A Sex Stimulant to the Male American Cockroach in the Compositae Plants

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It has been reported that several essential oils caused sexual excitement in male American cockroach, *Periplaneta americana* L.1) We wish to report isolation of the active principle which stimulate male American cockroach. The response was behaviourally identical to that elicited by the female sex pheromone extract of the species (Fig. 1).

Following to the bioassay procedure,2) green leaves of several species of plants were tested and consequently we found *Erigeron annus* (L.) Pers., *Eupatorium chinense* L. var *simplicifolium* (Makino) Kitamura and *Chrysanthemum morifolium* Ramat. were active among them. Stalks and leaves of *Erigeron annus* (L.) Pers. were extracted with ethanol and hydrocarbon fraction was obtained after concentration and column chromatography over silica gel and ‘Florisil.’ The main peak of the hydrocarbon fraction on GLC (10% Carbowax 20 M, 3 mm×75 cm, 110°C) was revealed to be C\textsubscript{15}H\textsubscript{24} by GC–MS [m/e 204 (M\textsuperscript{+}), 161 (M\textsuperscript{+}–43)]. The hydrocarbon was highly active against males and not females after purification by preparative GLC. In NMR spectrum, an isopropyl at δ 0.81 ppm (3H, doublet J=6 cps) and at δ 0.87 ppm (3H, doublet J=6 cps), a terminal methylene at δ ca. 4.8 ppm (2H), a methyl group on double bond at δ 1.52 ppm (3H, singlet) and three olefinic protons were confirmed as its partial constitutions.

From all informations mentioned above, the component was considered to be germacrene D (I). This was confirmed by comparison of IR spectrum with germacrene D which has been isolated and identified in the essential oils of *Pseudotsuga japonica*, *Pittosporum Tobira*, *Kadsura japonica* and *Piper Kadsura*.4) Furthermore, UV (λ\textsubscript{max}=259 nm, ε=4300) and [α]\textsubscript{D}=225° (c=1, n-hexane) data supported the identification. The isolated component was hydrogenated with Pd/C in ethanol to give a dihydro-compound which showed 206 (M\textsuperscript{+}) and 163 (M\textsuperscript{+}–43) in GC–MS.

*Eupatrium chinense* L. var *simplicifolium* (Makino) Kitamura gave 3 main sesquiterpene hydrocarbons which were assigned to be β-caryophyllene, germacrene D and humulene from their GLC (Fig. 2) and IR data. However, only germacrene D showed the activity on bioassay with male American cockroach. Ethanol extract of *Chrysanthemum morifolium* Ramat. also gave germacrene D as the active component.

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Fig. 1. Response of Male American Cockroaches to the Glass Plate (2×2 cm) Impregnated with 50 μg of Germacrene D.

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Threshold dose of germacrene D on bioassay was estimated to be about 10 µg on a glass plate (2 x 2 cm). By exposure of this amount, (+) can be applied for its assessment and further dilution did not elicit any excitement. More than 50 µg of the sample gave very high activity which correspond to (++) or (+++) on our description of the natural sex pheromone bioassay. This is extremely high dose compared with partially purified sex pheromone produced by females.

The bioassay was applied to another cockroach, Periplaneta japonica Karny and found male of this species showed identical response to germacrene D with female sex pheromone extract\(^5\) (Fig. 3). On the contrary, dihydrogermacrene D did not cause any response on males of both P. americana and P. japonica.

Nishino et al.\(^6\) recently found a cadinol as a stimulant of the American cockroach for both male and female in Solidago altissima L.\(^7\) However, germacrene D isolated from three Compositae plants elicited characteristic sexual excitement only in males of two Periplaneta species. Therefore, further survey of constituents of other plants as well as the Compositae plants and comparison of stimulatory activity to Periplaneta will reveal interesting interrelations among them.

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REFERENCES
7) From the extract of S. altissima L., germacrene D was also confirmed. Dr. R. Suemitsu, Doshisha University, Kyoto (Personal communication).