A New Phlorotannin from the Brown Alga *Ecklonia stolonifera*

Hye Sook KANG,[a] Hae Young CHUNG,[b] Jee Hyung JUNG,[b] Byeng Wha SON,[c] and Jae Sue CHOI*,[a]

[a] *Faculty of Food Science and Biotechnology, Pukyong National University; and* [c] *Department of Chemistry, Pukyong National University; Busan 608–737, Korea: and* [b] *College of Pharmacy, Pusan National University; Busan 609–735, Korea.* Received April 23, 2003; accepted June 2, 2003

A new phlorotannin, named eckstolonol (1), was isolated from the EtOAc soluble fraction of the methanolic extract of the brown alga, *Ecklonia stolonifera* OKAMURA, along with three known phlorotannins, eckol (2), phlorofucofuroeckol A (3), and dieckol (4). The structure of eckstolonol was identified as 5,8,13,14-tetraoxa-pentaphene-1,3,6,9,11-pentaol on the basis of spectroscopic evidence. The new compound was found to be a radical scavenger on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.

Key words phlorotannin; eckstolonol; 1,1-diphenyl-2-picrylhydrazyl radical; *Ecklonia stolonifera*

Ecklonia stolonifera OKAMURA is a member of the family of Laminariaceae, belonging to the order Laminariales as a perennial brown alga. The previous phytochemical investigations performed on this species resulted in the isolation of phloroglucinol,[1)] phlorotannins[2)] and ecklonialactones.[3,4)] In the course of a continuous study on the active principles of this alga, we isolated a new phlorotannin with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, along with three known ones, of the methanolic extract of *E. stolonifera*. Column chromatography of the EtOAc soluble part from the methanolic extract of this alga yielded four phlorotannins, compounds **1**—**4** in the order of increasing polarity. The structures of **2**, **3**, and **4** were identified by comparison with published spectral data as eckol, phlorofucofuroeckol A, and dieckol, respectively (Fig. 1).[5—8)]

Compound **1** was obtained as off-white amorphous powder. The molecular formula of **1** was determined as $C_{18}H_{10}O_9$ based on the NMR and HR-FAB-MS data [M^+, *m/z*: 370.0324 Calcd for $C_{18}H_{10}O_9$ *m/z*: 370.0325, Δ −0.1 mmu] indicating fourteen degrees of unsaturation. The infrared (IR) spectrum of **1** showed the absorption bands at 3243 (OH) and 1635 (aromatic C=C) cm^{-1}. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** indicated the presence of five non-substituted and thirteen O-bearing aromatic carbons, whereas the proton nuclear magnetic resonance (^{1}H-NMR) spectrum contained signals characteristic of five aromatic protons, *i.e.* two AB systems at δ 6.04 (1H, J=2.7 Hz) and 5.82 (1H, J=2.7 Hz), and δ 6.01 (1H, J=2.7 Hz) and 5.84 (1H, J=2.7 Hz), and a singlet at 6.10 (1H) as well as five singlets indicating phenolic hydroxy protons at δ 9.77, 9.64, 9.60, 9.27, and 9.26. These NMR spectral features are very similar to those of eckol (**2**) isolated from *Eisenia bicyclis* and *Ecklonia kurome*,[5,6)] indicating that **1** is composed of three phloroglucinol units. The only difference between the ^{1}H-NMR spectra of **1** and **2** is that the former lacks the signals for one phenolic hydroxyl proton and one aromatic proton, suggesting that **1** has an additional aryl-ether linkage. This was supported by the presence of a new oxygen-bearing carbon signal (δ 122.7), which is characteristic of an aromatic carbon with two oxygenated neighbors, and also by the formation of a pentaacetate (**1a**) on usual acetylation. Analysis of HMQC, HMBC and NOESY spectra of **1** allowed an unambiguous assignment of all the proton and carbon signals (Table 1, Fig. 2). In the HMBC spectrum, each cross peak between δ 9.77 and C-1 (δ 146.1), C-2 (δ 98.8), and C-14a (δ 122.3), and between δ 9.27 and C-2 (δ 98.9), C-3 (δ 153.3), and C-4 (δ 93.9) indicated the presence of the hydroxyl group at C-1 and C-3, respectively. Similarly, each cross peak between δ 9.26 and C-10 (δ 98.8), C-11 (δ 153.0), and C-12 (δ 93.9), and between δ 9.64 and C-8a (δ 122.7), C-9 (δ 146.0), and C-10 (δ 98.8) designated the existence of the hydroxyl groups at C-11 and C-9, respectively. Each cross peak between δ 9.60 and C-5a (δ 125.9), C-6 (δ 140.1), and C-7 (δ 97.6), established the presence of the hydroxyl group at C-6. The stereostructure of compound **1** was deduced to be planar and achiral by its specific rotation and a loss of additional anisotropic effect for the aromatic protons. It was found that specific rotation showed zero value and the similar chemical shifts not only between H-2 and H-10, but also between H-4 and H-12. Consequently, the structure of **1** was established as 5,8,13,14-tetraoxa-pentaphene-1,3,6,9,11-pentaol, named eckstolonol. Compounds **1**—**4** were found to be potent radical scavengers with the IC_{50} values of 8.8, 11.5, 4.6, and 6.2 μM, respectively. **1**, **3** and **4** were much stronger than that of a well-known antioxidant, L-ascorbic acid with an IC_{50} value of 10.3 μM.

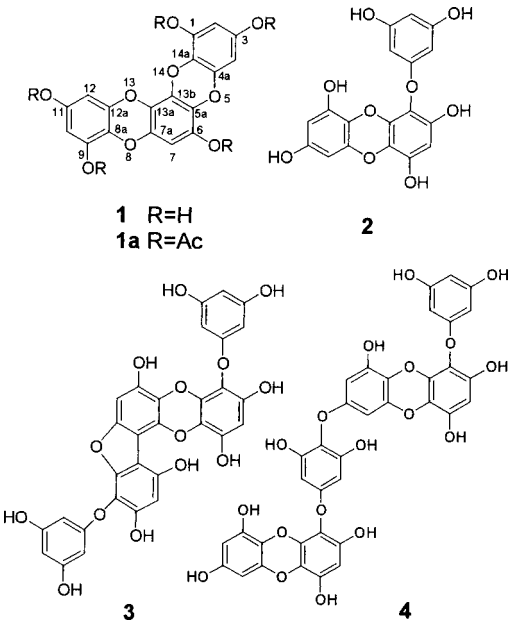

Fig. 1. The Structures of the Phlorotannins from *E. stolonifera*

* To whom correspondence should be addressed. e-mail: choijs@pknu.ac.kr

Table 1. NMR Spectral Data of **1** in DMSO-d_6 [a)]

Position	δ_C	DEPT	δ_H (J, Hz)[b)]	HMBC
1	146.1	C		
2	98.8	CH	6.04, d, (2.7)	C-1, C-3, C-14a
3	153.3	C		
4	93.9	CH	5.84, d, (2.7)	C-2, C-3, C-4a, C-14a
4a	142.1	C		
5a	125.9	C		
6	140.1	C		
7	97.6	CH	6.10, s	C-5a, C-6, C-7a, C-13a, C-13b
7a	137.2	C		
8a	122.7	C		
9	146.0	C		
10	98.8	CH	6.01, d, (2.7)	C-8a, C-9, C-11, C-12
11	153.0	C		
12	93.9	CH	5.82, d, (2.7)	C-8a, C-10, C-11, C-12a
12a	141.7	C		
13a	122.5	C		
13b	131.6	C		
14a	122.3	C		
1-OH			9.77, s	C-1, C-2, C-14a
9-OH			9.64, s	C-8a, C-9, C-10
6-OH			9.60, s	C-5a, C-6, C-7
3-OH			9.27, s	C-2, C-3, C-4
11-OH			9.26, s	C-10, C-11, C-12

a) Chemical shifts are referred to TMS. Multiplicities are indicated by usual symbols. Coupling constants (Hz) are in parentheses. *b*) These assignments were based on the evidence of HMQC measurements.

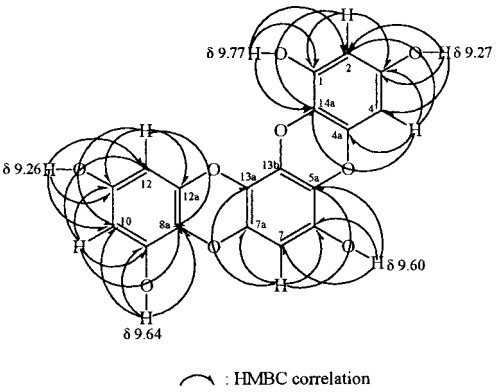

Fig. 2. Long-Range Correlations of **1** in the HMBC Spectrum

Experimental

General ^{1}H- and ^{13}C-NMR spectra were determined on a JNM ECP-400 spectrometer using CD_3OD, $CDCl_3$, and DMSO-d_6 with tetramethylsilane (TMS) as an internal standard. HMQC and HMBC spectra were recorded using pulsed field gradients. EI-MS, FAB-MS, IR and optical rotation were taken with a GC-MS QP-5050A (Shimadzu, Japan), a JMS-HX110A/HX110A Tandem mass spectrometer (JEOL), a FT-IR spectrometer spectrum 2000 (Perkin-Elmer Ltd., England), and a Perkin-Elmer polarimeter 341 (U.S.A.), respectively. Column chromatography was done with silica gel 60 (230—400 mesh, Merck, Germany), RP-18 Lichroprep (Merck, Germany), and Sephadex LH-20 (Sigma, st. Louis, MO, U.S.A.). TLC was carried out on a precoated Merck Kieselgel 60 F_{254} plate (0.25 mm) and a RP-18 F_{254s} plate (Merck, Art. 5685) and the spots were detected under UV light using 50% H_2SO_4 reagent. All the solvent for column chromatography was of a reagent grade from commercial sources.

Plant Material Leafy thalli of the *E. stolonifera* were collected at Gijang-gun in Busan, Korea in February 2000 and authenticated by Prof. H. G. Kim of the Faculty of Marine Bioscience and Technology, Kangnung National University. A voucher specimen (no. 20000228) has been deposited in the author's laboratory (J. S. Choi).

Extraction and Isolation The lyophilized powder (3 kg) was refluxed with MeOH (3×9 l) for 3 h. The extract (700 g) was suspended in water and partitioned with *n*-hexane (27.93 g), CH_2Cl_2 (25.58 g), EtOAc (24.99 g), *n*-BuOH (99.59 g), in sequence. The EtOAc fraction (24.99 g), which exhibited the most potent antioxidative effect on the DPPH radical, was applied to a silica gel (Merck, 70—230 mesh, 800 g) column (4×80 cm). The column was eluted using mixtures of EtOAc/MeOH under gradient conditions (50 : 1—5 : 1) to yield the 10 subfractions (F1—F10), *i.e.*, F1—F3; EtOAc/MeOH, 50 : 1 (5 l), F4—F6; EtOAc/MeOH, 10 : 1 (5 l), F7—F8; EtOAc/MeOH, 5 : 1 (5 l), and F9—F10; EtOAc/MeOH, 2 : 1 (2 l). The F1 was carried out further with a silica gel (70—230 mesh, 250 g) column (3×70 cm) chromatography (hexane/EtOAc, 1 : 1) to get the 11 subfractions (F1-1—F1-11). Compounds **1** (60 mg) and **2** (135 mg) were obtained from the RP-18 column chromatography (20% MeOH *ca.* 100% MeOH, gradient) of F1-5 (1.01 g). Compounds **3** and **4** in F1-6 (945 mg) were achieved by the RP-18 column chromatography using a 20% MeOH *ca.* 100% MeOH gradient, then purified by Sephadex LH-20 column chromatography with MeOH as a solevent, respectively.

Eckstolonol (**1**): Off-white powder, $[\alpha]_D^{20}$: 0° (c=0.008, MeOH) negative FAB-MS m/z: 369.0, HR-FAB-MS m/z: 370.0324 (Calcd for $C_{18}H_{10}O_9$, m/z 370.0325). IR (KBr) cm^{-1}: 3243, 1635, 1518, 1494, 1396, 1281, 1243, 1207, 1154, 1118, 1089, 1012, 810. ^{1}H- and ^{13}C-NMR: see Table 1.

Eckstolonol Pentaacetate (**1a**): A mixture of **1** (5 mg), acetic anhydride (0.3 ml), and pyridine (0.2 ml) was allowed to stand at room temperature for 24 h. The reaction mixture was evaporated the solvent with a N_2 gas stream to afford **1a** (7.8 mg). IR (KBr) cm^{-1}: 1769, 1506, 1477, 1371, 1193, 1079, 1021, 885. EI-MS m/z (R. int.): 580 (M^+, 21), 538 (45), 496 (47), 454 (72), 412 (52), 370 (100), 341 (30), ^{1}H-NMR (400 MHz, $CDCl_3$+DMSO-d_6) δ: 1.59 (3H, s), 1.61 (3H, s), 1.64 (3H, s), 1.65 (3H, s), 1.73 (3H, s), 5.80 (1H, s), 5.96 (1H, d, J=2.6 Hz), 5.97 (1H, d, J=2.6 Hz), 6.00 (1H, d, J=2.6 Hz), 6.02 (1H, d, J=2.6 Hz).

Eckol (**2**): Amorphous powder, Positive FAB-MS m/z: 372 $[M]^+$, $C_{18}H_{12}O_9$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 5.93 (3H, s), 5.94 (2H, s), 6.13 (1H, s). ^{13}C-NMR (100 MHz, CD_3OD) δ: 162.4, 160.7, 160.7, 150.0, 147.7, 147.6, 144.7, 143.8, 139.0, 126.1, 125.3, 125.1, 100.3, 99.9, 98.2, 96.3, 95.9, 96.9.

Phlorofucofuroeckol A (**3**): Amorphous powder, Positive FAB-MS m/z: 602 $[M]^+$, $C_{30}H_{18}O_{14}$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.63 (1H, s), 6.40 (1H, s), 6.26 (1H, s), 5.97 (2H, d, J=2.1 Hz), 5.94 (1H, t, J=1.9 Hz), 5.92 (1H, t, J=1.9 Hz), 5.88 (2H, d, J=2.1 Hz). ^{13}C-NMR (100 MHz, CD_3OD) δ: 162.67, 162.64, 161.00, 161.00, 160.97, 160.97, 153.99, 152.50, 151.96, 149.07, 149.03, 146.74, 144.71, 139.19, 136.15, 128.89, 125.86, 125.56, 123.17, 106.15, 106.11, 100.78, 100.22, 98.60, 98.48, 97.03, 96.24, 96.24, 96.21, 96.21.

Dieckol (**4**): Amorphous powder, Positive FAB-MS m/z: 742 $[M]^+$, $C_{36}H_{22}O_{18}$. ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.15 (1H, s), 6.13 (1H, s), 6.09 (2H, s), 6.06 (1H, d, J=2.9 Hz), 6.05 (1H, d, J=2.9 Hz), 5.98 (1H, d, J=2.8 Hz), 5.95 (1H, d, J=2.8 Hz), 5.92 (3H, s). ^{13}C-NMR (100 MHz, CD_3OD) δ: 162.67, 160.95, 160.95, 158.61, 156.81, 155.33, 153.19, 153.19, 148.14, 148.09, 147.92, 147.71, 145.10, 144.95, 144.20, 144.10, 139.44, 139.27, 127.28, 127.00, 126.46, 126.41, 125.67, 125.45, 125.38, 100.67, 100.56, 100.30, 100.19, 98.47, 97.02, 97.02, 96.65, 96.57, 96.17, 96.17.

DPPH Radical Scavenging Effect The DPPH radical scavenging effect was evaluated as previously described by Blois[9)] with minor modifications. A methanolic sample solution of 160 μl at several concentrations and 40 μl of the DPPH methanolic solution (1.5×10^{-4} M) were added to a 96-well microplate, in a total volume of 200 μl. After letting the reaction mixture stand at room temperature for 30 min, its absorbance was determined at 520 nm, in a microplate reader (VERSA max, Molecular device, CA, U.S.A.).

Acknowledgements The FAB-MS spectra were provided by the Korea Basic Science Institute. This study was supported by a grant from the "2002 Korea Sea Grant Program (2002 KSGP)", Ministry of Maritime Affairs and Fisheries (2002).

References

1) Lee J. H., Park J. C., Choi J. S., *Arch. Pharm. Res.*, **19**, 223—227 (1996).
2) Taniguchi K., Kurata K., Suzuki M., *Nippon Suisan Gakkaishi*, **57**, 2065—2071 (1991).
3) Kurata K., Taniguchi K., Shiraishi K., Hayama N., Tanaka I., Suzuki M., *Chem. Lett.*, 267—270 (1989).
4) Kurata K., Taniguchi K., Shiraishi K., Suzuki M., *Phytochemistry*, **33**, 155—159 (1993).
5) Nakamura T., Nagayama K., Uchida K., Tanaka R., *Fish. Sci.*, **62**, 923—926 (1996).

6) Fukuyama Y., Kodama M., Miura I., Kinzyo Z., Kido M., Mori H., Nakayama Y., Takahashi M., *Chem. Pharm. Bull.*, **37**, 349—353 (1989).
7) Fukuyama Y., Kodama M., Miura I., Kinzyo Z., Mori H., Nakayama Y., Takahashi M., *Chem. Pharm. Bull.*, **38**, 133—135 (1990).
8) Fukuyama Y., Miura I., Kinzyo Z., Mori H., Kido M., Nakayama Y., Takahashi M., Ochi M., *Chem. Lett.*, 739—742 (1985).
9) Blois M. S., *Nature* (London), **181**, 1199—1200 (1958).