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ACYLATED FLAVONOL GLYCOSIDES FROM *Sedum aizoon*

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*A new acylated flavonol glycoside (**1**) and a known compound (**2**) have been isolated from the whole plant of* Sedum aizoon *L. Their structures have been established as quercetin 3-*O*-[β-D-(2‴-*O*-(E)-feruloyl)-xylopyranosyl]-(1→6)-β-D-glucopyranoside (**1**) and rutin (**2**) by means of spectroscopic analysis and chemical methods.*

Keywords: *Sedum aizoon*, acylated flavonol glycosides, quercetin 3-*O*-[*β*-D-(2‴-*O*-(*E*)-feruloyl)-xylopyranosyl]-(1→6)-*β*-D-glucopyranoside.

Sedum aizoon L. is an endemic plant. It is useful for tranquilization, arresting bleeding, and relieving stasis, and as a decoction for counteracting toxicity. The plant has been used to treat pain or trauma and various hemorrhage and palpitation conditions [1, 2]. There are about 150 *Sedum* species distributed in China.

The phytochemical constituents of *Sedum* species, a large genus of the family Crassulaceae, have been extensively reported [3–6]. In our research on this plant, repeated column chromatographic separation of BuOH fractions of the MeOH extract resulted in the isolation of a new flavonol glycoside (**1**), as well as a known flavonol glycoside (**2**), whose structure was determined to be rutin (**2**) [7] by comparing its spectroscopic data with those in the literature.

Compound **1** (see structure in Fig. 1) was isolated as a yellowish amorphous powder. The HR-ESI-MS showed a quasi-molecular ion peak at *m/z* 773.1929 $[M + H]^+$ (calcd for 773.1937), corresponding to the molecular formula of $C_{36}H_{36}O_{19}$. The IR spectrum indicated the presence of hydroxyl groups, an ester carbonyl group, and an aromatic ring. Positive ESI-MS/MS of **1** showed a quasi-molecular ion $[M + H]^+$ at *m/z* 773.2 and fragment ions at *m/z* 641.2 $[M + H – 132]^+$ and 479.2 $[M + H – 132 – 162]^+$, indicating the loss of pentosyl and hexosyl moieties from the quasi-molecular ion. The molecular weight of the aglycone was *m/z* 302. Its ^{1}H NMR spectrum showed the presence of one chelated hydroxyl group (13.04, s, 5-OH), three ABX protons belonging to a 3,4-disubstituted phenyl group (δ 7.60, 1H, d, J = 1.8 Hz, H-2′; 6.89, 1H, d, J = 8.0 Hz, H-5′; 7.56, 1H, dd, J = 1.8, 8.0 Hz, H-6′), two protons typical of a 5,7-disubstituted ring A (δ 6.45, 6.84, each 1H, d, J = 2.0, H-8 and H-6), and two anomeric protons representing two glycosyl units (δ 5.15, d, J = 7.5 Hz and 4.43, d, J =7.5). These data, together with those of ^{13}C NMR spectroscopy, indicated the presence of a quercetin [8] and two glycosyl units [9]. After acid hydrolysis, quercetin was obtained as the aglycone. Glucose and xylose moieties were determined via acid hydrolysis and comparing with standard D-glucose and D-xylose. From the ^{1}H NMR spectrum, the coupling constant of Glc H-1″ (J = 7.5 Hz) and Xyl H-1‴ (J = 7.5 Hz) suggested a *β*-anomeric configuration for the glucose and xylose. The cross-peak between C-3 and H-1″ in the HMBC spectrum established the linkage point at quercetin and the glucosyl moiety. From the ^{13}C NMR spectral data, the C-6″ (Glc) was shifted downfield at δ 68.9, indicating that glycosylation was at this unit. In the HMBC spectrum, the cross-peak between C-6″ (Glc) and H-1‴ (Xyl) established the linkage point between the two sugar moieties (Fig. 1). The presence of the feruloyl moiety was deduced from the ABX spin system at δ 6.63 (1H, d, J = 8.0 Hz, H-5⁗), 6.91 (1H, dd, J = 1.8, 8.0 Hz, H-6⁗), and 7.14 (1H, d, J = 1.8 Hz, H-2⁗). The *E*-configuration of the double bond was confirmed by the large coupling constant of the coupled olefinic protons at δ 7.33 (1H, d, J = 15.9 Hz, H-7⁗) and 6.27 (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 147.9) in the HMBC spectrum (Fig. 1).

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TABLE 1. ^{1}H NMR and ^{13}C NMR Chemical Shifts of **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_H	δ_C	C atom	δ_H	δ_C
2		157.8	Xyl		
3		136.4	1‴	4.43 (d, J = 7.5)	104.7
4		179.5	2‴	4.62 (dd, J = 9.0, 9.0)	73.5
5		162.5	3‴	3.30 m	74.6
6	6.45 (d, J = 2.0)	99.8	4‴	3.40 m	69.8
7		163.1	5‴a	3.76 (dd, J = 12.0, 2.0)	65.8
8	6.84 (d, J = 2.0)	94.5	5‴b	3.04 (dd, J = 12.0, 4.0)	
9		157.6	*trans*-Feruloyl		
10		107.9	1⁗		125.5
1′		122.6	2⁗	7.14 (d, J = 1.8)	110.7
2′	7.60 (d, J = 1.8)	117.4	3⁗		147.9
3′		145.8	4⁗		145.3
4′		149.2	5⁗	6.63 (d, J = 8.0)	115.3
5′	6.89 (d, J = 8.0)	115.1	6⁗	6.91 (dd, J = 8.0, 1.8)	123.4
6′	7.56 (dd, J = 8.0, 1.8)	123.0	7⁗	7.33 (d, J = 15.9)	144.6
3-*O*-Glc		102.7	8⁗	6.27 (d, J = 15.9)	115.3
1″	5.15 (d, J = 7.5)	73.7	9⁗		168.7
2″	3.34 m	77.4	OMe	3.85 s	56.2
3″	3.30 m	70.3			
4″	3.10 m	76.2			
5″	3.58 m	68.9			
6″a	3.96 (dd, J = 12.2, 3.0)				
6″b	3.62 (dd, J = 12.2, 5.0)				

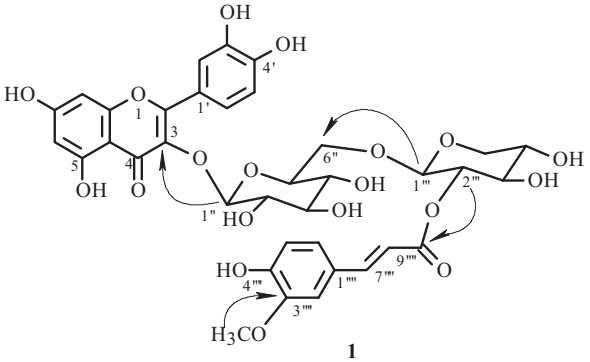

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Fig. 1. Key HMBC correlations of compound **1**.

The connection of the xylopyranosyl group through C-2‴ to the carbonyl group of *trans*-feruloyl at C-9⁗ was determined by the HMBC correlations, where the cross-peaks of H-2‴ (δ 4.62) to C-9⁗ (δ 168.7) were presented. Thus, the structure of **1** was elucidated to be quercetin 3-*O*-[*β*-D-(2‴-*O*-(*E*)-feruloyl)-xylopyranosyl]-(1→6)-*β*-D-glucopyranoside.

EXPERIMENTAL

General Experimental Procedures. Melting points were determined on an XT-4 digital melting-point apparatus with a binocular microscope and are uncorrected. UV and IR spectra were recorded respectively with a Jasco V-650 spectrophotometer and an FTS-40 infrared spectrometer with KBr pellets. HR-ESI-MS was measured on an Agilent 6520 Accurate-Mass Q-TOF LC/MS system. ^{1}H NMR (400 MHz) spectra were recorded on Bruker-400 spectrometer. ^{13}C NMR (100 MHz) and HMBC spectra were run on a Bruker-400 spectrometer with TMS as internal standard. Silica gel (Qingdao Marine Chemical Factory, 200–300 mesh) and Sephadex LH-20 (Pharmacia) and RP-18 (Merck, 40–60 μm) were used for column chromatography (CC). Reversed-phase HPLC separations were performed on a Shimadzu LC-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. Precoated plates of silica gel GF_{254} and silica gel RP-18 F_{254} (Merck) were used for TLC, and spots were detected under UV light or by heating after spraying with 98% H_2SO_4–EtOH (10:90, v/v).

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

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 day) 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, EtOAc, and *n*-BuOH successively. The petroleum ether layer was evaporated to give 25.4 g residue; the EtOAc fraction yielded 95.4 g, and the *n*-BuOH fraction yielded 230 g. The *n*-BuOH fraction was passed over a D_{101} macroporous resin CC (1 kg) eluted with a gradient of aqueous EtOH (0, 30, 60, 80, 95, v/v) to yield five fractions (1–5). Fraction 3 (11.3 g) was subjected to reversed-phase silica gel CC and eluted with a gradient of aqueous MeOH (0, 30, 45, 60, 75, 95, v/v) to give six fractions (3.1–3.6), Fraction 3.2 (890 mg) was subjected to Sephadex LH-20 CC (eluted with MeOH) and preparative HPLC (60% aqueous MeOH, 4 mL/min) to yield compound **1** (7.8 mg); fraction 3.3 (1.35 g) was also purified by Sephadex LH-20 CC (eluted with MeOH) to give compound **2** (10 mg).

Compound 1. Yellowish amorphous powder (MeOH), mp 201.3–202.1°C. $[\alpha]_D^{20}$–62° (*c* 0.2, MeOH). UV (MeOH, λ_{max}, nm): 256, 268, 328. IR (KBr, ν, cm^{-1}): 3434 (br), 2918, 1696, 1654, 1605, 1510, 1445, 1359, 1263, 1067, 812, and 595. For ^1H NMR and ^{13}C NMR data, see Table 1. ESI-MS *m/z*: 773.19 $[M + H]^+$, 795.18 $[M + Na]^+$; HR-ESI-MS *m/z*: 773.1929 $[M + H]^+$ (calcd for $C_{36}H_{36}O_{19}$, 773.1937).

Acid Hydrolysis of Compound 1. A solution of compound **1** (2.0 mg) 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 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 D-glucose and D-xylose by TLC [Si gel, BuOH–AcOH–H_2O (3:1:1)] with a standard sample (Merck).

Compound 2. Yellow amorphous powder, mp 185–188°C. ESI-MS (*m/z*): 611 $[M + H]^+$. ^1H NMR (400 MHz, acetone-d_6, δ, ppm, J/Hz): 12.58 (1H, s, 5-OH), 7.54 (1H, d, J = 1.8, H-2′), 7.52 (1H, dd, J = 8.4, 1.8, H-6′), 6.84 (1H, d, J = 8.4, H-5′), 6.37 (1H, d, J = 1.5, H-8), 6.18 (1H, d, J = 1.5, H-6), 5.34 (1H, d, J = 7.2, H-1″), 4.37 (1H, d, J = 1.6, H-1‴), 0.98 (3H, d, J = 6.0, H-6‴).

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