N^{ω}-ALKYLNITROSOCARBAMOYL-α,ω-DIAMINOCARBOXYLIC ACIDS. 3. SYNTHESIS AND ANTITUMOR ACTIVITY OF N^{ε}-NITROSO-N^{ε}-[N′-(2-CHLOROETHYL)CARBAMOYL]-*L*-LYSINE AND N^{ε}-[N′-(2-CHLOROETHYL)-N′-NITROSO-CARBAMOYL]-*L*-LYSINE

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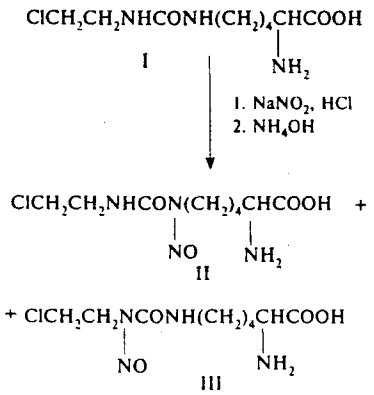
Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 30, No. 5, pp. 23 – 25, May, 1996.

Original article submitted September 21, 1995.

Previously [1] we described a series of newly synthesized N^{ω}-nitrosocarbamoyl derivatives of α,ω-diaminocarboxylic acids and showed that activity against experimental solid tumors and leukemia was observed for a mixture of N^{ε}-nitroso-N^{ε}-[N′-(2-chloroethyl)carbamoyl]-*L*-lysine (II) and N^{ε}-[N′-(2-chloroethyl)-N′-nitrosocarbamoyl]-*L*-lysine (III) obtained by nitrosation of N^{ε}-[N′-(2-chloroethyl)carbamoyl]-*L*-lysine (I) with $NaNO_2$ in diluted HCl. It is impossible to separate this mixture by conventional preparative methods because of the closely related physicochemical properties and high lability of compounds II and III, which are positional isomers with respect to the nitroso group.



In order to elucidate the special features of antitumor action and the contribution of each isomer to the antitumor activity of the mixture, we have synthesized and studied pure isomers II and III.

Since an isomer with the NO group at the nitrogen atom bound to the 2-chloroethyl (or methyl) residue was considered responsible for the antitumor effect of compounds of the nitrosoalkylurea (NAU) series, attempts were undertaken to synthesize III [2, 3]. Compound III was regioselectively synthesized with the use of dangerously explosive N-(2-chloroethyl)-N-nitrosocarbamoyl azide [2]. However, details of purification and yields were not presented. Another method [3], based on the nitrosation of a copper complex of compound I with excess $NaNO_2$ in absolute formic acid, followed by decomposition of the copper complex of isomer III and the isolation of III, did not ensure the isomer purity. It was established by the HPLC method that compound III synthesized under the conditions described in [3] contained a considerable fraction of isomer II.

To obtain isomer II, we used the difference in the hydrolytic stability of isomers II and III. Isomer II, whose content in the mixture is about 80% [1], is more stable in aqueous solutions than isomer III. The conditions were experimentally found when isomer III was virtually completely decomposed while only a partial decomposition took place for isomer II. Thus, isomer II was obtained after prolonged (120 – 140 h) holding of an aqueous solution of the isomer mixture at room temperature, followed by concentration of the solution and the isolation and recrystallization of the target product. The yield of compound II was 53%, as calculated for the initial amount of isomer II.

To synthesize isomer III, we used a regioselective pathway based on the use of "activated" N-alkylnitrosocarbamates, which were obtained according to the methods described in [4, 5].

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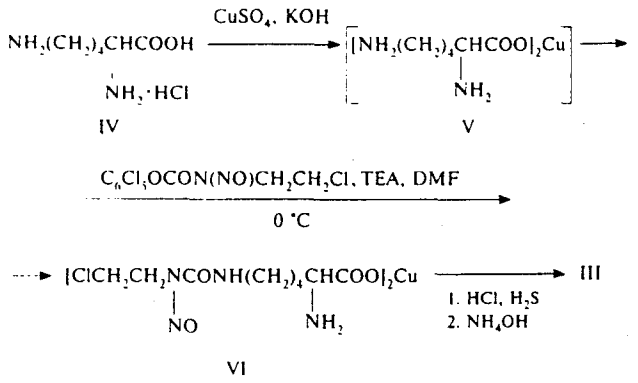

Copper complex VI, formed upon the carbamoylation of copper complex of *L*-lysine (V) with pentachlorophenyl ester of N-(2-chloroethyl)-N-nitrosocarbamic acid, was decomposed with hydrogen sulfide in diluted HCl, and then compound III was isolated after neutralization of the reaction mixture with aqueous ammonia. Compound III was purified by additional reprecipitation from diluted HCl with aqueous ammonia. The total yield of compound III was about 20%, as calculated for *L*-lysine hydrochloride.

Structures of compounds II and III were confirmed by IR, UV, and ^{1}H NMR spectroscopy data. Physicochemical characteristics are given in Table 1.

Compounds II and III as well as their mixture are yellowish-white crystalline substances melting above 100°C. The compounds have limited solubility in water, isomer II being somewhat more soluble than isomer III.

Thin-layer chromatography (TLC) in various solvent systems is unable to separate a mixture of isomers II and III, because they have equal R_f values. Separation of isomers II and III can be achieved only by using the HPLC method.

The UV spectra of isomers II and III display absorption maxima at λ 230 and 398 nm, which are due to the presence of the nitroso and carbonyl groups. As is seen from Table 1, solutions of isomers have virtually equal optical densities of the 230 nm peaks in the UV range. In the region of longer waves (240 – 270 nm), the UV spectrum of isomer II has (unlike the spectrum of isomer III) a small shoulder whose molecular extinction coefficient (for example, ε_{254}) is considerably higher than that for isomer III. On the contrary, in the visible range isomer III absorbs more intensively than isomer II.

There are no distinctions in the position of bands due to the nitrosourea carbonyl stretching vibrations at ν about 1700 cm^{-1} in IR spectra of isomers II and III. Some insignificant distinctions were observed in the positions of bands due to the stretching vibrations of NH groups.

The ^{1}H NMR spectra of compounds II and III showed distinctions in the signals from protons of the 2-chloroethyl group. Methylene protons of the 2-chloroethyl group of isomer II show a singlet at δ 3.86 ppm (4H), representing an example of the "accidental" equivalence of protons, i.e., of the coincidence in the chemical shifts of structurally nonequivalent nuclei. This is apparently due to the close magnitudes of the electron-induced effect of substituents on the shielding of methylene protons in isomer II. However, the influence of the NO group in isomer III results in a sharp distinction in chemical shifts of methylene protons of the 2-chloroethyl group (δ, ppm): 3.68 (t, $ClCH_2$) versus 4.25 (t, $CH_2N(NO)$). A similar phenomenon is observed for the other nitroso derivatives of N′-(2-chloroethyl)carbamoylamino acids [1]. The assignment of signals of CH_2 groups was made by the method of double $^1H\{^1H\}$ resonance.

EXPERIMENTAL CHEMICAL PART

The purity of the obtained compounds was checked by the TLC method on Silufol UV plates in the *n*-butanol–acetic acid–water (6 : 2 : 2) system, followed by visualization in UV light upon development with a ninhydrin solution. Melting points were determined on a Boetius heating device. UV spectra were recorded on a Specord UV-VIS spectrophotometer using water as solvent. The IR spectra were recorded in vaseline oil using a Specord IR-75 spectrophotometer. The ^{1}H NMR spectra were measured on a Bruker WH-360 spectrometer operated at a working frequency of 360 MHz, using D_2O as the solvent and as the internal standard (δ 4.80 ppm). The specific rotation angles of the polarization plane were determined on an A1-EPO polarimeter. The isomer purity of compounds obtained and the composition of isomer mixtures were determined by the HPLC method on a Milikhrom 4-UV chromatograph (detection at 230 nm; col-

TABLE 1. Physicochemical Characteristics of Compounds II and III

Compound	Solubility in water, 20°C, g/liter	α_D^{23}, grade (c. 2; 1 N HCl)	R_f (*n*-butanol–acetic acid–water)	HPLC: t_R, min	UV spectra λ_{max}, nm(ε)	^{1}H NMR spectra, D_2O, δ, ppm
II	15.2	+ 14.5	0.42	3.8 – 4.2	230(5740), 254(4610), 398(78.8)	1.37 m (2H, $CH_2CH_2CH(NH_2)COOH$), 1.54 m (2H, $CH_2CH_2CH_2CH(NH_2)COOH$), 1.89 m (2H, $CH_2CH(NH_2)COOH$), 3.75 t (1H, CH), 3.86 s (4H, CH_2CH_2Cl), 3.91 t (2H, $N(NO)CH_2$)
III	10.7	+ 15.0	0.42	4.9 – 5.2	230(5780), 254(4050), 398(86.1)	1.54 m (2H, $CH_2CH_2CH(NH_2)COOH$), 1.75 m (2H, $CH_2CH_2CH_2CH(NH_2)COOH$), 1.96 m (2H, $CH_2CH(NH_2)COOH$), 3.51 t (2H, $NHCH_2$), 3.68 t (2H, CH_2Cl), 3.79 t (1H, CH), 4.25 t (2H, $N(NO)CH_2$)

umn size, 62 × 2 mm; Silasorb C-18). The elution was performed with a mixture of ethanol and 0.01 M KH_2PO_4 (1 : 9) at a rate of 100 μl/min.

The elemental analysis data of compounds correspond to the calculated values.

N^{ε}-nitroso-N^{ε}-[N'-(2-chloroethyl)carbamoyl]-*L*-lysine (II). 25 g of a mixture of isomers II and III [1] containing 75% of isomer II were dissolved in 5 liters of water. The solution was allowed to stand at room temperature for 140 h. Water was distilled off under vacuum on a rotary evaporator at a temperature of 35 – 40°C until formation of a thick suspension. The reaction mixture was cooled to 0°C and the precipitate was filtered off, washed with water and ethanol, and dried in vacuum over P_2O_5 to obtain 12.8 g of compound II. Additionally, 2.5 g of compound II were isolated by evaporation of the filtrate and washing solutions. For the final purification, the product was recrystallized from 150 ml of 50% ethyl alcohol to yield 9.9 g of II (the total yield 53%).

N^{ε}-[N'-(2-chloroethyl)-N'-nitrosocarbamoyl]-*L*-lysine (III). A solution of 2.80 g (0.011 mole) of $CuSO_4 \cdot 5H_2O$ in 25 ml of water and then 20 ml of water and 15 ml of DMF were added under stirring to a solution of 4.10 g (0.022 mole) of IV and 2.51 g (0.045 mole) of KOH in 25 ml of water. The resulting solution of V was cooled to 0°C and added dropwise with stirring at 0 – 5°C for 45 min to a solution of 9.0 g (0.022 mole) of pentachlorophenyl ester of N-(2-chloroethyl)-N-nitrosocarbamic acid [4] in 90 ml of DMF; simultaneously, 2.25 ml (0.016 mole) of TEA were added in portions. The reaction mixture was held under cooling and stirring for 2 h. Then 150 ml of water were added, and the precipitate of VI was filtered off, washed several times with water and then with acetone, and dried in vacuum over P_2O_5 to obtain 4.9 g of VI (70%).

4.9 g (0.008 mole) of VI were thoroughly triturated and dissolved in 32 ml of 1 N HCl. Then H_2S was bubbled through the solution under cooling (0 – 5°C) until complete precipitation of CuS. The precipitate of CuS was filtered off and the filtrate was neutralized with NH_4OH to pH 6 on cooling and held at this pH and 0 – 5°C for 30 min. The precipitate was filtered off, washed with ethanol, and dried in vacuum over P_2O_5 to obtain 2.0 g of III (45%, as calculated for VI).

EXPERIMENTAL BIOLOGICAL PART

Experiments were carried out on C 57B1/DBA2 hybrid mice. Leukemia was induced in mice with an intraperitoneal injection of 1.0 – 1.2 × 10^6 cells, and solid tumors by subcutaneous injection of 50 mg of the tumor suspension into the axillary region. The antitumor activity was studied in mice with lymphoblast leukemia L 1210 and solid tumors, including mammary adenocarcinoma (Ca-755), large intestine cancer (AKATOL), Lewis lung carcinoma (LLC), cervical carcinoma (RShM-5), hepatoma (22s), and gastric cancer (OZh-5).

The therapeutic efficiency was evaluated by the tumor growth inhibition factor (TGI, %; 50% TGI is a minimum significant for the number of animals used), by the lifetime increase (LTI, %; 25% LTI is minimum significant), and by the percentage recovery (the animals were considered as cured after having no relapse over a period of not less than 3 months after complete tumor regression). Compounds were used in maximum tolerated doses. Results of the comparative study of the antitumor action of isomers II and III and their mixture are presented in Table 2.

As expected, compound III exhibited a high antitumor effect against the experimental tumors studied. Compound II at high doses (1000 – 1250 mg/kg) exhibits moderate antitumor activity against a number of tumors. The pure isomers exhibited a potentiation (synergistic) effect with respect to some tumors. For example, a protective effect was achieved in none of tumor localizations when II was used in doses (150 – 130 mg/kg) equivalent to the content in the II – III

TABLE 2. Antitumor Action of Isomers II and III and Their Mixture

Compound	Dose, mg/kg	Leukemia L 1210, % recovery	Solid tumors TGI, %					
			LLC	RShM-5	Ca-755	AKATOL	OZh-5	Hepatoma 22s
II	150		No effect		no effect	no effect		–
	600 – 800		No effect		76	no effect		–
	1000		49			51		29
	1250	(39 % LTI)	toxic	75(toxic)			38	–
	1500	(61 % LTI)					(17 % LTI)	–
III	40 – 45	96	98 (38 % recovery)		77	90	68 (18 % LTI)	55
	47.5 – 50	100	99.9 (100 % recovery)	99.6 (100 % recovery)	70 (69 % LTI)	80	toxic	–
II – III isomer mixture (75:25)	175 – 200 (isomer II, 130 – 150), (isomer III, 45 – 50)	100	99.9 (80 % recovery)	98.9 (94 % recovery)	84 (32 % LTI)	91	73 (42 % LTI)	52

mixture studied. Isomer III taken in doses of 40 – 45 mg/kg inhibited the growth of gastric cancer by 68% (with a decrease in effect) and increased the lifetime of animals only by 18%, and was toxic in a dose of 47.5 – 50 mg/kg. At the same time, the II – III isomer mixture in doses of 175 – 200 mg/kg (a dose of isomer III is 45 – 50 mg/kg) exhibited a stable antitumor effect: 73% TGI was retained over a period of 3 weeks after a single administration and the animal lifetime increased by 42%.

Therapeutic doses were determined for isomer III and the II – III isomer mixture, which produced 100% recovery of mice with leukemia L 1210. The range of therapeutic doses is 37.5 – 47.5 mg/kg for isomer III and increases twofold for the II – III mixture isomer, which corresponds to 26 – 57 mg/kg as calculated for isomer III.

Thus, the comparative study of the antitumor activity of the pure positional (with respect to the NO group) isomers II and III and their mixture allows us to conclude that the mixture of isomers possesses better therapeutic properties as compared to the active isomer III.

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