SYNTHESIS AND RADIOPROTECTIVE PROPERTIES OF SOME 2-PYRROLIDONE DERIVATIVES

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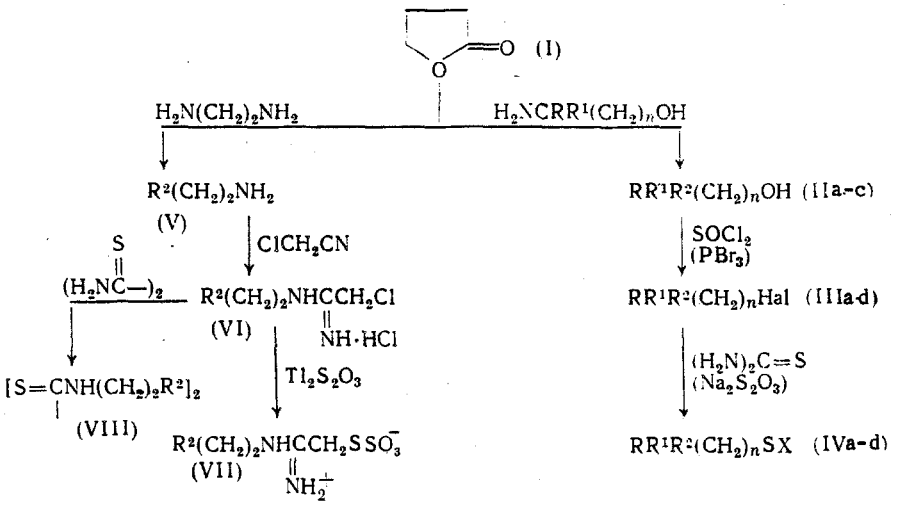
UDC 615.849.1.015.25:547.745].012.1

Interest in heterocyclic compounds as potential radioprotectors has recently increased significantly. The principal directions in the search for substances of this type have been illuminated in a review [1]. It is also known [11] that some pyrrolidine derivatives have high radioprotective activity (70-80%). A cyclic 2-pyrrolidinone (2-pyrrolidone) residue is often included in the composition of many pharmaceutical agents [4-6]. However, the literature does not contain information on the radioprotective activity of such compounds.

In order to study the radioprotective properties of 2-pyrrolidone derivatives we obtained IV, VII, and VIII.



R = R¹ = H (IIa, b, IIIa, b, d, IVa, b, d); Me (IIc, IIIc, IVc); R^2 = 2-pyrrolidin-1-onyl (IIa-c, IIIa-d, IVa-d, V—VIII); n = 1 (IIa, c, IIIa, c, IVa, c, d), 2 (IIb, IIIb, IVb); Hal = Cl(IIIa-c), Br (IIId); X=C(=NH)NH₂·HCl (IVa-c), SO_3Na (IVd).

The starting compound for obtaining IV is γ-butyrolactone (I), from which the corresponding N-(ω-hydroxyalkyl)-2-pyrrolidones IIa-c are obtained by reaction with amino alcohols. Compounds IIa-c are converted to N-(ω-haloalkyl)-2-pyrrolidones IIIa-d by treatment with $SOCl_2$ or PBr_3. The desired products are obtained by the reaction of IIIa-d with thiourea or sodium thiosulfate. In the treatment of IIa, b with $SOCl_2$ the HCl liberated does not enter into salt formation with IIIa, b. However, N-(1,1-dimethyl-2-chloroethyl)-2-pyrrolidone hydrochloride is liberated in the reaction of IIc with $SOCl_2$. This can evidently be explained by the effect of the R and R^1 substituents attached to the carbon atom of the ethyl group in the 1 position. When R = R^1 = H, the imino group of the pyrrolidone ring, like the amino group of carboxylic acid amides, loses its basicity. When R = R^1 = alkyl, the electron density on the nitrogen atom of the amino group increases due to the positive inductive effect of the alkyl radicals, and, as a result, the nitrogen atom acquires the ability to form a salt with HCl. The reaction mass is treated with $NaHCO_3$ to obtain free IIIc.

On the basis of N-(β-aminoethyl)-2-pyrrolidone (V), obtained by the reaction of γ-butyrolactone with ethylenediamine, we synthesized N-[β-(2-pyrrolidon-1-yl)ethyl]-α-thiosulfoacetamidine (VII) and N,N'-bis[β-(2-pyrrolidon-1-yl)ethyl]dithiooxamide (VIII).

Institute of Biophysics, Ministry of Public Health of the USSR, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 22, No. 6, pp. 705-710, June, 1988. Original article submitted February 23, 1987.

 0091-150X/88/2206-0472$12.50

TABLE 1. Characteristics of the 2-Pyrrolidone Derivatives

Compound	Yield, %	bp, °C/mm, mp, °C	n_D^{20}	Empirical formula	Found, %/calc., %					IR spectrum, ν_{max}, cm^{-1}
					C	H	N	Cl (Br)	S	
IIa	89	151 3/3	1,4893	$C_6H_{11}NO_2$	55,47 / 55,76	8,53 / 8,58	10,56 / 10,84		--	3640 (OH), 3400 (OH), 1680 (C=O)
IIb	79	138--40/1	1,4924	$C_7H_{13}NO_2$	58,42 / 58,68	9,03 / 9,15	9,86 / 9,78	--	--	3633 (OH), 3406 (OH), 1677 (C=O)
IIc	72	113 1/0,3	1,4886	$C_8H_{15}NO_2$	61,00 / 61,12	9,75 / 9,62	8,85 / 8,91		--	3640 (OH), 3420 (OH), 1680 (C=O)
IIIa	85	87 8/1	1,4962	$C_6H_{10}ClNO$	48,62 / 48,79	6,79 / 6,82	9,60 / 9,54	23,84 / 24,01	--	1720 (C=O), 680 (C—Cl)
IIIb	83	96--8/1	1,4945	$C_7H_{12}ClNO$	51,85 / 51,99	7,50 / 7,43	8,60 / 8,71	21,73 / 21,92	—	1725 (C=O), 676 (C=Cl)
IIIc	68	73 5/0,5	1,4890	$C_8H_{14}ClNO$	54,55 / 54,69	7,96 / 8,03	7,95 / 7,97	19,85 / 20,18	--	1715 (C=O), 680 (C—Cl)
IIId	75	91 2/0,8	1,5273	$C_6H_{10}BrNO$	36,65 / 37,51	5,30 / 5,25	7,25 / 7,29	41,71 / 41,59		1720 (C=O), 530 (C—Br)
IVa	75	212 13		$C_7H_{14}ClN_3OS$	37,60 / 37,57	6,25 / 6,30	18,90 / 18,88	16,13 / 15,84	13,90 / 14,32	1720 (C=O), 1690 (C=N) 1590 (NH_2), 680 (C—S)
IVb	82	149--50	--	$C_8H_{16}ClN_3OS$	40,45 / 40,40	6,75 / 6,78	17,80 / 17,67	15,31 / 14,90	13,80 / 13,48	1710 (C=O), 1685 (C=N) 1595 (NH_2) 685 (C—S)
IVc	72	190 2	--	$C_9H_{18}ClN_3OS$	43,00 / 42,93	7,30 / 7,20	16,80 / 16,69	13,79 / 14,08	12,42 / 12,73	1720 (C=O), 1680 (C=N) 1590 (NH_2) 680 (C—S)
IVd	65	79 81		$C_6H_{10}NO_4S_2Na$	28,73 / 29,13	4,10 / 4,07	5,70 / 5,69	--	25,48 / 25,98	1708 (C=O), 1220, 1160, 1030, 660 ($-SSO_3^-$)
VI	83	117 - 20		$C_8H_{15}Cl_2N_3O$	39,67 / 40,01	6,23 / 6,30	17,38 / 17,50	29,60 / 29,53	--	1680, 1500, 720 [$-NH-\overset{|}{C}(=NH_2)^{\oplus}$]
VII	70,7	150 (dec.)	--	$C_8H_{15}N_3O_4S_2$	34,24 / 34,15	5,10 / 5,37	14,85 / 14,93	--	22,57 / 22,79	1685, 1500, 705 [$-NH-\overset{|}{C}-(=NH_2)^{\oplus}$] 1230, 1160, 1010, 635 ($-SSO_3^-$)
VIII	68	180 (dec.)	--	$C_{14}H_{22}N_4O_2S_2$	48,93 / 49,09	6,50 / 6,47	16,40 / 16,36	--	18,60 / 18,72	1710 (C=O), 3210 (NH), 1386, 1369, 916 [$-NH-C(=S)$]

TABLE 2. Radioprotective Effectiveness and Acute Toxicity of 2-Pyrrolidone Derivatives

Compound	LD_{50}, mg/kg	Radioprotective effectiveness					
		dose, mg/kg	method of administration	administration time, min	total no. of animals	% surviving animals	ALT, days
IVa	340	115,0	A	20	20	0	9,4
		29,0	A	20	20	0	12,2
	1000*	300,0	B	30	18	0	11,9
			B	10	6	0	15,0
IVb	340	100,0	A	20	20	0	9,7
		25,0	A	20	18	0	10,0
	800*	300,0	B	30	16	23,3	16,0
		300,0	B	10	16	40,0	12,3
IVc	450	190,0	A	20	20	10,0	13,4
		47,5	A	20	20	0	13,6
IVd	1500*	300,0	A	20	20	10,0	13,0
VII	300	100,0	A	20	20	0	11,7
		250,0	A	20	20	0	13,4
VIII	1000*	300,0	A	20	19	0	12,5
Control	—	—	—	—	236	0	9.6

Note. A — indicates intraperitoneal administration, B — indicates administration per os, and ALT indicates average lifetime; an asterisk indicates no details upon administration of the given dose.

In the preparation of VII we selected thallium thiosulfate as the source of thiosulfate ion in the reaction with N-[β-(2-pyrrolidinon-1-yl)-ethyl]-α-chloroacetamidine hydrochloride (VI) in an aqueous medium. The thallium chloride formed as a result of the reaction is virtually insoluble in water and precipitates; this significantly simplifies the isolation of the desired product and increases its yield and purity.

Under conditions similar to those presented in [6] we accomplished the reaction of dithiooxamide with V and obtained the desired VIII in good yield.

The compositions and structures of II-VIII were confirmed by the results of elementary analysis and IR spectroscopic data. The properties of the compounds obtained and the results of analysis are presented in Table 1.

EXPERIMENTAL (CHEMICAL)

The IR spectra of microlayers (in the case of the liquid substances) and suspensions in mineral oil (in the case of the crystalline substances) were recorded with a Perkin—Elmer-225 spectrometer.

N-(ω-Hydroxyalkyl)-2-pyrrolidones (IIa-c). An autoclave was charged with 0.5 mole of γ-butyrolactone and 0.5 mole of the corresponding amino alcohol and heated for 6 h at 270°C. Fractionation of the reaction masses gave pure IIa-c. The yields, constants, results of elementary analysis, and IR spectra are presented in Table 1.

N-(γ-Chloropropyl)-2-pyrrolidone (IIIb). A 24.9-g (0.21 mole) sample of $SOCl_2$ was added dropwise with stirring at 15-30°C (with cooling) to a solution of 30 g (0.21 mole) of IIb in 25 ml of dry benzene, and the mixture was stirred for 3 h at room temperature. The solvent was removed in vacuo, and the residue was fractionated to give 28.1 g of IIIb.

N-(β-Chloroethyl)-2-pyrrolidone (IIIa) [9] was similarly obtained.

N-(1,1-Dimethyl-2-chloroethyl)-2-pyrrolidone (IIIc). A 15.3-g (0.128 mole) sample of $SOCl_2$ was added dropwise with stirring and cooling (10°C) to a solution of 20.2 g (0.128 mole) of IIc in 23 ml of dry benzene, and the mixture was stirred for 10 h at room temperature. The solvent was removed in vacuo, the solid residue was dissolved in 40 ml of ethanol, and the solution was neutralized with 15% aqueous $NaHCO_3$ solution. The solution was evaporated in vacuo to dryness, the residue was dissolved in dry ethanol, and the solid material was separated with a filter. The filtrate was fractionated to give 15.29 g of IIIc.

N-(β-Bromoethyl)-2-pyrrolidone (IIId). An 18-g (0.67 mole) sample of PBr_3 was added slowly dropwise with stirring and cooling (5°C) to a solution of 25.8 g (0.2 mole) of IIa in 50 ml of dry toluene, after which the cooling was discontinued, as a result of which the reaction heated up spontaneously to 50°C. The mixture was then stirred for 20 h at 40°C. Fractionation gave 28.8 g of IIId.

N-[S-β-(Isothiuroniaethyl)]-2-pyrrolidone Hydrochloride (IVa). A 1.86-g (0.024 mole) sample of finely ground thiourea was added to a solution of 3.6 g (0.024 mole) of IIIa in 30 ml of dry propanol, and the mixture was refluxed for 18 h. The small amount of precipitate was separated on the filter, the filtrate was evaporated in vacuo to the minimum volume, and 25 ml of dry ether was added to the concentrate. The precipitated crystals were removed by filtration and dried in vacuo over P_2O_5 to give 4.1 g of IVa.

N-[S-γ-(Isothiuroniapropyl)]-2-pyrrolidone hydrochloride (IVb) and N-(1,1-dimethyl-2-S-isothiuroniaethyl)-2-pyrrolidone hydrochloride (IVc) were similarly obtained.

N-(β-Thiosulfoethyl)-2-pyrrolidone Sodium Salt (IVd). A mixture of 4.2 g (0.022 mole) of IIId and 5.4 g (0.022 mole) of $Na_2S_2O_3 \cdot 5H_2O$ in 20 ml of water was refluxed for 1.5 h, after which it was cooled to room temperature, and the precipitate was separated on the filter. The filtrate was evaporated to dryness, the solid residue was extracted with 100 ml of hot ethanol, the extract was evaporated to the minimum volume, and 15 ml of ether was added to the concentrate. The liberated yellow oil was separated and triturated in methanol with ether until a white powder formed. The white powder was subjected to repeated similar treatment to give 3.5 g of IVd.

N-[β-(2-Pyrrolidon-1-yl)ethyl]-α-chloroacetamidine Hydrochloride (VI). A solution of 3.75 g (0.05 mole) of chloroacetonitrile in 20 ml of dry methanol was added dropwise to a stirred solution of MeONa (0.115 g of Na in 35 ml of dry MeOH), after which stirring at room temperature was continued for 2 h. A 5.9-g (0.046 mole) sample of V [10] was then added to the reaction mixture, after which it was acidified to pH 4.0 with a methanol solution of HCl. The reaction mixture was stirred for 6 h, the precipitate was separated on the filter, and the filtrate was evaporated in vacuo to dryness. The solid residue was recrystallized twice from propanol—ether to give 9.1 g of VI.

N-[β-(2-Pyrrolidon-1-yl)ethyl]-α-thiosulfoacetamidine (VII). A mixture of 3.5 g (0.0146 mole) of VI and 7.6 g (0.0146 mole) of $T_{12}S_2O_3$ in 50 ml of water was refluxed for 1 h, after which it was cooled to room temperature, and the precipitate was separated on the filter. The filtrate was evaporated in vacuo to dryness, and the residue in the form of an oil was triturated in isopropyl alcohol. The resulting pale-white powder was recrystallized from methanol—ether to give 2.9 g of VII.

N,N'-Bis[β-(2-pyrrolidon-1-yl)ethyl]dithiooxamide (VIII). A 7.7-g (0.06 mole) sample of V was added with stirring to a suspension of 3 g (0.025 mole) of dithiooxamide in 30 ml of ethanol, during which a reaction commenced, as one could judge from the liberation of ammonia. The reaction mass was heated without discontinuing stirring to 50°C in the course of 30 min. The resulting light-yellow precipitate was separated with a filter, washed with hot acetone, and dried in vacuo to give 5.8 g of VIII.

EXPERIMENTAL (BIOLOGICAL)

The toxic properties of the synthesized compounds were studied over a 3-day period of observation in white mongrel male mice with masses of 20-24 g. Aqueous solutions and suspensions of the substances were prepared ex tempore and administered in doses of the logarithmic scale at intervals of 0.1 intraperitoneally and orally. The doses that caused the death of the test animals were calculated by the Litchfield—Wilcoxon assay analysis method [2].

The radioprotective effectiveness of the compounds was evaluated with respect to male mice of the (CBA × C57Bl)F_1 strain with masses of 20-23 g. The preparations were administered in doses constituting 1/2 and 1/8 of the LD_{16} values intraperitoneally in the course of 20 min and orally in the course of 10 and 30 min prior to irradiation in the minimally absolutely lethal dose of 900 rad at a dose rate of 200-196 rad/min. The survival rate of the animals 30 days after irradiation served as the index of effectiveness. To obtain comparable results the radiation effect was realized for the same time of day (up to 12 h) [3]. The results were treated statistically by the method in [7].

The results of a study of the toxic and radioprotective properties of the compounds are presented in Table 2, from which it is apparent that in the series of isothiuronium derivatives of 2-pyrrolidone (IVa-c) neither the length of the hydrocarbon chain between the nitrogen atom and the S-containing residue nor its branching has a substantial effect on the acute toxicity of the compounds (the LD_{50} values for them in the case of intraperitoneal administration ranged from 340 to 450 mg/kg). Thiosulfate derivative IVd was virtually nontoxic, while VIII was less toxic than its acetamidine analog VII.

In the case of administration per os IVb protected 23-40% of the test animals, depending on the administration time, while the remaining investigated compounds had virtually no antiradiation activity.

Thus our studies have shown that, in contrast to pyrrolidine derivatives, the investigated 2-pyrrolidone derivatives have a small antiradiation effect.

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