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Mode of action of the plant-derived silphinenes on insect and mammalian $GABA_A$ receptor/chloride channel complex

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Abstract

The silphinenes are tricyclic sesquiterpenes that have antifeedant and toxic effects in insects and structural similarity to the known GABA antagonist, picrotoxinin. In murine synaptoneurosomes, silphinenes block GABA-stimulated influx of $^{36}Cl^-$ with EC_{50}s in the range of 10–30 μM. In insects, silphinenes were tested in neurophysiological recordings of central neurons from third instar *Drosophila melanogaster* larvae. Silphinenes reversed the blockage of neuronal firing induced by GABA, but had little effect below 100 μM. The structure–activity profile observed in the murine chloride flux assay was also observed in the larval neurophysiological assay, indicating little selectivity for the silphinenes. A reference silphinene was equally active on nerve preparations from the *rdl* strain of *D. melanogaster*, which is resistant to channel-blocking antagonists via an altered GABA receptor. This latter finding suggests that silphinenes interact with the insect GABA receptor in a manner somewhat different from PTX, and that *rdl* resistance in the field may have little effect on silphinene efficacy.

Keywords: GABA antagonist; *Senecio palmensis*; Insecticide; Antifeedant; *rdl*

1. Introduction

With increased reports of resistance development in insects to many insecticides currently in use, discovery of insecticidal molecules that act on entirely novel target sites or on existing target sites with different modes of action is a priority for insecticide toxicologists. In insects, the γ-aminobutyric acid (GABA)-gated receptor/chloride channel complex is a well established target site for many insecticidal compounds that act as agonists or antagonists [1–3]. Examples include the avermectins, as well as a number of structurally diverse channel-blocking compounds, including the polychlorocycloalkanes (e.g. cyclodienes and lindane), picrodendrins, and various analogs of EBOB [1–3].

The silphinenes (Fig. 1), a rare class of tricyclic sesquiterpenes derived from the aerial parts of the plant, *Senecio palmensis*, have potential as insect control agents or as leads in chemical synthesis programs. These compounds are especially effective against Coleopteran pests, such as the Western corn rootworm and the Colorado potato beetle [4–8], where they show both antifeedant and acutely lethal properties. Molecular modeling analysis observed a structural resemblance between the silphinenes and another plant-derived sesquiterpene compound, picrotoxinin (PTX), where there was steric overlap for a number of structural parameters [7].

PTX and synthetic organic insecticides, such as dieldrin, cause convulsions and death in insects and mammals by

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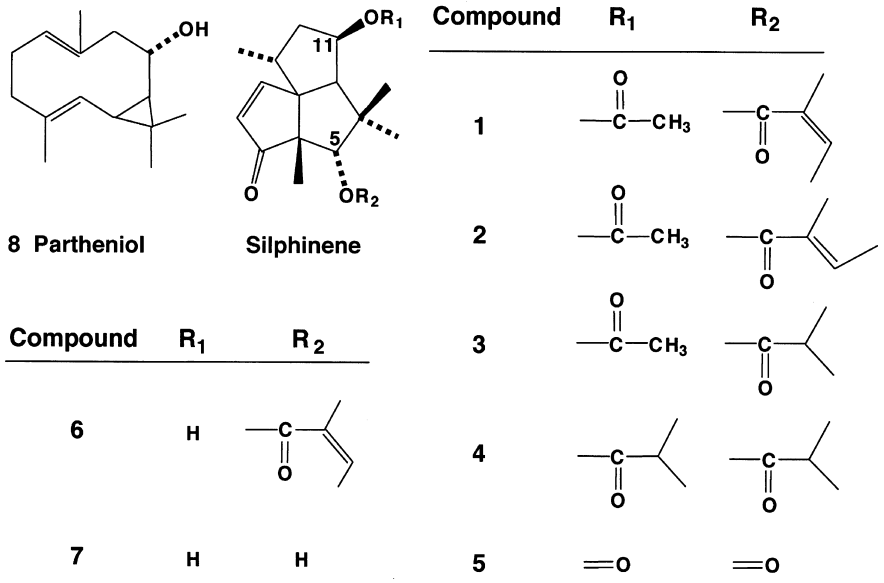

Fig. 1. Chemical structures of the silphinenes (**1**–**7**) and partheniol (**8**) used in these studies.

blocking inhibitory neurotransmitter receptors gated by GABA [9]. In addition, there are strains of insects resistant to PTX and dieldrin that have an altered GABA receptor structure with an Ala to Ser replacement within the M2 region of a GABA receptor subunit [10]. The purpose of the present study was to determine the mode of action and structure–activity relationships of silphinenes on mammalian and insect GABA receptor preparations and to determine whether neuronal preparations from cyclodiene-resistant (*rdl*) insects were also resistant to silphinene.

2. Materials and methods

2.1. Chemicals

The isolation and purification of parent silphinene (compound **1**) its derivatives (compounds **2**–**7**), were as previously described [5,6], and are shown in Fig. 1. Partheniol (**8**), provided by Prof. J.J. Hoffman (Univ. of Arizona, USA), was included as a reference sesquiterpene. All other chemicals used in this research were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Mouse brain synaptoneurosomal Cl^- uptake

GABA-stimulated $^{36}Cl^-$ uptake into mouse brain synaptoneurosomes was measured using well established procedures, essentially as described by Carlier et al. [11]. Vesicles from whole brain were pre-incubated with the silphinenes for 10 min at room temperature, then challenged with 100 μM GABA and $^{36}Cl^-$ (500 nCi/ml) for 15 s at 30 °C. Uptake was stopped with ice cold buffer, the incubate immediately filtered and then washed three times with additional cold buffer. Radioactivity trapped within the vesicles was quantified by liquid scintillation. For determination of inhibition kinetics, the concentration of GABA was varied in the presence and absence of a fixed concentration of silphinene **1**. Treatments were typically replicated at least three times on fresh synaptoneurosomes prepared on different days. Uptake data were fit to a four parameter logistic equation using Prism® (GraphPad Software, San Diego, CA), and differences in curve fit parameters were analyzed by *T*-test or ANOVA using InStat (GraphPad Software, San Diego, CA).

2.3. Insect neurophysiological studies

For neurophysiological recordings, the excised central nervous system (CNS) of wandering third-instar *Drosophila melanogaster* larvae was used as described by Bloomquist et al. [12]. Descending nerve activity was recorded with a suction electrode and converted to a spike frequency on-line using a Maclab (AD Instruments, Colorado Springs, USA). Preparations were treated with GABA (1 mM) to inhibit spontaneous nerve activity, and then challenged with silphinene. Compounds were applied to the preparations dissolved in saline (GABA) or DMSO (silphinene), and mixed by gentle pipetting. For analysis of nerve activity, the mean firing rate (±SEM) was calculated from a 60 s duration sample having the highest observed discharge following treatment with silphinene as a midpoint. This measure of spike activity was compared to a 60 s activity sample just before silphinene treatment. For each treatment group, data were obtained from at least three separate preparations and analyzed by *T*-test using InStat.

2.4. Toxicity bioassays

The most potent blocker of mammalian and insect GABA receptors in the above studies, the deacetylated sil-