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MODE OF ACTION OF THE SESQUITERPENE LACTONE, TENULIN, FROM *HELENIUM AMARUM* AGAINST HERBIVOROUS INSECTS

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ABSTRACT.—Tenulin [**1**], a sesquiterpene lactone from *Helenuim amarum*, is a potent antifeedant to the European corn borer *Ostrinia nubilalis*. At 3 μmol/g in artificial diets, **1** reduced growth and delayed larval development of *O. nubilalis* and the variegated cutworm *Peridroma saucia* larvae. An especially pronounced carry-over effect in *O. nubilalis* was substantial reduction in fecundity of adult moths resulting from treated larvae. The LD_{50} (lethal dose for 50% mortality) of **1** by injection in the migratory grasshopper *Melanoplus sanguinipes* was 0.88 μmol/insect. Toxicity in *M. sanguinipes* was antagonized by co-administration of cysteine, suggesting that the cyclopentenone group of tenulin undergoes Michael addition of biological nucleophiles in vivo. This mechanism was partially confirmed by the finding that only tenulin analogues capable of acting as electrophic acceptors had significant antifeedant activity.

Sesquiterpene lactones of the Asteraceae have a number of biological activities toward insects suggesting their evolution in plants as deterrents to insect herbivory. More than 50 compounds have been investigated, and the significant effects reported include feeding and oviposition deterrence, growth inhibition, lengthened development, and mortality (1). While feeding deterrence is especially notable, some studies have clearly indicated the role of these compounds as metabolic toxins as well. Direct injection of several sesquiterpene lactones into the hemocoel of the grasshopper *Melanoplus sanguinipes* caused mortality (2). One of the compounds examined, parthenin, also reduces heart rate in semi-isolated preparations from the same insect (3). In these and other studies (1) greatest activity has been linked to the presence of an α-methylene-γ-lactone group in the molecule, which can undergo Michael addition with biological nucleophiles. Evidence of this mechanism in insects was demonstrated by the reversal of toxicity when L-cysteine was co-administered with the sesquiterpene lactone (2,3).

Tenulin [**1**], a major constituent of bitterweed *Helenium amarum* (Raf.) H. Rock (Asteraceae) (4), has not been investigated seriously as a deterrent to herbivorous insects. However, the related compound desacetyl isotenulin from *Psathyrotes ramosissima* (Asteraceae) has reported molluscicidal activity (5). While many sequiterpene lactones possess the cyclopentenone moiety present in tenulin [**1**], all previously examined compounds with activity against insects have both the cyclopentenone and α-methylene-γ-lactone functionality or at least the latter. Tenulin [**1**] provides a model compound with only the cyclopentenone functionality for unambiguous investigation of the mode of action of this putative toxicophore in insects. The present study examines the effect of **1** on three insect herbivores: *Ostrinia nubilalis* (Lepidoptera, Pyralidae), which feeds on many composites; *Peridroma saucia* (Lepidoptera, Noctuidae), a highly polyphagous cutworm; and *Melanoplus sanguinipes* (Acrididae), a polyphagous grasshopper.

EXPERIMENTAL

Tenulin [**1**] was obtained from *H. amarum* according to published procedures (4,6). The preparations of the tenulin derivatives [**2-7**] discussed in this paper are described in Waddell *et al.* (6,7) and Lee *et al.* (8).

Adult male grasshoppers (*M. sanguinipes*), 7-10 days after the final molt, were obtained from a laboratory colony. Tenulin [**1**] was administered via intrahemocoelic injection as previously described (2). For

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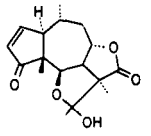

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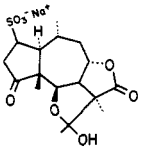

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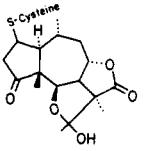

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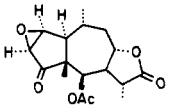

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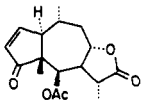

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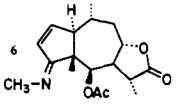

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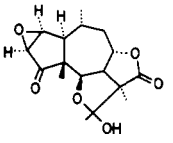

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the dose-response bioassay, 2 µl of Me_2CO served as the carrier. For the cysteine-antagonism experiment, the carrier was 3 µl of 50% aqueous Me_2CO.

Neonate variegated cutworms (*P. saucia*) were also obtained from a laboratory colony. Tenulin [**1**] was admixed with an artificial diet (No. 9795, Bioserv Inc., Frenchtown, NJ) and provided to cutworms as previously described (9).

European corn borer larvae (*O. nubilalis*) were obtained from a laboratory colony and antifeedant and growth studies conducted as described previously for azadirachtin (10).

RESULTS AND DISCUSSION

Although tenulin [**1**] is present in very high concentrations in leaves of bitterweed, *H. amarum*, (2.2% dry weight or approximately 15-25 µmol/g fresh weight) (4), biological activity towards herbivorous insects was detected at much lower levels. Tenulin [**1**] applied to leaf disks of corn reduced feeding of *O. nubilalis* with significance at 0.3 µmol/g fresh weight and above (Figure 1). While the relationship between consumption and concentration is not strictly linear, a PC_{50} (protective concentration for 50% reduction in feeding) of 0.2 µmol and a PC_{90} (90% reduction in feeding) of 8 µmol can be estimated. In spite of the potent antifeedant effects, larvae could be in-

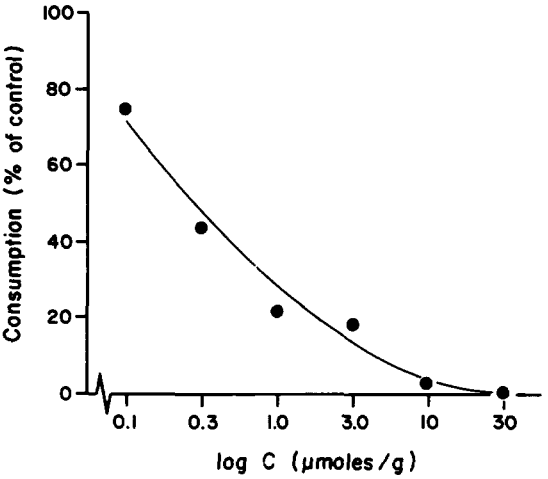

FIGURE 1. Consumption of corn leaf disk treated with different concentrations of tenulin by *Ostrinia nubilalis* neonate larvae (N=20).

duced to feed on artificial diets containing **1** at lower concentrations. Growth of both *O. nubilalis* and *P. saucia* larvae was reduced at 3 μmol/g tenulin [**1**] in diet but not appreciably at 0.3 μmol/g (Figure 2). *O. nubilalis* larvae reared through their life cycle (Table 1) exhibited some effects of tenulin at 3.0 μmol/g in diets, vis., lengthened time to pupation and lowered larval weights but no such effect at 0.3 μmol/g. One exceptional effect was on fecundity: The number of eggs per female was substantially reduced at both concentrations. This latent carry over effect at the F_1 level suggests toxic effects of **1** in addition to the observed feeding deterrence mechanism. These results contrast with a previous report that **1** at dietary concentrations as high as 8% dry weight has no significant effect on survival of the confused flour beetle, *Tribolium confusum* (11).

In order to determine the toxic effects of tenulin [**1**] without the complication of feeding deterrence, a standard injection procedure with *M. sanguinipes* was used (2). Tenulin [**1**] is clearly toxic, and its LD_{50} lies within the range of toxicities of sesquiter-

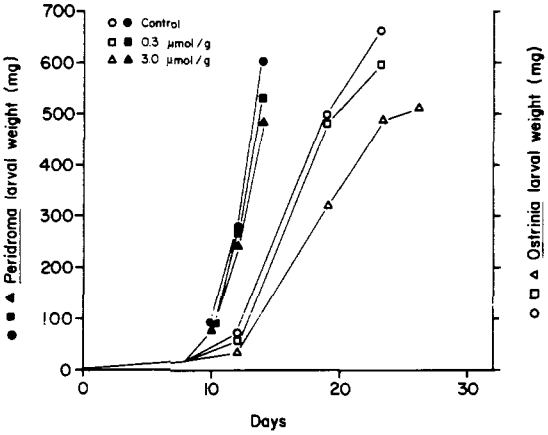

FIGURE 2. Growth of *Peridroma saucia* and *Ostrinia nubilalis* neonate larvae fed artificial diets containing tenulin (N=30).

TABLE 1. Effect of Tenulin [**1**] on Developmental Parameters of *Ostrinia nubilalis*[a]

Diet	Maximum larval weight (mg)	Number of days to pupation	Pupal Weight (mg) male/female		Duration of pupal period (days)	Percent pupation (%)	Percent larvae in diapause	Number of days to adult emergence	Percent adult emergence from pupae	Sex ratio of adults (female/total)	Number of eggs per female
Control	109.8 (20.9)	25.7 (2.1)	71.3 (9.4)	101.5 (9.3)	8.30 (1.12)	83.3	0	33.9 (2.2)	84.0	0.52	407
0.3 μmol/g . .	108.3 (22.7)	24.8 (2.7)	71.3 (6.7)	102.9 (12.1)	8.84 (1.54)	86.7	3.33	33.6 (2.6)	100	0.46	90
3 μmol/g . . .	107.4 (16.8)	30.0 (2.8)	69.0 (7.4)	86.3 (9.9)	8.67 (1.46)	86.7	3.33	38.2 (2.7)	92.3	0.58	17

[a]Each number is the mean result of 30 observations. (Standard deviation in parentheses.)

pene lactones possessing only the α-methylene-γ-lactone group (Table 2). Tenulin [**1**] lacks this moiety, instead possessing a cyclopentenone group. However, the most active compound, parthenin, possesses both functional groups. In a previous study (12) we found that hymenolin lacking both function groups was at least an order of magnitude less toxic to insects than parthenin.

TABLE 2. Comparison of Toxicity to *Melanoplus sanguinipes* and Chemical Functionality of Sesquiterpene Lactones[a]

	LD_{50}[b] μmoles/insect	α-methylene γ-lactone	cyclopentenone
tenulin [**1**]	0.88	−	+
parthenin	0.55	+	+
tetraneurin-A	0.68	+	−
coronopolin	0.68	+	−
confertin	1.22	+	−

[a]Excepting tenulin, data are from Isman (2).

[b]48 h LD_{50}s calculated following injection into hemocoel at doses of 0.25, 0.5, 0.75, 1.0 μmol/insect (N = 10-30 for each concentration).

Cysteine was co-administered with tenulin [**1**] to investigate if the cyclopentenone ring was capable of undergoing Michael addition in a manner similar to the α-methylene-γ-lactone moiety. The antagonism of tenulin toxicity observed is substantial (Table 3) but not as pronounced as the antagonism of tetraneurin A by cysteine (2).

A second test of the possibility that the cyclopentenone ring acts as an electrophilic acceptor to a biological nucleophile (S-H groups) was undertaken using a series of tenulin [**1**] analogues and derivatives [**2-7**]. Those analogues capable of acting as electrophilic acceptors [**4-7**] including tenulin [**1**] had significant antifeedant activity. Two derivatives incapable of acting as electrophilic acceptors, the cysteine [**3**] and HSO_3 [**2**] adducts, lacked significant antifeedant properties to *O. nubilalis* (Table 4). Our results confirm earlier reports that the exo-methylene group is not essential for insect antifeedant activity (13).

Previously tenulin [**1**] has received attention because of its activity as an inhibitor of tumor growth (7), an antihyperlipedemic agent (14), and an anti-inflammatory agent (15,16). In these studies, as in the present study with insects, investigation of structure activity relations using tenulin analogues revealed that retention of the cyclopentenone moiety was required for high in vivo activity (17). In addition, direct in vitro evidence that tenulin undergoes Michael addition of sulfhydryl agents such as glutathione and L-cysteine has been obtained (17). The present demonstration of antagonism of toxicity in grasshoppers suggests that a similar mechanism occurs in vivo.

The present study confirms that tenulin [**1**] is a potent deterrent in bitterweed to insect herbivores. The diversity of metabolic pathways that has evolved in the secondary metabolism of the Asteraceae can produce different molecular structures including the

TABLE 3. Antagonism of Tenuline Toxicity in Adult Grasshoppers by L-cysteine

Treatment	% mortality (N=30)
1.0 μmol tenulin [**1**]	63
1.0 μmol L-cysteine	0
1.0 μmol tenulin+L-cysteine	30
Carrier control	3

TABLE 4. Antifeedant Properties of Tenulin Analogues on Corn Leaf Disks to *Ostrinia nubilalis*

Treatment	% of leaf disk consumed
control	85.5[a]
tenulin [**1**]	13.9[b]
tenulin HSO_3 adduct [**2**]	64.0[a]
tenulin cysteine adduct [**3**]	42.6[a]
isotenulin oxide [**4**]	17.9[b]
isotenulin [**5**]	17.8[b]
isotenulin methyl imine [**6**]	16.8[b]
tenulin oxide [**7**]	10.6[b]

[a,b]Concentration of compound was 3 μmol/g leaf disk. Means followed by the same letter are not significantly different in Tukey's test ($p=0.05$).

α-methylene-γ-lactone and cyclopentenone functionalities. The convergent biological mode-of-action of these structures attests to the efficacy of this mechanism in plant defense.

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