Anticholinesterase and Antioxidant Constituents from *Gloiopeltis furcata*

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Activity-directed isolation of the ethyl acetate, methylene chloride and *n*-hexane fractions of *Gloiopeltis furcata* resulted in the isolation of 18 compounds. Their structures were elucidated as 2-(3-hydroxy-5-oxotetrahydrofuran-3-yl)acetic acid (1), glutaric acid (2), succinic acid (3), nicotinic acid (4), (*E*)-4-hydroxyhex-2-enoic acid (5), cholesterol (6), 7-hydroxycholesterol (7), uridine (8), glycerol (9), 5-(hydroxymethyl)-2-methoxybenzene-1,3-diol (10), (5*E*,7*E*)-9-oxodeca-5,7-dienoic acid (11), (*Z*)-3-ethylidene-4-methylpyrrolidine-2,5-dione (12), dehydrovomifoliol (13), loliolide (14), cholesteryl stearate (15), palmitic acid (16), *cis*-5,8,11,14,17-eicosapentaenoic acid (17) and α-linolenic acid (18) on the basis of spectroscopic and chemical evidences. Their anticholinesterase and antioxidant activities were evaluated *via* inhibitory activities on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) as well as scavenging activities on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and peroxynitrite ($ONOO^-$). All isolated compounds (1—18) exhibited moderate AChE inhibitory activities with IC_{50} values ranging from 1.14—12.50 μg/ml, whereas 1, 7, 9, 17, and 18 showed mild BChE inhibitory activities with IC_{50} values ranging from 5.57—15.89 μg/ml. Although most of the compounds isolated were lacking the scavenging activity on DPPH radical and $ONOO^-$, 5 and 10 showed good DPPH radical scavenging activity, and 5, 10, and 16 showed potent $ONOO^-$ scavenging activity.

Key words *Gloiopeltis furcata*; antioxidant activity; cholinesterase inhibition; 2-(3-hydroxy-5-oxotetrahydrofuran-3-yl)acetic acid; (5*E*,7*E*)-9-oxodeca-5,7-dienoic acid

Gloiopeltis furcata (Postels *et* Ruprecht) J. Agardh is a perennial red alga (Rhodophyta) which belongs to the Endocladiaceae family and distributed in the north coast of Pacific, including Korea and Japan. Many reports have been published highlighting the variety of its biological activities, such as cancer prevention,[1)] constipation improvement,[1)] radical scavenging,[2,3)] removement of cholesterol,[4)] recalcification of tooth,[5)] tyrosinase inhibition,[6)] hair growth inhibition,[7)] antitumor,[8)] antibacterial,[9)] antiaging and wrinkle prevention,[10)] anti oral bacteria,[11)] β-D-glucosidase inhibition,[12)] and blood anticoagulation[13)] from *G. furcata*. However, limited studies are available on the derived chemicals of this maritime plant, except for the studies on galactan and polysaccharides.[14)]

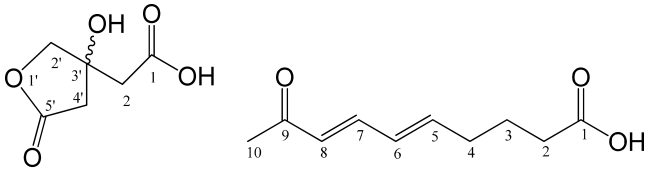
Alzheimer's disease (AD) is a chronic neurodegenerative disorder that damages the brain and results in cognitive impairment, including memory loss and learning disturbances commonly seen in the elderly.[15,16)] Many different theories have been postulated for the etiology of AD and one of the well-established theories suggests the involvement of cholinergic pathway, indicating that progressive decline in the levels of neurotransmitter acetylcholine as the culminating process for development of AD.[17)] AD is also characterized pathologically by the presence of amyloid plaques and tau-associated neurofibrillary tangles, which are commonly related to the states of oxidative stress, such as exposure to peroxynitrite ($ONOO^-$) and superoxide anion ($\cdot O_2^-$).[18)] Thus, this research was done to determine effectiveness as cholinesterases (ChEs) inhibitors by alleviating the cholinergic deficits improving neurotransmission and utilize its antioxidant property to ameliorate oxidative stress.

Limited studies have been conducted and reported that highlights the chemical constituents anticholinesterase and antioxidant activities of *G. furcate*. This work focuses on the isolation and characterization of bioactive constituents of *G. furcata* to evaluate of anticholinesterase and antioxidant activities *via* inhibitory activities on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) and scavenging activities on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and $ONOO^-$, respectively.

Results and Discussion

G. furcata was extracted with 95% ethanol (EtOH) and concentrated *in vacuo*. The EtOH extract of *G. furcata* was subsequently fractionated into five parts: *n*-hexane, methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), *n*-butanol (BuOH) and water. Anticholinesterase and antioxidant activities of these fractions were examined *via* inhibitory activities on AChE and BChE as well as scavenging activities on DPPH radical and $ONOO^-$. The results showed that the EtOAc fraction had the strongest DPPH radical and $ONOO^-$ scavenging activities; while *n*-hexane and EtOAc fractions had BChE inhibitory activity.

Chromatographic separation of the EtOAc, CH_2Cl_2, and *n*-hexane fractions yielded eighteen compounds, including **1**—**10** from the EtOAc fraction, **11**—**14** from the CH_2Cl_2 fraction, and **15**—**18** from the *n*-hexane fraction by RP-C18 and silica gel, Sephadex LH-20 gel column, and HPLC chromatographies.



1 **11**

Fig. 1. Structures of Compounds **1** and **11** Isolated from *Gloiopeltis furcata*

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The electron ionization-mass spectra (EI-MS) spectral analysis of **1** showed a $[M]^+$ at *m/z* 160 and HR-FAB-MS spectral analysis showed a $[M+H]^+$ at *m/z* 161.0448 corresponding to the molecular formula $C_6H_9O_5$ (Calcd 161.0450). The IR spectrum of **1** showed absorption bands at 3444 cm^{-1} (OH), 1719 cm^{-1} (ester), 1636 cm^{-1} (COOH), and 1067 cm^{-1} (C–O). The ^{1}H-NMR spectrum of **1** showed two doublets at δ 2.63 (1H, d, J=17.4 Hz) and δ 2.84 (1H, d, J=17.4 Hz), which were attributed to H-2a and H-2b. This spectrum also indicated two signals at δ 2.72 (1H, d, J=16.0 Hz) and δ 2.78 (1H, d, J=16.0 Hz), which could be assigned to H-4′a and H-4′b. Two doublets were presented at δ 4.30 (1H, d, J=10.0 Hz) and δ 4.38 (1H, d, J=10.0 Hz) integrating for two protons (H-2′a and H-2′b). The ^{13}C-NMR spectrum of **1** indicated the presence of one carboxyl carbon signal at δ 177.1 (C-1) and one ester carbon signal at δ 172.4 (C-5′). A hydroxylated carbon signal was presented at δ 74.4 (C-3′) and a carbon signal of C-2′ at δ 78.7. Additionally, two carbon peaks of C-2 and C-4′ were presented as one carbon signal at δ 41.7. Proton and carbon signals were assigned with the help of heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond connectivity (HMBC) and correlation spectroscopy (COSY) experiments. In HMBC spectrum (Fig. 2) of **1**, connectivity was observed between C-1 and H-2ab, between C-5′ and H-4′, and between C-3′ and H-2′ab, H-4′ab and H-2ab. The correlation in the COSY spectrum (Fig. 2) displayed connectivity between H-2′ab, H-2ab and H-4′ab. Therefore, the structure of **1** was determined as 2-(3-hydroxy-5-oxotetrahydrofuran-3-yl)acetic acid. The absolute configuration of **1** remains unknown. Compound **1** was first isolated from natural sources, and its physicochemical data have never been reported previously. Although isolation of methyl ester of **1** was reported, $[\alpha]_D^{25}$ −3.0° (c=0.3, $CHCl_3$), its absolute configuration remained also unknown.[19]

The FAB-MS spectral analysis of **11** showed a $[M+H]^+$ at *m/z* 183.1 and HR-FAB-MS spectral analysis of **11** showed a $[M+H]^+$ at *m/z* 183.1021 corresponding to the molecular formula $C_{10}H_{14}O_3$ (Calcd 183.1023). The IR spectrum of **11** showed absorption bands at 3434 cm^{-1} (O–H), 1719 cm^{-1} (C=O), 1654 cm^{-1} (COOH), and 1077 cm^{-1} (C–O). The ^{1}H-NMR spectrum of **11** showed four conjugated olefinic protons at δ 6.10 (1H, d, J=15.6 Hz), δ 6.18 (1H, dd, J=15.6, 10.0 Hz), δ 7.10 (1H, dd, J=15.2, 10.0 Hz) and δ 6.19 (1H, td, J=15.2, 7.2 Hz), which were attributed to H-8, H-7, H-6 and H-5. That spectrum also indicated two signals at δ 2.39 (2H, t, J=7.2 Hz) and δ 1.80 (2H, quintet, J=7.2 Hz), which could be assigned to H-2 and H-3. A proton H_2-4 was presented as multiplet at δ 2.25—2.30 and one singlet methyl proton was presented at δ 2.28. The ^{13}C-NMR spectrum of **11** indicated the presence of one carboxyl carbon signal at δ 179.1 (C-1), ketone carbon at δ 199.3 (C-9) and one methyl carbon at δ 23.8. Four doublet carbon signals were obtained at δ 144.0 (C-5), δ 130.0 (C-6), δ 143.9 (C-7) and δ 129.5 (C-8). Additionally, three carbon peaks of C-2, C-3 and C-4 were presented at δ 33.4, δ 23.8 and δ 32.4, respectively. Proton and carbon signals were assigned with the help of HSQC, HMBC and COSY experiments. In HMBC spectrum (Fig. 3) of **11**, connectivities were observed between C-1 and H-2 and H-3, between C-9 and H-7, H-8 and H-10, and between C-5 and H-7, H-6, H-4 and H-3, between C-8 and H-7, H-6 and H-10, and between C-1 and H-2 and H-3. The correlation in the COSY spectrum (Fig. 3) displayed connectivity between H-5 and H-4, H-6, between H-8 and H-7, H-6, between H-3 and H-2, H-4. Therefore, the structure of **11** was determined as (5*E*,7*E*)-9-oxodeca-5,7-dienoic acid. Compound **11** is a new compound.

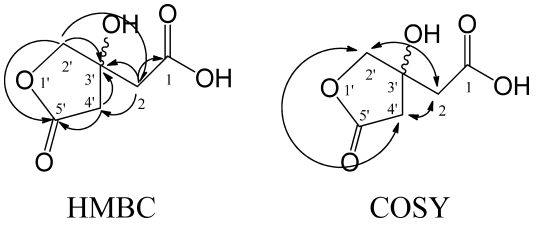

Fig. 2. Important HMBC and COSY Correlations of Compound **1**

Known compounds **2**—**10** and **12**—**18** were determined by direct comparison with authentic samples, or by comparing their physical and spectral data with those in the literature: 2-(3-hydroxy-5-oxotetrahydrofuran-3-yl)acetic acid[20] (**1**), glutaric acid[21] (**2**), succinic acid[22] (**3**), nicotinic acid[23] (**4**), (*E*)-4-hydroxyhex-2-enoic acid[24] (**5**), cholesterol[25] (**6**), 7-hydroxycholesterol[26] (**7**), uridine[27] (**8**), glycerol[28] (**9**), 5-(hydroxymethyl)-2-methoxybenzene-1,3-diol[29] (**10**), (*Z*)-3-ethylidene-4-methylpyrrolidine-2,5-dione[30] (**12**), dehydrovomifoliol[31] (**13**), loliolide[32] (**14**), cholesteryl stearate[33] (**15**), palmitic acid[34] (**16**), *cis*-5,8,11,14,17-eicosapentaenoic acid[35] (**17**) and α-linolenic acid[36] (**18**). All the compounds were isolated for the first time from this plant.

The anticholinesterase and antioxidant activities of **1**—**18**

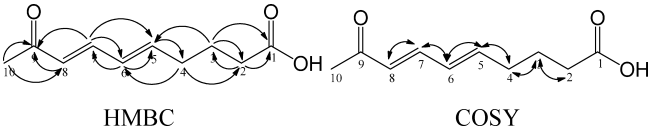

Fig. 3. Important HMBC and COSY Correlations of Compound **11**

Table 1. Anticholinesterase and Antioxidant Activities of **1**—**18**

Compounds	IC_{50} (μg/ml)			
	AChE	BChE	DPPH	$ONOO^-$
1	1.40±0.02	12.61±0.19	>50.0	>50.0
2	5.65±0.09	41.52±0.72	>50.0	>50.0
3	5.74±0.10	>100.0	>50.0	>50.0
4	1.14±0.08	20.86±0.43	>50.0	>50.0
5	12.29±0.22	31.49±0.62	37.81±0.19	1.10±0.06
6	1.15±0.06	>100.0	>50.0	>50.0
7	2.35±0.05	5.57±0.42	>50.0	>50.0
8	1.63±0.00	35.83±0.81	>50.0	>50.0
9	1.61±0.02	8.00±0.03	>50.0	>50.0
10	7.40±0.13	32.66±0.99	14.66±	0.78±0.01
11	5.68±0.18	95.98±1.12	>50.0	39.80±0.27
12	4.17±0.21	75.25±1.54	>50.0	>50.0
13	3.09±0.11	95.08±1.76	>50.0	46.75±0.23
14	7.57±0.17	>100.0	>50.0	>50.0
15	6.34±0.13	>100.0	>50.0	>50.0
16	8.69±0.23	>100.0	>50.0	7.30±0.01
17	11.53±0.43	6.56±0.34	>50.0	15.84±0.05
18	12.50±0.39	15.89±0.76	>50.0	16.24±0.09
Galanthamine	0.02±0.00	0.18±0.01	—	—
L-Ascorbic acid	—	—	2.66±0.08	—
L-Penicillamine	—	—	—	0.63±0.04

from *G. furcata* were evaluated *via* AChE and BChE inhibitory, DPPH radical and $ONOO^-$ scavenging assays. All of the compounds **1**—**18** showed moderate AChE inhibitory activities with IC_{50} values ranging of 1.14—12.50 μg/ml. Among them, **1**, **4**, **6**, **8**, and **9** exhibited good inhibitory activities with respective IC_{50} values of 1.40, 1.14, 1.15, 1.63, and 1.61 μg/ml, as compared with galantamine (IC_{50}=0.02 μg/ml) in the AChE assay. In the BChE assay, only **1**, **7**, **9**, **17**, and **18** showed moderate inhibitory activities with respective IC_{50} values of 12.61, 5.57, 8.00, 6.56, and 15.89 μg/ml, as compared to galanthamine (IC_{50}=0.18 μg/ml). In antioxidant assays, **5** and **10** showed moderate DPPH radical scavenging activities with respective IC_{50} values of 37.81 and 14.66 μg/ml, as compared with L-ascorbic acid (IC_{50}=2.66 μg/ml); **5** and **10** also exhibited strong $ONOO^-$ with respective IC_{50} values of 1.10 and 0.78 μg/ml, as compared to L-penicillamine (IC_{50}=0.63 μg/ml).

In conclusion, eighteen constituents, including a new (**11**) and sixteen known compounds (**1**—**10** and **12**—**18**) were first isolated from *G. furcata*. All isolated compounds **1**—**18** exerted moderate AChE inhibitory activities with IC_{50} values in the range of 1.14—12.50 μg/ml, whereas **1**, **7**, **9**, **17**, and **18** showed mild BChE inhibitory activities with IC_{50} values ranging from 5.57 to 15.89 μg/ml. Ahthough most of isolates were lacking in scavenging activity on DPPH radical and $ONOO^-$, **5** and **10** showed good DPPH radical scavenging activity, and **5**, **10**, and **16** displayed potent $ONOO^-$ scavenging activity.

Experimental

Plant Material *G. furcata* were purchased from Muan of JeollaNamdo of Korea in June 2007. A voucher specimen has been deposited at the College of Pharmacy, Catholic University of Daegu, Korea.

General Procedures Melting point was measured using Yanaco micro melting point apparatus (Kyoto, Japan). Optical rotation was measured using Jasco DIP-370 digital polarimeter (Tokyo, Japan). UV spectra were measured on Shimadzu UV-160A spectrometer (Kyoto, Japan). IR (KBr disk) spectra were measured on Mattson Genesis II (Madison, WI, U.S.A.) and Jasco-300E FT-IR spectrophotometers (Tokyo, Japan). EI-MS and HR-FAB-MS were performed with a Quattro II spectrometer (Micromass, Altrincham, U.K.). The NMR spectra were recorded in CD_3OD, $CDCl_3$ and C_5D_5N on Varian OXFORD-AS400 MHz instrument (Palo Alto, CA, U.S.A.).

Measurement of DPPH Radical Scavenging Activity The DPPH radical scavenging effect was evaluated using method reported earlier by Blois with suitable modifications.[37] The reduction of DPPH as indicated below was followed by monitoring its decrease in absorbance at a characteristic wavelength during the reaction. In its radical form, DPPH absorbs at 520 nm, but after reduction by an antioxidant or a radical species, the absorption disappears. One hundred sixty microliters of a MeOH solution (final concentration 50 μg/ml) was added to 40 μl DPPH methanol solution (1.5×10^{-4} M). After gentle mixing and allowing it to stand at room temperature for 30 min, the optical density was measured at 530 nm using a microplate reader spectrophotometer VERSAmax (Molecular Devices, CA, U.S.A.). The antioxidant activity of each sample was expressed in term of the IC_{50} (μg/ml required to inhibit DPPH radical formation by 50%), which was calculated from the log–dose inhibition curve. L-Ascorbic acid was used as a positive control.

Measurement of the $ONOO^-$ Scavenging Activity The $ONOO^-$ scavenging was measured by monitoring the oxidation of DHR 123 using the method described by Kooy *et al.* with suitable modifications.[38] DHR 123 (5 mM) in dimethylformamide, which was purged with nitrogen, was stored at −80 °C as a stock solution. This solution was then placed in ice and was protected from direct light until prior to the study. The samples were dissolved in 10% dimethyl sulfoxide (DMSO) (f.c. 50 μg/ml). The buffer used was consisted of 90 mM sodium chloride, 50 mM sodium phosphate, 5 mM potassium chloride at pH 7.4, and 100 μM diethylenetriaminepentaacetic acid (DTPA), each of which was prepared with high quality deionized water and was purged with nitrogen. The final concentration of DHR 123 was of 5 mM. The background and final fluorescent intensities were measured 5 min after treatment with and without adding authentic $ONOO^-$. DHR 123 was oxidized rapidly by authentic $ONOO^-$, and its final fluorescent intensity remained unchanged over time. The fluorescence intensity of oxidized DHR 123 was measured with a microplate fluorescence reader (FL 500, Bio-Tek Instruments) at the excitation and emission wavelengths of 480 nm and 530 nm, respectively. Results were expressed as means±S.E.M. (n=3) for the final fluorescence intensity minus background fluorescence. The effects were expressed as the percent inhibition of oxidation of DHR 123. Penicillamine was used as a positive control.

Measurement of Cholinesterases Inhibitory Activity For the inhibitory activities against ChEs were measured using the spectrophotometric method developed by Ellman *et al.*[39] ACh (acetylcholine) and BCh (butyrylcholine) were used as substrates to assay the inhibitions of AChE and BChE, respectively. The reaction mixture contained: 140 μl of sodium phosphate buffer (pH 8.0), 20 μl of test sample solution and 20 μl of either AChE or BChE solution, which were mixed and incubated for 15 min at room temperature. The reactions were initiated with the addition of 10 μl of dithiobisnitrobenzoate (DTNB) and 10 μl of either ACh or BCh, respectively. The hydrolysis of ACh or BCh was monitored after the formation of yellow 5-thio-2-nitrobenzoate anion at 412 nm for 15 min, which resulted from the reaction of DTNB with thiocholine, released by enzymatic hydrolysis of either ACh or BCh, respectively. Test samples and the positive control (galanthamine) were dissolved in 10% analytical grade ethanol. All reactions were performed in triplicate in 96-well microplates, using VERSAmax (Molecular Devices, CA, U.S.A.). The percentage (%) inhibition was calculated from $(E-S)/E\times100$, where E and S are the enzyme activities without and with the test sample, respectively. The ChEs inhibitory activity of each sample was expressed in terms of IC_{50} value (μg/ml required to inhibit the hydrolysis of the substrate; ACh or BCh, by 50%), as calculated from the log–dose inhibition curve.

Extraction and Isolation *G. furcata* (30 kg) was cut into small pieces and extracted with 95% EtOH at 80 °C to yield 1.9 kg of extract after removal of the solvent. It was partitioned between hexane–H_2O (1 : 1) to yield *n*-hexane-soluble fraction (119.0 g) and the H_2O solvent. The H_2O solvent was partitioned between CH_2Cl_2–H_2O (1 : 1) to yield the CH_2Cl_2-soluble fraction (4.0 g) and H_2O solvent. The H_2O solvent was partitioned between EtOAc–H_2O (1 : 1) to yield the EtOAc-soluble fraction (13.4 g) and H_2O solvent. The H_2O solvent was partitioned between *n*-BuOH–H_2O (1 : 1) to yield the *n*-BuOH-soluble fraction (38.8 g) and the H_2O soluble fraction (1.4 kg).

The EtOAc soluble fraction (13.4 g) was subjected to open column chromatography over silica gel (60 g) eluted with hexane–acetone and acetone–MeOH gradient. Fractions (GF-EA-A to GF-EA-Y) were collected and pooled according to their similar TLC patterns. Fraction GF-EA-XY (756 mg) was chromatographed on a reverse-phase column (2.0×50 cm, RP-C18) with MeOH–H_2O (gradient from 15 : 85 to 30 : 70) to afford GF-EA-XY-1, GF-EA-XY-2 and GF-EA-XY-20. GF-EA-XY-2 was purified with reverse-phase column (1.2×45 cm, RP-C18) with MeOH–H_2O (15 : 85) to afford 2-(3-hydroxy-5-oxotetrahydrofuran-3-yl)acetic acid (**1**, 20 mg). GF-EA-XY-20 was purified with reverse-phase column (1.2×45 cm, RP-C18) with MeOH–H_2O (15 : 85) to afford nicotinic acid (**4**, 37 mg). GF-EA-XY-1 was purified with reverse-phase column (1.2×45 cm, RP-C18) with MeOH–H_2O (20 : 80) to afford glycerol (**9**, 50 mg). Fraction GF-EA-Q (1.2 g) was chromatographed on a reverse-phase column (2.0×40 cm, RP-C18) with MeOH–H_2O (gradient from 15 : 85 to 100 : 0) to fractionated GF-EA-Q-A to GF-EA-Q-F. Fractions GF-EA-Q-B and GF-EA-R were recrystallized to afford glutaric acid (**2**, 50 mg) and succinic acid (**3**, 100 mg), respectively. Fraction GF-EA-Q-C was chromatographed with silica gel column (1.2×40 cm) with *n*-hexane–acetone (5 : 1) to afford 7-hydroxy cholesterol (**7**, 29 mg). Fraction GF-EA-Q-D (80 mg) was chromatographed on a Sephadex column (1.5×70 cm, LH-20) with MeOH–H_2O (gradient from 5 : 95 to 30 : 70) to afford GF-EA-Q-D-3 and GF-EA-Q-D-4. The GF-EA-Q-D-3 (65 mg) was purified with HPLC (Waters 600 pump, Waters 486 UV detector, Prep Nova-Pac HR-C18 7.8×300 mm column, MeOH : H_2O=15 : 85) to yield (*E*)-4-hydroxyhex-2-enoic acid (**5**, 10 mg). The GF-EA-Q-D-4 (180 mg) was purified with HPLC (Waters 600 pump, Waters 486 UV detector, Prep Nova-Pac HR-C18 7.8×300 mm column, MeOH : H_2O=15 : 85) to yield 5-(hydroxymethyl)-2-methoxybenzene-1,3-diol (**10**, 50 mg). Fraction GF-EA-G (50.1 mg) was chromatographed on a reverse-phase column (1.5×50 cm, RP-C18) with MeOH–H_2O (gradient from 20 : 80 to 50 : 50) to afford GF-EA-G-1. GF-EA-G-1 (39 mg) was purified with silica gel column (1.2×40 cm) with *n*-hexane–acetone (5 : 2) to afford cholesterol (**6**, 29 mg). Fraction GF-EA-Z (202.1 mg) was chromatographed on a silica gel column (1.5×70 cm) with *n*-hexane–acetone (7 : 1) to afford GF-EA-Z-12. GF-EA-

Z-12 (50 mg) was purified with silica gel column (1.3×45 cm) with *n*-hexane–acetone (4 : 1) to afford uridine (**8**, 10 mg).

The CH_2Cl_2-soluble fraction (4.0 g) was subjected to open column chromatography over silica gel (30 g) eluted with *n*-hexane–acetone gradient. Fractions (GF-MC-A to GF-MC-U) were collected and pooled according to their similar TLC patterns. GF-MC-G (176 mg) was chromatographed on a reverse-phase column (1.6×45 cm, RP-C18) with MeOH–H_2O (55 : 45) to afford GF-MC-G-1. GF-MC-G-1 was purified with silica gel column (1.5×45 cm) with *n*-hexane–acetone (8 : 1) to afford (5*E*,7*E*)-9-oxodeca-5,7-dienoic acid (**11**, 25 mg). GF-MC-E (175 mg) was chromatographed on a reverse-phase column (1.5×40 cm, RP-C18) with MeOH–H_2O (80 : 20) to afford GF-MC-E-2. GF-MC-E-2 was purified with silica gel column (1.2×40 cm) with *n*-hexane–acetone (10 : 1) to afford (*Z*)-3-ethylidene-4-methylpyrrolidine-2,5-dione (**12**, 10 mg). GF-MC-E (466 mg) was chromatographed on a reverse-phase column (2.0×45 cm, RP-C18) with MeOH–H_2O (60 : 40) to afford GF-MC-F-3 and GF-MC-F-4. GF-MC-F-4 was chromatographed on a reverse-phase column (1.2×40 cm, RP-C18) with MeOH–H_2O (75 : 25) to afford dehydrovomifoliol (**13**, 30 mg). GF-MC-F-3 was chromatographed on a reverse-phase column (1.2×40 cm, RP-C18) with MeOH–H_2O (75 : 25) to afford loliolide (**14**, 20 mg).

The *n*-hexane soluble fraction (119.1 g) was subjected to flash column chromatography over silica gel (2.0 kg) eluted with *n*-hexane–acetone gradient. Fractions (GF-HE-A to GF-HE-U) were collected and pooled according to their similar TLC patterns. GF-HE-C (12.0 g) was chromatographed on a silica gel column (7.8×50 cm) with *n*-hexane–acetone (gradient from 10 : 1 to 1 : 1) to afford GF-HE-C-1 and GF-HE-C-4. GF-HE-C-1 was chromatographed on a reverse-phase column (1.2×40 cm, RP-C18) with MeOH–H_2O (75 : 25) to afford cholesteryl stearate (**15**, 5 mg). GF-HE-C-4 was chromatographed on a reverse-phase column (3.0×50 cm, RP-C18) with MeOH–H_2O (77 : 23) to afford palmitic acid (**16**, 4.0 g). GF-HE-D (6.0 g) and GF-HE-E (6.1 g) were recrystallized to afford 1.0 g and 3.0 g of cholesterol (**6**), respectively. GF-HE-F (3.1 g) was chromatographed on a reverse-phase column (3.5×50 cm, RP-C18) with MeOH–H_2O (80 : 20) to afford GF-HE-F-11 and GF-HE-F-13. GF-HE-F-13 was chromatographed on a reverse-phase column (2.4×40 cm, RP-C18) with MeOH–H_2O (76 : 24) to afford *cis*-5,8,11,14,17-eicosapentaenoic acid (**17**, 60 mg). GF-HE-F-11 was chromatographed on a reverse-phase column (1.5×40 cm, RP-C18) with MeOH–H_2O (75 : 25) to afford α-linolenic acid (**18**, 45 mg).

Compound **1**: $[\alpha]_D^{25}$ −10.8° (*c*=0.10, MeOH). UV λ_{max} (MeOH) nm (log ε): 210 (2.41), 247 (1.77). IR (KBr) cm^{-1}: 3444 (O–H), 2930 (C–H), 1067 (C–O), 1719 (C=O), 1636 (C=O). EI-MS *m*/*z*: 160 $[M]^+$, 142 $[M-H_2O]^+$. FAB-MS *m*/*z*: 161 $[M+H]^+$. HR-FAB-MS *m*/*z*: 161.0448 $[M+H]^+$ (Calcd for $C_6H_9O_5$: 161.0450). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 2.63 (1H, d, *J*=17.4 Hz, H-2a), 2.72 (1H, d, *J*=16.0 Hz, H-4′a), 2.78 (1H, d, *J*=16.0 Hz, H-4′b), 2.84 (1H, d, *J*=17.4 Hz, H-2b), 4.30 (1H, d, *J*=10.0 Hz, H-2′a), 4.38 (1H, d, *J*=10.0 Hz, H-2′b). ^{13}C-NMR (CD_3OD, 100 MHz) δ: 41.7 (C-2, -4′), 74.4 (C-3′), 78.7 (C-2′), 172.4 (C-5′), 177.1 (C-1).

Compound **11**: UV λ_{max} (MeOH) nm (log ε): 209 (2.20), 265 (1.76). IR (KBr) cm^{-1}: 3434 (O–H), 2921 (C–H), 1719 (C=O), 1654 (C=O), 1077 (C–O). FAB-MS *m*/*z*: 183.1 $[M+H]^+$, 165.2 $[M-H_2O]^+$. HR-FAB-MS *m*/*z*: 183.1021 $[M+H]^+$ (Calcd for $C_{10}H_{14}O_3$: 183.1023). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.10 (1H, dd, *J*=15.2, 10.0 Hz, H-7), 6.19 (1H, td, *J*=15.2, 7.2 Hz, H-5), 6.18 (1H, dd, *J*=15.6, 10.0 Hz, H-6), 6.10 (1H, d, *J*=15.6 Hz, H-8), 2.39 (2H, t, *J*=7.2 Hz, H-2), 2.25—2.30 (2H, m, H-4), 2.28 (1H, s, H-10), 1.80 (2H, quintet, *J*=7.2 Hz, H-3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 199.3 (C-9), 179.1 (C-1), 144.0 (C-5), 143.9 (C-7), 130.0 (C-6), 129.5 (C-8), 33.4 (C-2), 32.4 (C-4), 27.4 (C-10), 23.8 (C-3).

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