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An unusual halogenated meroditerpenoid from *Stypopodium flabelliforme*: Studies by NMR spectroscopic and computational methods

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ABSTRACT

Meroditerpenoids, 2-[2′(*E*)-3′,7′,11′,15′-tetramethylhexadec-2-en-1′-yl]-6-methyl-1,4-benzohydroquinone diacetate and 4′-chlorostypotriol triacetate, along with eight known compounds isolated from the dichloromethane extract of the brown alga *Stypopodium flabelliforme* after peracetylation are reported. One of them, 2-(1-oxo-hexadecyl)-1,3,5-trihydroxybenzene, is described for the first time within this genus. Structural elucidation was carried out on the basis of spectroscopic data and theoretical studies using GIAO/DFT analysis at B3LYP/6-31G(d) and mPW1PW91/6-31G(d) levels of theory for 4′-chlorostypotriol. This isomer is the first metabolite from the *Stypopodium* genus possessing one halogen atom.

1. Introduction

Marine brown algae of the genus *Stypopodium* (Dictyotaceae, Phaeophyta) are localized mainly in tropical and subtropical seas. In Chile, *Stypopodium flabelliforme* is found in Easter Island. From the genus *Stypopodium*, several secondary metabolites with different biological activities have been reported (Gerwick and Fenical, 1981; Gonzalez et al., 1982; Gerwick et al., 1985; Rovirosa et al., 1992, 1994; Gil et al., 1995; Depix et al., 1998; Wessels et al., 1999; Dorta et al., 2002; Sabry et al., 2005). Those activities are related to their unique phytochemical structures such as chromenes, plastoquinones and polycyclic meroditerpenoids. However, some are unstable to air oxidation, such as stypotriol and tetraprenylhydroquinones (Gerwick and Fenical, 1981).

On the other hand, the gauge invariant atomic orbitals/density functional theory (GIAO/DFT) approach has been used for calculation of chemical shifts for a variety of natural products. This method is very helpful to assign NMR spectroscopic data of rigid molecular structures, as well as for determinations of relative conformations of flexible compounds (Bassarello et al., 2003; Da-Silva and Cunha-Neto, 2005; Chini et al., 2008; Braddock and Rzepa, 2008).

In the course of our studies for bioactive metabolites from algae from Chile, we re-investigated the brown alga *S. flabelliforme*. Herein, we report the structural elucidation using 1D and 2D NMR spectroscopy of two new meroditerpenoids including an unusual halogenated moiety. Besides, a comparison between experimental and theoretical chemical shifts was carried out for one of them in order to illustrate the reliability of the method as a valuable aid to the assignment of **7**.

2. Results and discussion

Fresh algae were extracted with CH_2Cl_2, with the extract so obtained concentrated under reduced pressure. Because of the instability of polyprenylhydroquinones, stypotriol and free phenols, the CH_2Cl_2 extract was immediately acetylated with Ac_2O/Py. Ten compounds were thus isolated as acetates from *S. flabelliforme* using chromatographic methods, and these metabolites were grouped as tetraprenylhydroquinones **1**–**2**, polycyclic meroditerpenoids **3**–**8**, one chromene **9** and one polyketide **10**, respectively, Fig. 1. The polyketide, compound **10**, was previously isolated from the brown alga *Lobophora papenfussii* (Dictyotales) (Gerwick and Fenical, 1982) but is reported here for the first time as a *S. flabelliforme* metabolite.

Compound **2** was obtained as pale yellow oil. It was characterized as 2-[2′(*E*)-3′,7′,11′,15′-tetramethylhexadec-2-en-1′-yl]-6-methyl-1,4-benzohydroquinone diacetate. Its molecular formula ($C_{31}H_{50}O_4$) was determined on the basis of HREIMS analysis. Its ^{1}H NMR spectrum (Table 1) displayed main proton signals at δ_H 6.98 (1H, *d*, *J* = 2.4, H-3), 6.77 (1H, *d*, *J* = 2.4, H-5), 5.30 (1H, *t*, *J* = 7.1, H-2′), 3.23 (2H, *d*, *J* = 7.1, H-1′) and 1.92 (3H, *s*, Me-7), along with five methyl groups in the side-chain at δ_H 1.51 (3H, *s*, Me-20′), 0.88 (3H, *d*, *J* = 6.8, Me-19′), 0.86 (9H, *d*, *J* = 6.8, Me-16′, 17′ and 18′), indicating that **2** is a prenylated hydroquinone. The

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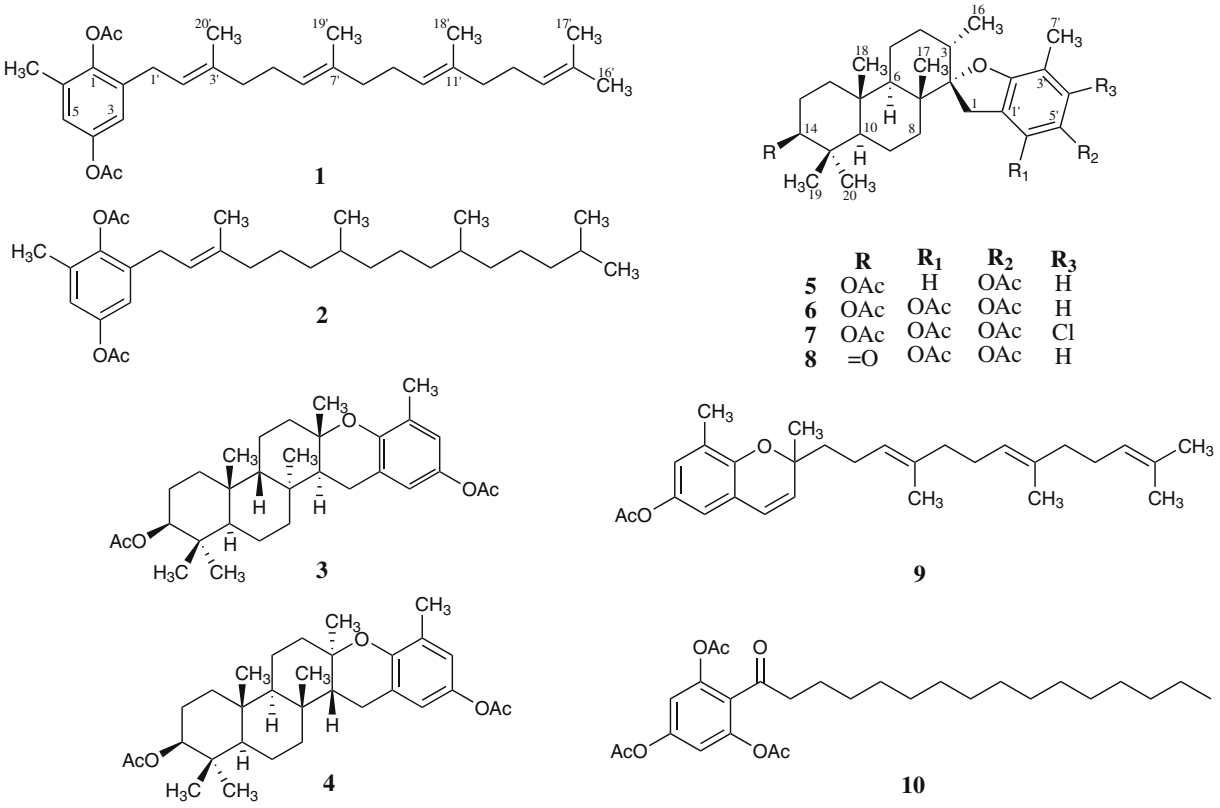


Fig. 1. Structure of compounds isolated from the brown seaweed *Stypopodium flabelliforme*.

Table 1
^{1}H and ^{13}C NMR (HMQC), ^{1}H–^{1}H COSY, and HMBC spectroscopic data of **2** (C_6D_6).

H	δ ^{1}H (*J*, Hz)	δ ^{13}C (DEPT)	HMBC
1	–	145.5 *s*	–
2	–	134.9 *s*	–
3	6.98 *d* (2.4)	120.2 *d*	C-1; C-4; C-1′
4	–	148.5 *s*	–
5	6.77 *d* (2.4)	121.2 *d*	C-1; C-4; C-7
6	–	131.6 *s*	–
7	1.92 *s*	16.1 *s*	C-5; C-6
1′	3.23 *d* (7.1)	28.8 *t*	C-1; C-2; C-3; C-4′
2′	5.3 *t* (7.1)	121.5 *d*	C-2; C-1′; C-4′; C-20′
3′	–	137.4 *s*	–
4′	1.92[a]	39.9 *t*	C-3′; C-5′; C-6′; C-20′
5′	1.41 *m*; 1.26 *m*	25.4 *t*	
6′	1.27 *m*; 1.22 *m*	36.7 *t*	C-8′
7′	1.37 *m*	32.7 *d*	C-19′
8′	1.28 *m*; 1.12 *m*	37.4 *t*	
9′	1.22[a]	24.9 *t*	
10′	1.07 *m*[b]	37.6 *t*[b]	C-18′
11′	1.37 *m*	32.9 *d*	C-10′; C-12′
12′	1.33 *m*[b]	37.5 *t*[b]	
13′	1.36 *m*; 1.21 *m*	24.6 *t*	
14′	1.13 *m*	39.4 *t*	C-13′; C-16′; C-17′
15′	1.48 *dq* (6.8)	27.9 *d*	C-16′; C-17′
16′	0.86 *d* (6.8)	22.5 *q*[c]	C-14′; C-15′
17′	0.86 *d* (6.8)	22.4 *q*[c]	C-14′; C-15′
18′	0.86 *d* (6.8)	19.5 *q*	C-11′
19′	0.88 *d* (6.8)	19.6 *q*	C-7′; C-8′
20′	1.51 *s*	15.7 *q*	C-2′; C-3′; C-4′
OAc	1.73 *s*	20.2 *q*	–
OAc	1.79 *s*	19.5 *q*	–
$COCH_3$	–	168.0 *s*	–
$COCH_3$	–	167.5 *s*	–

[a] Hidden signals.
[b,c] Assignments may be interchanged.

carbon signals in the ^{13}C NMR spectrum were similar to that of a well-known tetraprenyl toluoquinone derivative from *Taonia atomaria* (Tziveleka et al., 2005). However, some exceptions were observed in the ^{13}C NMR spectroscopic data; for instance one aromatic moiety was at δ_C 148.5 (C-4), 145.5 (C-1), 134.9 (C-2), 131.6 (C-6), 121.2 (C-5), 120.2 (C-3) and two acetate groups were at δ_C 168.0 and 167.5 instead of two quinone carbonyls. A combination of HMQC and HMBC experiments support this interpretation. The configuration of the carbon–carbon double bond was proposed to be *E* on the basis of their chemical shifts (mainly ^{13}C NMR), when compared with prenylquinone data from literature (Tziveleka et al., 2005; Mori et al., 2005; Seo et al., 2006). Compound **2** has previously been synthesized in investigations of the biosynthesis of α-tocopherols in spinach chloroplasts (Inoue et al., 1974). According to the Dictionary of Natural Products on CD-ROM, compound **2** has not been previously reported as a natural product.

Compound **7** was obtained as white powder. The ^{1}H NMR spectrum showed signals for one methine proton at δ_H 4.51 (1H, *dd*, *J* = 12.0, 5.0, H-14), one pair of doublets at δ_H 3.10 and 2.68 (1H, *d*, *J* = 16.4, H-1), three acetate methyl groups at δ_H 2.31 (3H, *s*), 2.26 (3H, *s*) and 2.10 (3H, *s*), one aromatic methyl at δ_H 2.25 (3H, *s*, Me-7′) and five aliphatic methyl groups at δ_H 0.93 (3H, *s*, Me-17), 0.87 (3H, *s*, Me-18), 0.84 (3H, *s*, Me-20), 0.83 (3H, *s*, Me-19) and 0.72 (3H, *d*, *J* = 6.4, Me-16). ^{13}C NMR spectroscopic analysis, including DEPT 135, established the presence of ten quaternary carbons, four methines, seven methylenes, six methyl groups and three acetate groups (Table 2). These analysis and chemical shift comparisons (data assigned by HMQC) were comparable to those previously reported for **6** (Gerwick and Fenical, 1981; Rovirosa et al., 1992). The main difference from **6** is the absence of C-4′ aromatic proton and addition of a chlorine atom in **7**. The above analysis was confirmed by HREIMS and ^{13}C NMR spectroscopic analysis thereby suggesting a molecular formula $C_{33}H_{45}ClO_7$ and therefore bearing 11 degrees of unsaturation. The EI mass spectrum indicated molecular ion $[M]^{+\cdot}$ isotopic peaks at *m*/*z* 588 and 590. The

Table 2
^{1}H and ^{13}C NMR (HMQC), and HMBC spectroscopic data of **7** ($CDCl_3$).

C	δ ^{1}H (*J*, Hz)	δ ^{13}C (DEPT)	HMBC
1	3.10 *d* (16.4); 2.68 *d* (16.4)	32.8 *t*	C-2; C-3; C-7; C-1′; C-2′; C-6′
2	–	97.4 *s*	–
3	1.38 *m*	37.0 *d*	C-1
4	1.46 *m*; 1.15 *m*	31.0 *t*	
5	1.68 *m*; 1.41 *m*	20.5 *t*	
6	1.52 *d* (12.6)	51.3 *d*	C-7; C-11
7	–	42.4 *s*	–
8	1.01 *dd* (15.1; 6.6)	32.8 *t*	C-9; C-17
9	1.47 *m*; 1.12 *m*	17.5 *t*	C-7; C-10; C-11
10	0.86 *m*[a]	55.0 *d*	C-11; C-12; C-14; C-15; C-18
11	–	37.0 *s*	–
12	1.75 *t* (13.1; 3.3); 1.45 *t* (13.1)	38.1 *t*	C-10; C-18
13	1.58 *t* (13.1; 3.3); 1.31 *m*	23.7 *t*	C-14; C-15
14	4.51 *dd* (12.0; 5.0)	80.8 *d*	C-13; C-15; C-19; C-20
15	–	37.8 *s*	–
16	0.72 *d* (6.4)	15.5 *q*	C-2; C-3; C-4
17	0.93 *s*	17.1 *q*	C-2; C-6; C-7; C-8
18	0.87 *s*	16.5 *q*	C-10; C-11; C-12
19	0.83 *s*	27.9 *q*	C-10; C-14; C-15; C-20
20	0.84 *s*	16.3 *q*	C-10; C-14; C-15; C-19
1′	–	118.7 *s*	–
2′	–	157.5 *s*	–
3′	–	115.5 *s*	–
4′	–	126.8 *s*	–
5′	–	132.1 *s*	–
6′	–	136.9 *s*	–
7′	2.25 *s*	12.9 *q*	C-2′; C-3′; C-4′
OAc	2.10	21.3 *q*	–
OAc	2.31	20.3 *q*	–
OAc	2.26	20.2 *q*	–
$COCH_3$	–	171.0 *s*	–
$COCH_3$	–	168.3 *s*	–
$COCH_3$	–	167.2 *s*	–

[a] Hidden signal.

isotopic pattern (3:1), as well as the mass fragments, confirmed the presence of one chlorine atom. The main connectivity in the HMBC spectrum showed the following ^{1}H, ^{13}C long-range correlations (Fig. 2): proton signals δ_H 3.10 (1H, *d*, H-1), 2.68 (1H, *d*, H-1) with carbon resonances δ_C 157.5 (C-2′), 136.9 (C-6′), 118.7 (C-1′), 97.4 (C-2), 42.4 (C-7) and 37.0 (C-3); proton signal δ_H 2.25 (3H, *s*, H-7′) with carbon resonances δ_C 157.5 (C-2′), 126.8 (C-4′) and 115.5 (C-3′). This established the position of the chlorine atom at C-4′. Analysis of the NOESY spectrum confirmed that **7** had the same relative configuration as stypotriol.

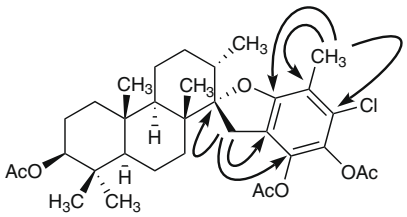
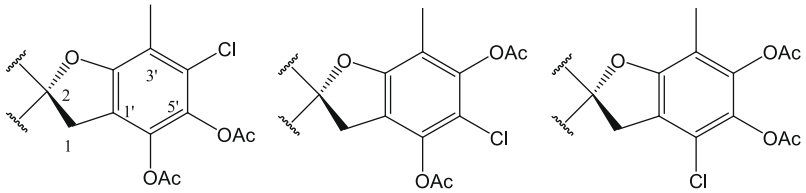


Fig. 2. The main correlations in the HMBC spectrum of compound **7**.

Finally, the theoretical ^{13}C NMR chemical shifts were calculated using B3LYP and mPW1PW91 functionals with a 6-31G(d) basis set including solvent effect for the isomers of **7**: 5′-chlorostypotriol triacetate (**7a**) and 6′-chlorostypotriol triacetate (**7b**). The results are summarized in Table 3, and Fig. 3 shows optimized structures. We have thus studied changes in different positions of the chlorine atom in the aromatic ring of **7**. The changes in the chemical shift for different positions represent the main information leading to theoretical NMR parameter assignments. The theoretical chemical shift for C-4′ are δ_C 130.3 and 128.4 for **7**; 142.1 and 141.9 for **7a** and 139.1 and 138.7 for **7b** at B3LYP/6-31G(d) and mPW1PW91/6-31G(d) level of theory, respectively. The same trend is observed for other carbons of the aromatic rings. Thus, the difference between theoretical and experimental values $\Delta\delta = |\delta_{exp} - \delta_{calc}|$ in the ^{13}C NMR spectra of C-4′ is shown as being $\Delta\delta_C$ about 2.6 and 3.5 at **7**, 31.3 and 35.1 at **7a** and 13.3 and 12.3, respectively. The large differences for **7a** and **7b** isomers predict that structure **7** has the best agreement between experimental and theoretical values. This is essentially a similarity index. In summary, our results show that GIAO/DFT calculations on the optimized structures indicate that isomer **7** is the one that agrees with the experimental ^{13}C NMR chemical shift.

In order to establish that halogenation of compound **7** was not an artifact arising from the isolation procedure, additional assays

Table 3
Calculated ^{13}C NMR chemical shifts of **7** and its isomers in $CDCl_3$ (only for dihydrobenzofurane rings).



C	**7**[a]	**7a**[a]	**7b**[a]	**7**[b]	**7a**[b]	**7b**[b]	Exp.
1	36.3	36.5	36.8	36.2	36.2	36.4	32.8
2	98.1	98.4	97.7	94.2	94.6	94.0	97.4
1′	115.8	115.4	119.0	116.1	115.3	119.2	118.7
2′	151.2	152.4	150.9	151.4	152.7	151.0	157.5
3′	111.4	108.4	109.3	111.2	108.3	109.4	115.5
4′	**130.3**	**142.1**	**139.1**	**128.4**	**141.9**	**138.7**	126.8
5′	127.8	112.5	128.2	126.7	110.7	127.8	132.1
6′	133.2	136.2	123.6	133.3	136.0	122.2	136.9
7′	13.9	11.8	11.2	14.0	11.8	11.4	12.9

[a] δ using B3LYP/6-31G(d) level.
[b] δ using PW1PW91/6-31G(d) level.

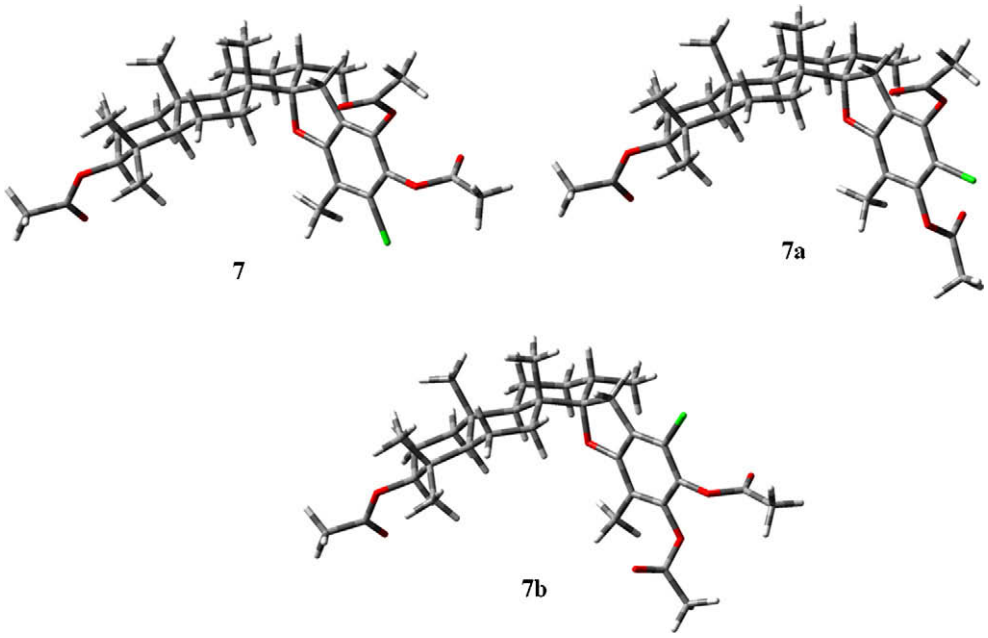


Fig. 3. Optimized geometries of **7**, **7a** and **7b**.

were carried out (see experimental) but with no formation of **7** being observed. Similar experiments were carried out with stypotriol triacetate and the results were the same: no reaction was observed in all of them. Taken together, these data indicated **7** is a natural product.

3. Concluding remarks

Halogenated metabolites are mainly known from marine organisms, prokaryotes and several higher plants. It is well accepted that the halometabolites isolated from marine invertebrates in many cases originate from incorporation of algal food. Seaweeds provide a rich source of structurally diverse secondary metabolites being mainly halogenated. Their functions are in defense against herbivores, fouling organisms and pathogens but they also play a role in reproduction, protection from UV radiation and as allelopathic agents. However, the variations in production of secondary metabolites in seaweeds is a function of life cycle and is genetically and environmentally controlled (Hay, 1996; Soares et al., 2003; Ianora et al., 2006). The occurrence of halogenated compounds is unusual in brown seaweed and there is no report of their presence in the *Stypopodium* genus.

4. Experimental

4.1. General experimental procedures

TLC was performed on Kieselgel 60 GF254 using *n*-hexane/EtOAc (7:3 and 1:1 v/v) as mobile phase. TLC spots were visualized by spraying the chromatograms with H_2SO_4–MeOH (5:95, v/v) and heating at 120 °C for 3 min. Column chromatography (CC) was performed over Merck Kieselgel 60, particle size 0.063–0.200 mm. All solvents were dried and purified before use according to standard procedures. Measurements of NMR spectra of compounds used a Bruker Avance AM-400 spectrometer equipped with 5 mm probes. All compounds were individually dissolved in $CDCl_3$ (1.0 ml) or C_6D_6 containing tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) were reported in ppm and coupling constants (J) in hertz. The pulse conditions were as follows: for the ^1H NMR spectrum, spectrometer frequency (SF) = 400.13 MHz, acquisition time (AQ) = 2.045 s, relaxation delay (RD) = 1.0 s, 30° pulse width = 6.5 μs, spectral width (SW) = 8012.8 Hz, line broadening (LB) = 0.3 Hz, Fourier transform (FT) size = 32K; for the ^{13}C NMR spectrum, SF = 100.61 MHz, AQ = 1.2 s, RD = 2.0 s, 30° pulse width = 14.0 μs, SW = 23980.8 Hz, LB = 1.0 Hz, FT size = 32K; for the H–H COSY spectrum, AQ = 0.13 s, RD = 1.0 s, SW = 4006.4 Hz, FT size = 1024 × 1024; for the HMBC spectrum, AQ = 0.13 s, RD = 1.0 s, SW = 4006.4 (^1H) and 30191.03 (^{13}C) Hz, FT size = 1024 × 2048, and 7.7 Hz long-range coupling constant; for the HMQC spectrum, parameters were very similar to those used in the HMBC experiments, except for the 145 Hz one-bond coupling constant. The NOESY spectrum was performed with eight scans, AQ = 0.106 s, RD = 1.0 s, SW = 4789.2 Hz, FT size = 1024 × 1024 and LB = 0.0 Hz. IR spectra were recorded on a Vector 22 FT-IR spectrometer with mass spectra acquired using a Thermo Finnigan MAT 95XP model spectrometer. Optical rotations were obtained in $CHCl_3$ on a Polartronic E polarimeter and UV spectra were recorded on a Shimadzu UV160 spectrophotometer.

4.2. Computational details

DFT calculations were carried out using the B3LYP and mPW1PW91 exchange-correlation functional, together with the standard 6-31G(d) basis set (Hehre et al., 1986; Lee et al., 1988). The ^{13}C NMR chemical shifts were calculated using gauge invariant atomic orbitals (GIAO) method at the same level (Wolinski et al., 1990). Solvent effects were evaluated by performing single-point B3LYP/6-31G(d) and mPW1PW91/6-31G(d) calculations at the gas-phase stationary points involved in the reaction using the polarizable continuum model (PCM) of Tomasi's group (Tomasi and Persico, 1994; Barone et al., 1998). A dielectric constant value was used for $CHCl_3$ (ε = 4.9). Relative chemical shifts were estimated by using the corresponding TMS shielding calculated at the same theoretical model and using PCM method to solvent effect. All calculations were carried out with the Gaussian 03 suite of programs (Frisch et al., 2004). All calculated data of the ^{13}C

NMR chemical shifts can be obtained upon request from the authors.

4.3. Plant material

The brown alga, *Stypopodium flabelliforme*, was collected intertidally near the Hanga Roa in Easter Island (South Pacific), V Región, Chile, in 2005 at a depth of 5–10 m. A voucher specimen (No. 0053) was deposited in the Museo Nacional De Historia Natural, Santiago, Chile and its identity was confirmed by Prof. M. Eliana Ramirez from the Museo de Historia Natural de Santiago, Chile.

4.4. Extraction and isolation

Wet specimens (2.0 kg) were frozen for transportation and later repeatedly extracted with CH_2Cl_2 (3 × 4 l). After filtration, the combined extracts were concentrated under reduced pressure. Then, the CH_2Cl_2 extract (60.5 g) was subsequently acetylated with Ac_2O/Py. The extract (60 g) was subjected to flash chromatography on silica gel (*n*-hexane/EtOAc/MeOH in gradient from 10:1:0 to 0:1:1 v/v/v) to produce nine fractions 1–9. Fraction 1 (20 g), was next subjected to silica gel CC and eluted with EtOAc–*n*-hexane (1:9, v/v), with fractions of interest being further purified by silica gel CC with a *n*-hexane/EtOAc gradient (0% up to 100% EtOAc) to yield sargaol acetate **9** (200 mg) (Numata et al., 1992), 2-(geranylgeranyl)-6-methyl-1,4-benzohydroquinone diacetate **1** (70 mg) (Gerwick and Fenical, 1981), 2-[2′(*E*)-3′,7′,11′,15′-tetramethylhexadec-2-en-1′-yl]-6-methyl-1,4-benzohydroquinone diacetate **2** (7 mg), epitaondiol diacetate **3** (2 g) (Sanchez-Ferrando and San-Martin, 1995) and a mixture of three compounds (1.0 g). An aliquot (150 mg) of this mixture was further separated by silica gel CC impregnated with $AgNO_3$ (1:9) with *n*-hexane/EtOAc as system solvent to give stypodiol diacetate **5** (30 mg) (Gerwick and Fenical, 1981; Abad et al., 1998), isoepitaondiol diacetate **4** (25 mg) (Rovirosa et al., 1992) and **3** (50 mg).

Fraction 2 (5 g) was applied to a silica gel column, this being eluted with EtOAc–*n*-hexane (1:4, v/v), with fractions of interest subjected to Sephadex LH-20 CC using MeOH to separate fatty acids, chlorophylls and pigments. Then, various fractions were further purified by silica gel CC, using a *n*-hexane/EtOAc gradient (0% up to 100% EtOAc) to afford 750 mg of a mixture and stypotriol triacetate **6** (800 mg) (Gerwick and Fenical, 1981). The mixture was subjected successively to further silica gel CC (*n*-hexane/EtOAc gradient of 10% up to 100% EtOAc) and Sephadex LH-20 (*n*-hexane/CH_2Cl_2/MeOH: 8/1/1 v/v/v) to afford 4′-chlorostypotriol triacetate **7** (2 mg) and 2-(1-oxo-hexadecyl)-1,3,5-trihydroxybenzene triacetate **10** (30 mg) (Gerwick and Fenical, 1982).

Fraction 3 (12 g) was subjected to silica gel CC, eluted with EtOAc–*n*-hexane (3:7, v/v), and then passed through a Sephadex LH-20 column using MeOH to separate chlorophyll. The fraction containing the meroditerpenoids applied to a silica gel CC (*n*-hexane–EtOAc gradient of 10% up to 100%) to afford **6** (5 g) and 14-ketostypotriol diacetate **8** (300 mg) (Rovirosa et al., 1994).

Fraction 4 (9 g) was subjected to silica gel CC, eluted with EtOAc–*n*-hexane (1:1, v/v), and contained pigments such as chlorophylls as evidenced by ^{1}H NMR and UV analysis.

Fractions 5–8, eluted with EtOAc–*n*-hexane (7:3, v/v); EtOAc; EtOAc–MeOH (1:1, v/v) and MeOH, respectively, were subjected to silica gel CC with a CH_2Cl_2/MeOH gradient (0% up to 100%), respectively. Some of those subfractions were analyzed for ^{1}H NMR with negative results for secondary metabolites.

4.5. Compound **2**

2-[2′(*E*)-3′,7′,11′,15′-Tetramethylhexadec-2-en-1′-yl]-6-methyl-1,4-benzohydroquinone diacetate: pale yellow oil. $[\alpha]_D^{20}$ +2.0° (*c* 0.01, $CHCl_3$). UV $[\lambda]_{max}^{MeOH}$ nm (log ε) 228 (4.0). HREIMS: calcd. for $C_{31}H_{50}O_4$ ($M^{+\cdot}$): 486.3709, found: 486.3718. FT-IR ν_{max} cm^{-1}: 2970, 2948, 1731, 1460, 1385, 1240, 1110.

4.6. Compound **7**

4′-Chlorostypotriol triacetate: white powder. $[\alpha]_D^{20}$ −5.0° (*c* 0.004, $CHCl_3$). UV $[\lambda]_{max}^{MeOH}$ nm (log ε) 289 (3.5). HREIMS: calcd. for $C_{33}H_{45}ClO_7$ ($M^{+\cdot}$): 588.2854, found: 588.2859. EI-MS: *m*/*z* (rel. int.%): 588 $[M]^{+\cdot}$/$[M+2]^{+\cdot}$ (6/2), 553 (2), 546 (100/33), 504 (21/7), 486 (8/3), 257 (39/13), 187 (14), 135 (8), 95 (8), 69 (9). FT-IR ν_{max} cm^{-1}: 2950, 1771, 1735, 1475, 1367, 1245, 1100.

4.7. Hydrolisis of stypotriol triacetate (**6**)

To a stirred solution of stypotriol acetate **6** (200 mg) in MeOH (20 ml), K_2CO_3 (catalytic amount) was added. The reaction mixture was stirred at room temperature under N_2 for 24 h, then rapidly concentrated and kept under N_2 atmosphere. Immediately, $FeCl_3$ (catalytic amount) was added to a stirred solution of stypotriol in CH_2Cl_2 (10 ml) under N_2. The resulting suspension was heated until reflux began, thus being maintained for one week and following the product was rapidly acetylated (Ac_2O/Py). The resulting mixture was then diluted with H_2O (20 ml) and filtered to yield a solid following solvent removed. The latter was re-dissolved in Et_2O for analysis. The same procedure without $FeCl_3$ was done as well. No halogenation of stypotriol was observed.

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