Cinnamacrins A−C, Cinnafragrin D, and Cytostatic Metabolites with α-Glucosidase Inhibitory Activity from *Cinnamosma macrocarpa*

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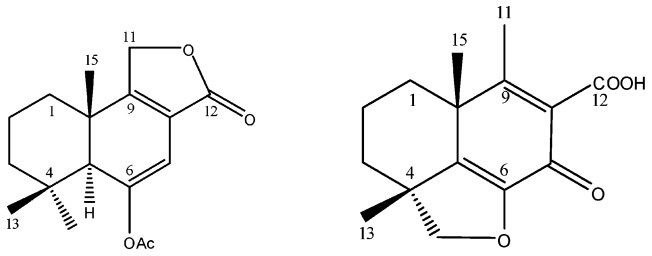
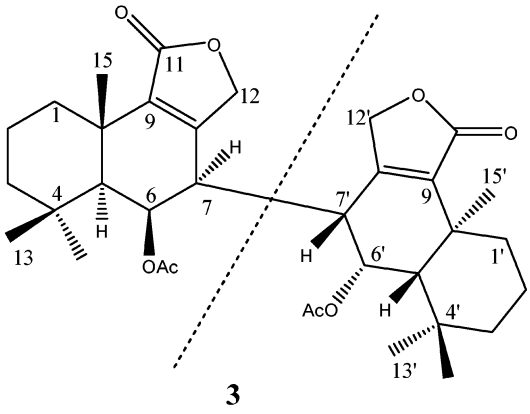
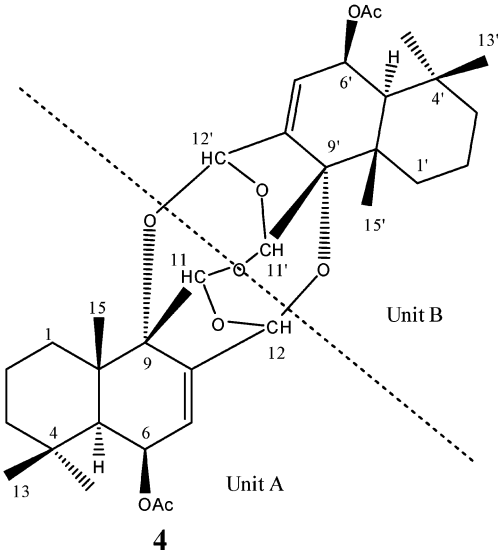
Two new monomeric and two new dimeric drimane sesquiterpenes, cinnamacrins A−C (**1**−**3**) and cinnafragrin D (**4**), along with bemadienolide (**5**), capsicodendrin (**6**), cinnamodial (**7**), cinnamolide (**8**), isopolygodial (**9**), and δ-tocotrienol (**10**), were isolated from *Cinnamosma macrocarpa*. The structures of the new compounds were determined by physical, chemical, and spectroscopic evidence. Capsicodendrin (**6**) and/or cinnamodial (**7**) are the major compounds in *C. fragran*s and *C. macrocarpa*, which are both widely used in Malagasy traditional medicine. The cytostatic activity as well as α-glucosidase inhibition and antiviral activities of the major constituents **6** and **7** and the compounds previously isolated from *C. fragrans* were evaluated.

Canellaceae is one of many unique plant families of which all of its species are widely used in traditional medicine where such plants grow. The family contains five genera: the South American *Capsicodendron* (=*Cinnamodendron*) and *Pleodendron*, the African *Warburgia*, the Malagasy *Cinnamosma*, and the American (Florida) *Canella*. Drimane-type sesquiterpenoids are found to be the chemosystematic markers of the family, and interestingly, all of the investigated species belonging to the five genera contain cinnamodial and/or warburganal.[1−3] The three species of *Cinnamosma* (*C. fragrans*, *C. macrocarpa*, and *C. madagascariensis*, all locally known as "mandravasarotra" = for every ailment) are traditionally used in the treatment of malarial symptoms, fatigue, and muscular aches.[4] The plants belonging to this genus are a rich source of drimane-type sesquiterpenoid dialdehyde and lactones, including the cytotoxic unsaturated dialdehyde cinnamodial (**7**), which is one of the compounds responsible for its pungent taste. Our recent investigation of the methanol extract of the bark of *C. fragrans*, a tree widely distributed in the northwestern and east central part of Madagascar, demonstrated the presence of dimeric and trimeric drimane sesquiterpenes, which can be converted to the bioactive compound **7**.[1] Unsaturated dialdehydes are reported to have a wide range of activities including antibacterial, antifungal, anti-inflammatory, and antiviral.[5,6] It is noteworthy that the δ-tocotrienol (**10**) content in *C. fragrans* is quite high (ca. 0.07%).[1] As mentioned earlier, *C. macrocarpa* is used in Malagasy pharmacopoea in the same way as *C. fragrans*, where the common compounds of both plants are undoubtedly the active constituents. The presence of δ-tocotrienol in the two species is one reason that they are used in traditional medicine.[1] In this report, the following three issues are addressed: (1) the phytochemical investigation of *C. macrocarpa*, collected from Ranomafana (Fianarantsoa, Madagascar), (2) the evaluation for cytostaticity and antiviral nature of the major compounds, and (3) the α-glucosidase inhibitory activity of the isolated metabolites compared with those previously found in *C. fragrans*.







Results and Discussion

1. Chemistry. The EtOAc extract of *Cinnamosma macrocarpa* was repeatedly chromatographed on Sephadex LH-20, silica gel, and ODS RP-18 column chromatography to give two new mono-

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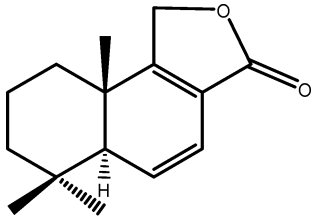

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Figure 1. Structures of compounds **1**−**5**.

meric (**1**, **2**) and two new dimeric sesquiterpene drimanes (**3**, **4**) (Figure 1), together with bemadienolide (**5**), capsicodendrin (**6**), cinnamodial (**7**), cinnamolide (**8**), isopolygodial (**9**), and δ-tocotrienol (**10**). The structures of the known compounds were identified through the interpretation of their physical and spectroscopical data and compared with those reported.[1,7−12] The structure of capsicodendrin (**6**) was revised in our recent investigation,[1] while that of compound **8** was confirmed by X-ray crystallographic analysis.

Positive HREIMS analysis of cinnamacrin A (**1**) showed a molecular ion at *m*/*z* 290.1511 $[M]^+$ corresponding to the molecular formula $C_{17}H_{22}O_4$. The IR spectrum suggested the presence of an α,β-unsaturated γ-lactone carbonyl and an *O*-acetyl group (ν_{max} 1779, and 1728 and 1244 cm^{-1}, respectively). The ^1H NMR spectrum (Table 1) displayed resonances for three quaternary methyl protons (δ 1.11, 1.14, and 1.15, each singlet), one deshielded acetoxy-methyl (δ 2.22, s), an olefinic proton (δ 6.11, d, $J = 2.6$ Hz, H-7), and two oxymethylene protons (δ 4.75, d, $J = 17.3$ Hz, H-11a and δ 4.83, d, $J = 17.3$ Hz, H-11b). The presence of 17 resonances, including one lactone and an *O*-acetyl carbonyl (δ 171.2, and 168.2, respectively), and four olefin carbons, one of which was bearing an oxygen atom (δ 151.5), was evident in the ^{13}C NMR spectrum. Comparison of the ^1H and ^{13}C NMR data of **1** (Tables 1 and 2) with those of bemadienolide (**5**) revealed that the presence of an *O*-acetyl group and the lack of an olefinic proton in **1** were the differences between the two compounds. To establish the full structure of **1**, extensive 2D NMR analysis was performed. HMBC correlations (Figure 2) between H-7 and C-5, C-6, C-8, and C-9 were observed, allowing the allocation of the proton at δ 6.11 to be at C-7. The location of the oxygen-bearing methylene at C-11 was substantiated by the long-range correlations between H-11a,b and C-10 and between H-11a,b and C-12. Thus, the only possibility for the attachment of the *O*-acetyl group must be at C-6. This was confirmed by the deshielded *O*-acetyl resonance at δ 2.22 (s). The absolute configuration of **1** was deduced to be the same as **5** ($[\alpha]_D^{19} = +36.3$) since both have positive optical rotation values.[9] From the above data, the structure of cinnamacrin A was established as 6-acetoxy-6,8-drimadien-12,11-olide.

Cinnamacrin B (**2**) exhibited a molecular formula of $C_{15}H_{18}O_4$, as indicated by the positive HREIMS mass (*m*/*z* 262.1208 $[M]^+$). The IR bands at ν_{max} 3392, 1730, and 1725 cm^{-1} suggested the presence of a carboxylic acid and a conjugated ketone in the six-membered ring. The ^1H NMR spectrum showed three methyl proton resonances, one of which is attached to an olefinic carbon (δ 1.59, s), and nonequivalent oxymethylene protons (δ 5.00, d, $J = 17.5$ Hz, H-14a and δ 5.06, d, $J = 17.5$ Hz, H-14b). The 15 resonances, including a carboxylic acid (δ 176.8), a conjugated ketone (δ 204.6), and an oxygen-bearing olefinic carbon (δ 150.8), in the ^{13}C NMR spectrum were ascribed to a drimane skeleton in which one of the methyl groups was replaced by an oxymethylene. The allocation of the ketone to be at C-7, the methyl at an sp^2 carbon (C-9), and the oxymethylene at C-14 was supported by interpretation of the COSY, HMBC, and NOESY data. The long-range correlations (Figure 3) between the methyl protons at δ 1.42 and C-10 and C-1 and between the most deshielded methyl (δ 1.59) and C-10 and C-8 substantiated the locations of the methyls at C-11 and C-15.

Table 1. ^1H NMR Data for Compounds **1**−**3** (600 MHz, in $CDCl_3$)[a]

position	**1**	**2**	**3**
1a	1.53 (m)	1.49 (m)	1.20 (m)
1b	1.68 (m)	2.01 (m)	1.75 (m)
2a	1.72 (m)	1.70 (m)	1.60 (m)
2b	1.92 (m)	1.95 (m)	1.82 (m)
3a	1.28 (td, 14.0, 4.4)	1.49 (m)	1.29 (td, 14.0, 4.2)
3b	1.48 (dm, 14.2)	2.62 (ddd, 14.0, 9.0, 4.9)	1.56 (m)
4			
5	2.64 (d, 2.7)		1.47 (s)
6			5.65 (brs)
7	6.11 (d, 2.6)		2.58 (brd, 9.3)
8			
9			
10			
11a	4.75 (d, 17.3)	1.59 (s)	
11b	4.83 (d, 17.3)		
12a			4.61 (d, 16.5)
12b			4.70 (d, 16.5)
13	1.15 (s)	1.41 (s)	1.02 (s)
14a	1.11 (s)	5.00 (d, 17.5)	1.05 (s)
14b		5.06 (d, 17.5)	
15	1.14 (s)	1.42 (s)	1.53 (s)
CH$_3$C=O	2.22 (s)		2.08 (s)
1′a			1.20 (m)
1′b			1.75 (m)
2′a			1.60 (m)
2′b			1.82 (m)
3′a			1.29 (td, 14.0, 4.2)
3′b			1.56 (m)
4′			
5′			1.47 (s)
6′			5.65 (brs)
7′			2.58 (brd, 9.3)
8′			
9′			
10′			
11′			
12′a			4.61 (d, 16.5)
12′b			4.70 (d, 16.5)
13′			1.02 (s)
14′			1.05 (s)
15′			1.53 (s)
CH$_3$C=O			2.08 (s)

[a] Assignment based on HSQC, COSY, and HMBC.

Hence, the location of the carboxylic acid must be at C-8. The cross-peak from the oxymethylene protons to C-6 and C-5 in the HMBC spectrum, together with the NOE correlation between H-14a and 13-CH_3 protons, allowed us to locate the oxymethylene at C-14 and the oxygenated olefinic carbon at C-6. The above data along with the observation of COSY correlations from H-1 to H-3 demonstrated that the ketone must be located at C-7. Therefore the structure of **2** was deduced to be 6,14-epoxy-5,8-drimadien-7-oxo-12-oic acid, named cinnamacrin B.

The dimeric nature of cinnamacrin C (**3**) was substantiated by its high-resolution FABMS, which exhibited a sodiated molecular ion at *m*/*z* 605.3198 $[M + Na]^+$ equivalent to a molecular formula of $C_{34}H_{46}O_8Na$ (requires *m*/*z* 605.3193). The IR spectrum showed the presence of an α,β-unsaturated γ-lactone carbonyl and an acetyl group (ν_{max} 1762, and 1729 and 1244 cm^{-1}, respectively). The ^{13}C NMR data, however, displayed 17 resonances (Table 2), suggesting that **3** was a symmetrical dimeric compound. In the ^1H NMR spectrum, the resonances of three quaternary methyls (δ 1.02, 1.05, and 1.53, each singlet), two methine protons, one of which was bearing an oxygen atom (δ 2.58, brd, $J = 9.3$ Hz, H-7 and δ 5.65, brs, H-6), and oxymethylene protons (δ 4.61, d, $J = 16.5$ Hz; δ 4.70, d, $J = 16.5$ Hz) could be ascribed to a drimane skeleton.[1,12] Interpretation of the COSY data revealed the presence of two partial

Table 2. ^{13}C NMR Data for Compounds **1**−**3** (150 MHz, in $CDCl_3$)

position	**1**	**2**	**3**
unit A			
1	33.9	36.8	37.2
2	18.0	16.2	18.4
3	43.3	27.5	43.2
4	33.1	36.4	33.9
5	53.9	145.8	49.4
6	151.5	150.8	73.1
7	106.9	204.6	37.6
8	122.0	146.0	158.0
9	167.0	170.0	138.5
10	38.8	40.3	35.0
11	67.8	26.4	172.3
12	171.2	176.8	69.5
13	15.8	27.6	33.6
14	22.8	67.3	22.9
15	34.2	27.2	20.4
CH$_3$C=O	21.8		21.5
CH$_3$*C*=*O*	168.2		171.6
unit B			
1′			37.2
2′			18.4
3′			43.2
4′			33.9
5′			49.4
6′			73.1
7′			37.6
8′			158.0
9′			138.5
10′			35.0
11′			172.3
12′			69.5
13′			33.6
14′			22.9
15′			20.4
CH$_3$C=O			21.5
CH$_3$*C*=*O*			171.6

structures: $-CH_2-CH_2-CH_2-$ and $-CH-CHO-CH-$. The observation of HMBC correlations between the two protons at δ 4.61 and 4.70 with C-7, C-8, C-9, and C-11 carbonyl allowed us to locate the oxymethylene protons at C-12 forming an α,β-unsaturated γ-lactone ring. The remaining oxygen-bearing carbon must be at C-6, since cross-peaks between H-6 and C-4 and between C-5 and C-8 were observed. The *O*-acetyl group was thus attached at C-6, with an axial orientation due to the coupling pattern (broad) and the NOESY correlations between H-6 and H-5, H-7, and the C-14 methyl protons. In the COSY spectrum, H-6 was the only proton that could correlate with the C-7 methine proton. However, the ^1H NMR spectrum showed H-7 as a broad doublet (J = 9.3 Hz) and the coupling between H-7 and H-7′ could be deduced since H-6 resonated as a broad singlet. This fact led to the conclusion that the two monomers were connected at C-7. Moreover, the axial orientation of H-7 (H-7′) was confirmed by the NOESY cross-peak between H-7 (H-7′) and H-5 (H-5′) and between H-7 (H-7′)

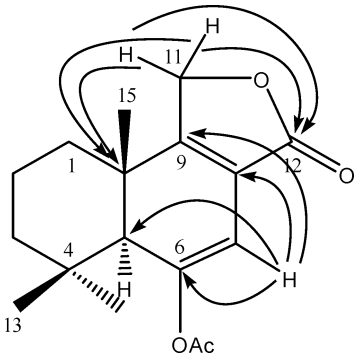

Figure 2. HMBC correlations observed in **1**.

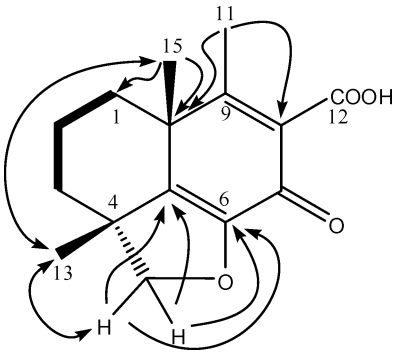

Figure 3. Correlations observed in **2** (bold lines: COSY; arrow: HMBC; double arrow: NOE).

and H-6 (H-6′), and the attachment of the two monomers was ascertained by the HMBC correlation between H-7 and C-7′ (and vice versa). The absolute configuration of **3**, similar to that of a normal drimane,[1] was substantiated from the negative Cotton effect ($\Delta\epsilon_{216}$ −3.2) by using the CD empirical rule for lactones and enones.[13,14] From the above data, the complete structure of cinnamacrin C was deduced as shown.

In the positive high-resolution FABMS analysis of compound **4** (cinnafragrin D), a sodiated molecular ion at *m*/*z* 621.3054 [M + Na]$^+$ corresponding to a molecular formula of $C_{34}H_{46}O_9Na$ (requires 621.3040) indicated that this compound corresponded to a dimeric cinnamodial (**7**) analogue. The IR data showed absorption of *O*-acetyl groups (ν_{max} 1732 and 1240 cm^{-1}), and the ^1H NMR spectrum displayed resonances of three quaternary methyl protons (δ 0.93, 1.00, 1.14, each singlet), an *O*-acetyl methyl (2.07, s), two acetal protons (δ 5.47, s), an oxymethine proton (δ 5.75, dd, J = 5.5, 3 Hz), and an olefinic methine proton (δ 5.91, d, J = 3 Hz). The above data coupled with 12 degrees of unsaturation as shown in HRFABMS data accounted for the symmetrical nature of **4**. In the ^{13}C NMR and DEPT spectra, 17 resonances (four methyls including one acetoxy-methyl, three methylenes, five methines, two of which are acetal carbons, and five quaternary carbons, Table 3) could be observed. The ^1H and ^{13}C NMR data were similar to those of cinnafragrin A (**11**), previously isolated from *C. fragrans*.[1] However only two acetal carbon resonances (δ 98.1, 99.6) and no resonance of an aldehyde carbon were observed in **4**, suggesting that acetalization at C-11 had occurred to give a symmetrical compound having two sets of two equivalent acetal carbons. The complete structure of **4** including the relative configurations of C-11, C-11′, C-12, and C-12′ was substantiated through the interpretation of the 2D NMR data including NOESY and HMBC. The presence of HMBC correlations between H-7 and C-5, C-6, C-8, C-9, and C-12, as well as that observed between H-6 and the *O*-acetyl carbonyl (δ 170.0), accounted for the location of the acetyl group at C-6 and C-6′. Because the HMBC experiment could not provide evidence to support the connectivity between the symmetrical acetals, the potential of NOESY correlations (Figure 4) was investigated. Thus, the most stable conformation of **4** was optimized by calculating the minimum energy (270 kcal·mol^{-1}) using MMFF-94 (Merck Molecular Force Field) parameters on MOE (molecular operating environment) software.[15] The NOE cross-peaks observed between H-11 (H-11′) and H-1a (H-1a′), H-12 (H-12′), and H-15 (H-15′); H-12′ (H-12) and H-5 (H-5′); C-14 (C-14′) methyl protons and H-12 (H-12′); and H-15 (H-15′) and H-11 (H-11′) and H-12 (H-12′) supported the location of the acetal groups at C-11 (C-11′) and C-12 (C-12′). Although the absolute configuration of **4** could not be determined, it is assumed to be the same as the drimane isolated from the same plants and that of cinnafragrins A−C (Figure 5) previously found in *C. fragrans*.[1] Interestingly, unlike cinnafragrin A (**11**) and capsicodendrin, cinnafragrin D (**4**) in pyridine

Table 3. ^{1}H and ^{13}C NMR Data for Compound **4** (In $CDCl_3$)[a]

position	H	C
unit A		
1a	1.28 (m)	30.5
1b	1.90 (td, 13.2, 4.4)	
2a	1.56 (m)	18.0
2b	1.63 (dt, 13.4, 3.3)	
3a	1.28 (m)	44.5
3b	1.57 (brd, 13.4)	
4		33.7
5	2.32 (d, 5.5)	44.1
6	5.75 (dd, 5.5, 3.0)	67.2
7	5.91 (d, 3.0)	124.9
8		141.6
9		72.3
10		38.0
11	5.47 (s)	99.6
12	5.47 (s)	98.1
13	1.14 (s)	24.6
14	0.93 (s)	16.8
15	1.00 (s)	32.1
CH$_3$C=O	2.07 (s)	21.6
CH$_3$*C*=*O*		170.0
unit B		
1′a	1.28 (m)	30.5
1′b	1.90 (td, 13.2, 4.4)	
2′a	1.56 (m)	18.0
2′b	1.63 (dt, 13.4, 3.3)	
3′a	1.28 (m)	44.5
3′b	1.57 (brd, 13.4)	
4′		33.7
5′	2.32 (d, 5.5)	44.1
6′	5.75 (dd, 5.5, 3.0)	67.2
7′	5.91 (d, 3.0)	124.9
8′		141.6
9′		72.3
10′		38.0
11′	5.47 (s)	99.6
12′	5.47 (s)	98.1
13′	1.14 (s)	24.6
14′	0.93 (s)	16.8
15′	1.00 (s)	32.1
CH$_3$C=O	2.07 (s)	21.6
CH$_3$*C*=*O*		170.0

[a] Assignment based on HSQC, COSY, and HMBC.

solution did not convert to its monomer (cinnamodial, **7**) after standing at room temperature overnight. This finding supported our suggestion on the conversion mechanism of capsicodendrin and **11**.[1] Further investigation concerning this topic is in progress.

2. Biological Activity. 2.1. α-Glucosidase Inhibitory Activity. All of the compounds (**1**−**10**) isolated from *C. macrocarpa* during the present investigation, together with those from *C. fragrans*,[1] were evaluated for their α-glucosidase inhibitory activity (Table 4). Capsicodendrin (**6**) and its monomer cinnamodial (**7**), the major compounds in both species, showed very strong inhibition (89.4% and 78.2%, respectively). Cinnafragrin A (**11**), the capsicodendrin epimer, however was inactive. The absence of activity of **11** might be interpreted to be due to the 12-*S* configuration. However, for the *R* configuration as in the case of capsicodendrin and cinnafragrin B, the distance between the aldehyde and the hydroxyl group at C-12′ is rather large. Collectively, the presence of the C-9 aldehyde group with the 12-*R* configuration seems to be important for the activity. This fact was supported by the observation of slight activity (26.5%) in cinnafragrin B (**12**), a methyl derivative of capsicodendrin. Examining all the data indicated that the C-12′ hydroxyl group plays an important role since the replacement of the C-12′ hydroxyl by a methoxyl group decreased the activity about 4-fold. The activity of **6** was intensified by the conversion of capsicodendrin to cinnamodial during the biological test. Interestingly, a small amount of **7** could be observed in the ^{1}H NMR spectrum of a solution of **6** in DMSO after allowing it to stand at room temperature overnight. The inhibitory activity of **6** is thus partially related to that of **7**.

In conclusion, the C-9 aldehyde group in drimane sesquiterpenes and C-12′ hydroxyl in cinnafragrins are necessary for strong α-glucosidase inhibition. Furthermore, since isopolygodial (**9**) did not show any activity, the β-orientation of the C-9 aldehyde group is deemed to be very important.

2.2. *In Vitro* Cytostatic and Antiviral Activity. Capsicodendrin (**6**) might be used by the plant as a reserve substance.[1] Compound **6** may convert to the bioactive compound cinnamodial (**7**) during the assay. Both compounds **6** and **7** exhibited strong cytostatic effects on the proliferation of murine leukemia L1210/0 and human T-lymphocyte Molt4/C8 and CEM/0 cells (Table 5), yet neither compound **6** nor **7** exhibited any specific antiviral effects against the different viruses evaluated [herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2), vaccinia virus (VV), vesicular stomatitis virus (VSV), Coxsackie B4 virus (CV-B4), or respiratory syncytial virus (RSV)] at concentrations that were not toxic to the host cells (Table 6).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ as solvent. IR spectra were measured on a Perkin-Elmer Spectrum One FT-IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity 600 NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) using $CDCl_3$ as a solvent. Chemical shifts are given relative to TMS (δ 0.00) as an internal standard (^{1}H) and δ 77.0 (ppm) from $CDCl_3$ as a standard (^{13}C). Mass spectra were recorded on a JEOL JMS AX-500 spectrometer. Column chromatography was carried out on Sephadex LH-20 (Amersham Pharmacia Biotech) and silica gel (Kieselgel 60: 0.040−0.063, Merck). Preparative HPLC was performed using a Cosmosil reversed-phase column, JASCO 880-PU pump, JASCO 875-UV UV detector, and ERC-7512 Erma CR Inc., RI detector.

Plant Material. *C. macrocarpa* was collected in Ranomafana (Fianarantsoa, Madagascar) in July 2005 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 (LivCM2005) was deposited at the Faculty of Pharmaceutical Sciences, Tokushima Bunri University.

Extraction and Isolation. Powdered *C. macrocarpa* (49 g) was extracted with EtOAc (500 mL) at room temperature for 2 months. The extract was filtered and concentrated *in vacuo* to yield a brown residue (5 g). The latter was divided into nine fractions by column chromatography on silica gel (solvent system: hexane/EtOAc gradient, from 8:2 to 100% EtOAc). ODS flash column chromatography of fraction 4 gave eight fractions. Compounds **2** (2.4 mg), **8** (22 mg), and **9** (3.1 mg) were obtained from the subfraction 4-1 by purifying it on ODS HPLC (70% aqueous CH_3CN), while fraction 4-7 afforded δ-tocotrienol (**10**, 80 mg). Fraction 5 was subjected to ODS RP-18 CC (solvent system: MeOH/H_2O, 9:1) to give seven subfractions. Capsicodendrin (**6**, 35 mg) precipitated from fraction 5-8. HPLC separation of fraction 5-1 (70% aqueous CH_3CN) afforded compounds **1** (6.7 mg), **5** (8.5 mg), and **7** (10.2 mg). Compound **3** (12.1 mg) was isolated from the fraction rich in fatty acid (fraction 5-5) by using HPLC (solvent system: 90% aqueous CH_3CN). Precipitation in MeOH of the fraction 5-6 afforded compound **4** (3.8 mg).

Cinnamacrin A (1): oil, $[\alpha]_D^{19}$ +123.2 (*c* 0.3, $CHCl_3$); IR 2940, 1779, 1728, 1244, 889 cm^{-1}; ^{1}H NMR and ^{13}C NMR spectra (see Tables 1 and 2); positive HREIMS *m*/*z* 290.1511 [M]$^+$ ($C_{17}H_{22}O_4$, requires 290.1504).

Cinnamacrin B (2): oil, $[\alpha]_D^{18}$ −108.3 (*c* 0.12, $CHCl_3$); IR 3392, 1730, 1725, 1519, 1134, 955 cm^{-1}; ^{1}H NMR and ^{13}C NMR spectra (see Tables 1 and 2); positive HREIMS *m*/*z* 262.1208 [M]$^+$ ($C_{15}H_{18}O_4$, requires *m*/*z* 262.1205).

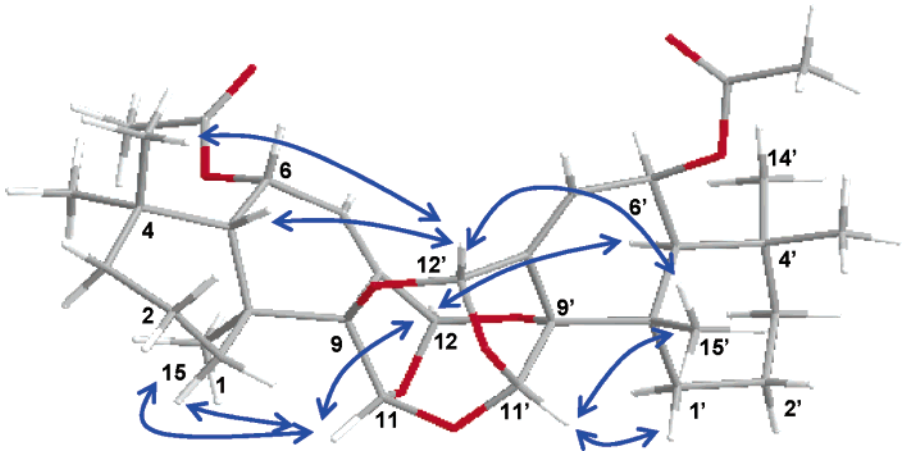

Figure 4. NOESY correlations observed in **4** optimized by MOE software.

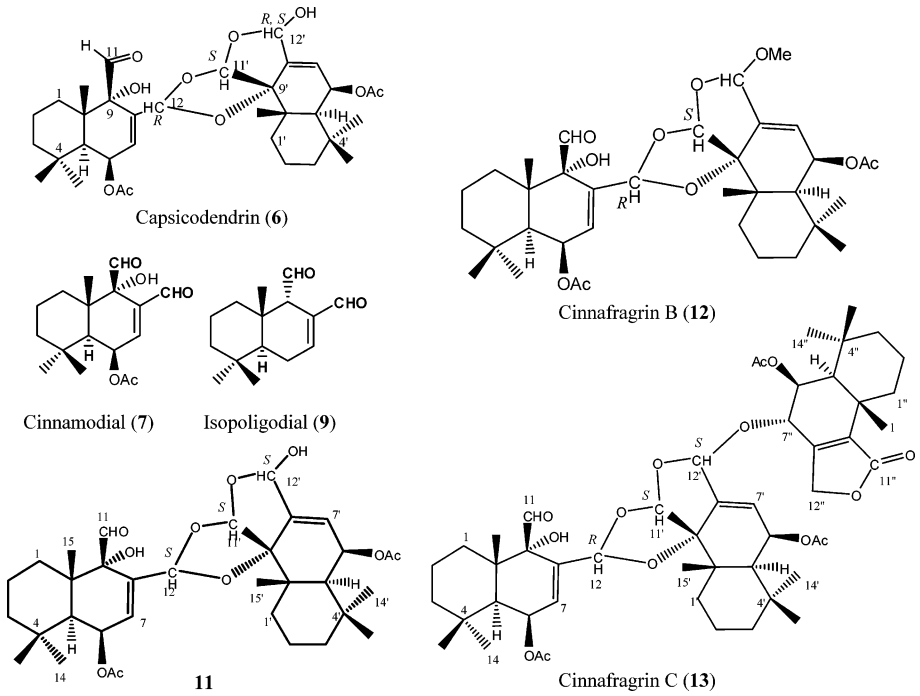

Figure 5. Structures of compounds **6**, **7**, **9**, and **11**−**13**.

Table 4. α-Glucosidase Inhibition Activities of Compounds **1**−**13**

compound[a]	α-glucosidase inhibition (%)
1	not active
2	not active
3	not active
4	not active
5	not active
6	89.4
7	78.2
8	not active
9	not active
10	not active
11	not active
12	26.5
13	not active
1-deoxynojirimycin	100

[a] At 1.20 mg/200 μL, 1-deoxynojirimycin as standard.

Cinnamacrin C (3): oil, $[\alpha]_D^{19}$ −47.5 (*c* 0.6, $CHCl_3$); IR 1762, 1729, 1375, 1244, 949 cm^{-1}; CD $\Delta\epsilon_{216}$ −3.2 (*c*, 0.1 MeOH); ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2); Positive HRFABMS *m*/*z* 605.3198 $[M + Na]^+$ ($C_{34}H_{46}O_8Na$, requires *m*/*z* 605.3193).

Table 5. Inhibitory Effects of Compounds **6** and **7** on the Proliferation of Murine Leukemia Cells (L1210/0) and Human T-Lymphocyte Cells (Molt4/C8, CEM/0)

	IC_{50} (μM)[a]		
compound	L1210/0	Molt4/C8	CEM/0
capsicodendrin (**6**)	1.21 ± 0.04	0.94 ± 0.16	0.86 ± 0.01
cinnamodial (**7**)	0.58 ± 0.06	1.51 ± 0.93	1.61 ± 1.03

[a] 50% inhibitory concentration or compound concentration required to inhibit tumor cell proliferation by 50%.

Cinnafragrin D (4): amorphous powder, $[\alpha]_D^{20}$ −71.3 (*c* 0.04, $CHCl_3$); IR 2920, 1732, 1240, 1022 cm^{-1}; ^1H NMR and ^{13}C NMR spectra (see Table 3); HRFABMS *m*/*z* 621.3054 $[M + Na]^+$ ($C_{34}H_{46}O_9Na$, requires 621.3040).

Bioassay. Enzyme Inhibition Assay. α-Glucosidase inhibitory activity was performed according to the method described by Oki and co-workers with slight modifications.[16] α-Glucosidase was purchased from TOYOBO Co. Ltd, Osaka, Japan. The enzyme solution (ES) was prepared by dissolving 0.6 U/mL of α-glucosidase in 100 mM

Table 6. Cytotoxicity and Antiviral Activities of Compounds **6** and **7** in E_6SM and HeLa Cell Cultures

compound	minimal cytotoxic concentration[a] (μM)	minimum inhibitory concentration[b] (μM)				
		HSV-1 (KOS)	HSV-2 (G)	VV	VSV	HSV-1 TK KOS ACV^r
6	≥1.03	>1.03	>1.03	>1.03	>1.03	>1.03
7	≥2.06	>2.06	>2.06	>2.06	>2.06	>2.06
acyclovir (μM)	>500	0.48	0.48	>500	>500	60
HeLa						

compound	minimum cytotoxic concentration[a] (μM)	minimum inhibitory concentration (μM)		
		VSV	CV B4	RSV
6	5.16	>1.03	>1.03	>1.03
7	10.31	>2.06	>2.06	>2.06
ribavirin (μM)	>500	300	>500	60

[a] Required to cause a microscopically detectable alteration of normal cell morphology. [b] Required to reduce virus-induced cytopathogenicity by 50%.

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 (SS). The ES (50 μL) and the test compounds (10 μL) were dissolved in DMSO at a concentration of 1 mM and then were mixed in each well of the microliter 96-well culture plates and measured spectrophotometrically (Abs 415 nm) at zero time by using a microplate reader (BIO-RAD model 550 microplate reader). The mixture was preincubated for 5 min at room temperature before SS addition (50 μM) followed by 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 test solution was replaced by DMSO. Experiments were performed in triplicate, and the averages were calculated and are presented in Table 4. 1-Deoxynojirimycin (Wako Pure Chemical Industries, Ltd.) 0.3 mM in DMSO was used as positive control.

***In Vitro* Cytostatic and Antiviral Activity.** Procedures for measuring cytotoxicity, cytostatic activity, and antiviral activity have been described previously.[17−19]

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Supporting Information Available: X-ray crystal structure of **8**, tables of crystal data and structure refinement, as well as the full list of bond lengths and angles from the X-ray crystallographic study of **8** are available free of charge via the Internet at http://pubs.acs.org.

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