SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 3,4-DISUBSTITUTED BENZENETHIOSULFONIC ACID ESTERS

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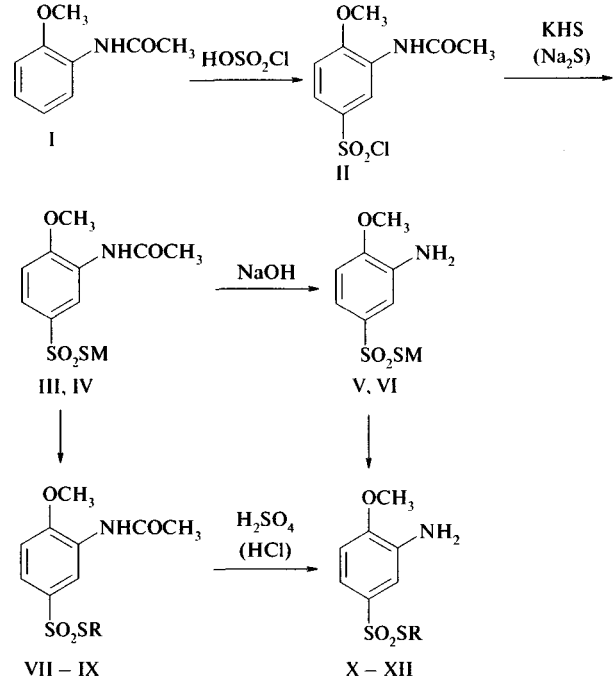
Thiosulfonates with the general formula R–SO_2–S–R′ occupy a special place among sulfur-containing compounds: combining a broad spectrum of antimicrobial action with low toxicity, these substances were recommended for use as drugs, preservatives, pesticides, and biocides providing protection against microbiological corrosion [1]. In particular, ethylsulfanylate in the the form of a 1% esulan ointment was suggested as a remedy for the treatment of tinea pedis and some other mycotic skin lesions. An advantage of the esulan ointment application is the keratolytic effect of this composition [2]. Some drugs based on thiosulfonates, capable of suppressing the anomalous development and degradation of erythrocytes, proved to be effective in the treatment of babesiasis and pyroplasmosis [3].

In this context, it was of interest to synthesize a series of new thiosulfonic acid esters and study their antimicrobial properties. This work was devoted to alkyl esters of 3,4-disubstituted benzenethiosulfonic acid synthesized by the following scheme.

In the initial stage of this synthesis, commercial *o*-acetylaminoanisole (I) was sulfochlorinated with technical-purity chlorosulfonic acid as described in [4] to obtain 3-acetylamino- 4-methoxybenzenesulfochloride (II). The subsequent interaction of sulfochloride II with aqueous solutions of potassium hydrosulfide or sodium sulfide led to the potassium or sodium salts of 3- acetylamino-4-methoxybenzenethiosulfonic acid (III and IV, respectively), converted into the corresponding salts 3-amino-4-methoxybenzenethiosulfonic acid (V, VI) by the hydrolysis of acetylamino group in a alkaline medium.

The alkylation of salts III – VI with alkylbromides or dimethyl sulfate led to esters VII – XII. In order to establish the optimum reaction conditions, the reactions were conducted at various temperatures (20, 40, 45, 60, 80°C) in various solvents (aqueous acetone, methanol, ethanol, chloroform, DMF). The duration of alkylation depends on the nature of the alkylating agent. The maximum yields of methyl (VII, X) and allyl (IX, XII) esters were observed at 20°C in aqueous acetone, while ethyl esters (VIII, IX) were obtained at a maximum yield at 45°C in methanol.



I II III, IV V, VI VII – IX X – XII

R = CH_3 (VII, X), C_2H_5 (VIII, XI), CH_2 = CH – CH_2 (IX, XII); M = K (III, V), Na (IV, VI).

The proposed structures of the synthesized compounds (VII – XII) were confirmed and their purity checked by TLC, ^{1}H NMR, and IR spectroscopic measurements and by elemental analyses. The reaction products appeared as white crystalline substances soluble in organic solvents and insoluble in water (Table 1).

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EXPERIMENTAL CHEMICAL PART

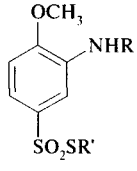
The IR absorption spectra were recorded on a Specord IR-75 spectrophotometer (Germany) using samples pelletized with KBr or suspended in a Vaseline oil. The ^{1}H NMR spectra were measured on a Varian VXR (300 MHz) spectrometer using TMS as the internal standard. Purity of the samples was checked by TLC on Silufol UV-252 plates eluted in an acetone – chloroform (10 : 1) system.

3-Acetylamino-4-methoxybenzenethiosulfonic acid potassium salt (III). To a mixture of 30.3 ml (0.228 mole) of a 42% aqueous solution of potassium hydrosulfide and 30 ml of water at 0 – 4°C was added 50 g (0.190 mole) of 3-acetylamino-4-methoxybenzenesulfochloride (II). The mixture was kept for 2 h at 0 – 4°C and heated to 65 – 70°C for about 1 h until complete dissolution of sulfur (pH 9 – 10). Then the reaction mixture was filtered hot and cooled. The precipitated crystals were filtered and dried to obtain 37.4 g (66%) of compound III; IR spectrum (ν_{max}, cm^{-1}): 1136, 1332 (SO_2), 1572, 1588, 1602 (Ar), 1620 (NH), 1662 (C = O).

3-Acetylamino-4-methoxybenzenethiosulfonic acid sodium salt (IV). Using a procedure analogous to that described above for compound III, compound IV was obtained proceeding from 54.6 g (0.228 mole) of a sodium sulfide nonahydrate and 50 g (0.190 mole) of sulfochloride IV; yield, 47.1 g (87.7%).

3-Amino-4-methoxybenzenethiosulfonic acid potassium salt (V). To a mixture of 50 g (0.17 mole) of potassium salt III and 50 ml of water at 20°C was added 13.4 g (0.34 mole) sodium hydroxide. The mixture was heated to 75 – 80°C and kept at this temperature for 2 h. Then the reaction mixture was filtered hot and cooled. The precipitated crystals were filtered and dried to obtain 32.6 g (75.8%) of compound V; IR spectrum (ν_{max}, cm^{-1}): 1128, 1330 (SO_2), 1574, 1586, 1600 (Ar), 1624 (NH), 3384, 3486 (NH_2).

TABLE 1. Yields and Melting Points of 3-Acetylamino- and 3-Amino- 4-methoxybenzenethiosulfonic Acid Esters



Compound	R	R'	Yield, %	M.p., °C (solvent for crystallization)
VII	CH_3CO	CH_3	82.4	161 (benzene)
VIII	CH_3CO	C_2H_5	68.1	118 (benzene)
IX	CH_3CO	$CH_2=CH-CH_2$	63.6	120 (CCl_4 – benzene, 1 : 1)
X	H	CH_3	35.0	101 (benzene)
XI	H	C_2H_5	44.2	Viscous
XII	H	$CH_2=CH-CH_2$	30.0	Viscous

3-Amino-4-methoxybenzenethiosulfonic acid sodium salt (VI). Using a procedure analogous to that described above for compound IV, compound VI was obtained proceeding from 50 g (0.18 mole) of compound IV and 14.1 g (0.36 mole) of sodium hydroxide; yield, 36.1 g (84.9%).

3-Acetylamino-4-methoxybenzenethiosulfonic acid methyl ester (VII). To a mixture of 12.2 g (43 mmole) of sodium salt IV with 30 ml of acetone and 3 ml of water at 20°C was added 3.4 ml (36 mmole) of dimethyl sulfate. After keeping the mixture for 2 h, the solvent was partly distilled off in vacuum and the precipitate was filtered, washed with water, and dried to obtain 8.1 g (82.4%) of compound VII; IR spectrum (ν_{max}, cm^{-1}): 1122, 1326 (SO_2), 1578, 1592, 1610 (Ar), 1628 (NH), 1668 (C = O); ^{1}H NMR spectrum in d_6-DMSO (δ, ppm): 2.200 (s, 3H, $COCH_3$), 2.567 (s, SCH_3), 4.005 (s, OCH_3), 7.261 (m, 3H, Harom).

3-Amino-4-methoxybenzenethiosulfonic acid methyl ester (X). Using a procedure analogous to that described above for compound VII, compound X was obtained proceeding from 12.9 g (0.043 mole) of salt V and 3.4 ml

TABLE 2. Sensitivity of Test Cultures with Respect to 3-Acetylmino-4-methoxybenzenethiosulfonic Acid Esters

Compound	Test culture	Incubation time	Drug concentration, mg/ml				
			2	1	0.5	0.2	0.1
VII	*Aeromonas sp.* 9615	24	–	–	–	–	–
		48	–	–	–	–	–
		120	–	–	–	–	–
	Escherichia coli C-600	24	–	–	–	–	+–
		48	–	–	–	–	+–
		120	–	–	–	–	+
	Candida albicans	24	–	+–	+–	+–	+
		48	+–	+–	+	+	+
		120	+	+	+	+	+
	Penicillium sp.	24	+–	+–	+–	+–	+
		48	+	+	+	+	+
		120	+	+	+	+	+
VIII	*Escherichia coli*	24	+	+	+	+	+–
		48	+	+	+	+	+–
	Candida albicans	24	+	+	+	+	+
		48	+	+	+	+	+–
IX	*Escnerinia coli*	24	+	+	+	+	+
		48	+	+	+	+	+
	Candida albicans	24	+	+–	+–	+–	–
		48	+	+–	+–	–	–

Note: (–) complete inhibition of the test culture growth; (+–) partial inhibition; (+) no inhibition, active growth of the microbe.

TABLE 3. Antimicrobial Activity of Compounds VII – IX: Minimum Bacteriostatic (BSC) and Bactericidal (BCC) Concentrations

Compound	Minimum BSC and BCC, μg/ml					
	Staphylococcus aureus		*Escherichia coli*		*Candida albicans*	
	BSC	BCC	BSC	BCC	BSC	BCC
VII	7.8	15.6	7.8	7.8	62.5	125
VIII	15.6	15.6	7.8	15.6	31.2	62.5
IX	15.6	31.2	15.6	31.2	125	125

(0.036 mole) of dimethyl sulfate in 30 ml of acetone and 3 ml of water; yield, 2.9 g (35%); IR spectrum (ν_{max}, cm^{-1}): 1128, 1320 (SO_2), 1576, 1596, 1604 (Ar), 1636 (NH), 3384, 3484 (NH_2).

3-Acetylamino-4-methoxybenzenethiosulfonic acid ethyl ester (VIII). To a mixture of 9 g (32 mmole) of sodium salt IV with 25 ml of methanol at 20°C was added 2.6 ml (26 mmole) of ethyl bromide. After heating the mixture for 6 h at 45°C, the solvent was distilled off in vacuum and the precipitate was filtered, washed with water, and dried to obtain 5.2 g (68.1%) of compound VIII; IR spectrum (ν_{max}, cm^{-1}): 1130, 1318 (SO_2), 1578, 1588, 1600 (Ar), 1612 (NH), 1668 (C = O).

3-Amino-4-methoxybenzenethiosulfonic acid ethyl ester (XI). Using a procedure analogous to that described above for compound VIII, compound XI was obtained proceeding from 9.7 g (35 mmole) of salt V and 2.8 ml (29 mmole) of ethyl bromide in 25 ml of methanol; yield, 3.2 g (44.2%); IR spectrum (ν_{max}, cm^{-1}): 1128, 1322 (SO_2), 1580, 1596, 1608 (Ar), 1632 (NH), 3384, 3486 (NH_2).

3-Acetylamino-4-methoxybenzenethiosulfonic acid allyl ester (IX). To a mixture of 12.7 g (42 mmole) of potassium salt III with 25 ml of acetone and 2.5 ml of water at 20°C was added 3.4 ml (35 mmole) of allyl bromide. After keeping the mixture for 2 h, the solvent was partly distilled off in vacuum and the precipitate was filtered, washed with water, and dried to obtain 6.8 g (63.6%) of compound IX; IR spectrum (ν_{max}, cm^{-1}): 1128, 1320 (SO_2), 1578, 1586, 1596 (Ar), 1626 (NH), 1650 (C = O).

3-Amino-4-methoxybenzenethiosulfonic acid allyl ester (XII). Using a procedure analogous to that described above for compound IX, compound XII was obtained pro-

TABLE 4. Antibacterial Activity of Compounds X – XII

Test culture	Ester concentration, %								
	X			XI			XII		
	0.1	0.01	0.001	0.1	0.01	0.001	0.1	0.01	0.001
Bacillus cereus 27A	–	–	+	–	+–	–	–	–	+
Bacillus mycoides 71d	–	+	+	–	+–	+	–	+–	+
Bacillus sp 5d	–	–	+	–	+–	–	–	–	–
Bacillus sp 9g	–	–	–	–	–	–	–	–	–
Bacillus licheniformis 7g	–	+–	+	–	+–	–	–	+–	+
Bacillus larvae 12g	–	+–	+	–	+–	–	–	–	+
Bacillus subtilis 26g	–	+–	+	–	+–	–	–	+–	+
Arthrobacter A_1G	–	+	+	–	+	+	–	+–	+
Arthrobacter A_2G	–	+	+	–	+–	+	–	+–	+
Arthrobacter A_3G	–	+	+	–	+–	+	–	+–	+
Arthrobacter A_4G	–	+–	+	–	+–	+	–	+–	+
Arthrobacter A_5G	–	–	+	–	+–	–	–	–	+
Pseudomonas putida 22A	–	–	+	–	+–	+	–	+–	+
Pseudomonas putida 37d	–	+–	+	–	+–	+	–	+–	+
Micrococcus 29g	–	+–	+	–	+–	+	–	+–	+
Micrococcus 25g	–	+	+	–	+–	+	–	+–	+
Micrococcus 31g	–	–	+	–	–	+	–	–	+
Micrococcus 23g	–	+–	+	–	+–	+	–	+	+
Staphylococcus epidermidis 30A	–	+	+	–	+	+	–	+	+
Rhodococcus rubrum 22g	–	–	+	–	–	–	–	–	+
Nocardia rubra 15g	–	–	+	–	–	–	–	–	+
Escherichia coli 36A	–	+	+	–	+–	+	–	+	+
Escherichia coli 44D	–	+–	+	–	–	+	–	+	+
Escherichia coli 395	–	–	+	–	–	+	–	+	+

Notes: (-) complete inhibition of the test culture growth; (+–) partial inhibition; (+) no inhibition, active growth of the microbe.

ceeding from 10.2 g (42 mmole) of salt VI and 3.4 ml (35 mmole) of allyl bromide in 25 ml of acetone and 2.5 ml of water; yield, 3.3 g (30%); IR spectrum (ν_{max}, cm^{-1}): 1130, 1324 (SO_2), 1572, 1598, 1610 (Ar), 1632 (NH), 3382, 3484 (NH_2).

EXPERIMENTAL BIOLOGICAL PART

The antimicrobial activity of the synthesized thioesters was determined by the conventional analytical methods of disks and double serial dilutions [5]. The tests were performed with the strains *Escherichia coli* C-600 and 1257, *Staphylococcus aureus* 209, *Aeromonas sp.* 9615, yeastlike fungi of the *Candida* genus, bacterial strains 9311, 9752, 9762, 97129, 9760, and 9767 (isolated from fish) possessing high DNAse activity, and micromycetes of *Penicillium* and *Aspergillus* genuses. The cultures of *E. coli*, *St. aureus*, and *Seromonas sp.* were grown in solid and liquid nutrient media (tryptose-soy agar, Difco broth, meat-infusion agar and meat-extract broth), while the *Candida* species and micromycetes were cultivated in Czapek and Sabouraud media. The experiments were performed with second-generation 18-h bacterial cultures, 48-h cultures of *Candida* species, and 72-h cultures of micromycetes.

It was established that thioesters VII – IX possess both antibacterial and antifungal (including anticandidiasic) activity.

As seen from Table 2, thioester VII inhibited the growth of *Aeromonas sp.* 9615 at all concentrations studied (0.1 – 2.0 mg/ml), while the most pronounced inhibition of *E. coli* C-600 was observed at 0.2 mg/ml. Similar results were obtained for compound VIII, while the minimum bacteriostatic concentration of compound IX with resect to *E. coli* was 1 mg/ml. The results of investigation of the antimicrobial activity of compounds VII – XII with respect to *E. coli* 1257, *Staphylococcus aureus* 209, and *Candida albicans* showed that compound VII exhibited the maximum bactericidal activity among the series of thioesters studied, while compound VIII showed the most pronounced fungicide activity with respect to yeastlike fungi (Table 3). As for the micromycete species studied, the fungistatic effect of compounds VII – IX was observed at higher concentrations (0.2 – 2 mg/ml).

Compounds X – XII were characterized only with respect to their antibacterial properties. As seen from Table 4, these compounds at a concentration of 0.1% completely suppress the growth of all test microbes; some strains of *Bacillus sp.* were effectively inhibited even at a concentration as small as 0.001%.

Thus, the results of our experiments show that 3,4-disubstituted benzenethiosulfonic acid alkyl esters (VII – XII) possess pronounced antimicrobial properties.

REFERENCES

1. B. G. Boldyrev, T. K. Bilozor, V. I. Lubenets, et al., *Biodamage in Industry. An Interinstitution Collection of Papers* [in Russian], Gorkii (1983), pp. 44 – 51.
2. B. G. Boldyrev, L. E. Kolmakova, G. M. Pershin, et al., *Khim.-Farm. Zh.*, **2**(4), 12 – 16 (1968).
3. French Patent No. 8,417,286 (1986).
4. *Organikum: 15 uberarbeitete Auflage*, VEB Deutscher Verlag der Wissenschaften, Berlin (1976), Bd. 1.
5. A. S. Labinskaya, *Microbiology and Techniques of Microbiological Investigation* [in Russian], Meditsina, Moscow (1972), p. 480.