18-Me), 0.93 (3H, *s*, 20-Me), CD (MeOH; *c* 1.000 mg/ml, *d* 2 mm, room temp.): 190 (0), 194 (−4.8), 195 (−2.9), 196 (−3.2), 210 (0), 242 (+2.0), 304 (+0.7).

Monacetate of rosthornin B (**5**). A soln of **2** (20 mg) in a mixture of pyridine (0.5 ml) and Ac_2O (0.5 ml) was allowed to stand at room temp. for 3 hr, then MeOH (4 ml) was added to the soln which was evapd to give a residue. This was purified by CC on silica gel to give **5** (13 mg). $C_{26}H_{36}O_8$. ν_{max}^{KBr} 3620, 1735, 1650, 1235, 1120, 1100, 1070, 1036, 980, 950 cm^{-1}; MS *m/z*: 476 $(M)^+$, 458, 448, 430, 416, 398, 380, 370, 356, 338, 328, 310, 295, 283, 265, 250, 149, 109, 43 (base peak). δ: 6.21 and 5.52 (each 1H, *br s*, 17-H_2), 5.40 (*br d*, 5 Hz, 11α-H), 4.36 (*dd*, 4, 12 Hz, 7β-H), 4.27 and 3.97 (each 1H, *d*, 11 Hz, 19-H_2), 2.14, 2.02 and 1.93 (each 3H, *s*, 3 × OAc), 1.48 (*br s*, 9β-H), 1.09 (3H, *s*, 18-Me), 0.96 (3H, *s*, 20-Me).

Diacetate of rosthornin B (**6**). A soln of **2** (20 mg) in Ac_2O–pyridine was sitrred at 70° for 72 hr, then treated in the same way as for **5** to give **6** (11 mg). $C_{28}H_{38}O_9$, ν_{max}^{KBr} 1735, 1645, 1235, 1090, 1035, 975, 946, 930 cm^{-1}; MS *m/z*: 434 [M − 2 × ketene]$^+$, 416, 398, 374, 356, 328, 314, 296, 283, 253, 109, 43 (base peak). δ: 6.22 and 5.76 (each 1H, *br s*, 17-H_2), 5.47 (*dd*, 4, 12 Hz, 7β-H), 5.40 (*br d*, 5 Hz, 11α-H), 4.25 and 3.93 (each 1H, *d*, 11 Hz, 19-H_2), 2.13, 2.04, 1.94 and 1.88 (each 3H, *s*, 4 × OAc), 1.48 (*br s*, 9β-H), 1.04 (3H, *s*, 18-Me), 0.95 (3H, *s*, 20-Me).

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DIKETOSTEROID FROM MARINE RED ALGA *HYPNEA MUSCIFORMIS*

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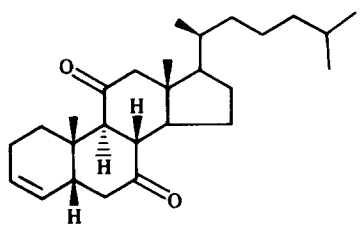
Key Word Index—*Hypnea musciformis*; Rhodophyta; red alga; diketo steroid; 5β-cholest-3-ene-7,11-dione.

Abstract—The isolation of a diketo steroid is reported from the hexane extract of the marine red alga *Hypnea musciformis*. The compound has been characterized as 5β-cholest-3-ene-7,11-dione based on 2D-NMR analysis.

INTRODUCTION

The major sterols of the red algae are C_{27} compounds. Cholesterol predominates, but in several species demosterol has been detected [1–10]. However, 22-dehydrocholesterol is reported to be present in relatively large amounts only in *Hypnea japonica* [11] and *Hypnea musciformis* [8]. Red algae also contain traces of C_{26}, C_{28} and C_{29} sterols [6, 7, 12]. Isolation of a 3-keto steroid [13, 14] and a 3, 6-diketo steriod [15] in some species is also documented. We now report, for the first time, the isolation of 7,11-diketo steroid from *Hypnea musciformis*.



1

RESULTS AND DISCUSSION

The hexane extract of air-dried seaweed was chromatographed over silica gel by gradient elution (ethyl acetate–hexane). A crystalline compound (**1**) was obtained by elution with 10% ethyl acetate in hexane.

The ^{1}H NMR spectrum and mass spectral fragmentation of compound **1** revealed that it was a steroid with a C_8H_{17} side chain. It gave a pink colour with the Komarowsky reagent [16], indicating it to be a keto-steroid. The ^{1}H NMR spectrum displayed signals at δ0.68 (3H, H_3-18) and δ0.93 (3H, H_3-19) for the two tertiary methyls, a signal at 0.98 (3H, *d*, J=6.5 Hz, H_3-21) and a signal for six protons at 0.84 which was assigned to the isopropyl group situated in the side chain. These signals are comparable to those in the spectrum of cholesterol (δ0.68, 3H, H_3-18, 1.00, 3H, H_3-19, 0.91, 3H, *d*, J=6.3 Hz, H_3-21 and 0.85, isopropyl).

The ^{1}H NMR spectrum of **1** also showed two multiplets at δ5.20 (*dd*), 5.31 (*dt*) and the ^{13}C NMR spectrum gave two signals at δ137.58 (*d*) and 126.56 (*d*) indicating a disubstituted double bond. The multiplicity of the olefinic proton signals showed the presence of the $-CH_2-CH=CH-CH<$ system which indicated the presence of a double bond between C-3, C-4 in ring A.

A sharp intense peak at 1705 cm^{-1} in the IR spectrum and ^{13}C NMR signals at δ211.09 and 208.95 indicated the presence of two six-membered cyclic keto groups. The $\lambda_{max}^{CHCl_3}$ at 213 nm showed that both keto groups and the double bond are not in conjugation. Thus it could not be a 2-keto compound. If compound **1** was to be 1-keto or 12-keto, the ^{13}C NMR signals should have appeared

Table 1. NMR spectral data of compound **1**

Position		^{1}H	COSY	DEPT	^{13}C	HeteroCOSY
1.	H_{eq}	2.37	(2.01, 1H_{ax}), (2.00, 2H_{ax}) (1.86, 2H_{eq})	CH_2	46.52	(2.37, 1H_{eq}) (2.01, 1H_{ax})
	H_{ax}	2.01	(2.37, 1H_{eq}), (2.00, 2H_{ax}) (1.86, 2H_{eq})			
2	H_{eq}	1.86	(2.00, 2H_{ax}), (2.37, 1H_{eq}) (2.01, 1H_{ax}), (5.20, 3H)	CH_2	30.02	(1.86, 2H_{eq}) (2.00, 2H_{ax})
	H_{ax}	2.00	(1.86, 2H_{eq}), (2.37, 1H_{eq}) (2.01, 1H_{ax})			
3	H	5.20	(5.31, 4H), (1.86, 2H_{eq})	CH	126.56	(5.20, 3H)
4	H	5.31	(5.20, 3H), (2.08, 5H)	CH	137.58	(5.31, 4H)
5	H	2.08	(5.31, 3H), (2.31, 6H_{ax}) (2.40, 6H_{eq})	CH	30.02	(2.08, 5H)
6	H_{ax}	2.31	(2.40, 6H_{eq}), (2.08, 5H)	CH_2	37.30	(2.31, 6H_{ax})
	H_{eq}	2.40	(2.31, 6H_{ax}), (2.08, 5H)			(2.40, 6H_{eq})
7	—	—	—	—	211.10	
8	H	1.27	(1.15, 14H)	CH	56.67	(1.27, 8H)
9	H	2.60	—	CH	57.44	(2.60, 9H)
10	—	—	—	—	41.18	—
11	—	—	—	—	208.96	
12	H_{ax}	2.29	(2.57, 12H_{eq})	CH_2	36.93	(2.29, 12H_{ax})
	H_{eq}	2.57	(2.29, 12H_{ax})			(2.57, 12H_{eq})
13	—	—	—	—	42.84	—
14	H	1.15	(1.27, 8H)	CH	55.80	(1.15, 14H)
15	H_{ax}	1.09	(1.52, 15H_{eq})	CH_2	23.44	(1.09, 15H_{ax})
	H_{eq}	1.52	(1.09, 15H_{ax})			(1.52, 15H_{eq})
16	H_{eq}	1.45	(1.67, 16H_{ax})	CH_2	21.61	(1.45, 16H_{ax})
	H_{ax}	1.67	(1.45, 16H_{eq})			(1.67, 16H_{ax})
17	H	1.45	(1.67, 16H_{ax}), (2.04, 20 H)	CH	53.43	(1.45, 17H)
18	3H	0.68	—	CH_3	12.15	(0.68, 18Me)
19	3H	0.93	—	CH_3	12.44	(0.93, 19Me)
20	H	2.04	(0.83, 21Me), (1.45, 17H) (2.05, 22H_a), (1.18 22H_b)	CH	39.97	(2.04, 20 H)
21	3H	0.98	(2.04, 20 H)	CH_3	20.74	(0.98, 21Me)
22	H_a	2.05	(1.18, 22H_b), (2.04, 20 H)	CH_2	39.22	(2.05, 22H_b)
	H_b	1.18	(2.05, 22H_a), (2.04, 20 H)			(1.18, 22H_a)
23	H_a	1.26	(1.87, 23H_b), (1.18, 22H_b)	CH_2	28.38	(1.26, 23H_b)
	H_b	1.87	(1.26, 23H_a)			(1.87, 23H_a)
24	H_a	1.57	(1.56, 25H), (1.84, 24H_b)	CH_2	30.02	(1.57, 24H_a)
	H_b	1.84	(1.57, 24H_a)			(1.84, 24H_a)
25	H	1.56	(0.84, 26, 27Me)	CH	28.38	(1.56, 25H)
26						
27	6H	0.84	(1.56, 25H)	2Me	22.24	(0.84, 26, 27Me)

above δ212. In the ^1H–^1H COSY spectrum the proton on C-5 showed cross peaks with that of C-6 indicating the absence of a 6-keto compound. Thus the two keto groups of **1** are located at the C-7 and C-11 positions.

The high resolution mass spectrum exhibited a molecular ion peak at *m/z* 398.3215 $[M]^+$ (61.8%) and other fragment ions at *m/z* 313 $[M-C_6H_{13}]^+$ (68.9%), 285 $[M-C_8H_{17}]^+$ (36%), 299 $[383-C_6H_{12}]^+$ (33.8%).

The ^{13}C NMR assignments of **1** (Table 1) were determined with the help of ^{13}C NMR coupled, decoupled, DEPT (Distortionless Enhancement of Polarisation Transfer) spectra and also with HeteroCOSY (^{13}C–^1H) experiments. The DEPT experiment with a flip angle of 135° revealed the presence of seven CH peaks (resonating at δ57.4, 56.6, 55.8, 53.4, 39.9, 30.0 and 28.38) and three Me peaks (resonating at δ22.2 and 12.2 for two Me each and δ20.7 for one Me) which gave signals in the positive direction. The eight peaks [resonating at δ46.5, 39.2, 37.3, 36.9, 30.02, 28.4, 23.4, 21.61, (30.02 corresponded for two CH_2 groups)] in the negative direction indicated the presence of nine CH_2 groups.

Proton connectivities were further deduced from the ^1H–^1H COSY spectrum (Table 1). H-3 and H-4 appeared as multiplets at δ5.31 and 5.20, respectively, and were coupled. H-3 (δ5.31) further showed cross peaks with H-2 (δ1.86) which again showed cross peaks with H-1 (δ2.01, 2.37). H-4 (δ5.20) gave connectivities with H-5 (δ2.08) which further showed cross peaks with H-6 (δ2.40, δ2.31).

The NOSEY spectrum further showed the through space connectivity between H-5 (δ2.08) and H_3-18 (δ0.68) which confirms the stereochemistry at H-5. The HeteroCOSY spectrum showed all the ^{13}C–^1H cross peaks (Table 1).

EXPERIMENTAL

The alga *Hypnea musciformis* was collected from the west coast of India (Lat. 22°28'N, Long. 69°05'E) during low tides in November 1986. The washed, air-dried, and pulverised alga (5 g) was extracted with hexane (3 × 5 l) at room temp. with the help of a mechanical stirrer. The solvent was removed in a rotavapour connected to aspirator, to yield a dark green extract (12 g). The concentrated hexane extract was chromatographed over silica gel and compound **1** was eluted with hexane–EtOAc (9:1) Compound **1** $[\alpha]_D^{20} = -20.5°$ ($CHCl_3$, *c* 5.03), UV $\lambda_{max}^{CHCl_3}$ nm: 213 IR λ_{max}^{KBr} cm^{-1}: 2950, 2910, 1705, 1460, 1425, 1385, 1375, 1360, 1345, 1325, 1300, 1280, 1265, 1255, 1240, 1165, 1135, 1085, 1070, 1045, 1015, 990, 970.

Chemical shifts are reported relative to TMS. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were measured in $CDCl_3$. EIMS were obtained at 70 eV.

The DEPT. experiments were performed using polarization transfer pulses of 45 and 135°, respectively, to obtain in the first case all $-CH$, $-CH_2$, $-Me$ groups and in the other case positive signals for -CH, and -Me and negative ones for $-CH_2$ groups. All 2-D NMR experiments (COSY, NOESY & Hetero-COSY) were performed on a Bruker 500 MHz FT-NMR spectrometer. The mixing time for the NOESY experiment was 800 msec.

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