Phytochemistry and Antifungal Properties of the Newly Discovered Tree *Pleodendron costaricense*

Virginie Treyvaud Amiguet,[†] Philippe Petit,[†] Chieu Anh Ta,[†] Ronaldo Nuñez,[§] Pablo Sánchez-Vindas,[§] Luis Poveda Alvarez,[§] Myron L. Smith,[‡] John Thor Arnason,[†] and Tony Durst*[,†]

Department of Biology and Chemistry, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5, Herbario Juvenal Valerio Rodriguez, Universidad Nacional, Heredia, Apartado 86-3000, Costa Rica, and Department of Biology, Carleton University, Ottawa, Ontario, Canada, K1S 5B6

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Gas chromatography analysis of the essential oils of leaves and bark collected from the newly discovered tree *Pleodendron costaricense* identified α-pinene, *β*-pinene, *β*-myrcene, *β*-thujene, and *β*-caryophyllene as their major constituents. Phytochemical analysis of *P. costaricense* parts led to the isolation and identification of *δ*-tocotrienol, *β*-sitosterol, four known drimane-type sesquiterpenes, cinnamodial (**1**), cinnamosmolide (**2**), polygodial (**3**), and mukaadial (**4**), and two new compounds, a drimane-type sesquiterpene, parritadial (**5**), and an eremophilane-type sesquiterpene, pleodendione (**6**). Antifungal assays with the two major compounds, **1** and **2**, were carried out, and results showed a high activity of **1** against *Alternaria alternata* (MIC = 3.9 *μ*g/mL), *Candida albicans* azole-resistant strain D10, and *Wangiella dermatitides* (MICs = 15.6 *μ*g/mL). Compound **2** showed less potent antifungal activities than **1** but was more effective against *Candida albicans* azole-resistant strain CN1A (MIC = 23.4 *μ*g/mL) and *Pseudallescheria boydii* (MIC = 78.1 *μ*g/mL). A combination of the dialdehyde sesquiterpenes with dillapiol showed a synergistic antifungal effect with **1** and an additive effect with **4** and **5**.

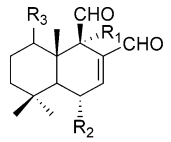
Canellaceae is a small tropical family endemic to East Africa, Madagascar, and tropical America. Watson and Dallwitz[1] described six genera, and the Missouri Botanical Garden[2] lists 26 species and five subspecies. Species from this family are mainly aromatic trees and can be identified by their thick, evergreen, and alternate leaves with glands containing volatile compounds. Their bark is also a source of essential oils.[3]

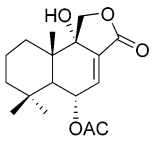
The genera *Canella* (tropical America) and *Warburgia* (East Africa) are the most studied members of the family, while only limited phytochemical data are available for other genera.[4] A detailed distribution of sesquiterpenes identified from six Canellaceae species (belonging to the genera *Canella*, *Capsicodendron*, *Cinnamosma*, and *Warburgia*) has listed a total of 19 drimane-type sesquiterpenes plus their derivatives.[5] Each species contained from six to 10 of these compounds. Two eremophilane-type sesquiterpenes were also identified, but each of them was only found in a single species.[5] They were mentioned to be widespread in Asteraceae[6] and similar to mycotoxins synthesized by some fungi such as *Penicillum roqueforti*.[7] During the last decade, no published data have described drimane-type sesquiterpenes in other terrestrial plants, but some have been identified in sponges and fungi.[5]

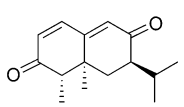
Despite being used for generations by indigenous people, plants of the Canellaceae are still little known and only a few have been developed commercially. In tropical America, *Canella alba* and *C. winterana* are used in traditional medicine as digestives, antirheumatic agents, emmenagogues, and tonics,[8−10] as well as food spices.[11] In Africa, *Warburgia salutaris* is used to treat cold, flu, respiratory and digestive problems, and malaria.[12] Hutchings et al.[13] determined the use of *W. salutaris* for inflammation, pain, and skin problems. Furthermore, it was reported that Swahili healers of East Africa considered the most efficient antifungal species of their traditional medicine to belong to the genus *Warburgia*.[14]

A new species of Canellaceae was recently discovered while surveying for a road in Costa Rica. It was identified by specialists of the Missouri Botanical Garden to belong to the genus *Pleodendron* and was recently named *P. costaricense*.[15] Only a few specimens have been located since the initial discovery of this tree. The present study is the first investigation of its phytochemical constituents and led to the isolation of four known (**1**−**4**) and two new sesquiterpenes (**5**, **6**). Accordingly, the leaves and bark of *P. costaricense* have afforded sesquiterpene dialdehydes related to the well-known warburganal. Since drimane-type sesquiterpenes, particularly the dialdehydes, such as polygodial or warburganal, have been reported to have promising antifungal activities,[16,17] minimum inhibitory concentrations (MIC) of the major dialdehyde cinnamodial (**1**) isolated from *P. costaricense* against nine pathogenic fungi were determined including drug-resistant and emerging opportunistic strains. Cinnamosmolide (**2**), the lactone analogue of **1**, was also tested to evaluate the importance of the dialdehyde groups in **1** in mediating the antifungal response.

Dillapiol, a cytochrome P450 inhibitor that is a major constituent of the essential oil of numerous aromatic plants, was tested in this study to see whether it enhanced the antifungal activity of the isolated dialdehydes against *C. albicans*. Previous studies have shown a synergistic effect with natural insecticides and antiparasitic agents.[18,19] As there are also P450 enzyme systems in fungi,[20] dillapiol was tested for its antifungal synergistic potential.







1 R_1 = OH, R_2 = OAc, R_3 = H **2** **6**
3 R_1 = H, R_2 = H, R_3 = H
4 R_1 = OH, R_2 = OH, R_3 = H
5 R_1 = OH, R_2 = OAc, R_3 = OAc (β)

Results and Discussion

Preliminary analysis of the essential oils of leaves and bark of *P. costaricense* was carried out to identify the major volatile components. The essential oils obtained by steam distillation and analyzed by gas chromatography (GC) contained six major volatile components. The compositions of the essential oils of the two plant

* To whom correspondence should be addressed. Tel: 613-562-5800, #6072. Fax: 613-562-5765. E-mail: tdurst@science.uottawa.ca.
[†] University of Ottawa.
[§] Universidad Nacional.
[‡] Carleton University.

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data ($CDCl_3$, 500 MHz) for Compounds **5** and **6** [δ in ppm (multiplicities, J in Hz)]

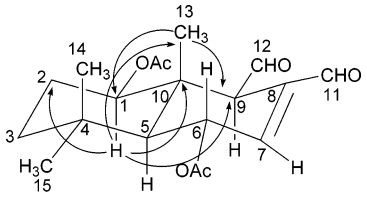
	5		**6**	
position	δ_C	δ_H	δ_C	δ_H
1	71.8	5.03 (dd, 4.8, 11.8)	142.3	6.96 (d, 9.8)
2	23.7	1.81 (m)	132.2	6.19 (d, 9.8)
2b		1.63 (m)		
3	40.8	1.49−1.37 (m)	200.0 or 200.2	
4	33.7		52.6	2.56 (q, 6.8)
5	45.2	2.11 (d, 5.1)	40.4	
6	65.9	5.88 (t, 4.9)	34.6	1.97 (dd, 12.9, 4.7)
6b				1.74 (t, 14.0)
7	148.3	6.95 (d, 4.7)	47.5	2.33 (ddd, 14.1, 4.6, 3.2)
8	141.1		200.0 or 200.2	
9	76.3	2.01 (s)	129.8	6.00 (s)
10	46.5		158.4	
11	199.7	9.81 (s)	26.2	2.55−2.63 (m)
12	192.7	9.42 (s)	17.9 or 20.4	0.82 (d, 7.0) (Me)
13	31.7	1.19 (s) (Me)	17.9 or 20.4	0.97 (d, 7.0) (Me)
14	24.6	1.03 (s) (Me)	18.6	1.12 (s) (Me)
15	13.4	1.41 (s) (Me)	7.2	1.14 (d, 7.0) (Me)
COMe	169.8			
	21.0	2.16 (s)		
	or 21.4			
COMe	169.9			
	21.0	1.95 (s)		
	or 21.4			

parts analyzed were very similar and showed high contents of α-pinene, β-pinene, β-myrcene, β-thujene, and β-caryophyllene with a lesser amount of linalool. The main difference between the volatile oils of the two plant parts was the content of β-caryophyllene, which was the second most abundant component in the leaves but almost absent in the bark.

As the odor of the essential oil of the leaves and bark of *P. costaricense* was perceived to be very similar to a mixture of cinnamon and pepper, their components were compared with the major volatile terpenes of cinnamon (*Cinnamomum zeylanicum* = *C. verum*; Lauraceae), namely, cinnamyl acetate, β-caryophyllene, and linalool,[21] as well as those of black pepper (*Piper nigrum*; Piperaceae), namely, α-pinene, β-pinene, limonene, and β-caryophyllene.[22] Phytochemical profiles obtained were consistent with the characteristic perceived fragrance.

Chromatographic separation of compounds contained in *P. costaricense* leaves and bark led to the isolation of two new sesquiterpenes (**5** and **6**) along with the four known sesquiterpenes cinnamodial (**1**),[23,24] cinnamosmolide (**2**),[23] polygodial (**3**),[25,26] and mukaadial (**4**),[27,28] as well as the known δ-tocotrienol[29] and β-sitosterol.[30] The structures of the known compounds were confirmed by comparison of their spectroscopic properties with the published data.

The structure of parritadial (**5**) was deduced by comparison of the ^{1}H and ^{13}C NMR spectra with **1**. The HRMS showed a molecular formula of $C_{19}H_{26}O_7$. All of the proton and carbon assignments were confirmed by a combination of DEPT, NOESY, ^{1}H−^{1}H COSY, and HMBC NMR correlations (Table 1). The ^{1}H NMR spectrum showed the presence of an additional acetoxy group in comparison to **1**. The ^{13}C NMR spectrum showed that **5** contained one less methylene and one more methine group compared to **1**. The placement of the additional acetoxy group at C-1, rather than at C-2 or C-3, was based on the HMBC spectrum, which showed connectivities between the hydrogens of the C-15 methyl group (δ_H 1.41) to C-9 (δ_C 76.3) and C-1 (δ_C 71.8) (Figure 1). This was confirmed by the H-1 (δ_H 5.01) correlation to C-15, C-2, C-9, and C-10. The 11.8 and 4.8 Hz coupling constants observed for H-1 in **5** were consistent with an axial−axial and an axial−equatorial relationship of this proton to the CH_2 methylene group at C-2. The axial placement of the remaining hydrogen at C-1 is also consistent with the significant enhancement of the signal for H-1 upon irradiation of H-5. Therefore, the structure of parritadial (**5**) was assigned as the 1α-acetoxycinnamodial.



5

Figure 1. Structure and selected HMBC correlations for compound **5**.

Compound **6**, named pleodendione, was shown to be an eremophilane-type sesquiterpene. The HRMS showed a molecular formula of $C_{15}H_{20}O_2$. The ^{13}C NMR (Table 1) showed 15 carbon signals, with those at δ_C 200.2 and 200.0 indicating two carbonyl groups and with four alkene carbons at δ_C 158.4 (C), 142.3 (CH), 132.2 (CH), and 129.8 (CH). The ^{1}H NMR spectrum (Table 1) indicated three alkene hydrogens at δ_H 6.96, 6.19, and 6.00 on α,β-unsaturated ketones. The signal at δ_H 6.00 was assigned to the β-hydrogen and the other two signals to the α-hydrogens. The HMQC spectrum confirmed these carbon−hydrogen assignments.

The ^{13}C NMR spectrum of **6** showed only one CH_2 group with nonequivalent hydrogens as part of an ABX system with δ_A 1.97, δ_B 1.74, and δ_X 2.55. The remaining proton peaks were assigned to four methyl peaks at δ_H 1.14, 1.12, 0.97, and 0.82, a quartet at δ_H 2.56, a ddd at δ_H 2.33, and a multiplet at δ_H 2.55. The ^{1}H and ^{13}C data are summarized in Table 1 and Figure 1.

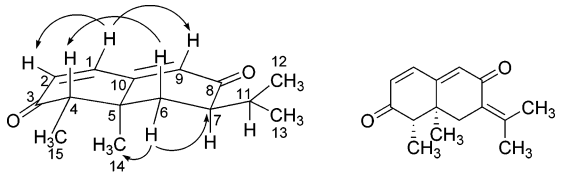
The mass spectrum of **6** showed a strong molecular ion at m/z = 232 (25%), consistent with the formula $C_{15}H_{20}O_2$. Prominent fragment ions were found at m/z = 217 (30%) and 190 (100%), signifying the loss of CH_3 and CH═CHCH$_3$, the latter being due to a very favorable McLafferty rearrangement. These fragmentations are consistent with the structure assignment of **6**. Other key fragmentations are recorded in the Experimental Section.

The ^{1}H NMR spectrum of **6** showed the required methyl singlet, a methyl doublet, and a set of diastereomeric methyl groups (C-12 and C-13) as doublets. The spin system involving the hydrogens at C-6, C-7, C-11, and the C-12 and C-13 methyl groups was readily apparent in the ^{1}H NMR spectrum, and each proton was reliably assigned. Thus, H-6_{ax} appeared as a triplet due to identical geminal and axial−axial coupling constants, and H-6_{eq} was observed as a doublet of doublets. The proton H-7_{ax} showed a large coupling to

Table 2. Minimum Inhibitory Concentrations (MICs, μg/mL) of Cinnamodial (**1**) and Cinnamosmolide (**2**) against Fungal Species and Strains Used in This Study

		MIC[b]			
		control		sesquiterpenes	
fungal species/strain	source[a]	berberine	ketoconazole	**1**	**2**
yeast-like					
Candida albicans					
Al-1 (wild type)	OGH-308-1329	31.3	0.4	62.5	1250.0
D10 (azole-resistant)	N. D. Lees	31.3	93.8	15.6	156.0
CN1A (azole-resistant)	N. D. Lees	15.6	93.8	62.5	23.4
Candida shehatae	NRC-2883	7.8	nt	31.3	625.0
Cryptococcus neoformans	OMH-FR2704	31.3	0.04	31.3	625.0
Saccharomyces cerevisiae	S288C	78.1	1.2	31.3	1250.0
Wangiella dermatitidis	OMH-FR2236	31.3	31.3	15.6	313.0
filamentous					
Pseudallescheria boydii	OMH-FR2625	62.5	93.8	125.0	78.1
Alternaria alternata	OMH-FR9884	125.0	nt	3.9	nt

[a] N. D. Lees, IUPUI, Indianapolis, IN; NRC, Natural Research Council of Canada, Ottawa, ON, Canada; OGH, Ontario General Hospital, Ottawa, ON, Canada; OMH, Ontario Ministry of Health, Toronto, ON, Canada. [b] Standard errors $\pm$ 0.00; $n = 3$; nt = not tested.



6 **7**

Figure 2. Structure and selected NOESY interactions for compound **6**, a new derivative of **7**.

H-6_{ax} and smaller but unequal couplings to H-6_{eq} and H-11. This confirms the β or equatorial stereochemistry of the isopropyl group. The NOESY NMR spectrum showed that the irradiation of H-1 enhanced the signals for H-2 and H-9, the irradiation of H-9 affected H-1, but not H-2, the irradiation of H-6_{ax} enhanced the signal for H-6_{eq} as well as for H-4, and finally, the irradiation of H-6_{eq} strongly affected the C-14 methyl group and the hydrogens H-6_{ax}, H-7, H-11, H-12, and H-13 but not H-4. These data confirmed the placement of H-4, H-7, and the C-14 methyl group as axial. The ^{13}C and the key NOESY NMR interactions are shown in Table 1 and Figure 2, respectively. Compound **6** is a derivative of warburgiadione (**7**) and can also be called 7,11-dihydrowarburgiadione.

The minimum inhibitory concentrations (MIC) of the two major sesquiterpenes, **1** and **2**, were determined against various fungi, and the results are presented in Table 2. The amounts of the new compounds **5** and **6** were not sufficient to carry out this bioassay, but previous antifungal tests have shown only weak activities. The results showed that the antifungal activities of cinnamodial (**1**) were similar to activity obtained with the natural compound berberine used as positive control,[31] against the yeast-like strains *Candida albicans* D10 (MIC = 15.6 μg/mL), *Saccharomyces cerevisiae* (MIC = 31.3 μg/mL), and *Wangiella dermatitidis* (MIC = 15.6 μg/mL) and especially against the filamentous strain *Alternaria alternata* (MIC = 3.9 μg/mL). The MIC against the azole-resistant D10 strain was 4-fold lower than ketoconazole. As expected, there was a reduction of the activity in the absence of aldehydes, as shown by cinnamosmolide (**2**) results, except for two species (azole-resistant *C. albicans* CN1A and *Pseudallescheria boydii*). Those results confirmed that the two aldehydes are important bioactive functionalities that often contribute toward the antifungal response. Taniguchi et al.[32] showed that the α,β-unsaturated aldehyde moiety in sesquiterpene dialdehyde molecules was responsible for their fungicidal action. Their mode of action is not completely known, but sesquiterpene dialdehydes such as polygodial (**3**) and warburganal lead to a structural disruption of the fungal cell membranes,[33] which seems to be due to the inhibition of the plasma membrane H^+-ATPase.[34] Kubo et al.[34] mentioned that the greater activity of the dialdehydes such as **3** and warburganal could be due to a balance between the hydrophilicity of the unsaturated aldehyde subunit and the hydrophobicity of the decalin portions of the molecule. They added that mukaadial (**4**) does not possess this balance due to its increased hydrophilicity and is therefore inactive. Interestingly, we observed significantly different sensitivities to **1** and **2** by the azole-resistant *C. albicans* strains CN1A and D10. CN1A has a mutation in erg2 and D10 in erg11, genes that encode enzymes involved in the biosynthesis of ergosterol, the major sterol of fungal cell membranes. Therefore, the morphology and physiology of the fungal cell membranes seem to play a major role in their resistance to such secondary active compounds as sesquiterpene dialdehydes.

Table 3. Antifungal Response of Cinnamodial (**1**), Mukaadial (**4**), and Parritadial (**5**) Used Alone and in Combination with Dillapiol

	MIC[a]			
compound	alone	+ dillapiol (1:5)	FICI[b]	results[c]
dillapiol	500			
berberine	31.3	15.6	0.5	S
1	62.5	15.6	0.3	S
4	1000	250	0.8	A
5	1000	250	1.5	A

[a] MIC: minimum inhibitory concentrations (μg/mL) against *Candida albicans* wild type (Al-1) are presented; standard errors $\pm$ 0.00; $n = 3$. [b] FICI: fractional inhibition concentration index. [c] S: synergist, A: additive.

We have tested the synergistic potential of the phenylpropanoid dillapiol in combination with the dialdehydes isolated in this study. Table 3 shows a 4-fold enhancement of antifungal activity when dillapiol was mixed with **1** and **4** individually. The antifungal activity of parritadial (**5**) was increased by 2-fold. To determine if these results were due to a synergism between the compounds, the fractional inhibition concentration indices (FICI), which take into consideration the MIC of the compounds used as synergists, were calculated (Table 3). The results show that dillapiol acts as a synergist with berberine and compound **1**, but only as an additive agent with **4** and **5**.

In conclusion, this first analysis of the chemical constituents of the newly discovered aromatic plant *P. costaricense* (Canellaceae) has shown the composition of its essential oil and led to the isolation of a variety of sesquiterpenes, which are characteristic of this plant family. Two new sesquiterpenes (**5** and **6**) were also identified. High antifungal activity was observed for cinnamodial (**1**), but no inhibition occurred with mukaadial (**4**) and parritadial (**5**) against *C. albicans*. As explained in a previous publication,[34] the lack of antifungal activity for mukaadial could be due to its higher hydrophilicity. The mode of action of those active compounds is very complex, and only a few studies have tried to demonstrate their antifungal mechanism. Further studies are ongoing to inves-

tigate the mode of action of cinnamodial against *Saccharomyces cerevisiae*. As *P. costaricense* is a very rare species, these results highlight the need for preservation and study of rare species such as *P. costaricense* that are potentially threatened by development in their habitats.

Experimental Section

General Experimental Procedures. Infrared spectra were recorded on a Shimadzu model FTIR-8400S spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on Bruker model Advance 300 and Brucker AMX-500 spectrometers with standard pulse sequences operating at 300 and 500 MHz in ^{1}H NMR and 125 MHz in ^{13}C NMR. $CDCl_3$ was used as solvent. EIMS and HREIMS data were recorded on a Kratos concept II H mass spectrometer. Gas chromatography was done on a Hewlett-Packard 5890A instrument with FID detector and an Agilent 6890 series autoinjector (Avondale, PA). The carrier gas was hydrogen with a linear velocity of 21 cm/s. The column was a DB-5 (60 m × 0.53 mm i.d. × 1.5 μm). The following method was used: pressure, 20 psi at 75 °C; sample injection, 2 μL; initial temperature, 75 °C; increase in temperature, 2 °C/min from 75 °C to 125 °C and 10 °C/min from 125 °C to 300 °C; final temperature hold, 10 min. Column chromatography was performed on silica gel (Merck, Darmstadt, Germany) (230−400 mesh), and recycling preparative HPLC was performed on a JAI LC-908 instrument (detectors: UV-254 and IR-5) equipped with a 500 × 22 mm Jordi Gel DVB 100A (Alltech) size exclusive column or a Luna 5 μm silica column (250 × 21 × 20 nm) connected to a 50 × 20 mm precolumn. Thin-layer chromatography (TLC) was performed on silica gel (precoated Kieselgel 60 F_{254} TLC, foil size 20 × 20 mm, thickness 0.25 mm, Merck), and spots were colored with a molybdate solution (ammonium molybdate (25 g), cerium sulfate (10 g) in 100 mL of sulfuric acid and 900 mL of distilled water).

Plant Material. Fresh leaves and bark of *Pleodendron costaricense* N. Zamora, Hammel & R. Aguilar were collected near Parrita (Costa Rica) in February 2000 and stored in 95% EtOH at 3 °C. A second collection with fresh and dried material has been carried out on in January 2004. The plant material was identified by two of the authors (P.S.-V. and L.P.A.). A voucher specimen (JVR-10679) is deposited in the Herbario Juvenal Valerio Rodriguez (UNA, Costa Rica).

Hydrodistillation. Leaves and bark (100 g) were immersed into 500 mL of distilled water and steam distilled for 30 min. The essential oils were extracted twice from the water phase with 1 mL of hexane, and the residual water was removed from the hexane phase with 0.5 mg of anhydrous $MgSO_4$. The distillate was filtered before GC analysis. A mixture of monoterpene standards was previously run, and peaks were identified to determine the components of the essential oils of *P. costaricense* based on the retention times.

Extraction and Isolation. February 2000 Collection. Fresh leaves stored in 95% EtOH or bark removed from a branch section (500 g) were ground and extracted in 50% EtOH for 24 h under moderate mechanical agitation (70 rpm). After filtration, the residue was extracted a second time with the same method, and the two fractions were pooled. This extract was rotoevaporated to dryness and redissolved in 50% EtOH. This was fractionated three times with equal volumes of hexane, giving three hexane fractions (pooled), and the hydro-alcoholic portion was fractionated again three times with EtOAc. All fractions were evaporated to dryness and freeze-dried. EtOAc extracts of leaves (5 g) or bark (4 g) were dissolved in CH_2Cl_2 and applied to an open silica gel column using solvent mixture of increasing polarity (hexane to 70% EtOAc−hexane, 5% to 10% every 200 mL). **Leaves**: Column chromatography of the leaf extract gave 11 fractions (I−XI). Fraction V was purified using a second silica gel column (same conditions). The major fraction (VI), corresponding to about 38% of the crude sample, was a mixture of two compounds of the same polarity, which were separated by size exclusive recycling preparative HPLC (flow rate, 2.5 mL/min; detection wavelength, 254 nm) to yield **1** (130 mg) and **2** (120 mg). Purified compounds were recrystallized by dissolution in a small amount of acetone before the addition of hexane. The solution was stored in the freezer for 30 min to allow the precipitation of the crystals. Crystals were then filtered and washed with cold hexane. Fraction VII yielded compound **1** (120 mg) with a light coloration removed by recrystallization. **Bark**: Column chromatography of the bark extract gave seven major fractions (I−VII). Fraction IV was purified by recrystallization to yield **6** (20 mg), and fraction VI, already pure, yielded compound **2** (1.2 g). **January 2004 collection**: Dried bark (350 g) was extracted with $CHCl_3$ for 2 days and with CH_2Cl_2 for another 2 days. The combined solutions were concentrated under reduced pressure. The residue (20 g) was fractionated by chromatography on a silica gel column using a solvent mixture of increasing polarity (hexane to EtOAc−hexane), to yield four fractions (I−IV). Fraction I (5.7 g), eluted with 10% EtOAc, was separated on a silica gel column using the same solvent system as mentioned earlier to yield three subfractions. The first subfraction (less polar) (1.8 g) was eluted with hexane and was mainly composed of fatty acids and monoterpenes. The second subfraction (490 mg), eluted with 5% EtOAc, was separated by normal-phase HPLC with CH_2Cl_2 (flow rate, 4.5 mL/min; detection wavelength, 254 nm) to yield δ-tocotrienol (20.7 mg) and β-sitosterol (2.5 mg).

The third subfraction, eluted with 10% EtOAc (250 mg), was separated by normal-phase HPLC with a mixture of CH_2Cl_2−CH_3CN (96:4) (flow rate, 4.5 mL/min; detection wavelength, 254 nm) to yield **1** (48.5 mg) and **3** (4.6 mg). Fraction II (6 g), eluted with 20% EtOAc, was subjected to a further silica gel column with a solvent mixture of increasing polarity (hexane to EtOAc−hexane) to yield more of compound **1** (4.9 g) and its lactone derivative **2** (0.7 g). Those two solids were purified by recrystallization. Fraction III (1.2 g), eluted with 30% EtOAc, was subjected to normal-phase HPLC with a mixture of CH_2Cl_2−CH_3CN (90:10) (flow rate, 4 mL/min; detection wavelength, 254 nm) to yield **1** (35 mg), **4** (30 mg), and **5** (26 mg).

Parritadial (5): amorphous, white solid; IR ν_{max} 3420, 2941, 2912, 2846, 1743, 1716, 1691, 1421, 1367, 1230, 1203 cm^{-1}; ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data, see Table 1; EIMS *m/z* 366 [M$^+$] (1), 337 (16), 235 (18), 162 (15), 123 (19), 88 (24), 86 (73), 84 (100), 47 (73), 43 (68); HREIMS *m/z* 366.1637 [M$^+$] (calcd for $C_{19}H_{26}O_7$ 366.1678).

Pleodendione (6): white solid; ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data, see Table 2; EIMS *m/z* 232 [M$^+$] (26), 217 (30), 190 (100), 175 (58), 161 (31), 91 (32); HREIMS *m/z* 232.1478 [M$^+$] (calcd for $C_{15}H_{20}O_2$ 232.1463).

Antifungal Activity Testing. Minimum inhibitory concentration (MIC) values were determined on the basis of a standardized method.[35] They were recorded as the lowest tested concentration of antifungal activity that resulted in a prominent reduction (≥80%) of fungal growth.[31] The strains and the sources of yeast-like and filamentous fungi used in this study are given in Table 2. They were cultured on Sabouraud dextrose (SD) agar medium (Difco) at 30 °C. One colony of yeast-like strains was inoculated into 100 mL of liquid SD broth and incubated overnight at 30 °C until reaching an OD_{600} between 0.6 and 0.9. Inocula of filamentous fungi were prepared by placing a 1 × 3 cm block of agar medium containing hyphae from the colony into 10 mL of SD broth and blending at high speed (sterile water-cooled Waring blender). Fungal inocula were then diluted with SD broth (1:5000), and 150 μL was dispensed into each microtiter well (Costar sterile plate, 96 V-shaped well). The tested substances were dissolved in 95% EtOH to make stock solutions of 20 mg/mL. Different concentrations of the tested compounds (50, 40, 30, 25, 20, 15, 10, 5 μL of the stock solution plus the corresponding amounts of liquid SD broth to maintain a total of 200 μL per well) were added to the eight different wells of the first column. A 4-fold serial dilution was performed across the microtiter plate with the last column used as a negative control. The plates were then incubated at 30 °C for 48 h. Berberine and ketoconazole were used as positive antifungal controls.

The evaluation of the synergistic effect of dillapiol was carried out using a similar MIC method. Toxicity of dillapiol alone against *Candida albicans* was determined previously at various concentrations. Due to low available amounts of the minor dialdehydes, a one-row 2-fold serial dilution was performed starting with a concentration of 1000 μg/mL. For each sesquiterpene, the assay was carried out with dillapiol alone, sesquiterpenes alone, and a combination of sesquiterpenes with dillapiol (1:5).

The fractional inhibitory concentration (FIC) of a drug was calculated as the MIC of the drug when used in combination with another drug divided by the MIC of the drug when used alone. The FIC index (FICI) value was calculated by adding the FIC of dillapiol to the FIC of each tested compounds pairwise. FICI values were interpreted as follows: FICI ≤ 0.5, synergistic; 0.5 < FICI ≤ 1, additive; 1 < FICI ≤ 4, indifferent, and FICI > 4, antagonistic.[36]

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