Insect Repellents from the Chinese Prickly Ash Zanthoxylum bungeanum

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INSECT REPELLENTS FROM THE CHINESE PRICKLY ASH *ZANTHOXYLUM BUNGEANUM*

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ABSTRACT.—A CH_2Cl_2 extract of *Zanthoxylum bungeanum* is highly repellent to insects. Using hplc and gc-ms together with repellent and deterrent bioassays we isolated and identified three active monoterpenes: piperitone, 4-terpineol, and linalool. Piperitone is more repellent than is the common insect repellent *N,N*-diethyl-*m*-toluamide (DEET).

The fruit of the Chinese prickly ash tree, *Zanthoxylum bungeanum* Maxim. (Rutaceae), is a "peppery" spice used in Chinese cooking (1). The pericarp of the seeds is used in Indian and Chinese traditional systems of medicine for the treatment of stomachache, toothache, abdominal pain, ascariosis, diarrhea, and dysentery (2,3).

Essential oil from the fruit of *Z. bungeanum* has been reported to be an insect repellent and a feeding deterrent (4,5). In this study we examined the repellent and feeding deterrent activities of *Z. bungeanum* fruit extract against ants of the genus *Crematogaster.* We report the isolation and identification of the insect repellent and deterrent components of the Chinese prickly ash.

Cc and hplc of *Z. bungeanum* extract gave two fractions with significant feeding deterrency for the *Crematogaster* ants (Figure 1). From these fractions we identified the monoterpenes 1,8-cineole, linalool, 4-terpineol, α-terpineol, piperitone, 4-terpinenyl acetate, α-terpinene, and α-terpinenyl acetate, and one sesquiterpene, caryophyllene. Several of these compounds have been reported previously in fruit of *Z. bungeanum* (1,6). However, α-terpinene, the terpinenyl acetates, and caryophyllene are newly reported natural components.

Authentic standards of the identified terpenes were assayed for repellency and feeding deterrence to the ants to determine which compounds from the *Z. bungeanum* extract are responsible for the observed biological activity. The activities of the identified natural products were also compared to those of the common insect repellent DEET (Figure 2). Of the active natural products, piperitone gave the strongest feeding deterrent activity (D_{80}=0.13 μg/μl), which is similar to that of DEET (D_{80}=0.16 μg/μl). Feeding deterrent activity of linalool is D_{80}=0.56 μg/μl, of 4-terpineol

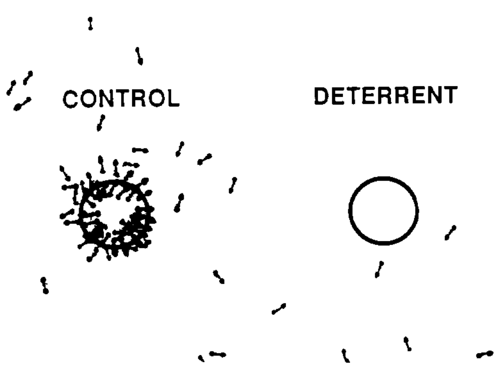

FIGURE 1. Feeding deterrency assay. Ants of the genus *Crematogaster* are deterred from feeding on a sucrose solution containing an active chromatographic fraction or pure monoterpene (right), while feeding on a solution of sucrose (left).

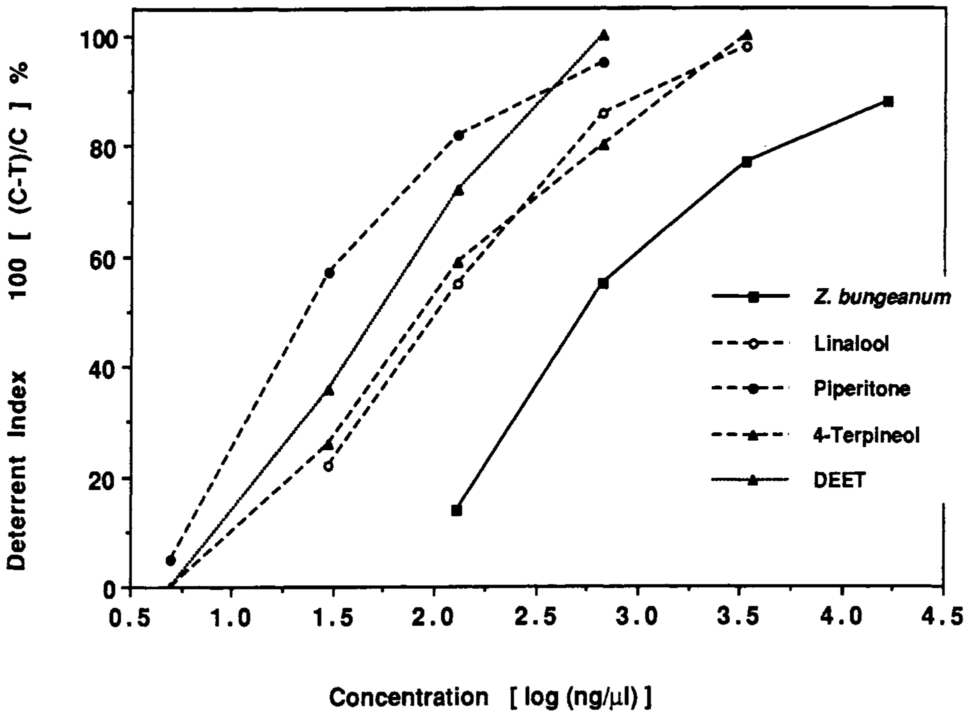

FIGURE 2. Feeding deterrency to *Crematogaster* spp. ants by *Zanthoxylum bungeanum* extract, terpene components of the extract, and DEET.

D_{80}=0.71 µg/µl, of 1,8-cineol D_{80}=0.64 µg/µl, and of α-terpineol D_{80}=0.45 µg/µl. We tested a mixture of linalool, 4-terpineol, and piperitone (1:1:1 by volume) to determine whether the activity of the deterrent compounds in combination was synergistic or additive. We find no evidence of synergism since the deterrent activity of the mixture (D_{80}=0.25 µg/µl) was comparable to the activity of each individual component (Table 1).

The repellent activities of the crude *Zanthoxylum* extract, chromatographic fractions, and authentic standards were compared with that of DEET (Table 1). Again, piperitone (R_{80}=8.91 µg/cm^2) had the strongest activity, about twice that of linalool (R_{80}=14.13 µg/cm^2) and 4-terpineol (R_{80}=20 µg/cm^2). All monoterpenes tested as space repellents showed higher activity than DEET (R_{80}=125.9 µg/cm^2), due perhaps to their higher volatility.

Piperitone is a major component of some essential oils (7) and is a feeding deterrent to the white pine weevil *Pissodes strobi* (8). Linalool is repellent to aphids (9), mosquitos (10), and cockroaches (11). 4-Terpineol is antifeedant to the locust and the Colorado potato beetle (12) and repellent to the ant *Messor structor* (12) and to mosquitoes (10).

Monoterpenes are widely distributed in the plant kingdom and are utilized as attractants and defensive and allelopathic agents. We believe that the monoterpenes identified in this study account for the deterrent and repellent activities of the *Z. bungeanum* extracts. In our evaluations piperitone is the most active compound in the feeding deterrent assays; however, 4-terpineol, linalool, and 1,8-cineole also contribute to the insect repellent and feeding deterrence of the Chinese prickly ash. Although DEET is an effective feeding deterrent, we find that the more volatile monoterpenes produced by *Z. bungeanum* are more effective repellents. Thus, the release of these volatile monoterpenes from the pericarp prob-

TABLE 1. Deterrency and Repellency of *Zanthoxylum bungeanum*, CH_2Cl_2 Extract, Terpene Components, and *N,N*-Diethyl-*m*-toluamide.

Sample	Deterrent Index D_{80} (μg/μl)	Repellency Index R_{80} (μg/cm²)
CH_2Cl_2 Extract	5.62	100.3
1,8 Cineol	0.64	17.8
Linalool	0.56	14.1
4-Terpineol	0.71	20.0
α-Terpineol	0.45	7.9
Piperitone	0.13	8.9
Standard mixture	0.25	14.4
N,N-Diethyl-*m*-toluamide	0.16	125.9

ably afford continuous protection from insect herbivory, and may be an especially effective protective mechanism for plants not dependent on pollination by insects.

EXPERIMENTAL

PLANT MATERIAL.—The dried fruit of *Z. bungeanum* was obtained as a commercial product of the People's Republic of China from the Wai Yan Trading Co., 8/F, Block A, 42-44 Ko Shing Street, Hong Kong.

CHEMICALS.—Authentic standards of piperitone (CAS #89-81-6) and 4-terpineol (CAS # 562-74-3) were purchased from Penta Mfg. Co., Fairfield, NJ. Linalool (CAS # 78-70-6) was obtained from Hoffman-La Roche, Nutley, NJ. *N,N*-Diethyl-*m*-toluamide (DEET) (CAS # 134-62-3) was purchased from Aldrich Chemical, Milwaukee. α-Terpineol (CAS #98-55-5) was purchased from Sigma Chemical, St. Louis. 1,8-Cineole (CAS # 470-82-6) was obtained from oil of eucalyptus supplied by the R.D. Webb Co., New York, NY.

EXTRACTION, ISOLATION, AND IDENTIFICATION.—Fruit of *Z. bungeanum* (128 g) was ground to a powder and stirred in 800 ml CH_2Cl_2 for 2 h. Following vacuum filtration through glass fiber paper, the cake was washed with 100 ml CH_2Cl_2, and the combined filtrates were evaporated in vacuo to give 13.39 g of extract. The CH_2Cl_2 extract was triturated with 200 ml Et_2O, and the Et_2O extract was evaporated to give 9.26 g of aromatic liquid, of which 2 g was chromatographed on 60 g of Florisil (deactivated with 7% H_2O) and eluted stepwise with hexane followed by 5, 15, 25, and 50% Et_2O in hexane (13). The 5% Et_2O/hexane fraction after evaporation gave 60 mg of repellent material, and the 15% Et_2O/hexane fraction produced 360 mg of highly repellent material. Hplc was performed on a 25 cm×4.6 mm column of 10 μ Si gel. Part (0.5 mg) of the active open column fraction was separated isocratically with 15% EtOAc in hexane at 1 ml/min and monitored by uv absorbance at 254 nm.

Gc-ms was performed on a Hewlett-Packard 5890 mass selective detector coupled with a 5890 gas chromatograph. The gc column was a 12 m HP-1 (methyl silicone) capillary, 0.2 mm diameter, 0.33 μ film thickness, using H_2 carrier gas. Fragmentation was by electron ionization at 70 eV. The repellent natural compounds were identified by superimposable mass spectra and coincident gc retention times compared to authentic standards. The other terpenes were identified by relative chromatographic retention times and their mass spectra.

BIOASSAYS.—For the deterrent bioassay all samples were dissolved in MeOH, and 10 μl was added to 20 μl of a 40% sucrose solution on a glass microscope slide. Final concentrations were 16.5, 3.3, 0.67, 0.13, 0.03, and 0.005 μg/μl. A 20 μl drop of the 40% sucrose solution plus 10 μl of MeOH on the same slide served as control. The slide was placed inside a colony of *Crematogaster* ants, and the number of ants feeding on the control (C) and on the treated (T) sucrose solutions were recorded over a period of 10 min. A deterrent index was then calculated as $100[(C-T)/C]\%$. Each concentration was replicated four times using four different ant colonies. The concentration which deterred 80% of the ants from feeding on the sucrose solution containing the experimental sample (D_{80}) was calculated for each compound or extract fraction.

In the repellency bioassay, samples were dissolved in MeOH and 10 μl of the test solution was applied to a filter paper (0.5 cm²) that was suspended 1 cm over a drop of 40% sucrose solution. The sample concentrations on the paper were 100, 20, 4, and 0.8 μg/cm². The control consisted of 10 μl of MeOH on paper suspended over the drop of sucrose solution. The dose that repelled 80% of the ants from feeding on the sucrose solution (R_{80}) was calculated as in the previous bioassay.

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