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Phytochemistry 67 (2006) 444–451
PHYTOCHEMISTRY
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Clerodane and labdane diterpenoids from *Nuxia sphaerocephala*

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Received 30 March 2005; received in revised form 17 November 2005
Available online 19 January 2006

Abstract

Seven diterpenoids including four clerodane and three labdane derivatives, (13*S*)-*ent*-7β-hydroxy-3-cleroden-15-oic acid (**1**), *ent*-7β-hydroxy-2-oxo-3-cleroden-15-oic acid (**2**), *ent*-2,7-dioxo-3-clero-den-15-oic acid (**3**), *ent*-18-(*E*)-caffeoyloxy-7β-hydroxy-3-cleroden-15-oic acid (**4**) (13*S*)-*ent*-18-(*E*)-coumaroyloxy-8(17)-labden-15-oic acid (**5**), *ent*-18-(*E*)-caffeoyloxy-8(17)-labden-15-oic acid (**6**), *ent*-15-(*E*)-caffeoyloxy-8(17)-labden-18-oic acid (**7**), have been isolated from an ethyl acetate extract of the leaves of *Nuxia sphaerocephala*, together with 17 known compounds. 3-Oxolup-20(29)-en-30-al (3-oxolupenal) (**8**) and 3β-hydroxylup-20(29)-en-30-al (3β-hydroxy-lupenal) (**9**) showed the best inhibitory activity against *Plasmodium falciparum* with the IC_{50} values between 1.55 and 4.67 μg/ml in vitro, respectively. The structure and the relative stereochemistry of the compounds were established on the basis of their spectroscopic properties. The absolute configuration at C-13 of **1** and **5** was determined by the PGME amide procedure.

Keywords: *Nuxia sphaerocephala*; Loganiaceae; Clerodanes; Labdanes; PGME amide method; Antiplasmodial activity

1. Introduction

Nuxia sphaerocephala Baker (Loganiaceae) is a shrub growing in the Eastern rainforests of Madagascar. Leaves are used in traditional medicine to treat splenomegaly associated with malaria, and also infantile hederosyphilis (Boiteau, 1999). Previous phytochemical studies on the genus *Nuxia* led to the isolation of the eight-carbon iridoid glucoside unedoside and its derivatives (Jensen et al., 1998; Frederiksen et al., 1999). To the best of our knowledge, no study has been reported on *N. sphaerocephala*. As part of our search for novel antimalarial agents from plants, we investigated the leaves of *N. sphaerocephala*, the ethyl acetate extract of which showed antiplasmodial activity with an IC_{50} value of 4.2 μg/ml (Rasoanaivo et al., 2004). Seven new compounds, including four clerodane diterpenoids (**1**, **2**, **3**, **4**) and three labdane diterpenoids (**5**, **6**, **7**) along with 17 known compounds were isolated, of which ten were identified as triterpenes namely 3-oxolupenal (3-oxolup-20(29)-en-30-al) (**8**), 3β-hydroxylupenal (3β-hydroxylup-20(29)-en-30-al) (**9**), lup-20(29)-ene-3β,30-diol (Wijeratne et al., 1981), 3-oxolupenol (30-hydroxylup-20(29)-en-3-one) (**10**) (Bohlmann and Jakupovic, 1979), lupeol, uvaol (Dehmlow et al., 1998), ursolic acid, 3β-acetylursolic acid (Houghton and Lian, 1986), 3β-acetyloleanolic acid (**11**) (Chavez and Delgado, 1994) and oleanolic acid (**12**); three were found to be diterpenes, *ent*-15-hydroxy-8(17)-labden-19-oic acid (**13**) (Zdero et al., 1991a), *ent*-18-hydroxy-8(17)-labden-15-oic acid (**14**) (Zdero et al., 1991b) and *ent*-18-

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✠ In memoriam.

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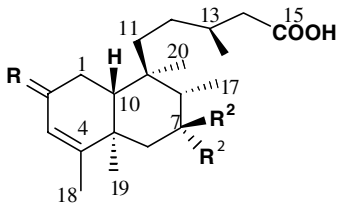
doi:10.1016/j.phytochem.2005.11.024

hydroxy-3-cleroden-15-oic acid (**15**) (Tsichritzis and Jakupovic, 1990), three were identified as flavones, 5-hydroxy-7-methoxyflavone (Mongkolsuk and Dean, 1964), 5-hydroxy-4′,7-dimethoxyflavone (Biftu and Stevenson, 1978) and 5,7-dihydroxy-4′-methoxyflavone (Gaydou and Bianchini, 1978) and one as a sterol, 3-*O*-β-D glycopyranosylsitosterol (Ahmad et al., 1992). All clerodane and labdane diterpenoids isolated from this plant were recognized as members of *ent*-series through their optical rotation values. In this paper, we describe the isolation and structural elucidation of **1**–**7** as well as the inhibitory activity of all isolated compounds against *Plasmodium falciparum*.

2. Results and discussion

A crude EtOH extract of leaves of *N. sphaerocephala* was partitioned successively with cyclohexane and ethyl acetate. The ethyl acetate soluble extract showed significant inhibitory activity against *P. falciparum* with an IC_{50} of 4.2 μg/ml and was subjected to a series of bioassay-guided column chromatographic purification steps over silica gel to afford seven new compounds **1**–**7** along with 17 known compounds.



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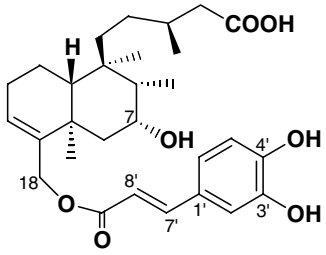
2. R = O; R^1 = OH; R^2 = H

3. R = O; R^1, R^2 = O



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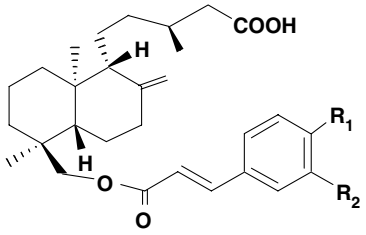
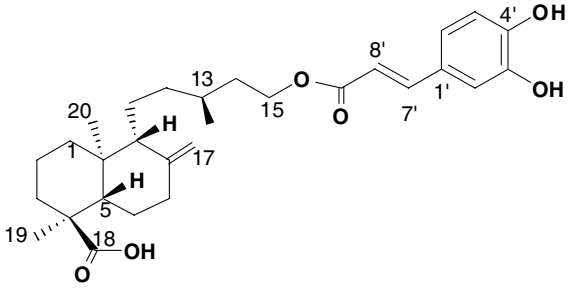


5 R^1 =OH; R^2 = H

6 R^1 = OH; R^2 = OH



7

Diterpenoids **1-7** from *N.sphaerocephala*

Compound **1** was obtained as colourless oil. Its molecular formula $C_{20}H_{34}O_3$ was established from its HRCIMS at *m/z* 340.2845 $[M + NH_4]^+$ and confirmed by ^{13}C NMR data which also revealed the presence of a trisubstituted double bond and a carboxyl group. The molecule is therefore bicyclic. The ^1H NMR spectrum (Table 2) displayed characteristic signals for an olefinic proton at δ 5.11 (1H, *m*), an oxymethine proton at δ 3.98 (1H, *q*, $J = 3.3$ Hz), two tertiary methyl singlets at δ 0.96 and 1.26 and three doublet methyl signals at δ 0.96 (*d*, $J = 6.9$ Hz), 0.98 (3H, *d*, $J = 7.2$ Hz) and 1.59 (*d*, $J = 1.4$ Hz) and suggested a clerodane skeleton. The ^{13}C NMR spectral data indicated that compound **1** contained 20 carbons, including five methyls, five methines, six methylenes and four quaternary carbons (Table 1). The COSY spectrum revealed three spin systems associated with ring A, ring B and the side chain as in **1**. HMBC correlations confirmed the clerodane nucleus and the position of the functionality. Thus H-3 showed correlations with C-2, C-5 and C-18 while H-7 correlated with C-5, C-6, C-8, C-9 and C-17. The structure of the side chain was established *inter alia* by the HMBC correlations from H-13 to C-11, C-12, C-14, C-15 and C-16. Other useful correlations of C-5 included those from Me-18 and Me-19. The configuration of the hydroxyl group at C-7 was deduced to be α (axial) on the basis of the small coupling constants of H-7. NOEs from Me-19 to Me-20 and from H-7 to Me-17 completed the relative stereochemistry of **1** apart from the A/B ring junction which was assumed to be *trans* on the basis of the lack of a NOE between Me-19 and H-10. The configuration at C-13 could not be resolved by spectroscopic means.

The absolute configuration at C-13 was determined by applying the phenylglycine methyl ester method (PGME) developed for carboxylic acids having a chiral center at

Table 1
^{13}C NMR data for compounds **1**–**7**

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[a]	**6**[b]	**7**[b]
1	17.8	34.7	35.0	18.8	38.5	39.8	39.5
2	26.7	200.6	198.9	27.5	18.6	19.7	19.5
3	119.9	125.0	126.0	126.4	36.0	37.2	38.4
4	144.9	173.1	169.5	145.1	37.0	38.3	48.0
5	37.5	39.4	44.3	38.6	49.5	50.8	51.2
6	42.8	41.4	51.2	43.6	24.3	25.6	28.0
7	73.9	73.3	211.3	74.0	38.0	39.2	39.1
8	39.2	38.8	50.1	40.6	148.1	149.6	149.0
9	38.0	38.1	44.3	39.2	56.8	58.4	58.7
10	46.5	45.9	45.7	47.8	39.5	40.6	40.0
11	36.6	35.9	35.3	37.8	20.9	22.0	22.2
12	29.5	29.2	29.2	30.7	35.6	36.9	36.9
13	30.8	30.7	30.5	32.2	30.7	32.1	31.7
14	41.3	41.1	40.8	42.7	41.8	43.3	37.0
15	178.6	177.0	176.0	177.1	178.6	177.6	63.9
16	19.9	19.9	19.9	20.3	19.5	20.0	20.3
17	12.4	12.3	7.7	13.0	106.6	107.2	107.4
18	18.0	19.0	18.8	66.0	72.8	73.7	182.2
19	21.8	20.2	19.4	23.7	17.6	18.0	17.1
20	20.2	19.7	19.1	20.6	14.9	15.4	15.2
1′				127.5	127.0	127.7	127.8
2′				115.2	130.0	115.2	115.2
3′				146.8	115.9	147.0	146.8
4′				149.6	157.9	149.5	149.5
5′				116.5	115.9	116.5	116.5
6′				122.9	130.0	123.0	122.9
7′				146.8	144.6	146.9	146.8
8′				115.4	115.5	115.0	115.3
CO				169.1	167.8	169.3	169.4

[a] Spectra were recorded in $CDCl_3$.
[b] Spectra were recorded in CD_3OD.

the β-position (Yabuuchi and Kusumi, 2000). Two portions of **1** (each 4 mg) were condensed in DMF with (*S*)- and (*R*)-PGME in the presence of PyBOP, HOBT, and *N*-methylmorpholine which afforded the (*S*)- and (*R*)-PGME amide derivatives (**1s** and **1r**). Assignments of the proton signals of PGME amides were based on the correlations of the ^{1}H–^{1}H COSY and NOESY spectra including HSQC and HMBC. The chemical shift difference values $\Delta\delta_{RS} = \delta_R - \delta_S$ of the individual protons of **1s** and **1r** are shown in Fig. 1. The systematic arrangement of positive and negative $\Delta\delta_{RS}$ values was observed and unambiguously established the absolute configuration of C-13 to be *S* as indicated by the structure **1** which belongs to the *enantio* series. Accordingly, compound **1** was assigned as (13*S*)-*ent*-7β-hydroxy-3-cleroden-15-oic acid.

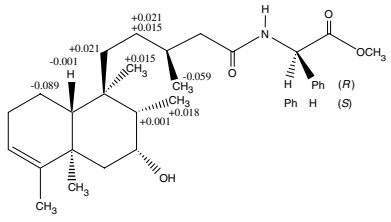


Fig. 1. $\Delta\delta(R - S)$ values (in ppm) for PGME amide derivatives of **1r** and **1s** in $CDCl_3$.

The FABMS spectrum of **2** exhibited a pseudomolecular ion peak at *m*/*z* 337 $[M + H]^+$ consistent with a molecular formula $C_{20}H_{32}O_4$ and five double bond equivalents. Its ^{13}C spectrum, UV and the IR spectra showed the presence of an α,β-unsaturated ketone (δ 200.6 (C-2), 125.0 (C-3) and 173.1 (C-4); ν_{max} 1657 cm^{-1}; λ_{max} 238 nm). The ^{1}H and ^{13}C NMR data for **2** (Tables 1 and 2) were similar to those of **1** apart from the presence of a ketone at C-2, confirmed by HMBC correlations from H-10 and 2H-1 to C-2. The relative stereochemistry of **2** was established in the same way as that of **1** with additional support coming from NOEs between H-6, H-8 and H-10 indicating that all these protons were axial (β). Thus, compound **2** was established as *ent*-7β-hydroxy-2-oxo-3-cleroden-15-oic acid.

The molecular formula of compound **3** was determined as $C_{20}H_{30}O_4$ from combined analysis of HRCIMS and ^{13}C data. Its ^{1}H and ^{13}C NMR data were similar to those of **1** except for the lack of an oxymethine proton (H-7) and the presence of two ketone carbonyl carbons at δ 198.8 and 211.3. Long-range correlations of H-6, H-8 and H_3-17 to the carbonyl at δ 211.3 allowed us to place the carbonyl group at C-7 and the influence of this group was observed by an upfield shift of C-17 (δ 7.7 instead of 12.4) in the ^{13}C spectrum. The second carbonyl group was located at C-2 as shown by the connectivities of H_2-1 and H-10 with this carbon in HMBC spectrum. The relative stereochemistry of **3** was assigned by analysis of NOESY correlations as for **1** and **2**. Me-20 showed strong correlations with 2H-11, Me-17 and Me-19 suggesting that they were all on the same face of the molecule. Compound **3** was therefore identified as *ent*-2,7-dioxo-3-cleroden-15-oic acid.

For compound **4**, the HRFABMS showed a pseudomolecular ion peak at *m*/*z* 523.2687 $[M + Na]^+$ (calcd. 507.2672 for $C_{29}H_{40}O_7Na$) in agreement with the molecular formula of $C_{29}H_{40}O_7$. Its ^{1}H NMR spectrum was markedly similar to that of **1** except for the presence of a caffeoyl moiety, a primary oxygenated carbon (δ_H 4.67 (*br s*, 2H-18), δ_C 66.0 (C-18)) and the lack of a vinyl methyl group. The spectroscopic data for the caffeoyl residue are in Tables 1 and 2 and in Section 3. An HMBC correlation from 2H-18 to the ester carbonyl at δ_C 169.1 established the connection of the caffeoyl group to the clerodane skeleton. The NOESY spectrum revealed the same relative stereochemistry as **1**. Hence, compound **4** was elucidated as *ent*-18-*E*-caffeoyloxy-7β-hydroxy-3-cleroden-15-oic acid.

Compound **5** (ν_{max} 3352, 1663, 1587 and 1416 cm^{-1}, λ_{max} 312, 296 and 226 nm), a colorless oil, contained ester, carboxylic acid and conjugated aromatic functionality. Its molecular formula $C_{29}H_{40}O_5$ was established from the $[M + NH_4]^+$ ion at *m*/*z* 486.3227 (calcd. 486.3219 for $C_{29}H_{44}O_5N$) in its HRCIMS spectrum. Its ^{1}H NMR spectrum (Table 3) revealed the presence of two tertiary methyls δ_H 0.84 and 0.7, a secondary methyl signal δ_H 0.96 (*d*, $J = 6.6$ Hz), an exomethylene group δ_H 4.47 and 4.80 (both *s*), an oxygenated methylene group δ_H 3.73, 3.96 (both *d*, $J = 10.9$ Hz) and a *trans p*-hydroxycinnamoyl moiety (cou-

Table 2
^{1}H NMR data of clerodane diterpenoids

Position	1[a]	2[a]	3[a]	4[b]
1	1.53, *m*	2.34, *dd* (3.9, 17.5) 2.44, *dd* (13.7, 17.5)	2.34, *m*	1.59, *m*
2	1.96, *m* 2.01, *m*			2.13, *m*
3	5.11, *m*	5.67, *d* (1.3)	5.77, *d* (1.2)	5.58, *br s*
6	1.37, *dd* (3.3, 14.1) 2.07, *dd* (2.6, 14.1)	1.50, *dd* (3.5, 14.0) 2.17, *dd* (2.8, 14.0)	2.34, *m* 2.51, *d* (11.9)	1.53, *dd* (3.1, 13.5) 2.10, *dd* (2.8, 13.5)
7	3.98, *q* (3.3)	4.06, *ddd* (2.8, 3.2, 3.5)		3.97, *ddd* (2.8, 3.1, 3.4)
8	1.51, *m*	1.53, *dd* (3.2, 7.2)	2.56, *q* (6.3, 12.8)	1.56, *m*
10	1.37, *m*	1.89, *dd* (3.9, 13.7)	2.46, *dd* (4.1, 11.2)	1.47, *dd* (2.1, 11.4)
11	1.28, *m*	1.25, *m*	1.26, *m* 1.41, *dd* (9.8, 12.6)	1.31, *m*
12	1.00, *m* 1.16, *ddd* (5.5, 12.3)	1.00, *m* 1.09, *m*	1.09, *m* 1.26, *m*	1.21, *m*
13	1.84, *m*	1.83, *m*	1.88, *m*	1.80, *m*
14	2.14, *dd* (7.9, 15.1) 2.33, *dd* (6.2, 15.1)	2.15, *dd* (8.7, 15) 2.29, *dd* (6.4, 15)	2.19, *dd* (7.3, 15.1) 2.28, *dd* (6.4, 15.1)	2.15, *dd* (14.8, 7.2) 2.23, *dd* (14.8, 7.0)
16	0.96, *d* (6.9)	0.95, *d* (6.7)	0.98, *d* (6.7)	0.97, *d* (6.7)
17	0.98, *d* (7.2)	1.00, *d* (7.2)	0.92, *d* (6.3)	1.00, *d* (6.1)
18	1.59, *d* (1.4)	1.89, *d* (1.3)	1.85, *d* (1.2)	4.67, *br s*
19	1.26, *s*	1.37, *s*	1.08, *s*	1.40, *s*
20	0.97, *s*	1.05, *s*	0.77, *s*	1.02, *s*
2′				7.03, *d* (2.0)
5′				6.77, *d* (8.2)
6′				6.93, *dd* (2.0, 8.2)
7′				7.52, *d* (15.9)
8′				6.25, *d* (15.9)

[a] Spectra were recorded in $CDCl_3$.
[b] Spectra were recorded in CD_3OD.

maroyl, see Tables 1 and 3). The COSY spectrum revealed spin systems associated with rings A and B and the side chain of a labdane skeleton which was confirmed by several correlations in the HMBC spectrum, in particular from H-7 to C-8, C-9 and C-17, H-5 to C-4, C-9, C-10 and C-20, H-1 to C-5, C-10 and C-20, H-3 to C-4 and C-19. Support for the side chain came from correlations of H-14 to C-15 and C-16 and from H-12 to C-9 and C-11. The oxymethylene protons clearly belonged to C-18 in view of HMBC correlations to C-3, C-4, C-5 and C-19. A further correlation of these to the coumarate ester carbonyl group at δ 167.8 confirmed the position of attachment of the ester. NOE interactions between Me-19 and Me-20, 2H-18 and H-5, and H-5 and H-9 indicated a typical A/B *trans* labdane system.

The absolute configuration at C-13 was also determined by applying the phenylglycine methyl ester method (PGME). The chemical shift difference values $\Delta\delta_{RS} = \delta_R - \delta_S$ of (*R*)-(**5**) and (*S*)-(**5**) PGME amides are shown in Fig. 2. From these data the absolute configuration of the chiral carbon at C-13 was identified to be *S* and compound **5** belongs to the *enantio* series. Thus, the structure of **5** was established as (13*S*)-*ent*-18-*E*-coumaroyloxy-8(17)-labden-15-oic acid (see Fig. 2).

The HRFABMS of compound **6** gave a pseudomolecular ion peak at *m*/*z* 507.2725 $[M + Na]^+$ (calcd. 507.2723 for $C_{29}H_{40}O_6Na$) corresponding to a molecular formula $C_{29}H_{40}O_6$. Its UV spectrum was characteristic of a caffeoyl moiety with absorption maxima at 327, 295, 245, 220 and 203 nm. The ^{1}H NMR spectrum of **6** resembled that of **5** apart differences in the aromatic region (Table 3). Examination of the proton and carbon data readily revealed that the coumaroyl moiety of **5** had been replaced by a caffeoyl moiety. The structure of **6** was determined as *ent*-18-*E*-caffeoyloxy-8(17)-labden-15-oic acid.

Compound **7** was obtained as colourless oil with a molecular formula of $C_{29}H_{40}O_6$ (*m*/*z* 507.2739) based on HRFABMS and ^{13}C data. The spectroscopic data, including the UV (λ_{max} 328, 293, 245, 220, 203 nm), indicated the presence of a caffeoyl moiety. The lack of 2H-14 resonances, present in **1**–**6** between δ_H 2.08 and 2.33, suggested that C-15 was no longer a carboxyl group but had been reduced to an alcohol. The spectroscopic properties indicated that **7** was a derivative of the C-4 epimer of *ent*-15-hydroxy-8(17)-labden-19-oic acid (Zdero et al., 1991a). The downfield shift 2H-15 and an HMBC correlation from 2H-15 to the caffeoyl carbonyl group revealed the position of attachment of the ester. NOEs between H-9 and H-5 and Me-19 and Me-20 established the *trans* nature of the ring junction and the presence of a C-18 carboxyl group. Thus compound **7** was assigned as 15-*E*-caffeoyloxy-8(17)-labden-18-oic acid.

All the isolated compounds were tested for their ability to inhibit in vitro the growth of the chloroquine-resistant strain FcB1 of *P. falciparum*. The antiplasmodial activity

Table 3
^{1}H NMR data of labdane diterpenoids

Position	5[a]	6[b]	7[b]
1	0.95, *m*	1.09, *m*	1.16, *dd* (4.7, 7.8)
	1.71, *m*	1.83, *m*	1.79, *m*
2	1.55, *m*	1.59, *m*	1.51, *m*
		1.70, *m*	
3	1.35, *m*	1.47, *m*	1.51, *m*
			1.71, *m*
5	1.40, *m*	1.51, *m*	1.96, *dd* (2.2, 12.5)
6	1.35, *m*	1.40, *m*	1.32, *m*
	1.40, *m*	1.69, *m*	1.44, *m*
7	1.96, *dd* (4.9, 13.2)	1.99, *m*	2.02, *dd* (4.7, 13.1)
	2.35, *m*	2.38, *m*	2.36, *m*
9	1.59, *d* (9.5)	1.65, *m*	1.65, *m*
11	1.35, *m*	1.42, *m*	1.44, *m*
	1.44, *m*	1.52, *m*	1.51, *m*
12	1.14, *m*	1.16, *m*	1.24, *m*
	1.31, *m*	1.37, *m*	1.32, *m*
13	1.90, *m*	1.89, *m*	1.51, *m*
14	2.12, *dd* (7.8, 15.1)	2.08, *dd* (7.7; 14.6)	1.51, *m*
	2.27, *dd* (6.2, 15.1)	2.24, *dd* (6.6, 14.6)	1.71, *m*
15			4.17, *m*
16	0.96, *d* (6.6)	0.96, *d* (6.6)	0.96, *d* (6.3)
17	4.47, *br s*	4.53, *s*	4.55, *s*
	4.80, *br s*	4.83, *s*	4.84, *s*
18	3.73, *d* (10.9)	3.75, *d* (10.8)	
	3.96, *d* (10.9)	3.99, *d* (10.8)	
19	0.84, *s*	0.89, *s*	1.12, *s*
20	0.70, *s*	0.77, *s*	0.74, *s*
2′	7.42, *d* (8.7)	7.06, *d* (2.0)	7.04, *d* (2.0)
3′	6.82, *d* (8.6)		
5′	6.82, *d* (8.6)	6.78, *d* (8.2)	6.77, *d* (8.3)
6′	7.42, *d* (8.7)	6.97, *dd* (2.0, 8.2)	6.94, *dd* (2.0, 8.3)
7′	7.59, *d* (15.9)	7.55, *d* (15.9)	7.52, *d* (15.9)
8′	6.30, *d* (15.9)	6.29, *d* (15.9)	6.24, *d* (15.9)

[a] Spectra were recorded in $CDCl_3$.
[b] Spectra were recorded in CD_3OD.

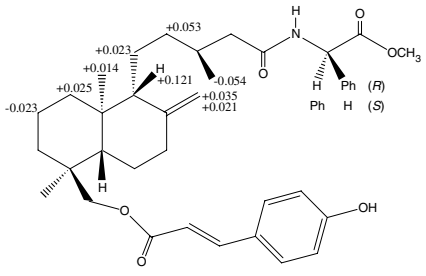
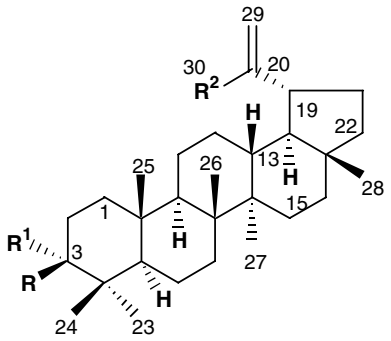


Fig. 2. $\Delta\delta(R - S)$ values (in ppm) for PGME amide derivatives of **5r** and **5s** in $CDCl_3$.

of new compounds **1**–**7** and the most active known compounds (**8**–**12**) are summarized in Table 4. The other compounds displayed IC_{50} value over 10 μg/ml. Compounds **8** and **9** displayed significant antimalarial activity with IC_{50} value between 1.55 and 4.67 μg/ml depending upon the *P. falciparum* strain tested (Fig. 3). In comparison to chloroquine which was used as a positive control, these compounds have to be considered as moderately active (Table 4).



8 R, R^1 = O , R^2 = CHO

9 R = OH, R^1 = H, R^2 = CHO

10 R, R^1 = O, R^2 = CH_2OH

Fig. 3. Structures of lupane derivatives **8**–**10**.

Table 4
In vitro antiplasmodial activity of compounds **1**–**12**

Compound	$IC_{50} \pm SD$ (μg/ml) FcB1	$IC_{50} \pm SD$ (μg/ml) FcM29
1	14.6 ± 1.4	
2	4.3 ± 0.9	
3	8.0 ± 0.2	
4	7.3 ± 0.8	
5	11.4 ± 1.1	
6	21.0 ± 1.85	
7	16.0 ± 0.87	
8	1.55 ± 0.06	4.67 ± 0.09
9	3.15 ± 0.07	4.06 ± 0.53
10	9.05 ± 1.06	15.56 ± 2.11
11	7.65 ± 0.49	
12	9.83 ± 3.1	
Chloroquine	0.05 ± 0.002	

Results are expressed as IC_{50} and IC_{90} values (μg/ml) ± standard deviations. All experiments were realised in triplicate.

For **8** and **9**, the C-30 aldehyde and the C-3 ketone probably have a major influence on the activity. The activity of **10**, with a C-30 hydroxyl group, was lower and with hydroxyls at both C-3 and C-30 the activity diminished considerably.

Ursolic acid exhibited antiplasmodial activity with IC_{50} value of 16 μg/ml which is consistent with that published (Azas et al., 2002). In vitro antiplasmodial activity of **12** has been previously reported with IC_{50} value similar to those found in the present work (Sairafianpour et al., 2003). The lupane triterpenoids betulinic acid and lupeol, as well as labdane diterpenoids have been reported to exhibit moderate antiplasmodial activity in vitro (Bringmann et al., 1997; Ziegler et al., 2002; Asili et al., 2004).

It has been demonstrated that terpenoids can be incorporated into the lipid bilayer of erythrocyte membranes irreversibly and induce shape transformation of this membrane (Ziegler et al., 2002; Asili et al., 2004). In this case,

the inhibition of parasite growth observed in vitro could be attributed to indirect effects due to stomatocytic or echinocytic modifications of the host cell membrane.

Non-parasitized erythrocytes were then incubated with increasing concentrations of compounds **8**, **9** and **10** under the same conditions as previously described (Ziegler et al., 2002). No lysis of cells and no change of erythrocyte membrane shape in echnocytic forms were observed at the concentrations up to 50 μg/ml by phase-contrast microscopy. There was also no evidence of stomatocytic forms. However, such modification might not be clearly detectable by photonic microscopy, further investigation using transmission electron microscopy will be required to confirm our observations.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Perkin–Elmer model 341 polarimeter at 20 °C. IR spectra were taken on a Nicolet Impact 400D spectrophotometer. The UV spectra were recorded on a Kontron spectrometer. ^{1}H and ^{13}C NMR spectra were recorded at 400.13 and 100.61 MHz, respectively, on a Bruker AVANCE-400 spectrometer at 298 K, equipped with ^{1}H-broad-band reverse gradient probehead. Temperature was controlled by a Bruker BCU-05 refrigeration unit and a BVT 3000 control unit. The ^{1}H and ^{13}C NMR chemical shifts were expressed in ppm relative to TMS, with coupling constants (*J*) given in Hz. High-resolution mass spectra and FAB-MS were recorded on a JEOL MS700 apparatus. Mass spectra data were recorded using an electrospray time of flight mass spectrometer (ESI-TOF-MS) operating in the positive mode (QSTAR Pulsar I of Applied Biosystems). TLC was carried out on precoated Si gel 60 F_{254} plates (Merck). Spots were detected under UV (254 and 366 nm) before spraying with phosphomolybdic acid solution in EtOH or Liebermann–Burchard reagent or vanillin-sulfuric solution followed by heating the plate at 110 °C. Column chromatography was performed on 200–400 mesh silica gel 60 (Merck). Preparative medium-pressure liquid chromatography (MPLC) was performed with a pump K-120 (Knauer) and Flashsmart cartridges (Si gel 20–40 μm, AIT, France).

3.2. Plant material

The plant material was collected in March 2000 in Ankazobe, middle West located at 100 km North from Antananarivo (Madagascar) and was identified by Armand Rakotozafy by comparison with authentic specimens held in the Department of Botany, Parc Botanique et Zoologique de Tsimbazaza, Antananarivo. A voucher specimen (ANKA 15/AR/2000) was deposited at the Institut Malgache de Recherches Appliquées.

3.3. Bioassay

The in vitro antiplasmodial tests, based on the inhibition of [^{3}H]-hypoxanthine uptake by *P. falciparum* cultured in human blood, were conducted as previously described (Frappier et al., 1996).

3.4. Extraction and isolation

Air-dried powdered leaves of *N. sphaerocephala* (600 g) was exhaustively extracted with ethanol (3 × 500 ml) at room temperature to give 31 g of crude extract. The ethanolic extract exhibited an IC_{50} value of 7.1 μg/ml against the growth of *P. falciparum*. A portion of the crude extract (14 g) was partitioned between ethyl acetate (3 × 500 ml) and water (300 ml). The organic fraction (10.8 g) showed an IC_{50} value of 4.2 μg/ml whereas the aqueous fraction was inactive (1.27 g; $IC_{50} > 25$ μg/ml). A portion (5 g) of the ethyl acetate extract was chromatographed on a silica gel column using a mixture of cyclohexane–EtOAc of increasing polarity as eluant to give 22 fractions. The purification of the most potent fractions F1 and F2 on silica gel column chromatography (cyclohexane–EtOAc 90:10) yielded 27 mg of compound **8**. Chromatography of F6 with cyclohexane–EtOAc 85:15 followed by crystallization (acetone) afforded 42 mg of compound **9**. Repeated chromatography of fraction F11 on MPLC column (AIT, France) with CH_2Cl_2–MeOH 98:2 furnished 8 mg of compound **1**. Chromatography of fraction F12 yielded additional compound **1**. From fraction F15, compound **5** (8 mg) was obtained after repeated purification on silica gel with cyclohexane–EtOAc 75:25 followed by CH_2Cl_2–MeOH 97:3. F16 was purified by silica gel column chromatography (CH_2Cl_2–MeOH 98:2) to afford 13 mg of compound **7**. Compound **6** (9 mg) was isolated from fraction F17 by successive chromatography on silica gel with cyclohexane–EtOAc 60:40 and on a Sephadex column using MeOH as eluting solvent. F18 was purified by silica gel column chromatography (cyclohexane–EtOAc 60:40 and CH_2Cl_2–MeOH 95:5) to afford 11 mg of compound **4**. Fraction F22 afforded compound **3** (2 mg) and compound **2** (5 mg) by further purification by column chromatography on silica gel eluting with CH_2Cl_2–MeOH 98:2.

3.4.1. (13*S*)-ent-7β-Hydroxy-3-cleroden-15-oic acid (**1**)

Colourless oil; $[\alpha]_D^{20}$ − 32.3 (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2925, 1712, 1470, 1066 cm^{-1}; UV (MeOH) λ_{max} (log ε) 237 (3.06), 203 (3.48) nm; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRCIMS, *m/z*: 340.2845 ($[M + NH_4]^+$) (calcd. for $C_{20}H_{38}O_3N$, 340.2852).

3.4.2. ent-7β-Hydroxy-2-oxo-3-cleroden-15-oic acid (**2**)

Colourless oil; $[\alpha]_D^{20}$ − 30 (*c* 0.195, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2925, 1657, 1470, 1029 cm^{-1}; UV (MeOH) λ_{max} (log ε) 281 (2.90), 238 (3.56), 203 (3.52) nm; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; HRFABMS, *m/z*: 337.2373 ($[M + H]^+$) (calcd. for $C_{20}H_{33}O_4$, 337.2379).

*3.4.3. ent-2,7-Dioxo-3-cleroden-15-oic acid (**3**)*

Colourless oil; $[\alpha]_D^{20}$ − 10 (*c* 0.215, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2931, 2372; 1663, 1458, 1079 cm^{-1}; UV (MeOH) λ_{max} (logε) 276 (2.70), 229 (3.29), 203 (3.47) nm; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; HRCIMS, *m/z*: 335.2224 ($[M + H]^+$) (calcd. for $C_{20}H_{31}O_4$, 335.2222).

*3.4.4. ent-18-E-Caffeoyloxy-7β-hydroxy-3-cleroden-15-oic acid (**4**)*

Colourless oil; $[\alpha]_D^{20}$ − 29 (*c* 0.165, MeOH); IR ($CHCl_3$) ν_{max} 2926, 1660, 1610, 1418, 1279, 1165, 1032, 797 cm^{-1}; UV (MeOH) λ_{max} (logε) 329 (4.02), 295 (3.91), 245 (3.81), 218 (3.97), 204 (4.02) nm; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; HRFABMS, *m/z*: 523.2687 ($[M + Na]^+$) (calcd. for $C_{29}H_{40}O_7Na$, 523.2672).

*3.4.5. (13S)-ent-18-E-Coumaroyloxy-8(17)-labden-15-oic acid (**5**)*

Colourless oil: $[\alpha]_D^{20}$ − 2.4 (*c* 0.365, MeOH); IR ($CHCl_3$) ν_{max} 2928, 1663, 1587, 1416, 1158, 1024 cm^{-1}; UV (MeOH) λ_{max} (logε) 312 (3.70), 296 (3.69), 226 (3.58), 203 (3.83) nm; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; HRCIMS, *m/z*: 486.3227 ($[M + NH_4]^+$) (calcd. for $C_{29}H_{44}O_5N$, 486.3219).

*3.4.6. ent-18-E-Caffeoyloxy-8(17)-labden-15-oic acid (**6**)*

Colourless oil; $[\alpha]_D^{20}$ − 8.9 (*c* 0.373, MeOH); IR ($CHCl_3$) ν_{max} 2371, 1640, 1270, 1165, 1038 cm^{-1}; UV (MeOH) λ_{max} (logε) 327 (3.93), 295 (3.84), 245 (3.76), 220 (3.96), 203 (4.15) nm; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; HRFABMS, *m/z*: 507.2725 ($[M + Na]^+$) (calcd. for $C_{29}H_{40}O_6Na$, 507.2723).

*3.4.7. ent-15-E-Caffeoyloxy-8(17)-labden-18-oic acid (**7**)*

Colourless oil; $[\alpha]_D^{20}$ − 21 (*c* 0.21, MeOH); IR ($CHCl_3$) ν_{max} 2371, 1659, 1273, 1170, 1026 cm^{-1}; UV (MeOH) λ_{max} (logε) 328 (3.78), 294 (3.84), 245 (3.64), 220 (3.83), 203 (4.00) nm; ^1H NMR and ^{13}C NMR data, see Tables 1 and 3; HRFABMS, *m/z*: 507.2739 ($[M + Na]^+$) (calcd. for $C_{29}H_{40}O_6Na$, 507.2723).

*3.4.8. ent-15-Hydroxy-8(17)-labden-19-oic acid (**13**)*

Colourless oil; $[\alpha]_D^{20}$ − 23 (*c* 0.165, $CHCl_3$), no lit. value available. ^1H NMR and ^{13}C NMR spectral data consistent with the literature values (Zdero et al., 1991a).

*3.4.9. ent-18-Hydroxy-8(17)-labden-15-oic acid (**14**)*

Colourless oil; $[\alpha]_D^{20}$ − 34 (*c* 0.40, $CHCl_3$); lit. $[\alpha]_D^{20}$ − 37 (*c* 2.3, $CHCl_3$) as methyl ester (Hugel et al., 1966). ^1H NMR and ^{13}C NMR spectral data consistent with the literature values (Zdero et al., 1991b).

*3.4.10. ent-18-Hydroxy-3-cleroden-15-oic acid (**15**)*

Colourless oil; $[\alpha]_D^{20}$ − 28.4 (*c* 0.25, $CHCl_3$), no lit. value available. ^1H NMR and ^{13}C NMR spectral data consistent with the literature values (Tsichritzis and Jakupovic, 1990).

*3.4.11. Preparation of the (R)- and (S)-PGME amide derivatives of **1** and **5***

(*R*)- and (*S*)-phenylglycine methyl ester were obtained by esterification of phenylglycine (Nagai and Kusumi, 1995).

Two portions of compound **1** (each 4 mg; 0.0124 mmol) were separately stirred with (*R*)-PGME (3.1 mg; 0.0155 mmol) and (*S*)-PGME (3.1 mg, 0.0155 mmol) in dry DMF (0.5 ml). To these solutions were added successively 1*H*-benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (6.5 mg, 0.0155 mmol), 1-hydroxybenzotriazole (2.4 mg, 0.0155 mmol) and *N*-methylmorpholine (5 μl) at 0 °C. After stirring at room temperature for 5 h, ethyl acetate was added to the reaction mixture which was successively washed with HCl 0.1 N, saturated $NaHCO_3$ solution and brine. The organic layers were dried over Na_2SO_4 and concentrated under vacuum. Purification on silica gel column chromatography (cyclohexane–EtOAc 75:25) afforded 3 mg of compound **1r** and **1s** in 52% yield. ^1H NMR data of the (*R*)-PGME amide derivative (**1r**): (400 MHz, $CDCl_3$) δ 7.341–7.312 (5H, *m*, Ar-H), 6.358 (1H, *d*, $J = 7.2$ Hz, NH), 5.570 (1H, *d*, $J = 7.2$ Hz, H-α), 5.113 (1H, *d*, $J = 1.4$ Hz, H-3), 3.971 (1H, *q*, $J = 3.3$ Hz, H-7), 3.708 (3H, *s*, CO_2CH_3), 2.249 (1H, *dd*, $J = 5.7$, 13.9 Hz, H-14a), 2.043 (1H, *dd*, $J = 2.7$, 14.2 Hz, H-6a), 1.952 (1H, *m*, H-2a), 1.930 (1H, *dd*, $J = 5.3$, 13.9 Hz, H-14b), 1.872 (1H, *m*, H-2b), 1.806 (1H, *m*, H-13), 1.590 (3H, *d*, $J = 1.3$ Hz, CH_3-18), 1.476 (3H, *m*, 2H-1, H-8), 1.312 (2H, *m*, H-6b, H-10), 1.255 (3H, *s*, CH_3-19), 1.233 (2H, *m*, 2H-11), 1.072 (1H, *m*, H-12a), 0.953 (3H, *d*, $J = 7.3$ Hz, CH_3-17), 0.949 (4H, *br s*, H-12b, CH_3-20), 0.859 (3H, *d*, $J = 6.5$ Hz, CH_3-16); ^1H NMR data of the (*S*)-PGME amide derivative (**1s**): (400 MHz, $CDCl_3$) δ 7.344–7.320 (5H, *m*, Ar-H), 6.327 (1H, *d*, $J = 7.1$ Hz, NH), 5.570 (1H, *d*, $J = 7.1$ Hz, H-α), 5.099 (1H, *d*, $J = 1.4$ Hz, H-3), 3.957 (1H, *q*, $J = 3.3$ Hz, H-7), 3.713 (3H, *s*, CO_2CH_3), 2.213 (1H, *dd*, $J = 5.7$, 14.1 Hz, H-14a), 2.037 (1H, *dd*, $J = 2.7$, 14.1 Hz, H-6a), 1.952 (3H, *m*, H-2a, H-14b), 1.864 (1H, *m*, H-2b), 1.824 (1H, *m*, H-13), 1.572 (3H, *d*, $J = 1.2$ Hz, CH_3-18), 1.485 (2H, *m*, 2H-1), 1.476 (1H, *m*, H-8), 1.313 (2H, *m*, H-6b, H-10), 1.248 (3H, *s*, CH_3-19), 1.212 (2H, *m*, 2H-11), 1.051 (1H, *m*, H-12a), 0.934 (7H, *m*, H-12b, CH_3-17, CH_3-20), 0.917 (3H, *d*, $J = 6.6$ Hz, CH_3-16).

*3.4.12. Preparation of the (R)- and (S)-PGME amide derivatives of **5***

Compound **5** (each 6 mg) was condensed with (*R*)- and (*S*)-PGME under the same conditions above to yield after purification on silica gel column chromatography (cyclohexane–EtOAc 80:20) 3 mg of **5r** and **5s**. ^1H NMR data of the (*R*)-PGME amide derivative (**5r**): (400 MHz, $CDCl_3$) δ 7.355–7.326 (5H, *m*, Ar-H), 7.273 (2H, *d*, $J = 8.4$ Hz, H-2′, H-6′), 6.939 (1H, *d*, $J = 12.4$ Hz, H-7′), 6.774 (2H, *d*, $J = 8.6$ Hz, H-3′, H-5′), 6.517 (1H, *d*, $J = 6.8$ Hz, NH), 5.768 (1H, *d*, $J = 12.4$ Hz, H-8′), 5.567 (1H, *d*, $J = 7.0$ Hz, H-α), 4.713 (1H, *br s*, H-17a), 4.363 (1H, *br s*,

H-17b), 3.795 (1H, *d*, $J = 11.4$ Hz, H-18a), 3.716 (3H, *s*, CO_2CH_3), 3.553 (1H, *d*, $J = 11.4$ Hz, H-18b), 2.199 (1H, *m*, H-7a), 2.152 (2H, *m*, 2H-14), 1.855 (1H, *m*, H-13), 1.630 (1H, *m*, H-7b), 1.579 (1H, *m*, H-1a), 1.480 (1H, *m*, H-3a), 1.420 (2H, *m*, 2H-2), 1.350 (1H, *m*, H-6a), 1.257 (2H, *m*, 2H-11), 1.176 (1H, *m*, H-6b), 1.154 (3H, *m*, H-9, 2H-12), 1.132 (1H, *m*, H-3b), 0.896 (1H, *m*, H-1b), 0.876 (1H, *m*, H-5), 0.860 (3H, *d*, $J = 6.5$ Hz, CH_3-16), 0.686 (3H, *s*, CH_3-19), 0.563 (3H, *s*, CH_3-20); ^{1}H NMR data of the (*S*)-PGME amide derivative (**5s**): (400 MHz, $CDCl_3$) δ 7.360–7.321 (5H, *m*, Ar-H), 7.113 (2H, *d*, $J = 8.3$ Hz, H-2′, H-6′), 6.922 (1H, *d*, $J = 12.5$ Hz, H-7′), 6.534 (2H, *d*, $J = 8.6$ Hz, H-3′, H-5′), 6.647 (1H, *d*, $J = 6.7$ Hz, NH), 5.754 (1H, *d*, $J = 12.5$ Hz, H-8′), 5.580 (1H, *d*, $J = 6.8$ Hz, H-α), 4.691 (1H, *br s*, H-17a), 4.328 (1H, *br s*, H-17b), 3.754 (1H, *d*, $J = 11.4$ Hz, H-18a), 3.722 (3H, *s*, CO_2CH_3), 3.539 (1H, *d*, $J = 11.4$ Hz, H-18b), 2.314 (1H, *m*, H-14a), 2.277 (1H, *m*, H-14b), 2.125 (2H, *m*, 2H-7), 1.873 (1H, *m*, H-13), 1.554 (2H, *m*, 2H-1), 1.482 (3H, *m*, 2H-2, H-3a), 1.267 (2H, *m*, 2H-6), 1.233 (2H, *m*, 2H-11), 1.089 (2H, *m*, 2H-12), 1.033 (1H, *m*, H-9), 0.913 (3H, *d*, $J = 6.5$ Hz, CH_3-16), 0.809 (1H, *m*, H-5), 0.669 (3H, *s*, CH_3-19), 0.548 (3H, *s*, CH_3-20).

Acknowledgment

This work was supported by a grant from the programme VIH-Pal, *Ministère Français de la Recherche*.

References

Ahmad, V.U., Ghazala, Uddin, S., 1992. A triterpenoid from *Zygophyllum propinquum*. Phytochemistry 31, 1051–1054.

Asili, J., Lambert, M., Ziegler, H.L., Staerk, D., Sairafianpour, M., Witt, M., Asghari, G., Inrahimi, I.S., Jaroszewski, J.W., 2004. Labdanes and isopimaranes from *Plactycladus orientalis* and their effects on erythrocyte membrane and on *Plasmodium falciparum* growth in the erythrocyte host cells. J. Nat. Prod. 67, 631–637.

Azas, N., Laurencin, N., Delmas, F., Di Gorgio, C., Gasquet, M., Laget, M., Timon-David, P., 2002. Synergistic in vitro antimalarial activity of plant extracts used as traditional herbal remedies in Mali. Parasitol. Res. 88, 165–171.

Biftu, T., Stevenson, R., 1978. Flavone and triterpenoid constituents of *Elaegia utilis*. J. Chem. Soc., Perkin Trans. I, 360–363.

Bohlmann, F., Jakupovic, J., 1979. Neue sesquiterpene, triterpene, flavanone und andere aromatische Verbindungen aus *Flourensia heterolepis*. Phytochemistry 18, 1189–1194.

Boiteau, P., 1999. Dictionnaire des Noms Malgaches des Végétaux, vol. II. Alzieu Editions, Grenoble, France, p. 281.

Bringmann, G., Saeb, W., Aké Assi, L., François, G., Narayanan, A.S., Peters, K., Peters, E.-M., 1997. Betulinic acid. Isolation from *Triphyophyllum peltatum* and *Ancistrocladus heyneanus*, antimalarial activity and crystal structure of the benzyl ester. Planta Med. 63, 255–257.

Chavez, M.I., Delgado, G., 1994. Isolation and relay synthesis of 11α-hydroperoxy-diacetylhederagenin, a novel triterpenoid derivative from *Serjania triquetra* (Sapindaceae). Biogenetic implications. Tetrahedron 50, 3869–3878.

Dehmlow, E.V., Ree, T.V., Jakupovic, J., Take, E., Künsting, H., 1998. Notes on activity tests and constituents of two supposed medicinal plants from South Africa, *Englerophytum magalismontanum* and *Diospyros lycioides des. Subsp. Sericea*. J. Chem. Res. (S) 5, 252–253.

Frappier, F., Jossang, A., Soudon, J., Calvo, F., Rasoanaivo, P., Ratsimamanga-Urverg, S., Saez, J., Schrével, J., Grellier, P., 1996. Bisbenzylisoquinolines as modulators of chloroquine resistance in *Plasmodium falciparum* and multidrug resistance in tumor cells. Antimicrob. Agents Chemother. 40, 1476–1481.

Frederiksen, L.B., Damtoft, S., Jensen, S.R., 1999. Biosynthesis of iridoids lacking C-10 and the chemotaxonomic implications of their distribution. Phytochemistry 52, 1409–1420.

Gaydou, E.M., Bianchini, J.-P., 1978. Etudes de composés flavoniques. Bull. Soc. Chim. Fr. II, 43–47.

Houghton, P.J., Lian, L.M., 1986. Triterpenoids from *Desfontainia spinosa*. Phytochemistry 25, 1939–1944.

Hugel, G., Oehlschlager, A.C., Ourisson, G., 1966. The structure and stereochemistry of diterpenes from *Trachylobium verrucosum*. Tetrahedron 22 (Suppl. 8), 203–216.

Jensen, S.R., Ravnkilde, L., Schripsema, J., 1998. Unedoside derivatives in *Nuxia* and their biosynthesis. Phytochemistry 47, 1007–1011.

Mongkolsuk, S., Dean, F.M., 1964. Pinostrobin and alpinetin from *Kaempferia pandurata*. J. Chem. Soc., 4654–4655.

Nagai, Y., Kusumi, T., 1995. New chiral anisotropic reagents for determining the absolute configuration of carboxylic acids. Tetrahedron Lett. 36, 1853–1856.

Rasoanaivo, P., Ramanitrahasimbola, D., Rafatro, H., Rakotondramanana, D., Robijaona, B., Rakotozafy, A., Ratsimamanga-Urverg, S., Labaïed, M., Grellier, P., Allorge, L., Mambu, L., Frappier, F., 2004. Screening extracts of Madagascan plants in search of antiplasmodial compounds. Phytother. Res. 18, 742–747.

Sairafianpour, M., Bahreininejad, B., Witt, M., Ziegler, H.L., Jaroszewski, J.W., Staerk, D., 2003. Terpenoids of *Salvia hydrangea*: two new, rearranged 20-norabietanes and the effect of oleanolic acid on erythrocyte membranes. Planta Med. 69, 846–850.

Tsichritzis, F., Jakupovic, J., 1990. Diterpenes and other constituents from *Relhania* species. Phytochemistry 29, 3173–3187.

Wijeratne, D.B.T., Kumar, V., Sultanbawa, M.U.S., 1981. 3-Oxolup-20(29)-en-30-al, a new lupane from *Gymnosporia emarginata* (Celastraceae). J. Chem. Soc., Perkin Trans. I, 2724–2726.

Yabuuchi, T., Kusumi, T., 2000. Phenylglycine methyl ester, a useful tool for absolute configuration determination of various chiral carboxylic acids. J. Org. Chem. 65, 397–404.

Zdero, C., Bohlmann, F., King, R.M., 1991a. Diterpenes and norditerpenes from the *Aristeguetia* group. Phytochemistry 30, 2991–3000.

Zdero, C., Bohlmann, F., King, R.M., 1991b. Guaianolides and labdanes from *Brickellia* species. Phytochemistry 30, 1591–1595.

Ziegler, H.L., Staerk, D., Christensen, J., Hviid, L., Hägerstrand, H., Jaroszewski, J.W., 2002. In vitro *Plasmodium falciparum* drug sensitivity assay: inhibition of parasite growth by incorporation of stomatocytogenic amphiphiles into the erythrocyte membrane. Antimicrob. Agents Chemother. 46, 1441–1446.