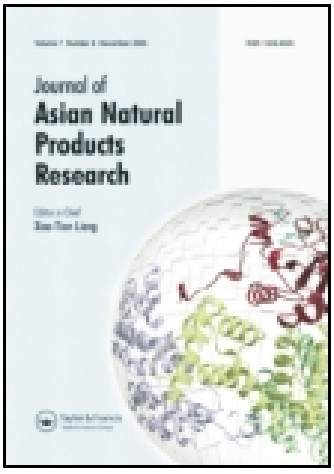

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Two new flavonoid glycosides from Semen Ziziphi Spinosae

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Published online: 01 Feb 2012.

To cite this article: Lin Zhang, Zhi-Lin Xu, Chun-Fu Wu, Jing-Yu Yang, Yoshihiro Kano & Dan Yuan (2012) Two new flavonoid glycosides from Semen Ziziphi Spinosae, Journal of Asian Natural Products Research, 14:2, 121-128, DOI: 10.1080/10286020.2011.637491

To link to this article: http://dx.doi.org/10.1080/10286020.2011.637491

Journal of Asian Natural Products Research
Vol. 14, No. 2, February 2012, 121–128

Two new flavonoid glycosides from *Semen Ziziphi Spinosae*

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(*Received 22 July 2011; final version received 30 October 2011*)

Two novel flavonoid glycosides, 6‴-dihydrophaseoylspinosin (**1**) and 6″,6‴-diferuloylspinosin (**2**), were isolated from the MeOH extract of *Semen Ziziphi Spinosae*, together with six known flavonoids, isovitexin-2″-*O*-*β*-(6-*O*-*E*-feruloyl)glucopyranoside (**3**), spinosin (**4**), isospinosin (**5**), 6‴-feruloylspinosin (**6**), swertisin (**7**), and isovitexin-2″-*O*-*β*-D-glucopyranoside (**8**). The structures of **1** and **2** were elucidated by spectroscopic methods including UV, IR, ESI-TOF-MS, 1D NMR, and 2D NMR experiments.

Keywords: *Semen Ziziphi Spinosae*; flavonoid glycosides; 6‴-dihydrophaseoylspinosin; 6″,6‴-diferuloylspinosin

1. Introduction

Semen Ziziphi Spinosae is botanically from the seeds of *Ziziphus jujuba* Mill var. spinosa (Bunge) Huex. H.F. Chou, which was used for tranquilizing mind, nourishing liver, soothing nerves, and arresting sweating in traditional Chinese medical therapy. Previous phytochemical studies on *Semen Ziziphi Spinosae* reported the isolation of many flavonoids [1–6], saponins [6–9], and alkaloids [10,11]. In a continuing search for new compounds from this plant, we obtained a series of flavonoids including two new flavonoid glycosides **1** and **2** and six known compounds **3**–**8** (Figure 1). In this paper, the isolation and structural elucidation of these compounds are described.

2. Results and discussion

The MeOH extract of the *Semen Ziziphi Spinosae* was prepared by reflux method. A series of column chromatography using silica gel, Sephadex LH-20, and MDS-5 reversed-phase packings led to two new compounds 6‴-dihydrophaseoylspinosin (**1**) and 6″,6‴-diferuloylspinosin (**2**), together with six known flavonoids, isovitexin-2″-*O*-*β*-(6-*O*-*E*-feruloyl)glucopyranoside (**3**), spinosin (**4**), isospinosin (**5**), 6‴-feruloylspinosin (**6**), swertisin (**7**), and isovitexin-2″-*O*-*β*-D-glucopyranoside (**8**). The structures of **1** and **2** were elucidated by 1D and 2D NMR, UV, IR, and MS techniques, and those of **3**–**8** were identified by comparing their NMR and MS spectral data with reported values [3,12].

Compound **1** was isolated as a yellowish amorphous powder. The molecular formula was determined to be $C_{43}H_{52}O_{19}$ from the $[M-H]^-$ ion peak at *m*/*z* 871.3026 in the ESI-TOF-MS. The UV absorption maxima at 219, 274, and 322 nm, together with a positive reaction in $AlCl_3$ reagent, revealed a flavone nucleus. The IR spectrum showed absorption bands due to a hydroxyl group (3398 cm^{-1}) and a carboxylic carbonyl

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ISSN 1028-6020 print/ISSN 1477-2213 online

http://dx.doi.org/10.1080/10286020.2011.637491
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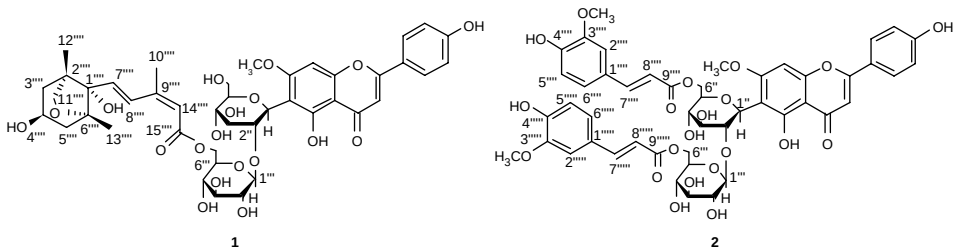

Figure 1. Chemical structures of compounds **1** and **2**.

($1705\,cm^{-1}$). The resonances for the carbons and protons located on the flavonoid framework had a close resemblance to those of the known spinosin, and they were assigned according to the literature values of the ^{1}H and ^{13}C NMR spectral data for spinosin [3] as well as its own HSQC spectrum. The spinosin moiety accounted for a partial molecular formula of $C_{28}H_{32}O_{15}$ with 13 degrees of unsaturation, and thus, the remaining molecular formula $C_{15}H_{21}O_4$ consisted structurally of one substituent, accounting for six degrees of unsaturation and being attached to the outer glucopyranosyl moiety. The resonances for the carbons and protons for the substituent in the ^{1}H and ^{13}C NMR as well as HSQC spectra included an ester carbonyl group (δ_C 164.6/164.8), as evidenced by the IR absorption band at $1705\,cm^{-1}$, three sp^2 methines (δ_H 5.51/5.50, 1H, s, δ_C 116.3/115.8; δ_H 6.42/6.40, 1H, d, $J = 15.6$ Hz, δ_C 135.6/135.5; δ_H 7.83/7.73, 1H, d, $J = 15.6$ Hz, δ_C 129.1/129.0), a sp^2-tertiary carbon (δ_C 129.1/129.0), four sp^3 carbons that should be directly attached to oxygen, including a methylene (δ_H 3.65, 3.58, 2H, m, δ_C 75.3/75.3) and a methine (δ_H 3.92, 1H, m, δ_C 63.8/63.8), two sp^3-tertiary carbons (δ_C 81.5/81.5; δ_C 85.7/85.7), two methylenes (δ_H 1.72, 1H, m, 1.54, 1H, m, δ_C 43.8/43.8; δ_H 1.89, 1H, m, 1.64, 1H, m, δ_C 45.4/45.4), a methyl (δ_H 1.86/1.91, 3H, s, δ_C 20.5/20.5) that should be directly attached to sp^2 carbon, two methyl groups (δ_H 0.83/0.82, 3H, s, δ_C 16.0/16.0; δ_H 1.00/0.97, 3H, s, δ_C 19.4/19.4), and an sp^3-tertiary carbons (δ_C 48.0/47.9). Thus, the substituent should be bicyclic moiety according to the degrees of unsaturation. The constitution of the substituent was deduced from the HMBC spectrum (Figure 2). The HMBC correlations of a hexacyclic moiety were determined between H-12$''''$ and C-1$''''$, C-2$''''$, and C-3$''''$, between H$_\beta$-3$''''$ and C-4$''''$, between H$_\beta$-5$''''$ and C-4$''''$, and between H-13$''''$ and C-1$''''$, C-5$''''$, and C-6$''''$. Those of a pentacyclic moiety were shown between H-11$''''$ and C-1$''''$, C-2$''''$, C-3$''''$, and C-6$''''$, and between H-12$''''$ and C-11$''''$. Thus, a bridge-cyclic moiety consisting of a hexacyclic ring and a pentacyclic ring was confirmed on the basis of the HMBC correlations mentioned above. A chain substituent ($C_6H_6O_2$) was confirmed on the basis of the HMBC correlations between H-14$''''$ and C-10$''''$, between H-8$''''$ and C-7$''''$, C-9$''''$, C-10$''''$, and C-14$''''$, and between H-7$''''$ and C-8$''''$ and C-9$''''$. And an ester carbonyl group assigned at C-14$''''$ was determined to be α,β-unsaturated lactone by a significant high-field shift of C-14$''''$ [13]. Its location at C-1$''''$ of the bridge-cyclic moiety was deduced from the correlations between H-7$''''$ and C-1$''''$, and between H-8$''''$ and C-1$''''$. In addition, a hydroxyl group could be also attached to C-1$''''$ because of the HMBC correlation between 1$''''$-OH and C-1$''''$ and C-7$''''$. The geometry of the *trans* C-7$''''$–C-8$''''$ double bond was confirmed on the basis of large coupling

constant 15.6 Hz, and that of the *cis* C-9‴–C-14‴ double bond was confirmed on the basis of the olefinic proton in a highfield shift relative to the corresponding signals of *trans* compounds [3]. Thus, the structure of the substituent was confirmed as a dihydrophaseoyl group [13]. It could be attached to C-6‴ for the chemical shift of C-6‴ shifted toward downfield by 1.3 ppm and that of C-5″ shifted toward upfield by 2.8 ppm relative to the corresponding signals of spinosin [3].

The relative configuration of **1** was determined from the 2D NOESY correlation (Figure 2). The strong NOESY correlations between α-H-4⁗ and α-H-11⁗, together with the rigid (2⁗,6⁗)-bridged ring indicated that both 11⁗-CH_2 and O-C (6⁗) had α-orientation. The β-H-5⁗ at δ_H 1.89 in the downfield region was indicated by the strong NOESY correlations between β-H-5⁗ and H-13⁗. Subsequently, the β-orientation of the chain substituent ($C_6H_6O_2$) at C-1⁗ was confirmed by the strong NOESY correlations between β-H-5⁗ and H-7⁗. The absolute configuration at the C-4⁗ position was elucidated to be S according to the literature values of the ^{1}H NMR data for 4⁗S-dihydrophaseic acid and 4⁗R-dihydrophaseic acid [14], and **1** possesses 1⁗S, 2⁗R, 4⁗S, 6⁗R absolute configuration [14]. Thus, all of the protons and functional groups were assigned and the complete structure of **1** was elucidated, named as 6‴-dihydrophaseoylspinosin. The HMBC, ^{1}H–^{1}H COSY, and NOESY results of **1** led to assignments for the ^{1}H and ^{13}C signals as shown in Table 1.

Compound **2** was isolated as a yellowish amorphous powder. The molecular formula was determined to be $C_{48}H_{48}O_{21}$ from the $[M-H]^-$ ion peak at *m/z* 959.2604 in the ESI-TOF-MS. The UV absorption maxima at 214, 258, and 327 nm, together with a positive reaction in $AlCl_3$ reagent, revealed a flavone skeleton. The IR spectrum showed absorption bands due to a hydroxyl group (3407 cm^{-1}) and a carboxylic carbonyl (1694 cm^{-1}). The resonances for the carbons and protons of the flavonoids framework had a close resemblance to those of the known spinosin, except for those of C-5″, C-6″, and C-6‴. They were assigned according to the literature values of the ^{1}H and ^{13}C NMR spectral data for spinosin [3] as well as its own HSQC spectrum. The downfield shift of C-6″ (δ_C 64.3/64.3, + 2.9) and C-6‴ (δ_C 62.5/62.5, + 1.9), and the upfield shift of C-5‴ (δ_C 73.7/73.2, − 2.5) [3], relative to the corresponding signals of spinosin,

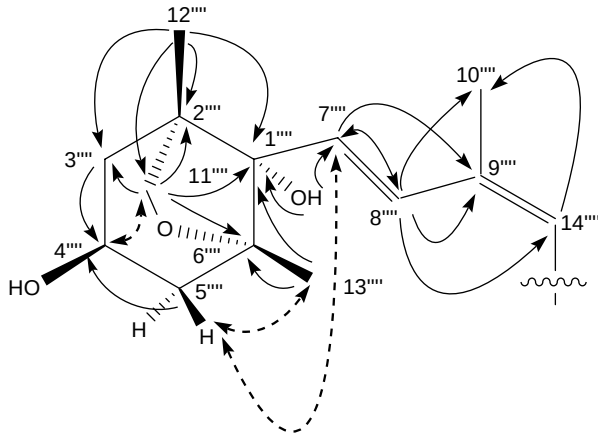

Figure 2. Selected HMBC (H → C) and NOESY (H ⇢ H) correlations of compound **1**.

Table 1. NMR spectral data of compound **1** in DMSO-d_6.

No.	^{1}H NMR (J, Hz)[a]	^{13}C NMR[a]	^{1}H–^{1}H COSY	HMBC[b]	NOESY
2		163.8/164.0			
3	6.78/6.82 (1H, s)	103.1/102.9		2, 4, 10	
4		182.1/181.8			
5		160.6/159.5			
6		108.8/108.6			
7		165.0/163.5			
8	6.75/6.73 (1H, s)	90.5/90.0		6, 9	7-OCH_3
9		156.9/156.8			
10		104.3/104.0			
1′		121.0/121.0			
2′, 6′	7.93/7.91 (2H, d, 8.4)	128.4/128.4	3′, 5′	4′, 2	
3′, 5′	6.91/6.91 (2H, d, 8.4)	115.8/114.9	2′, 6′	1′, 4′	
4′		161.1/161.1			
7-OCH_3	3.88/3.90 (3H, s)	56.3/56.0		7	8, 2″, 10‴, 3‴-H$_\alpha$, 5‴H$_\beta$,
1″	4.68/4.71 (1H, d, 9.6)	70.9/70.6	2″	2″, 6, 5, 7	5″
2″	4.48/4.26 (1H, m)	80.7/79.6	1″, 3″	1″, 1‴	7-OCH_3
3″	3.46/3.46 (1H, m)	78.6/78.3	2″, 4″		
4″	3.18 (1H, m)	70.4/70.3	3″		
5″	3.18 (1H, m)	81.8/81.5			1″
6″	3.72 (1H, m)	61.4/61.4			1‴-OH
	3.38 (1H, m)				1‴-OH
1‴	4.26/4.26 (1H, d, 7.8)	104.8/104.8	2‴		
2‴	2.84 (1H, m)	74.4/74.3	1‴, 3‴	1‴, 3‴	
3‴	3.06 (1H, m)	76.1/76.1	2‴, 4‴	2‴,	
4‴	2.95 (1H, m)	69.0/68.9	3‴, 5‴		
5‴	2.88 (1H, m)	73.4/73.4	4‴		
6‴	3.62 (1H, m) 3.82 (1H, m)	61.4/61.4			
1⁗		81.5/81.5			
1⁗-OH	4.50 (1H, s)			6⁗, 7⁗	6″
2⁗		48.0/47.9			
3⁗	H$_\beta$: 1.72 (1H, m)	43.8/43.8	H$_\alpha$	1⁗, 2⁗, 4⁗,	12⁗, 11⁗-H$_\alpha$
	H$_\alpha$: 1.54 (1H, m)		H$_\beta$	5⁗, 12⁗	7-OCH_3
4⁗	3.92 (1H, m)	63.8/63.8			11⁗-H$_\alpha$

$5'''$	H_β: 1.89 (1H, m)	45.4/45.4	H_α	$1'''$, $3'''$, $4'''$	$7'''$, 7-OCH_3, $13'''$
	H_α: 1.64 (1H, m)		H_β	$6'''$, $13'''$	
$6'''$		85.7/85.7			
$7'''$	6.40/6.42 (1H, 15.6)	135.6/135.5	$8'''$	$1'''$, $8'''$, $9'''$	$5'''$-H_β, $10'''$
$8'''$	7.83/7.73 (1H, 15.6)	129.1/129.0	$7'''$	$1'''$, $7'''$, $9'''$, $10'''$, $14'''$	
$9'''$		150.3/150.3			
$10'''$	1.86/1.91 (3H, s)	20.5/20.5		$8'''$, $9'''$, $14'''$	$7'''$, $14'''$, 7-OCH_3
$11'''$	H_α: 3.65 (1H, m)	75.3/75.3		$1'''$, $3'''$, $2'''$, $6'''$	$3'''$-H_β, $4'''$, $12'''$
	H_β: 3.58 (1H, m)				
$12'''$	0.83/0.82 (3H, s)	16.0/16.0		$1'''$, $2'''$, $3'''$, $11'''$	$3'''$-H_β, $11'''$-H_α
$13'''$	1.00/0.97 (3H, s)	19.4/19.4		$1'''$, $5'''$, $6'''$	$5'''$-H_β
$14'''$	5.51/5.50 (1H, s)	116.3/115.8		$10'''$	$10'''$
$15'''$		164.6/164.8			
5-OH	13.62/13.48 (1H, s)				

[a] Chemical shifts in ppm. Constant values *J* in Hz, obtained at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR.
[b] HMBC correlations, optimized for 8 Hz, are from H-atom (s) started to the indicated C-atom. All chemical shift assignments were carried out on the basis of HSQC and HMBC NMR techniques.

revealed the substitutions of C-6″ and C-6‴. The resonances for the carbons and protons of the substituents in the ^{1}H and ^{13}C NMR as well as HSQC spectra included two ABX aromatic proton systems, two ester carbonyl groups, four sp^2 methines, and two methoxyl moieties. The constitution of the substituents was deduced to be two feruloyl moieties from the HMBC spectrum (Figure 3), which was also supported by the fragment ion peaks at *m/z* 959 $[M - H]^-$, 783 $[M -$ feruloyl $- H]^-$, and 607 $[M -$ 2feruloyl $- H]^-$ in ESI-MS. The geometry of the *trans* C-7‴″–C-8‴″ double bond and *trans* C-7‴‴–C-8‴‴ double bond was confirmed on the basis of large coupling constant 15.6 Hz [3]. Hence, compound **2** was assigned as 6″,6‴-diferuloylspinosin.

The ^{1}H and ^{13}C NMR spectra of **1** and **2** showed the same phenomenon of separate signals because of rotational isomers which are produced by the rotational barriers 7-OCH_3 in flavone-6-C-glycoside. It was proved that the multiplicity collapsed to a first-order spectrum when measuring at high temperature [3]. Thus, structural elucidation of this type of compounds will be easy when measuring all spectra at high temperature (^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY).

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a JASCO P-1010 polarimeter. UV spectrum was obtained on a Shimadzu UV 1600 spectrophotometer. IR spectra were recorded on a Bruker IFS-55 infrared spectrometer. ESI-TOF-MS were obtained on a Micro TOF Bruker Daltonics mass spectrometer with a resolution of 25,000 (10% Valley). 1D NMR and 2D NMR were measured by a Bruker ARX-600 spectrometer with DMSO as the solvent and tetramethylsilane as an internal standard. HPLC analysis was carried out

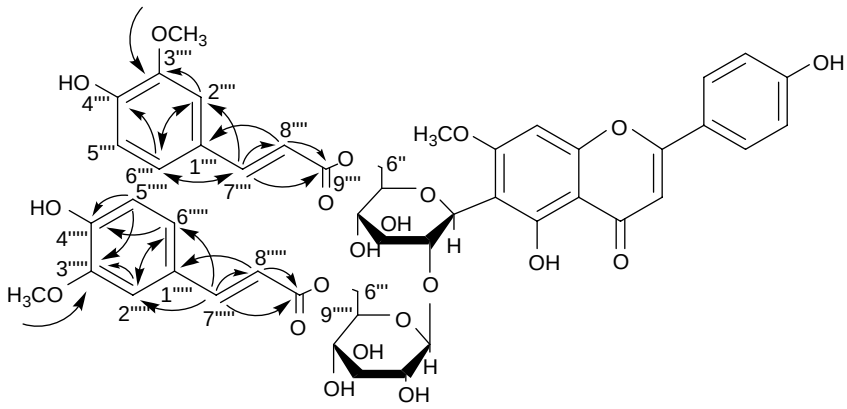

Figure 3. Selected HMBC correlations of compound **2** (H → C).

on a Kaseisorb LC NH2-60-5 column (Tokyo Kasei Co., Ltd, Tokyo, Japan, 4.6 mm i.d. × 250 mm) with an LC-6AD pump (Shimadzu, Kyoto, Japan), monitored by an optical rotation detector [Shodex OR-2 (Showa Denko Co., Ltd, Tokyo, Japan)]. Silica gel (200–300 mesh) was purchased from Qingdao Marine Chemical Ltd (Qingdao, China), Sephadex LH-20 from GE Healthcare (Stockholm, Sweden), and MDS-5 RP (200–300 mesh) from Beijing Medicine Technology Center (Beijing, China).

3.2 Plant material

The specimens of *Semen Ziziphi Spinosae* were collected from Chaoyang city of Liaoning Province in China (May 2008) and were identified by Prof. Dan Yuan, Shenyang Pharmaceutical University, China. A voucher specimen (No. 080518) has been deposited at the Department of Traditional Chinese Medicines, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

The air-dried, powdered *Semen Ziziphi Spinosae* (6.0 kg) was successively extracted with petroleum ether (48 liters) and MeOH (48 liters) for 2 × 1 h under reflux to give a petroleum ether-soluble fraction (800 g), and a MeOH-soluble fraction (470 g). The dried MeOH-soluble fraction was subjected to silica gel ($CHCl_3$–MeOH, 10:1 → 0:1) to obtain 15 fractions (Frs 1–15). Fr. 7 (3.5 g) was subjected to MDS-5 (MeOH–H_2O, 40:60 → 100:0) to afford Frs 7.1–7.3. Fr. 7.2 (59.0 mg) was further subjected to Sephadex LH-20 (MeOH) to give compound **8** (36.5 mg). Fr. 9 (3.9 g) was subjected to MDS-5 (MeOH–H_2O, 1:20 → 100:0) to obtain compounds **2** (7.0 mg) and **7** (8.0 mg). Compound **4** (5.0 g) was recrystallized from Fr. 11 (11.5 g) and the remaining Fr. 11 (6.5 g) was subjected to silica gel ($CHCl_3$–MeOH, 7:1 → 0:1) to give **6** (43.3 mg). Fr. 11.2 (3.5 g) was further separated by MDS-5 (MeOH–H_2O, 1:4 → 1:0) to provide compounds **1** (45.6 mg), **3** (22.6 mg), and **5** (80.4 mg).

3.3.1 Compound *1*

A yellowish amorphous powder with a molecular formula $C_{43}H_{52}O_{19}$, $[\alpha]_D^{20}$ −45.0 (*c* 0.1, MeOH). UV (MeOH) λ_{max} (log ε): 216 (1.62), 272 (1.52), 332 (1.29) nm. IR (KBr) ν_{max} (cm^{-1}): 3398, 2927, 1706, 1654, 1607, 1512, 1490, 1448, 840, and 779. For ^1H and ^{13}C NMR spectral data see Table 1. ESI-TOF-MS: *m/z*

Table 2. NMR spectral data of compound **2** in DMSO-d_6.

	^{1}H NMR (J, Hz)[a]	^{13}C NMR[a]	HMBC[b]		^{1}H NMR (J, Hz)	^{13}C NMR	HMBC
2		164.0/164.2		4‴	3.07/2.92 (1H, m)	69.0/68.7	
3	6.53/6.71 (1H, s)	103.2/103.0	2	5‴	2.94 (1H, m)	73.7/73.2	1‴
4		182.3/181.9		6‴	3.85 (2H, m)	62.5/62.5	
5		159.8/159.5		1⁗		125.6/125.4	
6		108.5/108.5		2⁗	7.30/7.30 (1H, brs.)	111.1/110.9	3⁗, 6⁗
7		165.2/163.9		3⁗		147.9/147.9	
8	6.69/6.67 (1H, s)	90.9/90.1	6, 7, 9, 10	4⁗		149.4/149.4	
9		157.1/157.0		5⁗	6.84/6.84 (1H, d, 8.4)	115.5/115.5	
10		104.6/104.1		6⁗	7.06/6.92 (1H, d, 8.4)	123.3/123.1	2⁗, 4⁗, 7⁗
1′		121.3/121.3		7⁗	7.51/7.51 (1H, d, 15.6)	145.4/145.4	2⁗, 6⁗, 8⁗, 9⁗
2′, 6′	7.81/7.80(2H, d, 8.4)	128.8/128.6	2	8⁗	6.47/6.44 (1H, d, 15.6)	114.4/114.1	1⁗, 9⁗
3′, 5′	6.90/6.89 (2H, d, 8.4)	115.8/115.8	1′	9⁗		166.4/166.4	
4′		161.3/160.9		OCH_3	3.84/3.86 (3H, s)	55.8/55.7	3⁗
5-OH	13.60/13.48 (1H, s)		5, 6, 10	1⁗′		128.6/128.8	
7-OCH_3	3.90/3.84 (3H, s)	56.3/56.5	7	2⁗′	7.30/7.30 (1H, brs.)	111.1/110.9	3⁗′, 6⁗′
1″	4.74/4.73 (1H, d, 9.6)	70.2/70.2	7, 2″	3⁗′		148.0/147.9	
2″	4.48/4.48 (1H, m)	80.0/80.0		4⁗′		149.3/149.3	
3″	3.50/3.50 (1H, m)	78.5/78.3	1″, 2″	5⁗′	6.77/6.75 (1H, d, 8.4)	115.8/115.8	3⁗′, 4⁗′
4″	4.90/4.90 (1H, m)	71.2/70.7		6⁗′	7.06/6.92 (1H, d, 8.4)	123.3/123.1	2⁗′, 4⁗′
5″	4.25/4.25 (1H, m)	81.7/81.7		7⁗′	7.52/7.52 (1H, d, 15.6)	130.4/130.4	2⁗′, 6⁗′, 8⁗′, 9⁗′
6″	4.52/4.52 (1H, m)	64.3/64.3		8⁗′	6.16/6.24 (1H, d, 15.6)	113.7/113.7	
	4.05/4.05 (1H, m)						
1‴	4.26/4.26 (1H, d, 7.8)	105.8/105.8	2″	9⁗′		166.8/166.8	
2‴	2.85 (1H, m)	74.5/74.4		OCH_3	3.84/3.84 (3H, s)	55.8/55.7	3⁗′
3‴	3.10/2.92 (1H, m)	76.4/76.4					

[a] Chemical shifts in ppm. Constant values J in Hz, obtained at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR.
[b] HMBC correlations, optimized for 8 Hz, are from H-atom (s) started to the indicated C-atom. All chemical shift assignments were carried out on the basis of HSQC and HMBC NMR techniques.

871.3026 $[M - H]^-$ (cacld for $C_{43}H_{51}O_{19}$, 871.3025).

3.3.2 Compound 2

A yellowish amorphous powder with a molecular formula $C_{48}H_{48}O_{21}$, $[\alpha]_D^{20}$ −97.0 (*c* 0.1, MeOH). UV (MeOH) λ_{max} (log ε): 214 (1.81), 275 (1.45), 326 (1.66) nm. IR (KBr) ν_{max} (cm^{-1}): 3408, 2927, 1694, 1653, 1605, 1514, 1491, 1449, 839, and 779. For ^1H and ^{13}C NMR spectral data see Table 2. ESI-MS: *m/z* 783 $[M - feruloyl - H]^-$, 607 $[M - 2feruloyl - H]^-$. ESI-TOF-MS: *m/z* 959.2604 $[M - H]^-$ (cacld for $C_{48}H_{48}O_{21}$, 959.2610).

3.4 Mild alkaline hydrolysis of 2

Solution of compound **2** (1 mg) in 0.05 N NH_4OH–50% MeOH (2 ml) was stirred at room temperature for 1 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and the resin was removed by filtration. The hydrolysate was extracted with EtOAc. After purification of the extract by Varian BOND ELUT® C_{18} column (1.0 × 4.0 cm, GL Science, Tokyo, Japan) eluting with a MeOH–H_2O gradient (20% MeOH → 50% MeOH → MeOH), two aromatic compounds were obtained, which were identified as spinosin and ferulic acid by co-chromatography with authentic samples.

3.5 Acid hydrolysis of 1 and 2

Compound **1** (1.0 mg) was dissolved in MeOH (9 ml) containing HCl (1 ml) and refluxed on a heated (80°C) water bath for 3 h. After cooling, the reaction mixture was concentrated and dried under reduced pressure, then was analyzed by TLC using the solvent system $CHCl_3$–MeOH–H_2O (20:10:1) for the identification of the complete reaction. The dried residues were dissolved in EtOAc, and partitioned with water. The water solution was analyzed and subjected to HPLC with optical detector using a Kaseisorb LC NH_2-60-5 column (4.6 mm i.d. × 250 nm) with CH_3CN–H_2O (v:v = 85:15, flow: 0.8 ml/min) as the mobile phase. Identification of D-glucose was carried out by comparison of the retention time (12.7 min) and OD (+) with authentic samples. Using the same method, the monosaccharide from **2** was also identified as D-glucose.

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