

Peroxyolasol and Mugipolasol: Two Novel Diterpenes from the Marine Sponge *Epipolasis* sp.

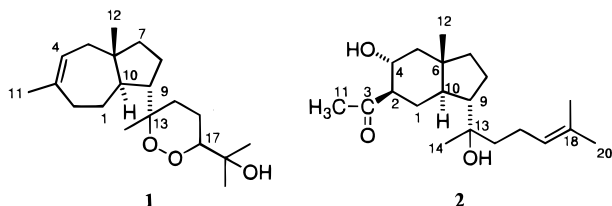
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Two novel diterpenes, peroxyolasol (**1**) and mugipolasol (**2**), have been isolated from the Japanese marine sponge *Epipolasis* sp. The structures of **1** and **2** were determined on the basis of spectroscopic data. Compound **1** is a diterpene with a peroxide ring in the side chain, and **2** is an unusual six/five-membered ring diterpene with an ethyl unit at C-2.

Marine sponges have been a rich source of unique types of diterpenes.¹ In 1987, Kashman *et al.* reported two new reduced azulene diterpenes, reishwiggins A and B, in a specimen of *Epipolasis reishwigi*,² and in 1991, they reported a third diterpene together with reishwiggins A and B from the same sponge.³ On the other hand, in 1990, Nakamura *et al.* described a reduced azulene diterpene.⁴ In our previous publication, we described the isolation and structure elucidation of three novel diterpenes, polasols A–C, from the Japanese marine sponge *Epipolasis* sp. (Jaspiidae).⁵ Further investigation of the EtOAc extract of the same sponge led to the isolation of two novel diterpenes, peroxyolasol (**1**) and mugipolasol (**2**). We now report the isolation and structure elucidation of **1** and **2**.



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 two novel diterpenes, peroxyolasol (**1**) and mugipolasol (**2**).

Peroxyolasol (**1**) was obtained as a colorless oil. The molecular formula C₂₀H₃₄O₃, as determined by HREIMS (*m/z* 288.2477; M–H₂O₂) and positive FABMS, suggested the presence of four degrees of unsaturation. The IR spectrum suggested that **1** possessed a hydroxyl group (3450 cm⁻¹). The ¹³C NMR spectrum indicated the presence of a double bond [δ 138.9 (s) and 122.4 (d)], an oxygenated carbon [δ 71.5 (s)], and two downfield-shifted oxygenated carbons [δ 86.6 (s) and 83.1 (d)]. These data require **1** to contain three rings, including a peroxide ring. The ¹H NMR spectrum contained five methyl singlets (δ 1.73, 1.21, 1.16, 1.12, and 0.76) and an olefinic proton [δ 5.36 (br d, *J* = 8.2 Hz)]. The ¹H–¹H COSY and HMQC experiments implied the partial structures **a** (CH₂CH₂–CHCHCH₂CH₂: from C-2 to C-7), **b** [CH₂CH=C(CH₃): from C-5 to C-11] and **c** (CH₂CH₂CH: from C-15 to C-17). An HMBC experiment revealed long-range couplings from

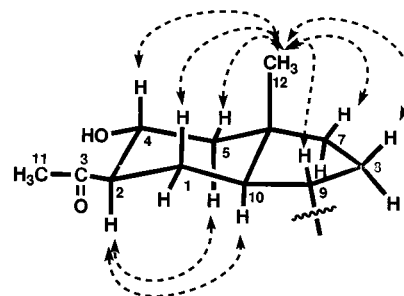


Figure 1. NOESY connectivities for **2**.

H-2 to C-11, from H-11 to C-3 and -4, from H-12 to C-5, -6, -7, and -10. This suggested a seven/five-membered ring (AB rings). Furthermore, the HMBC spectrum showed couplings from H-14 to C-9, -13, and -15; H-17 to C-19; H-19 to C-20; and H-20 to C-17, 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. The NOE between H-12/H-1 β , -4, -5 β , -7 β , and -9 β , H-5 α /H-2, -10 established the trans A/B ring junction, the β -orientation of H-9, and the α -orientation of H-10. Although the boat conformation for the peroxide ring was defined by the NOE between H-14 and H-17, the configuration at C-17 was undetermined because of an ambiguous configuration at C-13 (configurations at C-13/C-17; *S/R* or *R/S*). Compound **1** is a unique diterpene with a peroxide ring in the side chain.

Mugipolasol (**2**) was isolated as a colorless oil and was determined to have a molecular formula of C₂₀H₃₄O₃ by HRCIMS of the molecular ion at *m/z* 323.2605. The IR spectrum suggested that **2** possessed a hydroxyl group (3450 cm⁻¹) and a carbonyl group (1700 cm⁻¹). The ¹³C NMR spectrum indicated the presence of a carbonyl group [δ 212.9 (s)], a double bond [δ 131.9 (s) and 124.4 (d)], and two oxygenated carbons [δ 74.8 (s) and 67.6 (d)], which requires **2** to contain two rings. The ¹H NMR spectrum contained four methyl singlets (δ 1.70, 1.64, 1.19, and 0.82), an acetyl methyl group (δ 2.22), and an olefinic proton [δ 5.14 (br t, *J* = 7.0 Hz)]. The connectivity of the COSY and HMBC experiments (see Experimental Section) supported the proposed structure of **2**. The relative stereochemistry of **2**, except for that at C-13, was deduced by NOESY experiments (see Figure 1) and coupling constants. The β -acetyl group at C-2 and the α -OH group at C-4 could be assigned from the observed coupling constants for H-4 at δ 4.14 (ddd, *J* = 14.8, 9.9, 4.9 Hz) and for H-2 at δ 2.40 (ddd, *J* = 12.6, 9.9, 4.4 Hz), respectively. Thus, the

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structure of mugipolasol was assigned as **2**. To the best of our knowledge, this is the first isolation of a six/five-membered ring diterpene with an ethyl unit at C-2. Biosynthetically, **2** can be considered as being derived from a precursor having the same planar bicyclic system.

Experimental Section

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

Sponge Material. The marine sponge *Epipolasis* sp. (2.9 kg, wet wt) was obtained as previously described.⁵

Extraction and Isolation of Metabolites. The frozen sample (2.9 kg) was exhaustively extracted with $\text{MeOH}-\text{CH}_2\text{Cl}_2$ (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** (0.00021% wet wt) and **2** (0.00145%).

Peroxyolasol (1): colorless oil; $[\alpha]_{\text{D}}^{25} +21.5^\circ$ (c 0.60, CHCl_3); FT-IR (film) 3450 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.76 (3H, s, Me-12), 1.12 (3H, s, Me-19), 1.16 (3H, s, Me-14), 1.21 (3H, s, Me-20), 1.25 (1H, m, H-1), 1.27 (1H, m, H-7), 1.35 (1H, ddd, $J = 10.2, 10.2, 2.2$ Hz, H-5), 1.36 (1H, ddd, $J = 11.8, 8.0, 1.0$ Hz, H-10), 1.41 (1H, m, H-8), 1.65 (1H, m, H-15), 1.73 (3H, br s, Me-11), 1.78 (1H, m, H-15), 1.78 (1H, m, H-16), 1.80 (1H, m, H-8), 1.83 (1H, m, H-1), 1.88 (1H, m, H-16), 1.89 (1H, m, H-5), 1.93 (1H, ddd, $J = 11.8, 10.4, 4.9$ Hz, H-9), 2.01 (2H, m, H-2), 2.05 (1H, dd, $J = 14.8, 8.5$ Hz, H-5), 3.74 (1H, t, $J = 7.1$ Hz, H-17), 5.36 (1H, br d, $J = 8.2$ Hz, H-4); ^{13}C NMR (CDCl_3) δ 18.7 (q, C-12), 24.2 (q, C-14), 24.3 (q, C-19), 25.3 (t, C-8), 26.0 (t, C-16), 26.2 (t, C-1), 27.2 (q, C-11), 27.4 (q, C-20), 34.4 (t, C-2), 34.7 (t, C-15), 41.1 (t, C-5), 41.2 (t, C-7), 42.7 (s, C-6), 51.8 (d, C-9), 55.2 (d, C-10), 71.5 (s, C-18), 83.1 (d, C-17), 86.6 (s, C-13), 122.4 (d, C-4), 138.9 (s, C-3); COSY (H/H) 1/2, 1/10, 4/11 (4J), 4/5, 7/8, 8/9, 9/10, 15/16, 16/17; HMBC (H/C) 1/3, 2/1, 2/10, 2/11, 4/2, 4/5, 4/6, 4/11, 5/3, 5/4, 5/6, 5/7, 5/10, 5/12, 7/12, 8/13, 9/13, 11/3, 11/4, 12/5, 12/6, 12/7, 12/10, 14/9, 14/13, 14/15, 15/13, 15/17, 16/13, 16/17, 16/18, 17/16, 17/19, 17/20, 19/17, 19/18, 19/20, 20/17, 20/18, 20/19; HREIMS m/z 306.2571

(calcd for $\text{C}_{20}\text{H}_{34}\text{O}_3-\text{O}$, 306.2559), m/z 288.2477 (calcd for $\text{C}_{20}\text{H}_{34}\text{O}_3-\text{H}_2\text{O}_2$, 288.2453); positive FABMS m/z 345 $[\text{M}+\text{Na}]^+$.

Mugipolasol (2): colorless oil; $[\alpha]_{\text{D}}^{25} +31.7^\circ$ (c 1.71, CHCl_3); FT-IR (film) $3450, 1700\text{ cm}^{-1}$; ^1H NMR (CDCl_3) δ 0.82 (3H, s, Me-12), 1.19 (1H, m, H-5), 1.19 (1H, m, H-7), 1.19 (3H, s, Me-14), 1.29 (1H, m, H-1), 1.40 (1H, ddd, $J = 11.0, 11.0, 3.0$ Hz, H-10), 1.45 (1H, ddd, $J = 12.1, 8.5, 1.4$ Hz, H-7), 1.50 (2H, t, $J = 8.2$ Hz, H-15), 1.51 (1H, m, H-8), 1.64 (3H, d, $J = 1.0$ Hz, Me-20), 1.70 (3H, d, $J = 0.8$ Hz, Me-19), 1.77 (1H, ddd, $J = 11.0, 11.0, 6.0$ Hz, H-9), 1.83 (1H, m, H-8), 2.00 (1H, dd, $J = 12.1, 4.7$ Hz, H-5), 2.07 (2H, m, H-16), 2.21 (1H, m, H-1), 2.22 (3H, s, Me-11), 2.40 (1H, ddd, $J = 12.4, 9.9, 4.4$ Hz, H-2), 4.14 (1H, ddd, $J = 14.8, 9.9, 4.9$ Hz, H-4), 5.14 (1H, br t, $J = 7.0$ Hz, H-17); ^{13}C NMR (CDCl_3) δ 17.7 (q, C-20), 18.7 (q, C-12), 22.5 (t, C-16), 24.7 (t, C-8), 25.7 (q, C-19), 26.3 (q, C-14), 27.8 (t, C-1), 29.2 (q, C-11), 38.4 (t, C-7), 38.9 (t, C-15), 43.1 (s, C-6), 46.4 (t, C-5), 47.9 (d, C-10), 49.7 (d, C-9), 59.4 (d, C-2), 67.6 (d, C-4), 74.8 (s, C-13), 124.4 (d, C-17), 131.9 (s, C-18), 212.9 (s, C-3); COSY (H/H) 1/2, 1/10, 2/4, 4/5, 7/8, 8/9, 9/10, 15/16, 16/17, 17/19 (4J), 17/20 (4J); HMBC (H/C) 1/3, 1/2, 1/4, 1/6, 1/9, 1/10, 2/3, 4/3, 5/2, 5/4, 5/6, 5/10, 7/6, 7/9, 7/10, 7/12, 8/6, 8/7, 9/7, 9/8, 9/10, 9/13, 10/1, 10/6, 10/9, 10/13, 11/2, 11/3, 12/5, 12/6, 12/7, 12/10, 14/9, 14/13, 14/15, 15/9, 15/13, 15/14, 15/16, 15/17, 16/13, 16/15, 16/17, 16/18, 17/15, 17/16, 17/19, 17/20, 19/17, 19/20, 20/17, 20/19; HRCIMS m/z $[\text{MH}]^+$ 323.2605 (calcd for $\text{C}_{20}\text{H}_{34}\text{O}_3$, 323.2586).

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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, 1990; p 1197.
- (5) Umeyama, A.; Nozaki, M.; Arihara, S. *J. Nat. Prod.* **1998**, *61*, 945–947.

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