Journal of Chemical Ecology, Vol. 21, No. 10, 1995

AGGREGATION PHEROMONE OF COCONUT RHINOCEROS BEETLE, *Oryctes rhinoceros* (L.) (COLEOPTERA: SCARABAEIDAE)

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(Received October 31, 1994; accepted June 6, 1995)

Abstract—Male coconut rhinoceros beetles, *Oryctes rhinoceros* (L.), produce three sex-specific compounds, ethyl 4-methyloctanoate, ethyl 4-methylheptanoate, and 4-methyloctanoic acid, the first of which is an aggregation pheromone. Synthesis of these compounds involving conjugate addition of organocuprates to ethyl acrylate is reported. In field trapping experiments, (4*S*)-ethyl 4-methyloctanoate and the racemic mixture were equally attractive and 10 times more effective in attracting beetles than ethyl chrysanthemumate, a previously recommended attractant. Ethyl 4-methylheptanoate was as attractive as ethyl chrysanthemumate and more attractive than 4-methyloctanoic acid, but further studies are required before it can be classed as an aggregation pheromone. Compared to ethyl 4-methyloctanoate alone, combinations of the three male-produced compounds did not increase attraction, whereas addition of freshly rotting oil palm fruit bunches to pheromone-baited traps significantly enhanced attraction. With increasing dose, captures of *O. rhinoceros* increased, but doses of 6, 9, and 18 mg/day were competitive with 30 mg/day lures. Newly designed vane traps were more effective in capturing beetles than were barrier or pitfall traps. Results of this study indicate that there is potential for using ethyl 4-methyloctanoate in operational programs to control *O. rhinoceros* in oil palm plantations.

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Key Words—Coleoptera, Scarabaeidae, *Oryctes rhinoceros*, coconut rhinoceros beetle, aggregation pheromone, pheromone chirality, ethyl 4-methyloctanoate, ethyl 4-methylheptanoate, 4-methyloctanoic acid.

INTRODUCTION

The coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae), is one of the most important pests of coconut and oil palms in South and Southeast Asia (Ho and Toh, 1982; Zelazny and Alfiler, 1986). Adult rhinoceros beetles burrow into the growing point of palms and feed on unopened fronds, causing damage to inflorescences and reduction in photosynthetic area, which decreases or delays fruit production (Zelazny, 1979; Liau and Ahmad, 1991, 1993). Prolonged attacks can kill mature palms by defoliation and young palms if the growing point is destroyed. The wounds produced by the beetle provide entry points for lethal diseases and the palm weevils, *Rhynchophorus ferrugineus* Olivier and *R. vulneratus* Panzer (Bedford, 1980; Jacob and Bhumannavar, 1991).

O. rhinoceros breeds in decaying organic matter, such as felled rotting palms, and usually becomes a major problem in newly planted or replanted oil palm plantations. Covering the trunks with a rapidly growing ground cover (Wood, 1968) and/or shredding and burning of the felled trunks are common practices to minimize the build up of *O. rhinoceros* populations (Liau and Ahmad, 1991). Although effective, shredding and burning is very expensive and has been banned in some parts of Southeast Asia (Tajudin et al., 1993) to lower air pollution in this region of 4.5 million hectares of oil palm.

Treatment of breeding sites, such as stumps, with insecticidal drenches and routine application of granular insecticides (e.g., carbofuran) to the leaf axils of young oil palms are recommended (Ho and Toh, 1982). These techniques are currently considered economic (Liau and Ahmad, 1991) but are not very effective and present environmental and health risks. Manual removal of beetles from palms and larvae from decomposing trunks is costly and labor-intensive (Ho and Toh, 1982).

Limited success in managing *O. rhinoceros* populations has been achieved through introduction of the baculovirus, *Rhabdionvirus oryctes* Hüger (Bedford, 1986). Introduction of the baculovirus to the Philippines reduced *O. rhinoceros* populations to 10–20% of prerelease levels, but even low-level populations of *O. rhinoceros* can cause great damage (Zelazny and Alfiler, 1987, 1991). The baculovirus remains effective only if it infects new larval hosts or is repeatedly introduced, and the potential exists for *O. rhinoceros* to develop resistance to the baculovirus after prolonged exposure (Zelazny and Alfiler, 1991). Several compounds, including ethyl chrysanthemumate (EC, rhinolure) have been recommended as lures for trapping *O. rhinoceros* (Barber et al., 1971; Maddison

et al., 1973; Vander Meer et al., 1979), but they are only moderately attractive (Vander Meer et al., 1979; Young, 1986).

O. rhinoceros adults are gregarious. More than one beetle may attack a given palm at the same time while a neighboring tree is unattacked (Gressit, 1953). Aggregation of adults in decaying palm trunks to mate and the occurrence of both single and multiple pairs of adults in the same breeding site (Zelazny and Alfiler, 1991) suggest that *O. rhinoceros* is attracted to host kairomones and employs either an aggregation or sex pheromone or both. Sex pheromones have been identified in several scarabaeids: *Costelytra zealandica* White (Henzell and Lowe, 1970), *Popillia japonica* Newman (Tumlinson et al., 1977), *Kheper Lamarcki* MacLeay (Burger et al., 1983), *Anomala rufocuprea* Motschulsky (Tamaki et al., 1985), *A. cuprea* Hope (Leal, 1991; Leal et al., 1993a), *A. daimiana* Harold (Leal et al., 1993b), *A. schonfeldti* Ohaus (Hasegawa et al., 1993), *Blitopertha orientalis* (Leal, 1993), *A. octiescostata* Burmeister (Leal et al., 1994a), *A. albopilosa sakishimana* Nomura (Leal et al., 1994b), *Holotrichia parallela* Motschulsky (Leal et al., 1993c), *Exomala orientalis* Waterhouse (Leal et al., 1994c), and *A. orientalis* Waterhouse (Zhang et al., 1994). An aggregation pheromone, ethyl 4-methyloctanoate, has recently been reported for *Oryctes monoceros* Olivier (Gries et al., 1994a).

The candidate pheromones revealed for *O. rhinoceros* are 4-methyl alkanoic acids and esters (Figure 1). Because the methyl branch of these compounds is remote from the functional group, syntheses had to be developed that allowed introduction of chirality at remote locations. The candidate pheromones were synthesized through conjugate addition of organocuprates to ethyl acrylate, which provided a shorter route to racemic 4-alkyl substituted ethyl esters than previously reported. Use of readily available enantiomers of citronellol allowed synthesis of both chiral isomers of ethyl 4-methyloctanoate.

We report the structural elucidation, synthesis, and field testing of a male-produced aggregation pheromone and two other male-specific compounds that have the potential to be used in mass trapping of *O. rhinoceros* populations.

METHODS AND MATERIALS

Volatile Analysis and Bioassays

Larvae, pupae, or adults of *O. rhinoceros* were collected at Parungkuda, West Java, Indonesia. Fourteen female and 16 male adults were separately aerated for one week in modified Nalgene desiccators (#5311-0250) containing sugarcane (Oehlschlager et al., 1988, 1992). A water-driven, charcoal-filtered air stream (2 liters/min) was maintained through the chambers and volatiles were captured on Porapak Q held in a glass column (14 cm length × 13 mm OD). In a second series of aerations, 10 female and 10 male adults were aerated

separately. Volatiles were eluted from the Porapak Q with 175 ml of pentane, and the eluant was concentrated by distillation of solvent through a 30-cm Dufton column. The concentration of volatiles was adjusted so that 1 μl equaled 0.6 beetle hours of production.

Porapak Q-captured volatiles from both males and females were analyzed by gas chromatographic–electroantennographic detection (GC-EAD) (Arn et al., 1975) (Hewlett Packard 5890A), utilizing a custom-built amplifier with a passive low-pass filter and a cutoff frequency of 10 kHz, and by GC analyses (Hewlett Packard 5830A) and GC–mass spectrometry (GC-MS) (Hewlett Packard 5985B), employing either a fused silica (30 m $\times$ 0.25 mm ID) or glass (30 m $\times$ 0.5 mm ID) column coated with SP-1000 (Supelco, Mississauga, Ontario).

Bioassays were conducted with a white Plexiglas Y-shaped olfactometer (stem 5 $\times$ 5 $\times$ 20 cm, arms 5 $\times$ 5 $\times$ 15 cm) with a clear lid. Test compounds were added with a syringe to filter paper inside a glass cartridge placed in each arm of the olfactometer. Compressed air at 200 ml/min carried the volatiles through each arm towards beetles released singly into the stem of the olfactometer 10 cm from the Y junction. A baffle prevented mixing of the two stimuli in the Y junction. Beetles with intact antennae and walking ability were held 2–3 hr in separate containers without food prior to testing. Prior to each test, the paper lining on the olfactometer floor was replaced, and beetle and position of stimuli were randomly selected. A beetle that did not move forward within 5 min of introduction was classed as a nonresponder, whereas complete entrance of a beetle into one of the arms was recorded as a response. In two experiments, 10 μl of distillate from males (experiment 1) or females (experiment 2) were tested against 10 μl of hexane. In order to determine behavioral activity of two synthetic male-produced compounds, a 612-ng dose of ethyl 4-methyloctanoate, **1**, and ethyl 4-methylheptanoate, **3**, in a 100:2 ratio was tested against 10 μl of hexane (experiment 3).

Instruments and General Synthetic Procedures

Nuclear magnetic resonance (NMR) spectroscopy was conducted on a Bruker AMX-400 spectrometer at 400.13 and 100.62 MHz for ^{1}H and ^{13}C NMR spectra, respectively. ^{1}H chemical shifts are reported relative to TMS, and ^{13}C chemical shifts are referenced to $CDCl_3$. Gas chromatographic analyses were performed on Hewlett-Packard 5880A and 5890 instruments equipped with a flame ionization detector and a fused silica, DB-1 coated column (15 m $\times$ 0.25 mm ID; 0.25 mm film) (J & W Scientific, Folsom, California). Elemental analyses were performed using a Carbo Erba model-1106 Elemental Analyzer. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were freshly distilled from sodium-benzophenone-ketyl, and dichloromethane (CH_2Cl_2) was freshly distilled from CaH_2. Unless otherwise indicated, chemicals obtained from com-

mercial sources were used without further purification. All moisture- and air-sensitive reactions were conducted under argon. Column chromatography refers to flash chromatography using Silica Gel 60 (230–400 mesh, E Merck, Darmstadt, Germany) (Still et al., 1978). Thin-layer chromatography (TLC) was conducted on aluminum-backed plates precoated with Merck Silica Gel 60F-254 as the adsorbent, and visualized by treatment with an acidic solution of 1% $Ce(SO_4)_2$ and 1.5% molybdic acid followed by gentle heating.

Syntheses

(±)-Ethyl 4-methyloctanoate ***(1).*** Compound **1** (97% pure) (Figure 1) was synthesized as previously described (Gries et al., 1994a) or by ethyl ester formation from commercially available 4-methyloctanoic acid, **2** (CTC Organics, Atlanta, Georgia).

(±)-Ethyl 4-methylheptanoate ***(3).*** This compound was prepared by the conjugate addition of 2-pentyl magnesium cuprate to ethyl acrylate (Gries et al., 1994a). Compound **3** (Figure 1) was obtained as a colorless liquid (40% yield, 95% pure). ^{1}H NMR ($CDCl_3$, ppm): 0.88 (6H, m); 1.10 (1H, m); 1.27 (3H, t, J = 8 Hz); 1.30 (2H, m); 1.40 (3H, m); 1.66 (1H, m); 2.30 (2H, m); 4.10

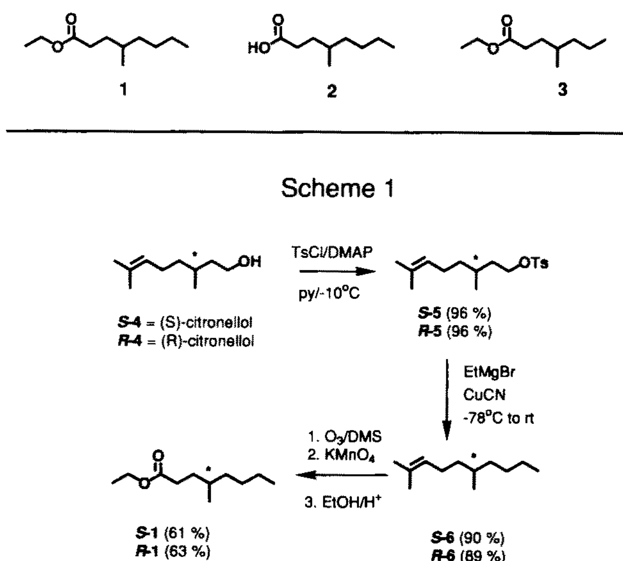

FIG. 1. Compounds **1–3** and scheme for synthesis of (4*S*)- and (4*R*)-ethyl 4-methyloctanoate.

(2H, q, J = 8 Hz); ^{13}C NMR ($CDCl_3$, ppm): 174.16, 60.11, 36.97, 32.17 (2C), 31.95, 31.16, 19.99, 19.23, 14.23. CI-MS m/z (relative intensity): 172 (M^+, 3), 127 (M^+-OEt, 30), 101 (M^+-CO_2Et, 100).

(3S)-3,7-Dimethyl-6-octenyltosylate **(*S*-5).** Enantiomer ***S*-5** (Figure 1, scheme 1) was prepared according to the procedure of Mori and Harashima (1993b) using (*S*)-citronellol, ***S*-4** (97.5% ee, Aldrich Chemical Co., Milwaukee, Wisconsin). Purification by column chromatography (9:1, pentane/Et_2O as eluant) yielded 5.70 g of ***S*-5,** (96% yield, 98% pure) as a pale yellow oil; ^{1}H NMR ($CDCl_3$, ppm): 0.80 (3 H, d, J = 8.8 Hz); 1.10 (1H, m); 1.20 (1H, m); 1.40 (1H, m); 1.50 (1H, m); 1.52 (1H, m); 1.56 (3H, s); 1.70 (3H, s); 1.90 (2H, m); 2.45 (3H, s), 4.02 (2H, m); 5.0 (1H, t, J = 8.8 Hz); 7.32 (2H, d, J = 8.8 Hz); 7.80 (2H, d, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$, ppm): 144.60, 133.41, 131.44, 129.79, 127.88, 124.34, 69.05, 36.72, 35.69, 28.93, 25.66, 25.27, 21.60, 19.06, 17.60; CI-MS m/z (relative intensity): 310 (M^+, 2), 138 (M^+-OSO_2-C_6H_4-CH_3, 50). ***R*-5** [from (*R*)-citronellol; 96% ee]: 5.61 g, 96% yield (98% pure).

(6R)-2,6-Dimethyl-2-decene **(*R*-6).** This intermediate compound was prepared according to the procedure of Fouquet and Schlosser (1974). CuCN (Aldrich Chemical Co.) was used as received. Column chromatography (pentane) afforded 2.42 g (89% yield, 97% pure) of ***R*-6** as a colorless liquid. ^{1}H NMR ($CDCl_3$, ppm): 0.88 (3H, d, J = 7.3 Hz); 0.90 (3H, t, J = 7.3 Hz); 1.10 (2H, m); 1.30 (7H, m); 1.60 (3H, s); 1.70 (3H, s); 1.90 (2H, m); 5.1 (1H, t, J = 8 Hz); ^{13}C NMR ($CDCl_3$, ppm): 130.83, 125.16, 37.19, 36.68, 32.46, 29.29, 25.62 (2C), 23.01, 19.60, 17.55, 14.05. CI-MS m/z (relative intensity): 168 (M^+, 40); IR (neat): 2926, 1673, 1458, 1377, 1122, 1094, 984, 826 cm^{-1}. Anal. calcd. for $C_{12}H_{24}$: C, 85.63; H, 14.37. Found: C, 85.69; H, 14.30. ***S*-6:** 2.39 g, 90% yield (98% pure). Anal. calcd. for $C_{12}H_{24}$: C, 85.63, H; 14.37. Found: C, 85.78; H, 14.57.

(4R)-Methyloctanal **(*R*-7).** Compound ***R*-7** was prepared according to the procedure of Mori and Harashima (1993a). Column chromatography (8:2, pentane/Et_2O as eluant, TLC R_f = 0.55) afforded ***R*-7** (1.68 g, 87% yield, 96% pure) as a colorless liquid. ^{1}H NMR ($CDCl_3$, ppm): 0.90 (6H, m); 1.12 (1H, m); 1.30 (5H, m); 1.40 (2H, m); 1.64 (1H, m); 2.64 (2H, m); 9.75 (1H, s); ^{13}C NMR ($CDCl_3$, ppm): 202.87, 41.70, 36.36, 32.38, 29.16, 28.93, 22.91, 19.35, 14.05; CI-MS m/z (relative intensity): 143 (M^+ + 1, 100); IR (neat): 2927, 2715, 1727, 1465, 1379, 1263, 1133, 1012, 896, 849, 729 cm^{-1}. Anal. calcd. for $C_9H_{18}O$: C, 75.00; H, 12.76. Found: C, 75.09; H, 12.48. ***S*-7:** 1.65 g, 85% yield (98% pure). Anal. calcd. for $C_9H_{18}O$: C, 75.00; H, 12.76. Found: C, 74.98; H, 12.66.

(4R)-4-Methyloctanoic acid **(*R*-2).** This compound was prepared by the oxidation of ***R*-7** (yield 1.78 g) according to the procedure of Furniss et al.

(1989). Compound ***R*-2** was used for the next step without further purification. ^{1}H NMR ($CDCl_3$, ppm): 0.90 (6H, m): 1.12 (1H, m); 1.14 (5H, m); 1.60 (2H, m); 1.80 (1H, m); 2.35 (2H, m), 11.30 (1H, br. s). ***S*-2:** 1.45 g.

(4R)-Ethyl-4-methyloctanoate **(*R-1*).** Enantiomer ***R*-2** was esterified using the Fisher method (Furniss et al., 1989). Column chromatography (9:1, pentane/Et_2O as eluant) gave 1.34 g of ***R*-1** (61% yield based on ***R*-7,** 98% pure). $[\alpha]_D^{20} = -1.67°$ (c = 1.345, $CHCl_3$); ^{1}H NMR ($CDCl_3$, ppm): 0.88 (3H, d, J = 8 Hz); 0.90 (3H, t, J = 8 Hz); 1.10 (1H, m); 1.24 (3H, t, J = 8 Hz); 1.30 (5H, m); 1.40 (2H, m); 1.66 (1H, m); 2.30 (2H, m); 4.10 (2H, q, J = 8 Hz); ^{13}C NMR ($CDCl_3$, ppm): 174.12, 60.14, 36.36, 34.42, 32.21, 31.97, 29.17, 22.94, 19.31, 14.25, 14.06; IR (neat): 2958, 2884, 1737, 1461, 1373, 1261, 1173, 1108, 1032, 932, 855, 779, 732 cm^{-1}. Anal. calcd. for $C_{11}H_{22}O_2$: C, 70.91; H, 11.91. Found: C, 70.99; H, 12.00. ***S*-1:** 1.36 g, 63% yield based on ***S*-7** (98% pure) $[\alpha]_D^{20} = +1.67°$ (c = 1.350, $CHCl_3$). Anal. calcd. for $C_{11}H_{22}O_2$: C, 70.91; H, 11.91. Found: C, 70.82; H, 11.98.

Chiral Determination

Enantiomeric excess of (*R*)- and (*S*)-citronellol was determined through GC analysis (DB-1) as the amides of D-(+)-α-phenylethylamine (Sigma, St. Louis, Missouri) of the corresponding citronellic acids (Sonnet and Gazzillo, 1990).

Analysis of ***S*-1** and ***R*-1** by GC on a fused silica, Cyclodex-B-coated column (30 m × 0.25 mm ID, J & W Scientific) failed to resolve the enantiomers. ^{1}H NMR spectra in the presence of tris[3-(heptanofluoropropylhydroxymethylene)-(−)-camphorato]europium(III) [Eu(hfc)$_3$] in CS_2 (1:1 or 1:2 ratio, ester: shift reagent) (McCreary et al., 1974; Valentine et al., 1976) or the chiral solvating agent (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol in CCl_4 (1:1 or 1:2 ratio, ester: anthrylethanol) (Pirkle et al., 1977; Pirkle and Sikkenga, 1977) failed to display diastereoisomeric complexes at different chemical shifts.

Field Experiments

Experiments were conducted in 1- or 2-year-old oil palm plantings at three P.T.P.P. London Sumatra Indonesia estates in North Sumatra, Indonesia. Specific locations are given either in Figures 5–10 below or in the text, if data are not presented in figures. All experiments were set up as randomized complete blocks with intertrap and interblock distances of at least 27 and 54 m, respectively. Traps were checked daily and captured beetles removed.

All compounds, except ***S*-1** and ***R*-1,** were released from heat-sealed, polymer membrane bag devices (Chem Tica International, Costa Rica) at constant rates. Release rates for each compound were determined by placing 10 sealed

devices containing 1 ml of neat compound in a thermostated chamber (25 ± 1°C, 60% relative humidity), allowing 48 hr equilibration, and then weighing (±0.1 mg) each device every two to three days for two weeks (devices releasing 30 mg/day) or four to five weeks (devices releasing <30 mg/day). Variability of release rates between devices was < ±5% and over the measurement period was < ±10%. Compound **1** was released at 0.9, 3, 6, 9, and 30 mg/day; **2** at 3 and 15 mg/day; **3** at 1 and 30 mg/day; and EC at 15 mg/day. Combinations of devices were used to achieve some release rates. *S*-**1** and *R*-**1** were released at 0.3 mg/day (at 25°C) from 1-mm-ID glass capillary tubes cut 1 cm above the meniscus and placed in capped 400-μl plastic centrifuge tubes with two 3-mm holes 1 cm below the top.

Behavioral Activity of Ethyl 4-methyloctanoate

A three-treatment, 10-replicate experiment (experiment 4) determined activity of the candidate pheromone, **1**. Black pitfall traps (Figure 2) were buried in the ground 1–1.5 m from palms and baited with either decomposing oil palm tissue (1–2 kg of leaf bases or empty fruit bunches), **1** (released at 30 mg per day), or both. Oil palm tissue or a cloth placed in traps baited with **1** only, was treated with 0.3% active ingredient solution of Basudin 60 EC (diazinon, Ciba-

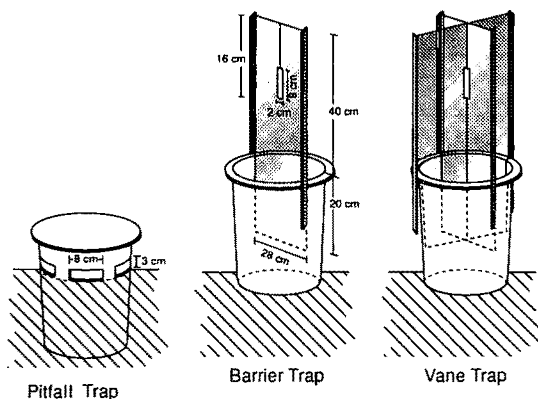

FIG. 2. Trap designs used for capturing *O. rhinoceros*. Pitfall trap: 19-liter black bucket buried in ground to allow beetles to enter through slots below rim; pheromone lure suspended within bucket from plywood lid. Barrier and vane traps: 20-liter white bucket with one or two unpainted sheet metal vanes extending 20 cm into bucket to prevent beetles from flying out; wooden slats on edge of vanes for reinforcement; lures suspended within slot to allow volatile dissemination in all directions.

Geigy). Beetles were captured because of contact with the insecticide-treated substrate or inability to exit the traps.

Trap Design Experiments

A three-treatment, 10-replicate experiment (experiment 5) compared trapping efficacies of pitfall to barrier and vane traps (Figure 2). In experiment 6 ($N = 9$), four types of vane traps were compared: unpainted (standard) vane traps (Figure 2), vane traps fitted with an internal funnel (15 cm opening) to prevent beetle escape, traps with matte black-painted vanes, and traps with both funnels and black-painted vanes (Dolok Estate, February 26–March 11, 1994). In both experiments 5 and 6, traps were baited with lures releasing **1** at 30 mg/day. No insecticide or organic matter was used in any of the traps.

Dose–Response of Ethyl 4-methyloctanoate

In a four-treatment, 18-replicate experiment (experiment 7) with standard vane traps at 36 m intervals, **1** was released at 0, 0.3, 3, or 30 mg/day. In experiment 8 ($N = 15$), standard vane traps were baited with **1** released at 0, 3, 6, 9, or 30 mg/day. In experiment 9 ($N = 12$), standard vane traps were baited with **1** released at 0, 6, 9, 18, or 30 mg/day. To minimize interference between treatments in experiments 8 and 9, intertrap and interblock distances were increased to 54 and ≥ 63 m, respectively.

Chiral Isomers of Ethyl 4-methyloctanoate

A four-treatment, 10-replicate experiment (experiment 10) tested attraction of beetles to racemic **1**, ***S*-1**, ***R*-1**, or a blank control in standard vane traps. *S*- or ***R*-1** were released at ~2 mg/day from eight capillary tubes and racemic **1** was released at ~4 mg/day from 16 capillary tubes.

Other Candidate Pheromones and Attractants

Attraction of beetles to three male-produced compounds, **1**, **2**, **3**, and to (±)-ethyl chrysanthemumate (EC, 95% pure, mixture of *cis* and *trans*, Aldrich Chemical Co.) was compared in experiment 11 ($N = 10$). All compounds were released at 30 mg/day. Experiment 12 ($N = 9$) compared attraction of beetles to **1** alone (30 mg/day) and in combination with **3** at ratios of 100:1, 100:10, and 100:100 (Rambong Sialang Estate, April 13–21, 1994). Experiment 13 ($N = 10$) compared attraction of beetles to **1** alone (10 mg/day) or in approximate natural ratios with either or both of **2** (30 mg/day) and **3** (0.1 mg/day) (Rambong Sialang Estate, June 9–17, 1994).

Pheromone–Host Material Interactions

The attraction of beetles to **1** (9 mg/day) alone, freshly milled empty fruit bunches (1/3 to 1/2 bunch per trap), or to both was examined in a three-treatment, 12-replicate experiment (experiment 14).

Statistical Analyses

The proportions of beetles responding to each stimulus in laboratory bioassays were compared by using the normal approximation to the binomial test, and differences between responses of female and male beetles were compared with χ^2 tests (Zar, 1984).

In all field experiments, no significant differences were found in the responses of male and female beetles and so catches were pooled by sex for analysis. Data were transformed by log $(x + 1)$ if they were not normally distributed and were subjected to analysis of variance (general linear modeling) (Minitab, 1989). If replicates were run at different times or locations, data were analyzed for time $\times$ treatment or location $\times$ treatment interactions. Following ANOVA, multiple pair comparisons were made using Bonferroni t tests. If homoscedasticity was not achieved by transformation, data were analyzed by χ^2 tests (Zar, 1984).

RESULTS

GC and GC-EAD analyses of Porapak Q-trapped volatiles obtained from aerations of either *O. rhinoceros* males or females revealed two abundant male-specific components (Figure 3), of which the early eluting volatile elicited antennal responses by male and female antennae (Figure 4). Retention and mass spectrometric characteristics of these two compounds were identical to ethyl 4-methyloctanoate, **1,** and 4-methyloctanoic acid, **2.** A second EAD-active compound (not visible in Figure 4) had a Kovats retention index (RI = 1379) suggestive of ethyl 4-methylheptanoate, **3.** GC-MS in both electron impact and chemical ionization modes of beetle-produced and authentic **3** confirmed this structural assignment.

In laboratory bioassays (experiments 1 and 2), male-produced volatiles were equally attractive to walking male and female *O. rhinoceros*, but female-produced volatiles were attractive only to males (Table 1). Behavioral activity of synthetic **1** plus **3** was demonstrated in experiment 3 (Table 2) and justified field testing of synthetic candidate pheromones. In field experiments, **1** at 30 mg/day alone or in combination with decomposing palm tissue attracted more beetles than palm tissue alone (Figure 5; experiment 4).

Both vane and barrier traps were superior to the pitfall trap (Figure 2), with

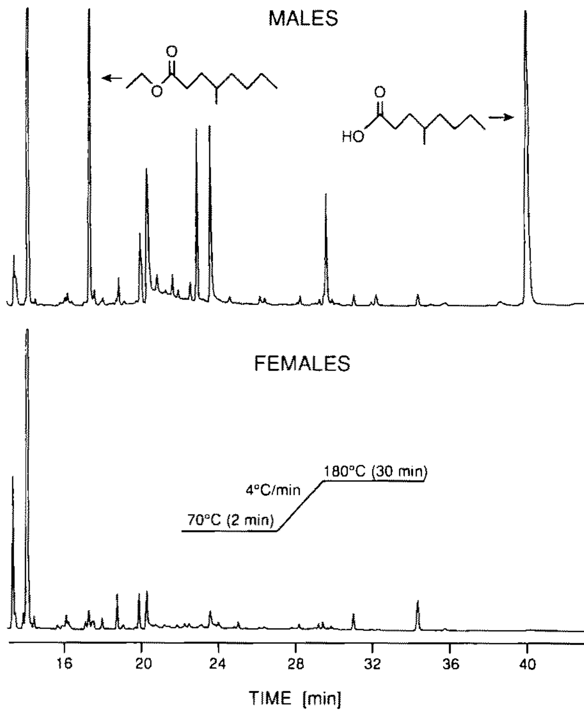

FIG. 3. Gas chromatograms of volatiles from 10 male and 10 female *O. rhinoceros* maintained in aeration chambers for one week with provision of sugarcane as food source. Chromatography: Hewlett Packard 5830A gas chromatograph (GC) equipped with a glass capillary column (30 m × 0.5 mm ID) coated with SP-1000.

vane traps capturing about three times more beetles than pitfall traps (Figure 6; experiment 5). Addition of a funnel to the vane trap and painting vanes black did not alter trap efficacy (experiment 6; ANOVA, $F = 1.19$, $df = 3$, $P = 0.341$).

Catches of *O. rhinoceros* increased with increasing release rates of the pheromone (Figure 7; experiments 7–9). A significant location × dose interaction was observed in experiment 7 ($F = 18.39$, $df = 1$, $P < 0.001$), which arose from the 3 mg/day lure being more attractive relative to the 30 mg/day lure at Rambong Sialang than at Dolok (mean catches in 3 mg/day traps were 64% and 32% of those in 30 mg/day traps, respectively). This result along with the prohibitive cost of 30 mg/day lures, prompted us to examine release rates between 3 and 30 mg/day. In experiments 8 and 9, lures releasing **1** at 6, 9, and 18 mg/day were competitive with 30 mg/day lures (Figure 7).

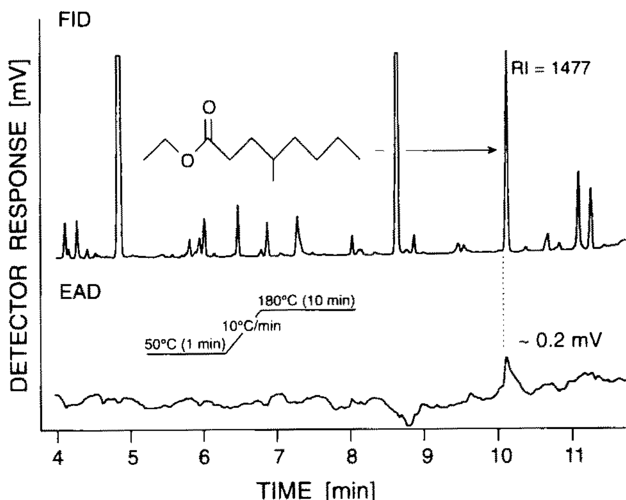

FIG. 4. FID and EAD responses to volatiles obtained from male *O. rhinoceros*. The antennal recording was carried out with an antenna of a female beetle. Chromatography: Hewlett Packard (HP) 5890A gas chromatograph (GC) equipped with a fused silica column (30 m × 0.25 mm ID) coated with SP-1000.

Racemic **1** and *S*-**1** were superior to ***R*-1** in attracting beetles (Figure 8; experiment 10). Racemic **1** was significantly more attractive than **2, 3,** or EC (Figure 9; experiment 11); **3** was significantly more attractive than **2,** but both compounds did not differ in attraction from EC. Traps baited with **1** alone or with **3** in different ratios were equally attractive (experiment 12; ANOVA, $F = 0.49$, $df = 3$, $P = 0.693$), as were traps baited with **1** alone, **1** plus **2,** or **1** plus **2** and **3** (experiment 13; ANOVA, $F = 2.28$, $df = 3$, $P = 0.105$).

Freshly milled oil palm fruit bunches alone were unattractive to rhinoceros beetles, but in combination with **1** they significantly enhanced pheromonal activity (Figure 10; experiment 14).

DISCUSSION

Methyl-branch-substituted pheromones are very common (Mori, 1992). There are several synthetic methods described for the synthesis of 4-methyl alkanoic acids and esters. Compounds **1** and **3** were prepared by Cason et al. (1944) by reaction of ethyl levulinate or levulinic acid with the corresponding Grignard reagent to produce a γ,γ-dialkylbutyrolactone. Treatment of the lactone with $SOCl_2$/ethanol/HCl followed by hydrogenation produced the respec-

TABLE 1. RESPONSES OF MALE AND FEMALE *Oryctes rhinoceros* TESTED INDIVIDUALLY TO PENTANE EXTRACT OF MALE- AND FEMALE-PRODUCED VOLATILES (10 μl = 5.6 BEETLE HOURS) VERSUS 10 μl OF HEXANE CONTROL IN Y-SHAPED OLFACTOMETER

Experiment	Sex	n	Number of responders to stimuli[a]		P^b
			Extract	Control	
1. Female extract *versus* hexane control	Male	20	9	2	0.006
	Female	20	5	8	0.393
2. Male extract *versus* hexane control	Male	15	12	1	0.0001
	Female	17	13	3	0.0014

[a] Responses to female extract in experiment 1 differ between males and females, $\chi^2 = 4.608$, $df = 1$, $P = 0.032$. Responses to male extract in experiment 2 are not significantly different between males and females, $\chi^2 = 0.738$, $df = 1$, $P = 0.39$.
[b] Determined by normal approximation to the binomial test (Zar, 1984).

TABLE 2. RESPONSES OF MALE AND FEMALE *Oryctes rhinoceros* TESTED INDIVIDUALLY TO 612 ng OF 100:2 BLEND OF SYNTHETIC ETHYL 4-METHYLOCTANOATE (**1**) AND ETHYL 4-METHYLHEPTANOATE (**3**) VERSUS 10 μl OF HEXANE CONTROL IN Y-SHAPED OLFACTOMETER (EXPERIMENT 3)

		Number of responders to stimuli[a]		
Sex tested	*N*	Synthetic blend	Hexane control	P^b
Male	29	17	7	0.025
Female	29	19	7	0.008

[a]Responses are not significantly different between males and females, $\chi^2 = 0.031$, $df = 1$, $P = 0.86$.

[b]Determined by normal approximation to the binomial test (Zar, 1984).

tive 4-methyl esters in overall yields of 50–60%. Vasi and Desai (1973) prepared 4-methylheptanoic acid from 2-methylbutyric acid using Arndt-Eistert synthesis. Mrowca (1981) reported the synthesis of carboxylic acids or esters by catalytic carboxylation or alcoxycarbonylation of unsaturated hydrocarbons (**2** was obtained in 86% yield). More recently, Sonnet and Baillargeon (1989) synthe-

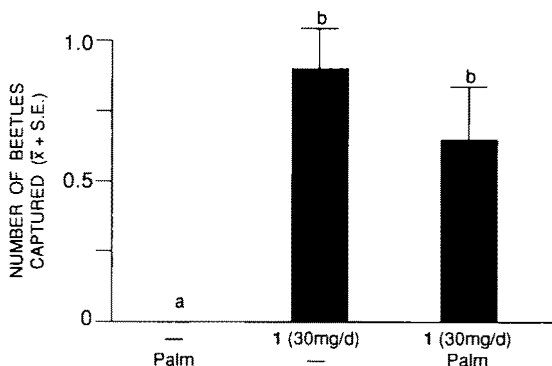

FIG. 5. Attraction of *O. rhinoceros* to ethyl 4-methyloctanoate, **1** (released at 30 mg/day), decaying oil palm tissue, or both together, in pitfall traps at Bah Lias and Rambong Sialang Estates, North Sumatra, Indonesia (October 14–22 and 16–23, 1993, respectively) (experiment 4). Data pooled as no locational differences were found ($\chi^2 = 2.2305$, $df = 1$, $P > 0.10$). Treatment differences for pooled data, $\chi^2 = 13.036$, $df = 2$, $P < 0.01$. Bars with the same letter are not significantly different, pairwise χ^2 tests, $P < 0.05$.

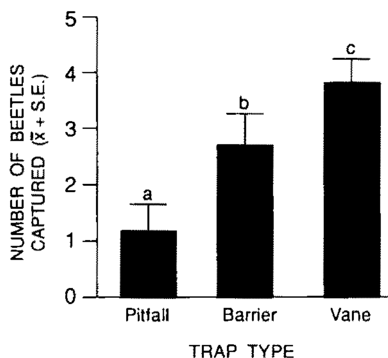

FIG. 6. Efficacy of three insecticide-free trap types for capturing *O. rhinoceros* (February 2–10, 1994), tested at Rambong Sialang Estate (experiment 5). All traps baited with **1** released at 30 mg/day. ANOVA, $F = 6.58$, $df = 2$, $P = 0.007$. Bars with the same letter are not significantly different, Bonferroni *t* test, $P < 0.05$.

sized 4-methyloctanoic acid, **2,** in 53% overall yield by methylation of the *N-t*-butylimine derivative of hexanal by condensation with malonic acid and hydrogenation.

In our study, both **1** and **3** were prepared by conjugate addition of organocuprates to ethyl acrylate (Corey and Boaz, 1985; Matsuzawa et al., 1989; Perlmutter, 1992; Gries et al., 1994a), a shorter synthetic procedure than in earlier reports. The required cuprates were prepared by adding 10 mol% CuCN to a solution of the corresponding Grignard reagent at −40°C. Subsequent addition of trimethylchlorosilane, HMPA, and ethyl acrylate (2:2:1 ratio) in THF or Et_2O produced **1** or **3** in 40–55% yield. Use of CuBr·DMS increased the yield of the conjugate addition by 10–15%. No further attempts to optimize the reaction yields were made because **1** can also be prepared by the esterification of commercially available **2** (CTC Organics, Atlanta, Georgia).

Syntheses of ***R*-2** and ***S*-2,** in high optical purity (95.4%) have been reported by Sonnet and Gazzillo (1990). Accordingly, alkylation of hexanoic acid produced 2-methylhexanoic acid, which was reacted with (*R*)- or (*S*)-α-phenylamine to give diastereoisomeric amides that were separated by crystallization. The optically pure amides were *N*-hydroxylated and reduced to the corresponding 2-methylhexanols, which were oxidized and converted to the methyl 4-methyl-2-octanoates via a Wittig reaction with carbomethoxymethylene triphenylphosphorane. Hydrogenation and saponification afforded ***R*-2** and ***S*-2** in ~10% overall yield (eight steps).

We envisioned a more efficient route using a highly enantiomerically enriched citronellol (Hanessian et al., 1990; Ho, 1992). The citronellane skel-

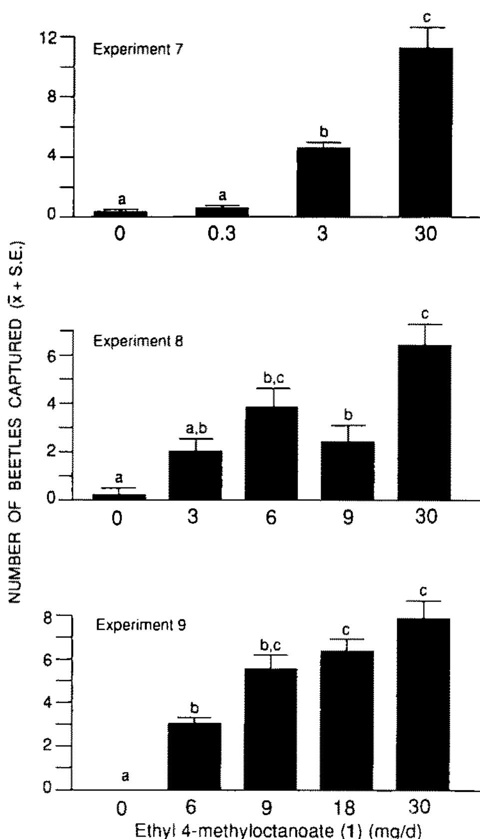

FIG. 7. Attraction of *O. rhinoceros* to **1** released at various rates from standard vane traps. Experiment 7, February 12–26 and February 21–March 4, 1994, Dolok and Rambong Sialang Estates, respectively; ANOVA, $F = 44.89$, $df = 3$, $P < 0.001$. Experiment 8, March 11–17, 1994, Dolok; ANOVA log $(x + 1)$ transformed data, $F = 12.13$, $df = 4$, $P < 0.001$. Experiment 9, May 24–June 8, 1994, Dolok; ANOVA $\log_{10} (x + 1)$ transformed data, $F = 49.84$, $df = 4$, $P < 0.001$. Bars with the same letter are not significantly different, Bonferroni *t* test, $P < 0.05$. Untransformed means presented.

eton is commonly used in the synthesis of natural products (e.g., Mori et al., 1991; Stork and Nakamura, 1983; Ho, 1992; Mori and Harashima, 1993a,b; Weinges et al., 1993; Mori and Murata, 1994; Paquette et al., 1995). Modifications at both termini of the citronellane skeleton can be performed without perturbing the chiral center (Ho, 1992). The synthesis of ***S*-1** and ***R*-1** (Figure 1, scheme 1) commenced with tosylation of (*R*)- or (*S*)-citronellol. Chain exten-

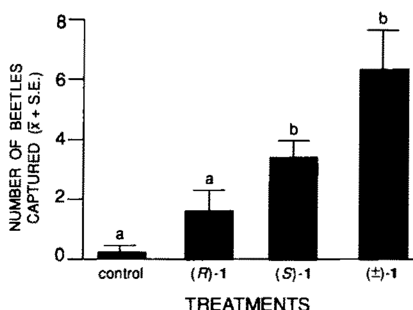

FIG. 8. Attraction of *O. rhinoceros* to standard vane traps containing stereoisomers of **1** at Rambong Sialang Estate (May 23–June 8, 1994) (experiment 10). ANOVA log ($x + 1$) transformed data, $F = 24.04$, $df = 3$, $P < 0.001$. Bars with the same letter are not significantly different, Bonferroni t test, $P < 0.05$. Untransformed means presented.

sion *via* cuprate displacement of the tosylate, **5** (Fouquet and Schlosser, 1974), produced the corresponding 2,6-dimethyl-2-decenes, **6**, in high yield. Ozonolysis followed by permanganate oxidation and esterification afforded ***S*-1** and ***R*-1** in yields of 45–47% over five steps. Attempts to determine the optical purity of **6**, **7**, ***S*-1**, or ***R*-1** by gas chromatography using a Cyclodex B column and NMR techniques were unsuccessful. Since the above procedures have significant precedent in the literature and involve transformations remote from the stereogenic carbon, we assumed that the chiral purity of ***S*-1** and ***R*-1** was identical to that of citronellol.

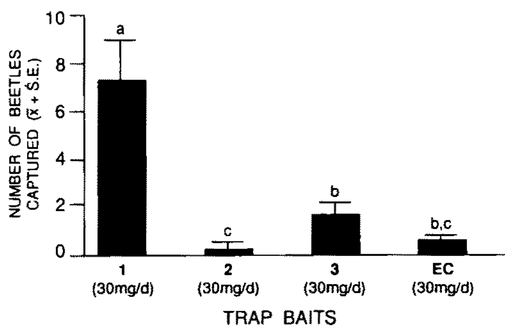

FIG. 9. Attraction of *O. rhinoceros* to **1**, **2**, **3**, and EC in standard vane traps at Rambong Sialang Estate (March 30–April 7, 1994) (experiment 11). ANOVA, log ($x + 1$) transformed data, $F = 35.77$, $df = 3$, $P < 0.001$. Bars with the same letter are not significantly different, Bonferroni t test, $P < 0.05$. Untransformed means presented.

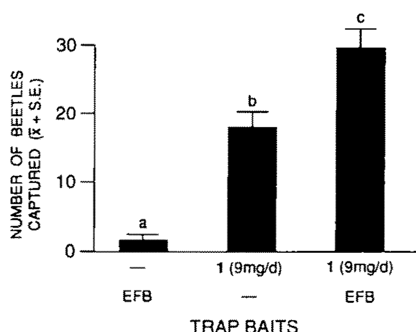

FIG. 10. Attraction of *O. rhinoceros* to standard vane traps containing **1** (released at 9 mg/day), freshly milled empty fruit bunches (EFB), or both together at Rambong Sialang and Dolok Estates (April 26–May 11 and May 3–18, 1994, respectively) (experiment 14). ANOVA, $F = 46.60$, $df = 2$, $P < 0.001$. Bars with the same letter are not significantly different, Bonferroni *t* test, $P < 0.05$.

Semiochemical communication in *O. rhinoceros* appears to involve both a male-produced aggregation pheromone and a female-produced sex pheromone (Table 1). Because aggregation pheromones have greater potential than sex pheromones for controlling *Oryctes* populations through mass trapping, research was focused on the identification of aggregation pheromones. Compound **1** was confirmed in field experiments as the major male-produced aggregation pheromone of *O. rhinoceros* (Figures 5 and 9). The same compound is a male-produced aggregation pheromone in the African rhinoceros beetle, *O. monoceros* (Gries et al., 1994a). Other geographically or temporally isolated scarabaeid beetles also utilize identical sex pheromones (Leal et al., 1993a,b).

Oryctes spp. respond to vertical silhouettes (Bedford, 1980). Above-ground light traps with vanes are effective in capturing *O. elegans* in the Arabian peninsula. Superior efficiency of vane and barrier traps for capturing *O. rhinoceros* (Figure 6) may mostly be attributed to the vertical silhouettes that are lacking in the pitfall trap. Because response to the vertical silhouette could not be enhanced further by use of nonreflective black vanes and the use of funnels did not improve retention of captured beetles in traps, insecticide-free, unpainted vane traps were adopted as the standard trap for *O. rhinoceros*.

As in *A. octiescostata* (Leal et al., 1994a), increase of pheromone release rate resulted in increasing numbers of captured beetles. Because traps baited with **1** released at 6, 9, 18, or 30 mg/day were similarly attractive, a release rate of 9 mg/day was adopted for operational trapping trials.

In the Japanese beetle, pheromonal attraction is strongly inhibited by the presence of the nonnatural enantiomer of its sex pheromone, (*R*,*Z*)-5-

(1-decenyl)dihydro-2(3H)-furanone (Tumlinson et al., 1977). We therefore investigated the response of *O. rhinoceros* to chiral isomers of **1**. Racemic and ***S*-1** were similarly attractive to *O. rhinoceros* (Figure 8), indicating that ***S*-1** is a naturally produced isomer and ***R*-1** is not repellent. Behavioral activity of ***R*-1** and enhanced attraction of *O. rhinoceros* to racemic **1** rather than ***S*-1** may indicate that both isomers are produced naturally. Accessible racemic **1** can be used operationally.

Synergism between aggregation pheromones and host compounds has recently been demonstrated for *A. octiescostata* (Leal et al., 1994d), and palm weevils, *Rhynchophorus* spp. (Oehlschlager et al., 1992; Gries et al., 1994b; Hallett et al., 1993; Giblin-Davis et al., 1994). Synergistic oil palm volatiles are apparently produced early in the decomposition (fermentation) process because freshly milled fruit bunches but not decomposed palm tissue enhanced pheromone attraction. Investigations are underway to identify palm kairomones and to determine the potential of mass-trapping *O. rhinoceros* in oil palm plantations.

Acknowledgments—This work was supported through an International Development Research Centre Young Canadian Researcher Award and a Natural Sciences and Engineering Research Council (NSERC) Postgraduate Fellowship to R.H.H., and NSERC grants to A.C.O. and J.H.B. The assistance of Dr. R.A. Syed in locating field sites, the generosity of P.T.P.P. London Sumatra Indonesia and its research and technical staff, and the University of Costa Rica for a fellowship to A.L.P. are also gratefully acknowledged.

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