Synthetic Antibacterials. I. Nitrofurylvinyl-s-triazine Derivatives

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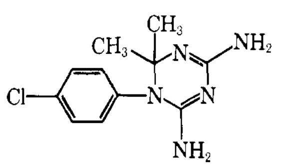
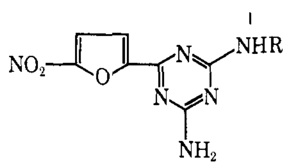
The preparation of several 2,4-disubstituted 6-[(5-nitro-2-furyl)vinyl]-*s*-triazines and 2,4-diamino-6-(5-nitro-2-furyl)-*s*-triazines is described. Some members of the series display high antibacterial activities *in vitro* against gram-positive and gram-negative organisms.

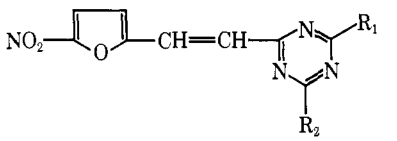
1-*p*-Chlorophenyl-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine (I), a metabolite of chlorguanide (N[1]-*p*-chlorophenyl-N[5]-isopropylbiguanide), was reported to have high antimalarial activity.[1] Some 2,4-diamino-6-(5-nitro-2-furyl)-*s*-triazines (II) showed antibacterial activity against gram-positive and gram-negative organisms.[2] These facts prompted us to investigate systematic syntheses of the *s*-triazine derivatives in an effort to obtain useful antibacterial agents. Our initial efforts have been directed toward the preparation of the vinylogs (III) of II in expectation of enhanced activity.[3] This paper describes the synthesis



I



II



III

of several 2,4-disubstituted 6-[(5-nitro-2-furyl)vinyl]-*s*-triazines (III), and the antibacterial testing data for these and other derivatives are discussed.

Chemistry.—The condensation of ethyl 5-nitro-2-furylacrylate with substituted biguanide was first tried to obtain III, but all attempts under various conditions were unsuccessful, starting materials being recovered in every case. Therefore, the general procedure, which consists of treatment of 5-nitrofurfural with *s*-triazines possessing an active methylene group, has been carried out to produce these vinylogs (III).

The key intermediates, *s*-triazine derivatives, were prepared by the following methods. 2,4-Dichloro-6-methyl-*s*-triazine (IV) was obtained from cyanuric chloride and MeMgBr according to the procedure described by Hirt, *et al.*[4] Amination of IV with amines at low temperature gave the 2-amino-4-chloro-6-methyl-*s*-triazines (V).[5] Displacement of the chloro group in V by alkoxides proceeds smoothly to give the 2-alkoxy-4-amino-6-methyl-*s*-triazines (VI). Compound V was converted to 2,4-diamino-6-methyl-*s*-triazines (VII) by further amination with appropriate amines in higher temperature. On the other hand, the general procedure of Overberger, *et al.*,[6] was also followed in the preparation of 2-alkyl-4,6-diamino-*s*-triazines (VII) from alkylbiguanides and esters. Condensation of 1,1-dimethylbiguanide hydrochloride and ethyl acetate in the presence of sodium ethoxide afforded the corresponding *s*-triazine along with a by-product, which proved to be identical with 1,1-dimethylbiguanide acetate. 2-Amino-4-cyanomethyl-6-dimethylamino-*s*-triazine was prepared by displacement of the chlorine of 2-amino-4-chloromethyl-6-dimethylamino-*s*-triazine[7] with KCN. The *s*-triazines that were prepared are listed in Table I.

Treatment of the *s*-triazines with 5-nitrofurfural led to the formation of desired vinylogs (III). The reaction was generally performed by heating the reactants in AcOH or Ac_2O in the presence or absence of catalysts such as concentrated H_2SO_4 or KOAc. Using the *s*-triazines substituted with primary amine as starting materials and Ac_2O for this condensation, the acetyl derivatives were isolated. The latter were hydrolyzed to the corresponding amino derivatives by boiling in alcoholic HCl. When 2,4-diamino-6-[α-alkyl-β-(5-nitro-2-furyl)vinyl]-*s*-triazines were refluxed with Ac_2O, the diacetyl derivatives were formed, which were converted to the monoacetyl derivatives by boiling for 16 hr in a mixture of water and DMF (see Scheme I). The physical and analytical properties of 2,4-disubstituted 6-[(5-nitro-2-furyl)vinyl]-*s*-triazines are summarized in Table II.

For the purpose of the comparison in activity with their respective vinylogs four nitrofuryl-*s*-triazines were prepared: 2,4-diamino-,[2a] 2-amino-4-methylamino-, 2-amino-4-dimethylamino-, and 2-amino-4-isopropylamino-6-(5-nitro-2-furyl)-*s*-triazines[2b] (Table III).

Screening Results.—The antibacterial activities of the compounds herein reported against *Escherichia coli* 0-55, *Staphylococcus aureus* 209P, *Bacillus subtilis* PCI 219, *Proteus vulgaris* HX-19, and *Aerobactor aerogenes* were tested *in vitro*. As can be seen from Table IV, most of these compounds possess activity against both gram-negative and gram-positive organisms. The data for 2,4-diamino(5-nitro-2-furyl)-*s*-triazines were listed in Table V for the comparison. From these data the following observations are apparent: (1) com-

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

(2) (a) R. U. Schock, U. S. Patent 2,885,400 (1959); (b) W. R. Sherman, *J. Org. Chem.*, **26**, 88 (1961).

(3) Several examples suggest the fact that the introduction of a conjugated double bond between the nitrofuryl group and the end group of the side chain may result to enhance the *in vitro* activity. For example, see T. Takahashi, H. Saikachi, S. Yoshina, and C. Mizuno, *Yakugaku Zasshi*, **69**, 284 (1949); *Chem. Abstr.*, **44**, 5372 (1950), and T. Sasaki, *Chem. Pharm. Bull.* (Tokyo), **2**, 104 (1954).

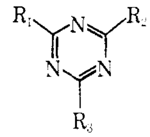
(4) R. Hirt, H. Nidecker, and R. Bechtold, *Helv. Chim. Acta*, **33**, 1365 (1950).

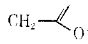
(5) R. Huffman and F. C. Schaeffer, *J. Org. Chem.*, **28**, 1816 (1963). They synthesized 2-amino-4-chloro-6-methyl-*s*-triazine by the cyclization of N,N′-dicyanoacetamidine using dry HCl in acetone.

(6) C. G. Overberger, F. W. Michelotti, and P. M. Carabateas, *J. Am. Chem. Soc.*, **79**, 941 (1957).

(7) S. L. Shapiro, E. S. Isaacs, V. A. Parrino and L. Freedman, *J. Org. Chem.*, **26**, 68 (1961).

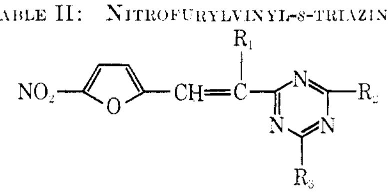
TABLE I: s-TRIAZINES

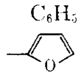


R_1	R_2	R_3	Yield, %	Mp,[f] °C	Recrystn solvent	Formula	Analyses
CH_3	NH_2	Cl	57[a]	204[g]	$CHCl_3$	$C_4H_5ClN_4$	C, H, N
CH_3	$N(CH_3)_2$	Cl	74[a]	85	$(CH_3)_2CO–H_2O$	$C_6H_9ClN_4$	C, H, N
CH_3	$NHCH_3$	NH_2	33[b]	210	MeOH	$C_5H_9N_5$	N
CH_3	$N(CH_3)_2$	NH_2	65[a] 30[b]	187–188[h]	MeOH	$C_6H_{11}N_5$	C, H, N
CH_3	$N(CH_3)_2$	$N(CH_3)_2$	86[a]	45[i]	Petr ether	$C_8H_{15}N_5$	
C_2H_5	$N(CH_3)_2$	NH_2	49[b]	146–147	EtOH	$C_7H_{13}N_5$	N
C_3H_7	$N(CH_3)_2$	NH_2	28[b]	121–122	C_6H_6	$C_8H_{15}N_5$	C, H, N
$CH_2C_6H_5$	$N(CH_3)_2$	NH_2	44[b]	140–141	MeOH	$C_{12}H_{15}N_5$	C, H, N
	$N(CH_3)_2$	NH_2	76[b]	125	$EtOH–H_2O$	$C_{10}H_{13}N_5O$	C, H, N
CH_2Cl	$N(CH_3)_2$	NH_2	52[b]	173[j]	EtOH	$C_6H_{10}ClN_5$	
CH_2CN	$N(CH_3)_2$	NH_2	85[c]	158	AcOEt	$C_7H_{10}N_6$	C, H, N
$CH(CH_3)_2$	$N(CH_3)_2$	NH_2	37[b]	150–151	$EtOH–H_2O$	$C_8H_{15}N_5$	C, H, N
CH_3	$N(CH_3)_2$	OCH_3	90[d]	87	$MeOH–H_2O$	$C_7H_{12}N_4O$	C, H, N
CH_3	$N(CH_3)_2$	OC_2H_5	92[d]	62–63	$MeOH–H_2O$	$C_8H_{14}N_4O$	C, H, N
CH_3	$N(CH_3)_2$	$OCH(CH_3)_2$	64[d]	59–61	$MeOH–H_2O$	$C_9H_{16}N_4O$	C, H, N
CH_3	$N(CH_3)_2$	OC_4H_9	58[d]	43	$MeOH–H_2O$	$C_{10}H_{18}N_4O$	C, H, N
C_2H_5	NH_2	NH_2	96[e]	300–302[k]	H_2O	$C_5H_9N_5$	
C_3H_7	NH_2	NH_2	52[e]	200–202[l]	H_2O	$C_6H_{11}N_5$	N

[a] Prepared by amination of 2,4-dichloro-6-methyl-s-triazine with appropriate amines. [b] Prepared by the condensation of an alkylbiguanide with an ester. [c] Prepared by the reaction of 2-amino-4-chloromethyl-6-dimethylamino-s-triazine with potassium cyanide. [d] Prepared by replacement of a chloro group of 2-chloro-4-dimethylamino-6-methyl-s-triazine with a sodium alkoxide. [e] Prepared by thermal cyclization of acylguanidine, see ref k. [f] All melting points are uncorrected. [g] Lit.[5] mp 206–207°. [h] Lit.[7] mp 192–193°. [i] Lit.[6] mp 45–46°. [j] Lit.[7] mp 176–179°. [k] J. K. Simons and W. Weaver [U. S. Patent 2,408,694 (1946)] reported subl > 230°. [l] Lit.[k] mp 195°.

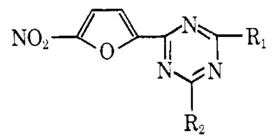
TABLE II: NITROFURYLVINYL-s-TRIAZINES



No.	R_1	R_2	R_3	Yield, %	Mp,[h] °C	Recrystn[j] solvent	Formula	Analyses
1	H	$NHCH_3$	NH_2	58[a]	219[i] dec	3% HCl–EtOH	$C_{10}H_{11}ClN_6O_3$[m]	N
2	H	$N(CH_3)_2$	$NHCOCH_3$	80[b]	199	EtOH[k]	$C_{13}H_{14}N_6O_4$	C, H, N
3	H	$N(CH_3)_2$	NH_2	86[c]	219[i] dec	EtOH[l]	$C_{11}H_{13}ClN_6O_3$[m]	C, H, N
4	H	$N(CH_3)_2$	$N(CH_3)_2$	30[b]	211	$EtOH–CHCl_3$	$C_{13}H_{16}N_6O_3$	N
5	CH_3	$N(CH_3)_2$	NH_2	28[d]	222–224	n-PrOH–$(CH_3)_2CO$[l]	$C_{12}H_{14}N_6O_3$	C, H, N
6	CH_3	$N(CH_3)_2$	$NHCOCH_3$	82[e]	124–125 dec	EtOH[k]	$C_{14}H_{16}N_6O_4$	N
7	C_2H_5	$N(CH_3)_2$	NH_2	11[d]	185 dec	EtOH[k]	$C_{13}H_{16}N_6O_3$	C, H, N
8	C_2H_5	$N(CH_3)_2$	$NHCOCH_3$	90[e]	139–140 dec	n-PrOH–MeOH	$C_{15}H_{18}N_6O_4$	C, H, N
9	C_6H_5	$N(CH_3)_2$	NH_2	78[d]	195 dec	n-PrOH[l]	$C_{17}H_{16}N_6O_3$	C, H, N
10		$N(CH_3)_2$	NH_2	66[f]	165	$EtOH–H_2O$	$C_{15}H_{14}N_6O_4$	C, H, N
11	Cl	$N(CH_3)_2$	NH_2	29[d]	244	C_6H_6	$C_{11}H_{11}ClN_6O_3$	C, H, N
12	CN	$N(CH_3)_2$	NH_2	34[d]	244	C_6H_6	$C_{12}H_{11}N_6O_3$	C, H, N
13	H	$N(CH_3)_2$	OCH_3	20[b]	244	$CHCl_3$	$C_{12}H_{13}N_5O_4$	C, H, N
14	H	$N(CH_3)_2$	OC_2H_5	33[b]	169	EtOH	$C_{13}H_{15}N_5O_4$	C, H, N
15	H	$N(CH_3)_2$	$OCH(CH_3)_2$	17[b]	174–175	EtOH	$C_{14}H_{17}N_5O_4$	N
16	H	$N(CH_3)_2$	OC_4H_9	15[b]	138	EtOH[j]	$C_{15}H_{19}N_5O_4$	C, H, N
17	H	NH_2	NH_2	66[d]	>300	DMF[l]	$C_9H_8N_6O_3$	C, H, N
18	CH_3	NH_2	NH_2	64[d]	>300	AcOH	$C_{10}H_{10}N_6O_3$	C, H, N
19	CH_3	$NHCOCH_3$	$NHCOCH_3$	87[e]	260	AcOH	$C_{14}H_{14}N_6O_5$	C, H, N
20	C_2H_5	NH_2	NH_2	69[d]	238 dec	EtOH[l]	$C_{11}H_{12}N_6O_3$	C, H, N
21	C_2H_5	NH_2	$NHCOCH_3$	94[g]	258	DMF	$C_{13}H_{14}N_6O_4$	C, H, N
22	C_2H_5	$NHCOCH_3$	$NHCOCH_3$	77[e]	260	EtOH	$C_{14}H_{16}N_6O_5$	N

[a] Condensation using Ac_2O and then hydrolysis with alcoholic 8% HCl. [b] Condensation using Ac_2O. [c] Hydrolysis of **2** with alcoholic 8% HCl. [d] Condensation using glacial AcOH and concentrated H_2SO_4. [e] Acetylation with Ac_2O. [f] Condensation using Ac_2O and KOAc. [g] Hydrolysis of **22** with aqueous DMF (1:1). [h] All melting points are uncorrected. [i] Hydrochloride. [j] All compounds were yellow needles unless noted otherwise. [k] Brown powder or crystals. [l] Yellow prisms or plates. [m] HCl salt.

TABLE III



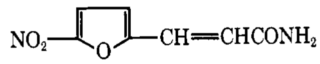
No.	R_1	R_2	Yield, %	Mp, °C	Recrystn solvent	Formula	Analyses
23	NH_2	NH_2	69	>300[d]	DMF[a]	$C_7H_6N_6O_3$	N
24	$NHCH_3$	NH_2	67	236–239	MeOH–Me_2CO[b]	$C_8H_8N_6O_3$	C, H, N
25	$N(CH_3)_2$	NH_2	41	287	MeOH–DMF[c]	$C_9H_{10}N_6O_3$	C, H, N
26	$NHCH(CH_3)_2$	NH_2	22	196–197[e]	EtOH[c]	$C_{10}H_{10}N_6O_3$	

[a] Yellow powder. [b] Brown powder. [c] Yellow needles. [d] Lit.[2a] mp >300°. [e] Lit.[2b] mp 197–197.5°.

TABLE IV
In Vitro ANTIBACTERIAL ACTIVITY OF 5-NITROFURYLVINYL-s-TRIAZINES

	Min inhib concn, μg/ml[a]				
No.	*E. coli* 0-55	*S. aureus* 209P	*B. subtilis* PCI 219	*P. vulgaris* HX-19	*A. aerogenes*
1	0.25	1	0.1	5	10
2	>20	2.5	2.5	40	>40
3	1	10	0.5	>5	>5
4	>1.56	>1.56	>1.56	>1.56	>1.56
5	3.13	1.56	0.78	>25	>25
6	6.25	0.78	0.195	>25	>25
7	>25	25	3.13	>25	>25
8	>25	6.25	1.56	>25	>25
9	>25	>25	6.25	...	...
10	>25	12.5	1.56	...	...
11	>12.5	>12.5	6.25	>12.5	...
12	>25	25	25	>25	...
13[b]	...	...	...	...	...
14	>12.5	6.25	1.56	>12.5	>12.5
15	>12.5	12.5	0.78	>12.5	>12.5
16	>3.13	>3.13	>3.13	>3.13	>3.13
17	0.5	0.25	0.5	5	2.5
18	1.56	0.78	0.195	12.5	3.13
19	3.13	1.56	1.56	>25	25
20	12.5	3.13	0.39	>25	25
21	>25	>25	>25	>25	>25
22	>25	6.25	0.78	>25	>25
5-Nitro-2-furanacrylamide[c]	1.56	3.13	0.78	25	6.25

[a] Minimum inhibitory concentration is the lowest concentration of compound that prevents visible growth after 48 hr of incubation at 37°. [b] Could not be tested due to low solubility in the common solvents. [c] This compound (see structure below) is stronger than nitrofurazone in the *in vitro* activity.



pounds **1**, **3**, **5**, **6**, **17–20** possess high antibacterial activity; the excellent activity of **1**, **17**, and **18** against *P. vulgaris* is especially noteworthy; (2) insertion of a vinyl group between the two hetero rings enhances clearly the *in vitro* activity (compare **1**, **3**, and **17** with **24**, **25**, and **23**); (3) there is no significant difference in activity between amino compounds and their acetyl derivatives.

TABLE V
In Vitro ANTIBACTERIAL ACTIVITY OF 5-NITROFURYL-s-TRIAZINES

	Min inhib concn, μg/ml[a]				
No.	*E. coli* 0-55	*S. aureus* 209P	*B. subtilis* PCI 219	*P. vulgaris* HX-19	*A. aerogenes*
23	12.5	12.5	25	>25	>25
24	5	5	2.5	50	25
25	>20	>20	1	>40	>40
26	50	10	2.5	50	50
5-Nitro-2-furanacrylamide[b]	1.56	3.13	0.78	25	6.25

[a] Minimum inhibitory concentration is the lowest concentration of compound that prevents visible growth after 48 hr of incubation at 37°. [b] See footnote *c* in Table IV.

Experimental Section

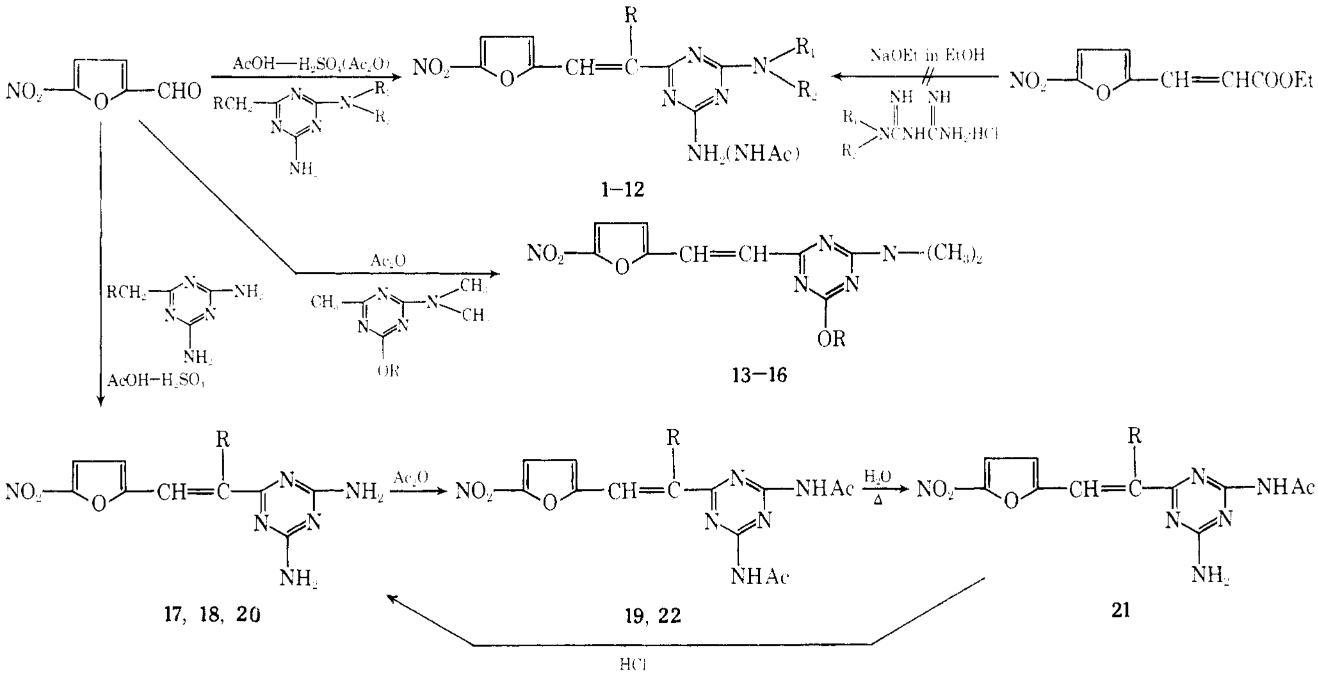
The preparation given below are the representative of the procedure indicated in Tables I–III.

2-Amino-4-chloro-6-methyl-s-triazine.—Alcoholic 12% NH_3 (18.5 g, 0.12 mole) was added dropwise over a period of 1.5 hr to a stirred solution of 2,4-dichloro-6-methyl-s-triazine (5 g, 0.03 mole) in 70 ml of C_6H_6. After stirring at room temperature for 2.5 hr longer, the precipitate which had separated was collected and crystallized from $CHCl_3$, providing a white powder (2.5 g, 57%).

2-Chloro-4-dimethylamino-6-methyl-s-triazine.—Aqueous 20% $NH(CH_3)_2$ (17 ml, 0.076 mole) was added dropwise over 1 hr to a stirred solution of 2,4-dichloro-6-methyl-s-triazine (10 g, 0.06 mole) in a mixture of 70 ml of Me_2CO and 60 ml of H_2O. The temperature of the reaction mixture was maintained below 5°. On adding K_2CO_3 (4.2 g, 0.03 mole) under stirring, crystals began to separate immediately. After stirring at 0–5° for 1 more hr, the product was filtered off and recrystallized from Me_2CO–H_2O; white needles (7.8 g, 74%) were obtained.

2-Amino-4-dimethylamino-6-methyl-s-triazine[7] **(a).**—1,1-

SCHEME I



Dimethylbiguanide hydrochloride (3.4 g, 0.02 mole) was neutralized with Na (0.46 g, 0.02 g-atom) in 35 ml of dry MeOH. The resulting NaCl was filtered, and AcOEt (1.8 g, 0.02 mole) was introduced and refluxed for 5 hr. Cooling gave colorless prisms (0.9 g, 30%) which were filtered off and recrystallized (MeOH). The filtrate was evaporated to dryness, and the residue was recrystallized from MeOH to give colorless prisms melting at 219°. This compound was ascertained to be 1,1-dimethylbiguanide acetate, an authentic sample of which was obtained from 1,1-dimethylbiguanide and AcOH. *Anal.* ($C_6H_{15}N_5O_2$) C, H, N.

(b) 2-Amino-4-chloro-6-methyl-*s*-triazine (2 g, 0.014 mole) was heated in alcoholic 20% Me_2NH (5 g, 0.02 mole) under pressure at 100° for 5 hr. The reaction mixture was evaporated to dryness, and the residue was recrystallized from MeOH to give colorless prisms (1.4 g, 65%).

2-Amino-4-dimethylamino-6-furfuryl-*s*-triazine.—Na (0.27 g, 0.012 g-atom) was dissolved in 40 ml of dry MeOH. 1,1-Dimethylbiguanide hydrochloride (2 g, 0.012 mole) was then added, the resulting NaCl was filtered, ethyl 2-furylacetate (1.8 g, 0.012 mole) was introduced, and the mixture was heated at reflux for 5 hr. It was then evaporated to dryness and the residue was recrystallized (C_6H_6) to give white crystals (2 g, 76%).

2-Amino-4-cyanomethyl-6-dimethylamino-*s*-triazine.—To a stirred suspension of 2-amino-4-chloromethyl-6-dimethylamino-*s*-triazine[7] (1.4 g, 0.0075 mole) in 100 ml of EtOH was added dropwise a solution of KCN (1.1 g, 0.008 mole) in 1 ml of H_2O. After heating at reflux for 4.5 hr, the reaction mixture was evaporated to dryness. Crystallization from AcOEt gave pale yellow crystals (1.1 g, 85%).

2-Amino-4-dimethylamino-6-[α-methyl-β-(5-nitro-2-furyl)-vinyl]-*s*-triazine (5).—2-Amino-4-dimethylamino-6-ethyl-*s*-triazine (3.1 g, 0.019 mole) was dissolved in 60 ml of AcOH including 6 ml of concentrated H_2SO_4. To this solution 5-nitrofurfural (2.7 g, 0.019 mole) was added and heated at 100° for 3 hr. The reaction mixture was diluted with 200 ml of H_2O and the separated sulfate was filtered off. The sulfate was neutralized with aqueous 10% NH_3 to yield free base, which was recrystallized from *n*-PrOH–Me_2CO to give yellow prisms (1.5 g, 28%).

2-Ethoxy-4-dimethylamino-6-[β-(5-nitro-2-furyl)vinyl]-*s*-triazine (14).—2-Ethoxy-4-dimethylamino-6-methyl-*s*-triazine (2 g, 0.011 mole) and 5-nitrofurfural (1.5 g, 0.011 mole) were dissolved in 15 ml of Ac_2O and the solution was heated for 4 hr at around 130°. On cooling, yellow crystals (1.19 g, 33%) separated. Crystallization (EtOH) gave yellow needles.

2,4-Diacetamido-6-[α-ethyl-β-(5-nitro-2-furyl)vinyl]-*s*-triazine (21).—2,4-Diamino-6-[α-ethyl-β-(5-nitro-2-furyl)vinyl]-*s*-triazine (0.2 g, 0.0007 mole) was heated under reflux with 5 ml of Ac_2O for 1.5 hr. On cooling, the diacetyl compound (0.2 g, 77%) separated. Recrystallization (EtOH) gave yellow crystals.

2-Acetamido-4-amino-6-[α-ethyl-β-(5-nitro-2-furyl)vinyl]-*s*-triazine (20).—A solution of 2,4-diacetamido-6[(α-ethyl-β-(5-nitro-2-furyl)vinyl]-*s*-triazine (0.17 g, 0.0005 mole) in 20 ml of H_2O–DMF (1:1) was heated at reflux for 16 hr. After cooling, the product was removed by filtration and purified by recrystallization from DMF to give pale yellow needles (0.15 g, 94%).

2-Amino-4-dimethylamino-6-(5-nitro-2-furyl)-*s*-triazine (23).—A solution of 1,1-dimethylbiguanide (0.65 g, 0.005 mole) and methyl 5-nitro-2-furoate (0.86 g, 0.005 mole) in 20 ml of MeOH was allowed to stand at room temperature for 3 hr. The yellow product which separated was collected by filtration and recrystallized from MeOH-DMF (1:3) to give yellow needles (0.5 g, 41%).

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