Notes

Secondary Metabolites of *Cinnamosma madagascariensis* and Their α-Glucosidase Inhibitory Properties

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Two new drimane-type sesquiterpenes, cinnamadin (**1**) and cinnamodial 11α,12*β*-dimethyl acetal (**2**), together with pereniporin B (**3**), ugandensolide (**4**), polygodial (**5**), capsicodendrin, cinnamodial (**6**), sitosterol, stigmasterol, lignoceric acid, cinnamosmolide (**7**), D-mannitol, and *δ*-tocotrienol were isolated from *Cinnamosma madagascariensis*. The structures of the new compounds were determined by physical, chemical, and spectroscopic evidence. Compound **7** and D-mannitol were isolated in high yield (5% and 1.36%, respectively). Evaluation of the α-glucosidase inhibitory properties of the isolated metabolites demonstrated that compounds **1** and **4** show moderate effects, while cinnamodial (**6**) exhibited the most potent activity. The chemosystematics of *Cinnamosma* species are also discussed.

Our interest in the phytochemical investigation of *Cinnamosma*, the Malagasy endemic genus belonging to the Canellaceae family, has been initiated by the isolation of drimane-type sesquiterpene mono-, di-, and trimers from *C. fragrans* and *C. macrocarpa*.[1,2] *Cinnamosma fragrans* is distributed in northern, central, and central-eastern parts of Madagascar, while *C. macrocarpa* and *C. madagascariensis* are found widely in the southeastern and in the southern part of this island. Although all three species are used in the Malagasy Pharmacopoea for many ailments, the prescriptions therein depend on the regions where they grow.[1] *Cinnamosma madagascariensis* has a more bitter taste compared to the other two species, which possess pungent tastes. The former is used in the southern part of the country mainly to treat cough and to strengthen the immune system.[1] Capsicodendrin and cinnamodial are the major biologically active constituents of *C. fragrans* and *C. macrocarpa*.[2,3]

Since the isolation of a quaternary aporphine alkaloid (chakranine),[4] and of N_b-*p*-coumaroyl- and N_b-feruloyltryptamine, (±)-lyoniresinol, and 5-methoxy-9*β*-xylopyranosyl-(−)-isolariciresinol from *C. madagascariensis*,[5] the taxonomic placement of the family Canellaceae in the order Magnoliiflorae has been confirmed. With the aim of isolating new active compounds from *Cinnamosma* species, we have carried out a phytochemical investigation of *C. madagascariensis* Danguy collected in Sakaraha, Madagascar. This report deals with the isolation and structural determination of metabolites of *C. madagascariensis*, the α-glucosidase inhibitory properties of the isolated metabolites, as well as a short discussion of the chemosystematics of the genus *Cinnamosma*.

The EtOAc-soluble fraction of *C. madagascariensis* bark was chromatographed repeatedly on silica gel, Sephadex LH-20, and ODS RP-18 columns to afford two new sesquiterpene drimanes (**1** and **2**), together with 10 known compounds identified as pereniporin B (**3**),[6] ugandensolide (**4**),[7] polygodial (**5**),[8] capsicodendrin,[2] cinnamodial (**6**),[2] sitosterol, stigmasterol, lignoceric acid, cinnamosmolide (**7**),[7] and *δ*-tocotrienol.[2,3] The structures of the known compounds were identified through the interpretation of their physical and spectroscopical data and by comparison with values reported in the literature.

Positive HRESIMS analysis of cinnamadin (**1**) exhibited a quasi-molecular ion peak at *m/z* 331.1511 $[M + Na]^+$, corresponding to the molecular formula $C_{17}H_{24}O_5$. The IR spectrum suggested the presence of a hydroxy, an α,*β*-unsaturated *γ*-lactone carbonyl, and an *O*-acetyl group (ν_{max} 3420, 1675 and 1239, and 1730 cm^{-1}, respectively). The ^1H NMR spectrum (Table 1) displayed signals for three quaternary methyl protons (*δ* 1.41, 0.99, and 0.98; each a singlet), one acetoxymethyl (*δ* 1.98, s), two oxygen-bearing methines (*δ* 5.38, brs; H-6 and *δ* 4.24, brs; H-7), and two oxymethylene protons (*δ* 4.79, dd, $J = 17.2$, 2.0 Hz, H-11a and *δ* 4.71, d, $J = 17.2$ Hz, H-11b). The ^{13}C NMR spectrum showed 17 resonances, including one lactone and one *O*-acetyl carbonyl (*δ* 169.9 and 170.0, respectively), and two quaternary sp^2 carbons, one of which was deshielded (*δ* 173.6 ppm). All of the above data were suggestive of the presence of a drimane sesquiterpene lactone.[2,3] Interpretation of the COSY spectrum led to the proposal of two partial structures: $-CH_2-CH_2-CH_2-$ and $-CH-CHO-CHO-$. The full structure of **1** was determined by careful analysis of the HSQC and HMBC spectra. Long-range correlations between H-6 and C-4 and between C-8 and C-10, on one hand, and between H-7 and C-5, C-9, and C-12 on the other hand allowed the oxygen-bearing methines to be positioned at C-6 and C-7, and thus the hydroxymethylene must be located at C-11. The relative configurations of the hydroxy group at C-7 and the oxygen-bearing methine at C-6 were substantiated by the coupling observed in the ^1H NMR spectrum as a broad singlet due to the dihedral angles between H-5 and H-6 and between H-6 and H-7 (*δ* 5.38, brs; H-6 and *δ* 4.24, brs; H-7). The HMBC correlation between the H-6 signal and the acetyl carbonyl resonance supported the location of the acetoxy group at C-6. Moreover, NOESY cross-peaks were observed between H-6 and both of the protons at C-5 and C-7. In order to determine the absolute structure of **1**, a chloro derivative (**8**) of cinnamosmolide (**7**) was prepared by treatment of **7** with thionyl

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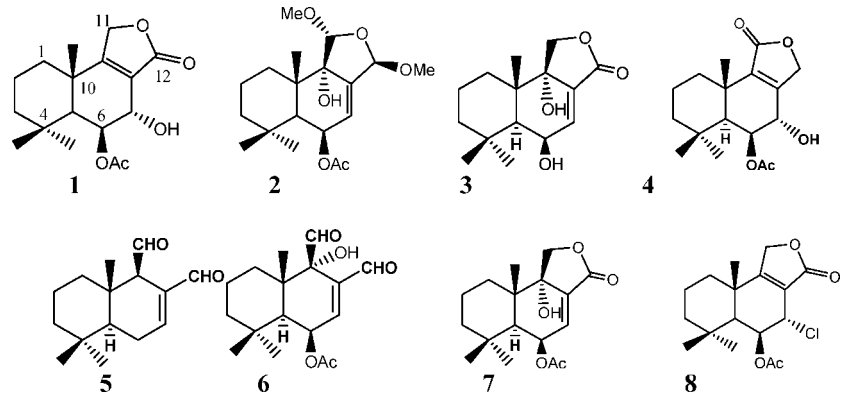

Table 1. ^{1}H NMR Data for Compounds **1** and **2** (400 MHz, in $CDCl_3$)[a]

position	1	2
5	1.65 (brs)	1.62 (d, 4.3)
6	5.38 (brs)	5.59 (brt, 4.5)
7	4.24 (brs)	6.71 (d, 4.0)
8		
9		
10		
11a	4.79 (dd, 17.2, 2)	4.90 (s)
11b	4.71 (d, 17.2)	
12		5.10 (s)
13	1.41 (s)	1.18 (s)
14	0.98[b] (s)	0.99 (s)
15	0.99[b] (s)	0.90 (s)
$\underline{CH_3}C{=}O$	1.98 (s)	1.99 (s)
OCH_3-11		3.52 (s)
OCH_3-12		3.33 (s)

[a] Assignment based on HSQC, COSY, and HMBC spectroscopic data. [b] Assignments can be interchanged.

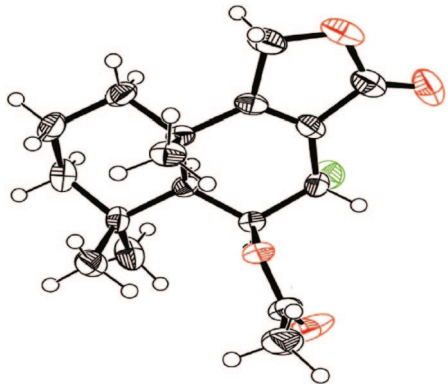

Figure 1. ORTEP drawing for compound **8**.

chloride and pyridine, as described in the previous literature.[9] The ^{1}H NMR data of **8** were very similar to those of **1**, indicating that **8** is a chloro derivative of **1**. The absolute stereostructure of **8** was confirmed by X-ray crystallography (Figure 1). The negative sign of the optical rotation of both **1** and **8** indicated that they have the same absolute configuration. From the above data, the structure of cinnamadin (**1**) was deduced as shown (Figure 2).

Cinnamodial 11α,12*β*-dimethyl acetal (**2**) exhibited a molecular formula of $C_{19}H_{30}O_6$, as designated by the positive HRESIMS (*m*/*z* 377.1934 $[M + Na]^+$). The IR band at ν_{max} 1725 cm^{-1} suggested the presence of an acetyl carbonyl group. The ^{1}H NMR spectrum showed three quaternary methyl proton resonances (*δ* 1.18, 0.99, and 0.90; each a singlet), two acetal proton signals (*δ* 5.10, H-12 and *δ* 4.90, H-11; both singlets), an acetoxyl methyl signal at *δ* 1.99, two methoxy groups (*δ* 3.52 and 3.33; both singlets), an oxygen-bearing methine (*δ* 5.59, brt, J = 4.5 Hz; H-6), and an olefinic methine at *δ* 6.71 (d, J = 4.0 Hz; H-7). The ^{13}C NMR data displayed 19 resonances that were very similar to those of 7-drimene-11α,12*β*-dimethyl acetal, previously isolated from *Canella winterana* (Canellaceae).[10] The allocations of the acetyl group at C-6, the two methoxyl groups at C-11 and C-12, and the C-11 and C-12 acetals were supported by careful interpretation of the HSQC, HMBC, and NOESY spectroscopic data. The long-range correlations observed between H-11 and C-8 and C-10, between H-12 and C-7 and C-9, and between the methoxyl protons at *δ* 3.52 and 3.33 and C-11 and C-12, respectively, substantiated the locations of the acetal groups at C-11 and C-12, where the methoxy groups are attached. The relative configurations of the C-11 and C-12 methoxy groups were deduced as follows. The cross-peaks observed between the H-11 and H-13 methyl protons, between H-11 and the C-12 methoxyl protons, and between H-5 and the C-11 methoxyl protons observed in the NOESY spectrum (Figure 2) provided evidence for the configuration of C-11 and C-12 as depicted for **2**. Furthermore, the *β*-orientation of the C-6 acetyl group was deduced by the NOE cross-peak between H-6 and H-5. Therefore the structure of **2** was concluded to be cinnamodial 11α,12*β*-dimethyl acetal.

α-Glucosidase (EC 3.2.1.20) is a well-known enzyme catalyzing the final step in the digestive process of carbohydrates. Its inhibitors can be thus very useful for the control of postprandial hyperglycemia, which a main cause of diabetes and obesity.[3] In a continuation of our systematic screening of α-glucosidase inhibitory activity of *Cinnamosma* metabolites, compounds **1**−**7** isolated from *C. madagascariensis*, together with compound **8**, were evaluated (Table 3). Apart from cinnamodial (**6**), which was reported to have a strong inhibition,[3] ugadensolide (**4**) showed moderate activity (46.2%). The α-glucosidase inhibitory properties of compounds **1**−**8** can be summarized as follows: (1) Substitution of the hydroxy group at C-7 of **1** by chlorine increases the activity by 2-fold. (2) The presence of a hydroxy group at a *β*-position to the lactone carbonyl decreases the activity. Unexpectedly, polygodial (**5**), which has a similar structure to **6**, showed only slight activity. As stated in a previous paper,[2] the presence of a C-9 aldehyde group in drimane-type sesquiterpenes and a C-12′ hydroxyl in the cinnafragrins are necessary for α-glucosidase inhibition. (3) Since polygodial (**5**) and isopolygodial[2] did not show any activity, the *β*-orientation of the C-9 aldehyde and the presence of both a C-9 hydroxy and a C-6 acetoxy groups seem to be very important for potent α-glucosidase inhibition within this class of compounds.

Cinnamosma is one of the five genera of the Canellaceae family. Thirty-one compounds have been isolated from three species of the genus *Cinnamosma* (Table S1, Supporting Information).[2–5,9,11,12] Their pungent taste is mainly due to the presence of cinnamodial (**6**), although polygodial is also isolated from *C. madagascariensis*. *Cinnamosma fragrans* displayed the highest amounts of **6**. Hence, this species is the most pungent. The bitter taste of the present sample of *C. madagascariensis* is due to its high content of **7** (5%). *C. madagascariensis* has been reported to contain chakranine,[4] N_b-*p*-coumaroyl- and N_b-feruloyltryptamine, (±)-lyoniresinol, and

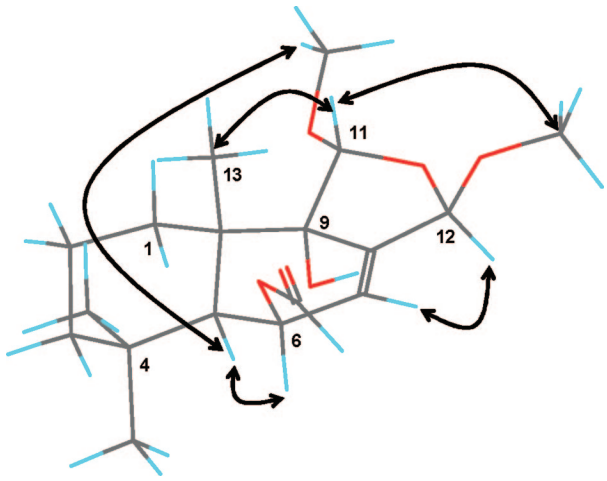

Figure 2. Important NOESY correlations observed in **2**.

Table 2. ^{13}C NMR Data for Compounds **1** and **2** (100 MHz, in $CDCl_3$)

position	1	2
1	38.4	31.9
2	18.5	19.6
3	42.8	44.5
4	33.5	33.6
5	48.5	44.9
6	72.3	67.3
7	63.6	134.9
8	122.7	131.5
9	173.6	76.7
10	37.2	38.9
11	68.5	104.3
12	169.9	104.1
13	22.5	32.9
14	23.1	24.2
15	33.0	18.0
$\underline{CH_3}C{=}O$	21.3	21.3
$CH_3\underline{C{=}O}$	170.0	170.1
OCH_3		56.6
OCH_3		54.9

Table 3. α-Glucosidase Inhibition Activities of Compounds **1**−**8**[a]

compound	α-glucosidase inhibition (%)
1	14.9
2	not active
3	7.2
4	46.2
5	2.1
6	83.7
7	20.0
8	28.1
1-deoxynojirimycin[b]	100

[a] At 0.1 mg/mL (final concentration). [b] Used as positive control.

5-methoxy-9β-xylopyranosyl-(−)-isolariciresinol.[5] During the present investigation, no alkaloids were detected in *C. madagascariensis* by spraying a developed TLC plate of the crude plant extract with Dragendorff reagent. Except for capsicodendrin, no dimeric compounds were present in *C. madagascariensis*. Polygodial (**5**) was isolated from *C. madagascariensis*, while isopolygodial has been detected only from *C. macrocarpa*. The present results represent the first detection of pungent unsaturated dialdehyde compound **5** apart from cinnamodial (**6**) in *Cinnamosma* species. The three species of *Cinnamosma* contain quite similar amounts of δ-tocotrienol. Lignoceric acid was isolated from *Cinnamosma* species for the first time.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 digital polarimeter. FT-IR spectra were recorded on a Horiba FT-710 spectrophotometer. UV spectra were measured with a JASCO V-520 UV/vis spectrometer. ^1H and ^{13}C NMR spectra were recorded on a JEOL α-400 spectrometer (400 and 100 MHz, respectively) with TMS as internal standard. HRESIMS were carried out on an Applied Biosystems QSTAR XL system mass spectrometer. Silica gel, Sephadex, column chromatography, and reversed-phase [octadecyl silica (ODS) gel] open column chromatography were performed on silica gel 60 (Merck, 70–230 mesh), Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Upsala, Sweden), and Cosmosil 75C18-OPN (Nacalai Tesque Co., Ltd., Kyoto, Japan). Preparative HPLC was performed using ODS-120T (TSK gel, $B = 10$ mm, $L = 28$ cm; Tosoh, Tokyo, Japan).

Plant Material. The bark of *C. madagascariensis* was collected in Sakaraha, near Tulear, Madagascar, in December 2006 by one of the authors (L.H.) and was identified by comparison with an authentic sample in the Herbarium of PBZT (Parc Botanique et Zoologique de Tsimbazaza, Antananarivo Madagascar). A voucher specimen (LivCINMAD2006) was deposited at the Graduate School Biomedical Sciences, Department of Pharmacognosy, Hiroshima University, Japan.

Extraction and Isolation. Powdered *C. madagascariensis* bark (292 g) was extracted with EtOAc (2 L) at room temperature for a week. The extract was filtered and concentrated in vacuo to yield a dark brown residue (23 g). The residue was dissolved in MeOH to obtain 3 g of white precipitate (D-mannitol). The remaining solution was evaporated and suspended in water before partition with ethyl acetate to afford 15.2 g of residue. White crystals (cinnamosmolide: **11**, 11.3 g) were obtained by dissolving the ethyl acetate fraction in hexane−ethyl acetate (7:3). The remaining solution was divided into three fractions by size-exclusion column chromatography on Sephadex LH-20 (solvent system: methanol−CH_2Cl_2, 9:1). ODS flash column chromatography of fraction 2 gave eight subfractions. Compounds **7** (1.5 mg), **11** (2.3 mg), and **4** (3 mg) were obtained from preparative TLC (solvent system: hexane−ethyl acetate, 4:1) of subfraction 2-1. Silica gel column chromatography of subfraction 2-7 (solvent system: hexane−ethyl acetate, 4:1) afforded compound **12** (17 mg). ODS HPLC (solvent system: 60% aqueous CH_3CN) of subfractions 2-2 and 2-3 afforded compounds **4** (32 mg), **11** (13 mg), **7** (19 mg), **8** (25 mg), **9** (3 mg), and **2** (4.2 mg). Lignoceric acid (**10**, 23 mg) was precipitated from subfraction 2-4. The mother liquor of the latter was subjected to ODS HPLC (solvent system: 90% aqueous MeOH) to give compound **5** (75 mg). Capsicodendrin (**6**, 40 mg) was crystallized from fraction 2-5. Purification of subfraction 2-7 by ODS HPLC (solvent system: 50% aqueous CH_3CN) yielded compounds **1** (5.4 mg) and **3** (3.5 mg).

Cinnamadin (1): amorphous powder, [α] −15.2 (*c* 0.2, $CHCl_3$); IR ν_{max} 3420, 2925, 2854, 1730, 1675, 1508, 1239, 1026 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 225 (4.22) nm; ^1H NMR and ^{13}C NMR spectra (see Tables 1 and 2); positive HRESIMS *m/z* 331.1511 $[M + Na]^+$ ($C_{17}H_{24}O_5Na$, requires 331.1521).

Cinnamodial 11α,12β-dimethyl acetal (2): amorphous powder, [α] −99.2 (*c* 0.28, MeOH); IR ν_{max} 1725, 1508, 1019 cm^{-1}; ^1H NMR and ^{13}C NMR spectra (see Tables 1 and 2); positive HRESIMS *m/z* 377.1934 $[M + Na]^+$ ($C_{19}H_{30}O_6Na$, requires *m/z* 377.1940).

X-ray Crystallographic Analysis of 8. The chloro derivative (**8**, 110 mg) of cinnamosmolide (**7**, 180 mg) was prepared following a literature procedure.[9] Crystal data: Colorless crystal; $C_{17}H_{23}ClO_4$, $M_r = 326.80$, orthorhombic, $P2_12_12_1$, $a = 7.9937(15)$ Å, $b = 13.664(3)$ Å, $c = 15.543(3)$ Å, $V = 1697.7(6)$ Å^3, Mo Kα radiation, $\lambda = 0.71073$, 10 353 reflections, 203 parameters; only coordinates of H atoms refined. Final *R* indices $[I > 2\sigma(I)]$ $R_1 = 0.0505$, $wR_2 = 0.1119$, *R* indices (all data) $R_1 = 0.0868$, $wR_2 = 0.1259$. The atomic ordinates and equivalent isotropic deplacement parameters, as well as a full list of bond distances and angles and the structure factor table, are deposited as Supporting Information at the Cambridge Crystallographic Data Centre (deposition number: CCDC 659441). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

α-Glucosidase Inhibition Assay. The assay was performed according to the method described by Oki and co-workers, with slight modifications.[13] α-Glucosidase was purchased from Toyobo Co., Ltd.

(Osaka, Japan). The enzyme solution was prepared by dissolving 0.6 U/mL of α-glucosidase in 100 mM phosphate buffer (pH 7) containing 2 g/L bovine serum albumin and 0.2 g/L NaN_3. *p*-Nitrophenyl-α-D-glucopyranoside (5 mM) in the same buffer solution (pH 7) was used as a substrate solution. The enzyme solution (50 μL) and the test compounds (10 μL), dissolved in DMSO to a final concentration of 0.1 mg/mL, were mixed in each well of the microliter 96-well culture plates and measured spectrophotometrically (absorbance 405 nm) at zero time, using a microplate reader (Bio-Rad model 550 microplate reader). The mixture was preincubated for 5 min at room temperature before the addition of substrate solution (50 μM) and followed by a 5 min incubation at room temperature. The increase in absorbance from zero time was measured. The inhibitory activity was expressed as 10 minus the relative absorbance difference (%) of test compounds to absorbance change of the control where the test solution was replaced by DMSO. Experiments were performed in triplicate, and the averages were calculated and are presented in Table 3. 1-Deoxynojirimycin (Wako Pure Chemical Industries, Ltd.) 0.3 mM in DMSO was used as a positive control.

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Supporting Information Available: Table showing distribution of the chemical constituents in *Cinnamosma* species and a figure showing the structures of known compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

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