Glycerides from the Aerial Parts of Garland (*Chrysanthemum coronarium* L.) and Their Inhibitory Effects on ACAT, DGAT, FPTase, and β-Secretase

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Abstract The aerial parts of garland (*Chrysanthemum coronarium* L.) were extracted in 80% aqueous methanol (MeOH) and the concentrated extract was then partitioned using ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), and H_2O, successively. EtOAc and *n*-BuOH fractions resulted in 4 glycerides with the application of octadecyl silica gel and silica gel column chromatography. The chemical structures of the glycerides were determined using several spectroscopic methods, including nuclear magnetic resonance (NMR) and mass spectrometry (MS) as (2*S*)-1-*O*-palmitoyl-*sn*-glycerol (**1**), (2*S*)-1-*O*-oleoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**2**), (2*S*)-1-*O*-palmitoyl-2-*O*-linoleoyl-3-*O*-phosphorouscholine-*sn*-glycerol (**3**), and (2*S*)-1-*O*-linolenoyl-2-*O*-palmitoyl-3-*O*-[α-D-galactopyrasyl-(1→6)-β-D-galactopyranosyl]-*sn*-glycerol (**4**). The free fatty acids of these glycerides were determined with gas chromatography (GC)-MS analysis following alkaline hydrolysis and methylation. These glycerides demonstrated an inhibitory effect on acyl-CoA: cholesterol acyltransferase (ACAT, compound **1**: 45.6±0.2% at 100 µg/mL), diacylglycerol acyltransferase (DGAT, compound **1**: 59.1±0.1% at 25 µg/mL), farnesyl protein transferase (FPTase, compound **2**: 98.0±0.1%; compound **3**: 55.2±0.1% at 100 µg/mL), and β-secretase (IC_{50}, compound **4**: 2.6 µg/mL) activity. This paper is the first report on the isolation of these glycerides from garland and their inhibitory activity on ACAT, DGAT, FPTase, and β-secretase.

Keywords: *Chrysanthemum coronarium*, glyceride, acyl-coenzyme A: cholesterol transferase (ACAT), acyl-coenzyme A: diglycerol acyltransferase (DGAT), farnesyl protein transferase (FPTase), β-secretase

Introduction

Vital functions, pharmacological activities, and nutritional values of isolated secondary metabolites of edible plants have been analyzed. During evaluation of the edible plants' biological activities, investigators have reported the isolation of secondary metabolites with biological functionality from several plants, including the fruit of *Euphoria longana* L (1), the flowers of *Campsis grandiflora* K. Schum. (2), the roots of *Ixeris dentata* forma *albiflora* (3), potatoes, pumpkin leaves, wild garlic, lettuce, and garland (*Chrysanthemum coronarium* L.) (4). Garland is a very popular food ingredient in various soups, seafood pot stews, and rice wrapped in leaves, among others. As a result, an investigation of the secondary metabolites of garland is pertinent.

Garland (Compositae) is an annual herb frequently included in the Korean diet because of its fragrant flavor and high nutritional value. Garland has also been used to protect against or remedy constipation and strengthen the spleen and stomach (4). Several constituents have been isolated from garland, including polyacetylenes (5,6), sesquiterpenoids (7,8), monoterpenoids, diterpenoids (9), and steroids (4). Thin layer chromatography (TLC)

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experiment, which revealed the presence of some compounds not previously reported in garland extracts, led to continuing research to isolate other compounds. Furthermore, their inhibitory activity on acyl-coenzyme A: cholesterol transferase (ACAT), acyl-coenzyme A: diglycerol acyltransferase (DGAT), farnesyl protein transferase (FPTase), and β-secretase was evaluated. It is very significant to identify the principal components manifesting pharmacological activity for development of new medicinal materials from natural sources.

ACAT, also referred to as sterol *O*-acyltransferase (SOAT; EC2.3.1.26), is responsible for the esterification of cholesterol with fatty acids. Previously, the inhibition of ACAT activity has been linked to a decrease in plasma cholesterol levels via the suppression of cholesterol absorption, and via a diminution of the assembly and secretion of apolipoprotein B-containing lipoproteins, such as very low density lipoprotein. Therefore, ACAT is an inhibitory target in the treatment of both hypercholesterolemia and atherosclerosis (10). DGAT [EC 2.3.1.201 catalyzes the reaction of acyl residue transfer from acyl-CoA to diacylglycerol to form triacylglycerol (11). Excess accumulation of triacylglycerol within certain organs and tissues of the body can cause high-risk conditions such as fatty liver, obesity, and hypertriglyceridemia, leading to the more serious diseases of atherosclerosis, diabetes, metabolic disorders, or the functional depression of some organs. DGAT is exclusively involved in triacylglycerol formation

and is therefore expected to be an effective inhibitory target in the prevention and treatment of these diseases.

Cancer remains the second leading cause of death in most countries, and there is a dire need for novel and effective treatments. Ras farnesyl protein transferase (FPTase) is an enzyme that catalyzes the transfer of the farnesyl group from farnesyl pyrophosphate (FPP) onto cysteine 186 at the C-terminal of the Ras protein (12). This is an essential step in the membrane association of Ras as it triggers the tumorigenic activity of the ras oncogene. When farnesylation of these proteins is blocked, their oncogenic activity is eliminated. Recent studies have demonstrated that specific inhibitors of FPTase showed excellent efficacy in vivo against solid tumors in mice (13).

Alzheimer's disease is a widespread, neurodegenerative, dementia-inducing disorder. Generation of amyloid β-peptide (Aβ) is regarded as a crucial step in the pathogenesis of Alzheimer's disease (14). To initiate Aβ formation, amyloid precursor protein (APP) is cleaved by β-secretase to generate a soluble NH_2-terminal fragment (APPsβ) and a 12-kDa COOH-terminal fragment (C99) which remains membrane bound. C99 is further cleaved by β-secretase, resulting in the production of pathogenic Aβ (15). In an alternate pathway, APP is cleaved by β-secretase within an Aβ sequence to generate a soluble NH_2-terminal fragment (APPsβ) and a 10-kDa membrane-bound COOH-terminal fragment (C83) (16). C83 is also cleaved by γ-secretase, resulting in the production of nonpathogenic p3 peptide. Because β- and γ-secretases play a critical role in Aβ formation, inhibitors of these proteases are promising therapeutics for Alzheimer's disease (17,18).

This paper describes not only the isolation of 4 glycerides from garland through repeated silica gel (SiO_2) and octadecyl silica gel (ODS) column chromatography (CC) but also structure determination of the glycerides through spectroscopic methods including nuclear magnetic resonance spectrometry (NMR), mass spectrometry (MS), and infrared spectrometry (IR). The type and composition of the fatty acids was determined with alkaline hydrolysis and methylation followed by gas chromatography (GC)-MS experiments. So, this paper suggests that garland contains several glycerides and phospholipids involved in the prevention of hyper-cholesterolemia, cancer, and dementia. Therefore, analysis of the functional compounds of garland could provide information to aid the treatment or prevention of several diseases.

Materials and Methods

Plant materials Garland (*Chrysanthemum coronarium* L.) was purchased from a farm located in Yangju, Korea in December, 2005, and identified by Prof. Dae-Keun Kim, Woosuk University, Jeonju, Korea. A voucher specimen (KHU050812) was reserved at the Laboratory of Natural Products Chemistry, KyungHee University, Suwon, Korea.

Instrumentation Infrared (IR) spectrum was run on a Perkin Elmer spectrum One Fourier transform (FT)-IR spectrometer (Perkin Elmer, Chicago, IL, USA). Electron impact (EI)-MS and fast atom bondardment (FAB)-MS were recorded on a Jeol JMS 700 (Jeol, Tokyo, Japan). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were

taken on a Varian Unity Inova AS 400 FT-NMR spectrometer (Varian, Palo Alto, CA, USA).

Isolation of glycerides The fresh aerial parts of garland (70 kg) were extracted at room temperature with 80% aqueous methanol (MeOH, 40 L×3) for 24 hr and filtered. The filtrate was concentrated in vacuo at 40°C to render the MeOH extracts. The extracts were suspended in water (2 L) and extracted by ethyl acetate (EtOAc, 2 L×3), and the aqueous layer was successively extracted by n-butanol (BuOH, 2 L×3). Each layer was concentrated using rotary vacuum evaporator to yield a EtOAc (CCE, 162 g), a BuOH (CCB, 115 g), and a water (CCW, 501 g) extracts. The EtOAc extract (160 g, CCE) was applied to SiO_2 (1,150 g) CC using chloroform ($CHCl_3$)-MeOH (30:1 20:1→16:1→14:1→12:1→8:1→4:1→2:1→1:1, 3 L of each) as eluents monitoring by TLC, which was sprayed by 10% aq. H_2SO_4 and heated at 120°C, to produce 17 fractions (CCE-1-CCE-17). CCE10 [15.1 g, elution volume/total volume (Ve/Vt) 0.63-0.70] was applied to the SiO_2 CC (300 g, n-hexane-EtOAc=4:1→3:1, 10 L→3 L) to yield 9 fractions. Fraction CCE10-7 (4.4 g, Ve/Vt 0.72-0.79) was subjected to the SiO_2 (200 g) CC (n-hexane-EtOAc=5:1, 3 L) and afforded 9 fractions. Fraction CCE10-7-4 (452 mg, Ve/Vt 0.31-0.38) was applied to the SiO_2 (130 g) CC (n-hexane-EtOAc =3:1→2:1→1:1, 2 L of each) to produce 6 fractions and to give compound 1 [CCE10-7-4-4, 200 mg, Ve/Vt 0.55-0.75, TLC (SiO_2 F_{254}) R_f 0.35, n-hexane-EtOAc=1:1]. Fraction CCE-10-7-8 (1.3 g, Ve/Vt 0.85-0.96) was chromatographed using the SiO_2 (150 g) column (n-hexane-EtOAc=4:1→3:1→2:1 →1:1, 1 L of each) to afford 7 fractions. Fraction CCB10-7-8-4 (315 mg, Ve/Vt 0.44-0.58) was subjected to the SiO_2 (100 g) CC and eluted with n-hexane-EtOAc (1:1→1:2, 1 L→2 L) resulting in 6 fractions. Fraction CCE10-7-4-8-4-2 (44 mg, Ve/Vt 0.11-0.30) was applied to the ODS (75 g) CC (acetone-acetonitrile=1:2, 1.5 L) to yield compound 2 [CCE10-7-8-4-2-6, 24 mg, Ve/Vt 0.60-0.71, TLC (ODS F_{254S}) R_f 0.33, acetone-acetonitrile=1:2] (Fig. 1).

Compound 1, Yellow oil; IR_v (KBr, 1/cm): 3,650, 1,736, 1,210, 1,028; positive FABMS m/z: 399 $[M1+H_2O+Na]^+$, 391 $[M3+K+Na]^+$, 377 $[M2+Na]^+$, 369 $[M3+K]^+$, 355 $[M2+H]^+$, 353 $[M3+Na]^+$: [M1: (2S)-1-O-stearoyl-sn-glycerol, M2: (2S)-1-O-linoleoyl-sn-glycerol, M3: (2S)-1-O-palmitoyl-sn-glycerol]; ^{1}H-NMR (400 MHz, CDCl$_3$, δ$_H$) 5.36 (4H, m, olefin of linoleoyl), 4.14 (2H, d, J=5.2 Hz, sn-H-1), 3.91 (1H, tdd, J=5.2, 6.0, 2.8 Hz, sn-H-2), 3.68 (1H, dd, J=11.2, 2.8 Hz, sn-H-3a), 3.58 (1H, d, J=11.2, 6.0 Hz, sn-H-3b), 2.80 (2H, allyl-metylene), 2.34 (2H, t, J=7.6 Hz, H-2 of linoleoyl), 2.05 (2H, allyl-methylene of linoleoyl), 1.62 (2H, methylene), 1.31 (16H, methylene), 0.97 (3H, t, J=7.6 Hz, methyl), 0.88 (3H, t, J=7.6 Hz, methyl); ^{13}C-NMR (100 MHz, CDCl$_3$, δ$_C$) See Table 3.

Compound 2, Yellow oil; IR_v (KBr, 1/cm): 3,665, 3,380, 2,930, 1,742, 1,216, 1,026; positive FABMS m/z:, 783 $[M1+H]^+$, 757 $[M2+H]^+$, 731 $[M3+H]^+$, 703 $[M4+H]^+$: [M1: (2S)-1-O-oleoyl-2-O-oleoyl-3-O-β-D-galactopyranosyl-sn-glycerol, M2: (2S)-1-O-oleoyl-2-O-palmitoyl-3-O-β-D-galactopyranosyl-sn-glycerol, M3: (2S)-1-O-palmitoyl-2-O-palmitoyl-3-O-β-D-galactopyranosyl-sn-glycerol, M4: (2S)-1-O-palmitoyl-2-O-myristoyl-3-O-β-D-galactopyranosyl-sn-glycerol]; ^{1}H-NMR (400 MHz, CDCl$_3$, δ$_H$) 5.34 (2H, m,
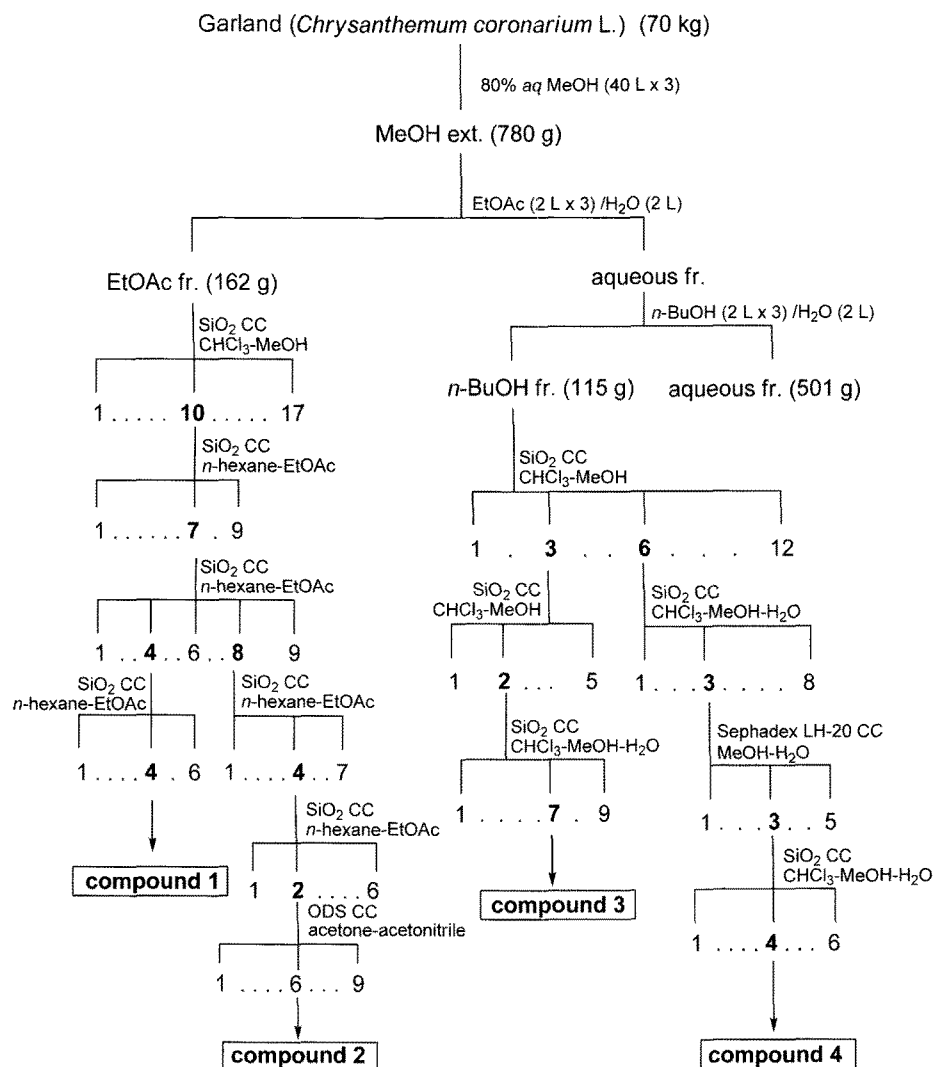
Fig. 1. Isolation procedure of compounds 1-4 from the aerial parts of garland. CC, column chromatography; ODS, a resin of octadecyl silica gel; SiO₂, a resin of silica gel.

olefin of oleoyl), 5.28 (1H, m, *sn*-2), 4.39 (1H, dd, *J*=11.6, 6.8 Hz, *sn*-1a), 4.25 (1H, d, *J*=11.6, 3.6 Hz, *sn*-1b), 4.22 (1H, d, *J*=7.6 Hz, H-1' of β-D-galactopyranosyl), 3.99 (1H, dd, *J*=11.2, 6.0 Hz, *sn*-3a), 3.68 (1H, dd, *J*=11.2, 7.6 Hz, *sn*-3b), 3.61-3.68 (6H, β-D-galactopyranosyl moieties), 2.80 (8H, dd, *J*=6.4, 6.4 Hz, allyl-methylene of oleoyl), 2.32 (4H, t, *J*=6.4 Hz, H-2 of oleoyl), 2.06 (8H, qd, *J*=7.6, 6.4 Hz, H-8,17 of oleoyl), 1.62 (8H, m, methylene), 1.31 (12H, m, methylene), 0.98 (3H, t, *J*=7.6 Hz, methyl), 0.94 (3H, t, *J*=7.6 Hz, methyl); ¹³C-NMR (100 MHz, CDCl₃, δ_C) See Table 3.

The *n*-BuOH fraction (90 g, CCB) was applied to the SiO₂ CC (800 g, CHCl₃-MeOH=10:1→9:1→8:1→7:1 →6:1, 3 L of each) to produce 12 fractions. Fraction CCB3 (2.0 g, Ve/Vt 0.15-0.22) was subjected to the SiO₂ CC (100 g, CHCl₃-MeOH=8:1, 3.6 L) and afforded 5 fractions. Fraciton CCB3-2 (564 mg, Ve/Vt 0.15-0.38) was applied to the SiO₂ CC (80 g, CHCl₃-MeOH-H₂O=16:3:1, 2 L of the lower layer) to yield compound **3** [CCB3-2-7, 70 mg, Ve/Vt 0.74-0.88, TLC (SiO₂ F₂₅₄) R_f 0.25, CHCl₃-MeOH-H₂O=12:3:1]. CCB6 (9.1 g, Ve/Vt 0.61-0.68) was

taken to the SiO₂ CC (200 g, CHCl₃-MeOH-H₂O=7:3:1, 4 L of the lower layer) resulting in 8 fractions. Fraction CCB6-3 (4.7 g, Ve/Vt 0.61-0.68) was chromatographed on a Sephadex LH-20 (100 g) column with 60% MeOH (2 L) as eluents to yield 5 fractions. Fraction CCB6-3-3 (3.8 g, Ve/Vt 0.25-0.78) was subjected to the SiO₂ (250 g) CC with CHCl₃-MeOH-H₂O (7:3:1, 2 L of the lower layer) as eluting solution to give compound **4** [CCB6-3-3-4, 2.0 g, Ve/Vt 0.34-0.92, TLC (SiO₂ F₂₅₄) R_f 0.42, CHCl₃-MeOH-H₂O=7:3:1] (Fig. 1).

Compound **3**, Yellow oil; IR_ν (KBr, 1/cm): 3,652, 2,936, 1,740, 1,330, 1,210, 1,132; positive FABMS *m/z*: 823 [M2+H₂O+Na]⁺, 801 [M2+H₂O]⁺, 722 [M1+H]⁺, 662 [M1-N(CH₃)₄]⁺, 568 [M1-phosphatidylcholine]⁺, 410 [M1-palmitic acid-ethylcholine+H₂O]⁺, 330 [M1-palmitic acid-phosphatidylcholine+H₂O]⁺: [M1: (2*S*)-1-*O*-palmitoyl-2-*O*-palmitoyl-3-*O*-phosphorouscholine-*sn*-glycerol, M2: (2*S*)-1-*O*-linoleoyl-2-*O*-linoleoyl-3-*O*-phosphorouscholine-*sn*-glycerol]; ¹H-NMR (400 MHz, CD₃OD, δ_H) 5.32 (4H, m, olefin of linoleoyl), 5.15 (1H, m, *sn*-2), 4.37 (1H, br. d, *J*=12.0 Hz, *sn*-1a), 4.15 (1H, dd, *J*=12.0, 6.4 Hz, *sn*-1b),
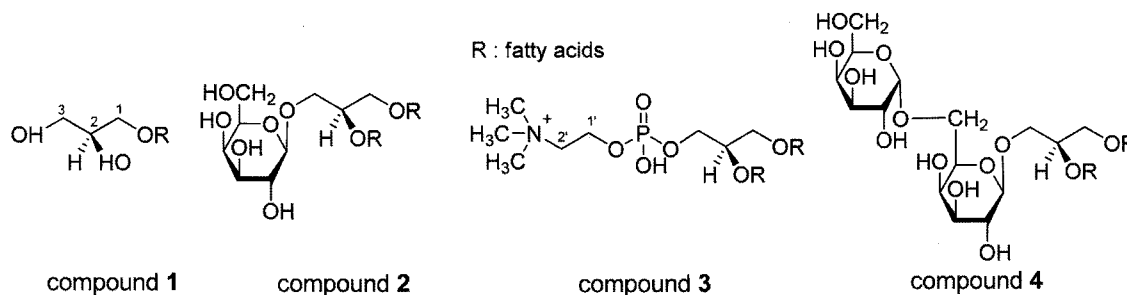
Fig. 2. Chemical structures of compounds 1-4 from the aerial parts of garland.

3.96 (2H, br. s, *sn*-3), 3.68 (9H, s, NCH$_3$ of choline), 3.54 (2H, t, *J*=7.2 Hz, H-2' of choline), 3.25 (2H, t, *J*=7.2 Hz, H-1' of choline), 2.78 (t, *J*=5.8 Hz, allyl-metylene), 2.62 (t, *J*=5.8 Hz, allyl-metylene), 2.29 (m, allyl-methylene), 2.06 (m, allyl-methylene), 1.58 (m, allyl-emthylene), 1.25-1.39 (methylene), 0.97 (3H, t, *J*=7.6 Hz, methyl), 0.88 (3H, t, *J*=7.6 Hz, methyl); ^{13}C-NMR (100 MHz, CD$_3$OD, δ_C) See Table 3.

Compound 4, Yellow oil; IR$_\nu$ (KBr, 1/cm): 3,655, 3,320, 2,924, 1,744, 1,238, 1,018; positive FABMS *m/z*: 937 [M1+H]$^+$, 915 [M2+H]$^+$, 893 [M3+H]$^+$: [M1: (2*S*)-1-*O*-linolenoyl-2-*O*-linolenoyl-3-*O*-[α-D-galactopyrasyl-(16)-β-D-galactopyranosyl]-*sn*-glycerol, M2: (2*S*)-1-*O*-linolenoyl-2-*O*-palmitoyl-3-*O*-[α-D-galactopyrasyl-(16)-β-D-galacto-pyranosyl]-*sn*-glycerol, M3: (2*S*)-1-*O*-palmitoyl-2-*O*-palmitoyl-3-*O*-[α-D-galactopyrasyl-(16)-β-D-galactopyranosyl]-*sn*-glycerol]; ^{1}H-NMR (400 MHz, CD$_3$OD, δ_H) 5.25 (4H, m, olefin of linolenoyl), 5.21 (1H, m, *sn*-2), 4.78 (1H, d, *J*=2.0 Hz, H-1' of α-D-galactopyranosyl), 4.34 (1H, dd, *J*=12.0, 2.8 Hz, *sn*-1a), 4.20 (1H, dd, *J*=12.4, 3.2 Hz, H-6'a of α-D-galactopyranosyl), 4.17 (1H, d, *J*=7.6 Hz, H-1" of -β-D-galactopyranosyl), 4.16 (1H, dd, *J*=12.4, 3.2 Hz, H-6'b of α-D-galactopyranosyl), 4.13 (1H, dd, *J*=12.0, 6.8 Hz, *sn*-1b), 3.41-3.86 (14H, galactopyranosyl and *sn*-3), 2.71 (t, *J*=5.8 Hz, allyl-metylene), 2.22 (m, allyl-methylene), 1.97 (m, allyl-methylene), 1.51 (m, allyl-emthylene), 1.22 (methylene), 0.87 (3H, t, *J*=7.6 Hz, methyl), 0.80 (3H, t, *J*=7.6 Hz, methyl); ^{13}C-NMR (100 MHz, CD$_3$OD, δ_C) See Table 3.

Acidic hydrolysis of glycosyldiglyceride Solutions of glycerides, compounds 2 and 4 (4.0 mg of each) in 3 mL 5% HCl (H$_2$O-MeOH=1:1), were refluxed for 1 hr, at which time TLC (CHCl$_3$-MeOH=5:1) indicated completion of the reaction. The reaction mixture was neutralized with Ag$_2$CO$_3$ and filtrated. The produced sugars were identified by directly comparing R$_f$ values on the silica gel TLC with those of authentic sugars. [Developing solvents: CHCl$_3$-MeOH-H$_2$O=6:4:1, R$_f$ value of methylgalactoside (0.53)]

Alkaline hydrolysis of glyceride and methylation Solutions of glyceride, compounds 1-4 (3.0 mg of each) in 4 mL 10% KOH/MeOH, were refluxed for 3 hr, at which time TLC (*n*-hexane-EtOAc=1:1) indicated the disappearance of the starting material. The reaction mixtures were neutralized with acidic cation-exchange resin (Dowex 50×W, H$^+$ form) and filtered. After each filtrate was evaporated *in vacuo* and extracted with EtOAc and H$_2$O,

then each EtOAc fr was evaporated *in vacuo*. Each concentrate in 3 mL MeOH was used in methyl-esterification with CH$_2$N$_2$/Et$_2$O for 1 day. Following evaporation of the methyl-esterified products, the concentrated products were dissolved in 3 mL of CHCl$_3$ and applied in gas chromatography analysis.

GC analysis A Shimadzu gas chromatograph (Model GC-14B; Kyoto, Japan) equipped with an on-column injection system and flame ionization detector (FID) was used. The column was fused silica DB-5 (30 m×0.32 mm i.d., film thickness 0.25-μm; J&W, Folsom, CA, USA). The operating conditions consisted of the following: carrier gas flow (N$_2$), 1.3 mL/min; H$_2$, 60 kPa; air, 50 kPa; make-up gas (N$_2$), 29 mL/min; injector, 300°C; detector, 300°C. The oven temperature was held at 100°C for 5 min and then raised to 250°C at 10°C/min and stabilized for 10 min. Three μL of each sample was injected directly into the inject port.

GC-MS A sector mass spectrometer was interfaced to a Hewlett-Packard 6890 gas chromatograph equipped with an on-column injector (J&W Scientific). A DB-5 capillary column (30 m×0.32 mm i.d., film thickness 0.25-μm) was used. The GC conditions were the same as previously described, with the exception of the linear velocity of carrier gas, which was 40 cm/sec. The mass spectra were taken over a range of *m/z* 30-1,000, utilizing an ionizing voltage of 70 eV. Fatty acid methyl esters were identified by comparing *m/z* values on the mass spectra and retention times on the chromatogram of authentic fatty acid methyl esters with those in the library of Wiley/NBS.

Acyl-CoA: cholesterol acyltransferase activity assay ACAT activity was measured according to methods indicated in Brecher and Chan (19), with slight modifications (20).

Diacylglycerol acyltransferase activity assay DGAT activity was measured according to methods indicated in Farese *et al.* (21), with some modifications (22).

Farnesyl protein transferase activity assay FPTase assays were measured according to the methods indicated in Kwon *et al.* (13).

β-Secretase activity assay β-Secretase assays were measured according to the methods detailed by Heo *et al.* (23).

Results and Discussion

To investigate the biologically active materials of garland, aerial parts of the plant were extracted with MeOH. Extracts were sequentially partitioned with EtOAc, *n*-BuOH, and H₂O. Repeated SiO₂ and ODS CC of the EtOAc and *n*-BuOH fractions led to the isolation of 4 glycerides.

Identification of glycerides 1-4 Compound **1** showed a dark brown color on the silica gel TLC by spraying with 10% *aq.* H₂SO₄ and heating, which indicated compound **1** might be a glyceride. An IR spectrum demonstrated absorption characteristics of hydroxyl (3,650/cm) and ester (1,736/cm) groups (Fig. 1). The ¹H- and ¹³C-NMR data supposed that compound **1** was a mixture of several glycerides, containing different fatty acids, but the procedure for structure determination worked primarily for major component's signals. The ¹H-NMR spectrum showed signals such as an olefine methine proton (δ_H 5.36, m), oxygenated methylene protons [δ_H 4.14 (*sn*-1), δ_H 3.68 (*sn*-3a) and δ_H 3.58 (*sn*-3b)], an oxygenated methine proton [δ_H 3.91 (*sn*-2)], methylene protons (δ_H 1.26-2.82), and methyl protons [δ_H 0.88 and δ_H 0.95], indicating that compound **1** was a glyceride composed of glycerol and fatty acids. The ¹³C-NMR spectrum yielded carbonyl carbon signals (δ_C 174.2 and δ_C 174.1), 4 olefine methine carbon signals (δ_C 130.0, δ_C 129.8, δ_C 127.9, and δ_C 127.7), 1 oxygenated methine carbon signal (δ_C 70.1), and 2 oxygenated methylene carbon signals (δ_C 64.95 and δ_C 63.28), of which the latter 3 were due to a glycerol. Several allyl-methylene and carbonated-methylene carbon signals at δ_C 20.53-34.38 and terminal methyl signals at δ_C 14.3 and δ_C 14.1 were observed, indicating the presence of fatty acids. Additionally, locations of functional groups were verified based on the gHMBC spectrum. The oxygenated methylene proton signal at δ_H 4.14 (*sn*-H-1) demonstrated a correlation with a carbonyl carbon signal at δ_C 174.1. As a result, compound **1** was identified as a mixture of monoglycerides, or a glycerol linked with fatty acids through ester bonds. The type and composition formula of the fatty acids included in the monoglyceride were determined using GC and GC-MS analyses for the methylated fatty acids, which were obtained through alkaline hydrolysis of compound **1** and esterification. Qualitative analysis and composition formula determination of the fatty acids was conducted by comparing retention time and the peak area of each peak with authentic chemicals in the GC experiment. This information was further confirmed by comparing the molecular ion peaks

(M⁺) and fragmentation ion peaks with those of the Wiley Library in the GC/MS experiment. Compound **1** was identified as a monoglyceride [(2*S*)-1-*O*-palmitoyl-*sn*-glycerol] containing palmitic acid (56.57%), linoleic acid (18.45%), and stearic acid (15.25%) (Table 1).

Compound **2** showed a dark brown color on the silica gel TLC. The IR spectrum demonstrated the absorption characteristics of hydroxyl (3,665, 3,380 1/cm) and ester (1,742/cm) groups. The ¹H- and ¹³C-NMR spectra revealed compound **2** to be similar to compound **1**, with the exception of β-D-galactopyranosyl moieties, including proton signals [δ_H 4.22 (H-1'), δ_H 3.61-3.68 (β-D-galactopyranosyl moieties)] and carbon signals [δ_C 103.7 (C-1'), δ_C 75.8 (C-5'), δ_C 73.3 (C-3'), δ_C 73.0 (C-2'), δ_C 71.2 (C-4'), and δ_C 63.5 (C-6')]. The chemical shift and coupling constant of H-1' (δ_H 4.22, d, *J*=7.6 Hz) as well as the chemical shift of C-1' (δ_C 103.7) indicated that the anomeric carbon had a β-configuration. In the gHMBC spectrum, an oxygenated methine proton signal at δ_H 5.28 (*sn*-2) demonstrated a cross-peak with a carbonyl carbon signal at δ_C 173.6, and other oxygenated methylene proton signals at δ_H 4.39 (*sn*-1a) and δ_H 4.25 (*sn*-1b) had cross-peaks with a carbonyl carbon signal at δ_C 173.1 via *J*₃ correlation. The anomeric proton signal of β-D-galactopyranosyl at δ_H 4.22 (H-1') demonstrated a cross-peak with an oxygenated methylene carbon signal of glycerol at δ_C 68.8 (*sn*-3). Therefore, compound **2** was identified as a mixture of monoglycosyldiglycerides, or a glycerol linked with fatty acids via ester bonds and a β-D-galactopyranose linked via an ether bond. Fatty acids of the glycosylglycerides were analyzed using the same procedure as that for compound **1**. Compound **2** was identified as monogalactosyldiglyceride [(2*S*)-1-*O*-oleoyl-2-*O*-oleoyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol] containing myristic acid (8.41%), palmitic acid (19.17%), and oleic acid (72.42%) (Table 1).

Compound **3** showed a dark brown color on the silica gel TLC. The IR spectrum demonstrated absorption characteristics of hydroxyl (3,660, 3,368 1/cm), ester (1,738/cm), and amine (1,330/cm) groups. The ¹H- and ¹³C-NMR spectra of compound **3** were similar to those of compound **2**, with the exception of nitrogenated and oxygenated ethyl moieties, including proton signals at δ_H 3.25 (H-1', OCH₂) and δ_H 3.54 (H-2', NCH₂), carbon signals at δ_C 56.8 (C-1') and δ_C 52.5 (C-2'), as well as *N*-methyls proton signals at δ_H 3.68 (NCH₃×3) and carbon signals at δ_C 51.8 (NCH₃×3). In the gHMBC spectrum, the nitrogenated methylene proton signal at δ_H 3.54 demonstrated a cross-peak with the *N*-methyl carbon signal at δ_C 51.8 via *J*₃ correlation. gCOSY revealed a vicinal

Table 1. Fatty acids in compound 1-4 from aerial parts of garland[1] (%)

No.	Myristic acid 14:0	Palmitic acid 16:0	Stearic acid 18:0	Oleic acid 18:1	Linoleic acid 18:2	Linolenic acid 18:3
1	-	56.57	24.98	-	18.45	-
2	8.41	19.17	-	72.42	-	-
3	-	74.07	19.23	-	6.70	-
4	-	38.88	5.14	-	7.94	48.04

[1]Retention times for each fatty acid: myristic acid methyl ester, 5.47 min; palmitic acid methyl ester, 6.55 min; linoleic acid methyl ester, 8.05 min; linolenic acid methyl ester, 8.18 min; oleic acid methyl ester, 8.32 min; stearic acid methyl ester, 8.55 min.

Table 2. Inhibitory effect of compounds 1-4 from aerial parts of garland on ACAT, DGAT, FPTase, and β-secretase[1] (%)

Compounds[2]	ACAT	DGAT	FPTase	β-Secretase[3]
1	45.6±0.2	59.1±0.1	12.3±0.2	NS
2	26.7±0.3	23.4±0.1	98.0±0.1	NS
3	26.0±0.1	13.5±0.2	55.2±0.1	NS
4	3.3±0.2	1.3±0.1	4.3±0.2	2.6
Positive control[4]	45.1±0.1	50.0±0.2	50.0±0.1	2.0

[1] The data are presented as the mean±SD of the triplicates.
[2] The treatment concentrations of compounds 1-4 were 100, 25, and 100 μg/mL for ACAT, DGAT, and FPTase activity assays, respectively.
[3] This indicates the IC_{50} value, which is the 50% inhibition concentration (μg/mL) that was calculated from regression lines using 5 different concentrations with triplicate determinations; NS, not significant.
[4] Positive controls included oleic acid anilide (0.11 μg/mL), xanthohumol (17.7 μg/mL), 2-hydroxycinnamaldehyde (17.9 μg/mL), and H-4848 for ACAT, DGAT, FPTase, and β-secretase activity assays, respectively.

coupling between δ_H 3.25 and δ_H 3.54 with a coupling constant of J=7.2 Hz. Therefore, compound **3** was identified as a glycerophospholipid, or a glycerol linked with 1 phosphorouscholine and 2 fatty acids. FABMS analysis was conducted to confirm the existence of phosphorus groups. The major ion peaks of the compound, which has 2 palmitic acids and phosphorouscholine, were observed at m/z 722 $[M1+H]^+$, m/z 662 $[M1-N(CH_3)_3+H]^+$, m/z 568 [M1-phosphorouscholine]$^+$, m/z 410 [M1-palmitic acid-choline+H₂O]$^+$, and m/z 330 [M1-palmitic acid-phosphorouscholine+H₂O]$^+$. Therefore, compound **3** was identified as phosphatidylcholine [(2S)-1-O-palmitoyl-2-O-palmitoyl-3-O-phosphorouscholine-sn-glycerol] containing palmitic acid (74.07%), stearic acid (19.23%), and linoleic acid (6.70%) (Table 1).

Compound **4** showed a dark brown color on silica gel TLC. The IR spectrum demonstrated absorption characteristics of hydroxyl (3,655, 3,320 1/cm) and ester (1,744/cm) groups. The ^{1}H- and ^{13}C-NMR spectra revealed that compound **4** was similar to compound **2**, with the exception of α-D-galactopyranosyl moieties, including proton signals [δ_H 4.78 (H-1"), δ_H 3.61-3.68] and carbon signals [δ_C 100.4 (C-1"), δ_C 74.3 (C-5"), δ_C 72.4 (C-3"), δ_C 70.9 (C-4"), δ_C 69.6 (C-2"), δ_C 62.7 (C-6")]. The configuration of the galactopyranose anomer carbon was confirmed with the coupling constant of the anomer proton signal (J=2.0 Hz). In the gHMBC spectrum, the oxygenated methine proton signal at δ_H 5.21 (sn-2) demonstrated a cross-peak with a carbonyl carbon signal at δ_C 174.6, and the oxygenated methylene proton signals at δ_H 4.34 (sn-1a) and δ_H 4.17 (sn-1b) demonstrated cross-peaks with the carbonyl carbon signal at δ_C 174.3 via J_3 correlation. The anomer proton signal of -β-D-galactopyranose at δ_H 4.17 (H-1') demonstrated a cross-peak with the oxygenated methylene carbon signal of glycerol at δ_C 68.7 (sn-3), and the anomer proton signal of α-D-galactopyranose at δ_H 4.78 (H-1") demonstrated a cross-peak with the oxygenated methylene carbon signal of -β-D-galactopyranose at δ_C 67.6 (H-6'). As a result, compound **4** was identified as a mixture of diglycosyl-diglycerides, or a glycerol linked with fatty acids through ester bonds and α-D-galactopyrasyl-(16)-β-D-galactopyranose linked through an ether bond. Fatty acids of the glycosylglyceride were analyzed using the same procedure as that of compound **1**. Compound **4** was identified as a digalactosyldiglycride [(2S)-1-O-linolenoyl-2-O-palmitoyl-

3-O-[α-D-galactopyrasyl-(16)-β-D-galactopyranosyl]-sn-glycerol] containing palmitic acid (38.88%), stearic acid (5.14%), linoleic acid (7.94%), and linolenic acid (48.04%) (Table 1).

Glycerides 1-4's inhibitory effects on ACAT, DGAT, FPTase, and β-secretase The 4 glycerides isolated from *C. cororarium* were evaluated for inhibitory effects on ACAT, DGAT, FPTase, and β-secretase activities (Table 2). Compound **1** demonstrated an inhibitory effect on ACAT of 45.6±0.2% at 100 μg/mL, and on DGAT of 59.1±0.1% at 25 μg/mL. The positive control, oleic acid anilide, inhibited ACAT activity by 45.1±0.1% at 0.11 μg/mL, and xanthohumol inhibited DGAT activity by 50.0±0.2% at 17.7 μg/mL. In a previous report, a polyacetylene from garland showed 64.4% inhibitory activity on ACAT at 100 μg/mL (25). Although compound **1** demonstrated a weak inhibitory effect on ACAT compared to oleic acid anilide, and nearly the same inhibitory effect as xanthohumol on DGAT, the lack of reports on naturally occurring ACAT and DGAT inhibitors gives compound **1** some significance as a natural inhibitor. Such a finding may lead to further study on the development of safe hypercholesterolemic and anti-atherogenic materials due to the considerable consumption of garland in Korea.

Compound **2** and **3** inhibited FPTase activity by 98±0.1 and 55.2±0.1% at 100 μg/mL, respectively, which was obviously inhibitory effect when compared with a well-known FPTase inhibitor, 2-hydoxycinnamaldehyde (IC_{50}= 17.9 μg/mL) (12). Two sterols and 2 polyacetylenes from garland showed the inhibitory activity on FPTase by 53.2, 96.6, 92.5, and 76.0%, respectively, at 50 μg/mL (25). As a result, compound **2** may be a viable novel compound for protection against cancer.

The IC_{50} value of compound **4** for inhibition of β-secretase activity was 2.6 μg/mL, while that of the positive control, H-4848 (Bachem, Switzerland), was 2.0 μg/mL. This result indicates that compound **4** has the potential to be a used in the prevention and treatment of dementia (24). In conclusion, 4 glycerides were isolated from garland, and these had inhibitory effects on ACAT, DGAT, FPTase, or β-secretase. This suggests that garland could be useful as a preventive and/or therapeutic material in the treatment of carcinogenesis, hypercholesterolemic atherosclerosis, and Alzheimer's disease.

Table 3. ¹³C-NMR chemical shifts (100 MHz, δ_C) of compounds 1-4 from aerial parts of garland¹⁾

	1	2	3	4
Fatty acids²⁾	174.2	173.6	173.4	174.6
	174.1	173.1	173.0	174.3
	130.0	131.9	172.8	132.6
	129.8	130.1	130.0	130.9
	127.9	128.2	129.8	129.1
	127.7	128.1	127.9	129.0
	34.1	127.7	127.7	128.7
	31.4	127.0	34.0	128.1
	29.7	34.9	32.6	30.9
	29.6	34.5	32.4	30.8
	29.5	34.4	31.8	30.8
	29.3	29.7	31.4	30.5
	29.1	29.6	29.7	30.4
	29.0	29.3	29.5	30.3
	28.9	29.2	29.3	30.3
	28.8	28.9	29.2	30.2
	27.2	27.3	29.1	30.1
	25.6	25.7	28.8	28.2
	25.5	25.6	27.2	26.6
	24.8	25.1	25.6	26.5
	22.6	24.9	24.8	26.0
	20.5	24.8	22.7	23.8
	14.3	20.6	22.6	21.5
	14.1	14.4	14.1	14.8
		13.9	14.0	14.3
Glycerol	70.1 (*sn*-C-2)	70.1 (*sn*-C-2)	70.3 (*sn*-C-2)	71.6 (*sn*-C-2)
	65,0 (*sn*-C-1)	68.8 (*sn*-C-3)	63.7 (*sn*-C-3)	68.7 (*sn*-C-3)
	63.3 (*sn*-C-3)	62.5 (*sn*-C-1)	62.6 (*sn*-C-1)	64.0 (*sn*-C-1)
O-β-D-Galactopyranose		103.7 (C-1')		105.1 (C-1')
		75.8 (C-5')		74.4 (C-5')
		73.3 (C-3')		72.2 (C-3')
		73.0 (C-2')		71.3 (C-2')
		71.2 (C-4')		70.0 (C-4')
		63.5 (C-6')		67.6 (C-6')
O-α-D-Galactopyranose				100.4 (C-1")
				74.3 (C-5")
				72.4 (C-3")
				70.9 (C-4")
				69.6 (C-2")
				62.7 (C-6")
Choline		56.8 (C-1')		
		52.5 (C-2')		
		51.8 (NCH₃)		

¹⁾The compounds 1 and 2 were dissolved in CDCl₃, and compounds 3 and 4 in CD₃OD.
²⁾All chemical shifts of fatty acids observed in the ¹³C-NMR spectra were described.

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