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SEX PHEROMONE OF THE AMERICAN COCKROACH, *PERIPLANETA AMERICANA* (L.).

I. ISOLATION TECHNIQUES AND ATTRACTION TEST FOR THE PHEROMONE IN A HEAVILY INFESTED ROOM.

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ABSTRACT

Y. S. Chow, Y. M. Lin, M. Y. Lee, Y. T. Wang and C. S. Wang (1976). *Sex Pheromone of the American Cockroach,* Periplaneta americana *(L.). I. Isolation Techniques and Attraction test for the Pheromone in a Heavily Infested Room.* Bull. Inst. Zool., Academia Sinica, **15**(2): 39-45 In a rice storage room, crude sex pheromone extract of female American cockroach, D-bornyl acetate and margarine oil can be used as attractants to the American cockroach. Except sex pheromones which are very attractive to adult males, D-bornyl acetate and margarine oil are attractive to nymphs and adults of both sexes. The chromatographic and some chemical properties of the sex pheromones were investigated and it was proved that there are more than 2 components consisting in the sex pheromones of American cockroach.

American cockroach, *Periplaneta americana* (L.), is an important pest of human habitations. In addition to actual spoilage, the insect is regarded as a vector of many diseases, causing deteriation for food, warehouses and homes. For the past 3 decades, people have relied heavily on synthetic organic compounds to control the roach and these synthetic compounds are very effective and widely used by people in many parts of the world. However overuse has led to environmental pollution, resulting in dangerous effect to living organisms. Thus, there is an urgent need for control systems that rely less on pesticides and more on other methods.

The sex pheromone of the American cockroach was first demonstrated by Wharton *et al.*[11] and was identified as 2, 2-dimethyl-3-isopropylidenecyclopropyl propionate by Jacobson *et al.*[5]. Later, Day and Whiting[3] synthesized this compound which was not biologically active when they tested with male roaches. Then Jacobson and Beroza[6] withdrew the structure and the subject was open again. Recently, Bowers and Bodenstein[1] proved that D-bornyl

acetate and B-santalal can cause partial male response at proper concentration. By using new techniques such as gas chromatography and mass spectrometry, Persoons *et al.*[8] have identified 2 elementary formulas $C_{15}H_{20}O_2$ and $C_{15}H_{20}O_3$, for the male excitants of the American cockroach. In Japan, Tahara *et al.*[10] also have isolated a cockroach sex stimulating compound germacrene Dl from plant. Although many scientists have investigated this subject intensively, the precise chemical structure of the compound has not been identified. In our laboratory, D-bornyl acetate has been synthesized and its response to the male roach has been tested. Comparing the activity of D-bornyl acetate with that of the true sex pheromone isolated from female roach, differences between the two were found. The purpose of this study was therefore aimed toward the investigation to find new isolation techniques of the pheromones and its biological activity in a heavily infested room.

MATERIALS AND METHODS

A. Attraction experiments of the synthetic chemicals, margarine oil and sex pheromone to the male roaches in Hwa-Shan rice barn:

In the first experiment, 2 grams of vaseline were applied to the inner entrance of the 2000ml flask to prevent the trapped roaches from excaping out of the flask. This flask was also used as a control group. Two-gram margarine oil, 10 μg synthetic D-bornyl acetate, 2-gram Lai-Lai-Lai oil like material (a merchandised product from Japan) and crude sex pheromone extract of adult females (7 F.E.) were mixed in the vaseline respectively and each mixture was applied to the neck of the individual flask. All flasks were placed in a corner of a heavily infestated house. The opening of the flask was directly contacted with the rough surface of the wall then roaches can easily go into the flask. The number of the trapped roaches was recorded every 3-4 days.

In the second experiment, margarine oil and D-bornyl acetate were tested alone and in combination to see if there is any synergistic effect. D-bornyl acetate was dissolved in margarine oil at a concentration of 20 ppm. Each time, two grams of this oil was used as an attractant and 2 gram margarine oil was used as control group.

B. Bioassay techniques:

Bioassay of the sex pheromone to the male roaches was carried out in a laboratory darkroom. The roaches were sexed first and only the adult males were placed in the woody box (30×30×30 cm) about 50-60 each. To reverse the biological clock of the male roaches, an electric timer-switch was used to control the light condition of the darkroom. The pheromone (0.1-10 F.E.) was applied to a tip of a pipette, when bioassayed, the pipette was puffed several times to the male under redlight in dark during office hours. If there is a fluttering of wings, or homosexual behavior, the response was recorded as percentage of the tested insects. The bioassay was repeated every 2 hours after the light was turned off. The bioassay technique of gas-chromatography was identical to previous one[2].

C. Isolation techniques of the female sex pheromone in laboratory:

Adult females collected from Hwa-Shan barn were dipped in methylene chloride and stored in a refrigerator. When extracting the pheromone, the whole bodies of the female roaches were thoroughly ground in a laboratory homogenizer. The liquid portion was filtrated and the residue was re-extracted with fresh methylene chloride. The Extracts were combined and concentracted to a definite volume. Then, the residue was applied to the top of a silicic acid column and the column was eluted with different percentage of ethyl ether in *n*-hexane according to previous method[2]. Biological active fraction from the above column was injected into a silicic acid column of a high pressure liquid chromatograph (Varian 4000), using a mixture of *n*-hexane, dichloromethane and methanol (90:10:2 V/V/V) as eluting solvent. Each of the eluted fractions was collected and bioassayed. The positive fraction was developed on a small

TLC. plate (7.4×4.0 cm) with *n*-hexane:benzene (1:2, V/V) as solvent system. Each spot was extracted with ethyl ether and bioassayed in the darkroom. The active fraction from high speed liquid chromatograph was injected into a gas-liquid-chromatograph, the retention times of the corresponding sex pheromone of different columns were also recorded as before[2]. The active fraction was reacted with many chemical reagents and the nature of this part would be described in detail in the next section.

RESULTS AND DISCUSSION

A. Attraction experiments:

In the room 4 of Hwa-Shan barn, a total of 1266 roaches have been trapped from August to December, 1975. The actual data were listed in Table I. And from these data, pure vaseline and Lai-Lai-Lai oil were not attractive to roaches and could be considered as our control groups. Margarine oil or D-bornyl acetatate alone was slightly attractive to roaches, especially to those of female adults and nymphs. The most attractive agent in this experiment was found to be female crude extract. This crude extract captured 41.5% of the total attracted roaches and by contrast to margarine oil and D-bornyl acetate, could trap a large number of adult male roaches (61.0%).

In the second experiment, the results presented in Table II, showed that there is a synergistic effect of D-bornyl acetate and margarine oil. When D-bornyl acetate was added to margarine oil, the total number of trapped insects increased from 3094 to 4749 in 19 replications, but the percentages of the trapped different stages (nymphs, adult males or females), were not statistically significant. Because the percentage of males trapped with D-bornyl acetate was 10% (Table II) which was too low comparing with that of crude sex pheromone of Table I (61%), D-bornyl acetate has no sex pheromone function. Since D-bornyl acetate and margarine oil were considered as a food attractant or a gregarious chemical, possibly they could be used as a survey tool for population density analysis. The data accumulated from May to August of 1975 are drawn in Fig. I. It is evident that the adult population increased from May to August whereas the nymph decreased at that period. One complete generation of American cockroach

TABLE I.

The attracted number of American cockrocach by female extract and chemicals from August to December of 1975 in Hwa-Shan rice barn.

Attractants	Vaseline (2 g)			Margarine (2 g) add Vaseline (2 g)			D-Bornyl AC (10 μg) add Vaseline (2 g)			Lai Lai Lai (2 g) add Vaseline (2 g)			Female ex. 7F. E. add Vaseline (2 g)		
	♂	♀	nymph	♂	♀	nymph	♂	♀	nymph	♂	♀	nymph	♂	♀	nymph
No. of attracted roach	19	22	24	54	58	50	120	231	89	8	6	7	355	133	90
Total number	65			162			440			21			578		
Repeats	13			5			13			8			13		
Mean	1.5	1.7	1.8	10.8	11.6	10	9.2	17.7	6.8	1	0.8	0.9	27.3	10.2	6.9
	5.0			32.4			23.7			2.7			44.4		
Percentage of ♂. ♀. & nymph	29	34	37	33	36	31	27	53	20	38	29	33	61	23	16
Total (%) percentage	4.6			29.5			21.9			2.5			41.5		

TABLE II

The attracted number and percentage of American cockroach by synthetic D-Bornyl acetate in Margarine in Hwa-Shan rice barn.

Attractant	D-Bornyl acetate in Margarine				Margarine			
	♂	♀	nymph	total	♂	♀	nymph	total
Numbers of attracted roach	477	1088	3184	4749	282	588	2224	3094
Percentage (%)	10	23	67		9	19	72	

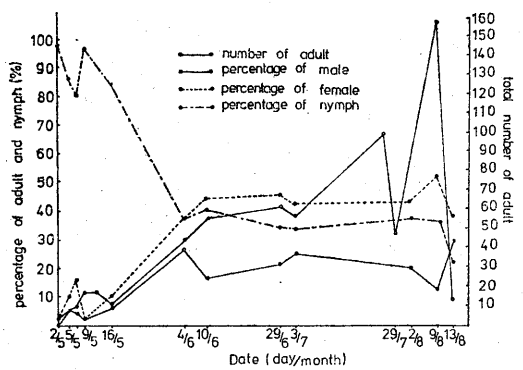

Fig. I. Percentage change of the adult male, female and nymph and fluctuation of the population density of adult American cockroach.

took one and helf years in subtropical conditions[4] and because they grew fast in summer than other seasons of the year; so more adult individuals could be trapped in the summer time. From this experiment, our data also suggested that the best period to control adult American cockroach at a large scale project is the summer time.

B. Bioassay results:

By using pipette method, behavioral response of the male roach described by many authors such as Wharton *et al.*[11] and Jacobson *et al.*[5] was confirmed. When the male was puffed under light condition, the percentage of the male with fluttering wings to total test males were inconsistent during different test period of the day in our laboratory. Other factor such as temperature was also found to be involved in the expressions of the copulatory behavior. They usually do not respond when the temperature drops below 20°C or above 35°C, no matter how high the concentration of sex pheromone is used. Therefore, the bioassayed data collected from a darkroom at 25°C under redlight were presented in Fig. II. Most males (70%) responded to sex pheromone (0.1 F. E.) when light was turned off 1-3 hours, then, the response decreased slowly to 30%. When light was turned on the response still decreased to a minimum (8%) and increased again. So the best copulatory period or the bioassay time for the American cockroach was 1 hour after the light off. Under room illumination, they do respond at concentration of 1 F. E. as shown in picture (Fig. III). In the gas-chromatographic bioassay, in addition to those data reported by Wharton[11], more than one peak was found to give male response

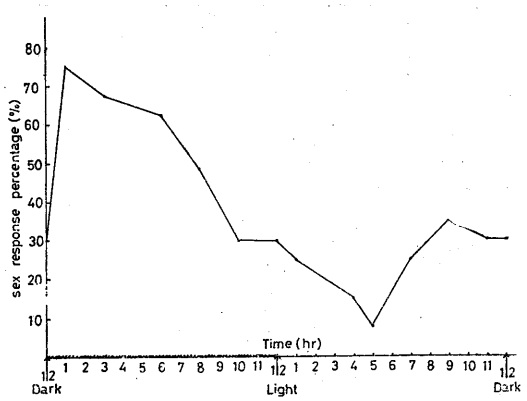

Fig. II. The sex response percentage of male American cockroach to female sex pheromone (1 F.E.) within one day.

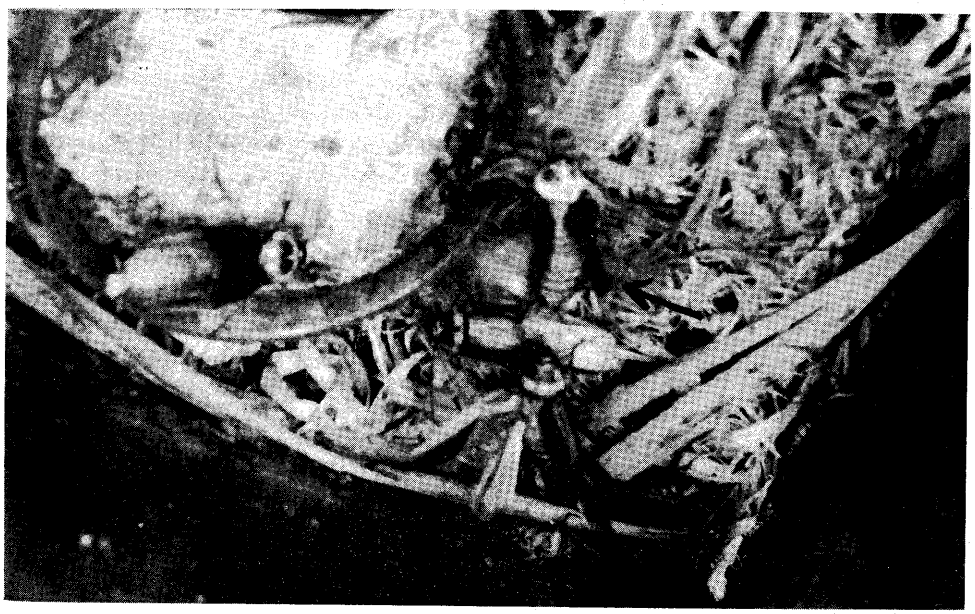

Fig. III. The sex response of male cockroach with pipette method. Arrow indicates the sex response of male cockroach

Adult male roaches when tested with D-bornyl acetate show excitation and try to fly in some extent. But when tested with the true sex pheromone, the male show more wing fluttering and always occur psudo-copulation.

C. Isolation of sex pheromone:

Crude female extract when bioassayed with male in the darkroom, highly positive response was obtained. The masking effect of the crude extract described by Jacobson and Smalls[7] was not confirmed. Silicic acid chromatography of the crude extract, gave 9 fractions. When bioassayed each fraction, only fraction 6 and 7 (eluted with 10% and 15% ethyl ether in *n*-hexane) gave positive response. Fraction 6 and 7 were combined and injected into a silicic column of a high pressure liquid chromatography (Varian 4000) using a refractive index as detector. Three elution fractions (C_A, C_B, C_C) were obtained and only fraction C_B contained active principles (Fig. IV). When C_B was spotted on a TLC plate, it showed 5 spots (C_1-C_5) (Fig. V). After each spot was eluted with ethyl ether and bioassayed with male, only spot 1 gave strong response. The elute from spot 1 was then injected into different columns of a gas-liquid chromatography and the retention times corresponding to sex pheromone are shown in Table III. From our data true sex pheromone of the American cockroach could be separated into at least 2 components with SP222PS and OV-17 columns. On the other hand, when using column SE-30, DEGS, OF-1 and SP1000, only one retention time was obtained. Possibly ester function group is involved in the sex

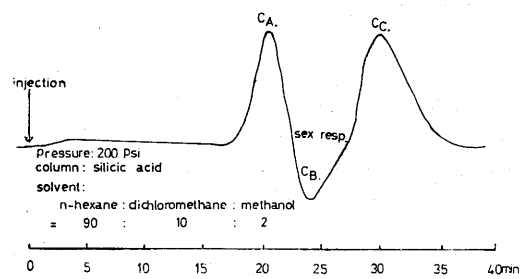

Fig. IV. High speed liquid chromatogram of female extract of American cockroach.

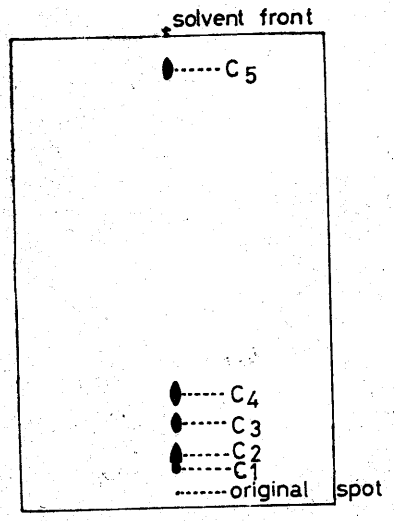

Fig. V. Thin layer chromatogram of partially purified female extract of American cockroach.

pheromone of cockroach since propyl cyclohexaneacetate was very attractive to German cockroach as reported by Sugawara *et al.*[9]. Recently, Persoons *et al.*[9] have obtained 2 excitants which could be separated by their DEGS and OV-210 columns, but not by columns OV-101, OV-17 and DC-200. It is evident, therefore, that the sex pheromone of the American cockroach is a mixture of at least 2 components.

D. Chemical tests on Crude extracts:

To the ether solution of crude extract, excess lithium aluminium hydride was added. After standing for 4 hrs, the reactant was treated as usual manner and bioassayed, the activity was diminished. The crude extract was also saponified with 1% sodium hydroxide in methanol, after saponification the activity was disappeared. Epoxidation the crude extract with *m*-chloroperbenzoic acid also eliminated activity. Acetylation the crude extract with acetyl chloride and pyridine did not affect the activity.

Therefore, the true sex pheromones are supposed to be unsaturated compounds with ester group and perhaps also carbonyl group, but do not contain hydroxyl group.

TABLE III

Retention times of sex pheromone of American cockroach compared to palmitic acetate in gas chromatographic analysis.

chemicals \ columns	DEGS[a]	SP1000[b]	SP222PS[c]	OV-17[d]	SE-30[e]
palmitic acetate	1.00	1.00	1.00	1.00	1.00
sex pheromone	2.11	0.97	3.00 4.34	1.00 1.49	0.87
D-Bornyl acetate	—	—	—	0.15	0.28

a. 120~180°C 4°C/min
b. 120~246°C 8°C/min
c. 120~183°C 4°C/min
d. 130~249°C 8°C/min
e. 120~238°C 8°C/min

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美洲蜚蠊性費洛蒙研究

I. 性費洛蒙之純化過程與誘蟲試驗

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在臺北華山倉庫內應用美洲蜚蠊雌性抽出物，D-bornyl acetate 及 Margarine 油都可以引誘蜚蠊。由誘蟲試驗之結果顯示含有性費洛蒙的雌性抽出物對雄性蜚蠊非常有效，而 D-bornyl acetate及 Margarine 油則可少許引誘兩性成蟲及幼蟲。在生物檢定上，雄性蜚蠊對其性費洛蒙及 D-bornyl acetate 都有反應，但其反應的行爲表現上亦有不同。性費洛蒙的色層純化步驟，本文亦有初步報導，並再證實美洲蜚蠊的性費洛蒙是由兩種以上的成份所組成。