New Triterpenoid Saponins from the Sponge *Erylus nobilis*

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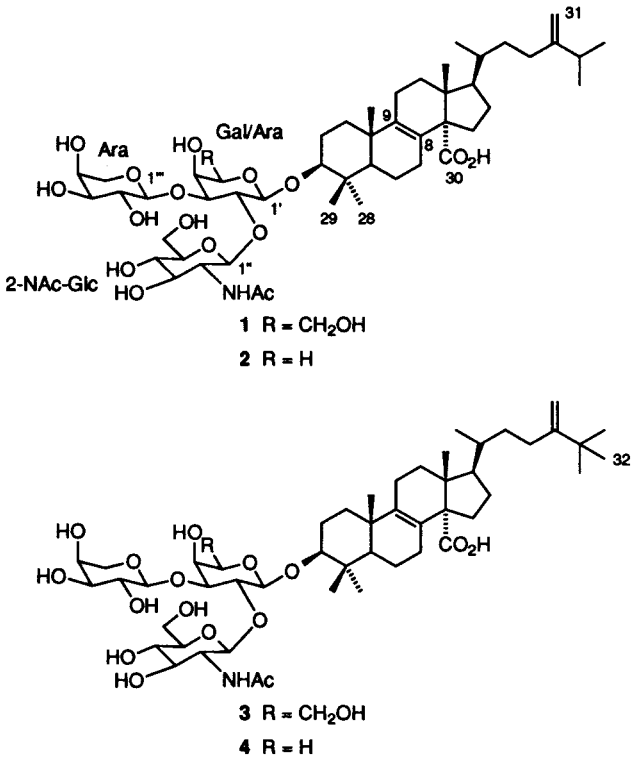
Received January 31, 2001

Erylosides G−J (**1**−**4**), four new triterpenoid saponins, were isolated from the sponge *Erylus nobilis* collected from Jaeju Island, Korea. On the basis of the results of combined chemical and spectral analyses, the structures of the aglycones were determined to be lanostane-based, modified penasterols. The oligosaccharide portions were composed of one unit each of L-arabinose, D-galactose, and 2-*N*-acetyl-D-glucosamine (**1** and **3**) or two units of L-arabinose and one unit of 2-*N*-acetyl-D-glucosamine (**2** and **4**). These compounds exhibited moderate cytotoxicty against a human leukemia cell line.

Steroidal and triterpenoid saponins, frequently encountered in starfish and sea cucumbers, are widely recognized as representative groups of echinoderm metabolites.[1,2] Several of these saponins have been reported to exhibit diverse bioactivities such as cytotoxic, antifungal, antiviral, and hemolytic activities. Despite their great abundance in echinoderms, however, saponins have been found in relatively few other marine organisms. Even in sponges, the most extensively investigated marine organisms, saponins are generally regarded as a minor structural group of metabolites.[1] However, a growing number of saponins, some possessing potent bioactivities, have been recently isolated from these animals.[3−16] Of the sponge-derived saponins, those possessing the lanostane-based penasterol or structurally related aglycones are mainly found in animals of the genus *Erylus* (order Astrophorida, family Geodiidae).[12−16]

During the course of our search for bioactive metabolites from marine invertebrates, we collected the sponge *Erylus nobilis* Thiele from Jaeju Island, Korea. The crude extract of these specimens exhibited moderate cytotoxicity (LC_{50} 317 μg/mL) against the human leukemia cell line K562. Herein we describe the structure and bioactivity of four new triterpenoid trisaccharides, designated as erylosides G−J (**1**−**4**), of the eryloside class. These compounds possessed C_{31} or C_{32} aglycones based on the carbon framework of lanostane. The sugar portions were proven to be branched assemblies of one unit each of L-arabinose, D-galactose, and 2-*N*-acetyl-D-glucosamine (**1** and **3**) or two units of L-arabinose and one unit of 2-*N*-acetyl-D-glucosamine (**2** and **4**).



Results and Discussion

The sponge was collected by hand using scuba off the coast of Seoguipo, Jaeju Island. The lyophilized specimens were repeatedly extracted with MeOH and CH_2Cl_2. Guided by the results of cytotoxicity tests and ^{1}H NMR analysis, the combined extracts were separated employing solvent-partitioning and reversed-phase vacuum flash chromatography. The fractions eluted with moderately polar solvents (20−0% aqueous MeOH) were repeatedly separated by silica column chromatography followed by reversed-phase HPLC to afford four saponins as pure metabolites.

Eryloside G (**1**) was isolated as a white amorphous solid which analyzed for $C_{50}H_{81}NO_{17}$ on the basis of combined HRFABMS and ^{13}C NMR analysis. The NMR spectra of this compound showed characteristic features of a saponin: a number of signals in the region of δ_H 5.0−3.0 and δ_C 110−60. The trisaccharide nature of this molecule was evident from the signals of three anomeric carbons at δ 107.0 (CH), 105.7 (CH), and 101.7 (CH) in the ^{13}C NMR data and those of the corresponding protons at δ 4.91, 4.38, and 4.37 in the ^{1}H NMR data (Table 1). The ^{13}C NMR spectra of **1** also contained two carbonyl and/or carboxyl carbons at δ 180.0 (C) and 173.4 (C) as well as those of two double bonds at δ 157.7 (C), 141.2 (C), 128.9 (C), and 106.9 (CH_2). Consideration of the molecular formula revealed the presence of four additional degrees of unsaturation, which was a characteristic feature of compounds possessing steroidal or related triterpenoid skeletons.

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10.1021/np010047d CCC: $20.00
Published on Web 06/02/2001

Table 1. ^{1}H and ^{13}C NMR Assignments for the Aglycone Moiety of Compound **1** in CD_3OD

position	δ_H	δ_C		HMBC (C → H)
1	1.76 m; 1.29 m	36.5	t	
2	2.00 m; 1.74 m	27.7	t	
3	3.18 dd (11.7, 4.4)	92.0	d	4, 28, 29, 1′(sugar)
4		40.6	s	
5	1.10 dd (12.7, 2.3)	51.7	d	
6	1.70 m; 1.53 m	19.3	t	5, 10
7	2.11 m; 1.98 m	28.8	t	
8		128.9	s	
9		141.2	s	
10		38.4	s	
11	2.14 m; 2.11 m	23.3	t	
12	2.25 m; 1.70 m	32.8	t	
13		48.0	s	
14		63.9	s	
15	2.06 m; 1.40 m	30.3	t	14
16	2.06 m; 1.58 m	28.9	t	13, 14
17	1.55 m	52.0	d	
18	0.80 s	18.2	q	12, 13, 14, 17
19	1.05 s	20.0	q	1, 5, 9, 10
20	1.48 m	37.1	d	
21	0.94 d (6.3)	19.1	q	17, 20, 22
22	1.55 m; 1.12 m	36.0	t	
23	2.08 m; 1.90 ddd (14.2, 10.7, 5.9)	32.0	t	20, 22, 24
24		157.7	s	
25	2.20 m	34.9	d	23, 24, 26, 27
26	1.02 d (6.8)	22.3	q	24, 25, 27
27	1.01 d (6.8)	22.5	q	24, 25, 26
28	1.08 s	28.2	q	3, 4, 5, 29
29	0.90 s	16.7	q	3, 4, 5, 28
30		180.0	s	
31	4.71 br s; 4.64 d (1.0)	106.9	t	23, 24, 25

The structure of the aglycone of **1** was determined by comparison of the NMR data with those of the previously reported saponins and a combination of ^{1}H COSY, TOCSY, gradient HSQC (*g*HSQC), and gradient HMBC (*g*HMBC) experiments.[13−16] In particular the ^2J and ^3J correlations of seven upfield methyl protons with neighboring carbons were very helpful to define the structure. The chemical shift and coupling pattern of the conspicuous H-3 at δ 3.18 (1H, dd, J = 11.7, 4.4 Hz) were consistent with those of a 3β-hydroxy-4,4-dimethyl sterol that was confirmed by the *g*HMBC correlations of the H-19, H-28, and H-29 methyls with C-1, C-3, C-4, C-5, and/or C-10.[13] The mutual *g*HMBC correlations between the methine at C-3 and an anomeric methine (δ_H 4.37, δ_C 105.7) allowed to place a glycosidic linkage at this position. The positioning of a double bond at $\Delta^{8(9)}$ was also secured by the long-range correlation of H-19 with C-9 at δ 141.2. A quaternary carbon at δ 63.9 in the ^{13}C NMR data was assigned to C-14 on the basis of its couplings with H-15, H-16, and H-18. The chemical shift of this carbon suggested the attachment of an electron-withdrawing group, the carboxyl carbon at δ 180.0, at this position, which was commonly observed in the penasterol type saponins derived from sponges of the genus *Erylus*.[12−17] Similarly, the *g*HMBC correlations of H-26, H-27, and the olefinic protons with neighboring carbons secured the presence of an exomethylene double bond at C-24. Thus, the aglycone of eryloside G (**1**) was determined as a lanostane-based C_{31} triterpenoid related to penasterol.

Eryloside G contained three sugar residues. The identification of each sugar unit as well as the arrangement of them was established by chemical degradation, GC analysis, and extensive NMR experiments. After the hydrolysis of **1** with 3 N HCl, the hydrolysate was trimethylsilated with HMDS and TMSCl, and GC retention times of each sugar were compared with those of the authentic samples prepared by the same manner. In this way, the sugar moieties of **1** were defined as one D/L-arabinose, one D/L-galactose, and one 2-*N*-acetyl-D-glucosamine. The D-configuration of 2-*N*-acetylglucosamine was further supported by measurement of the specific rotation of the sugar residue obtained by preparative TLC of the hydrolysate.

The absolute configurations of arabinose and galactose, undetermined by trimethysilation, were assigned by forming thiazolidine analogues. Treatment of the hydrolysate with L-cysteine methyl ester hydrochloride, followed by trimethylsilation and GC analysis could distinguish the D- and L-isomers of arabinose and galactose.[18] Hence, the sugar residues were defined as one L-arabinose, one D-galactose, and one 2-*N*-acetyl-D-glucosamine which were consistent with the results of NMR interpretation (Table 2). The pyranose nature of the sugars was determined on the basis of the *g*HMBC correlations between the anomeric methine and corresponding methylene (arabinose) or methine (galactose and 2-*N*-acetylglucosamine) at C-5′ (or C-5″ or C-5‴).

The ^{13}C NMR shifts (δ_C > 100 ppm) of the anomeric methine suggested that all of the sugars were connected through β-glycosidic linkages.[19] The arrangement of the sugar moieties was established by a combination of the *g*HMBC and ROESY experiments. The long-range correlations of H-2′ with C-1″ and H-1″ with C-2′ defined the linkage between the galactose and 2-*N*-acetylglucosamine. This interpretation was confirmed by the ROESY cross-peak between H-2′ and H-1″. Similarly, the long-range correlation of H-3′ with C-1‴ coupled with the ROESY cross-peak between H-3′ and H-1‴ assigned the linkage between the galactose and arabinose. Finally, the connectivity between C-1′ of the galactose and C-3 of the aglycone was evidenced by the *g*HMBC correlations of H-3 with C-1′ and H-1′ with C-3 as well as the ROESY cross-peak between H-3 and H-1′. Thus, the structure of eryloside G (**1**) was represented as 3β-*O*-[β-D-*N*-acetyl-2-amino-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-(3 → 1)-β-L-arbinopyranosyl]-14-carboxy-24-methyllanosta-8(9),24(31)-diene.

The molecular formula of eryloside H (**2**) was deduced as $C_{49}H_{79}NO_{16}$ by HRFABMS and ^{13}C NMR spectrometry. The spectral data of this compound were very similar to those of **1**. Careful examination of the ^{1}H and ^{13}C NMR data revealed that **2** was structurally identical to **1** with respect to the aglycone. The significant difference in the NMR spectra was the disappearance of a sugar methine that was traced to the replacement of the galactose by an arabinose on the basis of combined 2D NMR experiments (Table 2). The pyranose nature of this sugar was also determined by a long-range correlation between the C-1′ anomeric carbon at δ 106.1 and the H-5′ methylene at δ 3.53. This interpretation was confirmed by an acidic hydrolysis and GC analysis of **2** in which the L-configuration was also assigned for the newly associated arabinose.

The arrangement of sugar units was determined by *g*HMBC and ROESY experiments. Connectivity between the newly associated arabinose and 2-*N*-acetylglucosamine between C-2′ and C-1″ was assigned on the basis of long-range correlations of H-2′ with C-1″ and H-1″ with C-2′ as well as a ROESY cross-peak between H-2′ and H-1″. A long-range correlation of C-3′ with H-1‴ allowed the placement of the glycosidic linkage between C-3′ and C-1‴. The positioning of a linkage between the arabinose and aglycone was also assigned by mutual *g*HMBC correlations between the C-3 methine and the anomeric methine that

Table 2. ^{1}H and ^{13}C NMR Assignments for the Sugar Portions of Compounds **1** and **2** in CD_3OD

	1		**2**	
position	δ_H[a]	δ_C	δ_H[a]	δ_C
Gal/Ara				
1′	4.37 d (8.3)	105.7	4.35 d (7.8)	106.1
2′	3.95 dd (9.8, 8.3)	75.7	3.93 dd (9.3, 7.8)	75.9
3′	3.65 dd (9.8, 3.4)	86.3	3.67 dd (9.3, 3.4)	85.4
4′	4.12 m	70.5	4.05 m	70.4
5′	3.51 m	75.9	3.83 dd (12.7, 2.0); 3.53 brd (12.7)	66.6
6′	3.70 d (5.9, 2H)	62.4		
2-NAc-Glc				
1″	4.91 d (8.3)	101.7	4.89 d (8.3)	101.9
2″	3.73 dd (10.3, 8.3)	57.4	3.73 dd (10.3, 8.3)	57.4
3″	3.24 dd (10.3, 9.3)	77.6	3.25 dd (10.3, 8.8)	77.5
4″	3.10 dd (9.3, 9.3)	72.9	3.11 dd (9.8, 8.8)	72.8
5″	3.27 ddd (9.3, 7.7, 1.5)	78.4	3.30 ddd (9.8, 7.8, 1.5)	78.4
6″	3.83 dd (12.1, 1.5); 3.55 dd (12.1, 7.7)	63.9	3.83 dd (12.2, 1.5); 3.55 dd (12.2, 7.8)	63.7
Ac	2.02 s (3H)	23.4	2.01 s (3H)	23.3
		173.4		173.4
Ara				
1‴	4.38 d (7.8)	107.0	4.40 d (7.8)	106.8
2‴	3.68 dd (9.8, 7.8)	73.0	3.69 dd (9.8, 7.8)	72.9
3‴	3.50 dd (9.8, 3.4)	74.9	3.50 dd (9.8, 3.4)	74.8
4‴	3.82 m	70.2	3.81 m	70.1
5‴	3.86 dd (11.7, 2.4); 3.57 br d (11.7)	67.7	3.85 dd (12.2, 2.4); 3.56 br d (12.2)	67.6

[a] Splitting patterns and coupling constants were measured by combined ^{1}H COSY, 1D TOCSY, and *g*HSQC experiments.

was supported by a ROESY cross-peak between H-3 and H-1′. Thus, the structure of eryloside H (**2**) was determined as an analogue of eryloside G (**1**) in which the galactose was replaced by an arabinose residue.

A related metabolite, eryloside I (**3**), was isolated as a white amorphous solid, and the molecular formula was analyzed for $C_{51}H_{83}NO_{17}$ by combined HRFABMS and ^{13}C NMR spectrometry. The spectral data of this compound were highly compatible with those obtained for **1**. However, comparison of the NMR data revealed that signals of protons and carbons on the side chain were shifted considerably. The most significant difference in the ^{13}C NMR data was the replacement of the signal of the C-25 methine carbon of **1** by those of a quaternary carbon and a methyl carbon. Correspondingly signals of the H-26 and H-27 methyl protons observed as doublets (J = 6.8 Hz for each) in the ^{1}H NMR spectra of **1** collapsed to a singlet (δ 1.05, 12H) together with H-19 and new methyl protons in **3**. These spectral changes were readily accommodated by a methylation at C-25 of the aglycone, which was confirmed by combined 2D NMR experiments. The key evidence was provided by *g*HMBC data in which the new methyl signal at δ 1.05 showed correlations with C-24, C-25, and C-26 (or C-27) at δ 159.8, 37.2, and 29.8, respectively. The 2D NMR data also showed that the remaining portion of the molecule, including the sugar residues of **3**, was identical to **1**. Thus, the structure of eryloside I (**3**) was defined as a derivative of eryloside G possessing a lanostane-based C_{32} aglycone. A literature survey revealed that the carbon skeleton of the *tert*-butyl-bearing penasterol aglycone of **3** was previously found in eryloside E, a bioactive constituent of *E. goffrilleri* collected from the Caribbean Sea.[14]

The molecular formula of eryloside J (**4**) was deduced as $C_{50}H_{81}NO_{16}$ on the basis of HRFABMS and ^{13}C NMR analysis. The spectral data of this compound were highly compatible with those of other saponins. A combination of 2D NMR experiments revealed that the aglycone of **4** was the same as that of **3**, while the sugar residues as well as their arrangement pattern were totally identical to those of **2**. Thus, eryloside J (**4**) was defined as a triterpenoid trisaccharide possessing a modified penasterol aglycone.

Sponge-derived steroidal and triterpenoid saponins exhibit potent and diverse bioactivities.[1] For example, erylosides and structurally related penasterol saponins have been reported to exhibit cytotoxic and immunosuppressive activities and thrombin receptor antagonistic activity as well as inhibitory activity against platelet aggregation.[10,14,16] In our measurement, compounds **1**−**4** were mildly cytotoxic against the human leukemia cell line K562 with LC_{50} values of 22.1, 24.8, 17.9, and 21.8 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. IR spectra were recorded on a Mattson GALAXY spectrophotometer. NMR spectra were recorded in CD_3OD solutions containing Me_4Si as internal standard on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectra were provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside. Gas chromatography was performed on a HP5890-II plus chromatograph equipped with FID as a detector. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. The specimens of *Erylus nobilis* (sample number 97J-5) were collected by hand using scuba at 20−30 m depth off the coast of Seoguipo, Jaeju Island, Korea, in June 1997. The morphological characteristics of the specimens were in good agreement with those described previously.[20] The sponge was massive and had very firm consistency. The color in life was dark gray on the top, but beige underneath. The animal had five types of spicules: oxeas (470−1050 × 10−28 μm), orthotrianes (crad, 120−190 × 20−40 μm; rabdome, 200−510 × 23−42 μm), sterrasters (110−190 μm), micro-oxeas (40−70 μm), and oxyasters (30−50 μm). The voucher specimen (registry no. Por. 34) is on deposit at the Natural History Museum, Hannam University, under the curatorship of C.J.S.

Extraction and Isolation. The fresh collection was immediately frozen and kept at −25 °C until investigated chemically. The specimens were lyophilized (dry wt 850 g), macerated, and repeatedly extracted with MeOH (3 L × 2) and CH_2Cl_2 (3 L × 2). The combined crude extract (158.4 g) was partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was evaporated in vacuo, and the residue (25.7 g) was repartitioned between 15% aqueous MeOH (6.6 g) and *n*-hexane

(18.3 g). The aqueous MeOH layer was separated by C_{18} reversed-phase vacuum flash chromatography using stepped gradient mixtures of MeOH and H_2O as eluents (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, 100% MeOH) and finally acetone. The fractions eluted with 20−0% aqueous MeOH were combined and dried, and the residue (1.62 g) was subjected to Si gel gravity column chromatography using gradient mixtures of $CHCl_3$ and MeOH as eluents.

The fraction (100.8 mg) eluted with 50% $CHCl_3$ in MeOH was separated by reversed-phase HPLC (YMC ODS-A column, 10% aqueous MeOH) to afford erylosides H (**2**) and J (**4**) as white amorphous solids. Final purification was accomplished by reversed-phase HPLC (H_2O−MeOH−MeCN = 20:25:55, v/v) to yield 14.7 and 5.3 mg of **2** and **4**, respectively. The fraction (256.7 mg) eluted with 40% $CHCl_3$ in MeOH was separated by reversed-phase HPLC (10% aqueous MeOH) to yield erylosides G (**1**) and I (**3**). Purification was made by reversed-phase HPLC (H_2O−MeOH−MeCN = 20:25:55) to yield 38.5 and 9.6 mg of **1** and **3**, respectively.

Eryloside G (1): white amorphous solid; mp 187−191 (dec) °C; $[\alpha]^{25}_D$ −18.8° (*c* 0.09, MeOH); IR (KBr) ν_{max} 3350, 2945, 1645, 1565, 1455, 1375, 1075 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; *g*HMBC correlations of the sugar portion H-1′/C-3 (aglycone), C-3′; H-2′/C-1′, C-3′, C-1″; H-3′/C-1‴; H-4′/C-2′, C-3′; H-5′/C-1′, C-6′; H-1″/C-2′, C-3″; H-2″/C-1″, C-3″, NHAc (carbonyl); NHAc (CH_3)/NHAc (carbonyl); H-3″/C-2″, C-4″; H-4″/C-3″, C-5″, C-6″; H-5″/C-1″, C-6″; H-6″/C-5″; H-2‴/C-1‴, C-3‴; H-5‴/C-1‴, C-3‴, C-4‴; HRFABMS *m/z* 990.5401 $[M + Na]^+$ (calcd for $C_{50}H_{81}NO_{17}Na$, 990.5402, Δ −0.1 mmu).

Eryloside H (2): white amorphous solid; mp 208−210 °C; $[\alpha]^{25}_D$ −12.4° (*c* 0.07, MeOH); IR (KBr) ν_{max} 3350, 2940, 1645, 1565, 1455, 1375, 1080 cm^{-1}; ^1H NMR data of the aglycone (CD_3OD) δ 4.71 (1H, br s, H-31), 4.64 (1H, br s, H-31), 3.15 (1H, dd, *J* = 11.7, 4.4 Hz, H-3), 2.24 (1H, m, H-12), 2.20 (1H, m, H-25), 2.15 (1H, m, H-11), 2.12 (1H, m, H-11), 2.10 (2H, m, H-7, H-23), 2.07 (2H, m, H-15, H-16), 2.00 (1H, m, H-7), 1.90 (2H, m, H-2, H-23), 1.78 (1H, m, H-2), 1.75 (1H, m, H-1), 1.70 (2H, m, H-6, H-12), 1.58 (1H, m, H-16), 1.55 (2H, m, H-17, H-22), 1.53 (1H, m, H-6), 1.47 (1H, m, H-20), 1.39 (1H, m, H-15), 1.30 (1H, m, H-1), 1.13 (1H, m, H-22), 1.10 (1H, dd, *J* = 12.8, 2.3 Hz, H-5), 1.07 (3H, s, H-28), 1.04 (3H, s, H-19), 1.02 (3H, d, *J* = 6.8 Hz, H-26), 1.01 (3H, d, *J* = 6.8 Hz, H-27), 0.94 (3H, d, *J* = 6.4 Hz, H-21), 0.90 (3H, s, H-29), 0.79 (3H, s, H-18); ^1H NMR data of the sugar portion, see Table 2; ^{13}C NMR data of the aglycone (CD_3OD) δ 180.3 (s, C-30), 157.7 (s, C-24), 141.0 (s, C-9), 129.1 (s, C-8), 106.9 (t, C-31), 91.9 (d, C-3), 64.0 (s, C-14), 52.0 (d, C-17), 51.6 (d, C-5), 48.0 (s, C-13), 40.7 (C, C-4), 38.5 (s, C-10), 37.1 (d, C-20), 36.5 (t, C-1), 36.0 (t, C-22), 34.9 (d, C-25), 32.8 (t, C-12), 32.1 (t, C-23), 30.3 (t, C-15), 28.9 (t, C-16), 28.8 (t, C-7), 28.2 (q, C-28), 27.7 (t, C-2), 23.3 (t, C-11), 22.5 (q, C-27), 22.3 (q, C-26), 20.0 (q, C-19), 19.3 (t, C-6), 19.1 (q, C-21), 18.3 (q, C-18), 16.7 (q, C-29); ^{13}C NMR data of the sugar portion, see Table 2; HRFABMS *m/z* 960.5320 $[M + Na]^+$ (calcd for $C_{49}H_{79}NO_{16}Na$, 960.5297, Δ 2.3 mmu).

Eryloside I (3): white amorphous solid; mp 203−206 °C; $[\alpha]^{25}_D$ −18.0° (*c* 0.06, MeOH); IR (KBr) ν_{max} 3350, 2940, 1645, 1555, 1455, 1375, 1070 cm^{-1}; ^1H NMR data of the aglycone (CD_3OD) δ 4.84 (1H, br s, H-31), 4.65 (1H, d, *J* = 1.0 Hz, H-31), 3.18 (1H, dd, *J* = 11.7, 4.4 Hz, H-3), 2.25 (1H, m, H-12), 2.15 (1H, m, H-11), 2.12 (1H, m, H-23), 2.10 (2H, m, H-7, H-11), 2.07 (2H, m, H-15, H-16), 2.01 (1H, m, H-7), 1.99 (1H, m, H-2), 1.89 (1H, ddd, *J* = 15.1, 11.2, 4.9 Hz, H-23), 1.76 (1H, m, H-1), 1.74 (1H, m, H-2), 1.70 (2H, m, H-6, H-12), 1.60 (1H, m, H-17), 1.58 (2H, m, H-16, H-22), 1.54 (1H, m, H-6), 1.49 (1H, m, H-20), 1.40 (1H, m, H-15), 1.30 (1H, m, H-1), 1.17 (1H, m, H-22), 1.12 (1H, dd, *J* = 12.7, 2.0 Hz, H-5), 1.07 (3H, s, H-28), 1.05 (12H, s, H-19, H-26, H-27, H-32), 0.96 (3H, d, *J* = 6.4 Hz, H-21), 0.90 (3H, s, H-29), 0.80 (3H, s, H-18); Gal δ 4.40 (1H, d, *J* = 8.3 Hz, H-1′), 4.12 (1H, br d, *J* = 3.4 Hz, H-4′), 3.96 (1H, dd, *J* = 9.8, 8.3 Hz, H-2′), 3.70 (2H, d, *J* = 5.9 Hz, H-6′), 3.65 (1H, dd, *J* = 9.8, 3.4 Hz, H-3′), 3.50 (1H, m, H-5′); 2-NAc-Glc δ 4.91 (1H, d, *J* = 8.8 Hz, H-1″), 3.84 (1H, dd, *J* = 12.2, 2.4 Hz, H-6″), 3.73 (1H, dd, *J* = 10.3, 8.8 Hz, H-2″), 3.55 (1H, dd, *J* = 12.2, 7.8 Hz, H-6″), 3.27 (1H, ddd, *J* = 9.8, 7.8, 2.4 Hz, H-5″), 3.24 (1H, dd, *J* = 10.3, 8.8 Hz, H-3″), 3.10 (1H, dd, *J* = 9.8, 8.8 Hz, H-4″), 2.02 (3H, s, NHAc); Ara δ 4.41 (1H, d, *J* = 7.8 Hz, H-1‴), 3.85 (1H, dd, *J* = 12.8, 2.0 Hz, H-5‴), 3.83 (1H, m, H-4‴), 3.68 (1H, dd, *J* = 9.8, 7.8 Hz, H-2‴), 3.57 (1H, br d, *J* = 12.8 Hz, H-5‴), 3.50 (1H, dd, *J* = 9.8, 3.4 Hz, H-3‴); ^{13}C NMR data of the aglycone (CD_3OD) δ 180.7 (s, C-30), 159.8 (s, C-24), 140.8 (s, C-9), 129.2 (s, C-8), 106.7 (t, C-31), 92.1 (d, C-3), 64.0 (s, C-14), 52.0 (d, C-17), 51.7 (d, C-5), 48.0 (s, C-13), 40.6 (s, C-4), 38.4 (s, C-10), 37.6 (t, C-22), 37.5 (d, C-20), 37.2 (s, C-25), 36.5 (t, C-1), 32.8 (t, C-12), 30.4 (t, C-15), 29.8 (q × 3, C-26, C-27, C-32), 29.0 (t × 2, C-16, C-23), 28.8 (t, C-7), 28.2 (q, C-28), 27.7 (t, C-2), 23.4 (t, C-11), 20.0 (q, C-19), 19.3 (t, C-6), 19.2 (q, C-21), 18.3 (q, C-18), 16.7 (q, C-29); Gal δ 105.7 (d, C-1′), 86.3 (d, C-3′), 75.9 (d, C-5′), 75.8 (d, C-2′), 70.5 (d, C-4′), 62.4 (t, C-6′); 2-NAc-Glc δ 173.4 (s, NHAc), 101.7 (d, C-1″), 78.4 (d, C-5″), 77.6 (d, C-3″), 72.9 (d, C-4″), 57.4 (d, C-2″), 63.8 (t, C-6″), 23.4 (q, NHAc); Ara δ 107.0 (d, C-1‴), 74.9 (d, C-3‴), 73.0 (d, C-2‴), 70.2 (d, C-4‴), 67.7 (t, C-5‴); HRFABMS *m/z* 1004.5545 $[M + Na]^+$ (calcd for $C_{51}H_{83}NO_{17}Na$, 1004.5559, Δ −1.7 mmu).

Eryloside J (4): white amorphous solid; mp 193−196 °C; $[\alpha]^{25}_D$ −16.9° (*c* 0.06, MeOH); IR (KBr) ν_{max} 3350, 2935, 1645, 1565, 1460, 1375, 1075 cm^{-1}; ^1H NMR data of the aglycone (CD_3OD) δ 4.84 (1H, br s, H-31), 4.65 (1H, d, *J* = 1.0 Hz, H-31), 3.15 (1H, dd, *J* = 11.7, 4.4 Hz, H-3), 2.24 (1H, m, H-12), 2.15 (1H, m, H-11), 2.12 (1H, m, H-23), 2.10 (2H, m, H-7, H-11), 2.07 (2H, m, H-15, H-16), 2.00 (1H, m, H-7), 1.90 (2H, m, H-2, H-23), 1.76 (1H, m, H-1), 1.74 (1H, m, H-2), 1.69 (2H, m, H-6, H-12), 1.60 (1H, m, H-17), 1.58 (1H, m, H-22), 1.56 (1H, m, H-16), 1.54 (1H, m, H-6), 1.49 (1H, m, H-20), 1.39 (1H, m, H-15), 1.30 (1H, m, H-1), 1.17 (1H, m, H-22), 1.12 (1H, dd, *J* = 12.7, 2.0 Hz, H-5), 1.07 (3H, s, H-28), 1.05 (12H, s, H-19, H-26, H-27, H-32), 0.96 (3H, d, *J* = 6.4 Hz, H-21), 0.90 (3H, s, H-29), 0.80 (3H, s, H-18); Ara-I δ 4.35 (1H, d, *J* = 7.8 Hz, H-1′), 4.05 (1H, m, H-4′), 3.93 (1H, dd, *J* = 9.3, 7.8 Hz, H-2′), 3.83 (1H, dd, *J* = 12.7, 2.0 Hz, H-5′), 3.66 (1H, dd, *J* = 9.3, 3.4 Hz, H-3′), 3.53 (1H, br d, *J* = 12.7 Hz, H-5′); 2-NAc-Glc δ 4.89 (1H, d, *J* = 8.3 Hz, H-1″), 3.83 (1H, dd, *J* = 12.2, 2.0 Hz, H-6″), 3.73 (1H, dd, *J* = 10.3, 8.3 Hz, H-2″), 3.55 (1H, dd, *J* = 12.2, 8.3 Hz, H-6″), 3.30 (1H, ddd, *J* = 9.8, 8.3, 2.0 Hz, H-5″), 3.25 (1H, dd, *J* = 10.3, 8.8 Hz, H-3″), 3.11 (1H, dd, *J* = 9.8, 8.8 Hz, H-4″), 2.01 (3H, s, NHAc); Ara-II δ 4.40 (1H, d, *J* = 7.3 Hz, H-1‴), 3.85 (1H, dd, *J* = 12.2, 2.4 Hz, H-5‴), 3.81 (1H, m, H-4‴), 3.69 (1H, dd, *J* = 9.8, 7.3 Hz, H-2‴), 3.56 (1H, br d, *J* = 12.2 Hz, H-5‴), 3.50 (1H, dd, *J* = 9.8, 3.4 Hz, H-3‴); ^{13}C NMR data of the aglycone (CD_3OD) δ 180.1 (s, C-30), 159.8 (s, C-24), 141.1 (s, C-9), 129.0 (s, C-8), 106.7 (t, C-31), 91.9 (d, C-3), 63.9 (s, C-14), 52.0 (d, C-17), 51.7 (d, C-5), 48.0 (s, C-13), 40.7 (s, C-4), 38.5 (s, C-10), 37.6 (t, C-22), 37.5 (d, C-20), 37.2 (s, C-25), 36.5 (t, C-1), 32.7 (t, C-12), 30.3 (t, C-15), 29.8 (q × 3, C-26, C-27, C-32), 28.9 (t × 2, C-16, C-23), 28.8 (t, C-7), 28.2 (q, C-28), 27.7 (t, C-2), 23.3 (t, C-11), 20.0 (q, C-19), 19.3 (t, C-6), 19.2 (q, C-21), 18.2 (q, C-18), 16.7 (q, C-29); Ara-I δ 106.1 (d, C-1′), 85.5 (d, C-3′), 75.9 (d, C-2′), 70.4 (d, C-4′), 66.6 (t, C-5′); 2-NAc-Glc δ 173.4 (s, NHAc), 101.9 (d, C-1″), 78.4 (d, C-5″), 77.5 (d, C-3″), 72.8 (d, C-4″), 63.8 (t, C-6″), 57.4 (d, C-2″), 23.3 (q, NHAc); Ara-II δ 106.9 (d, C-1‴), 74.8 (d, C-3‴), 72.9 (d, C-2‴), 70.1 (d, C-4‴), 67.6 (t, C-5‴); HRFABMS *m/z* 974.5436 $[M + Na]^+$ (calcd for $C_{50}H_{81}NO_{16}Na$, 974.5453, Δ −1.7 mmu).

Acid Hydrolysis and GC Analysis of Eryloside G (I). A solution of **1** (2.1 mg) in 3 N HCl (0.5 mL) was stirred at 80 °C for 5 h. After being cooled to room temperature, the solution was concentrated by blowing with N_2. The residue was redissolved in a mixture of hexamethyldisilazane (HMDS) in TMSCl (50 μL, v/v = 2:1) in pyridine (0.5 mL), and the solution was stirred at 60 °C for 30 min. After drying the solution with a stream of N_2, the residue was separated with water and CH_2Cl_2 (1 mL, v/v = 1:1). The CH_2Cl_2 layer was analyzed by GC using an Omegawax column (0.32 mm × 30 m). Temperatures of injector and detector were 200 °C for both. A temperature gradient system was used for the oven; the initial temperature was maintained at 100 °C for 1 min, ramped to 200 °C at the rate of 5 °C/min, then maintained at 200 °C for 9 min. Peaks of the hydrolysate were detected at 6.42, 10.47, and 15.36 min, respectively. Retention times for authentic samples after being treated simultaneously with HMDS/

TMSCl were 6.34 (D- and L-arabinose), 10.50 (D- and L-galactose), and 15.45 min (2-*N*-acetyl-D-glucosamine), respectively.

The distinction between D- and L-isomers of arabinose and galactose was accomplished by forming diastereomeric thiazolidine analogues. After hydrolysis with HCl, the residue was redissolved with a solution of L-cysteine methyl ester hydrochloride (2.3 mg) in pyridine (0.5 mL), and the solution was stirred at 60 °C for 1.5 h. After being cooled and concentrated, the mixture was treated with HMDS/TMSCl in pyridine, stirred, and then separated by water and CH_2Cl_2 as described above. The GC analysis gave peaks at 20.35 and 23.36 min. Retention times for authentic samples were 20.40 (L-arabinose), 21.28 (D-arabinose), 23.23 (D-galactose), and 24.31 min (L-galactose), respectively. Co-injection of the hydrolysate with the authentic silyated L-arabinose and D-galactose gave single peaks at 20.42 and 23.45 min, respectively.

Acid Hydrolysis of Eryloside G (II). A solution of **1** (4.9 mg) in 3 N HCl (0.5 mL) was stirred at 80 °C for 3 h. After being cooled to room temperature and concentrated by blowing with N_2, the reaction mixture was separated by Si gel preparative TLC (Si gel 60, $CHCl_3$−MeOH−H_2O = 32:18:4) to yield pure 2-*N*-acetylglucosamine (0.2 mg); $[\alpha]_D$ +30.6° (*c* 0.04, H_2O); +40.5° (*c* 1, H_2O) for authentic sample (Aldrich).

Acid Hydrolysis and GC Analysis of Eryloside H. Analysis of **2** was carried out following the same procedures as for **1**. Treatment of **2** (1.7 mg) sequentially with 3 N HCl and HMDS/TMSCl followed by GC analysis gave peaks at 6.37 and 15.44 min, while those from the authentic samples were obtained at 6.41 (D- and L-arabinose) and 15.49 min (2-*N*-acetyl-D-glucosamine), respectively. Treatment with L-cysteine methyl ester hydrochloride before adding the solution of HMDS/TMSCl in pyridine gave single peak at 20.31 min in GC analysis. Co-injection of the hydrolysate with the authentic silyated L-arabinose gave a peak at 20.39 min.

Acknowledgment. The authors gratefully acknowledge Drs. Richard Kondrat and Ron New, Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside, for providing high-resolution mass data. This research was financially supported by the Ministry of Science and Technology (Grant PN-00410).

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NP010047D