Flavonoids from *Bauhinia glauca* subsp. *pernervosa*

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Seven flavonoids were isolated from *Bauhinia glauca* subsp. *pernervosa*, and their structures were elucidated as 6-methyl homoeriodictyol (1), bauhiniaside A (2), bauhiniasin (3), 2′,4′-dihydroxy-4-methoxydihydrochalcone-4′-*O*-*β*-D-glucopyranoside (4), farrerol (5), homoeriodictyol (6), and 2′,4′-dihydroxy-4-methoxydihydrochalcone (7) on the basis of spectroscopic analysis. Compounds 1, 2 and 3 are new flavonoids, compound 4 is a new natural product, its NMR data is reported for the first time. Compounds 5 and 7 were isolated from the genus *Bauhinia* for the first time, and compound 6 was isolated from the plant for the first time.

Key words *Bauhinia glauca* subsp. *pernervosa*; flavonoid; 6-methyl homoeriodictyol; bauhiniaside A; bauhiniasin

The genus *Bauhinia* belongs to the Leguminosae family and some species are used as traditional folk medicines in the treatment of diabetes.[1,2] *Bauhinia glauca* subsp. *pernervosa* is distributed widely in the southwest of China and is called "da-ye-guan-men." Their leaves and roots were used in folk medicine especially for the treatment of diabetes. The chemical studies resulted in the isolation and purification of three new flavonoids: 6-methyl homoeriodictyol (**1**), bauhiniaside A (**2**), bauhiniasin (**3**), together with four known flavonoids: 2′,4′-dihydroxy-4-methoxydihydrochalcone-4′-*O*-*β*-D-glucopyranoside (**4**), farrerol (**5**), homoeriodictyol (**6**), and 2′,4′-dihydroxy-4-methoxydihydrochalcone (**7**) from the EtOAc extract (Fig. 1). This paper deals with the isolation, structural elucidation of the three new compounds **1**—**3** and the new natural product **4** on the basis of spectroscopic methods including 2D NMR. Compounds **5** and **7** were isolated from the genus *Bauhinia* for the first time, and compound **6** was isolated from the plant for the first time.

Results and Discussion

Compound **1** was obtained as colorless needles. The molecular formula was assigned to be $C_{17}H_{16}O_6$ based on high resolution electrospray ionization mass spectroscopy (HR-ESI-MS) data *m*/*z* 339.0840 $[M+Na]^+$, 317.1020 $[M+H]^+$. The ultraviolet (UV) spectrum of **1** showed absorption maximum at 287 nm. The ^{1}H-NMR spectrum of **1** displayed three characteristic proton signals at δ 5.41 (lH, dd, J=12.9, 3.0 Hz), 3.21 (lH, dd, J=17.1, 12.9 Hz) and 2.72 (lH, dd, J=17.1, 3.0 Hz), the ^{13}C-NMR spectrum gave the characteristic carbon signals at δ 197.3 (C-4), 80.2 (C-2), and 43.7 (C-3), indicating that the skeleton of **1** was flavanone. The ^{1}H-NMR spectrum showed an aromatic proton signal at δ 6.04 (1H, s), suggesting the presence of a pentasubstituted A-ring. A typical ABX spin system of three aromatic protons at δ 7.19 (1H, d, J=1.8 Hz), 7.00 (1H, dd, J=8.4, 1.8 Hz), 6.87 (1H, d, J=8.1 Hz) was observed, indicating the presence of a 1,3,4-trisubstituted B-ring. The presence of one methoxy group, one methyl group, and a chelated hydroxy group were indicated by proton signals at δ 3.89 (3H, s), 1.98 (3H, s), 12.46 (1H, s), and carbon signals at δ 56.2 and 7.0. In the heteronuclear multiple bonding connectivity (HMBC) spectrum (Fig. 2), the correlations of δ_H 12.46 (OH-5) with δ_C 162.4 (C-5)/104.6 (C-6); δ_H 1.98 (CH_3) with δ_C 104.6 (C-6) were observed, indicating that the methyl group was attached to C-6. HMBC also showed the following correlations: δ_H 7.19 (H-2′) with δ_C 80.2 (C-2)/120.5 (C-6′)/147.8 (C-4′)/148.3 (C-3′); δ_H 7.00 (H-6′) with δ_C 80.2 (C-2)/111.1 (C-2′)/147.8 (C-4′); δ_H 6.87 (H-5′) with δ_C 131.4 (C-1′)/147.8 (C-4′)/148.3 (C-3′); δ_H 3.89 (OCH_3) with δ_C 148.3 (C-3′), indicating that the methoxy and the hydroxy groups were attached to C-3′ and C-4′, respectively. Furthermore, the nuclear Overhauser effect spectroscopy (NOESY) experiment showed the correlation of δ_H 3.89 (OCH_3) with δ_H 7.19 (H-2′), confirming that the methoxy group was attached to C-3′. The circular dichroism (CD) spectrum of **1** showed a positive Cotton effect at 336 nm ($\Delta\varepsilon$ +0.13), a strong negative Cotton effect at 292 nm ($\Delta\varepsilon$ −3.62), revealing that the absolute configuration of **1** was 2*S*.[3] Thus, the structure of **1** was determined to be 3′-methoxy-6-methyl-5,7,4′-trihydroxy-flavanone, named 6-methyl homoeriodictyol.

Compound **2** was obtained as colorless needles. The molecular formula of **2** was established to be $C_{22}H_{26}O_9$ based on HR-ESI-MS data *m*/*z* 457.1474 $[M+Na]^+$, 473.1214 $[M+K]^+$. The UV spectrum showed absorption maximum at 267 nm. The ^{1}H-NMR spectrum of **2** showed the presence of a 1,4-disubstituted benzene ring [δ 7.14 (2H, d, J=8.4 Hz), 6.81 (2H, d, J=8.7 Hz)], and a 1,2,4-trisubstituted benzene ring [δ 7.53 (1H, d, J=9.0 Hz), 6.60 (1H, d, J=1.8 Hz), 6.48 (1H, dd, J=8.4, 1.8 Hz)]. The presence of one methoxy

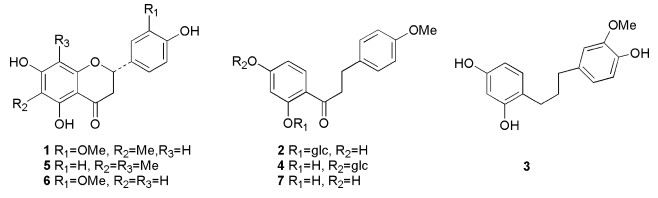

Fig. 1. Structures of Compounds **1**—**7**

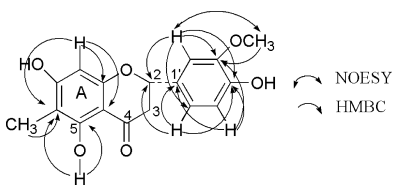

Fig. 2. Key HMBC and NOESY Correlations of **1**

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Table 1. ^{1}H- and ^{13}C-NMR Data of **2** and **4** in DMSO-d_6

No.	**2**		**4**	
	δ_H (m, J Hz)	δ_C	δ_H (m, J Hz)	δ_C
α	3.18 (t, 6.9)	44.6	3.31 (t, 7.2)	40.3
β	2.81 (t, 6.9)	29.0	2.88 (t, 7.2)	28.8
C=O	—	198.7	—	204.3
1	—	133.6	—	132.8
2, 6	7.14 (d, 8.4)	129.3	7.19 (d, 8.4)	127.3
3, 5	6.81 (d, 8.7)	113.6	6.84 (d, 8.7)	113.7
4	—	157.3	—	157.5
OCH_3-4	3.70 (s)	54.9	3.71 (s)	55.0
1′	—	119.7	—	114.4
2′	—	158.8	—	163.5
3′	6.60 (d, 1.8)	102.1	6.55 (d, 2.1)	103.4
4′	—	162.7	—	163.3
5′	6.48 (dd, 8.4, 1.8)	109.3	6.56 (dd, 8.7, 2.1)	108.3
6′	7.53 (d, 9.0)	131.7	7.93 (d, 9.0)	132.8
OH	10.29 (s)	—	12.48 (s)	—
1″	4.93 (d, 7.2)	100.7	4.99 (d, 7.2)	99.6
2″	3.29 (m)	73.3	3.31 (m)	73.1
3″	3.23 (m)	76.7	3.23 (m)	76.4
4″	3.17 (m)	69.5	3.34 (m)	69.5
5″	3.47 (m)	77.2	3.41 (m)	77.1
6″	3.74 (m), 3.52 (m)	60.5	3.67 (m), 3.46 (m)	60.5

group and one hydroxy group were indicated by proton signals at δ 3.70 (3H, s), 10.29 (1H, s). The ^{1}H-NMR spectrum also displayed proton signals of a –CH_2–CH_2– group at δ 3.18 (2H, t, J=6.9 Hz), and 2.81 (2H, t, J=6.9 Hz). The ^{13}C-NMR spectrum gave the characteristic carbon signals at δ 198.7 (C=O), 44.6 (C-α), 29.0 (C-β), twelve aromatic carbon signals belonging to the two benzene rings, indicating that the skeleton of **2** was dihydrochalcone (Table 1). The presence of a glucose moiety was determined by the ^{1}H- and ^{13}C-NMR data, together with the cross-peaks in the heteronuclear multiple quantum connectivity (HMQC) and HMBC spectra. Acid hydrolysis of **2** resulted in a release of glucose, which was identified by HPTLC comparison of the hydrolyzate with an authentic sample.[4] The absolute configuration of the glucose residue in **2** was assigned to D-form based on Massiot and Lavaud's assertion regarding the D-sugars commonly found in the plant kingdom: "The enantiomers of these sugars (glucose, galactose, *etc.*) are not found in plants, a fact used as a clue in the determination of these sugars."[5] The configuration of the glucose residue in **2** was assigned to be β- based on the coupling constant of the anomeric proton δ_H 4.93 (1H, d, J=7.2 Hz). The positions of the methoxy, hydroxy and the glucose moity were established by the HMBC experiments (Fig. 3). In the HMBC spectrum, the methene proton signal at δ 3.18 (H-α) correlated with carbon signals at δ 198.7 (C=O), 29.0 (C-β), and 119.7 (C-1′); the methene proton signal at δ 2.81 (H-β) correlated with carbon signals at δ 198.7 (C=O), 44.6 (C-α), 133.6 (C-1), and 129.3 (C-2, 6); the aromatic proton signal at δ 7.14 (H-2, 6) correlated with carbon signals at δ 157.3 (C-4), 129.3 (C-6, 2), and 29.0 (C-β); the aromatic proton signal at δ 6.81 (H-3, 5) correlated with carbon signals at δ 157.3 (C-4), 133.6 (C-1), and 113.6 (C-5, 3); the methoxy proton signal at δ 3.70 (OCH_3) correlated with carbon signal at δ 157.3 (C-4), indicating that the methoxy group was attached to C-4. This was also confirmed by the correlations of the methoxy proton signal at δ 3.70 (OCH_3) with the aromatic proton at δ 6.81 (H-3, 5) in the NOESY spectrum. HMBC spectrum also displayed correlations of δ_H 7.53 (H-6′) with δ_C 162.7 (C-4′)/158.8 (C-2′)/198.7 (C=O); δ_H 4.93 (H-1″) with δ_C 158.8 (C-2′), indicating that the glucose moiety was attached to C-2′ and the hydroxy group attached to C-4′. This was also confirmed by the correlation of δ 4.93 (H-1″) with δ 6.60 (H-3′) in the NOESY spectrum. Thus, the structure of **2** was determined to be 2′,4′-dihydroxy-4-methoxydihydrochalcone-2′-*O*-β-D-glucopyranoside, named bauhiniaside A.

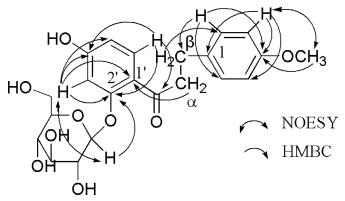

Fig. 3. Key HMBC and NOESY Correlations of **2**

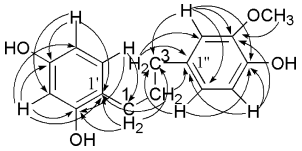

Fig. 4. Key HMBC Correlations of **3**

Compound **3** was obtained as brown oil. Its molecular formula was determined as $C_{16}H_{18}O_4$ on the basis of HR-ESI-MS data m/z 297.1099 $[M+Na]^+$, 275.1279 $[M+H]^+$. The UV spectrum showed absorption maximum at 279 nm. The ^{1}H-NMR spectrum displayed two ABX spin system of aromatic proton signals at δ 6.87 (1H, d, J=8.1 Hz), 6.37 (1H, d, J=2.4 Hz), 6.26 (1H, dd, J=8.1, 2.4 Hz) and δ 6.79 (1H, d, J=1.5 Hz), 6.71 (1H, d, J=8.1 Hz), 6.63 (1H, dd, J=7.8, 1.8 Hz). The ^{13}C-NMR spectrum also displayed twelve aromatic carbon signals belonging to two benzene rings. The presence of one methoxy and a –CH_2–CH_2–CH_2– moiety were confirmed by proton signals at δ 3.80 (3H, s), 2.54 (4H, t, J=7.2 Hz), 1.84 (2H, m), carbon signals at δ 56.1, 29.9, 33.0, 35.9, together with the cross-peaks in the HMQC and HMBC spectra. The skeleton of **3** was determined to be diarylpropane according to the above evidence. The positions of the hydroxy and methoxy groups were determined by the HMBC experiment (Fig. 4). In the HMBC spectrum, correlations of δ_H 2.54 (H-1) with δ_C 33.0 (C-2)/35.9 (C-3)/120.4 (C-1′)/131.2 (C-6′)/156.6 (C-2′); δ_H 1.84 (H-2) with δ_C 29.9 (C-1)/35.9 (C-3)/120.4 (C-1′)/134.8 (C-1″); δ_H 2.54 (H-3) with δ_C 29.9 (C-1)/33.0 (C-2)/134.8 (C-1″)/112.6 (C-2″)/121.4 (C-6″); δ_H 6.79 (H-2″) with δ_C 35.9 (C-3)/121.4 (C-6″)/145.3 (C-4″)/148.0 (C-3″); δ_H 6.63 (H-6″) with δ_C 35.9 (C-3)/145.3 (C-4″)/112.6 (C-2″); δ_H 6.71 (H-5″) with δ_C 134.8 (C-1″)/145.3 (C-4″)/148.0 (C-3″); δ_H 3.80 (OCH_3) with δ_C 148.0 (C-3″) were observed, indicating that the methoxy and the hydroxy groups were attached to C-3″ and C-4″, respectively. The HMBC spectrum also displayed correlations of δ_H 6.87 (H-6′) with δ_C 29.9 (C-1)/156.6 (C-2′)/157.2 (C-4′); δ_H 6.26 (H-5′) with δ_C 103.3 (C-3′)/120.4 (C-1)/157.2 (C-4′); δ_H 6.37 (H-3′) with δ_C 156.6 (C-2′)/157.2 (C-4′)/107.1 (C-5′)/120.4 (C-1), indicating that the two hydroxy groups were attached to C-2′ and C-4′, respectively. Thus, the structure of **3** was determined to be 1-(2,4-dihy-

droxyphenyl)-3-(4-hydroxy-3-methoxyphenyl)propane, named bauhiniasin.

Compound **4** was obtained as colorless needles. The molecular formula of **4** was determined to be $C_{22}H_{26}O_9$ on the basis of HR-ESI-MS data *m/z* 457.1468 $[M+Na]^+$, 473.1195 $[M+K]^+$. The UV spectrum showed absorption maximum at 267 nm. The ^{1}H- and ^{13}C-NMR spectra were quite similar to those of **2** (Table 1). The ^{1}H-NMR spectrum of **4** showed the presence of a 1,4-disubstituted benzene ring [δ 7.19 (2H, d, J=8.4 Hz) and 6.84 (2H, d, J=8.7 Hz)], a 1,2,4-trisubstituted benzene ring [δ 7.93 (1H, d, J=9.0 Hz), 6.56 (1H, dd, J=8.7, 2.1 Hz), and 6.55 (1H, d, J=2.1 Hz)], one methoxy group [δ 3.71 (3H, s)] and a chelated hydroxy group [δ 12.48 (1H, s)]. The ^{1}H-NMR spectrum also displayed proton signals of a $–CH_2–CH_2–$ group at δ 3.31 (2H, t, J=7.2 Hz), 2.88 (2H, t, J=7.2 Hz). The ^{13}C-NMR spectrum gave the characteristic carbon signals at δ 204.3 (C=O), 40.3 (C-α), 28.8 (C-β), twelve aromatic carbon signals belonging to the two benzene rings, indicating that the skeleton of **4** was also dihydrochalcone. The presence of D-glucose moiety was determined by the ^{1}H- and ^{13}C-NMR spectra, the cross-peaks in the HMQC and HMBC spectra, and acid hydrolysis of **4** on HPTLC.[4,5] The configuration of the glucose was assigned to be β- also based on the coupling constant of the anomeric proton δ_H 4.99 (1H, d, J=7.2 Hz). The positions of the methoxy, the hydroxy and the glucose moity were established by the HMBC experiment (Fig. 5). In the HMBC spectrum, the methene proton signal at δ 2.88 (H-β) correlated with carbon signals at δ 204.3 (C=O), 40.3 (C-α), 132.8 (C-1), and 127.3 (C-2, 6); the aromatic proton signal at δ 7.19 (H-2, 6) correlated with carbon signals at δ 157.5 (C-4), 28.8 (C-β); the aromatic proton signal at δ 6.84 (H-3, 5) correlated with carbon signals at δ 132.8 (C-1), 157.5 (C-4), 113.7 (C-5,3); the methoxy proton signal at δ 3.71 (OCH_3) correlated with carbon signal at δ 157.5 (C-4), indicating that the methoxy group was attached to C-4. This was also confirmed by the correlation of the methoxy proton signal at δ 3.71 (OCH_3) with the aromatic proton signal at δ 6.84 (H-3, 5) in the NOESY spectrum. The HMBC spectrum also displayed the following correlations: the chelated hydroxy proton signal at δ_H 12.48 (OH-2′) with δ_C 163.5 (C-2′)/103.4 (C-3′)/114.4 (C-1′); δ_H 7.93 (H-6′) with δ_C 204.3 (C=O)/163.5 (C-2′)/163.3 (C-4′); δ_H 6.56 (H-5′) with δ_C 114.4 (C-1′)/163.3 (C-4′); the anomeric proton signal at δ_H 4.99 (H-1″) with δ_C 163.3 (C-4′), indicating that the glucose moiety was attached to C-4′. This was also confirmed by the correlation of δ_H 4.99 (H-1″) with δ_H 6.55 (H-3′) and 6.56 (H-5′) in the NOESY spectrum. Thus, the structure of **4** was determined to be 2′,4′-dihydroxy-4-methoxydihydrochalcone-4′-*O*-β-D-glucopyranoside, which was synthesized[6] in 1978, however, it was isolated from natural resources for the first time, and its NMR data was reported here for the first time.

Compounds **5**—**7** were identified as farrerol,[7] homoeriodictyol,[8] and 2′,4′-dihydroxy-4-methoxydihydrochalcone,[9] respectively, by comparison of the NMR and mass spectroscopic data with the literature values.

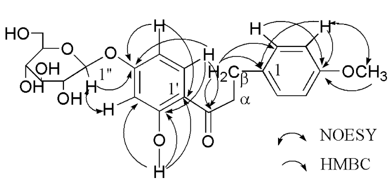

Fig. 5. Key HMBC and NOESY Correlations of **4**

Experimental

General Procedure Melting point was measured on an X-4 melting point apparatus. Optical rotations were determined on an AA-10R automatic polarimeter. UV spectra were measured on a Jasco MD-1501 multi-wavelength detector. CD spectra were measured on a Jasco J-810 spectropolarimeter. ESI-time of flight (TOF)-MS spectra were taken on a QSTAR mass spectrometer. HR-ESI-MS spectra were acquired using a Bruker APEX IV FT-MS (7.0T) mass spectrometer. 1D and 2D NMR spectra were measured with a Bruker VXR300 NMR spectrometer with tetramethylsilane (TMS) as internal standard. Column chromatograph (CC) was performed on silica gel G (200—300 mesh, Qingdao Marine Chemical Co., Ltd.), YMC GEL ODS-A (75 μm, YMC Co., Ltd.), and Sephadex LH-20 (GE Healthcare, Sweden).

Plant Material The caulis of *B. glauca* subsp. *pernervosa* were collected from Chongqing in 2004, and identified by Professor Yi Sirong, Institute of Medicinal Plantation of Chongqing. A specimen was deposited in the Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University Health Science Center.

Extraction and Isolation The air-dried powder of the caulis of *B. glauca* subsp. *pernervosa* (11.0 kg) were percolated with 95% aqueous EtOH at room temperature. After evaporation of the solvent under reduced pressure, the residues were suspended in water and then successively partitioned with petroleum ether, EtOAc and *n*-BuOH to afford 80.0 g, 350.0 g, and 480.0 g, respectively. The EtOAc extract (350.0 g) was subjected to silica gel column chromatography and eluted with $CHCl_3–CH_3OH–H_2O$ (20 : 1 : 0 to 7 : 3 : 1) to give 44 fractions (Fr. 1—Fr. 44). Fr. 22—27 (16.0 g) were further separated on silica gel column chromatography and eluted with $CHCl_3$–acetone (30 : 1 to 10 : 1) to give 11 fractions. Fr. 3—5 were subjected to reverse-phase column chromatography eluted with 50% MeOH–H_2O and then purified by Sephadex LH-20 column chromatography eluted with 60% MeOH–H_2O to yield compounds **1** (36.0 mg), **5** (20.0 mg), **6** (16.0 mg), and **7** (50.0 mg). Fr. 9—11 were separated on RP-18 and Sephadex LH-20 column chromatography eluted with 40% MeOH–H_2O and 80% MeOH–H_2O, respectively, to give compound **3** (13.0 mg). Fr. 39 and 40 were chromatographed on RP-18 and Sephadex LH-20 column eluted with 30% MeOH–H_2O and 80% MeOH–H_2O, respectively, to yield compounds **2** (18.0 mg) and **4** (10.0 mg).

6-Methyl homoeriodictyol (**1**): Colorless needles; mp 218—222 °C; $[\alpha]_D^{20}$ −78.6° (c=0.25, MeOH); UV λ_{max} (MeOH) nm: 287; CD (MeOH): θ_{336} +0.13, θ_{292} −3.62; HR-ESI-MS *m/z*: 339.0840 $[M+Na]^+$ (Calcd for $C_{17}H_{16}O_6Na$: 339.0839), 317.1020 $[M+H]^+$ (Calcd for $C_{17}H_{17}O_6$: 317.1020); positive ESI-TOF-MS *m/z*: 339 $[M+Na]^+$, 317 $[M+H]^+$, negative ESI-TOF-MS *m/z*: 315 $[M-H]^-$; ^{1}H-NMR (acetone-d_6, 300 MHz) δ: 12.46 (1H, s, OH-5), 7.19 (1H, d, J=1.8 Hz, H-2′), 7.00 (1H, dd, J=8.4, 1.8 Hz, H-6′), 6.87 (1H, d, J=8.1 Hz, H-5′), 6.04 (1H, s, H-8), 5.41 (1H, dd, J=12.9, 3.0 Hz, H-2), 3.89 (3H, s, OMe-3′), 3.21 (1H, dd, J=17.1, 12.9 Hz, H-3 *trans*), 2.72 (1H, dd, J=17.1, 3.0 Hz, H-3 *cis*), 1.98 (3H, s, Me-6); ^{13}C-NMR (acetone-d_6, 74.25 MHz) δ: 80.2 (C-2), 43.7 (C-3), 197.3 (C-4), 162.4 (C-5), 104.6 (C-6), 164.9 (C-7), 95.1 (C-8), 161.8 (C-9), 102.9 (C-10), 131.4 (C-1′), 111.1 (C-2′), 148.3 (C-3′), 147.8 (C-4′), 115.6 (C-5′), 120.5 (C-6′), 56.2 (OMe-3′), 7.0 (Me-6).

Bauhiniaside A (**2**): Colorless needles; $[\alpha]_D^{20}$ −57.0° (c=0.28, MeOH); UV λ_{max} (MeOH) nm: 267; HR-ESI-MS *m/z*: 457.1474 $[M+Na]^+$ (Calcd for $C_{22}H_{26}O_9Na$: 457.1469), 473.1214 $[M+K]^+$ (Calcd for $C_{22}H_{26}O_9K$: 473.1203); ^{1}H- and ^{13}C-NMR (300 MHz, DMSO-d_6) data see Table 1.

Bauhiniasin (**3**): Brown oil. UV λ_{max} (MeOH) nm: 279; HR-ESI-MS *m/z*: 297.1099 $[M+Na]^+$ (Calcd for $C_{16}H_{18}O_4Na$: 297.1097), 275.1279 $[M+H]^+$ (Calcd for $C_{16}H_{19}O_4$: 275.1278); ^{1}H-NMR (acetone-d_6, 300 MHz) δ: 6.87 (1H, d, J=8.1 Hz, H-6′), 6.79 (1H, d, J=1.5 Hz, H-2″), 6.71 (1H, d, J=8.1 Hz, H-5″), 6.63 (1H, dd, J=7.8, 1.8 Hz, H-6″), 6.37 (1H, d, J=2.4 Hz, H-3′), 6.26 (1H, dd, J=8.1, 2.4 Hz, H-5′), 3.80 (3H, s, OMe-3″), 2.54 (4H, t, J=7.2 Hz, H-1, 3), 1.84 (2H, m, H-2); ^{13}C-NMR (acetone-d_6, 75.45 MHz) δ: 29.9 (C-1), 33.0 (C-2), 35.9 (C-3), 120.4 (C-1′), 156.6 (C-2′), 103.3 (C-3′), 157.2 (C-4′), 107.1 (C-5′), 131.2 (C-6′), 134.8 (C-1″), 112.6 (C-2″), 148.0 (C-3″), 145.3 (C-4″), 115.4 (C-5″), 121.4 (C-6″), 56.1 (OMe).

2′,4′-Dihydroxy-4-methoxydihydrochalcone-4′-*O*-β-D-glucopyranoside (**4**): Colorless needles. $[\alpha]_D^{20}$ −63.0° (c=0.22, MeOH); UV λ_{max} (MeOH): 267 nm; HR-ESI-MS *m/z*: 457.1468 $[M+Na]^+$ (Calcd for $C_{22}H_{26}O_9Na$: 457.1469), 473.1195 $[M+K]^+$ (Calcd for $C_{22}H_{26}O_9K$: 473.1203); ^{1}H- and

^{13}C-NMR (300 MHz, DMSO-d_6) data see Table 1.

Acid Hydrolysis of 2 and 4 on HPTLC Plate Compounds **2** and **4** were individually spotted on a HPTLC precoated plate, and hydrolyzed with 6 N HCl vapor (60 °C water bath for 30 min) followed by co-TLC with authentic sugar, developing solvent EtOAc–MeOH–H_2O–HOAc (13 : 3 : 3 : 4). The TLC plate was sprayed with anilinium oxalate for detection. Glucose was detected with an *Rf* value of 0.4.

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References

1) Wu D. L., Chen B. Y., Wang Z. F., Chen D. Z., Hu J. Q., "Flora of China," Science Publisher, Beijing, 1988, p. 196.
2) Pepato M. T., Keller E. H., Baviera A. M., Kettelhut I. C., Vendramini R. C., Brunetti I. L., *J. Ethnopharmacol.*, **81**, 191—197 (2002).
3) Gaffield W., *Tetrahedron*, **26**, 4093—4108 (1970).
4) Li R. T., Li J. T., Wang J. K., Han Q. B., Zhu Z. Y., Sun H. D., *Chem. Pharm. Bull.*, **56**, 592—594 (2008).
5) Massiot G., Lavaud C., "Studies in Natural Products Chemistry," Vol. 15, Elsevier Sciences, Amsterdam, 1995, pp. 187—224.
6) Antus S., Farkas L., Gottsegen A., Nogradi M., Pfliegel T., *Acta Chim. Acad. Sci. Hung.*, **98**, 225—230 (1978).
7) Youssef Diaa T. A., Ramadan M. A., Khalifa A. A., *Phytochemistry*, **49**, 2579—2583 (1998).
8) Liu Y. L., Ho D. K., Cassady J. M., Cook V. M., Baird W. M., *J. Nat. Prod.*, **55**, 357—363 (1992).
9) Logendra S., Ribnicky D. M., Yang H., Poulev A., Ma J., Kennelly E. J., Raskin I., *Phytochemistry*, **67**, 1539—1546 (2006).