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Isolation and Structures of Erylosides from the Caribbean Sponge *Erylus goffrilleri*

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Eight new triterpene glycosides, erylosides R (**1**), S (**2**), T (**3**), U (**4**), F<sub>5</sub>–F<sub>7</sub> (**5**–**7**), and V (**8**), were isolated from the sponge *Erylus goffrilleri* collected near Arresife-Seko Reef (Cuba). Structures of **1** and **2** were determined as the corresponding monosides having aglycons related to penasterol with additional oxidation and methylation patterns in their side chains. Eryloside T (**3**) was structurally identified as the  $\Delta^7$ -isomer of **1**, containing an unusual (14 $\rightarrow$ 9)-lactone ring in the tetracyclic aglycon moiety, and eryloside U (**4**) was shown to be the 7,8-epoxide of **3**. Erylosides F<sub>5</sub>–F<sub>7</sub> (**5**–**7**) and V (**8**) contain new variants of carbohydrate chains with two (**5**–**7**) and three (**8**) sugar units, respectively.

Marine sponges of the genus *Erylus* (order Astrophorida, family Geodidae) are a source of various saponins, erylosides, belonging to the steroidal or triterpenoid series. Erylosides derived from 4 $\alpha$ -methyl-5 $\alpha$ -cholesta-8,14-dien-3 $\beta$ -ol or a related nortriterpenoid were isolated from *Erylus lendenfeldi*,<sup>1–3</sup> and glycosides derived from penasterol or its congeners were described from *E. formosus*,<sup>4–7</sup> *E. nobilis*,<sup>8,9</sup> *E. goffrilleri*,<sup>10</sup> and *Erylus* sp.<sup>11</sup> Recently, two steroid glycosides, sokodiosides A and B, with a novel carbon skeleton were isolated from *E. placenta*.<sup>12</sup> Erylosides from *Erylus* spp. have been reported to exhibit a wide spectrum of biological activities. For example, eryloside F was discovered to possess selective thrombin receptor antagonist activity and functional activity in a platelet aggregation assay.<sup>5</sup> Eryloside E exhibits immunosuppressive activity,<sup>10</sup> and nobiloside inhibits neuraminidase from the bacterium *Clostridium perfringens*.<sup>9</sup> Erylosides A, K, and L from *E. lendenfeldi*<sup>1–3</sup> and sokodiosides A and B from *E. placenta*<sup>12</sup> possessed antitumor and antifungal properties.

In continuation of our studies on glycosides from marine sponges,<sup>7,13</sup> we have isolated a series of new erylosides (**1**–**8**) from the ethanolic extract of the Caribbean sponge *Erylus goffrilleri*. We report herein the isolation and structural elucidation of eight new glycosides.

## Results and Discussion

The ethanolic extract of the sponge was separated by low-pressure reversed-phase column chromatography on Teflon powder Polycrome-1 followed by Si gel flash column chromatography and by several rounds of reversed-phase HPLC to yield individual glycosides **1**–**8** as colorless, amorphous solids.

The molecular formula of eryloside R (**1**) was determined as C<sub>38</sub>H<sub>64</sub>O<sub>9</sub> by a pseudomolecular ion at  $m/z$  687.4483 [M + Na]<sup>+</sup> in HRMALDIMS and by <sup>13</sup>C NMR analyses. A close inspection of the <sup>1</sup>H and <sup>13</sup>C NMR data (Tables 1, 4, and 5) of **1** by DEPT and HSQC revealed the presence of nine methyls; 11 methylenes, including one oxygen-bearing methylene; six oxygenated methines, including one methine linked to an anomeric carbon; and three tertiary and five saturated quaternary carbons. The remaining functionality, corresponding to the carbon signals at  $\delta$  178.2 (C), 139.8 (C), 127.9 (C), and 75.2 (C), suggested the presence of a carbonyl or carboxyl carbon, one tetrasubstituted double bond, and

**Table 1.** <sup>1</sup>H and <sup>13</sup>C NMR Data for the Carbohydrate Moieties of Erylosides **1**–**4**<sup>a</sup> in C<sub>5</sub>D<sub>5</sub>N

position	$\delta_C$	$\delta_H$ (J in Hz)	HMBC	NOESY
Gal (1 $\rightarrow$ C-3)				
1	107.4 CH	4.78 d (7.7)	C-3, C3-Gal	H-3, 28, H3,5-Gal
2	72.9 CH	4.44 dd (7.7, 9.5)	C1,3-Gal	
3	75.2 CH	4.16 dd (3.4, 9.5)	C2-Gal	H1-Gal
4	70.1 CH	4.60 brd (3.4)	C2,3-Gal	
5	76.5 CH	4.12 brt (6.0)	C1,4,6-Gal	H1-Gal
6	62.3 CH <sub>2</sub>	a: 4.47 dd (6.1, 10.9) b: 4.51 dd (6.0, 10.9)	C4,5-Gal C5-Gal	

<sup>a</sup> All assignment were given for **1**; spectra of **2**–**4** had only minor differences in chemical shift values.

one tertiary alcohol function. The IR spectrum of **1** exhibited a characteristic band attributable to a carboxyl (1687 cm<sup>–1</sup>) group. Interpretation of the COSY data gave rise to spin systems involving one anomeric proton, four oxymethines, and protons of a hydroxymethyl group. On the basis of these data, a triterpene monoside with a tetracyclic aglycon structure was suggested for **1**.

The correlation observed in the COSY and HSQC spectra of the aglycon part of **1** indicated the presence of the following distinct spin systems: –CHOH–CH<sub>2</sub>–CH<sub>2</sub>–(C-3–C-1), >CH–CH<sub>2</sub>–CH<sub>2</sub>–(C-5–C-7), CH<sub>2</sub>–CH<sub>2</sub>–(C-11–C-12), >CH–CH<sub>2</sub>–CH<sub>2</sub>–(C-17–C-15). These partial structures were further connected to each other by HMBC correlations: H<sub>3</sub>-19/C-1, C-5, C-9, and C-10; H<sub>3</sub>-28/C-3, C-4, C-5, and C-29; H<sub>3</sub>-29/C-3, C-4, C-5, and C-28; H-6/C-8 and C-10; H-12/C-9, C-13, C-14, and C-18; H<sub>3</sub>-18/C-12, C-13, C-14, and C-17; and H-15/C-8, C-13, and C-14. The position of a double bond at  $\Delta^{8,9}$  was evident from the long-range correlation of H<sub>3</sub>-19 with C-9 at 139.8. The chemical shift of C-14 at  $\delta$  62.7 and HMBC correlations of H<sub>2</sub>-15 ( $\delta$  1.69, 2.48) with C-30 ( $\delta$  178.2) indicated the attachment of a carboxyl function at C-14. The COSY and HMBC data allowed the assignment of the signal at  $\delta$  88.3 (C-3) to a secondary oxygen-bearing carbon, adjacent to a quaternary sp<sup>3</sup> carbon. This information together with the observed NOE correlations H<sub>3</sub>-19/H-2 $\beta$  ( $\delta$  1.90), H-6 $\beta$  ( $\delta$  1.55), and H-11 $\beta$  ( $\delta$  2.16), H-3/H-5 ( $\delta$  1.28) and H-28 ( $\delta$  1.24), H-6 $\alpha$ /H-28, H-6 $\beta$ /H-29 ( $\delta$  1.03), and H-12 $\alpha$ /H-17 ( $\delta$  2.07) indicated that **1** contained a 14-carboxylanost-8(9)-ene skeleton. In addition, the long-range COSY correlation between H<sub>3</sub>-19 and H-1 $\alpha$  and between H<sub>3</sub>-18 and H-12 $\alpha$ , H-17 confirmed this conclusion. The relative stereochemistry of the proton at C-3 was defined on the basis of the <sup>1</sup>H–<sup>1</sup>H coupling constants ( $J$  = 4.3, 11.8 Hz) observed between H-3 and H-2 $\alpha,\beta$  and assigned as axial. HMBC correlations of

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**Table 2.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for the Carbohydrate Moieties of Erylosides **5** and **6**<sup>a</sup> in  $\text{C}_5\text{D}_5\text{N}$ 

position	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	HMBC	NOESY
Gal (1 $\rightarrow$ C-3)				
1	104.8 CH	4.78 d (7.7)	C-3	H-3, H3, 5-Gal
2	80.8 CH	4.52 dd (7.7, 9.5)	1,3-Gal, 1-NAc-Glc	1-NAc-Glc
3	75.9 CH	4.18 dd (3.3, 9.5)		H1,5-Gal
4	69.8 CH	4.57 brd (3.5)	C2,3-Gal	H6a-Gal
5	76.2 CH	4.07 brt (6.2)	C4,6-Gal	H1-Gal
6	61.9 CH <sub>2</sub>	a: 4.43 dd (5.8, 9.9) b: 4.47 dd (6.3, 10.9)	C4,5-Gal	H4-Gal
NAc-Glc (1 $\rightarrow$ 2Gal)				
1	102.8 CH	5.39 d (8.3)	C2-Gal	H3,5-NAc-Glc, H2-Gal
2	59.3 CH	4.36 m	C3-NAc-Glc	
3	78.0 CH	4.13 t (9.5)	C2,4-NAc-Glc	H1,5-NAc-Glc
4	72.5 CH	4.17 t (9.2)	C3,6-NAc-Glc	
5	77.4 CH	3.60 m		H1,3-Nac-Glc
6	62.9 CH <sub>2</sub>	a: 4.29 dd (5.0, 11.4) b: 4.37 dd (3.2, 11.5)	C4,5-NAc-Glc C4-NAc-Glc	
NH		9.12 d (6.0)		
Ac	172.5 C 23.0 CH <sub>3</sub>	2.12 s		

<sup>a</sup> All assignment were given for **5**; spectra of **6** had only minor differences in chemical shift values.**Table 3.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data for the Carbohydrate Moieties of Erylosides **7** and **8** in  $\text{C}_5\text{D}_5\text{N}$ 

7					8				
position	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	NOESY	HBMC	position	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	NOESY	HBMC
Gal (1 $\rightarrow$ C-3)					Ara (1 $\rightarrow$ C-3)				
1	106.9 CH	4.79 d (7.7)	H-3, H3,5-Gal	C-3	1	105.0 CH	4.82 d (6.5)	H-3, H3,5a-Ara	C3,5-Ara
2	71.9 CH	4.62 dd (7.7, 9.6)		C1,3-Gal	2	77.4 CH	4.74 dd (6.5, 8.4)	H1-Gal	C1-Gal, C1,3-Ara
2	71.9 CH	4.62 dd (7.7, 9.6)		C1,3-Gal	2	77.4 CH	4.74 dd (6.5, 8.4)	H1-Gal	C1-Gal, C1,3-Ara
3	85.0 CH	4.26 dd (3.6, 9.6)	H1-Glc	C2-Gal	3	82.2 CH	4.28 dd (2.8, 8.7)	H1,5a-Ara, H1-Xyl	C2,4-Ara, C1-Xyl
4	69.7 CH	4.76 d (3.7)		C2,3Gal	4	68.5 CH	4.46 t (8.6)		
5	76.3 CH	4.07 brt (6.0)	H1-Gal	C1,4,6-Gal	5	65.4 CH <sub>2</sub>	a: 3.77 dd (2.1, 12.4) b: 4.28 m	H1,3-Ara	C4-Ara
6	62.2 CH <sub>2</sub>	a: 4.38 dd (6.0, 11.0) b: 4.42 dd (6.2, 11.0)		C4-Gal C4,5-Gal					
Glc (1 $\rightarrow$ 3) Gal					Xyl (1 $\rightarrow$ 3Ara)				
1	106.4 CH	5.40 d (7.8)	H3-Gal, H3,5-Glc	C3-Gal, C5-Glc	1	105.1 CH	5.17 d (7.5)	H3,5-Xyl, H3-Ara	C3-Xyl, C3-Ara
2	75.6 CH	4.04 dd (8.0, 9.0)		C1,3-Glc	2	74.8 CH	3.96 t (7.8)		C1,3-Xyl
3	78.2 CH	4.25 m	H1-Glc	C1,2,4-Glc	3	78.0 CH	4.10 t (8.7)	H1-Xyl	C2,4-Xyl
4	71.3 CH	4.25 m		C2,3,5-Glc	4	70.7 CH	4.17 dd (5.2, 9.8)		C2,3,5-Xyl
5	78.4 CH	3.97 m	H1-Glc		5	66.9 CH <sub>2</sub>	a: 3.64 dd (9.8, 11.1) b: 4.28 dd (5.2, 11.3)	H1-Xyl	C3,4-Xyl
6	62.4 CH <sub>2</sub>	a: 4.39 dd (6.0, 12.0) b: 4.51 dd (2.5, 11.8)							C3,4-Xyl
					Gal (1 $\rightarrow$ 2Ara)				
					1	104.9 CH	5.35 d (7.8)	H3,5-Gal, H2-Ara	C2-Ara
					2	73.3 CH	4.47 m		C1,3-Gal
					3	75.2 CH	4.12 dd (3.7, 9.5)	H1,5-Gal	C2-Gal
					4	69.4 CH	4.61 brd (3.7)		C2,3-Gal
					5	76.1 CH	3.81 brt (6.3)	H1,3-Gal <sub>1</sub>	C4,6-Gal
					6	61.1 CH <sub>2</sub>	4.28 m 4.48 m		C4,5-Gal

H<sub>3</sub>-21 ( $\delta$  1.10) with C-20 ( $\delta$  37.3) and C-17 ( $\delta$  51.7) established attachment of the side chain at C-17. COSY correlation between H-20 ( $\delta$  1.55), H<sub>2</sub>-22 ( $\delta$  1.20, 2.06), and H<sub>2</sub>-23 ( $\delta$  1.70, 1.76) along with HMBC correlations from H<sub>3</sub>-31 ( $\delta$  1.26) to C-23 ( $\delta$  33.4), C-24 ( $\delta$  75.2), and C-25 ( $\delta$  38.5), from the nine proton resonances observed for H-26, H-27, and H-32 ( $\delta$  1.14) to both C-24 and C-25, firmly established the structure of the side chain, including the location of the tertiary alcohol and *tert*-butyl group at C-24. NOESY

correlations H<sub>3</sub>-18/H-20 and H-12 $\beta$ /H<sub>3</sub>-21 determined the 20*R*\* configuration for C-20. Thus, eryloside R (**1**) was defined as a triterpenoid monoside possessing a modified pentasterol aglycone that was previously found in eryloside E, a bioactive constituent of *E. goffrilleri* collected from the Bahama Islands.<sup>10</sup>

The  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra of the sugar moieties of erylosides R, S, T, and U (**1–4**) showed a close similarity of all proton and carbon chemical shifts and proton multiplicities (Table 1). The acid

**Table 4.**  $^{13}\text{C}$  NMR Data for the Aglycon Moiety of Erylosides (**1–8**) in  $\text{C}_5\text{D}_5\text{N}$ 

position	1	2	3	4	5	6	7	8
1	35.5 $\text{CH}_2$	35.3 $\text{CH}_2$	30.2 $\text{CH}_2$	30.7 $\text{CH}_2$	35.3 $\text{CH}_2$	35.2 $\text{CH}_2$	35.3 $\text{CH}_2$	35.3 $\text{CH}_2$
2	27.0 $\text{CH}_2$	27.1 $\text{CH}_2$	26.0 $\text{CH}_2$	25.7 $\text{CH}_2$	27.0 $\text{CH}_2$	27.0 $\text{CH}_2$	27.0 $\text{CH}_2$	27.0 $\text{CH}_2$
3	88.3 CH	88.3 CH	88.2CH	88.0 CH	88.8 CH	88.7 CH	88.4 CH	88.4 CH
4	39.4 C	39.4 C	38.8 C	38.9 C	39.5 C	39.5 C	39.4 C	39.6 C
5	50.4 CH	50.3 CH	47.0 CH	42.6 CH	50.3CH	50.2 CH	50.2 CH	50.2 CH
6	18.3 $\text{CH}_2$	18.4 $\text{CH}_2$	21.7 $\text{CH}_2$	20.8 $\text{CH}_2$	18.4 $\text{CH}_2$	18.4 $\text{CH}_2$	18.4 $\text{CH}_2$	18.4 $\text{CH}_2$
7	27.9 $\text{CH}_2$	27.9 $\text{CH}_2$	120.2 CH	54.0 CH	27.9 $\text{CH}_2$	27.9 $\text{CH}_2$	27.9 CH	27.9 CH
8	127.9 C	127.9 C	140.2 C	64.4 C	127.9 C	127.9 C	127.9 $\text{CH}_2$	127.9 $\text{CH}_2$
9	139.8 C	139.9 C	88.6 C	85.8 C	139.9 C	139.9 C	139.9 C	139.9 C
10	37.4 C	37.4 C	38.1 C	37.3 C	37.3 C	37.3 C	37.3 C	37.3 C
11	22.5 $\text{CH}_2$	22.5 $\text{CH}_2$	25.9 $\text{CH}_2$	22.8 $\text{CH}_2$	22.5 $\text{CH}_2$	22.4 $\text{CH}_2$	22.5 $\text{CH}_2$	22.5 $\text{CH}_2$
12	31.7 $\text{CH}_2$	31.6 $\text{CH}_2$	34.3 $\text{CH}_2$	34.3 $\text{CH}_2$	31.8 $\text{CH}_2$	31.6 $\text{CH}_2$	31.7 $\text{CH}_2$	31.7 $\text{CH}_2$
13	46.9.0 C	46.9 C	45.9 C	46.7 C	46.9 C	46.9 C	46.9 C	46.9 C
14	62.7 C	62.8 C	65.1 C	59.6 C	62.8 C	62.8 C	62.7 C	62.8 C
15	28.3 $\text{CH}_2$	28.4 $\text{CH}_2$	22.2 $\text{CH}_2$	20.3 $\text{CH}_2$	28.3 $\text{CH}_2$	28.4 $\text{CH}_2$	28.3 $\text{CH}_2$	28.3 $\text{CH}_2$
16	29.6 $\text{CH}_2$	29.6 $\text{CH}_2$	28.0 $\text{CH}_2$	27.8 $\text{CH}_2$	29.5 $\text{CH}_2$	29.6 $\text{CH}_2$	29.5 $\text{CH}_2$	29.5 $\text{CH}_2$
17	51.7 CH	51.0 CH	53.0 CH	53.0 CH	51.6 CH	51.0 CH	51.5 CH	51.6 CH
18	18.0 $\text{CH}_3$	17.8 $\text{CH}_3$	14.0 $\text{CH}_3$	14.0 $\text{CH}_3$	17.9 $\text{CH}_3$	17.9 $\text{CH}_3$	18.0 $\text{CH}_3$	18.0 $\text{CH}_3$
19	19.7 $\text{CH}_3$	19.7 $\text{CH}_3$	16.9 $\text{CH}_3$	16.2 $\text{CH}_3$	19.5 $\text{CH}_3$	19.5 $\text{CH}_3$	19.5 $\text{CH}_3$	19.6 $\text{CH}_3$
20	37.3 CH	36.9 CH	36.5 CH	35.7 CH	37.4 CH	36.8 CH	37.4 CH	37.3 CH
21	18.8 $\text{CH}_3$	18.7 $\text{CH}_3$	18.5 $\text{CH}_3$	18.3 $\text{CH}_3$	18.8 $\text{CH}_3$	18.7 $\text{CH}_3$	18.8 $\text{CH}_3$	18.8 $\text{CH}_3$
22	30.4 $\text{CH}_2$	29.5 $\text{CH}_2$	30.1 $\text{CH}_2$	30.1 $\text{CH}_2$	30.4 $\text{CH}_2$	29.5 $\text{CH}_2$	30.4 $\text{CH}_2$	30.3 $\text{CH}_2$
23	33.4 $\text{CH}_2$	32.6 $\text{CH}_2$	33.1 $\text{CH}_2$	33.1 $\text{CH}_2$	33.5 $\text{CH}_2$	32.5 $\text{CH}_2$	33.5 $\text{CH}_2$	33.4 $\text{CH}_2$
24	75.2 C	87.4 C	75.1 C	75.2 C	75.3 C	87.4 C	75.2 C	75.3 C
25	38.5 C	34.0 CH	38.5 C	38.5 C	38.5 CH	34.0 CH	38.5 C	38.5 C
26	25.7 $\text{CH}_3$	17.1 $\text{CH}_3$	25.7 $\text{CH}_3$	25.7 $\text{CH}_3$	25.7 $\text{CH}_3$	17.1 $\text{CH}_3$	25.7 $\text{CH}_3$	25.7 $\text{CH}_3$
27	25.7 $\text{CH}_3$	16.9 $\text{CH}_3$	25.7 $\text{CH}_3$	25.7 $\text{CH}_3$	25.7 $\text{CH}_3$	16.9 $\text{CH}_3$	25.7 $\text{CH}_3$	25.7 $\text{CH}_3$
28	27.7 $\text{CH}_3$	27.6 $\text{CH}_3$	28.8 $\text{CH}_3$	28.1 $\text{CH}_3$	27.6 $\text{CH}_3$	27.5 $\text{CH}_3$	27.5 $\text{CH}_3$	27.4 $\text{CH}_3$
29	16.7 $\text{CH}_3$	16.6 $\text{CH}_3$	17.5 $\text{CH}_3$	16.6 $\text{CH}_3$	16.4 $\text{CH}_3$	16.4 $\text{CH}_3$	16.6 $\text{CH}_3$	16.4 $\text{CH}_3$
30	178.2 C	178.2 C	178.5 C	176.8 C	178.3 C	178.2 C	178.3 C	178.3 C
31	21.0 $\text{CH}_3$	19.8 $\text{CH}_3$	21.0 $\text{CH}_3$	21.0 $\text{CH}_3$	21.0 $\text{CH}_3$	19.7 $\text{CH}_3$	21.0 $\text{CH}_3$	20.9 $\text{CH}_3$
32	25.7 $\text{CH}_3$		25.7 $\text{CH}_3$	25.7 $\text{CH}_3$	25.7 $\text{CH}_3$		25.7 $\text{CH}_3$	25.7 $\text{CH}_3$
Ac		170.0 C 21.8 $\text{CH}_3$				169.8 C 21.9 $\text{CH}_3$		

hydrolysis of the sum of these glycosides gave D-galactose as the only sugar that was identified by GLC of the corresponding acetylated (–)-2-octyl glycoside, using authentic samples prepared from D- and L-galactose.<sup>14</sup>

A comparison of the  $^{13}\text{C}$  NMR spectra of **1–4** with published data for  $\alpha$ - and  $\beta$ -D-galactopyranosides, together with the magnitudes of  $^1\text{H}$ – $^1\text{H}$  spin coupling constants and NOE data, elucidated the presence of a  $\beta$ -D-galactopyranoside unit of C1 form in **1–4**.<sup>15–17</sup>

A long-range correlation H1-Gal ( $\delta$  4.78)/C-3 ( $\delta$  88.3) in the HMBC spectra of **1–4** and downfield chemical shift of C-3 ( $\delta$  88.3) revealed a linkage between H1-Gal and C-3 of the aglycon. This interpretation was confirmed by a strong NOESY cross-peak from H1-Gal to H-3. On the basis of the above data, the structure of eryloside R was established as 3-O-( $\beta$ -D-galactopyranosyl)-14-carboxy-24,25-dimethylstanost-8(9)-en-3 $\beta$ ,24-diol (**1**).

HRMALDIMS eryloside S (**2**) gave a quasimolecular ion at  $m/z$  715.4351  $[\text{M} + \text{Na}]^+$ . These data, coupled with  $^{13}\text{C}$  NMR spectral data, established the molecular formula of **2** as  $\text{C}_{39}\text{H}_{64}\text{O}_{10}$ . The general features of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Tables 4, 5) of the aglycon part of **2** closely resemble those of eryloside R (**1**), with the exception of proton and carbon signals belonging to the side chain. The difference in the NMR spectra was the appearance of the signals of the acetoxy group ( $\delta$  2.01 s, 170.0 C, 21.8  $\text{CH}_3$ ) and downfield chemical shift of a quaternary oxygenated carbon (C-24,  $\delta$  87.4). Furthermore, the interpretation of the COSY and HSQC data revealed two additional isolated spin systems:  $>\text{CH}-\text{CH}_3$  (C-25–C-26, C-27). This information and a COSY correlation from H-20 ( $\delta$  1.52), H-22 ( $\delta$  1.05, 1.30) to H-23 ( $\delta$  1.97, 2.04) along with HMBC correlations from H<sub>3</sub>-31 ( $\delta$  1.37) to C-23 ( $\delta$  32.6), C-24 ( $\delta$  87.4), and C-25 ( $\delta$  34.0), from the doublet signals ( $J = 7.0$  Hz for each) observed for H-26 ( $\delta$  0.85) and H-27 ( $\delta$  0.92) to both C-24 and C-25, established the structure of the side chain, including the C-24 tertiary acetoxy group. Thus, the structure of eryloside S (**2**) was determined as 3-O-( $\beta$ -D-galactopyranosyl)-14-carboxy-24-acetoxy-24-methylstanost-8(9)-en-3 $\beta$ -ol.

Eryloside T (**3**) exhibited the molecular formula  $\text{C}_{38}\text{H}_{62}\text{O}_9$  as deduced from its HRMALDIMS ( $[\text{M} + \text{Na}]^+$ ,  $m/z$  685.4331) and  $^{13}\text{C}$  NMR spectra. A close inspection of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data (Tables 1, 4, and 6) of **3** revealed the presence of a galactoside with a triterpenoid aglycon structure possessing a secondary alcohol function at  $\delta$  88.2, two quaternary oxygenated carbons at  $\delta$  75.1 and 88.6, and one carbonyl carbon and one trisubstituted double bond at  $\delta$  178.5 (C), 120.2 (CH), and 140.2 (C). The IR spectrum of **3** exhibits a characteristic band at 1748  $\text{cm}^{-1}$  attributable to a five-membered lactone. A major portion of the tetracyclic backbone was assembled on the basis of COSY-45, HSQC, and 1D-TOCSY experiments and through interpretation of key HMBC correlations from four methyl singlets ( $\delta$  0.77 H<sub>3</sub>-18, 0.97 H<sub>3</sub>-19, 1.26 H<sub>3</sub>-28, 1.09, H<sub>3</sub>-29) and from H-7 ( $\delta$  5.58, t,  $J = 4.8$  Hz) to C-5, C-6, C-9, and C-14 and from H-17 ( $\delta$  1.99, brq,  $J = 9.5$  Hz) to C-12, C-13, C-16, C-18, and C-20. Localization of the trisubstituted double bond at  $\Delta^{7(8)}$  was evident from the COSY-45 and 1D-TOCSY data and HMBC correlations of H-5 ( $\delta$  1.35, t,  $J = 7.5$  Hz) with C-6 and C-7 and of H-11 ( $\delta$  1.71, 1.82) and H-15 ( $\delta$  1.70) with C-8. A quaternary carbon at  $\delta$  65.1 in the  $^{13}\text{C}$  NMR spectrum was assigned to C-14 on the basis of its coupling with H-7, H-15, and H<sub>3</sub>-18. The chemical shift of this carbon and HMBC correlations from H<sub>2</sub>-15 ( $\delta$  1.70, 1.77) to C-30 ( $\delta$  178.5) suggested the attachment of a carbonyl function at C-14. The positioning of a  $\gamma$ -lactone at C-14 and C-9 was established by long-range correlation of H<sub>3</sub>-19 with the signal of C-9 ( $\delta$  88.6).

The relative configuration of the aglycon part of **3** was assigned on the basis of a NOESY experiment and  $^1\text{H}$ – $^1\text{H}$  coupling constants. The magnitudes of the vicinal coupling constants (4.0, 11.6) between H-3 ( $\delta$  3.39) and H-2 $\alpha,\beta$  ( $\delta$  1.88, 2.39) revealed an equatorial configuration of the oxygen function at C-3. Observed NOE correlations H<sub>3</sub>-19/H-29 ( $\delta$  1.09), H-1 $\beta$  ( $\delta$  1.56); H-2 $\beta$ /H-29; and H-3 $\alpha$ /H-5 $\alpha$  ( $\delta$  1.35), H-28 ( $\delta$  1.26) indicated a *trans*-ring fusion of the A and B rings, as well as the axial orientation of H<sub>3</sub>-19. The axial orientation of H<sub>3</sub>-18 was assignable on the basis of long-range COSY correlations H<sub>3</sub>-18/H-12 $\alpha$  ( $\delta$  1.59), H-17 ( $\delta$

**Table 5.**  $^1\text{H}$  NMR Data for the Aglycon Moieties of **1**,<sup>a</sup> **5**, **7**, **8**,<sup>b</sup> and **6** in  $\text{C}_5\text{D}_5\text{N}$ 

H	1			2		
	$\delta_{\text{H}}$ (J, Hz)	HMBC	NOESY	$\delta_{\text{H}}$ (J, Hz)	HMBC	NOESY
1	$\alpha$ : 1.30 m $\beta$ : 1.68 m	C-3		$\alpha$ : 1.30 m $\beta$ : 1.70 m		H-3 H-19
2	$\alpha$ : 2.32 m $\beta$ : 1.90 m	C-10		$\alpha$ : 2.31 m $\beta$ : 1.88 m	C-3	
3	3.25 dd (4.3, 11.8)	C-4,28,29	H-19,29 H-5,28, H1-Gal	3.25 dd (4.3, 11.5)	C-4,28,29, C1-Gal	H-19,29 H-5,28, H1-Gal
5	1.28 m	C-10,19,28,29	H-3	1.28 m	C-4,19,29	H-3,6 $\alpha$
6	$\alpha$ : 1.70 m $\beta$ : 1.55 m	C-5,8	H-19,29	$\alpha$ : 1.72 m $\beta$ : 1.55 m	C-8,10	H-28
7	2.35 m 2.23 m	C-8		2.35 m 2.25 m		
11	$\alpha$ : 2.42 m $\beta$ : 2.16 m	C-8,9	H-19	2.42 m 2.16 m		
12	$\alpha$ : 2.86 dt (11.6) $\beta$ : 1.86 dd (8.5, 13.0)	C-13,18 C-9,13,14,18	H-17 H-18,21	$\alpha$ : 2.84 m $\beta$ : 1.84 m	C-18 C-9,13,14,18	
15	$\alpha$ : 2.48 m $\beta$ : 1.69 m	C-13,14,30 C-8,14,30		$\alpha$ : 2.55 m $\beta$ : 1.79 m	C-13,14,30 C-14,30	H-18
16	$\alpha$ : 2.37 m $\beta$ : 1.48 m		H-18	2.46 m 1.57 m		
17	2.07 brq (9.3)	C-13,16,18,20	H-12 $\alpha$ ,21	2.05 m	C-13,16,18,20	H-21
18	0.90 s	C-12,13,14,17	H-12 $\beta$ ,15 $\beta$ , 16 $\beta$ ,20	0.91 s	C-12,13,14,17	H-12 $\beta$ ,20
19	1.08 s	C-1,5,9,10	H-2 $\beta$ ,6 $\beta$ ,11 $\beta$	1.09 s	C-1,5,9,10	H-2 $\beta$
20	1.55 m		H-18	1.52 m		H-18
21	1.10 d (6.5)	C-17,20,22	H-12 $\beta$ ,17	1.05 d (6.5)	C-17,20,22	H-12 $\beta$ ,17
22	2.06 m			1.30 m		
23	1.20 m 1.76 m 1.70 m			1.05 m 2.04 m 1.97 m		H-31 H-31
25				2.43 m	C-23,24,26,31	H-26,27
26	1.14 s	C-24,25,27,32		0.85 d (7.0)	C-24,25,27	H-27,31
27	1.14 s	C-24,25,26,32		0.92 d (7.0)	C-24,25,26	H-26,31
28	1.24 s	C-3,4,5,29	H-3,6 $\alpha$ ,29, H1-Gal	1.25 s	C-3,4,5,29	H-3,6 $\alpha$ ,29, H1-Gal
29	1.03 s	C-3,4,5,28	H-2 $\beta$ ,6 $\beta$ ,28	1.03 s	C-3,4,5,28	H-2 $\beta$ ,28
31	1.26 s	C-23,24,25		1.37 s	C-23,24,25	H-21,26,27,Ac
32	1.14 s	C-24,25,26,27				
Ac				2.01 s		

<sup>a</sup> All assignment were given for **1**; spectra of **5**, **7**, and **8** had only minor differences in chemical shift values. <sup>b</sup> All assignment were given for **2**; spectra of **6** had only minor differences in chemical shift values.

1.99, brq,  $J = 9.5$ ) as well as an NOE between H<sub>3</sub>-18 and H-11 $\beta$  ( $\delta$  1.71). Finally, NOE cross-peaks H<sub>3</sub>-18/H-20 and H-12 $\alpha$ /H-21 showed the  $R^*$  configuration of C-20 and the  $\beta$ -orientation for the side chain. The structure of the side chain was established to be the same as that of eryloside R on the basis of COSY and HMBC correlations. The  $\alpha$ -orientation of a  $\gamma$ -lactone in **3** was assigned on the basis of the upfield shift of the C-5 signal (47.0) when compared with that for lanost-7-en-3 $\beta$ ,17 $\alpha$ -diol (50.0).<sup>15</sup> Eryloside T (**3**) was thus unambiguously determined as 3-*O*-( $\beta$ -D-galactopyranosyl)-14,9-lactone-24,25-dimethyl lanost-7(8)-en-3 $\beta$ ,24-diol. Thus, eryloside T exhibits a unusual structural feature among known marine sponge glycosides, a 14,9-carbolactone.

The molecular formula of eryloside U (**4**) was determined to be  $\text{C}_{38}\text{H}_{62}\text{O}_{10}$  by a HRMALDIMS peak at  $m/z$  701.4263 and was in accordance with  $^{13}\text{C}$  NMR data. The IR spectrum of **4** exhibited a characteristic band at  $1748\text{ cm}^{-1}$  attributable to a five-membered lactone. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the aglycon part of **4** (Tables 4 and 6) indicated the presence of a  $\beta$ -equatorial secondary alcohol function at  $\delta$  88.0 (CH), a  $\gamma$ -lactone at 176.8 (C) and 85.8 (C), two quaternary oxygenated carbons at 64.4 (C) and 75.2 (C), and one secondary oxygenated carbon at 54.0 (CH). The placement of a  $\gamma$ -lactone at C-14 and C-9 was evident from the long-range correlation of H<sub>3</sub>-19 ( $\delta$  1.01) with C-9 ( $\delta$  85.8) and of H<sub>3</sub>-18 ( $\delta$  1.04), H-15 ( $\delta$  1.25) with C-14 ( $\delta$  59.6). The COSY-45 and HSQC spectra of **4** revealed the connectivity sequence of the protons in ring B ( $>\text{CH}$  (5)– $\text{CH}_2$  (6)– $\text{CHO}$  (7)–). Further, the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra showed signals corresponding to a trisubstituted epoxy methine at  $\delta_{\text{H}}$  3.13 (1H, brd,  $J = 6.4$  Hz) and  $\delta_{\text{C}}$  54.0. These data

and HMBC correlations of H-7 with C-5 ( $\delta$  42.6), C-6 ( $\delta$  20.8), and C-8 ( $\delta$  64.4), of H-6 with C-5, C-7 ( $\delta$  54.0), and C-8, and of H-11 with C-8 (Table 6) indicated the location of an epoxy group at C-7, C-8. The structure of the side chain was established to be the same as that of eryloside R on the basis of COSY and HMBC correlations. The orientation of an epoxy group in **4** was assigned as  $\alpha$ , because the upfield shift of the C-5 signal (42.6) when compared with that for **3** (47.0) may be explained by the  $\gamma$ -effect of an axial oxygen function at C-7. This shift is predictable on the basis of analysis of spectral data of lanostane derivatives.<sup>15–18</sup> The above data defined the structure of **4** as 3-*O*-( $\beta$ -D-galactopyranosyl)-7,8-epoxy-14,9-lactone-24,25-dimethyl lanostan-3 $\beta$ ,24-diol. This agrees with the molecular mass difference of 16 mass units between eryloside T and **4**.

Taking into consideration that five biosides, namely, erylosides F and F<sub>1</sub>–F<sub>4</sub>, were previously described,<sup>5,7</sup> we have designated glycosides **5**–**7** as erylosides F<sub>5</sub>–F<sub>7</sub>. The NMR spectra of erylosides F<sub>5</sub> (**5**) and F<sub>6</sub> (**6**) indicated that both compounds contained identical carbohydrate moieties (Table 2). Initial examination of the 1-D proton and one-bond correlation NMR data suggested the presence of two sugars (anomeric signals at  $\delta_{\text{H}}$  4.77,  $\delta_{\text{C}}$  104.8 and  $\delta_{\text{H}}$  5.42,  $\delta_{\text{C}}$  102.8). Interpretation of the  $^1\text{H}$ – $^1\text{H}$  COSY and 1D-TOCSY spectra gave rise to a spin system for these monosaccharides, which were assigned as  $\beta$ -galactopyranose (Gal) and  $\beta$ -2-*N*-acetylglucosamine by analysis of  $^{13}\text{C}$  NMR, HSQC, HMBC, and NOESY data as well as by the  $^3J_{\text{H-H}}$  coupling constant of ring protons.<sup>19,21</sup> The acid hydrolysis of the sum of these glycosides gave D-galactose and D-*N*-acetylglucosamine, which were identified by capillary GC



**Table 6.**  $^1\text{H}$  NMR Data for the Aglycon Moieties of **3** and **4** in  $\text{C}_5\text{D}_5\text{N}$ 

H	<b>3</b>			<b>4</b>		
	$\delta_{\text{H}}$ (J, Hz)	HBMC	NOESY	$\delta_{\text{H}}$ (J, Hz)	HBMC	NOESY
1	$\alpha$ : 2.00 m $\beta$ : 1.56 m		H-19	$\alpha$ : 1.91 m $\beta$ : 1.49 m	C-10 C-3,5,10	H-19
2	$\alpha$ : 2.39 m $\beta$ : 1.88 m		H-29	$\alpha$ : 2.35 m $\beta$ : 1.83 m	C-1,3,4,10	H-19
3	3.39 dd (4.0, 11.6)	C-4,28,29	H-5,28, H1-Gal	3.36 dd (3.9,11.8)	C-4,28,29, C1-Gal	H-5,28, H1-Gal
5	1.35 t (7.5)	C-4,6,7,10, 19,28	H-3	1.58 dd (4.0, 13.5)	C-4,6,9, 10,28,29	H-3,28
6	1.95 t (6.2)	C-5,7,8,10	H-19,28,29	$\alpha$ : 1.93 ddd (4.0, 6.6, 15.0) $\beta$ : 1.72 t (14.6)	C-5,7,10 C-5,7,8,10	H-7,28 H-7,29
7	5.58 t (4.8)	C-5,6,9,14	H-15	3.13 brd (6.4)	C-5,6,8,14	H-6 $\alpha$ , $\beta$ ,15
11	$\alpha$ : 1.82 m $\beta$ : 1.71 m	C-8 C-8	H-18	2.02 m 1.87 m	C-12 C-8,12,13	H-19 H-19
12	$\alpha$ : 1.59 m $\beta$ : 1.84 m		H-21 H-18	$\alpha$ : 1.63 m	C-11,13,14, 17,18	
15	1.77 m 1.70 m	C-13,14,30 C-14,30	H-7	$\beta$ : 1.95 m 1.64 m	C-9,11,14 C-30	H-18,21
16	2.28 m 1.42 m			1.25 m 2.23 m	C-8,14,30 C-13,14,15,17	
17	1.99 brq (9.5)	C-12,13,16, 18,20	H-21	1.35 m 1.93 m		
18	0.77 s	C-12,13,14,17	H-11 $\beta$ ,12 $\beta$ , 20		C-12,13,14,17	H-12 $\beta$ ,20
19	0.97 s	C-1,5,9,10	H-1 $\beta$ ,6, H-29	1.01 s	C-1,5,9,10	H-1 $\beta$ ,6 $\beta$ ,11
20	1.44 m		H-18	1.45 m		H-18
21	0.96 d (7.0)	C-17,20,22		0.94 s	C-17,20,22	H-12 $\beta$
22	2.02 m 1.23 m			1.99 m 1.22 m		
23	1.74 m 1.70 m			1.71 m		
26	1.15 s	C-24,25,27,32		1.14 s	C-24,25,27,32	
27	1.15 s	C-24,25,26,32		1.14 s	C-24,25,26,32	
28	1.26 s	C-3,4,5,29	H-3,6 H1-Gal	1.22 s	C-3,4,5,29	H-3,5,6 $\alpha$ ,29, H1-Gal
29	1.09 s	C-3,4,5,28	H-6	0.98 s	C-3,4,5,28	H-6 $\beta$ ,28
31	1.29 s	C-23,24,25		1.28 s	C-23,24,25	
32	1.15 s	C-24,25,26,27		1.14 s	C-24,25,26,27	

of the corresponding acetylated (–)- and (+)-2-octyl glycosides using authentic samples prepared from D- and L-galactose and D-*N*-acetylglucosamine.<sup>13</sup> The arrangement of the sugar units was determined by HMBC and NOESY experiments. A long-range  $^1\text{H}$ – $^{13}\text{C}$  correlation (H1-Gal ( $\delta$  4.78)/C-3 ( $\delta$  88.8)) (Tables 2 and 4) as well as the NOESY cross-peak between H1-Gal and H-3 revealed a linkage between the galactose and aglycone. Similarly, a long-range correlation of H-1-NAcGlc ( $\delta$  5.39)/C2-Gal ( $\delta$  80.8) and the NOESY cross-peak between H1-NAcGlc and H2-Gal ( $\delta$  4.52) assigned the linkage between these two sugar units.

The HRMALDIMS of eryloside **F**<sub>5</sub> (**5**) showed the quasimolecular ion at  $m/z$  890.5214 [ $\text{M} + \text{Na}$ ]<sup>+</sup>, consistent with the molecular formula  $\text{C}_{46}\text{H}_{77}\text{O}_{14}$ . The structure of the aglycon moiety of **5** was found by extensive NMR spectroscopy ( $^1\text{H}$  and  $^{13}\text{C}$  NMR, COSY, HSQC, HMBC, and NOESY) (Tables 4 and 5) to be the same as that of eryloside R. All the above data confirmed the structure of eryloside **F**<sub>5</sub> (**5**) as 3-*O*-[2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-galactopyranosyl]-14-carboxy-24,25-dimethylallanost-8(9)-en-3 $\beta$ ,24-diol.

The molecular formula of eryloside **F**<sub>6</sub> (**6**) was determined as  $\text{C}_{47}\text{H}_{77}\text{O}_{15}$  on the basis of a high-resolution MALDIMS peak at  $m/z$  918.5226 and was in accordance with  $^{13}\text{C}$  NMR data. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data observed for the aglycon part of **6** (Tables 4 and 5) matched those reported for eryloside S. Thus, the structure of eryloside **F**<sub>6</sub> (**6**) was represented as 3-*O*-[2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-galactopyranosyl]-14-carboxy-24-acetoxy-24-methylallanost-8(9)-en-3 $\beta$ -ol.

Eryloside **F**<sub>7</sub> (**7**) was analyzed for  $\text{C}_{44}\text{H}_{74}\text{O}_{14}$  on the basis of HRMALDIMS and NMR data. The disaccharide nature of **7** was

evident from its  $^{13}\text{C}$  and DEPT spectra (Table 3), which exhibited two signals for anomeric carbons at  $\delta$  106.4 (CH) and 106.9 (CH) and those of the corresponding protons at  $\delta$  5.40 (d,  $J$  = 7.8 Hz) and 4.79 (d,  $J$  = 7.7 Hz) in the  $^1\text{H}$  NMR data. Interpretation of the  $^{13}\text{C}$  NMR, COSY, HSQC, and 1D-TOCSY spectra gave rise to spin systems for these monosaccharides, which were assigned as *galacto*- and *gluco*- $\beta$ -configured residues on the basis of chemical shifts and coupling constants of ring protons.<sup>19,21–23</sup> This deduction was further corroborated by intraresidual NOE connectivity between anomeric methines and H3,5-Gal, H3,5-Glu found in the NOESY spectrum (Table 3). The absolute configurations of the galactose and glucose were determined after acid hydrolysis of **7** by preparation of acetylated (–)-2-octyl glycosides followed by GC and comparison with corresponding authentic samples obtained from D- and L-galactose and D- and L-glucose.<sup>14</sup> The arrangement of the sugar moieties in **7** was established by a combination of the HMBC and NOESY spectra. A long-range  $^1\text{H}$ – $^{13}\text{C}$  correlation (H1-Gal ( $\delta$  4.79)/C-3 ( $\delta$  88.4)) (Tables 3 and 4) as well as the NOESY cross-peak between H1-Gal and H-3 revealed a linkage between the galactose and aglycone. Similarly, a long-range correlation of H1-Glc ( $\delta$  5.40)/C3-Gal ( $\delta$  85.0) and the NOESY cross-peak between H1-Glc and H3-Gal ( $\delta$  4.26) assigned the 1,3-linkage between glucose and galactose. A close inspection of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **7** (Tables 4 and 5) revealed that eryloside **F**<sub>7</sub> was structurally identical to eryloside R with respect to the aglycon. All the above data confirmed the structure of eryloside **F**<sub>7</sub> (**7**) as 3-*O*-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-galactopyranosyl]-14-carboxy-24,25-dimethylallanost-8(9)-en-3 $\beta$ ,24-diol.

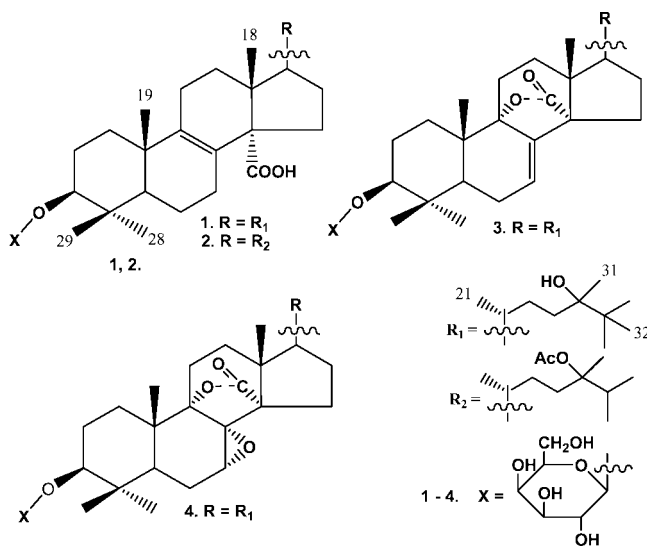


Figure 1. Structures of erylosides 1–4.

The molecular formula of eryloside V (**8**) was determined to be  $C_{48}H_{80}O_{17}$  by high-resolution MALDIMS ( $m/z$  951.5258,  $[M + Na]^+$ ) and  $^{13}C$  NMR analyses. Its  $^1H$  and  $^{13}C$  NMR spectra (Table 3) revealed three anomeric protons at  $\delta$  4.82 (d,  $J = 6.5$  Hz), 5.17 (d,  $J = 7.5$  Hz), and 5.35 (d,  $J = 7.8$  Hz), which correlated with the anomeric carbon signals at  $\delta$  105.0 (CH), 105.1 (CH), and 104.9 (CH). Acid hydrolysis of **8** gave L-arabinose, D-xylose, and D-galactose, which were identified by GC of the corresponding acetylated (–)-2-octyl glycosides, using authentic samples prepared from the standard monosaccharides.<sup>14</sup> The identification of each sugar as well as their sequence, interglycosidic linkage, and configuration of glycosidic bonds ( $\beta$  for xylose and galactose and  $\alpha$  for arabinose) in **8** were determined by 1D and 2D NMR, including HMBC, HMQC, NOESY, and  $^1H$ – $^1H$  coupling constant values.<sup>19,21–23</sup> The correlation observed in the HMBC spectrum between H1-Ara and C-3 as well as the NOESY cross-peak H-3/H1-Ara assigned the connectivity between arabinose and C-3 of the aglycon. The long-range correlations of H2-Ara with C1-Gal and H3-Ara with C1-Xyl coupled with NOESY cross-peaks between H2-Ara and H1-Gal and between H3-Ara and H1-Xyl defined the 1,2-linkage between galactose and arabinose and 1,3-linkage between xylose and arabinose. The aglycon moiety of **8** was found by extensive NMR spectroscopy (Tables 4 and 5) to be the same as that of eryloside R. On the basis of all the data above, the structure of eryloside V (**8**) was established as 3-*O*-{[ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 2)]-[ $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 3)]- $\alpha$ -L-arabinopyranosyl]-14-carboxy-24,25-dimethyl-8(9)-en-3 $\beta$ ,24-diol.

Erylosides R, S, T, V, F<sub>6</sub>, and F<sub>7</sub> exhibited cytotoxic action against tumor cells of Ehrlich carcinoma ( $IC_{50} = 20$ – $40 \mu M$ ) in vitro.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured using a Perkin-Elmer 343 polarimeter. The  $^1H$  and  $^{13}C$  NMR spectra were recorded in  $C_3D_5N$  on Bruker Avance 500 and Avance 600 spectrometers at 500 and 125.8 MHz and 600 and 150.9 MHz, respectively, using tetramethylsilane as an internal standard. HR MALDI-TOF mass spectra were recorded on a Bruker Biflex III laser desorption mass spectrometer coupled with delayed extraction using a  $N_2$  laser (337 nm) and  $\alpha$ -cyano-4-hydroxycinnamic acid as matrix. GC analyses were performed on an Agilent 6850 Series GC system equipped with a HP-5MS column using a temperature program of 100 to 250  $^{\circ}C$  at 5  $^{\circ}C$   $min^{-1}$ ; temperatures of injector and detector were 150 and 270  $^{\circ}C$ , respectively. Low-pressure liquid column chromatography was performed using Polychrome-1 (Teflon powder, Biolar, Latvia) and Si gel L (40/100  $\mu m$ , Chemapol, Praha, Czech Republic). Glass plates (4.5  $\times$  6.0 cm) precoated with Si gel (5–17  $\mu m$ , Sorbfil,

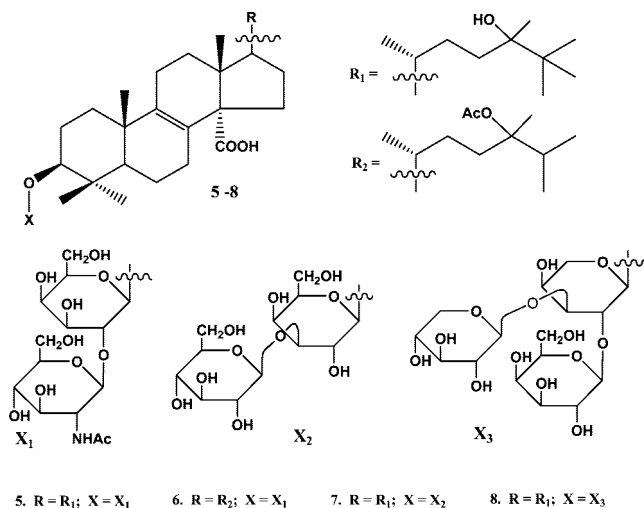


Figure 2. Structures of erylosides 5–8.

Russia) were used for TLC. Preparative HPLC was carried out on a Beckman-Altex chromatograph, using Diasphere-110-C18 (5  $\mu m$ , 10  $\times$  250 mm) and YMC-Pack ODS-A (5  $\mu m$ , 10  $\times$  250 mm) columns with an RIDK refractometer detector.

**Animal Material.** The sponge was collected in February 1998 near Arresife-Seko Reef (Cuba) by scuba diving at depths of 15–20 m. The sponge was cut and lyophilized immediately after collection. A voucher specimen (PIBOC 001-059) is on deposit in the collection of the Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia. The sponge was identified as *Erylus goffrilleri* Wiedenmayer, 1977 (family Geodiidae). The sponge was massively encrusting, up to 30 mm thick. Texture is firm, slightly compressible; surface is smooth and gently wrinkled. A layer of aspidasters and microoxes form a detachable crust (up to 0.8 mm thick). The rare orthotriangles are radially arranged to surface. The rhabdus are 650 by 10  $\mu m$ , the clads 240 by 8  $\mu m$ . The choanosomal oxes are slightly curved (1 mm by 15  $\mu m$ ). The thin aspidasters are irregularly rhomb shaped 150  $\mu m$  in length and 90  $\mu m$  in width. The microoxes/microstrongyles are slightly centrotlyte and curved (30  $\mu m$  by 2  $\mu m$ ). There are small calthrop-like oxyasters with 4–6 acanthose rays (14  $\mu m$  in diameter). There is some resemblance to *Erylus formosus* Sollas widely distributed in the Caribbean, but clearly different by the morphology of the aspidasters.

**Extraction and Isolation.** The lyophilized specimens (0.1 kg) were macerated and extracted with EtOH (4  $\times$  500 mL) and 70% EtOH (2  $\times$  500 mL). The combined extracts were concentrated to dryness and separated by low-pressure RP CC (20  $\times$  8 cm column) on Polychrome-1 Teflon powder in  $H_2O$  and 50% EtOH. After elution of inorganic salts and highly polar compounds by  $H_2O$ , 50% EtOH was used to obtain the fraction of amphiphilic compounds, including the erylosides. After evaporation of the solvent, half of the residual material (4.5 g) was subjected to Si gel flash CC (7  $\times$  13 cm) with a solvent gradient system of increasing polarity from 5% to 30% EtOH in  $CHCl_3$  (total volume 3 L). Fractions of 10 mL were collected and combined by TLC examination to obtain two subfractions. Subfraction I (540 mg) was further purified and separated by RP HPLC on a Diasphere-110-C18 column eluting with MeOH– $H_2O$  (90:10) and repeatedly chromatographed on a YMC-Pack ODS-A column in the same system to yield erylosides R (**1**) (180 mg), S (**2**) (5.5 mg), T (**3**) (3.0 mg), and U (**4**) (3 mg). Subfraction II (240 mg) was subjected to HPLC on a Diasphere-110-C18 column with MeOH– $H_2O$  (85:15) and then on a YMC-Pack ODS-A column using MeOH– $H_2O$ – $CHCl_3$  (75:25:5) to give erylosides A<sub>5</sub> (**5**) (3.5 mg), A<sub>6</sub> (**6**) (12.0 mg), A<sub>7</sub> (**7**) (10.0 mg), and V (**8**) (8 mg).

**Eryloside R (1):** colorless, amorphous solid; 180 mg;  $[\alpha]_D^{20} -38.0$  (c 0.1, MeOH); IR ( $CD_3OD$ ) 1687  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR data, see Tables 1, 4, 5; HR MALDI TOF MS  $m/z$  687.4483  $[M + Na]^+$ , calcd for  $C_{38}H_{64}O_9Na$  687.4448.

**Eryloside S (2):** colorless, amorphous solid; 5.5 mg;  $[\alpha]_D^{20} -24.5$  (c 0.2, MeOH); IR ( $CD_3OD$ ) 1685  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR data, see Tables 1, 4, 5; HR MALDI TOF MS  $m/z$  715.4351  $[M + Na]^+$ , calcd for  $C_{39}H_{64}O_{10}Na$  715.4397.

**Eryloside T (3):** colorless, amorphous solid; 3.0 mg;  $[\alpha]_D^{20} -14.0$  (c 0.1, MeOH); IR ( $CD_3OD$ ) 1748  $cm^{-1}$ ;  $^1H$  and  $^{13}C$  NMR data, see

Tables 1, 4, 6; HR MALDI TOF MS  $m/z$  685.4331  $[M + Na]^+$ , calcd for  $C_{38}H_{62}O_9Na$  685.4292.

**Eryloside U (4):** colorless, amorphous solid; 3.0 mg;  $[\alpha]_D^{20}$   $-55.0$  ( $c$  0.1, MeOH); IR (CD<sub>3</sub>OD) 1748  $cm^{-1}$ ; <sup>1</sup>H and <sup>13</sup>C NMR data, see Tables 1, 4, 6; HR MALDI TOF MS  $m/z$  701.4263  $[M + Na]^+$ , calcd for  $C_{38}H_{62}O_{10}Na$  701.4241.

**Eryloside F<sub>5</sub> (5):** colorless, amorphous solid; 4.0 mg;  $[\alpha]_D^{20}$   $-41.0$  ( $c$  0.1, MeOH); <sup>1</sup>H and <sup>13</sup>C NMR data, see Tables 1, 3; HR MALDI TOF MS  $m/z$  890.5214  $[M + Na]^+$ , calcd for  $C_{46}H_{77}O_{14}NNa$  890.5242.

**Eryloside F<sub>6</sub> (6):** colorless, amorphous solid; 12.0 mg;  $[\alpha]_D^{20}$   $-35.0$  ( $c$  0.25, MeOH); <sup>1</sup>H and <sup>13</sup>C NMR data, see Tables 4, 5; HR MALDI TOF MS  $m/z$  918.5226  $[M + Na]^+$ , calcd for  $C_{47}H_{77}O_{15}NNa$  918.5191.

**Eryloside F<sub>7</sub> (7):** colorless, amorphous solid; 10.0 mg;  $[\alpha]_D^{20}$   $-29.5$  ( $c$  0.2, MeOH); <sup>1</sup>H and <sup>13</sup>C NMR data, see Tables 4, 5; HR MALDI TOF MS  $m/z$  849.4898  $[M + Na]^+$ , calcd for  $C_{44}H_{74}O_{14}Na$  849.4976.

**Eryloside V (8):** colorless, amorphous solid; 8.0 mg;  $[\alpha]_D^{20}$   $-24.52$  ( $c$  0.2, MeOH); <sup>1</sup>H and <sup>13</sup>C NMR data, see Tables 4, 5; HR MALDI TOF MS  $m/z$  951.5258  $[M + Na]^+$ , calcd for  $C_{48}H_{80}O_{17}Na$  951.5293.

**Acidic Hydrolysis of Erylosides R–U (1–4).** A solution of a mixture of compounds 1–4 (each 1.5 mg) in 0.2 M TFA (0.5 mL) was heated in a stoppered reaction vial at 100 °C for 1 h. The H<sub>2</sub>O layer was extracted with CHCl<sub>3</sub> and then neutralized with Dowex (HCO<sub>3</sub><sup>−</sup>). The residue obtained after evaporation of the H<sub>2</sub>O layer was purified on a Zorbax NH<sub>2</sub> column (5  $\mu$ m, 4.6  $\times$  150 mm) eluting with CH<sub>3</sub>CN–H<sub>2</sub>O (90:10) to yield 1.1 mg of galactose. The monosaccharide was treated with (−)-2-octanol (0.2 mL) in the presence of trifluoroacetic acid (1 drop) in a stoppered reaction vial at 130 °C overnight.<sup>13</sup> The mixture was evaporated to dryness and acetylated with Ac<sub>2</sub>O in pyridine. The acetylated (−)-2-octyl glycoside was analyzed by GC using the corresponding authentic samples prepared from D- and L-galactose.

**Acidic Hydrolysis of Erylosides F<sub>5</sub> and F<sub>6</sub> (5, 6).** A solution of a mixture of compounds 5 and 6 (each 4.0 mg) in 2 N HCl (1 mL) was heated in a stoppered reaction vial at 100 °C for 2 h. The residue obtained after evaporation of the H<sub>2</sub>O layer was separated on a Zorbax NH<sub>2</sub> column (5  $\mu$ m, 4.6  $\times$  250 mm) eluting with CH<sub>3</sub>CN–H<sub>2</sub>O (90:10) to yield 0.8 mg of galactose and 0.7 mg of 2-*N*-acetylglucosamine. The absolute configurations of the monosaccharides were determined by GC of the acetylated (−)-2-octyl glycosides using the corresponding authentic samples prepared from D- and L-galactose. Retention time for the L-GlcNAc derivative was determined for (+)-2-octyl glycoside of the corresponding D-sugar according to Leontein.<sup>13</sup>

**Acidic Hydrolysis of Eryloside F<sub>7</sub> (7).** Compound 7 (6.0 mg) was hydrolyzed as described above for erylosides F<sub>5</sub> and F<sub>6</sub>. The absolute configurations of the monosaccharides were determined by GC of the acetylated (−)-2-octyl glycosides using the corresponding authentic samples prepared from D- and L-galactose and D- and L-glucose.<sup>14</sup>

**Acidic Hydrolysis of Eryloside V (8).** Compound 7 (6.0 mg) was hydrolyzed as described above for erylosides F<sub>5</sub> and F<sub>6</sub>. The absolute configurations of the monosaccharides were determined by GC of the acetylated (−)-2-octyl glycosides using the corresponding authentic samples prepared from D- and L-galactose, D- and L-arabinose, and D- and L-xylose.<sup>14</sup>

**Bioassay.** Ehrlich carcinoma cells were grown intraperitoneally in albino mice, 18–20 g in weight. Cells were harvested on the seventh to tenth day after inoculation and washed twice by centrifugation (450 g, 10 min) in cold phosphate-buffered saline (PBS). Then 100  $\mu$ L of

the cell suspension (final cell concentration (2–5)  $\times$  10<sup>6</sup> cells/mL) was placed into wells of a 96-well microplate containing 10  $\mu$ L solutions of tested compounds. The incubation was conducted within 1 h at 37 °C. Then, 10  $\mu$ L of an aqueous solution of propidium iodide (final concentration 2.5  $\mu$ g/mL) was added to each well, and the microplate was incubated additionally for 10 min at 37 °C. The fluorescence intensity was measured at  $\lambda_{ex}$  = 485 nm,  $\lambda_{em}$  = 620 nm.

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