Natural Product Sciences
8(3) : 103-107 (2002)

Cytotoxic Activities of Brominated Sesquiterpenes from the Red Alga *Laurencia okamurae*

Geonseek Ryu, Soo Hee Park, Byoung Wook Choi, Nam Ho Lee,[1] Hye Jung Hwang,[2] Shi Yong Ryu,[3] and Bong Ho Lee*

Regional Research Center and Department of Chemical Technology, Hanbat National University, 16-1 Dukmyung-dong, Yusung-ku, Taejon, 305-719, Korea
[1]Department of Chemistry, Cheju National University, 1 Ara 1-dong, Cheju, 690-756, Korea
[2]Livechem, INC., Hanbat National University, 16-1 Dukmyung-dong, Yusung-ku, Taejon, 305-719, Korea
[3]Korea Research Institute of Chemical Technology, P.O. Box 107, Yusung-ku, Taejon, 305-600, Korea

Abstract – Four known sesquiterpenes, laurinterol (**1**), isolaurinterol (**2**), aplysinal (**3**) and aplysin (**4**) were isolated from the Korean red alga *Laurencia okamurae* off Cheju Island, Korea. Their structures were identified by comparison with the literature data. Compounds **1-4** showed potent cytotoxicity against A549, SK-OV-3, SK-MEL-2, XF498, and HT15 cell lines with EC_{50} values ranging from 1.2 to 17.6 μg/mL.
Keywords – Marine red algae, *Laurencia okamurae*, Aplysins, Cytotoxicity.

Introduction

Some halogenated metabolites from marine organisms are believed to play the role of chemical defense substances against marine herbivores (Vairappan *et al.*, 2001; Biswas *et al.*, 1990). Red algae in genus *Laurencia* (Ceramiales, Rhodomelaceae) are a rich source of the secondary metabolites including halogenated sesquiterpenes with diverse structural features depending on the species and localities (Hay *et. al.*, 1987.; Kurata *et al.*, 1998). Among the chemical constituents isolated from the algal extracts were the aplysins unusual tricyclic sesquiterpenes (Hay *et al.*, 1987; Kurata *et al.*, 1998; Yamamura *et al.*, 1963; Selover *et al.*, 1980; Blunt *et al.*, 1984; Shizuri *et al.*, 984; Irie *et al.*, 1964; McMillan *et al.*, 1976; Ohta *et al.*, 1977; Crews *et al*, 1986; Suzuki *et al.*, 1976). The co-occurrence of brominated and non-brominated aplysins in all natural sources prompted speculation that the debromo-analogues scavenged reactive halogens from the marine environment before they could damage on the host. The aplysins are also believed to act as anti-feedant preventing the predatory advances of other marine organisms (Biswas *et al.*, 1990). As a part of our continuing searches for antitumor agents from Korean marine algae, we found potent cytotoxic activity in the methanol extract of *Laurencia* sp. We herein report the isolation, structural characteristics and cytotoxic activity of compounds **1-4**.

*Author for correspondence, E-mail: lbh011@hanbat.ac.kr

Experimental

General – UV spectrum was recorded with a HP8453 UV/VIS spectrophotometer. IR spectrum was performed on a Perkin-Elmer model 1750 FT-IR spectrophotometer. MS spectra were measured on a JEOL JMX-SX 102 mass spectrometer. High resolution mass measurement was done with a JEOL AX-505H mass spectrometer at high resolution. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at 25°C on a Brucker ARX-400 NMR spectrometer. Chemical shifts (**δ**) are given relative to TMS, using the solvent peaks [$CDCl_3$ (δ_H 7.26, δ_C 77.1)] as an internal standard. TLC was performed on precoated Kiesel gel 60 F254 plates (Merck). The silica gel used for column chromatography was Kiesel gel 60 (70-230 mesh, Merck).

Plant materials – The Brown Alga *Laurencia okamurae* was collected off Cheju island, Korea in 2000. A voucher specimen (GD007) of the algae has been deposited at the Laboratory of Natural Products Chemistry, Hanbat National University.

Cytotoxic assay – Sulforhodamin B (SRB) was used for cytotoxicity evaluation (Monsks *et al.*, 1991): TCA-fixed cells were stained for 30 minutes with 0.4% (wt/vol) SRB dissolved in 1% acetic acid. At the end of the staining period, SRB was removed and cultures were quickly rinsed four times with 1% acetic acid to remove unbound dye. The

acetic acid was poured directly into the culture wells from a beaker. This procedure permits rinsing to be performed quickly so that desorption of protein-bound dye would not occur. Residual wash solution was removed by sharply flicking plates over a sink, which ensured complete removal of rinsing solution. Because of the strong capillary action in 96-well plates, draining by gravity alone often fails to remove the rinse solution when plates are simply inverted. After being rinsed, the cultures were air dried until no standing moisture was visible. Bound dye was solubilized with 10 mM unbuffered Tris base (pH 10.5) for 5 minutes on a gyratory shaker.

OD was read in either an UVmax microtiter plate reader (Molecular Devices, Menlo Park, CA) or a Beckman DU-70 spectrophotometer. For maximum sensitivity, OD was measured at 564 nm. Since readings are linear with dye concentrations only below 1.8 OD units, suboptimal wavelengths are generally used, so that all samples in an experiment remained within the linear OD range. With most cell lines, wavelengths of approximately 490-530 nm worked well for this purpose.

Extraction and isolation – The MeOH extract (6.4 g) of the dried algal sample (50 g) was suspended with 90% MeOH and extracted with *n*-hexane three times. The lower layer was concentrated *in vacuo* and further partitioned between 30% MeOH and $CHCl_3$. The $CHCl_3$ phase was fractionated by flash column chromatography (C_{18} and SiO_2) as well as prep. TLC with MeOH/$CHCl_3$ (2:98) to give laurinterol **1** (320 mg). The upper layer (*n*-hexane phase) was purified by silica column and prep. TLC to afford isolaurinterol (**2**, 28 mg), aplysinal (**3**, 17 mg) and aplysin (**4**, 40 mg).

Laurinterol (1) – colorless oil; $[\alpha]_D^{23}$ +14.5° (*c*2.4, $CHCl_3$); UV λ_{max} (log ε) 225 nm (3.8), 283 (4.3); IR (film) ν_{max} cm^{-1} 3450 (OH), 1610, 1580 (aromatic), and 1150 (C-O); EIMS *m/z* 294 $[M]$,$^+$ 296 $[M+2]^+$; HREIMS *m/z* 294.0614 $[M]^+$, calcd for $C_{15}H_{19}BrO$, 294.0619; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.67 (1H, s, H-12), 6.62 (1H, s, H-9), 2.32 (3H, s, 15-Me), 2.14 (1H, dd, 13.2, 8.0 Hz, Ha-2), 2.03-1.94 (1H, m, Ha-1), 1.70 (1H, dd, 12.4, 8.0 Hz, Hb-1), 1.45 (3H, s, 13-Me), 1.36 (3H, s, 14-Me), 1.33 (1H, s, m, Hb-2), 1.19 (1H, m, H-3), 0.63-0.57 (2H, m, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2.

Isolaurinterol (2) – colorless oil; UV λ_{max} (log ε) 285 nm (3.3); IR (film) ν_{max} cm^{-1} 2950 (C_{sp3}-H), 1640 (aromatic); EIMS *m/z* 294 $[M]$,$^+$ 296 $[M+2]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ7.37 (1H, s, H-11), 6.65 (1H, s, H-8), 5.03 (1H, d, 2.0, Ha-14), 4.86 (1H, d, 2.0, Hb-14), 2.76 (1H, m, H-3), 2.23 (3H, s, 15-Me), 2.14 (1H, m, Ha-1), 1.97 (1H, m, Ha-2), 1.51 (1H, m, Hb-1), 1.38 (3H, s, 13-Me), 1.38-1.28 (1H, m, Hb-2), 1.13 (3H, d, 7.0 Hz, 12-Me); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2.

Aplysinal (3)-colorless solid; UV λ_{max} (log ε) 296 nm (3.5), 234 (3.7); IR (film) ν_{max} cm^{-1} 2950 (aliphatic C-H), 1730 (carbonyl) and 1610 (aromatic); EIMS *m/z* 308 $[M]^+$, 310 $[M+2]^+$, 281 $[M\text{-}CHO]^+$, 239; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.68 (1H, s, aldehyde), 7.09 (1H, s, H-11), 6.72 (1H, s, H-8), 2.28 (3H, s, 15-Me), 1.85-1.65 (4H, m, H-1 and H-2), 1.42-1.18 (1H, m, H-3), 1.22 (3H, s, 13-Me), 0.94 (3H, d, 6.7 Hz, 12-Me); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2.

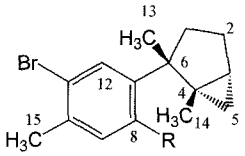
Aplysin (4) – colorless solid; UV λ_{max} (log ε) 296 nm (3.5), 234 (3.7); IR (film) ν_{max} cm^{-1} 2950 (aliphatic C-H), 1580 (aromatic); EIMS *m/z* 294 $[M]$,$^+$ 296 $[M+2]^+$; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.06 (1H, s, H-11), 6.55 (1H, s, H-8), 2.23 (3H, s, 15-Me), 1.80-1.48 (4H, m, H-1 and H-2), 1.23 (3H, s, 13-Me), 1.20 (3H, s, 14-Me), 1.11-1.06 (1H, m, H-3), 1.03 (3H, d, 6.8 Hz, 12-Me); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2.

Acetylation of 1 – A mixture of laurinterol (**1**, 16 mg), Ac_2O (1.0 mL), and pyridine (1.0 mL) was stirred at room temperature overnight. The reagents were evaporated *in vacuo* and the residue was subjected to SiO_2 column chromatography with $CHCl_3$ to yield an acetyl laurinterol (**5**, 14 mg).

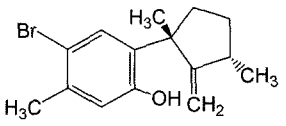
Compound 5 – colorless oil; EIMS *m/z* 336 $[M]$,$^+$ 338 $[M+2]$,$^+$ 321, 323; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82 (1H, s, H-12), 6.88 (1H, s, H-9), 2.34 (3H, s, OCH_3), 2.30 (3H, s, 15-Me), 2.14 (3H, s, $COCH_3$), 1.96-1.63 (4H, m, H-1 and H-2), 1.43 (3H, s, 13-Me), 1.33 (3H, s, 14-Me), 1.14 (1H, m, H-3), 0.88-0.49 (2H, m, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 2.

Results and Discussion

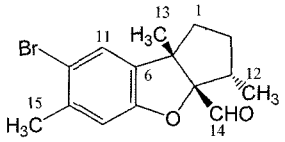
Compound **1** was obtained as a colorless oil. Its molecular formula was established as $C_{15}H_{19}BrO$ on the basis of HREIMS (*m/z* 294.0614 $[M]^+$, Δ-0.5 mmu) and ^{13}C NMR data. Its IR spectrum revealed absorptions due to a hydroxyl group (3450 cm^{-1}), aromatic C=C bands (1610, 1580), and C-O band (1150). The ^{1}H NMR spectrum showed two singlet aromatic protons (δ 7.67, 6.62), three singlet methyls (δ 2.32, 1.45, 1.36), cyclopropanyl protons (δ 0.63-0.57). The ^{13}C NMR spectrum combined with the DEPT spectrum of **1** suggested the presence of four quaternary olefinic carbons, two olefinic methine carbons, two quaternary sp^3-carbons, an sp^3-methines, three sp^3-methylenes, and three methyls (Table 2). Acetylation of **1** with Ac_2O in pyridine gave monoacetyl laurinterol (**5**), indicative of the presence of a hydroxyl group in **1**. The same intensive M^+



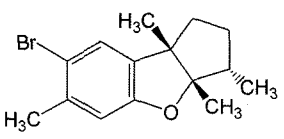
1 R=OH
5 R=OAc



2



3

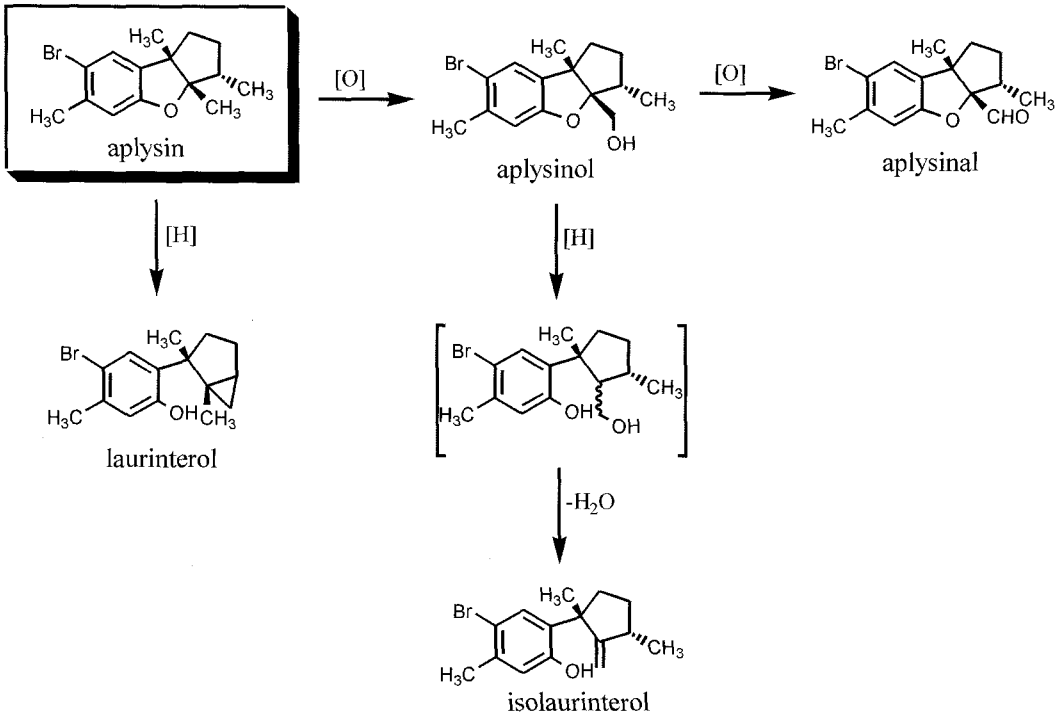


4

and $(M+2)^+$ ions at *m/z* 294 and 296 in the EIMS spectrum, respectively, also confirmed the presence of a bromine in compound **1**. A variety of 2D NMR experiments allowed the entire carbon framework to be constructed as **1**. In particular, an HMBC experiment of compound **1** provided the connection between a proton resonance system ($CH_2CH_2CHCH_2$) and a tetrasubstituted benzene skeleton through 13-methyl protons. The observation of correlations between H-13 and H-14, between H-13 and Ha-2, between Hb-2 and H-5, and between H-14 and H-3 in the NOESY spectrum of **1** provided strong evidence for the *cis* configuration between 13-CH_3 and 14-CH_3. Therefore, compound **1** was identified as laurinterol (Irie *et al.*, 1966).

Compound **2** was obtained as a colorless oil and displayed two molecular isotope ion peaks at *m/z* 294 and 296 in the EIMS spectrum. In the ^1H and ^{13}C NMR spectra of **2**, the signals were similar to those of **1**, except for the appearance of an exo-methylene signal (δ 4.86, 5.03; δ 107.3) and a doublet methyl (δ 1.13) instead of a singlet methyl in **1**. The NMR spectral and physical data of **2** were in good agreement with those reported in the literatures (Irie *et al.* 1970). Thus, compound **2** was identified as isolaurinterol. Compound **3** was obtained as a colorless solid and showed two molecular isotope ion peaks at *m/z* 308 and 310 in EIMS spectrum. The IR spectrum of **3** exhibited a strong band at 1730 cm^{-1}, indicative of the presence of a carbonyl group in **3**. As expected, the ^1H and ^{13}C NMR spectra of **3** were characterized by an aldehyde signal at 9.86 ppm (singlet) and 202.8 ppm, respectively. Compound **3** was determined as aplysinal by comparison with the literature data (Ohta *et al.*, 1977). Compound **4** was obtained as a colorless solid and revealed two molecular isotope ion peaks at *m/z* 294 and 296 in EIMS spectrum. The ^1H NMR spectrum of **4** showed three singlet methyls (δ 2.23, 1.23, 1.20) and a doublet methyl (δ 1.03). The DEPT spectrum of **4** further exhibited four methyl carbons and only two methylene carbons. By comparison of its spectral data with those of the literature values (Winkler *et al.*, 1962), compound **4** was identified as aplysin.

The biogenesis of the aplysins may be imagined to involve an aplysin precursor, possibly via significant deviation by



Scheme 1. A putative biosynthetic pathway for aplysin-related compounds.

Table 1. Cytotoxicities of the aplysins against human cancer cell lines

Compound	ED_{50} (μg/mL)				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	12.6	3.4	10.2	2.4	3.6
2	10.4	2.1	6.5	4.7	6.6
3	17.6	12.4	8.2	4.6	10.6
4	6.7	2.4	6.2	1.2	4.4
Adriamycin	0.18	0.21	0.32	0.22	2.4

ED_{50} value of the compounds against each cancer cell line was defined as a concentration (μg/mL) that caused 50% inhibition of cell growth, and adriamycin was used as a reference.

Table 2. ^{13}C NMR Data of Compounds 1-5 in $CDCl_3$

Position	1	2	3	4	5
1	25.4	31.7	31.8	31.5	25.7
2	36.1	39.5	43.0	42.9	36.2
3	24.6	38.1	42.4	46.4	24.2
4	29.7	165.4	103.9	100.1	29.9
5	16.4	50.2	58.8	54.6	16.6
6	48.2	133.1	134.3	136.5	48.3
7	134.3	153.4	158.5	158.5	136.5
8	153.4	120.8	111.5	111.2	148.6
9	119.0	137.5	138.0	137.3	126.3
10	136.1	115.9	115.5	114.3	140.0
11	115.1	131.7	126.4	126.8	121.6
12	132.5	21.5	13.0	13.4	132.8
13	23.6	28.1	24.0	23.5	22.7
14	18.8	107.3	202.8	23.7	19.0
15	16.4	22.6	23.0	20.3	21.9
$COCH_3$					169.7
$COCH_3$					24.1

reductase or oxidase from routine that lead to laurinterol, isolaurinterol, aplysinol (McMillan *et al.*, 1976) and aplysinal, as shown in Scheme 1. It might also be thought that the debromo-aplysins scavenged reactive bromines from the marine environment before they could inflict damage on the host (Harrowven *et al.*, 2001). The cytotoxicities of the compounds were tested by SRB bioassay against five cultured human tumor cells. Compounds **1-4** exhibited significant cytotoxicity against cultured human tumor cell lines, A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian), SK-MEL-2 (skin melanoma), XF498 (CNS), and HCT15 (colon), as shown in Table 1. These compounds, however, exhibited no antiviral activity against herpes simplex virus.

Acknowledgments

This work was supported by the Korea Science and Engineering Foundation (KOSEF) through the Advanced Material Research Center for Better Environment at Hanbat National University. We thank Professor Y. P. Lee of Cheju National University for identification of the algae. We are also debt to Dr. E. C. Lee, Korea Ginseng & Tobacco Research Institute for nuclear magnetic resonance and mass measurements.

References

Biswas, S., Ghosh, A. and Kurosawa, E., Stereocontrolled synthesis of (±)debromoalysin, (±)aplysin, (±)debromoaplysinol, (±)aplysonol, and (±) isoaplysinol, *J. Org. Chem.*, **55**, 3498-3502 (1990).

Blunt, J. W., Lake, R. J., Munro, M. H. G., Sesquiterpenes from the marine red alga *Laurencia distichophylla. Phytochem.*, **23**, 1951-1954 (1984).

Crews, P. and Selover, S. J., Comparison of the sesquiterpenes from the seaweed *Laurencia pacifica* and its epiphyte *Erythrocystis saccata. Phytochem.*, **25**, 1847-1852 (1986).

Hay, M.E. and Fenical, W., Gustafson, K., Chemical defence against diverse coral-reef herbivores. *Ecology*, **68**, 1581-1591 (1987).

Harrowven, D. C., Lucas M. C. and Howes P. D., The synthesis of a natural product family: from debromoisolaurinterol to the aplysins. *Tetrahedron*, **57**, 791-804 (2001).

Irie, T., Suzuki, M., Kurata, K. and Hayakawa, Y., Constituents of marine plants. XII. Isolation of aplysin, debromoaplysin, and aplysinol from *Laurencia okamurai. Bull. Chem. Soc. Jap.*, **42**, 843-844 (1964).

Irie, T., Suzuki, M., Kurosawa, E. and Masamune, T., Laurinterol and debromolaurinterol, constituents from *Laurencia intermedia, Tetrahedron Lett.*, **17**, 1837-1840 (1966).

Irie, T., Suzuki, M., Kurosawa, E. and Masamune, T., Constituents from marine plants. XVI. Laurinterol, debromolaurinterol, and isolaurinterol, constituents of *Laurencia intermedia. Tetrahedron*, **26**, 3271-3277 (1970).

Kurata, K., Taniguchi, K., Agatsuma, Y. and Suzuki, M. Diterpenoid feeding-deterrents from *Laurencia saitoi. Phytochem.* **47**, 363-369 (1998).

McMillan, J.A., Paul, I.C., Caccamese, S. and Rinehart Jr K. R.,. Aplysinol from *Laurencia decidua*: crystal structure and Absolute stereochemistry, *Tetrahedron Lett.*, **17**, 4219-4222 (1976).

Monsks, A., Scudiro, D., Skehan, P., Shoemaker, R., Paull, K., Vistica D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell H., Mayo J. and Booyd M., Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.* **83**, 757-766 (1991).

Ohta, K. and Takagi, M., Halogenated sesquiterpenes from the marine red alga *Marginisporum aberrans. Phytochem.*, **16**, 1062-1063 (1977).

Selover, S. J., Crews, P. and Kylinone, a new sesquiterpene skeleton from the marine alga *Laurencia pacifica. J. Org. Chem.*, **45**, 69-72 (1980).

Shizuri, Y., Yamada, K. and Yamada, A.,Laurequinone, a cyclolaurane sesquiterpene from the red alga *Laurencia nidifica*. *Phytochem.*, **23**, 2672-2673 (1984).

Suzuki, M. and Kurosawa, E. New bromo compounds from *Laurencia glandulifera* kützing, *Tetrahedron Lett.*, **17**, 4817-4818 (1976).

Vairappan, C. S., Suzyki, S., Abe, T. and Masuda, M., Halogenated metabolites with antibacterial from the Okinawan *Laurencia* species. *Phytochem.*, **58**, 517-523 (2001).

Winkler, L. R., Tilton, B. E. and Hardinge, M. G., A cholinergic agent extracted from sea hares. *Arch. Intern. Pharmacodyn.*, **137**, 76-83 (1962).

Yamamura, S. and Hirata, Y., Structure of aplysin and aplysinol, naturally occurring bromocompounds. *Tetrahedron*, **19**, 1485-1496 (1963).

(Accepted September 14, 2002)