

MODIFICATION OF ALKYL ESTERS OF 4-AMINOBENZENETHIOSULFONIC ACID BY S-TRIAZINE FRAGMENT AND INVESTIGATION OF THEIR GROWTH-REGULATIVE ACTIVITY

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Abstract. The acylation of aminogroup of alkyl S-esters 4-aminobenzethiosulfoacid with cyanurichloride was investigated. Series of esters with triazine fragment were synthesized and characterized by elemental analysis, ¹H NMR and IR spectroscopy. The growth-regulative activity of the synthesized compounds was researched. Some appropriate effect of thiosulfoesters structure upon their growth-regulative activity was determined.

Keywords: thiosulfoacid derivatives, thiosulfoesters, s-triazine, growth-regulative activity.

1. Introduction

The application of plant growth regulators in agriculture is an important factor in improving the quality and yield of crop production. Most functions performed in one way or another are aimed at plant protection. The use of growth regulators allows purposefully to regulate the most important processes of plant growth and development to prevent grains from falling flat, to accelerate ripening and forming fruits, to interrupt resting phases of seeds, and to prevent the fruit loss.

In addition, growth regulators increase plants resistance to adverse natural or anthropogenic factors as critical temperature differences, moisture deficit, phytotoxicity of pesticides, pests and diseases of fungal, bacterial and viral origin.

Today, the following growth-regulating drugs as Nitrozyme, Maxicrop and Sea Magic are used in agriculture in which active substances are natural (endogenous) compounds, phytohormones in particular. Based on the results of researches on endogenous growth

regulators and their participation in metabolism, synthetic (exogenous) growth regulators were made, such as Ivin, Poteitin, and Lucis, which are also widely used in agriculture.

s-Triazine derivatives are of interest in terms of finding new growth regulators [1-3]. 1,3,5-Triazines or s-triazines have been known for over 100 years. Their derivatives are used as herbicides, plant protection products, drugs, reagents for condensation in peptide synthesis, dye synthesis, at modification of polysaccharides, *etc.* [3-8]. Not so long ago, substituted triazine was isolated from algae, and characterized by a number of physical chemical methods [9].

Derivatives of thiosulfonic acids are no less of interest in this aspect of research. In particular, thiosulfoesters exhibiting high growth-stimulating activity in relation to crops are quite known [10-13]. Aforementioned compounds are getting more of interest due to the fact that thiosulfoesters are structural analogues of natural antibacterial substances [14-16].

It is known that synthetic esters of thiosulfoacids possess the biological activity, which often exceeds the efficiency of natural analogues [17]. Quite a few of them are offered as medicines, preservatives of fruit and vegetables, effective remedies for plant protection, growth regulators, biocidal additives, insecticides and radioprotector. [17-21]. In addition, thiosulfoacid esters are effective sulfenilating and sulfonylating reagents in organic synthesis, and also valuable objects for solving complex issues of molecular biology and biochemistry [17, 22, 23].

Among thiosulfoacid derivatives with a wide range of biological activity at relatively low toxicity (LD₅₀ = 2500 mg/kg), alkyl S-esters of 4-aminobenzethiosulfoacids are pointed out [17, 21].

Therefore, in order to search for new promising growth regulators and making comparative researches on determination of “structure and biological activity”

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regularities of thiosulfonates, it is certainly important and interesting to synthesize and research growth-regulative properties of the above-mentioned thiosulfoesters modified by the *s*-triazine moiety because they are the closest structural analogues of alkyl *S*-esters of 4-aminobenzenethiosulfoacids.

2. Experimental

2.1. General Experimental Details

All melting points were determined in open capillary tubes and were uncorrected. ^1H NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer (500 MHz) in DMSO- d_6 , the chemical shifts were measured relative to tetramethylsilane. IR spectra were recorded on SPECORD M80 spectrophotometer in tablets with KBr. Monitoring of the reactions and individuality of compounds were performed by TLC method on Silufol UV254 plates.

2.2. Synthesis of Main Compounds

Alkyl esters of 4-aminobenzenethiosulfoacid 1a-c. Methods of obtaining the compound and its characteristics are presented in the previous work [24].

General procedure of aminogroup acylation of alkyl S-esters 4-aminobenzethiosulfoacid 1a-c with cyanurichloride. The pyridine (0.0027 mol) was added to the cooled (268–273 K) solution (0.0027 mol) of cyanuric chloride in acetone (10 ml) under intensive stirring. The mixture was stirred for 10 min and solution (0.0027 mol) of appropriate alkyl *S*-ester of 4-aminobenzenethiosulfoacid in acetone was gradually added at the temperature not higher than 273 K. After 0.5 h the reaction mass was filtered, and the filtrate was poured onto ice. The resulting precipitate was filtered off and recrystallized from methanol.

S-methyl 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino] benzenesulfonothioate 2a. Yield 67.5 %, mp: 499–500 K; IR (KBr, cm^{-1}): 688-704 $^{\beta***}$, 808 $^{\gamma***}$, 1016-1080 ** , 1416-1548 $^{\gamma}$ (triazine ring), 848, 1168, 1256, 1292 (C-Cl), 1136 $_{\gamma_s}$, 1328 $_{\gamma_{as}}$ (SO $_2$), 1584, 1598 (C=C $_{ar}$), 1616 (C=N), 3304 (NH), ^1H NMR (500 MHz, DMSO- d_6): δ 2.02 s (3H, CH $_3$), 7.28 d (2H, J=7.8 Hz, Ar-H), 7.88 d (2H, J=7.8 Hz, Ar-H), 8.1 s (1H, NH), MS (EI) m/z 352.43[M+1]. Anal. calcd for C $_{10}$ H $_8$ Cl $_2$ N $_4$ O $_2$ S $_2$: C, 34.19; H, 2.29; Cl, 20.19; N, 15.95; S, 18.25; found: C, 33.85; H, 2.42; Cl, 19.84; N, 15.62; S, 17.98.

S-ethyl 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino] benzenesulfonothioate 2b. Yield 75%, mp: 497–498 K; IR (KBr, cm^{-1}): 688-720 $^{\beta***}$, 810 $^{\gamma***}$, 1016-1100 ** , 1424-1548 $^{\gamma}$ (triazine ring), 848, 1156, 1270, 1296 (C-Cl), 1136 $_{\gamma_s}$, 1320 $_{\gamma_{as}}$ (SO $_2$), 1580, 1600 (C=C $_{ar}$), 1612(C=N), 3304 (NH), ^1H NMR (500 MHz, DMSO- d_6): δ 1.4 t (3H, J = 7.4 Hz, CH $_3$), 3.1 q (2H, J = 7.3 Hz, CH $_2$), 7.2 d (2H, J=7.8 Hz, Ar-H), 7.9 d (2H, J=7.8 Hz, Ar-H), 8.12 s (1H, NH), MS (EI) m/z 366.33 [M+1]. Anal. calcd for C $_{11}$ H $_{10}$ Cl $_2$ N $_4$ O $_2$ S $_2$: C, 36.17; H, 2.76; Cl, 19.41; N, 15.34; S, 17.56; found: C, 35.86; H, 2.93; Cl, 19.10; N, 15.11; S, 17.95.

S-propyl 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino] benzenesulfonothioate 2c. Yield 61.8 %, mp: 455–456 K; IR (KBr, cm^{-1}): 700-720 $^{\beta***}$, 806 $^{\gamma***}$, 1024-1100 ** , 1420-1550 $^{\gamma}$ (triazine ring), 848, 1162, 1260, 1296 (C-Cl), 1120 $_{\gamma_s}$, 1304 $_{\gamma_{as}}$ (SO $_2$), 1588, 1600 (C=C $_{ar}$), 1612(C=N), 3314 (NH), ^1H NMR (500 MHz, DMSO- d_6): δ 1.2 t (3H, J = 6.8 Hz, CH $_3$), 1.89 m (2H, CH $_2$ -CH $_3$), 2.7 t (2H, J = 6.3 Hz, S-CH $_2$), 6.6 d (2H, J=7.8 Hz, Ar-H), 7.8 d (2H, J=7.9 Hz, Ar-H), 8.06 s (1H, NH), MS (EI) m/z 379.29 [M+1]. Anal. calcd for C $_{12}$ H $_{12}$ Cl $_2$ N $_4$ O $_2$ S $_2$: C, 38.00; H, 3.19; Cl, 18.69; N, 14.77; S, 16.91; found: C, 37.72; H, 3.43; Cl, 18.36; N, 14.39; S, 16.63.

2.3. Biology

2.3.1. Grow-regulating activity research

Grow-regulating activity of the researched compound is studied according to the standard methods modified by T. Sergejeva [25]. The experiment is carried out on agar medium of the following composition (g/l): MgSO $_4$ ·7H $_2$ O – 1; K $_2$ HPO $_4$ – 1; FeSO $_4$ ·7H $_2$ O – 0.02; agar – 8. Some amount of compound solution is added to medium which is put into Petri plates.

Monocotyledons (oats) and dicotyledons (cress) seeds are used in the test. Seed samples are couched on agar medium for 3 days in thermostat at 295 K with further germinants growing for 4 days in the dry box by artificial light at 292–293 K.

Seed germination and linear size of plant components are defined at the end of the test. The results are presented in percentage concerning control samples. Forcing is defined at the index exceeding 100 % and growth inhibition is defined at the index under 100 %. Tests are thrice-repeated.

3. Results and Discussion

3.1. Chemistry

Despite detailed study and widespread use of *s*-triazines in organic synthesis and particularly the use of

** "Breathing" oscillations of the ring.

*** β – plane deformation oscillations of the ring; γ – off-planar deformation oscillations of the ring;

cyanurchloride just as a reagent for thiosulfoesters [26], there is quite not enough information on thiosulfonate 1,3,5-triazine. Obtaining of thiosulfoesters with a triazine fragment by the heterylation of salts of aliphatic thiosulfonic acids with cyanurchloride [27] is a well-known fact.

Taking into account the synthetic capabilities of mono- and di-substituted derivatives of 1,3,5-triazine, we investigated the interaction of alkyl S-esters of 4-aminobenzenethiosulfoacid with cyanurchloride in aprotic and protic solvents in the conditions of nucleophilic catalysis in the presence of tertiary amines.

Within the temperature range of 268–273 K, in the presence of acetone, pyridine forms an intermediate adduct with cyanurchloride that precipitates. The newly formed adduct easily interacts with an acetone solution of alkyl S-esters of 4-aminobenzenethiosulfoacid. The monosubstitution of chlorine in cyanurchloride when it interacts with thiosulfoesters **1a-c** in the above conditions, results in 60–75 % yield of S-alkyl 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzenesulfonothioates **2a-c**.

Acylation of ethyl S-esters of 4-aminobenzenethiosulfoacid **1b** with cyanurchloride occurs at 273–278 K in a non-polar solvent (benzene) in the presence of triethylamine with an equimolar ratio of reagents followed by the formation of monosubstitution product **2b** with a significantly lower yield (25 %).

3.2. Growth-Regulative Activity

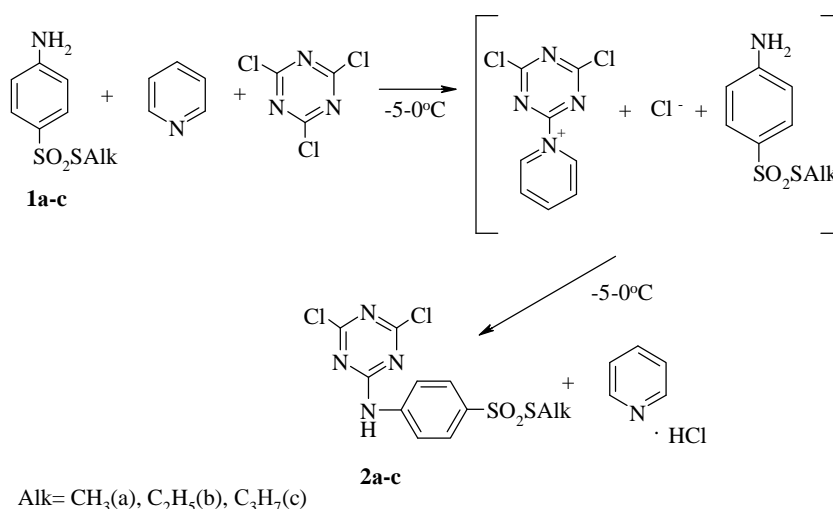
In order to find new growth regulators for plants that are competitive with commercial drugs, the growth regulating activity of synthesized thiosulfoester derivatives of s-triazine **2a-c** has been researched (Table).

The results of the researches indicate that at the concentration of 100 mg/l, S-methyl 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzenesulfonothioate **2a** tends

to stimulate oat root growth by 12 % and stem growth by 2 %. The sprouting of oat at the same concentration as compared to the control is higher by 37 %. At the concentration of 100 mg/l, compound **2a** activates the cress root growth by 41 %. The sprouting of the cress and the control is the same. With the decrease in the concentration of the compound **2a** in the medium, there is stimulation of both cress root growth (at 1 mg/l by 55 %), oat stem and root growth (at 1 mg/l by 30 %). Consequently, thiosulfoester **2a** contributes to the oat and cress growth mostly in all researched concentrations in relation to the control. It is quite typical that the growth of plants increases while the concentration decreases.

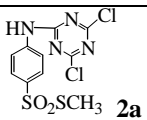
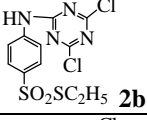
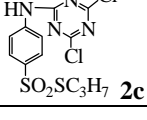
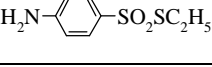
S-ethyl 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzenesulfonothioate **2b** stimulates oat stem and roots, as well as cress growth to some extent as well. At the concentration of 100 mg/l, compound **2b** contributes to the oat stem and roots growth by 18–19 %, and at the concentration of 1 mg/l – by 58 and 74 %, respectively. The sprouting of oat exceeds the sprouting in the control sample by 37 % at the concentration of 100 mg/l. In the amount of 100 mg/l thiosulfoester **2b** stimulates the cress root growth by 2 % (sprouting to the control is 40 % lower), and at the concentration of 1 mg/l – by 10 % (sprouting is 24 % lower). The sprouting of the cress is lower by 40–24 % compared to the control.

As can be seen from Table, at the concentration of 100 mg/l, S-propyl 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzenesulfonothioate **2c** is a stimulating factor of oat growth (*i.e.* root and stem). At the concentration of 100 mg/l, the oat root and stem growth is higher by 20 and 10 %, respectively, while the sprouting is 21 % higher than in the control. Mostly, ester **2c** contributes to the oat growth with a decrease in concentration (at the concentration of 1 mg/l the root and stem growth is higher by 54–56 %).



Table

Growth-regulative activity of thiosulfoesters

Compound	Conc., mg/l	Growth-regulative activity, % to control		Sprouting, %	Growth-regulative activity, % to control	
		oat			cress	Sprouting, %
		root	stem			
 2a	100	112.0±2.0	102.0±1.5	137.5±1.9	141.1±2.4	100.2±1.2
	10	125.1±1.6	128.3±2.0	84.2±1.1	145.0±1.9	88.0±1.1
	1	130.0±1.9	128.0±2.3	147.4±1.5	155.5±1.8	104.0±1.6
 2b	100	118.0±1.5	119.2±1.5	137.0±1.0	102.0±1.1	60.4±1.1
	10	154.4±1.3	159.0±1.8	121.0±1.7	105.0±0.9	72.3±1.0
	1	158.3±1.2	174.2±2.8	168.4±2.2	110.4±1.5	76.6±1.4
 2c	100	120.0±1.7	110.4±1.1	121.0±2.4	119.7±1.0	64.8±1.1
	10	130.3±0.9	121.0±1.7	111.3±1.7	157.3±1.9	84.2±1.5
	1	156.0±1.0	154.0±1.9	126.0±1.9	161.1±1.3	108.0±1.0
 1b	100	66.0±0.9	84.3±1.1	80.1±1.0	80.0±0.9	70.0±0.9
	10	109.0±1.1	103.7±0.9	96.0±1.1	115.0±1.5	115.0±1.1
	1	95.3±2.1	95.2±1.6	101.0±1.5	118.2±1.2	120.4±1.7
Terpal (growth-inhibition preparation)	100	52.4±2.3	83.3±2.0	114.4±1.9	42.0±1.6	102.2±2.1
	10	76.0±1.5	103.1±2.1	105.0±1.3	59.2±0.9	102.4±1.5
	1	90.0±1.7	101.0±1.1	117.0±2.0	79.5±1.2	98.0±1.4
Ivin (growth-promotional preparation)	100	107.1±2.4	107.0±0.9	100.3±0.9	111.0±1.6	95.4±1.1
	10	108.0±1.9	105.0±1.3	104.4±1.5	117.0±2.1	93.2±1.6
	1	116.0±1.0	111.4±1.6	95.0±1.4	131.3±2.1	95.0±1.1

To compare the effects of the *s*-triazine fragment introduction into the structure of alkyl esters of 4-aminobenzenethiosulfoacid on their growth regulatory activity, the aforementioned **1b** compound activity has been researched. At the concentration of 10 mg/l thiosulfonester **1b** stimulates the oat root growth by 9 % and the stem growth by 3 %. There is 16 % and 34 % inhibition of the stem and oat root growth, respectively, at the concentration of 100 mg/l. At the concentration of 1 mg/l, there is 5 % inhibition of the stem and oat root growth. The sprouting of oat is lower than in the control by 20 % (100 mg/l) and by 4 % (10 mg/l). Ethylthiosulfonylate stimulates the growth of cress at the concentration of 10 mg/l by 15 % and at the concentration of 1 mg/l by 18 %. The sprouting of cress is 30 % lower than in the control sample at the concentration of 100 mg/l, and 20 % higher at the concentration of 1 mg/l.

The obtained results of the researches indicate that the introduction of the *s*-triazine fragment into the structure of compound **1b** contributes to its growth stimulating activity.

4. Conclusions

Thus, the acylation of the amino group of alkyl S-esters of 4-aminobenzenethiosulfoacid with 2,4,6-

trichloro-1,3,5-triazine was investigated, and it was determined that in the polar aprotic solvent in the presence of organic bases at low temperatures of 273–278 K, the target S-alkyl 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzenesulfonothioates **2a-c** can be obtained with the yield of 60–75 %.

All the researched compounds exhibit a stimulating effect on the growth of both oat stem and root and the cress root, and it is higher compared to the well-known growth-stimulating drug Ivin.

In addition, compounds **2a-c** are of a following typical regularity: with a decrease in the concentration of the substance there is an increase in the growth of the oat stem and root and of the cress root. That is, the greatest effect on plant growth is typical of low concentrations (1 mg/l).

The following compounds are of the best stimulating activity: S-ethyl 4-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzenesulfonothioate **2b** at the concentration of 1 mg/l; S-propyl 4-[4,6-dichloro-1,3,5-triazin-2-yl)amino]benzenesulfonothioate **2c** at the concentration of 1 mg/l – relative to the cress.

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**МОДИФІКАЦІЯ АЛКІЛОВИХ ЕСТЕРІВ
4-АМІНОБЕНЗЕНТІОСУЛЬФОКИСЛОТИ
СИМ-ТРИАЗИНОВИМ ФРАГМЕНТОМ
ТА ДОСЛІДЖЕННЯ ЇХ РІСТРЕГУЛЮВАЛЬНОЇ
АКТИВНОСТІ**

Анотація. Досліджено ацилювання аміногрупи алкілових *S*-естерів 4-амінобензентіосульфокислоти ціанурхлоридом. Серію естерів із триазиновим фрагментом було синтезовано та охарактеризовано елементарним аналізом, ¹H-ЯМР та ІЧ-спектроскопією. Досліджено рістрегулювальну активність синтезованих сполук. Визначено певний вплив структури тіосульфоестерів на їх рістрегулювальну активність.

Ключові слова: тіосульфокислотні похідні, тіосульфоестери, *s*-триазин, рістрегулювальна активність.