Two New Flavanone Glycosides of *Jasminum lanceolarium* and Their Anti-oxidant Activities

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Two new flavanone glucosides, (2*S*)-5,7,3′,5′-tetrahydroxy-flavanone 7-*O*-*β*-D-allopyranoside (1) and (2*S*)-5,7,3′,5′-tetrahydroxy-flavanone 7-*O*-*β*-D-glucopyranosie (2) were isolated from the stems and leaves of *Jasminum lanceolarium*, along with five known compounds: Betulinaldehyde (3), betulinic acid (4), betulin (5), syringin (6) and Liriodendrin (7). Their structures were determined on the basis of spectroscopic and chemical methods. The isolated compounds were screened for their *in vitro* antioxidant activity through DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. Compounds 2 demonstrated significant radical scavenging activity.

Key words *Jasminum lanceolarium*; flavanone glycoside; antioxidative activity

Jasminum is a genus of about 200 species, several members of which are known for their medicinal application in Chinese folklore. About 47 species of *Jasminum* have been reported to occur in China.[1,2] *Jasminum lanceolarium* is a climbing shrub distributed over thickets at low altitudes from south-eastern China to India.[3] Its steams and roots are used in Chinese medicine for the treatment of fever and rheumatic pain. The leaves are also used as an antiinflammatory agent for the release of pain in the eyes.[4] Previous phytochemical investigation resulted in the isolation of several secoiridoid glycosides.[4,5] This paper reports the isolation and structural elucidation of two new flavanone glucosides, (2*S*)-5,7,3′,5′-tetrahydroxy-flavanone 7-*O*-*β*-D-allopyranoside (**1**) and (2*S*)-5,7,3′,5′-tetrahydroxy-flavanone 7-*O*-*β*-D-glucopyranosie (**2**), along with five known compounds obtained from the stems and leaves of this plant and their DPPH radical scavenging activities.

Results and Discussion

The ethanolic extract of the shade-dried stems and leaves of *J. lanceolarium* was evaporated *in vacuo*, then the residue was suspended in H_2O and successively partitioned with petroleum (60—90 °C), $CHCl_3$, EtOAc and *n*-BuOH. Compound **3** was isolated from the petroleum (60—90 °C)-soluble fraction and compounds **4** and **5** were obtained from the $CHCl_3$-soluble fraction, while compounds **1**, **2**, **6** and **7** were isolated from the EtOAc-soluble fraction of *J. lanceolarium* by successive use of column chromatography. Of the seven compounds elucidated, the five known compounds were subsequently identified as Betulinaldehyde (**3**),[6] betulinic acid (**4**),[7] betulin (**5**),[7] syringin (**6**)[8] and Liriodendrin (**7**)[9] by comparisons of their spectral data (UV, IR, NMR and MS) with those reported previously. All of these known compounds were isolated from *J. lanceolarium* for the first time.

Compound 1 Compound **1** was obtained as a pale yellow powder. Its molecular formula was established as $C_{21}H_{22}O_{11}$ by HR-FAB-MS at *m/z* 451.1220 $[M+H]^+$ (Calcd 451.1240). The IR absorption bands at 3383, 1643, 1578 and 1068 cm^{-1} were consistent with the presence of hydroxyl, carboxyl, aromatic ring, and ether groups respectively. The characteristic UV absorption bands (225, 283, 322 nm) showed compound **1** to be a flavanone. That was confirmed by the ABX system protons at δ_H 2.75 (1H, dd, *J*=17.2, 3.6 Hz), 3.23 (1H, m), and 5.44 (1H, dd, *J*=12.8, 3.6 Hz) in its ^{1}H-NMR spectrum and at δ_C 78.9 and 42.3 in its ^{13}C-NMR spectrum (Table 1).[10] The ^{1}H-NMR spectrum showed three phenolic hydroxyl group at δ_H 12.05 , 9.06 and 9.02 (D_2O exchangeable). The ^{13}C-NMR spectrum (Table 1) resolved 21 carbon signals, corresponding to a flavanone skeleton bearing three hydroxyl group and one sugar moiety. These signal patterns were similar to those of 5,7,3′,5′-tetrahydroxy-flavanone except for the sugar moiety.[11] In the aromatic region of the ^{1}H-NMR spectrum, the remaining five protons occurred as a set of meta coupled doublets at δ_H 6.13 (1H, d, *J*=2.0 Hz) and 6.11 (1H, d, *J*=2.0 Hz) for the A-ring protons, and three singlets δ_H 6.89 (1H, br s), 6.76 (1H, br s

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data of Compounds **1** and **2**, δ (ppm) in DMSO-d_6

Position	**1**		**2**	
	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C
2	5.44 dd (12.8, 3.6)	78.9	5.44 dd (12.8, 3.2)	78.7
3	2.75 dd (17.2, 3.6 H *cis*) 3.23 m (H *trans*)	42.3	2.72 dd (17.2, 3.2 H *cis*) 3.23 m (H *trans*)	42.1
4		197.3		197.2
5		163.1		162.9
6	6.11 d (2.0)	96.5	6.13 d (2.0)	96.4
7		165.7		165.2
8	6.13 d (2.0)	95.8	6.14 d (2.0)	95.4
9		162.9		162.7
10		103.4		103.2
1′		129.4		129.2
2′	6.76 br s	118.2	6.75 br s	118.1
3′		145.9		145.8
4′	6.89 br s	114.6	6.88 br s	114.4
5′		145.4		145.2
6′	6.76 br s	115.5	6.75 br s	115.3
1″	5.14 d (7.2)	98.3	4.97 d (7.6)	99.5
2″	3.41—3.45 m	70.2	3.13—3.25 m	73.0
3″	3.91 br s	71.6	3.13—3.25 m	76.3
4″	3.41—3.45 m	67.1	3.13—3.25 m	69.5
5″	3.71 m	75.0	3.13—3.25 m	77.0
6″	3.41—3.45, 3.67 m	61.0	3.40 m, 3.65 dd (10.4, 5.2)	60.6
5-OH	12.05 s		12.05 s	
3′-OH	9.06 s		9.09 s	
5′-OH	9.02 s		9.03 s	

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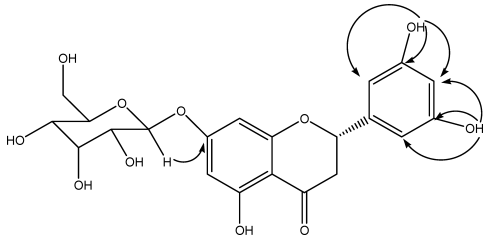

Fig. 1. Important HMBC Correlations of **1**

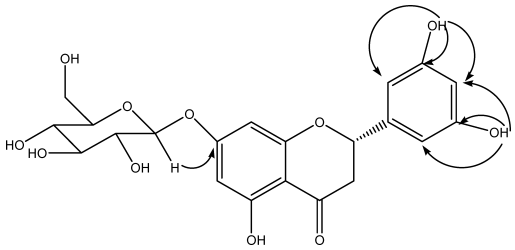

Fig. 2. Important HMBC Correlations of **2**

and 6.76 (1H, br s) for the B-ring protons. These data indicated that the substitution pattern of the A-ring was 5-hydroxy-substituted and 7-oxygenated, which of the B-ring was 3′,5′-dihydroxy-substituted, and the latter three signals were assigned to the C-4′, C-2′ and C-6′ protons, respectively, from their chemical shifts and coupling patterns. This was confirmed by the HMBC experiment, long-range correlations were observed between the following protons and carbons: 5-OH and 5, 6, 10-C; 3′-OH and 2′, 3′, 4′-C; 5′-OH and 4′, 5′, 6′-C (Fig. 1). One sugar moiety was D-allose obtained from aqueous acid hydrolysis of compound **1** by comparison of TLC analysis with an authentic sample and confirmed by comparing the ^{13}C-NMR data (δ_C 98.3, 75.0, 71.6, 70.2, 67.1, 61.0) with those reported in the literatures.[12] Its configuration was determined to be β-oriented as judged by the coupling constants of one anomeric protons at δ_H 5.14 (1H, d, J=7.2 Hz) in the ^{1}H-NMR spectrum. In the HMBC spectrum (Fig. 1), the proton at δ_H 5.14 (1H, d, J=7.2 Hz, H-1′) was correlated with C-7 (δ_C 165.7), suggesting that one β-D-allose moiety was located at C-7. The circular dichroism (CD) spectrum showed a positive cotton effect at 325 nm and a negative one at 284 nm, consistent with the *S*-configuration at C-2.[13,14] On the basis of the above evidence, compound **1** was determined to be (2*S*)-5,7,3′,5′-tetrahydroxy-flavanone 7-*O*-β-D-allopyranoside.

Compound 2 Compound **2** was also obtained as a pale yellow powder. Its molecular formula was established as $C_{21}H_{22}O_{11}$ by HR-FAB-MS at *m/z* 451.1234 $[M+H]^+$ (Calcd 451.1240). The characteristic UV absorption bands, ^{1}H- and ^{13}C-NMR spectra (Table 1) were resembled to those of compound **1** except for the appearance of six signals due to a sugar moiety. It was D-glucose obtained from aqueous acid hydrolysis of compound **2** by comparison of TLC analysis with an authentic sample and confirmed by the ^{13}C-NMR data (δ_C 99.5, 77.0, 76.3, 73.0, 69.5, 60.6).[15] Similarly, the β-oriented glucose moiety was assigned to C-7 by the coupling constants of one anomeric protons at δ_H 4.97 (1H, d, J=7.6 Hz) in the ^{1}H-NMR spectrum and HMBC analysis (Fig. 2). The stereochemistry at C-2 of compound **2** was determined to be *S* due to the presence of a positive cotton effect at 334 nm and a cotton negative effect at 284 nm in the CD spectrum.[15,16] Therefore, compound **2** was established as (2*S*)-5,7,3′,5′-tetrahydroxy-flavanone 7-*O*-β-D-glucopyranosie.

Seven isolated compounds **1**—**7** were assessed for antioxidant activity using DPPH assay, in which compounds **1**, **2** and **5** exhibited a radical scavenging activity against DPPH with EC_{50} values of 29.27, 2.00 and 478.00 μg/ml respectively. Among them, compound **2** was more potent than the positive control, ascorbic acid (EC_{50}, 3.32 μg/ml). 5,7,3′,5′-tetrahydroxy-flavanone and blumeatin (5,3′,5′-trihydroxy-7-methoxy-flavanone) exhibited a radical scavenging activity against DPPH with EC_{50} values of 97.30 and 90.80 μg/ml respectively reported in the literature.[17] So we can believe that the antioxidant activities of them decreased in the order: compound **2**>compound **1**>blumeatin>5,7,3′,5′-tetrahydroxy-flavanone. The result may be due to the difference of the 7-substituted group, of which the steric effect is decreased in the order: D-glucose>D-allose>methoxy group>hydroxy group. It is possible that the change of the steric effect of the 7-substituted group affects the anti-oxidant activities of them.

Experimental

General Melting points were determined on a Fisher–Johns apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Perkin-Elmer 983G spectrometer. NMR spectra were measured in DMSO-d_6 on a Bruker AM-500 spectrometer, using TMS as internal standard. NMR experiments included the HMQC and HMBC pulse sequences. Coupling constants (*J* values) were given in Hz. An Autospec-Ultima ETOF spectrometer was used to record the FAB-MS and HR-FAB-MS. CD data was recorded on a JASCO J-715 instrument. Column chromatography was performed on silica gel H (10—40 μm), silica gel GF254 sheets (0.20—0.25 mm) (both from Qingdao Haiyang Chemical Group Co., Qingdao, Shandong Province, People's Republic of China) and Sephadex LH-20 (Amersham Biosciences, Piscataway, NJ, U.S.A.). D-Allose, D-glucose and ascorbic acid were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.).

Plant Material The stems and leaves of *Jasminum lanceolarium* were collected in Jiujiang, Jiangxi Province, China, in September of 2002. The identity of the plant material was verified by Professor Ceming Tan, and a voucher specimen (XC-02-0917) was deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation The stems and leaves (9.7 kg) of *Jasminum lanceolarium* were shade-dried, ground, and extracted with refluxing 95% EtOH successively (46 l, 3 h, 2 times). The EtOH extract was evaporated *in vacuo* to yield a semisolid (1400 g), 1350 g of which was suspended in H_2O (5000 ml) and partitioned successively with petroleum ether (3×5 l), $CHCl_3$ (3×5 l), EtOAc (3×5 l), *n*-BuOH (3×5 l) to yield 29 g, 47 g, 83 g, and 505 g, respectively. The petroleum ether extract (27 g) on purification over a silica gel column with petroleum ether–EtOAc (9 : 1) afforded **3** (34 mg). The $CHCl_3$ extract (42 g) was column chromatographed over silica gel using petroleum ether and EtOAc step gradient as eluents. The petroleum ether and EtOAc (9 : 1, 8 : 2) eluates were purified individually by repeated columns over silica gel to yield **4** (52 mg) and **5** (15 mg). The EtOAc extract (80 g) was subjected to column chromatography on silica gel eluted with $CHCl_3$–MeOH (100 : 0, 95 : 5, 90 : 10, 80 : 20, 70 : 30, 60 : 40, 1 : 1) and MeOH to yield fractions 1—8. Fraction 4 (5.65 g) was chromatographed over silica gel ($CHCl_3$–EtOAc–MeOH–H_2O, 2 : 1 : 1.35 : 0.15) to give fractions 4a—f. Fraction 4b was purified successively with 50% MeOH over Sephadex LH-20 to afford **1** (51 mg) and **2** (11 mg). Fraction 4c was crystallized repeatedly with MeOH to provide **6** (71 mg). Fraction 7 (8.65 g) was chromatographed over silica gel ($CHCl_3$–EtOAc–MeOH–H_2O, 2 : 1 : 1.80 : 0.20) to give fractions 7a—e. Fraction 7d was purified through column chromatography over silica gel using $CHCl_3$–EtOAc–MeOH–H_2O (2 : 1 : 1.80 : 0.20) and crystallized repeatedly with MeOH to afford compound **7** (43 mg).

(2*S*)-5,7,3′,5′-Tetrahydroxy-flavanone 7-*O*-β-D-Allopyranoside (**1**): Pale yellow amorphous powder (MeOH); mp 197—199 °C; $[\alpha]_D^{20}$ −2.68°

(c=1.14, MeOH). UV λ_{max} [MeOH, nm (log ε)]: 225 (3.62), 283 (3.58), 322 (2.85); IR (KBr) cm^{-1}: 3383, 2922, 1643, 1578, 1525, 1449, 1380, 1296, 1198, 1171, 1069, 1042, 1022; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H and ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C given in Table 1. Positive-ion FAB-MS m/z (%): 451 $[M+H]^+$ (16), 374 (13), 289 (25), 282 (100), 256 (40), 165 (22), 137 (42); HR-FAB-MS: m/z 451.1220 $[M+H]^+$ (Calcd for $C_{21}H_{22}O_{11}$: 451.1240).

(2*S*)-5,7,3′,5′-Tetrahydroxy-flavanone 7-*O*-*β*-D-Glucopyranosie (**2**): Pale yellow amorphous powder (MeOH); mp 219—221 °C; $[\alpha]_D^{20}$ −2.74° (c= 1.15, MeOH). UV λ_{max} [MeOH, nm (log ε)]: 225 (3.59), 282 (3.56), 322 (2.85); IR (KBr) cm^{-1}: 3392, 2921, 1643, 1578, 1524, 1502, 1381, 1295, 1198, 1170, 1069, 1041, 1022; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H and ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C given in Table 1. Positive-ion FAB-MS m/z (%): 451 $[M+H]^+$ (14) , 318 (8), 289 (19), 274 (29), 185 (41), 137 (16), 93 (100); HR-FAB-MS: m/z: 451.1234 $[M+H]^+$ (Calcd for $C_{21}H_{22}O_{11}$: 451.1240).

Acid Hydrolysis of 1 and 2 Compounds **1** and **2** (each 2 mg) were refluxed with 10% HCl in 75% EtOH (3 ml) for 6 h. After cooling, the reaction mixture was extracted with EtOAc (3 ml). The water layers were concentrated and checked by TLC analysis [system 1: silica-gel, *n*-BuOH–C_5H_5N–H_2O (8.0 : 4.0 : 3.0), *Rf*: D-glucose, 0.25; D-allose, 0.36; system 2: silica-gel, EtOAc–MeOH–H_2O–AcOH (6.5 : 2.0 : 1.5 : 1.5), *Rf*: D-glucose, 0.42; D-allose, 0.55] respectively. Those indicated that one sugar moiety was D-allose obtained from aqueous acid hydrolysis of compound **1** and the other sugar moiety was D-glucose obtained from aqueous acid hydrolysis of compound **2**.

Free Radical-Scavenging Activity Assay The DPPH radical scavenging activity of the samples was estimated according to the method of Hatano *et al.*[16] Samples in EtOH (2.5 ml) added to a solution of DPPH radical in EtOH (0.2 mM, 0.5 ml), and the reaction mixture was left to stand for 30 min at room temperature in the dark. The scavenging activity of samples was estimated by measuring the absorption of the mixture at 515 nm, which reflects the amount of DPPH radical remaining in the solution. The scavenging activity was expressed as the EC_{50}, the concentration of samples required for scavenging 50% of DPPH radical in the solution. Ascorbic acid was used as a standard agent.

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