Bioactive Metabolites from Sponges

- New Triterpenoid Saponins from the Sponge *Erylus nobilis*

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SUMMARY: Four new triterpenoid saponins, erylosides G-J (**1-4**), were isolated from the sponge *Erylus nobilis* collected from Jaeju Island, Korea. Based upon 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 cytotoxicity against a human leukemia cell-line K562.

KEY WORDS: sponge, triterpenoid saponins, *Erylus nobilis*, cytotoxicity

INTRODUCTION

Steroidal and triterpenoid saponins are widely recognized as a representative group of echinoderm metabolites.^{1,2} Except for these echinoderms, only a few marine organisms have produced saponins. However, a number of these compounds have been recently isolated from sponges.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 Choristida, family Geodiidae).¹²⁻¹⁶

During the course of our search for bioactive metabolites from marine invertebrates, we collected the sponge *Erylus nobilis* from Jaeju Island, Korea. The crude extract of these specimens exhibited moderate cytotoxicity (LC₅₀ 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.

MATERIALS AND METHODS

Animal Material

The specimens of *Erylus nobilis* were collected by hand using SCUBA at 20-30 m depth off the coast of Seoguipo, Jaeju Island, Korea in June 1997. The sponge was massive and had very firm consistency. The color in life was dark gray on the top, but beige underneath. The voucher specimen 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, macerated, and repeatedly extracted with MeOH and CH₂Cl₂. The combined crude extract was partitioned

between *n*-BuOH and H₂O. The *n*-BuOH layer was evaporated in vacuo and the residue was re-partitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer was separated by C₁₈ reversed-phase vacuum flash chromatography using stepped gradient mixtures of MeOH and H₂O as eluents. The fractions eluted with 20-0% aqueous MeOH were combined, dried and the residue was subjected to Si gel gravity column chromatography using gradient mixtures of CHCl₃ and MeOH as eluents.

The fraction eluted with 30% CHCl₃ in MeOH was separated by reversed-phase HPLC (10% aqueous MeOH) to afford erylosides H (2) and J (4) as white amorphous solids. The fraction eluted with 40% CHCl₃ in MeOH was separated by reversed-phase HPLC (H₂O:MeOH:MeCN = 20:25:55) to yield erylosides G (1) and I (3).

Eryloside G (1): mp 187–191°C; $[\alpha]_D^{25}$ -18.8° (c 0.09, MeOH); IR (KBr) $\nu_{\max}$ 3350, 2945, 1645, 1565, 1455, 1375, 1075 cm⁻¹; ¹H and ¹³C NMR data, see Tables 1 and 2; HRFABMS *m/z* 990.5401 [M + Na]⁺ (calcd for C₅₀H₈₁NO₁₇Na, 990.5402).

Eryloside H (2): mp 208–210°C; $[\alpha]_D^{25}$ -12.4° (c 0.07, MeOH); IR (KBr) $\nu_{\max}$ 3350, 2940, 1645, 1565, 1455, 1375, 1080 cm⁻¹; ¹H NMR data of the aglycone (CD₃OD) δ 4.71 (1H), 4.64 (1H), 3.15 (1H), 2.24 (1H), 2.20 (1H), 2.15 (1H), 2.12 (1H), 2.10 (2H), 2.07 (2H), 2.00 (1H), 1.90 (2H), 1.78 (1H), 1.75 (1H), 1.70 (2H), 1.58 (1H), 1.55 (2H), 1.53 (1H), 1.47 (1H), 1.39 (1H), 1.30 (1H), 1.13 (1H), 1.10 (1H), 1.07 (3H), 1.04 (3H), 1.02 (3H), 1.01 (3H), 0.94 (3H), 0.90 (3H), 0.79 (3H); Ara-I δ 4.35 (1H), 4.05 (1H), 3.93 (1H), 3.83 (1H), 3.67 (1H), 3.53 (1H); 2-Nac-Glc δ 4.89 (1H), 3.83 (1H), 3.73 (1H), 3.55 (1H), 3.30 (1H), 3.25 (1H), 3.11 (1H), 2.01 (3H); Ara-II δ 4.40 (1H), 3.85 (1H), 3.81 (1H), 3.69 (1H), 3.56 (1H), 3.50 (1H); ¹³C NMR data of the aglycone (CD₃OD) δ 180.3, 157.7, 141.0, 129.1, 106.9, 91.9, 64.0, 52.0, 51.6, 48.0, 40.7, 38.5, 37.1, 36.5, 36.0, 34.9, 32.8, 32.1, 30.3, 28.9, 28.8, 28.2, 27.7, 23.3, 22.5, 22.3, 20.0, 19.3, 19.1, 18.3, 16.7; Ara-I δ 106.1, 85.4, 75.9, 70.4, 66.6; 2-Nac-Glc δ 173.4, 101.9, 78.4, 77.5, 72.8, 63.7, 57.4, 23.3; Ara-II δ 106.9, 74.8, 72.9, 70.1, 67.6; HRFABMS *m/z* 960.5320 [M + Na]⁺ (calcd for C₄₈H₇₉NO₁₆Na, 960.5297).

Eryloside I (3): mp 203–206°C; $[\alpha]_D^{25}$ -18.0° (c 0.06, MeOH); IR (KBr) $\nu_{\max}$ 3350, 2940, 1645, 1555, 1455, 1375, 1070 cm⁻¹; ¹H NMR data of the aglycone (CD₃OD) δ 4.84 (1H), 4.65 (1H), 3.18 (1H), 2.25 (1H), 2.15 (1H), 2.12 (1H), 2.10 (2H), 2.07 (2H), 2.01 (1H), 1.99 (1H), 1.89 (1H), 1.76 (1H), 1.74 (1H), 1.70 (2H), 1.60 (1H), 1.58 (2H), 1.54 (1H), 1.49 (1H), 1.40 (1H), 1.30 (1H), 1.17 (1H), 1.12 (1H), 1.07 (3H), 1.05 (12H), 0.96 (3H), 0.90 (3H), 0.80 (3H); Gal δ 4.40 (1H), 4.12 (1H), 3.96 (1H), 3.70 (2H), 3.65 (1H), 3.50 (1H); 2-Nac-Glc δ 4.91 (1H), 3.84 (1H), 3.73 (1H), 3.55 (1H), 3.27 (1H), 3.24 (1H), 3.10 (1H), 2.02 (3H); Ara δ 4.41 (1H), 3.85 (1H), 3.83 (1H), 3.68 (1H), 3.57 (1H), 3.50 (1H); ¹³C NMR data of the aglycone (CD₃OD) δ 180.7, 159.8, 140.8, 129.2, 106.7, 92.1, 64.0, 52.0, 51.7, 48.0, 40.6, 38.4, 37.6, 37.5, 37.2, 36.5, 32.8, 30.4, 29.8, 29.0, 28.8, 28.2, 27.7, 23.4, 20.0, 19.3, 19.2, 18.3, 16.7; Gal δ 105.7, 86.3, 75.9, 75.8, 70.5, 62.4; 2-Nac-Glc δ 173.4, 101.7, 78.4, 77.6, 72.9, 57.4, 63.8, 23.4; Ara δ 107.0, 74.9, 73.0, 70.2, 67.7; HRFABMS *m/z* 1004.5545 [M + Na]⁺ (calcd for C₅₁H₈₃NO₁₇Na, 1004.5559).

Eryloside J (4): mp 193–196°C; $[\alpha]_D^{25}$ -16.9° (c 0.06, MeOH); IR (KBr) $\nu_{\max}$ 3350, 2935, 1645, 1565, 1460, 1375, 1075 cm⁻¹; ¹H NMR data of the aglycone (CD₃OD) δ 4.84 (1H), 4.65 (1H), 3.15 (1H), 2.24 (1H), 2.15 (1H), 2.12 (1H), 2.10 (2H), 2.07 (2H), 2.00 (1H), 1.90 (2H), 1.76 (1H), 1.74 (1H), 1.69 (2H), 1.60 (1H), 1.58 (1H), 1.56 (1H), 1.54 (1H), 1.49 (1H), 1.39 (1H), 1.30 (1H), 1.17 (1H), 1.12 (1H), 1.07 (3H), 1.05 (12H), 0.96 (3H), 0.90 (3H), 0.80 (3H); Ara-I δ 4.35 (1H), 4.05 (1H), 3.93 (1H), 3.83 (1H), 3.66 (1H), 3.53 (1H); 2-Nac-Glc δ 4.89 (1H), 3.83 (1H), 3.73 (1H), 3.55 (1H), 3.30 (1H), 3.25 (1H), 3.11 (1H), 2.01 (3H); Ara-II δ 4.40 (1H), 3.85 (1H), 3.81 (1H), 3.69 (1H), 3.56 (1H), 3.50 (1H); ¹³C NMR data of the aglycone (CD₃OD) δ 180.1, 159.8, 141.1, 129.0, 106.7, 91.9, 63.9, 52.0, 51.7, 48.0, 40.7, 38.5, 37.6, 37.5, 37.2, 36.5, 32.7, 30.3, 29.8, 28.9, 28.8, 28.2, 27.7, 23.3, 20.0, 19.3, 19.2, 18.2, 16.7; Ara-I δ 106.1, 85.5, 75.9, 70.4, 66.6; 2-Nac-Glc δ 173.4, 101.9, 78.4, 77.5, 72.8, 63.8, 57.4, 23.3; Ara-II δ 106.9, 74.8, 72.9, 70.1, 67.6; HRFABMS *m/z* 974.5436 [M + Na]⁺ (calcd for C₅₀H₈₁NO₁₆Na, 974.5453).

Table 1. ¹H and ¹³C NMR Assignments for the Aglycone Moiety of Compound 1 in CD₃OD.

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

Acid Hydrolysis and GC Analysis of Eryloside G

Compound 1 (2.1 mg) was treated with a 3 N HCl (0.5 mL) at 80°C for 5 hr. After being concentrated, the residue was redissolved in a mixture of hexamethyldisilazane (HMDS) with TMSCl (50 μ L, v:v=2:1) in pyridine (0.5 mL) and the solution was stirred at 60°C for 30 min. The resulting derivatives were analyzed by GC (Omegawax column; 0.32 mm x 30 m, injector and detector temperature; 200°C, temperature gradient system; 5°C/min) to identify arabinose (*t_R*=6.34 min), galactose (*t_R*=10.47 min) and 2-N-acetylglucosamine (*t_R*=15.36 min).

The distinction between D- and L- isomers of arabinose and galactose were accomplished by forming

diastereomeric thiazolidine analogs. After hydrolysis with HCl, the residue was reacted with L-cysteine methyl ester hydrochloride (2.3 mg) in pyridine (0.5 mL) at 60°C for 1.5 hr. After being concentrated, the mixture was treated with HMDS/TMSCl in pyridine, and then analyzed as described above. The GC analysis gave peaks at 20.35 (L-arabinose) and 23.36 (D-galactose) min.

RESULTS AND DISCUSSION

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 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 1H COSY, TOCSY, gradient HSQC (gHSQC), and gradient HMBC (gHMBC) 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 of this carbon suggested the attachment of an electron-withdrawing group, the carboxyl carbon at δ 180.0, at this position that was commonly observed in the penasterol type saponins derived from sponges of the genus *Erylus*.¹²⁻¹⁷ 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 trimethylsilylated 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, that 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 trimethylsilylation, were assigned by forming thiazolidine analogs.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 gHMBC correlations between the anomeric methine and corresponding methylene (arabinose) or methine (galactose and 2-N-acetyl-glucosamine) at C-5' (or C-5'' or 5''').

The ^{13}C NMR shifts ($\delta_c > 100$ 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 gHMBC and ROESY experiments. The structure of eryloside G (**1**) was represented as β -O-[β -D-N-acetyl-2-

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

Position	δ_H	δ_C
Gla		
1'	4.37 d (8.3)	105.7
2'	3.95 dd (9.8, 8.3)	75.7
3'	3.65 dd (9.8, 3.4)	86.3
4'	4.12 m	70.5
5'	3.51 m	75.9
6'	3.70 d (5.9, 2H)	62.4
2-NAc-Glc		
1''	4.91 d (8.3)	101.7
2''	3.73 dd (10.3, 8.3)	57.4
3''	3.24 dd (10.3, 9.3)	77.6
4''	3.10 dd (9.3, 9.3)	72.9
5''	3.27 ddd (9.3, 7.7, 1.5)	78.4
6''	3.83 dd (12.1, 1.5); 3.55 dd (12.1, 7.7)	63.9
Ac	2.02 s (3H)	23.4
		173.4
Ara		
1'''	4.38 d (7.8)	107.0
2'''	3.68 dd (9.8, 7.8)	73.0
3'''	3.50 dd (9.8, 3.4)	74.9
4'''	3.82 m	70.2
5'''	3.86 dd (11.7, 2.4); 3.57 br d (11.7)	67.7

aminoglucopyranosyl-(1 $\rightarrow$ 2)- β -D-galactopyranosyl-(3 $\rightarrow$ 1)- β -L-arabinopyranosyl]-14-carboxy-24-methyl-24- β -lanosta-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 1H 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. The arrangement of sugar units was determined by gHMBC and ROESY experiments. The structure of eryloside H (**2**) was determined as an analog 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 signal of the C-25 methine carbon of **1** by those of a quaternary carbon and a methyl carbon. These spectral changes were readily accommodated by a

methylation at C-25 of the aglycone, which was confirmed by combined 2D NMR experiments. 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.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.

In our measurement of bioactivity, 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 $\mu g/mL$, respectively.

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