Isolation and Structures of Erylosides from the Carribean Sponge *Erylus formosus*

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Nine new triterpene glycosides, erylosides F_1−F_4 (**1**−**4**), M (**5**), N (**6**), O (**7**), P (**8**), and Q (**9**), along with previously known erylosides F (**10**) and H (**11**) were isolated from the sponge *Erylus formosus* collected from the Mexican Gulf (Puerto Morelos, Mexico). Structures of **1**−**4** were determined as the corresponding biosides having aglycons related to penasterol with additional oxidation patterns in their side chains. Erylosides **5**−**9** contain new variants of carbohydrate chains with three (**5**, **6**), four (**7**), and six (**8**, **9**) sugar units, respectively. Erylosides **5**, **7**, **8**, and **6**, **9** contain 14-carboxy-24-methylenelanost-8(9)-en-3β-ol and penasterol as aglycons, respectively. In contrast with its epimer **2**, the compound **3** induced the early apoptosis of Ehrlich carcinoma cells at a concentration of 100 μg/mL, while **1** and **10** activated the Ca^{2+} influx into mouse spleenocytes (130% of the control) at the same doses.

The triterpene glycosides erylosides have been reported from several marine sponges belonging to the genus *Erylus*.[1−6] Some glycosides of this structural group were described using other names, for example, nobiloside from *Erylus nobilis*,[7] formoside from *Erylus formosus*,[8] and feroxosides A and B from *Ectyoplasia ferox*.[9] There are two series of erylosides differing from each other by the aglycon structure, namely, (a) glycosides derived from penasterol or congeners carrying additional substituents at C-24, C-25, or both and (b) glycosides derived from 4α-methyl-5α-cholesta-8,14-dien-3β-ol or related nortriterpenoids. Penasterol or 14-carboxylanosta-8,24-dien-3β-ol was originally isolated from the sponges *Penares* sp.[10] and *Penares incrustans*[11,12] and showed potent antileukemic activity and inhibitory action against IgE-dependent histamine release from rat mast cells.

Erylosides also exhibit a wide spectrum of biological activities. For example, eryloside A possesses antitumor and antifungal properties.[1] Eryloside E is an antagonist of C5a-receptor binding.[3] Eryloside F was reported as a potent thrombin receptor antagonist and activator of intacellular calcium influx.[4] Erylosides G−J exhibit moderate cytotoxicity against a human leukemia cell line.[5] Nobiloside inhibits neuroaminidase from the bacterium *Clostridium perfringes*.[7] In addition, erylosides and related triterpene glycosides from marine sponges appear to have multiple ecological functions. It was shown that the top layers and swabs of the surfaces of the sponge *Erylus formosus* contained sufficiently high concentrations of erylosides to deter enemies such as reef fishes, bacterial settlement, and fouling.[13,14] It is of special interest that a mixture of triterpene glycosides protected *E. formosus* from predatory reef fishes, but when these compounds were separated into fractions, they failed to deter feeding at natural concentrations.[1]

As a rule, erylosides are present in sponges as very complicated mixtures of closely related glycosides. Probably, the individual glycosides differ significantly from each other in their physiological and ecological actions. Moreover, the minor constituents appear to be more potent than the major glycosides, as was suggested by Pawlik and collaborators.[14]

In continuation of our studies on glycosides from marine sponges,[15,16] we have isolated a series of new erylosides (**1**−**9**) from the alcoholic extract of the Caribbean sponge *Erylus formosus* along with the previously known erylosides F (**10**)[4] and H (**11**).[5] In this paper we described the isolation and structural elucidation of nine new glycosides.

Results and Discussion

The ethanolic extract of the sponge *E. formosus* collected off Puerto Morelos (the Caribbean Sea, Mexico) was evaporated *in vacuo* and separated by flash column chromatography on silica gel L followed by HPLC on a Supelco Sil and Supelco C-18 columns to give four new biosides (**1**−**4**), two new triosides (**5**, **6**), one new tetraoside (**7**), and two new hexaosides (**8**, **9**) as white, amorphous solids, along with the previously known bioside (**10**) and trioside (**11**). A portion of the bioside glycoside mixture containing **1**−**4** and **10** was hydrolyzed with 10% hydrochloric acid with heating. D-galactose and L-arabinose were identified as the major sugars after their isolation by preparative TLC and comparison of optical rotation data with those of standard monosaccharides. Taking into consideration that one of the biosides, namely, eryloside F (**10**), was previously described,[4] we have designated glycosides **1**−**4** as erylosides F_1−F_4. A preliminary inspection of NMR spectra (Tables 1, 4, 5) of **1**−**4** suggested the glycotriterpene nature of all of these compounds.

Structures of **1**−**4** were determined by extensive application of spectroscopic methods, especially 2D NMR techniques. Analysis of NMR spectra established that **1**−**4** had identical carbohydrate moieties and the same tetracyclic core of aglycon as in the previously known eryloside F.[4] Actually, these spectra showed characteristic features of biosides, namely, a series of signals at δ_H 3.0−5.0 and two signals of anomeric carbons at δ_C 104.6 (CH) and 106.7 (CH). Interpretation of ^1H−^1H COSY spectrum gave rise to spin systems of the sugars in **1**−**4**, which were assigned as α-arabinopyranose (Ara) and β-galactopyranose (Gal) by analysis of HSQC, HMBC, and NOESY data as well as by ^1H−^1H coupling constant values obtained from 1D-TOCSY experiments and $J_{(C1H1)}$ values[17] (Table 1). The pyranose nature of the sugars in **1**−**4** was determined on the basis of HMBC correlations between the anomeric methines and the C5-Ara methylene group and C5-Gal methine group. All sugars were connected through β-glycosidic linkages for those belonging to the D-series and α- for L-arabinose as followed from ^{13}C NMR shifts (δ_C > 100 ppm) and spin-decoupling constants of anomeric protons. Taking into consideration that L-arabinose and D-galactose were identified in the hydrolysate of the glycoside fraction, HMBC correlations H1-Ara/C-3 and H1-Gal/C2-Ara implied the sequence β-D-galactopyranosyl-(1→2)-α-L-arabinopyranosyl in the carbohydrate moiety as well as the attachment of this fragment to C-3 of an aglycon.

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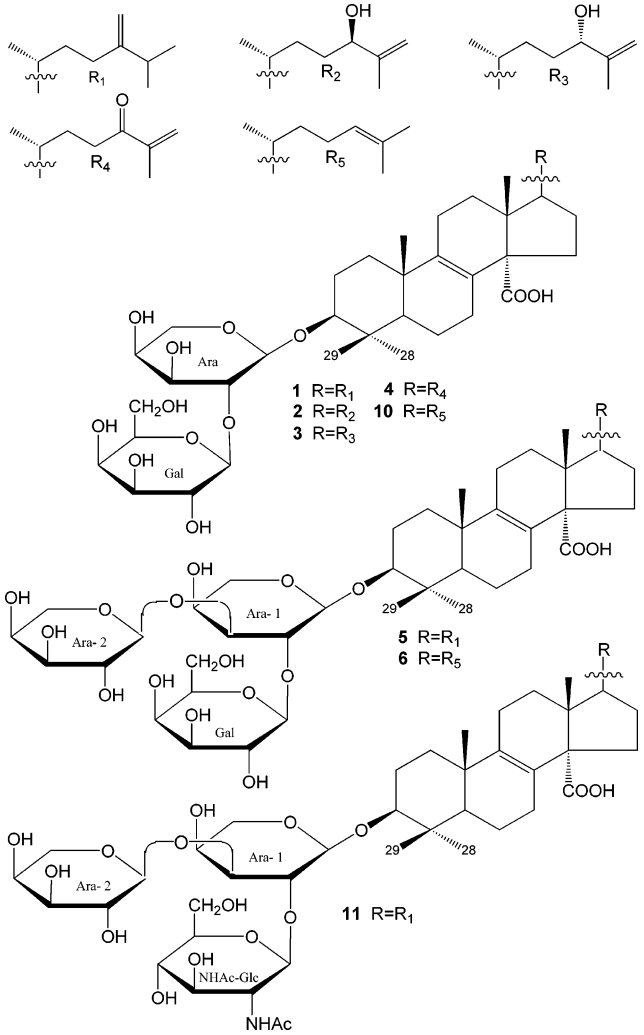

Figure 1. Structures of erylosides **1**−**6** and **11**.

Inspection of COSY-45 spectra of **1**−**4** allowed us to detect several distinct spin systems belonging to the tetracyclic aglycon moiety. The H(3)−H_2(2)−H_2(1) spin system and the H_2(11)−H_2-(12) sequence were revealed from signals H-3 [δ_H 3.12 dd] and H_2-12 (δ_H 1.83 and 2.83 m), respectively. The latter signal gave a cross-peak with H_3-18 (δ_H 0.92s). The proton sequence H(17)−H_2(16)−H_2(15) was indicated from the signal H-17 [δ_H 2.03 brq (9.9)], which gave cross-peaks with H-20 [δ_H 1.56 m)] and H_3-18. Finally, the proton sequence H(5)−H_2(6)−H_2(7) was indicated from the signals of H-5 [δ_H 1.24 dd (12.8, 2.0)]. The positioning of a double bond at $\Delta^{8(9)}$ was established by long-range correlation of H_3-19 with the signal of C-9 at δ_C 139.8. The chemical shifts of C-14 at 62.7−62.8 and the carboxyl carbonyl at 178.2[5] along with other HMBC results, given in Table 5, indicated that **1**−**4** contain a 14-carboxylanost-8(9)-ene skeleton system. This was confirmed by the results of NOESY experiments, which gave cross-peaks H-3/H-5, H-3/H_3-28, H-1/H-11, and H-12/H-17. Cross-peaks H-20/H_3-18 and H-12/H_3-21 showed the *R*-configuration of C-20.[15,16]

Erylosides F_1−F_4 differed from each other in side chain structures only. Eryloside F_1 (**1**) analyzed for $C_{42}H_{68}O_{12}$ by combined HRESTOFMS (+) (pseudomolecular peak at *m*/*z* 787.4464 [M + Na]$^+$) and ^{13}C NMR data. The structure of its side chain followed from ^{13}C and ^{1}H NMR spectra (Tables 4, 5), indicating the same characteristic features as those of the side chain of 24-methylenecholesterol. Actually, the signals of an exo-methylene group were observed at δ_H 4.81 d (1.4) and 4.84 s as well as at δ_C 106.4 s (C-24) and 106.4 (C-31). Along with key HMBC correlations such as H-31 (δ_H 4.81 d, 4.84 s)/C-23 (31.4), C-24, C-25 (33.8) and H_3-26 (δ_H 1.03 d, 1.04 d)/C-24, C-25, all these data confirmed the structure of eryloside F_1 (**1**) as 3β-*O*-[β-D-galactopyranosyl-(1→2)-α-L-arabinopyranosyl]-14-carboxy-24-methylenelanost-8(9)-en-3β-ol.

The molecular formula of eryloside F_2 (**2**) was deduced as $C_{41}H_{66}O_{13}$ by HRTOFMSES (+) (pseudomolecular peak at *m*/*z* 789.4371 [M + Na]$^+$) and ^{13}C NMR spectra. Spectroscopic data of **2** were very similar to those of **1** and suggested that **2** had an additional hydroxyl group, and a double bond in the side chain was in another position when compared with **1**. The difference in the NMR spectra was the appearance of the CH−O signals at δ_H 4.35 m and δ_C 75.4 and the 25(26) double-bond signals at δ_H 4.95 m, 5.25 d (H_2-26) and δ_C 149.7 s (C-25), 109.9 t (C-26) instead of those of the 24(28) double bond. Positions of the hydroxyl group at C-24 and the double bond as $\Delta^{25(26)}$ were confirmed by HMBC cross-peaks H-26/C-24, C-27 and H_3-27/C-24, C-25, C-26.

Eryloside F_3 (**3**) was shown to be a glycoside epimer of **2** at C-24. Its molecular formula was also deduced as $C_{41}H_{66}O_{13}$ by HRTOFMSES (+) (pseudomolecular peak at *m*/*z* 789.4390 [M + Na]$^+$]) and ^{13}C NMR spectroscopy. The significant difference was only in the chemical shifts of proton and carbon atoms flanking the hydroxyl group in the side chain. The chemical shifts of side chain carbons of two epimeric 12-*O*-(β-D-glucopyranosyl)-(20*S*,24*R*,*S*)-3-ketodammaran-25(26)-ene-20,24-diols, containing similar side chains, whose structures were established by X-ray analysis,[18] were earlier reported by Malinovskaya et al.[19] These data indicated that the signals of C-24 and C-26 were low-field shifted, while the signal of C-27 was higher field shifted in the spectrum of the 24*S*-isomer, when compared with that of the 24*R*-isomer. Similar, consistent patterns were found by us on comparison of the ^{13}C NMR spectra of **2** and **3** (Table 4). On this basis, we assigned the configuration of C-24 as *R* in **2** and as *S* in **3**. Thus, structures of **2** and **3** were established as (24*R*)- and (24*S*)-3β-*O*-

Table 1. ^{1}H and ^{13}C NMR Data for Carbohydrate Moieties of Erylosides **1**−**4**[a] in C_5D_5N

position	δ_C ($J_{C,H}$ in Hz)	δ_H (J in Hz)	HMBC	NOESY
Ara (1→C−3)				
1	104.6 CH (162.6)	4.91 d (5.6)	C-3, C2,5-Ara	H-3, H3-Ara
2	81.3 CH	4.56 dd (5.5, 7.4)		
3	73.2 CH	4.33 dd (3.6, 7.6)		H1-Ara
4	68.0 CH	4.36 m		
5	64.7 CH_2	3.80 dd (2.5, 11.9)	C1-Ara	
		4.31 dd (4.9, 11.7)	C3,4-Ara	
Gal (1→2Ara)				
1	106.7 CH (156.9)	5.05 d (7.7)	C5-Gal, C2-Ara	H3,5-Gal
2	73.7 CH	4.55 dd (7.6, 9.5)	C3-Gal, C1-Ara	
3	75.0 CH	4.13 dd (3.4, 9.5)	C2-Gal	H1-Gal
4	69.4 CH	4.67 brd (3.1)	C3-Gal	
5	76.6 CH	3.98 brdd (6.0, 7.4)	C4,6-Gal	H1-Gal
6	61.1 CH_2	4.37 dd (5.6, 10.6)	C4,5-Gal	
		4.57 dd (8.0, 10.3)	C4,5-Gal	

[a] All assignment were given for **1**; spectra of **2**−**4** had only minor differences in chemical shift values.

Table 2. ^{1}H and ^{13}C NMR Data for Carbohydrate Moieties of Erylosides **5** and **6**[a] in C_5D_5N

position	δ_C	δ_H (J in Hz)	HMBC	NOESY
Ara1 (1→C-3)				
1	104.9 CH	4.83 d (5.5)	C-3, C5-Ara1	H-3, H3,5-Ara1
2	77.0 CH	4.72 dd (5.9, 8.4)	C3-Ara1, C1-Gal	H1-Gal
3	81.3 CH	4.32 dd (3.2, 8.4)	C2-Ara1	H1-Ara1, H1-Ara2
4	68.0 CH	4.49 m		
5	65.1 CH_2	3.75 m		H1-Ara1
		4.28 m	C3,4-Ara1	
Ara2 (1→3Ara1)				
1	104.7 CH	5.17 d (7.0)	C3,5-Ara2, C3-Ara-1	H3,5-Ara1
2	72.3 CH	4.49 brt (8.0)		
3	74.2 CH	4.15 dd (3.1, 8.7)	C2-Ara2	H1-Ara2
4	69.0 CH	4.31 m	C2,3-Ara2	
5	66.2 CH_2	3.74 m	C3-Ara2	H1-Ara2
		4.30 m	C4-Ara2	
Gal (1→2Ara1)				
1	105.0 CH	5.30 d (7.8)	C2-Ara1	H3,5-Gal, H2-Ara1
2	73.2 CH	4.48 brt (8.9)	C4-Gal	
3	75.3 CH	4.09 dd (3.4, 9.6)	C2-Gal	H1-Gal
4	69.5 CH	4.61 brd (3.9)	C2,3-Gal	
5	76.1 CH	3.75 m	C1,4,6-Gal	H1-Gal
6	61.3 CH_2	4.27 m	C4,5-Gal	
		4.47 m	C4,5-Gal	

[a] All assignments were given for **5**; spectra of **6** had only minor differences in chemical shift values.

[β-D-galactopyranosyl-(1→2)-α-L-arabinopyranosyl]-14-carboxyl-anosta-8,25-diene-3β,24-diols, respectively.

Eryloside F_4 (**4**) was analyzed for the molecular formula $C_{41}H_{64}O_{13}$ by HRTOFMSES (+) (pseudomolecular peak at *m/z* 787.4272 $[M + Na]^+$) and ^{13}C NMR data. In accordance with NMR data (Tables 4, 5) glycoside **4** differed from **2** and **3** in the presence of a 24-ketone instead of a 24-hydroxyl group. The chemical shift of the carbonyl carbon at δ_C 202.0 and HMBC correlations H-26 (δ_H 5.70 s, 5.97 s)/C-24 (δ_C 202.0), C-27 (δ_C 17.6) and H_3-27 (δ_H 1.90 s)/C-24, C-25 (δ_C 144.4) and C-26 (δ_C 124.3) confirmed this suggestion. From all these data, eryloside F_4 (**4**) was structurally identified as 3β-*O*-[β-D-galactopyranosyl-(1→2)-α-L-arabinopyranosyl]-14-carboxy-3β-hydroxy-lanosta-8,25-dien-24-one.

Both new triosides designated as erylosides M (**5**) and N (**6**) contained identical carbohydrate chains (Table 2). The trisaccharide nature of **5** and **6** was evident from the signals of three anomeric carbons at δ_C 104.7 (CH), 104.9 (CH), and 105.0 (CH) and those of the corresponding protons at δ_H 5.17, 4.83, and 5.30 in the NMR spectra. The pyranose nature of the sugars and β-glycosidic linkages between monosaccharide units (α- for L-arabinoses) as well as the identification of sugar units as L-arabinose and D-galactose (2:1) were determined in the same manner as for **1**−**4**. Their arrangement was established by extensive NMR experiments. The long-range correlations of H2-Ara1 with C1-Gal and H3-Ara1 with C1-Ara2 along with NOESY cross-peaks between H2-Ara1 and H1-Gal, H3-Ara1, and H1-Ara2 defined the 1,3-linkage between the two arabinoses and 1,2-linkage between galactose and Ara1 (Table 2). Thus, this moiety was shown to be a branched assembly of two units of L-arabinose and one unit of D-galactose, containing an additional L-arabinose unit attached to C-3 of Ara1 when compared with the carbohydrate chains of **1**−**4** (Table 1).

Eryloside M (**5**) analyzed for $C_{47}H_{76}O_{16}$ by combined HR-MALDI-TOFMS (*m/z* 919.5138 $[M + Na]^+$) and ^{13}C NMR spectrometry. The spectroscopic data of the aglycon moiety of **5** were highly compatible with those obtained for **1**. The positioning of two double bonds as $\Delta^{8(9)}$ and $\Delta^{24(31)}$ as well as the presence of a carboxyl group at C-14 were established in the same manner as described above for eryloside F_1 (Tables 4, 5). The attachment of a carbohydrate chain to C-3 was confirmed by cross-peaks H-3 (δ_H 3.12 dd)/H1-Ara1 (δ_H 4.83 dd) and H1-Ara1/C-3 (δ_C 88.4−88.5) in the NOESY and HMBC spectra, respectively. Therefore, the structure of eryloside M (**5**) was represented as 3β-*O*-{[β-D-galactopyranosyl-(1→2)]-[α-L-arabinopyranosyl-(1→3)]-α-L-arabinopyranosyl}-14-carboxy-24-methylenelanost-8(9)-en-3β-ol.

Eryloside N (**6**) had the molecular formula $C_{46}H_{74}O_{16}$, which was determined by high-resolution MALDI-TOFMS (*m/z* 905.4870 $[M + Na]^+$) and spectroscopic data. The general features of the ^{1}H and ^{13}C NMR spectra of the aglycon part of **6** closely resembled those of eryloside M (**5**) (Tables 4−6) with the exception of proton and carbon signals belonging to the side chain. Positioning of the double bond in the side chain as $\Delta^{24(25)}$ followed from HMBC cross-peaks H-24/C-26 (δ_C 17.4), C-27 (δ_C 25.5) and H_3-26 (δ_H 1.60 brs)/C-24 (δ_C 125.5), C-25 (δ_C 130.6), and C-27. Stereochemical peculiarities of the lanosterol-derived core of **6** were confirmed by NOESY correlations H-5 (δ_H 1.25 m)/H-3 (δ_H 3.12 dd) and H_3-18/H-20 (δ_H 1.56 m) and H_3-21 (δ_H 1.05 d)/H-12 (δ_H 1.82 m). On the basis of all these data and comparison with literature[7,8] the aglycon of **6** was identified as penasterol. The attachment of the carbohydrate chain to C-3 was confirmed by cross-peaks H-3 (δ_H 3.12 dd)/H1-Ara1 (δ_H 4.83 d) in the NOESY spectrum. Taking into account that **5** and **6** had identical carbohydrate chains, the structure of **6** was represented as 3β-*O*-{[β-D-galactopyranosyl-(1→2)]-[α-L-arabinopyranosyl-(1→3)]-α-L-arabinopyranosyl}-14-carboxylanosta-8,24-dien-3β-ol.

Eryloside O (**7**) analyzed for $C_{54}H_{86}NO_{20}$ on the basis of combined HR-MALDI-TOFMS (*m/z* 1092.5827 $[M + Na]^+$) and ^{13}C NMR analysis. NMR spectra exhibited the tetrasaccharide nature of this molecule. In fact, there were signals of four anomeric carbons at δ_C 101.6 (CH), 105.1 (CH), 106.2 (CH), and 107.6 (CH) and those of the corresponding protons at δ_H 5.50, 4.67, 5.01, and 5.07 (Table 3). Identifications of sugars as three arabinose units and one 2-*N*-acetylglucosamine unit were based on GC and NMR data. The D-configuration of 2-*N*-acetylglucosamine and the L-configurations of arabinoses were determined after acid hydrolysis by preparation of acetylated (−)- and (+)-2-octylglycosides followed by GC and comparison with those obtained from standard sugars.[20] The pyranose nature of the sugars and configurations of glycosidic bonds were determined in the same manner as described above. The arrangement of sugar units was established by extensive NMR experiments. Assignments of proton and carbon signals of the carbohydrate chain were carried out by DEPT, HSQC, 1D-TOCSY, and COSY-45 NMR spectra. With this information in hand, NOESY and HMBC data were used to link carbohydrate units together. The long-range correlations of H1-2Ara with C3-Ara1 and H1-NAc-Glc with C2-Ara1 along with NOESY cross-peaks between H2-Ara1 and H1-NAc-Glc, H3-Ara1 and H1-Ara2 (Table 3) defined the 1,3-linkage between arabinose-2 and arabinose-1 and the 1,2-linkage between 2-*N*-acetyl-D-glucosamine and arabinose-

Table 3. ^{1}H and ^{13}C NMR Data for the Carbohydrate Moiety of Eryloside **7** in C_5D_5N

position	δ_C	δ_H (*J* in Hz)	HMBC	NOESY
Ara1 (1→C−3)				
1	105.1 CH	4.67 d (8.1)		H-3, H5-Ara1
2	76.1 CH	4.67 m	C2-Ara1	H1-NAc-Glc
3	84.6 CH	4.22 dd (2.6, 8.6)	C2-Ara1	H1-Ara1, H1-Ara2
4	69.3 CH	4.58 brs		
5	66.4 CH_2	4.22 m	C1,4-Ara1	
		3.76 brd (11.3)	C1,4-Ara1	H1-Ara1
Ara2 (1→3Ara1)				
1	106.2 CH	5.01 d (7.7)	C3-Ara−1	H3,5-Ara2, H3-Ara1
2	73.2 CH	4.43 brt (9.5)	C1,3-Ara2	
3	74.6 CH	4.15 brd (10.5)		H1-Ara2
4	**79.8** CH	4.40 brd (3.6)	C2-Ara2, C1-Ara3	H1-Ara-3
5	66.7 CH_2	4.54 dd (2.0, 12.5)	C1,3,4-Ara2	
		3.89 brd (11.6)	C1,4-Ara2	H1-Ara2
Ara3 (1→4Ara2)				
1	107.6 CH	5.07 d (7.6)	C4-Ara2	H3,5-Ara3, H4-Ara2
2	72.9 CH	4.51 dd (7.7, 9.1)	C1-Ara3	
3	74.5 CH	4.12 dd (3.5, 9.2)	C2-Ara3	H1-Ara3
4	69.6 CH	4.28 brs	C2-Ara3	
5	67.4 CH_2	4.33 dd (2.2, 12.4)	C1,3,4-Ara3	
		3.78 brs (11.6)	C1,4-Ara3	H1-Ara3
NAc-Glc (1→2Ara1)				
1	101.6 CH	5.50 d (8.5)	C2-Ara1	H3,5-NAc-Glc, H2-Ara1
2	57.2 CH	4.63 brq (9.0)	C1,3-NAc-Glc	
3	78.1 CH	4.00 t (9.8)	C2,4-NAc-Glc	H1,5-NAc-Glc
4	72.9 CH	4.07 t (9.2)	C3,5,6-NAc-Glc	
5	76.6 CH	3.21 m		H1,3-NAc-Glc
6	63.2 CH_2	4.24 m		
		4.14 m		
NH		8.66 d (9.4)		
Ac	23.5			
	170.3			

Table 4. ^{13}C NMR Data in C_5D_5N for the Aglycon Moiety of Erylosides (**1**−**9**)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	35.3 CH_2	35.3 CH_2	35.3 CH_2	35.3 CH_2	35.3 CH_2	35.3 CH_2	35.3 CH_2	35.3 CH_2	35.3 CH_2
2	26.8 CH_2	26.8 CH_2	26.8 CH_2	26.8 CH_2	26.9 CH_2	26.9 CH_2	26.9 CH_2	26.9 CH_2	26.9 CH_2
3	88.4 CH	88.4 CH	88.4 CH	88.4 CH	88.5 CH	88.4 CH	88.8 CH	88.6 CH	88.6 CH
4	39.4 C	39.4 C	39.4 C	39.4 C	39.6 C	39.6 C	39.5 C	39.6 C	39.6 C
5	50.1 CH	50.2 CH	50.2 CH	50.2 CH	50.3CH	50.2 CH	50.3 CH	50.2 CH	50.3 CH
6	18.4 CH_2	18.4 CH_2	18.4 CH_2	18.4 CH_2	18.4 CH_2	18.4 CH_2	18.4 CH_2	18.4 CH_2	18.6 CH_2
7	27.9 CH_2	27.9 CH_2	27.9 CH_2	27.9 CH_2	27.9 CH_2	27.9 CH_2	27.9 CH	27.9 CH	27.9 CH
8	128.0 C	128.0 C	128.0 C	128.0 C	128.0 C	127.9 C	128.0 CH_2	127.9 CH_2	127.9 CH_2
9	139.8 C	139.8 C	139.8 C	139.8 C	139.9 C	139.9 C	139.9 C	139.9 C	139.9 C
10	37.4 C	37.4 C	37.4 C	37.4 C	37.4 C	37.4 C	37.4 C	37.4 C	37.4 C
11	22.5 CH_2	22.5 CH_2	22.5 CH_2	22.5 CH_2	22.5 CH_2	22.5 CH_2	22.5 CH_2	22.5 CH_2	22.5 CH_2
12	31.7 CH_2	31.7 CH_2	31.7 CH_2	31.6 CH_2	31.7 CH_2	31.7 CH_2	31.7 CH_2	31.7 CH_2	31.7 CH_2
13	47.0 C	46.9 C	46.9 C	46.9 C	47.0 C	46.9 C	47.0 C	46.9 C	46.9 C
14	62.8 C	62.8 C	62.8 C	62.7 C	62.8 C	62.8 C	62.8 C	62.8 C	62.8 C
15	28.3 CH_2	28.3 CH_2	28.3 CH_2	28.3 CH_2	28.3 CH_2	28.3 CH_2	28.3 CH_2	28.3 CH_2	28.3 CH_2
16	29.5 CH_2	29.5 CH_2	29.5 CH_2	29.4 CH_2	29.5 CH_2	29.5 CH_2	29.5 CH_2	29.5 CH_2	29.5 CH_2
17	51.0 CH	51.2 CH	51.1 CH	51.0 CH	51.0 CH	51.0 CH	51.0 CH	51.0 CH	51.0 CH
18	17.9 CH_3	17.9 CH_3	17.9 CH_3	17.9 CH_3	17.9 CH_3	17.9 CH_3	17.9 CH_3	17.9 CH_3	17.9 CH_3
19	19.6 CH_3	19.6 CH_3	19.6 CH_3	19.5 CH_3	19.6 CH_3	19.6 CH_3	19.5 CH_3	19.6 CH_3	19.6 CH_3
20	36.3 CH	36.3 CH	36.4 CH	36.0 CH	36.3 CH	36.0 CH	36.3 CH	36.3 CH	36.0 CH
21	18.6 CH_3	18.8 CH_3	18.8 CH_3	18.4 CH_3	18.6 CH_3	18.5 CH_3	18.6 CH_3	18.6 CH_3	18.6 CH_3
22	35.0 CH_2	32.3 CH_2	32.4[a] CH_2	31.1 CH_2	35.0 CH_2	36.3 CH_2	35.0 CH_2	35.0 CH_2	36.3 CH_2
23	31.4 CH_2	32.6 CH_2	32.5[a] CH_2	34.7 CH_2	31.4 CH_2	25.0 CH_2	31.4 CH_2	31.4 CH_2	25.0 CH_2
24	156.4 C	75.4 CH	75.9 CH	202.0 C	156.5 C	125.5 C	156.5 C	156.4 C	125.5 CH
25	33.8 CH	149.7 C	149.7 C	144.4 C	33.8 CH	130.6 C	33.8 CH_3	33.8 CH_3	130.6 C
26	21.7 CH_3	109.9 CH_2	110.2 CH_2	124.3 CH_2	21.7 CH_3	17.4 CH_3	21.7 CH_3	21.7 CH_3	17.4 CH_3
27	21.9 CH_3	17.9 CH_3	17.5 CH_3	17.6 CH_3	21.9 CH_3	25.5 CH_3	21.8 CH_3	21.9 CH_3	25.6 CH_3
28	27.6 CH_3	27.6 CH_3	27.6 CH_3	27.6 CH_3	27.6 CH_3	27.5 CH_3	27.5 CH_3	27.5 CH_3	27.5 CH_3
29	16.4 CH_3	16.4 CH_3	16.4 CH_3	16.4 CH_3	16.4 CH_3	16.4 CH_3	16.4 CH_3	16.4 CH_3	16.4 CH_3
30	178.3 C	178.2 C	178.2 C	178.2 C	178.2 C	178.3 C	178.2 C	178.2 C	178.2 C
31	106.4 CH_2				106.4 CH_2		106.4 CH_2	106.4 CH_2	

[a] Interchangeable signals.

1. The HMBC cross-peak H1-Ara3 (δ_H 5.07)/C4-Ara2 (δ_C 79.8) as well as the NOESY correlation H1-Ara3/H4-Ara2 (δ_H 4.40) showed that arabinose-3 was bonded with arabinose-2 by a 1,4-linkage. On the basis of these data the carbohydrate moiety was shown to be a branched assembly of three units of L-arabinose and one unit of 2-*N*-acetyl-D-glucosamine. It contains an additional L-arabinose unit attached to C-4 of arabinose-2 and 2-*N*-acetyl-D-glucosamine instead of galactose, when compared with the carbohydrate chains of **5** and **6**.The spectroscopic data of the aglycon moiety of **7** were highly compatible with those obtained for **1** and

Table 5. ^{1}H NMR Data in C_5D_5N for the Aglycon Moiety of **1**−**5**

	1		**2**		**3**		**4**		**5**		
	δ_H (*J*, Hz)	HMBC	δ_H (*J*, Hz)	HMBC	δ_H (*J*, Hz)	HMBC	δ_H (*J*, Hz)	HMBC	δ_H (*J*, Hz)	NOESY	HMBC
1	1.28 m		1.28 m		1.29 m		1.28 m		1.71 m	H-11	
	1.73 m		1.72 m		1.73 m		1.72 m		1.29 m		
2	1.88 m		1.88 m		1.86 m		1.87 m		2.09 m		
	2.14 m		2.13 m		2.14 m		2.13 m		1.86 m		
3	3.12 dd (4.5, 12.0)		3.11 dd (4.5, 11.9)		3.11 dd (4.4, 11.9)		3.12 dd (4.3, 11.7)		3.12 dd (4.2,11.7)	H-5, H1-Ara1	C-4,28,29
5	1.24 dd (2.0, 12.8)		1.24 dd (2.1, 12.6)		1.24 dd (2.0, 12.4)		1.24 dd (2.0, 12.5)		1.26 dd (2.1,12.5)	H-3	
6	1.55 m		1.54 m		1.53 m		1.54 m		1.70 m		
	1.70 m		1.68 m		1.68 m		1.68 m		1.54 m		
7	2.26 m		2.25 m		2.24 m		2.25 m		2.34 m		
	2.33 m		2.32 m		2.32 m		2.31 m		2.24 m		
11	2.17 m		2.16 m		2.17 m		2.15 m		2.42 m	H-1	
	2.41 m		2.41 m		2.41 m		2.40 m		2.16 m		
12	1.83 m		1.82 m		1.82 m		1.80 m		2.83 m	H-17	
	2.83 m		2.82 m		2.82 m		2.80 m		1.83 m	H_3-21	
15	1.76 m	C-30	1.75 m	C-30	1.74 m	C-30	1.73 m	C-30	2.53 m		C-30
	2.52 m		2.51 m		2.50 m		2.50 m		1.75 m		C-30
16	1.52 m		1.57 m		1.56 m		1.57 m		2.40 m		
	2.40 m		2.44 m		2.43 m		2.40 m		1.52 m		
17	2.03 brq		2.06 brq		2.06 brq		2.02 brq		2.03 brq	H-12	
	(9.9)		(10.0)		(9.5)		(10.0)		(9.7)		
18	0.92 s	C-12,13,14,17	0.91 s	C-12,13,14,17	0.90 s	C-12,13,14,17	0.89 s	C-12,13,14,17	0.91s	H-20	C-12,13,14,17
19	1.09 s	C-1,5,9,10	1.09 s	C-1,5,9,10	1.09 s	C-1,5,9,10	1.08 s	C-1,5,9,10	1.08s		C-1,5,9,10
20	1.56 m		1.63 m		1.61 m		1.55 m		1.56 m	H-18	
21	1.06 d (7.0)	C-17,20,22	1.08 d (6.5)	C-17,20,22	1.07 d (6.5)	C-17,20,22	1.01 d (6.5)	C-17,20,22	1.05 d (6.5)	H-12	C-17,20,22
22	1.20 m		1.41 m		1.19 m		1.38 m		1.69 m		
	1.69 m		1.74 m		1.88 m		1.96 m		1.20 m		
23	1.96 m		1.70 m		1.75 m		2.67 m		2.20 m		
	2.20 m		1.94 m		1.93 m		2.78 m		1.96 m		
24			4.35 m		4.34 m						
25	2.24 m								2.23 m		C-23,24,26,31
26	1.04 d (6.5)	C-24,25,27	4.95 m	C-27, C-24	4.94 m	C-27, C-24	5.70 s	C-24, C-27	1.04 d (6.5)		
			5.25 d (2.5)	C-24, C-27	5.20 d (2.5)	C-24, C-27	5.97 s	C-27, C-24			
27	1.03 d (6.5)		1.90 s	C-24,25,26	1.90 s	C-24,25,26	1.90 s	C-24,25,26	1.03 d (6.5)		C-24,25,26
28	1.19 s	C-3,4,5,29	1.18 s	C-3,4,5,29	1.18 s	C-3,4,5,29	1.18 s	C-3,4,5,29	1.23 s		C-3,4,5,29
29	1.11 s	C-3,4,5,28	1.11 s	C-3,4,5,28	1.11 s	C-3,4,5,28	1.11 s	C-3,4,5,28	1.17 s		C-3,4,5,28
31	4.81 d (1.4)	C-23,24							4.84 brs		C-23,24,25
	4.84 s	C-25							4.81 brd		C-23,24,25
									(1.4)		

Table 6. ^{1}H NMR Data in C_5D_5N for the Aglycon Moiety of **6**−**9**

	6			**7**			**8**			**9**		
	δ_H	NOESY	HMBC	δ_H	NOESY	HMBC	δ_H	NOESY	HMBC	δ_H	NOESY	HMBC
1	1.71 m	H-11		1.70 m			1.72 m	H_3-19		1.72 m		
	1.29 m			1.26 m			1.29 m			1.28 m		
2	2.09 m			2.07 m			2.10 m			2.09 m	H-11	
	1.87 m			1.84 m			1.88 m			1.87 m		
3	3.12 dd (4.3, 11.8)	H-5, H1-Ara1	C-4,28,29	3.07 dd (4.4, 11.9)	H-5, H1-Ara1	C3-Ara1 C-28,29	3.11 dd (4.2,11.8)	H-5, H1-Ara1	C-4,28,29	3.1 dd (4.0,11.5)	H-5 H1-Ara1	C-4,28,29
5	1.25 dd (2.0, 12.4)	H-3		1.24 m	H-3		1.26 m	H-3		1.25 m	H-3	
6	1.70 m			1.72 m			1.70 m			1.70 m		
	1.54 m			1.54 m	H_3-29		1.54 m	H-29		1.54 m		
7	2.34 m			2.36 m			2.33 m			2.34 m	H-15	
	2.25 m			2.26 m			2.25 m			2.25 m		
11	2.42 m	H-1		2.42 m			2.41 m			2.41 m	H-1	
	2.16 m			2.15 m			2.15 m			2.14 m		
12	2.82 m	H-17		2.82 m	H-17		2.82 m	H_3-21	C-18	2.81 m	H-17	C-13,18
	1.82 m	H_3-21		1.83 m	H_3-21		1.83 m	H_3-21	C-13,14,18	1.82 m	H_3-21	
15	2.53 m		C-30	2.53 m		C-13,30	2.52 m		C-13,14,30	2.52 m		C-13
	1.74 m		C-30	1.76 m			1.75 m		C-8,30	1.75 m		
16	2.40 m			2.40 m			2.40 m			2.39 m	H-7	
	1.52 m			1.51 m			1.52 m			1.52 m		
17	2.01 brq (9.4)	H-12		2.04 m	H-12	C-13,18	2.03 brq (9.0)	H-12	C-13,20,21	2.01 brq (9.4)	H-12	C-13,16,18,20
18	0.90s	H-20	C-12,13,14,17	0.90 s	H-20	C-12,13,14,17	0.90 s	H-20	C-12,13,14,17	0.90 s	H-20	C-12,13,14,17
19	1.07s		C-1,5,9,10	1.06 s		C-1,5,9,10	1.08 s	H-1	C-1,5,9,10	1.08 s		C-1,5,9,10
20	1.56 m	H-18		1.56 m	H_3-18		1.56 m			1.56 m	H_3-18	
21	1.05 d (6.5)	H-12	C-17,20,22	1.05 d (6.5)	H-12	C-17,20,22	1.05 d (6.5)			1.05 d (6.5)	H-12	C-17,20,22
22	1.56 m			1.70 m			1.71 m			1.56 m		
	1.12 m			1.22 m			1.22 m			1.10 m		
23	2.13 m			2.20 m			2.20 m			2.12 m		
	1.95 m			1.97 m		C-31	1.97 m			1.96 m		
24	5.21 brt (7.0)		C-22,26,27							5.20 brt (7.0)		C-23 26,27
25				2.24 m		C-23,24,26,31	2.24 m		C-23,24,26,31			
26	1.60 brs		C-24,25,27	1.04 d (6.5)		C-24,25,27	1.04 d (6.5)		C-24,25,27	1.60 brs		C-24,25,27
27	1.68 brs		C-24,25,26	1.03 d (6.5)		C-24,25,26	1.03 d (6.5)		C-24,25,26	1.68 brs		C-24,25,26
28	1.23 s		C-3,4,5,29	1.21 s		C-3,4,5,29	1.24 s		C-3,4,5,29	1.23 s		C-3,4,5,29
29	1.17 s		C-3,4,5,28	1.13 s	H-6	C-3,4,5,28	1.18 s		C-3,4,5,28	1.17 s		C-3,4,5,28
31				4.84 brs		C-23,25	4.84 brs		C-23,24,25			
				4.81 brs		C-23,25	4.81 brs		C-23,24,25			

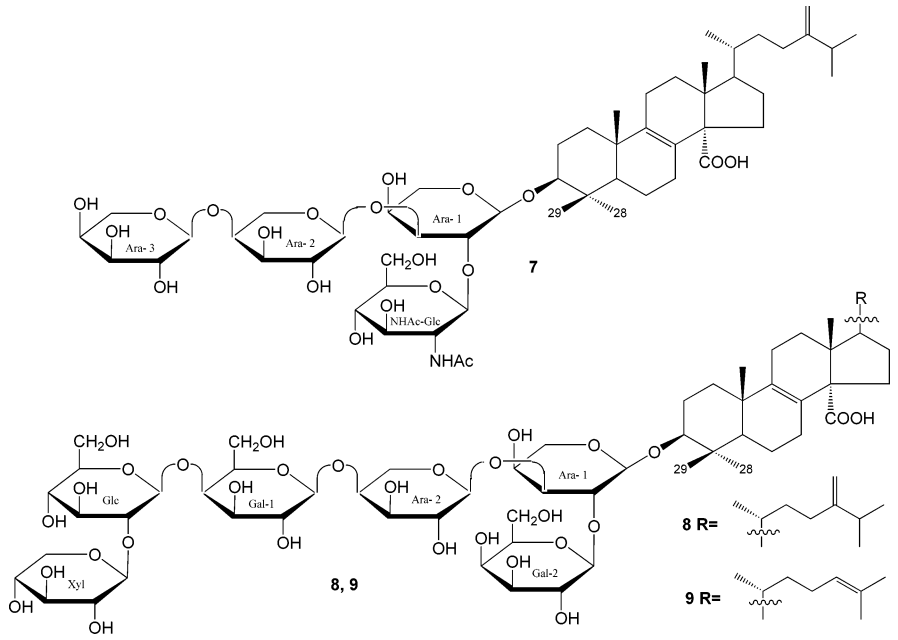

Figure 2. Structures of erylosides **7**, **8**, and **9**.

5. The identity of aglycons in these glycosides was confirmed by HMBC and NOESY data (Table 6). The attachment of a carbohydrate chain to C-3 was indicated from the H-3 (δ_H 3.07)/H1-Ara1 (δ_H 4.67) correlation in the NOESY spectrum. Thus, the structure of eryloside O (**7**) was represented as 3*β*-*O*-{[α-L-arabinopyranosyl-(1→4)-α-L-arabinopyranosyl-(1→3)]-[*β*-D-2-*N*-acetyl- D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-14-carboxy-24-methylenelanost-8(9)-en-3*β*-ol.

The molecular formulas of erylosides P (**8**) and Q (**9**) were deduced as $C_{64}H_{104}O_{30}$ and $C_{63}H_{102}O_{30}$, respectively, by HR-MALDI-TOFMS (*m*/*z* 1375.6443 $[M + Na]^+$ and 1361.6402 $[M + Na]^+$, respectively) and ^{13}C NMR spectra. The NMR spectra of **8** and **9** indicated that both compounds contained identical carbohydrate moieties (Table 7). Analysis of ^{13}C and ^1H NMR spectra revealed the presence of six signals attributable to anomeric carbons (δ_C 105.1, 104.6, 106.2, 105.0, 107.7, and 104.8) along with six signals of the corresponding protons [δ_H 4.79 d (5.7), 5.21 d (6.8), 5.10 d (7.7), 5.16 d (7.8), 4.91 d (7.5), 5.32 d (7.9)], indicating the hexasaccharide nature of this carbohydrate chain. Hydrolysis of these compounds gave arabinose, galactose, glucose, and xylose, 2:2:1:1, identified by GC in the form of their corresponding peracetates of the aldononitriles. The D-configurations of galactose and xylose and L-configuration of arabinose were determined after acid hydrolysis by preparation of acetylated (−)-2-octyl glycosides followed by GC and comparison with those obtained from standard D- and L-sugars.[20] All sugars were in the pyranose forms and attached to each other by *β*-glycosidic bonds beside L-arabinose units which had α-glycosidic bonds. This was established on the basis of NMR data in the same manner as was used for the structure elucidation of **1**−**7** (Table 7). The arrangement of sugar units as well as positions of the attachment of carbohydrate moieties to aglycons were established by a combination of the HMBC and NOESY experiments. The mutual HMBC correlations between the methine at C-3 (88.6) and an anomeric methine (δ_H 4.79) in the NMR spectra of both glycosides allowed us to place a glycosidic linkage at this position. Two-dimensional NMR experiments including COSY, DEPT, HSQC, HMQC-TOCSY, 1D-TOCSY, HMBC, and NOESY indicated that the carbohydrate chain includes the same trisaccharide fragment as in **5** and **6**. Actually, the 1,2-linkage between galactose-2 and arabinose-1 was confirmed by cross-peaks H1-Gal2 (δ_H 5.32)/C2-Ara1 (δ_C 77.0) and H1-Gal2 and H2-Ara1 (δ_H 4.74) in the HMBC and NOESY experiments, respectively. The 1,3-linkage between arabinose-2 and arabinose-1 was established by mutual HMBC correlations between two methines at C1-Ara2 (δ_H 5.21) and C3-Ara1 (δ_C 81.9) and confirmed by a cross-peak of H1-Ara2 (δ_H 5.21)/H3-Ara1 (δ_H 4.28) in the NOESY spectrum. In addition, the 1,2-linkage between the end xylose and glucose-4 was established by cross-peaks of H2-Glc (δ_H 4.13)/C1-Xyl (δ_C 107.7) and H1-Xyl (δ_H 4.91)/H2-Glc in the HMBC and NOESY spectra, respectively. The 1,4-linkage between glucose-4 and galactose-1 was indicated by the corresponding correlations H4-Gal1 (δ_H 4.52)/C1-Glc (δ_C 105.0) and H1-Glc (δ_H 5.16)/H4-Gal1. Finally, the presence of a 1,3-linkage between galactose-1 and arabinose-2 followed from the HMBC cross-peak H1-Gal1 (δ_H 5.10)/C3-Ara2 (δ_C 83.2) and NOESY cross-peak H1-Gal1/H3-Ara2 (Table 7). Therefore, the carbohydrate moiety proved to be a branched assembly of two D-galactoses, two L-arabinoses, one D-glucose, and one D-xylose, containing an additional trisaccharide fragment attached to C-3 of arabinose-2, when compared with the carbohydrate chains of **5** and **6**.

The aglycon moieties of **8** and **9** were deduced by extensive NMR spectroscopy (^1H and ^{13}C NMR, $^1H-^1H$ COSY, HMBC, and NOESY) (Tables 2−4) to be the same as that of **1** (either **5** or **7**) and **6**, respectively. Thus, the structures of erylosides P and Q were established as 3*β*-*O*-{[*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→3)]-[*β*-D-galactopyranosyl-(1→2)]-α-L-arabinopyranosyl}-14-carboxy-24-methylenelanost-8(9)-en-3*β*-ol (**8**) and 3*β*-*O*-{[*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl-(1→3)-α-L-arabinopyranosyl-(1→3)]-[*β*-D-galactopyranosyl-(1→2)]-α-L-arabinopyranosyl}-14-carboxylanosta-8,24-dien-3*β*-ol (**9**).

The structures of oligoglycosides from some marine invertebrates, such as holothurians, are used as chemotaxonomic markers on different taxonomic levels (species, genus, subfamily). As it was established by us earlier, holothurians belonging to the same taxon show characteristic structural features of glycosides independent of location of collection and season.[21] It seems that similar taxonomic specificity exists also for erylosides. Actually, all the glycosides from *E. formosus* collected in different geographic areas[8] demonstrate structural similarity to each other. They contain the related 14-carboxylated aglycons of the lanostane series and carbohydrate chains, comprising L-arabinose as the first monosaccharide unit. Similar structural features were found for glycosides from some other species belonging to the same genus,[5] in contrast

Table 7. ^{13}C and ^{1}H NMR Data for Carbohydrate Moieties of Erylosides **8** and **9**[a] in C_5D_5N

position	δ_C	δ_H (*J* in Hz)	NOESY	HMBC		δ_C	δ_H (*J* in Hz)	NOESY	HMBC
Ara1 (1→C-3)					Glc (1→4Gal1)				
1	105.1 CH	4.79 d (5.7)	H-3, H5-Ara1	C-3	1	105.0 CH	5.16 d (7.8)	H4-Gal1, H3,5-Glc	C4-Gal1
2	77.0 CH	4.74 m	H6-Ara1	C1,3-Ara1	2	86.2 CH	4.13 t (8.4)	H1-Xyl	C1-Glc, C1-Xyl
3	81.9 CH	4.28 dd (2.9, 8.7)	H1-Ara2		3	77.7[b]CH	4.27 m	H1-Glc	C4-Glc
4	68.3 CH	4.44 m			4	72.0 CH	3.99 m		
5	65.3 CH_2	3.74 brd (10.4)	H1-Ara1	C1-Ara1	5	77.8[b] CH	3.99 m	H1-Glc	C4-Glc
		4.24 m		C4-Ara1					
					6	63.0 CH_2	4.13 m		
							4.66 m		
Ara2 (1→3Ara1)					Xyl (1→2Glc)				
1	104.6 CH	5.21 d (6.8)	H3-Ara1, H3,5-Ara2	C3-Ara1	1	107.7 CH	4.91 d (7.5)	H3,5-Xyl, H2-Glc	C2-Glc
2	71.0 CH	4.64 dd (6.7, 8.8)		C1,3-Ara2	2	76.3 CH	3.97 t (8.2)		C1,3-Xyl
3	83.2 CH	4.24 dd (2.8, 8.9)	H1-Ara2, H1-Gal1	C1-Gal1	3	77.6 CH	3.92 t (8.8)	H1,5-Xyl	C2,4-Xyl
4	68.3 CH	4.44 brd (4.0)			4	70.1 CH	3.87 m		C3-Xyl
5	66.3 CH	3.71 brd (11.1)	H1-Ara2	C1-Ara2	5	66.9 CH_2	3.39 dd (9.5, 11.5)	H1,3-Xyl	C1,3,4-Xyl
		4.23 m		C3-Ara2			4.29 dd (5.1, 11.1)		
Gal1 (1→3Ara2)					Gal2 (1→2Ara1)				
1	106.2 CH	5.10 d (7.7)	H3,5-Gal1, H3-Ara2	C2-Gal1	1	104.8 CH	5.32 d (7.9)	H3,5-Gal2, H2-Ara1	C2-Ara1
2	73.4 CH	4.40 dd (7.7, 9.7)		C1,4-Gal1	2	73.3 CH	4.45 t (8.3)		C3-Gal2
3	75.0 CH	4.02 dd (4.2, 9.8)	H1- Gal1		3	75.2 CH	4.05 dd (4.0, 9.6)	H1-Gal2	C2-Gal2
4	80.1 CH	4.52 brd (3.9)	H1-Glc	C1-Glc, C2-Gal1	4	69.4 CH	4.55 brd (3.6)		C2-Gal2
5	75.3 CH	3.93 m	H1-Gal3	C4,6-Gal1	5	76.0 CH	3.79 brt (6.5)	H1-$Gal2_1$	C1,4,6-Gal2
6	60.0 CH	4.15 m		C4,5-Gal1	6	61.2 CH_2	4.27 m		C4-Gal2
		4.72 t (9.7)		C4,5-Gal1			4.47 m		C5-Gal2

[a] All assignments were given for **8**; spectra of **9** had only minor differences in chemical shifts and spin-coupling values. [b] Interchangeable signals.

to another group of species including *E. lendenfeldi*,[1,22] *E. goffrilleri*,[3] and probably other related species.

In contrast with its epimer **2**, compound **3** induced apoptosis of Ehrlich carcinoma cells at a concentration of 100 μg/mL. Compounds **1** and **10** at a concentration of 100 μg/mL were found to activate Ca^{2+} influx into mouse spleenocytes up to 130% of the control.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Perkin-Elmer 343 polarimeter. The ^{1}H and ^{13}C NMR spectra were recorded in C_5D_5N on a Bruker DRX-500 spectrometer at 500 and 125.8 MHz, respectively, using tetramethylsilane as an internal standard. HRESIMS spectra were recorded on a Micromass Q-TOF MICRO spectrometer coupled with an HPLC Waters Alliance 2695 equipped with a Waters 2996 photodiode and a Rheodyne 7725i injector. The instrument was calibrated for high-resolution measurements by using a PEG mixture from 200 to 1000 MW (resolution specification 5000 fwhm, deviation <5 ppm rms in the presence of a known lock mass). 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 α-cyano-4-hydroxycinnamic acid as matrix. GC analyses were performed on an Agilent 6850 Series GC system equipped with a HP-5MS column at a temperature program of 100 to 250 °C at 5 °C min^{-1}; temperatures of injector and detector were 150 °C and 270 °C, respectively. Low-pressure column liquid chromatography was performed using Polychrome-1 (powder Teflon, Biolar, Latvia), Sephadex LH-20 (Sigma, Chemical Co), and silica gel L (40/100 μm, Chemapol, Praha, Czech Republic). Glass plates, 4.5 × 6.0 cm, precoated with silica gel (5−17 μm, Sorbfil, Russia) were used for TLC. Preparative HPLC was carried out on a Dupont-8800 chromatograph, using Silasorb-ODS (10 μm, 9.6 × 200 mm), Diasphere-110-C18 (5 μm, 10 × 250 mm), and YMC-Pack ODS-A (5 μm, 10 × 250 mm) columns with an RIDK refractometer detector.

Animal Material. The sponge *Erylus formosus* was collected off Puerto Morelos (the Caribbean Sea, Mexico) by scuba diving from a depth of 15 m. A voucher specimen is on deposit in the collection of the Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia.

Extraction and Isolation. The lyophilized specimens (0.3 kg) were macerated and extracted with EtOH (4 × 1 L). The EtOH extract concentrated *in vacuo* was separated by low-pressure reversed-phase CC (the column 20 × 8 cm) on Teflon powder Polychrome-1 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. The obtained fraction after evaporation *in vacuo* (40 g) was subjected to silica gel flash CC (7 × 10 cm) with a solvent gradient system of increasing polarity from 3% to 30% MeOH in $CHCl_3$ (total volume 3 L). Fractions of 10 mL were collected and combined by TLC examination to obtain four subfractions. The subfraction I (140 mg) was further purified and separated by normal-phase HPLC on a Supelco Sil column using $CHCl_3$−MeOH (65:7) as eluent and then by reversed-phase HPLC on a Supelco C-18 column eluting with MeOH−H_2O (72:28) and repeatedly with MeOH−H_2O (80:20) to yield erylosides F_1 (**1**) (3 mg), F_2 (**2**) (2 mg), F_3 (**3**) (3.5 mg), F_4 (**4**) (6 mg), and F (**10**) (4.5 mg.). Subfraction II (320 mg) was subjected to HPLC on a Supelco C-18 column with MeOH−H_2O (90:10) to give erylosides M (**5**) (11.0 mg), N (**6**) (13.0 mg), and H (**11**) (3.0 mg). Erylosides O (**7**) (9 mg), P (**8**) (21 mg), and Q (**9**) (34 mg) were obtained from subfraction III (540 mg) by HPLC on a Supelco C-18 column using the solvent system MeOH−H_2O (95:5) and then by HPLC on a Supelco Sil using $CHCl_3$−MeOH (65:30) as the solvent system.

Eryloside F_1 (1): amorphous solid, 3.0 mg (0.005% of dry weight); $[\alpha]_D^{25}$ −23.0 (*c* 0.10, MeOH); ^{1}H and ^{13}C NMR data, see Tables 1−3; HR (+) ESTOFMS *m*/*z* 787.4464 $[M + Na]^+$, calcd for $C_{42}H_{68}O_{12}Na$ 787.4603.

Eryloside F_2 (2): amorphous solid, 2.0 mg (0.0033% of dry weight); $[\alpha]_D^{25}$ −32.0 (*c* 0.10, MeOH); ^{1}H and ^{13}C NMR data, see Tables 1−3; HR (+) ESTOFMS *m*/*z* 789.4371 $[M + Na]^+$, calcd for $C_{41}H_{66}O_{13}Na$ 789.4401.

Eryloside F_3 (3): amorphous solid, 3.5 mg (0.0059% of dry weight); $[\alpha]_D^{25}$ −25.0 (*c* 0.10, MeOH); ^{1}H and ^{13}C NMR data, see Tables 1−3; HR (+) ESTOFMS *m/z* 789.4390 $[M + Na]^+$, calcd for $C_{41}H_{66}O_{13}Na$ 789.4401.

Eryloside F_4 (4): amorphous solid, 6.0 mg (0.01% of dry weight); $[\alpha]_D^{25}$ −12.0 (*c* 0.10, MeOH); ^{1}H and ^{13}C NMR data, see Tables 1−3; HR (+) ES TOF MS *m/z* 787.4272 $[M + Na]^+$, calcd for $C_{41}H_{64}O_{13}Na$ 787.4245.

Eryloside M (5): amorphous solid, 11.0 mg (0.018% of dry weight); $[\alpha]_D^{25}$ −10.2 (*c* 0.25, MeOH); ^{1}H and ^{13}C NMR data, see Tables 1−3; HR-MALDI-TOF MS *m/z* 919.5138 $[M + Na]^+$, 875.4 $[M + Na - CO_2]^+$, calcd for $C_{47}H_{76}O_{16}Na$ 919.5026.

Eryloside N (6): amorphous solid, 13.0 mg (0.022% of dry weight); $[\alpha]_D^{25}$ −14.0 (*c* 0.25, MeOH); ^{1}H and ^{13}C NMR data, see Tables 4, 5; HR-MALDI-TOF MS *m/z* 905.4740 $[M + Na]^+$, calcd for $C_{46}H_{74}O_{16}Na$ 905.4870, 861.5 $[M + Na - CO_2]^+$.

Eryloside O (7): amorphous solid, 21.0 mg (0.035% of dry weight); $[\alpha]_D^{25}$ −7.0 (*c* 0.25, MeOH); ^{1}H and ^{13}C NMR data, see Tables 4, 5; HR-MALDI-TOF MS *m/z* 1092.5827 $[M + Na]^+$, calcd for $C_{54}H_{76}O_{87}O_{20}NNa$ 1092.5714.

Eryloside P (8): amorphous solid, 9.0 mg (0.015% of dry weight); $[\alpha]_D^{25}$ −10.2 (*c* 0.25, MeOH); ^{1}H and ^{13}C NMR data, see Tables 4, 5; HR-MALDI-TOF MS *m/z* 1375.6443 $[M + Na]^+$, calcd for $C_{64}H_{104}O_{30}Na$ 1375.6506.

Eryloside Q (9): amorphous solid, 34 mg (0.057% of dry weight); $[\alpha]_D^{25}$ −11.0 (*c* 0.25, MeOH); ^{1}H and ^{13}C NMR data, see Tables 4, 5; HR-MALDI-TOF MS *m/z* 1361.6402 $[M + Na]^+$, calcd for $C_{63}H_{102}O_{30}Na$ 1361.6349, 1317.5 $[M + Na - CO_2]^+$, 1185.5 $[M + Na - CO_2 - C_5H_8O_4]^+$.

Eryloside F (10): amorphous solid, 4.5 mg (0.0075% of dry weight); $[\alpha]^{20}_D$ −41.0 (*c* 0.1, MeOH); HR-MALDI-TOF MS *m/z* 1361.6502 $[M + Na]^+$, calcd for $C_{63}H_{102}O_{30}Na$ 1361.6349. ^{1}H and ^{13}C NMR spectra were identical with those reported in the literature.[4]

Eryloside H (11): amorphous solid, 3.0 mg; $[\alpha]^{20}_D$ −11.2 (*c* 0.1, MeOH); HR-MALDI-TOF MS *m/z* 1361.6502 $[M + Na]^+$, calcd for $C_{63}H_{102}O_{30}Na$ 1361.6349. ^{1}H and ^{13}C NMR spectra were identical with those reported in the literature.[5]

Acidic Hydrolysis of Erylosides F_1−F_4 (1−4). A solution of compounds **1**−**4** (each 1.5 mg) in 2 N HCl (0.5 mL) was heated in a stoppered reaction vial at 100 °C for 2 h. The water layer was extracted with $CHCl_3$ and then neutralized with Dowex (HCO_3^-). The residue obtained after evaporation of the H_2O layer was separated on an Agilent ZORBAX carbohydrate analysis column (5 μm, 4.6 × 250 mm) eluting with CH_3CN-H_2O (75:25) to yield 1.0 mg of D-galactose, $[\alpha]^{20}_D$ +79.0 (*c* 0.1, H_2O) and 0.8 mg of L-arabinose, $[\alpha]^{20}_D$ +101.0 (*c* 0.08, H_2O).

Acidic Hydrolysis Followed by GC Monosaccharide Analysis of Eryloside O (7). A solution of compound **7** (3.5 mg) in 1 N TFA (1 mL) was heated in a stoppered reaction vial at 100 °C for 2 h. The residue obtained after evaporation of the H_2O layer was separated on an Agilent ZORBAX carbohydrate analysis column (5 μm, 4.6 × 250 mm) eluting with CH_3CN-H_2O (75:25) to yield 1.0 mg of galactose, 0.7 mg of arabinose, and 0.8 mg of 2-*N*-acetylglucosamine. The monosaccharides were treated with (−)-2-octanol (0.2 mL) in the presence of trifluoroacetic acid (1 drop) in a stoppered reaction vial at 130 °C overnight. The mixtures were evaporated to dryness and acetylated with Ac_2O in pyridine. The acetylated (−)-2-octyl glycosides were analyzed by GC using the corresponding authentic samples prepared from D- and L-galactose, and D- and L-arabinose. Retention time for the L-GlcNAc derivative was determined for (+)-2-octyl glycoside of the corresponding D-sugar according to Leontein.[20]

Acidic Hydrolysis and GC Analysis of Monosaccharide Composition of Eryloside P (8). Compound **8** (6.5 mg) was hydrolyzed as described above for erylosides F_1−F_4. 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.[20]

Bioassays. Ehrlich carcinoma cells were grown intraperitoneally in albino mice, 18−20 g of 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 μL of the cell suspension (final cell concentration (2−5) × 10^6 cells/mL) was placed into wells of a 96-well microplate containing 10 μL solutions of tested compounds. The incubation was conducted within 1 h at 37 °C. Then 10 μL of an aqueous solution of propidium iodide (final concentration 2.5 μg/mL) was added to each well, and the microplate was incubated additionally 10 min at 37 °C. The intensity of fluorescence was measured at λ_{ex} = 485 nm, λ_{em} = 620 nm.

A suspension of Ehrlich carcinoma cells (200 μL in each well of a 96-well microplate containing 20 μL solutions of tested compound) was incubated for 1 h at 37 °C. Then 10 μL of Hoechst 33342 water solution was added to each well (final concentration 5 μM). After 5 min of incubation at room temperature the fluorescence of the cell suspension was measured at λ_{ex} = 355 nm and λ_{em} = 460 nm. The induction of chromatin condensation (early apoptosis) was determined by comparison of fluorescence intensity of Hoechst 33342 (apoptotic cells) and propidium iodide (necrotic cells) in the cell suspension.

Mouse lymphocytes (spleenocytes) were obtained from a mouse spleen. For this purpose a spleen was isolated and cut with scissors into small-sized slices in PBS (pH 7.4) and then pressed through nylon gauze (280 mesh). The obtained suspension was washed twice in PBS by centrifugation (2000 rpm, 10 min). The final concentration of cells in the incubation medium was (2−5) × 10^6 cells/mL. A solution of calcium green-1/AM (final concentration 10 μM) was added to the suspension, and the cells were incubated with the fluorescent probe during 60−90 min at 37 °C. Then the suspension of spleenocytes was washed in PBS by a centrifugation at 1500 rpm, and 100 μL of the suspension was placed in wells of a 96-well microplate including 10 μL solutions of tested compounds. The incubation was conducted during 5 min at 37 °C, and the intensity of the fluorescence was measured at λ_{ex} = 485 nm, λ_{em} = 518 nm. The intensity of the fluorescence in all experiments was measured with a Fluoroscan Ascent (ThermoLabsystems, Finland) fluorescent plate reader.[23]

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Supporting Information Available: 2D NMR spectra of compounds **1**−**9**. This material is available free of charge via the Internet at htpp://pubs.acs.org.

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