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Flavonoids from the Whole Plants of *Orostachys japonicus*

Hee Juhn Park, Han Suk Young§, Kun Young Park*, Sook Hee Rhee*, Hae Young Chung and Jae Sue Choi**

College of Pharmacy, Pusan National Univeristy, Pusan 609-735, Korea
**Department of Food Science and Nutrition, Pusan National University, Pusan 609-735, Korea*
***Department of Nutrition and Food Science, National Fisheries University of Pusan, Pusan 608-737, Korea*
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Abstract □ From the whole plants of *Orostachys japonicus*, kaempferol, quercetin, astragalin, quercitrin, isoquercitrin, cynaroside, afzelin, 3-*O*-α-L-rhamnosyl-7-*O*-β-D-glucosyl kaempferol, and 3,7-di-*O*-β-D-glucosyl kaempferol were isolated and characterized by spectral data.

Keywords □ Flavonoids, *Orostachys japonicus*, ^{13}C-NMR

Orostachys japonicus (Crassulaceae) is a perennial herb which is fairly distributed over Korea, and the herbs have been used as a Chinese crude drug for the treatment of fever, breding and intoxication and used in folk medicine as an anti-cancer agents[1]. Since its chemistry has not yet been investigated, we have examined the herbs and here report the results.

EXPERIMENTAL METHODS

All melting points were measured on a Thomas Hoover 6406-H apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Shimadzu IR-400 spectrophotometer and the UV spectra were runned with CE 599 Universal automatic scanning spectrophotomer. The ^{1}H- and ^{13}C-NMR spectra were obtained on either a Bruker AM-300 or a Jeol-GX 400 spectrometer using TMS as an internal standard. The FAB mass spectrum was taken with Kratos MS 25 RFA spectrometer. For TLC, Kieselgel 60 F_{254} sheets (Merck) were used.

Plant material

The *O. japonicus* used was purchased from the Chinese herb medicine shop at the Pyongwha market, Pusan, Korea. A voucher specimen is deposited in the herbarium of College of Pharmacy, Pusan National University, Pusan, Korea.

Extraction, fractionation and isolation

Dried whole plants of commercially available *O. japonicus* were extracted with MeOH under reflux. The MeOH extract (102 g) was partitioned with *n*-hexane (13 g), $CHCl_3$ (27 g), EtOAc (10 g), *n*-BuOH (15 g) and H_2O (4 g) sucessively. The EtOAc extract (10 g) was chromatographed over silica gel using $CHCl_3$:MeOH:H_2O (65:35:10, lower phase) mixture to give **1** (0.24 g), **2** (0.14 g), **3** (0.13 g), **4** (0.15 g), **5** (0.125 g), and **6** (0.25 g). The *n*-BuOH extract (13 g) was subjected to chromatography using SiO_2 (solvent; EtOAc:MeOH:H_2O=600:99:81) column to give **7** (0.03 g), **8** (0.55 g) and **9** (0.015 g).

Compound 1 (kaempferol)

Yellowish needless from MeOH, mp. 277-279℃, $FeCl_3$, Mg/HCl, Zn/HCl; positive. IR ν_{max}^{KBr} (cm^{-1}): 3350 (-OH), 1667 (α, β-unsaturated ketone), 1620, 1575, 1510 (aromatic C=C), 1375, 1245, 1175, 810. UV λ_{max}^{MeOH} nm: 257 (sh.), 269, 300 (sh.), 330, 370, $\lambda_{max}^{MeOH+NaOMe}$ nm: 280, 320, 420, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 270, 308, 350, 428, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 258 (sh.), 270, 308, 350, 427, $\lambda_{max}^{MeOH+NaOAc}$ nm: 274, 310, 380,

§To whom all correspondence should be addressed

$\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm: 270, 298, 324, 370.

Compound 2 (quercetin)

Yellowish needles from MeOH-H_2O (1:1), mp. 310-313℃, $FeCl_3$, Mg/HCl, Zn/HCl; positive, IR ν_{max}^{KBr} (cm^{-1}): 3380, 3300 (-OH), 1670 (α, β-unsaturated ketone), 1610, 1510, (aromatic C=C), 1360, 1315, 1240, 1160, 1090, 995, 817. UV λ_{max}^{MeOH} nm: 258, 305 (sh.), 375, $\lambda_{max}^{MeOH+NaOMe}$ nm: 248 (sh.), 335, 420 (dec.), $\lambda_{max}^{MeOH+AlCl_3}$ nm: 275, 340 (sh.), 460, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 270, 307 (sh.), 365, 435, $\lambda_{max}^{MeOH+NaOAc}$ nm: 260 (sh.), 278, 328, 388, $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm: 243, 285 (sh.), 372.

Compound 3 (afzelin)

Yellowish needles from MeOH-H_2O (1:1), mp. 173-178℃, $FeCL_3$, Mg/HCl, Zn/HCl, Molisch test; positive. IR ν_{max}^{KBr} (cm^{-1}): 3400-3100 (broad, -OH), 1655 (α, β-unsaturated ketone), 1605 (aromatic C=C), 1355, 1170, 1100-1000 (glycosidic linkage). UV λ_{max}^{MeOH} nm: 268, 357, $\lambda_{max}^{MeOH+NaOMe}$ nm: 276, 402, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 274, 351, 403, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 274, 351, 403, $\lambda_{max}^{MeOH+NaOAc}$ nm: 273, 361, $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm: 268, 350. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.81 (3H, d, J=5.6 Hz, rha-CH_3), 5.32 (1H, d, J=1.47 Hz, anomeric), 6.21 (1H, d, J=2 Hz, H-6), 6.41 (1H, d, J=2 Hz, H-8), 6.92 (2H, d, J=8.8 Hz, H-3′ and H-5′), 7.76 (2H, d, J=8.8 Hz, H-2′ and H-6′), 12.63 (1H, s, H_5-OH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: see Table I.

Compound 4 (astragalin)

Pale yellowish needles from MeOH, mp. 230-233℃, $FeCl_3$, Mg/HCl, Zn/HCl, Molish test: positive. IR ν_{max}^{KBr} (cm^{-1}): 3500-3100 (broad, -OH), 1560 (α, β-unsaturated ketone), 1650, 1575, 1505 (aromatic C=C), 1350, 1170, 1180, 1100-1000 (glycosidic linkage). UV λ_{max}^{MeOH} nm: 267, 300 (sh.), 352, $\lambda_{max}^{MeOH+NaOMe}$ nm: 275, 327, 400, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 269, 295 (sh.), 306, 352, 398, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 276, 296 (sh.), 304, 347, 398, $\lambda_{max}^{MeOH+NaOAc}$ nm: 276, 307, 370, $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm: 261, 379. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 5.54 (1H, d, J=7.2 Hz, anomeric), 6.21 (1H, d, J=2.1 Hz, H-6), 6.43 (1H, d, J=2.1 Hz, H-8), 6.89 (2H, d, J=8.8 Hz, H-3′ and H-5′), 8.04 (2H, d, J=8.8 Hz, H-2′ and 6′).

Compound 5 (quercitrin)

Yellowish needles from MeOH, mp. 186-187℃, $FeCl_3$, Mg/HCl, Zn/HCl, Molisch test: positive. IR ν_{max}^{KBr} (cm^{-1}): 3500-3100 (broad, -OH), 1650 (α, β-unsaturated ketone), 1600, 1570, 1505, (aromatic C=C), 1360, 1275, 1175, 1100-1000 (glycosidic linkage). UV λ_{max}^{MeOH} nm: 256, 265 (sh.), 301 (sh.), 350, $\lambda_{max}^{MeOH+NaOMe}$ nm: 270, 326, 393, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 276, 304 (sh.), 333, 430, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 272, 303 (sh.), 353, 401, $\lambda_{max}^{MeOH+NaOAc}$ nm: 272, 322 (sh.), 372, $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm: 260, 300 (sh.), 367. ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 0.80 (3H, d, J=5.8 Hz, rha-CH_3), 5.28 (1H, d, J=1.47 Hz, anomeric), 6.23 (1H, d, J=2.1 Hz, H-6), 6.39 (1H, d, J=2.1, H-8), 6.88 (1H, d, J=8.3 Hz, H-5′), 7.27 (1H, dd, J=2.1 Hz and 8.3 Hz, H-6′), 7.32 (1H, d, J=1.9 Hz, H-2′). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: see Table I.

Compound 6 (isoquercitrin)

Yellowish needles from MeOH, mp. 234-236℃, $FeCl_3$, Mg/HCl, Zn/HCl, Molisch test: positive. IR λ_{max}^{KBr} (cm^{-1}): 3500-3100 (broad, -OH), 1650 (α, β-unsaturated ketone), 1596, 1590, 1480 (aromatic C=C), 1350, 1285, 1195, 1100-1000 (glycosidic linkage). UV λ_{max}^{MeOH} nm: 258, 359, $\lambda_{max}^{MeOH+NaOMe}$ nm: 273, 412, $\lambda_{max}^{MeOH+AlCl_3}$ nm: 276, 435, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 271, 404, $\lambda_{max}^{MeOH+NaOAc}$ nm: 275, 376, $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm: 262, 380. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 5.46 (1H, d, J=7.9 Hz, anomeric), 6.20 (1H, d, J=1.8 Hz, H-6), 6.40 (1H, d, J=1.8 Hz, H-8), 6.85 (1H, d, J=9.2 Hz, H-5′), 7.58 (1H, d, J=2.4 Hz, H-2′), 7.58 (1H, dd, J=2.4 and 9.2 Hz, H-6′). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: see Table I.

Compound 7 (cynaroside)

Pale yellowish powder from MeOH, mp. 259-260℃, $FeCl_3$, Mg/HCl, Zn/HCl, Molisch test: positive. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 5.06 (1H, d, J=7.2 Hz, anomeric), 6.44 (1H, d, J=2.1 Hz, H-6), 6.71 (1H, s, H-3), 6.78 (1H, d, J=2.1 Hz, H-8), 6.89 (1H, d, J=8.4 Hz, H-5′), 7.41 (1H, dd, J=2.1 and 8.4 Hz, H-6′), 7.45 (1H, d, J=2.1 Hz, H-2′). ^{13}C-NMR (DMSO-d_6, 100 MHZ) δ: see Table I.

Compound 8 (3-O-α-L-rhamnosyl-7-O-β-D-glucosyl kaempferol)

White needles from MeOH-H_2O, mp. 256-258℃, $FeCl_3$, Mg/HCl, Zn/HCl, Molisch test: positive. IR ν_{max}^{KBr} cm^{-1}: 3500-3100 (broad, -OH), 1650 (α, β-unsaturated ketone), 1602, 1510, 1494 (aromatic C=C), 1450, 1210, 1181, 1100-1000 (glycosidic linkgae).

Table I. ^{13}C-NMR spectral data of compound 1-9 and related compounds in DMSO-d_6

Carbon No.	1[a]	2[a]	3	4	5	6	7	8	9	A[a,b]	B[a,b]
2	146.8	146.9	157.3	156.4	157.2	156.4	164.9	157.7	156.8	147.9	156.8
3	135.6	135.6	134.3	133.2	134.2	133.4	103.1	134.5	133.5	136.0	133.8
4	175.9	175.7	177.8	177.4	177.7	177.4	181.8	177.9	177.6	176.1	177.6
5	160.7	160.7	161.4	161.2	161.3	161.3	161.1	160.9	160.9	160.5	160.9
6	98.2	98.2	98.8	98.7	98.6	98.7	99.5	99.3	99.3	99.2	99.4
7	163.9	163.9	164.3	164.2	164.2	164.4	162.9	162.9	162.8	162.9	162.8
8	93.5	93.4	93.8	93.6	93.5	93.6	94.7	94.6	94.4	94.8	94.5
9	156.2	156.2	156.6	156.2	156.4	156.2	156.9	156.1	156.0	156.0	156.0
10	103.1	103.0	104.2	104.0	104.0	103.9	105.3	105.8	105.7	105.0	105.8
1′	121.7	122.0	120.7	120.9	121.0	121.6	121.3	120.3	120.8	121.7	120.9
2′	129.5	115.3	130.6	130.8	115.4	115.3	113.5	130.6	131.0	129.6	130.7
3′	115.4	145.0	115.5	115.1	145.1	144.8	145.7	115.4	115.1	115.6	115.0
4′	159.2	147.6	160.0	159.9	148.4	148.5	149.8	160.1	160.1	159.4	160.0
5′	115.4	115.6	115.5	115.1	115.6	116.2	115.9	115.4	115.1	115.6	115.0
6′	129.5	120.0	130.6	130.8	120.7	121.2	119.1	130.6	131.0	129.6	130.7
3-Rha											
1			101.8		101.8			101.8			
2			70.6*		70.0*			70.0*			
3			70.2*		70.3*			70.2*			
4			71.3		71.2			71.1			
5			70.6*		70.5*			70.6*			
6			17.5		17.4			17.4			
3-Glc											
1				101.0		100.9			100.7		
2				74.2		74.1			74.2		
3				76.4		76.5			76.4		
4				69.9		69.9			69.9		
5				77.4		77.6			77.5		
6				60.9		61.0			60.8		
7-Glc											
1							99.9	99.9	99.7	100.5	
2							73.0	73.1	73.1	73.4	
3							76.3	76.4	76.4	76.7	
4							69.2	69.6	69.6	70.1	
5							77.1	77.2	77.1	77.3	
6							60.6	60.6	60.6	61.25	

*Values with the same symbol may be interchanged in the vertical column
[a]data taken from ref. 7. [b]A: kaempferol 7-··-glucoside. B: kaempferol 3-*O*-glucosyl-7-*O*-rhamnoside.

UV λ_{max}^{MeOH} nm (log ε): 232 (sh. 4.42), 267 (4.54), 320 (4.31), 346 (4.38), $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε): 248 (4.46), 272 (4.46), 385 (4.49), $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε): 230 (4.47), 276 (4.59), 304 (4.25), 354 (4.43), 400 (4.38), $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε): 228 (4.49), 276 (4.56), 302 (4.27), 345 (4.40), 396 (4.28), $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε): 268 (4.54), 320 (sh. 4.23), 351 (4.36), $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm (log ε): 267 (4.58), 318 (sh., 4.36), 346 (4.43). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.82 (3H, d, J=5.6 Hz, rha-CH$_3$), 5.06 (1H, d, J=7.2 Hz, anomeric), 5.34 (1H, d, J=1.5 Hz, anomeric), 6.46 (1H, d, J=2 Hz, H-6), 6.76 (1H, d, J=2 Hz, H-8), 6.93 (2H, d, J=8.9 Hz, H-3′ and 5′), 7.79 (2H, d, J=8.9 Hz, H-2′ and 6′). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: see Table I FABMS (*m/z*, %): 617 [(M+Na)$^+$, 21], 595 [(M+H)$^+$, 40].

Compound 9 (3,7-di-glucosyl kaempferol)

Yellowish needles from MeOH, mp. 147-148℃, $FeCl_3$, Mg/HCl, Zn/HCl, Molisch test: postive. IR ν_{max}^{KBr} cm $^{-1}$: 3500-3100 (broad, -OH), 1650 (α, β-unsaturated ketone), 1604 (aromatic C=C), 1356, 1170, 1100-1000 (glycoside). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ: 5.07 (1H, d, J=7.0 Hz, anomeric), 5.47 (1H, d, J=7.0 Hz, anomeric), 6.44 (1H, d, J=1.8 Hz, H-6), 6.78 (1H, d, J=1.8 Hz, H-8), 6.89 (2H, d, J=8.8 Hz, H-3′ and 5′), 8.06 (2H, d, J=8.8 Hz, H-2′ and 6′). ^{13}C-NMR (DMSO-d_6, 75.5 MHz) δ: see Table I.

Acid hydrolysis of 3,4,5,6,7,8 and 9

Forty mg of each compound was refluxed with 5%-H_2SO_4 (50 ml) for 3 hrs. After cooling, the reaction mixture was filtered. The aglycone was crystallized from MeOH to give kaempferol from **3**,**4**,**8** and **9**, quercetin from **5** and **6** and luteolin from **7** which were confirmed by direct comparisons with authentic samples (TLC, mmp, and UV). The filtrate was neutralized with $BaCO_3$, filtered and concentrated *in vacuo*. L-rhamnose from **3** and **5**, D-glucose from **4**, **6**, **7** and **9**, L-rhamnose and D-glucose from **8** were detected by TLC.

Enzymatic hydrolysis of 8

Thirty mg of **8** was incubated with β-glucosidase for 1 hr. The reaction mixture was filtered and the filtrate was partitioned, concentrated *in vacuo* and crystallized from MeOH-H_2O to give afzelin as yellow needles, mp. 234-236℃. It was confirmed by direct comparisons with compound **3** (TLC, mmp, and UV). The water layer was concentrated *in vacuo*. D-glucose was identified by TLC.

Enzymatic hydrolysis of 9

Thirty mg of **9** was incubated with β-glucosidase for 1 hr. The reaction mixture was filtered and the filtrate was partitioned, concentrated *in vacuo* and crystallized from MeOH to give astragalin as yellow needles, mp. 230-233℃. It was confirmed by direct comparisons with compound **8** (TLC, mmp, UV). The water layer was concentrated *in vacuo*. D-glucose was identified by TLC.

RESULTS AND DISCUSSION

Silica gel column chromatography of the ethyl acetate and *n*-BuOH soluble portions of the MeOH extract yielded nine compounds (**1**-**9**) in the order of increasing polarity. Compounds **1**, **2** and **4**-**7** were readily elucidated as kaempferol, quercetin, astragalin, quercitrin, isoquercitrin and cynaroside, respectively by comparison of reported spectroscopic data[2-5] and finally confirmed by comparison with authentic samples.

Compounds **3**, **8** and **9** showed positive results in Molisch tests besides flavonoid color reactions and showed absorption bands for glycoside linkages (1,000-1,100 cm $^{-1}$) in their IR spectra. On acid hydrolysis yielded all compounds gave kaempferol as the aglycone and L-rhamnose from **3**, L-rhamnose and D-glucose from **8** and D-glucose from **9** as the sugar. The ^{1}H-NMR spectrum of **3** showed only one anomeric proton signal indicating the presence of one mole of L-rhamnose in **3**. The glycosidic position at C-3 was determined by the UV maxima at 350-360 nm. This was further confirmed by the inspection of ^{13}C-NMR spectrum (see Table I). The configuration and conformation of sugar moiety was determined by the J value of the anomeric proton signal (see Experimental). Thus, the structure of **3** was elucidated as kaempferol 3-*O*-α-L-rhamnopyranoside (afzelin). The ^{1}H-NMR spectra of **8** and **9** showed two anomeric proton signal, indicating the presence of two mole of sugar in each compound. The band **2** in the UV spectra of each compound was not affected by an addition of NaOAc, indicating that 7-hydroxy group must be glycosylated[6]. Additionally, enzymatic treatment of **8** with β-glucosidase gave a product which was identified by UV and ^{1}H-NMR as compound **3**. These results indicated the linkage of L-rhamnose to the 3-*O*-position and D-glucose at the 7-*O*-position in **8**. In the same way, enzymatic treatment of **9** with β-glucosidase gave a product which was identified as compound **4**. The linkage of each D-glucose to the 3- and 7-*O*-position in **9** was also indicated. These were further confirmed by the inspection of the ^{13}C-NMR spectra (see Table I). Glycosylation with L-rhamnose at C-3 appeared to have a more marked effect on the C-3 signal (0.7-1.3 ppm) than with other sugars and this difference has a diagnostic value[7]. As shown in Table I, the signal of **8** was deshielded when compared with **9**. Thus, the structures of **8** and **9** were elucidated as 3-*O*-α-L-rhamnosyl-7-*O*-D-glucosyl kaempferol and 3,7-di-*O*-β-D-

glucosyl kaempferol, respectively.

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