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DETERMINING THE ACTIVE COMPONENT IN
1,3,3-TRIMETHYL-2-OXABICYCLO [2,2,2] OCTANE (CINEOLE) THAT
REPELS THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA*¹

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ABSTRACT. The compound 1,3,3-trimethyl-2-oxabicyclo [2,2,2] octane, more commonly known as cineole or eucalyptol, present in bay leaves, is a natural repellent to the American cockroach, *Periplaneta americana* L. It was found that the isopropyl-oxygen-isopropyl fragment of the compound is the smallest effective portion, and that the cyclohexane plus oxygen subgroup has the greatest effectiveness.

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INTRODUCTION

Work by Verma and Meloan (1981) isolated 6 compounds from the bay leaf (cineole, geraniol, linalool, alpha phellandrene, piperazine and phenyl hydrazine) that repelled the American cockroach (*Periplaneta americana*, Linneus), to various degrees. Of those compounds, clearly the most effective was 1,3,3-trimethyl-2-

oxabicyclo [2,2,2] octane (cineole). The structure and numbering system of this compound is shown in fig. 1. If this compound is to be used to help understand the basic mechanism of the olfactory process then it is important to know if the entire molecule is needed for the repellent effect. It was the purpose of this project to determine if smaller portions of the molecule could be effective. It was found only a small portion of the molecule was necessary for activation in previous work (Scriven and Meloan 1984), and therefore it was

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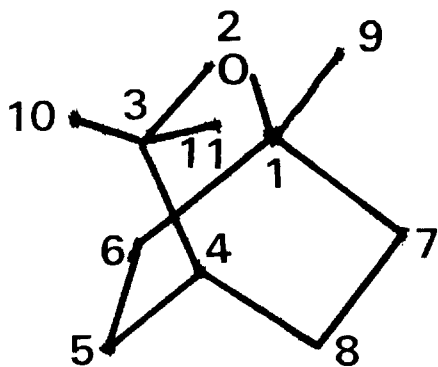


FIGURE 1. Structure and numbering system for the cineole molecule.

thought a similar situation might exist with this compound.

METHODS AND MATERIALS

EQUIPMENT. The following instruments were used to identify and determine the purity of the compounds used: Gas chromatograph-Tracor-560 with FID detector. The column was 1.8 m \times 3 mm o.d. SS packed with 3% SE-30 on Chromasorb W 60/80 mesh. Infrared-Perkin-Elmer 237 and 437. Nuclear Magnetic Resonance-Varian T-60.

CHEMICALS. All chemicals were reagent grade and redistilled before being used unless indicated otherwise: Ethanol, Diethyl ether, Diisopropyl alcohol, Isopropyl alcohol, *t*-Butanol, Cyclohexane, Methylcyclohexane, Cyclohexanol, 1-Hexanol, Di-*t*-butyl ketone, and Di-*t*-butyl ether (prepared by a method suggested by J. Pauksrelis (pers. comm.).

INSECTS. American cockroaches were obtained from a colony maintained by the Department of Entomology at Kansas State University. Adults of both sexes were used.

BIOASSAY. A choice test was used. Two crystallizing dishes (16 \times 9 cm) were modified by having two 3-cm diameter holes placed in the rim of the dish so that when the dishes were inverted the cockroaches could enter the dish.

Each dish was wrapped with a piece of cardboard around the sides and a cardboard lid was placed on the top of the dish after it was inverted. This provided a dark environment, which is preferred by cockroaches, and the removable lid made it easy to count the cockroaches.

The test compound (10 or 100 μ l) was placed on a small piece of cotton and held up out of the way inside of the dishes by a piece of screen wire. The control was prepared in the same manner but without the test compound. The 2 dishes were placed in a large glass jar 25.5 cm tall and 40 cm in diameter. A 5-cm wide band of stopcock grease was placed on the top inside of the jar to prevent the cockroaches from escaping. The entire apparatus was placed in a

hood with the windows covered to provide a dark environment.

The cockroaches were placed in the jar. Then the test compound was added and the dishes adjusted. A fluorescent lamp was turned on to encourage the cockroaches to go inside the darkened dishes. Counts were taken every 2 h for 8 h. The dishes were lifted and the cockroaches scattered after each count. It was found that it took about 4 h for the cockroaches to become adjusted to the test dishes. The tests were repeated at least 6 times using 25 cockroaches each time. Cockroaches that did not enter either chamber were not counted as being repelled.

RESULTS AND DISCUSSION

Table 1 shows the data which includes an attractancy-repellency factor (Leonard and Erhman 1977).

$$A = \frac{N_c - N_b}{N_t}$$

where A = attractancy; N_c = number of insects in the test chamber; N_b = number of insects in the blank chamber; N_t = total number of insects.

This can be used further if desired to calculate χ^2 .

$$\chi^2 = A^2 N_t$$

The significance level of the χ^2 test can be obtained from any statistics manual.

Negative values indicate that the insects were repelled by the test compound and the greater the deviation from 0 toward the minimum of -1 the greater the repellency. The reverse is true for attractiveness. In this type of bioassay more than 50% of the insects have to be repelled for the test to be considered positive.

The ethoxy fragment (O—C₁—CH₃) as represented by ethanol was not a repellent, and it was, in fact, a weak attractant.

The diethoxy ether fragment as tested by diethyl ether was ineffective. Because of its high volatility, extreme care was taken when making the early measurements, but no significant positive response was noticed.

Isopropyl alcohol, representing the isopropoxy fragment [(C₁₀, C₁₁)C₃—O] produced a very good repellent response from

TABLE 1

*Effects of the various groups within cineole.**(Totals of 6 tests at 2, 4, 6, and 8 h. Cockroaches that did not enter either chamber were not counted.)*

Compound with carbon combinations	Sample size	Number of cockroaches		Repellency factor
		Test	Control	
Cineole	100 μ l	5	225	-0.96
Ethanol	100 μ l	131	80	+0.24
(1,9)				
Diethyl ether	100 μ l	128	109	+0.08
(1,9,3,10)				
Isopropyl alcohol	100 μ l	90	82	+0.05
(10,3,11)				
Diisopropyl ether	100 μ l	10	110	-0.83
(10,3,11 and 9,1,7)	10 μ l	27	72	-0.45
<i>t</i> -Butanol	100 μ l	90	96	+0.01
(3,10,11,4 or 1,6,7,9)				
Di- <i>t</i> -butyl ether	100 μ l	129	138	- .03
(3,10,11,4,1,6,7,9)				
Di- <i>t</i> -butyl ketone	100 μ l	25	98	-0.59
Cyclohexane	100 μ l	97	96	+0.01
(1,7,8,4,5,6,)				
Methylcyclohexane	100 μ l	111	302	-0.46
(1,7,8,4,5,6,9)				
Cyclohexanol	100 μ l	1	193	>-0.99
(1,7,8,4,5,6)	10 μ l	12	164	-0.86
1-Hexanol	100 μ l	78	136	-0.27

about 3-6 h, then the effect decreased markedly.

The diisopropoxy ether fragment, as tested by diisopropyl ether, gave a marked repellent response. Previous work by Bodenstern and Fales (1976) in which hundreds of compounds were screened for roach repellency, indicated that diisopropyl cumene peroxide was an excellent cockroach repellent. This compound has 2 isopropoxy fragments.

There are 2 *t*-butoxy fragments attached to the oxygen in the cineole molecule. Based on the results of the isopropoxy fragment which includes an oxygen atom, the *t*-butoxy fragment was tested with *t*-butanol. This group was not effective. These results are likely to be distorted because of the nature of the molecule. While *t*-butanol boils at 82°C, it is a solid (M.P. 25.5°C) at room temperature and therefore has a lower vapor pressure compared to the other compounds tested.

The compound di-*t*-butyl ether is a very strained molecule and not easy to prepare. It is not commercially available. A close look at cineole shows that it is actually di-*t*-butyl ether with 2 additional carbons (5 and 8 of fig. 1). It is possible that the truly effective group is the di-*t*-butoxyl ether fragment but, because of its lack of stability due to steric strain, the 2 carbons were added to hold the molecule together. When di-*t*-butyl ether was synthesized and tested it was found not to be effective. Di-*t*-butyl ketone, a much less strained molecule, was reasonably effective.

Cyclohexane had no measureable effect. Methylcyclohexane was reasonably effective. Cyclohexanol was quite effective. When the test concentrations of both diisopropyl ether and cyclohexanol were decreased to 10 μ l the cyclohexanol was more effective. Cyclohexanol contains the isopropoxy fragment but is held in a somewhat more rigid position than the free iso-

propoxy fragment (C's 3, 10, 11) described previously.

For comparison purposes 1-hexanol was tested and was not very effective. The addition of a linear four-carbon chain to ethanol made the compound more effective but not nearly as much as placing a second methyl group in a gem position (isopropyl) on ethanol.

The above results indicate that the smallest fragment present in the cineole molecule that has a repellent effect on the American cockroach, *P. americana*, is the isopropyl-oxygen-isopropyl combination. A methyl group attached to cyclohexane was somewhat effective, but the most effective subgroup is an oxygen attached to a cyclohexane ring.

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