Natural Product Sciences
10(6) : 253-261 (2004)

Bioactive Metabolites from the Brittle Star *Ophioplocus japonicus*

Weihong Wang[1], Jongki Hong[2], Chong-Ok Lee[3], Hee Young Cho[3], Sook Shin[4], and Jee H. Jung[1]*

[1]*College of Pharmacy, Pusan National University, Busan 609-735, Korea*
[2]*Basic Science Institute, Seoul, Korea*
[3]*Korea Research Institute of Chemical Technology, Daejon, Korea*
[4]*Sahmyook University, Seoul, Korea*

Abstract – Ten terpenes (**1-10**), two sterols (**11** and **12**), and two unusual phenylpropanoids (**13** and **14**) were isolated from the brittle star *Ophioplocus japonicus* (Family Ophiuridae). Their structures were identified by analyses of the spectral data and by comparison with the literature data. The terpenes (**1-10**) and phenylpropanoids (**13** and **14**) are first encountered in brittle stars. These compounds were evaluated for cytotoxicity against a small panel of human solid tumor cell lines. Compounds **6-10** and **14** displayed moderate to significant cytotoxicity. The compounds were evaluated for antibacterial activity against 20 clinically isolated strains. Compound 5 exhibited antibacterial activities against three *Streptococcus* and three *Staphylococcus* strains.
Keywords – brittle star, *Ophioplocus japonicus*, cytotoxicity, antibacterial activity

Introduction

The brittle stars (Ophiuroidea) are the largest group of echinoderms in the number of species, and they are probably the most abundant also (Hickman *et al.*, 1984). However, the brittle stars have received less attention than the starfish (Asteroidea) and sea cucumbers (Holothuroidea) during the last three decades. Only sporadic papers dealing with their chemical components have been published (D'Auria *et al.*, 1991; Minale *et al.*, 1995; Maier *et al.*, 2000), and there are only a few reports concerning their bioactivity (Roccatagliata *et al.*, 1996). In our screening process of the marine organisms for their brine shrimp lethality, we noticed the toxicity of the extract of the brittle star *Ophioplocus japonicus* H. L. Clark (LD_{50} 36 μg/mL). Guided by the ^{1}H NMR monitoring and the brine shrimp lethality, we have isolated ten terpenes (**1-10**), two sulfated sterols (**11** and **12**), and two unusual phenylpropanoids (**13** and **14**). Most of the previously reported compounds from the brittle stars are sterols (Minale *et al.*, 1995; Maier *et al.*, 2000). Others are steroidal glycosides, carotenoid sulfates, and naphthoquinones (D'Auria *et al.*, 1991; Minale *et al.*, 1995). The terpenes (**1-10**) are first encountered in brittle stars and the phenylpropanoids (**13** and **14**) are the first examples of additional class of compounds isolated from brittle stars. The terpenes (**1-10**) have been previously isolated from algae or sea hares which feed on algae (Bouaicha *et al.*, 1993; Jongaramruong *et al.*, 2002). The unusual phenylpropanoids (**13** and **14**) were previously reported only from the cyanobacterium *Kyrtuthrix maculans* (Lee *et al.*, 1998). Although compound **11** is a common sulfated sterol, which is widely distributed in higher animals (Liu *et al.*, 2003; Strott *et al.*, 2003), the 3β-sulfate substitution was first encountered in sterols from brittle stars. Compound **12** was previously reported from some species of starfish and brittle stars (D'Auria *et al.*, 1995; Ivanchina *et al.*, 2003), though it is first reported from *O. japonicus*.

Experimental

Animal Material – The brittle star *O. japonicus* was collected in October 2002, off the coast of Jeju Island, Korea. Arms are almost three and half times longer than the diameter of disk. Disk is closely covered with small scales, the largest one of which is located in the vicinity of the radial shields, and the primary plate is hardly distinguishable. Dorsal arm plates are consisted of eight to twelve plates, five large ones of which are along the proximal border of each arm joint. Interbrachial spaces are covered with a close scaling, which is finest along the genital slits and close to the oral shield. Genital slits are long, extending from the oral shield more than

*Author for correspondence
Fax: +82-51-510-2803; E-mail: jhjung@pusan.ac.kr

halfway to disk margin. Oral shields are more or less pentagonal, with all angles, except the most proximal rounded one. Adoral plates, meeting within, are somewhat wider at outer end than at inner end; oral plates are well marked, but smaller than adoral plates. There are usually five oral papillae on each side, with outset part much the widest. Teeth, about five, are very broad, flat, and rounded. The first ventral arm plate is more or less triangular and much smaller than the second one, and the second and succeeding plates are quadrilateral, wider than length. Side arm plates, with three short, thick, and blunt arm spines, are small and low. Two tentacle scales are on proximal side of pore, and another two or often only one, is on distal side. The brittle star is dark brown, with arms irregularly banded with light and dark brown color. The voucher specimen (J04M-C) was deposited in the Marine Natural Product Laboratory, Pusan National University, Busan, Korea.

General Procedures – Optical rotations were recorded using a JASCO DIP-370 digital polarimeter. UV spectra were obtained using a Shimadzu mini 1240 UV-vis spectrophotometer. IR spectra were recorded on a JASCO FT/IR-410 Spectrometer. FABMS data were obtained on a JEOL JMS-SX-102A double-focusing spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AC200 or a Varian Inova 500 instruments. Chemical shifts were reported with reference to the respective solvent peaks and residual solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD, δ_H 7.27 and δ_C 77.0 for $CDCl_3$). HPLC was performed with a C18-5E Shodex packed column (250 × 10 mm I.D., 5 μm, 100 Å) and an YMC-Pack ODS column (250 × 10 mm I.D., 5 μm, 80 Å) using a Shodex RI-71 detector.

Extraction and Isolation – The frozen brittle stars (390 g, wet wt) were chopped into small pieces and extracted with MeOH at room temperature. Guided by the brine shrimp lethality assay, the MeOH extract was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane to afford aqueous MeOH (832 mg) and *n*-hexane soluble (1.3 g) fractions. The aqueous MeOH fraction was subjected to a reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å 500/400 mesh) eluting with MeOH-H_2O (gradient, 3:2 → 1:0) to obtain 11 fractions (1−11). Fractions 6-9 were active in the brine shrimp lethality assay (LD_{50}, 21-150 μg/mL) and were subjected to repeated chromatographic separation on a C18-5E shodex packed column eluting with 75% MeOH to afford 14 compounds. Compound **2** was obtained from fraction 6. Compounds **1**, **3**, **4**, and **8** were obtained from fraction 8. Compounds **7** and **12-14** were obtained from fraction 7. Compounds **5**, **6**, and **9-11** were obtained from fraction 9.

Preparation of MTPA esters. Compound **14** (ca. 1 mg) was treated with (*R*)-(−)-and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (4 μL) in dry pyridine (25 μL) for 24 h at room temperature to afford (*S*)-(−)-MTPA ester and (*R*)-(+)-MTPA ester, respectively. The reaction was monitored by TLC (ODS, MeOH) and stopped when the spot of the starting material disappeared. After removal of solvent, the product was purified by reversed-phase HPLC (YMC-Pack ODS column, MeOH), and analyzed by ^{1}H NMR.

Pacifenol (1) – white needles; $[\alpha]^{21}_D$ – 3.5° (*c* 0.34, $CHCl_3$); EIMS *m/z*: 408 (1.5) / 410 (3.4) / 412 (2.3) [M – H_2O]$^+$, 329 (77) / 331 (100) / 333 (26), 249 (13) / 251 (28) / 253 (20); HREIMS *m/z*: 411.9496 (calcd for $C_{15}H_{19}O^{81}Br_2{}^{35}Cl$, 411.9450); ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 2.18 (1H, dd, *J* = 14.5, 13.5 Hz, H-1), 2.35 (1H, dd, *J* = 14.5, 2.0 Hz, H-1), 5.45 (1H, dd, *J* = 13.5, 3.8 Hz, H-2), 2.36 (1H, dd, *J* = 15.0, 14.0 Hz, H-4), 2.70 (1H, dd, *J* = 15.0, 5.3 Hz, H-4), 4.69 (1H, dd, *J* = 13.5, 5.0 Hz, H-5), 1.88 (1H, s, OH-7), 5.40 (1H, d, *J* = 10.0 Hz, H-8), 6.07 (1H, d, *J* = 10.0 Hz, H-9), 1.12 (3H, s, H-12), 1.31 (3H, s, H-13), 1.52 (3H, s, H-14), 1.78 (3H, s, H-15).

Johnstonol (2) – white needles; ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 2.13 (1H, dd, *J* = 15.0, 13.0 Hz, H-1), 2.23 (1H, dd, *J* = 15.0, 2.0 Hz, H-1), 4.31 (1H, dd, *J* = 13.0, 2.5 Hz, H-2), 2.24 (1H, dd, *J* = 14.5, 13.5 Hz, H-4), 2.57 (1H, dd, *J* = 14.5, 5.0 Hz, H-4), 4.67 (1H, dd, *J* = 14.0, 5.0 Hz, H-5), 3.21 (1H, s, H-8), 4.04 (1H, d, *J* = 1.5 Hz, H-9), 2.60 (1H, d, *J* = 1.5 Hz, OH-9), 1.17 (3H, s, H-12), 1.29 (3H, s, H-13), 1.46 (3H, s, H-14), 1.75 (3H, s, H-15).

Prepacifenol epoxide (3) – white power; $[\alpha]^{21}_D$ + 115° (*c* 0.09, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 2.16 (1H, ddd, *J* = 14.5, 4.0, 2.5 Hz, H-1), 2.56 (1H, t, *J* = 14.0 Hz, H-1), 4.75 (1H, dd, *J* = 13.8, 3.8 Hz, H-2), 2.04 (1H, m, H-4), 2.54 (1H, m, H-4), 4.05 (1H, sext, *J* = 3.0 Hz, H-5), 3.09 (1H, s, H-8), 3.68 (1H, s, H-9), 1.00 (3H, s, H-12), 1.55 (3H, s, H-13), 1.50 (3H, s, H-14), 1.93 (3H, s, H-15).

2,10-Dibromo-3-chlorochamigran-7-en-9-ol (4) – wh ite power; $[\alpha]^{21}_D$ + 30.0° (*c* 0.11, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 2.24 (2H, m, H-1), 4.91 (1H, dd, *J* = 12.0, 6.5 Hz, H-2), 2.33 (2H, m, H-4), 1.73 (1H, m, H-5), 2.10 (1H, ddd, *J* = 14.0, 13.5, 5.5 Hz, H-5), 5.45 (1H, dd, *J* = 3.0, 1.5 Hz, H-8), 4.27 (1H, ddd, *J* = 9.0, 3.0, 2.0 Hz, H-9), 4.37 (1H, d, *J* = 9.0 Hz, H-10), 0.99 (3H, s, H-12), 1.23 (3H, s, H-13), 2.06 (3H, t, *J* = 1.8 Hz, H-14), 1.71 (3H, s, H-15).

Laurinterol (5) – colorless oil; $[\alpha]^{21}_D$ + 15.0° (*c* 0.19, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 1.31 (1H, m, H-1), 2.10 (1H, dd, *J* = 13.3, 8.3 Hz, H-1), 1.68 (1H, dd, *J* = 12.3, 8.3 Hz, H-2), 1.97 (1H, tdd, *J* = 12.3, 8.0, 4.5 Hz, H-2), 1.15 (1H, dt, *J* = 8.5, 4.0 Hz, H-3), 0.57 (2H, m, H-5), 5.09 (1H, s, OH-8), 6.63 (1H, s, H-9), 7.62 (1H, s, H-12), 1.42 (3H, s, H-13), 1.34 (3H, t, *J* = 1.8 Hz, H-14), 2.31 (3H, s, H-15).

10-Acetoxy-18-hydroxy-2,7-dolabelladiene (6) – colorless oil; IR (film) ν_{max} 3449, 2960, 1740, 1646 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 5.10 (1H, d, *J* = 16.5 Hz, H-2), 5.25 (1H, dd, *J* = 16.5, 7.5 Hz, H-3), 2.15 (1H, m, H-4), 1.45 (1H, m, H-5), 1.63 (1H, m, H-5), 2.15 (2H, m, H-6), 5.13 (1H, t, *J* = 8.0 Hz, H-7), 2.06 (1H, m, H-9), 2.38 (1H, t, *J* = 11.5 Hz, H-9), 4.83 (1H, dt, *J* = 11.0, 2.5 Hz, H-10), 1.79 (1H, dd, *J* = 10.3, 2.8 Hz, H-11), 2.31 (1H, td, *J* = 10.8, 4.0 Hz, H-12), 1.42 (1H, m, H-13), 1.89 (1H, m, H-13), 1.45 (1H, m, H-14), 1.63 (1H, m, H-14), 0.89 (3H, s, H-15), 0.96 (3H, d, *J* = 7.0 Hz, H-16), 1.66 (3H, br s, H-17), 1.27 (3H, s, H-19), 1.20 (3H, s, H-20), 2.08 (3H, s, CH_3CO).

Dihydroxycrenulide (7) – colorless oil ; $[\alpha]^{21}_D$ + 29.0° (*c* 0.25, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 nm (3.78), 208 nm (3.90); IR (film) ν_{max} 3350, 2955, 1750, 1668, 1450, 1290 cm^{-1}; FABMS *m/z*: 357 $[M + Na]^+$, 335 $[M + H]^+$; HRFABMS *m/z*: 357.2045 (calcd for $C_{20}H_{30}O_4Na$, 357.2042); ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 3.15 (1H, d, *J* = 9.5 Hz, H-3), 4.46 (1H, q, *J* = 2.5 Hz, H-4), 1.78 (1H, m, H-5), 1.94 (1H, m, H-5), 1.31 (1H, m, H-6), 0.97 (1H, m, H-7), 0.42 (1H, q, *J* = 5.5 Hz, H-8), 1.04 (1H, m, H-8), 1.45 (1H, m, H-9), 1.95 (1H, m, H-10), 1.15 (1H, m, H-11), 1.33 (1H, m, H-11), 1.98 (1H, m, H-12), 2.09 (1H, m, H-12), 5.09 (1H, br t, *J* = 7.3 Hz, H-13), 1.61 (3H, s, H-15), 1.70 (3H, s, H-16), 1.08 (3H, d, *J* = 6.5 Hz, H-17), 5.95 (1H, d, *J* = 2.0 Hz, H-18), 1.04 (3H, d, *J* = 7.5 Hz, H-20).

Dictyolactone (8) – colorless oil ; $[\alpha]^{21}_D$ – 150° (*c* 0.40, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 225 nm (3.80), 204 nm (3.10); IR (film) ν_{max} 2960, 1745, 1670, 1289 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 2.74 (1H, d, *J* = 7.5 Hz, H-2), 1.69 (1H, m, H-3), 1.50 (1H, m, H-4), 1.76 (1H, m, H-4), 1.97 (1H, m, H-5), 2.26 (1H, m, H-5), 5.39 (1H, dd, *J* = 11.3, 3.3 Hz, H-7), 2.93 (1H, ddd, *J* = 17.0, 7.5, 4.0 Hz, H-8), 3.14 (1H, ddt, *J* = 17.0, 11.8, 2.0 Hz, H-8), 6.97 (1H, dt, *J* = 8.0, 2.3 Hz, H-9), 1.64 (1H, m, H-10), 1.21 (1H, m, H-11), 1.93 (1H, m, H-12), 5.06 (1H, tt, *J* = 7.5, 1.0 Hz, H-13), 1.60 (3H, s, H-15), 1.70 (3H, s, H-16), 0.96 (3H, d, *J* = 6.0 Hz, H-17), 4.55 (1H, d, *J* = 10.0 Hz, H-18), 4.05 (1H, dd, *J* = 10.0, 7.3 Hz, H-18), 1.75 (3H, s, H-20).

Pachydictyol A (9) – colorless oil; ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 2.68 (1H, q, *J* = 9.3 Hz, H-1), 2.23 (1H, ddq, *J* = 16.0, 8.5, 1.5 Hz, H-2), 2.50 (1H, m, H-2), 5.34 (1H, s, H-3), 2.33 (1H, m, H-5), 3.93 (1H, br d, *J* = 7.0 Hz, H-6), 1.56 (1H, m, H-7), 1.54 (1H, m, H-8), 2.12 (1H, m, H-9), 2.63 (1H, dt, *J* = 17.0, 3.0 Hz, H-9), 1.56 (1H, m, H-11), 1.22 (1H, m, H-12), 1.53 (1H, m, H-12), 1.96 (1H, m, H-13), 2.05 (1H, m, H-13), 5.13 (1H, br t, *J* = 6.5 Hz, H-14), 1.70 (3H, s, H-16), 1.62 (3H, s, H-17), 1.01 (3H, d, *J* = 6.0 Hz, H-18), 1.82 (3H, br s, H-19), 4.75 (1H, br s, H-20), 4.76 (1H, br s, H-20).

Dictyol E (10) – colorless oil; ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 2.59 (1H, q, *J* = 9.3 Hz, H-1), 2.23 (1H, ddq, *J* = 15.5, 8.5, 1.5 Hz, H-2), 2.52 (1H, m, H-2), 5.36 (1H, br s, H-3), 2.39 (1H, br t, *J* = 7.0 Hz, H-5), 4.21 (1H, dd, *J* = 8.0, 3.0 Hz, H-6), 1.66 (1H, m, H-7), 1.70 (1H, m, H-8), 1.76 (1H, m, H-8), 2.14 (1H, m, H-9), 2.70 (1H, ddd, *J* = 14.0, 5.3, 2.3 Hz, H-9), 1.75 (1H, t, *J* = 8.3 Hz, H-12), 2.04 (1H, m, *J* = 7.5 Hz, H-13), 2.12 (1H, m, H-13), 5.17 (1H, tt, *J* = 7.3, 1.5 Hz, H-14), 1.70 (3H, s, H-16), 1.63 (3H, s, H-17), 1.25 (3H, s, H-18), 1.83 (3H, br s, H-19), 4.77 (1H, br s, H-20), 4.80 (1H, br s, H-20).

Cholesterol sulfate (11) – white powder; FABMS (+ve) *m/z*: 511 $[M + Na]^+$; ESIMS (–ve) *m/z*: 465 $[M–Na]^-$; ^{1}H-NMR (500 MHz, CD_3OD) δ : 4.12 (1H, m, H-3), 0.71 (3H, s, H-18), 1.03 (3H, s, H-19), ^{13}C-NMR (50 MHz, CD_3OD) δ : 38.5 (C-1), 30.0 (C-2), 79.8 (C-3), 40.4 (C-4), 141.6 (C-5), 123.3 (C-6), 33.2 (C-7), 33.1 (C-8), 51.6 (C-9), 37.7 (C-10), 22.2 (C-11), 41.1 (C-12), 43.5 (C-13), 58.1 (C-14), 25.3 (C-15), 29.3 (C-16), 57.5 (C-17), 12.3 (C-18), 19.8 (C-19), 37.1 (C-20), 19.2 (C-21), 37.4 (C-22), 24.9 (C-23), 40.7 (C-24), 29.2 (C-25), 23.2 (C-26), 23.2 (C-27).

(20R)-5α-chlostane-3α,21-diyl disulfate (12) – white powder; FABMS *m/z*: 609 $[M + H]^+$; ^{1}H-NMR (500 MHz, CD_3OD) δ : 4.64 (1H, m, H-3), 0.75 (3H, s, H-18), 0.85 (3H, s, H-19), 3.98 (1H, dd, *J* = 9.5, 5.5 Hz, H-21), 4.16 (1H, dd, *J* = 9.5, 4.0 Hz, H-21); ^{13}C-NMR (50 MHz, CD_3OD) δ : 33.2 (C-1), 28.0 (C-2), 76.7 (C-3), 33.9 (C-4), 41.5 (C-5), 28.5 (C-6), 33.1 (C-7), 34.7 (C-8), 55.9 (C-9), 36.9 (C-10), 22.0 (C-11), 40.5 (C-12), 43.6 (C-13), 57.8 (C-14), 25.0 (C-15), 29.6 (C-16), 52.5 (C-17), 13.0 (C-18), 11.9 (C-19), 40.7 (C-20), 69.9 (C-21), 31.1 (C-22), 24.6 (C-23), 40.7 (C-24), 29.0 (C-25), 23.0 (C-26), 23.1 (C-27).

Maculalactone A (13) – colorless oil; UV (MeOH) λ_{max} (log ε) 211 nm (4.24); IR (film) ν_{max} 3025, 2935, 1752, 1603 cm^{-1}; FABMS *m/z*: 377 $[M + Na]^+$ (100), 285 (12), 221 (11), 91 (11); HRFABMS *m/z*: 377.1516 (calcd for $C_{25}H_{22}O_2Na$, 377.1517); ^{1}H-NMR (500 MHz, $CDCl_3$)

δ : 4.96 (1H, t, *J* = 5.0 Hz, H-4), 3.57 (1H, d, *J* = 15.5 Hz, H-1a), 3.63 (1H, d, *J* = 15.5 Hz, H-1a), 6.91 (2H, dd, *J* = 7.3, 2.0 Hz, H-3a,7a), 7.19 (2H, dd, *J* = 7.5, 2.5 Hz, H-4a,6a), 7.18 (1H, m, H-5a), 3.50 (1H, d, *J* = 16.0 Hz, H-1b), 3.94 (1H, d, *J* = 16.0 Hz, H-1b), 7.05 (2H, br d, *J* = 7.0 Hz, H-3b,7b), 7.32 (2H, m, H-4b,6b), 7.27 (1H, m, H-5b), 2.85 (1H, dd, *J* = 14.5, 6.0 Hz, H-1c), 3.25 (1H, dd, *J* = 14.5, 4.0 Hz, H-1c), 7.16 (2H, dd, *J* = 6.5, 3.0 Hz, H-3c,7c), 7.27 (2H, m, H-4c,6c), 7.25 (1H, m, H-5c); 13C-NMR (50 MHz, $CDCl_3$) δ : 173.5 (C-1), 128.6 (C-2), 161.6 (C-3), 81.6 (C-4), 29.4 (C-1a), 137.7 (C-2a), 128.2, 128.6, 129.1, 129.5 (C-3a,4a,6a,7a, 3b,4b,6b,7b, and 3c,4c,6c,7c), 126.3 (C-5a), 135.9 (C-2b), 127.3 (C-5b), 134.9 (C-2c), 127.1 (C-5c).

Maculalactone E (14) – colorless needles; FABMS *m/z*: 525 $[M + Na]^+$ (100), 470 (12), 434 (4), 370 (2); HR FABMS *m/z*: 525.2057 (calcd for $C_{34}H_{30}O_4Na$, 525.2042); ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 4.67 (1H, br s, H-5), 2.09 (1H, dt, *J* = 14.5, 4.0 Hz, H_{eq}-6), 3.21 (1H, td, *J* = 14.5, 2.5 Hz, H_{ax}-6), 3.45 (1H, dd, *J* = 14.5, 4.3 Hz, H-7), 2.49 (1H, d, *J* = 16.0 Hz, H-1a), 3.01 (1H, d, *J* = 16.0 Hz, H-1a), 6.03 (2H, d, *J* = 8.0 Hz, H-3a,7a), 6.94 (2H, dd, *J* = 7.5, 7.5 Hz, H-4a,6a), 7.01 (1H, dd, *J* = 7.5, 7.5 Hz, H-5a), 7.31 (4H, m, H-2b,3b,5b,6b), 7.24 (1H, dd, *J* = 7.5, 7.5 Hz, H-4b), 7.54 (2H, d, *J* = 7.5 Hz, H-2c,6c), 7.43 (2H, dd, *J* = 7.5, 7.5 Hz, H-3c,5c), 7.31 (1H, m, H-4c), 2.71 (1H, d, *J* = 14.0 Hz, H-1d), 3.65 (1H, d, *J* = 14.0 Hz, H-1d), 7.40 (2H, dd, *J* = 8.0, 8.0 Hz, H-3d,7d), 7.30 (3H, m, H-4d-6d); ^{13}C-NMR (50 MHz, $CDOl_3$) δ : 172.5 (C-2), 131.5 (C-3), 77.6 (C-4), 74.2 (C-5), 31.4 (C-6), 49.8 (C-7), 91.4 (C-8), 160.6 (C-9), 29.3 (C-1a), 137.6 (C-2a), 127.4 (C-3a,7a), 127.9 (C-4a,6a), 125.5 (C-5a), 141.2 (C-1b), 128.1 (C-2b,3b,5b,6b), 128.8 (C-4b), 138.3 (C-1c), 129.5 (C-2c,6c), 128.3 (C-3c,5c), 127.6 (C-4c), 38.7 (C-1d), 135.7 (C-2d), 131.1 (C-3d,7d), 127.9 (C-4d,6d), 126.7 (C-5d).

(*S*)-MTPA ester of 14 – white power; ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 5.92 (1H, t, *J* = 2.5 Hz, H-5), 2.301 (1H, dt, *J* = 15.0, 4.0 Hz, H_{eq}-6), 3.233 (1H, td, *J* = 15.0, 2.0 Hz, H_{ax}-6), 2.75 (1H, dd, *J* = 14.0, 4.0 Hz, H-7), 1.90 (1H, d, *J* = 16.0 Hz, H-1a), 2.95 (1H, d, *J* = 16.0 Hz, H-1a), 5.81 (2H, d, *J* = 7.5 Hz, H-3a,7a), 2.61 (1H, d, *J* = 13.5 Hz, H-1d), 3.59 (1H, d, *J* = 13.5 Hz, H-1d).

(*R*)-MTPA ester of 14 – white power; ^{1}H-NMR (500 MHz, $CDCl_3$) δ : 5.93 (1H, t, *J* = 2.5 Hz, H-5), 2.299 (1H, dt, *J* = 15.0, 4.0 Hz, H_{eq}-6), 3.204 (1H, td, *J* = 15.0, 2.0 Hz, H_{ax}-6), 2.82 (1H, dd, *J* = 13.5, 4.5 Hz, H-7), 1.97 (1H, d, *J* = 16.5 Hz, H-1a), 3.01 (1H, d, *J* = 16.5 Hz, H-1a), 5.83 (2H, d, *J* = 7.5 Hz, H-3a,7a), 2.63 (1H, d, *J* = 14.0 Hz, H-1d), 3.60 (1H, d, *J* = 14.0 Hz, H-1d).

Results and Discussion

Compound **1** was isolated as white needles. The molecular formula was deduced to be $C_{15}H_{21}O_2Br_2Cl$ on the basis of the HREIMS data and NMR data. The $[M - H_2O]^+$ ion was observed at *m/z* 411.9496 ($C_{15}H_{19}O^{81}Br_2{}^{35}Cl$, Δ + 4.6 mmu). The ^{1}H NMR spectrum showed four singlet methyl signals and eight methylene or methine proton signals. The ^{1}H NMR data, combined with the ^{13}C NMR data displaying 15 carbon signals, were immediately reminiscent of a sesquiterpene skeleton. A combination of 2D NMR experiments allowed the entire carbon framework to be constructed for **1**. In particular, the HMBC correlations of C-6 (δ 53.4) with H-1 (δ 2.18 and 2.35), H-2 (δ 5.45), H-4 (δ 2.36 and 2.70), H-5 (δ 4.69), H-8 (δ 5.40), H-12 (δ 1.12), H-13 (δ 1.31), and H-14 (δ 1.52) were observed, suggesting the connection of two cyclohexane rings through the quaternary carbon at C-6. Thus, compound **1** was identified as pacifenol (Argandona *et al.*, 1993). Pacifenol is widespread in red algae of the genus *Laurencia* and also was found in some species of sea hares of the genus *Aplysia* that feed on the algae.

Compound **2** was isolated as white needles. The ^{1}H NMR spectrum of **2** was similar to that of **1**, except for the presence of an epoxymethine signal at δ 3.21 and a hydroxymethine signal at δ 4.04 instead of two olefinic proton signals as in **1**. The NMR data of **2** were in accordance with the partly defined data of johnstonol (Kimura *et al.*, 1999). The full assignment established by exhaustive 2D NMR experiments is presented in this paper. Analysis of the NMR data of compounds **3** and **4** suggested that they are halochamigrene derivatives structurally related to compounds **1** and **2**. Comparison of the physical properties of **3** and **4** with those of previously reported for halochamigrene derivatives showed that compound **3** is prepacifenol epoxide (McPhail *et al.*, 1999), and **4** is 2,10-dibromo-3-chlorochamigran-7-en-9-ol (Suzuki *et al.*, 1988). Compounds **2** and **3** were previously found in the red algae of the genus *Laurencia* and in the sea hares of the genera *Aplysia* and *Dolabella* that feed on the algae. Compound **4** was only found in the red algae of the genus *Laurencia*.

Compound **5** was isolated as a colorless oil. The ^{1}H NMR spectrum showed two singlet aromatic proton signals at δ 6.63 and 7.62, three singlet methyl signals at δ 1.34, 1.42 and 2.31, and a cyclopropyl methylene signal at δ 0.57. The ^{13}C NMR spectrum showed a total of 15 carbons including six aromatic carbons. On the basis of the ^{1}H and ^{13}C NMR data together with the COSY, HSQC, and HMBC experiments, compound **5** was identified as

Table 1. ^{13}C NMR Data of **1-10** (CD_3OD, 50 MHz)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	34.3	34.2	34.0	39.0	36.2	46.8	133.5	135.5	46.1	46.2
2	59.4	58.5	62.2	62.5	25.5	134.4	165.0	43.8	33.9	33.8
3	69.0	68.4	71.5	70.6	24.6	135.0	49.0	47.1	124.0	124.4
4	46.1	45.7	47.3	40.5	29.8	37.6	70.5	28.6	141.4	140.9
5	74.2	75.2	70.0	31.5	16.4	35.7	48.5	40.2	60.5	60.4
6	53.4	51.0	50.2	48.0	48.3	27.0	28.6	136.5	75.1	74.4
7	77.2	61.8	61.0	143.0	134.2	130.8	26.0	122.8	47.8	48.8
8	134.3	60.3	56.8	124.0	153.5	127.2	8.6	30.3	23.5	21.6
9	132.5	75.2	55.6	73.0	119.1	45.3	10.4	139.6	40.6	40.6
10	99.7	114.0	75.6	71.1	136.2	71.7	32.4	32.6	152.6	152.0
11	52.0	50.0	46.8	45.5	115.3	55.4	36.5	37.2	34.8	76.3
12	23.6	18.8	24.3	18.1	132.6	49.1	25.6	25.6	35.1	40.9
13	24.7	25.2	27.3	25.0	23.8	26.7	123.6	124.0	25.6	23.3
14	25.1	22.1	22.1	26.0	18.9	39.2	132.3	131.1	124.7	124.4
15	33.7	31.7	28.0	24.1	22.5	19.3	17.7	17.6	131.5	131.8
16						21.2	25.8	25.4	25.7	25.7
17						18.1	17.3	17.2	17.7	17.7
18						73.0	97.0	67.8	17.5	25.4
19						31.8	172.0	172.4	15.9	15.8
20						22.7	23.8	17.3	107.1	107.6
CH_3CO						21.5				
CH_3CO						169.1				

laurinterol (Ryu *et al.*, 2002), which was previously isolated from the red algae *Chondria crassicaulis, Marginisporum aberrans*, and *Laurencia* spp., and sea hares of the genus *Aplysia* which feed on the algae (Ryu et al., 2002; Jongaramruong *et al.*, 2002).

Compound **6** was isolated as a colorless oil. The strong HMBC correlation between the carbonyl carbon signal at δ 169.1 and the methyl proton signal at δ 2.08, together with an IR absorption at 1740 cm^{-1}, suggested the presence of an acetate group. The two singlet methyl signals at δ 1.20 and 1.27 in the ^1H NMR spectrum, together with the oxygenated quarternary carbon signal at δ 73.0 in the ^{13}C NMR spectrum, indicated the presence of an isopropyl alcohol moiety. In addition, the ^1H NMR spectrum also showed another three methyl proton signals at δ 0.89 (s), 0.96 (d, J = 8.0 Hz), and 1.66 (br s), and vinyl proton signals at δ 5.10 (d, J = 16.5 Hz), 5.13 (t, J = 8.0 Hz), and 5.25 (dd, J = 16.5, 7.5 Hz). The IR and NMR data of **6** were identical to those reported for 10-acetoxy-18-hydroxy-2,7-dolabelladiene (Ireland *et al.*, 1977), which was previously isolated from several brown algae and sea hares of the genus *Dolabella* that feed on the algae (Ireland *et al.*, 1977; Amico *et al.*, 1980).

Compound **7** was isolated as a colorless oil. Its molecular formula was established as $C_{20}H_{30}O_4$ on the basis of the pseudomolecular ion peak at *m/z* 357.2045 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_4Na$, 357.2042, Δ +0.3 mmu) and NMR data. IR spectrum showed a hydroxyl absorption at 3350 cm^{-1} and a carbonyl stretching band at 1750 cm^{-1}. These observation, combined with the ^{13}C NMR signals at δ 172.0 (C-19), 165.0 (C-2), 133.5 (C-1), 97.0 (C-18) and the UV absorption at 230 nm, suggested the presence of a γ-hydroxy-α,β-unsaturated-γ-lactone. In the ^1H NMR spectrum, two vinyl methyl signals were observed at δ 1.61 and 1.70, and two doublet methyl signals were observed at δ 1.04 and 1.08. Two cyclopropane protons were found as one-proton multiplets at δ 0.42 and 1.04. Based upon comparison of its spectral data with those reported (Midland *et al.*, 1983), compound **7** was identified as dihydroxycrenulide, which was originally isolated from the sea hare *Aplysia vaccaria.*

Compound **8** was isolated as a colorless oil. This compound is also a bicyclic diterpene with the same side chain as **7**. The downfield olefinic proton signal at δ 6.97, the carbon signals at δ 172.4 (C-19), 139.6 (C-9), and 135.5 (C-1), along with the IR absorption at 1745 cm^{-1} and the UV absorption at 225 nm, indicated that compound **8** is a lactone with an exocyclic α,β-unsaturation. Compound

8 was identified as dictyolactone by comparison of its spectral data with those reported (Finer *et al.*, 1979). Compound **8** was previously obtained from several brown algae and sea hares of the genus *Aplysia* that feed on the algae (Finer *et al.*, 1979; Bouaicha *et al.*, 1993).

Compound **9** was isolated as a colorless oil. The ^{1}H NMR spectrum showed a methyl doublet at δ 1.01, three broad methyl singlets at δ 1.62, 1.70, and 1.82, two trisubstituted olefinic signals at δ 5.13 and 5.34, and two exomethylene signals at δ 4.75 and 4.76. The proton signal at δ 3.93 was assigned to an oxymethine proton. All the NMR data were in good agreement with those reported for pachydictyol A (De Rosa *et al.*, 1986). Compound **10** was isolated as a colorless oil. Comparison of the NMR data with those of **9** indicated that **10** is the 11-oxy derivative of **9**, that is dictyol E (Amico *et al.*, 1980). Compounds **9** and **10** were previously isolated from a few brown algae and sea hares of the genus *Aplysia* which feed on the algae (Minale *et al.*, 1976; Amico *et al.*, 1980).

Compound **11** was isolated as a white powder. The molecular formula of **11** was established as $C_{27}H_{45}NaO_4S$ by combined analyses of the MS and NMR data. The $[M + Na]^+$ ion was observed at *m/z* 511 in the FABMS spectrum. The NMR data at δ_C 79.8 (δ_H 4.12) of C-3 suggested the presence of the 3β-sulfate substituent (Kapustina *et al.*, 2001). Compound **11** was identified as cholesterol sulfate by analysis of the spectral data and comparison with those of sterol sulfates (Riccio *et al.*, 1985; Kapustina *et al.*, 2001). Compound **12** was isolated as a white powder. The ^{1}H NMR data (δ 4.64, 4.16, and 3.98) indicated that it was a disulfated sterol. The

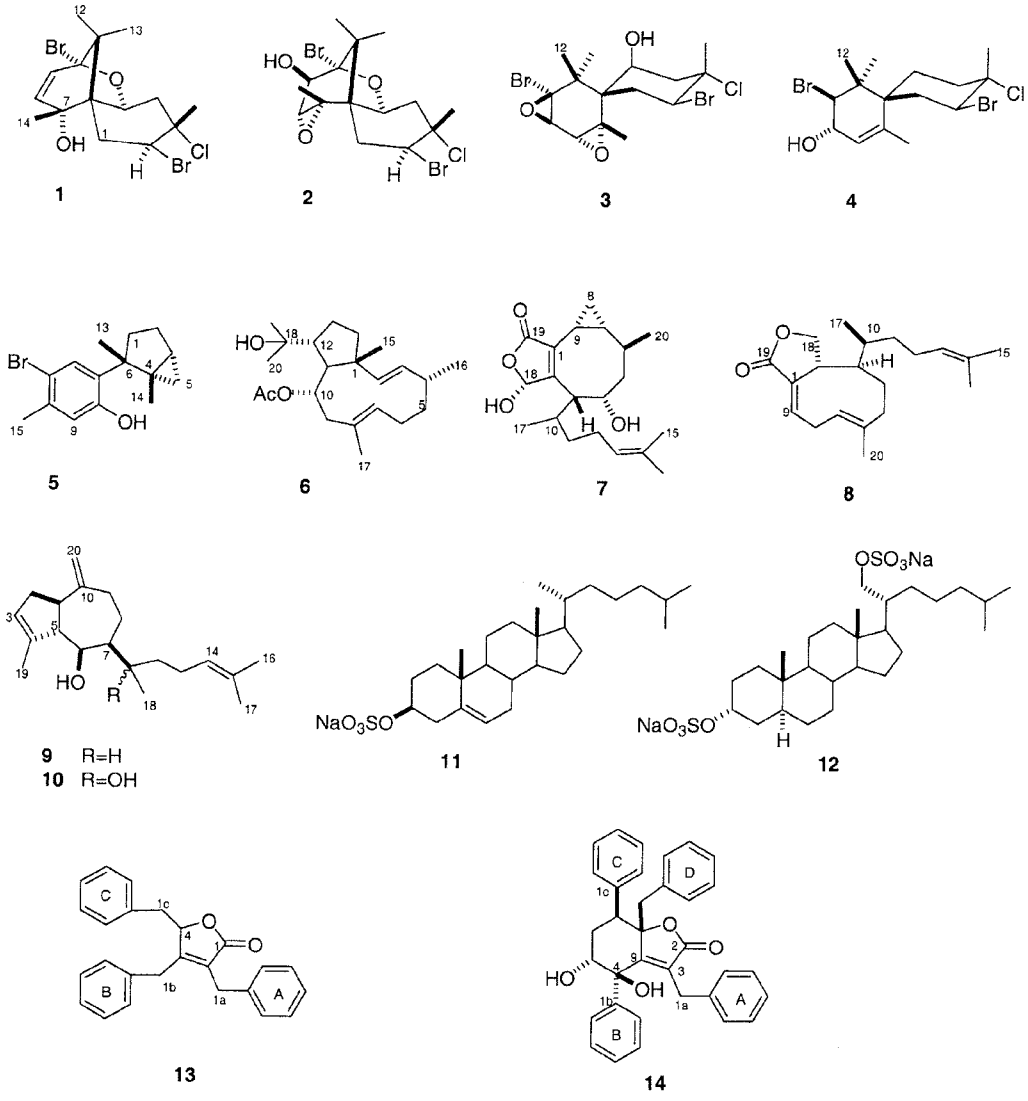

Fig. 1. Structures of compounds 1-14.

oxymethine proton signal at δ 4.64 was assigned to H-3β. The two coupled oxymethylene signals at δ 3.98 and 4.16 were attributed to H-21. The spectral data of **12** were identical to those reported (Riccio *et al.*, 1985). Compound **12** was previously isolated from the brittle star *Ophioderma longicaudum*, *Ophiopholis aculeata*, *Ophiura sarsi*, and *Stegophiura brachiactis* (Riccio *et al.*, 1985; Fedorov *et al.*, 1994).

Compound **13** was isolated as a colorless oil. The molecular formula of **13** was established as $C_{25}H_{22}O_2$ on the basis of the pseudomolecular ion peak at *m/z* 377.1516 $[M+Na]^+$ (calcd for $C_{25}H_{22}O_2Na$, 377.1517). The ^1H NMR signal at δ 4.96, the four ^{13}C NMR signals at δ 173.5 (C-1), 161.6 (C-3), 128.6 (C-2), and 81.6 (C-4), along with the IR absorption at 1752 cm^{-1}, indicated the presence of an α,β-unsaturated-γ-lactone. The ^{13}C NMR data also suggested the presence of three mono-alkyl-substituted benzene rings. The signals at δ 134.9, 135.9, and 137.7 were assigned to the alkyl-substituted quaternary aromatic carbons. The relatively upfield-shifted signals at δ 126.3, 127.1, and 127.3 were due to the aromatic methines *para* to the substituents. Several overlapped peaks at δ 128.2-129.5 corresponded to the *ortho*- and *meta*-carbons. Each of the quaternary and *ortho* aromatic carbons of the three benzene rings correlated with different pairs of methylene protons. These observations supported the presence of three aromatic groups as benzyl substituents. Compound **13** was identified as maculalatone A by further analysis of the 2D NMR data and comparison with those reported (Lee *et al.*, 1998).

Compound **14** was isolated as colorless needles. The molecular formula of **14** was established as $C_{34}H_{30}O_4$ on the basis of the pseudomolecular ion peak at m/z 525.2057 $[M+Na]^+$ (calcd for $C_{34}H_{30}O_4Na$, 525.2042). The ^1H and ^{13}C NMR data suggested the presence of four benzene groups. Analysis of the 2D NMR data established the 4-hydroxy-4,5,6,7-tetrahydrobenzofuran-2-one nucleus substituted by two phenyl groups at C-4 and C-7, and by two benzyl groups at C-3 and C-8, respectively. The fully assigned NMR data were identical to those of maculalatone E (Lee *et al.*, 1998). The relative stereochemistry of **14** was determined by ^1H-^1H coupling constants and NOESY correlations as described previously (Lee *et al.*, 1998). The H-5 proton (δ 4.67) was assigned at an equatorial position because it was observed as a broad singlet ($W_{1/2}$ = 7.7 Hz), without diaxial coupling to H_{ax}-6. The H-7 proton was in an axial position based on a diaxial coupling (J = 14.5 Hz) to H_{ax}-6. The H_{ax}-6 signal also showed correlations with the *ortho* protons of C-phenylring, and H-1d in the NOESY experiment, which established the relative stereochemistry of the D-benzyl ring (Fig. 2). The hydroxyl groups at C-4 and C-5 were presumed to be trans to each other because the ^1H and ^{13}C chemical shifts of H-4,5 and C-4,5 matched with those reported, otherwise, the chemical shift would be significantly different. In addition, the location of the bulky B-phenyl ring at equatorial position renders the molecule more stability. The 5*R* absolute configuration was confirmed by modified Mosher's method on the basis of the Δδ (δ_S-δ_R) value (+0.029 ppm) of H_{ax}-6, and accordingly the absolute stereochemistry of **14** was established for the first time as shown in Fig. 1. Compounds **13** and **14** were previously obtained only from the cyanobacterium *Kyrtuthrix maculans* (Lee *et al.*, 1998).

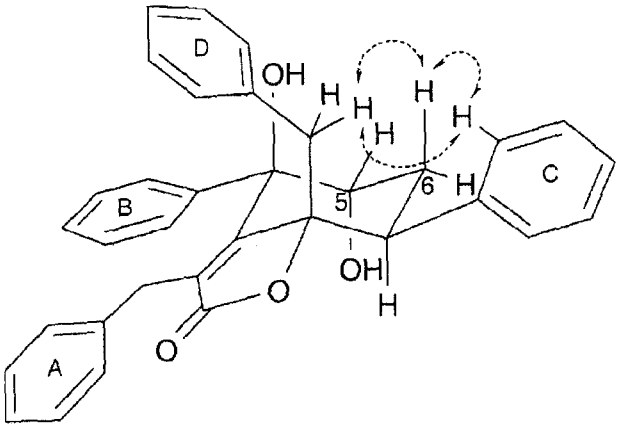

Fig. 2. Key NOESY correlations of **14**.

Table 2. Cytotoxicity Data of **1-4, 6-10, 13**, and **14** against Human Solid Tumor Cells[a,b]

compounds	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	>30	>30	>30	>30	>30
2	>30	>30	>30	>30	>30
4	20.4	8.0	8.7	11.4	18.6
6	5.1	5.5	4.9	4.4	4.6
8	4.4	0.8	4.1	4.0	4.3
9	3.8	4.2	2.8	3.3	2.5
10	4.7	11.0	4.9	4.2	4.3
doxorubicin	0.03	0.03	0.01	0.03	0.05
3	>30	28.8	>30	17.9	>30
7	7.3	4.2	8.0	6.8	4.9
doxorubicin	0.09	0.16	0.07	0.04	0.13
13	>30	>30	>30	48.9	15.9
14	3.7	3.6	3.6	2.8	3.4
doxorubicin	0.14	0.17	0.13	0.13	0.14

[a] Data as expressed in ED_{50} values (μg/mL). A549: human lung cancer; SK-OV-3: human ovarian cancer; SK-MEL-2: human skin cancer; XF498: human CNS cancer; HCT 15: human colon cancer. [b] Compounds were assayed in three separate batches.

Table 3. Antibacterial Activity Data of Compound **5** against Methicillin Resistant Strains[a,b]

Strains	5	Imipenem	Meropenem
Streptococcus Pyogenes 308 A	1.563	0.004	0.013
Streptococcus Pyogenes 77 A	3.125	0.004	0.007
Streptococcus faecium MD 8b	3.125	0.781	6.250
Staphylococcus aureus SG 511	3.125	0.013	0.049
Staphylococcus aureus 285	3.125	0.013	0.195
Staphylococcus aureus 503	3.125	0.013	0.049

[a] Data as expressed *in* MIC values *(μg/mL).* [b] MIC values >25.0 μg/mL were determined for *Escherichia coli* 078, *Escherichia coli* DC0, *Escherichia coli* DC2, *Escherichia coli* TEM, *Escherichia coli* 1507E, *Pseudomonas aeruginosa* 9027, *Pseudomonas aeruginosa* 1592E, *Pseudomonas aeruginosa* 1771, *Pseudomonas aeruginosa* 1771M, *Salmonella typhimurium*, *Klebsiella oxytoca* 1082E, *Klebsiella aerogenes* 1522E, *Enterobacter cloacae* P99, *and Enterobacter cloacae* 1321E.

Compounds **1-4**, **6**, **8-10**, **13**, and **14** were evaluated for cytotoxicity against a small panel of five human solid tumor cell lines (Table 2). Compounds **4**, **6-10,** and **14** showed moderate to significant cytotoxicity. Compound **3** was unstable and one of the epoxide rings was cleaved to give **2** during incubation in cytotoxicity evaluation. The cytotoxicity of compound **5** against the five human solid tumor cell lines was previously reported (Ryu *et al.*, 2002). Compounds **1**, **2**, **4-8**, and **10-12** were also tested for their antibacterial activity against 20 clinically isolated strains. Only compound **5** showed antibacterial activity against three *Streptococcus* and three *Staphylococcus* strains (Table 3). Other compounds displayed no antibacterial activity against these 20 clinically isolated strains up to the concentration of 25.0 μg/mL.

Acknowledgments

This study was supported by a grant from Korea Science and Engineering Foundation.

References

Amico, V., Oriente, G., Piattelli, M., Tringali, C., Fattorusso, E., Magno, S., Mayol, L. Diterpenes based on the dolabellane skeleton from *Dictyota dichotoma. Tetrahedron* **36**(10), 1409-1414 (1980).

Argandona, V. H., San-Martin, A., Rovirosa, J. Halogenated sesquiterpenes pacifenol and pacifenol derivatives on the aphid *Schizaphis graminum. Phytochemistry* **32**(5), 1159-1161 (1993).

Bouaicha, N., Tringali, C., Pesando, D., Mallea, M., Roussakis, C., Verbist, J. F. Bioactive diterpenoids isolated from *Dilophus ligulatus. Planta Med.* **59**(3), 256-258 (1993).

D'Auria, M. V., Minael, L., Riccio, R., Uriarte, E. Dehy droophioxanthin, a new acetylenic carotenoid sulfate from the ophiuroid *Ophiocomina nigra. J. Nat. Prod.* **54**(2), 606-608 (1991).

D'Auria, M. V., Gomez-Paloma, L., Minale, L., Riccio, R., Zampella, A. On the composition of sulfated polyhydroxysteroids in some ophiuroids and the structure determination of six new constituents. *J. Nat. Prod.* **58**(2), 189-196 (1995).

De Rosa, S., De Stefano, S., Zavodnik, N. Chemical studies of North Adriatic seaweeds. Part 2. Hydroazulenoid diterpenes from the brown alga *Dictyota dichotoma var. implexa. Phytochemistry* **25**(9), 2179-2181 (1986).

Fedorov, S. N., Levina, E. V., Kalinovsky, A. I., Dmitrenok, P. S., Stonik, V. A. Sulfated steroids from Pacific brittle stars *Ophiopholis aculeata*, *Ophiura sarsi*, and *Stegophiura brachiactis. J. Nat. Prod.* **57**(12), 1631-1637 (1994).

Finer, J., Clardy, J., Fenical, W., Minale, L., Riccio, R., Battaile, J., Kirkup, M., Moore, R. E. Structures of dictyodial and dictyolactone, unusual marine diterpenoids. *J. Org. Chem.* **44**(12), 2044-2047 (1979).

Hickman, C. P., Roberts, C. S., Hickman, F. M. Integrated Principles of Zoology. 7th edition. Times Mirror/Mosby College Publishing, U. S. A., pp. 476(1984).

Ivanchina, N. V., Kicha, A. A., Kalinovsky, A. I., Dmitrenok, P. S., Stonik, V. A. Hemolytic steroid disulfates from the Far Eastern starfish *Pteraster pulvillus. J. Nat. Prod.* **66**(2), 298-301 (2003).

Jongaramruong, J., Blackman, A. J., Skelton, B. W., White, A. H. Chemical relationships between the sea hare *Aplysia parvula* and the red seaweed *Laurencia filiformis* from Tasmania. *Aust. J. Chem.* **55**(4), 275-280 (2002).

Kapustina, I. I., Ponomarenko, L. P., Moiseenko, O. P., Stonik, V. A. Free and sulfated sterols of two far-east *Leptasterias* starfish. *Chem. Nat. Comp.* **37**(6), 515-519 (2001).

Kimura, J., Kamada, N., Tsujimoto, Y. Fourteen chamigrane derivatives from a red alga, *Laurencia nidifica. Bull. Chem. Soc. Jpn.* **72**(2), 289-292 (1999).

Koenig, G. M., Wright, A. D., Sticher, O., Angerhofer, C. K., Pezzuto, J. M. Biological activities of selected marine natural products. *Planta Med.* **60**(6), 532-537 (1994).

Lee, S., Williams, G. A., Brown, G. D. Tribenzylbutyrolactones and dibenzyldiphenyl-4,5,6,7-tetrahydrobenzofuranones from *Kyrtuthrix maculans. J. Nat. Prod.* **61**(1), 29-33 (1998).

Liu, S., Sjoevall, J., Griffiths, W. J. Neurosteroids in rat brain: extraction, isolation, and analysis by nanoscale liquid chromatography-electrospray mass spectrometry. *Anal. Chem.* **75**(21), 5835-5846 (2003).

Maier, M. S., Araya, E., Seldes, A. M. Sulfated polyhy droxysteroids from the antarctic ophiuroid *Gorgonocephalus chilensis. Molecules* (Electronic Publication) **5**(3), 348-349 (2000).

McPhail, K. L., Davies-Coleman, M. T., Copley, R. C. B., Eggleston, D. S. New halogenated sesquiterpenes from South African specimens of the circumtropical sea hare *Aplysia dactylomela. J. Nat. Prod.* **62**(12), 1618-1623 (1999).

Midland, S. L., Wing, R. M., Sims, J. J. New crenulides from the

sea hare, *Aplysia vaccaria*. *J. Org. Chem.* **48**(11), 1906-1909 (1983).

Minale, L. Riccio, R. Constituents of the digestive gland of the molluscs of the genus Aplysia. I. Novel diterpenes from *Aplysia depilans*. *Tetrahedron Lett.* **31**, 2711-2714 (1976).

Minale, L., Riccio, R., Zollo, F. Structural studies on chemical constituents of echinoderms. *Stud. Nat. Prod. Chem.* **15**, 43-110 (1995).

Riccio, R., D'Auria, M. V., Minale, L. Unusual sulfated marine steroids from the ophiuroid *Ophioderma longicaudum*. *Tetrahedron* **41**(24), 6041-6046 (1985).

Roccatagliata, A. J., Maier, M. S., Seldes, A. M., Pujol, C. A., Damonte, E. B. Antiviral sulfated steroids from the ophiuroid *Ophioplocus januarii*. *J. Nat. Prod.* **59**(9), 887-889 (1996).

Ryu, G., Park, S. H., Choi, B. W., Lee, N. H., Hwang, H. J., Ryu, S. Y., Lee, B. H. Cytotoxic activities of brominated sesquiterpenes from the red alga *Laurencia okamurae*. *Nat. Prod. Sci.* **8**(3), 103-107 (2002).

Strott, C. A., Higashi, Y. Cholesterol sulfate in human physiology: What's it all about? *J. Lipid Res.* **44**(7), 1268-1278 (2003).

Suzuki, M., Kurosawa, E., Furusaki, A. Constituents of marine plants. 73. The structure and absolute stereochemistry of a halogenated chamigrene derivative from the red alga *Laurencia* species. *Bull. Chem. Soc. Jpn.* **61**(9), 3371-3373 (1988).

Vairappan, C. S., Suzuki, M., Abe, T., Masuda, M. Halogenated metabolites with antibacterial activity from the Okinawan *Laurencia* species. *Phytochemistry*, **58**(3), 517-523 (2001).

(Accepted November 8, 2004)