Flavonoid Constituents from the Roots of *Acanthopanax brachypus*

Hao-bin Hu* and Ji-hua Zhu

College of Chemistry and Chemical Engineering, Longdong University; South Street 137, Xifeng District, Qingyang 745000, Gansu Province, China.

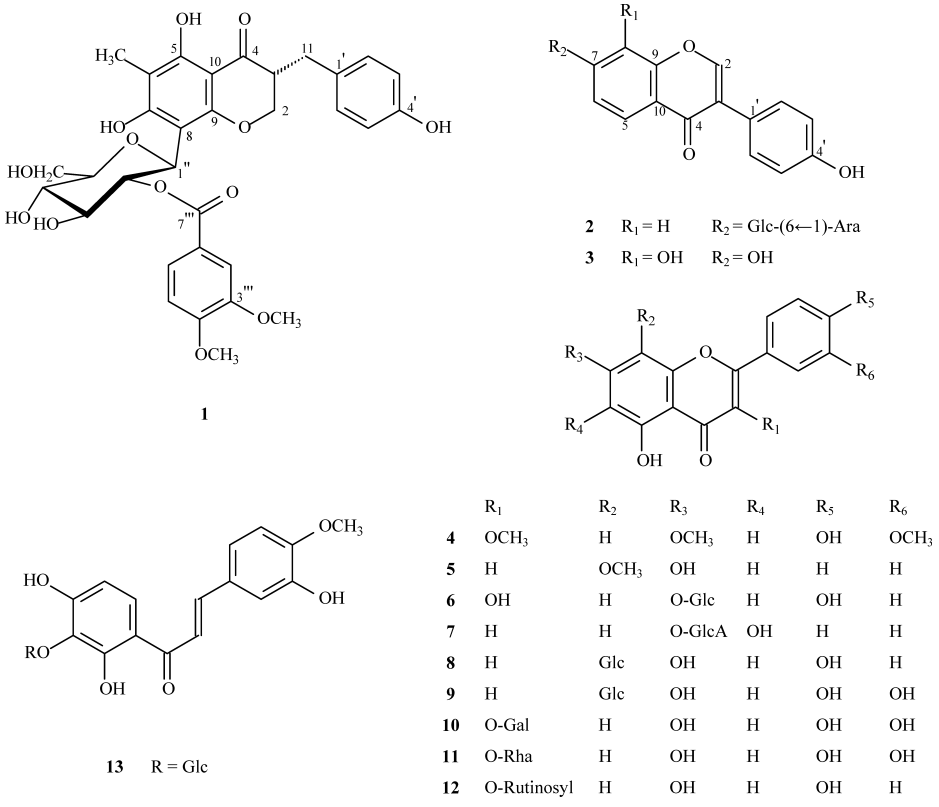
Received September 21, 2010; accepted October 19, 2010; published online October 21, 2010

Two new natural products, (3*R*)-5,7-dihydroxy-8-(2″-*O*-veratroyl-β-D-glucopyranosyl)-3-(4′-hydroxyphenyl)-6-methylchroman-4-one (1) and 4′-hydroxyisoflavone-7-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside] (2), along with eleven other known flavonoids, were isolated from the roots of *Acanthopanax brachypus*. Their structures were elucidated on the basis of spectroscopic and chemical evidence.

Key words *Acanthopanax brachypus*; Araliaceae; flavonoid; (3*R*)-5,7-dihydroxy-8-(2″-*O*-veratroyl-β-D-glucopyranosyl)-3-(4′-hydroxyphenyl)-6-methylchroman-4-one; 4′-hydroxyisoflavone-7-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside]

As an endemic Asian genus, *Acanthopanax* species are herbaceous genus of the family Araliaceae that are widely distributed in East-Asia, such as China, Korea, Russia, Japan and so on.[1] The root and stem bark of these plants have been clinically used for a long time as a tonic and sedative, as well as for the treatment of rheumatism, diabetes, chronic bronchitis, hypertension, anti-stress and ischemic heart disease, tumor and gastric ulcer.[2] Until now, many studies on the triterpenoid constituents of *Acanthopanax* species have been carried out, with more than 120 triterpenoids being reported,[3,4] but there have few reports on flavonoid constituents.[5—8] As a group of natural benzo-γ-pyran derivatives, flavonoids are ubiquitous in photosynthesising cells, and play important roles in plant biochemistry and physiology as well as mammalian metabolism.[9] Research into their chemistry, synthesis, natural distribution and biological function is widely conducted.[10] Flavonoids have come under investigation in the last three decades for use as natural antioxidants as well as for their health promoting properties in humans.[11]

Acanthopanax brachypus is distributed in a narrow geographical area, most in the loess plateau of the northwest of China.[12] Different parts of the plant have been employed for various therapeutic purposes in China and Korea.[13] However, to date, the research has mainly concentrated on the reproductive biology and ecology, and there have been few studies on the chemical composition and biological activity.[14] In a continuing search for the phytochemical constituents in *A. brachypus*, we describe here the isolation and structural determination of thirteen flavonoid constituents from the roots of *A. brachypus*.



	R1	R2	R3	R4	R5	R6
4	OCH_3	H	OCH_3	H	OH	OCH_3
5	H	OCH_3	OH	H	H	H
6	OH	H	O-Glc	H	OH	H
7	H	H	O-GlcA	OH	H	H
8	H	Glc	OH	H	OH	H
9	H	Glc	OH	H	OH	OH
10	O-Gal	H	OH	H	OH	OH
11	O-Rha	H	OH	H	OH	OH
12	O-Rutinosyl	H	OH	H	OH	H

Fig. 1. Compounds **1**—**13** Isolated from *Acanthopanax brachypus*

* To whom correspondence should be addressed. e-mail: huhaobin_88@yahoo.com.cn

Results and Discussion

Two new and eleven known compounds were isolated from the roots of *A. brachypus*. The known compounds were identified as 7,8,4′-trihydroxyisoflavone (**3**),[15] pachypodol (**4**),[16] wogonin (**5**),[17,18] kaempferol-7-*O*-*β*-D-glucopyranoside (**6**),[19] baicalin (**7**),[20] vitexin (**8**),[21,22] orientin (**9**),[23,24] hyperoside (**10**),[25,26] quercitrin (**11**),[7] kaempferol-3-*O*-rutinoside (**12**)[27] and 3′-*O*-*β*-D-glucopyranosylokanin 4-methyl ether (**13**)[28,29] on the basis of spectroscopic analysis, chemical evidence and comparison of spectral data with the literature values. Among these known compounds, compounds **3**—**9** and **13** were isolated for the first time from this genus, and compounds **10**—**12** were isolated for the first time from *A. brachypus*.

Compound **1** was obtained as a pale yellow powder with molecular formula $C_{32}H_{34}O_{13}$ and corresponding to 16 degrees of unsaturation, as established by the molecular ion peak at *m/z* 627.2081 $[M+H]^+$ ($C_{32}H_{35}O_{13}$, Calcd for 627.2078) in its high resolution (HR)-FAB-MS. Its UV spectrum displayed the characteristic absorption of homoisoflavanones (at 205, 220, 292, 345 nm).[30] The IR spectrum showed the presence of hydroxyl groups (3328 cm^{-1}), carbonyl groups (1701, 1638 cm^{-1}) and aromatic rings (1605, 1524, 1469 cm^{-1}). The ^{1}H-NMR spectrum revealed the presence of two sets of an ABX-type spin system [δ_H 4.27 (1H, dd, J=11.2, 4.1 Hz), 4.07 (1H, dd, J=11.2, 7.8 Hz), 2.88 (1H, m), 3.16 (1H, dd, J=13.1, 4.7 Hz), 2.65 (1H, dd, J=13.1, 9.8 Hz)], an aromatic A_2B_2-type spin system [δ_H 7.04 and 6.68 (each 2H, d, J=8.2 Hz)] for a symmetrical *para*-substituted aromatic ring B. Consistent with the ^{1}H-NMR spectral analysis, its ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra also exhibited three sp^3 carbon signals [δ_C 69.5 (CH_2), 46.1 (CH) and 31.1 (CH_2)], one carbonyl carbon signal [δ_C 197.9 (C)], and ten aromatic carbon signals [δ_C 158.1 (C), 103.0 (C), 162.4 (C), 102.1 (C), 154.8 (C), 103.9 (C), 129.0 (C), 129.7 (CH), 115.3 (CH) and 155.6 (C)]. These spectral characteristics were typical for 3-benzyl-4-chromanone, namely homoisoflavanone.[31,32] In addition to above signals, the ^{1}H-NMR spectrum also showed signals due to two aromatic methoxyl groups [δ_H 3.73 and 3.76 (each 3H, s)], an ABX-type spin system [δ_H 7.31 (1H, dd, J=8.4, 2.0 Hz), 6.95 (1H, d, J=8.4 Hz) and 7.20 (1H, d, J=2.0 Hz)] for a 1,3,4-trisubstituted aryl ring, an anomeric proton [δ_H 4.87 (1H, d, J=10.0 Hz)], an aromatic *C*-methyl [δ_H 2.01 (3H, s)] and three aromatic hydroxyl [δ_H 12.15, 9.78 and 9.54 (each 1H, s)] groups, moreover δ_H 12.15 was assigned to the chelated hydroxyl group linked to the C-5 of the homoisoflavanone aglycone. Accordingly, the ^{13}C-NMR and DEPT spectra revealed 30 carbon signals, of which 15 were assigned to the homoisoflavanone skeleton, 6 to the glucosyl group [δ_C 71.2, 72.8, 75.9, 70.6, 81.7 (each CH), 61.8 (CH_2)], and the remaining 9 were assigned to the veratroyl group [δ_C 122.1 (C), 111.7 (CH), 148.6 (C), 152.1 (C), 110.8 (CH), 122.9 (CH), 164.8 (C), 55.5 (CH_3) and 55.8 (CH_3)],[33] suggesting that **1** was typical of homoisoflavanone *C*-glycoside with a veratroyl group. The glucose was determined to be *β*-D-configuration by its $^3J_{H1-H2}$ value (J=10.0 Hz), as well as TLC and GC analysis after derivatization.[34]

The position of substituents in compound **1** were determined by analysis of the ^{13}C-NMR, heteronuclear multiple bond connectivity (HMBC) and nuclear Overhauser effect spectroscopy (NOESY) spectra (see Fig. 2). The glycosidation position was determinged at C-8 of the aglycone by the downfield shift of C-8 ($\Delta\delta$ *ca.* +7.1) and the HMBC correlation between H-1″ (δ_H 4.87) of the glucosyl with C-8 (δ_C 102.1), C-7 (δ_C 162.4) and C-9 (δ_C 154.8) of the aglycone. The position of the acyl group at C-2″ was evident from the upfield shifts of C-1″ ($\Delta\delta$ *ca.* −2.2) and C-3″ ($\Delta\delta$ *ca.* −2.8) of the glucosyl in the ^{13}C-NMR spectrum.[35] The HMBC correlation between H-2″ (δ_H 5.51) of the glucosyl with C-7‴ (δ_C 164.8) of the veratroyl also confirmed that the veratroyl group was attached to the C-2″ of the glucosyl. The same conclusion with regard to the glucosyl and veratroyl sequence was also drawn from the fragment ions at *m/z* 463 $[M+H-164]^+$ and 301 $[M+H-164-162]^+$ in the mass spectrum. Similarly, the position of one methyl group at C-6 and three hydroxyl groups at C-5, C-7 and C-4′ of the aglycone, respectively, were established from the following HMBC correlations: between 6-CH_3 (δ_H 7.8) with C-5 (δ_C 158.1), C-6 (δ_C 103.0) and C-7 (δ_C 162.4), between 5-OH (δ_H 12.15) with C-10 (δ_C 103.9), C-5 (δ_C 158.1) and C-6 (δ_C 103.0), 7-OH (δ_H 9.78) with C-6 (δ_C 103.0), C-7 (δ_C 162.4) and C-8 (δ_C 102.1), between 4′-OH (δ_H 9.54) with C-3′ (δ_C 115.3) and C-4′ (δ_C 155.6). The circular dichroism (CD) spectrum of **1** displayed positive Cotton effects at 250 and 316 nm, while a negative Cotton effect at 291 nm, which suggests the absolute configuration of C-3 to be *R*.[36,37] The assignments of all protons and carbon signals were determined by DEPT, ^{1}H–^{1}H correlation spectroscopy (COSY), HMBC and NOESY spectra. Thus, the structure of **1** was determined to be (3*R*)-5,7-dihydroxy-8-(2″-*O*-veratroyl-*β*-D-glucopyrano-

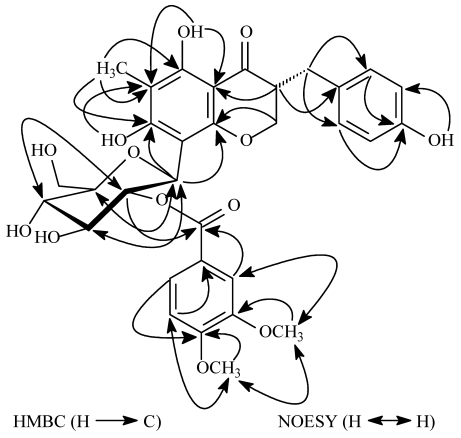


Fig. 2. Selected HMBC and NOESY Correlations of **1**

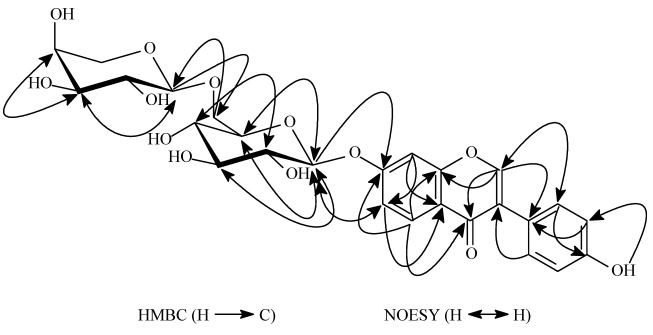


Fig. 3. Selected HMBC and NOESY Correlations of **2**

syl)-3-(4′-hydroxyphenyl)-6-methylchroman-4-one.

Compound **2** was obtained as a pale yellow powder with the molecular formula $C_{26}H_{28}O_{13}$, which indicated 13 degrees of unsaturation, as determined from the data of the positive (HR)-FAB-MS (*m/z* 549.1613 $[M+H]^+$, Calcd for $C_{26}H_{29}O_{13}$, 549.1608). The absorption band at 257 and 326 nm in the UV spectrum in combination with a singlet resonance at δ_H 8.35 and corresponding olefinic oxymethine signal at δ_C 153.7 in NMR spectra (Table 1) due to H-2 and C-2, respectively, suggested this compound to have an isoflavone skeleton.[38—40] The IR spectrum displayed the characteristic absorptions for hydroxyl groups (3410 cm^{-1}, br s), carbonyl functions (1630 cm^{-1}), aromatic rings (1605, 1531, 1452 cm^{-1}) and glycosidic bond (1055 cm^{-1}).

The ^{13}C-NMR spectrum with the aid of DEPT experiment displayed 24 carbon signals, including 15 methines, two methylenes and seven quarternary carbons, suggesting the presence of symmetrical structure in the molecule. Among these, six oxygenated carbon signals at δ_C 101.2, 73.1, 76.3, 70.1, 75.7 (each CH) and 67.9 (CH_2) were assigned to a glucopyranosyl unit, likewise, δ_C 103.1, 72.4, 73.2, 69.3 (each CH), 67.2 (CH_2) were the characteristic signals of the arabinopyranosyl unit.[41] Analysis of the ^{1}H-NMR spectrum of **2** revealed the presence of an aromatic ABX-type spin system [δ_H 8.07 (d, J=8.8 Hz), 7.13 (dd, J=8.8, 2.2 Hz) and 7.21 (d, J=2.2 Hz)] for the A-ring, an aromatic A_2B_2-type spin system [δ_H 7.52 and 6.95 (each 2H, d, J=8.5 Hz)] for the B-ring, one hydroxyl group at δ_H 9.18 (s), two anomeric protons at δ_H 5.01 (d, J=7.5 Hz) and 4.76 (d, J=7.8 Hz) arising from the glucosyl and arabinosyl units, respectively. The coupling constants ($^3J_{H1-H2}$=7.5, 7.8 Hz) determined the glucose and arabinose to be β and α-configuration, respectively. Acid hydrolysis of **2** gave D-glucose and L-arabinose (in the ratio of 1 : 1) as component sugars, which were identified by TLC and GC analysis after derivatization.[34]

The hydroxyl group at C-4′ could be deduced from the downfield shift ($\Delta\delta$ *ca.* +14.0) of C-4′ (δ_C 158.4, C) and the HMBC correlation of 4′-OH (δ_H 9.18) with C-3′ (δ_C 113.9, CH). The HMBC correlation of H-1‴ (δ_H 4.76) with C-6″ (δ_C 67.9) and the downfield shift ($\Delta\delta$ *ca.* +7.0) of C-6″ indicated the arabinosyl unit linked to C-6″ of the glucosyl unit. The glycosidation at C-7 of the aglycone was unambiguously determined by the HMBC correlation of H-1″ (δ_H 5.08) of the glucosyl with C-7 (δ_C 163.2) of the aglycone and the NOESY correlation of H-1″ (δ_H 5.08) with H-6 (δ_H 7.13) of the aglycone, and this was also evident from a significant

Table 1. ^{1}H- (100 MHz) and ^{13}C-NMR (400 MHz) Data of Compounds **1** and **2** in DMSO-d_6

Position	**1**		**2**	
	δ_C (DEPT)	δ_H (J in Hz)	δ_C (DEPT)	δ_H (J in Hz)
Aglycone				
2	69.5 (CH_2)	4.27 dd (11.2, 4.1)/4.07 dd (11.2, 7.8)	153.7 (CH)	8.35 s
3	46.1 (CH)	2.88 m	123.9 (C)	
4	197.9 (C)		178.5 (C)	
5	158.1 (C)		127.1 (CH)	8.07 d (8.8)
6	103.0 (C)		115.8 (CH)	7.13 dd (8.8, 2.2)
7	162.4 (C)		163.2 (C)	
8	102.1 (C)		103.4 (CH)	7.21 d (2.2)
9	154.8 (C)		159.2 (C)	
10	103.9 (C)		119.5 (C)	
11	31.1 (CH_2)	3.16 dd (13.1, 4.7)/2.65 dd (13.1, 9.8)		
1′	129.0 (C)		124.1 (C)	
2′	129.7 (CH)	7.04 d (8.2)	130.3 (CH)	7.52 d (8.5)
3′	115.3 (CH)	6.68 d (8.2)	113.9 (CH)	6.95 d (8.5)
4′	155.6 (C)		158.4 (C)	
5′	115.3 (CH)	6.68 d (8.2)	113.9 (CH)	6.95 d (8.5)
6′	129.7 (CH)	7.04 d (8.2)	130.3 (CH)	7.52 d (8.5)
6-CH_3	7.8 (CH_3)	2.01 s		
5-OH		12.15 s		
7-OH		9.78 s		
4′-OH		9.54 s		9.18 s
Sugar				
1″	71.2 (CH)	4.87 d (10.0)	101.2 (CH)	5.01 d (7.5)
2″	72.8 (CH)	5.51 dd (10.0)	73.1 (CH)	3.41 m
3″	75.9 (CH)	3.61 m	76.3 (CH)	3.57 m
4″	70.6 (CH)	3.51 m	70.1 (CH)	3.48 m
5″	81.7 (CH)	3.41 m	75.7 (CH)	3.52 m
6″	61.8 (CH_2)	3.85 m/3.63 m	67.9 (CH_2)	4.12 m/3.78 m
1‴	122.1 (C)		103.1 (CH)	4.76 d (7.8)
2‴	111.7 (CH)	7.20 d (2.0)	72.4 (CH)	3.61 m
3‴	148.6 (C)		73.2 (CH)	3.75 m
4‴	152.1 (C)		69.3 (CH)	3.87 m
5‴	110.8 (CH)	6.95 d (8.4)	67.2 (CH_2)	3.86 dd (12.0, 1.8)/3.58 d (12.0)
6‴	122.9 (CH)	7.31 dd (8.4, 2.0)		
7‴	164.8 (C)			
3‴-OCH_3	55.5 (CH_3)	3.73 s		
4‴-OCH_3	55.8 (CH_3)	3.76 s		

downfield shift ($\Delta\delta$ *ca.* +20) of C-7 (δ_C 163.2) when compared to that of the common isoflavone.[42] These above conclusions were supported by the mass spectral data, which showed the fragment ion peaks at *m/z* 387 $[M+H-162]^+$ and 255 $[M+H-162-132]^+$ due to the successive loss of the glucosyl and arabinosyl units from the molecule, thus suggesting the linkage of arabinosyl-(1→6)-glucosyl-(1→7)-aglycone. The NOESY correlations of H-2 (δ_H 8.35) with H-2′ (δ_H 7.52), H-1″ (δ_H 5.01) with H-6 (δ_H 7.13), H-3″ (δ_H 3.57) and H-5″ (δ_H 3.52), H-3‴ (δ_H 3.75) with H-1‴ (δ_H 4.76) and H-4‴ (δ_H 3.87), as well as H-2″ (δ_H 3.41) with H-4″ (δ_H 3.48) were also observed.

The assignments of all protons and carbons were made by DEPT, ^1H–^1H COSY, HMBC and NOESY experiments. Thus, the structure of **2** was determined to be 4′-hydroxyisoflavone-7-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside].

Experimental

General Procedures Melting points were determined on X-4 digital micro-melting point apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 341 digital polarimeter. UV spectra were obtained on a Shimadzu UV-2401 spectrometer. IR spectra were recorded with KBr pellets on a Perkin-Elmer 1700 spectrometer. CD spectra were performed with a JASCO J-715 spectropolarimeter. GC analysis were recorded on a Hewlett Packard 6890 Series gas chromatograph equipped with an H_2 flame ionization detector. The MS were recorded on a VG Autospec-3000 mass spectrometer, and the NMR spectra were recorded with a Bruker AMX-400 (400 MHz for ^1H-NMR and 100 MHz for ^{13}C-NMR). Chemical shifts were given in δ (ppm) with tetramethylsilane (TMS) as an internal standard and coupling constants (*J*) were reported in Hertz (Hz). Column chromatography was performed with Silica-gel H (Qingdao Haiyang Chemical Plant, P. R. China), Diaion HP-20 (Mitsubishi Chemical, Japan), Sephadex LH-20 (Pharmacia, Sweden), and Macroporous resin D101 (0.3—1.2 mm, Shanghai Sinopharm Chemical Reagent Company, P. R. China). Thin-layer chromatography (TLC) employed precoated Silica-gel GF_{254} plates (Qingdao Haiyang Chemical Plant, P. R. China) and detection was achieved by 10% H_2SO_4–EtOH, and aniline-phthalate reagents for sugars.

Plant Material The roots of *A. brachypus* were collected in August of 2007 from Qingyang of Gansu Province, P. R. China, and identified by Prof. Xiao-qiang Guo, Department of Life-Sciences, Longdong University, P. R. China. A voucher specimen (10732) was deposited in the Herbarium of the Department of Life-Sciences, Longdong University.

Extraction and Isolation The air-dried and pulverized roots of *A. brachypus* (15.0 kg) were extracted three times with 75% aqueous EtOH (15 l×7 d, each time) at room temperature, and then the extracts were combined and concentrated under reduced pressure at 60 °C to yield 735 g of a brown viscous residue. The EtOH extract was suspended in distilled water (1500 ml) and partitioned successively with *n*-hexane (750 ml), EtOAc (750 ml) and *n*-BuOH (saturated with water, 750 ml). The concentrated EtOAc-soluble extract was subjected to silica gel column, polarity gradient with *n*-hexane, $CHCl_3$, acetone and MeOH (pure or in mixtures) to give fractions E_1—E_{25} (TLC control). Fraction E_5 was rechromatographed over silica gel and further purified by preparative TLC (MeOH–$CHCl_3$–*n*-hexane, 1 : 5 : 2) to afford compound **4** (24.3 mg) and **5** (19.2 mg). Fraction E_6 was chromatographed on Sephadex LH-20 column with MeOH as eluent to give compound **3** (12.2 mg). Fraction E_8 was chromatographed over silica gel with a gradient mixture of $CHCl_3$–MeOH (from 2 : 1 to 1 : 4) to give six subfractions E_{801}—E_{806}. Subfraction E_{802} was chromatographed on Sephadex LH-20 column with MeOH, and further recrystallized with MeOH to provide compound **6** (19.8 mg). Similarly, subfraction E_{804} was chromatographed on Sephadex LH-20 column with MeOH, and then recrystallized with MeOH to yield compound **8** (23.2 mg). Subfraction E_{806} was recrystallized with MeOH to provide compound **9** (16.4 mg). Fraction E_{11} was chromatographed over silica gel with a gradient mixture of EtOAc–MeOH (from 8 : 1 to 1 : 7) to yield 13 mg of crude material, which was repeatedly subjected to silica gel column with a gradient mixture of $CHCl_3$–MeOH (from 3 : 1 to 1 : 10) to afford **7** (34.7 mg). Fraction E_{14} was further purified on silica gel column eluting stepwise with $CHCl_3$–MeOH (from 5 : 1 to 1 : 8), and then on Sephadex LH-20 column eluting with MeOH to obtain compound **10** (22.7 mg) and **11** (15.3 mg), respectively. Fraction E_{16} was further purified by preparative thin-layer chromatography to give compound **1** (16.1 mg). Fraction E_{20} was purified as aforementioned on Sephadex LH-20 column with MeOH to afford compound **2** (9.5 mg). Fraction E_{22} was further purified by preparative TLC to furnish compound **12** (8.2 mg).

(3*R*)-5,7-Dihydroxy-8-(2″-*O*-veratroyl-β-D-glucopyranosyl)-3-(4′-hydroxyphenyl)-6-methylchroman-4-one (**1**): Pale yellow powder. $[\alpha]_D^{20}$ −43.5° (*c*=0.15, MeOH). UV (MeOH) λ_{max} nm: 205, 220, 292 (sh), 345. IR (KBr) ν_{max} cm^{-1}: 3328, 2959, 1701, 1638, 1605, 1524, 1469, 1452, 1385, 1232, 1174, 1086, 1022, 939, 843, 758. ^1H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (DMSO-d_6, 100 MHz): see Table 1. (HR)-FAB-MS (positive) *m/z* 627.2081 $[M+H]^+$ (Calcd for $C_{32}H_{35}O_{13}$, 627.2078). FAB-MS (positive) *m/z* 463 $[M+H-164]^+$, 301 $[M+H-164-162]^+$. CD ($c=5.12\times10^{-5}$ mol/l, MeOH) $\Delta\varepsilon^{20}$ (nm): +2.31 (250), 0 (268), −4.07 (291), 0 (302), +1.67 (316).

4′-Hydroxyisoflavone-7-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside] (**2**): Pale yellow powder. UV (MeOH) λ_{max} nm: 257, 326 (sh). IR (KBr) ν_{max} cm^{-1}: 3410, 2923, 1630, 1605, 1590, 1531, 1452, 1369, 1306, 1055. ^1H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR (DMSO-d_6, 100 MHz): see Table 1. (HR)-FAB-MS (positive) *m/z* 549.1613 $[M+H]^+$ (Calcd for $C_{26}H_{29}O_{13}$, 549.1608). FAB-MS (positive) *m/z* 387 $[M+H-162]^+$, 255 $[M+H-162-132]^+$.

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharide A solution of compounds **1** or **2** (2.0 mg) in 2 mol/l HCl–MeOH (1 : 2, 3 ml) was refluxed in a water bath at 90 °C for 5 h. After cooled, the solution was extracted with EtOAc (2 ml×3). The aqueous layer was neutralized by passing through an Amberlite MB-3 resin column eluted with H_2O, then concentrated and dried to furnish a monosaccharide residue. Then, the sugars were detected by TLC analysis [*n*-BuOH–AcOH–H_2O (4 : 1 : 5), detection solution: aniline-phthalate, *Rf*: 0.18 (glucose), 0.25 (arabinose)] against the standard samples. The residue was dissolved in pyridine (0.2 ml), and then a pyridine solution (0.2 ml) of L-cysteine methyl ester hydrochloride (5 mg) was added to the solution. The mixture was kept at 60 °C for 1.5 h, dried *in vacuo*, and trimethylsilylated with 1-trimethylsilylimadazole (0.1 ml) at 60 °C for 2 h. After being partitioned between *n*-hexane and H_2O (0.5 ml each), the *n*-hexane extract was concentrated and analyzed by GC under the following conditions: HP-5 MS fused silica capillary column (30 m×0.25 mm, film thinkness 0.25 μm), column temperature at 230 °C, injection temperature at 250 °C, N_2 as carrier gas. The sugars were confirmed by the comparison of the retention times of their derivatives with standard samples [retention time, D-glucose (18.3 min), and L-arabinose (11.5 min)]. The presence of D-glucose in **1**, D-glucose and L-arabinose in **2** were detected.

Acknowledgements This work was financially supported by the Science and Technology Support Program of Gansu Province (Grant No. 090NKCM128) and the Scientific Research Foundation of Longdong University (Grant No. XYZK0802).

References

1) Delectis Florae Reipublicae Popularis Sinicae, Agendae Academiae Sinicae Edita, "Florae Reipublicae Popularis Sinicae," Vol. 54, Science Press, Beijing, 1980, pp. 104—106.
2) Ni N., Liu X. Q., *Chin. Tradit. Herb. Drugs*, **37**, 1895—1900 (2006).
3) Chang S. Y., Yook C. S., Nohara T., *Chem. Pharm. Bull.*, **46**, 163—165 (1998).
4) Yook C. S., Kim I. H., Hahn D. R., Nohara T., Chang S. Y., *Phytochemistry*, **49**, 839—843 (1998).
5) Chen M. L., Song F. R., Guo M. Q., Liu Z. Q., Liu S. Y., *Chem. J. Chin. Univ.*, **23**, 805—808 (2002).
6) An Q., Yang C. J., Song Y., Yu K., Xiong Z. L., Li F. M., *Nat. Prod. Res. Dev.*, **20**, 765—769 (2008).
7) Shirasuna K., Miyakoshi M., Mimoto S., Isoda S., Satoh Y., Hirai Y., Ida Y., Shoji J., *Phytochemistry*, **45**, 579—584 (1997).
8) Sawada H., Miyakoshi M., Isoda S., Ida Y., Shoji J., *Phytochemistry*, **34**, 1117—1121 (1993).
9) Havsteen B., *Biochem. Pharmacol.*, **32**, 1141—1148 (1983).
10) Shahidi F., Wanasundra P. K., *Crit. Rev. Food Sci. Nutr.*, **45**, 17—21 (1992).
11) Bekker M., Bekker R., Brandt V. E., *Phytochemistry*, **67**, 818—823 (2006).
12) Wang Z. L., Liu L. D., Tian G. W., Shen J. H., *Biodiv. Sci.*, **5**, 251—256 (1997).
13) Yi J. M., Kim M. S., Seo S. W., Lee K. N., Yook C. S., Kim H. M.,

Clin. Chim. Acta, **32**, 163—168 (2001).
14) Wei P., Yu, G. M., *Chin. Tradit. Herb. Drugs*, **20**, 152—154 (1989).
15) Esaki H., Onozaki H., Morimitsu Y., Kawakishi S., Osawa T., *Biosci. Biotechnol. Biochem.*, **62**, 740—746 (1998).
16) Itokawa H., Suto K., Takeya K., *Chem. Pharm. Bull.*, **29**, 254—256 (1981).
17) Harrison L. J., Sia G. L., Sim K. Y., *Planta Med.*, **60**, 493—494 (1994).
18) Wang H. Y., Xiao L. H., Liu L., Xu S. X., *J. Shengyang Pharm. Univ.*, **20**, 339—341 (2003).
19) Li N., Li X., Yang S. L., Wang J. H., Wang N., *J. Shengyang Pharm. Univ.*, **21**, 105—108 (2004).
20) Ishimaru K., Nishikawa K., Omoto T., Asai I., Yoshihira K., Shimomura K., *Phytochemistry*, **40**, 279—281 (1995).
21) Zhou X., Peng J. Y., Fan G. R., Wu Y. T., *J. Chromatogr. A*, **1092**, 216—221 (2005).
22) Soltis D. E., Bohm B. A., *J. Nat. Prod.*, **45**, 415—417 (1982).
23) Wagner H., Iyengar M. A., Beal J. L., *Phytochemistry*, **10**, 2553—2554 (1971).
24) Kang S. W., Yu Y. F., Wang P., *Chin. Tradit. Herb. Drugs*, **15**, 7—9 (1984).
25) Markham K. R., Ternai B., Stanley R., Geiger H., Mabry T. J., *Tetrahedron*, **34**, 1389—1397 (1978).
26) Xu B. A., Shu H., Zhang M. Z., *Acta Sci. Nat. Univ. Pekinensis*, **32**, 699—702 (1996).
27) Sawada H., Miyakoshi M., Isoda S., Ida Y., Shoji J., *Phytochemistry*, **34**, 1117—1121 (1993).
28) Bernhard H., Josef H., *Planta Med.*, **54**, 52—54 (1988).
29) Wang J. P., Wang M. Z., Gao Y. M., Lu J., Zhang H. Y., Qin H. Y., *Chin. Tradit. Herb. Drugs*, **26**, 68—70 (1995).
30) Nguyen A. T., Fontaine J., Malonne H., Duez P., *Phytochemistry*, **67**, 2159—2163 (2006).
31) Silayo A., Ngadjui B. T., Abegaz B. M., *Phytochemistry*, **52**, 947—955 (1999).
32) Nguyen T. H. A., Tran V. S., Andrea P., Katrin F., Ludger A. W., *Phytochemistry*, **62**, 1153—1158 (2003).
33) Zou J. H., Yang J. S., Zhou L., *J. Nat. Prod.*, **67**, 664—667 (2004).
34) Zhao J., Nakamura N., Hattori M., Yang X. W., Komatsu K., Qiu M. H., *Chem. Pharm. Bull.*, **52**, 230—237 (2004).
35) Zou J. H., Yang J. S., Dong Y. S., Zhou L., Lin G., *Phytochemistry*, **66**, 1121—1125 (2005).
36) Namikoshi M., Saitoh T., *Chem. Pharm. Bull.*, **35**, 3597—3602 (1987).
37) Jiang H. B., Tian X. Q., Hu X. B., Huang J., Sun Q., Chen Y. *West China J. Pharm. Sci.*, **21**, 416—419 (2006).
38) Zhang Y. M., Xu X. D., Hu B. H., Liu Q., Hou C. Y., Liu Y. L., Yang J. S., *Acta Pharm. Sin.*, **32**, 301—304 (1997).
39) Li W., Asada Y., Yoshikawa T., *Phytochemistry*, **55**, 447—456 (2000).
40) Qin M. J., Ji W. L., Wang Z. T., Ye W. C., *J. Integr. Plant Biol.*, **47**, 1404—1408 (2005).
41) Markham K. R., Stanley B. T., Geiger H., *Tetrahedron*, **34**, 1389—1397 (1978).
42) Li D. P., Ouyang M. A., Chaweewan J., Yang C. R., *Acta Pharm. Sin.*, **34**, 43—45 (1999).