NEW ANTITUMOR AGENT — SPIROBROMIN

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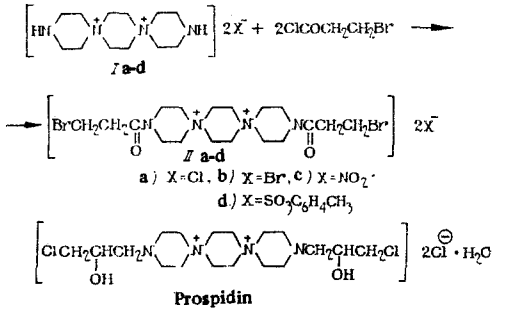
In continuation of studies [1, 2] on the search for new antitumor agents in the area of N',N''-dispirotripiperazinium derivatives, routes of synthesis of the previously unknown N,N'''-di-(β-bromopropionyl)-N',N''-dispirotripiperazinium salts (IIa-d) have been investigated.

Substances of this type differ from the antitumor preparation prospidin by the presence of β-bromopropionyl residues on the terminal nitrogen atoms in place of γ-chloro-β-hydroxypropyl groups and are of interest for the clarification of the principles of the connection between structure and biological activity.

With this aim the interaction has been studied of N',N''-dispirotripiperazinium dichloride (Ia) with the acid chloride of β-bromopropionic acid. Reaction was effected in aqueous medium or a medium of water and ether with basic agents such as LiOH or $NaHCO_3$ used as acceptors to bind the hydrogen chloride formed in the reaction process. N,N'''-Di(β-bromopropionyl)-N',N''-dispirotripiperazinium dichloride (IIa) was obtained in this way. To prepare other bis-quaternary salts, particularly the bromide, nitrate, and toluene-p-sulfonate (IIb-d) the initial (Ia) was first converted into the appropriate salt by treatment with an aqueous solution of sodium bromide, silver nitrate, or toluene-p-sulfonic acid respectively and acylation with β-bromopropionic acid chloride was carried out under the conditions indicated above (Table 1).



Compounds (IIa-d) were white crystalline substances having no characteristic melting points. Of them compound (IIa) was readily soluble in water, (IIb-d) moderately soluble, but compounds (IIa-d) were practically insoluble in organic solvents.

The structure of the quaternary salts (IIa-d) was confirmed by physicochemical methods, by IR and PMR spectroscopy, and also by chemical conversions. A medium absorption band was observed in the 1650-1670 cm^{-1} region of the IR spectra of compounds (IIa-d) which was assigned to the amide CO groups. There were characteristic proton signals in the PMR spectrum of (IIa) at 3.14 ppm from the $COCH_2$ group, a triplet at 3.67 ppm from CH_2Br, a multiplet at 4.00 ppm from methylene protons of the two side rings, and a singlet at 4.26 ppm from the methylene protons of the central ring.

Compounds (IIa, b, and d) were subjected to a biological study. The antitumor activity was studied in experiments on animals with transplanted tumors (Table 2) by the generally accepted procedure of [3]. When studying the toxic properties of the compounds the LD_{50} was determined according to Kerber [4]. A cumulative index of toxic action (in percent) and the intoxication index were calculated [1]. The results of experiments showed that all the compounds proved to have distinct antitumor action, substances (IIa, b) being the most active of them. These compounds administered at the maximum tolerated dose reduced the growth of the

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TABLE 1. Characteristics of Compounds (IIa-d)

Compound	Found, %					Empirical formula	Calculated, %					Yield, %
	C	H	N	Cl	Br		C	H	N	Cl	Br	
IIa	38,1	5,6	9,8	12,6	28,1	$C_{18}H_{32}Br_2Cl_2N_4O_2$	38,1	5,6	9,9	12,5	28,2	35,0
IIb	33,2	4,9	8,8	—	48,8	$C_{18}H_{32}Br_2N_4O_2$	32,9	4,9	8,5	—	48,7	78,4
IIc	—	—	—	—	25,9	$C_{18}H_{32}Br_2N_6O_8$	—	—	—	—	25,8	61,0
IId	—	—	—	—	19,2	$C_{32}H_{46}Br_2N_4O_8S_2$	—	—	—	—	19,1	50,1

Jensen sarcoma by 99 and 100%, of sarcoma M-1 by 99 and 97%, and of sarcoma 45 by 99 and 100%. It should be recorded that a reduction of approximately 80-fold in the dose of compound (IIa) led to an insignificant drop in antitumor effect. Although compound (IIa) at a dose of 160 mg/kg caused a 99% inhibition of the growth of Jensen sarcoma the administration of it to rats at a dose of 2 mg/kg led to 69% inhibition of tumor growth. Transplanted tumors of mice also proved to be sensitive. The application of the maximum tolerated dose of compounds (IIa, b) led to inhibition of growth of sarcoma 180 by 66 and 52%, of sarcoma 37 by 88 and 60%, and of AK sarcoma by 87 and 81%.

Study of toxicity showed that compound (IIa) was less toxic than compound (IIb). The LD_{50} of compound (IIa) on single intraperitoneal injection to mice was 1924 mg/kg and of compound (IIb) 1150 mg/kg under the same experimental conditions.

Thus compound (IIa) proved to be highly active in the presence of low toxicity and has been studied in more detail under the name "spirobromin."

It was established that spriobromin possessed high antileukemic activity. Application of it to mice with leukamia L_α increased the life span of treated animals by a factor of 3 in comparison with controls. In addition spirobromin increased the life span of mice with leukemias P-388 and L 1210 by 2- and 1.03-fold respectively.

A comparative study has been carried out on spirobromin and prospidin which revealed several special features. Spirobromin has a different spectrum of antitumor action (see Table 2), it is more active in relation to sarcoma 45 and 536 in rats and to sarcoma 180 in mice. A characteristic special feature was the high antileukemic activity in relation to leukemia L_α, while prospidin did not influence the development of the leukemic process in mice. In addition spirobromin proved to be more active than prospidin in relation to leukemia P-388.

The great breadth of the therapeutic action must be considered as an appreciable advantage of spirobromin over prospidin. This may be judged by the so-called chemotherapeutic index (ratio of maximum tolerated to minimum effective doses, see Table 2).

A study of the toxic properties of spirobromin in comparison with prospidin revealed its low toxicity. The LD_{50} for mice on single interaperitoneal injection was 1924 mg/kg, the maximum tolerated dose (MTD) was 1613 mg/kg. Under the same experimental conditions the LD_{50} for prospidin was 1000 mg/kg and the MTD 900 mg/kg. The intoxication index for spirobromin possessed somewhat more marked cumulative properties than prospidin (Table 3).

Spirobromin is therefore less toxic than prospidin, it displays significant antileukemic and antitumor activity, and has a different spectrum of antitumor action. Spirobromin has a great breadth of therapeutic action, its chemotherapeutic index on Jensen sarcoma was two times greater than the chemotherapeutic index of prospidin.

On the basis of the obtained experimental data spirobromin was recommended for the treatment of patients with malignant neoplasia. Clinical study confirmed the low toxicity and high effectiveness of spriobromin in the treatment of patients with acute leukemia, malignant lymphoma, throat cancer, skin reticulosis, cancer of the neck of the womb, and certain other tumors. At the present time spirobromin has been authorized by the Ministry of Public Health of the USSR for medical use.

EXPERIMENTAL

IR spectra of the synthesized compounds were taken in nujol on a Perkin-Elmer 457 instrument (USA), UV spectra in water in a Perkin-Elmer 575 spectrophotometer (USA), and PMR spectra

TABLE 2. Spectrum of the Antitumor Action of Spirobromin in Comparison with Prospidin

Preparation	Jensen sarcoma	Sarcoma 45	Sarcoma M-1	Sarcoma 536	Sarcoma 180	Sarcoma 37	Sarcoma AK	NK Carcinoma	Melanoma B16	Lewis lung cancer	Leukemia			Chemotherapeutic index for the Jensen sarcoma
											L_a	P-388	1210	
Spirobromin	++++	++++	++++	++	++	+++	+++	+++	+++	+++	+++	++	+	60
Prospidin	++++	+++	++++	0	+				+++	++	0	+	++	27

Note. ++++) Inhibition of tumor growth by 95-100%, +++) by 80-95%, ++) by 60-80%, +) by 30-60%, 0) effect absent.

TABLE 3. Toxic Properties of Spirobromin in Comparison with Prospidin

Preparation	Single injection		Index of cumulative toxic action, %	Index of intoxication
	LD_{50}	MTD		
	mg/kg			
Spirobromin	1924	1613	82	0,4
Prospidin	1000	900	58	0,3

in D_2O in a Jeol instrument (Japan) with an operating frequency of 100 MHz, internal standard was tetramethylsilane, and proton signals are given on the δ scale.

Chromatography was effected on plates of size 20 × 20 mm with an applied mixture of cellulose, gypsum, and water (5.0:0.35:40.0) in the system butanol–pyridine–acetic acid–water (4:1:1:2) of pH 4.7-4.8. Visualizing agent was Dragendorf's reagent. The acid chloride of β-bromopropionic acid was obtained by the reaction of acrylonitrile with NaBr and subsequent treatment of β-bromopropionic acid with $SOCl_2$.

Information on the synthesized compounds is given in Table 1.

N,N'"-Di(β-bromopropionyl)-N',N"-dispirotripiperazinium Dichloride (IIa). A solution of β-bromopropionyl chloride (5.2 g:0.03 mole) was added slowly with vigorous stirring at 5-10°C to a mixture of compound (Ia) (3.0 g:0.01 mole), LiOH (1.8 g:0.04 mole), water (0.2 ml), and ether (6 ml). The reaction mixture was stirred at 15-20°C for 3 h. The solid was filtered off and dissolved in water (30 ml). CH_3OH (100 ml) was poured into the obtained solution which was left to stand in the refrigerator for 20-22 h. The precipitated solid was filtered off, washed with alcohol, and dried to constant weight. Compound (IIa) was obtained of R_f 0.63. UV spectrum (in water), λ_{max}, nm (log ε): 198 (4.17).

Compound (IId) was obtained under analogous conditions. Compound (IIc) was obtained with the only difference that the process was carried out at 0-5°C.

N,N'"-Di(β-bromopropionyl)-N',N"-dispirotripiperazinium Dichloride (IIb). A solution of sodium bicarbonate (7.5 g:0.09 mole) was added to a suspension of (Ib) (15.0 g:0.04 mole) in water (40 ml). β-Bromopropionyl chloride (13.38 g:0.08 mole) was added with vigorous stirring. The reaction mixture was stirred for 2 h at 18-20°C, the solid filtered off, washed with water, with alcohol, and dried to constant weight. Compound (IIb) was obtained.

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