Note

Fucophloretol C, a phlorotannin as a lipoxygenase inhibitor

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Abbreviations: NDGA, nordihydroguaiaretic acid; LOX, lipoxygenase.

Abstract

Fucophlorethol C, a phlorotannin, was isolated from the brown alga *Colpomenia bullosa* (Scytosiphonaceae) as a novel lipoxygenase inhibitor. It was obtained as a free form from natural origin for the first time. The compound inhibited a soybean lipoxygenase to the same extent as the known inhibitor nordihydroguaiaretic acid.

Key words: lipoxygenase; inhibitor; *Colpomenia bullosa;* phlorotannin; fucophlorethol C.

Lipoxygenase (LOX) mediates formation of hydroperoxides of unsaturated fatty acids. The hydroperoxides are converted to leukotrienes, related to various diseases, psoriasis, asthma, rhintis, and arthritis.[1] LOX inhibitor is expected to suppress such diseases. The inhibitors have been obtained from terrestrial plants.[2,3] Among them, nordihydroguaiaretic acid (NDGA) is a known inhibitor isolated from Zygophyllaceae plant.[4] However, NDGA shows toxic effect when patients use this plant and NDGA during long time.[5] Meanwhile, a few studies have been performed on LOX inhibitors originated from algae.[6,7] Recently we have isolated alkapolyenes and pheophytin *a* as LOX inhibitors from the brown alga *Sargassum thunbergii* and the red alga *Odonthalia corymbifera*, respectively.[8] In the present study, fucophlorethol C (**1**, Fig. 1), a phlorotannin, was isolated as a novel LOX inhibitor from the brown alga *Colpomenia bullosa* (Scytosiphonaceae). This is the first report on isolation of fucophlorethol C as a free form.

LOX assay was performed by the previously described method.[8] *C. bullosa* (250.6 g, air-dried), collected at the coast of Hakodate, Japan, was washed with tap water and extracted with methanol at room temperature for a week. The methanolic extract (4.6 g) was separated by a series of organic solvent partitioning to obtain ethyl acetate-soluble (415 mg) and *n*-butanol-soluble (168 mg) fractions which inhibited a soybean LOX. . Both inhibitory fractions were combined because their profiles were similar on analytical TLC. The combined fraction was chromatographed on silica gel eluted with chloroform-methanol (3:1, v/v) to obtain semi-purified fraction. The fraction was finally purified by using HPLC on Mightysil RP-18 column (Kanto Chemical Co., Ltd, Tokyo, Japan) using 3% formic acid-methanol (9:1, v/v) as a eluent to afford inhibitor **1** (49.2 mg).

Inhibitor **1** showed positive response for 2,4-dimethoxybenzaldehyde reagent,[9] used for detecting *meta*-diphenolic or phloroglucinol moieties, to be categorized as phlorotannin. Therefore an aliquot of compound **1** was acetylated with acetic anhydride/pyridine to obtain peracetylated derivative **2**. NMR data for **1** and **2** are shown in Table 1. All proton and carbon signals were assigned as aromatic signals, except for acetyl signals of **2**. FD-HRMS of **1** gave a molecular ion at *m/z* 374.06192, calculated 374.06378 for $C_{18}H_{14}O_9$. Index of hydrogen deficiency of **1** is calculated as 12. Thus compound **1** could consist of three aromatic rings. FD-MS of **2** gave a molecular ion at *m/z* 710. Thus compound **1** possesses eight free phenolic hydroxyl groups. Residual one oxygen atom forms an ether linkage. The inhibitor **1** is assumed to be one of fucophlorethols (Fig. 1).[10-12] Only a correlation between H-3b and H-5b was observed in COSY of **1**. The NMR signals were assigned in each aromatic ring from the results of HSQC and HMBC experiments. Compound **1** consists of symmetric 2,4,6-trioxygenated phenyloxy, symmetric 2,4,6-trioxygenated phenyl, and asymmetric 4,6-dioxygenated 2-aryloxyphenyl moieties

from consideration of NMR data. Unfortunately, connectivity of three aromatic rings was unclear because no HMBC correlation was observed between the rings. Fucophlorethol A (**A**) is rejected because compound **A** consists of three symmetric aromatic rings. Fucophlorethol B (**B**) is also rejected because the meta-coupling between two proton signals with an integral value corresponding to one proton of **1** suggested that these protons locate in the same aromatic ring. Therefore compound **1** is identified as fucophlorethol C. This identification is supported by NMR data of compound **2**, which coincided with literature data.[12] Peracetylated fucophlorethol C have been isolated from the brown algae *Himanthalia elongate*,[12] *Analipus japonicus*,[13] and *Cystophora torulosa*.[14] To best our knowledge, this is the first report on isolation of fucophlorethol C as a free form.

Fucophlorethol C (**1**) inhibited soybean LOX reaction with a K_i value of 137 μM. Inhibition mode of **1** was deduced as a mixed inhibition manner from the results of Lineweaver-Burk plots (Fig. S1). Its inhibitory activity, an IC_{50} value of 215 μM, was almost identical value (285 μM) with the known inhibitor NDGA, at the substrate concentration of 1.25 mM. Acetylated derivative **2** showed no inhibition against the LOX reaction. Free phenolic hydroxyl groups in fucophlorethol C are important for inhibition of LOX. Fucophlorethols and their derivatives, except for fucophlorethol C, have been reported various functions, such as antibacterial,[15] antioxidant,[16,17] and cytotoxic[18] effects. However this is the first report on a function of fucophlorethol C.

In conclusion, we isolated fucophlorethol C in a free form as a soybean LOX inhibitor from the brown alga *C. bullosa*. The compound **1** inhibited the enzyme to the same extent as the known inhibitor NDGA.

Author Contribution

H. K. made experimental plans of the manuscripts, interpreted experimental data and organized all of the studies. R. K. conceived and performed the experiments. K. T. participated in their designs and helped to draft the manuscript. All authors read and approved the final manuscript.

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References

[1] Bell RL, Young PR, Albert D, Lanni C, Summers JB, Brooks DW, Rubin P, Carter GW. The discovery and development of zileuton-an orally active 5-lipoxygenase inhibitor. Int. J. Immunopharmacol. 1992; 14, 505-510.

[2] Schneider I, Bucar F. Lipoxygenase inhibitors from natural plant sources. Part 1: Medical plants with inhibitory activity on arachidonate 5-lipoxygenase and 5-lipoxygenase/cyclooxygenase. Phytother. Res. 2005; 19, 81-102.

[3] Schneider I, Bucar F. Lipoxygenase inhibitors from natural plant sources. Part 2: Medical plants with inhibitory activity on arachidonate 12-lipoxygenase, 15-lipoxygenase and leukotriene receptor antagonists. Phytother. Res. 2005; 19, 263-272.

[4] Whitman S, Gezginci M, Timmermann BN, Holman TR. Structure-activity relationship studies of nordihydroguaiaretic acid inhibitors toward soybean, 12-human, and 15-human lipoxygenase. J. Med. Chem. 2002; 45, 2659-2661.

[5] Artega S, Andrade-Cetto A, Cardenas R. *Larrea tridentata* (Creosote bush), an abundant plant of Mexican and US-American deserts and its metabolite nordihydroguaiaretic acid. J. Ethnopharmacol. 2005; 98, 231-239.

[6] Matsukawa R, Dubinsky Z, Kishimoto E, Masaki K, Masuda Y, Takeuchi T, Chihara M, Yamamoto Y, Niki E, Karube I. A comparison of screening methods for antioxidant activity in seaweeds. J. Appl. Phycol. 1997; 9, 29-35.

[7] Shibata T, Nagayama K, Tanaka R, Yamaguchi K, Nakamura T. Inhibitory effects of brown algal phlorotannins on secretory phospholipase A_{2s}, lipoxygenases and cyclooxygenases. J. Appl. Phycol. 2003; 15, 61-66.

[8] Kurihara H, Kagawa Y, Konno R, Kim SM, Takahashi K. Lipoxygenase inhibitors derived from marine macroalgae. Bioorg. Med. Chem. Lett. 2014; 24, 1383-1385.

[9] Stern JL, Hagerman AE, Steinberg PD, Winter FC, Estes JA. A new assay for quantifying brown algal

phlorotannins and comparisons to previous methods. J. Chem. Ecol. 1996; 22, 1273-1293.

[10] Glombitza K-W, Rauwald H-W, Eckhardt G. Fucophloretholes, polyhydroxyoligophenyl ethers from *Fucus vesiculosus*. Planta Med. 1977; 32, 33-45.

[11] Glombitza K-W, Wiedenfeld G, Eckhardt G. Antibiotica aus Algen, XX. Niedermolekulare Phlorotannine aus *Cystoseira baccata*. Arch. Pharm. 1978; 311, 393-399.

[12] Glombitza K-W, Grosse-Damhues J. Antibiotics from algae XXXIII: phlorotannins of the brown alga *Himanthalia elongata*. Planta Med. 1985; 51, 42-46.

[13] Glombitza K-W, Zieprath G. Phlorotannins from the brown alga *Analipus japonicus*. Planta Med. 1989; 55, 171-175.

[14] Glombitza K-W, Hauperich S, Keusgen M. Phlorotannins from the brown algae *Cystophora torulosa* and *Sargassum spinuligerum*. Nat. Toxins. 1997; 5, 58-63.

[15] Sandsdalen E, Haug T, Stensvag K, Styrvold OB. The antibacterial effect of a polyhydroxylated fucophlorethol from the marine brown alga, *Fucus vesiculosus*. World J. Microbiol. Biotechnol. 2003; 19, 777-782.

[16] Cerantola S, Breton F, Gall EA, Deslandes E. Co-occurrence and antioxidant activities of fucol and fucophlorethol classes of polymeric phenols in *Fucus spiralis*. Bot. Mar. 2006; 49, 347-351.

[17] Wang T, Jonsdottir R, Liu H, Gu L, Kristinsson G, Raghavan S, Olafsdottir G. Antioxidant capacities of phlorotannins extracted from the brown algae *Fucus vesiculosus*. J. Agric. Food Chem. 2012; 60, 5874-5883.

[18] Li Y, Qian Z-J, Kim M-M, Kim S-K. Cytotoxic activities of phlorethol and fucophlorethol derivatives isolated from Laminariaceae *Ecklonia cava*. J. Food Biochem. 2011; 35, 357-369.

135 Table 1. NMR data for compound **1** and its acetylated derivative **2**.

	Compound **1**[a]		Derivative **2**[b]		Literature data for **2**[12)]
Position	δ_H	δ_C	δ_H	δ_C	δ_H
1a		101.8		115.8	
2a, 6a		157.3 (2C)		149.6 (2C)	
3a, 5a	6.08 (s, 2H)	96.6 (2C)	6.95 (s, 2H)	113.6 (2C)	6.97 (2H)
4a		158.9		150.5	
1b		102.3		111.6	
2b		159.0[d]		155.2[g]	
3b	5.72 (d, 2.2, 1H)[c]	93.3[e]	6.39 (d, 2.1, 1H)[f]	105.6[h]	6.41 (1H)
4b		159.2		151.0	
5b	6.10 (d, 2.2, 1H)[c]	97.8[e]	6.70 (d, 2.1, 1H)[f]	111.0[h]	6.72 (1H)
6b		157.8[d]		149.6[g]	
1c		124.1		135.4	
2c, 6c		151.8 (2C)		142.9 (2C)	
3c, 5c	5.93 (s, 2H)	95.8 (2C)	6.88 (s, 2H)	114.8 (2C)	6.90 (2H)
4c		156.4		146.3	
Ac			2.23 (s, 3H)	168.6	2.26 (3H)
			2.20 (s, 3H)	168.5	2.23 (3H)
			2.18 (s, 3H)	168.4	2.20 (3H)
			2.03 (s, 6H)	168.1 (2C)	2.04 (6H)
			1.99 (s, 6H)	168.0	2.02 (6H)
			1.96 (s, 3H)	167.5 (2C)	1.98 (3H)
				21.0	
				20.9	
				20.8	
				20.5 (2C)	
				20.4	
				20.3 (2C)	

136 Note: [a]measured in methanol-d_4. [b]measured in chloroform-*d*. [c-h]interchangeable within same letters.

Table 2. HMBC correlations of **1**.

H	C
2H-3a, 5a	C-1a; 2C-2a, 6a; 2C-3a, 5a[a]; C-4a
H-3b	C-1b; C-2b; C-4b; C-5b
H-5b	C-1b; C-3b; C-4b; C- 6b
2H-3c, 5c	C-1c; 2C-2c, 6c; 2C-3c, 5c[a]; C-4c

[a] ${}^{3}J_{CH}$ coupling between positions 3 and 5.

Figure caption

Fig. 1. Structures of fucophlorethol C (**1**) and its acetylated derivative **2**, along with fucophlorethols A (**A**) and B (**B**).

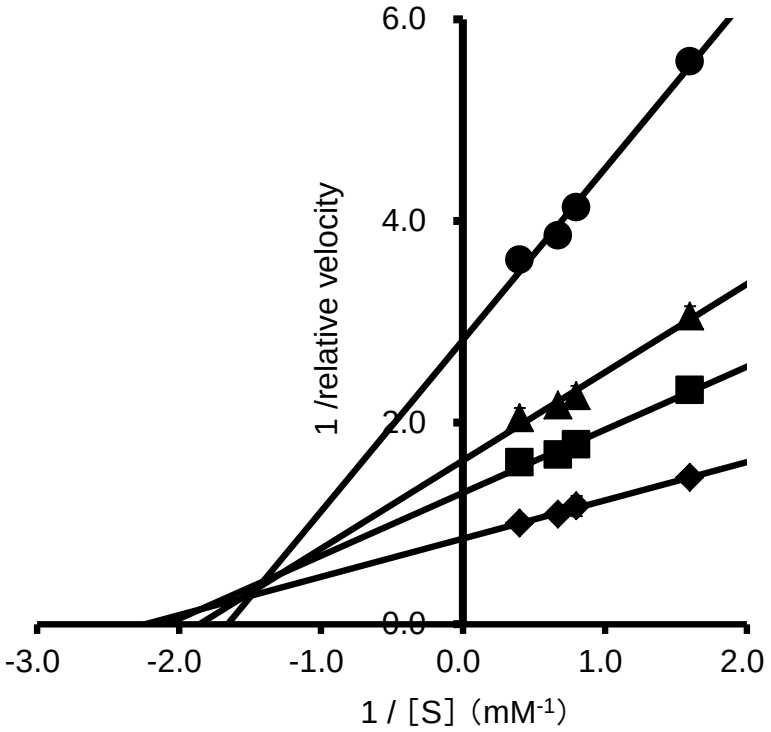
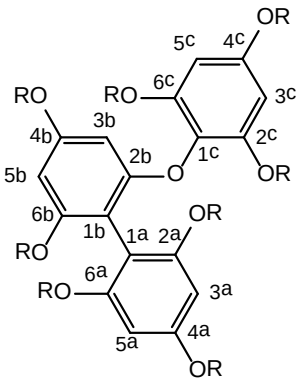
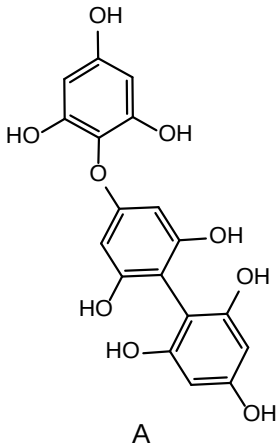


Fig. S1. Lineweaver-Burk plots of inhibition of **1** against a soybean lipoxygenase.

Relative velocity is divided by the lipoxygenase reaction velocity under absence of **1** and the maximum substrate concentration of 2.50 mM. Symbols are represented as final concentrations of **1**: diamond, 0 μM; square, 100 μM; triangle, 200 μM, and circle, 500 μM.


OR
5c
4c
RO
RO
6c
3c
3b
4b
2b
1c
2c
5b
O
OR
6b
OR
1b
1a
2a
RO
6a
RO
3a
4a
5a
OR

1 R=H
2 R=Ac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O
OH
OH
HO
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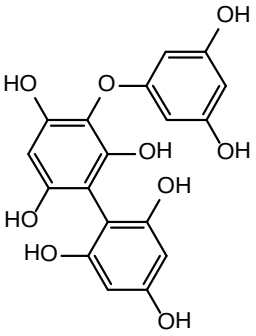
OH

A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HO
O
OH
OH
OH
HO
HO
OH

B