

## Isolation of a New Phlorotannin, Fucodiphlorethol G, from a Brown Alga *Ecklonia cava*

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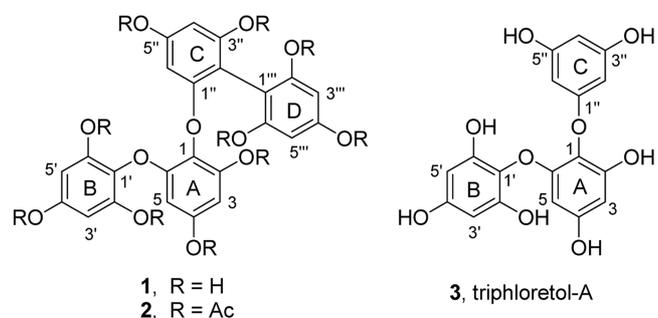
*Ecklonia cava* is a brown alga (Alariaceae), widely distributed offshore in Jeju Island. In search of anti-oxidative and anti-tyrosinase components from natural products in Jeju, we have been working on *E. cava*.<sup>1</sup> During the phytochemical study on the methanol extract of *E. cava*, a new phlorotannin-type compound **1**, which we named fucodiphlorethol G, was isolated. Phlorotannins are oligomeric compounds using phloroglucinol (1,3,5-trihydroxybenzene) as a basic unit. Some phlorotannins have been identified as the bioactive components in *Ecklonia* species such as *E. cava*,<sup>2</sup> *E. kurome*<sup>3</sup> and *E. stolonifera*.<sup>4</sup> Described here are the isolation and structure identification of the compound **1**.

The seaweed, *E. cava* was collected offshore in Jeju Island, and air dried and cut into small pieces. The dried *E. cava* powder (4.0 kg) was extracted using 80% MeOH to provide the oily material (1.0 kg). The extract was partitioned between water and ethyl acetate. The obtained ethyl acetate fraction was successively column chromatographed using celite and then sephadex LH-20 to give 11 fractions. The eighth fraction was further purified by using Prep-LC (LC-9104, JAI) system, yielded compound **1** (140 mg) as off-white amorphous powder.

By analysis of the <sup>1</sup>H and <sup>13</sup>C NMR spectra in **1**, only aromatic proton and carbon peaks were observed. Therefore it was tentatively assumed that this compound is a phloroglucinol oligomer, a class of common compounds detected in this alga. Further investigation of <sup>13</sup>C and DEPT NMR data suggest that **1** has a structure of phloroglucinol tetramer. The molecular formula of **1** was determined as C<sub>24</sub>H<sub>18</sub>O<sub>12</sub> (unsaturation number 16) on the HR-FAB-MS data [m/z 499.0860 (M+H)<sup>+</sup>, calcd for C<sub>24</sub>H<sub>19</sub>O<sub>12</sub> 499.0877, Δ -1.7 mmu].

However, investigation of the <sup>13</sup>C NMR spectrum showed only 20 carbon signals, which implies that two symmetric benzene units are involved in compound **1**. By the examination of <sup>1</sup>H and <sup>13</sup>C NMR data, it was found that structure of compound **1** is similar to that of triphlorethol-A (**3**), a trimeric phlorotannin previously isolated.<sup>1d</sup> In the case of **1**, it was assumed that one additional phloroglucinol unit was attached to triphlorethol-A framework. Comparative examination of NMR δ values revealed that A and B rings in **1** are relatively unchanged compared to **3**. However, some difference was observed in C ring in **1**. For example, <sup>1</sup>H

doublet at δ<sub>H</sub> 6.04 (2H, H-2'' and H-6'') in **3** was converted to a doublet at δ<sub>H</sub> 6.03 (1H, H-6'') in **1**. The proton signal corresponding to H-2'' in **3** was disappeared. In addition, four carbon signals from symmetric C ring in **3** was changed to six carbon signals in **1**, indicating the loss of ring symmetry. Therefore, it was clear that additional phloroglucinol unit is attached to in C ring at C-2'' (or C-6'') position in **3**. The attachment of additional phlorotannin unit could be made through either direct carbon-carbon or carbon-oxygen connections. Inspection of <sup>1</sup>H NMR spectrum of **1** revealed that only a singlet signal (2H, H-3''' and H-5''') was appeared in D ring, which means that the aromatic carbon in D ring is directly connected to C-2'' in C ring. Chemical shift of C-2'' at δ<sub>C</sub> 102.0 in **1** also supported this assignment since, if an oxygen is attached, chemical shift of C-2'' should be shifted to 20-30 ppm more down field compared to δ<sub>C</sub> 102.0.



We also have tried to confirm the connection of C-D ring by HMBC long range correlation using NMR. However, we could not observe any cross peak between C and D rings in compound **1**. Therefore, we decided to prepare the per-acetylated compound **2**. The compound **2** was prepared by the treatment of acetic anhydride and pyridine, and its NMR data is also summarized in Table 1. In contrast to the case of **1**, the synthetic derivative **2** provided desired HMBC correlation data. Unusually long range <sup>5</sup>J<sub>CH</sub> connectivity between H-6'' and C-2''' (and C-6''') was appeared in **2**. Presumably, this observation was possible due to the conjugated π-system in the C-D ring.

In the case of acetylated phlorotannins, Glombitza has reported a structure prediction method based on calculated <sup>13</sup>C NMR data considering their substitution types and steric effects around aromatic rings.<sup>5</sup> When this protocol was

**Table 1.** Comparative NMR data for compounds **1**, **2** and **3**

no.	<b>1<sup>a</sup></b>		<b>2<sup>b</sup></b>			<b>3<sup>a</sup></b>	
	$\delta_C$	$\delta_H$ (mult, <i>J</i> in Hz)	$\delta_C$	$\delta_H$ (mult, <i>J</i> in Hz)	HMBC (H $\rightarrow$ C)	$\delta_C$	$\delta_H$ (mult, <i>J</i> in Hz)
1	124.9	–	133.4	–	–	127.5	–
2	157.5	–	144.2	–	–	152.7	–
3	98.0	5.69 (d, 2.7)	108.5	6.40 (d, 2.6)	C-1, C-5	98.0	5.78 (d, 2.7)
4	153.7	–	147.5	–	–	156.1	–
5	94.5	6.02 (d, 2.7)	112.1	6.67 (d, 2.6)	C-1, C-3	94.9	6.09 (d, 2.9)
6	152.0	–	149.9	–	–	153.7	–
1'	124.3	–	136.6	–	–	124.6	–
2'	152.1	–	143.8	–	–	152.1	–
3'	96.0	5.91 (s)	115.1	6.85 (s)	C-1', C-5'	96.2	5.93 (s)
4'	157.9	–	146.9	–	–	156.4	–
5'	96.0	5.91 (s)	115.1	6.85 (s)	C-1, C-3'	96.2	5.93 (s)
6'	152.1	–	143.8	–	–	152.1	–
1''	159.4	–	156.6	–	–	162.4	–
2''	102.0	–	112.2	–	–	95.4	6.04 (d, 2.2)
3''	159.2	–	151.3	–	–	160.3	–
4''	97.5	6.10 (d, 2.2)	104.8	6.30 (d, 2.2)	C-2'', C-6''	97.5	5.98 (t, 2.2)
5''	159.5	–	150.5	–	–	160.3	–
6''	94.3	6.03 (d, 2.2)	110.7	6.70 (d, 2.2)	C-2'', C-4'', C-2''', C-6'''	95.4	6.04 (d, 2.2)
1'''	101.8	–	116.0	–	–	–	–
2'''	156.3	–	149.8	–	–	–	–
3'''	96.4	6.05 (s)	113.8	6.90 (s)	C-1''', C-5'''	–	–
4'''	159.2	–	151.0	–	–	–	–
5'''	96.7	6.05 (s)	113.8	6.90 (s)	C-1''', C-3'''	–	–
6'''	156.3	–	149.8	–	–	–	–

<sup>a</sup>Recorded in CD<sub>3</sub>OD. <sup>b</sup>Recorded in CDCl<sub>3</sub>

subjected to **2**, experimental  $\delta_C$  values for **2** were well matched to the calculated  $\delta_C$  values, *i.e.*, the difference between the values were within 3.2 ppm range for all carbons.

The nomenclature system for the marine phlorotannins was originally introduced by Glombitza.<sup>6</sup> According to this system, tetrameric phlorotannins (C<sub>24</sub>) composed of difucol

**Table 2.** Analysis of fucodiphlorethols A-F based on their connection pattern between difucol and diphlorethol

Difucol

Diphlorethol

Fucodiphlorethol (C <sub>24</sub> )	Atoms connected each other	
	Difucol (C <sub>12</sub> )	Diphlorethol (C <sub>12</sub> )
A	O-4	C-10
B	C-3	O-2
C	C-3	O-9
D	C-3	O-4
E	O-2	C-8
F <sup>a</sup>		C-10, C-10

<sup>a</sup>C-10 of diphlorethol is coupled each other to form a dimer.

(C<sub>12</sub>, C<sub>6</sub>-C<sub>6</sub> form) and diphlorethol (C<sub>12</sub>, C<sub>6</sub>-O-C<sub>6</sub> form) are named as fucodiphlorethol. In the literature, six different types of fucodiphlorethols, named fucodiphlorethol A-F, have been isolated and characterized in the form of acetylated derivatives.<sup>6</sup> For example, in the case of fucodiphlorethol A (C<sub>24</sub>), O-4 in difucol (C<sub>12</sub>) is connected to C-10 in diphlorethol (C<sub>12</sub>). The same analysis can be applied to the other fucodiphlorethols (see Table 2). In the case of phlorotannin **1**, we can describe that O-2 in difucol (C<sub>12</sub>) is coupled to C-8 in diphlorethol (C<sub>12</sub>). It is clear that this connection pattern is different from the previous known fucodiphlorethols A-F. Thus, new phlorotannin **1** is named as fucodiphlorethol G.

In conclusion, we have isolated a new compound **1** from the methanol extract of *Ecklonia cava*, a brown alga collected offshore in Jeju Island. The tetrameric phlorotannin **1**, named fucodiphlorethol G, has strong radical scavenging effect (IC<sub>50</sub> 0.60  $\mu$ M) on the DPPH test.<sup>7</sup>

## Experiment Section

**Reagent and Equipment.** Thin layer chromatography was performed on Merck prepared plates (silica gel 60 F-254 on aluminum). Column chromatography was performed using Merck silica gel 60 (230-400 mesh). Sephadex LH-20 (25-100  $\mu$ m) for Gel filtration chromatography (GFC) was

obtained from Fluka. The UV absorbance was performed with a Biochrom Libra S22 UV-visible spectrophotometer.  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  (100.60 MHz) NMR spectra were recorded on a JEOL, JNM-LA400 instrument, with chemical shift data reported in ppm relative to the solvent used. 2D NMR spectra were recorded on the same instrument using field gradient FG2 (inverse) probe. HPLC separation was performed using LC-9140 (JAI) equipped with ODS-column. General laboratory solvents were distilled from glass before use.

**Isolation of Compound 1.** The seaweed, *E. cava* was collected in the seashore of Gwideog-ri, Jeju Island, and air dried and cut into small pieces.

The dried *E. cava* powder (4.0 kg) was suspended in 16 L of 80% MeOH and mechanically stirred for 24 h at room temperature. The solution was filtered and the filtrate was concentrated under reduced pressure to give the oily extract (1.0 kg). After the extract was suspended on 1 L of distilled water, organic soluble fraction (220 g) was obtained by treating ethyl acetate. The obtained ethyl acetate fraction was poured in a celite column (5 cm  $\times$  40 cm), and the column was sequentially eluted with *n*-hexane (3 L),  $\text{CH}_2\text{Cl}_2$  (6 L), diethyl ether (9 L) and methanol (5 L). Based on NMR analysis, phlorotannins were observed in diethyl ether fraction (14 g), which was further purified by sephadex LH-20 column chromatography using stepwise gradient  $\text{CHCl}_3$ /methanol (2/1 to 1/1 to 0/1) solvents system to give 11 fractions based on TLC analysis. The compound **1** (140 mg) was obtained by further purification of the fraction 8 (514 mg) using Prep-LC (LC-9104, JAI) system equipped with ODS column in methanol solvent.

**Fucodiphloretho G (1).** Off-white powder; UV ( $\text{CH}_3\text{OH}$ ) 280 ( $\epsilon$  32,500) 269 ( $\epsilon$  8,000); HR-FAB-MS data [ $m/z$  499.0860 ( $\text{M}+\text{H}$ ) $^+$ , calcd for  $\text{C}_{24}\text{H}_{19}\text{O}_{12}$  499.0877,  $\Delta$  -1.7 mmu]. For  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1.

**Acetylation of Compound 1.** In a vial were placed the compound **1** (52 mg), pyridine (1 mL) and acetic anhydride (1 mL). After the resulting mixture was stirred for 4 h at rt, the solution was partitioned between chloroform and water.

The chloroform layer was concentrated, and purified using short path  $\text{SiO}_2$  column chromatography to give decaacetylated **2** (7.1 mg). For  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1. Proton NMR peaks for the acetyl groups are showing up at  $\delta_{\text{H}}$  2.21 (3H), 2.20 (3H), 2.16 (3H), 2.13 (3H), 2.08 (3H), 1.90 (3H), 1.89 (6H), 1.80 (3H), 1.50 (3H). Due to peak overlap, only six  $^{13}\text{C}$  peaks for the C=O in the acetyl groups are appeared at  $\delta_{\text{C}}$  168.8, 168.7, 168.6, 168.4, 168.2, 168.1 ppm.

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