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Isojaspisin: A Novel Styryl Sulfate from a Marine Sponge, *Jaspis* sp., That Inhibits Hatching of Sea Urchin Embryos

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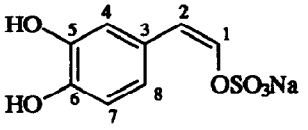
Abstract: A novel styryl sulfate, isojaspisin (1), was isolated from a marine sponge, *Jaspis* sp. The structure was determined to be sodium (Z)-5,6-dihydroxystryryl sulfate on the basis of its spectroscopic data. This sulfate inhibited hatching of sea urchin embryos with a minimum inhibitory concentration of 60 μM.

In the course of our search for biologically active compounds from marine organisms,[1-3] we found that a methanolic extract of the marine sponge *Jaspis* sp. strongly inhibits hatching of sea urchin (*Hemicentrotus pulcherrimus*) embryos. Bioassay-guided purification of the crude extract resulted in the isolation of a novel styryl sulfate, which was designated isojaspisin (1). In this paper, we report the structure of **1** which has been deduced from its spectroscopic data.

The marine sponge *Jaspis* sp. (1 kg, wet weight) was collected off the coast of Okino-shima Island, Kohchi Prefecture, Japan. The methanolic extract was suspended in ethyl acetate and insoluble material was collected by centrifugation. The material was subjected to chromatography on Diaion HP-20 (H_2O) and subsequently on Sephadex LH-20 (H_2O) to afford **1** (30 mg) as a viscous oil.



1

Compound **1**[4] has a sulfate group, which was deduced by a strong IR absorption at 1,230 cm^{-1}. The sulfate was present as a sodium salt, which was indicated by an ion peak at *m/z* 115 ([matrix+Na]$^+$) in the positive FABMS of **1** (matrix: glycerol only). Compound **1** has a molecular formula, $C_8H_7O_6SNa$, which was determined by negative high resolution FAB mass spectrometry (*m/z* 230.9981 [M-Na]$^-$, Δ +1.8 mmu). The IR spectrum (film) of **1** suggested the presence of hydroxyl groups (3,280 cm^{-1}), a vinyl group (1,660 cm^{-1}), and a benzene ring (1,607 and 1,528 cm^{-1}) in addition to the sulfate group.[4] The UV spectrum (H_2O, pH 4.5) showed absorption maxima at 212 nm (ε 18,000), 257 nm (ε 12,700) and 296 nm (ε 3,400), suggesting that **1** is a styryl derivative. ^1H NMR spectrum (D_2O) of **1** exhibited the presence of three aromatic protons and two vinyl protons.[4] The spin coupling pattern of the three aromatic protons indicated that **1** has a 1,3,4-trisubstituted benzene ring system. The ^{13}C NMR spectrum (D_2O) of **1** exhibited the presence of three

hydrogen-bearing aromatic carbon atoms at δ 116.1 (d, C-4), 115.7 (d, C-7) and 121.7 (d, C-8), two oxygen-bearing aromatic carbon atoms at δ 143.3 (s, C-5) and 143.2 (s, C-6), an aromatic carbon atom at δ 126.3 (s, C-3), a hydrogen-bearing vinyl carbon atom at δ 112.1 (d, C-2), and an oxygen-bearing vinyl carbon atom at δ 134.7 (d, C-1). The C-H COSY spectrum of **1** allowed the assignments of all of the protonated carbon atoms. The vinyl proton at δ 5.52 (H-2) showed NOE's with the aromatic protons H-4 (δ 7.05) and H-8 (δ 6.85) in the NOESY spectrum. This suggested that the vinyl group is located at C-3. This assignment was further supported by the HMBC[5] correlation of the vinyl proton H-1 (δ 6.41) to C-3 (δ 126.3) and that of the other vinyl proton H-2 to C-1 (δ 134.7), C-4 (δ 116.1) and C-8 (δ 121.7). The H-1 proton at δ 6.41 showed no NOE correlation to the aromatic protons at δ 7.05 (H-4) and 6.85 (H-8). This suggested that the geometry of the vinyl group is *Z*. This assignment was also supported by the vicinal coupling constant of 7.3 Hz between the two vinyl protons H-1 and H-2. Because **1** did not react with 2,4-dinitrophenylhydrazine in aqueous HCl, the sulfate group is attached to C-1, and two hydroxyl groups to C-5 and C-6. Thus the structure of **1** was determined to be sodium (*Z*)-5,6-dihydroxystyryl sulfate.

Although many compounds containing sulfate groups have been obtained from marine organisms,[6] there are very few enol sulfates among them.[7]

When fertilized sea urchin eggs were cultured from fertilization in the presence of **1** at a concentration of 60 μM or greater, they blastulated normally after passing through a rapid cleavage period, and formed cilia on schedule; the ciliated blastulae were unable to hatch, however. Furthermore, **1** at 30 μM inhibited by 50% the hatching enzyme activity when the activity was assayed by the dissolution of the fertilization envelope of methanol-fixed, 2-to 4-cell stage embryos. The use of **1** offers much promise for analyzing the degradation of the fertilization envelope and related extracellular matrix components of an embryo.

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4. **1**: ^{1}H NMR (500 MHz, D_2O, chemical shifts expressed downfield of external TMS) δ 5.52 (d, *J* = 7.3 Hz, H-2), 6.41 (d, *J* = 7.3 Hz, H-1), 6.69 (d, *J* = 8.3 Hz, H-7), 6.85 (dd, *J* = 8.3 and 1.8 Hz, H-8), and 7.05 (d, *J* = 1.8 Hz, H-4); (-)FABMS *m/z* 323 $[M+glycerol-Na]^-$, 231 $[M-Na]^-$, and 97 $[HSO_4]^-$.
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