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# SHORT NOTE

# SEX PHEROMONE OF THE BROWN COCKROACH, *PERIPLANETA BRUNNEA* BURMEISTER[1]

## I. Isolation

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**H. Y. Ho, H. T. Yang, R. Kou and Y. S. Chow** (1992) Sex pheromone of the brown cockroach, *Periplaneta brunnea* Burmeister. I. Isolation. *Bull. Inst. Zool., Academia Sinica* **31**(3): 225-230. The midguts of 102 unmated females and 102 unmated males of the brown cockroach, *Periplaneta brunnea*, were dissected; the sex pheromone isolated from these midguts was analyzed using gas chromatography-mass spectrometry (GC/MS). By comparing GC/MS data from the hexane extract of the midguts, the peak occurring only in the female extract (which induced sexual responses toward male adults) was tentatively identified. Its structure was proposed as being (2Z, 6E, 8S)—1(14), 2, 5(15),6—germacratetraene, and it was given the name periplanene-Br (PeBr).

**Key words:** *Periplaneta brunnea*, Sex pheromone, Gas chromatography-mass spectrometry (GC/MS), (2Z, 6E, 8S)—1(14),2,5(15),6—Germacratetraene, Periplanene-Br (PeBr).

The sexual behavior of cockroaches in the genus *Periplaneta* has been established by several investigators who suggested the existence of volatile sex pheromones in female cockroaches (Roth and Willis, 1952; Schafer, 1977). However, identification of the chemical structure of these pheromones has only been made for two cockroaches: the American cockroach, *Periplaneta americana* (L.), whose pheromones were named periplanone—A (PA), periplanone—B (PB), periplanone—C (PC), periplanone—D (PD) (Persoons *et al.*, 1990 and references therein); and the Japanese cockroach, *Periplaneta japonica* Karny, whose pheromone was identified and named as periplanone—J (PJ) (Takegawa and Takahashi, 1989).

The brown cockroach, *Periplaneta brunnea* Burmeister, shares biological similarities in form, coloration, habitat, and body size with *P. americana*. The existence of a female sex pheromone in this cockroach was reported by Schafer (1977), and its electroantennogram (EAG) response was documented by Nishino and Manabe (1985); however, there has been no previous chemical identification of the sex pheromone of this cockroach. In this

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report, the sex pheromone of *P. brunnea* has tentatively been designated as periplanene—Br (PeBr) according to a comparison of GC/MS data from female and male extracts.

## MATERIALS AND METHODS

### Insects

Colonies of *P. brunnea* were reared under normal room temperature, relative humidity (RH), and photoperiod conditions. Cockroaches were fed water and commercial dry dog food *ad lib.* Males and females were separated after ecdysis to the final nymphal stage. Females were reared under normal conditions, but males were reared in a controlled environment (26±2°C, 55±5% RH, 16 hr day/8 hr night photoperiod).

### Behavioral test

A total of twenty one-month-old unmated male brown cockroaches housed in a 30×30×30 cm glass container were used for our behavioral tests. Using a glass dropper, testing samples of approximately 0.001 FE in dosage were continuously added inside the container for 1 min. The sample which induced sexual responses in the males was chosen for further chemical separation and analysis. A dramatic increase in physical activity, with 15 out of 20 gathering at the point where the sample was introduced, and 1 out of 20 showing wing raising, were considered as giving a positive response.

### Purification of the sex pheromone of *P. brunnea*

The midguts of 102 virgin female and 102 unmated male cockroaches were dissected and separately immersed in hexane for about 30 minutes. The hexane extracts were then concentrated with a rotoevaporator; 101 mg samples from males and 153 mg samples from females were collected and subjected to column chromatography on 430 g silica gel (Merck silica gel 60 mesh 70-230). Thirty milliliters each of the following solvents were used: 100% hexane, 5% ether in hexane, 10% ether in hexane, 20% ether in hexane, 30% ether in hexane, 40% ether in hexane, 50% ether in hexane, and 100% ether.

High-performance liquid chromatography was conducted using a Shimadzu 6A HPLC fitted with a Merck LiChrospher Si-60 column (250×4 mm) eluted with 2% ethyl acetate in hexane at 2 ml/min. The GC/MS analysis was conducted using a Finnigan—MAT INCOS 50 coupled with a Varian GC 3400 using fused silica capillary column DB-5 (30 m×0.25 mm I. D.). Operating conditions were as follows: column temperature—50°C holding for 5 min., then 50°-280° at 8°C/min.; ionization voltage—70 ev; ion source temperature—180°C.

Hydrogenation was carried out by adding palladium on charcoal as a catalyst to the sample, then bubbling the solution with $H_2$ gas for about 5 min.

## RESULTS AND DISCUSSION

Hexane extracts from the midguts of female *P. brunnea* induced sexual responses in males but the male extract did not induce sexual responses from tested males. After separation by column chromatography, the fractions of female extract eluted at 40% ether in hexane elicited responses from male cockroaches. These and corresponding fractions of male extract were collected and concentrated, then approximately 80 mg of female and 45 mg of male extract were collected. Further purification was carried out through high performance liquid chromatography (HPLC). Fractions (2ml) were collected, as were the most active


Fig. 1. Photographs of male sexual response of the brown cockroach showing wing—raising behavior (arrow) in sequence. A. begin raising; B. half raising; C. fully raising and exposing its abdomen.

female extract fractions were; corresponding fractions of male extract were also combined, then both fractions were subjected to separate GC/MS analyses. Although the GC/MS results showed more than 30 peaks, those peaks showing identical retention times and mass spectra in both the female and male extracts were not considered to be sex pheromone. After screening according to these criteria, one peak which only occurred in the female extract was identified as

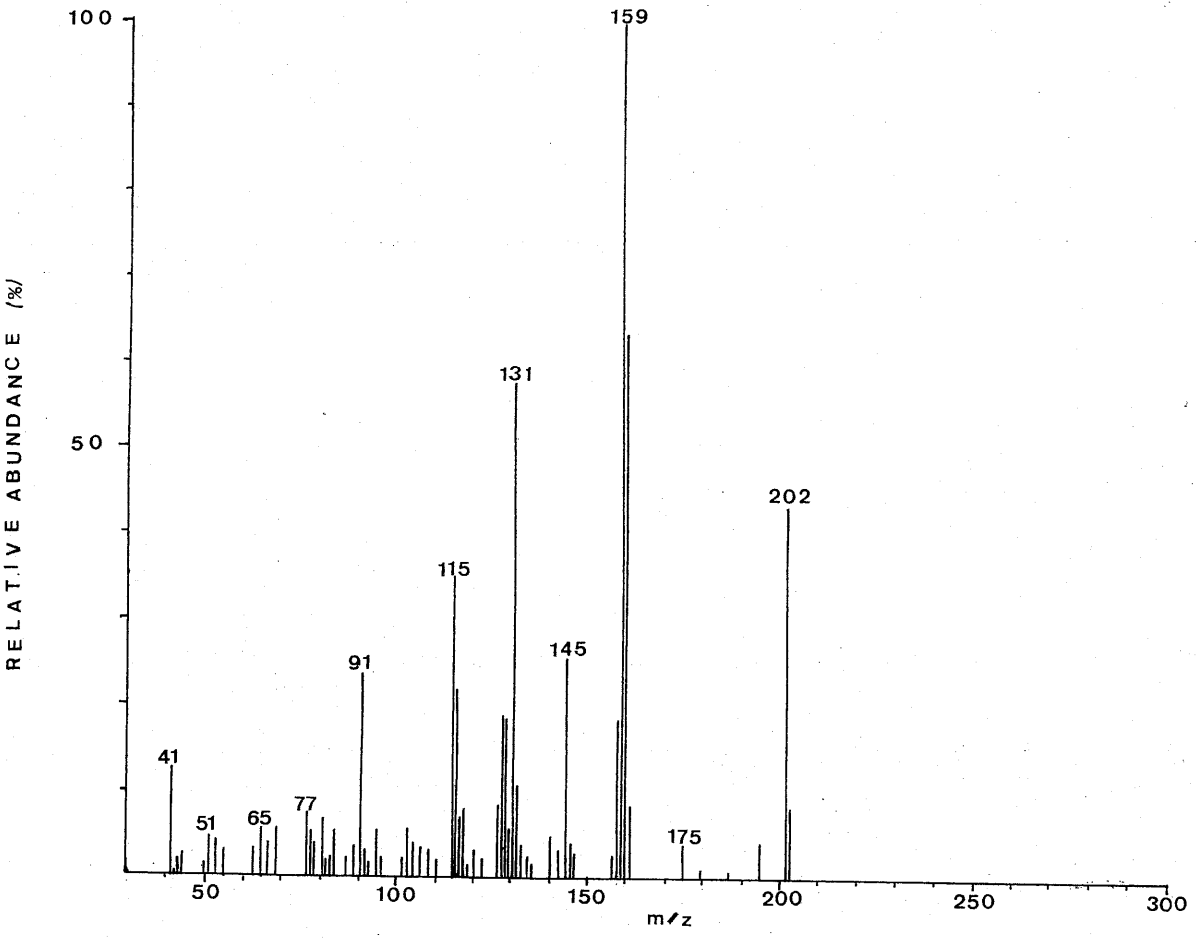



Fig. 2. Mass spectrum of periplanene—Br.

the sex pheromone of *P. brunnea* and named as periplanene—Br (PeBr).

Further confirmation that this peak was the sex pheromone of the brown cockroach was made by means of the following: first, GC/MS analysis of the inactive female extract fractions showed that there was no PeBr peak. Second, the fact that the active fraction of female extract lost its effectiveness after hydrogenation with $H_2$/Pd indicated that there was double bond unsaturation in the sex pheromone of *P. brunnea*. Finally, the disappearance of the PeBr peak according GC/MS analysis of the hydrogenated products of the active fraction taken from the female extract was further evidence that the peak was indeed the sex pheromone of *P. brunnea*.

The electron impact (EI) mass spectrum of PeBr is shown in Fig. 2. An apparent molecular ion ($M^+$) was observed at m/z 202. The profile of the spectrum of this compound is quite similar to that of germacrene—D. The relative intensities of the prominent fragment ion peaks of this compound at m/z 159, 145, 131 are similar to those of germacrene—D with only a two mass unit difference. The fact that the molecular ion peak of this compound (m/z 202) is only two mass units less than that of germacrene—D (m/z 204) indicates that this compound has one more double bond unsaturation than germacrene—D. The retention times for germacrene—D, PA, PB, and PeBr were

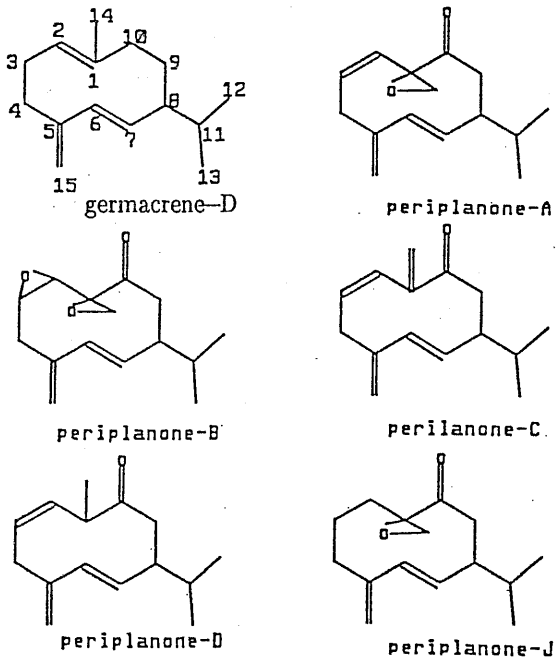
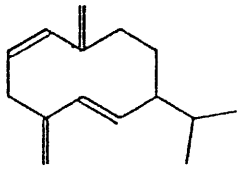



Fig. 3. Structures of germacrene—D, periplanone—A, periplanone—B, periplanone—C, periplanone—D, and periplanone—J (ref. Persoons, C. J. *et al.* 1990 and Takegawa, H. and S. Takahashi, 1989).

20′26″, 23′46″, 25′32″, and 23′54″ on column DB—5 under the conditions described in the material and methods section, respectively.

Nishino and Manabe (1985) reported that the sex pheromone of *P. brunnea* has a very close relationship with PB by EAG. Since *P. brunnea* are attracted by PB (Takahashi *et al.*, 1988b) and PA (Takahashi *et al.*, 1988a), and because our present results show that the retention times of PA and PeBr are quite similar, we propose that the sex pheromone of the brown cockroach has a similar structure as that of *P. americana*. After comparing the structures of germacrene—D with PA, PB, PC, PD and PJ (Fig. 3), we tentatively assigned the unsaturated double bonds of PeBr between C1, 14; C2, 3; C5, 15; and C6, 7. The structure of PeBr is proposed as being (2Z, 6E, 8S)—1(14),2,5(15),6—germacratetraene.



periplanene—Br

Our laboratory is currently working on further purification of this peak from the hexane extract of midguts, a bioassay, and EAG response.

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# 棕色蜚蠊 *Periplaneta brunnea* Burmeister 性費洛蒙之研究

## I. 分　　離

賀孝雍　楊琇婷　寇　融　周延鑫

由棕色蜚蠊雌雄各 102 隻，分別用正已烷萃取其中腸成分中具有性反應者。經由氣相層析質譜分析法比較雌雄蜚蠊之成分異同，發現其中一個成分只雌蟲具有，提純後對雄性表現生物活性反應。經初步鑑定，其化學構造爲 (2Z, 6E, 8S)-1(14),2,5(15),6-germacratetraene，命名爲 periplanene-Br (PeBr)。