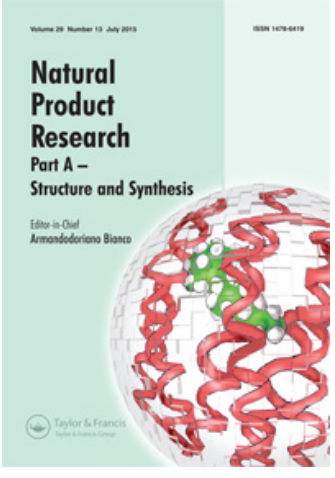

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A new caffeoylgluconic acid derivative from the nearly ripe fruits of *Evodia rutaecarpa*

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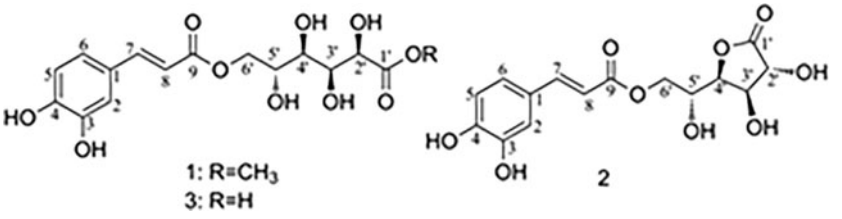

A new caffeoylgluconic acid derivative, *trans*-caffeoyl-6-*O*-D-gluconic acid methyl ester (**1**), together with two known compounds named *trans*-caffeoyl-6-*O*-D-glucono-γ-lactone (**2**) and *trans*-caffeoyl-6-*O*-D-gluconic acid (**3**), was isolated from the nearly ripe fruits of *Evodia rutaecarpa* (Juss.) Benth.. These compounds were isolated by various separation methods associated with the UPLC-Q-TOF-MS technique. Their structures were elucidated on the basis of extensive spectroscopic methods.

Keywords: *Evodia rutaecarpa*; UPLC-Q-TOF-MS; caffeoylgluconic acid derivatives

1. Introduction

The nearly ripe fruits of *Evodia rutaecarpa* (Juss.) Benth., known as 'WuZhuYu' in Traditional Chinese Medicine, have been used to treat headache, cold pain in the stomach duct and abdomen, stomachache, colic, dysmenorrhoea, vomiting and diarrhoea (Chinese Pharmacopoeia Commission 2010). Various compounds, including indole alkaloids (Zuo et al. 2000, 2003; Wang et al. 2010), quinolone alkaloids (Tang et al. 1996; Huang et al. 2012), limonin (Zhang et al. 2005; Yang et al. 2008), flavonoids (Pan et al. 2004) and others (Zhao & Yang 2008; Wang et al. 2011; Cai et al. 2012), have been isolated and identified from *E. rutaecarpa*. However, most of the previous studies focused on fat-soluble substances, instead of water-soluble substances. A new indoloquinazoline alkaloidal glucoside, rutaecarpine-10-*O*-β-D-glucopyranoside, from this plant has been reported (Zhang et al. 2013). In our subsequent investigation, a number of phenolic acids, including caffeoylgluconic acid derivatives, feruloylgluconic acid derivatives and chlorogenic acid and its isomers, were identified from the H_2O extract of 'WuZhuYu' by using the UPLC-Q-TOF-MS technique. To the best of our knowledge, only two new acylgluconic acid compounds, *trans*-feruloylgluconic acid and *trans*-caffeoylgluconic acid,

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have been obtained from *E. rutaecarpa* (Zhao & Yang 2008) so far. In this paper, we described the isolation and structural elucidation of a new caffeoylgluconic acid derivative, *trans*-caffeoyl-6-*O*-D-gluconic acid methyl ester (**1**), and two known compounds named *trans*-caffeoyl-6-*O*-D-glucono-γ-lactone (**2**) (Wu et al. 2005) and *trans*-caffeoyl-6-*O*-D-gluconic acid (**3**) (Zhao & Yang 2008) (Figure 1) from the H_2O extract of *E. rutaecarpa.* Their structures were confirmed by NMR spectroscopic and mass spectrometric (HR-ESI-MS) techniques. This was the first report of *trans*-caffeoyl-6-*O*-D-glucono-γ-lactone (**2**) isolated from the genus *Evodia*.

2. Results and discussion

Compound **1** was a yellowish amorphous powder with negative optical rotation $[\alpha]_D^{20}$ − 2.7 ($c = 0.36$, MeOH). The molecular formula was $C_{16}H_{20}O_{10}$, deduced from HR-ESI-MS with a quasi-molecular ion peak at *m/z* 373.1136 $[M + H]^+$. The UV spectrum showed λ_{max} (MeOH) at 217, 245, 303 and 329 nm. The IR spectrum indicated the presence of hydroxyl groups at 3263 cm^{-1}, carbonyl groups at 1703 and 1743 cm^{-1}, an olefinic bond at 1632 cm^{-1} and aromatic double bonds at 1599, 1516 and 1445 cm^{-1}. The ^1H NMR spectrum revealed signals of three aromatic protons at δ 7.06 (1H, br s), 7.00 (1H, d, $J = 7.2$ Hz) and 6.78 (1H, d, $J = 7.2$ Hz), a methoxyl group at δ 3.65 (3H, s), complex sugar-like signals from δ 3.51 to 4.33 and two *trans*-olefinic protons at δ 7.51 (1H, d, $J = 15.6$ Hz) and 6.27 (1H, d, $J = 15.6$ Hz), both displaying HMBC correlations with a carbonyl ester at δ_C 167.2. The ^{13}C NMR data of **1** also supported the presence of a caffeoyl moiety and sugar-like carbon signals. The signal patterns at δ 126.0, 115.2, 146.1, 148.8, 116.3, 121.7, 145.4, 114.7 and 167.2 showed the existence of the caffeoyl moiety (Pauli et al. 1999) in compound **1**. The signal patterns at δ 173.5, 73.2, 71.0, 72.1, 69.1 and 66.6 indicated that the sugar was a monosaccharic acid (Horton et al. 1983). Comparison of the ^{13}C NMR spectral data of **1** with those of D-gluconyl (Ramos & Gil 2004), D-galactonyl (Ramos et al. 1997a) and L-mannonyl (Ramos et al. 1997b) ester suggested that the monosaccharic acid moiety of **1** was a D-gluconyl group. Furthermore, D-gluconic acid and caffeic acid were detected after acid catalysed hydrolysis of compound **1** and compared with authentic samples on TLC. The ^1H and ^{13}C NMR spectra of **1** were similar to those of **3**, *trans*-caffeoyl-6-*O*-D-gluconic acid, except for additional signals due to a methoxyl group. The HMBC correlation of H-6′ with C-9 proved that the caffeoyl moiety was linked to C-6′. The position of the methoxyl group linked to gluconic acid was deduced by the HMBC correlation observed between the methyl protons (δ 3.65) and C-1′ (δ 173.5). Therefore, the structure of compound **1** was assigned as *trans*-caffeoyl-6-*O*-D-gluconic acid methyl ester, which was a new compound.

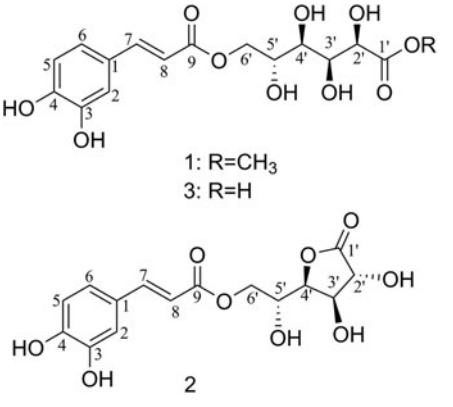

Figure 1. The structures of compounds **1**–**3**.

Compound **2** was a yellowish amorphous powder with $[\alpha]_D^{20}$ + 31.58 (c = 0.38, MeOH). Its molecular formula of $C_{15}H_{16}O_9$ was determined from HR-ESI-MS data at *m/z* 341.0870 $[M + H]^+$. The UV spectra showed maximum absorptions at 218, 242, 303 and 330 nm, suggesting the presence of conjugated groups. The IR spectrum showed absorptions for hydroxyl groups (3394 cm^{-1}), an olefinic bond (1632 cm^{-1}) and α,β-unsaturated ester bond (1689, 1280–1180 cm^{-1}), as well as aromatic double bonds (1601, 1522 and 1446 cm^{-1}). The ^1H NMR spectrum showed a *trans*-caffeoyl moiety due to an ABX system at δ 7.06 (1H, d, J = 2.4 Hz), 7.01 (1H, dd, J = 2.4, 8.4 Hz) and 6.78 (1H, d, J = 8.4 Hz); two *trans*-olefinic protons at δ 7.53 (1H, d, J = 15.6 Hz) and 6.29 (1H, d, J = 15.6 Hz), both displaying HMBC correlations with a carbonyl ester at δ_C 167.0. ^1H and ^{13}C NMR signals in the aliphatic region constructed a gluconic acid skeleton due to resonances at δ 4.13 (1H, d, J = 4.2 Hz), 4.21 (1H, m), 4.49 (1H, dd, J = 4.8, 6.6 Hz), 4.08 (1H, m), 4.32 (1H, dd, J = 3.0, 11.4 Hz) and 4.20 (1H, dd, J = 6.0, 11.4 Hz) and δ 175.9, 73.5, 73.0, 80.4, 67.1 and 66.1. The HMBC correlation of H-6′ with C-9 proved that the caffeoyl moiety was linked to C-6′. The downfield-shifted H-4′ and the HMBC connectivities of H-2′ to H-4′ with C-1′ proved that the gluconic acid cyclised to a γ-lactone. Based on the above results, the structure of compound **2** was elucidated as *trans*-caffeoyl-6-*O*-D-glucono-γ-lactone, which has been isolated from *Vittaria anguste-elongata* (Wu et al. 2005).

3. Experimental

3.1. *General experimental procedures*

UV spectra were measured on a WFZ UV-2102PCS UV-visible photometer (UNICO, Dayton, NJ, USA). IR spectra were recorded on a Spectrum 65 spectrometer (Perkin-Elmer, Waltham, MA, USA). NMR spectra were recorded on Bruker AVIII 400 and 600 MHz spectrometers (Bruker, Zurich, Switzerland). The HR-ESI-MS data were recorded on a Synapt G2 mass spectrometer (Waters, Milford, MA, USA). Preparative HPLC was performed using an Agilent 1260 system, composed of a quaternary pump, a DAD detector and a ZORBAX SB-C18 PrepHT column (Agilent, Santa Clara, CA, USA). UPLC was carried out using an ACQUITY UPLC instrument equipped with a quaternary pump, a UV detector and an ACQUITY UHPLC BEH C18 column (Waters). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), ODS silica gel (120 A, 50 μm, YMC, Kyoto, Japan), Sephadex LH-20 gel (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and D101 macroporous resin (Tianjin Haiguang Chemical Co., Ltd, Tianjin, China) were used for column chromatography. Precoated plates with GF_{254} silica gel (Qingdao Haiyang Chemical Co., Ltd) were used for TLC analysis.

3.2. *Plant material*

The dried fruits of *E. rutaecarpa* were obtained from Anguo county, Hebei province of P.R. China, and authenticated by Prof. Tian-Xiang Li, Tianjin University of Traditional Chinese Medicine (Tianjin, China). A voucher specimen (No. 10523266) was deposited in the herbarium of pharmacognosy, Tianjin University of Traditional Chinese Medicine.

3.3. *Extraction and isolation*

The dried nearly ripe fruits of *E. Rutaecarpa* (3 kg) were refluxed with H_2O twice. The H_2O extract was subjected to D101 macroporous resin column chromatography with 95% EtOH to remove the impurities, then eluted with 0.1% formic acid–H_2O. The eluent was evaporated and concentrated to yield a crude extract (197 g), 40 g of which were exposed to Sephadex LH-20 column chromatography and eluted with MeOH–H_2O (90:10, 100:0) to afford 22 fractions (Fr.1–Fr.22) and 16 fractions (Fr.23–Fr.38), respectively. Frs9–38 (35 g) were separated by

ODS column chromatography, eluted with 10%, 15%, 20%, 30%, 40% and 50% MeOH as eluants to get 76 fractions. The target compounds were found in Frs12–36 on the basis of UPLC-MS. Then Frs12–36 were purified by preparative HPLC with MeOH–0.1% formic acid (23%–30%, 30 min) as mobile phase to give compounds **1**–**3**.

3.3.1. *trans-Caffeoyl-6-O-D-gluconic acid methyl ester (**1**)*

$C_{16}H_{20}O_{10}$, yellowish amorphous powder, $[\alpha]_D^{20}$ − 2.7 ($c = 0.36$, MeOH). HR-ESI-MS *m/z*: 373.1136 $[M + H]^+$ (calcd for $C_{16}H_{21}O_{10}$, 373.1135). UV (MeOH) λ_{max} nm (log ε): 217 (4.19), 245 (4.0), 303 (4.11), 329 (4.23). IR (KBr) ν cm^{-1}: 3263, 1743, 1703, 1632, 1599, 1515, 1445, 1282, 1161. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.06 (1H, br s, H-2), 6.78 (1H, d, $J = 7.2$ Hz, H-5), 7.00 (1H, d, $J = 7.2$ Hz, H-6), 7.51 (1H, d, $J = 15.6$ Hz, H-7), 6.27 (1H, d, $J = 15.6$ Hz, H-8), 3.65 (3H, s, OCH_3), 4.25 (1H, br s, H-2′), 3.95 (1H, m, H-3′), 3.51 (1H, m, H-4′), 3.77 (1H, m, H-5′), 4.04 (1H, m, H-6′), 4.33 (1H, d, $J = 9.6$ Hz, H-6′). ^{13}C NMR (150 MHz, DMSO-d_6): δ 126.0 (C-1), 115.2 (C-2), 146.1 (C-3), 148.8 (C-4), 116.3 (C-5), 121.7 (C-6), 145.4 (C-7), 114.7 (C-8), 167.2 (C-9), 173.5 (C-1′), 73.2 (C-2′), 71.0 (C-3′), 72.1 (C-4′), 69.1 (C-5′), 66.6 (C-6′), 51.9 (OCH_3).

3.3.2. *trans-Caffeoyl-6-O-D-gluconic-γ-lactone (**2**)*

$C_{15}H_{16}O_9$, yellowish amorphous powder, $[\alpha]_D^{20}$ + 31.58 ($c = 0.38$, MeOH). HR-ESI-MS *m/z*: 341.0870 $[M + H]^+$ (calcd for $C_{15}H_{17}O_9$, 341.0873). UV (MeOH) λ_{max} nm (log ε): 218 (4.22), 242 (4.02), 303 (4.12), 330 (4.23). IR (KBr) ν cm^{-1}: 3394, 1689, 1632, 1601, 1522, 1446, 1280, 1180. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.06 (1H, d, $J = 2.4$ Hz, H-2), 6.78 (1H, d, $J = 8.4$ Hz, H-5), 7.01 (1H, dd, $J = 2.4$, 8.4 Hz, H-6), 7.53 (1H, d, $J = 15.6$ Hz, H-7), 6.29 (1H, d, $J = 15.6$ Hz, H-8), 4.13 (1H, d, $J = 4.2$ Hz, H-2′), 4.21 (1H, m, H-3′), 4.49 (1H, dd, $J = 4.8$, 6.6 Hz, H-4′), 4.08 (1H, m, H-5′), 4.32 (1H, dd, $J = 3.0$, 11.4 Hz, H-6′), 4.20 (1H, dd, $J = 6.6$, 11.4 Hz, H-6′). ^{13}C NMR (100 MHz, DMSO-d_6): δ 126.0 (C-1), 115.3 (C-2), 146.1 (C-3), 148.9 (C-4), 116.2 (C-5), 121.8 (C-6), 145.8 (C-7), 114.3 (C-8), 167.0 (C-9), 175.9 (C-1′), 73.5 (C-2′), 73.0 (C-3′), 80.4 (C-4′), 67.1 (C-5′), 66.1 (C-6′).

3.3.3. *trans-Caffeoyl-6-O-D-gluconic acid (**3**)*

$C_{15}H_{18}O_{10}$, yellowish amorphous powder. HR-ESI-MS *m/z*: 359.0978 $[M + H]^+$ (calcd for $C_{15}H_{19}O_{10}$, 359.0978). UV (MeOH) λ_{max} nm (log ε): 218 (4.48), 244 (4.30), 301 (4.43), 329 (4.53). IR (KBr) ν cm^{-1}: 3395, 1689, 1632, 1603, 1518, 1445. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.05 (1H, br s, H-2), 6.76 (1H, d, $J = 8.0$ Hz, H-5), 6.98 (1H, d, $J = 8.0$ Hz, H-6), 7.50 (1H, d, $J = 16.0$ Hz, H-7), 6.26 (1H, d, $J = 16.0$ Hz, H-8), 3.57 (1H, m, H-2′), 3.95 (1H, m, H-3′), 3.77 (1H, m, H-4′), 4.00 (1H, m, H-5′), 4.01 (1H, m, H-6′), 4.33 (1H, d, $J = 10.8$ Hz, H-6′). ^{13}C NMR (100 MHz, DMSO-d_6): δ 125.5 (C-1), 114.2 (C-2), 148.3 (C-3), 144.9 (C-4), 115.8 (C-5), 121.2 (C-6), 145.6 (C-7), 114.7 (C-8), 166.7 (C-9), 175.2 (C-1′), 72.7 (C-2′), 68.6 (C-3′), 71.9 (C-4′), 70.2 (C-5′), 66.3 (C-6′).

3.4. *Acid hydrolysis of compounds 1–3*

Compounds **1**–**3** (each 2 mg) were dissolved in 1 M HCl (50 mL), and then heated in a H_2O bath at 80°C for 1 h. After cooling to room temperature, each reaction mixture was extracted by EtOAc three times (Zhang et al. 2013). The extract of EtOAc was evaporated under vacuum to obtain a residue, which was dissolved in MeOH and then analysed by silica gel GF_{254} TLC ($CHCl_3$/MeOH = 4:1, $R_f = 0.31$). Caffeic acid was identified in the above residue in comparison with an authentic sample. The aqueous layer was neutralised with Ag_2CO_3,

centrifuged and evaporated to dryness, which was dissolved in MeOH–H_2O (2:1, v/v) and analysed by silica gel TLC (MeOH/H_2O/HAc = 6:4:1, $R_f = 0.50$) together with authentic D-gluconic acid. TLC detection was obtained by soaking into the solution of the lead tetraacetate/2,7-dichlorofluorescein followed by heating, which appeared as yellow elliptic spots.

4. Conclusion

So far, few caffeoylgluconic acid compounds have been reported from *E. rutaecarpa*. In this study, three caffeoylgluconic acid derivatives were isolated from the nearly ripe fruits of *E. rutaecarpa* by using the UPLC-Q-TOF-MS technique as guidance to find target compounds. Our study indicated that the UPLC-Q-TOF-MS analytical system in combination with traditional separation methods is more rapid and reliable to obtain new/target compounds. Moreover, the biological activity of acylgluconic acid compounds is worth to be investigated in the future.

Disclosure statement

No potential conflict of interest was reported by the authors.

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