The compounds were administered as aqueous solutions or Tween emulsions, intraperitoneally, 10-15 min before irradiation. Radioprotectant activity was assessed by the survival of the animals to the 30th day following irradiaton.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF CARBOXYLIC ACID DERIVATIVES CARRYING SULFUR-CONTAINING GROUPS IN THE β-POSITION

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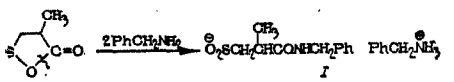
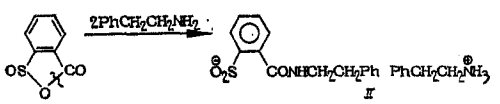
We have previously reported [3] the synthesis of new heterocyclic compounds: 1,2-oxathiolan-5-one 2-oxides and 2,1-benzoxathiol-3-one 1-oxide. In the present work, we have studied reactions involving splitting of these compounds by certain nucleophilic reagents. As in the case of cyclic anhydrides of β-sulfocarboxylic acids [9], their splitting by nucleophiles does not occur at the O–SO bond, but at the O–CO bond with the formation of the corresponding derivatives of β-(hydroxysulfinyl)carboxylic acids. Thus, reactions of 4-methyl-1,2-oxathiolan-5-one 2-oxide and 2,1-benzoathiol-3-one 1-oxide with amines led to the corresponding amides of β-(hydroxysulfinyl)isobutyric and o-(hydroxysulfinyl)benzoic acids (I-II) in the form of salts with these amines (Table 1).

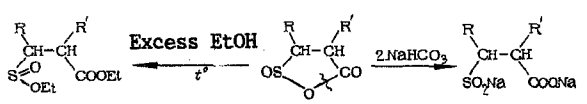


I



II

The splitting of 1,2-oxathiolan-5-one 2-oxide by alcohol in ether also proceeds at the O–CO bond with the formation of ethyl ester of β-(hydroxysulfinyl)propionic acid, which under more rigorous conditions in the presence of a large excess of alcohol is esterified to a diester (III). As should have been expected, anhydrides of β-(hydroxysulfinyl)carboxylic acids are readily saponified by sodium bicarbonate with the formation of the corresponding disodium salts (IV-V) (see Table 1)



III IV, V

III: R = R′ = H; IV: R = CH_3, R′ = H; V: R = H, R′ = CH_3

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TABLE 1. Physicochemical Properties of Compounds I-V, VII

Compound	Yield, %	mp, °C (solvent) or bp, °C/mm Hg	Calculated, %				Empirical formula	Found, %				Equivalent (oxidimetric, $KMnO_4$)	
			C	H	N	S		C	H	N	S	calculated	found
I	82	141—145[1]	62,07	6,90	—	9,19	$C_{18}H_{24}N_2O_3S$	61,58	6,72	—	8,72	—	—
II	99	159 (alcohol)[1,2]	—	—	6,83	7,80	$C_{23}H_{26}N_2O_3S$	—	—	7,12	8,10	205	180
III	66	58—60/0,04[3]	43,29	7,21	—	16,49	$C_7H_{14}O_4S$	42,57	7,01	—	16,69	—	—
IV	94	~340 (dec)[4]	24,49	3,06	—	16,32	$C_4H_6Na_2O_4S$	24,09	3,02	—	16,07	98,0	98,3
V	73	~340 (dec)[5]	24,49	3,06	—	16,32	$C_4H_6Na_2O_4S$	24,40	2,99	—	15,69	98	99
VII	100	147 (dec) (EA)[6]	40,75	5,66	5,28	24,14	$C_9H_{15}NO_4S_2$	41,25	5,98	5,48	23,86	—[7]	—

1) Sparingly soluble in alcohol. 2) Soluble in aqueous alcohol. 3) n_D^{24} 1.4560; soluble in water and organic solvents. 4) Does not melt; soluble in water, insoluble in alcohol, acetone, chloroform. 5) Does not melt; soluble in water. 6) Moderately soluble in water (pH 4), soluble in alcohol, chloroform and DMSO. 7) For acid VII neutralization equivalent: calculated 265, found 260.

TABLE 2. Data on IR Spectra of Compounds I-V and VII

Compound	ν, cm⁻¹						
	C=O	S=O	N–H	$\overset{+}{N}H_3$	C–O	CH_3	other bands
I	1635	955–1020	3265	1550, 1565 (deform. + Ar); 2800–3100 (stretch.)	—	1375 2950–2980	—
II	1635–1640	955, 1010	3250	1540–1570 (deform. + Ar); 2600–3100 (stretch.)	—	—	—
III	1740	1030, 1130	—	—	1190–1240	1379, 2950 2990	722 (CH_2–O) 1250–1275 (CH_2)
IV	1430, 1570–1590	990–1040	—	—	1240	1370, 2952, 2985	—
V	1415–1430. 1570–1590	995–1030	—	—	1255	1380, 2965, 2980	—
VII	1550, 1630 (CON); 1710 (COOH); 1737 (COS)	—	3350	—	1260	1375, 2940, 2980	2400–3100 (OH)

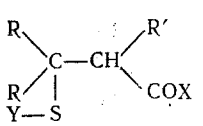
TABLE 3. Data on PMR Spectra of Compounds III and VII

Compound	δ, ppm			
	CH_3	CH_3CO	CH_2CH_2	other signals
III[a]	1.34 t (CO_2Et) 1.42 t (SO_2Et) $J_{CH_3-CH_2}$ 7.3 Hz	—	2,53–3,2 m	3,91–4.44 (two quadr.) $SOCH_2+COCH_2$)
VII[b]	1,25 s; 1.32 s	2,17 s (AcN) 2.33 s (AcS)	—	4,56 d (CH) 8,11 d (NH) J_{NH-CH}=8,0 Hg

[a] In CCl_4.
[b] In CF_3COOH.

The splitting of 1,2-oxathiolan-5-one 2-oxides by nucleophilic reagents is conveniently followed spectroscopically according to the disappearance of the anhydride band ν_{COO}: 1807-1812 cm^{-1}. The structure of the reaction products I-V was confirmed by IR and PMR spectra (Table 2). In the IR spectra of the salts of sulfinic acids I-II, IV-V, strong absorption is observed in the 955-1040 cm^{-1} region, characteristic of a sulfinate anion.

It was of interest to compare the biological activity of the carboxylic acid derivatives carrying a hydroxysulfinyl group in the β-position with analogous derivatives containing a divalent S atom at the β-position. Since the biological activity of β-acetylthio- and β-acetyldithioalkanoic acids has previously not been investigated, we synthesized benzylamide of β-(acetylthio)isobutyric acid (VI) and α-acetylamino-β-acetyldithioisovaleric acid (VII).



VI: Y = Ac, R = H, R′ = Me, X = $NHCH_2Ph$; VII: Y = AcS, R = Me, R′ = NHAc, X = OH

Benzylamide VI was synthesized by aminolysis of the corresponding acid chloride [2], while acid VII was obtained by the reaction of acetylsulfenyl chloride with N-acetyl-D,L-penicillinamine in the presence of triethylamine. Compound VI was characterized by spectra and titration (see Tables 1, 2).

TABLE 4. Radioprotective Activity and Toxicity of Carboxylic Acid Derivatives Carrying a Sulfinate, Acetylthio, and Acetyldithio Group in the β-Position

Compound	Method of administration	LD_{50}, mg/kg	Radioprotective action		
			dose, mg/kg	number of animals	
				total	% surviving
I	A	1844	540	10	10
			135	10	0
II	A	1640	410	10	40
			410	9	22
			105	10	10
III	A	912	280	10	0
			70	9	0
IV	A	>1568	390	10	10
			785	10	0
V	A	>1568	390	10	0
			785	10	0
VI	B	1130	350	10	20
			90	5	0
VII	A	610	240	20	0
			55	20	0
Control				50	0

Note. A) Intraperitoneally 20-30 min before irradiation; B) perorally 20-30 min before irradiation.

EXPERIMENTAL (CHEMICAL)

The IR spectra were run on a UR-10 spectrophotometer (GDR) in KBr tablets (for solid compounds) or in liquid film; the PMR spectra were obtained on a "Perkin-Elmer R-12" spectrometer (60 (MHz), using HMDS as internal or external standard. The purity of sulfinates I-II, IV-V was controlled by oxidimetric titration, as described in [3]. The yields, physical constants, solubility, elemental analysis and titration data of compounds I-VII obtained are given in Table 1, the IR and PMR spectra data, in Tables 2 and 3.

All the reactions were carried out in absolute solvents.

Benzylammonium salt of β-(hydroxysulfinyl)isobutyric acid benzylamide (I). A solution of 2.18 g (20 mmoles) of benzylamine in 10 ml of ether is added to a solution of 1.26 g (9 mmoles) of 4-methyl-1,2-oxathiolan-5-one 2-oxide [3] in 20 ml of ether; the reaction is exothermic. An oil precipitates, which solidifies on standing. On the following day, the precipitate is filtered, washed with ether,and dried in a vacuum-exsiccator. The yield of I is 2.66 g.

2-Phenylethylammonium salt of o-(hydroxysulfinyl)benzoic acid 2-phenylethylamide (II) was obtained in a similar way from 10 mmoles of 2,1-benzoxathiol-3-one 1-oxide [3] and 20 mmoles of 2-phenylethylamine in 35 ml of $CHCl_3$, after evaporation of the reaction mixture in vacuo and crystallizatin of the residue.

Diethyl Ester of β-(Hydroxysulfinyl)propionic Acid (III). A 150 ml portion of ethanol is added to 6 g (50 mmoles) of 1,2-oxathiolan-5-one 2-oxide [3]; the reaction is exothermic. On the following day, the reaction mixture is boiled under reflux condenser for 3-4 h, and then evaporated in vacuo, and the residue is fractionated. Yield, 5.9 g of III.

Disodium Salt of β-(Hydroxysulfinyl)butyric Acid (IV). A 4.3 g portion (32 mmoles) of 3-methyl-1,2-oxathiolan-5-one 2-oxide [3] is added slowly, with stirring to a solution of 5.4 g (64 mmoles) of $NaHCO_3$ in 10 ml of water. On the following day, the neutral reaction mixture is evaporated to dryness, and the solid residue is washed with alcohol, and dried in vacuo to a constant weight. Yield 5.9 g of IV.

Disodium salt of β-(hydroxysulfinyl)isobutyric acid (V) is obtained in a similar to IV from 4-methyl-1,2-oxathiolan-5-one 2-oxide [3].

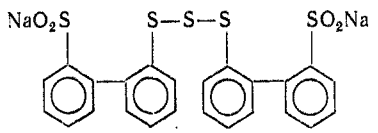
Benzylamide of β-(acetylthio)isobutyric acid (VI) is obtained by condensation of β-(acetylthio)isobutyryl chloride with benzylamine according to [2]; the physical constants and spectra of VI coincide with those given in [2].

α-Acetylamino-β-acetyldithioisovaleric acid (VII). A 2.3 g portion (21 mmoles) of acetylsulfenyl chloride is added dropwise at −40°C, with stirring, to a solution of 4 g (21 mmoles) of N-acetylpenicillamine in 60 ml of chloroform, containing 2.9 ml (21 mmoles) of triethylamine. After 2 h (20°C), 150 ml of ether are added to the reaction mixture, the precipitate of triethylamine hydrochloride is filtered, and the mother liquor is evaporated to dryness. Yield, 5.55 g of VII.

EXPERIMENTAL (BIOLOGICAL)

The radioprotective effectiveness of the compounds was studied on hybrid mice F_1 (CBA × C57Bl) weighing 19-23 g each. The compounds were administered intraperitoneally to the animals in the form of aqueous solutions or suspensions with Tween-80, in a volume of 0.2 ml, 20-30 min before the irradiation. The irradiation was carried out on a gamma-apparatus IGUR in a dose of 900 rd and at a dose rate of 208-210 rd/min. The effectiveness of the compounds was indicated by the survival on the 30th day after irradiation. The toxicity of the compounds was studied on white unpedigreed male mice, weighing 19-26 g each, and was determined by the method of V. I. Suslikov et al. [4].

The results of the radiobiological tests of the compounds obtained are presented in Table 4. All the compounds I-VII are nontoxic. Among them, aryl sulfinate II (in a dose of 410 mg/kg) has a moderate radioprotective action and acetyl sulfide VI (in a dose of 350 mg/kg) has a very moderate radioprotective action. Alkane sulfinates I and IV give a weak radioprotective effect. The presence of radioprotective activity in phenylethylamide II and benzylamide IV is best explained by the influence of the amine residue, since, for example, in contrast to other inactive derivatives of β-mercaptopropionic acid, its benzylamide $HSCH_2CH_2CONHCH_2Ph$ has the property of prolonging the radioprotective action [1]. However, it is known that the sulfinate group imparts special radioprotective properties to the $-S(CH_2)_4SO_2Na$ residue, compared with the inactive −SBu group. A highly active nontypical (not containing an amino group) radioprotector is butane sulfinate, containing the trisulfide group $S[S(CH_2)_4SO_2Na]_2$ [5-8]. Aryl sulfinate also exhibits a high radio-



protective activity (90%), evenin small doses (from 4.6 to 20 mg/kg) [7, 8].

The comparison of the data obtained with the literature data shows that although the combination of the sulfinate and carboxylic groups in one molecule is less effective than the combination of the sulfinate and trisulfide groups, nevertheless the prospect of a search for new radioprotectors among bifunctional sulfinate compounds is obvious [7].

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