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CHEMICAL ATTRACTANTS FOR THE SMALLER EUROPEAN ELM BARK BEETLE *Scolytus multistriatus* (COLEOPTERA: SCOLYTIDAE)[1]

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Abstract—The secondary attractant for the smaller European elm bark beetle *Scolytus multistriatus* is a mixture of three compounds: (-)4-methyl-3-heptanol (I); 2,4-dimethyl-5-ethyl-6,8-dioxabicyclo(3.2.1) octane (II); and (-)α-cubebene (III). The novel structure assigned to compound II was confirmed by synthesis. All three compounds were isolated from the volatile compounds collected on Porapak Q by aerating elm bolts infested with virgin female beetles. The GLC fractionations were monitored by two laboratory bioassays. Individually, each compound was inactive in the laboratory bioassays, but a mixture of all three showed activity nearly equivalent to that of the of the original Porapak extract. A mixture of synthetic I and II plus natural III (from cubeb oil) was highly attractive to beetles in preliminary field tests.

Key Words—aggregation pheromone, α-cubebene, 2,4-dimethyl-5-ethyl-6,8-dioxabicyclo(3.2.1)octane, Dutch elm disease, elm bark beetle, insect attractant, 4-methyl-3-heptanol, multilure, multistriatin, *Scolytus multistriatus*.

INTRODUCTION

The smaller European elm bark beetle *Scolytus multistriatus* (Marsham)

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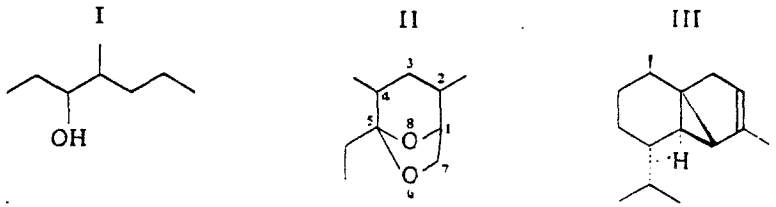
is the principle vector for the Dutch elm disease pathogen *Ceratocystis ulmi*. The disease has devastated elm populations in the northeastern United States and presently threatens American elms throughout their natural and cultivated range. The chemical elucidation of the aggregation pheromone for *S. multistriatus* has been motivated by the possibility that mortality traps baited with the attractant might be used for the survey and control of elm bark beetle populations as an integral part of future Dutch elm disease control programs.

Investigations by Martin (1936), Meyer and Norris (1967), and Peacock et al. (1971) have shown that adult elm bark beetles are weakly attracted to uninfested elm wood. The report by Peacock et al. (1971) also demonstrated that secondary mass attack by both sexes of beetles on potential breeding sites is directed by an aggregation pheromone produced by virgin females boring into the phloem-cambial region of weakened elm trees. The attractiveness of the virgin females is greatest during days 4 through 7 after initial infestation and is markedly reduced after mating. We report here that the chemical attractant is comprised of at least three compounds (I–III) that in



combination elicit beetle responses in both laboratory and field assays: 4-methyl-3-heptanol (I) and 2,4-dimethyl-5-ethyl-6,8-dioxabicyclo(3.2.1) octane (II) are beetle-produced pheromones, while α-cubebene (III) is a host-produced synergist.

ATTRACTANT SOURCE AND CHEMICAL ISOLATION

The total attractant source was a pentane extract of Porapak Q[5], a solid absorbent used to collect organic volatiles from aerated chambers containing virgin female-infested elm bolts.[5] A recent report by Byrne et al. (1975) demonstrated the general applicability of Porapak Q as a trapping agent for collection of insect pheromones by aeration. Initial attempts to isolate the pheromone utilized virgin female frass as a source (Peacock et al., 1973) since the frass of other scolytids is known to contain aggregation pheromones (Silverstein, 1970; Vité, 1970; Borden and Stokkink, 1971).

[5] Mention of a product does not imply endorsement by the Forest Service or the USDA.

TABLE 1. LABORATORY BIOASSAYS OF ATTRACTANT COMPONENTS

Test	Materials tested[a]	Dosage per replicate[b] (ng) Compounds I	II	III	Mean response (%) Bioassay 1[c]	Bioassay 2[d]
A	I	25	—	—	0	7.0
B	II	—	19	—	1.3	3.0
C	III	—	—	50	2.7	9.0
D	I+II	25	19	—	22.7	18
E	I+III	25	—	50	17.3	15
F	II+III	—	19	50	2.7	13
G	I+II+III	25	19	50	46.0	44
H	Porapak extract	25	19	50	45.3	54
I	*n*-Hexane[e]	—	—	—	0	0

[a] The Porapak extract used in this test was quantitatively analyzed by GLC for compounds I, II, and III. Test solutions A–H were prepared with *n*-hexane such that a 25-μl aliquot delivered the quantities indicated in the table.

[b] Each 25-μl aliquot of test solutions A–H represented 50 beetle hours (BH). The BH was the unit of biological quantitation. For example, a Porapak aeration extract of 7 days (168 hours) on 4000 ♀♀ represented: $168 \times 4000 = 6.7 \times 10^5$ BH.

[c] Peacock et al. (1973). Mean % responses of 3 replicates of 25 beetles each for tests A–F; 6 replicates of 25 for tests G and H.

[d] Moeck (1970). Mean % responses of 3 replicates of 25 for tests E and F; 4 replicates for tests A, B, C, and D; 7 replicates for test G; and 8 replicates for test H.

[e] Baker "Analyzed Reagent."

Subsequent laboratory and field comparisons of frass, frass extracts, and Porapak aeration extracts showed the Porapak extract to be a superior source of attractive material (Peacock et al., 1975).

Each infestation of 4,000–7,000 virgin females was aerated continuously for 7 days beginning at the 3rd day after introduction in order to coincide with the period of maximum pheromone production. The Porapak extracts were concentrated under a fractional distillation column (glass bead packing) and the concentrates were fractionated by preparative GLC. The fractionation schemes were monitored in the laboratory by the arrestant-excitant bioassay (Peacock et al., 1973) and by an attractant bioassay with an olfactometer described by Moeck (1970).

Biological activity of the original Porapak extract could be approximated only by recombination of 3 of the 7 initial GLC fractions (SE-30), each of which yielded 1 of the 3 active components after successive GLC fractionation on Carbowax 20M and Apiezon L columns. The activities of all possible combinations of compounds I, II, and III relative to the Porapak extract were determined by the bioassays mentioned above (Table 1). Thus, the laboratory

bioassays clearly indicate that none of the compounds is active individually, and that all 3 are necessary and sufficient to yield activity nearly equivalent to that of the Porapak extract.

BIOLOGICAL SOURCES AND RELEASE RATIOS

Virgin female *S. multistriatus* beetles retrieved from elm bolts within 3–5 days after infestation were mixed with powdered dry ice and mascerated with a mortar and pestle. A pentane extract of the mascerated beetle tissue was analyzed by GLC for compounds I, II, and III. Positive identifications for compounds I and II were obtained on two analytical columns (6.1-m Carbowax 20M, 6.1-m Apiezon L), but only a trace of III was observed. Similar analyses of a hexane-Waring blender extract of uninfested elm tissue (xylem and phloem strips) and a Porapak extract from the aeration of uninfested elm bolts yielded compound III, but no detectable quantities of I or II. On the basis of these results, we conclude that I and II are beetle-produced pheromone components and that III is a host-produced component.[6]

Quantitative GLC analyses of several Porapak extracts from aeration of virgin females on logs indicated that the release ratio of I to II was consistently 1 :1. The ratio of I or II to III, however, was variable between 1 :2 and 1 :10.

IDENTIFICATION AND SYNTHESIS

Compound I was identified as (-)4-methyl-3-heptanol ($[\alpha]_D^{26} - 15°$) by comparison of its MS, IR, and NMR spectra with those of a synthetic sample prepared by sodium borohydride reduction of 4-methyl-3-heptanone (Aldrich Chemical Co.). Since 4-methyl-3-heptanol contains two chiral centers, the synthetic material exists as two diastereomeric forms; however, only one form is produced by the female beetle. The synthetic diastereomers are separable by gas chromatography, with the natural alcohol corresponding to the diastereomer of shorter retention time on a Carbowax 20M column. Compound I was shown to be a single enantiomer by comparison of the ^1H and ^{19}F NMR spectra of the Mosher derivatives[7] of racemic I with those of natural I.

The novel structure assigned to compound II is consistent with the MS, IR, and NMR spectra. The carbon skeleton of II was determined by hydro-

[6] The possible role of microorganisms in the production of bark beetle attractants has not been investigated.

[7] The esters of R(+)α-methoxy-α-trifluoromethylphenylacetic acid (Dale et al., 1969).

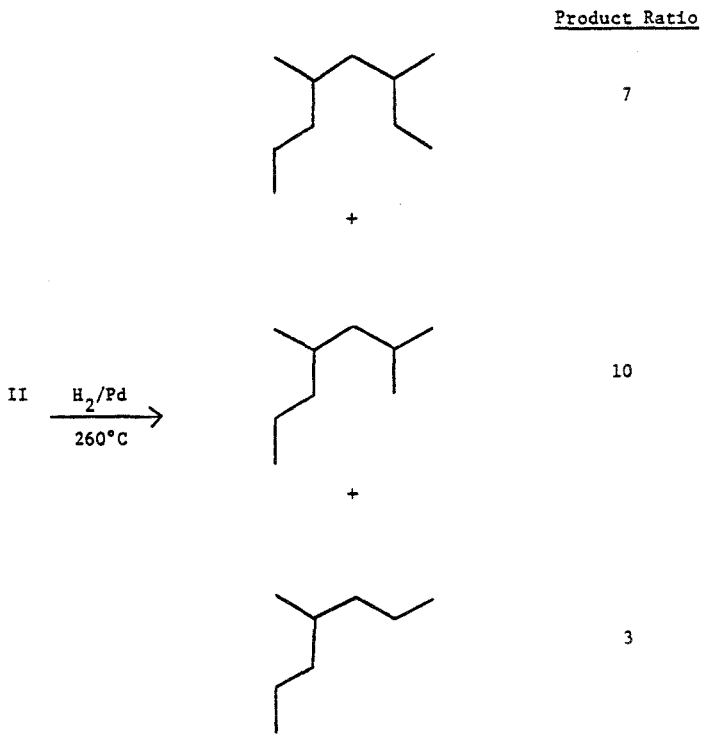
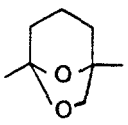
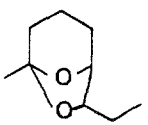


FIG. 1. Hydrogenolysis of α-multistriatin (II).

genolysis on palladium (Figure 1). The mass spectra of the hydrogenolysis products separated by gas chromatography were identical with those of authentic samples of 3,5-dimethyloctane, 2,4-dimethylheptane, and with the published mass spectrum of 4-methylheptane. The ring structure of II is significant since it is identical to the ring structures of two other scolytid aggregation pheromones, frontalin (Kinzer et al., 1969) and brevicomin (Silverstein et al., 1968).



Frontalin



Brevicomin

The spectra of a fourth compound isolated from the Porapak extract and inactive in the laboratory bioassay were also consistent with the ketal structure assigned to II. Structure II can theoretically exist as four diastereomers by inversions of stereochemistry at carbons 2 and 4 relative to carbon 1,

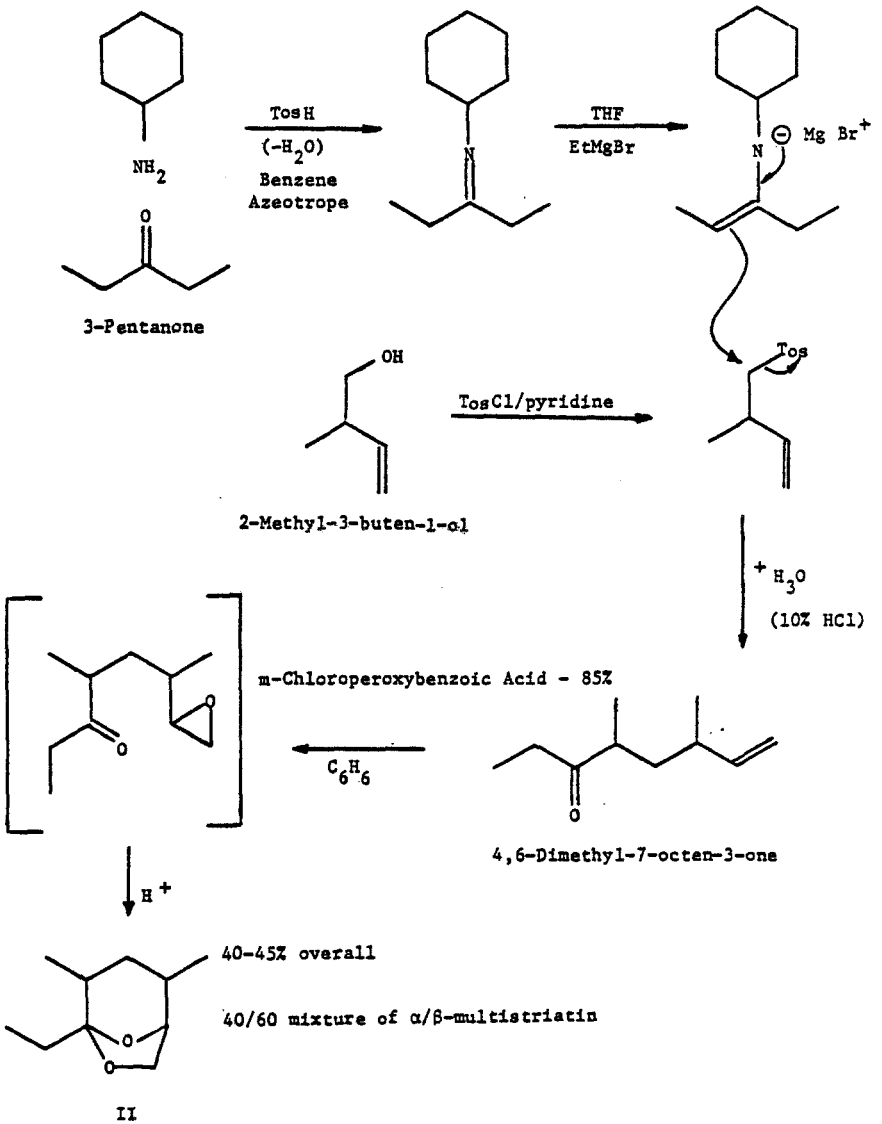


FIG. 2. Synthesis of α- and β-multistriatin.

thereby suggesting that the fourth compound is a diastereomer of II. The active and inactive ketals have been assigned the trivial names of α- and β-multistriatin, respectively.

The gross structure of α- and β-multistriatin was confirmed by synthesis (Figure 2). The final synthetic product was distilled through a short-path

microstill (bp 81–84°C, 22 mm Hg) in 40–45% overall yield from 2-methyl-3-buten-1-ol, and consisted of 90% total ketals with a 40/60 ratio of α/β multistriatin. The MS, IR, and NMR spectra of natural α- and β-multistriatin were congruent with those of the synthesized compounds. Results of investigations into the relative stereochemistry of α- and β-multistriatin will be reported in a later publication.

Compound III ($[\alpha]_D^{25} -24°$) was identified as (-)α-cubebene, a known sesquiterpene[8,9] whose structure and absolute stereochemistry have been rigorously determined (Ohta et al., 1966; Piers et al., 1971; Tanaka et al., 1972). The MS, IR, and NMR spectra of isolated III matched those of an authentic sample of (-)α-cubebene[10] and the specific rotation was in accord with the literature value ($[\alpha]_D^{30} -20.0°$) (Ohta et al., 1966; Vlakhov et al., 1967). In addition, GLC comparison by coinjection on 100-ft Carbowax and 50-ft Apiezon L SCOT columns further supported the assignment of III as α-cubebene. Quantities of (-)α-cubebene sufficient for field tests were obtained by distillation of 75 ml of cubeb oil[11] through a 40-plate spinning band fractional distillation column, with the purest fraction (1 ml, bp 30–32°C, 1.3 mm Hg) containing 90% (-)α-cubebene.

FIELD TEST OF SYNTHETIC ATTRACTANTS

The field tests were conducted in a residential section of Charlotte, North Carolina during April 30 and May 1, 1974. The test was designed to indicate the relative attractiveness of three pheromone preparations. These were:

(1) Porapak aeration extract (PE)

(2) Attractant mixture (AM).

(a) synthetic 4-methyl-3-heptanol (>99%), a 50/50 mixture of diastereomers.

(b) GLC-purified synthetic α-multistriatin (99%).

(c) (-)α-cubebene (90%), distilled from cubeb oil.

(3) "Multilure," crude attractant mixture (CAM).

(a) 4-methyl-3-heptanol (>99%) (Aldrich Chemical Co.), a 55/45 mixture of diastereomers.

[8] A major constituent of the essential oil of the fruits of cubeb (*Piper cubeba* L.) (Ohta et al., 1966); also isolated from Bulgarian peppermint oil (*Mentha piperita*) (Vlakhov et al., 1967) and several citrus oils (Veldhuis and Hunter, 1967).

[9] This compound was also isolated from 4–7-day virgin female frass.

[10] An authentic sample of α-cubebene was graciously supplied by Dr. Y. Hirose, Institute of Food Chemistry, Dojimanaka, Kita-ku, Osaka, Japan.

[11] Fritzsche Dodge & Olcott, Inc., New York, New York 10011.

TABLE 2. COMPARATIVE FIELD EVALUATION OF ATTRACTANT MIXTURES FOR *Scolytus multistriatus*[a]

Date	Paired comparisons					
	PE[b] vs. AM[c]		PE vs. multilure[c]		AM vs. multilure	
4/30	690	766	1164	843	929	1268
5/1	356	724	465	897	865	645
Total	1046	1490	1629	1740	1794	1913

Total beetles caught on: PE (20 traps); 2675
AM (20 traps); 3284
Multilure (20 traps); 3653

[a] Each entry is a total for the 10 traps used for each material indicated. Fresh screens were employed with the original vial for the second night's test.
[b] Dosage: Unanalyzed Porapak extract; 1×10^5 BH per vial (50 μl PE).
[c] Dosage: 1×10^5 BH corresponds to the following quantities of attractants per vial. 0.1 mg 4-methyl-3-heptanol (0.05 mg of active diastereomer). 0.05 mg α-multistriatin: 0.125 mg of α/β mixture in multilure. 0.2 mg (-)α-cubebene (90%): 2.0 mg of cubeb oil in multilure. The components were mixed prior to loading, and diluted with hexane so that 50 μl of solution was introduced per vial.

(b) crude synthetic multistriatin (90%), a 40/60 mixture of α- and β-multistriatin.

(c) cubeb oil, containing 10% (-)α-cubebene.

A paired comparison test was used to evaluate the relative attractiveness of the three attractant preparations. A total of 30 trapping sites were employed with 10 sites for each of the following paired comparisons: PE vs. AM, PE vs. multilure, AM vs. multilure. Each trap site consisted of two traps hung at one tree on opposite sides of the main bole. The traps were 31-cm-square hardware cloth (6-mm × 6-mm mesh) coated with Stikem Special®[12] baited at the center with 2.5-ml polyethylene snap-cap vials[13] containing one of the three attractant preparations. The vials were loaded and sealed with the attached cap 3 days prior to placement in the field. Positional effects were minimized by exchanging the positions of the traps at each site after the first day of trapping. Trap catches for the 48-hour test period are summarized in Table 2.

The following observations can be extracted from the field test data:

(1) The three attractant preparations appear to be equally attractive in the field.

[12] Michel and Pilton Co., Emeryville, California 94608.
[13] Bel Art Products, Pequannock, New Jersey 07440 (Catalogue No. F-17561).

(2) The inactive diastereomers of I and II do not inhibit responses of flying beetles.

(3) The remaining constituents of cubeb oil likewise do not deter attraction to the crude synthetic mixture.

CONCLUSIONS

Compounds I, II, and III appear to be the principal components of the secondary attractant for *Scolytus multistriatus*. Furthermore, multilure is a practical, inexpensive, and highly attractive mixture that may prove useful in large-scale trapping of beetles on a city-wide basis. A study is presently under way in Detroit, Michigan, to determine if Dutch elm disease incidence can be reduced by mass trapping of *S. multistriatus*.

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