SYNTHESIS AND ANTIVIRAL ACTIVITY OF ALKOXYCARBONYL-METHOXYBENZO-2,1,3-THIADIAZOLES AND THEIR DERIVATIVES

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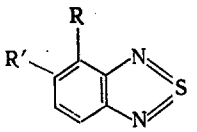
Some plant-growth regulators, both natural and synthetic, are known to exhibit antiviral action; for example, gibberellin and benzamidazole are both plant-growth stimulators [3, 5] and inhibitors of viral activity [4, 8].

In the present work, the antiviral activity of a new group of synthetic plant-growth regulators, alkoxycarbonylmethoxybenzo-2,1,3-thiadiazoles [7, 10], and some of their derivatives has been studied.

The reaction of 4- and 5-hydroxybenzo-2,1,3-thiadiazoles (I, II) with ethyl monobromoacetate in DMFA in the presence of sodium or potassium carbonate gave 4- and 5-carbethoxymethoxybenzo-2,1,3-thiadiazoles (III, IV).



I—IV

I: R=OH, R′=H; II: R=H, R′=OH; III: R=OCH_2COOEt, R′=H; IV: R=H, R′=OCH_2COOEt

Compound V, 5,7-dichloro-3-carbethoxymethoxybenzo-2,1,3-thiadiazole, could not be obtained by this method and was synthesized as described in [9]. Chlorination of compound III with SO_2Cl_2 or Cl_2 (3-5 min) in acetic acid or DMFA gave a mixture of V and 5-chloro-4,7-dioxo-4,7-dihydrobenzo-2,1,3-thiadiazole (VI), which could not be separated by crystallization or vacuum distillation.

Under these conditions, Cl_2 and SO_2Cl_2 are not only chlorinating agents but also oxidizing agents.

The quinone VI, used for comparison, was obtained by the oxidation of compound I by the method given in [1].

Chlorination of compound III for 1 h with Cl_2 in acetic acid in the presence of I_2 gave a mixture of VI and 5,6-dichloro-4,7-dioxo-4,7-dihydrobenzo-2,1,3-thiadiazole (VII). Further chlorination of this mixture with Cl_2 in acetic acid in the presence of I_2 gave the quinone VII, identical with that described in [1].

TABLE 1. Esters of 4-Chloro-5-carboxymethoxybenzo-2,1,3-thiadizole (IX-XII)

Compound	Yield, %	mp, °C	Found, %		Empirical formula	Calc., %		R_f
			N	S		N	S	
IX	85	95—7	10,20	11,63	$C_{10}H_9ClN_2O_3S$	10,28	11,74	0,87
X	34	110—1	10,47	...	$C_9H_7ClN_2O_3S$	10,83	12,38	0,86
XI	90	84—5	7,90	9,27	$C_{15}H_{11}ClN_2O_3S$	8,37	9,57	0,84
XII	82	36—9	9,90	10,84	$C_{12}H_{13}ClN_2O_3S$	9,32	10,65	0,83

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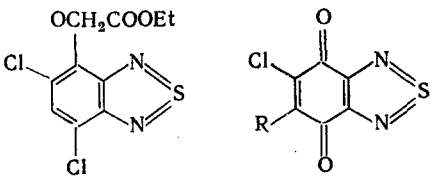
TABLE 2. Effect of Preventive Injection of Compounds III-VII, IX-XI, and Rimantadine on Influenza A2/Texas Virus in Chick Embryos

Compound	Concentration of compound, mg/ml	Number of chick embryos with virus, %	Prevention coefficient (K_p)	Prevention index (I_p)	Geometric mean of titer
III	2,1	83,33	1	0	1:479
IV	2,1	83,33	1	0	1:632
V	2,1	83,33	1	0	1:588
VI	0,35	33,33	2,5	60	1:13
	2,1	0	—	100	—
VII	0,35	16,67	5	80	1:3,5
	2,1	0	—	100	—
IX	2,1	16,67	5	80	1:3,5
X	2,1	66,67	1,25	20	1:120
XI	2,1	83,33	1	0	1:512
Rimantadine	2,1	16,67	5	80	1:21,1
Control, influenza A2/Texas virus		83,33	—	—	1:588

Note. Dilution of virus, 10^{-4}; number of embryos, 12; solvent, DMSO; single injection into chorion allantoic cavity, 1 h after injection of virus.

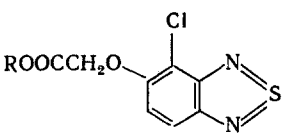


V VI, VII

VI: R=H; VII: R=Cl

Hydrolysis of 4-chloro-5-carbethoxymethoxybenzo-2,1,3-thiadiazole (IX) with hydrochloric acid gave 4-chloro-5-carboxymethoxybenzo-2,1,3-thiadiazole (VIII). It was found that the ester IX was hydrolyzed in 5 min, in contrast to [9], where hydrolysis with H_2SO_4 took 1 h.

On reaction with an alkyl halide in DMFA in the presence of potassium or sodium carbonate, compound VIII gave the ester (X-XII).



VIII: R=H; IX: R=Et; X: R=Me; XI: R=CH_2Ph; XII: R=Bu

The conditions for the alkylation of hydroxy compounds and acids are thus identical.

EXPERIMENTAL CHEMICAL SECTION

The course of the reaction and examination of the products were carried out by TLC on Silufol UV-254 plates in acetone–$CHCl_3$–hexane, 2:1:2 (system A), or in benzene–acetone–acetic acid, 100:50:1 (system B); spots were developed in UV light or NH_3 vapor. IR spectra were taken on a Perkin Elmer 577 spectrophotometer in CCl_4 or $CHCl_3$.

4-Carbethoxymethoxybenzo-2,1,3-thiadiazole (III). To a mixture of 1 g (6.58 mmoles) of I and 1 g (7.25 mmoles) of potassium carbonate in 10 ml of DMFA heated to 60°C was added 0.75 ml (6.67 mmoles) of ethyl monobromoacetate. The reaction mixture was stirred for 30 min at 80-90°C, then cooled and 50 ml of water added. The precipitated material was filtered

TABLE 3. Effect of Compounds IX and I on A2/Leningrad Influenza Virus

Compound	Concentration of compounds, mg/ml	Number of chick embryos	Number with virus		Protection coefficient (K_p)	Protection index (I_p)	Geometric mean of titer
			abs.	%			
	Preventive injection						
IX	2,1	12	1	8,33	10,00	90,00	1:0,5
	1,05	12	5	41,67	2,00	50,00	1:13
I	2,1	12	5	41,67	2,00	50,00	1:22,6
	1,05	12	7	58,83	1,43	30,07	1:56
	Simultaneous injection						
IX	2,1	12	2	16,67	5,0	80	1:3,5
	1,05	—	8	66,67	1,25	20	1:104
I	2,1	—	7	58,33	1,54	30,07	1:79
	1,05	—	9	75	1,11	9,9	1:256
	Remedial injection						
IX	2,1	12	1	8,33	10,0	90,00	1:2
	1,05	—	6	50	1,67	40,12	1:30
I	2,1	—	8	66,67	1,25	20	1:169
	1,05	—	9	75	1,11	9,9	1:315
Control virus — A2/ Leningrad		12	10	83,33	—	—	1:479

Note. Dilution of virus, 10^{-3}; solvent, DMSO; $I_p = \frac{K_p - 1}{K_p}$;

$$K_p = \frac{\%\text{embryos with virus in control}}{\%\text{embryos with virus in test}}$$

off, washed with water, and dried, giving 1.18 g (75.4%) of III, mp 7-72°C (literature value mp 74-75°C [9]). R_f 0.80 (system A).

5-Carbethoxymethoxybenzo-2,1,3-thiadiazole (IV). Using the same method, 1 g (6.58 mmoles) of II, 1 g (7.25 mmoles) of potassium carbonate, and 0.75 ml (6.67 mmoles) of ethyl monoacetate in 10 ml of DMFA gave 1.3 g (83%) of IV, mp 108-109°C, R_f 0.79 (system A); a sample obtained by the method given in [9] had the same R_f value.

5,7-Dichloro-4-carbethoxymethoxybenzo-2,1,3-thiadiazole (V) and 4,7-Dioxo-4,7-dihydrobenzo-2,1,3-thiadiazole (VI). A. A solution of 1 g (4.2 mmoles) of III in 8 ml of DMFA (or 15 ml of glacial acetic acid) at room temperature was saturated with Cl_2 gas for 2.5 min. The solution was then cooled, poured into water, and the viscous product obtained (1 g) distilled in vacuum (bp 195-198°C at 6 mm) to give 0.8 g of a yellow material which was a mixture of V (R_f 0.92) and VI (R_f 0.88) (system A).

B. To 1.124 g (4.72 mmoles) of III in 5 ml of glacial acetic acid or DMFA was added 0.95 ml (11.7 mmoles) of SO_2Cl_2 and the reaction mixture mixed for 20 min at 50-60°C, then cooled and poured into water. The viscous product (0.96 g) was separated and distilled in vacuum (bp 195-198°C at 6 mm) to give 0.74 g of a yellow material which was a mixture of V (R_f 0.92) and VI (R_f 0.88) (system A).

5,6-Dichloro-4,7-dioxo-4,5-dihydrobenzo-2,1,3-thiadiazole (VII). To a solution of 1 g (4.20 mmoles) of III in 15 ml of glacial acetic acid at 60-65°C was added 1 g (3.94 mmoles) of I_2, and Cl_2 bubbled through the reaction mixture. Chlorine treatment was continued for 1 h during which the mixture was allowed to reflux. The reaction mixture was then cooled and poured onto ice. The precipitate was filtered off, washed with water, and dried to give 0.2 g of a mixture of quinones VI and VII. This mixture was dissolved in 5 ml of glacial acetic acid, 0.19 g (0.75 mmoles) of I_2 added, and gaseous Cl_2 passed for 2.5 h with refluxing. The reaction mixture was cooled and the precipitate filtered off, washed with water, and dried to give 0.13 g (13.2%) of VII (based on III), which was crystallized from dichloroethane, sublimation temperature 189-190°C, R_f 0.8 (system A). In the infrared, the C=O of VII absorbed at 1705 cm^{-1}; the R_f and the IR spectrum of the quinone VII agree with that of a sample obtained by the method given in [1].

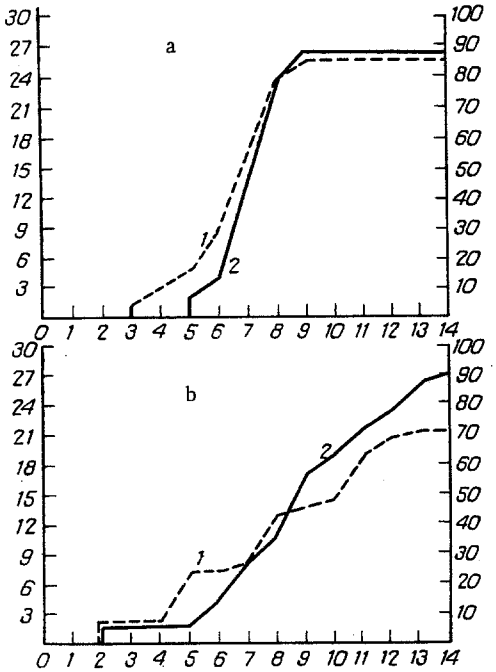


Fig. 1. Effect of ester IX in doses of 2 mg (a) and 6 mg (b) on mice infected with 5 LD_{50}'s (a) and 3 LD_{50}'s (b). Plotted along x axis: days after infection; plotted along y axis: at left, number of animals lost; at right, toxicity (%). 1) Control, 2) test.

4-Chloro-5-carboxymethoxybenzo-2,1,3-thiadiazole (VIII). To 0.2 g of IX, obtained by the method given in [9] and recrystallized from hexane, was added 10 ml of water and 11 ml of concentrated HCl, and the mixture refluxed for 5 min. The solution was cooled and the precipitated material filtered off, washed with water, and dried to give 0.13 g (75%) of VIII with R_f 0.56 (system B), and mp 193-194°C; this material gave no depression of melting point on admixture with the acid VIII obtained as in [9].

Esters of 4-Chloro-5-carboxymethoxybenzo-2,1,3-thiadiazole (X-XII). To 2 mmoles of VIII and 1 g (9.4 mmoles) of sodium carbonate in 5 ml of DMFA was added 2 mmoles of the corresponding alkyl halide. The mixture was stirred for 25 min at 120-140°C, then cooled, and poured into 70 ml of water. The precipitate was filtered off, washed with water, and dried to give the esters (Table 1).

EXPERIMENTAL PHARMACOLOGICAL SECTION

The esters III-V and IX-XI and the quinones VI and VII were tested for antiviral activity against influenza A2/Texas virus by standard methods using chick embryos.

Table 2 shows that of all the tested compounds, the quinones VI and VIII and the ester IX were the most effective. An acute-toxicity study, carried out by Litchfield and Wilcoxon's method, showed that the quinones VI and VII were 10 times more toxic than the ester IX; therefore, only the ester IX (LD_{50} 600 mg/kg), the activity of which was comparable with that of rimantadine, was studied.

The ester IX was shown (Table 3) to possess antiviral activity when injected 1 h before an injection of virus (preventive injection), at the same time as the virus, or 4 h after the virus (remedial injection).

Comparison of the antiviral activities of the ester IX and 4-oxybenzo-2,1,3-thiadiazole against Venezuelan equine encephalitis and vaccinia viruses [2] showed that the ester IX was 1.8 times more effective than compound I in its preventive action and 4.5 times more active when used remedially.

The antiinfluenza action of the ester IX on an experimental influenza infection in mice was carried out as described in [6].

Control and test groups, chosen randomly, consisted of 30 nonpedigree white mice weighing 12-15 g. The experimental infection was introduced nasally, as 0.05 ml of dilute allantoic liquid containing 3-5 LD_{50}'s of A0/32 (H0N1) influenza virus, into animals lightly anesthetized with ether. The ester IX (0.2 ml of a suspension containing 2 or 6 mg of the ester) was given via a stomach probe; the mice received the drug at 24 h and 1 h before infection, and also at 24, 48, and 72 h after infection. Control animals received only water. The animals were observed for 14 days after infection.

Curves plotted (Fig. 1) of the number of animals lost against time show that at first the ester IX somewhat suppressed the loss of infected animals, but this protective action disappeared later. Moreover, an injection of 6 mg of the preparation at the end of the observation period (14th day) resulted in a greater loss of animals in the test group ($P < 0.05$) than in the control group.

The very high antiviral effect of the ester IX *in ovo*, and the absence of this effect in tests on animals can presumably be explained by the fact that in the chick-embryo allantoic cavity, the ester IX acts directly on the extracellular virion, while in mice, the effect is mediated by pharmacological mechanisms.

LITERATURE CITED

1. I. A. Belen'kaya, G. P. Krokhina, and S. A. Andronati, Khim. Geterotsikl. Soedin., No. 10, 1344-1346 (1982).
2. I. A. Belen'kaya, N. P. Chizhov, and N. G. Chigareva, Khim.-Farm. Zh., No. 8, 66-72 (1978).
3. V. P. Borisenko, N. A. Malichenko, and N. I. Zhurvskaya, Physiologically Active Substances [in Russian], Kiev, No. 13 (1981), pp. 26-29.
4. L. K. Zherebchuk, Antiviral Substances [in Russian], Riga (1982). pp. 26-27.
5. N. N. Mel'nikov, K. V. Novozhilov, and T. N. Pylova, Handbook of Chemical Agents for Plant Protection (Pesticides) [in Russian], Moscow (1980), p. 41.
6. V. I. Il'enko, Methods of Testing and Evaluating the Antiviral Activity of Chemical Compounds against Influenza Virus [in Russian], Leningrad (1977), pp. 19-33.
7. V. E. Sovetkina, D. Kh. Shashenkova, and I. A. Belen'kaya, Khim. Sel'sk. Khoz. 18, No. 7, 43-45 (1980).
8. I. Tamm, Chemotherapeutic Strategy [in Russian], Moscow (1960), pp. 210-245.
9. J. J. Van Daalen and J. Daams, Swedish Patent No. 562817 (1975).
10. J. J. Van Daalen and J. Daams, Naturwissenschaften, 57, 395 (1970).