New Isomalabaricane Triterpenes from the Marine Sponge *Stelletta globostellata* That Induce Morphological Changes in Rat Fibroblasts[1]

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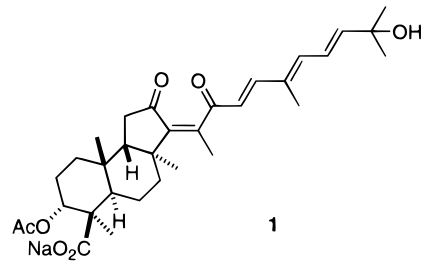
Three new isomalabaricane triterpenes, 29-hydroxystelliferin D (**2**), 3-*epi*-29-hydroxystelliferin E (**3**), and 3-*epi*-29-hydroxystelliferin A (**4**), were isolated from the marine sponge *Stelletta globostellata*. Their structures, including absolute stereochemistry, were determined on the basis of spectral data and chemical methods. Rat fibroblasts treated with 0.2 μM of **2**−**4** exhibited unusual morphological characteristics, followed by death in 5 days.

The marine sponge *Stelletta globostellata*[2] contains cytotoxic triterpenes of the isomalabaricane class.[3,4] In a newly developed assay system using rat 3Y1 fibroblasts, by which we aim to discover marine metabolites acting on molecules involved in the regulation of cytoskeleton formation, the same sponge induced unusual morphological changes in the cells. However, the major cytotoxin, globostellatic acid A (**1**)[3] showed no activity in the assay, which prompted us to search for the active constituents. Bioassay-guided isolation afforded five isomalabaricane terpenoids that induced characteristic morphological changes in 3Y1 rat fibroblasts at a concentration of 0.2 μM.



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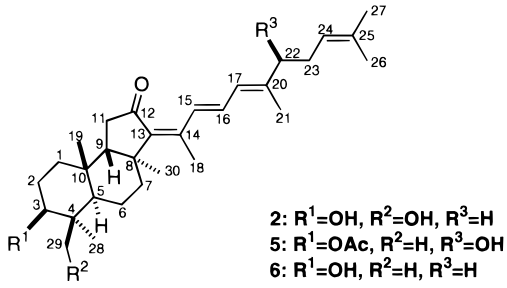
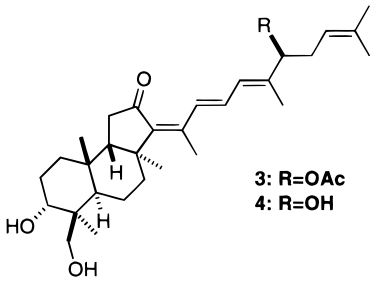


2: R^1=OH, R^2=OH, R^3=H
5: R^1=OAc, R^2=H, R^3=OH
6: R^1=OH, R^2=H, R^3=H



3: R=OAc
4: R=OH

Results and Discussion

The EtOH extract of the frozen specimens (10 kg) was fractionated by solvent partitioning to provide *n*-hexane, MeOH−H_2O (9:1), and H_2O fractions. The active aqueous MeOH fraction was successively separated by ODS flash chromatography and gel filtration followed by repeated reversed-phase HPLC to yield 29-hydroxystelliferin D (**2**), 3-*epi*-29-hydroxystelliferin E (**3**), and 3-*epi*-29-hydroxystelliferin A (**4**), together with the known stelliferin A (**5**) and stelliferin D (**6**)[5] in yields of 4.2, 23.8, 5.3, 127.3, and 1.5 mg, respectively. Because compounds **2**−**4** are readily photoisomerized, as are other isomalabaricane triterpenes, we shielded the glassware from light by using aluminum foil.

29-Hydroxystelliferin D (**2**) has a molecular formula of $C_{30}H_{46}O_3$ on the basis of HRFABMS. It exhibits UV absorption maxima at 239 and 353 nm, reminiscent of isomalabaricane terpenoids. The ^{1}H NMR spectrum (Table 1) exhibits four olefinic protons [δ 8.70 (d, J = 15.4 Hz), 6.97 (dd, J = 11.0, 15.4 Hz), 6.25 (br d, J = 11.2 Hz), and 5.15 (tsept, J = 6.9, 1.4 Hz)], four olefinic methyls (δ 1.81, 1.72, 1.64, and 1.52), an oxygenated methine [δ 3.11 (dd, J = 5.0, 11.5 Hz)], an oxygenated methylene [δ 4.09 (d, J = 11.2 Hz), 3.26 (dd, J = 11.2, 1.2 Hz)], and three aliphatic methyls [δ 1.26 (3H, s), 1.04 (3H, s), 0.60 (3H, s)]. These data, together with a ketone signal at δ 206.0 in the ^{13}C NMR spectrum, implied that **2** was an isomalabaricane triterpene.[6] Furthermore, the ^{1}H and ^{13}C NMR spectra were almost superimposable on those of the co-occurring stelliferin D (**6**), except for the signals arising from the vicinity of C-4.[7] A hydroxymethyl group on C-4 in **2**, instead of a methyl group in **6**, was inferred from HMBC data; hydroxylation of 29-Me was indicated by NOESY data (Figure 1).

The relative stereochemistry of the tricyclic core was substantiated by ^{1}H−^{1}H coupling constants and NOESY data. The J value of 11.5 Hz between H-3 and H-2β indicated axial orientation of H-3. NOESY correlations (Me-19/H-9, Me-19/H_2-29, H-5/H-3, H-5/H-1α, H-5/Me-30) were consistent with the isomalabaricane skeleton in which ring B adopts a twist-boat conformation.[6] The chemical shift value of δ 8.70 for H-15 supported (13*Z*)-geometry,[8] while the coupling constant between H-15 and H-16 (J = 15.4 Hz), chemical shift for C-21 (δ 17.0), and NOESY correlations (H-15/H-17, H-16/Me-18, and H-16/Me-21) secured (15*E*,17*E*)-geometries.

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Table 1. ^{1}H and ^{13}C NMR Data for **2**−**4** in C_6D_6

	2		**3**		**4**	
position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	33.0	0.925 ddd (13.0, 4.1, 3.0) 1.070 m	29.4	0.77 dt (12.8, 4.1) 1.45 m	29.4	0.78 dt (12.6, 4.0) 1.45 m
2	29.5	1.47 m 1.61 m	24.7	1.62 m (2H)	24.7	1.63 m (2H)
3	80.6	3.11 dd (5.0, 11.5)	73.8	5.14 t (3.3)	73.8	5.14 t (3.5)
4	43.7		43.2		43.2	
5	47.2	1.45 dd (12.3, 1.2)	42.3	2.16 dd (12.7, 1.2)	42.3	2.16 dd (12.7, 1.2)
6	18.7	1.075 m 1.48 m	19.2	1.23 m 1.49 m	19.2	1.23 m 1.51 m
7	38.6	1.62 m 1.73 dd (13.1, 8.1)	39.0	1.78 m 1.80 m	39.1	1.78 m 1.82 m
8	44.5		44.5		44.5	
9	50.0	1.38 dd (15.2, 7.5)	50.1	1.48 dd (14.8, 7.9)	50.2	1.50 dd (14.7, 7.7)
10	35.2		35.3		35.3	
11	36.8	2.00 m (2H)	36.7	2.05 m 2.06 m	36.8	2.03 m 2.08 m
12	206.0		205.0		205.2	
13	145.8		146.7		146.3	
14	142.0		141.2		141.7	
15	131.2	8.70 d (15.4)	133.7	8.73 d (15.4)	132.9	8.73 d (15.8)
16	131.0	6.97 dd (11.0, 15.4)	129.6	6.87 dd (15.4, 11.0)	130.3	6.94 dd (15.4, 11.2)
17	127.1	6.25 dq (11.2, 1.3)	128.4	6.51 dq (11.0, 1.5)	126.7	6.40 dq (11.0, 1.2)
18	15.8	1.81 s	15.8	1.79 s	15.8	1.82 s
19	22.5	0.60 s	22.2	0.68 s	22.2	0.68 s
20	142.3		139.0		143.7	
21	17.0	1.72 d (1.5)	13.6	1.78 d (1.5)	12.6	1.79 d (1.5)
22	40.5	2.04 m (2H)	78.4	5.38 t (6.6)	77.0	3.95 dd (7.9, 5.2)
23	27.0	2.06 m (2H)	32.3	2.30 m 2.45 m	34.7	2.24 m 2.33 m
24	124.4	5.15 sept (6.9, 1.4)	119.6	5.14 sept (7.3, 1.4)	120.8	5.19 sept (7.2, 1.4)
25	131.6		134.3		134.0	
26	17.7	1.52 d (1.2)	17.9	1.51 d (1.2)	17.9	1.52 d (1.5)
27	25.8	1.64 d (1.2)	25.8	1.59 d (1.2)	25.9	1.62 d (1.5)
28	23.8	1.26 s	22.0	1.02 s	22.0	1.02 s
29	64.0	3.26 dd (11.2, 1.2) 4.09 d (11.2)	65.8	3.28 d (11.0) 3.37 d (11.0)	65.8	3.27 d (11.2) 3.37 d (11.2)
30	24.8	1.04 s	24.2	1.19 s	24.2	1.21 s
3-Ac			20.7 169.6	1.73 s	20.7 169.6	1.72 s
22-Ac			20.7 169.4	1.67 s		

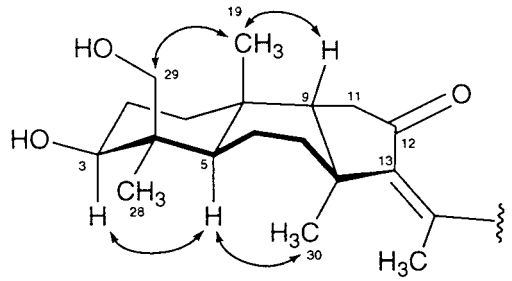

Figure 1. Selected NOESY correlations for 29-hydroxystelliferin D (**2**).

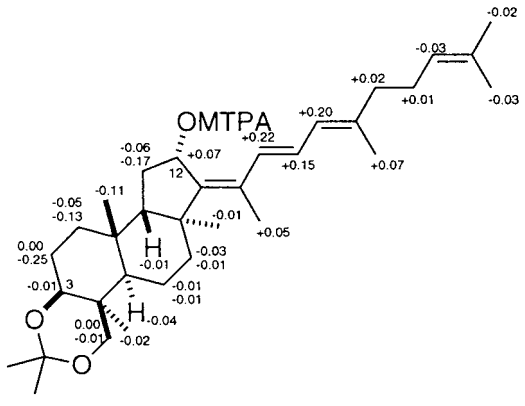

Figure 2. Distribution of Δδ values for MTPA esters of **7**.

The absolute stereochemistry of 29-hydroxystelliferin D (**2**) was determined by the advanced Mosher method.[9] Because the presence of proximate hydroxyl groups on C-3 and C-29 was not favorable for the application of this method,[9] we opted to reduce the ketone group on C-12 prior to derivatization. At first, the 3,29-diol was converted to the 3,29-acetonide, which was treated with DIBAL to afford **7** as a single product. The stereochemistry at C-12 was established by comparing ^{1}H NMR data with that of the DIBAL reduction product of stelliferin A (**5**) (see below). MTPA (methoxytrifluoromethylphenylacetic acid) esters of **7**[10] showed Δδ values as indicated in Figure 2, which resulted in 12*S*, thereby establishing (3*S*,4*R*,5*R*,8*S*,9*S*,10*S*)-stereochemistry. This is in accordance with the stereochemistry proposed for a jaspiferal derivative.[11]

The molecular formula of 3-*epi*-29-hydroxystelliferin E (**3**) was determined by HRFABMS as $C_{34}H_{50}O_6$. Spectral data were consistent with an isomalabaricane triterpene possessing a triene moiety conjugated with a ketone group. There are two oxygenated methines (δ_C 78.4 and 73.8), an oxygenated methylene (δ_C 65.8), and two acetates [δ_H 1.73 and 1.67; δ_C 169.6, 169.4, and 20.7 (2C)] in the NMR spectra; two acetates could be placed on C-3 and C-22 on the basis of HMBC data (δ 5.13/169.6 and 5.38/169.4) as

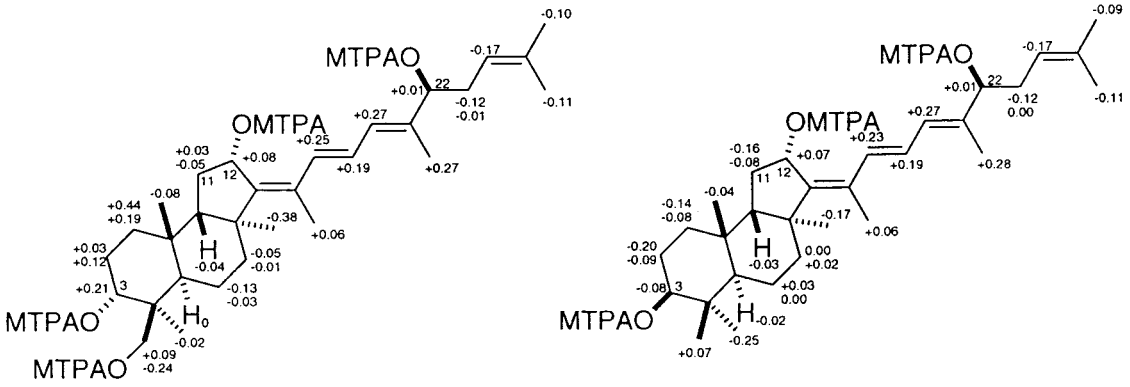

Figure 3. Δδ Distributions calculated for MTPA esters of **8** (left) and those of **9** (right).

well as other 2D NMR data. The splitting pattern of H-3, which was significantly different from that of **2**, indicated that H-3 is coupled to C-2 methylene protons by 3.3 Hz each, thereby implying an equatorial orientation.

To apply the advanced Mosher method, compound **3** was reduced with DIBAL to the tetraol **8**,[12] which again gave a single isomer. MTPA esters of **8** implied (22*S*)-stereochemistry. We did not use this method to assign the configuration at C-3, since it is not recommended for axial alcohols.[9] Although the Δδ values for H-15, H-16, and H-18 implied (12*S*)-stereochemistry, the Δδ values for the methylene protons on C-11 did not support this assignment; one proton gave a positive value, while another gave a negative value. To obtain reference data, stelliferin A (**5**) was converted to the triol **9**. The 12-OH group of **9** was established to be α oriented by the NOESY cross-peak between H-9 and H-12. Triol **9** was then esterified to give tris-MTPA esters, which clearly indicated (12*S*,22*S*)-stereochemistry. The Δδ values for MTPA esters of **9** were almost identical with those for MTPA esters of **8** in the side chain (Figure 3). Therefore, the stereochemistry at C-12 and, hence, that of the tricyclic portion of **3** were established. The Δδ values observed for the C-11 methylene protons were likely due to an anomalous effect of the axial MTPA group on C-3.[9]

3-*epi*-29-Hydroxystelliferin A (**4**) has a molecular formula of $C_{32}H_{48}O_5$, which is smaller than **3** by a C_2H_2O unit, corresponding to the desacetyl derivative of **3**. The UV and ^{1}H NMR spectra are superimposable with those of **3** except for the downfield shift of H-22 and the absence of a signal for an acetyl group. Therefore, compound **4** is the C22-desacetyl derivative of **3**, which was confirmed by converting both compounds to the common triacetate **10**.

3Y1 rat embryonic fibroblasts exposed to 0.2 μM of compounds **2**−**6** lost pseudopodia, which affected their ability to attach to substrates, thereby becoming long and narrow cylinder shapes with an uneven surface. The effect was reversible; the affected cells regained viability after being washed with fresh medium. If unwashed, most cells died in 5 days. Similar effects were observed for rubrosides isolated from the marine sponge *Siliquariaspongia japonica*,[13] but stelliferins did not induce vacuolation of cytoplasm. It is noted that the related stellettins exhibit cytotoxicity profiles that do not match any NCI standard agents.[14,15] GI_{50} values of **2**−**6**, **9**, and **11** (triacetate of **9**, data not shown) against 3Y1 and P388 cells were determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] tetrazolium method (Table 2). Globostellatic acid A (**1**) was not cytotoxic to either cell line at 18 μM in the present assay. Interestingly, triol **9** and triacetate **11**, prepared from stelliferin A (**5**), were significantly less cytotoxic, indicating the importance of a ketone group on C-12 for activity. Stelliferins **2**−**6** showed no antimicrobial activity against three fungi (*Candida albicans*, *Mortierella ramanniana*, and *Penicillium chrysogenum*) and four bacteria (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) at 100 μg/disk.

Table 2. Cytotoxicity of Stelliferin Derivatives against 3Y1 and P388 Cells (GI_{50}: nM)

	2	**3**	**4**	**5**	**6**	**9**	**11**	adriamycin[a]
3Y1	60	52	46	34	18	1300	1300	3.8
P388	190	160	180	21	24	980	1600	12

[a] Positive cytotoxicity control.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a JEOL A600 NMR spectrometer. ^{1}H and ^{13}C NMR chemical shifts were referenced to solvent peaks: δ 7.15 and 128.0 for benzene-d_6, and δ 2.04 and 39.8 for acetone-d_6. FABMS were measured on a JEOL JMX-SX102/SX102 tandem mass spectrometer using 3-nitrobenzyl alcohol (3-NBA) or triethanolamine (TEA) as a matrix. Optical rotations were obtained on a JASCO DIP-1000 digital polarimeter, IR spectra on a JASCO FT/IR-5300 infrared spectrometer, and UV spectra on a Hitachi 330 spectrophotometer.

Extraction and Isolation. *Stelletta globostellata* Carter, 1883 (Stellettidae)[2] was collected using scuba at a depth of 3 m off Mage-jima Island (30° 43′ N, 130° 52′ E), 1000 km southwest of Tokyo. The sponge was immediately frozen and kept at −20 °C until extraction. The frozen sponge (10 kg) was extracted with EtOH, and the concentrated aqueous solution was extracted with EtOAc. The organic phase was further partitioned between MeOH/H_2O (9:1) and *n*-hexane. The aqueous MeOH fraction was flash-chromatographed on ODS eluting with MeOH/H_2O (1:1 and 3:1), MeOH, and $CHCl_3$−MeOH/H_2O (7:3:0.5). The MeOH eluate was gel-filtered on Sephadex LH-20 [MeOH/CH_2Cl_2 (1:1)] followed by ODS flash chromatography (MeCN/H_2O) to afford active fractions that were eluted with MeCN/H_2O (8:2) and MeCN/H_2O (95:5) in yields of 3.39 and 2.61 g, respectively. The former fraction was further purified by repetitive reversed-phase HPLC [(1) ODS; MeCN/H_2O (95:5), (2) ODS; MeCN/50 mM NH_4OAc in H_2O (80:20), (3) ODS; MeOH/50 mM NH_4OAc (88:12 or 86:14), and (4) phenylhexyl; MeCN/H_2O (76:24)] to yield 3-*epi*-29-hydroxystelliferin A (**3**; 5.3 mg, 5.3 × 10^{-5} %) and 3-*epi*-29-hydroxystelliferin E (**2**; 23.8 mg, 2.4 × 10^{-4} %). The latter fraction was similarly separated to afford stelliferin A (**4**; 127.3 mg, 1.3 × 10^{-3} %), 29-hydroxystelliferin D (**1**; 4.2 mg, 4.2 × 10^{-5} %), and stelliferin D (**5**; 1.5 mg, 1.5 × 10^{-5} %).

29-Hydroxystelliferin D (2): a pale yellow solid; $[\alpha]^{25}_D$ −34° (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 239 (3.88), 353 (4.46) nm; IR (CCl_4) ν_{max} 3364, 2932, 2876, 1690, 1632, 1586, 1555, 1445, 1377, 1200, 1167, 1073, 1026, 974, 793 cm^{-1};

HRFABMS (3-NBA) *m/z* 455.3554 $(M + H)^+$ (calcd for $C_{30}H_{47}O_3$, 455.3525); ^1H and ^{13}C NMR data, see Table 1.

3-*epi*-29-Hydroxystelliferin E (3): a pale yellow solid; $[\alpha]^{25}_D$ −113° (*c* 0.69, MeOH); UV (MeOH) λ_{max} (log ϵ) 234 (4.27), 339 (4.55) nm; IR (CCl_4) ν_{max} 3505, 2957, 2880, 1738, 1694, 1561, 1451, 1373, 1242, 1167, 1020, 976, 762 cm^{-1}; HRFABMS (3-NBA) *m/z* 577.3516 $(M + Na)^+$ (calcd for $C_{34}H_{50}O_6Na$, 577.3505); ^1H and ^{13}C NMR data, see Table 1.

3-*epi*-29-Hydroxystelliferin A (4): a pale yellow solid; $[\alpha]^{25}_D$ −84° (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 238 (3.75), 345 (4.34) nm; HRFABMS (3-NBA) *m/z* 513.3584 $(M + H)^+$ (calcd for $C_{32}H_{49}O_5$, 513.3580); ^1H and ^{13}C NMR data, see Table 1.

DIBAL Reduction of Stelliferin A (5). A 10-mg portion of stelliferin A (**5**) was dissolved in THF (1 mL), to which excess 1 M DIBAL in toluene (300 μL) was added at room temperature. The initial yellow color disappeared immediately. The mixture was stirred for 5 min and quenched by addition of saturated aqueous NH_4Cl (2 mL). The aqueous suspension was extracted with EtOAc (2 mL × 5) and the combined organic layers were evaporated, passed through a short SiO_2 column (1.3 × 3 cm) with EtOAc, and purified by SiO_2 HPLC [Cosmosil SL-II, 10 × 250 mm, *n*-hexane−EtOAc (1:1)] to yield triol **9** (6.9 mg) as a colorless solid.

Triol 9: HRFABMS (3-NBA) *m/z* 479.3503 $(M + Na)^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3501); ^1H NMR (C_6D_6) δ 7.00 (1H, d, J = 15.4 Hz, H-15), 6.62 (1H, dd, J = 11.0, 15.2 Hz, H-6), 6.38 (1H, dd, J = 11.2, 1.2 Hz, H-17), 5.24 (1H, tsept, J = 7.2, 1.4 Hz, H-24), 4.72 (1H, dt, J = 1.3, 7.6 Hz, H-12), 4.04 (1H, br t, J = 13.0 Hz, H-22), 3.07 (1H, dd, J = 11.5, 5.9 Hz, H-3), 2.37 (1H, m, H-23a), 2.31 (1H, m, H-23b), 1.98 (1H, m, H-11β), 1.83 (1H, m, H-7β), 1.83 (3H, d, J = 1.5 Hz, H-18), 1.82 (3H, d, J = 1.2 Hz, H-21), 1.72 (1H, m, H-7α), 1.64 (3H, d, J = 1.2 Hz, H-27), 1.57 (1H, m, H-2α), 1.54 (3H, d, J = 1.2 Hz, H-26), 1.52 (1H, m, H-2β), 1.47 (1H, m, H-6a), 1.47 (1H, d, J = 11.5 Hz, H-5), 1.45 (1H, m, H-11α), 1.36 (1H, dt, J = 4.1, 12.8 Hz, H-1α), 1.23 (3H, s, H-30), 1.22 (1H, m, H-1β), 1.17 (1H, m, H-6b), 1.13 (1H, dd, J = 5.6, 14.5 Hz, H-9), 1.02 (3H, s, H-28), 0.81 (3H, s, H-19), 0.78 (3H, s, H-29); ^{13}C NMR (C_6D_6) δ 133.4 (C-15), 126.5 (C-17), 124.0 (C-16), 121.2 (C-24), 79.2 (C-3), 77.2 (C-22), 72.2 (C-12), 52.0 (C-9), 47.1 (C-5), 38.5 (C-7), 35.0 (C-23), 34.1 (C-1), 31.8 (C-11), 29.7 (C-2), 29.3 (C-28), 25.9 (C-27), 25.7 (C-18), 22.3 (C-19), 18.6 (C-6), 18.0 (C-26), 16.1 (C-29), 15.3 (C-18), 12.8 (C-21); chemical shift values of the quaternary carbons were not assigned.

Acetonide 7. To a CH_2Cl_2 solution (1 mL) of 29-hydroxystelliferin D (**2**) (4.8 mg) were added 2,2-dimethoxypropane (1.0 mL) and pyridinium *p*-toluenesulfonate (10.5 mg), and the mixture was stirred at room temperature for 15 min. After evaporating the solvent, the residue was passed through a SiO_2 column [1.3 × 3 cm, *n*-hexane/EtOAc (2:1)], and the eluent was subjected to DIBAL reduction as described above to afford 3,-29-*O*,*O*-isopropylidene-12-hydroxyl derivative **7** (3.6 mg): HRFABMS (3-NBA) *m/z* 519.3819 $(M + H)^+$ (calcd for $C_{33}H_{52}O_3Na$, 519.3814); ^1H NMR (C_6D_6) δ 6.94 (1H, d, J = 15.0 Hz, H-15), 6.65 (1H, dd, J = 10.8, 15.0 Hz, H-16), 6.20 (1H, d, J = 10.8 Hz, H-17), 5.21 (1H, br t, J = 6.6 Hz, H-24), 4.73 (1H, br t, J = 7.5 Hz, H-12), 3.95 (1H, d, J = 12.0 Hz, H-29a), 3.54 (1H, br t, J = 4.2 Hz, H-3), 3.11 (1H, d, J = 12.0 Hz, H-29b), 2.17 (2H, m, H-23), 2.15 (2H, m, H-22), 2.01 (1H, m, H-11a), 1.85 (1H, m, H-2a), 1.83 (3H, d, J = 1.5 Hz, H-18), 1.79 (1H, dd, J = 8.3, 13.3 Hz, H-7a), 1.77 (3H, d, J = 1.5 Hz, H-21), 1.66 (1H, d, J = 1.2 Hz, H-27), 1.64 (1H, m, H-7b), 1.64 (1H, m, H-2b), 1.58 (1H, m, H-1a), 1.55 (3H, d, J = 1.2 Hz, H-26), 1.53 (1H, overlapped, H-5), 1.47 (1H, m, H-11b), 1.42 (3H, s, acetonide-CH_3), 1.39 (3H, s, acetonide-CH_3), 1.33 (1H, m, H1b), 1.32 (3H, s, H-30), 1.24 (1H, dd, J = 8.4, 13.8 Hz, H-6a), 1.17 (1H, dd, J = 5.7, 14.7 Hz, H-9), 1.16 (3H, s, H-19), 1.07 (3H, s,H-28).

Preparation of MTPA Esters. To a 1-mg portion of an alcohol were added 60 μL of dry pyridine and either (−)- or (+)-MTPACl (10 μL). The mixture was kept at room temperature for 30 min. After the pyridine was removed by lyophilization, the residue was passed through a SiO_2 column (1.3 × 2 cm) with EtOAc and purified by HPLC [on SiO_2, 10 × 250 mm, *n*-hexane/EtOAc or *n*-hexane/ether system)] to furnish the MTPA esters.

(−)-MTPA ester of 7: ^1H NMR (acetone-d_6) δ 6.57 (1H, dd, J = 15.0, 10.8 Hz, H-16), 6.33 (1H, d, J = 15.0 Hz, H-15), 6.16 (1H, t, J = 7.3 Hz, H-12), 5.91 (1H, d, J = 10.8 Hz, H-17), 5.09 (1H, m, H-24), 3.91 (1H, d, J = 11.5 Hz, H-29a), 3.53 (1H, t, J = 4.4 Hz, H-3), 3.06 (1H, d, J = 11.5 Hz, H-29b), 2.48 (1H, m, H-11a), 2.12 (2H, m,H-22), 2.12 (2H, m, H-23), 2.03 (1H, m, H-7a), 1.94 (1H, m, H-7b), 1.88 (3H, d, J = 1.5 Hz, H-18), 1.84 (1H, m, H-2a), 1.82 (3H, d, J = 1.2 Hz, H-21), 1.81 (1H, m, H-2b), 1.72 (1H, dd, J = 1.5, 12.7 Hz, H-5), 1.64 (1H, m, 1-Ha), 1.63 (3H, d, J = 1.2 Hz, H-27), 1.58 (3H, d, J = 1.2 Hz, H-26), 1.57 (1H, m, H-6a), 1.52 (1H, dd, J = 5.8, 14.6 Hz, H-9), 1.45 (1H, dd, J = 12.0, 6.3 Hz, H-11b), 1.38 (1H, m, H-6b), 1.37 (3H, s, H-30), 1.31 (1H, m, H-1b), 1.28 (3H, s, acetonide-CH_3), 1.26 (3H, s, acetonide-CH_3), 1.16 (3H, s,H-19), 1.09 (3H, s, H-28).

(+)-MTPA ester of 7: ^1H NMR (acetone-d_6) δ 6.42 (1H, dd, J = 14.7, 11.0 Hz, H-16), 6.11 (1H, d, J = 14.4 Hz, H-15), 6.09 (1H, t, J = 7.3 Hz, H-12), 5.71 (1H, d, J = 10.0 Hz, H-17), 5.12 (1H, m, H-24), 3.92 (1H, d, J = 11.5 Hz, H-29a), 3.54 (1H, t, J = 4.4 Hz, H-3), 3.06 (1H, d, J = 11.0 Hz, H-29b), 2.54 (1H, m, H-11a), 2.11 (2H, m, H-23), 2.10 (2H, m, H-22), 2.02 (1H, m, H-7a), 1.97 (1H, m, H-7b), 1.86 (1H, m, H-2a), 1.83 (3H, s, H-18), 1.81 (1H, m, H-2b), 1.77 (1H, m, H-1a), 1.76 (1H, overlapped, H-5), 1.75 (3H, s, H-21), 1.65 (3H, s, H-27), 1.62 (1H, m, H-11b), 1.61 (3H, s, H-26), 1.58 (1H, m, H-6a), 1.53 (1H, dd, J = 5.9, 14.7 Hz, H-9), 1.39 (1H, m, H-6b), 1.38 (3H, s, H-30), 1.36 (1H, m, H-1b), 1.29 (3H, s, acetonide-CH_3), 1.29 (3H, s, acetonide-CH_3), 1.27 (3H, s, H-19), 1.11 (3H, s, H-28).

Tetraol 8: 3-*epi*-29-Hydroxystelliferin E (**3**) (2.5 mg) was reduced with DIBAL and worked up as described above to afford tetraol **8** (0.7 mg): ^1H NMR (C_6D_6) δ 7.03 (1H, d, J = 15.0 Hz, H-15), 6.62 (1H, dd, J = 15.2 Hz, 11.0, H-16), 6.38 (1H, d, J = 10.8 Hz, H-17), 5.22 (1H, tsept, J = 7.3, 1.5 Hz, H-24), 4.71 (1H, br t, J = 7.7 Hz, H-12), 3.99 (1H, dd, J = 8.1, 5.8 Hz, H-22), 3.73 (1H, dd, J = 6.9, 3.1 Hz, H-3), 3.39 (1H, d, J = 10.4 Hz, H-29), 3.23 (1H, d, J = 10.4 Hz, H-29), 2.35 (1H, m, H-23a), 2.27 (1H, m, H-23b), 2.10 (1H, d, J = 12.3 Hz, H-5), 1.91 (1H, m, H-11a), 1.81 (1H, m, H-7a), 1.81 (3H, d, J = 1.2 Hz, H-18), 1.79 (3H, d, J = 1.5 Hz, H-21), 1.76 (1H, m, H-1a), 1.71 (1H, m, H-7b), 1.62 (3H, d, J = 1.2 Hz, H-27), 1.57 (1H, m, H-2a), 1.52 (3H, d, J = 1.2 Hz, H-26), 1.48 (1H, m, H-2b), 1.45 (1H, m, H-11b), 1.42 (1H, m, H-6a), 1.22 (3H, s, H-30), 1.18 (1H, dd, J = 15.0, 5.3 Hz, H-9), 1.12 (1H, m, H-6b), 1.03 (3H, s, H-28), 0.99 (1H, m, H-1b), 0.75 (3H, s, H-19).

(−)-Tetrakis-MTPA ester of 8: ^1H NMR (acetone-d_6) δ 6.55 (1H, d, J = 15.2, 10.6 Hz, H-16), 6.51 (1H, d, J = 15.0 Hz, H-15), 6.25 (1H, dd, J = 10.6, 1.3 Hz, H-17), 6.12 (1H, t, J = 6.9 Hz, H-12), 5.45 (1H, dd, J = 6.4, 7.8 Hz, H-22), 5.17 (1H, t, J = 2.9 Hz, H-3), 4.93 (1H, br t, J = 7.1 Hz, H-24), 4.52 (1H, d, J = 11.5 Hz, H-29), 4.24 (1H, d, J = 11.5 Hz, H-29), 2.50 (1H, m, H-23a), 2.41 (1H, m, H-11a), 2.31 (1H, m, H-23b), 2.25 (1H, m, H-2a), 2.15 (1H, d, J = 11.9 Hz, H-5), 1.91 (1H, m, H-7b), 1.91 (1H, m, H-2), 1.86 (3H, d, J = 1.5 Hz, H-18), 1.86 (3H, d, J = 1.2 Hz, H-21), 1.81 (1H, m, H-7b), 1.63 (3H, d, J = 1.2 Hz, H-27), 1.63 (1H, m, H-1a), 1.53 (3H, d, J = 1.5 Hz, H-26), 1.48 (1H, dd, J = 13.7, 8.7 Hz, H-6a), 1.42 (1H, dd, J = 14.8, 5.2 Hz, H-9), 1.37 (1H, dd, J = 11.7, 6.4 Hz, H-11b), 1.28 (1H, m, H-1b), 1.25 (1H, m, H-6b), 1.02 (3H, s, H-30), 0.95 (3H, s, H-19), 0.68 (3H, s, H-28).

(+)-Tetrakis-MTPA ester of 8: ^1H NMR (acetone-d_6) δ 6.39 (1H, dd, J = 15.2, 11.0 Hz, H-16), 6.26 (1H, d, J = 15.0 Hz, H-15), 6.04 (1H, br t, J = 6.9 Hz, H-12), 5.98 (1H, dd, J = 11.0, 1.4 Hz, H-17), 5.44 (1H, dd, J = 8.5, 6.2 Hz, H-22), 5.10 (1H, br t, J = 7.2 Hz, H-24), 4.96 (1H, t, J = 2.7 Hz, H-3), 4.76 (1H, d, J = 11.5 Hz, H-29), 4.15 (1H, d, J = 11.5 Hz, H-29), 2.62 (1H, m, H-23a), 2.46 (1H, m, H-11a), 2.32 (1H, m, H-23b), 2.15 (1H, d, J = 11.5 Hz, H-5), 2.13 (1H, m, H-2a), 1.92 (1H, m, H-7a), 1.88 (1H, m, H-2b), 1.86 (1H, m, H-7b), 1.80 (3H, d, J = 1.5 Hz, H-18), 1.70 (3H, d, J = 1.2 Hz, H-27), 1.64 (3H, d, J = 1.5 Hz, H-26), 1.59 (3H, d, J = 1.2 Hz, H-21), 1.51 (1H, m, H-6a), 1.46 (1H, dd, J = 14.8, 5.6 Hz, H-9), 1.40 (3H, s, H-30), 1.38 (1H, m, H-6b), 1.34 (1H, m, H-11b), 1.19 (1H, m, H-1a), 1.09 (1H, m, H-1b), 0.96 (3H, s, H-28), 1.03 (3H, s, H-19).

(−)-Tris-MTPA ester of 9: ^{1}H NMR (acetone-d_6) δ 6.60 (1H, dd, J = 15.2, 10.6 Hz, H-16), 6.53 (1H, d, J = 15.0 Hz, H-15), 6.26 (1H, dd, J = 1.5, 9.4 Hz, H-17), 6.16 (1H, br t, J = 6.9 Hz, H-12), 5.45 (1H, dd, J = 6.2, 1.9 Hz, H-22), 4.93 (1H, sept, J = 7.3, 1.5 Hz, H-24), 4.80 (1H, dd, J = 5.0, 11.5 Hz, H-3), 2.50 (1H, m, H-23a), 2.50 (1H, m, H-11β), 2.32 (1H, m, H-23b), 2.07 (1H, m, H-7β), 2.02 (1H, m, H-7α), 1.92 (1H, 3H, d, J = 1.2 Hz, H-18), 1.91 (1H, m, H-2β), 1.87 (3H, d, J = 1.2 Hz, H-21), 1.81 (1H, dd, J = 1.2, 12.3 Hz, H-5), 1.73 (1H, m, H-2α), 1.71 (1H, m, H-6a), 1.60 (3H, d, J = 1.2 Hz, H-27), 1.58 (1H, m, H-1α), 1.54 (1H, dd, J = 5.8, 15.0 Hz, H-9), 1.53 (3H, d, J = 1.2 Hz, H-26), 1.47 (1H, m, H-6b), 1.47 (1H, m, H-1β), 1.45 (1H, m, H-11α), 1.23 (3H, s, H-30), 1.03 (3H, s, H-19), 1.01 (3H, s, H-28), 0.87 (3H, s, H-29).

(+)-Tris-MTPA ester of 9: ^{1}H NMR (acetone-d_6) δ 6.41 (1H, dd, J = 15.2, 11.0 Hz, H-16), 6.30 (1H, dd, J = 15.0 Hz, H-15), 6.09 (1H, br t, J = 7.1 Hz, H-12), 5.99 (1H, dd, J = 1.5, 9.4 Hz, H-17), 5.44 (1H, dd, J = 6.0, 8.3 Hz, H-22), 5.10 (1H, br t, J = 7.2 Hz, H-24), 4.88 (1H, dd, J = 5.4, 11.5 Hz, H-3), 2.62 (1H, m, H-23a), 2.58 (1H, m, H-11β), 2.32 (1H, m, H-23b), 2.05 (1H, m, H-7β), 2.02 (1H, m, H-7α), 2.00 (1H, m, H-2β), 1.93 (1H, m, H-2α), 1.86 (3H, d, J = 1.5 Hz, H-18), 1.83 (1H, dd, J = 12.3, 1.5 Hz, H-5), 1.72 (1H, m, H-1α), 1.69 (3H, d, J = 1.5 Hz, H-27), 1.68 (1H, m, H-6a), 1.64 (3H, d, J = 1.2 Hz, H-26), 1.61 (1H, m, H-11α), 1.59 (1H, dd, J = 1.2 Hz, H-21), 1.57 (1H, overlapped, H-9), 1.55 (1H, m, H-1β), 1.47 (1H, m, H-6b), 1.40 (3H, s, H-30), 1.07 (3H, s, H-19), 0.86 (3H, s, H-28), 0.80 (3H, s, H-29).

Acetylation of 2 and 3. To a solution of either **2** or **3** (1.0 mg) in pyridine (1 mL) was added 0.5 mL of Ac_2O, and the mixture was stirred at room temperature for 15 h. Excess Ac_2O was quenched by addition of H_2O (1 mL), and the mixture was extracted with Et_2O (1 mL × 4). The organic extract was purified by SiO_2 HPLC [Cosmosil SL-II, 10 × 250 mm, *n*-hexane/Et_2O (1:1)] to furnish the same triacetate **10** (0.9 mg and 0.8 mg, respectively).

Triacetate 10 from 2: $[\alpha]^{24}_D$ −75° (*c* 0.05, MeOH); HR-FABMS (3-NBA) *m*/*z* 619.3601 $(M + Na)^+$ (calcd for $C_{36}H_{52}O_7$, 619.3611); ^{1}H NMR (C_6D_6) δ 8.75 (1H, d, J = 15.4 Hz, H-15), 6.89 (1H, dd, J = 15.4, 11.2 Hz, H-16), 6.52 (1H, dd, J = 11.2, 1.2 Hz, H-17), 5.38 (1H, t, J = 6.7 Hz, H-22), 5.14 (1H, tsept, J = 6.9, 1.5 Hz, H-24), 5.13 (1H, t, J = 3.3 Hz, H-3), 4.21 (1H, d, J = 11.5 Hz, H-29), 3.91 (1H, d, J = 11.5 Hz, H-29), 2.45 (1H, m, H-23a), 2.30 (1H, m, H-23b), 2.18 (1H, dd, J = 12.9, 2.5 Hz, H-5), 2.01 (1H, m, H-11a), 1.97 (1H, m, H-11b), 1.77 (6H, overlapped s, H-18 and -21), 1.76 (1H, m, H-7a), 1.69 (1H, m, H-7b), 1.63 (1H, m, H-2a), 1.59 (3H, d, J = 1.2 Hz, H-27), 1.58 (1H, m, H-2b), 1.51 (3H, d, J = 1.2 Hz, H-26), 1.42 (1H, m, H-6a), 1.41 (1H, dd, J = 14.6, 8.1 Hz, H-9), 1.39 (1H, m, H-1a), 1.17 (3H, s, H-30), 1.14 (1H, m, H-6b), 0.99 (3H, s, H-28), 0.70 (1H, ddd, J = 9.6, 4.6, 3.5 Hz, H-1b), 0.63 (3H, s, H-19). 1.71, 1.69, and 1.66; 3-, 22-, and 29-OCOCH_3.

Triacetate 10 from 3: $[\alpha]^{24}_D$ −50° (*c* 0.05, MeOH); HR-FABMS (3-NBA) *m*/*z* 619.3601 $(M + Na)^+$ (calcd for $C_{36}H_{52}O_7$, 619.3611); the ^{1}H NMR spectrum was superimposable on that of the triacetate from **2**.

Rat 3Y1 Fibroblast Assay. Rat embryonic fibroblasts 3Y1 B clone 1−6 were grown in a DMEM (Gibco) medium supplemented with 10% fetal calf serum, gentamycin (Gibco), antibiotic−antimycotic (Gibco), and 40 mM $NaHCO_3$ (adjusted to pH 7.1 with 2N HCl). Cell cultures were passaged twice a week using trypsin (Difco) to detach the cells from their culture dish (150 mm, Corning) and maintained as a subconfluent monolayer in a humidified atmosphere of 5% CO_2−95% air at 37 °C. The cell suspensions (500 μL each), at a density of 2×10^4 cells/mL, were inoculated in 24-well cell culture plates, and the plates were kept in an incubator for 24 h before exposure to test samples. Sample solutions (5 μL) dissolved in MeOH were added to the cell culture, and morphology of the cells was observed after 1, 5, and 24 h, and on day 5 by a phase contrast microscope.

Cytotoxicity Tests. Cytotoxicity of each agent was evaluated as GI_{50} by using the modified MTT-microculture tetrazolium assay. 3Y1 cells were inoculated at a density of 300 cells/well (200 μL by volume) into wells of 96-well microtiter plates. After incubation for 24 h, 2 μL of sample solutions were applied, and plates were incubated at 37 °C for 72 h. Then 20 μL of MTT solution (5 mg/mL in PBS without Ca^{2+} and Mg^{2+}) was added, and the plates were incubated at 37 °C for 4 h. After removal of the supernatant by aspiration, the dyed cells were solubilized by adding 50 μL of DMSO and shaking for 5 min, and the absorbance of the solution at 540 nm was measured. The tetrazolium assay using P388 cells was carried out according to our previous method[16] with slight modification.

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References and Notes

(1) Bioactive Marine Metabolites Series. 96. Part 95: Matsunaga, S.; Yamashita, T.; Tsukamoto, S.; Fusetani, N. *J. Nat. Prod*. **1999**, *62*, 1202−1204.
(2) Although *Stelletta globostellata* (often called *Rhabdastrella* or *Aurora globostellata*) and *Jaspis stellifera*, both of which contain isomalabaricane triterpenes, are closely related, they are apparently different. It is most likely that the latter sponge does not belong to the genus *Jaspis* (van Soest, Rob W. M., personal communication).
(3) Ryu, G.; Matsunaga, S.; Fusetani, N. *J. Nat. Prod*. **1996**, *59*, 512−514.
(4) Rao, Z.; Deng, S.; Wu, H.; Jiang, S. *J. Nat. Prod*. **1997**, *60*, 1163−1164.
(5) Tsuda, M.; Ishibashi, M.; Agemi, K.; Sasaki, T.; Kobayashi, J. *Tetrahedron* **1991**, *47*, 2181−2194.
(6) McCabe, T.; Clardy, J.; Minale, L.; Pizza, C.; Zollo, F.; Riccio, R. *Tetrahedron Lett*. **1982**, *23*, 3307−3310.
(7) We use the numbering system used for stelliferins to avoid confusion.
(8) In Ryu et al.[3], the structures of globostellatic acids B−D were drawn with (13*Z*)-geometry. They have (13*E*)-geometry on the basis of the chemical shift values for H-15. We thank Professor M. Valeria D'Auria, University of Naples, for pointing out the misassignment.
(9) Otani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc*. **1991**, *113*, 4092−4096.
(10) The crude MTPA esters gave only one spot on a TLC plate. After purification by HPLC they gave four spots on TLC, but exhibited a clean ^{1}H NMR spectrum.
(11) Kobayashi, J.; Yuasa, K.; Kobayashi, T.; Sasaki, T.; Tsuda, M. *Tetrahedron* **1996**, *52*, 5745−5750.
(12) Only the 12α-alcohol was produced.
(13) Sata, N. U.; Wada, S.; Matsunaga, S.; Watabe, S.; van Soest, R. W. M.; Fusetani, N. *J. Org. Chem*. **1999**, *64*, 2331−2339.
(14) McKee, T. C.; Bokesch, H. R.; McCormick, J. L.; Rashid, M. A.; Spielvogel, D.; Gustafson, K. R.; Alavanja, M. M.; Cardellina, J. H., II; Boyd, M. R. *J. Nat. Prod*. **1997**, *60*, 431−438.
(15) McCormick, J. L.; McKee, T. C.; Cardellina, J. H., II; Leid, M.; Boyd, M. R. *J. Nat. Prod*. **1996**, *59*, 1047−1050.
(16) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem*. **1995**, *60*, 608−614.

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