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Rhopaloic Acid A: A Novel Norsesterterpene from a Marine Sponge, *Rhopaloeides* sp., Which Inhibits Gastrulation of Starfish Embryos

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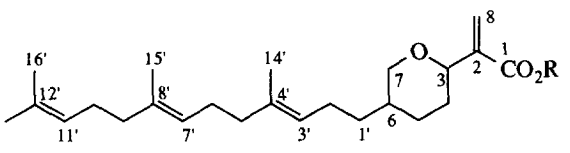
Abstract: A novel norsesterterpene, rhopaloic acid A, was isolated from a marine sponge, *Rhopaloeides* sp. The structure was determined to be 2-methylene-6-(4,8,12-trimethyl-3,7,11-tridecatrienyl)-3,7-epoxyheptanoic acid on the basis of its spectroscopic data. Rhopaloic acid A inhibited gastrulation of starfish embryos and also exhibited potent cytotoxicities *in vitro* against human myeloid K-562 cells, human MOLT-4 leukemia cells, and murine L1210 leukemia cells.

In the course of our search for biologically active compounds from marine organisms,[1-3] we found that an acetone-chloroform extract of the marine sponge *Rhopaloeides* sp. potently inhibits gastrulation of starfish (*Asterina pectinifera*) embryos. Bioassay-guided purification of the crude extract resulted in the isolation of a novel norsesterterpene, which was designated rhopaloic acid A (**1**). In this paper, we report the structure of **1** which has been deduced from its spectroscopic data.

The marine sponge *Rhopaloeides* sp. (500 g, wet weight) was collected off the coast of Sada-misaki, Ehime Prefecture, Japan, and extracted with methanol. The residue was subsequently extracted with acetone-chloroform (1:1, v/v). The bioactive acetone-chloroform extract (4.9 g) was partitioned between hexane and water. The hexane layer (2.0 g) was subjected to chromatography on silica gel using 10–80% ethyl acetate in hexane as eluent. The bioactive fraction was subsequently chromatographed on silanised silica gel (Merck) (MeOH-H_2O, 4:1, v/v) to afford **1** (3.1 mg) as a viscous colorless oil.

Rhopaloic acid A (**1**),[4] $[\alpha]_D^{25}$ +40° (*c* 0.47, $CHCl_3$), had a molecular formula, $C_{24}H_{38}O_3$, which was determined by high resolution electron impact (HREI) mass spectrometry (*m/z* 374.2836, M^+, Δ +1.5 mmu). The ^{13}C NMR[4] and DEPT spectra revealed that the 24 carbons in **1** consisted of a carbonyl carbon, eight olefinic carbons, two oxygenated sp^3 carbons, a methine, eight methylenes, and four methyl groups. Considering that the molecular formula requires 6 degrees of unsaturation, **1** must contain a ring structure.



1 R = H
2 R = Et

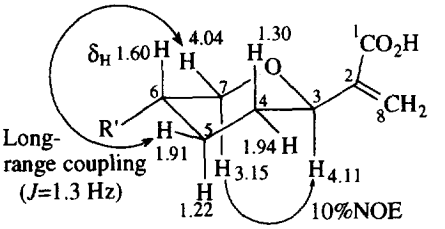


Fig. 1. Relative stereochemistry of the tetrahydropyran moiety in rhopaloic acid A (**1**).

The ^{13}C NMR and ^{1}H-^{1}H COSY spectra of **1** exhibited the presence of an (*E,E*)-farnesyl group. The geometry of the carbon-carbon double bonds of the farnesyl group was determined by the ^{13}C chemical shifts of the methyl carbons, C-13', C-14', C-15', and C-16'.[5,6] Treatment of **1** with ethyl iodide in the presence of Na_2CO_3 gave an ethyl ester (**2**; EIMS, *m/z* 402, M^+). In the ^{13}C NMR spectrum of **2**,[7] the chemical shifts ascribable to a carbonyl carbon (C-1) and an exo-methylene (C-8) differed significantly from those of **1**, supporting the existence of an α-methylene carboxyl group in **1**. The H_2-8 exo-methylene protons of **1** showed HMBC[8] cross peaks with C-1 (δ 170.7), C-2 (δ 141.9) and C-3 (δ 76.0), and the H-3 methine proton with C-1, C-2 and C-8 (δ 125.8), thus revealing that the α-carbon (C-2) is attached to C-3. The ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectra revealed the presence of a segment C-3–C-7 and the connection of the farnesyl group through a methylene (C-1') to the C-6 methine. The observation of an HMBC correlation between the H-7 proton at δ 4.04 and C-3 indicated that the C-3 and C-7 carbons linked to the same oxygen atom to form a tetrahydropyran ring. The H-3 proton signal showed 10% NOE with the H-7 proton signal at δ 3.15, as shown in Fig. 1. A long-range coupling (J = 1.3 Hz) was observed between the H-7 proton signal at δ 4.04 and the H-5 methylene proton signal at δ 1.91. In addition to these observations, careful analysis of ^{1}H vicinal coupling constants of the H-3 and H-7 proton signals indicated a diaxial arrangement for H-3 and H-6, consistent with the relative stereochemistry of the tetrahydropyran moiety as shown in Fig. 1. At present the absolute stereochemistry of the tetrahydropyran moiety remains to be determined. Thus, the structure of **1** was determined to be 2-methylene-6-(4,8,12-trimethyl-3,7,11-tridecatrienyl)-3,7-epoxyheptanoic acid. Although many biologically active compounds have been obtained from marine organisms,[9] there are very few, if any, norsesterterpenoids among them.[10] Furthermore, chemicals carrying an α-methylene carboxyl functionality such as sinularic acid[11] are rarely obtained from marine organisms.

When fertilized starfish eggs were cultured from fertilization in the presence of **1** at a concentration of 0.5 μmol/l or greater, they blastulated normally after passing through a rapid cleavage period, and hatched on schedule; the gastrulation was selectively inhibited, however. Futhermore, **1** affected neither fertilization of starfish gametes nor early embryonic development of fertilized eggs up to the pre-gastrula stage even at the concentration of 20 μmol/l. Rhopaloic acid A (**1**) also exhibited potent cytotoxicities against human myeloid K-562 cells, human MOLT-4 leukemia cells, and murine L1210 leukemia cells with IC_{50} values of 0.04, 0.05, and 0.10 μmol/l, respectively.

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REFERENCES AND NOTES

1. Ohta, S.; Okada, H.; Kobayashi, H.; Oclarit, J. M.; Ikegami, S. *Tetrahedron Lett.*, **1993**, *34*, 5935-5938.
2. Ohta, S.; Kobayashi, H.; Ikegami, S. *Tetrahedron Lett.*, **1994**, *35*, 4579-4580.
3. Ikegami, S.; Kobayashi, H.; Myotoishi, Y.; Ohta, S.; Kato, K. H. *J. Biol. Chem.*, **1994**, *269*, 23262-23267.
4. **1**: IR (film) 3300, 1700 (CO_2H), 1630 cm^{-1} (C=C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.15 (2H, m, H-1'), 1.22 (m, H-5), 1.30 (m, H-4), 1.60 (9H, s, H_3-14', H_3-15', H_3-16'), 1.60 (m, H-6), 1.68 (3H, s, H_3-13'), 1.91 (m, H-5), 1.94 (m, H-4), 1.99 (4H, m, H_2-5' and H_2-9'), 2.00 (2H, m, H_2-2'), 2.06 (4H, m, H_2-6' and H_2-10'), 3.15 (t, J=11.0 Hz, H-7), 4.04 (ddd, J=11.0, 3.8, and 1.6 Hz, H-7), 4.11 (d, J=10.1 Hz, H-3), 5.10 (3H, m, H-3', H-7', and H-11'), 5.88 (brs, H-8), 6.31 (brs, H-8), 10.0 (br, CO_2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7 (C-1), 141.9 (C-2), 135.3 (C-8'), 135.0 (C-4'), 131.2 (C-12'), 125.8 (C-8), 124.4 (C-11'), 124.2 (C-3' and C-7'), 76.0 (C-3), 73.9 (C-7), 39.7 (C-5' and C-9'), 35.3 (C-6), 32.5 (C-1'), 32.1 (C-4), 30.4 (C-5), 26.8 (C-10'), 26.6 (C-6'), 25.7 (C-13'), 25.0 (C-2'), 17.7 (C-16'), 16.0 (C-14' and C15'); SIMS *m/z* 375 $[M+H]^+$ and 397 $[M+Na]^+$.
5. Tanaka, Y.; Sato, H.; Kageyu, A. *Polymer*, **1982**, *23*, 1087-1090.
6. Ibata, K.; Mizuno, M.; Takigawa, T.; Tanaka, Y. *Biochem. J.*, **1983**, *213*, 305-311.
7. **2**: ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.0 (C-1), 123.9 (C-8).
8. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.*, **1986**, *108*, 2093-2094.
9. Faulkner, D. J. *Nat. Prod. Rep.*, **1995**, *12*, 223-269.
10. (a) Butler, M. S.; Capon, R. J. *Nat. Prod. Lett.*, **1992**, *1*, 171-178. (b) Tanaka, J.; Higa, T.; Suwanborirux, K.; Kokpol, U.; Bernardinelli, G.; Jefford, C. W. *J. Org. Chem.*, **1993**, *58*, 2999-3002.
11. Kobayashi, M.; Hamaguchi, T. *Chem. Pharm. Bull.*, **1988**, *36*, 3780-3786.

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