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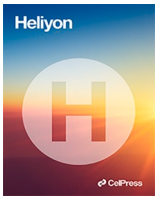

Research article

Cytotoxic *ent*-abietane diterpenoids, banyangmbolides A-E, from the leaves of *Suregada occidentalis*

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ABSTRACT

The chemical investigation of a leaf extract from a herbarium specimen of *Suregada occidentalis* collected in Banyang Mbo Wildlife Sanctuary, Southwest Region, Cameroon, yielded five undescribed *ent*-abietane diterpenoids, banyangmbolides A-E, (**1**–**5**), and four known diterpenoids, gelomulides A (**6**), B (**7**), D (**8**) and O (**9**). The structures of the isolated compounds were determined using NMR, IR, ECD and HRESIMS. Compounds **5**, **7** and **8**, showed 48–55% inhibition at 200 μM against FM-55-M1 human melanoma cells.

1. Introduction

Suregada Roxb. ex Rottler (Euphorbiaceae) is a genus of shrubs or trees with 32 accepted species, eight of which occur in continental Africa [1]. Previously known as *Gelonium* Roxb. ex Willd. [1,2], species of this genus are found from West Africa to Madagascar, India, southern China, Philippines, New Guinea and Northern Australia. In Cameroon only a single species, *Suregada occidentalis* (Hoyle) Croizat is recorded [3], with occurrence in Ivory Coast as well [2]. Plants of *S. occidentalis* were encountered during a survey for botanical conservation prioritisation of the Banyang Mbo Wildlife Sanctuary in SW Region Cameroon. Banyang Mbo comprises highly species-diverse lowland and submontane evergreen forest (rainfall c. 3 m p.a.), and has been the source of numerous new species to science all of which are threatened [4–8], and new records of threatened species previously thought to be restricted to the adjacent Bakossi forests [9–11]. *S. occidentalis* is unique in the genus in having 4-winged stems with sessile, subcordate leaves. It is an understorey shrub growing up to 3 m tall in dense lowland forest. No ethnomedical uses were recorded for the species in Banyang Mbo, nor are such recorded in the rest of its range [12].

The chemistry of *S. occidentalis* has not been previously investigated. However, other members of the genus predominantly yielded *ent*-abietane diterpenoids, that possess an α-methyl-α,β-unsaturated γ-lactone moiety, and to a lesser extent kaurane diterpenoids, triterpenoids, flavonoids and pyrrolidine alkaloids [13]. Following results from *Suregada* species, in this study we report undescribed *ent*-abietane diterpenoids banyangmbolides A-E (**1**–**5**) and four known diterpenoids, gelomulides A (**6**) [14,15], B (**7**) [15], D (**8**) [15], and O (**9**) [16] (Fig. 1).

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1.1. Results and discussion

The leaf extracts of *S. occidentalis* were subjected to repeated column chromatography to yield five undescribed *ent*-abietane diterpenoids banyangmbolides A-E (**1**–**5**) and four known diterpenoids, gelomulides A (**6**) [14,15], B (**7**) [15], D (**8**) [15], and O (**9**) [16] (Fig. 1).

The HRESIMS of **1** gave an $[M+H]^+$ ion peak at *m/z* 391.2117 (calcd. for $C_{22}H_{30}O_6$ + H, *m/z* 391.2120). The FTIR spectrum presented absorptions for *α,β*-unsaturated *γ*-lactone carbonyl at 1750 cm^{-1}, and two carbonyl groups at 1732 and 1716 cm^{-1} for a lactone and an acetate group. The ^{13}C NMR, DEPT and ^1H NMR spectroscopic data of compound **1** showed a 20-carbon diterpenoid skeleton, an acetoxy group and an *α*-methyl-*α,β*-unsaturated *γ*-lactone group typical of compounds previously isolated from the *Suregada* genus [13]. The 3H-17, methyl group on the α,β-unsaturated γ-lactone moiety exhibited a resonance at δ_H 1.97 (d, J = 2.1 Hz), the methyl of an acetoxy group at δ_H 2.09 (s), and in addition three singlet methyl group resonances were observed at δ_H 1.14 (s), 0.99 (s) and 0.91 (s). Furthermore, the ^1H NMR spectrum supported four oxy-methine proton resonances at δ_H 5.08 (m), 4.78 (t, J = 3.3 Hz), 3.88 (dd, J = 11.2, 5.0 Hz) and 3.78 (s). The singlet at δ_H 3.78 integrating to 1H attached to the carbon at δ_C 56.3 in the HSQCDEPT spectrum, was assigned to an allylic epoxy group, typical at C-14 for *ent*-abietane diterpenoids isolated from the *Suregada* genus [17,18].

The HMBC spectrum for **1** showed correlations between the H_3-17 with the carbonyl group at δ_C 174.4 for C-16, and the carbon resonances at δ_H 156.3 and 129.1 for C-13 and C-15 respectively. C-13 and C-15 showed correlations in the HMBC spectrum with H-14 allylic epoxy proton resonance at δ_H 3.78 (s). The H-14 proton resonance showed correlations in the HMBC spectrum with carbon resonances at δ_C 75.9, 61.5, 49.7 and 34.5 for C-12, C-8, C-9, and C-7 respectively. The corresponding H-12 at δ_H 5.08 (m) showed correlations in the HMBC spectrum with C-9, C-13, C-14, C-15 and C-17, and was coupled in the COSY spectrum with the methylene group at δ_H 1.47 (m), and the unusually downfield resonance at δ_H 3.20 (ddd, J = 12.6, 5.4, 1.7 Hz), for anisotropically effected proton for H_2-11. H_2-11 showed correlation in the COSY spectrum with H-9 at δ_H 2.20 (br d, J = 10.4 Hz) for H-9 (Fig. 2). The NMR data supported the presence of an *α,β*-unsaturated *γ*-lactone ring D typically observed for *ent*-abietane diterpenoids consistently found in the *Suregada* genus [14,15,19].

For this compound the H_3-18 and H_3-19 methyl proton resonances were at δ_H 0.91 (s) and 0.99 (s), and their corresponding carbon resonances were δ_C 28.3 and 22.1 respectively. The two methyl group proton resonances showed correlations in the HMBC spectrum with carbon resonances at δ_C 78.3 for C-3, δ_C 49.0 for C-5 and δ_C 36.8 for C-4. The corresponding H-3 proton resonance was at δ_H 4.78 (t, J = 3.3 Hz), typically deshielded due to acetylation at this position. This H-3 resonance showed a correlation with an acetoxy carbonyl resonance at δ_C 170.6 in the HMBC spectrum, and in turn, showed coupling with the overlapped 2H-2 proton resonance at δ_H 1.88 (m) in the COSY spectrum. The 2H-2 proton resonance, in turn, showed coupling in the COSY spectrum with an oxy-methine proton resonances at δ_H 3.88 (dd, J = 11.2, 5.0 Hz) that was assigned to H-1. The corresponding C-1 resonance was assigned as δ_C 73.0, and in turn, showed a correlation in the HMBC spectrum with the H_3-20 at δ_H 1.14 (s). The H_3-20 resonance showed correlations in the HMBC spectrum with C-5 (δ_c 49.0), C-9 (δ_C 49.7) and C-10 (δ_C 45.0) (Fig. 2).

The above spectroscopic data supported a 1-hydroxy-3-acetoxy substitution for compound **1**. The use of NOESY spectrum showed that H-1 was β-configured, due to its correlation with H-9 and H-5, therefore, the 1-OH was assigned as α. H-3 showed a correlation with 3H–20 hence the acetoxy group was assigned as β-configured (Fig. 2). In addition, the examination of the coupling constants for H-1 and H-3 (Table 1) supported the equatorial hydroxy at C-1 and axial acetate at C-3. Compound **1** was determined to be a C-1 de-acetylated derivative of gelomulide N [20], and was determined as 3β-acetoxy-1α-hydroxy-8β,14β-epoxy-*ent*-abiet-13,15-en-16, 12-olide, and trivially named banyangmbolide A.

Compounds **2** and **3** were determined to be isomers possessing α-methyl-α,β-unsaturated γ-lactone, the characteristic features of a

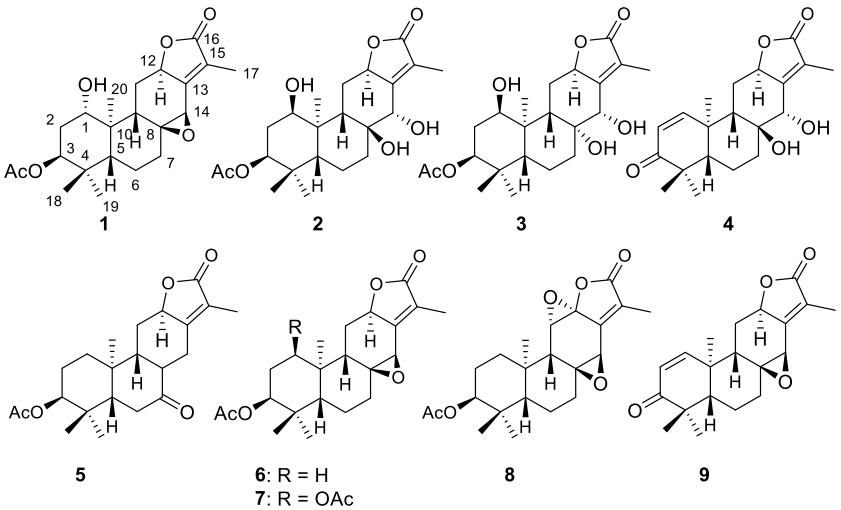

Fig. 1. Compounds isolated from *S. occidentalis*.

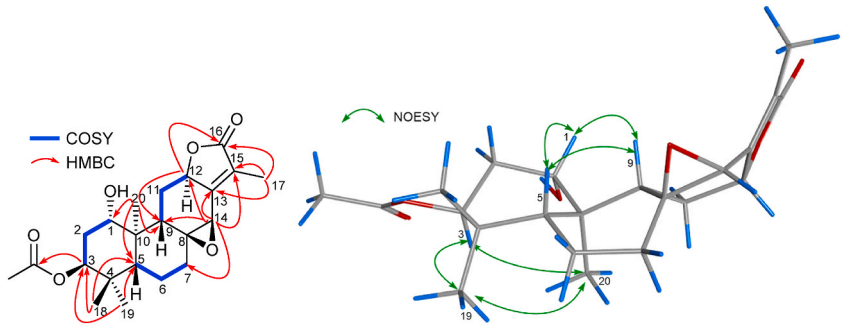

Fig. 2. Key COSY, HMBC and NOESY correlations for compound **1**.

Suregada ent-abietane as observed in compound **1**. They both gave molecular formulae of $C_{22}H_{32}O_7$ due to $[M+H]^+$ ions at *m/z* 409.2221 and 409.2222 in the HRESIMS, respectively (calcd. for $C_{22}H_{32}O_7 + H$, *m/z* 409.2226). The two compounds presented similar IR spectra with absorptions for hydroxy groups (3427 and 3441 cm^{-1}), and broad absorptions at 1731 and 1724 cm^{-1} respectively for α,β-unsaturated γ-lactone and acetate carbonyls. Compound **2** showed similar NMR spectroscopic data with those of the previously reported gelomulide S [20], except for the ^{13}C NMR data for ring A, and the absence of a second acetoxy group. The ^1H NMR spectrum for **2** showed an acetoxy methyl resonance at δ_H 2.07 (s, δ_C 21.2), and the corresponding methine group appearing at δ_H 4.73 (t, $J = 3.2$ Hz, δ_C 80.5). HMBC correlations between two methyl proton resonances at δ_H 0.99 and δ_H 0.89 for H_3-19 and H_3-18 with the oxymethine carbon resonance at δ_C 80.5 observed above for C-3, suggested that the acetoxy group was at C-3 position. Coupling between H-3 with a pair of methylene proton resonances at δ_H 2.02 (m) and 1.76 (m) for H_2-2, which in turn, were coupled with an oxymethine proton resonance at δ_H 3.72 (dd $J = 11.8$, 4.4 Hz) in the COSY spectrum, allowed for the placement of a hydroxy group at C-1 (δ_C 77.8). C-1 showed a correlation in the HMBC spectrum with H_3-20 (δ_H 1.31, s). The NOESY spectrum showed correlation between H-1/H-3, H-1/H_3-20, H_3-20/H-12, H-5/H-9 and H-9/H-14. Furthermore, analysis of compound **2** in pyridine (Table S1.2), allowed for 2-OH, 8-OH and 14-OH at δ_H 6.23, 5.95 and 7.78. These resonances in NOESY supported a correlation between H_3-20 with 8-OH, hence the assigned configurations that were like those of gelomulide S [16]. Compound **2** was assigned 3β-acetoxy-1α-hydroxy-8β, 14β-*ent*-abiet-13,15-en-16,12-olide, trivially named as banyangmbolide B. On the other hand, compound **3**, an isomer of **2**, showed similar arrangement of substituents as compound **2**, however, the ^{13}C and ^1H NMR resonances differed at positions 1, 6, 7, 8, 9, 10, 15, 17 and 20 (Table 1 and S1.2). Furthermore, the use of NOESY spectrum showed that the hydroxy group at C-8 for **2** was β-configured, whereas the hydroxy of C-8 for **3** was α-configured. The NOESY spectrum showed correlations for H-1/H-3, H-3/H_3-20, 8-OH/H_3-20, 14-OH/H-9, and H_3-20/H-12. Compound **3** was determined as 3β-acetoxy-1α-hydroxy-8α,14β-*ent*-abiet-13,15-en-16,12-olide, and trivially named as banyangmbolide C.

Compound **4** showed a $[M+H]^+$ ion at *m/z* 347.1849 ($C_{20}H_{26}O_5$+H, calcd. *m/z* 347.1859) in its HRESIMS. The IR spectrum for compound **4** showed absorptions for hydroxy (3439 cm^{-1}) and α,β-unsaturated γ-lactone carbonyl (1731 cm^{-1}), as seen in **1**, **2** and **3**, together with one absorption ascribable to double bond (1666 cm^{-1}). The ^{13}C NMR, DEPT and ^1H NMR spectroscopic data of compound **4** showed a ketone carbon resonance at δ_C 206.5 and an extra pair of double bond carbon resonances at δ_C 160.2 (δ_H 7.34, d, $J =$ 10.3 Hz) and δ_C 124.6 (δ_H 5.86, d, $J = 10.3$ Hz). The two methyl proton resonances for H_3-18 (δ_H 1.17) and H_3-19 (δ_H 1.15) showed a correlation in the HMBC spectrum with the ketone carbon resonance, hence assignable to the C-3 position. In addition, the H_3-20 methyl proton resonance at δ_H 1.43 (s) showed a correlation in the HMBC spectrum with a methine carbon double bond resonance at δ_C 160.2 assignable to the C-1 position. In addition, the coupled H-1 and H-2, showed a correlation in the HMBC spectrum with C-3, hence the double bond in ring A. The spectroscopic data for ring B, C and those of the γ-lactone group were comparable to those of compound **2**. Therefore, compound **4**, was determined as 3β-oxo-8β,14β-dihydroxy-*ent*-abiet-2,13(15)-dien-16,12-olide, and trivially named as banyangmbolide D.

Compound **5** had a molecular formula of $C_{22}H_{30}O_5$ due to a molecular ion peak at *m/z* 375.2164 (calcd. for $C_{22}H_{30}O_5 + H$, *m/z* 375.2171), determined from its HRESIMS. The IR spectrum for **5** showed absorption bands, at 1754, 1726 and 1707 cm^{-1} for a ketone, γ-lactone carbonyl and an acetate carbonyls. The ^{13}C NMR spectrum showed 22 carbon resonances including carbon resonances at δ_C 209.4 for a ketone group, δ_C 175.0 and 170.5 for γ-lactone carbonyl and an acetate carbonyl, δ_C 160.6 and 122.3 for a double bond, δ_C 77.4 (δ_H 4.87 m) and 77.0 (δ_H 4.74 dd, $J = 2.8$, 2.5 Hz) for two oxygenated carbon resonances. As in compound **1**, the presence of an acetoxy group and an α-methyl-α,β-unsaturated γ-lactone were typical of compounds previously isolated from the *Suregada* genus. The HMBC correlations, H_3-18/C-3, H_3-19/C-3, H-3/C of acetate carbonyl, and the methyl of the acetate with is carbonyl, supported placement of acetoxy group at C-3 position. In addition, C-5 carbon resonance showed correlations in the HMBC spectrum with H_3-18, H_3-19 and H_3-20, and its corresponding proton resonance was at 1.71 (m). The H-5 proton resonance was coupled in the COSY spectrum with an overlapped and deshielded methylene resonance at δ_H 2.41 for 2H-6. Both H-5 and 2H-6 showed correlations in the HMBC spectrum with the ketone carbon resonance hence, assignable to C-7. In addition, the C-7 ketone carbon resonance showed correlation with H-8 (δ_H 2.63 m), H-9 (δ_H 1.50 m), and the uncharacteristic and overlapped methylene resonance at δ_H 2.86 (m) assignable to 2H-14. This is an unprecedented report of a 2H-14 in *Suregada* genus, with an epoxy or dihydroxylation at C-8 and C-14 previously reported. Compound **5**, was determined as 3β-acetoxy-7-oxo-*ent*-abiet-13(15)-en-16,12-olide, and trivially named as banyangmbolide E. In addition to the identification of banyangmbolide A-E (**1**–**5**), four known diterpenoids, gelomulides A (**6**) [14,

Table 1
^{1}H and ^{13}C NMR chemical shifts in $CDCl_3$ for compounds **1**–**5** isolated from *S. occidentalis*.

No.	1		2		3		4		5	
1α	73.0		77.8		76.3		160.2	7.34 d (10.3)	31.4	1.28 m
β		3.88 dd (11.2, 5.0)		3.72 dd (11.8, 4.4)		3.62 dd (11.9, 4.3)		–		1.51 m
2α	33.8	1.88 m	34.5	2.02 m	36.6		124.6	5.86 d (10.3)	22.6	1.89 m
β		1.88 m		1.76 m				–		1.73 m
3	78.3	4.78 t (3.3)	80.5	4.73 t (3.2)	80.2	4.72 t (2.9)	206.5	–	77.4	4.87 m
4	36.8		37.9		37.8		44.6	–	37.3	–
5	49.0	1.67 m	49.2	1.50 dd (12.1, 2.5)	50.9	1.30 m	51.7	1.83 m	48.5	1.71 m
6α	20.7	1.69 m	21.1	1.91 m	28.1	1.85 m	20.8	1.65 m	38.5	2.41 m
β		1.69 m		1.55 m		1.52 m		1.65 m		2.41 m
7α	34.5	2.00 m	41.8	2.26 dt (13.1, 3.7)	47.8	1.71 m	41.2	2.33 m	209.4	–
β		1.68 m		1.65 m		1.27 m		1.70 m		
8	61.5	–	76.6		78.4		75.8	–	44.5	2.63 m
9	49.7	2.20 br d (10.4)	57.7	1.84 m	49.4		50.8	1.89 m	49.8	1.50 m
10	45.0	–	45.7		44.4		41.0	–	37.1	–
11α	26.6	3.20 ddd (12.6, 5.4, 1.7)	32.2	3.54 m	32.1	2.79 m	34.9	2.25 m	28.3	2.30 m
β		1.47 m		1.71 m		1.66 m		1.79 m		1.56 m
12	75.9	5.08 m	79.6	5.27 m	80.6	5.43 m	77.1	5.24 m	77.0	4.74 dd (2.8, 2.5)
13	156.3	–	165.7		166.4		163.5	–	160.6	–
14	56.3	3.78 s	74.1	4.39 s	74.8	4.09 br s ($W_{1/2} = 4.7$)	72.1	4.47 s	24.1	2.86 m
										2.86 m
15	129.1	–	123.0		125.1		121.6	–	122.3	–
16	174.4	–	177.7		178.1		176.2	–	175.0	–
17	9.1	1.97 d (2.1)	8.4	1.84 d (1.8)	9.5	1.93 dd (1.7, 1.7)	7.3	1.85 d (1.8)	8.7	1.83 br m ($W_{1/2} = 5.17$)
18	28.3	0.91 s	28.8	0.89 s	28.5	0.91 s	28.0	1.17 s	27.5	0.85 s
19	22.1	0.99 s	22.4	0.99 s	22.7	1.01 s	20.7	1.15 s	21.3	0.98 s
20	12.3	1.14 s	12.2	1.31 s	10.4	1.20 s	20.6	1.43 s	13.3	1.15 s
Ac–C	170.6	–	172.5		172.4		–		170.5	–
Ac-CH_3	21.4	2.09 s	21.2	2.07 s	21.1	2.05 s	–		21.3	2.4 s

15], B (**7**) [15], D (**8**) [15], and O (**9**) [16] (Fig. 1) were isolated from this plant, in this study.

The ECD spectra of **1**, **2**, **4** and **5** showed a positive Cotton effect at ca. 250 nm (n → π*) and a negative effect at ca. 215 nm (π → π*), which suggested that the configuration at C-12 is *R*, due to the α,β-unsaturated γ-lactone chromophore, showing left-handed chirality as previously reported [16]. For **3**, the negative Cotton effect at 214 nm was observed, and the second Cotton effect at 246 nm was also negative differing to **2**, and this effect was attributable to the hydroxy group at C-8 being α-configured.

The isolation of compounds with similar structural features of **1**–**5** from the *Suregada* genus is common [13], and hydroxy groups and epoxy groups at C-8 and C-14 have been previously reported, this is typified by co-isolation of the four known diterpenoids, gelomulides A (**6**) [14,15], B (**7**) [15], D (**8**) [15], and O (**9**) [16].

Compounds **1**–**9** were tested against human melanoma cells, FM-55-M1 (ECACC 13012546), in a 48-h cell viability assay using CellTiter reagent as described previously [21]. The most active compounds, **5**, **7** and **8**, showed 48–55% inhibition at 200 μM (Fig. 3). None of the compounds analysed showed inhibition below a concentration of 40 μM, the cytotoxic compounds etoposide and comptothecin showed expected activity. The effects of the test compounds were also examined on serum starved human dermal fibroblast cells over 48 h to look for signs of stimulation of cell proliferation. Cells were counted using live cell cytometry before addition of test compounds and at 48 h. Fibroblast cells treated with 10% foetal bovine serum showed increased cell proliferation as anticipated, however none of the test compounds **1**–**9**, showed signs of stimulation.

2. Experimental section

2.1. General experimental procedures

The spectroscopic and spectrometric analysis used in this study are those described in Onanae et al., 2023 [21], and are follows: measurement of IR to determine functional groupds in compounds, were done using a PerkinElmer Frontier/Spotlight 200 spectrometer. NMR experiments to obtain 1D (^{1}H, ^{13}C and DEPT spectra) and 2D (COSY, NOESY, HSQCDEPT and HMBC) NMR spectra were recorded on a 400 MHz Bruker AVANCE NMR instrument at room temperature using $CDCl_3$. The chemical shifts (δ) on the 1D spectra are expressed in ppm and were referenced against the trace chloroform solvent resonances centred at δ_H 7.26 and δ_C 77.23 ppm for ^{1}H and ^{13}C NMR. High resolution mass spectrometry, for resolving molecular formulae of compounds, were measured using a Thermo Scientific Orbitrap Fusion spectrometer. An Applied Photophysics Chirascan CD spectrometer was used to acquire ECD spectra of the compounds. The compounds were dissolved using CH_3CN and measured in a 1 mm precision cell. Fractions and pure compounds from column chromatography were monitored using thin layer chromatography (TLC) plates, pre-coated aluminium-backed plates (silica gel 60 F_{254}, Merck). The TLC plates were visualised by UV radiation at 254 nm and stained, using an anisaldehyde spray reagent (1% *p*-anisaldehyde:2% H_2SO_4: 97% cold MeOH) followed by heating. Purifications of compounds were achieved using preparative thin layer chromatography (Merck 818133) and gravity column chromatography, carried out on a 2 cm diameter column packed with silica gel (Merck Art. 9385) in pre-determined solvent systems.

2.2. Plant material

The leaves of *Suregada occidentalis* were obtained from excess duplicates of herbarium specimens kept at the Royal Botanic Gardens, Kew initially collected from live plants at or adjacent to the Banyang Mbo Wildlife Sanctuary, SW Region Cameroon in November, and December 2000. Collection of specimens were done using the patrol method [22] and dried up in ventilated herbarium presses over gas stoves. The specimens were collected, and the top set deposited at YA, The National Herbarium of Cameroon, and duplicates transferred to Kew herbarium, following agreement captured in a series of Memoranda of Understanding between RBG, Kew and the

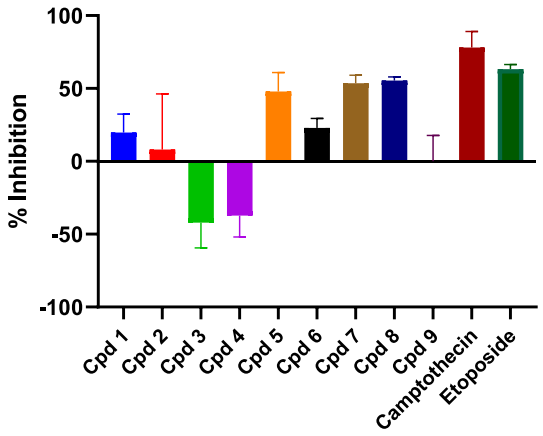

Fig. 3. In vitro growth inhibition of FM-55-M human melanoma cells. Inhibition was measured at a concentration of 200 μM after 48 h of incubation. Cell viability was determined relative to no compound controls after subtracting no cell background readings. Data of three independent experiments shown as the average ± standard deviation.

IRAD-National Herbarium of Cameroon, Yaoundé. MC identified the plant against authenticated reference herbarium specimens at the Kew Herbarium. Voucher specimens for the material used in this study include *Cheek* 10683 (K, MO, WAG, YA) and *Sonké* 2391 (K, YA). Herbarium codes follow Thiers [23].

2.3. Extraction and isolation

The leaves of *S. occidentalis* were freeze-dried and ground to fine powder using a juice blender. The powdered leaves (2.65 g) were successively extracted using methylene chloride (CH_2Cl_2) and methanol solvent (MeOH) to obtain CH_2Cl_2 (0.106 g) and MeOH (0.196 g) extracts respectively. TLC analysis of the CH_2Cl_2 and MeOH extracts were different; therefore, each extract was subjected to column chromatography. The CH_2Cl_2 extract was subjected to gravity column chromatography packed with silica gel merck 9385 soaked in 1:1 hexane: CH_2Cl_2 and eluted using a step gradient, firstly, using 100% hexane and increasing amounts of CH_2Cl_2 to achieve ratios of 1:1, 1, 4, and 100% CH_2Cl_2, and thereafter adding EtoAc to CH_2Cl_2 to achieve ratios of 1:24, 1:10, 1:20 and 1:30 (Table S1.1), collecting 5 ml. The fractions were monitored using ^{1}H NMR and TLC and fractions with the same retention times were pooled. Combined fractions 55–60 gave compound **8**, and combined fractions 64–70 was repurified using 100% CH_2Cl_2 in silica gel to give compound **6**. Combined fractions 75–80 was repurified using 5% Ethyl acetate in CH_2Cl_2 to give a semi pure fraction, that was further purified using sephadex eluted with 1:1 CH_2Cl_2:MeOH to give compounds **5** and **9**. Fractions 89–90 gave compound **7**, whereas fraction 103 gave compound **1**, and fractions 123–125 gave compound **4**. The MeOH extract was subjected to gravity column chromatography packed with a 1:1 blend of silica gel merck 9385 packed with silica gel merck 9385 soaked in CH_2Cl_2 and eluted using a step gradient initially using 100% CH_2Cl and increasing amounts of EtOAc to achieve ratios of 1:24 and 1:10, and thereafter adding MeOH to CH_2Cl_2 to achieve ratios of 0.1:10, 0.2:10, 0.3:10, 1:10 and 2:10, (Table S1.1), collecting 5 ml. Fractions 1–80 gave semi-pure fractions of the compounds identified in the CH_2Cl_2 extracts, but fraction 85 gave compound **3** and fraction 90 gave compound **2**.

2.4. Compound characterization

3β-acetoxy-8β,14β-epoxy-1β-hydroxy-13(15)-abieten-16,12-olide ***(1)***. Colourless oil; IR (NaCl) νmax (cm^{-1}): 3473, 3056, 2951, 2850, 1750, 1739, 1733, 1247, 1179, 1025; ECD (CH_3CN; *c* 0.1 mg/ml) λ (Δε) 255 nm (+16.7), 213 nm (−35.8); ^{1}H and ^{13}C NMR are given in Table 1; HRESIMS *m/z* 391.2117 (calcd. for $C_{22}H_{30}O_6$ + H, *m/z* 391.2120).

3β-acetoxy-1β,8β,14β-trihydroxy-13(15)-abieten-16,12-olide ***(2)***. Colourless oil; IR (NaCl) νmax (cm^{-1}): 3427, 2927, 1731, 1263, 1026; ECD (CH_3CN; *c* 0.1 mg/ml) λ (Δε) 273 nm (+2.6), 223 nm (−50.7); ^{1}H and ^{13}C NMR are given in Table 1 and S1.2; HRESIMS *m/z* 409.2221 (calcd. for $C_{22}H_{32}O_7$ + H, *m/z* 409.2226).

3β-acetoxy-1β,8β,14α-trihydroxy-13(15)-abieten-16,12-olide ***(3)***. Colourless oil; IR (NaCl) νmax (cm^{-1}): 3441, 2954, 1725, 1263, 1261, 1038; ECD (CH_3CN; *c* 0.1 mg/ml) λ (Δε) 246 nm (−13.1), 211 nm (−17.5); ^{1}H and ^{13}C NMR are given in Table 1 and S1.2; HRESIMS *m/z* 409.2222 (calcd. for $C_{22}H_{32}O_7$ + H, *m/z* 409.2226).

8β,14α-dihydroxy-3-oxo-1,13(15)-abietadien-16,12-olide ***(4)***. Colourless oil; IR (NaCl) νmax (cm^{-1}): 3440, 2927, 1731, 1666, 1248, 1032; ECD (CH_3CN; *c* 0.1 mg/ml) λ (Δε) 343 nm (+5.2), 276 nm (+11.3), 226 nm (−40.4); ^{1}H and ^{13}C NMR are given in Table 1; HRESIMS *m/z* 347.1849 (calcd. for $C_{20}H_{26}O_5$ + H, *m/z* 347.1853).

3β-acetoxy-7-oxo-13(15)-abieten-16,12-olide ***(5)***. Colourless oil; IR (NaCl) νmax (cm^{-1}): 2929, 1754, 1726, 1707, 1245, 1035; ECD (CH_3CN; *c* 0.1 mg/ml) λ (Δε) 245 nm (+7.0), 221 nm (−25.1); ^{1}H and ^{13}C NMR are given in Table 1; HRESIMS *m/z* 375.2164 (calcd. for $C_{22}H_{30}O_5$ + H, *m/z* 375.2166).

2.5. FM55-M1 cytotoxicity assay and stimulation of human dermal fibroblasts

Compounds **1**–**9** were tested for cytotoxic effects on the human melanoma cell line FM-55-M1. They were also tested for their ability to stimulate the proliferation of serum starved human dermal fibroblasts. Both these assays were carried out as described previously with minor modifications [21]. Detailed methods for both assays are provided in the supplementary section.

CRediT authorship contribution statement

Yanisa Olaranont: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Eduard Mas-Claret:** Writing – review & editing, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Martin Cheek:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Thomas A.K. Prescott:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Jean Michel Onana:** Writing – review & editing, Supervision, Resources, Project administration, Formal analysis, Conceptualization. **Moses K. Langat:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest on the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25917.

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