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Identification of new diterpene esters from green Arabica coffee beans, and their platelet aggregation accelerating activities

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ABSTRACT

Eight new *ent*-kaurane diterpene fatty acid esters, namely caffarolides A–H (**1**–**8**), were isolated from green beans of *Coffea arabica*. Their chemical structures were confirmed by extensive spectroscopic analysis including 1D, 2D NMR (HSQC, HMBC, ^{1}H–^{1}H COSY, and ROESY), HRMS, IR and CD spectra and by GC-FID analysis. Interestingly, the diterpene moiety of these new compounds first occurred in genus *Coffea*. All the isolates were evaluated for platelet aggregation activity *in vitro*. As the results, caffarolides C, D and F (**3**, **4** and **6**) showed induction effect for platelet aggregation and the possible structure-activity relationships have been discussed briefly.

1. Introduction

Coffea arabica (Arabica coffee) is the most economic importance specie around 120 species of the genus *Coffea* (Rubiacea), occupying 61% of the world's coffee production (ICO, 2018). Coffee is one of the most consumed beverages worldwide which is prepared by the ripe seeds from the coffee plants. Chemical investigations showed that coffee is rich in bioactive compounds, such as caffeine, trigonelline, chlorogenic acids, phenolic compounds, diterpenes and melanoidins (Ludwig, Clifford, Lean, Ashihara, & Crozier, 2014). They showed the activities of neuroprotective (Machado-Filho et al., 2014), anti-oxidant (Moreira, Nunes, Domingues, & Coimbra, 2012; Zhou, Zhou, & Zeng, 2013), hepatoprotective (Baeza et al., 2015) and anti-cancer (Cavin et al., 2002). Therefore, long-time coffee consumption will improve several related chronic diseases, such as cardiovascular diseases (Miranda, Steluti, Fisberg, & Marchioni, 2017; Ranheim and Halvorsen, 2005), , liver diseases (Muriel & Arauz, 2010; Saab, Mallam, Ii, & Tong, 2014), cognition disorders (Carman, Dacks, Lane, Shineman, & Fillit, 2014; Nehlig, 2016), cancers (Bohn, Blomhoff, & Paur, 2014; Vitaglione, Fogliano, & Pellegrini, 2012) and diabetes (Chu et al., 2011; Pan, Tung, Yang, Li, & Ho, 2016).

The chemical constituents of coffee brews are directly impacted by the chemical compositions of green coffee beans, because green coffee beans contain all the active components (or their precursors) that existed in coffee brews. However, the studies that characterized the chemical compositions of green coffee beans mainly focused on water-soluble fraction (Chu et al., 2016; Shu et al., 2014), but a few on the lipid fraction, despite that the lipid content of green Arabica coffee beans reached 15% (Kurzrock & Speer, 2001).

The main compositions of coffee lipid consist of triacylglycerols, diterpene esters, sterols, sterol esters, and free diterpenes (Durán, Filho, & Maciel, 2010; Kurzrock & Speer, 2001; Speer and Speer, 2006). Among them, cafestol and kahweol are the well-known diterpenes in coffee with cholesterol-rising (Urgert et al., 1995) and anti-cancers (Cavin et al., 2002). However, diterpenes in coffee are rarely present in the form of free (0.4% of the coffee lipid), most of them are esterified with different fatty acids (18% of the coffee lipid) (Kurzrock & Speer, 2001). Until now only a few diterpene esters have been reported, including cafestol, kahweol, 16-*O*-methylcafestol and 16-*O*-methylkahweol with fatty acids C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, C_{20}, and C_{22} (Speer and Speer, 2006). Cafestol and kahweol palmitate are the main two diterpene esters that have been reported to enhance glutathione S-

Abbreviations: 1D NMR, proton and carbon nuclear magnetic resonance; 2D NMR, two-dimensional nuclear magnetic resonance; HSQC, heteronuclear single quantum coherence; HMBC, heteronuclear multiple bond connectivity; ^{1}H–^{1}H COSY, ^{1}H–^{1}H homonuclear chemical shift correlated spectroscopy; ROESY, rotating frame nuclear overhauser and exchange spectroscopy; HRMS, high resolution mass spectrometry; IR, infrared spectroscopy; CD, circular dichroism; GC-FID, gas chromatography with flame ionization detector; TLC, thin layer chromatography; P-TLC, preparative thin layer chromatography; CC, column chromatography; MeOH, methanol; PRP, platelet-rich plasma; PPP, platelet-poor plasma; ADP, adenosine diphosphate

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transferase (Huber et al., 2010), inhibit angiogenesis (Moeenfard et al., 2016) and COX-2 (Muhammada et al., 2007) activities. The presence of diterpene eaters in coffee brew is influenced by roasting degree and methods of preparation. In general, the content of total diterpene esters in coffee beans decreased with an increase degree of roasting (Kurzrock & Speer, 2001; Speer and Speer, 2006). Meanwhile, the concentrations of total diterpene esters in different coffee brews were in the order boiled coffee > French press coffee > espresso > filtered coffee and instant coffee due to different contact time, grind degree, filter paper and temperature (Moeenfard, Erny, and Alves, 2016; Ratnayake, Hollywood, O'Grady, & Stavric, 1993).

Recently, several studies found that coffee extracts can inhibit platelet aggregation *in vitro* (Bydlowski, Yunker, Rymaszewski, & Subbiah, 1987; Naito, Yatagai, Maruyama, & Sumi, 2011), which is a key step in the development of thrombosis and other cardiovascular diseases. Further studies demonstrated that these water-soluble compounds like phenolic acids (Natella et al., 2008), pyridinium compounds (Kalaska et al., 2014) may be responsible for the antithrombotic effect of coffee extract, but the effect of coffee lipids on platelet aggregation has been poorly studies.

Therefore, as part of our systematic chemical investigations on *Coffee arabica*, the aims of the study presented here were to isolate and structurally characterize new diterpene esters from coffee lipid fraction and to evaluate their activities of platelet aggregation (Fig. 1).

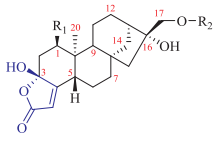
2. Material and methods

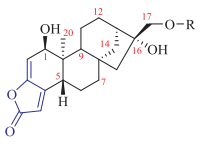
2.1. General

A Jasco P-1020 polarimeter (Jasco, Japan) was used to obtain optical rotations. Ultraviolet spectra were measured by UV-2401 PC spectrophotometers (Shimadzu, Japan). A Bruker Tensor-27 instrument (Bruker, German) was used for recording infrared spectra by using KBr pellets and HRMS data were measured by an API QSTAR Pulsar spectrometer (Waters, UK). The Bruker AM-400, and DRX-600 instruments (Bruker, Zurich, Switzerland) with transcranial magnetic stimulation (TMS) were used to detect ^{1}H and ^{13}C NMR spectra. Circular dichroism spectra were scanned by a Chirascan spectropolarimeter (Applied Photophysics, UK). Semi-preparative HPLC was performed on an Agilent HP1100 or 1260 series instrument with a UV L-2400 detector (Agilent, USA) and an ZORBAX SB C-18 column (5 μm, 9.4 mm × 250 mm, wavelength detection at 220 and 290 nm). The analysis of fatty acid methyl esters was acomplished by an Agilent 6890N gas chromatograph (Agilent Technologies, Germany) equipped with FID detector. The green coffee beans were ground into 50–300 mesh powder by YB-2500B grinder (Yunbang, Yongkang, China).

2.2. Chemical and reagents section

TLC detection was performed on TLC plates (200–250 μm thickness, F254 Si gel 60, Qingdao Marine Chemical, Inc., China). The ordinary column chromatographic materials include Lichroprep RP-18 (40–63 μm, Fuji, Japan), Sephadex LH-20 (20–150 μm, Pharmacia, USA) and Silical gel (200–300 mesh, Qingdao Marine Chemical, Inc., China). The industrial-grade methanol, chloroform, ethyl acetate, acetone, petroleum ether were purchased from Tianjing Chemical Reagents Co. (Tianjing, China). The analytical-grade acetonitrile, tetrahydrofuran, hydrochloric acid, sodium hydroxide, methyl palmitate (16:0), methyl linoleate (18:2), methyl oleate (18:1), methyl nonadecadienoate (19:2), and methyl eicosadienoate (20:2) were purchased from Aladdin Industrial Corporation (Shanghai, China).





	R_1	R_2
1	H	$CH_3(CH_2)_{14}CO$
2	H	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO$
3	H	$CH_3(CH2)_7CH{=}CH(CH_2)_7CO$
4	OH	$CH_3(CH_2)_{14}CO$
5	OMe	$CH_3(CH_2)_{14}CO$
6	OMe	$CH_3(CH2)_4CH{=}CHCH_2CH{=}CH(CH_2)_8CO$

	R
7	$CH_3(CH_2)_{14}CO$
8	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_9CO$

Fig. 1. Structures of diterpene esters isolated from green Arabica coffee beans.

2.3. Plant material

The green coffee beans of *Coffea arabica* cultivated in Yunnan province (P. R. China), with a subtropical monsoon climate at the location of 22°68′ north altitude and 100.94′ east longitude, were harvested in June 2014. The material was authenticated by Ming-Hua Qiu, Kunming Institute of Botany, Chinese Academy of Sciences. A specimen was deposited in State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

2.4. Extraction and isolation of the lipid fraction of green Arabica coffee beans

The powder of dried coffee green beans (50–300 mesh, 10 kg) was soaked in acetone for four days at room temperature, then the acetone extract was evaporated under reduced pressure. The residue (963 g) was dissolved with $CHCl_3$, filtered with a Buchner funnel and recrystallized for removing caffeine. Then, the $CHCl_3$ layer (63 g) was subjected to Silica gel column chromatography (CC, 20.0 × 120 cm) and eluted in a step gradient manner with petroleum ether/acetone (20:1, 5:1, 1:1, 0/100, v/v) to yield four fractions (Fr.): Fr. 1 (16 g), Fr. 2 (9 g), Fr. 3 (5 g), and Fr. 4 (30 g).

Fr. 1 (16 g) was then further separated on a RP C-18 (15.0 × 100 cm) column and eluted in a gradient of MeOH/H_2O (55 → 80%, v/v) to yield three sub-fractions (Fr. 1-1-1-3). Fr. 1-1 (5 g) was subjected to Silica gel CC (5.0 × 70 cm), eluted with a $CHCl_3$/MeOH (80:1 → 20:1, v/v) gradient system to afford four minor fractions (Fr. 1-1-1-1-1-4) on the basis of TLC analysis. Fr. 1-1-2 (205 mg) was separated by preparative thin layer chromatography (P-TLC, eluting with $CHCl_3$/MeOH, 30:1, v/v) to gain **7** (14 mg).

Fr. 2 (9 g) was applied to Silica gel CC (5.0 × 100 cm) and eluted in a gradient of MeOH/H_2O (50 → 75%, v/v) to yield four fractions (Fr. 2-1-2-4). Fr. 2-1 (3 g) was separated by Silica gel CC (5.0 × 70 cm, eluted with $CHCl_3$/MeOH, 40:1, v/v), to afford five minor fractions (Fr. 2-1-1-2-1-5). Fr. 2-1-2 (330 mg) was subjected to chromatography over RP C-18 (2.0 × 20 cm, eluted with MeOH/H_2O, 40–60%, v/v) to obtain **2** (10 mg) and **5** (8 mg). Fr. 2-2 (2 g) was chromatographed on a Silica gel column (5.0 × 50 cm), eluted with $CHCl_3$/MeOH (20:1, v/v) to yield four minor fractions (Fr. 2-2-1-2-2-4). Then, Fr. 2-2-3 (400 mg) was separated by P-TLC, eluted with $CHCl_3$/MeOH (30:1, v/v) to gain minor fractions, then separated by reverse-phase semi-preparative HPLC (CH_3CN/H_2O: 40 → 70%, 30 min, flow rate = 3.0 mL/min, UV 220, 290 nm) to get **3** (11 mg, t_R = 11.5 min), **6** (9 mg, t_R = 14.7 min), and **8** (8 mg, t_R = 23.4 min). Fr. 2-3 (3 g) was separated by use of Sephadex LH-20 (5.0 × 200 cm, eluted with MeOH, 100%, 2 L) and divided into three fractions (Fr. 2-3-1-2-3-3), then Fr. 2-3-1 (506 mg) was applied to Silica gel CC (2.0 × 50 cm), eluted with $CHCl_3$/MeOH (10:1, v/v) to afford four minor fractions (Fr. 2-3-1-1-2-3-1-4). Then, Fr. 2-3-1-2 (88 mg) was treated by reverse-phase semi-preparative HPLC (CH_3CN/H_2O: 45 → 80%, 35 min, flow rate = 3.0 mL/min, UV 220, 290 nm) to get **1** (20 mg, t_R = 15.5 min), **4** (9 mg, t_R = 21.3 min).

2.4.1. Caffarolide A (1)

White amorphous powder, $[\alpha]^{25}d$ −246.9 (c = 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 239 (1.59), 201(1.04),193 (1.05) nm; IR (KBr)