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Cinnacassides A–E, five geranylphenylacetate glycosides from *Cinnamomum cassia*

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ABSTRACT

Cinnacassides A–E (**1**–**5**), five novel glycosides with a unique geranylphenylacetate carbon skeleton, were isolated from the stem bark of *Cinnamomum cassia*. Each of the cinnacassides A–D (**1**–**4**) possesses one of the four stereoisomers in the aglycone. Their structures were established by extensive spectroscopic analysis and chemical and chiroptical methods. A plausible biosynthetic route to **1**–**5** was also proposed.

1. Introduction

Cinnamomum cassia Presl (Lauraceae) is an economically important plant growing in South China. Its stem bark has been widely applied in the Traditional Chinese Medicine (TCM) to induce perspiration and to treat tussis, gastrointestinal neurosis, diarrhea, amenorrhea, dysmenorrhea, impotency, arthralgia, edema, and cardiac palpitation.[1] Pharmacological investigations showed that the crude extract or compounds isolated from this species possessed a wide variety of activities including insecticidal, antimicrobial, antiucler, anti-inflammatory, vasodilatory, immunesuppressive, and neuronal death prevention, tyrosinase inhibition and anticancer, antioxidant and free radical scavenging, as well as antidiabetic and aldose reductase inhibition activities.[2] Phytochemical studies of this plant showed the presence of monoterpenoids, sesquiterpenoids, diterpenoids, sterols, cinnamaldehyde and its analogues, and flavan-3-ols and their oligomers.[2] In our search for structurally diverse and biologically active molecules, five novel geranylphenylacetate glycosides, cinnacassides A–E (**1**–**5**), along with nine known ones were isolated from the stem bark of *C. cassia*. This paper describes the isolation and structural determination of these compounds.

2. Results and discussion

Separation of the EtOAc-soluble fraction of an EtOH extract of the stem barks of *C. cassia* through extensive column and planar chromatography furnished cinnacassides A–E (**1**–**5**) (Fig. 1) and nine known compounds. The known compounds were identified as anhydrocinnzeylanine,[3] anhydrocinnzeanol,[3] cinnzeylanine,[3] cinncassiol A,[3] cinnzeylanol,[3] (+)-syringaresinol,[4] (+)-pinoresinol,[5] 5,7,3′-tri-*O*-methyl-(−)-epicatechin,[6] and 5,3′-di-*O*-methyl-(−)-epicatechin[6] by comparison of their spectral data with those reported in the literature.

Cinnacasside A (**1**), a white amorphous powder, showed a molecular formula of $C_{25}H_{36}O_{11}$ with eight double bond equivalents as determined by the HRESIMS pseudo-molecular ion peak at *m*/*z* 535.2122 $[M+Na]^+$ (calcd for $C_{25}H_{36}O_{11}Na$, 535.2155). The IR absorption bands suggested the presence of hydroxyl (3408 cm^{-1}), carbonyl (1720 cm^{-1}), and aromatic (1597 and 1456 cm^{-1}) functionalities. Apart from the proton resonances for three tertiary methyls (δ 1.42, 1.19, 1.17, each 3H, s) and one methoxyl at δ 3.69 (3H, s), one disubstituted trans double bond (δ 7.09, d, J=16.2 Hz; 6.20, d, J=16.2 Hz) was easily identified in the ^{1}H NMR spectrum (Table 1, Fig. 3). The UV absorption maximum at 307 nm (log ε 3.51) suggested that the trans double bond was probably conjugated with the aromatic moiety. The NMR data of **1** (Tables 1 and 2) showed the presence of a β-pyranoglucose moiety, whose anomeric proton resonated at δ 4.56 (d, J=7.6 Hz). All the carbons in the molecular formula were resolved as 25 resonances in the ^{13}C NMR spectrum (Table 2).

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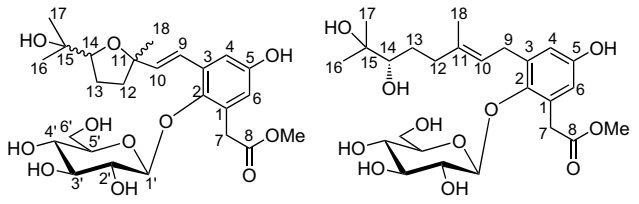


Figure 1. Structures of compounds **1**–**5**.

Furthermore, 2D NMR techniques were extensively used to assign the structure of **1**. The HMQC spectrum first allowed the assignment of all the protons to their bonding carbons. The ^{1}H–^{1}H COSY and HMBC spectra (Fig. 2a) were then applied to construct the planar structure of **1**. From the ^{1}H–^{1}H COSY spectrum, one hexose moiety (C-1′ to C-6′) and two subunits (C-9 to C-10 and C-12 to C-14) drawn with bold bonds were established. The key HMBC correlations enabled us to assemble the three proton bearing subunits with the quaternary carbons and other functionalities. The HMBC correlations of C-11 with H_3-18, H-10, and H-12 not only connected C-10 and C-12 to C-11 but also placed C-18 at C-11. The HMBC correlations from H_3-16 (or H_3-17) to C-14 and C-15 allowed the attachment of the hydroxyisopropanyl group to C-14. A geranyl carbon skeleton (from C-9 to C-18) was thus established. This geranyl moiety was located at C-3 by the HMBC correlations from H-9 to C-2, C-3, and C-4. The HMBC correlations from both H_2-7 and O*Me* to C-8 suggested the presence of a methoxycarbonylmethyl group, which was connected to C-1 by the HMBC correlations from H_2-7 to C-1, C-2, and C-6. The carbon signal at δ_C 155.5 correlating with H-4 and H-6 in the HMBC was assigned to C-5 bearing a hydroxyl. A *meta*-geranylphenylacetate carbon skeleton was thus established for the aglycone of **1**.

The above functionalities accounted for seven double bond equivalents, and the remaining one required the presence of an additional ring in **1**. Two downfield shifted oxygenated carbons C-11 at δ 85.2 and C-14 at δ 87.4 in the ^{13}C NMR spectrum indicated that a tetrahydrofuran ring was formed between C-11 and C-14 via an ether bond. The HMBC correlation between H-1′ and C-2 readily placed the sugar moiety at C-2. The planar structure of **1** was therefore constructed.

Table 2
^{13}C NMR data (δ) of cinnacassides A–E (**1**–**5**) in CD_3OD

Position	1	2	3	4	5
1	132.1	132.0	132.0	132.0	131.2
2	147.1	147.1	147.2	147.2	147.5
3	133.0	133.0	133.0	133.2	131.2
4	112.7	112.7	112.5	112.8	117.0
5	155.5	155.5	155.5	155.5	155.4
6	118.2	118.2	118.1	118.2	116.3
7	37.3	37.3	37.2	37.3	37.3
8	175.6	175.6	175.6	175.6	175.7
9	124.1	124.0	123.3	124.1	29.7
10	138.1	137.9	138.0	138.5	125.2
11	85.2	85.4	85.1	85.2	137.8
12	38.8	39.1	39.6	39.5	38.3
13	28.2	28.0	27.9	28.5	31.1
14	87.4	87.5	87.0	87.4	79.4
15	73.1	73.3	73.2	73.1	74.3
16	26.0	26.3	26.4	26.3	25.3
17	26.5	26.4	26.9	26.7	26.3
18	27.7	27.9	26.9	26.7	17.0
1′	106.9	106.9	107.0	106.9	106.9
2′	76.0	76.1	76.1	76.1	76.0
3′	78.5	78.5	78.1	78.5	78.4
4′	72.2	72.0	71.8	72.1	72.1
5′	78.6	78.6	78.5	78.6	78.6
6′	63.3	63.2	63.1	63.3	63.3
OMe	52.9	52.9	52.9	52.9	52.9

The ESIMS, UV, IR, ^{1}H and ^{13}C NMR (Tables 1 and 2) spectra of cinnacassides B–D (**2**–**4**) were almost identical to those of **1**, the minor differences being the chemical shifts of the protons around the tetrahydrofuran ring (see Fig. 3). These observations suggested that cinnacassides A–D (**1**–**4**) were possibly four stereoisomers with different configurations only occurring at the tetrahydrofuran ring. Analysis of the 1D NMR, HMQC, and HMBC spectra suggested that **1**–**4** shared the same planar structure, and each of them bore a β-pyranoglucose moiety at C-2. The ROESY correlations of H-14/H-9 and H_3-18/H_3-17(16) in **1** (Fig. 4a) and **2** (Fig. 4b) indicated that **1** and **2** had a *trans*-oxide, while the ROESY correlations of H_3-18/H-14 in **3** (Fig. 4c) and **4** (Fig. 4d) suggested that both **3** and **4** possessed a *cis*-oxide. Since the β-pyranoglucose in this series of

Table 1
^{1}H NMR data of cinnacassides A–E (**1**–**5**) in CD_3OD at 400 MHz

Position	δ_H (mult, J_{HH} in Hz)				
	1	2	3	4	5
4	6.86 (d, 2.8)	6.86 (d, 2.8)	6.88 (d, 2.9)	6.87 (d, 2.9)	6.55 (d, 2.9)
6	6.56 (d, 2.8)	6.56 (d, 2.8)	6.56 (d, 3.0)	6.57 (d, 2.9)	6.47 (d, 3.3)
7a	3.90 (d, 16.3)	3.89 (d, 16.8)	3.89 (d, 16.4)	3.90 (d, 16.2)	3.91 (d, 16.3)
7b	3.69 (d, 16.3)	3.70 (d, 16.8)	3.70 (d, 16.4)	3.70 (d, 16.2)	3.73 (d, 16.3)
9	7.09 (d, 16.2)	7.12 (d, 16.1)	7.20 (d, 16.2)	7.11 (d, 16.5)	3.48 (dd, 2H, 7.0, 5.8)
10	6.20 (d, 16.2)	6.18 (d, 16.1)	6.29 (d, 16.2)	6.29 (d, 16.5)	5.34 (t, 6.6)
12a/α	1.79 (ddd, 11.9, 8.5, 6.8)	1.79 (m)	1.88–1.98 (m)	1.88 (m)	2.30 (m)
12b/β	2.13 (ddd, 11.9, 7.8, 6.3)	2.06 (ddd, 11.8, 6.2, 5.4)	1.88–1.98 (m)	2.11 (m)	2.09 (m)
13a/α	1.89 (m)	1.87–1.95 (m)	1.88–1.98 (m)	1.91–2.04 (m)	1.79 (m)
13b/β	1.98 (m)	1.87–1.95 (m)	1.88–1.98 (m)	1.91–2.04 (m)	1.39 (m)
14	3.90 (dd, 7.3, 7.1)	4.03 (dd, 7.5, 6.9)	3.89 (m)	3.90 (dd, 7.4, 6.9)	3.25 (dd, 10.5, 1.5)
16	1.17 (s)	1.19 (s)	1.18 (s)	1.17 (s)	1.13 (s)
17	1.19 s	1.20 (s)	1.24 (s)	1.20 (s)	1.16 s
18	1.42 (s)	1.42 (s)	1.40 (s)	1.42 (s)	1.74 (s)
1′	4.56 (d, 7.6)	4.55 (d, 7.6)	4.53 (d, 7.6)	4.53 (d, 7.7)	4.57 (d, 7.4)
2′	3.45 (dd, 8.7, 7.6)	3.45 (m)	3.41 (dd, 8.8, 7.6)	3.45 (dd, 9.1, 7.7)	3.43 (dd, 7.9, 7.4)
3′	3.38 (dd, 8.8, 8.7)	3.38 (m)	3.37 (m)	3.37 (dd, 9.1, 8.6)	3.39 (dd, 8.7, 8.6)
4′	3.32 (dd, 8.8, 8.7)	3.34 (m)	3.35 (m)	3.32 (m)	3.34 (m)
5′	3.08 (ddd, 8.7, 5.8, 2.4)	3.07 (ddd, 9.5, 5.3, 2.4)	3.06 (ddd, 8.7,5.5, 2.3)	3.07 (ddd, 8.1, 4.7, 2.2)	3.13 (ddd, 9.0,5.5,2.4)
6′a	3.74 (dd, 12.0, 2.4)	3.73 (dd, 12.1, 2.4)	3.76 (dd, 11.9, 2.3)	3.73 (dd, 12.0, 2.2)	3.76 (dd, 12.0, 2.4)
6′b	3.60 (dd, 12.0, 5.8)	3.61 (dd, 12.1, 5.3)	3.61 (dd, 11.9, 5.5)	3.60 (dd, 12.5, 4.7)	3.63 (dd, 12.0, 5.5)
OMe	3.69	3.69	3.69	3.69	3.69

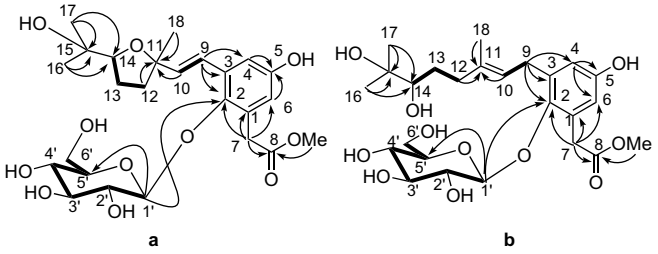


Figure 2. ^{1}H–^{1}H COSY (▬) and selected HMBC (H→C) correlations of cinnacassides A (**1**) (a) and E (**5**) (b).

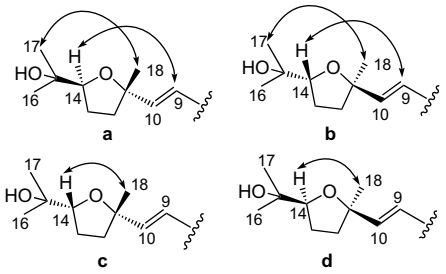


Figure 4. Key ROESY (↔) correlations of **1**–**4** (a–d).

compounds all possessed a D-configuration,[7] compounds **1** and **2** were therefore two diastereoisomers with enantiomeric aglycones, so were compounds **3** and **4**.

The CD spectra of **1**–**4** did not provide an unambiguous evidence for the absolute configurations, and lack of crystallinity of **1**–**4** limited the application of single crystal X-ray diffraction for this purpose. Attempt to obtain the aglycones of **1**–**4** by β-glucosidase hydrolysis failed probably due to the steric hindrance at C-2, while those by hydrochloric acid hydrolysis resulted in overall decomposition of the aglycones. By using Hudson's rules of isorotation,[8] the molecular rotations[9] of the aglycones (**1a**–**4a**) of compounds **1**–**4** were readily calculated ($[M]_D$: **1a**: 30.7; **2a**: −30.7; **3a**: −71.7; **4a**: 71.7) from the measured specific rotations of **1**–**4**.[10] The absolute configurations of **1a**–**4a** were thus assigned to be (11*S*,14*S*), (11*R*,14*R*), (11*S*,14*R*), and (11*R*,14*S*), respectively, by correlating their molecular rotations with those of the selected models, four linalyl oxides (**1m**–**4m**) (Fig. 5).[11] The structures of **1**–**4** were thus fully established as depicted.

The HRESIMS ion peak at *m*/*z* 537.2330 (calcd for $C_{25}H_{38}NaO_{11}$ $[M+Na]^+$, 537.2312) suggested that cinnacasside E (**5**) had two more hydrogen atoms than **1**–**4**. The ^{1}H NMR spectrum (Fig. 3) of **5** bore a resemblance to that of **1**, the major differences being the replacement of the two olefinic doublets in **1** by an upfield-shifted olefinic triplet (δ 5.34) in **5** and the downfield shifted Me-18 resonance (δ 1.74) in **5**. These observations suggested that a Δ^{10} or a Δ^{11} double bond was present in **5** with the tetrahydrofuran ring opened. The HMBC correlations of C-11 (δ 137.8) with H_3-18, H-10, and H-12 (Fig. 2b) were consistent with a Δ^{10} double bond, while the upfield-shifted carbon resonance of C-18 at δ 17.0 indicated that the double bond had an *E*-geometry.[12] 2D NMR (^{1}H–^{1}H COSY, HSQC, and HMBC) analysis further confirmed the structure of **5**. The calculated molecular rotation of the aglycone (**5a**) ($[M]_D$: −66.6)[10] suggested that the only asymmetric center (C-14) of **5a** had an *S*-configuration, different from that of the fungal metabolite colletochlorin (model compound, **5m**)[13] (Fig. 5). The structure of **5** was thus defined as depicted.

A plausible biosynthetic pathway to cinnacassides A–E (**1**–**5**) has been proposed in Scheme 1. Epoxidation of the $\Delta^{14,15}$ double bond of the geranylphenylacetate precursor **i** followed by hydrolysis of the newly formed 14,15-epoxides would generate **5** and **ii**. Subsequent epoxidation of the $\Delta^{10,11}$ double bond of **5** or **ii** followed by nucleophilic attack of OH-14 on the newly generated 10,11-epoxides would form the tetrahydrofuran rings. In the last step, dehydration would occur between the OH-10 and H-9 to give cinnacassides A–D (**1**–**4**), respectively.

In conclusion, the new compounds **1**–**5** are structurally related to a rare structural class of geranylhydroquinones isolated frequently from various marine[14] or fungal[13] organisms, but they are distinct from the marine or fungal metabolites by possessing a β-D-glucose and a methoxycarbonylmethyl moiety at the hydroquinone ring. To the best of our knowledge, this is the first report of a *meta*-geranylphenylacetate carbon skeleton. Besides, secondary metabolites of or related to this class in a plant origin have been very rarely found, and the isolation of these compounds from a well-known TCM plant is very interesting. Compounds **1**–**4** representing all the four stereoisomers of a 2,5-trisubstituted tetrahydrofuran ring in the aglycone with determined absolute configurations and complete spectroscopic data may be useful for both synthetic and natural product chemists.

The three major cinnacassides (**1**, **3**, and **5**) were evaluated for neuroprotective[15] and antimicrobial[16,17] activities, but none showed obvious effects.[18] Since the well-known TCM showed a wide range of biological activities, what role these peculiar structures play in the TCM remains to be determined.

3. Experimental

3.1. General experimental procedures

Melting points were measured with a SGW X-4 apparatus (Shanghai Precision & Scientific Instrument Co., Ltd.) and were uncorrected. Optical rotation was determined on a Perkin–Elmer

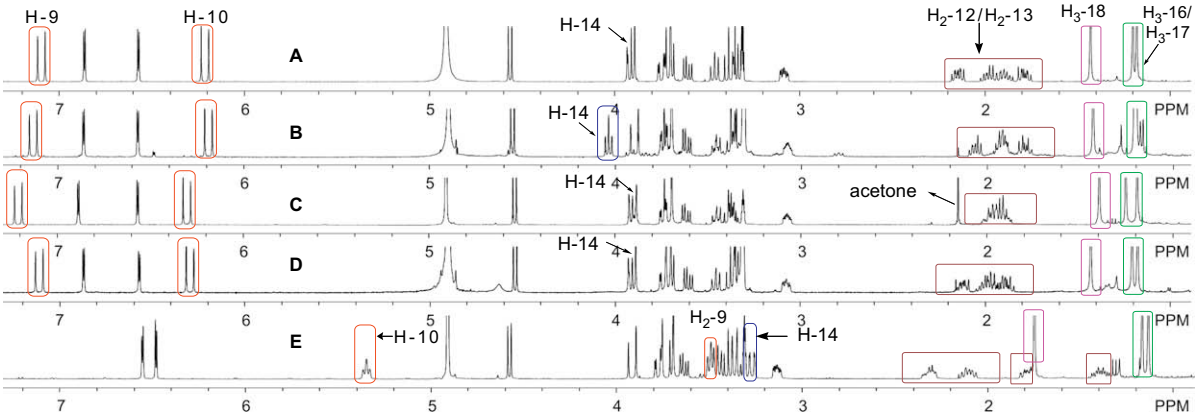


Figure 3. ^{1}H NMR spectra of **1**–**5** (A–E) in CD_3OD at 400 Hz.

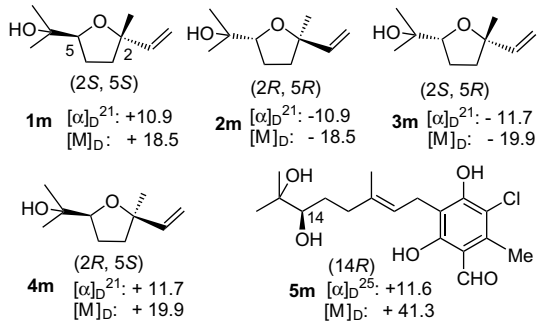
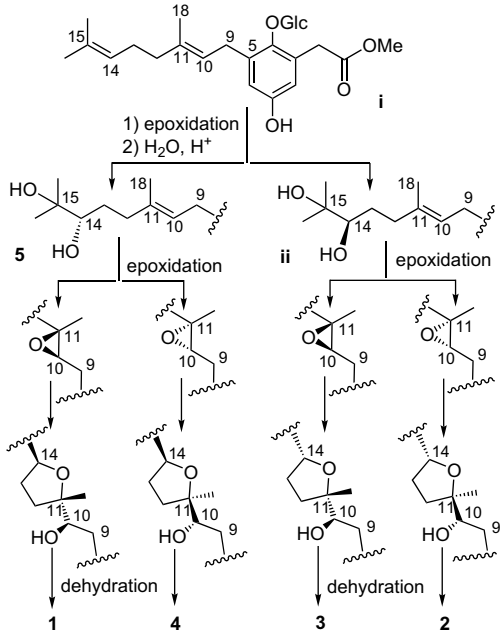


Figure 5. Specific rotations and calculated molecular rotations of five model compounds (**1m**–**5m**), and values for **1m** and **3m** were deduced from those of **2m** and **4m**, respectively.

341 polarimeter. UV was measured on Shimadzu UV-210A. UV and CD spectra were measured on a JASCO J-810 instrument. IR spectra were recorded on a Perkin–Elmer 577 spectrometer. NMR spectra were measured on a Bruker AM-400 spectrometer. ESIMS were carried out on an Esquire 3000 plus LC–MS instrument. All solvents used were of analytical grade (Shanghai Chemical Plant). Silica gel (200–300 mesh), silica gel H, DA-201 macroporous absorption resin, C18 reversed-phase silica gel (250 mesh, Merck), and MCI gel (CHP20P, 75–150 μ, Mitsubishi Chemical Industries Ltd.) were used for column chromatography. Pre-coated silica gel GF_{254} plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for TLC. YMC-Pack ODS-A column (250×10 mm) was used for preparative HPLC (monitor wavelength: 254 nm).

3.2. Plant material

The barks of *C. cassia* Presl were purchased from the Shanghai Pharmaceutical Corporation Limited and identified by Professor You-Kai Xu from the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (accession number: RGCC-02Y).



Scheme 1. Plausible biogenetic pathway to cinnacassides A–E (**1**–**5**).

3.3. Extraction and isolation

The air-dried powder of the stem barks of *C. cassia* (10 kg) was extracted three times with 95% EtOH under reflux. Evaporation of the solvent under reduced pressure provided the EtOH extract (1525 g). After removing the oil (petroleum ether/acetone, 15:1; v/v), MeOH was used as eluent to give a mixture (745 g), which was then extracted with EtOAc. Fractionation of the EtOAc-soluble fraction (335 g) with silica gel column chromatography (CC) (petroleum ether/acetone, 9:1→petroleum ether/isopropanol, 1:3; v/v) gave three major fractions, 1–3. Initial column chromatographic enrichment of fraction 2 by DA-201 macroporous absorption resin ($MeOH/H_2O$, 9:1, v/v) followed by fractionation by MCI column ($MeOH/H_2O$, 9:1, v/v) afforded three fractions, 2a–2c. Fraction 2b obtained from 50–60% MeOH in H_2O was further fractionated by CC (petroleum ether/EtOAc, 9:1→EtOAc→EtOAc/MeOH, 5:1) to give five fractions 2b1–2b5. Silica gel ($CHCl_3$/MeOH, 200:1→50:1) and reversed-phase C18 ($MeOH/H_2O$, 3:7→7:3, v/v) column chromatographic enrichment of fraction 2b2 (0.955 g) afforded a diterpenoid fraction (0.188 g), column chromatographic separation (Si gel, petroleum ether/EtOAc, 1:1→1:1.5) of which yielded cinnzeylanine (52 mg) and anhydrocinnzeanol (18 mg). Separation of fraction 2b3 (1.01 g) by reversed-phase C18 CC ($MeOH/H_2O$, 3:7→7:3, v/v) afforded fractions 2b3a (70 mg) and 2b3b (61 mg), purification of which by Sephadex LH-20 (MeOH) gave cinnzeylanol (5 mg) and anhydrocinnzeylanine (19 mg), respectively. Separation of fraction 2b4 (1.452 g) by silica gel CC ($CHCl_3$/MeOH, 200:1→50:1) yielded (+)-syringaresinol (781 mg), while that of fraction 2b5 (1.385 g) by reversed-phase C18 CC ($MeOH/H_2O$, 3:7→6:4, v/v) afforded fractions 2b5a–2b5b Column chromatographic separation of fraction 2b5a (0.205 g) (Sephadex LH-20, MeOH; silica gel, $CHCl_3$/MeOH, 12:1→8:1) gave cinncassiol A (15 mg) and **5** (15 mg). Fraction 2b5b (0.256 g) was subjected to silica gel CC ($CHCl_3$/MeOH, 10:1→9:1) to give fractions 2b5b1 and 2b5b2. Purification of 2b5b1 (54 mg) yielded **3** (22 mg), while separation of fraction 2b5b2 (57 mg) by PTLC ($CHCl_3$/MeOH 6:1) gave **1** (18 mg) and another fraction, purification of the latter by reversed-phase C18 CC ($MeOH/H_2O$, 4:6, v/v) gave **2** (1.8 mg) and **4** (3.8 mg). Silica gel column chromatographic separation of fraction 2c (5.152 g) ($CHCl_3$/MeOH, 50:1) gave 5,7,3′-tri-*O*-methyl-(−)-epicatechin (18 mg). Chromatographic enrichment by macroporous absorption resin column ($MeOH/H_2O$, 90:10, v/v) followed by separation by MCI ($MeOH/H_2O$, 5:5→9:1, v/v) and silica gel CC (petroleum ether/isopropanol, 10:1→3:1; v/v) of fraction 3 afforded (+)-pinoresinol (18 mg) and fraction 3b. Purification of the latter by silica gel CC (petroleum ether/EtOAc, 5:1→2:1; v/v) yielded 5,3′-di-*O*-methyl-(−)-epicatechin (46 mg).

3.3.1. Cinnacasside A (*1*)

White powder; $[\alpha]_D^{21}$ +19 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.24), 254 (4.02), 307 (3.51) nm; IR (KBr) ν_{max} 3408, 2974, 1720, 1597, 1456, 1331, 1203, 1072 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100 MHz), see Table 2; positive-ion ESIMS *m*/*z* 535.3 $[M+Na]^+$, 1047.3 $[2M+Na]^+$; negative-ion ESIMS *m*/*z* 557.3 $[M+HCOOH-H]^-$, 1023.9 $[2M-H]^-$; HRESIMS *m*/*z* 535.2122 (calcd for $C_{25}H_{36}O_{11}Na$ $[M+Na]^+$, 535.2155).

3.3.2. Cinnacasside B (*2*)

White powder; $[\alpha]_D^{21}$ +7 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.18), 255 (4.01), 306 (3.48) nm; IR (KBr) ν_{max} 3421, 2972, 2928,1722, 1599, 1456, 1383, 1331, 1203, 1072 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100 MHz), see Table 2; positive-ion ESIMS *m*/*z* 535.4 $[M+Na]^+$, 1047.3 $[2M+Na]^+$; negative-ion ESIMS *m*/*z* 557.2 $[M+HCOOH-H]^-$, 1023.3 $[2M-H]^-$.

*3.3.3. Cinnacasside C (**3**)*

White powder; $[\alpha]_D^{21}$ −4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.13), 253 (4.10), 307 (3.63) nm; IR (KBr) ν_{max} 3415, 2972, 1722, 1597, 1456, 1333, 1203, 1072 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100 MHz), see Table 2; positive-ion ESIMS *m/z* 535.3 $[M+Na]^+$, 1069.4 $[2M+2Na-H]^+$; negative-ion ESIMS *m/z* 511.2 $[M-H]^-$, 557.2 $[M+HCOOH-H]^-$, 1023.5 $[2M-H]^-$; HRESIMS *m/z* 535.2109 (calcd for $C_{25}H_{36}NaO_{11}$ $[M+Na]^+$, 535.2155).

*3.3.4. Cinnacasside D (**4**)*

White powder; $[\alpha]_D^{21}$ +24 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.15), 254 (4.05), 307 (3.58) nm; IR (KBr) ν_{max} 3425, 2972, 2926, 1722, 1599, 1458, 1383, 1333, 1203, 1074 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100 MHz), see Table 2; positive-ion ESIMS *m/z* 535.3 $[M+Na]^+$, 1047.3 $[2M+Na]^+$; negative-ion ESIMS *m/z* 557.6 $[M+HCOOH-H]^-$, 1023.7 $[2M-H]^-$.

*3.3.5. Cinnacasside E (**5**)*

White powder; $[\alpha]_D^{21}$ −3 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.32), 224 (3.91), 284 (3.39) nm; IR (KBr) ν_{max} 3425, 2924, 1720, 1618, 1462, 1203, 1072 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz), see Table 1; ^{13}C NMR (CD_3OD, 100 MHz), see Table 2; positive-ion ESIMS *m/z* 537.3 $[M+Na]^+$; HRESIMS *m/z* 537.2330 (calcd for $C_{25}H_{38}NaO_{11}$ $[M+Na]^+$, 537.2312).

3.4. General procedure for acid hydrolysis of the three major glycosides 1, 3, and 5

To a mixture of concentrated HCl (0.5 mL), H_2O (1.5 mL) and dioxane (3 mL), the glucoside (5 mg) was added and the mixture was refluxed for 2 h. After completion of the reaction (TLC for monitoring), H_2O was added to the reaction mixture, which was then extracted with $CHCl_3$ (3×5 mL). The aqueous layer was neutralized with $NaHCO_3$ and then concentrated to dryness under reduced pressure. The hydrolyzate was purified by Sephadex LH-20 and then determined to be D-glucose by direct comparison with an authentic sample (co-TLC and specific rotation) {TLC, silica gel, $CHCl_3$/MeOH/H_2O (1:1:0.1), R_f value: 0.47; $[\alpha]_D^{20}$ +34 (*c* 0.50, MeOH)}.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.11.041.

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9. Molecular rotation $[M]_D$=MW×$[\alpha]_D$/100, where MW is the molecular weight and $[\alpha]_D$ is the specific rotation.
10. $[M]_{D(\text{geranylphenylacetate glycoside})}=[M]_{D(\text{geranylphenylacetate})}+[M]_{D(\text{sugar})}$. Assuming that **1a** and **2a** (or **3a** and **4a**) were enantiomers, namely, $[M]_{D(\mathbf{1a})}=-[M]_{D(\mathbf{2a})}$; $[M]_{D(\mathbf{3a})}=-[M]_{D(\mathbf{4a})}$, the $[M]_D$ values of the four aglycones (**1a**–**4a**) and β-D-glucose could be caculated as follows:
$[M]_{D\ (\mathbf{1})}=[M]_{D(\mathbf{1a})}+[M]_{D(\text{sugar})}$ (1)
$[M]_{D\ (\mathbf{2})}=[M]_{D(\mathbf{2a})}+[M]_{D(\text{sugar})}$ (2)
Thus, $[M]_{D(\mathbf{1a})}=-[M]_{D(\mathbf{2a})}\approx+30.7$, and $[M]_{D(\text{sugar})}\approx+66.6$ (*)
Analogously,
$[M]_{D\ (\mathbf{3})}=[M]_{D(\mathbf{3a})}+[M]_{D(\text{sugar})}$ (3)
$[M]_{D\ (\mathbf{4})}=[M]_{D(\mathbf{4a})}+[M]_{D(\text{sugar})}$ (4)
So, $[M]_{D(\mathbf{3a})}=-[M]_{D(\mathbf{4a})}\approx-71.7$, and $[M]_{D(\text{sugar})}\approx+51.2$ (**)
Similarly, $[M]_{D\ (\mathbf{5})}=[M]_{D(\mathbf{5a})}+[M]_{D(\text{sugar})}$, but since no $[M]_{D(\text{sugar})}$ for β-D-glucose in MeOH was available and the $[M]_{D(\text{sugar})}$ for β-D-glucose in H_2O was +34 (see Ref. 8b), the $[M]_{D(\text{sugar})}\approx+51.2$ obtained in (**) was used in this case for calculation. Thus, $[M]_{D(\mathbf{5a})}=[M]_{D(\mathbf{5})}-[M]_{D(\text{sugar})}=514\times(-3)/100-51.2\approx-66.6$.
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18. In the evaluation for protection against H_2O_2-induced cell damage, **3**, **5**, and **7** did not show any obvious effects at 1 and 10 μM, while in the antimicrobial assays against a range of pathogenic microbes, *Helicobacter pylori* SS1, *H. pylori* ATCC43504, *Candida albicans*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Bacillus subtilis*, and *Escherichia coli*, none of the compounds showed an MIC of less than 50 μg/mL.