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Monoglycerides from the brown alga *Sargassum sagamianum*: Isolation, synthesis, and biological activity

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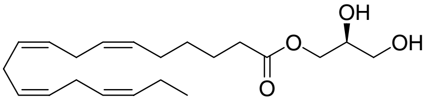
ABSTRACT

Polyunsaturated fatty acid-derived monoglycerides were characterized from the marine brown alga *Sargassum sagamianum*, collected from Jeju Island, Korea. A new compound of this structural class was isolated and determined to be 1-octadecatetraenoyl glycerol, by combined spectroscopic methods. Based on the structures and bioactivity of these compounds, a series of monoglycerides were synthesized using glycerol and various fatty acids. Several compounds exhibited moderate to significant inhibition of phospholipase A_2 and cyclooxygenase-2.

Marine brown algae (division Phaeophyta) produce a wide variety of secondary metabolites, including terpenoids, oxylipins, phlorotannins, volatile hydrocarbons, and mixed biogenetic products.[1] Several of these compounds exhibit a wide spectrum of pharmacological activities such as cytotoxic, antimicrobial, and antioxidant activities as well as inhibitory activity against various enzymes. During the course of our search for anti-inflammatory natural products from marine organisms from Korea, we encountered the brown alga *Sargassum sagamianum*, the crude extract of which exhibited significant inhibitory activity (>45% at 100 μg/mL) against cyclooxygenase-2 (COX-2).

Bioassay-guided separation of the crude extract using diverse chromatographic methods yielded a fraction consisting primarily of polyunsaturated fatty acid-containing monoglycerides. One compound was successfully isolated and determined to be 1-octadecatetraenoyl glycerol (**1**, Fig. 1), a novel natural product. This finding prompted us to design and synthesize a series of monoacyl glycerides. Here, we report the synthesis, structural determination, and bioactivity of fatty acid-derived monoacyl glycerides.



1

Figure 1. Structure of natural **1**.

Sargassum sagamianum was collected in the intertidal zone off of Jeju Island, South Korea, in April 2004. The specimens were lyophilized (dry weight, 0.2 kg), macerated, and repeatedly extracted with MeOH (3× 2 L) and CH_2Cl_2 (2× 2 L). The combined crude extract (10.2 g) was partitioned between 15% aqueous MeOH and *n*-hexane. The aqueous MeOH layer (7.9 g) was separated by C_{18} reversed-phase vacuum flash chromatography, using sequential mixtures of MeOH and H_2O as eluents. The combined fractions (270 mg), eluting with 20 and 10% aqueous MeOH, were separated by C_{18} reversed-phase HPLC (YMC ODS-A column, 25% aqueous MeCN) to yield four fractions active against COX-2 (48–96% inhibition at 12.5 μg/mL). Proton NMR analysis revealed that all of the fractions consisted primarily of mixtures of monoglycerides bearing polyunsaturated fatty acids at their side chains. Purification of the most bioactive fraction was accomplished by reversed-phase

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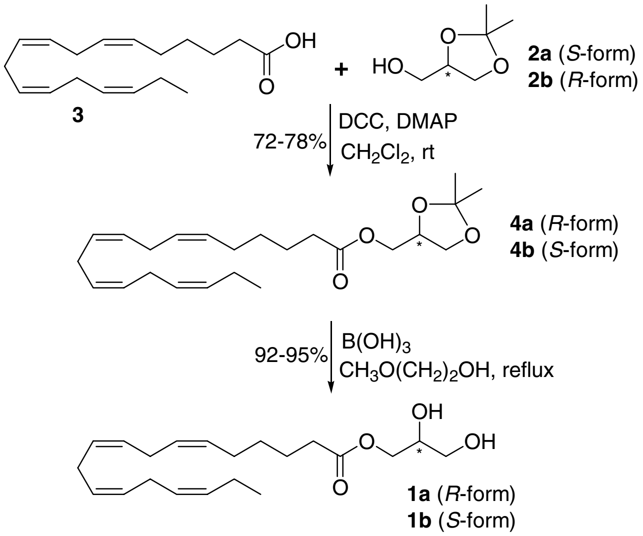
HPLC (15% aqueous MeOH) to afford a single compound **1** as a colorless gum (8.4 mg). Compound **1** was determined to be $C_{21}H_{34}O_4$ by combined HRFABMS and ^{13}C NMR spectrometry: observed, 373.2350 (calculated for $C_{21}H_{34}O_4Na$, 373.2355); UV λ_{max}, no maximum; IR (KBr), 3450, 2930, 1740, and 1425 cm^{-1}. A carbonyl, four double bonds, and three oxygenated carbons were revealed by signals at δ 175.4 (1× C), 133–128 (6× CH), and 72–64 (2× CH_2 and 1× CH), respectively, in the ^{13}C NMR spectra and corresponding signals in the ^1H NMR spectra (Table 1).

Using this information, the structure of **1** was elucidated by combined 2-D NMR analyses. The ^1H COSY correlations among the alkoxy protons in the region δ *4.14–3.53, in conjunction with the HSQC correlations of these protons with the carbons bearing them, revealed the presence of a glycerol moiety. Long-range correlations of a carbonyl carbon at* δ 175.4 with the oxymethylene protons at δ 4.14 and 4.06 indicated the attachment of the carbonyl at C-1 of the glycerol. A combination of the ^1H COSY, TOCSY, and HMBC data showed that a linear chain of four methylenes was directly attached to the carbonyl carbon. In addition, an allylic ethyl group was placed at the terminus of the molecule by combined ^1H COSY and HMBC analyses.

The remaining portion, four double bonds and three bis-allylic methylenes, was alternately arranged in linear mode between the alkyl chain and allylic ethyl group, on the basis of chemical shifts of the allylic methylenes and the lack of an absorption maximum in the UV spectra. Thus, the structure of **1** was determined to be 1-octadecatetraenoyl glycerol, a monoglyceride. Although fatty acid-bearing glycerol compounds have frequently been isolated from nature, to our knowledge, 1-octadecyltetraenoyl glycerol is unprecedented.

To determine the absolute stereochemistry of natural monoglyceride **1**, we synthesized each enantiomer of **1** from optically pure 1,2-isopropylidene glycerol **2**, according to a known procedure.[2] Octadecatetraenoic acid (ODTA, **3**) was condensed with **2** in the presence of DCC and DMAP to give **4** in high yield (Scheme 1). The ketal protecting group of **4** was successfully removed by treatment with boric acid in 2-methoxyethanol at reflux, to give the desired 1-monoacylglycerols **1a** and **1b** in 92–95% yield and sufficient purity (>95%). The impurity was most likely 2-monoacylglycerol, a 1,2-acyl-migrated isomer. In this hydrolysis process, acids other than boric acid, including TFA and Dowex, were not useful, because the chemical yield was low and the chromatographically isolated product was contaminated with significant amounts of unidentified impurities and an acyl-migrated isomer. The NMR spectroscopic data for the obtained **1a** and **1b** were identical with those of the isolated natural form. The optical rotation of synthetic **1a** was $[\alpha]_D^{20}$ −3.1 (*c* 1.1, MeOH) and that of **1b** was $[\alpha]_D^{20}$ +3.7 (*c* 0.93, MeOH). Although the optical rotation values of natural **1** $\{[\alpha]_D^{20} + 1.09$ (*c* 0.14, MeOH)$\}$ and synthetic **1b** were slightly different, it seemed plausible that the absolute stereochemistry of the natural monoglyceride **1** was *S*. The two enantiomers exhibited a very similar degree of inhibitory activity against COX-2, with 35–36% inhibition at 50 μM. This result suggests that the stereochemistry of the glycerol moiety is not important for biological activity.



Scheme 1. Synthesis of 1-octadecatetraenoyl glycerol.

Table 1

^1H and ^{13}C NMR assignments for compound **1**[a,b]

Position	δ_H	δ_C	
1	4.14, dd (11.4, 4.4) 4.06, dd (11.4, 6.3)	66.5	CH_2
2	3.81, m	71.2	CH
3	3.56, dd (11.3, 5.3) 3.53, dd (11.3, 5.7)	64.1_2	CH_2
1′		175.4	C
2′	2.38, t (7.8)	34.8_2	CH_2
3′	1.64, tt (7.8, 7.3)	25.6	CH_2
4′	1.41, p (7.3)	30.2	CH_2
5′	2.09, m	27.9	CH_2
6′	5.39, m	132.6	CH
7′	5.38–5.32, m	129.0[c]	CH
8′	2.83, m	26.6[d]	CH_2
9′	5.38–5.32, m	129.4[c]	CH
10′	5.38–5.32, m	129.0[c]	CH
11′	2.83, m	26.5[d]	CH_2
12′	5.38–5.32, m	129.3[c]	CH
13′	5.38–5.32, m	129.2[c]	CH
14′	2.83, m	26.4[d]	CH_2
15′	5.31, m	128.2	CH
16′	5.38–5.32, m	132.8	CH
17′	2.08, m	27.9	CH_2
18′	0.97, t (7.5)	14.6	CH_3

[a] Measured in $CDCl_3$ solution.

[b] Assignments were aided by a combination of ^1H COSY, gHSQC, and gHMBC experiments.

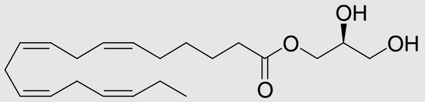
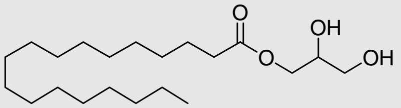
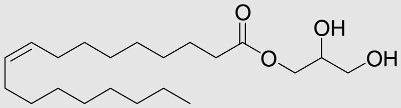
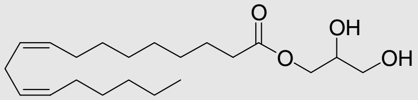
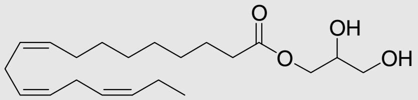
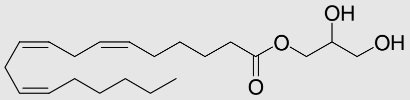
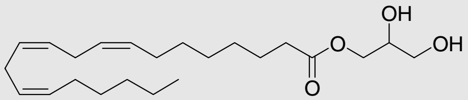
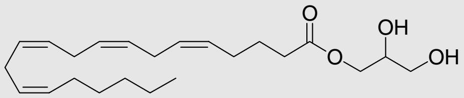
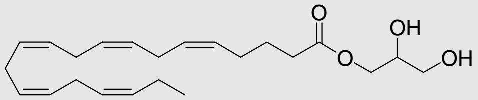
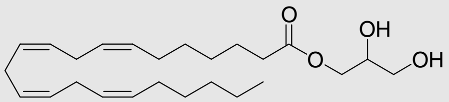
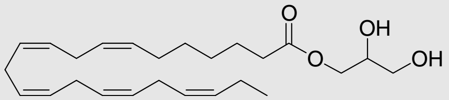
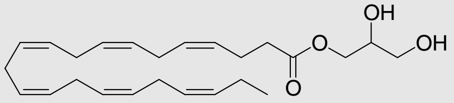
[c,d] Interchangeable signals.

The other COX-2-active fractions from *S. sagamianum* seemed to contain various polyunsaturated fatty acid-containing 1-monoglycerides. However, the isolation and identification of those compounds were not easy tasks, primarily due to their instability under chromatographic conditions and low natural abundance. Instead of overcoming these isolation problems, we decided to synthesize various 1-monoacylglycerols with fatty acids of different chain lengths and unsaturation to find out which fatty acid residue(s) was more effective biologically. Thus, we prepared 11 analogues of **1** in their racemic forms, according to the above-mentioned procedure. Most of the prepared monoacylglycerols have been previously synthesized or isolated from natural sources.[3] However, their biological functions have not been systematically studied.

The prepared monoacylglycerols **5**–**15** were evaluated for inhibitory activity against COX-2 and group IIA secretory phospholipase A_2 ($sPLA_2$-IIA). $sPLA_2$ is a pivotal enzyme in the biosynthesis of prostaglandins (PGs) and catalyzes the hydrolysis of glycerol-phospholipids to release arachidonic acid.[4] The released free arachidonic acid is metabolized by COX to PGH_2, which is further converted to various PGs. Several lines of evidence suggest that the overproduction of PGs is involved in a variety of pathophysiological processes, including inflammation, Alzheimer's disease, hypertension, heart failure, and carcinogenesis.[5] Among the $sPLA_2$ and COX members, $sPLA_2$-IIA and COX-2, respectively, have attracted the most interest over past decades and have been considered as attractive drug targets.[6] COX-2 is up-regulated by sPLA$_2$-IIA, and the two are functionally coupled. Thus, we tested our synthesized compounds against COX-2 and sPLA$_2$-IIA, according to previously described procedures.[7,8]

The inhibitory potencies, expressed as percentage inhibition, of the synthesized monoacylglycerols are compared with that of natural **1** shown in Table 2. These data reveal interesting trends in the structural features of monoacylglycerols. The presence and position of the double bond and the chain length were found to influence the activity. The removal of the double bonds of ODTA of **1** resulted in diminished inhibitory activity in the COX-2 assay (**5–9**). For example, compounds containing 0–2 double bonds, such as **5–7**, were less active than **1**, and those containing three cis double bonds (**8** and **9**) were associated with increased COX-2 activity. A subset of 22-carbon polyunsaturated fatty acid-containing derivatives (**13**–**15**), regardless of the number of cis double bonds, exhibited appreciable COX-2 inhibitory activities, which were considerably higher than that of **1**. On the other hand, the COX-2 inhibitory activities of compounds **10**–**12**, which have fatty acid chains of 20 carbon atoms, were sensitive to the number of cis double bonds. Compound **12** with five cis double bonds showed high inhibitory activity, but compounds **10** and **11** produced increased COX-2 activity.

Table 2
Enzyme inhibitory activity of natural **1** and synthetic compounds

	Compound	COX-2 inhibition, %[a] (at 50 μM)	sPLA$_2$-IIA inhibition, %[a] (at 10 μM)
1		35.6	26.1
5		16.5	32.8
6		24.7	60.6
7		20.5	38.1
8		−12.8	32.0
9		−6.5	38.8
10		−10.5	53.9
11		−20.5	33.6
12		71.9	37.4
13		86.8	37.9
14		92.9	37.4
15		74.2	41.7

[a] Values are means of three experiments.

In the $sPLA_2$-IIA assay, all the tested compounds exhibited inhibitory activity similar to or higher than **1**, with oleic acid-derived compound **6** having the most appreciable inhibitory activity. Compounds **5** and **7**, which differ from **6** by only one cis double bond in the fatty acid moiety, were about half as active as **6**. The polyunsaturated fatty acid-containing compounds generally exhibited modest activity, regardless of the number of cis double bonds. However, dihomo-γ-linolenic acid-derived compound **10** showed inhibitory activity against $sPLA_2$-IIA comparable to that of **6**.

These results suggest that the appropriate presentation of the cis double bond may be important in the inhibitory activity of monoacylglycerols against COX-2 and $sPLA_2$-IIA. Although our results do not provide conclusive information on the structure-activity relationships, they suggest the possibility of developing potent and well-balanced dual inhibitors of both enzymes, as well as specific inhibitors of selected PG biosynthetic enzymes. The development of such compounds is likely to be feasible, considering that compounds **12–15** exhibited appreciable inhibitory potency against both COX-2 and $sPLA_2$-IIA, whereas **10** showed inhibitory activity against only $sPLA_2$-IIA.

In conclusion, we isolated and determined the structure of monoglycerides from the brown alga *S. sagamianum*. Based on the COX-2 inhibitory activity of a novel compound, 1-octadecatetraenoyl glycerol, we synthesized a series of monoglycerides. Bioactivity tests revealed that several compounds of this structural class had moderate to significant inhibitory activity toward COX-2 and $sPLA_2$-IIA. It is well recognized that the polyunsaturated fatty acids are chemically and metabolically unstable. Therefore, the monoglycerides would not be good potential lead compounds for drug development. However, the information obtained may facilitate the design of novel inhibitors of COX-2 and $sPLA_2$-IIA.

Acknowledgments

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8. For the $sPLA_2$ assay, see: Jimenez, M.; Cabanes, J.; Gandia-Herrero, F.; Escribano, J.; Garcia-Carmona, F.; Perez-Gilabert, M. *Anal. Biochem.* **2003**, *319*, 131. Phospholipase A_2 activity was determined by means of a coupled enzyme assay using DL-PC as a substrate and lipoxygenase as a coupling enzyme. The linoleic acid released by phospholipase activity was oxidized by lipoxygenase, giving rise to the corresponding hydroperoxide derivative. The phospholipase activity was followed by measuring the absorbance at 234 nm.