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Steroids from the red alga *Acanthophora spicifera*

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1. Subject and source

The alga *Acanthophora spicifera* (Vahl) Borgesen (Ceramiales: Rhodophyta) is a rhodophycean alga with wide distribution throughout the tropics and subtropics (Kilar and McLachlan, 1986) and one of the most abundant red algal species found on reef flats (Joikel and Morrissey, 1986). Investigation with this species is rather scarce (Aihara and Yamamoto, 1968; Wahidulla et al., 1986, 1991, 1998; Wahidulla and Kamat, 1991; Prakash et al., 1989; Wang et al., 1998, 2003). Therefore, as a part of the systematic chemical research with rhodophycean algae, we report here the results concerning the main sterols isolated from *A. spicifera*, an abundant alga in Southern Brazil.

2. Previous work

In previous phytochemical studies, 5α-cholestane-3,6-dione (Wahidulla et al., 1991), cholest-4-ene-3-one (Wahidulla and Kamat, 1991), 11α-hydroxy-5α-cholestane-3,6-dione (Prakash et al., 1989) cholest-5-en-3α-ol (Wahidulla et al., 1986) and cholest-4-ene-3α,6β-diol (Wahidulla et al., 1998) from *A. spicifera* were described. Flavonoids such as quercetin, (−)−catechin and tiliroside (Wang et al., 1998, 2003), acanthophorin A and B (Zeng et al., 2001), acid derivates (Wahidulla et al., 1986; Wang et al., 2003), dipeptides (Wahidulla et al., 1991) and antheraxanthin (Aihara and Yamamoto, 1968) were also described.

3. Materials and methods

The material used in this investigation was collected in December 2005 at Lagoa da Conceição, Florianópolis, Brazil and the systematic identification was performed at the Phycology Laboratory, Federal University of Santa Catarina, Brazil.

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Fresh material (4 kg) was extracted by maceration (7 days) with ethanol. After filtration, the extract was evaporated to dryness under reduced pressure at 60 °C. The extract (120 g) was dissolved in water and was further extracted with ethyl acetate three times (400 mL). The solvent was removed under reduced pressure at 60 °C to yield 14.7 g of ethyl acetate fraction.

Part of this material (8 g dry weight) was submitted to column chromatography over silica gel (Merck 60), collecting fractions of 50 mL, starting with 20% ethyl acetate in *n*-hexane (fractions 1–30); 40% ethyl acetate in *n*-hexane (fractions 31–70); 60% ethyl acetate in *n*-hexane (fractions 71–105) and 100% ethyl acetate (fractions 105–120). Fractions 98–102 afforded a residue of 2 g containing two main components (1 and 2), detected using anisaldehyde·H_2SO_4 reagent. Compound **1** was purified by silica gel column eluted with a gradient of *n*-hexane–chloroform, with increasing polarity (from 80 to 100% $CHCl_3$), resulting in 23 mg of the pure compound. Compound **2** was purified by silica gel column eluted with chloroform–ethyl acetate (8:2) resulting 20 mg of the pure compound.

The structures of the compounds were established through ^1H and ^{13}C NMR (Bruker Avance 2), IR (Shimadzu – IR Prestige-21) and MS (Shimadzu QP-5000) analysis and comparison with literature data.

4. Results and discussion

Compound **1** was obtained as a white powder. MS data showed a molecular ion peak at *m/z* 398 corresponding to the molecular formula $C_{27}H_{42}O_2$. The IR spectrum (KBr) revealed the presence of carbonyl groups (ν_{max} 1680 cm^{-1}). The ^{13}C NMR spectrum showed the presence of 27 carbons, suggesting a steroidal structure. The ^1H NMR and ^{13}C NMR spectra displayed signals for five methyl resonances suggesting a cholestane structure (Migliuolo et al., 1990): singlets at δ 0.72 and 1.16 (H-18 and H-19, respectively) and doublets at δ 0.92, 0.87 and 0.86 (H-21, H-26 and H-27, respectively). A singlet at δ 6.17 was assigned to an olefinic proton. This was confirmed by the ^{13}C NMR spectrum, which showed the presence of two carbonyl groups (δ 199.5, 202.3) besides a carbon–carbon double bond conjugated to carbonyl (δ 161.0, 125.4). Cholestane derivatives were already described for *A. spicifera* (Wahidulla et al., 1987, 1991, 1998). Comparison with literature data (Wahidulla et al., 1987) indicated similarity with cholestane-3,6-dione, except for the assignments of atoms near or related to the double bond. Detailed analysis of a complete set of 2D NMR spectra revealed compound **1** as cholest-4-en-3,6-dione (Fig. 1).

Compound **2** was also obtained as a white powder. MS spectra showed a molecular ion peak at *m/z* 400 corresponding to a molecular formula of $C_{27}H_{44}O_2$. The IR spectrum (KBr) revealed the presence of carbonyl (ν_{max} 1683 cm^{-1}) and hydroxyl groups (ν_{max} 3475 cm^{-1}). The ^1H NMR spectrum, considered together with the ^{13}C NMR clearly indicated a steroidal structure similar to compound **1** with five methyl groups: two singlets at δ 0.74 and 1.38 (H-18 and H-19, respectively) and three doublets at δ 0.92, 0.87 and 0.86 (H-21, H-26 and H-27, respectively). A sharp singlet at δ 5.81 was due to an olefin proton, and a sharp pseudo-triplet (double doublet) at δ 4.35 was assigned to a proton geminal to a hydroxyl group. The ^{13}C NMR spectrum showed the presence of a carbonyl group at δ 200.53 ppm and a carbon bearing a hydroxyl group at δ 73.3 ppm. Besides, the spectrum indicated the presence of a double bond conjugated to carbonyl (δ 168.4, 126.3). Careful spectral analysis revealed compound **2** as 6β-hydroxy-cholest-4-en-3-one (Fig. 1). Bultel-Poncé et al. (2002) described 6α-hydroxy-cholest-4-en-3-one from the red alga *Hypnea musciformis*. The signal of H6 at δ 4.35 ppm in this work was described as follows: "H6 resonating as a sharp singlet implies a weak coupling constant value with H-7 so H-6 is in the α or equatorial position". Despite the H6 assignment as in the α position, the stereochemistry of the hydroxyl at C-6 was incorrectly described in the article as 6α-hydroxy.

The ^1H NMR and ^{13}C NMR spectral data are listed in Table 1. Complete assignment of the signals was performed by interpretation of DEPT, COSY, HSQC and HMBC spectra.

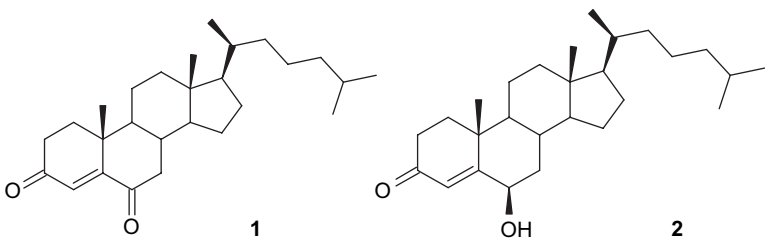

Fig. 1. Structures of the characterized steroids cholest-4-en-3,6-dione (**1**) and 6β-hydroxy-cholest-4-en-3-one (**2**).

Table 1
^{1}H and ^{13}C assignments (δ ppm) of sterols 1 and 2 in $CDCl_3$ (500 and 125 MHz, respectively)

C	1		2	
	^{1}H (multiplicity, J Hz)	^{13}C	^{1}H (multiplicity, J Hz)	^{13}C
1	1.91–2.15	35.5	1.72–2.04	37.1
2	2.46–2.52	33.9	2.38–2.51	34.2
3	–	199.5	–	200.5
4	6.17	125.4	5.81	126.3
5	–	161.0	–	168.4
6	–	202.3	4.35 (dd)	73.3
7	2.03–2.66	46.8	1.25–2.01	38.5
8	1.89	34.2	1.26	29.7
9	1.37	50.9	0.91	53.6
10	–	39.8	–	37.9
11	1.51–1.62	20.8	1.51	20.9
12	1.26–2.13	39.1	2.05	39.6
13	–	42.5	–	42.5
14	1.15	55.9	1.02	55.9
15	1.61	23.9	1.60	24.1
16	1.52–1.88	27.9	1.85	28.1
17	1.17	56.5	1.12	56.1
18	0.72 (s)	11.9	0.74 (s)	11.9
19	1.16 (s)	17.5	1,38 (s)	19.5
20	1.39	35.6	1.38	35.7
21	0.94 (d, 6.6)	18.6	0.92 (d, 6.6)	18.6
22	1.34	36.0	1.00–1.34	36.1
23	1.13–1.33	23.8	1.15–1.34	23.8
24	1.13	39.4	1.14	39.5
25	1.25	29.7	1.52	27.9
26	0.86 (d, 6.6)	22.5	0.86 (d, 6.6)	22.8
27	0.86 (d, 6.6)	22.8	0.86 (d, 6.6)	22.5

The steroids cholest-4-en-3,6-dione (**1**) and 6β-hydroxy-cholest-4-en-3-one (**2**) are here reported for the first time for the genus *Acanthophora* and to the best of our knowledge are here first reported for the order Ceramiales. Compound **2** was formerly isolated from the red alga *H. musciformis* (Bultel-Poncé et al., 2002), but its configuration was described erroneously as 6α-hydroxy. Compound **1** was previously isolated from the marine sponges *Geodia cydonium* (Migliuolo et al., 1990) and *Cinachyra tarentina* (Aiello et al., 1991). However, this compound was not previously isolated from marine algae. Otherwise, there are some reports of the occurrence of similar cholesterol oxidated derivatives in *A. spicifera* (Wahidulla et al., 1986, 1991, 1998; Wahidulla and Kamat, 1991; Prakash et al., 1989) and *H. musciformis* (Bultel-Poncé et al., 2002). Therefore, it seems that the sterol profile in algae can show specific features that can be of taxonomic significance and deserve more detailed research.

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