# Antileishmanial Compounds from *Cordia fragrantissima* Collected in Burma (Myanmar)

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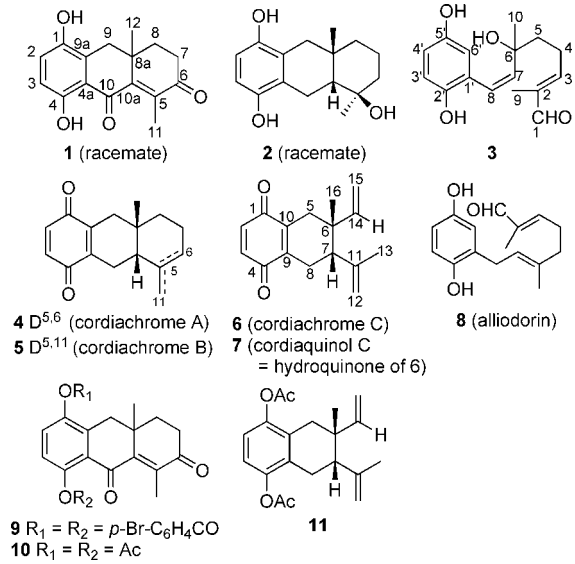
A methanol extract of the wood of *Cordia fragrantissima*, collected in Burma (Myanmar), was found to exhibit significant activity against *Leishmania major*. Bioassay-guided fractionation of this extract using several chromatographic techniques afforded three new compounds (**1**–**3**) and five known compounds (**4**–**8**). The structures of the new compounds were revealed on the basis of spectroscopic data interpretation and by X-ray crystallographic analysis. Interestingly, the new compounds, despite the presence of asymmetric carbons, were found to be racemates. The activities of the isolates from *C. fragrantissima* and several derivatives were evaluated against the promastigote forms of *Leishmania major*, *L. panamensis*, and *L. guyanensis*.

Leishmaniasis is a parasitic disease caused by protozoal species of the genus *Leishmania*, of which over 20 are known to be pathogenic to humans.[1] *Leishmania* spp. are all transmitted by small biting sandflies (*Phlebotomus* spp.). Twelve million people in 88 countries are afflicted by this disease.[2] *Leishmania major*, the causative agent of cutaneous leishmaniasis, is a digenetic parasite that exists as an extracellular promastigote within the insect vector, and as a nonmotile intracellular amastigote within the phagolysosome of macrophages and other cells of the reticuloendothelial system of the mammalian host.[3] Pentavalent antimonials are currently used for basic treatment of the disease, but these drugs are extremely toxic and generally expensive.[4] More economical and less toxic drugs seem to be necessary.

As part of a search for plant compounds that are active against *Leishmania* spp., it was found that a methanol extract of the wood of *Cordia fragrantissima* Kurz. (Boraginaceae), a plant from Burma (Myanmar), exhibited significant in vitro antileishmanial activity. This report deals with the isolation and structural determination of the active antileishmanial components of this plant and the activity of their derivatives of these naturally occurring compounds.




## Results and Discussion

In a preliminary screening, a methanol extract of the wood of *C. fragrantissima* (local name "Sandawa"), used in Burma (Myanmar) for making furniture, was found to exhibit potent activity against *Leishmania major* [MLC (minimum lethal concentration): 25 $\mu$g/mL; MIC (minimum inhibitory concentration): 12.5 $\mu$g/mL].[5] Although secondary metabolites of other *Cordia* species such as *C. alliodora* and *C. multispicata* have been investigated previously,[6–12] the chemical composition of *C. fragrantissima*has not yet been reported.

Timber from *C. fragrantissima* was shaved, and the collected flakes were extracted with methanol. Bioassay-guided (in vitro leishmanicidal assay using *L. major*) fractionation of the methanol extract was performed by reversed-phase flash column chromatography, medium-pressure liquid chromatography (MPLC), and HPLC, yielding three new compounds, **1**–**3**, and five known compounds, cordiachromes A (**4**),[13,14] B (**5**),[13,14] and C (**6**),[11] cordiaquinol C (**7**),[8] and alliodorin (**8**).[7]

Compound **1** was obtained as a yellow, amorphous solid. Its molecular formula was established as $C_{16}H_{16}O_4$ by ESITOFMS (observed *m*/*z* 271.0952, calculated 271.0970, $[M - H]^-$). The 16 carbon signals present in the $^{13}C$ NMR spectrum were characterized by DEPT-90 and 135 spectra as two carbonyls [$\delta$ 195.8 (C-10), 199.6 (C-6)], two olefinic carbons [$\delta$ 138.8 (C-5), 150.1 (C-10a)], and four aromatic quaternary carbons [$\delta$ 117.3 (C-9a), 125.8 (C-4a), 144.8 (C-1), 157.6 (C-4)], as well as two aromatic methines [$\delta$ 116.1 (C-2), 125.2 (C-3)], three methylenes [$\delta$ 33.7 (C-7), 36.2 (C-8), 37.6 (C-9)], an $sp^3$-quaternary carbon [$\delta$ 37.8 (C-8a)], and two methyl carbons [$\delta$ 13.5 (C-11), 23.9 (C-12)]. The $^{13}C$ NMR chemical shifts of all hydrogenated carbons were assigned unambiguously using the HSQC spectrum. The $^1H$ NMR spectrum of **1** ($CDCl_3$) showed signals for two methyl groups at $\delta$ 1.25 (3H, s, H-12) and 2.12 (3H, s, H-11), two aromatic proton signals at $\delta$ 6.77 (1H, d, $J$ = 9.0 Hz, H-3) and 7.01 (1H, d, $J$ = 9.0 Hz, H-2), and four signals due to methylene protons at $\delta$ 2.08 (2H, t, $J$ = 5.6 Hz, H-8), 2.61 (2H, t, $J$ = 5.6 Hz, H-7), 2.82 (1H, d, $J$ = 16.8 Hz, H-9b), and 3.06 (1H, d, $J$ = 16.8 Hz, H-9a). Complete elucidation

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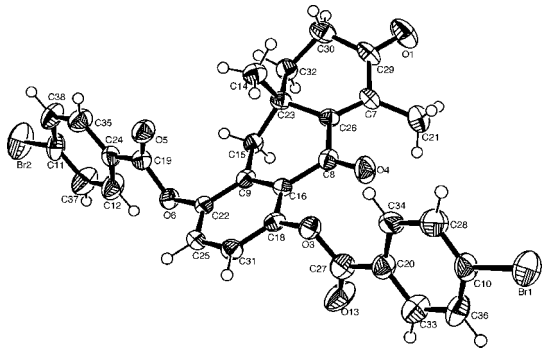


**Figure 1.** ORTEP drawing of **9**, the di-*p*-bromobenzoate derivative of **1**, as determined by X-ray analysis. Only one form of the racemate is shown.

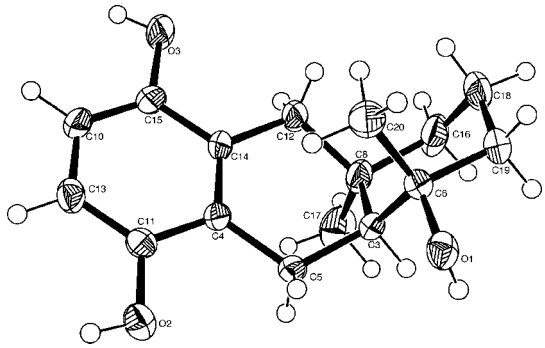


**Figure 2.** ORTEP drawing of **2** as determined by X-ray analysis. Only one form of the racemate is shown.

of the structure of **1**was achieved on the basis of HMBC correlations. $^{1}H/^{13}C$: Me-11/C-5, C-6, and C-10a; Me-12/C-8, C-9, and C-10a; H-7/C-8; H-8/C-7 and C-8a; H-9/C-1, C-4a, C-9a, and C-10a; H-2/C-1, C-4, and C-9a; H-3/C-4 and C-4a. In this way, a tricyclic structure including a hydroquinone moiety was established.

Compound **1** has an asymmetric carbon at position C-8a. In an attempt to elucidate its absolute configuration, **1** was converted to the di-*p*-bromobenzoate (**9**, obtained as yellow crystals) by addition of *p*-bromobenzoyl chloride in pyridine. An X-ray crystallographic analysis of **9** (Figure 1) confirmed the structure of **1**. Surprisingly, this work revealed that **9** exists as a racemate (space group $P\bar{1}$), which explained its optical sign: $[\alpha]_D$ −0.25 (*c* 0.13, $CHCl_3$). On acetylation under the usual conditions, compound **1** formed a diacetate derivative (**10**).

Compound **2**, $[\alpha]_D$ −0.84 (*c* 0.31, MeOH), was obtained as pale pink crystals. Its molecular formula, $C_{16}H_{22}O_3$, determined by ESITOFMS (observed *m/z* 285.1439, calculated 285.1467, $[M + Na]^+$), implied that this compound might be an analogue of **1**. This turned out to be the case; compound **2** was found to contain a *p*-hydroquinone moiety [$^{13}C$: $\delta$ 112.3 (d), 112.5 (d), 125.0 (s), 125.2 (s), 148.7 (s), 149.1 (s)] and two methyl groups [$^{1}H$: $\delta$ 0.92 (3H, s), 1.02 (3H, s)]. Although no carbonyls ($^{13}C$) were evident in **2**, a quaternary oxygenated carbon ($^{13}C$: $\delta$ 74.0) was present. The planar structure of **2** was elucidated by integrated analysis of the 1D and 2D NMR spectra. The following HMBC correlations were observed: Me-11 ($\delta$ 0.92)/C-5, C-6, and C-10a; Me-12 ($\delta$ 1.02)/C-8, C-9, C-8a, and C-10a; H-6 ($\delta$ 1.51)/C-5 and C-10a; H-7 ($\delta$ 1.69)/C-5 and C-8a; H-9 ($\delta$ 1.42)/C-12 and C-8a; H-8 ($\delta$ 2.27)/C-12, C-8a, and C-10a; H-10 ($\delta$ 3.18)/C-5, C-8a, and C-10a; H-10a ($\delta$ 1.65)/C-5, C-9, C-10, C-11, C-12, and C-8a; H-2 and H-3 ($\delta$ 6.47: overlapped)/C-1 and C-4, C-4a, and C-9a. The structure and relative configuration of **2** were eventually established by X-ray crystallography (Figure 2). Again the X-ray analysis indicated that compound **2** exists as a racemate (space group *Cc*), which is consistent with its small optical rotation value. The structures of compounds **1** and **2** are closely related to those of **4** and **5**, containing a benzocogeijerene skeleton (terpenoid benzoquinone), which have been isolated from other *Cordia* plants.[14] The present results represent the first examples of X-ray analysis of benzocogeijerene-type compounds. The monomethyl ether of **2** at the C-4 hydroxy group was obtained previously by acid treatment of a mushroom component.[15]

Compound **3**, $[\alpha]_D$ −1.80 (*c* 0.11, $CHCl_3$), $C_{16}H_{20}O_4$ (observed *m/z* 281.1151, calculated 281.1154, $[M + Na - H_2O]^+$) (ESITOFMS), was obtained as a pale yellow, amorphous solid. The $^{1}H$ NMR spectrum of **3**($CDCl_3$) showed signals similar to those of compounds **1** and **2**, with two aromatic proton signals at $\delta$ 6.53 (2H, overlapped), together with a new aromatic proton signal at $\delta$ 6.47 (1H, d, $J$ = 2.0 Hz), suggesting the presence of a monosubstituted *p*-hydroquinone moiety. The presence of the *p*-hydroquinone moiety was evident from the carbon signals at $\delta$ 113.8 (d), 116.2 (d), 116.5 (d), 122.9 (s), 147.2 (s), and 150.9 (s), which confirmed that the *p*-hydroquinone group is monosubstituted.

Together with this moiety, the presence of the following groups was deduced from the NMR data ($^{1}H$; $^{13}C$): an aldehyde [$\delta$ 9.30 (1H, s); $\delta$ 197.2 (d) (C-1)], an olefin [$\delta$ 6.61 (1H, tq, $J$ = 7.8, 1.3 Hz); $\delta$ 140.3 (C-2) and 157.0 (C-3)] conjugated with the aldehyde, another olefin [$\delta$ 5.64 and 6.33 (AB pattern, $J_{AB}$ = 10.0 Hz); $\delta$ 124.5 (C-8) and 131.0 (C-7)], a methylene [$\delta$ 2.51 (2H, q, $J$ = 8.0 Hz); $\delta$ 25.2 (C-4)], another methylene [$\delta$ 1.48 (2H, t, $J$ = 8.0 Hz); $\delta$ 40.3 (C-5)], two quaternary methyls [$\delta$ 1.36 (3H, s) and 1.64 (3H, d, $J$ = 1.3 Hz); $\delta$ 9.0 (C-9) and 26.5 (C-10)], and a quaternary carbon bearing an oxygen ($\delta$ 78.9). COSY correlations between the proton signals at H-3 ($\delta$ 6.61), H-4 (2H, $\delta$ 2.51), and H-5 (2H, $\delta$ 1.48) and between H-7 ($\delta$ 5.64) and H-8 ($\delta$ 6.33) allowed partial elucidation of the structure. Complete elucidation of this structure was achieved by an HMBC experiment. $^{1}H/^{13}C$: H-1/C-2; H-3/C-9; H-4/C-3; H-5/C-4 and C-6; H-7/C-1′; H-8/C-6, C-2′, and C-6′; Me-9/C-2 and C-3; Me-10/C-6 and C-7; H-3′/C-5′; H-4′/C-5′. The structure of **3** is related to that of alliodorin (**8**). The small $[\alpha]_D$ value of **3** suggests that this compound may also exist as a racemate.

Of the known compounds isolated in the present investigation, **4**–**7** have asymmetric carbons. These compounds have been obtained previously from other *Cordia* species.[7,8,11,13,14] Since the $[\alpha]_D$ values of **4**–**7** (>99% pure by HPLC), which were obtained in this study, are all small (−0.26, −1.06, −1.06, and +0.02, respectively), they may be racemates, as in the case of compounds **1**–**3**. The $[\alpha]_D$ value reported for **6** is −1.11,[11] although the values of **4**, **5**, and **7** have not been reported.

The possible biosynthetic pathway[16] of the compounds obtained by this work is outlined in Figure S1, Supporting Information. Geranylhydroquinone (**a**) is oxidized to **b**, a precursor of **3** and **8**, the cyclization of which gives rise to a bicyclic diene (**c**). Cope rearrangement of **c** [**c**(**Cope**)] gives rise to a diene (**d**), which produces **6** and **7**. On the other hand, an ene-reaction of **c** [**c**(**Ene**)] would result in a tricyclic compound (**e**), a precursor of **1**, **2**, **4**, and **5**. The fact that an achiral precursor (**c**) is an intermediate in these reactions explains why compounds **1**, **2**, and **4**–**7** were obtained as racemates.

The activities of the new compounds (**1**–**3**) and the known compounds (**4**–**8**), together with some synthetic derivatives (**9**–**11**), were tested against the promastigote form of *Leishmania major*, *L. panamensis*, and *L. guyanensis*. All the tested samples exhibited leishmanicidal activity with $IC_{50}$ values ranging from 1.4 to 81.4 $\mu$g/mL (Table 1). *Leishmania guyanensis* and *L. panamensis* seem to be more sensitive to these compounds than *L. major*, which is the easiest to handle (giving rapid and stable growth) and was used in the bioassay-guided isolation. In the *L. major* assay, **2**, **4**, **5**, **7**, **8**, and **11** showed good activity (1.4–7.0 $\mu$g/mL). The presence of a tricyclic framework or benzoquinone or hydroquinone functionality does not seem essential for leishmanicidal activity. No significant difference of the activities between natural (**1**–**8**) and synthetic

**Table 1.** In Vitro Leishmanicidal Activity and Cytotoxicity of Compounds **1**–**11**

| | $IC_{50}$ ($\mu$g/mL) | | | $IC_{50}$ ± SD ($\mu$g/mL; $\mu$M)[d] | |
|---|---|---|---|---|---|
| compd | *L. major* | *L. guyanensis* | *L. panamensis* | COS-7 | HuH-7 |
| **1** | 81.4 | 7.0 | 13.0 | 51.7 ± 0.8 | 71.8 ± 1.7 |
| **2** | 2.7 | 3.0 | 1.8 | >100 | 41.7 ± 1.2 |
| **3** | >25 | NT[a] | NT | >100 | >100 |
| **4** | 4.1 | NT | NT | 22.5 ± 0.3 | 61.1 ± 3.2 |
| **5** | 2.5 | NT | NT | 14.6 ± 0.3 | 71.5 ± 0.3 |
| **6** | 21.1 | 6.0 | 5.5 | NT | NT |
| **7** | 4.5 | NT | NT | NT | NT |
| **8** | 7.0 | 2.0 | 1.8 | 31.9 ± 7.2 | 44.8 ± 1.2 |
| **9** | 23.5 | NT | NT | >100 | >100 |
| **10** | 80.4 | NT | NT | 50.7 ± 0.7 | 65.5 ± 1.7 |
| **11** | 1.4 | NT | NT | 20.1 ± 0.3 | 60.9 ± 1.9 |
| AmB[b] | <0.1 | <0.1 | <0.1 | | |
| MG132[c] | | | | <10 | <10 |

[a] NT = not tested. [b] AmB = amphotericin B. Positive control for antileishmanial assay. [c] Positive control for cytotoxicity assay. [d] Concentration in $\mu$g/mL for compounds **1**–**11** and AmB, and $\mu$M for MG132.

(**9**–**11**) compounds was found. The cytotoxicities of **1**–**5** and **8**–**11** were tested against COS-7 (African green monkey kidney cells, epithelial-like) and HuH-7 (human liver cancer cells, epithelial-like) cells (Table 1). None of these compounds was cytotoxic.

## Experimental Section

**General Experimental Procedures.** Optical rotations were taken on a JASCO 1010 polarimeter. IR spectra were measured on a JASCO FT/IR-6300 spectrophotometer. UV spectra were taken on a JASCO International V-530 spectrophotometer. The 1D and 2D NMR spectra were obtained on Bruker AVANCE 400 MHz, 700 MHz, and Varian Unity INOVA 500 MHz spectrometers. ESITOFMS were measured on a JASCO International Q-TOF Micro mass spectrometer. For MPLC, reversed-phase material (Ultrapak, Yamazen Co., Ltd.) was used. ODS-flash column chromatography was carried out on Cosmosil $C_{18}$ (Nakalai Tesque Co., Ltd.). For high-performance liquid chromatography (HPLC), columns of Shiseido Capcell pak $C_{18}$ MG 5 $\mu$m, 20 × 250 mm, and $C_{18}$ UG120 5 $\mu$m, 10 × 250 mm, Waters XTerra MS $C_{18}$ 5 $\mu$m, 20 × 150 mm, Atlantis $dC_{18}$ 5 $\mu$m, 20 × 100 mm, and Intact Unison UK-$C_{18}$ 3 $\mu$m, 10 × 250 mm, and the HPLC system of JASCO Co., Ltd., were used. TLC was conducted on precoated silica gel 60 $F_{254}$ (Merck) and/or RP-18 $F_{254s}$ (Merck), and the spots were detected by heating after spraying with *p*-methoxybenzaldehyde−$H_2SO_4$ reagent.

**Plant Material.** The wood of *Cordia fragrantissima* was produced and kindly donated by the Ministry of Forestry of Myanmar in November 2004 and identified by Dr. Nyan Tun, taxonomist at the Institute of Forestry, Forest Department, Ministry of Forestry, Union Myanmar, where voucher specimens are deposited (accession number: 2.17-1b/cu. ft. 50).

**Extraction and Isolation.** The shaved timber of *C. fragrantissima* (850 g) was soaked in MeOH and extracted at 40 °C for 4 h three times. The MeOH extract was concentrated under reduced pressure to give a residue (70 g). The residue was treated with $H_2O$. The resultant aqueous suspension was partitioned with ethyl acetate. The EtOAc extract (64 g) was partitioned between hexane (18 g) and 90% MeOH (36 g). A part of the hexane extract (4.7 g) was subjected to silica gel column chromatography eluting with hexane−EtOAc (98:2 to 6:4) to give 10 fractions (Frs. 1–10). One third of fraction 4 (42 mg) was purified by recycle-HPLC [MeOH−distilled $H_2O$ (95:5)] to afford cordiachrome A (**4**) (3 mg), $[\alpha]^{26}_D$ −0.26 (*c* 0.16, MeOH), cordiachrome B (**5**) (7 mg), $[\alpha]^{26}_D$ −1.06 (*c* 0.16, MeOH), and cordiachrome C (**6**) (5 mg), $[\alpha]^{26}_D$ −1.06 (*c* 0.19, MeOH) [lit. $[\alpha]^{23}_D$ −1.11 (*c* 0.27, $CHCl_3$)].[11] One third of the 90% MeOH extract (26 g) was subjected to ODS-flash column chromatography, eluting with MeOH−distilled $H_2O$ (3:7 to 9:1), to give five fractions (Frs. 1-1–1-5). Fraction 1-2 (1.0 g) was applied to ODS-flash column chromatography with MeOH−distilled $H_2O$ (1:1) to give five fractions (Frs. 2-1–2-5). One third of fraction 2-2 (100 mg) was purified by HPLC [MeOH−distilled $H_2O$ (1:1)] to afford a new hydroquinone, cordiaquinol J (**2**, 3 mg). One third of the fraction 2-4 (55 mg) was purified by HPLC [MeOH−distilled $H_2O$ (1:1)] to afford alliodorin (**8**) (3 mg). Fraction 2-5 (115 mg), mainly consisting of **8**, was also purified in a similar manner. Fraction 1-3 (2.1 g) was purified by MPLC with MeOH−distilled $H_2O$ (7:3) to give three fractions (Frs. 3-1–3-3). Fraction 3-2 (467 mg) was purified by preparative HPLC with MeOH−distilled $H_2O$ (1:1) to give eight fractions (Frs. 4-1–4-8). Fractions 4-4 and 4-6 constituted a new hydroquinone, cordiaquinol I (**1**, 60 mg), and cordiaquinol C (**7**) (648 mg), $[\alpha]^{27}_D$ +0.02 (*c* 0.19, MeOH), respectively, in pure states. One third of fraction 4-2 (11 mg) was purified by preparative HPLC with MeOH−distilled $H_2O$ (6:4) to afford a new hydroquinone aldehyde, cordiaquinol K (**3**, 2 mg).

**Cordiaquinol I (1):** yellow, amorphous solid; $[\alpha]^{27}_D$ +0.82 (*c* 0.36, $CHCl_3$); IR $\nu_{max}$ (NaCl) 1683, 1670 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 294 (3.86) nm; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 12.13 (1H, s, OH-4, hydrogen-bonded with C═O-10), 7.01 (1H, d, *J* = 9.0 Hz, H-2), 6.77 (1H, d, *J* = 9.0 Hz, H-3), 3.06 (1H, d, *J* = 16.8 Hz, H-9a), 2.82 (1H, d, *J* = 16.8 Hz, H-9b), 2.61 (2H, t, *J* = 5.6 Hz, H-7), 2.12 (3H, s, Me-11), 2.08 (2H, t, *J* = 5.6 Hz, H-8), 1.25 (3H, s, Me-12); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 199.6 (C-6), 195.8 (C-10), 157.6 (C-4), 150.1 (C-10a), 144.8 (C-1), 138.8 (C-5), 125.8 (C-4a), 125.2 (C-3), 117.3 (C-9a), 116.1 (C-2), 37.8 (C-8a), 37.6 (C-9), 36.2 (C-8), 33.7 (C-7), 23.9 (C-12), 13.5 (C-11); HRESITOFMS (negative) *m*/*z* 271.0952 [M − H]$^-$ (calcd for $C_{16}H_{15}O_4$, 271.0970).

**Cordiaquinol J (2):** pale pink crystals; mp 205–208 °C; $[\alpha]^{26}_D$ −0.84 (*c* 0.31, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 294 (4.02) nm; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 6.47 (2H, ABq, *J* = 9.2 Hz, H-2, H-3), 3.18 (1H, d, *J* = 19.1 Hz, H-10eq), 2.68 (1H, d, *J* = 17.6 Hz, H-9eq), 2.63 (1H, dd, *J* = 7.8, 19.1 Hz, H-10ax), 2.27 (1H, d, *J* = 19.1 Hz, H-9ax), 1.74 (1H, m, H-6), 1.69 (2H, m, H-7), 1.65 (1H, d, *J* = 7.8 Hz, H-10a), 1.62 (1H, m, H-8), 1.51 (1H, dt, *J* = 4.5 and 12.0 Hz, H-6), 1.42 (1H, dt, *J* = 5.2 and 12.6 Hz, H-8), 1.02 (3H, s, Me-12), 0.92 (3H, s, Me-11); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 149.1 (C-1), 148.7 (C-4), 125.2 (C-9a), 125.0 (C-4a), 112.5 (C-2), 112.3 (C-3), 74.0 (C-5), 50.1 (C-10a), 43.5 (C-6), 40.7 (C-8), 33.4 (C-8a), 32.6 (C-9), 31.6 (C-12), 22.8 (C-11), 21.7 (C-10), 21.2 (C-7); HRESITOFMS (positive) *m*/*z* 285.1439 [M + Na]$^+$ (calcd for $C_{16}H_{22}O_3Na$, 285.1467).

**X-ray Crystallographic Analysis Data of 2.** Crystal size, 0.30 × 0.30 × 0.10 mm; molecular formula, $C_{18}H_{28}O_4$; molecular formula moiety, $C_{16}H_{22}O_3$, $C_2H_6O$; crystal system, monoclinic; space group, *Cc*; unit cell dimensions (*a*, *b*, *c*), 11.474(3) Å, 13.935(3) Å, 10.813(2) Å; $\alpha$ = 90°, $\beta$ = 104.93(2)°, $\gamma$ = 90°, volume, 1670.5(7) Å$^3$; *Z* = 4; density, 1.226 mg $m^{-3}$; absorption coefficient, 0.085 $mm^{-1}$; *F*(000) = 672.0; diffractometer used, Rigaku RAXIS-RAPID; radiation ($\lambda$) Mo K$\alpha$ (0.71073 Å); 2$\theta$ max 54.9°; reflections collected, 8225; independent reflections, 1911; observed reflections, 1612 [*R*(int) = 0.022]; final *R* indices, *R* = 0.0430 (obsd data), wR2 = 0.1080 (in-depth data); goodness of fit, 0.995; *T* = 173(1) K. The structure was solved by direct methods and refined by full matrix least-squares on $F^2$.[17]

**Cordiaquinol K (3):** dark yellow oil; $[\alpha]^{27}_D$ −1.80 (*c* 0.11, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 264 (3.67) nm; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.30 (1H, s, H-1), 6.61 (1H, tq, *J* = 7.8, 1.3 Hz, H-3), 6.53 (2H, overlapped, H-3′and 4′), 6.47 (1H, d, *J* = 2.0 Hz, H-6′), 6.33 (1H, d, *J* = 10.0 Hz, H-8), 5.64 (1H, d, *J* = 10.0 Hz, H-7), 2.51 (2H, q, *J* = 8.0 Hz, H-4), 1.84 (2H, t, *J* = 8.0 Hz, H-5), 1.64 (3H, d, *J* = 1.3 Hz, Me-9), 1.36 (3H, s, Me-10); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 197.2 (C-1), 157.0 (C-3), 150.9 (C-5′), 147.2 (C-2′), 140.3 (C-2), 131.0 (C-7), 124.5 (C-8), 122.9 (C-1′), 116.5, 116.2 (C-3′, C-4′), 113.8 (C-6′), 78.9 (C-6), 40.3 (C-5), 26.5 (C-10), 25.2 (C-4), 9.0 (C-9); HRESITOFMS (positive) *m*/*z* 281.1151 [M + Na − $H_2O$]$^+$ (calcd for $C_{16}H_{18}O_3Na$, 285.1154).

**1,4-*p*-Dibromobenzoylcordiaquinol I (9).** To a solution of compound **1** (14 mg, 0.05 mmol) in pyridine (4.0 mL) was added *p*-bromobenzoyl chloride (58 mg, 0.26 mmol), and the reaction mixture was stirred at room temperature for 24 h. Workup as usual gave a residue, which was purified by silica gel column chromatography (hexane−EtOAc, 4:1) to give **9** (25 mg) as yellow crystals in hexane−$CH_2Cl_2$: mp 172–180 °C; $[\alpha]^{23}_D$ −0.25 (*c* 0.13, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.10 (4H, d, *J* = 8.4 Hz, benzoyl), 7.72, 7.69 (each 2H, d, *J* = 8.4 Hz, benzoyl), 7.46 (1H, d, *J* = 8.4 Hz, H-3), 7.21 (1H, d, *J* = 8.4 Hz H-2), 3.01 (1H, d, *J* = 16.8 Hz, H-9eq), 2.83 (1H, d, *J* = 16.8 Hz, H-9ax), 2.55 (2H, t, *J* = 5.6 Hz, H-7), 2.01 (2H, m, H-8), 1.94 (3H, s, Me-11), 1.28 (3H, s, Me-12); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 199.3 (C-6), 188.9 (C-10), 164.7 (benzoyl) 163.8 (benzoyl), 150.5 (C-10a), 148.1 (C-4), 146.4 (C-1), 137.5 (C-5), 134.7 (C-4a), 132.3 (benzoyl), 132.0 (benzoyl), 131.9 (benzoyl), 131.8

(benzoyl), 129.6 (benzoyl), 128.8 (benzoyl), 128.5 (benzoyl), 128.2 (C-3), 127.4 (benzoyl), 126.4 (C-9a), 123.1 (C-2), 37.6 (C-8a), 37.3 (C-9), 35.7 (C-8), 33.7 (C-7), 24.5 (C-12), 13.1 (C-11); HRESIMS (positive) *m/z* 660.9672 $[M + Na]^+$ (calcd for $C_{30}H_{22}O_6Na^{79}Br^{81}Br$, 660.9660).

**X-ray Crystallographic Analysis Data of 9.** Crystal size, 0.25 × 0.10 × 0.08 mm; molecular formula, $C_{30}H_{22}Br_2O_6$; crystal system, triclinic; space group, $P\bar{1}$; unit cell dimensions (*a*, *b*, *c*), 8.889(3) Å, 11.238(2) Å, 14.179(4) Å; $\alpha = 94.10(3)°$, $\beta = 100.838(5)°$, $\gamma = 109.55(3)°$, volume, 1297.0(7) $Å^3$; $Z = 2$; density, 1.634 mg $m^{-3}$; absorption coefficient, 3.169 $mm^{-1}$; $F(000) = 640.0$; diffractometer used, Rigaku RAXIS-RAPID; radiation ($\lambda$) Mo K$\alpha$ (0.71073 Å); $2\theta$ max 55.0°; reflections collected, 12 761; independent reflections, 5919; observed reflections, 2778 [$R$(int) = 0.050]; final $R$ indices, $R = 0.0480$ (obsd data), wR2 = 0.0730 (indept data); goodness of fit, 1.070; $T = 173(1)$ K. The structure was solved by direct methods and refined by full matrix least-squares on $F^2$.[17]

**Acetylcordiaquinol I (10).** Compound **1** (8 mg, 0.03 mmol) was treated with pyridine (0.5 mL) and acetic anhydride (0.5 mL), and the mixture was allowed to stand overnight. The reagents were evaporated off, and the residue was purified by passage over an ODS cartridge (Waters Sep-Pak, $C_{18}$, MeOH−distilled $H_2O$), giving **10** (6 mg) as an oil; $[\alpha]^{23}_D$ −5.55 (*c* 0.18, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 7.30 (1H, d, $J = 8.7$ Hz, H-2), 7.03 (1H, d, $J = 8.7$ Hz, H-3), 2.93 (1H, d, $J = 16.5$ Hz, H-9a), 2.78 (1H, d, $J = 16.5$ Hz, H-9b), 2.59 (2H, m, H-7), 2.41 (3H, s, OCO*CH*$_3$), 2.37 (3H, s, OCO*CH*$_3$), 2.05 (2H, m, H-8), 2.02 (3H, s, Me-11), 1.23 (3H, s, Me-12); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 199.3 (C-6), 189.1 (C-10), 168.7 (O*CO*CH$_3$), 168.1 (O*CO*CH$_3$), 150.6 (C-10a), 148.0 (C-4), 146.2 (C-1), 137.4 (C-5), 134.4 (C-4a), 128.1 (C-2), 126.0 (C-9a), 122.9 (C-3), 37.6 (C-9), 37.3 (C-8a), 35.7 (C-8), 33.7 (C-7), 24.3 (C-12), 21.1 (OCO*CH*$_3$), 20.8 (OCO*CH*$_3$), 13.1 (C-11); HRESIMS (positive) *m/z* 379.1133 $[M + Na]^+$ (calcd for $C_{20}H_{20}O_6Na$, 379.1158).

**Acetylcordiaquinol C (11).** Cordiaquinol C (**7**) (83 mg, 0.34 mmol) was treated with pyridine (2.0 mL) and acetic anhydride (2.0 mL), and the mixture was allowed to stand overnight. The reagents were evaporated off, and the residue was purified by passage over an ODS cartridge (Waters Sep-Pak, $C_{18}$, MeOH−distilled $H_2O$), giving **11** (58 mg); $[\alpha]^{23}_D$ −1.59 (*c* 0.73, $CHCl_3$); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 6.89 (2H, brs, H-2, H-3), 5.94 (1H, dd, $J = 10.8$ and 17.6 Hz, H-14), 4.98 (1H, d, $J = 17.6$ Hz, H-15a), 4.91 (1H, d, $J = 10.8$ Hz, H-15b), 4.89 (1H, s, H-12a), 4.78 (1H, s, H-12b), 2.66 (1H, d, $J = 17.2$, H-5$\beta$), 2.63 (2H, d, $J = 6.8$ Hz, H-8), 2.46 (1H, d, $J = 17.2$ Hz, H-5$\alpha$), 2.45 (2H, m, H-7), 2.30 (3H, s, OCO*CH*$_3$), 2.29 (3H, s, OCO*CH*$_3$), 1.77 (3H, s, Me-13), 1.14 (3H, s, Me-16); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 168.0 (O*CO*CH$_3$), 166.2 (O*CO*CH$_3$), 146.3 (C-4), 146.2 (C-1), 141.6 (C-14), 146.0 (C-11), 130.3 (C-9), 130.0 (C-10), 119.7 (C-15), 119.5 (C-12), 113.5, 113.2 (C-2, C-3), 49.8 (C-7), 37.7 (C-5), 37.7 (C-6), 27.2 (C-8), 26.0 (C-16), 23.1 (C-13), 20.7 (OCO*CH*$_3$), 20.7 (OCO*CH*$_3$); HRESITOFMS (positive) *m/z* 351.1563 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_4Na$, 351.1572).

**Cultivation of *Leishmania* Promastigotes.** Medium 199 was used for cultivation of promastigotes of *Leishmania major*, *L. guyanensis*, and *L. panamensis*. Promastigotes were cultured in medium [supplemented with heat-inactivated (56 °C for 30 min) fetal bovine serum (10%)] at 27 °C, in an atmosphere of 5% $CO_2$ in an incubator.

**Leishmanicidal Assay.** The leishmanicidal effects of the samples were assessed by an improved [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide] (MTT) method as follows. Cultured promastigotes were seeded at $4 \times 10^5$ per 50 $\mu$L of medium per well in 96-well microplates, and then 50 $\mu$L samples of the test compounds at different concentrations, dissolved in a mixture of DMSO and medium, were added to each well. Each concentration was tested in triplicate. The microplate was incubated at 27 °C in 5% $CO_2$ for 48 h. Tetra Color One (10 $\mu$L) [a mixture of WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt) and 1-methoxy-PMS (1-methoxy-5-methylphenazinium methyl sulfate)] was added to each well, and the plates were incubated at 27 °C in 5% $CO_2$ for 6 h. Optical density values (test wavelength 450 nm, reference wavelength 630 nm) were measured using a Viento XS multispectrophotometer (Dainippon Pharmaceutical). Leishmanicidal activities were expressed as a minimum lethal concentration (MLC) and a minimum inhibitory concentration (MIC). The $IC_{50}$ (50% inhibitory concentration) values for compounds were estimated from graphs. As positive control, amphotericin B was used.

**Cytotoxicity Assay.** Compounds were assayed for cytotoxicity against COS-7 and HuH-7 cells using the XTT method.[18–21] The $IC_{50}$ is the concentration of agent that reduced cell growth by 50% under the experimental conditions. MG132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal) was used as positive control.

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**Supporting Information Available:** Figure S1, showing the principal HMBC correlations observed in compounds **1**–**3**. Figure S2, showing a biogenetic pathway of compounds obtained in the present study. This material is available free of charge via the Internet at http://pubs.acs.org.

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