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HEMOLYTIC AND CYTOTOXIC ACTIVITY OF DAMMARANE-TYPE TRITERPENOIDS

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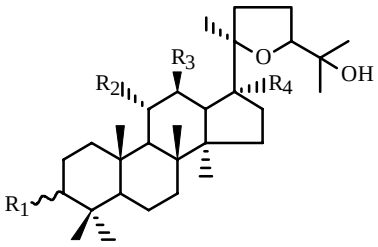
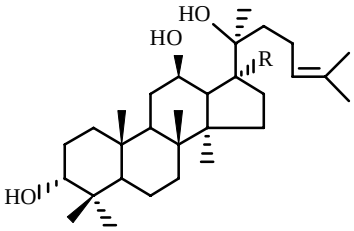
The relationship between chemical structure and hemolytic and cytotoxic activity was studied for dammarane-type triterpenoids isolated from leaves of various Betula *species. It was shown that an acyclic sidechain (betulafolienetriol and betulafolienetetraol) imparted significantly higher hemolytic and cytotoxic activity than a cyclic sidechain. The activity of epoxydammaranetriol with an 11α-OH group was slightly higher than that of epoxydammaranetriol with a 12β-OH group. The activity of C-3 epimeric epoxydammaranetriols and -tetraols with a 12β-OH group was independent of the configuration of the C-3 hydroxyl. Epoxydammaranetriols with an 11α-OH group and epoxydammaranediols with a 3α-OH group were more active than those with a 3β-OH group. The effect of the most active compounds on the microviscosity of tumor-cell membranes was determined.*

Key words: dammarane-type triterpenoids, erythrocytes, tumor cells, hemolytics, cytostatics, membrane microviscosity.

Dammarane-type triterpenoids isolated from various *Betula* species are interesting because their structures are similar to the aglycons of ginseng glycosides. This makes dammarane triterpenoids useful as starting material for the synthesis of ginsenoside analogs [1]. It was shown earlier that these triterpenoids possess hemolytic [2, 3] and cytotoxic activity toward tumor cells [4-6] and inhibit multiplication of Gram-positive bacteria [2]. Betulafolienetetraol (**8**) modifies the structure—activity properties of erythrocyte and model membranes [7].



1, 2, 4 - 7, 9, 10



3, 8

1, 4, 6, 9: R_1 =  **2, 5, 7, 10:** R_1 = 

3: R = H
8: R = OH

1, 2: $R_2 = R_3 = R_4 = H$; **4, 5:** $R_2 = R_4 = H$, $R_3 = OH$
6, 7: $R_2 = OH$, $R_3 = R_4 = H$; **9, 10:** $R_2 = H$, $R_3 = R_4 = OH$

*Deceased.

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TABLE 1. Hemolytic and Cytotoxic Activity of **1**-**10**

Compound	HC_{50}, μg/mL*					IC_{50}, μg/mL**
	37°C			42°C		
	pH 7.4	pH 6.5	pH 5.5	pH 7.4	pH 6.5	
20(*S*),24(*R*)-Epoxydammaran-3α,25-diol (epiocotillol) (**1**)	>100	36.0±5.5	12.0±1.3	12.0±4.5	6.0±2.4	12.0±3.5
20(*S*),24(*R*)-Epoxydammaran-3β,25-diol (ocotillol) (**2**)	>100	>100	>100	>100	50.0±10.5	50.0±5.0
Dammar-24-en-3α,12β,20(*S*)-triol (betulafolienetriol) (**3**)	>100	45.0±0.1	3.0±5.5	20.0±3.8	3.0±1.7	6.0±1.5
20(*S*),24(*R*)-Epoxydammaran-3α,12β,25-triol (betulafolienetriol oxide) (**4**)	>100	>100	30.0±3.7	80.0±7.5	30.0±7.5	20.0±4.0
20(*S*),24(*R*)-Epoxydammaran-3β,12β,25-triol (pyxinol) (**5**)	>100	>100	35.0±4.5	70.0±8.0	30.0±5.5	20.0±4.5
20(*S*),24(*R*)-Epoxydammaran-3α,11α,25-triol (**6**)	>100	>100	15.0±2.5	40.0±6.4	20.0±4.6	12.0±3.5
20(*S*),24(*R*)-Epoxydammaran-3β,11α-25-triol (**7**)	>100	>100	30.0±2.0	50.0±4.6	40.0±8.5	16.0±2.6
Dammar-24-en-3α,12β,17α,20(*S*)-tetraol (betulafolienetetraol) (**8**)	50±6.3	12.0±1.8	2.0±0.3	6.5±2.2	0.50±0.05	1.5±0.5
20(*S*),24(*R*)-Epoxydammaran-3α,12β,17α,25-tetraol (betulafolienetetraol oxide) (**9**)	>100	>100	43.0±6.5	>100	45.0±6.8	25.0±5.0
20(*S*),24(*R*)-Epoxydammaran-3β,12β,17α,25-tetraol (**10**)	>100	>100	40.0±6.0	>100	50.0±7.5	25.0±5.0
Cauloside C				15.0±4.2	6.0±2.0	

*Concentration causing 50% hemolysis in 30 min, **concentration causing 50% lysis of tumor cells. Data from three determinations are given.

TABLE 2. Effect of **3** and **8** on Tumor-Cell Membrane Viscosity (pH 7.4)

Compound	μmol	I_{480}/I_{394}	%
Control		0.70±0.03	100
Betulafolienetriol (**3**)	20.0	0.58±0.02	72
	40.0	0.42±0.04	60
Betulafolienetetraol (**8**)	20.0	0.55±0.03	78
	40.0	0.38±0.02	54
Cauloside C	30.0	0.65±0.02	82

Data from three determinations are given.

The present work continues the study of the structure—activity relationship of this class of compounds. We investigated the hemolytic and cytotoxic activity of ten dammarane-type triterpenoids as a function of chemical structure and the effect of the most active compounds on the microviscosity of tumor-cell membranes. Tables 1 and 2, respectively, give the results.

We found previously that the membrane activity of certain dammarane-type triterpenoids was a function of pH and medium temperature [3, 8]. Table 1 shows that the temperature and pH also have a substantial influence on the membrane activity for all studied compounds. At pH 7.4 and 37°C, only betulafolienetetraol (**8**) had moderate hemolytic activity. The other compounds did not exhibit hemolytic activity at concentrations up to 100 μg/mL. Shifting the reaction medium to the weakly acid region and increasing the temperature increased the hemolytic activity of the studied compounds by several times. Triterpenoids **3** and **8**, which have an open sidechain, were most active. The activity of tetraol **8** was four times greater than that of triol **3**. The hemolytic activity of **3** (pH 6.5, 42°C) was ten times greater than that of its analog **4** with a closed sidechain. The activity of **8** was two orders of magnitude greater than that of the corresponding analog **9**. Epiocotillol (**1**) is a triterpenoid

with a tetrahydrofuran in the sidechain and an activity similar to that of **3**. The remaining compounds exhibited moderate hemolytic activity either at pH 5.5 (37°C) or at elevated temperature (42°C).

A comparison of the C-3 epimers of epoxydammaranetriols **4** and **5** with epoxydammaranetetraols **9** and **10** with 12β-OH groups showed that the configuration of the C-3 hydroxyl had no noticeable effect on the hemolytic and cytotoxic activity. Because the HC_{50} and IC_{50} values for these pairs of epimers were similar, we determined the hemolysis kinetics, which turned out also to be similar. The hemolysis delay times for **4** and **5** (25.0 μg/mL, pH 5.5, 42°C) were 130 and 100 s; the hemolysis rates, 0.08 and 0.06 s^{-1}, respectively. The configuration of the C-3 hydroxyl in **9** and **10**, like for **4** and **5**, also had little effect on the hemolysis kinetic parameters (data not given). On the other hand, the activity of the epoxydammaranetriol epimers with an 11α-OH group, i.e., **6** and **7**, and epoxydammaranediols **1** and **2** did depend on the configuration of the C-3 hydroxyl. Compounds with a 3α-OH group were more active than the analogs with a 3β-OH group. The activity of **6** and **7** with an 11α-OH group was slightly greater than that of **4**, **5**, **9**, and **10** with a 12β-OH group.

Compounds **1**-**10** exhibited cytotoxic activity for tumor cells in vitro. Table 1 shows that the cytotoxic and hemolytic activities of the triterpenoids were directly correlated. Of the studied compounds, **3** and **8** had the highest cytotoxic activity.

It was demonstrated earlier that the biological activity of dammarane triterpenoids is based on their ability of modify membrane properties [9, 10]. We measured the membrane microviscosity of tumor cells treated with the most active compounds, **3** and **8** (Table 2). These compounds (**3** and **8**, 20 μmol) reduced the degree of excimerization of pyrene incorporated into tumor-cell membranes. At concentrations of 40 μmol, the degree of pyrene excimerization was even less, 40 and 46%, respectively. Increasing the concentrations of **3** and **8** further increased the overall microviscosity of the bilayer due to saturation of the membrane with triterpenoids. Besides this, it was found that the membrane microviscosity of tumor cells treated with **3** and **8** was practically independent of the reaction medium. Thus, the degree of pyrene excimerization was insignificantly lower at pH 6.5 and 5.5 than at pH 7.4 (data not given).

Thus, the results indicated that dammarane-type triterpenoids may be interesting for further studies of their use in antitumor chemotherapy.

EXPERIMENTAL

Compounds **1**-**10** were isolated from the unsaponified part of the ether extract of various *Betula* species as previously described [11-16].

Determination of Hemolytic Activity. Hemolytic activity was determined by the literature method [9]. A suspension of erythrocytes with optical density 1.0 at 700 nm in phosphate buffer (66 mM, pH 7.4, 6.5, and 5.5) was used. An alcohol solution (0.02 mL) of the test compound was mixed with erythrocyte suspension (1.98 mL) and incubated at 37 or 42°C for 30 min. After incubation, the optical density of the suspension was measured at 700 nm on a Specord UV—Vis Specord spectrophotometer. We determined the concentration of the compound that caused 50% lysis (HC_{50}) of the erythrocytes. The triterpene glycoside cauloside C isolated from *Caulophyllum robustum* Maxim. was used as a positive control [17].

Determination of Cytotoxic Activity. Ethanol solutions of the tested compounds (1 μL of various concentrations) were added to Ehrlich carcinoma ascites cells (EAC, 0.1 mL) in medium 199 containing bovine serum (10%, 0.7×10^6 cells/mL) in order to determine the cytotoxic activity. The mixtures were incubated at 37°C for 24 h. After incubation, the vitality of EAC cells was estimated from staining by a trypan blue solution (0.17%) using a microscope. Table 1 gives the concentration causing 50% lysis of the tumor cells (IC_{50}) [6].

Measurement of Membrane Microviscosity. The microviscosity of EAC was estimated by spectrophotometry using the degree of pyrene excimerization I_{480}/I_{394} [18]. Tumor cells (5×10^6 cells/mL) in medium (NaCl, 120.0 mmol; KCl, 6.9; Na_2HPO_4, 5.0; glucose, 5.5; $CaCl_2$, 1.0; Tris, 10.0; pH 7.4, 6.5, and 5.5) were treated successively with pyrene in alcohol (1 μmol) and the tested compounds at various concentrations and incubated at 37°C for 5 min. Fluorescence spectra were recorded on a M 850 spectrofluorimeter (Hitachi, Japan). Table 2 gives the membrane microviscosity expressed as pyrene excimerization I_{480}/I_{394} and in percent of the control, which was taken as 100%.

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