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Sesquiterpene lactones from the endemic Cape Verdean *Artemisia gorgonum*

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ABSTRACT

Leaves and flowers of *Artemisia gorgonum* (Asteraceae) collected in Fogo, Cape Verde islands, were phytochemically investigated and resulted in isolation and characterization of three guaianolides **1**, **2**, **5**, and a secoguaianolide **4**, in addition to eight known guaianolides **6**–**11** and two known germacranolides **12**, **13**. Structures were elucidated by 1D and 2D NMR experiments. Careful examination of the ^{13}C NMR spectrum led to revision of the structure of a previously described guaianolide from **2** to **3**. Most compounds exhibited mild antiplasmodial activities, ridentin (**13**) being the most interesting with an IC_{50} of 3.8 ± 0.7 μg ml^{-1} against *Plasmodium falciparum* FcB1 and weak cytotoxicity in a vero cell line (IC_{50} 71.0 ± 3.9 μg ml^{-1}).

1. Introduction

The large Asteraceae family contains 25,000–30,000 species belonging to over 1000 genera. The chemistry of members of this family has been studied intensively and more than 28,000 substances have been identified so far (Quan-Xiang et al., 2006). The genus *Artemisia*, usually represented by small herbs and shrubs, is one of the largest and most widely distributed genera of the Asteraceae. Different families of sesquiterpene lactones have been reported from this genus, eudesmanolides and guaianolides being the most common (Tan et al., 1998). Among the large therapeutical applications of this class of compounds, artemisinin from *Artemisia annua*, is undoubtedly a lead compound as a potent antimalarial agent (White, 2008). In combination with other therapeutics, artemisinin is nowadays widely used to fight malaria and it has been qualified by the WHO as 'hope for the future' in 2001.

The endemic Cape Verdean *Artemisia gorgonum* Webb is used in local folk medicine as a treatment for symptoms associated with fever. The fact that this species had never been studied previously prompted us to undertake a full chemical study in the search for new antimalarial compounds. Its chemical investigation afforded the new 5α-hydroxy-6-epileukodin (**1**), 1,10-dioxo-1,10-deoxy-1,10-secogorgonolide (**4**), and 3β,4β-epoxy-1β,10β-epiarborescin (**5**) together with six known sesquiterpene lactones 5α-hydroxyleukodin (**6**) (Balboul et al., 1997), arborescin (**7**) (Bates et al., 1963; Ando et al., 1982), 2α-hydroxyarborescin (**8**) (Bohlmann et al., 1985), 1β,10β-epoxy-2α-hydroxykauniolide (**9**) (de Gutierrez et al., 1990), 1α,4α,10α-trihydroxy-5α,11βH-guaia-2-en-12,6α-olide (**10**), 1β,4α,10α-trihydroxy-5α,11βH-guaia-2-en-12,6α-olide (**11**) (Bohlmann et al., 1985), ridentin (**12**) (Irwin et al., 1969), and hanphyllin (**13**) (Mata et al., 1985) (Fig. 1). The previously proposed structure for gorgonolide (**2**) (Bohlmann et al., 1985) was revised to 1β,10α-dihydroxy-1,10-deoxygorgonolide (**3**). Modest antiplasmodial bioactivities were associated with most of the isolated compounds.

2. Results and discussion

2.1. Isolation

The EtOH extract of leaves and flowers of *A. gorgonum* was partitioned between CH_2Cl_2/H_2O and the CH_2Cl_2 extract exhibiting antimalarial activity (IC_{50} 3.6 μg ml^{-1}) was subjected to normal phase flash chromatography (SiO_2). The most bioactive fractions were studied further by being subjected to a normal phase column chromatography fractionation followed by C_{18} reverse-phase HPLC purification of the bioactive fractions which allowed us to isolate the sesquiterpene lactones **1**–**13** (Fig. 1).

2.2. Structure determination

Compound **1** was obtained as a colourless gum. The molecular formula $C_{15}H_{18}O_4$ was determined by HRESI-MS analysis ($[M+H]^+$ 263.12787 Δ 1.8 uma). The IR spectrum showed absorption bands

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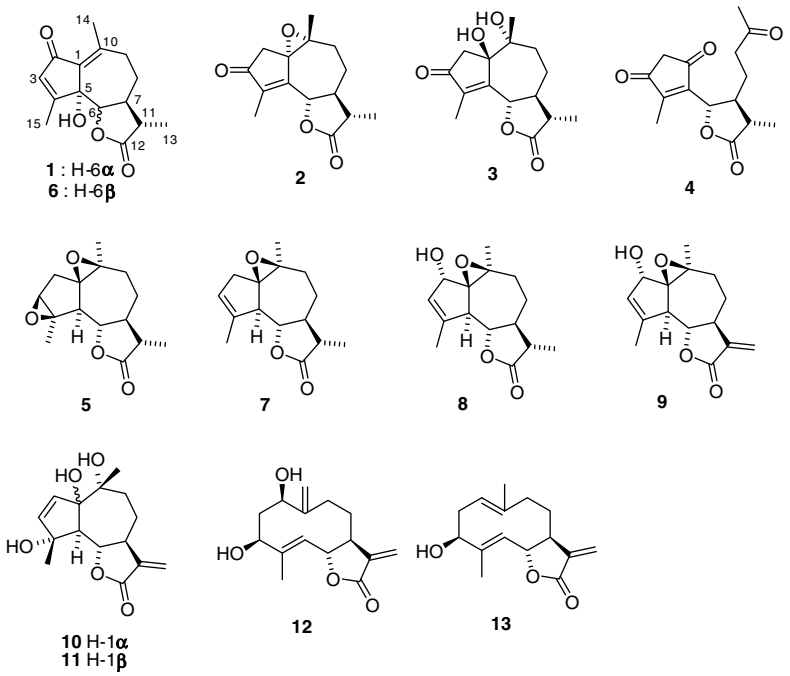

Fig. 1. Structures of compounds **1**–**13**.

at 3428, 1773, and 1633 cm^{-1} assignable to hydroxyl, lactone, and unsaturated ketone functions respectively. From the seven unsaturations related to the molecular formula of **1**, three were assigned to a bisunsaturated ketone as suggested by characteristic ^{13}C NMR signals at δ 196.5 (C-2), 131.5 (C-1), 133.8 (C-3), 154.1 (C-10), 171.3 (C-4) (Table 2). The quaternary carbon resonating at δ 180.2 (C-12) confirmed the lactone moiety and the presence of three cycles was then deduced. The ^1H NMR spectrum revealed three methyl groups: one placed on a saturated carbon at δ 1.18 (3H, *d*, *J* = 7.0 Hz, H_3-13) and two on an unsaturation at δ 2.02, 2.21 (3H each, both *s*, H_3-15, H_3-14) (Table 1). The molecular formula and all these NMR data clearly indicated that **1** was a sesquiterpene lactone similar to leukodin (Martinez et al., 1988). The lack of H-5 suggested a 5-hydroxyleukodin but because NMR chemical shifts were slightly different from those described the assessment of the relative stereochemistry was undertaken (Jakupovic et al., 1992; Balboul et al., 1997). The low coupling constant $J_{6,7}$ = 7.5 Hz indicated that H-6 and H-7 were *cis*. Because of the downfield shift of H-6 at δ_H 4.70 ppm and also a strong H-O/H-6 NOESY correlation, H-O and H-6 were also *cis* to each other. Compound **1** was first thought to be 5α-hydroxyachillin isolated by Bohlmann et al. (1982) but H-6/H-7/H_3-13 NOESY correlations indicated that the methyl substituent at C-11 was α oriented as in leukodin, thus the new compound **1** was then named 5α-hydroxy-6-epileukodin. Isolation of the known 5α-hydroxyleukodin (**6**) from the same extract allowed us to compare NMR data for both compounds and therefore to confirm the structure of **1** (Balboul et al., 1997, Tables 1 and 2).

Compound **2** was obtained as a colourless gum. The molecular formula $C_{15}H_{18}O_4$ was determined by HRESI-MS analysis ($[M+H]^+$ 263.12778 Δ −0.03 uma). The IR spectrum showed absorption bands at 1770 and 1710 cm^{-1} assignable to lactone and unsaturated ketone functions. The ^{13}C NMR data revealed the presence of a monosubstituted ketone at δ 203.0 (C-3), 141.4 (C-4), 158.9 (C-5) (Table 2). The last unsaturation was due to an epoxide function at C-10/C-1 evidenced by ^{13}C NMR signals at δ 65.9 (C-10) and 68.2 (C-1). Low downfield doublets at δ 2.73 (1H, *d*, *J* = 18.5 Hz, H-2a) and δ 2.59 (1H, *d*, *J* = 18.5 Hz, H-2b) were characteristic of a methylene at C-2. Similar chemical shifts were observed for an oxidized derivative of arborescin isolated by Bohlmann et al. (1985). ^1H and ^{13}C NMR data of the known compound were however slightly different from those of **2** but identical with a more polar compound **3** also isolated in the course of

Table 1
^1H NMR data of compounds **1** and **6** in DMSO-d_6 and **2**–**5** in $CDCl_3$ (500 MHz).

No.	1	6	2	3	4	5
2a	–	–	2.73 *d* (18.5)	2.57 *d* (18.0)	2.96 *d* (2.5)	2.33 *dd* (15.5, 1.0)
2b			2.59 *d* (18.5)	2.48 *d* (18.0)		1.90 *d* (15.5)
3	6.05 *q* (1.5)	6.14 *q* (1.5)	–	–	–	3.29 *br s*
5		–	–	–	–	2.36 *d* (10.5)
6	4.70 *d* (7.5)	3.95 *dd* (10.0, 0.5)	4.84 *br d* (10.5)	5.27 *br d* (11.0)	5.06 *d* (9.0)	4.12 *t* (10.5)
7	2.61 *td* (7.5, 3.5)	2.56 *dtd* (12.0, 10.0, 3.5)	1.62 *m*	1.76 *m*	2.29 *m*	1.30 *m*
8a	2.45 *m*	1.92 *br d* (12.5)	1.86 *dt* (13.5, 4.0)	1.88 *m*	1.86 *m*	1.59 *br d* (13.5)
8b	1.41 *m*	1.37 *q* (12.5)	1.60 *m*	1.78 *m*	1.83 *m*	1.41 *br q* (12.5)
9a	2.79 *ddd* (18.0, 6.0, 4.0)	2.77 *t* (13.0)	2.36 *m*	2.15 *m*	2.51 *m*	2.10 *ddd* (15.5, 5.5, 2.5)
9b	2.21 *ddd* (18.0, 10.0, 4.0)	2.06 *dd* (13.0, 5.5)	2.02 *m*	1.50 *m*	2.49 *m*	1.84 *ddd* (15.5, 12.5, 3.0)
11	2.36 *dq* (7.5, 7.0)	2.39 *dq* (13.0, 7.0)	2.30 *dq* (11.5, 7.0)	2.38 *dq* (12.0, 6.5)	2.43 *dq* (10.5, 7.0)	2.19 *dq* (12.0, 7.0)
13	1.18 *d* (7.0)	1.09 *d* (7.0)	1.26 *d* (7.0)	1.26 *d* (6.5)	1.35 *d* (7.5)	1.18 *d* (7.0)
14	2.21 *s*	2.33 *s*	1.43 *s*	1.29 *s*	2.13 *s*	1.28 *s*
15	2.02 *d* (1.5)	2.12 *d* (1.5)	2.03 *d* (1.5)	1.87 *br s*	2.12 *s*	1.64 *s*
OH	5.90 *s*	5.90 *s*				

Table 2
^{13}C NMR data of compounds **1**–**6** (125 MHz, $CDCl_3$).

Position	1	6	2	3	4	5
1	131.5	134.5	68.2	80.6	198.5	69.3
2	196.5	195.2	40.8	46.8	41.2	36.6
3	133.8	134.6	203.0	205.2	199.4	62.3
4	171.3	173.3	141.4	137.6	158.6	65.5
5	79.1	79.1	158.9	165.1	152.9	50.0
6	87.2	86.9	82.2	78.1	75.5	80.5
7	43.1	47.6	50.2	52.7	47.8	54.4
8	23.2	27.3	24.5	23.4	25.4	22.7
9	34.3	35.3	33.6	34.5	40.3	33.6
10	154.1	155.4	65.9	74.2	207.0	59.4
11	39.4	41.2	41.4	42.9	41.4	41.3
12	180.2	178.7	177.3	177.8	177.7	178.7
13	12.8	12.2	12.9	13.0	15.2	12.5
14	20.9	22.2	25.7	26.4	30.1	22.9
15	14.8	15.5	9.3	8.6	9.7	18.8

this work (Tables 1 and 2). We further observed a slow transformation of **2** into **3** in the NMR tube. Due to the different polarities of both compounds, we assumed that **3** was formed by hydrolysis of **2** resulting in the opening of the instable epoxide at C-1/C-10. This result was confirmed by the key H_3-14/C-1 and H_3-14/C-10 HMBC correlations which indicated clear downfield chemical shifts of C-1 (δ 80.6) and C-10 (δ 74.2) for compound **3** which is characteristic of a vicinal diol and not an epoxide. In fact, the compound isolated by Bohlmann et al. (1985) was diol **3** and not epoxide **2**. Looking now at the stereochemistry which appeared troublesome in previous studies (Collado et al., 1987), key H_3-14/H-6/H-9a NOESY correlations placed the epoxide of **2** in an α-orientation and **2** was named gorgonolide. The relative stereochemistry assigned to **3**, 1β,10α-dihydroxy-1,10-deoxygorgonolide, was based on H_3-14/H-6/H-11/H-9a NOESY correlations.

Compound **4** was obtained as a colourless gum. The molecular formula $C_{15}H_{18}O_5$ was determined by HRESI-MS analysis ($[M+H]^+$ 279.12241 Δ 1.05 uma). The IR spectrum showed absorption bands at 1775 and 1707 cm^{-1} also assignable to lactone and conjugated ketone functions respectively. ^{13}C NMR data of **4** indeed revealed the presence of three keto groups at δ 198.5 (C-1), 199.4 (C-3), 207.0 (C-10), two of them being conjugated (δ 152.9 (C-5), 158.6 (C-4)). A new doublet (δ 2.96, 2H, J = 2.5 Hz) was assigned to H_2-2 and the key H_2-2/C-1 and H_2-2/C-3 HMBC correlations suggested presence of a cyclopentenedione. The NMR frequencies of the H-8 and H-9 methylenes appeared almost equivalent and then confirmed an opening of the seven membered ring leading to a methylketone and a 1,10-secoguaianolide. Comparison with similar compounds described in the literature allowed us to confirm this assumption (Jakupovic et al., 1988; Tan et al., 1991). Compound **4** was named 1,10-dioxo-1,10-deoxy-1,10-secogorgonolide and could be formed biosynthetically by oxidative cleavage of the vicinal diol of **3**.

Compound **5** was obtained as a colourless gum. The molecular formula $C_{15}H_{20}O_4$ was determined by HRESI-MS analysis ($[M+H]^+$ 265.14337 Δ −0.24 uma). Key ^1H NMR signals at δ 4.12 (1H, *t*, J = 10.5 Hz, H-6) and δ 2.36 (1H, *d*, J = 10.5 Hz, H-5) indicated that **5** belongs to the large arborescin family (Bohlmann et al., 1985). We deduced the presence of two epoxides at C-1/C-10 and C-3/C-4, because vinylic protons or carbons were absent from the ^1H and ^{13}C NMR spectra but also because of the presence of four characteristic ^{13}C NMR signals at δ 59.4 (C-10), 62.3, (C-3), 65.5 (C-4), and 69.3 (C-1). The only previously isolated compound containing two epoxides at these positions was isoepoxyestafiatin (Bohlmann and Zdero, 1972). The stereochemistry of compound **5** was assigned by comparison with compounds synthesized in the course of the total synthesis of isoepoxyestafiatin (Ando and Yoshimura, 1993) and confirmed by the key H-5/H_3-15, H-6/H-8a, and H-8b/H_3-14 NOESY correlations. The configuration of the C-3/C-4 epoxide was thus opposite to that naturally occurring estafiatin derivatives but identical with the intermediates in the synthesis of estafiatin derivatives (Ando and Yoshimura, 1993).

The ability of 11 compounds to inhibit the growth of *Plasmodium falciparum* strain *in vitro* was evaluated following the method of Desjardins et al. (1979). The results provided in Table 3 indicate that arborescin (**7**) is the most promising compound. Ridentin (**12**) and hanphyllin (**13**) also exhibited interesting bioactivity which may be attributed to the exomethylene group of the lactone function. These compounds were further evaluated for their cytotoxicity on mammalian VERO cell line growth using the dimethylthiazolyldiphenyl tetrazolium (MTT)-based assay (Prado et al., 2007). All compounds were only weakly cytotoxic (IC_{50} > 50 μg ml^{-1}). When compared with their antiplasmodial activity this suggests a selective effect against *P. falciparum* growth. Further structure–activity relationship studies will be carried out in order to identify the biologically important structural features of this series of compounds as *in vivo* assays will be made in order to determine their potential as new anti-malarial drugs.

3. Experimental

3.1. General experimental techniques

Preparative HPLC was performed on a Waters equipment with a 600E pump, an autoinjector 417 and Photodiode Array Detector 996 coupled with a SEDEX 55 Evaporative Light Scattering Detector (ELSD). NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer. Chemical shift values (δ) were reported in parts per million (ppm) relative to appropriate internal solvent standard and coupling constants (J-values) are given in Hertz. ESI Mass spectra were recorded on a Bruker Esquire 3000 Plus spectrometer. HRESIMS spectra were recorded on a LTQ Orbitrap hybrid mass

Table 3
Antimalarial activity of compounds **2**–**10** and **12**–**13**.

Compound	IC_{50} *P. falciparum* FcB1 (μg ml^{-1}) ± SD (n = 3)	IC90 *P. falciparum* FcB1 (μg ml^{-1}) ± SD (n = 3)	IC_{50} Vero cells (μg ml^{-1}) ± SD (n = 3)
2	15.5 ± 7.6	33.3 ± 9.1	87.9 ± 8.9
3	19.2 ± 2.5	19.2 ± 6.38	>100
4	12.5 ± 2.5	22.9 ± 1.6	63.8 ± 7.5
5	13.3 ± 3.8	27.0 ± 2.8	87.5 ± 8.6
6	> 25	ND	> 100
7	3.8 ± 0.7	9.8 ± 4.5	71.0 ± 3.9
8	> 25	ND	> 100
9	5.8 ± 1.5	11.8 ± 1.1	44.3 ± 14.8
10	8.6 ± 2.0	16.1 ± 2.9	ND
12	5.4 ± 1.2	13.4 ± 6.0	67.37 ± 7.8
13	2.3 ± 0.9	5.4 ± 0.3	26.4 ± 5.4
Chloroquine	0.02 ± 0.01	0.05 ± 0.02	>100

spectrometer (Thermo). IR spectra were recorded on a Nicolet 360FT-IR spectrophotometer and UV spectra on a Varian CARY 300. Specific rotations were measured on a Jasco P1010 polarimeter. For biological assays see references of Prado et al. (2007) and Desjardins et al. (1979).

3.2. Plant material

The leaves and flowers of *A. gorgonum* were collected in Fogo, Cape Verde islands in March 2006. Taxonomical identification was performed by Izildo Gomes. A voucher specimen was deposited in the Herbarium of INIDA, Santiago, Cape Verde.

3.3. Extraction and isolation

The air-dried aerial parts of *A. gorgonum* (140 g) were extracted at r.t. with EtOH (2 × 0.5 l) for 10 days. The combined extract was concentrated *in vacuo* to give 38 g of a residue. 20 g of the residue were then partitioned between CH_2Cl_2 and H_2O (4 × 250 ml) to afford, after solvent removal, the organic fraction (7.3 g). The CH_2Cl_2 fraction was fractionated by flash chromatography over silica gel (100 g) with solvent mixtures of increasing polarities: cyclohexane (100%, 0.5 l); cyclohexane/CH_2Cl_2 (8:2, 0.5 l); cyclohexane/CH_2Cl_2 (5:5, 0.5 l); CH_2Cl_2 (0.5 l); CH_2Cl_2/EtOAc (5:5, 0.5 l); EtOAc (0.5 l); EtOAc/MeOH (5:5, 0.5 l); MeOH (0.5 l). Evaporation of the CH_2Cl_2/EtOAc 5:5 fraction led to 1.9 g of residue which was fractionated by column chromatography over silica gel with a gradient solvent system: cyclohexane (350 ml); cyclohexane/CH_2Cl_2 (5:5, 400 ml); CH_2Cl_2 (400 ml); CH_2Cl_2/EtOAc (9:1, 200 ml); CH_2Cl_2/EtOAc (8:2, 400 ml); CH_2Cl_2/EtOAc (7:3, 200 ml); CH_2Cl_2/EtOAc (6:4, 200 ml); CH_2Cl_2/EtOAc (5:5, 400 ml); EtOAc (400 ml); EtOAc/MeOH (8:2, 400 ml); EtOAc/MeOH (5:5, 400 ml); MeOH (200 ml) to afford 185 fractions of 25 ml each. Fractions 85–90 were combined and evaporated *in vacuo* to give a residue (104 mg) which was purified by semi-preparative reverse-phase HPLC (C_{18}, Luna Phenomenex, 250 × 10 mm, 5A) using an isocratic solvent system MeOH/H_2O 45:55 to give ridentin (**12**, 3.2 mg), 2α-hydroxyarborescin (**8**, 1.1 mg), 1β,10β-epoxy-2α-hydroxykauniolide (**9**, 1.5 mg) and hanphyllin (**13**, 1.3 mg). Fractions 91–103 were combined and evaporated *in vacuo* to give a residue (158 mg) which was submitted to HPLC (C_{18}, Luna Phenomenex, 250 × 10 mm, 5A) with an isocratic solvent system MeOH/H_2O 45:55 to afford 5α-hydroxy-6-epileukodin (**1**, 1.8 mg), 5α-hydroxyleukodin (**6**, 1.6 mg) and 1α,4α,10α-trihydroxy-5α,11βH-guaia-2-en-12,6α-olide (**10**, 1.8 mg). Evaporation of the CH_2Cl_2 fraction gave 0.5 g of a residue which was submitted to HPLC (C_{18}, Luna Phenomenex, 250 × 10 mm, 5A) with an isocratic solvent system ACN/H_2O 80:20. Gorgonolide (**2**, 1.5 mg), 1β,10α-dihydroxy-1,10-deoxygorgonolide (**3**, 1.6 mg), 1,10-dioxo-1,10-deoxy-1,10-secogorgonolide (**4**, 1.3 mg), 3β,4β-epoxy-1β,10β-epiarborescin (**5**, 1.4 mg), arborescin (**7**, 9.8 mg) and 1β,4α,10α-trihydroxy-5α,11βH-guaia-2-en-12,6α-olide (**11**, 1.0 mg) were thus obtained.

3.4. 5α-Hydroxy-6-epileukodin (*1*)

Colourless oil; $[\alpha]_D^{25}$ −7.0° (*c* 0.04, MeOH); UV (CH_3OH) λ_{max}: (log ε) 267 (3.4) nm; IR ν_{max} (thin film) cm^{-1}: 3428, 1773, 1633, 1261; ^1H NMR (500 MHz, DMSO-d_6) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-MS *m*/*z* 285.3 $[M+Na]^+$.

3.5. Gorgonolide (*2*)

Colourless oil; $[\alpha]_D^{25}$ +8.1° (*c* 0.48, MeOH); UV (CH_3OH) λ_{max}: (log ε) 234 (3.2) nm; IR ν_{max} (thin film) cm^{-1}: 1770, 1711, 1455, 1378, 1234, 1171; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-MS *m*/*z*: 285.1 $[M+Na]^+$.

3.6. 1β,10α-Dihydroxy-1,10-deoxygorgonolide (*3*)

Colourless oil; $[\alpha]_D^{25}$ −31.7° (*c* 0.44, MeOH); UV (CH_3OH) λ_{max}: (log ε) 234 (3.3) nm; IR ν_{max} (thin film) cm^{-1}: 1760, 1700, 1455, 1378, 1240, 1171; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-MS *m*/*z*: 303.1 $[M+Na]^+$.

3.7. 1,10-Dioxo-1,10-deoxy-1,10-secogorgonolide (*4*)

Colourless oil; $[\alpha]_D^{25}$ −17.5° (*c* 0.60, MeOH); UV (CH_3OH) λ_{max}: (log ε) 233 (5.03) nm; IR ν_{max} (thin film) cm^{-1}: 1774, 1707, 1460, 1378, 1249, 1169; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-MS *m*/*z*: 301.0 $[M+Na]^+$.

3.8. 3β,4β-Epoxy-1β,10β-epiarborescin (*5*)

Colourless oil; $[\alpha]_D^{25}$ +12.0° (*c* 0.54, MeOH); UV (CH_3OH) λ_{max}: (log ε) 240 sh (2.02) nm; IR ν_{max} (thin film) cm^{-1}: 1775, 1454, 1380, 1233, 1172; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-MS *m*/*z*: 287.1 $[M+Na]^+$.

3.9. 5α-Hydroxyleukodin (*6*)

Colourless oil; ^1H NMR (500 MHz, $CDCl_3$) see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$) see Table 2; ESI-MS *m*/*z*: 285.3 $[M+Na]^+$.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2008.09.022.

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