4-hydroxy-5-pyrimidineacetate——1) To a mixture of 4.5 g. of 1-phenylbiguanide hydrochloride and 0.5 g. of Na in 30 ml. of anhyd. EtOH, 4 g. of freshly distilled diethyl formylsuccinate was added dropwise at a room temperature. The mixture was stirred for 5 hr. and then refluxed for 1 hr. After cooling, the precipitates deposited were collected and recrystallized from EtOH-pyridine.

2) A mixture of 1 g. of 2-cyanamino-4-hydroxy-5-pyrimidineacetic acid and 0.7 g. of anilinium chloride in 30 ml. of H_2O was heated under reflux for 10 hr. After cooling, the precipitates deposited were collected, washed with H_2O and dried. The product was then suspended in 30 ml. of 5% ethanolic HCl and the mixture was heated under reflux for 3 hr. After cooling, the precipitates separated were collected and dissolved in H_2O. The aqueous solution was made weakly alkaline with 10% NaOH. The precipitates separated were recrystallized from pyridine.

General Method for Ethyl 2-(Substituted guanidino)-4-hydroxy-5-pyrimidineacetate——1) To a mixture of 0.025 mole of 1-substituted biguanide hydrochloride and 0.5 g. of Na in 30 ml. of anhyd. EtOH, 4 g. of freshly distilled diethyl formylsuccinate was added dropwise at 20-25°. The solution was stirred for 5 hr. at this temperature, and then warmed at 50~55° for 1 hr. After cooling, the reaction mixture was filtered. The filtrate was evaporated *in vacuo* and a small amount of AcOEt was added. The precipitates deposited were recrystallized from EtOH. 2-Cyclohexylguanidino derivative was purified by the recrystallization from MeOH-pyridine.

2) A solution of 1 g. of 2-cyanamino-4-hydroxy-5-pyrimidineacetic acid and 0.005 mole of amine hydrochloride in 30 ml. of pyridine was heated under reflux for 15 hr. The reaction mixture was concentrated to dryness *in vacuo*. The residue was then heated with 30 ml. of 5% ethanolic HCl for 3 hr. After removal of the solvent, the precipitates were dissolved in H_2O. The aqueous solution was made weakly alkaline with 10% NaOH and the precipitates separated were recrystallized from EtOH.

Synthesis of 2-Cyanamino-4-hydroxy-5-pyrimidineacetic Acid——A mixture of 3.5 g. of dicyanodiamide, 8 g. of diethyl formylsuccinate and 0.9 g. of Na in 100 ml. of anhyd. EtOH was heated under reflux for 3 hr. After cooling, the precipitates deposited were collected and dissolved in H_2O. The aqueous solution was made acidic with HCl and the precipitates separated were recrystallized from EtOH to colorless plates, m.p. 247~250° (decomp.). Yield, 5 g. *Anal.* Calcd. for $C_7H_6O_3N_4$: N, 28.86. Found : N, 29.05.

General Method for Ethyl 4-(Substituted amino)-6-amino-*s*-triazine-2-propionate and 2,2′-Ethylenebis[4-(substituted amino)-6-amino-*s*-triazine]——To a mixture of 0.025 mole of 1-substituted biguanide hydrochloride and 1.2 g. of Na in 50 ml. of anhyd. EtOH, was added 4.4 g. of diethyl succinate. The mixture was refluxed for 5 hr. and allowed to cool. The reaction mixture was filtered by suction. The ethanolic filtrate was evaporated and the residue was treated with H_2O. Ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propionate was obtained in colorless prisms. Yield, 1~1.6%.

The precipitates were treated with H_2O and the H_2O insoluble part was removed by filtration. The aqueous filtrate, when it was made acidic with AcOH, deposited 4-(substituted amino)-6-amino-*s*-triazine-2-propionic acid, which was recrystallized from EtOH or EtOH-H_2O to colorless needls. Yield, ca. 25%. The above *s*-triazine-2-propionic acid was esterified by heating with 5% ethanolic HCl for 3 hr. Yield, ca. 70% (see Table III).

The H_2O insoluble part was recrystallized from EtOH-H_2O to 2,2′-ethylenebis[4-(substitued amino)-6-amino-*s*-triazine]. Yield, ca. 22%.

2,2′-Ethylenebis[4-(substituted amino)-6-amino-*s*-triazine] were also prepared by the following method. To a mixture of 0.01 mole of 1-substituted biguanide hydrochloride and 0.4 g. of Na in 50 ml. of anhyd.

EtOH, was added 0.01 mole of ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propionate having same substituent to that of the former. The mixture was refluxed for 10 hr. After cooling, the colorless crystals were collected and recrystallized from EtOH-H_2O. Yield, ca. 10%.

General Method for 2-(Substituted guanidino)-4-hydroxy-5-pyrimidineacetic Acid Hydrazide——A mixture of 1 g. of ethyl 2-(substituted guanidino)-4-hydroxy-5-pyrimidineacetate and 1 g. of $NH_2NH_2 \cdot H_2O$ in 30 ml. of EtOH or pyridine (see Table II) was refluxed for 2 hr. The solution allowed to stand overnight. The precipitates deposited were collected and recrystallized from EtOH-H_2O.

General Method for 4-(Substituted amino)-6-amino-*s*-triazine-2-propionic Acid Hydrazide——A mixture of 0.01 mole of ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propionate and 2 ml. of $NH_2NH_2 \cdot H_2O$ was heated at 100° for several hours on a water bath. After cooling, the crude hydrazide deposited was collected, washed with H_2O and recrystallized from EtOH to colorless needles.

General Method for Guanidinium Salt of 4-(Substituted amino)-6-amino-*s*-triazine-2-propionic Acid——1) A mixture of 0.04 mole of ethyl 4-(substituted amino)-6-amino-*s*-triazine-2-propionate and 0.5 g. of the free base guanidine in anhyd. EtOH was heated under reflux for 5 hr. After removal of the solvent *in vacuo*, a small amount of Et_2O was added. The precipitates deposited were collected and recrystallized from EtOH-Et_2O.

2) To a ethanolic solution of guanidine, prepared from 0.5 g. of guanidinium chloride and 0.1 g. of Na in 20 ml. of anhyd. EtOH, was added 0.5 g. of 4-(substituted amino)-6-amino-*s*-triazine-2-propionic acid. The mixture was warmed for several min. on a water bath and then filtered. The filtrate was evaporated *in vacuo* and the residue was treated with a small amount of Et_2O. The precipitates deposited were recrystallized from EtOH-Et_2O.

Summary

Condensation of 1-substituted biguanide with diethyl formylsuccinate gave ethyl 2-(substituted guanidino)-4-hydroxy-5-pyrimidineacetate, which was also prepared through the reaction of 2-cyanamino-4-hydroxy-5-pyrimidineacetic acid with amine hydrochloride. The ethyl esters of 5-pyrimidineacetic acids were converted into corresponding hydrazides with hydrazine hydrate.

Condensation of 1-substituted biguanide with diethyl succinate gave 4-(substituted amino)-6-amino-*s*-triazine-2-propionic acid, its ethyl ester and 2,2′-ethylene-bis[4-(substituted amino)-6-amino-*s*-triazine]. The ethyl esters of *s*-triazine-2-propionic acids gave corresponding hydrazides with hydrazine hydrate. The reaction of ethyl esters of *s*-triazine-2-propionic acids with guanidine did not afford any objective acid guanidides, but gave guanidinium salt of 4-(substituted amino)-6-amino-*s*-triazine-2-propionic acid.

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