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Bafoudiosbulbins F and G, further clerodane diterpenoids from *Dioscorea bulbifera* L. var *sativa* and revised structure of Bafoudiosbulbin B

Rémy Bertrand Teponno [a], Azefack Léon Tapondjou [a,*], Eliane Abou-Mansour [b], H. Stoeckli-Evans [b], Pierre Tane [a], Luciano Barboni [c]

[a] *Department of Chemistry, Faculty of Science, University of Dschang, Box 183, Dschang, Cameroon*
[b] *Institute of Chemistry, University of Neuchâtel, Av. Bellevaux 51, CH-2000 Neuchâtel, Switzerland*
[c] *Dipartimento di Scienze Chimiche, Università di Camerino, Via S. Agostino 1, 62032 Camerino, MC, Italy*

A B S T R A C T

From the bulbils of *Dioscorea bulbifera* L. var *sativa*, two clerodane diterpenoids, Bafoudiosbulbins F (**1**) and G (**2**), together with five known compounds: Bafoudiosbulbins A–C, 3,5,4′-trihydroxy-3′-methoxybibenzyl, and kaempferol were isolated. Their structures were established by spectroscopic techniques, including ^{1}H, ^{13}C NMR, NOESY, ROESY, COSY, TOCSY, HSQC, and HMBC. The relative stereochemistry of compounds **1** and **2** was assigned on the basis of X-ray crystallographic diffraction analysis. Furthermore, the structure of Bafoudiosbulbin B was revised using extensive 2D NMR techniques as well as chemical transformation.

Keywords:
Dioscorea bulbifera L. var *sativa*
Dioscoreaceae
Clerodane diterpenoids
Bafoudiosbulbins F–G
X-ray diffraction analysis

1. Introduction

The Dioscoreaceae, distributed throughout the tropics and some temperate regions, constitute a family consisting mainly of tropical climbers. *Dioscorea bulbifera* L. var *sativa* grows wild and its bitter tubers are used by tribal people in Bangladesh for treatment of leprosy and tumours (Murray et al., 1984). In our previous papers, we described the isolation and structural elucidation of five clerodane diterpenoids (Bafoudiosbulbins A–E) from this plant (Teponno et al., 2006; Teponno et al., 2007). In order to increase the quantities of the isolated compounds for biological screenings and chemical transformations, the bulbils of this plant were recollected. Extensive phytochemical study of its EtOAc extract led to the isolation of two new clerodane diterpenoids together with the known Bafoudiosbulbins A–C, 3,5,4′-trihydroxy-3′-methoxybibenzyl, and kaempferol. Furthermore, the structure of Bafoudiosbulbin B was revised using 2D NMR as well as chemical transformation.

2. Results and discussion

The dried and pulverized bulbils of *D. bulbifera* L. var *sativa* were extracted two times (each time for 24 h) with 80% MeOH. The filtrate obtained was concentrated under reduced pressure to yield a dark residue which was suspended in water and extracted with EtOAc. The EtOAc soluble portion was fractionated and chromatographed repeatedly on silica gel columns to give the new clerodane diterpenoids **1** and **2** together with the known Bafoudiosbulbins A–C (Teponno et al., 2006, 2007), 3,5,4′-trihydroxy-3′-methoxybibenzyl (Leong et al., 1999), and kaempferol (Jain et al., 1990).

Compound **1** was obtained as colourless crystals from CH_2Cl_2–MeOH, m.p.: 265–266 °C, $[\alpha]_D^{21}$ −5° (c 0.6, CH_2Cl_2). Its HRESIMS showed the pseudomolecular ion peak at *m*/*z* 439.8583 $[M+Cl]^-$. This was confirmed by the ESIMS which displayed the pseudomolecular ion peak at *m*/*z* 439 corresponding to the molecular formula $C_{21}H_{24}O_8$. The IR spectrum showed absorption bands for hydroxyl (3400 cm^{-1}), carbonyl (1740 cm^{-1}), and furan (870 cm^{-1}) functionalities. The ^{13}C NMR spectrum revealed signals due to three carbonyl groups of the ester type at δ 176.9 (C-19), 172.6 (C-18) and 171.9 (C-17) (Table 1). Other salient features of this spectrum included signals due to a β-substituted furan (δ 144.3 (C-15), 140.9 (C-16), 125.6 (C-13) and 109.5 (C-14)), two oxygenated sp^3 methines (δ 73.6 (C-2), 70.5 (C-12)) and one oxygenated quaternary sp^3 carbon δ 74.7 (C-8).

The ^{1}H NMR spectrum showed the characteristic signals of a β-substituted furan ring (Simirgiotis et al., 2000) at δ 7.68 (1H, *brs*, H-16), 7.64 (1H, *brs*, H-15), 6.51 (1H, *brs*, H-14) and two methyl signals at δ 3.68 (3H, *s*, H-21) and 0.81 (3H, *s*, H-20). The one proton resonance at δ 5.47 (1H, *dd*, *J* = 12.9, 3.9 Hz, H-12) together with the signals at δ 1.60 (1H, *dd*, *J* = 14.3, 12.9 Hz, H-11α), and 2.21 (1H, *dd*, *J* = 14.3, 3.9 Hz, H-11β) were consistent with the ABX system formed by H-12 and the C-11 methylene protons.

* Corresponding author. Tel.: +237 500 48 26; fax: +237 345 17 35.
E-mail address: tapondjou2001@yahoo.fr (A.L. Tapondjou).

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data in DMSO, HMBC correlations of compound **1**

C/H atom	δ ^{13}C (ppm)	DEPT	δ ^{1}H (mult, *J*(Hz))	HMBC (H → C)
1	27.0	CH_2	1.60 *m*; 1.90 *m*	
2	73.6	CH	4.84 *brt* (4.8)	
3	29.1	CH_2	2.20 *m*	
4	41.7	CH	2.88 *dd* (10.8, 8.6)	
5	42.9	C		H-4
6	25.5	CH_2	1.84 *m*; 1.97 *m*	
7	27.0	CH_2	1.52 *m*; 2.16 *m*	OH
8	74.7	C		H-20
9	39.3	C		H-20, OH
10	38.0	CH	1.89 *dd* (11.1, 7.9)	H-20, H-4
11	34.9	CH_2	1.60 *dd* (14.3, 12.9)	H-20
			2.21 *dd* (14.3, 3.9)	
12	70.5	CH	5.47 *dd* (12.9, 3.9)	H-11
13	125.6	C		H-15, H-14
14	109.5	CH	6.51 *brs*	H-16
15	144.3	CH	7.64 *brs*	H-16, H-14
16	140.9	CH	7.68 *brs*	H-15, H-14
17	171.9	C		H-7, OH
18	172.6	C		H-4, H-21
19	176.9	C		H-2, H-4, H-10
20	17.4	CH_3	0.81 *s*	
21	52.8	CH_3	3.68 *s*	
O–H	–	–	6.20 *s*	

Furthermore, the downfield signal observed at δ 4.84 (1H, *brt*, *J* = 4.8 Hz, H-2) was ascribed to the methine proton H-2 (Table 1). Complete assignment of the ^{1}H NMR signals was achieved by inspection of the ^{1}H–^{1}H COSY spectrum combined with the TOCSY data, starting from the easily distinguished protons. The HSQC spectrum correlated all the proton signals with those of the corresponding one-bond coupled carbons. The HMBC correlations proved **1** to have all the expected connectivities for the proposed clerodane skeleton. Final proof of the structure and stereochemistry of compound **1** was obtained from a single crystal X-ray analysis using direct methods. The ORTEP diagram of the crystal structure of this compound (Fig. 1) clearly shows that the δ-lactone ring bridging C-2 and C-5, and the proton H-10 are placed on the α face of the molecule. It also shows that the carboxymethyl group at C-4, the hydroxyl group at C-8, the methyl group C-20, and the furan moiety are β-oriented. Consequently, compound **1** is methyl 15,16-epoxy-8β-hydroxycleroda-13(16),14-diene-17,12*S*;19,2α-diolide-18-carboxylate (Bafoudiosbulbin F).

Compound **2** was obtained as colourless crystals in Hexane–EtOAc–MeOH, m.p.: 199–200 °C, $[\alpha]_D^{20}$ −46° (c 0.9, CD_3OD) with a molecular formula $C_{23}H_{26}O_{10}$ determined from the HRESIMS ($[M+Na]^+$ 485.13960). This was confirmed by the ESIMS which showed the pseudomolecular ion peaks at *m*/*z* 485 $[M+Na]^+$ and 503 $[M+Na+H_2O]^+$. The IR spectrum of **2** indicated the presence of hydroxyl group (3500 cm^{-1}), carbonyl groups (1740 and 1730 cm^{-1}), and furan moiety (872 cm^{-1}). The ^{1}H and ^{13}C NMR spectra (Table 2) showed typical signals for a diterpene with a clerodane-type skeleton (Guo et al., 2007). The ^{1}H NMR spectrum showed the characteristic signals of a β-substituted furan ring at δ 7.72 (1H, *brs*, H-16), 7.65 (1H, *brs*, H-15) and 6.55 (1H, *brs*, H-14). Furthermore, three downfield signals were observed at δ 5.69 (1H, *dd*, *J* = 12.6, 3.2 Hz, H-12), 5.05 (1H, *dd*, *J* = 3.9, 1.8 Hz, H-6) and 4.82 (1H, *brt*, *J* = 4.1 Hz, H-2) corresponding to three oxygenated methines, as well as three methyl singlets at δ 3.63 (3H, *s*, H-21), 1.80 (3H, *s*, CH_3CO) and 1.17 (3H, *s*, H-20). The ^{13}C NMR of **2** exhibited signals characteristic of furoclerodanes (Kapingu et al., 2000) at δ 144.0 (C-15), 140.6 (C-16), 125.1 (C-13) and 109.0 (C-14). Other salient features of this spectrum were four deshielded quaternary carbon signals of the ester type at δ 172.4 (C-19), 171.8 (C-18), 170.0 (C-17) and 168.5 (CH_3CO). The complete assignment of all ^{1}H and ^{13}C resonances were achieved by COSY, HSQC and HMBC (Table 2). It was clear that the ^{1}H and ^{13}C NMR spectra exhibited an additional *O*-acetyl group besides several signals similar to those of **1**, indicating **2** to be an *O*-acetylated derivative of **1**. In the HMBC spectrum, cross-peak correlation between H-6 (δ 5.05) and the *O*-acetyl carbonyl (δ 168.5) showed that the *O*-acetyl group was link at C-6. The C-12 configuration was deduced from careful comparison of the ^{1}H and ^{13}C data of **2** with those of related compounds (Teponno et al., 2007; Murray et al., 1984) and was corroborated by the axial orientation of H-12 which showed coupling constants of 12.6 and 3.2 Hz to the axial and equatorial protons at C-11, respectively. The absolute stereochemistry of other chiral centres was established by ROESY experiment. The lack of ROESY correlation between H-12 (δ 5.69) and Me-20 (δ 1.17) as well as between H-10 (δ 2.64) and Me-20, H-10 and H-2 (δ 4.82) indicated their *trans* relationships, respectively. However, ROESY crosspeaks were observed between H-12 and H-10, OH (δ 6.16) and Me-20, and between H-6 (δ 5.05) and OMe (δ 3.63). The relative stereochemistry of this structure was confirmed on the basis of single-crystal X-ray crystallographic analysis (Fig. 2). Thus, the structure of **2** was therefore concluded to be methyl 15,16-epoxy-8β-hydroxy-6α-*O*-acetylcleroda-13(16),14-diene-17,12*S*;19,2α-diolide-18-carboxylate (Bafoudiosbulbin G).

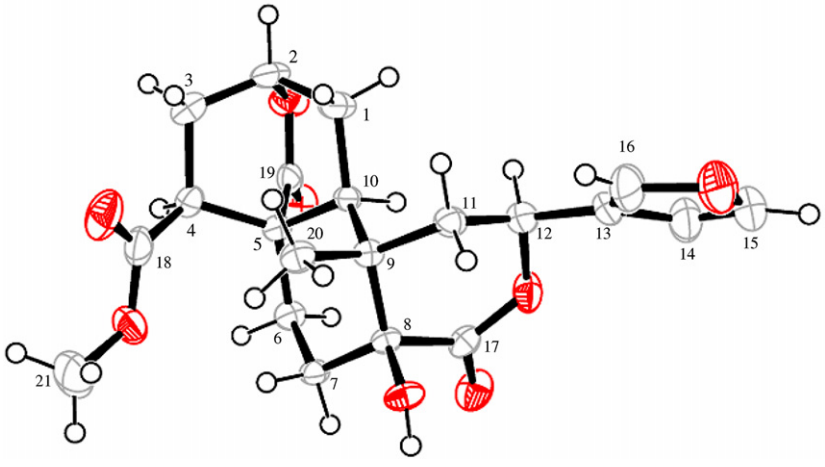


Fig. 1. ORTEP diagram of the crystal structure of compound **1**.

Table 2
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data in DMSO, HMBC correlations of compound **2**

C/H atom	δ ^{13}C (ppm)	DEPT	δ ^{1}H (mult, J(Hz))	HMBC (H → C)
1	25.6	CH_2	1.67 *dd* (13.9, 5.6) 2.28 *brt* (13.9)	
2	72.8	CH	4.82 *brt* (4.1)	H-4
3	28.8	CH_2	2.10 *o*; 2.20 *brd* (14.6)	
4	38.7	CH	3.55 *dd* (10.5, 4.5)	
5	44.9	C		H-6, H-4
6	67.1	CH	5.05 *dd* (3.9, 1.8)	
7	30.6	CH_2	1.93 *dd* (14.5, 1.8) 2.48 *dd* (14.5, 4.1)	
8	71.5	C	–	OH, H-6
9	44.9	C	–	
10	30.8	CH	2.64 *dd* (11.8, 5.5)	
11	36.2	CH_2	1.75 *dd* (14.8, 3.4) 2.10 *o*	
12	69.7	CH	5.69 *dd* (12.6, 3.2)	H-14
13	125.1	C	–	H-12, H-14, H-15
14	109.0	CH	6.55 *brs*	H-16
15	144.0	CH	7.65 *brs*	H-16, H-14
16	140.6	CH	7.72 *brs*	H-15, H-14
17	170.0	C	–	H-7, OH
18	171.8	C	–	H-4, H-21
19	172.4	C	–	H-2, H-4, H-10
20	18.7	CH_3	1.17 *s*	
21	52.3	CH_3	3.63 *s*	
CH_3CO	168.5	**C**	–	H-6
CH_3CO	20.6	CH_3	1.80 *s*	
O–H	–	–	6.16 *s*	

o: overlapped.

The structure of Bafoudiosbulbin B was initially proposed as **5** (Teponno et al., 2006). Extensive 2D NMR techniques as well as chemical transformation proved the true structure of Bafoudiosbulbin B to be **3**. No reaction was achieved upon acetylation of this compound by standard procedure (acetic anhydride–pyridine at room temperature). This observation allowed us to conclude that the free hydroxyl group in Bafoudiosbulbin B was tertiary and not primary as previously proposed. We then decided to carry out acetylation under forcing conditions as described by Kilonda et al. (2003) to react sterically crowded alcohol group and Bafoudiosbulbin B acetate **4** was obtained in 23% yield. The main difference between the ^{13}C NMR spectra of **3** and **4** was the chemical shift of C-8, which appeared at δ 79.4 in compound **4** instead of appearing at δ 74.7 in compound **3** due to the acetylation shift (Manguro et al., 2006). Extensive examination of the HMBC spectrum of Bafoudiosbulbin B further supported that the δ-lactone bridge was between C-5 and C-20 instead of being between C-5 and C-8 as previously described because it showed a cross-peak correlation between one of the oxymethylene protons at δ 4.55 and the carbon at δ 172.5 (C-19). Many other important correlations were also observed (Fig. 3). In the NOESY spectrum, the lack of correlation between H-12 (δ 5.50) and the oxymethylene protons H-20 (δ 4.55, 4.72) as well as between H-20 and H-10 (δ 2.60) indicated their *trans* relationships, respectively. However, cross-peak correlations were observed between H-12 (δ 5.50) and H-10 (δ 2.60), H-10 and H-4 (δ 3.46), H-1 (δ 1.95) and H-20 (δ 4.88), and between H-11 (δ 2.70) and H-20 (δ 4.55) (Fig. 3). From these data, the A ring conformation of compound **3** was deduced to be of boat form as in compounds **1** and **2**. The correct structure of Bafoudiosbulbin B was then elucidated as 15,16-epoxy-8β-hydroxycleroda-13(16),14-diene-17,12*S*;18,2β;19,20β-triolide **3**.

3. Experimental

3.1. General experimental procedures

Melting points were determined using the REICHERT AUSTRIA Microscope and on the Gallenkamp Melting Point Apparatus. Optical rotations were measured on a Perkin–Elmer 241 MC Polarimeter. IR spectra were measured as a film on a KBr pellet using a FTIR-8400S Shimadzu spectrometer. ESIMS was carried out on a Hewlett–Packard HP-1100 series LC–MSD system and on the mass spectrometer Brucker FTMS4.7T, BIOAPEXII. ^{1}H NMR spectra were recorded in deuterated solvents (DMSO, acetone, and pyridine) on a Varian Mercury Plus Spectrometer at 400 and 500 MHz while ^{13}C NMR spectra were recorded in the same solvents and the same apparatus at 100 and 125 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 or 0.063–0.2 mm). TLC and preparative TLC were carried out on precoated Kieselgel 60 F_{254} (Merck) plates developed with CH_2Cl_2:MeOH (95:5, 96:4 or 98:2). TLC plates were viewed with

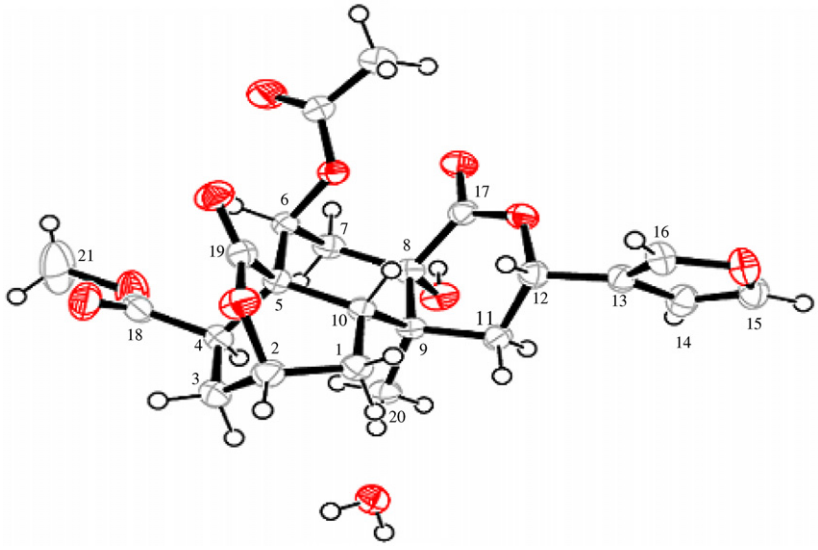


Fig. 2. ORTEP diagram of the crystal structure of compound **2** · H_2O.

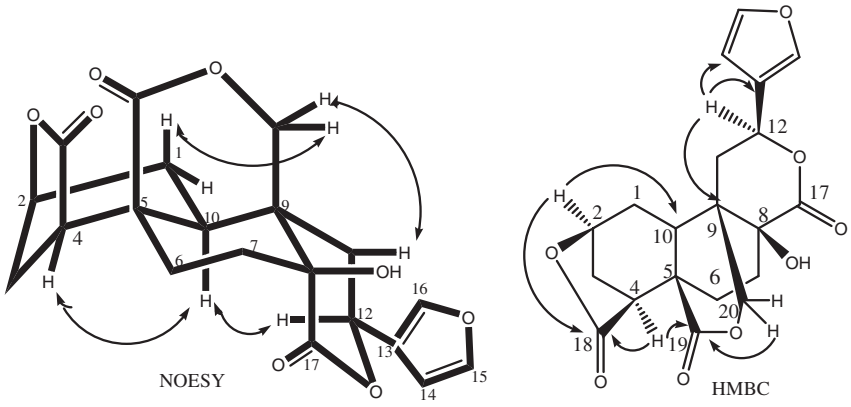


Fig. 3. Important NOESY and HMBC correlations for Bafoudiosbulbin B.

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 bulbils of *D. bulbifera* L. var *sativa* were collected in Bafou village near Dschang (West province of Cameroon) in February 2007. The plant was identified by Dr G. Achoundong, Cameroon National Herbarium, Yaoundé, where a voucher specimen (Ref. 22211/SRF/CAM) was deposited.

3.3. Extraction and isolation

The dried and pulverized bulbils of *D. bulbifera* L. var *sativa* (12 kg) were extracted two times (each time for 24 h) with MeOH–H_2O (8–2). The filtrate obtained was concentrated under reduced pressure to yield a dark residue (530 g). Part of this extract (502 g) was suspended in water (1000 ml) and extracted with EtOAc. The EtOAc layer was concentrated to dryness under reduced pressure to afford 210 g of residue. Part of this residue (192 g) was subjected to column chromatography over silica gel (Hexane–EtOAc with increasing polarity) yielding seven main fractions A–G. Fraction C (4.5 g) (Hexane–EtOAc (1–1)) was rechromatographed on silica gel column chromatography using CH_2Cl_2–MeOH (98–2) as eluent, to afford Bafoudiosbulbin F (175 mg) and a mixture mainly consisting of two compounds. Fraction B (15.8 g) (Hexane–EtOAc (7–3)) and the above mixture were combined mainly on the basis of TLC patterns. The resulting fraction was subjected to repeated silica gel column chromatography (CH_2Cl_2–MeOH (96–4)) to yield kaempferol (45 mg), as well as an inseparable mixture which was repeatedly subjected to silica gel preparative TLC using CH_2Cl_2–MeOH (95–5) to afford 8 mg of 3,5,4′-trihydroxy-3′-methoxybibenzyl. Fraction D (13.2 g) (Hexane–EtOAc (4–6)) mainly yielded Bafoudiosbulbin A (557 mg) upon recrystallisation in MeOH. Filtration of fraction E (14.4 g) (Hexane–EtOAc (3–7)) yielded a white powder (8.5 g) composed of Bafoudiosbulbins A and B. The filtrate obtained was crystallised with Hexane–EtOAc–MeOH to afford pure crystals of Bafoudiosbulbin G (678 mg). The above powder was purified by silica gel column chromatography eluted with CH_2Cl_2–MeOH (97–3) to yield Bafoudiosbulbin B (2.2 g) and Bafoudiosbulbin A (1.7 g) as well as a mixture of these compounds (3.6 g). Repeated silica gel column chromatography of fraction F (27.6 g) (Hexane–EtOAc (3–7)) with CH_2Cl_2–MeOH (95–5) yielded Bafoudiosbulbin C (135 mg).

3.4. X-ray crystallographic analysis of Bafoudiosbulbin F (*1*) and Bafoudiosbulbin G (*2*)

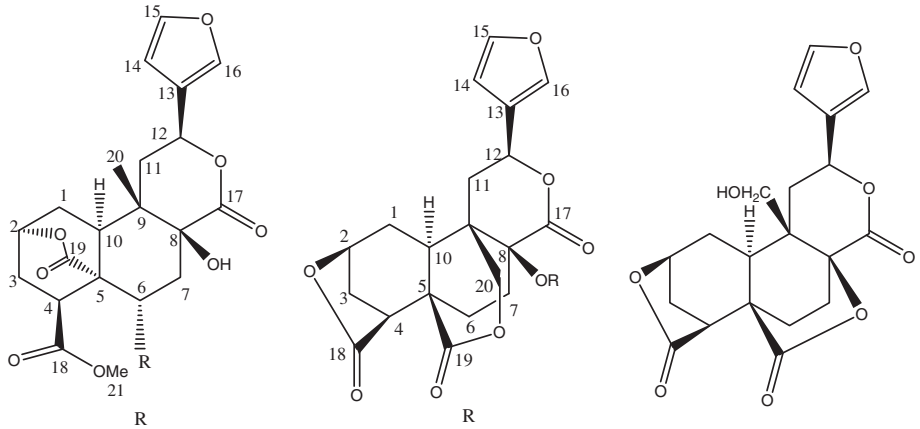
Suitable crystals of compounds **1** and **2** · H_2O were obtained as colourless blocks from CH_2Cl_2–MeOH and Hexane–EtOAc–MeOH solutions, respectively. The intensity data were collected at 173 K (−100 °C) on a Stoe Mark II-Image Plate Diffraction System equipped with a two-circle goniometer and using Mo Kα graphite monochromated radiation. Compound **1**: $C_{21}H_{24}O_8$, M = 404.40; crystallized in the non-centrosymmetric orthorhombic space group $P2_12_12_1$, a = 7.0460 (7), b = 15.7928 (19), c = 17.3891 (16), Z = 4. Image plate distance 130 mm, ω rotation scans 0–180° at ϕ 216°, step $\rho\omega$ = 1.5°, with an exposure time of 6 min per image, 2θ range 1.76–52.59°, $d_{min} - d_{max}$ = 23.107 − 0.802 Å. The final reliability factors are: R = 0.0374, wR = 0.0715 and the goodness of fit on F^2 was equal to 0.905. Compound **2**: $C_{23}H_{28}O_{11}$, M = 480.45; crystallized as a monohydrate in the non-centrosymmetric orthorhombic space group $P2_12_12_1$, a = 9.6296 (5), b = 11.7509 (5), c = 20.2697 (11), Z = 4. Image plate distance 130 mm, ω rotation scans 0–156° at ϕ 306°, 14–64° at ϕ 336° and 174–176° at ϕ 160°, step $\rho\omega$ = 1°, with an exposure time of 2 min per image, 2θ range 1.76–52.59°, $d_{min} - d_{max}$ = 23.107 – 0.802 Å. The final reliability factors are: R = 0.0264, wR = 0.0665 and the goodness of fit on F^2 was equal to 1.039. The structures were solved by Direct methods using the programme SHELXS-97 (Sheldrick, 2008). The refinement and all further calculations were carried out using SHELXL-97 (Sheldrick, 2008). 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.

3.5. Compound *1*: Bafoudiosbulbin F

Colourless crystals, m.p. 265–266 °C (CH_2Cl_2–MeOH); $[\alpha]_D^{21}$ −5° (c 0.6, CH_2Cl_2); IR ν_{max} (KBr) cm^{-1}: 3400, 1740, 870; ^1H NMR (DMSO, 400 MHz): Table 1; ^{13}C NMR (DMSO, 100 MHz): Table 1; ESIMS: m/z 439 $[M+Cl]^-$ (100).

3.6. Compound *2*: Bafoudiosbulbin G

Colourless crystals, m.p. 199–200 °C (Hexane–EtOAc–MeOH); $[\alpha]_D^{20}$ −46° (c 0.9, CD_3OD); IR ν_{max} (KBr) cm^{-1}: 3500, 1740, 1730, 872; ^1H NMR (DMSO, 500 MHz): Table 2; ^{13}C NMR (DMSO, 125 MHz): Table 2; ESIMS: m/z 485 $[M+Na]^+$ (100), 503 $[M+Na+H_2O]^+$ (60).



*3.7. Compound **3**: Bafoudiosbulbin B*

White powder, m.p. 312.9 °C (MeOH); $[\alpha]_D^{21}$ +52 (c 0.01, pyridine); IR ν_{max} (KBr) cm^{-1}: 3428, 2949, 1786, 1758, 1024, 874; ^1H NMR (pyridine, 400 MHz): 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), 5.50 (1H, *dd*, *J* = 12.7, 3.4 Hz, H-12), 4.88 (1H, *m*, H-2), 4.72 (1H, *d*, *J* = 13.7 Hz, H-20a), 4.55 (1H, *d*, *J* = 13.7 Hz, H-20b), 3.46 (1H, *dd*, *J* = 12.5, 7.3 Hz, H-4), 3.10 (1H, *m*, H-7a), 2.70 (1H, *dd*, *J* = 14.1, 12.7 Hz, H-11β), 2.60 (1H, *brd*, *J* = 9.8 Hz, H-10), 2.51 (1H, *m*, H-3a), 2.42 (1H, *m*, H-7b), 2.30 (2H, overlapped, H-1a, H-6a), 2.16 (1H, *m*, H-3b), 2.10 (1H, *m*, H-6b), 1.95 (1H, *m*, H-1b), 1.70 (1H, *dd*, *J* = 14.1, 3.4 Hz, H-11α); ^{13}C NMR (pyridine, 100 MHz): 175.1 (C-18), 172.5 (C-19), 172.1 (C-17), 144.6 (C-15), 141.1 (C-16), 125.7 (C-13), 109.5 (C-14), 74.7 (C-8), 73.8 (C-2), 70.8 (C-12), 67.5 (C-20), 45.1 (C-4), 44.2 (C-5), 40.2 (C-9), 36.5 (C-10), 30.6 (C-11), 28.9 (C-7), 27.5 (C-3), 27.3 (C-6), 27.1 (C-1); ESIMS: *m*/*z* (rel. int.) 387 $[M-H]^-$ (100), 343 $[M-H-CO_2]^-$ (10), 329 $[M-H-CO-CH_2O]^-$ (30).

3.8. Acetylation of Bafoudiosbulbin B

To 50 mg of Bafoudiosbulbin B was added 6 ml of acetic anhydride–pyridine (1–1). After adding catalytic amount of DMAP (2 mg), the resulting mixture was heated at 60 °C for 12 h. The reaction process was monitored by TLC plates which showed the formation of products. Evaporation to dryness under reduced pressure after adding 5 ml of MeOH afforded a brown gum which was purified by column chromatography over silica gel (CH_2Cl_2–MeOH (98–2)) to yield 13 mg (23%) of **4** and 17 mg of the starting substrate (Bafoudiosbulbin B).

*3.9. Compound **4**: Bafoudiosbulbin B acetate*

White amorphous powder; IR ν_{max} (KBr) cm^{-1}: 2949, 1786, 1758, 1024, 874; ^1H NMR (acetone, 400 MHz): 7.66 (1H, *brs*, H-16), 7.56 (1H, *brs*, H-15), 6.63 (1H, *brs*, H-14), 5.63 (1H, *dd*, *J* = 12.5, 4.1 Hz, H-12), 4.95 (1H, *brs*, H-2), 4.81 (1H, *d*, *J* = 14.3 Hz, H-20b), 4.30 (1H, *d*, *J* = 14.3 Hz, H-20a), 3.11 (1H, *dd*, *J* = 12.7, 7.2 Hz, H-4), 2.94 (1H, *brd*, *J* = 8.3 Hz, H-7b), 2.64 (1H, *m*, H-3b), 2.48 (2H, overlapped, H-1b, H-10), 2.34 (1H, *dd*, *J* = 14.2, 12.5 Hz, H-11b), 2.25 (1H, *dd*, *J* = 14.6, 7.2 Hz, H-3a), 2.16 (3H, *s*, MeCO), 2.02-2.13 (3H, overlapped, H-1a, H-6b, H-7a), 1.87 (1H, *dd*, *J* = 14.2, 4.1 Hz, H-11a), 1.83 (1H, *d*, *J* = 11.2 Hz, H-6a); ^{13}C NMR (acetone, 100 MHz): 173.8 (C-18), 171.0 (C-19), 169.0 (Me*CO*), 166.7 (C-17), 143.7 (C-15), 140.7 (C-16), 124.7 (C-13), 109.0 (C-14), 79.4 (C-8), 73.0 (C-2), 70.9 (C-12), 66.6 (C-20), 44.4 (C-4), 44.4 (C-9), 38.9 (C-5), 35.3 (C-10), 30.6 (C-11), 27.2 (C-7), 26.7 (C-3), 26.5 (C-1), 25.5 (C-6), 20.3 (*Me*CO), ESIMS: *m*/*z* 465 $[M+Cl]^-$ (100).

4. Supplementary material

Crystallographic data for structures **1** and **2** have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 682270 (**1**) and CCDC 682271 (**2**). Copies of the data can be obtained free of charge on application to CCDS, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) +44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

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