

## Enantioselective synthesis and vanilloid activity evaluation of 1-β-(*p*-methoxycinnamoyl)polygodial, an antinociceptive compound from *Drymis winteri* barks

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This work is dedicated to the memory of Prof. G. Sodano.

**Abstract**—A simple strategy is outlined for preparation of the antinociceptive 1-β-(*p*-methoxycinnamoyl)polygodial, isolated from *Drymis winteri* barks. The synthesized compound showed vanilloid activity.

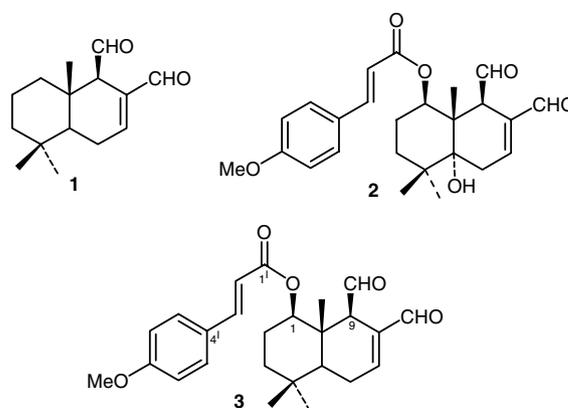
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*Drymis winteri* is a plant (Winteraceae) used in folk medicine, in many countries of South America, for treatment of inflammatory and dolorous processes.<sup>1</sup> Recently it has been shown that some drimane compounds, isolated from *D. winteri* barks, such as polygodial (**1**), drimanial (**2**) and 1-β-(*p*-methoxycinnamoyl)polygodial (**3**), exhibit significant antinociceptive activity.<sup>2</sup> Moreover, antifungal properties, well known for polygodial **1**, were also recorded for its cinnamoyl derivative **3**.<sup>3</sup>

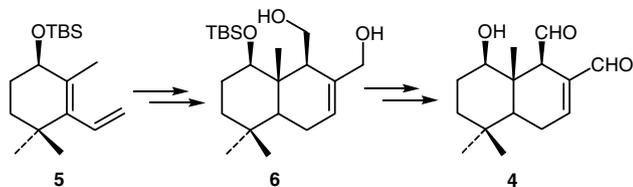
Recent studies have been focused only on compounds **1** and **2**, showing evidences for the involvement of glutamatergic<sup>4</sup> and vanilloid<sup>5,6</sup> receptors in their antinociceptive activity. These activities have stimulated our interest to afford a synthetic strategy for **3** in order to assay its vanilloid activity. In the course of our studies on the vanilloid activity of terpenoidic dialdehydes, we have recently prepared 1(*R*)-hydroxypolygodial (**4**) using a synthetic strategy (Scheme 1) involving a stereoselective Diels–Alder reaction of a chiral diene **5** prepared using a Corey–Bakshi–Shibata oxazaborolidine-mediated reduction.<sup>7</sup>

**Keywords:** Polygodial; *Drymis winteri*; Antinociception; Vanilloid activity.

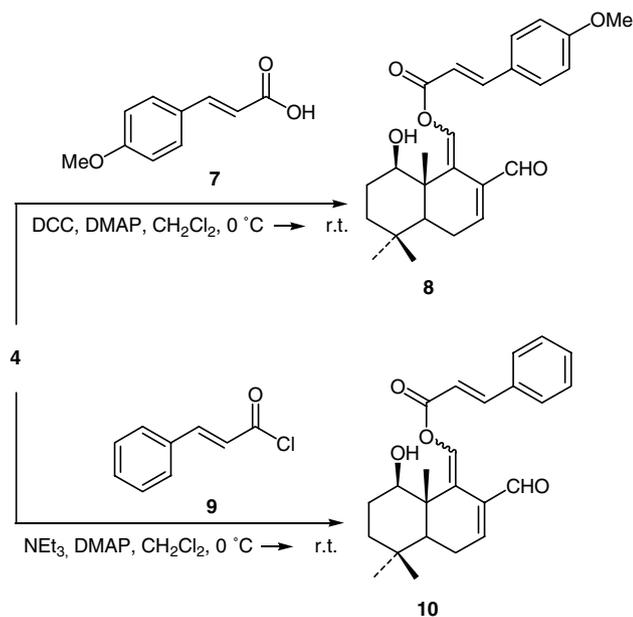
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The synthesis of **3** was first investigated starting from 1(*R*)-hydroxypolygodial (**4**). However, our initial efforts did not allow the installation of the cinnamoyl moiety at 1-position in effective and convenient way. In fact, a preliminary study on direct acylation showed that 1-hydroxypolygodial (**4**), under typical acylation conditions, gave rise to a complex mixture of reaction products (Scheme 2). The main obtained products were the enolacyl derivatives **8**<sup>8</sup> (90%) and **10** (80%), respectively, using commercially available compounds **7** and **9**. Very low yields of the desired acylated product at C-1 were achieved. In both cases, enolization at C-9 and subse-



**Scheme 1.** Synthetic strategy for the preparation of 1(*R*)-hydroxypolygodial (**4**).

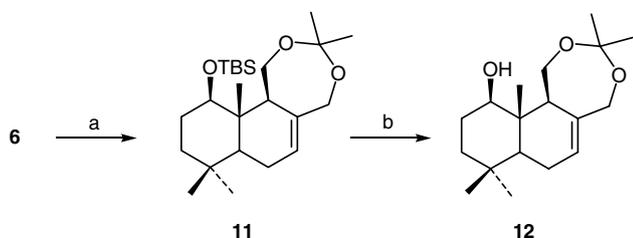


**Scheme 2.** Direct acylation of 1-hydroxypolygodial (**4**).

quent acylation of the resulting enol was faster than acylation at C-1, even controlling reaction times and conditions.

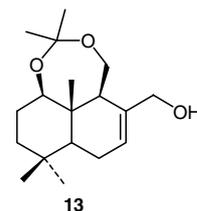
Therefore, the synthetic scheme, previously developed to obtain **4**, was modified starting from diol **6**, as shown in Scheme 3.

Before transformation of the hydroxy group at C-1 of **6**, it was required to install appropriate protecting groups at diol moiety. Thus, both primary hydroxy groups were protected by preparation of acetonide **11** (93% yield). Removal of TBS protecting group (TBAF, 88% yield) furnished alcohol **12**. Careful manipulation of compound **12** was needed because of its lability in acidic



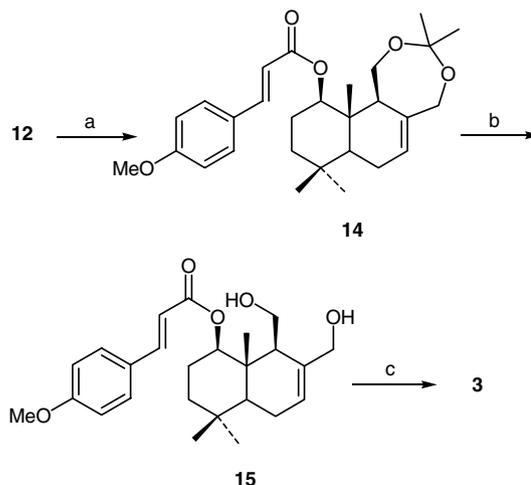
**Scheme 3.** Reagents and conditions: (a) (CH<sub>3</sub>O)<sub>2</sub>C(CH<sub>3</sub>)<sub>2</sub>, *p*-TsOH, rt, 2 h, 93%; (b) TBAF, THF, 0 °C → rt, 16 h, 88%.

conditions, which caused acetonide migration to afford primary alcohol **13**.

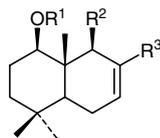


The transformation of **12** into the desired ester **14** was quantitatively carried out by treatment of **12** with *p*-methoxycinnamic acid (**7**) in the presence of DCC and DMAP (Scheme 4). Deprotection of the acetonide moiety was initially performed using catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) in THF/MeOH. However, this reaction was not very clean, affording a mixture containing the expected ester **15**<sup>9</sup> (33%) together with isomeric products, derived by migration of the acyl moiety, respectively, to the alcohol groups at C-11 and at C-12. To circumvent this problem, the acetonide **14** was finally deprotected using pyridinium *p*-toluenesulfonate (PPTS) in THF/MeOH to afford **15** along with unreacted starting acetonide. The recovered **14** was exposed again to the deprotection conditions affording **15** in 79% overall yield. Oxidation of primary alcohols in **15** to the corresponding dialdehyde using Swern conditions completed the synthesis of 1-β-(*p*-methoxycinnamoyl)polygodial (**3**). The spectroscopic data of our synthetic sample<sup>10</sup> were identical with those of the natural compound.<sup>11</sup>

Finally, the synthesized compound was tested for its vanilloid activity. Furthermore, in order to evaluate the influence of some structural requirements, the vanilloid activity of **3** was compared with those of 1(*R*)-hydroxypolygodial (**4**) and diol **15** (Table 1).



**Scheme 4.** Reagents and conditions: (a) **7**, DCC, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C → rt, 12 h, quant; (b) PPTS (6%), THF/MeOH (1:1), 2 × 30 h, 79%; (c) DMSO, (COCl)<sub>2</sub>, NEt<sub>3</sub>, -78 °C → rt, 86%.

**Table 1.** Vanilloid activity assays for compounds **3**, **4**, and **15** (1  $\mu$ M)

Compound	R <sup>1</sup>	R <sup>2</sup> , R <sup>3</sup>	% VA <sup>a</sup> (RA) <sup>b</sup>
<b>3</b>	<i>p</i> -Methoxycinnamoyl	CHO	40.4 (22)
<b>4</b>	H	CHO	0
<b>15</b>	<i>p</i> -Methoxycinnamoyl	CH <sub>2</sub> OH	0

<sup>a</sup> Results are reported as a percent of the maximum possible absolute effect obtained with 4  $\mu$ M ionomycin.

<sup>b</sup> Residual Ca<sup>2+</sup> influx activity after treatment with IRTX.

Vanilloid activity was evaluated by measuring the entry of Ca<sup>2+</sup> (the concentration of internal calcium [Ca<sup>2+</sup>]<sub>i</sub>) before and after the addition of test compounds) into human embryonic kidney HEK-293 cells transfected with the human TRPV1, a typical TRPV1-mediated effect.<sup>12</sup> Compound **3** caused Ca<sup>2+</sup> influx activity in these assays. However, this activity was only partially due to the interaction with vanilloid receptor because calcium mobility was not completely inhibited with the selective TRPV1 antagonist 5-iodo-resiniferatoxin (IRTX). Compounds **4** and **15** resulted not active, showing that the presence in the molecule of dialdehyde moiety may be important but not sufficient for the vanilloid activity.

In conclusion, the synthesis of 1- $\beta$ -(*p*-methoxycinnamoyl)polygodial (**3**) has been accomplished starting from chiral diene **5**.<sup>7</sup> The vanilloid activity showed by the synthesized compound may furnish an insight into its antinociceptivity. Further application of this strategy to the synthesis of other biologically active compounds for the studies of structure–activity relationship is currently underway in our laboratory.

### Acknowledgments

Financial support from Università di Salerno and from MIUR-COFIN 2004 “La chemorecezione gustativa: progettazione razionale di nuove molecole, modellazione dell’attività biologica e applicazioni nell’industria farmaceutica ed alimentare” is gratefully acknowledged.

### References and notes

- Houghton, P. J.; Manby, J. *Ethnopharmacology* **1985**, *13*, 89.
- Malheiros, A.; Filho, V. C.; Schmitt, C. B.; Santos, A. R. S.; Scheidt, C.; Calixto, J. B.; Delle Monache, F.; Yunes, R. A. *Phytochemistry* **2001**, *57*, 103.
- Malheiros, A.; Filho, V. C.; Schmitt, C. B.; Yunes, R. A.; Escalante, A.; Svetaz, L.; Zacchino, S.; Delle Monache, F. *J. Pharm. Pharmacol. Sci.* **2005**, *8*, 335.
- Scheidt, C.; Santos, A. R. S.; Ferreira, J.; Malheiros, A.; Cechinel-Filho, V.; Yunes, R. A.; Calixto, J. B. *Neuropharmacology* **2002**, *43*, 340.

- Andre, E.; Ferreira, J.; Malheiros, A.; Yunes, R. A.; Calixto, J. B. *Neuropharmacology* **2004**, *46*, 590.
- André, E.; Campi, B.; Trevisani, M.; Ferreira, J.; Malheiros, A.; Yunes, R. A.; Calixto, J. B.; Geppetti, P. *Biochem. Pharm.* **2006**, *71*, 1248.
- Della Monica, C.; Della Sala, G.; Izzo, I.; De Petrocellis, L.; Di Marzo, V.; Spinella, A. *Tetrahedron* **2007**, *63*, 6866.
- Compound **8**: *R*<sub>f</sub> = 0.20 (30% ethyl acetate in hexane). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.91 (3H, s, CH<sub>3</sub>), 0.96 (3H, s, CH<sub>3</sub>), 1.00 (3H, s, CH<sub>3</sub>), 1.37 (1H, m, H-3<sub>a</sub>), 1.53 (1H, ddd, *J* = 3.1, 3.1, 13.4 Hz, H-3<sub>b</sub>), 1.66 (1H, dd, *J* = 5.6, 11.5 Hz, H-5), 1.78–1.84 (2H, m, H<sub>2</sub>-2 overlapped), 2.44 (1H, ddd, *J* = 3.4, 11.4, 21.2 Hz, H-6<sub>a</sub>), 2.63 (1H, ddd, *J* = 4.8, 5.6, 21.2 Hz, H-6<sub>b</sub>), 3.86 (3H, s, CH<sub>3</sub>O), 4.22 (1H, t-like, *J* = 9.0 Hz, H-1), 6.34 (1H, d, *J* = 15.9 Hz, H-2'), 6.79 (1H, dd, *J* = 3.4, 4.8 Hz, H-7), 6.93 (2H, d, *J* = 8.8 Hz, H-6' and H-8'), 7.54 (2H, d, *J* = 8.8 Hz, H-5' and H-9'), 7.84 (1H, d, *J* = 15.9 Hz, H-3'), 7.89 (1H, s, H-11), 9.50 (1H, s, OHCC-8). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 62.9 MHz):  $\delta$  13.5 (CH<sub>3</sub>), 21.5 (CH<sub>3</sub>), 26.2 (CH<sub>2</sub>), 26.7 (CH<sub>2</sub>), 32.1 (CH<sub>3</sub>), 33.5 (C), 39.7 (CH<sub>2</sub>), 44.9 (C), 47.6 (CH), 55.4 (CH<sub>3</sub>), 73.4 (CH), 112.6 (CH), 114.4 (2 $\times$ ) (CH), 122.3 (C), 126.5 (C), 130.4 (2 $\times$ ) (CH), 130.9 (CH), 137.3 (C), 147.9 (CH), 152.9 (CH), 162.1 (C), 162.9 (C), 192.3 (CH). [ $\alpha$ ]<sub>D</sub><sup>21</sup> –9.2 (*c* = 0.33, CHCl<sub>3</sub>). ESIMS: *m/z* 411 [M+H]<sup>+</sup>. Anal. Calcd for C<sub>25</sub>H<sub>30</sub>O<sub>5</sub>: C, 73.15; H, 7.37; O, 19.49. Found: C, 73.55; H, 7.67; O, 19.89.
- Compound **15**: *R*<sub>f</sub> = 0.60 (60% ethyl acetate in petroleum ether). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.90 (3H, s, CH<sub>3</sub>), 0.94 (3H, s, CH<sub>3</sub>), 1.05 (3H, s, CH<sub>3</sub>), 1.34 (1H, dd, *J* = 4.8, 11.4 Hz, H-5), 1.45–1.49 (2H, m, H<sub>2</sub>-3 overlapped), 1.67–1.79 (2H, m, H<sub>2</sub>-2 overlapped), 1.95–2.13 (2H, m, H<sub>2</sub>-6 overlapped), 2.39 (1H, m, H-9), 3.71 (1H, dd, *J* = 6.8, 11.0 Hz, H-11<sub>a</sub>), 3.85 (3H, s, CH<sub>3</sub>O), 3.97 (1H, br d, *J* = 12.3 Hz, H-12<sub>a</sub>), 4.15 (1H, br d, *J* = 11.0 Hz, H-11<sub>b</sub>), 4.30 (1H, br d, *J* = 12.3 Hz, H-12<sub>b</sub>), 4.92 (1H, dd, *J* = 4.9, 10.9 Hz, H-1), 5.85 (1H, m, H-7), 6.35 (1H, d, *J* = 15.9 Hz, H-2'), 6.91 (2H, d, *J* = 8.7 Hz, H-6' and H-8'), 7.50 (2H, d, *J* = 8.7 Hz, H-5' and H-9'), 7.67 (1H, d, *J* = 15.9 Hz, H-3'). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>, 100 MHz):  $\delta$  10.7 (CH<sub>3</sub>), 22.4 (CH<sub>3</sub>), 23.8 (CH<sub>2</sub>), 26.0 (CH<sub>2</sub>), 33.3 (CH<sub>3</sub>), 33.6 (C), 40.3 (CH<sub>2</sub>), 41.2 (C), 50.5 (CH), 55.1 (CH), 55.8 (CH<sub>3</sub>), 62.2 (CH<sub>2</sub>), 66.8 (CH<sub>2</sub>), 83.5 (CH), 115.3 (2 $\times$ ) (CH), 117.2 (CH), 125.8 (CH), 128.1 (C), 130.9 (2 $\times$ ) (CH), 139.5 (C), 145.0 (CH), 162.6 (C), 166.8 (C). [ $\alpha$ ]<sub>D</sub><sup>25</sup> –42.0 (*c* = 1.0, acetone). ESIMS: *m/z* 415 [M+H]<sup>+</sup>. Anal. Calcd for C<sub>25</sub>H<sub>34</sub>O<sub>5</sub>: C, 72.43; H, 8.27; O, 19.30. Found: C, 72.03; H, 8.57; O, 19.70.
- Compound **3**: *R*<sub>f</sub> = 0.5 (40% ethyl acetate in petroleum ether). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.96 (3H, s, CH<sub>3</sub>), 1.01 (3H, s, CH<sub>3</sub>), 1.06 (3H, s, CH<sub>3</sub>), 1.42 (1H, dd, *J* = 5.2, 11.2 Hz, H-5), 1.47–1.60 (2H, m, H<sub>2</sub>-3 overlapped), 1.68 (1H, dddd, *J* = 4.0, 11.5, 12.8, 12.8 Hz, H-2<sub>a</sub>), 1.88 (1H, dddd, *J* = 3.8, 4.0, 4.0, 12.8 Hz, H-2<sub>b</sub>), 2.35–2.51 (2H, m, H<sub>2</sub>-6 overlapped), 3.34 (1H, m, H-9), 3.84 (3H, s, CH<sub>3</sub>O), 4.87 (1H, dd, *J* = 4.0, 11.5 Hz, H-1), 6.26 (1H, d, *J* = 15.9 Hz, H-2'), 6.90 (2H, d, *J* = 8.8 Hz, H-6' and H-8'), 7.06 (1H, m, H-7), 7.48 (2H, d, *J* = 8.8 Hz, H-5' and H-9'), 7.62 (1H, d, *J* = 15.9 Hz, H-3'), 9.33 (1H, s, OHCC-8), 9.81 (1H, d, *J* = 2.7 Hz, OHCC-9). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  10.6 (CH<sub>3</sub>), 22.2 (CH<sub>3</sub>), 24.1 (CH<sub>2</sub>), 24.5 (CH<sub>2</sub>), 32.6 (CH<sub>3</sub>), 32.8 (C), 39.3 (CH<sub>2</sub>), 42.3 (C), 48.9 (CH), 55.4 (CH<sub>3</sub>), 59.2 (CH), 81.5 (CH), 114.3 (2 $\times$ ) (CH), 115.2 (CH), 126.9 (C), 130.0 (2 $\times$ ) (CH), 140.4 (C), 145.4 (CH), 151.9 (CH), 161.5 (C), 166.4 (C), 192.3 (CH), 200.4 (CH). [ $\alpha$ ]<sub>D</sub><sup>25</sup> +21.6 (*c* = 1.0, acetone). ESIMS: *m/z* 433 [M+Na]<sup>+</sup>. Anal. Calcd for C<sub>25</sub>H<sub>30</sub>O<sub>5</sub>: C, 73.15; H, 7.37; O, 19.49. Found: C, 72.85; H, 7.67; O, 19.79.

11. We thank Prof. Yunes, R. A. (Federal University of Santa Catarina, Florianópolis, SC, Brazil) for providing spectral data of natural 1- $\beta$ -(*p*-methoxycinnamoyl)polygodial (**3**).
12. The effect of the substances on the influx of  $\text{Ca}^{2+}$  into cells was determined by using Fluo-4 (4  $\mu\text{M}$  Molecular Probes), a selective intracellular fluorescent probe for  $\text{Ca}^{2+}$ .