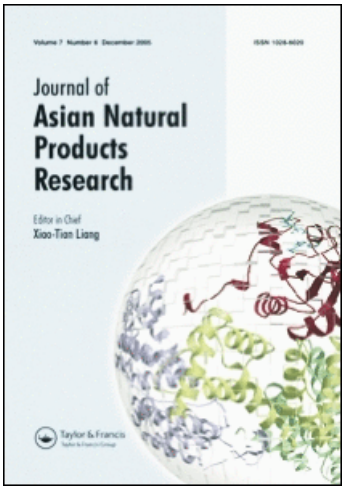

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BIOACTIVE STEROIDS FROM THE BROWN ALGA *SARGASSUM CARPOPHYLLUM*

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By activity-guided fractionation, two new sterols, 3β,28ξ-dihydroxy-24-ethylcholesta-5,23*Z*-dien (**1**) and 2a-oxa-2-oxo-5α-hydroxy-3,4-dinor-24-ethylcholesta-24(28)-ene (**2**), together with five known steroids, fucosterol (**3**), 24-ethylcholesta-4,24(28)-dien-3,6-dione (**4**), 24ξ-hydroperoxy-24-vinylcholesterol (**5**), 24-ketocholesterol (**6**), 24*R*,28*R*- and 24*S*, 28*S*-epoxy-24-ethylcholesterol (**7**), were isolated from the brown alga *Sargassum carpophyllum* as active compounds causing morphological abnormality of *Pyricularia oryzae* mycelia. Compounds **1**, **3**, **4**, **5** and **7** also exhibited cytotoxic activity against various cancer cell lines. The IC_{50} values for **1** and **5** against HL-60 were 7.8 and 8.5 μg/ml, **3** and **4** against P-388 were 0.7 and 0.8 μg/ml, whereas **7** against MCF-7, HCT-8, 1A9, HOS and PC-3 were 4.0, 8.8, 10.0, 10.0 and 7.2 μg/ml, respectively.

Keywords: Brown alga; *Sargassum carpophyllum*; Steroids; *Pyricularia oryzae*

Morphological deformation of mycelia or conidia of microorganisms, such as curling, swelling, hyper-divergency, bead formation, and so on, along with inhibition of germination, are often induced in the presence of bioactive substances. *Pyricularia oryzae* P-2b, a plant pathogenic fungus, has been used as a test microorganism for the primary screening of antineoplastic and antifungal agents [1] such as rhizoxin [2], fusarielin A [3] from natural sources. We applied the bioassay method [4] to marine algae for the first time. So far, 15 species of algae from East China Sea and South China Sea have been collected and bioassayed. The ethanolic extract of *Sargassum carpophyllum* J. Agargh showed strong activity.

The brown algae of Sargassaceae are abundant seaweeds growing along the coast of South China Sea. Sheu reported the isolation of six new cytotoxic oxygenated fucosterols from a species of the family, *Turbinaria conoides* [5]. However, only a few phytochemical work have been performed on the title alga up to date [6]. Bioactivity-guided fractionation of the EtOAc-soluble portion of the ethanolic extract led to the isolation and identification of two new sterols (**1**, **2**), along with five known steroids, which were identified as fucosterol (**3**) [7], 24-ethylcholesta-4,24(28)-dien-3,6-dione (**4**) [5], 24ξ-hydroperoxy-24-vinyl-cholesterol (**5**) [7], 24-ketocholesterol (**6**) [8],

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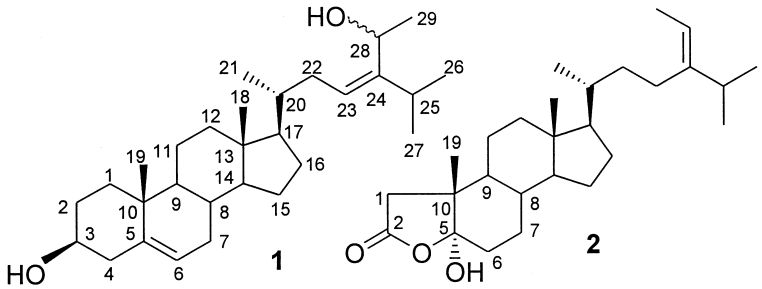
24*R*,28*R*- and 24*S*,28*S*-epoxy-24-ethylcholesterol (**7**) [9] by comparison of their physical and spectral data (MS, ^{1}H NMR, and ^{13}C NMR) with literature values.

RESULTS AND DISCUSSION

Compound **1** was obtained as pale solid of composition $C_{29}H_{48}O_2$ as determined by HREIMS. The ^{1}H NMR spectrum of **1** displayed a olefinic proton signal at δ5.35 (brd,



SCHEME 1 Structures of sterols 1 and 2.

TABLE I ^{1}H and ^{13}C NMR data of **1** and **2*** in $CDCl_3$ (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR)

	1		**2**	
Position	^{1}H	^{13}C	^{1}H	^{13}C
1	1.09, 1.86†	37.2	2.38 d (16.8) 2.63 d (16.8)	41.2
2	1.50, 1.83†	31.6		177.1
3	3.52 m	71.8		
4	2.30†	42.3		
5		140.7		108.2
6	5.35 brd(5.2)	121.7	1.72 td (14.0, 4.4) 2.03 dt (14.0, 3.0)	31.9
7	1.52, 2.00†	31.9	1.12, 1.60†	27.0
8	1.45†	31.9	1.24†	34.8
9	0.95†	50.1	0.88†	47.6
10		36.5		45.7
11	1.46, 1.51†	21.1	1.29, 1.46†	22.4
12	1.17, 2.02†	39.7	1.14, 2.00†	39.5
13		42.4		42.5
14	1.02†	56.7	1.01†	55.6
15	1.07, 1.60†	24.3	1.08, 1.60†	24.1
16	1.27, 1.85†	28.4	1.29, 1.87†	28.2
17	1.12†	56.2	1.12†	55.5
18	0.70 s	11.9	0.69 s	12.0
19	1.01 s	19.4	1.11 s	13.2
20	1.45 m	36.9	1.38†	36.4
21	0.89 d(6.4)	18.7	0.98 d (6.8)	18.6
22	1.84, 2.17†	33.5	1.08, 1.40†	35.1
23	5.25 dd(8.4, 6.0) and 5.49 dd(8.4, 6.0)	123.0 and 123.3	1.84, 2.03†	25.7
24		149.0 and 149.6		46.9
25	2.50 m and 2.77 m	27.9	2.19 m	34.8
26	1.05 d(6.4)‡	24.5¶	0.97 d(6.0)	22.1§
27	1.07 d(6.4)‡	24.7¶	0.97 d(6.0)	22.2§
28	4.32 q(6.4) and 4.76 q(6.4)	66.7 and 67.7	5.18 q(6.8)	115.6
29	1.27 d(6.4)	21.5 and 21.7	1.57 d(6.8)	13.2

* Chemical shifts in ppm rel. to TMS. Coupling constants (*J*) in Hz.
† Obscured by other signals: coupling constants could not be accurately determined.
‡,¶,§ Assignments with the same superscripts may be interchanged.

$J = 5.2$ Hz, 6-H), a multiplet at δ3.52 (3-H) and two three-proton singlets at δ0.70 and 0.89, which were quite similar to those of **3** [7], suggesting that **1** possesses the 5-en-3β-ol steroid skeleton as **3**. A close inspection of the ^{1}H and ^{13}C NMR spectra of **1** (see Table I) by DEPT and HMQC experiments revealed the presence of six methyl groups, nine methylenes, 10 methines and four quaternary carbons. Compared with the spectral data of **3**, some notable difference was observed in the side chain. The presence of a trisubstituted double bond in the side chain of **1** was indicated by a olefinic proton signal [δ5.25 and 5.49 (total 1H, each dd, $J = 8.4$, 6.0 Hz, 23-H)] and two sets of two olefinic carbon signals [(δ123.0 and 123.3, C-23) and (δ149.0 and 149.6, C-24)] in the ^{1}H and ^{13}C NMR spectra. The two quartets at δ4.32 and 4.76 (total 1H, $J = 6.4$ Hz, 28-H), a three-proton doublet at δ1.27 (29-CH_3), two sets of two carbon signals [(δ66.7 and 67.7, C-28) and (δ21.5 and 21.7, C-29)] suggested that an oxygen-bearing carbon attached with a secondary methyl group and a quaternary carbon should be present in the side chain. Three doublets at δ0.89, 1.05 and 1.07 in the ^{1}H NMR spectrum were due to 21-, 26- and 27-CH_3, respectively. Based on the above data, **1** was assumed to be a mixture of epimers, probably at C-28. In accordance with the molecular formula of **1**, one hydroxy group should be bonded to C-28 in both *R* and *S* configurations. 2D-NMR spectra (HMQC, HMBC) strongly supported these assignments (see Table II), so the planar structure of **1** was deduced. The geometry of the double bond at C-23 was determined by NOESY experiment. The NOE from 23-H to 26- or 27-CH_3 rather than to 29-CH_3 was observed, suggesting the presence of a *cis*-double bond at C-23(24). The 17β-orientation of the side chain was disclosed by NOESY cross-peaks 12β-H/21-CH_3 and 18-CH_3/20-H. Hence **1** was assigned as 3β,28ξ-dihydroxy-24-ethylcholesta-5,23*Z*-dien.

Compound **2** was obtained as an amorphous solid, which was shown to have the molecular formula $C_{27}H_{44}O_3$ by HREIMS. The ^{13}C NMR data was reminiscent of fucosterol skeleton [7], as regards the C-12 to C-18 and C-20 to C-29, except for the absence of signals for ring A carbons and the presence of signals for an ester (δ177.1) and a ketal (δ108.2). The presence of a γ-lactone ring was supported by an IR band at 1758 cm^{-1} as well as ^{1}H NMR signals at δ2.38 and 2.63 (both d, $J = 16.8$ Hz). The ^{1}H NMR showed the signals due to six methyl groups at δ0.69 (s, 18-CH_3), 1.11(s, 19-CH_3), 0.98(d, $J = 6.8$ Hz, 21-CH_3), 0.97(d, $J = 6.0$ Hz, 26,27-CH_3), 1.57(d, $J = 6.8$ Hz, 29-CH_3), virtually the identical chemical shifts found for fucosterol [7]. The five-membered lactol moiety having an α-methylene group is possible only for a 3,4-dinor structure with a hemiketal at C-5, which was also supported by molecular formula. Thus, **2** was assigned as 2a-oxa-2-oxo-5-hydroxy-3,4-dinor-24-ethylcholesta-24(28)-ene. The HMQC spectrum correlated the C-6 signal at δ31.9 with the ^{1}H NMR signals at δ1.72 (td, $J = 14.0$, 4.4 Hz, 6β-H) and 2.03 (dt, $J = 14.0$, 3.0 Hz, 6α-H). The HMBC spectrum supported these assignments (see Table II). The stereochemistry at C-5 was assigned to α based on the following evidence: the ^{1}H NMR of **2** in pyridine-d_5 showed pyridine-induced shifts [10] for the 6α-equatorial proton ($\Delta\delta = \delta_{C5D5N} - \delta_{CDCl3}$,

TABLE II Key HMBC correlations of **1** and **2**

1		**2**	
17-H	C-21, C-20, C-15, C-13	1-H	C-19, C-10, C-9, C-5, C-2
18-H	C-17, C-14, C-13	7-H	C-6
21-H	C-22, C-20	8-H	C-6
22-H	C-24, C-23, C-20	9-H	C-11
23-H	C-28, C-25	19-H	C-10, C-9, C-5, C-1
25-H	C-28, C-27, C-26, C-24, C-23	25-H	C-28, C-27, C-26, C-24
26-,27-H	C-27, C-26, C-25, C-24	26-, 27-H	C-27, C-26, C-25, C-24
28-H	C-25	28-H	C-29, C-25, C-23
29-H	C-28, C-24	29-H	C-28, C-24

TABLE III Bioactivity* of steroids **1–7**

Compound	*Pyricularia oryzae*	Cell line† P-388	HL-60	MCF-7	HCT-8	1A9	HOS	PC-3
1	63		7.8					
2	250			>20.0	>20.0	>20.0	>20.0	>20.0
3	250	0.7						
4	250	0.8						
5	63		8.5					
6	31			>10.0	>10.0	>10.0	>10.0	>10.0
7	63			4.0	8.8	10.0	10.0	7.2
5-fluorouracil‡	5							
10-hydroxycam-ptothecine¶		<0.1	<0.1					

* Bioactivity against *P. oryzae* is expressed as minimum morphological deformation concentrations (MMDC, μg/ml), and an MMDC value of >500 μg/ml is considered inactive. Cytotoxicity against various cancer cell lines is expressed as IC_{50} values (μg/ml), and an IC_{50} value of ≤4.0 μg/ml is required for significant activity of pure compounds.
† Key to cell lines used: P-388=mouse lymphocytic leukemia; HL-60=human promyelocytic leukemia; MCF-7=human breast cancer; HCT-8=human ilececal cancer; 1A9=human ovarian cancer; HOS=human bone tumor; PC-3=human prostate cancer.
‡,¶ Positive control compounds.

+0.36) and one of the C-1 methylene protons (δ2.38, +0.54), but the signal of 19-CH_3 showed only small shift (+0.13). The 6β-H signal was shifted upfield and overlapped in other signals. These facts indicated that 6β-H is *anti* and the 6α-H is *gauche* to the 5-OH, which, in turn, takes near 1,3-synperiplanar position with 1α-H, thus indicating the A/B-*trans* ring fusion. Kobayashi and Murata have reported the isolation of 2a-oxa-2-oxo-5α-hydroxy-3,4-dinor cholestane from the red alga *Laurencia obtuse* [11], **2** is the second example of the ring A-dinorsteroid isolated from the natural source.

Steroids **1**–**7** were evaluated for bioactivity of inducing morphological deformation of *P. oryzae* mycelia [4], and cytotoxic activity against several cultured cancer cell lines [12–14], and the results are shown in Table III. The data showed that all the steroids exhibited activities causing morphological abnormality of *P. oryzae* mycelia. Compounds **3** and **4** exhibited significant cytotoxicity toward P-388 cancer cells whereas **1** and **5** showed mild activity against the growth of HL-60 cancer cells assayed by the MTT colorimetric method [12,13]. In the antitumor screen using a panel of human cell lines and established protocols [14], only the epoxy sterol **7** showed some cytotoxicity against several human cell lines, whereas **2** and **6** were inactive. **2**, **6** and **7** were also evaluated for HIV growth inhibition activity in H9 lymphocytes [15]. The EC_{50} and IC_{50} values for **6** were <0.500 and 0.975 μg/ml, whereas **2** and **7** were inactive.

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined on a micromelting point apparatus and are uncorrected; IR spectra were measured on X-zoom Cursor and Bruker Vector 22 IR spectrometers; NMR spectra were recorded on a Varian Inova-400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C, in $CDCl_3$ using TMS as internal standard; EIMS and HREIMS were obtained with a Varian MAT 212 instrument; Semipreparative HPLC was performed on Waters Millennium 2010 instrument with a Whatman partisil 10 ODS column (9.4 × 500 mm) using UV(PDA 996) as a detector; Silica gel (10-40 μm) and Sephadex LH 20 (Pharmacia) were used for CC; TLC was performed on precoated $HSGF_{254}$ silica gel plates and detection was achieved by spraying with 20% H_2SO_4 followed by heating.

Plant Material

Sargassum carpophyllum was collected along the coast of South China Sea located in Beihai, Guangxi Province, China, in March 1999, and identified by Prof. Jin-Xin Hang of the Shanghai Museum of Natural Science. A voucher specimen (No. HN005) is deposited in the School of Pharmacy, Second Military Medical University.

Extraction and Isolation

The air-dried alga (40 kg) was extracted repeatedly with 80% EtOH (100 l × 3). The combined ethanolic extracts were evaporated to dryness *in vacuo* (835 g), then partitioned between EtOAc and H_2O and the organic layer (613 g) was shown to be bioactive with the test mode of *P. oryzae*. A portion of the extract (105 g) was chromatographed on a silica gel column (1500 g, 10–40 μm) using a gradient (1000ml each eluant) of 0–50% MeOH in $CHCl_3$ (0, 2, 5, 10, 20, 50%, 15,000 ml each). Fractions were pooled based on TLC analysis (24 combined fractions). Fraction 4 (3.9 g) eluted with 2% MeOH/$CHCl_3$ proved to be most

active against *P. oryzae* and was further separated on a silica gel column (200 g, 10–40 μm) using a gradient of 5–30% EtOAc/petroleum ether (5, 10, 20, 30%, 1500 ml each) to yield 12 fractions (A–L), of which fractions B (0.3 g), D (1.4 g), E (0.2 g), H (1.0 g) and J (0.1 g) were confirmed as active against *P. oryzae*. Fraction B was subjected to HPLC eluted with 98% MeOH to give compounds **2** (t_R: 12.5 min, 4 mg) and **4** (t_R: 14.9 min, 36 mg). Fraction D and H were separated on Sephadex LH 20 (2.5 × 100 cm) with $CHCl_3$/MeOH (1:1) as eluting solvent, respectively. Fraction D afforded **3** (between 180 and 220 ml, 640 mg) and **7** (between 260 and 285 ml, 20 mg), and fraction H yielded **5** (between 200 and 235 ml, 40 mg). Fraction E was placed on CC over 30 g silica gel (10–40 μm) with petroleum ether/Me_2CO (10:1, 400 ml) to give **6** (35 mg). Fraction J was separated on CC over 25 g silica gel (10–40 μm) with CH_2Cl_2/MeOH (40:1, 400 ml) to afford **1** (32 mg). TLC [silica gel HSGF254, solvents: petroleum ether/EtOAc (5:1) and petroleum ether/Me_2CO (5:1), respectively]: R_f **1**: 0.19 and 0.22, **2**: 0.39 and 0.56, **3**: 0.35 and 0.50, **4**: 0.45 and 0.60, **5**: 0.22 and 0.32, **6**: 0.24 and 0.37, **7**: 0.30 and 0.48. Copies of the original spectra are obtainable from the author of correspondence.

3β,28ξ-dihydroxy-24-ethylcholesta-5,23Z-dien(***1***): pale solid, mp 139–141°C. IR(KBr)ν_{max} 3323, 1670, 1462, 1375, 1062, 838 cm^{-1}; ^1H and ^{13}C NMR data, see Table I; EIMS m/z $[M]^+$ 428 (2), 411 (5), 410 (12), 395 (3), 385 (5), 367 (4), 315 (6), 301 (19), 283 (47), 271 (100), 255 (10), 215 (3). HREIMS m/z $[M]^+$ 428.3548 (cacld for $C_{29}H_{48}O_2$, 428.3654).

2a-oxa-2-oxo-5α-hydroxy-3,4-dinor-24-ethylcholesta-24(28)-ene(***2***): amorphous solid, mp 130–133°C. IR (ATR) ν_{max}3348, 1758, 1455, 1374, 1142, 918 cm^{-1}; ^1H and ^{13}C NMR (in $CDCl_3$) data, see Table I; ^1H NMR (C_5D_5N) δ5.25 (q, $J = 6.6$ Hz, 28-H), 2.92 (d, $J = 16.1$ Hz, 1-H), 2.57 (d, $J = 16.8$ Hz, 1-H), 2.39 (m, 6α-H), 1.64 (d, $J = 6.4$ Hz, 29-CH_3), 1.24 (s, 19-CH_3), 1.04 (9H, d, $J = 6.8$ Hz, 21-, 26- and 27-CH_3), 0.64 (s, 18-CH_3). EIMS m/z $[M]^+$ 416 (6), 401 (6), 398 (5), 388 (6), 383 (3), 357 (66), 345 (16), 318 (100), 303 (13), 275 (22). HREIMS m/z $[M]^+$ 416.3297(cacld for $C_{27}H_{44}O_3$, 416.3290).

P. oryzae bioassay was performed with the reported method [4]. The cytotoxic activity of **3**–**5** and **1** was evaluated by an MTT assay procedure as described in the previous papers [12,13]. The cytotoxicity data for **2**, **6** and **7** were obtained from a panel of human cancer lines using established protocol [14] and the anti-HIV assay was carried out according to a published method [15].

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References

[1] Hu, K., Dong, A.J., Yao, X.S., Kobayashi, H. and Iwasaki, S. (1996), *Planta Med.* **62**, 573–575.
[2] Tsuruo, T., Oh-hara, T., Iida, H., Tsukagoshi, S., Sato, Z., Matsuda, I., Iwasaki, S., Okuda, S., Shimizu, F., Sasagawa, K., Fukami, M., Fukuda, K. and Arakawa, M. (1986), *Cancer Res.* **46**, 381–385.
[3] Kobayashi, H., Sunaga, K., Furihata, K., Morisaki, N. and Iwasaki, S. (1995), *J. Antibiotics* **48**, 42–52.
[4] Kobayashi, H., Namikoshi, M., Yoshimoto, T. and Yokochi, T. (1996), *J. Antibiotics* **49**, 873–879.
[5] Sheu, J.H., Wang, G.H., Sung, P.J. and Duh, C.Y. (1999), *J. Nat. Prod.* **62**, 224–227.
[6] Wang, C.J., Yan, S.J., Zeng, L.M. and Su, J.Y. (1997), *Chinese J. Chromatography* **15**, 396–399.
[7] Sheu, J.H., Wang, G.H., Sung, P.J., Chiu, Y.H. and Duh, C.Y. (1997), *Planta Med.* **63**, 571–572.
[8] Kurata, K., Taniguchi, K., Shiraishi, K. and Suzuki, M. (1990), *Phytochemistry* **29**, 3678–3680.
[9] Fujimoto, Y., Murakami, K. and Ikekawa, N. (1980), *J. Org. Chem.* **45**, 566–569.

[10] Demarco, P.V., Farkas, E., Doddrell, D., Mylari, B.L. and Wenkert, E. (1968), *J. Am. Chem. Soc.* **90**, 5480–5486.
[11] Kobayashi, M. and Murata, O. (1992), *Tetrahedron Lett.* **33**, 519–520.
[12] Sheu, J.H., Liaw, C.C. and Duh, C.Y. (1995), *J. Nat. Prod.* **58**, 1521–1526.
[13] Mimaki, Y., Kuroda, M., Fukasawa, T. and Sashida, Y. (1999), *J. Nat. Prod.* **62**, 194–197.
[14] Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S. and Boyd, M.R. (1990), *J. Natl. Cancer Inst.* **82**, 1107–1112.
[15] Xie, L., Takeuchi, Y., Cosentino, L.M., McPhail, A.T. and Lee, K.H. (2001), *J. Med. Chem.* **44**, 664–671.