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STEROIDS AND TRITERPENOIDS FROM THE BROWN ALGA *Kjellmaniella crassifolia*

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Kjellmaniella crassifolia (Miyabe) is a special macro-brown alga that is used as a medicinal, is edible, and is of economic importance. It is widely distributed around the southern sea area of Hokkaido, Japan. It has been reported that the aqueous extract of *K. crassifolia* exhibits antitumor activity against Ehrlich carcinoma and Meth-A fibrosarcoma [1]. So far, however, no secondary metabolite has been reported from this species, except for the polysaccharide constituents, for example, the affluent fucoidan [2] with multifunctional properties including anti-thrombotic, anti-inflammatory, and antitumor activities [3].

Plant Material. The whole plant of *K. crassifolia* was collected from Hokkaido, Japan, 2007. The voucher specimen (JP-HK-20070006) was deposited in the Key Laboratory of Marine Drugs, Ministry of Education, Ocean University of China, Qingdao, China.

Extraction and Isolation. The sliced alga *K. crassifolia* (4.7 kg, dry weight) was extracted exhaustively with 95% ethanol (4000 mL × 5) at room temperature. The organic layer was filtered and concentrated under vacuum and then partitioned between EtOAc and H_2O. The solvent-free EtOAc extract (76.0 g) was subjected to column chromatography on silica gel and eluted with EtOAc in petroleum (0–100%, gradient) to yield nine fractions. The subfractions were then isolated by successive column chromatography on silica gel and Sephadex LH-20 and purified by semi-preparative HPLC, leading to the isolation of compounds **1** (15.6 mg), **2** (4.0 mg), **3** (2.5 mg), **4** (4 mg), **5** (22.6 mg), **6** (40.2 mg), **7** (7.4mg), **8** (6.0 mg), **9** (5.3 mg), **10** (7.1 mg), and **11** (3.6mg).

Recently, in the course of our search for bioactive substances from marine alga, 11 compounds including eight steroids (**1**–**8**) and three triterpenoids (**9**–**11**) were isolated and identified from *K. crassifolia*, and all of these compounds were obtained from this species for the first time.

On the basis of extensive spectroscopic analyses and by comparison with those reported in the literatures, the structures of compounds **1**–**11** were established as cholesterol (**1**) [4], ergosta-5,22-dien-3-ol (**2**) [5], lobophytol A (**3**) [6], 24-methylenecholest-5-en-3-ol (**4**) [7], saringosterol (**5**) [8], fucosterol (**6**) [9], 24-ethylcholesta-4,24(28)-dien-3-one (**7**) [10], 24-ethyl-5α-cholesta-24(28)*Z*,25-dien-3β-ol (**8**) [11], 24,25-dihydrocimicifugenol (**9**) [12], 3-epicyclomusalenol (**10**) [13], and cyclosadol (**11**) [14]. Cycloartane-type triterpenoids, which are always found in higher plants such as *Tillandsia fasciculata* [15], are very scarce in alga. It was reported that they served as membrane components [16]. The three cycloartane-type triterpenoids **9**–**11** were isolated from algae for the first time.

All the isolated compounds were evaluated by the lethality assay on brine shrimp *Artemia salina* [17] and fish-toxicity assay on embryo of zebrafish *Danio rerio* [18, 19]. Compounds **1** and **3** exhibited moderate lethality toward brine shrimp *A. salina* at a concentration of 50 μg/mL. Compounds **2** and **3** showed fish toxicity at 50 μg/mL, inhibiting the incubation of zebrafish embryo. Compounds **4**–**6** were selected to test for cytotoxic activity on tumor cell lines of HL-60 and A-549, and no obvious cytotoxicity was found. In addition, compounds **10** and **11** were previously reported to have moderate cancer chemopreventive effects [20].

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24-Methylenecholest-5-en-3-ol (4). $C_{28}H_{46}O$, white powder, mp 141–142°C. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.35 (1H, br.d, H-6), 4.71 (1H, d, J = 1.2, H-28a), 4.65 (1H, d, J = 1.2, H-28b), 3.52 (1H, m, H-3), 2.31–1.05 (26H, m), 1.02 (6H, d, J = 6.6, H-26, 27), 1.00 (3H, s, H-19), 0.94 (3H, d, J = 6.6, H-21), 0.68 (3H, s, H-18).

Saringosterol (5). $C_{29}H_{48}O_2$, colorless needle crystal, mp 160–161°C. ^{1}H NMR (600 MHz, $(CD_3)_2CO$, δ, ppm, J/Hz): 5.79 (1H, ddd, J = 17.4, 10.8, 8.4, H-28), 5.34 (1H, br.d, H-6), 5.17 (1H, ddd, J = 17.4, 3.6, 1.2, H-29a), 5.11 (1H, ddd, J = 10.8, 3.6, 1.2, H-29b), 3.51 (1H, m, H-3), 2.30–1.04 (26H, m), 0.99 (3H, s, H-19), 0.91 (3H, dd, J = 6.6, 3.6, H-26), 0.89 (3H, dd, J = 6.6, 4.2, H-27), 0.85 (3H, d, J = 7.2, H-21), 0.67 (3H, s, H-18). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 142.6 (C-28), 140.8 (C-5), 121.8 (C-6), 113.0 (C-29), 77.8 (C-24), 71.9 (C-3), 56.8 (C-14), 55.9 (C-17), 50.2 (C-9), 42.4 (C-4), 42.4 (C-13), 39.8 (C-12), 37.3 (C-1), 36.6 (C-10), 36.2 (C-20), 36.0 (C-25), 34.6 (C-8), 32.0 (C-7), 32.0 (C-23), 31.7 (C-2), 29.2 (C-22), 28.3 (C-16), 24.4 (C-15), 21.2 (C-11), 19.5 (C-19), 18.9 (C-21), 17.7 (C-27), 16.6 (C-26), 11.9 (C-18).

Fucosterol (6). $C_{29}H_{48}O$, colorless needle crystal, mp 122–124°C, ESI-MS *m/z*: 413 $[M + H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.35 (1H, br.d, H-6), 5.18 (1H, q, J = 6.6, H-28), 3.52 (1H, m, H-3), 2.28–1.60 (10H, m), 1.57 (3H, d, J = 6.6, H-29), 1.52–1.06 (16H, m), 1.03 (3H, d, J = 6.6, H-21), 1.02 (3H, s, H-19), 0.99 (6H, d, J = 7.2, H-26, 27), 0.67 (3H, s, H-18). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 147.0 (C-24), 140.8 (C-5), 121.7 (C-6), 115.6 (C-28), 71.9 (C-3), 56.8 (C-14), 55.8 (C-17), 50.2 (C-9), 42.4 (C-4), 42.4 (C-13), 39.8 (C-12), 37.3 (C-1), 36.6 (C-10), 36.5 (C-20), 35.3 (C-22), 34.8 (C-25), 31.9 (C-7), 31.9 (C-8), 31.7 (C-2), 28.3 (C-23), 21.1 (C-11), 24.4 (C-15), 25.7 (C-16), 22.3 (C-26), 22.2 (C-27), 19.4 (C-19), 18.8 (C-21), 13.2 (C-29), 11.9 (C-18).

24-Ethylcholesta-4,24(28)-dien-3-one (7). $C_{29}H_{46}O$, white powder, mp 83–84°C. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.72 (1H, br.s, H-4), 5.17 (1H, q, J = 6.6, H-28), 2.45–1.60 (15H, m), 1.56 (3H, d, J = 6.6, H-29), 1.54–1.37 (5H, m), 1.18 (3H, s, H-19), 1.17–1.00 (6H, m), 0.99 (6H, d, J = 6.6, H-26, 27), 0.96 (3H, d, J = 7.2, H-21), 0.71 (3H, s, H-18).

24-Ethyl-5α-cholesta-24(28)*Z*,25-dien-3β-ol (8). $C_{29}H_{48}O$, white powder. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.63 (1H, q, J = 7.2, H-28), 4.96 (1H, s, H-26a), 4.85 (1H, s, H-26b), 3.52 (1H, m, H-3), 2.37–1.95 (5H, m), 1.87 (3H, s, H-27), 1.85–1.80 (3H, m), 1.71 (3H, d, J = 7.2, H-29), 1.56–1.05 (16H, m), 1.02 (3H, d, J = 6.6, H-21), 1.00 (3H, s, H-19), 0.96–0.85 (4H, m), 0.68 (3H, s, H-18).

24,25-Dihydrocimicifugenol (9). $C_{30}H_{50}O$, colorless crystal. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.60 (1H, dd, J = 4.8, 3.0, H-16), 3.28 (1H, m, H-3), 1.31 (3H, d, J = 1.8, H-18), 2.35–1.67 (8H, m), 1.30–0.98 (14H, m), 0.96 (3H, s, H-30), 0.89 (3H, d, J = 7.2, H-21), 0.87 (3H, s, H-28), 0.85 (6H, d, J = 7.2, H-26, 27), 0.85–0.82 (2H, m), 0.81 (3H, s, H-29), 0.55 (1H, d, J = 4.2, H-19a), 0.32 (1H, d, J = 4.2, H-19b).

3-Epicyclomusalenol (10). $C_{30}H_{50}O$, colorless needle crystal, mp 121–122°C. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.71 (1H, br.s, H-27a), 4.66 (1H, br.s, H-27b), 3.27 (1H, m, H-3), 2.25–1.70 (7H, m), 1.68 (3H, s, H-26), 1.64–1.05 (18H, m), 1.02 (3H, d, J = 6.6, H-28), 0.96 (3H, s, H-30), 0.89 (3H, d, J = 6.6, H-29), 0.87 (3H, d, J = 7.2, H-21), 0.81 (3H, s, H-18), 0.78 (1H, m), 0.55 (1H, d, J = 4.2, H-19a), 0.32 (1H, d, J = 4.2, H-19b).

Cyclosadol (11). $C_{31}H_{52}O$, colorless needle crystal, mp 121–124°C. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.10 (1H, t, J = 7.2, H-23), 3.27 (1H, m, H-3), 2.26–1.73 (9H, m), 1.60 (3H, s, H-28), 1.58–1.06 (17H, m), 1.02 (3H, d, J = 6.6, H-21), 0.96 (6H, s, H-29, 30), 0.94 (3H, s, H-31), 0.88 (6H, d, J = 7.2, H-26, 27), 0.81 (3H, s, H-18), 0.55 (1H, d, J = 4.2, H-19a), 0.32 (1H, d, J = 4.2, H-19b).

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