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New Alkoxyglycerols from the Jellyfish *Nemopilema nomurai*

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Abstract – The great economic and social damage caused by unusual explosion of jellyfish population has attracted the attention of researchers. A chemical study on the bioactive components of the giant jellyfish *Nemopilema nomurai* led to the isolation of two new (**1** and **2**) and three known alkoxyglycerols (**3** - **5**), along with known monoglycerides (**6** - **7**) and fatty acids. Based on NMR and MS data, the structures of compounds **1** and **2** were elucidated as 1-*O*-[(*Z*)-tetradec-3-enyl]-*sn*-glycerol and 1-*O*-[(*Z*)-octadec-10-enyl]-*sn*-glycerol, respectively. The absolute configurations of compounds **1** - **7** were determined by comparison of specific optical rotation values with those reported. The isolated compounds were evaluated for suppressive effect on the pro-inflammatory mediators (NO, IL-6, and TNF-α) in murine macrophage cells. However, they were found inactive upto the concentration of 100 μM.
Keywords – Jellyfish, *Nemopilema nomurai*, Alkoxyglycerol

Introduction

Nemopilema nomurai (Cnidaria, Scyphozoa), one of the largest jellyfish species, is distributed primarily in the waters between China and Japan, primarily centralized in the northern parts of the East China Sea, Yellow Sea, and Bohai Sea. Over the past few years, unusual explosion of population of this giant jellyfish has caused economic and social damages, and there have been widely disseminated presumptions to the cause of the population increase, such as over-fishing of natural predators, global warming and industrialization, but no definite explanation is available (Yasuda, 2004; Kawahara *et al.*, 2006). To utilize jellyfish in a productive manner, some trials were attempted to promote jellyfish as food source or fertilizer. In addition, various proteins found in this jellyfish, such as collagens and mucin (a glycoprotein), have been the subject of researches (Miura and Kimura, 1985; Masuda *et al.*, 2007).

In our chemical study on bioactive compounds from the jellyfish *Nemopilema nomurai*, a series of lipids were isolated, including two new alkoxyglycerols (**1** and **2**). The compounds were investigated for their anti-inflammatory activity through the evaluation of their inhibitory effects on the production of major pathophysiological mediators (NO, IL-6, and TNF-α) in lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophage cells. However, none of the compounds showed suppressive effect on the pro-inflammatory mediators upto the concentration of 100 μM.

Experimental

General – Optical rotations were measured in MeOH using a JASCO P-1020 digital polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on UNITY 400 and Varian INOVA 500 instruments. Chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 7.26 and δ_C 77.0 for $CDCl_3$, δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS data were obtained on a JEOL JMS SX-102A. HRFABMS data were obtained on a JEOL JMS SX-101A. HPLC was performed with an YMC ODS-H80 column (250 × 10 mm i.d., 4 μm, 80 Å) using a Shodex RI-71 detector.

Animal material – The jellyfish was collected in June

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2007, off the southern coast of Korea. The specimen was identified by Dr. Won Duk Yoon, National Fisheries Research and Development Institute, Busan, Korea. The voucher specimen of the jellyfish was deposited in the Marine Natural Product Laboratory, Pusan National University, Busan, Korea.

Extraction and isolation – The frozen jellyfish (146.2 kg) was exhaustively extracted with EtOAc at room temperature to afford the EtOAc layer which was concentrated by rotary evaporator. Guided by the lethality to brine shrimp larvae (LD_{50} 269.0 - 325.7 μg/mL), the EtOAc extract was further partitioned between aqueous MeOH and *n*-hexane. Aqueous MeOH fraction was subjected to a stepped gradient MPLC (ODS-A, 120 Å, S-30/50 mesh) eluting with 60 to 100% MeOH to afford 12 fractions. Fraction 4 was separated by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm, 4 μm, 80 Å) eluting with 70% AcCN to afford 12 subfractions. Subfraction 12 was purified by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm, 4 μm, 80 Å) eluting with 90% AcCN to yield compounds **1** (1.0 mg) and **6** (1.4 mg). Compound **3** (0.8 mg) was isolated by purifying subfraction 10 on reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm, 4 μm, 80 Å) eluting with 90% AcCN. Fraction 5, one of the bioactive fractions (LD_{50} 25.8 μg/mL), was subjected to reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm, 4 μm, 80 Å) eluting with 93% MeOH + 0.4% HCOOH (v/v) to afford 7 subfractions. Compound **4** (0.9 mg) was obtained by purifying subfraction 3 on reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm, 4 μm, 80 Å) eluting with 95% AcCN. Fraction 6 was separated by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm, 4 μm, 80 Å) eluting with 90% AcCN to afford 8 subfractions. Subfraction 7 was purified by reversed-phase HPLC (YMC ODS-H80, 250 × 10 mm, 4 μm, 80 Å) eluting with 93% MeOH to yield compounds **2** (0.7 mg), **5** (2.0 mg) and **7** (1.7 mg) (Fig. 1).

1-*O*-[(*Z*)-Tetradec-3-enyl]-*sn*-glycerol (1) – Colorless oil. $[\alpha]_D^{25}$ +1.60 (*c* = 0.13, MeOH); ^1H NMR and ^{13}C NMR data see Table 1; HRFABMS *m/z* 309.2411 [M + Na]$^+$ (calcd for $C_{17}H_{34}O_3Na$, 309.2406).

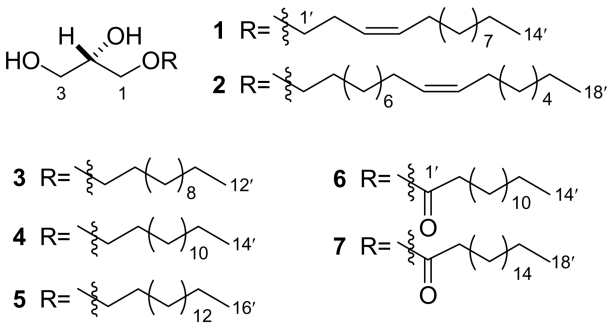

Fig. 1. Structures of compounds isolated from *Nemopilema nomurai*.

Table 1. NMR data of compounds **1** and **2** (in $CDCl_3$)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	3.55 (dd, 10.0, 4.0)	72.8	3.54 (dd, 10.0, 4.0)	72.6
2	3.84 (m)	70.5	3.85 (m)	70.4
3a	3.64 (dd, 11.2, 5.2)	64.5	3.65 (dd, 11.5, 5.0)	64.4
3b	3.70 (dd, 11.2, 5.2)	64.5	3.72 (dd, 11.5, 5.0)	64.4
1'	3.47 (m)	71.5	3.48 (m)	71.9
2'	2.32 (m)	28.0	1.57 (m)	31.0
3'	5.34 (m)	125.2	1.26 (br s)	26.1
4'	5.45 (m)	132.7	1.26 (br s)	29.0-29.8
5'	2.02 (m)	27.6	1.26 (br s)	29.0-29.8
6'-8'	1.24 (br s)	29.6-31.2	1.26 (br s)	29.0-29.8
9'	1.24 (br s)	29.6-31.2	2.01 (m)	27.3
10'	1.24 (br s)	29.6-31.2	5.35 (m)	129.9
11'	1.24 (br s)	29.6-31.2	5.35 (m)	129.9
12'	1.24 (br s)	32.1	2.01 (m)	27.3
13'	1.24 (br s)	22.9	1.26 (br s)	29.0-29.8
14'	0.86 (t, 7.2)	14.3	1.26 (br s)	29.0-29.8
15'-17'	–	–	1.26 (br s)	29.0-29.8
18'	–	–	0.88 (m)	14.2

1-*O*-[(*Z*)-Octadec-10-enyl]-*sn*-glycerol (2) – Colorless oil. $[\alpha]_D^{25}$ +3.80 (*c* = 0.05, MeOH); ^{1}H NMR and ^{13}C NMR data see Table 1; HRFABMS *m/z* 365.3033 $[M + Na]^+$ (calcd for $C_{21}H_{42}O_3Na$, 365.3032). FAB-CID-MS/MS *m/z* 365 $[M + Na]^+$ (100), 349 (0.03), 335 (0.05), 321 (0.07), 307 (0.19), 293 (0.20), 279 (0.13), 265 (0.12), 253 (0.03), 239 (0.16), 225 (0.07), 211 (0.11), 183 (0.11), 169 (0.12), 113 (0.13), 98 (0.12), 84 (0.13).

1-*O*-Dodecyl-*sn*-glycerol (3) – White, amorphous solid. $[\alpha]_D^{25}$ +3.50 (*c* = 0.02, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, t, *J* = 7.0 Hz, H-12'), 1.26 (18H, br s, H-3'~11'), 1.58 (2H, m, H-2'), 3.47 (2H, m, H-1'), 3.53 (2H, m, H-1), 3.66 (1H, dd, *J* = 11.5, 4.0 Hz, H-3a), 3.73 (1H, dd, *J* = 11.5, 4.0 Hz, H-3b), 3.86 (2H, m, H-2); FABMS *m/z* 261 $[M + H]^+$, 305 $[M + 2Na - H]^+$, 283 $[M + Na]^+$.

1-*O*-Tetradecyl-*sn*-glycerol (4) – White, amorphous solid. $[\alpha]_D^{25}$ +6.62 (*c* = 0.07, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, t, *J* = 7.0 Hz, H-14'), 1.26 (22H, br s, H-3'~13'), 1.57 (2H, m, H-2'), 3.47 (2H, m, H-1'), 3.54 (2H, m, H-1), 3.66 (1H, m, H-3a), 3.73 (1H, m, H-3b), 3.86 (2H, m, H-2); FABMS *m/z* 333 $[M + 2Na - H]^+$, 311 $[M + Na]^+$.

1-*O*-Hexadecyl-*sn*-glycerol (5) – White, amorphous solid. $[\alpha]_D^{25}$ +1.56 (*c* = 0.17, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, m, H-16'), 1.26 (20H, br s, H-3'~15'), 1.57 (2H, m, H-2'), 3.46 (2H, m, H-1'), 3.52 (1H, m, H-1), 3.65 (1H, m, H-3a), 3.72 (2H, m, H-3b), 3.85 (2H, m, H-2); FABMS *m/z* 317 $[M + H]^+$, 339 $[M + Na]^+$.

1-*O*-Tetradecanoyl-*sn*-glycerol (6) – White, amorphous solid. $[\alpha]_D^{25}$ +1.59 (*c* = 0.11, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3H, m, H-14'), 1.23 (20H, br s, H-4'~13'), 1.61 (2H, m, H-3'), 2.33 (2H, t, *J* = 7.2 Hz, H-2'), 3.58 (1H, dd, *J* = 11.2, 6.0 Hz, H-3a), 3.68 (1H, dd, *J* = 11.2, 6.0 Hz, H-3b), 3.92 (2H, m, H-2), 4.13 (2H, dd, *J* = 11.6, 6.0 Hz, H-1a), 4.19 (2H, dd, *J* = 11.6, 6.0 Hz, H-1b); FABMS *m/z* 303 $[M + H]^+$, 325 $[M + Na]^+$.

1-*O*-Octadecanoyl-*sn*-glycerol (7) – White, amorphous solid. $[\alpha]_D^{25}$ +6.51 (*c* = 0.17, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (3H, t, *J* = 7.5 Hz, H-18'), 1.25 (28H, br s, H-4'~17'), 1.63 (2H, m, H-3'), 2.35 (2H, t, *J* = 7.0 Hz, H-2'), 3.60 (1H, dd, *J* = 11.2, 6.0 Hz, H-3a), 3.70 (1H, dd, *J* = 11.2, 6.0 Hz, H-3b), 3.93 (2H, m, H-2), 4.15 (2H, dd, *J* = 11.5, 6.0 Hz, H-1a), 4.21 (2H, dd, *J* = 11.5, 6.0 Hz, H-1b); FABMS *m/z* 381 $[M + Na]^+$.

Cell culture – Anti-inflammatory assay was performed at Cheju National University. The mouse macrophage RAW 264.7 was purchased from ATCC (Rockville, MD) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-activated fetal bovine serum, streptomycin (100 μg/mL), and penicillin (100 U/mL) at 37 °C atmosphere and 5% CO_2.

Nitrite assay – The production of nitric oxide (NO) was measured, as previously described by using the Griess reagent (Sigma, MO) (Ryu *et al*., 2000). Briefly, the RAW 264.7 cells were stimulated with LPS (1 μg/mL), and 100 μL of the supernatant was mixed with 100 μL of the Griess reagent (0.1% naphthlyene diamine dihydrochloride, 1% sulfanilamide, 2.5% H_3PO_4). This mixture was incubated for 10 min at room temperature (light protected). Absorbance at 540 nm was measured using an ELISA reader (Amersham Pharmacia Biotech, UK), and the results were compared against a calibration curve using sodium nitrite as the standard.

Measurement of the production of pro-inflammatory cytokines (IL-6 and TNF-α) – The inhibitory effects of the isolated compounds on IL-6 and TNF-α production were determined by the method previously described (Cho *et al.*, 2000). The samples were dissolved with EtOH and diluted with Dulbecco's Modified Eagle's Medium (DMEM). The final concentration of chemical solvents did not exceed 0.1% in the culture medium. At these conditions, none of the solubilized solvents altered IL-6 and TNF-α production in RAW 264.7 cells. Before stimulation with LPS (1 μg/mL) and test materials, RAW 264.7 cells were incubated for 18 h in 24-well plates under the same conditions. Lipopolysaccharide (LPS) and the test materials were then added to the cultured cells. The medium was used for IL-6 and TNF-α assay using mouse ELISA kits (R & D Systems Inc., MN).

Results and Discussion

Compound **1** was isolated as a colorless oil. Its molecular formula was established as $C_{17}H_{34}O_3$ on the basis of HRFABMS. The exact mass of the $[M + Na]^+$ ion at *m/z* 309.2411 matched well with the expected formula $C_{17}H_{34}O_3Na$ (Δ +0.5 mmu). The presence of a glycerol moiety in **1** was deduced by the characteristic proton signals at δ_H 3.64 (H-3a), 3.70 (H-3b), 3.84 (H-2), and 3.55 (H-1). Furthermore, the HMBC correlation between the oxymethylene proton signal at δ_H 3.47 (H-1') and the carbon signal at δ_C 72.8 (C-1) established the ether linkage in the molecule. The ^{1}H NMR spectrum showed the presence of two multiplets at δ_H 5.34 and δ_H 5.45, corresponding to vicinal olefinic protons. In the COSY spectrum, two-proton signal at δ_H 3.47 (H-1') was correlated with an allylic proton resonance at δ_H 2.32 (H-2'), which was further coupled with the olefinic proton at δ_H 5.34. Thus, the double bond was shown to be located

at C-3', and the geometry of the double bond was deduced to be *cis*, since the allylic carbons resonated at δ_C 27.6 and 28.0 (Mansoor *et al.*, 2005; Mansoor *et al.*, 2007). The absolute configuration at C-2 was established as *S* from the positive optical rotation (Mancini *et al.*, 1997; Seo *et al.*, 1999). Accordingly, the chemical structure of **1** was determined to be 1-*O*-[(*Z*)-tetradec-3-enyl]-*sn*-glycerol.

Compound **2** was also isolated as a colorless oil. Its molecular formula was established as $C_{21}H_{42}O_3$ on the basis of HRFABMS. The exact mass of the $[M + Na]^+$ ions at *m/z* 365.3033 matched well with the expected molecular formula of $C_{21}H_{42}O_3Na$ (Δ +0.01 mmu). The NMR data were found to be nearly identical to those of **1**, which indicated that **2** was also an alkoxyglycerol. The position of the double bond was determined by collision-induced-dissociation fast-atom-bombardment tandem mass spectrometry (FAB-CID-MS/MS) analysis of the $[M + Na]^+$ ion at *m/z* 365. Allylic cleavages were observed as enhanced peaks at *m/z* 225 and 279, indicating the location of the double bond at C-10' (Fig. 2). The geometry of the double bond was established as *cis* on the basis of C^{13} NMR chemical shifts of the allylic carbons. The configuration at C-2 was defined as *S* from the positive optical rotation. Thus, the structure of **2** was determined to be 1-*O*-[(*Z*)-octadec-10-enyl]-*sn*-glycerol.

The ^1H NMR spectral data of **3 - 5** were very similar to those of **1**. The only significant difference in the NMR spectra was the absence of signals for olefinic group. The length of the linear chain was deduced from the FABMS data. The configuration of the glyceryl moiety of three compounds was found to be the same as that of **1** on the basis of positive optical rotation. Though alkoxyglycerols are found frequently in humans, cows, and the family of fish to which sharks belong, this is the first report on the isolation of them from a jellyfish.

The molecular formula for **6** was deduced as $C_{17}H_{34}O_4$ by FABMS and NMR data. Although the spectral data for this compound were very similar to those of alkoxyglycerols, the absence of oxygenated proton signal at ca. δ_H 3.5 (H-1') and a triplet at δ_H 2.33 (H-2') revealed the ester linkage in the molecule. The configuration at C-2 was established from the positive optical rotation, which is a general feature of the long-chain 1-*O*-acyl-*sn*-glycerols (Burgos *et al.*, 1987; Gaffney and Reese, 1997; Ungur *et al.*, 1999; Zhao *et al.*, 2003; Mansoor *et al.*, 2007). Thus, the structure of **6** was determined to be 1-*O*-tetradecanoyl-*sn*-glycerol. The only difference between **7** and **6** was the length of acyl chain which was defined by FABMS analysis.

Alkoxyglycerols have shown various beneficial effects for health. The oral administration of alkoxyglycerols reduces the negative effects of radiations received in radiation therapies (Brohult *et al.*, 1970; Andreesen, 1988). Some Alkoxyglycerols can suppress the growth of solid tumor when administered through various routes (Ando *et al.*, 1972). It has been observed in experiments for both animals and humans that they are able to improve the immune response (Palmblad *et al.*, 1990). The alkoxyglycerols also showed significant anti-inflammatory activity when given orally in rats (Burford *et al.*, 1968).

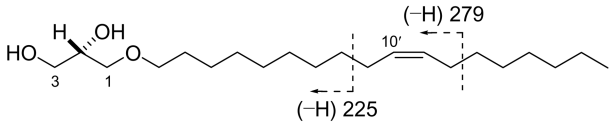

Fig. 2. Key FAB-CID tandem mass fragmentations of the $[M + Na]^+$ ion of **2**.

In our study, the isolated compounds were evaluated for anti-inflammatory activity by determining their inhibitory effects on the production of major pathophysiological mediators (NO, IL-6, and TNF-α) in lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophage cells, but they were found to be inactive upto the concentration of 100 μM.

Acknowledgments

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