SYNTHESIS AND ANTITUMOR PROPERTIES OF 3(4)-CHLORO-4(3)-(2-CHLOROETHYLTHIO)BUTYRIC ACID DERIVATIVES

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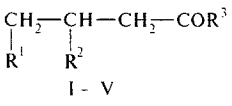
Previously we have studied some derivatives of 2(3)-chloro-3(2)-chloroethylthiocarboxylic acids possessing antitumor activity [1 – 3].

In continuation of the search for new compounds possessing a still higher antitumor activity, we have synthesized a series of 3(4)-chloro-4(3)-(2-chloroethylthio)butyric acids and their amides (I – V):



I - V

I: R^1 = Cl; R^2 = SCH_2CH_2Cl; R^3 = OH;
II: R^1 = SCH_2CH_2Cl; R^2 = Cl; R^3 = OH;
III: R^1 = Cl; R^2 = SCH_2CH_2Cl; R^3 – NHC_6H_5;
IV: R^1 = SCH_2CH_2Cl; R^2 = Cl; R^3 = NHC_6H_5;
V: R^1 = SCH_2CH_2Cl; R^2 = Cl; R^3 = $NHCHCH_2C_6H_5$.
COOH

Compounds I, III, and V were synthesized via interactions of the corresponding vinylacetic acid derivatives with chloroethylsulfene chloride in carbon tetrachloride [4].

4-Chloro-3-(2-chloroethylthio)butyric acid (I) was synthesized by the interaction of vinylacetic acid with chloroethylsulfene chloride at 0°C. The isomeric 4-(2-chloroethylthio)-3-chlorobutyric acid (II) was obtained by boiling acid I in nitromethane.

In a similar manner, 4-chloro-3-(2-chloroethylthio)butyric acid anilide (III) was synthesized from the corresponding vinylacetic acid anilide and 2-chloroethylsulfene chloride at 3°C, and the isomeric 4-(2-chloroethylthio)-3-chlorobutyric acid anilide (IV) was obtained by boiling acid III in nitromethane.

In order to establish a relationship between the chemical structure and biological activity, the antitumor properties of compounds I – V were compared to those of 2-chloro-3-(2-chloroethylthio)isobutyric acid (VI) [3] (see Table 1).

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EXPERIMENTAL CHEMICAL PART

The ^{1}H NMR spectra were measured on a Hitachi R-22 spectrometer (working frequency, 90 MHz) using TMS as the internal standard and deuterated acetone as the solvent. The data of elemental analyses agree with the results of analytical calculations according to the empirical formulas.

4-Chloro-3-(2-chloroethylthio)butyric acid (I). To a solution of 0.86 g (0.01 mole) of vinylacetic acid in 15 ml of carbon tetrachloride at – 15°C was added dropwise 1.31 g (0.01 mole) of chloroethylsulfene chloride in 10 ml carbon tetrachloride and the reaction mixture was allowed to stand for 24 h at 0°C. Then the solvent was evaporated in vacuum and the residue recrystallized from an ether – dioxane mixture to obtain 1.8 g (85%) of acid I; m.p. = 52 – 54°C; $C_6H_{10}Cl_2O_2S$; ^{1}H NMR spectrum (δ, ppm): 3.68 (m, 2H, $ClCH_2$), 2.96 (m, 2H, CH_2S), 3.80 (m, 2H, $ClCH_2$), 3.38 (m, 1H, SCH), 2.68 (m, 1H, CH_2CO), 2.58 (m, 1H, CH_2CO).

4-(2-Chloroethylthio)-3-chlorobutyric acid (II). A solution of 2.16 g (0.01 mole) of acid I in 50 ml of anhydrous nitromethane was boiled for 6 h. Then the solvent was evaporated and the residue recrystallized from dioxane to obtain 1.3 g (60%) of acid II; m.p. = 31 – 33°C; $C_6H_{10}Cl_2O_2S$; ^{1}H NMR spectrum (δ, ppm): 3.62 (m, 2H, $ClCH_2$), 2.98 (m, 2H, CH_2S), 2.92 (m, 2H, SCH_2), 4.34 (m, 1H, CHCl), 2.15 (m, 1H, CH_2CO), 2.78 (m, 1H, CH_2CO).

TABLE 1. Toxicity and Antitumor Activity of Compounds I – VI

Compound	LD_{100}, mg/kg	MTD, mg/kg	Tumor growth inhibition, %	
			WCS (*p*)	C45 (*p*)
I	20	10	91.0 (< 0.001)	46.7 (< 0.05)
II	10	5	90.3 (< 0.001)	42 (< 0.5)
III	20	10	98.5 (< 0.001)	63 (= 0.05)
IV	35	20	99.0 (< 0.001)	72 (= 0.05)
V	100	90	68.0 (< 0.05)	65.6 (< 0.01)
VI	25	15	24 (> 0.05)	29 (= 0.05)

4-Chloro-3-(2-chloroethylthio)butyric acid anilide (III). To a solution of 4.83 g (0.03 mole) of vinylacetic acid anilide in 30 ml of carbon tetrachloride at – 30°C was added dropwise 3.93 g (0.03 mole) of chloroethylsulfene chloride in 20 ml carbon tetrachloride and the reaction mixture was allowed to stand for 13 h at 3°C. Upon termination of the reaction (indicated by vanishing of the characteristic color of sulfene chloride), the solvent was evaporated in vacuum and the residue recrystallized from absolute ether to obtain 7.0 g (80%) of compound III; m.p. = 84 – 85°C; $C_{12}H_{15}Cl_2NOS$; ^1H NMR spectrum (δ, ppm): 3.70 (m, 2H, $ClCH_2$), 2.94 (m, 2H, CH_2S), 3.40 (m, 1H, SCH), 2.72 (m, 1H, CH_2CO), 2.61 (m, 1H, CH_2CO), 3.87 (m, 2H, CH_2Cl), 7.07 – 7.58 (m, 5H, C_6H_5).

4-(2-Chloroethylthio)-3-chlorobutyric acid anilide (IV). Anilide III (2.92 g, 0.01 mole) was boiled for 4.5 h with 60 ml of anhydrous nitromethane. Then the solvent was evaporated and the residue recrystallized from absolute ether to obtain 1.75 g (60%) of compound IV; m.p. = 87 – 89°C; $C_{12}H_{15}Cl_2NOS$; ^1H NMR spectrum (δ, ppm): 3.62 (m, 2H, $ClCH_2$), 2.98 (m, 2H, CH_2S), 2.92 (m, 2H, SCH_2), 4.54 (m, 1H, CHCl), 2.83 – 3.15 (m, 2H, CH_2CO), 7.03 – 7.55 (m, 5H, C_6H_5).

N-[4-(2-Chloroethylthio)-3-chlorobutanoyl]phenylalanine (V). To a solution of 2.4 g (0.01 mole) of N-(vinylacetyl)phenylalanine in 45 ml of carbon tetrachloride at – 20°C was added dropwise 1.4 g (0.01 mole) of chloroethylsulfene chloride and the reaction mixture was allowed to stand for 8 h at 0°C. Then the solvent was evaporated in vacuum and the residue recrystallized from a hexane – ether mixture to obtain 1.9 g (50%) of amide V; m.p. = 112 – 116°C; $C_{15}H_{19}Cl_2NO_3S$; ^1H NMR spectrum (δ, ppm): 4.15 (m, 1H, C-H), 4.32 (m, 1H, CHCl), 4.01 (m, 2H, $ClCH_2$), 2.98 (m, 2H, CH_2S), 2.93 (m, 2H, CH_2S), 2.83 – 3.30 (m, 2H, CH_2CO), 7.12 (m, 5H, C_6H_5).

EXPERIMENTAL BIOLOGICAL PART

The antitumor activity of the synthesized compounds was studied by conventional techniques [5] on a group of female rats weighing 90 – 110 g. The test animals were inoculated with Walker carcinosarcoma (WCS) or sarcoma 45 (C45) cells. Each animal in the test group was injected with 0.5 ml of a 20% tumor cell suspension in physiological solution. The tumor treatment began 5 days after the tumor inoculation. For this purpose, compounds I – V suspended in vegetable oil were injected intraperitoneally over 10 days at a daily dose of 1/10 LD_{100}. The therapeutic effect was evaluated by the degree of tumor growth inhibition.

The toxicity and antitumor activity of compounds I – V were compared to the action of 2-chloro-3-(2-chloroethylthio)isobutyric acid ethyl ester VI studied previously [3].

The experimental data were statistically processed as described in [6]. The results were considered as reliable for $p < 0.05$.

It was established that 3(4)-chloro-4(3)-(2-chloroethylthio)butyric acid derivatives I – V possess antitumor properties, exceeding in this respect the known compound VI (see Table 1). The WCS model was more sensitive than C45 to the presence of all substances studied in this work.

A comparison of the new results with the previous data [3] confirmed our suggestion that antitumor activity is inherent in the compounds structurally analogous to di(2-chloroethyl)sulfide, an agent known to interact with DNA [7]. The 3(4)-chloro-4(3)-(2-chloroethylthio)butyric acid derivatives I – V have proved to be more active compared to 2-chloro-3-(2-chloroethylthio)isobutyric acid (VI).

The high antitumor properties of the butyric acid derivatives I – V indicates that it would be expedient to continue the search for new active agents in this series of compounds.

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