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Phytochemical Constituens 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-3β-ol (**2**), 24-hydroperoxycycloart-25-en-3β-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*-(9z, 12z, 15z-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 ED_{50} values ranging from 2.66 to 11.25 μM.

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.

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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 Schimadzu UV-1601 UV-Visible spectrophotometer, respectively. The EI-MS spectrum was measured on JMS700 (JEOL, JAPAN). The ^{1}H- and ^{13}C-NMR spectra were recorded with Varian UNITY INOVA 500 and Brucker AMX 500 spectrometer. GC-MS was Hewlett-Packard 6890 Gas Chromatography-5973 Mass Selective Detector and connected Ultra-2 capillary column (25 m × 200 μm I.D., 0.11 μm d_f) or HP-5MS (30 m × 250 μm I.D.). The preparative HPLC was Knauer preparative HPLC with UV and Refractive dual detector system and connected Econosil® silica 10 u (10 × 250 mm) column. Low-pressure liquid column chromatography (LPLC) was caried out over Lichroprep Si 60 Lobar®-A (Merck, 40-63 μm) and Lichroprep RP-18 Lobar®-A (Merck, 40-63 μm) with a FMI LAB PUMP MODEL QSY (U.S.A.). TLC was performed on precoated Kiesel gel 60F_{254}

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 specemen (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 CH_2Cl_2 three times at room temperature. The concentrated CH_2Cl_2 extract (50 g) was chromatographed over a silica gel column using a gradient solvent system of CH_2Cl_2-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 (CH_2Cl_2-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 CH_2Cl_2-acetone (20:1) to give six subfractions (F80~F85). The F81 subfraction (720 mg) was chromatographed over Sephadex LH-20 (CH_2Cl_2-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 CH_2Cl_2-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 (CH_2Cl_2-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-CH_2Cl_2-MeOH, 4:2:2:1) and further purified with silica gel Lobar®-A column chromatography (n-hexane-EtOAc-CH_2Cl_2-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; $[\alpha]_D$: +0.35°(c=0.5, EtOH) ; UV λ_{max} (EtOH) nm (log ε) : 292 (3.74), 215 (4.32) ; IR ($CHCl_3$) ν_{max}^{-1} : 3439 (OH), 1620 (C=C) ; EI-MS m/z (rel. int): 430 $[M]^+$ (44), 205 (9), 165 (100); ^{1}H-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 (×2), 0.91 (each 3H, s, 4'a, 8'a, 12'a, 13') ; ^{13}C-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-3β-ol (2). White powder; $[\alpha]_D$: +30°(c=0.3, $CHCl_3$) ; mp. : 138°C ; IR ($CHCl_3$) 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); ^{1}H-NMR ($CDCl_3$, 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-3β-ol (3). White powder; $[\alpha]_D$: +46°(c=0.4, $CHCl_3$) ; mp. : 128°C ; IR ($CHCl_3$) ν_{max}^{-1} : 3455 (OH, OOH), 1617 (C=C) ; EI-MS m/z (rel. int.) : 458 $[M]^+$ (4), 203 (39), 175 (71), 161 (40), 147 (50), 135 (64), 121 (64), 107 (80), 95 (100), 81 (71); ^{1}H-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°(c=4.2, $CHCl_3$) ; mp. : 35°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 $[M]^+$ (7), 158 (100), 152 (63), 91 (70), 71 (62), 55 (63) ; ^{1}H-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°(c=0.3, $CHCl_3$) ; UV λmax (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 $[M]^+$ (7), 278 (5), 123 (32), 81 (37), 71 (100), 57 (61) ; ^{1}H-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 (CH_2, 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; ^{1}H-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, -(CH_2)-× 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; ^{1}H-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, -(CH_2)-× 7), 0.89 (3H, t, *J* = 7.0 Hz, Me-18)

Hexadecanoic acid (8). Colorless gum; ^{1}H-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)- × 12), 0.89 (3H, t, J = 7.0 Hz, Me-16)

Sitosterol-3-*O*-[6'-*O*-6", 9"-octadacadienoyl]-β-D-glucopyranoside (9). Colorless gum; UV λ_{max} (EtOH) nm (log ε) : 245 (3.57), 203 (4.13) ; IR ($CHCl_3$) ν_{max}^{-1} : 3449 (OH) 1744 (ester linkage), 1078 (glycosidic C-O) ; Negative-mode FAB-MS m/z (rel. int.) : 838 $[M-H]^+$; ^{1}H-NMR ($CDCl_3$, 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) ; ^{13}C-NMR ($CDCl_3$, 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 Ac_2O (500 μl) 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; ^{1}H-NMR ($CDCl_3$, 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); ^{13}C-NMR ($CDCl_3$, 125 MHz) : δ 173.8 (C-1"), 170.6~169.6 (3 × 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 × 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)

(2*R*)-1, 2-*O*-(9Z, 12Z, 15Z-Dioctadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol (10). Colorless gum; ^{1}H-NMR ($CDCl_3$, 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, H-2", 2"'), 2.06 (8H, m, 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"'), ^{13}C-NMR ($CDCl_3$, 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°(c=0.1, MeOH).

Sitosterol-3-*O*-β-D-glucopyranoside (11). White powder; $[\alpha]_D$: -41.7°(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) ; ^{1}H-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) ; ^{13}C-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, ^{1}H- and ^{13}C-NMR spectral data and the comparison of those in the previous papars (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 $C_{30}H_{50}O_3$ based on ^{13}C-NMR (C30) and molecular ion peak at m/z 458 in EI-MS spectrum. The ^{1}H-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 ^{13}C-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 $C_{30}H_{50}O_3$ based on ^{13}C-NMR (C30) and molecular ion peak at m/z 458 in EI-MS spectrum. The ^{1}H- and ^{13}C-NMR spectra of **3** were similar with those of **2**. The major differences between **2** and **3** in the ^{1}H-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 ^{13}C-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 ^{13}C-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 papars (Cabrera & Seldes, 1995; Kato *et al.*, 1997; Inada *et al.*, 1997), the structure of **3** was established as 24-epimeric mixture of 24-hydrope-roxycycloart-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, ^{1}H-NMR and ^{13}C-NMR spectral data, the molecular formula was deduced to be $C_{15}H_{20}O_2$. 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 ^{1}H- and ^{13}C-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 papars (Hikino *et al.*, 1967; Yuuya *et al.*, 1999), the structure of **4** was established as mokko lactone [4(15), 10(14)-guaiadien-12, 6-olide].

Compound **5** was obtained as colorless oil. On the basis of the EI-MS, ^{1}H- and ^{13}C-NMR spectral data and the comparison of the data in the previous papars (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 ^{1}H-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 ^{1}H- and ^{13}C-NMR spectra indicated the presence of sterol skeleton, which was confirmed by comparing the ^{1}H- and ^{13}C-NMR data with those of β-sitosterol (Guevara *et al.*, 1989). The ^{1}H-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 ^{13}C-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 mutiplet at δ 2.35 (2H, m, H-2") in the ^{1}H-NMR spectrum was indicative

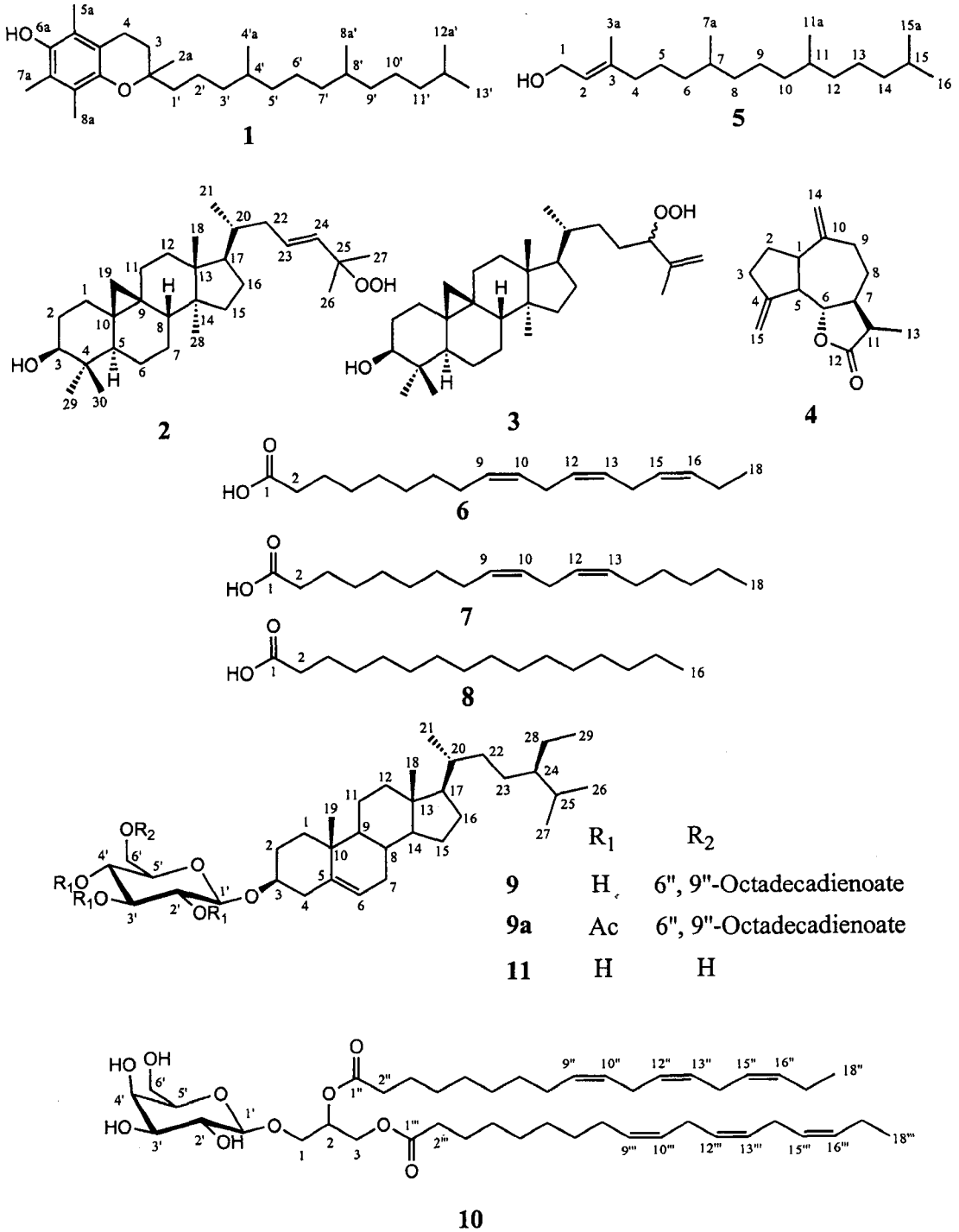

Fig. 1. The structures of compounds **1~11** from *Cirsium setidens*

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 46.3 : 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}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}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}H-NMR spectrum suggested a glycerol moiety (Jung *et al.*, 1996; Kobayashi *et al.*, 1992). In ^{13}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 mehtyl 9, 12, 15-octadecatrienoate by GC-MS analysis, and the latter, $[\alpha]_D$ -9.2° (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}C-NMR data (Jung *et al.*, 1996). Based on the above consideration and the comparison of the data in the previous papars, the structure of **10** was established as (2*R*)-1, 2-*O*-(9z, 12z, 15z-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}H- and ^{13}C-NMR spectra data and the comparison of the data in the previous papars (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 (ED_{50} > 30 μM).

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