New 24-Isopropylcholesterol and 24-Isopropenylcholesterol Sulfate from the Marine Sponge *Epipolasis* Species

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Two novel steroids, polasterol A (**1**) and polasterol B sulfate (**2**), along with two known compounds, 22-(*E*),24-isopropylcholesta-5,22-dien-3β-ol (**3**) and halistanol sulfate (**4**), have been isolated from the Japanese marine sponge *Epipolasis* sp. The structures of **1** and **2** were determined as 3β-hydroxy-24-isopropylcholesta-5,22(*E*)-dien-7-one and 24ξ-isopropenylcholesta-22(*Z*),28(29)-diene-2β,3α,6α-triyl trisodium sulfate on the basis of spectroscopic investigations and a chemical conversion.

Marine sponges continue to be a rich source of unique steroids with unusual side chains and nuclei.[1,2] Steroids with an additional isopropyl group at C-24 are relatively rare and were first reported in 1979 from sponges, a *Pseudaxinyssa* sp.[3] and a *Verongia* cauliformis.[4] Recently, Ishibashi *et al*. reported topsentinols A−J from the marine sponge *Topsentia* sp.[5] As part of an ongoing investigation of metabolites isolated from marine organisms, it was found that extracts of the sponge *Epipolasis* sp. contained two new steroids with an additional isopropyl or isopropenyl group at C-24, polasterol A (**1**) and polasterol B sulfate (**2**), along with two known compounds, 22(*E*),24-isopropylcholesta-5,22-dien-3β-ol (**3**)[3,4,] and halistanol sulfate (**4**).[6] We now describe the isolation and structure elucidation of polasterol A (**1**) and polasterol B sulfate (**2**).

The sponge *Epipolasis* sp. was extracted with MeOH and MeOH/CH_2Cl_2 (3:1). The combined extract was divided into EtOAc-, *n*-BuOH-, and H_2O-soluble portions. The EtOAc- and *n*-BuOH-soluble portions were chromatographed on Sephadex LH-20 and Si gel columns, respectively. Final purification by reversed-phase HPLC afforded **1** and **3** from the EtOAc-soluble portion and **2** and **4** from the *n*-BuOH-soluble portion.

Polasterol A (**1**) was obtained as an amorphous powder. The molecular formula of **1** was established as $C_{30}H_{48}O_2$ on the basis of HREIMS and corresponds to seven degrees of unsaturation. The IR spectrum contained bands at 3395 cm^{-1} (hydroxyl group) and 1680 cm^{-1} (α,β-unsaturated ketone), and the UV absorption at 238 nm (log ϵ 3.87) confirmed the presence of the α,β-unsaturated ketone. The ^{1}H NMR spectrum contained a signal at δ 5.69 (d, J = 2.2 Hz), which is appropriate for an α-proton on an α,β-unsaturated ketone, and two methyl singlets at δ 0.70 and 1.20; five methyl doublets at δ 0.77 (J = 6.9 Hz), 0.78 (J = 6.9 Hz), 0.84 (J = 6.9 Hz), 0.85 (J = 6.9 Hz), and 1.04 (J = 6.9 Hz); and one oxygenated methine proton at δ 3.68 (dddd, J = 11.3, 11.3, 4.4, 4.4 Hz). A DEPT experiment indicated seven methyl, seven methylene, 12 methine, and four quaternary carbons. The ^{13}C NMR spectrum indicated the presence of a disubstituted double bond at δ 139.1 (d) and 127.2 (d), a trisubstituted double bond at δ 165.1 (s) and 126.1 (d), a carbonyl group at δ at 202.2 (s), and a carbon bearing a hydroxy group at δ 70.5 (d). This indicated that **1** had an additional isopropyl group on a usual steroidal structure. An HMQC experiment established the C−H connectivities, and HMBC and COSY experiments were used to determine the C−C connectivities. The COSY spectrum clearly showed cross-peaks (H-24/H-25, H-24/H-28, H-25/H-26, H-25/H-27, H-28/H-29, H-28/H-30) that indicated the presence of a 24-isopropyl group (Experimental Section). The HMBC spectrum showed that the H-3 methine proton was coupled to C-1 and C-5, the H-4 methylene protons to C-6, and the H-9 methine proton to the α,β-unsaturated carbonyl C-7 (Experimental Section). Thus, the planar structure of **1** was determined.

The relative stereochemistry of **1** was established by NOESY experiments and coupling constants. The NOE between H-18/H-8, -15, and -20 and between H-19/H-2β, -4β, -8, and -11β suggested a common sterol nucleus. The β-OH group at C-3 could be assigned from the observed coupling constants for H-3 (J = 11.3, 11.3, 4.4, 4.4 Hz). The 17β-orientation of the side chain was disclosed by NOESY cross-peaks H-12/H-21 and H-18/H-20. The geometry of the Δ^{22}-double bond was deduced to be *E* from the coupling constant (J = 15.4 Hz). The CD spectrum [$\Delta\epsilon$ −5.64 (237 nm)] of **1** showed a negative Cotton effect, ensuring the absolute stereochemistry of a sterol nucleus.[7] Finally, polasterol A (**1**) can be designated as 3β-hydroxy-24-isopropylcholesta-5,22(*E*)-dien-7-one.

Polasterol B sulfate (**2**) was isolated as a white powder, and the molecular formula $C_{30}H_{47}Na_3O_{12}S_3$ was determined by HREIMS (m/z 404.3434; M − 3NaHSO$_4$) and negative FABMS (m/z 741 [M − Na]$^-$ and 455 [M − 3SO$_3$Na]$^-$). The IR spectrum suggested that **2** possessed sulfate groups at 1220 and 1250 cm^{-1}. The ^{13}C NMR spectrum indicated the presence of two double bonds at δ 111.4 (t), 129.7 (d), 138.6 (d), and 149.4 (s) and three oxygenated carbons at δ 75.5 (d) × 2 and 78.7 (d). The ^{1}H NMR spectrum contained three methyl singlets at δ 0.73, 1.04, and 1.63; three methyl doublets at δ 0.83 (J = 6.9 Hz), 0.86 (J = 6.9 Hz), and 0.90 (J = 6.6 Hz); three oxymethine protons at δ 4.18 (ddd, J = 11.0, 11.0, 4.4 Hz), 4.74 (ddd, J = 2.5, 2.5, 2.5 Hz), and 4.79 (br s); a terminal methylene proton at δ 4.66 (s) and 4.67 (s), and olefinic protons at δ 5.20 (2H, m). The steroidal feature of **2** was inferred from COSY, HMQC, and HMBC data (Experimental Section). Oxygenation at C-2, C-3, and C-6 in ring A was straightforward by interpretation of 2D NMR data. The H-2 methine resonance at δ 4.79 and the H-3 methine resonance at δ 4.74 showed HMQC correlations to the same methine carbon resonance at δ 75.5. Three sulfate groups could be accommodated on C-2, C-3, and C-6 as judged by the lowfield resonance of H-2, H-3, and H-6. The relative stereochemistry of **2** was deduced by NOESY experiments and coupling constants. All trans

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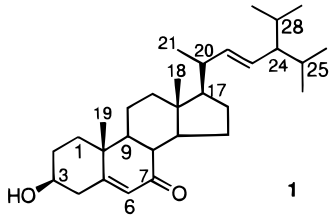
Table 1. ^{1}H NMR Data for **1**, **2**, and **5**

	1 (in $CDCl_3$)	**2** (in CD_3OD)	**5** (in C_5D_5N)
C no.	δ_H mult, *J* (Hz)	δ_H mult, *J* (Hz)	δ_H mult, *J* (Hz)
1	1.93 m	2.07 br d, 14.6	2.15 dd, 13.7, 1.9
	1.19 m	1.43 dd, 14.3, 3.3	2.00 dd, 13.7, 3.6
2	1.94 m	4.79 br s	4.57 br s
	1.60 m		
3	3.68 dddd, 11.3, 11.3, 4.4, 4.4	4.74 ddd, 2.5, 2.5, 2.5	4.60 br d, 2.5
4	2.51 ddd, 14.0, 4.4, 2.5	2.26 br d, 14.8	2.88 br d, 13.5
	2.40 ddd, 14.0, 11.3, 2.2	1.78 ddd, 14.8, 11.0, 2.7	2.40 ddd, 12.9, 12.9, 2.5
5		1.62 ddd, 13.5, 11.0, 2.7	2.32 m
6	5.69 d, 2.2	4.18 ddd, 11.0, 11.0, 4.4	3.90 ddd, 10.4, 10.4, 4.1
7		2.34 ddd, 12.1, 4.4, 4.4	2.34 m
		1.02 m	1.34 m
8	2.25 dd, 12.6, 11.0	1.52 m	1.62 m
9	1.50 m	0.75 m	0.93 m
11	1.57 m	1.54 m	1.68 m
		1.32 ddd, 13.1, 13.1, 3.5	1.40 m
12	2.01 ddd, 12.9, 3.6, 3.6	1.98 ddd, 12.5, 3.5, 3.5	1.95 ddd, 12.4, 3.3, 3.3
	1.13 m	1.16 m	1.12 m
14	1.33 m	1.10 m	1.08 m
15	2.38 m	1.58 m	1.64 m
	1.24 m	1.10 m	1.15 m
16	1.76 m	1.71 m	1.77 m
	1.27 m	1.22 m	1.34 m
17	1.13 m	1.18 m	1.17 m
18	0.70 s	0.73 s	0.79 s
19	1.20 s	1.04 s	1.47 s
20	2.07 m	2.51 m	2.64 m
21	1.04 d, 6.9	0.90 d, 6.6	1.05 d, 6.6
22	5.14 dd, 15.4, 8.8	5.20 m	5.34 dd, 10.7, 10.7
23	5.03 dd, 15.4, 9.9	5.20 m	5.27 dd, 10.7, 10.7
24	1.32 m	2.67 dd, 8.5, 8.5	2.83 dd, 9.3, 9.3
25	1.67 m	1.66 m	1.66 m
26	0.78 d, 6.9	0.83 d, 6.9	0.93 d, 6.6
27	0.85 d, 6.9	0.86 d, 6.9	0.86 d, 6.9
28	1.67 m		
29	0.84 d, 2.5	4.67 s	4.86 d, 2.5
		4.66 s	4.80 dd, 2.5, 1.6
30	0.77 d, 6.9	1.63 s	1.67 s

junctions from A to D rings were confirmed by NOESY cross-peaks (H-1α/H-5, H-1α/H-9, H-4*β*/H-19, H−5/H-7α, H-5/H-9, H-6*β*/H-19, H-7α/H-9, H-8/H-18, H-9/H-14, H-11*β*/H-18, H-15*β*/H-18, H-16*β*/H-18). These 2α-, 3*β*-, and 6*β*-sulfate groups on rings A and B could be assigned from the observed coupling patterns for H-2 at *δ* 4.79 (br s), H-3 at *δ* 4.74 (ddd, *J* = 2.5, 2.5, 2.5 Hz), and H-6 at *δ* 4.18 (ddd, *J* = 11.0, 11.0, 4.4 Hz), respectively. The 17*β*-orientation of the side chain was disclosed by NOESY cross-peaks H-12*β*/H-21 and H-18/H-20.

Upon treatment of **2** with dioxane and pyridine, **5** was obtained. The alcohol **5** was fully characterized by spectral data (COSY, HMQC, HMBC), and complete NMR assignments are reported in Tables 1 and 2. Although the coupling pattern for H-22 and H-23 in **2** could not be unambiguously interpreted, its *Z* geometry in **5** was established by the coupling constant (*J* = 10.7 Hz). Furthermore, the *Z* geometry of the Δ^{22}-double bond for **2** was implied by ^{13}C NMR signal *δ* 20.6 at C-21 (*δ* 20.5 in **5**; *δ* 21.4 in **1** [*E* geometry]).[6] Thus, the structure of polasterol B sulfate (**2**) was concluded to be 24*ξ*-isopropenylcholesta-22(*Z*),28(29)-diene-2*β*,3α,6α-triyl trisodium sulfate.



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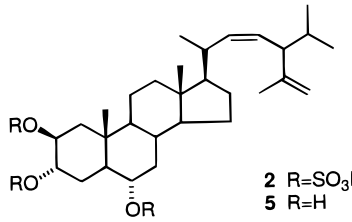

2 R=SO_3Na
5 R=H

Experimental Section

General Experimental Procedures. The following instruments were used: a JASCO FT/IR-5300 (IR), a JASCO DIP-360 polarimeter (optical rotation), a JASCO J-500 (CD), a JEOL JMS-HX-100 mass spectrometer (HRMS), and a Varian UNITY 600 NMR spectrometer (^{1}H and ^{13}C NMR).

Animal Material. The marine sponge *Epipolasis* sp. (1.3 kg, wet wt) was collected off the coast of Tokushima prefecture [(134° 30′E, 33° 35′N) by netting at a depth between 10 and 70 m] and was kept frozen (−20 °C) until used; it was identified by Professor P. R. Bergquist of Auckland University. The voucher sample (TS8103) of the organism under consideration is deposited in the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University. The sponge is a large curving lamellate, 2−2.5 cm thick, 10 cm high, with a broad attachment base; color in life, pale brown. Oscular and poral faces are differentiated. The deeper part of the skeleton is a confused mass of oxeas of two size categories, while the superficial skeleton is a palisade of vertical or obliquely disposed small oxeas. Spicules were 250−400 *μ*m long × 8−10 *μ*m thick and 600−750 *μ*m long × 12−18 *μ*m thick. The texture of the sponge is rock hard.

Table 2. ^{13}C NMR Data[a] for **1**, **2**, and **5**

C no.	**1** (in $CDCl_3$)	**2** (in CD_3OD)	**5** (in C_5D_5N)
1	36.3 (t)	39.2 (t)	41.3 (t)
2	31.2 (t)	75.5 (d)	71.6 (d)
3	70.5 (d)	75.5 (d)	70.9 (d)
4	41.8 (t)	25.1 (t)	26.9 (t)
5	165.1 (s)	45.3 (d)	47.1 (d)
6	126.1 (d)	78.7 (d)	68.8 (d)
7	202.2 (s)	40.0 (t)	43.0 (t)
8	45.4 (d)	35.1 (d)	34.3 (d)
9	49.9 (d)	55.9 (d)	55.4 (d)
10	38.3 (s)	37.6 (s)	37.3 (s)
11	21.2 (t)	21.8 (t)	21.3 (t)
12	38.6 (t)	41.0 (t)	40.3 (t)
13	43.0 (s)	43.7 (s)	42.8 (s)
14	50.0 (d)	57.6 (d)	56.7 (d)
15	26.4 (t)	25.1 (t)	24.6 (t)
16	29.2 (t)	29.3 (t)	28.6 (t)
17	54.7 (d)	57.8 (d)	56.8 (d)
18	12.2 (q)	12.8 (q)	12.6 (q)
19	17.3 (q)	15.3 (q)	16.1 (q)
20	40.4 (d)	35.7 (d)	35.0 (d)
21	21.4 (q)	20.6 (q)	20.5 (q)
22	139.1 (d)	138.6 (d)	138.0 (d)
23	127.2 (d)	129.7 (d)	128.8 (d)
24	56.1 (d)	54.9 (d)	53.6 (d)
25	28.54 (d)	31.3 (d)	30.4 (d)
26	19.2 (q)	21.2 (q)	20.9 (q)
27	21.7 (q)	21.4 (q)	21.1 (q)
28	28.50 (d)	149.4 (s)	148.5 (s)
29	21.6 (q)	111.4 (t)	111.3 (t)
30	19.0 (q)	19.3 (q)	19.4 (q)

[a] Multiplicity inferred from a DEPT experiment.

Extraction and Isolation of Metabolites. The frozen sample was exhaustively extracted with MeOH/CH_2Cl_2 (3:1) (2 L × 4) at room temperature for 1 day. The extract was concentrated, and the resulting residue was extracted with EtOAc (500 mL × 3).

The EtOAc-soluble portion (17.5 g) was repeatedly subjected to Sephadex LH-20 column chromatography (MeOH/CH_2Cl_2) and Si gel flash column chromatography (using increasing concentrations of MeOH in CH_2Cl_2 as eluent) to give **1** (0.00067% wet wt) and **3** (0.00633%). The *n*-BuOH-soluble portion (125.0 g) was repeatedly subjected to Si gel flash column chromatography (using increasing concentrations of 95% MeOH in CH_2Cl_2 as eluent), followed by reversed-phase HPLC (27−30% MeOH) to give **2** (0.00423% wet wt) and **4** (0.06202%).

Polasterol A (1): white amorphous powder; mp 122−125 °C; $[\alpha]^{21}_D$ −73.7° (*c* 0.43, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 238 (3.87) nm; CD $\Delta\epsilon$ −5.64 (237 nm) (*c* 3.1 × 10^{-5} M, MeOH); FT−IR (film) ν_{max} 3395,1680 cm^{-1}; COSY (H/H) 1/2, 2/3, 3/4, 4/6 (^4J), 8/9, 8/14, 9/11, 11/12, 14/15, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 24/25, 24/28, 25/26, 25/27, 28/29, 28/30; HMBC (H/C) 1/2, 1/3, 1/5, 1/19, 2/1, 2/3, 2/4, 2/10, 4/2, 4/3, 4/5, 4/6, 4/10, 6/4, 6/8, 6/10, 8/7, 8/9, 8/10, 8/14, 8/15, 9/1, 9/7, 9/8, 9/10, 9/11, 9/19, 11/8, 11/9, 11/12, 11/13, 12/11, 12/13, 12/14, 12/18, 14/8, 14/13, 14/15, 14/18, 15/13, 15/16, 15/17, 16/13, 16/17, 17/16, 17/20, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 20/16, 20/17, 21/17, 21/20, 21/22, 22/17, 22/20, 22/21, 22/23, 22/24, 23/20, 23/22, 23/24, 23/25, 23/28, 24/22, 24/23, 24/26, 24/27, 24/29, 24/30, 26/24, 26/25, 26/27, 27/24, 27/25, 27/26, 29/24, 29/28, 29/30, 30/24, 30/28, 30/29; EIMS *m*/*z* 440 $[M]^+$ (37), 397 (100), 287 (50); HREIMS *m*/*z* 440.3650 (calcd for $C_{30}H_{48}O_2$, 440.3655).

Polasterol B sulfate (2): white amorphous powder; mp 185−187 °C; $[\alpha]^{21}_D$ +12.8° (*c* 2.73, MeOH); FT−IR (film) ν_{max} 1220, 1250 (sh) cm^{-1}; COSY (H/H) 1/2, 2/3, 3/4, 4/5, 5/6, 6/7, 7/8, 8/9, 8/14, 9/11, 11/12, 14/15, 15/16, 16/17, 17/20, 20/21, 20/22, 23/24, 24/25, 25/26, 25/27, 29/30 (^4J); HMBC (H/C) 1/5, 1/10, 1/19, 2/3, 2/4, 2/10, 3/1, 3/2, 3/5, 4/3, 4/5, 4/6, 4/10, 6/4, 6/5, 6/10, 7/5, 7/6, 7/8, 7/9, 8/7, 8/9, 8/10, 8/14, 8/15, 9/1, 9/7, 9/8, 9/10, 9/11, 9/19, 11/8, 11/9, 11/12, 11/13, 12/11, 12/13, 12/14, 12/18, 14/8, 14/13, 14/15, 14/18, 15/13, 15/16, 15/17, 16/13, 16/17, 17/16, 17/20, 17/22, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 20/16, 20/17, 20/21, 20/22, 20/23, 21/17, 21/20, 21/22, 22/17, 22/21, 22/24, 23/25, 23/28, 24/25, 24/26, 24/27, 24/28, 24/29, 24/30, 26/24, 26/25, 26/27, 27/24, 27/25, 27/26, 29/24, 29/28, 29/30, 30/24, 30/28, 30/29; EIMS *m*/*z* 404 (67), 392 (34), 380 (56), 361 (34), 253 (33); negative FABMS *m*/*z* 741 [M − Na]$^-$, 455 [M − $3SO_3Na]^-$, 403; HREIMS *m*/*z* [M − $3NaHSO_4]^+$ 404.3434 (calcd for $C_{30}H_{44}$, 404.3443).

Polasterol B (5): white amorphous powder; mp 138−141 °C; $[\alpha]^{21}_D$ −5.82° (*c* 0.52, MeOH); FT−IR (film) ν_{max} 3330 cm^{-1}; COSY (H/H) 1/2, 2/3, 3/4, 4/5, 5/6, 6/7, 7/8, 8/9, 8/14, 9/11, 11/12, 14/15, 15/16, 16/17, 17/20, 20/21, 20/22, 22/23, 23/24, 24/25, 25/26, 25/27, 29/30 (^4J); HMBC (H/C) 1/5, 1/10, 1/19, 2/3, 2/4, 2/10, 3/1, 3/2, 3/5, 4/3, 4/5, 4/6, 4/10, 6/4, 6/5, 6/10, 7/5, 7/6, 7/8, 7/9, 8/7, 8/9, 8/10, 8/14, 8/15, 9/1, 9/7, 9/8, 9/10, 9/11, 9/19, 11/8, 11/9, 11/12, 11/13, 12/11, 12/13, 12/14, 12/18, 14/8, 14/13, 14/15, 14/18, 15/13, 15/16, 15/17, 16/13, 16/17, 17/16, 17/20, 17/22, 18/12, 18/13, 18/14, 18/17, 19/1, 19/5, 19/9, 19/10, 20/16, 20/17, 20/21, 20/22, 20/23, 21/17, 21/20, 21/22, 22/17, 22/20, 22/21, 22/24, 23/20, 23/24, 23/25, 23/28, 24/25, 24/26, 24/27, 24/28, 24/29, 24/30, 26/24, 26/25, 26/27, 27/24, 27/25, 27/26, 29/24, 29/28, 29/30, 30/24, 30/28, 30/29; positive FABMS *m*/*z* $[M + Na]^+$ 481.3630 (calcd for $C_{30}H_{50}O_3Na$ 481.3657); EIMS *m*/*z* 426 (10), 408 (100), 365 (53); HREIMS *m*/*z* $[M - O_2]^+$ 426.3893 (calcd for $C_{30}H_{50}O$, 426.3882).

Solvolysis of 2: A solution of compound **2** (12 mg) in dioxane (1.5 mL) and pyridine (1.5 mL) was heated at 120° for 2 h in a stoppered reaction vial. The residue was purified by HPLC (reversed-phase C_{18}) to give the desulfated compound **5** (4 mg).

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References and Notes

(1) Giner, J.-L. *Chem. Rev.* **1993**, *93*, 1735−1752.
(2) D'Auria, M. V.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839−1896.
(3) Hofheinz, W.; Oesterhelt, G. *Helv. Chim. Acta* **1979**, *62*, 1307−1309.
(4) Kokke, W. C. M. C.; Pak, C. S.; Fenical, W.; Djerassi, C. *Helv. Chim. Acta* **1979**, *62*, 1310−1318.
(5) Ishibashi, M.; Yamagishi, E.; Kobayashi, J. *Chem. Pharm. Bull.* **1997**, *45*, 1435−1438.
(6) Fusetani, N.; Matsunaga, S.; Konosu, S. *Tetrahedron Lett.* **1981**, 22, 1985−1988.
(7) Koreeda, M.; Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1974**, *96*, 266−268.

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