Journal of Chemical Ecology, Vol. 24, No. 11, 1998

MALE-RELEASED SEX PHEROMONE OF THE STINK BUG *Piezodorus hybneri*

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(Received November 5, 1997; accepted June 30, 1998)

Abstract—Male-released semiochemicals of the stink bug *Piezodorus hybneri* (Heteroptera: Pentatomidae) elicit attraction of male and female bugs and homosexual behavior in males. Three active components were isolated from the airborne volatiles of males by flash chromatography, with the activity monitored by GC-EAD and behavioral bioassay. The pheromone system was characterized as a mixture of β-sesquiphellandrene, (*R*)-15-hexadecanolide, and methyl 8-(*Z*)-hexadecenoate (ratio: 10:4:1), and the activity of the semiochemicals was assessed with authentic samples. Enantiomerically pure samples of the *R* and *S* macrolactones were obtained by Yamaguchi's and Mitsunobu's macrolactonization of a key intermediate, (*R*)-15-hydroxyhexadecanoic acid. The nonnatural *S* stereoisomer was neither a beneficial nor a behavioral antagonist. Individual constituents or binary mixtures were active, but the optimal male response was elicited only by the full mixture. Behavioral observation and the fact that the onset of pheromone production is coincident with ovarian development strongly suggest that these semiochemicals are, in fact, sex pheromones.

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0098-0331/98/1100-1817/$15.00/0

Key Words—Homosexual behavior, aggregation pheromone, β-sesquiphellandrene, (*R*)-15-hexadecanolide, methyl 8-(*Z*)-hexadecenoate.

INTRODUCTION

Chemical communication requires the release of specific chemicals from a producer (emitter), the transmission of these chemicals to a receiver, and the processing of these signals leading to appropriate behavioral responses in the receiver (Roelofs, 1995). Among the insect groups in which chemical attraction is a major means of sexual recruitment, namely Lepidoptera, Coleoptera, Hymenoptera, Orthoptera, Diptera, and Homoptera, females predominantly are the emitters and males the receivers (Cardé and Baker, 1984) of the signal referred to as a sex pheromone. When a member of one sex produces a signal that attracts others of the same sex, the cue is termed an aggregation signal, such as the aggregation pheromone of bark beetles (Borden, 1985). However, the assumption that male signals have evolved to attract other males deserves close scrutiny (Thornhill and Alcock, 1983).

Hitherto, male-released pheromones of true bugs (Heteroptera) have been identified for a few species (Aldrich et al., 1984, 1991, 1994; Baker et al., 1987; Oliver et al., 1972; Leal et al., 1995, 1996c; Sugie et al., 1996) and are referred to as aggregation pheromones, implying a male-to-male attraction. We describe here the characterization of a male-released sex pheromone of a stink bug, *Piezodorus hybneri* (Heteroptera: Pentatomidae). Interestingly, the semiochemicals elicit a clear precopulatory behavior in males, which in the absence of females is directed toward males.

METHODS AND MATERIALS

Extracts and Isolation of Pheromone. Airborne volatiles from laboratory-raised adult *P. hybneri* (25°C, 16 hr light, 8 hr dark photoperiod) were trapped on a Super Q column (Alltech, Deerfield, Illinois) set in an all-glass aeration apparatus (Leal et al., 1996a). The column was washed with hexane and the extracts concentrated to 0.1 insect equivalent per microliter. Crude male extracts were subjected to flash column chromatography on silica gel (Wakogel C-200, Tokyo, Japan) by successive elution with hexane–ether mixtures in the following order: 100:0, 95:5, 90:10, 80:20, 50:50, 0:100.

Bioassays. The activity of crude extracts and of fractions separated by column chromatography was monitored in a wind tunnel (2 m long × 30 cm ID). Five females per experiment were placed in the downwind end of the tunnel and observed for 15 min at an airflow of 30 cm/sec at 25°C. Behavioral responses of male and female bugs were also observed in a Y-olfactometer (long arm, 24

cm; side arms, 16 cm; 2.6 cm ID; airflow, 300 ml/min). Behavioral data were statistically analyzed with JMP Software, Version 2 (SAS Institute, Cary, North Carolina).

Analytical Procedures. Gas chromatography (GC) was carried out on Hewlett-Packard 5890 II Plus instruments equipped with split/splitless injector, electronic pressure control, flame ionization detector, and HP 3365 Series II Chemstation. High-resolution GC analyses were performed with polar and nonpolar capillary columns (0.25 mm × 0.25 μm). The polar columns, HP-Innowax (30 m) and BP-20 (25 m), were operated at 50°C for 1 min, increased to 150°C at a rate of 4°C/min, held at this temperature for 1 min, increased to 230°C at a rate of 10°C/min, and finally held at this temperature for 10 min. The nonpolar columns HP-5MS and DB-5 (30 m × 0.22 mm × 0.25 μm) were operated at 50°C for 1 min, increased to 180°C at 5°C/min, held for 1 min, increased to 270°C at 10°C/min, and finally held at this temperature for 10 min. Chiral resolution of the enantiomers of 15-hexadecanolide was achieved on a CP-Chirasil-DEX CB capillary column (25 m × 0.25 mm: 0.25 μm, Chrompack), operated at 150°C and with a (helium) head pressure of 1.17 kg/cm^2 (2.39 ml/min; 61.4 cm/sec). Low-resolution mass spectrometry (MS) was carried out with HP 5890 II Plus GCs linked either to a mass selective detector MSD 5972, a Mass Engine 5989B, or a GC electron ionization detector GCD (Hewlett-Packard) and with a HP 5890 GC linked to a Finnigan-MAT (San Jose, California) ion-trap detector 800. Vapor-phase Fourier transform infrared (FTIR) spectra were recorded with a GC HP6890 (Hewlett-Packard) coupled to a light pipe interface, FTS-40A, GC/C32 (Bio-Rad). GC coupled with an electroantennographic detector (GC-EAD) was carried out on a previously described system (Leal et al., 1996b). ^{1}H NMR spectra were recorded on Varian XL-400 and Jeol JNM-A500 spectrometers.

Chemical Derivatizations. Dimethyl disulfide (DMDS) adducts of the monoene methyl ester were obtained by reaction of dimethyl disulfide DMDS (70 μl) with the isolated natural product (20 μl; ca. 10 ng of the natural ester per microliter of sample), catalyzed by iodine. The mixture was stirred for 1 hr at 85°C in a 1-ml conical vial containing a magnetic spin vane and closed with a Teflon-lined cap. After the mixture was cooled to room temperature, hexane (100 μl) was added, and iodine was removed by washing with sodium thiosulfate. The organic phase was dried over anhydrous sodium sulfate, concentrated to 10 μl, and analyzed by GC-MS. Methyl palmitoleate was derivatized by the same procedure as a standard. The macrolactones (ca. 5 μg) were converted to the corresponding hydroxy methyl esters by overnight reaction with boron trifluoride diethyl etherate (8 μl) in methanol (100 μl) at 70°C. After cooling to room temperature and addition of hexane (100 ml), the reaction mixture was washed (water, 2 × 50 μl), dried over anhydrous sodium sulfate, and concentrated. After GC-MS analyses, the mixture was dried under argon and redis-

solved with CH_2Cl_2 (200 μl). Pyridinium dichromate (PDC; 1.5 mg) was added and the mixture was stirred for 50 min. This was diluted with hexane (500 μl) and filtered through a Pasteur pipet loaded with silica gel.

Synthesis. Methyl 8-(Z)-hexadecenoate. 1-Nonyne was coupled with 1-bromo-5-chloropentane to give 1-chloro-6-tetradecyne. The isolated product reacted with the anion obtained by the reaction of diethyl malonate with sodium hydride in dimethyl sulfoxide (DMSO) to yield diethyl 6-tetradecynylmalonate. Hydrolysis of the malonate followed by heating (135°C) gave 8-hexadecynoic acid, which was esterified with boron trifluoride diethyl etherate in methanol to generate methyl 8-tetradecynoate. Its catalytic hydrogenation (5% $Pd/BaSO_4$; quinoline) in methanol gave the desired methyl 8-(Z)-hexadecenoate (43% yield from 1-nonyne).

(R)-15-Hexadecanolide (Asymmetric Methylation). A mixture of cyclopentadecanone (0.69 g, 3 mmol) and (*S*)-(−)-1-amino-2-methoxymethylpyrrolidine (SAMP) (0.4 g, 3 mmol) was heated at 70°C for 20 hr under an argon atmosphere. After cooling to room temperature, the reaction mixture was dissolved in methylene chloride (20 ml) and dried over $MgSO_4$. The solution, after removal of $MgSO_4$ by filtration, was concentrated by vacuum to provide the hydrazone product (1 g, 99%).

To a solution of lithium diisopropylamide (LDA), prepared by addition of *n*-BuLi (1.6 M in hexane, 0.6 ml 1 mmol) to a solution diisopropylamine (200 mg, 2 mmol) in ether (5 ml), was slowly injected the above hydrazone (327 mg, 1 mmol) in ether (1 ml) at 0°C under an argon atmosphere. After stirring for 10 hr at 0°C, the mixture was cooled to ca. −110°C by an ethanol–liquid N_2 bath. Methyl iodide (150 mg, 1.2 mmol) was injected slowly, and the reaction mixture was allowed to stand at this temperature for 2 hr. After the reaction mixture was warmed to 25°C, methyl iodide (1 ml) was added, and the reaction mixture was heated to about refluxing temperature for 20 hr. Removal of the excess methyl iodide gave a brown residue that was further treated with HCl (3 N, 4 ml)–pentane (20 ml) for 3 hr at 25°C. The organic layer was separated and concentrated to give a crude product that was further purified by flash chromatography over silica gel (2% ethyl acetate in hexane) to provide (*R*)-2-methylcyclopentadecanone (64 mg, 27%) and recovered cyclopentadecanone (105 mg).

To a solution of (*R*)-2-methylcyclopentadecanone (10 mg, 0.04 mmol) in methylene chloride (1 ml) was added *m*-chloroperbenzoic acid (20 mg). After heating to ca. 60°C for 48 hr, the reaction mixture was subjected to flash chromatography separation over silica gel (2% ethyl acetate in hexane) to provide the desired product (4.4 mg, 42%).

(R)- and (S)-15-Hexadecanolide (Linear Approach). (*R*)-3-(*t*-Butyldimethylsilyloxy)butanal, obtained in two steps from ethyl (*R*)-3-hydroxybutyrate in 63% yield (*tert*-buthyldimethylsilyl chloride, imidazole, dimethylformamide;

diisobutylaluminium hydride, toluene), was subjected to the Wittig reaction with the ylid prepared by treating a solution of (11-carboxyundecyl)triphenylphosphonium bromide in tetrahydrofuran–hexamethylphosphoramide (4:1) with potassium bis(trimethylsilyl)amide (0.5 M in ether, 2 eq) to give an olefinic product with *Z*-geometry (Dawson and Vasser, 1977). Deprotection (HF, acetonitrile) and catalytic hydrogenation (H_2, Pd-C, EtOH) of the product gave (*R*)-15-hydroxyhexadecanoic acid (62% in three steps). Macrolactonization of the hydroxy acid by the Yamaguchi method (Inanaga et al., 1979) (2,4,6-trichlorobenzoyl chloride, Et_3N, 4-dimethylaminopyridine, toluene) afforded (*R*)-(−)-15-hexadecanolide ($[\alpha]_D^{25}$ − 18.2 (c = 1.2, hexane) (literature $[\alpha]_D^{20}$ = −16.5 (c = 1.029) (Kaiser and Lamparsky, 1978) in 84% yield, while Mitsunobu lactonization conditions (Kurihara et al., 1976) gave rise to the concomitant inversion of configuration to give (*S*)-(+)-15-hexadecanolide in 31% yield ($[\alpha]_D^{25}$ = +18.7 (c = 1, hexane).

Further detail of organic synthesis will be published elsewhere (Kuwahara et al., 1998).

RESULTS AND DISCUSSION

In order to study chemical communication in *P. hybneri*, we collected airborne volatiles from groups of males and females and tested the activity of these extracts in the wind tunnel. Females clearly responded to whole extracts of airborne volatiles from male bugs (36 ± 4% attraction; control 0%; N = 5), but not a single male was attracted to female extracts in comparable tests.

In order to isolate the semiochemicals involved in this chemical communication system, we separated the whole extract of airborne volatiles from males on a silica gel column and monitored the activity of each fraction in a wind tunnel. Females were attracted only to the hexane–ether (90:10 and 95:5) fractions. The response to the former fraction was clear-cut (42 ± 9%; N = 3). Although the attraction of the latter fraction was weaker (7 ± 5%), this fraction elicited wing fanning in females (45 ± 10%), a precopulatory behavior.

GC-EAD experiments showed electrophysiological activity of two male-specific compounds that were eluted in the hexane–ether (90:10) fraction. Interestingly, both male and female antennae responded to these semiochemicals. In the course of this study, one of us (H.H.) observed in field experiments with caged *P. hybneri* that males induced aggregation of both males and females (Higuchi, 1996). In our indoor bioassays, males also responded to the same semiochemicals involved in male-female attraction.

The two male-specific EAD-active compounds [retention times (R_t) 18.46 and 18.74 min] appeared only in the hexane–ether (90:10) fraction, whereas the other biologically active fraction [hexane–ether (95:5)] also had a male-specific compound (R_t 14.75 min), which did not give any EAD response.

Considering that these three compounds were male-specific, we decided to identify all of them and to assess biological activity of synthetic samples.

The compound with R_t 14.75 min gave a MS with the base peak at *m/z* 69 and the molecular ion at 204 (12%), and matched closely with the spectrum of (1′*R*, 6*S*)-2-methyl-6-(4′-methylenecyclohex-2′-enyl)-hept-2-ene (β-sesquiphellandrene) in the Wiley library. Other fragments were: 41 (59%), 55 (25), 77 (34), 91 (49), 93 (60), 109 (23), 120 (25), 133 (26), and 161 (32). A pure sample of this sesquiterpene from the oil of gingiber, *Zingiber officinale* (Connell and Sutherland, 1966), was indistinguishable from the natural product in terms of retention time as well as the MS profile. Although we did not determine analytically the absolute configuration of the bug-derived sesquiterpene, it is likely that it has the same stereochemistry as the plant-derived β-sesquiphellandrene (see bioassay below).

The MS [base peak, 55; 41 (75%), 43 (56), 69 (61), 74 (75), 87 (48), 97 (41), 110 (23), 123 (17), 137 (11), 141 (8), 152 (14), 194 (17), 236 (14), 237 (12), and M^+ 268 (3)] and IR [$\nu_{C=O}$, 1760 cm^{-1}, medium; ν_{CH} 2937 strong; $\nu_{=CH}$ (*cis*), 3013, weak] data for the EAD-active peak at 18.74 min suggested that the natural product was a methyl hexadecenoate, with the double bond in *cis* configuration. Although these data were similar to those of methyl palmitoleate, the two compounds showed slightly different retention times. DMDS derivatization of the pheromone, followed by MS analyses showed that these two compounds were in fact isomers. The DMDS adduct of the semiochemical gave a MS with the base peak at *m/z* 203, an outstanding peak at 159, and the molecular ion peak at 362. Interpretation of these data suggests that the original double bond was located in position 8. A sample of synthetic methyl 8-(*Z*)-hexadecenoate was identical to the natural product in the MS, IR, and GC data; the synthetic ester was also biologically active (see below).

Finally, the MS and IR data for the second EAD-active compound (R_t 18.46 min) suggested that it was either 15- or 16-hexadecanolide. MS data: base peak, 105; 41 (83%), 43 (54), 69 (53), 71 (32), 83 (42), 97 (41), 111 (22), 125 (13), 152 (11), 192 (9), 194 (7), 210 (10), 236 (17), and M^+ 254 (2). IR data: $\nu_{C=O}$ 1747 cm^{-1}, medium; ν_{CH} 2937, strong. Derivatization of this macrolactone with boron trifluoride diethyl etherate in methanol gave a product with a peak at *m/z* 45, suggesting the occurrence of a secondary alcohol in the product. This was confirmed by further oxidation of this derivative product with pyridinium dichromate, which gave a peak at *m/z* 58 (McLafferty rearrangement). This evidence for the presence of a methyl ketone moiety suggested that the original macrolactone would be 15-hexadecanolide. A sample of this macrolactone was prepared by asymmetric methylation of a hydrazone obtained by condensation of cyclopentadecanone with (*S*)-(−)-1-amino-2-(methoxymethyl)pyrrolidine (SAMP), followed by hydrazone cleavage and Baeyer-Villiger

oxidation of the resulting 2-methylcyclopentadecanone. The synthetic and natural macrolactones were indistinguishable by GC, MS, and IR.

The method of chiral amplification (Shi et al., 1996) was used to determine the absolute configuration of the natural product. The original macrolactone was derivatized with trifluorobromide in methanol to generate a related secondary alcohol. Diastereoisomers of the methyl 15-hydroxyhexadecanoate were obtained by reaction with (*R*)- and (*S*)-α-methoxy(α-trifluoromethyl)phenylacetyl chloride (Mosher's reagent). Unfortunately, diastereoisomeric resolution was not achieved on GC, probably due to the high molecular weight, and, consequently, long retention time. This problem could be overcome by the use of high-performance liquid chromatography (HPLC) or by the resolution of the macrolactones on a chiral column.

Enantiomeric resolution was achieved on a CP-Chirasil-DEX CB capillary column. The synthetic lactone obtained by asymmetric methylation as the key step showed a low enantiomeric purity (Figure 1A and B). The *R* enantiomer

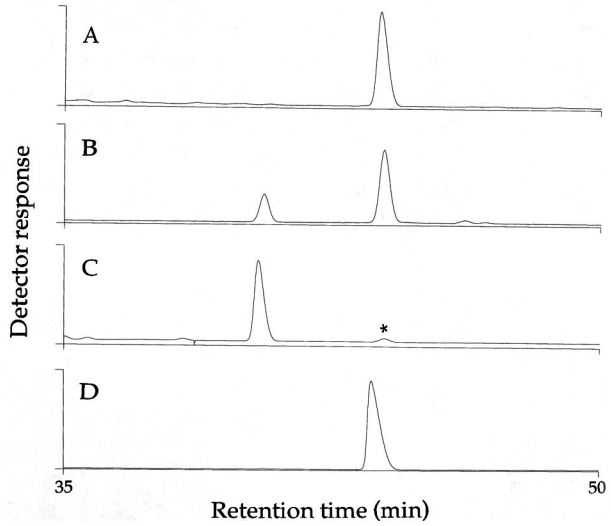

FIG. 1. Enantiomeric resolution of 15-hexadecanolide on a CP-Chirasil-DEX CB capillary column at 150°C. The natural product (A) gave a single peak that corresponded to the major peak of the synthetic sample (B) that gave predominantly (*R*)-15-hexadecanolide. (*S*)-15-Hexadecanolide obtained by an unambiguous synthesis (C) confirmed the identity of the earlier eluting peak. The chemical impurity (*) was removed by further purification on a silica gel column. (D) Enantiomerically pure (*R*)-15-hexadecanolide obtained by a linear approach.

gave the same retention time (R_t 43.93 min) as the natural product whereas the *S* stereoisomer appeared at 40.56 min.

Enantiomerically pure stereoisomers were prepared by Yamaguchi or Mitsunobu macrolactonization of (*R*)-15-hydroxyhexadecanoic acid, which was obtained from ethyl (*R*)-3-hydroxybutyrate via the Wittig chain-elongation reaction. As demonstrated by chiral GC (Figure 1 C and D), both enantiomers were obtained in high optical purity.

In the olfactometer bioassay, both males (Figure 2A) and females (Figure 2B) were significantly attracted to a mixture of β-sesquiphellandrene, (*R*)-15-hexadecanolide, and methyl 8-(*Z*)-hexadecenoate (500 ng of a 10:4:1 mixture). Interestingly, males were not only attracted to the pheromone source, but they also displayed a clear precopulatory behavior. While walking towards the pheromone source, males mounted other males they encountered and tried to effect copulation. When males were released individually, they displayed a sequence of behavior in response to the pheromone: walking towards the pheromone source, searching around the filter paper impregnated with these compounds, and exposing the pygophor (Figure 3).

In order to investigate whether all these semiochemicals are involved in chemical communication of *P. hybneri*, bioassays were conducted in the olfactometer with all combinations of the three compounds. Males were attracted more strongly to the full mixture than to any binary combination or individual component (Figure 4A). Although (*R*)-15-hexadecanolide was not attractive per se, it is required for the optimal male response. The precopulatory behavior (exposure of male's pygophor) on the other hand, is elicited even by individual components with responses equivalent to the full mixture (Figure 3B). The

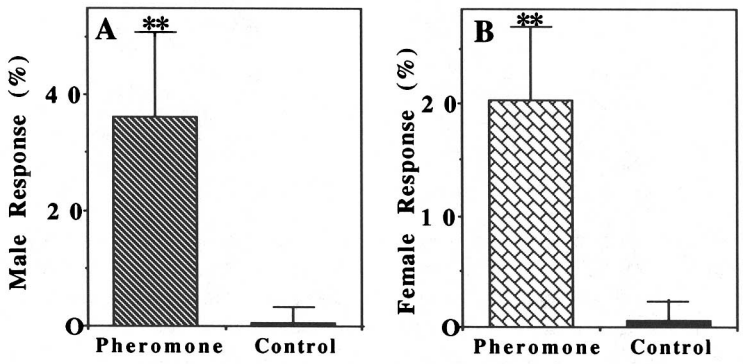

FIG. 2. Responses of *P. hybneri* males (A, $N = 13$) and females (B, $N = 8$) to the synthetic pheromone mixture (ca. 2 male equivalents) in the olfactometer. $^{**}P < 0.01$ (*t* test).

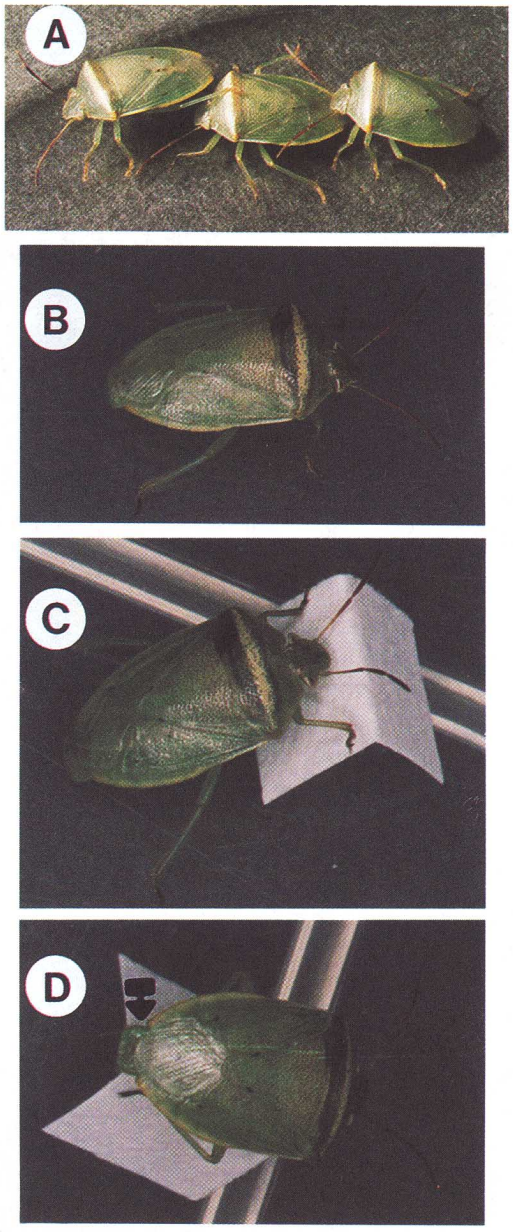

FIG. 3. Responses of *P. hybneri* males elicited by the male-released sex pheromone. (A) Males trying to mount other males they encountered en route to the pheromone source. (B) Male walking towards the pheromone source, (C) touching the pheromone-laden filter paper and starting searching behavior, and (D) showing a precopulatory behavior by exposure of the pygophor (arrow).

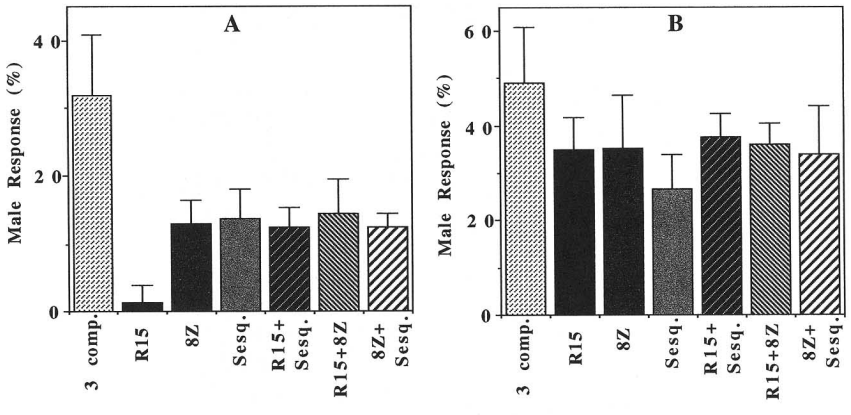

FIG. 4. Behavioral responses of *P. hybneri* males to various combinations of the semiochemicals isolated from bugs of the same sex ($N = 4$ for each candidate lure). (A) Attraction in the olfactometer and (B) precopulatory behavior characterized by the exposure of pygophor.

nonnatural (*S*)-15-hexadecanolide did not elicit any response in males, but it was not a behavioral antagonist. Male responses to the full mixture containing the (*R*)-15-hexadecanolide were not significantly different from those displayed when the sample was spiked with an equimolar amount of the *S* enantiomer, i.e., a racemic mixture of the lactone (Figure 5A and B).

In conclusion, the sex pheromone system for the stink bug *P. hybneri* is composed of at least three constituents, β-sesquiphellandrene, methyl 8-(*Z*)-hexadecenoate, and (*R*)-15-hexadecanolide, that are released by males in a 10:4:1 ratio. The observation that *P. hybneri* males responded to the male-released sex pheromone system with a clear precopulatory behavior sheds light on the evolution of chemical communication in this group of insects and strongly suggests that referring to semiochemicals that attract both sexes as aggregation pheromones may be misleading.

Because laboratory bioassays with true bugs often do not consistently yield any quantifiable orientation behavior, one is normally tempted to carry out field tests with candidate chemicals and look at the overall response in terms of catches in pheromone-baited traps (in comparison to control traps) (Aldrich et al., 1984, 1991; Leal et al., 1995, 1996c; Sugie et al., 1996). It seems that the male–male sexual behavior elicited by male-released pheromones has not been observed before in other hemipterans because no one looked for it. This behavior, which was first observed in aposematic beetles, *Lycus loripes* (Eisner and Kafatos, 1962), strongly suggests that the semiochemicals are involved in sexual recruitment.

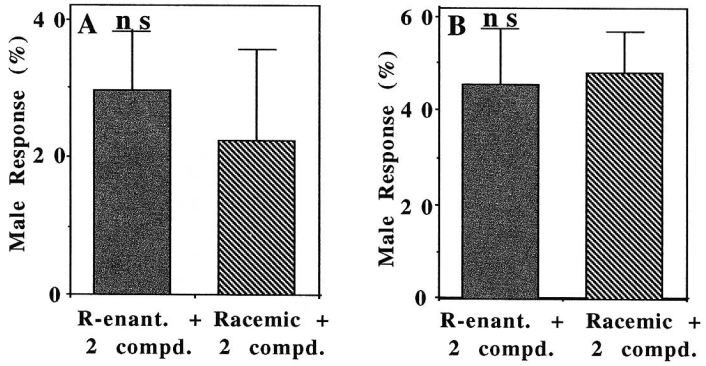

FIG. 5. Effect of 15-hexadecanolide stereochemistry on male responses: (A) attraction, (B) precopulatory behavior. (ns, not significant by Wilcoxon/Kruskal-Wallis test; $N = 5$).

In various species for which females are the pheromone-emitters, sex pheromone may elicit homosexual behavior. Males responding to synthetic chemicals try to copulate with each other in the absence of females. The fact that the male-released pheromone of *P. hybneri* elicited male–male sexual behavior suggests that the semiochemicals are sex pheromone constituents. In addition, the onset of pheromone production in *P. hybneri* males is coincident with the ovarian development in females. Pheromone production starts with 6- to 8-day-old bugs (40 ng/male/day), increases to 250 ng/male/day (at 13 days old) and remains at this level throughout the observation time (up to 25 days old). At 26°C and under the same light conditions at which we collected the airborne volatiles from males, oviposition of *P. hybneri* starts six days after emergence (or at seven days at 24°C) (A. Kikuchi, personal communication).

It is not known how males evolved the ability to respond to their own semiochemicals, but it is likely that responding males are exploiting the signal emitted by other males for the recruitment of females. This scenario may be an example of an alternative mating strategy for males to pool their pheromonal resource in order to increase their individual chances of getting a mate. It would be interesting to pursue further studies on the chemical communication of *P. hybneri* to address the questions of whether any one male could be either a "caller" or a "joiner" at different times in the field, the factors that determine which strategy will be pursued, and the comparative mating success of a joiner versus a caller.

Acknowledgments—The NISES component of this research was supported by a special coordination fund for promoting science and technology by the Science and Technology Agency, Japan, and by the Program for Promotion of Basic Research Activities for Innovative Biosciences (BRAIN).

During his sabbatical leave at Cornell, W. S. L. held a Johnson & Johnson fellowship in chemical ecology. The partial support of this research by NIH grant GM 53830 is acknowledged. S. B. Krasnoff reviewed an earlier version of this manuscript.

REFERENCES

ALDRICH, J. R., KOCHANSKY, J. P., and ABRAMS, C. B. 1984. Attractant for a beneficial insect and its parasitoids: Pheromone of the predatory spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae). *Environ. Entomol.* 13:1031–1036.

ALDRICH, J. R., HOFFMANN, M. P., KOCHANSKY, J. P., LUSBY, W. R., EGER, J. E., and PAYNE, J. A. 1991. Identification and attractiveness of a major pheromone component for Nearctic *Euschistus* spp. stink bugs (Heteroptera: Pentatomidae). *Environ. Entomol.* 20:477–483.

ALDRICH, J. R., OLIVER, J. E., LUSBY, W. R., KOCHANSKY, J. P., and BORGES, M. 1994. Identification of male-specific volatiles from Nearctic and Neotropical stink bugs (Heteroptera: Pentatomidae). *J. Chem. Ecol.* 20:1103–1111.

BAKER, R., BORGES, M., COOKE, N. G., and HERBERT, R. H. 1987. Identification and synthesis of (*Z*)-(1′*S*,3′*R*,4′*S*)-(−)-2-(3′,4′-epoxy-4′-methylcyclohexyl)-6-methylhepta-2,5-dione, the sex pheromone of the southern green stinkbug, *Nezara viridula* (L.). *J. Chem. Soc. Chem. Commun.* 1987: 414–416.

BORDEN, J. H. 1985. Aggregation pheromones, pp. 257–285, *in* G. A. Kerkut and L. A. Gilbert (eds.). Comprehensive Insect Physiology, Biochemistry and Pharmacology, Pergamon Press, Oxford.

CARDÉ, R. T., and BAKER, T. C. 1984. Sexual communication with pheromones, pp. 355–383, *in* W. J. Bell and R. T. Cardé (eds.). Chemical Ecology of Insects, Chapman and Hall, New York.

CONNELL, D. W., and SUTHERLAND, M. D. 1966. Terpenoid chemistry. XI. (−)-β-Sesquiphellandrene. *Aust. J. Chem.* 19:283–288.

DAWSON, M. I., and VASSER, M. 1977. Synthesis of prostaglandin synthetase analogues. 1. (*Z*)-14-Hydroxy-12,13-methano-8-nonadecenoic acid. *J. Org. Chem.* 42:2783–2785.

EISNER, T., and KAFATOS, F. C. 1962. Defense mechanisms of arthropods. X. A pheromone promoting aggregation in an aposematic distasteful insect. *Psyche* 69:53–61.

HIGUCHI, H. 1996. Male-attraction in *Piezodorus hybneri*. Proceedings, 40th Annual Meeting of the Society of Applied Entomology and Zoology of Japan, p. 119 (in Japanese).

INANAGA, J., HIRATA, K., SAEKI, H., KATSUKI, T., and YAMAGUCHI, M. 1979. A rapid esterification by means of mixed anhydride and its application to large-ring lactonization. *Bull. Chem. Soc. Jpn.* 52:1989–1993.

KAISER, VON R., and LAMPARSKY, D. 1978. Neue macrolide und einige Sesquiterpen-Derivate aus dem *Galbanum*-Harz. *Helv. Chim. Acta* 61:2671–2680.

KURIHARA, T., NAKAJIMA, Y., and MITSUNOBU, O. 1976. Synthesis of lactones and cycloalkanes. Cyclization of ω-hydroxy acids and ethyl α-cyano-ω-hydroxycarboxylates. *Tetrahedron Lett.* 2455–2458.

KUWAHARA, S., TSURUTA, T., LEAL, W. S., and KODAMA, O. 1998. Synthesis of both enantiomers of 15-hexadecanolide, a sex pheromone component of the stink bug, *Piezodorus hybneri*. *Biosci. Biotechnol. Biochem.* 62:1261–1263.

LEAL, W. S., HIGUCHI, H., MIZUTANI, N., NAKAMORI, H., KADOSAWA, T., and ONO, M. 1995. Multifunctional communication in *Riptortus clavatus* (Heteroptera: Alydidae): Conspecific nymphs and egg parasitoid *Ooencyrtus nezarae* use the same adult attractant pheromone as chemical cue. *J. Chem. Ecol.* 21:973–985.

LEAL, W. S., HASEGAWA, M., SAWADA, M., ONO, M., and TADA, S. 1996a. Scarab beetle *Anomala*

albopilosa albopilosa utilizes a more complex sex pheromone system than a similar species *A. cuprea*. *J. Chem. Ecol.* 22:2011–2020.

LEAL, W. S., KUWAHARA, S., ONO, M., and SAKAE, K. 1996b. (*R*, Z)-7,15-Hexadecadien-4-olide, sex pheromone of the yellowish elongate chafer, *Heptophylla picea*. *Bioorg. Med. Chem.* 4:315–321.

LEAL, W. S., UEDA, Y., and ONO, M. 1996c. Attractant pheromone for male rice bug, *Leptocorisa chinensis*: Semiochemicals produced by both male and female. *J. Chem. Ecol.* 22:1429–1437.

OLIVER, J. E., ALDRICH, J. R., LUSBY, W. R., WATERS, R. M., and JAMES, D. G. 1992. A male-produced pheromone of the spined citrus bug. *Tetrahedron Lett.* 33:891–894.

ROELOFS, W. L. 1995. The chemistry of sex attraction, pp. 103–117, *in* T. Eisner and J. Meinwald (eds.). Chemical Ecology: The Chemistry of Biotic Interaction. Natioal Academy Press, Washington, D.C.

SHI, X., LEAL, W. S., and MEINWALD, J. 1996. Assignment of absolute stereochemistry to an insect pheromone by chiral amplification. *Bioorg. Med. Chem.* 4:297–303.

SUGIE, H., YOSHIDA, M., KAWASAKI, K., NOGUCHI, H., MORIYA, S., TAKAGI, K., FUKUDA, H., FUJIE, A., YAMANAKA, M., OHIRA, Y., TUTSUMI, T., TSUDA, K., FUKUMOTO, K., YAMASHITA, M., and SUZUKI, H. 1996. Identification of the aggregation pheromone of the brown-winged green bug, *Plautia stali* Scott (Heteroptera: Pentatomidae). *Appl. Entomol. Zool.* 31:427–431.

THORNHILL, R., and ALCOCK, J. 1983. The Evolution of Insect Mating Systems, Harvard University Press, Cambridge, Massachusetts, pp. 145–181.