Natural Product Sciences
24(2) : 132-138 (2018)
https://doi.org/10.20307/nps.2018.24.2.132

Phytochemical Constituents of *Capsella bursa-pastoris* and Their Anti-inflammatory Activity

Joon Min Cha[1], Dong Hyun Kim[1], Tae Hyun Lee[1], Lalita Subedi[2], Sun Yeou Kim[2], and Kang Ro Lee[1,*]

[1]Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, 2066 Seobu-Ro, Jangan-gu, Suwon, Gyeonggi-do 440-746, Republic of Korea
[2]Gachon Institute of Pharmaceutical Science, Gachon University, 191 Hambakmoero, Yeonsu-gu, Incheon 21936, Republic of Korea

Abstract – Phytochemical investigation of 80% MeOH extract of the aerial parts of *Capsella bursa-pastoris* yielded fourteen compounds (**1 - 14**). The structures of the compounds were elucidated by spectroscopic methods to be methyl-1-thio-*β*-D-glucopyranosyl disulfide (**1**), 10-methylsulphinyl-decanenitrile (**2**), 11-methyl-sulphinyl-undecanenitrile (**3**), 1-*O*-(lauroyl)glycerol (**4**), phytene-1, 2-diol (**5**), (3*S*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmen-9-one (**6**), loliolide (**7**), *β*-sitosterol (**8**), 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (**9**), 1-feruloyl-*β*-D-glucopyranoside (**10**), pinoresinol-4'-*O*-*β*-D-glucopyranoside (**11**), luteolin (**12**), quercetin-3-*O*-*β*-D-glucopyranoside (**13**), and luteolin 6-*C*-*β*-glucopyranoside (**14**). Although compound **1** was reported as synthetic compound, **1** was first isolated from natural source. NMR spectral data assignments of **1**, **2** and **3** were reported for the first time, and compounds **1 - 14** were for the first time reported from this plant source. The anti-inflammatory effects of **1 - 14** were evaluated in lipopolysaccharide (LPS)-stimulated murine microglia BV-2 cells. Compounds **12** exhibited strong inhibitory effects on nitric oxide production in LPS-activated BV-2 cells with IC_{50} values of 9.70 μM.
Keywords – *Capsella bursa-pastoris*, Cruciferae, Sulfur compound

Introduction

Capsella bursa-pastoris (L.) Medik (Cruciferae), commonly known as Shepherd's purse, is widely distributed throughout the world.[1] The root of this plant was edible and has been used in Korean folks medicine for the treatment of edema and hypertension.[2] Previous phytochemical investigation on this plant reported the isolation of flavonoids, terpenoids and phenolic compounds,[2-4] with their biological studies, such as anti-microbial, anti-bacterial, anti-tumor, and liver catalase activities.[5-7] As parts of our search for biologically active compounds from Korean natural plant sources, we investigated the constituents of the aerial parts of *C. bursa-pastoris* and reported the isolation of phenolic glycosides and their anti-inflammatory effects.[8] In our continuing study on this plant source, we further isolated fourteen compounds, including three sulfur compounds (**1 - 3**). The structures of these compounds were elucidated by physicochemical and spectroscopic methods. The isolated compounds (**1 - 14**) were evaluated for their potential inhibitory effects on NO production in LPS-activated microglia BV-2 cell line.

Experimental

General experimental procedures – Melting point was determined on Gallenkamp melting point apparatus. Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. HR-FAB-MS spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra were recorded on a Bruker AVANCE III 700 NMR spectrometer at 700 MHz (^{1}H) and 175 MHz (^{13}C). Preparative high performance liquid chromatography (HPLC) was conducted using a Gilson 306 pump with Shodex refractive index detector and Phenomenex-Luna-10u-silica-(2) column (250 × 10.00 mm) or YMC J'sphere ODS-M80 column (250 × 10.00 mm). Low-pressure liquid chromatography (LPLC) was carried out on a Merck LiChroprep Lobar®-A RP-C_{18} and Si 60 column (240 × 10

*Author for correspondence
Kang Ro Lee, Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, 2066Seobu-Ro, Jangan-ku, Suwon, Gyeonggi-do 440-746, Republic of Korea.
Tel: +82-31-290-7710; E-mail: krlee@skku.edu

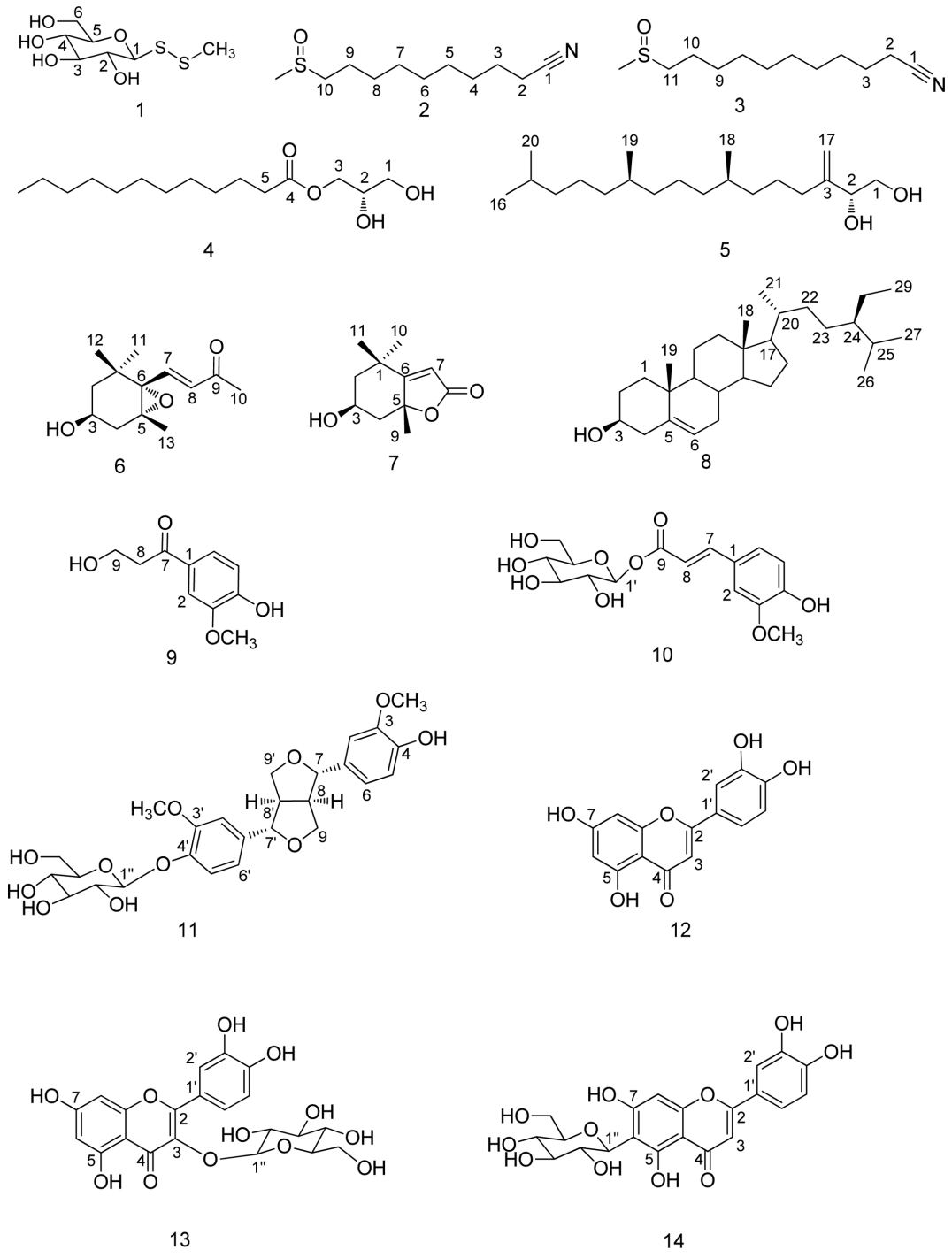

Fig. 1. The structures of **1 - 14** isolated from *C. bursa-pastoris*.

mm) with an FMI QSY-0 pump (ISCO). Silica gel 60 (Merck, 70 – 230 and 230 - 400 mesh) and RP-C18 silica gel (Merck, 230 - 400 mesh) were used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia Co.). TLC was performed using Merck precoated Silica gel F254 plates and RP-18 F254s plates. Spots were detected by thin layer chromatography (TLC) under UV light or by heating after spraying with anisaldehyde–sulfuric acid.

Plant materials – Whole plants of *C. bursa-pastoris* (2.5 kg) were purchased from Anmyeon-Island, Chungcheongnam-do, Korea in March 2015. A voucher specimen (SKKU-NPL 1410) has been deposited in the herbarium

of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation – The whole plants of *C. bursa-pastoris* (2.5 kg) were extracted three times with 80% aqueous MeOH. The filtrate was evaporated under reduced pressure to give a MeOH extract (280 g), which was suspended in distilled water (800 mL) and solvent-partitioned to give*n*-hexane (33.0 g), $CHCl_3$ (5.0 g), EtOAc (4.0 g), and *n*-BuOH (26.0 g). The $CHCl_3$ layer (4.0 g) was chromatographed on a silica gel column ($CHCl_3$-MeOH = 40:1 → 1:1) to yielded six fractions (C1–C6). Fraction C1 (1.1 g) was separated over a silica gel column ($CHCl_3$-MeOH = 40:1 → 1:1) to give eight subfractions (C11–C18). Fraction C13 (248 mg) was subjected to Sephadex LH-20 column chromatography eluted with 100% MeOH as to give seven subfractions (C131–C137). Subfraction C137 (20 mg) was purified with a RP-C_{18} prep HPLC (100% MeOH) to yield **5** (20 mg). Fraction C2 (671 mg) was separated on a silica gel column ($CHCl_3$-MeOH = 100:1 → 1:1) to give eight subfractions (C21–C28). Subfraction C26 (207 mg) was chromatographed over RP-C_{18} silica Lobar®-A (80% MeOH) and a RP-C_{18} prep HPLC (65% MeOH) to yield **2** (48 mg), **3** (20 mg) and **4** (5 mg). Subfraction C27 (116 mg) was chromatographed over RP-C_{18} silica Lobar®-A (80% MeOH) and a RP-C_{18} prep HPLC (40% MeOH) to yield **6** (5 mg), **7** (5 mg) and **9** (5 mg). Subfraction C28 (20 mg) was purified with a RP-C_{18} prep HPLC (100% MeOH) to yield **5** (5 mg). The EtOAc layer (4.0 g) was separated on a RP-C_{18} silica gel column with 40~100% MeOH to yielded sixteen fractions (E1–E16). Fraction E3 (120 mg) was chromatographed over silica Lobar®- A ($CHCl_3$-MeOH-H_2O = 4:1:0.1) and a RP-C_{18} prep HPLC (10% MeCN) to yield **1** (15 mg) and **10** (17 mg). Fraction E5 (150 mg) was chromatographed over a Sephadex LH-20 column (85% MeOH) to yield five subfractions (E51–E55). Subfraction E55 (31 mg) was purified with a RP-C_{18} prep HPLC (20% MeCN) to yield **14** (9 mg). Fraction E8 (116 mg) was chromatographed over silica Lobar®-A (EtOAc-MeOH-H_2O = 5:1:0.1) and a RP-C_{18} prep HPLC (25% MeCN) to yield **11** (10 mg). Fraction E11 (150 mg) was chromatographed over a Sephadex LH-20 column (100% MeOH) and a RP-C_{18} prep HPLC (10% MeCN) to yield **13** (38 mg). Fraction E13 (208 mg) was chromatographed over silica Lobar®-A ($CHCl_3$-MeOH-H_2O = 7:1:0.1) and a RP-C_{18} prep HPLC (30% MeCN) to yield **12** (11 mg).

Methyl-1-thio-β-D-glucopyranosyl disulfide (1) – Yellowish gum. $[\alpha]_D^{19}$: -78.0 (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3412, 2875, 1642, 1031 cm^{-1}; ^1H, ^{13}C NMR see Table 1; HR-FAB-MS (positive-ion mode) *m/z*: 265.0178 $[M+Na]^+$ (calcd for $C_7H_{14}O_5S_2Na$, 265.0180).

Table 1. ^1H and ^{13}C NMR data of **1** in CD_3OD (δ in ppm, 700 MHz for ^1H and 175 MHz for ^{13}C)

Position	**1**	
	δ_H (*J* in Hz)	δ_C
1	4.37 d (9.4)	92.3
2	3.50m	72.8
3	3.40t (8.8)	79.6
4	3.30 m	71.6
5	3.30 m	82.61
6	3.64 dd (12.0, 5.5)	63.2
	3.86 dd (12.0, 1.8)	63.2
S-CH_3	2.50 m	24.9

[a]^1H and ^{13}C NMR data were recorded at 700 and 175 MHz, respectively.
Coupling constants (in Hz) are given in parentheses.

10-Methylsulphinyl-decanenitrile (2) – Colorless gum. $[\alpha]_D^{19}$: -8.3 (*c* 0.01, $CHCl_3$); IR (KBr) ν_{max}: 3420, 3010, 2204, 1665, 1483, 1035, 984, 671 cm^{-1}; ^1H, ^{13}C NMR see Table 2; HR-FAB-MS (positive-ion mode) *m/z*: 216.1424 $[M+H]^+$ (calcd for $C_{11}H_{22}NOS$, 216.1422).

11-Methylsulphinyl-undecanenitrile (3) – Colorless gum. $[\alpha]_D^{24}$: -10.1 (*c* 0.01, $CHCl_3$); IR (KBr) ν_{max}: 3213, 3102, 2306, 1664, 1427, 1028, 991, 598 cm^{-1}; ^1H, ^{13}CNMR see Table 2; HR-FAB-MS (positive-ion mode) *m/z*: 230.1580 $[M+H]^+$ (calcd for $C_{12}H_{24}NOS$, 230.1579).

1-*O*-(Lauroyl)glycerol (4) – Colorless gum. ^1H NMR ($CDCl_3$, 700 MHz): δ 4.14 (1H, dd, *J* = 11.3, 3.9 Hz, H-3a), 4.05 (1H, dd, *J* = 10.2, 5.7 Hz, H-3b), 3.81 (1H, m, H-1b), 3.66 (1H, m, H-2), 3.56 (1H, dd, *J* = 11.1, 5.8 Hz, H-1a), 2.30 (1H, t, *J* = 7.3Hz, H-5), 1.62 (2H, m, H-6), 1.31 (18H, brs, H-7 to 14), 0.88 (3H, t, *J* = 6.7 Hz, H-15); ^{13}C NMR ($CDCl_3$, 175 MHz): δ 173.0 (C-4), 70.1 (C-2), 64.8 (C-3), 63.3 (C-1), 33.7 (C-5), 31.5 (C-13), 28.9 (C-11), 28.9 (C-10), 28.9 (C-9), 28.8 (C-12), 28.8(C-8), 28.7 (C-7), 25.1 (C-6), 22.3 (C-14), 13.8 (C-15); FABMS *m/z* 273.1 $[M+H]^+$.

Phytene-1,2-diol (5) – Colorlessgum. ^1H NMR ($CDCl_3$, 700 MHz): δ 5.00 (1H, s, H-17a), 4.84 (1H, s, H-17b), 4.02 (1H, dd, *J* = 7.0, 3.2 Hz, H-2), 3.71 (1H, dd, *J* = 6.5, 4.0 Hz, H-1a), 3.42 (1H, dd, *J* = 6.5, 2.1 Hz, H-1b), 2.20 – 2.01 (2H, m, H-4), 1.57-1.06 (19H, m), 0.91 (3H, d, *J* = 7.0 Hz, H-16), 0.88 (6H, d, *J* = 7.0 Hz, H-18, 20), 0.85 (3H, d, *J* = 7.0 Hz, H-19); ^{13}C NMR ($CDCl_3$, 175 MHz): δ 151.0 (C-3), 109.1 (C-17), 74.3 (C-2), 65.5 (C-1), 38.2 (C-14), 37.0 (C-8), 36.9 (C-10, 12), 36.8 (C-6), 33.1 (C-4), 31.0 (C-7, 11), 28.5 (C-15), 26.4 (C-5), 23.5 (C-13), 23.1 (C-9), 22.4 (C-20), 22.5 (C-16), 19.0 (C-19),

Table 2. ^{1}H and ^{13}C NMR data of **2** and **3** in $CDCl_3$ (δ in ppm, 700 MHz for ^{1}H and 175MHz for ^{13}C)

Position	**2**		**3**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		119.8		119.9
2	2.28 t (7.0)	17.1	2.32t (7.1)	17.2
3	1.59 m	25.3	1.64m	25.3
4	1.23-1.30 m	28.9	1.25-1.29 m	29.2
5	1.38 m	28.6	1.38 m	28.8
6	1.38 m	28.7	1.38 m	28.9
7	1.23-1.30 m	28.5	1.25-1.29 m	28.7
8	1.23-1.30 m	29.0	1.25-1.29 m	28.7
9	1.70 m	22.5	1.25-1.29 m	29.3
10	2.66 m	54.7	1.74 m	22.6
	2.58 m			
11			2.73 m	54.6
			2.65 m	
CH_3SO	2.51 s	38.6	2.57 s	38.6

[a]^{1}H and ^{13}C NMR data were recorded at 700 and 175 MHz, respectively. Coupling constants (in Hz) are given in parentheses.

18.9 (C-18); FABMS *m/z* 313.3 $[M+H]^+$.

(3*S*,5*R*,6*S*,7*E*)-5,6-Epoxy-3-hydroxy-7-megastigmen-9-one (6) – Colorless gum. $[\alpha]_D^{23}$: -53.4 (*c* 0.01, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 700 MHz): δ 7.05 (1H, d, *J* = 15.6 Hz, H-7), 6.31 (1H, d, *J* = 15.6 Hz, H-8), 3.93 (1H, m, H-3), 2.41 (1H, dd, *J* = 9.2, 5.0 Hz, H-4a), 2.31 (3H, s, CH_3-10), 1.68 (1H, dd, *J* = 14.6, 9.2 Hz, H-4b), 1.60 (1H, dd, *J* = 12.2, 4.2 Hz, H-2a), 1.30 (1H, dd, *J* = 12.2, 10.2 Hz, H-2b), 1.22 (3H, s, CH_3-13), 1.20 (3H, s, CH_3-11), 1.00 (3H, s, CH_3-12); ^{13}C NMR ($CDCl_3$, 175 MHz): δ 197.6 (C-9), 142.6 (C-7), 132.8 (C-8), 69.6 (C-6), 67.4 (C-5), 64.2 (C-3), 46.8 (C-4), 40.8 (C-2), 35.3 (C-1), 29.5 (C-11), 28.5 (C-10), 25.2 (C-12), 20.0 (C-13); FABMS *m/z* 225.1 $[M+H]^+$.

Loliolide (7) – Colorless gum. $[\alpha]_D^{21}$: -32.1 (*c* 0.01, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 700 MHz): δ 5.72 (1H, s, H-7), 4.36 (1H, m, H-3), 2.49 (1H, td, *J* = 13.8 Hz, H-4α), 2.00 (1H, dd, *J*=14.4 Hz, H-2α), 1.81 (3H, s, H-9), 1.74 (1 H, d, *J* = 3.3 Hz, H-4β), 1.60 (1 H, d, *J* = 3.6 Hz, H-2β), 1.50 (3H, s, H-10), 1.30 (3H, s, H-11); ^{13}C-NMR ($CDCl_3$, 175 MHz): δ 185.6 (C-8), 171.4 (C-6), 112.5 (C-7), 87.6 (C-5), 65.2 (C-3), 48.3 (C-2), 47.0(C-4), 38.1 (C-1), 32.5 (C-9), 27.3 (C-10), 27.0 (C-11); FABMS *m/z* 197.1 $[M+H]^+$.

***β*-Sitosterol (8)** – White powder, mp. 145 ºC; ^{1}H-NMR ($CDCl_3$, 700 MHz): δ 5.35 (1H, d, *J* = 5.0 Hz, H-6), 3.53 (1H, m, H-3), 1.06 (3H, s, H-19), 0.95 (3H, d, *J* = 6.5 Hz, H-21), 0.92 (3H, d, *J* = 6.5 Hz, H-26), 0.80 (3H, t, *J* = 6.5 Hz, H-29), 0.76 (3H, s, H-18). ^{13}C-NMR ($CDCl_3$, 175 MHz): δ 141.7 (C-5), 120.9 (C-6), 73.4 (C-3), 58.0 (C-14), 56.3 (C-17), 51.2 (C-9), 47.0 (C-24), 46.1 (C-4), 43.5 (C-13), 40.3 (C-12), 38.2 (C-1), 35.1 (C-10), 36.2 (C-20), 34.0 (C-22), 32.8 (C-2), 31.5 (C-8), 29.4 (C-25), 27.9 (C-16), 26.5 (C-23), 25.8 (C-15), 23.4 (C-28), 20.6 (C-11), 20.0 (C-27), 19.3 (C-26),19.1(C-21), 19.0 (C-19), 12.0 (C-29), 11.9 (C-18); FABMS *m/z* 415.3 $[M+H]^+$.

3-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (9) – Colorless gum. ^{1}H-NMR (CD_3OD, 700 MHz): δ 7.55 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 7.53 (1H, d, *J* = 2.0 Hz, H-2), 6.95 (1H, d, *J* = 8.5Hz, H-5), 4.02 (2H, t, *J* = 5.5Hz, H-5), 3.96 (3H, s, 3-OCH_3), 3.18 (2H, t, *J* = 5.5Hz, H-5); ^{13}C-NMR (CD_3OD, 175 MHz): δ 199.3 (C-7), 152.4 (C-3), 148.4 (C-4), 131.0 (C-1), 123.8 (C-6), 115.4 (C-5), 111.9 (C-2), 58.5 (C-9), 56.5 (OCH_3), 50.8 (C-3), 41.6 (C-8); FABMS *m/z* 197.0 $[M+H]^+$.

1-Feruloyl-*β*-D-glucopyranoside (10) – Yellowish gum. ^{1}H NMR (CD_3OD, 700 MHz): δ 7.72 (1H, d, *J* = 15.9 Hz, H-8), 7.19 (1H, d, *J* = 1.9 Hz, H-2), 7.09 (1H, dd, *J* = 8.2, 1.8 Hz, H-6), 6.82 (1H, d, *J* = 8.3 Hz, H-5), 6.39 (1H, d, *J* = 15.9 Hz, H-7), 5.58 (1H, d, *J* = 7.9 Hz, H-1'), 3.86 (1H, s, 3-OCH_3), 3.83 (1H, m, H-6'), 3.70 (1H, m, H-3'), 3.43 (1H, m, H-5'), 3.31 (1H, m, H-2'), 3.23 (1H, m, H-4'); ^{13}C NMR (CD_3OD,175 MHz): δ 151.1 (C-9), 149.3 (C-4), 147.6 (C-3), 147.4 (C-7), 137.6 (C-1), 133.9(C-6), 120.2 (C-8), 119.9 (C-2), 95.9 (C-1'), 78.9 (C-3'), 78.2 (C-5'), 74.2 (C-2'), 71.9 (C-4'), 62.3 (C-6'), 56.9 (3-OCH_3); FABMS *m/z* 357.1 $[M+H]^+$.

Pinoresinol-4'-*O*-*β*-D-glucopyranoside (11) – Yellowish

gum. $[\alpha]_D^{22}$: +21.3 (*c* 0.01, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 7.14 (1H, d, *J* = 8.3 Hz, H-5), 7.02 (1H, d, *J* = 1.9 Hz, H-2), 6.94 (1H, d, *J* = 1.8 Hz, H-2'), 6.91 (1H, dd, *J* = 8.3, 1.9 Hz, H-6), 6.81 (1H, dd, *J* = 8.1, 1.9 Hz, H-6'), 6.77 (1H, d, *J* = 8.1 Hz, H-5'), 4.88 (1H, d, *J* = 7.8 Hz, H-1"), 4.76 (1H, d, *J* = 4.1 Hz, H-7), 4.70 (1H, d, *J* = 4.5 Hz, H-7'), 4.24 (2H, m, H-9a, 9'a), 3.86 (3H, s, 3-OCH_3), 3.85 (3H, s, 3'-OCH_3), 3.82 (2H, m, H-9b, 9'b), 3.70 - 3.26 (5H, m, sugar-H), 3.12 (2H, m, H-8, 8'); ^{13}C NMR (CD_3OD, 175 MHz): δ 151.1 (C-4), 149.3 (C-4'), 147.6 (C-3'), 147.4 (C-3), 137.6 (C-1), 133.9(C-1'), 120.2 (C-6'), 119.9 (C-6), 118.2 (C-5), 116.2 (C-5'), 111.8 (C-2), 111.2 (C-2'), 102.9 (C-1"), 87.6 (C-7'), 87.2 (C-7), 78.3 (C-5"), 78.0 (C- 3"), 75.1 (C-2"), 72.8 (C-9'), 72.8 (C-9), 71.4(C-4"), 62.6 (C-6"), 56.9 (3-OCH_3), 56.6 (3'-OCH_3), 55.6 (C-8'), 55.5 (C-8); FABMS *m/z* 521.1 $[M+H]^+$.

Luteolin (12) – Yellowish gum. ^{1}H NMR (CD_3OD, 700 MHz): δ 7.40 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 7.38 (1H, d, *J* = 2.0 Hz, H-2'), 6.89 (1H, d, *J* = 8.4 Hz, H-5'), 6.53(1H, s, H-3), 6.43 (1H, s, H-8), 6.19 (1H, s, H-6).; ^{13}C NMR (CD_3OD, 175 MHz): δ 184.1 (C-4), 166.3 (C-7), 166.1 (C-2), 163.2 (C-5), 159.5 (C-9), 151.7 (C-4'), 147.2 (C-3'), 123.1 (C-1'), 120.4 (C-6'), 116.9 (C-5'), 114.3 (C-2'), 105.4 (C-10), 104.5 (C-3), 100.2 (C-6), 95.1 (C-8); FABMS *m/z* 287.0 $[M+H]^+$.

Quercetin-3-*O*-*β*-D-glucopyranoside (13) – Yellowish gum. $[\alpha]_D^{19}$: -20.3 (*c* 0.01, MeOH); ^{1}H NMR (CD_3OD, 700 MHz): δ 7.72 (1H, br s, H-2'),7.59 (1H, d, *J* = 8.2 Hz, H-6'), 6.87 (1H, d, *J* = 8.2 Hz, H-5'), 6.37 (1H, s, H-6), 6.19 (1H, s, H-8), 5.24 (1H, s, *J* = 7.3 Hz, H-1"), 3.72 (1H, dd, *J* = 14.1, 1.30 Hz, H-6"), 3.58 (1H, dd, *J* = 11.5, 5.3 Hz, H-3"), 3.50 (1H, td, *J* = 10.5, 7.1 Hz, H-5"), 3.36 (1H, m, H-2"), 3.23 (1H, m, H-4"); ^{13}C NMR (CD_3OD, 175 MHz): δ 173.6 (C-4), 166.2 (C-7), 163.2 (C-5), 153.1 (C-9), 158.6 (C-2), 150 (C-4'), 146.1 (C-3'), 135.7 (C-3), 123.3 (C-6'), 123.2 (C-1'), 117.7 (C-5'), 116.5 (C-2'), 105.8 (C-10), 104.5 (C-1"), 100.1 (C-6), 94.8 (C-8), 78.5 (C-3"), 78.2 (C-5"), 75.8 (C-2"), 71.3 (C-4"), 62.7 (C-6"); FABMS *m/z* 465.0 $[M+H]^+$.

Luteolin 6-C-*β*-glucopyranoside (14) – Yellowish gum. ^{1}H NMR (CD_3OD, 700 MHz): δ 7.36 (2H, br s, H-2', 6'), 6.89 (1H, d, *J* = 8.3 Hz, H-5'), 6.54 (1H, s, H-3), 6.48 (1H, s, H-8), 4.90 (1H, d, *J* = 9.9 Hz, H-1"), 4.16 (1H, t, *J* = 9.1, 8.4 Hz, H-2"), 3.88 (1H, dd, *J* = 12.1, 1.8 Hz, H-6"a), 3.74 (1H, dd, *J* = 12.1, 5.1 Hz, H-6"b), 3.48 (1H, m, H-5"), 3.42 (1H, m, H-4"), 3.41 (1H, m, H-3"); ^{13}C NMR (CD_3OD, 175 MHz): δ 184.1 (C-4),165.1 (C-7), 162.2 (C-5), 158.8 (C-9), 151.2 (C-4'), 147.2 (C-3'), 123.7 (C-1'), 120.5 (C-6'), 116.9 (C-5'), 116.4 (C-2), 105.3 (C-10), 95.3 (C-8), 82.7 (C-5"), 80.3 (C-3"), 75.5 (C-1"), 72.7 (C-4"), 71.9 (C-2"), 63.1 (C-6"); FABMS *m/z* 449.1 $[M+H]^+$.

Measurement of nitric oxide production and cell viability – BV 2 cells were plated into a 96-well plate (3×10^4 cells/well). After 24 h, cells were pretreated with compounds **1 - 14** for 30 min, and then stimulated with 100 ng/ml of LPS for another 24 h. Nitrite, a soluble oxidation product of NO, was measured in the culture media using the Griess reaction. The supernatant was harvested and mixed with an equal volume of Griessreagent (1% sulfanilamide, 0.1% N-1 napthylethylenediamine dihydrochloride in 5% phosphoric acid). After 10min, the absorbance at 570 nm was measured using a microplate reader. Sodium nitrite was used as a standard to calculate the NO2- concentration. Cell viability was assessed by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. NG-mono- methyl-L-arginine (L-NMMA, Sigma, St.Louis, MO, USA), a well-known nitric oxide synthase (NOS) inhibitor, was tested as a positive control.

Result and Discussion

Structures of compounds **4 - 14** were determined by comparing ^{1}H, ^{13}C NMR, and MS spectral data with those in the literatures to be 1-*O*-(lauroyl)glycerol (**4**),[9] phytene-1,2-diol (**5**),[10] (3*S*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-7-megastigmen-9-one (**6**),[11] loliolide (**7**),[12] *β*-sitosterol (**8**),[13] 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone (**9**),[14] 1-feruloyl-*β*-D-glucopyranoside (**10**),[15] pinoresinol-4'-*O*-*β*-D-glucopyranoside (**11**),[16] luteolin (**12**),[17] quercetin-3-*O*-*β*-D-glucopyranoside (**13**),[18] and luteolin 6-*C*-*β*-glucopyranoside (**14**).[19] Compounds **1 - 14** were first isolated from this sources. The following describes the structure elucidation of **1**, which was first isolated from natural source, although it was reported as synthetic compound.[20] Since NMR spectral data of **2** and **3** have not been reported, the NMR data were explained and assigned (Table 2).

Compound **1** was obtained as a yellowish gum. From the HR-FAB-MS (positive-ion mode) *m/z*: 265.0178 $[M+Na]^+$ (calcd for $C_7H_{14}O_5S_2Na$, 265.0180), the molecular formula was deduced to be $C_7H_{14}O_5S_2$. The IR spectrum of **1** indicated the presence of hydroxyl (3412 cm^{-1}) and C-O functional groups (1031 cm^{-1}). The ^{1}H NMR spectrum of **1** showed signals for a methyl group [δ_H 2.50 (3H, s, S-CH_3)], four oxygenated methine proton signals [δ_H 3.50 (1H, m, H-2), 3.40 (1H, t, *J* = 8.8 Hz, H-3), and 3.69 (2H, m, H-4, 5)] and two oxygenated methylene proton signals [δ_H 3.86 (1H, dd, *J* = 16.0, 5.5 Hz, H-6a), and 3.64 (1H, dd, *J* = 16.0, 1.8Hz, H-6b)] and an anomeric proton adjacent to disulfide group [δ_H 4.37 (1H, d, *J* = 9.4Hz, H-

1)]. The ^{13}C NMR spectrum showed 7 carbon signals, including one methyl carbon [δ_C 24.9 (S-CH_3)], four oxygenated methine carbons [δ_C 82.6 (C-5), 79.6 (C-3), 72.8 (C-2), and 71.6 (C-4)], an anomeric carbon [δ_C 92.3 (C-1)]. The β-form of D-glucose was confirmed by the large coupling constant (9.4 Hz, **1**; 5.4 Hz, α-form; 9.1 Hz, β-form).[21] This spectroscopic data were very similar to those of ethyl 1-thio-β-D-glucopyranosyl disulfide[20] except that the absence of a methylene group [δ_H 2.86; δ_C 33.8]. Based on the above evidences, the structure of **1** was elucidated as methyl-1-thio-β-D-glucopyranosyl disulfide.

Compound **2** was isolated as a colorless gum. The molecular formula was determined to be $C_{11}H_{21}NOS$ from HR-FAB-MS (positive-ion mode) *m/z*: 216.1424 $[M+H]^+$ (calcd for $C_{11}H_{22}NOS$, 216.1422). The IR spectrum of **2** displayed characteristic absorption band of C≡N (2204 cm^{-1}). The ^1H-NMR spectrum showed the presence of a methylene signals [δ_H 2.66 (1H, m, H-10a), and 2.58 (1H, m, H-10b)] and a methyl group [δ_H 2.51 (3H, s, Me-12)]. Additionally, eight methylene protons were shown [δ_H 2.28 (2H, t, J = 7.1 Hz, H-2), 1.70 (2H, m, H-9), 1.59 (2H, m, H-3), 1.38 (4H, m, H-5, 6), and 1.23 - 1.30 (6H, m, H-4, 7, 8)]. The ^{13}C NMR spectrum showed 11 carbon signals, including a nitrile carbon at δ_C 119.8 (C≡N), a methyl sulfoxide at δ_C 38.6 (CH_3SO), two characteristic methylene carbons [δ_C 54.7 (C-10), and 17.1 (C-2)], and seven methylene carbons [δ_C 29.0 (C-8), 28.9 (C-3), 28.7 (C-6), 28.6 (C-5), 28.5 (C-7), 25.3(C-3), and 22.5 (C-9)]. These spectroscopic data was very similar to those of 9-(methylsulphinyl)-nonanenitrile[22] except that the presence of a methylene group [δ_H 1.23; δ_C 28.6]. Thus, the structure of **2** was elucidated as 10-methylsulphinyl-decanenitrile.

Compound **3** was isolated as a colorless gum. The molecular formula was determined to be $C_{12}H_{23}NOS$ from HR-FAB-MS (positive-ion mode) *m/z*: 230.1580 $[M+H]^+$ (calcd for $C_{12}H_{24}NOS$, 230.1579). The IR spectrum of **3** displayed C≡N (2306 cm^{-1}) functional group. Its ^1H and ^{13}C-NMR data were very similar to those of **2** except for an additional methylene group [δ_H 1.25; δ_C 28.7]. Thus, the structure of **3** was elucidated as11-methylsulphinyl-undecanenitrile.[22]

Anti-inflammatory activity of the isolates (**1 - 14**) was evaluated by measuring the levels of nitric oxide (NO) production in lipopolysaccharide (LPS)-activated microglia BV-2 cells. Compound **12** significantly inhibited NO levels with IC_{50} value of 9.70 μM better than positive control (17.40 μM). Compounds **1** and **4** showed moderate NO product inhibitory (44.10 μM and 32.60 μM, respectively), but the other compounds showed little effects (Table 3).

Table 3. Effects of compounds **1 - 14** on NO production in LPS-activated BV-2 cells

Compound	IC_{50}[a] (mM)	Cell viability [b] (%)
1	44.10	118.28 ± 6.54
2	75.23	136.44 ± 5.13
3	144.64	117.91 ± 8.44
4	32.60	136.20 ± 11.20
5	153.71	152.92 ± 3.50
6	167.24	117.44 ± 2.83
7	>500	114.70 ± 8.48
8	77.12	119.36 ± 6.1
9	259.50	135.42 ± 10.68
10	63.55	112.15 ± 2.94
11	266.61	107.41 ± 2.63
12	9.70	137.66 ± 3.11
13	77.17	119.36 ± 6.13
14	146.69	120.36 ± 3.88
[c]L-NMMA	17.40	110.21 ± 4.56

[a]The IC_{50} value of each compound was defined as the concentration (μM) that caused 50% inhibition of NO production in LPS-activated BV-2 cells; [b]Cell viability after treatment with 20 μM of each compound was determined by the MTT assay and is expressed as a percentage (%). The results are averages of three independent experiments, and the data are expressed as mean ± SD; [c] L-NMMA was used as a positive control

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A5A2A 28671860). We are thankful to the Korea Basic Science Institute (KBSI) for the measurements of NMR and MS spectra.

References

(1) Lee, Y. N.; Flora of Korea; Kyohaksa: Korea, 1996; p250.

(2) Song, N.; Xu, W.; Guan, H.; Liu, X.; Wang, Y.; Nie, X. *Asian. J. Tradit. Med.* **2007**, *2*, 218-222.

(3) Grosso, C.; Vinholes, J.; Silva, L. R.; de Pinho, P. G.; Gonçalves, R. F.; Valentão, P.; Jäger, A. K.; Andrade, P. B. *Braz. J. Pharmacog.* **2011**, *21*, 635-644.

(4) Selenu, M. B.; Carrus, F.;Bonsignore, L. *Boll. Chim. Farm.* **2005**, *144*, 66-78.

(5) Park, C. J.; Park, C. B.; Hong, S. S.; Lee, H. S.; Lee, S. Y.; Kim, S. C. *Plant Mol.Biol*. **2000**, *44*, 187-197.

(6) Kuroda, K.; Akao, M. *Jpn. J. Cancer. Res.* **1981**, *72*, 777-782.

(7) Kuroda, K.; Akao, M. *Jpn. J. Cancer. Res.* **1975**, *66*, 461-462.

(8) Cha, J. M.; Suh, W. S.; Lee, T. H.; Subedi, L.; Kim, S. Y.; Lee, K. R. *Molecules* **2017**, *22*, 1023.

(9) Reif, D. W.; McCreedy, S. A. *Arch. Biochem. Biophys.* **1995**, *320*, 170-176.

(10) Batovska, D., I.; Tsubota, S.; Kato, Y.; Asano, Y.; Ubukata, M. *Tetrahedron* **2004**, *15, 3551-3559.*
(11) Wong, H. F.; Brown, G.D. *J. Chem. Res.* **2002**, *5*, 30-33.
(12) Duan, H.; Takaishi, Y.; Momota, H.; Ohmoto, Y.; Taki, T. *Phytochemistry* **2002**, *59*, 85-90.
(13) Kimura, J.; Maki, N. *J. Nat. Prod.* **2002**, *65*, 57-58.
(14) Chang, Y. C.; Chang, F. R.; Wu, Y. C. *J. Chin. Chem. Soc.* **2000**, *47*, 373-380.
(15) Achenbach, H.; Stöcker, M.; Constenla, M. A. *Phytochemistry* **1988**, *27*, 1835-1841.
(16) Kim, J. S.; Kwon, Y. S.; Sa, Y. J.; Kim, M. J. *J. Agric. Food. Chem*. **2011**, *59*, 138-144.
(17) Kim, D. K.; Lim, J. P.; Kim, J. W.; Park, H. W.; Eun, J. S. *Arch. Pharm. Res.* **2005**, *28*, 39-43.
(18) Li, Y. L.; Li, J.; Wang, N. L.; Yao, X. S. *Molecules* **2008**, *13*, 1931-1941.
(19) Kajjout, M.; Rolando, C. *Tetrahedron* **2011**, *67*, 4731-4741.
(20) Rayyan, S.; Fossen, T.; Nateland, H. S.; Andersen, Ø. M. *Phytochem. Anal.* **2005**, *16*, 334-341.
(21) Gamblin, D. P.; Garnier, P.; van Kasteren, S.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G. *Angew. Chem. Int. Ed.* **2004**, *43*, 828-833.
(22) Angles d'Ortoli, T.; Widmalm, G. *Tetrahedron* **2016**, *72*, 912-927.
(23) Repčak, M.; Imrich, J.; Pihlaja, K.; Kal'atová, M. *Phytochemisty* **1998**, *47*, 1219-1221.

Received February 7, 2018
Revised April 2, 2018
Accepted April 2, 2018