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Isolation and characterization of two new phenolic acids from cultured cells of *Saussurea involucrata*

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ABSTRACT

Two new phenolic acids, 1, 5-*O*-dicaffeoyl-3-*O*-(4-maloyl)-quinic acid (**1**) and 3, 5-di-*O*-caffeoyl-1-*O*-(2-*O*-caffeoyl-4-maloyl)-quinic acid (**2**), were isolated from cultured cells of *Saussurea involucrata*. Their structures were elucidated using 2D NMR spectroscopy and MS. Further *in vitro* bioactive investigations demonstrated that 3, 5-di-*O*-caffeoyl-1-*O*-(2-*O*-caffeoyl-4-maloyl)-quinic acid (**2**) had significant scavenging activities against radicals 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) and 2, 2′-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS).

1. Introduction

Saussurea involucrata Kar.et Kir., the herbal name "Tianshan-xuelian", is a well-known Chinese herbal medicine plant distributed mainly in Xinjiang Uighur Autonomous Region of China, which is used for the treatment of rheumatic arthritis, gynopathy, headache, stomachache, several female disorders in Chinese folk medicine (Yi et al., 2010). This herb now becomes endangered due to the long period of its regeneration and over-harvesting. Therefore, it is highly desired to use cultured cells of *S. involucrata* (CCS) as an alternative material, which could theoretically generate comparable secondary metabolites and may thus serve as a new source of drug for clinical usage. Indeed, previous studies reported the CCS had anti-fatigue effects (Jia and Wu, 2008), radio-protective effect (Liu et al., 2011), anti-inflammatory, analgesic activities (Jia et al., 2005), and phytochemical investigations revealed CCS contained terpenes (Chen et al., 2009), flavones and lignans (Li et al., 2007), reflecting its potential values for replacing natural *S. involucrata*.

In this study, we reported the identification of two new caffeoyl maloyl quinic acid type phenolic acids, 1, 5-*O*-dicaffeoyl-3-*O*-(4-maloyl)-quinic acid (**1**)and 3, 5-di-*O*-caffeoyl-1-*O*-(2-*O*-caffeoyl-4-maloyl)-quinic acid (**2**). They were further assayed for free radical scavenging activities using vitamin C as a positive control. The new compounds showed much lower IC_{50} during the radical scavenging activity tests.

2. Results and discussion

2.1. Structural elucidation of new compounds

Compound **1** was obtained as white amorphous power with optical rotation $[\alpha]_D^{20}$ + 28.0 (*c* 0.2, MeOH); mp 205–206 °C. In the UV spectrum, the absorption peaks were at 330 (4.31), 300 (sh, 4.18), 245 nm (4.02), which reflected the features of caffeic acid substituted compounds (Lee et al., 2010). Its IR (KBr disc) spectrum suggested the presence of hydroxy (3300 cm^{-1}), carbonyl (1680 cm^{-1}), arom double bond (1596, 1525 cm^{-1}), trans double bond (1260, 972 cm^{-1}). The pseudo molecular ion peak was at *m*/*z* 631.1294($[M-H^+]^-$), in correspondence with the molecular formula of $C_{29}H_{28}O_{16}$ ($[M-H^+]^-$ = 631.1305). Moreover, ESI-MS/MS spectrum had fragment ions of *m*/*z* 469.0981 ($[M-\text{caffeoyl}-H^+]^-$, $M_{[\text{caffeic acid}]^-}$ = 180.0423), 353.0875 ($M_{[\text{caffeoyl-quinic acid}-H^+]^-}$ = 353.0878), 307.0667 ($[M-2\times\text{caffeoyl}-H^+]^-$, 307.0671), 191.0563

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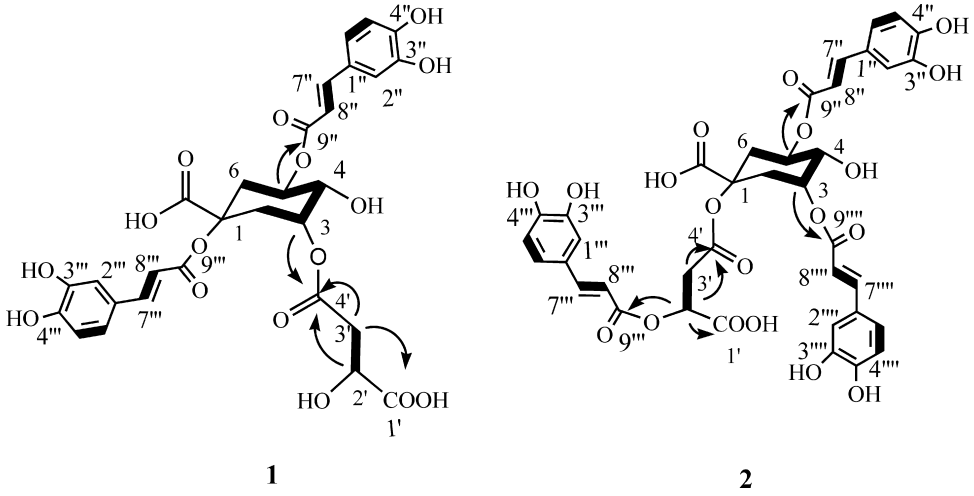

Fig. 1. ^{1}H–^{1}H COSY (bold) and HMBC correlations (arrows) of compound **1** and **2**.

($M_{[quinic\ acid-H^+]^-}$ = 191.0561), suggesting the existence of a dicaffeoyl-quinic acid moiety. Interestingly, ion at *m/z* 307.0667 was also presented in the MS^1 spectra, reflecting the thermal fragmentation of this compound in the ESI source. Tandem MS/MS spectrum of this ion presented product ions at *m/z* 191.0563 ([quinic acid−H^+]$^-$) and 133.0145 ($M_{[maloyl]^-}$ = 133.0145), thus suggesting the fragmentation of [maloyl-quinic acid−H]$^-$. ^{1}H NMR and ^{13}C NMR spectra exhibited four doublets with coupling constants of 15.9 Hz characteristic for trans olefinic protons (δ_H 7.61, δ_H 6.30, δ_H 7.62, δ_H 6.35). The coupling pattern of two 1,3,4-trisubstituted benzenes [δ_H 6.79 (d, 8.2), δ_H 6.97 (d, 8.2), δ_H 6.80 (d, 8.2), δ_H 6.99 (d, 8.2)] indicated the presence of two caffeic acid moieties, which could be confirmed in ^{13}C spectrum by compared with caffeic acid reported in a previous study (Lee et al., 2010). The carboxyl, δ_C 173.3 (C-1′) and δ_C 171.7 (C-4′) suggested that there was a 4-esterified-malic acid group, rather than a 1-esterified-malic acid group in previous studies (Dang et al., 2007; Yang et al., 2005). The rest signals of ^{1}H NMR and ^{13}C NMR were attributed to a quinic acid moiety (Pauli and Poetsch Nahrstedt, 1998). The low-field-shifted signals of δ_H 5.42 (1H, ddd, *J* = 4.2, 4.0, 4.2, H-5), and δ_H 5.48 (1H, m, H-3), indicated that 5-OH and 3-OH were substituted. The ^{1}H NMR and ^{13}C NMR data of caffeoyl and quinic acid groups were highly similar with those of 1,3,5-tri-*O*-caffeoyl-quinic acid (Cui et al., 2009), which had three acyl groups on C-1, 3, 5 of a quinic acid. In the HMBC spectrum, the presence of the correlation of δ_H 5.42 (1H, ddd, 4.2, 4.0, 4.2, H-5) to δ_C 168.7 (C-9″) indicated there was a substitution of a caffeoyl on the C-5 of the quinic acid; The correlation of δ_H 5.48 (1H, m, H-3) to the carbonyl carbon signal at δ_C 171.7 (C-4′) indicated a substitution of a maloyl on the C-3 of the quinic acid (Fig. 1). It was worthy to note that the correlation of δ_H 2.75 (2H, m, H-3′) to δ_C 171.7 (C-4′) was stronger than the one from δ_H 4.45 (1H, m, H-2′) to δ_C 171.7 (C-4′), verifying the signal at δ_C 171.7 belonged to C-4′. Conclusively, the other caffeoyl group was unambiguously connected to 1-OH. Therefore, compound **1** was identified as 1, 5-*O*-dicaffeoyl-3-*O*-(4-maloyl)-quinic acid (MDCQA). ^{1}H NMR and ^{13}C NMR spectra data are shown in Table 1.

Compound **2** was obtained as white amorphous power with optical rotation $[\alpha]_D^{20}$ + 81.76 (*c* 0.17, MeOH); mp 233–234 °C;

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of **1** and **2** (CD_3OD, TMS).

Position	Compound **1**		Compound **2**	
	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
1		80.8		80.9
2a	2.84 (m) **A**	33.1	2.82 (d) **B**	32.9
2b	2.46 (dd,15.4, 3.0)		2.47 (d,14.4)	
3	5.48 (m)	73.3	5.50 (m) **C**	73.6
4	3.95 (dd,3.5,9.1)	71.7	3.96 (dd,3.2,9.4)	71.8
5	5.42 (ddd,4.2,4.0,4.2)	71.3	5.48 (ddd) **C**	71.3
6a	2.03 (dd, 13.4,10.3)	40.7	2.00 (m)	38.1
6b	2.64 (br. d,12.3)		2.64 (br. d,12.6)	
7		174.4		174.6
1‘		173.3		172.7
2‘	4.45 (m)	68.2	5.40 (m)	70.0
3‘a	2.75 (m) **A**	37.7	2.88 (dd,4.5,16.6) **B**	37.6
3‘b			3.03 (dd,6.8,16.6)	
4‘		171.7		170.8
1″/1‴/1⁗		127.7;127.8		127.6;127.8
2″/2‴/2⁗	7.06 (d,1.9); 7.10(d,1.9)	115.0;115.2	7.07 (m)	115.1;114.4;115.0
3″/3‴/3⁗		146.8		146.7;146.8
4″/4‴/4⁗		149.7;149.8		149.6;149.7
5″/5‴/5⁗	6.79 (d, 8.2) 6.80 (d, 8.2)	116.55;116.6	6.76-6.80 (m)	116.5
6″/6‴/6⁗	6.97 (d, 8.2) 6.99 (d, 8.2)	123.1;123.3	6.93-6.97 (m)	123.3;123.1;123.2
7″/7‴/7⁗	7.61 (dd, 15.9); 7.62(dd, 15.9)	147.5;147.95	7.53-7.63 (m)	148.0;147.5;147.8
8″/8‴/8⁗	6.30 (d,15.9); 6.35(d,15.9)	115.3;115.4	6.25-6.36 (m)	115.3;115.4
9″/9‴/9⁗		167.8;168.7		168.8;167.8;168.1

Note: the chemical shifts in the same letters were overlapped.