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Clerodane and 19-norclerodane diterpenoids from the tubers of *Dioscorea antaly*

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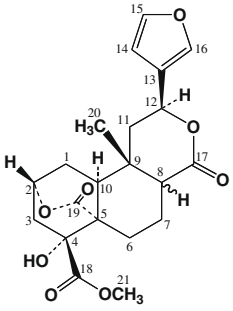
ABSTRACT

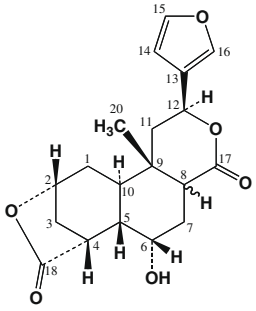
Two clerodane diterpenoids, antadiosbulbins A and B and two 19-norclerodane diterpenes, 8-epidiosbulbins E and G along with the known diosbulbin E as well as nine known phenolics including five phenanthrenes and stilbenes and four flavonoids were isolated from the ethyl acetate soluble part of the methanolic extract of the tubers of *Dioscorea antaly*, a yam endemic to Madagascar. Structures were determined by analysis of the spectral data, mainly 2D-NMR and mass spectrometry.

1. Introduction

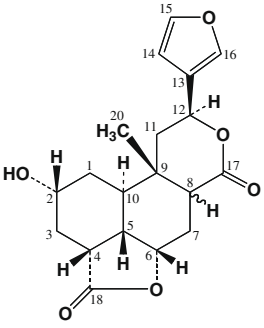
Dioscorea antaly Jum. and H. Perrier (Dioscoreaceae) is a liana endemic to Madagascar and found in the West and North-West regions. In times of scarcity, its tubers are used as food after prerequisite detoxification to remove bitters and toxic principles. As a part of our research program devoted to chemical knowledge and possible commercial use of Madagascar yams, we have chemically investigated this species. The present article describes the isolation from the EtOAc soluble part of the methanol extract of the tubers, and structure determination of the four new diterpenoids, two clerodanes, antadiosbulbins A (**1**) and B (**2**), and two furanoid 19-norclerodanes, 8-epidiosbulbin E (**3**) and 8-epidiosbulbin G (**4**) together with the complete ^{1}H and ^{13}C NMR assignments for the known diterpenoid diosbulbin E (**5**) (Ida et al., 1978a). In addition, nine known phenolics were isolated, which were five stilbene or phenanthrene derivatives, i.e. 3,7-dihydroxy-2,4-dimethoxyphenanthrene (Leong et al., 1997), (*E*)-piceatannol (Brinker and Seigler, 1991), 3,4,3′,5′-tetrahydroxy-dihydrostilbene or dihydropiceatannol (Mannila et al., 1993; Matsuda et al., 2001), the latter being obtained for the first time from a natural source, cassigarol D (Baba et al., 1992), scirpusin B (Nakajima et al., 1978), and four flavonoids, catechin (Davies et al., 1996), 3-*O*-[*β*-D-glucose-(6 → 1)-*α*-L-rhamnose]-kaempferol (Kartnig and Bucar-Stachel, 1991; Markham et al., 1978), kaempferol and quercetin.



Antadiosbulbin A (**1**): 8α-H
Antadiosbulbin B (**2**): 8β-H



8-Epidiosbulbin E (**3**): 8β-H
Diosbulbin E (**5**): 8α-H



8-Epidiosbulbin G (**4**): 8β-H
Diosbulbin G (**7**): 8α-H

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Table 1
NMR data for antadiosbulbins A (**1**) and B (**2**) (400 MHz for ^{1}H).

Position	Antadiosbulbin A (**1**) in CD_3OD				Antadiosbulbin A (**1**) in $CDCl_3$				Antadiosbulbin B (**2**) in CD_3OD				Antadiosbulbin B (**2**) in $CDCl_3$			
	δ_C	δ_H	*Mult.*	*J* (Hz)	δ_C	δ_H	*Mult.*	*J* (Hz)	δ_C	δ_H	*Mult.*	*J* (Hz)	δ_C	δ_H	*Mult.*	*J* (Hz)
1a	28.1	2.32	*dddd*	13.4; 11.2; 5.2; 2.1	27.1	2.31	*m*		28.0	2.34	*dddd*	13.5; 11.2; 5.2; 2.2	27.1	2.27	*m*	
b		1.63	*dd*	13.4; 8.2		1.57	*dd*	13.3; 8.8		1.62	*dd*	13.5; 8.3		1.57	*m*	
2	74.3	4.88	*dd*	5.2; 5.2	72.1	4.90	*dd*	5.2; 5.2	74.1	4.92	dd	5.2; 5.2	71.8	4.90	*dd*	5.2; 5.2
3a	40.2	2.77	*d*	14.8	38.6	2.70	*ddd*	14.9; 1.1; 1.1	39.8	2.76	*d*	15.0	38.2	2.50	*dd*	15.0; 0.7
b		2.13	*ddd*	14.8; 5.2; 2.1		2.26	*m*			2.16	*ddd*	15.0; 5.2; 2.2		2.31	*m*	
4	76.5	–			75.9	–			77.0	–			76.1	–		
5	53.4	–			52.2	–			53.3	–			51.8	–		
6eq	24.1	2.62	*ddd*	15.1; 3.9; 3.0	23.1	2.34	*m*		21.6	2.40	*dddd*	14.8; 3.5; 3.5; 0.8	20.5	2.10	*m*	
ax		2.06	*ddd*	15.1; 14.5; 5.0		2.14	*m*			1.90	*ddd*	14.8; 14.2; 4.0		2.10	*m*	
7eq	19.7	1.91	*dddd*	14.8; 5.0; 3.0; 3.0	18.3	2.04	*dddd*	14.2; 4.8; 3.2; 3.2	19.9	2.30	*dddd*	14.3; 4.0; 3.5; 3.3	19.0	2.39	*dddd*	14.0; 3.8; 3.8; 3.8
ax		1.56	*dddd*	14.8; 14.5; 12.4; 3.9		1.44	*dddd*	14.2; 14.2; 12.0; 3.2		1.75	*dddd*	14.3; 14.2; 4.5; 3.5		1.55	*m*	
8	48.0	2.78	*dd*	12.4; 3.0	46.7	2.48	*dd*	12.0; 3.2	48.1	2.53	*dd*	3.5; 3.3	47.3	2.36	*m*	
9	37.2	–			36.0	–			36.3	–			35.0	–		
10	47.8	2.20	*dd*	11.2; 8.2	46.2	2.09	*m*		38.8	2.08	*dd*	11.2; 8.3	37.1	2.11	*m*	
11eq	44.0	2.07	*dd*	14.5; 6.3	43.6	1.94	*dd*	14.1; 6.4	42.2	2.09	*dd*	14.8; 3.8	41.7	2.03	*dd*	14.8; 3.5
ax		1.86	*dd*	14.5; 12.0		1.83	*dd*	14.1; 11.0		1.86	*dd*	14.8; 12.5		1.75	*dd*	14.8; 12.3
12	71.6	5.50	*dd*	12.0; 6.3	69.9	5.33	*dd*	11.0; 6.4	72.3	5.48	*dd*	12.5; 3.8	70.2	5.27	*dd*	12.3; 3.5
13	125.9	–			124.0	–			126.5	–			124.6	–		
14	109.7	6.48	*dd*	1.8; 0.8	108.4	6.37	*dd*	1.9; 0.9	109.6	6.53	*dd*	1.9; 0.9	108.3	6.38	*dd*	1.9; 0.9
15	145.1	7.48	*dd*	1.8; 1.6	143.8	7.40	*dd*	1.9; 1.7	144.9	7.48	*dd*	1.9; 1.7	143.7	7.39	*dd*	1.9; 1.7
16	141.6	7.56	*ddd*	1.6; 0.9; 0.8	139.6	7.42	*ddd*	1.7; 0.9; 0.9	141.5	7.59	*ddd*	1.7; 0.9; 0.8	139.8	7.43	*ddd*	1.7; 0.9
17	176.7	–			172.9	–			174.5	–			171.0	–		
18	174.0	–			173.9	–			174.0	–			173.6	–		
19	177.2	–			172.1	–			177.0	–			172.3			
20	20.6	0.90	*s*		20.2	0.83	*s*		24.0	0.98	*s*		24.1	0.98	*s*	
OMe	53.3	3.80	*s*		53.5	3.83	*s*		53.4	3.82	*s*		53.4	3.85	*s*	
4-OH	–	–			–	3.45	*s*		–	–			–	3.44	*s*	

2. Results and discussion

The dried and ground tubers of *D. antaly* were defatted with cyclohexane and then extracted with MeOH at room temperature. After concentration to dryness, the MeOH extract was partitioned between EtOAc and water. The EtOAc soluble part on fractionation by a combination of column and MPL chromatographies on silica gel, C-18 reverse phase and Sephadex LH-20, led to the isolation of the four new compounds antadiosbulbins A (**1**, 3 mg) and B (**2**, 3 mg), 8-epidiosbulbins E (**3**, 534 mg) and G (**4**, 26 mg), together with the known diosbulbin E (**5**, 16 mg) and the nine phenolic substances.

Compound **1** was an optically active $[\alpha]_D^{20}$ −45° (*c* 0.7, $CHCl_3$) colourless amorphous solid. Its HR-ESI-MS showed the protonated molecular ion $[M+H]^+$ at *m/z* 405.1519 (calc. for $C_{21}H_{25}O_8$: 405.1548) a molecular formula indicative of ten degrees of unsaturation. The IR spectrum of **1** displayed absorption bands at 1740 (–COO–), 3447 (OH), and 3150 and 1506 cm^{-1} suggesting the presence of a furan ring. The ^{13}C *J*-modulated NMR spectrum (CD_3OD) of **1** exhibited the resonances of 21 carbons consisting of one methyl, one methoxyl at δ_C 53.3, five methylenes, seven methines including three sp^2 carbons at δ_C 109.7, 141.6 and 145.1, seven quaternary carbons including three carbonyls at δ_C 174.0, 176.7 and 177.2 and one ethylenic carbon at 125.9 (Table 1). Taking into account the ten degrees of unsaturation, compound **1** should include five rings. The ^1H NMR spectrum (CD_3OD) displayed typical signals of a methyl singlet at δ_H 0.90, the methoxyl of an ester at δ_H 3.80, three ethylenic protons at δ_H 7.56, 7.48 and 6.48 and two sp^3 oxymethine protons at δ_H 4.88 and 5.50 (Table 1).

Detailed analysis of the ^1H–^1H COSY and HSQC spectra established the presence of the four sub-structures: **a** (>CH–CH_2–CH(O)–CH_2–), **b** (–CH_2–CH_2–CH<), **c** (–CH_2–CH(O)–) and **d** (a monosubstituted furan ring) which are marked with bold bonds in Fig. 1, and further assembled from the cross-peaks observed in the HMBC spectrum. The C=O signal at δ_C 174.0 (C-18) was assigned as a methyl ester at C-18 because of the correlations with protons at δ_H 2.77 (H-3eq) and 4.88 (H-2; $^4J_{H-C}$ *W* coupling) and the methoxyl at 3.80 (CH_3-21) pointing C(O)-4 at δ_C 76.5. Similarly the C=O at δ_C 177.2 was assigned as C-19 due to the correlations with protons at δ_H 2.62 and 2.06 (CH_2-6) and 2.20 (CH-10), and the correlation with the oxymethine proton at 4.88 (H-2) indicated the lactone ring closure to C-2. The second lactone C=O at δ_C 176.7 was assigned to C-17 because it correlated with protons at δ_H 1.91, 1.56 (CH_2-7) and 2.78 (H-8), while its correlation with the proton at δ_H 5.50 (H-12) indicated the second lactone ring closure to C-12. The protons of the methyl at δ_H 0.90 (C-20) were strongly correlated with carbons at δ_C 48.0 (C-8), 37.2 (C-9), 47.8 (C-10) and 44.0 (C-11) indicating this methyl to be linked to C-9, hence sub-structures **a**–**c** could be assembled as shown in Fig. 1. The furan group was linked to C-12, because the quaternary carbon of this ring at δ_C 125.9 (C-13) correlated with the protons at δ_H 5.50 (H-12) and 1.86 (H-11ax). Other HMBC data confirmed this assemblage of sub-structures (**a**–**d**) to form the proposed planar structure for **1** (Fig. 1). This structure was confirmed by analysis of the 1D- and 2D-NMR spectra of **1** in solution in $CDCl_3$ and especially the linkage of the free hydroxyl group at δ_H 3.45 to C-4 (Table 1). The large coupling constant of H-12 with H-11ax (^3J = 11.0 Hz), indicated they were in a *trans* relationship on the half-chair C-ring. A *trans*-diaxial disposition was observed for H-8, which showed a large coupling (^3J = 12.4 Hz) with H-7ax and a gauche coupling (^3J = 3.0 Hz) with H-7eq. The large coupling constant of H-10 (^3J = 11.2 Hz) with one of the CH_2-1 protons indicated it was axial on the B-ring. Methine H-2 gave gauche couplings with only one proton of each of its vicinal methylenes at C-1 (H-1a, at lower fields) and C-3 (H-3b, at higher fields) and in addition a *W* coupling (2.1 Hz) was observed between these two protons (Table 1). These

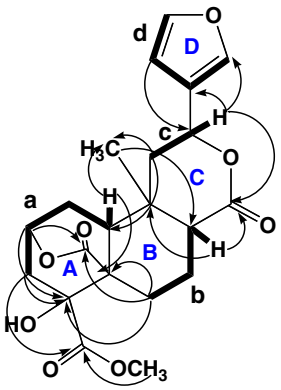

Fig. 1. Sub-structures (**a**–**d**), key COSY (bold bonds) and HMBC (arrows) correlations for antadiosbulbin A (**1**).

data, together with the NOEs observed in both solvents ($CDCl_3$ and CD_3OD) between α-face protons (H-8 with H-7eq, H-10 and H-12; H-10 with H-6ax), or the β-face protons (CH_3-20 with H-1b, H-3a, H-7ax, H-11eq and H-14) whereas H-6eq was correlated with the hydroxyl group at C-4, and the OCH_3-21 with H-3a, H-7ax and CH_3-20, led to the relative configuration proposed in Fig. 2. Hence the structure (relative configuration) of **1** was established as methyl 15,16-epoxy-4-hydroxyclero-13(16),14-diene-17,12;19,2-diolide-18-carboxylate and the name antadiosbulbin A was proposed for this novel furano-clerodane with two δ-lactone rings bridging carbonyl C-19 to C-2 and carbonyl C-17 to C-12.

Antadiosbulbin B (**2**) was an optically active $[\alpha]_D^{20}$ −28° (*c* 0.4, $CHCl_3$) colourless amorphous solid. It depicted the same molecular formula $C_{21}H_{24}O_8$ as antadiobulbin A (**1**) and had similar IR and NMR (in CD_3OD as well as in $CDCl_3$) spectra, suggesting they were isomers (Table 1). Analysis of the COSY spectrum of **2** led to the same sub-structures **a**–**d** as **1** (Fig. 1). When compared with those of antadiosbulbin A, the chemical shifts of carbons C-6, C-9, C-10, C-11 and C-17 of **2** (in $CDCl_3$) were shifted upfield, with $\Delta\delta_C$ = −2.6, −1.0, −9.1, −1.9 and −1.9 ppm, whereas those of C-7, C-8 and C-20 were shifted downfield with $\Delta\delta_C$ +0.6, +1.6 and +3.9, respectively (Table 1), with the upfield shift of C-10 being notable. The second main distinction between the two compounds were the coupling constants of H-8 (CD_3OD), one large and one small for **1** (^3J = 12.4 and 3.0 Hz) and two small for **2** (^3J = 3.5 and 3.3 Hz) due to its β-equatorial disposition with respect to the B-ring in **2**, instead of α-axial as in **1**. This equatorial disposition of H-8 on the β-face was confirmed from the NOESY spectrum where this proton did not give cross-peak with H-12, but gave a strong one with the methyl CH_3-20 which was also correlated with H-1b, H-3a, H-7ax (Fig. 3). The other NOE data indicated for the various chiral centers the same relative configurations as for antadiosbulbin A. With a *cis*-fused A(cyclohexane)/B-ring and a *cis*-fused B/C-ring the structure of **2** was established as methyl 15,16-epoxy-4-hydroxyclero-13(16),14-diene-17,12;19,2-diolide-18-carboxylate and the name antadiosbulbin B was proposed for this new compound **2**, which is thus the 8-epimer of antadiosbulbin A.

Compound **3** was a colourless amorphous solid, $[\alpha]_D^{20}$ −16° (*c* 0.3, $CHCl_3$), with the molecular formula $C_{19}H_{22}O_6$ based on the protonated molecular ion $[M+H]^+$ at *m*/*z* 347.1485 (calc. 347.1493 for $C_{19}H_{23}O_6$) indicative of nine degrees of unsaturation. Detailed analyses of the 2D-NMR spectra allowed full assignment of protons and carbons and indicated that **3** was a furanoid 19-norclerodane derivative (Table 2). The spin systems observed on the ^1H–^1H COSY spectrum (Fig. 4) were connected from the HMBC spectrum to form the norclerodane nucleus and determine the functionalities location. From the NOE data and ^1H–^1H vicinal coupling constants, proton H-2 was β-equatorial due to its gauche *J* values ($J_{H2-H1eq}$ = 4.9 and $J_{H2-H3eq}$ = 5.5 Hz) and H-4 was also β-equatorial because of its small coupling constants (*J* = 5.1 and 1.1 Hz) with H-3eq and H-5ax on the A-ring which is now a chair (Fig. 5). The coupling of H-5 with H-10 (*J* = 12.5 Hz) and the lack of NOE interaction between them indicated they were in a *trans*-diaxial disposition and hence the junction between the A- and B-rings was *trans*. Proton H-12 was α-axial because of its large coupling constant (*J* = 12.3 Hz) with β-axial H-11. The small coupling constants of H-6 and H-8 with the two protons at CH_2-7 indicated that they were both β-equatorial. Finally NOEs were observed between β-face protons (H-8 with CH_3-20, H-7ax and H-11ax; CH_3-20 with H-5 and H-1ax; H-1ax with H-3ax; H-6 with H-4 and H-5), and between α-face protons H-10 and H-12 (Fig. 5). The structure was confirmed by analysis of the 2D-NMR spectra in C_5D_5N (Table 2). Hence compound **3** was 15,16-epoxy-6α-hydroxy-19-nor-clero-13(16),14-diene-17,12;18,2-diolide, the epimer at C-8 of diosbulbin E (**5**), a substance previously isolated from *Dioscorea bulbifera* L. (*forma spontanea* Makino et Nemoto) (Ida et al., 1978a), and thus named 8-epidiosbulbin E. Acetylation of **3** by acetic anhydride in pyridine afforded in good yield the mono-acetate **6**, which had spectral data identical with those of 8-epidiosbulbin E acetate previously

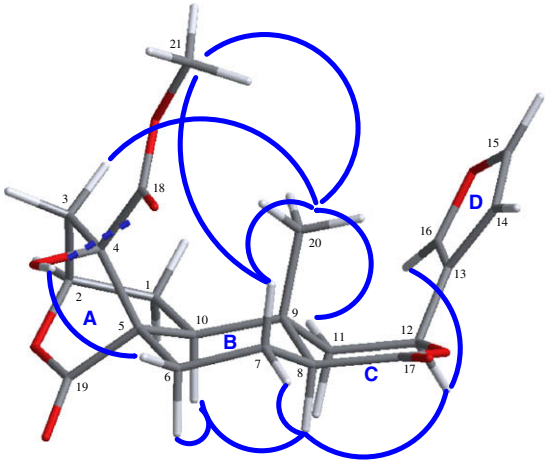

Fig. 2. Selected NOESY correlations (blue lines) and proposed 3D-structure for antadiosbulbin A (**1**). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

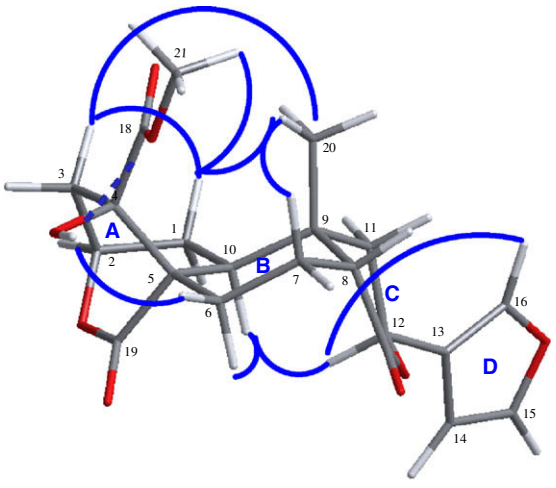

Fig. 3. Selected NOESY correlations (blue lines) and proposed 3D-structure for antadiosbulbin B (**2**). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
NMR data for 8-epidiosbulbin E (**3**) and diosbulbin E (**5**) (400 MHz for ^{1}H).

Position	8-Epidiosbulbin E (**3**) in $CDCl_3$				8-Epidiosbulbin E (**3**) in C_5D_5N				Diosbulbin E (**5**) in $CDCl_3$			
	δ_C	δ_H	*Mult.*	*J* (Hz)	δ_C	δ_H	*Mult.*	*J* (Hz)	δ_C	δ_H	*Mult.*	*J* (Hz)
1eq	28.2	2.10	*dddd*	13.3; 6.0; 4.9; 1.7	28.4	1.97	*dddd*	12.3; 6.0; 5.0; 1.5	28.5	2.12	*brd*	13.2
ax		1.45	*ddd*	13.3; 12.2; 0.9		1.31	*ddd*	12.3, 12.1; 1.2		1.42	*ddd*	13.2; 12.5; 0.7
2	78.0	4.85	*dd*	5.5; 4.9	77.6	4.73	*dd*	5.5; 5.0	78.4	4.92	*brdd*	5.3; 5.0
3eq	38.3	2.52	*dddd*	11.6; 5.5; 5.1; 1.7	38.6	2.32	*dddd*	11.5; 5.5; 5.5; 1.5	38.6	2.53	*dddd*	11.7; 5.5; 5.3; 1.9
ax		1.77	*d*	11.6		1.62	*d*	11.5		1.78	*brd*	11.7
4	43.3	2.65	*brdd*	5.1; 1.1	43.6	2.63	*dd*	5.5; 1.8	43.6	2.68	*brdd*	5.4; 1.5
5	42.7	1.80	*ddd*	12.5; 2.0; 1.1	43.4	1.75	*ddd*	12.1; 1.8; 1.5	42.9	1.72	*ddd*	12.4; 2.0; 1.5
6	69.4	4.14	*ddd*	2.5; 2.2; 2.0	68.8	4.19	*brdddd*	3.4; 2.2; 1.5; 1.5	69.4	4.23	*ddd*	2.6; 2.6; 2.0
7eq	29.7	2.75	*ddd*	14.7; 3.2; 2.2	31.2	2.99	*ddd*	14.2; 3.4; 2.0	28.9	2.14	*brdd*	14.6; 2.6
ax		1.88	*ddd*	14.7; 6.3; 2.5		1.91	*ddd*	14.2; 6.5; 2.2		1.75	*ddd*	14.6; 12.2; 2.6
8	45.5	2.25	*dd*	6.3; 2.1	45.9	2.41	*dd*	6.5; 2.0	41.0	3.20	*dd*	12.2; 3.6
9	34.6	–			34.8	–			35.9	–		
10	29.7	2.71	*ddd*	12.5; 12.2; 6.0	30.1	3.09	*ddd*	12.1; 12.1; 6.0	39.0	2.39	*ddd*	12.5; 12.4; 5.5
11eq	39.6	2.08	*dd*	15.4; 3.1	39.5	2.04	*dd*	14.6; 3.1	42.3	2.13	*dd*	15.0; 6.1
ax		1.74	*dd*	15.4; 12.3		1.80	*dd*	14.6; 12.6		1.77	*dd*	15.0; 11.2
12	69.4	5.49	*dd*	12.3; 3.1	69.5	5.56	*dd*	12.6; 3.1	70.0	5.36	*dd*	11.2; 6.1
13	125.0	–			126.4	–			124.2	–		
14	108.5	6.40	*dd*	1.8; 0.9	109.4	6.53	*dd*	1.9; 0.8	108.5	6.39	*dd*	1.7; 1.0
15	143.5	7.37	*dd*	1.8; 1.7	143.9	7.56	*brs*		143.7	7.40	*dd*	1.7; 1.7
16	139.7	7.45	*ddd*	1.7; 0.9; 0.5	140.4	7.59	*brs*		139.6	7.44	*dd*	1.7; 1.0
17	171.8	–			171.8	–			174.2	–		
18	179.2	–			178.3	–			180.0	–		
20	22.0	1.10	*s*		21.9	0.98	*s*		18.3	0.98	*s*	
OH	–	2.30	*brs*		–	5.69	*s*		–	2.35	*brs*	

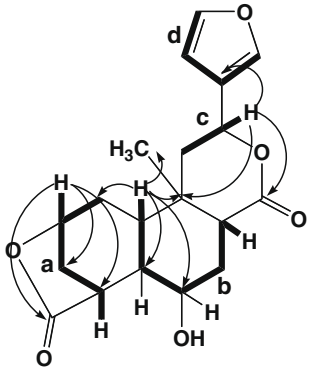

Fig. 4. Sub-structures (**a**–**d**) and key COSY (bold bonds) and HMBC (arrows) correlations for 8-epidiosbulbin E (**3**).

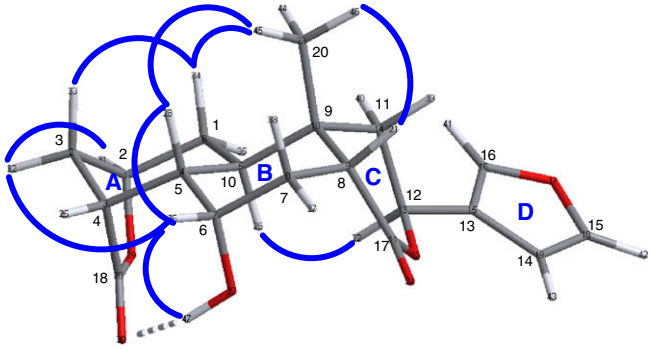

Fig. 5. Selected NOESY correlations (blue lines) and proposed 3D-structure for 8-epidiosbulbin E (**3**). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

isolated from *D. bulbifera* L. var. *sativa* (Murray et al., 1984; Shriram et al., 2008). Diosbulbin E (**5**) has been also isolated in the present work and its absolute configuration was established earlier by circular dichroism (Ida et al., 1978a). However, no detailed NMR data for **5** has so far appeared in the literature and we now report its complete ^{1}H and ^{13}C NMR assignments (Table 2). Compared with 8-epidiosbulbin E (**3**), C-7, C-8 and C-20 of **5** (in $CDCl_3$) were shifted upfield by −0.8, −4.5 and −3.7 ppm, whereas C-9, C-10, C-11 and C-17 were shifted downfield by +1.3, +9.3, +2.7 and +2.4 ppm (Table 2). Again, a change from H-8β to H-8α results in a shift of about δ_C +10 ppm for C-10. The NMR data for 6-*O*-coumaroyl derivative of diosbulbin E recently isolated from *D. bulbifera*, are in agreement with the above observation (Wang et al., 2009). Thus **3** is the 8β-epimer of diosbulbin E (**5**).

Compound **4**, another optically active colourless amorphous solid, $[\alpha]_D^{20}$ −20° (*c* 0.3, $CHCl_3$) with molecular formula $C_{19}H_{22}O_6$ exhibited the protonated molecular ion $[M+H]^+$ at *m*/*z*: 347.1403 (calc. 347.1493 for $C_{19}H_{23}O_6$). All the 2D-NMR data indicated that **4** had the same planar structure as diosbulbin G (**7**) (Ida et al., 1978b). The junction of the A- and B-chair rings was *trans* due to the *trans*-diaxial disposition of H-5 and H-10 ($^3J_{H\text{-}5/H\text{-}10}$ = 12.2 Hz). NOEs were depicted between equatorial H-2 and its geminal-hydroxyl and its vicinal protons H-1eq, H-1ax, H-3eq and H-3ax (Fig. 6). NOEs were also observed between β-face protons (H-6 with H-4eq, H-5ax and H-7ax; H-4eq with H-5ax and H-3ax; CH_3-20 with H-1ax, H-5, H-8) and between α-face protons (H-12 with H-10). Thus compound **4** differed from diosbulbin G in the configuration of C-8, H-8 being β in **4** instead of α. The full ^{13}C NMR assignment of diosbulbin G (**7**) in Pyr-d_5, has been published by Ternai and co-workers (Lentini et al., 1986). It is interesting that the chemical shift of C-10 in **4** where H-8 is β, is more than 10 ppm downfield ($\Delta\delta_C$ +13.5) from its shift in diosbulbin G where it is α. The β-orientation of H-8, which implies the α-orientation of the C-17 carbonyl group in 8-epidiosbulbin G (**4**), induces steric constraints which are responsible for this shift, is also true for antadiosbulbin B and 8-epidiosbulbin E. Compound **4** may by thought of as an isomer of 8-epidiosbulbin E (**3**) from which it differs in the direction of cyclization of the C-18 carboxyl which forms a γ-lactone involving C-6 instead of C-2.

Dioscorea species are known to biosynthesize diterpenoids of the clerodane or of the 19-norclerodane types which are responsible of the bitter taste, in addition to alkaloids such as dioscorine and steroid sapogenins responsible for their toxicity (Sautour

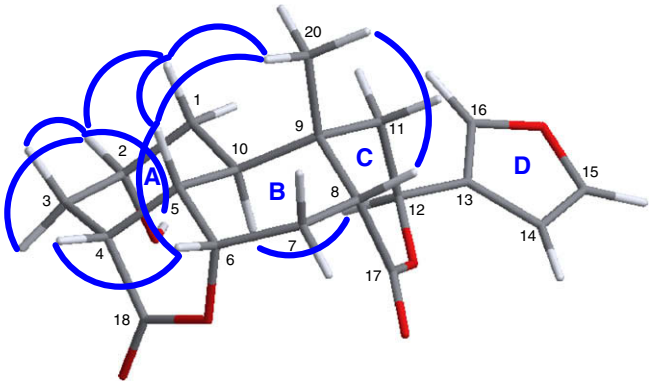

Fig. 6. Selected NOESY correlations (blue lines) and proposed 3D-structure for 8-epidiosbulbin G (**4**). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2007). The bitter taste of some Nepalese species has been assigned to bitter components identified as furanoid norditerpenes, especially to diosbulbin B (Bhandari and Kawabata, 2005). Only diterpenoids were isolated from *D. antaly* in the course of our work and no saponins were detected, which suggests that *D. antaly* and *D. bulbifera* are close taxonomically and distinct from other *Dioscorea* species. Only one saponin, a pennogenin glycoside, has been isolated from *D. bulbifera* var. *sativa* (Teponno et al., 2006), all other phytochemical investigations on *D. bulbifera* have not produced any steroid sapogenins (Wang et al., 2009). The water-soluble extract of the tubers of *D. antaly* was slightly toxic when medaka fishes were incubated in a medium containing this extract, with LD_{50} around 0.86 mg/ml (Rakotobe et al., 2010).

In summary, two C-8 epimers antadiosbulbins A and B, as well as two new 19-norclerodanes, 8-epidiosbulbins E and G have thus been isolated from the tubers of *D. antaly* in addition to the known diosbulbin E. A general scheme for the biosynthesis of *Dioscorea* diterpenes from geranyl–geranyl diphosphate might lead to a trihydroxylated tri-carboxylic acid intermediate, in which the asymmetric center at C-8, α to an acid function might undergo racemization (Fig. 7). Cyclization could then yield antadiosbulbins A and B. After decarboxylation, the resulting 19-norclerodane might further be cyclized to yield either diosbubin E and 8-epidiosbulbin E by forming of a lactone ring between the carboxylic function at C-18 and the alcohol at C-2 or diosbulbin G and its epimer at C-8 by cyclization with the alcohol group at C-6.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a Perkin Elmer model 341 polarimeter at 20 °C and the $[\alpha]_D$ values are given in deg cm^2 g^{-1}. IR spectra were taken on a Shimadzu FTIR-8400S spectrophotometer. Mass spectra data were recorded using on an API Q-STAR Pulsar I of Applied Biosystems. ^{13}C NMR spectra were recorded on an AC 300 BRUKER spectrometer operating at 75.47 MHz (for ^{13}C). ^{1}H and 2D-NMR spectra were on an Avance-400 BRUKER spectrometer operating at 400.13, equipped with ^{1}H-broad-band reverse gradient probe head. The ^{1}H and ^{13}C NMR chemical shifts are given in ppm relative to TMS, with coupling constants (*J*) reported in Hz. For the HMBC experiments, the delay (1/2*J*) was 70 ms and for the NOESY experiments the mixing time was 150 ms. TLC was carried out on precoated Si gel 60 F_{254} plates (Merck). Spots were detected under UV (254 and 366 nm) before spraying with phosphomolybdic acid solution in EtOH or Liebermann–Burchard reagent or vanillin–sulfuric solution followed by heating the plate at 110 °C. Column chromatography was performed on 200–400 mesh silica gel 60 (Merck). Preparative medium-pressure liquid chromatography (MPLC) was performed with a pump K-120 (Knauer) and Flashsmart cartridges (Si and C-18 gels 20–40 μm, AIT, France).

3.2. Plant material

Tubers of *D. antaly* Jum. and H. Perrier were collected in May 2004 in the Menabe region near Morondava (Beroboka) located 600 km South-West Antananarivo (Madagascar). The plant was identified by Prof. V.H. Jeannoda and voucher specimens (MT 066 to MT 069) were deposited at the Herbarium of the Department of Botany, University of Antananarivo.

3.3. Extraction and isolation

Tubers were washed, cut into small pieces, air-dried and milled. The powdered plant material (546 g) was extracted successively with cyclohexane (4 × 250 ml) and MeOH (4 × 250 ml) at rt and concentrated to dryness under reduced pressure. The crude methanolic extract (15.7 g) was partitioned between EtOAc (200 ml) and water (300 ml) to yield EtOAc (6.2 g) and aqueous (9 g) extracts after evaporation of solvents. The EtOAc-soluble extract (6.2 g) was further chromatographed over silica gel column (300 g) eluted with a cyclohexane/EtOAc gradient of increasing polarity, followed by EtOAc–MeOH gradient to afford 27 fractions (500 ml each). Fractions 11, 12 and 13 (290 mg) which showed similar profiles on TLC were grouped together and purified on Sephadex LH-20 eluted with MeOH/CH_2Cl_2:90/10 to yield 3,7-dihydroxy-2,4-dimethoxyphenanthrene (17 mg).

Purification of fraction 16 (560 mg) by CC on Sephadex LH-20 eluted with MeOH and then by MPLC yielded dihydroxypiceatannol (25 mg), piceatannol (20 mg), compound **1** (7 mg) and a mixture of compound **1** and **2** (161 mg). This mixture was further separated by isochratic MPLC using CH_2Cl_2/EtOAc:98/2 at flow rate of 1 ml/min and furnished further 25 mg of compound **2**. Fraction 17 (1.18 g) was subjected to repeated column chromatography on Sephadex LH-20 eluted with MeOH to furnish 23 sub-fractions (90 ml each) from which sub-fractions 17-7 and 17-23 contained pure catechin (150 mg) and cassigarol D (162 mg), respectively. Flash separation of sub-fraction 17-3 on MPLC with gradient elution CH_2Cl_2/MeOH:99/1 afforded diosbulbin E (**5**, 16 mg), antabiosbulbins A (**1**, 3 mg) and B (**2**, 3 mg). Purification of sub-fraction 17-16 (15 mg) on a C-18 reverse phase flash column (3 g) on MPLC using ACN/H_2O + 0.1% TFA (20:80) yielded scircupsin B (1 mg).

Recrystallisation of fraction 19 (1473 mg) in MeOH yielded 8-epidiosbulbin E (**3**, 534 mg) and that of fraction 20 (559 mg) yielded 8-epidiosbulbin G (**4**, 26 mg). By successive repurification on Sephadex LH-20 followed by MPLC of fraction 21 afforded (3-*O*-[β-D-glucose-(6 → 1)-α-L-rhamnose]-kaempferol) (32 mg). Kaempferol (12 mg) and quercetin (6 mg) were obtained from fraction 22 (313 mg) by chromatography on Sephadex LH-20 eluted with MeOH.

3.3.1. Antadiosbulbin A (*1*)

Colourless amorphous solid; $[\alpha]_D^{20}$ −45° (*c* 0.7, $CHCl_3$); HR-ESI-MS positive mode, *m/z*: 405.1519 $[M+H]^+$, $C_{21}H_{25}O_8$ (calc.: 405.1548); IR ($CHCl_3$) ν_{max} (cm^{-1}): 3447, 3150, 2953, 2928, 1740, 1506, 1448, 1368, 1265, 1157, 1115, 1082; ^{1}H and ^{13}C NMR data, see Table 1.

3.3.2. Antadiosbulbin B (*2*)

Colourless amorphous solid; $[\alpha]_D^{20}$ −28° (*c* 0.4, $CHCl_3$); HR-ESI-MS positive mode, *m/z*: 405.1527 $[M+H]^+$, $C_{21}H_{25}O_8$ (calc.: 405.1548); IR ($CHCl_3$) ν_{max} (cm^{-1}): 3447, 3150, 2953, 1750, 1734, 1505, 1464, 1437, 1204, 1113; ^{1}H and ^{13}C NMR data, see Table 1.

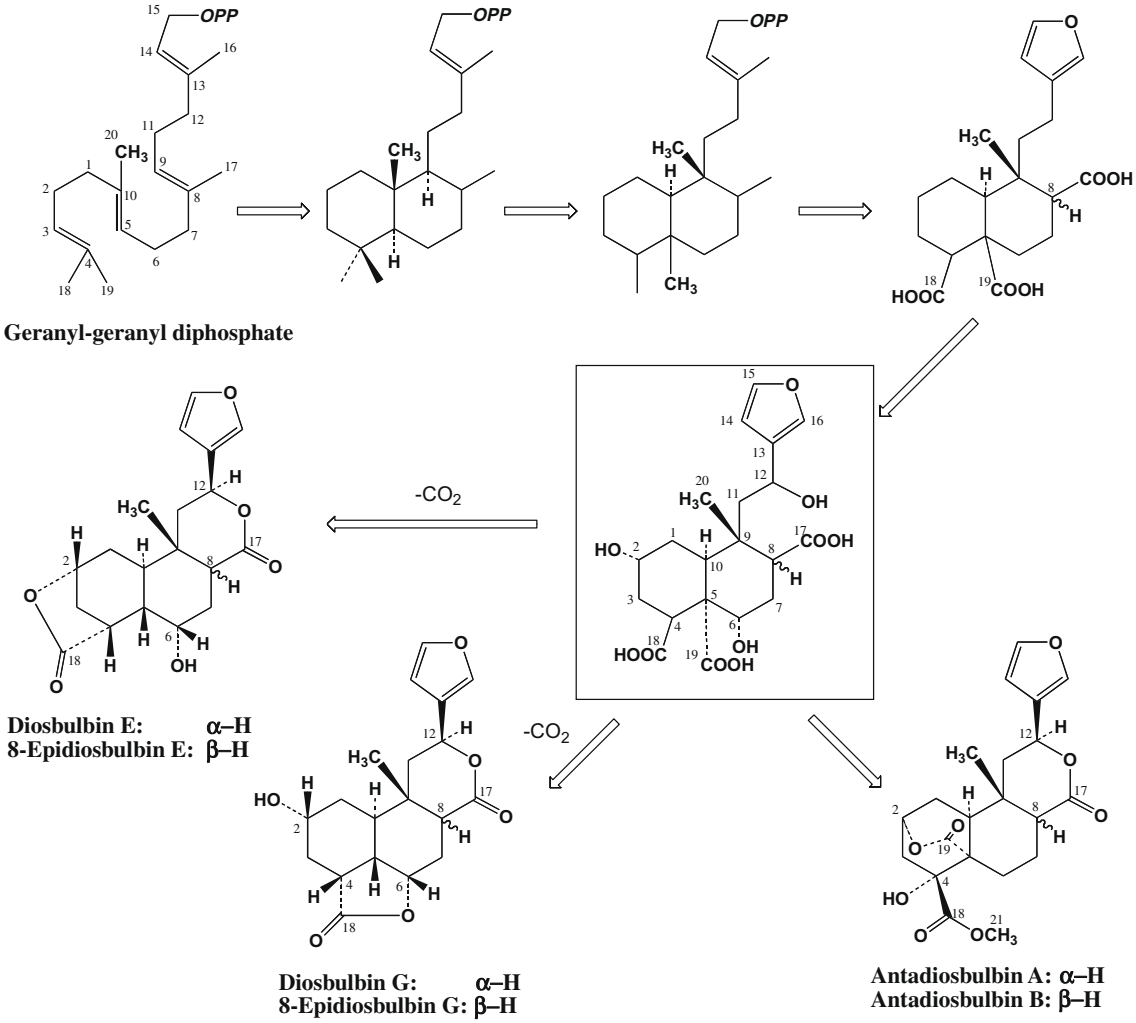

Fig. 7. Plausible pathway for the biosynthesis of the *Dioscorea* diterpenoids.

3.3.3. 8-Epidiosbulbin E (**3**)

Colourless amorphous solid; $[\alpha]_D^{20}$ −16° (*c* 0.3, $CHCl_3$); HR-ESI-MS positive mode, *m*/*z*: 347.1485 $[M+H]^+$, $C_{19}H_{23}O_6$ (calc.: 347.1493); IR ($CHCl_3$) ν_{max} (cm^{-1}): 3636, 3150, 2926, 1744, 1725, 1505, 1204, 1109; ^1H and ^{13}C NMR data, see Table 2.

3.3.4. 8-Epidiosbulbin G (**4**)

Colourless amorphous solid; $[\alpha]_D^{20}$ −20° (*c* 0.3, $CHCl_3$); HR-ESI-MS positive mode, *m*/*z*: 347.1497 $[M+H]^+$, $C_{19}H_{23}O_6$ (calc.: 347.1493); ^1H and ^{13}C NMR data see Table 3.

3.3.5. Diosbulbin E (**5**)

Colourless amorphous solid; HR-ESI-MS positive mode, *m*/*z*: 347.1496 $[M+H]^+$, $C_{19}H_{23}O_6$ (calc.: 347.1493); IR ($CHCl_3$) ν_{max} (cm^{-1}): 3484, 3150, 2930, 1769, 1718, 1503, 1298, 1209, 1147, 1076, 1024; ^1H and ^{13}C NMR data, see Table 2.

3.3.6. 8-Epidiosbulbin E acetate (**6**)

Compound **3** (5 mg) was added to a mixture of 1 ml of pyridine and 1 ml of Ac_2O at room temperature and stirred for 24 h. To accelerate the acetylation, 7 mg of dimethylaminopyridine (DMAP) were added to the reaction mixture and stirring was maintained for 24 h; then 10 ml of aqueous HCl (5%) were added to the reaction mixture. After extraction with CH_2Cl_2 and washing with

Table 3
^{13}C and ^1H NMR data for 8-epidiosbulbin G (**4**) in Pyr-d_5 (400.13 MHz for ^1H).

Position	8-Epidiosbulbin G (**4**)			
	δ_C	δ_H	*Mult.*	*J* (Hz)
1eq	30.6	1.88	*dm*	12.7
ax		1.16	*ddd*	12.7; 12.2; 2.2
2	64.5	4.35	*m*	
3eq	30.3	2.54	*dm*	14.8
ax		1.79	*ddd*	14.8; 7.8; 3.7
4	40.5	2.85	*ddd*	7.8; 6.2; 1.5
5	38.5	2.22	*ddd*	12.2; 6.2; 4.9
6	76.2	4.48	*ddd*	5.9; 4.9; 4.0
7eq	25.8	3.00	*ddd*	15.3; 5.9; 4.0
ax		2.18	*ddd*	15.3; 5.9; 5.9
8	46.3	2.51	*dd*	5.9; 5.9
9	33.5	–		
10	28.7	2.65	*ddd*	12.2; 12.2; 2.2
11eq	40.4	2.23	*dd*	14.6; 2.8
ax		1.83	*dd*	14.6; 12.5
12	70.2	5.82	*dd*	12.5; 2.8
13	126.1	–		
14	109.3	6.51	*dd*	2.0; 1.0
15	144.0	7.54	*dd*	2.0; 1.8
16	140.4	7.59	*brs*	
17	172.1	–		
18	178.2	–		
20	21.6	0.97	*s*	
2-OH	–	5.10	*s*	

10 ml H_2O the organic layers were dried over Na_2SO_4 and concentrated under vacuum. Purification on silica gel column chromatography (cyclohexane/EtOAc:95/5) afforded 3 mg of 8-epidiosbulbin E acetate (**6**).

Compound **6**: colourless amorphous solid; ESI-MS positive mode, *m*/*z*: 389.1607 $[M+H]^+$, $C_{21}H_{25}O_7$ (calc.: 389.1599); ^{13}C NMR ($CDCl_3$): 28.2 (C-1), 76.4 (C-2), 38.6 (C-3), 42.0 (C-4), 41.2 (C-5), 69.0 (C-6), 26.8 (C-7), 45.6 (C-8), 34.5 (C-9), 31.6 (C-10), 39.7 (C-11), 69.6 (C-12), 124.9 (C-13), 108.6 (C-14), 143.9 (C-15), 139.9 (C-16), 170.8 (C-17), 175.7 (C-18), 21.9 (C-20), 170.7 (C-21), 20.9 (C-22). ^1H NMR ($CDCl_3$): 2.10 (1H, *brddd* 13.3, 6.0, 5.0, H-1a), 1.46 (1H, *ddd* 13.3, 12.0, 1.1, H-1b); 4.85 (1H, *dd* 5.5, 4.7, H-2); 2.51 (1H, *dddd* 11.4, 5.7, 5.5, 1.7, H-3a); 1.77 (1H, *d* 11.5, H-3b); 2.57 (1H, *dd* 5.8, 1.6, H-4); 1.92 (1H, *ddd* 12.3, 2.0, 2.0, H-5); 5.15 (1H, *brddd* 2.8, 2.7, 2.7, H-6); 2.83 (1H, *ddd* 15.1, 3.1, 2.1, H-7a); 1.87 (1H, *ddd* 15.1, 6.2, 2.6, H-7b); 2.31 (1H, *dd* 6.2, 2.1, H-8); 2.60 (1H, *ddd* 12.3, 12.0, 4.0, H-10); 2.11 (1H, *dd* 14.7, 3.3, H-11a); 1.78 (1H, *dd* 14.7, 12.6, H-11b); 5.51 (1H, *dd* 12.6, 3.3, H-12); 6.41 (1H, *brdd* 1.9, 0.8, H-14); 7.40 (1H, *brdd* 1.9, 1.6, H-15); 7.48 (1H, *ddd* 1.6, 0.8, 0.8, H-16); 1.16 (1H, *s*, H-20); 1.99 (1H, *s*, H-22).

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