

Phytochemical Constituents of *Cirsium setidens* Nakai and Their Cytotoxicity against Human Cancer Cell Lines

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Five terpenes (**1**–**5**), three fatty acids (**6**–**8**), two sterols (**9** and **11**), and a monogalactosyldiacyl glycerol (**10**) were isolated from the methylene chloride extract of the aerial part of *Cirsium setidens*. Their chemical structures were determined to be α -tocopherol (**1**), 25-hydroperoxycycloart-23-en-3 β -ol (**2**), 24-hydroperoxycycloart-25-en-3 β -ol (**3**), mokko lactone (**4**), *trans*-phytol (**5**), 9, 12, 15-octadecatrienoic acid (**6**), 9, 12-octadecadienoic acid (**7**), hexadecanoic acid (**8**), acylglycosyl β -sitosterol (**9**), (2*R*)-1, 2-*O*-(9*z*, 12*z*, 15*z*-dioctadecatrienoyl)-3-*O*- β -D-galactopyranosyl glycerol (**10**) and β -sitosterol glucoside (**11**) by spectral evidences. Compound **3** exhibited significant cytotoxic activity against five human cancer cell lines with its ED₅₀ values ranging from 2.66 to 11.25 μ M.

Key words: *Cirsium setidens*, Compositae, Triterpene hydroperoxide, Cytotoxicity

INTRODUCTION

Cirsium setidens Nakai (Compositae), a perennial herb, is distributed mainly in Kangwon province, Korea, and *Cirsium* species have been used to treat edema, bleeding and hemoptysis. (Lee, 1985; Lee, 1966; Kim, 1984) Flavonoids (Morita *et al.*, 1964; Morita *et al.*, 1973; Lim *et al.*, 1978), aplotaxane (Christensen, 1992) and furan derivatives (Shen & Mu, 1990) were reported from *Cirsium* species, while no phytochemical and pharmacological study for *C. setidens* have been performed. As part of our systematic study for Korean Compositae medicinal plants, the terpene compounds from methylene chloride (MC) extract of *Cirsium setidens* were investigated. The repeated column chromatographic separation of the MC extract afforded two cycloartane-type triterpene hydroperoxides (**2** and **3**), two acyclic diterpenes (**1** and **5**), a sesquiterpene lactone (**4**), three fatty acids (**6**–**8**), an acylglycosyl sterol (**9**), a monogalactosyldiacyl glycerol (**10**) and a sterol glycoside (**11**). Compounds **1**–**5** and **9**–**11** were first reported from *Cirsium* species. The cytotoxic activities of the isolated compounds were investigated against five

cultured human cancer cell lines. The present paper describes the isolation, structure elucidation and cytotoxic activities of these compounds.

MATERIALS AND METHODS

Instruments and reagents

Melting points were determined on a Gallenkamp melting point apparatus and were uncorrected. Optical rotations were measured on a JASCO P-1020 instrument. The IR and UV spectra were measured on Bruker Vector[®] 22 FT-IR spectrometer and Shimadzu UV-1601 UV-Visible spectrophotometer, respectively. The EI-MS spectrum was measured on JMS700 (JEOL, JAPAN). The ¹H- and ¹³C-NMR spectra were recorded with Varian UNITY INOVA 500 and Bruker AMX 500 spectrometer. GC-MS was Hewlett-Packard 6890 Gas Chromatography-5973 Mass Selective Detector and connected Ultra-2 capillary column (25 m \times 200 μ m I.D., 0.11 μ m d_f) or HP-5MS (30 m \times 250 μ m I.D.). The preparative HPLC was Knauer preparative HPLC with UV and Refractive dual detector system and connected Econosil[®] silica 10 u (10 \times 250 mm) column. Low-pressure liquid column chromatography (LPLC) was carried out over Lichroprep Si 60 Lobar[®]-A (Merck, 40–63 μ m) and Lichroprep RP-18 Lobar[®]-A (Merck, 40–63 μ m) with a FMI LAB PUMP MODEL QSY (U.S.A.). TLC was performed on precoated Kiesel gel 60F₂₅₄

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precoated plate (Art. 5554, Merck). Silica gel for column chromatography was Kiesel gel 60 (70-230 and 230-400 mesh, ASTM Art. 7734 and 9385, Merck) and packing materials for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia).

Plant materials

The aerial parts of *Cirsium setidens* Nakai were collected at Mt. Taebaek, Korea in July 1998. A voucher specimen (SKK-98-002) was deposited at the college of pharmacy, Sungkyunkwan University, Korea.

Test for cytotoxicity *in vitro*

Sulforhodamin B bioassay (SRB) was used as cytotoxicity screening method (Skehan *et al.*, 1990). Cytotoxic activities of each compound were performed against five cultured human tumor cells at Korea Research Institute of Chemical Technology; A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), XF498 (CNS cancer cells) and HCT15 (colon cancer cells) *in vitro*.

Extraction, separation and purification of compounds

Dried and chopped aerial parts of *Cirsium setidens* (2.1 kg) were extracted with CH₂Cl₂ three times at room temperature. The concentrated CH₂Cl₂ extract (50 g) was chromatographed over a silica gel column using a gradient solvent system of CH₂Cl₂-MeOH (1:0~0:1) to give seventeen fractions (F1~F17). The F1 fraction (1.4 g) was subjected to a silica gel column chromatography eluted with n-hexane-EtOAc (10:1) to give three subfractions (F11~F13). The F12 subfraction (120 mg) was purified with silica Lobar[®]-A column chromatography (n-hexane-EtOAc, 15:1) to afford **1** (60 mg). The F4 fraction (350 mg) was subjected to a silica gel column chromatography eluted with n-hexane-EtOAc (7:1) to give three subfractions (F41~F43). The F42 subfraction (80 mg) was purified with the Sephadex LH-20 (CH₂Cl₂-MeOH, 1:1) and silica Lobar[®]-A column chromatography (n-hexane-EtOAc, 6:1) to afford **2** (9 mg) and **3** (6 mg). The F5 fraction (130 mg) was purified with silica Lobar[®]-A (n-hexane-EtOAc, 7:1) and RP Lobar[®]-A column chromatography (MeOH) to afford **4** (7 mg) and **5** (10 mg). The F8 fraction (9 g) was subjected to a silica gel column chromatography eluted with CH₂Cl₂-acetone (20:1) to give six subfractions (F80~F85). The F81 subfraction (720 mg) was chromatographed over Sephadex LH-20 (CH₂Cl₂-MeOH, 1:1) to give three subfractions (F821~F823). The F822 subfraction (200 mg) was subjected to RP Lobar[®]-A column chromatography (95% MeOH) to give two subfractions (F8221 and F8222). The F8221 subfraction (120 mg) was purified with RP Lobar[®]-A column

chromatography (90% MeOH) to afford **6** (30 mg) and **7** (15 mg). The F8222 subfraction (50 mg) was further purified with silica Lobar[®]-A column chromatography (n-hexane-EtOAc, 4:1) to afford **8** (18 mg). The F9 fraction (1 g) was subjected to a silica gel column chromatography eluted with CH₂Cl₂-MeOH (20:1) to give two subfractions (F91 and F92). The F91 (450 mg) was further rechromatographed with silica gel column chromatography (n-hexane-EtOAc-MeOH, 5:5:1) to give five subfractions (F911~F915) and the F911 subfraction (80 mg) was purified with preparative HPLC (n-hexane-EtOAc-MeOH, 10:5:1, flow rate 3.0 ml/min) to afford **9** (30 mg, Rt=8.8 min). The F11 fraction (2 g) was subjected to a silica gel column chromatography eluted with n-hexane-EtOAc-MeOH (4:1~0:1) and a Sephadex LH-20 column chromatography (CH₂Cl₂-MeOH, 1:1) to give three subfractions (F11-1~F11-3). The F11-3 subfraction (350 mg) was rechromatographed with the silica gel Lobar[®]-B column chromatography (n-hexane-EtOAc-CH₂Cl₂-MeOH, 4:2:2:1) and further purified with silica gel Lobar[®]-A column chromatography (n-hexane-EtOAc-CH₂Cl₂-MeOH, 4:2:2:1) to afford **10** (15 mg). The F13 fraction (3.5 g) was subjected to a silica gel column chromatography eluted with n-hexane-EtOAc-MeOH (4:1~0:1) to give five subfractions (F13-1~F13-5). The F13-3 subfraction was chromatographed over RP flash column using a gradient solvent system of water-tetrahydrofuran (4:1~0:1) to give three subfractions (F13-31~F13-33). The F13-33 (160 mg) was purified by washing with MeOH to afford **11** (110 mg).

α-Tocopherol (1). Pale yellowish oil; [α]_D: +0.35°(c=0.5, EtOH); UV λ_{max} (EtOH) nm (log ϵ): 292 (3.74), 215 (4.32); IR (CHCl₃) ν_{max}^{-1} : 3439 (OH), 1620 (C=C); EI-MS m/z (rel. int): 430 [M]⁺ (44), 205 (9), 165 (100); ¹H-NMR (Acetone-*d*₆, 500MHz): δ 2.61 (2H, t, *J* = 7.2 Hz, H-4), 2.16, 2.12, 2.09 (each 3H, s, Me-7a, 8a, 5a) 1.80 (2H, m, H-3), 1.24 (3H, s, Me-2a), 0.89, 0.90 (\times 2), 0.91 (each 3H, s, 4'a, 8'a, 12'a, 13'); ¹³C-NMR (Acetone-*d*₆, 125MHz): δ 146.2 (C-9), 145.8 (C-6), 122.7 (C-10), 122.3 (C-8), 120.3 (C-7), 117.6 (C-5), 74.7 (C-2), 40.2 (C-1'), 40.0 (C-11'), 37.64 (C-3'), 37.56 (C-5'), 37.50 (C-7'), 37.4 (C-9'), 32.9 (C-4'), 32.8 (C-8'), 31.9 (C-3), 28.1 (C-12'), 25.0 (C-10'), 24.5 (C-6'), 23.6 (C-2a), 22.5 (C-12'a), 22.4 (C-13'), 21.1 (C-2'), 20.8 (C-4), 19.6 (C-4'a), 19.5 (C-8'a), 12.2 (C-7a), 11.5 (C-8a), 11.3 (C-5a)

25-Hydroperoxycycloart-23-en-3 β -ol (2). White powder; [α]_D: +30°(c=0.3, CHCl₃); mp.: 138°C; IR (CHCl₃) ν_{max}^{-1} : 3452 (OH, OOH), 1650 (C=C); EI-MS m/z (rel. int.): 458 [M]⁺ (10), 255 (14), 203 (32), 187 (33), 175 (52), 161 (40), 147 (53), 135 (70), 121 (83), 107 (87), 95 (100), 87 (73); ¹H-NMR (CDCl₃, 500MHz): δ 7.26 (1H, s, OOH), 5.70 (1H, ddd, *J* = 15.6, 8.5, 5.9 Hz, H-23), 5.53 (1H, d, *J* =

Table 1. ^{13}C -NMR Spectral data of Compounds **2** and **3** (CDCl_3 , 125MHz, δ ppm)

Position	Compound 2	Compound 3
1	32.7	32.7
2	31.1	31.1
3	79.5	79.5
4	41.2	41.2
5	47.8	47.8
6	21.8	21.8
7	26.7	26.7
8	48.6	48.6
9	20.7	20.7
10	26.8	26.8
11	27.1	27.1
12	33.5	33.5
13	46.0	46.0
14	49.5	49.5
15	36.3	36.2
16	28.8	28.8
17	52.8	52.8; 52.7 [†]
18	18.8	18.7
19	30.5	30.6
20	37.0	36.7; 36.5 [†]
21	19.0	18.9; 18.8 [†]
22	40.0	28.3; 28.0 [†]
23	131.4	32.7
24	135.1	91.1; 90.9 [†]
25	83.0	144.6; 144.3 [†]
26	25.1 [†]	114.9; 115.4 [†]
27	25.0 [†]	17.6; 17.9 [†]
28	26.1	26.1
29	14.7	14.7
30	20.0	20.0

[†]Signals for C-24 epimer

15.6 Hz, H-24), 3.29 (1H, m, $W_{1/2}$ = 17.0 Hz, H-3), 1.34 (6H, s, H-26 and H-27), 0.98 (3H, s, H-18)^{*}, 0.97 (3H, s, H-28)^{*}, 0.89 (3H, s, H-30), 0.87 (3H, d, J = 6.5 Hz, H-21), 0.81 (3H, s, H-29), 0.56 (1H, d, J = 4.1 Hz, H-19a), 0.34 (1H, d, J = 4.1 Hz, H-19b) ^{*}assignment may be exchangeable; ^{13}C -NMR (CDCl_3 , 125MHz): Table. I

24-Hydroperoxycycloart-25-en-3 β -ol (3). White powder; $[\alpha]_D$: +46 $^\circ$ ($c=0.4$, CHCl_3); mp.: 128 $^\circ\text{C}$; IR (CHCl_3) ν_{max}^{-1} : 3455 (OH, OOH), 1617 (C=C); EI-MS m/z (rel. int.): 458 $[\text{M}]^+$ (4), 203 (39), 175 (71), 161 (40), 147 (50), 135 (64), 121 (64), 107 (80), 95 (100), 81 (71); ^1H -NMR (CDCl_3 , 500MHz): δ 7.73 (1H, s, OOH), 5.03 (1H, m, $W_{1/2}$ = 9.3 Hz, H-26a), 5.02 (1H, br. s, H-26b), 4.28 (1H, dt, J = 6.7, 2.0 Hz, H-24), 3.29 (1H, m $W_{1/2}$ = 17.9 Hz), 1.75 (3H, s, H-27), 0.97 (3H, s, H-18)^{*}, 0.96 (3H, s, H-28)^{*}, 0.89 (3H, d, J = 1.5 Hz, H-30), 0.87 (3H, d, J = 6.5 Hz, H-21), 0.81 (3H, s, H-29), 0.56 (1H, d, J = 4.1 Hz, H-19a), 0.34 (1H, d,

J = 4.1 Hz, H-19b) ^{*}assignment may be exchangeable.; ^{13}C -NMR (CDCl_3 , 125MHz): Table. I

4(15), 10(14)-Guaiadien-12, 6-olide (mokko lactone) (4). White powder; $[\alpha]_D$: +18 $^\circ$ ($c=4.2$, CHCl_3); mp.: 35 $^\circ\text{C}$, UV λ_{max} (EtOH) nm (log ϵ): 203 (3.74); IR (CHCl_3) ν_{max}^{-1} : 1772 (γ -lactone), 1620 (C=C); EI-MS m/z (rel. int.): 232 $[\text{M}]^+$ (7), 158 (100), 152 (63), 91 (70), 71 (62), 55 (63); ^1H -NMR (CDCl_3 , 500MHz): δ 5.21 (1H, d, J = 2.1 Hz, H-15a), 5.06 (1H, d, J = 2.1 Hz, H-15b), 4.89 (1H, br.s, H-14a), 4.79 (1H, br.s, H-14b), 3.93 (1H, t, J = 9.5 Hz, H-6), 2.89 (1H, dt, J = 8.1, 4.5 Hz, H-1), 2.81 (1H, br.dd, J = 9.5, 8.1 Hz, H-5), 2.49 (3H, m, H-3, 11), 2.22 (1H, dd, J = 12.0, 7.1 Hz, H-9), 2.12 (1H, m, H-7), 2.05 (1H, dt, J = 12.0, 5.1 Hz, H-9), 1.95 (1H, m, H-2), 1.94 (1H, m, H-8), 1.87 (1H, m, H-2), 1.32 (1H, m, H-8), 1.25 (3H, d, J = 6.8 Hz, H-13); ^{13}C -NMR (CDCl_3 , 125MHz): δ 179.0 (C-12), 152.0 (C-4), 150.2 (C-10), 112.1 (C-14), 109.5 (C-15), 85.6 (C-6), 52.2 (C-5), 50.1 (C-11), 47.3 (C-1), 42.3 (C-7), 37.9 (C-9), 32.8 (C-3, 8), 30.5 (C-2), 13.5 (C-13)

trans-Phytol (5). Colorless oil; $[\alpha]_D$: +0.2 $^\circ$ ($c=0.3$, CHCl_3); UV λ_{max} (EtOH) nm (log ϵ): 233 (2.36), 204 (3.76); IR (CHCl_3) ν_{max}^{-1} : 3443 (OH), 1667 (C=C); EI-MS m/z (rel. int.): 296 $[\text{M}]^+$ (7), 278 (5), 123 (32), 81 (37), 71 (100), 57 (61); ^1H -NMR (CDCl_3 , 500 MHz): δ 5.42 (1H, tq like, J = 6.8, 1.2 Hz, H-2), 4.16 (2H, d, J = 6.8 Hz, H-1), 2.00 (2H, m), 1.68 (3H, s, H-3a), 1.61.0 (CH₂, CH), 0.880.85 (12H, m, H-7a, 11a, 15a, 16); ^{13}C -NMR (CDCl_3 , 125 MHz): δ 140.6 (C-3), 123.3 (C-2), 59.7 (C-1), 40.1, 39.7, 37.7, 37.6, 37.5, 36.9, 33.1, 32.9, 28.2, 25.4, 25.1, 24.7, 23.0, 22.9, 20.01, 20.00, 16.4

9, 12, 15-Octadecatrienoic acid (6). Colorless gum; ^1H -NMR (CDCl_3 , 500 MHz): δ 5.36 (6H, m, H-9, 10, 12, 13, 15, 16), 2.80 (4H, br.t, J = 5.5 Hz, H-11, 14), 2.34 (2H, t, J = 7.3 Hz, H-2), 2.06 (4H, m, H-8, 17), 1.62 (2H, m, H-3), 1.31 (8H, m, $-(\text{CH}_2)_4 \times 4$), 0.97 (3H, t, J = 7.3 Hz, Me-18); ^{13}C -NMR (CDCl_3 , 125MHz): δ 180.6 (C-1), 132.2, 130.5, 128.52, 128.48, 128.0, 127.4 (C-9, 10, 12, 13, 15, 16), 34.4 (C-2), 19.9, 29.4, 29.3, 29.2 (C-4, 5, 6, 7), 27.4 (C-8), 25.9, 25.8 (C-11, 14), 24.9 (C-3), 20.8 (C-17), 14.6 (C-18)

9, 12-Octadecadienoic acid (7). Colorless gum; ^1H -NMR (CDCl_3 , 500MHz, ppm): δ 5.36 (4H, m, H-9, 10, 12, 13), 2.77 (2H, t, J = 6.8 Hz, H-11), 2.34 (2H, t, J = 7.7 Hz, H-2), 2.05 (4H, dd, J = 15.4, 7.3 Hz, H-8, 14), 1.63 (2H, m, H-3), 1.32 (14H, m, $-(\text{CH}_2)_7 \times 7$), 0.89 (3H, t, J = 7.0 Hz, Me-18)

Hexadecanoic acid (8). Colorless gum; ^1H -NMR (CDCl_3 , 500MHz, δ ppm): δ 2.35 (2H, t, J = 7.3 Hz, H-2),

1.63 (2H, m, H-3), 1.25 (24H, m, $-(CH_2)_m \times 12$), 0.89 (3H, t, $J = 7.0$ Hz, Me-16)

Sitosterol-3-O-[6'-O-6'', 9''-octadecadienoyl]- β -D-glucopyranoside (9). Colorless gum; UV λ_{max} (EtOH) nm (log ϵ): 245 (3.57), 203 (4.13); IR (CHCl₃) ν_{max}^{-1} : 3449 (OH), 1744 (ester linkage), 1078 (glycosidic C-O); Negative-mode FAB-MS m/z (rel. int.): 838 [M-H]⁺; ¹H-NMR (CDCl₃, 500 MHz): δ 5.37 (1H, br.s, H-6), 5.36 (m), 4.49 (1H, dd, $J = 12.1, 4.8$ Hz, H-6'), 4.39 (1H, d, $J = 7.7$ Hz, H-1'), 4.26 (1H, dd, $J = 12.1, 2.2$ Hz, H-6'), 3.57 (2H, m, $W_{1/2} = 15.6$ Hz, H-3', 5'), 3.45 (1H, ddd, $J = 9.9, 4.7, 2.2$ Hz, H-3), 3.37 (2H, dd, $J = 19.0, 9.2$ Hz, H-2', 4'), 2.35 (2H, m, H-2''), 1.27 (br.s, $-(CH_2)_m$), 1.01 (3H, s, Me-19), 0.92 (3H, d, $J = 6.2$ Hz, Me-21), 0.88 (3H, t, $J = 6.9$ Hz, Me- $(CH_2)_m$), 0.86-0.81 (9H, m, Me-26, 27 and 29), 0.68 (3H, s, Me-18); ¹³C-NMR (CDCl₃, 125 MHz): δ 175.0 (C-1''), 140.5 (C-5), 122.4 (C-6), 101.4 (C-1'), 79.8 (C-3), 76.2 (C-3'), 74.2 (C-5'), 73.8 (C-2'), 70.3 (C-4'), 63.4 (C-6'), 57.0 (C-14), 56.3 (C-17), 50.4 (C-9), 46.0 (C-24), 42.6 (C-13), 40.0 (C-12), 39.1 (C-4), 37.5 (C-1), 37.0 (C-10), 36.4 (C-20), 34.5 (C-22), 34.2 (C-2''), 32.2 (C-7), 32.1 (C-8), 30.0~29.2 ($-(CH_2)_m$), 29.8 (C-2), 29.6 (C-25), 28.5 (C-16), 26.3 (C-23), 24.5 (C-15), 23.3 (C-28), 21.3 (C-11), 20.1 (C-26), 19.6 (C-19), 19.3 (C-27), 19.0 (C-21), 14.4 ($(CH_2)_m-CH_3$), 12.2 (C-29), 12.1 (C-18)

Alkaline hydrolysis of 9

A solution of **9** (1 mg) with 2.3 g dry NaOMe in MeOH (1 ml) was stirred at room temperature for 12 hours. The reaction mixture was neutralized with 1N HCl and partitioned between MeOH and *n*-hexane. The *n*-hexane layer was evaporated *in vacuo* to yield the mixture of fatty acid methyl ester. The mixture of fatty acid methyl ester was identified as the mixture of 6, 9-octadecadienoate, 9-octadecenoate and hexadecanoate at a ratio of 47 : 26 : 27 by GC-MS analysis.

Acetylation of 9

9 (20 mg) was acetylated with pyridine (3 ml) and Ac₂O (500 μ l) for 12 hours to give a triacetate (15 mg). A triacetate was purified with silica Lobar[®]-A column chromatography (*n*-hexane:EtOAc=5 : 1) to afford **9a** (10 mg).

Sitosterol-3-O-[2', 3', 4'-O-triacetyl-6'-O-6'', 9''-octadecadienoyl]- β -D-glucopyranoside (9a). White powder; ¹H-NMR (CDCl₃, 500 MHz): δ 5.36 (1H, br.s, H-6), 5.21 (1H, t, $J = 9.6$ Hz, H-3'), 5.05 (1H, t, $J = 9.6$ Hz, H-4'), 4.96 (1H, dd, $J = 9.6, 8.1$ Hz, H-2'), 4.59 (1H, d, $J = 8.1$ Hz, H-1'), 4.23 (1H, dd, $J = 12.2, 5.4$ Hz, H-6'a), 4.14 (1H, dd, $J = 12.2, 2.5$ Hz, H-6'b), 3.68 (1H, ddd, $J = 9.6, 5.4, 2.5$ Hz, H-5'), 3.48 (1H, m, H-3), 2.33 (2H, t, $J = 7.5$ Hz, $-CH_2-CO_2-$), 2.06, 2.03, 2.01 (each 3H, s, OAc), 1.27 (br.s, $-(CH_2)_m$),

1.00 (3H, s, Me-19), 0.93 (3H, d, $J = 4.2$ Hz, Me-21), 0.88 (3H, t, $J = 7.0$ Hz, Me- $(CH_2)_m$), 0.86-0.82 (9H, d and t, $J = 6.8$ Hz, Me-26, 27 and 29), 0.69 (3H, s, Me-18); ¹³C-NMR (CDCl₃, 125 MHz): δ 173.8 (C-1''), 170.6~169.6 ($3 \times CH_3CO$), 140.6 (C-5), 122.4 (C-6), 99.9 (C-1'), 80.4 (C-3), 73.2 (C-5'), 72.0 (C-3'), 71.7 (C-2'), 68.9 (C-4'), 62.3 (C-6'), 57.0 (C-14), 56.3 (C-17), 50.4 (C-9), 46.1 (C-24), 42.6 (C-13), 40.0 (C-12), 39.2 (C-4), 37.5 (C-1), 37.0 (C-10), 36.4 (C-20), 34.4 (C-22), 34.2 (C-2''), 32.2 (C-7), 32.1 (C-8), 30.029.2 ($-(CH_2)_m$), 29.8 (C-2), 29.6 (C-25), 28.5 (C-16), 26.3 (C-23), 24.5 (C-15), 23.3 (C-28), 21.3 (C-11), 21.0~20.9 ($3 \times CH_3CO$), 20.1 (C-26), 19.6 (C-19), 19.3 (C-27), 19.0 (C-21), 14.4 ($-(CH_2)_m-CH_3$), 12.2 (C-29), 12.1 (C-18)

(2R)-1, 2-O-(9Z, 12Z, 15Z-Dioctadecatrienoyl)-3-O- β -D-galactopyranosyl glycerol (10).

Colorless gum; ¹H-NMR (CDCl₃, 500 MHz): δ 5.36 (12H, m, H-9'', 9''', 10'', 10''', 12'', 12''', 13'', 13''', 15'', 15''', 16'', 16'''), 5.30 (1H, m, H-2), 4.39 (1H, dd, $J = 12.1, 3.5$ Hz, H-1a), 4.28 (1H, d, $J = 7.7$ Hz, H-1'), 4.21 (1H, dd, $J = 12.1, 6.6$ Hz, H-1b), 4.02 (1H, d, $J = 2.9$ Hz, H-4'), 3.99 (1H, dd, $J = 11.8, 6.0$ Hz, H-6'a), 3.91 (1H, dd, $J = 11.2, 5.3$ Hz, H-3a), 3.89 (1H, dd, $J = 11.8, 3.7$ Hz, H-6'b), 3.75 (1H, dd, $J = 11.2, 6.4$ Hz, H-3b), 3.65 (1H, dd, $J = 9.5, 7.5$ Hz, H-2'), 3.60 (1H, dd, $J = 9.5, 2.9$ Hz, H-3'), 3.55 (1H, br.dd, $J = 4.8$ Hz, H-5'), 2.80 (8H, m, H-11'', 11''', 14'', 14'''), 2.32 (4H, dd, $J = 15.4, 8.1, 8.1, 2.06$ Hz, H-8'', 8''', 17'', 17'''), 1.61 (4H, m, H-3'', 3'''), 1.30 (16H, m, H-4'', 4''', 5'', 5''', 6'', 6''', 7'', 7'''), 0.97 (6H, t, $J = 7.7$ Hz, H-18'', 18'''), ¹³C-NMR (CDCl₃, 125 MHz): δ 174.0, 173.7 (C-1'', C-1'''), 132.2, 130.46, 130.45, 128.54, 128.46, 128.01, 127.99, 127.34 (9'', 9''', 10'', 10''', 12'', 12''', 13'', 13''', 15'', 15''', 16'', 16'''), 104.0 (C-1'), 74.7 (C-5'), 73.7 (C-3'), 72.0 (C-2'), 70.4 (C-2), 69.8 (C-4'), 68.7 (C-3), 63.2 (C-1), 62.9 (C-6'), 34.5, 34.4 (C-2'', C-2'''), 29.8, 29.43, 29.37, 29.34, 29.28 (4''~7'', 4''''~7'''''), 27.5 (8'', 8'''), 25.9 (11'', 11''', 14'', 14'''), 25.12, 25.07 (C-3'', C-3'''), 20.8 (17'', 17'''), 14.6 (18'', 18''')

Alkaline hydrolysis of 10

A solution of **10** (7 mg) with 2.3 g dry NaOMe in MeOH (2 ml) was stirred at room temperature for 12 hours. The reaction mixture was neutralized with 1N HCl and partitioned between MeOH and *n*-hexane. The *n*-hexane layer was evaporated *in vacuo* to yield fatty acid methyl ester. The fatty acid methyl ester was analyzed by GC-MS. The MeOH layer was concentrated under reduced pressure and purified by the Sephadex LH-20 column chromatography (MeOH only) and RP Lobar[®]-A column chromatography (83% MeOH) to afford a glycerol galactoside (2 mg), $[\alpha]_D -9.2^\circ$ (c=0.1, MeOH).

Sitosterol-3-O- β -D-glucopyranoside (11). White powder; $[\alpha]_D -41.7^\circ$ (c=0.2, pyridine); mp.: 293°C; EI-MS m/z

(rel. int.) : 576 [M]⁺ (7), 414 (16), 396 (100), 255 (27), 175 (11), 147 (34), 85 (21) ; ¹H-NMR (DMSO-*d*₆, 500 MHz) : δ 5.36 (1H, br.s, H-6), 4.88 (1H, d, *J* = 4.5 Hz, Sugar-OH), 4.85 (2H, m, 2 × Sugar-OH), 4.40 (1H, t, *J* = 5.8 Hz, 6'-OH), 4.22 (1H, d, *J* = 7.8 Hz, H-1'), 3.65 (1H, dd, *J* = 11.5, 5.8 Hz, H-6'a), 3.46, (1H, m, H-3), 3.42 (1H, dt, *J* = 11.5, 5.8 Hz, H-6'b), 3.14~3.00 (3H, m, H-3', 4', 5'), 2.89 (1H, m, H-2'), 0.96 (3H, s, Me-19), 0.91 (3H, d, *J* = 6.3 Hz, Me-21), 0.83-0.76 (9H, m, Me-26, 27 and 29), 0.65 (3H, s, Me-18) ; ¹³C-NMR (DMSO-*d*₆, 125 MHz) : δ 140.4 (C-5), 121.1 (C-6), 100.8 (C-1'), 76.9 (C-3), 76.73 (C-3'), 76.68 (C-5'), 73.4 (C-2'), 70.1 (C-4'), 61.1 (C-6'), 56.1 (C-14), 55.4 (C-17), 49.6 (C-9), 45.1 (C-24), 41.8 (C-13), 39.1 (C-12), 38.3 (C-4), 36.8 (C-1), 36.2 (C-10), 35.5 (C-20), 33.3 (C-22), 31.4 (C-7), 31.3 (C-8), 29.2 (C-2), 28.7 (C-25), 27.7 (C-16), 25.4 (C-23), 23.8 (C-15), 22.6 (C-28), 20.6 (C-11), 19.7 (C-26), 19.0 (C-19), 18.9 (C-27), 18.6 (C-21), 11.7 (C-29), 11.6 (C-18)

RESULTS AND DISCUSSION

Compound **1** was obtained as yellowish oil. Based on the EI-MS, ¹H- and ¹³C-NMR spectral data and the comparison of those in the previous papers (Pouchert, 1993a; Pouchert & Behnke, 1993b; Al-Malaika, 2001; Windholz *et al.*, 2001) the structure of **1** was established as α-tocopherol.

Compound **2** was obtained as white powder and positive with peroxide reagent (Lee, 1991). The molecular formula was assigned as C₃₀H₅₀O₃ based on ¹³C-NMR (C30) and molecular ion peak at *m/z* 458 in EI-MS spectrum. The ¹H-NMR spectrum showed four tertiary [δ 0.81 (3H, s), 0.89 (3H, s), 0.97 (3H, s), 0.98 (3H, s)], a secondary [δ 0.87 (3H, d, *J* = 6.5 Hz)] and two equivalent methyl group signals [δ 1.34 (6H, s)]. A pair of doublets at δ 0.34 and 0.56 (each 1H, *J* = 4.1 Hz) was indicative of a cyclopropane ring (Pavia *et al.*, 1996) and the multiplet at δ 3.29 was due to a carbinol proton. Coupling constants of olefinic protons at δ 5.70 (1H, ddd, *J* = 15.6, 8.5, 5.9 Hz, H-23) and 5.53 (1H, d, *J* = 15.6 Hz, H-24) were attributed to *trans* double bond. 30 carbons composed of two olefinic carbons at δ 131.4 and 135.1, a carbinol carbon at δ 79.5 and a carbon bearing hydroperoxy group at δ 83.0 in the ¹³C-NMR spectrum suggested a hydroperoxycycloartane skeleton for **2** (Cabrera & Seldes, 1995). Based on the above consideration and the comparison of the data in the previous papers (Kato *et al.*, 1997; Inada *et al.*, 1997), the structure of **2** was established as 25-hydroperoxycycloart-23-en-3β-ol.

Compound **3** was obtained as white powder and positive with peroxide reagent (Lee, 1991). The molecular formula of **3** was assigned as C₃₀H₅₀O₃ based on ¹³C-NMR (C30) and molecular ion peak at *m/z* 458 in EI-MS

spectrum. The ¹H- and ¹³C-NMR spectra of **3** were similar with those of **2**. The major differences between **2** and **3** in the ¹H-NMR spectrum were the presence of an exomethylene proton signals [δ 5.03 (1H, m), 5.02 (1H, br.s)] and a vinylic methyl group signal (δ 1.75) in **3**. In the ¹³C-NMR spectrum, signals for the double bond and the carbon with hydroperoxy group appeared at the different chemical shift region (**2** : δ 135.1, 131.4 and 83.0; **3** : δ 144.6, 114.9 and 91.1). The ¹³C-NMR spectrum of **3** showed in doublets at signals of the side-chain at C-17 (Table 1.), suggesting that **3** was actually a mixture of two epimers (24*R* and 24*S* form). Based on the above consideration and the comparison of the data in the previous papers (Cabrera & Seldes, 1995; Kato *et al.*, 1997; Inada *et al.*, 1997), the structure of **3** was established as 24-epimeric mixture of 24-hydroperoxycycloart-25-en-3β-ol.

Compound **4** was obtained as white powder. The IR spectrum showed bands corresponding to a γ-lactone ring (1772 cm⁻¹) and double bond (1620 cm⁻¹). From the EI-MS, ¹H-NMR and ¹³C-NMR spectral data, the molecular formula was deduced to be C₁₅H₂₀O₂. The signals at δ 3.93 (1H, t, *J* = 9.5 Hz) and 2.05 (1H, dt, *J* = 12.0, 5.1 Hz) was indicative of a lactone ring. The ¹H- and ¹³C-NMR spectra showed the typical pattern of guaiane-type sesquiterpene lactone (Kwon *et al.*, 2001). Based on the above consideration and the comparison of the data in the previous papers (Hikino *et al.*, 1967; Yuuya *et al.*, 1999), the structure of **4** was established as mokko lactone [4(15), 10(14)-guaidiene-12, 6-olide].

Compound **5** was obtained as colorless oil. On the basis of the EI-MS, ¹H- and ¹³C-NMR spectral data and the comparison of the data in the previous papers (Goodman *et al.*, 1973; Sims & JR. Pettus, 1976), the structure of **5** was established as *trans*-phytol [(2*E*)-3,7,11,15-tetramethyl-2-hexadecen-1-ol].

The compounds **6**, **7** and **8** were identified to be 9, 12, 15-octadecatrienoic acid, 9, 12-octadecadienoic acid and hexadecanoic acid by ¹H-NMR data and GC-MS analysis, respectively.

Compound **9** was obtained as colorless gum. The IR spectrum showed bands corresponding to ester group (1744 cm⁻¹), a glycosidic C-O (1078 cm⁻¹) and hydroxyl group (3449 cm⁻¹). The ¹H- and ¹³C-NMR spectra indicated the presence of sterol skeleton, which was confirmed by comparing the ¹H- and ¹³C-NMR data with those of β-sitosterol (Guevara *et al.*, 1989). The ¹H-NMR spectrum also showed the signals corresponding to a sugar moiety at δ 4.39 (d, *J* = 7.7 Hz) and δ 4.49~3.37. The ¹³C-NMR spectrum showed the signals for a sugar moiety at δ 101.4, 76.2, 74.2, 73.8, 70.3, and 63.4 indicated of the presence of a β-D-glucose (Mahato *et al.*, 1982). The broad signals at 1.27 and the multiplet at δ 2.35 (2H, m, H-2'') in the ¹H-NMR spectrum was indicative

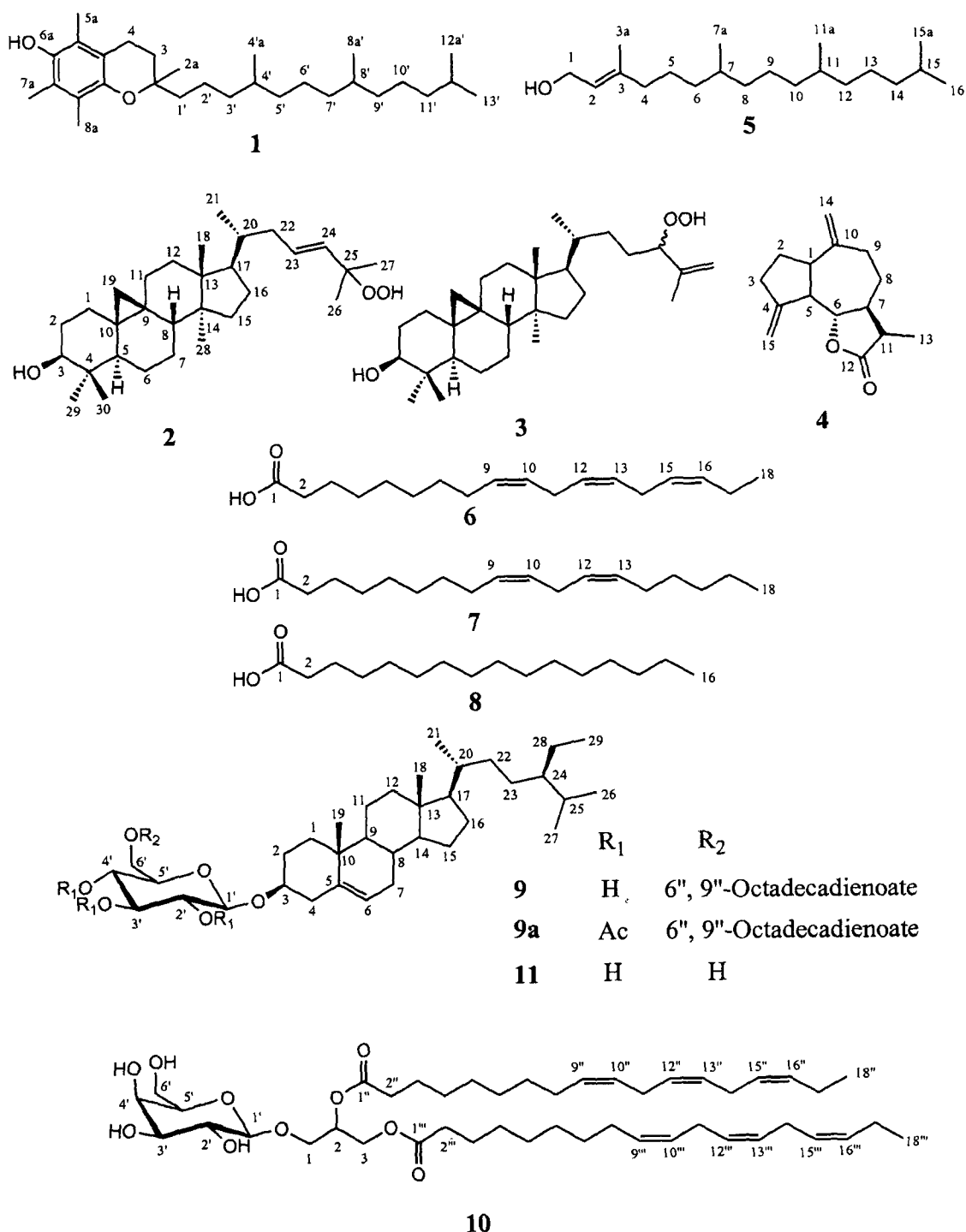


Fig. 1. The structures of compounds 1-11 from *Cirsium setidens*

of fatty acid unit. The GC-MS analysis of the mixture of fatty acid methyl ester obtained from the alkaline hydrolysis of **9** confirmed the presence of 6, 9-octadecadienoate, 9-octadecenoate and hexadecanoate at a ratio of 46.8 : 26.2 : 27.0. The signals of H-6' methylene group in the glucose moiety appeared at δ 4.49 ($J = 12.1, 4.8$ Hz) and 4.26 ($J = 12.1, 2.2$ Hz). In $^1\text{H-NMR}$ spectrum of

9a obtained by acetylation of **9**, the signals for H-2', H-3' and H-4' appeared at downfield than those of **9** and the signal for H-6' was significantly shifted upfield, clearly indicated that the fatty acid was connected with an ester linkage to the hydroxyl at C-6' in the glucose moiety (Agrawal, 1992). Based on the above consideration and the comparison of the data in the previous papers (Muhammad

et al., 2002; Geng et al., 1988; Hashimoto et al., 1991; Rubnov et al., 2001; Greca et al., 1991; Cho et al., 1992), the major component of **9** was assigned as sitosterol-3-O-(6'-O-6", 9"-octadacadienoyl)- β -D-glucopyranoside, while the minor components were sitosterol-3-O-(6'-O-9"-octadecenoyl)- β -D-glucopyranoside and sitosterol-3-O-(6'-O-hexadecanoyl)- β -D-glucopyranoside.

Compound **10** was obtained as colorless gum. The spectral data of **10** showed the presence of a sugar and an aliphatic long chain with double bonds. This results indicated of a glycolipid (Dey & Harbone, 1990). The $^1\text{H-NMR}$ signals of the sugar moiety [δ 4.28 (d, $J = 7.7$ Hz), 4.02 (d, $J = 2.9$ Hz), 3.99 (dd, $J = 11.8, 6.0$ Hz), 3.89 (dd, $J = 11.8, 3.7$ Hz), 3.65 (dd, $J = 9.5, 7.5$ Hz), 3.60 (dd, $J = 9.5, 2.9$ Hz), 3.55 (br.dd, $J = 4.8$ Hz)] indicated of the presence of a β -D-galactopyranose (Jung et al., 1996; Kobayashi et al., 1992). An ABMXY coupling system connected to oxygenated carbons (δ 70.4, 68.7 and 63.2) in the $^1\text{H-NMR}$ spectrum suggested a glycerol moiety (Jung et al., 1996; Kobayashi et al., 1992). In $^{13}\text{C-NMR}$ spectrum, two carbonyl carbon signals at δ 174.0 and 173.7 suggested two acyl group moieties. The alkaline hydrolysis of **10** afforded the fatty acid methyl ester and glycerylgalactoside. The former was identified as methyl 9, 12, 15-octadecatrienoate by GC-MS analysis, and the latter, $[\alpha]_D^{20} -9.2^\circ$ ($c=0.1$, MeOH), as (2*R*)-1-O-glyceryl- β -D-galactopyranoside (Jung et al., 1996; Kobayashi et al., 1992). The geometry of double bonds of acyl group moieties was determined to be *cis*-form by the coupling constant and $^{13}\text{C-NMR}$ data (Jung et al., 1996). Based on the above consideration and the comparison of the data in the previous papers, the structure of **10** was established as (2*R*)-1, 2-O-(9*z*, 12*z*, 15*z*-dioctadecatrienoyl)-3-O- β -D-galactopyranosyl glycerol.

Compound **11** was obtained as white powder and showed a molecular ion peak at m/z 576. Based on the $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectra data and the comparison of the data in the previous papers (Kim et al., 2001; Cho et al., 1992), the structure of **11** was established as sitosterol-3-O- β -D-glucopyranoside.

Compounds **1~5** and **9~11** were first reported from *Cirsium* species. The *in vitro* cytotoxicity of compounds **1~11** against five cultured human cancer cell lines, A549 (non small cell lung adenocarcinoma), SK-OV-3 (ovarian cancer cells), SK-MEL-2 (skin melanoma), XF498 (CNS cancer cells) and HCT15 (colon cancer cells), using Sulforhodamin-B (SRB) Bioassay, was studied. Compound **2** exhibited moderate cytotoxicity against A549, SK-MEL-2 and HCT15 (ED_{50} : 17.53, 21.84 and 27.91 μM , respectively), compound **4** weak cytotoxicity against SK-MEL-2 and HCT15 (ED_{50} : 20.27 and 20.06 μM , respectively), and compound **3** significant cytotoxicity against SK-OV-3 and SK-MEL-2 (ED_{50} : 4.24 and 2.66 μM , respectively).

The compound **10** showed the selective cytotoxicity against SK-MEL-2 (ED_{50} : 6.64 μM). The other compounds showed little cytotoxic activity against any of the human cancer cell lines tested ($\text{ED}_{50} > 30 \mu\text{M}$).

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REFERENCES

- Agrawal, P. K., Review Article Number 70 ; NMR Spectroscopy in the Structural Elucidation of Oligosaccharides and Glycosides. *Phytochemistry*, 31, 3307-3330 (1992).
- Al-Malaika, S. and Issenhuth, S., The antioxidant role of vitamin E in polymers. . Reaction products of DL- α -tocopherol with lead dioxide and with polyolefins. *Polymer*, 42, 2915-2939 (2001).
- Cabrera, G. M. and Seldes, A. M., Hydroperoxycycloartanes from *Tillandsia recurvata*. *J. Nat. Prod.*, 58(12), 1920-1924 (1995).
- Cho, Y. K., Lee, M. W., Kang, H. M., Lee, H. K. and Kang, S. S., Acylglucosyl Sterols from the Roots of *Caragana chamlagu*. *Kor. J. Pharmacogn.*, 23(1), 14-19 (1992).
- Christensen, L. P., Aplotaxene derivatives from *Cirsium helenioides*. *Phytochemistry*, 31, 2039-2041 (1992).
- Dey, P. M. and Harborne, J. B., Methods in Plant Biochemistry (Vol. 4). Academic Press, London, pp. 72, (1990).
- Geng, P. W., Fukuyama, Y., Wang, R., Bao, J. and Nakagawa, K., An Acylated Sitosterol Glucoside from *Alisma plantago-aquatica*. *Phytochemistry*, 27(6), 1895-1896 (1988).
- Goodman, R. A., Oldfield, E. and Allerhand, A., Assignments in the Natural-Abundance Carbon-13 Nuclear Magnetic Resonance Spectrum of Chlorophyll a and a Study of Segmental Motion in Neat Phytol. *J. Am. Chem. Soc.*, 95, 7553-7558 (1973).
- Greca, M. D., Molinaro, A., Monaco, P. and Previtara, L., Acylglycosyl Sterols from *Pistia stratiotes*. *Phytochemistry*, 30(7), 2422-2424 (1991).
- Guevara, A. P., Lim-Syllianco, C. Y., Dayrit, F. M and Finch, P., Acylglucosyl sterols from *Momordica charantia*. *Phytochemistry*, 28, 1721-1724 (1989).
- Hashimoto, T., Tori, M. and Asakawa, Y., Piscidal Sterol Acylglucosides from *Edgeworthia chrysantha*. *Phytochemistry*, 30(9), 2927-2931 (1991).
- Hikino, H., Meguro, K., Kusano, G. and Takemoto, T., Structure of Mokko Lactone. *Yakugaku Zasshi*, 87(1), 70-74 (1967).
- Inada, A., Murata, H., Inatomi, Y., Nakanishi, T. and Darnaedi, D., Pregnanes and Triterpenoid Hydroperoxides from the

- Leaves of *Aglaia grandis*. *Phytochemistry*, 45(6), 1225-1228 (1997).
- Jung, J. H., Lee, H. K. and Kang, S. S., Diacyl-glycerylgalactosides from *Arisaema amurense*. *Phytochemistry*, 42(2), 447-452 (1996).
- Kato, T., Frei, B., Heinrich, M. and Sticher, O., Antibacterial Hydroperoxysterols from *Xanthosoma robustum*. *Phytochemistry*, 41(4), 1991-1995 (1997).
- Kim, H. J., Le, Q. K., Lee, M. H., Kim, T. S., Lee, H. K., Kim, Y. H., Bae, K. H. and Lee, I. S., A Cytotoxic Secocycloartenoid from *Abies koreana*. *Arch. Pharm. Res.*, 24(6), 527-531 (2001).
- Kim, J. G., Illustrated Natural Drugs Encyclopedia (Color Edition) (Vol. 1). Seoul, Nam San Dang, Seoul, pp. 37, (1984).
- Kobayashi, M., Hayashi, K., Kawazoe, K. and Kitagawa, I., Marine Natural Products. . Heterosigma-glycolipid I, II, III, and IV, Four Diacylglyceroglycolipids Possessing ω 3-Polyunsaturated Fatty Acid Residues, from the Raphidophycean Dinoflagellate *Heterosigma akashiwo*. *Chem. Pharm. Bull.*, 40(6), 1404-1410 (1992).
- Kwon, H. C., Choi, S. Z., Lee, W. B., Min, Y. D., Yang, M. C., Chung, A. K., Lee, K. H. and Lee, K. R., Sesquiterpene Lactones of *Artemisia sylvatica*. *Yakhak Hoeji*, 45(2), 147-152 (2001).
- Lee, K. R., Peroxide Constituents in the Natural Product Research. *Kor. J. Pharmacogn.*, 22, 145-155 (1991).
- Lee, S. J., Korean Folk Medicine. Seoul National University Press, Seoul, pp. 145-146 (1966).
- Lee, T. B., Illustrated Flora of Korea. HyangMunSa, Seoul, pp. 769, (1985).
- Lim, C. N., Arisawa, M., Shimizu, M. and Morita, N., The constituents of *Cirsium japonicum* D.C. var. *takaoense* Kitamura. Isolation of two new flavonoids, Cirsitakaoside (IV) and Cirsitakaogenin (VI). *Chem. Pharm. Bull.*, 26, 2036-2039 (1978).
- Mahato, S. B., Ganguly, N. P. and Sahu, N. P., Review ; Steroid Saponins. *Phytochemistry*, 21, 959-978 (1982).
- Morita, N., Fukuta, M. and Shimizu, M., Studies on the medicinal resources. XXIII. Flavonoids of *Cirsium* Plants (Compositae) in Japan. (4). *Syoyakugaku Zasshi*, 18, 9-11 (1964).
- Morita, N., Shimizu, M. and Arisawa, M., Two new flavone glycosides from *Cirsium lineare*. *Phytochemistry*, 12, 421-423 (1973).
- Muhammad, S. A., Muhammad, S., Waqar, A., Masood, P. and Raghav, Y., A chlorinated monoterpene ketone, acylated β -sitosterol glycoside from *Mentha longifolia* (Lamiaceae). *Phytochemistry*, 59, 889-895 (2002).
- Pavia, D. L., Lampman, G. M. and Kriz, G. S., Introduction to Spectroscopy ; A Guide for Students of Organic Chemistry. Saunders College Publishing, U.S.A., pp. 117, (1996).
- Pouchert, C. J., The Aldrich library of FT-IR spectra (Vol. 1). Aldrich Chemical Company, Inc., U.S.A., pp. 1103, (1993a).
- Pouchert, C. J. and Behnke, J., The Aldrich library of ^{13}C and ^1H FT NMR spectra (Vol. 1). Aldrich Chemical Company, Inc., U.S.A., pp. 297, (1993b).
- Rubnov, S., Kashman, Y., Rabinowitz, R., Schlesinger, M. and Mechoulam, R., Suppressors of Cancer Cell Proliferation from Fig (*Ficus carica*) Resin : Isolation and Structure Elucidation. *J. Nat. Prod.*, 64, 993-996 (2001).
- Shen, Y. M. and Mu, Q. Z., New Furans from *Cirsium chlorolepis*. *Planta Med.* 56, 472-474 (1990).
- Sims, J. J. and JR. Pettus, J. A., Isolation of free *cis* and *trans*-Phytol from the red Alga *Gracilaria andersoniana*. *Phytochemistry*, 15, 1076-1077 (1976).
- Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.*, 82, 1107-1112 (1990).
- Windholz, M., Budavari, S., Blumetti, R. F. and Otterbein, E. S., The Merck Index ; An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck & Co., Inc., U.S.A., pp. 9573, (2001).
- Yuuya, S., Hagiwara, H., Suzuki, T., Ando, M., Yamada, A., Suda, K., Kataoka, T. and Nagai, K., Guaianolides as Immunomodulators. Synthesis and Biological Activities of Dehydrocostus Lactone, Mokko Lactone, Eremanthin, and Their Derivatives. *J. Nat. Prod.*, 62, 22-30 (1999).