Aragusterol B and D, New 26,27-Cyclosterols from the Okinawan Marine Sponge of the Genus *Xestospongia*

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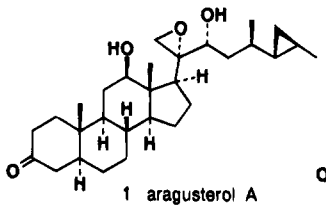
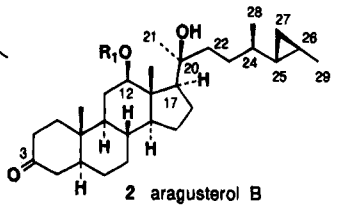
Received December 17, 1993 (Revised Manuscript Received September 2, 1994)

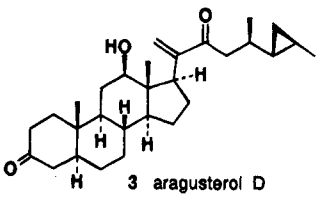
Marine sponges are sources of various novel sterols, particularly in terms of unique side chain structures such as those with high degrees of alkylation and unusual functionalization.[1] As a continuation of research[2] on biologically active substances of Okinawan marine invertebrates, we isolated aragusterol A (**1**), a potent antitumor sterol from the sponge of the genus *Xestospongia*. The complete structure of **1** was determined from spectroscopic analysis, chemical conversion, and synthesis.[3] The compound was characterized by the rare 26,27-cyclo structure in the side chain. In further investigation on congeners of **1** from the sponge, new members of 26,27-cyclosterol, aragusterol B (**2**) and D (**3**), were isolated. This paper describes structural elucidation based on spectroscopic analysis, chemical conversion and X-ray crystallographic analysis.



1 aragusterol A



2 aragusterol B



3 aragusterol D

The methanol extract of wet specimens of the sponge,[4] collected on the coral reef of Aragusuku Island (Okinawa, Japan), was partitioned between ethyl acetate and water. Repeated chromatographic separation of the ethyl acetate soluble portion gave aragusterol B (**2**) (0.65% yield based on the ethyl acetate soluble portion) and D (**3**) (0.088% yield based on the ethyl acetate soluble portion).

The IR spectrum of **2** ($C_{29}H_{48}O_3$) showed absorptions due to hydroxyl (3420 cm^{-1}) and carbonyl (1718 cm^{-1}) groups. ^1H and ^{13}C NMR data (Table 1) indicated the presence of a ketone (δ_C 211.8), a secondary hydroxyl group [δ_H 3.37 (1H, dd), δ_C 77.8], a tertiary hydroxyl group (δ_C 75.7), and a 1,2-disubstituted cyclopropyl group [δ_H 0.11 (1H, m), 0.18 (2H, m), 0.49 (1H, m)]. In addition to the signals of these groups, ^1H and ^{13}C NMR spectra showed signals due to five methyls, 10 methylenes, six methines, and two quaternary carbons. The NMR spectra of **2** were closely related to those[3] of aragusterol A (**1**), suggesting the structure of aragusterol B to be that shown as **2**.

The structure of **2**, except for stereochemistry at C-20 bearing the hydroxyl group, was established by the following chemical conversion. Dehydration of **2** with concentrated hydrochloric acid in MeOH under reflux gave trisubstituted olefin **4**. The *E* configuration of the carbon–carbon double bond was indicated by the ^{13}C chemical shift (δ 15.5 ppm)[5] of the olefinic methyl group at C-20. Treatment of **4** with ozone followed by reduction with dimethyl sulfide gave triketone **5** and diketone **6**. The formation of **5** can be rationalized by the reaction path shown in Scheme 1. Triketone **5** was identical with the compound previously converted from aragusterol A (**1**),[3] indicating the structure of the steroidal nucleus of **2**. The structure of the side chain was elucidated by treatment of **4** with ozone followed by sodium borohydride reduction and *p*-nitrobenzoylation to give **7**, which was also converted from **1**.[3]

The stereochemistry at C-20 bearing the tertiary hydroxyl group was difficult to determine by spectroscopy or a chemical method. X-ray crystallographic analysis of **2** was thus conducted. Although all crystals of **2** obtained by recrystallization of the pure crystals in several solvent systems were shown to be inadequate for measurement, good crystals were unexpectedly obtained by the slow evaporation of a solution of the chromatographic fraction during isolation. The structure was elucidated by a direct method (SHELXS 86) refined by full-matrix least-squares to $R = 0.052$. The computer-generated perspective drawing shown in Figure 1 established the full structure of **2** involving absolute stereochemistry at C-20. Interestingly the crystals consisted of two conformers differing in side chain conformation as shown in Figure 1.

The IR spectrum of aragusterol D (**3**, $C_{29}H_{44}O_3$) showed absorptions due to a hydroxyl group (3350 cm^{-1}), nonconjugated carbonyl group (1710 cm^{-1}), and conjugated enone group (1660, 1620 cm^{-1}). The presence of the conjugated enone group (CH_2=CCO–) was demonstrated by UV [λ_{max} 225 nm (ϵ 5700)], ^1H NMR [δ_H 5.86 (1H, s), 6.20 (1H, s)], and ^{13}C [δ_C 125.3 (CH_2), 149.8 (C), 207.5 (C)] spectra. ^1H and ^{13}C NMR spectra (Table 1) also showed the signals due to a ketone [δ_C 211.6 (C)], a secondary hydroxyl group [δ_H 3.62 (1H, td), δ_C 77.8 (CH)], and 1,2-disubstituted cyclopropyl group [δ_H 0.15 (1H, m), 0.25 (2H, m), 0.49 (1H, m)]. The ^1H and ^{13}C NMR spectra of **3** were closely related to those of aragusterol B (**2**) except for signals of the CH_2=CCO– group instead of those of the $CH_3C(OH)CH_2$– group in **2**, suggesting the structure of aragusterol D to be **3**. The structure was confirmed by the following chemical conversion.

Reduction of **3** with sodium borohydride in the presence of cerium(III) chloride[6] gave diastereomeric allylic alco-

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1993**, *10*, 497 and previous papers in this series.

(2) Recent examples: (a) Iguchi, K.; Shimada, Y.; Yamada, Y. *J. Org. Chem.* **1992**, *57*, 522. (b) Iguchi, K.; Kitade, M.; Kashiwagi, T.; Yamada, Y. *Ibid.* **1993**, *58*, 5690.

(3) Iguchi, K.; Fujita, M.; Nagaoka, H.; Mitome, H.; Yamada, Y. *Tetrahedron Lett.* **1993**, *34*, 6277.

(4) The sponge was identified as the genus *Xestospongia* by Prof. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam. Specimens are on deposit in his collection (registered number: ZMA Por. 7842).

(5) Stothers, J. B. *Carbon-13 NMR Spectroscopy*; Academic Press: New York, 1972; Chapter 11. The assignment of this signal of **4** was confirmed by HMQC measurements.

(6) Luche, J.-L. *J. Am. Chem. Soc.* **1978**, *100*, 2226.

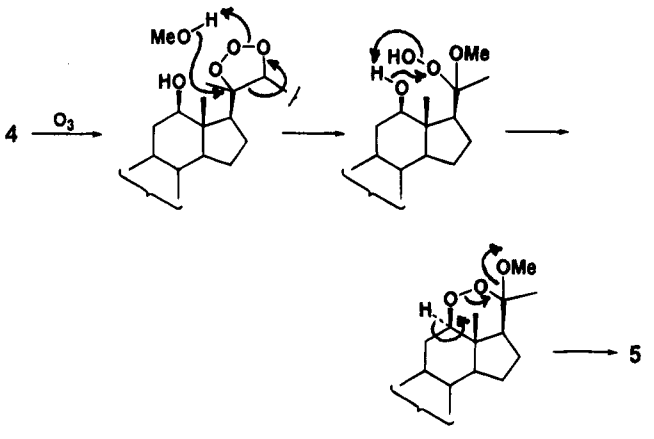
0022-3263/94/1959-7499$04.50/0

Table 1. NMR Data for Aragusterol B and D

	aragusterol B (2)		aragusterol D (3)	
no.	^{13}C[a]	^{1}H (J in Hz)[b]	^{13}C[a]	^{1}H (J in Hz)[b]
1	38.5 (CH_2)	2.03 (1H, ddd, 2.3, 6.5, 13.3)	38.4 (CH_2)	2.01 (1H, ddd, 2.4, 6.3, 13.2)
2	38.2 (CH_2)	2.32 (1H, brd, 13.5)	38.1 (CH_2)	2.35 (1H, dt, 6.4, 13.4)
		2.36 (1H, dt, 6.5, 13.5)		
3	211.8 (C)		211.3 (C)	
4	44.8 (CH_2)	2.09 (1H, ddd, 2.0, 5.8, 14.2)	44.5 (CH_2)	2.10 (1H, ddd, 2.0, 3.9, 14.1)
		2.26 (1H, t, 14.2)		2.25 (1H, t, 14.1)
5	46.7 (CH)		46.5 (CH)	
6	29.0 (CH_2)		28.9 (CH_2)	
7	31.2 (CH_2)		31.3 (CH_2)	
8	33.9 (CH)		35.0 (CH)[d]	
9	52.7 (CH)		52.6 (CH)	
10	35.7 (C)		35.6 (C)	
11	29.3 (CH_2)		30.0 (CH_2)	
12	77.8 (CH)	3.37 (1H, dd, 4.5, 11.1)	77.8 (CH)	3.61 (1H, td, 4.2, 11.2)[g]
				2.68 (1H, d, 4.2, OH)
13	48.8 (C)		49.3 (C)	
14	54.8 (CH)		48.2 (CH)[e]	
15	23.5 (CH_2)[c]		23.9 (CH_2)[f]	
16	25.1 (CH_2)[c]		28.9 (CH_2)[f]	
17	64.3 (CH)	1.63 (1H, m)	54.7 (CH)[e]	2.82 (1H, t, 10.1)
18	9.7 (CH_3)	0.86 (3H, s)	11.3 (CH_3)	1.01 (3H, s)
19	11.5 (CH_3)	1.02 (3H, s)	11.3 (CH_3)	1.01 (3H, s)
20	75.7 (C)		149.8 (C)	
21	28.8 (CH_3)	1.17 (3H, s)	125.3 (CH_2)	5.86 (1H, s), 6.20 (1H, s)
22	34.0 (CH_2)	1.82 (1H, dt, 4.1, 14.1)	207.5 (C)	3.97 (1H, dd, 3.3, 11.1)
23	31.8 (CH_2)		46.3 (CH_2)	2.61 (1H, dd, 8.2, 15.5)
				2.87 (1H, dd, 5.5, 15.5)
24	39.1 (CH)	0.67 (1H, m)	35.6 (CH)[d]	
25	27.2 (CH)	0.18 (1H, m)	27.1 (CH)	0.25 (1H, m)
26	13.0 (CH)	0.49 (1H, m)	12.4 (CH)	0.49 (1H, m)
27	11.6 (CH_2)	0.11 (1H, m)	12.3 (CH_2)	0.15 (1H, m)
		0.18 (1H, m)		0.25 (1H, m)
28	20.3 (CH_3)	0.93 (3H, d, 6.7)	19.0 (CH_3)	0.94 (3H, d, 6.7)[h]
29	19.2 (CH_3)	1.03 (3H, d, 6.7)	19.8 (CH_3)	0.96 (3H, d, 6.5)[h]

[a] 125 MHz, $CDCl_3$. [b] 500 MHz, $CDCl_3$. [c–f,h] The signals may be interchanged in each column. [g] The signal changed to 3.61 (dd) on adding D_2O.

Scheme 1



hols **8** (24% yield) and **9**[7] (10% yield). The stereochemistry of the new chiral center at C-22 was not clear at this stage. Each compound was converted to epoxide by oxidation with *m*-chloroperbenzoic acid. Physical data for the epoxides were compared with those of **10**[7] obtained by reduction of aragusterol A (**1**) with sodium borohydride. The epoxide from **8** was shown to be identical with **10**, indicating the complete structure of aragusterol D as that shown by **3**. This finding also demonstrated the stereochemistry at C-22 in **8** and **9**.

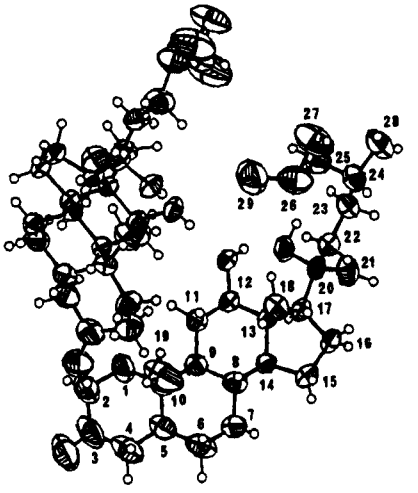
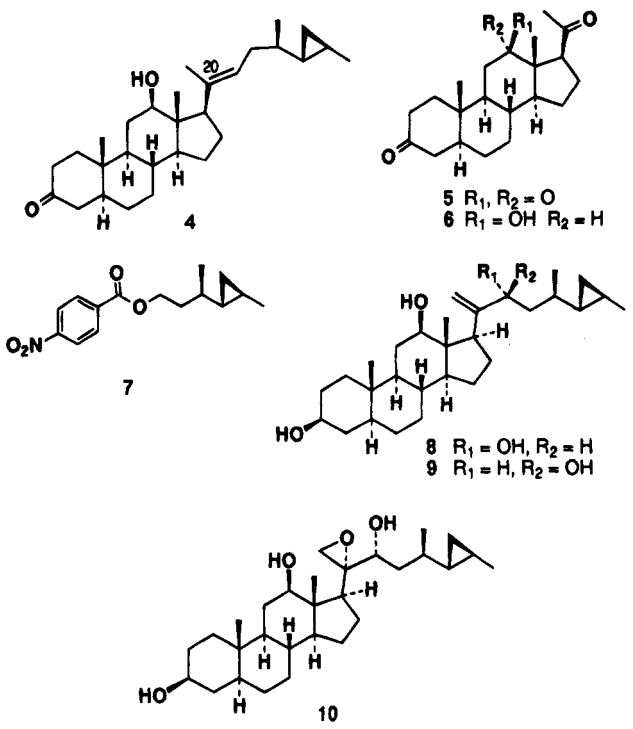


Figure 1.

Kobayashi *et al.* reported xestokerol C,[8] a steroid from the Okinawan marine sponge (*Xestospongia* sp.), with the structure similar to that of aragusterol D (**3**). However, the absolute configurations at the C-24, -25 and -26 positions in the side chain of xestokerol C were undetermined. Although the sample of xestokerol C was not obtained from Professor Kobayashi because of an insufficient quantity of material and it was not possible to

(7) The methyl signals at C-24 in the side chain appeared as each singlet at δ 0.95 ppm in the ^{1}H NMR spectra (in $CDCl_3$) of **9** and **10**, although the corresponding methyl signals appeared as a doublet in other compounds such as **2**, **3**, and **8**. This was also observed in the ^{1}H NMR spectrum of aragusterol A (**1**) [δ 0.95 (s) ppm], but the signal appeared as a doublet (δ 1.00 ppm) in C_6D_6 solution.[3] Though not examined, the methyl signals of **9** and **10** could appear as a doublet in C_6D_6 solution.

(8) Kobayashi, J.; Ishida, K.; Naitoh, K.; Shigemori, H.; Mikami, Y.; Sasaki, T. *J. Nat. Prod.* **1993**, *56*, 1350.



make a direct comparison of the two compounds, the ^{13}C NMR spectrum (provided by Professor Kobayashi) of xestokerol C was identical with that of aragusterol D (**3**).

Aragusterol B (**2**) showed moderate antiproliferative activity (IC_{50} 3.3 μg/mL) toward KB cells *in vitro*. Unexpectedly, aragusterol D (**3**) did not show antiproliferative activity toward KB cells even though it has a conjugated enone system.

Experimental Section

^1H NMR (400 and 500 MHz) and ^{13}C NMR (100 and 125 MHz) spectra were recorded in $CDCl_3$ solution. ^1H chemical shifts are given in δ ppm based on $CHCl_3$ (7.26 ppm). ^{13}C chemical shifts are given in δ ppm based on the solvent used (77.1 ppm for $CDCl_3$). Numbers of attached protons for ^{13}C signals were determined by DEPT experiments.

Extraction and Isolation. Wet specimens[4] of the sponge of the genus *Xestospongia* (44.8 kg), collected on the coral reef of Aragusuku Island (Okinawa, Japan) in May 1992, were extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O to give an EtOAc soluble portion (267 g). A part (51.3 g) of the EtOAc soluble portion was chromatographed on a silica gel column to give three fractions; fraction 1 eluted with hexane–EtOAc = 5:1 (3 L), fraction 2 eluted with hexane–EtOAc = 1:1 (1 L), and fraction 3 eluted with EtOAc (1 L) and then MeOH (1 L). The remaining EtOAc soluble portion was divided into four portions, each of which was similarly chromatographed on a silica gel column. The combined fraction 2 (67.8 g) was divided into five portions which were each chromatographed on an active charcoal column to give fraction 1 eluted with MeOH (1 L), fraction 2 eluted with EtOAc (1 L), and fraction 3 eluted with $CHCl_3$ (2 L).

The combined fraction 3 (27.3 g) from the charcoal column was divided into six portions, all of which were separately chromatographed on a silica gel column to give fraction 1 eluted with hexane–EtOAc = 2:1 (200 mL), fraction 2 eluted with hexane–EtOAc = 2:1 (150 mL), fraction 3 eluted with hexane–EtOAc = 2:1 (200 mL), fraction 4 eluted with hexane–EtOAc = 2:1 (150 mL), fraction 5 eluted with hexane–EtOAc = 2:1 (650 mL), fraction 6 eluted with hexane–EtOAc = 2:1 (700 mL), and fraction 7 eluted with MeOH (300 mL). ^1H NMR analysis of the fractions showed that fractions 4 and 5 contained aragusterol A (**1**) and B (**2**) and fraction 2 aragusterol D (**3**). Combined fractions 4 (8.76 g) and 5 (10.52 g) were subjected to repeated flash chromatography (eluted with hexane–acetone = 2:1 for the first chromatography, and hexane–acetone = 4:1 for the second one) to give crude crystals of aragusterol B (1.84 g from the fraction 4 and 1.94 g from the fraction 5). The combined crude crystals were recrystallized from hexane–EtOAc to give colorless needles of aragusterol B (**2**, 1.74 g): mp 194–195 °C; $[\alpha]_D$ +4.0° (*c* 1.56, $CHCl_3$); IR (KBr) 3420, 1718 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; EIMS *m/z* 444 (M^+), 426 ($M^+ - H_2O$), 408 ($M^+ - 2H_2O$); HREIMS $M^+ - 2H_2O$ *m/z* obsd 408.3381, $C_{29}H_{44}O$ required 408.3392. Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found: C, 77.90; H, 10.85.

Combined fraction 2 (2.16 g) was subjected to flash chromatography (eluted with hexane–AcOEt = 3:1) to give crude aragusterol D (720 mg). Recrystallization of the crude crystals from MeOH gave colorless needles of aragusterol D (**3**, 234 mg): mp 152.5–153.5 °C; $[\alpha]_D$ −61.3° (*c* 0.30, $CHCl_3$); IR ($CHCl_3$) 3350, 1710, 1660, 1620 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; EIMS *m/z* 440 (M^+), 422 ($M^+ - H_2O$); HREIMS M^+ *m/z* obsd 440.3308, $C_{29}H_{44}O_3$ required 440.3290. Anal. Calcd for $C_{29}H_{44}O_3$: C, 79.04; H, 10.07. Found: C, 78.68; H, 9.98.

Dehydration of Aragusterol B (2). A solution of aragusterol B (**2**, 92 mg, 0.21 mmol) in a mixture (25 mL) of concentrated HCl and MeOH (prepared by adding 0.6 mL of concentrated HCl to 100 mL of MeOH) was refluxed for 30 min. The mixture was diluted with ether, washed with saturated $NaHCO_3$ solution and then saturated NaCl solution, dried over anhyd Na_2SO_4, and concentrated under reduced presuure. The residue (101 mg) was dissolved in 80% AcOH solution (1 mL), and the mixture was stirred for 25 min at room temperature. After the mixture was concentrated under reduced pressure, the residue was purified by silica gel column chromatography (hexane–CH_2Cl_2–acetone = 25:25:1 as an eluent) to give olefin **4** (72 mg), which was recrystallized from hexane to give colorless plates of **4**: mp 110 °C; $[\alpha]_D$ −29.1° (*c* 0.06, $CHCl_3$); ^1H NMR (400 MHz) δ 0.10(1H, m), 0.17 (2H, m), 0.48 (1H, m), 0.77 (3H, s), 0.92 (3H, d, J = 6.7 Hz), 0.99 (3H, d, J = 5.9 Hz), 1.02 (3H, s), 1.70 (3H, br s), 3.61 (1H, dd, J = 4.8, 10.6 Hz), 5.55 (1H, t, J = 7.0 Hz); EIMS *m/z* 426 (M^+), 408 ($M^+ - H_2O$); ^{13}C NMR (100 MHz) δ 8.8 (CH_3), 11.5 (CH_3), 11.9 (CH_2), 12.7 (CH), 15.5 (CH_3), 19.1 (CH_3), 19.7 (CH_3), 24.4 (CH_2), 25.4 (CH_2), 27.0 (CH), 29.0 (CH_2), 29.2 (CH_2), 31.3 (CH_2), 34.4 (CH), 35.8 (C), 35.9 (CH_2), 38.2 (CH_2), 38.6 (CH_2), 39.0 (CH), 44.7 (CH_2), 46.7 (CH), 49.4 (C), 52.8 (CH), 53.4 (CH), 60.9 (CH), 80.2 (CH), 127.4 (CH), 139.2 (C), 211.5 (C); HREIMS M^+ *m/z* obsd 426.3491, $C_{29}H_{46}O_2$ required 426.3498.

Ozonolysis of Olefin 4. Formation of Triketone 5 and Diketone 6. O_3 was passed through a stirred solution of olefin **4** (4 mg) in CH_2Cl_2 and MeOH (1:1, 2 mL) at −78 °C until the color of the solution turned pale blue. Following removal of excess O_3 in the solution by passage of O_2, dimethyl sulfide (0.1 mL) was added and the temperature raised to room temperature. The mixture was concentrated under reduced pressure. The residue was chromatographed on a silica gel column (hexane–EtOAc = 2:3 as an eluent) to give triketone **5** (0.9 mg) and diketone **6** (1.4 mg).

Triketone **5**: colorless crystals (recrystallized from hexane–EtOAc); $[\alpha]_D$ +156.4° (*c* 0.13, $CHCl_3$); ^1H NMR (400 MHz) δ 0.98 (3H, s), 1.10 (3H, s), 2.27 (3H, s), 2.58 (1H, t, J = 13.3 Hz), 3.32 (1H, t, J = 9.4 Hz).

Diketone **6**: colorless cubics (recrystallized from hexane–EtOAc); mp 157–160 °C; $[\alpha]_D$ +47.6° (*c* 0.08, $CHCl_3$); ^1H NMR (400 MHz) δ 0.74 (3H, s), 1.02 (3H, s), 2.21 (3H, s), 3.45 (1H, dd, J = 4.7, 10.9 Hz).

***p*-Nitrobenzoate 7.** O_3 was passed through a stirred solution of olefin **4** (44.2 mg, 0.10 mmol) in CH_2Cl_2 and MeOH (1:1, 8 mL) at −78 °C until the color of the solution turmed pale blue. After removal of excess O_3 in the solution by passage of O_2, $NaBH_4$ (18 mg, 0.48 mmol) was added at −78 °C and the temperature raised to room temperature. The mixture was diluted with diethyl ether (30 mL), washed with H_2O (three times) and then saturated NaCl solution, dried over anhyd Na_2SO_4, and concentrated under reduced pressure (prolonged concentrating under reduced pressure cause considerable loss of product). The residue was dissolved in $CHCl_3$ (1.2 mL), and to this solution were added Et_3N (0.4 mL), *p*-nitrobenzoyl chloride (120 mg, 0.65 mmol), and 4-(dimethylamino)pyridine (6 mg). After being stirred for 20 h at room temperature, *p*-nitrobenzoyl chloride (170 mg) was added. Stirring was

conducted for 28 h at room temperature, the mixture was diluted with ether, washed successively with H_2O, saturated $CuSO_4$ solution, H_2O, saturated $NaHCO_3$ solution, H_2O, and saturated NaCl solution, dried over anhyd Na_2SO_4, and cencentrated under reduced pressure. The residue was chromatographed on a silica gel column (pentane–diethyl ether = 25:1 as an eluent) to give crude *p*-nitrobenzoate (7.3 mg), which was purified by normal phase HPLC (silica gel, hexane–EtOAc = 25:1, UV 254 nm) to give *p*-nitrobenzoate **7** (4.0 mg): colorless oil; $[\alpha]_D$ −20.3° (*c* 0.07, $CHCl_3$); ^1H NMR (400 MHz) δ 0.18 (1H, m), 0.24 (2H, m), 0.50 (1H, m), 0.94 (1H, m), 1.02 (3H, d, J = 6.9 Hz), 1.04 (3H, d, J = 6.0 Hz), 1.85 (2H, br q, J = 6.9 Hz), 4.47 (1H, td, J = 7.1, 11.6 Hz), 4.48 (1H, td, J = 6.9, 11.6 Hz), 8.20 (2H, d, J = 8.9 Hz), 8.28 (2H, d, J = 8.9 Hz).

X-ray Crystallography. All crystals (colorless needles) of aragusterol B (**2**) obtained by recrystallization of the pure crystals in several solvent systems were colorless needles and inadequate for measurement of X-ray crystallographic analysis. Good crystals (colorless cubics) were unexpectedly obtained by the slow evaporation of half the concentrated solution of one of the fractions in the flash chromatography (eluted with hexane–EtOAc = 2:1) of combined fraction 4 (see Extraction and Isolation): monoclinic, *C*2, a = 34.532(9) Å, b = 8.181(2) Å, c = 22.785(5) Å, V = 5547(2) Å^3, Z = 8, D_{calcd} = 1.06 g cm^{-3}, crystal size = 0.60 × 0.40 × 0.20 mm^3. Diffraction data were obtained using a Mac Science M × 18 diffractometer at room temperature with Cu Kα radiation. The structure was solved by direct methods (SHELXS 86) and refined by full-matrix least-squares techniques.

$NaBH_4$ Reduction of Aragusterol D (3). To a solution of aragusterol D (**3**, 50 mg, 0.11 mmol) in MeOH (4 mL) were added $CeCl_3$ (380 mg, 1.0 mmol) and $NaBH_4$ (125 mg, 3.4 mmol) at 0 °C. After being stirred for 30 min at 0 °C, saturated NH_4Cl (0.15 mL) and acetone (0.3 mL) were added. The mixture was diluted with ether, washed with H_2O and saturated NaCl solution, dried over anhyd $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, hexane–EtOAc = 1:1 as an eluent) to give crystalline **9** and **8** in this order. Recrystallization of each compound from hexane–EtOAc gave pure **8** (11.7 mg) and **9** (4.8 mg), respectively.

8: colorless needles; mp 198–200 °C; HREIMS $M^+ - H_2O$ m/z obsd 426.3467, $C_{29}H_{46}O_2$ required 426.3498; ^1H NMR (400 MHz) δ 0.74 (3H, s), 0.83 (3H, s), 0.95 (3H, d, J = 6.5 Hz), 1.03 (3H, d, J = 6.0 Hz), 3.48 (1H, dd, J = 4.8, 11.2 Hz), 3.58 (1H, m), 4.33 (1H, dd, J = 5.6, 8.1 Hz), 4.92 (1H, s), 5.10 (1H, s).

9: colorless cubics; mp 132–135 °C; HREIMS M^+ m/z obsd 444.3616, $C_{29}H_{48}O_3$ required 444.3603; ^1H NMR (400 MHz) δ 0.68 (3H, s), 0.82 (3H, s), 0.95 (3H, s), 1.03 (3H, d, J = 5.9 Hz), 3.52 (1H, dd, J = 4.8, 10.9 Hz), 3.59 (1H, m), 4.21 (1H, dd, J = 5.4, 7.9 Hz), 4.99 (1H, s), 5.31 (1H, s).

Epoxidation of Allylic Alcohol 8. To a solution of **8** (3.5 mg, 0.008 mmol) in CH_2Cl_2 (0.3 mL) were added Na_2HPO_4 (35 mg, 0.25 mmol) and *m*-CPBA (7.5 mg, 0.043 mmol). The mixture was stirred for 50 min at 0 °C. After the same amount of *m*-CPBA was again added, the mixture was stirred for 55 min at 0 °C and then for 15 min at room temperature. The mixture was diluted with ether, washed successively with H_2O, 3 N NaOH solution, H_2O, and saturated NaCl solution, dried over anhyd $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, CH_2Cl_2–EtOH = 25:1 as an eluent) to give **10** (2.3 mg): colorless amorphous substance; $[\alpha]_D$ +13.0° (*c* 0.20, $CHCl_3$); HREIMS M^+ m/z obsd 460.3558, $C_{29}H_{48}O_4$ required 460.3355. The ^1H NMR (400 MHz) of **10** coincided with that of alcohol **10** from aragusterol A (**1**).

$NaBH_4$ Reduction of Aragusterol A (1). To a solution of aragusterol A (**1**, 20 mg, 0.044 mmol) in MeOH (2 mL) was added $NaBH_4$ (13 mg, 0.34 mmol) at 0 °C, and the mixture was stirred for 30 min at 0 °C. Following the addition of acetone (15 μL), the mixture was diluted with ether, washed with H_2O and saturated NaCl solution, dried over anhyd $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, $CHCl_3$–EtOH = 25:1) to give alcohol **10** (5.8 mg): colorless amorphous substance; $[\alpha]_D$ +13.3° (*c* 0.15, $CHCl_3$); HREIMS M^+ m/z obsd 460.3581, $C_{29}H_{48}O_4$ required 460.3355; ^1H NMR (400 MHz) δ 0.70 (3H, s), 0.81 (3H, s), 0.95 (3H, s), 1.02 (3H, d, J = 6.0 Hz), 2.91 (1H, d, J = 3.9 Hz), 3.07 (1H, d, J = 3.9 Hz), 3.37 (1H, dd, J = 4.6, 11.0 Hz), 3.48 (1H, dd, J = 2.4, 10.5 Hz), 3.58 (1H, m).

Acknowledgment. We thank Prof. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam, for identification of the sponge, and Prof. J. Kobayashi, Faculty of Pharmaceutical Sciences, Hokkaido University, for providing NMR spectra of xestokerol C. We also thank Taisho Pharmaceutical Co., Ltd. for measurement of the biological activity.

Supplementary Material Available: Copies of the ^1H NMR spectra of **2**–**10** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.