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A Polyacetylene and Flavonoids from *Cirsium rhinoceros*

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Cirsium rhinoceros Nakai (Compositae) is a herbaceous perennial native to Korea, whole plant of which has been used in folklore medicine. *C. rhinoceros* was extracted by a standard extraction procedure. Its *n*-hexane, $CHCl_3$ and *n*-BuOH extracts were fractionated by column chromatography to provide a polyacetylene, a coumarin and five flavonoids. Ciryneol C, scopoletin, acacetin, cirsimarin were isolated for the first time from this plant.

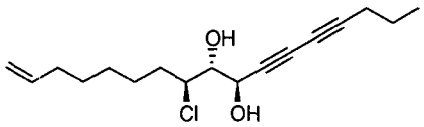
Key words: *Cirsium rhinoceros*, Compositae, Polyacetylene, Coumarin, Flavonoid

INTRODUCTION

Cirsium rhinoceros Nakai (Compositae) is a herbaceous perennial, which grows indigenously in Jeju Island, Korea (Lee *et al.*, 1982). This plant has been used in folklore medicine for the treatment of hematemesis, hematuria and hemorrhage (Kim *et al.*, 1984; Do *et al.*, 1994; Lee *et al.*, 1994).

Various flavones (Harborne *et al.*, 1975; Yun *et al.*, 1978; Do *et al.*, 1994), triterpenes (Dutta *et al.*, 1972), wax (Tulloch *et al.*, 1982), polyolefins, and some widespread acetylenes (Bohlmann *et al.*, 1973; Morita *et al.*, 1973; Yano, 1977; Katsumi *et al.*, 1980; Bohlmann *et al.*, 1981; Takano *et al.*, 1987; Takaishi *et al.*, 1990; Takaishi *et al.*, 1991; Binder *et al.*, 1992) have been isolated from the genus *Cirsium*. Recent studies on *C. rhinoceros* revealed the presence of flavonoids (Lee *et al.*, 1994; Chung *et al.*, 2002), however, no extensive chemical investigation has been performed on *Cirsium rhinoceros*.

As part of our research program on the chemical composition of this plant, we have isolated seven compounds from its whole plant. *C. rhinoceros* was extracted by a standard extraction method. Its *n*-hexane, $CHCl_3$ and *n*-BuOH extracts were fractionated by column chromatography to give ciryneol C (**1**), scopoletin (**2**), acacetin (**3**), cirsimaritin (**4**), cirsimarin (**5**), pectolinarigenin 7-*O*-β-D-glucopyranoside (**6**), and pectolinarin (**7**). The structures of compounds



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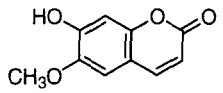

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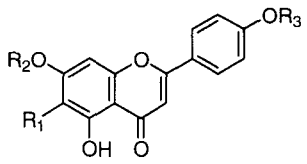

3 $R_1 = R_2 = H$, $R_3 = Me$
4 $R_1 = OMe$, $R_2 = Me$, $R_3 = H$
5 $R_1 = OMe$, $R_2 = Me$, $R_3 = Glc$
6 $R_1 = OMe$, $R_2 = Glc$, $R_3 = Me$
7 $R_1 = OMe$, $R_2 = Rha(1\rightarrow6)Glc$, $R_3 = Me$

Fig. 1. Structures of compounds **1** through **7**

(Fig. 1) were established on the basis of MS, IR, UV and elaborate NMR spectral analyses.

MATERIALS AND METHODS

Experimental procedures

^1H- (500 MHz) and ^{13}C-(125 MHz)NMR spectra were obtained on a Varian Unity INOVA 500 spectrometer (Varian, Inc., U.S.A.). Chemical shifts were expressed in parts per million (ppm) relative to TMS as the internal standard, and coupling constants (*J*) were given in Hz. MS were obtained

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on a Varian Saturn 4D mass spectrometer (Varian, Inc., U.S.A.) and JEOL JMS HX-110/110A tandem mass spectrometer (JEOL Ltd., Japan). IR spectra were obtained on a Jasco FT/IR-300E spectrometer (Jasco Corp., Japan) and UV spectra were recorded on a Jasco V-530 UV/Vis spectrophotometer (Jasco Corp., Japan). HPLC was performed on a Waters LC 600E pump using a Waters μPorasil™ column (5 μm, 300×7.8 i.d.) and Supelco Silica column (3 μm, 150×4.6 i.d.). TLC was carried out on Merck Silica gel F_{254}-precoated glass plates and RP-18 F_{254s} plates. MPLC was carried out with Silica gel 60 (230-400 mesh) and Lobar® A (240-10) Lichroprep® RP-18 (40-63 μm) prepacked columns (Merck).

Plant material

C. rhinoceros was collected in October, 2001 at Mt. Halla Jeju Island, Korea, and dried at room temperature for 2 weeks. This plant was identified by Prof. H. T. Im of Chonnam National University. A voucher specimen was deposited at the College of Pharmacy, Chonnam National University.

Extraction and isolation

C. rhinoceros (827.2 g) was dried and extracted with MeOH at room temperature. The MeOH extract was concentrated *in vacuo* to give a dark green residue (95.9 g). This was diluted with H_2O, and extracted with *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH, successively. The *n*-hexane extract was subjected to silica gel column chromatography (cc) with a $CHCl_3$-MeOH gradient system (95:5 → 85:15) to provide 18 fractions. Fraction 2 (347.5 mg) was rechromatographed on a silica gel MPLC using *n*-hexane-EtOAc (7:3) as an eluent to give compound **1** (26.5 mg). Fraction 14 (550.5 mg) was purified by HPLC (Supelco, $CHCl_3$-MeOH = 20:1) to afford compound **2** (3.5 mg). The $CHCl_3$ extract (5.40 g) was chromatographed on silica gel with *n*-hexane-EtOAc (60:1 → 1:1) to provide 18 fractions. Fraction 5 (405.0 mg) was subjected to silica gel cc by eluting with $CHCl_3$-MeOH (95:5 → 1:2, MeOH 100%) to yield six fractions. The purification of subfraction 3 afforded compound **3** (7.2 mg). Subfraction 2 (261.0 mg) was rechromatographed with $CHCl_3$-MeOH (90:10 → 1:1, MeOH 100 %) to give two fractions. Fraction 2 was purified by HPLC (μPorasil™ 5 μm, 7.8×300, $CHCl_3$ 100%) to afford compound **4** (8.3 mg). The *n*-BuOH extract (15.0 g) was chromatographed on silica gel with $CHCl_3$-MeOH-H_2O ternary gradient system (20:2.5:0.4 → 20:50:10). Compounds **5** (53.4 mg), **6** (13.8 mg) and **7** (80.0 mg) were recrystallized using MeOH.

Ciryneol C (1)

Colorless oil; UV λ_{max} nm: 217, 230, 242, 256; IR ν_{max} (KBr) 2230, 1640, 916 cm^{-1}; EIMS *m/z*: 297 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$) δ: 5.81 (1H, *ddt*, *J* = 17.0, 9.8, 6.8 Hz, H-2), 5.00 (1H, *ddt*, *J* = 17.0, 1.6, 1.4 Hz, H-1t), 4.94 (1H, *ddt*, *J* = 9.8, 1.6, 1.4 Hz, H-1c), 4.52 (1H, *t*, *J* = 6.7 Hz, H-10), 4.28 (1H, *ddd*, *J* = 7.8, 4.5, 4.5 Hz, H-8), 3.71 (1H, *ddd*, *J* = 7.8, 6.7, 4.0 Hz, H-9), 2.27 (2H, *td*, *J* = 7.1, 0.7 Hz, H-15), 2.06 (2H, *q*, *J* = 7.0 Hz, H-3), 1.57 (2H, *sextet*, *J* = 7.0 Hz, H-16), 1.2-1.8 (8H, *m*, H-4~H-7), 1.00 (3H, *t*, *J* = 7.3 Hz, H-17); ^{13}C-NMR ($CDCl_3$) δ: 138.8 (C-2), 114.4 (C-1), 82.3 (C-14), 75.6 (C-9), 72.8 (C-11), 72.1 (C-12), 64.4 (C-8, 10), 64.3 (C-13), 34.5 (C-7), 33.6 (C-3), 28.7 (C-5), 28.4 (C-4), 26.2 (C-6), 21.6 (C-16), 21.2 (C-15), 13.5 (C-17).

7-Hydroxy-6-methoxy-2*H*-1-benzopyran-2-one (2; scopoletin)

Colorless needles; UV λ_{max} nm: 346, 298, 260, 254; IR ν_{max} (KBr) 3280 (OH), 1705, 1560, 1293, 1146 cm^{-1}; EIMS *m/z*: 192 $[M]^+$.

5,7-Dihydroxy-4'-methoxyflavone (3; acacetin)

Yellow needles; UV λ_{max} nm: 268, 327; IR ν_{max} (KBr) 3432, 1656, 1606, 1510 cm^{-1}; EIMS *m/z*: 284 $[M]^+$; ^{1}H-NMR (pyridine-d_5): δ 7.96 (2H, *d*, *J* = 9.0 Hz, H-2', 6'), 7.10 (2H, *d*, *J* = 9.0 Hz, H-3', 5'), 6.95 (1H, *s*, H-3), 6.84 (1H, *d*, *J* = 2.5 Hz, H-8), 6.78 (1H, *d*, *J* = 2.5 Hz, H-6), 3.76 (3H, *s*, 4'-OMe); ^{13}C-NMR (pyridine-d_5) δ: 183.1 (C-4), 166.4 (C-2), 164.4 (C-7), 163.5 (C-4'), 163.3 (C-5), 158.9 (C-9), 128.9 (C-2', 6'), 124.2 (C-1'), 115.2 (C-3', 5'), 105.4 (C-10), 104.9 (C-3), 100.4 (C-6), 95.3 (C-8), 55.9 (4'-OCH_3).

5,4'-Dihydroxy-6,7-dimethoxyflavone (4; cirsimaritin)

Yellow needles; UV λ_{max} nm: 331, 275; IR ν_{max} (KBr) 3401, 1656, 1571, 1509 cm^{-1}; EIMS *m/z*: 315 $[M+H]^+$; ^{1}H-NMR (pyridine-d_5) δ: 13.82 (1H, *s*, 5-OH), 7.95 (2H, *d*, *J* = 8.5 Hz, H-2', 6'), 7.08 (2H, *d*, *J* = 8.5 Hz, H-3', 5'), 6.92 (1H, *s*, H-8), 6.89 (1H, *s*, H-3), 3.96 (3H, *s*, 6-OMe), 3.74 (3H, *s*, 7-OMe); ^{13}C-NMR (pyridine-d_5) δ: 183.2 (C-4), 164.1 (C-2), 162.9 (C-4'), 158.9 (C-7), 154.2 (C-5), 153.7 (C-9), 132.2 (C-6), 128.6 (C-2', 6'), 124.1 (C-1'), 114.9 (C-3', 5'), 105.4 (C-10), 104.1 (C-3), 95.3 (C-8), 60.3 (6-OCH_3), 55.5 (7-OCH_3).

5,4'-Dihydroxy-6,7-dimethoxyflavone 4'-glucoside (5; cirsimarin)

White amorphous powder; UV λ_{max} nm: 324, 276; IR ν_{max} (KBr) 3368, 1656, 1605, 1461, 1098 cm^{-1}; FABMS *m/z*: 477 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6) δ: 12.90 (1H, *s*, 5-OH), 8.08 (2H, *d*, *J* = 9.0 Hz, H-2', 6'), 7.21 (2H, *d*, *J* = 9.0 Hz, H-3', 5'), 6.97 (1H, *s*, H-8), 6.96 (1H, *s*, H-3), 5.41 (1H, *d*, *J* = 4.5 Hz, 2"-OH), 5.15 (1H, *d*, *J* = 4.2 Hz, 3"-OH), 5.08 (1H, *d*, *J* = 4.5 Hz, 4"-OH), 5.05 (1H, *d*, *J* = 6.5 Hz, H-1"), 4.62 (1H, *t*, *J* = 5.4 Hz, 6"-OH), 3.93 (3H, *s*, 7-OCH_3), 3.75 (3H, *s*, 6-OCH_3), 3.71-3.20 (6H, sugar H); ^{13}C-NMR (DMSO-d_6) δ: 182.2 (C-4), 163.3 (C-2), 160.3 (C-4'), 158.6 (C-7), 152.6 (C-9), 151.9 (C-5), 131.8 (C-6), 128.1 (C-2', 6'), 123.8

(C-1'), 116.5 (C-3', 5'), 105.1 (C-10), 103.5 (C-3), 99.7 (C-1"), 91.6 (C-8) 77.1 (C-5"), 76.5 (C-3"), 73.1 (C-2"), 69.6 (C-4"), 60.6 (C-6"), 60.0 (6-OCH_3), 56.4 (7-OCH_3).

Pectolinarigenin 7-*O*-β-D-glucopyranoside (6)

White amorphous powder; UV λ_{max} nm: 328, 276; IR ν_{max} (KBr) 3399, 1660, 1614, 1573, 1070 cm^{-1}; FABMS *m/z*: 477 $[M+H]^+$; ^{1}H-NMR (DMSO-d_6) δ: 12.91 (1H, *s*, 5-OH), 8.06 (2H, *d*, *J* = 9.0 Hz, H-2', 6'), 7.13 (2H, *d*, *J* = 9.0 Hz, H-3', 5'), 7.05 (1H, *s*, H-8), 6.95 (1H, *s*, H-3), 5.43 (1H, *d*, *J* = 5.1 Hz, sugar OH), 5.11-5.16 (2H, sugar OH), 5.08 (1H, *d*, *J* = 5.4 Hz, anomeric H of glc.), 4.63 (1H, *t*, *J* = 5.6 Hz, sugar 6"-OH), 3.87 (3H, *s*, 6-OCH_3), 3.77 (3H, *s*, 4'-OCH_3), 3.15-3.74 (sugar H); ^{13}C-NMR (DMSO-d_6) δ: 182.2 (C-4), 163.7 (C-2), 162.4 (C-4'), 156.4 (C-7), 152.3 (C-5), 152.1 (C-9), 132.4 (C-6), 128.3 (C-2', 6'), 122.7 (C-1'), 114.5 (C-3', 5'), 105.7 (C-10), 103.3 (C-3), 100.1 (C-1"), 94.3 (C-8), 77.2 (C-5"), 76.6 (C-3"), 73.1 (C-2"), 69.5 (C-4"), 60.5 (C-6"), 60.2 (6-OCH_3), 55.5 (4'-OCH_3).

Pectolinarigenin 7-*O*-rutinoside (7; Pectolinarin)

Pale yellow amorphous powder; UV λ_{max} nm: 332, 276; IR ν_{max} (KBr) 3397, 1664, 1614, 1561, 1075 cm^{-1}; FABMS *m/z*: 645 $[M+Na]^+$; ^{1}H-NMR (DMSO-d_6) δ: 12.96 (1H, *s*, 5-OH), 8.05 (2H, *d*, *J* = 9.0 Hz, H-2', 6'), 7.17 (2H, *d*, *J* = 9.0 Hz, H-3', 5'), 6.95 (1H, *s*, H-8), 6.94 (1H, *s*, H-3), 5.24 (1H, *d*, *J* = 5.1 Hz, anomeric H of glc.), 5.20 (1H, *d*, *J* = 4.5 Hz, sugar OH), 5.13 (1H, *d*, *J* = 7.5 Hz, sugar OH), 4.71 (1H, *d*, *J* = 5.7 Hz, sugar OH), 4.59 (1H, *d*, *J* = 4.2 Hz, sugar OH), 4.57 (1H, *d*, *J* = 1.2 Hz, anomeric H of rha.), 4.43 (1H, *J* = 6.0 Hz, sugar OH), 3.87 (3H, *s*, 6-OCH_3), 3.78 (3H, *s*, 4'-OCH_3), 3.10-3.69 (sugar H), 1.06 (3H, *d*, *J* = 6.0 Hz, -CH_3 of rha.); ^{13}C-NMR (DMSO-d_6) δ: 182.3 (C-4), 164.0 (C-2), 162.3 (C-4'), 156.4 (C-7), 152.4 (C-5), 152.1 (C-9), 132.6 (C-6), 128.4 (C-2', 6'), 122.6 (C-1'), 114.7 (C-3', 5'), 105.8 (C-10), 103.3 (C-3), 94.3 (C-8), 60.2 (6-OCH_3), 55.5 (4'-OCH_3); Glc.: 100.3 (C-1"), 76.4 (C-3"), 75.7 (C-5"), 73.1 (C-2"), 69.4 (C-4"), 65.9 (C-6"); Rha.: 100.3 (C-1'"), 71.9 (C-4'"), 70.7 (C-2'"), 70.4 (C-3'"), 68.2 (C-5'"), 17.7 (C-6'").

RESULTS AND DISCUSSION

Fractionation and separation of the extract of *C. rhinoceros* led to the isolation of seven major compounds.

Compound **1** was isolated from *n*-hexane extract with column chromatography. It showed absorptions at 3434 (hydroxyl), 2230 (acetylene) and 1640, 916 (vinyl) cm^{-1} in the IR spectrum. The UV spectrum also showed conjugated triple bonds at 230, 242 and 256 nm. The EI mass spectrum of **1** showed the peak for $[M+H]^+$ at *m/z* 297 ($C_{17}H_{25}ClO_2$). The ^{1}H-NMR spectrum of **1** showed the typical spin system of terminal vinyl methylene group (C-1 ~C-3) at δ 5.00, 4.94, 5.81 and 2.06, a chloride in the molecule attached at δ 4.28 (C-8), methine (C-9, C-10) protons attached to a secondary hydroxyl at δ 3.71 and 4.52, methylene protons of a straight hydrocarbon chain (C-15, C-16) at δ 2.27 and δ 1.57, and the corresponding terminal methyl group protons at δ 1.00. The correlation of these protons was confirmed by 2D NMR spectrum. The ^{13}C-NMR spectrum of **1** showed the typical aliphatic methylene carbons at δ 33.6, 28.4, 28.7, 26.2, 34.5, 21.2 and 21.6, the terminal methyl carbon of the straight aliphatic chain at δ 13.5, the two carbons of the terminal vinyl group at δ 114.4 and δ 138.8, the four carbons of the conjugated triple bonds at δ 72.8, 72.1, 64.3 and 82.3, two carbons attached to the oxygen functionality at δ 75.6, 64.4 and the one carbon attached to the chloride at δ 64.4. The compound **1** was identified as ciryneol C. The relative stereochemistry of ciryneol C at C-8, C-9, C-10 was proposed, although its complete stereochemistry is yet to be unambiguously determined (Takaishi *et al.*, 1990; Baek *et al.*, 1995).

Compound **2** was identified as scopoletin by direct comparison of IR, NMR and UV spectral data with those reported in the literature (Pouchert *et al.*, 1993).

MS, ^{1}H- and ^{13}C-NMR spectra of compound **3** suggested a 5,7-dihydroxy-4'-methoxyflavone having a methoxyl group. It showed absorption at 3432 (hydroxyl), 1656 (carbonyl group), 1606 and 1510 (aromatic alkenes) cm^{-1} in the IR spectrum. The UV spectrum of **3** showed the presence of a flavone at 268, 327 nm. The EI mass spectrum of **3** showed the peak for $[M]^+$ at *m/z* 284 ($C_{16}H_{12}O_5$). The ^{1}H-NMR spectrum of **3** showed a methoxyl group at δ 3.76 (4'-OMe) and 4'-substituted B ring protons at δ 7.10 (H-3', 5') and 7.96 (H-2', 6'). In addition, the presence of the ring A is indicated by three resonance peaks [a quaternary (C-2) at δ 166.4, a methine (C-3) at δ 104.9 and a carbonyl (C-4) at δ 183.1] in ^{13}C-NMR, and a singlet [δ 6.95 (1H, H-3)] in ^{1}H-NMR data. Compound **3** was identified as acacetin by analysis of its spectral data and comparison with literature values (Sinha *et al.*, 1981; Kim *et al.*, 1995).

The UV spectroscopy of compounds **4** and **5** pointed toward flavones. Subsequent MS and NMR analyses identified compounds **4** and **5** as cirsimaritin (Chung *et al.*, 2002) and cirsimaritin 4'-*O*-glucoside (Yun *et al.*, 1978; Park *et al.*, 1995; Hasrat *et al.*, 1997), respectively. It showed absorption at 3401, 3368 (hydroxyl), 1656, 1656 (carbonyl group), 1571, 1606 and 1509, 1461 (aromatic alkenes) cm^{-1} in the IR spectrum, respectively. The FAB mass spectrum of compound **5** showed a $[M+H]^+$ ion at *m/z* 477, indicating a molecular weight of 476, and an intense peak at *m/z* 315 corresponding to the loss of one hexose unit, suggesting an *O*-glucoside (Li *et al.*, 1994). The ^{1}H-NMR spectrum of compound **5** indicated a glycosylated flavonoid with 4'-monosubstitution in the B ring and two methoxyl groups. ^{13}C-NMR spectra of **4** and **5** led to the identification of the

sugar moiety as glucose and the 4'-OH as the glycosylation site. ^{1}H- and ^{13}C-NMR data of the aglycone **4** were in agreement with published data for cirsimaritin (Hasrat *et al.*, 1997). These data indicate that compound **5** is the glucoside of compound **4**.

Compounds **6** and **7** were identified as pectolinarigenin 7-*O*-β-D-glucopyranoside (Williams, 1979) and pectolinarigenin 7-*O*-rutinoside (Lee *et al.*, 1975; Lin *et al.*, 1978; Do *et al.*, 1994), respectively, by direct comparison of IR, NMR and UV spectral data with those reported in the literature (Lee *et al.*, 1994). The peaks at *m/z* 477 and 645 shown in FABMS of **6** and **7** were assigned the molecular formula $C_{23}H_{24}O_{11}$ and $C_{29}H_{34}O_{15}$, respectively.

Cinineol C (**1**), scopoletin (**2**), acacetin (**3**) and cirsimarin (**5**) were isolated for the first time from this plant.

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REFERENCES

Baek, N. -I., Park, J. D., Lee, Y. H., Jeong, S. Y., and Kim, S. I., A novel polyacetylene from *Cirsium* spp. *Yakhak Hoeji*, 39, 268-275 (1995).

Bindel, R. G., Benson, M., Haddon, W. F., and French, R. C., Aplotaxene derivatives from *Cirsium arvense*. *Phytochemistry*, 31, 1033-1034 (1992).

Bohlmann, F. and Abraham, W. -R., Aplotaxene epoxide from *Cirsium hypoleucum*. *Phytochemistry*, 20, 855-856 (1981).

Bohlmann, F., Burkhardt, T., and Zdero, C., *Naturally Occurring Acetylenes*. Academic Press, London (1973).

Chung, A. K., Kwon, H. C., Choi, S. Z., Min, Y. D., Lee, S. O., Lee, W. B., Yang, M. C., Lee, K. H., Nam, J. H., Kwak, J. H., and Lee, K. R., Norisoprenoids from *Cirsium rhinoceros*. *Kor J. Pharmacogn.*, 33, 81-84 (2002).

Do, J. C. Jung, K. Y., and Son, K. H., Isolation of pectolinarin from the aerial parts of *Cirsium nipponicum*. *Kor. J. Pharmacogn.*, 25, 73-75 (1994).

Dutta, C. P., Ray, L. P., and Roy, D. N., Taraxasterol and its derivatives from *Cirsium arvense*. *Phytochemistry*, 11, 2267-2269 (1972).

Harborne J. B., Mabry, T. J., and Mabry, H., *The Flavonoids*. Chapman & Hall, London (1975).

Hasrat, J. A., Pieters, L., Claeys, M., Vlietinck, A., De Backer, J. P., and Vauquelin, G., Adenoside-1 active ligands: cirsimarin, a flavone glycoside from *Microtea debilis*. *J. Nat. Prod.*, 60, 638-641 (1997).

Katsuri, Y., A new acetylenic alcohol from *Cirsium japonicum*. *Phytochemistry*, 19, 1864-1866 (1980).

Kim, J. G., *Illustrated Natural Drugs Encyclopedia (Vol. 1)*. Nam San Dang, Seoul, p. 37 (1984).

Kim, Y. C. and Kingston, D. G. I., Phenolic compounds from *Frullania nisquallensis*. *Kor. J. Pharmacogn.*, 26, 248-252 (1995).

Lee, H. B., Kwak, J. H., Zee, O. P., and Yoo, S. J., Flavonoids from *Cirsium rhinoceros*. *Arch. Pharm. Res.*, 17, 273-277 (1994).

Lee, T. B., *Illustrated Flora of Korea*. Hyang Moon Sa, Seoul, p. 768 (1982).

Lee, Y. C., Lee, Y. W., and Kim, C. K., Chemical studies on the *Cirsium* species (3). Studies on the *Cirsium rhinoceros* Nakai. *Report of Institute of Science and Technology, Sung Kyun Kwan University*, pp. 69-73 (1975).

Li, Q. M. and Claeys, M., Characterization and differentiation of diglycosyl flavonoids by positive ion fast atom bombardment and tandem mass spectrometry. *Biol. Mass Spectrom.*, 23, 406-416 (1994).

Lin, 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).

Morita, N., Shimazu, M., and Arisawa, M., Two new flavone glycosides from *Cirsium lineare*. *Phytochemistry*, 12, 421-423 (1973).

Park, J. C., Lee, J. H., and Choi, J. S., A flavone diglycoside from *Cirsium japonicum*. *Phytochemistry*, 39, 261-262 (1995).

Pouchert, C. J. and Behnke, J., *The Aldrich Library of ^{13}C and ^{1}H-FT NMR spectra (Edition I, Vol. 2)*. Aldrich Chemical Company, Inc., p. 1314 (1993).

Sinha, N. K., Seth, K. K., Pandey, V. B., Dasgupta, B., and Shah, A. H., Flavonoids from the flowers of *Clerodendron infortunatum*. *Planta Med.*, 42, 296-298 (1981).

Takaishi, Y., Okuyama, T., Masuda, A., Nakano, K., Murakami, K., and Tomimatsu, T., Acetylenes from *Cirsium japonicum*. *Phytochemistry*, 29, 3849-3852 (1990).

Takaishi, Y., Okuyama, T., A., Nakano, Masuda, K., Murakami, K., and Tomimatsu, T., Absolute configuration of a triolacetylene from *Cirsium japonicum*. *Phytochemistry*, 30, 2321-2324 (1991).

Takano, S. and Kawaminami, S., Eight aliphatic aldehydes from *Cirsium dipsacolepis* and their stereoselective synthesis. *Phytochemistry*, 26, 435-438 (1987).

Tulloch, A. P. and Hoffman, L. L., Epicuticular wax of *Cirsium arvense*. *Phytochemistry*, 21, 1639-1642 (1982).

Williams, C. A., The leaf flavonoids of the Orchidaceae. *Phytochemistry*, 18, 803-813 (1979).

Yano, K., Hydrocarbons from *Cirsium japonicum*. *Phytochemistry*, 16, 263-264 (1977).

Yun, H. S. and Chang, I. M., Separation and identification of cirsimarin from *Cirsium pendulum*. *Kor. J. Pharmacog.*, 9, 145-147 (1978).