SYNTHESIS AND RADIOPROTECTIVE ACTIVITY OF THE PRODUCTS OF REACTIONS BETWEEN CYCLIC ANHYDRIDES OF β-(OXYSULFINYL)CARBOXYLIC ACIDS AND AMINES

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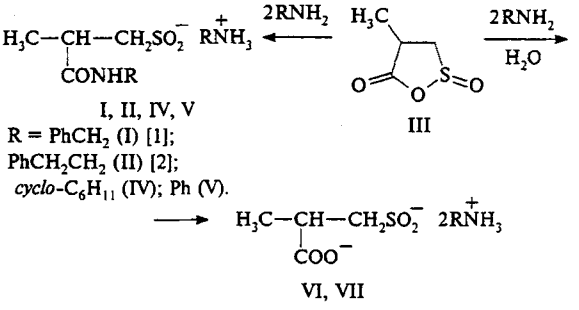
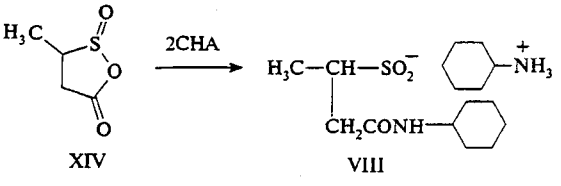
Previously we have studied the pharmacological activity of some derivatives of β-(oxysulfinyl)carboxylic acids, including their sodium and ammonium salts, esters and amides, in which the radioprotective properties were observed only in amine salts and amides [1, 2]. The radioprotective activity and toxicity of these compounds were dependent on the structure of both acid and amine. Among the sulfinocarboxylic acids studied, the most promising base compound for the creation of potential radioprotective drugs was β-(oxysulfinyl)isobutyric acid. The radioprotective properties of the ammonium salts of amides of this acid depend on the amine part: benzylamide (I) was nontoxic and of low activity [1], while the corresponding phenylethylamide (II) was highly active but at a subtoxic dose [2]. In continuation of that work, we have studied the radioprotective activity of the products of reactions between cyclic anhydrides of β-(oxysulfinyl)carboxylic acids and other amines, including aniline, cyclohexylamine (CHA), 1-adamantylamine (1-AdNH$_2$), and bis(dimethylamino)methane. Our interest in the cycloaliphatic amines is stimulated by the fact that radioprotective properties were observed in derivatives of adamantane [3] and CHA [4]. As is known, the acylation of amines sometimes leads to compounds possessing pronounced radioprotective activity [4].

In this work, the acylating agent was represented by 4-methyl-1,2-oxathiolan-5-one-2-oxide (III) – an internal anhydride of β-(oxysulfinyl)isobutyric acid. Interaction of compound III with CHA or aniline in an anhydrous medium leads to the corresponding ammonium salts of β-(oxysulfinyl)isobutyric acid amides (IV, V). Reactions of compound III with CHA or 1-AdNH$_2$ in the presence of water yield, instead of the amides, the bisammonium salts of the same acid (VI, VII). By analogy with the synthesis of compound IV, the reaction of CHA with 3-methyl-1,2-oxathiolan-5-one-2-oxide (XIV) led to cyclohexylamide (VIII).



R = *cyclo*-C_6H_{11} (VI), 1-Ad (VII).



Amides of *o*-(oxysulfinyl)isobutyric acid were obtained, in the form of salts (IX, X) with amines, by the action of 2 moles of CHA or 1-AdNH$_2$ on 1 mole of benzo-2,1-oxathiol-3-one-1-oxide (XI). As is known, interaction of bis(dimethylamino)methane (XII) with anhydride PhC(O)OS(O)Ph leads to scission of the O–CO bond to yield $PhCONMe_2$ and the salt $PhSO_2^- \cdot Me_2N^+{=}CH_2$ [5]. A similar reaction takes place between cyclic anhydride XI and an equimolar amount of XII with the formation of an immonium salt (XIII).

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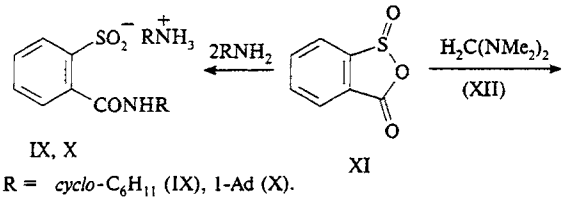
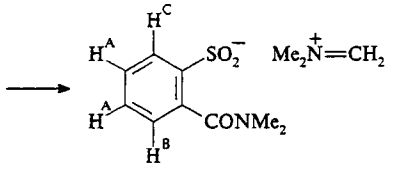


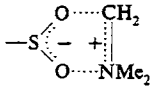
IX, X XI

R = *cyclo*-C_6H_{11} (IX), 1-Ad (X).



XIII

The proposed structures of synthesized compounds were confirmed by IR spectra, and the structure of immonium salt XIII was also confirmed by the ^1H NMR data. The IR spectra of salts IV – X show strong absorption bands at 930 – 1050 cm^{-1} characteristic of the sulfinate anions. For compound XIII, this band exhibits a high-frequency shift ($\nu_{SO_2^-}$, 1035 – 1100 cm^{-1}), which is probably due to coordination of the sulfinate anion to an electron-acceptor resonance immonium cation of the type



EXPERIMENTAL CHEMICAL PART

IR spectra were measured on an UR-20 spectrophotometer using samples prepared as KBr disks or thin films. The ^1H NMR spectra were obtained on a Bruker WP-200 SY (200 MHz) spectrometer using HMDS as the internal standard. All reactions (except VI and VII) were performed in anhydrous solvents and reagents. The reaction yields, physicochemical constants, solubilities, and IR data for compounds IV – X and XIII are listed in Table 1. The initial anhydrides III, XI, and XIV were obtained as described in [6]. The results of elemental analyses of the synthesized compounds agreed with the analytically calculated values.

Cyclohexylammonium salt of cyclohexylamide of β-(oxysulfinyl)isobutyric acid (IV). Compound IV was obtained using 2.2 g of anhydride III and 3.25 g CHA under conditions used for the synthesis of benzylamide I [1]. Yield of compound IV, 3.8 g.

Anilinium salt of anilide of β-(oxysulfinyl)isobutyric acid (V). To 5.96 g (0.064 mole) of aniline in 30 ml of ether was added with stirring 4.2 g (0.031 mole) of compound III in 20 ml of ether and the mixture was allowed to stand overnight. Then the precipitate was filtered, washed with petroleum ether, and dried over P_2O_5 to obtain 8.6 g of compound V; decomposition temperature 142 – 145°C. After recrystallization yield of compound V, 6.8 g.

Bis(cyclohexylammonium) salt of β-(oxysulfinyl)isobutyric acid (VI). To 2.5 g (0.0187 mole) of compound III in 30 ml of technical-grade ether was added 3.7 g (0.037 mole) of CHA in 30 ml of the same solvent. After keeping the mixture for 1 – 2 days at 0 – 4°C, the precipitate was filtered and dried to obtain 5.5 g of compound VI.

Bis(1-adamantylammonium) salt of β-(oxysulfinyl)isobutyric acid (VII). Compound VII was obtained similarly to VI, proceeding from 2.2 g of compound III and 4.95 g of 1-$AdNH_2$. Yield of compound VII, 6.3 g.

Cyclohexylammonium salt of cyclohexylamide of β-(oxysulfinyl)isobutyric acid (VIII). Compound VIII was

TABLE 1. Physicochemical Properties of Compounds IV – X and XIII

Compound	Yield, %	T_{decomp}, °C (solvent for crystallization)	Empirical formula	IR spectrum (ν_{max}, cm^{-1})				
				C=O	SO_2^-	N–H	$\nu(N^+H_3)$	$\delta(N^+H_3)$, δ(CON), and / or δ(Arom.)
IV[1)]	70	115	$C_{16}H_{32}N_2O_3S$	1635	955 (s), 1010 – 1023 (as)	3255	2600 – 3100	1550 – 1560
V[1),2)]	87	142 – 145 (ethanol)	$C_{16}H_{20}N_2O_3S$	1665	960 – 1050	3300	2400 – 3100	1540 – 1600
VI[3)]	84	180 – 190	$C_{16}H_{34}N_2O_4S$	1410, 1545 – 1575	970 (s), 1023 (as)	–	2600 – 3100	1580
VII[4)]	85	215 (ethanol)	$C_{24}H_{42}N_2O_4S$	1380 – 1415, 1560 – 1575	965 (s), 1022 (as)	–	2500 – 3200	1590
VIII[5)]	47	155 – 162 (ethanol – petroleum ether, 1:3)	$C_{16}H_{32}N_2O_3S$	1632	970, 1010, 1030	3260	2600 – 3100	1540 – 1557
IX[6)]	93	190 – 194	$C_{19}H_{30}N_2O_3S$	1638 – 1643	940 – 967 (s), 1010 – 1038 (as)	3230 – 3270	2700 – 3100	1540 – 1560
X[7)]	47	210 – 220 (ethanol or ethanol – ether)	$C_{27}H_{38}N_2O_3S$	1650	930 – 950 (s), 995 – 1045 (as)	3270	2600 – 3100	1515 – 1545
XIII[5),8)]	99	Thick oil	$C_{12}H_{12}N_2O_3S$	1635 – 1645	1035 – 1110	–	–	1490 – 1510

Notes. [1)] moderately soluble in water; [2)] soluble in ethanol and DMF, poorly soluble in acetone; [3)] soluble in water, $CHCl_3$, and ethanol, insoluble in ether, hexane, and acetone; [4)] soluble in ethanol and aqueous ethanol; [5)] soluble in water and ethanol; [6)] well soluble in water; [7)] soluble in $CHCl_3$ and ethanol, insoluble in water and acetone; [8)] soluble in $CHCl_3$, insoluble in ether.

TABLE 2. Acute Toxicity and Radioprotective Activity of β-(Oxysulfinyl)carboxylic Acids and Their Amides

Compound	Method of administration	LD_{50}, mg/kg	Dose, mg/kg	Survival, % of control
I	A	1844	540	10 [1]
	A		135	0 [1]
II	A	260	200	80 [2]
	A		100	60 [2]
IV	A	950	300	0
	A		100	0
V	A	> 1500	800	50
	A		400	40
VI	A	1098	450	0
VII	A	250	150	13
	A		50	0
VIII	A	750	300	15
	A		200	0
	A		100	10
	B		500	15
IX	A	900	500	13
	A		300	20
	B		500	0
X	A	≫ 1000	150	45
	A		50	15
	B		300	25
	B		150	0
XIII	C	≫ 1000	600	0
	D		600	24
	C		300	0
	D		300	7

Note. A: i.p. (30 min before irradiation); B: p.o. (1 h); C: i.p. (20 min); D: i.p. (1 h).

obtained similarly to V (but at a temperature of 4°C), proceeding from 2.73 g of compound XIV and 4.46 g of CHA. The oily product was purified by dissolving in 20 ml of boiling absolute ethanol, followed by precipitation with petroleum ether (60 ml). The precipitate was dried over P_2O_5 to obtain 3.15 g of compound VIII in the form of white crystals.

Cyclohexylammonium salt of cyclohexylamide of *o*-(oxysulfinyl)isobutyric acid (IX). To 2.52 g (0.015 mole) of anhydride XI in 20 ml of $CHCl_3$ was added 2.97 g (0.03 mole) of CHA in 30 ml of the same solvent. After keeping the mixture for 12 h at 20°C, the precipitate was filtered and dried over P_2O_5 in vacuum to obtain 5.1 g of compound IX.

1-Adamantylammonium salt of 1-adamantylamide of *o*-(oxysulfinyl)isobutyric acid (X). Compound X was obtained similarly to IX, proceeding from 2.52 g of compound XI and 4.53 g of 1-$AdNH_2$. After evaporation of the reaction solution, the glassy residue was mixed with 13 ml of boiling ethanol, the insoluble impurity separated, and the mother liquor cooled. The precipitate was filtered, washed with ether, and dried over P_2O_5 to obtain 1.9 g of compound X; adding ether to the filtrate yields another 1.4 g of the target product.

N,N-Dimethylmethyleneimmonium salt of dimethylamide of *o*-(oxysulfinyl)isobutyric acid (XIII). To 1.76 g (0.017 mole) of anhydride XII in 10 ml of ether at 4°C was added 2.9 g (0.017 mole) of compound XI in 50 ml of the same solvent. After keeping the mixture overnight, the solvent was evaporated under vacuum, and the residue kept over P_2O_5 in vacuum until dried to constant weight. Yield, 4.6 g of compound XIII in the form of a thick oil (n_D^{22}, 1.5460) decomposing during distillation in vacuum (0.04 Torr).

Oxidimetric equivalent: found ($KMnO_4$ titration), 69; calcd. (M/4), 67.5; ^1H NMR spectrum, $CDCl_3$ (δ, ppm): 2.65 (s, Me_2N^+), 2.92 (s, $CONMe_2$), 3.19 (s, $CONMe_2$), 4.42 (s, CH_2=), 7.38 (d, H^B), 7.56 – 7.73 (m, $2H^A$), 8.08 (d, H^C, $J_{AB} = J_{AC} = 9.5$ Hz).

EXPERIMENTAL BIOLOGICAL PART

The acute toxicity (LD_{50}) of the synthesized compounds was determined on white mongrel mice, and the radioprotective activity was studied on a group of hybrid (CBA × C57B1)F_1 mice weighing 19 – 23 g. The compounds were introduced either by intraperitoneal injections of aqueous solutions (0.2 ml) 20 – 60 min before irradiation or perorally 1 h before the exposure. The animals were irradiated on an IGUR setup with a ^{137}Cs β-radiation source, at a dose rate of 210 r/min to a total dosage of 850 – 900 r. The efficacy of the drugs was evaluated from the increase in survival of a group of 15 – 20 irradiated animals within a time period of 30 days. The loss of control animals under these conditions was 100%.

The results of biological tests are presented in Table 2. For comparison, we have also reproduced analogous data obtained previously for compounds I and II. It was established that most of the newly synthesized amides are of low toxicity. Acylation with anhydrides III, XI, and XIV decreases the toxicity of CHA (LD_{50} = 129 mg/kg [4]) to 1/6 – 1/7 of the initial level. For example, the LD_{50} of cyclohexylamides IV, VIII, and IX fall within 750 – 950 mg/kg. The binary salts of β-(oxysulfinyl)alkanoic acids with CHA (adduct VI) or 2-$SdNH_2$ (adduct VII) are 5 times less toxic compared to bisphenylethylammonium salt $HO_2S(CH_2)_2COOH \cdot 2Ph(CHC_2)_2NH_2$ (XV) (LD_{50} = 50 mg/kg [2]). However, the radioprotective activity of the latter salt was also markedly higher: 47 – 53% [2] against 0 – 13% for the binary salts VI and VII. In contrast to what was reported in [4], cyclohexylamides IV, VIII, and IX obtained by the acylation of CHA are inactive or exhibit a weak radioprotective activity. Immonium salt XIII produced a small protective effect (not exceeding 24%) only when introduced 1h before irradiation.

The maximum therapeutic ratio (> 10 – 20) among the synthesized compounds was observed for 1-adamantylammonium salt of 1-adamantylamide of *o*-sulfinobenzoic acid X, possessing a moderate radioprotective activity (below 45%) at small doses (50 – 150 mg/kg). The analogous product of reaction between anhydride XI and β-phenylethy-

lamine (a salt of β-phenylethylamide of *o*-sulfinobenzoic acid) was less effective, showing a radioprotective effect of 22 – 40% at a dose of 410 mg/kg [1]. A rather unexpected result was the radioprotective activity (40 – 50%) of anilide V, since no radioprotectors among the aniline derivatives were reported so far. This is probably a particular case related to alternation of the biological activity of phenylalkylammonium salts of the phenylalkylamides of β-(oxysulfinyl)isobutyric acid, $Ph(CH_2)_nNHCOCH(Me)CH_2SO_2^- Ph(CH_2)_n\overset{+}{N}H_3$, depending on the chain length. Indeed, compounds with an even number of units, V ($n = 0$) of II ($n = 2$), are active, whereas those with an odd number of units are inactive [I ($n = 1$)] (Table 2). Verification of this hypothesis would require the synthesis of compounds with $n > 3$. Elongation of the two-monomer-unit hydrocarbon chain in well-known radioprotectors, such as $H_2N(CH_2)_nSX$ (X = H or SO_3H), to 3 – 6 monomer units leads to the loss of radioprotective activity [7].

Thus, most promising potential radioprotectors among the products of aminolysis of the cyclic anhydrides of β-(oxysulfinyl)carboxylic acids are phenylalkylamides and adamantylamides.

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