Two new flavonoid glycosides from the halophyte Limonium franchetii

Na-Na Kong, Sheng-Tao Fang, Jian-Hua Wang, Zhen-Hua Wang & Chuan-Hai Xia

a Key Laboratory of Coastal Biology and Biological Resources Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
b College of Resource and Environment, University of the Chinese Academy of Sciences, Beijing 100049, China
c College of Life Sciences, Yantai University, Yantai 264005, China

Published online: 06 Mar 2014.

To cite this article: Na-Na Kong, Sheng-Tao Fang, Jian-Hua Wang, Zhen-Hua Wang & Chuan-Hai Xia (2014) Two new flavonoid glycosides from the halophyte Limonium franchetii, Journal of Asian Natural Products Research, 16:4, 370-375, DOI: 10.1080/10286020.2014.884081

To link to this article: http://dx.doi.org/10.1080/10286020.2014.884081
Two new flavonoid glycosides from the halophyte *Limonium franchetii*

Na-Na Kong\textsuperscript{ab}, Sheng-Tao Fang\textsuperscript{a*}, Jian-Hua Wang\textsuperscript{a}, Zhen-Hua Wang\textsuperscript{c} and Chuan-Hai Xia\textsuperscript{a*}

\textsuperscript{a}Key Laboratory of Coastal Biology and Biological Resources Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China; \textsuperscript{b}College of Resource and Environment, University of the Chinese Academy of Sciences, Beijing 100049, China; \textsuperscript{c}College of Life Sciences, Yantai University, Yantai 264005, China

(Received 18 October 2013; final version received 12 January 2014)

Two new flavonoid glycosides, named quercetin-3-O-(2\textsuperscript{00}-O-tigloyl)-\(\alpha\)-L-rhamnopyranoside (1) and quercetin-3-O-(3\textsuperscript{00}-O-tigloyl)-\(\alpha\)-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\textsuperscript{00}-O-tigloyl)-\(\alpha\)-L-rhamnopyranoside (1) and quercetin-3-O-(3\textsuperscript{00}-O-tigloyl)-\(\alpha\)-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\textsubscript{26}H\textsubscript{26}O\textsubscript{12} was established by HR-EI-MS at \textit{m}/\textit{z} 530.1428 [M]\textsuperscript{+}. The UV spectrum of 1 showed the absorption maxima at 211,
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 \(\alpha,\beta\)-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 \(d_H 12.54\) (1H, br s) was attributed to hydroxyl group at C-5, and an ABX spin system at \(d_H 7.33\) (1H, d, \(J = 2.2\) Hz, H-2\(^0\)), 6.88 (1H, d, \(J = 8.3\) Hz, H-5\(^0\)), and 7.28 (1H, dd, \(J = 8.3, 2.2\) Hz, H-6\(^0\)) characterized an ortho-disubstituted B ring of the flavone aglycon. In addition, a pair of meta-coupled doublets at \(d_H 6.21\) (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 \(d_H 5.44\) (1H, d, \(J = 1.6\) Hz, H-1\(^0\)) and 0.88 (3H, d, \(J = 5.8\) Hz, H-6\(^0\)) belonging to a \(\alpha\)-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 [\(\delta_C 166.6\) (s, C-1\(^{\prime\prime}\)), 128.4 (s, C-2\(^{\prime\prime}\)), 138.3 (d, C-3\(^{\prime\prime}\)), 14.6 (q, C-4\(^{\prime\prime}\)), and 12.4 (q, C-5\(^{\prime\prime}\))]. 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\(^0\) from \(\delta_H 3.99\) to 5.34 (\(\Delta + 1.35\) ppm) in the \(^1\)H NMR spectrum, the upfield shifts of C-1\(^{\prime\prime}\) (from \(\delta_C 102.3\) to 98.7, \(\Delta - 3.6\) ppm) and C-3\(^{\prime\prime}\) (from \(\delta_C 71.1\) to 68.9, \(\Delta - 2.2\) ppm), and the downfield shift of C-2\(^{\prime\prime}\) (from \(\delta_C 70.8\) 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\(^{\prime\prime}\) of the sugar moiety. This was also confirmed by the HMBC correlations of H-2\(^0\) with C-1\(^{\prime\prime}\). In addition, the HMBC correlations from H-1\(^{\prime\prime}\) to C-3 (Figure 2) suggested that the \(\alpha\)-rhamnopyranosyl moiety was attached to C-3 of flavone skeleton. Thus, the structure of 1 was characterized as quercetin-3-\(O-\text{(2}\(^{\prime\prime}\)-O-tigloyl)-\(\alpha\)-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\(^{\prime\prime}\) (\(\Delta + 1.35\) ppm) in the \(^1\)H NMR spectrum.
and the upfield shifts of C-2\(^{00}\) (\(\Delta -2.7\) ppm) and C-4\(^{00}\) (\(\Delta -2.9\) ppm), and the downfield shift of C-3\(^{00}\) (\(\Delta +3.2\) ppm) in the \(^{13}\)C NMR spectrum (Figure 2), indicated that the tigloyl unit is located at C-3\(^{00}\) in 2. This was also confirmed by the HMBC correlations from H-3\(^{00}\) to C-1\(^{000}\) (Figure 2). Therefore, compound 2 was

| No. | 1  | 2  | 3  |
|-----|----|----|----|
| No. | \(\delta_H\) (mult., \(J\) in Hz) | \(\delta_C\) | \(\delta_H\) (mult., \(J\) in Hz) | \(\delta_C\) | \(\delta_H\) (mult., \(J\) in Hz) | \(\delta_C\) |
| 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 | 156.9 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 | 167.4 s |
| 2'''' | 128.4 s | 128.8 s | 128.8 s |
| 3'''' | 6.79 (m) | 138.3 d | 6.89 (dd, 6.9, 1.2) | 137.6 d | 6.79 (m) | 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) |

Figure 2. Selected HMBC correlations (H \(\rightarrow\) 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 hepatoma 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 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% H₂SO₄ 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 × 201). 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 CH₂Cl₂–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–H2O; 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–H2O 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 CH2Cl2–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; [α]D24,8 = 47.2 (c = 0.21, MeOH); UV (MeOH) λmax nm (log e): 211 (4.50), 257 (4.28), 350 (4.14); IR (KBr) νmax (cm⁻¹): 3357, 3251, 1701, 1651, 1604, 1504, 1446, 1362, 1265, 1200, 1161, 1084; for 1H and 13C 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-ESI-MS: m/z 530.1418 [M]⁺ (calcd for C26H26O12, 530.1424).

3.3.2 Quercetin-3-O-(3′′-O-tigloyl)-α-L-rhamnopyranoside (2)
 Pale yellow powder; [α]D24,5 = 120.2 (c = 0.23, MeOH); UV (MeOH) λmax nm (log e): 209 (4.50), 257 (4.25), 350 (4.11); IR (KBr) νmax (cm⁻¹): 3371, 3286, 1701, 1655, 1604, 1508, 1446, 1362, 1273, 1200, 1165, 1084; for 1H and 13C 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-ESI-MS: m/z 530.1418 [M]⁺ (calcd for C26H26O12, 530.1424).

Acknowledgments
This work was financially supported by the Projects of Natural Key Technology Research and Development Program of the Ministry of Science and Technology of China (No. 2011BAC02B04), and the National Natural Science Foundation of China (No. 21202198).

References
[1] K. Zhao, J. Song, G. Feng, M. Zhao, and J. Liu, Plant Soil. 342, 495 (2011).
[2] Editorial Board of Flora of China, Flora of China (Science Press, Beijing, 1987), Vol. 60, p. 32.
[3] Editorial Committee of Compilation of Chinese Herb Medicine, Compilation of Chinese Herb Medicine, 2nd ed., (People’s Medical Publishing House, Beijing, 1996), p. 407.
[4] L.C. Lin, Y.C. Kuo, and C.J. Chou, Planta Med. 66, 333 (2000).
[5] L.C. Lin and C.J. Chou, Planta Med. 66, 382 (2000).
[6] A.P. Murray, S. Rodriguez, M.A. Frontera, M.A. Tomas, and M.C. Mulet, Z. Naturforsch 59c, 477 (2004).
[7] G.E. Zhusupova and S.A. Abilkaeva, Chem. Nat. Compd. 42, 112 (2006).
[8] G. Ye and C. Huang, Chem. Nat. Compd. 42, 232 (2006).
[9] F.E. Kandil, K.M. Ahmed, H.A. Hussieny, and A.M. Soliman, Arch. Pharm. Pharm. Med. Chem. 333, 275 (2000).
[10] J.I. Lee, C.S. Kong, M.E. Jung, J.W. Hong, S.Y. Lim, and Y. Seo, Biotechnol. Bioprocess Eng 16, 992 (2011).
[11] C.X. He, North. Hortic. 2013, 182 (2013).
[12] S.T. Fang, X. Liu, N.N. Kong, S.J. Liu, and C.H. Xia, Chin. Tradit. Herb. Drugs 44, 2035 (2013).
[13] K.R. Markham, B. Ternai, R. Stanley, H. Geiger, and T.J. Mabry, Tetrahedron 34, 1389 (1978).
[14] G. Nicollier and A.C. Thompson, J. Nat. Prod. 46, 112 (1983).
[15] D. Sun, Z. Zhao, Y.F. Lai, and W. Herbert, Chem. Ind. Forest Prod. 4, 251 (1991).
[16] H. Guo and J. Yuan, Chin. Tradit. Herb. Drugs 25, 398 (1994).
[17] B.B. Xie, F.Q. Xu, L.B. Li, and C.X. Chen, Acta Bot. Yunnan. 27, 232 (2005).
[18] F. Feng, W.Y. Liu, Y.S. Chen, J.H. Liu, and S.X. Zhao, J. Chin. Pharm. Univ. 34, 119 (2003).
[19] J. Han, M. Ye, X. Qiao, W.Y. Wu, G.Q. Qu, and D.A. Guo, J. Chin. Pharm. Sci. 16, 307 (2007).
[20] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. House, J. Langley, P. Cronse, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, and M. Boyd, J. Natl Cancer Inst. 83, 757 (1991).