Published in Phytochemistry 67, issue 17, 1957-1963, 2006
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Bafoudiosbulbins A, and B, two anti-salmonellal clerodane diterpenoids from *Dioscorea bulbifera* L. var *sativa*

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Abstract

Two clerodane diterpenoids, Bafoudiosbulbins A **1**, and B **2**, together with five known compounds: tetracosanoic acid, 1-(tetracosanoyl)-glycerol, *trans*-tetracosanylferulate, β-sitosterol and 3-*O*-β-D-glucopyranosyl-β-sitosterol were isolated from the tubers of *Dioscorea bulbifera* L. var *sativa*. Their structures were established by spectroscopic methods (1D and 2D-NMR, MS) and X-ray crystallographic diffraction analysis of compound **1**. The CH_2Cl_2-soluble portion of the crude extract and the two clerodanes were screened for anti-bacterial activity using both agar diffusion and broth dilution techniques against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B. They both showed significant activities against *P. aeruginosa*, *S. typhi*, *S. paratyphi* A and *S. paratyphi* B.

Keywords: *Dioscorea bulbifera* L. var *sativa*; Dioscoreaceae; Clerodane; Anti-salmonellal activity; Anti-typhoid

1. Introduction

Dioscorea bulbifera L. var *sativa* (Dioscoreaceae) usually grows wild and its bitter tubers are used by tribal people in Bangladesh for the treatment of leprosy and tumors (Murray et al., 1984). Earlier chemical investigation of its tubers afforded two norclerodane diterpenoids (Murray et al., 1984). Clerodane class of diterpenes is a group of compounds that has attracted considerable interest because of problems associated with their stereochemistry and because of the diverse biological activities shown by some members (Roengsunran et al., 2002; Komori, 1997; Ortega et al., 1982; Fayos et al., 1984). They are known to possess anti-tumor, anti-bacterial, anti-feedant, anti-fungal and hallucinogenic activities (Urones et al., 1995; Biswanath et al., 2005; Harding et al., 2006). In our continuous search of potentially interesting novel and/or known compounds from Cameroonian medicinal plants (Tapondjou et al., 2003; Tapondjou et al., 2005; Djoukeng et al., 2005), we have examined the extract from the tubers of *D. bulbifera* L. var *sativa* growing in the western highlands of Cameroon for its anti-typhoid activity. Typhoid fever is caused by *Salmonella typhi*, whereas paratyphoid fevers are caused by *Salmonella paratyphi* A and *Salmonella paratyphi* B (Cheesbrough, 1991; Gatsing et al., 2006). Typhoid fever continues to be a marked public health problem in developing countries in general and in Sub-saharan Africa in particular, where it is endemic. The greater prevalence of resistance to all three first-line anti-microbials (ampicillin, chloramphenicol and co-trimoxazole) has been established

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(WHO, 1981). This paper deals with the bioassay-guided isolation and structural elucidation of two new anti-salmonellal clerodane diterpenoids together with the known tetracosanoic acid, 1-(tetracosanoyl)-glycerol, *trans*-tetracosanylferulate, β-sitosterol and 3-*O*-β-D-glucopyranosyl-β-sitosterol from the extract of the tubers of *D. bulbifera* L. var *sativa*. The anti-microbial activity of the extract and the above two clerodanes was investigated against several bacteria among which *S. typhi*, *S. paratyphi* A and *S. paratyphi* B.

2. Results and discussion

The air-dried and pulverized tubers of *D. bulbifera* L. var *sativa* were extracted with 80% MeOH at room temperature for 24 h followed by heating for 20 min. The aqueous methanolic extract was concentrated under reduced pressure to yield a dark residue which was suspended in water and submitted to successive partition with CH_2Cl_2 and *n*-butanol. The CH_2Cl_2 soluble portion was fractionated and chromatographed repeatedly on silica gel and sephadex LH-20 columns to give two new clerodane diterpenoids **1** and **2** together with the known tetracosanoic acid (Salas et al., 2005), 1-(tetracosanoyl)-glycerol (Sultana et al., 1999), *trans*-tetracosanylferulate (Jih-Jung et al., 2004), β-sitosterol (Flamini et al., 2001) and 3-*O*-β-D-glucopyranosyl-β-sitosterol (Flamini et al., 2001).

Compound **1** was isolated as white needles in MeOH, m.p.: 252.8 °C, $[\alpha]_D^{21}$ −64.6 (*c* 0.025, DMSO). Its HRESIMS (positive mode) displayed a pseudomolecular ion peak at *m/z* 425.12071 $[M + Na]^+$ corresponding to the molecular formula $C_{21}H_{22}O_8$ (calc. 425.12). This was confirmed by the ESIMS (positive mode) which showed the pseudomolecular ion peak at *m/z* 403 $[M + H]^+$ and two important peaks at *m/z* 375 $[M + H - CO]^+$ and 357 $[M + H - CO - H_2O]^+$. Compound **1** exhibited also in its IR spectrum two particularly broad and intense bands at 1741 and 1787 cm^{-1} which are characteristic of δ-lactone and γ-lactone, respectively (Murray et al., 1984) and another band at 875 cm^{-1} suggesting the presence of a furan moiety. The 100 MHz ^{13}C NMR of **1** exhibited 21 signals, confirming the mass data. Signals for three methines at δ 143.9 (C-15), 141.9 (C-16) and 111.2 (C-14) are characteristic of furoclerodanes (Kapingu et al., 2000). The last furan carbon signal was observed at δ 126.4 (C-13). Other salient features of this spectrum were three deshielded quaternary signals of the ester type at δ 177.4 (C-17), 173.2 (C-18) and 171.0 (C-19). The remaining peaks were due to two methyl, four methylene, five methine groups and three quaternary carbons (Table 1).

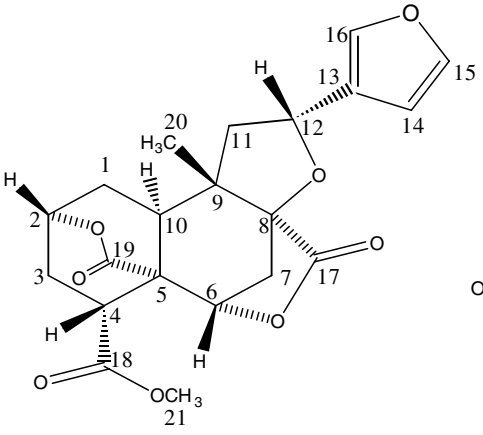
The 400 MHz ^1H NMR spectrum of compound **1** displayed three furan signals at δ 7.68 (1H, *brs*, H-16), 7.60 (1H, *brs*, H-15), 6.82 (1H, *brs*, H-14), and two methyl peaks at δ 3.67 (3H, *s*, H-21) and 1.38 (3H, *s*, H-20). Furthermore, three downfield signals were observed at δ 5.30 (1H, *dd*, $J = 5.0$, 10.9 Hz, H-12), 4.89 (1H, *m*, H-2) and 4.85 (1H, *brd*, $J = 5.3$ Hz, H-6) corresponding to three oxygenated methines. The complete assignments of all ^1H and ^{13}C resonances were achieved by COSY, HSQC, and HMBC (Table 1). Final proof of the structure and stereochemistry of compound **1** was obtained from a single crystal X-ray

Table 1
^1H and ^{13}C NMR data in DMSO, COSY and HMBC correlations of compound **1**

C/H atom	δ ^{13}C (ppm)	DEPT	δ ^1H (mult, *J* (Hz))	COSY (H ↔ H)	HMBC (C → H)
1	27.5	CH_2	1.72 *m*		
			2.21 *m*		
2	74.3	CH	4.89 *m*	Hα-3, Hβ-3	
3	31.0	CH_2	2.15 *m*		H-2
			2.18 *m*		
4	39.5	CH	3.65 *nd*		
5	46.2	C	/		H-6
6	75.0	CH	4.85 br*d* (5.3)	Hα-7, Hβ-7	
7	32.6	CH_2	2.64 *dd* (5.3, 12.4)	Hα-7	
			2.49 *d* (12.4)	Hβ-7	
8	88.9	C	/		H-20, H-6, Hβ-7
9	46.6	C	/		H-20, H-12, H-6
10	38.8	CH	2.08 *brd* (7.8)	Hα-1, Hβ-1	
11	43.9	CH_2	1.89 *dd* (10.9, 11.4)	Hβ-11	
			2.00 *dd* (5.0, 11.4)		
12	75.0	CH	5.30 *dd* (5.0, 10.9)	Hα-11, Hβ-11	Hβ-7
13	126.4	C	/		H-16, H-12, H-14
14	111.2	CH	6.82 *brs*	H-15	H-15, H-12
15	143.9	CH	7.60 *brs*	H-14	H-14
16	141.9	CH	7.68 *brs*		H-15, H-12, H-14
17	177.4	C	/		Hα-7, H-6
18	173.2	C	/		H-21
19	171.0	C	/		H-2
20	18.7	CH_3	1.38 *s*		
21	53.3	CH_3	3.67 *s*		

nd: not determined.

analysis using direct methods. The ORTEP diagram of the crystal structure of this compound (Fig. 1) clearly shows that the γ-lactone ring bridging C-6 and C-8, the δ-lactone ring bridging C-2 and C-5, the carboxymethyl group at C-4 and the furan moiety are placed on the α face of the molecule. It also shows that the proton H-10 and the methyl group C-20 have the α and β-orientations, respectively. Compound **1** is then elucidated as methyl 8,12*R*,15,16-diepoxyclerodane-13(16),14-diene-17,6α;19,2α-diolide 18-oate. To the best of our knowledge its structure has not been reported previously.



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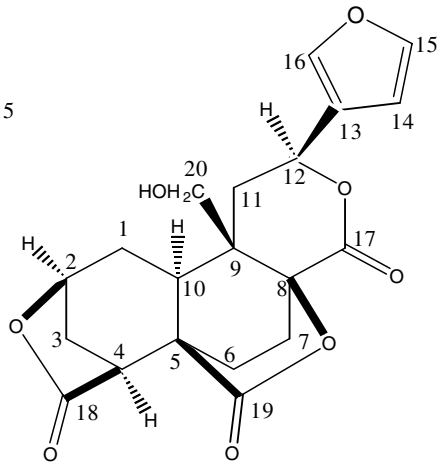

2

Compound **2** was isolated as white crystals in MeOH, m.p.: 312.9 °C, $[\alpha]_D^{21}$ +52 (*c* 0.010, pyridine). Its ESI mass spectrum (negative mode) displayed a molecular ion peak at *m/z* 387 $[M - H]^-$ establishing the formula $C_{20}H_{20}O_8$, and two important peaks at *m/z* 343 $[M - H - CO_2]^-$ and 329 $[M - H - CO - CH_2O]^-$. The IR spectrum of **2** was almost superimposable to that of **1**, the only difference being the presence of an intense band at 3428 cm^{-1}, characteristic of hydroxyl group in compound **2**. The ^{13}C NMR spectrum of compound **2** was very informative because it revealed the presence of 20 resonances. Among these, three carbonyl groups of the lactone type were observed at δ 172.1 (C-17), 172.5 (C-19) and 175.1 (C-18). Four signals observed at δ 144.6 (C-15), 141.1 (C-16), 125.7 (C-13) and 109.5 (C-14) are characteristic of furoclerodanes (Kapingu et al., 2000; Tane et al., 1997; Fayos et al., 1984). Combination of ^{13}C NMR and DEPT spectra showed the presence of 7 methines, 6 methylenes and 7 quaternary carbons. Signals at δ 73.8 (C-2), 70.8 (C-12), 74.7 (C-8) and 67.5 (C-20) indicated the presence of two oxygenated methine groups, one oxygenated quaternary carbon and one hydroxymethylene group, respectively.

The 400 MHz ^1H NMR spectrum of **2** displayed, in addition to the furan signals [δ 7.70 (1H, *d*, *J* = 0.7 Hz, H-16); 7.51 (1H, *d*, *J* = 1.7 Hz, H-15), 6.57 (1H, *dd*, *J* = 1.7, 0.7 Hz, H-14)], one extra AB system of two protons at δ 4.72 (1H, *d*, *J* = 13.7 Hz) and 4.55 (1H, *d*, *J* = 13.7 Hz) ascribed to the two non-equivalent protons of the hydroxymethylene group at C-20. Furthermore, two downfield signals integrating each for one proton were observed at δ 4.88 (1H, *m*, H-2) and 5.50 (1H, *dd*, *J* = 12.7, 3.4 Hz, H-12) corresponding to two oxygenated methine protons. Proximity relationships between ^1H–^1H were

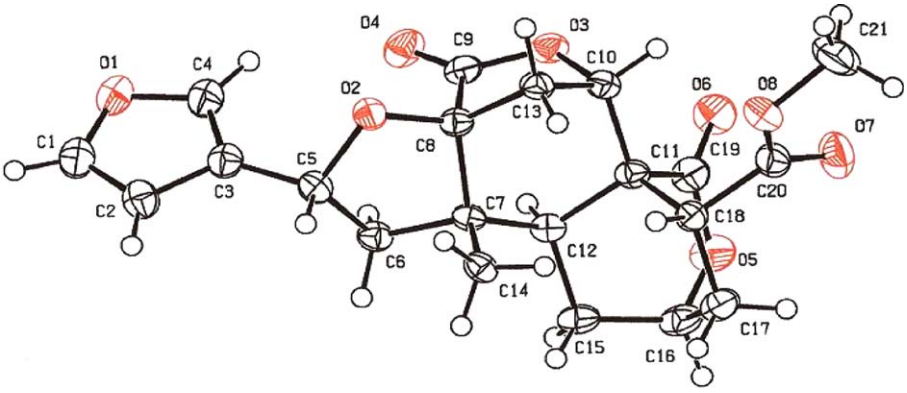

Fig. 1. ORTEP diagram of the crystal structure of compound **1**.

Table 2
^{1}H and ^{13}C NMR data in C_5H_5N-d_5, COSY and HMBC correlations of compound **2**

Positions	δ ^{13}C (ppm)	DEPT	δ ^{1}H (mult, J (Hz))	COSY (H ↔ H)	HMBC (C → H)
1	27.1	CH_2	1.95 *m*	H-2, H-10	
			2.30 *m*		
2	73.8	CH	4.88 *m*	Hα-3, Hβ-3	
3	27.5	CH_2	2.16 *m*		H-4
			2.51 *m*		
4	45.1	CH	3.46 *dd* (12.5, 7.3)	Hα-3, Hβ-3	H-1, Hα-6, Hβ-3
5	44.2	C	/		H-20, Hα-7, Hβ-11
6	27.3	CH_2	2.10 *m*		Hα-7
			2.30 *m*		
7	28.9	CH_2	2.42 *m*		Hα-6, Hβ-6
			3.10 *m*	Hα-6, Hβ-6	
8	74.7	C	/		Hα-7, Hβ-11, H-20
9	40.2	C	/		Hα-7, Hα-6, H-4
10	36.5	CH	2.60 *brd* (9.8)	Hα-1, Hβ-1	Hβ-11, H-4, H-2
11	30.6	CH_2	1.70 *dd* (14.1, 3.4)	Hβ-11	H-20
			2.70 *dd* (14.1, 12.7)		
12	70.8	CH	5.50 *dd* (12.7, 3.4)	Hα-11, Hβ-11	Hβ-11
13	125.7	C	/		H-12, Hβ-11, H-14, H-16
14	109.5	CH	6.57 *dd* (1.7, 0.7)	H-15	H-12, H-15, H-16
15	144.6	CH	7.51 *d* (1.7)	H-14	H-14
16	141.1	CH	7.70 *d* (0.7)		H-14
17	172.1	C	/		H-20, Hβ-7, Hα-7
18	175.1	C	/		H-2, H-4, Hβ-3
19	172.5	C	/		H-4, Hβ-3
20	67.5	CH_2	4.55 *d* (13.7)		H-10, Hβ-11
			4.72 *d* (13.7)		

achieved by COSY experiment while direct C → H correlations were established using the HSQC spectrum data (Table 2). The HMBC sequence provided information on the long-range connectivities between various ^{1}H and ^{13}C atoms. It showed ^{2}J, ^{3}J and ^{4}J interactions between H-4 (δ 3.46) and C-19, C-18, C-10, C-9 and C-3, between H-2 (δ 4.88) and C-18, C-10 and C-4, between Hβ-11 (δ 2.70) and C-5, C-8, C-10, C-12 and C-20. Many other correlations were also observed (Table 2). The C-12 configuration was deduced from careful comparison of the ^{1}H and ^{13}C data with those of related compounds (Kapingu et al., 2000; Fayos et al., 1984; Ortega et al., 1982; Murray et al., 1984). This was corroborated by the axial nature of H-12 which showed couplings of 12.7 and 3.4 Hz to the axial and equatorial protons at C-11, respectively (Murray et al., 1984). The relative stereochemistry of **2** was determined by NOESY experiment. Significant correlations were depicted on its NOESY spectrum (Fig. 2) between H-12 (δ 5.50) and H-10 (δ 2.60), H-10 and H-2 (δ 4.88), H-10 and H-4 (δ 3.46), H-4 and Hα-3 (δ 2.51), Hα-3 and H-2, H-2 and Hα-1 (δ 2.30). The interaction between Hβ-1 (δ 1.95) and one of the C-20 hydroxymethylene protons (δ 4.72) allowed us to deduce the C-9 configuration. The inspection of the structure of **2** made by molecular model suggested that such NOESY correlations are expected when the δ-lactone ring bridging C-5 and C-8 is placed on the β face of the molecule (Fig. 2). Consequently, compound **2** is 15,16-epoxy-20-hydroxyclerodane-13(16),14-diene-17,12*S*;18,2β;19,8β-triolide and to the best of our knowledge, its structure is hereby reported for the first time.

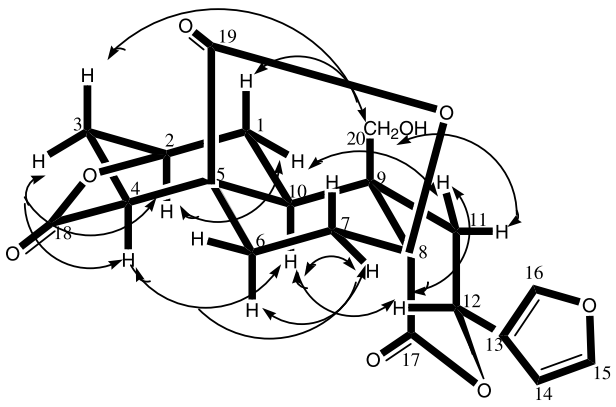

Fig. 2. Significant NOESY correlations for compound **2**.

The various extracts (crude aqueous methanolic extract, CH_2Cl_2 extract and the *n*-butanol extract) were initially tested against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *S. typhi*, *S. paratyphi* A and *S. paratyphi* B. The *n*-butanol extract showed no activity against all the seven bacteria strains, whereas the crude aqueous methanolic extract showed very weak activity against *P. aeruginosa*, *S. typhi*, *S. paratyphi* A and *S. paratyphi* B and no activity against *E. coli*, *K. pneumoniae* and *S. aureus*. The CH_2Cl_2 extract showed remarkable activity against *P. aeruginosa*, *S. typhi*, *S. paratyphi* A and *S. paratyphi* B and no activity against *E. coli*, *K. pneumoniae* and *S. aureus* (Table 3). Thereafter the CH_2Cl_2

Table 3
Diameters of inhibition of *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* by the CH_2Cl_2-extract and compounds **1** and **2**

	Disc potency	Bacteria and diameters of zones of inhibition (mm)						
		ST	SPA	SPB	PA	EC	SA	KP
CH_2Cl_2-extract	5 mg	7	7	12	9	NA	NA	NA
Compound **1**	100 μg	13	11	9	12	NA	NA	NA
Compound **2**	100 μg	11	13	9	15	NA	NA	NA
Chloramphenicol	100 μg	18	20	25	NT	NT	NT	NT
Amoxicillin	100 μg	NT	NT	NT	18	17	19	14

Key. NA = not active, NT = not tested.
ST: *Salmonella typhi*, SPA: *Salmonella paratyphi* A, SPB: *Salmonella paratyphi* B, PA: *Pseudomonas aeruginosa*, EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, SA: *Staphylococcus aureus*.

extract was fractionated and purified to yield mainly compounds **1** and **2** which were also tested against all the seven bacteria strains. The data obtained showed that compounds **1** and **2** were also very active against *P. aeruginosa*, *S. typhi*, *S. paratyphi* A and *S. paratyphi* B (Table 3).

Compounds **1** and **2**, which showed anti-bacterial activity against *P. aeruginosa*, *S. typhi*, *S. paratyphi* A and *S. paratyphi* B were further studied using broth dilution technique in order to determine the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values (Table 4).

Anti-microbial substances are considered as bactericidal agents when the ratio MBC/MIC $\leqslant 4$ and bacteriostatic agents when the ratio MBC/MIC > 4 (Carbonnelle et al., 1987; Gatsing et al., 2006). For compounds **1** and **2**, these ratios were $\leqslant 4$ (Table 4) suggesting that they may be classified as bactericidal agents. All these results suggest that the CH_2Cl_2 extract and the two new clerodanes essentially exhibited anti-salmonellal activity, thus indicating that this plant could be used in the treatment of typhoid fevers.

Table 4
Inhibition parameters (MIC, MBC) of the isolated compounds against *Salmonella typhi*, *Salmonella paratyphi* A, *Salmonella paratyphi* B and *Pseudomonas aeruginosa*

	Parameters	Bacteria strains			
		ST	SPA	SPB	PA
Compound **1**	MIC (μg/ml)	50	50	25	50
	MBC (μg/ml)	200	100	100	200
	MBC/MIC	4	2	4	4
Compound **2**	MIC (μg/ml)	50	25	25	25
	MBC (μg/ml)	200	100	100	100
	MBC/MIC	4	4	4	4
Chloramphenicol	MIC (μg/ml)	2.5	2.5	2.5	/
	MBC (μg/ml)	20	20	20	/
	MBC/MIC	8	8	8	/
Amoxicillin	MIC (μg/ml)	/	/	/	12.5
	MBC (μg/ml)	/	/	/	25
	MBC/MIC	/	/	/	2

Key. ST: *Salmonella typhi*, SPA: *Salmonella paratyphi* A, SPB: *Salmonella paratyphi* B, PA: *Pseudomonas aeruginosa*, MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

3. Experimental

3.1. General experimental procedures

Melting points were determined using the Gallenkamp Melting Point Apparatus. Optical rotations were measured on a Perkin–Elmer 241 Polarimeter, IR spectra were measured as a film on a KBr pellet using a FTIR-8400S Shimadzu spectrometer. HRESIMS were taken on the mass spectrometer Brucker FTMS4.7T, BIOAPEXII. ^{1}H NMR spectra were recorded in deuterated solvents (DMSO and C_5H_5N) on a Brucker AMX-400 Spectrometer at 400 MHz while ^{13}C NMR spectra were recorded in the same solvents and the same apparatus at 100 MHz. All chemical shifts (δ) are given in ppm units with reference to tetramethylsilane (TMS) as internal standard and the coupling constants (J) are in Hz. Column chromatography was performed using silica gel 60 Merck (0.040–0.063 mm) and sephadex LH-20. TLC were carried out on precoated Kieselgel 60 F_{254} (Merck) plates developed with hexane:AcOEt (7:3 or 1:1) and AcOEt:MeOH (98:2 or 95:5). TLC plates were viewed with an ultraviolet lamp MULTIBAND UV-254/365 nm for fluorescent spots. They were also visualised by spraying with 50% H_2SO_4 and heating for 10 min at 110 °C.

3.2. Plant material

The tubers of *D. bulbifera* L. var *sativa* were collected in Bafou village near Dschang (West province of Cameroon) in March 2005. Specimens documenting the collection are deposited in the Cameroon National Herbarium in Yaoundé (Ref.: 22211/SRF/CAM).

3.3. Test bacteria and culture media

The test microorganisms, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *S. aureus*, *S. typhi*, *S. paratyphi* A and *S. paratyphi* B, were obtained from the Medical Bacteriology Laboratory of Pasteur Centre, Yaoundé, Cameroon. The culture media used, namely Nutrient agar and Nutrient Broth (used for the culture of *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. aureus*), Salmonella-Shigella agar (SS agar) and Selenite Broth (for culture of *S. typhi*, *S. paratyphi* A

and *S. paratyphi* B) were supplied by International Diagnostics Group PLC, Topley House, 52 Wash Lane, Bury, Lancashire BL9 6AU, UK.

3.4. Anti-microbial assay

The anti-bacterial activity was determined using both agar diffusion and broth dilution techniques as previously described by Cheesbrough (1991) and Gatsing et al. (2006).

Agar diffusion susceptibility testing was done using the disc method. A disc of blotting paper was impregnated with 10 μl of a 500 mg/ml (for crude extract) or 10 mg/ml (for pure compounds) solution of each sample dissolved in DMSO. Thus the disc potencies were 5 mg and 100 μg for crude extract and pure compounds, respectively. Amoxicillin and chloramphenicol (Sigma) were used as the standard drugs. After drying, the disc was placed on a plate of sensitivity testing agar inoculated with the test organism. The petridishes were left at room temperature for about 45 min to allow the extract or the compounds to diffuse from the disc into the medium. They were then incubated at 37 °C for 24 h, after which the zones of no growth were noted and their diameters recorded as the zones of inhibition.

For the broth dilution susceptibility testing, the solutions (maximum concentration) of the active compounds (i.e. the compounds that induced zones of inhibition) were prepared in DMSO, serially (2-fold) diluted and 0.5 ml of each dilution was introduced into a test tube containing 4.4 ml of Selenite broth; then 0.1 ml of bacteria suspension (5×10^5 cfu/ml) was added and the mixture was homogenized. The total volume of the mixture was 5 ml, with the test-compound concentrations in the tube ranging from 200 to 6.25 μg/ml and those of the standard compounds, i.e. amoxicillin and chloramphenicol, ranging from 100 to 3.125 μg/ml and 40 to 0.625 μg/ml, respectively. After 24 h of incubation at 37 °C, the MIC was reported as the lowest concentration of anti-microbial that prevented visible growth. The MBC was determined by sub-culturing the last tube to show visible growth and all the tubes in which there was no growth on already prepared plates containing nutrient agar or SS agar medium. The plates were then incubated at 37 °C for 24 h and the lowest concentration showing no growth was taken as the MBC.

3.5. Extraction and isolation

The air-dried tubers of *D. bulbifera* L. var *sativa* (1.8 kg) were pulverized and extracted four times (each time for 24 h followed by heating for 20 min) with 80% MeOH. The aqueous methanolic extract was concentrated under reduced pressure to yield a dark residue (75 g), which was suspended in water (200 ml) and submitted to successive partition with CH_2Cl_2 and *n*-butanol. The CH_2Cl_2 soluble portion (33.5 g) was subjected to column chromatography over silica gel. Elution using hexane, hexane–EtOAc mixtures, EtOAc–MeOH mixtures and MeOH afforded 10 main fractions, A–J. From fraction G (hexane–EtOAc 40%) (4.00 g), Bafoudiosbulbins A (450 mg) and B (410 mg) were obtained after further purification over silica gel (eluted with hexane–EtOAc 1–1) and sephadex (eluted with CH_2Cl_2–MeOH 1–1) column chromatography followed by recrystallisation with MeOH. Successive column chromatography over silica gel of fraction B (hexane–EtOAc 10%) (6.00 g) using hexane–EtOAc 9–1, 9–2 and 9–3 afforded tetracosanoic acid (50 mg), *trans*-tetracosanyl-ferulate (25 mg) and β-sitosterol (150 mg), respectively. Column chromatography of fraction D (hexane–EtOAc 20%) (5.50 g) over silica gel (eluted with hexane–EtOAc 7–3) yielded 1-(tetracosanoyl)-glycerol (230 mg) while 3-*O*-β-D-glucopyranosyl-β-sitosterol (100 mg) was obtained as white powder after recrystallisation of fraction I (EtOAc) (1.5 g).

3.6. X-ray crystallographic analysis of Bafoudiosbulbin A

Suitable crystals of Bafoudiosbulbin A were obtained as colourless rods from a chloroform/methanol/acetone solution. The compound crystallized in the chiral orthorhombic space group $P2_12_12_1$. Crystal data: $C_{21}H_{22}O_8$, $M = 402$. The intensity data were collected at 173 K (−100 °C) on a Stoe Mark II-Image Diffraction System equipped with a two-circle goniometer and using MoKα graphite monochromated radiation. Image plate distance 100 mm, ω rotation scans 0–180° at ϕ 0°, 0–74° at ϕ 90°, step $\rho\omega = 1.0°$, with an exposure time of 4 min per image, 2θ range 2.29–59.53°, $d_{min} - d_{max} = 17.779$–$0.716$ Å. The structure was solved by direct method using the program SHELXS. The refinement and all further calculations were carried out using SHELXL-97. The H-atoms were included in calculated positions and treated as riding atoms using SHELXL default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2.

3.7. Compound *1*: Bafoudiosbulbin A

White needles, m.p. 252.8 °C (MeOH); $[\alpha]_D^{21}$ −64.6 (*c* 0.025, DMSO); IR ν_{max} (KBr) cm^{-1}: 2916, 1787, 1741, 1062, 875; ^1H NMR (DMSO, 400 MHz): Table 1; ^{13}C NMR (DMSO, 100 MHz): Table 1; HRESIMS: *m/z* 425.12071 $[M + Na]^+$; ESIMS: *m/z* (rel. int.) 403 $[M + H]^+$ (25), 375 $[M + H - CO]^+$ (90), 357 $[M + H - CO - H_2O]^+$ (10).

3.8. Compound *2*: Bafoudiosbulbin B

White crystals, m.p. 312.9 °C (MeOH); $[\alpha]_D^{21}$ +52 (*c* 0.010, pyridine); IR ν_{max} (KBr) cm^{-1}: 3428, 2949, 1786, 1758, 1024, 874; ^1H NMR (pyridine, 400 MHz): Table 2; ^{13}C NMR (pyridine, 100 MHz): Table 2; ESIMS: *m/z* (rel. int.) 387 $[M - H]^-$ (100), 343 $[M - H - CO_2]^-$ (10), 329 $[M - H - CO - CH_2O]^-$ (30).

Acknowledgements

This research was supported by the Committee on Scientific and Technological Cooperation of the Organization of Islamic Conference (COMSTECH), Islamabad, Pakistan and the International Foundation for Science (IFS), Stockholm, Sweden, through a grant to Prof. Tapondjou A. Léon.

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