[Chem. Pharm. Bull. 11 (10) 1247 ~ 1250]

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# 200. Mitsuru Furukawa, Shigeshi Toyoshima, and Takeo Ueda : Antiviral Effect and Syntheses of 4-Amino-6-alkylamino-*s*-triazine-2-carboxylic Acid Derivatives. Researches on Chemotherapeutic Drugs against Viruses. XXXVI.[1]

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As described in the preceding paper,[2] many compounds of 4,6-diamino-*s*-triazine-2-carboxamide derivatives were synthesized to screen as to their antiviral activity. Recently, our group[3] found that N-amidino-4-amino-6-morpholino-*s*-triazine-2-carboxamide showed a remarkable inhibitory effect on polio and measles viruses in tissue culture, a chemotherapeutic effect on influenza virus in mice and a very low toxicity.

This finding prompted us to synthesize the other derivatives of 4-amino-6-alkylamino-*s*-triazine-2-carboxylic acid.

However, criterion is raised as for the importance of morpholino group in N-amidino-4-amino-6-morpholino-*s*-triazine-2-carboxamide. Among compounds of 1-substituted biguanide, 1,1-dimethylbiguanide and 1-isopropylbiguanide[4] showed effects nearly equal to that of 1,1-(2,2′-oxydiethyl)biguanide (ABOB)[5] against influenza virus, though the effectiveness of ABOB was proved to be negligibly slight in dembryonated egg-culture and mice. This finding suggested that morpholino group might be replaceable with other groups to give rise to antiviral activity.

On the other hand, Ueda, *et al.* have obtained many antiviral compounds by introducing alkylamino groups to variable structures. This fact indicated that new antiviral agents might be obtainable by introducing alkyl groups into 4,6-diamino-*s*-triazine-2-carboxylic acid. Thus, compounds of 4-amino-6-alkylamino-*s*-triazine-2-carboxylic acid were conceived to synthesize and examine as to their antiviral activity.

This paper is concerned with synthesese of 4-amino-6-alkylamino-*s*-triazine-2-carboxylic acid derivatives.

**Synthesis of N-Amidino-4-amino-6-alkylamino-*s*-triazine-2-carboxamide**

Compounds of this series were anticipated to be synthesized by reacting 1-alkylbiguanide in anhydrous methanol with diethyl oxalate and condensing the resulting product, ester of 4-amino-6-alkylamino-*s*-triazine-2-carboxylic acid, with guanidine. In the former reaction, 1-alkyl-3-(4,5-dioxo-2-imidazolidinylidene)guanidine might be formed as an intermediate, referred to the synthetic reaction of 4-amino-6-alkylamino-*s*-triazine-2-carboxylic acid ester.

At first, the synthesis of 1-alkylbiguanide were investigated : Alkylbiguanide derivatives having alkyl-chain higher than propyl were, with ease, prepared in accordance with the method of Bamburger, *et al.*[6] by the fusion of dicyandiamide with alkylamine hydrochloride at 160~170° for 1 hour. The methyl and ethyl homologs, however, were found unobtainable by this method. Because, the decomposition might occur due to the hasty elevation of the reaction temperature in this fusion.

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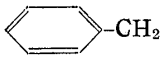
1) Part XXXV. M. Furukawa, S. Toyoshima : This Bulletine, **11**, 518 (1963).
2) M. Furukawa, T. Ueda : *Ibid.*, **11**, 596 (1963))
3) The paper presented at the 82nd Annual Meeting of Pharmaceutical Society of Japane, November, 1962.
4) K. S. Pilcher, K. F. Soikes : Antibiotics and Chemotherapy, **11**, 881 (1961).
5) B. Melander : *Ibid.*, **10**, 34 (1960); Toxicology and Applied Pharmacol., **2**, 274 (1960).
6) E. Bamburger, W. Dieckmann : Ber., **25**, 545 (1892).

The methyl and ethyl homologs were, with success, obtained through the reaction between dicyandiamide and alkylamine in the presence of copper sulfate catalyst under pressure, according to the method of Slotta, *et al.*[7] When equimolar quantities of 1-alkylbiguanide and diethyl oxalate were reacted in anhydrous methanol at room temperature, white precipitates were found immediately in good yields and in fairly pure states. The structure of the products thus obtained was not justified evidently because of the impossibility to convert into the characterizing derivatives due to instability. As can be seen in previous papers,[2,8] they were, however, inferred to have five-membered rings from infrared spectra and the identifying reaction.

The properties and infrared absorptions of C=O of these five-membered ring compounds synthesized are shown in Table I.

Table I. R-NH-C(=NH)-N=C(NH-CO)(NH-CO)

| R | m.p. (°C) | Formula | Anal. N% Calcd. | Anal. N% Found | $\nu_{C=O}$ cm$^{-1}$ |
|---|---|---|---|---|---|
| $CH_3$ | 213~214 | $C_5H_7O_2N_5$ | 41.41 | 41.30 | 1720, 1761 |
| $C_2H_5$ | 219~220 | $C_6H_9O_2N_5$ | 38.24 | 38.19 | 1720, 1762 |
| $C_3H_7$ | 210~211 | $C_7H_{11}O_2N_5$ | 35.52 | 35.33 | 1721, 1763 |
| iso-$C_3H_7$ | 218~219 | $C_7H_{11}O_2N_5$ | 35.52 | 35.90 | 1722, 1754 |
| $C_4H_9$ | 203~204 | $C_8H_{13}O_2N_5$ | 33.16 | 33.39 | 1717, 1763 |
| $C_6H_{13}$ | 212~213 | $C_{10}H_{17}O_2N_5$ | 29.27 | 29.36 | 1722, 1760 |
| $C_8H_{17}$ | 198~199 | $C_{12}H_{21}O_2N_5$ | 26.20 | 26.38 | 1704, 1765 |
|  | 214~215 | $C_{11}H_{11}O_2N_5$ | 28.56 | 28.69 | 1713, 1760 |

After refluxing the five-membered ring compounds inferred as 1-alkyl-3-(4,5-dioxo-2-imidazolidinylidene)guanidine, in anhydrous methanol or ethanol until they were completely dissolved, the products of N-amidino-4-amino-6-alkylamino-*s*-triazine-2-carboxamide were readily prepared by treating with guanidine under reflux for ca. 10 hours without the isolation of the intermediates of ethyl or methyl 4-amino-6-alkylamino-*s*-triazine-2-carboxylate. The synthetic process of the objective compounds are illustrated in Chart 1.

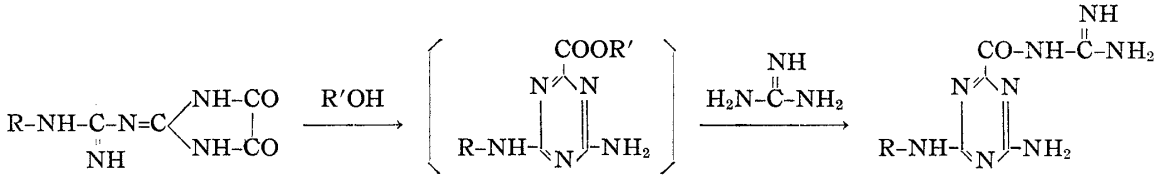


Chart 1.

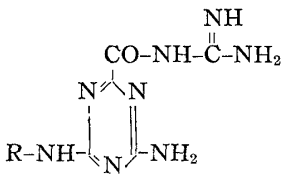
The properties and elementary analytical data of these synthesized compounds of this series are shown in Table II.

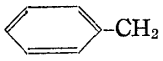
**Synthesis of 4-Amino-6-alkylamino-*s*-triazine-2-carboxylic Acid**

Overberger, *et al.*[9] reported that 4-amino-6-anilino-*s*-triazine-2-carboxylic acid was obtained, when an intermediate, which was formed by condensing 1-phenylbiguanide with diethyloxalate, was hydrolysed with water under reflux. According to this method, 4-amino-6-alkylamino-*s*-triazine-2-carboxylic acid could be, in a good yield,

7) K.H. Slotta, R. Tschesche : Ber., **62**, 1396 (1929).
8) M. Furukawa : This Bulletin, **10**, 1215 (1962).
9) C.G. Overberger, S.L. Shapiro : J. Am. Chem. Soc., **76**, 93 (1954).

Table II.



| R | m.p. (°C) | Formula | Anal. N% Calcd. | Found |
|---|---|---|---|---|
| $CH_3$ | >300 | $C_6H_{10}ON_8$ | 53.31 | 53.19 |
| $C_2H_5$ | >300 | $C_7H_{12}ON_8$ | 50.42 | 50.11 |
| $C_3H_7$ | 293~296 | $C_8H_{14}ON_8$ | 47.03 | 46.89 |
| iso-$C_3H_7$ | >300 | $C_8H_{14}ON_8$ | 47.03 | 47.32 |
| $C_4H_9$ | 270~271 | $C_9H_{16}ON_8$ | 44.42 | 44.23 |
| $C_6H_{13}$ | 222~223 | $C_{11}H_{20}ON_8$ | 39.92 | 40.10 |
| $C_8H_{17}$ | 280~282 | $C_{13}H_{24}ON_8$ | 36.33 | 36.39 |
|  | >300 | $C_{12}H_{14}ON_8$ | 39.14 | 39.01 |

synthesized by the treatment of 1-alkyl-3-(4,5-dioxo-2-imidazolidinylidene)guanidine, which was intermediately produced from the reaction of 1-alkylbiguanide with diethyl oxalate, with water as illustrated in Chart 2.

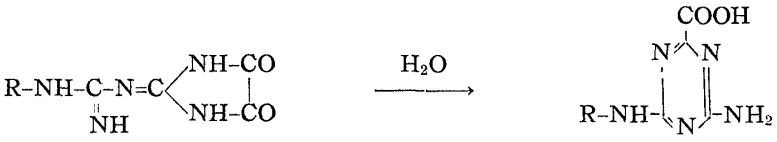


Chart 2.

In this hydrolysis, the higher alkyl homologs than butyl were found difficultly obtainable, apparently because of their insolubility in water in the course of this reaction. However, their sodium salts were obtained by neutralizing 1-alkyl-3-(4,5-dioxo-2-imidazolidinylidene)guanidine with equimolar amounts of aqueous sodium hydroxide solution and then heating the mixtures.

When an excess amount of sodium hydroxide was employed for this reaction, the decarboxylation of the formed 4-amino-6-alkylamino-*s*-triazine-2-carboxylic acid take place to give 4-amino-6-alkylamino-*s*-triazine, as shown in Chart 3.

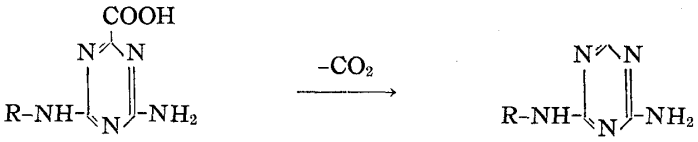


Chart 3.

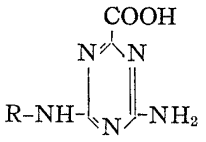
4-Amino-6-alkylamino-*s*-triazine-2-carboxylic acid could be also prepared according to the other methods shown in previous paper,[8] *i.e.* the hydrolysis of ethyl or methyl 4-amino-6-alkylamino-*s*-triazine-2-carboxylate with alkaline solution and the reaction of 1-alkylbiguanide with oxalic monoester. The properties of these compounds synthesized are listed in Table III.

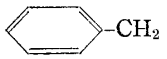
As described above, the authors obtained new compounds of N-amidino-4-amino-6-alkylamino-*s*-triazine-2-carboxamide and 4-amino-6-alkylamino-*s*-triazine-2-carboxylic acid, related to an antiviral compound, N-amidino-4-amino-morpholino-*s*-triazine-2-carboxamide.

**Screening Tests of Compounds Synthesized**

The compounds of N-amidino-4,6-diamino-*s*-triazine-2-carboxamide and 4,6-diamino-*s*-triazine-2-carboxylic acid series were screened for their antiviral effect on both of

TABLE III.



| R | m.p. (°C) | Formula | Anal. N% Calcd. | Found |
|---|---|---|---|---|
| $CH_3$ | 251～252 | $C_5H_7O_2N_5$ | 41.41 | 41.10 |
| $C_2H_5$ | 229～230 | $C_6H_9O_2N_5$ | 38.24 | 38.08 |
| $C_3H_7$ | 237～238 | $C_7H_{11}O_2N_5$ | 35.52 | 35.51 |
| $C_4H_9$ | 211～212 | $C_8H_{13}O_2N_5$ | 33.16 | 33.20 |
| $C_6H_{13}$ | 215～216 | $C_{10}H_{17}O_2N_5$ | 29.27 | 29.57 |
| $C_8H_{17}$ | 222～223 | $C_{12}H_{20}O_2N_5$ | 26.20 | 26.24 |
|  | 212～213 | $C_{11}H_{11}O_2N_5$ | 28.56 | 28.33 |

type-1 strain of adeno virus and Mahoney strain of polio virus by tissue culture method according to the same experimental procedures as those described in the previous report. The result of these test showed that N-amidino-4,6-diamino-*s*-triazine-2-carboxamide has an inhibitory effect on multiplication of $100 \times TCD_{50}$ of the polio virus and none of the other compounds showed any effect.

This fact suggests that morpholino group of N-amidino-4-amino-6-morpholino-*s*-triazine-2-carboxamide is not always essential for the generation of the antiviral effect.

## Experimental

**General Procedure for Synthesis of 1-Alkyl-3-(4,5-dioxo-2-imidazolidinylidene)guanidine**——A solution of Na-alcohoxide prepared by dissolving 0.01 mole of Na in small amount of abs. MeOH or EtOH was added with stirring into the solution of 0.01 mole of 1-alkylbiguanide hydrochloride in abs. MeOH or EtOH. After removing the deposited NaCl by filtration, 0.01 mole of diethyl oxalate was added into the filtrate at room temperature. The resulting white precipitates were collected by suction and washed with hot MeOH.

**General Procedure for Synthesis of N-Amidino-4-amino-6-alkylamino-*s*-triazine-2-carboxamide**——A suspension of 0.01 mole of 1-alkyl-3-(4,5-dioxo-2-imidazolidinylidene)guanidine in 200 ml. of abs. MeOH or EtOH was refluxed for about 5 to 10 hr. until it was completely dissolved. Without isolation of methyl or ethyl 4-amino-6-alkylamino-*s*-triazine-2-carboxylate thus produced, 0.01 mole of guanidine was added into the solution and the reaction mixture was continued to reflux for 5 to 10 hr., by which white precipitates were gradually seperated. The mixture was concentrated and the resulting white precipitates were collected by filtration, washed with MeOH and recrystallized from MeOH to prisms.

**General Procedure for Synthesis of 4-Amino-6-alkylamino-*s*-triazine-2-carboxylic Acid and its Sodium Salts**——1) A suspension of 0.01 mole of 1-alkyl-3-(4,5-dioxo-2-imidazolidinylidene)guanidine in suitable amount of $H_2O$ or aqueous solution of 0.01 mole of NaOH was heated on a water bath until it was completely dissolved. On concentration of the solution, the resulting precipitates were collected by suction and recrystallized from $H_2O$.

2) A small amount of methanolic solution, in which dissolved 0.01 mole of Na, was added with stirring into the solution of 0.01 mole of 1-alkylbiguanide hydrochloride in 20 ml. of abs. MeOH, and deposited NaCl was removed by filtration. Into the filtrate potassium methyl oxalate was added and the mixture was refluxed with stirring for about 5 hr. The resulting precipitates were collected by suction and recrystallized from $H_2O$.

## Summary

New compounds of 4-amino-6-alkylamino-*s*-triazine-2-carboxylic acid and N-amidino-4-amino-6-alkylamino-*s*-triazine-2-carboxamide were synthesized from 1-alkyl-3-(4,5-dioxo-2-imidazolidinylidene)guanidine prepared by condensation of 1-alkylbiguanide with diethyl oxalate and examined as to their antiviral effect. Among these compounds, N-amidino-4,6-diamino-*s*-triazine-2-carboxamide was found to possess an inhibitory effect on polio virus.

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