PREPARATION OF SOME DERIVATIVES OF 3-MERCAPTOPROPIONIC ACID, CYSTAMINE, AND CYSTEAMINE AND STUDY OF THEIR RADIATION PROTECTION PROPERTIES

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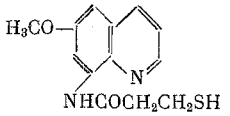
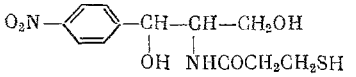
A large number of substances of various chemical types are now known which have prophylactic radiation protection activity. The most effective of these are bivalent sulfur compounds (cysteamine, cystamine, 2-(2-aminoethyl)-2-thiopseudourea, and others). Of the large number of preparations, only a few have begun to be subjected to clinical tests. A considerable obstacle is their high toxicity and small range of therapeutic action. The object of the present work was the search for new antiradiation preparation free from these disadvantages.

It has been shown previously that N-benzyl-3-mercaptopropionamide has a prolonged radiation protection power [1]. For the purpose of the study of radiation protection activity we have now synthesized a number of substituted 3-mercaptopropionamides, 3-mercapto-2-methylpropionamides, and 3-mercapto-2,2-diphenylpropionamides, and also some derivatives of cystamine [2,2'-dithiobisethylamine] and cysteamine [2-aminoethanethiol]. Thus, by the acylation of 8-amino-6-methoxyquinoline with 3-mercaptopropionic acid at a high temperature we obtained 3-mercapto-N-(6-methoxy-8-quinolyl)propionamide (I). By the acylation of 2-amino-1-p-nitrophenyl-1,3-propanediol in presence of chloroformic ester and triethylamine we obtained N-[β-hydroxy-α-(hydroxymethyl)-p-nitrophenethyl]-3-mercaptopropionamide (II).

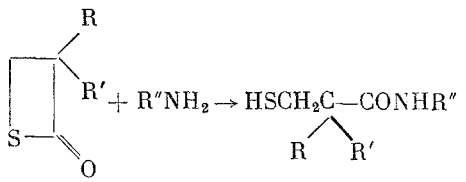


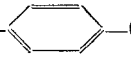
(I)



(II)

Here the intermediate formation of a mixed anhydride or a β-thiolactone is possible. We have shown previously [2, 3] that β-thiolactones are active acylating agents and can be used for the introduction of β-mercapto acyl groups into amino acids. By the use of this method we have prepared a number of new N-acyl derivatives of some amino acids (III)-(V).



(III)—R=R'=C_6H_5, R"=CH(COOH)$CH_2CH_2SCH_3$; (IV)—R=H, R'=CH_3, R"=  —CH_2COOH; (V)—R=H, R'=CH_3, R"=  —COOH

The oxygen analogs of β-thiolactones (β-lactones) are split by various salts at the bond between O and the alkyl residue [4]. As we have shown for the case of 2,2-diphenyl-β-propiolactone, the cleavage of β-lactones with trisodium phosphorothioate makes it possible to prepare S-substituted β-mercapto acids (VI):

Institute of Heteroorganic Compounds, Academy of Sciences of the USSR. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 2, pp. 334-341, February, 1967. Original article submitted October 1, 1964.

TABLE 1. Protective Action of Derivatives of 3-Mercaptopropionic Acid, Cystamine, and Cysteamine for the Irradiation of White Mice in a Dose of 700 R

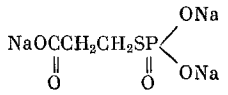
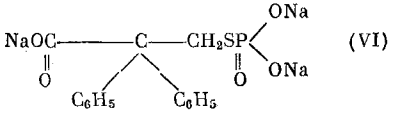
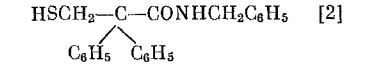
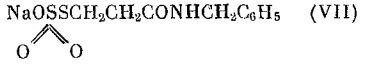
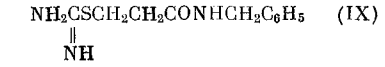
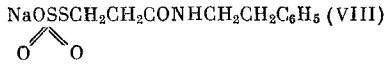
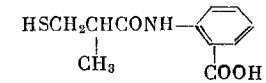
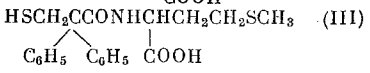
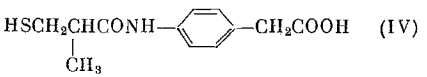
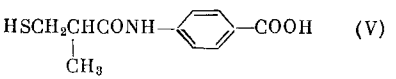
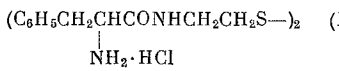
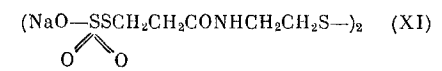
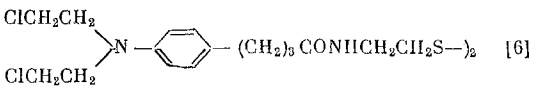
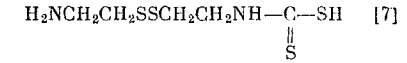
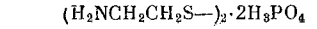
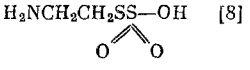
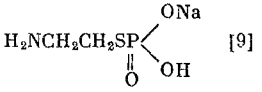
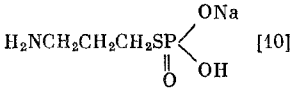
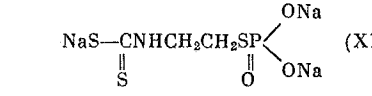
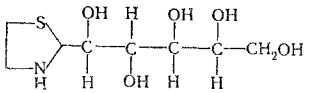
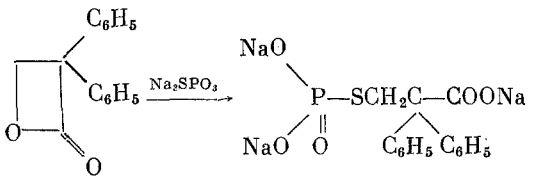
Substance	Dose, mg/kg	Method of introducing preparation	Number of animals	Survival rate, %	Mean life of fallen animals, days
 [5]	200	Intraperitoneally 15 min beforehand	10	—	6
	300	" 15 min beforehand	10	—	7
	300	" 24 h beforehand	10	—	6
 (VI)	150	" 15 min beforehand	10	—	10
	300	" 15 min beforehand	10	—	12
	300	" 24 h beforehand	10	—	9
 [2]	500	By mouth 1 h beforehand	10	—	13
	900	" " 1 h beforehand	20	20	16
	900	" " 24 h beforehand	10	—	7
 (VII)	300	Intraperitoneally 15 min beforehand	10	—	5
	300	" 4 h beforehand	30	10	12
	300	" 24 h beforehand	30	3	9
 (IX)	100	" 15 min beforehand	10	—	10
	100	" 24 h beforehand	10	—	9
 (VIII)	300	" 15 min beforehand	10	—	7
	300	" 4 h beforehand	30	30	11
	300	" 24 h beforehand	30	16.7	12
 [3]	200	" 15 min beforehand	10	33.3	4
	400	" 15 min beforehand	10	20	4
	300	" 24 h beforehand	10	—	11
 (III)	500	By mouth 1 h beforehand	10	—	14
	800	" " 1 h beforehand	10	—	13
	700	" " 24 h beforehand	10	—	12
 (IV)	700	Intraperitoneally 15 min beforehand	10	—	9
 (V)	200	" 15 min beforehand	10	10	11
 (XIII)	200	" 15 min beforehand	10	—	10
	300	" 15 min beforehand	10	10	6
 (XI)	500	" 15 min beforehand	10	20	14
	500	" 24 h beforehand	10	—	4
 [6]	500	By mouth 1 h beforehand	10	—	17

Table 1 (continued)

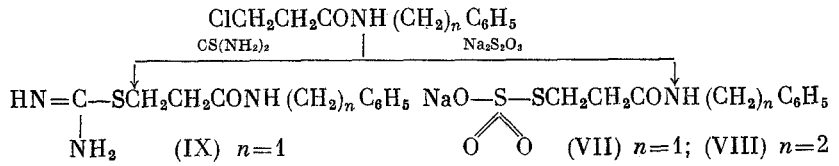
 [7]	500	By mouth 1 h beforehand	10	—	11
	500	" " 4 h beforehand	50	40	12
	500	" " 15 min beforehand	10	10	9
	500	" " 24 h beforehand	55	29	13
	350	Intraperitoneally 15 min beforehand	9	11	13
	600	" 1 h beforehand	19	21	14
 [8]	400	Intraperitoneally 15 min beforehand	20	40	12
	400	Subcutaneously 15 min beforehand	20	60	9
	700	By mouth 2 h beforehand	20	35	11
 [9]	500	Intraperitoneally 15 min beforehand	20	85	15
	400	Subcutaneously 15 min beforehand	20	85	8
	700	By mouth 1 h beforehand	20	65	11
 [10]	250	Intraperitoneally 15 min beforehand	20	30	16
	350 *	" 15 min beforehand	20	25	—
 (XIV)	150	" 15 min beforehand	20	5	13
	300	" 15 min beforehand	20	20	12
	500	" 15 min beforehand	20	35	15
	300	By mouth 1 h beforehand	20	—	12
	500	" " 1 h beforehand	20	5	10
	700	" " 1 h beforehand	20	5	10
 [11]	2000	Intraperitoneally 15 min beforehand	10	—	19
	2000	" 15 min beforehand	10	—	13
Cystamine hydrochloride (control)	150	" 15 min beforehand	20	55	15
	400	By mouth 1 h beforehand	20	40	13
Physiological solution (control)		Intraperitoneally	100	3	9

*Nine mice died immediately after the irradiation.

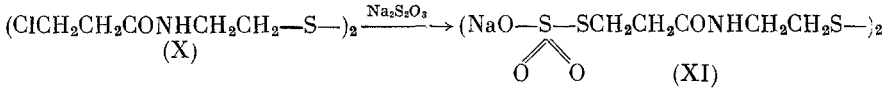


(VI)

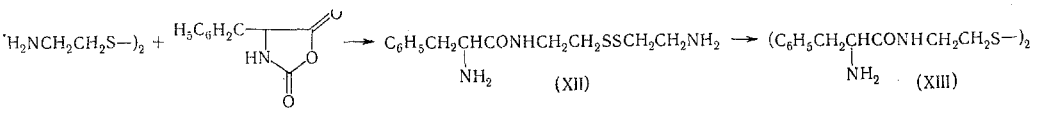
By the reaction of substituted 3-chloropropionamides with sodium thiosulfate or thiourea the corresponding derivatives of 3-mercaptopropionic acid (VII)-(IX) are obtained:



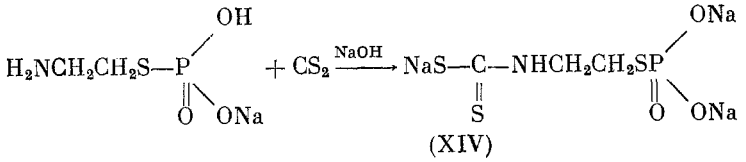
Analogously, from N,N'-bis-3-chloropropionylcystamine (X) and sodium thiosulfate we obtained the corresponding Bunte salt (XI):



By the acylation of cystamine with N-carboxyphenylalanine anhydride we obtained mono- and bis-(phenylalanyl)cystamines (XII) and (XIII):



The trisodium salt of S-{2-[(dithiocarboxy)amino]ethyl} dihydrogen phosphorothioate (XIV) was prepared in accordance with the scheme



The toxicities of most of the compounds obtained were relatively low with the exception of 3-(amidinothio)-N-benzylpropionamide. The results of the study of the radiation protection properties of a number of the compounds obtained, and also of some previously described substances, are given in Table 1. As will be seen from Table 1, the highest protective effect was given by sodium S-2-aminoethyl hydrogen phosphorothioate, which with intraperitoneal and subcutaneous application gave a survival rate of up to 85%.* Of the other substances studied interest is presented by sodium S-3-aminopropyl hydrogen phosphorothioate, sodium S-2-aminoethyl thiosulfate, the trisodium salt of S-{2-[(dithiocarboxy)amino]ethyl} dihydrogen phosphorothioate (XIV), and also by preparations with a prolonged action, such as sodium S-[2-(phenethylcarbamoyl)ethyl] thiosulfate (VIII) and N-(dithiocarboxy)cystamine, which show protective action when applied a relatively long time before the exposure to radiation (4-24 h).

Comparison of the radiation protection effects of the compounds studied reveals a certain superiority of phosphorus-containing compounds. Since phosphoric acid itself has no protective action, in this case it probably plays the part of a "transportation group." It is evident that the introduction of new transportation groups may intensify the radiobiological effect of antiradiation substances. Work along these lines continues.

*The results of a detailed study of the radiobiological activity of this preparation are published in [12].

EXPERIMENTAL

3-Mercapto-N-(6-methoxy-5-quinolyl)propionamide (I). A mixture of 3 g of 8-amino-6-methoxyquinoline and 5.4 g of 3-mercaptopropionic acid was heated at 145-150° for 1 h, cooled, and made alkaline with aqueous sodium bicarbonate solution. (I) was extracted with ether with heating, the ether was evaporated, and the residue was again dissolved in a little ether with heating. We obtained 1.1 g of product, m.p. 120-121°. The combined ethereal mother solutions were extracted with 2 N NaOH. By acidifying the alkaline solution with dilute HCl we obtained a further 0.69 g of (I). Found %: C 59.57; H 5.53; S 12.15. $C_{13}H_{14}N_2O_2S$. Calculated %: C 59.54; H 5.34; S 12.21.

N-[β-Hydroxy-α-(hydroxymethyl)-p-nitrophenethyl]-3-mercaptopropionamide (II). 1.46 g of triethylamine was added to a solution of 1.5 g of 3-mercaptopropionic acid in 120 ml of dry ether, the mixture was cooled to −15°, 2.1 g of isobutyl chloroformate was added with stirring, and the mixture was left overnight at −15°. Triethylamine hydrochloride was filtered off, and a suspension of 3 g of 2-amino-1-p-nitrophenyl-1,3-propanediol in 40 ml of dry ether was added to the filtrate. On the next day the precipitate was filtered off and dissolved in 2 N NaOH, and the alkaline solution was acidified and extracted with ether. We obtained 0.47 g of (II), m.p. 129-130° (ethyl acetate–petroleum ether). Found %: C 48.17; H 5.36; S 10.05. $C_{12}H_{16}N_2O_5S$. Calculated %: C 48.00; H 5.33; S 10.66.

N-(3-Mercapto-2,2-diphenylpropionyl)methionine (III). 0.8 g of methionine was dissolved in alcoholic alkali (0.24 g of NaOH in 60 ml of alcohol), and the solution was added to 0.96 g of 2,2-diphenyl-β-propiothiolactone in 60 ml of alcohol. The solution was boiled for 5 h and then evaporated in a stream of nitrogen; the residue was dissolved in water and acidified with dilute HCl. The precipitate formed (1.1 g) was dried in a vacuum desiccator and reprecipitated from diethyl ether with petroleum ether. The yield of (III) was 0.7 g; m.p. 115-116°. Found %: C 61.21; H 5.98; N 3.91; S 15.9. $C_{20}H_{23}NO_3S_2$. Calculated %: C 61.70; H 5.91; N 3.60; S 16.45.

[p-(3-Mercapto-2-methylpropionamide)phenyl]acetic Acid (IV). 0.049 mole of 2-methyl-β-propiothiolactone in 50 ml of acetonitrile was added to 0.049 mole of (p-aminophenyl)acetic acid in 80 ml of acetonitrile. The mixture was boiled for 18 h and then vacuum-evaporated, and the residue was precipitated with HCl from its solution in $NaHCO_3$ solution. We obtained (IV), m.p. 149-150° (water). Found %: C 56.90; H 5.97; N 5.85; S 12.64. $C_{12}H_{15}NO_3S$. Calculated %: C 56.91; H 5.92; N 5.53; S 12.64.

Analogously, from 0.049 mole of p-aminobenzoic acid and 0.049 mole of 2-methyl-β-propiothiolactone (boiling for 6 h) we obtained p-(3-mercapto-2-methylpropionamido)benzoic acid (V) in 22.2% yield; m.p. 260° (50% alcohol). Found %: C 55.17; H 5.44; N 6.35; S 13.37. $C_{11}H_{13}NO_3S$. Calculated %: C 55.23; H 5.43; N 5.85; S 13.39.

Trisodium Salt of S-(2-Carboxy-3,3-diphenylethyl) Dihydrogen Phosphorothioate (VI). A solution of 0.5 g of trisodium phosphorothioate in 14 ml of water was added dropwise with stirring to a solution of 0.93 g of 1,1-diphenyl-β-propiolactone in 7 ml of dimethylformamide at 40-50°. Stirring was continued until the test for Na_3SPO_3 with $AgNO_3$ was negative. Unchanged lactone (0.31 g) was separated, a large amount of alcohol was added to the filtrate, and the mixture was left overnight. The precipitate formed (1.16 g) was stirred with 15 ml of absolute methanol, and the mixture was filtered. Ether was added to the filtrate. The precipitate of (VI) was washed with ether; the substance did not melt. Found %: C 32.80; H 5.04; P 6.11. $C_{15}H_{12}Na_3O_5PS \cdot 8H_2O$. Calculated %: C 32.80; H 5.10; P 5.65.

N,N'-Bis-3-chloropropionylcystamine (X). A solution of 1 g of cystamine in 15 ml of 1 N NaOH was added dropwise with stirring to a solution of 1.67 g of 3-chloropropionyl chloride in 10 ml of dioxane (the temperature of the mixture must not rise above 5°). The mixture was made alkaline with 1 N NaOH (15 ml) and stirred for 30 min with cooling and for 30 min at 20°. The precipitate was filtered off, and we obtained 0.96 g of (X), m.p. 134-135° (alcohol). Found %: C 36.53; H 5.51; Cl 21.88; N 8.71; S 18.36. $C_{10}H_{18}Cl_2N_2O_2S_2$. Calculated %: C 36.36; H 5.40; Cl 21.33; N 8.41; S 19.20.

Sodium S-[2-(Benzylcarbamoyl)ethyl] Thiosulfate (VII). 3 g of N-benzyl-3-chloropropionamide in 2 ml of alcohol was mixed with 1.35 g of sodium thiosulfate in 2 ml of water. The mixture was boiled for 6 h and then cooled, and alcohol was added until a turbidity appeared. On the next day the precipitate was separated and the filtrate was vacuum-evaporated to dryness. The residue was dissolved in a little absolute alcohol, and the solution was filtered. From the filtrate (VII) was precipitated with ether; yield 1.15 g; m.p. 175-177° (alcohol–ether). Found %: C 40.41; H 4.07; N 4.61; S 21.15. $C_{10}H_{12}NNaO_4S_2$. Calculated %: C 40.40; H 4.04; N 4.71; S 21.54.

Analogously, from 1.5 g of N,N'-bis-3-chloropropionylcystamine (X) we obtained 1.85 g of disodium S,S'-[dithiobis(methyleneiminocarbonylethyl)] bisthiosulfate (XI). Found %: C 21.73; H 3.79; N 4.76. $C_{10}H_{18} \cdot N_2Na_2O_8S_6$. Calculated %: C 22.55; H 3.38; N 5.20.

From 6 g of 3-chloro-N-phenethylpropionamide we obtained 3.6 g of sodium S-[2-(phenethylcarbamoyl)ethyl] thiosulfate (VIII), m.p. 163-165° (alcohol–ether). Found %: C 42.37; H 4.28; N 4.19. $C_{11}H_{14}N \cdot NaO_4S_2$. Calculated %: C 42.44; H 4.50; N 4.50.

3-(Amidinothio)-N-benzylpropionamide (IX). 7.6 g of thiourea was added to a solution of 20.7 g of N-benzyl-3-chloropropionamide in 60 ml of absolute alochol, and the mixture was boiled for 3 h. The mixture was left for a long time and then evaporated, the residue was treated with water, and the undissolved substance was washed with ether and crystallized from alcohol and then from ethyl acetate We obtained (IX), m.p. 132°. Found %: C 54.62; H 6.35; S 12.05. $C_{11}H_{15}N_3OS$. Calculated %: C 55.70; H 6.32; S 13.08.

Cystamine Phosphate. 0.03 mole of phosphoric acid was added to 0.01 mole of cystamine in 25 ml of alcohol, and the precipitate formed was reprecipitated from water with alcohol; m.p. 192-193° (decomp.). Found %: C 14.69; H 4.80; N 8.21; S 18.04. $C_4H_{18}N_2O_8P_2S_2$. Calculated %: C 13.8; H 5.11; N 8.04; S 18.40.

N-(Phenylalanyl)cystamine Dihydrochloride (XII). 1.5 g of N-carboxyphenylalanine anhydride in 10 ml of ethyl acetate was added to a solution of 2.64 g of cystamine in 15 ml of ethyl acetate at −70°. The mixture was kept for 2.5 h at −70° and 1 h at room temperature. Ethyl acetate was removed by decantation, the oily residue was extracted with alcohol, the extract was combined with the ethyl acetate solution, and the whole was vacuum-evaporated to dryness. Alcoholic HCl and dry ether were added to the residue, and absolute alcohol was added to dissolve the precipitate. Dry ether was added to the filtrate until (XII) had been precipitated completely; yield 1.45 g. The substance deliquesces in air. Found %: C 41.90; H 6.3; N 10.61. $C_{13}H_{21}N_3OS_2 \cdot 2HCl$. Calculated %: C 41.93; H 6.17; N 11.29.

N,N'-Bisphenylalanylcystamine (XIII). 1.8 g of cystamine in 20 ml of ethyl acetate was added to a solution of 2 g of N-carboxyphenylalanine anhydride in 15 ml of ethyl acetate at −70°. The mixture was kept for 2 h at −70° and 1 h at room temperature. The precipitate formed was separated, washed with ethyl acetate, and extracted with alcohol; the extract was filtered and vacuum-evaporated, and the residue was rubbed out with ether. We obtained 0.85 g of (XIII), m.p. 88-90°. From the ethyl acetate solution after evaporation and similar treatment we obtained a further 0.32 g of (XIII), m.p. 102-104° (ethyl acetate–ether). Found %: C 59.11; H 6.74; N 12.45; S 14.08. $C_{22}H_{30}N_4O_2S_2$. Calculated %: C 59.11; H 6.72; N 12.32; S 14.32.

Trisodium Salt of S{2-[(Dithiocarboxy)amino]ethyl} Dihydrogen Phosphorothioate (XIV). A solution of 0.33 g of CS_2 in 1 ml of alcohol was added to a solution of 0.5 g of sodium S-2-aminoethyl hydrogen phosphorothioate, a solution of 0.22 g of NaOH in 4 ml of water was then added in portions with stirring, stirring was continued further for 1 h, and 40 ml of alcohol was added. On the next day the precipitated oil was separated from the solution and rubbed out with several portions of alcohol. The precipitate formed was filtered off rapidly, washed with alcohol, and dried in a vacuum desiccator. The substance did not melt. Found %: C 12.09; H 2.01; N 5.02; P 10.46. $C_3H_5NNa_3O_3PS_3$. Calculated %: C 12.04; H 1.70; N 4.68; P 10.36.

CONCLUSIONS

A number of new derivatives of 3-mercaptopropionic acid, cystamine, and cysteamine were synthesized, and their radiation protection properties were studied.

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