Antiplasmodial Triterpenoids from *Ekebergia capensis*

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From the stem bark of *Ekebergia capensis*, 10 new triterpenoid compounds, ekeberins A (**1**), B (**2**), C_1 (**3**), C_2 (**4**), C_3 (**5**), D_1 (**6**), D_2 (**7**), D_3 (**8**), D_4 (**9**), and D_5 (**10**), were isolated together with 17 known compounds. The structures of these new compounds were elucidated on the basis of the results of spectroscopic analysis, and the absolute configuration of compounds **6**−**10** were determined by partial synthesis from known compounds and using the Mosher ester method. Several of these compounds were screened in vitro against both chloroquine (CQ)-sensitive and -resistant *Plasmodium falciparum* isolates and were found to exhibit moderate antiplasmodial activity, with compounds **20** (7-deacetoxy-7-oxogedunin) and **27** (2-hydroxymethyl-2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene) showing IC_{50} values of 6 and 7 μM, respectively. Compound **27** at a dose of 500 mg/kg showed moderate parasitemia suppression of 52.9% against *P. berghei* NK 65 in a mouse model.

We have reported the antiplasmodial activities[1] of the chloroform, ethyl acetate, methanol, and water extracts of the stem bark from *Ekebergia capensis* Sparrm. (Meliaceae) previously, a tree growing up to 30 m in height that is widely distributed in many regions of Kenya. The bark of this tree is used as an emetic for heartburn and for respiratory complaints, to treat abscesses and boils, and for pimples. It is also used to treat heart ailments and infertility.[2]

The methanol extract of the stem bark of *E. capensis* was suspended in water and extracted with ether and ethyl acetate, sequentially. Antiplasmodial screening of the fractions showed that the activity was mainly concentrated in the ether and ethyl acetate layers. Previous investigators on the chemical constituents of *E. capensis* have reported four limonoids[3,4] and two acyclic triterpenoids.[5,6]

In the present study, two known coumarins, ekersenin (=pereflorin) (**11**)[7] and 4,6-dimethoxy-5-methylcoumarin (**12**),[8] six known triterpenoids, oleanolic acid (**13**),[9] 3-epioleanolic acid (**14**),[9] oleanoic acid (**15**),[9] 3,11-dioxoolean-12-en-28-oic acid (**16**),[10] melliferone (**17**),[11] and 3-oxo-11,13(18)-oleandien-28-oic acid (**18**),[12] a new triterpenoid, ekeberin A (**1**), a known pregnane, (*Z*)-volkendousin (**19**),[13] a new pregnane, ekeberin B (**2**), six known limonoids, 7-deacetoxy-7-oxogedunin (**20**),[14] 7-acetylneotrichilenone (**21**),[15] proceranolide (**22**),[16] mexicanolide (**23**),[17] swietenolide (**24**),[16] and methylangolensate (**25**),[16] three new limonoids, ekeberins C_1 (**3**), C_2 (**4**), and C_3 (**5**), two known acyclic triterpenoids, 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (3*R*,22*R*) (**26**)[5,6] and 2-hydroxymethyl-2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene (2*R*,3*R*,22*R*) (**27**),[5,6] and five new acyclic triterpenoids, ekeberins D_1 (**6**), D_2 (**7**), D_3 (**8**), D_4 (**9**), and D_5 (**10**), were isolated from the ether and ethyl acetate layer of the methanol extract of the stem bark. The structures of these new compounds were elucidated on the basis of spectroscopic evidence and by chemical transformation. For the new acyclic triterpenoids, their absolute configuration was determined by the use of the Mosher ester method. The antiplasmodial activity of isolated compounds was tested in vitro against both chloroquine (CQ)-sensitive (FCR-3, originally from Gambia) and -resistant (K-1, originally from Thailand) *Plasmodium falciparum* isolates. Also compounds **26** and **27**, which were obtained in sufficient yields for in vivo antimalarial assays, were screened in mice against artificially induced CQ-tolerant *P. berghei* NK 65.

Results and Discussion

Ekeberin A (**1**) was isolated as a colorless, amorphous solid with the ^{1}H and ^{13}C NMR data assigned as shown in Table 1. On the basis of HRFABMS and the ^{13}C NMR data, the molecular formula $C_{30}H_{48}O_3$ was deduced. The ^{1}H NMR spectrum displayed five singlet methyl signals at δ 0.91, 0.96, 1.03, 1.06, and 1.08, two doublet methyl signals at δ 0.88 (6H), a methine proton signal at δ 1.69 as a septet (J = 7 Hz), an oxymethine proton signal at δ 3.54 (dd, J = 10, 1.5 Hz), and oxymethylene proton signals at δ 3.43 (dd, J = 8.5, 1.5 Hz) and 4.24 (dd, J = 8.5, 3 Hz), suggesting this compound to be a cyclic triterpenoid.[9] The ^{13}C NMR spectrum displayed a ketone carbon signal at δ 217.7 and three oxygenated carbon signals at δ 69.2 (methylene), 78.7 (methine), and 97.5 (quaternary). The last-mentioned carbon atom was assumed to be attached to two oxygen atoms due to its chemical shift. The septet methine proton signal at δ 1.69 suggested the presence of an isopropyl residue for which the spin system was isolated from a further spin system. This methine proton and two doublet methyl protons were long-range coupled with the carbon at δ 97.5, which was also long-range coupled with the protons at δ 3.43, 3.54, and 4.24. The NMR data of the A, B, and C rings were similar to those of a 3-keto-type triterpenoid.[9] In a NOE experiment, NOEs were observed at the methine proton signal (δ 3.54), on irradiation at the H_3-27 methyl protons signal (δ 0.91) and at the methine proton signal (δ 1.88), and the oxymethylene proton signal (δ 4.24) on irradiating at the H_3-26 methyl proton signal (δ 1.06). The methine proton signal at δ 1.88 (overlapped with other signals) was coupled with the proton signal at δ 3.54 by 10 Hz and appeared as a double double-doublet signal having three coupling constants (J = 10, 10, 3.5 Hz) in the difference HOHAHA spectrum. The oxymethine proton signal at δ 3.54 showed *W*-type long-range coupling with the oxymethylene proton signal at δ 4.24, while the oxymethylene proton signal at δ 3.43 also gave a *W*-type long-range coupling

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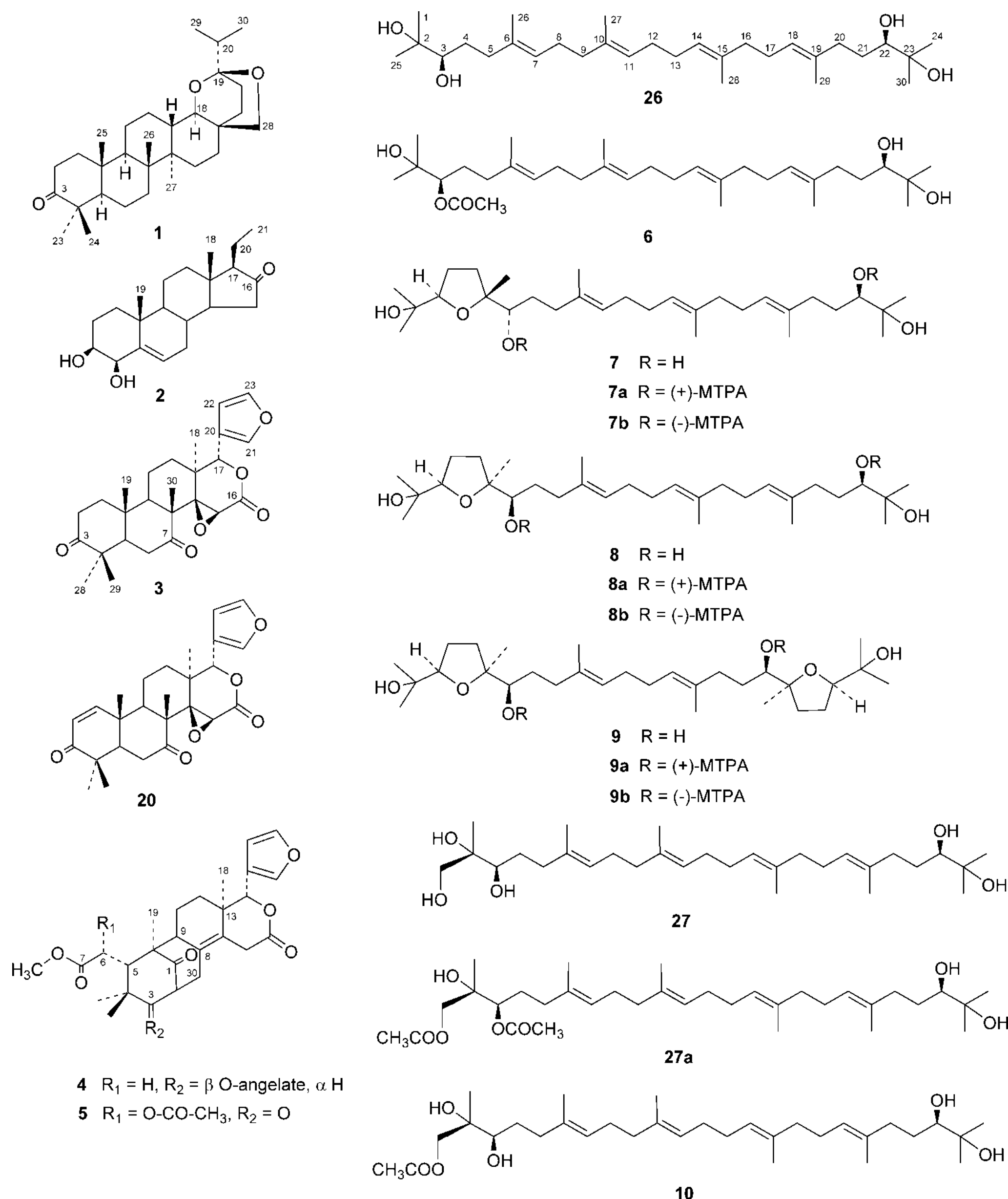
Table 1. NMR Spectroscopic Data (400 MHz, $CDCl_3$) for Compounds **1–5**

	1		**2**		**3**		**4**		**5**	
position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	1.49, overlapped 1.97, overlapped	39.8	1.08, m 1.88, m	36.4		38.5		218.2		212.6
2	2.43, ddd (15.5, 8, 4.5) 2.49, ddd (15.5, 9.5, 7.5)	34.0	1.66, m 1.90, m	24.9		32.5	3.24, m	48.2	3.25, dd (6, 3.5)	57.8
3		217.7	3.57, ddd (12, 3.5, 3.5)	72.1		214.5	4.85, d (10)	79.4		209.3
4		47.2	4.15, d (3.3)	76.8		47.5		38.3		49.4
5	1.35, overlapped	55.0	1,4,5,7,9,10,23,24,25	142.7		56.0	3.32, m	40.7	2.95, brd (1.5)	43.7
6	1.40, overlapped 1.47, overlapped	19.5	5.68, dd (5, 2.5)	127.5	2.34, dd (14, 4) 2.84, dd (14, 14)	37.1	2.38, dd (16.5, 4) 2.42, d (16.5)	33.6	5.57, brs	72.8
7	1.40, overlapped 1.40, overlapped	32.9	1.66, m 2.09, m	31.7		208.9		174.1		170.7
8		40.7	1.68, m	30.6		53.0		127.6		125.2
9	1.45, overlapped	50.3	1.10, m	49.9	2.00, m	51.8	2.04, m	52.3	2.14, m	51.9
10		36.9		35.9		38.5		53.1		54.6
11	1.34, overlapped 1.56, m	21.3	1.54, m 1.66, m	19.7	1.42, m 1.72, m	17.4	1.81, m	18.8	1.81, m 1.83, m	18.5
12	1.11, ddd (13, 13, 4.5) 2.03, brddd (13, 7, 4)	24.1	1.40, m 1.93, m	38.1	2.55, m 2.58, m	33.2	1.10, m 1.73, m	29.2	1.87, m 1.91, m	28.4
13	1.88, overlapped	39.4		41.5		37.5		38.3		38.2
14		41.4	1.44, m	50.6		65.4		132.0		134.9
15	1.05, overlapped 1.49, overlapped	26.4	1.81, m 2.24, m	37.7	3.77, s	53.5	3.28, m 3.58, brd (21.5)	32.8	3.44, ddd (21.5, 2.5, 2.5) 3.57, ddd (21.5, 2, 2)	33.0
16	1.35, overlapped 1.23, m	28.5		218.9		166.8		169.6		169.4
17		31.4	1.66, m	64.8	5.45, s	78.1	5.57, s	80.8	5.31, brs	80.5
18	3.54, dd (10, 1.5)	78.7	0.73, s	13.0	1.13, s	21.0	1.02, s	17.5	1.01, s	17.5
19		97.5	1.22, s	20.7	1.18, s	16.8	1.17, s	16.7	1.04, s	17.8
20	1.69, sept (7)	35.4	1.27, m 1.66, m	17.3		120.4		120.8		120.4
21	1.86, overlapped 1.93, overlapped	27.6	1.03, t (6.5)	13.1	7.40, m	141.0	7.54, m	141.7	7.41, dd (1.5, 1.5)	142.4
22	1.47, overlapped 1.62, ddd (11, 6, 5)	32.8			6.36, m	109.8	6.47, m	110.0	6.47, m	109.9
23	1.08, s	26.7			7.39, m	143.0	7.40, dd (2, 2)	142.8	7.55, ddd (1, 1, 1)	141.6
24	1.03, s	20.9								
25	0.96, s	16.2								
26	1.06, s	15.6								
27	0.91, s	14.2								
28	3.43, dd (8.5, 1.5) 4.24, dd (8.5, 3)	69.2			1.10, s	26.4	0.84, s	20.5	1.14, s	20.5
29	0.88, d (7)	16.9			1.09, s	20.3	0.82, s	23.9	1.08, s	20.9
30	0.88, d (7)	17.1			1.17, s	16.5	2.12, m 2.62, dd (15.5, 2.5)	33.6	2.36, m 3.20, dd (14, 2.5)	36.3
OMe							3.71, s	52	3.77, s	53.3
								167.2		
1′										169.5
								127.3		
2′									2.18, s	20.9
							6.20, qq (7, 1.5)	140.1		
3′										
							2.07, dq (7, 1.5)	15.9		
4′										
							1.94, dq (1.5, 1.5)	20.7		
5′										

Table 2. NMR Spectroscopic Data (400 MHz, $CDCl_3$) for Compounds **6–10**

	6		**7**		**8**		**9**		**10**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.18, s	24.5	1.13, s	24.0	1.21, s	27.8	1.13, s	25.3	1.15, s	19.6
2		72.2		70.5		72.3		71.9		73.8
3	4.78, dd (10, 2.5)	79.5	3.76, dd (10, 6)	87.7	3.77, dd (7, 7)	84.7	3.81, dd (7.5, 7.5)	84.5	3.48, dd (10, 2.5)	73.8
4	1.70, m	27.7[d]	1.85, m	26.6	1.87, m	26.7	1.90, dd (7.5, 7.5)	26.9	1.55, m	28.2
					1.87, m		1.92, m		1.60, m	
5		35.8	1.56, m	31.5	1.46, m	31.6	1.50, m	31.5	2.23, m	36.4
			2.09, m		2.05, m		2.11, m		2.23, m	
6		133.6[d]		86.1	86.7		86.3		134.9[h]	
7	5.19, m[a]	124.0[c]	3.50, dd (10, 1.5)	76.2	3.51, dd (10.5, 2)	77.0	3.55, dd (10.5, 2)	76.6	5.19, m	124.4[i]
8		26.4[e]	1.40, m	29.9	1.34, m	30.1	1.38, m	30.3	2.10, m	26.0[j]
			1.60, m		1.55, m		1.59, m			
9		39.3	2.03, m	36.6	1.99, m	37.0	2.11, m	36.6	2.00, m	39.7
			2.26, m		2.21, m		2.24, m			
10		134.6[d]		135.0		135.2		135.1		134.9[h]
11	5.14, m[a]	124.2[c]	5.20, m	124.9	5.14, m	125.1	5.20, m	125.0	5.14, m	124.4[j]
12		27.9[f]	2.03, m	28.2	1.19, m	28.4	2.05, m	28.1		28.8
13		27.9[f]	2.03, m	28.2	1.19, m	28.4	2.05, m	28.1		28.2
14	5.14, m[a]	124.7[c]	5.14, m	124.5	5.09, m	124.7	5.20, m	125.0	5.14, m	125.0[i]
15		134.7[d]		134.9		135.2		135.1		134.8[h]
16		39.3	2.03, m	40.0	1.19, m	40.0	2.11, m	36.6		39.6
			2.09, m		2.05, m		2.24, m			
17		26.3[e]	2.09, m	26.4	1.34, m	30.0	1.38, m	30.3	2.10, m	26.5[k]
							1.59, m			
18	5.12, m[a]	124.8[c]	5.20, m	125.1	5.16, m	125.2	3.55, dd (10.5, 2)	76.6	5.19, m	125.2[j]
19		134.5[d]		134.9		135.1		86.3		134.6[h]
20		36.5	2.05, m	36.8	1.19, m	37.2	1.50, m	31.5	2.23, m	36.8
			2.25, m		2.21, m		2.11, m		2.23, m	
21	1.42, m	29.4	1.40, m	29.7	1.39, s	30.8	1.90,dd (7.5, 7.5)	26.9	1.45, m	29.7
			1.56, m		1.45, m		1.92, dd (7.5, 7.5)		1.55, m	
22	3.35, dd (10, 1.5)	78.0	3.34, dd (10.5, 2)	78.3	3.30, dd (10.5, 2)	78.5	3.81, dd (7.5, 7.5)	84.5	3.35, dd (10.5, 2)	78.3
23		72.6		73.0			73.3	71.9		73.0
24	1.15, s	23.0	1.15, s	23.4	1.11, s	24.0	1.13, s	25.3	1.15, s	23.3
25	1.19, s	26.1	1.22, s	27.7	1.09, s	25.6	1.25, s	27.5	4.00, d (11.5)	69.0
									4.14, d (11.5)	
26	1.62, brs[b]	15.6[g]	1.14, s	23.8	1.12, s	23.6	1.15, s	23.6	1.62, brs	15.9[l]
27	1.60, brs[b]	15.6[g]	1.60, brs	16.0	1.57, brs	16.3	1.61, s	16.0	1.60, brs	15.9[l]
28	1.60, brs[b]	15.7[g]	16.0, brs	16.0	1.55, brs	16.2	1.61, s	16.0	1.60, brs	16.0[l]
29	1.60, brs[b]	15.7[g]	1.62, brs	15.9	1.57, brs	16.3	1.15, s	23.6	1.62, brs	16.0[l]
30	1.19, s	26.4	1.20, s	26.5	1.15, s	26.6	1.25, s	27.5	1.20, s	26.4[k]
C=O									171.3	
CH_3	2.01, s								2.10, s	20.8

[a–k] Assignments are interchangeable.



with the proton at δ 1.47. These data indicated that the H-13 and H-18 are β and α, respectively. The CD spectrum of **1** showed a positive Cotton effect at 291 nm.[18] From these data, the structure of **1** was deduced as shown.

The molecular formula of ekeberin B (**2**) was formulated as $C_{21}H_{32}O_3$ by HRFABMS and from the ^{13}C NMR data. The ^1H NMR spectrum displayed two singlet methyl signals at δ 0.73 and 1.22, two oxymethine proton signals at δ 3.57 (ddd, J = 12, 3.5, 3.5 Hz) and 4.15 (d, J = 3.5 Hz), and an olefinic proton signal at δ 5.68 (dd, J = 5, 2.5 Hz). The ^1H and ^{13}C NMR spectroscopic data were very similar to those of **19** except for the lack of one olefinic proton signal. The ^{13}C NMR spectrum disclosed three sp^2 carbons and 18 sp^3 carbons, namely, two sp^2 carbons less and two sp^3 carbons more than those of **19**. A ketone carbon signal at δ 218.9 was shifted downfield by 10.7 ppm relative to that of **19**. These data suggested that **2** has no double bond between C-17 and C-20. The configuration at C-17 was deduced as being *R* by observing the NOE correlations between δ 1.27 (m) and 1.66 (m), which were assigned to methylene proton signals at C-20 by the connection with a triplet methyl signal at δ 1.03 on irradiation of H_3-18 (δ 0.73, s).

The HRFABMS of ekebergin C_1 (**3**) furnished a $[M + H]^+$ at m/z 441.2274, consistent with a molecular formula of $C_{26}H_{32}O_6$. The ^1H NMR spectrum displayed four singlet methyl signals at δ 1.09, 1.10, 1.17, and 1.18, two oxymethine proton signals at δ 3.77 (s) and 5.45 (s), and three olefinic proton signals at δ 6.36 (m), 7.39 (m), and 7.40 (m), which were assigned to the protons of a 3-monosubstituted furan ring. The ^1H and ^{13}C NMR spectra were very close to those of **20** except for the lack of two olefinic proton signals that were conjugated with a carbonyl group and the downfield shift of a ketone carbon at δ 214.5 by 11.5 ppm. From these data the structure of **3** was decided to be the dihydro analogue of **20**.

The ^1H NMR spectrum of ekeberin C_2 (**4**) displayed four singlet methyl signals at δ 0.82, 0.84, 1.02, and 1.17, two olefinic methyl signals at δ 1.94 (dq, J = 1.5, 1.5 Hz) and 2.07 (dq, J = 1.5, 1.5 Hz), a carbomethoxy proton signal at δ 3.71, two oxymethine proton signals at δ 4.85 (d, J = 10 Hz) and 5.57 (s), and three olefinic proton signals at δ 6.47 (m), 7.40 (m), and 7.54 (m), which were assigned to the protons of a 3-monosubstituted furan ring. The ^{13}C NMR spectrum displayed 12 sp^2 carbon signals and 20 sp^3 carbon signals, and of the sp^2 carbons, one was a ketone carbon, three were ester carbons, and eight were olefinic carbons. The ^1H and ^{13}C NMR data closely matched those reported for khayasin T (**22a**).[16] However, the ^{13}C NMR data of the ester moiety were different. Two methyl carbon signals were shifted downfield to δ

Table 3. NMR Spectroscopic Data (400 MHz, $CDCl_3$) for **7a**, **7b**, **8a**, **8b**, **9a**, and **9b**

	7a		**7b**		
position	δ_H (*J* in Hz)	(H to H)	δ_H (*J* in Hz)	(H to H)	**a** − **b**
1	1.08, s	3	1.09, s	3, 30	−0.01
3	3.65, dd (8.5, 6.5)		3.67, dd (9, 3.5)		−0.02
26	1.15, s	7	1.18, s	7	−0.03
25	1.16, s	3	1.19, s	1, 3	−0.03
7	5.09, dd (9.5, 2)		5.10, dd (9.5, 2.5)		
8	1.60, m		1.53, m		+0.07
	1.66, m		1.58, m		+0.08
21	1.58, m		1.66, m		−0.08
	1.70, m		1.80, m		−0.10
22	4.98, dd (10, 2.5)		4.98, dd (10, 2.5)		
24	1.16, s	22	1.13, s	22	+0.03
30	1.22, s	22	1.17, s	22	+0.04

	8a		**8b**		
position	δ_H (*J* in Hz)	NOE (H to H)	δ_H (*J* in Hz)	NOE (H to H)	**a** − **b**
1	1.12, s		1.11, s		+0.01
3	3.80, dd (7.5, 7.5)		3.79, dd (7.5, 7.5)	1, 26, 30	+0.01
26	1.17, s	3	1.17, s		+0.03
25	1.20, s		1.14, s	7	−0.03
7	5.03, dd (10, 2)		5.03, br d (9)		
8	1.61, m		1.68, m		−0.07
	1.64, m		1.70, m		−0.06
21	1.56, m		1.65, m		−0.09
	1.70, m		1.80, m		−0.10
22	4.98, dd (10, 2.5)		4.99, dd (10, 2)		
24	1.16, s	22	1.13, s	22	+0.03
30	1.22, s	22	1.17, s		+0.05

	9a	**9b**		
position	δ_H (*J* in Hz)	δ_H (*J* in Hz)	(H to H)	**a** − **b**
1, 30	1.13, s	1.11, s		+0.02
3, 22	3.82, dd (7, 7)	3.79, dd (7, 7)		+0.03
26, 29	1.18, s	1.14, s	3, 7	+0.04
24, 25	1.21, s	1.17, s		+0.04
7, 18	5.03, dd (10, 2)	5.03, dd (10, 2)		
8, 17	1.60, m	1.67, m		−0.07
	1.64, m	1.70, m		−0.06
27, 28	1.53, br s	1.57, d (1)		−0.04

20.7 (α-methyl to C=O) and 15.9 (*β*-methyl to C=O) in comparison with those of **22a**, consistent with the acyl group being an angelate.

The ^1H NMR spectrum of ekebergin C_3 (**5**) displayed four singlet methyl signals at δ 1.01, 1.04, 1.08, and 1.14, one acetyl methyl signal at δ 2.18, one carbomethoxyl signal at δ 3.77, two oxymethine proton signals at δ 5.31 (brs) and 5.57 (brs), and three furanoid proton signals at δ 6.47 (m), 7.41 (dd, $J = 1.5, 1.5$ Hz), and 7.55 (ddd, $J = 1, 1, 1$ Hz). The ^1H and ^{13}C NMR data suggested that **5** is also limonoid like compounds **4** and **22**−**24**, bearing an acetyl group [δ 2.18 (3H, s) and δ 20.9, (CH_3) 169.5 (COO)]. The HRFABMS of **5** furnished a $[M + H]^+$ peak at *m*/*z* 527.2302, consistent with the molecular formula $C_{29}H_{34}O_9$. In the ^1H NMR spectrum, the H-6 signal was shifted downfield to δ 5.57 (brs) by 1.03 ppm in comparison with the same signal of **24**. These results suggested that the position of the acetyl group in **5** is at C-6. The HMBC correlations also supported this conclusion.

The molecular formula of ekeberin D_1 (**6**) was found to be $C_{32}H_{56}O_5$, from the HRFABMS and ^{13}C NMR data. The ^1H NMR spectrum displayed four singlet methyl proton signals at δ 1.15, 1.18, and 1.19 (2 × CH_3), four olefinic methyl proton signals at δ 1.60 (2 × CH_3) and 1.62 (2 × CH_3) as a broad singlet, an acetyl methyl at δ 2.10, two oxymethine proton signals at δ 3.35 (dd, $J = 10, 1.5$ Hz) and 4.78 (dd, $J = 10, 2.5$ Hz), and four olefinic proton signals at δ 5.12 (m), 5.14 (m, 2H), and 5.19 (m). The ^1H NMR data were similar to those of **26** except for the presence of an acetyl methyl signal at δ 2.10 and a downfield-shifted methine proton signal at δ 4.78 (dd, $J = 10, 2.5$ Hz). These data suggested that **6** is a monoacetate of **26** at the C-3 secondary hydroxyl group. Thus, **26** was acetylated with acetic anhydride under mild conditions to afford **6** in good yield. Accordingly, the absolute configurations of the two carbons were decided to be *R*.

The HRFABMS of **7** furnished a $[M + Na]^+$ peak at *m*/*z* 517.3881, consistent with a molecular formula of $C_{30}H_{54}O_5$. The ^{13}C NMR spectrum displayed peaks corresponding to 30 carbons, which were classified into six sp^2 carbons and 24 sp^3 carbons. Of these sp^3 carbons, six carbons were judged to be oxygenated from their chemical shifts. In the ^1H NMR spectrum, five singlet methyl signals (δ 1.13, 1.14, 1.15, 1.20, and 1.22), three broad singlet methyl signals (δ 1.60 × 2, 1.62), three olefinic proton signals [δ 5.14 (m), 5.20 (m, 2H)], and three oxymethine proton signals [δ 3.34 (dd, $J = 10.5, 2$ Hz), 3.50 (dd, $J = 10, 1.5$ Hz), 3.76 (dd, $J = 10, 6$ Hz)] were observed. These data suggested that **7** contains a tetrahydrofuran ring. Next, **26** was treated with *m*-chloroperbenzoic acid to afford several products.[19] One of these products gave the same spectroscopic data as **7**, suggesting the absolute configurations of C-3 and C-22 to be *R*. In a NOE experiment, NOEs were observed at δ 1.13 (H_3-1), 1.22 (H_3-25), and δ 3.50 (H-7) on irradiating at δ 3.76 (H-3), at δ 1.14 (H_3-26) on irradiating at δ 3.50 (H-7), and at δ 1.15 (H_3-24) and 1.20 (H_3-30) on irradiating at δ 3.34 (H-22). From these NOE data, the absolute configuration of C-6 was deduced as being *R*. The configuration of C-7 was deduced as *S* by the use of the Mosher ester method (Table 3).[19,20]

Table 4. 50% Inhibitory Concentrations (IC_{50} values, in μM) of Acyclic Triterpenoids Isolated from the Stem Bark of *E. capensis*

compound	FCR-3 (CQ-susceptible)	K-1 (CQ-resistant)
9	40	ND[a]
10	55	137
20	6	ND[a]
26	55	59
27	18	7
27a	113	97
CQ	28 (nM)	420 (nM)

[a] ND, not determined. [b] CQ refers to chloroquine as reference drug.

Table 5. Parasitemia Suppression on Day 4 Postinfection for Mice[a] Treated with Compounds **26** and **27**

compound (mg/kg)	mean parasitemia (±SD)	parasitemia suppression (%)
26 100	2.5 ± 0.9	26.5
250	2.5 ± 03[b]	26.5[b]
500	2.7 ± 0.7	20.5
27 100	3.5 ± 1.1	NS[c]
250	2.0 ± 07[b]	41.2[b]
500	1.6 ± 0.5	52.9[b]
water control	3.4 ± 0.5	

[a] *P. berghei* NK65-infected ICR mice were treated orally from day 0 p.i. with compounds **26** and **27** at a dose of 100, 250, and 500 mg/kg body weight once a day for 4 days. Each group consisted of 5 mice. [b] Significant, $p < 0.05$ considered significant [c] NS, no suppression.

The spectroscopic data of ekeberin D_3 (**8**) were very similar to those of **7** and were the same as those of the product from the reaction of **26** with *m*-chloroperbenzoic acid, suggesting 3*R* and 22*R* configurations. Therefore, **8** was assumed to be a stereoisomer of **7**. In a NOE experiment, NOEs were observed at δ 1.21 (H_3-1), 1.09 (H_3-25), and 1.12 (H_3-26) on irradiating at δ 3.77 (H-3) and at δ 1.12 (H_3-26) on irradiating at δ 3.51 (H-7). These NOE data suggested the configuration of C-6 to be *S*. The absolute configuration at C-7 was decided to be *R* by the Mosher ester method.

The molecular formula of ekeberin D_4 (**9**) was found to be $C_{30}H_{54}O_6$ by HRFABMS. The ^{13}C NMR spectrum showed 15 carbon signals including two sp^2 carbon signals and 13 sp^3 carbon signals, suggesting that **9** has a symmetric structure. Of these sp^3 carbons, four carbons were oxygenated from their chemical shifts. This compound was found to be the same as the product from the reaction of **26** with *m*-chloroperbenzoic acid, suggesting the C-3*R* and C-22*R* configurations. In a NOE experiment, NOEs were observed at δ 1.13 (H_3-1), 1.25 (H_3-25), and 1.15 (H_3-26) on irradiating at δ 3.81 (H-3) and at δ 1.15 (H_3-26) on irradiating at δ 3.55 (H-7). These data showed C-6*S*, 19*S* configurations, with those at C-7 and C-18 decided as both *R* using the Mosher ester method.

The spectroscopic data of ekeberin D_5 (**10**) were similar to those of **27** except for the presence of an acetyl methyl proton signal at δ 2.10 and downfield-shifted methylene proton signals at δ 4.00 (d, J = 11.5 Hz) and 4.14 (d, J = 11.5 Hz). Acetylation of **9** with acetic anhydride under mild conditions afforded **10** in good yield. Accordingly, the structure of **10** was determined as shown.

Compounds **26** and **27** were already reported by Nishiyama et al.[5,6] from this plant, but the other compounds were isolated for the first time from this plant.

Antiplasmodial activities of natural compound **20** as well as the acyclic triterpenoid compounds **6**−**10**, **20**, **26**, and **27** and a semisynthesized compound **27a** were evaluated in vitro against both the CQ-sensitive (FCR-3) and -resistant (K-1) *P. falciparum* isolates, respectively (Table 4). Also, compounds **26** and **27** were assayed against an artificially induced CQ-tolerant malaria parasite, *P. berghei* NK 56, in mice (Table 5). Whereas compounds **6**−**8** were inactive (data not shown), the other compounds had good to moderate activities, with **20** and **27** showing potent IC_{50} activities as low as 6 and 7 μM, respectively. Compound **27** showed strong in vivo parasitemia suppression of 52.9%, and this finding is consistent with the compound's good activity of 18 μM (against FCR-3, CQ-sensitive) and 7 μM (against K-1, CQ-resistant) in the in vitro test used.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. The circular dichroism spectra were measured on JASCO J-20A spectrometer. ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM α-400 FT-NMR spectrometer, and chemical shifts are given as δ values with TMS as an internal standard at 35 °C. Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C\text{-}H}$ = 145 Hz) and HMBC (optimized for $^nJ_{C\text{-}H}$ = 8 Hz) pulse sequences with a pulse field gradient. HRFABMS data were obtained on a JEOL JMS 700 mass spectrometer, using a *m*-nitrobenzyl alcohol matrix. Preparative HPLC was performed on a JASCO 800 instrument.

Plant Material. *Ekebergia capensis* was collected in January 2004 from the Mount Kenya Forest in the Nanyuki area in central Kenya and identified by Mr. Geoffrey M. Mungai, a taxonomic botanist from the East Africa Herbarium in Nairobi. Voucher specimens (200408105) were deposited at the Herbarium, University of Shizuoka.

Extraction and Isolation. The powdered stem bark of *E. capensis* (886 g) was extracted with hot methanol (5 L) twice. The methanol extract was concentrated at reduced pressure and the extract (210 g) suspended in water (1.5 L) and extracted with ether (1 L) and with ethyl acetate (1 L) sequentially, using an Aldrich modified convertible liquid–liquid continuous extractor, to give two brown syrups (ether layer, 26 g, and ethyl acetate layer, 78 g). These two extracts were combined and subjected to normal-phase chromatography using a silica gel (Fuji Silysia, PSQ 100B, 100 μm) column (9 × 40 cm), eluting with hexane−acetone (90:10 → 40:60), to afford 24 fractions (Frs. A−X). From Fr. F (15.030 g), compound **15** (5.303 g) was afforded as colorless needles from methanol. From Fr. I (2.468 g), compound **14** (1.560 g) was obtained as colorless needles from methanol. From Frs. P, Q, and R (27.497 g) compound **26** was isolated as a colorless, viscous oil. From Fr. V (3.091 g), compound **27** was obtained as a colorless, viscous oil. Fr. C (142 mg) was subjected to preparative TLC on silica gel using hexane−acetone (9:1) as a solvent, to give compound **1** (43 mg). Fr. D (240 mg) was subjected to semipreparative HPLC [column, Nomura Chemical, Develosil 60, 2 × 25 cm; solvent, hexane−chloroform (60:40); detector, UV 205 nm] to yield compound **17** (7 mg). Fr. J (620 mg) was subjected to semipreparative HPLC [column, GL Sciences, Inertsil ODS-3, 3 × 50 cm; solvent, methanol–water (80:20); detector, UV 205 nm] to give **4** (14 mg), **12** (7 mg), **13** (82 mg), **16** (54 mg), and **21** (6 mg). Fr. K (2.800 g) was subjected to preparative HPLC [column, Tosoh, TSKgel ODS-120A, 5.5 × 60 cm × 2; solvent, acetonitrile−water (54:46 → 20:80); detector, UV 205 nm], to give 18 fractions (Frs. K_A−K_R). From Frs. K_H (21 mg), K_K (18 mg), K_O (683 mg), and K_Q (11 mg) compounds **12**, **14**, and **18** were obtained, respectively. Fr. K_G (8 mg) was subjected to semipreparative HPLC [column, Shiseido, Capcell pak C_{18}, 2 × 25 cm; solvent, acetonitrile−water (40:60), detector, UV 205 nm] to give compound **11** (1 mg). Fr. O (994 mg) was subjected to preparative HPLC [column, Tosoh, TSKgel ODS-120A, 5.5 × 60 cm × 2; solvent, methanol–water (80:20 → 89:11); detector, UV 205 nm] to give 19 fractions (Frs. O_A−O_S). From Frs. O_E (26 mg), O_K (84 mg), O_O (18 mg), and O_Q (51 mg), compounds **2**, **8**, **20**, and **26** were obtained, respectively. Frs. O_G and O_H (27 mg) were subjected to semipreparative HPLC [column, Shiseido, Capcell pak C_{18}, 2 × 25 cm; solvent, methanol–water (72:28); detector, UV 205 nm] to give compounds **3** (5 mg), **5** (2 mg), **23** (2 mg), and **25** (2 mg). Fr. O_I (32 mg) was subjected to semipreparative HPLC [column, Shiseido, Capcell pak C_{18}, 2 × 25 cm; solvent, methanol–water (75:25); detector, UV 205 nm] to give compound **22** (20 mg). Fr. O_J (12 mg) was subjected to semipreparative HPLC [column, Shiseido, Capcell pak $C_{18,}$ 2 × 25 cm; solvent, methanol–water (72:28); detector, UV 205 nm] to give compound **19** (3 mg). Fr. O_L (40 mg) was subjected to semipreparative HPLC [column, Shiseido, Capcell pak $C_{18,}$ 2 × 25 cm; solvent, methanol–water (84:16); detector, UV 205 nm] to give compound **9** (3 mg). Fr. O_N (22 mg) was subjected to semipreparative HPLC [column, Shiseido, Capcell pak C_{18}, 2 × 25 cm; solvent, methanol–water (85:15); detector, UV 205 nm] to give compound **7** (7 mg). Fr. O_R (26

mg) was subjected to semipreparative HPLC [column, Shiseido, Capcell pak C_{18}, 2 × 25 cm; solvent, methanol–water (90:10); detector, UV 205 nm] to give compound **6** (15 mg). Fr. S (1.791 g) was subjected to preparative HPLC [column, Tosoh, TSKgel ODS-120A, 5.5 × 60 cm × 2; solvent, methanol–water (50:50 → 100:0); detector, UV 205 nm] to give 14 fractions (S_A–S_N). From Frs. S_K (18 mg) and S_M (100 mg) compounds **10** and **26** were obtained. Fr. S_D (48 mg) was subjected to semipreparative HPLC [column, Shiseido, Capcell pak C_{18}, 2 × 25 cm; solvent, methanol–water (60:40); detector, UV 205 nm] to give compound **24** (6 mg).

Ekeberin A (1): colorless, amorphous solid; [α] +79.3 (*c* 1.74, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 1; HRFABMS *m/z* 457.3676 (calcd for $C_{30}H_{49}O_3$, 457.3684).

Ekebergin B (2): colorless, amorphous solid; [α] −89.7 (*c* 2.10, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 1; HRFABMS *m/z* 371.1992 (calcd for $C_{21}H_{33}O_3K$, 371.1989).

Ekebrrin C$_1$ (3): colorless, amorphous solid; [α] −61.8 (*c* 0.14, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 1; HRFABMS *m/z* 441.2274 (calcd for $C_{26}H_{33}O_6$ 441.2278).

Ekeberin C$_2$ (4): colorless, amorphous solid; [α] −178.1 (*c* 1.38, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 1; HRFABMS *m/z* 553.2787 (calcd for $C_{32}H_{41}O_8$, 553.2802).

Ekeberin C$_3$ (5): colorless, amorphous solid; [α] −61.5 (*c* 036, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 1; HRFABMS *m/z* 527.2302 (calcd for $C_{29}H_{34}O_4$, 527.2282).

Ekebergin D$_1$ (6): colorless, viscous oil; [α] +13.9 (*c* 2.32, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 2; HRFABMS *m/z* 543.4026 (calcd for $C_{32}H_{56}O_5Na$, 543.4025).

Ekebergin D$_2$ (7): colorless, viscous oil; [α] +7.2 (*c* 0.53, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 2; HRFABMS *m/z* 517.3881 (calcd for $C_{30}H_{54}O_5Na$, 517.3871).

Ekebergin D$_3$ (8): colorless, viscous oil; [α] +15.5 (*c* 0.55, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 2; HRFABMS *m/z* 517.3868 (calcd for $C_{30}H_{54}O_5Na$, 517.3869).

Ekebergin D$_4$ (9): colorless, viscous oil; [α] +8.0 (*c* 0.42, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 2; HRFABMS *m/z* 533.3813 (calcd for $C_{30}H_{54}O_6Na$, 533.3828).

Ekebergin D$_5$ (10): colorless, viscous oil; [α] +15.5 (*c* 0.43, $CHCl_3$); ^1H NMR and ^{13}C NMR, Table 2; HRFABMS *m/z* 555.3967 (calcd for $C_{32}H_{56}O_6Na$, 559.3975).

Acetylation of 26. Compound **26** (50 mg) was acetylated with acetic anhydride (1 mL) at 45 °C for 5 h. The reaction mixture was subjected to semipreparative HPLC [column, Capcell pak C_{18}, 2 × 25 cm; solvent, MeOH−H_2O (87:13); detector UV, 205 nm] to give **6** (23 mg).

Acetylation of 27. Compound **27** (220 mg) was acetylated with acetic anhydride (2 mL) at room temperature for 3 days. The reaction mixture was subjected to semipreparative HPLC [column, Cosmosil ODS, 2 × 25 cm; solvent, MeOH−H_2O (80:20); detector UV, 205 nm] to give **10** (59 mg) and **27a** (18 mg) as a colorless viscous oil. **27a**: ^1H NMR ($CDCl_3$) 1.15 (3H, s), 1.18 (3H, s), 1.20 (3H, s), 1.60 (9H, brs), 1.62 (3H, brs), 2.10 (6H, s), 3.48 (1H, dd, $J = 10, 2.5$ Hz), 4.00 (1H, d, $J = 11.5$ Hz), 4.15 (1H, d, $J = 11.5$ Hz), 4.78 (1H, dd, $J = 10, 3$ Hz), 5.12 (1H, m), 5.14 (2H, m), 5.19 (1H, m).

Cyclization of 26. To a chloroform solution (3 mL) of compound **26** (300 mg) was added *m*-chloroperbenzoic acid (160 mg), and the mixture was stirred for 15 h at room temperature. The reaction mixture was washed with sodium bicarbonate-saturated water (15 mL) and water (20 mL). The chloroform solution was concentrated and subjected to semipreparative HPLC [column, Inertsil ODS-3, 3 × 50 cm; solvent, methanol–water (80:20); detector UV, 205 nm] to give **7** (5.3 mg), **8** (5.5 mg), and **9** (4.2 mg).

MTPA Esters of 7, 8, and 9. To a solution of triterpene alcohol (each 2 mg) in pyridine (30 μL) was added (+)-MTPA chloride or (−)-MTPA chloride (4 μL), and the solution was left overnight. 3-[(Dimethyamino)prolyl]amine (3 μL) was added and the mixture left for 1 h. The solvent was evaporated and the mixture was subjected to preparative HPLC [column, YMC ODS, 1 × 25 cm; solvent, acetonitrile−water (95:5); detector UV, 205 nm] to give (+)-MTPA esters (**7a**, **8a**, **9a**, each 2 mg) and (−)-MTPA esters (**7b**, **8b**, **9b**, each 2 mg) as a colorless, viscous oil. ^1H NMR: Table 3.

Cultivation of *P. falciparum*. A chloroquine (CQ)-susceptible *P. falciparum* strain, FCR-3 (originally from Gambia), whose 50% inhibitory concentration (IC_{50}) of CQ was 28 nM, and a CQ-resistant strain, K-1 (originally from Thailand), whose IC_{50} of CQ was 420 nM, were grown synchronously by the modified method of Mitamura et al.[21] In brief, RPMI 1640 medium was used with glutamine supplemented with 10% human serum, 25 mM HEPES/NaOH (pH 7.2), 23.8 mM $NaHCO_3$, 3.4 mM L-glutamine, 50 μg/mL hypoxanthine, 25 μg/mL gentamicin, and 2% hematocrit human type O^+ red blood cells (RBCs) in disposable sterile dishes and a controlled atmosphere of 5% CO_2, 5% O_2, 90% N_2 at 37 °C.

Antiparasitic Agents. Nine test samples (**6–10**, **20**, **26**, **27**, and **27a**) were reconstituted in DMSO, whose final concentration was less than 0.2% in the stock solution,[22] and were then diluted with the supplemented RPMI 1640 medium in a 2-fold serial dilution system. A 50 μL amount of each diluted solution was added to the wells of a 96-well microculture plate that contained 50 μL of K-1- or FRC-3-infected RBCs (0.5% parasitemia) in supplemented RPMI 1640 medium (2% hematocrit).

Evaluation and Determination of IC_{50} Values. Parasitized RBCs showing an initial parasitemia level of 0.5% were incubated for 52 h, when the schizonts in the control wells were all ruptured and transformed into new ring forms. Then the culture plates were removed from the incubator, and thin-smear specimens were prepared and stained with Giemsa solution. The numbers of ring-infected RBCs were counted under a microscope, and the effects of the drugs on parasite growth were evaluated by the observation of decreased numbers of ring forms per equal number of RBCs counted previously in the control cultures. The growth inhibition effect (in percentage) was calculated as follows: [test well ring-form infected RBC count/control well ring-form infected RBC count] × 100. The amount of the test samples was limited, and thus the samples were tested at each concentration in a single assay. The IC_{50} values of the test samples were calculated by the probit method.

Animals and Test Parasites. The handling and care of animals was done as recommended in the *Guide for the Care and Use of Laboratory Animals* manual of Hamamatsu University School of Medicine. Outbred SPF male ICR mice, 7 weeks old (purchased from Japan SLC Inc., Hamamatsu, Japan), were used as a host. The chloroquine-tolerant malaria parasite, *P. berghei* (strain NK 65), was maintained by serial blood passage in mice, and blood-stage parasites were stored in a −80 °C deep freezer.

In Vivo Evaluation of the Compounds for Antimalarial Activity. Compounds **26** and **27** obtained in sufficient quantities were evaluated for in vivo antiplasmodial activity using the 4-day suppressive protocol described by Peters et al.[23] For experiments, blood-stage parasites from frozen stock were inoculated intraperitoneally into two donor mice. Six days after parasite inoculation, *P. berghei* NK 65-parasitized blood was obtained from the donor mice under ether anesthesia. Experimental mice were infected intraperitoneally with 10^5 parasitized erythrocytes and were divided into seven groups of five mice per cage. All mice were fed ad libitum on a commercial diet (LabDiet, PMI Nutrition International, St. Louis, MO) and water. The compounds were solubilized in DMSO and then diluted with distilled water such that the final concentration of DMSO was less than 0.2%. The initial (day 0) treatment of the experimental groups was done between 2 and 4 h postinfection (p.i.) with the parasite, with each mouse within a group receiving the respective test compound at a dose of 100, 250, and 500 mg/kg body weight, once a day for 4 days using a metal catheter. The untreated control group received a corresponding volume of distilled water only. Twenty-four hours after the last treatment (i.e., on day 4 p.i.), thin blood smears were prepared by bleeding via the tail vein and were stained with Giemsa, and parasitemia was determined microscopically.

Data and Statistical Analysis. For comparison of mean parasitemia, F-test (two-sample for variances) and Student's *t*-test (two-tailed) were employed (Microsoft Excel 2004), with $p < 0.05$ being considered significant.

Supporting Information Available: The structures of all compounds and HMBC and ROE data of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Muregi, F. W.; Chhabra, S. C.; Njagi, E. N. M.; Lang'at-Thoruwa, C. C.; Njue, W. M.; Orago, A. S. S.; Omar, S. A.; Ndiege, I. O. *Phytother. Res.* **2004**, *18*, 379–384.

(2) Mulholland, D. A.; Coombes, P. H.; Iourine, S. E.; Mahomed, H.; Raynor, M. W.; Raidoo, D. M. *Proceedings of the 7th NAPRECA*

Symposium on Natural Products, Dar es Salaam, Tanzania,1997; 1998; Vol. 10, pp 41–48.
(3) Taylor, D. H. A. *Phytochemistry* **1981**, *20*, 2263–2265.
(4) Mulholland, D. A.; Iourine, S. *Phytochemistry* **1998**, *47*, 1357–1361.
(5) Nishiyama, Y.; Moriyasu, M.; Ichimaru, M.; Tachibana, Y.; Kato, A.; Mathende, S. G.; Nganga, J. N.; Juma, F. D. *Phytochemistry* **1996**, *42*, 803–807.
(6) Nishiyama, Y.; Moriyasu, M.; Ichimaru, M.; Kato, A.; Mathende, S. G.; Nganga, J. N.; Juma, F. D. *Phytochemistry* **1999**, *52*, 1593–1596.
(7) Bohlmann, F.; Zdero, C. *Phytochemistry* **1977**, *16*, 239–242.
(8) Okogun, J. I.; Enyenihi, V. U.; Ekong, D. E. U. *Tetrahedron* **1978**, *34*, 1221–1224.
(9) Ahmad, V. U.; Atta-ur-Rahman. *Handbook of Natural Products Data*: Elsevier: New York, 1994; Vol. 2.
(10) Fukuda, Y.; Yamada, T.; Wada, S.; Sakai, K.; Matsunaga, S.; Tanaka, R. *J. Nat. Prod.* **2006**, *69*, 142–144.
(11) Ito, J.; Chang, F.-R.; Wang, H.-K.; Park, Y. K.; Ikegaki, M.; Kilgore, N.; Lee, K.-H. *J. Nat. Prod.* **2001**, *64*, 1278–1281.
(12) Fukuyama, Y.; Minami, H.; Fuji, H.; Tajima, M. *Phytochemistry* **2002**, *60*, 765–768.
(13) Rogers, L. L.; Xeng, L.; McLaughlin, J. L. *J. Org. Chem.* **1998**, *63*, 3781–3785.
(14) Bevan, C. W. L.; Powell, J. W.; Taylor, D. A. H. *J. Chem. Soc.* **1963**, *980*, 982.
(15) Kraus, W.; Cramer, R.; Sawitzki, G. *Phytochemistry* **1981**, *20*, 117–120.
(16) Kadota, S.; Marpaung, L.; Kikuchi, T.; Ekimoto, H. *Chem. Pharm. Bull.* **1990**, *38*, 639–651.
(17) Connolly, J. D.; McCrindle, R.; Overton, K. H. *Tetrahedron* **1968**, *24*, 1497–1502.
(18) Lischewski, M.; Ty, P. D.; Kuschabsky, L.; Pfeiffer, D.; Phiet, H. V.; Preiss, A.; Sung, T. V.; Adam, G. *Phytochemistry* **1985**, *24*, 2355–2357.
(19) Kodama, M.; Yoshio, S.; Tabata, T.; Deguchi, Y.; Sekiya, Y.; Fukuyama, Y. *Tetrahedron Lett.* **1997**, *38*, 4627–4630.
(20) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(21) Mitamura, T.; Hanada, K.; Ko-Mitamura, E. P.; Nishijima, M.; Horii, T. *Parasitol. Int.* **2000**, *49*, 219–229.
(22) Elueze, E. I.; Croft, S. L.; Warhust, D. C. *J. Antimicrob. Chemother.* **1996**, *37*, 511–518.
(23) Peters, W.; Portus, J. H.; Robinson, B. L. *Ann. Trop. Med. Parasitol.* **1975**, *69*, 155–171.

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