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Eryloside E From An Atlantic Sponge *Erylus goffrilleri*

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Abstract: A glycoside, which we call eryloside E (1), was isolated from the marine sponge *Erylus goffrilleri* and characterized by spectroscopic methods. Eryloside E possesses a rare penasterol nucleus with a *t*-butyl substituent on the side chain and three sugar molecules attached through carbons 3 and 30.

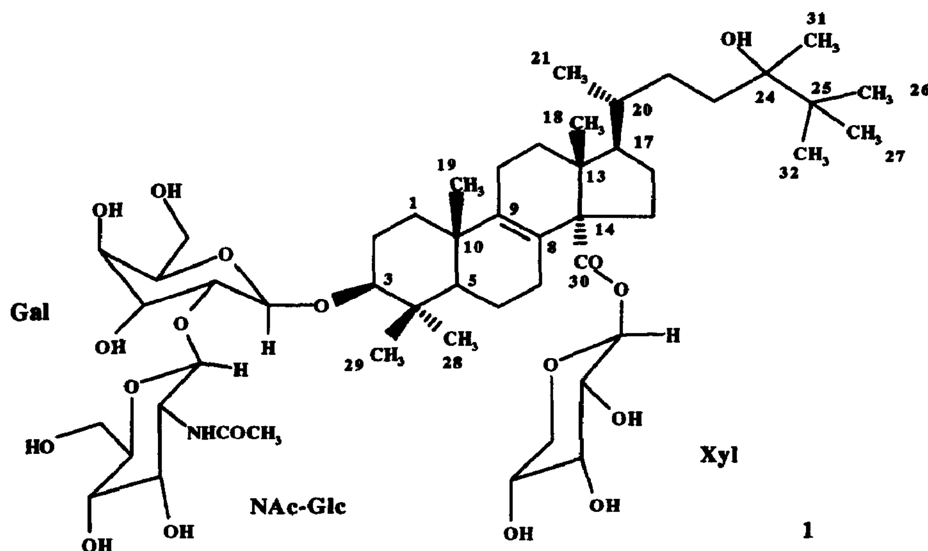
The erylosides, which are terpene glycosides possessing either steroidal or penasterol aglycones with two or four sugar substituents, have been reported from several marine sponges.[1,2] A crude extract of *Erylus goffrilleri* Wiedenmayer,[3] 1977 (Demospongiae, Choristida, Geodiidae), which was collected from Port Nelson, Rum Cay, Bahama Islands by Scuba at a depth of 120 feet on a fore reef escarpment, was shown to inhibit binding of ^{125}[I]-Bolton Hunter labeled C5a (1-74) to its receptor. Bioassay guided fractionation of the extract led to the isolation of a series of terpene glycosides which are very weakly active in this assay. The structure of the major component of the extract was determined through spectroscopic methods. This paper will describe the isolation and structure elucidation of this metabolite, which we call eryloside E, 1.

The sponge was successively extracted with MeOH, EtOAc, and CH_2Cl_2. The combined extracts were concentrated to dryness and partitioned between *n*-BuOH and H_2O. The *n*-BuOH partition was chromatographed by vacuum column chromatography on an RP-18 stationary phase. The active fraction eluted with H_2O/CH_3OH (1/9) was successively chromatographed by HPLC using an RP-2 column eluted with H_2O/CH_3OH (2/8) and H_2O/CH_3CN (64/26) to yield eryloside E[4,5] (0.6 % wet wt, Tables I - III).

The presence of a number of resonances between 3.0 and 5.5 ppm in the ^{1}H NMR spectrum of 1 which were attributable to protons on carbons bearing hetero atom substitution (including three anomeric proton resonances) and the presence of five methyl resonances [δ 1.09 (s, H-28), 1.04 (s, H-19), 0.89 (s, H-29), 0.80 (s, H-18) and 0.91 (s, H-21)] suggested that 1 is a terpene glycoside. High resolution FABMS indicated a molecular formula of $C_{51}H_{85}NO_{18}$ for 1. Comparison with the previously published data [2] suggested that the aglycone is a rare penasterol derivative with a side chain possessing a *t*-butyl group [δ 0.92 (9H s; H-26, H-27, H-32)]. The ^{13}C NMR data for the terpene nucleus of compound 1 (Table II) matched that reported previously for the penasterol aglycone except for C-14, C-15, C-16 and the side chain carbons. The *t*-butyl group was located at C-24 based upon correlations observed in the HMBC experiment between the nine proton resonance observed for H-26, H-27 and H-32 and both C-24 and C-25. The C-31 methyl group was also placed on C-24 based upon correlations observed in the HMBC experiment between H-31 and C-23, C-24 and C-25. The aglycone unit was further confirmed after hydrolysis of 1 with 6N HCl followed by isolation of compound 2 which by HREIMS had the molecular formula $C_{32}H_{52}O_3$. The ^{13}C (Table IV) and ^{1}H NMR data[6] observed for 2 matched that reported for the

penasterol nucleus.[7] During hydrolysis, the hydroxyl group at C-24 underwent elimination to form a double bond between C-23 and C-24. The side chain of 2 was assigned based upon interpretation of the HMQC and HMBC data in which the following correlations were observed: H-21 to C-17, C-20 and C-22; H-31 to C-23, C-24 and C-25; H-26, H-27 and H-32 to C-24 and C-25.

The sugars were identified as galactose, xylose and N-acetylglucose based upon their ^1H and ^{13}C chemical shift values (Table I and III respectively) and through interpretation of the ^1H-^1H COSY spectra



and homonuclear decoupling experiments. All of the sugars are in the pyranose form. In the first sugar unit, the observed scalar couplings, $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, $J_{4,5}$ and $J_{5,6}$ were found to be 7.7, 9.5, 3.4, 1.0, and 6.3 Hz respectively which identified a galactopyranose unit in compound 1 where H-1, H-2, H-3 and H-5 are axial while H-4 is equatorial. The same type of analysis for the second sugar unit in which $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, were 7.3, 8.5 and 8.5 Hz, respectively, while $J_{4,5a}$ $J_{4,5e}$, and $J_{5a,5e}$ were 9.5, 5.1 and 11.7 Hz, respectively, led to the assignment of H-1, H-2, H-3 and H-4 as axial which confirmed the presence of a xylopyranose unit. The scalar couplings for the third sugar unit, $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, $J_{4,5}$ and $J_{5,6}$ were found to be 8.3, 10.4,

Table I. ^1H NMR Chemical Shifts[a] for Sugars of Eryloside E (in CD_3OD)

	Gal	Xyl	NAc-Glc
H-1	4.35 (1H, d, 7.7)	5.35 (1H, d, 7.3)	4.85 (1H, d, 8.3)
H-2	3.85 (1H, dd, 7.7, 9.5)	3.29 (1H, dd, 7.3, 8.5)	3.64 (1H, dd, 8.3, 10.4)
H-3	3.55 (1H, dd, 9.5, 3.4)	3.37 (1H, dd, 8.5, 8.5)	3.39 (1H, dd, 8.7, 10.4)
H-4	3.80 (1H, dd, 3.4, 1.0)	3.51 (1H, ddd, 8.5, 5.1, 9.5)	3.15 (1H, dd, 8.7, 9.8)
H-5	3.47 (1H, ddd, 1.0, 6.3, 6.3)	3.28 (1H, dd, 9.5, 11.7)	3.25 (1H, ddd, 9.8, 2.6, 7.4)
		3.87 (1H, dd, 5.1, 11.7)	
H-6	3.71 (2H, d, 6.3)	--	3.58 (1H, dd, 7.4, 12.0)
			3.85 (1H, dd, 2.6, 12.0)

[a] proton # chemical shift (multiplicity, coupling constant J in Hz)

8.7, 9.8, 2.6 and 7.4 Hz respectively, which suggested that H-1, H-2, H-3, H-4 and H-5 were all axial and identified the presence of a glucopyranose moiety. Interpretation of an HMQC spectrum allowed for the assignment of all ^{13}C NMR resonances (Table III). Comparison of the observed values to those reported in the literature for NAc-Glc[8] and Xyl[9] matched with C-1 substituted forms while the Gal[2] matched with a C-1 and C-2 substituted form as drawn in 1. HMBC correlations observed between the N-acetyl carbonyl (174.1 ppm) and the sugar proton observed at δ 3.64 allowed for the placement of the $NHCOCH_3$ group at C-2 of the glucopyranoside. Other HMBC correlations which allowed for the placement of the sugar units were Gal H-1 to C-3 of the aglycone; Xyl H-1 to C-30 of the aglycone and NAc-Glc H-1 to Gal C-2.

Table II. ^{13}C NMR Chemical Shifts for Aglycone of Eryloside E (in CD_3OD)

C-1	36.4(t)	C-9	142.1(s)	C-17	52.4(d)	C-25	39.3(s)
C-2	27.6(t)	C-10	38.6(s)	C-18	18.5(q)	C-26	26.0(q)
C-3	91.5(d)	C-11	23.3(t)	C-19	19.1(q)	C-27	26.0(q)
C-4	40.5(s)	C-12	28.9(t)	C-20	38.2(d)	C-28	28.4(q)
C-5	51.4(d)	C-13	not obs.	C-21	19.8(q)	C-29	16.8(q)
C-6	19.4(t)	C-14	64.3(s)	C-22	32.7(t)	C-30	176.0(s)
C-7	28.6(t)	C-15	31.0(t)	C-23	33.9(t)	C-31	23.0(q)
C-8	128.4(s)	C-16	30.0(t)	C-24	77.5(s)	C-32	26.0(q)

Table III. ^{13}C NMR Chemical Shifts for Sugars of Eryloside E (in CD_3OD)

	Gal	Xyl	NAc-Glc
C-1	105.6(d)	96.4(d)	102.2(d)
C-2	77.7(d)	74.0(d)	58.2(d)
C-3	76.9(d)	76.2(d)	77.9(d)
C-4	70.5(d)	71.0(d)	72.9(d)
C-5	76.2(d)	67.4(t)	78.2(d)
C-6	62.4(t)	--	63.7(t)
CO	--	--	174.1(s)
CH_3	--	--	20.7(q)

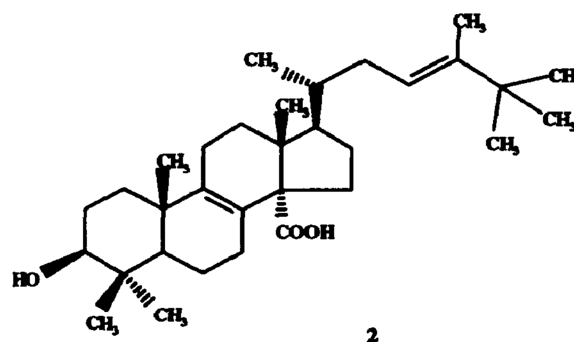


Table IV. ^{13}C NMR Chemical Shifts for the Hydrolysed Aglycone 2 (in DMSO-d_6)

C-1	34.9(t)	C-9	139.0(s)	C-17	50.2(d)	C-25	35.8(s)
C-2	27.5(t)	C-10	37.0(s)	C-18	17.6(q)	C-26	28.9(s)
C-3	76.7(d)	C-11	21.6(t)	C-19	19.2(q)	C-27	28.9(q)
C-4	38.5(s)	C-12	27.2(t)	C-20	36.6(d)	C-28	28.1(q)
C-5	49.7(d)	C-13	46.2(s)	C-21	18.4(q)	C-29	15.8(q)
C-6	17.9(t)	C-14	61.2(s)	C-22	34.1(t)	C-30	176.4(s)
C-7	28.7(t)	C-15	30.8(t)	C-23	119.2(d)	C-31	12.7(q)
C-8	127.1(s)	C-16	27.5(t)	C-24	143.1(s)	C-32	28.9(q)

Further confirmation of the substitution pattern of the sugars was obtained by recording the ^{13}C NMR spectra for 1 in CD_3OD and CD_3OH under identical conditions and observing the deuterium isotope shifts of the carbon resonances.[10] All of the sugar carbons bearing OH groups shifted to low field by about 0.1-0.2 ppm leaving Gal: C-1, C-2 and C-5; NAc-Glc: C-1, C-5 and Xyl: C-1, C-5 unchanged. The same magnitude shift was observed for C-24 indicating the presence of a free OH at C-24. The stereochemistry of the OH at C-3 was assigned as β based on the axial H-3 (dd, J = 4.5, 11.7 Hz). The remainder of the stereochemistry was assigned, based upon comparison to the data reported for penasterol. To the best of our knowledge eryloside E is the first compound in this series to have sugar substitution at C-30 through an ester linkage in combination with the rare *t*-butyl substitution of the side chain. Eryloside E weakly inhibited binding of ^{125}[I]-Bolton Hunter labelled C5a to its receptor (IC_{50} > 10 μM). Due to the weak binding activity, eryloside E was not pursued further for C5a activity. Eryloside E also showed immunosuppressive activity with an EC_{50} of 1.3 μg/mL in the mixed lymphocyte reaction assay and a TC_{50} of 12.3 μg/mL in a lymphocyte viability assay,[11] resulting in a TI [therapeutic index (TC_{50}/EC_{50})] of 9.5. This indicates that the immunosuppressive effect is specific and is not due to general cytotoxic effects.

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REFERENCES AND NOTES

1. Carmely, S.; Roll, M; Loya, Y.; Kashman, Y. *J. Nat. Prod.* **1989**, *52*, 167-170.
2. D'Auria, M.V.; Paloma, L.G. ; Minale, L.; Riccio, R.; Debitus, C. *Tetrahedron* **1992**, *48*, 491-498.
3. a) Wiedenmayer, F. *Experientia Suppl. 28*: *Shallow-water sponges of the western Bahamas*; Birkhauser Verlag: Basel, 1977, pp. 1- 287. b) A taxonomic voucher spaecimen is deposited in the Harbor Branch Oceanographic Museum, catalog number 003:00902.
4. The NMR measurements were recorded on a Bruker instrument operating at 360 MHz for ^{1}H and 90 MHz for ^{13}C. The 2D NMR spectra were recorded on a Bruker instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. The IR spectra were recorded on a MIDAC M series instrument. The optical activity was measured on a JASCO DIP-360 digital polarimeter. Mass spectra were recorded on a Kratos MS-80RFA instrument.
5. Eryloside E: $[\alpha]_D$ -23.4° (C = 0.44 in MeOH); IR (film on KBr) ν_{max} 3388 (brd), 2939, 1717, 1639, 1559, 1374, 1069 cm^{-1}; HRFABMS *m/z* $(M+Na)^+$ 1022.5650, calcd. 1022.5664 for $C_{51}H_{85}NO_{18}Na$ (Δ 1.4 mmu); FABMS *m/z* 1022 (7.1), 844 (0.6), 498 (0.6), 467 (1.6), 423 (4.9); ^{1}H NMR (aglycone; CD_3OD) δ 3.19 (1H, dd, J = 4.5, 11.7 Hz, H-3), 1.09 (3H, s, H-28), 1.05 (3H, s, H-31), 1.04 (3H, s, H-19), 0.92 (9H, s, H-26, H-27, H-32), 0.91 (3H, d, J = 6.5 Hz, H-21), 0.89 (3H, s, H-29), 0.80 (3H. s, H-18), 1.98 (3H, s, CH_3CONH); See tables I, II and III for ^{1}H and ^{13}C NMR.
6. Compound 2 :- $[\alpha]_D$ -36.7° (C = 0.23 in MeOH); IR (film on KBr) ν_{max} 3347 (brd), 2958, 2272, 1687, 1463, 1373, 1040 cm^{-1}; HREIMS *m/z* M^+ 484.3923, calcd. 484.3916 for $C_{32}H_{52}O_3$ (Δ 0.7 mmu); EIMS *m/z* 484 (1.3), 438 (1.7), 372 (0.9), 313 (1.0); ^{1}H NMR (DMSO-d_6) δ 5.14 (1H, brd t, J = 6.9 Hz, H-23), 2.98 (1H, m, H-3), 1.54 (3H, s, H-31), 0.98 (9H, s, H-26,27 and 32), 0.94 (3H, s, H-19), 0.89 (3H, s, H-28), 0.83 (3H, d, J = 6.7 Hz, H-21), 0.69 (6H, s, H-18, H-29); See table IV for ^{13}C NMR.
7. Cheng, J.-F.; Kobayashi, J.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T. *J. Chem. Soc. Perkin Trans. I* **1988**, 2403-2406.
8. Tachibana, K.; Sakaitani, M.; Nakanishi, K. *Tetrahedron* **1985**, *41*, 1027-1037.
9. Kalinowoski, H.-O.; Berger, S. *Carbon-13 NMR Spectroscopy*; John Wiley and Sons: New York, 1988; p 441.
10. Zabriskie T.M.; Mayne C.L.; Ireland C.M. *J. Am. Chem. Soc.* **1988**, *110*, 7919-7920.
11. Longley, R.; Caddigan, O.; Harmody, D.; Gunasekera, M.; Gunasekera, S. *Transplantation* **1991**, *52*, 650-656.

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