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Flavonoids from *Ziziphus jujuba Mill var. spinosa*

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Abstract—Eight flavonoid compounds were isolated from the seeds of *Ziziphus jujuba Mill var. Spinosa*. On the basis of chemical and spectral analyses their structures were elucidated as swertish (**1**), puerarin (**2**), 6‴-feruloylspinosin (**3**), apigenin-6-*C*-β-D-glucopyranoside (**4**), spinosin (**5**), 6‴-feruloylisospinosin (**6**), isospinosin (**7**), and isovitexin-2″-*O*-β-D-glucopyranoside (**8**). Flavonoids **6** and **7** are novel compounds. Rotamers exist for compounds **1**, **3** and **5**, which are reported for the first time. Compounds **2**, **4** and **8** were isolated from this plant for the first time. Spinosin and swertish possess significant sedative activity.

Introduction

The seeds of *Ziziphus jujuba Mill var. spinosa (Bunge)* Huex. H.F. Chou are used as a sedative medicine in China. In our recent research, a 95% aqueous EtOH extract of the seeds were separated by repeated chromatagraphy to give eight flavonoid compouds. On the basis of chemical and spectral analyses their structures were elucidated as swertish (**1**), puerarin (**2**), 6‴-feruloylspinosin (**3**), apigenin-6-*C*-β-D-glucopyranoside (5, 7, 4′- trihydroxyflavone-6-*C*-β-D-glucopyranoside, **4**), spinosin (**5**), 6‴-feruloylisospinosin (**6**), isospinosin (**7**), isovitexin-2″-*O*-β-D-glucopyranoside (5, 7, 4′-trihydroxyflavone-6-*C*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside, **8**) (Fig.1). Flavonoids **6** and **7** are novel compounds named 6‴-feruloylisospinosin and isospinosin. Rotamers exist for compounds **1**, **3** and **5**, which are reported for the first time. Compounds **2**, **4** and **8** were isolated from this plant for the first time. Spinosin and swertish possess significant sedative activity. The oral administration of spinosin and swertish (4×10^{-5} mol/Kg) prolonged pentobarbital induced sleeping time by 29–31% compared to the control group. Swertish used to study the sedative activity was afforded by the acidic hydrolysis of **5**.

Results and Discussion

The seeds of *Ziziphus jujuba Mill var. spinosa* were extracted with 95% aqueous EtOH. The extract was dissolved in 50% aqueous EtOH and extracted with petroleum ether. The 50% aqueous EtOH layer was concentrated and the residue obtained was extracted with *n*-BuOH. The butanolic extract was isolated using silica gel column, Sephadex LH-20 column and HPLC to obtain compounds **1**–**8** (Fig. 1).

Compound **5**, yellow powder, mp 237–240°C. UV λmax (nm) MeOH: 271, 334; NaOMe: 271, 389; $AlCl_3$: 281, 301, 352, 383; $AlCl_3$/HCl: 281, 302, 350, 382; NaOAc: 271, 390. The UV spectrum showed **5** had the structure of 5,4′-dihydroxyflavonoid. NFAB-MS gave a quasi molecular ion peak at *m/z*: 607$[M-1]^-$. ^{1}H NMR and ^{13}C NMR spectra possess separate signals (Experimental and Table 1), which were almost superimposable on those of the mixture of spinosin and zivulgarin reported by Zeng Lu[1] the structure of spinosin is 5,4′-dihydroxy-7-methyoxyflavone-6-*C*-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside, zivulgarin is 5,4′-dihydroxy-7-methyoxyflavone-6-*C*-β-D-glucopyranosyl (1→4)-β-D-glucopyranoside. Compound **5** also had the same R_f value as the mixture of spinosin and zirulgorin on TLC. In a comparison of the ^{1}H and ^{13}C NMR spectra of **5** with those of spinosin,[2] except for the phenomenon of separate signals all of the signals of **5** were almost superimposable on those previously reported for spinosin. One- and two-dimensional NMR techniques (^{1}H NMR, ^{13}C NMR, COSY, HMBC) permitted assignments of all the ^{1}H and ^{13}C signals of **5**. HMBC experiments showed correlation of the H-1 of the terminal glucose with C-2 of the internal glucose, and H-1 of the internal glucose with C-6 of the aglycone (Fig. 1, spinosin). These results permitted us to conclude two glucose linked together at C-2 and the sugar chain bound to C-6 of the aglycone of **5**.

Keywords: *Ziziphus jujuba Mill var. spinosa*, flavonoid, flavone-C-glycoside; conformers; spinosin; 6‴-feruloylisospinosin; isospinosin; sedative activity.

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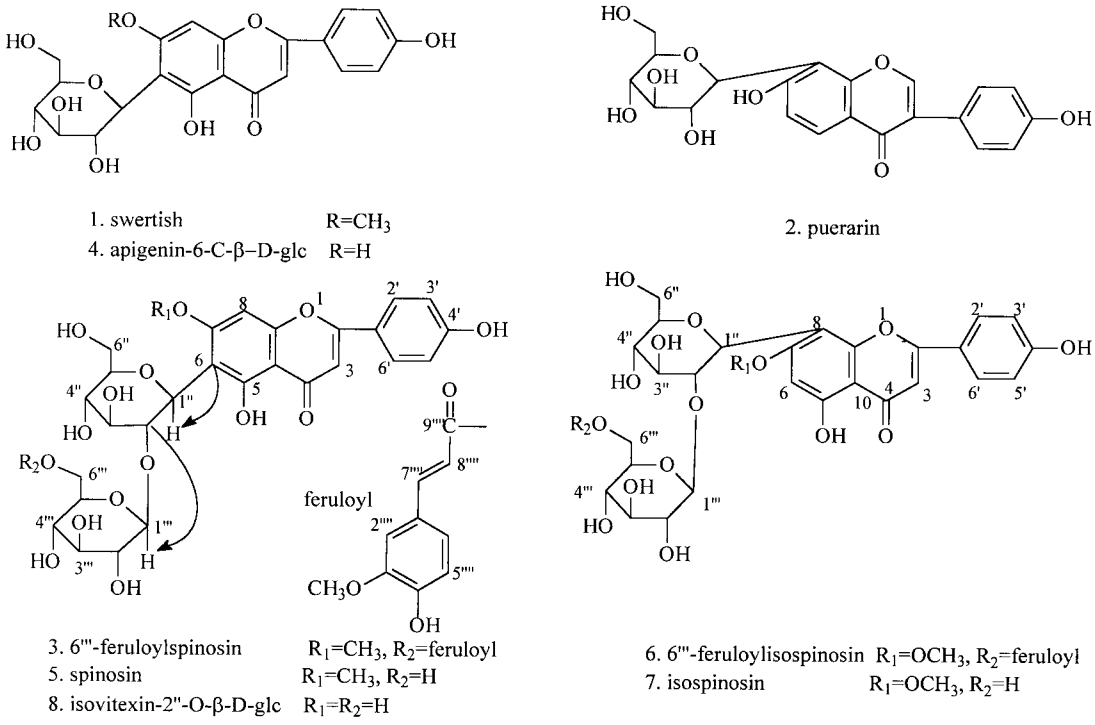


Figure 1. The structure of compounds **1**–**8**.

Table 1. ^{13}C NMR data of compounds **1**, **3**, **5** (125 Mz, DMSO-d_6) (*B=spinosin)

No.	*B[2]	**5** (298 K)>		**5** (393 K)	**1** (298 K)		**3**(298 K)	
Aglycone 2	163.7	163.66	163.66	163.59	163.7	163.8	163.6	163.8
3	102.8	102.90	102.99	102.77	103.0	103.0	102.9	103.1
4	181.6	181.82	182.15	181.48	181.8	182.2	181.7	182.2
5	159.9	159.57	160.42	159.68	159.5	160.2	159.4	160.7
6	108.7	108.58	108.58	109.00	109.6	109.6	108.7	108.7
7	164.3	163.72	164.94	164.11	164.9	164.9	165.1	164.1
8	90.2	90.20	90.67	90.30	90.1	91.0	89.9	90.6
9	156.9	156.85	156.96	156.58	156.7	156.8	156.8	157.0
10	104.1	104.09	104.36	104.11	104.1	104.5	103.9	104.4
1′	120.9	120.92	120.92	120.89	121.0	121.0	121.1	121.2
2′ 6′	127.9	128.34	128.34	127.73	128.4	128.4	128.4	128.5
3′ 5′	115.6	115.59	115.89	115.59	115.8	115.9	115.70	115.75
4′	161.1	160.78	161.22	160.78	161.2	161.2	161.1	161.1
OCH_3		55.99	56.42	55.89	56.2	56.4	56.3	56.0
Inner glc1″	70.7	70.64	70.99	70.72	70.8	70.9	70.62	70.96
2″	80.3	80.58	81.06	80.06	72.5	72.8	80.1	81.5
3″	78.2	78.19	78.55	78.09	79.0	79.0	78.5	78.7
4″	70.3	70.41	70.41	70.45	69.6	70.3	70.2	70.3
5″	81.0	81.42	81.67	80.60	81.5	81.7	81.9	81.9
6″	61.3	61.40	61.40	61.39	61.7	61.7	61.4	61.4
Outer glc1‴	104.6	105.08	105.22	104.26			105.1	105.6
2‴	74.3	74.48	74.62	74.22			74.3	74.3
3‴	76.1	76.25	76.25	76.04			76.2	76.2
4‴	69.6	69.23	69.52	69.85			68.2	68.9
5‴	75.9	76.25	76.25	75.73			73.1	73.3
6‴	60.5	60.10	60.59	60.68			62.0	62.4
Feruloyl 1‴′							125.3	125.5
2‴′							110.8	110.9
3‴′							147.77	147.85
4‴′							149.2	149.2
5‴′							115.3	115.4
6‴′							122.9	129.0
7‴′							144.6	144.7
8‴′							113.6	114.0
9‴′							166.1	166.2
OCH_3							55.6	55.6

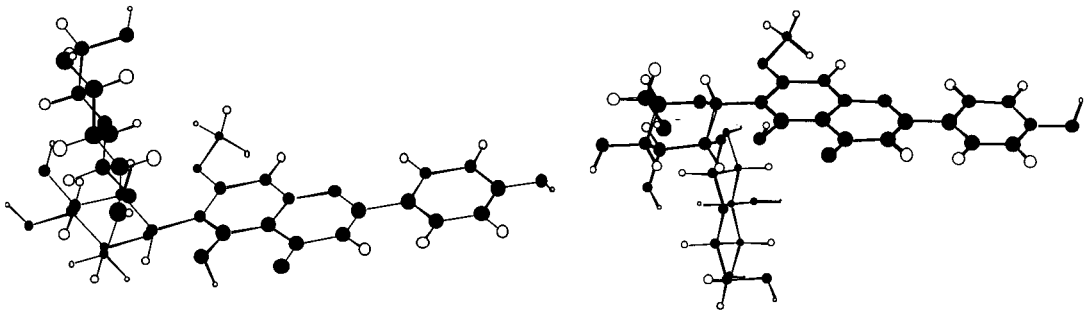


Figure 2. Two conformers of spinosin from CS Chem 3D Pro.

When ^{1}H and ^{13}C NMR spectra of **5** were measured at high temperature (393 K, 120°C in DMSO-d_6), the multiplicity collapsed to a first order spectrum, and on decreasing to room temperature multiplicity was observed again. On the basis of these observations, the structure of **5** was established as spinosin and it is proposed that rotational isomers (Fig. 2) must exist in spinosin. Thus the report of the mixture of spinosin and zivulgarin[1] is incorrect. The compound is not a mixture but instead rotamers of spinosin. A very interesting phenomenon was observed. Like spinosin, swertish (**1**) and 6‴-ferulloylspinosin (**3**) also had the same NMR phenomenon -serial separate signals. All these compounds have 5-OH and 7-OCH_3, 6-C-glycose, but in compounds 2, 4 and 6–8 the separate signals were not observed. Obviously, two conformers are produced by the rotational barrierd 7-OCH_3 in flavone-6-C-glycoside.

As mentioned above, compound **5** showed two stable conformers in NMR spectroscopy. In order to uncover the factors stabilizing these two conformers at room temperature, conformational analysis was performed on compound A (**5**), B (**8**) and C (See Fig. 3).

The conformational analysis was performed using the MSI INSIGHT II software package.[3] The consistent Force Field (CFF91)[4] was chosen to assign parameters to the compounds. CFF91 is optimised to suit the prediction of gas-phase geometries, vibrational frequencies, conformational energies, torsion barriers and crystal structures of small models. The CFF91 forcefield employs quartic polynomials for bond stretching and angle bending and a three-term Fourier expansion for torsions. The out-of-plane (also called inversion) coordinate is defined according to Wilson et al.[5] The van der Waals interactions use an inverse 9th-power term for the repulsive part rather than the more customary 12th-power term.

Grid Scan was used as the method of conformer search. This procedure enables systematic exploration of conformations by stepwise alteration of selected torsion angles over specified range of values. The spatial hindrance of the methoxyl group on the aglycone moiety to the free rotation of the link bond between the aglycone part and the saccharide part seems to be the main reason of the existence of two similar conformers. So the torsion 1 and torsion 2 were consideed in the confomational search. Torsion 3 was added in order to compare compounds A, B and C to show the difference between the effects of methoxyl group and hydroxyl group on the whole molecular conformation in total. All 3 torsions were set to rotate from 0 to 350° and once per 30°, thus 1728 conformations in total were generated in the Grid Scan. To avoid the irrational interatom distance generated by the rotation, which will cause system energy rise to an unreasonable level, each conformer obtained in the rotation was minimized from its current point to a reasonable conformer. Thus, the energies of those conformers are appropriate and comparable.

For each compound, an energy vs. conformer graph was generated after rotation of each torsion and minimization of each conformer. For compound A, the lowest energy is 202.67 kcal/mol (conformer 679), and the highest energy is 294.53 kcal/mol (conformer 1055). For compound B, the lowest energy is 193.59 kcal/mol (conformer 1520), and the highest energy is 247.54 kcal/mol (conformer 291). For compound C, the lowest energy is 234.41 kcal/mol (conformer 1552), and the highest energy is 319.30 kcal/mol (conformer 1326). It must be mentioned here that since the structures of these three compounds are different,

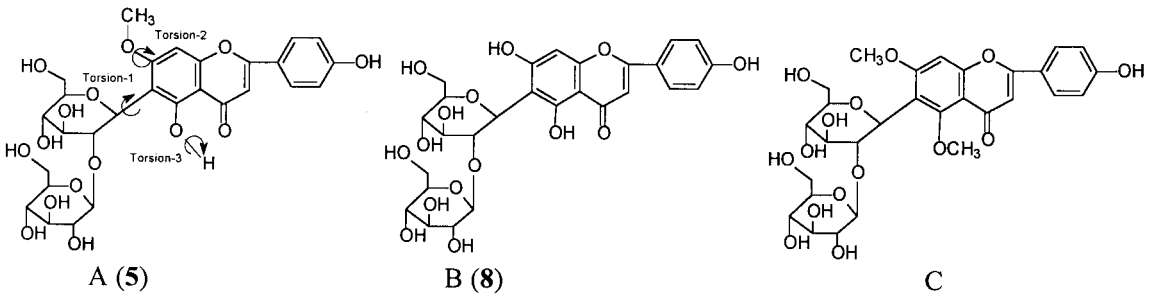
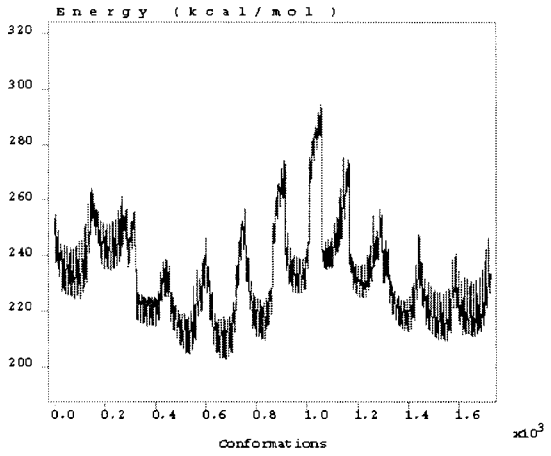
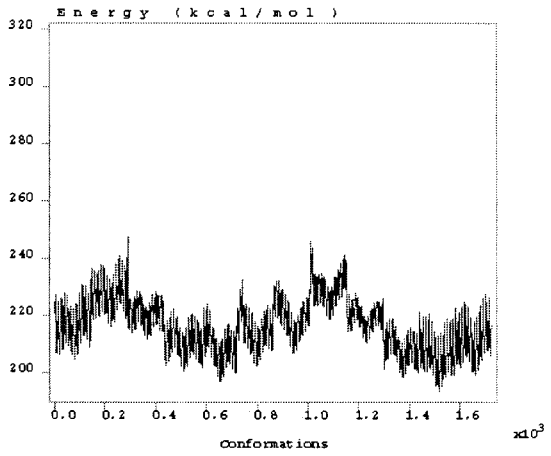


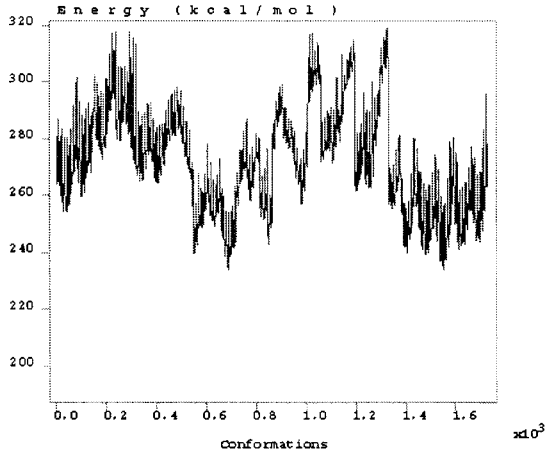
Figure 3. The structures of the compounds used in the conformational analysis. (The torsions used in conformation analysis are indicated on the compound A(5) and are similar for the other two compounds.)



1. Compound A



2. Compound B



3. Compound C

Figure 4. The conformational analysis results of Compounds A, B and C.

the absolute energy can not useful for comparison. Only the energy difference between conformers of the same compound is meaningful.

In Fig. 4.1, there is an obvious energy barrier between two energy minima, which agrees with the existence of the two stable conformers of Compound A in NMR spectroscopy. As for compound B, the substitution of a methoxyl group with a hydroxyl group reduced the spatial hindrance to the rotation of the link bond (Torsion-1). In the conformational analysis Fig. 4.2, the energy barrier is much lower than for compound A. Thus compound B dose not show iso-conformers in NMR spectroscopy at room temperature with so smooth a barrier between the two potential minima. When the two hydroxyl groups in the aglycone moiety are both methylated to form compound C, the conformation analysis also reflects the enhanced hindrance caused by two methoxyl group. The energy barrier in Fig. 4.3 is deeper when compared to Fig. 4.1, which confirms that two conformers of compound C can exist.

Compound **6** was obtained as a yellow powder, mp 200–201°C, UV λmax (nm): 271, 325; NaOMe: 248, 381; NaOAc: 269, 331 (sh); $AlCl_3$: 278, 304, 334, 380; $AlCl_3$/HCl: 278, 304, 334, 381, the UV spectum showed **6** had a structure of 5,4′-dihydroxyflavonoid. FAB-MS gave a pseudo-molecular ion peak at (*m*/*z*) 785$[M+1]^+$. HR-MS showed a molecular formula $C_{38}H_{40}O_{18}$, calcd 784.7232, obs. 807.2135 $[M+Na]^+$. In the ^{13}C NMR the signals of **6** were in good agreement with those of 6‴-feruloylspinosin[6] except for the signals at 109.4 and 90.6 for C-6 and C-8. The two ^{13}C NMR signals of **6** were observed at 94.9 and 104.8, which were identical with the signals at 94.5 and 105.2 due to C-6 and C-8 of 5, 4′-dihydroxy-7-methoxyflavone-8-*C*-β-D-glucosid.[7] Based on these observation the structure of **6** was determined to be 5,4′-dihydroxy-7-methyoxyflavone-8-C-6‴-feruloyl-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside. Compound **6** is a novel compound named 6‴-feruloyl-isospinosin.

Compound **7** was obtained as yellow powder. UV λmax (nm) MeOH: 269, 329; NaOMe: 272, 380; NaOAc: 269, 358; $AlCl_3$: 276, 304, 342, 385; $AlCl_3$/HCl: 277, 303, 339, 382, the UV spectrometer showed **7** had the structure of 5,

4′-dihydroxyflavonoid. TOF-MS and FAB-MS gave the same quasimolecular ion peak at *m/z* 609[M+1]$^+$ as spinosin (**5**). Negative HR-MS showed a molecular formula $C_{28}H_{31}O_{15}$, calcd 607.1668, obs. 607.1670 [M−1]$^-$. In the ^{13}C NMR of **7** the signals for the aglycone were in good agreement with those of compound **6**, the signals for the sugars were identical with those of **5**(120°C). The structure of **7** was identified as 5,4′-dihydroxy-7-methoxyflavone-8-*C*-β-D-glucopyranosyl(1→2) -β-D-glucopyranoside. Compound **7** is a novel compound named isospinosin.

In the ^{13}C NMR spectra of compounds **1**, **3**, **4** and **8**, signals were found to be identical with those of known compounds swertish,[2] 6‴-feruloylspinosin,[6] apigenin-6-*C*-β-D-glucopyranoside[8] and isovitexin-2″-*O*-β-D-glucopyranoside, respectively,[9] but the multiplicity caused by rotatrenal isomeism in compounds **1** and **3** was also observed in their NMR spectra.

Experimental

General experimental procedures

Melting points were determined on an X_4 apparatus and are uncorrected. UV spectra were taken in MeOH on a Shimadzu UV 260 spectrometer. ^{1}H NMR and ^{13}C NMR, H–H COSY, HMQC and HMBC spectra were recorded with a Bruker AM-500 instrument. FAB-MS was taken on a KYKY-ZHP-5# spectrometer, TOF-MS was recorded on MALDI-TOF instrument. Silica gel was purchased from Marine Chemical Factory in Qingdao. Sephadex LH-20, RP-18(Chemical Reagent Factory, Tian Jin) were used. TLC was performed on a RP-18 precoated layer (Merck).

Plant material

The seeds of *Ziziphus jujuba Mill var. spinosa* were collected in Baoji, Shaanxi Province of China and were identified by Professor Zheng Junhua in Division of Pharmacognosy and deposited in Division of Natural Medicinal Chemistry, Beijing Medical University.

Extraction and isolation

The seeds of the plant were extracted with 95% aqueous EtOH. The extract was dissoved in 50% aqueous EtOH and was extracted with petrol. The 50% aqueous EtOH layer was concentrated and the residue obtained was extracted with *n*-BuOH. The *n*-BuOH extract was fractioned by silica gel column eluting with gradient $CHCl_3$–MeOH–H_2O to give fractions A–C. Fraction A was purified using Sephadex LH-20 and separated by HPLC (43% aqueous MeOH) to yield **1** (10 mg).Fraction B was fractioned by Sephadex LH-20(MeOH) to afford fractions 1–3. Fraction 1 was subjected to CC on silica gel eluting with EtOAc–EtOH–H_2O=10:1:5, to give **2** (13 mg) and **3** (40 mg). Fraction 2 and 3 were each separated by HPLC (43% aqueous MeOH), to give **4** (15 mg) and **6** (18 mg). Fraction C was crystallised ($CHCl_3$–EtOH) to give **5** (2.4 g). The mother-liquor was subjected to HPLC (52% aqueous MeOH) to afford **7** (12 mg) and **8** (8 mg).

Acidic hydrolysis of 5

A solution of **5** in 2 M HCl/H_2O was refluxed at 100°C for 6 h. The reaction mixture was extracted with *n*-BuOH. The *n*-BuOH layer was washed with H_2O and evaporated to dryness to obtain a yellow solid, its mp, R_f and ^{13}C NMR data were identical with an authentic sample of swertish.

Identification

Compound 1 swertish. Yellow powder mp 225–228°C, UV λ_{max} (nm): MeOH: 270, 329, NaOMe: 270, 390, $AlCl_3$: 278, 302, 343, 373. HRFAB MS *m/z*: 445.1131[calcd for $C_{22}H_{21}O_{10}$(M−H)$^-$,445.1140].^{1}H NMR (500 MHz DMSO-d_6), (splitting caused by rotational isomerism): 7.97 (2H, d, *J*=7.9 Hz, 2′, 6′-H); 6.93 (2H, d, *J*=7.9 Hz, 3′, 5′-H); 6.87,6.85 (1H, s, 8-H); 6.83, 6.82 (s, 3-H). ^{13}C NMR data see Table 1.

Table 2. ^{13}C NMR data of compounds **4**, **6**–**8** (125 MHz,DMSO-d_6) (*A: 5, -4′-dihydroxy-7-methoxyflavon-8-*C*-β-D-glycopyranoside)

No.	*A[7]	4	6	7	8	No.	6	7	8
2	164.0	163.0	163.3	163.5	163.5	1‴	104.2	104.9	105.3
3	102.6	102.6	102.5	102.7	102.8	2‴	74.4	74.5	74.7
4	181.8	181.9	182.0	182.3	182.0	3‴	75.8	76.2	76.4
5	161.0	160.5	161.1	161.2	161.2	4‴	68.9	69.3	69.4
6	95.4	108.9	94.9	95.1	108.0	5‴	73.6	76.2	76.5
7	163.1	163.0	164.0	164.3	163.0	6‴	62.4	60.2	60.5
8	105.2	93.9	104.8	104.9	93.0	1‴′	125.6		
9	155.3	156.3	155.2	155.3	156.4	2‴′	111.1		
10	104.6	102.6	104.2	104.3	103.9	3‴′	147.9		
1′	123.1	121.1	121.5	121.7	121.2	4‴′	149.2		
2′ 6′	128.8	128.2	128.9	129.1	128.5	5‴′	115.5		
3′ 5′	115.8	115.9	115.8	115.9	116.0	6‴′	123.0		
4′	161.0	161.1	161.4	161.5	181.2	7‴′	144.7		
OCH_3			56.3	56.0		8‴′	114.3		
1″	73.1	73.2	71.4	71.4	70.5	9‴′	166.3		
2″	70.7	70.5	79.8	80.9	80.5	OCH_3	56.6		
3″	78.7	78.9	78.2	78.2	78.3				
4″	70.4	70.2	70.2	70.1	70.4				
5″	81.7	81.3	81.8	81.9	81.7				
6″	61.2	61.3	60.9	60.9	61.4				

Compound 2 puerarin. White powder, mp 203–205°C, UV λ_{max} (nm): MeOH: 250, 303(sh), NaOAc: 258, 342, $AlCl_3$: 250, 305(sh)° ^{1}H NMR, (500 MHz DMSO-d_6): 8.3 (1H, s, 2-H), 7.9 (1H, d, J=8.7 Hz, 5-H), 7.4 (2H, d, J=8.4 Hz, 2′, 6′-H), 7.0 (1H, d, J=8.7 Hz, 6-H),6.8 (2H, d, J=8.4 Hz, 3′,5′-H), 4.8 (1H, d, J=9.8 Hz 1″-H). FAB-MS, *m/z*: 439, 417, 386, 328, 307, 296, 288, 273, 258, 245, 225. HRFAB MS *m/z*: 415.1036 [calcd for $C_{21}H_{19}O_9(M-H)^-$, 415.1035].^{13}C NMR (δppm, C-2–10; 1′–6′; 1″–6″): 152.5, 120.3, 174.8, 126.1, 114.9, 161.0, 112.6, 156.1, 116.7; 122.6, 129.9, 114.9, 157.1, 114.9, 129.9; 73.4, 70.8, 78.6, 70.4, 81.7, 61.3. On the basis of the spectral analysis the structure of compound **2** was identified as puerarin (7,4′-dihydroxy-isoflavone-8-*C*-β-D-glucopyranoside).

Compound 3 (6‴-feruloylspinosin). Yellow powder, mp 224°C (dec.), UV λ_{max} (nm) MeOH: 274, 328, NaOMe: 269, 380, $AlCl_3$: 284, 302, 336, 385, NaOAc: 273, 333° ^{1}H NMR (500 MHz DMSO-d_6), (splitting caused by rotational isomerism): 7.81 (d, J=7.9 Hz 2′,6′-H), 7.18, 7.06, (d, J=15.7 Hz, 7‴′-H), 7.17, 7.04 (d,J=2.0 Hz, feruloyl- 2‴′-H), 6.89 (d, J=7.9 Hz, 3′,5′-H), 6.85, 6.83 (s, 3-H), 6.77, 6.67 (s, 8-H), 6.72 (dd, J=8.1,2.0 Hz, 6‴′-H),6.67 (d, J=8.1 Hz, 5‴′-H), 6.23, 6.15 (d, J=15.7 Hz, 8‴′-H),4.67 (t, J=9.3 Hz, glc1″-H), 4.27, 4.23 (t, J=7.9 Hz, glc1‴-H) FAB-MS, *m/z*: 783 $[M-1]^-$, 612, 607, 459, 443, 409, 379, 352, 339. HRFAB MS *m/z*: 783.2135 [calcd for $C_{38}H_{39}O_{18}(M-H)^-$,783.2142]. ^{13}C NMR data see Table 1.

Compound 4 (5, 7, 4′-trihydroxyflavone-6-*C*-β-D-glucoside). Yellow powder, mp 242–244°C, UV λ_{max} (nm): MeOH: 271, 329; NaOMe: 279(sh), 380; $AlCl_3$: 277, 301, 343, 380; $AlCl_3$/HCl=$AlCl_3$; NaOAc; 278, 329, 395. ^{1}H NMR (500 MHz DMSO-d_6): 13.5 (1H, brs, 5-OH), 7.88 (2H, d, J=8.6 Hz, 3′, 5′-H), 6.91 (2H, d, J=8.6 Hz, 2′, 6′-H), 6.69 (1H, s, 3-H), 6.41 (1H, s, 8-H), 4.57 (1H, d, 9.9 Hz, 1″-H). ^{13}C NMR data see Table 2.

Compound 5 (spinosin). Yellow powder, mp 237–240°C, UV λ_{max} (nm) MeOH: 271, 334; NaOMe: 271, 389; $AlCl_3$: 281, 301, 352, 383; $AlCl_3$–HCl: 281, 302, 350, 383; NaOAc: 271, 390. NFAB-MS *m/z*: 607$[M-1]^-$. HRFAB MS *m/z*: 607.1657 [calcd for $C_{28}H_{31}O_{15}(M-H)^-$, 607.1663]. ^{1}H NMR (500 MHz DMSO-d_6) (at room temperature): 7.97 (2H, d, J=8.7 Hz, 2′, 6′-H), 6.95 (2H, d, J=8.7 Hz, 3′, 5′-H), 6.83, 6.84 (1H, s, 3-H), 6.67, 6.80 (1H, s, 8-H), 4.67, 4.69 (1H, d, J=9.8 Hz, glc 1″-H), 4.15, 4.17(1H, d, J=8.5 Hz, glc 1‴-H). ^{1}H NMR (at 120°C):7.88 (2H, d, J=8.7 Hz, 2′, 6′-H), 6.98 (2H, d, J=8.7 Hz, 3′, 5′-H), 6.73 (1H, s, 3-H), 6.67 (1H, s, 8-H), 4.77 (1H, d, J=9.7 Hz, glc 1″-H), 4.25 (1H, d, J=7.7 Hz, glc 1‴-H), 3.92 (3H, s, OCH_3),3.89 (3H, s, OCH_3), ^{13}C NMR data see Table 1.

Compound 6 (6‴-feruloylisospinosin). Yellow powder, mp 200–201°C, FAB-MS, *m/z*: 785$[M+1]^+$ HRFAB MS *m/z*: 807.2135 [calcd for $C_{38}H_{40}O_{18}Na$ $[M+Na]^+$, 807.2112]. IR (KBr) ν_{max} cm^{-1}: 3316, 2918, 1669, 1642, 1605, 1589, 1507, 1486, 1446, 1431, 1396, 1348, 1276, 1202, 1171, 1083, 1057, 1025. UV λ_{max} (nm): MeOH: 271, 325; NaOAc: 269, 331(sh); $AlCl_3$: 278, 304, 334, 380. ^{1}H NMR (500 MHz DMSO-d_6): 7.98 (2H, d, J=8.6 Hz, 2′, 6′-H), 7.48 (1H, d, J=15.9 Hz, 7‴′-H), 7.30 (1H, d, J=1.5 Hz, 2‴′-H), 7.08 (1H, dd, J=8, 1.5 Hz, 6‴′-H), 6.85 (2H, d, J=8.6 Hz, 3′, 5′-H), 6.81 (1H, d, J=8.2 Hz, 5‴′-H), 6.70 (1H, s, 6-H), 6.43 (1H, s, 3-H), 6.29 (1H, d, J=15.9 Hz, 8‴′-H), 4.82 (1H, d, J=9.6 Hz, glc 1″-H), 4.03 (1H, d, J=7.6 Hz, glc 1‴-H), 3.84 (3H, s, 7-OCH_3),3.82 (3H, s, feruloyl-OCH_3). ^{13}C NMR data see Table 2.

Compound 7 (isospinosin). Yellow powder, mp 215–216°C IR (KBr) $\nu_{max}cm^{-1}$: 3330, 2923, 1643, 1601, 1512, 1480, 1447, 1410, 1379, 1345, 1299, 1252, 1195, 1178, 1167, 1115, 1069, 1018. UV λ_{max} (nm) MeOH: 269, 329; NaOMe: 272, 380; NaOAc: 269, 358; $AlCl_3$: 276, 304, 342, 385; $AlCl_3$/HCl: 277, 303, 339, 382, TOF-MS and FAB-MS: 609$[M+1]^+$. NHR-MS showed a molecular formula $C_{28}H_{31}O_{15}$, calcd 607.1663, obs. 607.1670 $[M-1]^-$.^{1}H NMR (500 MHz DMSO-d_6): 13.3 (s, 5-OH), 8.01 (2H, d, J=8.6 Hz, 3′, 5′-H), 6.89 (2H, d, J=8.6 Hz, 2′, 6′-H), 6.77 (1H, S, 6-H), 6.46 (1H, S 3-H), 4.82 (1H, d, J=10.0 Hz, glc 1″-H), 4.03 (1H, d, J=7.5 Hz, glc 1‴-H).3.84 (3H, s, OCH_3), ^{13}C NMR data see Table 2.

Compound 8 (isovitexin-2″-*O*-β-D-glucopyranoside). Yellow powder, mp 206–208°C TOF-MS: 617 $[M+Na]^+$, 633 $[M+K]^+$. ^{1}H NMR (500 MHz DMSO-d_6) 13.6 (s, 5-OH), 7.91 (2H, d, J=8.8 Hz, 2′, 6′-H), 6.92 (2H, d, J=8.8 Hz, 3′, 5′-H), 6.75 (1H, s, 8-H), 6.48 (1H, s, 3-H), 4.64 (1H, d, J=9 Hz, glc 1″-H), 4.15 (1H, d, J=7.6 Hz, glc 1‴-H). ^{13}C NMR data see Table 2.

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