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TWO NEW FLAVONOL GLYCOSIDES FROM *SEDUM AIZOON L.*

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Abstract – Two new flavonol glycosides, Sedacins C (**1**) and D (**2**), and two known compounds (**3**-**4**), have been isolated from the whole plant of *Sedum aizoon L*. Their structures have been established as 6''-*O*-(*E*)-feruloylquercetin (**1**) and 6''-*O*-(*E*)-feruloylisorhamnetin (**2**) by means of spectroscopic analysis and chemical methods.

Sedum aizoon L. is an endemic plant, named as 'tusanqi' in folk medicine. It is useful in tranquilization and arresting bleeding and relieving stasis and decoction for counteracting toxicity, which has been used to treat pain or traumatic, various hemorrhage and palpitation.[1-2] There are about 150 Sedum species distributed in China, it has light green leaves on thick stems and a yellow flower blooming in the summer. Previously, the phytochemical constituents of Sedum species, a large genus of family Crassulaceae, have been extensively reported.[3-6] In our recent research on this plant, The repeated column chromatographic separation of the ethyl acetate and BuOH fractions of the MeOH extract resulted in the isolation of two new flavonol glycosides (**1**-**2**), as well as two known flavonol glycosides (**3**-**4**), The structures of the known compounds were determined to kaempferol-7-*O*-*β*-D-glucoside (**3**),[7] luteolin-7-*O*-*β*-D-glucoside (**4**)[8] by comparing their spectroscopic data with those in the literatures.

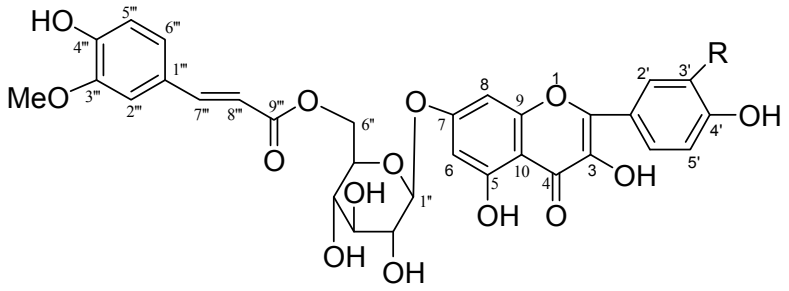
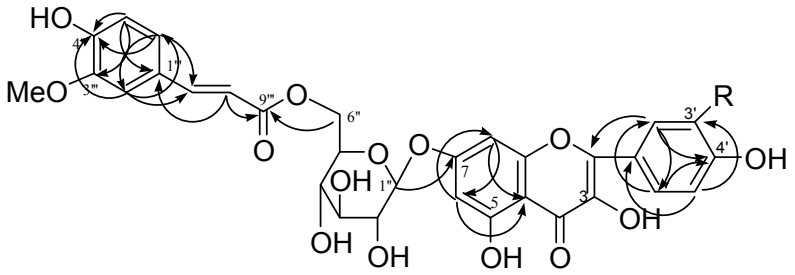


Figure 1. Structures of compounds **1** and **2**

Compound **1** (structure given in Figure 1) was isolated as yellowish powder. The HRESIMS showed a quasi-molecular ion peak at *m/z* 677.1572 $[M+Na]^+$ (calcd for 677.1585), corresponding to a molecular formula of $C_{32}H_{30}O_{15}$. The IR spectrum indicated the presence of hydroxyl groups, an ester carbonyl group and an aromatic ring. Its ^{1}H NMR spectrum showed the presence of one chelated hydroxyl group (δ 13.09, s, 5-OH), three ABX protons belonging to a 3,4-disubstituted phenyl group (δ 7.48, 1H, d, J = 2.3, H-2'; δ 6.87, 1H, d, J = 8.0 Hz, H-5'; δ 7.51, 1H, dd, J = 2.3, 8.0 Hz, H-6'), two protons typical of a 5,7-disubstituted ring A (δ 6.42, 6.81, each 1H, d, J = 2.1 Hz, H-8 and H-6), and an anomeric proton representing a glycosyl unit (δ 5.18, d, J = 7.9 Hz), These data, together with those of the ^{13}C NMR spectroscopy, indicated the presence of a quercetin[9] and a hexose unit.[10] After acid hydrolysis, quercetin was obtained as the aglycone, glucose moiety was detected by comparison with authentic samples. Meanwhile a glucopyranosyl group was also deduced by ^{1}H and ^{13}C NMR data (Table 1), where the coupling constant of H-1" (J = 7.9 Hz) suggested a β-anomeric configuration for the glucosyl and its D-configuration was determined via acid hydrolysis comparing with standard D-glucose, the HMBC crosspeak between C-7 and H-1" established the linkage point quercetin and the glucosyl moiety (Figure 2). The presence of a feruloyl moiety was deduced from an ABX spin system at δ 6.81 (1H, d, J = 8.5 Hz, H-5'''), δ 7.05 (1H, dd, J = 2.0, 8.0 Hz, H-6'''), and δ 7.19 (1H, d, J = 2.0 Hz, H-2'''). The *E*-configuration of the double bond was recognized by the large coupling constant of the coupled olefinic protons at δ 7.64 (1H, d, J = 15.9 Hz, H-7''') and δ 6.39 (1H, d, J = 15.9 Hz, H-8'''). The methoxy group at δ 3.85 (3H, s, OCH_3) was determined at C-3''' of the feruloyl, which was evidenced by the cross-peaks of the *O*-methyl hydrogens to C-3''' (δ 149.1) in HMBC spectrum (Figure 2). The connection of the glucopyranosyl group through C-6" to the carbonyl group of *trans*-feruloyl at C-9''' was determined by the HMBC correlations, where the cross-peaks of H-6" (δ 4.64 and 4.24) to C-9''' (δ 168.7) were presented. Thus, the structure of **1** was elucidated to be 7-*O*-(6"-*O*-*trans*-feruloyl)-*β*-D-glucopyranosyl-3,5,3',4'-tetrahydroxyflavone[6"-*O*-(*E*)-feruloylquercetin] and named Sedacin C.



1 R=OH
2 R=OMe

Figure 2. Key HMBC correlations of compounds **1** and **2**

Compound **2** (structure given in Figure 1) was obtained as yellowish powder. The molecular formula was determined to be $C_{33}H_{32}O_{15}$ from HRESIMS, which showed a quasi-molecular ion peak at *m/z* 691.1741 $[M + Na]^+$. Its IR, UV, and NMR spectroscopic data showed patterns very similar to those of 1, except for a 3', 4'-di-*O*-substituted B ring as indicated by the ^{1}H NMR signals of δ 7.52 (1H, d, J = 2.1 Hz, H-2'), δ 6.95 (1H, d, J = 8.0 Hz, H-5'), and δ 7.42 (1H, dd, J = 8.0, 2.1 Hz, H-6'). For the two methoxy groups, one at δ 3.72 (3H, s, OCH_3) was assigned at C-3' by observed long-range correlation between the *O*-methyl hydrogens and C-3' (δ 147.8) in the HMBC spectrum. The other at δ 3.81 (3H, s, OCH_3) was determined at C-3''' for a feruloyl, where the cross-peaks of the *O*-methyl hydrogens to C-3''' (δ 149.3) in HMBC was observed (Figure 2). The presence of a β-glucopyranosyl group was confirmed by ^{13}C NMR (Table 1). The glycosidic site and the position of the feruloyl group were the same as for **1** and were determined by the HMBC in which the correlations of H-1'' to C-7 (δ 165.7) and H-6'' (δ 4.62, 4.21) to C-9''' (δ 167.0) were observed (Figure 2). On the basis of the NMR analysis (Table 1), **2** was identified as 7-*O*-(6''-*O*-*trans*-feruloyl)-β-D-glucopyranosyl-3,5,4'-trihydroxy-3'-methoxyflavone[6''-*O*-(*E*)-feruloylisorhamnetin] and named Sedacin D.

EXPERIMENTAL

General. Melting points were determined on an XT digital melting-point apparatus with a microscope and are uncorrected. UV and IR spectra were recorded respectively with a Jasco V-650 spectrophotometer and a Thermo Nicolet 5700 infrared spectrometer with KBr pellets. HR ESIMS was measured on an Agilent 6520 Accurate-Mass Q-TOF LC/MS. NMR spectra were recorded on a Varian Mercury-400 spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C), with TMS as internal standard. Silica gel (Qingdao Marine Chemical Factory, 160-200 and 200-300 mesh), Sephadex LH-20 (Pharmacia) and RP-18 (Merck, 40-60 μm) were used for column chromatography (CC). Precoated plates of silica gel GF_{254} and silica gel RP-18 F_{254} (Merck) were used for TLC, and detected under UV light or by heating after spraying with

98% H_2SO_4-EtOH (10:90, *v/v*). Reversed-phase HPLC separations were performed on a Shimadzu 6A system, detected by a UV detector at 254 nm and equipped with a YMC semipreparative C_{18} column (10 μm, 10 × 250 mm) running with a flow rate of 4 ml/min.

Plant material. The plant of *Sedum aizoon L*. was collected from Taihang Mountain, in Henan province, P. R. China, in September 2007, and identified by professor Chen-Ming Dong, Henan College of Traditional Chinese Medicine. A voucher specimen (No. 200798) had been deposited at the Herbarium of Pharmacy College of Xinxiang Medical University.

Extraction and isolation. The dry whole plant tissue of *Sedum aizoon L*. (4.5 kg) was macerated at room temperature with MeOH for 48 h, four times (4 ×10 L, 7 days) and filtered. The combined methanolic extract was concentrated *in vacuo* to yield a crude extract (350 g), which was dissolved in water (2.0 L) and then partitioned with petroleum ether (4 × 1 L), EtOAc (4 × 1 L), and *n*-BuOH (4 × 1 L) successively. The EtOAc layer was evaporated to give 95.4 g residue, the EtOAc residue (95.4 g) was chromatographed over a silica gel column eluted with a solvent system of petroleum ether-acetone and MeOH. The fraction (4.2 g) of petroleum ether-acetone (7:3) continued silica gel CC elution with $CHCl_3$/MeOH (100:0 to 0:1) to give 10 subfractions. Subfraction 2 of $CHCl_3$/MeOH (9:1, 724 mg) was subjected to a Sephadex LH-20 column chromatography using $CHCl_3$-MeOH (1:1) to obtain a target portion (246 mg), and purified by preparative RP-HPLC with an ODS column (MeOH:H_2O 75:25) to afford compound **1** (12 mg, flow rate 4.0 mL/min), Subfraction 3 of $CHCl_3$/MeOH (8:2, 324 mg) was purified by Sephadex LH-20 CC using $CHCl_3$-MeOH (1:1) and preparative HPLC (80% aqueous MeOH, 4 mL/min) to yield compound **2** (9 mg, flow rate 4.0 mL/min) . The 80 g of the BuOH fraction (230 g) passed over a D101 macroporous resin CC (1 kg) eluted with a gradient of aqueous EtOH (0, 30, 60, 75, 100%, v/v) to yield five fractions (1–5). Fraction 3 (6.3 g) was subjected to reversed-phase silica gel (100 g) CC and eluted with gradient aqueous MeOH (0, 30, 60, 95%, *v*/*v*) to give four fractions (3.1–3.4), Fraction 3.2 (740 mg) was purified by Sephadex LH-20 CC (eluted with MeOH) to yield compound **3** (5.4mg), Fraction 3.3 (0.6 g) was also purified by Sephadex LH-20 CC (eluted with MeOH) to give compound **4** (7.5mg).

6''-O-(*E*)-Feruloylquercetin: Yellowish powder (MeOH); mp 235.3–235.1 °C; UV(MeOH) *λmax (nm)*: 256, 285, 364; $[\alpha]_D^{20}$ − 72.5 (c 0.2, MeOH); IR (KBr) *v*: 3320, 1609, 1513, 1457, 1350, 1266, 1165 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): and ^{13}C NMR (DMSO-d_6, 100MHz) data see Table 1; ESIMS *m/z*: 655.2 $[M+H]^+$, 677.2 $[M+Na]^+$; HRESIMS *m/z*: 677.1572 $[M+Na]^+$ (calcd for $C_{32}H_{30}NaO_{15}$, 677.1585).

6''-O-(*E*)-Feruloylisorhamnetin: Yellowish powder (MeOH); mp 258.3–259.0 °C; UV(MeOH) λmax(nm): 254, 287, 378; $[\alpha]_D^{20}$ − 63.5 (c 0.2, MeOH); IR (KBr) *v*: 3328, 1619, 1543, 1427, 1350, 1266, 1145 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): and ^{13}C NMR (DMSO-d_6, 100MHz) data see Table 1; ESIMS *m/z*: 669.2 $[M+H]^+$, 691.2 $[M+Na]^+$; HRESIMS *m/z*: 691.1741 $[M+Na]^+$ (calcd for $C_{33}H_{32}NaO_{15}$, 691.1756).

Acid Hydrolysis of compounds 1-2

A solution of compounds **1-2** (3.0 mg, each) in 1 M HCl (1.0 mL) was heated under reflux for 3 h. After cooling, the reaction mixture was extracted with EtOAc, and the residues from the organic phase were identified as quercetin (isorhamnetin) and ferulic acid by comparative TLC [Si gel, $CHCl_3$-MeOH (8:2)] with authentic samples available in our laboratory. The aqueous phase was concentrated, and the sugar was identified as *β*-D-glucose by TLC [Si gel, BuOH-AcOH-H_2O (3:1:1)] with a standard sample (Merck).

Table 1. ^1H NMR and ^{13}C NMR chemical shifts of **1**–**2**

Position	**1**		**2**	
	δ_H (*J* Hz)	δ_C	δ_H (*J* Hz)	δ_C
2		133.5		132.7
3		122.4		122.4
4		187.0		186.5
5		158.3		154.3
6	6.42 d (2.1)	98.7	6.52 d (2.0)	98.7
7		166.7		165.7
8	6.81 d (2.1)	94.8	6.75 d (2.0)	95.2
9		159.9		160.0
10		104.2		104.2
1'		122.7		122.7
2'	7.48 d (2.3)	116.8	7.52 d (2.1)	116.8
3'		146.5		147.8
4'		148.9		146.7
5'	6.87 d (8.0)	115.6	6.95 d (8.0)	115.6
6'	7.52 dd (2.3, 8.0)	121.4	7.42 dd (2.1, 8.0)	119.5

1"	5.18 d (7.9)	102.5	4.96 d (7.9)	102.3
2"	3.62 m	73.8	3.69 m	74.8
3"	3.56 m	76.2	3.56 m	76.2
4"	3.34 m	72.1	3.38 m	73.1
5"	3.75 m	75.1	3.72 m	76.1
6"a	4.64 dd (12.0, 1.5)	63.8	4.62 dd (12.0, 1.5)	64.8
6"b	4.24 dd (12.0, 5.0)		4.21 dd (12.0, 5.0)	
1"'		128.4		128.4
2"'	7.19 d (2.0)	113.5	7.19 d (2.0)	114.7
3"'		149.1		149.3
4"'		142.4		142.8
5"'	6.81 d (8.5)	116.4	6.80 d (8.4)	117.3
6"'	7.05 dd (2.0, 8.5)	119.8	7.05 dd (2.0, 8.4)	120.4
7"'	7.64 d (15.9)	142.8	7.66 d (15.9)	143.5
8"'	6.39 d (15.9)	117.6	6.41 d (15.9)	119.1
9"'		165.0		167.0
3-OH	9.46 s		9.57 s	
5-OH	13.09 s		12.59 s	
3'- OMe			3.72 s	58.3
4'- OH	10.12 s		10.15 s	
3"'- OMe	3.85 s	56.3	3.81 s	57.9

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