Globostellatic Acids A−D, New Cytotoxic Isomalabaricane Triterpenes from the Marine Sponge *Stelletta globostellata*[1]

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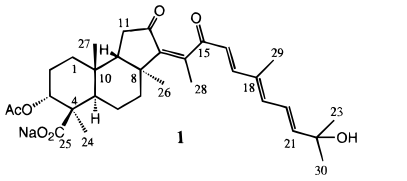
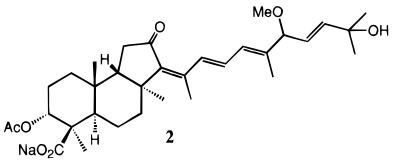
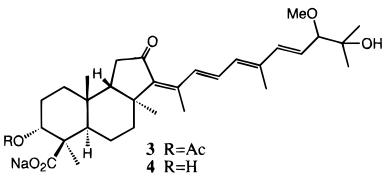
Four new cytotoxic triterpenes, globostellatic acids A−D (**1**−**4**), have been isolated from the marine sponge *Stelletta globostellata*. Their structures have been determined by interpretation of spectral data. They exhibited cytotoxicity against P-388 murine leukemia cells with IC_{50} values of 0.1−0.46 μg/mL.

Triterpenoids are a minor group of sponge metabolites;[2] among these, malabaricane/isomalabaricane triterpenoids are known only from two Indo-Pacific sponges *Jaspis stellifera*,[3−5] a Somalian *Stelletta* sp.,[6] and the Chinese *Stelletta tenuis*.[7] In our continuing search for cytotoxic/antitumor metabolites from Japanese marine invertebrates, we found cytotoxic activity against P-388 murine leukemia cells in the MeOH extract of the marine sponge *Stelletta globostellata* Carter, 1883 (Stellettidae) collected from southern Japan. Bioassay-guided isolation afforded four new active triterpenes, globostellatic acids A−D (**1**−**4**). In this paper we describe the isolation and structure elucidation of these metabolites.

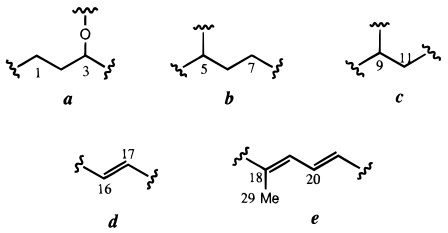






The EtOH extract of the frozen sponge (1.0 kg) was partitioned between H_2O and Et_2O; the ether phase was further partitioned between *n*-hexane and MeOH/H_2O (9:1). The aqueous MeOH fraction was repeatedly fractionated by ODS flash chromatography (MeOH/H_2O, 6:4, to MeOH), gel-filtration on Sephadex LH-20 (CH_2Cl_2/acetone, 1:4), and reversed-phase HPLC with MeOH/0.1M $NaClO_4$ (77:23) and MeCN/0.1M $NaClO_4$ (47:53) to afford glabostellatic acids A−D as bright yellow solids (**1**, 21 mg, 2.1 × 10^{-5}% yield based on wet weight; **2**, 12 mg, 1.2 × 10^{-5}%; **3**, 18 mg, 1.8 × 10^{-5}%; **4**, 8.0 mg, 8.0 × 10^{-6}%).

Globostellatic acid A (**1**) had a molecular formula of $C_{32}H_{43}O_7Na$ as determined by HRFABMS and ^{13}C-NMR data. The IR (3450, 1735, 1715, and 1690 cm^{-1}) and UV spectra [326 nm (ϵ 56 000) and 226 (24 000)], together with ^{1}H-NMR data [five methyls on sp^3 quaternary carbons (δ 1.06, 1.16, 1.31, 1.31, 1.51), two olefinic methyls (1.91, 1.96), an acetyl (2.06), an oxygenated methine (5.41), and five olefinic protons] were reminiscent of a malabaricane or isomalabaricane triterpene. In fact, the gross structure **1** was assigned by interpretation of 2D NMR data. Structural units ***a***−***e*** were readily obtained by tracing COSY cross peaks, and they were connected on the basis of HMQC and HMBC data.[8] Connectivity of C3 (unit ***a***) and C5 (unit ***b***) via C4, which was linked to methyl (δ 1.16) and carboxyl (δ 181.2) groups, was deduced from HMBC cross peaks: CH_3-24/C3, C4, C5, C25. C1 (unit ***a***), C5 (unit ***b***), and C9 (unit ***c***) could be connected to C10, which carried a methyl group (δ 1.06) as rationalized by HMBC cross peaks CH_3-27/C1, C5, C9, C10. Connectivities of C7 (unit ***b***) and C9 (unit ***c***) through C8 were justified by HMBC cross peaks CH_3-26/C7, C8, C9, C13, which also implied that C8 was linked to an sp^2 carbon (δ 148.7). HMBC cross peaks H11α/C13, C12 substantiated that C13 was connected to C11 through the C12 ketone (δ 206.9). HMBC cross peaks CH_3-23 (CH_3-30)/C21, C22 indicated that C21 was linked to a dimethyl carbinol unit. An acetoxy functionality was placed at C3 by an HMBC cross peak between H3 and the acetoxy carbonyl carbon at δ 172.5, thus constructing gross structure **1** belonging to the malabaricane or isomalabaricane class of triterpenoids.



Values of coupling constants $J_{16,17}$ = 16.0, $J_{20,21}$ = 15.5 and the chemical shift (δ 12.7) of CH_3-29 suggested 16*E*,18*E*,20*E*-geometries, whereas 13*Z*-geometry was assigned by a NOESY cross peak[9] between CH_3-28 and CH_3-26. ^{13}C-NMR data for the tricyclic portion, espe-

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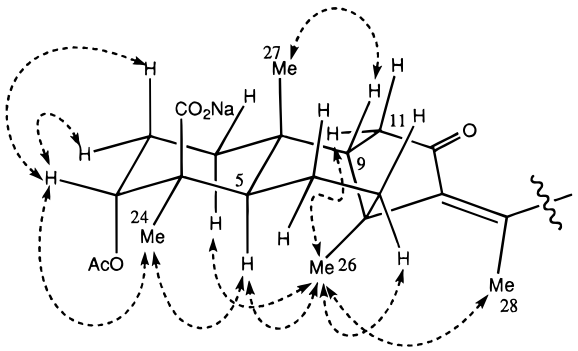

Figure 1. NOESY correlations for globostellatic acid A (**1**).

cially the chemical shifts of carbons in ring B, indicated that **1** had an isomalabaricane skeleton,[5] which was substantiated by NOESY data (Figure 1). An intense cross peak between CH_3-26 and H5 suggested that ring B was in the boat conformation. Another intense cross peak, CH_3-26/H1α, and a weaker but distinct cross peak, CH_3-26/H11α and CH_3-26/H7α, led to assignment of diastereotopic methylene protons, whereas CH_3-27 was only correlated with H9. Therefore, the *trans-syn-trans* stereochemistry was established. A NOESY cross peak CH_3-24/H5 revealed an α-disposition of the methyl group at C4, whereas small coupling constants observed between H3 and both of the C2 methylene protons indicated that H3 was equatorial. The relative stereochemistry is thus assigned as shown; the absolute stereochemistry was not determined.

Globostellatic acid B (**2**) revealed an $(M - H)^-$ ion *m/z* 577.3131 in its HRFABMS spectrum, establishing a molecular formula of $C_{33}H_{46}O_7Na$. The molecular formula and ^{13}C-NMR data of **2** indicated that one of the ketones in **1** was replaced by a methine linked to an *O*-methyl group in **2**. In fact, placement of the methoxy group at C19 was straightforward by 2D NMR analysis, while the vinylic methine nature of C15 was apparent. Spectral data indicated the same relative stereochemistry of the tricyclic portion as that of **1**.

Globostellatic acid C (**3**) was an isomer of **2**. The conjugated system in **3** was longer than that of **2** as revealed by UV data [**3**, 257 nm (ϵ 9200), 368 (25 000); **2**, 233 (15 800), 341 (51 000)]. NMR data of **3** were superimposable on those of **2**, except for chemical shifts of the terminal portion of the side chain. An HMBC cross peak CH_3-23/C21 (δ 90.9) placed an *O*-methyl group at C21.

Globostellatic acid D (**4**) had a molecular formula of $C_{31}H_{44}O_6Na$, suggesting a deacetyl derivative of **3**. An identical chromophore as in **3** was revealed by the UV spectrum. NMR data of **4** were quite similar to those of **3** except for the absence of the acetyl group in **4**, which was apparent from an upfield shift of the C3 proton to δ 4.08. Therefore, compound **4** was the deacetyl derivative of **3**, which was in agreement with 2D NMR data.

The bright yellow interior of this sponge apparently comes from globostellatic acids, which constitute additional examples of isomalabaricane triterpenes. Although two carboxylic acid methyl esters of malabaricane triterpenes were reported from the Great Barrier Reef collection of *Jaspis stellifera*,[3] they seem to have the isomalabaricane skeleton as do other isomers of sponge origin. Globostellatic acids A−D were cytotoxic against P-388 murine leukemia cells, with IC_{50} values of 0.1, 0.1, 0.46, and 0.1 μg/mL, respectively.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Hitachi 330 spectrophotometer. IR spectra were measured with a JASCO FT/IR-5300 infrared spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL A500 NMR spectrometer. ^{1}H and ^{13}C chemical shifts were referenced to solvent peaks: δ_H 3.3 and δ_C 49.9 for CD_3OD. MS were obtained with a JEOL SX 102 mass spectrometer. HRFABMS were measured using a dual target inlet probe. Optical rotations were determined with a JASCO DIP-371 digital polarimeter.

Animal Material. The sponge samples were collected by scuba at depths of 3−10 m off Mage Island near Kagoshima, 1000 km south of Tokyo; frozen immediately after collection; and kept frozen until used. The sponge was identified as *Stelletta globostellata* Carter, 1883 (class Dosmospongia, order Astrophorida, family Ancorinidae) by Dr. Rob van Soest, University of Amsterdam. A voucher specimen (ZMA POR 11016) was deposited at the Zoological Museum of the University of Amsterdam, The Netherlands.

Extraction and Isolation. The frozen sponge (1.0 kg, wet wt) was extracted by blending with EtOH (2 L × 3). The combined extracts were concentrated and partitioned between H_2O (1 L) and Et_2O (1 L × 3). The ether-soluble portion was further partitioned between *n*-hexane and 90% MeOH. The aqueous MeOH layer (6.8 g) was fractionated by flash chromatography on an ODS column (70/230 mesh) with 60%, 80%, 90%, and 100% MeOH. The cytotoxic 80% MeOH fraction (2.1 g) was gel-filtered on Sephadex LH-20 with CH_2Cl_2/Me_2CO (1:4) to afford an active fraction (870 mg), which was repeatedly purified by reversed-phase HPLC (ODS, 10 × 250 mm; flow rate 2.0 mL/min; UV detection at 220 nm), with MeOH/0.1 M $NaClO_4$ (77:23) and MeCN/0.1M $NaClO_4$ (47:53), followed by desalting with ODS to yield globostellatic acids A−D (**1**, 21 mg, 2.1 × 10^{-3}% yield based on wet weight; **2**, 12 mg, 1.2 × 10^{-3}%; **3**, 18 mg, 1.8 × 10^{-3}%; **4**, 8.0 mg, 8.0 × 10^{-4}%).

Globostellatic acid A (1): yellow amorphous solid; $[\alpha]^{23}_D$ −45.7° (*c* 0.35, MeOH); IR (film) 3450 (br), 2970, 2930, 1735, 1715, 1630, 1600, 1575, 1245, 754 cm^{-1}; UV (MeOH) 326 nm (ϵ 56 000), 226 (24 000); FABMS *m/z* 561 [$(M - H)^-$]; HRFABMS *m/z* 561.2836 calcd for $C_{32}H_{42}O_7Na$ (Δ + 0.7 mmu); ^{1}H NMR (CD_3OD) δ 7.02 (1H, d, *J* = 16.0 Hz, H17), 6.65 (1H, dd, *J* = 11.5, 15.5 Hz, H20), 6.44 (1H, d, *J* = 11.5 Hz, H19), 6.13 (1H, d, *J* = 16.0 Hz, H16), 6.12 (1H, d, *J* = 15.5 Hz, H21), 5.41 (1H, br s, H3), 2.38 (1H, m, H5), 2.34 (1H, m, H2*β*), 2.26 (1H, m, H7*β*), 2.24 (1H, m, H11α), 2.11 (1H, m, H7α), 2.09 (1H, m, H11*β*), 2.06 (3H, s, AcO), 1.99 (1H, m, H9), 1.98 (2H, m, H6), 1.96 (3H, s, Me28), 1.91 (3H, s, Me29), 1.79 (1H, m, H1α), 1.68 (1H, br d, 14.5, H2α), 1.51 (3H, s, Me26), 1.31 (6H, s, Me23, Me30), 1.18 (1H, m, H1*β*), 1.16 (3H, s, Me24), 1.06 (3H, s, Me27); ^{13}C NMR (CD_3OD) see Table 1.

Globostellatic acid B (2): pale yellow amorphous solid; $[\alpha]^{23}_D$ +126.6° (*c* 0.54, MeOH); IR (film) 3450 (br), 2970, 2930, 1735, 1685, 1580, 1555, 1245, 756 cm^{-1}; UV (MeOH) 233 nm (ϵ 15 800), 341 (51 000); FABMS *m/z* 577 [$(M - H)^-$]; HRFABMS *m/z* 577.3131 calcd for $C_{33}H_{46}O_7Na$ (Δ − 1.0 mmu); ^{1}H NMR (CD_3OD) δ 6.96 (1H, dd, *J* = 11.0, 15.0 Hz, H16), 6.65 (1H, d, *J* = 15.0 Hz, H15), 6.28 (1H, br d, *J* = 11.0 Hz, H17), 5.83 (1H,

Table 1. ^{13}C-NMR Data for Globostellatic acids A−D (**1**−**4**) in CD_3OD at 300K[a]

compd	**1**	**2**	**3**	**4**
1	31.2	30.7	30.5	30.1
2	26.6	26.2	26.2	28.7
3	77.3	76.8	75.6	71.4
4	49.9	48.9	48.1	49.1
5	43.7	43.4	43.7	41.6
6	21.4	21.3	21.4	21.4
7	38.6	41.3	41.1	41.3
8	44.8	46.1	46.1	46.2
9	52.3	51.2	50.9	50.9
10	37.4	36.8	37.2	37.1
11	36.1	37.6	37.6	38.0
12	206.9	210.1	209.7	209.9
13	148.7	147.7	148.1	148.2
14	144.3	143.2	143.3	143.0
15	204.2	133.6	134.2	134.2
16	125.6	133.3	133.5	133.5
17	150.6	128.4	132.9	133.1
18	134.8	142.8	139.4	139.3
19	141.3	87.9	139.8	140.1
20	123.9	126.1	128.7	128.6
21	149.1	142.2	90.9	90.8
22	71.8	70.7	73.9	73.4
23	30.1	30.0	26.0	25.3
24	24.6	24.1	23.7	24.3
25	181.2	180.7	180.3	181.8
26	25.2	26.0	25.7	26.0
27	21.0	20.8	20.2	20.6
28	18.1	14.9	14.7	14.8
29	12.7	12.8	12.9	13.1
30	30.1	30.0	25.8	26.7
OAc	172.5	172.3	172.0	
	21.5	21.1	20.8	
OCH_3		56.7	57.3	57.2

[a] Chemical shifts were determined by tracing HMQC and HMBC.

d, J = 15.5, 1.0 Hz, H21), 5.53 (1H, dd, J = 16.0, 6, 5 Hz, H20), 5.34 (1H, br s, H3), 4.09 (1H, br d, J = 6.5 Hz, H19), 3.22 (3H, s, OCH_3), 2.37 (1H, br d, J = 12.0 Hz, H5), 2.25 (3H, s, Me28), 2.23 (2H, m, H2β and H11α), 2.16 (1H, m, H7β), 2.11 (1H, m, H7α), 2.08 (1H, m, H11β), 2.02 (3H, s, AcO), 1.88 (2H, m, H6), 1.84 (1H, m, H9), 1.78 (1H, m, H1α), 1.75 (3H, s, Me29), 1.65 (1H, br d, J = 15.0 Hz, H2α), 1.42 (3H, s, Me26), 1.24 (3H, s, Me23), 1.23 (3H, s, Me30), 1.15 (1H, m, H1β), 1.14 (3H, s, Me24), 0.98 (3H, s, Me27); ^{13}C NMR (CD_3OD) see Table 1.

Globostellatic acid C (3): yellow amorphous solid; $[\alpha]^{23}_D$ +15.2° (*c* 0.82, MeOH); IR (film) 3450, 2930, 1735, 1685, 1570, 1540, 1245, 754 cm^{-1}; UV (MeOH) 257 nm (ϵ 9200), 368 (25 000); FABMS m/z 577 [(M − H)$^-$]; HRFABMS m/z 577.4368 calcd for $C_{33}H_{46}O_7Na$ (Δ − 0.9 mmu); ^{1}H NMR (CD_3OD) δ 7.12 (dd, J = 15.0, 11.0 Hz, H16), 6.75 (1H, d, J = 15.0 Hz, H15), 6.43 (1H, d, J = 16.0 Hz, H19), 6.36 (1H, d, J = 11.0 Hz, H17), 5.74 (1H, dd, J = 16.0, 8.5 Hz, H20), 5.34 (1H, br s, H3), 3.46 (1H, d, J = 8.5 Hz, H21), 3.29 (3H, s, OMe), 2.47 (1H, br d, J = 12.0 Hz, H5), 2.31 (3H, s, Me28), 2.27 (1H, m, H11α), 2.26 (1H, m, H7β), 2.23 (1H, m, H2β), 2.14 (1H, m, H7α), 2.13 (1H, m, H11β), 2.08 (3H, s, OAc), 1.99 (3H, br s, Me29), 1.95 (2H, m, H6), 1.89 (1H, m, H9), 1.81 (1H, m, H1α), 1.71 (1H, m, H2α), 1.47 (3H, s, Me26), 1.23 (3H, s, Me24), 1.17 (1H, m, H1β), 1.15 (3H, s, Me30), 1.14 (3H, s, Me23), 0.99 (3H, s, Me27); ^{13}C NMR (CD_3OD) see Table 1.

Globostellatic acid (4): yellow amorphous solid; $[\alpha]^{23}_D$ +135.7° (*c* 1.1, MeOH); IR (film) 3450, 2970, 2930, 1735, 1685, 1570, 1545, 1450, 1205, 750 cm^{-1}; UV (MeOH) 257 nm (ϵ 9800), 368 (27 000); FABMS m/z 535 [(M − H)$^-$]; HRFABMS m/z 535.2264 calcd for $C_{31}H_{44}O_6$-Na (Δ +2.8 mmu); ^{1}H NMR (CD_3OD) δ 7.12 (1H, dd, J = 15.0, 11.5 Hz, H16), 6.75 (1H, d, J = 15.0 Hz, H15), 6.42 (1H, d, J = 15.0 Hz, H19), 6.33 (1H, br d, J = 11.5 Hz, H17), 5.75 (1H, dd, J = 16.0, 8.0 Hz, H20), 4.08 (1H, br s, H3), 3.46 (1H, d, J = 8.0 Hz, H21), 3.30 (3H, s, OMe), 2.51 (1H, br d, J = 12.0 Hz, H5), 2.32 (3H, s, Me28), 2.27 (1H, m, H11α), 2.24 (1H, m, H7β), 2.20 (1H, m, H2β), 2.14 (1H, m, H7α), 2.13 (1H, m, H11β), 2.00 (3H, d, J = 1.5 Hz, Me29), 1.92 (1H, m, H1α), 1.88 (2H, m, H6), 1.87 (1H, m, H6), 1.87 (1H, m, H9), 1.68 (1H, m, H2α), 1.46 (3H, s, Me26), 1.27 (3H, s, Me24), 1.16 (3H, s, Me23), 1.15 (3H, s, Me30), 1.11 (1H, m, H1β), 0.98 (3H, s, Me27); ^{13}C NMR (CD_3OD) see Table 1.

Cytotoxicity Tests. Cytotoxicity was tested against P-388 murine leukemia cells as reported previously.[10]

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