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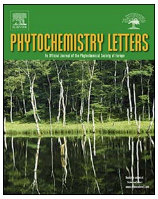

Short communication

Antiproliferative constituents from *Aphananthe aspera* leaves

Jia Sun[a,1], Quan Gao[b,1], Xia-Bing Li[a,1], Feng Tang[a,*], Cheng-Xiang Li[b]

[a] *State Forestry Administration Key Open Laboratory, International Centre for Bamboo and Rattan, Beijing 100102, China*
[b] *School of Plant Protection, Anhui Agricultural University, Hefei 230036, China*

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5-*O*-caffeoyl quinic acid-(7′*R*,8′*S*,7″*E*)-3′,4′,3″-dihydroxy-4″,7′-epoxy-8′,5″-neolign-7′-ene-9-carboxyl
Anti-proliferative activity

ABSTRACT

Extensive screening for the antiproliferative activity of different compounds found in trees was performed by extracting the leaves of *Aphananthe aspera* (Thunb.) Planch and then using chromatographic separation to afford 2 new compounds, (2*S*,4*R*)-2-carboxy-4-(*E*)-*p*-caffeoyl-1-methyl-hydroxyproline (**1**) and 5-*O*-caffeoyl quinic acid-(7′*R*,8′*S*,7″*E*)-3′,4′,3″-dihydroxy-4″,7′-epoxy-8′,5″-neolign-7′-ene-9- carboxyl (**2**). In addition, 6 known compounds were discovered from the leaves of this plant. The structural determination of all compounds, including their absolute configurations, was established by UV, IR, HRESIMS, 1D and 2D NMR, and CD spectroscopy. The novel compound **1** showed strong antiproliferative activity against human breast adenocarcinoma cells MCF-7 and MDA-MB-231.

1. Introduction

Aphananthe aspera (Thunb.) Planch is an indigenous tree species in East Asia (China flora editorial board, 1998), and the bark of the stem and root is used in traditional Chinese medicine (Chinese herbal medicine company, 1994). however, research on the chemical composition of the leaves is relatively scarce. In our on-going study aimed at discovering new anticancer compounds in certain Asian tree species, we screened an extract of *A. aspera* (Thunb.) Planch leaves for its anti-proliferative activity. We now report the isolation and identification of 8 compounds from the leaves of this plant using gradient column chromatography separation as well as their anti-proliferative activity against human breast adenocarcinoma MCF-7 and MDA-MB-231 cells. Two novel compounds, (2*S*,4*R*)-2-carboxy-4-(*E*)-*p*-caffeoyl-1-methyl-hydroxyprolinehydroxyproline (**1**) and 5-*O*-caffeoyl quinic acid-(7′*R*,8′*S*,7″*E*)-3′,4′,3″-dihydroxy-4″,7′-epoxy-8′,5″-neolign-7′-ene-9-carboxyl (**2**), were isolated (Fig. 1).

2. Results and discussion

2.1. Compound characterisation

Compound **1** was obtained as a colourless powder. $[\alpha]_D$ −95.2° (*c* 0.1, methanol). The molecular formula was determined to be $C_{15}H_{17}NO_6$ according to its quasi-molecular ion peak at *m/z* 308.1115 $[M + H]^+$ in the HRESIMS (calcd for $C_{15}H_{18}NO_6$, 308.1034). The UV spectrum exhibited absorption maxima at 214.7, 247.6 and 323.6 nm (see Supplementary material). The IR spectrum showed absorption bands for hydroxyl groups (3430 cm^{-1}), carbonyl groups (1712 cm^{-1}), and aromatic groups (1620 cm^{-1}). The ^1H NMR spectrum (Table 1) showed one ABX system at δ_H 7.06 (s, H-2′), 7.01 (d, J = 8.0 Hz, H-6′) and 6.77 (d, J = 8.0 Hz, H-5′), indicating the presence of a 1,3,4-trisubstituted phenyl group. The presence of one *trans*-double bond was supported by two olefinic protons at δ_H 7.51 (d, J = 16.0 Hz, H-7′) and δ_H 6.26 (d, J = 16.0 Hz, H-8′). Two methylene protons at δ_H 2.23 (ddd, J = 8.0, 11.0, 14.5 Hz, H-3α) and δ_H 2.15 (dddd, J = 3.0, 8.0, 11.0, 14.5 Hz, H-3*β*), another two methylene protons at δ_H 3.53 (dd, J = 6.0, 11.0 Hz, H-5α) and δ_H 2.62 (dd, J = 3.0, 11.0 Hz, H-5*β*), one methine proton at δ_H 3.32 (t, J = 8.0 Hz, H-2), one oxymethine proton at δ_H 5.18 (dddd, J = 3.0, 6.5, 8.0, 11.0 Hz, H-4) and one N-methyl proton at δ_H 2.46 (s, 3H) were also observed in the ^1H NMR spectrum. The ^{13}C NMR spectroscopic data, in conjunction with the DEPT experiments, exhibited the presence of 15 resonances, which were classified as one caffeoyl, one hydroxyproline and one N-methyl group. The hydroxyproline moiety was established by ^1H–^1H COSY correlations from H-2 to H-3, H-3 to H-4 and H-4 to H-5. Additionally, HMBC correlations from H-7 to C-2/5 placed the methyl group at N-1, while H-4 to C-9′ identified C-4 to be the site of acylation (Fig. 2). The relative stereochemistries at C-2 and C-4 were based on the analysis of the ROESY spectrum in which no correlation was observed between H-2 with H-4 indicating their *trans*-relationship (see Supplementary material). The absolute configuration of compound **1** was established by electronic circular dichroism (ECD) measurement and comparison with the calculated ECD data using time-dependent density functional theory at the

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[*] Corresponding author.
E-mail address: fengtang@icbr.ac.cnv (F. Tang).
[1] These authors contributed equally to this work.

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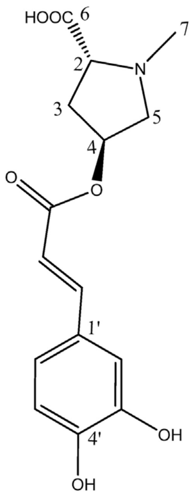

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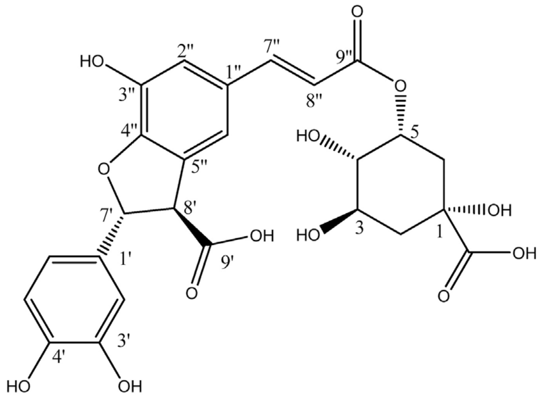

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Fig. 1. Structures of novel compounds **1** and **2**.

Table 1
^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** (at 500 MHz for ^{1}H and 125 MHz for ^{13}C, measured in DMSO-d_6) ^{a}.

No.	1		No.	2	
	δ_C	δ_H (*J* in Hz)		δ_C	δ_H (*J* in Hz)
2	67.1, CH	3.32 (1H, t, 8.0)	1	77.2, C	
3	36.5, CH_2	2.23 (1H, ddd, 8.0, 11.0, 14.5)	2	37.6, CH_2	1.96 (2H, m)
		2.15 (1H, dddd, 3.0, 8.0, 11.0, 14.5)	3	69.9, CH	3.98 (1H, d, br, 12.5)
4	72.8, CH	5.18 (1H, dddd, 3.0, 6.0, 8.0, 11.0)	4	71.5, CH	3.58 (1H, br s)
5	60.9, CH_2	3.53 (1H, dd, 6.0, 11.0)	5	73.2, CH	5.11 (1H, t, br, 5.0)
		2.62 (1H, dd, 3.0, 11.0)	6	37.7, CH_2	2.02 (2H, m)
6	172.7, C		7	175.5, C	
7	40.8, CH_3	2.46 (3H, s)	1′	131.0, C	
1′	125.8, C		2′	113.8, CH	6.77 (1H, s)
2′	115.4, CH	7.06 (1H, s)	3′	145.9, C	
3′	146.1, C		4′	146.2, C	
4′	149.1, C		5′	115.9, CH	6.75 (1H, d, 8.0)
5′	116.3, CH	6.77 (1H, d, 8.0,)	6′	117.1, CH	6.68 (1H, d, 8.0)
6′	121.8, CH	7.01 (1H, d, 8.0)	7′	86.9, CH	5.86 (1H, d, 7.5)
7′	146.1, CH	7.51 (1H, d, 16.0)	8′	55.6, CH	4.26 (1H, d, 7.5)
8′	114.1, CH	6.26 (1H, d, 16.0)	9′	170.3	
9′	166.5, C		1″	128.3	
			2″	117.7, CH	7.07 (1H, s)
			3″	142.0	
			4″	149.2	
			5″	126.8	
			6″	116.8, CH	7.24 (1H, s)
			7″	144.9, CH	7.49 (1H, d, 16.0)
			8″	116.0, CH	6.26 (1H, d, 16.0)
			9″	166.1, C	

B3LYP/6-31G(d) level, $\sigma = 0.4$ eV. The ECD calculation was performed after the optimization of the conformers **1**a and **1**b. Comparison of the experimental and theoretically calculated ECD curves permitted the assignment of the absolute configuration of **1** as 2*S*,4*R* (see Supplementary material). Moreover, D-hydroxyproline does not exist in nature (Xu., 2004), the (2*S*,4*R*)-configuration of compound **1** was further confirmed. Thus, the structure of **1** was established as (2*S*,4*R*)-2-carboxy-4-(*E*)-*p*-caffeoyl-1-methylhydroxyproline.

Compound **2** was obtained as a white powder with a positive optical rotation $[\alpha]_D$ +36.9° (*c* 0.1, methanol). Its molecular formula was determined to be $C_{25}H_{24}O_{13}$ by HRESIMS (*m/z* 531.1154 $[M-H]^-$, calcd for $C_{25}H_{23}O_{13}$, 531.1139). The UV spectrum exhibited absorption maxima at 219.4, 250.0 and 328.3 nm (Supplementary material). The IR spectrum showed absorption bands for hydroxyl groups (3446 cm^{-1}), methylene (2949 cm^{-1}), carbonyl (1712 cm^{-1}) and aromatic groups (1631 cm^{-1}). The ^{1}H NMR spectrum (Table 1) exhibited signals for a 1,3,4-trisubstituted phenyl moiety, which included three aromatic protons signals at δ_H 6.77 (s, H-2′), 6.75 (d, *J* = 8.0 Hz, H-5′) and 6.68 (d, *J* = 8.0 Hz, H-6′); The presence of one *trans*-double bond was supported by proton signals at δ_H 7.49 (d, *J* = 16.0 Hz, H-7″) and 6.26 (d, *J* = 16.0 Hz, H-8″). Two additional singlets at δ_H 7.24 (s, H-6″) and 7.07 (s, H-2″) indicated the presence of a tetra-substituted phenyl ring in the molecule. Additionally, five oxymethine protons at δ_H 5.86 (d, *J* = 7.5 Hz, H-7′), 4.26 (d, *J* = 7.5 Hz, H-8′), 5.11 (t, *J* = 5.0 Hz, H-5), 3.98 (d, *J* = 12.5 Hz, H-3) and 3.58 (*br s*, H-4) as well as two oxymethylene protons at δ_H 2.02 (m, H-6) and δ_H 1.96 (m, H-2) were observed in the ^{1}H NMR spectrum. The ^{13}C NMR and DEPT spectra showed 25 carbon signals corresponding to the chemical units described above, and a caffeoylquinic acid group was indicated by ^{1}H–^{1}H COSY (see Supplementary material) signals for H-2/H-3, H-3/H-4, H-4/H-5 and H-5/H-6. Additionally, two oxymethines at δ_C 86.9 (C-7′) and δ_C 55.6 (C-8′) were attributed to a benzofuran unit, which suggested that compound **2** was a benzofuranoid neolignan group, as indicated by HMBC correlations of H-7′ to C-2′/9′/4″ and H-8′ to C-1′/4″/5″ (Beguma et al., 2009; Xu et al., 2006). The coupling constant (*J* = 7.5 Hz) of H-7′ and H-8′ supported a *trans* configuration between C-7′ and C-8′ (Lee et al., 2006; Ouyang et al., 2007; Jiang et al., 2005). Based on the reversed helicity rule of the $^{1}L_b$ band, CD for the 7-methoxy-2,3-dihydrobenzo[b]furan chromophore showed a negative Cotton effect at 279 nm in the CD spectrum of **2,** indicating that it had a 7*R*,8*S* configuration (see Supplementary material) (Jensen et al., 2002; Warashina et al., 2005; Jiang et al., 2001; Dou et al., 2009). Therefore, compound **2** was determined to be 5-*O*-caffeoyl quinic acid-(7′*R*,8′*S*,7″*E*)-3′,4′,3″-dihydroxy-4″,7′-epoxy-8′,5″-neolign-7′-ene-9-carboxyl.

The known compounds (**3**-**8**) were identified by comparison of their MS and NMR spectroscopic data with the literature as *L*-epicatechin (**3**) (Tian et al., 2017), (+)-catechin (**4**) (Mei et al., 2017), 4-propylbenzene-1,2-diol (**5**) (Liang et al., 2016), 4-dicaffeoylquinic acid (**6**) (Amakura et al., 2013), 1-(3,4-dihydroxyphenyl)-1,2-dihydro-6,7-dihydroxy-bis(5-carboxy-2,3,5-trihydroxycyclohexyl) ester,[1*R*-[1α[1*R**, 2*R**(1*R**,2*R**,3*R**,5*S**)],2*β*,3*β*,5*β*]]-2,3-naphthalenedicarboxylic acid (**7**) (Kashiwada et al., 1995) and 3,4-dihydroxy-benzaldehyde (**8**) (Zhang et al., 2016).