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Flavonoids from *Cirsium rhinoceros*

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Six flavonoids were isolated from the aerial parts of *Cirsium rhinoceros*. The flavonoids were identified as apigenin, luteolin, pectolinarigenin-7-O-β-D-glucopyranoside, linarin, pectolinarin and hispidulin-7-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside on the basis of chemical and spectral evidence.

Key words: *Cirsium rhinoceros*, Compositae, Flavones, Flavone glycosides

INTRODUCTION

Cirsium rhinoceros Nakai, one of the Compositae, is a perennial herb which has been used as folkloric medicine for the treatment of edema, hemorrhage and hematemesis together with other *Cirsium* genus plants (Lee, 1966; Kim, 1984).

This plant is a endemic herb which is only distributed in Jeju Island, Korea (Lee, 1982). Since pectolinarin has been isolated from this plant(Lee *et al.*, 1975), no further phytochemical and biological investigations were carried out. As part of a continuing investigation of the flavonoid compounds in plants and their value as chemotaxonomic markers, we have examined the phytochemical constituents of the aerial parts of this species. This paper deals with the isolation and identification of six flavonoids from *Cirsium rhinoceros*.

MATERIALS AND METHODS

General Experimental Procedures

Melting points were measured on Gallenkamp melting point apparatus and are uncorrected. The NMR spectra were measured with a Bruker AC-200 spectrometer or a Bruker AM-300 spectrometer in DMSO-d_6 or $CDCl_3$ solutions with TMS as internal standard. IR spectra were determined on a Shimadzu IR-435 Infrared spectrophotometer. UV spectra were obtained on a Shimadzu UV 240 UV-Visible recording spectrophotometer. EI-MS were determined on a Hewlett-Packard 5985B GC/MS system equipped with direct inlet system.

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Plant Materal

Aerial parts of *Cirsium rhinoceros* were collected on Mt. Halla of Jejudo, Korea in August 1992. A voucher speciman is deposited in the herbarium of college of Pharmacy, Sungkyunkwan University.

Extraction and Isolation

The dried aerial parts (1.2 kg) were extracted with CH_2Cl_2 at room temperature (2 times). The extracts were combined and concentrated *in vacuo* to give a residue. The marc was extracted with MeOH for 8 h (3 times, at 50 ℃) and concentrated *in vacuo*. The MeOH extract (75 g) was suspended in H_2O and partitioned with CH_2Cl_2, EtOAc and n-BuOH, successively. The EtOAc extract (6.5 g) and n-BuOH extract (15 g) were chromatographed on silica gel with $CHCl_3$-MeOH-H_2O (40 : 10 : 1, 70 : 30 : 3 and 60 : 40 : 4, successively) to give 12 subfractions (F1-F12) on the basis of TLC characteristics. Subfraction F2 was rechromatographed on silica gel with hexane-EtOAc-MeOH (10 : 10 : 1). Further gel filtration on Sephadex LH-20 (Pharmacia) with MeOH afforded Compound **1** (8 mg) and Compound **2** (4 mg). Subfraction F3 was chromatographed on silica gel with $CHCl_3$-MeOH-H_2O (35 : 10 : 1) and was recrystallized from MeOH to give Compound **3** (15 mg). Subfraction F6 and F7 were rechromatographed on a silica gel column by elution with $CHCl_3$-MeOH-H_2O (70 : 30 : 3) to give Compound **4** (58 mg). Compound **5** (23 mg) was obtained from gel filtration of subfraction F8 on Sephadex LH-20 (MeOH : pyridine : H_2O=7 : 2 : 1). Repeated silica gel column chromatography ($CHCl_3$: MeOH : H_2O=70 : 30 : 3) of the

subfraction F10 gave Compound **6** (45 mg).

Compound 1—Yellow amorphous powder, mp. 288-293 ℃; $FeCl_3$, Mg/HCl and Zn/HCl tests: positive.

IR ν_{max} (KBr) cm^{-1} 3400 (OH), 1650 (C=O), 1600, 1500 (aromatic C=C); UV λ_{max} (MeOH) nm: 267, 335, 395sh, λ_{max} (MeOH+NaOMe) nm: 274, 325, 392, λ_{max} (MeOH+$AlCl_3$) nm: 273, 300, 348, 382, λ_{max} (MeOH+$AlCl_3$+HCl) nm: 276, 298, 342, 380, λ_{max} (MeOH+NaOAc) nm: 274, 307, 386, λ_{max} (MeOH+NaOAc+H_3BO_3) nm: 268, 320sh, 342; ^{1}H-NMR (DMSO-d_6) δ 6.18 (1H, d, J=2.1 Hz, H-6), 6.47 (1H, d, J=2.1 Hz, H-8), 6.78 (1H, s, H-3), 6.91 (2H, d, J=8.8 Hz, H-3′,5′), 7.92 (2H, d, J=8.8 Hz, H-2′,6′), 12.96 (1H, s, 5-OH).

Compound 2—Yellow amorphous powder, mp. 288-293 ℃; $FeCl_3$, Mg/HCl and Zn/HCl tests: positive.

IR ν_{max} (KBr) cm^{-1} 3400 (OH), 1660 (C=O), 1605, 1550 (aromatic C=C); UV λ_{max} (MeOH) nm: 253, 266, 287sh, 344, λ_{max} (MeOH+NaOMe) nm: 269, 315sh, 398, λ_{max} (MeOH+$AlCl_3$) nm: 272, 295sh, 340sh, 417, λ_{max} (MeOH+$AlCl_3$+HCl) nm: 262sh, 274, 293, 354, 383, λ_{max} (MeOH+NaOAc) nm: 270, 320, 395, λ_{max} (MeOH+NaOAc+H_3BO_3) nm: 262, 365; ^{1}H-NMR (DMSO-d_6) δ 6.20 (1H, d, J=2 Hz, H-6), 6.46 (1H, d, J=2 Hz, H-8), 6.66 (1H, s, H-3), 6.90 (1H, d, J=8 Hz, H-5′), 7.40 (2H, dd, J=8 Hz, 2Hz, H-2′,6′), 12.9 (1H, s, 5-OH).

Compound 3—White amorphous powder, mp. 177-180 ℃; $FeCl_3$, Mg/HCl, Zn/HCl and Molisch tests: positive.

IR ν_{max} (KBr) cm^{-1} 3400 (OH), 1660 (C=O), 1605, 1575 (aromatic C=C), 1065 (glycosidic C-O); UV λ_{max} (MeOH) nm: 277, 326, λ_{max} (MeOH+NaOMe) nm: 295, 323sh, λ_{max} (MeOH+$AlCl_3$) nm: 263sh, 288sh, 299, 353, 387sh, λ_{max} (MeOH+$AlCl_3$+HCl) nm: 263sh, 288sh, 298, 348, 385sh, λ_{max} (MeOH+NaOAc) nm: 277, 327, λ_{max} (MeOH+NaOAc+H_3BO_3) nm: 277, 327; ^{1}H-NMR (DMSO-d_6) δ 3.10-3.70 (sugar H), 3.75 (3H, s, 4′-OCH_3), 3.85 (3H, s, 6-OCH_3), 5.08 (1H, br. d, J=5.8 Hz, anomeric H of glc.), 6.96 (1H, s, H-3), 7.04 (1H, s, H-8), 7.12 (2H, d, J=9.0 Hz, H-3′,5′), 8.06 (2H, d, J=9.0 Hz, H-2′,6′), 12.91 (1H, s, 5-OH); ^{13}C-NMR see Table I.

Compound 4—Pale yellow amorphous powder, mp. 242-248 ℃; $FeCl_3$, Mg/HCl, Zn/HCl and Molisch tests: positive.

IR ν_{max} (KBr) cm^{-1} 3400 (OH), 1660 (C=O), 1605, 1575 (aromatic C=C), 1065 (glycosidic C-O); UV λ_{max} (MeOH) nm: 268, 326, λ_{max} (MeOH+NaOMe) nm: 292, 370sh, λ_{max} (MeOH+$AlCl_3$) nm: 277, 299, 347, 380sh, λ_{max} (MeOH+$AlCl_3$+HCl) nm: 278, 298, 343, 380sh, λ_{max} (MeOH+NaOAc) nm: 268, 327, λ_{max} (MeOH+NaOAc+H_3BO_3) nm: 268, 328; ^{1}H-NMR (DMSO-d_6) δ 1.07 (3H, d, J=5.8 Hz, rha. -CH_3), 3.08-3.80 (sugar H), 3.87 (3H, s, 4′-OCH_3), 4.58 (1H, br.s, anomeric H of rha.), 5.22 (1H, d, J=6.5 Hz, anomeric H of glc.), 6.47 (1H, d, J=2 Hz, H-6), 6.81 (1H, d, J=2 Hz, H-8), 7.18 (2H, d, J=8.9 Hz, H-3′,5′), 8.07 (2H, d, J=8.9 Hz, H-2′,6′), 12.93 (1H, s, 5-OH); ^{13}C-NMR see Table I.

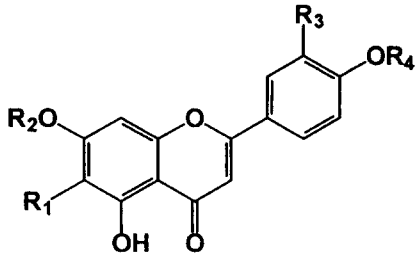
Table I. ^{13}C-NMR spectral data of Compound **3**, **4**, **6** and **6a** in DMSO-d_6

Carbon No.	3	4	6	6a
C-2	165.0	163.0	164.3	163.8
C-3	104.0	104.0	102.7	102.4
C-4	183.3	182.4	182.3	182.2
C-5	153.0[a]	157.0	152.5	152.9
C-6	133.2	100.5[b]	132.8	131.3
C-7	157.2	164.2	155.8	157.2
C-8	95.4	94.8	94.4	94.3
C-9	152.8[a]	161.2	152.0	152.5
C-10	106.4	104.5	105.8	104.1
C-1′	123.5	123.0	121.1	121.2
C-2′	129.4	128.5	128.6	128.5
C-3′	115.5	114.8	116.0	116.0
C-4′	163.5	162.5	161.4	161.2
C-5′	115.5	114.8	116.0	116.0
C-6′	129.4	128.5	128.6	128.5
6-OCH_3	61.3		60.3	60.0
4′-OCH_3	56.3	55.6		
glc. C-1	100.8	100.4[b]	100.0	
C-2	73.7	73.2	75.8[d]	
C-3	76.9	76.8[c]	77.2[d]	
C-4	70.3	69.7	70.5[e]	
C-5	77.7	76.3[c]	77.6[d]	
C-6	61.3	66.3	60.5	
rha. C-1		100.4[b]	97.9	
C-2		70.8	70.3[e]	
C-3		70.4	67.7[e]	
C-4		72.1	72.0	
C-5		68.6	68.6	
C-6		18.2	18.1	

[a,b,c,d,e] Assignments bearing the same superscript in any one spetrum may be reversed in each column

Compound 5—Pale yellow amorphous powder, mp. 262-266 ℃; $FeCl_3$, Mg/HCl, Zn/HCl and Molisch tests: positive.

IR ν_{max} (KBr) cm^{-1} 3400 (OH), 1655 (C=O), 1605, 1565 (aromatic C=C), 1060 (glycosidic C-O); UV λ_{max} (MeOH) nm: 275, 332, λ_{max} (MeOH+NaOMe) nm: 272, 388, λ_{max} (MeOH+$AlCl_3$) nm: 285sh, 300, 358, λ_{max} (MeOH+$AlCl_3$+HCl) nm: 287sh, 298, 353, λ_{max} (MeOH+NaOAc) nm: 272, 340sh, 392, λ_{max}(MeOH+NaOAc+H_3BO_3) nm: 275, 336; ^{1}H-NMR (DMSO-d_6) δ 1.04 (3H, d, J=6.0 Hz, rha. -CH_3), 3.15-3.72 (sugar H), 3.75 (3H, s, 4′-OCH_3), 3.85 (3H, s, 6-OCH_3), 4.57 (1H, br.s, anomeric H of rha.), 5.24 (1H, d, J=5.3 Hz, anomeric H of glc.), 6.93 (1H, s, H-3), 6.94 (1H, s,



1 $R_1=R_2=R_3=R_4=H$

2 $R_1=R_2=R_4=H$, $R_3=OH$

3 $R_1=OCH_3$, $R_2=Glc.$, $R_3=H$, $R_4=CH_3$

4 $R_1=R_3=H$, $R_2=Rha.^{1\rightarrow6}Glc.$, $R_4=CH_3$

5 $R_1=OCH_3$, $R_2=Rha.^{1\rightarrow6}Glc.$, $R_3=H$, $R_4=CH_3$

6 $R_1=OCH_3$, $R_2=Rha.^{1\rightarrow2}Glc.$, $R_3=R_4=H$

H-8), 7.16(2H, d, J=8.9 Hz, H-3',5'), 8.04 (2H, d, J=8.9 Hz, H-2',6'), 12.95 (1H, s, 5-OH).

Compound 6—Yellow amorphous powder, mp. 178-185 ℃; $FeCl_3$, Mg/HCl, Zn/HCl and Molisch tests: positive.

IR ν_{max} (KBr) cm^{-1} 3400 (OH), 1655 (C=O), 1605, 1565 (aromatic C=C), 1060 (glycosidic C-O); UV λ_{max} (MeOH) nm: 275, 332, λ_{max} (MeOH+NaOMe) nm: 272, 388, λ_{max} (MeOH+$AlCl_3$) nm: 285sh, 300, 358, λ_{max} (MeOH+$AlCl_3$+HCl) nm: 287sh, 298, 353, λ_{max} (MeOH+NaOAc) nm: 272, 340sh, 392, λ_{max} (MeOH+NaOAc+H_3BO_3) nm: 275, 336; ^{1}H-NMR (DMSO-d_6) δ 1.12 (3H, d, J=6 Hz, rha. -CH_3), 3.12-3.78 (sugar H), 3.80 (3H, s, 6-OCH_3), 5.22 (1H, br. d, J=1.1 Hz, anomeric H of rha.), 5.30 (1H, d, J=7.2 Hz, anomeric H of glc.), 6.87 (1H, s, H-3), 6.95 (2H, d, J=8.8 Hz, H-3',5'), 7.05 (1H, s, H-8), 7.97 (2H, d, J=8.8 Hz, H-2',6'), 12.96 (1H, s, 5-OH); ^{13}C-NMR see Table I.

Acid hydrolysis of Compound 3, 4 and 5

Compound **3** (8 mg), 4 (15 mg) and 5 (8 mg) in 20% H_2SO_4 (10, 10 and 7 ml) were separately refluxed for 3 h. After cooling, the reaction mixtures were filtered. Each precipitate was purified by recrystallization from MeOH to afford pectolinarigenin (3 mg) from 3, acacetin (6 mg) from 4 and pectolinarigenin (3 mg) from 5, which were identified by direct comparision with authentic samples (Co-TLC, MS, NMR). Each filtrate was neutralized with $BaCO_3$, filtered and concentrated *in vacuo*. D-glucose from 3, and D-glucose and L-rhamnose from 4 and 5 were detected by TLC (precoated cellulose plate, pyridine : EtOAc : HOAc : H_2O= 36 : 36 : 7 : 21).

Acid hydrolysis of Compound 6

Acid hydrolysis of Compound **6** (15 mg) was performed by refluxing with 30% H_2SO_4 (10 ml) for 5 h. Aglycone (Compound **6a**, 5 mg) was obtained from reaction mixture. Compound **6a** was identified as hispidulin on the basis of spectral data. D-glucose and L-rhamnose from the filtrate were detected by TLC. Compound **6a** was recrystallized from MeOH to give yellow amorphous powder, mp. 288-293 ℃; IR ν_{max} (KBr) cm^{-1} 3400 (OH), 1660 (C=O), 1610, 1570 (aromatic C=C); UV λ_{max} (MeOH) nm: 273, 333, λ_{max} (MeOH+NaOMe) nm: 276, 327, 393, λ_{max} (MeOH+$AlCl_3$) nm: 264sh, 280sh, 302, 358, λ_{max} (MeOH+$AlCl_3$+HCl) nm: 263sh, 284sh, 300, 353, 385sh, λ_{max} (MeOH+NaOAc) nm: 274, 302sh, 332sh, 384, λ_{max} (MeOH+NaOAc+H_3BO_3) nm: 276, 327sh, 340, 395sh; MS m/z (rel. int.) 300 (M^+, 100), 285 (M^+-CH_3, 100), 282 (M^+-H_2O, 100), 271 (20.6), 257 (100), 254 (44.3), 167 (16.2), 139 (23.4), 119 (23.8); ^{1}H-NMR (DMSO-d_6) δ 3.73 (3H, s, 6-OCH_3), 6.58 (1H, d, H-3), 6.78 (1H, s, H-8), 6.90 (2H, d, J=8.8 Hz, H-3',5'), 7.91 (2H, d, J=8.8 Hz, H-2',6'), 13.07 (1H,s, 5-OH); ^{13}C-NMR see Table I.

Acetylation of Compound 6

Compound **6** (20 mg) was acetylated with Ac_2O/pyridine at room temperature (for 24 h). The reaction mixture was poured into crushed ice and filtered to give a acetate. Compound **6** acetate (16 mg) was crystallized from MeOH as white powdery crystal, mp. 134-139 ℃; IR ν_{max} (KBr) cm^{-1} 1750, 1230 (acetoxyl), 1610 (aromatic C=C), 1040 (glycosidic C-O); UV λ_{max} (MeOH) nm: 260, 303; ^{1}H-NMR ($CDCl_3$) δ 1.13 (3H, d, J=6.2 Hz, rha. -CH_3), 1.94, 1.95 (×2), 2.03, 2.10, 2.12 (each 3H, s, OAc×6), 2.33 (3H, s, 4'-OAc), 2.47 (3H, s, 5-OAc), 3.87 (3H, s, 6-OCH_3), 3.90-4.30, 4.90-5.40 (sugar H), 5.10 (1H, br.s, anomeric H of rha.), 5.34 (1H, d, J= 5.1 Hz, anomeric H of glc.), 6.57 (1H, s, H-3), 7.08 (1H, s, H-8), 7.24 (2H, d, J=8.7 Hz, H-3',5'), 7.88 (2H, d, J=8.7 Hz, H-2',6').

RESULTS AND DISCUSSION

Chromatographic isolation of the EtOAc and n-BuOH soluble fractions of MeOH extract afforded six compounds (1-6). All compounds showed positive results in $FeCl_3$, Mg/HCl and Zn/HCl tests. The UV spectra exhibited characteristic absorptions for flavones (Markham, 1982).

Compound **1** and **2** were identified as well-known flavones, apigenin and luteolin, by comparision of IR, NMR spectral data and the UV spectral response to shift reagents with those of the reported in literature (Lee *et al.*, 1988) and finally confirmed by direct comparision with authentic samples.

Compound **3** gave positive results to $FeCl_3$, Mg/HCl, Zn/HCl and Molisch tests, indicating to be flavonoid glycoside. The UV spectrum exhibited typical absorp-

tion maxima of flavone at 277 and 326nm. The bathochromic shift of band I in the presence of $AlCl_3$ and $AlCl_3$/HCl indicated the presence of free 5-hydroxyl group. The ^{1}H-NMR spectrum of Compound **3** showed two methoxyl group at δ 3.75 (4'-OCH_3) and 3.93 ppm (6-OCH_3) together with 4'-substituted B ring protons [δ 7.12 (2H, d, J=9.0 Hz, H-3',5'); 8.17 (2H, J=9.0 Hz, H-2',6')] and a singlet at δ 7.04 ppm (1H, H-8). In addition, a singlet at δ 6.96 ppm (1H) assigned to the proton at C-3. Acid hydrolysis of Compound **3** yielded an aglycone and D-glucose as the sugar. The aglycone was identified as pectolinarigenin on the basis of spectral data and by direct comparision with authentic sample(Lee *et al.*, 1975). The ^{13}C-NMR data of Compound **3** supported that glucopyranosyl moiety was attached to 7-position of pectolinarigenin. Furthermore, the ^{1}H-NMR spectrum showed anomeric proton signal of glucose at δ 5.08 ppm(1H, br.d, J=5.8 Hz), thus configuration of D-glucose was determined to be β-linkage. From these evidences, Compound **3** was identified as pectolinarigenin-7-O-β-D-glucopyranoside which was isolated from *Eria javanica* (Williams, 1979).

Compound **4** gave a positive reaction in Molisch test and showed glycosidic C-O bond (1065 cm^{-1}) in IR spectrum. Acid hydrolysis yielded acacetin as aglycone, D-glucose and L-rhamnose. The band II shift by the effect of NaOAc in the UV spectra was not observed, thus 7-hydroxyl group must be glycosylated (Mabry *et al.*, 1970). From carbon signals for D-glucose and L-rhamnose of Compound **4**, L-rhamnose was implying a terminal. The signal for C-6 carbon of the glucose was appeared at the low field (66.3 ppm), indicating that the disaccharide should be rutinose (Markhnam *et al.*, 1976; Markham *et al.*, 1978). Thus Compound **4** was identified as linarin and confirmed by direct comparision with authentic sample.

Compound **5** was identified as pectolinarin, previosuly isolated from the plant, by comparision of spectral and chemical data, and confirmed by direct comparision with authentic sample.

Compound **6**, a yellow amorphous powder, was positive to $FeCl_3$, Mg/HCl, Zn/HCl and Molisch tests. The UV spectrum showed absorption bands at 275 and 332 nm and bathochromic shifts were observed after adding NaOMe, $AlCl_3$, $AlCl_3$HCl and NaOAc reagents which indicated that Compound **6** was a flavone glycoside with the presence of 5,4'-hydroxyl groups and the absence of free hydroxyl groups at C-3 and C-7 (Markham, 1982). The ^{1}H-NMR spectrum of Compound **6** showed a methoxyl group at δ 3.80 (6-OCH_3) and 4'-substituted B ring protons [δ 6.95 (2H, d, J=8.8 Hz, H-3',5'); 7.97 (2H, d, J=8.8 Hz, H-2',6')]. In addition, singlets at δ 6.87 (1H) and δ 7.05 (1H) assigned to the proton at C-3 and C-8, respectively. The ^{1}H-NMR spectrum of its acetate showed six aliphatic (δ 1.94, 1.95 (×2), 2.03, 2.10, 2.12 ppm) and two aromatic acetyl signals (δ 2.33, 2.47 ppm). Acid hydrolysis of Compound **6** yielded an aglycone, D-glucose and L-rhamnose as the sugar. The aglycone was identified as hispidulin on the basis of spectral data (IR, UV, MS and NMR) and confirmed by comparision with spectral data of hispidulin reported in literature (Collado *et al.*, 1985; Liu *et al.*, 1992). Comparision of UV spectra of Compound **6** and **6a** (hispidulin) indicated that the position of glycosylation is at C-7 hydroxyl group (Mabry *et al.*, 1970). The ^{13}C-NMR data of Compound **6** and 6a supported that sugar moiety was attached to 7-position of hispidulin and interglycosidic linkage was determined by ^{13}C-NMR data. It showed ^{13}C-NMR signals for terminal L-rhamnopyranose moiety and glycosidation shift for C-2 chemical shift of inner D-glucopyranose. Thus the interglycosidic linkage is rhamnose 1→2 glucose (neohesperidose) (Markham *et al.*, 1976; Markham *et al.*, 1978). Moreover the ^{1}H-NMR spectrum showed anomeric proton signals of glucose and rhamnose at δ 5.22 (1H, br.d, J=1.1 Hz) and 5.30 ppm (1H, d, J=7.2 Hz). The configurations of D-glucose and L-rhamnose were determined to be β- and α-linkages, respectively. On the basis of the above results, the structure of Compound **6** was characterized as hispidulin-7-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

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