Macrophyllin-Type Bicyclo[3.2.1]octanoid Neolignans from the Leaves of *Pleurothyrium cinereum*

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Four new macrophyllin-type bicyclo[3.2.1]octanoid neolignans, (7*S*,8*R*,3′*S*,5′*R*)-Δ$^{8'}$-5,5′,3′-trimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-2′,4′-dioxo-7.3′,8.5′-neolignan (cinerin A), **1**, (7*R*,8*R*,3′*S*,4′*R*,5′*R*)-Δ$^{8'}$-4′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-2′-oxo-7.3′,8.5′-neolignan (cinerin B), **2**, (7*S*,8*R*,3′*R*,4′*S*,5′*R*)-Δ$^{8'}$-4′-hydroxy-5,5′,3′-trimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-2′-oxo-7.3′,8.5′-neolignan (cinerin C), **3**, and (7*S*,8*R*,2′*R*,3′*S*,5′*R*)-Δ$^{8'}$-2′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-4′-oxo-7.3′,8.5′-neolignan (cinerin D), **4**, along with the known diterpene kaurenoic acid **5**, were isolated from the leaves of *Pleurothyrium cinereum*. The structures and configuration of these compounds were determined by extensive spectroscopic analysis. Cinerins A−D (**1**−**4**) were tested for their inhibition efficacy of platelet activating factor (PAF)-induced aggregation of rabbit platelets. Compound **3** was the most potent PAF antagonist. Compounds **1**−**5** were tested against *Mycobacterium tuberculosis* (H_{37}Rv strain) using the MABA method. Compound **5** induced 91.3% growth inhibition at 50 μg mL^{-1}. Compounds **1**−**5** showed no significant inhibitory activity against some Gram-positive and Gram-negative bacteria by the agar-well diffusion method.

Pleurothyrium cinereum van der Werff. (Lauraceae) is a native plant from the south of Colombia, whose primary use is focused on timber purposes due to its resistant hardwood. The Lauraceae plants (preferably the genera including the Ocotea complex[1]) are known to contain bicyclo[3.2.1]octanoid neolignans, an important class of natural products,[2] which are further subdivided into the guianin and the macrophyllin classes.[3] Although the macrophyllin-type compounds are the least common among the known bicyclooctanoids, most of these have exhibited excellent PAF[4] (platelet-activating factor) antagonistic activities.[5] Numerous cells and tissues synthesize PAF (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) upon suitable stimulation, which was discovered to be a lipid mediator of hypersensitivity and inflammation. Medical studies have proposed that PAF antagonists may be of clinical benefit in many inflammatory disorders including asthma, atherosclerosis, endotoxin shock, and multiple sclerosis among others.[6] In earlier work carried out on *P. cinereum*, we isolated two known macrophyllin-type neolignans, macrophyllin B[7] and its oxidation product,[7] along with two furofuran and three aryltetralin lignans, five 2,3-dihydrobenzofuran neolignans, one dihydrochalcone, two oxoaporphine alkaloids, and one diterpene.[7,8] As part of our research on the chemistry of neolignans of Lauraceae plants, we describe herein a phytochemical exploration of the leaves of *P. cinereum*, affording four new macrophyllin-type bicyclo[3.2.1]octanoid neolignans, **1**−**4** (named cinerins A−D, respectively), and kaurenoic acid, **5**,[9] whose structures (see Figure 1) have been established on the basis of spectroscopic analysis. In addition, compounds **1**−**5** were tested for antimycobacterial, PAF-antagonistic, and antibacterial activities.

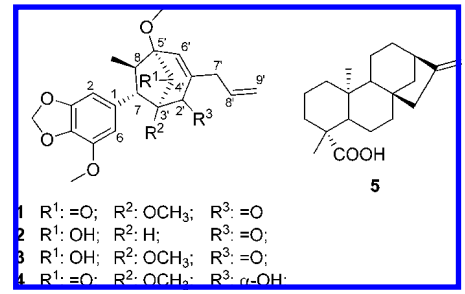

Figure 1. Macrophyllin-type bicyclo[3.2.1]octanoid neolignans **1**−**4** and kaurenoic acid (**5**) isolated from *P. cinereum*.

Results and Discussion

The molecular formula of compound **1** was assigned as $C_{22}H_{24}O_7$ by HRESIMS analysis ([M + H]$^+$ *m*/*z* 401.1608, calcd for $C_{22}H_{25}O_7$, 401.1600). The IR spectrum showed absorption bands at 1768 and 1685 cm^{-1} due to a strained ketone and an α,β-unsaturated ketone moiety, respectively. The ^{1}H NMR spectrum (see Experimental Section) indicated the presence of three methoxy groups, i.e., one methoxy group attached to an aromatic carbon [δ_H 3.84 (3H, s)] and two methoxy groups attached to aliphatic carbons [δ_H 3.48 (3H, s), 3.34 (3H, s)], a methylenedioxy group [δ_H 5.94 (2H, s)], an allyl group [δ_H 5.19−5.15 (2H, m), 5.88−5.82 (1H, m), 3.09−3.07 (2H, m)], a methyl group [δ_H 1.09 (3H, d, J = 6.8 Hz)], a 3,4,5-trioxyphenyl group [δ_H 6.72 (1H, d, J = 1.3 Hz), 6.53 (1H, d, J = 1.3 Hz)], an olefinic proton as part of an enone system [δ_H 7.09 (1H, s)], and two overlapping methine protons [δ_H 2.44−2.42 (2H, m)]. The ^{13}C NMR spectrum of **1** (Table 1) revealed the presence of two carbonyl groups (δ_C 203.4 and 198.6), a 3,4,5-trioxyphenyl group (δ_C 149.1, 143.3, 134.8, 131.5, 110.2, 104.5), two carbinyl carbons (δ_C 89.9 and 88.6), an aromatic *O*-methyl group (δ_C 56.3), and two methoxy groups (δ_C 54.6 and 54.0) attached to aliphatic carbon atoms. The spectroscopic information was used to establish the core structure as a bicyclo[3.2.1]octane derivative,[10] which was confirmed by HMBC data (Figure 2). The HMBC spectrum of **1** showed a long-range correlation between overlapping H-7, H-8 [δ_H 2.44−2.42 (2H, m)], and C-4′ (δ_C 203.4). The C-7′ methylene protons [δ_H 3.09−3.07 (2H, m)] and H-6′ [δ_H 7.09 (1H, s)] were correlated with the C-2′ carbonyl carbon (δ_C 198.6); both correlations confirmed the connection of two C_6−C_3 moieties. The ^{1}H and ^{13}C NMR chemical shifts of the C-8 methyl group were considered as key signals for determining the relative configuration of **1**. The *trans* relationship between the C-7 aryl group and C-8 methyl group was assigned on the basis of the ^{1}H NMR chemical shift of H_3-9 [δ_H 0.99 (3H, d, J = 6.8 Hz)].[11] The *endo* orientation of the C-8 methyl group was established by its ^{13}C NMR chemical shift [δ_C 13.2], which correlates with literature values for similar compounds.[12] The

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Table 1. ^{13}C NMR Chemical Shifts (δ) of Compounds **1**−**4** (100 MHz, $CDCl_3$)

carbon	1	2	3	4
1	131.5	132.2	131.7	132.5
2	104.5	104.3	104.8	104.9
3	149.1	148.2	148.8	148.4
4	134.8	135.1	134.4	134.2
5	143.3	143.5	143.1	143.0
6	110.2	109.8	110.5	110.8
7	53.5	52.4	56.4	56.8
8	48.2	47.2	47.8	48.2
9	13.8	13.2	13.4	14.2
1′	140.4	139.9	139.6	140.6
2′	198.6	197.8	198.3	76.8
3′	88.6	53.5	87.2	88.8
4′	203.4	75.4	75.3	204.2
5′	89.9	89.8	89.9	89.2
6′	147.4	149.1	149.3	127.1
7′	32.7	32.5	32.6	36.5
8′	134.1	134.3	134.4	135.0
9′	118.0	118.0	117.9	117.3
OCH_2O-3,4	101.2	101.3	101.5	101.4
OMe-5	56.3	56.2	56.7	56.6
OMe-5′	54.6	53.9	54.6	54.5
OMe-3′	54.0		53.8	53.5

absolute configuration of **1** was defined by comparison of chiroptical properties with those of related molecules. The circular dichroism (CD) data of **1** were identical to those of denudadiones A and B. These two macrophyllin-type bicyclo[3.2.1]octane neolignans isolated from *Magnolia denudata*[10] have negative Cotton effects at 330 and 270 nm and a positive Cotton effect at 305 nm. On the basis of the ketone and the α,β-unsaturated ketone chromophores, the values were interpreted in terms of the empirical rules for defining the absolute configuration.[10] Additionally, oxidation of **3** and **4** (*vide infra*) using MnO_2 in $CHCl_3$[13] afforded a derivative possessing identical spectroscopic and chiroptical properties to that of **1**. Thus, the structure of **1** was determined as (7*S*,8*R*,3′*S*,5′*R*)-Δ$^{8′}$-5,5′,3′-trimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-2′,4′-dioxo-7.3′,8.5′-neolignan (cinerin A).

Compound **2** has the molecular formula $C_{21}H_{24}O_6$, as deduced from HRESIMS analysis ([M + H]$^+$ *m*/*z* 373.1638, calcd for $C_{21}H_{25}O_6$, 373.1651). The IR spectrum showed absorption bands at 3455 and 1688 cm^{-1} due to a hydroxy group and an α,β-unsaturated ketone moiety, respectively. The ^1H NMR spectrum of **2** exhibited a similar signal pattern to that of **1** except for the presence of the C-4′ carbinol proton [δ_H 4.21 (1H, s)] and the C-3′ methine proton [δ_H 3.08 (1H, s)] and the absence of a methoxy group attached to an aliphatic carbon. The ^{13}C NMR spectrum of **2** (Table 1) was also similar to that of cinerin A (**1**), except for the presence of a carbinol carbon (δ_C 75.4) instead of carbonyl group and the absence of a methoxy group attached to an aliphatic carbon. The ^1H and ^{13}C NMR data of the bicyclo[3.2.1]octane part of **2** was in agreement with the reported data for related molecules.[10,14] HMBC correlations of **2** were similar to those of **1**: H-7 [δ_H 2.40 (1H, d, J = 7.5 Hz)] and H-8 [δ_H 2.64−2.68 (1H, m)] exhibited long-range correlation with C-4′ (δ_C 75.4). The C-7′ methylene protons [δ_H 3.02−3.06 (2H, m)] as well as H-6′ [δ_H 7.09 (1H, s)] were correlated with the C-2′ carbonyl group (δ_C 197.8). Interestingly, H-4′ [δ_H 4.21 (1H, s)] and H-3′ [δ_H 3.08 (1H, s)] resonated as singlets, which indicated dihedral angles for H-4′···H-3′ and H-3′···H-7 of approximately 90°.[10,14] The *trans* relationship between the C-7 aryl group and the C-8 methyl group and the *endo* orientation of the latter were assigned in analogy with **1** (^1H NMR chemical shift of the C-8 methyl group [δ_H 0.99 (3H, d, J = 6.8 Hz)][11] and ^{13}C NMR chemical shift [δ_C 13.2][12]). The absolute configuration of C-4′ of **2** was determined to be *R,* on the basis of the corresponding (*R*)- and (*S*)-MTPA esters, following the reported methodology for stereochemical assignments.[15] In addition, on comparing the CD data of **2** (negative Cotton effects at 260 and 337 nm) with the literature, compound **2** was found to have the same absolute configuration as kadsurenin C, a bicyclo[3.2.1]octanoid neolignan isolated from *Piper kadsura*,[14] whose absolute configuration was previously established by single-crystal X-ray analysis.[16] From these facts, the structure of **2** was determined as (7*R*,8*R*,3′*S*,4′*R*,5′*R*)-Δ$^{8′}$-4′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-2′-oxo-7.3′,8.5′-neolignan (cinerin B).

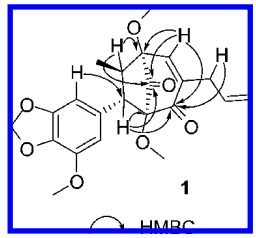

Figure 2. Key HMBC correlations of compound **1**.

The molecular formula of compound **3** was assigned as $C_{22}H_{26}O_7$ by HRESIMS analysis ([M + H]$^+$ *m*/*z* 403.1742, calcd for $C_{22}H_{27}O_7$, 403.1757). The IR spectrum showed absorption bands at 3434 and 1690 cm^{-1} corresponding to a hydroxy group and an α,β-unsaturated ketone moiety, respectively. The ^1H and ^{13}C NMR spectra of **3** exhibited a similar signal pattern to that of **2**, except for the presence of a methoxy group [δ_H 3.47 (3H, s); δ_C 53.8] instead of a methine proton. In addition, ^1H and ^{13}C NMR spectra of the bicyclo[3.2.1]octane moiety of **3** were similar to those reported for related molecules.[10,14] The relative configuration of **3** including the *endo* orientation of the C-8 methyl group and the *trans* relationship between the C-7 aryl group and the C-8 methyl group were again assigned on the basis of the ^1H and ^{13}C NMR chemical shifts of the C-8 methyl group [δ_H 0.90 (3H, d, J = 6.8 Hz); δ_C 13.4].[11,12] The absolute configuration of **3** was identical to that of **2** by comparison of its CD data (negative Cotton effects at 332 and 263 nm), which were also similar to those of the related kadsurenin C.[14,16] Furthermore, the absolute configuration of **1** was confirmed by oxidation of **3** using MnO_2 in $CHCl_3$,[13] which afforded a product possessing the same spectroscopic and chiroptical features as cinerin A (**1**). Thus, the structure of **3** was determined as (7*S*,8*R*,3′*R*,4′*S*,5′*R*)-Δ$^{8′}$-4′-hydroxy-5,5′,3′-trimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-2′-oxo-7.3′,8.5′-neolignan (cinerin C).

Compound **4** has the same molecular formula as compound **3** [HRESIMS analysis ([M + H]$^+$ *m*/*z* 403.1734, calcd for $C_{22}H_{27}O_7$, 403.1757)]. However, the IR spectrum showed absorption bands at 3468 and 1757 cm^{-1} due to a hydroxy group and a strained ketone moiety, respectively. The ^1H NMR spectrum of **4** displayed a similar signal pattern to that of **3**, except for the chemical shifts of the carbinol proton [δ_H 4.90 (1H, s)] and the olefinic proton [δ_H 5.68 (1H, s)]. The ^{13}C NMR data of **4** (Table 1) were also similar to those of **3**, indicating that **3** and **4** are regioisomers. The HMBC spectrum of **4** exhibited long-range correlations between the overlapping protons H-7, H-8, [δ_H 2.50−2.42 (2H, m)] and the carbonyl carbon [δ_C 204.2] and between both protons H_2-7′ [δ_H 3.05 (2H, d, J = 7.2 Hz] and H-6′ [δ_H 5.68 (1H, s)] and the carbinol carbon [δ_C 76.8]. This indicated a C-4′ carbonyl group and C-2′ carbinol carbon. The *endo* methyl group [δ_H 1.04 (3H, d, J = 6.8 Hz)] and the aryl-C-7/methyl-C-8 *trans* relationship were determined as in compounds **1**−**3**. The relative configuration of C-2′ was deduced from the NOESY correlation between H-2′ [δ_H 4.90 (1H, s)] and H-7 [δ_H 2.50−2.42 (2H, m)], which indicated a C-2′ α-OH group (supported by the deshielding of C-7 (δ_C 56.8) as a result of the so-called γ-effect[12]). Finally, the absolute configuration was deduced from the CD data (negative Cotton effects at 247 and 308 nm) and from the CD data of its oxidation product (MnO_2 in $CHCl_3$[13]), which were identical to those of cinerin A (**1**). The structure of **4** was therefore determined as (7*S*,8*R*,2′*R*,3′*S*,5′*R*)-Δ$^{8′}$-2′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-4′-oxo-7.3′,8.5′-neolignan (cinerin D).

Table 2. PAF-Antagonistic Activity of Compounds **1**−**4**

compound	IC_{50} μg/mL	IC_{50} μM	potency (%)[a]
cinerin A, **1**	6.7	16.8	6.7
cinerin B, **2**	1.0	1.5	73.3
cinerin C, **3**	0.45	1.1	100
cinerin D, **4**	2.7	6.6	17.0
ginkgolide B[c]	0.39	0.93	118
kadsurenone[b]		0.12	

[a] Potency of inhibition was calculated as the IC_{50} (μM). The effect of cinerin C was arbitrarily set at 100%. [b] PAF-antagonistic activity of kadsurenone reported in ref 18. [c] Ginkgolide B (BN52021) as positive control.

Table 3. Growth Inhibition of Compounds **1**−**5** Tested by MABA Method against *M. tuberculosis* $H_{37}Rv$

	growth inhibition		
compound	100 μg/mL	50 μg/mL	25 μg/mL
cinerin A, **1**	8.1	7.2	2.5
cinerin B, **2**	19.5	20.9	13.9
cinerin C, **3**	49.4	49.8	43.8
cinerin D, **4**	7.3	6.2	1.3
kaurenoic acid, **5**	94.2	91.3	88.6
rifampin			97.6
isoniazid			94.2

Cinerins A−D (**1**−**4**) were tested for PAF-antagonistic activity on rabbit platelets by inhibition of the PAF-induced aggregation according to Koch.[6] Cinerin C (**3**) was found to be the most potent antagonist (IC_{50} 1.1 μM), with cinerins A, B, and D having only about 7%, 73%, and 17% of its potency (Table 2). Kadsurenin C, whose structure is similar to that of **3**, was found to have an IC_{50} of 5.1 μM,[17] corresponding to 22% of the potency of **3**. However, **3** showed lower activity than kadsurenone (IC_{50} 0.12 μM), a recognized potent PAF antagonist isolated from Chinese *Piper* species,[18] but similar to the positive control gingkolide B (BN 52021, IC_{50} 0.93 μM).

Cinerins A−D (**1**−**4**) and kaurenoic acid (**5**) were tested against the *Mycobacterium tuberculosis* (MTB) strain $H_{37}Rv$ (ATCC-27294) by the microplate Alamar Blue assay (MABA)[19] method (see Table 3). Compound **5** induced 91.3% growth inhibition at 50 μg mL^{-1}, whereas **3** had a moderate activity (49.8% growth inhibition at 50 μg mL^{-1}). The action of compound **5** had not been tested before against MTB, and its activity was found to be comparable to those of the drugs rifampin (RMP) and isoniazid (NIH).

Compounds **1**−**5** were also tested for antibacterial activity against some Gram-positive and Gram-negative bacteria by the agar-well method reported by Lehrer et al.[20] All compounds exhibited no significant antibacterial activity with growth inhibition of <30%.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus without correction. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter in $CHCl_3$ at 25 °C. CD spectra were obtained using a JASCO J-715 spectropolarimeter. IR spectra were recorded on a Thermo Nicolet 6700 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer, using TMS as internal shift reference. EIMS and HRMS were determined on a Finnigan SSQ 710 (70 eV) and qTOF-micro Micromass (Waters Inc.) (with an ESI source and in the positive ion mode) mass spectrometers, respectively. Column chromatography (CC) was carried out with silica gel (70−230 and 230−400 mesh, Merck).

Plant Material. The whole plant of *P. cinereum* was collected in the indigenous reservation Awá at Alto Albí, Tumaco County (Department of Nariño, Colombia), in November 2005. The plant material was identified by biologyst Ayda Lucia Patino. A voucher specimen (number COL518334) was deposited at Herbario Nacional Colombiano, Universidad Nacional de Colombia.

Extraction and Isolation. Air-dried leaves of *P. cinereum* (250 g) were extracted with EtOH (3 L) at room temperature. The extract was filtered and concentrated to afford a residue (25.8 g). The residue was fractionated in a Soxhlet apparatus using different solvents: petroleum ether fraction (5.2 g), $CHCl_3$ fraction (3.9 g), EtOAc fraction (1.1 g), and MeOH fraction (9.0 g). The petroleum ether-soluble fraction 1 was subjected to column chromatography on silica gel (eluent: toluene/isopropyl acetate (gradient elution by changing from 95:5 to 30:70)) to give 15 fractions (1.1.−1.15). Further purification of fraction 1.8 (84 mg) by CC on silica gel using 20% EtOAc in petroleum ether provided compound **1** (7.5 mg). Fraction 1.9 (126 mg) was subjected to CC on siliga gel using 5% MeOH and 5% EtOAc in toluene as mobile phase to obtain four fractions (1.9.1−1.9.4). Compound **2** (8.0 mg) was obtained from fraction 1.9.3 by CC on silica gel using 2% MeOH and 3% EtOAc in $CHCl_3$ as eluent. Compound **5** (22.2 mg) was obtained from fraction 1.10 (218.3 mg) by CC on silica gel using a mixture of 40% isopropyl acetate in toluene. The $CHCl_3$-soluble fraction 2 was subjected to CC on silica gel eluted with toluene/isopropyl acetate (gradient elution by changing from 9:1 to 1:1), affording nine fractions (2.1−2.9). Compound **3** (12.4 mg) was obtained from fraction 2.4 (315 mg) by CC on silica gel using 30% EtOAc in toluene as mobile phase. Fraction 2.5 (176 mg) was subjected to CC on silica gel eluted with 10% MeOH in $CHCl_3$, affording compound **4** (10.3 mg).

Cinerin A [(7*S*,8*R*,3′*S*,5′*R*)-$\Delta^{8'}$-5,5′,3′-trimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-2′,4′-dioxo-7.3′,8.5′-neolignan] (1): slightly yellowish, viscous oil; $[\alpha]^{25}_D$ −18.8 (*c* 0.23, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (4.22), 223 (4.07), 275 (3.65); CD (*c* 0.05, MeOH) $[\theta]_{265}$ −15234, $[\theta]_{305}$ +6799, $[\theta]_{335}$ −11 125; IR (film) ν_{max} 1768, 1685 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.09 (1H, s, H-6′), 6.72 (1H, d, J = 1.3 Hz, H-6), 6.53 (1H, d, J = 1.3 Hz, H-2), 5.94 (2H, s, OCH_2O), 5.88−5.82 (1H, m, H-8′), 5.15−5.19 (2H, m, H-9′), 3.84 (3H, s, OCH_3-5), 3.48 (3H, s, OCH_3-5′), 3.34 (3H, s, OCH_3-3′), 3.09−3.07 (2H, m, H-7′), 2.44−2.42 (2H, m, H-7, H-8), 1.09 (3H, d, J = 6.8 Hz, H-9); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 400 [M^+] (100), 385 (12), 372 (9), 354 (13), 331 (18), 235 (55), 192 (62); HRESIMS *m*/*z* 401.1608 $[M + H]^+$ (calcd for $C_{22}H_{25}O_7$, 401.1600).

Cinerin B [(7*R*,8*R*,3′*S*,4′*R*,5′*R*)-$\Delta^{8'}$-4′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-2′-oxo-7.3′,8.5′-neolignan] (2): colorless, viscous oil; $[\alpha]^{25}_D$ −13.2 (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 214 (4.48), 248 (4.17), 288 (3.40); CD (*c* 0.09, MeOH) $[\theta]_{260}$ −14 216, $[\theta]_{337}$ −9271; IR (film) ν_{max} 3455 (br), 1688 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.86 (1H, s, H-6′), 6.68 (1H, s. br, H-6), 6.48 (1H, s. br, H-2), 5.93 (2H, d, J = 1.0 Hz, OCH_2O), 5.81−5.85 (m, 1H, H-8′), 5.14−5.17 (2H, m, H-9′), 4.21 (1H, s, H-4′), 3.86 (3H, s, OCH_3-5), 3.46 (3H, s, OCH_3-5′), 3.08 (1H, s, H-3′), 3.02−3.06 (2H, m, H-7′), 2.64−2.68 (1H, m, H-8), 2.51 (1H, s, 4-OH), 2.40 (1H, d, J = 7.5 Hz, H-7), 0.99 (3H, d, J = 6.8 Hz, H-9); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 372 [M^+] (79), 357 (8), 354 (6), 338 (10), 326 (21), 235 (50), 194 (100), 192 (42), 180 (26); HRESIMS *m*/*z* 373.1638 $[M + H]^+$ (calcd for $C_{21}H_{25}O_6$, 373.1651).

Cinerin C [(7*S*,8*R*,3′*R*,4′*S*,5′*R*)-$\Delta^{8'}$-4′-hydroxy-5,5′,3′-trimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-2′-oxo-7.3′,8.5′-neolignan] (3): white crystals (Me_2CO); mp 176−177 °C; $[\alpha]^{25}_D$ −13.2 (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 215 (4.55), 245 (4.12), 285 (3.40); CD (*c* 0.06, MeOH) $[\theta]_{263}$ −16 070, $[\theta]_{332}$ −9889; IR (KBr) ν_{max} 3434, 1690 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.87 (1H, s, H-6′), 6.70 (1H, d, J = 1.2 Hz, H-6), 6.50 (1H, d, J = 1.2 Hz, H-2), 5.93 (2H, s, OCH_2O), 5.87−5.83 (1H, m, H-8′), 5.14−5.19 (2H, m, H-9′), 4.23 (1H, s, H-4′), 3.87 (3H, s, OCH_3-5), 3.47 (3H, s, OCH_3-5′), 3.34 (3H, s, OCH_3-3′), 3.08 (2H, mdd, J = 16.2, 9.4 Hz, H-7′), 2.88 (1H, m, H-8), 2.64 (1H, s, 4-OH), 2.43 (1H, d, J = 8.5 Hz, H-7), 0.90 (3H, d, J = 6.8 Hz, H-9); ^{13}C NMR data, see Table 1; EIMS *m*/*z* 402 [M^+] (67), 384 (5), 370 (7), 210 (21), 194 (100), 192 (35), 150 (9), 135 (5); HRESIMS *m*/*z* 403.1742 $[M + H]^+$ (calcd for $C_{22}H_{27}O_7$, 403.1757).

Cinerin D [(7*S*,8*R*,2′*R*,3′*S*,5′*R*)-$\Delta^{8'}$-2′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxy-2′,3′,4′,5′-tetrahydro-4′-oxo-7.3′,8.5′-neolignan] (4): colorless needles (*n*-hexane); mp 104−105 °C; $[\alpha]^{25}_D$ −10.3 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (4.72), 236 (3.88), 289 (3.52); CD (*c* 0.04, MeOH) $[\theta]_{247}$ −27 810, $[\theta]_{308}$ −15 450; IR (KBr) ν_{max} 3468, 1757 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.59 (1H, d, J = 1.4 Hz, H-6), 6.54 (1H, d, J = 1.4 Hz, H-2), 5.97 (2H, s, OCH_2O), 5.85−5.95 (1H, m, H-8′), 5.68 (1H, s, H-6′), 5.00−5.20 (2H, m, H-9′), 4.90 (1H, s, H-2′), 3.81 (3H, s, CH_3-5), 3.55 (3H, s, OCH_3-5′), 3.40 (3H, s, OCH_3-3′), 3.05 (2H, d, J = 7.2 Hz, H-7′), 2.50−2.42 (2H, m,

H-7, H-8), 1.04 (3H, d, $J = 6.8$ Hz, H-9); ^{13}C NMR data, see Table 1; EIMS *m/z* 402 [M$^+$] (100), 387 (10), 384 (29), 370 (12), 306 (25), 210 (30), 194 (75), 192 (44), 150 (12); HRESIMS *m/z* 403.1734 [M + H]$^+$ (calcd for $C_{22}H_{27}O_7$, 403.1757).

Preparation of (*R*)- and (*S*)-MTPA Esters of 2. (*R*)- and (*S*)-MTPA ester derivatives of **2** were prepared by reacting **2** (0.6 mg) in pyridine (30 μL) with (*R*)- and (*S*)-MTPA chlorides (1.5 μL), respectively, at room temperature overnight. The solvent was removed by evaporation, affording the ester as a colorless oil in good yield, which was dissolved in $CDCl_3$ and placed into an NMR tube. The assignment was made according to the reported method.[15]

(*R*)-MTPA Ester of 2: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.84 (1H, s, H-6′), 6.66 (1H, s, H-6), 6.49 (1H, s, H-2), 5.94 (2H, d, $J = 1.0$ Hz, OCH_2O), 5.82−5.86 (m, 1H, H-8′), 5.13−5.16 (2H, m, H-9′), 5.50 (1H, s, H-4′), 3.85 (3H, s, OCH_3-5), 3.58 (3H, s br, OCH_3-7″), 3.44 (3H, s, OCH_3-5′), 3.24 (1H, s, H-3′), 3.00−3.05 (2H, m, H-7′), 2.60−2.64 (1H, m, H-8), 2.48 (1H, d, $J = 7.4$ Hz, H-7), 0.97 (3H, d, $J = 6.7$ Hz, H-9); HRESIMS *m/z* 589.2058 [M + H]$^+$ (calcd for $C_{31}H_{32}F_3O_8$, 589.2049).

(*S*)-MTPA Ester of 2: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (1H, s, H-6′), 6.65 (1H, s, H-6), 6.44 (1H, s, H-2), 5.95 (2H, d, $J = 1.0$ Hz, OCH_2O), 5.80−5.84 (m, 1H, H-8′), 5.11−5.15 (2H, m, H-9′), 5.52 (1H, s, H-4′), 3.83 (3H, s, OCH_3-5), 3.60 (3H, s br, OCH_3-7″), 3.51 (3H, s, OCH_3-5′), 3.12 (1H, s, H-3′), 3.01−3.06 (2H, m, H-7′), 2.66−2.70 (1H, m, H-8), 2.37 (1H, d, $J = 7.6$ Hz, H-7), 1.08 (3H, d, $J = 6.6$ Hz, H-9); HRESIMS *m/z* 589.2055 [M + H]$^+$ (calcd for $C_{31}H_{32}F_3O_8$, 589.2049).

Oxidation of 3 and 4.[13] A dispersion of MnO_2 (500 mg) in a solution of either **3** or **4** (3 mg) in $CHCl_3$ (5 mL) was stirred under N_2 for 6 h and filtered over silica gel using $CHCl_3$, and the solvent was removed. The product (2 mg) was found to be the same starting from either **3** or **4**, which exhibited identical spectroscopic and chiroptical properties to those of compound **1**.

Inhibition of PAF-Induced Aggregation in the Rabbit Platelet Assay. Anti-PAF activity was carried out according to the method reported by Koch.[6] Briefly, convenient platelet-rich plasma (PRP) suspensions from rabbit's blood were stirred at 800 rpm and maintained at 37 °C. Samples of PRP were preincubated for 5 min at 37 °C with tested compounds in dimethyl sulfoxide (DMSO). Aggregation was induced by the addition of 10 μL of diluted PAF. The final PAF concentration was 1 ng/mL for PRP. In order to eliminate the effect of the solvent on the aggregation, the final concentration of DMSO was fixed at 0.5%, which did not affect the aggregation measured. Inhibition of platelet aggregation versus a solvent control was calculated as a percentage. Half-maximal inhibition concentrations (IC_{50}) were determined by nonlinear regression analysis using the software GraphPad prism 5.00 (GraphPad software, San Diego, CA).

Alamar Blue Susceptibility Test (MABA). Anti-MTB activity evaluation was performed *in vitro* according to the methodology proposed by Collins and Franzblau,[19] using the H_{37}Rv strain of *M. tuberculosis*. Briefly, initial compound dilutions were prepared in DMSO. A convenient prepared bacteria plate of H_{37}Rv was incubated at 37 °C. Starting at day 4 of incubation, 20 mL of Alamar Blue solution (Alamar Biosciences/Accumed, Westlake, OH) and 12.5 mL of 20% Tween 80 were added, and the plates were reincubated at 37 °C. Wells were observed at 12 and 24 h for a color change from blue to pink and for a reading of ≥50.000 fluorescence units (FU). Fluorescence was measured in a Cytofluor II microplate fluorometer (PerSeptive Biosystems, Framingham, MA) in bottom-reading mode with excitation at 530 nm and emission at 590 nm. Plates were then incubated at 37 °C, and results were recorded at 24 h post-reagent addition. Percent inhibition was defined as 1 − (test well FU/mean FU of triplicate wells) × 100.

Antibacterial Assay. Antibacterial activity was tested according to the methodology described by Lehrer et al.[20] using Gram-positive (*Staphylococcus aureus* ATCC-65380, *Enteroccocus faecalis* ATCC-29212) and Gram-negative (*Escherichia coli* ATCC-25922, *Salmonella typhimurium* ATCC 14028s, MS 7953, and EG10627) strains.

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of new compounds **1**−**4**, ^{1}H NMR spectra of (*S*)- and (*R*)-MTPA ester derivatives, and HMBC and NOESY of compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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