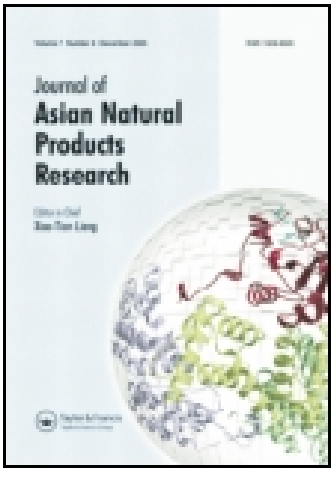

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Two new flavonoid glycosides from the halophyte Limonium franchetii

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Two new flavonoid glycosides from the halophyte *Limonium franchetii*

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Two new flavonoid glycosides, named quercetin-3-*O*-(2″-*O*-tigloyl)-α-L-rhamnopyranoside (**1**) and quercetin-3-*O*-(3″-*O*-tigloyl)-α-L-rhamnopyranoside (**2**), together with 10 known flavonoids (**3**–**12**), were isolated from the whole plant of the halophyte *Limonium franchetii*. Their structures were elucidated on the basis of extensive spectroscopic analysis including 2D NMR and HR-EI-MS. In addition, primary bioassays showed that compound **1** had moderate cytotoxic activity against rat C6 glioma cell lines.

Keywords: *Limonium franchetii*; flavonoid glycosides; halophyte; cytotoxic activity

1. Introduction

Limonium franchetii is a halophytic plant belonging to the family Plumbaginaceae [1], and only grows in saline soil or hillsides of coastal areas in Shandong, Liaoning, and Jiangsu Provinces of China [2]. Some *Limonium* species in China such as *L. sinense* and *L. bicolor* have been used in Chinese folk medicines for the treatment of hemorrhage, menstrual disorders, and gastrohelcoma for centuries [3]. Previous phytochemical investigations on *Limonium* plants suggested that the flavonoids were main active ingredients and showed antitumor, antioxidant, antiviral, and antimicrobial activities [4–11]. Although the chemical constituents of the genus of *Limonium* plants have been extensively studied, there is no report on the chemical constituents and biological activities of the species of *L. franchetii*. As a part of our continuing search for bioactive constituents of the halophytic plants of Shandong Province in China [12], the chemical constituents of the whole plant of *L. franchetii* had been investigated. We now report the isolation and structural elucidation of two new flavonoids, quercetin-3-*O*-(2″-*O*-tigloyl)-α-L-rhamnopyranoside (**1**) and quercetin-3-*O*-(3″-*O*-tigloyl)-α-L-rhamnopyranoside (**2**), along with 10 known flavonoids (**3**–**12**) from this plant in this paper. Their structures were elucidated on the basis of extensive spectroscopic analysis and by comparison with previously reported physical and spectral data of the known compounds (Figure 1). In addition, the cytotoxicities of all isolated compounds against HepG2, BGC-823, and rat C6 cell lines were evaluated.

2. Results and discussion

Compound **1** was obtained as a pale yellow powder, and its molecular formula $C_{26}H_{26}O_{12}$ was established by HR-EI-MS at *m/z* 530.1428 $[M]^+$. The UV spectrum of **1** showed the absorption maxima at 211,

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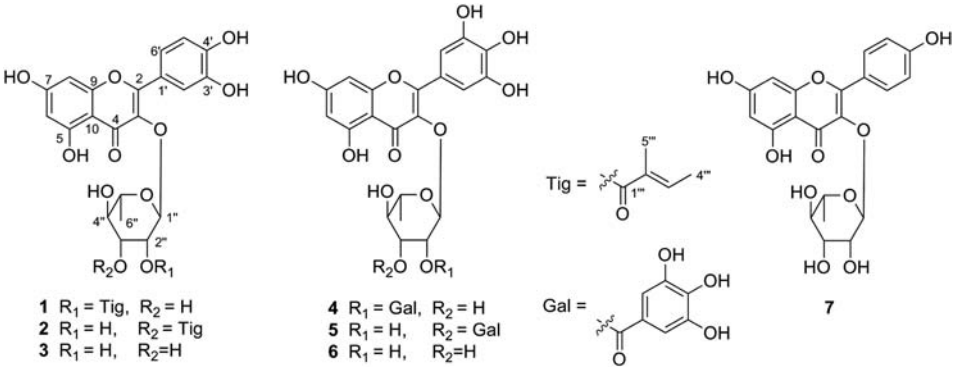

Figure 1. Structures of compounds **1**–**7**.

257, and 350 nm, suggesting the presence of a flavone skeleton, and the IR spectrum showed the presence of hydroxyl (3375 and 3251 cm^{-1}), benzene ring (1604, 1504, and 1446 cm^{-1}), conjugated carbonyl (1651 cm^{-1}), and α,β-unsaturated ester (1701 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** (Table 1) showed the typical signal pattern for a quercetin derivative. A downfield signal at δ_H 12.54 (1H, br s) was attributed to hydroxyl group at C-5, and an ABX spin system at δ_H 7.33 (1H, d, $J = 2.2$ Hz, H-2′), 6.88 (1H, d, $J = 8.3$ Hz, H-5′), and 7.28 (1H, dd, $J = 8.3$, 2.2 Hz, H-6′) characterized an ortho-disubstituted B ring of the flavone aglycon. In addition, a pair of meta-coupled doublets at δ_H 6.21 (1H, d, $J = 1.9$ Hz) and 6.40 (1H, d, $J = 1.9$ Hz) were assigned to H-6 and H-8 of ring A, respectively. Moreover, the proton signals at δ_H 5.44 (1H, d, $J = 1.6$ Hz, H-1″) and 0.88 (3H, d, $J = 5.8$ Hz, H-6″) belonging to a α-rhamnopyranosyl moiety were also observed in the ^{1}H NMR spectrum of **1**. The ^{13}C NMR spectral data of **1** (Table 1) revealed the presence of 26 carbons, including 15 olefinic carbons of the flavone skeleton, 6 carbons of a sugar, and 5 other carbons belonging to a tigloyl unit [δ_C 166.6 (s, C-1‴), 128.4 (s, C-2‴), 138.3 (d, C-3‴), 14.6 (q, C-4‴), and 12.4 (q, C-5‴)]. From the above information, we found that the NMR data of **1** were very similar to those of **3** [13], except for the extra signals of tigloyl group and the chemical shift changes of the sugar signals in **1**. From the detailed comparison of the NMR spectral data of **1** with **3**, the downfield shift of H-2″ from δ_H 3.99 to 5.34 ($\Delta + 1.35$ ppm) in the ^{1}H NMR spectrum, the upfield shifts of C-1″ (from δ_C 102.3 to 98.7, $\Delta -3.6$ ppm) and C-3″ (from δ_C 71.1 to 68.9, $\Delta -2.2$ ppm), and the downfield shift of C-2″ (from δ_C 70.8 to 72.3, $\Delta + 1.5$ ppm) in the ^{13}C NMR spectrum (Figure 2) were observed. It is indicated that the tigloyl unit is located at C-2″ of the sugar moiety. This was also confirmed by the HMBC correlations of H-2″ with C-1‴. In addition, the HMBC correlations from H-1″ to C-3 (Figure 2) suggested that the α-rhamnopyranosyl moiety was attached to C-3 of flavone skeleton. Thus, the structure of **1** was characterized as quercetin-3-*O*-(2″-*O*-tigloyl)-α-L-rhamnopyranoside.

Compound **2** was isolated as a pale yellow powder, giving the molecular formula $C_{26}H_{26}O_{12}$ by the HR-EI-MS at *m/z* 530.1418 $[M]^+$, which was the same as that of **1**. The NMR spectral data of **2** (Table 1) were very similar to those of **1**, except for the location of the tigloyl group. Comparison of the ^{1}H and ^{13}C NMR spectral data of **2** with those of **3** (Table 1) [13], the downfield shift of H-3″ ($\Delta + 1.35$ ppm) in the ^{1}H NMR spectrum

Table 1. ^{1}H and ^{13}C NMR (500/125 MHz) spectral data of compounds **1**–**3** in DMSO-d_6.

	1		**2**		**3**	
No.	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
2		157.8 s		157.7 s		156.9 s
3		133.8 s		134.9 s		134.7 s
4		177.9 s		178.2 s		178.2 s
5		161.7 s		161.8 s		161.8 s
6	6.21 (d, 1.9)	99.2 d	6.22 (d, 2.0)	99.2 d	6.22 (d, 2.0)	99.2 d
7		164.8 s		164.7 s		164.7 s
8	6.40 (d, 1.9)	94.1 d	6.41 (d, 2.0)	94.1 d	6.40 (d, 2.0)	94.1 d
9		156.9 s		156.9 s		157.8 s
10		104.5 s		104.5 s		104.5 s
1′		121.9 s		121.1 s		121.6 s
2′	7.33 (d, 2.2)	116.1 d	7.29 (d, 2.2)	115.9 d	7.31 (d,2.0)	116.1 d
3′		145.7 s		145.8 s		145.7 s
4′		149.0 s		149.0 s		148.9 s
5′	6.88 (d, 8.3)	116.0 d	6.88 (d, 8.2)	115.9 d	6.88 (d, 8.3)	115.9 d
6′	7.28 (dd, 8.3, 2.2)	121.6 d	7.31 (dd, 8.2, 2.2)	121.8 d	7.26 (dd, 8.3, 2.0)	121.2 d
1″	5.44 (d, 1.6)	98.7 d	5.22 (d, 1.5)	102.4 d	5.26 (d, 1.1)	102.3 d
2″	5.34 (dd, 3.2, 1.7)	72.3 d	4.23 (br s)	68.1 d	3.99 (br s)	70.8 d
3″	3.73 (dd, 8.8, 3.2)	68.9 d	4.87 (dd, 9.4, 3.2)	74.3 d	3.52 (dd, 9.0, 3.0)	71.1 d
4″	3.18 (m)	72.1 d	3.44 (m)	68.7 d	3.15 (m)	71.6 d
5″	3.20 (m)	71.1 d	3.41 (m)	71.2 d	3.23 (m)	70.5 d
6″	0.88 (d, 5.8)	18.0 q	0.88 (d, 5.9)	17.9 q	0.82 (d, 6.1)	18.0 q
1‴		166.6 s		167.4 s		
2‴		128.4 s		128.8 s		
3‴	6.79 (m)	138.3 d	6.89 (dd, 6.9, 1.2)	137.6 d		
4‴	1.77 (d, 6.0)	14.6 q	1.79 (d, 7.1)	14.6 q		
5‴	1.77 (s)	12.4 q	1.82 (s)	12.5 q		
5-OH	12.54 (br s)		12.63 (br s)		12.66 (s)	

and the upfield shifts of C-2″ (Δ −2.7 ppm) and C-4″ (Δ −2.9 ppm), and the downfield shift of C-3″ (Δ + 3.2 ppm) in the ^{13}C NMR spectrum (Figure 2), indicated that the tigloyl unit is located at C-3″ in **2**. This was also confirmed by the HMBC correlations from H-3″ to C-1‴ (Figure 2). Therefore, compound **2** was

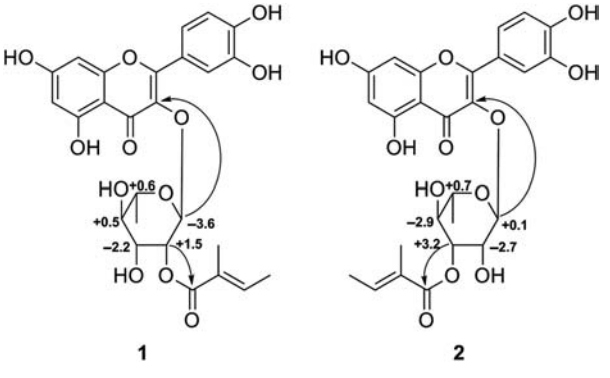

Figure 2. Selected HMBC correlations (H → C) of compounds **1** and **2**, and the chemical shifts ($\Delta\delta_C$) for sugar carbons by comparison with **3**.

determined as quercetin-3-*O*-(3″-*O*-tigloyl)-α-L-rhamnopyranoside.

In addition to the above-mentioned new flavonoids, 10 known compounds including quercetin-3-*O*-α-L-rhamnopyranoside (**3**) [13], myricetin-3-*O*-(2″-*O*-galloyl)-α-L-rhamnopyranoside (**4**) [5,14,15], myricetin-3-*O*-(3″-*O*-galloyl)-α-L-rhamnopyranoside (**5**) [5,15], myricetin-3-*O*-α-L-rhamnopyranoside (**6**) [16], kaempferol-3-*O*-α-L-rhamnopyranoside (**7**) [17], apigenin (**8**) [13], luteolin (**9**) [13], quercetin (**10**) [13], myricetin (**11**) [15], and dihydrokaempferol (**12**) [18] were also isolated and identified by comparing their NMR and MS spectral data with those reported in the literature.

Compounds **1**–**12** were tested *in vitro* for cytotoxicity against human gastric carcinoma cell lines BGC-823 using the MTT method [19], and against human heptoma cell lines HepG2 and rat glioblastoma C6 cell lines using the SRB method [20] with DMSO as negative controls, in which only compound **1** exhibited a proliferation inhibition ratio of 77.09% against rat C6 cell lines at 100 μg ml^{-1}. However, the other isolated compounds did not show significant cytotoxic activity against BGC-823, HepG2, and rat C6 cancer cells.

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a Jasco P-1020 automatic digital polarimeter (Jasco, Tokyo, Japan). UV spectra were recorded on a Nanodrop ND-2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA). IR spectra were measured on a Jasco FT/IR-4100 spectrometer (Jasco) with KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker Avance III 500 instruments (Bruker, Fällanden, Switzerland) with TMS as an internal standard. ESI-MS were determined on a LCQ Fleet ion trap mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). HR-EI-MS were measured on an AutoSpec Primier P776 mass spectrometer (Waters, Milford, CT, USA). Column chromatography was performed over silica gel (200–300 mesh; Yantai Xinde Chemical Co., Ltd, Yantai, China), ODS gel (YMC, Kyoto, Japan), and Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden). TLC was performed on the silica gel plates (Yantai Xinde Chemical Co., Ltd), and spots were visualized by spraying with 10% H_2SO_4 in EtOH, followed by heating. Fractions were separated by preparative MPLC (Puriflash 450, Interchim Company, Montlucon, France) on the flash chromatographic columns (Santai Technologies, Inc., Changzhou, China).

3.2 Plant material

The whole plant of *L. franchetii* was collected from the sandy seashore of Yantai, Shandong Province of China, in June 2012, and identified by Associate Prof. Fuhua Bian, College of Life Sciences, Yantai University. A voucher specimen (No. 20120606-1) was deposited at the Key Laboratory of Coastal Biology and Biological Resources Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried and powdered whole plant of *L. franchetii* (2.6 kg) was extracted with MeOH at room temperature (4 × 20 l). The combined extracts were concentrated under reduced pressure to yield a residue (251 g). Then, the residue was suspended in water and extracted by petroleum ether (PE), ethyl acetate (EtOAc), and *n*-BuOH, successively. The EtOAc extract (39.1 g) was subjected to column chromatography on silica gel eluted with a gradient eluent of PE–acetone system (15:1 to 1:5, v/v) to provide 10 fractions (A–J). Fraction B (1.15 g) was performed on Sephadex LH-20 eluting with CH_2Cl_2–MeOH (1:1, v/v)

to give four subfractions B1–B4. Subfraction B1 (120.1 mg) was submitted to silica gel column chromatography eluted with PE–EtOAc (1:1, v/v) to yield compound **3** (10.0 mg). Subfraction B3 (57.6 mg) was purified by reversed-preparative MPLC (MeOH–H_2O; 1:1, v/v) to afford compound **8** (10.2 mg). The fraction C (1.13 g) was chromatographed on Sephadex LH-20 and repeated silica gel columns to afford compounds **9** (20.2 mg) and **10** (32.2 mg). Fraction D (1.89 g) was further subjected to an ODS column eluted with MeOH–H_2O gradient (1:4 to 1:0, v/v) to afford eight subfractions (D1–D8). Compounds **1** (8.1 mg), **2** (8.2 mg), and **7** (14.2 mg) were obtained from the subfractions D5 (23.5 mg), D6 (36.2 mg), and D7 (53.5 mg) by silica gel columns eluted with CH_2Cl_2–MeOH (10:1, v/v), respectively. Subfraction D8 (50.9 mg) led to the isolation of compound **11** (23.8 mg) by Sephadex LH-20 column. Compounds **6** (20.2 mg), **12** (30.8 mg), and the mixture of **4** and **5** (20.2 mg) were isolated from Fraction E (2.45 g) by Sephadex LH-20 and repeated silica gel columns.

*3.3.1 Quercetin-3-O-(2″-O-tigloyl)-α-L-rhamnopyranoside (**1**)*

Pale yellow powder; $[\alpha]_D^{24.8}$ − 47.2 (c = 0.21, MeOH); UV (MeOH) λ_{max} nm (log ε): 211 (4.50), 257 (4.28), 350 (4.14); IR (KBr) ν_{max} (cm^{-1}): 3357, 3251, 1701, 1651, 1604, 1504, 1446, 1362, 1265, 1200, 1161, 1084; for ^1H and ^{13}C NMR spectral data, see Table 1; positive ESI-MS: *m/z* 553 $[M + Na]^+$, 1083 $[2M + Na]^+$; negative ESI-MS: *m/z* 529 $[M - H]^-$; HR-EI-MS: *m/z* 530.1428 $[M]^+$ (calcd for $C_{26}H_{26}O_{12}$, 530.1424).

*3.3.2 Quercetin-3-O-(3″-O-tigloyl)-α-L-rhamnopyranoside (**2**)*

Pale yellow powder; $[\alpha]_D^{24.5}$ − 120.2 (c = 0.23, MeOH); UV (MeOH) λ_{max} nm (log ε): 209 (4.50), 257 (4.25), 350 (4.11); IR (KBr) ν_{max} (cm^{-1}): 3371, 3286, 1701, 1655, 1604, 1508, 1446, 1362, 1273, 1200, 1165, 1084; for ^1H and ^{13}C NMR spectral data, see Table 1; positive ESI-MS: *m/z* 553 $[M + Na]^+$, 1083 $[2M + Na]^+$; negative ESI-MS *m/z* 529 $[M - H]^-$; HR-EI-MS: *m/z* 530.1418 $[M]^+$ (calcd for $C_{26}H_{26}O_{12}$, 530.1424).

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