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Flavonoids of *Elscholtzia cristata*

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Abstract □ Apigenin, apigenin-7-O-glucoside, luteolin-7-O-glucoside and linarin were isolated from *Elscholtzia cristata* (Labiatae).

Keywords □ *Elscholtzia cristata,* Labiatae, flavonoids, apigenin apigenin glucoside, luteolin glucoside, linarin.

Elsholtzia cristata(= *E. patrinii*) is an annual herb which is wide spread in Korea. The entire plant is mentioned as astringent, stomachic, diuretic and febrifuge and is recommended in treating abdominal pains, kidney trouble and cholerine; a decoction is prescribed for dropsy, nosebleed and nausea[1].

The chemistry of this plant has not been extensively studied, apart from reports on essential oil, which contains elsholtzia ketone or dehydro-elsholtzia ketone as the main constituent[2,3]. We herein wish to describe the results of the investigation on flavonoid constituents of this plant.

Column chromatography of the ethylacetate soluble fraction of the methanol extract and crystallization yielded four compounds (**1-4**). All compounds showed positive results in $FeCl_3$, Zn + HCl and Mg + HCl and UV spectra characteristic of flavones.

Compound **1** was identified as a well know compound, apigenin by comparison of reported IR and NMR data and the UV spectral response to shift reagents and finally confirmed by direct comparison of an authentic sample.

Compound **2, 3** and **4** gave a positive reaction in Molisch test besides flavone color reactions and showed glycoside bonds (1000-1100 cm^{-1}) in IR spectra. Acid hydrolysis yielded apigenin and glucose from **2**, luteolin and glucose from **3** and acacetin, glucose and rhamnose from **4**. The band II shift of compound **2-4** by the effect of NaOAc was not observed, thus 7-hydroxyl group must be glycosilated. The sugar-sugar linkage of disaccharide in compound **4** was deduced from its ^{13}C-NMR (Table I). All the carbon signals for rhamnose were similar to those of methylrhamnoside, implying a terminal sugar and the signal for primary alcohol carbon of the glucose was appeared at the low field (66.0 ppm), indicating that the disaccharide should be rutinose. Idendity of compound **2, 3** and **4** with apigenin-7-O-glucoside, luteolin-7-O-glucoside and linarin, respectively was confirmed by direct comparison of authentic samples.

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EXPERIMENTAL

Isolation of the flavonoids

The powdered dry whole plants of *E. cristata* collected in the Seoul National University Yun Keun Dong campus was refluxed with MeOH. The MeOH extract was partitioned with $CHCl_3$ and EtOAc, successively. The EtOAc soluble fraction was chromatographed over SiO_2 column eluting $CHCl_3$-MeOH-H_2O (25:6:0.7) to give compounds **1-4**.

Compound 1 (apigenin)

mp, 296-298°. IR ν_{max}^{KBr} cm^{-1}: 3200-3400(OH), 1650(C=O), 1600, 1495(C=C). UV λ_{max}^{MeOH} nm (log ε): 269(3.99), 338(4.07); with NaOMe 276.5 (4.18), 327.5(3.93), 393(4.29); with $AlCl_3$ 277.5 (4.01), 302(3.99), 346(4.09), 384(4.00); with $AlCl_3$ + HCl 278(4.02), 301(4.01), 342.5(4.09), 382.5(3.96); with NaOAc 276(4.10), 301(3.91), 372 (3.98); with NaOAc + H_3BO_3 269.5(4.02), 341 (4.05). MS m/z(%): 270(M^+, 100), 242(M^+ -CO, 45.7), 152(RDA fragment with A ring, 32.5), 118

Table I. ^{13}C NMR chemical shifts of Compound 2, 3, 4 and acacetin(5) (20MHz, DMSO-d_6)

Carbon	2	3	4	5
2	164.3	164.5	163.8	163.9
3	102.8	103.1	103.7	103.9
4	181.7	181.7	181.8	182.3
5	161.5	161.1	161.0	162.2
6	99.5	99.5	99.8	99.4
7	162.8	162.9	162.3	164.8
8	94.9	94.7	94.8	94.3
9	156.8	156.9	156.8	158.0
10	105.4	105.2	105.4	104.4
1′	121.3	121.4	122.6	123.5
2′	128.4	113.5	128.3	128.4
3′	116.0	145.7	114.6	114.8
4′	161.1	149.8	162.9	162.8
5′	116.0	116.0	114.6	114.8
6′	128.4	119.1	128.3	128.4
OCH_3			55.4	55.5
1″	100.2	100.0	100.0	
2″	73.1	73.1	73.0	
3″	76.5	76.4	76.3	
4″	69.8	69.6	69.6	
5″	77.1	77.1	75.7	
6″	61.0	60.6	66.0	
1‴			100.4	
2‴			70.3	
3‴			70.7	
4‴			72.0	
5‴			68.2	
6‴			17.6	

(RDA fragment with B ring, 19.3), 124(152-CO, 40.3). ^{1}H-NMR (80 MHz, DMSO-d_6) : 6.19(1H, d, J=2Hz, H-6), 6.48(1H, d, J=2, H-8), 6.76(1H, s, H-3), 6.92(2H, d, J=8.8 Hz, H-3′, 5′), 7.92(2H, d, J=8.8Hz, H-2′, 6′), 12.94(1H, s, 5-OH).

Compound 2 (apigenin-7-O-glucoside)

mp 192-198°. IR ν_{max}^{KBr} cm^{-1}: 3400(OH), 1670 (C=O), 1610, 1498(C=C), 1000-1100 (glycoside). UV λ_{max}^{MeOH} nm(log ε); 269.5(4.43), 332(4.48); with NaOMe 268(4.35), 391(4.70); with $AlCl_3$ 277.5 (4.37), 300(4.29), 348(4.50), 385(4.42); with $AlCl_3$ + HCl 277.5(4.37), 300(4.29), 343(4.48), 382(4.34); with NaOAc 269.5(4.38), 351(4.38), 387(4.38); with NaOAc + H_3BO_3 269.5(4.43), 339(4.52).

^{1}H-NMR (80MHz, DMSO-d_6) δ : 5.03(1H, d, J=7Hz, anomeric H), 6.44(1H, d, J=2Hz, H-6), 6.83(1H, d, J=2Hz, H-8), 6.84(1H, s, H-3), 6.93 (2H, d, J=8.8Hz, H-3′,5′), 7.95(2H, d, J=8.8Hz, H-2′,6′), 12.92(1H, s, 5-OH).

Compound 3 (luteolin-7-O-glucoside)

mp 250-258°. IR ν_{max}^{KBr} cm^{-1}: 3200-3400(OH), 1650(C=O), 1595, 1490(C=C), 1000-1100 (glycoside). UV λ_{max}^{MeOH} nm (log ε); 256(4.32), 267(4.28), 358(4.41); with NaOMe 264(4.34), 397 (4.45); with $AlCl_3$ 275(4.39), 296.5(4.04), 428 (4.53); with $AlCl_3$ + HCl 275(4.30), 296.5(4.08), 359.5(4.30), 367.5(4.32); with NaOAc 260.5(4.37), 402.5(4.37); with NaOAc + H_3BO_3 260(4.48), 374 (4.47).

^{1}H-NMR (80MHz, DMSO-d_6) δ : 5.07(1H, d, J=7Hz, anomeric H), 6.44(1H, d, J=2Hz, H-6), 6.72(1H, s, H-3), 6.77(1H, d, J=2Hz, H-8), 6.89 (1H, d, J=7Hz, H-5′), 7.39(1H, d, J=2Hz, H-2′), 7.44(1H, dd, J=7 and 2Hz, H-6′), 12.5(1H, s, 5-OH).

Compound 4 (linarin)

mp 248-250°. IR ν_{max}^{KBr} cm^{-1}: 3400(-OH), 1660 (C=O), 1605, 1580, 1570(C=C) 1000-1100 (glycoside). UV λ_{max}^{MeOH} nm (log ε); 270(4.30), 330(4.40); with NaOMe 294(4.43), 370(3.93); with $AlCl_3$ 276(4.26), 301(4.21), 347(4.42), 383(4.32); with $AlCl_3$ + HCl 279(4.28), 301(4.27), 340(4.42), 383(4.22); with NaOAc 270(4.30), 330(4.40); with NaOAc + H_3BO_3 270(4.30), 330(4.40).

^{1}H-NMR(80MHz, DMSO-d_6) δ ; 1.06(3H, d, J=6Hz, CH_3 of rhamnose), 3.83(3H, s, OCH_3), 4.53(1H, brs, anomeric H of rhamnose), 5.04(1H, d, J=5.5Hz, anomeric H of glucose), 6.85(1H, s, H-3) 6.44(1H, d, J=2Hz, H-6), 6.78(1H, d, J=2Hz, H-8), 7.12(2H, d, J=9Hz, H-3′ & 5′), 8.02(2H, d, J=9Hz, H-2′ & 6′), 12.9(1H, s, 5-OH).

Acid hydrolysis of compounds 2, 3 and 4

Each flavonoid (20mg) was refluxed with 5% H_2SO_4 in MeOH for 4hr. Workup in the usual way, followed by crystallization from MeOH afforded apigenin, luteolin and acacetin from compound **2**, **3** and **4**, respectively.

Luteolin

mp 330°. IR ν_{max}^{KBr} cm^{-1}: 3200-3400(OH), 1660 (C=O), 1605, 1498(C=C) UV λ_{max}^{MeOH} nm (log ε); 253(4.29), 267(4.01), 349(4.38); with NaOMe 266(4.59), 401(4.63); with $AlCl_3$ 274(4.53), 426 (4.59); with $AlCl_3$ + HCl 275(4.23), 355(4.24), 385

(4.25); with NaOAc 269(4.39), 384(4.32); with NaOAc + H_3BO_3 259(4.40), 370(4.35).
MS m/z(%): 286(M^+, 100), 152(RDA fragment with A ring, 4) 134(RDA fragment with B ring, 12).
^{1}H-NMR (80MHz, DMSO-d_6) δ : 6.12(1H, d, J=2Hz, H-6), 6.45(1H, d, J=2Hz, H-8), 6.72(1H, s, H-3), 6.83(1H, d, J=7Hz, H-5′), 7.26(1H, d, J=2Hz, H-2′), 7.32(1H, dd, J=7 and 2Hz, H-6′), 12.5(1H, s, 5-OH).

Acacetin

mp: 246-248°. IR ν_{max}^{KBr} cm^{-1}: 3400(OH), 1650 (C=O), 1600, 1580, 1560(C=C). UV λ_{max}^{MeOH} nm (log ε): 271(4.23), 330(4.26); with NaOMe 278 (4.44), 297(Sh. 4.27), 369(4.14); with $AlCl_3$ 278 (4.21), 297(Sh. 4.18), 304(4.22), 346(4.27), 384 (4.16); with $AlCl_3$ + HCl 280(4.20), 297(Sh. 4.19), 304(4.22), 340(4.24), 382(4.03); with NaOAc 278 (4.38), 298(Sh. 4.24), 352(4.12); with NaOAc + H_3BO_3 271(4.23), 330(4.25).
MS m/z(%): 284(M^+, 100), 252(M^+-CO, 4.9), 152(RDA fragment with A ring, 10.8), 132(RDA fragment with B ring, 31.4), 124(152-CO, 11.8), 117 (132-CH_3, 10.8).
^{1}H-NMR (80MHz, DMSO-d_6) δ : 3.86(3H, s, -OMe), 6.21(1H, d, J=2Hz, H-6), 6.51(1H, d, J=2Hz, H-8), 6.85(1H, s, H-3), 7.11(2H, d, J=9Hz, H-3′ & H-5′), 8.04(2H, d, J=9Hz, H-2′ & H-6′), 12.9(1H, s, 5-OH).

The sugars in each aqueous residue free from an aglycone, after removal of acid and concentration, were identified by TLC (cellulose, pyridine-EtOAc-HOAc-H_2O = 36:36:7:21) comparison with authentic sugars as glucose, glucose and glucose + rhamnose for compound **2**, **3** and **4**, respectively.

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LITERATURE CITED

1. Perry, L.M.: Medicinal plants of East and Southeast Asia. MIT, Cambridge, p. 186 (1980).
2. Kobold, V., Vostrowsky, O., Bestmann, H.J., Bisht, J.C., Pant, A.K., Melkani, A.B. and Mathela, C.S.: Terpenoids from Elsholtzia species. II. Constituents of essential oil form a new chemotype of *Elsholtzia cristata*. *Planta Med.* **53**, 268 (1987).
3. Simonov, I.N., Pavlova, T.A. and Demyanov, P.I.: Accumulation of essential oils in *Elsholtzia patrinii* and *Lophanthus anisatus*. Izv. *Timiryazevsk. S-kh. Akad.* **195**, (1987)[CA, 107, 4322g].