

Polasols A–C, Three New Diterpenes from the Marine Sponge *Epipolasis* sp.

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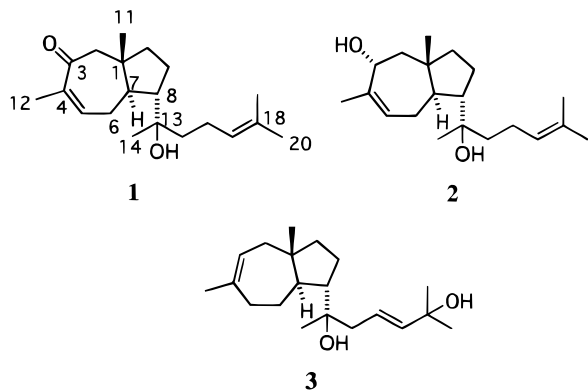
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Three new diterpenes, polasols A–C (**1–3**), have been isolated from the Japanese marine sponge *Epipolasis* sp. The structures of **1–3** were assigned primarily by 2D NMR methods.

Marine sponges have been a rich source of unique types of diterpenes.¹ In 1987, Kashman et al. reported the two new reduced azulene diterpenes, reiswigins A and B, in a specimen of *Epipolasis reiswigi*.² In 1991, they reported a third diterpene, isoreiswigin, from the same sponge,³ in 1990, Nakamura et al. reported a related diterpene.⁴ Our chemical interest in marine organisms led to the isolation of three new reduced azulene diterpenes, polasols A–C (**1–3**), from the Japanese marine sponge *Epipolasis* sp (Jaspidae), and we now report their isolation and structure elucidation.

A MeOH–CH₂Cl₂ (3:1) extract of the sponge was divided into EtOAc- and H₂O-soluble portions. The EtOAc-soluble portion was chromatographed on Sephadex LH-20 and Si gel columns. Final purification by reversed-phase HPLC afforded three novel diterpenes, polasols A–C (**1–3**).



Polasol A (**1**) was obtained as a colorless oil. The molecular formula C₂₀H₃₂O₂, as determined by HREIMS, suggested the presence of five degrees of unsaturation. The IR spectrum suggested that **1** possessed a hydroxyl group at 3300–3500 cm⁻¹ and an α,β -unsaturated carbonyl group at 1645 cm⁻¹. The ¹³C NMR spectrum indicated the presence of one carbonyl group at δ 202.7 (s); two double bonds at δ 139.4 (d), 137.1 (s), 132.0 (s), and 124.3 (d); and one carbon bearing a hydroxyl group at δ 75.3 (s), which requires that **1** must contain two rings. The ¹H NMR spectrum contained five methyl singlets at δ 1.85, 1.70, 1.64, 1.18, and 0.93 and two olefinic protons at δ 6.30 (1H, br d, J = 7.1 Hz) and 5.14 (1H, br t, J = 7.0 Hz). These data suggested that **1** was a bicyclic diterpene. The ¹H–¹H

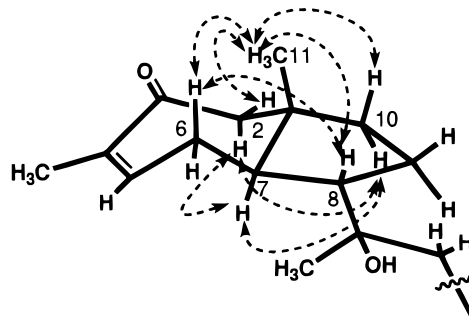


Figure 1.

COSY and HMQC experiments implied the partial structures **a** (=CHCH₂CHCHCH₂CH₂: from C-5 to C-10) and **b** (CH₂CH₂CH=: from C-15 to C-17). The HMBC experiment revealed long-range couplings from H-2 to C-1, -3, -4, -7, and -10; from H-5 to C-3 and -12; from H-11 to C-1, -2, -7, and -10; and from H-12 to C-3, -4, and -5, suggesting that the AB ring included partial structure **a**. Furthermore, the HMBC spectrum showed couplings from H-14 to C-8, -13, and -15, and from H-17 to C-19 and -20, establishing the connectivity between the AB ring and the side chain. Thus, the planar structure of **1** was determined. The relative stereochemistry of **1** was established by NOESY experiments (see Figure 1). The NOEs between H-2 α /H-7, H-2 α /H-10 α , H-2 β /H-11, H-6 β /H-8, H-6 β /H-11, H-7/H-10 α , H-8/H-11, and H-10 β /H-11 established the trans A/B ring junction, the α -orientation of H-7, and the β -orientation of H-8. The CD spectrum [$\Delta \epsilon$ -1.38 (245 nm)] of **1** showed a negative Cotton effect, indicating that the absolute stereochemistries at C-1, C-7, and C-8 were (S), (S), and (R), respectively.

Polasol B (**2**) was isolated as a colorless oil and was determined to have a molecular formula of C₂₀H₃₄O₂ by HREIMS, differing from the molecular formula of **1** by the addition of H₂. Comparison of the physicochemical data of **2** with those of **1** revealed that the only difference was that **2** had a hydroxyl group at C-3 instead of a carbonyl group as in **1**. The connectivity of the COSY and HMBC experiments (see Experimental Section) supported the proposed structure of **2**. The relative stereochemistry of **2** was determined by coupling constants and NOESY experiments. The α -OH group at C-3 could be assigned from the observed coupling constants for H-3 at δ 4.34 (br d, J = 11.0 Hz). The NOEs between H-2 α /H-10 α , H-2 β /H-10 α , H-2 β /H-10 β , H-3/H-6 β , H-3/H-11, H-5/H-7, H-6 α /H-8, H-6 β /H-8, H-6 β /H-11, H-7/H-10 α , H-8/H-11, and H-10 β /H-11

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established the trans A/B ring junction, the α -orientation of H-7, and the β -orientation of H-8. The configurations of the asymmetric carbons at C-1, C-7, and C-8 were found to be the same as in **1**. Thus, the structure of polasol B was assigned as **2**.

Polasol C (**3**) was isolated as a colorless oil, and its molecular formula was determined as $C_{20}H_{34}O_2$ by HREIMS. The physicochemical data of **3** resembled those of **2**. The ^{13}C NMR spectrum indicated the presence of one disubstituted double bond at δ 142.1 (d) and 122.3 (d) [instead of one trisubstituted double bond at δ 131.8 (s) and 124.5 (d) in **2**] and one carbon bearing a tertiary hydroxyl group at δ 70.8 (s) [instead of one carbon bearing a secondary hydroxyl group at δ 70.3 (d) in **2**]. A combination of COSY and HMBC experiments (see Experimental Section) enabled us to construct the structure of **3**. The relative stereochemistry of **3** was established by NOESY experiments and coupling constants. The NOEs between H-2 α /H-7, H-2 α /H-10 α , H-6 β /H-11, H-8/H-11, H-9 β /H-11, and H-10 β /H-11 established the trans A/B ring junction, the α -orientation of H-7, and the β -orientation of H-8. The configurations of the asymmetric carbons at C-1, C-7, and C-8 were found to be the same as in **1**. The 1H NMR spectrum of **3** in C_6D_6 gave signals for a disubstituted double bond at δ 5.76 (1H, ddd, $J = 15.5, 7.7, 7.7$ Hz) and δ 5.57 (1H, d, $J = 15.5$ Hz), and the (*E*)-configuration was assigned to this double bond on the basis of the coupling constant of 15.5 Hz. Thus, the structure of polasol C was determined to be **3**.

Experimental Section

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

Sponge Material. The marine sponge *Epipolasis* sp. (2.9 kg, wet wt) was collected off the coast of Tokushima prefecture, Japan, [(134° 30'E: 33° 35'N) by netting at a depth between 10 and 70 m], and was kept frozen (−20 °C) until used. The sponge was identified by Professor P. R. Bergquist of Auckland University. The voucher sample (TS076) of the organism under consideration is deposited in the Herbarium of the Department of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan. The sponge body is massive, irregular, roughly hemispherical, 13 cm in diameter, 8 cm high, and the texture is hard, not at all compressible. The surface is pitted and undulating, with prominent exhalant canals roofed by only a surface membrane through which rows of small oscules (0.5–1 mm in diameter) extend. Each osculum has a prominent contractile lip. The spicules are oxeas, evenly tapering and straight or slightly curved. Rare spicules show a monotriaene modification. Their dimensions are 1120–1260 μm long, 5–10 μm wide. The interior of the body is cavernous, and the arrangement of the skeleton is confused even at the surface where a weak radial orientation occurs. This species is unique within *Epipolasis* in having oxeas in only one size category of oxeas. However, better material is required before a full description can be attempted.

Extraction and Isolation of Metabolites. The frozen sample (2.9 kg) was exhaustively extracted with

Table 1. 1H NMR Data for Polasols A–C (**1–3**) in $CDCl_3$

position	1	2	3
2	2.51 (d, 15.7)	1.46 (m)	1.92 (dd, 15.0, 2.5)
	2.75 (d, 15.7)	2.04 (dd, 13.0, 2.4)	2.04 (dd, 15.0, 8.0)
3		4.34 (br d, 11.0)	5.36 (dd, 8.0, 2.5)
	5	6.30 (br d, 7.1)	5.51 (br d, 8.2)
6	2.20 (ddd, 19.0, 11.8, 2.5)	1.91 (br dd, 17.0, 15.5)	2.08 (br t, 15.0, 15.0)
	2.78 (ddd, 19.0, 7.1, 3.5)	2.40 (ddd, 17.0, 8.2, 2.7)	1.25 (m)
	1.98 (ddd, 11.8, 8.2, 3.5)	1.48 (m)	1.84 (m)
7	1.98 (ddd, 11.8, 8.2, 3.5)	1.48 (m)	1.45 (ddd, 10.2, 10.2, 2.2)
8	1.84 (m)	1.76 (m)	1.80 (ddd, 10.2, 5.0, 5.0)
9	1.58 (m)	1.77 (m)	1.75 (m)
		1.48 (m)	1.49 (m)
10	1.38 (m)	1.77 (m)	1.75 (m)
		1.29 (ddd, 20.0, 11.0, 11.0)	1.26 (m)
11	1.48 (m)	1.42 (m)	1.35 (m)
12	0.93 (s)	0.89 (s)	0.76 (s)
14	1.85 (br s)	1.83 (br s)	1.74 (s)
15	1.18 (s)	1.16 (s)	1.15 (s)
16	1.50 (m)	1.49 (m)	2.21 (m)
			2.26 (m)
17	2.07 (m)	2.05 (m)	5.71 (m)
19	5.14 (br t, 7.0)	5.13 (br t, 7.0)	5.71 (s)
20	1.64 (br s)	1.63 (br s)	1.33 (s)
	1.70 (br s)	1.69 (br s)	1.33 (s)

Table 2. ^{13}C NMR Data^a for Polasols A–C (**1–3**) in $CDCl_3$

position	1	2	3
1	41.6 (s)	43.1 (s)	42.8 (s)
2	58.1 (t)	50.2 (t)	41.2 (t)
3	202.7 (s)	70.3 (d)	122.4 (d)
4	137.1 (s)	140.0 (s)	138.9 (s)
5	139.4 (d)	124.3 (d)	34.3 (t)
6	34.1 (t)	30.3 (t)	26.09 (t)
7	48.1 (d)	48.6 (d)	54.7 (d)
8	54.2 (d)	52.6 (d)	52.3 (d)
9	25.3 (t)	24.4 (t)	24.4 (t)
10	42.0 (t)	42.0 (t)	41.0 (t)
11	18.6 (q)	17.8 (q)	18.8 (q)
12	22.0 (q)	23.0 (q)	27.2 (q)
13	75.3 (s)	75.4 (s)	75.2 (s)
14	25.66 (q)	25.6 (q)	26.12 (q)
15	39.0 (t)	39.5 (t)	42.7 (t)
16	22.5 (t)	22.5 (t)	122.3 (d)
17	124.3 (d)	124.5 (d)	142.1 (d)
18	132.0 (s)	131.8 (s)	70.8 (s)
19	17.7 (q)	17.7 (q)	29.9 (q)
20	25.72 (q)	25.7 (q)	29.8 (q)

^a Multiplicity determined by a DEPT experiment.

MeOH– CH_2Cl_2 (3:1) (2 L \times 4) at room temperature for 1 day. The extract was concentrated, and the resulting residue was extracted with EtOAc (500 mL \times 3). The EtOAc-soluble portion (21.0 g) was repeatedly subjected to Si gel flash column chromatography (using increasing concentrations of MeOH in CH_2Cl_2 as eluent), followed by reversed-phase HPLC (60–70% MeOH) to give **1** (10.3 mg, 0.00036% wet wt), **2** (30.5 mg, 0.00105%), and **3** (13.4 mg, 0.00046%).

Polasol A (1): colorless oil; $[\alpha]_D^{25} + 60.1^\circ$ (c 0.44, $CHCl_3$); FT-IR (film) 3300–3500, 1645 cm^{-1} ; CD $\Delta\epsilon -1.38$ (245 nm) (c 0.015, MeOH); UV (MeOH) λ_{max} 240 (log ϵ 2.90) nm; 1H NMR, see Table 1; ^{13}C NMR, see Table 2; COSY (H/H) 5/6, 5/12 (4J), 6/7, 6/12 (5J), 7/8, 8/9, 9/10, 15/16, 16/17, 16/19 (5J), 16/20 (5J), 17/19 (4J), 17/20 (4J); HMBC (H/C) 2/3, 2/4, 2/7, 2/10, 2/11, 5/3,

5/6, 5/7, 5/12, 6/4, 6/5, 6/7, 7/13, 8/13, 8/14, 9/1, 9/7, 9/8, 9/10, 9/13, 10/1, 10/2, 10/7, 10/8, 10/9, 10/11, 11/1, 11/2, 11/7, 11/10, 12/3, 12/4, 12/5, 14/8, 14/13, 14/15, 15/8, 15/13, 15/14, 15/16, 15/17, 16/13, 16/17, 16/18, 17/15, 17/16, 17/19, 17/20, 19/17, 19/18, 19/20, 20/17, 20/18, 20/19; HREIMS obsd m/z 304.2422 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Polasol B (2): colorless oil; $[\alpha]^{25}_D + 11.4^\circ$ (c 0.56, $CHCl_3$); FT-IR (film) 3300–3500 cm^{-1} ; 1H NMR, see Table 1; ^{13}C NMR, see Table 2; COSY (H/H) 2/3, 3/5 (4J), 5/6, 5/12 (4J), 6/7, 6/12 (5J), 7/8, 8/9, 9/10, 15/16, 16/17, 16/19 (5J), 16/20 (5J), 17/19 (4J), 17/20 (4J); HMBC (H/C) 2/1, 2/3, 2/4, 2/7, 2/10, 5/3, 5/7, 5/12, 6/1, 6/4, 6/5, 6/7, 8/6, 8/10, 8/13, 8/15, 9/7, 10/7, 10/8, 10/11, 11/1, 11/2, 11/7, 11/10, 12/3, 12/4, 12/5, 14/8, 14/13, 14/15, 16/17, 16/18, 17/16, 17/19, 17/20, 19/17, 19/18, 19/20, 20/17, 20/18, 20/19; NOESY $2\alpha/10\alpha$, $2\beta/10\alpha$, $2\beta/10\beta$, 2/11, 3/6 β , 3/11, 3/12, 5/7, 5/12, 6 $\alpha/8$, 6 $\beta/8$, 6 $\beta/11$, 6 $\alpha/14$, 7/10 α , 7/14, 8/11, 8/14, 8/15, 8/16, 9/11, 10/11, 15/17, 16/19, 17/20, 19/20; HREIMS m/z $[M - H_2O]^+$ 288.2460 (calcd for $C_{20}H_{34}O_2 - H_2O$, 288.2453).

Polasol C (3): colorless oil; $[\alpha]^{25}_D - 5.37^\circ$ (c 0.62, $CHCl_3$); FT-IR (film) 3300–3500 cm^{-1} ; 1H NMR, see Table 1; ^{13}C NMR, see Table 2; COSY (H/H) 2/3, 4/11 (4J), 5/6, 5/11 (5J), 6/7, 7/8, 8/9, 9/10, 15/16, 16/17; HMBC (H/C) 2/1, 2/3, 2/4, 2/7, 2/10, 2/11, 5/4, 5/12, 6/1,

6/4, 6/5, 7/1, 7/2, 7/5, 7/6, 7/8, 7/11, 7/13, 8/9, 8/13, 8/14, 9/1, 9/8, 9/10, 9/13, 10/1, 10/2, 10/7, 10/8, 10/9, 10/11, 11/1, 11/2, 11/7, 11/10, 12/3, 12/4, 12/5, 14/8, 14/13, 14/15, 15/8, 15/13, 15/14, 15/16, 15/17, 16/15, 16/18, 17/15, 17/18, 17/19, 17/20, 19/17, 19/18, 20/17, 20/18; NOESY $2\alpha/7$, $2\alpha/10\alpha$, $2\beta/11$, 3/11, 3/12, 5 $\beta/12$, 6 $\beta/11$, 8/11, 8/14, 8/15, 9 $\alpha/15$, 9 $\beta/11$, 10 $\beta/11$, 14/15, 15/17, 17/19, 17/20; HREIMS m/z $[M - H_2O]^+$ 288.2424 (calcd for $C_{20}H_{34}O_2 - H_2O$, 288.2453).

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References and Notes

- (1) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259–302 and earlier reviews cited therein.
- (2) Kashman, Y.; Hirsch, S.; Koehn, F.; Cross, S. *Tetrahedron Lett.* **1987**, *28*, 5461–5464.
- (3) Kashman, Y.; Hirsch, S. *J. Nat. Prod.* **1991**, *54*, 1430–1432.
- (4) Nakamura, H.; To, S.; Takamatsu, M.; Kobayashi, J.; Ohizumi, Y.; Kondo, K.; Hirata, Y. 59th Annual Meeting of Chemical Society of Japan, Yokohama, Symposium Papers, P. 1197, 1990.

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