Hydroxyalkenylresorcinols from *Stylogyne turbacensis*

Carlos Jiménez-Romero,[†] Daniel Torres-Mendoza,[†] Luis David Ureña González,[‡] Eduardo Ortega-Barría,[‡] Kerry L. McPhail,[§] William H. Gerwick,[⊥] and Luis Cubilla-Rios*[,†]

Laboratory of Tropical Bioorganic Chemistry, Faculty of Natural, Exact Sciences and Technology, Apartado 0824-10835, University of Panama, Panama City, Republic of Panama, Institute for Advance Scientific Investigation and High Technology Services, Secretariat of Science and Technology, Clayton, Ancon, Republic of Panama, College of Pharmacy, Oregon State University, Corvallis, Oregon 97331, and Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093

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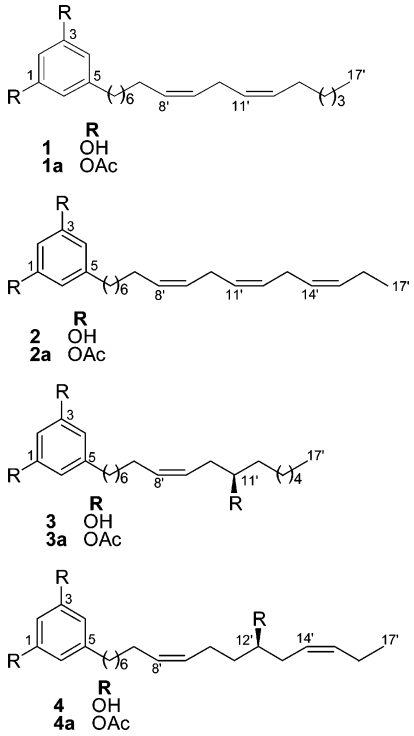
Two new compounds, 5-(11′(*S*)-hydroxy-8′-heptadecenyl)resorcinol (**3**) and 5-(12′(*S*)-hydroxy-8′,14′-heptadecadienyl)-resorcinol (**4**), were isolated from the leaves of *Stylogyne turbacensis* together with the known analogue metabolites **1** and **2**. Compounds **3** and **4** showed the strongest activity in the leishmania assay, 7 and 3 μM, respectively, while compounds **1**, **2**, and **4** showed moderate activity against a drug-resistant strain of *Trypanosoma cruzi* with IC_{50} values of 30, 25, and 22 μM, respectively. Additional testing in MCF-7 and NCI-H460 was performed for compounds **3** and **4**. The structures of compounds **1**−**4** were elucidated using NMR, MS, and other spectroscopic data. The absolute stereochemistry of compounds **3** and **4** was also investigated using the Mosher ester approach. Peracetylated derivatives of these four metabolites were produced and their activities determined in the *Trypanosoma cruzi* assay.

As part of the Panama ICBG project, which uses an ecological approach to discover new treatments for parasitic diseases and cancer from tropical plants,[1] we report the isolation of two new hydroxyalkenylresorcinols (**3**, **4**) from the leaves of *Stylogyne turbacensis* Lundell (Myrsinaceae). Alkylresorcinols are found in many different living organisms, such as lower and higher plants, algae, fungi, bacteria, and animals, and are important in multiple aspects of cellular biochemistry and physiology.[2] Recently, 5-alkylresorcinols have been isolated from the mushroom *Merulius incarnates*. These compounds were found to inhibit the growth of methicillin-resistant *Staphylococcus aureus* (MRSA).[3] Although there are no ethnopharmacologic uses for this plant, the genus *Stylogyne* has often been confused with plants of the genera *Geissanthus* J. D. Hooker and *Ardisia* Swartz,[4] and the latter genus has been investigated for its traditional use in human medicine.[5] Related alkenylresorcinols[6] and quinones with unsaturated alkyl chains have been obtained from the genus *Ardisia*.[7]

Results and Discussion

The EtOAc/MeOH (1:1) extract of mature leaves of *S. turbacensis* was fractionated by vacuum liquid chromatography (VLC) using a polarity gradient with mixtures of *n*-hexane, EtOAc, and MeOH into five main fractions (1−5) according to their TLC profile. Compounds **1**, **2** and **3** were isolated from fraction 2 (IC_{50} 4.5 μg/mL), and compound **4** was isolated from fraction 3 (IC_{50} 4.3 μg/mL) using silica gel column chromatography.

Compounds **1** and **2** yielded similar ^{1}H NMR spectra, and both showed the presence of three *meta*-coupled aromatic protons at δ 6.24 (d, J = 2.3 Hz, 2H) and 6.17 (t, J = 2.3 Hz, 1H) and a broad olefinic proton multiplet centered at δ 5.36 (4H for **1** and 6H for **2**). Signals due to a bis-allylic methylene group were centered at δ 2.77 (t, J = 6.7, 2H) in compound **1** and at δ 2.81 (t, J = 5.4 Hz, 4H) in compound **2**, while benzylic and homobenzylic protons appeared at δ 2.46 (t, J = 7.7 Hz, 2H) and δ 1.56 (m, 2H), respectively. Both spectra also showed signals due to a long alkenyl side chain at δ 1.29 (overlapped, 14H for **1** and 8H for **2**) and a terminal methyl at δ 0.89 (t, J = 7.0 Hz, **1**) and δ 0.97 (t, J = 7.5 Hz, **2**). The ^{13}C NMR spectra showed 23 carbon atoms were present in both compounds. Four olefinic methine carbons for compound **1** and six for compound **2** suggested that they possessed C_{17} di- and triunsaturated alkenyl chains, respectively. The identification of compounds **1** and **2** was confirmed by comparing the ^{1}H and ^{13}C NMR and MS data with literature values for these known substances.[7,8]



Compound **3** was found to have a molecular formula of $C_{23}H_{38}O_3$ by HRCIMS ($[M + H]^+$ m/z 362.2887, calcd for 362.2899), which was consistent with the carbon and proton count from ^{13}C NMR and DEPT spectra. The IR spectrum indicated the presence of phenolic and OH groups (3398 and 3260 cm^{-1}) and olefins (1598 cm^{-1}). The ^{1}H NMR spectrum showed signals similar to those for

* To whom correspondence should be addressed. Tel: (507) 6676 5824. Fax: (507) 264 4450. E-mail: lucr@ancon.up.ac.pa.
[†] University of Panama.
[‡] Institute for Advance Scientific Investigation and High Technology Services.
[§] Oregon State University.
[⊥] University of California at San Diego.

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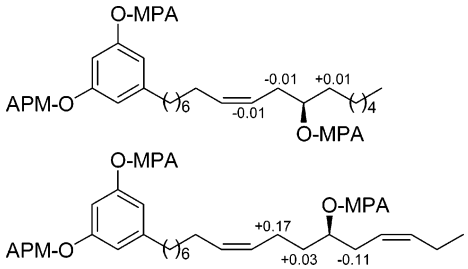

Figure 1. $\Delta\delta^{RS}$ values for the MPA esters of compounds **3** and **4**.

compound **1**, with notable differences being the absence of a bisallylic methylene at δ 2.77 (H_2-10′ in **1**), appearance of a multiplet at δ 3.66 (H-11′), and increased resolution of the olefinic protons at δ 5.59 and 5.51. The ^{13}C NMR spectrum showed two fewer olefinic carbons with respect to **1** and the appearance of a methine resonance at δ 71.9 (HC-11′) and a methylene at δ 35.4 (HC-12′). These data suggested that **3** had a C_{17} monounsaturated side chain containing an OH group.

The connectivities in the hydroxy alkenyl chain in **3** were assigned on the basis of HSQC, HMBC, and COSY data. The HMBC spectrum showed correlation of the terminal methyl at δ 14.2 (C-17′) with two methylenes at δ 22.8 (C-16′) and 32.0 (C-15′). The HMBC also showed correlations between a methine hydroxyl-bearing carbon at δ 71.9 (C-11′) and one olefinic carbon at δ 125.0 (C-9′) as well as two methylenes at δ 35.4 (C-12′) and 25.8 (C-13′). The COSY spectrum correlated a proton signal at δ 5.59 (H-8′) with signals at δ 2.05 (H-7′) and 5.41 (H-9′), while H-9′ (δ5.41) showed correlations to H-8′ (δ 5.59) and H-10′ (δ 2.23). Additionally, H-11′ (δ 3.66) was coupled to H-10′ (δ 2.23) and H-12′ (δ 1.46). Hence compound **3** was identified as 5-(11′-hydroxy-8′-heptadecenyl)resorcinol.

HRCIMS of compound **4** gave a molecular formula of $C_{23}H_{36}O_3$ ($[M + H]^+$ *m/z* 360.2749, calcd for 360.2743), which was consistent with ^{13}C NMR and DEPT spectra. The IR spectrum showed a broad band for phenolic and OH groups (3330 cm^{-1}) and olefins (1602 cm^{-1}). While the ^{1}H NMR spectrum was similar to that for compound **2**, differences in the spectrum for **4** included two new olefinic proton signals at δ 5.40 (H-14′) and 5.36 (H-15′), a broad allylic methylene signal at δ 2.17 (H-10′), and a terminal methyl triplet at δ 0.95 (J = 7.5 Hz). The ^{13}C NMR spectrum showed 23 carbon atoms including four olefinic carbons. The HMBC spectrum showed correlation of the ^{1}H signal at δ 3.68 (H-12′) with C-10′ (δ 23.5), C-11′ (δ 30.6), and C-15′ (δ 132.4). Additionally, the terminal methyl at δ 0.95 was correlated to C-16′ (δ 20.5). The COSY spectrum for **4** showed that the ^{1}H signal at δ 3.68 (H-12′) was coupled with signals at δ 2.24 (H-13′) and 1.54 (H-11′). In turn, the H-13′ signal (δ 2.24) coupled to proton signals at δ 3.68 (H-12′) and 5.40 (H-14′), while the proton at δ 5.36 (H-15′) correlated with signals at δ 5.40 (H-14′) and 2.01 (H-16′). Therefore, compound **4** was characterized as 5-(12′-hydroxy-8′,14′-heptadecadienyl)resorcinol.

The absolute configuration of hydroxyalkenylresorcinols **3** and **4** was addressed through the use of a modified Mosher ester method.[9] Both (*R*)- and (*S*)-methoxyphenylacetic acid (MPA) esters of **3** and **4** were prepared and purified by simple column chromatography and normal-phase HPLC. All of the protons of these derivatives were characterized by ^{1}H NMR, and the $\Delta\delta$ ($\delta_R - \delta_S$) values are shown in Figure 1, indicating that the absolute configuration at C-11′ of **3** is *S*. Also, the Mosher ester analysis of C-12′ in **4** was consistent with an *S* configuration.

This is the first biological evaluation of alkenyl resorcinols against the intracellular form of the parasite *Trypanosoma cruzi*, the causative organism of Chagas disease, and against *Leishmania donovani*, a unicellular kinotoplastid protozoan parasite, the causative agent for human visceral leishmaniasis. As shown in Table 2, metabolites **1**, **2**, and **4** are nearly equipotent at inhibiting the growth of the intracellular form of the parasite. However, the Vero cell toxicity increases with the presence of a hydroxyl group in the alkyl chains of compounds **3** and **4**. For all four compounds, their activity was reduced by acetylation, and therefore, free phenolic hydroxyl groups appear to be required for biological response in this compound series. Compound **3** is less active against the parasite, and this may be due to the absence of the omega-3 double bond in the alkyl portion of the molecule.

A comparison of activities between the phenolic compound cathecol[10] and the alkenylresorcinols revealed that antileishmanial activity of compounds **3** (7 μM) and **4** (3 μM) was stronger than catechol against *Leishmania donovani* (31.78 μM); in the same way **4** was more active than catechol against *T. cruzi* (68.1 μM). The stronger activity of the alkenylresorcinols reported here could be due to the presence of an aliphatic chain and the number of double bonds. The OH group on the aliphatic chains of **3** and **4** appears to play a role in the inhibitory activity in the case of leishmaniasis. Additionally, compound **4** is 10 times more active than cytotoxic (30 μM).

Table 1. NMR Data (300 MHz, $CDCl_3$) for Metabolites **3** and **4**

	3		**4**	
position	δ_C mult.	δ_H (J in Hz)	δ_C mult.	δ_H (J in Hz)
1	156.8 $_q$C		156.8 $_q$C	
2	100.3 CH	6.14, t (2.3)	100.3 CH	6.17, d, (2.1)
3	156.8 $_q$C		156.8 $_q$C	
4	107.9 CH	6.23, d (2.3)	107.9 CH	6.22, d (2.1)
5	145.8 $_q$C		145.8 $_q$C	
6	107.9 CH	6.23, d (2.3)	107.9 CH	6.22, d (2.1)
1′	35.6 CH_2	2.48, t (7.4)	35.5 CH_2	2.44, t (7.5)
2′	36.8 CH_2	1.55, br	36.5 CH_2	1.58, br
3′	29.0[a] CH_2	1.29, br	29.0[a] CH_2	1.27, br
4′	28.8[a] CH_2	1.29, br	28.7[a] CH_2	1.27, br
5′	29.1[a] CH_2	1.29, br	28.9[a] CH_2	1.27, br
6′	29.5[a] CH_2	1.29, br	29.4[a] CH_2	1.27, br
7′	27.5 CH_2	2.05, t (7.0)	27.3 CH_2	2.06, t (6.7)
8′	133.9 CH	5.59, br	133.9 CH	5.54, br
9′	125.0 CH	5.41, br	128.4 CH	5.32, br
10′	35.4 CH_2	2.23, t (7.2)	23.5 CH_2	2.17, br
11′	71.9 CH	3.66, br	30.6 CH_2	1.54
12′	30.6 CH_2	1.46	71.5 CH	3.68, br
13′	25.8 CH_2	1.29, br	35.2 CH_2)	2.24
14′	29.5[a] CH_2	1.29, br	124.8 CH	5.40, br
15′	32.0 CH_2	1.29, br	132.4 CH	5.36, br
16′	22.8 CH_2	1.29, br	20.5 CH_2	2.01, t (7.5)
17′	14.2 CH_3	0.87, t (7.0)	14.3 CH_3	0.95, t (7.5)

[a] These assignments are interchangeable.

Table 2. Antitrypanosomal, Antileishmanial, and Cytotoxic Activities of Alkenylresorcinols **1**−**4** and Derivatives **1a**−**4a**

	IC$_{50}$ (μM)[a]				
compound	*T. cruzi*	*L. donovani*	MCF-7	NCI-H460	cytotoxicity[b]
1	30 ± **7**	0.6	8.7	8.7	148 ± 11
2	25 ± **0.1**	0.6	8.7	8.7	104 ± 74
3	138 ± 0.00	7	14	16	76 ± 67
4	22 ± **4**	3	22	>10[d]	30 ± 2
1a	117 ± 0.00	ND	ND	ND	124 ± 0.6
2a	117 ± 0.00	ND	ND	ND	138 ± 0.3
3a	102 ± 0.00	ND	ND	ND	75 ± 4.0
4a	107 ± 0.00	ND	ND	ND	82 ± 46
nifurtimox	6 ± **0.2**	ND	ND	ND	ND
amphotericin B	ND[c]	0.09	ND	ND	ND

[a] Results show the IC$_{50}$ value ± SD (n = 2). [b] Experiments performed with Vero cells. [c] ND = not determined. [d] Inactive (<50% inhibition) at 20 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured with an Autopol III 6971 automatic polarimeter. IR spectra were

taken on a Perkin-Elmer FT-IR RXI spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) with TMS as an internal standard. FABMS and HRCIMS were recorded on a JEOL MSRoute instrument. HPLC was carried out on a Waters LC system, including a 600 pump and a 996 photodiode array detector.

Plant Material. Mature leaves of *S. turbacensis* Lundell were collected from Barro Colorado Nature Monument in Gatún Lake in the Republic of Panama in April 2004. The material was identified by Professor Mireya Correa of the University of Panama and the Smithsonian Tropical Research Institute. Vouchers of the plant have been deposited in the herbarium of the University of Panama (PMA 54008).

Extraction and Isolation. Upon collection, mature leaves were transferred to sealed plastic bags, kept at 15 °C, and processed within 6 h. After removal of the stems, 0.386 kg of fresh leaves was homogenized and concentrated to yield 23.3 g of crude extract (IC_{50} = 9.0 μg/mL).[9] Four aliquots of 5.84 g of the crude extract of mature leaves were chromatographed by VLC on Si gel (7GF, J.T. Baker) eluting with a solvent gradient of *n*-hexane/EtOAc/MeOH (10:0:0, 8:2:0, 6:4:0, 4:6:0, 2:8:0, 0:10:0, 0:5:5, 0:0:10). Fractions were combined according to their TLC profile into five main fractions (1−5). Fraction 2 was subjected to Si gel (37−75 μm) column chromatography and eluted with 1.4 L of 3:1 *n*-hexane/EtOAc and 250 mL of acetone to yield nine fractions (2a−2i), where fraction 2d showed only a single spot on TLC. ^{1}H NMR spectroscopy of this fraction showed olefinic protons indicative of a mixture of similar compounds with different degrees of unsaturation, while TLC impregnated with 15% $AgNO_3$[11] of this fraction showed two spots. Fraction 2d was chromatographed on Si gel 7GF impregnated with 15% $AgNO_3$ eluted with 7:3 *n*-hexane/EtOAc to yield compounds **1** (60 mg) and **2** (14.5 mg). Fraction 2i was chromatographed on the same stationary phase as 2d (6:4 *n*-hexane/EtOAc) to yield **3** (79.5 mg). Fraction 3 was subjected to Si gel 7GF eluted with *n*-hexane/EtOAc, 6:4, 1:1, 3:7, and EtOAc, to yield nine fractions (3a−3i). Fraction 3d was subjected to Si gel impregnated with 15% $AgNO_3$ column chromatography (6:4 *n*-hexane/EtOAc) to yield compound **4** (36.4 mg).

5-(11′(*S*)-Hydroxy-8′-heptadecaenyl)resorcinol (3): $[\alpha]^{25}_D$ +6.5 (*c* 0.80, $CHCl_3$); IR (film) ν_{max} 3398, 3260, 2924, 1598, 1332, 1154, 996 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 362 $[M]^+$ (24), 345 (29), 307 (100), 289 (58), 217 (22), 163 (20); HRCIMS (CH_4) *m*/*z* 363.2887 $[M + H]^+$ (calcd for $C_{23}H_{39}O_3$, 363.2899).

5-(12′(*S*)-Hydroxy-8′, 14′-heptadecadienyl)resorcinol (4): $[\alpha]^{25}_D$ +22.7 (*c* 0.26, $CHCl_3$); IR (film) ν_{max} 3330, 3006, 2928, 1602, 1342, 1154, 1000 cm^{-1}; for ^{1}H and ^{13}C NMR data, see Table 1; FABMS *m*/*z* 361.2 $[M + H]^+$ (37), 343.2 (46), 307.1 (100), 289.1 (68), 257.0 (25), 235.0 (31), 220.9 (51), 188.9 (64), 163.0 (92); HRCIMS (CH_4) *m*/*z* 361.2750 $[M + H]^+$ (calcd for $C_{23}H_{37}O_3$, 361.2743).

Acetylation of Compounds 1−4. Separately, to 18.4 mg of **1**, 8.1 mg of **2**, 11 mg of **3**, and 15 mg of **4** were added 2 mL of acetic anhydride and 2 drops of pyridine. The reaction mixtures were stirred overnight at RT, at which time ice was added and they were extracted with CH_2Cl_2. The organic layers were washed successively with 2 N HCl, 6% Na_2CO_3, and H_2O and then dried over anhydrous Na_2SO_4. After removal of the solvent by rotary evaporation, 16.4 and 9.0 mg of the diacetates **1a** and **2a** and 12.5 and 17.0 mg of the triacetates **3a** and **4a** were obtained. Diacetate **1a**: IR (film) ν_{max} 2930, 2858, 1770, 1616, 1370, 1202, 1022, 900 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.79 (2H, d, *J* = 2.3 Hz, H-4, H-6), 6.74 (1H, s, H-2), 5.36 (2H, br, H-8′, H-9′), 5.31 (2H, br, H-11′, H-12′), 2.76 (2H, t, *J* = 6.0 Hz, H-10′), 2.59 (2H, t, *J* = 7.8 Hz, H-1′), 2.27 (s, *CH*$_3$CO-1, *CH*$_3$CO-3), 2.04 (2H, br t, *J* = 6.0 Hz, H-7′, H-13′), 1.58 (2H, br, H-2′), 1.28 (14H, overlapped, H-3′, H-4′, H-5′, H-6′, H-14′, H-15′, H-16′), 0.88 (3H, t, *J* = 7.0 Hz, H-17′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.0 (Me*C*O-1, Me*C*O-3), 150.9 (2C, C-1, C-3), 145.4 (C, C-5), 130.2 (CH, C-8′), 130.1 (CH, C-9′), 128.0 (CH, C-11′), 127.9 (CH, C-12′), 118.9 (2CH, C-4, C-6), 112.6 (CH, C-2), 35.7 (CH_2, C-1′), 31.5 (CH_2, C-15′), 30.8 (CH_2, C-2′), 29.6 (CH_2, C-6′), 29.3 (2CH_2, C-5′ , C-14′), 29.2 (2CH_2, C-3′, C-4′), 27.2 (2CH_2, C-7′, C-13′), 25.6 (CH_2, C-10′), 22.6 (CH_2, C-16′), 21.1 (*C*H$_3$CO-1, *C*H$_3$CO-3), 14.0 (CH_3, C-17′); FABMS *m*/*z* 427.3 $[M + 1]^+$ (100), 387.3 (45), 345.2 (85), 321.2 (27), 307.1 (36), 289.1 (43), 277.1 (22), 247.0 (31), 235.0 (32), 219.0 (40), 204.9 (63), 190.9 (44), 178.0 (47), 165.0 (38); HRCIMS (CH_4) *m*/*z* 429.3007 $[M + 1]^+$ (calcd for $C_{27}H_{41}O_4$, 429.3005). Diacetate **2a**: IR (film) ν_{max} 2930, 2858, 1768, 1618, 1370, 1200, 1022, 902 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.79 (2H, d, *J* = 2.3 Hz, H-4, H-6), 6.73 (1H, s, H-2), 5.36 (12H, overlapped, H-8′, H-9′, H-11′, H-12′, H-14′, H-15′), 2.80 (4H, t, *J* = 5.6 Hz, H-10′, H-13′), 2.59 (2H, t, *J* = 8.0 Hz, H-1′), 2.27 (s, *CH*$_3$CO-1, *CH*$_3$CO-3), 2.06 (4H, br, H-7′, H-16′), 1.59 (2H, br, H-2′), 1.28 (8H, br, H-3′, H-4′, H-5′, H-6′), 0.97 (3H, t, *J* = 7.5 Hz, H-17′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.1 (Me*C*O-1, Me*C*O-3), 150.9 (2C, C-1, C-3), 145.2 (C, C-5), 132.0 (CH, C-8′), 130.3 (CH, C-15′), 128.3 (2CH, C-9′, C-11′), (CH, C-12′), 127.1 (CH, C-14′), 118.9 (2CH, C-4, CH, C-6), 112.6 (CH, C-2), 35.6 (CH_2, C-1′), 30.8 (CH_2, C-2′), 29.6 (CH_2, C-3′), 29.4 (CH_2, C-6′), 29.2 (2CH_2, C-4′, C-5′), 27.2 (CH_2, C-16′), 25.6 (CH_2, C-10′), 25.5 (CH_2, C-13′), 21.1 (*C*H$_3$CO-1, *C*H$_3$-CO-3), 20.5 (CH_2, C-7′), 14.2 (CH_3, C-17′); HRCIMS (CH_4) *m*/*z* 427.2847 $[M + 1]^+$ (calcd for $C_{27}H_{39}O_4$, 427.2849). Triacetate **3a**: $[\alpha]^{25}_D$ +6.3 (*c* 0.58, $CHCl_3$); IR (film) ν_{max} 2930, 2858, 1772, 1736, 1370, 1198, 1024, 905 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.79 (2H, d, *J* = 2.3 Hz, H-4, H-6), 6.73 (1H, s, H-2), 5.46 (1H, br, H-8′), 5.32 (1H, br, H-9′), 4.87 (1H, br, H-11′), 2.58 (2H, t, *J* = 7.5 Hz, H-1′), 2.27 (2H, br, H-10′), 2.27 (s, *CH*$_3$CO-1, *CH*$_3$CO-3), 2.02, (2H, br, H-7′), 2.02 (s, *CH*$_3$CO-11′), 1.59 (2H, br, H-2′), 1.56 (2H, br, H-12′), 1.27 (16H, overlapped, H-3′, H-4′, H-5′, H-6′, H-13′, H-14′, H-15′, H-16′), 0.87 (3H, t, *J* = 7.0 Hz, H-17′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.8 (Me*C*O-11′), 169.0 (Me*C*O-1, Me*C*O-3), 150.9 (2C, C-1, C-3), 145.3 (C, C-5), 132.6 (CH, C-8′), 124.2 (CH, C-9′), 118.9 (2CH, C-4, C-6), 112.6 (CH, C-2), 74.0 (CH, C-11′), 35.7 (CH_2, C-1′), 33.6 (CH_2, C-12′), 31.9 (CH_2, C-10′), 31.7 (CH_2, C-15′), 30.8 (CH_2, C-2′), 29.6−29.1 (5CH_2, C-3′, C-4′, C-5′, C-6′, C-14′), 27.3 (CH_2, C-7′), 25.3 (CH_2, C-13′), 22.6 (CH_2, C-16′), 21.2 (*C*H$_3$CO-11′), 21.0 (*C*H$_3$CO-1, *C*H$_3$-CO-3), 14.0 (CH_3, C-17′); FABMS *m*/*z* 489.4 $[M + 1]^+$ (9), 429.4 (30), 387.4 (55), 345.3 (100), 326.9 (16), 280.9 (34), 266.8 (18), 246.8 (14), 206.7 (43), 190.7 (21), 162.8 (28); HRCIMS (CH_4) *m*/*z* 489.3230 $[M + 1]^+$ (calcd for $C_{29}H_{45}O_6$, 489.3216). Triacetate **4a**: $[\alpha]^{25}_D$ +23.1 (*c* 0.41, $CHCl_3$); IR (film) ν_{max} 2930, 2856, 1772, 1738, 1370, 1200, 1024, 905 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.79 (2H, d, *J* = 2.1 Hz, H-4, H-6), 6.73 (1H, s, H-2), 5.47 (1H, br, H-8′), 5.38 (1H, br, H-15′), 5.34 (1H, br, H-14′), 5.28 (1H, br, H-9′), 4.88 (1H, br, H-12′), 2.59 (2H, t, *J* = 7.7 Hz, H-1′), 2.27 (s, *CH*$_3$CO-1, *CH*$_3$CO-3), 2.27 (2H, br, H-13′), 2.02 (*CH*$_3$CO-12′), 2.06 (2H, br, H-7′), 2.02 (2H, br, H-16′), 1.59 (2H, br, H-2′), 1.57 (2H, br, H-11′), 1.27 (8H, overlapped, H-3′, H-4′, H-5′, H-6′), 0.95 (3H, t, *J* = 7.5 Hz, H-17′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.7 (Me*C*O-12′), 169.0 (Me*C*O-1, Me*C*O-3), 150.9 (2C, C-1, C-3), 145.3 (C, C-5), 132.8 (CH, C-8′), 132.3 (CH, C-15′), 127.9 (CH, C-9′), 124.0 (CH, C-14′), 118.9 (2CH, C-4, C-6), 112.6 (CH, C-2), 73.6 (CH, C-12), 35.7 (CH_2, C-1′), 33.6 (CH_2, C-13′), 31.9 (CH_2, C-2′), 30.8 (CH_2, C-11′), 29.7−29.2 (4CH_2, C-3′, C-4′, C-5′, C-6′), 27.3 (CH_2, C-7′), 23.1 (CH_2, C-10′), 21.2 (*C*H$_3$CO-12′), 21.1 (*C*H$_3$CO-1, *C*H$_3$CO-3), 20.5 (CH_2, C-16′), 14.2 (CH_3, C-17′); FABMS 487.3 $[M + 1]^+$ (7), 461.0 (7), 443.3 (9), 427.3 (100), 385.3 (19), 343.2 (48), 315.1 (9), 301.0 (8), 280.9 (12), 246.8 (10), 232.8 (11), 204.7 (12), 188.7 (14), 162.8 (38); HRCIMS (CH_4) *m*/*z* 487.3075 $[M + 1]^+$ (calcd for $C_{29}H_{43}O_6$, 487.3060).

Assignment of Absolute Stereochemistry. The MPA esters of the alkenylresorcinols **3** and **4** were prepared by treatment with the corresponding (*R*)- and (*S*)-MPA in the presence of DCC and DMAP in CH_2Cl_2. The mixtures were filtered to remove the dicyclohexylurea by flash chromatography on Si gel eluting with CH_2Cl_2. The MPA esters were purified using a semipreparative normal-phase HPLC (YMC-Pack SIL, 5 μm, 150 × 10 mm; 1.0 mL/min 8:2 *n*-hexane/EtOAc). The spectra of the resulting MPA esters were recorded, the signals assigned, and the $\Delta\delta^{RS}$ values calculated (Figure 1). The model designed by the Riguera group was used for configuration assignment of the secondary alcohols.[9]

5-[(*R*)-Methoxyphenyl acetate](8′-heptadecaenyl)resorcinol: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.67 (1H, d, *J* = 6.7 Hz, H-6), 6.67 (1H, d, *J* = 6.7 Hz, H-4), 6.55 (1H, t, *J* = 2.1 Hz, H-2), 5.28 (1H, br, H-9′), 5.03 (1H, br, H-8′), 4.90 (1H, m, H-11′), 2.51 (2H, t, *J* = 8.5, Hz, H-1′) 2.16 (1H, t, *J* = 7.5, Hz, H-10′), 1.41 (2H, q, *J* = 6.6, 15.5 Hz, H-12′), 1.04 (2H, m, H-16′) 0.86 (3H, t, *J* = 6.7 Hz, H-17′).

5-[(*S*)-Methoxyphenyl acetate](8′-heptadecaenyl)resorcinol: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.68 (1H, d, *J* = 2.1 Hz, H-6), 6.68 (1H, d, *J* = 2.1 Hz, H-4), 6.55 (1H, t, *J* = 2.2, 4.3 Hz, H-2), 5.29 (1H, br, H-9′), 5.04 (1H, br, H-8′), 4.91 (1H, m, H-11′), 2.51 (2H, t, *J* = 6.5,

Hz, H-1′) 2.17 (1H, t, $J = 7.4$ Hz, H-10′), 1.40 (2H, q, $J = 7.6$, 16.1 Hz, H-12′), 1.05 (2H, m, H-16′) 0.86 (3H, t, $J = 6.6$, Hz, H-17′).

5-[(*R*)-Methoxyphenyl acetate](8′,14′-heptadecadienyl)resorcinol: ^{1}H NMR ($CDCl_3$, 400 MHz) δ6.68 (1H, s, H-6) 6.68 (1H, s, H-4) 6.56 (1H, s, H-2) 5.36 (1H, m, H-14′) 5.31 (1H, m, H-9′) 5.25 (1H, m, H-15′) 5.06 (1H, q, H-8′) 4.91 (1H, quint, H-12′) 2.51 (2H, t, $J = 7.6$ Hz, H-1′) 2.19 (2H, m, H-13′) 1.87 (2H, t, $J = 6.4$ Hz, H-10′) 1.51 (2H, t, $J = 7.8$ Hz, H-11′) 0.91 (3H, t, $J = 7.3$ Hz, H-17′).

5-[(*S*)-Methoxyphenyl acetate](8′,14′-heptadecadienyl)resorcinol: ^{1}H NMR ($CDCl_3$, 400 MHz) δ6.67 (1H, s, H-6) 6.67 (1H, s, H-4) 6.55 (1H, s, H-2) 5.46 (1H, m, H-14′) 5.30 (1H, m, H-9′) 5.24 (1H, m, H-15′) 5.09 (1H, m, H-8′) 4.90 (1H, q, H-12′) 2.51 (2H, t, $J = 7.8$ Hz, H-1′) 2.30 (2H, m, H-13′) 1.70 (2H, m, H-10′) 1.48 (2H, t, $J = 6.8$ Hz, H-11′) 0.84 (3H, t, $J = 7.5$ Hz, H-17′).

***Trypanosoma cruzi* Bioassay.** A β-galactosidase-expressing transgenic *T. cruzi* (Tulahuen strain, clone C4) was used to detect antitrypanosomal activity.[12] Growth of the intracellular stage of *T. cruzi* was determined from the cleavage of the colorimetric substrate chlorophenol red-β-D-galactoside.

Antileishmanial Bioassay. Leishmaniasis bioassays were performed using a method previously employed in our Laboratory, based on parasite DNA fluorescence.[13] In this latter assay, amphotericin B was used as the positive control and had an IC_{50} of 0.09 μM.

Cytotoxicity Assay. The cytotoxic activity against MCF-7 (human breast cancer cell line) was performed following the standard protocol of the National Cancer Institute.[14]

Vero Cell Cytotoxicity Bioassay. Vero cells, derived from kidneys of the African green monkey, were adhered to 96-well plates and used to evaluate the toxicity of the compounds purified from *S. turbacensis* on the basis of reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO).[15] After treatment with the test compound and 4 h incubation at 37 °C, cell viability was evaluated in an ELISA reader at 570 nm.

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References and Notes

(1) Coley, P. D.; Heller, M. V.; Aizprúa, R.; Araúz, B.; Flores, N.; Correa, M.; Gupta, M.; Solís, P. N.; Ortega-Barría, E.; Romero, L.; Gómez, B.; Ramos, M.; Cubilla-Rios, L.; Capson, T. L.; Kursar, T. A. *Front. Ecol. Environ.* **2003**, *1*, 421−428.
(2) Kozubek, A.; Tyman, J. H. P. *Chem. Rev.* **1999**, *1*, 1−25.
(3) Jin, W.; Zjawiony, J. K. *J. Nat. Prod.* **2006**, *69*, 704−706.
(4) (a) Huang, P.; Chen, W.; Hu, Y. *Yao Hsueh Tung Pao* **1980**, *15*, 39. (b) Huang, B.; Chen, W.; Hu, Y.; Qiu, C.; Zhang, W.; Liang, B. *Yao Hsueh Hsueh Pao* **1981**, *16*, 27−30.
(5) Kobayashi, H.; de Mejia, E. *J. Ethnopharmacol.* **2005**, *96*, 347−354.
(6) Kusumi, T.; Kakisawa, H.; Chen, Y.; Lyn, J.; Hsu, H. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 943−944.
(7) (a) Barrow, R. A.; Capon, R. J. *Aust. J. Chem.* **1991**, *44*, 1393−1405. (b) Reffstrup, T.; Hammershoy, O.; Boll, P. *Acta Chem. Scand. B* **1982**, *36*, 291−294.
(8) (a) Scannell, R. T.; Barr, J. R.; Murty, V. S.; Sambi Reddy, K.; Hecht, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 3650−3651. (b) Barr, J. R.; Murty, V. S.; Yamaguchi, K.; Singh, S.; Smith, D. H.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *1*, 204−207. (c) Lytollis, W.; Scannell, R. T.; An, H.; Murty, V. S.; Sambi Reddy, K.; Barr, J. R.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 12683−12690. (d) Starck, S. R.; Deng, J.; Hecht, S. M. *Biochemistry* **2000**, *39*, 2413−2419.
(9) Seco, J. M.; Latypov, S.; Riguera, R. *Tetrahedron: Asymmetry* **2001**, *12*, 2915−2925.
(10) Tasdemir, D.; Kaiser, M.; Brun, R.; Yardley, V.; Schmidt, T. J.; Tosun, F.; Redi, P. *Antimicrob. Agents Chemother.* **2006**, *50* (4), 1352−1364.
(11) Torres-Mendoza, D.; Ureña González, L. D.; Ortega-Barría, E.; Capson, T. L.; Cubilla-Rios, L. *J. Nat. Prod.* **2003**, *66*, 928−932.
(12) (a) Touchstone, J. C.; Dobbins, M. F. *Practice of Thin Layer Chromatography*, 2nd ed.; John Wiley & Sons Inc.: New York, 1983; p 23. (b) Hostettmann, K.; Marston, A.; Hostettmann, M. *Preparative Chromatography Techniques: Applications in Natural Product Isolation*; 2nd ed.; Springer: New York, 1997; pp 57−58.
(13) Calderon, A.; Romero, L.; Ortega-Barria, E.; Brun, R.; Correa, M. A.; Gupta, M. P. *Panama Pharm. Biol.* **2006**, *44*, 1−16.
(14) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757−766.
(15) Chiari, E. Camargo, E. In *Genes and Antigens of Parasites. A Laboratory Manual*; Morel, C. M., Ed.; Fundacão Oswaldo Cruz: Río de Janeiro, Brazil, 1984; pp 23−28.

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