Short Communication

Behavioural and Electroantennogram Responses of Male Cigarette Beetle (*Lasioderma serricorne* F.) to Optically Active Serricornins

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Serricornin "4,6-dimethyl-7-hydroxy-nonan-3-one"¹⁾ is the unique sex pheromone produced by the female cigarette beetle (*Lasioderma serricorne* F.), a cosmopolitan pest of cured tobacco leaves.

Recently, the stereochemistry of the three chiral centers in the natural serricornin molecule at C₄, C₆ and C₇, was established to be (4*S*,6*S*,7*S*) by the syntheses of several serricornin stereoisomers, (4*RS*,6*R*,7*S*)-²⁾, (4*RS*,6*R*,7*R*)-³⁾ and (4*S*,6*R*,7*R*)-⁴⁾serricornins, in addition to the synthesis of the natural sex pheromone itself, (4*S*,6*S*,7*S*)-serricornin.⁵⁾

With regard to the stereochemistry-pheromone activity relationship of the serricornins, it was demonstrated that the *threo* relative configuration at C₆ and C₇ was essential to elicit pheromone activity by a comparison of the pheromone activity between (±)-6,7-*threo*- and (±)-*erythro*-serricornins⁶⁾ in a behavioural bioassay.

In this communication, we wish to report on the pheromone activity of several optically active 6,7-*threo*-serricornins, (4*S*,6*S*,7*S*)-, (4*S*,6*R*,7*R*)- and (4*R*,6*R*,7*R*)-serricornins, and (6*S*,7*S*)-anhydroserricornin (Fig. 1).

(4*S*,6*S*,7*S*)-, (4*R*,6*R*,7*R*)- and (4*S*,6*R*,7*R*)-Serricornins were synthesized stereoselectively from (2*R*,3*R*)- and (2*S*,3*S*)-3-methylaspartic acids, and (−)-α-D-glucose, respectively. (6*S*,7*S*)-Anhydroserricornin [(5*S*,6*S*)-2,6-diethyl-3,5-dimethyl-3,4-dihydro-2*H*-pyrane]⁹⁾ was obtained as a minor product in the course of the synthesis of (4*S*,6*S*,7*S*)-serricornin,^{5a)} $[\alpha]_D^{23} - 53.1^\circ$ ($c = 0.065$, hexane), $[\alpha]_{577} - 62.6^\circ$, $[\alpha]_{546} - 66.6^\circ$, $[\alpha]_{435} - 105.1^\circ$, $[\alpha]_{365} - 140.6^\circ$, MS (m/z); 168(28)=M⁺ (C₁₁H₂₀O), 41(70), 43(78), 55(67), 86(30), 99(100), 111(7), 125(19), 139(15), 153(4), PMR $\delta_{CDCl_3}^{TMS}$: 0.89 (3H, t, $J = 7$ Hz), 0.92 (3H, d, $J = 7$ Hz), 1.02 (3H, t, $J = 7$ Hz), 1.57 (3H, s), 1.3~1.6 (3H, m), 1.9~2.3 (4H, m), 3.57 (1H, m), CMR $\delta_{CDCl_3}^{TMS}$: 10.53 (q), 12.23 (q), 13.63 (q), 17.49 (q), 23.51 (t 2), 29.78 (d), 35.34 (t), 79.04 (d), 98.93 (s), 148.13 (s). The chemical purities of these serricornin stereoisomers were 93.9% for (4*S*,6*S*,7*S*)-isomer

FIG. 1. Structures of Optically Active Serricornins.

which contained 3.4% of (4*S*,6*R*,7*R*)-, 2.6% of (4*R*,6*S*,7*S*)- and 0.1% of (4*R*,6*R*,7*R*)-isomer, 87.4% for (4*R*,6*R*,7*R*)-isomer which contained 8.6% of (4*S*,6*R*,7*R*)-, 3.6% of (4*R*,6*S*,7*S*)- and 0.4% of (4*S*,6*S*,7*S*)-isomer, $\approx 99.9\%$ for (4*S*,6*R*,7*R*)-isomer and 96.5% for (6*S*,7*S*)-anhydroserricornin which contained 3.5% of (6*R*,7*R*)-anhydroserricornin.

The cigarette beetles were reared at 28°C and 60% RH on corn-flour containing 5% brewer's yeast powder. Virgin males (7~10 days old) for behavioural bioassay and electroantennogram (EAG) experiments were prepared by sexing at the pupal stage. Typical pheromone responses⁷⁾ such as antennal elevation, attractiveness and homosexual mounting were evaluated by two behavioural bioassay systems as described in our previous communication.⁶⁾

For the preparation for EAG recording, a head of male cigarette beetle with antennae was cut away from the body and the head with antennae was placed on the sponge stand in a small petri dish filled with Ringer's solution. EAGs were recorded with an Ag-AgCl glass microcapillary electrode filled with Ringer's solution (recording electrode). The terminal segment of the antenna was inserted into the recording electrode and an Ag-AgCl indifferent electrode was positioned in the Ringer's

solution. The EAG signals generated were amplified with amplifiers (DPZ-12 and DPA-100F, DIA Medical Co.) via a high impedance probe (DPZ-110, DIA Medical Co.) and visible on a monitor scope with wave memory (DPZ-202, DIA Medical Co.). The signals were also recorded on a chart recorder. Two μl of a hexane solution containing $10^{-2} \mu\text{g}$ of each serricornin was applied to a $\phi 5 \text{ mm}$ filter paper disk and the filter paper was placed in a 3 ml-glass syringe for 10 sec. One ml of the vapor from the syringe was injected manually onto the antenna with the aid of a continuous air stream (1 liter/min). The stimulus duration was approximately 0.5 sec. To correct any changes in the antennal responses during the experimental period, the responses were normalized with respect to the responses elicited by $10^{-2} \mu\text{g}$ of ($\pm$)-6,7-*threo*-serricornin.⁸⁾

The results of the behavioural bioassay by the 5 ml-vial tube method (bioassay I) indicated that more than a 90% level of antennal elevation response was elicited by $10^{-3} \mu\text{g}$ of (4*S*,6*S*,7*S*)-serricornin, but amounts less than $10^{-3} \mu\text{g}$ of (4*S*,6*R*,7*R*)- and (4*R*,6*R*,7*R*)-serricornins, and (6*S*,7*S*)-anhydroserricornin failed to elicit the typical responses (Fig. 2).

From all the behavioural parameters estimated by the $\phi 65 \text{ mm}$ -petri dish method (bioassay II) such as the number of males attract-

FIG. 2. Results of Bioassay (I).

FIG. 3. Results of Bioassay (II).

FIG. 4. Relative EAG Responses to Optically Active Serricornins.

ed to the sample, the number of homosexual couples and the number of males responding among 10 tested males, it is clear that the pheromone activity of (4*S*,6*S*,7*S*)-serricornin is much greater than that of other serricornin stereoisomers, although the testing chemicals contained other stereoisomers as impurities (Fig. 3).

These behavioural bioassay results were consistent with the results from EAG studies (Fig. 4). The amplitude of ≈ 2 mV EAG response could be observed at a dose of 10^{-2} μ g of (4*S*,6*S*,7*S*)-serricornin. This dose level is considered to be approximately the same as that produced by a female cigarette beetle.*¹ It is evident that (4*S*,6*S*,7*S*)-serricornin has the strongest pheromone activity among the tested serricornin stereoisomers.*²

These results demonstrated that the high specificity of the stereochemistry-pheromone activity relationship was associated with the configuration at the chiral centers at C₆ and C₇ in the serricornin molecule.

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*¹ The amount of serricornin produced by a female cigarette beetle was expected to be approximately 2×10^{-2} μ g by a calculation from the amount isolated from the extract of the beetle.

*² EAG responses recorded from the antenna of female cigarette beetle were very weak even at a dosage level of 10 μ g of these serricornins.