Antimicrobial and Antiparasitic (+)-*trans*-Hexahydrodibenzopyrans and Analogues from *Machaerium multiflorum*

Ilias Muhammad,* Xing-Cong Li, Melissa R. Jacob, Babu L. Tekwani, D. Chuck Dunbar, and Daneel Ferreira

National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, Mississippi 38677

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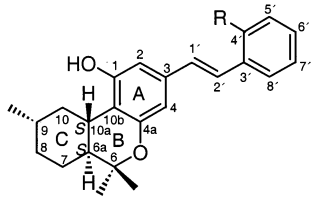
Machaerium multiflorum yielded two additional new (+)-*trans*-hexahydrodibenzopyrans (HHDBP's), machaeriol C (**1**) and machaeriol D (**2**), and three new 5,6-*seco*-HHDBP's, machaeridiol A (**3**), machaeridiol B (**4**), and machaeridiol C (**5**). Their structures and stereochemistries were determined by 1D and 2D NMR data, including HMBC, NOESY, and circular dichroism experiments. Machaeriol C (**1**) demonstrated in vitro antibacterial activity against *Staphylococcus aureus* (IC_{50} 0.65 μg/mL) and methicillin-resistant *S. aureus* (MRSA) (IC_{50} 0.70 μg/mL), while its corresponding 5,6-*seco*-analogues machaeridiol A (**3**) and machaeridiol B (**4**) showed antibacterial activity against *S. aureus* and MRSA (IC_{50} 1.0−2.6 μg/mL) and antifungal activity against *Candida albicans* (IC_{50}, 2.0−3.5 μg/mL). In addition, machaeridiol B (**4**) demonstrated antiparasitic activities against *Plasmodium falciparum* D6 and W2 clones and *Leishmania donavani* with IC_{50} values of 0.64, 0.22, and 0.9 μg/mL, respectively.

In our previous report,[1] we described the structures of two new antimalarial and antibacterial (+)-6a*S*,9*S*,10a*S*-*trans*-hexahydrodibenzopyrans (HHDBP's), namely, machaeriol A (**6**) and machaeriol B (**7**), isolated from the stem bark of *Machaerium multiflorum* Spruce[2] (Fabaceae). The native Amazonian liana found in Loreto, Peru, was investigated on the basis of its initial antimicrobial and antiparasitic activities. Earlier chemical investigation of other species has revealed the presence of triterpenes, benzoquinones, and flavonoids, including the antiinflammatory and anti-HIV isoflavonoids, isoflavans, and lectins from *M. vestitum*, *M. villosum*, and *M. biovulatuml*.[3−8] In addition, antigiardial isoflavonoids from *M. aristulatum* and biologically active procyanidins from *M. floribundum* have been reported.[9,10] In continuation of the previous investigation,[1] examination of the CH_2Cl_2 fraction of *M. multiflorum* led to the isolation and characterization of five additional structurally related compounds, namely, the two HHDBP's **1** and **2**, as well as three 5,6-*seco*-HHDBP's, **3**−**5**.



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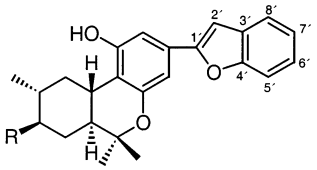

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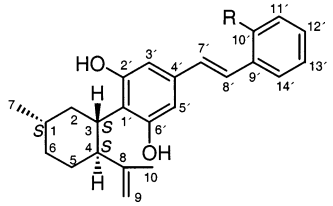

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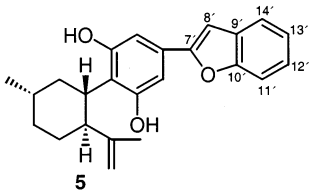

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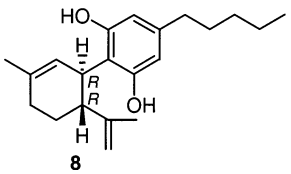

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Results and Discussion

Repeated chromatography of the antibacterial CH_2Cl_2-soluble fraction of the EtOH extract of *M. multiflorum* resulted in the isolation of compounds **1**−**5**. Machaeriol C (**1**), $C_{24}H_{28}O_3$, had a conjugated stilbene chromophore (UV λ_{max} 214, 316 nm; IRν_{max} 1620, 1510 cm^{-1}) and hydroxyl group(s) (IR ν_{max} 3560 cm^{-1}). A hexahydrodibenzopyran base skeleton[1,11−14] was suggested from the NMR data (Table 1). The ^1H and ^{13}C NMR spectra of **1** were in close agreement with those of machaeriol A (**6**),[1] except for the presence of an additional hydroxyl group at C-4′ (δ_C 153.4). The DQF-COSY spectrum established the *trans*-coupled AB system (δ 6.85, d and 7.22, d; each J = 16.2 Hz) and ABCD protons [δ 6.76 (d, J = 7.9 Hz), 7.08 (dd, J = 7.9, 7.7 Hz), 6.88 (dd, J = 7.7, 7.6 Hz), 7.43 (br d, J = 7.7 Hz)] of the 4′-hydroxystyrene unit (Table 1). The gross structure of **1** was assigned using 2D NMR HMQC and HMBC experiments. HMBC established the assignment of the C-6 and C-9 methyl groups by ^3J-correlations between δ_{C-6a} 49.5 and δ 1.05, 1.37 (C-6-Me's), and between δ_{C-10} 39.3, δ_{C-8} 35.9, and δ 0.92 (C-9-Me). The location of the 4′-hydroxystyrene substituent was established by the correlation

* To whom correspondence should be addressed. Tel: (662) 915-1051. Fax: (662) 915−7989. E-mail: milias@sunset.backbone.olemiss.edu.

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **2** (δ, ppm in $CDCl_3$)

	1		**2**	
C/H	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
1		155.7 s		157.5 s
2	6.41 br s	105.8 d	6.78 br s	106.5 s
3		137.4 s		129.8 s
4	6.56 br s	109.2 d	6.80 br s	104.0 s
4a		155.7 s		156.3 s
6		77.8 s		77.0 s
6a	1.44 ddd (2.0, 11.2, 11.4)	49.5 d	1.56 br t (9.0, 11.2)	48.0 d
7α	1.82 m	28.5 t	2.07 m	37.7 t
7β	1.09 m		1.13 m	
8	1.82 m; 1.09 m	35.9 t	3.24 br ddd (4.7, 10.3, 10.4)	76.1 d
9	1.61 m	33.3 d	1.54 m	37.0 d
10α	0.75 dd (12.2, 12.6)	39.3 t	0.83 br t (12.8, 13.4)	40.7 t
10β	3.02 br d (12.6)		3.20 dt (2.6, 13.4)	
10a	2.47 ddd (2.3, 11.2, 12.6)	36.4 d	2.51 ddd (2.6, 11.1, 12.8)	35.9 d
10b		113.5 s		113.9 s
6α-Me	1.05 s	19.4 q	1.09 s	18.7 q
6β-Me	1.37 s	28.1 q	1.38 s	27.5 q
9α-Me	0.92 d (6.5)	23.0 q	1.04 d (6.5)	18.9 q
1′	6.85 d (16.2)	128.9 d		155.9 s
2′	7.22 d (16.2)	123.1 d	7.09 s	101.4 d
3′		125.2 s		129.8 s
4′		153.4 s		155.1
5′	6.76 d (7.9)	116.5 d	7.41 br d (8.1)	111.2 d
6′	7.08 dd (7.7, 7.9)	127.5 d	7.24 dd br (7.3, 8.1)	124.6 d
7′	6.88 dd (7.6, 7.7)	121.5 d	7.21 dd (1.2, 7.3, 7.7)	123.4 d
8′	7.43 br d (7.7)	129.8 s	7.50 br d (7.7)	121.3 d
OH	5.76, 5.51 (2 × br s)		8.61 br s	

[a] Coupling constants (J values in Hz) are in parentheses. [b] Multiplicities of carbon signals were determined by DEPT (135°) and HMQC experiments.

between δ_{C-3} 137.4 and δ 7.22 (H-2′). Finally, the oxygenated carbons at C-1 and C-4a were placed by the ^3J-correlations between δ 2.47 (H-10a) and δ_C 155.7 (C-1 and C-4a).

The absolute stereochemistry at C-6a, C-9, and C-10a was established using optical rotation, NOESY, and circular dichroism experiments. Comparison of the NMR data and $[\alpha]_D$ values of **1** (+117.4°) with machaeriol A (**6**) (+115.4°),[1] as well as enantiomeric HHDBP's, (+)- and (−)-hexahydrocannabinol (+79.5° and −73.2°, respectively),[11−14] indicates that **1** is presumably an analogue of the dextrorotatory series of HHDBP's, i.e., exhibiting a 6a*S*, 10a*S* absolute configuration. On the basis of this assumption the spatial orientation of the relevant protons and methyl groups was confirmed by the NOESY spectrum, which showed correlations between H-9 (δ 1.61), H-10β (δ 3.02), H-10a (δ 2.47), and C-6β-Me (δ 1.37), indicating that these protons are β-cofacially oriented. The NOESY also showed correlations between H-6a (δ 1.44), H-10α (δ 0.75), and C-9 methyl (δ 0.92), thereby confirming both the α-cofacial arrangement of the equatorial H-6a, the C-9-equatorial methyl group, and hence the *trans*-fused B/C ring junction. This was further evident from the large *trans* coupling ($J_{6a,10a}$ = 11.2 Hz) between H-6a and H-10a.[15] Collectively these data permitted tentative assignment of a 6a*S*, 9*S*, 10a*S* absolute configuration for compound **1**. This was unequivocally confirmed by CD data (vide infra).

The ^{1}H and ^{13}C NMR spectra of machaeriol D (**2**), $C_{24}H_{26}O_4$, were generally similar to those observed for **1** (Table 1), except for the differences associated with the presence of a hydroxyl group at C-8 (δ 3.24, ddd, J = 4.7, 10.3, 10.4 Hz; δ_C 76.1, d) and a benzo[*b*]furan substituent at C-3. The ^{1}H NMR spectrum revealed four aromatic protons (δ 7.41, 7.24, 7.21, and 7.50; H-5′−H-8′, respectively) for an ABCD system, as well as one proton singlet at δ 7.09 (H-2′), suggesting the presence of a C-1′ substituted benzo[*b*]furan moiety, as observed in machaeriol B (**7**).[1] The linkage between C-1′ of the benzo[*b*]furan side chain and C-3 of HHDBP was also established by HMBC, which revealed cross-peaks between δ_{C-3} 129.8 and H-2′ and between $\delta_{C-1'}$ 155.9 and δ 6.78 (H-2) and δ 6.80 (H-4). The location of the hydroxyl group at C-8 was established by COSY and HMBC experiments; the former showed the presence of the system $-CH_2-CH(OH)-CH(CH_3)-CH_2-$, while the latter revealed ^3J correlations between H-8 (δ 3.24), C-9-Me (δ_C 18.9), and C-6a (δ_C 48.0).

The positive $[\alpha]_D$ value of +80.2° of compound **2** indicated that it probably too belongs to the dextrorotatory series of HHDBP's, hence displaying 6a*S*, 10a*S* absolute stereochemistry. The stereochemical assignment of the C-8 hydroxyl group was determined by the NOESY spectrum, which showed that H-6a (δ 1.56), H-8 (δ 3.24), and the C-9 methyl group (δ 1.04) were cofacial, thereby confirming the α-orientation of H-8 and the C-9 equatorial methyl group. The NOESY experiment also showed correlations between H-9β (δ 1.54), H-10aβ (δ 2.51), and C-6β-Me (δ 1.38) as observed in **1**. These data again permitted tentative assignment of 6a*S*, 8*R*, 9*R*, 10a*S* absolute stereochemistry for compound **2**, which was unambiguously confirmed by CD data.

The availability of machaeriols A−D (**6**, **7**, **1**, **2**) offered the opportunity to assess the potential of circular dichroism for defining the absolute stereochemistry of the benzylic C-10a stereocenter by application of the aromatic quadrant rule.[16,17] All four compounds displayed high- and low-amplitude positive Cotton effects in the 220−230 and 290−300 nm regions, respectively, of their CD spectra. When placed in the quadrants formed between the nodal plane of the aromatic ring (A) and the symmetry plane passing through C-3, C-10b, and the benzylic C-10a, and viewed through the same carbon atoms, C-10 and C-9 are in the positive right lower quadrant, C-6 lies in the positive left upper quadrant, while only C-6a is in the negative left lower quadrant. Collectively, these quadrant projections then explain the high-amplitude positive Cotton effect for the $A_{1g} \rightarrow B_{1u}$ aromatic transition of machaeriols A−D and hence their 10a*S* absolute configuration. Conspicuously, the CD spectrum of Δ^9-tetrahydrocannabinol, with its 10a*R* absolute configuration, displays a near mirror image relationship with those of **1**, **2**, **6**, and **7** in the aforementioned regions. Such unambiguous confirmation of the absolute configuration at the benzylic C-10a stereocenter of HHDBP's via chiroptical data in conjunction with appropriate NOESY experimentation (vide supra) then permits the ready assessment of absolute stereochemistry of this group of natural products.

Three additional compounds, namely, machaeridiol A−C (**3**−**5**), were obtained as amorphous powders and analyzed for the molecular formulas $C_{24}H_{28}O_2$, $C_{24}H_{28}O_3$, and $C_{24}H_{26}O_3$, respectively, by HRESIMS. Their 5,6-*seco*-HHDBP carbon skeleton was suggested from ^{1}H and ^{13}C NMR data (Table 2).[18] Compounds **3** and **4** contained styrene and *O*-hydroxystyrene substituents, respectively; thus their side chains are analogous to those of machaeriol A (**6**) and machaeriol C (**1**). The ^{1}H and ^{13}C NMR data of **3** and **4** (Table 2) were generally similar to those reported for **6**[1] and **1**, respectively, except for the differences associated with the presence of an isopropenyl group at C-4 and a

Table 2. ^{1}H and ^{13}C NMR Data for Compounds **3**−**5** (δ, ppm in $CDCl_3$)

	3		**4**		**5**	
C/H	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
1	1.40 qdd (3.4, 6.5, 11.9)	33.7 d	1.42 qdd (3.1, 6.4, 12.3)	33.7	1.47 qdd (3.5, 7.0, 12.0)	33.4
2α	1.50 m	39.6 t	1.54 m	39.3	1.45 m	39.5
2*β*	1.69 m		1.78 m		1.69 m	
3	3.14 ddd (3.0, 11.7, 14.0)	39.2 d	3.22 ddd (3.2, 11.6, 14.7)	38.4	3.18 ddd (4.0, 11.7, 14.5)	39.2
4	3.87 ddd (3.2, 11.7, 14.0)	48.0 d	3.06 ddd (3.1, 11.6, 14.7)	47.4	2.93 ddd (3.9, 11.7, 14.5)	47.9
5α	1.55 m	33.5 t	1.49 m	33.4	1.51 m	33.7
5*β*	1.75 m		1.69 m		1.75 m	
6α	1.11 ddd (3.4, 11.9, 15.4)	35.6 t	1.08 m	35.6	1.11 ddd (3.5, 11.8, 15.6)	35.4
6*β*	1.78 m		1.73 m		1.78 m	
7	0.91 d (6.5)	22.9 q	0.89 d (6.4)	22.6	0.95 d (7.0)	22.9
8		150.3 s		150.2		150.3
9	4.67 br s	109.9 t	4.61 d (2.4)	108.9	4.67 d (1.5)	109.9
	4.50 br s		4.37 s		4.50 br s	
10	1.58 s	19.9 q	1.57 br s	18.5	1.61 s	19.9
1′		118.9 s		118.7		119.8
2′		156.1 s		157.6		157.3
3′	6.40 br s[c]	106.9 d[c]	6.43 br s[c]	106.0[c]	6.77 br s[c]	106.3[c]
4′		136.6 s		136.6		129.5
5′	6.47 br d (1.3)[c]	107.9 d[c]	6.39 br d (1.3)[c]	104.9[c]	6.85 br s[c]	105.3[c]
6′		154.6 s		155.7		155.6
7′	6.95 d (16.3)	128.0 d	6.88 d (16.4)	128.2		154.7 s
8′	7.85 d (16.3)	129.1 d	7.27 d (16.4)	122.4	6.89 br s	101.6
9′		137.7 s		125.1		129.4 s
10′	7.42 d (7.5)	126.9 d		154.9 s		155.1 s
11′	6.32 br t (7.5)	129.1 d	6.76 br d (8.1)	115.6	7.45 d (7.8)	111.4
12′	7.22 br t (7.5, 7.5)	128.3 d	7.01 ddd (1.3, 8.1, 8.5)	126.3	7.28 br t (7.6, 7.8)	124.6
13′	6.32 br t (7.5)	129.1 d	6.78 br t (7.7, 8.5)	119.9	7.25 br t (7.5, 7.6)	123.3
14′	7.42 d (7.5)	126.9 d	7.44 br d (7.7)	128.7	7.55 d (7.5)	121.2
OH	4.67 br s		5.40 s		4.88 br s	

[a] Coupling constants (J values in Hz) are in parentheses. [b] Multiplicities of carbon signals were determined by DEPT (135°) and HMQC experiments. [c] Interchangeable signals.

hydroxyl group at C-6′ (δ_{C-8} 150.3, δ_{C-9} 109.9, δ_{C-10} 19.9, $\delta_{C-6'}$ 154.6 for **3** and δ 150.2, 108.9, 18.5, 157.7 for **4**). The presence of the C-4′ styrene units for both compounds was inferred from the spectroscopic data (δ 6.95, 7.85; each d, J = 16.3 Hz for **3**, δ 6.88, 7.27; each d, J = 16.4 Hz, for **4**). The gross structures for **3** and **4** were established by extensive 2D NMR COSY, HMQC, and HMBC experiments. The HMBC experiment on **3** established the assignments of the C-3 and C-4 methine carbons by ^3J-correlations between $\delta_{C-2'}$ 156.1, $\delta_{C-6'}$ 154.6, and δ 3.14 (H-3); and δ_{C-9} 109.9, δ_{C-10} 19.9, and δ 3.87 (H-4). The location of the styrene substituent was established by cross-peaks between $\delta_{C-9'}$ 137.7 and δ 6.95 (H-7′), and $\delta_{C-4'}$ 136.6 and δ 7.85 (H-8′). A similar HMBC correlation pattern was observed for **4**, thus establishing the position of the *O*-hydroxystyrene substituent by cross-peaks between $\delta_{C-10'}$ 154.9 and δ 7.44 (H-14′) and δ 7.01 (H-12′); $\delta_{C-9'}$ 125.1 and δ 6.88 (H-7′); and $\delta_{C-4'}$ 136.6 and δ 7.27 (H-8′).

The stereochemical assignments of the stereocenters of machaeridiol A (**3**) were resolved using NOESY, CD spectra, and optical rotation. The CD spectrum of **3** exhibited a positive Cotton effect at 250 nm ([θ] = $+2.37 \times 10^3$), in MeOH, which is opposite of that observed for (−)-3*R*,4*R*-cannabidiol (**8**) {λ 240 nm ([θ] = -1.25×10^4)}. Machaeridiol A (**3**) thus possesses a 3*S*, 4*S* absolute configuration. Comparison of the $[\alpha]_D$ values of **3** (+17.8°) with (−)-cannabidiol[19] (−118.3°) also indicates that **3** is an analogue of the dextrorotatory series of 5,6-seco-HHDBP's with a 1*S*,3*S*,4*S* absolute configuration. A NOESY experiment showed correlations between H-1 (δ 1.40), H-3 (δ 3.14), and the C-10 methyl protons (δ 1.58), hence indicating their cofacial disposition and β-orientation. The NOESY also showed cross-peaks between H-2α (δ 1.50) and the C-1 methyl group (δ 0.91), as well as between H-4 (δ 3.87) and H-6α (δ 1.11), thereby confirming the α-orientation of the C-1 methyl group and β-orientation of the C-4 isoprenyl substituent. The NOESY spectra and $[\alpha]_D$ value (+23.6°) of machaeridiol B (**4**) were similar to those observed for **3**, hence both possessing the same 1*S*, 3*S*, 4*S* absolute stereochemistry.

Finally, the ^{1}H and ^{13}C NMR data of machaeridiol C (**5**) were generally similar to those of **3** (Table 2), except for the differences associated with the presence of a benzo[*b*]-furan substituent at C-4′ instead of the C-4′-styrene moiety in **3**. Its structure was determined by extensive 2D NMR spectral data (Table 2). The relative stereochemistry at the C-1, C-3, and C-4 stereocenters of **5** was similar to those observed for **3** and **4**. Thus, the NOESY spectrum showed that H-1 (δ 1.47), H-3 (δ 3.18), and the C-4 prenyl group were cofacially located, while H-2α (δ 1.45) was correlated with the C-1 methyl protons (δ 0.95), and H-5α (δ 1.51) with H-4 (δ 2.93). Therefore, H-4 and the C-1 methyl group should occupy the α-face of the molecule, opposite of the C-4 isoprenyl group. A close comparison of the NMR spectral data and $[\alpha]_D$ values (+18.3°) of **5** with those of **3** and **4** indicates that **5** is an analogue of the dextrorotatory series of 5,6-*seco*-HHDBP's, also possessing 1*S*, 3*S*, 4*S* absolute stereochemistry. A conspicuous feature of the ^{1}H and ^{13}C NMR spectra of compounds **3**−**5** is the magnetic nonequivalence of both 3′- and 5′-H and of 3′- and 5′-C (Table 2). This presumably reflects restricted rotation about the $C_3-C_{1'}$ bond with an energy barrier sufficiently high to observe two rotamers on the NMR time scale.

Machaeriol C (**1**) demonstrated in vitro antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) with IC_{50}/MIC/MBC values at 0.65/1.56/6.25 and 0.70/1.56/12.5 μg/mL, respectively (Table 3), while its 5,6-*seco*-analogues machaeridiol A−C (**3**−**5**) showed similar antibacterial potency, with MBC values in a range of 3.13−12.5 μg/mL (Table 4). Machaeriol D (**2**) was only marginally active against *S. aureus* and MRSA (IC_{50} 25 and 30 μg/mL, respectively). In addition, com-

Table 3. Antimicrobial Activities (IC_{50}/MIC in μg/mL) of Compounds **1**−**5**

	C. albicans	*C. neoformans*	*S. aureus*	*MRSA*	*M. intracellularae*	*A. fumigatus*
1	20/NT	40/NT	0.65/1.56	0.70/1.56	20/NT	NT/100
2	−[a]	−[a]	25/50	30/−[a]	−[a]	−[a]
3	3.5/50	15/−[a]	1.0/1.56	1.0/1.56	7.0/12.5	NT/12.5
4	2.0/6.25	3.5/NT	2.6/6.25	2.0/6.25	−[a]	NT/10
5	45/NT	25/NT	3.0/6.25	3.5/6.25	10/25	−[a]
AMB	0.10/0.31	0.35/0.63	NT	NT	NT	NT/1.25
CIP	NT	NT	0.15/1.25	0.10/0.31	0.45/0.63	NT

[a] Inactive. AMB: amphotericin B. CIP: ciprofloxacin. MRSA: methicillin-resistant *S. aureus*. NT: not tested.

Table 4. Minimum Fungicidal/Bactericidal Concentrations (MFC/MBC in μg/mL) of Compounds **1** and **3**−**5**

	C. albicans	*S. aureus*	MRSA	*M. intracellularae*	*A. fumigatus*
1	NT	6.25	12.5	NT	−[a]
3	−[a]	3.13	3.13	50	−[a]
4	−[a]	6.25	6.25	NT	−[a]
5	NT	12.5	12.5	−[a]	NT
CIP	NT	0.31	0.31	NT	NT

[a] Inactive at 50 μg/mL. NT: not tested. CIP: ciprofloxacin.

Table 5. Antimalarial, Antileishmanial, and Cytotoxic Activities (IC_{50}/TC_{50} in μg/mL) of Compounds **1**, **4**, and **5**

	P. falciparum D6 clone[a]		*P. falciparum* W2 clone[b]		*L. donavani*		cytotoxicity (VERO)
	IC_{50}	SI[c]	IC_{50}	SI[c]	IC_{50}	IC_{90}	TC_{50}
1	3.0	1.6	3.7	1.3	6.0	12	NC[d]
4	0.64	>7.4	0.22	>22	0.9	8.0	NC[d]
5	3.3	>1.4	2.5	>1.9	3.0	10	NC[d]
chloroquine	0.015	>34	0.14	>251	NT	NT	NT
pentamidine	NT		NT		0.42	1.5	NT

[a] Chloroquine-sensitive clone. [b] Chloroquine-resistant clone. [c] Selectivity index = IC_{50} VERO cells/IC_{50} *P. falciparum*. [d] No cytotoxicity at 4.7 μg/mL. NT: not tested.

pounds **1** and **3**−**5** showed antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, as well as antibacterial activity against *Mycobacterium intracellulare* and *Aspergillus fumigatus* (Tables 3 and 4). When tested for antiparasitic activity (Table 5), machaeridiol B (**4**) demonstrated strong inhibition against chloroquine-sensitive (D6) and the chloroquine-resistant (W2) *Plasmodium falciparum* clones and against *Leishmania donavani* with IC_{50} values of 0.64, 0.22, and 0.9 μg/mL, respectively.

Various HHDBP's and 5,6-*seco* HHDBP's have previously been synthesized, including (+)- and (−)-hexahydrocannabinols (HHC)[11−13] and dihydrocannabidiol,[18] but to our knowledge only one HHC (cannabiripsol)[20] and seven 5,6-*seco* HHC's (cannabidiols)[21,22] have been isolated from *Cannabis* species as natural products. Cannabinoids and their precursors, cannabidiols, are restricted to *Cannabis sativa* and its variants in higher plants.[21,22] The only bibenzyl analogue of Δ^9-THC, perrottetinen, had previously been reported from the liverwort *Radula perrottetii*.[23] It is intriguing to note that the antimicrobial and antiparasitic activity of Peruvian *M. multiflorum* is contributed by HHDBP's[1] and their 5,6-*seco* analogues.

Experimental Section

General Experimental Procedures. UV spectra were obtained in MeOH, using a Hewlett-Packard 8452A spectrophotometer, and IR spectra were taken as KBr disks on a Ati Mattson (Genesis Series) FTIR spectrophotometer. The NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$, using residual solvent as standard. Multiplicity determinations (DEPT) and 2D NMR spectra (gradient DQF-COSY, HMQC, gradient HMBC, and NOESY) were run using a standard Bruker pulse program. HRMS were obtained by direct injection using a Bruker Bioapex-FTMS with electro-spray ionization (ESI) in positive mode. Optical rotation measurements were taken on JASCO DIP-370 digital polarimeter in MeOH at ambient temperature, and CD spectra were recorded on a JASCO J-715 spectrometer. Centrifugal preparative TLC (CPTLC, using Chromatotron, Harrison Research Inc. Model 8924): 1 or 2 mm Si gel GF Chromatotron rotors (Analtech, Inc.). Solvent: $CHCl_3$−*n*-hexane (9:1), using a N_2 flow rate of 4 mL min^{-1}. HPLC: Waters LC module I plus, using semi-preparative C-18 column. TLC was carried out on Si gel F254 with the solvent system CH_2Cl_2−*n*-hexane (8:2). The isolated compounds were visualized by observing under UV-254 nm, followed by spraying with anisaldehyde−H_2SO_4 reagent.

Plant Material. The stem bark of *M. multiflorum* was collected in November, 1997, from open sandy forest near Loreto (Maynas), Peru, and was identified by Dr. Sidney T. McDaniel. A voucher specimen (IBE 12161) has been deposited at the Herbarium of Mississippi State University.

Extraction and Bioassay. The powdered stem bark of *M. multiflorum* (0.5 kg) was extracted by percolation with 95% EtOH (3 × 2 L), and the combined extracts were evaporated under reduced pressure and then freeze-dried (yield 17.7 g). A portion of the dried EtOH extract (15 g) was percolated with *n*-hexane, followed by CH_2Cl_2, and finally the residual extract was washed with MeOH (each 200 mL × 3). The *n*-hexane, CH_2Cl_2, and MeOH fractions were separately filtered and dried, which afforded 3.8, 8.9, and 4.5 g, respectively. Antiparasitic and antibacterial screenings (vide infra) of these fractions showed that the antiparasitic activity resided in the *n*-hexane-soluble fraction (IC_{50} 0.4 μg/mL [SI >119] against *P. falciparum* D-6 clone), while the antimicrobial activity was detected in the CH_2Cl_2 fraction (IC_{50} <20 μg/mL against *S. aureus* and MRSA).

Isolation of Compounds. The CH_2Cl_2 fraction (3.5 g) was subjected to column chromatography on silica gel, using gradient elution with CH_2Cl_2−EtOAc. All the chromatographic fractions and fractions containing pure compounds were tested for antimicrobial and antimalarial activities. Elution with 5% EtOAc−CH_2Cl_2 afforded compound **1** (20 mg) and fraction A, containing the mixture of compounds **3** and **5** (120 mg). Further elution with 10% EtOAc−CH_2Cl_2 yielded fraction B (150 mg), which was subjected to centrifugal preparative TLC

(using a Chromatotron, 2 mm silica gel P-254 disk), using 5% EtOAc−CH_2Cl_2 as eluant, affording **2** (18 mg) followed by **4** (40 mg). Fraction A was separated by semipreparative RP-HPLC (column: ODS prodigy 10μ, 250 × 10 mm; detector: UV-254 nm), using 90% MeCN−H_2O as solvent, which afforded **5** [16 mg; R_f 0.53, silica gel, solvent: *n*-hexane−CH_2Cl_2 (8:2)], followed by **3** (10 mg; R_f 0.50).

Machaeriol C (6a*S*,7,8,9*S*,10,10a*S*-hexahydro-1-hydroxy-6,6,9-trimethyl-3-[2-(2-hydroxyphenyl)]ethenyl-6*H*-dibenzo[*b*,*d*]pyran) (1): amorphous solid; $[\alpha]_D$ +117.4° (*c* 1.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.45), 316 (4.52) nm; IR (KBr) ν_{max} 3560 (OH), 2980−2820, 1620, 1510, 1470, 1440, 1300, 1285, 1265, 740 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 365.2093 $[MH]^+$ (calcd for $C_{24}H_{29}O_3$, 365.2038); CD (MeOH) λ_{max} ([θ]) 223 (+4.01 × 10^3), 298 (+4.72 × 10^2) nm.

Machaeriol D (6a*S*,7,8,9*R*,10,10a*S*-hexahydro-1,8*R*-dihydroxy-6,6,9-trimethyl-3-benzo[*b*]furan-1-yl-6*H*-dibenzo[*b*,*d*]pyran) (2): amorphous solid; $[\alpha]_D$ +80.2° (*c* 0.347, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.6), 240 (4.38), 304 (4.50) nm; IR (KBr) ν_{max} 3500 (OH), 2921, 2865, 1620, 1560, 1453, 1245, 1141, 950, 749 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 379.1940 $[MH]^+$ (calcd for $C_{24}H_{27}O_4$, 379.1831); CD (MeOH) λ_{max} ([θ]) 225 (+3.96 × 10^3), 299 (+4.8 × 10^2) nm.

Machaeridiol A (2-[1*S*,2*S*-(1-methylethenyl)-5*S*-methylcyclohexyl]-5-phenylethenyl-1,3-benzenediol) (3): amorphous solid; $[\alpha]_D$ +17.8° (*c* 0.53, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (4.2), 228 (3.62), 290 (4.18) nm; IR (KBr) ν_{max} 3550 (OH), 2980−2820, 1630, 1515, 1480, 1440, 1330 1285, 1265, 740 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 349.2146 $[MH]^+$ (calcd for $C_{24}H_{29}O_2$, 349.2084); CD (MeOH) λ_{max} ([θ]) 250 (+2.37 × 10^3), 316 (−3.15 × 10^2) nm.

Machaeridiol B (2-[1*S*,2*S*-(1-methylethenyl)-5*S*-methylcyclohexyl]-5-(4-hydroxy)phenylethenyl-1,3-benzenediol) (4): amorphous solid; $[\alpha]_D$ +23.6° (*c* 0.9, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.0),230 (3.75), 292 (4.33) nm; IR (KBr) ν_{max} 3550 (OH), 2980−2820, 1620, 1510, 1450, 1440, 1300, 1285, 740 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 365.2111 $[MH]^+$ (calcd for $C_{24}H_{29}O_3$, 365.2038).

Machaeridiol C (2-[1*S*,2*S*-(1-methylethenyl)-5*S*-methylcyclohexyl]-5-benzo[*b*]furan-1-yl-1,3-benzenediol) (5): amorphous solid; $[\alpha]_D$ +18.3° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.45), 235 (3.65), 314 (4.52) nm; IR (KBr) ν_{max} 3560 (OH), 2980−2820, 1625, 1515, 1470, 1450, 1300, 740 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS *m*/*z* 363.1954 $[MH]^+$ (calcd for $C_{24}H_{27}O_3$, 363.1882).

CD Spectra and Optical Rotation of Cannabidiol (8). CD (MeOH) λ_{max} ([θ]) 240 (−1.25 × 10^4), 281 (+3.05 × 10^3) nm; $[\alpha]_D$ −118° (*c* 0.1, MeOH) (lit.[19] $[\alpha]_D$ −120 ± 1°).

CD Spectra and Optical Rotation of Δ^9-10a*R*-Tetrahydrocannabinol. CD (MeOH) λ_{max} ([θ]) 234 (−1.1 × 10^4), 280 (−3.0 × 10^3) nm; $[\alpha]_D$ −148° (*c* 0.1, $CHCl_3$).

Antimicrobial Assay. All organisms are obtained from the American Type Culture Collection (Manassas, VA) and include *C. albicans* ATCC 90028, *C. neoformans* ATCC 90113, *A. fumigatus* ATCC 90906, *S. aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 43300 (MRSA), *P. aeruginosa* ATCC 27853, and *M. intracellulare* ATCC 23068. Susceptibility testing was performed using a modified version of the NCCLS methods.[24−27] Susceptibility testing of *M. intracellulare* was done using the modified Alamar Blue procedure of Franzblau et al.[28] Samples (in DMSO) are serially diluted using 0.9% saline and transferred in duplicate to 96-well microplates. Microbial inocula are prepared after comparison of the OD_{630} to the 0.5 McFarland standard by diluting in broth [Sabouraud Dextrose and cation-adjusted Mueller-Hinton (Difco)] for the fungi and bacteria, respectively, and 5% Alamar Blue (BioSource International) in Middlebrook 7H9 broth with OADC enrichment for *M. intracellulare* to afford recommended inocula. The *A. fumigatus* inoculum is prepared via comparison with a standard curve and dilution of spores in YM broth to afford a final target inoculum of 4.0 × 10^4 CFU/mL. Microbial inocula are added to the samples to achieve a final volume of 200 μL and final sample concentrations starting with 200 μg/mL for crude extracts and 50 μg/mL for pure compounds. Growth, solvent, and media controls are included on each test plate. Except for *A. fumigatus*, which is inspected visually, all other organisms are read at either 630 nm or 544ex/590em, gain = 25 (*M. intracellulare*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies) prior to and after incubation. For organisms read on the plate reader, percent growth is calculated and plotted versus test concentration to afford the IC_{50}. MIC is defined as the lowest test concentration that allows no detectable growth. The minimum fungicidal or bactericidal concentrations (MFC/MBCs) are determined by removing 5 μL from each clear well, transferring to agar, and incubating until growth is seen. The MFC/MBC is defined as the lowest test concentration that kills 100% of the organism.

Antimalarial/Parasite LDH Assay. The in vitro antimalarial assay procedure[29−31] used was described previously.[1]

Antileishmanial Assays. Compounds and extracts were screened for in vitro antileishmanial activity against *L. donovani* promastigotes. A transgenic cell line of *L. donovani* promastigotes showing stable expression of luciferase was used as the test organism. Cells in the 200 μL of growth medium (L-15 with 10% FCS) were plated at a density of 2 × 10^6 cells per mL in a clear 96 cell well microplate. The plates were incubated at 26 °C for 72 h. An aliquot of 50 μL was transferred from each well to a fresh opaque/black microplate, and 40 μL of Steadyglo reagent was added to each well. The plates were read in a Polar Star Galaxy microplate luminometer. IC_{50} and IC_{90} were calculated from the dose−response graphs. Pentamidine was tested as a standard antileishmanial.

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