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Five labdane diterpenoids from the seeds of *Aframomum zambesiacum*

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Abstract

Five labdane diterpenoids, (**3**–**5**), zambesiacolactone A (**7**) and zambesiacolactone B (**8**), were isolated from the seeds of *Aframomum zambesiacum* (Baker) K. Schum., along with five known labdanes and a linear sesquiterpene, nerolidol. Their structures were elucidated by spectroscopic analysis. Their antiplasmodial activity was evaluated in vitro against *Plasmodium falciparum*. Compound **3** was the most active with an IC_{50} value of 4.97 μM.

Keywords: *Aframomum zambesiacum*; Zingiberaceae; Labdane diterpenoids; Antiplasmodial activity; *Plasmodium falciparum*

1. Introduction

The genus *Aframomum* of the Zingiberaceae family includes 40 species and is most common in tropical and subtropical regions (Thomas et al., 1989). Twenty species are found in Cameroon, where they are widely used in traditional medicine, for spiritual purposes and as spices (Thomas et al., 1989). The compounds isolated from plants of this genus include flavonoids (De Bernardi et al., 1976; Ayafor and Connolly, 1981), diaryl heptanoids (Kamnaing et al., 2003), sesquiterpenes (Ayafor and Connolly, 1981) and labdane diterpenoids, specially in *Aframomum alboviolaceum* (Abreu and Noronha, 1997), *Aframomum aulacocarpos* (Ayafor et al., 1994a), *Aframomum daniellii* (Kimbu et al., 1979, 1987), *Aframomum escapum* (Ayimele et al., 2004), and *Aframomum sceptrum* (Tomla et al., 2002). A great deal of interest has been focused on the labdanes from *Aframomum* species, some of which exhibit antifungal, cytotoxic, and other biological activity (Ayafor et al., 1994a,b). In general, many labdanes from terrestrial plants and marine sources show antibacterial, antifungal, anti-inflammatory, antileishmanial, cardiotonic, cytotoxic, enzyme inhibitory (Singh et al., 1999), and trypanocidal (Scio et al., 2003) activities. Several *Aframomum* species (i.e., *Aframomum angustifolium*, *A. danielli*, *Aframomum sanguineum*, and*Aframomum sulcatum*) were traditionally used to treat fevers in Africa (Iwu, 1993), and recently, the antiplasmodial activity of some labdanes from *A. sceptrum* and *Aframomum latifolium* has been investigated (Duker-Eshun et al., 2002).

This paper describes the first phytochemical investigation of the seeds of *Aframomum zambesiacum* (Baker) K. Schum. This species was selected in the framework of a

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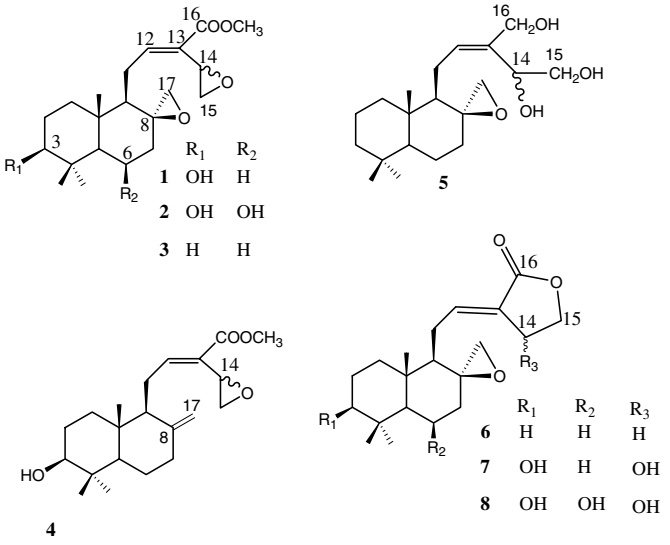
screening program to discover novel active compounds from Cameroonian medicinal plants. The structural elucidation of the isolated compounds was followed by evaluation of their in vitro antiplasmodial activity against *Plasmodium falciparum*.

2. Results and discussion

The dry, powdered seeds of *A. zambeciacum* were successively extracted with petroleum ether, chloroform, and methanol. Chromatographic purification of the two less polar extracts afforded 10 labdanes (**1**–**10**), five of which are new, and nerolidol (**11**) which was previously isolated from *Aframomum pruinosum* (Ayafor and Connolly, 1981) and *A. escapum* (Ayimele et al., 2004). The structures were assigned by analysis of spectroscopic data and by comparison with literature values. The five known labdanes were identified as aulacocarpin A (**1**) and aulacocarpin B (**2**), previously isolated from *Aframomum aulacocarpus* (Ayafor et al., 1994a) and *A. escapum* (Ayimele et al., 2004), and galanolactone (**6**), aframodial (**9**) and galanal A (**10**), isolated from *Alpinia galanga* (Morita and Itokawa, 1988).



	R_1	R_2	R_3
1	OH	H	
2	OH	OH	
3	H	H	
6	H	H	H
7	OH	H	OH
8	OH	OH	OH

Compound **3** was obtained as a white powder. Its molecular formula, $C_{21}H_{32}O_4$, was established by positive HRESI-MS (*m*/*z* $[M + Na]^+$ 371.2196). The strong IR absorptions at υ_{max} 1724 and 1645 cm^{-1} suggested the presence of an α,β-unsaturated ester. This is in agreement with the three ^{13}C NMR resonances for sp^2 carbons corresponding to an ester carbonyl (δ 166.8) and a trisubstituted olefin (δ 127.5 (CH) and δ 150.7 (C)). Thus, the compound is tetracyclic. The ^1H NMR spectrum revealed three tertiary methyls (δ 0.87, 0.9, 0.93) and a methyl ester (δ 3.75). Other proton signals included a deshielded vinyl proton [δ 6.81 (*t*, *J* = 6.5 Hz, H-12)], a monosubstituted epoxide [δ 3.60 (*brm*, H-14), 2.81 (*dd*, *J* = 5.5, 2.8 Hz, H-15a) and 2.99 (*dd*, *J* = 5.5, 4.3 Hz, H-15b)], a disubstituted epoxide [δ 2.30 (*d*, *J* = 3.9 Hz, H-17a) and 2.51 (*d*, *J* = 3.9 Hz, H-17b)]. These data suggested that compound **3** was a labdane diterpenoid related to aulacocarpin A (**1**) and B (**2**) (Ayafor et al., 1994a). All ^1H and ^{13}C NMR signals for **3** were assigned by analysis of COSY, HSQC, and HMBC spectra. The ^1H and ^{13}C NMR data (Table 1) of **3** were almost identical to those of aulacocarpin A (**1**). In the ^{13}C NMR spectrum, the main difference was the replacement of the C-3 methine (δ 78.8) of **1** by a methylene (δ 42.1) in **3**. This difference was also evident in the ^1H NMR spectrum, where H-3 (δ 3.24, *dd*) of **1** was replaced by two methylene protons at δ 1.17 and 1.41 (both *m*). The relative configuration at C-8 was deduced from a NOESY correlation between H-17 and H-9 and by chemical shift comparison with aulacocarpin A (**1**) and B (**2**) (Ayafor et al., 1994; Morita and Itokawa, 1988). As in the case of **1**, the *E* configuration of the 12,13 double bond was deduced from NMR data (Ayafor et al., 1994a). Thus, compound **3** is 3-deoxyaulacocarpin A, or methyl-8β,17:14ξ,15-diepoxy-12*E*-labden-16-oate.

Compound **4**, $C_{21}H_{32}O_4$ (*m*/*z* $[M + Na]^+$ 371.2211 HRESI-MS in positive mode), a colorless oil, had bands in its IR spectrum at υ_{max} 3417 cm^{-1}, 1714, and 1644 cm^{-1} in agreement with the presence of a hydroxyl and an α,β-unsaturated ester. The ^1H and ^{13}C NMR spectral data of **4** (Table 1) were very similar to those of **1**. The only significant differences were the absence of the signals of the H_2-17 epoxide protons of **1** and the presence of an 8,17-*exo*methylene group [δ 4.86 (*d*, *J* = 0.9 Hz) and 4.49 (*d*, *J* = 0.9 Hz)] in **4**. The β-orientation of the hydroxyl group at C-3 was deduced from the coupling constants of H-3 [δ 3.27 (*dd*, *J* = 11.7 and 4.3 Hz)] and from the NOESY spectrum, while the *E* configuration of the 12,13 double bond follows from the deshielded nature of H-12. Therefore, **4** is methyl-14ξ,15-epoxy-3β-hydroxy-8(17),12*E*-labdadien-16-oate. The corresponding 3-deoxy-derivative has been reported from the seeds of *A. danielli* (Kimbu et al., 1987).

Compound **5**, $C_{20}H_{34}O_4$ (*m*/*z* $[M + Na]^+$ 361.2349 HRESI-MS in positive mode), was obtained as white powder, m.p. 154–155 °C, and showed an hydroxyl absorption (υ_{max} 3402 cm^{-1}) in its IR spectrum. The ^1H and ^{13}C NMR data (Table 1) of **5** indicated that it was also a labdane diterpenoid. The ^1H NMR spectrum showed three methyl singlets at δ 0.88, 0.92, and 0.92, the protons of three oxymethylene groups [δ_H 3.50, (*dd*, *J* = 11.3, 7.6 Hz, H-15a) and 3.60 (*dd*, *J* = 11.3, 4.6 Hz, H-15b), δ_H 4.04 (*d*, *J* = 12.9 Hz, H-16a) and 4.15 (*d*, *J* = 12.9 Hz, H-16b), and 2.27 (*d*,*J* = 4.1 Hz, H-17a) and δ_H 2.69 (*d*, *J* = 4.1 Hz, H-17b)] and a proton of an oxymethine (δ_H 4.6 (*dd*, *J* = 7.8, 4.3 Hz, H-14)). Comparison of the ^{13}C NMR data of **5** with those of **3** showed that the monosubstituted epoxide had been replaced by an oxymethylene (δ_C 66.1, C-15) and an oxymethine (δ_C 71.9, C-14). Further analyses of the NMR spectra led to the assignments of all protons

Table 1
^{1}H and ^{13}C NMR data of **3**, **4**, **5**, **7** and **8** in $CDCl_3$

	3		**4**		**5**[a]		**7**		**8**[b]	
	δ_H	δc	δ_H	δc	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1ax	0.96 *dd* (13.0, 3.4)	39.3	1.27 *td* (13.1, 3.5)	37.2	1.03 *td* (12.9, 3.1)	40.3	1.15 *td* (12.9, 3.9)	37.6	1.12 *dd* (12.9, 3.7)	39.9
1eq	1.78 *brd* (13.0)	39.3	1.79 *dd* (13.1, 3.5)	37.2	1.83 *dd* (12.7, 3.6)		1.79 *dt* (12.9, 3.4)		1.73 *dt* (13.1, 3.6)	
2ax	1.43 *m*	18.7	1.60 *qd* (13.3, 3.4)	28.0	1.48 *dq* (13.6, 3.4)	19.7	1.62 *qd* (13.3, 3.6)	27.3	1.60 *m*	26.5
2eq	1.58 *m*		1.72 *dq* (13.3, 3.7)		1.65 *m*		1.69 *m*		1.65 *m*	
3ax/3	1.17 *td* (13.6, 4)	42.1	3.27 *dd* (11.7, 4.3)	78.8	1.24 *td* (13.5, 3.7)	43.1	3.27 *dd* (11.5, 4.4)	78.8	3.12 *dd* (11.2, 4.6)	78.6
3eq	1.41 *m*				1.43 *dtd* (13.1, 3.2, 1.5)					
4		33.7	–	39.4	–	34.4	–	39.3	–	40.0
5	1.02 *dd* (12, 2.8)	55.2	1.12 *dd* (12.5, 2.6)	54.7	1.10 *dd* (12.3, 2.3)	56.2	1.02 *dd* (12.5, 3.5)	54.2	0.94 *d* (1.9)	55.8
6ax	1.58 *m*	20.2	1.41 *qd* (13.1, 4.2)	23.9	1.61 *m*	21.2	1.72 *m*	19.9	4.46 *td* (3, 1.8)	67.9
6eq	na		1.75 *m*		1.72 *m*		1.72 *m*			
7ax	1.36 *dq* (13.8, 2.5)	36.0	2.00 *td* (13, 4.9)	37.9	1.31 *dq* (1.39, 2.6)	37.1	1.40 *ddd* (13.9, 3.7, 2.6)	35.9	1.15 *dd* (14.8, 2.5)	43.8
7eq	1.93 *td* (13.8, 5.3)		2.41 *dq* (13, 2.3)		1.97 *td* (13.7, 5)		1.94 *td* (13.9, 5.8)		2.18 *dd* (14.8, 3.6)	
8	–	57.7	–	147.8	–	59.3	–	58.1	–	57.2
9	1.57 *m*	53.0	1.77 *m*	56.6	1.60 *d* (10.6)	54.6	1.66 *m*	52.3	1.66 *m*	52.5
10	–	40.0	–	39.5	–	40.9	–	39.6	–	39.8
11a	2.02 *m*	21.0	2.50 *ddd* (16, 11.6, 7.6)	23.8	1.78 *brt* (8.3)	20.7	2.08 *ddd* (16.5, 9.1, 7.8)	22.6	2.10 *m*	22.4
11b	2.34 *dd* (18.4, 6.1)		2.61 *ddd* (16.6, 6.3, 2.8)		1.98 *m*		2.27 *brdd* (16.5, 6.8)		2.31 *d* (6.6)	
12	6.81 *t* (6.5)	150.7	6.87 *t* (7.00)	149.6	5.44 *ddd* (8.1, 4.7, 1)	133.3	6.85 *td* (7.1, 1.6)	149.2	6.77 *td* (6.9, 1.8)	148.7
13	–	127.4	–	127.5	–	138.8	–	128.4	–	128.8
14	3.60 *brs*	48.6	3.65 *t* (3.4)	49	4.6 *dd* (7.8, 4.3)	71.9	5.03 *t* (5.6)	66.1	4.92 *d* (6.1)	65.4
15a/15	2.81 *dd* (5.5, 2.8)	47.9	3.00 *dd* (5.5, 4.4)	47.8	3.5 *dd* (11.3, 7.6)	66.1	4.27 *dd* (10.4, 2)	74.5	4.18 *dd* (10.2, 4.6)	75.0
15b	2.99 *dd* (5.5, 4.3)		2.78 *dd* (5.5, 2.8)		3.6 *dd* (11.3, 4.6)		4.47 *dd* (10.4, 6.1)		4.41 *dd* (10.2, 6.2)	
16a/16	–	166.8	–	166.8	4.04 *d* (12.9)	63.9	–	170.2	–	171.3
16b					4.15 *d* (12.9)					
17a	2.30 *d* (3.9)	49.2	4.49 *d* (0.9)	108.2	2.27 *d* (4.1)	50.3	2.34 *d* (3.6)	49.4	2.27 *d* (3.5)	47.4
17b	2.51 *d* (3.9)		4.86 *d* (0.9)		2.69 *d* (4.1)		2.73 *d* (3.6)		2.75 *d* (3.5)	
18	0.90 *s*	33.7	0.79 *s*	28.5	0.92 *s*	34.0	1.04 *s*	28.5	1.06 *s*	28.0
19	0.87 *s*	21.9	1.00 s	15.6	0.88 *s*	22.2	0.85 *s*	15.7	1.22 *s*	16.8
20	0.93 *s*	14.8	0.74 *s*	14.6	0.92 *s*	15.2	0.95 *s*	14.9	1.17 *s*	16.7
O-CH_3	3.75 *s*	52.1	3.73 *s*	51.9						

na: not assigned.
[a] Measured in CD_3OD.
[b] Measured in $CDCl_3$ with some drops of CD_3OD.

and carbons, the *E* configurations of the double bond, and the β configuration of the 8,17-epoxide. Thus, structure **5** is 8β,17-epoxy-12*E*-labdene-14ξ,15,16-triol.

Compound **7**, $C_{20}H_{30}O_5$ (*m/z* $[M+Na]^+$ 373.1974 HRESI-MS in positive mode) a white powder, showed IR absorption bands at υ_{max} 3415 and 1743 cm^{-1}. Its ^1H and ^{13}C NMR spectra data were similar to those of **6** (Morita and Itokawa, 1988) except for the absence of two methylene signals (C-3 and C-14) and the appearance of two oxymethine carbon resonances at δ 66.1 (C-14) and 78.8 (C-3) in the ^{13}C NMR spectrum of **7**. H-3 appeared at δ 3.27 (*dd*, $J = 11.5,4.4$ Hz) indicating that the hydroxyl attached to C-3 is β. Thus compound **7**, zambesiacolactone A, is 8β,17-epoxy-3β,14ξ-dihydroxy-12(*E*)-labden-16,15-olide.

Analysis of the ^1H and ^{13}C NMR spectral data (Table 1) of compound **8**, $C_{20}H_{30}O_6$ (*m/z* $[M+Na]^+$ 389.1956 HRESI-MS in positive mode; υ_{max} 3417 and 1739 cm^{-1}), indicated that it was closely related to compound **7**. The main difference was the presence of signals for an oxymethine [δ_H 4.46 (*td*, $J = 3.0,1.8$ Hz, H-6); δ_C 67.9 (C-6)] in **8** replacing the C-6 resonances in **7**. The β axial orientation of the C-6 hydroxyl of **8** was deduced from the small coupling constant ($J = 1.9$ Hz) between H-5 and H-6. Furthermore, the upfield resonance of H-5 (δ 0.94) is reminiscent of some 6β-hydroxylated *Scapania* labdane derivatives (Huneck et al., 1986). Thus, compound **8**, zambesiacolactone B, was assigned the structure 8β,17-epoxy-3β,6β,14ξ-trihydroxy-12(*E*)-labden-16,15-olide.

The relative configuration at C-14 remains undetermined in all the new compounds, as in aulacocarpin A (**1**) and aulacocarpin B (**2**) (Ayafor et al., 1994a).

The in vitro antiplasmodial activity of the labdanes **1**–**9** was determined against an FCB1 chloroquine-resistant strain of *P. falciparum* relative to artemisinine, chloroquine, and quinine (Table 2). The amount of compound **10** was insufficient to allow evaluation of its biological activity. Compounds **1**, **3**, **7**, and **8** showed moderate activity with IC_{50} values between 4 and 20 μM. Amongst the active compounds, compound **3**, the least polar compound, was the most active with an IC_{50} of 4.97 μM (1.73 μg/ml).

Table 2
In vitro antiplasmodial activity of compounds **1**–**9**, quinine, chloroquine, and artemisinine on FCB1 line of *Plasmodium falciparum*

compound	IC_{50} (μM)	n[a]
Aulacocarpin A (**1**)	13.68 ± 6.89	3
Aulacocarpin B (**2**)	21.10 ± 4.55	2
3	4.97 ± 2.27	2
4	39.94 ± 12.58	2
5	>133.13	2
Galanolactone (**6**)	92.79 ± 12.83	2
Zambesiacolactone A (**7**)	17.20 ± 3.05	3
Zambesiacolactone B (**8**)	15.51 ± 4.20	3
Aframodial (**9**)	>94.33	3
Quinine	0.55 ± 0.10	3
Chloroquine	0.30 ± 0.03	5
Artemisinine	0.01 ± 0.00	3

[a] n = number of experiments.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Reichert apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 500 (^1H at 500 MHz and ^{13}C at 125 MHz). 2D experiments were performed using standard Bruker microprograms. ESI-MS and HRESI-MS were obtained on a Micromass Q-TOF micro spectrometer. IR spectra were obtained with a Nicolet AVATAR 320 FT-IR spectrophotometer. Optical rotations were determined in MeOH or $CHCl_3$ with a Perkin–Elmer 241 polarimeter. Centrifugal TLC was carried out on a chromatotron, Model 7924T (Harrison Research). The plate was coated with silica gel 60 F_{254} Merck. TLC was performed on pre-coated silica gel 60 F_{254} Merck and detection was achieved by spraying with a vanillin sulphuric reagent containing (3 g Vanillin, 100 ml EtOH, and 3 ml H_2SO_4). CC was carried out on Kieselgel 60 (63–200 mesh) Merck.

3.2. Plant material

Seeds of *A. zambesiacum* (Baker) K. Schum. were collected in Nyassosso, a small locality of the district of Tombel in south-west of Cameroon in November 2003. A voucher specimen (accession number 37737HNY) has been deposited at the National Herbarium Yaoundé Cameroon. The identification was confirmed by Dr. Tchiengu and P. Mezili, botanists of the Cameroon National Herbarium.

3.3. Extraction and isolation

Dried and finely powdered seeds (97 g) were extracted successively with petroleum ether (2.5 l), chloroform (2.5 l), and methanol (2.5 l) at room temperature by percolation in an open column after a maceration step for 36 h. The extracts were concentrated to yield respective residues 3.55 g (petroleum ether **I**), 6.78 g (chloroform **II**) and 3.54 g (methanol **III**).

The petroleum ether extract **I** (3.55 g) was subjected to column chromatography (CC) on silica gel with a mixture of hexane/EtOAc of increasing polarity to give 15 fractions (Fr. 1–15). Fr. 3 (50 mg) eluted with hexane/EtOAc (98/2) was purified by preparative TLC in hexane/EtOAc (8/2) to give nerolidol (**11**) (10 mg). Fr. 6 (60 mg) eluted with hexane/EtOAc (92.5/7.5) was purified by preparative TLC using the same conditions as above to give compound **3** (30 mg). Aframodial (**9**) (50 mg) was obtained from Fr. 8 (70 mg) eluted with hexane/EtOAc (9/1) after preparative TLC in hexane/EtOAc (8/2).

The chloroform extract **II** (6.78 g) was fractionated on silica gel CC eluting with a mixture of hexane/EtOAc/MeOH of increasing polarity to give 22 fractions (Fr. 1–22). Fr. 8 eluted with hexane/EtOAc (8/2) was further subjected to a Sephadex LH20 column, eluting with MeOH/

CH_2Cl_2 (1/1) and purified by preparative TLC in hexane/EtOAc (7/3) to give galanolactone (**6**) (14 mg). Galanal A (**10**) (15 mg) and compound **4** (3.8 mg) were obtained from Fr. 9 (20 mg) eluted with hexane/EtOAc (8/2), and Fr. 11 (70 mg) eluted with hexane/EtOAc (75/25), respectively, after purification with CC over Sephadex LH20 eluting with MeOH/CH_2Cl_2 (1/1) and preparative TLC in hexane/EtOAc (6/4). Fr. 13 (73 mg) eluted with hexane/EtOAc (75/25) was subjected to silica CC eluting with hexane/EtOAc (6/4) to give aulacocarpin A (**1**) (42 mg). Aulacocarpin B (**2**) (70 mg) was obtained from Fr. 17 (100 mg) eluted with hexane/EtOAc (6/4) by recrystallization in a mixture hexane/EtOAc (1/1). Fr. 19 (80 mg) eluted with hexane/EtOAc (3/7) was fractioned on Sephadex LH20 CC eluting with CH_2Cl_2/MeOH (1/1) and compound **7** (5.8 mg) was purified by centrifugal TLC using $CHCl_3$/MeOH (99/1) and preparative TLC in CH_2Cl_2/acetone (8/2). Fr. 20 (224 mg) eluted with EtOAc/MeOH (95/5) was fractioned on Sephadex LH20 as above to give Fr. a (37 mg) and Fr. b (34 mg) which were respectively purified by centrifugal TLC using a mixture $CHCl_3$/MeOH (98/2) to yield compound **5** (20 mg) by recrystallisation in MeOH and compound **8** (10 mg) after a preparative TLC in CH_2Cl_2/acetone (6/4).

3.4. Aulacocarpin A (*1*)

ESI-MS (positive ion mode) m/z 365 $[M + Na]^+$; spectroscopic data as in Ayafor et al. (1994a).

3.5. Aulacocarpin B (*2*)

ESI-MS (positive ion mode) m/z 381 $[M + Na]^+$; spectroscopic data as in Ayafor et al. (1994a).

3.6. 3-Deoxyaulacocarpin A (*3*)

White powder: m.p. 88–89 °C; $[\alpha]_D^{20}$ +40.3° ($CHCl_3$, *c* 1.14); IR (KBr) υ_{max} 2952, 2923, 1724, 1645, 1433, 1389, 1262, 1245, 1214 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$): see Table 1; HRESI-MS: $[M + Na]^+$ Calc. 371.2198; found 371.2196; ESI-MS (positive ion mode) m/z 371 $[M + Na]^+$.

3.7. Methyl-14ξ,15-epoxy-3β-hydroxy-8(17),12E-labdadien-16-oate (*4*)

Colorless oil: $[\alpha]_D^{20}$ + 28.2° ($CHCl_3$, *c* 1.13); IR (film) υ_{max} 3496, 2940, 2848, 1714, 1644, 1439, 1386, 1260 cm^{-1} ^1H and ^{13}C NMR ($CDCl_3$): see Table 1; HRESI-MS: $[M + Na]^+$ Calc. 371.2198; found 371.2211; ESI-MS (positive ion mode) m/z 371 $[M + Na]^+$.

3.8. 8β,17-Epoxy-12E-labdene-14ξ,15,16-triol (*5*)

White powder: m.p. 154–155 °C; $[\alpha]_D^{20}$ + 13.2° (MeOH, *c* 1.19); IR (KBr) υ_{max} 3402, 2929, 1647, 1434, 1387, 1218 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD): see Table 1; HRESI-MS: $[M + Na]^+$ Calc. 361.2355; found 361.2349; ESI-MS (positive ion mode) m/z 361 $[M + Na]^+$.

3.9. Galanolactone (*6*)

ESI-MS (positive ion mode) m/z 341 $[M + Na]^+$; spectroscopic data as in Morita and Itokawa (1988).

3.10. Zambesiacolactone A (*7*)

White powder: m.p. 164–166 °C; $[\alpha]_D^{20}$ +73.6° ($CHCl_3$, *c* 0.8); IR (KBr) υ_{max} 3415, 2938, 2868, 1743, 1673, 1460, 1214 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$): see Table 1; HRESIMS: $[M + Na]^+$ Calc. 373.1991; found 373.1974 ESI-MS (positive ion mode) m/z 373 $[M + Na]^+$.

3.11. Zambesiacolactone B (*8*)

White powder: m.p. 141–142 °C; $[\alpha]_D^{20}$ +73.6° (MeOH, *c* 0.8); IR (KBr) υ_{max} 3417, 2929, 2868, 1739, 1673, 1463, 1421, 1364, 1215 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$/CD_3OD): see Table 1; HRESI-MS: $[M + Na]^+$ Calc. 389.1940; found 389.1956 ESI-MS (positive ion mode) m/z 389 $[M + Na]^+$.

3.12. Aframodial (*9*)

ESI-MS (positive ion mode) m/z 341 $[M + Na]^+$; spectroscopic data as in Morita and Itokawa (1988).

3.13. Galanal (*10*)

ESI-MS (positive ion mode) m/z 341 $[M + Na]^+$; spectroscopic data as in Morita and Itokawa (1988).

3.14. Antiplasmodial assays

Continuous cultures of asexual erythrocytic stages of an FCB1 chloroquine-resistant strain of *P. falciparum* were maintained following the procedure of Trager and Jensen (1976) and as described previously (Frédérich et al., 2002). Artemisinin (Sigma, Bornem, Belgium), chloroquine diphosphate (Sigma C6628), and quinine base (Aldrich 14590-4) were used as antimalarial references. Each test sample was applied in a series of eight fourfold dilutions (final concentrations ranging from 20 to 0.0012 μg/ml) and was tested in duplicate and triplicate. Parasite growth was estimated by determination of lactate dehydrogenase activity as described by Delhaes et al. (1999) and Makler et al. (1993) and slightly modified. Briefly, in a new microtiter plate, a 20 μl subsample of the contents of each well was mixed with 100 μl of a substrate solution containing 1 mg lithium L-lactate (Sigma), 0.2 mg 3-acetyl pyridine adenine dinucleotide (APAD, Sigma), 0.2 μl Triton X-100 (Sigma), 10 μg saponine (Merck) in TRIS buffer (pH 8, Sigma). After incubation for 20 min, 20 μl of a mix of nitroblue tetrazolium (NBT, 2 mg/ml in TRIS pH 8 buffer, SIGMA) and phenazine ethosulfate (PES, 0.1 mg/ml in

TRIS pH 8 buffer, Sigma) was added to each well. After another 30 min of incubation, the formation of the reduced form of APAD was measured at 595 nm.

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