The results we obtained indicate that ACF have a significantly greater capacity to absorb cholesterol and atherogenic complexes than do GAC. Moreover, the presence of carboxyl groups on the ACF surface affords a charcoal fiber sorbent a distinct specificity for atherogenic complexes of cholesterol and significantly increases ACF hemocompatibility. This latter fact gives us reason to believe that the controlled modification of ACF will significantly improve their properties.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF MERCAPTOTHIOCARBOXYLIC ACIDS AND THEIR CYCLIC ANALOGS

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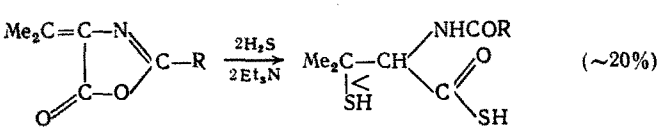
Certain derivatives of 1,2-dithiolanes (α-lipoic acid) play an important role in metabolism [3]. However, the radioprotective activity of 1,2-dithiolan-3-ones or their acyclic analogs, the β-acetyldithiocarboxylic acids, has not been studied. It is also known that certain β-mercaptoamino acids (cysteine) [9] and aminothiolic acid [10] possess radioprotective activity.

In order to study their radioprotective activity, we developed a synthesis of the thioderivative of cysteine and penicillamine and, for comparison, β-mercaptocarboxylic acids, as well as their cyclic analogs the 1,2-dithiolan-3-ones.

The N-acylthiopenicillamines (I and II) were obtained earlier in low yield by reaction of 4-isopropylidineoxazolones with H_2S [11, 12]:



(~20%)

I: R = Me, II: R = Ph

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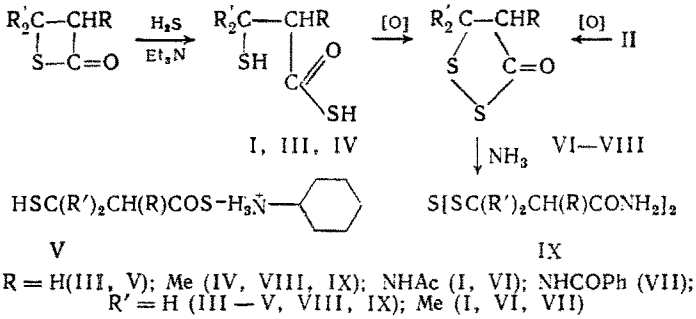
This method, however, is limited because of the poor availability and instability of the starting materials. We developed an original means of synthesis of not only the α-acylamino compound I, but also the previously unknown β-mercaptothiolalkanoic acids (III, IV), by the reaction of β-propiothiolactone with H_2S in the presence of triethylamine. The compounds I were obtained in high yield (78%) by this method (Table 1). β-Mercaptothiolpropionic acid III was also characterized in the form of its highly water-soluble cyclohexylammonium salt (V).

The β-mercaptothiocarboxylic acids obtained are convenient starting materials for the synthesis of five-membered cyclic disulfides, the 1,2-ditholan-3-ones (VI-VIII). Reaction of compound VIII with ammonia gave the corresponding aminotrisulfide IX.



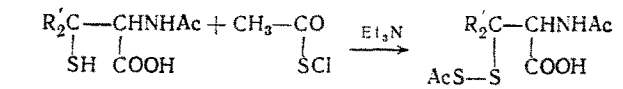
I, III, IV

V

IX

R = H(III, V); Me (IV, VIII, IX); NHAc (I, VI); NHCOPh (VII);
R′ = H (III — V, VIII, IX); Me (I, VI, VII)

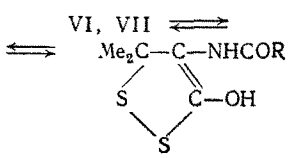
The earlier-unknown β-acetyldisulfide derivatives of L-cysteine (X) and D,L-penicillamine (XI) were obtained by reaction of the corresponding amino acid with acetylsulfenyl chloride:



R′ = H (X); Me (XI)

X, XI

The yields and the physicochemical characteristics of the compounds prepared are presented in Table 1. The structures of the reaction products I-XI were verified by IR and PMR spectra (Table 2). The spectral data established that the acylaminodithiolanes VI and VII exist in equilibrium with the hydroxydithioles:



The type of isomer present depends upon the solvent: in CF_3COOH the equilibrium is displaced to the left, and in dimethylsulfoxide the solution contains about 25% enol.

The results of the toxicity studies and the radioprotective properties of these compounds are presented in Table 3. The salt V and the disulfide VIII were toxic, and the remaining compounds were of low toxicity.

Usually the mercaptoderivatives (cysteamine or cysteine) are more active than their oxidation products, the corresponding disulfides (cystamine or cystine) [9]. Nevertheless, we discovered that, even though compound I was not active its oxidation product, the cyclic disulfide VI, shows a radioprotective effect at a dose of 300 mg/kg (30%). The existence of radioprotective activity in VI may be explained by its ability to trap radicals. The analogous 1,2-dithiolan-3-one containing an amino group in the 4-m position blocked with benzoyl (VII) rather than acetyl is not as active as disultide VIII, with no amino group. S-Acetylsulfide derivatives of mercaptoaminoacids (X-XI), salt V, and trisulfide IX also showed no radioprotective activity.

It is probable that the removal of the acetyl blocking groups in compounds VI leads to an increase in radioprotective activity, but attempts to hydrolyze the acylaminodithiolanones VI and VII in acid resulted in opening of the rings and the production of sulfur.

TABLE 1. Physicochemical Characteritics and Titration Data for Compounds I-XI

Compound	Yield	mp, °C, bp., °C/mm	n_D (t °C)	Calculated, %				Empirical formula	Found, %				Equivalent	
				C	H	S	N		C	H	S	N	calculated	found
I	78	95—8 (benzene- hex)	—	40,58	6,28	30,92	—	$C_7H_{13}NO_2S_3$	40,83	6,32	30,71	—	207*, 103,5**	211*, 105**
III	42	70—3/10	1,5542 (23)	29,51	4,92	52,46	—	$C_3H_6OS_2$	30,31	5,02	52,25	—	122*, 61**	121,9*, 57**
IV	45	58/3	1,5378 (21,5)	35,29	5,88	47,06	—	$C_4H_8OS_2$	36,04	5,55	47,05	—	68**	64**
V	100	56 (in sealed capillary)	—	—	—	29,00	6,33	$C_9H_{19}NOS_2$	—	—	29,22	6,37	110,5**	112**
VI	88	148(alcohol); lit.[12] mp 146-147°C	—	40,98	5,37	31,22	—	$C_7H_{11}NO_2S_2$	41,27	5,46	31,10	—	—	—
VII	94	125—6 (aq.MeOH); lit.[11] mp 124-126°C	—	53,93	4,87	23,97	5,24	$C_{12}H_{13}NO_2S_2$	53,90	4,76	24,57	4,73	—	—
VIII	70	58—60/1****	1,5770 (20)***	—	—	—	—	—	—	—	—	—	—	—
IX	91	53****	—	—	—	—	—	—	—	—	—	—	—	—
X	71	70 ($CHCl_3$— ether)	—	35,44	4,64	27,00	5,91	$C_7H_{11}NO_4S_2$	36,00	4,89	26,89	6,02	237*	239*
XI	100	147(dec.) (ethyl acetate)	—	40,75	5,66	24,14	5,28	$C_9H_{15}NO_4S_2$	41,25	5,98	23,86	5,48	265*	260*

*with NaOH; **with iodine; ***bp 57°C/1 mm, n_D^{20} 1.5790 [4]; ****cf. [2] — not determined.

TABLE 2. IR and PMR Spectral Data for the Compounds Prepared

Compound	IR spectrum (ν_{max}, cm^{-1})					
	C=O	N—H	S—H	O—H	S—S	other bands
I	1693 (COS); 1545, 1660 (CON)	3270	2540	—	—	—
III	1691, 1710	—	2568	—	—	—
IV	1691, 1708	—	2570	—	—	—
V	1445—1552	—	2510, 2568	—	—	2800—3000 ($\overset{+}{N}H_2$)
VI	1710 (COS); 1635, 1540 (CON)	3270	—	2900—3080*	500, 535	1450, 1555 (C=C)*
VII	1710 (COS); 1650, 1535 (CON)	3325	—	2900—3000*	524, 570	1453, 1520 (C=C*, Ar)
X	1710 (COOH), 1738 (COS); 1620, 1570 (CON)	3355	—	2400—3100	595	1260 (C—O)
XI	1710 (COOH), 1737 (COS); 1630, 1550 (CON)	3350	—	2400—3100	555, 605	1375, 2940, 2980 (CH_3); 1260 (C—O)

Compound	PMR spectrum (δ, ppm; J, Hz)						
	CH_3	CH_3CO	CH_2	CH	NH	SH	other signals
I[1]	1,47s; 1,73s	2,28s	—	3,61—3,87m	4,73d J_{NH-CH} = 9,3	2,48 s	2,61s (COSH)
III[2]	—	—	2,6—3,2m	—	—	1,71 t. . J_{SH-CH_2} = 8,0	4,69s (COSH)
IV[2]	1,51d; J_{CH_3-CH} = 6,7	—	2,59—3,24m		—	1,78 t. J_{CH-CH_2} = 8,7	4,75 s (COSH)
VI[3]	1,30s; 1,36s	2,13s	—	4,91d. J_{CH-NH} = 9,3	7,19d	—	—
VI[4]	1,42s	2,02s	—	3,53—4,12 m	4,9d	—	4,9s (NH+OH)[5]
X[3]	—	2,17s (AcN) 2,29 s (AcS)	2,89—3,27 dd	4,51—4,89m J_{CH-NH} = 9,3	8,31d	—	—
XI[3]	1,25s; 1,32s	2,17s (AcN) 2,33 s (AcS)	—	4,56 d J_{NH-CH} = 8,0 J_{NH-CH} = 8,0	8,11d	—	—

Notes. *Enol bands: [1]in $CDCl_3$; [2]in CCl_4; [3]in CF_3COOH; [4]in DMSO; [5]signals from 25% enol isomer.

TABLE 3. Radioprotective Activity and Toxicity of the Compounds Synthesized

Compound	Method of introduction	LD_{50}, mg/kg	Radioprotective effect			
			dose, mg/kg	number of animals		avg. surv. time (ast), days
				total	% surviving	
I	A	>1000	300	20	0	9,3
	B	>1000	300	20	0	8,1
V	A	14,5	5,5	20	0	8,8
			1,4	20	0	7,7
VI	A	>1000	300	19	30	11,3
VIII	A	730	290	20	0	9,7
			72,5	20	0	8,2
VIII	A	15,0	6,3	20	0	8,4
			1,6	20	0	8,8
	B	110	35	20	0	7,9
			8,9	20	0	8,4
IX	A	>1000	300	14	0	8,6
	B	>1000	300	20	0	8,1
X	A	>800	300	20	0	8,6
	B	>1000	300	20	0	7,5
XI	A	610	240	20	0	8,2
Control				230	0	9,3

Note. A – Intraperitoneal introduction 20 min before irradiation; B – Peroral introduction 10 and 30 min before irradiation.

Thus, the present work describes the synthesis of β-mercaptothio- and β-acetylthiocarboxylic acids and their cyclization products, the 1,2-dithiolan-3-ones, among which 4-acetylamino-1,2-dithiolan-3-one showed radioprotective activity.

EXPERIMENTAL CHEMICAL

IR spectra were recorded with an UR-10 instrument in KBr pellets (for solids) and as liquid films. PMR spectra were obtained with a Perkin-Elmer R-12 instrument (60 MHz) with internal TMS. Yields, constants, elemental analytical data, and titrations for the compounds obtained (I-XI) are presented in Table 1, and IR- and PMR-spectral data are given in Table 2.

α-Acetylamino-β-mercaptothiolisovaleric Acid (I). To a flask fitted with a bubbler and condenser, and cooled in dry ice/acetone was added 3.46 g (0.02 mole) of α-acetylamino-β,β-dimethyl-β-propiolactone [5] in 40 ml of dry $CHCl_3$. Hydrogen sulfide was passed into the solution for 0.5 h, then 2.32 g (0.023 mole) of Et_3N in 10 ml of dry $CHCl_3$ was added and H_2S addition was continued for 7 h. On the following day the reaction mixture was extracted with water (10 ml × 3). The aqueous extract was acidified with 10% HCl to pH 1-2, extracted with $CHCl_3$ (15 ml × 3), dried over Na_2SO_4, and the solvent was removed under vacuum (N_2) to give 3.23 g of I, mp 95°C, (Lit. 11, mp 99°C). Acid I was moderately soluble in water, and soluble in aqueous alcohol, ethanol, acetone, MeOH, and $CHCl_3$.

β-Mercaptothiolpropionic Acid (III) and β-mercaptothiolisobutyric Acid (IV) were prepared analogously to I from 0.1 mole of β-propiothiolactone or α-methyl-β-propiothiolactone and 10.1 g (0.1 mole) of Et_3N.

Cyclohexyl β-Mercaptothiolpropionate (V). To a solution of 3.66 g (0.03 mole) of acid III in 15 ml of dry ether at 0°C under N_2 was added 3.27 g (0.033 mole) of cyclohexylamine in 15 ml of dry ether. After 3 h (20°C), the resulting precipitate was filtered, washed with ether, and dried in a vacuum desiccator over P_2O_5 to give 6.7 g of V, soluble in water and ethanol, insoluble in acetone and ether.

4-Methyl-1,2-dithiolan-3-one (VIII). To a solution of 2.72 g (0.02 mole) of acid IV in 40 ml of MeOH at 0°C was gradually added with stirring ~0.5 liter of 0.05 M $FeCl_3 \cdot 6H_2O$. After 1.5 h (20°C) the reaction mixture was extracted with ethyl acetate (50 ml × 3), the organic layer was dried with Na_2SO_4, and concentrated to give 1.88 g of VIII, the IR spectrum of which confirmed with that in [4].

4-Acetylamino-5,5-dimethyl-1,2-dithiolan-3-one (VI) was prepared analogously to VIII from acid I (cf. [11]). Compound VI was soluble in ethyl acetate, $CHCl_3$, MeOH, ethanol, acetone, and hot water, and insoluble in CCl_4.

4-Benzoylamino-5,5-dimethyl-1,2-dithiolan-3-one (VII) was prepared analogously to VI by acidification of α-benzoylamino-β-mercaptothiolisobutyric acid II, as described in [12].

4,5,6-Trithianonan-2,8-dicarboxamide (IX) was prepared by ammonolysis of VIII, as described in [2].

α-Acetylamino-β-acetyldithiopropionic Acid (X). To a solution of 6.49 g (39.8 mmoles) of N acetyl-L-cysteine in 90 ml of absolute $CHCl_3$ containing 4.02 g (39.8 mmoles) of Et_3N at −40°C was added with stirring 4.4 g (39.8 mmoles) of acetylsulfenyl chloride in 10 ml of absolute $CHCl_3$. After 2 h (20°C), 200 ml of ether was added to the reaction mixture which was then kept in the refrigerator. The following day the resulting triethylammonium chloride was filtered off, and the filtrate was evaporated to dryness under vacuum. The viscous oil (~10 g) obtained was carefully triturated with absolute ether to give X as a white powder (6.7 g), soluble in water, ethanol, $CHCl_3$, and ethyl acetate, insoluble in ether and benzene.

α-Acetylamino-β-acetyldithioisovaleric acid (XI) was obtained analogously to X from N-acetyl-D,L-penicillamine and acetylsulfenyl chloride; XI was moderately soluble in water, soluble in ethanol, $CHCl_3$, and dimethylsulfoxide.

EXPERIMENTAL BIOLOGICAL

Studies of the acute toxicity and radioprotective effectiveness of the compounds were carried out according to [7]. Toxicity was determined by intraperitoneal and peroral introduction of the compounds into nonhybrid, male white mice weighing 20-24 g. Aqueous solutions or suspensions of the compounds were prepared as needed and introduced in logarithmic-scaled doses. The results were treated statistically according to the method of [1]. The radioprotective effectiveness of the compounds was studied in male (CBA × C57B1)F_1 mice weighing 20-23 g. The preparations were introduced intraperitoneally 20 min after and perorally 10 and 30 min after γ-irradiation with ^{137}Cs in a dose of 900 R at a dose rate of 213-215 R/min. To obtain comparable results [6] the radiation treatments were all carried out on the same day. The data obtained were treated statistically by means of tables [8].

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