The Structural Determination of a New Steroidal Metabolite from the Brown Alga *Sargassum asperifolium*

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An investigation of the natural products chemistry of the brown alga *Sargassum asperifolium* from the red sea yielded a new steroidal metabolite, 24-vinyl cholest -4-ene -24- ol -3 one, saringosterone (**3**), a known steroidal metabolite, 24-vinyl cholest -5-ene -3b, 24-diol, saringosterol (**4**), and known diterpene with a hydroazulene skeleton, dictyone (**1**). The identification of the isolated metabolites was established mainly by spectral methods and chemical transformation of the dictyone (**1**) to its acetate (**2**).

Key words: Sargassum, Steroids, Saringosterone

Introduction

Marine organisms have yielded a variety of secondary metabolites that possess novel chemical structures and interesting pharmacological activities (Stonik and Elyalov, 1986). Recently, researchers have described a wide range of biological activities for algal compounds including antibiotic, anti-HIV, anticoagulant, anticonvulsant, anti-inflamnatory, antineoplastic, and antitumor (Ayyad *et al.*, 2002; Lincolon *et al.*, 1991). A number of diterpenes and sterols have been isolated from the brown algae (Ayyad *et al.*, 2001; Banaigs *et al.*, 1983; Combaut *et al.*, 1980; Enoki *et al.*, 1982; Faulkner *et al.*, 1977; Franciso *et al.*, 1977).

In the course of our investigation on the biologically active components of the sargassaceae algae, we report, the isolation and characterization of a new saringosterone (**3**), a known saringosterol (**4**), and a known diterpene dictyone (**1**) from the brown alga *Sargassum asperifolium.*

Results and Discussion

An ethanolic extract of the brown alga Sargassum asperifolium was fractionated on silica gel using a gradient of hexane-ethyl acetate as gradient solvent. The fractions were monitored by TLC using hexane-ethyl acetate afford in order of elution, three compounds (**1,3,4**, Fig. 1). The structures of known compounds **1**, and its acetate **2** (Ayyad *et al.*, 2001; Enoki *et al.*, 1982) and **4** (Tsuda *et al.*, 1958) were established by comparing their physical and spectral data with those in the literature.

Compound **3**, showed in its EI MS a molecular ion at *m/z* 426, which, together with ^{13}C-NMR and HREI MS suggested a molecular formula of $C_{29}H_{46}O_2$ (*m/z* 426.3413; calcd. 426.3349). The double doublet at δ 5.75 (J = 18, 13 Hz), doublet at 5.29 (J = 13 Hz), and doublet at 5.17 (J = 18 Hz) in the ^{1}H-NMR and signals at δ 137.79 and 117.10 in the ^{13}C NMR attributable to terminal vinyl protons, three methyl doublets at 0.96, 0.87, 0.85 in the ^{1}H-NMR spectrum. These data, together with the presence in the mass spectrum of a fragment at *m/z* 271 due to the loss of the side-chain $C_{10}H_{19}O$, suggest a stigmastane skeleton with unsaturation at C-28.

The IR spectrum showed strong bands at 3420 and 1675 cm^{-1}. The above data, together with the presence of signals at δ 200.38, 172.38, 124.42 and 89.75 in the ^{13}C NMR spectrum and a signal at δ 5.74 in the ^{1}H-NMR spectrum, suggested the presence of α,β-unsaturated carbonyl group and a tertiary hydroxyl function in the molecule.

As the compound contains two double bonds and two oxygen atoms one as carbonyl and the second as tertiary hydroxyl, also has seven degrees of unsaturation revealed by mass spectrometry, it must be a tetracyclic product. Once again differences for H(3, 4 and 6) and C(3, 4, 5 and 6) between **3** and known **4** (Tsuda *et al.*, 1958) were

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Table I. ^{13}C-NMR data for compounds **1**–**4** in $CDCl_3$.

No. of C	Dictyone	Dictyone acetate	Saringosterone	Saringosterol
	1	2	3	4
1	46.31	46.62	36.55	37.15
2	34.43	34.63	34.65	32.55
3	124.02	125.70	200.38	72.47
4	143.01	140.74	124.42	37.91
5	59.71	58.00	172.38	141.41
6	74.30	79.87	33.61	122.38
7	49.74	46.95	32.68	32.33
8	24.35	23.53	36.34	36.86
9	41.22	40.42	54.43	50.76
10	153.25	152.60	39.26	37.91
11	34.70	35.31	21.67	21.73
12	38.36	39.28	40.24	40.40
13	27.66	31.02	43.06	42.99
14	217.01	215.81	56.28	57.40
15	41.54	41.52	24.83	24.96
16	18.88	18.91	28.88	29.04
17	16.78	16.00	56.51	56.54
18	107.45	108.36	12.61	12.51
19	18.00	16.48	17.32	17.33
20	18.65	18.90	36.82	36.51
21			18.37	18.37
22			32.55	32.32
23			28.93	28.92
24			89.75	89.83
25			29.03	29.16
26			19.48	20.05
27			18.04	19.54
28			137.79	137.84
29			117.10	117.00
OAc		22.42 171.50		

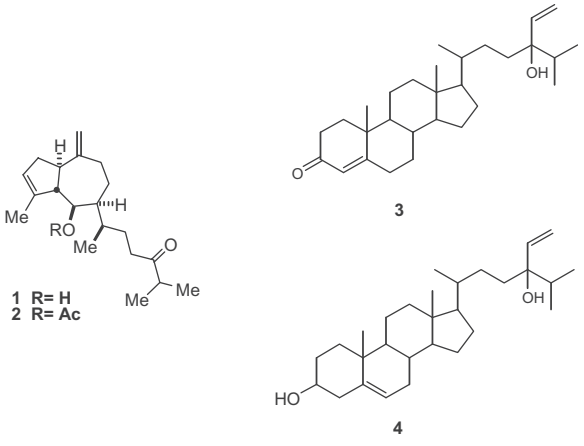

Fig. 1. Structures of new (**3**-saringosterone) and known (**1**-dictyone, **2**-dictyone acetate and **4**-saringosterol) compounds isolated from *Sargassum asperifolium.*

consistent with the former being the oxidation of the 3β OH and rearrangement of the double bond at C(5) of the latter. Thus, deshielding of C(3, 4 and 5) from 72.47, 37.91 and 141.41 in **4** to 200.38, 124.42 and 172.38 in **3** and shielding of C(6) from 122.38 in **4** to 33.61 in **3**, led to the assignment of structure **3** as α,β-unsaturated ketone, saringosterone (Fig. 1).

Experimental

Plant material, apparatus and methods

^{1}H NMR spectra were recorded at 300 or 500 MHz and ^{13}C NMR at 75 MHz. Department of Chemistry, University of Minnesota. Chemical shifts are given in (ppm) relative to TMS as internal standard. Overlapped protons in the region of 1.2–2.3 are not listed; only discrete resonances from that region of the spectrum are listed. Infra-

red spectra were determined on thin films cast from $CHCl_3$, recorded on a Protégé-400 (S. S. P.) spectrophotometer (Department of Chemistry, University of Minnesota). Electron impact mass spectra were determined at 70 eV on a Kratos MS-25 instrument. Thin-layer chromatography was performed on silica gel (kieselgel 60, F254) of 0.25 mm layer thickness. Preparative thin-layer chromatography (PTLC) was performed on silica gel plates (20 cm × 20 cm) of 500 m thickness. The alga *Sargassum asperfolium* was collected at Hurgada, just south of the Suez Gulf. A voucher sample was identified by Dr. Mohamed, A. Diayb, Department of Botany, Dammietta Faculty of Science, and Mansoura University and deposited at the Botany Department, Dammietta Faculty of Science, and Mansoura University.

Extraction and isolation

The alga was air dried in the shade at room temperature and grounded to a powder with a mortar and pestle. This powder (1 kg) was slurred in ethanol (~ 4 l) and allowed to stand at room temperature for several days. Filtration and concentration of the filtrate provided a crude extract that was dissolved in (100 ml) methanol, stored at ~ 0 °C overnight, and filtered to remove lipids. The filtrate was again evaporated under reduced pressure to afford a dark brown viscous oily residue (~ 10 g, ~ 1% of the dry weight of the alga). This residue was chromatographed on a silica gel column using a hexane- EtOAc gradient. Fractions of ~ 50 ml were collected. The fractions containing a single spot on TLC were combined and further purified by preparative TLC to afford the compounds in the following order:

*Compound (**1**)*

Identified as dictyone. Fractions 6–9 were combined. PTLC using mixtures of hexane-EtOAc (17:3 v/v) afforded dictyone **1** as a pale yellow oil (10 mg, 0.001% dry wt). IR (cm^{-1}): 3480 (OH), 1713 (C = O), 1644 (C = C); EIMS *m/z* (rel. int.): 304 (3) [M^+, $C_{20}H_{32}O_2^+$], 286 (35) [M^+-H_2O, $C_{20}H_{30}O^+$], 159 (100) [$C_{12}H_{15}^+$], 145 (15), 107 (30), 71 (40), 43 (50) [$C_3H_7^+$]. ^{1}H NMR ($CDCl_3$) 5.31 [1H, br s, H(3)], 4.73 [2H, br s, H(18), H(18′)], 4.04 [1H, dd, *J* = 8.1, 3.3 Hz, H(6)], 1.85 [3H, br s, Me(17)], 1.10 [6H, d, *J* = 6.9, Me(16), Me(20)], and 0.97 [3H, d, *J* = 6.3, Me(19)]. ^{13}C-NMR (Table I). The spectral properties are identical to those reported for **1** (Ayyad *et al.*, 2001, Enoki *et al.*, (1982).

*Compound (**2**)*

Acetylating of **1** a solution of dictyone (5 mg) in a mixture of acetic anhydride (500 µl) and a few drops of pyridine were heated for about 4 h in a water bath and then cooled. It was poured into water and extracted with ethyl ether. The ether extract was washed with water and dried over anhydrous sodium sulfate. The solvent free residue was purified by PTLC using hexane: benzene: EtOAc (10:10:3 v/v/v) to afford dictyone acetate **2** as a colorless oil. IR (cm^{-1}): 1733 (OAc), 1710 (C = O), 1644 (C = C); EIMS *m/z* (rel. int.): 286 (14) [M^+-AcOH, $C_{20}H_{30}O^+$], 159 (90) [$C_{12}H_{15}^+$], 43 (100) [$C_3H_7^+$]. ^{1}H NMR ($CDCl_3$) 5.32 [1H, br s, H (3)], 5.31[1H, dd, *J* = 8.5, 2.5 Hz, H (6)], 4.75 [1H, br s, H (18)], 4.74 [1H, br s, H (18′)], 2.04 [3H, s, Me (Ac)], 1.65 [3H, br s, Me (17)], 1.01 [6H, d, *J* = 7.0, Me (16), Me (20)], and 0.84 [3H, d, *J* = 6.3, Me (19)]. ^{13}C-NMR (Table I): The spectral properties are identical to those reported for **2** (Ayyad *et al.*, 2001).

*Compound (**3**)*

Fractions 10–14 were combined. PTLC using hexane-EtOAc (8:2 v/v) afforded saringosterone 3 as a colorless oil (30 mg, 0.003% dry wt). IR (cm^{-1}): 3420 (OH), 1675 (C = O), 1630 (C = C); HREIMS: *m/z* 426.3413 (calcd. 426.3349762) $C_{29}H_{46}O_2$; EIMS *m/z* (rel. int.): 426 (12) [M^+] [$C_{29}H_{46}O_2^+$], 383 (21) [M^+-C_3H_7], 313 (30), 271(35), 269 (100). ^{1}H NMR ($CDCl_3$) 5.75 [1H, dd, *J* = 18, 13 Hz, H-28], 5.74 [1H, br s, H-4], 5.29 [1H, d, *J* = 13 Hz, H-29], 5.17 [1H, d, *J* = 18 Hz H-29′], 1.18 [3H, s, Me-19], 0.96 [3H, d, *J* = 6.5 Hz, Me-21], 0.87 [3H, d, *J* = 7.0 Hz, Me-26], 0.85 [3H, d, *J* = 7.0 Hz, Me-27], 0.72 [3H, s, Me-18]. ^{13}C-NMR (Table I).

*Compound (**4**)*

Fractions 22–25 were combined. PTLC using mixtures of hexane-EtOAc (3:1 v/v) afforded saringosterol **4** as a colorless oil (20 mg, 0.002% dry wt). IR (cm^{-1}): 3440 (OH), 1640 (C = C); EIMS *m/z* (rel. int.): 428 (12) [M^+, $C_{29}H_{48}O_2^+$], 410 (6)

$[M^+-H_2O]$, 314 (40), 273(20), 271 (100), 255 (28), 228 (22), 213 (40), 145 (64). ^1H NMR ($CDCl_3$) 5.74 [1H, dd, J = 18, 13 Hz, H-28], 5.35 [1H, br s, H-6], 5.29 [1H, d, J = 13 Hz, H-29], 5.18 [1H, d, J = 18 Hz H-29′], 3.51 [1H, m, H-3], 1.01 [3H, s, Me-19], 0.96 [3H, d, J = 6.6 Hz, Me-21], 0.89 [3H, d, J = 6.6 Hz, Me-26], 0.88 [3H, d, J = 6.6 Hz, Me-27], 0.69 [3H, s, Me-18], 13C-NMR (Table I). The spectral properties are identical to those reported for **4** (Tsuda *et al.*, 1958).

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