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Isolation and identification of active compounds from *Drimys winteri* barks

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**Abstract**

The barks of *Drimys winteri* are used in folk medicine as a remedy to treat several diseases, including dolorous processes. Previous pre-clinical experiments carried out in our laboratories revealed that the hydroalcoholic extract of this plant showed anti-allergenic, anti-inflammatory and antinociceptive properties. Such promising results led us to determine the analgesic compounds present in *D. winteri*. Through conventional chromatographic procedures with fractions of CH₂Cl₂ and EtOAc obtained from methanolic extract, it was found that polygodial (1), 1-β-(p-methoxy-cinnamyl) polygodial (2), taxifolin (3) and astilbin (4), are the main components of these fractions. Compounds 1 and 2 exhibited marked antinociceptive action by intraperitoneal and oral routes against acetic acid-induced abdominal constrictions in mice, suggesting that they are responsible, at least partially, for the antinociceptive effects of this plant. In addition, both compounds were notably more potent than aspirin and acetaminophen, two well-known drugs used here as comparison. © 1998 Published by Elsevier Science Ireland Ltd. All rights reserved.

**Keywords:** *Drimys winteri*; Folk medicine; Antinociception; Mice; Terpenes; Flavonoids

**1. Introduction**

*Drimys winteri* (Winteraceae) is a medicinal plant, employed in Brazil and many countries, in
folk medicine against a variety of ailments, especially for the treatment of fevers, ulcers, cancers, pains, affections of respiratory tract, among others (Houghton and Manby, 1985; Gupta, 1995; Graves, 1996). Such plant has been phytochemically studied and different authors have demonstrated the occurrence of sesquiterpenes, lactones (Appel and Dohr, 1958; Aasen et al., 1977; Cortés and Oyarzún, 1981; Sierra et al., 1986; Brown, 1994) and flavonoids (Cruz et al., 1973; Reyes et al., 1990; Torres et al., 1992).

We have previously shown that hydroalcoholic extract, obtained from D. winteri bark, inhibited the contraction caused by several mediators involved in asthma and allergy in guinea-pig trachea (Sayah et al., 1997). This extract also exhibited anti-inflammatory and antinociceptive activities in different pharmacological models (Mata et al., 1994; Tratsk et al., 1997).

In the present study, we have investigated other components present in D. winteri bark and evaluated their possible antinociceptive properties against acetic acid-induced abdominal constriction in mice. Moreover, the potency of some of these compounds was compared to aspirin and acetaminophen, two well-known anti-inflammatory and analgesic drugs.

2. Material and methods

2.1. Plant material

Drimys winteri J.R. et Forster was collected in Bom Retiro, State of Santa Catarina, Brazil, in January 1994 and identified by Professor Leila da Graça Amaral (Department of Botany, Universidade Federal de Santa Catarina). A voucher specimen was deposited in the ‘Herbarium FLOR’ (UFSC) under number 26313.

2.2. Isolation and identification of active compounds

Barks of D. winteri were dried at 45–50°C for 2 days. The dried material (750 g) was cut into small pieces and macerated with commercial methanol at room temperature for 10 days. The extract was then concentrated under reduced pressure to desired volume, suspended in water and then successively partitioned with CH₂Cl₂ and EtOAc, respectively. The CH₂Cl₂ fraction (1.8 g) was chromatographed on a silica gel column, eluted with a hexane:acetone gradient. After monitoring by TLC, fractions were combined and re-chromatographed as described above, given polygodial (1) (240 mg) and 1-β-(p-methoxy-cynnamyl) polygodial (2) (20 mg). Both compounds were identified by spectral data (IR, ¹H NMR and ¹³C NMR), which were in good accordance to those previously described (McCallion et al., 1982; Ciccio, 1984; Ferreto et al., 1988) and by direct comparison with authentic samples.

The AcOEt fraction (5.5 g) was submitted to column chromatography on silica gel eluted with chloroform:methanol gradient, affording taxifolin (3) (45 mg) and astilbin (4) (1487 mg), two well-known flavonoids already studied in our laboratory (Carneiro et al., 1993; Cechinel Filho, 1995). They were identified by direct comparison with authentic samples.

2.3. Pharmacological assay: writhing test in mice

Male Swiss mice, 25–30 g, were kept in a temperature controlled environment (23 ± 2°C) with a 12-h light–dark cycle. Food and water were freely available. The abdominal constriction resulting from intraperitoneal injection of acetic acid (0.6%), consisting of a contraction of the abdominal muscle together with a stretching of hind limbs, was carried out according to procedures previously described (Collier et al., 1968; Santos et al., 1995). Animals were pretreated with the extracts or compounds 30 (i.p.) and 60 (p.o.) min prior to acetic acid injection. For the purpose of comparison, other groups of animals received aspirin or acetaminophen given either i.p. or p.o., 30 and 60 min before testing. Control animals received a similar volume of 0.9% NaCl (10 ml/kg). All experiments were carried out at 20–22°C. After challenge, pairs of mice were placed in separate boxes and the number of abdominal constrictions was cumulatively counted over a period of 20 min.
3. Results and discussion

In order to determine the active constituents present in *D. winteri* barks, we have analysed the chloroform and ethyl acetate fractions, which were obtained by successive partition from the crude methanolic extract and exhibited the best antinociceptive effects (results not shown). Chloroform fraction was chromatographed on silica gel column and eluted with hexane-ethyl acetate gradient, as described in Section 2. Such procedure led to isolation of polygodial (1) and 1-β-(p-methoxy-cynnamyl) polygodial (2) (Fig. 1). Compound 1 is a well-known antimicrobial (McCallion et al., 1982; Kubo and Tanigushi, 1988; Samuelsson, 1992) and antifeedant agent (Jair and Tripathi, 1993), being previously isolated from several plants, including *D. winteri* (McCallion et al., 1982; Cortés and Oyarzún, 1981) and *D. granadensis* (Ciccio´, 1984). However, compound 2 was only detected previously in *D. granadensis* (Ferreto et al., 1988).

The structures of 1 and 2 were elucidated by spectroscopic methods (1H NMR and 13C NMR, IR) in comparison with data reported in the literature (McCallion et al., 1982; Ciccio´, 1984; Ferreto et al., 1988) and co-TLC with authentic samples.

The antinociceptive effects of polygodial (1) and 1-β-(p-methoxy-cynnamyl) polygodial (2) are indicated in Table 1. As can be observed, both compounds, given intraperitoneally or orally, exhibited significant and dose-related antinociceptive action against acetic acid-induced abdominal constriction in mice. The calculated ID50 values

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID50 (μmol/kg, i.p.)</th>
<th>MI (%)</th>
<th>ID50 (μmol/kg, p.o.)</th>
<th>MI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.6 (2.6–5.0)</td>
<td>90 ± 3</td>
<td>164 (133–203)</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>2</td>
<td>16 (12–21)</td>
<td>93 ± 3</td>
<td>&gt;131</td>
<td>47 ± 5</td>
</tr>
<tr>
<td>Aspirin</td>
<td>133 (104–250)</td>
<td>83 ± 1.4</td>
<td>605 (516–705)</td>
<td>82 ± 5</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>125 (104–250)</td>
<td>88 ± 1</td>
<td>1145 (708–1846)</td>
<td>59 ± 4</td>
</tr>
</tbody>
</table>

Each group represents the mean ± S.E.M. of six experiments.

* With their respective 95% confidence limits

* Maximal inhibition.
(µmol/kg, i.p.) were 3.6 and 16, respectively. At ID$_{50}$ level, compound 1, administered intraperitoneally, was ≈35 to 37-fold more potent than acetaminophen and aspirin, whereas compound 2 was ≈8-fold more potent than standard drugs (Table 1). When given orally, compound 1 presented a ID$_{50}$ of 164 µmol/kg, being 3.7 and 7-fold more potent than aspirin and acetaminophen, respectively. Because of the limited quantity of compound 2, it was not possible to determine its exact ID$_{50}$ value orally, but it is ≈131 µmol/kg, since it inhibited 47% the abdominal constrictions at this same dose.

An important result consisted in the determination of absence of drimenol in chloroform fraction, once this sesquiterpene was previously isolated from this same plant with a high yield (6%) (Appel, 1948). This fact suggests that environmental factors may be acting on the plant.

Chromatographic procedures using ethyl acetate fraction (see Section 2), furnished two known flavonoids, identified as taxifolin 3 and astilbin 4 (Fig. 1). These compounds were earlier studied in our laboratory, showing different pharmacological properties, such as antinociceptive and also anti-inflammatory (Carneiro et al., 1993; Cechinel Filho, 1995).

In summary, our results demonstrated the presence of some active principles in D. winteri barks, which could explain at least partially the pharmacological properties of this plant. Presently, the pharmacological studies are in progress in order to analyse whether some of the compounds reported here, especially polygodial, are also responsible for the anti-allergic and anti-inflammatory effects previously described for the hydroalcoholic extract of D. winteri (Mata et al., 1994; Sayah et al., 1997; Tratsk et al., 1997).

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**References**


