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Antiplasmodial sesquiterpenes from the seeds of *Salacia longipes* var. *camerunensis*

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ABSTRACT

Phytochemical investigation of the seeds of *Salacia longipes* var. *camerunensis* led to the isolation of four sesquiterpenoid derivatives, salaterpene A (**1**) (1α,2β,8β-triacetoxy-6β,9β-dibenzoyloxy-4β-hydroxy-dihydro-β-agarofuran), salaterpene B (**2**) (1α,2β,8β-triacetoxy-9β-benzoyloxy-6β-cinnamoyloxy-4β-hydroxy-dihydro-β-agarofuran), salaterpene C (**3**) (1α,2β-diacetoxy-6β,9β-dibenzoyloxy-4β-hydroxy-dihydro-β-agarofuran) and salaterpene D (**4**) (2β-acetoxy-1α,6β-dibenzoyloxy-4β-hydroxy-9β-nicotinoyloxy-dihydro-β-agarofuran) together with two known compounds (**5** and **6**). The structures of the compounds were established by means of NMR spectroscopy. Compounds **1**–**4** and **6** were tested *in vitro* for their antiplasmodial activity against *Plasmodium falciparum* chloroquine-resistant strain W2. All the tested compounds exhibited a moderate potency with IC_{50} below 2.7 μM.

1. Introduction

Plants of the Celastraceae family are generally trees, shrubs and lianas widely distributed in tropical Africa including Cameroon where they are used for the treatment of several ailments such as blenorrhagia, fever and malaria (Gessler et al., 1994; Chhabra et al., 1989). Previous phytochemical investigation of plants of this family reported the presence of bioactive dihydro-β-agarofuranoid sesquiterpenes (Chen et al., 2006; Spivey et al., 2002; Gao et al., 2007) as major compounds, benzenoids (Chen et al., 2008) and triterpenes (Wang et al., 2007). To the best of our knowledge no phytochemical or pharmacological studies have been done on *Salacia longipes* var. *camerunensis*. In our continuing search for bioactive compounds from Cameroonian medicinal plants, we have investigated the CH_2Cl_2–MeOH (1:1) extract of the seeds of *S. longipes* var. *camerunensis* which was found to be active *in vitro* against *Plasmodium falciparum* chloroquine-resistant strain W2 in a preliminary screening with IC_{50} of 2.28 μg/mL. We report herein on the isolation and the structure elucidation of four new sesquiterpenoids **1**–**4** together with the antiplasmodial activity of some of the isolated compounds.

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2. Results and discussion

Extensive chromatographic purification of the CH_2Cl_2–MeOH (1:1) extract of the seeds of *S. longipes* var. *camerunensis* afforded four new sequiterpenoids, salaterpenes A–D (**1**–**4**) together with two known compounds 1α,6β-diacetoxy-8β,9β-dibenzoyloxy-4β-hydroxy-2-oxo-dihydro-β-agarofuran (**5**) (Takaishi et al., 1992a,b) and 2β-acetoxy-1α,6β,9β-tribenzoyloxy-4β-hydroxy-dihydro-β-agarofuran (**6**) (González et al., 1993) (Fig. 2).

2.1. Characterization of salaterpene A (*1*)

Compound **1** was obtained as colorless crystals, m.p. 190–191 °C, $[\alpha]_D^{20}$ +17.5 (*c* 0.5, $CHCl_3$). Its molecular formula $C_{35}H_{40}O_{12}$ was determined from the NMR data and its positive HRESIMS which showed the pseudo-molecular ion peak $[M+H]^+$ at *m/z* 653.2586 (calcd 653.2598 for $C_{35}H_{41}O_{12}$). UV absorptions at 244 and 276 nm suggested the presence of aromatic moieties. The IR spectrum showed absorption bands for hydroxyl (3515 cm^{-1}) and ester carbonyl (1755 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) of compound **1** showed three single signals of four methyl groups at δ_H 1.49 (3H, H-14), 1.59 (6H, H-12; H-15) and 1.68 (3H, H-13), three acetyl groups at δ_H 1.78, 1.90 and 1.95 (8-, 1- and 2-OAc) and one methylene group at δ_H 2.10 (m, H-3). In addition, six methine groups, five of which were oxygenated [δ_H 2.64 (br d, *J* = 3.2 Hz, H-7), 5.01 (dt,

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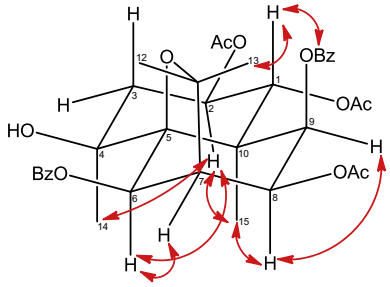

Fig. 1. nOE effects for compound **1**.

J = 6.8 and 10.4 Hz, H-2), 5.41 (d, J = 6.0 Hz, H-9), 5.69 (d, J = 10.4 Hz, H-1), 5.70 (br s, H-6) and 5.74 (dd, J = 3.2 and 6.0 Hz, H-8)], one hydroxyl group at δ_H 3.20 (br s, 4-OH) and two A_2MX_2 spin systems specific to two benzoyl groups [δ_H 8.21, 8.13 (2H each, d both, J = 8.6 Hz, H-2′/6′ and H-2″/6″), 7.61 (2H, t, J = 8.6 Hz, H4′ and H-4″) and 7.50 (4H, t, J = 8.6 Hz, H-3′/5′ and H-3″/5″)] were observed. The ^{13}C NMR spectrum (Table 2) of compound **1** showed signals of 35 carbons which were sorted by DEPT and HSQC into eleven quaternary carbons including five carbonyl groups (δ_C 165.6, 165.9, 168.9, 169.0 and 170.3), sixteen methines, one methylene and seven methyl groups. All these data indicated that **1** was a pentasubstituted polyester sesquiterpene with a dihydro-β-agarofuran skeleton (Brüning and Wagner, 1978). The positions of acetate groups were determined using the HMBC experiment from correlations observed between the protons H-2 (δ_H 5.01), H-1 (δ_H 5.69) and H-8 (δ_H 5.74) and the carbonyls of the acetate groups at δ_C 170.3, 169.0 and 168.9, respectively (Fig. 3). From the same experiment, the benzoate groups were found to be attached to C-6 and C-9, from correlations between the protons H-6 (δ_H 5.70) and H-9 (δ_H 5.41), and the benzoate carbonyls at δ_C 165.6 and 165.9, respectively.

The relative stereochemistry of **1** was established on the basis of the ^{1}H NMR coupling constants together with ^{1}H–^{1}H COSY and NOESY experiments from where resonances at δ_H 5.01 (H-2), 5.41 (H-9), 5.69 (H-1), 5.70 (H-6) and 5.74 (H-8) were assigned as H-2$_{ax}$, H-9$_{eq}$, H-1$_{ax}$, H-6$_{ax}$ and H-8$_{ax}$. The NOESY experiment (Fig. 1) showed the NOE effect between H-1 and H-13, H-1 and the benzoyl protons H-2′/H-6′, which supports the axial orientation of H-1 and the C-9 benzoate moiety. Other dipolar interactions were also observed, like H-2/H-6, H-2/H-14 and H-2/H-15; H-6/H-7; H-8/H-9 and H-8/H-15. H-9 couples with H-8 only and the coupling constant J = 6.0 Hz suggests an axial/equatorial relationship. In addition H-8 couples to H-7 in an axial/equatorial relationship with J = 3.2 Hz. These assignments are in agreement with the relative configurations observed at these positions in this class of natural products (Chou et al., 2007; Takaishi et al., 1992a; González et al., 1990; Muñoz-Martínez et al., 2005). As previously shown for similar derivatives, a coupling between the equatorial proton H-7 and the axial proton H-6 is small or not observed at all (Muñoz-Martínez et al., 2005). According to the literature on some dihydro-β-agarofuran sesquiterpenes (Muñoz-Martínez et al., 2005), H-1 resonating as a doublet with J = 3.6 Hz is indicative for an axial/equatorial relationship while a doublet with J = 11.0 Hz, is typical for an axial-axial relationship. In the ^{1}H NMR spectrum of compound **1**, H-1 resonates as a doublet with J = 10.4 Hz, indicating axial orientations of both H-1 and H-2. Thus compound **1** is assigned as 1α,2β,8β-triacetoxy-6β,9β-dibenzoyloxy-4β-hydroxy-dihydro-β-agarofuran, named salaterpene A.

Table 1
^{1}H NMR (δ, $CDCl_3$, J in Hz in parentheses, 400 MHz) data of compounds **1**–**4**.

Position	1	2	3	4
1	5.69 d (10.4)	5.63 d (10.4)	5.65 d (10.4)	5.90 d (10.4)
2	5.01 td (10.4, 6.8)	4.96 td (10.4, 6.4)	4.98 td (10.4, 6.8)	5.13 td (10.4, 7.2)
3	2.10 m	2.04 m	2.07 m	2.13 m
6	5.70 br s	5.59 br s	5.65 br s	5.69 br s
7	2.64 br d (3.2)	2.52 br d (2.8)	2.36 br t (3.2)	2.39 br dd (3.2, 2.8)
8	5.74 dd (6.0, 3.2)	5.64 dd (6.4, 2.8)	2.22 br dd (16.4, 2.8) 2.55 ddd (16.4, 6.4, 3.2)	2.23 br dd (16.8, 3.2) 2.55 ddd (16.8, 6.8, 3.2)
9	5.41 d (6.0)	5.35 d (6.4)	5.08 br d (6.4)	5.08 br d (6.8)

Table 2
^{13}C NMR (δ, $CDCl_3$, 100 MHz) data of compounds **1**–**4**.

Position	1	2	3	4
1	71.8	71.9	73.9	72.8
2	68.7	68.8	70.5	68.7
3	44.4	44.3	45.5	44.6
4	72.0	70.9	72.5	71.2
5	90.9	90.9	92.3	91.1
6	78.1	77.6	81.8	80.2
7	53.8	53.8	50.4	48.9
8	68.7	68.7	32.9	31.7
9	71.9	71.8	74.3	73.4
10	50.3	50.3	53.3	52.1
11	85.3	85.3	86.5	85.0
12	30.4	30.4	30.7	29.7
13	26.5	26.5	26.9	25.9
14	24.7	24.8	25.9	24.9
15	20.3	20.3	21.4	20.6

2.2. Characterization of salaterpene B (*2*)

Compound **2** was obtained as colorless crystals, m.p. 204–205 °C, $[\alpha]_D^{20}$ +30 (c 0.5, $CHCl_3$). Its HRESIMS showed the molecular ion $[M+H]^+$ at m/z 679.2742, supporting the formula $C_{37}H_{42}O_{12}$ (calcd for $C_{37}H_{43}O_{12}$, 679.2754), consistent with seventeen double bond equivalents. This value is 26 mass units higher than that of compound **1**, suggesting the presence of an additional C_2H_2 unit in compound **2**. UV absorptions at 242 and 282 nm suggested the presence of aromatic moieties. The IR spectrum showed absorption bands for hydroxyl (3515 cm^{-1}) and ester carbonyl (1747 and 1712 cm^{-1}) groups. The spectroscopic data of compound **2** point out a high similarity with the structure of **1**. The major difference between **1** and **2** was the replacement of the C-6 benzoyloxy group in **1** by a *trans*-cinnamoyloxy group [δ_H 6.43 and 7.83 (1H, d, J = 16.0 Hz, each, ethylenic protons); and δ_H 7.32–7.50 (5H, m, aromatic protons)] in **2**. This was further confirmed by the HMBC spectrum of compound **2** where a correlation between the proton H-6 (δ_H 5.59) and the carbonyl of the cinnamoyloxy group at δ_C 165.7 was observed (Fig. 3).

The relative stereochemistry of **2** was determined based on the ^{1}H NMR and NOESY studies. In fact, the coupling constant value $J_{8,9}$ = 6.4 Hz observed between H-8 and H-9 indicated an axial/equatorial orientation of these two protons. The NOESY experiment showed proximity between H-7/H-6 and H-7/H-8; H-2/H-8; H-6/H-14; H-1/9-OBz and H-9/H-15. According to the above data, the stereostructure of **2** was established as 1α,2β,8β-triacetoxy-9β-benzoyloxy-6β-cinnamoyloxy-4β-hydroxy-dihydro-β-agarofuran, named salaterpene B.

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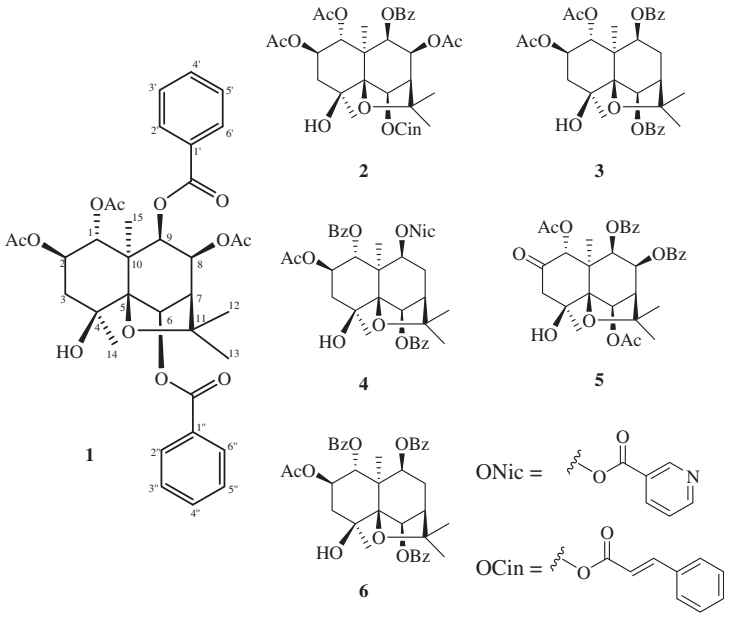

Fig. 2. Chemical structures of compounds **1**–**6**.

2.3. Characterization of salaterpene C (**3**)

Compound **3** was isolated as colorless crystals, m.p. 279–280 °C, $[\alpha]_D^{20}$ −5.2 (*c* 0.5, $CHCl_3$). Its molecular formula, $C_{33}H_{38}O_{10}$, with fifteen double bond equivalents, was deduced from the HRESIMS, which showed a quasi-molecular ion peak $[M+Na]^+$ at *m/z* 617.2349 (calcd: 617.2365 for $C_{33}H_{38}O_{10}Na$). This value is 58 mass units lower than that of compound **1**, suggesting that one acetate group was replaced by a hydrogen atom in compound **3**. Its UV spectrum showed absorptions at λ_{max} 241 and 275 nm, and the IR spectrum showed absorption bands for hydroxyl (3494 cm^{-1}) and ester carbonyl (1751 and 1713 cm^{-1}) groups. The spectroscopic data of compound **3** were similar to those of **1**. The ^1H NMR spectrum of **3** indicated the replacement of a methine by a methylene group [δ_H 2.22 (1H, br dd, *J* = 2.8 and 16.4 Hz, H-8_{eq}) and 2.55 (1H, ddd, *J* = 3.2, 6.4 and 16.4 Hz, H-8_{ax})], which clearly indicated that the acetate group at C-8 is absent in **3**. This was confirmed by the HMBC spectrum of **3** (Fig. 3), where correlations were observed between H-2 (δ_H 4.98) and H-1 (δ_H 5.65), and the carbonyls at δ_C 171.3 and 171.1 (acetate groups), respectively; and between H-6 (δ_H 5.65) and H-9 (δ_H 5.08) and the carbonyls at δ_C 167.7 and 167.2 (benzoate groups), respectively.

The relative stereochemistry of compound **3** was established from a careful study of coupling constants in the ^1H NMR spectrum which showed an axial/axial relationship between H-1 and H-2, $J_{1,2}$ = 10.4 Hz, and an equatorial/axial relationship between H-9 (δ_H 5.08) and H-8_{ax} (δ_H 2.55), $J_{8ax,9}$ = 6.4 Hz. This was confirmed by the NOESY experiment which showed dipolar interactions between H-6/H-7, H-8/H-9, one of the 9-OBz protons and H-1, and H-2/H-14. The latter NOE cross-peak especially confirmed that H-2 is axial. Thus compound **3** was assigned as 1α,2β-diacetoxy-6β,9β-dibenzoyloxy-4β-hydroxy-dihydro-β-agarofuran, named salaterpene C.

2.4. Characterization of salaterpene D (**4**)

Compound **4**, colorless crystals, m.p. 216–217 °C, $[\alpha]_D^{20}$ +82.5 (*c* 0.5, $CHCl_3$), has the molecular formula $C_{37}H_{39}NO_{10}$ (HRESIMS *m/z* 658.2657, $[M+H]^+$ (calcd for $C_{37}H_{40}NO_{10}$, 658.2653). UV absorptions at 243 and 263 nm suggested the presence of aromatic moieties. The IR spectrum exhibited signals for hydroxyl (3515 cm^{-1}) and ester carbonyl (1714 cm^{-1}) groups. The ^1H, ^{13}C NMR and DEPT spectra (Tables 1 and 2) clearly indicated that compound **4** has four quaternary methyls [δ_H 1.50 (H-12), 1.51 (H-14), 1.56 (H-13) and 1.64 (H-15)]; two methylenes [δ_H 2.13 (m, H-3); 2.23 (br dd, *J* = 3.2 and 16.8 Hz, H-8_{eq}) and 2.55 (ddd, *J* = 3.2, 6.8 and 16.8, H-8_{ax})], five methines [δ_H 2.39 (dd, *J* = 2.8 and 3.2 Hz, H-7), 5.08 (d, *J* = 6.8 Hz, H-9), 5.13 (dt, *J* = 7.2 and 10.4 Hz, H-2), 5.69 (br s, H-6), and 5.90 (d, *J* = 10.4 Hz, H-1)], one acetate group (δ_H 1.76, 2-OAc), two benzoate and one nicotinate groups (δ_H 7.23–9.09), one hydroxyl group (δ_H 3.23, br s) and four quaternary carbons [δ_C 52.1 (C-10), 71.2 (C-4), 85.0 (C-11) and 91.1 (C-5)]. These data indicated that its parent structure was a dihydro-β-agarofuran sesquiterpene polyester (Brüning and Wagner, 1978). The HMBC experiment showed correlations between the proton at δ_H 5.13 (H-2) and the carbonyl at δc 170.3 (acetate group). The two benzoate groups were attached to C-1 and C-6 according to the cross-peaks observed between H-1 (δ_H 5.90) and the carbonyl at δ_C 164.9, and between H-6 (δ_H 5.69) and the carbonyl at δ_C 166.0 (Fig. 3). The nicotinate group was located at position C-9 as proven by the correlation of H-9 (δ_H5.08) with the carbonyl at δ_C 163.9.

The relative configuration of **4** was determined using the NOESY experiment which showed correlations between H-9/H-15, H-6/H-7, H-2/H-14 and H-2/H-15. This was further confirmed in the ^1H NMR spectrum by the coupling constant values of $J_{1,2}$ = 10.4 Hz and $J_{8ax,9}$ = 6.8 Hz, indicating a *trans*-diaxial relationship between H-1 and H-2, and an axial/equatorial orientation for H-8_{ax} and H-9. Correlations observed in the same NOESY spectrum between H-1 and H-2′ (δ_H 9.09) of the nicotinate group confirmed its axial orientation. Therefore, **4** was concluded to be 2β-acetoxy-1α, 6β-dibenzoyloxy-4β-hydroxy-9β-nicotinoyloxy-dihydro-β-agarofuran, named salaterpene D.

All the isolated compounds are sesquiterpene esters based on a dihydro-β-agarofuran (5,11-epoxy-5β-10α-eudesm-4(14)-ene skeleton type, confirming the fact that this class of compounds is a chemotaxonomic indicator of the Celastraceae family (Chen et al., 2007).

2.5. Biological activity

The isolates were evaluated *in vitro* for their antiplasmodial activity against *P. falciparum* W2 strain, the protozoa responsible for malaria, which is resistant to chloroquine and other antimalarial

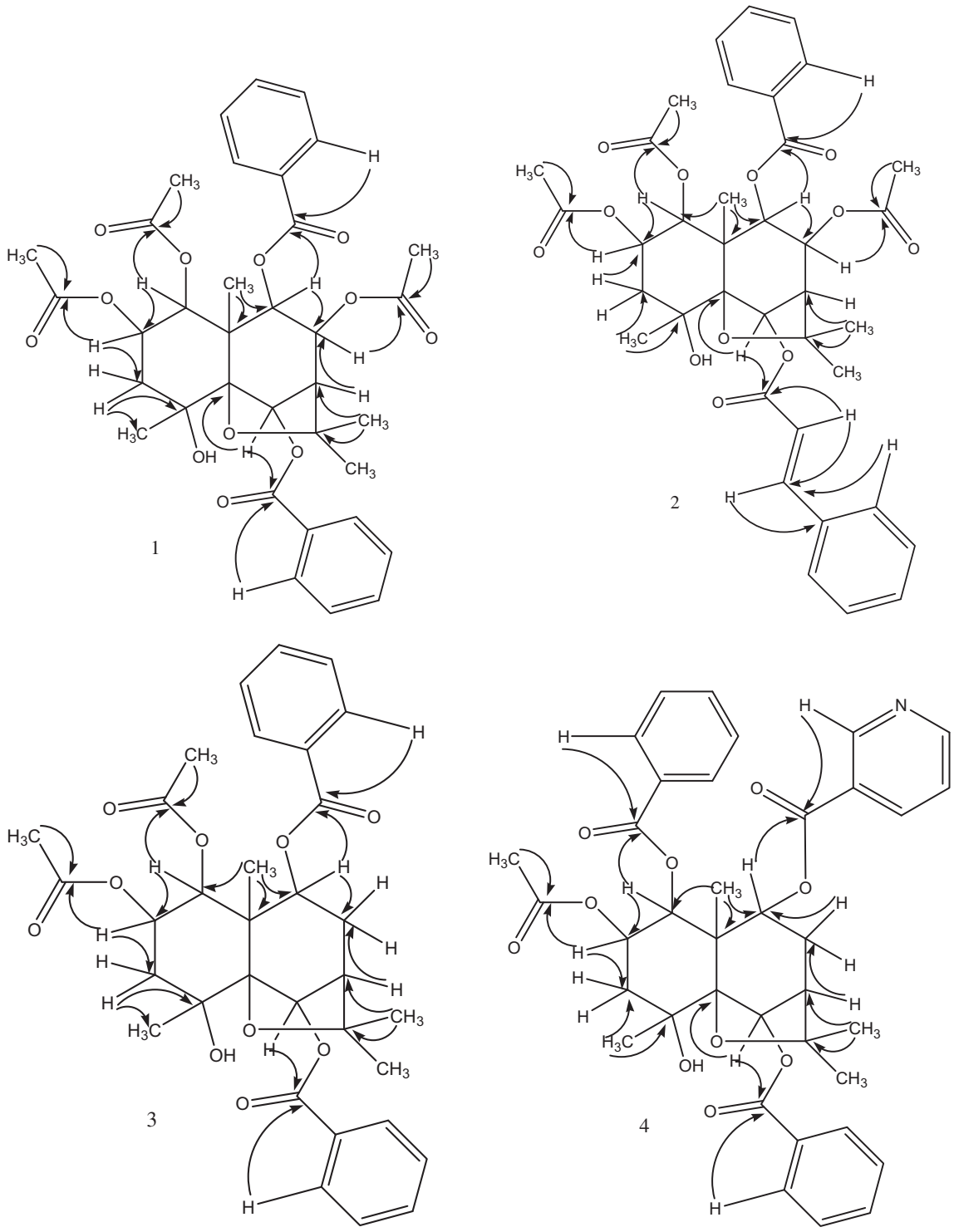

Fig. 3. Selected HMBC correlations for compounds **1**–**4**.

drugs (Singh and Rosenthal, 2001) (Table 3). Compounds **1**–**4** and **6** were found to exhibit a moderate antiplasmodial activity *in vitro* with IC_{50} values below 2.7 μM. All the tested compounds are oxidized at different positions of the dihydro-β-agarofuran sesquiterpene nucleus. The presence of an additional benzoate (in compound **6**) or cinnamate (in compound **2**) moieties could positively influence activity. Dihydro-β-agarofuran sesquiterpenoids are known to possess antioxidant (Chen et al., 2006), immunosuppressive (Zheng et al., 1989), cytotoxic (Kuo et al., 1994), insecticidal (Wu et al. 2001), anti-HIV (Duan et al., 1999), antitumor-promoting (González et al., 2000), antitubercular (Chen et al., 2008) and anti-inflammatory (Jin et al., 2002) activities, and to display an influence on multidrug-resistance (Kennedy et al., 2001).

3. Concluding remarks

We report the first phytochemical investigation of the seeds of *S. longipes*. Four compounds, namely salaterpene A–D (**1–4**) were isolated and their structures established. These dihydro-β-agarofuran sesquiterpenoids showed moderate antiplasmodial activities

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Table 3
Antiplasmodial activity of extracts and compounds **1**–**4** and **6** against *P. falciparum* W2 strain.

Extracts and compounds	IC_{50}	
	(μg/mL ± SD[a])	μM
Seeds extract	2.28 ± 0.07	–
Pericarp extract	>10	–
1	1.32 ± 0.16	2.02 ± 0.25
2	1.23 ± 0.10	1.81 ± 0.15
3	1.56 ± 0.07	2.63 ± 0.12
4	1.57 ± 0.28	2.38 ± 0.42
6	1.12 ± 0.04	1.71 ± 0.06
Chloroquine	0.06 ± 0.01	0.11 ± 0.02

[a] Standard deviation.

against the W2 strain of *P. falciparum in vitro*. The antiplasmodial property of this class of secondary metabolites is reported here for the first time. Our study has demonstrated the antiplasmodial potency of the seeds extracts and constituents of *S. longipes*. The interesting results obtained in this study highlight the bioactive potency of dihydro-*β*-agarofuran sesquiterpenoids and contribute to the validation of the seeds of plants of the Celastraceae family as a source of bioactive compounds.

4. Experimental section

4.1. General experimental procedures

Melting points were determined on a ThermoFisher Scientific Digital M.P., serial IA 9000 melting point apparatus. Optical rotations were measured on a JASCO P-2000 spectropolarimeter. UV spectra were recorded on a Carry 300 spectrophotometer. IR spectra were recorded on a JASCO Fourier Transform IR spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance 400 spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C), respectively; a Mercury-300 spectrometer operating at 300 MHz (^1H) and INOVA-500 operating at 125 MHz (^{13}C), with TMS as internal standard. HRESIMS were recorded on a micrOTOF 10237 and SCA Pharma Stbg QToF. Silica gel 230–400 mesh (Merck) and silica gel 70–230 mesh (Merck) were used for flash and column chromatography, while percolated aluminum silica gel 60 F_{254} sheets were used for TLC with different mixtures of *n*-hexane–ethyl acetate, and dichloromethane–methanol as eluents. Spots were visualized with UV light (254 and 365 nm) or using MeOH–H_2SO_4 reagent.

4.2. Plant material

S. longipes var. *camerunensis* was collected in December 2008 at Mount Kala (Yaoundé) in the Centre region of Cameroon and identified by Mr. Nana Victor, botanist at the National Herbarium of Cameroon where a voucher specimen has been deposited (N° 28963/SRF/Cam).

4.3. Extraction and separation

The seeds of *S. longipes* var. *camerunensis* (1.5 kg), separated from the fruit pericarp, were pulverized and extracted at room temperature with a mixture of CH_2Cl_2–MeOH (1:1), (2 × 2 L, 48 h each). The solvent was removed under reduced pressure to afford 139.2 g of extract. The ground pericarp (1 kg) was extracted at room temperature with a mixture of CH_2Cl_2–MeOH 1:1 (2 × 1 L, 24 h each). The solvent was removed under reduced pressure to yield 45.2 g of extract. These two extracts were screened for their antiplasmodial activity *in vitro*. The extract from the seeds showed moderate antiplasmodial activity (IC_{50} of 2.28 μg/mL) while that from the pericarp showed no significant activity. The seed extract (137.1 g) was chromatographed on silica gel using mixtures of *n*-hexane–ethyl acetate of increasing polarity as eluent. Seventy fractions of 400 mL each were collected and combined on the basis of TLC analysis to yield five main fractions labeled A (28.0 g), B (20.5 g), C (32.0 g), D (22.4 g) and E (30.0 g).

Fractions A (28.0 g) and B (20.5 g) were essentially oils that were not further investigated. Fraction C (32.0 g) was subjected to column chromatography over silica gel (70–230 mesh), eluting with *n*-hexane–ethyl acetate gradient mixtures resulting in the collection of 290 fractions of 100 mL each, which were combined on the basis of TLC analysis. Further purification of sub-fractions 75–90 afforded salaterpene B (**2**, 18.1 mg) and **6** (30.4 mg). Sub-fractions 119–127 yielded salaterpene C (**3**, 17.5 mg). Chromatography of sub-fractions 153–175 afforded salaterpene A (**1**, 40.1 mg) and that of sub-fractions 253–259 afforded **5** (15.7 mg). Fraction D (22.4 g) was subjected to column chromatography over silica gel (70–230 mesh), eluting with *n*-hexane–ethyl acetate (80:20–30:70) to yield salaterpene D (**4**, 35.2 mg). The fraction E (30.0 g) was a complex mixture that was not further studied. The pericarp extract which showed no significant activity on the *P. falciparum* W2 strain was not further studied.

4.4. Spectroscopic data

4.4.1. 1α,2β,8β-Triacetoxy-6β,9β-dibenzoyloxy-4β-hydroxy-dihydro-β-agarofuran (**1**)

Colorless crystals; m.p. 190-191°C; $[\alpha]_D^{20}$ +17.5 (*c* 0.5, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log *ε*) 244 (4.03), 276(3.43) nm; IR (KBr) ν_{max} 3515 (OH), 1755 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.49 (3H, s, Me-14), 1.59 (6H, s, Me-12,15), 1.68 (3H, s, Me-13), 1.78 (3H, s, 8-OAc), 1.90 (3H, s, 1-OAc), 1.95 (3H, s, 2-OAc), 3.20 (br s, 4-OH), 7.50–8.21 (10H, m, 6 and 9-OBz), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C OBz × 2 [128.2 (d × 2), 128.7 (d × 2),129.5 (s), 130.0 (s), 130.2 (d × 2), 130.4 (d × 2), 133.3 (d), 133.6 (d)], 165.6 (s, 6-OBz), 165.9 (s, 9-OBz), [168.9 (s) and 20.4 (q), 8-OAc], [169.0 (s) and 20.5 (q), 1-OAc], [170.3 (s) and 20.9 (q), 2-OAc], for other signals, see Table 2; HRESIMS: $[M+H]^+$, *m*/*z* 653.2586 (calcd. for $C_{35}H_{41}O_{12}$; 653.2598).

4.4.2. 1α,2β,8β-Triacetoxy-9β-benzoyloxy-6β-cinnamoyloxy-4β-hydroxy-dihydro-β-agarofuran (**2**)

Colorless crystals; m.p. 204–205 °C; $[\alpha]_D^{20}$ +30 (*c* 0.5, $CHCl_3$); UV(CH_2Cl_2) λ_{max}(log *ε*) 242 (4.12), 282 (4.43) nm; IR (KBr) ν_{max} 3515 (OH), 1747, 1712, 1650 (C=C) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.44 (3H, s, Me-14), 1.53 (3H, s, Me-15), 1.57 (3H, s, Me-12), 1.63 (3H, s, Me-13), 1.73 (3H, s, 8-OAc), 1.86 (3H, s, 1-OAc), 1.90 (3H, s, 2-OAc), 3.08 (br s, 4-OH), 6.43 (1H, d, *J* = 16.5 Hz, ethylene), 7.83 (1H, d, *J* = 16.5 Hz, ethylene), OCin and OBz [7.36 (3H, m), 7.47 (2H, m), 7.50 (3H, m), 8.08 (2H, m)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C OCin and OBz [117.4 (d), 128.2 (d × 2), 128.4 (d × 2), 128.9 (d × 2), 129.5 (s), 130.4 (d × 2), 130.7 (d), 133.3 (d), 134.1 (s), 146.9 (d)], 165.7 (s, 6-OCin), 165.9 (s, 9-OBz), [169.0 (s) and 20.8 (q), 8-OAc], [169.1 (s) and 20.6 (q), 1-OAc], [170.3 (s) and 20.9 (q), 2-OAc], for other signals see, Table 2; HRESIMS: $[M+H]^+$, *m*/*z* 679.2742 (calcd for $C_{37}H_{43}O_{12}$; 679.2754).

4.4.3. 1α,2β-Diacetoxy-6β,9β-dibenzoyloxy-4β-hydroxy-dihydro-β-agarofuran (**3**)

Colorless crystals, m.p. 279-280°C; $[\alpha]_D^{20}$ −5.2 (*c* 0.5, $CHCl_3$); UV(CH_2Cl_2) λ_{max} (log *ε*) 241 (3.48), 275 (2.55) nm; IR (KBr) ν_{max} 3494 (OH), 1751, 1713 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.47 (3H, s, Me-14), 1.49 (3H, s, Me-15), 1.50 (3H, s, Me-12), 1.56 (3H, s, Me-13), 1.70 (3H, s, 1-OAc), 1.91 (3H, s, 2-OAc), 3.23 (s, 4-OH),

OBz × 2 [7.45 (4H, m), 7.55 (2H, m), 8.08 (2H, m), 8.19 (2H, m)], for other signals, see Table 1; ^{13}C NMR (MeOD/$CDCl_3$, 100 MHz) δ_C OBz × 2 [129.5 (d × 2), 129.9 (d × 2),130.8 (s), 130.9(s), 131.4 (d × 2), 131.6 (d × 2), 134.7 (d), 134.9 (d)], 167.2 (s, 9-OBz), 167.7 (s, 6-OBz),[171.1 (s) and 21.5 (q), 1-OAc], [172.3 (s) and 21.8 (q), 2-OAc], for other signals, see Table 2; HRESIMS: $[M+Na]^+$, m/z 617.2349 (calcd. for $C_{33}H_{38}O_{10}Na$; 617.2365).

4.4.4. 2β-Acetoxy-1α,6β-dibenzoyloxy-4β-hydroxy-9β-nicotinoyloxy-dihydro-β-agarofuran (**4**)

Colorless crystal; m.p. 216–217 °C; $[\alpha]_D^{20}$ +82.5 (*c* 0.5, $CHCl_3$); UV (CH_2Cl_2) λ_{max} (log ε) 243 (4.12), 263 (3.73) nm; IR (KBr) ν_{max} 3515 (OH), 1714 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.50 (3H, s, Me-13), 1.51 (3H, s, Me-14), 1.56 (3H, s, Me-12), 1.64 (1H, s, Me-15), 1.76 (3H, s, 2-OAc), 3.23 (s, 4-OH), ONic and OBz × 2 [7.23 (2H, m), 7.42 (3H, m), 7.50 (4H, m), 8.20 (2H, m), 8.28 (1H, m), 8.78 (1H, d, 3.6 Hz), 9.09 (1H, s)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C ONic and OBz × 2 [123.1 (d), 125.7 (s), 128.3 (d × 2), 128.7 (d × 2), 129.1 (d × 2), 129.5 (s), 129.6 (s), 130.2 (d × 2), 133.1 (d), 133.5 (d), 137.9 (d), 151.3 (d), 153.1 (d)], 163.9 (s, 9-ONic), 164.9 (s, 1-OBz), 166.0 (s, 6-OBz), [170.3 (s) and 20.8 (q), 2-OAc], for other signals, see Table 2; HRESIMS: $[M+H]^+$, m/z 658.2657 (calcd for $C_{37}H_{40}NO_{10}$; 658.2653).

4.5. Antiplasmodial activity assay

Antiplasmodial activity was determined using the W2 strain of *P. falciparum* which is resistant to chloroquine and other antimalarials and was cultured in sealed flasks at 37 °C, in a 3% O_2, 5% CO_2 and 91% N_2 atmosphere in RPMI 1640, 25 mM HEPES, pH 7.4, supplemented with heat inactivated 10% human serum and human erythrocytes to achieve a 2% hematocrit. Parasites were synchronized in the ring stage by serial treatment with 5 % sorbitol (SIGMA) (Lambros and Vanderberg, 1979) and studied at 1 % parasitemia.

Compounds were prepared to 10 μM stock solutions in DMSO, diluted as needed for individual experiments, and tested in triplicate. The stock solutions were diluted in supplemented RPMI 1640 medium so as to have at most 0.2% DMSO in the final reaction medium. An equal volume of 1% parasitemia, 4% hematocrit culture was thereafter added and gently mixed thoroughly. Negative controls contained equal concentrations of DMSO. Positive controls contained 1 μM chloroquine phosphate (sigma). Cultures were incubated at 37 °C for 48 H (1 parasite erythrocytic life cycle). Parasites at the ring stage were thereafter fixed by replacing the serum medium by an equal volume of 1% formaldehyde in PBS. Aliquots (50 μL) of each culture were then added to 5 ml round-bottom polystyrene tubes containing 0.5 mL 0.1% Triton X-100 and 1 nM YOYO nuclear dye (Molecular Probes) in PBS, and parasitemias of treated and controls cultures were compared using a Becton–Dickinson FACSort flow cytometer to count nucleated (parasitized) erythrocytes. Data acquisition was performed using CellQuest software. These data were normalized to percent control activity and 50% inhibitory concentrations (IC_{50}) were calculated using Prism 3.0 software (GraphPad) with data fitted by non linear regression to the variable slope sigmoidal dose response formula, $y = 100/[1 + 10^{(\log IC_{50} - x)H}]$, where H is the Hill coefficient or slope factor (Singh and Rosenthal, 2001).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytochem.2013.06.022.

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