Stelliferin Riboside, a Triterpene Monosaccharide Isolated from the Fijian Sponge *Geodia globostellifera*

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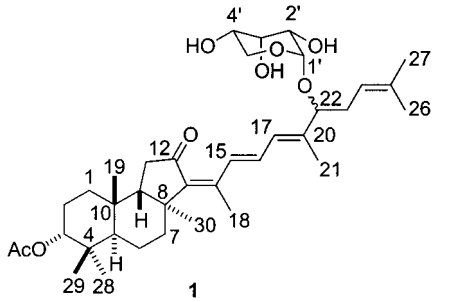
A triterpene monosaccharide, stelliferin riboside (**1**), was isolated from the Fijian marine sponge *Geodia globostellifera*. In addition, stellettins A and B (**2**, **3**) were also isolated. This is the first report of a stelliferin from this species and the first example of a saccharide derivative of a stelliferin.

Chemical investigations of sponges in the genus *Geodia* have resulted in the isolation of steroids, alkaloids, nucleosides, polypeptides, and cyclic peptides with interesting biological activities.[1−6] There have been no reports, however, of terpenoids from this genus. The rare isomalabaricane class of triterpenoids have only been reported from sponges of the genera *Jaspis*[7−10] and *Stelletta*.[11−15] During our investigations of the sponge *Geodia globostellifera*, we have isolated an unusual cytotoxic triterpene, **1**, consisting of an isomalabaricane skeleton with an α-ribopyranose moiety together with two known isomalabaricane triterpenoid compounds, stellettins A (**2**) and B (**3**).



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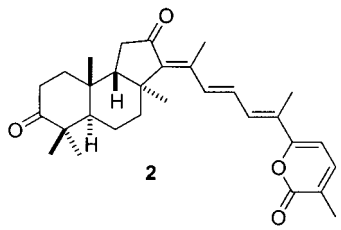

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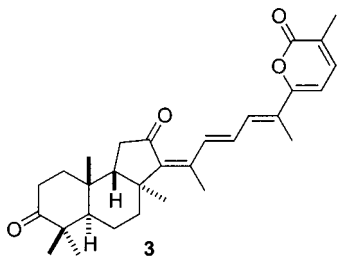

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Geodia globostellifera (Carter 1880, Geodiidae) was collected at a depth of about 5 m from Cakaulevu reef, in the district of Wainunu, in the island of Vanua Levu, Fiji Islands. A small portion of the crude methanol extract was subjected to anticancer bioassays, which showed an IC_{50} of 0.017 and 0.01 μg/mL for ovarian tumor (A2780) and leukemia (K562) cells, respectively, which warranted further investigation. A sample of the sponge was freeze-dried and shipped to the Department of Chemistry, University of Aberdeen, where it was extracted and a solvent partition was carried out. Of the four solvent partition fractions, the CH_2Cl_2 fraction displayed the highest IC_{50} values for ovarian tumor (A2780) and leukemia (K562) cells, 0.14 and 0.4 μg/mL, respectively. This fraction was further subjected to Sephadex LH-20 chromatography followed by reversed-phase flash chromatography and HPLC to yield compounds **1**, **2**, and **3**. In addition, the hexane partition fraction also yielded **2** and **3**.

Inspection of the ^1H, ^{13}C NMR, DEPT-135, and MS data of compounds **2** and **3** indicated that they were the known compounds stellettin A and B, respectively, previously isolated from sponges of the genera *Jaspis* and *Stelletta*. Interpretation of the ^1H and ^{13}C and DEPT-135 NMR spectra of compound **1** (Table 1) indicated the presence of nine methyls, seven methylenes, 12 methines, and nine quaternary carbons, giving a CH count of $C_{37}H_{53}$. The ^{13}C NMR spectrum also indicated oxygenated functionalities: an acetate group, a conjugated ketone, and a pentose sugar moiety adding a further O_8H_3, giving a molecular formula of $C_{37}H_{56}O_8$, which was confirmed by the high-resolution electrospray MS *m/z* 651.3876 ($[M + Na]^+$, Δ −0.3 mmu). The protonated carbons were all assigned using an HSQC NMR experiment in combination with DEPT-135 experiments. An unsaturation number of 10, in conjunction with the presence of the acetate and ketone carbonyl groups and four double bonds, suggested the presence of four rings.

The UV spectrum of **1** (λ_{max} = 346 nm, EtOH) suggested the presence of a trienone chromophore,[16] which was further supported by a strong IR absorption band at 1687 cm^{-1} typical of α,β-unsaturated ketones.[17] The splitting pattern and the respective *J* values of the protons in the olefinic region of the ^1H spectrum indicated proton H-16 (δ 6.76 ppm) to be coupled to both protons H-15 (δ 7.96 ppm) and H-17 (δ 6.20 ppm) in a trans geometry typical of carotenoids. A substructure consisting of C-13 to C-27 was constructed, which was further supported by $^1H-^1H$ COSY correlations H-17−H-21, H-22−H-23, H-26−H-27. The HMBC NMR spectrum showed connectivity from the H-18 to C-13 and C-14, indicating methyl 18 was attached at C-14. Similarly, H-21 showed HMBC correlations to C-22 and C-17, which suggested that the methyl 21 was attached at C-20. Further HMBC connectivities were found from C-14 to H-15 and H-16; C-17 to H-15 and H-22; C-24 to H-22, H-23, H-26, and H-27; and C-25 to H-23, H-26, and

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Table 1. ^{1}H and ^{13}C NMR Spectral Data in $CDCl_3$ at 400/100 MHz for Compound **1**

atom no.	^{13}C	^{1}H (δ/ppm, m, J (Hz))	HMBC data (^{13}C → ^{1}H)
1	31.5 (t)	0.90 (1H, m)	H-3, H-19
		1.05 (1H, m)	
2	25.4 (t)	1.68 (1H, m)	
		1.90 (1H, m)	
3	79.0 (d)	4.72 (1H, dd, 3.2)	H-1, H-5, H-28, H-29
4	38.4 (s)		H-28, H-29
5	42.6 (d)	2.32 (1H, m)	H-3, H-19, H-28, H-29
6	19.1 (t)	0.96 (1H, m)	
		1.40 (1H, m)	
7	39.6 (t)	1.30 (2H, m)	H-30
8	45.7 (s)		H-9, H-11, H-30
9	51.5 (d)	1.78 (1H, m)	H-11, H-19
10	36.6 (s)		H-19
11	37.7 (t)	2.14 (2H, m)	
12	208.1 (s)		H-11
13	147.8 (s)		H-7, H-15, H-18, H-30
14	143.2 (s)		H-15, H-16, H-18
15	134.2 (d)	7.96 (1H, d, 15.6)	H-17, H-18
16	130.4 (d)	6.76 (1H, dd, 11.0, 15.3)	
17	131.7 (d)	6.20 (1H, d, 11.2)	H-15, H-21, H-22
18	17.0 (q)	1.98 (3H, s)	H-15
19	23.4 (q)	0.98 (3H, s)	
20	139.1 (s)		H-16, H-22
21	12.8 (q)	1.73 (3H, s)	H-17, H-22
22	83.0 (d)	4.15 (1H, t, 7.2)	H-17, H-21, H-23, H-1′
23	33.5 (t)	A 2.18 (1H, m)	H-22
		B 2.36 (1H, m)	
24	120.5 (d)	5.00 (1H, bm)	H-22, H-23A, H-23B, H-26, H-27
25	135.4 (s)		H-23A, H-23B, H-26, H-27
26	26.8 (q)	1.64 (3H, s)	
27	19.0 (q)	1.57 (3H, s)	
28	28.8 (q)	0.88 (3H, s)	H-29
29	22.5 (q)	0.84 (3H, s)	H-3, H-28
30	25.2 (q)	1.36 (3H, s)	
AcO (CH_3)	22.3 (q)	2.02 (3H, s)	
(C=O)	171.5 (s)		H-3, CH_3C=O
1′	98.9 (d)	4.37 (1H, d, 4.8)	H-22, H-5′A, H-5′B,
2′	72.2 (d)	3.57 (1H, dd, 4.6, 6.4)	H-3′, H-5′A, H-5′B
3′	73.7 (d)	3.67 (1H, t, 6.0)	H-2′
4′	70.8 (d)	3.71 (1H, dm, 5.5)	H-2′
5′	64.0 (t)	A 3.34 (1H, dd, 6.0, 12.1)	H-1′, H-5′A
		B 4.00 (1H, dd, 3.4, 12.1)	H-5′B

H-27, thus confirming the C-13−C-27 substructure. NOE difference experiments as well as cross-peaks in the T-ROESY spectrum (T_{mix} = 0.3 s) for H-16−H-18 and H-21, H-15−H-17, H-24−H-27 firmly established the *Z, E, E, E* configuration of the C-13, C-15, C-17, C-24 decatetraene system. Further data came from the low-field resonances of H-15 (δ 7.96) and H-16 (δ 6.76) in the ^{1}H NMR spectrum of **1**. This suggested that they were in the deshielding region of the carbonyl group, which could happen only if the orientation of the C-13−C-14 double bond was *Z*.[7]

The ^{1}H−^{1}H COSY spectrum also showed that four of the deshielded methines (H-1′, H-2′, H-3′, H-4′) and the deshielded methylene H-5′ were in the same spin system, which suggested the presence of a pentose sugar moiety. A literature search showed ^{13}C chemical shifts most closely resembled those of a ribose sugar in the pyranose form (lit. C-1′−C-5′ 96.3, 72.8, 72.0, 70.0, 65.7 ppm, exptl 98.9, 72.2, 73.7, 70.8, 64.0 ppm).[18] A molecular mechanics minimization of α-ribopyranose using the Amber force field and calculation of the coupling constant using the Altona equation gave a calculated value of $J_{(H-1'-H2')}$ = 4.5 Hz, which is close to the experimentally observed value of 4.8 Hz.[19] NOEs confirming the pentose sugar was α-ribopyranose were found between H-1′−H-2′; H-1′−H-3′; H-1−H-5′B; H-2′−H-4′; H2′−H-5′B; H3′−H-5′B; and H-4−H5′B. HMBC connectivities from H-22 to C-1′ as well as C-22 and H-1′ indicated the position of attachment of the α-ribopyranose moiety.

The remainder of the molecule was constructed by referring to the NMR data for **2** and **3**, as well as the use of HMBC correlations. The conjugation requirement of the ketone carbonyl relegated it to the only remaining logical position, C-12. This was confirmed by HMBC correlation from C-12 to H-11. The position of the acetoxy group was found by HMBC correlations from the methyl group at δ_H 2.02 ppm to the ester carbonyl at δ_C 171.5 ppm, which was shown to be coupled to an oxymethine proton signal at δ 4.72 ppm (H-3), indicating an acetoxy group was present at C-3. Apart from correlating to each other, both H-28 and H-29 and H-5 correlated to C-3, allowing the placement of C-3. HMBC correlations from H-30 to C-7 and C-8 and H-7, H-15, H-18, and H-30 to C-13 indicate that the unsaturated side chain is joined to the tricyclic system at C-13.

HMBC correlations from H-11 and H-9 to C-8 allowed the placement of H-9. C-5 was found to correlate to H-3, H-19, H-28, and H-29, indicating the position of C-5. Positioning of the other three remaining methylene (C-1, C-2, and C-6) positions was hampered by overlapping signals. However, this was accomplished by comparing ^{13}C chemical shifts with those of known isomalabaricanes found in the literature.[9,12]

The relative stereochemistry of **1** was determined by a T-ROESY experiment (T_{mix} = 0.3 s) and by comparison of ^{13}C chemical shift data with the previously isolated triterpenoid isomalabaricanes found in the literature.[7,10] A

Table 2. Anticancer Activities (IC_{50} Values, μg/mL) of **1**, **2**, and **3**

compound	A2780 ovarian tumor	K562 leukemia
1	38.1	>50.0
2	1.52	3.82
3	0.0051	0.93

useful model was described by Tsuda et al.,[9] where the presence of a ^{13}C chemical shift of ca. δ 22.5 for the 4β-Me (C-29) is indicative of the presence of a 3α-acetoxy group since the chemical shift is generally lower field than for a 3β-acetoxy group (δ_C 17.0 for 4β-Me is generally associated with a 3β-acetoxy).

To determine the stereochemistry of the ring junctions of the tricyclic fragment, ^{13}C chemical shift values of δ 42.6, 23.4, 25.2, and 51.5 for C-5(α), C-19(β), C-30(α), and C-9(β), respectively, agree well with published data. This was confirmed by key cross-peaks in the T-ROESY spectrum between H-5−H-30 and the acetoxy-Me and H-9−H-19 and H-19−H-29, which suggested a trans−syn−trans stereochemistry, consistent with the crystal structure of known isomalabaricane skeletons.[20]

This is the first ever report of a stelliferin from the marine sponge *Geodia globostellifera* and also the first example of a stelliferin type molecule containing a sugar moiety. The sugar moiety at C-22 could be an important precursor in the biosynthetic pathway for the biosynthesis of stelliferins.

The biological activity of these three compounds is shown in Table 2. Stelliferin riboside (**1**) was found to show moderate cytotoxicity against ovarian cancer cells. The major activity was due to the presence of stellettin A (**2**) and B (**3**), with the latter being very potent against ovarian cancer cells. Stelletin A and B differ only in the geometry of the C-13 and C-14 bond but differ by a significant magnitude in anticancer activities. The activity of stellettin A has been reported by Su et al.[11] with an IC_{50} of 0.001 μg/mL against P388 leukemia cells. The NCI's 60-cell line assay[12] reported GI_{50} concentrations for a mixture of the two isomers (stellettin A and B) in the lower to mid nanomolar range.

Experimental Section

General Experimental Procedures. Low-resolution electrospray mass spectra were obtained on a Finnigan Masslab Navigator, and high-resolution mass data were obtained on a Finnigan MAT-95. ^1H, ^{13}C, and all NMR 2D experiments were recorded on a Varian Unity INOVA 400 MHz spectrometer, in $CDCl_3$ solution. Chemical shifts are reported in parts per million (δ) downfield relative to residual $CHCl_3$ at 7.27 ppm. HPLC separations were carried out using a Spectra series P100 isocratic pump and monitored using a Hewlett-Packard HP 1050 Series variable wavelength UV detector and a Waters reversed-phase (ODS (10 × 250 mm)) column. UV and IR were taken on a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer and Ati Mattson Genesis Series FTIR machine, respectively.

Animal Material. The sample of *Geodia globostellifera* (2.99 kg dry wt), collection number 9712SD156, was collected in December 1997 at a depth of about 5 m by free diving from Cakaulevu reef, in the district of Wainunu, on the island of Vanua Levu, Fiji Islands (17° 2.609′; 178° 54.694′ E). It was identified by John Hooper of the Queensland Centre for Biodiversity, and a voucher specimen (No. 9712SD114) is preserved at the Regional Herbarium, School of Pure and Applied Sciences, University of the South Pacific, Fiji.

Extraction and Isolation. The sponge was stored in ice and transported to the Department of Chemistry, University of the South Pacific, where it was frozen before processing. The sponge was cut into small pieces and then freeze-dried. Freeze-dried samples were stored in plastic bags and sent by courier mail to the Department of Chemistry, University of Aberdeen. The sponge was extracted with MeOH (3×) and CH_2Cl_2 (3×), the solvent was removed under reduced pressure, and the extracts were combined. The crude oil was partitioned between water and CH_2Cl_2. The aqueous layer was then extracted with *s*-BuOH to give a yellow-colored oil. The solvent was removed from the CH_2Cl_2 layer, and the resulting oil was partitioned between *n*-hexane and 10% aqueous MeOH. The MeOH layer was then phase adjusted to 50% aqueous MeOH and extracted with CH_2Cl_2. The CH_2Cl_2 fraction was purified by Sephadex LH-20 chromatography (MeOH−CH_2Cl_2, 1:1) followed by reversed-phase flash chromatography (MeOH). Further purification was achieved by reversed-phase HPLC (ODS, MeOH) to afford 10.4 mg of compound **1**, 14.7 mg of compound **2**, and 7.3 mg of compound **3**. The *n*-hexane fraction was also subjected to Sephadex LH-20 chromatography using (CH_2Cl_2−*n*-hexane, 1:1). Further purification by reversed-phase HPLC (ODS, MeOH) afforded 9.6 mg of **2** and 8.3 mg of **3**.

Stelliferin riboside (1): light yellowish oil; UV (100% EtOH) λ_{max} 338 (ϵ 47 640); $[\alpha]_D$ −119 (*c* 0.02 g/mL, MeOH); IR 2954, 2923, 1733, 1687, 1584, 1559, 1458, 1373, 1243, 1163, 1033, 986; LRESIMS *m*/*z* 651.3 $[M + Na]^+$; HRESIMS *m*/*z* 651.3876 $[M + Na]^+$ Δ −0.3 mmu calcd for $C_{37}H_{56}O_8Na^+$; NMR data, see Table 1.

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