New Dammarane and Malabaricane Triterpenes from *Caloncoba echinata*

Hanne L. Ziegler,[†] Dan Stærk,[†] Jette Christensen,[†] Carl Erik Olsen,[‡] Archibald A. Sittie,[§] and Jerzy W. Jaroszewski*,[†]

Department of Medicinal Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark, Department of Chemistry, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg, Denmark, and Centre for Scientific Research into Plant Medicine, P.O. Box 73, Mampong-Akwapim, Ghana

Received April 19, 2002

Three novel triterpenes, (11*R*,20*R*)-11,20-dihydroxy-24-dammaren-3-one (**1**), (17*S*,20*R*,24*R*)-17,25-dihydroxy-20,24-epoxy-14(18)-malabaricen-3-one (**2**), and (17*R*,20*S*,24*R*)-17,25-dihydroxy-20,24-epoxy-14(18)-malabaricen-3-one (**3**), were isolated from leaves of *Caloncoba echinata*. The structures were established using mainly 800 MHz NOESY and HMBC connectivities. The absolute stereochemistry of C-11 in **1** and that of C-17 in **2** were established by the Mosher method. The stereochemistry of the side chains of the malabaricanes is compatible with their biosynthesis by a cascade opening of diepoxides. The isolated triterpenes inhibited growth of *Plasmodium falciparum* parasites in vitro apparently via incorporation into erythrocyte membrane, as suggested by transformation of erythrocytes into stomatocytes at a concentration level at which the growth inhibition was observed.

Caloncoba Gilg is a small genus of Flacourtiaceae[1,2] (Kiggelariaceae[3]) represented in tropical Africa. The genus is scarcely known from the chemical point of view. *Caloncoba* belongs to the cyanide-producing section of Flacourtiaceae.[4] *Caloncoba echinata* (Oliv.) Gilg is indigenous to West Africa and is a source of gorli oil, which contains cyclopentanoid fatty acids and has been used for the treatment of leprosy similarly as chalmoogra oil.[5,6] Cyclopentanoid amino acids[7] and friedelane-type triterpenes[8−10] have been isolated from *Caloncoba* species.

In our investigation directed toward identification of novel antiplasmodial natural products from plants, extracts of *C. echinata* leaves were found to cause almost complete inhibition of growth of *Plasmodium falciparum* parasites in vitro at 12 μg/mL. Bioactivity-guided fractionation of the extract afforded three new triterpenes described below.

Results and Discussion

Diethyl ether extract of *C. echinata* leaves was partitioned between light petroleum and methanol containing 10% of water. Only the methanol fraction showed antiplasmodial activity. This fraction was subjected to vacuum liquid chromatography (VLC) followed by normal-phase preparative HPLC, to afford three compounds.

The FAB mass spectrum (positive mode) of the first compound (**1**) showed a peak at *m*/*z* 459 compatible with $C_{30}H_{50}O_3$. ^{13}C NMR spectra revealed 30 carbon resonances: eight tertiary methyl groups, nine methylene groups, and six methine groups, including one oxygenated (δ 71.20) and one olefinic (δ 124.50) methine carbon, as well as seven quaternary carbon resonances, including one olefinic (δ 131.89) and one oxygenated (δ 75.67) quaternary carbon, and one carbonyl group (δ 218.72). The ^{1}H NMR coupling pattern of the olefinic hydrogen present in the spectrum (triple septet, δ 5.13) demonstrated that the double bond was substituted with one methylene group and two geminal methyl groups. These features indicated a tetracyclic triterpene oxygenated at C-20 and having a double bond between C-24 and C-25. The keto group was placed at C-3 on the basis of an isolated $-CH_2CH_2-$ spin system observed in an 800 MHz COSY spectrum and HMBC connectivities from C-3 to H-1, H-2, H-28, and H-29.

The oxygenated methine hydrogen (δ 3.99) showed couplings to another methine group (δ 1.55) and to a methylene group (δ 1.42 and 2.23). The latter methine group did not show any further COSY connectivities, whereas the methylene group was coupled to yet another methine group (at δ 1.88). HSQC and HMBC connectivities showed that this $-CH-CH(OH)-CH_2-CH-$ spin system corresponded to the C-9−C-11−C-12−C-13 fragment of a dammarane-type triterpenoid. In particular, the methine carbon C-9 (δ 54.69) bearing the hydrogen at δ 1.55 (HSQC) showed HMBC connectivities to two methyl groups mutually correlated in an 800 MHz NOESY spectrum, which were identified as the 1,3-diaxial methyl groups attached to C-8 and C-10. The vicinal couplings observed for H-11 (two diaxial couplings, J = 10.7 Hz, and one axial−equatorial coupling, J = 4.9 Hz) demonstrated that the 11-hydroxy group was equatorial.

Use of the generalized Mosher method[11−13] allowed the determination of the absolute configuration of C-11. Thus, epimeric (*S*)- and (*R*)-methoxy(trifluoromethyl)phenylacetates (MTPA esters) of **1** esterified at the 11α-hydroxy group were prepared, and the chemical shift differences $\Delta\delta = \delta_S - \delta_R$ were determined for all hydrogens that could be assigned using NOESY experiments. For H-12eq, H-12ax, H-13, H-18, and H-22 the positive $\Delta\delta$ values were observed, whereas the $\Delta\delta$ values for H-19, H-28, H-29, and H-30 were negative (see Experimental Section). Therefore, the absolute configuration at C-11 is *R*.[11−13]

The configuration of C-20 in 20-hydroxydammaranes can be derived from ^{13}C NMR chemical shifts of the surrounding carbons, in particular C-21 and C-22.[14] In the 20*S*-series, the resonance of C-21 has a higher δ value and that of C-22 has a lower δ value than in the 20*R*-series.[14] A compound identified as the 20*S* epimer of **1** was described by Tofern et al.[15] The ^{13}C NMR chemical shift differences between the latter and **1** were less than 0.3 ppm (in most cases much less) for all carbons, except for C-16, C-17, and C-20 to C-23, the differences for C-21 and C-22 ($\Delta\delta = \delta_S - \delta_R$ 2.3 and −1.7 ppm, respectively) being the largest and

* To whom correspondence should be addressed. Tel: (45) 35306372. Fax: (45) 35306040. E-mail: jj@dfh.dk.
[†] Royal Danish School of Pharmacy.
[‡] Royal Veterinary and Agricultural University.
[§] Centre for Scientific Research into Plant Medicine.

10.1021/np0201826 CCC: $22.00
Published on Web 10/11/2002

Table 1. 800 MHz ^{1}H NMR Spectral Data for Compounds **1**−**3** ($CDCl_3$)[a]

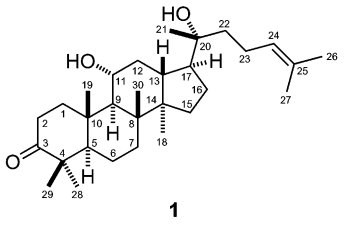
hydrogen	**1**	**2**	**3**[b]
H-1	eq: 2.67 (ddd, $J_{1eq,1ax}$ = 13.9, $J_{1eq,2ax}$ = 8.3, $J_{1eq,2eq}$ = 5.6) ax: 1.69 (m)	eq: 1.78 (ddd, $J_{1eq,1ax}$ = 13.4, $J_{1eq,2ax}$ = 7.6, $J_{1eq,2eq}$ = 3.5) ax: 1.51 (m)	eq: 1.78 (ddd, $J_{1eq,1ax}$ = 13.3, $J_{1eq,2ax}$ = 7.6, $J_{1eq,2eq}$ = 3.5) ax: NA
H-2	eq: 2.49 (ddd, $J_{2eq,2ax}$ = 15.3, $J_{2eq,1ax}$ = 8.9, $J_{2eq,1eq}$ = 5.6) ax: 2.41 (ddd, $J_{2ax,2eq}$ = 15.3, $J_{2ax,1ax}$ = 8.3, $J_{1ax,2eq}$ = 8.9)	eq: 2.39 (ddd, $J_{2eq,2ax}$ = 16.1, $J_{2eq,1ax}$ = 7.3, $J_{2eq,1eq}$ = 3.5) ax: 2.55 (ddd, $J_{2ax,2eq}$ = 16.1, $J_{2ax,1ax}$ = 10.9, $J_{2ax,1eq}$ = 7.6)	eq: 2.39 (ddd, $J_{2eq,2ax}$ = 16.1, $J_{2eq,1ax}$ = 7.3, $J_{2eq,1eq}$ = 3.5) ax: 2.56 (ddd, $J_{2ax,2eq}$ = 16.1, $J_{2ax,1ax}$ = 10.9, $J_{2ax,1eq}$ = 7.6)
H-5	1.52 (m)	1.26 (m)	NA
H-6	eq: 1.52 (m) ax: 1.47 (m)	1.53 (m)	NA
H-7	eq: 1.55 (m) ax: 1.27 (m)	eq: 1.63 (m) ax: 1.15 (m)	NA
H-9	1.55 (m)	1.40 (dd, $J_{9,11A}$ = 7.7, $J_{9,11B}$ = 12.7)	NA
H-11	3.99 (ddd, $J_{11,12ax}$ = $J_{11,9}$ = 10.7, $J_{11,12eq}$ = 4.9)	1.51 (m)	NA
H-12	eq: 2.23 (ddd, $J_{12eq,13}$ = 3.9, $J_{12eq,11}$ = 4.9, $J_{12eq,12ax}$ = 12.2) ax: 1.42 (m)	α: 1.59 (m) β: 2.01 (m)	NA
H-13	1.88 (m)	2.18 (m)	NA
H-15	α: 1.10 (m) β: 1.40 (m)	A: 2.13 (m) B: 2.18 (m)	NA
H-16	α: 1.35 (m) β: 1.79 (m)	A: 1.43 (m) B: 1.49 (m)	NA
H-17	1.79 (m)	3.52 (dd, $J_{17,16A}$ = 10.2, $J_{17,16B}$ = 2.0)	3.57 (dd, $J_{17,16A}$ = 10.2, $J_{17,16B}$ = 2.2)
H-18	0.93 (d, $J_{18,15\beta}$ = 1)	A: 4.62 (br s)[c] B: 4.91 (q, $J_{18,15A}$ = $J_{18,15B}$ = $J_{18,13}$ = 1.2)[c]	A: 4.62 (br s)[c] B: 4.91 (q, $J_{18,15A}$ = $J_{18,15B}$ = $J_{18,13}$ = 1.2)[c]
H-19	1.08 (br s)	0.98 (s)	0.98 (s)
H-21	1.14 (s)	1.14 (s)	1.15[e]
H-22	1.48 (m)	A: 1.56 (m) B: 2.10 (m)	NA
H-23	2.07 (m)	1.87 (m)	NA
H-24	5.13 (tsp, $J_{24,23}$ = 7.2, $J_{24,26}$ = $J_{24,27}$ = 1.4)	3.77 (dd, $J_{24,23A}$ = 5.4, $J_{24,23B}$ = 10.6)	3.83 (t, $J_{24,23A}$ = $J_{24,23B}$ = 7.4)
H-26	1.69 (br s)	1.13 (s)[d]	1.17[e]
H-27	1.63 (br s)	1.22 (s)[d]	1.27[e]
H-28	1.10 (s)	1.07 (s)	1.07 (s)
H-29	1.07 (s)	1.04 (s)	1.04 (s)
H-30	1.02 (s)	1.03 (s)	1.02 (s)

[a] Multiplicity of signals is given in parentheses: s, singlet; d, doublet; t, triplet; sp, septet; m, multiplet; br, broad; coupling constants (apparent splittings) are reported as numerical values in Hz; eq and ax designate equatorial and axial hydrogen atom, α and β hydrogen below and above the ring plane, respectively, and A and B designate magnetically nonequivalent geminal hydrogens; the assignments for **1** and **2** are based on 800 MHz COSY and NOESY spectra. [b] From a 400 MHz ^{1}H NMR spectrum; NA: not assigned. [c] A and B designate H-18 protons trans and cis to C-15, respectively. [d] Non-stereospecific assignment; the geminal methyl groups exhibit equally intense NOEs to H-24. [e] These assignments may be interchanged.

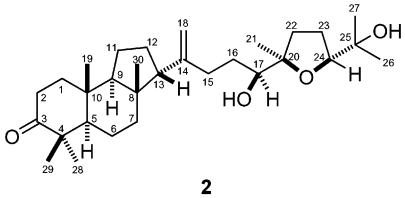
in agreement with the rule[14] mentioned above. Consistent chemical shifts were reported for other 20*S*-hydroxydammaranes.[16−19] Thus, **1** has the *R*-configuration at C-20. The ^{1}H and ^{13}C NMR data are shown in Tables 1 and 2. A list of 2D NMR connectivities is included as Supporting Information.

FAB mass spectra of the two other compounds (**2** and **3**) showed ions at *m*/*z* 475, indicating the molecular formula $C_{30}H_{50}O_4$. The base-peak observed for each compound (*m*/*z* 457) corresponded to loss of a water molecule from the molecular ion. The malabaricane[20] structure of the carbon skeleton of **2** was established by analysis of 800 MHz HMBC and NOESY spectra (see Tables 1 and 2 and Supporting Information). NOE interactions were observed between H-19 and the 1,3-diaxially juxtaposed H-29 and H-30, between H-28 and H-29, and between H-28 and H-5. This confirmed the relative stereochemistry of rings A−C and excluded the isomalabaricane skeleton.[21,22] Compound **3** exhibited ^{13}C NMR signals remarkably similar ($\Delta\delta$ < 0.05) to those of the tricyclic system of **2** (Table 2). This indicated that **2** and **3** differ only in the stereochemistry of the side chain.

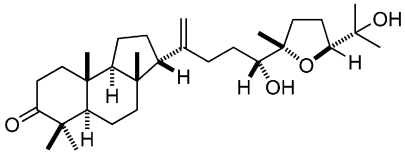
Analysis of 800 MHz COSY and HMBC spectra of **2** disclosed the presence of two hydroxy groups and a tetrahydrofuran ring in the malabaricane side chain. The



1



2



3

presence of a tertiary alcohol is in agreement with the easy loss of water in FABMS. The oxygenation pattern present

Table 2. 100 MHz ^{13}C NMR Spectral Data for Compounds **1**−**3** ($CDCl_3$)[a]

carbon	1	2	3
C-1	41.99	39.14	39.12
C-2	34.20	34.17	34.17
C-3	218.72	217.71	217.74
C-4	47.73	47.47	47.47
C-5	55.28	55.32	55.31
C-6	19.64	20.34	20.34
C-7	35.14	35.75	35.76
C-8	40.62	45.14	45.14
C-9	54.69	54.67	54.65
C-10	38.21	36.22	36.22
C-11	71.20	20.78	20.77
C-12	39.83	27.55	27.51
C-13	40.89	55.75	55.80
C-14	49.72	154.19	154.20
C-15	30.66	36.08	36.00
C-16	25.46	30.07	30.63
C-17	49.01	75.91	76.36
C-18	16.12	109.57	109.64
C-19	16.79	15.10	15.09
C-20	75.67	86.18	86.41
C-21	23.42	23.97	23.62
C-22	41.92	31.23	31.35
C-23	22.27	26.61	27.00
C-24	124.50	87.76	84.32
C-25	131.89	70.49	72.08
C-26	25.75	23.97[b]	25.42[b]
C-27	17.76	27.74[b]	27.51[b]
C-28	27.48	26.61	26.61
C-29	20.77	21.09	21.08
C-30	16.32	24.21	24.19

[a] The assignments for **1** and **2** are based on 800 MHz HSQC and HMBC experiments. [b] Non-stereospecific assignment of the geminal methyl groups.

can result from a cascade opening[23−27] of two epoxide rings. This partial structure has been found in several other natural products, including quassiols,[28−30] squalene-derived polyethers from red algae,[31−35] and terrestrial terpenoids.[36−38] There are four possible stereoisomeric diepoxides derived from 14(18),17(20)*E*,24-malabaricatriene that can serve as precursors for the skeleton present in **2** and **3** (17*R*,20*R*,24*R*, 17*S*,20*S*,24*S*, 17*S*,20*S*,24*R*, and 17*R*,20*R*,-24*S*). Regardless whether the cascade reaction involves a nucleophilic attack at C-17 or C-25 of any of the diepoxides, the only possible tetrahydrofurans that can be formed are the cis stereoisomers with the relative configuration 17*R**,-20*S**,24*R** and the trans stereoisomers with the relative configuration 17*S**,20*R**,24*R** (the 17*R**,20*R**,24*R** diastereomers cannot result from a concerted cascade reaction). Two previously described algal polyethers, thyrsiferol and venustatriol, have side chains with stereochemistry corresponding to 17*S*,20*R*,24*R* and 17*R*,20*S*,24*R*, respectively, as unequivocally established by X-ray crystallography[31,33] and enantioselective syntheses.[39−41] The ^{13}C chemical shifts of C-16, C-17, and C-20 to C-27 in **2** (Table 2) were closely similar to those of the corresponding carbons in thyrsiferol[42] and epidehydrothyrsiferol,[34] showing that **2** has the 17*S**,20*R**,24*R** configuration. This relative stereochemistry was confirmed by a NOE between H-17 and H-24 (800 MHz, 700 ms NOESY spectrum), demonstrating that these hydrogens are on the same side of the ring. Further evidence was provided by NOEs to the diastereotopic hydrogens attached to C-22. Thus, unlike the two hydrogens attached to C-23, which had closely similar chemical shifts, the two H-22 hydrogens were well resolved, with chemical shifts of δ 1.56 and 2.10 (Table 1), as evident from HSQC and NOESY experiments. The high-field H-22 hydrogen showed a distinct NOE to H-21, whereas the low-field H-22 hydrogen gave a NOE to H-24. Thus, the relative stereochemistry of the tetrahydrofuran ring of **2** is proved. On the other hand, the respective ^{13}C chemical shifts of **3** matched very well those in quassiol B[29] and kuhistanol,[38] showing the 17*R**,20*S**,24*R** configuration of **3**.[30] Moreover, the ^{1}H NMR spectra of **2** and **3** matched very well those of thyrsiferol, venustatriol, and model compounds.[40] Hashimoto et al. have found[40] that in the isomers with the two largest substituents of the tetrahydrofuran ring in the trans orientation a hydrogen corresponding to H-24 appeared as a doublet (J = 5.3 and 10.2 Hz), whereas a triplet (J = 7.3 Hz) was observed in the cis diastereomers.[40] This was indeed also observed in the spectra of **2** and **3** (Table 1). Finally, the absolute configuration of C-17 in **2** was established by the Mosher method. The observed $\Delta\delta$ values were positive for H-21 and H-24 and negative for both H-18 hydrogens, H-15 and H-16. The absolute configuration of C-17 in **2** is thus *S*, and the side chain stereochemistry is 17*S*,20*R*,24*R*. The Mosher derivatives of **3** could not be obtained because of scarcity of the material. However, under the assumption that **3** originated from the same biosynthetic precursor as **2**, its absolute configuration can be tentatively formulated as 17*R*,20*S*,24*R*.

The compounds **2** and **3** are novel representatives of a rather small group of triterpenes with the malabaricane skeleton. Although rare, the malabaricanes appear to be broadly distributed, being found in many flowering plant families including Anacardiaceae,[43] Compositae,[44] Leguminosae,[37] Simaroubaceae,[20] and now Flacourtiaceae, as well as in ferns (Polypodiaceae[45,46]). Isomalabaricanes, on the other hand, appear to be restricted to marine organisms.

The remaining issue is the antiplasmodial activity of **1**−**3**. As recently found, certain natural products inhibit growth of *Plasmodium* parasites in vitro apparently not via action on the parasites themselves as required for a potential antimalarial drug but via their incorporation into erythrocyte membrane.[47,48] Incorporation of chemicals into the erythrocyte membrane causes stomatocytic or echinocytic changes of cell curvature, which can be observed microscopically. If such erythrocyte cell shape changes take place at the same concentration as the inhibition of *Plasmodium* parasites growth, the observed antiplasmodial activity may be a trivial indirect effect.[47,48] It was, therefore, of interest to investigate whether **1**−**3** cause erythrocyte membrane changes. In fact, *C. echinata* fractions containing **1**−**3** caused profound transformation of erythrocytes into stomatocytes at concentrations where the *Plasmodium falciparum* growth-inhibitory activity was observed (IC_{50} < 12 μg/mL). Because stomatocytic transformation of erythrocytes was earlier linked to indirect antiplasmodial effects,[47] studies of antiplasmodial activity of these compounds were discontinued.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at 25 °C with a Bruker AMX 400 or a Varian Unity Inova 800 spectrometer (proton frequency 400.13 and 799.809 MHz, respectively) in $CDCl_3$, using TMS as internal reference. NOESY spectra were obtained with mixing times of 700 ms. HMBC experiments were optimized for $^{n}J_{C,H}$ = 5 Hz. Mass spectra were obtained on a JEOL JMS-AX505W double-focusing mass spectrometer operating in EI or FAB mode. Preparative HPLC separations were performed on a 25 × 1.6 cm i.d. column packed with Lichrosorb Si-60, 7 μm, eluted with 95:5 heptane−ethanol, 6 mL/min, with spectrophotometric detection at 205 nm. Column chromatography (CC) was performed using Matrex silica gel 60A, 35−70 μm

(Millipore). Merck silica gel 60 H was used for VLC separations. Merck silica gel 60 F_{254} plates were used for thin-layer chromatography (TLC), the spots being visualized by spraying with 7% phosphomolybdic acid hydrate in EtOH, followed by heating.

Plant Material. Leaves of *Caloncoba echinata* (Oliv.) Gilg were collected in Atewa Range Forest Reserve, Ghana, in October 1999. Mr. Patrick Ekpe, Department of Botany, University of Ghana, authenticated the material. Voucher specimens were deposited in Herbarium GC (Ghana Herbarium, Department of Botany, University of Ghana, Legon; voucher number GC47674) and in Herbarium C (Botanical Museum, University of Copenhagen, Copenhagen, voucher number DFHJJ26).

Extraction and Isolation. Finely ground leaves of *Caloncoba echinata* (136 g) were extracted for 4 h in a Soxhlet apparatus using 1.5 L of diethyl ether (12 cycles). The extract was evaporated to dryness, and the residue (8.2 g) was distributed between methanol−water (9:1) and light petroleum (bp 40−65 °C), to give 2.0 g of residue from the methanol−water and 6.0 g from the petroleum ether fraction. The antiplasmodial activity was associated with the former fraction (IC_{50} < 12 μg/mL). This material was subjected to VLC on a 5 × 7 cm i.d. column eluted with 5 × 50 mL of CH_2Cl_2, 5 × 50 mL of CH_2Cl_2 containing 3% of MeOH, 10 × 50 mL of CH_2Cl_2 containing 5% of MeOH, and 5 × 50 mL of CH_2Cl_2 containing 10% of MeOH, collecting 50 mL fractions. Fractions 10−13 (0.72 g) had IC_{50} < 12 μg/mL. After treating with charcoal in CH_2Cl_2, the fraction was repeatedly chromatographed by normal-phase HPLC to give 10 mg (0.007%) of **1**, 10 mg (0.007%) of **2**, and 4 mg (0.003%) of **3**, each as a colorless gum.

(11*R*,20*R*)-11,20-Dihydroxy-24-dammaren-3-one (1): $[\alpha]_D^{25}$ +47° (*c* 0.46, $CHCl_3$); HREIMS *m*/*z* 440.3630 (100%, $[M - H_2O]^+$), $[C_{30}H_{50}O_3 - H_2O]^+$ requires 440.3649; FABMS *m*/*z* 459 (45%, $[MH]^+$), 441 (36%, $[(M - H_2O) + H]^+$), 423 (100%, $[(M - 2H_2O) + H]^+$).

(17*S*,20*R*,24*R*)-17,25-Dihydroxy-20,24-epoxy-14(18)-malabaricen-3-one (2): $[\alpha]_D^{25}$ +24° (*c* 0.49, $CHCl_3$); HREIMS *m*/*z* 456.3575 (100%, $[M - H_2O]^+$), $[C_{30}H_{50}O_4 - H_2O]^+$ requires 456.3598; FAB MS *m*/*z* 475 (20%, $[MH]^+$), 457 (100%, $[(M - H_2O) + H]^+$).

(17*R*,20*S*,24*R*)-17,25-Dihydroxy-20,24-epoxy-14(18)-malabaricen-3-one (3): HRFABMS *m*/*z* 475.3842 (35%, MH^+), $[C_{30}H_{50}O_4 + H]^+$ requires 475.3782, 457 (100%, $[(M - H_2O) + H]^+$). Optical rotation was not determined due to the small amount of material available.

Determination of the Absolute Configuration of 1 by Mosher's Method. A sample of **1** (3.4 mg, 0.007 mmol) was treated with (*S*)-methoxy(trifluoromethyl)phenylacetyl chloride (13 μL, 0.007 mmol) in 50 μL of pyridine for 24 h under nitrogen at room temperature, and the resulting *R*-ester was purified by CC (6 × 0.9 cm i.d. silica gel column eluted with CH_2Cl_2). The *S*-ester was obtained similarly from (*R*)-methoxy-(trifluoromethyl)phenylacetyl chloride. The observed δ values (400 MHz ^{1}H NMR, $CDCl_3$) were as follows for the *S*- and the *R*-ester, respectively: H-11, 5.309 and 5.340; H-12eq, 2.459 and 2.392; H-12ax, 1.475 and 1.381; H-13, 1.933 and 1.898; H-18, 0.936 and 0.922; H-19, 0.629 and 0.844; H-22, 1.470 and 1.459; H-24, 5.104 and 5.117; H-28, 0.997 and 1.043; H-29, 0.926 and 1.002; H-30, 1.052 and 1.071. Differences for the remaining, assigned hydrogens were less than 0.01 ppm.

Determination of the Absolute Configuration of 2 by Mosher's Method. A sample of **2** (4.2 mg, 0.009 mmol) was dissolved in 50 μL of pyridine, 8 μL (0.043 mmol) of (*R*)-methoxy(trifluoromethyl)phenylacetyl chloride was added under nitrogen, and the mixture was allowed to stand for 24 h at room temperature. The resulting *S*-ester was purified by CC (8 × 0.9 cm i.d. silica gel column eluted with CH_2Cl_2 followed by CH_2Cl_2−EtOAc, 19:1). The *R*-ester was obtained similarly from 5 mg (0.011 mmol) of **2** and 9 μL (0.048 mmol) of (*S*)-methoxy(trifluoromethyl)phenylacetyl chloride. The observed δ values (400 MHz ^{1}H NMR, $CDCl_3$) for unequivocally assigned hydrogens were as follows for the *S*- and the *R*-ester, respectively: H-16, 1.804 and 1.841; H-18A, 4.620 and 4.642; H-18B, 4.909 and 4.928; H-21, 1.173 and 1.156; H-24, 3.667 and 3.646. Differences for the remaining, assigned hydrogens were less than 0.01 ppm.

Assay for Antiplasmodial Activity. The assay was performed using chloroquine-sensitive *Plasmodium falciparum* 3D7 strain as previously described.[47,49]

Erythrocyte Membrane Transformations. Concentrates containing **1**−**3** (0.625−100 μg/mL) were incubated with non-parasitized erythrocytes for 48 h exactly as in the assay for antiplasmodial activity.[47] After the incubation period, 20 μL samples were spread on microscope slides, allowed to dry, fixed with methanol, stained with Giemsa, and examined microscopically. The results showed changes of the erythrocytes toward the stomatocytic form. In the concentration range of 25−100 μg/mL lysis was seen and spherostomatocytes as well as stomatocytes of type S2 and S1[50] were observed. Ghost cells were observed at 50−100 μg/mL. At 12.5 μg/mL there was no sign of lysis, the dominating cell shape being S1. At the concentration 6.25 μg/mL normal discocytes dominated, but the cells were slightly perturbed and more of S1 and S2 type stomatocytes were present than in the control sample. The cells treated with 0.625 μg/mL were identical with control cells.

Acknowledgment. We thank Mr. Partick Ekpe and Mr. Daniel K. Abbiw (Department of Botany, University of Ghana, Legon, Ghana) for the plant material used in this work, and Ms. Dorte Brix and Ms. Heidi L. Døring (Department of Medicinal Chemistry, Royal Danish School of Pharmacy) for technical assistance. The 800 MHz ^{1}H NMR spectra were obtained using the Varian Unity Inova spectrometer of the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules.

Supporting Information Available: 2D NMR connectivities (800 MHz COSY, NOESY, HMBC) for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Miller, R. B. *J. Arnold Arbor*. **1975**, *56*, 20−102.
(2) Lemke, D. E. *Aliso* **1988**, *12*, 29−43.
(3) Bernhard, A.; Endress, P. K. *Plant Syst. Evol*. **1999**, *215*, 141−155.
(4) Spencer, K. C.; Seigler, D. S. *Biochem. Syst. Ecol*. **1985**, *13*, 421−431.
(5) Cole, H. I.; Cardoso, H. T. *J. Am. Chem. Soc*. **1938**, *60*, 617−619.
(6) Mangold, H. K.; Lundberg, W. O. *J. Oil Techn. Assoc. India* **1980**, 108−113.
(7) Clausen, V.; Frydenvang, K.; Koopmann, R.; Jørgensen, L. B.; Abbiw, D. K.; Ekpe, P.; Jaroszewski, J. W. *J. Nat. Prod*. **2002**, *65*, 542−547.
(8) Tchuendem, M. H. K.; Ayafor, J. F.; Connolly, J. D. *Nat. Prod. Lett*. **1996**, *9*, 27−32.
(9) Giner, R. M.; Gray, A. I.; Gibbons, S.; Waterman, P. G. *Phytochemistry* **1993**, *33*, 237−239.
(10) Giner-Pons, R. M.; Gray, A. I.; Lavaud, C.; Massiot, G.; Gibbons, S.; Waterman, P. *Phytochemistry* **1991**, *31*, 223−225.
(11) Ohtani, I.; Kusumi, T.; Ishitsuka, M. O.; Kakisawa, H. *Tetrahedron Lett*. **1989**, *30*, 3147−3150.
(12) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc*. **1991**, *113*, 4092−4096.
(13) Kusumi, T.; Ohtani, I. I. In *The Biology-Chemistry Interface*; Cooper, R., Snyder, J. K., Eds.; Dekker: New York, 1999; pp 103−137.
(14) Asakawa, J.; Kasai, R.; Yamasaki, K.; Tanaka, O. *Tetrahedron* **1977**, *33*, 1935−1939.
(15) Tofern, B.; Jenett-Siems, K.; Siems, K.; Jakupovic, J.; Eich, E. *Z. Naturforsch*. **1999**, *54c*, 1005−1010.
(16) Tori, M.; Matsuda, R.; Sono, M.; Asakawa, Y. *Magn. Reson. Chem*. **1988**, *26*, 581−590.
(17) Bianchini, J.-P.; Gaydou, E. M.; Rafaralahitsimba, G.; Waegell, B.; Zahra, J.-P. *Phytochemistry* **1988**, *27*, 2301−2304.
(18) Pokhilo, N. D.; Denisenko, V. A.; Uvarova, N. I. *Khim. Prirod. Soedin*. **1991**, 145−146.
(19) Fuchino, H.; Satoh, T.; Yokochi, M.; Tanaka, N. *Chem. Pharm. Bull*. **1998**, *46*, 169−170.
(20) Chawla, A.; Dev, S. *Tetrahedron Lett*. **1967**, 4837−4843.
(21) McCabe, T.; Clardy, J.; Minale, L.; Pizza, C.; Zollo, F.; Riccio, R. *Tetrahedron Lett*. **1982**, *23*, 3307−3310.
(22) Meragelman, K. M.; McKee, T. C.; Boyd, M. R. *J. Nat. Prod*. **2001**, *64*, 389−392.
(23) Cane, D. E.; Celmer, W. D.; Westley, J. W. *J. Am. Chem. Soc*. **1983**, *105*, 3594−3600.
(24) Dolle, R. E.; Nicolaou, K. C. *J. Am. Chem. Soc*. **1985**, *107*, 1691−1694.
(25) Still, W. C.; Romero, A. G. *J. Am. Chem. Soc*. **1986**, *108*, 2105−2106.
(26) Schreiber, S. L.; Sammakia, T.; Hulin, B.; Schulte, G. *J. Am. Chem. Soc*. **1986**, *108*, 2106−2108.

(27) Taber, D. F.; Bhamidipati, R. S.; Thomas, M. L. *J. Org. Chem*. **1994**, *59*, 3442−3444.
(28) Tinto, W. F.; McLean, S.; Reynolds, W. F.; Carter, C. A. G. *Tetrahedron Lett*. **1993**, *34*, 1705−1708.
(29) Miller, S. L.; Tinto, W. F.; McLean, S.; Reynolds, W. F.; Yu, M.; Carter, C. A. G. *Tetrahedron* **1995**, *51*, 11959−11966.
(30) Kodama, M.; Yoshio, S.; Tabata, T.; Deguchi, Y.; Sekiya, Y.; Fukuyama, Y. *Tetrahedron Lett*. **1997**, *38*, 4627−4630.
(31) Blunt, J. W.; Hartshorn, M. P.; McLennan, T. J.; Munro, M. H. G.; Robinson, W. T.; Yorke, S. C. *Tetrahedron Lett*. **1978**, 69−72.
(32) Gonzalez, A. G.; Arteaga, J. M.; Fernandez, J. J.; Martin, J. D.; Norte, M.; Ruano, J. Z. *Tetrahedron* **1984**, *40*, 2751−2755.
(33) Sakemi, S.; Higa, T.; Jeford, C. W.; Bernardinelli, G. *Tetrahedron Lett*. **1986**, *27*, 4287−4290.
(34) Norte, M.; Fernandez, J. J.; Souto, M. L.; Garcia-Gravalos, M. D. *Tetrahedron Lett*. **1996**, *37*, 2671−2674.
(35) Norte, M.; Fernandez, J. J.; Souto, M. L.; Gavin, J. A.; Garcia-Gravalos, M. D. *Tetrahedron* **1986**, *53*, 3173−3178.
(36) Herz, W.; Sharma, R. P. *J. Org. Chem*. **1975**, *40*, 192−199.
(37) Faini, F.; Castillo, M.; Torres, R.; Monache, G. D.; Gacs-Baitz, E. *Phytochemistry* **1995**, *40*, 885−890.
(38) Chen, B.; Kawazoe, K.; Takaishi, Y.; Honda, G.; Itoh, M.; Takeda, Y.; Kodzhimatov, O. K.; Ashurmetov, O. *J. Nat. Prod*. **2000**, *63*, 362−365.
(39) Corey, E. J.; Ha, D.-C. *Tetrahedron Lett*. **1988**, *29*, 3171−3174.
(40) Hashimoto, M.; Kan, T.; Nozaki, K.; Yanagiya, M.; Shirahama, H.; Matsumoto, T. *J. Org. Chem*. **1990**, *55*, 5088−5107.
(41) Gonzalez, I. C.; Forsyth, C. J. *J. Am. Chem. Soc*. **2000**, *122*, 9099−9108.
(42) Blunt, J. W.; McCombs, J. D.; Munro, M. H. G.; Thomas, F. N. *Magn. Reson. Chem*. **1989**, *27*, 792−795.
(43) Marner, F.-J.; Freyer, A.; Lex, J. *Phytochemistry* **1991**, *30*, 3709−3712.
(44) Jakupovic, J.; Eid, F.; Bohlmann, F.; El-Dahmy, S. *Phytochemistry* **1987**, *26*, 1536−1538.
(45) Arai, Y.; Hirohara, M.; Ageta, H. *Tetrahedron Lett*. **1989**, *30*, 7209−7212.
(46) Masuda, K.; Shiojima, K.; Ageta, H.; *Chem. Pharm. Bull*. **1989**, *37*, 1140−1142.
(47) Ziegler, H. L.; Stærk, D.; Christensen, J.; Hviid, L.; Hägerstrand, H.; Jaroszewski, J. W. *Antimicrob. Agents Chemother*. **2002**, *46*, 1441−1446.
(48) Ziegler, H. L.; Jensen, T. H.; Christensen, J.; Stærk, D.; Hägerstrand, H.; Sittie, A. A.; Olsen, C. E.; Staalsø, T.; Ekpe, P.; Jaroszewski, J. W. *Planta Med*. **2002**, *68*, 547−549.
(49) Sairafianpour, M.; Christensen, J.; Stærk, D.; Budnik, B. A.; Kharazmi, A.; Bagherzadeh, K.; Jaroszewski, J. W. *J. Nat. Prod*. **2001**, *64*, 1398−1403.
(50) Bessis, M. In *Red Cell Shapes, An Illustrated Classification and its Rationale*; Bessis, M., Weed, R. I., Leblond, P. F., Eds.; Springer-Verlag: Heidelberg, 1974; pp 1−24.

NP0201826