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Hippospongic Acid A: An Unusual Triterpenoic Acid from a Marine Sponge, *Hippospongia* sp., Which Inhibits Gastrulation of Starfish Embryos

Shinji Ohta* **and Mihoko Uno**
Instrument Center for Chemical Analysis, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739, Japan.

Munetaka Tokumasu and Yoshikazu Hiraga
Department of Chemistry, Faculty of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739, Japan.

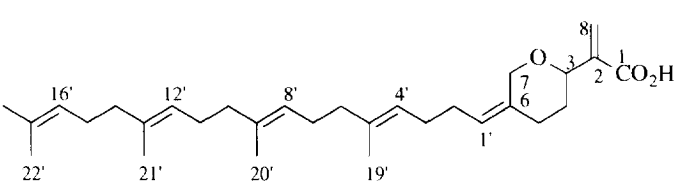
Susumu Ikegami*
Department of Applied Biochemistry, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima 739, Japan.

Abstract: An unusual triterpenoic acid, hippospongic acid A (**1**), was isolated from the marine sponge *Hippospongia* sp. and the structure was elucidated on the basis of its spectroscopic data. Compound **1** selectively inhibited the gastrulation of starfish embryos with a minimum inhibitory concentration of 14 μM. Copyright © 1996 Elsevier Science Ltd

Gastrulation is a fundamental process that occurs during embryonic development of multicellular animals. The process involves the transformation of the simple hollow ball of epithelial cells into a multilayered structure with a mesendodermal archenteron produced by tucking cells from the exterior into the interior. There are few, if any, selective inhibitors of gastrulation.[1-3] In the course of our search for biologically active compounds from marine organisms,[4-6] we found that an acetone-$CHCl_3$ extract of the marine sponge *Hippospongia* sp. potently inhibits gastrulation of starfish (*Asterina pectinifera*) embryos. Bioassay-guided purification of the crude extract resulted in the isolation of a novel terpenoic acid, which was designated hippospongic acid A (**1**). In this paper, we report the structure of **1** which has been deduced from its spectroscopic data.

The marine sponge *Hippospongia* sp. (500 g, wet weight) was collected off the coast of Sada-misaki, Ehime Prefecture, Japan, and extracted with MeOH. The residue was subsequently extracted with acetone-$CHCl_3$ (1:1, v/v). The bioactive acetone-$CHCl_3$ extract (4.9 g) was partitioned between hexane and H_2O. The hexane layer (2.0 g) was successively fractionated by silica gel column chromatography using 10–80% EtOAc in hexane as eluent and reversed phase HPLC (MeOH) to afford **1** (1.2 mg) as a viscous colorless oil.

Hippospongic acid A (**1**),[7] $[\alpha]_D^{25}$ +37° (*c* 0.22, $CHCl_3$), had the molecular formula, $C_{30}H_{46}O_3$, which was determined by high resolution electron impact (HREI) mass spectrometry (*m/z* 454.3449, M^+, Δ +0.2 mmu). The ^{13}C NMR[7] and DEPT spectra revealed that the 30 carbons in **1** consisted of a carbonyl, twelve olefinic, two oxygenated sp^3, ten methylene, and five methyl carbons. Considering that the molecular formula requires 8 degrees of unsaturation, it is inferred that **1** must contain a ring structure.



1

The ^{1}H NMR, ^{13}C NMR and ^{1}H-^{1}H COSY spectra of **1** exhibited the presence of an α-methylene carboxyl moiety (C-1, C-2, and C-8) and an (*E,E,E*)-homogeranylgeranyl group (C-2'–C-22'). The geometry of the three carbon-carbon double bonds ($\Delta^{4'}$, $\Delta^{8'}$, and $\Delta^{12'}$) of the homogeranylgeranyl group was determined by the ^{13}C chemical shifts (δ 16.0–16.1) of the methyl carbons, C-19', C-20', and C-21'.[8,9] The NOE and HMBC[10] data indicated the presence of a pyran ring (C-3–C-7), as shown in Fig. 1. They also revealed the connection of the α-carbon (C-2) to C-3 and that of C-2' through C-1' to the C-6 quaternary carbon. Irradiation of H-1' (δ 5.24) caused enhancement of the H-5 signal (δ 2.40), indicating that the geometry of the C-6-C-1' double bond was *Z*. At present, the absolute configuration of C-3 remains to be determined. Thus, the structure of **1** was determined to be 2-methylene-6-(5,9,13,17-tetramethyl-4,8,12,16-octadecatetraenyl-idene)-3, 7-epoxyheptanoic acid.

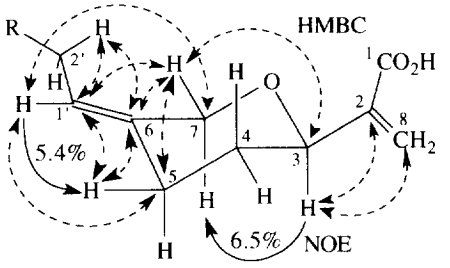


Fig. 1. Selected NOE and HMBC data of **1**.

Compounds containing an α-methylene carboxyl or formyl functionality attached to a tetrahydropyran ring have been isolated from marine organisms: brevetoxin-A,[11] -B,[12] and hemibrevetoxin-B[13] from the red tide alga, *Gymnodinium breve*, TX2[14] from the greenshell mussel, *Perna canaliculus*, and rhopaloic acid A[15] from the marine sponge, *Rhopaloeides* sp. However, the biosynthesis of **1** can be envisioned as derived via a quite different route involving tail-to-tail condensation of geranyl diphosphate and geranylgeranyl diphosphate, or an elimination of a terminal C_{10} unit from lycopersene.[16]

When fertilized starfish eggs were cultured from fertilization in the presence of **1** at a concentration of 14 μmol/l or greater, they blastulated normally after passing through a rapid cleavage period, and hatched on schedule; the gastrulation was selectively inhibited, however. Furthermore, **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 110 μmol/l.

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7. **1**: IR (film) 3350, 1700 (CO_2H), 1630 cm^{-1} (C=C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50 (m, H-4), 1.60 (12H, s, H_3-19', H_3-20', H_3-21', H_3-22'), 1.68 (3H, s, H_3-18'), 1.95–2.10 (16H, m, H-4, H-2', H_2-3', H_2-6', H_2-7', H_2-10', H_2-11', H_2-14', H_2-15'), 2.15 (m, H-2'), 2.40 (2H, m, H_2-5), 3.91 (d, *J*=12.8 Hz, H-7), 4.32 (d, *J*=11.0 Hz, H-3), 4.72 (d, *J*=12.8 Hz, H-7), 5.10–5.15 (4H, m, H-4', H-8', H-12', H-16'), 5.24 (t, *J*=7.3 Hz, H-1'), 5.91 (brs, H-8), 6.40 (brs, H-8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.0 (C-1), 140.2 (C-2), 135.2, 134.9, 134.3 (C-5', C-9', C-13'), 132.2 (C-6), 131.3 (C-17'), 127.0 (C-8), 125.3 (C-1'), 125.0, 124.4, 124.3, 124.2 (C-4', C-8', C-12', C-16'), 76.2 (C-3), 67.2 (C-7), 39.7×3 (C-6', C-10', C-14'), 33.5 (C-4), 32.8 (C-5), 28.3, 28.2 (C-3', C-7'), 26.8, 26.7 (C-11', C-15'), 25.7×2 (C-2', C-18'), 17.7 (C-22'), 16.1, 16.0×2 (C-19', C-20', C-21'); EIMS *m/z* (rel. int.) 454 (M^+, 20), 411 (8), 385 (2), 180 (59), 69 (100).
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