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SYNTHESIS AND BIOLOGICAL ACTIVITY OF DISPIROTRIPIPERAZINIUM DERIVATIVES

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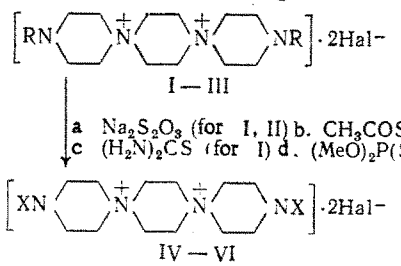
New antitumor preparations based on dispirotripiperazinium [2, 6-8] have been found and studied in recent years. A distribution study of tagged compounds has shown that these preparations are readily absorbed and stored in various organs and tissues [2, 9]. The high penetrability of these substances is probably due to a dispirotripiperazinium transport fragment in their structure. We used this fragment in the synthesis of potential radioprotectors in which case we introduced typical radioprotector sulfur-containing groups into compounds I-III. In order to study the possible effect that the substances' structure has on their activity, we synthesized compounds with a variously structured hydrocarbon chain between the nitrogen and sulfur atoms and the identical sulfur-containing substituents (IV and Va, b).



I: R = $BrCH_2CH_2CO$, Hal = Br (spirobromine); II: R = $ClCH_2CH_2$, Hal = Cl (spiraziné); III: R = H, Hal = Cl (dispirotripiperazinium dichloride); IVa: X = $^-OSO_2SCH_2CH_2CO$, Hal = 0 (internal salt); IVb: X = $CH_3COSCH_2CH_2CO$, Hal = Br; IVc: X = $HBr \cdot H_2N(HN)CSCH_2CH_2CO$, Hal = Br; Va: X = $^-OSO_2SCH_2CH_2$, Hal = 0 (internal salt) Vb X = $CH_3COSCH_2CH_2$, Hal = Cl; VI: X = $(MeO)_2P(S)$, Hal = Cl.

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Reactions under mild conditions between compounds I and II and sodium thiosulfate, lithium thioacetate, and thiourea resulted in high yields of the corresponding bisthiosulfate (IVa and Va), thioacetyl (IVb and Vb) and isothiouronium (IVa) derivatives of the dispirotripiperazinium series in the form of dichlorides, dibromides, and internal salts (in the case of IVa and Na).

N,N"'-bis-(O,O-dimethylthiophosphate)-N'N"-dispirotripiperazinium dichloride (VI) was obtained by reacting compound III with chloro-O,O-dimethylthiophosphate in the presence of lithium acetate. All of the compounds are crystalline and water soluble except IVa and Va, and have been described by element analysis data.

Compounds IV-VI were tested for acute toxicity and radioprotective activity. These compounds contain a potential mercapto group and a new transport fragment of dispirotripiperazinium which enables substances to pass through the blood-brain barrier. Acute toxicity ranges between 16 and 1000 mg/kg and is probably directed related to the *in vivo* release rate of the mercapto group. Thus, the least toxic substances are the thiosulfate derivatives IVa and Va (LD_{50} > 800 mg/kg) whose hydrolysis occurs slowly. The isothiouronium and acetyl derivatives of the thiols VIb, c, and Vb are hydrolyzed much more easily and they are much more toxic. The particularly high toxicity of IVb (LD_{50} 16.3 mg/kg) warrants attention. It is generally recognized that animals can be significantly sensitized to these substances due to the thiol-induced release of histamine [3]. No radioprotective activity was detected in compounds IV-VI.

EXPERIMENTAL CHEMICAL PART

The previously known starting compounds I-III [6] were kindly made available to us by Doctor of Chemical Sciences T. S. Safonova (S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry).

N,N"'-Di-[β-(thiosulfate)propionyl]-N',N"-dispiropiperazinium (IVa). A solution of 3.72 g (0.0075 mole) of $Na_2S_2O_3 \cdot 5H_2O$ was added to a solution of 2.84 g (0.005 mole) of I in 25 ml of water. After 5 h at 20°C, the resultant precipitate was filtered off and thoroughly washed with water and ethyl alcohol, and then air dried. Yield of IVa 2.33 g (84%), mp 300°C with decomposition. Calculated, %: C 38.6: H 5.72; N 10.0; S 22.9. $C_{18}H_{32}N_4O_8S_4$. Found, %: C 38.2: H. 5.74; N 9.8; S 22.6.

N,N"'-Di-[β-(thiosulfate)ethyl]-N',N"-dispiropiperazinium (Va) was obtained under similar conditions from 2.11 g (0.005 mole) of II and 3.72 g (0.0075 mole) of $Na_2S_2O_3 \cdot 5H_2O$ in 20 ml of water. Yield was 2.27 g (90%) of Va, mp 260°C. Calculated, %: C 38.1; H 6.35; N 11.1; S 25.4. $C_{16}H_{32}N_4O_6S_4$. Found, %: C 37.7; H 6.19; N 10.8; S 25.6.

N,N"'-Di-[β-(acetylthio)propionyl]-N',N"-dispirotripiperazinium Dibromide (IVb). A solution of 0.93 g (0.022 mole) of $LiOH \cdot H_2O$ and 1.67 g (0.022 mole) of thiolacetic acid in 10 ml of 50% ethyl alcohol was added to a solution of 5.67 g (0.01 mole) of I in 20 ml of water. After 3 h at 20°C 20 ml of ethyl alcohol were added to the reaction mixture. After cooling with ice water the resultant precipitate was filtered off, washed with alcohol and air dried. Yield of IVb was 4.92 g (76%), mp 280°C with decomposition. Calculated, %: 40.9; H 5.90; Br 24.8; N 8.6; S 9.9. $C_{22}H_{38}Br_2N_4O_4S_2$. Found, %: C 40.5; H 5.88; Br 24.9; N 8.8; S 9.5.

N,N"'-Di-[β-(acetylthio)ethyl]-N',N"-dispirotripiperazinium Dichloride (Vb). A solution of 0.93 g (0.022 mole) of $LiOH \cdot H_2O$ and 1.67 g (0.022 mole) of thiolacetic acid in 5 ml of 50% ethyl alcohol was added to a solution of 4.22 g (0.01 mole) of II in 10 ml of water. After 24 h at 20°C the reaction was evaporated to dryness and the solid residue was recrystallized from 50% alcohol. Yield was 4.83 g (96%) of Vb, mp 298°C. Calculated, %: C 47.9; H 7.58; Cl 14.2; N 11.2; S 12.8. $C_{20}H_{38}Cl_2N_4O_2S_2$. Found, 5: C 47.5: H 7.60: Cl 14.5; N 11.2: S 12.2.

N,N"'-Di-[β-(isothiouronium)propionyl]-N,N"-dispirotripiperazinium Dichloride Dibromide (IVc). A solution of 1.29 g (0.009 mole) of thiourea in 20 ml of ethyl alcohol was added to a solution of 4.25 g (0.0075 mole) of I in 15 ml of water. After 24 h at 20°C the reaction mixture was evaporated to dryness and the residue was recrystallized from 50% alcohol. Yield was 5.2 g (96%) of IVc, mp 265°C. Calculated, %: 33.4: H 5.56; Cl 9.9; Br 22.2; N 15.6; S 8.9. $C_{20}H_{40}Cl_2Br_2N_8O_2S_2$. Found, %: C 33.2; H 5.49; Cl 9.5; Br 21.9: N 15.5; S 8.7.

N,N"'-Di-[(O,O-dimethylthiophosphate)]-N,N"-dispirotripiperazinium Dichloride (VI). A solution of 3.55 g (0.022 mole) of chloro-O,O-dimethylthiophosphate in 10 ml of acetone was

TABLE 1. Toxicity and Radioprotective Efficacy of Dispirotripiperazinium Derivatives in Intraperitoneal Administration to Mice

Compound	LD_{50}, mg/kg	Preparation's radioprotective efficacy		
		dose of preparation, mg/kg	number of animals	survival, %
IVa	800	300	19	0
IVb	155	53; 13	40	0
IVc	16,3	10; 5	40	0
Va	1000	300	10	0
Vb	50*	5; 20	160	0
VI	1000**	300	40	0
Control			243	0

*Preparation was administered 20, 30, 45, and 60 min prior to irradiation.

**Preparation was administered 20 and 60 min prior to irradiation.

added to a solution of 2.97 g (0.01 mole) of spiran III, 0.93 g of $LiOH \cdot H_2O$, and 1.32 g of CH_3COOH (0.022 mole each) in 20 ml of water and 10 ml of acetone. After 2 h at 20°C the resultant precipitate was filtered off, washed with acetone, and air dried. Yield of VI was 3.3 g (60.5%), mp 270°C. Calculated, %: C 35.2; H 6.61; Cl 13.0; N 10.3; P 11.4; S 11.8. $C_{16}H_{36}Cl_2N_4P_2S_2$. Found, %: C 35.2; H 6.80; Cl 12.7; N 9.9; P 11.4; S 11.7.

EXPERIMENTAL BIOLOGICAL PART

The acute toxicity and radioprotective activity of the represented compounds was studied in accordance with [5]. Acute toxicity was determined by the compounds' ip and peroral administration to white mongrel male mice weighing 20-24 g. Aqueous solutions or suspension were prepared ex tempore in a 1% amyl mucilage of the compounds and administered at logarithm scale doses. The results were statistically processed by the Litchfield and Wilcoxon method as modified by M. L. Belen'kii [1].

The radioprotective activity of the substances was tested on male mice of breed (CBA × $C_{57}Bl)F_1$ weighing 20-23 g. The preparations were administered ip 20 min before and perorally 10 and 30 min before γ-irradiation by Cs^{137} at a dose of 900 R and a dosage rate of 215-213 R/min.

In order to obtain comparable results the animals were irradiated at the same time of day [4]. The data was statistically tabulated [10]. The results of the study of the dispirotripiperazinium derivatives' toxic and radioprotective properties are given in the Table.

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SYNTHESIS AND STUDY OF THE ANTIRADIATION PROPERTIES OF SOME DERIVATIVES OF N-ALLYL-N'-SUBSTITUTED THIOCARBAMIDES

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It is known that N-allylthiocarbamide has pronounced radioprotective activity in experiments with model systems [6]; however, the activity of this compound or its derivatives in experiments with animals has not been studied. The pronounced radioprotective activity of isothiuronium derivatives in experiments with animals is also known [2].

For a systematic study of the dependence of the biological activity on the chemical structure it seemed of interest to synthesize and study, in radiobiological experiments, N'-substituted derivatives of N-allylthiocarbamide, as well as isothiuronium derivatives with linear and cyclic structures, that contain one or several thiocarbamide fragments.

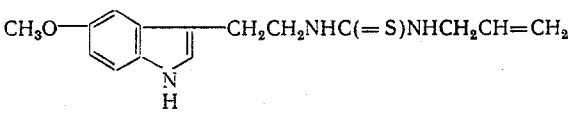
N, N'-Bis(allylthiocarbamoyl)ethylenediamine (I), N,N',N''-tris(allylthiocarbamoyl)diethylenetriamine (II), and N,N'-bis(allylthiocarbamoyl)-1,4-diaminobutane-2,3-diol (III), respectively, were obtained by the reaction of allyl isothiocyanate with ethylenediamine, diethylenetriamine, and 1,4-diamino-2,3-dihydroxybutane:

$CH_2=CHCH_2NHC(=S)NHCH_2CH_2NHC(=S)NHCH_2CH=CH_2$
I

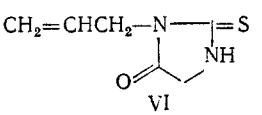
$[CH_2=CHCH_2NHC(=S)NHCH_2CH_2]_2NC(=S)NHCH_2CH=CH_2$
II

$CH_2=CHCH_2NHC(=S)NHCH_2CH(OH)CH(OH)CH_2NHC(=S)NHCH_2CH=CH_2$
III

N-Allyl-N'-[2-(5-methoxy-3-indolyl)ethyl]thiocarbamide (IV) was synthesized by the reaction of allyl isothiocyanate with 3-(2-aminoethyl)-5-methoxyindole hydrochloride in the presence of sodium ethoxide. N-Allyl-N'-carbethoxymethylthiocarbamide (V) could not be obtained when glycine ethyl ester hydrochloride was subjected to a similar reaction. Instead, 1-allyl-5-oxoimidazolidine-2-thione (VI) was isolated.


IV

$CH_2=CHCH_2NHC(=S)NHCH_2COOC_2H_5$
V


VI

This is evidently associated with the ability of V to undergo intramolecular cyclization with splitting out of a molecule of alcohol in the presence of sodium ethoxide. It is interesting to note that the described cyclization is not observed if an alcohol solution of

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