Antimalarial Compounds from *Rhaphidophora decursiva*

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Bioassay-directed fractionation led to the isolation of 14 compounds, six of which possess antimalarial activity, from the dried leaves and stems of *Rhaphidophora decursiva*. Polysyphorin (**1**) and rhaphidecurperoxin (**6**) showed strong activities against *Plasmodium falciparum*. Rhaphidecursinol A (**2**), rhaphidecursinol B (**3**), grandisin (**4**), and epigrandisin (**5**) were less active against the same organism. Among the isolates, rhaphidecursinol A (**2**) and rhaphidecursinol B (**3**) were determined to be new neolignans, and rhaphidecurperoxin (**6**) is a new benzoperoxide. Known compounds isolated include polysyphorin (**1**), grandisin (**4**), epigrandisin (**5**), (+)-medioresinol, (−)-pinoresinol, (−)-syringaresinol, (+)-glaberide I, (+)-dehydrovomifoliol, (−)-liliolide, (−)-hydroxydihydrobovolide, and *N*-butylbenzamide, of which compound **1** appears worthy of further evaluation as an antimalarial agent. Structure elucidation and identification were accomplished by spectroscopic means including 1D and 2D NMR analyses.

Malaria is a disease that affects nearly 40% of the world's population.[1] Following tuberculosis and AIDS,[2] this disease is one of the most onerous global health problems, responsible for 1−2 million deaths each year.[1,2] Malaria is endemic to 90 countries worldwide[3] and is caused by protozoan parasites of the genus *Plasmodium*. Despite an international campaign in the mid-20th century to control and eliminate malaria,[4−6] the incidence of infection continues to increase at a significant rate.[1,7] A major contributing factor to this increase is the growing spread of resistance to standard antimalarial drug regimens.[8,9] Although several research programs are focused on various strategies to control malaria,[7,10] drug discovery is one of the main areas of concentrated effort.[11−13]

Participation in the International Cooperative Biodiversity Group (ICBG) project has afforded an opportunity to discover antimalarial compounds from plants. The ICBG was established in part to foster collaborations between countries with technological capabilities and countries with biological diversity, to pursue the goals of drug discovery, biodiversity conservation, and economic growth of developing nations.[14] Through collaborations established with several institutes in Vietnam, Laos, and the United States, we share the common goal of finding antimalarial and anti-HIV agents from higher plants.[15] Several hundred plant samples have been collected from Vietnam and Laos, and extracts have been tested for antimalarial and cytotoxic potential using in vitro model systems. By testing these two activities, both the efficacy and selectivity of a particular extract can be assessed. Extracts are chosen for further investigation if they inhibit plasmodial growth ($IC_{50} \leq 4\ \mu g/mL$) while having no apparent toxic effect on human cancer cells in culture ($ED_{50} > 20\ \mu g/mL$).

Leaves/stems extracts of *Rhaphidophora decursiva* Schott (Araceae), a perennial, evergreen, semisucculent epiphytic vine found in the Cuc Phuong National Park (Nho Quan District, Ninh Binh Province, Vietnam), were shown to be active against both the D6 and W2 clones of *Plasmodium falciparum* with no apparent toxicity. A search of the literature revealed no prior phytochemical or pharmacological reports for this plant.

Bioassay-directed fractionation of a combined sample of the dried leaves and stems led to the isolates (+)-medioresinol, (−)-pinoresinol, (−)-syringaresinol, (+)-glaberide I, (+)-dehydrovomifoliol, (−)-liliolide, (−)-hydroxydihydrobovolide, and *N*-butylbenzamide and a very active but chemically complex minor fraction (0.49 mg from 5.03 kg of starting plant material). None of these isolates, which were tested in the antimalarial assay at a concentration of 10 μg/mL, displayed activity, while the complex minor fraction inhibited parasite growth by 50% at a concentration of 10 ng/mL. Subsequently, a larger sample (20.1 kg) of re-collected dried leaves and stems of *R. decursiva* was subjected to phytochemical separation employing comparative chromatographic methods to afford a fraction having TLC and HPLC profiles similar to the previously defined active fraction. Workup of this fraction by semipreparative HPLC resulted in the isolation of three novel and three previously identified compounds with varying degrees of antimalarial activity. Polysyphorin (**1**) is a known neolignan that is currently shown to be active as an antimalarial agent. Both rhaphidecursinol A (**2**) and B (**3**) are new neolignans with marginal activities, while rhaphidecurperoxin (**6**) is a new a 13-membered ring benzoperoxide capable of mediating significant activities. Two previously identified tetrahydrofuran lignans, which proved to be marginally active, are grandisin (**4**) and epigrandisin (**5**). The present paper describes the isolation and biological evaluation of the isolates as well as the identification of the known compounds and the structure elucidation of the novel entities obtained.

Results and Discussion

Bioassay-directed fractionation of the antimalarial active $CHCl_3$-soluble fraction from a 5.3 kg sample of leaves and stems of *R. decursiva* by gravity and reversed-phase flash column chromatography led to the isolation of eight known compounds, (+)-medioresinol,[16] (−)-pinoresinol,[16] (−)-sy-

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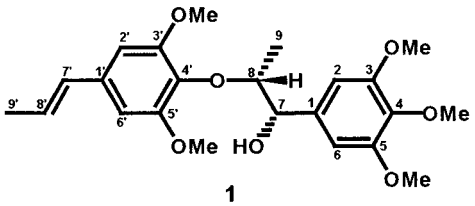

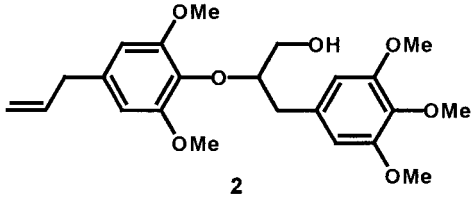

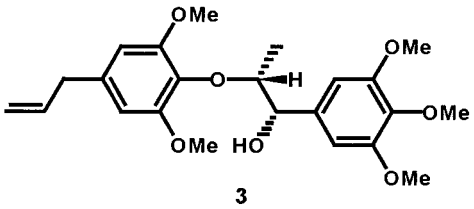

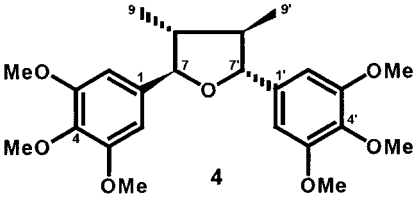

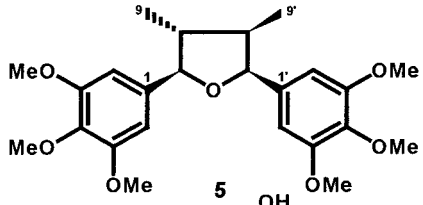

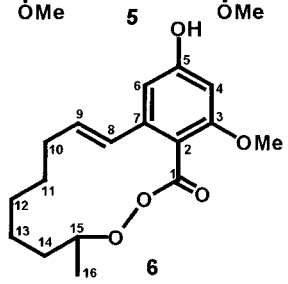

ringaresinol,[16] (+)-glaberide I,[17] (+)-dehydrovomifoliol,[18] (−)-liliolide,[19] (−)-hydroxydihydrobovolide,[20] and *N*-butylbenzamide,[21] identified by comparison of their spectroscopic properties with those reported in the literature. None of these isolates were found to be active against the protozoan parasite (IC_{50} values > 10 μg/mL). Also obtained was a very active, but chemically complex mixture, the paucity (0.49 mg) of which precluded bioassay-directed isolation ($IC_{50} \approx$ 10 ng/mL). Phytochemical separation of a larger sample (20.1 kg) of recollected dried leaves and stems of *R. decursiva* guided by TLC and HPLC profiles afforded six active isolates, polysyphorin (**1**), rhaphidecursinol A (**2**), rhaphidecursinol B (**3**), grandisin (**4**), epigrandisin (**5**), and rhaphidecurperoxin (**6**). Rhaphidecursinols A and B (**2**, **3**) are novel neolignans, and rhaphidecurperoxin (**6**) is a new benzoperoxide. Polysyphorin (**1**), a known neolignan,[22] and grandisin (**4**)[23] and epigrandisin (**5**),[24] two known tetrahydrofuran lignans, were identified by comparison of their spectroscopic properties to those found in the literature. The isolates are minor constituents, with compounds **1** and **3** being obtained with yields of less than 1 mg (5×10^{-6}% of dried plant material); the yields of isolates **2** and **4**−**6** were not more than 2×10^{-5}%.

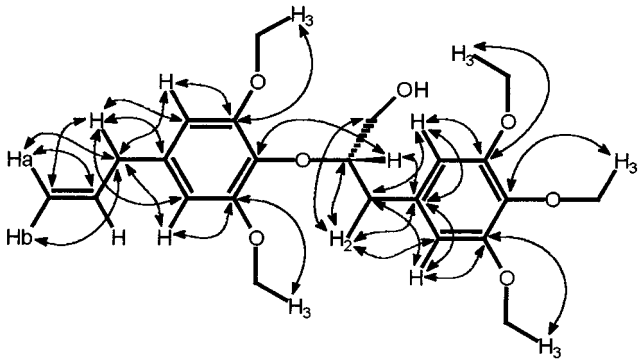

Figure 1. $^1H-^{13}C$ long-range correlations of **2** observed by HMBC (500 MHz, $CDCl_3$).

Polysyphorin (**1**), a known neolignan,[22] was shown to have a molecular formula of $C_{23}H_{30}O_7$ by FABMS ([M + Na]$^+$ *m*/*z* 441), which was consistent with ^{13}C NMR and DEPT experiments. A 1,2,3-trimethoxybenzyl group was observed, as expected, in the ^1H and ^{13}C NMR spectra [δ_H 6.56 (2H, s), 3.83 (6H, s, 2 × OMe), and 3.79 (3H, s, OMe); δ_C 153.1 (2C, s), 137.5 (s), 136.4 (s), 104.3 (2C, d), 56.1 or 56.0 (2C, q), and 60.8 (q)], and the attachment of a dioxypropyl group to the aromatic molecule was evidenced by signals at δ_H 4.57, 3.94, and 1.20 and δ_C 86.5 (d), 79.4 (d), and 17.7 (q) ppm, thus confirming the presence of a phenylpropyl derivative. The ^1H and ^{13}C NMR data of **1** also revealed the presence of a 3-dimethoxy-4-substituted benzyl group [δ_H 6.57 (2H, s), and 3.87 (6H, s, 2 × OMe); δ_C 152.8 (2C, s), 136.4 (s), 133.9 (s), 102.9 (2C, d), and 56.1 or 56.0 (2C, q)] to which is attached a propenyl group [δ_H 6.32, 6.15, and 1.87; δ_C 130.7 (d), 125.7 (d), and 18.4 (q)]. These data indicated the presence of a second phenylpropyl moiety, leading to **1** being a neolignan. The final structure of **1** was confirmed by $^1H-^1H$ COSY and HMBC data, and the configuration of the two chiral carbons (C-7 and C-8) was considered to be *threo* according to the coupling constant (J = 8.17 Hz) between H-7 and H-8.[25] Accordingly, **1** was confirmed to be *threo*-2-(4-propenyl-2,6-dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)propan-1-ol, previously isolated from *Piper polysyphorum* C. DC. A comparison of the NMR data to those published by Ma et al. confirmed its identity.[22]

Rhaphidecursinol A (**2**) has the same molecular formula as **1** ($C_{23}H_{30}O_7$) based on HRFABMS ([M + Na]$^+$ *m*/*z* 441.1899). Analysis of the ^1H and ^{13}C NMR data showed that **2** had spectral data very similar to those of **1**, indicating a close structural relationship. Compound **2** differs from **1** by a change in the location of the double bond from C-7′(8′) in **1** to C-8′(9′) in **2** [δ_H 5.93, 5.09, 5.08, 3.32; δ_C 137.0 (d), 116.2 (t), and 40.5 (t)] and by a change of the hydroxy group position at C-7 in **1** to C-9 in **2** [δ_H 4.19, 3.56, 3.44, 3.21, and 2.97; δ_C 84.2 (d), 62.4 (t), and 38.1 (t)]. The observation of long-range correlation between H-8 and C-4′ in the HMBC spectrum of **2** confirmed the connection of the two phenylpropyl moieties, which was not observed in the HMBC spectrum of **1** (Figure 1). Thus, **2** was determined to be a new neolignan, 2-(4-allyl-2,6-dimethoxyphenoxy)-3-(3,4,5-trimethoxyphenyl)propan-1-ol, and assigned the trivial name rhaphidecursinol A.

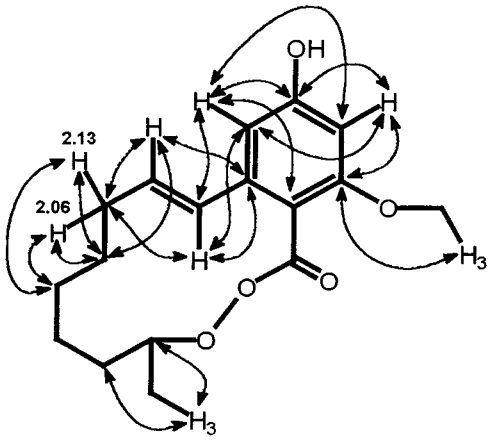

Figure 2. $^1H-^{13}C$ long-range correlations of **6** observed by HMBC (500 MHz, $CDCl_3$).

Rhaphidecursinol B (**3**) was determined to be an additional analogue of **1,** with the same molecular formula of $C_{23}H_{30}O_7$ by HRFABMS $[M]^+$ *m/z* 418.1971. The structure of **3** was found to be composed of one-half the structure of **1** and one-half the structure of **2** from the ^1H and ^{13}C NMR data. One-half of the structure of **3** showed NMR data [δ_H 6.56, 4.57, 3.92, 3.83 (6H, s, 2×OMe), 3.80 (3H, s, OMe), and 1.20; δ_C 152.7 (2C, s), 137.3 (s), 135.9 (s), 104.3 (2C, d), 86.4 (d), 79.4 (d), 56.1 or 56.0 (2C, q), 60.8 (q), and 17.7 (q)] identical to those of the 1-(3,4,5-trimethoxyphenyl)-propan-2-oxy group in **1**. The other half structure of **3** possessed indentical NMR data [δ_H 6.43, 5.95, 5.11, 5.09, 3.85 (6H, s, 2 × OMe), and 3.33; δ_C 153.1 (2C, s), 137.0 (d), 136.0 (s), 134.5 (s), 105.4 (2C, d), 116.2 (t), 56.11 or 56.0 (2C, q), and 40.5 (t)] with those of the 4-allyl-2,6-dimethoxyphenoxy group in **2**. The connection of the two substructures was determined by observation of the long-range correlation between H-8 and C-4′. Thus, **3** was determined to be another new neolignan, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(3,4,5-trimethoxyphenyl)propan-1-ol, and was assigned the trivial name rhaphidecursinol B.

Rhaphidecurperoxin (**6**) had a molecular formula of $C_{17}H_{22}O_5$ as determined by HRFABMS (*m/z* 307.1542 $[M + 1]^+$) and supported by ^{13}C NMR and DEPT spectra. The ^1H and ^{13}C NMR data of **6** revealed a 2,4,6-trisubstituted phenol group [δ_H 6.83, 6.73, and 3.64 (3H, s, OMe); δ_C 168.3 (s, C═O), 161.1 (s), 158.6 (s), 139.6 (s), 115.5 (s), 106.7 (d), 98.9 (d), and 55.8 (q, OMe)]. It also contains an *E*-double bond [δ_H 6.56, and 5.90; δ_C 134.5 (d), and 130.1 (d)], a mono-oxyethyl group [δ_H 5.27 and 1.28; δ_C 72.0 (d) and 20.3 (q)], and five non-oxymethylenes [δ_C 33.5 (t), 32.1 (t), 27.6 (t), 25.0 (t), and 21.9 (t)]. The *E*-double bond was attached to the 2,4,6-trisubstituted phenol group based on the long-range correlations in the HMBC experiment between the NMR signals of the two functional groups (Figure 2). Linkage of the mono-oxyethyl group and the five non-oxymethylenes to the *E*-double bond, forming a nine-carbon alkyl moiety, was then deduced. The unusual downfield shift of H-15 (δ_H 5.27) suggested that the oxy group on C-15 was esterified rather than a free hyroxyl group. On the basis of the molecular formula, the structure of **6** can be speculated to contain seven double bond equivalents, which suggests the presence of a relatively large ring. This ring was therefore formed between C-15 and C-1 bridged by a peroxide group. The structure of **6** was thus elucidated as 2-(5,6,7,8,9-pentahydro-4-methyl)-4-hydroxy-6-methoxybenzoperoxotridecan-1-one and assigned the trivial name rhaphidecurperoxin.

The initial extract of *R. decursiva* and all fractions and isolates were subjected to biological evaluation for antimalarial and cytotoxic activities. With each level of separation, the activity profile revealed greater antiplasmodial potency, but with a concomitant increase in toxicity. These activity values are given for the extract and isolates in Table 3. Chloroquine, artemisinin, and quinine, which served as positive controls, effectively inhibited plasmodial growth in the ng/mL range with no toxicity. The selectivity index (SI), calculated by dividing the ED_{50} value for the cancer cell line by the IC_{50} value of each of the *Plasmodium* clones, serves to estimate whether the test substance has efficacy against the parasite while retaining selectivity and, as such, gives an indication of a therapeutic window. All compounds presented in Table 3 displayed some level of antimalarial activity, with **1** and **6** being the most potent (IC_{50} values of approximately 400 and 500 ng/mL, respectively). Compound **4** and **5** are *epi*-isomers but vary significantly in activity levels (IC_{50} values of 1500 and 5000 ng/mL, respectively). Potency in this range is generally not regarded of sufficient magnitude to warrant further investigation. Although the one major structural difference among compounds **1**, **2**, and **3** is the position of the double bond, compound **1** ($\Delta^{7',8'}$) is approximately 10-fold more active than either **2** ($\Delta^{8',9'}$) or **3** ($\Delta^{8',9'}$). One possible explanation for this difference in activity is that **1** has a conjugated double bond, allowing easy formation of free radicals, which damage essential cellular components.

Compound **1** appears worthy of further evaluation in an in vivo antimalarial model. Finally, it is interesting to note that **6** contains a peroxide ester, a domain structurally similar to the endo peroxide bridge of artemisinin, which is required for antimalarial activity.[26] However, due to the poor SI value, this compound is not considered of great interest as an antimalarial agent.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer model 241 polarimeter. UV spectra were obtained with a Beckman DU-7 spectrometer. IR spectra were run on a Jasco FT/IR-410 spectrometer as a film on a KBr plate. 1D and 2D NMR spectra were recorded on a Bruker DRX-500 MHz spectrometer. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. All NMR experiments were obtained by using standard pulse sequences supplied by the vendor. Column chromatography was carried out on Si gel (200−400 mesh, Natland International Corporation). Reversed-phase flash chromatography was accomplished with RP-18 Si gel (40−63 μm, EM Science), and reversed-phase HPLC was carried out on a Waters 510 delivery system pump, equipped with a Waters 486 UV detector and a YMC packed ODS column (250 × 20 mm, 5 μm, 120 Å) or a GROM-SIL ODS column (120 Å, 5 μm, 300 × 20 mm), at a flow rate of 5 mL/min, with the UV detector set at 202 nm. Thin-layer chromatography was performed on Whatman glass-backed plates coated with 0.25 mm layers of Si gel 60. FABMS and HRFABMS spectra were recorded on a VG 7070-HF spectrometer.

Plant Material. The initial collection of leaves and stems of *R. decursiva* was made at the Cuc Phuong National Park, with voucher herbarium specimens represented by living collections in the park. Due to conservation considerations, the first recollection of leaves and stems (SVA-5005, 5.03 kg) was made outside of the park, in Hatinh, Central Vietnam, on December 3, 1999, with voucher herbarium specimens, *Soejarto et al. 11220*. At a later date, through a continuing search for this species inside the 20 000-hectare Cuc Phuong National Park, large populations were located, which made it possible to collect the required quantity of sample (SVB-5005, 20.1 kg)

Table 1. ^{1}H NMR Data of Compounds **1**−**3** (500 MHz, $CDCl_3$, *J* in Hz)

H	**1**	**2**	**3**
H-2	6.56 s	6.51 s	6.56 s
H-6	6.56 s	6.51 s	6.56 s
H-7	4.57 d (8.17)		4.57 d (8.35)
H-7a		3.21 dd (13.57, 5.39)	
H-7b		2.97 dd (13.64, 8.39)	
H-8	3.94 m	4.19 m	3.92 m
H-9	1.20 d (6.28)		1.20 d (6.27)
H-9a		3.56 brd (11.39)	
H-9b		3.44 m	
H-2′	6.57 s	6.41 s	6.43 s
H-6′	6.57 s	6.41 s	6.43 s
H-7′	6.32 dq (15.67, 1.57)	3.32 d (6.69)	3.33 brd (6.72)
H-8′	6.15 dq (15.62, 6.51)	5.93 ddt (16.91, 10.12, 6.80)	5.95 ddt (16.84, 10.05, 6.75)
H-9′	1.87 dd (6.62, 1.56)		
H-9′a		5.09 dq (17.04, 1.61)	5.11 dq (16.98, 1.69)
H-9′b		5.08 dq (10.03, 1.24)	5.09 dq (10.05, 1.26)
3,5-OMe	3.83 s	3.83 s	3.83 s
4-OMe	3.79 s	3.80 s	3.80 s
3′,5′-OMe	3.87 s	3.81 s	3.85 s

Table 2. ^{13}C NMR Data of Compounds **1**−**3** (125 MHz, $CDCl_3$)

C	**1**	**2**	**3**
C-1	136.4 s	134.2 s	135.9 s
C-2	104.3 d	106.5 d	104.3 d
C-3	153.1 s	153.0 s	152.7 s
C-4	137.5 s	136.2 s	137.3 s
C-5	153.1 s	153.0 s	152.7 s
C-6	104.3 d	106.5 d	104.3 d
C-7	79.4 d	38.1 t	79.4 d
C-8	86.5 d	84.2 d	86.4 d
C-9	17.7 q	62.4 t	17.7 q
C-1′	133.9 s	136.2 s	136.0 s
C-2′	102.9 d	105.6 d	105.4 d
C-3′	152.8 s	153.2 s	153.1 s
C-4′	136.4 s	133.6 s	134.5 s
C-5′	152.8 s	153.2 s	153.1 s
C-6′	102.9 d	105.6d	105.4 d
C-7′	130.7 d	40.5 t	40.5 t
C-8′	125.7 d	137.0 d	137.0 d
C-9′	18.4 q	116.2 t	116.2 t
3,5-OMe	56.1 q or 56.0 q	56.08 q or 56.06 q	56.1 q or 56.0 q
4-OMe	60.8 q	60.9 q	60.8 q
3′,5′-OMe	56.1 q or 56.0 q	56.08 q or 56.06 q	56.1 or 56.0 q

Table 3. Bioactivities of Compounds **1**−**6**

	KB	D6		W2	
compound	ED_{50} (μg/mL)	IC_{50} (ng/mL)	SI[a]	IC_{50} (ng/mL)	SI[a]
methanolic extract	>20	2106 ± 89	>6	6840 ± 43	>3
1	2	404 ± 65	5	368 ± 50	6
2	12	3007± 292	4	1736 ± 226	5
3	10	5372 ± 1100	1	4678 ± 1623	2
4	14	1510 ± 665	7	1485 ± 590	8
5	16	>10 000	<2	3339 ± 117	4
6	4	540 ± 50	0.7	420 ± 70	1
chloroquine	17.4	3 ± 0.8	5800	38 ± 3	460
artemisinin	>20	2 ± 0.5	>10 000	2 ± 0.5	>10 000
quinine	>20	9 ± 0.5	>2000	41 ± 12	>490

[a] SI = selectivity index = ED_{50} KB/IC_{50} *P. falciparum*.

for complete reisolation work; this quantity was pooled from samples gathered from numerous locations inside the park, with voucher herbarium specimens, *Soejarto et al. 11291*. Voucher specimens of both samples are on deposit at the herbaria of Cuc Phuong National Park, Institute of Ecology and Biological Resources (National Center for Science and Technology, Hanoi), and the Field Museum of Natural History (Chicago, IL).

Antimalarial Assays. Cultures of *P. falciparum* (chloroquine-sensitive clone D6 derived from CDC Sierra Leone and chloroquine-resistance clone W2 derived from CDC Indochina III) were maintained in human erythrocytes as described by Trager and Jensen.[27] Parasites were inoculated into type A+ human erythrocytes at a hematocrit of 6% in culture medium [RPMI-1640 (Gibco) supplemented with 32 mM $NaHCO_3$ (Gibco), 35 mM HEPES (Sigma), 7.3 μM hypoxanthine (Sigma), and 10% Albumax II (Gibco)]. Parasitemia was maintained below 10% under an atmosphere of 5% O_2, 5% CO_2, and 90% N_2 in 25 cm^2 tissue culture flasks at 37° C.

Antimalarial activity was evaluated using an in vitro radioisotope method according to the protocol of Desjardins et al.[28] Test substances, diluted from an initial concentration of 4 mg/mL, were assayed in triplicate in standard 96-well microtiter plates (Corning). Standard antimalarial compounds (chloroquine, artemisinin, and quinine) were included in each experiment and were assayed over a concentration range of 0.3−250 ng/mL. *P. falciparum* cultures were added to the microtiter plate at a parasitemia of 0.5−1% and a hematocrit of 1%. Test plates were then incubated in a sealed chamber under an atmosphere of 5% O_2, 5% CO_2, and 90% N_2 at 37 °C for 24 h. Subsequently, 0.5 μCi [^{3}H]hypoxanthine (NEN Research Products) was added to each well, and the plates were returned to the sealed chamber for an additional incubation period of 18 h. The assay was terminated by harvesting the contents of each well onto a glass fiber filter (Wallac) using a Tomtec Mach III harvestor (Wallac). The filters were dried and placed in polyethylene bags with 4 mL of scintillation fluid. Radioactivity was measured using a Wallac Micro-Beta liquid scintillation counter. Concentrations of test compounds and positive controls that inhibited parasite-specific [^{3}H]hypoxanthine incorporation by 50% (IC_{50}) were determined using nonlinear regression analyses. Zero-drug controls defined 100% incorporation.

Cytotoxicity Assays. One human cancer cell line (the oral epidermoid cancer line KB) was used to evaluate the toxicity of the fractions generated from *R. decursiva* as described previously.[29] Each culture was grown to 60−70% confluency, whereupon media were changed. The cells were used the following day for testing. Test samples, initially dissolved in DMSO to a concentration of 4 mg/mL, were diluted 10-fold with water. Serial dilutions were performed using 10% aqueous DMSO, and tests were performed in triplicate with standard 96-well microtiter plates. Cell suspensions were added to the test plates (KB: 3×10^4 cells/mL), and incubations were performed for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. The incubations were terminated with the addition of 100 μL of cold 20% aqueous trichloroacetic acid to fix the cells onto the plastic substratum. Fixed cells were stained with 0.4% sulforhodamine B (w/v) dissolved in 1% acetic acid for 30 min and then washed with 1% acetic acid. Unbuffered Tris base, pH 10 (200 μL), was added to each, and absorbance was determined at 515 nm. A zero-day control was included in all experiments by adding an equivalent number of cells to several wells of a 96-well plate and incubating 30−60 min. The cells

were fixed, stained, and processed as described above. Finally, absorption values of each treatment procedure were averaged, and the average value of the zero-day control was subtracted. These values were then expressed as a percentage relative to the solvent-treated control incubations, and ED_{50} values were calculated using nonlinear regression analysis (percent survival vs concentration).

Extraction and Isolation. The dried, milled plant material (5.03 kg) was extracted with MeOH, and the extract (280 g) was subsequently defatted with *n*-hexane and partitioned with $CHCl_3$. The $CHCl_3$-soluble fraction (20.1 g) was chromatographed over a Si gel column (400 g), which was developed by gradient elution with $CHCl_3$ and increasing concentrations of Me_2CO to afford nine fractions. Bioassay localized the antimalarial activity in fractions F-2 and F-3, which were pooled (1.35 g) and subjected to flash column chromatography on Si gel (gradient elution with $CHCl_3$ and Me_2CO) to yield the active fraction F-14 (527 mg). F-14 was subjected to flash chromatography on successive C-18 reversed-phase (RP-18) columns (elution with Me_2CO and H_2O), leading to fraction F-29. Chromatography of F-29 (216 mg) on C-18 reversed-phase (RP-18) columns (elution with Me_2CO and H_2O) followed by a series of semipreparative HPLC separations on a YMC C-18 column (solvent systems: MeOH/H_2O, 1:1; 3:2) led to the isolation of (+)-medioresinol (7.2 mg), (−)-pinoresinol (1.2 mg), (−)-syringaresinol (11.6 mg), (+)-glaberide I (1.7 mg), (+)-dehydrovomifoliol (1.3 mg), (−)-liliolide (8.9 mg), (−)-hydroxydihydrobovolide (1.7 mg), *N*-butylbenzamide (0.8 mg), and a very active, but chemically complex mixture (F87, 0.49 mg).

In an attempt to isolate the active consitutents contained in fraction F87, a second batch of dried, milled plant material (20.1 kg) was acquired and subjected to phytochemical extraction and separation. Accordingly, the plant material was extracted with MeOH, and the extract was concentrated, defatted, and partitioned with $CHCl_3$ as previously described. The $CHCl_3$-soluble fraction was subjected to a series of Si gel column, reversed-phase flash column, and preparative HPLC separations, employing F87 as the phytochemical marker to afford a fraction (BF25) displaying a similar HPLC profile. Fraction BF25 (0.15 g) was subjected to a series of semipreparative HPLC separations (YMC C18 column; elution solvent systems, MeOH−H_2O/7:3; MeCN−H_2O/6:4) to afford polysyphorin (**1**) (0.45 mg), rhaphidecursinol A (**2**) (1.84 mg), rhaphidecursinol B (**3**) (0.96 mg), grandisin (**4**) (3.90 mg), epigrandisin (**5**) (1.26 mg), and rhaphidecurperoxin (**6**) (3.21 mg).

Polysyphorin (1): $[\alpha]^{20}_D$ 0° (*c* 0.007, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.66), 268 (3.83) nm; IR ν_{max} (film) 2925.5, 2854.1, 1591.0, 1504.2, 1460.8, 1419.4, 1326.8, 1233.3, 1125.3 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; FABMS *m/z* 441 $[M + Na]^+$ (31), 413 (11), 329 (27), 207 (7), 209 (5), 216 (8), 194 (24), 176 (100), 154 (55), 136 (39), 107 (16), 69 (27), 55 (46).

Rhaphidecursinol A (2): $[\alpha]^{20}_D$ +2.61° (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (3.99) nm; IR ν_{max} (film) 2957.3, 2932.2, 2872.5, 1592.0, 1507.1, 1461.8, 1419.4, 1331.6, 1234.2, 1127.2, 1009.6 cm^{-1}. ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; FABMS *m/z* 441 $[M + Na]^+$ (30), 413 (6), 329 (31), 307 (15), 289 (7), 225 (10), 176 (100), 154 (77), 136 (59), 107 (17), 89 (16), 77 (19), 63 (10); HRFABMS *m/z* 441.1899 (calcd for $C_{23}H_{30}NaO_7$, 441.1889).

Rhaphidecursinol B (3): $[\alpha]^{20}_D$ 0° (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.01) nm; IR ν_{max} (film) 3475.1, 2935.1, 2841.6, 1590.0, 1503.2, 1459.9, 1421.3, 1327.8, 1233.3, 1126.2, 1038.5 cm^{-1}; ^1H ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Tables 1 and 2; FABMS *m/z* 418 $[M]^+$ (14), 401 (15), 307 (17), 289 (12), 224 (30), 194 (100), 154 (85), 136 (52), 107 (20), 89 (23), 77 (28); HRFABMS *m/z* 418.1971 (calcd for $C_{23}H_{30}O_7$, 418.1992).

Rhaphidecurperoxin (6): $[\alpha]^{20}_D$ +44.53 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 230 (3.62), 290 (2.71) nm; IR ν_{max} (film) 3346.9, 2926.5, 2854.1, 1719.2, 1600.6, 1457.0, 1352.8, 1260.3, 1162.9, 1084.8 cm^{-1}; ^1H (pyridine-d_5, 500 MHz) δ 12.0 (1H, brs, COO), 6.83 (1H, d, J = 2.01 Hz, H-6), 6.73 (1H, d, J = 2.05 Hz, H-4), 6.56 (1H, d, J = 15.78 Hz, H-8), 5.90 (1H, dt, J = 15.71, 7.59 Hz, H-9), 5.27 (1H, m, H-15), 3.64 (3H, s, OMe), 2.13 (1H, m, H-10a), 2.06 (1H, m, H-10b), 1.81 (1H, m, H-14a), 1.54 (2H, m, H_2-13), 1.46 (2H, m, H-11a and H-12a), 1.35 (1H, m, H-14b), 1.33 (1H, m, H-11b), 1.31 (1H, m, H-12b), 1.28 (3H, d, J = 6.29 Hz, Me-16); ^{13}C NMR (pyridine-d_5, 125 MHz) δ 168.3 (s, C-1), 161.1 (s, C-5), 158.6 (s, C-3), 139.6 (s, C-7), 134.5 (d, C-9), 130.1 (d, C-8), 115.5 (s, C-2), 106.7 (d, C-6), 98.9 (d, C-4), 72.0 (d, C-15), 55.8 (q, OMe), 33.5 (t, C-14), 32.1 (t, C-10), 27.6 (t, C-12), 25.0 (t, C-11), 21.9 (t, C-13), 20.3 (q, Me-16); FABMS *m/z* 329 $[M + Na + 1]^+$ (100), 307 $[M + 1]^+$ (87), 289 (64), 177 (33), 155 (27), 138 (78), 120 (21), 105 (36), 91 (70), 65 (81); HRFABMS *m/z* 307.1542 $[M + 1]^+$ (calcd for $C_{17}H_{23}O_5$, 307.1546).

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