Benzoic Acid Derivatives from *Piper* Species and Their Fungitoxic Activity against *Cladosporium cladosporioides* and *C. sphaerospermum*

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Piper crassinervium, *P. aduncum*, *P. hostmannianum*, and *P. gaudichaudianum* contain the new benzoic acid derivatives crassinervic acid (**1**), aduncumene (**8**), hostmaniane (**18**), and gaudichaudianic acid (**20**), respectively, as major secondary metabolites. Additionally, 19 known compounds such as benzoic acids, chromenes, and flavonoids were isolated and identified. The antifungal activity of these compounds was evaluated by bioautographic TLC assay against *Cladosporium cladosporioides* and *C. sphaerospermum*.

Piperaceae species have been extensively investigated as a source of new natural products with potential antitumoral, antimicrobial, antifungal, and insecticidal activities.[1−3] The phytochemical profile in *Piper* species is characterized by the production of typical classes of compounds such as amides, benzoic acids, and chromenes in addition to lignans, neolignans, and a few alkaloids.[4−6]

As part of our research aiming to uncover new antifungal compounds in Brazilian Piperaceae species, we have previously reported the occurrence of pyrrolidine amides in *P. hispidum*[7] and piperidine, isobutyl, and pyrrolidine amides in *P. tuberculatum*[8] and *P. arboreum*.[9] Additionally, antifungal prenylated hydroquinones and flavanones from leaves of *P. crassinervium*[10] have been described as well. The search for new antifungal compounds will contribute to establish a model for further investments in this field in order to preserve, to study, and to exploit rationally the remaining flora of São Paulo State.

In the course of the Biota Program (http://www.biota.sp), a large number of *Piper* species collected in the state of São Paulo had their extracts assayed by means of bioautography using *Cladosporium cladosporioides* (Fresen) de Vries and *C. sphaerospermum* (Perzig). Since the extracts of *Piper crassinervium* Kunth, *P. aduncum* L., *P. hostmannianum* (Miquel) C. DC., and *P. gaudichaudianum* Kunth showed high growth inhibitory activity, they were subjected to dereplication procedures using chromatographic techniques associated with the bioautographic assay. Thus, the major aim of this paper is to describe the isolation and structural determination of the new antifungal compounds from Piperaceae species.

Results and Discussion

The MeOH extract from leaves of *P. crassinervium* yielded three prenylated 4-hydroxybenzoic acids [4-hydroxy-3-(3′,7′-dimethyl-3′-hydroxy-1′-oxo-6′-octenyl)benzoic acid (crassinervic acid, **1**), 4-hydroxy-(3′,7′-dimethyl-1′-oxo-octa-2′-*E*-6′-dienyl)benzoic acid[11] (**2**), and 4-hydroxy-(3′,7′-dimethyl-1′-oxo-octa-2′-*Z*-6′-dienyl)benzoic acid[11] (**3**)], 3,4,5-trimethoxydihydrocinnamic acid[12] (**4**), 1,4-dihydroxy-2-(3′,7′-dimethyl-1′-oxo-octa-2′-*E*-6′-dienyl)benzene[10] (**5**), and two flavanones [5,7-dihydroxy-4′-methoxyflavanone[13] (naringenin 4′-methyl ether, **6**) and 5,4′-dihydroxy-7-methoxyflavanone[14] (sakuranetin, **7**)].

The CH_2Cl_2- and hexane-soluble parts of the MeOH extract of the leaves of *P. aduncum* yielded one new prenylated benzoic acid [methyl 2-methoxy-5-(3′-methyl-1′-oxo-2′-butenyl)benzoate (aduncumene, **8**)], four known chromenes [methyl 2,2-dimethyl-8-(3′-methyl-2′-butenyl)-2*H*-1-benzopyran-6-carboxylate (**9**), methyl 2,2-dimethyl-2*H*-1-benzopyran-6-carboxylate (**10**), methyl 8-hydroxy-2,2-dimethyl-2*H*-1-benzopyran-6-carboxylate (**11**), and 2,2-dimethyl-2*H*-1-benzopyran-6-carboxylic acid[15,16] (**12**)], 4-methoxy-3-(3′,7′-dimethylocta-2′-*E*-6′-dienyl)benzoic acid[15] (**13**), and two dihydrochalcones [2′,6′-dihydroxy-4′-methoxydihydrochalcone[15] (**14**) and piperaduncine B[17] (**15**)].

The CH_2Cl_2−MeOH (2:1) extract of the leaves of *P. hostmannianum* yielded methyl 2,2-dimethyl-2*H*-1-benzopyran-6-carboxylate[16] (**10**), three prenylated methyl hydroxybenzoate derivatives [methyl 4-hydroxy-3-(3′-methyl-2′-butenyl)benzoate[18,19] (**16**), methyl 4-hydroxy-3-(3′-methyl-2′-hydroxy-3′-butenyl)benzoate[16] (**17**), and the new derivative methyl 4-hydroxy-3-(2′,3′-dihydroxy-3′-methylbutyl)benzoate (hostmaniene, **18**)], and pinocembrin[20,21] (**19**).

The CH_2Cl_2−MeOH (2:1) extracts of the leaves and roots of *P. gaudichaudianum* yielded two chromenes [2-methyl-2-(4′-methyl-3′-pentenyl)-8-(3″-methyl-2″-butenyl)-2*H*-1-benzopyran-6-carboxylic acid (gaudichaudianic acid, **20**) and methyl 2,2-dimethyl-1-oxo-2*H*-1-benzopyran-6-carboxylate[22] (**21**)], three prenylated benzoic acids [4-methoxy-3-(3′,7′-dimethylocta-2′-*E*-6′-dienyl)benzoic acid[15] (**13**), 4-hydroxy-3-(3′,7′-dimethylocta-2′-*E*-6′-dienyl)benzoic acid[23] (**22**), and methyl 4-hydroxy-3-(3′-methyl-1′-oxo-2′-butenyl)benzoate[24] (methyl taboganate, **23**)], and pinocembrin[20,21] (**19**).

Compound **1** was isolated from the leaves of *P. crassinervium* as a white amorphous solid. Its molecular formula was established as $C_{17}H_{22}O_5$ by analysis of its HREIMS spectrum (*m/z* 306.1459, calcd 306.1467). The IR spectrum indicated the presence of hydroxyl groups (3380 cm^{-1}), two carbonyl groups (1696, 1611 cm^{-1}), and an aromatic ring (1489, 1421 cm^{-1}). The ^1H NMR spectrum exhibited signals

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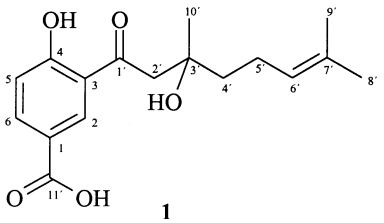

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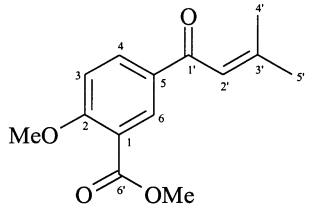

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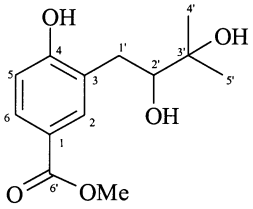

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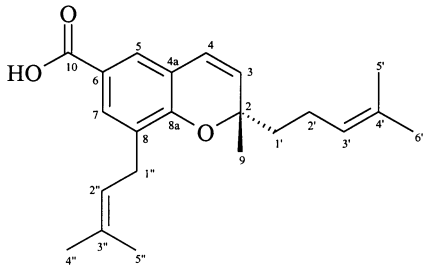

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for three aromatic hydrogens at δ 6.93 (d, J = 8.9 Hz), 8.11 (dd, J = 8.9 and 2.4 Hz), and 8.56 (d, J = 2.4 Hz), indicative of a 3,4-disubstituted benzoic acid derivative. Additional signals included three methyl singlets (δ 1.37, 1.50, 1.58), two doublets at δ 2.77 (1H, J = 16.5 Hz) and 2.64 (1H, J = 16.5 Hz), characteristic of an isolated α-carbonyl methylene group, and a triplet at δ 4.98 (1H, J = 7.2 Hz), indicating the presence of a double bond in the side chain. The ^{13}C NMR spectra (BBD and DEPT 135°) showed signals for 17 carbon atoms assigned as two carbonyl (δ 191.5, 170.6), six aromatic (δ 163.7, 137.3, 129.9, 122.1, 119.9, 118.8), two olefinic (δ 132.5, 122.9), three methyl (δ 25.6, 23.9, 17.5), one tertiary carbinolic (δ 82.3), and three aliphatic methylenic carbons (δ 47.2, 39.3, 22.2). The assignments of the signals for aromatic carbons were based on comparison with ^{1}H and ^{13}C NMR data reported for known benzoic acids analogues.[11] The connectivity between aromatic and aliphatic moieties was revealed by analysis of the HMBC spectrum. The correlations between signals at δ 8.56 (H-2)/2.77 (H-2′a)/2.64 (H-2′b) with 191.5 (C-1′) as well as between δ 8.56 (H-2)/8.11 (H-6) with 170.6 (CO_2H) (Figure 1) indicated that compound **1** contains one carbonyl group at C-1′ and one carboxyl group attached to C-1. The double bond at the side chain was assigned on the basis of the observed cross-peaks between δ 4.98 (H-6′) and 25.6 (C-8′)/17.5 (C-9′) in the HMBC spectrum and between signals at δ 4.98 (H-6′), 2.03 (H-5′), 1.58 (H-8′), and 1.50 (H-9′) in the DQF-COSY spectrum. Thus, the hydroxyl group on a tertiary carbon atom was placed at C-3′ of the side chain, in agreement with the HMBC correlations observed between the hydrogen signals at δ 2.77 (H-2′a)/2.64 (H-2′b), 1.70 (H-4′), and 1.37 (H-10′) with the carbon signal at δ 82.3 (C-3′). Analysis of the DQF-COSY, HMQC, and HMBC NMR spectra allowed full assignment for all hydrogen and carbon atoms, and thus, the structure of crassinervic acid (**1**) was elucidated as 4-hydroxy-3-(3′,7′-dimethyl-3′-hydroxy-1′-oxo-6′-octenyl)benzoic acid.

Compound **8** was isolated from the leaves of *P. aduncum* as a yellow amorphous solid. The molecular formula $C_{14}H_{16}O_4$ was determined by LRESIMS and elemental analysis. The IR spectrum exhibited absorption bands at 1700, 1610, and at 1445 cm^{-1}, assignable to two carbonyl groups and aromatic double bonds, respectively. The structure of the prenylated benzoic acid derivative was recognized by analysis of the ^{1}H NMR spectrum, which showed signals at δ 8.18 (1H, d, J = 1.9 Hz), 8.06 (1H, dd, J = 8.5 and 1.9 Hz), and 6.93 (1H, d, J = 8.5 Hz). This spectrum also showed a set of signals including two methyl groups at δ 1.96 (3H, d, J = 1.5 Hz)/2.20 (3H, d, J = 1.5 Hz) and a multiplet at 6.53 (1H), which are characteristics of an isoprene unit, and additionally, two methoxyl groups at δ 3.90 (3H, s) and 3.86 (3H, s). The ^{13}C NMR spectra (BBD and DEPT 135°) exhibited, in the low-field region, one carbomethoxyl group at δ 166.6, six aromatic carbons at δ 161.2, 134.1, 132.0, 131.2, 122.9, 111.4, and two signals corresponding to the double-bond carbons at δ 125.1 (CH) and 156.7 (C). These two signals, associated with the carbonyl signal at δ 192.7, indicated an α,β-unsaturated carbonyl system.[22,25,26] The high-field region exhibited signals assignable to two methyl groups at δ 28.3 and 21.6 and two methoxyl groups at δ 52.5 and 56.2. The assignment of all signals observed in the ^{13}C NMR spectrum of **8** was corroborated by HMQC and HMBC experiments (Figure 1). The HMBC spectrum showed the correlation of the signal at δ 192.2 (C-1′) with the hydrogens at δ 8.18 (H-6), 8.06 (H-4), and 6.53 (H-2′) and between δ 166.6 (C-6′) and 8.18 (H-6) and also with 3.86 (OCH_3). Finally, the cross-peaks observed between δ 156.7 (C-3′) and 2.20 (H-4′) and 1.96 (H-5′) confirmed the structure of **8** as methyl 2-methoxyl-5-(3′-methyl-1′-oxo-2′-butenyl)benzoate, named aduncumene.

Compound **18** was obtained from the leaves of *P. hostmannianum*, as a white amorphous powder. Its LRESIMS data associated with ^{13}C NMR spectra (BBD and DEPT 135°) and elemental analysis indicated a molecular formula of $C_{13}H_{18}O_5$. The IR spectrum indicated the presence of hydroxyl groups (3463 cm^{-1}), an aromatic ring (1613, 1440 cm^{-1}), and a carbonyl group (1715 cm^{-1}). The ^{1}H NMR spectrum showed signals assignable to three coupled aromatic hydrogens at δ 6.76 (d, J = 9.0 Hz), 7.72 (dd, J = 9.0 and 2.0 Hz), and 7.77 (d, J = 2.0 Hz), characteristic of the 3,4-disubstituted benzoic acid derivative. In addition, this spectrum displayed three singlets at δ 1.27 (3H), 1.30 (3H), and 3.80 (3H) assigned, respectively, to two methyl groups attached to an oxygen-bearing carbon and to one methoxyl group. The presence of an oxymethine hydrogen at δ 3.77 (dd, J = 6.0 and 4.5 Hz), associated with the signals at δ 3.02 (1H, dd, J = 17.0 and 4.5 Hz) and 2.73 (1H, dd, J = 17.0 and 6.0 Hz), suggested a hydroxylated isoprene side chain at C-3 of the benzoic acid nucleus. The HMBC spectrum of **18** (Figure 1) showed correlation between the signals at δ 31.1 (C-1′) and 7.77 (H-2), between δ 69.2 (C-2′) and 1.27 (H-4′)/1.30 (H-5′)/3.02 (H-1′a)/2.73

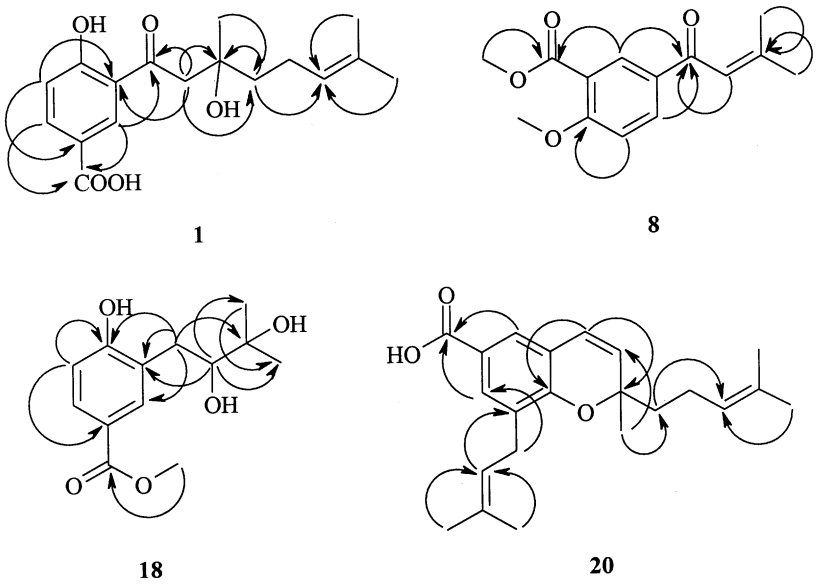

Figure 1. Key HMBC correlations observed for compounds **1**, **8**, **18**, and **20**.

(H-1′b), between δ 25.0 (C-4′) and 1.30 (H-5′), and between δ 22.0 (C-5′) and 3.77 (H-2′)/1.27 (H-4′), confirming the positioning of the vicinal hydroxyl groups at C-2′ and C-3′. The methoxyl group was determined as a methyl ester, considering that a cross-peak between the signals at δ 166.9 (C-6′) and 3.80 (OCH_3) was observed in the HMBC spectrum. Therefore, the structure of **18** was determined as methyl 4-hydroxy-3-(2′,3′-dihydroxy-3′-methylbutyl)benzoate, named hostmaniane.

Compound **20** was isolated from the leaves of *P. gaudichaudianum* as an amorphous solid. The molecular formula $C_{22}H_{28}O_3$ was deduced by LREIMS and elemental analysis. The IR spectrum showed absorption bands at 3500, 1681, and 1601 cm^{-1} assignable to a hydroxyl, conjugated carbonyl, and aromatic ring, respectively. The ^1H NMR spectrum showed two doublets at δ 5.58 (1H, J = 10.0 Hz) and 6.38 (1H, J = 10.0 Hz) and four singlets at δ 1.41 (3H, s), 1.67 (3H, s), 1.56 (3H, s), and 1.73 (6H, s), which were associated with the chromene/isoprene moieties.[15,27] Two low-field doublets at δ 7.59 (1H, J = 2.1 Hz) and 7.75 (1H, J = 2.1 Hz), assignable to the *meta* aromatic hydrogens, indicated a 3,4,5-trisubstituted benzoic acid derivative, which was corroborated by analysis of the ^{13}C NMR spectra. A carboxyl group, with a signal at δ 172.1, was placed between the two aromatic hydrogens (C-6). The chromene moiety was confirmed by the presence of signals at δ 79.9, 129.5, and 121.9 assignable to the carbons C-2, C-3, and C-4, respectively.[27] The DEPT 135° spectrum confirmed these previous assignments and showed the presence of three methylenes at δ 41.9 (C-1′), 22.7 (C-2′), and 28.2 (C-1″), which were associated with the respective protons at δ 1.78 (H-1′), 2.25 (H-2′), and 3.28 (H-1″) by means of the HMQC spectrum. The linkage positions of the two isoprene side chains to the chromene/aromatic ring were determined by an HMBC experiment (Figure 1), in which correlations between the signals at δ 128.9 (C-8) and 5.28 (H-2″), between δ 131.8 (C-7) and 3.28 (H-1″), between δ 41.9 (C-1′) and 1.41 (H-9), and between δ 123.9 (C-3′) and 1.78 (H-1′) were observed. Thus, the complete assignments of NMR data of **20** were made by analysis of HMQC and HMBC data and by comparison with those obtained for similar compounds isolated from *P. lhotzkyanum* and *P. aduncum*.[11,28] Therefore, the structure of **20** was determined as 2-methyl-2-(4′-methyl-3′-pentenyl)-8-(3″-methyl-2″-butenyl)-2*H*-1-benzopyran-6-carboxylic acid, named gaudichaudianic acid. The optical rotation, $[\alpha]^{25}_D$ +21 (c 0.1, $CHCl_3$), was similar to that observed for sargatriol,[29] but this compound has two additional chiral carbons (C5′ and C6′) in its side chain and is not appropriate for a direct comparison. Nevertheless, the CD curve obtained for **20** was fully opposite to that described for sargatriol including the positive Cotton effect at 260−280 nm (styrene chromophore). Thus, despite the differences in the substitution pattern in the aromatic ring for **20**, the *S* configuration at C-2 was suggested.

The antifungal activities of compounds **1**−**23** isolated from these four *Piper* species were evaluated against *C. cladosporioides* and *C. sphaerospermum*[7] (Table 1). The fungitoxic potential for the compounds **5**, **6**, **7**, **17**, **19**, and **21** has been previously reported.[10,22,26,27] As can be seen, the chromene **9**, crassinervic acid (**1**), and hostmaniane (**18**) showed high potential as antifungal compounds and should be further evaluated against other fungi. Several compounds, **2**, **3**, **5**, **7**, **16**, and **19**, showed potency (1.0 μg) similar to that of the control. In the prenylated benzoic acid series (**1**−**3**) the introduction of a hydroxyl group in the side chain, as in the case of **1**, doubled the activity, while the changes in the double-bond configuration (**2** and **3**) did not cause significant improvement.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 digital polarimeter (Na filter, λ = 588 nm) and CD spectrum in $CHCl_3$ with a JASCO ORD/UV-6 spectropolarimeter. IR spectra were measured in KBr pellets on a Perkin-Elmer infrared spectrometer model 1750. UV spectra were recorded on a HP 8452 A spectrophotometer using MeOH as solvent. LREIMS and HREIMS spectra were measured at 70 eV on a HP 5990/5988 A and a VG Autospec spectrometer, respectively, while LRESIMS spectra were recorded on a VG Platform II spectrometer. ^1H and ^{13}C NMR spectra were recorded at 500/300 and 125/75 MHz, respectively, on Bruker DRX-500 and Bruker DPX-300 spectrometers. $CDCl_3$ (Aldrich) was used as solvent and TMS as internal standard. Chemical shifts were reported in δ units (ppm) and coupling constants (J) in Hz. Elemental analyses were obtained on a Perkin-Elmer elemental analyzer model 2400 CHN. Silica gel (Merck, 230−400 mesh) and Sephadex LH-20 (Sigma) were used for column chromatographic separations, while silica gel 60 PF_{254} (Merck) was used for analytical

Table 1. Antifungal Activities of the Compounds Isolated from *Piper crassinervium* (**1**−**7**), *P. aduncum* (**8**−**15**), *P. hostmanniannm* (**10**, **16**−**19**), and *P. gaudichaudianun* (**13**, **19**−**23**) and Positive Controls against *Cladosporium cladosporioides* and *C. sphaerospermum*

	antifungal activity (μg)[a]	
compound	*C. cladosporioides*	*C. sphaerospermum*
1	0.5	0.5
2	1.0	1.0
3	1.0	1.0
4	10.0	10.0
5	1.0	1.0
6	1.0	5.0
7	1.0	1.0
8	5.0	5.0
9	0.1	0.1
10	5.0	5.0
11	5.0	5.0
12	5.0	5.0
13	*b*	*b*
14	*b*	*b*
15	*b*	*b*
16	1.0	1.0
17	5.0	5.0
18	0.5	0.5
19	1.0	1.0
20	10.0	10.0
21	1.0	1.0
22	5.0	5.0
23	*b*	*b*
nystatin	1.0	1.0
miconazole	1.0	1.0

[a] Minimum amount required for the inhibition of fungal growth on thin-layer chromatography plates (TLC). [b] Inactive at 100 μg.

TLC (0.25 mm), preparative TLC (1.0 mm), and circular chromatography (4.0 mm). HPLC separations were performed on a Varian model SD-1, using a reversed-phase column (C-18 Supelcosil, 10 μm, 25 cm × 10 mm), with a flow rate of 2 mL/min and detection at 270 nm.

Plant Material. Leaves from *P. crassinervium* Kunth were collected on the campus of the Universidade de São Paulo, Brazil, and identified by Dr. Guillermo E. D. Paredes (Universidad Nacional Pedro Ruiz Gallo, Peru). The voucher specimen (Kato-0084) was deposited in the Herbarium of Instituto de Botânica, São Paulo, Brazil. Leaves from *P. aduncum* were collected at Reserva Ripasa, Ibaté, São Paulo, Brazil, and identified by Dr. Elsie F. Guimarães (Jardim Botânico, Rio de Janeiro, Brazil). The voucher specimen (PA22) was deposited in the Herbarium of the Instituto de Botânica, São Paulo, Brazil. Leaves from *P. hostmannianum* were collected in Manaus (State of Amazonas) and identified by Dr. Guillermo E. D. Paredes. The voucher specimen (Kato-0156) was deposited at the Herbarium of the Instituto de Biociências, USP, São Paulo, Brazil. Leaves and roots from *P. gaudichaudianum* were collected on the campus of the Universidade de São Paulo (Brazil) and were identified by Dr. Elsie F. Guimarães. The voucher specimen (Kato-0093) was deposited in the Herbarium of the Instituto de Botânica, São Paulo, Brazil.

Extraction and Isolation. Fresh leaves from *P. crassinervium* were air-dried, and the ground powder (320 g) was extracted by maceration with MeOH three times (3 × 2 L) at room temperature. The resulting solution was concentrated in a vacuum to yield 6.0 g of crude extract, which was submitted to column chromatography over silica gel (250 g), using a gradient elution from hexane to EtOAc and from EtOAc to MeOH, yielding 14 fractions. Fraction 4 (524 mg) was submitted to silica gel chromatography using gradient mixtures of EtOAc in hexane, yielding three fractions (I−III). Fraction II (94 mg) was purified by preparative TLC [CH_2Cl_2−EtOAc, 9:1, three elutions] to yield 51 mg of **5**. Compounds **2** (41 mg) and **6** (124 mg) were obtained by purification of fraction 6 (1083 mg) in column chromatography over silica gel, eluted with mixtures of hexane−EtOAc in a gradient mode. Fraction 7 (312 mg) was purified by silica gel chromatography using a gradient elution from CH_2Cl_2 to EtOAc, yielding 57 mg of a fraction composed of **6** and **7**. Compound **3** (23 mg) was obtained from fraction 9 (149 mg) after flash silica gel column chromatography eluting with CH_2Cl_2−EtOAc (9:1, 8:2, and 7:3). Fraction 10 (812 mg) was chromatographed on a silica gel column using a gradient from hexane to EtOAc and from EtOAc to MeOH, yielding four fractions (I−IV). Fraction IV (354 mg) was submitted to gel permeation column chromatography on Sephadex LH-20 (60 × 2 cm) using hexane−CH_2Cl_2 (4:1) and CH_2Cl_2−Me_2CO (1:1) as eluent, to yield **1** (115 mg). Compound **4** (14 mg) was purified from fraction 11 (102 mg) after preparative TLC [CH_2Cl_2−EtOAc, 8:2, three elutions].

Fresh leaves from *P. aduncum* were air-dried, and the powder (1300 g) was extracted by maceration with EtOH three times (3 × 3 L) at room temperature. The resulting solution was partitioned between hexane and EtOH−H_2O (8:2) to yield the hexane-soluble solution. The EtOH−H_2O solution was extracted with CH_2Cl_2, yielding a CH_2Cl_2-soluble solution (14.9 g), which was applied to a silica gel column and eluted with hexane containing increasing volumes of EtOAc, yielding 19 fractions (1−19). Fraction 2 yielded **14** (579 mg). Fraction 1 (5.5 g) was subjected to silica gel column chromatography eluting with hexane containing increasing concentrations of EtOAc, yielding 20 fractions (I−XX). Fraction V (37.2 mg) was subjected to preparative TLC [hexane−EtOAc (7:3), two elutions], yielding 6 mg of compound **11**, and the fraction XII (1.12 g) was applied to a silica gel column, eluted with hexane containing increasing amounts of EtOAc, to yield 16 fractions (A−L). Fraction C yielded **8** (119 mg). The hexane-soluble part (15.3 g) was applied to a silica gel column and eluted with hexane containing increasing amounts of EtOAc, yielding seven fractions (1−7). Fraction 5 (1.44 g) was applied to a silica gel column, eluted with hexane containing increasing amounts of EtOAc, yielding 15 fractions (I−XV). Fraction I (20 mg) was subjected to preparative TLC [hexane−EtOAc (7:3), two elutions], yielding **9** (5 mg). Fraction IV (48 mg) was subjected to preparative TLC [hexane−EtOAc (7:3), two elutions], yielding **10** (38 mg). Fraction VII (97 mg) was subjected to reversed-phase HPLC (MeOH−H_2O, 65:35), yielding **13** (33 mg). Fraction 7 (395 mg) was applied to a silica gel column, eluted with hexane containing increasing amounts of EtOAc, yielding 14 fractions (I−XIV). Fraction XII (100 mg) was subjected to preparative TLC [hexane−EtOAc (7:3), two elutions], yielding five fractions (A−E). Fraction B (50 mg) was subjected to reversed-phase HPLC (MeOH−H_2O, 65:35), yielding **15** (13 mg). The air-dried and powdered leaves from *P. aduncum* (300 g) were extracted with EtOAc three times (3 × 1 L). The resulting extract (28.3 g) was dissolved in $CHCl_3$ (100 mL) and partitioned with a saturated solution of $NaHCO_3$ (3 × 50 mL). The $NaHCO_3$ solution was neutralized with HCl (6 M), extracted with EtOAc (3 × 50 mL), dried over Na_2SO_4, and concentrated, yielding 764 mg of residue. This residue was applied to a silica gel column and eluted with hexane containing increasing amounts of EtOAc, yielding five fractions (1−5). Fraction 3 (80 mg) was subjected to preparative TLC [$CHCl_3$−MeOH (99:1), three elutions], yielding **12** (15 mg).

The dried and powdered leaves from *P. hostmannianum* (550 g) were extracted three times with a mixture of CH_2Cl_2−MeOH (2:1) (3 × 1 L) at room temperature. The resulting CH_2Cl_2−MeOH extract was concentrated in vacuo to yield 8 g of the crude extract. Part of this extract (6.5 g) was submitted to silica gel column chromatography and eluted with increasing amounts of EtOAc in hexane, yielding seven fractions (1−7). Fraction 1 (233 mg) was applied on a silica gel column and was eluted with CH_2Cl_2 containing increasing amounts of EtOAc, yielding **10** (10 mg) and **16** (100 mg). Fraction 3 (120 mg) was purified by reversed-phase column chromatography eluting with MeOH−H_2O (95:5) to yield 12 mg of **19**. Fraction 4 (123 mg) was submitted to preparative TLC [hexane−EtOAc (8:2), three elutions] to yield **17** (24 mg) and **18** (16 mg).

Dried ground leaves (20 g) and roots (12 g) from *P. gaudichaudianum* were extracted twice with CH_2Cl_2−MeOH

(2:1) (2 × 1 L), yielding 5.5 and 2.9 g of crude extracts, respectively. Part of the extract from the leaves (4.5 g) was suspended in MeOH−H_2O (8.5:1.5), filtered in a bed of Celite, and concentrated in vacuo to yield 2.5 g of the chlorophyll-free leaves extract. This extract was subjected to fractionation on a silica gel column using hexane with increasing amounts of EtOAc as eluent, yielding 15 fractions (1−15). Fraction 7 (50 mg) was subjected to preparative TLC [CH_2Cl_2−hexane−EtOAc−AcOH, 7.7:1.5:0.5:0.3, three elutions] to yield **20** (15 mg) and **23** (3 mg). Part of the crude CH_2Cl_2−MeOH extract from leaves (0.9 g) was dissolved in EtOAc (50 mL) and partitioned with a saturated solution of $NaHCO_3$ (3 × 50 mL). The $NaHCO_3$ fraction was neutralized with HCl (6 M), extracted with EtOAc (3 × 50 mL), and dried over Na_2SO_4, yielding a residue (20 mg). This residue was subjected to preparative TLC [hexane−EtOAc, 9:1, three elutions], yielding **13** (4 mg) and **22** (7 mg). Part of the extract from roots (245 mg) was separated by circular chromatography (layer of 4 mm) and eluted with hexane with increasing amounts of EtOAc, yielding **23** (77 mg) and **21** (35 mg).

Crassinervic acid [4-hydroxy-3-(3′,7′-dimethyl-3′-hydroxy-1′-oxo-6′-octenyl)benzoic acid] (**1**) was obtained as a white amorphous solid: $[\alpha]^{25}_D$ −6.70 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 262 (3.90), 320 (3.28) nm; IR (KBr) ν_{max} 3380, 1696, 1611, 1489, 1421 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.56 (1H, d, J = 2.4 Hz, H-2), 6.93 (1H, d, J = 8.9 Hz, H-5), 8.11 (1H, dd, J = 8.9, 2.4 Hz, H-6), 2.77 (1H, d, J = 16.5 Hz, H-2′a), 2.64 (1H, d, J = 16.5 Hz, H-2′b), 1.70 (2H, m, H-4′), 2.03 (2H, m, H-5′), 4.98 (1H, t, J = 7.2 Hz, H-6′), 1.58 (3H, br s, H-8′), 1.50 (3H, br s, H-9′), 1.37 (3H, s, H-10′), 12.3 (1H, s, OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 119.9 (C, C-1), 129.9 (CH, C-2), 122.1 (C, C-3), 163.7 (C, C-4), 118.8 (CH, C-5), 137.3 (CH, C-6), 191.5 (C, C-1′), 47.2 (CH_2, C-2′), 82.3 (C, C-3′), 39.3 (CH_2, C-4′), 22.2 (CH_2, C-5′), 122.9 (CH, C-6′), 132.5 (C, C-7′), 25.6 (CH_3, C-8′), 17.5 (CH_3, C-9′), 23.9 (CH_3, C-10′), 170.6 (C, C-11′); LRESIMS *m/z* 329 $[M + Na]^+$, 307 $[M + H]^+$; HREIMS *m/z* 306.1459 (calcd for $C_{17}H_{22}O_5$ 306.1467).

Aduncumene [methyl 2-methoxy-5-(3′-methyl-1′-oxo-2′-butenyl)benzoate] (**8**) was obtained as yellow amorphous solid: UV (MeOH) λ_{max} (log ϵ) 254 (2.89) nm; IR (KBr) ν_{max} 1700, 1610, 1445 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.18 (1H, d, J = 1.9 Hz, H-6), 6.93 (1H, d, J = 8.5 Hz, H-3), 8.06 (1H, dd, J = 8.5, 1.9 Hz, H-4), 6.53 (1H, m, H-2′), 2.20 (3H, d, J = 1.5 Hz, H-4′), 1.96 (3H, d, J = 1.5 Hz, H-5′), 3.90 (3H, s, OC*H*$_3$-2), 3.86 (3H, s, OC*H*$_3$-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.2 (C, C-1), 122.9 (C, C-2), 111.4 (CH, C-3), 134.1 (CH, C-4), 131.2 (C, C-5), 132.0 (CH, C-6), 192.7 (C, C-1′), 125.1 (CH, C-2′), 156.7 (C, C-3′), 28.3 (CH_3, C-4′), 21.6 (CH_3, C-5′), 166.6 (C, C-6′), 52.2 (CH_3, O*C*H$_3$/C-2), 56.2 (CH_3, O*C*H$_3$/C-6′); LRESIMS *m/z* 249 $[M + H]^+$, 193 $[M^+ - C_4H_7]$; *anal.* C 66.04%, H 5.58%, calcd for $C_{14}H_{16}O_4$, C 66.70%, H, 5.45%.

Hostmaniane [methyl 4-hydroxy-3-(2′,3′-dihydroxy-3′-methylbutyl)benzoate] (**18**) was obtained as a white amorphous powder: $[\alpha]^{25}_D$ −36.2 (*c* 0.78, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (3.42), 290 (3.31) nm; IR (KBr) ν_{max} 3463, 1715, 1613, 1440 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (1H, d, J = 2.0 Hz, H-2), 6.76 (1H, d, J = 9.0 Hz, H-5), 7.72 (1H, dd, J = 9.0, 2.0 Hz, H-6), 3.02 (1H, dd, J = 17.0, 4.5 Hz, H-1′a), 2.73 (1H, dd, J = 17.0, 6.0 Hz, H-1′b), 3.77 (1H, dd, J = 6.0, 4.5 Hz, H-2′), 1.27 (3H, s, H-4′), 1.30 (3H, s, H-5′), 3.80 (3H, s, OC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 122.4 (C, C-1), 132.2 (CH, C-2), 118.7 (C, C-3), 157.1 (C, C-4), 117.2 (CH, C-5), 129.5 (CH, C-6), 31.1 (CH_2, C-1′), 69.2 (CH, C-2′), 77.8 (C, C-3′), 25.0 (CH_3, C-4′), 22.0 (CH_3, C-5′), 166.9 (C, C-6′), 51.8 (CH_3, OCH_3); LREIMS *m/z* 236 $[M - H_2O]^+$ (46), 205 (12), 187 (17), 178 (17), 165 (72), 147 (7), 134 (14), 107 (23), 91 (11), 71 (57), 59 (19), 43 (100); LRESIMS *m/z* 255 $[M + H]^+$ (100); *anal.* C 61.23%, H, 7.30%, calcd for $C_{13}H_{18}O_5$, C 61.40%, H 7.13%.

Gaudichaudianic acid [(2*S*)-2-methyl-2-(4′-methyl-3′-pentenyl)-8-(3″-methyl-2″-butenyl)-2*H*-1-benzopyran-6-carboxylic acid] (**20**) was obtained as an amorphous solid: $[\alpha]^{25}_D$ +21.0 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 240 (4.26) nm; IR (KBr) ν_{max} 3500, 1681, 1601 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 5.58 (1H, d, J = 10.0 Hz, H-3), 6.38 (1H, d, J = 10.0 Hz, H-4), 7.59 (1H, d, J = 2.1 Hz, H-5), 7.75 (1H, d, J = 2.1 Hz, H-7), 1.41 (3H, s, H-9), 1.78 (2H, m, H-1′), 2.25 (2H, m, H-2′), 5.09 (1H, m, H-3′), 1.56 (3H, s, H-5′), 1.67 (3H, s, H-6′), 3.28 (2H, d, J = 7.3 Hz, H-1″), 5.28 (1H, m, H-2″), 1.73 (6H, s, H-4″ and H-5″); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 79.9 (C, C-2), 129.5 (CH, C-3), 121.9 (CH, C-4), 120.6 (C, C-4a), 126.7 (CH, C-5), 120.8 (C, C-6), 131.8 (CH, C-7), 128.9 (C, C-8), 155.8 (C, C-8a), 26.9 (CH_3, C-9), 172.1 (C, C-10), 41.9 (CH_2, C-1′), 22.7 (CH_2, C-2′), 123.9 (CH, C-3′), 131.8 (C, C-4′), 17.6 (CH_3, C-5′), 25.6 (CH_3, C-6′), 28.2 (CH_2, C-1″), 121.9 (CH, C-2″), 132.6 (C, C-3″), 25.8 (CH_3, C-4″), 17.3 (CH_3, C-5″); LREIMS *m/z* 340 $[M]^+$ (34), 325 (12), 257 (100), 69 (33); *anal.* C 77.01%, H 7.98%, calcd for $C_{22}H_{28}O_3$, C 77.65%, H 8.24%.

Bioassay Evaluation. The microorganisms used in the antifungal assays, *C. cladosporioides* (Fresen) de Vries SPC 140 and *C. sphaerospermum* (Perzig) SPC 491, have been maintained at the Instituto de Botânica, São Paulo, Brazil.

For the antifungal assay 10.0 μL of solutions corresponding to 100.0 μg of crude extracts or semipurified fractions were applied to precoated Si gel TLC plates, developed with hexane−EtOAc (7:3), and dried for complete removal of solvents. For the pure compound 10.0 μL of solutions corresponding to 50.0, 10.0, 5.0, 1.0, 0.5, and 0.1 μg were applied to precoated Si gel TLC plates. The chromatograms were sprayed with a spore suspension of *C. cladosporioides* or *C. sphaerospermum* in glucose and salt solution and incubated for 72 h in darkness in a moistened chamber at 25 °C, following the previously reported procedure.[10,30,31] Fungal growth inhibition appeared as clear zones against a dark background, indicating the minimum amount of compounds **1**−**25** required for it (Table 1). Nystatin and miconazole were used as positive controls, whereas ampicillin and chloramphenicol were used as negative controls.

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