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Flavonoid Glycosides from the Fronds of *Pyrrosia lingua*

Jae Chul Do, Keun Young Jung and Kun Ho Son*

College of Pharmacy, Yeungnam University, Kyongsan 712-749 and
*Department of Food and Nutrition, Andong National University, Andong 760-749, Korea

Abstract—Two flavonoid glycosides, astragalin(**1**) and liquiritin(**2**), were isolated from the fronds of *Pyrrosia lingua*.

Keywords—*Pyrrosia lingua* · Polypodiaceae · flavonoid glycoside · astragalin · liquiritin

Pyrrosia lingua Farwell(Polypodiaceae) is a medicinal plant which has been used as a remedy for anuria and gonorrhea.[1] The isolation of kaempferol, quercetin, isoquercetin, trifolin, β-sitosterol, sucrose and chlorogenic acid from the dried sample of *P. lingua* was previously reported.[2] The present paper deals with the isolation of one flavanone and one flavonol glycosides from this plant.

Experimental Method

The mps were taken on a Yanaco micro-melting point apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Perkin-Elmer 841 spectrophotometer and the UV spectra were run with a Varian DMS 200 UV-Vis spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AM-300 spectrometer with TMS as an internal standard and chemical shifts are given as ppm. TLC chromatography was performed on precoated Kieselgel 60 F_{254} plates(Merck, 5715).

Plant Material—The dried fronds of *P. lingua* were purchased from a crude drug market in Daegu and a voucher specimen is deposited in College of Pharmacy, Yeungnam University.

Extraction, Fractionation and Isolation—Dried fronds of *P. lingua* (500 g) were extracted with MeOH under reflux. The MeOH extract (97 g) was partitioned with n-hexane (17 g), $CHCl_3$ (3 g), EtOAc (3 g) and n-BuOH (10 g), successively. The EtOAc extract was subjected to flash column chromatography over silica gel using $CHCl_3$—MeOH gradient(0 to 10%) elution system to give 10 fractions. Fraction 5(6% fraction) was rechromatographed over silica gel with EtOAc-MeOH-H_2O(20 : 1 : 0.5, upper phase), yielding compound **1**. Fraction 6(7% fraction) was subjected to Sephadex LH-20 column chromatography eluted with MeOH to yield compound **2**.

Astragalin(**1**)—Yellowish needles from MeOH, mp 183~185°, $FeCl_3$, Mg/HCl, and Molisch tests: positive. IR, ν_{max}^{KBr} 3435(OH), 1660(α,β-unsaturated C=O), 1605, 1575, 1505(C=C), 1100~1000(glycosidic C-O)cm^{-1}; UV, λ_{max}^{MeOH} nm (log ε) 266(4.18), 306(3.96), 357(4.12); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε) 277(4.31), 329(4.09), 403 (4.38); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε) 278(4.24), 303 (3.96), 352(4.13), 400(4.15); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε) 278(4.24), 302(3.99), 348(4.15), 394(4.02); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε) 277(4.37), 308 (4.02), 381(4.10); $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm (log ε) 268(4.25), 297(3.98), 355(4.12); ^{1}H-NMR (DMSO-d_6) δ : 5.42(1H, d, J=7.2 Hz, anomeric

Table I. ^{13}C-NMR Spectral data for **1** and **2** in DMSC-d_6

Carbon No.	1	2	Carbon No.	1	2
C-2	156.3[a]	78.6	Glc C-1″	100.9	100.3
C-3	133.2	43.1	C-2″	74.1	73.2
C-4	177.4	189.8	C-3″	76.4	76.6
C-5	161.1	128.3	C-4″	69.9	69.7
C-6	98.6	110.5	C-5″	77.3	77.0
C-7	164.0	164.6	C-6″	60.8	60.7
C-8	93.5	102.5			
C-9	156.1[a]	163.0			
C-10	103.9	113.5			
C-1′	120.8	132.3			
C-2′	130.8	127.9			
C-3′	115.0	116.2			
C-4′	159.8	157.4			
C-5′	115.0	116.2			
C-6′	130.8	127.9			

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

H), 6.21(1H, d, J=2.0 Hz, H-6), 6.43(1H, d, J=2.0 Hz, H-8), 6.89(2H, d, J=8.9 Hz, H-3′ and 5′), 8.03 (2H, d, J=8.9 Hz, H-2′ and 6′); ^{13}C-NMR: see Table I.

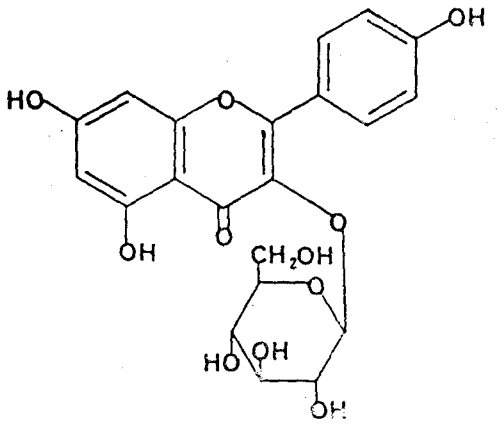
Acid Hydrolysis of Compound 1—Solution of **1** (30mg) in 5% methanolic H_2SO_4 was refluxed for 30 min, and the reaction mixture was diluted with ice water. The precipitate was collected by filtration and purified by recrystallization from MeOH to afford yellowish needles, mp 279~280°, which was identified as kaempferol by direct comparison with an authentic sample (co-TLC and mmp). The filtrate was neutralized with Ag_2CO_3, filtered and concentrated in vacuo. D-glucose from **1** was detected by TLC.

Liquiritin (2)—White amorphous from MeOH, mp 209~211°, $FeCl_3$, Mg/HCl and Molisch tests: positive. IR, ν_{max}^{KBr} 3337(OH), 1652(α,β-unsaturated C=O), 1608, 1558, 1512 (C=C), 1100~1000(glycosidic C-O) cm^{-1}; UV, λ_{max}^{MeOH} nm (log ε) 275(4.10), 310(3.94); $\lambda_{max}^{MeOH+NaOMe}$ nm (log ε) 253(4.59), 336(4.33); $\lambda_{max}^{MeOH+AlCl_3}$ nm (log ε) 276(4.12), 311(3.92), 400(4.15); $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm (log ε) 277(4.13), 310(3.91), 394(4.02); $\lambda_{max}^{MeOH+NaOAc}$ nm (log ε) 253(3.94), 274(3.90), 335(4.25); $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ nm (log ε) 274(4.11), 312(3.92); ^{1}H-NMR(DMSO-d_6) δ : 2.68(1H, dd, J=17.0 and 2.8 Hz, H-3b) and 3.12(1H, dd, J=17.0 and 12.6 Hz, H-3a), 4.88(1H, d, J=7.2 Hz, anomeric H), 5.53(1H, dd, J=12.6 and 2.8 Hz, H-2), 6.35(1H, d, J=2.0 Hz, H-8), 6.51(1H, dd, J=8.7 and 2.0Hz, H-6), 7.07(2H, d, J=8.7 Hz, H-3′ and 5′), 7.44(2H, d, J=8.7 Hz, H-2′ and 6′), 7.65(1H, d, J=8.7 Hz, H-5); ^{13}C-NMR: see Table I.

Results and Discussion

Compound **1** gave positive $FeCl_3$, Mg-HCl and Molisch tests and showed absorption bands for glycoside linkage(1000~1100cm^{-1}) in its IR spectrum, indicating to be a flavonoid glycoside. Acid hydrolysis of **1** yielded kaempferol and D-glucose. The ^{1}H-NMR spectrum of **1** showed one anomeric proton signal indicating the presence of one mole of D-glucose in **1**. Compound **1** showed the UV maxima at 357nm[3] and on the comparison of the ^{13}C-NMR chemical shifts of **1** with those of kaempferol,[4] the signals corresponding to C-2, C-3 and C-4 of **1** revealed glycosidation shifts[5] at C-2(+9.5ppm), C-3(−2.5ppm) and C-4(+1.5ppm), suggesting that glucose unit was attached at C-3 of kaempferol. The configuration of sugar moiety was determined by J value of the anomeric proton signal (see Experimental). Thus, the structure of **1** was elucidated as kaempferol 3-O-β-D-glucopyranoside (astragalin) and direct comparison (co-TLC and mmp) with an authentic standard[4] supported this conclusion.

Compound **2** showed positive result in Molisch test besides flavonoid color reactions and showed



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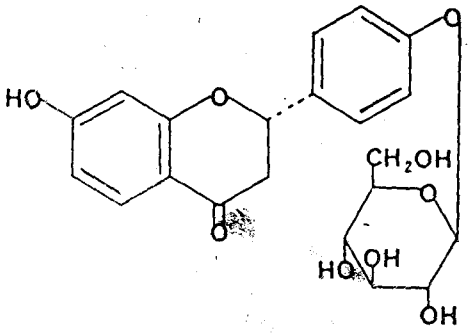

2

absorption bands for glycoside linkage (1000-1100 cm^{-1}) in its IR spectrum. The UV spectrum exhibited typical absorption maxima (band II) of flavanone at 275nm. From the bathochromic UV shifts observed in MeOH+NaOMe (61nm), and MeOH+NaOAc (60nm), it was evident that **2** possessed one free hydroxyl group at C-7.[3] The ^{1}H-NMR spectrum of **2** showed typical signal pattern ascribable to rings A and C of flavanone with one hydroxyl group at C-7. Besides, the signals at δ 7.07 (2H, J=8.7 Hz) and 7.44 (2H, J=8.7 Hz) were resolved into AA'BB' system due to a *para*-substituted benzene ring, and the signal of one anomeric proton was observed at δ 4.88 (d, J=7.2 Hz). Finally, the absolute stereochemistry at C-2 was assumed to be *S* on the basis of couplings recorded for H-2 (J=12.6 and 2.8 Hz) showing this proton with the axial orientation.[6] These data indicated that **2** was a 7, 4'-oxygenated flavanone (liquiritigenin) with one sugar. In the ^{13}C-NMR spectrum of **2**, the signals of β-glucopyranosyl moiety were observed. On comparison of the ^{13}C-NMR data of **2** with that of liquiritigenin,[7] the signals due to C-3', C-4', and C-5' of **2** revealed significant glycosidation shifts[5] at C-3' (+2.3ppm), C-4' (−2.2ppm), and C-5' (+2.3ppm), indicating that glucose unit was attached at C-4' of liquiritigenin. In the light of the above evidence, the structure of **2** could be assigned as liquiritigenin 4'-O-β-D-glucopyranoside (liquiritin) and the comparison with literature data[8] established its identity.

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