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C-GLUCOSYL FLAVONES FROM THE SEEDS OF *Ziziphus jujuba* var. *spinosa*

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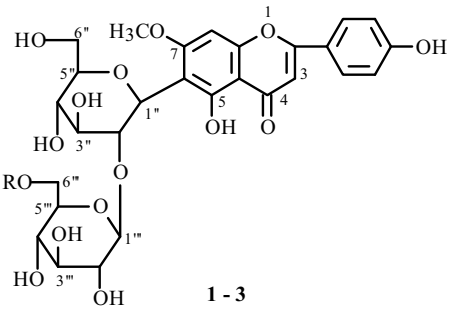
*A new C-glucosyl flavone, 6‴-vanilloylspinosin (**1**), was isolated from the seeds of* Ziziphus jujuba *var.* spinosa*, together with three known C-glucosyl flavones, spinosin (**2**), 6‴-feruloylspinosin (**3**), and isospinosin (**4**). The structure of **1** was elucidated by spectral methods (1D NMR, ESI-MS, HR-MS, 2D NMR).*

Keywords: 6‴-vanilloylspinosin, flavone, *Ziziphus jujuba* var. *spinosa*.

Ziziphus jujuba var. *spinosa* (Bunge) Huex. H. F., a thorny rhamnaceous plant, is widely distributed in north China. Its dried fruits have been used in traditional Chinese medicine as an immune system stimulant and antitumor. The seeds have sedative, demulcent, and hypnotic effects, as well as hypotensive. In recent years, new saponins [1, 2], flavonoids [3], and alkaloids [4, 5] have been reported from this plant. In our present research, a new C-glucosyl flavone **1**, together with three known compounds, spinosin (**2**) [3], 6‴-feruloylspinosin (**3**) [3], and isospinosin (**4**) [3], were isolated from a 95% aqueous EtOH extract of the seeds by repeated chromatography. The structure of **1** was assigned as apigenin 7-methyl-ether-6-[2-*O*-[6-*O*-(4-hydroxyl-3-methoxybenzoyl)-C-β-D-glucopyranosyl]-C-β-D-glucopyranoside by spectroscopic methods, named as 6‴-vanilloylspinosin.



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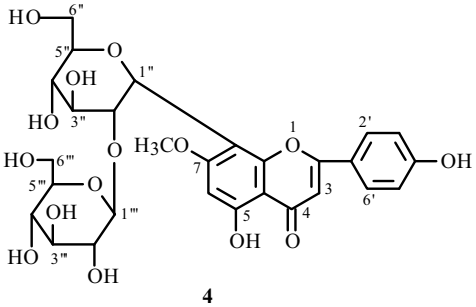

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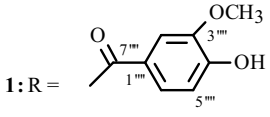

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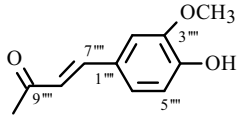

3: R =

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TABLE 1. ^{13}C NMR Data of Compounds **1–4** (DMSO-d_6)

C atom	**1**	**2**	**3**	**4**
2	163.9, 164.1 (s)	163.6, 163.7	163.8, 163.9	163.5
3	102.5, 102.6 (d)	102.8, 103.0	102.6, 102.9	102.7
4	181.6, 182.1 (s)	181.8, 182.2	182.2, 181.7	182.3
5	159.5, 160.7 (s)	159.6, 160.4	160.1, 160.9	161.2
6	108.5, 108.7 (s)	108.6, 108.6	108.7, 108.9	95.1
7	163.4, 165.1 (s)	163.8, 165.0	164.5, 164.9	164.3
8	89.7, 90.4 (d)	90.6, 90.9	90.1, 90.6	104.9
9	157.0, 157.1 (s)	156.8, 157.0	157.7, 158.1	155.3
10	103.9, 104.4 (s)	104.0, 104.4	104.3, 104.4	104.3
1′	123.1, 123.3 (s)	121.0, 121.3	122.0, 121.4	21.8
2′,6′	128.3, 128.5 (d)	128.4, 128.5	128.6, 128.7	129.0
3′,5′	116.0, 117.0 (d)	115.6, 115.9	116.1, 116.2	115.9
4′	162.3, 162.3 (s)	160.8, 161.2	161.4, 164.4	161.6
OCH_3	56.0, 56.3 (q)	55.9, 56.5	56.3, 56.4	56.1
Glc-1″	70.7, 71.0 (d)	70.5, 71.0	71.1, 71.2	71.4
2″	79.7, 80.6 (d)	80.4, 81.0	79.9, 80.1	80.9
3″	78.5, 78.9 (d)	77.9, 78.2	78.6, 78.9	78.2
4″	70.3, 70.4 (d)	70.2, 70.4	70.3, 70.5	70.1
5″	81.8, 81.9 (d)	81.4, 81.7	81.9, 81.9	81.8
6″	61.4, 61.5 (t)	61.4, 61.9	61.0, 61.3	60.9
Glc-1‴	104.9, 105.5 (d)	105.0, 105.2	104.6, 104.9	104.9
2‴	74.4, 74.4 (d)	74.5, 74.7	74.4, 74.5	74.5
3‴	76.2, 76.2 (d)	76.2, 76.3	76.0, 76.4	76.1
4‴	68.9, 69.0 (d)	69.1, 69.4	68.7, 68.9	69.3
5‴	73.2, 73.3 (d)	76.3, 76.3	73.5, 73.8	76.2
6‴	62.1, 62.6 (t)	60.1, 60.6	62.4, 62.9	60.3
	Vanilloyl		Feruloyl	
1⁗	119.8, 119.9 (s)		125.1, 125.5	
2⁗	112.2, 112.4 (d)		111.8, 112.0	
3⁗	147.2, 147.2 (s)		147.6, 147.8	
4⁗	151.9, 152.1 (s)		149.9, 149.9	
5⁗	114.9, 115.0 (d)		115.1, 115.4	
6⁗	123.1, 123.3 (d)		123.4, 123.6	
7⁗	165.0, 165.1 (s)		144.4, 144.9	
8⁗			114.0, 114.7	
9⁗			165.3, 165.4	
OCH_3	55.1, 55.3 (q)		55.5, 55.9	

The UV spectrum (MeOH) absorptions maximum of **1** at 276 and 335 nm, were characteristic of flavonoids. The IR spectrum implied the existence of hydroxyl (3340 cm^{-1}) and ester carbonyl (1725 and 1278 cm^{-1}) groups, as well as a benzene ring (1608, 1585, 1502, and 1483 cm^{-1}). The acid hydrolysis (2 M HCl) of compound **1** afforded swertisin, D-glucose, and vanillic acid in the mixture, which were determined by gas-liquid chromatography [6]. As was introduced [3], the multiplicity caused by rotational isomerism in **1** was also observed in its NMR spectra. The ^1H and ^{13}C NMR spectra showed the presence of an apigenin 7-methyl ether moiety and two sugar residues which were in good agreement with those of 6‴-feruloylspinosin (**3**) [3]. This was further supported by the HMBC correlation peaks observed (Fig. 1). The correlations between the anomeric proton of inner glucose at δ 4.64, 4.65 (d, J = 10.0 Hz) (H-1″) and the carbon at δ 108.5, 108.7 (C-6) showed the inner glucose group should be attached to C-6. The position of the outer glucose group was determined by the correlations between the anomeric proton of outer glucose at δ 4.10, 4.24 (d, J = 8.0 Hz) (H-1‴) and the carbon at δ 79.7, 80.6 (C-2″). Then the correlations between H-6‴ at δ 3.94, 3.95; 3.89, 3.99 (m) and the carbon at δ 165.0, 165.1 (C-7⁗) suggested the acylation of the vanilloyl moiety and 6‴-OH. Therefore, **1** was identified as apigenin 7-methyl-ether-6-[2-*O*-[6-*O*-(4-hydroxy-3-methoxybenzoyl)]-*β*-D-glucopyranosyl]-C-*β*-D-glucopyranoside unambiguously, and named as 6‴-vanilloylspinosin.

TABLE 2. ^{1}H NMR Data of Compounds **1–4** (DMSO-d_6, J/Hz)

C atom	**1**	**2**	**3**	**4**
3	6.40, 6.61 (s)	6.83,6.84 (s)	6.83, 6.85 (s)	6.47 (s)
6				6.77 (s)
8	6.50, 6.60 (s)	6.67, 6.80 (s)	6.67, 6.77 (s)	
2′,6′	7.77 (d, J = 8.8)	7.97 (d, J = 8.7)	7.81 (d, J = 7.9)	8.03 (d, J = 8.4)
3′,5′	6.86 (d, J = 8.8)	6.95 (d, J = 8.7)	6.89 (d, J = 7.9)	6.91 (d, J = 8.4)
OCH_3	3.77, 3.83 (s)	3.86 (s)	3.86 (s)	3.86 (s)
Glc-1″	4.64, 4.65 (d, J = 10.0)	4.67, 4.69 (d, J = 9.8)	4.67, 4.70 (t, J = 9.3)	4.83 (d, J = 10.0)
2″	4.22, 4.45 (m)			
3″	3.41, 3.41 (m)			
4″	3.18, 3.18 (m)			
5″	3.18; 3.19 (m)			
6″	3.36, 3.67; 3.34, 3.65 (m)			
Glc-1‴	4.10, 4.24 (d, J = 8.0)	4.15, 4.17 (d, J = 8.5)	4.23, 4.27 (t, J = 7.9)	4.04 (t, J = 8.8)
2‴	2.87, 2.91 (m)			
3‴	3.07, 3.11 (m)			
4‴	3.05, 3.11 (m)			
5‴	2.95, 3.18 (m)			
6‴	3.94, 3.95; 3.89, 3.99 (m)			
	Vanilloyl		Feruloyl	
2⁗	7.00, 7.10 (d, J = 1.6)		7.04, 7.17 (d, J = 2.0)	
5⁗	6.64, 6.73 (d, J = 8.0)		6.67 (d, J = 8.1)	
6⁗	7.06, 7.13 (dd, J = 8.0, 1.6)		6.72 (dd, J = 8.1, 2.0)	
7⁗			7.06, 7.18 (d, J = 15.7)	
8⁗			6.15, 6.23 (d, J = 15.7)	
OCH_3	3.56, 3.65 (s)		3.55, 3.65 (s)	

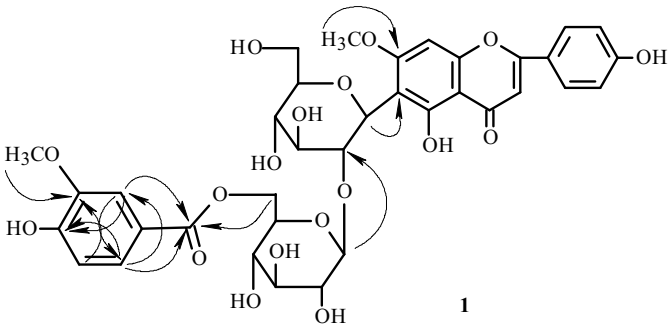

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Fig. 1. Key HMBC correlations of compound **1**.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined by an X_4 apparatus. UV spectra were taken in MeOH by a UV-2401 PC spectrometer. ^{1}H NMR and ^{13}C NMR, HMQC, and HMBC spectra were recorded with a Bruker AV 400 instrument, with TMS as an internal standard. Mass spectra were obtained on an MS Agilent 1100 Series LC/MS Trap mass spectrometer (ESI-MS) and a Micro Q-TOF MS (HR-ESI-MS), respectively. Silica gel (200~300 mesh) was purchased from Qingdao Ocean Chemical Group Co. in China. Polyamide was bought from Taizhou, Zhejiang. Diaion HP-20 (Mitsubishi Chemical), Sephadex LH-20 (Pharmacia), and RP-18 (ODS-A, 50 m, YMC) were used. TLC was performed on HPTLC plates (Alltech), RP-18 F_{254S} precoated plate (Merck), and polyamide plate (from Tianjin).

Plant Material. The seeds of *Ziziphus jujuba* var. *spinosa* (11.0 kg) were collected in November 2009 in Tianjin City of China and were identified by Prof. Minjian Qin of the Department of Natural Resource, China Pharmaceutical University. A voucher specimen has been deposited in China Pharmaceutical University (specimen No. Z-007).

Extraction and Isolation. The seeds of *Ziziphus jujuba* var. *spinosa* were extracted with 95% aqueous EtOH (3 × 20 L). After removal of the solvent under reduced pressure, the extract was dissolved in water and partitioned with petroleum, ethyl acetate, and *n*-BuOH, successively. The *n*-BuOH extract (252 g) was fractioned by HP-20 (2.5 kg) eluted with 20%, 50%, and 80% aqueous EtOH to give fractions A (20%), B (50%), and C (80%), respectively. Fractions B (30 g) was further fractionated by a silica gel column (250 g) eluted with gradient $CHCl_3$–MeOH (10:1→8:2→6:4→2:8) to give four subfractions (B 1-4). Subfraction B2 was subjected to column chromatography on polyamide eluted with gradient $CHCl_3$–MeOH to give fractions B 2-1 to B 2-3. Fraction B 2-2 was further separated on a RP-18 column using gradient aqueous MeOH, followed by a Sephadex LH-20 column eluted with aqueous MeOH to afford **1** (4 mg), **2** (85 mg), **3** (230 mg), and **4** (18 mg), respectively.

Hydrolysis and GC Chromatography. Two mg of compound **1** was refluxed with 5 mL 2M HCl for 2 h at 100°C. The reaction mixture was extracted with ethyl acetate. The residue was evaporated to dryness. The gas-liquid chromatography experiment was finished using the reported method [6].

Compound **1** (6‴-vanilloylspinosin), yellow powder, mp 225–228°C. UV spectrum (MeOH, λ_{max}, nm): 276, 335; (NaOMe, λ_{max}, nm): 276, 392; (NaOAc, λ_{max}, nm): 276, 393; ($AlCl_3$, λ_{max}, nm): 284, 302, 351, 385; ($AlCl_3$-HCl, λ_{max}, nm): 284, 302, 351, 385. IR spectrum (KBr, ν_{max}, cm^{-1}): 3340, 2928, 1725, 1608, 1585, 1502, 1483, 1380, 1345, 1278, 1200, 1175, 1093, 1078, 1021. ESI-MS *m/z* 757 $[M - H]^-$, HR-ESI-MS *m/z* 781.1953, calcd for $C_{36}H_{38}O_{18}Na$ $[M + Na]^+$ 781.1956. For ^1H NMR (400 MHz, DMSO-d_6) (300 K) spectral data, see Table 2, and for ^{13}C NMR (100 MHz, DMSO-d_6) (300 K) spectral data, see Table 1.

Compound **2** (spinosin), yellow powder, mp 237–240°C. UV (MeOH, λ_{max}, nm): 270, 334; ESI-MS *m/z* 607 $[M - H]^-$. For ^1H NMR (400 MHz, DMSO-d_6) (300 K) spectral data, see Table 2, and for ^{13}C NMR (100 MHz, DMSO-d_6) (300 K) spectral data, see Table 1.

Compound **3** (6‴-feruloylspinosin), yellow powder, mp 224°C (dec.). UV (MeOH, λ_{max}, nm): 273, 328; ESI-MS *m/z* 783 $[M - H]^-$. For ^1H NMR (400 MHz, DMSO-d_6) (300 K) spectral data, see Table 2, and for ^{13}C NMR (100 MHz, DMSO-d_6) (300 K) spectral data, see Table 1.

Compound **4** (isospinosin), yellow powder, mp 215–216°C. UV (MeOH, λ_{max}, nm): 268, 330. ESI-MS *m/z* 607 $[M - H]^-$. For ^1H NMR (400 MHz, DMSO-d_6) (300 K) spectral data, see Table 2, and for ^{13}C NMR (100 MHz, DMSO-d_6) (300 K) spectral data, see Table 1. For comparison with the literature [3], we modified its ^1H NMR spectral data.

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REFERENCES

1. M. Yoshikawa, T. Murakami, A. Ikebata, S. Wakao, N. Murakami, H. Matsuda, and J. Yamahara, *Chem. Pharm. Bull.*, **45**, 1186 (1997).
2. H. Matsuda, T. Murakami, A. Ikebata, J. Yamahara, and M. Yoshikawa, *Chem. Pharm. Bull.*, **47**, 1744 (1997).
3. G. Cheng, Y. J. Bai, Y. Y. Zao, J. Tao, Y. Liu, G. Z. Tu, L. B. Ma, N. Liao, and X. J. Xu, *Tetrahedron*, **56**, 8915 (2000).
4. B. H. Han, M. H. Park, and Y. N. Han, *Phytochemistry*, **29**, 3315 (1997).
5. M. H. Park, D. Y. Suh, and B. H. Han, *Phytochemistry*, **43**, 701 (1996).
6. S. Hara, H. Okabe, and K. Mihashi, *Chem. Pharm. Bull*., **35**, 501 (1987).