CHEMICAL CONSTITUENTS FROM *Clematis terniflora*

Lin Zhang, Xiaoling Luo, and Jingkui Tian*

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*One new flavonol glycoside named terniflonoside A (**1**) and four known flavonol glycosides were isolated from the whole plants of* Clematis terniflora. *The structure was determined by 1D and 2D NMR, ESI-MS techniques, and chemical methods.*

Key words: *Clematis terniflora*, terniflonoside A.

Clematis terniflora Tamura is a folk medicinal plant that grows in southeastern China. The whole plant is used for treating rheumatoid arthritis [1]. In the course of our ongoing search for anti-inflammatory compounds, we have isolated some compounds from *Ranunculus ternatus* [2, 3]; here we report the isolation and structural elucidation of five flavonol glycosides, kaempferol-7-*O*-(6-caffenic-)-glucosyl-(1→3)-rhamnoside (**1**), kaempferol-3-*O*-glucoside (**2**), kaempferol-3-*O*-rutinoside (**3**), kaempferol-3,7-*O*-dirhamnoside (**4**), and rutin (**5**) from *Clematis terniflora*. Compound **1** was a new compound, named terniflonoside A.

Compound **1**, a yellow amorphous solid, showed molecular formula $C_{36}H_{36}O_{18}$ as determined from its HR-ESIMS (*m/z* 757.1969 $[M+H]^+$, calcd. 757.1974). In the positive and negative ESIMS, it showed signals of quasi-molecular ion peaks at *m/z* 757 $[M+H]^+$ and 755 $[M-H]^-$ respectively, The fragment ion peaks at *m/z* 595 [M–162(caffeoyl)+H]$^+$, 433 [595–162 (glucosyl)]$^+$and 287 [433–146 (rhamnosyl)]$^+$ indicated the presence of a rhamnosyl inner unit. D-Glucose and L-rhamnose were detected by GC analysis after acid hydrolysis and preparation of their thiazolidine derivatives. The UV spectrum of **1** showed two absorption maxima at 335 and 265 nm, indicating the presence of substituted aromatic rings and α, β-unsaturated ketones in the molecule. The ^{1}H NMR spectrum of compound **1** (Table 1) showed the typical pattern of a flavonol with a kaempferol aglycon together with signals ascribable to sugar moieties and acyl residue. The two anomeric protons arising from the sugar moieties appeared at δ 5.48 (1H, d, J = 2.5 Hz) and 4.52 (1H, d, J = 7.8 Hz), which correlated respectively with signals at 98.5 and 105.2 ppm in the HMQC spectrum. The ^{1}H NMR spectrum showed also the presence of a caffeoyl residue (Table 1). All the ^{1}H and ^{13}C NMR signals of **1** were assigned using ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY experiments.

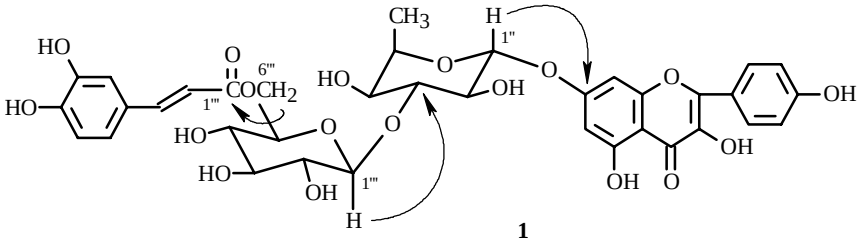

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Fig. 1. Structure and key HMBC correlations of compound **1**.

Institute of Modern Traditional Chinese Medicine, Zhejiang University, Hangzhou, 310058, People's Republic of China, e-mail: tjk@zju.edu.cn. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 108-110, March-April, 2007. Original article submitted December 27, 2006.

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TABLE 1. NMR, HMBC, and NOESY Spectral Data of **1** (^{1}H, 400 MHz; ^{13}C, 100 MHz; δ ppm, J/Hz, DMSO-d_6)

Atom	δ_H	δ_C	HMBC	NOESY
2		147.9		
3		136.4		
4		176.4		
5		161.4		
6	6.34 (d, 1H, 2.0)	99.2	C-5, 7, 8, 10	H-1″, 8
7		161.6		
8	6.70 (d, 1H, 2.0)	94.8	C-6, 7, 9, 10	H-1″, 6
9		156.0		
10		105.2		
1′		121.9		
2′,6′	8.06 (d, 2H, 8.2)	130.1	C-2, 1′, 3′, 4′, 5′	H-3′, 5′
3′,5′	6.90 (d, 2H, 8.2)	115.9	C-1′, 2′, 4′, 6′	H-2′, 6′
4′		159.7		
Rha-1″	5.48 (d, 1H, 2.5)	98.5	C-7, 2″,3″	H-6, 8, 2″
2″	4.08	69.6		
3″	3.78 (dd, 1H, 7.2, 6.0)	81.5	C-1″,2″,4″,5″,1‴	H-1‴, 2″, 4″
4″	3.20	70.7		
5″	3.39	70.0		
6″	1.12 (d, 3H, 6.0)	18.2	C-4″, 5″	
Glc-1‴	4.52 (d, 1H, 7.8)	105.2	C-3″, 2‴, 3‴	H-3″, 2‴
2‴	3.52	74.1		
3‴	3.30	76.6		
4‴	3.44	70.6		
5‴	3.13	74.2		
6‴	4.42 (dd, 1H, 12.5, 6.8)	63.9	C-4‴, 5‴, 1⁗	
	4.18 (dd, 1H, 12.5, 6.8)			
Caffeoyl-1⁗		166.9		
2⁗	6.25 (d, 1H, 16.0)	114.0	C-1⁗, 3⁗, 4⁗	H-5⁗, 9⁗
3⁗	7.44 (d, 1H, 16.0)	145.8	C-1⁗, 2⁗, 4⁗, 5⁗, 9⁗	H-5⁗, 9⁗
4⁗		125.7		
5⁗	6.91 (d, 1H, 2.0)	115.3	C-3⁗, 4⁗, 6⁗, 7⁗, 9⁗	H-2⁗, 3⁗, 9⁗
6⁗		145.7		
7⁗		148.7		
8⁗	6.52 (d, 1H, 8.0)	115.8	C-4⁗, 6⁗, 7⁗, 9⁗	H-9⁗
9⁗	6.82 (dd, 1H, 8.0, 2.0)	121.3	C-3⁗, 4⁗, 5⁗ 7⁗, 8⁗	H-2⁗, 3⁗, 5⁗, 8⁗

Two sugar units were obtained from the HMQC spectrum of **1**, in which their anomeric protons at δ 5.48 (1H, d, J = 2.5 Hz) and 4.52 (1H, d, J = 7.8 Hz) were correlated with carbon signals at δ 98.5 and 105.2, respectively. The spin systems associated with monosaccharides were identified by a HMQC experiment with the aid of ^{1}H–^{1}HCOSY and NOESY spectra. All carbon signals of the sugar moieties were assigned as shown in Table 1. Combined with spin-spin couplings and GC analysis after the acid hydrolysis, the two sugar units were identified as one *β*-D-glucopyranoside and one *α*-L-rhamnoside.

The sugar and caffeoyl sequences of the oligosaccharide chain as well as the glycoside sites were subsequently determined by the HMBC and NOESY spectra. In the HMBC spectrum of **1** (Fig. 1), correlations could be achieved between the anomeric proton of rhamnosyl at δ 5.48 (1H, d, J = 2.5 Hz) and C-7 of aglycone at δ 161.6, the anomeric proton of glucosyl at δ 4.52 (1H, d, J = 7.8 Hz) and the C-3 of rhamnosyl at δ 81.5, and the H-6 of glucosyl at δ 4.42, 4.18 (2H, dd, J = 12.5, 6.8 Hz) and the C-1 of caffeoyl at δ 166.9, respectively, suggesting the sugar and caffeoyl sequences of the oligosaccharide chain as shown in Fig. 1. The NOESY correlations between H-6, H-8, and H-1″, H-3″ and H-1‴, and analysis of ESIMS also showed sugar sequences as the above analysis.

TABLE 2. ^{13}C NMR Spectral Data of **2-5** (100 MHz; δ ppm, DMSO-d_6)

Atom	**2**	**3**	**4**	**5**
2	157.3	157.3	156.5	156.9
3	138.6	133.6	135.0	133.7
4	178.5	177.8	178.4	177.7
5	161.5	161.6	161.4	161.6
6	98.9	99.1	98.9	99.0
7	164.8	164.6	162.1	164.4
8	94.1	94.2	95.0	93.9
9	157.3	157.0	160.6	156.8
10	105.7	104.5	106.2	104.3
1′	121.8	121.2	120.8	121.9
2′	135.6	131.2	131.1	115.6
3′	115.7	115.6	115.8	145.1
4′	159.7	160.3	158.2	148.8
5′	115.7	115.6	115.8	116.6
6′	135.6	131.2	131.1	121.5
Sugar	Glc at C-3	Glc at C-3	Rha at C-3	Glc at C-3
1″	101.3	101.7	102.3	101.5
2″	73.6	74.5	71.0	74.4
3″	76.8	76.7	70.6	76.8
4″	70.0	70.3	72.0	70.3
5″	77.6	76.1	70.4	76.3
6″	61.0	67.3	18.3	67.3
Sugar		Rha at C-6″	Rha at C-7	Rha at C-6″
1‴		101.2	99.9	101.1
2‴		70.7	70.7	70.7
3‴		71.0	70.5	70.9
4‴		72.2	71.5	72.2
5‴		68.6	70.2	68.6
6‴		17.8	17.9	18.1

Thus, the structure of the compound **1** was established as kaempferol-7-*O*-(6-caffenic-)-glucosyl-(1→3)-rhamnoside, named terniflonoside A.

EXPERIMENTAL

The *Clematis terniflora* was collected in Zhejiang province, China, and identified by Dr. Jing-kui Tian, Department of Chinese Medicine Science and Engineering, Zhejiang University.

Melting points were measured on an X4 apparatus and uncorrected. NMR spectra were recorded on a Bruker AC-80 (400 MHz) instrument. ESIMS were obtained on a Thermo Finnigan LC/MS spectrometer; HPLC was performed using an Agilent 1100 pump with DAD detector. For column chromatography, D101 resin (Tianjin Nankai), silica gel (200–300 mesh, Qingdao Haiyang), and ODS C_{18} (50 μm, Beijing Huide) were used. TLC and HPTLC (silica gel GF_{254} precoated plates, Qingdao Haiyang) detection was performed by spraying with 10% H_2SO_4 following heating.

The whole plants of *Clematis terniflora* 5 kg were refluxed with 95% EtOH, and the total EtOH extract was concentrated. The reside was dissolved in pure water and patitioned with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH, successively. *n*-BuOH extract was chromatographed over a D-101 resin column, eluting with H_2O and 20, 60, and 95% EtOH. The 60% EtOH eluate was chromatographed on a Si gel column, eluting with $CHCl_3$–MeOH (containing 5% H_2O) from 100 to 30:70 in a gradient manner divided into 30 fractions. Fraction 17 (4.2 g) was separated on a repeat Si gel column to afford **2** (36 mg). Fraction 20 (2.8 g) was separated on a Si gel column and ODS column to afford **4** (280 mg), Fraction 2 (5.5 g) was separated on a Si gel column and ODS column to afford **3** (78 mg) and **5** (350 mg), Fraction 26 (4.7 g) was separated on a Si gel column and ODS column to afford **1** (220 mg).

Compound 1, yellow amorphous powder, mp 191–194°C; UV (λ_{max}, nm): 265, 335; for ^{1}H NMR and ^{13}C NMR, see Table 1; (+)-ESI-MS *m/z* 757 $[M+H]^+$, HR-ESIMS (*m/z* 757.1969 $[M+H]^+$, calcd. 757.1974).

Compound 2, yellow amorphous powder, mp 240–244°C; UV (λ_{max}, nm): 265, 345; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, J/Hz): 7.43 (2H, d, J = 8.0, H-2′,6″), 6.87 (2H, d, J = 8.0, H-3′,5′), 6.77 (1H, d, J = 2.4, H-8), 6.42 (1H, d, J = 2.4, H-6), 5.06 (1H, d, J = 7.2, H-1″); for ^{13}C NMR, see Table 2; (–)-ESI-MS *m/z* 447 $[M-H]^-$. The above data show that compound **2** was kaempferol-3-*O*-glucoside.

Compound 3, yellow amorphous powder, mp 171–173°C; UV (λ_{max}, nm): 260, 340; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, J/Hz): 7.99 (2H, d, J = 8.0, H-2′,6″), 6.88 (2H, d, J = 8.0, H-3′,5′), 6.43 (1H, d, J = 2.0, H-8), 6.22 (1H, d, J = 2.0, H-6), 5.61 (1H ,d, J = 3.0, H-1‴), 4.99 (1H, d, J = 7.2, H-1″), 0.99 (3H, d, J = 6.4, H-6‴); for ^{13}C NMR, see Table 2; (–) -ESI-MS *m/z* 593 $[M-H]^-$. It was identified as kaempferol-3-*O*-rutinoside (**3**).

Compound 4, yellow amorphous powder, mp 217–223°C; UV (λ_{max}, nm): 265, 340; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, J/Hz): 7.76 (2H, d, J = 8.4, H-2′,6″), 6.89 (2H, d, J = 8.4, H-3′,5′), 6.77 (1H, d, J = 2.0, H-8), 6.44 (1H, d, J = 2.0, H-6), 5.61 (1H, d, J = 3.0, H-1″), 5.10 (1H, d, J = 3.5, H-1‴), 1.10 (3H, d, J = 6.0, H-6‴), 0.99 (3H, d, J = 6.5, H-6‴); for ^{13}C NMR, see Table 2; (–)-ESI-MS *m/z* 577 $[M-H]^-$. The above data show that compound **4** was kaempferol-3,7-*O*-dirhamnoside.

Compound 5, yellow amorphous powder, mp 190–193°C; UV (λ_{max}, nm): 265, 345; ^{1}H NMR (DMSO-d_6, 400 MHz, δ, J/Hz): 7.53 (1H, dd, J = 8.0, 2.5, H-6″), 7.04 (1H, d, J = 2.5, H-2″) 6.81 (1H, d, J = 8.0, H-5′), 6.48 (1H, d, J = 2.0, H-8), 6.17 (1H, d, J = 2.0, H-6), 5.32 (1H ,d, J = 3.5, H-1‴), 5.08 (1H, d, J = 8.0, H-1″), 1.03 (3H, d, J = 6.0, H-6‴); for ^{13}C NMR, see Table 2; (–)-ESI-MS *m/z* 609 $[M-H]^-$. it was identified as rutin (**5**).

Acid Hydrolysis 1. Compound **1** (5 mg) dissolved in water (100 mL) and 2 M HCl (100 mL) was heated at 100°C for 1 h. The water was passed through an Amberlite IRA-60E column (6×50 mm) and the eluate was concentrated. The residue was dissolved in pyridine (25 mL) and stirred with D-cysteine methyl ester (4.0 mg) for 1.5 h at 60°C. To the reaction mixture, hexamethyldisilazane (10 mL) and trimethylsilyl chloride (10 mL) were added and the mixture was stirred for 30 min at 60°C. The supernatant was then analyzed by GC [Column: DB-50, 25 mm × 30 m, column temperature; 235°C; carrier gas: N_2, retention time D-Glc (16.5 min), L-Glc (16.1 min), D-Rha (13.2 min), L-Rha (12.9 min). From the new saponins D-glucose and L-rhamnose were detected.

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