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Abstract
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Keywords
Blattella germanica, catnip, nepetalactone, repellency

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Behavioral Activity of Catnip (Lamiaceae) Essential Oil Components to the German Cockroach (Blattodea: Blattellidae)

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ABSTRACT The essential oil of catnip, *Nepeta cataria* L., contains two isomers of nepetalactone, *E,Z*- and *Z,E*-nepetalactone, and was tested for repellent activity to adult male German cockroaches, *Blattella germanica* (L.), in a choice-test arena. The two isomers of nepetalactone were purified by using preparative thin-layer chromatography and tested for behavioral activity in the choice-test arena. Significant differences due to concentration were detected by analysis of variance, and the responses were compared by least-squared means analysis. The activities of the essential oil and purified isomers were compared with *N,N*-diethyl-3-methylbenzamide (DEET) by a paired *t*-test. *E,Z*-Nepetalactone was the most active of the compounds tested, being significantly more active to this species than equivalent doses of DEET, the essential oil, or *Z,E*-nepetalactone. Antennectomized insects showed no response to concentrations that were active against intact insects.

KEY WORDS *Blattella germanica*, catnip, nepetalactone, repellency

CATNIP, *Nepeta cataria* L., is well known for its intoxicating effects on cats. The essential oil of catnip contains the monoterpene-derived iridodial compound nepetalactone (5,6,7,7*a*-tetrahydro-4,7-dimethylcyclopenta[*e*]pyran-1-(4*a*H)-one) (McElvain et al. 1941), which exists in the plant as two isomers, *Z,E*- and *E,Z*-nepetalactone (Fig. 1). The *E,Z*-isomer is the more attractive of the two isomers to cats (Bates and Sigel 1963). Catnip has folk uses as an insect repellent and some uses have been confirmed scientifically. Nepetalactone vapors were shown to be repellent to insect species in 13 families (Eisner 1964). Nepetalactone is an important component of the defensive secretions of the coconut stick insect, *Graffea crouani* Le Guillou (Smith et al. 1979), and the lubber grasshopper, *Romalea guttata* (Houttuyn) (Snook et al. 1993). Other essential oils and individual monoterpenoids repel German cockroaches, *Blattella germanica* (L.) (Inazuka 1982, 1983; Karr and Coats 1988; Coats et al. 1991), as do various plant extracts (Scheffler and Dombrowski 1992) and plant products (Appel and Mack 1989).

In the current study, we describe the isolation and purification of the two isomers of nepetalactone and their behavioral effects against the German cockroach. Ethanol and ethyl ether extracts of catnip were previously reported as being repellent to this species, but there was no mention of the activity of the isomers (Bodenstein and Fales 1976). We compared the activity of the catnip essential oil and the two individual isomers against *N,N*-diethyl-3-methylbenzamide (DEET, formerly *N,N*-diethyl-*m*-toluamide). First synthesized in the early 1950s (McCabe et al. 1954), worldwide use of DEET exceeded 200,000,000 persons in 1980 (EPA 1980). DEET can occasionally have severe adverse effects on mammals (Miller 1982, Roland et al. 1985, Snyder et al. 1986, Qiu et al. 1998). We tested the effect of antennectomy on the behavioral response of *B. germanica* to doses of extracts and compounds active to intact *B. germanica.*

Materials and Methods

Plant Collection and Steam Distillation. The aerial portions of *N. cataria* plants were collected from unsprayed areas of the Iowa State University campus, Ames, IA, as needed during the growing season. Plants not distilled immediately were frozen at –80°C. Plant leaves and stems were cut by using scissors, placed into a 5-liter, three-necked boiling flask, and steam distilled according to the method of Pavia et al. (1988). The collected distillate was washed three times with one volume each of hexane. The hexane was removed by using rotary evaporation at 500 mm Hg vacuum at 25°C.

Extract Analysis. A portion of the liquid obtained from rotary evaporation was diluted to 1 μl/ml with hexane and subjected to chromatographic analysis. Gas chromatographic analyses were conducted on a Varian 3700 gas chromatograph (Palo Alto, CA) with a 2-m packed OV-101 column, a nitrogen carrier, an

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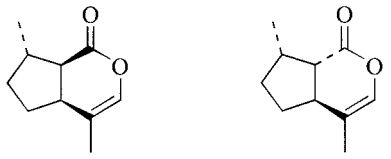

Fig. 1. Nepetalactone isomers from catnip.

FID detector, an injection temperature of 250°C, an injection volume of 1.5 μl, and with an initial column temperature of 70°C ramped at 5°C/min to 150°C and held for 8 min. High-performance liquid chromatography (HPLC) was conducted by using a Hewlett–Packard 1100 HPLC (Palo Atlo, CA) with a Pirkle Covalent Phenylglycine hi-chrom preparative column (25 cm by 10 mm i.d., 5 μm S5NH Modified Shereosorb, Regis Technologies, Morton Grove, IL), with a mobile phase of 9:1 hexane/ethyl acetate at 2.5 ml/min flow rate, and detection by using a Spectroflow 757 UV-detector (Chestnut Ridge, NY) at 254 nm.

Separation of Nepetalactone Isomers. The two isomers of nepetalactone were separated by using silica gel preparative thin-layer chromatography plates (20 by 20 cm, 1,000 μm in thickness, Whatman, Hillsboro, OR) with a solvent system of 19:1 hexane/ethyl ether. The plates were run seven times and allowed to dry completely between runs. The products were visualized under 254-nm UV light, and the silica gel was scraped off the plates and washed with three washings of ethyl ether. The ether was removed by rotary evaporation, and the purity of the isomers was assessed by using HPLC.

Instrumental Analysis of Isomers. Gas chromatography/mass spectroscopy of the nepetalactone isomers was conducted on a Varian 3400 gas chromatograph, with a DB-5 ms nonpolar 30-m capillary column (0.25 mm i.d., J. & W. Scientific, Folsom, CA). The injector temperature was 250°C and the column temperature was held at 150°C (isothermal). The gas chromatograph was coupled to a Finnigan TSQ 700 triple quadrupolemass spectrometer (San Jose, CA), with an electron impact of 70 eV.

Cockroach Bioassay. Adult male *B. germanica* were obtained from a colony reared in our laboratory for several years. Male German cockroaches have been found by us (data not shown) and others (Scheffler and Dombrowski 1992) to be more sensitive than females to olfactory stimuli. The catnip essential oil and the nepetalactone isomers were dissolved in hexane, and DEET (Aldrich, Milwaukee, WI) was dissolved in acetone. A 12.5 cm round filter paper was cut in half. One side was treated with 1 ml of the test compound solution, and the other side was treated with either acetone or hexane, depending upon which solvent was used to dissolve the test material. The papers were allowed to dry for ≈2 min before being placed in a 15 cm petri dish arena. The position of the treated side (to the right or to the left) was randomized by using a random number table. The top of the petri dish had a hole cut in the center for introduction of the insect directly into the center of the arena. One insect at a time was introduced. The hole was stopped by using a small piece of tape to prevent escape of the insect. Immediately after the introduction of the insect, the number of seconds it spent on the treated or untreated side in a total of 300 s (5 min) was timed with two stopwatches. Filter papers and cockroaches were used once then discarded. Repellency values were calculated by subtracting the number of seconds the insect spent on the treated side from the number of seconds spent on the untreated side, dividing by the total number of seconds (300), and then multiplying that value by 100 to obtain a percentage. Each test was replicated 10 times. All tests were run between 1000 and 1600 hours (CST) with overhead florescent lighting at ambient temperature (20–25°C) and humidity (50–70% RH).

For the tests that used antennectomized cockroaches, a razor blade was used to remove the antennae at the scape. The cockroaches were allowed to recover from the procedure for 24 h before being exposed to the test compounds by the method outlined in the previous paragraph.

Significance due to concentration was determined by using ANOVA, and means for each dose were compared by least-squares means analysis (SAS Institute 1991) to determine dose–response relationships. Comparisons between compounds or treatments were made by using a paired *t*-test.

Results and Discussion

By using gas chromatography, we determined that the catnip essential oil contained *Z,E*-nepetalactone and *E,Z*-nepetalactone in a 6:1 ratio, or roughly 85:15%. Bates and Sigel (1963) reported a ratio of 3:1 *Z,E*-nepetalactone to *E,Z*-nepetalactone, or 75:25%. These isomers together comprised 98% of the steam distillate, and minor components were not identified.

Mass spectral analysis revealed that the two isomers are indistinguishable. *Z,E*-Nepetalactone eluted off the DB-5 ms column at 2.45 min and showed ions at m/z 166 [M^+] (100%), m/z 123 (78.5%), m/z 109 (46.3%), m/z 95 (58.8%), m/z 81 (62.2%), and m/z 69 (46.7%). *E,Z*-Nepetalactone eluted off the column at 2.65 min and had the following mass spectrum: m/z 166 [M^+] (100%), m/z 123 (99.9%), m/z 109 (51.8%), m/z 95 (66.6%), m/z 81 (67.0%), and m/z 69 (50.2%).

Significance due to concentration was observed by two-tailed ANOVA for DEET ($F = 4.83$; df = 5, 54; $P = 0.001$), *Z,E*-nepetalactone ($F = 20.00$; df = 4, 45; $P = 0.0001$), and *E,Z*-nepetalactone ($F = 41.08$; df = 2, 27; $P = 0.0001$). Significance due to concentration was not seen for the catnip essential oil at the 0.05 two-tailed significance level, but was seen at the 0.1 significance level ($F = 3.44$; df = 3, 36; $P = 0.0267$). Repellency values (% ± SEM) were calculated and means were compared by using least-squares means analysis (Table 1). All DEET concentrations tested at <1,600 μg/cm^2 were not significantly different from the control by least-squared means analysis ($\alpha = 0.05$). The be-

Table 1. Percentage repellency (±SEM) of DEET and catnip compounds to male *B. germanica* in a choice-test assay

Test solution	Dose (μg/cm^2)	% repellency ±SEM
DEET	1,600	58.3 ± 10.5b
	800	25.8 ± 9.5a
	160	20.4 ± 9.2a
	80	15.5 ± 5.4a
	16	15.4 ± 5.9a
	0	5.2 ± 7.5a
Catnip essential oil		
	800	55.6 ± 9.8b
	160	27.7 ± 13.1ab
	80	33.7 ± 15.7ab
	16	31.7 ± 8.1ab
	0	2.9 ± 3.7a
Z,E-Nepetalactone		
	800	68.2 ± 5.7b
	160	56.8 ± 7.8b
	80	15.4 ± 6.9a
	16	16.1 ± 7.4a
	0	2.9 ± 3.7a
E,Z-Nepetalactone		
	80	79.4 ± 3.5c
	16	46.4 ± 11.0b
	0	2.9 ± 3.7a

For each test solution, repellency values followed by the same letter are not significantly different by least-squares means analysis (α = 0.05). Repellency = [(no. of seconds spent on untreated side − no. of seconds spent on treated side)/300] × 100.

havioral response to catnip essential oil was significantly different from the control at a dose of 800 μg/cm^2. The response to *Z,E*-nepetalactone was significantly different from the control at doses of 160 μg/cm^2 and higher. The response to *E,Z*-nepetalactone was significantly different from the control at all doses tested (down to 16 μg/cm^2).

Paired *t*-test comparisons (α = 0.05, df = 9) between the different compounds at equivalent doses were made. The response of the cockroaches to catnip essential oil differed significantly from DEET at 800 μg/cm^2, and did not differ from it at lower doses. The response to *Z,E*-nepetalactone differed from equivalent doses of DEET above 80 μg/cm^2 and the response to *E,Z*-nepetalactone differed significantly from DEET at all concentrations tested, down to 16 μg/cm^2. The response to *Z,E*-nepetalactone, which comprised ≈85% of the essential oil, did not differ significantly from the response to catnip essential oil at any of the concentrations tested. *E,Z*-Nepetalactone was more active than the catnip essential oil at 80 μg/cm^2. Both *Z,E*- and *E,Z*-nepetalactone were compared at 80 and 16 μg/cm^2, and *E,Z*-nepetalactone was significantly more active than the *Z,E*- isomer at both concentrations. Catnip essential oil should be more active than the *Z,E*-isomer alone because the oil contains ≈15% of the more active *E,Z*-isomer. This was not observed, perhaps because the variability inherent in behavioral tests obscures statistical determination of differences. Visual examination of the results indicates that the essential oil may be more active than the *Z,E*-isomer at the two lowest doses (80 and 16 μg/cm^2), and more rigorous testing may statistically reveal the expected differences.

Antennectomy of adult male cockroaches resulted in a diminished response to the test compounds (paired *t*-test, α = 0.05, df = 9) (Table 2). In the three comparisons, the amount of time the antennectomized insects spent on either the treated or untreated side did not differ significantly by the paired *t*-test. This indicates that the chemoreceptors involved in this behavioral response are probably located on the antennae.

The results presented herein indicate that catnip essential oil and the isomers of nepetalactone cause German cockroaches to spend less time in treated areas. These compounds may be useful in the development of exclusion barriers to prevent entry of insects into sensitive areas; e.g., kitchens, children's nurseries, and hospital rooms. Using such compounds in shipping containers may reduce the incidence of accidental pest introduction to novel areas. That these compounds are volatile and the response was olfactory (rather than contact) may be important in providing protection over a large area, because the insects will not have contact with the treated surfaces. Volatility, however, may shorten the effective time. This problem may be alleviated by special formulations, such as microincapsulation, to reduce volatile loss. Using these compounds as part of a "push-pull" system, repelling the insects out of one area and luring into an attract-and-kill system in another, also may be possible.

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Table 2. Results of behavioral assay of antennectomized male German cockroaches, and paired *t* test comparison with nonantennectomized male cockroaches tested at the same concentration

	% repellency[a] ± SEM		
Treatment, μg/cm^2	Antennectomized	Nonantennectomized	Calculated *t* value
1600 DEET	1.7 ± 11.4	58.3 ± 10.5	3.03*
160 *Z,E*-Nepetalactone	19.8 ± 7.0	56.8 ± 7.8	3.40*
80 *E,Z*-Nepetalactone	−1.3 ± 10.2	79.4 ± 3.5	7.84*

% Repellency = [(no. of seconds spent on untreated side − no. of seconds spent on treated side)/300] × 100.
*, Difference is significant by two-tailed paired *t* test at α = 0.05, df = 9.

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