A New Phlorotannin from the Brown Alga *Ecklonia stolonifera*

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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 phlorotannins2) and ecklonialactones.3,4) In a course of a continuous study on the active principles of this alga, we isolated a new phlorotannin with 1,1-diphenyl-2-picrylhydrazyl 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).3—6)

Compound 1 was obtained as off-white amorphous powder. The molecular formula of 1 was determined as C18H10O9 based on the NMR and HR-FAB-MS data [M+, m/z: 370.0324 Calcd for C18H10O9 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=O) cm−1. The carbon-13 nuclear magnetic resonance (13C-NMR) spectrum of 1 indicated the presence of five non-substituted and thirteen O-bearing aromatic carbons, whereas the proton nuclear magnetic resonance (1H-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 1H-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 HMGC, 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 IC50 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 IC50 value of 10.3 μM.

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

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Experimental

General 1H and 13C-NMR spectra were determined on a JNM ECP-400 spectrometer using CD3OD, CDC13, and DMSO-d6 with tetramethylsilane (TMS) as an internal standard. HMOC 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 (Perkin-Elmer Ltd., England), and an FT-IR spectrometer using CD3OD, CDCl3, and DMSO-d6 as a solvent. HMQC and HMBC spectra were recorded by using 50% H2SO4 reagent. All the solvent for column chromatography was eluted using mixtures of EtOAc/MeOH, 20:1 (1 l), F1—F2; EtOAc/MeOH, 10:1 (5 l), F3—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 (20—230 mesh, 250 g) column (3 X 70 cm) chromatography (hexane/EtOAc, 1:1) to get the 11 subfractions (F1—F11). Compounds 1 (60 mg) and 2 (135 mg) were obtained from the RP-18 column chromatography using a 20% MeOH gradient (20% MeOH ca. 100% MeOH gradient, respectively) then purified by Sephadex LH-20 column chromatography with MeOH as a solvent, respectively.

Dieckol (1): Open-white powder, [α]25D 0° (c = 0.008, MeOH) negative FAB-MS m/z 369.0, HR-FAB-MS m/z 370.0324 (Caled for C18H10O9, m/z 370.0325). IR (KBr) cm−1: 3430, 1653, 1518, 1494, 1396, 1281, 1243, 1207, 1154, 1118, 1089, 1012, 810. 1H- and 13C-NMR: see Table 1. Eckstolonol (2): A mixture of I (5 mol), acetic anhydride (0.3 mol), and pyridine (0.5 ml) was allowed to stand at room temperature for 24 h. The reaction mixture was evaporated with a N2 gas stream to afford 1a (7.8 mg). IR (KBr) cm−1: 1769, 1506, 1477, 1371, 1109, 1021, 885. EI-MS m/z (R int.): 580 (M+ 1, 21), 538 (45), 496 (47), 454 (72), 412 (52), 370 (100), 341 (30), 1H-NMR (400 MHz, CDCl3, δ; 0.19 (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). Eckstolonol Pentaacetate (3a): A mixture of I (5 mol), acetic anhydride (0.3 mol), and pyridine (0.5 ml) was allowed to stand at room temperature for 24 h. The reaction mixture was evaporated with a N2 gas stream to afford 3a (8.2 mg). IR (KBr) cm−1: 1769, 1731, 1642, 1577, 1477, 1476, 1447, 1348, 1339, 126.1, 125.1, 125.1, 125.2, 100.3, 99.9, 98.2, 96.3, 95.9, 96.9.

Dieckol (4): Aromatic powder, Positive FAB-MS m/z 372 [M] +, C18H10O9. 1H-NMR (400 MHz, CD3OD, δ; 5.93 (3H, s), 5.94 (2H, s), 6.13 (1H, s). 13C-NMR (100 MHz, CD3OD, δ; 162.4, 160.7, 160.7, 150.0, 147.7, 147.6, 144.7, 134.8, 133.9, 126.1, 125.1, 125.1, 100.3, 99.9, 98.2, 96.3, 95.9, 96.9.

Pentaacetate (5): Aromatic powder, Positive FAB-MS m/z 742 [M] +, C18H10O9. 1H-NMR (400 MHz, CD3OD, δ; 6.15 (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). 13C-NMR (100 MHz, CD3OD, δ; 162.67, 162.64, 161.00, 161.00, 160.97, 160.97, 159.39, 152.50, 151.96, 149.07, 149.03, 146.74, 144.71, 139.19, 136.15, 128.51, 128.56, 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.

DPPH Radical Scavenging Effect

The DPPH radical scavenging effect was evaluated as previously described by Blois3 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.).

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References