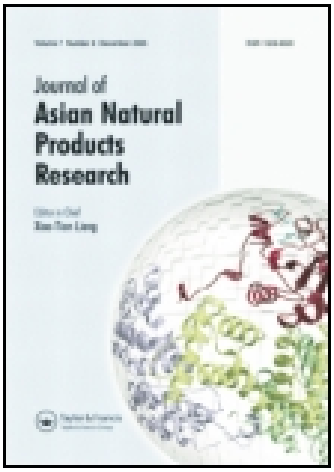

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A new acylated flavonol glycoside from the aerial parts of Cardamine tangutorum

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A new acylated flavonol glycoside from the aerial parts of *Cardamine tangutorum*

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A new acylated flavonol glycoside, kaempferol-3-*O*-β-D-(2-feruloylglucopyranosyl) (1 → 6)-[β-D-glucopyranosyl(1 → 2)]-β-D-glucopyranoside, named tangutorumoside **A** (**1**), together with 12 known compounds, was isolated from 50% acetone extract of *Cardamine tangutorum*. Their structures were elucidated by NMR and MS experiments. In addition, compound **1** could promote the proliferation of splenic lymphocytes and thymic lymphocytes with ConA *in vitro*.

Keywords: *Cardamine tangutorum*; acylated flavonol glycoside; Cruciferae

1. Introduction

Cardamine tangutorum (Cruciferae family) is a perennial herb and mainly distributed in the mountains and uplands of north and southwest of China. *Cardamine* consists of about 130 species in the world, of which 42 species are found in China [1]. Most herbs of Cruciferae family have obvious biological activities, such as antibiosis, antiviral, and antiendotoxin [2–4]. Some glycosidated compounds, such as megastigmane glucopyranosides and kaempferol glycosides, have been isolated from this genus [5–6]. However, few of the phytochemical studies of the plant have been reported previously. In our search for bioactive compounds from this medicinally important genus, a new acylated flavonol glycoside named tangutorumoside A, together with 12 known compounds, kaempferol-3-*O*-β-D-glucopyranosyl(1 → 6)-[β-D-glucopyranosyl(1 → 2)] -β-D-glucopyranoside (**2**) [7], 5,4′-dihydroxy-6,7-dimethoxy-8-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavone (**3**) [8], kaempferol-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (**4**) [9], 5,4′-dihydroxy-7-methoxy-6-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavone (**5**) [10], 5,4′-dihydroxy-7-methoxy-8-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavone (**6**) [10], 5,7,4′-trihydroxy-6-methoxy-8-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavone (**7**) [11], 5,7,4′-trihydroxy-8-methoxy-6-*C*-[β-D-xylopyranosyl-(1 → 2)]-β-D-glucopyranosyl flavone (**8**) [11], quercetin-3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranoside (**9**) [12], *p*-hydroxyphenylethanol (**10**) [13], hydroferulic acid (**11**) [14], isovanillic acid (**12**) [15], and methyl *p*-hydroxycinnamate (**13**) [16], was isolated from 50% acetone extract of *C. tangutorum*. All these known compounds are reported from this plant for the first time. In this paper, we report the isolation and structural elucidation of these compounds and the MTT proliferation

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activity of compound **1** against the splenic lymphocytes and thymic lymphocytes.

2. Results and discussion

Compound **1** was isolated as a yellow amorphous powder. Its molecular formula was deduced as $C_{43}H_{48}O_{24}$ from the $[M + Na + H]^+$ peak at *m/z* 972.2473 using HR-ESI-MS. The IR spectrum exhibited the absorptions of hydroxyl (3403 cm^{-1}), carbonyl (1654 cm^{-1}), and aromatic rings (1604 and 1513 cm^{-1}). The UV spectrum showed absorption maxima at 269 and 327 nm. Upon acid hydrolysis with 1 M HCl, **1** afforded kaempferol as its aglycone, which was identified by comparison of its NMR spectral data with those in the literature [17], and D-glucose as a sugar component which was identified by gas–liquid chromatographic analysis. The ^{1}H NMR spectrum of **1** (Table 1) exhibited nine aromatic proton signals, four of which resonated at δ 8.13 (2H, d, $J = 8.8$ Hz, H-2′, 6′), 6.85 (2H, d, $J = 8.8$ Hz, H-3′, 5′), being ascribed to a set of AA′BB′ system of flavone B-ring. Two deshielded one-proton doublets at δ 6.19 and 6.43 with coupling interactions of 1.3 Hz each were assigned to aromatic ring A *meta*-coupled H-6 and H-8, respectively. The rest of the signals at δ 6.83 (1H, d, $J = 8.0$ Hz, H-5′′′′′), 7.02 (1H, dd, $J = 8.0$, 1.1 Hz, H-6′′′′′), and 7.21 (1H, d, $J = 1.1$ Hz, H-2′′′′′) as well as a *trans*-double bond at δ 7.54 (1H, d, $J = 15.8$ Hz, H-7′′′′′) and 6.16 (1H, d, $J = 15.8$ Hz, H-8′′′′′) were assigned to the feruloyl moiety. In addition, 21 proton signals were found in the range of δ 3.13–5.35, which suggested the existence of three glucose moities. The ^{13}C NMR spectrum of **1** showed important signals for two carbonyl carbons at δ 179.7 (C-4) and 168.5 (C-9′′′′′), four double band carbons at δ 158.8 (C-2), 147.4 (C-7′′′′′), 134.8 (C-3), and 115.3 (C-8′′′′′), three aromatic rings at δ 95.4–167.0, as well as three glucose moieties (δ 62.2–104.6), respectively.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of compound **1** (CD_3OD, δ ppm).

No.	δ_H (*J*, Hz)	δ_C
2		158.8
3		134.8
4		179.7
5		163.0
6	6.19 (d, $J = 1.3$)	100.6
7		167.0
8	6.43 (d, $J = 1.3$)	95.4
9		158.5
10		105.5
1′		122.5
2′, 6′	8.13 (d, $J = 8.8$)	132.5
3′, 5′	6.85 (d, $J = 8.8$)	116.4
4′		161.7
Glc″		
1″	5.35 (d, $J = 7.5$)	101.5
2″	4.04 (m)	79.7
3″	3.60 (m)	74.5
4″	3.73 (m)	70.3
5″	3.58 (m)	78.2
6″	3.74 (m), 3.70 (m)	68.5
Glc‴		
1‴	4.72 (d, $J = 6.9$)	104.6
2‴	3.38 (m)	75.2
3‴	3.37 (m)	77.9
4‴	3.39 (m)	71.3
5‴	3.37 (m)	77.4
6‴	3.80 (m), 3.77 (m)	62.6
Glc⁗		
1⁗	4.39 (d, $J = 8.0$)	101.9
2⁗	4.58 (m)	75.4
3⁗	3.13 (m)	75.9
4⁗	3.34 (m)	71.5
5⁗	3.50 (m)	77.3
6⁗	3.59 (m), 3.69 (m)	62.2
Feruloyl⁗′		
1⁗′		127.9
2⁗′	7.21 (d, $J = 1.1$)	111.9
3⁗′		150.7
4⁗′		149.4
5⁗′	6.83 (d, $J = 8.0$)	116.5
6⁗′	7.02 (dd, $J = 8.0$, 1.1)	124.2
7⁗′	7.54 (d, $J = 15.8$)	147.4
8⁗′	6.16 (d, $J = 15.8$)	115.3
9⁗′		168.5
$-OCH_3$	3.95 (s)	56.7

Further analysis of the ^{1}H and ^{13}C NMR spectra of **1** revealed the presence of three sugars at δ 5.35 (1H, d, $J = 7.5$ Hz, H-1″), 4.72 (1H, d, $J = 6.9$ Hz, H-1‴), and 4.39 (1H, d, $J = 8.0$ Hz, H-1⁗), which were

correlated in the HSQC spectrum with three anomeric carbons at δ 101.5, 104.6, and 101.9, respectively. The coupling constants of three anomeric protons demonstrated that the glucoses were in β-orientation. Complete assignment of each glycosidic proton system was realized from the combined analysis of $^{1}H-^{1}H$ COSY, TOCSY, HSQC, and HMBC spectra. Specifically, the $^{1}H-^{1}H$ COSY and HSQC spectra of **1** confirmed the presence of CH(1″)-CH(2″), CH(1‴)-CH(2‴), and CH(1⁗)—CH(2⁗) units. The sequence of the glycosidic chain in **1** was determined by the analysis of the 2D NMR spectroscopic data. The key HMBC correlations from Glc-H-1″ (δ 5.35) to C-3 (δ 134.8) indicated unambiguously that the Glc″ residue was linked to C-3 of the aglycone. The long-range correlations observed between the anomeric proton at δ 4.72 (Glc-H-1‴) and the carbon at δ 79.7 (Glc-C-2″), and between Glc-H-1⁗ at δ 4.39 and Glc-C-6″ at δ 68.5 indicated that the trisaccharide residue at C-3 of the aglycone is β-D-glucopyranosyl-(1 → 6)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranoside. In addition, the HMBC correlation from Glc-H-2⁗ (δ 4.58) to C-9⁗⁗ (δ 168.5) confirmed that the feruloyl moiety was connected to C-2⁗ of the glucosyl moiety. Thus, the structure of **1** was determined to be kaempferol-3-*O*-β-D-(2-feruloylglucopyranosyl)(1 → 6)-[β-D-glucopyranosyl(1 → 2)]-β-D-glucopyranoside (Figure 1).

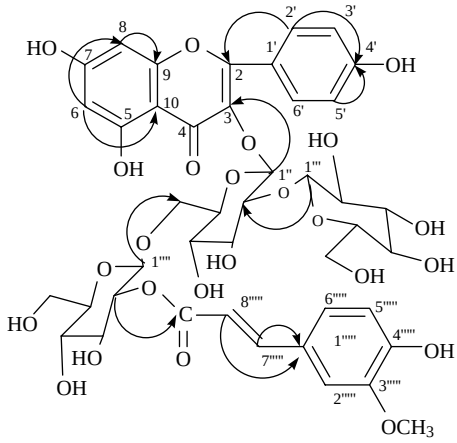

Figure 1. The chemical structure and selected HMBC correlations of compound **1**.

The cell viability of compound **1** was tested on Balb/c mice splenic lymphocytes and thymic lymphocytes with ConA by the MTT method *in vitro*. According to the results (Figure 2), compound **1** could promote the proliferation of splenic lymphocytes and thymic lymphocytes.

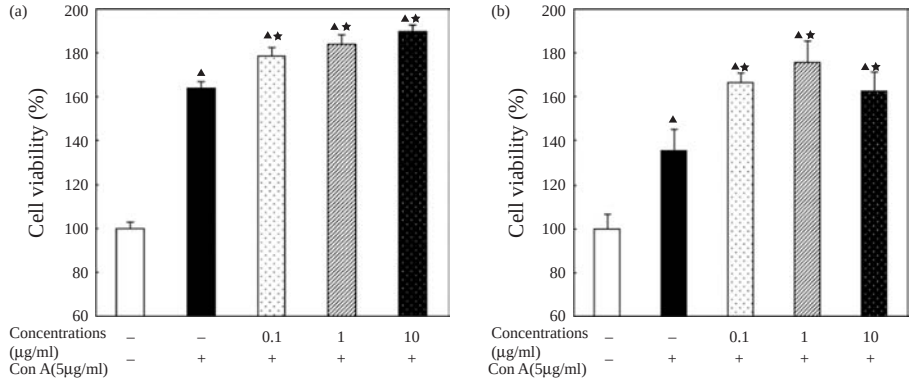

Figure 2. Effects of compound **1** on proliferation of splenic lymphocytes and thymic lymphocytes by MTT assay *in vitro*. Thymic lymphocytes (a) and splenic lymphocytes (b) were both treated with vehicle, Con A (5 μg/ml), 0.1, 1, 10 μg/ml of compound **1**, along with Con A (5 μg/ml) as indicated (▲, $p < 0.01$, compared to the vehicle-treated cells; ★, $p < 0.01$, compared to the Con A-treated cells).

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained on a Perkin-Elmer 341 polarimeter (PerkinElmer Corporation, Waltham, MA, USA). UV spectra were measured with a Shimadzu UV–vis 2201 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR spectra were measured with a Shimadzu FTIR-8201 PC spectrometer (Shimadzu Corporation). ^{1}H and ^{13}C NMR spectra were obtained on a Bruker DPX-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) with TMS as an internal reference (Bruker Corporation, Ettlingen, Germany). HR-ESI-MS were recorded on a Bruker APEX II spectrometer in positive-ion mode (Bruker Corporation). Gas chromatography (GC) data were recorded on an Agilent 7890A instrument (Agilent Technologies Corporation, Santa Clara, CA, USA). Column chromatography was carried out on Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (160–200 mesh, Qingdao Marine Chemical Industry, Qingdao, China), Toyopearl HW-40, and Sephadex LH-20 (TOSOH Corporation, Tokyo, Japan). TLC was conducted on self-made silica gel G (Qingdao Marine Chemical Industry) plates. Cell viability was quantitatively measured by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay with a BIO-RAD Model 680 micro-plate reader (BIO-RAD Corporation, Hercules, CA, USA). The chemical reagents were supplied by Beijing Chemical Plant (Beijing, China) and Tianjin No. 3 Reagent Plant (Tianjin, China).

3.2 Plant material

Air-dried aerial plants of *C. tangutorum* were collected in May 2010 from Xixia County, Henan Province of China, and identified by Prof. Cheng-Ming Dong of the Henan University of Traditional Chinese Medicine. A voucher specimen (No. 20100702) has been deposited in our laboratory.

3.3 Extraction and isolation

Dried aerial parts of *C. tangutorum* (2.5 kg) were extracted with 50% of aqueous acetone twice at room temperature, and concentrated under reduced pressure below 45°C. The water-soluble part (150 g) was chromatographed over Diaion HP-20 with H_2O containing increasing amounts of MeOH to afford H_2O eluate 21.5 g (A), 10% MeOH eluate 8.32 g (B), 30% MeOH eluate 20.3 g (C), and 40% MeOH eluate 18.5 g (D). Fraction A was chromatographed on Toyopearl HW-40 (coarse grade) developing with 10% MeOH–50% MeOH. The 10% MeOH eluate (6.4 g) of A was rechromatographed on Toyopearl HW-40 (50% MeOH) to yield compound **3** (10.1 mg). The 20% MeOH eluate (4.3 g) of A was rechromatographed on Toyopearl HW-40 (70% MeOH) and on silica gel ($CHCl_3$–MeOH–H_2O, 6:1:0.1) to yield compounds **4** (15.2 mg) and **5** (25.2 mg). Fraction B was chromatographed on Toyopearl HW-40 (coarse grade) developing with 10% MeOH–50% MeOH. The 10% MeOH eluate (7.1 g) of B was rechromatographed on Toyopearl HW-40 (70% MeOH) and on silica gel ($CHCl_3$–MeOH, 10:1) to yield compounds **6** (11 mg) and **7** (15 mg). The 20% MeOH eluate (5.4 g) of B was rechromatographed on Toyopearl HW-40 (MeOH) and on silica gel ($CHCl_3$–MeOH, 8:1:0.05) to yield compounds **8** (25 mg), **9** (11 mg), and **10** (11 mg). Fraction C was chromatographed on Toyopearl HW-40 (coarse grade), developing with 10% MeOH–50% MeOH. The 10% MeOH eluate (6.8 g) of C was rechromatographed on Toyopearl HW-40 (MeOH) and on silica gel (EtOAc–EtOH–H_2O, 12:2:1) to yield compounds **1** (12 mg), **2** (21 mg), and **13** (15 mg). Fraction D was chromatographed on Toyopearl HW-40 (coarse grade), developing with 10% MeOH–50% MeOH. The 10% MeOH eluate (15.6 g) of D was

rechromatographed on Toyopearl HW-40 (MeOH) to yield compounds **11** (12 mg) and **12** (12 mg).

*3.3.1 Kaempferol-3-O-β-D-(2-feruloylglucopyranosyl)(1 → 6)-[β-D-glucopyranosyl(1 → 2)]-β-D-glucopyranoside (**1**)*

Yellow amorphous powder. $[\alpha]_D^{20}$ −17 ($c = 0.12$, MeOH); IR (KBr) ν_{max}: 3403, 2925, 1654, 1604, 1513, 1178, 1076 cm^{-1}; UV (MeOH) λ_{max}: 269, 327 nm. ^1H and ^{13}C NMR spectral data are shown in Table 1. HR-ESI-MS: *m/z* 972.2473 $[M + Na + H]^+$ (calcd for $C_{43}H_{49}O_{24}Na$, 972.2512).

3.4 Acid hydrolysis of compound 1

Compound **1** (2 mg) was dissolved in 1 M HCl (aq) (5 ml) and heated at 90°C for 2 h under constant stirring. After extraction with EtOAc (3 × 5 ml), the aqueous layer was evaporated and cryodesiccated. The residue was dissolved in dry pyridine (1 ml), and then L-cysteine methyl ester hydrochloride (2 mg) was added. The mixture was stirred at 60°C for 2 h, and then 0.2 ml of *N*-trimethylsilylimidazole was added, followed by heating to dryness at 60°C for 2 h. The dried reactant was partitioned between *n*-hexane and H_2O (0.2 ml), and the *n*-hexane fraction was subjected to GC (column: DM-5, 0.25 mm × 30 m × 25 μm; detector: FLD; temperature: 280°C; injector temperature: 250°C; carrier: N_2 gas). The sugar from the reactant was identified by comparison of the retention time with those for authentic standards [t_R: 19.84 min for D-glucose].

3.5 Bioassay

The effects of compound **1** on cell proliferation in Balb/c mouse primary thymic lymphocytes and splenic lymphocytes were evaluated in accordance with a reported protocol [18]. Briefly, the splenic lymphocytes and thymic lymphocytes were isolated from the Balb/c mice in sterile conditions. Then, the lymphocytes were suspended by treatment with trypsin (0.25%) and were maintained in 1640 medium. After that, the cells were plated into 96-well (30,000 and 40,000 cells/well, respectively) tissue confluence plates. The medium was replaced by new medium containing compound **1** (0.1, 1, and 10 μg/ml, respectively) after the cells reached confluence. To observe the cell proliferation, MTT assay was carried out after 24 h and 48 h cultivation, respectively.

Acknowledgments

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References

[1] T.Y. Zhou, et al. *Flora of China* (Science Press, Beijing, 1987), p. 189.
[2] J.L. Zheng, M.H. Wang, and X.Z. Yang, *Chin. J. Microecol.* **15**, 18 (2003).
[3] E. Praphoveanu, *Virology* **38**, 115 (1987).
[4] Y.H. Liu, Q.X. Li, and Y.M. Shi, *J. Chin. Med. Mater.* **17**, 36 (1994).
[5] L.K. Lee, K.H. Kim, and S.Y. Lee, *Chem. Pharm. Bull.* **59**, 773 (2011).
[6] F. Yang, Y.F. Su, and Y.P. Bi, *Helv. Chim. Acta* **93**, 536 (2010).
[7] G.L. Qu, J. Ouyang, and D.Y. Kong, *Chin. Pharm. J.* **17**, 1300 (2008).
[8] W.S. Feng, X.K. Zheng, and Y.B. Liu, *Acta. Pharm. Sin.* **39**, 110 (2004).
[9] K.R. Markham, B. Ternai, and R. Stanley, *Tetrahedron* **34**, 1389 (1978).
[10] S. Rayyan, T. Fossen, and Q.M. Andersen, *Agric. Food Chem.* **53**, 10057 (2005).
[11] W.S. Feng, X.K. Zheng, Y.B. Liu, and H.X. Kuang, *J. Asian Natl Prod Res.* **9**, 85 (2007).
[12] L.M. Larsen, J.K. Nlelsen, and H. Sorensen, *Phytochemistry* **21**, 1029 (1982).

[13] B. Wu, L.J. Wu, L. Zhang, and Z.S. Jin, *J. Shenyang Pharm. Univ.* **21**, 341 (2009).
[14] X.L. Wang, N.L. Wang, M.S. Wong, and X.S. Yao, *J. Shenyang Pharm. Univ.* **25**, 25 (2008).
[15] Y. Chen, G.G. Zhang, D.S. Mao, and Y.N. Li, *Chin. J. Med. Chem.* **18**, 48 (2008).
[16] L. Jia, F. Xi, N. Wang, L.L. Jing, and D.Y. Kong, *Chin. J. Pharm.* **41**, 98 (2010).
[17] S.S. Kang, Y.S. Chang, and J.S. Kim, *Chem. Pharm. Bull.* **48**, 1242 (2000).
[18] M. Yamaguchi, I. Hasegawa, N. Yahagi, Y. Ishigaki, F. Takano, and T. Ohta, *Agric. Food Chem.* **58**, 8566 (2010).