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SYNTHESIS AND INVESTIGATION OF α-NITROSOUREIDOCARBONIC ACIDS WITH POTENTIAL ANTITUMOR ACTIVITY

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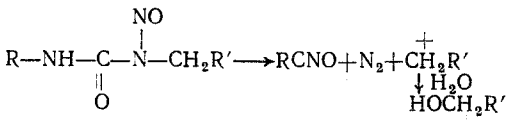
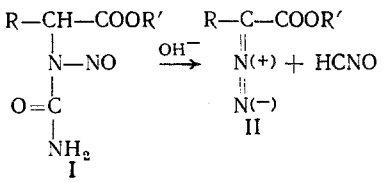
After the antitumor activity of a series of derivatives of nitrosourea was established against a broad spectrum of experimental tumors, and since some representatives of this group of compounds such as 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), and N-methyl-N-nitrosourea found wide application in oncological practice [1 - 3], an extensive search developed for their structural analogs. The introduction of the nitrosourea group into various classes of organic compounds has extended to the present time, and in a number of cases has resulted in highly active substances [4].

It is known that the antitumor activity of nitrosoalkylurea depends on their ability to decompose under the body's conditions and to form cytotoxic carbamylating and alkylating fragments [5]. However, as has been shown experimentally [6], the alkylating ability of nitrosoureas is small, inasmuch as in the course of their decomposition, carbonium ions are formed which are highly reactive and are rapidly stabilized (mainly as a result of the reaction with water which is found in large excess in the cellular and intercellular fluid), and are converted into biologically inactive alcohols [5].

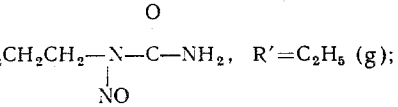


R and R' = H, alkyl, aryl, and other radicals.

In order to increase the alkylating ability of nitrosoureas, we synthesized unsubstituted α-nitrosoureidocarboxylic acids and their esters, with the general formula shown (I). These compounds, upon decomposition *in vivo*, by analogy with the decomposition of known nitrosoalkylureas, can be expected to form comparatively stable diazoesters of α-aminoacids (II), which possess considerable alkylating ability [6].



I: R=H, R'=C_2H_5 (a); R=CH$(CH_3)_2$, R'=C_2H_5 (b);
R=$CH_2CH_2COOC_2H_5$, R'=C_2H_5 (c); R=CH_2CH_2COOH, R'=H (d);
R=$CH_2C_6H_5$, R'=C_2H_5 (e); R=$CH_2C_6H_5$, R'=H (f)



R=$CH_2CH_2CH_2CH_2$—N(NO)—C(=O)—NH_2, R'=C_2H_5 (g);

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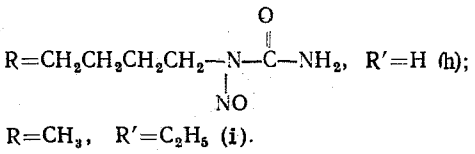
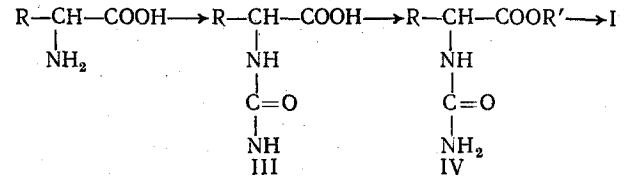


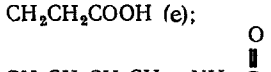
R=$CH_2CH_2CH_2CH_2$—N(NO)—C(=O)—NH_2, R′=H (h);

R=CH_3, R′=C_2H_5 (i).

Compounds (I) were obtained from α-aminoacids, according to the following chemical scheme:



III: R=H (a); $CH(CH_3)_2$ (b);
$CH_2C_6H_5$ (c); CH_3 (d);
CH_2CH_2COOH (e);



$CH_2CH_2CH_2CH_2$—NH—C(=O)—NH_2 (f).

As starting materials we used glycine, L-α-alanine, L-valine, β-phenyl-L-α-alanine, L-lysine, and L-glutamic acid, differing in the chemical nature of side chains (containing acidic and basic functional groups, an aromatic ring, hydrophobic and hydrophillic chains). We obtained the α-ureidocarboxylic acids (III) as colorless crystalline compounds after treatment of the aqueous solutions of the sodium salts of the corresponding α-aminoacids with potassium isocyanate, or by boiling with excess urea and subsequent acidifying of the reaction mixture to a pH of 2.0. Compounds III were esterified by absolute ethanol in the presence of thionyl chloride as a catalyst. The esters of ureidoacids (IV), which are not water soluble, were nitrated further with sodium nitride in anhydrous formic acid, and the water soluble compound IIIf was treated with sodium nitrite in dilute hydrochloric acid.

At the end of the reaction the nitroso-derivatives (I) precipitated as yellow solids or oils which were extracted with appropriate organic solvents. The oils obtained by either methods were further purified by chromatography.

The pure nitrosoureidocarboxylic acids and their esters are either pale-yellow crystalline solids or yellowish viscous oils. Their composition and structure were confirmed by elemental analysis data, and by IR and UV spectroscopy. In the UV spectra of all nitrosoderivatives (I) there is a characteristic maximum around 400 nm, with fine structure, and dependent on the $n\rightarrow\pi^*$ transition, and an intensive maximum around 235 nm, associated with a $\pi\rightarrow\pi^*$ transition.

In the IR spectra of the nitroso compounds investigated we found distinct bands characteristic of the vibrations of the N—NO group (1420 - 1410 cm^{-1}), N—N ($\sim$1000 cm^{-1}), CO in the amide (1670 - 1640 cm^{-1}), and also the CO of the carboxyl group or of the ester compound (1720 - 1710 and 1740 - 1730 cm^{-1}, respectively). The characteristic data for these compounds are listed in Table 1.

We investigated the chemical affinity of the decomposition of ethyl esters of nitrosoureidocarboxylic acids (I) under conditions similar to physiologic conditions (pH 6.9, temperature 37°C) by taking as an example the decomposition of nitrosoureidoacetic acid in a solution of Tris-HCl buffer and ethyl alcohol (1:1, by volume). Based on the data of thin-layer chromatography (TLC) and UV spectra taken over 9 h, under those conditions the complete decomposition of the starting material occurs with the formation of a compound which was identified as a diazoacetic ester.

The formation of diazoacetic acid ester from the decomposition of compound Ia was proven by comparison of the chromatogram, UV and IR spectra of the decomposition product, and the diazoacetic acid ester obtained by direct synthesis from glycine ethyl ester hydrochloride [7]. In the electronic spectra of both products we noted maxima around 395 nm which are characteristic of diazocompounds, and in the IR spectra these groups correspond to intensive bands at 2125 - 2120 cm^{-1}. In summary, the spectra of the two compounds proved identical.

TABLE 1. Properties of α-Nitrosoureidocarboxylic Acids and Their Esters

Compound	Yield, %	Melting point, °C	R_f	$[\alpha]_D^{22}$ (C ~ 1.0, ethanol)	Found, %			Molecular formula	Calculated, %			IR spectra ν, cm^{-1}
					C	H	N		C	H	N	
Ia	42	60	0,41	—	35,03	4,91	24,10	$C_5H_9N_3O_4$	34,99	5,14	24,00	3410 (NH), 1745 (COOR), 1660 (CONH), 1430 (N—NO), 1020 (N—N)
Ib	43	Oil	0,38	—23,2	44,66	6,82	19,20	$C_8H_{15}N_3O_4$	44,24	6,91	19,35	3350 (NH), 1740 (COOR), 1665 (CONH), 1420 (N—NO), 990 (N—N)
Ic	14,0	"	0,42	—25,8	43,63	6,18	15,24	$C_{10}H_{17}N\ O_6$	43,64	6,44	15,21	3350 (NH), 1740 (COOR), 1410 (N—NO), 980 (N—N)
Id	19,5	"	0,11	+10,6	32,62	4,28	19,09	$C_6H_9N_3O_6$	32,88	4,11	19,18	3380 (NH), 1710 (COOH), 1660 (CONH), 1420 (N—NO), 1000 (N—N)
Ie	22	"	0,46	11,0	54,34	5,66	16,85	$C_{12}H_{15}N_3O_4$	54,54	5,58	15,60	1740 (COOR), 1660 (CONH), 1510 (C—N), 1410 (N—NO), 980 (N—N)
If	12,5	"	0,38	—38,0	50,63	4,64	17,72	$C_{10}H_{11}N_3O_4$	50,48	4,66	17,44	3405 (NH), 1710 (COOH), 1660 (CONH), 1415 (N—NO), 990 (N—N)
Ig	41	Oil	0,33	—10,2	37,48	5,44	26,34	$C_{10}H_{18}N_6O_6$	37,74	5,66	26,42	3350 (NH), 1745 (COOR), 1650 (CONH), 1425 (N—NO), 990 (N—N)
Ih	19,5	55 (decomp.)	0,71	—8,9	33,0	4,99	28,69	$C_8H_{14}N_6O_6$	33,10	4,83	28,97	3345 (NH), 1710 (COOH), 1660 (CONH), 1410 (N—NO), 995 (N—N)
Ii	29	102 (decomp.)	0,56	+2,71	38,62	5,82	22,22	$C_6H_{11}N_3O_4$	38,56	5,67	22,44	3350 (NH), 1740 (COOR), 1655 (CONH), 1420 (N—NO), 1020 (N—N)

TABLE 2. Rate Constants of the Decomposition Reaction and Half-life for the Ethyl Esters of Nitrosoureidocarboxylic Acids

Compound	Parameter	
	k, h^{-1}	$t_{1/2}$, h
Ia	0,180	3,89
Ib	0,0044	168
Ic	0,177	3,97
Id	0,022	34,1
Ie	0,148	4,76
If	0,0038	214
Ig	0,04	17,5
Ih	0,30	2,4
Ii	0,021	31,4

In order to determine the relationship between the rate of decomposition of the synthesized compounds and their biological activity, we studied the kinetics of decomposition of ethyl esters of nitrosoureidocarboxylic acids (Ia-i) in a solution of Tris-HCl buffer at a pH of 6.9 and a temperature of 37°C, and their antitumor activity in experiments *in vitro* and *in vivo*. The change in the concentration of nitrosoureido esters during the decomposition process was determined spectrophotometrically through changes in the absorption in the 400 nm wavelength in the maximum absorption of the nitroso group. It was established that the decomposition reaction of the compounds investigated follows a first-order reaction rate. On the basis of these results we calculated the decomposition reaction rate constants (k) and the half-life ($t_{1/2}$) of the substances synthesized [8] (Table 2).

The data obtained indicate that, under conditions similar to physiologic ones, all the synthesized nitrosoureido-derivatives (I) can decompose, with the formation of diazoalkyl esters (II), which appear to be active alkylating agents. However, the rate of decomposition of these compounds is considerably slower than that of the known nitrosoalkyl ureas. According to previous reports [5], the half-life of BCNU and CCNU are 57 and 64 min, respectively, while the half-life of the synthesized compounds under similar conditions range from 2.4 to 214 h.

The antitumor activity of compound Ia - Ii was investigated on tissue culture C-37, lymphadenoma NK/Ly, Fischer and Ehrlich tumors. This activity was evaluated by the inhibition by these compounds of nucleic acid synthesis [9]. The compound's activity was characterized by the average effective dose ED_{50} producing a lowering of nucleic acid synthesis by 50% (Table 3).

The antitumor activity of the synthesized compounds *in vivo* in animal experiments was investigated by the following scheme: For the majority of these compounds the LD_{50} ranged from 200 to 1000 mg/kg. The antitumor activity was investigated on mice tumors of the L-1210 strain, lung cancer of Lewis (LLC), adenocarcinoma 755, sarcomas 180 and 37. In view of the

TABLE 3. Antitumor Activity of Nitrosoureidocarboxylic Acids and Their Esters

Compound	in vitro				in vivo				
	ED_{50}, μg/ml				LD_{50}, mg/kg	Tumor strain	mice	single dose, mg/kg	inhibition, %
	NK/Ly	Ehrlich tumor	C-37	Fischer tumor					
Ia	—	10	10	50	300	C-37	Unbred (female)	60	65
						C-180	" "	60	5
						LLC	$C57Bl_6$ (male)	60	16
Ib	n/e	—	—	n/e	800	C-180	Unbred (male)	150	31
						C-37	Unbred (female)	150	+41
						LLC	$C57Bl_6$ (male)	200	+7
						Ca2755	$C57Bl_6$ (female)	200	8
Ic	—	n/e	—	—	1200	C-37	Unbred (female)	300	+36
						L-1210	DBA × $C57Bl_6$ (male)	300	0
Id	100	n/e	—	—	300	C-180	Unbred (male)	50	15
						C-37	Unbred (female)	50	33
						Ca-755	$C57B_{16}$ (female)	50	5
						LLC	$C57B_{16}$ (male)	50	+28
Ie	—	n/e	—	—	600	C-37	Unbred (female)	150	+5
						LLC	$C57Bl_6$ (male)	150	+19
						Ca-755	$C57Bl_6$ (female)	150	+6
						L-1210	DBA×$C57Bl_6$ (male)	150	0
If	100	—	—	n/e	200	C-180	Unbred (male)	150	9
						C-180	" "	200	13
						Ca-755	$C57Bl_6$ (female)	200	21
Ig	—	10	—	50	250	C-180	Unbred (female)	50	+10
						Ca-755	$C57Bl_6$ (female)	50	+60
						LLC	$C57Bl_6$ (male)	50	2
						C-37	Unbred (female)	50	2
Ih	—	—	—	n/e	600	Ca-755	$C57Bl_6$	100	7
						LLC	$C57Bl_6$	100	+11
						C-37	Unbred	100	15
Ii	—	n/e	—	—	1000	C-37	Unbred	200	22
						Ca-755	$C57Bl_6$	200	21

Note: n/e = no effect. Sign + indicates a stimulation of tumor growth.

low water solubility of the group of compounds investigated, and since most of them are actually viscous oils, the latter were introduced as alcohol-oil suspensions in starch sizing. All substances, except compound I-d, were introduced intaperitoneally five times during a 24 h period, with observations following within a week. Compound I-d was introduced four times, with 48 h intervals between injections. The results of the investigation of antitumor activity are listed in Table 3. It can be seen that only compound Ia and Ig exhibited a substantially high activity in *in vitro* experiments against C-37 and Ehrlich tumors. Compound Ia showed a measurable activity (65% inhibition) against C-37 in experiments on mice. The remaining compounds appeared inactive.

In our opinion, the reason for such low activity by these synthetic compounds is their fairly slow decomposition with the formation of cytotoxic fragments. Apparently, it is not accidental that among these nitrosoureidocarboxylic acids and their esters, the highest activity was exhibited by those compounds with the lowest half-lives for decomposition (compounds Ia and Ig). In this way the investigation of the synthesis, kinetics of decomposition, and the biological activity of nitrosoureidocarboxylic acids and their esters, enables one make conclusions about further search for active compounds. With that in view, one should aim in the direction of obtaining compounds with faster rates of decomposition with the formation of cytotoxic fragments.

EXPERIMENTAL

For TLC we used Silufol and Silufol UV-254 plates. The solvent system employed was benzene—acetic acid—methanol (45:4:8). IR spectra were obtained with a UR-20 spectrophotometer with paraffin oil mulls or in solutions of methylene chloride. UV spectra were obtained on a Specord UV Vis spectrophotometer. The optical activity was measured in a 241MC (Perkin-Elmer) polarimeter in alcohol, dimethylformamide, or 10% potassium hydroxide solutions.

α-Ureidocarboxylic Acids (III). A. To a suspension of 0.1 mole L-α-aminoacid in 100 ml of water we added 0.1 mole of sodium carbonate. After all the solids completely dissolved, we added 0.5 mole of urea, and the solution was refluxed for 5 h. The reaction mixture was then acidified to a pH of 2.0 with concentrated hydrochloric acid; then cooled, and the precipitate filtered and purified by recrystallization from water. Compounds IIIb, IIIc, and IIIf were obtained by this method.

B. A mixture of 0.1 mole L-α-aminoacid and 0.1 mole potassium isocyanate was dissolved in 100 ml of 1 N potassium hydroxide and kept at room temperature for 16 h. The reaction mixture was acidified with concentrated hydrochloric acid to a pH of 2.0 and then chilled. The colorless precipitate that formed was filtered and recrystallized from water. Compounds IIIa, IIIc, and IIIe were obtained in this fashion.

Ethyl Esters of α-Ureidocarboxylic Acids (IV). We suspended 0.1 mole of ureidocarboxylic acid in absolute ethanol, chilled the suspension to 0 - 5°C, and added dropwise freshly distilled thionyl chloride (0.12 mole). The temperature was raised to 45°C, and the reaction mixture maintained at that temperature for 5 h and then allowed to stand overnight. The alcohol was vacuum distilled, and to the residue we added water and the reaction mixture was neutralized with sodium carbonate to a pH of 7.0 - 8.00. Compounds IV precipitated as colorless crystalline substances which were recrystallized from water or 50% alcohol. Compound IVd did not precipitate after the neutralization of the reaction mixture, and it was extracted from the reaction mixture with dioxane (after the water from the mixture had been removed under vacuum).

Ethyl Ester of α-Nitrosoureidocarboxylic Acid (Ia). We dissolved 6 g (0.05 mole) of IVa in 15 ml of anhydrous formic acid, and chilled the mixture to 5°C. As soon as the temperature dropped to 10°C, we added 6 g (0.07 mole) of sodium nitrite. The solution was maintained at 5 - 10°C for 1 h and was then chilled by the addition of ice. Compound Ia precipitated as a yellowish solid; it was filtered and washed with water. Compound Ib to Ig and Ii were obtained in a similar way. Compounds Ib, c, and e however, formed yellowish oils upon chilling the reaction mixture. The latter oils were decanted, dissolved in ether, the ether solution was washed with water, dried over magnesium sulfate, and evaporated under vacuum. The residue was purified by column chromatography, with the column filled with silica gel mesh 40/100 μ (Chemapol); mobile phase: benzene—acetic acid—methanol. Compounds If, Id, Ig, and Ii did not precipitate from the reaction mixture on cooling, and they

were extracted with ethyl acetate and subsequently isolated and purified by chromatography by the method used for Ib, c, and e.

Nitroso-α, ε-Diureidocaproic Acid (Ih). We dissolved 4 g (0.014 mole) of IIIf in 25 ml of water and 15 ml of concentrated hydrochloric acid. The reaction mixture was cooled to 5°C, and to it we promptly added 8 g (0.95 mole) of sodium citrate while maintaining the temperature at 10°C during the addition. The solution was then maintained at 4 - 6°C for 2 h. The yellowish precipitate that formed was filtered, washed with cold water, and dried under vacuum.

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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 2-MERCAPTOIMIDAZOLE DERIVATIVES

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Substituted 2-mercaptoimidazoles are of definite interest in the planned search of new biologically active substances and as intermediates for the synthesis of condensed heterocyclic systems based upon them.

It is the purpose of this work to synthesize new derivatives of the 2-mercaptoimidazole series and to investigate their biological activity.

We studied the reaction of 2-substituted mercaptoimidazoles (I, II) with alkyl halides, β-halogenated alcohols, α-epoxides, α-halo ketones, and esters of haloacetic acids. It was established that, in contrast with 2-methylmercapto-benzimidazole [1] and 2-methylmercaptonaphth[1,2-d]-imidazole [2], compounds I and II react only with alkyl halides and α-epoxides. That reaction takes place on heating of the starting materials in an alcohol medium in the presence of alkaline reagents (sodium alcoholate or alkali hydroxides), and leads to the formation of corresponding N-substituted 2-methyl(benzyl)mercapto-4,5-diphenylimidazoles (III, V - IX) (See Table 1).

However, under the conditions investigated, we failed to react I with ethylene oxide, and 1-(β-hydroxyethyl)-2-methylmercapto-4,5-diphenylimidazole (IV) was obtained by the methylation of 1-(β-hydroxyethyl)-2-mercapto-4,5-diphenylimidazole (XVI) by using methyl iodide in low-molecular-weight alcohols in the presence of an equivalent amount of an alkaline reagent. Compound XVI was synthesized by substituting bromine in 1-(β-hydroxyethyl)-2-bromo-4,5-diphenylimidazole [3] by a mercapto group, using the method previously reported [4].

From the reaction of I and II with unsymmetrical derivatives of ethylene oxide we isolated only one isomer which, in accordance with Krassuski's rule and our data [1, 2], has the structure of a secondary alcohol.

We obtained 1-acetylmethyl-2-mercaptoimidazole (X) by methylating 1-phenacyl-2-mercapto-4,5-diphenylimidazole which had been previously described [4].

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