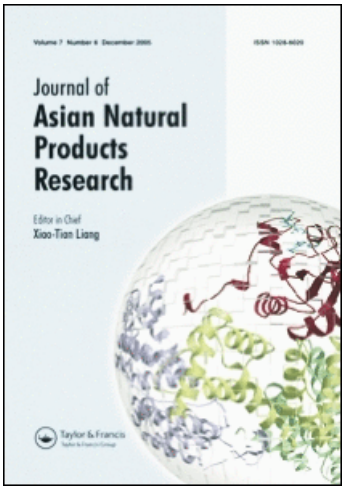

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Two new acylgluconic acids from the nearly ripe fruits of *Evodia rutaecarpa*

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Two new acylgluconic acids from the nearly ripe fruits of *Evodia rutaecarpa*

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Two new acylgluconic acids, *trans*-feruloylgluconic acid (**1**) and *trans*-caffeoylgluconic acid (**2**), together with three known compounds, *myo*-inositol (**3**), phthalic acid dibutyl ester (**4**), and wuzhuyuamide-I (**5**), were isolated from the water soluble part of the dried nearly ripe fruits of *Evodia rutaecarpa* (Juss.) Benth. Their structures were determined by spectroscopic methods, including IR, UV, ESITOFMS, HRSIMS, 1D and 2D NMR spectral analyses.

Keywords: *Evodia rutaecarpa*; acylgluconic acids; *trans*-feruloylgluconic acid; *trans*-caffeoylgluconic acid

1. Introduction

The evodiae fructus, from the nearly ripe fruits of *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae), has been used in Chinese medicine for two millenary in the treatment of headache, abdominal pain during menstruation, weakness and edema of the legs, diarrhoea occurring before dawn daily, hypertension, dysentery, *post partum* haemorrhage and amenorrhea, and external use for ulcers in the mouth [1]. Various alkaloids of indolequinazoline and quinoline [2–4], and limonoids [5] from the nearly ripe fruits of *E. rutaecarpa* have been reported. Some of them exhibit anti-inflammatory effect [6,7], inhibition of acetylcholinesterase activity [8,9], cytotoxic activity [10,11], and eradication of *Helicobacter pylori*,[12] etc. The aim of our work was to further investigate the chemical constituents of the nearly ripe fruits of *E. rutaecarpa*. Herein, we describe the isolation and structural elucidation of two new acylgluconic acids, named *trans*-feruloylgluconic acid (**1**) and *trans*-caffeoylgluconic acid (**2**), together with three known compounds, *myo*-inositol (**3**) [13], phthalic acid dibutyl ester (**4**) [14], and wuzhuyuamide-I (**5**) [3], as shown in Figure 1. The compounds **3** and **4** were isolated from the title plant for the first time.

2. Results and discussion

Compound **1** was obtained as yellowish amorphous powder with $[\alpha]_D^{20}$ − 8.8 (*c* 0.22, H_2O). The molecular formula was inferred as $C_{16}H_{20}O_{10}$ from HRFTICRMS, HSQC and ^{13}C NMR (Table 1) spectral data. The IR spectrum showed absorption bands at 3389 (OH), 1696 and 1274-1179 (α,β-unsaturated ester), 1629 (olefinic), 1598, 1516, 1429 (aromatic double bond) cm^{-1} and the UV spectrum showed absorption maxima at 206 (log ε, 3.20), 234 (3.98), 292 (3.95), and 325 (3.70) nm, suggesting the presence of a feruloyl moiety in **1** [15]. The ^1H and ^{13}C NMR data of **1** (Table 1) exhibited the characteristic pattern of a *trans*-ferulate moiety: [16] three aromatic protons at δ 7.22

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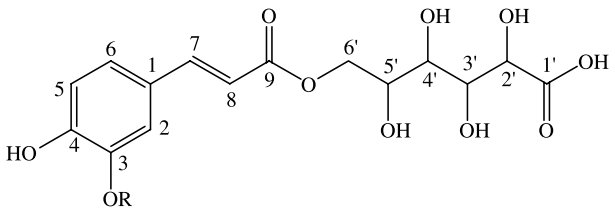

Figure 1. Structures of compounds **1** and **2**.

(1H, br s), 7.01 (1H, d, $J = 8.1$ Hz), and 6.77 (1H, d, $J = 8.1$ Hz), a methoxyl proton at δ 3.76 (3H, s), and two *trans*-olefinic protons at δ 7.54 (1H, d, $J = 15.6$ Hz) and 6.41 (1H, d, $J = 15.6$ Hz), both displaying HMBC correlations with a carbonyl ester at δ_C 167.8. The upfield region from δ 3.60 to 4.40 displayed complex sugar-like signals. The ^{13}C NMR data (Table 1) of **1** also supported the presence of the feruloyl moiety [16] and sugar-like carbon signals. The presence of a set of the carbon signals at δ 176.2, 73.6, 72.9, 70.6, 69.2, and 66.9 indicated that the sugar was a monosaccharic acid [13]. In the ESITOFMS of **1**, positive mode gave quasi-molecular ion peaks at *m/z* 373 $[M + H]^+$ and 395 $[M + Na]^+$, and negative mode gave quasi-molecular ion peak at *m/z* 371 $[M - H]^+$, suggesting that **1** should have a molecular mass of 372. The positive HRFTICRMS of **1** gave the quasi-molecular ion peaks at *m/z* 373.1123 $[M + H]^+$ and 395.0941 $[M + Na]^+$, corresponding to the molecular formula $C_{16}H_{20}O_{10}$ of **1**. These findings led to a conclusion that a monosaccharic acid moiety of **1** had the molecular formula as $C_6H_{12}O_7$. Therefore, comparison of the ^{13}C NMR spectral data of **1** with those of D-gluconyl,[17] D-galactonyl [18] and L-mannonyl [19] ester suggest the monosaccharic acid moiety of **1** was D-gluconyl group. Thereafter, D-gluconic acid was detected after the alkaline hydrolysis and compared with authentic sample on PC. On the other hand,

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** and **2** in DMSO-d_6 (δ values).

	^{1}H NMR (δ_H, *J*/Hz)				^{13}C NMR (δ_C)		
H	**1**	**2**	CSH[a]	C	**1**	**2**	CSH [21]
1		–	–	1	126.3	125.6	125.6
2	7.22 (br s)	7.04 (br s)	7.07 (s)	2	111.5	114.2	114.7
3		–	–	3	148.5	148.5	148.4
4		–	–	4	149.7	145.0	145.6
5	6.77 (d, 8.1)	6.75 (d, 8.0)	6.78 (d, 7.9)	5	115.5	115.8	115.8
6	7.01 (d, 8.1)	6.96 (d, 8.0)	7.01 (d, 7.9)	6	123.6	121.3	121.4
7	7.54 (d, 15.6)	7.48 (d, 15.5)	7.52 (d, 15.8)	7	145.7	145.7	145.1
8	6.41 (d, 15.6)	6.25 (d, 15.5)	6.29 (d, 15.8)	8	114.7	114.7	114.1
9		–	–	9	167.8	166.9	166.7
OCH_3	3.76 (s)	–	–		56.2	–	–
1′			3.28 (br s)	1′	176.2	176.6	62.8
2′	3.63 (br d, 8.5)	3.60 (br d, 8.4)	–	2′	73.6	73.4	102.6
3′	3.90 (m)	3.96 (m)	3.85	3′	69.2	68.6	76.1
4′	3.75 (m)	3.78 (m)	4.08	4′	72.9	72.2	75.9
5′	4.00 (m)	3.98 (m)	3.58 (t, 5.8)	5′	70.6	70.2	81.7
6′	4.03 (m) 4.36 (d, 9.2)	4.02 (m) 4.34 (d, 9.3)	3.83	6′	66.9	66.5	69.9
7′			4.23 (dd, 3.0, 11.2)	7′			65.6

[a] CSH: 7-Caffeoylsedoheptulose.

trans-ferulic acid was also detected in the mild alkaline hydrolysis solution of **1** by comparison with the authentic sample on the silica gel $60F_{254}$ TLC analysis, this spot on the plate was observed under UV light and visualized by spraying with 5% ethanolic solution of molybdophosphoric acid reagent followed by heating. The above spectral evidence indicated that **1** comprised a D-gluconic acid attached to a feruloyl moiety. By comparing the ^{13}C NMR spectral data of **1** with those of authentic D-gluconic acid, it was observed that C-6′ signal at δ 65.0 [20] of D-gluconic acid was downfield shifted to δ 66.9, which was in accordance with esterification chemical shift rule [16], suggesting carboxylic group of *trans*-ferulic acid is bound by ester linkages to C_6–OH of D-gluconic acid. Additionally, the linkage position of the feruloyl moiety to the D-gluconic acid moiety was established by HMBC spectroscopy in which the signals of H-6′ at δ 4.03 (1H, m) and 4.36 (1H, d, $J = 9.2$ Hz) correlated with the ester carbonyl carbon signal at δ 167.8. Therefore, the structure of **1** was assigned as *trans*-feruloyl-6-*O*-gluconic acid. It is a new compound.

Compound **2** was also obtained as yellowish amorphous powder with $[\alpha]_D^{20} - 7.6$ (*c* 0.56, H_2O). Its IR spectrum displayed the strong hydroxyl band (3382 cm^{-1}), carbonyl absorptions at ν_{max} 1720 and 1640 cm^{-1}, and aromatic double bond absorptions at ν_{max} 1599, 1528 and 1446 cm^{-1}. Its UV spectrum showed the absorption maxima at 219 (3.04), 232 (3.40), 294 (3.32), and 327 (2.95) nm, which was similar to those of **1**. The ^1H and ^{13}C NMR data of **2** (Table 1) exhibited characteristic pattern of a *trans*-caffeate moiety: three aromatic protons at δ 7.04 (1H, br s), 6.96 (1H, d, $J = 8.0$ Hz), and 6.75 (1H, d, $J = 8.0$ Hz), and two *trans*-olefinic protons at δ 7.48 (1H, d, $J = 15.5$ Hz) and 6.25 (1H, d, $J = 15.5$ Hz), both displaying HMBC correlations with a carbonyl ester at δ_C 166.9, which were well in agreement with those of *trans*-caffeate moiety in 7-caffeoylsedoheptulose [21]. In addition, a set of the carbon signals of D-gluconyl group at δ 176.6, 73.4, 72.2, 70.2, 68.6, and 66.5 were also observed in the ^{13}C NMR spectrum of **2**. By comparing the ^{13}C NMR spectral data of **2** with those of **1**, it was suggested that the carboxylic group of *trans*-caffeic acid is bound by ester linkages to C_6–OH of D-gluconic acid, which was also approved by the HMBC correlation between the signal of H-6′ at δ_H 4.02 (1H, m), 4.34 (1H, d, $J = 9.3$ Hz), and C-9 at δ_C 166.9. The confirmation of D-gluconyl group was also made by alkaline hydrolysis of **2** with 1*N* NaOH, which yielded D-gluconic acid and caffeic acid. Accordingly, compound **2** was formulated as *trans*-caffeoyl-6-*O*-gluconic acid. The positive ESITOFMS *m/z* 359 $[M + H]^+$, 381 $[M + Na]^+$, 397 $[M + K]^+$, and HRFTICRMS *m/z* 381.07887 $[M + Na]^+$ (calcd for $C_{15}H_{18}NaO_{10}$, 381.07922) of **2** unhesitatingly supported the above conclusion. The *trans*-caffeoyl-6-*O*-gluconic acid is a new compound.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 243B polarimeter with H_2O as solvent. UV spectra were obtained on a Varian Cary-300 UV-visible photometer in MeOH solution. IR spectra were taken on a Thermo Nicolet Nexus 470 FT-IR spectrometer. Mass spectra were recorded on a TRACE 2000 GC-MS (for EIMS), a MDS SCIEX API ASTAR (for ESITOFMS), and a APEX II FTICRMS (for HRSIMS) spectrometer. 1D and 2D NMR spectra were recorded on a JEOL AL-300 OV (300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR) using DMSO-d_6 as solvent and TMS as internal standard. Open column chromatography was carried out using silica gel (200–300 mesh, Qingdao Marine Chemical Co., Qingdao, China) and D_{101} macroporous resin (Tianjin Chem. Ind. Co. Ltd Tianjin, China) as stationary phase. Silica gel TLC and paper chromatography (PC) were conducted on silica gel GF_{254} plates (Merck, New Jersey, NJ, USA) and Waterman No. 1 paper for qualitative analysis. D-Gluconic acid (99.9%) was purchased from Jiangxixinhuanghai Medicine, Food and Chemical Industry Ltd

Co. (Zhangshu City, Jiangxi Province, China) *trans*-Ferulic acid (0773-9910) and *trans*-caffeic acid (8852-200001) were purchased from National Institute for the Control of Pharmaceutical and Biological Products of People's Republic of China.

3.2 Plant material

The nearly ripe fruits of *E. rutaecarpa* were obtained in Xiangtan City, Hunan Province of China, in December 2000, and authenticated by Professor Xiu-wei Yang. The voucher specimen (No. 19990901) of this plant is deposited in the Herbarium of School of Pharmaceutical Sciences, Peking University.

3.3 Extraction and isolation

The extract procedure has been previously reported [2,5]. The water soluble part (299.5 g) was dissolved in H_2O and was then subjected to D_{101} macroreticular resin column and eluted successively with 10, 30, 60, and 95% EtOH, giving four fractions (Fr.1–Fr.4), respectively. The Fr.1 was fractionated on silica gel column chromatography eluting with $CHCl_3$–MeOH gradient mixtures to give 48 fractions (ca. 100 ml each), which were combined on the basis of TLC analysis leading to three main fractions (Fr.1-1, Fr.1-2, and Fr.1-3). The Fr.1-1 was crystallized with MeOH–H_2O to afford compound **3** (8 g). The Fr.1-2 was subjected to polyamide column and eluted with MeOH–H_2O to give compound **1** (10 mg). The Fr.1-3 was deposited with EtOH–H_2O to yield compound **2** (200 mg). Compounds **4** (8 mg) and **5** (10 mg) were obtained from Fr. 3 and Fr. 4, respectively, separated, and purified by silica gel chromatographic column eluted with $CHCl_3$–MeOH as gradient eluent.

3.3.1 trans-*Feruloyl-6*-O-*gluconic acid* (***1***)

Yellowish amorphous powder; $C_{16}H_{20}O_{10}$; $[\alpha]_D^{20}$ − 8.8 (*c* 0.22, H_2O); UV (MeOH) λ_{max} nm (log ε): 206 (3.20), 234 (3.98), 292 (3.95), and 325 (3.70); IR (KBr) ν_{max} (cm^{-1}): 3389, 1696, 1629, 1598, 1516, 1429, 1385, 1274, 1179, 1124, 1089, 1031, 816, 602, and 571; ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectral data were shown in Table 1; positive ESITOFMS *m/z* 373 $[M + H]^+$, 395 $[M + Na]^+$; negative ESITOFMS *m/z* 371 $[M - H]^+$; positive HRFTICRMS *m/z* 373.11231 $[M + H]^+$ (calcd for $C_{16}H_{21}O_{10}$, 373.11293); $[M + Na]^+$ 395.09413 ($C_{16}H_{20}NaO_{10}$, 395.09487).

3.3.2 trans-*Caffeoyl-6*-O-*gluconic acid* (***2***)

Yellowish amorphous powder; $C_{15}H_{18}O_{10}$; $[\alpha]_D^{20}$ − 7.6 (*c* 0.56, H_2O); UV (MeOH) λ_{max} nm (log ε): 219 (3.04), 232 (3.40), 294 (3.32), and 327 (2.95); IR (KBr) ν_{max} (cm^{-1}): 3382, 1720, 1682, 1640, 1599, 1528, 1446, and 1090; ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6) spectral data were shown in Table 1; positive ESITOFMS *m/z* 359 $[M + H]^+$, 381 $[M + Na]^+$, 397 $[M + K]^+$; HRFTICRMS *m/z* 381.07887 $[M + Na]^+$ (calcd for $C_{15}H_{18}NaO_{10}$, 381.07922).

3.3.3 myo-*Inositol* (***3***)

Colorless crystals (H_2O); IR (KBr) ν_{max} (cm^{-1}): 3420, 2923, 2854, and 1707; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.57 (1H, d, $J = 4.2$ Hz), 4.51 (2H, d, $J = 4.5$ Hz), 4.48 (1H, d, $J = 5.4$ Hz), 4.37 (2H, d, $J = 5.4$ Hz), 3.70 (1H, q, $J = 3.0$ Hz), 3.34 (2H, td, $J = 4.5$, 9.0 Hz), 3.13 (1H, dd, $J = 2.4$, 5.1 Hz), 3.10 (1H, dd, $J = 2.7$, 5.7 Hz), 2.90 (1H, td, $J = 4.2$, 9.0 Hz); ^{13}C NMR (75.5 Hz, DMSO-d_6) δ: 75.3 (C-5), 72.8 (C-1), 72.8 (C-3), 72.7 (C-2), 71.9 (C-4), and 71.9 (C-6); positive ESITOFMS *m/z* 181 $[M + H]^-$; ^{13}C NMR spectral data was in agreement with the reported data for *myo*-inositol [13].

3.3.4 *Phthalic acid dibutyl ester* (***4***)

$C_{16}H_{22}O_4$; EI-MS *m/z*: 278 $[M]^+$, 149; positive ESITOFMS *m/z* 301 $[M + Na]^+$; IR, UV, and NMR spectral data were in agreement with the reported data for phthalic acid dibutyl ester [14].

*3.3.5 Wuzhuyuamide-I (**5**)*

Colorless needle ($CHCl_3$–MeOH), mp 261–262°C; EIMS, IR, and NMR spectral data were in agreement with the reported data for wuzhuyuamide-I [3].

3.4 Alkaline hydrolysis of compounds 1 and 2

Each solution of compounds **1** or **2** (each 1.0 mg) in 1 N NaOH aq. (4 ml) was refluxed for 1.5 h. The hydrolysate was allowed to cool and then acidified by HCl to pH 2.0. The acidic solution was evaporated under vacuum to afford a residue, which was dissolved in MeOH–H_2O (2:1, v/v) and subjected to PC ($CHCl_3$ saturated with H_2O–MeOH, 1:1.5, $R_f = 0.55$) together with authentic D-gluconic acid. PC detection was obtained by spraying bromocresol green reagent followed by heating, which appeared as yellow spots. In addition, ferulic acid ($CHCl_3$: MeOH = 4:1, $R_f = 0.42$) or caffeic acid ($CHCl_3$: MeOH = 4:1, $R_f = 0.31$) was also identified in the above residue by silica gel TLC in comparison with an authentic sample.

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