Polygodial, a drimane sesquiterpenoid dialdehyde purified from *Drimys winteri*, inhibits voltage-gated sodium channels

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**ABSTRACT**

*Drimys winteri* J.R.Forst. & G.Forst, a South American evergreen shrub that is used by the Mapuche people for treatment of several painful conditions, contains polygodial, a lipophilic drimane-type sesquiterpene dialdehyde with known activity at transient receptor potential channel family members including TRPA1 and TRPV1. We sought to assess the activity of polygodial at NaV1.7 and NaV1.8, two key isoforms of the voltage-gated sodium channel family involved in nociception. Polygodial was isolated from *D. winteri* by thin-layer chromatography and analysed structurally by 1D and 2D nuclear magnetic resonance (NMR) spectroscopy. Activity at heterologously expressed NaV1.7 and NaV1.8 was assessed using automated whole-cell patch-clamp electrophysiology. Here, we show that polygodial inhibits members of the voltage-gated sodium channel family, specifically NaV1.7 and NaV1.8, without changing the voltage-dependence of activation or inactivation. Activity of polygodial at voltage-gated sodium channels may contribute to the previously reported antinociceptive properties.

**Abbreviations:** TRPA1: transient receptor potential Ankyrin 1; TRPV1: transient receptor potential Vanilloid 1; NaV1.7: voltage-gated sodium channel isoform 1.7; NaV1.8: voltage-gated sodium
1. Introduction

Plants are a valuable source of bioactive compounds and are still being used for the treatment of many ailments. In Chile, the indigenous Mapuche people use the Winter’s bark or Canelo tree *Drimys winteri* J.R.Forst. & G.Forst for several medicinal purposes, including in the treatment of toothache, ulcers and labour pain (Houghton and Manby 1985). The main antinociceptive component of *D. winteri* is polygodial, an \( \alpha,\beta \)-unsaturated 1,4-dialdehyde that has also been isolated from *Drimys brasiliensis* Miers (Winteraceae) (Cechinel Filho et al. 1998; Mecchi and Lago 2013). The antinociceptive properties of polygodial have also been utilised by other cultures, including the Māori of New Zealand, who use the polygodial-producing Horopito shrub (*Pseudowintera colorata*) as an analgesic and for a range of skin conditions in topical applications. Polygodial is known to act at ion channels expressed in peripheral sensory neurons, including the transient receptor potential (TRP) channels TRPV1 and TRPA1, as well as human two-pore domain potassium channels TASK-1, TASK-3, and TRESK (Andre et al. 2006; Iwasaki et al. 2009; Beltran et al. 2013; Mathie et al. 2017). Activity at TRP channels, in particular activation of TRPV1 and TRPA1, likely account for the pungency of polygodial-containing plants. In this context, polygodial is a particularly potent agonist at human TRPA1 channels with an EC\(_{50}\) of 59 nM, while activity at rat TRPV1 is partial and less potent (EC\(_{50}\) 5 \( \mu \)M) (Iwasaki et al. 2009). However, reports of numbness and dysesthesia after consumption of *D. winteri* (Ravindran et al. 2012), combined with the clear antinociceptive effects, motivated us to also assess activity of polygodial at the voltage-gated Na\(^+\) channel subtypes Na\(_{\alpha}1.7\) and Na\(_{\alpha}1.8\), which are known analgesic targets and also frequently modulated by natural products. Here we describe for the first time that polygodial, isolated from *D. winteri*, is a blocker of voltage-gated Na\(^+\) channels.

2. Results and discussion

2.1. Chemical characterization of polygodial purified from the bark of *D. winteri*

Isolation of polygodial (structure in Suppl. Figure S1) from the bark of *D. winteri* produced a yellow oil with 0.37% yield and purity of 98.2% by Gas chromatography–mass spectrometry (Suppl. Figures S8 and S9). The structure of polygodial was confirmed by 1D and 2D NMR (Suppl. Figures S2–S6), with excellent agreement to reported data (Rodríguez et al. 2005). The compound is formed by a central trans-decalin ring functionalized with an \( \alpha,\beta \)-unsaturated 1,4-dialdehyde moiety. The aldehyde C-11 has a carbon chemical shift of 202.0 ppm and the proton at 9.53 ppm as \( d, J = 4.4 \) Hz, while C-12 is the second aldehyde shifted...
at 193.2 ppm and his proton as singlet at 9.46 ppm. The unsaturated C-7 has a shift of 154.5 ppm, and C8a shift of 138.4 ppm. This C7-C8-C12 conjugated system gives increased reactivity and a planar configuration to this group.

### 2.2. Polygodial inhibits voltage-gated sodium channels

Polygodial concentration-dependently inhibited peak current of the analgesic target Na\textsubscript{V}1.8 (Figure 1A) with an IC\textsubscript{50} of 16±8 μM (Figure 1B). To assess if this inhibition was state-dependent, we used an 8 s conditioning voltage step to −40 mV to inactivate approximately half of the available channels. Using this protocol, the IC\textsubscript{50} of polygodial was 12±1 μM, indicating that unlike local anesthetics, it shows minimal preference for the inactivated state (Figure 1B). To assess if polygodial is selective for the Na\textsubscript{V}1.8 isoform, we also tested activity at Na\textsubscript{V}1.7. Polygodial inhibited Na\textsubscript{V}1.7 peak current with an IC\textsubscript{50} of 57±7 μM (Figure 1C), indicating it has minimal selectivity for Na\textsubscript{V}1.8 (~4 fold), and likely inhibits all Na\textsubscript{V} isoforms non-selectively, although this remains to be determined. To gain insights into mechanism of Na\textsubscript{V} channel block, we next assessed the effect of polygodial on the voltage-current relationship of Na\textsubscript{V}1.8 (Figure 1D). At a
concentration of 20 μM, polygodial caused a minor shift of the voltage-dependence of activation (V1/2: control 3.0 ± 1.3 mV, polygodial 10.0 ± 0.4 mV; P < 0.05 paired t-test; Figure 1E). At the same concentration, polygodial had no significant effect on the voltage-dependence of steady-state fast inactivation (V1/2: control –29.0 ± 0.9 mV, polygodial –32.4 ± 1.6 mV; P > 0.05 paired t-test; Figure 1F).

2.3. Discussion
Polygodial-producing plants have been widely recognised for their analgesic activity and pungency, with several cultures globally using the leaves, flower buds or bark of *D. winteri*, *Tasmannia lanceolata*, *Pseudowintera colorata*, *Acmella oleracea*, and *Persicaria hydropiper* for culinary or medicinal purposes. Polygodial, along with the related drimanial, warburganal, isogadial, and miogadial (Suppl. Figure S1), is an α,β-unsaturated 1,4-dialdehyde sesquiterpenoid that displays promiscuous activity at several ion channels expressed in sensory neurons. Specifically, activity at the transient receptor potential channels TRPV1 and TRPA1, as well as the two-pore domain potassium channels TASK-1, TASK-3, and TRESK has previously been reported (Andre et al. 2006; Iwasaki et al. 2009; Beltran et al. 2013; Mathie et al. 2017). TRPA1 is the target of a number of phytochemicals found in spicy or pungent foods, including allyl isothiocyanate, which is found in horseradish and wasabi. Activity at this ion channel likely accounts for the pungency of polygodial-containing plants. The isothiocyanates, as well as other electrophiles such as cinnamaldehyde, activate TRPA1 via covalent or non-covalent cysteine modification involving thiol-Michael adduct formation, bimolecular nucleophilic substitution or thiol-α,β-unsaturated aldehyde reactions (Hinman et al. 2006, Macpherson et al. 2007, Bahia et al. 2016). In contrast, polygodial as well as warburganal and 1β-acetoxy-9-deoxyisomuzigadial likely modulate TRPA1 via covalent lysine modification (Mathie et al. 2017). While TRPA1 is a well-established analgesic target, and desensitization of TRPA1, or TRPA1-expressing sensory neurons, could plausibly contribute to the analgesic effects of polygodial, this activity is inconsistent with reports of numbness following consumption (Ravindran et al. 2012). We thus assessed the effects of polygodial on NaV1.7 and NaV1.8, two subtypes of the family of voltage-gated Na⁺ channels that are selectively expressed in peripheral nerve terminals and that are targeted by local anaesthetics. We found that polygodial blocked both NaV1.7 and NaV1.8 with low micromolar IC₅₀, albeit with lower potency compared to activity at TRPA1. Inhibition of NaV1.7 and NaV1.8 is consistent with the reported analgesic effects of polygodial, although activity at additional pharmacological targets cannot be ruled out. For example, the related terpenoid drimanial also inhibits [³H]glutamate binding in cerebral cortical membranes with an IC₅₀ of 4.39 μM (Scheidt et al. 2002), and activity at glutamatergic systems could also contribute to in vivo analgesic activity.

Although the precise binding site and mechanism of NaV inhibition by polygodial remain to be determined, our experiments suggest that polygodial acts as a pore blocker, rather than gating modifier, as voltage-dependence of activation and inactivation were unchanged or only minimally affected (Figure 1). In addition, NaV inhibition by polygodial was distinct from the pharmacological effects of local anaesthetics,
which show strong state-dependent effects. In light of the above-mentioned putative covalent modification of lysine residues by polygodial, it is conceivable that similar mechanisms could be involved in Na\textsubscript{V} channel block. The highly conserved nature of Na\textsubscript{V} pore domain would also explain lack of subtype selectivity of polygodial, although this remains to be assessed in detail.

3. Conclusions

Overall, we show that in addition to TRP and two-pore domain channels, polygodial also has activity at Na\textsubscript{V} subtypes expressed on peripheral nociceptors, which may contribute to its analgesic activity.

Acknowledgments

The authors would like to thank Professor Bernd Schmidt from University of Potsdam for the scientific support given to CP.

Disclosure statement

The authors declare no conflict of interest.

Funding

This research was funded by ANID of Chile government with the grant number Fondecyt 11181076 to CP. J.R.D. and I.V. were supported by research fellowships from the Australian National Health and Medical Research Council (APP1162503 and APP1139961).

Author contributions

Molecular purification and structure analysis, C.P.; extraction of D. winteri, L.S., bioassays, J.R.D.; manuscript writing and editing, C.P., J.R.D., I.V.

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