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Flavonoids from the Leaves of *Polygala japonica*

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Abstract—From the leaves of *Polygala japonica*, kaempferol (**1**), astragalin (**2**), kaempferol 3-O-(6″-O-acetyl)-β-D-glucopyranoside (**3**) and kaempferol 3,7-di-O-β-D-glucopyranoside (**4**), have been isolated and characterized by chemical and spectral means.

Keywords—*Polygala japonica* • Polygalaceae • kaempferol • astragalin • kaempferol 3-O-(6″-O-acetyl)-β-D-glucopyranoside • kaempferol 3,7-di-O-β-D-glucopyranoside

Polygala japonica Houtt. (Polygalaceae) is a perennial herb which has been used as folkloric medicine for the treatment of asthmatic attacks and menstrual irregularity, and used as a nutritious tonic, sedative and expectorant.[1] The previous authors reported on the isolation of triterpenoidal sapogenin and saponins from the aerial parts of *P. japonica*.[2-4] The present paper deals with the isolation and identification of kaempferol derivatives from the leaves of this plant.

Experimental

All melting points were measured on a Yanaco apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Mattson Polaris TM FT-IR spectrophotometer and the UV spectra were run with Varian DMS 200 UV-Vis spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded with Bruker AM-300 spectrometer with TMS as an internal standard. Chemical shifts are given as ppm. TLC was carried out on precoated Kieselgel 60 F_{254} sheets (Merck) and detection was achieved by UV (254nm) and spraying 10% H_2SO_4 followed by heating. Sugars were run on precoated cellulose plates (Merck) and detected by aniline phthalate.

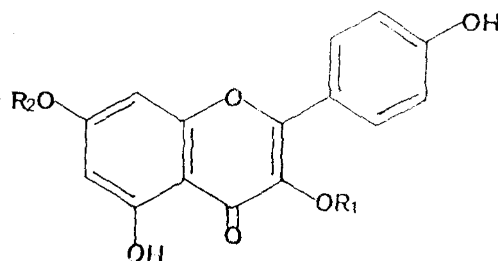
Plant Material—The dried whole plant of *P. japonica* was purchased from a crude drug market in Daegu and a voucher specimen is deposited in College of Pharmacy, Yeungnam University.

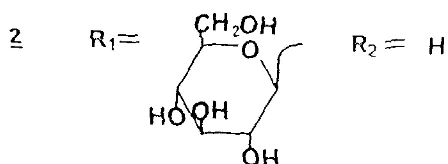
Extraction and Isolation—The dried leaves (1.5 kg) of *P. japonica* were refluxed with hot MeOH. The MeOH extract (278g) was partitioned with n-hexane (43 g), $CHCl_3$ (6.5 g), EtOAc (22 g) and n-BuOH (39 g), successively. The EtOAc extract was chromatographed over a SiO_2 column using $CHCl_3$-MeOH (gradient) followed by Sephadex LH-20 column with MeOH to yield **1**, **2**, and **3**. The n-BuOH extract was subjected to SiO_2 chromatography with EtOAc saturated with H_2O-MeOH (gradient) to give **4**.

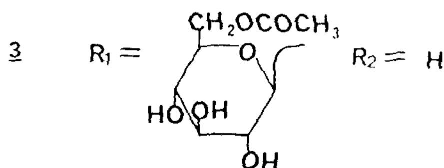
Compound 1 (kaempferol)—Yellowish needles from MeOH, mp 279~280°; $FeCl_3$, Mg/HCl, Zn/HCl test: positive; IR, $\nu_{max}^{KBr}(cm^{-1})$:

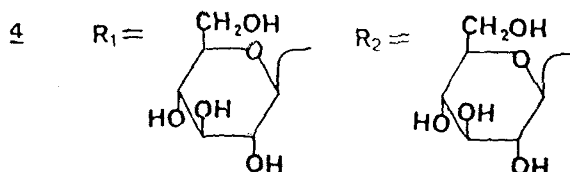
3370 (OH), 1661 (α,β-unsaturated ketone), 1614, 1570, 1510 (aromatic C=C); UV, λ_{max} (MeOH) nm (log ε): 268 (4.20), 325 (4.09), 366 (4.37); λ_{max} (MeOH+NaOMe) nm (log ε): 282 (4.35), 315 (4.02), 427 (4.40); λ_{max} (MeOH+$AlCl_3$) nm (log ε): 272 (4.30), 306 (3.86), 353 (4.02), 425 (4.43); λ_{max} (MeOH+$AlCl_3$+HCl) nm (log ε): 272 (4.28), 304 (3.86), 350 (4.06), 423 (4.38); λ_{max} (MeOH+NaOAc) nm (log ε): 275 (4.34), 309 (4.07), 390 (4.29); λ_{max} (MeOH+NaOAc+H_3BO_3) nm (log ε): 269 (4.22), 305 (4.02), 369 (4.34); ^{1}H-NMR, (DMSO-d_6) δ: 6.21 (1H, d, J=2.1Hz, H-6), 6.45 (1H, d, J=2.1Hz, H-8), 6.94 (2H, d, J=8.8Hz, H-3′ and 5′), 8.06 (2H, d, J=8.8Hz, H-2′ and 6′); ^{13}C-NMR: see Table I.

Compound 2 (astragalin)—Pale yellowish needles from MeOH, mp 183~185°; $FeCl_3$, Mg/HCl, Zn/HCl, Molisch test: positive; IR, ν_{max}^{KBr} (cm^{-1}): 3439 (OH), 1665 (α,β-unsaturated ketone), 1610, 1565, 1510 (aromatic C=C), 1059 (glycosidic C—O); UV, λ_{max} (MeOH) nm (log ε): 267 (4.29), 309 (4.07), 350 (4.21); λ_{max} (MeOH+NaOMe) nm (log ε): 275 (4.39), 326 (4.14), 401 (4.43); λ_{max} (MeOH+$AlCl_3$) nm (log ε): 274 (4.31), 305 (4.02), 352 (4.19), 397 (4.20); λ_{max} (MeOH+$AlCl_3$+HCl) nm (log ε): 275 (4.31), 302 (4.04), 346 (4.17), 396 (4.10); λ_{max} (MeOH+NaOAc) nm (log ε): 275 (4.43), 309 (4.09), 384 (4.23); λ_{max} (MeOH+NaOAc+H_3BO_3) nm (log ε): 268 (4.30), 305 (4.06), 353 (4.24); ^{1}H-NMR,









(DMSO-d_6) δ : 5.46 (1H, d, J=7.2Hz, anomeric H), 6.21 (1H, d, J=2.1Hz, H-6), 6.43 (1H, d, J=2.1Hz, H-8), 6.89 (2H, d, J=9.0Hz, H-3′ and 5′), 8.04 (2H, d, J=9.0Hz, H-2′ and 6′); ^{13}C-NMR: see Table I.

Compound 3 [kaempferol 3-O-(6″-O-acetyl)-β-D-glucopyranoside]—Yellowish powder from MeOH, mp 262~264°; $FeCl_3$, Mg/HCl, Zn/HCl, Molisch test: positive; IR, ν_{max}^{KBr} (cm^{-1}) : 3440 (OH), 1718 (ester), 1660 (α,β-unsaturated ketone), 1570, 1520 (aromatic C=C), 1075 (glycosidic C—O); UV, λ_{max} (MeOH) nm (log ε): 268 (4.18), 349 (4.25); λ_{max} (MeOH+NaOMe) nm (log ε): 276 (4.32), 326 (4.15), 402 (4.43); λ_{max} (MeOH+$AlCl_3$) nm (log ε): 275 (4.20), 306 (4.01), 355 (4.19), 399 (4.21); λ_{max} (MeOH+$AlCl_3$+HCl) nm (log ε): 276 (4.21), 304 (4.03), 347 (4.19), 397 (4.13); λ_{max} (MeOH+NaOAc) nm (log ε): 275 (4.37), 309 (4.13), 389 (4.24); λ_{max} (MeOH+NaOAc+H_3BO_3) nm (log ε): 295 (4.11), 355 (4.29); ^{1}H-NMR, (DMSO-d_6) δ : 1.76 (3H, s, OAc), 5.35 (1H, d, J=7.2Hz, anomeric H), 6.21 (1H, d, J=2.0Hz, H-6), 6.44 (1H, d, J=2.0Hz, H-8), 6.88 (2H, d, J=8.8Hz, H-3′ and 5′), 8.00 (2H, d, J=8.8Hz, H-2′ and 6′); ^{13}C-NMR: see Table I.

Compound 4 (kaempferol 3,7-di-O-β-D-glucopyranoside)—Yellowish powder from MeOH, mp 160~163°; $FeCl_3$, Mg/HCl, Zn/HCl, Molisch test: positive; IR, ν_{max}^{KBr} (cm^{-1}) : 3400 (OH), 1650 (α,β-unsaturated ketone), 1595, 1490 (aromatic C=C), 1080 (glycosidic C—O); ^{1}H-NMR, (DMSO-d_6) δ : 5.07 (1H, d, J=7.4Hz, anomeric H), 5.47 (1H, d, J=7.3Hz, anomeric H), 6.44 (1H, d, J=2.0Hz, H-6), 6.79 (1H, d, J=2.0Hz, H-8), 6.89 (2H, d, J=8.8Hz, H-3′ and 5′), 8.06 (2H, d, J=8.8Hz, H-2′ and 6′); ^{13}C-NMR: see Table I.

Acid hydrolysis of 2, 3 and 4—Forty mg of each compound was refluxed with 5% methanolic H_2SO_4 (20ml) for 1hr. After cooling, the reaction mixture was filtered. The precipitates were crystallized from MeOH to give the common aglycone, kaempferol (**1**), which was identified by direct comparison with an authentic sample (co-TLC and mmp). Each filtrate was neutralized with Ag_2CO_3, filtered and concentrated in vacuo. D-Glucose from **2**, **3** and **4** was detected by TLC.

Alkaline hydrolysis of 3—A mixture of 3

Table I. ^{13}C-NMR spectral data for **1**~**4** in DMSO-d_6.

Carbon No.		1	2	3	4
	2	146.8	156.4	156.6	156.8
	3	135.7	133.2	133.1	133.5
	4	175.9	177.9	177.3	177.6
	5	160.7	161.3	161.2	160.8
	6	98.2	98.7	98.7	99.3
	7	163.9	164.2	164.2	162.8
	8	93.5	93.7	93.6	94.5
	9	156.2	156.3	156.4	156.0
	10	103.0	104.0	103.9	105.6
	1′	121.7	120.9	120.7	120.8
	2′	129.5	130.9	130.8	130.9
	3′	115.4	115.1	115.0	115.1
	4′	159.2	160.0	160.0	160.1
	5′	115.4	115.1	115.0	115.1
	6′	129.5	130.9	130.8	130.9
3-Glc	1″		101.0	101.2	100.7
	2″		74.2	74.0[a]	74.2
	3″		77.5	76.1	76.4
	4″		69.9	69.7	69.9
	5″		76.4	73.9[a]	77.5
	6″		60.9	62.7	60.8[a]
7-Glc	1‴				99.8
	2‴				73.1
	3‴				76.4
	4‴				69.7
	5‴				77.2
	6‴				60.6[a]
O$\underline{C}$OCH$_3$				169.7	
OCO$\underline{C}$H$_3$				20.1	

[a] Assignment may be reversed in the vertical column.

(10mg) and 0.5N KOH in EtOH (5ml) was refluxed for 1hr, and then neutralized with 0.5N aqueous H_2SO_4. The resulting mixture was partitioned between EtOAc and water. The EtOAc layer was evaporated in vacuo and purified by SiO_2 column chromatography to give astragalin (2).

Results and Discussion

Chromatographic isolation of the EtOAc and n-BuOH soluble fractions yielded four compounds (1-4). All compounds showed positive results in $FeCl_3$, Zn/HCl and Mg/HCl tests, and UV spectra exhibited characteristic absorptions for flavonols.[5]

Compound 1 was identified as a well-known flavonol, kaempferol, by comparison of IR, NMR data, and the UV spectral response to shift reagents in literature[6] and finally by comparison with an authentic standard.

Compounds 2, 3, and 4 gave positive Molisch and flavonoid color reactions, and showed absorption bands for glycoside linkages (1000~1100 cm^{-1}) in their IR spectra. On acid hydrolysis each compound gave glucose as the common sugar and kaempferol as the common aglycone. The ^{1}H-NMR spectrum of 2 showed one anomeric proton signal indicating the presence of one mole of glucose in 2. The glycosidic position at C-3 was determined by the UV maxima at 350~360nm.[5] This was further confirmed by the inspection of ^{13}C-NMR spectrum. The configuration of glucosidic linkage was determined by the J value of the anomeric proton signal. Thus the structure of 2 was elucidated as kaempferol 3-O-β-D-glucopyranoside (astragalin).

Compound 3 gave quite similar UV and ^{1}H-NMR spectra to those of 2 except for the presence of one acetoxy methyl singlet at δ1.76 in the ^{1}H-NMR spectrum. The alkaline hydrolysis of 3 gave astragalin, which was identified by direct comparison with an authentic sample. The position of an acetyl group in 3 was determined as follows. In the ^{1}H-NMR spectrum, the glucosyl methylene protons (6''-H_2) of 3 [δ 3.96(1H, dd, J=11.8, 5.9Hz) and 4.12 (1H, dd, J=11.8, 2.0Hz)] resonated downfield from the corresponding signals for usual glucosides.[7] Furthermore, in comparison of the ^{13}C-NMR spectral data of 3 with those of 2, C-6'' and C-5'' chemical shifts were shifted downfield by 1.8 ppm and upfield by 2.5 ppm, respectively, due to the acylation shifts. These data suggested the presence of a (6-O-acetyl)-D-glucoside moiety in 3. From the above findings, the structure of 3 was elucidated as kaempferol 3-O-(6''-O-acetyl)-β-D-glucopyranoside. The ^{1}H-NMR spectrum of 4 showed two anomeric proton signals, indicating the presence of two moles of glucose in 4. On comparison of the ^{13}C-NMR spectrum of 4 with that of astragalin (2), the C-7 signal of 4 exhibited upfield shift (−1.4 ppm) than that of 2, and the C-6, -8 and -10 signals of 4 showed downfield shifts (+0.6ppm, +0.8ppm and +1.6ppm, respectively). These results indicated that one mole each of glucose was linked to the C-3 and C-7 hydroxyl groups of 4.[8] Finally, the structure of 4 was assigned to be kaempferol 3,7-di-O-β-D-glucopyranoside.

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