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Xanthine oxidase inhibitory activity of constituents of *Cinnamomum cassia* twigs

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ABSTRACT

A methanol extract of the twigs of *Cinnamomum cassia* was found to inhibit xanthine oxidase. Purification of the methanol extract afforded three new phenolic glycosides, cinnacasolide A–C (**11–13**), together with 10 known compounds (**1–10**). The structures of the three new compounds were determined by interpretation of spectroscopic data. Cinnamaldehyde derivatives **1**–**5** and **7** were significant inhibitors of xanthine oxidase, with IC_{50} values ranging from 7.8 to 36.3 μg/mL. The results indicate that the acyl group of these cinnamaldehyde derivatives plays an important role in the inhibition of xanthine oxidase.

Ramulus cinnamomi, the dried young stem of *Cinnamomum cassia* (Lauraceae), is distributed in Vietnam, Myanmar, Laos and the southern part of mainland China. Ramulus cinnamomi has been used as traditional Chinese medicines for treating dyspepsia, gastritis, blood circulation disturbances, and inflammatory diseases.[1] Previous investigations on the bark and the twig of *C. cassia* showed the presence of a variety of monoterpenoids, sesquiterpenoids, diterpenoids, sterols, cinnamaldehyde and its analogues, and flavan-3-ols and their oligomers as well as coumarins, and polyphenols.[2,3] Pharmacological investigations showed that the crude extract or compounds isolated from this species possessed a wide variety of activities including anti-allergy, insecticidal, antimicrobial, antiulcer, anti-inflammatory, vasodilatory, immune-suppressive, and neuronal death prevention, tyrosinase inhibition and anticancer, antioxidant and free radical scavenging, as well as antidiabetic and aldose reductase inhibition activities.[4–9] In the course of our program to screening for xanthine oxidase inhibitors from medicinal plants, it was found that a methanol extract of the twigs of *C. cassia* exhibited a strong inhibitory activity (>85% inhibition at 100 μg/mL).

Xanthine oxidase (XOD), which catalyzes the oxidation of xanthine and hypoxanthine into uric acid, plays a vital role in disorders such as hyperuricemia and gout. Among the many known XOD inhibitors, allopurinol, oxypurinol, and febuxostat have been used widely for the treatment of hyperuricemia and gout. The inhibition of XOD reduces both vascular oxidative stress and circulating levels of uric acid. In addition, the superoxide anion radicals generated by XOD are involved in various pathological processes such as hepatitis, inflammation, aging, carcinogenesis, and ischemia-reperfusion. Thus, XOD inhibitors may be useful for treatment of many other diseases.[10,11] The present study describes the isolation and characterization of thirteen phenolic compounds, including three new phenolic glucosides (**11–13**) and ten known compounds (**1–10**), from the MeOH extract of the twigs of *C. cassia* and their inhibitory activity against XOD.

The twigs of *C. cassia* was purchased from Yenbai Province, Vietnam in November 2010, and authenticate by Professor Pham Thanh Ky, Department of Pharmacognosy, Hanoi College of Pharmacy, Vietnam where the voucher specimens were deposited.

The MeOH extract of the twigs of *C. cassia* was partitioned into *n*-hexane-, EtOAc-, and BuOH-soluble fractions. Repeated column chromatography of those fractions resulted in the purification of compounds **1**–**13** (Fig. 1). The 10 known compounds were identified as cinnamaldehyde (**1**),[12] 2-methoxycinnamaldehyde (**2**),[13] 2-hydroxycinnamaldehyde (**3**),[14] cinnamic acid (**4**),[15] coniferaldehyde (**5**),[16] cinnamic alcohol (**6**),[17] *O*-coumaric acid (**7**),[18] dihydromelilotoside (**8**),[19] methyl dihydromelilotoside (**9**),[20] and rosavin (**10**).[21] Three new metabolites cinnacasolide A (**11**), cinnacasolide B (**12**), and cinnacasolide C (**13**) were determined by

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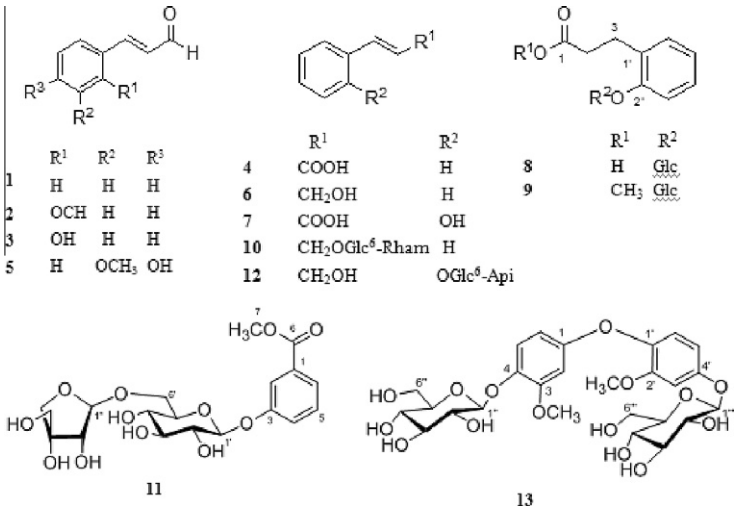

Figure 1. Structures of isolated compounds **1**–**13**.

spectroscopic data interpretation such as UV, IR, ^{1}H NMR, ^{13}C NMR, HMQC, HMBC, COSY, and ROESY, as well as high-resolution mass spectrometry (HREIMS).

Compound **11** was obtained as a colorless powder, with $[\alpha]_D^{25}$ +27.5. Its infrared (IR) absorbance spectrum showed a band at 1675 cm^{-1} due to a carboxyl group and strong absorption bands at 3445 and 1123 cm^{-1}, which suggest a glycosidic structure. The molecular formula was established as $C_{19}H_{26}O_{12}$ from a molecular ion peak at *m/z* 469.1326 $[M+Na]^+$ in the HREIMS spectrum (cacld for $C_{19}H_{26}O_{12}Na$, 469.1322). ^{1}H and ^{13}C NMR spectra (Table 1) of **11** indicated the presence of a β-D-glucopyranosyl moiety, a β-D-apiofuranosyl moiety, and methyl 3-hydroxybenzoate resonances. The downfield shift of C-6′ (δ_C 68.9) indicated a linkage to C-1″ of an apiofuranosyl moiety. Analysis of the HBMC spectrum of **11** showed long-range correlations between H-1′ and C-3 and between H-1″ and C-6′ (Fig. 2), indicative of connections between the β-D-glucopyranosyl group and the 3-hydroxy benzoyl at C-3 and between the β-D-apiofuranosyl group and the β-D-glucopyranosyl group at C-6′. Consequently, compound **11** was methyl 3-hydoxybenzoyl-3-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside and was named cinnacasolide A.

Compound **12** was purified as an amorphous solid. The molecular formula, $C_{20}H_{28}O_{11}$, was determined from a molecular ion peak at *m/z* 443.1645 $[M]^-$ in the HREIMS spectrum (cacld for $C_{20}H_{28}O_{11}$, 443.16348). The IR spectrum showed strong bands at 3885 and 1112 cm^{-1}, suggestive of a glycosidic structure. Combined analyses of the ^{1}H, ^{13}C, DEPT and HMQC NMR spectra of **12** revealed the presence of an *o*-hydroxycinnamic alcohol skeleton. The data further contained an oxygenated methylene signal, two *trans*-olefinic resonances, and *o*-hydroxyphenyl signals. The ^{1}H and ^{13}C NMR data (Table 1) also suggested the presence of two sugar moieties, a β-D-glucopyranosyl unit and a β-D-apiofuranosyl unit, similar to those in **11**. The HMBC spectrum revealed key correlations between the aromeric proton H-1′ and C-2 and

Table 1
NMR spectroscopic data for **11**–**13** (in MeOD)

No.	11		12		No.	13	
	δ_H (*J* in Hz)	δ_C, mult	δ_H (*J* in Hz)	δ_C, mult		δ_H (*J* in Hz)	δ_C, mult
1	—	122.4	—	128.4	1	—	152.2
2	7.39 d (2.4)	119.2	—	156.2	2	6.46 d (2.7)	102.1
3	—	154.7	7.18 t (8.0)	117.3	3	—	155.1
4	7.57 dt (1.6, 8.0)	123.8	7.23 dt (1.6, 8.0)	130.5	4	—	141.2
5	7.13 t (8.0)	135.4	6.99 t (7.2)	123.7	5	7.00 d (8.7)	120.7
6	7.76 dd (1.6, 8.0)	132.2	7.51 dd (1.2, 7.2)	127.4	6	6.30 dd (2.7, 8.7)	107.8
7	—	168.4	7.07 d (16.0)	126.5	7-OCH_3	3.81 s	56.6
8	3.89 s	53.0	6.34 td (5.6, 16.0)	130.5	1′	—	143.1
9	—	—	4.24 dd (1.6, 5.6)	64.2	2′	—	149.5
1′	4.86 d (7.2)	104.0	4.86 d (7.2)	102.9	3′	6.81 d (2.7)	103.9
2′	3.48 m	75.1	3.48 m	75.1	4′	—	152.9
3′	3.56 m	77.7	3.56 m	77.1	5′	6.58 dd (2.7, 8.7)	110.8
4′	3.31 m	71.5	3.31 m	71.7	6′	6.69 d (8.7)	116.2
5′	3.45 m	78.1	3.45 m	78.2	7″-OCH_3	3.82 s	56.7
6′	4.01 d (10.0); 3.60 m	68.9	4.01 d (10.0); 3.60 m	68.9	1″	4.70 d (7.5)	104.6
1″	5.00 d (2.4)	111.1	4.89 d (2.4)	111.1	2″	3.44 m	75.2
2″	3.91 d (2.4)	77.3	3.91 d (2.4)	78.2	3″	3.56 m	78.2
3″	—	80.6	—	80.7	4″	3.31 m	71.6
4″	3.96 d (9.6); 3.74 d (9.6)	75.1	3.96 d (9.6); 3.74 d (9.6)	75.1	5″	3.45 m	78.4
5″	3.57 s	65.5	3.57 s	65.7	6″	3.90 d (10.5); 3.66 m	62.8
					1‴	4.74 d (7.5)	104.0
					2‴	3.45 m	75.1
					3‴	3.56 m	78.0
					4‴	3.31 m	71.8
					5‴	3.45 m	78.3
					6‴	3.68 d (10.5); 3.65 m	62.7

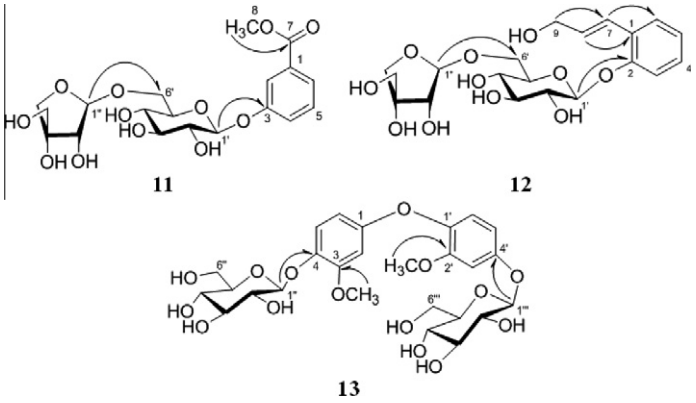

Figure 2. Selected HMBC correlations of **11**–**13**.

between H-1″ and C-6′. These indicated linkages between β-D-apiofuranosyl unit and C-6′ and between the β-D-glucopyranosyl group and C-2 (Fig. 2). Accordingly, **12** was elucidated as 2-hydroxy cinnamic alcohol 2-*O*-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside and was named cinnacasolide B.

Compound **13** was obtained as a colorless powder with $[\alpha]_D^{25}$ 36.5. The molecular formula $C_{26}H_{34}O_{15}$ was established from an HREIMS ion peak at *m/z* 609.1869 $[M+Na]^+$ (cacld for $C_{19}H_{26}O_{12}Na$, 609.1862). The IR spectrum contained absorption peaks at 3354 and 1087 cm^{-1}, indicative of a glycosidic structure. The ^1H NMR spectrum of **13** contained peaks corresponding to two individual β-glucopyranosyl groups with aromeric protons at δ_H 4.70 (H-1′) and 4.74 (H-1″) and signals corresponding to a 1,3,4-trisubstituted benzene ring at δ_H 6.46 (d, *J* = 2.7 Hz, H-2), 7.00 (d, *J* = 8.7 Hz, H-5), and 6.30 (dd, *J* = 2.7, 8.7 Hz, H-6). Also evident were a 1,2,4-trisubtituted benzene ring at δ_H 6.81 (d, *J* = 2.7 Hz, H-3′), 6.58 (dd, *J* = 2.7, 8.7 Hz, H-5′), 6.69 (d, *J* = 8.7 Hz, H-6′) and two resonances corresponding to methoxy groups at δ_H 3,81 and 3.82 for 7-OCH_3 and 7′-OCH_3, respectively. The ^{13}C NMR spectrum of **13** indicated twelve aromatic carbon atoms (6 CH and 6 C), two methoxy carbon atoms, and two β-glucopyranose units. Two de-shielded ^{13}C signals at δ_C 152.2 (C-1) and 143.1 (C-1′) in benzyl rings suggested the presence of a C–*O*–C moiety. NMR data (Table 1) indicated that **13** is an arbutin dimer derivative with a structure very similar to that of pyrocallianthaside A.[22,23] HMQC, COSY, and HMBC spectra revealed the linkage positions of the methoxy and glucose groups to aromatic rings. Correlations were observed between 7-OCH_3 and C-3, and between 7′-OCH_3 and C-2′, indicating bonds between two OCH_3 groups and C-3 and C-2′, respectively. The $^3J_{C,H}$ correlations between H-1″ and C-4 and between H-1‴ and C-4′ support the respective linkage of the two *β*-D-glucopyranosyl units to C-4 and C-4′ (Fig. 2). Accordingly, compound **13** was identified as 1-*O*,1′-didehydrobis[3,2′-dimethoxy phenyl β-glucopyranoside] and was named cinnacasolide C.

All of the isolated compounds **1**–**13**,[24] together with allopurinol as a positive control,[11] were examined for their inhibitory effects on XOD activity in an in vitro assay.[25] Table 2 shows the percent inhibitory activity of each compound at a dose of 50 μg/mL. Compounds **1**–**4**, obtained from the hexane-soluble fraction of the extract, exhibited strong potential activity with percentages of inhibition greater than 90%. Compounds **5**–**7** were purified from the EtOAc-soluble fraction and also displayed significant inhibition of XOD with percent inhibition values of 72.5 for **5**, 47.6 for **6**, and 73.2 for **7**. Conversely, the phenolic glycosides **8**–**13**, which were isolated from the BuOH-soluble fraction, yielded weak or negligible inhibitory activity against XOD. The isolated compounds **1**–**13** showed significant differences with regard to biological activity and chemical structure. Cinnamaldehyde derivatives **1**–**3** and **5** were strong inhibitors of XOD with IC_{50} values ranging from 7.8 to 36.3 μg/mL. The cinnamic acids **4** and **7** showed remarkable degrees of inhibition, with IC_{50} values of 26.4 and 32.2 μg/mL, respectively. Other compounds, including the six phenolic glucosides **8**–**13** and cinnamic alcohol (**6**) showed only weak inhibitory effects, with percent inhibition values from 18.7% to 47.6% at a dose of 50 μg/mL. These data suggest that an acyl group is an essential structural component that helps determine the XOD inhibitory activity of a given compound. An aldehyde group played an important role in the inhibition of XOD, as indicated by a comparison of cinnamic aldehyde (**1**) (IC_{50} 7.8 μg/mL), cinnamic acid (**7**) (IC_{50} 26.4 μg/mL), and cinnamic alcohol (**10**) (IC_{50} >50 μg/mL). These data indicate that cinnamaldehyde derivatives, isolated from twigs of *C. cassia*, can significantly inhibit the activity of xanthine oxidase.

Table 2
Inhibition effects of isolated compounds **1**–**13** against XOD

Compounds	Xanthine oxidase[a]	
	% Inhibition at (50 μg/mL)	IC_{50} (μg/mL)
Cinnamaldehyde (**1**)	96.7 ± 2.3	7.8 ± 1.1
2-Methoxycinnamaldehyde (**2**)	95.8 ± 1.6	13.8 ± 1.5
2-Hydroxycinnamaldehye (**3**)	94.2 ± 2.1	14.6 ± 2.0
Cinnamic acid (**4**)	93.4 ± 2.3	26.4 ± 1.2
Coniferaldehyde (**5**)	72.5 ± 2.5	36.3 ± 1.9
Cinnamic alcohol (**6**)	47.6 ± 3.4	>50
o-Coumaric acid (**7**)	73.2 ± 1.6	32.2 ± 2.1
Dihydromelilotoside (**8**)	45.4 ± 2.3	>50
Methyldihydromelilotoside (**9**)	22.4 ± 2.0	>50
Rosavin (**10**)	18.7 ± 2.1	>50
Cinnacasolide A (**11**)	22.2 ± 2.0	>50
Cinnacasolide B (**12**)	35.4 ± 1.7	>50
Cinnacasolide C (**13**)	28.2 ± 2.1	>50
Allopurinol[b]	N.D	0.53 ± 0.07

[a] Values present mean ± S.D. of triplicate experiments.
[b] Positive control. N.D.: Not determined.

In summary, three new phenolic glycosides, cinnacasolide A (**11**), cinnacasolide B (**12**), and cinnacasolide C (**13**), and 10 known compounds (**1** – **10**), were isolated from the twigs of *C. cassia* and evaluated for their inhibition of xanthine oxidase. Cinnamaldehyde derivatives **1**–**5** and **7** were the most potent inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.051.

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24. The sliced twigs of *C. cassia* (20 kg) were extracted with hot MeOH (3 × 40 L), and then the MeOH extract was concentrated. The residue (1260 g) was suspended in H_2O (4 L) and then fractionated successively with *n*-hexane, ethyl acetat (EtOAc), and *n*-butanol (BuOH), producing a hexane-soluble (310 g), EtOAc-soluble (175 g), and *n*-BuOH-soluble (85 g) fractions, respectively. The hexane-soluble fraction (310 g) was subjected to silica gel column chromatography, eluted with hexane and EtOAc gradient mixtures (100:1 → 0:100) to give seven fractions H1−H7. Fraction H2 was chromatographed on silica gel, eluting with hexane–EtOAc (25:1), as well as ODS silica gel with MeOH−H_2O (55:45), to afford **1** (3.0 g), and **2** (1.0 g). Fraction H6 was subjected to silica gel column chromatography, eluting with hexane−EtOAc (10:1), and then purification by medium-pressure liquid chromatography (MPLC) (YMC-ODS 15 × 300 mm, 5 μm), eluted with MeOH−H_2O (60:40), to give **3** (1.2 g). Compound **4** (2.0 g) was isolated from fraction H7 using YMC-ODS column chromatography, with MeOH−H_2O (60:40) as eluent and and then purified with MPLC (YMC-ODS 15 × 300 mm, 5 μm) and MeOH−H_2O (50:50) as mobile phase. The EtOAc-soluble fraction (175 g) was chromatographed on a silica gel column with a stepwise gradient of $CHCl_3$ and MeOH (60:1 to 0:1), to separate eight fractions E1−E8. Subfraction E2 was chromatographed on a silica gel column with mixtures of hexane−EtOAc (10:1 to 2:1) as the eluting solvent systems, and preparative HPLC (YMC-ODS, 10 × 200 mm, 5 μm), MeOH−H_2O (40:60) as eluting mobile-phase, to afford **5** (10 mg). Subfraction E4 was supplied to silica gel column chromatography, using hexane–EtOAc (4:1 ∼2:1) as the eluting gradient systems, and then purification by MPLC (YMC-ODS, 15 × 300 mm, 5 μm), eluted with MeOH−H_2O (60:40) to give **6** (250 mg). Subfraction E5 was subjected to a silica gel column chromatography, eluted with hexane–acetone (2:1) to give **7** (36 mg) after purifying by YMC ODS column chromatography, eluting with MeOH–H_2O (50:50). The *n*-BuOH-soluble fraction (85 g) was suspended in H_2O and then applied to a Diaion HP-20 column with a H_2O and MeOH gradient eluent to give fractions B1−B8. Fraction B5 was subjected to passage over silica gel, using $CHCl_3$–MeOH−H_2O (10:1:0.1 to 1:1:0.1) for elution, to yield nine subfractions B5.1–B5.9. Sub-fraction B5.5 was applied to a Sephadex LH-20 column, eluted with MeOH−H_2O (1:5), and then YMC-ODS column chromatography with the mobile phase system, MeOH−H_2O (1:4), to give **12** (5 mg). Fraction B5.6 was chromatographed on a Sephadex LH-20 column with MeOH−H_2O (1:5) as solvent, and then purified by reversed-phase HPLC (YMC-ODS, 10 × 250 mm, 5 μm), with MeOH−H_2O (1:5) as mobile phase, and yielded **8** (15 mg), **9** (10 mg), **10** (42 mg). Fraction B7 was chromatographed on a silica gel column, using $CHCl_3$–MeOH−H_2O (10:1:0.1 to 3:1:0.1) to give five subfractions B7.1–B7.5. Subfraction B7.3 was subjected to passage over LH-20 column with MeOH−H_2O (1:5) as solvent, and then purified by reversed-phase HPLC (YMC-ODS, 10 × 250 mm, 5 μm), with MeOH−H_2O (1:4) as mobile phase to give **11** (7.5 mg), and **13** (2.9 mg).
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