

LONG-TERM EFFECTS ON FOOD CHOICE OF LAND  
SNAIL *Arianta arbustorum* MEDIATED BY PETASIN AND  
FURANOPETASIN, TWO SESQUITERPENES FROM  
*Petasites hybridus*

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**Abstract**—Sesquiterpenes (STs) from the Senecioneae have been found to be potent snail repellents. We investigated the range of activity of the STs petasin, isopetasin, furanopetasin, kablicin, and cacalol, which were isolated from *Petasites hybridus*, *P. kablikianus*, and *Adenostyles alpina*. We found the petasin content of leaves of *P. hybridus* to lie within the range of deterrence of the isolated compound. Furthermore, leaf extracts containing petasin proved to be deterrent, and leaf discs with low petasin content were preferred over discs with higher petasin content. The cacalol-containing fraction of a leaf extract of *A. alpina* was not deterrent to the snails. When the snails had experience with the relevant ST one week before a choice test, their sensitivity towards petasine and furanopetasine increased whereas for the other ST it remained at the same level. We speculate that this sensitivity increase could be the result of a rapid long-term associative learning process, but there is also the possibility that these STs are directly interfering with the feeding motor program of the snails, thereby eliciting a direct neurophysiological sensitization reaction which prevents them from further feeding.

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## INTRODUCTION

When the botanist Stahl (1888) published his comprehensive study about plant compounds and mollusk herbivory, he was one of the first to argue that the function of the diversity of compounds found in plants was to deter herbivores. Although his ideas were disputed by Heikertinger (1914) and other zoologists (Schmid, 1929), they still persist, and most researchers nowadays would agree with this position (Rausher, 1992). Since then, however, most research in plant-herbivore interactions has been done with insects or mammals (Fraenkel, 1959; Ehrlich and Raven, 1964; Freeland and Janzen, 1974; Crawley, 1983; Strong et al., 1984). Mollusk herbivory is generally considered to be of ecological importance only during seedling recruitment (Hanely et al., 1995a,b). In suitable mollusk habitats, such as along creeks and riverbeds, however, mollusk biomass is high, and they may well be a major basal component of the food chain. Consequently, mollusk herbivory in these habitats should not only affect seedlings but also exert some pressure on fully grown plants. One of the very abundant plants along humid riverbeds, *Petasites hybridus* (L.), is widespread all over Europe and, due to its tall growing wide leaves, it is an important contributor to green plant biomass in its habitat. Although not usually heavily affected by herbivory, occasionally snails have stripped the plants completely by the end of the growing season (M. Klemm, personal communication).

As potentially protective secondary plant compounds, sesquiterpenes of the (furano-) eremophilane type (hereafter STs) have been found also in the leaves of *P. hybridus*, as they are typical to its tribe, the Senecioneae (Asteraceae) (Seaman, 1982). Two different chemovariants of the species, containing furanopetasin or petasin as their major compound, have been described (Stoll et al., 1956; Novotny et al., 1961, 1966). Since STs were found to deter snails (Hägele, 1992; Speiser et al., 1992; Hägele et al., 1996), we wanted further to establish their range of activity and possible effects on snail food choice over time. To help bridge the gap between the deterrent activity of an isolated compound in laboratory experiments and the effect of that compound on the behavior of an animal in its natural environment, we also tested plant extracts containing the ST together with other unknown plant compounds. Finally, we tested intact leaf tissue of *P. hybridus* to see whether snails are indeed distinguishing between leaf discs containing different concentrations of petasin.

*Adenostyles alpina*, another senecionean plant, was rarely found to suffer from snail herbivory (Hägele, 1992). Since the transformed ST cacalol was identified as being a major constituent of *A. alpina* leaves (J. Harmatha, unpub-

lished), we tested its range of activity and the cacalol containing plant extracts. Thereby, we aimed to discover whether STs are responsible for the limitation of herbivory on *P. hybridus* and the observed almost complete lack of mollusk herbivory on *A. alpina*.

#### METHODS AND MATERIALS

Choice experiments with pure substances and plant extracts were conducted with wheat wafers. Squares of about 25 mg dry weight were cut from commercially available wafers (Back-Oblaten, Küchle GmbH & Co.), consisting of wheat flour and starch. After cutting, the squares were weighed and placed on a glass plate where the test and control solutions were applied. After complete evaporation of the solvent (methanol), one treatment and one control square were placed into each experimental container. Then the snail was added and after 16–18 hr it was removed and the remains of the squares were recovered, dried, and weighed for estimation of consumption. Trials were started 3–4 hr before dark, lasted overnight (10 hr), and 3–4 hr into the light period of the next morning. During that period the animals would usually eat not more than half of the food presented. The masses eaten from the treatment and the control squares were compared by a paired *t* test. The tests with furanopetasin were conducted one year before the other tests and therefore followed a protocol that used leaf discs of lettuce (*Lactuca sativa*) as the test substrate (for details see Hägele et al., 1996). “Naive” snails always experienced the test substance for the first time, although they might have experienced another substance before. Care was taken in order to assure that snails tested with either petasin, isopetasin, or furanopetasin were not retested with another substance from this group.

Organs of *P. hybridus* were extracted and their petasin content was quantified by HPLC as described in Wildi (1997). Leaves and rhizomes of *A. alpina* were extracted as described in Novotny et al. (1972). The quantity of cacalol in relevant fractions was determined by HPLC (Hägele, 1996).

Clones of *P. hybridus* were regenerated from tissue cultures and kept in the garden when fully grown. On the morning of the experiment one leaf from each clone was harvested, put into a plastic bag and kept refrigerated until the experiment. The choice between leaves was provided by cutting discs from one half of the test leaves and pinning them to the ground of the experimental container. After the experimental period (as above), all leftovers were recovered, glued on a paper sheet, dried, and plant area was measured with a video-based image analysis system. The second half of the leaf was stored at  $-20^{\circ}\text{C}$  until it was further processed for petasin quantification (Wildi, 1997).

The STs tested (Figure 1), were isolated from *P. hybridus* [furanopetasin (FP) and petasin (P)], from *P. kablikianus* [kablicin (KB) and isopetasin (IP)],

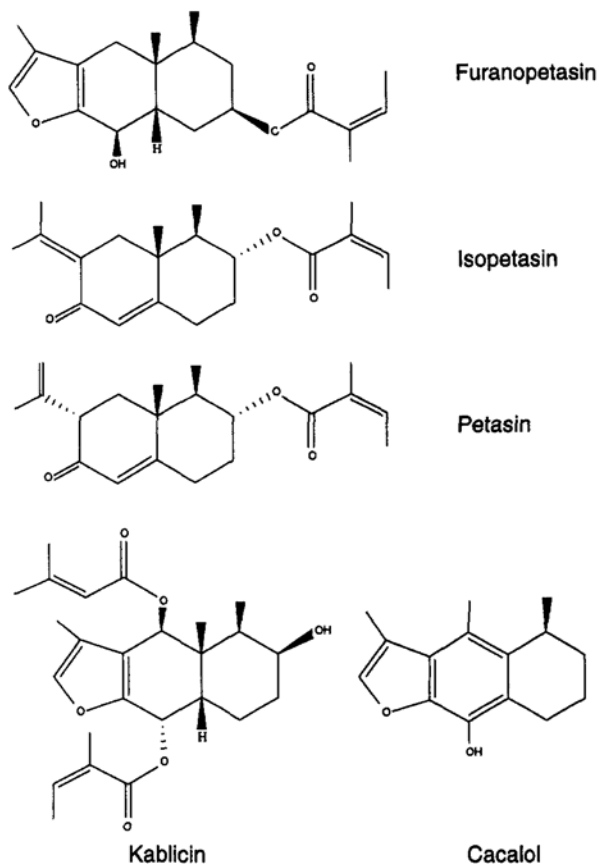


FIG. 1. Structural formulas of sesquiterpenes tested.

and from *Adenostyles alpina* [cacalol (CA)] (Novotny et al., 1972, 1987; J. Harmatha, unpublished results). All plant names correspond to the nomenclature of the *Flora Europaea* (Tutin et al., 1976).

All tests were made with adult specimen of *Arianta arbustorum* L. (Gastropoda, Helicidae). *A. arbustorum* is an omnivorous snail that includes green plants, decaying plant material, and fungi in its diet (Hägele, 1992). It is the commonest helioid snail in central Europe and is present in high densities in habitats where *P. hybridus* and *A. alpina* occur (Kerney et al., 1983; Hägele, 1992). All snails were collected as adults near Zastler (Germany, Black Forest) and kept individually on CaCO<sub>3</sub>-enriched soil in plastic containers of 9 cm diameter in an incubator at 15°C and a 14L:10D regime. Snails were maintained

on leaves of fresh lettuce (*L. sativa*) and discs of carrot roots (*Daucus carota*). For experiments, snails were transferred to separate containers of the same size that had a moist plaster bottom and were lined with filter paper. Due to the high relative humidity in the containers, the wafers stuck to the filter paper so that there was no risk that the snails would displace them during feeding. Leaching of test compounds into the filter paper was unlikely, since all test compounds are of low polarity and therefore not water soluble.

## RESULTS

The amount of P, IP, FP, and KB necessary to elicit food choice behavior in naive snails was 0.5% dry weight content (Figure 2A). The amount of CA in the test substrate necessary to elicit choice behavior was 0.05% dry weight content (Figure 2A). One week later the sensitivity of the same snails towards IP, KB, and CA was unchanged, whereas the amount of P that was now necessary to elicit choice behavior had decreased to 0.05%, and the amount of FP decreased to 0.1% (Figure 2B).

When we tested extracts from different tissues of *P. hybridus*, which all contained the P fraction, all extracts except the petiole extract were deterrent to the snails (Figure 3). The leaf extract of *A. alpina*, which contained CA (0.048% dry weight), was not deterrent to the snails. The CA-containing rhizome extract (0.375% dry weight), however, was strongly deterrent to the snails (Figure 3).

When we provided naive snails with a choice of leaf discs of *P. hybridus*, the discs that originated from clone C1, which had the lowest P content, were preferred over discs from clones C6 and C7 (Figure 4). No preference was apparent between discs from clones C6 and C7, which each had a different but relatively high P content (Figure 4). When we tested leaves from different individual plants of the same clones 11 days later, their leaf P content had changed drastically. C1 now had almost twice as much P in its leaves, whereas the level of P in C6 and C7 had fallen 6- to 10-fold to about the same low value. The experienced snails now ate more of the leaves with the low level of petasin (Figure 4), although only in the choice experiment of C1 versus C7 did they show a clear preference for C1 leaf discs (Figure 4).

## DISCUSSION

The deterrence range for pure P on wheat wafers lies well within the range of leaf P content in the investigated *P. hybridus* leaves. We found P content in leaves to be 0.071–0.716% dry weight content, and deterrence to occur at 0.05% for experienced snails. Furthermore, the reaction of snails to leaf extracts con-

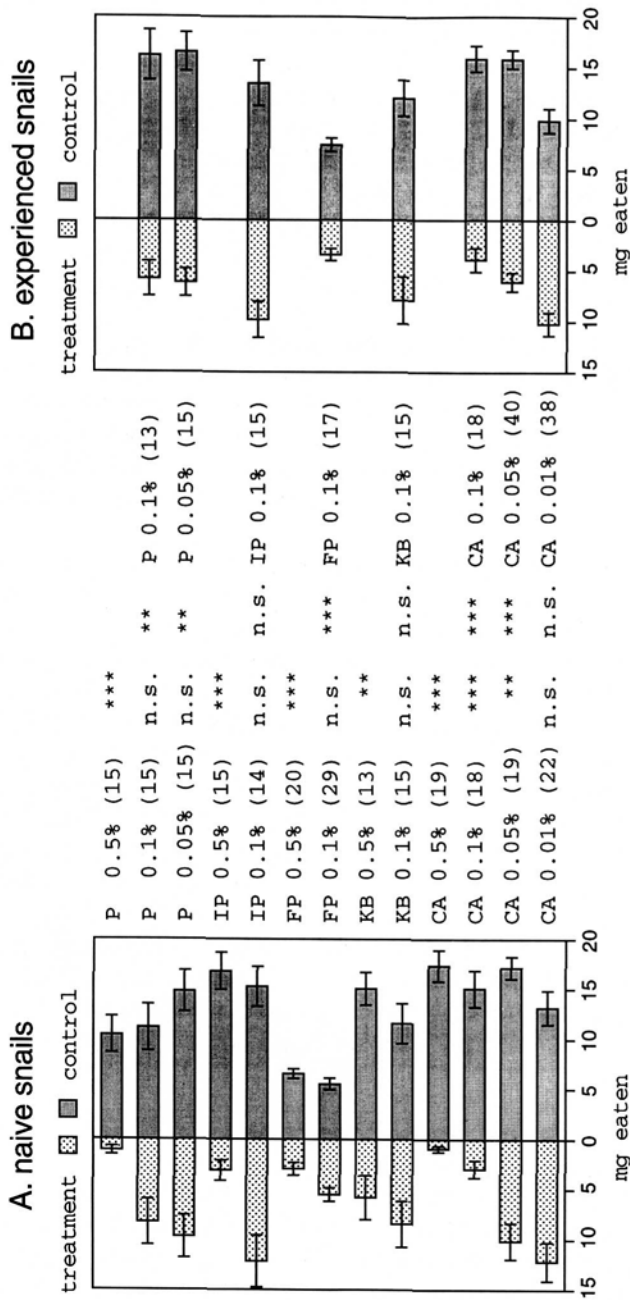


FIG. 2. Dry mass of treatment and control wafers eaten by snails when naive (A), or when tested again seven days after the first test (B). Means and standard errors are shown. Sesquiterpenes (STs) on treatment wafers were petasin (P), isopetasin (IP), kabalicin (KB), and cacalol (CA). Furanopetasin (FP) was tested by applying its solution on lettuce leaf discs. Means of FP experiments are square centimeters of discs eaten. The amount of ST applied is given as percent of dry weight of the test substrate, and the number of snails tested is shown in parentheses. Significant differences ( $P < 0.05$ ) between treatments and controls are indicated by asterisks (\*, \*\*, \*\*\* correspond to  $P < 0.05$ , 0.01, 0.001, respectively), n.s. indicates nonsignificant differences ( $P > 0.05$ ).

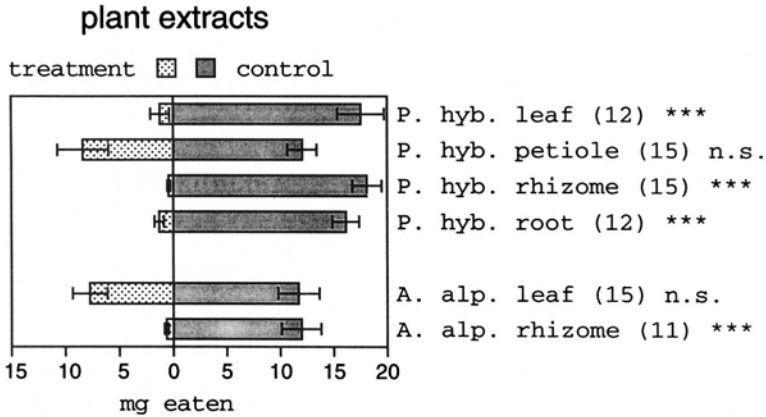


FIG. 3. Dry mass of treatment and control wafers eaten by snails, when treatments were extracts from different organs of *P. hybridus* and *A. alpina* containing petasin and cacalol, respectively. Means and standard errors are given. The concentration of petasin (in *P. hybridus* extracts) and cacalol (in *A. alpina* extracts) is given as percent of tissue dry weight, and the number of snails tested is shown in parentheses. Significant differences ( $P < 0.05$ ) between treatments and controls are indicated by asterisks (\*\*\* $P < 0.001$ ), n.s. indicates nonsignificant differences ( $P > 0.05$ ).

### Leaf disc choice test

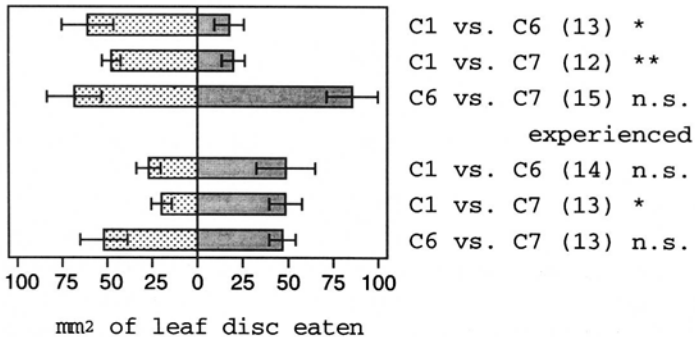


FIG. 4. Area of leaf discs from *P. hybridus* clones eaten by naive snails and the same (experienced) snails 11 days later. Means and standard errors are given. Petasin content of clones is given as percent of tissue dry weight, and the number of snails tested is shown in parentheses. Significant differences ( $P < 0.05$ ) between leaf disc area eaten from different clones are indicated by asterisks (\*, \*\* correspond to  $P < 0.05$ , 0.01, respectively), n.s. indicates nonsignificant differences ( $P > 0.05$ ).

taining P fit the overall picture that P might indeed be the major snail deterrent in *P. hybridus*. The choice experiment with leaf discs of *P. hybridus* clones with different P content further showed that naive snails preferred the leaf with the lower P content if the difference was large (0.464%) and the P content of the alternative leaf was relatively low (Figure 4, upper part).

Whether FP is also the main effective snail deterrent in the FP chemovariant of *P. hybridus* remains an open question because we lack data about the range of FP leaf content in this chemovariant. However, since FP seems to have a deterrence similar to P (tests were made on lettuce leaves), and since a sensitivity change also occurred, we might well expect it to be as effective as P in its snail-detering properties.

CA proved to be deterrent at 0.05% dry weight content in the wheat wafer experiment, but no sensitivity change occurred that would have lowered the deterrent concentration even further. CA content in *A. alpina* leaves was found to vary between populations from means of 0.008% to 0.022% dry weight content (Hägele 1996). Thus it seems unlikely that CA is the major snail deterrent compound in *A. alpina*. Furthermore, fractionation of *A. alpina* leaf extracts produced several snail-deterrent fractions other than the CA-containing one (Hägele et al., unpublished), so that the observed overall deterrence of the leaf might well be the result of additive or synergistic actions of several compounds (Adams and Bernays, 1978; Woodhead and Bernays, 1978). However, CA could still be of importance for deterring rhizome-feeding snails, since it is present in rhizomes in much higher concentrations (~0.38% dry weight) than in other tissues, and the CA-containing rhizome extract proved to be snail-deterrent (Figure 3). In addition, adenostylone and neoadenostylone, two related STs that have been shown to possess snail-deterrent activity (Hägele et al., 1996), are both also present in high quantities in *A. alpina* rhizomes (~0.46–0.25% dry weight; J. Harmatha, unpublished).

The slug *Limax maximus* is known to reject bitter foods after an initial taste contact (Carew and Sahley, 1986). Since sesquiterpene lactones and some other specific derivatives are known for their bitter taste, bitterness might well be the basis for the deterrent activity of the STs tested. The basis for the observed increase in sensitivity towards P and FP after an initial experience is still unknown. However, since the observed sensitivity effect in experienced snails is due to a failure to ingest larger amounts of the test substrate rather than to a failure to approach the test substrate (consumed mass of test substrate is significantly different from zero), we put forward two possibilities about the mechanisms in action.

Since P is known as a muscle-relaxing substance (Aebi et al., 1958) and is used pharmaceutically, it might well have postingestive effects in snails as well. If P is also muscle relaxing in snails, a partial immobilization would make them much more vulnerable to desiccation. Such an effect might be the basis of a learned aversion towards these substances.

Another possibility is that P and FP act directly at the neurological level of transmitter substances and receptors. Dopamine elicits the feeding motor program in *L. maximus* in a dose-dependent manner (Wieland and Gelperin, 1983), and P, IP, and FP inhibited binding of [<sup>3</sup>H]spiperone (a ligand to the dopamine receptor) to the vertebrate dopamine-D<sub>2</sub> receptor (Berger et al., 1998). It thus might well be that the observed sensitivity change towards P and FP is the result of sensitization that involves direct long-term facilitation of the neurological pathways due to a partial blocking of dopamine receptors. However, at this stage it is not clear why the treatment with IP did not show the same sensitivity characteristics as with P and FP, since it was also able to block the dopamine receptor (Berger et al., 1998). So the exact mode of action of P and FP still has to be established, especially whether they also affect serotonergic neurons, which have been shown to participate in adult snail feeding sensitization (Zakharov and Balaban, 1987).

In the nutritionally more complex context, when experienced snails were feeding from whole leaf tissue, their reaction to different concentrations of P in the leaf tissue was less clear. Only in the choice C1 vs. C7 could we see a clear preference for the leaf disc containing less P, whereas in the choice C1 vs. C6, which was a similar choice regarding P content (Figure 4), we could not detect any differences. This reminds us that the action of deterrents on feeding is context dependent (Schoonhoven et al., 1992) and other factors such as different phagostimulants or learned associations might have influenced the actual food choice. It is therefore possible that from the previous experiment the snails learned to associate characteristics of plant C1 with low P content and characteristics of C6 with high P content. Subsequently, at their second experience with leaves from the same plants (clones), the snails only adjusted their behavior towards the now changed P content after initially choosing the "wrong" (C1) disc, which now had a higher P content than before. A single trial has been reported as sufficient for *L. maximus* to acquire a learned aversion (Sahley et al., 1981, 1990), and Delaney and Gelperin (1986) reported that learned post-ingestive food aversions could last for several months.

The dynamics of ST change are remarkable. Leaves from the same clone of *P. hybridus* varied up to 10-fold in their P content from week to week. Together with the observed change in sensitivity after experience with P, there is potentially a complicated interaction between P leaf content and snail herbivory. In the beginning of the growing season, when young leaf tissue has to be especially protected from herbivory, a high P content would deter snails from feeding on it. Through possible sensitization and associative learning, this effect could then be reinforced and maintained over the season even when the leaf P content starts to decline. A specific feature of the P-snail system might be that it involves not only a substance that is possibly bitter tasting and therefore learned to be avoided, but perhaps one of the rare cases where a plant compound has a direct neurophysiological influence on the feeding behavior itself.

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