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Bark Beetle Conversion of a Plant Compound to a Sex-Specific Inhibitor of Pheromone Attraction

Abstract. Both sexes of the bark beetle *Dendroctonus brevicomis* convert the (+) and (−) enantiomers of the tree terpene α -pinene to the corresponding enantiomers of *trans*-verbenol at about equal rates. (−)-*trans*-Verbenol inhibited the response of females, but not of males, to a mixture of attractive pheromone components. Since the female initiates the attack on a pine tree, (−)-*trans*-verbenol may play a role in reducing intraspecific competition for breeding areas.

Many species of bark beetle have pheromones that incite individuals to collect in a mass attack on a tree for the purposes of finding mates and locating suitable breeding areas; this often results in the death of the tree. Some species use an olfactory mechanism to avoid interspecific competition for hosts (1-3) as well as to reduce intraspecific competition (4). The Western pine beetle *Dendroctonus brevicomis* LeConte is a destructive species infesting ponderosa pine, the predominant forest tree in California. Male and female beetles are equally attracted to a mixture of three pheromone components: *exo*-brevicomin, produced by females; frontalin, produced by males; and myrcene, derived from tree resin (5).

Racemic α -pinene, a major monoterpene of the pine tree (6), is apparently hydroxylated by *D. brevicomis* females to form *trans*-verbenol (7), which accumulates in the hindguts at the beginning of colonization (2, 8). However, despite several attempts, no behavioral or ecological function has been established for *trans*-verbenol (9, 10). I have found that the (−) enantiomer of *trans*-verbenol acts to inhibit the attraction of females, but not of males, to pheromone components. Since the female begins the attack on a tree, this inhibitory response appears to play a role in regulating the density of colonization and intraspecific competition. Renwick *et al.* (11) reported that the cohabiting bark beetle *Ips paraconfusus* converted the (+) enantio-

mer of α -pinene to *trans*-verbenol and converted the (−) enantiomer to an attractive pheromone component, *cis*-verbenol. In contrast, I found that both sexes of *D. brevicomis* use both enantiomers of α -pinene to synthesize the corresponding enantiomers of *trans*-verbenol (12) and synthesize only trace amounts, if any, of *cis*-verbenol.

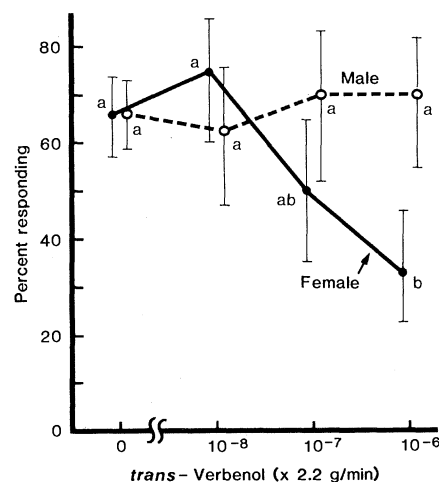


Fig. 1. Effect of increasing release rates of (−)-*trans*-verbenol on the attraction response of walking male and female *Dendroctonus brevicomis* to a 1:1:1 mixture of pheromone components *exo*-brevicomin (> 95 percent), frontalin (> 95 percent), and myrcene (> 99 percent) (Chemical Samples Company), each released at 2.2×10^{-9} g/min (18 October 1976). Points with the same letter were not significantly different ($\alpha = .01$, χ^2). The brackets represent 95 percent confidence limits for proportions.

Adult *D. brevicomis* were reared in the laboratory from the bark of larva-infested ponderosa pine trees (Sierra National Forest, California) and were used in experiments shortly after they emerged. The beetles were exposed for 18 hours to vapors of either the (+) or (−) enantiomer of α -pinene (Aldrich), purified > 99.8 percent by gas-liquid chromatography (GLC). The quantities of *trans*-verbenol, verbenone, and myrtenol in the hindguts were determined by GLC (13) (Table 1). The amounts of *trans*-verbenol produced from each enantiomer of α -pinene were similar in males and females, as were the amounts of myrtenol (Table 1). Females did not produce verbenone in detectable levels, whereas males produced significant amounts after being held in a jar for 18 hours at room temperature, whether or not they were exposed to α -pinene vapors. A beetle of either sex contained no more than 0.5×10^{-7} g of *cis*-verbenol (quantified by GLC) after exposure to (−)- α -pinene, not significantly different from the amount in unexposed beetles—but exposure to (+)- α -pinene resulted in about 5×10^{-7} g of a compound having the retention time of *cis*-verbenol.

Samples of *trans*-verbenol were collected in ethanol from the gut extracts (diethyl ether) by condensation on glass beads from the GLC effluent (14). The concentrations were quantified by GLC, and the optical rotations (± 5 percent) were obtained with an electrobalancing polarimeter (Autopol III). A comparison of the specific rotations of the (+)- and (−)- α -pinene with the specific rotations of (+)- and (−)-*trans*-verbenol produced in males ($[\alpha]_D^{22} = +105.2^\circ$, -89.5°) and females ($[\alpha]_D^{22} = +104.9^\circ$, -91.5°) indicated that both sexes converted both enantiomers, but each only to the corresponding enantiomer of *trans*-verbenol (15). The GLC-purified samples of *trans*-verbenol were then subjected to analysis by gas chromatography-mass spectrometry (V.G. Micromass 7070F mass spectrometer with computerized data system) on a 40-m capillary column (SCOT; OV101) at 115°C. The *trans*-verbenol extracted from males and females had mass spectra identical to the mass spectrum of a standard purified > 99.8 percent by GLC (Glidden Organics) (16).

Walking beetles were tested with a laboratory olfactometer for their response to a mixture of pheromone components containing increasing concentrations of *trans*-verbenol, $[\alpha]_D^{22} = -131^\circ$ (Glidden Organics) (3). Only the response of females to pheromone components was inhibited at the highest release

rate of *trans*-verbenol (Fig. 1). Females were further tested to determine whether both enantiomers could inhibit attraction. The predominantly (+)- or predominantly (-)-*trans*-verbenol obtained from the females exposed to the respective enantiomers of α -pinene was released at 6.6×10^{-7} g/min in the olfactometer with a mixture of the attractive pheromone components (each released at 1.2×10^{-8} g/min). A female response of 68.3 percent ($N = 180$) to the pheromone mixture was unaffected by (+)-*trans*-verbenol (68.9 percent, $N = 90$), but attraction was reduced by (-)-*trans*-verbenol (52.2 percent, $N = 90$, $P < .05$, χ^2).

A field test, on 5 to 12 August 1980, in which five pairs of traps were placed in various areas of the Sierra National Forest (elevation 1000 m), was designed to determine whether (-)-*trans*-verbenol could influence the sex ratio of beetles landing and entering beetle-sized holes in a paper carton containing pheromone components. The carton (19 cm in diameter and 21.5 cm high) had 48 holes (2.5 mm) evenly spaced around the sides and an adhesive coating (Stickem Special) (2) on the inside floor. Glass vials containing the three pheromone components, each released at about 2 mg/day, and two vials containing (-)-*trans*-verbenol, released at about 0.5 mg/day (2), were placed inside the carton. A 13-cm² wire screen (6-mm mesh) coated with adhesive was placed immediately below the carton. Both carton and screen were attached to a wooden stake 1.5 m above ground and 8 m away from the control traps, which did not contain *trans*-verbenol. The sticky screen on the control traps caught 95 males and 93 females. This sex ratio was similar to that of beetles caught on screens below cartons containing *trans*-verbenol (61 males and 64 females), an indication that *trans*-verbenol did not significantly affect the long-distance attraction to pheromone. Inside the control cartons there were 75 males and 56 females, and this sex ratio was not significantly different from the ratio of those caught on the screens below ($P > .05$, χ^2). However, the sex ratio of beetles caught inside the cartons releasing *trans*-verbenol (93 males and 21 females) was significantly different from the sex ratio of beetles on the screens below and from the ratio of beetles caught on the inside of the control cartons ($P < .001$, χ^2). This result supports the laboratory finding that *trans*-verbenol inhibits the response of females, since females appeared less likely than males either to enter a hole from which *trans*-verbenol

was being released or to remain on the outside of the cartons.

Each of the two enantiomers of α -pinene is converted by both sexes of *D. brevicomis* to the comparable enantiomer of *trans*-verbenol, but only the (-) enantiomers of precursor and product appear to be involved in the behavioral ecology of the beetle. In the cohabiting bark beetle *I. paraconfusus* the biosynthetic system which produces *cis*- and *trans*-verbenol from α -pinene seems fundamentally different from that in *D. brevicomis* (11, 17). Both *cis*- and *trans*-verbenol have been produced in culture by a bacterium isolated from *I. paraconfusus* (18), but when these beetles were fed an antibiotic at concentrations that abolished synthesis of two other pheromone components, ipsenol and ipsdienol, there was no apparent effect on verbenol production (19). In contrast, *D. brevicomis* produced no *cis*-verbenol or only trace amounts from (-)- α -pinene, and only a small peak, about 4 percent relative to *trans*-verbenol, was detected after exposure to (+)- α -pinene. This may be because *D. brevicomis* appears to use (+)-*cis*-verbenol for detecting and avoiding areas of the tree infested with *I. paraconfusus* in order to reduce competition (2, 3). Both species probably use (-)- α -pinene to make both attractive and inhibitory pheromone components because this enantiomer predominates in ponderosa pine resin (6).

The largest quantities of *trans*-verbenol are found in female *D. brevicomis*. These quantities are comparable to the amounts of attractive components produced by the female when gallery construction under bark is begun (2, 20). The release of *trans*-verbenol may cause females to avoid areas with high densities of boring females and would thus regulate intraspecific competition. The amount of *trans*-verbenol in the hindguts of both males and females diminishes gradually over a 3-week period, whereas attractive pheromone components decline more rapidly (20). The change in the ratio of these substances may indicate to females arriving later in the attack sequence that the tree is fully colonized. It would be advantageous for males not to be inhibited by *trans*-verbenol—as they apparently were not—since they seek mates and sources of food and protection. However, it would benefit a male to maximize his reproductive success by complementing the female's production of *trans*-verbenol, since his progeny would be as much affected by any intraspecific competition as that of his mate.

The present study provides a method of producing relatively pure enantiomers of *trans*-verbenol for use in behavioral experiments with other bark beetles such as the Southern pine beetle, mountain pine beetle, and Douglas-fir beetle, which use the compound as an aggrega-

Table 1. Amounts of *trans*-verbenol, verbenone, and myrtenol in hindguts of male and female *D. brevicomis* when exposed or not exposed to vapors of each enantiomer of α -pinene. There were no significant differences between males and females in the amount of *trans*-verbenol or myrtenol produced from each enantiomer of α -pinene or in the amounts of *trans*-verbenol or myrtenol produced from each enantiomer by either sex ($P > .05$). Significantly more *trans*-verbenol and myrtenol were produced in males and females exposed to enantiomers of α -pinene than in controls not exposed to vapor for 18 hours ($P < .001$).

Treatment	Production per beetle ($\times 10^{-7}$ g)		
	<i>trans</i> -Verbenol	Verbenone	Myrtenol
<i>Male</i>			
(+)- α -pinene*	58.5 \pm 9.8	6.4 \pm 1.5	20.5 \pm 2.9
(+)- α -pinene†	135.3 \pm 4.8	13.3 \pm 2.7	44.9 \pm 4.8
(-)- α -pinene*	74.6 \pm 10.8	12.4 \pm 0.6	10.7 \pm 1.5
(-)- α -pinene†	218.1 \pm 20.6	22.9 \pm 5.1	28.6 \pm 3.4
None‡	0.5 \pm 0.1	6.2 \pm 2.4	0.2 \pm 0.1
No vapor (18 hours)‡	3.0 \pm 0.5§	27.9 \pm 3.6	0.8 \pm 0.3
<i>Female</i>			
(+)- α -pinene*	65.9 \pm 13.9	< 0.1	29.0 \pm 6.4
(+)- α -pinene†	139.9 \pm 16.7	< 0.1	45.2 \pm 5.9
(-)- α -pinene*	89.7 \pm 9.4	< 0.1	17.7 \pm 1.4
(-)- α -pinene†	208.8 \pm 28.9	< 0.1	37.6 \pm 5.5
None‡	0.7 \pm 0.1	< 0.1	0.1 \pm 0.1
No vapor (18 hours)‡	6.6 \pm 1.3§	< 0.1	0.6 \pm 0.1

*Two groups of 100 beetles of each sex were exposed to each enantiomer of α -pinene ($[\alpha]_D^{25} = +45.8^\circ$; $[\alpha]_D^{25} = -41.6^\circ$) at $4.9 \pm 0.6 \times 10^{-6}$ g per milliliter of air for 18 hours on 15 December 1977 (13). †Six groups of 20 beetles of each sex were exposed to each enantiomer of α -pinene at $7.8 \pm 0.8 \times 10^{-6}$ g per milliliter of air for 18 hours (two groups each on 13 October and 16 November 1977 and on 3 May 1980).

‡Two groups of 15 beetles of each sex were not exposed to vapors at any time, or for 18 hours, on each of the three dates above. §Both sexes contained significantly more *trans*-verbenol and males more verbenone after being kept in a jar for 18 hours at room temperature than corresponding beetles that had emerged and were immediately refrigerated (3°C) for 18 hours until extraction ($P < .001$). || Females did not produce detectable amounts of verbenone.

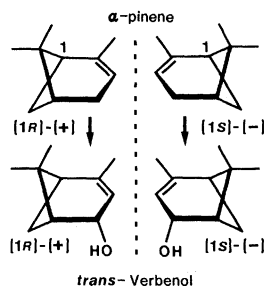
tion signal (21). The field trap design illustrates a means of determining the behavioral activity of repellent or inhibitory pheromones under conditions similar to those in nature. *trans*-Verbenol, alone or in combination with other inhibitors, such as verbenone (10, 22), ipsdienol (14), or *cis*-verbenol (3), may be useful in protecting trees from attack by *D. brevicomis*.

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12. Hydroxylation of (1R)-(+)- and (1S)-(–)- α -pinene enantiomers (non-superimposable mirror images) to (1R)-(+)- and (1S)-(–)-*trans*-verbenol enantiomers in *D. brevicomis*:



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15. The same proportions of (+) and (–) enantiomers found in the samples of (+) and (–)- α -pinene were found in the beetle-produced (+)- and (–)-*trans*-verbenol, as judged by the highest reported rotations for (–)-*trans*-verbenol ($[\alpha]_D^{22} = -129.6^\circ$) and (+)- α -pinene ($[\alpha]_D^{22} = +52.4^\circ$) (11).
16. The *trans*-verbenol had a molecular weight of 152 and mass spectra (mass-to-charge ratio) in decreasing magnitude from a base peak of 109: 94 (59 percent of base peak), 91, 81, 119, 95, 69, 83, 137, 92, 84, 41, 67, 107, 79, and 134 which were in agreement with spectra of other samples of *trans*-verbenol obtained by G. Odham, University of Lund, and G. Bergström, University of Göteborg, Sweden. However, the spectra were not in agreement with those obtained by J. A. A. Renwick (8), who reported a base peak of 119 and major fragments of 91 and 134 for *trans*-verbenol obtained from *D. brevicomis*.
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Recognition Sites for Norepinephrine Uptake: Regulation by Neurotransmitter

Abstract. Recognition sites for the uptake of norepinephrine on adrenergic neurons in the brain and periphery were labeled with [3 H]desipramine. The number of these uptake sites varied with the concentration of transmitter; depletion of norepinephrine with reserpine reduced the number of uptake sites, whereas increasing the concentration of norepinephrine induced by treatment with monoamine oxidase inhibitors raised the number of binding sites. These dynamic alterations in norepinephrine uptake recognition sites may regulate synaptic function homeostatically, providing less inactivation of reuptake when the synaptic concentration of the transmitter is low and increased inactivation when it is high.

The ability to label neurotransmitter receptors by their binding of radioactive drugs has enhanced our understanding of synaptic processes (1). The number of neurotransmitter receptor binding sites varies with the concentration of synaptically active transmitter, usually increasing in parallel with the functional supersensitivity that follows a reduction in the synaptic transmitter and decreasing with increased transmitter (2).

Biogenic amine and amino acid neurotransmitters are inactivated after they

are released from synapses by specific uptake systems at nerve terminals (3). Recognition sites for the uptake of γ -aminobutyric acid (GABA) and glutamate can be labeled by [3 H]GABA and [3 H]glutamate, respectively, in the presence of sodium (4). Tricyclic antidepressant drugs potentially inhibit the uptake of norepinephrine, dopamine, and serotonin (5). The uptake sites for serotonin and norepinephrine can be labeled with [3 H]imipramine (6) and [3 H]desipramine (7–11), respectively, thereby permitting

Table 1. Effects of reserpine on central and peripheral adrenergic parameters. Reserpine (5 mg/kg) was given subcutaneously once a day for 5 days. On day 6, rats were decapitated and binding of [3 H]desipramine (2 nM), [3 H]dihydroalprenolol (1 nM), [3 H]WB-4101 (1 nM), and *p*-[3 H]aminoclonidine (1 nM) to cerebral cortex membranes and salivary gland (submaxillary and sublingual) membranes and the uptake of [3 H]norepinephrine in crude synaptosomal preparations were determined (7, 8). Binding data are expressed as picomoles per gram of tissue, uptake data as picomoles per gram of tissue in 5 minutes, and norepinephrine level as nanomoles per gram of tissue. The numbers in parentheses show the percentage of control values. Results are the mean \pm standard error of four separate determinations performed in triplicate. N.D., not determined.

Parameter	Cerebral cortex		Salivary gland	
	Control	Reserpine	Control	Reserpine
[3 H]Desipramine binding (norepinephrine uptake site)	2.69 \pm 0.19	1.58 \pm 0.21† (59)	5.61 \pm 0.26	2.79 \pm 0.04† (50)
[3 H]Norepinephrine accumulation	108.4 \pm 2.4	27.5 \pm 4.78‡ (25)	N.D.	N.D.
Norepinephrine level	2.35 \pm 0.14	<0.01 (0.4)	N.D.	N.D.
[3 H]Dihydroalprenolol binding (β -receptor)	2.64 \pm 0.17	3.70 \pm 0.12† (140)	2.43 \pm 0.21	4.19 \pm 0.27† (172)
[3 H]WB-4101 binding (α_1 -receptor)	4.57 \pm 0.17	5.62 \pm 0.20† (123)	2.60 \pm 0.22	2.54 \pm 0.17 (89)
<i>p</i> -[3 H]Aminoclonidine binding (α_2 -receptor)	2.43 \pm 0.11	2.81 \pm 0.13 (115)	0.72 \pm 0.08	1.12 \pm 0.12 (150)

* $P < .05$. † $P < .02$. ‡ $P < .005$.