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# 5α,8α-Epidioxysterol sulfate from a diatom *Odontella aurita*

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**Abstract**

A 5α,8α-epidioxysterol sulfate was isolated from the cultured diatom *Odontella aurita* (NIES 589), and its structure was elucidated by spectroscopic methods.


*Keywords:* *Odontella aurita*; Diatom; 5α,8α-Epidioxysterol; Sulfate

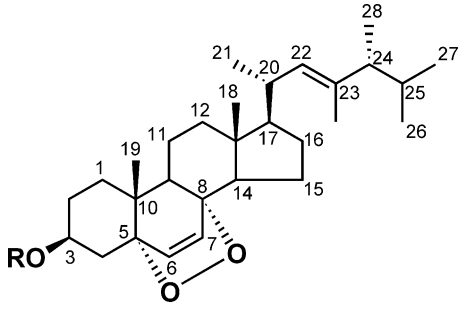
## 1. Introduction

*Odontella aurita* (Lyngbye) Agardh (Eupodiscaceae) is a diatom, which looks like a bobbin with a length of 10–95 μm and grows in northern coasts (Chihara and Murano, 1997). No studies have been reported on the chemical constitutents of *O. aurita*, while several fatty acids and sterols were reported as constituents in *O. weissflogii* (Skerratt et al., 1998). During our search for bioactive natural products from microalgae, we recently investigated the chemical constituents of the cultured *O. aurita* (NIES 589). Here we describe isolation and structure elucidation of a new sterol sulfate (**1**).

## 2. Results and discussion

The diatom *O. aurita* (NIES 589) was unialgally cultured statically at 25 °C for 3 weeks in a seawater medium enriched with f/2 supplement. The EtOAc-soluble portion of the MeOH extract of the harvested algal cells was subjected to column chromatography over silica gel eluted with $CHCl_3$/MeOH (4:1 to 1:1), followed by purification using reversed-phase HPLC on ODS (80% MeOH) to afford a 5α,8α-epidioxysterol sulfate (**1**).

Compound **1** was obtained as colorless amorphous solid and was shown to have the molecular formula $C_{29}H_{46}O_6SNa$ from the observation of a quasi-molecular ion peak in high-resolution (HR) FABMS. The IR absorption band of **1** at 1240 $cm^{-1}$ was indicative of the presence of a sulfate group, and no particular UV absorption was observed for **1**. The $^1H$ NMR spectrum of **1** showed signals due to seven methyl groups: four secondary methyls [$\delta_H$ 0.94, 3H, *d*, $J=6.3$ Hz ($H_3$-21); $\delta_H$ 0.87, 3H, *d*, $J=6.6$ Hz, ($H_3$-26); $\delta_H$ 0.80, 3H, *d*, $J=6.6$ Hz ($H_3$-27); $\delta_H$ 0.96, 3H, *d*, $J=6.6$ Hz ($H_3$-28)] and two tertiary methyls [$\delta_H$ 0.88, 3H, *s* ($H_3$-18); $\delta_H$ 0.92, 3H, *s* ($H_3$-19)], and one vinyl methyl group [$\delta_H$ 1.53, 3H, *d*, $J=1.3$ Hz ($H_3$-29)]. The $^1H$ NMR aided spectrum, with the $^{13}C$ NMR spectral data, suggested the presence of two olefins (one disubstituted and one trisubstituted ones). Olefin protons [$\delta_H$ 6.60, 1H, *d* (H-7); $\delta_H$ 6.23, 1H, *d*, (H-6)] with *cis*-coupling ($J=8.5$ Hz), together with two oxygenated quaternary carbons on C-5 ($\delta_C$ 83.3) and C-8 ($\delta_C$ 80.7), were suggestive of the presence of a peroxide structure. The $^1H$–$^1H$ COSY spectrum of **1** revealed the following five partial structures for H-1–H-4, H-6–H-7, H-9–H-12, H-14–H-22, and H-24–H-28.




**1** R = $SO_3Na$

**2** R = H

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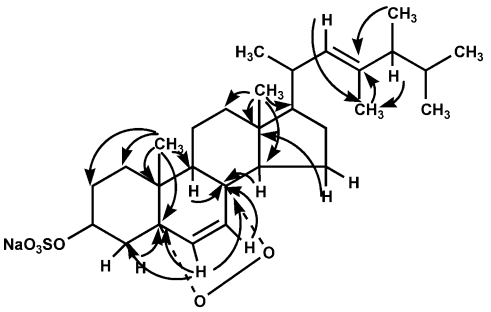


Fig. 1. Selected HMBC correlations.

The HMBC spectrum of **1** afforded long-range $^{1}H$–$^{13}C$ correlations shown in Fig. 1. These structural features were similar to those of a desulfated derivative (**2**), which was previously obtained from an edible mushroom *Lentinus edodes* (Yaoita et al., 1998). From the detailed comparison of the spectral data between **1** and **2**, compound **1** was assigned as a sulfate ester at C-3 hydroxyl group of **2** [the $^{13}C$ chemical shift of C-3: $\delta_C$ 75.0 for **1** and $\delta_C$ 66.3 for **2**].

A sterol sulfate, hymenosulfate, was isolated from a marine haptophyte *Hymenomonas* sp. (Kobayashi et al., 1989), whereas 5α,8α-epidioxysterols were obtained from sea hare *Aplysia juliana* (Miyamoto et al., 1988). No 5β,8β-isomer of **1** was obtained from this diatom. Compound **1** showed no antimicrobial activity against *Bacillus subtilis* and no toxicity against *Artemia salina*.

## 3. Experimental

### 3.1. General

Optical rotations were recorded on a JASCO J-20. UV spectra were obtained on a Hitachi U-3400 spectrophotometer. IR spectra were measured from samples on KBr disks in a Hitachi 260-10 infrared spectrophotometer. NMR spectra were recorded on Jeol JNM GSX-A400 and ecp600 spectrometers. HR-FAB–MS were acquired on a JMS HX-110 mass spectrometer.

### 3.2. Extraction and isolation

A voucher specimen of *O. aurita* (NIES 589) is deposited at National Institute for Environmental Studies, Tsukuba, Japan. The diatom *O. aurita* (NIES 589) was unialgally cultured at 25 °C for 3 weeks statically in a seawater medium enriched with f/2 supplement (Erata, 1997). EtOAc-soluble portion of the MeOH extract of the harvested algal cells (120 g, wet weight, from 340 l of culture) was subjected to CC over silica gel eluted with $CHCl_3$/MeOH (4:1 to 1:1), followed by purification using reversed-phase HPLC on ODS (80% MeOH) to afford a 5α,8α-epidioxysterol sulfate (**1**).

### 3.3. Compound *1*

Colorless amorphous solid; [α] −1.2° (*c* 0.23, MeOH), [α] −5.3° (*c* 0.23, MeOH), IR (KBr) $\nu_{max}$ 1735, 1650, and 1240 $cm^{-1}$; $^{1}H$ NMR (in $CD_3OD$): $\delta_H$ 6.60 (1H, *d*, *J* = 8.5 Hz; H-7), 6.23 (1H, *d*, *J* = 8.5 Hz; H-6), 4.93 (1H, *d*, *J* = 9.9 Hz; H-22), 4.51 (1H, *m*; H-3), 1.53 (3H, *d*, *J* = 1.3 Hz; $H_3$-29), 0.96 (3H, *d*, *J* = 6.6 Hz; $H_3$-28), 0.94 (3H, *d*, *J* = 6.3 Hz; $H_3$-21), 0.92 (3H, *s*; $H_3$-19), 0.88 (3H, *s*; $H_3$-18), 0.87 (3H, *d*, *J* = 6.6 Hz; $H_3$-26), and 0.80 (3H, *d*, *J* = 6.6 Hz; $H_3$-27); $^{13}C$ NMR (in $CD_3OD$) $\delta_C$ 137.0 (C-23), 136.6 (C-6), 132.6 (C-22), 131.6 (C-7), 83.3 (C-5), 80.7 (C-8), 75.0 (C-3), 58.3 (C-17), 53.0 (C-14), 53.0 (C-24), 51.7 (C-9), 45.7 (C-6), 39.2 (C-10), 38.0 (C-12), 35.7 (C-1), 35.3 (C-4), 35.3 (C-20), 32.0 (C-25), 28.5 (C-2), 28.5 (C-16), 24.3 (C-15), 22.2 (C-27), 21.5 (C-11), 20.9 (C-25), 20.5 (C-26), 18.5 (C-19), 17.4 (C-28), 13.4 (C-18), and 13.4 (C-29); FAB–MS *m*/*z* 545 $(M+H)^+$ and 567 $(M+Na)^+$; HR-FAB–MS *m*/*z* 545.2899 $(M+H)^+$ [calc. for C29H46O6SNa, (M + H) 545.2885].

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