

## Two New Aurones from Marine Brown Alga *Spatoglossum variable*

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**Two new aurones, 4'-chloro-2-hydroxyaurone (1) and 4'-chloroaurone (2) were isolated from *Spatoglossum variable*. The structures of these compounds were elucidated by modern spectroscopic techniques.**

**Key words** *Spatoglossum variable*; Dictyoaceae; aurone; 4'-chloro-2-hydroxyaurone; 4'-chloroaurone

In recent years several new bioactive compounds have been isolated from marine organisms.<sup>1)</sup> However, despite recent progress in the area of marine chemistry, organisms of the Arabian Sea are little explored, which stimulated us to investigate the chemical constituents of such marine organisms. *Spatoglossum variable* FIGARI *et De* NOTAR. is a brown alga belonging to the family Dictyoaceae.<sup>2)</sup> *S. variable* grows on the mid and littoral rocks along the coastline of Pakistan near Karachi city.<sup>3)</sup> Previously we have isolated two new compounds, spatozoate and varninasterol along with four known compounds, fucosterol, cholesta-5-ene-23-yne-3 $\beta$ -ol, apiole and nothoapiole from this alga.<sup>4)</sup> This paper describes the isolation of two aurone derivatives (**1**, **2**) from the methanolic extracts of this seaweed.

The HREI-MS (high resolution electron impact mass spectrum) of compound **1** showed the  $M^+$  at  $m/z$  274.5231, corresponding to the molecular formula  $C_{15}H_{11}O_3Cl$  (Calcd 274.5238) indicating ten degrees of unsaturation in the molecule. The mass spectrum of **1** also showed the base peak at  $m/z$  139.9977, which was due to the fragment  $C_8H_9Cl$ . Some other significant ions were present at  $m/z$  256.0236 [ $C_{15}H_9O_2Cl$ ] $^+$ , 228.0305 [ $C_{14}H_9OCl$ ] $^+$  and 118.0426 [ $C_8H_6O$ ] $^+$ . The prominent fragment ions are presented in Chart 1.

The UV (ultraviolet) spectrum exhibited absorptions at 248, 221, 202 nm which indicated the presence of an aromatic conjugated system.<sup>5)</sup> The IR (infrared) spectrum of **1** showed strong absorptions at 804, 1011, 1695 and 3401  $cm^{-1}$  for C–Cl, C–O, C=O and OH, respectively.<sup>5)</sup> The hydrogen bonding resulted in the lowering of the carbonyl frequency (*i.e.* 1695  $cm^{-1}$ ).

Analysis of the  $^1H$ -NMR spectrum of **1** indicated the presence of two sets of signals for a total of eight aromatic protons and two benzylic methylene protons. This showed the aurone nature of the compound **1**.<sup>6,7)</sup> Signals in the aromatic region indicated the presence of two benzene rings in the molecule. The protons of ring A resonating at  $\delta$  8.15 (1H, dd,  $J_{4,5}=7.0$  Hz,  $J_{4,6}=1.0$  Hz), 7.53 (1H, dt,  $J_{5,4}=7.0$  Hz,  $J_{5,7}=1.0$  Hz), 7.39 (1H, dt,  $J_{6,5}=7.0$  Hz,  $J_{6,4}=1.0$  Hz) and 7.24 (1H, dd,  $J_{5,6}=7.0$  Hz,  $J_{7,5}=1.0$  Hz) were assigned to H-4, H-5, H-6 and H-7, respectively. The aromatic protons on the ring C appeared as a pair of *ortho*-coupled doublets at  $\delta$  7.93 (H-2', H-6') and 7.43 (H-3', H-5'). The C-2 methylene protons, on the other hand, resonated as a singlet at  $\delta$  4.60. The COSY-45 $^\circ$  spectrum of **1** exhibited coupling between H-4 ( $\delta$  8.15), H-5 ( $\delta$  7.53), H-6 ( $\delta$  7.39) and H-7 ( $\delta$  7.24) of ring A. Couplings between H-2'/H-6' and H-3'/H-5' were

also observed.

The  $^{13}C$ -NMR, BB (broad-band decoupled) and DEPT (distortionless enhancement by polarization transfer) spectra of **1** showed resonances for all fifteen carbon atoms comprising one methylene, eight methine and six quaternary carbon atoms. Two downfield carbon signals at  $\delta$  103.4 (C-2) and 169.0 (C-3) indicated the presence of one quaternary hydroxyl and one ketone function in the molecule, respectively.

In the HMQC (heteronuclear multiple quantum coherence) spectrum, the protons of ring A were observed at  $\delta$  8.15 (H-4), 7.53 (H-5), 7.39 (H-6) and 7.24 (H-7) and were found to be coupled with the carbon atoms resonating at  $\delta$  135.4, 134.3, 127.4 and 128.9. In addition, the protons of ring C resonating at  $\delta$  7.93 (H-2'/H-6') and 7.43 (H-3'/H-5') were also found to be coupled with the carbons which resonated at  $\delta$  128.8 (C-2'/C-6') and 129.5 (C-3'/C-5'), respectively. The HMBC (heteronuclear multiple bond connectivity) spectrum of **1** displayed correlations of H-4 ( $\delta$  8.15) with C-9 ( $\delta$  121.2), C-8 ( $\delta$  157.8) and C-4 ( $\delta$  135.4) which indicated the close proximity of H-4 to the carbonyl carbon and ether functions. H-7 ( $\delta$  7.24) showed HMBC interactions with C-9 ( $\delta$  121.2) and C-8 ( $\delta$  157.8), whereas H<sub>2</sub>-10 ( $\delta$  4.60) displayed interactions with C-2 ( $\delta$  103.4), C-1 ( $\delta$  122.8) and C-2/C-6 ( $\delta$  128.8). These interactions further supported the proposed structure **1** for 4'-chloro-2-hydroxyaurone (**1**).

Compound **1** is a racemic mixture of *R* and *S* epimers resulting from the epimerization of OH group at C-2. This was

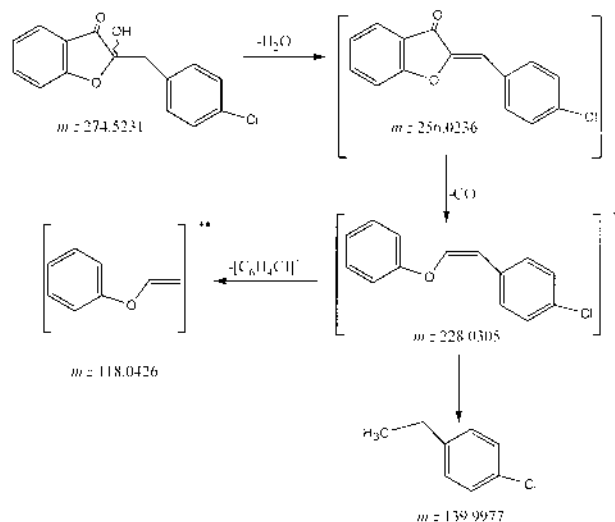
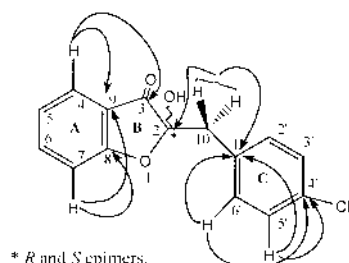
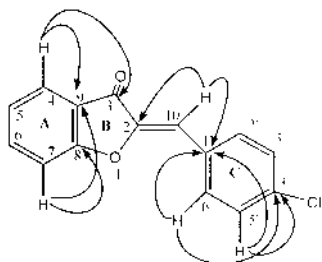


Chart 1. The Mass Fragmentation in Compound **1**

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Table 1.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR (500 MHz and 75 MHz in  $\text{CDCl}_3$ ) Data of Compounds **1** and **2**

Carbon No.	<b>1</b>		<b>2</b>	
	$^1\text{H}$ -NMR, $\delta$ (ppm), ( $J$ =Hz)	$^{13}\text{C}$ -NMR, $\delta$ (ppm)	$^1\text{H}$ -NMR, $\delta$ (ppm), ( $J$ =Hz)	$^{13}\text{C}$ -NMR, $\delta$ (ppm)
2	—	103.4	—	152.7
3	—	169.0	—	182.0
4	8.15 (dd, $J_{4,5}=7.0$ , $J_{4,6}=1.0$ )	135.4	8.28 (dd, $J_{4,5}=7.0$ , $J_{4,6}=1.0$ )	129.6
5	7.53 (dt, $J_{5,4}=7.0$ , $J_{5,7}=1.0$ )	134.3	7.69 (dt, $J_{5,4}=7.0$ , $J_{5,7}=1.1$ )	135.0
6	7.39 (dt, $J_{6,5}=7.0$ , $J_{6,4}=1.0$ )	127.4	7.47 (dt, $J_{6,5}=7.0$ , $J_{6,4}=1.1$ )	126.0
7	7.24 (dd, $J_{7,6}=7.0$ , $J_{7,5}=1.0$ )	128.9	7.49 (dd, $J_{7,6}=7.0$ , $J_{7,5}=1.1$ )	128.4
8	—	157.8	—	162.0
9	—	121.2	—	120.7
10	4.60 (s)	44.8	6.91 (s)	102.2
1'	—	122.8	—	130.5
2'	7.93 (d, $J_{2',3'}=8.5$ )	128.8	7.79 (d, $J_{2',3'}=8.5$ )	126.5
3'	7.43 (d, $J_{3',2'}=8.5$ )	129.5	7.42 (d, $J_{3',2'}=8.5$ )	129.1
4'	—	136.7	—	137.3
5'	7.43 (d, $J_{5',6'}=8.5$ )	129.5	7.42 (d, $J_{5',6'}=8.5$ )	129.1
6'	7.93 (d, $J_{6',5'}=8.5$ )	128.8	7.79 (d, $J_{6',5'}=8.5$ )	126.5

Fig. 1. Important HMBC Interactions in **1**Fig. 2. Important HMBC Interactions in **2**

inferred from the optical rotation measurement ( $[\alpha]_D$ ) which was found to be zero.

Compound **2**,  $\text{C}_{15}\text{H}_9\text{O}_2\text{Cl}$ , differs from compound **1** only by the presence of a double bond at C-2/C-10 instead of a hydroxyl group at C-2, *i.e.* compound **2** may result naturally from the dehydration of compound **1**. The structure was further supported by mass,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR experiments. The  $^1\text{H}$ -NMR spectrum of **2** displayed a vinylic signal at  $\delta$  6.91 which was assigned to H-10, whereas its  $^{13}\text{C}$ -NMR spectrum showed two downfield signals at  $\delta$  102.2 and 152.7, which were assigned to olefinic C-10 and C-2, respectively.

The *Z*-geometry across the C-2/C-10 double bond in compound **2** was suggested on the basis of the calculation of heat of formations of both the *Z* and *E* isomers by AM1 (the Austin Model 1) method.<sup>8,9</sup> The result indicated that the *Z* isomer is more stable than the *E* isomer by 1.98 kcal/mole.

Since compound **2** is probably produced through the dehydration of compound **1**, the formation of thermodynamically more stable *Z* isomer is more likely.<sup>8,9</sup>

#### Experimental

The IR spectra were recorded on a JASCO A-302 spectrophotometer. The UV spectra were recorded on a Hitachi U-3200 spectrophotometer. Optical rotation was measured on a JASCO-DIP-360 digital polarimeter in the  $\text{CHCl}_3$ . The EI (electron impact), FAB (fast atom bombardment) and HREI-MS were recorded on JMS HX110 with data system DA 5000 and on a MAT 112S mass spectrometer. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR were recorded at 400 and 125 MHz, respectively, and in  $\text{CDCl}_3$  on a Bruker AM 400 and an AMX 500 MHz spectrometer.

**Plant material** *S. variabile* FIGARI *et* DE NOTAR. (35 kg) was collected from the Buleji coastline of Karachi in December 1996. A voucher specimen has been deposited in the Department of Botany, University of Karachi.

**Extraction and Isolation** The alga was washed with water, air-dried for five days and then soaked in methanol (50l) for one week. The methanolic extract of the alga was filtered and then evaporated under vacuum. The residue (512.0 g) was triturated with distilled water and successively fractionated with *n*-hexane,  $\text{CHCl}_3$ , EtOAc and *n*-BuOH. The concentrated EtOAc extract (49.2 g) was loaded onto a silica gel (804 g) column and then subjected to gradient elution with mixtures of *n*-hexane- $\text{CHCl}_3$  and  $\text{CHCl}_3$ -MeOH. The fraction obtained on elution with  $\text{CHCl}_3$ -MeOH (9:1) was subjected to preparative TLC using  $\text{CHCl}_3$ -MeOH- $\text{NH}_4\text{OH}$  (9.5:0.5:3 drops) as the solvent system to afford the new compounds **1** and **2**.

**4'-Chloro-2-hydroxyaurone (1):** Colorless solid (14.8 mg,  $2.8 \times 10^{-3}\%$ ). mp 225 °C. IR ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ : 804 (C-Cl), 1011 (C-O), 1695 (C=O), 3401 (OH). UV  $\lambda_{\text{max}}$   $\text{CHCl}_3$ : 202 (log  $\epsilon=5.02$ ) nm.  $[\alpha]_D^{25}=0$  ( $\text{CHCl}_3$ ).  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ ) and  $^{13}\text{C}$ -NMR (Table 1). EI MS:  $m/z$  274.0, 256.9, 228.0, 139.0, 111.0. HREI MS:  $m/z$  274.5231 ( $\text{C}_{15}\text{H}_{11}\text{O}_3\text{Cl}$ , Calcd 274.5238), 256.0236 ( $\text{C}_{15}\text{H}_9\text{O}_2\text{Cl}$ ), 228.0305 ( $\text{C}_{14}\text{H}_9\text{OCl}$ ), 139.9977 ( $\text{C}_8\text{H}_9\text{Cl}$ ), 118.0426 ( $\text{C}_8\text{H}_6\text{O}$ ).

**4'-Chloroaurone (2):** Colorless solid compound (17.3 mg,  $3.3 \times 10^{-3}\%$ ). mp 206 °C, IR ( $\text{CHCl}_3$ )  $\text{cm}^{-1}$ : 792 (C-Cl), 1011 (C-O), 1721 (C=O). UV  $\lambda_{\text{max}}$   $\text{CHCl}_3$ : 204 (log  $\epsilon=4.90$ ) nm.  $^1\text{H}$ -NMR ( $\text{CDCl}_3$ ) and  $^{13}\text{C}$ -NMR (Table 1). EI-MS:  $m/z$  256, 228.0, 165.0, 139.0, 111.0. HREI-MS:  $m/z$  256.0236 ( $\text{C}_{15}\text{H}_9\text{O}_2\text{Cl}$ , Calcd 256.0231), 139.9977 ( $\text{C}_8\text{H}_9\text{Cl}$ ), 228.0305 ( $\text{C}_{14}\text{H}_9\text{OCl}$ ), 118.0426 ( $\text{C}_8\text{H}_6\text{O}$ ).

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