OXIDATION PRODUCTS OF PINENE IN THE BARK BEETLE, 
**DENDROCTONUS FRONTALIS**

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Abstract—Southern pine beetles were collected as they emerged from infested pine bolts and exposed in Petri dishes to α- and β-pinene for 20 hr. Changes in the volatile contents of the beetles' hindguts were detected by gas chromatography, and previously unidentified components were characterized by mass, i.r., and n.m.r. spectroscopy. A prominent compound in hindguts of both sexes before and after treatment was identified as myrtenol, and a less conspicuous, male-specific compound proved to be myrtenal. Upon exposure to α-pinene, males produced cis- and trans-verbenol, and 4-methyl-2-pentanol was found in both sexes. Compounds present after treatment with β-pinene vapour were identified as trans-pinocarveol in both sexes, and pinocarvone in the males.

INTRODUCTION

Many bark beetle species are known to produce pheromones which guide their colonization of host trees. Aggregation pheromones can be detected in the hindguts of attacking beetles during their period of attraction (Vité and Renwick, 1970), and other compounds released by defaecation may serve to regulate the sex ratio or control the pattern of attack (Renwick and Vité, 1970). Pheromones may be present in the emergent adults, or feeding may be necessary for their production. However, Hughes (1973a, b) recently found that pheromone production in Dendroctonus can result from exposure of beetles to host oleoresin or to individual terpene components of the resin. This phenomenon has also been demonstrated in the genus Ips (Vité et al., 1972).

The olfactory guidance of aggregation of the southern pine beetle, Dendroctonus frontalis Zimm., is largely dependent on one female-produced pheromone, frontalin (Kinzer et al., 1969; Renwick and Vité, 1969). However, males or females of other Dendroctonus species have been found to produce additional compounds that supplement the attraction of a key pheromone (Vité and Pitman, 1969; Pitman, personal communication).

The purpose of this study was to identify several unknown volatile components of hindguts from both sexes of D. frontalis before and after exposure to α-pinene and β-pinene, the most abundant monoterpenes in southern pines.

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EMERGENT beetles collected from the insectary were placed in Petri dishes where they were held at room temperature in the dark for a period of 20 hr. For exposure to terpene vapours, a container of α-pinene (98%) or β-pinene (98%) was enclosed in the Petri dishes as described by Vitte et al. (1972). After separation of the sexes, hindguts were removed and stored in 1 ml centrifuge tubes held in dry ice. For comparative gas chromatography–mass spectrometry studies, 500 hindguts from each sex were used. For complete spectral analysis of a major compound in unexposed beetles, 4000 males and 2000 females were used. Hindgut samples were extracted with ethyl ether and the extracts were carefully concentrated to a small volume for gas chromatography.

The gas chromatograph used was a Perkin–Elmer Model 990 with a flame ionization detector. A Carbowax 20M support-coated open tubular (SCOT) column, 50 ft x 0.02 in. i.d., was used. The nitrogen flow rate was 6 ml/min and the temperature was programmed from 120 to 150°C at 2°C/min. Mass spectra were obtained with a Hitachi–Perkin–Elmer RMU-6E coupled to an Aerograph Model 204 gas chromatograph, and a jet separator was used for sample enrichment. The column in this case was 5 ft x ⅜ in. o.d. stainless steel packed with 3% Carbowax 20M on 80/100 Varaport 30 (Varian Aerograph), and the helium flow rate was 25 ml/min. Samples for i.r. and n.m.r. spectroscopy were collected from an Aerograph Model 700 using a 6 ft x ⅜ in. o.d. stainless steel column packed with 10% Carbowax 20M on 70/80 Varaport 30, at a helium flow rate of 40 ml/min.

Infrared spectra were obtained in carbon tetrachloride solution with a Perkin–Elmer Model 221. Nuclear magnetic resonance spectra were run in deuterated chloroform with a Jeolco C-60HL using a micro-cell, with TMS as the internal standard.

RESULTS AND DISCUSSION

The major volatile components of hindguts from emergent D. frontalis have been identified previously as trans-verbenol in females and verbenone in males (Renwick, 1967). However, another relatively prominent compound was detected in both sexes (peak 7, Fig. 1). Comparative gas chromatography and mass spectrometry confirmed the identity of this compound from the two sources, so that the subsequent isolation and characterization did not require separation of the sexes.

The mass spectrum of peak 7 indicated a molecular weight of 152. The base peak was at m/e 79, with other major fragments at m/e 91 (89%), 41 (64%), 108 (60%), 39 (50%), 77 (47%), 93 (32%), 27 (31%), 119 (30%), 43 (27%), and 53 (27%). The parent ion was prominent (19%), with fragments at m/e 137 and 134, indicating the loss of CH₃ and H₂O, respectively. This fragmentation pattern is characteristic of a terpene alcohol, and a lack of u.v. absorption suggested a mono-unsaturated, bicyclic structure. The i.r. spectrum (in CCl₄) gave a sharp band at 3063 cm⁻¹ (OH), a weak band at 1667 cm⁻¹ (C=O), a strong doublet at 1382
and 1372 cm$^{-1}$ ($\text{C}<\text{CH}_3$), and other strong bands at 1054, 980, and 887 cm$^{-1}$.

The n.m.r. spectrum showed absorption bands at 4.6$\tau$ (1 proton), 6.14$\tau$ (2 protons), a multiplet from 6.5 to 8.05$\tau$ (5 protons), 8.52$\tau$ (1 proton), 8.73$\tau$ (3 protons), and 9.17$\tau$ (3 protons). These spectral data were consistent with the structure of myrtenol, a terpene alcohol found in the oil of certain plants, including myrtle. This identification was confirmed when a sample of myrtenol synthesized by the method of DUPONT et al. (1934) had spectral and chromatographic properties identical to those of the natural material.

![Gas chromatograms of volatiles from hindguts of male D. frontalis.](image)

**Fig. 1.** Gas chromatograms of volatiles from hindguts of male *D. frontalis*. (A) Untreated. (B) Exposed to $\alpha$-pinene. (C) Exposed to $\beta$-pinene.

Another compound was consistently found in emergent males (peak 3, Fig. 1) but not in female beetles. The mass spectrum of this material was similar to that of myrtenol in that the base peak was at $m/e$ 79. The molecular weight was apparently 150, the parent ion having a relative intensity of 11%. Other prominent fragments were at $m/e$ 107 (72%), 77 (64%), 39 (54%), 91 (46%), 41 (45%), 106 (38%), 105 (34%), 108 (31%), 27 (31%), 53 (30%), 29 (27%), 51 (25%), 65
(23%), 66 (22%), 93 (22%), and 78 (21%). This spectrum suggested the structure of myrtenal, and matched that of the authentic aldehyde obtained by oxidation of α-pinene (DUPONT et al., 1934). In addition, the gas chromatographic retention times of the synthetic and natural compounds were identical.

After exposure to α-pinene, an additional compound was produced by both males and females of *D. frontalis*. The new component was detected in the region of the monoterpene hydrocarbons in chromatograms of hindgut volatiles (peak 1, Fig. 1). The mass spectrum of this compound was indicative of an acyclic alcohol with a molecular weight of 102. No parent ion was observed, but fragments were obtained at m/e 87 (—CH₃) and 84 (—H₂O). The base peak was at m/e 45, with other fragments at m/e 43 (35%), 41 (27%), and 69 (25%). Comparisons of the spectral and chromatographic data with those of authentic alcohols (Chemical Samples Co., Columbus, Ohio) indicated that the compound was 4-methyl-2-pentanol.

Female beetles exposed to α-pinene produced no other obvious new volatiles, although quantitative changes were apparent. The concentrations of cis- and trans-verbenol in hindguts were considerably higher. On the other hand, male beetles appeared to produce cis- and trans-verbenol (peaks 4α and 5, Fig. 1), which were not present before treatment. The identity of each isomer of verbenol was confirmed by its mass spectrum and gas chromatographic retention times.

Treatment of beetles with β-pinene vapour resulted in the production of two additional compounds. One of these (peak 2β, Fig. 1) was detected in the males only, and appeared to be present in low concentrations before treatments. The mass spectrum of this component indicated a molecular weight of 150. The base peak was at m/e 53, with major fragments at m/e 41 (88%), 39 (80%), 107 (67%), 79 (63%), 27 (55%), 106 (43%), 77 (40%), and 69 (29%). The parent ion had a relative intensity of 57%, with fragments at m/e 135 (27%) and 122 (17%), typical of a terpene ketone. The fragmentation pattern matched that of pinocarvone, and the retention times of synthetic pinocarvone (HARTSHORN and WALLIS, 1964) were also identical to those of the natural material.

The other compound detected after β-pinene treatment was present in both sexes (peak 4β, Fig. 1). Its mass spectrum was characteristic of a terpene alcohol with a molecular weight of 152. The base peak was at m/e 41, with fragments at m/e 92 (87%), 55 (85%), 91 (75%), 39 (69%), 70 (63%), 43 (50%), 27 (48%), 83 (48%), 81 (46%), and 53 (45%). The parent ion was small (1%), but diagnostic peaks were obtained at m/e 137 (3%), 134 (42%), and 119 (28%). The mass spectral and chromatographic data were in exact agreement with those of a synthetic sample of trans-pinocarveol (HARTSHORN and WALLIS, 1964).

Compounds with retention times longer than that of myrtenol were not identified. Additional minor components also remain to be characterized, and at least one peak was found to be a mixture. However, the identities of the major chromatographic peaks in Fig. 1 are listed on p. 1739.

The results indicate that males of *D. frontalis* are capable of producing more oxidation products of α- and β-pinene than the females (Table 1). The only
female-specific terpenoids in emergent beetles, *cis*- and *trans*-verbenol, are synthesized by males on exposure to α-pinene. However, the females do not produce significant amounts of the male-specific compounds, verbenone, myrtenal, and pinocarvone. It appears, therefore, that the males have an oxidation system to produce alcohols, and further oxidize these to the corresponding carbonyl compounds. But the oxidation process in females is restricted essentially to the synthesis of the alcohols.

It is not yet known how many of these oxygenated terpenes might play a rôle as pheromones in the aggregation of bark beetles, but the importance of verbenone, *trans*-verbenol, and *cis*-verbenol has already been demonstrated (Silverstein et al., 1979).

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<tr>
<th>Compounds detected after treatment</th>
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<tr>
<td><strong>Male</strong></td>
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<td>None</td>
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<td>α-Pinene</td>
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**Table 1—Major volatile components of D. frontalis hindguts identified after exposure to α-pinene and β-pinene**
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1966; Pitman and Vité, 1969; Renwick and Vité, 1969; Renwick and Vité, 1972). The production of cis-verbenol, an essential component of the pheromone system of Ips calligraphus, was recently shown to be dependent on exposure of the attacking beetles to host resin vapour (Vité et al., 1972). It is conceivable, therefore, that studies on the compounds produced upon exposure of beetles to resin components could provide valuable clues in the search for additional bark beetle pheromones.

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REFERENCES


