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Aragusterols E-H, New 26,27-Cyclosterols from the Okinawan Marine Sponge of the Genus *Xestospongia* and Absolute Configurations of Xestokerols A and B

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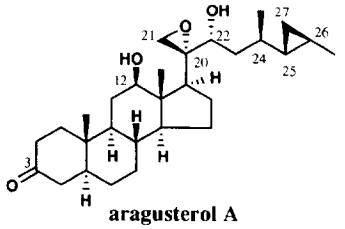
Abstract: New 26, 27-cyclosterols, aragusterols E-H, were isolated from the Okinawan marine sponge of the genus *Xestospongia*. Their structures were determined by spectroscopic measurement and chemical conversion. The absolute configurations of xestokerols A and B were determined by chemical conversion. © 1997 Elsevier Science Ltd.

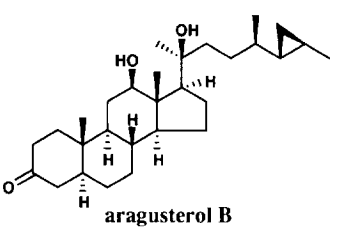
From marine sponges, various novel sterols, particularly in terms of unique side chain structures such as those with high degree of alkylation and unusual functionalization, can be obtained.[2] In the course of our studies on the chemical constituents of Okinawan marine invertebrates,[3] we reported isolation and structural determination of aragusterols A, B, C and D, potent antitumor steroids from the marine sponge of the genus *Xestospongia*.[4] These compounds were characterized by the 26,27-cyclo structure in side chain. Aragusterol related compounds, xestokerols A, B and C[5] and aragusteroketals A and C,[6] from Okinawan marine sponge (*Xestospongia* sp.) have also been repoted. The absolute configurations at the side chain of xestokerols A and B have so far not been determined. In searching for related steroids from the sponge, we isolated new members of 26,27-cyclosterol, aragusterols E-H (**1**-**4**) in addition to xestokerols A (**5**) and B (**6**). In the present study, structural elucidation of aragusterols E-H was carried out based on spectroscopic evidence and chemical conversion and the structures of xestokerols A and B were completely determined.

Specimens of the sponge (wet wt 9.3 kg), from the coral reef of Aragusuku Island, Okinawa, Japan, in May 1992, were immersed successively in MeOH and EtOAc. The MeOH and EtOAc extracts were combined and partitioned between EtOAc and H_2O. The EtOAc-soluble portion was partitioned between hexane and 80% MeOH to give hexane-soluble portion (55.0 g) and 80% MeOH-soluble portion (26.0 g). Repeated chromatographic separation of the hexane-soluble portion gave aragusterol E (**1**) (0.044 % yield based on the hexane-soluble portion). Repeated chromatographic separation of the 80% MeOH-soluble portion gave aragusterols F (**2**), G (**3**) and H (**4**) (0.31, 0.038 and 0.050 % yield, respectively, based on the 80% MeOH-soluble portion), together with xestokerols A (**5**) and B (**6**) (0.52 and 0.45 % yield, respectively, based on the 80% MeOH-soluble portion).

Aragusterol E (**1**) was found to have the molecular formula $C_{29}H_{46}O_3$ based on high resolution mass measurement. The IR spectrum of **1** showed absorptions at 3405 cm^{-1} due to the hydroxyl group and 1706



aragusterol A



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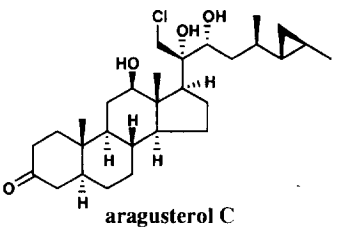
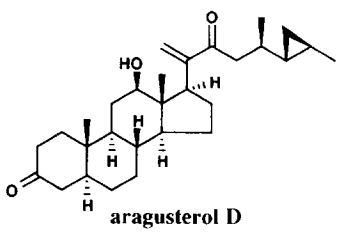
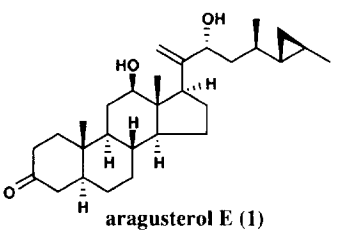


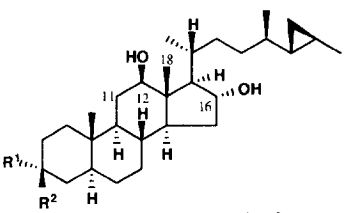
aragusterol C



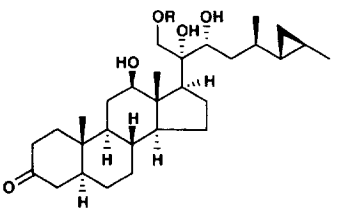
aragusterol D



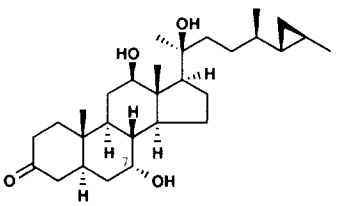
aragusterol E (1)



aragusterol F (2) : R^1, R^2=O
aragusterol G (3) : R^1=OH, R^2=H
aragusterol H (4) : R^1=H, R^2=OH



xestokerol A (5) : R=H
10 : R=Ac



xestokerol B (6)

cm^{-1} due to the carbonyl group. All twenty nine carbons appeared in the ^{13}C NMR spectrum. Examination of the DEPT spectrum disclosed the presence of four methyls, ten sp^3 methylenes, one sp^2 methylene, ten sp^3 methines, two sp^3 quaternary carbons and two sp^2 quaternary carbons. ^1H and ^{13}C NMR correlations evident from the HMQC spectrum. The ^1H and ^{13}C NMR spectra indicated a ketone [δ_C 211.8 (C)], two secondary hydroxyl groups [δ_H 3.48 (1H, dd, 4.6, 11.2 Hz), 4.33 (1H, dd, 2.7, 7.8 Hz), δ_C 78.5 (CH), 76.8 (CH)], a 1,2-disubstituted cyclopropane group [δ_H 0.14 (1H, m), 0.22 (1H, m), 0.22 (1H, m), 0.56 (1H, m)] and *exo*-methylene [δ_H 4.94 (1H, s), 5.11 (1H, s), δ_C 113.7 (CH_2), 151,0 (C)]. The NMR spectra of **1** were closely related to those of aragusterol A[4a] except for signals of the *exo*-methylene group instead of 20, 21-epoxide in aragusterol A, suggesting the structure of aragusterol E to be **1**. The stereochemistry of **1** was confirmed by chemical conversion of aragusterol E to aragusterol A. The epoxidation of aragusterol E (**1**) with *m*CPBA in CH_2Cl_2 at 0°C afforded aragusterol A, mp 158-161 °C, $[\alpha]_D$ +38.0° ($CHCl_3$), as a sole product. Physical data for aragusterol A thus obtained were in agreement with those of corresponding natural aragusterol A in all respects.[4a] The structure of aragusterol E is thus shown to be **1**.

Aragusterol F (**2**) was shown to have the molecular formula $C_{29}H_{48}O_3$ based on high resolution mass measurement. The IR spectrum of **2** showed absorptions at 3448 cm^{-1} due to the hydroxyl group and 1708 cm^{-1} due to the carbonyl group. All twenty nine carbons appeared in the ^{13}C NMR spectrum; the DEPT spectrum indicated the presence of five methyls, ten sp^3 methylenes, eleven sp^3 methines, two sp^3 quaternary carbons and one sp^2 quaternary carbon. ^1H and ^{13}C NMR correlations were evident from the HMQC spectrum. ^1H and ^{13}C NMR spectra showed a ketone [δ_C 211.5 (C)], two secondary hydroxyl groups [δ_H 3.53 (1H, dd, 4.4, 10.8 Hz), 4.32 (1H, t, 7.0 Hz), δ_C 79.4 (CH), 74.0 (CH)] and a 1,2-disubstituted

Table 1 NMR data for aragusterols E and F

	aragusterol E (1)			aragusterol F (2)		
	^{13}C NMR[a]		^{1}H NMR[b]	^{13}C NMR[a]		^{1}H NMR[b]
1	38.5	(CH_2)	1.99 (1H, m)	38.3	(CH_2)	1.98 (1H, ddd, 2.1, 6.1, 13.4)
2	38.1	(CH_2)	2.35 (1H, m)	38.0	(CH_2)	2.32 (1H, m)
						2.36 (1H, dt, 6.5, 15.4)
3	211.8	(C)		211.5	(C)	
4	44.6	(CH_2)	2.09 (1H, ddd, 1.8, 3.5, 14.3)	44.5	(CH_2)	2.08 (1H, m)
			2.26 (1H, t, 14.3)			2.23 (1H, t, 14.3)
5	46.6	(CH)	1.50 (1H, m)	46.5	(CH)	1.50 (1H, m)
6	28.8	(CH_2)		28.8	(CH_2)	
7	31.3	(CH_2)		31.1	(CH_2)	
8	35.0	(CH)		33.6	(CH)	
9	52.9	(CH)		52.6	(CH)	0.85 (1H, m)
10	35.6	(C)		35.6	(C)	
11	30.1	(CH_2)		31.2	(CH_2)	
12	78.5	(CH)	3.48 (1H, dd, 4.6, 11.2)	79.4	(CH)	3.53 (1H, dd, 4.4, 10.8)
13	49.1	(C)		49.2	(C)	
14	54.9	(CH)	1.15 (1H, m)	51.1	(CH)	1.15 (1H, m)
15	24.1	(CH_2)		36.1	(CH_2)	
16	32.0	(CH_2)	1.61 (1H, m)	74.0	(CH)	4.32 (1H, t, 7.0)
			1.87 (1H, m)			
17	47.9	(CH)		67.7	(CH)	
18	8.3	(CH_3)	0.76 (3H, s)	9.1	(CH_3)	0.72 (3H, s)
19	11.4	(CH_3)	1.02 (3H, s)	11.4	(CH_3)	0.99 (3H, s)
20	151.0	(C)		32.4	(CH)	1.83 (1H, m)
21	113.7	(CH_2)	4.94 (1H, s)	22.5	(CH_3)	1.07 (3H, d, 6.9)
			5.11 (1H, s)			
22	76.8	(CH)	4.33 (1H, dd, 2.7, 7.8)	32.7	(CH_2)	
23	44.9	(CH_2)		36.2	(CH_2)	
24	34.8	(CH)		38.7	(CH)	0.63 (1H, m)
25	27.3	(CH)	0.22 (1H, m)	27.3	(CH)	0.15 (1H, m)
26	12.9	(CH)	0.56 (1H, m)	12.8	(CH)	0.44 (1H, m)
27	11.7	(CH_2)	0.14 (1H, m)	11.5	(CH_2)	0.08 (1H, m)
			0.22 (1H, m)			0.15 (1H, m)
28	20.1	(CH_3)	0.94 (3H, d, 6.6)	20.0	(CH_3)	0.89 (3H, d, 6.7)
29	19.0	(CH_3)	1.02 (3H, d, 4.9)	19.1	(CH_3)	0.99 (3H, d, 5.3)

[a] ^{13}C NMR spectra were recorded at 100 MHz in $CDCl_3$. Carbon multiplicities were determined by DEPT experiments.
[b] ^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$. Proton and carbon assignments were made based on the results of HMQC and HMBC experiments.

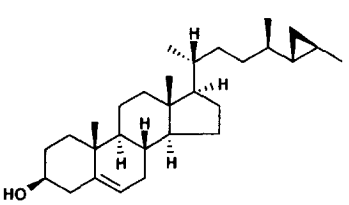
cyclopropane group [δ_H 0.08 (1H, m), 0.15 (1H, m), 0.15 (1H, m), 0.44 (1H, m)]. The presence of two secondary hydroxyl groups was confirmed by PCC oxidation of aragusterol F. Treatment of **2** with PCC in CH_2Cl_2 at room temperature gave triketone **7**, $[\alpha]_D$ -19.7° ($CHCl_3$), [δ_C 210.4, (C), 211.5 (C), 215.6 (C)]. The hydroxyl group was attached to the C-16 position as indicated by the HMBC spectrum, suggesting the structure of aragusterol F to be **2**. The β configuration of the hydroxyl group at C-12 was determined based on the coupling constants between H_2-11 and H-12 (J = 4.4, 10.8 Hz). The α configuration of the hydroxyl group at C-16 was determined from the NOESY correlation between H-16 and Me-18. The stereochemistry of the side chain including absolute configuration was determined by the following chemical conversions. Petrosterol,[7] whose absolute configuration was determined by X-ray previously, was converted to ketone **8**, $[\alpha]_D$ +40.4° ($CHCl_3$), by hydrogenation of the olefin and oxidation of the hydroxyl group. Aragusterol F (**2**) was converted to ketone **8**, $[\alpha]_D$ +40.0° ($CHCl_3$), via diketone **9** as follows: i) ketalization of ketone, ii) PCC oxidation of two hydroxyl groups to give diketone **9**, iii) formation of ditosylhydrazone and then $NaBH_4$ reduction[8] to give alcohol,[9] iv) PCC oxidation of the hydroxyl group. Physical data for ketone **8** from petrosterol were in agreement with those of corresponding ketone **8** from aragusterol F. The structure of aragusterol F including the absolute configuration is thus shown to be **2**.

Table 2 NMR data for aragusterols G and H

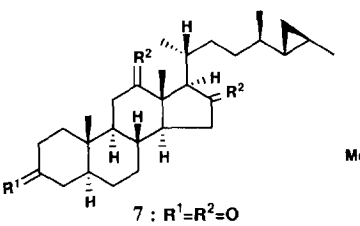
	aragusterol G (**3**)			aragusterol H (**4**)		
	^{13}C NMR[a]		^{1}H NMR[b]	^{13}C NMR[a]		^{1}H NMR[b]
1	32.1	(CH_2)		36.8	(CH_2)	
2	28.9	(CH_2)		31.4	(CH_2)	
3	66.4	(CH)	4.00 (1H, m)	71.2	(CH)	3.59 (1H, tt, 4.7, 11.0)
4	35.8	(CH_2)		38.0	(CH_2)	
5	39.0	(CH)		44.8	(CH)	
6	28.4	(CH_2)		28.5	(CH_2)	
7	31.5	(CH_2)		31.5	(CH_2)	
8	33.6	(CH)		33.7	(CH)	
9	53.1	(CH)		53.1	(CH)	
10	36.0	(C)		35.4	(C)	
11	30.7	(CH_2)		31.1	(CH_2)	
12	79.9	(CH)	3.53 (1H, dd, 4.6, 10.9)	79.9	(CH)	3.51 (1H, dd, 4.7, 10.9)
13	49.2	(C)		49.3	(C)	
14	51.4	(CH)		51.3	(CH)	
15	36.1	(CH_2)		36.2	(CH_2)	
16	74.1	(CH)	4.32 (1H, t, 6.8)	74.1	(CH)	4.32 (1H, t, 6.9)
17	67.8	(CH)		67.8	(CH)	
18	9.1	(CH_3)	0.71 (3H, s)	9.1	(CH_3)	0.71 (3H, s)
19	11.1	(CH_3)	0.78 (3H, s)	12.2	(CH_3)	0.80 (3H, s)
20	32.3	(CH)	1.84 (1H, m)	32.3	(CH)	1.82 (1H, m)
21	22.6	(CH_3)	1.09 (3H, d, 6.9)	22.6	(CH_3)	1.08 (3H, d, 6.9)
22	32.7	(CH_2)		32.7	(CH_2)	
23	36.2	(CH_2)		36.2	(CH_2)	
24	38.7	(CH)	0.65 (1H, m)	38.7	(CH)	0.67 (1H, m)
25	27.3	(CH)	0.15 (1H, m)	27.3	(CH)	0.15 (1H, m)
26	12.8	(CH)	0.46 (1H, m)	12.8	(CH)	0.46 (1H, m)
27	11.5	(CH_2)	0.09 (1H, m)	11.6	(CH_2)	0.08 (1H, m)
			0.15 (1H, m)			0.15 (1H, m)
28	20.0	(CH_3)	0.91 (3H, d, 6.7)	20.0	(CH_3)	0.91 (3H, d, 6.7)
29	19.1	(CH_3)	1.00 (3H, d, 6.0)	19.1	(CH_3)	1.00 (3H, d, 6.0)

[a] ^{13}C NMR spectra were recorded at 100 MHz in $CDCl_3$. Carbon multiplicities were determined by DEPT experiments.
[b] ^{1}H NMR spectra were recorded at 400 MHz in $CDCl_3$. Proton and carbon assignments were made based on the results of HMQC and HMBC experiments.

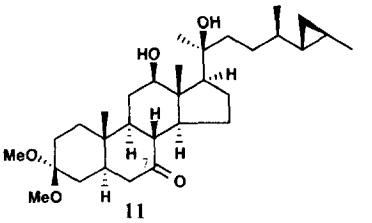
Aragusterols G (**3**) had the molecular formula $C_{29}H_{50}O_3$ based on the high resolution mass measurement. The IR spectrum of **3** showed absorptions at 3342 cm^{-1} due to the hydroxyl group. All twenty nine carbons appeared in the ^{13}C NMR spectrum and the DEPT spectrum indicated five methyls, ten sp^3 methylenes, twelve sp^3 methines, two sp^3 quaternary carbons to be present. ^{1}H and ^{13}C NMR correlations were demonstrated by the HMQC spectrum. The ^{1}H and ^{13}C NMR spectra indicated three secondary hydroxyl groups [δ_H 3.53 (1H, dd, 4.6, 10.9 Hz), 4.00 (1H, m), 4.32 (1H, t, 6.8 Hz), δ_C 79.9 (CH), 66.4 (CH), 74.1 (CH)] and a 1,2-disubstituted cyclopropane group [δ_H 0.09 (1H, m), 0.15 (1H, m), 0.15 (1H, m), 0.46 (1H, m)]. The NMR spectra of **3** were closely related to those of aragusterol F except for signals of the hydroxyl group instead of the ketone at C-3 in aragusterol F. The structure of aragusterol G would thus appear to be **3**. The α configuration of the hydroxyl group at C-3 was determined based on the half-width of the signal of the proton[10] and chemical shift[11] [δ_H 4.00 (1H, m, $W_{1/2}$ 6.9 Hz)]. The β configuration of a hydroxyl group at C-12 was determined from the coupling constants (J = 4.6, 10.9 Hz). The α configuration of the hydroxyl group at C-16 was determined based on the NOESY correlation between H-16 and Me-18. The absolute configuration of side chain was determined by the following chemical conversions. PDC oxidation of aragusterol G (**3**) afforded triketone **7**, $[\alpha]_D$ -20.4° ($CHCl_3$). Physical data for triketone **7** from **3** were in agreement with those of corresponding synthesized triketone **7** from aragusterol F. The structure of aragusterol G including absolute configuration was thus concluded to be **3**.



petrosterol



7 : R^1=R^2=O
8 : R^1=O, R^2=H_2
9 : R^1=$(OMe)_2$, R^2=O



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Aragusterols H (**4**) had the molecular formula $C_{29}H_{50}O_3$ based on the high resolution mass measurement. The IR spectrum of **4** showed absorptions at 3307 cm^{-1} due to the hydroxyl group. All twenty nine carbons appeared in the ^{13}C NMR spectrum and the DEPT spectrum indicated five methyls, ten sp^3 methylenes, twelve sp^3 methines and two sp^3 quaternary carbons to be present. ^1H and ^{13}C NMR correlations were indicated by the HMQC spectrum. ^1H and ^{13}C NMR spectra indicated three secondary hydroxyl groups [δ_H 3.51 (1H, dd, 4.7, 10.9 Hz), 3.59 (1H, tt, 4.7, 11.0 Hz), 4.32 (1H, t, 6.9 Hz), δ_C 79.9 (CH), 71.2 (CH), 74.1 (CH)] and a 1,2-disubstituted cyclopropane group [δ_H 0.08 (1H, m), 0.15 (1H, m), 0.15 (1H, m), 0.46 (1H, m)]. The NMR spectra of **4** were closely related to those of aragusterol G, suggesting the structure of aragusterol H to be that shown as **4**. The β configuration of the hydroxyl groups at C-3 and C-12 were determined from the coupling constants. The α configuration of the hydroxyl group at C-16 was indicated by NOESY correlation between H-16 and Me-18. The absolute configuration of the side chain was determined by PDC oxidation of aragusterol H (**4**) to afford triketone **7**, $[\alpha]_D$ -20.0° ($CHCl_3$). Physical data for triketone **7** obtained from **4** were in agreement with those of corresponding triketone **7** synthesized from aragusterol F. The structure of aragusterol H including absolute configuration is thus shown to be **4**.

The absolute configurations at the side chain of xestokerols A (**5**) and B (**6**) were undetermined. The stereochemistry of the side chain in **5** was determined from the chemical conversion of aragusterol A to xestokerol A (**5**). Treatment of aragusterol A with AcONa in the presence of AcOH in hexamethylphosphoramide (HMPA) at room temperature gave monoacetate **10** followed by methanolysis of the acetate afforded xestokerol A, $[\alpha]_D$ +30.9° ($CHCl_3$). Physical data for xestokerol A obtained from aragusterol A was in agreement with those of the corresponding natural xestokerol A in all respects. The absolute configuration of xestokerol A is thus shown to be **5**.

The stereochemistry of the side chain of xestokerol B (**6**) was determined by removal of the hydroxyl group at C-7 in **6** to give aragusterol B. Xestokerol B (**6**) was converted to ketone **11** by ketalization of the ketone at C-3 and regioselective PDC oxidation of the hydroxyl group at C-7. Reduction of ketone **11** by the modified Wolff-Kishner procedure,[12] followed by hydrolysis of the ketal afforded aragusterol B, $[\alpha]_D$ +4.1° ($CHCl_3$). Physical data for aragusterol B thus produced were in agreement with those of the corresponding natural aragusterol B in all respects.[4c] The complete structure of xestokerol B is thus shown to be **6**.

Aragusterols E, F, G and H showed antiproliferative activity toward KB cells with IC_{50} 8.05, 4.58, 6.61 and 6.48 μg/mL, respectively.

Experimental

Melting points were measured on a Yazawa BY-2 micro melting point apparatus without correction. Optical rotation was measured with a JASCO DIP-360 automatic polarimeter. Infrared (IR) spectra were recorded

with a Perkin-Elmer FT-IR 1710 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian Gemini-300, Bruker AM-400 or Bruker AM-500. Chemical shifts were expressed on a δ (ppm) scale with tetramethylsilane (TMS) as the internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Electron impact mass spectra (EIMS), fast atom bombardment mass spectra (FABMS) and high resolution electron impact mass spectra (HREIMS) were obtained with a Hitachi M-80 or VG Auto Spec spectrometer. Elemental analysis was conducted using a Perkin-Elmer 240B.

Animal Material. The Marine sponge was collected on the coral reef of Aragusuku Island (Okinawa, Japan) in May 1992. The sponge identified as the genus *Xestospongia* by Prof. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam. The specimens are on deposit in his collection (registered number: ZMA Por. 7842).

Extraction and Isolation. Wet specimens (9.3 kg) were immersed successively in MeOH (25 L) and EtOAc (25 L x 2). The MeOH and EtOAc extracts were combined and partitioned between AcOEt (1 L x 5) and H_2O (1 L). The EtOAc-soluble portion (81.0 g) was partitioned between hexane (500 mL x 4) and 80% MeOH (1 L) to give the hexane-soluble portion (55.0 g) and 80% MeOH-soluble portion (26.0 g).

The hexane-soluble portion (55.0 g) was chromatographed on a silica gel column to give four fractions: fraction 1 (3.0 g) eluted with hexane-EtOAc (7:1), fraction 2 (30.0 g) eluted with hexane-EtOAc (2:1), fraction 3 (7.0 g) eluted with hexane-EtOAc (1:1) and fraction 4 (13.0 g) eluted with EtOAc. ^{1}H NMR analysis of the fractions showed fraction 4 to contain aragusterol A and C. Fraction 2 was subjected to repeated flash chromatography on silica gel with hexane-EtOAc (5:1), hexane-EtOAc (3:1), hexane-EtOAc (2:1) and Et_2O-hexane (3:1) and to repeated flash chromatography on ODS with acetone-H_2O (10:1) and MeOH-H_2O (10:1) to give crude aragusterol E. Further purification was carried out by gel-filtration on Sephadex LH-20 with $CHCl_3$-MeOH (1:1) to give aragusterol E (**1**) (24 mg) as colorless glassy solids.

The 80% MeOH-soluble portion (26.0 g) was chromatographed on a silica gel column with hexane-EtOAc (1:1) to give fraction 1 (1.9 g), fraction 2 (3.5 g), fraction 3 (9.1 g), fraction 4 (4.0 g) and fraction 5 (6.0 g). Fraction 3 was crystallized from hexane-EtOAc to give crude crystals (1.4 g) and mother liquids (7.7 g). The crude crystals were subjected to flash chromatography on silica gel with hexane-EtOAc (5:1) to give crude crystalline xestokerol B. The crude crystals were recrystallized from EtOAc to give colorless needles of xestokerol B (**6**) (135mg). The mother liquids underwent to repeated flash chromatography on silica gel with hexane-acetone (5:1), $CHCl_3$-MeOH (20:1) on ODS with MeOH-H_2O (5:1) and on silica gel with $CHCl_3$-MeOH (25:1) to give crude crystalline xestokerol A. The crude crystals were recrystallized from hexane-EtOAc to give colorless needles of xestokerol A (**5**) (117 mg). Fraction 2 was subjected to chromatography on silica gel with hexane-EtOAc (1:1) to give fraction 2-1 (1.8 g), fraction 2-2 (1.1 g) and fraction 2-3 (300 mg). Fraction 2-1 by repeated flash chromatography on silica gel with hexane-acetone (5:1), on ODS with MeOH-H_2O (5:1) and on silica gel with $CHCl_3$-acetone (10:1) gave crude crystalline aragusterol F. The crude crystals were recrystallized from hexane-EtOAc to give colorless plates of aragusterol F (**2**) (81 mg). Fraction 2-2 by repeated flash chromatography on ODS with MeOH-H_2O (1:1) and on silica gel with hexane-acetone (3:1) gave fraction 2-2-1 (160 mg), fraction 2-2-2 (650 mg) and fraction 2-2-3 (220 mg). Fraction 2-2-2 underwent to repeated flash chromatography on silica gel with $CHCl_3$-acetone (3:1) and on ODS with MeOH-H_2O (6:1) to give aragusterol G (**3**) (10 mg) as colorless glassy solids. Fraction 2-2-3 was subjected to repeated flash chromatography on silica gel with $CHCl_3$-MeOH (20:1), $CHCl_3$-acetone (5:1), on ODS with MeOH-H_2O (6:1) and on silica gel with hexane-EtOAc (1:1) to give aragusterol H (**4**) (13 mg) as colorless rods.

Aragusterol E (1): colorless glassy solids: $[\alpha]_D$ +12.1° (*c* 0.24, $CHCl_3$); IR ($CHCl_3$) 3405, 1706 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; EIMS m/z 442(M^+); HREIMS: Calcd for $C_{29}H_{46}O_3$ (M^+) 442.3447: Found: 442.3444.

Aragusterol F (2): colorless plates: mp 168-170 °C; $[\alpha]_D$ +20.3° (*c* 0.79, $CHCl_3$); IR (KBr) 3448, 1708 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 1; EIMS m/z 444(M^+); HREIMS: Calcd for $C_{29}H_{48}O_3$ (M^+) 444.3603: Found: 444.3607.

Aragusterol G (3): colorless glassy solids: $[\alpha]_D$ +1.6° (*c* 0.50, $CHCl_3$); IR (KBr) 3342 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 2; EIMS m/z: 446(M^+); HREIMS: Calcd for $C_{29}H_{50}O_3$ (M^+) 446.3760: Found: 446.3772.

Aragusterol H (4): as colorless rods: mp 189-190 °C; $[\alpha]_D$ -1.5° (*c* 0.13, $CHCl_3$); IR (KBr) 3307 cm^{-1}; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) see Table 2; EIMS m/z 446(M^+); HREIMS: Calcd for $C_{29}H_{48}O_3$ (M^+) 446.3760: Found: 446.3758.

Xestokerol A (5): colorless needles: mp 200-202 °C; $[\alpha]_D$ +30.9° (*c* 1.17, $CHCl_3$); $[\alpha]_D$ +30.3° (*c* 1.1, MeOH). (lit. colorless oil; $[\alpha]_D^{21}$ +30.0° (*c* 1.1, MeOH)).[5]

Xestokerol B (6): colorless needles: mp 246-247 °C; $[\alpha]_D$ -5.0° (*c* 0.40, MeOH). (lit. colorless amorphous powder: mp 218-221 °C; $[\alpha]_D^{21}$ -5.2° (*c* 1.1, MeOH)).[5]

Epoxidation of aragusterol E (1) to aragusterol A. To a solution of aragusterol E (**1**) (20 mg, 0.045 mmol) in CH_2Cl_2 (1.0 mL) were added Na_2HPO_4 (64 mg, 0.45 mmol) and *m*CPBA (20 mg, 0.12 mmol). The mixture was stirred for 6 h at 0 °C. Dimethyl sulfide (0.05 mL) was added and the mixture was stirred for 1 h at room temperature. The mixture was diluted with ether, washed successively with saturated $NaHCO_3$ solution, H_2O and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-EtOAc (3:2) to give aragusterol E (4.0 mg, 20% recovered) and aragusterol A (11.0 mg, 53 % yield), which was recrystallized from EtOAc-hexane to give colorless needles of aragusterol A: mp 158-161 °C; $[\alpha]_D$ +38.0° (*c* 0.11, $CHCl_3$). (lit. mp 157-158 °C; $[\alpha]_D$ +37.6° (*c* 1.06, $CHCl_3$)).[4a]

PCC oxidation of aragusterol F (2) to triketone 7. To a solution of aragusterol F (**2**) (18.0 mg, 0.041 mmol) in $CHCl_3$ (1.0 mL) were added powdered molecular sieves 4A (56 mg) and PCC (106 mg, 0.49 mmol) at room temperature. The mixture was stirred at room temperature for 1 h, diluted with $CHCl_3$ (10 mL) and filtered through silica gel. The filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-EtOAc (2:1) to give triketone **7** (8.5 mg, 48 % yield): colorless amorphous; $[\alpha]_D$ -19.7° (*c* 0.43, $CHCl_3$); EIMS m/z 440(M^+); HREIMS: Calcd for $C_{29}H_{44}O_3$ (M^+) 440.3290: Found: 440 3265; IR ($CHCl_3$) 1735, 1705 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (1H, m), 0.15 (2H, m), 0.46 (1H, m), 0.65 (1H, m), 0.91 (3H, d, *J* = 6.7 Hz), 0.96 (3H, d, *J* = 6.9 Hz), 1.00 (3H, d, *J* = 6.0 Hz), 1.13 (3H, s), 1.21 (3H, s), 2.71 (1H, t, *J* = 13.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.2, 11.6, 12.7, 13.4, 19.1, 19.1, 19.8, 27.3, 28.4, 31.0, 31.5, 32.6, 34.1, 35.2, 36.5, 37.6, 37.6, 37.7, 38.5, 38.8, 44.3, 46.0, 51.2, 56.3, 56.9, 60.8, 210.4, 211.5, 215.6.

Conversion of petrosterol to ketone 8. A mixture of petrosterol (50 mg, 0.121 mmol), 5% Pd-C (10 mg) and EtOH-AcOEt (1:1, 1.0 mL) was stirred under H_2 (1 atom) for 3 h. The reaction mixture was filtered through celite, the filtrate was concentrated under reduced pressure to give crude petrostanol[13] (50 mg). The crude product was used for subsequent reaction without further purification.

To a solution of the above crude petrostanol in 1,2-dichloroethane (1.0 mL) were added powdered molecular sieves 4A (32 mg) and PCC (32 mg, 0.15 mmol) at room temperature. The mixture was stirred at room temperature for 2 h, diluted with ether (25 mL) and filtered through silica gel. The filtrate was

concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-Et_2O (3:1) to give ketone **8** (40 mg, 80 % yield (2 steps)): colorless plate; mp 136-137 °C; $[\alpha]_D$ +40.4° (*c* 0.9, $CHCl_3$); EIMS m/z 412(M^+); HREIMS: Calcd for $C_{29}H_{48}O$ (M^+) 412.3705: Found: 412.3695; IR (KBr) 1734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (1H, m), 0.14 (2H, m), 0.45 (1H, m), 0.60 (1H, m), 0.68 (3H, s), 0.73 (1H, m), 0.89 (3H, d, *J* = 6.7 Hz), 0.91 (3H, d, *J* = 6.5 Hz), 1.01 (3H, d, *J* = 6.0 Hz), 1.01 (3H, s), 1.70 (1H, m), 1.84 (1H, m), 2.01 (2H, m), 2.08 (1H, m), 2.26 (1H, m), 2.36 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.9, 12.0, 12.5, 13.2, 19.1, 19.6, 20.3, 21.9, 24.6, 27.8, 28.7, 29.4, 32.1, 33.9, 34.3, 35.8, 36.1, 36.3, 38.6, 39.0, 39.1, 40.3, 43.0, 45.2, 47.1, 54.2, 56.6, 56.7, 212.6.

Conversion of aragusterol F (2) to diketone 9. To a solution of aragusterol F (**2**) (10 mg, 0.023 mmol) in 0.1% PPTS solution in MeOH (0.2 mL) was added trimethyl orthoformate (0.005 mL) at room temperature. The mixture was stirred at room temperature for 12 h. To the mixture was added pyridine (0.05 mL) and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-EtOAc (1:1) to give dimethylketal (10.5 mg).

To a solution of dimethylacetal (10.5 mg, 0.021 mmol) in 1,2-dichloroethane (0.2 mL) were added powdered molecular sieves 4A (25 mg) and PDC (25 mg, 0.064 mmol) at room temperature. The mixture was stirred at room temperature for 12 h, diluted with ether (10 mL) and filtered through silica gel. The filtrate was concentrated under reduced pressure to give diketone **9** (7.3 mg, 67 % yield (2 steps)):colorless plate: mp 134-135 °C; $[\alpha]_D$ -36.0° (*c* 0.2, $CHCl_3$); EIMS m/z 486(M^+); HREIMS: Calcd for $C_{31}H_{50}O_4$ (M^+) 486.3709: Found: 486.3695; IR (KBr) 1739, 1721 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (1H, m), 0.16 (2H, m), 0.46 (1H, m), 0.65 (1H, m), 0.91 (3H, d, *J* = 6.8 Hz), 0.92 (3H, s), 0.96 (3H, d, *J* = 6.9 Hz), 0.99 (3H, d, *J* = 6.0 Hz), 1.17 (3H, s), 2.29 (1H, dd, *J* = 13.0, 5.0 Hz), 2.33 (1H, dd, *J* = 18.1, 7.0 Hz), 2.47 (1H, d, *J* = 7.0 Hz), 2.61 (1H, t, *J* = 13.0 Hz), 3.13 (3H, s), 3.19 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.3, 11.6, 12.7, 13.4, 19.2, 19.2, 19.8, 27.3, 28.0, 28.1, 31.3, 31.5, 32.7, 34.2, 34.4, 35.2, 35.3, 36.7, 37.6, 38.5, 38.9, 42.2, 47.5, 47.6, 51.4, 56.7, 56.9, 60.8, 100.1, 212.3, 216.1.

Conversion of diketone 9 to ketone 8. To a solution of diketone **9** (5.0 mg, 0.0010 mmol) in MeOH (0.1 mL) was added *p*-toluenesulfonhydrazide (3.0 mg, 0.016 mmol) and stirred at room temperature for 4 h. at 50 °C for 2 h. To the mixture was added $NaBH_4$ (4.0 mg, 0.1 mmol) and refluxed for 6 h. The reaction mixture was cooled to room temperature, diluted with ether (5 mL) and washed successively with saturated H_2O and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The crude alcohol was used for subsequent reaction without purification.[9]

To the above crude alcohol in 1,2-dichloroethane (0.1 mL) were added powdered molecular sieves 4A (5 mg) and PCC (5 mg, 0.01 mmol) at room temperature. The mixture was stirred at room temperature for 3 h, diluted with ether (5 mL) and filtered through silica gel. The filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-Et_2O (3:1) to give ketone **8** (0.55 mg, 13 % yield (3 steps)): $[\alpha]_D$ +40.0° (*c* 0.03, $CHCl_3$).

PDC oxidation of aragusterol G (3) to triketone 7. To a solution of aragusterol G (**3**) (3.0 mg, 0.0067 mmol) in CH_2Cl_2 (0.1 mL) were added powdered molecular sieves 4A (10.1 mg) and PDC (10.1 mg, 0.027 mmol) at room temperature. The mixture was stirred at room temperature for 3 h, diluted with Et_2O (5 mL) and filtered through silica gel. The filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-EtOAc (2:1) to give triketone **7** (2.7 mg, 91 % yield):

colorless amorphous; $[\alpha]_D$ -20.4° (*c* 0.27, $CHCl_3$); HREIMS: Calcd for $C_{29}H_{44}O_3$ (M^+) 440.3290: Found: 440.3283.

PDC oxidation of aragusterol H (4) to triketone 7. To a solution of aragusterol H (**4**) (2.0 mg, 0.0045 mmol) in CH_2Cl_2 (0.1 mL) were added powdered molecular sieves 4A (6.8 mg) and PDC (6.8 mg, 0.018 mmol) at room temperature. The mixture was stirred at room temperature for 3 h. The mixture was diluted with Et_2O (5 mL) and filtered through silica gel. The filtrate was concentrated under reduced pressure and the residue was chromatographed on a silica gel column with hexane-EtOAc (2:1) to give triketone **7** (1.0 mg, 51 % yield): colorless amorphous; $[\alpha]_D$ -20.0° (*c* 0.10, $CHCl_3$); HREIMS: Calcd for $C_{29}H_{44}O_3$ (M^+) 440.3290: Found: 440.3270.

Conversion of aragusterol A to monoacetate 10. To a solution of aragusterol A (15 mg, 0.033 mmol) in HMPA (0.5 mL) were added AcONa (120 mg, 1.46 mmol) and AcOH (0.38 mL) at room temperature. The mixture was stirred at room temperature for 7 d, diluted with ether, washed successively with saturated H_2O and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-AcOEt (1:2) to give monoacetate **10** (14 mg, 83 % yield): colorless amorphous; $[\alpha]_D$ +28.0° (*c* 0.3, $CHCl_3$); EIMS m/z 500 (M^+-H_2O); HREIMS: Calcd for $C_{31}H_{48}O_5$ (M^+-H_2O) 500.3501: Found: 500.3521; IR ($CHCl_3$) 3420, 1713 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.16 (1H, m), 0.24 (3H, m), 0.51 (1H, m), 0.93 (6H, s), 1.01 (3H, d, *J* = 6.6 Hz), 1.02 (3H, s), 2.10 (3H, s), 3.41 (1H, dd, *J* = 4.4, 11.2 Hz), 3.58 (1H, d, *J* = 10.7 Hz), 4.21 (1H, d, *J* = 11.8 Hz), 4.49 (1H, d, *J* = 11.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 8.9, 11.4, 12.3, 12.5, 18.6, 19.2, 21.0, 22.5, 23.4, 27.9, 28.8, 29.3, 31.0, 33.8, 35.0, 35.6, 36.6, 38.1, 38.4, 44.5, 46.5, 49.0, 52.4, 54.0, 55.0, 65.7, 70.2, 76.1, 77.2, 171.5, 211.8.

Conversion of monoacetate 10 to xestokerol A (5). To a solution of monoacetate **10** (3.0 mg, 0.0059 mmol) in MeOH (0.3 mL) was added K_2CO_3 (3.0 mg, 0.022 mmol) followed by stirring at room temperature for 5 m. The reaction mixture was diluted ether and filtered though silica gel. The filtrate was concentrated under reduced pressure and the residue was chromatographed on a silica gel column with hexane-AcOEt (1:4) to give xestokerol A (1.8 mg, 65 % yield): $[\alpha]_D$ +30.9° (*c* 0.12, $CHCl_3$).

Conversion of xestokerol B (6) to ketone 11. To a solution of xestokerol B (**6**) (100 mg, 0.22 mmol) in 0.1% PPTS solution in MeOH (10 mL) was added trimethyl orthoformate (1.0 mL) at room temperature. The mixture was stirred at room temperature for 1 h. To the mixture was added pyridine (1.0 mL) and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-EtOAc (2:1) to give dimethylketal (70 mg, 64 % yield): colorless powder: mp 217-218 °C; $[\alpha]_D$ -16.3° (*c* 0.48, $CHCl_3$); EIMS m/z 475(M^+-OMe); HREIMS: Calcd for $C_{30}H_{51}O_4$ (M^+-OMe) 475.3787: Found: 475.3745; IR (KBr) 3383, 2951 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (1H, m), 0.17 (2H, m), 0.49 (1H, m), 0.66 (1H, m), 0.80 (3H, s), 0.83 (3H, s), 0.92 (3H, d, *J* = 6.7 Hz), 1.02 (3H, d, *J* = 6.0 Hz), 1.16 (3H, s), 3.13 (3H, s), 3.19 (3H, s), 3.35 (1H, dd, *J* = 4.7, 10.2 Hz), 3.85 (1H, s).

To a solution of dimethylketal (50 mg, 0.099 mmol) in CH_2Cl_2 (7.0 mL) were added powdered molecular sieves 4A (80 mg) and PDC (50 mg, 0.064 mmol) at room temperature. The mixture was stirred at room temperature for 30 m, and diluted with ether, washed successively with saturated $CuSO_4$ solution, H_2O and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-Et_2O (1:3) to give ketone **11** (26.3 mg, 53 % yield): mp 56-58 °C; $[\alpha]_D$ -0.27° (*c* 0.33, MeOH); EIMS m/z 437(M^+-OMe-$2H_2O$); HREIMS: Calcd for $C_{30}H_{45}O_2$ (M^+-OMe-$2H_2O$) 437.3420: Found: 437.3415; IR (KBr) 3285, 2952, 1709 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (1H,

m), 0.16 (2H, m), 0.50 (1H, m), 0.68 (1H, m), 0.82 (3H, s), 0.92 (3H, d, J = 6.7 Hz), 1.03 (3H, d, J = 5.9 Hz), 1.07 (3H, s), 1.18 (3H, S), 3.10 (3H, s), 3.19 (3H, s), 3.30 (1H, dd, J = 4.2, 11.0 Hz).

Conversion of ketone 11 to aragusterol B. To a solution of ketone **11** (89.7 mg, 0.178 mmol) in di(ethylene glycol) (6.0 mL) was added hydrazine monohydrate (40 mL) and the mixture was heated at 130°C for 2 h. After adding KOH (320 mg, 5.7 mmol), the temperature was raised to 220°C by distilling out the low-boiling material. After 13 h, the mixture was diluted with ether, washed successively with saturated H_2O and saturated NaCl solution, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed on a silica gel column with hexane-Et_2O (1:2) to give ketal (34.5 mg, 40 % yield): mp 78-80 °C; $[\alpha]_D$ +0.15° (*c* 0.13, $CHCl_3$); EIMS m/z 458(M^+-MeOH); HREIMS: Calcd for $C_{30}H_{50}O_3$ (M^+-MeOH) 458.3760: Found: 458.3734; IR (KBr) 3264, 2951 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (1H, m), 0.17 (2H, m), 0.49 (1H, m), 0.66 (1H, m), 0.80 (3H, s), 0.83 (3H, s), 0.92 (3H, d, J = 6.7 Hz), 1.03 (3H, d, J = 5.9 Hz), 1.15 (3H, s), 3.14 (3H, s), 3.19 (3H, s), 3.35 (1H, dd, J = 4.4, 11.0 Hz).

A solution of the ketal (13 mg, 0.027 mmol) in 80% AcOH was stirred at room temperature for 23 h and then concentrated under pressure. The residue was chromatographed on PTLC with Et_2O to give aragusterol B (6.0 mg, 51 % yield): colorless needles: mp 194-195 °C; $[\alpha]_D$ +4.1° (*c* 0.39, $CHCl_3$). (lit. mp 194-195 °C; $[\alpha]_D$ +4.0° (*c* 1.56, $CHCl_3$)).[4c]

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References and Notes

1. Present address: *School of Life Science, Tokyo University of Pharmacy and Life Science*
2. Faulkner, D.J. *Nat. Prod. Rep.*, **1996**, *13*, 75-125, and previous papers in this series.
3. Recent examples: a) Iguchi, K.; Kajiyama, K.; Miyaoka, H; Yamada, Y. *J. Org. Chem.*, **1996**, *61*, 5998-6000. b) Miyaoka, H.; Taira, S.; Mitome, H.; Iguchi, K.; Matsumoto, K.; Yokoo, C.; Yamada, Y. *Chem. Lett.*, **1996**, 239-240.
4. a) Iguchi, K.; Fujita, M.; Nagaoka, H.; Mitome, H.; Yamada, Y. *Tetrahedron Lett.*, **1993**, *34*, 6277-6280. b) Shimura, H.; Iguchi, K.; Yamada, Y.; Nakaike, S.; Yamagishi, T.; Matsumoto, K.; Yokoo, C. *Experientia*, **1994**, *50*, 134-136. c) Iguchi, K.; Shimura, H.; Taira, S.; Yokoo, C.; Matsumoto, K.; Yamada, Y. *J. Org. Chem.*, **1994**, *59*, 7499-7502.
5. Kobayashi, J.; Ishida, K.; Naitoh, K.; Shigemori, H.; Mikami, Y.; Sasaki, T. *J. Nat. Prod.*, **1993**, *56*, 1350-1355. Xestokerol C seems to be identical with aragusterol D.
6. Kobayashi, M.; Chen, Y.-J.; Higuchi, K.; Aoki, S.; Kitagawa, I. *Chem. Pharm. Bull.*, **1996**, *44*, 1840-1842.
7. a) Sica, D.; Zollo, F. *Tetrahedron Lett.*, **1978**, 837-838. b) Mattia, C.A.; Mazzarella, L.; Puliti, R.; Sica, D.; Zollo, F. *Tetrahedron Lett.*, **1978**, 3953-3954. c) Ravi, B.N.; Kokke, W.C.M.C.; Delseth, C.; Djerassi, C. *Tetrahedron Lett.*, **1978**, 4379-4380.
8. Caglioti, L. *Tetrahedron*, **1966**, *22*, 487-493.
9. Diketone **9** was treated with *p*-toluenesulfonhydrazide, followed by $NaBH_4$ to give 3-hydroxysterol, which was resulted by deprotection of the ketal at C-3 and reduction of the ketone.
10. Kawazoe, Y.; Sato, Y.; Okamoto, T.; Tsuda, K. *Chem. Pharm. Bull.*, **1963**, *11*, 328-332.
11. Kirk, D.N.; Toms, H.C.; Douglas, C.; White, K.A.; Smith, K.E.; Latif, S.; Hubbard, R.W.P. *J. Chem. Soc., Perkin Trans. 2*, **1990**, 1567-1594.
12. Mukawa, F.; Nagata, W. *Bull. Chem. Soc. Jpn.*, **1972**, *45*, 574-577.
13. Cho, J.-H.; Djerassi, C. *J. Chem. Soc., Perkin Trans. 1*, **1987**, 1307-1318.

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