

## Acylphloroglucinols from *Elaphoglossum crassipes*: Antidepressant-like Activity of Crassipin A

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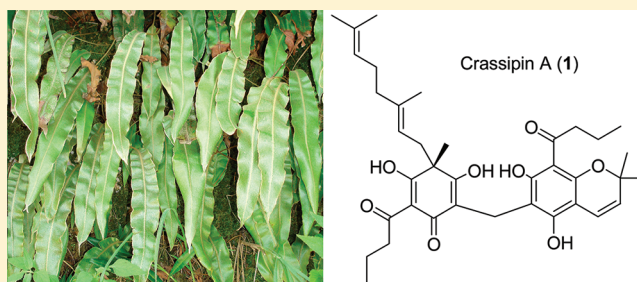
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### S Supporting Information

**ABSTRACT:** Nine new terpenylated acylphloroglucinols, crassipins A–I, were isolated from the rhizomes and roots of the fern *Elaphoglossum crassipes*, and their structures were elucidated by analysis of spectroscopic data and chemical derivatization. The absolute configurations of some of the compounds were established by CD and VCD in combination with a quantum mechanical method. Crassipin A (**1**), the major acylphloroglucinol of the Et<sub>2</sub>O extract of *E. crassipes*, as well as its peracetylated derivative (**8**), displayed antidepressant-like activity in a mouse forced-swimming test when administered orally at a dose of 15 mg/kg.



The presence of acylphloroglucinols in herbarium specimens of ferns belonging to the genus *Elaphoglossum* (*E. erinaceum* and *E. paleaceum*) (Dryopteridaceae) was detected more than 25 years ago.<sup>1</sup> The authors described them as “unknown phenolics” that were “clearly not identical with those occurring in *Dryopteris* and some other related genera”. It was not until 2009 that the first phloroglucinol derivatives were isolated from *E. piloselloides* and structurally characterized.<sup>2</sup> Moreover, preliminary analysis by NMR spectroscopy of the Et<sub>2</sub>O extracts of six out of seven Argentine *Elaphoglossum* species showed the presence of acylphloroglucinols. These compounds exhibit characteristic singlets in the range of 12 to 19 ppm that can be detected easily in a routine <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> of even a complex mixture such as an extract. In previous publications, the isolation and characterization of 18 new acylphloroglucinols from *E. piloselloides*, *E. gayanum*, *E. yungense*, and *E. lindbergii* have been reported.<sup>2–5</sup> They resemble structurally those of *Hypericum*<sup>6–8</sup> (flowering plants), rather than those of *Dryopteris*<sup>9–11</sup> (ferns).

As ancient remedies effective against helminthiasis, *Dryopteris* ferns have been the subject of chemical investigations since the end of the 19th century.<sup>12</sup> Up to the present, several bioactive acylphloroglucinols from *Dryopteris* and other sources, particularly *Hypericum* whole plants and *Elaphoglossum* rhizomes and roots, have been isolated.<sup>2–8,13,14</sup> One of the most remarkable examples is hyperforin, a phloroglucinol isolated from St. John’s wort (*Hypericum perforatum* L.) (Clusiaceae), which displays antidepressant activity.<sup>15,16</sup> Hyperfoliatin, isolated from *H. perforatum*, possesses an antidepressant-like effect in a rodent model.<sup>17</sup> As conventional antidepressants are associated frequently with a series of side effects, as well as poor

clinical responses,<sup>18</sup> natural products may offer an alternative approach to develop innovative antidepressant drugs.

Continuing with a search for bioactive constituents from Argentine ferns,<sup>2–5,13,19,20</sup> the isolation and structural characterization of nine new geranylated acylphloroglucinols from the scaly rhizomes and roots of *Elaphoglossum crassipes* (Hieron.) Diels are described herein. The evaluation of the antidepressant-like activity of the major constituent, crassipin A (**1**), as well as of its peracetylated derivative (**8**) in a mouse forced-swimming test is also reported.

### RESULTS AND DISCUSSION

The air-dried plant material of *E. crassipes* was ground and extracted by maceration at room temperature with Et<sub>2</sub>O. The extract was fractionated, and the fractions were further processed as described in the Experimental Section to yield the new acylphloroglucinols crassipins A–I.

The HRFABMS of crassipin A (**1**) showed a pseudomolecular ion peak [M + H]<sup>+</sup> at *m/z* 621.3450, consistent with a molecular formula of C<sub>37</sub>H<sub>48</sub>O<sub>8</sub>, with 14 degrees of unsaturation. The <sup>1</sup>H NMR spectrum of acylphloroglucinol **1** (Table 1) exhibited a signal at a very low field ( $\delta$  18.69) assigned to an enolizable  $\beta$ -triketonic system, as was previously reported for phloroglucinol derivatives carrying an acylfilicin acid-type moiety from *Hypericum* species and *Elaphoglossum* ferns.<sup>2,4,7,8</sup> The <sup>1</sup>H NMR spectrum showed duplicated signals, suggesting the presence of tautomers in solution. This evidence, along with the <sup>13</sup>C NMR signals at  $\delta$  189.7 and 50.8 assigned to

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Table 1. <sup>1</sup>H NMR Data of Acylphloroglucinols 1, 2, 3a, and 3b (acetone-*d*<sub>6</sub>, 500 MHz)

position	1	2	3a	3b
7	3.54, d, <i>J</i> = 16.0 3.51, d, <i>J</i> = 16.0	3.55, d, <i>J</i> = 15.5 3.52, d, <i>J</i> = 15.5	3.54, d, <i>J</i> = 15.5 3.51, d, <i>J</i> = 15.5	3.54, d, <i>J</i> = 16.0 3.51, d, <i>J</i> = 16.0
8	1.54, s	1.52, s	1.54, s	1.54, s
9	2.76, dd, <i>J</i> = 13.2, 9.0 2.59, dd, <i>J</i> = 13.2, 7.0	2.78, dd, <i>J</i> = 13.0, 9.5 2.58, dd, <i>J</i> = 13.0, 6.0	2.76, dd, <i>J</i> = 13.0, 9.5 2.58, dd, <i>J</i> = 13.0, 6.5	2.76, dd, <i>J</i> = 13.0, 10.0 2.59, dd, <i>J</i> = 13.0, 6.5
10	4.65, brt, <i>J</i> = 7.5	4.62, brs	4.64, brt, <i>J</i> = 7.5	4.65, brt, <i>J</i> = 7.8
12	1.64–1.56 <sup>a</sup>	2.28, m 2.14, m	1.67–1.54 <sup>a</sup>	1.68–1.56 <sup>a</sup>
13	1.64–1.56 <sup>a</sup>	5.17, dt, <i>J</i> = 16.0, 7.5	1.70–1.57 <sup>a</sup>	1.68–1.56 <sup>a</sup>
14	4.86, brs	5.33, d, <i>J</i> = 16.0	4.85, brs	4.87, brs
16	1.58, s	1.14, s	1.577, s	1.58, s
17	1.46, s	1.13, s	1.46, s	1.47, s
18	1.37, s	1.37, s	1.35, s	1.37, s
20	3.20, ddd, <i>J</i> = 16.5, 7.5, 7.0 3.15–3.04 <sup>a</sup>	3.18, t, <i>J</i> = 7.5	3.20, dt, <i>J</i> = 16.5, 7.5 3.16–3.08 <sup>a</sup>	3.20, dt, <i>J</i> = 16.0, 7.5 3.16–3.08 <sup>a</sup>
21	1.69, sext., <i>J</i> = 7.5	1.68, sext., <i>J</i> = 7.5	1.69, brsext., <i>J</i> = 7.5	1.69, sext., <i>J</i> = 7.5
22	0.99, t, <i>J</i> = 7.5	0.99, t, <i>J</i> = 7.5	0.99, t, <i>J</i> = 7.5	0.99, t, <i>J</i> = 7.5
3'	5.57, d, <i>J</i> = 10.0	5.59, d, <i>J</i> = 10.0	5.55, d, <i>J</i> = 10.0	5.55, dd, <i>J</i> = 10.0, 3.0
4'	6.63, d, <i>J</i> = 10.0	6.66, d, <i>J</i> = 10.0	6.68, d, <i>J</i> = 10.0	6.69, d, <i>J</i> = 10.0
11'	1.522, s	1.53, s	1.47, s	1.47, s
12'	1.516, s	1.51, s	1.88, ddd, <i>J</i> = 14.0, 11.0, 6.0 1.79, ddd, <i>J</i> = 14.0, 12.0, 6.0	1.88, ddd, <i>J</i> = 13.5, 11.0, 5.0 1.78, ddd, <i>J</i> = 13.5, 11.0, 5.0
13'			2.19, brtdd, <i>J</i> = 12.5, 7.5, 6.0 2.14, brtdd, <i>J</i> = 12.5, 7.5, 6.0	2.19, brtdd, <i>J</i> = 12.0, 7.5, 5 2.13, brqd, <i>J</i> = 12.0, 7.5, 5
14'			5.14, t, <i>J</i> = 7.5	5.14, t, <i>J</i> = 7.5
16'			1.65, s	1.64, s
17'			1.584, s	1.57, s
2''	3.12, dt, <i>J</i> = 15.5, 7.5 3.08, dt, <i>J</i> = 15.5, 7.5	3.15, ddd, <i>J</i> = 16.0, 8.0, 7.0 3.09, ddd, <i>J</i> = 16.0, 8.0, 7.0	3.16–3.08 <sup>a</sup>	3.16–3.08 <sup>a</sup> 3.08, dt, <i>J</i> = 16.5, 7.5
3''	1.72, sext., <i>J</i> = 7.5	1.710, d sext., <i>J</i> = 15.5, 7.0 1.708, d sext., <i>J</i> = 15.5, 7.0	1.73, brsext., <i>J</i> = 7.5	1.72, sext., <i>J</i> = 7.5
4''	1.01, t, <i>J</i> = 7.5	1.01, t, <i>J</i> = 7.0	1.01, t, <i>J</i> = 7.5	1.01, t, <i>J</i> = 7.5
OH-3	9.94, s	9.88, s	9.98, s	9.98, s
OH-5	18.69, s	18.68, s	18.73, s	18.73, s
OH-5'	11.42, s	11.42, s	11.44, s	11.44, s
OH-7'	16.28, s	16.26, s	16.36, s	16.35, s

<sup>a</sup>Overlapping signals.

C-1 and C-4, respectively, and comparison with literature data, allowed identification of a prenylated acylflicinic acid moiety (Table 2).<sup>4,6,21</sup> The prenyl moiety at C-4 was identified as geranyl ( $\delta$  2.76, 1H, dd, *J* = 13.2, 9.0 Hz, H-9a; 2.59, 1H, dd, *J* = 13.2, 7.0 Hz, H-9b; 4.65, 1H, brt, *J* = 7.5 Hz, H-10; 1.64–1.56, 4H, overlapping signals, H-12 and H-13; 4.86, brs, H-14; 1.58, 3H, s, H-16; 1.46, 3H, s, H-17; 1.37, 3H, s, H-18).<sup>4</sup> Signals for a methylene bridge linking two rings were detected at  $\delta$  3.54 and 3.51 (1H each, d, *J* = 16.0 Hz). The position of these signals indicated that the bridge linked an aromatic and an acylflicinic acid-type ring.<sup>4,7,21</sup> In addition, two acyl residues were clearly detected from the <sup>13</sup>C NMR signals at  $\delta$  208.5 and 207.6 (Table 2), providing further evidence of the presence of two rings since in fern acylphloroglucinols each ring carries only one acyl residue. Assignment of the ring carbons was accomplished by analysis of the HMBC correlations of these carbons with the methylene bridge, the hydroxy protons, and H-4' (Figure 1). Signals for a *cis*-disubstituted double bond at  $\delta$  6.63 and 5.57 (1H each, d, *J* = 10 Hz) were present in the <sup>1</sup>H NMR spectrum of **1** (Table 1). These 1D-NMR observations made for crassipin A resembled those for yungensin A, an acylphloroglucinol previously isolated from *E. yungense*,<sup>4</sup> but with a

different acyl moiety attached to C-6. In the NMR spectra of crassipin A (**1**), signals for two butanoyl residues were assigned unambiguously.<sup>5</sup> Thus, compound **1** was assigned as 2-[[5,7-dihydroxy-2,2-dimethyl-8-butanoyl-6-chromenyl]methyl]-3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-butanoyl-2,5-cyclohexadien-1-one.

The circular dichroism (CD) spectrum of crassipin A (**1**) showed positive Cotton effects at  $\Delta\epsilon_{210} +9.85$ ,  $\Delta\epsilon_{258} +1.94$ , and  $\Delta\epsilon_{314} +6.16$  and negative Cotton effects at  $\Delta\epsilon_{238} -4.59$  and  $\Delta\epsilon_{360} -11.34$ . The absolute configuration at C-4 was established as *R* by comparing the experimental circular dichroism spectrum with the simulated electronic circular dichroism (ECD) spectra of both enantiomers (Figure 2). Geometries of the molecules with *R* and *S* configurations at C-4 were optimized with the B3LYP level of theory and 6-31G\* basis set. Then, TD-DFT calculations were performed at the optimized geometries using the same combination of functional and basis set. The outcome of these calculations was used to simulate the CD spectra of the enantiomers. The simulated spectrum for the 4*R* enantiomer showed positive bands centered at 208, 268, and 314 nm and negative bands at 238 and 370 nm. Peracetylation of compound **1** gave the

Table 2.  $^{13}\text{C}$  NMR Data of Compounds 1–6 (acetone- $d_6$ , 125 MHz)

position	$\delta_{\text{C}}$ [ppm], mult.							
	1	2	3a	3b	4	5a	5b	6
1	189.7, C	189.6, C	189.7, C	189.6, C	189.8, C	189.8, C	189.8, C	191.1, C
2	115.2, C	115.2, C	115.2, C	115.2, C	115.2, C	115.3, C	115.4, C	110.4, C
3	171.8, C	171.9, C	171.8, C	171.8, C	171.2, C	171.2, C	171.2, C	176.2, C
4	50.8, C	50.6, C	50.9, C	50.8, C	50.8, C	50.8, C	50.7, C	54.5, C
5	199, C	199.7, C	199.9, C	199.9, C	199.8, C	199.8, C	199.7, C	197.3, C
6	111.8, C	111.8, C	111.9, C	111.8, C	111.8, C	111.9, C	111.9, C	107.8, C
7	18.0, CH <sub>2</sub>	17.9, CH <sub>2</sub>	18.0, CH <sub>2</sub>	18.0, CH <sub>2</sub>	18.2, CH <sub>2</sub>	18.3, CH <sub>2</sub>	18.3, CH <sub>2</sub>	15.3, CH <sub>2</sub>
8	24.0, CH <sub>3</sub>	23.9, CH <sub>3</sub>	23.9, CH <sub>3</sub>	24.0, CH <sub>3</sub>	23.8, CH <sub>3</sub>	24.0, CH <sub>3</sub>	23.9, CH <sub>3</sub>	25.3, CH <sub>3</sub>
9	40.0, CH <sub>2</sub>	40.1, CH <sub>2</sub>	40.1, CH <sub>2</sub>	40.0, CH <sub>2</sub>	40.1, CH <sub>2</sub>	39.9, CH <sub>2</sub>	40.0, CH <sub>2</sub>	40.1, CH <sub>2</sub>
10	118.8, CH	119.2, CH	118.8, CH	118.8, CH	118.6, CH	118.8, CH	118.8, CH	120.4, CH
11	141.5, C	140.5, C	141.6, C	141.5, C	141.8, C	141.9, C	141.7, C	139.5, C
12	41.3, CH <sub>2</sub>	43.2, CH <sub>2</sub>	41.2, CH <sub>2</sub>	41.3, CH <sub>2</sub>	41.3, CH <sub>2</sub>	41.4, CH <sub>2</sub>	41.4, CH <sub>2</sub>	41.4, CH <sub>2</sub>
13	28.4, CH <sub>2</sub>	124.6, CH	28.3, CH <sub>2</sub>	28.4, CH <sub>2</sub>	28.4, CH <sub>2</sub>	27.2, CH <sub>2</sub>	28.6, CH <sub>2</sub>	28.4, CH <sub>2</sub>
14	125.8, CH	143.1, CH	125.8, CH	125.8, CH	125.7, CH	125.8, CH	125.7, CH	126.0, CH
15	132.7, C	71.0, C	132.6, C	132.7, C	132.7, C	132.7, C	132.8, C	132.6, C
16	26.8, CH <sub>3</sub>	31.4, CH <sub>3</sub>	26.84, CH <sub>3</sub>	26.8, CH <sub>3</sub>	26.8, CH <sub>3</sub>	26.8, CH <sub>3</sub>	26.8, CH <sub>3</sub>	26.8, CH <sub>3</sub>
17	18.6, CH <sub>3</sub>	31.4, CH <sub>3</sub>	18.72, CH <sub>3</sub>	18.7, CH <sub>3</sub>	18.5, CH <sub>3</sub>	18.6, CH <sub>3</sub>	18.5, CH <sub>3</sub>	18.6, CH <sub>3</sub>
18	17.1, CH <sub>3</sub>	17.6, CH <sub>3</sub>	17.1, CH <sub>3</sub>	17.1, CH <sub>3</sub>	17.2, CH <sub>3</sub>	17.4, CH <sub>3</sub>	17.3, CH <sub>3</sub>	17.2, CH <sub>3</sub>
19	207.6, C	207.8, C	207.6, C	207.6, C	207.7, C	207.6, C	207.7, C	204.2, C
20	44.3, CH <sub>2</sub>	44.4, CH <sub>2</sub>	44.3, CH <sub>2</sub>	44.3, CH <sub>2</sub>	44.4, CH <sub>2</sub>	44.3, CH <sub>2</sub>	44.4, CH <sub>2</sub>	43.2, CH <sub>2</sub>
21	19.8, CH <sub>2</sub>	19.6, CH <sub>2</sub>	19.8, CH <sub>2</sub>	19.8, CH <sub>2</sub>	19.6, CH <sub>2</sub>	19.7, CH <sub>2</sub>	19.7, CH <sub>2</sub>	20.4, CH <sub>2</sub>
22	15.1, CH <sub>3</sub>	15.1, CH <sub>3</sub>	15.1, CH <sub>3</sub>	15.1, CH <sub>3</sub>	15.2, CH <sub>3</sub>	15.3, <sup>a</sup> CH <sub>3</sub>	15.3, CH <sub>3</sub>	15.3, CH <sub>3</sub>
1'								108.5, C
2'	80.2, C	80.2, C	83.0, C	83.0, C	82.5, C	83.2, C	83.4, C	162.9, C
3'	126.9, CH	127.2, CH	125.5, CH	125.5, CH	126.7, CH	69.6, CH	70.3, CH	108.4, C
4'	118.6, CH	118.6, CH	119.3, CH	119.2, CH	118.2, CH	27.2, CH <sub>2</sub>	27.3, CH <sub>2</sub>	160.0, C
5'	161.0, C	161.0, C	161.0, C	161.0, C	161.8, C	164.5, C	164.4, C	110.4, C
6'	108.0, C	108.2, C	107.8, C	107.9, C	108.1, C	107.7, C	107.8, C	162.0, C
7'	163.4, C	163.3, C	163.3, C	163.3, C	162.3, C	159.9, C	160.0, C	207.5, C
8'	106.0, C	106.1, C	105.9, C	105.9, C	107.1, C	106.1, C	106.3, C	46.6, CH <sub>2</sub>
9'	157.6, C	157.5, C	157.8, C	157.8, C	156.7, C	157.0, C	156.8, C	19.8, CH <sub>2</sub>
10'	105.5, C	105.4, C	105.1, C	105.1, C	103.7, C	101.5, C	101.8, C	15.3, CH <sub>3</sub>
11'	29.0, CH <sub>3</sub>	29.0, CH <sub>3</sub>	27.9, CH <sub>3</sub>	27.9, CH <sub>3</sub>	29.1, CH <sub>3</sub>	31.4, CH <sub>3</sub>	31.4, CH <sub>3</sub>	
12'	29.0, CH <sub>3</sub>	29.1, CH <sub>3</sub>	43.2, CH <sub>2</sub>	43.2, CH <sub>2</sub>	28.5, CH <sub>3</sub>	22.5, CH <sub>3</sub>	21.2, CH <sub>3</sub>	
13'			25.0, CH <sub>2</sub>	24.9, CH <sub>2</sub>				
14'			125.8, CH	125.8, CH				
15'			133.2, C	133.2, C				
16'			26.80, CH <sub>3</sub>	26.8, CH <sub>3</sub>				
17'			18.69, CH <sub>3</sub>	18.7, CH <sub>3</sub>				
1''	208.5, C	208.5, C	208.4, C	208.4, C	208.8, C	208.7, C	208.7, C	29.5, CH
2''	47.4, CH <sub>2</sub>	47.5, CH <sub>2</sub>	47.6, CH <sub>2</sub>	47.5, CH <sub>2</sub>	48.1, CH <sub>2</sub>	48.0, CH <sub>2</sub>	48.0, CH <sub>2</sub>	36.0, CH <sub>2</sub>
3''	20.1, CH <sub>2</sub>	20.1, CH <sub>2</sub>	20.0, CH <sub>2</sub>	20.0, CH <sub>2</sub>	20.0, CH <sub>2</sub>	20.0, CH <sub>2</sub>	20.0, CH <sub>2</sub>	78.8, C
4''	15.2, CH <sub>3</sub>	15.2, CH <sub>3</sub>	15.2, CH <sub>3</sub>	15.2, CH <sub>3</sub>	15.3, CH <sub>3</sub>	15.2, <sup>a</sup> CH <sub>3</sub>	15.2, CH <sub>3</sub>	39.2, CH <sub>2</sub>
5''								23.5, CH <sub>2</sub>
6''								47.6, CH
7''								89.1, C
8''								25.6, CH <sub>3</sub>
9''								30.9, CH <sub>3</sub>
10''								29.6, CH <sub>3</sub>

<sup>a</sup>Signals with the same letter may be exchanged.

pentaacetylated derivative, **8**, with spectroscopic features (Experimental Section) in good agreement with the structure proposed. As depicted, acetylation occurred at C-19, indicating that the reaction conditions favor the enol form in the butanoyl residue attached to C-6. The configuration at the newly formed double bond between C-19 and C-20 was deduced to be *E* from the NOESY correlation observed between H-20 and the CH<sub>3</sub> group of the acetyl residue attached to C-19.

The molecular formula of crassipin B (**2**), C<sub>37</sub>H<sub>48</sub>O<sub>9</sub>, was deduced from a pseudomolecular ion peak  $[\text{M} + \text{Na}]^+$  at  $m/z$  659.3185 in the HRFABMS (calcd for C<sub>37</sub>H<sub>48</sub>O<sub>9</sub>Na, 659.3197). Comparison of the elemental formula of **2** with that of **1** revealed an additional oxygen atom. A signal for an oxygen-bearing quaternary carbon was observed at  $\delta$  71.0 in the  $^{13}\text{C}$  NMR spectrum of **2**. Analysis of its NMR spectra indicated that **2** carries a hydroxylated prenyl chain attached to C-4, which

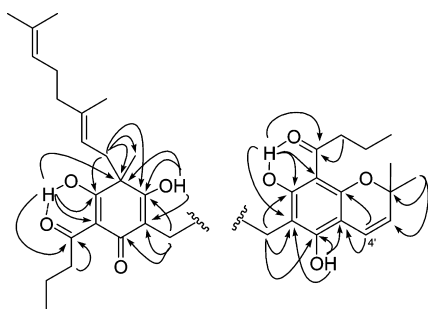


Figure 1. Key HMBC correlations of crassipin A (1).

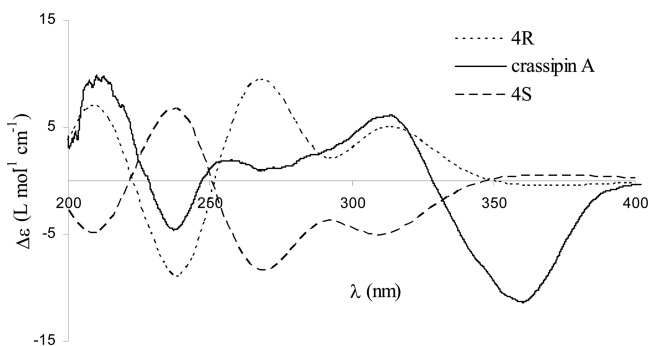


Figure 2. Experimental CD spectrum of crassipin A (1) in ethanol and simulated ECD spectra for its enantiomers.

was identified as a 7-hydroxy-3,7-dimethyl-2,5-octadienyl unit from the  $^1\text{H}$  NMR signals observed at  $\delta$  2.78 (1H, dd,  $J = 13, 9.5$  Hz, H-9a), 2.58 (1H, dd,  $J = 13, 6$  Hz, H-9b), 4.62 (1H, brs, H-10), 2.28 (1H, m, H-12a), 2.14 (1H, m, H-12b), 5.17 (1H, dt,  $J = 16, 7.5$  Hz, H-13), 5.33 (1H, d,  $J = 16$  Hz, H-14), 1.14 (3H, s, H-16), 1.13 (3H, s, H-17), and 1.37 (3H, s, H-18). The position of this side chain at C-4 was established through H–C long-range correlations detected between H-9 and C-3, C-4, and C-5. On the basis of this evidence, the structure of crassipin B (2) was established as 2-[[5,7-dihydroxy-2,2-dimethyl-8-butanoyl-6-chromenyl]methyl]-3,5-dihydroxy-4-methyl-4-(7-hydroxy-3,7-dimethyl-2,5-octadienyl)-6-butanoyl-2,5-cyclohexadien-1-one. The absolute configuration of compounds 2 and 4–7 at C-4 was assumed to be *R*, as in crassipin A (1).

The HREIMS of crassipin C (3a) disclosed the molecular formula  $\text{C}_{42}\text{H}_{36}\text{O}_8$  ( $m/z$  688.3968, calcd 688.3977). The NMR data of compound 3a (Tables 1 and 2) showed close similarities to those of crassipin A (1), with the exception of additional signals due to a 4-methyl-3-pentenyl group ( $\delta$  1.88, 1H, ddd,  $J = 14, 11, 6$  Hz; 1.79, 1H, ddd,  $J = 14, 12, 6$  Hz; 2.19, 1H, brtdd,  $J = 12.5, 7.5, 6$  Hz; 2.14, 1H, brtdd,  $J = 12.5, 7.5, 6$  Hz; 5.14, 1H, t,  $J = 7$  Hz; 1.65, 3H, s; 1.584, 3H, s). The location of this residue at C-2' was established through long-range H–C correlations observed between  $\text{CH}_2$ -12' and carbons 2' and 11'. Compound 3a was then elucidated as 2-[[5,7-dihydroxy-2-methyl-2-(4-methyl-3-pentenyl)-8-butanoyl-6-chromenyl]methyl]-3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-butanoyl-2,5-cyclohexadien-1-one. The CD spectrum of 3a was identical to that of crassipin A (1) (Figure 3), indicating that (a) this compound has the same absolute configuration (*R*) at C-4 and (b) the additional chiral center at C-2' has no relevant contribution to the CD spectrum, and thus the above-mentioned technique cannot be used to establish its absolute configuration.

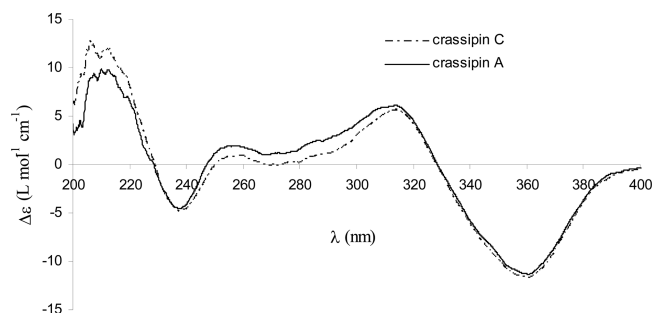


Figure 3. Comparison of the experimental CD spectra of compounds 1 and 3a in ethanol.

The vibrational circular dichroism (VCD) spectrum of 3a was then measured with the aim of establishing the absolute configuration at C-2'. Both isomers at C-2' were modeled with Hyperchem.<sup>22</sup> A conformer search was carried out for each epimer at the molecular mechanics level of theory. Conformers with energy within 1 kcal/mol of the lowest energy conformer were selected. Their geometry was optimized, and then frequencies, vibrational strengths, and rotational strengths were calculated using Gaussian 03.<sup>23</sup> The VCD spectrum of each conformer was built using Lorentzian bands. Contributions of each conformer to the final VCD spectrum were calculated using the Boltzmann distribution equation. The VCD spectrum used for comparison with the experimental VCD was obtained as a sum of the Boltzmann-weighted VCD spectra of selected conformers. The results are shown in Figure 4. Both isomers

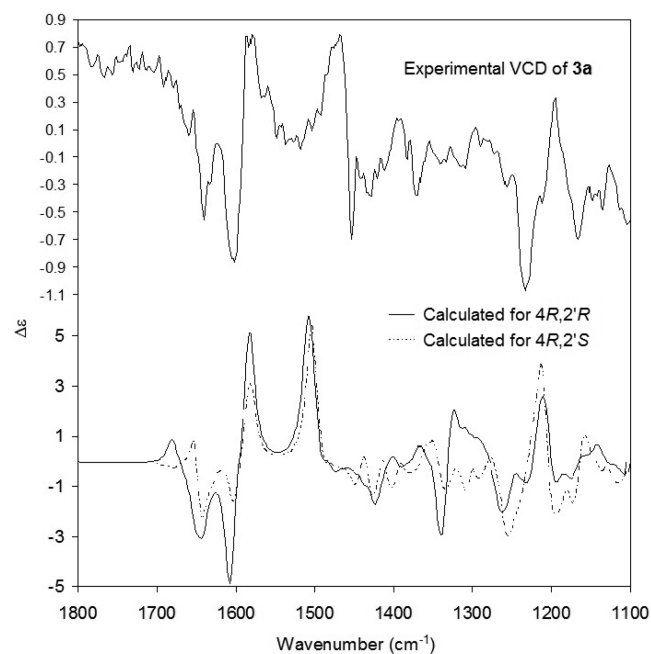
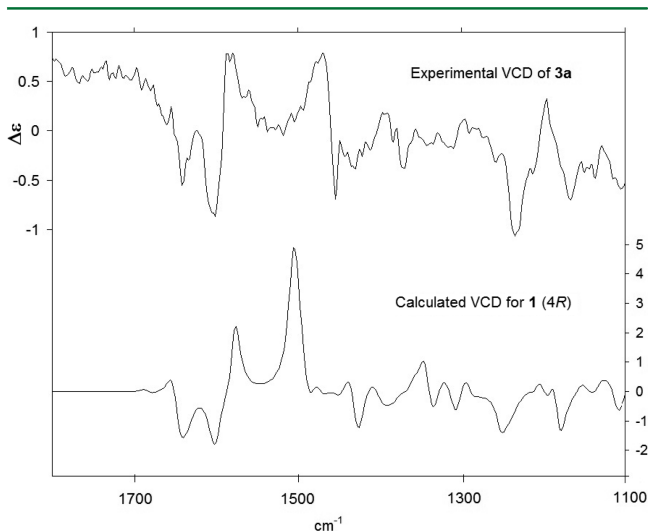


Figure 4. Experimental VCD spectrum of compound 3a ( $\text{CHCl}_3$  solution) in comparison with the calculated ECD spectra (B3LYP/6-31G\*) of both hypothetical diastereomers (4*R*,2'*R* and 4*R*,2'*S*).

gave similar spectra, indicating once more that the stereogenic center at C-2' has no significant contribution to the VCD spectrum at the range studied. Further evidence was obtained from comparison of the simulated VCD spectra of crassipin A (1) with the experimental VCD of crassipin C (3a). VCD spectra were calculated using the method described previously.

The spectrum of the 4*R* isomer was similar to that of crassipin C (**3a**) (Figure 5), confirming that (a) the absolute configuration



**Figure 5.** Simulated VCD spectrum of crassipin A (**1**) in comparison with the experimental VCD of compound **3a** ( $\text{CHCl}_3$  solution).

of crassipin A (**1**) at C-4 is *R* and (b) the chiral center at C-2' has no relevant contribution to the VCD spectrum of **3a**. Thus, the absolute configuration of **3a** at C-2' could not be established.

Crassipin D (**3b**) gave the same molecular formula as **3a**, indicating that these two compounds are isomers. The 500 MHz  $^1\text{H}$  NMR spectrum of crassipin D (**3b**) showed small but noticeable differences from that of crassipin C (**3a**), not only in the chemical shifts but in the shape of the signals, indicating that **3a** and **3b** are different compounds. A significant difference was observed in the signal assigned to H-3' ( $\delta$  5.55, dd,  $J$  = 10.0, 3.0 Hz) in the proton-NMR spectrum of **3b**, while the corresponding signal was a doublet ( $J$  = 10.0 Hz) for **3a**. Moreover, the specific rotation values were very different for **3b** (+70.0) and for **3a** (+12.4), suggesting that these substances are epimeric at C-2'. The experimental CD and VCD spectra of **3b** were identical to those of **3a** (S37 and S38, Supporting Information).

The HRFABMS of crassipin E (**4**) indicated the same molecular formula as compound **1**. The 1D-NMR data of this compound (Tables 2 and 3) were very similar to those of **1** except for the chemical shifts of the signals assigned to OH-5' ( $\delta$  14.21) and OH-7' ( $\delta$  11.77), suggesting that cyclization to form the 2,2-dimethylchromene unit occurs at the hydroxy group close to the methylene bridge. The signal for the OH-5' proton at  $\delta$  14.21 clearly suggested its location close to the carbonyl group attached to C-6'. Further evidence was obtained from the HMBC spectrum of **4**, which showed a correlation between OH-5' and C-1". Thus, the structure of compound **4** was established as 2-[[5,7-dihydroxy-2,2-dimethyl-6-butanoyl-8-chromenyl]methyl]-3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-butanoyl-2,5-cyclohexadien-1-one. Acetylation of crassipin E (**4**) with  $\text{Ac}_2\text{O}$  in pyridine gave a penta-acetylated derivative (**9**), for which the spectroscopic data were consistent with the structure proposed for **4**.

An accurate mass measurement of the molecular ion of crassipin F (**5a**) in the positive-ion HREIMS gave  $m/z$  638.3427, consistent with a molecular formula of  $\text{C}_{37}\text{H}_{50}\text{O}_9$ .

The  $^1\text{H}$  NMR spectrum of this compound (Table 3) was very similar to that of **4**, except for the doublets assigned to the vinyl protons of the chromene-type ring at  $\delta$  6.69 and 5.65 (1H each, d,  $J$  = 10 Hz) in compound **4**, which were absent in the  $^1\text{H}$  NMR spectrum of **5a**. Additional signals of the adjacent methylene and methine groups were detected at  $\delta$  3.90 (1H, dt,  $J$  = 6.5, 5 Hz), 2.54 (1H, dd,  $J$  = 17, 5 Hz), and 2.58 (1H, dd,  $J$  = 17, 6.5 Hz) in the  $^1\text{H}$  NMR spectrum of **5a**. Accordingly, the elemental formula of this compound accounted for 13 degrees of unsaturation, compared to 14 for compound **4**. The carbinol vicinal to C-2' was evident from the signal at  $\delta$  69.5 in the  $^{13}\text{C}$  NMR spectrum of **5a** (Table 2). The  $^1\text{H}$  NMR spectrum of crassipin F showed a doublet at  $\delta$  4.41 (1H, d,  $J$  = 5.0 Hz) assigned to a proton not bound to any carbon, as deduced from its HSQC spectrum, and was thus assigned to the C-2' hydroxy proton. Long-range correlations were observed between  $\text{CH}_2$ -4' and C-10', C-2', and C-3'. Additional HMBC cross-peaks were observed between Me-11' and Me-12', and the signals were assigned to C-2' and C-3', supporting the location of the hydroxy group at C-3'. The proposed structure was confirmed by comparison of the spectroscopic data of **5a** with those of sarothralen C, an acylphloroglucinol with a chromanol-type ring isolated from *Hypericum japonicum*.<sup>11</sup> Hence, the structure of crassipin F (**5a**) was elucidated as 2-[[3,5,7-trihydroxy-2,2-dimethyl-6-butanoyl-8-chromanyl]-methyl]-3,5-dihydroxy-4-methyl-4-(3,7-dimethyl-2,6-octadienyl)-6-butanoyl-2,5-cyclohexadien-1-one. The absolute configuration of this acylphloroglucinol at C-3' was not established.

Crassipin G (**5b**) was obtained as a yellow oil. Its molecular formula was deduced as  $\text{C}_{37}\text{H}_{50}\text{O}_9$  from a molecular ion peak at  $m/z$  638.3457 in the HREIMS (calcd 638.3456). The 1D-NMR spectra of this compound (Tables 2 and 3) resembled those of **5a**. In the  $^{13}\text{C}$  NMR spectrum of **5b**, a slight difference occurred in the chemical shift of the carbinol carbon located at  $\delta$  70.3 and that of a methyl carbon of the dimethyl chromane ( $\delta$  21.2). Differences were also detected in the  $^1\text{H}$  NMR spectrum of **5b** compared to that of **5a** in the environment of the stereogenic center ( $\text{CH}_2$ -4', Me-11', and Me-12', Table 3). On the basis of the aforementioned evidence, crassipin G (**5b**) was determined to be the epimer of **5a** at C-3'.

The molecular formula of crassipin H (**6**),  $\text{C}_{42}\text{H}_{58}\text{O}_9$ , was obtained from its HREIMS ( $m/z$  706.4042, calcd 706.4082) and accounted for 14 degrees of unsaturation. Spectroscopic evidence indicated that the acylfilicin acid-type ring of this acylphloroglucinol was the same as that of the compounds previously described. Signals assigned to protons of a methylene bridge ( $\delta$  3.96 and 3.49, 1H each, d,  $J$  = 14.5 Hz) and to two carbonyl carbons at  $\delta$  207.5 and 204.2 were observed in the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **6**, respectively (Tables 3 and 2), indicating the presence of a second ring. Evidence for the presence of a terpenoid moiety was clear from the  $^1\text{H}$  NMR spectrum of this acylphloroglucinol (Table 3). The unsaturations associated with the rings and acyl groups added up to 12. Thus, there were two unsaturations that could be attributed to the terpenoid moiety, accounting for two additional rings. Two oxygen-bearing quaternary carbons were detected in the  $^{13}\text{C}$  NMR spectrum, at  $\delta$  89.1 and 78.8 (Table 2), while the HSQC spectrum of **6** indicated that the remaining carbons included three  $\text{CH}_3$ , three  $\text{CH}_2$ , and two CH groups. COSY correlations between the protons of the side chain were used to build a part of the chain (Figure 6a). Both methine protons showed correlations with protons linked to two carbon atoms each, so it was inferred that they are also bonded to a

Table 3. <sup>1</sup>H NMR Data of Compounds 4–6 (acetone-*d*<sub>6</sub>, 500 MHz)

position	4	5a	5b	6
7	3.60, d, <i>J</i> = 15.8 3.55, d, <i>J</i> = 15.8	3.57, d, <i>J</i> = 15.5 3.53, d, <i>J</i> = 15.5	3.56, d, <i>J</i> = 15.5 3.52, d, <i>J</i> = 15.5	3.96, d, <i>J</i> = 14.5 3.49, d, <i>J</i> = 14.5
8	1.56, s	1.56, s	1.56, s	1.30, s
9	2.76, dd, <i>J</i> = 13.2, 9.0 2.60, dd, <i>J</i> = 13.2, 7.0	2.72, dd, <i>J</i> = 13.5, 8.0 2.66, dd, <i>J</i> = 13.5, 8.0	2.76, dd, <i>J</i> = 13.2, 9.0 2.62, dd, <i>J</i> = 13.2, 7.0	2.56, dd, <i>J</i> = 13.5, 7.8 2.47, dd, <i>J</i> = 13.5, 7.8
10	4.66, brt, <i>J</i> = 7.5	4.70, t, <i>J</i> = 8.0	4.68, t, <i>J</i> = 7.5	4.69, td, <i>J</i> = 7.5, 1.0
12	1.61–1.48 <sup>a</sup>	1.62–1.51 <sup>a</sup>	1.63–1.46 <sup>a</sup>	1.67–1.62 <sup>a</sup>
13	1.61–1.48 <sup>a</sup>	1.62–1.51 <sup>a</sup>	1.63–1.46 <sup>a</sup>	1.76–1.69 <sup>a</sup>
14	4.82, t, <i>J</i> = 7.0	4.86, brs	4.83, t, <i>J</i> = 7.0	4.92, tt, <i>J</i> = 7.0, 1.5
16	1.56, s	1.58, s	1.57, s	1.60, s
17	1.43, s	1.46, s	1.44, s	1.49, s
18	1.38, s	1.39, s	1.38, s	1.39, s
20	3.23, dt, <i>J</i> = 16.0, 7.5 3.11–3.02 <sup>a</sup>	3.22, ddd, <i>J</i> = 15.5, 8.0, 7.0 3.10, ddd, <i>J</i> = 15.5, 8.0, 7.0	3.22, ddd, <i>J</i> = 15.5, 8.0, 7.0 3.09, ddd, <i>J</i> = 15.5, 8.0, 7.0	3.20, ddd, <i>J</i> = 16.0, 8.5, 6.5 2.95, brdt, <i>J</i> = 16.0, 7.5
21	1.69, sext., <i>J</i> = 7.5	1.75–1.63 <sup>a</sup>	1.73–1.64 <sup>a</sup>	1.67–1.59 <sup>a</sup>
22	0.98, t, <i>J</i> = 7.5	0.98, t, <i>J</i> = 7.0	0.98, t, <i>J</i> = 7.0	0.96, t, <i>J</i> = 7.5
3'	5.65, d, <i>J</i> = 10.0	3.90, dt, <i>J</i> = 6.5, 5.0	3.86, dt, <i>J</i> = 8.0, 5.5	
4'	6.69, d, <i>J</i> = 10.0	2.84, dd, <i>J</i> = 17.0, 5.0 2.58, dd, <i>J</i> = 17.0, 6.5	2.90, dd, <i>J</i> = 16.8, 5.5 2.50, dd, <i>J</i> = 16.8, 8.0	
8'				3.20, ddd, <i>J</i> = 16.5, 8.5, 6.5 2.95, ddd, <i>J</i> = 16.5, 8.5, 6.5
9'				1.76–1.67 <sup>a</sup>
10'				0.99, t, <i>J</i> = 7.5
11'	1.66, s	1.49, s	1.29, s	
12'	1.54, s	1.48, s	1.41, s	
1''				2.75, brt, <i>J</i> = 2.0
2''	3.15, ddd, <i>J</i> = 17.0, 8.5, 6.5 3.11–3.02 <sup>a</sup>	3.16, ddd, <i>J</i> = 17.0, 8.0, 6.5 3.07, ddd, <i>J</i> = 17.0, 8.0, 6.5	3.15, ddd, <i>J</i> = 17.0, 8.5, 6.5 3.06, ddd, <i>J</i> = 17.0, 8.5, 6.5	2.10–2.05 <sup>a</sup> 2.02, dd, <i>J</i> = 13.2, 2.0
3''	1.68, brsxt., <i>J</i> = 7.5	1.75–1.63 <sup>a</sup>	1.73–1.64 <sup>a</sup>	
4''	0.99, t, <i>J</i> = 7.5	0.99, t, <i>J</i> = 7.0	0.99, t, <i>J</i> = 7.5	1.79–1.72 <sup>a</sup> 1.64–1.56 <sup>a</sup>
5''				1.41–1.35 <sup>a</sup> 0.74, brqd, <i>J</i> = 13.5, 6.0
6''				2.26, ddd, <i>J</i> = 11.5, 5.0, 2.5
8''				1.62, s
9''				1.16, s
10''				1.39, s
OH-3	9.12, s	9.30, s	9.29, s	9.68, s
OH-5	18.68, s	18.65, s	18.66, s	19.42, s
OH-3'		4.41, d, <i>J</i> = 5.0	4.52, d, <i>J</i> = 5.5	
OH-4'				15.29, s
OH-5'	14.21, s	14.18, s	14.18, s	
OH-7'	11.77, s	11.38, s	11.40, s	

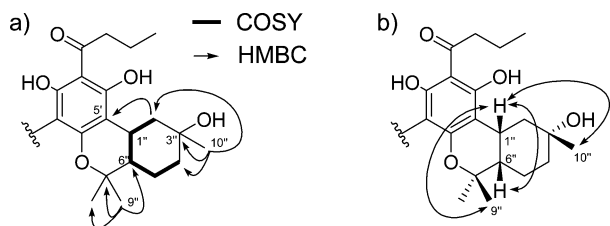
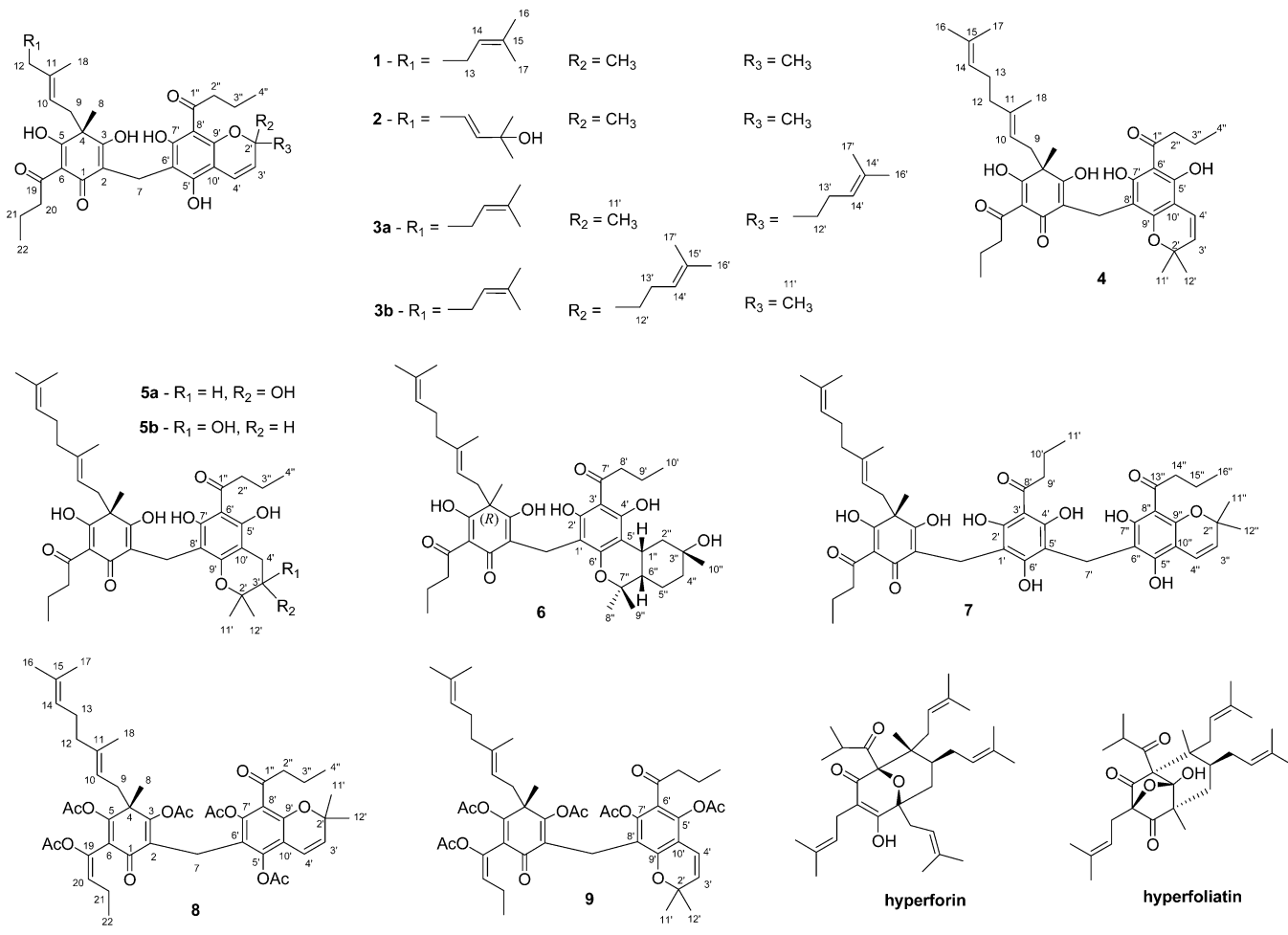
<sup>a</sup>Overlapping signals.

quaternary carbon. A similar situation was observed for methylenes 2'' and 4'', which displayed correlations with protons linked to one carbon only, and thus the other carbon to which they were bonded is quaternary. <sup>3</sup>J H–C correlations observed between the methyl groups at C-3'' and C-7'' (i.e., CH<sub>3</sub>-8'', CH<sub>3</sub>-9'', and CH<sub>3</sub>-10'') were key to the assignment of the terpenoid moiety. HMBC cross-peaks were observed between CH<sub>3</sub>-10'' and C-2'', C-3'' and C-4''. Other <sup>3</sup>J H–C correlations were detected between both H-8'' and H-9'' and C-6'' and C-7'' (Figure 6a). The relative stereochemistry of 6, as proposed, was established by a two-dimensional NOESY experiment. Specifically, correlations were observed between H-1'' and the signals assigned to H-6'', CH<sub>3</sub>-9'', and CH<sub>3</sub>-10'' (Figure 6b).

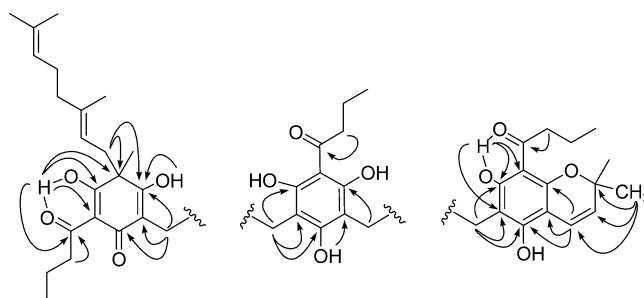
Crassipin I (7) gave a molecular ion peak at *m/z* 828.4096 in its HRFABMS, indicating a molecular formula of C<sub>48</sub>H<sub>60</sub>O<sub>12</sub>

(calcd 828.4086). Signals for two methylene bridges were observed ( $\delta$  3.59, 1H, d, *J* = 15.5 Hz; 3.54, 1H, d, *J* = 15.5 Hz; 3.78, 2H, s) in the <sup>1</sup>H NMR spectrum of 7. The signals assigned to CH<sub>2</sub>-7 were characteristic of a methylene group linked to a hexadienone and an aromatic ring ( $\delta$  3.59, 1H, d, *J* = 15.5 Hz and 3.54, 1H, d, *J* = 15.5 Hz), while the signal at  $\delta$  3.78 indicated that the other methylene bridge connects two aromatic rings. The 1D-NMR spectroscopic data of 7 resembled those of yungensin G, a compound previously isolated from *Elaphoglossum yungense*,<sup>4</sup> with the only difference being the signals assigned to the acyl moiety attached to C-6. In compound 7 this group was identified as a butanoyl residue, as signals for three methyl groups were observed at  $\delta$  0.98, 1.00, and 1.01 (3H each, t, *J*  $\approx$  8.0 Hz). The chemical shifts of these signals indicated that they arise from methyl groups of butanoyl

Chart 1



**Figure 6.** Significant 2D NMR correlations of compound 6: (a) COSY and HMBC, (b) NOESY.



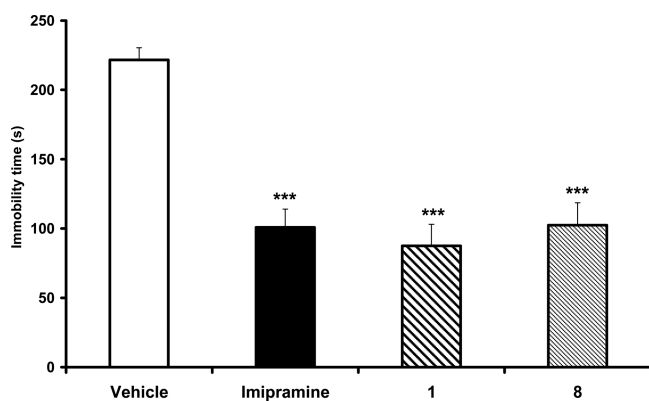
**Figure 7.** Relevant HMBC correlations of crassinin I (7).

moieties, as was reported previously for other acylphloroglucinols.<sup>5</sup> Relevant HMBC correlations observed for compound 7 are shown in Figure 7. Thus, the structure of crassinin I (7) was elucidated as depicted.

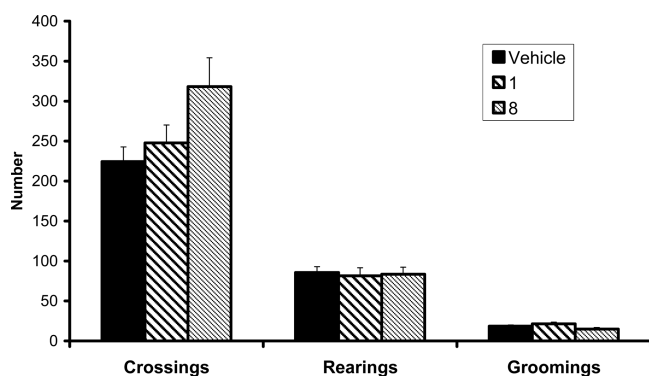
From the five Argentine species of the genus *Elaphoglossum* analyzed thus far, 26 acylphloroglucinols have been isolated. It is noteworthy that no single phloroglucinol derivative has been found to be present in more than one species.

The results illustrated in Figure 8 show that crassinin A (1) and its acetylated derivative (8) (15 mg/kg, po) significantly reduced the immobility of mice in a forced-swimming test (FST) (ANOVA;  $p < 0.001$ ) and were compared with imipramine (20 mg/kg, po), an established antidepressant drug. Immobility in the FST is considered as an expression of “behavioral despair”, which could be a component of depression syndrome.<sup>24</sup> Furthermore, these two phloroglucinol derivatives did

not alter the numbers of crossing, rearing, and grooming in a subsequent open field test, as shown in Figure 9, revealing that their anti-immobility effect was not related to a nonspecific behavioral stimulation. This observation points to an antidepressant-like effect of these dimeric acylphloroglucinols, which can represent a new chemical scaffold related to antidepressant activity. It is also important to note that 1 and 8 exhibit dimeric structures consisting of an acylphloroglucinol moiety and an acylfilicin acid-type ring that differs from hyperforin, which exhibits a bicyclononane skeleton with several isoprene chains.<sup>25</sup> Structural differences between 1 and 8 and hyperforin could be especially relevant since the latter has been considered to be responsible for serious drug interactions, which restrict the clinical use of *H. perforatum*.<sup>26</sup> It is noteworthy that crassinin A (1) constituted 19.3% w/w of



**Figure 8.** Effect of single doses of crassipin A (**1**) and its acetylated derivative (**8**) (15 mg/kg po); imipramine (20 mg/kg po); or vehicle (1 mL/100 g po) in the mouse forced swimming test. The results are presented as means  $\pm$  SEM ( $n = 8$ –10 mice/group). Significantly different values were detected by one-way ANOVA followed by a Student–Newman–Keuls test. \*\*\* $p < 0.001$  when compared to the vehicle group.



**Figure 9.** Effect of single doses of crassipin A (**1**) and its acetylated derivative (**8**) (15 mg/kg po) or vehicle (1 mL/100 g po) on the number of crossing, rearing, and grooming events assessed in the mouse open field test. The results are presented as means  $\pm$  SEM ( $n = 8$ –10 mice/group).

the Et<sub>2</sub>O extract of *E. crassipes* and 1.2% w/w of the plant material.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were measured on a JASCO P-1030 digital polarimeter. UV and CD spectra were recorded on a JASCO V-550 spectrometer and a JASCO J-810 spectropolarimeter, respectively. VCD spectra were registered on a ChiralIR 2X VCD spectrometer (BioTools, Inc.) equipped with a Dual PEM, with 4 cm<sup>-1</sup> resolution. Infrared spectra were recorded on a Shimadzu FT/IR-8400S spectrometer by the diffuse reflectance method. NMR spectra were measured at 600 or 500 MHz for <sup>1</sup>H and 150 or 125 MHz for <sup>13</sup>C on a Varian Unity 600 and a Varian Unity 500, using acetone-*d*<sub>6</sub> or CDCl<sub>3</sub> as solvent and TMS as internal standard for the latter. MS analyses were conducted on a JEOL JMS AX-500 spectrometer. Column chromatography was carried out over silica gel 60 (70–230 mesh, Merck), using an *n*-hexane–EtOAc gradient as mobile phase. The eluate was monitored by TLC on glass precoated plates F<sub>254</sub>. Godin reagent was used to visualize the spots.<sup>27</sup> Preparative HPLC was carried out on a Gilson equipment, using a silica gel column (Chemcopak; Chemcosorb 5 Si–U, 5  $\mu$ m, 250  $\times$  10 mm i.d.) and ultraviolet and refractive index detectors in parallel.

**Plant Material.** *Elaphoglossum crassipes* was collected near La Banderita, Tucumán, Argentina, in December 2007. The plant material was identified by Lic. Marcela Hernández de Terán, and a voucher

specimen (LIL 609962) has been deposited at the Herbarium of the Instituto Miguel Lillo, Tucumán, Argentina.

**Extraction and Isolation.** The dried and ground rhizomes and roots of *E. crassipes* (92 g) were extracted with Et<sub>2</sub>O to afford 5.8 g of an extract. This crude extract was subjected to column chromatography over silica gel using a *n*-hexane–EtOAc gradient as mobile phase to give four phloroglucinol-containing fractions. A portion (240 mg) of fraction I (1.65 g) was purified by normal-phase HPLC (*n*-hexane–EtOAc, 99:1, 3.0 mL/min), yielding **1** (163 mg), **3a** (14.4 mg), and **3b** (13.4 mg). The processing of fraction II (223 mg) by normal-phase HPLC (*n*-hexane–EtOAc, 97:3, 3.0 mL/min) led to the isolation of phloroglucinol **4** (63.6 mg). Fraction III (223 mg), on purification by HPLC (*n*-hexane–EtOAc, 92:8, 3.0 mL/min), yielded compounds **6** (1.4 mg) and **7** (8.2 mg). Chromatography of fraction IV (180 mg) by HPLC (*n*-hexane–EtOAc, 85:15, 3.0 mL/min) gave **2** (8.2 mg), **5a** (6.6 mg), and **5b** (8 mg).

**Crassipin A (1):** yellow oil; [ $\alpha$ ]<sub>D</sub><sup>22</sup> +38.7 (*c* 1.0, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3161, 3057, 2706, 2644, 1641, 1603, 1458, 1431, 1363, 1192, 1132 cm<sup>-1</sup>; UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 358 (4.40), 290 (4.52), 225 (4.60) nm; CD (1.83  $\times 10^{-5}$  M, EtOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 210 (+9.85), 238 (–4.59), 258 (+1.94), 314 (+6.16), 360 (–11.34) nm; <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>) data, see Table 1 and Figure S1, Supporting Information; <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>) data, see Table 2 and Figure S2, Supporting Information; HRFABMS *m/z* 621.3450 [M + H]<sup>+</sup> (calcd for C<sub>37</sub>H<sub>48</sub>O<sub>8</sub>, 621.3429).

**Crassipin B (2):** orange oil; [ $\alpha$ ]<sub>D</sub><sup>22</sup> +3.4 (*c* 1.0, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3427, 3192, 2723, 2650, 1643, 1606, 1462, 1429, 1373, 1134 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>) data, see Table 1; <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>) data, see Table 2; HRFABMS *m/z* 659.3185 [M + Na]<sup>+</sup> (calcd for C<sub>37</sub>H<sub>48</sub>O<sub>9</sub>Na, 659.3197).

**Crassipin C (3a):** yellow oil; [ $\alpha$ ]<sub>D</sub><sup>22</sup> +12.4 (*c* 1.0, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3165, 3060, 2712, 2648, 1641, 1601, 1429, 1375 cm<sup>-1</sup>; UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 360 (4.22), 292 (4.36), 225 (4.46) nm; CD (2.62  $\times 10^{-5}$  M, EtOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 206 (+14.29), 213 (+13.34), 238 (–5.32), 259 (+1.18), 314 (+6.38), 360 (–12.92) nm; VCD (CHCl<sub>3</sub>, 28 mg/mL), see Figure 4; <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>) data, see Table 1; <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>) data, see Table 2; HREIMS *m/z* 688.3968 [M]<sup>+</sup> (calcd for C<sub>42</sub>H<sub>56</sub>O<sub>8</sub>, 688.3977).

**Crassipin D (3b):** yellow oil; [ $\alpha$ ]<sub>D</sub><sup>21</sup> +70.0 (*c* 1.0, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3167, 3057, 2710, 2648, 1641, 1601, 1429, 1373 cm<sup>-1</sup>; UV (EtOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 360 (4.26), 292 (4.40), 225 (4.50) nm; CD (2.91  $\times 10^{-5}$  M, EtOH)  $\lambda_{\max}$  ( $\Delta\epsilon$ ) 207 (+9.23), 212 (+8.96), 237 (–3.74), 254 (+1.58), 312 (+7.16), 359 (–11.48) nm; VCD (CHCl<sub>3</sub>, 27 mg/mL), see Figure 4; <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>) data, see Table 1; <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>) data, see Table 2; HREIMS *m/z* 688.3973 [M]<sup>+</sup> (calcd for C<sub>42</sub>H<sub>56</sub>O<sub>8</sub>, 688.3977).

**Crassipin E (4):** yellow oil; [ $\alpha$ ]<sub>D</sub><sup>22</sup> +6.2 (*c* 1.0, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3242, 3053, 2731, 2656, 1643, 1605, 1462, 1435, 1194, 1142 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>) data, see Table 3; <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>) data, see Table 2; HRFABMS *m/z* 621.3434 [M + H]<sup>+</sup> (calcd for C<sub>37</sub>H<sub>48</sub>O<sub>8</sub>, 621.3429).

**Crassipin F (5a):** orange oil; <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>) data, see Table 3; <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>) data, see Table 2; HREIMS *m/z* 638.3427 [M]<sup>+</sup> (calcd for C<sub>37</sub>H<sub>50</sub>O<sub>9</sub>, 638.3456).

**Crassipin G (5b):** orange oil; [ $\alpha$ ]<sub>D</sub><sup>22</sup> –4.3 (*c* 1.0, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3387, 3240, 2731, 2660, 1641, 1616, 1460, 1431, 1377, 1128 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>) data, see Table 3; <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>) data, see Table 2; HREIMS *m/z* 638.3457 [M]<sup>+</sup> (calcd for C<sub>37</sub>H<sub>50</sub>O<sub>9</sub>, 638.3456).

**Crassipin H (6):** yellow oil; <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>) data, see Table 3; <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>) data, see Table 2; HREIMS *m/z* 706.4042 [M]<sup>+</sup> (calcd for C<sub>42</sub>H<sub>58</sub>O<sub>9</sub>, 706.4082).

**Crassipin I (7):** yellow oil; [ $\alpha$ ]<sub>D</sub><sup>24</sup> –2.6 (*c* 1.0, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3230, 2632, 1636, 1608, 1558, 1454, 1375, 1171, 1134 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, acetone-*d*<sub>6</sub>) data, see Table 4; <sup>13</sup>C NMR (125 MHz, acetone-*d*<sub>6</sub>) data, see Table 4; HRFABMS *m/z* 828.4096 [M]<sup>+</sup> (calcd for C<sub>48</sub>H<sub>60</sub>O<sub>12</sub>, 828.4086).

**Acetylation of 1.** Compound **1** (17 mg) was dissolved in 1 mL of pyridine, with 1 mL of Ac<sub>2</sub>O added, and the mixture stirred at room temperature overnight. The reaction mixture was dried, and the product



Table 4. 1D NMR Data of Compound 7 (acetone-*d*<sub>6</sub>, 500 MHz)

position	$\delta$ [ppm], multiplicity, <i>J</i> [Hz]	$\delta_C$ [ppm], mult.	position	$\delta$ [ppm], multiplicity, <i>J</i> [Hz]	$\delta_C$ [ppm], mult.
1		190.1, C	5'		105.2, C
2		115.2, C	6'		161.9, C
3		172.1, C	7'	3.78, s	17.6, CH <sub>2</sub>
4		51.0, C	8'		209.6, C
5		200.2, C	9'	3.15, brt, <i>J</i> = 7.5	47.6, CH <sub>2</sub>
6		111.7, C	10'	1.72, sext., <i>J</i> = 7.5	20.0, CH <sub>2</sub>
7	3.59, d, <i>J</i> = 15.5	18.6, CH <sub>2</sub>	11'	1.01, t, <i>J</i> = 7.5	15.2, CH <sub>3</sub>
	3.54, d, <i>J</i> = 15.5		2''		80.3, C
8	1.54, s	23.9, CH <sub>3</sub>	3''	5.60, d, <i>J</i> = 10.0	127.5, CH
9	2.73, dd, <i>J</i> = 13.5, 8.8	40.2, CH <sub>2</sub>	4''	6.63, d, <i>J</i> = 10.0	118.3, CH
	2.57, dd, <i>J</i> = 13.5, 6.5		5''		159.9, C
10	4.60, brs	118.7, CH	6''		107.9, C
11		141.6, C	7''		163.3, C
12	1.52–1.41 <sup>a</sup>	41.3, CH <sub>2</sub>	8''		106.4, C
13	1.52–1.41 <sup>a</sup>	28.4, CH <sub>2</sub>	9''		157.6, C
14	4.74, brs	125.6, CH	10''		105.2, C
15		132.7, C	11''	1.507, s	29.0, CH <sub>3</sub>
16	1.54, s	26.8, CH <sub>3</sub>	12''	1.514, s	29.0, CH <sub>3</sub>
17	1.38, s	18.5, CH <sub>3</sub>	13''		208.7, C
18	1.27, s	16.9, CH <sub>3</sub>	14''	3.11, dd, <i>J</i> = 8.0, 6.5	47.5, CH <sub>2</sub>
19		207.7, C	15''	1.71, brsxt., <i>J</i> = 7.5	20.0, CH <sub>2</sub>
20	3.24, dt, <i>J</i> = 15.5, 8.0	44.4, CH <sub>2</sub>	16''	0.98, t, <i>J</i> = 7.5	15.2, CH <sub>3</sub>
	3.09, dt, <i>J</i> = 15.5, 8.0		OH-3	9.95, s	
21	1.69, sext., <i>J</i> = 8.0	19.6, CH <sub>2</sub>	OH-5	18.70, s	
22	1.00, t, <i>J</i> = 8.0	15.2, CH <sub>3</sub>	OH-2'	16.38, brs	
1'		108.2, C	OH-4'	9.83, brs	
2'		163.3, C	OH-6'	11.42, s	
3'		107.2, C	OH-5''	9.59, s	
4'		160.3, C	OH-7''	16.28, s	

<sup>a</sup>Overlapping signals.

was purified by normal-phase HPLC (*n*-hexane–AcOEt, 7:3, 2.5 mL/min) to afford 13 mg of 8.

**Compound 8:** yellow oil; [ $\alpha$ ]<sub>D</sub><sup>22</sup> +44.3 (*c* 1.0, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  3018, 2967, 2934, 2875, 1780, 1690, 1648, 1363, 1184 cm<sup>-1</sup>; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  3.67 (1H, d, *J* = 14.8 Hz, H-7a), 3.20 (1H, d, *J* = 14.8 Hz, H-7b), 1.18 (3H, s, H-8), 2.47 (1H, dd, *J* = 14.0, 8.8 Hz, H-9a), 2.28 (1H, dd, *J* = 14.0, 7.5 Hz, H-9b), 4.73 (1H, brt, *J* = 5.0 Hz, H-10), 1.87 (2H, t, *J* = 8.0 Hz, H-12), 1.97 (2H, m, H-13), 5.03 (1H, tt, *J* = 6.7, 1.4 Hz, H-14), 1.67 (3H, brs, H-16), 1.57 (3H, s, H-17), 1.48 (3H, s, H-18), 5.37 (1H, t, *J* = 7.4 Hz, H-20), 2.03 (2H, quint., *J* = 7.4 Hz, H-21), 0.99 (3H, t, *J* = 7.4 Hz, H-22), 5.61 (1H, d, *J* = 9.9 Hz, H-3'), 6.10 (1H, d, *J* = 9.9 Hz, H-4'), 1.46 (3H, s, H-11'), 1.45 (3H, s, H-12'), 2.89 (1H, dt, *J* = 17.0, 7.7 Hz, H-2'a), 2.77 (1H, ddd, *J* = 17.0, 8.0, 6.6 Hz, H-2'b), 1.71–1.64 (2H, m, H-3''), 0.96 (3H, t, *J* = 7.4 Hz, H-4''), 2.28, 2.24, 2.20, 2.19, 2.10 (3H each, s, CH<sub>3</sub>C=O); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  183.3 (C, C-1), 128.8 (C, C-2), 161.6 (C, C-3), 47.8 (C, C-4), 163.4 (C, C-5), 125.2 (C, C-6), 18.2 (CH<sub>2</sub>, C-7), 21.6 (CH<sub>3</sub>, C-8), 35.9 (CH<sub>2</sub>, C-9), 117.2 (CH, C-10), 139.0 (C, C-11), 39.8 (CH<sub>2</sub>, C-12), 26.7 (CH<sub>2</sub>, C-13), 124.1 (CH, C-14), 131.5 (C, C-15), 25.7 (CH<sub>3</sub>, C-16), 17.7 (CH<sub>3</sub>, C-17), 16.0 (CH<sub>3</sub>, C-18), 136.2 (C, C-19), 127.4 (CH, C-20), 19.9 (CH<sub>2</sub>, C-21), 13.2 (CH<sub>3</sub>, C-22), 77.5 (C, C-2'), 130.9 (CH, C-3'), 116.0 (CH, C-4'), 146.2 (C, C-5'), 118.6 (C, C-6'), 145.3 (C, C-7'), 121.6 (C, C-8'), 149.4 (C, C-9'), 113.2 (C, C-10'), 28.1 (CH<sub>3</sub>, C-11'), 27.8 (CH<sub>3</sub>, C-12'), 202.9 (C, C-1''), 45.6 (CH<sub>2</sub>, C-2''), 17.2 (CH<sub>2</sub>, C-3''), 13.8 (CH<sub>3</sub>, C-4''), 168.4, 168.2, 167.3, 166.6 (CH<sub>3</sub>C=O), 21.2, 20.9, 20.6, 20.4 (CH<sub>3</sub>C=O); HRFABMS *m/z* 853.3793 [M + Na]<sup>+</sup> (calcd for C<sub>47</sub>H<sub>58</sub>O<sub>13</sub>Na, 853.3776).

**Acetylation of 4.** Compound 4 (10 mg) was acetylated using the method described for the acetylation of compound 1. The reaction mixture was dried, and the product was purified by normal-phase HPLC (*n*-hexane–AcOEt, 7:3, 3.0 mL/min) to afford 7.2 mg of 9.

**Compound 9:** yellow oil; [ $\alpha$ ]<sub>D</sub><sup>23</sup> +24.3 (*c* 1.0, CHCl<sub>3</sub>); IR (neat)  $\nu_{\max}$  2966, 2935, 2876, 1776, 1693, 1649, 1366, 1182, 1144 cm<sup>-1</sup>; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  3.58 (1H, d, *J* = 15.5 Hz, H-7a), 3.53 (1H, d, *J* = 15.5 Hz, H-7b), 1.19 (3H, s, H-8), 2.47 (1H, dd, *J* = 14.6, 8.8 Hz, H-9a), 2.26 (1H, dd, *J* = 14.6, 6.8 Hz, H-9b), 4.87 (1H, brt, *J* = 6.8 Hz, H-10), 1.87 (2H, m, H-12), 1.97 (2H, m, H-13), 5.02 (1H, hept., *J* = 6.9, 1.4 Hz, H-14), 1.66 (3H, d, *J* = 1.4, H-16), 1.57 (3H, s, H-17), 1.53 (3H, s, H-18), 5.30 (1H, t, *J* = 7.4 Hz, H-20), 2.02 (2H, quint., *J* = 7.4 Hz, H-21), 0.98 (3H, t, *J* = 7.4 Hz, H-22), 5.63 (1H, d, *J* = 10.1 Hz, H-3'), 6.14 (1H, d, *J* = 10.1 Hz, H-4'), 1.38 (3H, s, H-11'), 1.38 (3H, s, H-12'), 2.64 (2H, t, *J* = 7.4 Hz, H-2''), 1.63 (2H, sext., *J* = 7.4 Hz, H-3''), 0.92 (3H, t, *J* = 7.4 Hz, H-4''), 2.26, 2.22, 2.20, 2.15, 2.10 (3H each, s, CH<sub>3</sub>C=O); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>)  $\delta$  183.3 (C, C-1), 128.4 (C, C-2), 160.7 (C, C-3), 47.7 (C, C-4), 163.8 (C, C-5), 125.6 (C, C-6), 18.4 (CH<sub>2</sub>, C-7), 21.9 (CH<sub>3</sub>, C-8), 35.8 (CH<sub>2</sub>, C-9), 117.5 (CH, C-10), 138.9 (C, C-11), 40.0 (CH<sub>2</sub>, C-12), 26.8 (CH<sub>2</sub>, C-13), 124.1 (CH, C-14), 131.4 (C, C-15), 25.6 (CH<sub>3</sub>, C-16), 17.7 (CH<sub>3</sub>, C-17), 16.0 (CH<sub>3</sub>, C-18), 136.2 (C, C-19), 127.1 (CH, C-20), 20.0 (CH<sub>2</sub>, C-21), 13.2 (CH<sub>3</sub>, C-22), 77.6 (C, C-2'), 131.5 (CH, C-3'), 115.6 (CH, C-4'), 141.8 (C, C-5'), 121.1 (C, C-6'), 145.8 (C, C-7'), 119.9 (C, C-8'), 153.2 (C, C-9'), 112.5 (C, C-10'), 27.8 (CH<sub>3</sub>, C-11'), 27.7 (CH<sub>3</sub>, C-12'), 200.9 (C, C-1''), 45.4 (CH<sub>2</sub>, C-2''), 17.2 (CH<sub>2</sub>, C-3''), 13.7 (CH<sub>3</sub>, C-4''), 168.4, 168.3, 167.4, 167.1, 166.0 (CH<sub>3</sub>C=O), 20.90, 20.88, 20.7, 20.62, 20.60 (CH<sub>3</sub>C=O); HRFABMS *m/z* 853.3812 [M + Na]<sup>+</sup> (calcd for C<sub>47</sub>H<sub>58</sub>O<sub>13</sub>Na, 853.3776).

**Computational Details.** Two pairs of epimers with configuration *R* and *S* at C-4 were modeled for crassipin C (3). For each isomer, a conformer search was made using the computer program Hyperchem<sup>22</sup> with a molecular mechanics force field. Geometries of conformers within 1 kcal/mol of the lowest energy conformer were optimized at the B3LYP/6-31G(d) level of theory with Gaussian 03.<sup>23</sup> PCM was used to include solvent effects. IR and VCD frequencies and

intensities were calculated with the same combination of method and basis set at the optimized geometries. Theoretical frequencies were scaled by 0.98, and intensities were converted to Lorentzian bands with 8 cm<sup>-1</sup> half-width. The contribution of each conformer to the simulated spectra was calculated using the Boltzmann distribution equation. The sum of the Boltzmann-weighted spectra of the selected conformers afforded the simulated spectra used for comparison with experimental IR and VCD.

**Forced-Swimming Test.** All behavioral experimental protocols were approved by The Animal Care Local Ethical Committee (CEUA UFRGS; Protocol 18518) and performed according to guidelines of The National Research Ethical Committee (published by National Health Council–MS, 1998) and Brazilian law,<sup>28</sup> which are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the International Guidance Principles for Biomedical Research Involving Animals.<sup>29</sup>

Male CF1 mice (25–30 g) were habituated to laboratory conditions 1 h before being exposed to the forced-swimming test. The experiment was maintained under artificial lighting, at a temperature of 23 ± 2 °C. The FST was conducted using the method described by Porsolt and associates<sup>24</sup> with minor modifications previously standardized and validated.<sup>30</sup> Mice were forced individually to swim in a cylinder pool (10 cm diameter, 13 cm height, 22 ± 1 °C); the total duration of immobility during a 6 min test was scored and determined by a blinded observer. Each mouse was considered to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. Crassipin A (**1**) and its acetylated derivative (compound **8**) were suspended in saline (NaCl 0.9%) with 2% polysorbate 80 immediately before the administration. Different groups of mice (*n* = 8–10) were treated by gavage (10 mL/kg) with crassipin A (**1**) (15 mg/kg), its acetylated derivative (**8**) (15 mg/kg), imipramine (20 mg/kg), or vehicle (NaCl 0.9% plus 2% of polysorbate 80) and submitted to the FST 60 min later. Imipramine (99.9% pure) was purchased from Henrifarma Produtos Químicos e Farmacêuticos LTDA (São Paulo-SP, Brazil).

**Open-Field Test.** In order to rule out any unspecific locomotor effect of crassipin A (**1**) and its acetylated derivative (**8**), mice were treated with doses that produced anti-immobility effects in the FST and evaluated in an open-field test 60 min later. Briefly, mice were placed individually in an acrylic box (40 × 30 × 30 cm), with the floor divided into 24 equal squares. Animals were acclimated for 5 min, and then the number of crossings in the squares with the four paws were measured during a period of 15 min by a blinded human observer.

**Statistical Analysis.** Data obtained in pharmacological experiments were evaluated by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test when appropriate; *p* values less than 0.05 were considered as statistically significant. The analyses were performed using Sigma Stat 2.03 software (Jandel Scientific Corporation).

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

NMR spectra of compounds 1–8, computational details, and a photograph of the scaly rhizomes of *Elaphoglossum crassipes* are available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES

- (1) Widén, C.-J.; Sarvela, J.; Britton, D. M. *Ann. Bot. Fennici* **1983**, *20*, 407–417.
- (2) Socolsky, C.; Borkosky, S. A.; Asakawa, Y.; Bardón, A. *J. Nat. Prod.* **2009**, *72*, 787–790.
- (3) Socolsky, C.; Borkosky, S. A.; Hernández de Terán, M.; Asakawa, Y.; Bardón, A. *J. Nat. Prod.* **2010**, *73*, 901–904.
- (4) Socolsky, C.; Arena, M. E.; Asakawa, Y.; Bardón, A. *J. Nat. Prod.* **2010**, *73*, 1751–1755.
- (5) Socolsky, C.; Cartagena, E.; Asakawa, Y.; Bardón, A. *Arkivoc* **2011**, *2011*, 450–460.
- (6) Jayasuriya, H.; Clark, A. M.; McChesney, J. D. *J. Nat. Prod.* **1991**, *54*, 1314–1320.
- (7) Ishiguro, K.; Nagata, S.; Fukumoto, H.; Yamaki, M.; Isoi, K. *Phytochemistry* **1994**, *35*, 469–471.
- (8) Jayasuriya, H.; McChesney, J. D.; Swanson, S. M.; Pezzuto, J. M. *J. Nat. Prod.* **1989**, *52*, 325–331.
- (9) Widén, C.-J.; Fraser-Jenkins, C.; Reichstein, T.; Gibby, M.; Sarvela, J. *Ann. Bot. Fennici* **1996**, *33*, 69–100.
- (10) Widén, C.-J.; Fraser-Jenkins, C. R.; Reichstein, T.; Sarvela, J. *Acta Bot. Fennica* **1999**, *164*, 1–56.
- (11) Widén, C.-J.; Fraser-Jenkins, C. R.; Reichstein, T.; Sarvela, J. *Ann. Bot. Fennici* **2001**, *38*, 99–138.
- (12) Euw, J. v.; Reichstein, T.; Widén, C.-J. *Helv. Chim. Acta* **1985**, *68*, 1251–1275.
- (13) Socolsky, C.; Borkosky, S.; Bardón, A. *Nat. Prod. Commun.* **2011**, *6*, 387–391.
- (14) Ishiguro, K.; Nagata, S.; Fukumoto, H.; Yamaki, M.; Isoi, K. *Phytochemistry* **1994**, *35*, 469–471.
- (15) Di Carlo, G.; Borrelli, F.; Ernst, E.; Izzo, A. A. *Trends Pharmacol. Sci.* **2001**, *22*, 292–297.
- (16) Zanolli, P. *CNS Drugs* **2004**, *10*, 203–218.
- (17) Do Rego, J. C.; Benkiki, N.; Chosson, E.; Kabouche, Z.; Seguin, E.; Costentin, J. *Eur. J. Pharmacol.* **2007**, *569*, 197–203.
- (18) Lam, R. W.; Wan, D. D.; Cohen, N. L.; Kennedy, S. H. *J. Clin. Psychiat.* **2002**, *63*, 685–693.
- (19) Socolsky, C.; Salvatore, A.; Asakawa, Y.; Bardón, A. *Arkivoc* **2003**, *2003*, 347–355.
- (20) Socolsky, C.; Muruaga, N.; Bardón, A. *Chem. Biodiversity* **2005**, *2*, 1105–1108.
- (21) Rocha, L.; Marston, A.; Potterat, O.; Kaplan, M. A. C.; Hostettmann, K. *Phytochemistry* **1996**, *42*, 185–188.
- (22) *HyperChem Professional 7.51*; Hypercube, Inc.: Gainesville, FL, 2009.
- (23) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.;

- Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; and Pople, J. A. *Gaussian 03*, Revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.
- (24) Porsolt, R. D.; Bertin, A.; Jalfre, M. *Arch. Int. Pharmacodyn. Ther.* **1977**, *229*, 327–336.
- (25) Kieser, M.; Szegedi, A. *Pharmacopsychiatry* **2005**, *38*, 194–200.
- (26) Kasper, S.; Caraci, F.; Forti, B.; Drago, F.; Aguglia, E. *Eur. Neuropsychopharmacol.* **2010**, *20*, 747–765.
- (27) Godin, P. *Nature* **1954**, *174*, 134.
- (28) Lei no. 11.794, de 8 de Outubro de 2008. DOU, Ministério Público, Brasil, 9.10.2008.
- (29) Council for International Organizations of Medical Sciences (CIOMS). In *International Guiding Principles for Biomedical Research Involving Animals*; Bankowski, Z., Howard-Jones, N., Eds.; Geneva, 1986.
- (30) Viana, A. F.; Do Rego, J. C.; Von Poser, G.; Ferraz, A.; Heckler, A. P.; Constantin, J.; Kuze-Rates, S. M. *Neuropharmacology* **2005**, *49*, 1042–1052.