Anti-diabetic nephropathy compounds from *Cinnamomum cassia*

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**Abstract**

**Ethnopharmacological relevance:** The bark of *Cinnamomum cassia*, called ‘Rou-Gui’, a traditional spice and medicine in China, is used to treat diseases resulted from kidney yang deficiency, including diabetic nephropathy. The aim of this study is to investigate the anti-diabetic nephropathy activity of Rou-Gui and the active compounds in it.

**Materials and methods:** The air-dried bark of *C. cassia* was extracted with 90% EtOH, the obtained residue was successively partitioned by petroleum ether, EtOAc, and n-BuOH followed by concentrating to give petroleum ether (RG-1), EtOAc (RG-2), n-BuOH (RG-3), and water fraction (RG-4), respectively. The anti-diabetic nephropathy activity of fraction (RG-1–4) was evaluated in vitro by inhibiting the expression of fibronectin, monocyte chemoattractant protein-1 and interleukin-6 in high-glucose-induced mesangial cells. By bioassay screenings, repeated column chromatography on fractions of RG-1, 2, and 3, led to the isolation of 23 compounds, whose structures were determined by extensive spectroscopic analyses, and the anti-diabetic nephropathy activity of the isolated compounds was also evaluated.

**Results:** Four new sesquiterpenoids, cinnamoids A–D (1–4), a new natural product (5), and 18 known compounds (6–23) were isolated from the EtOH extract of the bark of *C. cassia* under the bioassay-guided screenings. The anti-diabetic nephropathy activity assay showed that fractions of RG-1, 2, and 3 could significantly inhibit the production of fibronectin, monocyte chemoattractant protein-1 and interleukin-6 in high-glucose-stimulated mesangial cells at the concentration of 50 μg/ml; and sesquiterpenoids 5, 6, 14 and compound 20 could significantly inhibit the expression of fibronectin, monocyte chemoattractant protein-1 and interleukin-6 at the concentration of 50 μM.

**Conclusions:** The results revealed that sesquiterpenoids may be the active compounds in *C. cassia* bark on diabetic nephropathy which provided new evidences for the traditional use of this herb to treat diabetic nephropathy and associated kidney diseases.

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1. Introduction

Diabetic nephropathy, a common complication of diabetes, is the most common cause of chronic kidney failure and end-stage kidney disease in the United States. Nearly all diabetes patients with more than 5 years’ disease history have the possibility of getting diabetic nephropathy, and around 40% kidney failure are caused by diabetes (Wild et al., 2004). With the rapid increase of diabetes patients in both developed and emerging countries, diabetic nephropathy has become a growing global threat (Ha et al., 2008). However, no ideal solutions on diabetic nephropathy therapy are available currently, especially in the late period, which makes earlier prevention to be more effective. There are piles of evidences that multiple factors, including extracellular matrix, cytokines and chemokines, are involved in the pathogenesis of diabetic nephropathy acting by driving inflammatory and fibrotic process. The excessive accumulation of extracellular matrix (such as fibronectin) and cytokine (such as interleukin-6) are thought to be harmful to the mesangial cells in kidney (Kolset et al., 2012). Monocyte chemoattractant protein-1 (MCP-1) is a potent chemokine for monocyte/macrophage and T cells. MCP-1 is the strongest known chemotactic factor for monocytes and is upregulated in diabetic nephropathy. Recent studies have shown that the excessive expression of MCP-1 is one of the main contributing factors for diabetic nephropathy (Tesch, 2008). Therefore, research targeting on the above pathogenic factors may contribute to the intervention of diabetic nephropathy (Ha and Kim, 1995).

It is well-known that some traditional Chinese medicines (TCMs) have been used for the treatment of diabetic nephropathy in China since ancient time. In our previous studied, some bioactive compounds against diabetic nephropathy have been successfully characterized from *Acorus tatarinowii*, *Brachystemma calycinum*, *Euryale ferox*, and *Rosa laevigata* (Cheng et al., 2011; Li et al., 2012; Song et al., 2011; Tong...
et al., 2010). In ancient China, diabetic nephropathy is categorized as ‘Shen-Xiao’, ‘Xiao-Shen’ or ‘Xia-Xiao’. Although the pathogenesis of ‘Shen-Xiao’ is quite complex, kidney ‘Yang’ deficiency is distinctly implicated in it especially in the late period of ‘Shen-Xiao’. The ‘Yin–Yang’ theory is an ancient Chinese philosophy that underlies the practice of TC. According to the pathogenic process of ‘Shen-Xiao’, one important therapeutic strategy for the treatment of diabetic nephropathy in clinical practice is to invigorate ‘Yang’ using Chinese herbs with warm property to restore a balance of ‘Yin’ and ‘Yang’. A statistical study on 295 TCM prescriptions showed that the bark of Cinnamomum cassia has been frequently used for the treatment of diabetic nephropathy since ancient time (Liang, 2014). C. cassia (Lauraceae) is an evergreen tree originating in southern China, and widely cultivated in the countries of southern and eastern Asia (India, Indonesia, Laos, Malaysia, Thailand, and Vietnam) (Li et al., 2013). The dried bark of C. cassia, a common flavour spice, called ‘Rou-Gui’ in Chinese, is mainly produced in Guangxi, Guangdong, Fujian, and Yunnan Provinces in China. ‘Rou-Gui’ was first described in ‘Shen-Nong-Ben-Cao-Jing’ written in the Hou-Han Dynasty (AD. 25–220) and considered as a tonic for ‘Yang’ deficiency of kidney. It always occurs together with Poria cocos Wolf or Alisma plantago-aquatica or Rehmannia glutinosa when it is used for the treatment of diabetic nephropathy. Pharmacological studies have demonstrated that ‘Rou-Gui’ possessed the effects of anti-diabetes (Andrews, 2013), anti-hyperlipid, anti-bacteria, anti-tumor, and analgesia (Wu and Jia, 2012). Previous chemical investigations revealed the presence of volatile oil, phenylpropanoids, terpenoids, flavonoids, and polyphenols (Fang, 2007). Cinnamaldehyde, one main compound in the C. cassia volatile oil, was reported to ameliorate metabolic disorder and relieve renal damage induced by diabetes by targeting Nrf2 activation in recent years (Zheng et al., 2011). However, the other bioactive substances responsible for the anti-diabetic nephropathy activity in C. cassia bark remain much unknown. As a part of our ongoing work on finding active compounds against diabetic nephropathy, we undertook a phytochemical study on C. cassia bark, and the anti-diabetic nephropathy activity of the isolated compounds was also evaluated by inhibiting the secretion of fibroinectin, MCP-1 and interleukin-6 in high-glucose-induced mesangial cells.

2. Materials and methods

2.1. General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 polarimeter. UV spectra were collected on a Shimadzu double-beam 210A spectrometer. IR spectra were recorded on a Tensor 27 spectrometer with KBr pellets. NMR spectra were determined on a Bruker AV-400 or a DRX-500, or an Avance III 600 spectrometer. Mass spectra were obtained using KBr pellets. NMR spectra were determined on a Bruker AV-400 spectrometer. UV spectra were collected on a Shimadzu double-beam 210A spectrophotometer. Column chromatography (CC) was performed on silica gel obtained on a VG Auto Spec-3000 mass spectrometer (VG, Manchester, England). Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., PR China), RP-18 (40–60 μm, Daiso Co., Japan), MCI gel CHP 20P (75–150 μm, Tokyo, Japan), and Sephadex LH-20 (Amersham Biosciences, Sweden). Semipreparative HPLC was carried out using an Agilent 1200 liquid chromatograph, the column was used as a 250 mm x 9.4 mm i.d., 5 μm, Zorbax SB-C18.

2.2. Plant material

The banks of C. cassia were purchased from Yunnan Xianghi Biological Technology Co. Ltd. (YNXHBT), Kunming, Yunnan Province, PR China, in July 2010, and were authenticated by Mr. Fu-Shou Xie at YNXHBT. A voucher specimen (CHXY00173) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China of Kunming Institute of Botany.

2.3. Extraction and isolation

The powdered C. cassia barks (50 kg) were extracted three times with 80% ETOH (90 l x 3) under reflux. The extract was concentrated under reduced pressure to give a residue (5.5 kg) which was suspended in water and successively partitioned with petroleum ether, EtOAc, and n-BuOH followed by concentrating to give petroleum ether (RG-1), EtOAc (RG-2), n-BuOH (RG-3), and water extracts (RG-4), respectively.

The petroleum ether extract (RG-1, 300 g) was subjected to CC over silica gel (200–300 mesh), eluted with petroleum ether/EtOAc (from 10:1 to 0:1) to give fractions (A–E). Fraction D (40 g) was subjected to Sephadex LH-20 CC (CHCl3/MeOH, 6:4) to provide four portions (D1–D4). Fraction D4 (2.3 g) was further purified by silica gel CC eluted with petroleum ether/Me2CO (10:1) to give compounds 21 (150 mg), 22 (35 mg), and 23 (41 mg). Fraction E (11 g) was further purified using Sephadex LH-20 CC (MeOH) and then chromatographed over silica gel eluted with petroleum ether/Me2CO (8:1) to give compound 9 (38 mg). Fraction E4 (1.3 g) was divided into four fractions (E4.1–E4.4) by RP-18 CC (aqueous MeOH, 50–90%) and then silica gel CC eluted with petroleum ether/Me2CO (5:1) to give compound 5 (2.5 mg).

The EtOAc extract (RG-2, 625 g) was applied to silica gel CC and eluted with increasing amounts (2–100%) of MeOH in CHCl3 to furnish six fractions (A–F). Fraction B (63 g) was divided into five portions (B1–B5) by MCI gel CHP 20P eluted with gradient aqueous MeOH (30–90%). Fraction B2 (7 g) was subjected to Sephadex LH-20 CC (MeOH) and followed by RP-18 CC (aqueous MeOH, 40–50%) to provide six portions (B2.1–B2.6). Fraction B3 (130 mg) was further purified using Sephadex LH-20 CC (MeOH) and then chromatographed over silica gel eluted with petroleum ether/Me2CO (10:1) to give compound 19 (12.8 mg). Fraction C (97.8 g) was divided into five portions (C1–C5) by MCI gel CHP 20P CC eluted with gradient aqueous MeOH (30–100%). Fraction C2 (8 g) was chromatographed over Sephadex LH-20 CC (MeOH) and followed by RP-18 CC (MeOH/H2O, 30–50%) to provide six fractions (C2.1–C2.6). Fraction C2.3 (1.3 g) was chromatographed over silica gel CC eluted with petroleum ether/Me2CO (8:1), and then purified by Sephadex LH-20 CC (MeOH) and preparative TLC eluting with (CHCl3/Me2CO, 4:1) to give compounds 16 (24.5 mg), 17 (10 mg), and 18 (27.5). Fraction C2.5 (0.5 g) was chromatographed over RP-18 CC (aqueous MeOH, 50–60%) and silica gel CC eluted with petroleum ether/Me2CO (12:1) to give compounds 3 (4.3 mg), 8 (2.5 mg), and 14 (11.2 mg). Fraction C2.6 (1.1 g) was chromatographed over silica gel CC eluted with petroleum ether/Me2CO (8:1) and RP-18 CC (aqueous MeOH, 50–60%) to give compounds 12 (12 mg), 13 (6.8 mg), and a mixture, which was further purified by using successive semipreparative HPLC (aqueous MeOH, 75%) to yield compounds 2 (3.1 mg), 6 (1.3 mg), and 7 (1.8 mg). Fraction C3 (7 g) was divided into nine portions (C3.1–C3.9) by RP-18 column (aqueous MeOH, 35–60%) and silica gel CC eluted with CHCl3/Me2CO (12:1), which was further purified by successive semipreparative HPLC (acetonitrile/H2O, 50%) to yield compounds 11 (1.9 mg), 4 (1.5 mg), and 1 (2.0 mg). Fraction D (18 g) was divided into five fractions (D1–D5) by MCI gel CHP 20P CC eluted with gradient aqueous MeOH (20–80%). Fraction D2 (1.1 g) was chromatographed over Sephadex LH-20 CC (MeOH) and followed by RP-18 CC (aqueous MeOH, 30–50%), and then purified by silica gel CC eluted with petroleum ether/’PrOH (8:1) to yield compound 15 (8.4 mg).

The n-BuOH extract (RG-3, 1 kg) was divided into nine portions (A–I) by MCI gel CHP 20P CC eluted with gradient aqueous MeOH (5–50%). Fraction F (35 g) was chromatographed over Sephadex LH-20 CC (MeOH) and followed by RP-18 CC (aqueous MeOH, 15–30%) to provide six fractions (F1–F6). Fraction F3 (4 g) was subjected to Sephadex LH-20 CC (MeOH) and silica gel CC eluted
with CHCl₃/MeOH (12:1) to give compounds 10 (1.0 mg) and 20 (8.5 mg).

2.3.1. Cinnamoid A (1)
White solid. [α]D23 = -4.3 (c 0.10, MeOH); IR (KBr) νmax: 3424, 2959, 2922, 2850, 1629, 1460, 1349, 1063, 1038 cm⁻¹; ¹H and ¹³C NMR data see Table 1; El-MS: m/z 238 [M]+; HREI-MS: m/z 238.1921 [M]+ (calcd for C₁₅H₂₂O₂, 238.1795).

2.3.2. Cinnamoid B (2)
White solid. [α]D23 = -379 (c 0.38, MeOH); UV (MeOH) λmax (log ε): 204 (2.46); IR (KBr) νmax: 3442, 2959, 2925, 2855, 1630, 1453, 1384, 1031 cm⁻¹; ¹H and ¹³C NMR data see Table 1; El-MS: m/z 252 [M]+; HREI-MS: m/z 252.1711 [M]+ (calcd for C₁₅H₂₄O₃).

2.3.3. Cinnamoid C (3)
Colourless oil. [α]D23 = -54.9 (c 0.26, MeOH); UV (MeOH) λmax (log ε): 205 (2.41); IR (KBr) νmax: 3432, 2955, 2923, 2850, 1632, 1454, 1333, 1060 cm⁻¹; ¹H and ¹³C NMR data see Table 1; El-MS: m/z 252 [M]+; HREI-MS: m/z 252.1719 [M]+ (calcd for C₁₅H₂₄O₃).

2.3.4. Cinnamoid D (4)
White solid. [α]D23 = +11.2 (c 0.05, MeOH); UV (MeOH) λmax (log ε): 206 (3.04); IR (KBr) νmax: 3424, 2948, 2925, 2845, 1641, 1452, 1384, 1278, 1062 cm⁻¹; ¹H and ¹³C NMR data see Table 2; El-MS: m/z 236 [M]+; HREI-MS: m/z 236.1795 [M]+ (calcd for C₁₅H₂₂O₂).

2.3.5. Cinnamoid E (5)
White solid. [α]D23 = -219 (c 0.20, MeOH); UV (MeOH) λmax (log ε): 257 (2.94), 210 (2.91); IR (KBr) νmax: 3421, 2967, 2937, 2870, 1666, 1620, 1462, 1440, 1376, 1171, 1128 cm⁻¹; ¹H and ¹³C NMR data see Table 2; El-MS: m/z 234 [M]+; HREI-MS: m/z 234.1605 [M]+ (calcd for C₁₅H₂₂O₂).

2.4. Biological activity

2.4.1. Inhibition of fibronectin, MCP-1, and interleukin-6 secretion
Rat mesangial cells (HBZY-1, purchased from Life-Science Academy of Wuhan University, PR China) were cultured in DMEM (Gibco, catalogue number C11885500BT), supplemented with 10% foetal bovine serum (FBS, Invitrogen). Rat mesangial cells were grown in DMEM containing 5.6 mM D-glucose (normal glucose, NG), supplemented with 20% bovine serum (FBS, Invitrogen). After the mesangial cells reached 80% confluence, their growth was arrested
in 0.5% FCS for 24 h. Exposure of the rat mesangial cells to medium containing high-concentration glucose induced the overproduction of interleukin-6, fibronectin, as described in the previous reports (Min et al., 2009; Xia et al., 2006). To determine whether the compound inhibited the fibronectin, MCP-1, and interleukin-6 overproduction triggered by high glucose in a dose-dependent manner, the mesangial cells were pretreated with different concentrations of compounds for 1 h and then exposed to 30 mM (high glucose, HG) D-glucose for 24 h. The levels of supernatant fibronectin, MCP-1, and interleukin-6 were measured with a solid-phase quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit for rat fibronectin (Assaypro). Similar protocols were used to rat interleukin-6 (Dakewe), rat MCP-1 (Raybiotech).

### 2.4.2. MTT assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to measure cell viability. Briefly, cells were seeded at $10^4$ cells/well in 96-well plates. Before experiments, the medium was removed and replaced with serum-free medium for 24 h incubation. Cells were incubated in the presence or absence of compounds (10 μM and 50 μM) for 48 h. Then 50 μl of MTT (5 mg/ml) was added to each well and incubation continued at 37 °C for additional 4 h. The medium was then carefully removed, so as not to disturb the formazan crystals formed. Dimethyl sulphoxide (DMSO, 150 μl), which solubilized the formazan crystals, was added to each well and the absorbance of solubilized blue formazan read at wavelength of 570 nm using a microplate reader. The reduction in optical density was used as a measurement of cell proliferation, normalized to cells incubated in a control medium.

### 2.4.3. Statistical analysis

The differences were tested using ANOVA. All values were expressed as mean ± S.D., and statistical significance was defined as $P < 0.05$.

### 3. Results and discussion

#### 3.1. Structure elucidation of compounds

The known compounds were identified as (−)-15-hydroxy-T-muurolol (6) (Ding et al., 2009), 15-hydroxy-α-cadinol (7) (Kuo et al., 2002), (4α,10β)-4,10-dihydroxy cadin-1(6)-en-5-one (8) (Chyu et al., 2007), mustakone (9) (Nyasse et al., 1988), (35,5R,6S,7E)-3,5,6-trihydroxy-7-megastigmen-9-one (10) (Park et al., 2011), (2E,9E)-6,7-cis-dihydroxyhumulan-2,9-diene (11) (Namikawa et al., 1978), clovane-2β,9α-diol (12) (Heymann et al., 1994), caryolane-1,9β-diol (13) (Heymann et al., 1994), ent-4β,10α-dihydroxy aromadendrane (14) (Gilabert et al., 2011), cinnzeylanol (15) (Gonzalez-Coloma et al., 1996), cinnzeylanine (16) (Gonzalez-Coloma et al., 1996), cinnzeylanone (17) (Gonzalez-Coloma et al., 1996), anhydrocinnzeylanol (18) (Fraga et al., 2001), anhydrocinnzeylanine (19) (Isogai et al., 1977), and cinnacasside A (20) (Liao (Fig. 1). Chemical structures of compounds 1–23 isolated from the barks of *Cinnamomum cassia*.
et al., 2009), by comparison of their spectroscopic data to previously reported values. Compounds 21–23 were each directly identified as cinnamaldehyde (21), cinnamic acid (22), and cinnamyl alcohol (23) by NMR and MS experiments (Fig. 1).

Compound 1 had the molecular formula C_{15}H_{26}O_{2} deduced from its HREI-MS. The IR absorption at 3424 cm^{-1} is characteristic of the hydroxyl functional group. The 1H NMR spectrum (Table 1) of 1 showed the presence of an isopropyl (δ_{H} 0.80, d; δ_{C} 17.1, C-15) suggested the dihedral angles of H-6 and H-13, and it also indicated the isopropyl was linked to C-7. The molecular formula of compound 1 is characteristic of the isopropyl moiety in the molecule, and it also indicated the isopropyl was linked to C-7.

The HMBC correlations (Fig. 2) from H-9, H-3 and H-6, H-6/12-CH_{3} and 13-CH_{3} (Fig. 1) indicated that 15-CH_{3}, C-13 (δ_{C} 82.5), respectively, indicated by their chemical shifts in 13C NMR spectrum (Table 1) displayed 15 carbons resonances, including four methyl, two methylene, six methine, and two quaternary carbons, indicating a sesquiterpenoidal skeleton. The 1H–1H COSY spectrum (Fig. 2) revealed the proton spin systems of H-3/H-2/H-1/H-6/H-7/H-8/H-9/H-7/H-11/H-12, and H-11/H-13. The construction of the molecule, and it also indicated the isopropyl was linked to C-7. The HMBC correlations from 12-CH_{3} to C-7 (δ_{C} 21.2), and correlations from 13-CH_{3} to C-7, C-11, C-12 (δ_{C} 20.7) confirmed the presence of the isopropyl moiety in the molecule, and it also indicated the isopropyl was linked to C-7.

The two OH groups were deduced to locate at C-3 (δ_{C} 70.2) and C-5 (δ_{C} 82.5), respectively, indicated by their chemical shifts in 13C NMR spectrum, as well as the HMBC correlations from H-2 (δ_{H} 2.08, 1.16) to C-3, 15-CH_{3} to C-3 and C-5, 14-CH_{3} to C-5 (Fig. 2).

The relative configuration of 1 was assigned by ROESY spectrum (Fig. 2) and molecular modelling. The ROESY correlations of 15-CH_{3}/H-3 and H-6, H-6/12-CH_{3} and 13-CH_{3} (Fig. 1) indicated that 15-CH_{3}, H-3, H-6 are on the same side, possessing α-orientation, while H-7 was β-oriented. H-1 was assigned to be β-oriented from the 1H NMR spectrum. The small coupling constants of H-7 and H-6/H-1 suggested that the dihedral angles of H-6-C-6-C-7-H-7 and H-6-C-6-C-7-H-1 were both approached to 90°, corresponding to a cis-relationship of H-1/H-6. In the molecular modelling study, we noted that H-1 and H-6 are naturally at the same orientation once the rigid rings were formed. The α-orientation of 14-CH_{3} and β-orientation of C-5 were deduced from the ROESY correlations of 14-CH_{3}/H-1, H-7/H-5. The ROESY correlations of H-3/H-2/C-2, H-2/C-14 further confirmed the α-orientation of 14-CH_{3}. The structure of 1 was thus determined as shown, and named as cinnamoid A. Notably, compound 1 is a new sesquiterpenoid possessing an unprecedented carbon skeleton which is probably derived from the cadinane sesquiterpenoid. The possible biosynthesis pathway of 1 was carried out by the cleavage of C-5 and C-6, and then followed by the construction of C-4 with C-6 and C-5 with C-10.

Compound 2 was isolated as colourless oil, its molecular formula C_{15}H_{26}O_{2} was assigned from its HREI-MS. The IR absorption bands at 3442 and 1630 cm^{-1}, indicative of the presence of hydroxyl and carbonyl groups. The 1H NMR spectrum (Table 1) showed the signals accounting for one isopropyl (δ_{H} 0.80, d, J=6.9 Hz, 6H, H-12, H-13; δ_{C} 167.6, C-1H, H-11), two methyl (δ_{H} 1.04, d, J=6.8 Hz, 3H, H-14; δ_{C} 130.0, s, H-15) and two olefinic protons (δ_{H} 5.93, dd, J=10.0, 11.1 Hz, H-2; δ_{C} 5.99, d, J=10.0 Hz, H-3). The 13C NMR spectrum (Table 1) displayed 15 carbons resonances, including four methyl, two methylene, six methine (of which two were olefinic ones), and three quaternary carbons (one carbonyl and two oxygenated sp³ carbons). The 1H and 13C NMR spectroscopic data of compound 2 were similar with those of (4α,10β)-4,10-dihydroxycadin-1(6)-en-5-one (Chyu et al., 2007), a cadinane-type sesquiterpenoid, except for the double bond between C-1 and C-6 in (4α,10β)-4,10-dihydroxycadin-1(6)-en-5-one was moved to between C-2 and C-3, which was deduced from the chemical shift at δ_{C} 128.4 (CH, C-2) and δ_{C} 138.1 (CH, C-3) in the 13C NMR spectrum, in accordance with the signals of two olefinic protons revealed in the 1H NMR spectrum. This presumption was further confirmed by the HMBC correlations (Fig. 1) from 15-CH_{3} to C-3, C-4 (δ_{C} 70.6), and C-5 (δ_{C} 210.8), and the 1H–1H COSY correlation (Fig. 2) of H-2/H-3. The HMBC correlations (Fig. 1) from 15-CH_{3} to C-4 and C-5 also confirmed that the C-4 was an oxygenated quaternary carbon and the ketoene carbonyl was located at C-5. Besides, the HMBC correlations (Fig. 1) from 14-CH_{3} to C-1 (δ_{C} 77.4), C-9 (δ_{C} 32.1), and C-10 (δ_{C} 42.7) and correlations from H-2 and H-3 to C-10 indicated that C-10 was attached to a hydroxyl group (Fig. 2). The other correlations in the 1H–1H COSY spectrum (Fig. 2) further confirmed the above conclusion.

The relative configuration of 2 was assigned by ROESY spectrum (Fig. 2). The correlations of 1-OH (in acetone-d_{6})/H-6, H-10, and 15-CH_{3} suggested the β-orientation of 1-OH, H-6, 15-CH_{3}, and H-10, which was further confirmed by the correlation of H-6/H-10. The α-orientation of H-7 was established by the correlations of H-6/12-CH_{3}. Therefore, compound 2 was identified as (1β,4α,10αc)-1,4-dihydroxy cadin-2(3)-en-5-one, and named as cinnamoid B.

Compound 3 had the molecular formula C_{15}H_{24}O_{3} by analysis of its HREI-MS, the same as compound 2, indicating they are isomers. The 1H and 13C NMR spectroscopic data of 3 (Table 1) were highly similar to those of 2, except for the upfield shift of C-2 (Δδ_{C} –0.8 ppm), C-4 (Δδ_{C} –2.3 ppm), C-5 (Δδ_{C} –2.1 ppm), and 15-CH_{3} (Δδ_{C} –1.3 ppm), which might be arose from the different configurations of 4-OH in compound 3. In the ROESY spectrum, the correlations of H-10/H-6, 12-CH_{3} indicated the α-orientation of H-7. The β-orientation of 4-OH was assigned from the key ROESY correlation of H-7/15-CH_{3}. Thus, compound 3 was determined to be (1β,4β,10αc)-1,4-dihydroxycadin-2(3)-en-5-one, and named as cinnamoid C.

The molecular formula of compound 4 was determined as C_{15}H_{24}O_{3} from its HREI-MS. The 1H NMR spectrum (Table 2) displayed the presence of isopropyl (δ_{H} 0.83, d, J=6.7 Hz, 3H, H-12; δ_{C} 85.3, d, J=6.7 Hz, 3H, H-13; δ_{H} 1.48, m, 1H, H-11), a single methyl (δ_{H} 0.88, s, H-15), and two terminal olefinic protons (δ_{H} 5.07, d, J=2.0 Hz, 4.82, d, J=2.0 Hz, H-14). The 13C NMR spectrum (Table 2) showed 15 carbons signals attributed to three methyl, three methylene, seven
methine (two oxygenated ones), and two quaternary carbons. The
\textsuperscript{1}H-\textsuperscript{1}H COSY spectrum (Fig. 1) exhibited three spin systems consisting
HMBC correlations (Fig. 1) from H-14 to C-3 (δ\textsubscript{C} 75.6), C-4 (δ\textsubscript{C} 154.4),
C-5 (δ\textsubscript{C} 60.6) confirmed the double bond was located between C-4 and
C-14, and correlations from 15-CH\textsubscript{3} to C-1 (δ\textsubscript{C} 45.3), C-5, C-9 (δ\textsubscript{C} 37.8),
and C-10 (δ\textsubscript{C} 41.7) suggested that 15-CH\textsubscript{3} was connected to C-10. The
two OH groups were established to attach to C-2 and C-3, respectively,
by the chemical shifts of C-2 (δ\textsubscript{C} 80.3) and C-3, which was further
confirmed by the HMBC correlation (Fig. 1) from H-14 to C-3 and
\textsuperscript{1}H-\textsuperscript{1}H COSY correlations (Fig. 2) of H-1/H-2, H-2/H-3. Thus, the planar
structure of 4 was elucidated as shown, a rearranged cadinane-type
sesquiterpenoid. The ROESY correlations (Fig. 1) of 15-CH\textsubscript{3}/H-1, H-3
indicate that these protons are at the same side of the cyclohexane
ring. The ROESY correlation of H-2/H-6 indicated that these two
protons are spatially vicinal and are on the other side of the
cyclohexane ring. The large \textit{J}_{HH} W coupling (J = 6.0 Hz) is character-
istic of a cis-relationship of H-1 with H-5 at the cyclobutane ring

(Ghosal and Vishwakarma, 1997). H-6 behaving as a singlet indicated
that the dihedral angle of H-6-C-6-C-1-H-1, H-6-C-6-C-5-H-5, and
H-6-C-6-C-7-H-7 were all approached to 90°, the cis-relationship
of H-7 with H-5 relative to the cyclohexane ring was thus assigned. As
a result, the structure of 4 was determined as shown, and named as
cinnamoid D.

The molecular formula of 5 was determined to be C\textsubscript{15}H\textsubscript{22}O\textsubscript{2},
from its HREI-MS. The NMR data of 5 (Table 2) are in accordance
with those of aglycone of tinocordiside, which has been obtained
by hydrolysis of glycoside (Ghosal and Vishwakarma, 1997),
suggesting that 5 is a new natural product. Similar to 4, the typical
\textit{W} coupling (J\textsubscript{HH} = 6.6 Hz) between H-1 and H-5, and the typical
singlet of H-6 indicated the relative configuration of 5 is same as
that of aglycone of tinocordiside. The structure of 5 was thus
determined as shown, and named as cinnamoid E.
3.2. Anti-diabetic nephropathy activity

C. cassia bark extract has been found to have anti-diabetic effect (Wu and Jia, 2012). The anti-diabetic nephropathy activity of the fractions from C. cassia bark was evaluated by inhibition on the expression of fibronectin, MCP-1 and interleukin-6 in high-glucose-stimulated mesangial cells. At the concentration of 50 μg/ml, RG-1, RG-2 and RG-3 could significantly inhibit the expression of fibronectin, MCP-1 and interleukin-6, respectively. In contrast, the inhibitory effect of RG-4 was unmeasurable (Fig. 3). The activity of the isolated compounds 1–23 were also detected, among them, compounds 5, 14, and 20 markedly decreased the expression of fibronectin, MCP-1 and interleukin-6 at the concentration of 50 μM in the high-glucose-stimulated mesangial cells (Fig. 4). In addition, cell viability assay was carried out, and the result showed that all the samples exhibited no toxic effects in mesangial cells at 50 μg/ml for the extracts, and 10 μM or 50 μM for the compounds (data not shown).

C. cassia as a frequently consumed spice and traditional medicine has attracted great attention in the past. Water-soluble polyphenols have been considered to have anti-diabetic activity (Anderson et al., 2004; Chen et al., 2012). This study revealed that multiple substances including nonpolar sesquiterpenoids in the spice showed reno-protective effects by inhibiting the expression of fibronectin, MCP-1 and interleukin-6 in high-glucose-induced mesangial cells, which provided partial evidences for the clinic practices of Rou-Gui in diabetic nephropathy prevention and treatment. Further work is needed to promote the reasonable usage and development of this spice as drugs or foods for diabetic nephropathy and related kidney diseases.

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References


