JOURNAL OF NATURAL PRODUCTS

Article

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Diterpenoids with Immunosuppressive Activities from *Cinnamomum cassia*

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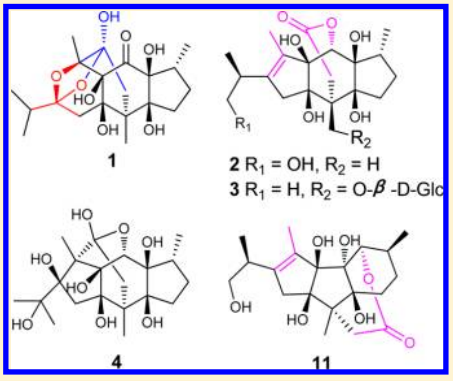
Supporting Information

ABSTRACT: Three new diterpenoids with unprecedented carbon skeletons, cinncassiols F (**1**) and G (**2**) and 16-*O*-*β*-D-glucopyranosyl-19-deoxycinncassiol G (**3**), a new isoryanodane diterpenoid, 18-hydroxyperseanol (**4**), six known isoryanodane diterpenoids, **5**−**10**, and a known ryanodane diterpenoid, **11**, were isolated from the stem bark of *Cinnamomum cassia*. Compound **1** possesses an 11,13:12,13-diepoxy-6,11-epoxy:12,13-disecoisoryanodane diterpenoid skeleton bearing ketal and hemiketal functionalities, whereas compounds **2** and **3** feature an 11,12-secoisoryanodane diterpenoid skeleton with an 11,6-lactone moiety. The structures of the four new diterpenoids, **1**−**4**, and their absolute configurations were established using HRESIMS, NMR, ECD, single-crystal X-ray diffraction, and chemical methods. Compounds **2** and **11** significantly inhibited the proliferation of murine T cells induced by ConA.



Plants are a rich source of natural immunomodulators, which play pivotal roles in the treatment or prevention of autoimmune diseases, tumorigenesis, and chronic inflammatory disorders.[1] Concanavalin A (ConA) and lipopolysaccharide (LPS) can specifically induce the proliferation of T and B cells, respectively, and the ConA/LPS-induced splenocyte proliferation assay is a sensitive method for evaluating the immunomodulatory activities of natural products.[2]

Cinnamomum cassia Presl. (Lauraceae) originates in South China and is widely cultivated in the Yunnan, Guangdong, Fujian, Guangxi, Hainan, and Guizhou Provinces of Mainland China, as well as in Taiwan, India, Vietnam, Laos, Indonesia, Thailand, and Malaysia.[3] The dried bark of this plant, which is also known as cortex cinnamomi, is used not only as a spice and flavoring agent but also as a traditional Chinese medicine to treat amenorrhea, cardiac palpitations, gastrointestinal neurosis, diarrhea, dysmenorrhea, edema, impotency, rheumatoid arthritis, and tussis.[4] The aqueous extract of *C. cassia* bark has been reported to exhibit potent anticomplement [5] and immunosuppressive activities,[6] and its 80% MeOH extract has also shown anti-inflammatory activity.[7] In addition, *trans*-cinnamaldehyde and 2-methoxycinnamaldehyde from the essential oils of *C. cassia* bark have been reported to inhibit NF-*κ*B,[7] and cinnamaldehyde has been shown to possess potential in vitro and in vivo anti-inflammatory activities.[8]

During a search for new immunomodulators from the medicinal plants of China,[9] the 95% EtOH extract of *C. cassia* was found to significantly inhibit the proliferation of T cells induced by ConA. The *n*-BuOH partition fraction exhibited the most potent inhibitory activity, with an inhibition rate of 89.1% at a concentration of 20 *μ*g/mL (see Supporting Information, Table S1), and was thus selected for further study. Fractionation of this active fraction led to the isolation of four new diterpenoids, cinncassiols F (**1**) and G (**2**), 16-*O*-*β*-D-glucopyranosyl-19-deoxycinncassiol G (**3**), and 18-hydroxyperseanol (**4**), and seven known diterpenoids, perseanol (**5**),[10] cinncassiols D_1 (**6**),[11] D_1 glucoside (**7**),[11] D_2 (**8**),[11] D_3 (**9**),[11] and D_4 glucoside (**10**),[12] and cinnacasol (**11**).[13] Compound **1** possesses a rare 11,13:12,13-diepoxy-6,11-epoxy:12,13-disecoisoryanodane diterpenoid skeleton bearing ketal and hemiketal functionalities, whereas compounds **2** and **3** feature an unprecedented 11,12-secoisoryanodane diterpenoid skeleton with an 11,6-lactone moiety. This is the first report of the presence of the ryanodane diterpenoid perseanol (**5**) in *C. cassia*.

RESULTS AND DISCUSSION

Cinncassiol F (**1**), which was obtained as colorless prisms from MeOH (mp 184−186 °C), exhibited an $[M + Na]^+$ quasi-molecular ion peak at *m*/*z* 421.1822 in the HRESIMS, which, in conjunction with the ^{13}C NMR data, suggested a molecular

Received: June 9, 2014
Published: August 4, 2014

formula of $C_{20}H_{30}O_8$ (calcd for $C_{20}H_{30}O_8Na$, 421.1838), requiring six indices of hydrogen deficiency. The absorption bands at 3394 and 1709 cm^{-1} in the IR spectrum indicated the presence of hydroxy and carbonyl functional groups, respectively. The ^1H NMR data (Table 1) of **1** showed

Table 1. ^1H NMR Data (Methanol-d_4, 400 MHz) for Compounds 1−4, J in Hz

position	1	2	3	4
2	2.33 m	2.08 m	2.07 (m)	1.96 (m)
3α	1.18 m	0.92 m	0.94 (m)	1.55 (m)
3β	1.96 m	1.70 m	1.70 (m)	1.60 (m)
4α	2.04 ddd (13.1, 7.2, 5.7)	2.31 m	2.66 (m)	2.20 (m)
4β	1.64 ddd (13.1, 6.8, 6.0)	1.62 m	1.66 (m)	1.57 (m)
6		4.40 s	4.40 (s)	3.89 (s)
10a	1.81 d (15.6)	2.65 d (20.2)	3.04 (d, 20.4)	2.28 (d, 14.7)
10b	1.33 d (15.6)	2.56 d (20.2)	2.59 (d, 20.4)	1.81 (d, 14.7)
14a	2.25 d (13.7)	2.81 dq (17.7, 1.1)	3.36 (dq, 18.0, 1.4)	2.99 (d, 15.6)
14b	1.71 d (13.7)	2.37 dq (17.7, 2.3)	2.30 (dq, 18.0, 2.5)	1.76 (d, 15.6)
15	1.16 d (7.4)	1.21 d (7.0)	1.20 (d, 7.0)	1.11 (d, 6.9)
16a	1.31 s	1.18 s	4.32 (d, 10.7)	1.17 (s)
16b			3.99 (d, 10.7)	
17	1.31 s	1.67 t (1.8)	1.65 (s)	1.37 (s)
18	1.92 m	2.74 m	2.72 (m)	
19a	1.01 d (7.0)	3.46 dd (10.3, 7.4)	0.97 (d, 6.9)	1.18 (s)
19b		3.41 dd (10.3, 6.7)		
20	1.01 d (7.0)	1.0 d (7.0)	1.00 (d, 6.9)	1.33 (s)
1′			4.33 (d, 7.9)	
2′			3.23 (dd, 7.9, 7.8)	
3′			3.37 (m)	
4′			3.32 (m)	
5′			3.29 (m)	
6′a			3.88 (dd, 1.8, 11.9)	
6′b			3.70 (dd, 5.0, 11.9)	

resonances for three secondary methyls (δ_H 1.01, 6H, d, H_3-19, H_3-20; 1.16, 3H, d, H_3-15), two tertiary methyls (δ_H 1.31, 6H, s, H_3-16, H_3-17), and two methylenes (δ_H 1.81, d, H-10a; 1.33, d, H-10b; 2.25, d, H-14a; 1.71, d, H-14b). The ^{13}C NMR spectrum displayed 20 carbon resonances (Table 2), which were assigned by DEPT and HSQC spectra to five methyls (δ_C 15.8, C-15; 16.4, C-17; 17.2, C-19; 17.6, C-20; 20.4, C-16), four methylenes (δ_C 42.3, C-10; 38.8, C-14; 37.2, C-4; 30.5, C-3), two methines (δ_C 46.1, C-2; 35.1, C-18), a ketocarbonyl carbon (δ_C 209.6, C-6), a ketal carbon (δ_C 108.0, C-13), a hemiketal carbon (δ_C 102.8, C-11), five oxygenated quaternary carbons (δ_C 89.9, C-12; 78.5, C-8; 78.7, C-7; 87.5, C-5; 84.9, C-1), and an aliphatic quaternary carbon (δ_C 42.1, C-9). The carbonyl group accounts for one index of hydrogen deficiency, and the remaining five indices of hydrogen deficiency suggested the presence of five additional rings in **1**.

The HSQC and COSY spectra (Figure 1) of **1** suggested the presence of two partial structures: CH_3-15−CH-2−CH_2-3−

Table 2. ^{13}C NMR Data (Methanol-d_4, 400 MHz) for Compounds 1−4

position	1	2	3	4
1	84.9	82.8	83.3	81.5
2	46.1	47.8	47.0	47.3
3	30.5	30.2	30.3	30.3
4	37.2	37.2	38.0	37.8
5	87.5	83.4	82.2	85.3
6	209.6	78.6	78.5	78.7
7	78.7	86.8	87.3	89.7
8	78.5	82.5	83.2	83.0
9	42.1	43.2	46.3	45.5
10	42.3	41.7	37.4	46.1
11	102.8	173.1	173.2	105.2
12	89.9	134.3	131.3	63.6
13	108.0	145.4	148.9	84.9
14	38.8	42.6	41.8	48.3
15	15.8	12.7	12.7	13.0
16	20.4	18.7	71.6	18.4
17	16.4	10.4	10.1	11.4
18	35.1	36.3	28.3	76.5
19	17.2	66.4	20.5	26.4
20	17.6	15.0	20.7	27.8
1′			104.7	
2′			75.1	
3′			78.6	
4′			71.7	
5′			78.0	
6′			62.6	

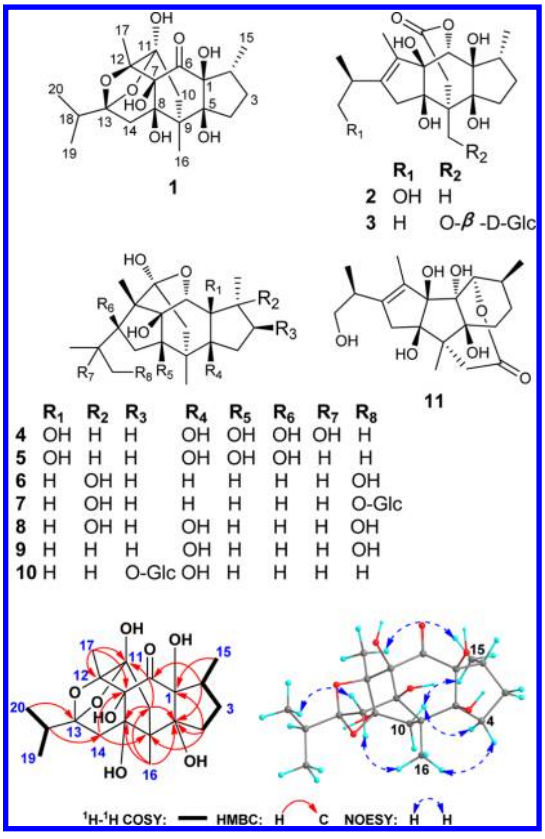


Figure 1. ^1H−^1H COSY, key HMBC, and key NOESY correlations of cinncassiol F (**1**).

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CH_2-4 and the CH_3-19−CH-18−CH_3-20 isopropyl group. The HMBC correlations (Figure 2) of H_2-4/H-2 to C-5 and H_2-4/

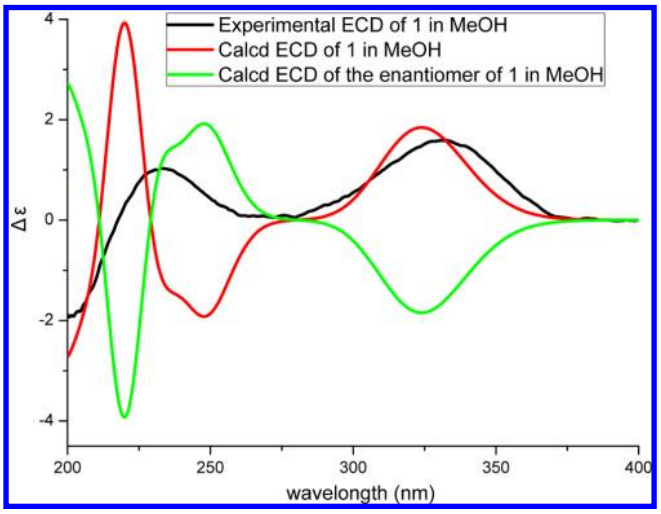

Figure 2. Experimental ECD spectrum of cinncassiol F (**1**) and the calculated ECD spectra for (1*S*,2*R*,5*S*,7*S*,8*R*,9*S*,11*R*,12*S*,13*R*)-cinncassiol F (**1**) and its enantiomer.

H_3-15/H-2 to C-1 indicated a five-membered ring with the C-1−C-2−C-3−C-4−C-5 sequence. These partial structures and other NMR resonances revealed that the structure of **1** is similar to that of the isoryanodane diterpenoid perseanol (**5**).[10] By comparing their NMR data, it was concluded that the structure of **1** differs from that of **5** by the presence of a ketocarbonyl (δ_C 209.6) at C-6, which replaces an oxymethine group (δ_C 77.8) in **5**. In addition, the C-12 (δ_C 89.9) and C-13 (δ_C 108.0) resonances in **1** are significantly deshielded compared to those in **5** (δ_C 63.1, C-12; 82.3, C-13), which suggests that the presence of an oxygen atom between C-12 and C-13 in **1** is responsible for the chemical shifts of C-12 (δ_C 89.9) and C-13 (δ_C 108.0). This conclusion was supported by the HMBC correlations of H_3-19/H-18/H_3-20 to C-13 and by the lack of a H_3-17 to C-13 HMBC correlation. To meet the requirements of the molecular formula and six indices of hydrogen deficiency, an additional ether linkage between C-13 and C-11 is required in **1**; thus, a ketal and a hemiketal functionality are present.

To establish the absolute configuration of **1**, the electronic circular dichroism (ECD) spectra of **1** and of its enantiomer were calculated using time-dependent density functional theory (TDDFT).[14] A comparison of the calculated and experimental ECD spectra (Figure 2) allowed the absolute configuration of **1** to be assigned as (1*S*,2*R*,5*S*,7*S*,8*R*,9*S*,11*R*,12*S*,13*R*).

A prism crystal of **1** suitable for single-crystal X-ray diffraction was obtained from MeOH. The crystal structure (Figure 3) revealed that **1** possesses an 11,13:12,13-diepoxy-6,11-epoxy:12,13-disecoisoryanodane diterpenoid skeleton bearing ketal and hemiketal functionalities. The assigned absolute configuration of **1** was further supported by the Flack parameter of 0.05(8)[15] and the Hooft parameter of 0.03(2) for 2752 Bijvoet pairs[16] for the resulting X-ray structure.

Cinncassiol G (**2**) was obtained as colorless prisms (MeOH) with a melting point of 195−196 °C. The HRESIMS ion peak at *m*/*z* 383.2066 and the ^{13}C NMR data inferred a molecular formula of $C_{20}H_{30}O_7$ for **2** (calcd for $C_{20}H_{31}O_7$, 383.2070). The

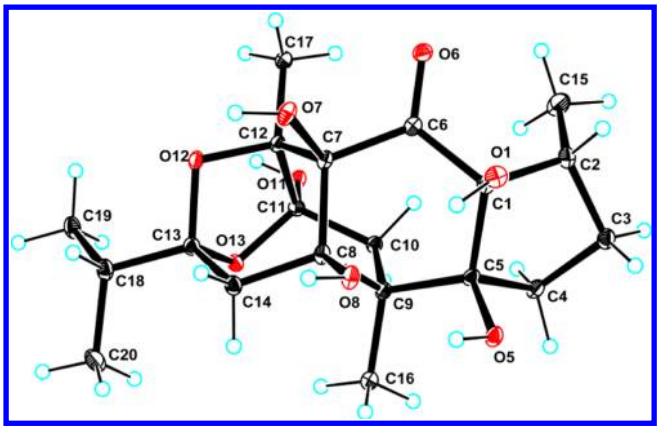

Figure 3. ORTEP drawing of cinncassiol F (**1**).

NMR data for **2** (Tables 1 and 2) resembled those of perseanol (**5**),[10] and the major differences are the presence of an ester carbonyl (δ_C 173.1, C-11) and a tetrasubstituted double bond (δ_C 134.3, C-12; 145.4, C-13) in **2** in place of a hemiketal (δ_C 104.5, C-11), a quaternary carbon (δ_C 63.1, C-12), and an oxygenated tertiary carbon (δ_C 82.3, C-13) in **5**. These differences suggested that there is not a bond between C-11 and C-12 in **2** and that the hemiketal group at C-11 in **5** is replaced by an ester carbonyl in **2**. This finding was supported by the HMBC correlations of H-6 and H_2-10 to C-11 in **2** and by the lack of an HMBC correlation between H_3-17 and C-11. Furthermore, HMBC correlations of H_3-17 to C-13 (δ_C 145.4) and C-12 (δ_C 134.3) confirmed the location of the tetrasubstituted C-12−C-13 double bond. In addition, CH_3-19 (δ_C 20.5) in **5** is replaced with an oxygenated methylene group, CH_2-19 (δ_H 3.46, 3.41; δ_C 66.4), in **2**, which was supported by the HMBC correlations of H_2-19 to C-18 (δ_C 36.3), C-20 (δ_C 15.0), and C-13 (δ_C 145.4). Analyses of the HSQC, ^{1}H−^{1}H COSY, and HMBC spectra confirmed the planar structure of compound **2**.

NOESY correlations between H-2β/H-4β, H-10/H-4α/H_3-16, H-6/H_3-15, H_3-16/H-14α/H-10, and H_3-17/H-6 established the relative configuration of **2**, which is the same as that of **5**. By comparing the calculated and experimental ECD spectra (Figure 4), the absolute configuration of cinncassiol G (**2**) was determined to be 1*R*,2*R*,5*S*,6*R*,7*S*,8*R*,9*S*,18*S*. Single-crystal X-ray diffraction using Cu Kα radiation was employed to confirm the absolute configuration of **2**. The Flack parameter of 0.05(13) and the Hooft parameter of 0.09(4) for 1337 Bijvoet pairs obtained from the single-crystal X-ray diffraction analysis confirmed the absolute configuration of cinncassiol G (**2**). The crystal structure (Figure 5) revealed that **2** possesses an unprecedented 11,12-secoisoryanodane diterpenoid skeleton with an 11,6-lactone moiety and that all junctions of the 5/6/5 ring system possessed the *cis* relative configuration.

Compound **3**, 16-*O*-β-D-glucopyranosyl-19-deoxycinncassiol G, was obtained as a colorless oil. The molecular formula of **3** was assigned as $C_{26}H_{40}O_{12}$ based on the HRESIMS peak at *m*/*z* 567.2385 $[M + Na]^+$ (calcd for $C_{26}H_{40}O_{12}Na$, 567.2418) and the ^{13}C NMR data. Comparison of the NMR data of diterpenoids **3** and **2** (Tables 1 and 2) revealed that **3** is a glucosidic derivative of **2**. With the exception of the sugar moiety, the primary differences between compounds **3** and **2** were the replacement of the oxygenated methylene (δ_H 3.46, dd, 3.41, dd, H_2-19; δ_C 66.4, C-19) in **2** with a methyl group (δ_H 0.97, d, H_3-19) in **3** and the replacement of the methyl

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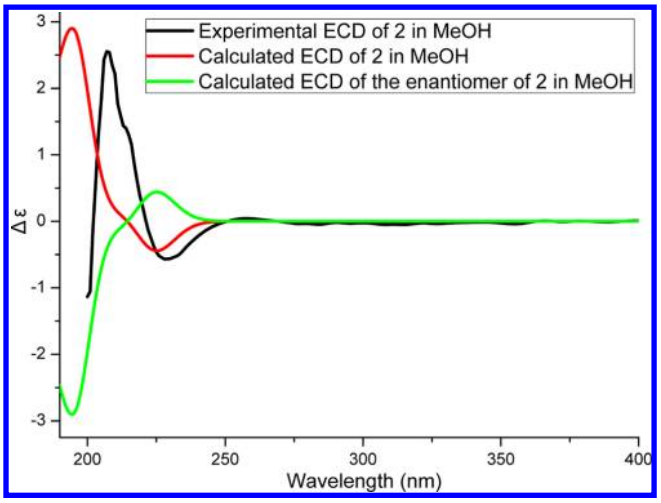

Figure 4. Experimental ECD spectrum of cinncassiol G (**2**) and the calculated ECD spectra for (1*R*,2*R*,5*S*,6*R*,7*S*,8*R*,9*S*,18*S*)-cinncassiol G (**2**) and its enantiomer.

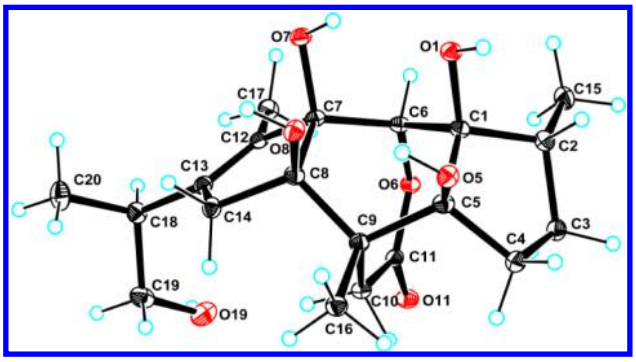

Figure 5. ORTEP drawing of cinncassiol G (**2**).

group (δ_H 1.18, s, H_3-16) in **2** by an oxygenated methylene (δ_H 4.32, d, 3.99, d, H_2-16; δ_C 71.6, C-16) in **3**. The sugar moiety was assigned as a β-glucopyranose by ^{1}H NMR resonances at δ_H 4.33 (d, J = 7.9 Hz, H-1′), 3.23 (dd, H-2′), 3.37 (m, H-3′), 3.32 (m, H-4′), 3.29 (m, H-5′), 3.88 (dd, H-6′a), and 3.70 (dd, H-6′b) and by ^{13}C NMR resonances at δ_C 104.7 (C-1′), 75.1 (C-2′), 78.6 (C-3′), 71.7 (C-4′), 78.0 (C-5′), and 62.6 (C-6′). To determine the absolute configuration of the glucopyranose, **3** was hydrolyzed with 10% HCl, and the trimethylsilylthiazolidine derivatives of the hydrolysate and standards, D- and L-glucose, were prepared. By comparing the retention times of the three trimethylsilylthiazolidine derivatives obtained from GC analysis, the absolute configuration of the glucose in **3** was determined to be D. HMBC correlations from H-1′ of the glucose to C-16 (δ_C 71.4) and from H-16 to C-1′ of the glucose determined that glucosidation had occurred at C-16. Except for the sugar unit, **2** and **3** shared the same relative and absolute configurations, based on the close similarities in their NOESY and ECD spectra. Thus, the structure of compound **3** was defined as 16-*O*-*β*-D-glucopyranosyl-19-deoxycinncassiol G.

Compound **4**, 18-hydroxyperseanol, a colorless oil, has a molecular formula of $C_{20}H_{32}O_8$ as determined by the $[M + Na]^+$ ion peak at m/z 423.1979 in the HRESIMS and the ^{13}C NMR data. Its NMR data (Tables 1 and 2) closely resembled those of **5**.[10] The major differences between **4** and **5** are that **4** has two methyl singlets (δ_H 1.18, s, Me-19; 1.33, s, Me-20) in the ^{1}H NMR spectrum, whereas the spectrum of **5** indicates an isopropyl group, and C-18 (δ_C 76.5) of **4** was deshielded compared to that of **5** (δ_C 35.2). Thus, compound **4** was determined to be the 18-hydroxy derivative of compound **5**, which was supported by the HMBC correlations from H_3-19 and H_3-20 to C-18. The planar structure of **4** was confirmed by ^{1}H−^{1}H COSY, HSQC, and HMBC analyses. Cross-peaks of H_3-15/H-6, H-6/H_3-17, H-4α/H-10a, H_3-16/H-10b, H-10b/H-14a, and H-14b/H-19 in the NOESY spectrum suggested that the relative configuration of **4** was the same as that of **5**.

To date, a total of 22 diterpenoids have been isolated from *C. cassia*, and five types of carbon skeletons (Figure 6), those of

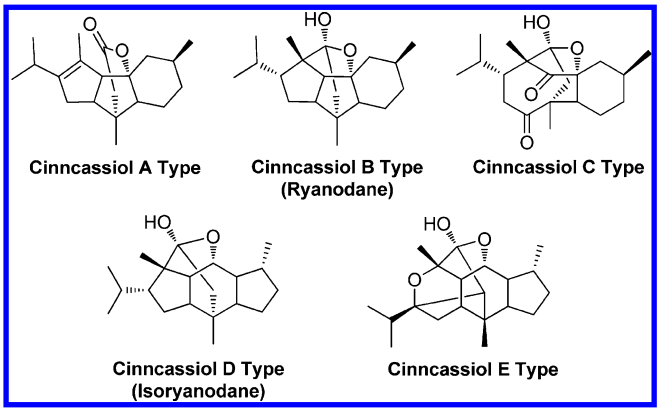

Figure 6. Five known carbon skeletal types of diterpenoids from *C. cassia*.

cinncassiols A,[5] B,[17] C,[18] D,[11] and E,[19] have been reported. Skeletons of cinncassiols B and D are known as the ryanodane and isoryanodane diterpenoids, respectively. Only 15 isoryanodane diterpenoids have hitherto been isolated from nature.[20] Compound **1** represents the first example of an 11,13:12,13-diepoxy-6,11-epoxy:12,13-disecoisoryanodane diterpenoid skeleton bearing ketal and hemiketal functionalities, and compounds **2** and **3** are the first examples of an 11,12-secoisoryanodane diterpenoid skeleton with an 11,6-lactone moiety. Compounds **1** and **2** are distinct from the five diterpenoid skeleton types reported to date; therefore, they are herein designated cinncassiols F and G, respectively.

Considering their structural similarities, compounds **1** and **2** should be oxidation derivatives of isoryanodane diterpenoids. Herein, we propose that the isoryanodane diterpenoid perseanol (**5**) is the biosynthetic precursor of **1** and **2**. Furthermore, plausible biosynthetic pathways for **1** and **2**, which involve an enzyme-mediated oxidation and the formation of ketal and hemiketal functionalities, are proposed in Schemes 1 and 2.

The bark of *C. cassia* has been used as a Chinese medicine to treat the autoimmune disease rheumatoid arthritis.[4] The bark extract has been reported to possess potent immunosuppressive activity.[6] In our ConA/LPS-induced splenocyte proliferation assay, the 95% EtOH extract inhibited 78.5% of T cell proliferation induced by ConA at a concentration of 100 μg/mL (Supporting Information, Table S1). The *n*-BuOH, EtOAc, $CHCl_3$, and petroleum ether partition fractions from the 95% EtOH extract exhibited significant inhibitory activities at concentrations of 20 μg/mL, with inhibition rates of 82.7%, 86.3%, 78.8%, and 89.1%, respectively. Notably, the *n*-BuOH partition fraction was the most potent. Compounds **1**−**11** from the *n*-BuOH partition fraction demonstrated different effects on the proliferation of T and B cells induced by ConA and LPS,

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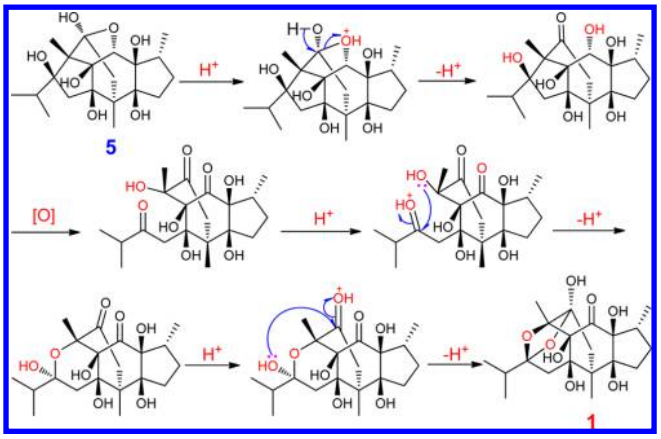
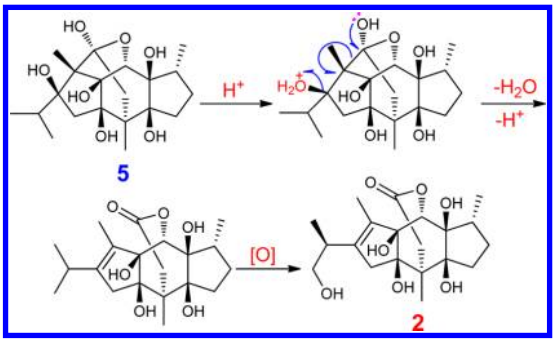
Scheme 1. Proposed Biosynthetic Pathway for Cinncassiol F (1)



Scheme 2. Proposed Biosynthetic Pathway for Cinncassiol G (2)



respectively (Supporting Information, Table S2). Of these, compounds **2** and **11** significantly inhibited the proliferation of ConA-induced T cells (Figure 7) and LPS-induced B cells

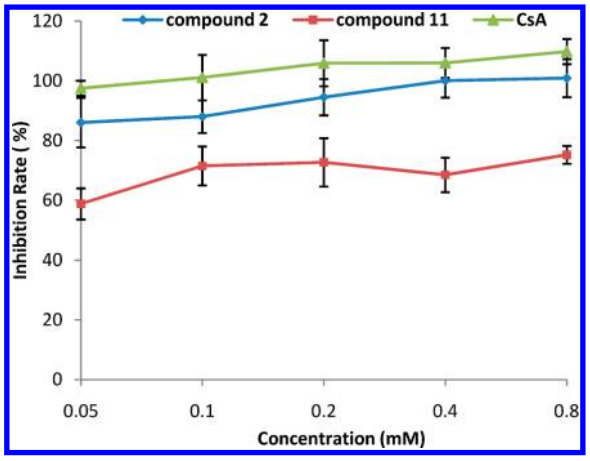


Figure 7. Inhibition rates of compounds **2**, **11**, and CsA against ConA-induced murine T cell proliferation. Data are expressed as the mean ± SD (n = 3).

(Figure 8) in a dose-dependent manner. Interestingly, the inhibition rate of compound **2** (94.5%) on the ConA-induced murine T cell proliferation approached that of cyclosporine A (CsA, 101.1%), which is a potent immunosuppressive agent, at a concentration of 100 μM. Compounds **2** and **11** significantly inhibited T cell proliferation even at 50 μM, with inhibition rates of 86.1% and 58.8%, respectively. Thus, compounds **2** and

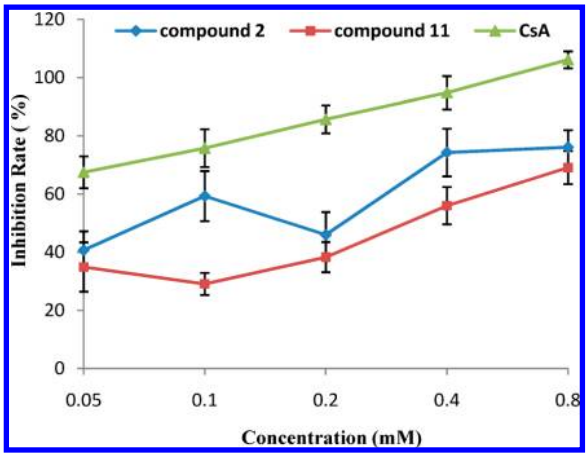


Figure 8. Inhibition rates of compounds **2**, **11**, and CsA against LPS-induced murine B cell proliferation. Data are expressed as the mean ± SD (n = 3).

11 are the active components in the polar n-BuOH partition fraction. More importantly, both of these compounds are nontoxic against murine lymphocytes at 50 μM with cell viabilities of 104.8% and 97.8%, respectively. This result suggests that the immunosuppressive activities of **2** and **11** do not result from their cytotoxicity. Analysis of the structures and activities of compounds **1**–**11** revealed that the Δ^{12}-11,6-lactone and Δ^{12}-11,1-lactone moieties may be essential for activity in these compounds. However, glucosidation at C-11 in compound **3** might reduce its inhibitory potency. Therefore, compounds **2** and **11** may have potential as immunosuppressive agents. Isoryanodane and ryanodane diterpenoids have been reported to exhibit anticomplement and antifeedant activities;[20] however, this is the first evaluation of their immunomodulatory activities against murine lymphocytes.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured using a Beijing Tech X-5 micromelting point apparatus without correction. Optical rotations were determined in MeOH using a Perkin-Elmer 341 polarimeter. UV spectra were recorded with a Varian Cary 50 spectrometer. ECD spectra were obtained with a JASCO J-810 spectrometer. IR spectra were recorded using a Bruker Vertex 70 instrument. NMR spectra were acquired on a Bruker AM-400 spectrometer, and the ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peaks for methanol-d_4 at δ_H 3.31 and δ_C 49.15. HRESIMS was conducted in the positive-ion mode on a Thermo Fisher LC-LTQ-Orbitrap XL spectrometer. The crystallographic data were collected on a Bruker SMART APEX-II CCD diffractometer using graphite-monochromated Cu Kα radiation (λ = 1.54178 Å). HPLC was performed with an RP C_{18} column (10 × 250 mm, 5 μm) on a Dionex quaternary system with a diode array detector at a flow rate of 2 mL/min. GC analysis was performed with a capillary column (30 m × 0.32 mm × 0.5 μm) on an Agilent 7890A GC.

Plant Material. The stem bark of *C. cassia* was collected in Qujing, Yunan Province, China, in July 2010 and was identified by Prof. C.-G. Zhang at the School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology. A voucher specimen (No. 2010-0703) has been deposited at the Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology.

Extraction and Isolation. The powdered stem bark of *C. cassia* (25 kg) was soaked three times at room temperature in 95% EtOH (3 × 100 L). The solvent was removed under vacuum, and the residue (2.6 kg) was successively partitioned with petroleum ether, $CHCl_3$,

dx.doi.org/10.1021/np500465g | J. Nat. Prod. 2014, 77, 1948–1954

EtOAc, and *n*-BuOH. The *n*-BuOH partition fraction was concentrated under vacuum to afford a brown syrup, which was subsequently extracted using MeOH. The MeOH-soluble extract (250 g) was fractionated using silica gel CC eluting with $CHCl_3$−MeOH from 15:1 to 3:1 to afford seven fractions: 1−7. Fraction 2 (16 g) was chromatographed on an RP C_{18} column eluting with MeOH−H_2O (from 0:100 to 50:50) to afford six subfractions: Fr.2A−2F. Fr.2B was successively fractionated using silica gel CC ($CHCl_3$−MeOH, 15:1), Sephadex LH-20 CC (MeOH), and RP C_{18} HPLC (MeOH−H_2O, 30:70) to afford compound **1** (20.0 mg, t_R 19.5 min). Compounds **4** (t_R 33 min, 3.0 mg) and **5** (t_R 29 min, 4.0 mg) were obtained from subfraction Fr.2C using RP C_{18} HPLC (CH_3CN−H_2O, 20:80). Subfraction Fr.2D was subjected to RP C_{18} CC (MeOH−H_2O, 10:90) and further separated using RP C_{18} HPLC (MeOH−H_2O, 35:65) to afford compound **11** (50.0 mg, t_R 19.5 min). Fraction 3 (20 g) was purified using RP C_{18} CC (MeOH−H_2O, from 0:100 to 50:50) to afford five subfractions: Fr.3A−3E. Subfraction Fr.3B was fractionated using silica gel CC (petroleum ether−EtOAc, 1:2) and RP C_{18} HPLC (MeOH−H_2O, 30:70) to afford compound **6** (t_R 20.0 min, 30.0 mg). Compound **2** (t_R 30 min, 4.0 mg) was obtained from subfraction Fr.3C using silica gel CC (petroleum ether−EtOAc, 1:3) and RP C_{18} HPLC (MeOH−H_2O, 32:68). Fraction 4 (22.0 g) was separated on an RP C_{18} column (MeOH−H_2O, from 0:100 to 50:50), to afford five subfractions: Fr.4A−4E. Subfraction Fr.4B was further fractionated using Sephadex LH-20 CC (MeOH) and RP C_{18} HPLC (MeOH−H_2O, 20:80) to afford compound **8** (t_R 22.0 min, 9.0 mg). Compound **9** (t_R 16 min, 30.0 mg) was isolated from subfraction Fr.4C using silica gel (eluting with 30:1 $CHCl_3$−MeOH) and Sephadex LH-20 (MeOH) CC and RP C_{18} HPLC (MeOH−H_2O, 30:70). Fraction 5 (25.0 g) was separated using RP C_{18} CC (MeOH−H_2O, from 10:90 to 50:50) to afford six subfractions: Fr.5A−5F. Subfraction Fr.5C was separated using silica gel (eluting with 6:1 $CHCl_3$−MeOH) and Sephadex LH-20 (MeOH) CC and RP C_{18} HPLC (MeOH−H_2O, 45:55) to obtain compound **10** (t_R 31.0 min, 7.5 mg). Compound **7** (t_R 32.0 min, 10.0 mg) was obtained from subfraction Fr.5D using silica gel CC ($CHCl_3$−MeOH, 10:1) and RP C_{18} HPLC (MeOH−H_2O, 30:70). Similarly, compound **3** (t_R 28.0 min, 3.0 mg) was isolated from subfraction Fr.5E using RP C_{18} HPLC (MeOH−H_2O, 45:55).

*Cinncassiol F (**1**):* colorless prisms (MeOH); mp 184−186 °C; $[\alpha]^{25}_D$ −11 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 279 (2.7) nm; ECD (MeOH) 233 ($\Delta\varepsilon$, +1.03), 332 ($\Delta\varepsilon$, +1.588) nm; IR (KBr) ν_{max} 3394, 2968, 2881, 1709, 1445, 1388, 1367, 1331, 1257, 1086, 1039, 999, 851, 787, 758, 683 cm^{-1}; ^1H NMR (methanol-d_4, 400 MHz), Table 1; ^{13}C NMR (methanol-d_4, 100 MHz), Table 2; HRESIMS *m*/*z* 421.1822 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_8Na$, 421.1838).

*Cinncassiol G (**2**):* colorless prisms (MeOH); mp 195−196 °C; $[\alpha]^{25}_D$ +25 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 279 (2.5) nm; ECD (MeOH) 208 ($\Delta\varepsilon$, +2.68), 229 ($\Delta\varepsilon$, −0.58) nm; IR (KBr) ν_{max} 3418, 2969, 2886, 2115, 1641, 1581, 1412, 1388, 1269, 1103, 1026, 833 cm^{-1}; ^1H NMR (methanol-d_4, 400 MHz), Table 1; ^{13}C NMR (methanol-d_4, 100 MHz), Table 2; HRESIMS *m*/*z* 383.2066 $[M + H]^+$ (calcd for $C_{20}H_{31}O_7$, 383.2070).

*16-O-β-D-Glucopyranosyl-19-deoxycinncassiol G (**3**):* colorless oil; $[\alpha]^{25}_D$ +2 (*c* 0.4, MeOH); ECD (MeOH) 224 ($\Delta\varepsilon$, −0.83) nm; IR (KBr) ν_{max} 3391, 2960, 2931, 1716, 1629, 1459, 1376, 1240, 1214, 1165, 1081, 1027, 943, 869, 774, 693 cm^{-1}; ^1H NMR (methanol-d_4, 400 MHz), Table 1; ^{13}C NMR (methanol-d_4, 100 MHz), Table 2; HRESIMS *m*/*z* 567.2385 $[M + Na]^+$ (calcd for $C_{26}H_{40}O_{12}Na$, 567.2418).

*18-Hydroxyperseanol (**4**):* colorless oil; $[\alpha]^{25}_D$ +15 (*c* 0.1, MeOH); ^1H NMR (methanol-d_4, 400 MHz), Table 1; ^{13}C NMR (methanol-d_4, 100 MHz), Table 2; HRESIMS *m*/*z* 423.1979 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_8Na$, 423.1995).

Single-Crystal X-ray Diffraction Analysis and Crystallographic Data of Cinncassiols F (1) and G (2). The intensity data collection, reduction, and refinement were performed according to the procedure and software described in our previously published papers.[9] The crystallographic data of **1** (deposition number CCDC 898422) and **2** (deposition number CCDC 906642) have been deposited in the Cambridge Crystallographic Data Centre.

*Crystallographic data of cinncassiol F (**1**):* $C_{40}H_{60}O_{16}$ [2 × ($C_{20}H_{30}O_8$)], *M* = 796.88, *T* = 100(2) K, λ = 1.511 78 Å, colorless prism, size 0.13 × 0.13 × 0.60 mm^3; monoclinic, space group $P2_1$; *a* = 8.6352(10) Å, *b* = 9.5547(10) Å, *c* = 22.1075(3) Å, β = 96.19°, $\alpha = \gamma$ = 90.00°, *V* = 1813.38 (4) $Å^3$, *Z* = 2, D_c = 1.459 g/cm^3, μ = 0.937 mm^{-1}; *F*(000) = 856, 15 535 reflections and 6192 independent reflections (R_{int} = 0.0235) were collected in the θ range 2.01° ≤ θ ≤ 69.59° with index ranges of *h*(−8/9), *k*(−11/11), *l*(−25/26), completeness θ_{max} = 94.4%, data/restraints/parameters 6192/1/526, GOF = 1.046. Final *R* indices: R_1 = 0.0287, wR_2 = 0.0749. *R* indices (all data): R_1 = 0.0288, wR_2 = 0.0750. Largest difference peak and hole = 0.302 and −0.205 e $Å^{-3}$. Flack parameter of 0.05(8) and Hooft parameter of 0.03(2) for 2752 Bijvoet pairs.

*Crystallographic data of cinncassiol G (**2**):* $C_{20}H_{30}O_7$, *M* = 382.44, *T* = 100(2) K, λ = 1.511 78 Å, colorless prism, size 0.13 × 0.18 × 0.18 mm^3; orthorhombic, space group $P2_12_12_1$; *a* = 9.5101(2) Å, *b* = 13.9479(3) Å, *c* = 14.2836(3) Å, $\alpha = \beta = \gamma$ = 90.00°, *V* = 1894.66(7) $Å^3$, *Z* = 4, D_c = 1.341 g/cm^3, μ = 0.833 mm^{-1}; *F*(000) = 824, 9500 reflections and 3279 independent reflections (R_{int} = 0.0258) were collected in the θ range 4.43° ≤ θ ≤ 69.04° with index ranges of *h*(−11/11), *k*(−16/16), *l*(−16/16), completeness θ_{max} = 95.7%, data/restraints/parameters 3279/0/253, GOF = 1.063. Final *R* indices: R_1 = 0.0330, wR_2 = 0.0840. *R* indices (all data): R_1 = 0.0332, wR_2 = 0.0842, largest difference peak and hole = 0.261 and −0.225 e $Å^{-3}$. Flack parameter of 0.05(13) and Hooft parameter of 0.09(4) for 1337 Bijvoet pairs.

Computational Methods. The quantum chemical computations were performed using the Gaussian 09 software (Gaussian, Inc., Pittsburgh, PA, 2009)[9a] at the Supercomputing Center of CNIC, CAS. The input geometries of cinncassiols F (**1**) and G (**2**) were obtained directly from their crystal structures, which were further optimized using the DFT method at the B3LYP/6-311G(d,p) level. To ensure that the obtained structure is the local minimum, harmonic frequency analysis at the same computational level was also performed. TDDFT calculations were performed at the same level of theory for geometry optimization. Calculation of the ECD spectra was performed using the GaussSum 2.2.5 program.[21] When the fwhm (full width at half-maximum) for each peak was set at 0.5 eV, the electronic transitions were extended as Gaussian curves, which provided a better fit to the measured widths of the bands.

Acid Hydrolysis and Absolute Configuration Determination of the Sugar Moiety of 16-*O*-β-D-Glucopyranosyl-19-deoxycinncassiol G (3). Acid hydrolysis of compound **3** and the preparation of the trimethylsilylthiazolidine derivatives of the hydrate and the standards D- and L-glucose were performed according to the published method and procedure.[22] The trimethylsilylthiazolidine derivative of the hydrolysate of **3** showed a retention time of 17.94 min using the described GC conditions.[22] The trimethylsilylthiazolidine derivatives of the standards D- and L-glucose exhibited retention times of 17.93 and 18.53 min, respectively.

Lymphocyte Proliferation Tests. The in vitro lymphocyte proliferation bioassays on compounds **1**−**11** were performed according to the reported procedure.[9]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

X-ray crystallographic data of **1** and **2**; HRESIMS, UV, ECD, IR, and NMR spectra of **1**−**4**; lymphocyte bioassay data for four fractions and compounds **1**−**11**; ECD calculation data of **1** and **2**; and GC analyses of sugar derivatives from **3** and standards. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

■ ACKNOWLEDGMENTS

We are grateful to Professors F. D. Horgen and Y. Chen at Hawaii Pacific University for their editing of the manuscript, and the Analytical and Testing Center at Huazhong University of Science and Technology for assistance in conducting ECD and IR analyses. This project was financially supported by the National Natural Science Foundation of China (31370372, 31170323, 81001403), Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry of China (to G.Y.), the Program for Youth Chutian Scholar of Hubei Province of China (to G.Y.), and the Fundamental Research Funds for the Central Universities (HUST: 2014QN131, to G.Y.).

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