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SYNTHESIS AND ANTITUMOR PROPERTIES OF 13-DIHYDRO-14-HYDROXYCARMINOMYCIN

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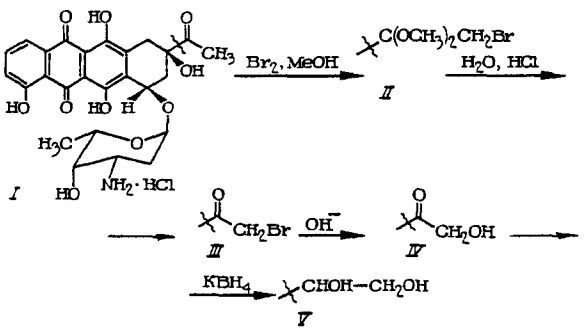
Reduction of the carbonyl group C(13) in 14-hydroxycarminomycin by the action of potassium borohydride yielded 13-dihydro-14-hydroxycarminomycin. Its antitumor activity was studied in comparison with carminomycin and doxorubicin for four tumors grafted to mice, namely, lymphadenosis HK/Li, ascitic Ehrlich carcinoma, hemocytoblastosis La, and leucemia P-388. The new compound was shown to have a high antitumor activity and a broad spectrum of antitumor action which is more pronounced than that of carminomycin and doxorubicin, while with respect to hemocytoblastosis La it is equal to the latter. Its effect on leukemia P-388 is lower than that of carminomycin. The drug exhibits considerable selectivity in its antitumor effect with respect to lymphadenosis HK/Li, exceeding the selectivity of doxorubicin and carminomycin. The selectivity of the antitumor effect of the drug with respect to Ehrlich carcinoma is virtually the same as that of carminomycin, but lower than that of doxorubicin.

The natural antibiotic of the anthracycline series carminomycin (I) exhibits antitumor activity and is used at clinics for treating a number of cancers [1]. We developed a number of methods for the chemical transformation of compound I to obtain drugs with improved chemotherapeutic properties [2, 3]. In particular, it was shown that the bromination of compound I proceeds via the formation of a dimethylketal of 14-bromocarminomycin (II) whose acid hydrolysis leads to 14-bromocarminomycin (III) [4]. The alkaline hydrolysis of III yields 14-hydroxycarminomycin (IV), exhibiting high antitumor activity [3, 5]. However, compound IV in the form of a hydrochloride or base, unlike I, is virtually insoluble in water and organic solvents, except for DMSO, apparently because of the presence of strong hydrogen bonds between the molecules.

The 13-dihydro derivatives of daunorubicin and doxorubicin containing the fragment 9-$CH(OH)CH_3$ in comparison with the starting antibiotics have a higher solubility in water and a quite pronounced, although somewhat lower, antitumor activity [3]. Considering these circumstances, in the present work to obtain a drug with an improved solubility in water, we reduced the carbonyl group at C(13) in compound IV bytreatment with potassium borohydride. As a result, we obtained a novel semisynthetic derivative of I – 13-dihydro-14-hydroxycarminomycin (V) with a yield of 80%. In the form of the hydrochloride, it dissolves well in water.



To avoid the separation of the sparingly soluble compound IV, we developed two more methods for obtaining compound V, namely: from I without the separation of intermediates II – IV, and from the bromide III without separation of the intermediate IV. The latter is the optimal way of synthesizing compound V. It makes it possible to synthesize V with a yield of about 30% (calculation based on the starting compound I).

We studied the physicochemical properties of V, used spectral methods to confirm its chemical structure, and studied its antitumor properties in tests on animals.

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In the IR spectra of V there is no absorption band from the carbonyl group C-13 (present in the IR spectrum of IV at 1730 cm^{-1}), but the absorption band of C=O from he quinone carbonyls at 1610 cm^{-1} remains.

The reduction of the carbonyl group results in a new asymmetric center in he molecule at C-13 and to the formation of a mixture of R- and S-epimers, respectively. When thin-layer and column chromatography as well as high-performance liquid chromatography were used, no separation of the epimers was observed. The presence in compound V of a mixture of epimers is indicated by the NMR ^1H spectrum, containing a double set of the proton signals 8-H and 10-H, as by the NMR ^{13}C spectrum, containing double signals from C-8 and C-10. For example, in the NMR ^1H spectrum signals were observed for protons 8-He at 2.47 ppm (br. d, J = 15.1 Hz) and 2.28 ppm (br. d, J = 15.1 Hz). The group of lines in the region of 2.30 – 1.90 ppm is the superposition of the H-8a signals from different epimers and 2H-2′. The signals from the 10-Ha protons yielded two sets of doublets at 2.82 and 2.78 ppm (J = 18.7 Hz), while the signals from the 10-Hb protons yielded doublets at 3.06 and 3.03 ppm (J = 18.7 Hz). The signals from the 13-H protons from different epimers at 3.59 ppm are poorly resolved. In the region of 3.76 and 3.97 ppm, the superposition of the 14-Ha and 14-Hb signals from different epimers with respect to C-13 was observed. The signals from the remaining protons had the same chemical shifts as in the spectrum of I [6]. In the NMR ^{13}C spectrum, doubling of the signals from the C-8 and C-10 carbons, belonging to two different epimers with respect to C-13, was observed.

CHEMICAL EXPERIMENTAL PART

The NMR signals were recorded on a VXR-400 (Varian, USA). To assign the signals, we used the methods COSY and HETCOR. The IR spectra (KBr) were obtained in an SP-1100 spectrophotometer (Pye Unicam, Great Britain). The melting point was determined on a Buchi SMP-20 (Switzerland) and was not corrected. The angle of rotation was determined in a Perkin – Elmer 241 polarimeter in a cuvet 1 dm long. Thin-layer chromatography was performed on Silufol plates (Kavalier, CFSR) in the systems chloroform – methanol – water, 13 : 6 : 1 (A) and chloroform – benzene – methanol, 10 : 1 : 2 (B). The found values of the elemental analyses correspond to the calculated ones.

13-Dihydro-14-hydroxycarminomycin (V). *a.* To a suspension of 630 mg (1.1 mmole) of the hydrochloride of 14-hydroxycarminomycin (IV) in a mixture of 100 ml of methanol and 100 ml of chloroform we added 115 mg (2.1 mmole) of potassium borohydride (in portions). With stirring at 20°C for 3.5 h, the mixture becomes homogeneous. We evaporated the reaction mixture under vacuum to a volume of 100 ml, added 50 ml of water, and acidified it with 10 ml of 0.1 N HCl to pH 7.0 – 7.5. We extracted the product with a mixture of chloroform and methanol, 4 : 1 (4 × 250 ml). We dried the extract over Na_2SO_4, filtered it, and acidified it with 1.5 ml of 0.7 N HCl in methanol. We evaporated the acidified solution to a volume of 100 ml, added 30 ml of propanol, again evaporated it to 30 ml, and precipitated the product by adding 150 ml of ether. We filtered out the precipitate, washed it on the filter with ether, and dried it under vacuum. We obtained 504 mg of V, $C_{26}H_{29}NO_{11}HCl$ (yield 80%), m. p. 201 – 203°C. $[\alpha]_D^{20}$ + 130°C (c 0.1, methanol), IR spectrum (KBr), ν_{max}, cm^{-1}: 1610, 3300 – 3600. UV spectrum (methanol): λ_{max}, nm ($E_{1\ cm}$, %) : 490 (182), 230 (462). PMR spectrum (ppm, CD_3OD): 7.78 (d, J = 7.6 Hz, 1H, H-1), 7.74 (dd, J = 8.5, 7.6 Hz, 1H, H-2), 7.28 (d, J = 8.5 Hz, 1H, H-1); 5.45 (m, 1H, H-1′), 5.09 (m, H, H-7), 4.29 (d, J = 6.6 Hz, 1H, H-5′), 3.95 (m, 1H, Ha-14), 3.78 (m, 1H, Hb-14), 3.68 (br. s, 1H, H-4′), 3.60 (m, H, H-13), 3.55 (m, 1H, H-3′), 3.05 and 3.02 (d, J = 18.7 Hz, 1H, H-10a), 2.81 and 2.78 (d, J = 18.7 Hz, 1H, H-10b), 2.29 and 2.48 (br. d, J = 14.9 Hz, 1H, He-8), 2 and 2.01 (dd, J = 14.9, 5.1 and 14.9 Hz, 1H, Ha-8, superposed on signals 2H-2′), 2 (br. d, 1H, Hb-2′, overlap with H-8), 1.92 (br. d, J = 12.5 Hz, 1H, Ha), 1.33 (d, J = 6.6 Hz, 3H, H-6′).

NMR ^{13}C spectrum (ppm, CD_3OD): 16.93 (C-6′), 29.41 (C-2′), 63.48 (C-14), 67.88 (C-4′), 67.99 (C-5′), 71.78 (C-7), 72.39 (C-9), 77.72 (C-13), 100.99 (C-1′), 111.19 (C-11a), 111.98 (C-5a), 120.43 (C-3), 120.44 (C-4a), 125.71 (C-1), 134.54 (C-6a), 136.07 (C-12a), 136.13 (C-10a), 138.33 (C-2), 157.84 (C-11), 158.87 (C-6), 163.48 (C-4), 187.22 (C-12), 191.59 (C-5), K_f = 0.47 (A) and 0.22 (B).

b. To a solution of 1.5 g (2.7 mmole) of the hydrochloride of I in a mixture of 40 ml of methanol and 20 ml of dioxane, we added 4 ml of the ethyl ester of orthoformic acid and then a solution of 0.18 ml (2.9 mmole) of bromine in 18 ml of dioxane. We monitored the course of the reaction with the aid of thin-layer chromatography in system B. After 50 min, we added 30 ml of 0.1 N HBr and held the mixture for 3 h at 37°C, controlling the course of hydrolysis of the ketal group by thin-layer chromatography in system A. After 3 h, we added 27 ml of a 10% solution of sodium formate and kept the reaction mixture in a thermostat at 37°C for 24 h. After this, we added a saturated solution of $NaHCO_3$ in an amount of 40 ml to pH 8.0 – 8.5 and introduced 76 mg of KBH_4 into the mixture in portions with stirring. After stirring for 4 h, we acidified the solution by adding 1 N HCl to a pH of 8.0 – 8.5, and extracted the product with a chloroform-methanol mixture (4 : 1) until the aqueous layer lost its color. We evaporated the organic extract under vacuum down to a volume of about 50 ml, added 50 ml of 0.1 N HCl; the product passes over into the aqueous layer. The aglycone and some of the impurities were separated by extraction with a chloroform-methanol mixture (4 : 1). We added 125 ml of butanol to the aqueous layer and removed all the water by azeotropic distillation under vacuum. We precipitated the product from the butanol solution by adding 100 ml of ether. The yield was 385 mg of V (yield 25.7%).

Preparation of 14-bromorubomycin (III). To a solution of 2.6 g (4.7 mmole) of the hydrochloride of I in a mixture of

TABLE 1. Antitumor Activity and Toxicity of Compounds V, I, and Doxorubicin in Treating Mice with Lymphadenosis NK/Li and Ehrlich Carcinoma

Index	Lymphadenosis NK/Li			Ehrlich carcinoma		
	V	I	doxorubicin	V	I	doxorubicin
ED_{10}	0.32	0.49 (0.38 – 0.63)	1.3 (0.9 – 1.88)	0.45	0.48 (0.38 – 0.60)	0.8 (0.55 – 1.04)
ED_{50}	0.76 (0.58 – 0.99)	0.90 (0.79 – 1.02)	2.3 (1.93 – 2.74)	0.76 (0.59 – 0.98)	0.64 (0.56 – 0.73)	1.75 (1.54 – 2.22)
ED_{90}	1.85	1.60 (1.24 – 2.06)	4.1 (2.83 – 5.95)	1.3	0.86 (0.68 – 1.09)	4.5 (3.28 – 6.17)
LD_{10}	1.45 (1.23 – 1.71)	0.77 (0.56 – 1.05)	3.3 (2.87 – 3.80)	1.25 (1.02 – 1.52)	1.05 (0.94 – 1.17)	3.4 (2.81 – 4.11)
LD_{50}	1.90 (1.77 – 2.04)	1.17 (1.02 – 1.35)	4.1 (3.73 – 4.51)	1.55 (1.38 – 1.74)	1.20 (1.13 – 1.27)	5.2 (4.56 – 5.93)
LD_{90}	2.45 (2.07 – 2.89)	1.75 (1.28 – 2.39)	5.2 (4.52 – 5.98)	1.95 (1.59 – 2.38)	1.43 (1.27 – 1.60)	8.2 (6.78 – 9.92)
Chemotherapeutic index LD_{50}/ED_{50}	2.5 (1.91 – 3.27)	1.3 (1.08 – 1.57)	1.8 (1.49 – 2.18)	2.0 (1.51 – 2.64)	1.8 (1.63 – 2.15)	2.8 (2.24 – 3.50)

TABLE 2. Antitumor Activity of Compounds V, I, and Doxorubicin in Treating Mice with Hemocytoblastosis La and Leukemia P-388

Drug	Dose		Mean lifespan of animals, % of control	
	in fractions of LD_{50}	in mg/kg	hemoblastosis La	leukemia P-388
V	0.3	1.1	160	137
	0.4	1.5	200	153
	0.5	1.8	236	174
	0.6	2.2	263	208
	0.7	2.6	258	217
	0.8	3.0	–	225
I	0.3	1.0	141	173
	0.4	1.4	153	251
	0.5	1.7	163	>287*
	0.6	2.1	148	>175*
	0.7	2.4	–	108
Doxorubicin	0.3	4.0	141	116
	0.4	5.4	174	138
	0.5	6.7	140	153
	0.6	8.1	85	174
	0.7	11.4	–	210

* 35% of the animals survived on the 90th day of observation.

40 ml of methanol and 20 ml of dioxane we added 7.5 ml of the ethyl ester of orthoformic acid and then 0.3 ml of bromine in 20 ml of dioxane. After 50 min, we added 150 ml of 0.1 N HCl to the mixture and held it at 40°C for 4 h. After this we distilled off the methanol from the reaction mixture under vacuum at not over 50°C, from the remaining solution we extracted the formed aglycones with chloroform (3 × 100 ml), and then extracted the hydrochloride of III from the aqueous layer with butanol (3 × 200 ml). We added 100 ml of water to the butanol solution and distilled the azeotropic water-butanol mixture under vacuum at 50°C up to complete removal of the water. About 50 ml of the hydrochloride of III crystallizes from the remaining butanol solution. We filtered it off, washed it on the filter with ether, and dried it under vacuum. We obtained 1.95 g (75%) of III, melting at 169 – 170°C.

Preparation of compounds V from III. To a solution of 1.9 g (3 mmole) of the hydrochloride of III in a mixture with 150 ml of water, 150 ml of methanol, and 300 ml of dioxane, we added 110 ml of a 0.2 N solution of Na_2CO_3 and held it in a nitrogen atmosphere for 45 min. After this, the solution containing 14-hydroxycarminomycin IV was acidified with 40 ml of 1 N HCl to pH 4.0 – 5.0 and evaporated to half of its initial volume. We filtered off the precipitate (mainly a mixture of aglycones), added 10 ml of a saturated solution of $NaHCO_3$ (pH 8.0) to the mother liquor, then 290 mg of KBH_4, and stirred the solution for 4 h. We extracted the resulting compound V with a mixture of chloroform and methanol 4 : 1 (4 × 250 ml). We dried the extract over Na_2SO_4, and added 5 ml of 0.6 N HCl in methanol to transform the base V into the hydrochloride, evaporated down to a volume of 50 ml, and precipitated the product by adding 100 ml of ether. We obtained 1.09 g of the hydrochloride of V (yield 56% based on the amount of the hydrochloride of III used).

BIOLOGICAL EXPERIMENTAL PART

The antitumor activity of compound V with intravenous administration was studied in comparison with compound I, and also with doxorubicin with respect to four tumors grafted to mice: lymphodenosis NK/Li, ascitic Ehrlich carcinoma, hemocytoblastosis La, and leukemia P-388. The experiments with lymphadenosis NK/Li and Ehrlich carcinoma were performed with nonpedigree white male mice; the hemocytoblastosis La experiments were done with male mice of the line $C_{57}B1/6$; and the experiments with leukemia P-388 were done with male mice BDF_1 ($C_{57}B1/6 \times DBA_2$). The mass of the animals was 18 – 20 g. In the experiments with lymphadenosis NK/Li and Ehrlich carcinoma, we injected the drugs four times at 48-h intervals. In the experiments with the remaining tumors, we injected the drug twice with an interval of 120 h. In all cases, the drug was introduced the first time 24 h after implantation of the tumor. We implanted lymphadenosis NK/Li, Ehrlich carcinoma, and leukemia P-388 by intraperitoneal injection of 9×10^6, 5×10^6, and 1×10^6 tumor cells in Hank's solution, respectively. We implanted hemocytoblastosis La intraperitoneally using 0.2 ml of a homogenate containing 5×10^6 spleen cells in 1 ml of Hank's solution for each animal.

The efficacy of the tested drugs in the experiments with leukemia P-388 and hemocytoblastosis La was assayed by the

change in the mean lifespan of the animals in comparison with the lifespan of the control animals in percent. The results of the experiments with lymphadenosis NK/Li and Ehrlich carcinoma were considered according to the inhibition of tumor growth in the treated animals in comparison with the control animals in percent. When working with these tumor models, we processed the experimental results using the probit analysis method of Litchfield and Wilcoxon [7]. We tested each drug dose in all the experiments on 10 mice. The control groups included 10 – 15 mice each, depending on the number of experimental groups.

Studying the antitumor activity of compound V with respect to lymphodenosis NK/Li showed that unlike the starting compound I, it exhibits considerable selectivity in the antitumor effect (Table 1). A comparative determination of the antitumor activity of compound V and I revealed that the difference between them is insignificant, but compound V is 1.6 (1.39 – 1.89) times less toxic than compound I. The value of the chemotherapeutic index LD_{50}/ED_{50} for I indicates that it exhibits practically no selectivity in the antitumor effect, whereas compound V not only exhibits a considerable selectivity in its antitumor effect, but is also superior to doxorubicin in its effect.

With respect to Ehrlich carcinoma (see Table 1), compound V exhibits an antitumor activity and toxicity virtually equal to that of compound I, and as a result exhibits equal selectivity in its antitumor effect. However, compound V is inferior to doxorubicin with respect to selectivity of the antitumor effect.

In the treatment of hemocytoblastosis La, the drug V is much superior to I and also to doxorubicin. For example, in doses of 0.5 and 0.6 of LD_{50}, the mean lifespan of mice treated with V is 236 and 263% of the control values, respectively. The mean lifespan of mice treated with compound I in the same doses is 163 and 148%, respectively. Consequently, even in a dose of 0.5 of LD_{50} (not inducing the maximum therapeutic effect), for compound V the mean lifespan of the animals increases by 73% in comparison with the starting compound I used in an equivalent dose. The maximum therapeutic effect for doxorubicin is observed in a dose of 0.4 of LD_{50} and is 174% of control (Table 2).

As regards leukemia P-388, drug V exhibits a considerable therapeutic effect virtually equal to that of doxorubicin, but the maximum therapeutic effect of V is observed in a dose considerably exceeding the maximum tolerated dose — 0.8 of LD_{50}. The maximum therapeutic effect of I, in turn, is observed in a dose of 0.5 of LD_{50} and it also exceeds the therapeutic effect of V (see Table 2).

Our results show drug V to exhibit high antitumor activity and a broad spectrum of antitumor effects. It should be noted that this modification of molecule I changes the range of the antitumor effect of the antibiotic, and results in considerable selectivity of the antitumor effect with respect to lymphadenosis NK/Li that is not typical of the starting compound I. It exhibits a therapeutic effect with respect to hemocytoblastosis La greater than that of compound I and doxorubicin, and also an effect equal to that of doxorubicin (although inferior to I) with respect to leukemia P-388.

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