A New Sulfated Alkene from the Ophiuroid *Ophiocoma echinata*

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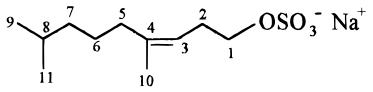
A new sulfated alkene (**1**) has been isolated from the ophiuroid *Ophiocoma echinata*, and its structure was determined by spectroscopic methods to be 4,8-dimethylnon-3-ene-1-sodium sulfate. This is the first report of a sulfated alkene from an ophiuroid.

The echinoderms are a rich source of sulfated metabolites. Starfish and sea cucumbers contain saponins, having both triterpenoid (sea cucumber) and steroidal (starfish) sulfated aglycons. Starfish also contain polyhydroxylated steroidal glycosides sulfated at the steroidal skeleton or the carbohydrate. Ophiuroids are characterized by their content of polar sulfated steroidal polyols and the lack of saponins.[1] These types of compounds have shown a broad spectrum of biological activities, such as antiviral properties,[2] cytotoxic action, and inhibition of protein tyrosine kinases.[3]

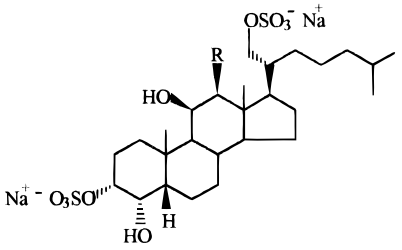
To date, only a few examples of sulfated alkanes and alkenes have been reported from marine organisms.[4,5] Three sulfated hydrocarbons isolated from the sponge *Toxadocia cylindrica* showed potent inhibitory activity against thrombin,[6] while four antibacterial and antifungal sulfated alkanes and alkenes were reported from the ascidian *Halocynthia roretzi*.[7] Among echinoderms, Findlay *et al.* have reported the isolation of two sulfated hydrocarbons from the starfish *Asterias forbesi*,[8] and from the sea cucumber *Cucumaria frondosa*.[9]

In a continuation of our studies on the metabolites of echinoderms,[10] we have examined the ophiuroid *Ophiocoma echinata* Lamark (Ophiuroidea) and have isolated a new sulfated alkene (**1**), together with two sulfated polyhydroxysteroids, 5β-cholestane-3α,4α,11β,12β,21-pentol 3,21-disulfate (**2**) and 5β-cholestane-3α,4α,11β,-21-tetrol 3,21-disulfate (**3**), previously reported from this organism.[11]



1

The MeOH extract of the ophiuroid (1.3 kg, wet wt), collected off Neguange Bay, Colombia (at a depth of 1 m) was evaporated and the residue partitioned between H_2O and *n*-hexane. The aqueous residue was then lyophilized, and the solid material (13.9 g) thus obtained was subjected to vacuum dry-column chromatography on reversed-phase (35−75μ) Si gel. Final purification of the fraction containing the sulfated compounds was accomplished by reversed-phase HPLC to give the pure compounds **1** (10.6 mg), **2** (14.2 mg), and **3** (7.6 mg). The known compounds (**2** and **3**) were identified by comparison of their ^{1}H-, ^{13}C-NMR, FABMS, and optical rotation data with published data.[11]



2 R = OH
3 R = H

The ^{13}C-NMR spectrum of **1** showed 11 signals, and DEPT measurements revealed the presence of three methyl groups, five methylene groups, one methine group, and a trisubstituted double bond. The presence of a methylene signal at δ 68.8 and the high polarity of **1** suggested the presence of a primary alcohol esterified with sulfuric acid. This was further corroborated by strong IR (KBr) absorption bands at 1241 and 1215 cm^{-1}, typical for the sulfate moiety.[12] Negative-ion FABMS of **1** showed a quasi-molecular anion peak at *m*/*z* 249, corresponding to a formula $C_{11}H_{21}SO_4$, while the positive FABMS exhibited two molecular ion species at *m*/*z* 295 ($C_{11}H_{21}SO_4Na$ + Na) and 311 ($C_{11}H_{21}SO_4Na$ + K).

The ^{1}H-NMR spectrum exhibited a doublet at δ 0.88 (6H, J = 6.6 Hz) being part of an isopropyl group that was coupled with a methine at δ 1.58, which in turn correlated with a methylene at δ 1.22 in the ^{1}H−^{1}H COSY spectrum. The triplet at δ 3.92 (2H, J = 7.0 Hz) was assigned to H_2-1 from the HETCOR spectrum and was consistent with the presence of a $-CH_2OSO_3Na$ unit. From the ^{1}H−^{1}H COSY spectrum it was determined that H_2-1 was coupled to the broad quartet at δ 2.36 (2H, J = 7.0 Hz), assigned to H_2-2, which in turn was coupled to Me-10 at δ 1.68 and to the broad triplet at δ 5.17 (H-3). This latter olefinic proton was coupled to allylic methylene protons at δ 2.04 (H-5) and to the allylic methyl group at δ 1.68. Based on the multiplicities of the signals for H-2 and H-5 and the NOESY crosspeak δ 2.04/1.68 (H_2-5/Me-10), the position of Me-10 was assigned as C-4.

^{13}C-NMR chemical shifts of all carbons were assigned from the HETCOR spectrum. The geometry of the double bond was deduced to be *Z* from the ^{13}C-NMR chemical shift of the allylic methyl group (δ 23.6)[13] and NOESY crosspeak δ 2.04/2.36 (H_2-5/H_2-2).

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The finding of **1** from *O. echinata* is the first report of a sulfated alkene from an ophiuroid.

Experimental Section

General Experimental Procedures. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker ACE-200 instrument. FABMS were obtained on a VG-ZAB mass spectrometer. IR spectra were recorded on a Nicolet Magna-550 FT-IR spectrometer. Preparative HPLC was carried out on a SP liquid chromatograph equipped with a Spectra Series P100 solvent delivery system, a Rheodyne manual injector, and a refractive index detector using a C_{18} Bondclone 10-μ column (30 cm × 7.8 mm i.d.); flow rate 2 mL/min. TLC was performed on precoated Si gel F_{254} and C_{18} reversed-phase plates.

Animal Material. Specimens of *O. echinata* (1.3 kg) were collected off Neguange Bay, Colombia. The animals were identified by Dr. Sven Zea, and a voucher specimen of the organism is preserved at the Instituto de Investigaciones Marinas de Punta Betín, Santa Marta, Colombia (INV 6296-EQU 179).

Extraction and Isolation. The animals, frozen prior to storage, were homogenized in MeOH (2 L) for 12 h and centrifuged. The MeOH was evaporated, and the residue was partitioned between H_2O and *n*-hexane. This aqueous residue was lyophilized, and the solid material (13.9 g) thus obtained was subjected to vacuum dry-column chromatography on Davisil C_{18} reversed-phase (35−75 μ) using H_2O, H_2O−MeOH mixtures with increasing amounts of MeOH, and finally MeOH. Each fraction was analyzed by TLC on SiO_2 in *n*-BuOH−HOAc−H_2O (4:5:1) (upper layer) and by C_{18} reversed-phase TLC [MeOH−H_2O (65:35)] and detected by spraying with H_2SO_4. Final purification of the fraction containing the sulfated compounds was accomplished by HPLC on a C_{18} Bondclone column with MeOH−H_2O (50:50), to give the pure compounds **1** (10.6 mg), **2** (14.2 mg), and **3** (7.6 mg).

4,8-Dimethylnon-3-ene-1-sodium sulfate (1): was obtained as a white powder; IR (KBr) ν_{max} 2960−2863 (CH, aliphatic and alkene), 1650 (C=C, alkene), 1241, 1215 (sulfate moieties), 1071, 992; ^{1}H NMR (CD_3OD, 200.1 MHz) δ 0.88 (6H, d, J = 6.6 Hz, Me-9, Me-11), 1.22 (2H, m, H-7), 1.38 (2H, m, H-6), 1.58 (1H, m, H-8), 1.68 (3H, br s, Me-10), 2.04 (1H, t, J = 7.0 Hz, H-5), 2.36 (2H, q, J = 7.0 Hz, H-2), 3.92 (2H, t, J = 7.0 Hz, H-1), 5.17 (1H, br t, J = 7.0 Hz, H-3); ^{13}C NMR (CD_3-OD, 50.3 MHz) δ 139.3 (s, C-4), 121.0 (d, C-3), 68.8 (t, C-1), 40.0 (t, C-7), 32.9 (t, C-5), 29.1 (d, C-8), 29.0 (t, C-2), 26.8 (t, C-6), 23.6 (q, C-10), 23.0 (q, C-9 and C-11).

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