New Polyhydroxy Sterols: Proteasome Inhibitors from a Marine Sponge *Acanthodendrilla* sp.

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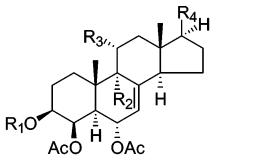
Seven new polyhydroxylated sterols, as well as the known agosterols A, C, and D_2 (**8**−**10**), were isolated from a marine sponge *Acanthodendrilla* sp. as proteasome inhibitors. Their structures were elucidated on the basis of their spectral data, and they were characterized as agosterol congeners, which had been initially isolated from a marine sponge *Spongia* sp. and reported to reverse multidrug resistance in tumor cells. Among them, agosterol C (**9**) most strongly inhibited chymotrypsin-like activity of the proteasome with an IC_{50} value of 10 μg/mL.

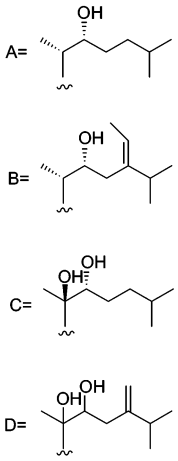
The ubiquitin−proteasome proteolytic pathway plays a major role in selective protein degradation and regulates various cellular events including cell growth and apoptosis.[1−4] Ubiquitin, a highly conserved protein composed of 76 amino acids and found only in eukaryotes, attaches to a target protein prior to degradation. The polyubiquitin chains tagged to the target protein are recognized by the 26S proteasome, an intracellular high molecular weight protease subunit complex, and the protein portion is degraded by the proteolytic active sites in a cavity in the 26S proteasome.[1,2] The potential of specific proteasome inhibitors, which act as anticancer agents, is now under intensive investigation. Several proteasome inhibitors show antitumor activity against various tumor cells that are resistant to conventional chemotherapeutic agents. In addition, proteasome inhibitors are considered to inhibit NF-κB transcriptional activity to induce apoptosis.[5] To date, synthetic peptides, such as MG132 and PS-341, and natural products, including lactacystin and epoxomycin, have been reported to inhibit proteasome activity.[6,7] Among them, PS-341 shows antitumor activity in a variety of tumor types, and phase II clinical study using this inhibitor in hematologic malignancies is now in progress.[8] Interestingly, different classes of proteasome inhibitors can differentially affect the degradation of various proteasome substrates, which result in diverse cellular responses.[5] During a search for natural products exhibiting new biological activities,[9,10] we screened extracts derived from marine organisms for proteasome inhibitory activity and isolated new agosterol congeners from a marine sponge *Acanthodendrilla* sp. Agosterols were initially isolated from a marine sponge *Spongia* sp. and found to reverse multidrug resistance in tumor cells.[11,12] In this paper, we report the isolation, structure elucidation, and biological activities of new agosterol congeners.

Results and Discussion

Specimens of *Acanthodendrilla* sp. (0.8 kg) were collected from Toyama Bay in the Japan Sea and kept frozen until extraction with MeOH. After evaporation of the solvent, the resulting aqueous residue was extracted with EtOAc. The EtOAc layer showed proteasome inhibitory and cytotoxic activities and was fractionated by a combination of silica gel and ODS chromatographies followed by reversed-phase HPLC to furnish seven new agosterol congeners, agosterols E (**1**, 29.2 mg), E_3 (**2**, 4.3 mg), C_2 (**3**, 102.4 mg), C_7 (**4**, 9.1 mg), F (**5**, 18.8 mg), G (**6**, 14.2 mg), and A_3 (**7**, 15.5 mg), together with the known agosterols A (**8**, 188.4 mg), C (**9**, 86.3 mg), and D_2 (**10**, 29.6 mg).[11,12]



	R_1	R_2	R_3	R_4
1	Ac	OH	H	A
2	Ac	OH	H	B
3	H	H	H	C
4	H	H	H	D
5	H	H	OH	A
6	H	H	OAc	A
7	Ac	H	OH	B
8	Ac	H	OH	A
9	H	H	H	A
10	Ac	H	H	C



Agosterol E (**1**) has a molecular ion peak at *m*/*z* 599 [M + Na]$^+$ in the FABMS, which matched a formula of $C_{33}H_{52}O_8$. The ^{1}H NMR spectrum (Table 1) revealed five methyl signals [δ 0.56 (s), 0.88 (d, J = 6.8 Hz), 0.90 (d, J = 6.8 Hz), 0.92 (d, J = 6.8 Hz), and 1.22 (s)], three acetyl methyl signals [δ 1.96 (s), 2.02 (s), and 2.07 (s)], methylene and methine signals (δ 1.2−2.3), three oxymethine signals [δ 3.60 (br d, J = 11.5 Hz), 4.80 (dt, J = 12.6, 3.3 Hz), 5.28 (dt, J = 10.8, 3.3 Hz), and 5.48 (t, J = 3.3 Hz)], and an olefin signal at δ 5.18 (br s) (Table 1). The steroidal nature of **1** was readily implied from ^{13}C NMR, COSY, HMQC, and HMBC data (Table 1) and supported by the molecular formula. Oxygenation at C-3, C-4, and C-6 in rings A and B and C-22 in the side chain was straightfoward by analysis of 2D NMR data; three acetyl groups could be placed on C-3, C-4, and C-6 as judged by the low-field

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Table 1. NMR Data for **1** in $CDCl_3$

no.	δ_H		δ_C	HMBC
1	1.20 m		29.9 t	
	1.90 m			
2	1.70 m		26.6 t	
	1.89 m			
3	4.80 dt	12.6, 3.3	71.9 d	
4	5.48 t	3.3	67.0 d	C-5
5	2.22 dd	10.8, 3.3	40.8 d	C-4, C-6, C-7, C-10, C-19
6	5.28 dt	10.8, 3.3	67.3 d	C-5, C-7, C-8
7	5.18 br s		121.6 d	C-5, C-9, C-14
8			142.9 s	
9			73.90 s	
10			40.2 s	
11	1.57 m		21.9 t	C-13
	1.57 m			
12	1.20 m		35.4 t	
	1.97 m			C-9, C-13
13			44.2 s	
14	2.28 m		50.6 d	C-7, C-8, C-18
15	1.41 m		22.9 t	C-17
	1.57 m			C-17
16	1.52 m		27.1 t	
	1.80 m			C-13, C-17
17	1.32 m		52.9 d	C-12, C-13, C-16, C-18
18	0.56 s		11.1 q	C-12, C-13, C-14, C-17
19	1.22 s		17.9 q	C-1, C-5, C-9, C-10
20	1.67 m		42.5 d	
21	0.92 d	6.8	12.6 q	C-17, C-22
22	3.60 br d	11.5	73.86 d	C-17, C-21
23	1.22 m		27.2 t	C-22
	1.35 m			
24	1.22 m		36.1 t	
	1.37 m			
25	1.52 m		28.1 d	
26	0.88 d	6.8	22.4 q	C-24, C-25, C-27
27	0.90 d	6.8	22.9 q	C-24, C-25, C-26
3-OAc	1.96 s		20.95 q	3-O*C*OCH$_3$
			170.2 s	
4-OAc	2.07 s		21.0 q	4-O*C*OCH$_3$
			170.3 s	
6-OAc	2.02 s		20.86 q	6-O*C*OCH$_3$
			171.2 s	

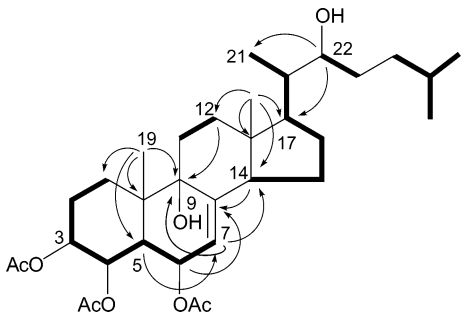

Figure 1. COSY (bold lines) and HMBC (arrows) correlations observed for **1**.

resonances of H-3 (δ 4.80), H-4 (δ 5.48), and H-6 (δ 5.28), which was substantiated by HMBC cross-peaks, δ 4.80/δ 170.2, δ 5.48/δ 170.3, and δ 5.28/δ 171.2. The presence of a hydroxy group at C-22 was suggested by the COSY and HMBC [δ 3.60 (H-22)/δ 12.6 (C-21) and 52.9 (C-17)] data (Figure 1). The $\Delta^{7(8)}$ double bond [δ_H 5.18 (H-7); δ_C 121.6 (d, C-7) and 142.9 (s, C-8)] was consistent with a COSY cross-peak, δ 5.28 (H-6)/δ 5.18 (H-7), and HMBC cross-peaks, δ 2.28 (H-14) and 5.28 (H-6)/δ 142.9 (C-8), δ 5.18 (H-7)/δ 50.6 (C-14), and δ 2.22 (H-5)/δ 121.6 (C-7) (Figure 1). These NMR data are almost superimposable on those of the major metabolite, agosterol A (**8**), except for the presence of a quaternary carbon at δ 73.9 (s) in **1** instead of an oxymethine carbon at δ 69.0 (d, C-11) and a downfield hydrogen resonance at δ 3.98 (H-11) in **8**. The position of the quaternary carbon in **1** was established to be C-9 by HMBC cross-peaks from H_3-19 (δ 1.22, 3H, s) as well as from H-12 (δ 1.97, m) and H-7 (Figure 1). Thus, the structure of **1** was established.

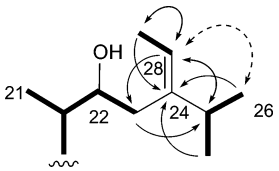

Figure 2. HMBC (arrows) and NOE correlations (dashed arrows) observed for **2**.

Agosterol E_3 (**2**) has a molecular formula of $C_{35}H_{54}O_8$ as established by HRFABMS, a C_2H_2 unit more than **1**. NMR data (Table 2) of **2** showed that the steroidal nucleus and the 22*S*-hydroxy group in the side chain of **2** were the same as that of **1**. The signals, δ_H 5.53 (q, J = 6.8 Hz, H-28)/δ_C 120.0 (d, C-28) and δ_C 142.95 (s, C-24), indicated the presence of a double bond in the side chain. The COSY spectrum showed that the olefin signal (δ 5.53) was coupled with a methyl group at δ 1.65 (d, J = 6.8 Hz, H_3-29) (Figure 2). The olefin signal exhibited HMBC correlation with the carbons at δ 13.6 (q, C-29), 29.5 (t, C-23), and 33.7 (d, C-25), and the allyl methyl signal (δ 1.65, H_3-29) showed correlation with the carbons at δ 120.0 (C-28) and 142.95 (C-24). These data resulted in the presence of a moiety of $\Delta^{24(28)}$ unsaturation in **2** as in fucosterol, and the *E* configuration was confirmed by NOE correlation between the olefin signal (H-28) and methyl signals (H_3-26 and H_3-27), which was supported by the magnitude of the chemical shift of a methine signal at δ 2.23 (m, H-25); in the case of the 25*Z* configuration as in isofucosterol the methine signal was shifted low-field to δ 2.8.[13] Thereby, the structure of **2** was determined as shown.

A molecular formula of agosterol C_2 (**3**) $C_{31}H_{50}O_7$ was established for **3** by HRFABMS, an oxygen atom more than agosterol C (**9**). The ^{1}H and ^{13}C NMR spectra revealed that **3** had the same steroidal nucleus as **9**, although their side chains were different (Table 2). The analysis of the 2D NMR spectrum of **3** showed the same side chain as agosterol D_2 (**10**). Agosterol C_7 (**4**) has a molecular formula of $C_{32}H_{50}O_7$, a carbon atom more than **3**. The presence of an exomethylene group at C-24 [δ_H 4.84 (s, H-28) and 4.93 (s, H-28); δ_C 109.9 (t, C-28) and 153.1 (s, C-24)] in **4** was consistent with HMBC cross-peaks δ 4.84 and 4.93/δ 37.4 (C-23), 153.1 (C-24), and 33.3 (C-25). Thus, the structures of **3** and **4** were determined as shown. The stereochemistry at C-20 and C-22 in **4** was not determined.

Similarly, the structures of the remaining compounds, agosterols F (**5**), G (**6**), and A_3 (**7**), were elucidated on the basis of FABMS and 2D NMR data. The structure of agosterols F (**5**) was identical with that of 3-*O*-deacetylagosterol A, which was previously prepared from agosterol A (**8**).[12] However, this is the first report of the isolation from nature. Agosterol G (**6**) has a molecular formula of $C_{33}H_{52}O_8$, a C_2H_2O unit more than **5**. The ^{1}H NMR spectrum of **6** was almost superimposable on that of **5** except for the presence of one more acetyl methyl signal and a low-field resonance of H-11 (δ 3.97 in **5**; δ 5.18 in **6**). The analysis of 2D data confirmed **6** was 11-*O*-acethylagosterol F. Agosterol A_3 (**7**) had the same steroidal nucleus as **8**, and the side chain matched that of agosterol E_3 (**2**).

Although several polyhydroxylated sterols with a 9α-hydroxy group were isolated from marine sponges,[14,15] agosterols E (**1**) and E_3 (**2**) are the first 9α-hydroxysteroids in agosterol congeners.[11,12] This is the second report of

Table 2. NMR Data for **2**, **3**, and **4** in $CDCl_3$

	2			**3**			**4**		
no.	δ_H		δ_C	δ_H		δ_C	δ_H		δ_C
1	1.55 m		30.0 t	1.24 m		36.9 t	1.24 m		37.0 t
	2.00 m			1.86 dt	13.2, 3.4		1.86 m		
2	1.72 m		26.7 t	1.71 m		25.0 t	1.57 m		25.0 t
	1.88 m			1.75 m			1.77 m		
3	4.82 dt	11.7, 3.9	71.9 d	3.70 m		71.4 d	3.71 m		71.5 d
4	5.50 br s		67.0 d	5.34 t	3.2	69.8 d	5.34 t	3.4	69.8 d
5	2.24 m		40.87 d	1.67 dd	10.3, 3.2	47.8 d	1.67 dd	10.8, 3.4	47.8 d
6	5.30 br d	10.8	67.3 d	5.38 br d	10.3	68.1 d	5.39 br d	10.8	68.2 d
7	5.20 br s		121.7 d	5.10 d	1.5	118.9 d	5.11 d	1.5	118.8 d
8			142.88 s			142.1 s			142.2 s
9			74.0 s	1.77 m		50.0 d	1.77 m		50.0 d
10			40.3 s			35.6 s			35.6 s
11	1.48 m		21.92 t	1.53 m		20.8 t	1.55 m		21.1 t
	1.62 m			1.56 m			1.60 m		
12	1.24 m		35.4 t	1.33 dd	12.7, 4.9	39.4 t	1.32 dt	4.9, 12.7	39.5 t
	1.96 m			2.15 br d	12.7		2.16 br d	12.7	
13			44.3 s			44.3 s			44.2 s
14	2.32 m		50.7 d	1.84 m		54.8 d	1.84 m		54.90 d
15	1.44 m		23.0 t	1.50 m		21.1 t	1.48 m		21.2 t
	1.60 m			1.62 m			1.55 m		
16	1.40 m		27.1 t	1.47 m		22.3 t	1.58 m		21.8 t
	1.85 m			1.85 m			1.60 m		
17	1.38 m		52.7 d	1.60 m		54.7 d	1.63 m		54.93 d
18	0.59 s		11.1 q	0.72 s		13.5 q	0.74 s		13.6 q
19	1.23 s		17.9 q	1.10 s		15.4 q	1.10 s		15.4 q
20	1.84 m		40.85 d			77.2 s			76.6 s
21	1.024 d	6.8	12.6 q	1.20 s		20.6 q	1.23 s		20.5 q
22	3.75 dt	11.2, 3.4	70.6 d	3.38 br d	8.8	76.3 d	3.59 dd	10.8, 2.0	73.1 s
23	1.94 m		29.5 t	1.22 m		29.2 t	1.95 dd	14.2, 10.8	37.4 t
	2.36 dd	13.7, 11.2		1.42 m			2.30 br d	14.2	
24			142.95 s	1.22 m		36.2 t			153.1 s
				1.44 m					
25	2.23 m		33.7 d	1.55 m		28.1 d	2.25 m		33.3 d
26	1.016 d	6.8	22.9 q	0.89 d	6.8	22.3 q	1.08 d	6.8	22.2 q
27	1.05 d	6.8	21.96 q	0.90 d	6.8	22.5 q	1.05 d	6.8	22.6 q
28	5.53 q	6.8	120.0 d				4.84 s		109.9 t
							4.93 s		
29	1.65 d	6.8	13.6 q						
3-OAc	1.98 s		20.9 q						
			170.25 s						
4-OAc	2.08 s		21.0 q	2.10 s		21.0 q	2.11 s		21.6 q
			170.29 s			172.2 s			172.2 s
6-OAc	2.04 s		21.1 q	2.05 q		21.2 q	2.05 s		20.9 q
			171.2 s			171.1 s			171.1 s

Table 3. Biological Activities of **1**−**10**

compound	proteasome inhibition IC_{50} (μg/mL)	cytotoxicity IC_{50} (μg/mL)
1	*a*	25
2	*a*	14
3	23	>25
4	20	>25
5	35	16
6	100	8.8
7	*a*	10
8	50	25
9	10	22
10	90	23

[a] Inactive at 100 μg/mL.

isolation of compounds from sponges of the genus *Acanthodendrilla* sp.[16]

Inhibitory activities of agosterol derivatives against the proteasome and their cytotoxic activities against HeLa cells are shown in Table 3. Agosterol C (**9**) is the most potent proteasome inhibitor, with an IC_{50} value of 10 μg/mL, and three of them are inactive even at 100 μg/mL. Despite their structural similarities, their inhibitory potencies are different from one another. In addition, agosterols exhibit moderate cytotoxic activity, but the ranking of cytotoxic activity among agosterols is not correlated with that of their potency as proteasome inhibitors. Since agosterols have different structural features from those of other known proteasome inhibitors,[6,7] elucidation of their inhibitory mechanisms is an interesting issue to be clarified.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Horiba SEPA-300 high sensitive polarimeter. IR spectra were recorded on a Shimadzu IR-460 infrared spectrophotometer. The fluorescence intensity was measured (excitation, 380 nm; emission, 460 nm) using a Shimadzu spectrofluorophotometer RF-5000. NMR spectra were recorded on a JEOL GSX500 NMR spectrometer in $CDCl_3$. Chemical shifts were referenced to the residual solvent peaks (δ_H 7.26 and δ_C 77.0). Mass spectra were measured on a JEOL SX-102 mass spectrometer.

Animal Material. The marine sponge was collected by snorkeling at a depth of 2 m in Toyama Bay in the Japan Sea, frozen immediately, and kept frozen until processed. The sponge was identified as *Acanthodendrilla* sp. (class Demospongiae, order Dendroceratida, family Dictyodendrillidae). The sponge is finger-shaped, colored dark brown outside and light beige inside. The surface is densely conulose with sharp conules of about 1−2 mm high, 2 mm apart. The skeleton consists of an irregular reticulation of laminated spongin fibers cored predominantly by broken spicules, but also with sand

grains and other debris. Pith is visible in the fibers in variable quantities. Fibers 25−105 μm in diameter form meshes of 200−700 μm. There are no matching descriptions in the literature. The voucher is deposited in the collections of the Zoological Museum of the University of Amsterdam under registration number POR. 17001.

Extraction and Isolation. The frozen sponge (0.8 kg, wet wt) was extracted with MeOH. The extract was concentrated under reduced pressure and extracted with EtOAc. The EtOAc layer (3.5 g) was subjected to silica gel chromatography with a stepwise gradient of hexane/EtOAc. The first fraction (909.1 mg) eluted with hexane/EtOAc (1:1) was purified by ODS chromatography with 80% MeOH/H_2O followed by reversed-phase HPLC with 80% MeOH/H_2O to afford agosterols E (**1**, 29.2 mg, 3.7 × 10^{-3}%, wet wt), E_3 (**2**, 4.3 mg, 5.4 × 10^{-4}%), and A_3 (**7**, 15.5 mg, 1.9 × 10^{-3}%) together with the known agosterols A (**8**, 188.4 mg, 2.4 × 10^{-2}%), C (**9**, 86.3 mg, 1.1 × 10^{-2}%), and D_2 (**10**, 29.6 mg, 3.7 × 10^{-3}%). The second fraction (467.2 mg) eluted with hexane/EtOAc (1:2) was purified by reversed-phase HPLC with 80% MeOH/H_2O to afford agosterols C_2 (**3**, 102.4 mg, 1.3 × 10^{-2}%) and C_7 (**4**, 9.1 mg, 1.1 × 10^{-3}%). The third fraction (462.0 mg) eluted with hexane/EtOAc (1:4) was purified by reversed-phase HPLC with 80% to afford agosterols F (**5**, 18.8 mg, 2.4 × 10^{-3}%) and G (**6**, 14.2 mg, 5.3 × 10^{-4}%).

Agosterol E (1): $[\alpha]^{25}_D$ +21.8° (*c* 0.73, $CHCl_3$); IR (film) ν_{max} 3525, 2950, 1735, 1360, 1250 cm^{-1}; NMR data, see Table 1; HRFABMS (positive) *m/z* 599.3539 $[M + Na]^+$ (calcd for $C_{33}H_{52}O_8Na$, Δ −2.0 mmu).

Agosterol E_3 (2): $[\alpha]^{26}_D$ +12.6° (*c* 0.132, $CHCl_3$); IR (film) ν_{max} 3525, 2950, 1735, 1240 cm^{-1}; NMR data, see Table 2; HRFABMS (positive) *m/z* 625.3715 $[M + Na]^+$ (calcd for $C_{35}H_{54}O_8Na$, Δ −1.0 mmu).

Agosterol C_2 (3): $[\alpha]^{24}_D$ +41.2° (*c* 1.56, $CHCl_3$); IR (film) ν_{max} 3440, 2960, 1740, 1345, 1250 cm^{-1}; NMR data, see Table 2; HRFABMS (positive) *m/z* 557.3452 $[M + Na]^+$ (calcd for $C_{31}H_{50}O_7Na$, Δ −2.0 mmu).

Agosterol C_7 (4): $[\alpha]^{25}_D$ +34.2° (*c* 0.336, $CHCl_3$); IR (film) ν_{max} 3430, 2950, 1720, 1360, 1250 cm^{-1}; NMR data, see Table 2; HRFABMS (positive) *m/z* 569.3474 $[M + Na]^+$ (calcd for $C_{32}H_{50}O_7Na$, Δ +1.9 mmu).

Agosterol F (5): $[\alpha]^{26}_D$ +43.0° (*c* 0.712, $CHCl_3$); IR (film) ν_{max} 3430, 2950, 1735, 1370, 1250, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.56 (3H, s, H_3-18), 0.89 (3H, d, J = 6.4 Hz, H_3-27), 0.90 (3H, d, J = 6.4 Hz, H_3-26), 0.94 (3H, d, J = 6.8 Hz, H_3-21), 1.18 (1H, m, H-24), 1.20 (3H, s, H_3-19), 1.20 (1H, m, H-23), 1.28 (1H, m, H-17), 1.32 (1H, m, H-12), 1.35 (1H, m, H-23), 1.36 (1H, m, H-1), 1.43 (1H, m, H-15), 1.43 (1H, m, H-16), 1.43 (1H, m, H-24), 1.56 (1H, m, H-15), 1.56 (1H, m, H-25), 1.67 (1H, m, H-2), 1.67 (1H, m, H-20), 1.69 (1H, m, H-5), 1.71 (1H, m, H-2), 1.79 (1H, m, H-16), 1.89 (1H, m, H-14), 2.05 (3H, s, 6-OAc), 2.10 (3H, s, 4-OAc), 2.32 (1H, dd, J = 11.7, 4.9 Hz, H-12), 2.49 (1H, dt, J = 14.2, 3.4 Hz, H-1), 3.59 (1H, br d, J = 11.2 Hz, H-22), 3.70 (1H, dt, J = 11.2, 4.4 Hz, H-3), 3.97 (1H, dt, J = 4.9, 10.3 Hz, H-11), 5.17 (1H, d, J = 2.0 Hz, H-7), 5.36 (1H, br d, J = 11.2 Hz, H-6), 5.37 (1H, br s, H-4); ^{13}C NMR ($CDCl_3$) δ 12.6 (q, C-21), 12.8 (q, C-18), 15.5 (q, C-19), 21.06 (q, 6-OCO*C*H_3), 21.13 (q, 4-OCO*C*H_3), 22.4 (q, C-27), 22.8 (t, C-15), 22.9 (q, C-26), 25.2 (t, C-2), 27.1 (t, C-16), 27.7 (t, C-23), 28.1 (d, C-25), 36.0 (t, C-24), 36.5 (s, C-10), 38.7 (t, C-1), 42.5 (d, C-20), 43.6 (s, C-13), 47.9 (d, C-5), 50.8 (t, C-12), 52.9 (d, C-17), 54.4 (d, C-14), 57.6 (d, C-9), 67.7 (d, C-6), 68.9 (d, C-11), 69.9 (d, C-4), 71.2 (d, C-3), 73.8 (d, C-22), 120.8 (d, C-7), 139.4 (s, C-8), 171.1 (s, 6-O*C*OCH$_3$), 172.1 (s, 4-O*C*OCH$_3$); HRFABMS (positive) *m/z* 557.3463 $[M + Na]^+$ (calcd for $C_{31}H_{50}O_7Na$, Δ +0.9 mmu).

Agosterol G (6): $[\alpha]^{25}_D$ +41.0° (*c* 0.108, $CHCl_3$); IR (film) ν_{max} 3475, 2950, 1735, 1370, 1240, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.62 (3H, s, H_3-18), 0.89 (3H, d, J = 6.4 Hz, H_3-27), 0.90 (3H, d, J = 6.4 Hz, H_3-26), 0.92 (3H, d, J = 6.4 Hz, H_3-21), 1.16 (3H, s, H_3-19), 1.18 (1H, m, H-24), 1.22 (1H, m, H-23), 1.27 (1H, m, H-15), 1.28 (1H, m, H-12), 1.29 (1H, m, H-17), 1.35 (1H, m, H-23), 1.42 (1H, m, H-24), 1.43 (1H, m H-1), 1.44 (1H, m, H-16), 1.54 (1H, m, H-25), 1.55 (1H, m, H-2), 1.65 (1H, m, H-20), 1.67 (1H, m, H-15), 1.68 (1H, m, H-2), 1.72 (1H, dd, J = 11.7, 3.4 Hz, H-5), 1.80 (1H, dt, J = 13.7, 3.4 Hz, H-1), 1.81 (1H, m, H-16), 1.93 (1H, m, H-14), 2.03 (3H, s, 11-OAc), 2.06 (3H, s, 6-OAc), 2.12 (3H, s, 4-OAc), 2.14 (1H, br d, J = 9.8 Hz, H-9), 2.40 (1H, dd, J = 12.2, 5.9 Hz, H-12), 3.59 (1H, br d, J = 7.3 Hz, H-22), 3.70 (1H, dt, J = 15.2, 3.4 Hz, H-3), 5.18 (1H, dt, J = 5.4, 10.7 Hz, H-11), 5.22 (1H, br s, H-7), 5.36 (1H, br s, H-4), 5.37 (1H, br d, J = 9.8 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 12.4 (q, C-18), 12.6 (q, C-21), 15.6 (q, C-19), 21.0 (q, 4-OCO*C*H_3), 21.1 (q, 6-OCO*C*H_3), 21.9 (q, 11-OCO*C*H_3), 22.4 (q, C-26), 22.87 (t, C-15), 22.92 (q, C-27), 25.1 (t, C-2), 27.1 (t, C-16), 27.8 (t, C-23), 28.2 (d, C-25), 36.0 (t, C-24), 36.7 (s, C-10), 37.8 (t, C-1), 42.5 (d, C-20), 43.3 (s, C-13), 45.5 (t, C-12), 47.6 (d, C-5), 52.8 (d, C-17), 53.9 (d, C-9), 54.2 (d, C-14), 67.5 (d, C-6), 69.7 (d, C-4), 71.0 (d, C-11), 71.2 (d, C-3), 73.8 (d, C-22), 121.2 (d, C-7), 139.0 (s, C-8), 169.9 (s, 11-O*C*OCH$_3$), 171.0 (s, 6-O*C*OCH$_3$), 172.1 (s, 4-O*C*OCH$_3$); HRFABMS (positive) *m/z* 599.3563 $[M + Na]^+$ (calcd for $C_{33}H_{52}O_8Na$, Δ +0.3 mmu).

Agosterol A_3 (7): $[\alpha]^{26}_D$ +32.0° (*c* 0.428, $CHCl_3$); IR (film) ν_{max} 3570, 2950, 1740, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.59 (3H, s, H_3-18), 1.01 (3H, d, J = 6.8 Hz, H_3-21), 1.03 (3H, d, J = 6.8 Hz, H_3-26), 1.04 (3H, d, J = 6.8 Hz, H_3-27), 1.24 (3H, s, H_3-19), 1.34 (1H, m, H-17), 1.36 (1H, m, H-12), 1.43 (1H, m, H-1), 1.45 (1H, m, H-15), 1.45 (1H, m, H-16), 1.65 (1H, m, H-2), 1.65 (1H, m, H-15), 1.65 (1H, d, J = 6.8 Hz, H_3-29), 1.74 (1H, dd, J = 10.8, 3.2 Hz, H-5), 1.80 (1H, br d, J = 10.5 Hz, H-9), 1.84 (1H, m, H-20), 1.90 (1H, m, H-2), 1.90 (1H, m, H-16), 1.90 (1H, m, H-23), 1.92 (1H, m, H-14), 1.97 (3H, s, 3-OAc), 2.04 (3H, s, 6-OAc), 2.08 (3H, s, 4-OAc), 2.21 (1H, septet, J = 6.8 Hz, H-25), 2.34 (1H, m, H-23), 2.36 (1H, dd, J = 11.7, 4.9 Hz, H-12), 2.57 (1H, dt, J = 14.0, 3.8 Hz, H-1), 3.73 (1H, dt, J = 11.2, 3.4 Hz, H-22), 3.98 (1H, m, H-11), 4.80 (1H, dt, J = 12.2, 3.4, H-3), 5.17 (1H, br s, H-7), 5.33 (1H, br d, J = 10.8 Hz, H-6), 5.47 (1H, br s, H-4), 5.53 (1H, q, J = 6.8 Hz, H-28); ^{13}C NMR ($CDCl_3$) δ 12.6 (q, C-21), 12.8 (q, C-18), 13.6 (q, C-29), 15.5 (q, C-19), 20.9 (q, 3-OCO*C*H_3), 21.0 (q, 4-OCO*C*H_3), 21.2 (q, 6-OCO*C*H_3), 22.0 (t, C-2), 22.5 (q, C-26), 22.8 (q, C-27), 22.9 (t, C-15), 27.1 (t, C-16), 29.5 (t, C-23), 33.7 (d, C-25), 36.5 (s, C-10), 38.7 (t, C-1), 40.8 (d, C-20), 43.7 (s, C-13), 47.9 (d, C-5), 50.8 (t, C-12), 52.7 (d, C-17), 54.4 (d, C-14), 57.6 (d, C-9), 66.8 (d, C-4), 67.4 (d, C-6), 69.0 (d, C-11), 70.5 (d, C-22), 72.0 (d, C-3), 120.1 (d, C-28), 120.9 (d, C-7), 139.1 (s, C-8), 142.9 (s, C-24), 170.30 (s, 3-O*C*OCH$_3$), 170.33 (s, 4-O*C*OCH$_3$), 171.2 (s, 6-O*C*OCH$_3$); HRFABMS (positive) *m/z* 625.3715 $[M + Na]^+$ (calcd for $C_{35}H_{54}O_8Na$, Δ −0.1 mmu).

Proteasome Inhibition Assay. Proteasome used in this study was partially purified from rat liver. Liver was dissected and homogenized in ice-cold lysis buffer consisting of 20 mM HEPES, pH 7.5, 5 mM KCl, 1.5 mM $MgCl_2$, 1 mM dithiothreitol, 2 mM ATP, and 10% glycerol at 4 °C for 5 min. The extract was filtered through cheesecloth, and the filtrate was immediately centrifuged at 10 000 rpm for 5 min. The supernatant was centrifuged at 105 000*g* for 20 min, and the resultant supernatant was further centrifuged at 300 000*g* for 2 h. The precipitates thus obtained were suspended in lysis buffer containing 50% glycerol and used as the crude proteasome-enriched preparation. The fluorogenic substrate succinyl-leucyl-leucyl-valyl-tyrosine 4-methylcoumaryl-7-amide (MCA) (Peptide Institute, Inc., Osaka) was used as a substrate for chymotrypsin-like activity of the proteasome. The proteasome in a mixture (0.5 mL) that contained 50 mM Tris-HCl, pH 7.8, 1 mM dithiothreitol, 5 mM EDTA, and 0.02% SDS was preincubated with each inhibitor at 30 °C for 10 min. Then, 0.05 mM substrate was added to the mixture, and the mixture was further incubated at 30 °C for 1 h. The reaction was stopped by adding 0.5 mL of 10% SDS, and the fluorescence intensity owing to 7-amino-4-methylcoumarin (AMC) was measured (excitation, 380 nm; emission, 460 nm). The value of IC_{50}, the concentration required for 50% inhibition of proteasome inhibitory activity, was calculated from the data of duplicate measurements.

Cytotoxicity Test. A cytotoxicity test was carried out with HeLa cells. HeLa cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (50 units/mL), and streptomycin (50 μg/mL) under a humidified atmosphere of 5% CO_2 at 37 °C. The cells were

seeded into 96-well microplates (3×10^3 cells/well) and precultured for a day. The medium was replaced with that containing test compounds at various concentrations, and the cells were further cultured at 37 °C for 3 days. The medium was then replaced with 50 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.2 mg/mL in medium), and the cells were incubated under the same conditions for 4 h. After addition of 200 μL of DMSO, the optical density at 570 nm was measured with a microplate reader.

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Supporting Information Available: Color photograph of a marine sponge of the genus *Acanthodendrilla*. This material is available free of charge via the Internet at http://pubs.acs.org.

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