Agric. Chem. Biotechnol. 44(3), 140-142 (2001)

Determination of Ant Repellents Activity of Cineol, α-Terpineol, Linalool, and Piperitone

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Recevied October 11, 2000

Quantitative gas chromatographic method for determining the ant repellent activity of cineol, α-terpineol, linalool, and piperitone which usually found in Chinese Prickly Ash *Zanthoxylum piperitum* DC. was developed. These monoterpenes showed higher ant repellent activities than DEET due perhaps to their volatility. Gas chromatographic method quantified the volatility of the four monoterpenes and DEET.

Key words: *quantitative GC analysis, ant repellent, Chinese Prickly Ash,* Zanthoxylum piperitum, *monoterpene, DEET.*

Many natural insect repellents and feeding deterrents were isolated from plants, and the repellent activities of these essential oils have been well documented. Oils obtained from steam distillate of *Vitex negundo* leaves was fractionated through column chromatography. The mosquito repellent activity against *Aedes aegypti* was mainly confined to the most polar fractions, and the protection period against mosquito bites by polar fractions ranged between 1-3 h.[1)] Quwenling, which is a mosquito repellent product of China derived from extracts of the lemon eucalyptus plant (*Eucalyptus maculata citriodon*), was evaluated.[7)]

A hexane extract of the gum of an African plant, *Commiphora erythraea* Engler (Burseraceae), has larvicidal and repellent activity against the lone star tick, *Amblyomma americanum* (L.), and American dog tick, *Dermacentor variabilis* (Say). Adult deer ticks, *Ixodes dammini* Spielman, Clifford, Piesman, and Corwin, were also repelled by the extract.[2)] Sesquiterpenoid ant repellent from fruit of *Dysoxylum spectabile* was reported. The hexane soluble portion of the whole fruit extract was fractionated through silica gel flash chromatography, sephadex LH20 column chromatography, and centrifugal TLC to give (2S,3R)-2,3-dimethyl-3-(4-methyl-3-pentenyl)-2- norbornanol, a repellent against ants.[6)] Several limonoids with insect antifeeding activities have been isolated from the leaves of *D. richii* and *D. binectariferum*.[5,9)] Essential oil from the fruit of *Zanthoxylum piperitum*, an insect repellent and feeding deterrent, has been isolated.[3,8)] The essential oil of *Zanthoxylum piperitum* of Chinese origin cultivated in Urbino, Italy were collected through hydrodistillation and capillary gas chromatography showing 28 peaks, and 24 compounds were identified.[10)]

Insect repellent and deterrent compounds from the fruit of *Zanthoxylum piperitum* were isolated, and the biological activity against ants of the genus *Crematogaster* was examined. The activities of the identified compounds were compared to those of *N,N*-Diethyl-*m*-toluamide, DEET.[1)] The Bowers group found that the extract of *Z. piperitum* showed high repellency against the ants of the genus *Crematogaster.* Three active monoterpenes, piperitone, α-terpineol, and linalool, were isolated and identified. Piperitone had higher repellency than the common insect repellent *N,N*-Diethyl-*m*-toluamide, DEET. In this bioassay, the repellent activities of monoterpenes and DEET were tested as space repellents. In general monoterpene compounds are highly volatile. The purpose of this study was to determine the volatility of four monoterpenes and DEET in respect to their long-lasting effectiveness using gas chromatography.

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Abbreviations: DEET, N,N-diethyl-m-toluamide.

Materials and Methods

Materials. *N,N*-Diethyl-*m*-toluamide (DEET) (Cas # 134-62-3), Piperitone (Cas # 89-81-6), α-terpineol (Cas # 98-55-5), linalool (Cas # 78-70-6), and 1,8-cineol (Cas # 470-82-6), were purchased from Aldrich Chemical Company, Milwaukee, WI.

Methyl alcohol (Cas # 67-56-1) was purchased from Duksan Chemical Company, Korea. and filter paper (CT No 1001658, size 1 cm) was from Whatman, England.

Gas chromatography (GC). A Hewlett-Packard 4890A (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector and HP-1 column (Hewlett-Packard, Avondale, PA, 30 m × 0.53 mm i.d., 1.5 μm film thickness) was used and peak areas were quantified using a HP3395 integrator. GC operating conditions: injector temperature, 200°C; detector temperature, 280°C; injector operated in splitless mode; temperature program, 50°C (10 min) then ramped at 10°C/min to 200°C.

Bioassay. For a deterrent bioassay, 10 μl^{-1} of monoterpenes were added to 20 μ*l* of a 40 % sucrose solution on a glass micro scope slide. Final concentrations were 16.5, 3.3, 0.67, 0.13, 0.03 and 0.005 $\mu g \cdot \mu l^{-1}$. A 20 μ*l* drop of the 40% sucrose solution plus 10 μ*l* of MeOH on the sucrose slide was used as a control. The slide was placed inside a colony of Crematogaster ants and the number of ants feeding on the control(C) and on the terated (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.

For a repellency assay samples were dissolved in MeOH same deterrent bioassay and 10 μ*l* of the test solution was applied to a filter paper thet was suspended 1 cm over a drop of 40% sucrose solution. The control consisted of 10 μ*l* of MeOH on the paper. Samples that repelled 80% of the ant from feeding on the control sucrose solution (R_{80}) were recorded over a period of 10 min.

Volatility measurement. Five microliter of 0.1 M DEET solution in MeOH (95.5 μg of DEET/μ*l*) was applied on to a filter paper and it was put directly into the vial which contained 1 m*l* of MeOH (0 min sample). Another treated filter paper was air-dried for 10 min and was put into a vial which contained 1 m*l* of MeOH (10 min sample). In a similar fashion, 20, 30, and 40 min samples were prepared and one microliter of each samples (0, 10, 20, 30, and 40 min) were injected for the analysis. Samples for α-terpineol, piperitone, linalool, and cineol were prepared following the same procedure as above, and 1 μ*l* of each sample was injected (Table 1).

Results and Discussion

The piperitone showed the most effective feeding deterrent activity (D_{80} = 0.13 $\mu g \cdot \mu l^{-1}$), followed by in decreasing order by DEET (D_{80} = 0.16 $\mu g \cdot \mu l^{-1}$), α-terpineol (D_{80} = 0.45 $\mu g \cdot \mu l^{-1}$), linalool (D_{80} = 0.56 $\mu g \cdot \mu l^{-1}$), and cineol (D_{80} = 0.64 $\mu g \cdot \mu l^{-1}$). These results for the deterrent assay do not depend on the volatility of monoterpenes and DEET due to the bioassay method. However, the repellency assay may possibly depend on the volatility due to the repellency assay method used. α-Terpineol (R_{80} = 7.9 μg/cm^2) had the most effective repellent activity, followed in decreasing order by piperitone (D_{80} = 8.9 μg/cm^2), linalool (D_{80} = 14.1 μg/cm^2), cineol (D_{80} = 17.8 μg/cm^2), and DEET (D_{80} = 125.9 μg/cm^2). Based on these results, the more volatile monoterpenes produced by *Zanthoxylum piperitum* were found to be more effective repellents than DEET. In this case, the repellent activities of monoterpenes and DEET were tested as space repellents. As such, these results should be recalculated as the repellent activity per mole of monoterpenes and DEET. From GC peak areas the actual quantity and mole of each peak were calculated (Table 1).

Table 1. Actual quantity, and mole of four monoterpenes and DEET for different time periods.

sample	time (min)	actual quantity (ng · μl^{-1})	mole ($\times 10^{-1}$ nmol)
DEET	0	95.5	5.00
	10	91.9	4.80
	20	88.0	4.61
	30	86.5	4.53
	40	84.3	4.41
α-terpineol	0	77.0	5.00
	10	60.8	3.95
	20	53.8	3.49
	30	52.5	3.41
	40	51.0	3.31
piperitone	0	76.0	5.00
	10	61.4	4.04
	20	60.4	3.97
	30	55.5	3.65
	40	54.9	3.61
linalool	0	77.0	5.00
	10	59.1	3.83
	20	53.7	3.49
	30	51.5	3.34
	40	49.9	3.24
cineol	0	77.0	5.00
	10	25.9	1.68
	20	25.1	1.63
	30	24.8	1.61
	40	25.0	1.62

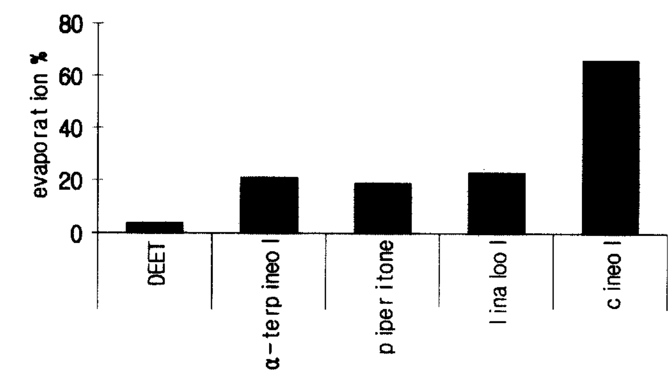

Fig. 1. Evaporation of four monoterpenes and DEET during a period of 10 minutes.

Table 2. Repellent activities of four monoterpenes in comparison with DEET.

sample	repellency index[a] R_{80} ($\mu g/cm^2$)	rel. repellent activity vs. DEET	evaporation rate vs. DEET	actual repellent activity vs. DEET
cineol	17.8	7.07	16.6	0.43
linalool	14.1	8.93	5.85	1.53
α-terpineol	7.90	15.9	5.25	3.03
piperitone	8.90	14.0	4.80	2.94
DEET	125.9	1.00	1.00	1.00

[a]Bowers's bioassay results (J. of Natural Products, 1993, 56, 935-938)

As expected, the quantity of DEET for different evaporation time periods decreased.

The quantity of the four monoterpenes for different evaporation time periods also decreased. In this ant repellency study, the actual quantity and mol of each peak for 10 min evaporation time were determined. Evaporation data of these five samples after 10 min show that cineol was the most volatile monoterpene followed in decreasing order by linalool, α-terpineol, piperitone and DEET (Table 1, Fig. 1). As illustrated previously, in the repellency assay of the Bowers et al. study, the relative repellent activities of cineol, linalool, α-terpineol and piperitone in comparison to DEET were 7.07-, 8.93-, 15.9-, and 14.1-fold higher, respectively. Therefore, the relative evaporation rates of monoterpenes which were 16.6-, 5.85-, 5.25-, and 4.80-fold higher than DEET for cineol, linalool, α-terpineol, and piperitone, respectively, should be considered. From these results, we could obtain the actual repellent activities of the four monoterpenes considering their volatilities were 0.43-, 1.53-, 3.03-, and 2.94-fold higher than DEET for cineol, linalool, α-terpineol, and piperitone, respectively (Table 2).

In summary, cineol and linalool showed similar repellent activities to DEET and α-terpineol and piperitone showed ca. three-fold higher repellent activities. However, in respect to their long-lasting effectiveness, these monoterpenes were found to be too volatile as insect repellents.

Acknowledgments. This work was supported by the Ministry of Agriculture and Forestry through the R & D Promotion Center for Agriculture and Forestry toY.D.K.

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