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Labdane diterpenoids from *Aframomum sceptrum*: NMR study and antiparasitic activities

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ABSTRACT

Two novel labdane diterpenoids, 15ξ-methoxy-labdan-8(17),11(*E*),13(14)-trien-15,16-olide (**1**) and 12(*S*)-hydroxy-15ξ-methoxy-labdan-8(17),13(14)-dien-15,16-olide (**2**) were isolated from the rhizomes of *Aframomum sceptrum* K. Schum (Zingiberaceae). Their structures were established on the basis of their spectroscopic data. Stigmast-4-en-6β-ol-3-one and caryophylene oxide were also obtained. *In vitro* trypanocidal and leishmanicidal activities of labdanes **1** and **2** were evaluated. Compound **2** exhibited activity similar to that of reference drugs against *Leishmania donovani*.

1. Introduction

Aframomum is an economically and medicinally important genus (Kerharo, 1974) of the Zingiberaceae family, which includes about 60 species from tropical and subtropical Africa. The rhizomes of *Aframomum sceptrum* K. Schum are traditionally used in Ivory Coast for infectious diseases including human African trypanosomiasis (sleeping sickness) (Okpekon et al., 2004). The species was however poorly studied. Previous works reported the isolation of labdanes from the seeds and fruits of the plant (Duker-Eshun et al., 2002; Tomla et al., 2002). After a previous investigation (Cheikh-Ali et al., 2011), we conducted a bioguided fractionation of rhizomes of the plant.

2. Results and discussion

A methanolic extract of the rhizomes of *A. sceptrum* was partitioned using MeOH/H_2O/cyclohexane. The organic partition exhibited greater antileishmanial and trypanocidal activities (IC_{50} = 25 μg/mL at 72 h [concentration inhibiting parasite growth by 50%] and LC_{100} = 25 μg/mL at 24 h [lethal concentration 100%], respectively) in regards to the crude extract (IC_{50} > 50 μg/mL and LC_{100} = 100 μg/mL, respectively). It was subjected to Sephadex® LH-20 and silica gel column chromatographies to furnish two new compounds **1** and **2** (Fig. 1). They were identified on the basis of their chemical characteristics and spectral data.

Compound **1** was obtained as a dark yellow oil. HR-MALDI-ToF MS analysis showed a sodium adduct $[M+Na]^+$ at *m*/*z* 353.2108, in accordance with the molecular formula $C_{21}H_{30}O_3$. IR spectrum evidenced an exo-methylene (ν_{max} 3040, 1643, 892 cm^{-1}) and an α,β-unsaturated γ-lactone (ν_{max} 1769 cm^{-1}), as suggested by a positive Kedde reaction. The ^1H NMR spectrum obtained in $CDCl_3$ (Table 1) confirmed the presence of an exo-methylene (δ 4.70 and δ 4.42 ppm), and showed three methyl groups (δ 0.78, 0.81 and 0.83 ppm), consistent with a labdane-type diterpene. A methoxy group (δ 3.51 ppm) was also observed. Furthermore, the presence of a double bond was observed (H-12, δ 6.03, *d* and H-11, δ 6.91 ppm, *dd*), with a *trans* configuration, according to measured coupling constant (*J* = 15.9 Hz). H-11 coupled with an allylic proton H-9 (δ 2.31 ppm; *J* = 10.2 Hz), as shown by the ^1H–^1H COSY spectrum. H-11 was deshielded because the *trans*-olefinic group is directly linked to the α,β-unsaturated γ-lactone. Furthermore, COSY showed a correlation between H-14 and H-15 (Table 2).

The ^{13}C NMR spectrum obtained in $CDCl_3$ (Table 1) exhibited resonances for 21 carbons, including a lactone carbonyl at δ 174.1 (C-16), a methoxy at δ 56.8/56.9 (C-21) and three methyl groups at δ 33.5 (C-18), 21.9 (C-19) and 15.1 ppm (C-20). Two double bonds were characterized by signals at δ 139.5 (C-11) and 120.3 ppm (C-12), and at δ 132.9 (C-13) and 139.2 ppm (C-14). Two other unsaturated carbons at 149.2 and 108.5 ppm are in agreement

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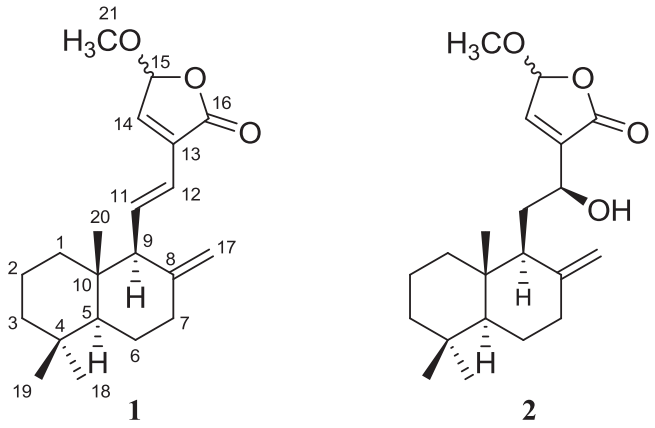

Fig. 1. Sceptrumlabdalactones A (**1**) and B (**2**).

with the exo-double bond at C-8/C-17. The presence of the α-β unsaturated γ-lactone was confirmed by the HMBC spectrum, with key correlation between both H-12 (δ 6.03 ppm) and H-14 (δ 6.72 ppm) and the lactonic carbonyl at C-16. Other correlations between H-12 and C-9 or H-7 and C-9 (Table 2) provided to the gross structure **1**. Additionally, NOESY data obtained in $CDCl_3$ permitted the determination of the relative configuration of **1**, with typical features of a labdane diterpene (Fig. 2). NOESY spectrum obtained in DMSO-d_6 showed a correlation between H-11 and H-14 which could not be observed in $CDCl_3$.

In the ^{1}H NMR spectrum of **1** recorded in $CDCl_3$, the signal corresponding to H-11 was split, suggesting the probable co-existence of two rotamers in a 4:6 approximate ratio (Fig. 3a). To allow rapid conversion between these rotamers and observation of one form only, $CDCl_3$ was replaced by DMSO-d_6 in order to increase temperature. Even without heating, no more splitting of signal corresponding to H-11 was observed (Fig. 3b). Remarkable differences in chemical shifts of H-14 and H-15 were noticed (from δ 6.72 and 5.70 in $CDCl_3$ to 7.33 and 6.00 ppm in DMSO-d_6, respectively for H-14 and H-15), probably because of anisotropic effects due to interaction of DMSO with the lactone ring. No significant shifts were observed in the ^{13}C NMR spectrum for the corresponding carbons (from δ 139.2 and 101.9 in $CDCl_3$ to 142.8 and 102.7 ppm in DMSO-d_6 for C-14 and C-15, respectively). Other ^{13}C NMR shifts were also unchanged in DMSO-d_6 (Reichardt, 2003).

Structure of compound **1** was established as 15ξ-methoxy-labdan-8(17),11(*E*),13(14)-trien-15,16-olide. **1** was named sceptrumlabdalactone A.

Compound **2** was obtained as a colourless oil. Its HR-MALDI-ToF MS spectrum showed a sodium adduct $[M+Na]^+$ at *m*/*z* 371.2198 consistent with the molecular formula $C_{21}H_{32}O_4$. As in compound **1**, absorption bands of an α,β-unsaturated γ-lactone (ν_{max} 1769 cm^{-1}) and of an exo-methylene (ν_{max} 3040, 1643, 892 cm^{-1}) were observed in IR spectrum. An additional band corresponding to a hydroxyl group (3620 cm^{-1}) was observed, consistently with MS analysis (+18 amu). NMR data evidenced a structure similar to that of compound **1**, the only difference being the hydration of the double bond at C-11 and C-12 (Tables 1 and 2). The relative stereochemistry of **2** was established from NOESY experiments (Fig. 2), with ambiguity for C-12 in regard to the *chair*/*chair* decaline, due to free rotation of the branched substituent (C-11–C-16). Bell et al. (1972) reported the synthesis of epimeric hydroxy derivatives in the labdane series and showed some differences in the shifts of the H-17 protons depending of the configuration at C-12 (*R*: δ H-17a: 4.48, H-17b: 4.86; *S*: δ H-17a: 4.72, H-17b: 4.88 ppm in $CDCl_3$). Chemical shifts of H-17a at 4.73 and H-17b at 4.90 ppm thus strongly suggested a *S* configuration for C-12 (Chen et al., 2008; Waridel et al., 2003).

Structure of compound **2** was established as 12(*S*)-hydroxy-15ξ-methoxy-labdan-8(17),13(14)-dien-15,16-olide. **2** was named sceptrumlabdalactone B. Labdanes **1** and **2** are closely related to those obtained by Tomla et al. (2002) from seeds of the species. Chemically diverse analogues are frequently encountered within the genus (39 labdanes isolated so far) and Zingiberaceae family (Ayimèle et al., 2004; Kenmogne et al., 2006; Sob et al., 2007; Tsopmo et al., 2002; Wabo et al., 2006). Analogues were recently isolated from *Curcuma* and *Hedychium* spp. (Zingiberaceae) (Chokchaisiri et al., 2010; Zhao et al., 2008).

For both **1** and **2**, absolute configuration at C-15 could not be determined but the presence of both epimers is suspected, on the basis of pairs of resonance for C-18 (OMe; 56.8, 56.9 for **1**, 57.1, 57.2 for **2**). It is noteworthy that similar compounds from

Table 1
NMR spectroscopic data (400 MHz, *J* in Hz) of compounds **1** and **2**.

Position	Compound **1**			Compound **2** ($CDCl_3$)	
	δ_H ($CDCl_3$)	δ_H (DMSO-d_6)	δ_C ($CDCl_3$)	δ_H	δ_C
1	1.34 *m*/0.98 *m*		40.8	1.34 *m*/1.02 *m*	39.0
2	1.32 *m*		19.1	1.50 *m*	19.3
3	1.33 *m*/1.05 *m*		42.2	1.33 *m*/1.20 *m*	42.1
4			33.5		33.6
5	1.01 *m*		54.7	1.18 *m*	55.5
6	1.35 *m*/1.66 *m*		23.3	1.25 *m*/1.75 *m*	24.3
7a, *ax.*	2.02 *m*		36.7	2.00 *m*	38.2
7b, *eq.*	2.37 *br d* (*13.2*)			2.42 *br d* (*11.4*)	
8			149.2		148.1
9	2.31 *d* (*10.2*)		62.2	2.03 *m*	51.9
10			39.4		39.0
11	6.96 *dd* (*15.9, 10.2*)	6.85 *dd* (*16.0, 12.0*)	139.5	1.48 *m*	30.4
12	6.03 *d* (*15.9*)	6.13 *d* (*16.0*)	120.3	4.56 *d* (*10.2*)	65.8
13			132.9		141.9
14	6.72 *br s*	7.33 *br s*	139.2	6.96 *br s*	141.5
15	5.70 *br s*	6.00 *br s*	101.9	5.78 *br s*	102.8
16			174.1		170.1
17a	4.70 *br s*	4.75 *br s*	108.5	4.90 *br s*	107.3
17b	4.42 *br s*	4.42 br s		4.73 *br s*	
18-Me	0.83 *s*	0.88 *s*	33.5	0.88 *s*	33.6
19-Me	0.81 *s*	0.81 *s*	21.9	0.80 *s*	21.7
20-Me	0.78 *s*	0.80 *s*	15.1	0.68 *s*	14.6
21-OMe	3.51 *s*	3.45 *s*	56.8/56.9	3.58 *s*	57.1/57.2

Table 2
COSY (^{1}H to ^{1}H) and HMBC (^{1}H to ^{13}C) correlations for compounds **1** and **2** in $CDCl_3$.

Position	Compound **1**		Compound **2**	
	COSY	HMBC	COSY	HMBC
H-1		C-5		
H-2				
H-3		C-4, C-20		
H-5				C-19, C-20, C-21
H-6				C-7
H-7	H-6	C-6, C-9	H-6	
H-9	H-11	C-8, C-10	H-17a, H-17b	
H-11	H-9, H-12	C-13	H-9, H-12	
H-12	H-11	C-9, C-11, C-16	H-11	
H-14	H-15	C-12, C-15, C-16	H-15	C-15
H-15	H-14	C-18	H-14	C-16, C-18
H-17a		C-9, C-7		C-9, C-7
H-17b		C-9, C-7		C-9, C-7
H-18		C-3, C-5, C-18		C-3, C-4, C-5, C-19
H-19		C-3, C-5, C-18		C-3, C-4, C-5, C-18
H-20		C-1, C-5, C-9		C-9, C-10
H-21		C-15		C-15

Renealmia alpinia (Zingiberaceae) were isolated as mixture of C-15 epimers (Yang et al., 1999). Artifactual nature for **1** and **2** is however possible. It remains ambiguous, in regard to hemiacetalic and acetalic analogues obtained by others using MeOH (Boalino et al., 2004).

In the course of this work, caryophyllene oxide and stigmast-4-en-6β-ol-3-one were also obtained. Another batch of plant material allowed cholesterol, campesterol, β-sitosterol, stigmasterol and stigmast-4-en-3-ol.

Several labdanes were shown to exert antiplasmodial *in vitro* activity (Duker-Eshun et al., 2002; Yang et al., 1999). Leishmanicidal activity was also observed (Singh et al., 1999), and moderate trypanocidal activity was reported for labdanes of *Aframomum aulacocarpos* (Sob et al., 2007). In accordance with the traditional use of the plant, compounds **1** and **2** were tested against bloodstream forms of *Trypanosoma brucei brucei* [minimum lethal concentration (MLC) values determined after 24 h]. They were also evaluated against promastigotes of *Leishmania donovani* [concentration inhibiting parasite growth by 50% (IC_{50}) determined at 72 h]. Compound **1** showed moderate activity against *L. donovani*, and was poorly active against *T. b. brucei*. Compound **2** was reasonably active against *T. b. brucei*, and strongly active against *L. donovani*, with IC_{50} close to that of pentamidine (Table 3).

3. Experimental

3.1. General experimental procedures

For column chromatography, Merck Silica 60 and Sephadex® LH-20 (Pharmacia) gels were used. TLC were carried out on aluminium plates coated with silica gel 60 F254 (Merck), and visualized with UV light, vanillin-H_2SO_4 and Kedde [3,5-dinitrobenzoic acid 2% (MeOH, *m*/*v*) then KOH 2 N (MeOH)] reagents after heating. Optical rotations

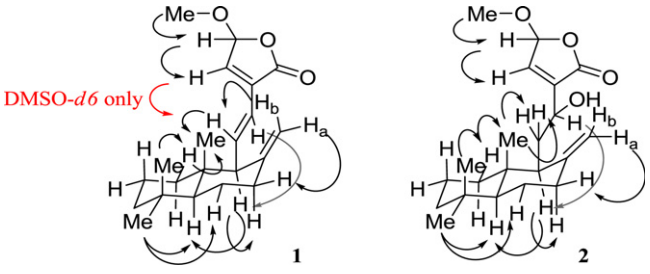

Fig. 2. Key NOESY correlations observed for labdanes **1** and **2**.

Table 3
Antiparasitic activities of labdanes **1** and **2**.

Compound	*T. brucei brucei* MLC (μM)	*L. donovani* IC_{50} (μM)
1	204.0	25.0 ± 0.7
2	35.7	5.7 ± 0.9
Pentamidine	12.5	2.5 ± 0.2
Miltefosine		3.0 ± 0.0

Experiments were performed in triplicate. IC_{50} values are expressed as mean ± SD.

were measured on a PolAAr 32 polarimeter (Optical activity Ltd., Ramsey, UK). IR spectra were recorded on a Bruker Vector-22 spectrometer (Champs-sur-Marne, France). UV spectra were obtained in MeOH on a Biochrom WPA lightwave II$^+$ spectrometer (Cambridge, UK). ^{1}H NMR and ^{13}C NMR 1D and 2D spectra were recorded in $CDCl_3$ and DMSO-d_6 on a Bruker AM-400 spectrometer (Champs-sur-Marne, France). Mass spectrometry analyses were performed with a Voyager-DE STR MALDI-ToF spectrometer (Perseptive biosystems, Framingham, MA, USA) equipped with a digital oscilloscope Tektronix TDS 540C (Beaverton, USA) and a N_2 laser (λ = 337 nm) and with a CI-MS Nermag-Sidar R10-10C spectrometer (Argenteuil, France).

3.2. Plant material

The rhizomes of *A. sceptrum* K. Schum were harvested in Ivory Coast, in 2007. A voucher specimen (reference number: AFTAK2007) was deposited in the UFR Sciences des Structures de la Matière et Technologie, Cocody-Abidjan University.

3.3. Extraction and isolation

The extraction of the dried rhizomes of *A. sceptrum* was performed with MeOH using a Soxhlet apparatus for 96 h. Removal of solvent *in vacuo* afforded 207 g of a brown-black, pungent extract (Yield: 7.7%). Liquid/liquid extraction was performed using MeOH/H_2O/cyclohexane (15:40:45), affording 5.8 g of cyclohexanic fraction (yield: 10% of the crude MeOH extract), which was subjected to silica gel CC (cyclohexane/CH_2Cl_2/EtOAc gradient) to yield eight major sub-fractions (F1–F8). F1 (142 mg) was chromatographed with cyclohexane/EtOAc (gradient), yielding 3 sub-fractions (F1-1–F1-3). Fraction F1-3 (20 mg) was purified by silica gel CC (cyclohexane/EtOAc 95:5) to give compound **1** (7 mg).

F2 (1.4 g) was subjected to gel permeation CC through Sephadex® LH-20 (MeOH/CH_2Cl_2 1:1) to give three sub-fractions (F2-1–F2-3). F2-3 (351 mg) was further chromatographed on silica gel (cyclohexane/EtOAc gradient). The third sub-fraction, obtained from F2-3 with EtOAc 10–30% (20 mg), was purified by silica CC (cyclohexane/EtOAc, 85:15). It allowed compound **2** (8 mg). Caryophyllene oxide and Stigmast-4-en-6β-ol-3-one were obtained from F2 and F7, respectively.

3.3.1. 15ξ-methoxy-labdan-8(17),11(E),13(14)-trien-15,16-olide (*1*)

Dark yellow oil; $[\alpha]_D^{28}$ = +24.9 (c 0.41, $CHCl_3$); UV (MeOH) λ_{max} 213 nm; IR (film, $CHCl_3$) ν_{max} 3040, 2925, 1769, 1643, 1460, 1090, 1027, 892, 752 cm^{-1}; MALDI-ToF HRMS *m*/*z* [M+Na]$^+$, 353.2108 (calcd for $C_{21}H_{30}O_3Na$: 353.2093); APCI-MS *m*/*z* (+ve) 331 [M+H]$^+$, 299 [M-OCH_3]$^+$, 191 [$C_{14}H_{23}$]$^+$, 149 [$C_{11}H_{17}$]$^+$; ^{1}H NMR, ^{13}C NMR, HMBC, COSY ($CDCl_3$, DMSO-d_6, 400 MHz): see Tables 1 and 2.

3.3.2. 12(S)-hydroxy-15ξ-methoxy-labdan-8(17),13(14)-dien-15,16-olide (*2*)

Colourless oil; $[\alpha]_D^{28}$ = +17.4 (c 0.40, $CHCl_3$); UV (MeOH) λ_{max} 210 nm; IR (film, $CHCl_3$) ν_{max} 3620, 2930, 1769, 1643, 1458, 1128, 1024, 892, 733 cm^{-1}; MALDI-ToF HRMS *m*/*z* [M+Na]$^+$ 371.2198 (calcd for $C_{21}H_{32}O_4Na$: 371.2205); APCI-MS *m*/*z* 349 [M+H]$^+$, 331

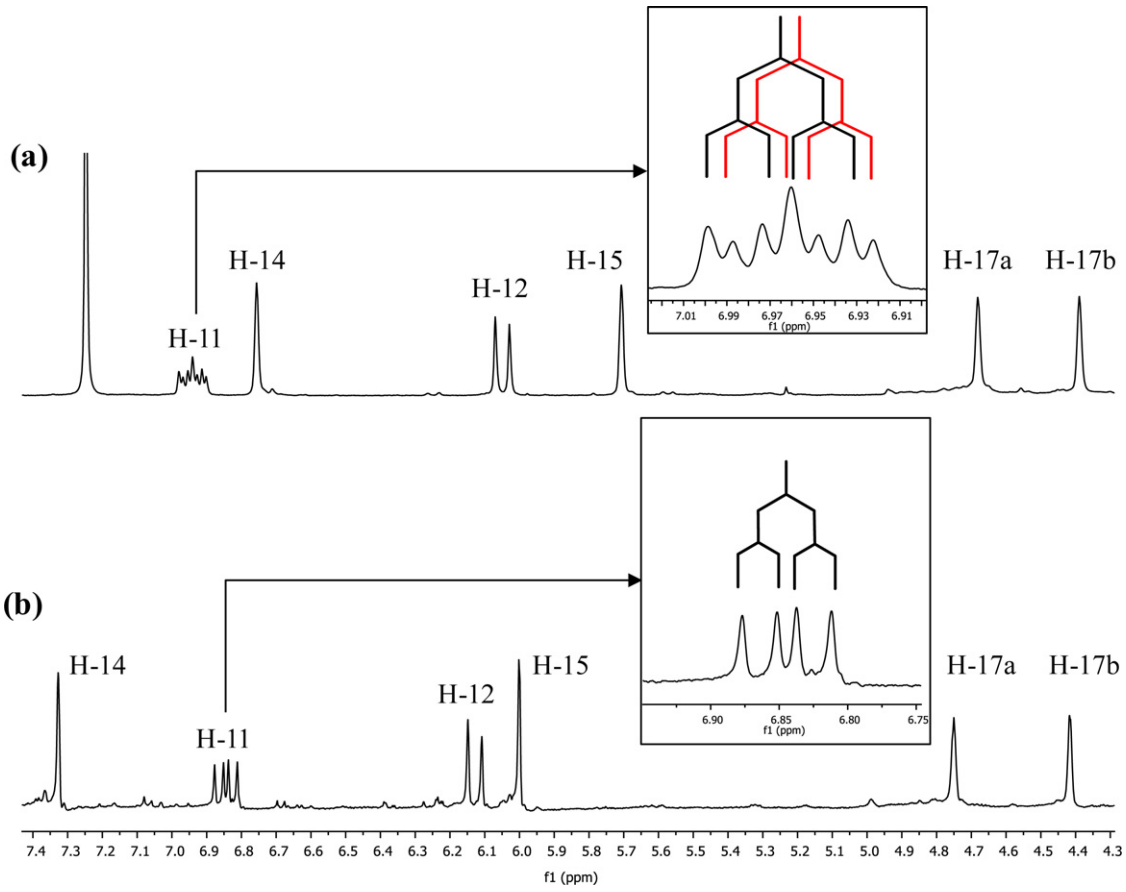

Fig. 3. ^{1}H NMR spectra (400 MHz) for compound **1** in $CDCl_3$ (a) and DMSO-d_6 (b).

$[M\text{-}H_2O+H]^+$, 299 $[M\text{-}OCH_3\text{-}H_2O]^+$, 241 $[C_{18}H_{25}]^+$, 191 $[C_{14}H_{23}]^+$; ^{1}H NMR, ^{13}C NMR, HMBC, COSY ($CDCl_3$, 400 MHz): see Tables 1 and 2. NMR spectra of compounds **1** and **2** are available as Supporting information.

3.4. Antiparasitic assays

Trypanocidal activity was assessed using the method described by Loiseau et al. (2000). The bloodstream forms of *T. b. brucei* (10^6 parasites/mL) were maintained *in vitro* in the dark at 37 °C in a 5% CO_2 atmosphere, in minimum essential medium (Gibco-BRL) with a series of concentrations of tested compounds. MLC (minimum concentration at which no viable parasite was observed microscopically) was measured after 24 h of incubation. Pentamidine was used as positive control. Antileishmanial assay was performed as described in Desrivot et al. (2007), with promastigotes of *L. donovani* (10^7 parasites/mL). After 72 h incubation, growth inhibition was measured, and results were expressed as the concentration inhibiting parasite growth by 50% (IC_{50}). Miltefosine was the positive control. All experiments were performed in triplicate, using 3 wells per condition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2011.04.006.

References

Ayimèle, G.A., Tane, P., Connolly, J.D., 2004. Aulacocarpin A and B, nerolidol and β-sitosterol glucoside from *Aframomum escapum*. Biochem. Syst. Ecol. 32, 1205–1207.
Bell, R.A., Gravestock, M.B., Taguchi, V.Y., 1972. Synthesis of lambertianic acid. Can. J. Chem. 50, 3749–3760.
Boalino, D.M., McLean, S., Reynolds, W.F., Tinto, W.F., 2004. Labdane diterpenes of *Leonurus sibiricus*. J. Nat. Prod. 67, 714–717.
Chen, L., Zhu, H., Wang, R., Zhou, K., Jing, Y., Qiu, F., 2008. *Ent*-labdane diterpenoid lactone stereoisomers from *Andrographis paniculata*. J. Nat. Prod. 71, 852–855.
Cheikh-Ali, Z., Adiko, M., Boutier, S., Bories, C., Okpekon, T., Champy, P., Poupon, E., 2011. Composition, antimicrobial and remarkable antiprotozoal activities of essential oil of rhizomes of *Aframomum sceptrum* K. Schum (Zingiberaceae). Chem. Biodiv. 6, 658–667.
Chokchaisiri, R., Chaneiam, N., Svasti, S., Fucharoen, S., Vadolas, J., Suksamrarn, A., 2010. Labdane diterpenes from the aerial parts of *Curcuma comosa* enhance fetal hemoglobin production in an erythroid cell line. J. Nat. Prod. 73, 724–728.
Desrivot, J., Waikedre, J., Cabalion, P., Herrenknecht, C., Bories, C., Hocquemiller, R., Fournet, A., 2007. Antiparasitic activity of some New Caledonian medicinal plants. J. Ethnopharmacol. 112, 7–12.
Duker-Eshun, G.W., Jaroszewski, J., Asomaning, W.A., Oppong-Boachine, F., Olsen, C.E., Christensen, S.B., 2002. Antiplasmodial activity of labdanes from *Aframomum latifolium* and *Aframomum sceptrum*. Planta Med. 68, 642–644.
Kenmogne, M., Prost, E., Harakat, D., Jacquier, M.-J., Frédérich, M., Sondengam, L.B., Zèches, M., Waffo-Téguo, P., 2006. Five labdane diterpenoids from the seeds of *Aframomum zambesiacum*. Phytochemistry 67, 433–438.
Kerharo, J., 1974. La Pharmacopée Sénégalaise Traditionnelle. Vigot Frères, Paris.
Loiseau, P.M., Lubert, P., Wolf, J.G., 2000. Contribution of dithiol ligands to *in vitro* and *in vivo* trypanocidal activities of dithiaarsanes and investigation of ligand exchange in an aqueous solution. Antimicrob. Agents Chemother. 44, 2954–2961.
Okpekon, T., Yolou, S., Gleye, C., Roblot, F., Loiseau, P., Bories, C., Grellier, P., Frappier, F., Laurens, A., Hocquemiller, R., 2004. Antiparasitic activities of medicinal plants used in Ivory Coast. J. Ethnopharmacol. 90, 91–97.
Reichardt, C., 2003. Solvents and Solvent Effects in Organic Chemistry, 3rd ed. John Wiley & Sons, New York.
Singh, M., Pal, M., Sharma, R.P., 1999. Biological activity of the labdane diterpenes. Planta Med. 65, 2–8.

Sob, S.V.T., Tane, P., Ngadjui, B.T., Connolly, J.D., Ma, D., 2007. Trypanocidal labdane diterpenoids from the seeds of *Aframomum aulacocarpos* (Zingiberaceae). Tetrahedron 63, 8993–8998.

Tomla, C., Kamnaing, P., Ayimele, G.A., Tanifum, E.A., Tsopmo, A., Tane, P., Ayafor, J.F., Connolly, J.D., 2002. Three labdane diterpenoids from *Aframomum sceptrum* (Zingiberaceae). Phytochemistry 60, 197–200.

Tsopmo, A., Ayimele, G.A., Tane, P., Ayafor, J.F., Connolly, J.D., Sterner, O., 2002. A norbislabdane and other labdanes from *Aframomum sulcatum*. Tetrahedron 58, 2725–2728.

Wabo, H.K., Tane, P., Connolly, J.J.D., 2006. Diterpenoids and sesquiterpenoids from *Aframomum arundinaceum*. Biochem. Syst. Ecol. 34, 603–605.

Waridel, P., Wolfender, J.-L., Lachavanne, J.-B., Hostettmann, K., 2003. *Ent*-Labdane diterpenes from the aquatic plant *Potamogeton pectinatus*. Phytochemistry 64, 1309–1317.

Yang, S.W., Zhou, B.N., Malone, S., Werkhoven, M.C.M., Van Troon, F., Wisse, J.H., Kingston, D.G.I., 1999. A new labdane diterpenoid from *Renealmia alpinia* collected in the Suriname rainforest. J. Nat. Prod. 62, 1173–1174.

Zhao, Q., Qing, C., Hao, X.-J., Han, J., Zuo, G.-Y., Zou, C., Xu, G.-L., 2008. Cytotoxicity of labdane-type diterpenoids from *Hedychium forrestii*. Chem. Pharm. Bull. 56, 210–212.