Journal of Chemical Ecology, Vol. 11, No. 4, 1985

# *Lasioderma* CHEMISTRY
# Sex Pheromone of Cigarette Beetle (*Lasioderma serricorne* F.)

TATSUJI CHUMAN, KEIKO MOCHIZUKI, MASATAKA MORI, MASAHIRO KOHNO, KUNIO KATO, and MASAO NOGUCHI

*Central Research Institute*
*The Japan Tobacco and Salt Public Corporation*
*6-2 Umegaoka, Midori-ku Yokohama 227, Japan*

(Received March 1, 1984; accepted July 31, 1984)

**Abstract**—A chemical study of the sex pheromone of the cigarette beetle was carried out. Seven components were isolated from active fractions of column chromatography of the female extract, and their structures were elucidated by spectroscopic evidence and confirmed by synthesis to be (4*S*,6*S*,7*S*)-4,6-dimethyl-7-hydroxynonan-3-one (serricornin) (I), 2,6-diethyl-3,5-dimethyl-3,4-dihydro-2H-pyran (anhydroserricornin) (II), 4,6-dimethylnonan-3,7-dione (III), 4,6-dimethylnonan-3,7-diol (IV), 4,6-dimethyl-7-hydroxy-4-nonen-3-one (V), (2*S*,3*R*)-2,3-dihydro-3,5-dimethyl-2-ethyl-6-(1-methyl-2-oxobutyl)-4H-pyran-4-one (serricorone) (VI) and (2*S*,3*R*)-2,3-dihydro-3,5-dimethyl-2-ethyl-6-(1-methyl-2-hydroxybutyl)-4H-pyran-4-one (serricorole) (VII).

These structural features suggested that the occurrence of these components might be related to the polyketide biosynthesis. The behavioral bioassay and EAG experiments revealed the biological role of each component in the copulatory behavior of this insect.

**Key Words**—Sex pheromone, *Lasioderma serricorne* F., cigarette beetle, Coleoptera, Anobiidae, serricornin, polyketide biosynthesis.

## INTRODUCTION

The cigarette beetle (*Lasioderma serricorne* F.) is a serious cosmopolitan pest of not only cured tobacco leaves but also of nearly all dry food stuffs. The difficulty in detecting the infestation of this pest until their population has increased beyond the economic threshold level because of their clandestine nature and the deficiency of pesticide-dependent control methods led us to investigate



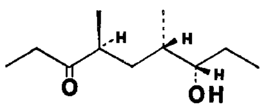
0098-0331/85/0400-0417$04.50/0 

the sex pheromone of this insect. The goal of this investigation was the development of efficient tools for integrated pest control.

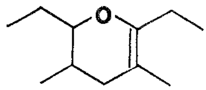
The existence of the sex pheromone produced by female cigarette beetles has been previously reported (Burkholder, 1970). A simple quantitative laboratory bioassay technique has also been developed (Coffelt and Burkholder, 1972). It was revealed that the male responses to the sex pheromone consisted of a sequence of copulatory behaviors such as an antennal elevation with leg extension, rapid locomotion to the pheromone source, and copulatory attempts with other test males.

No chemical studies had been reported when our study on the sex pheromone of this insect started. The structural elucidation and the synthesis of serricornin (Chuman et al., 1979a,b), determination of the absolute configuration of serricornin by stereoselective syntheses (Chuman et al., 1981; K. Mori et al., 1981, 1982; M. Mori et al., 1982a,b), the structure–pheromone activity relationship of serricornin (Chuman et al., 1982a,b) has already been reported. Recently, the structural elucidations of serricorone and serricorole were also reported (Chuman et al., 1983).

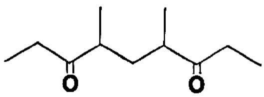
In this article, we wish to describe the isolation, structural elucidation, syntheses, and sex pheromone activity of these components in detail, although some parts of this study have already been reported in the short communications, and to discuss the occurrence of the sex pheromone components related to the polyketide biosynthesis (Figure 1).



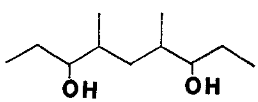
I



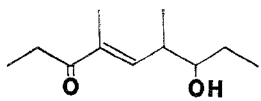
II



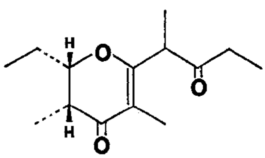
III



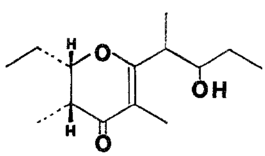
IV



V



VI



VII

FIG. 1. Structures of the sex pheromone components.

## METHODS AND MATERIALS

Cigarette beetles were reared on corn flour containing 5% brewer's yeast powder at 28°C and 60% relative humidity with a 1:1 light–dark photoperiod. Virgin females for extraction and the males for bioassay and EAG experiments were sexed at the pupal stage by the tail characteristic. The pheromone activity was estimated by behavioral bioassy and EAG experiments as described previously (Chuman et al., 1982a,b).

### *Analysis*

IR spectra refer to films and were determined on a Jasco IRA-1 spectrometer. The 100-MHz FT-PMR and CMR spectra were recorded in $CDCl_3$ with TMS as an internal standard on a JEOL-FX-100 spectrometer unless otherwise mentioned. Optical rotations were measured on a Jasco DIP-4 polarimeter. Capillary GC analyses were performed on a Shimadzu Minim-1 GC using OV-101 (30 m, 0.25 mm ID). Preparative GC was performed on a Hitachi 063 GC using OV-101 (1 m, 3 mm ID). The GC-MS analyses were carried out on JEOL-D-300, Hitachi 50GC, and Hitachi M80 mass spectrometers.

### *Preexamination of Pheromone Isolation*

To examine the chemical properties of the pheromone prior to the large scale isolation, about 100 virgin females were extracted with hexane. The extract could elicit the strong sex pheromone activity on attractiveness and sex stimulation for the male. When the extract was reacted with 2,4-dinitrophenylhydrazine or with acetic anhydride and pyridine, nearly all the activity disappeared. The activity could be recovered by hydrolysis of the acetylated material with 3% KOH–methanol. Column fractionation on silicic acid with hexane–ether mixtures yielded a low-polarity fraction, eluted with 2–5% ether/hexane, that was very active and a high-polarity fraction, eluted with 100% ether, that was somewhat active. The activity of the low-polarity fraction was relatively stable to alkali, but unstable to acid. It was also unstable to heat as demonstrated by loss of activity on attempted preparative GC purification. This instability to preparative GC purification could be avoided by acetylation of the 2–5% ether/hexane fraction from column chromatography.

### *Large-Scale Isolation*

The 260,000 cigarette beetles (mixed population, F/M ratio = 1:1) were extracted with hexane, and the crude extract was chromatographed on a silicic acid column with hexane–ether mixtures as a solvent to furnish two active fractions, which corresponded to the 2–5% ether/hexane and 100% ether fractions. The 2–5% ether/hexane fraction, which was the main active fraction, was acetylated with acetic anhydride and pyridine. Final purification by preparative GC gave compound I (3.1 mg) as a main component, compound III (1.0 mg), and compound V (1.8 mg) from the acetylated 2–5% ether/hexane fraction, and compound VI (1.2 mg) and compound VII (0.7 mg) from the 100% ether fraction. Compound II and compound IV were detected as minor components in the 2–5% ether/hexane fraction by GC-MS analysis.

*Syntheses of Components* (Fig. 2).

*4,6-Dimethyl-7-hydroxy-nonan-3-one (Serricornin) (I) and 2,6-Diethyl-3,5-dimethyl-3,4-dihydro-2H-pyran (Anhydroserricornin) (II) (Figure 2).* Crude serricornin could be obtained by the method described previously (Ono et al., 1980). The crude serricornin (100 g) was refluxed in benzene in the presence of TsOH and distilled to furnish pure 2,6-diethyl-3,5-dimethyl-3,4-dihydro-2H-pyran (anhydroserricornin, II, 54 g) in 60% yield. Boiling point 37–38° C/1 mm Hg, MS ($m/z$): 168($M^+$, 38), 41(46), 43(54), 55(58), 57(42), 69(42), 86(31), 99(100), 111(12), 125(19), 139(15), 153(4); IR ($cm^{-1}$, film): 2970(s), 2930(s), 2840(s), 1682(s), 1460(s), 1380(s), 1350(m), 1270(s), 1230(s), 1170(s), 1050(s), 983(s); PMR ($CDCl_3$, $\delta$ TMS): 0.8–1.0 (9H, m), 1.2–1.8 (5H, m), 1.56 (3H, bds), 2.0 (2H, q), 3.2–3.6 (1H, m); CMR ($CDCl_3$, $\delta$, TMS): 9.5 (t), 10.6 (c), 12.2 (c,t), 13.5 (c,t), 17.5 (c), 17.7 (t), 23,6(cx2), 23.9 (t), 25.4 (t), 30.1 (c), 31.4 (t), 35.7 (c), 35.9 (t), 79.0 (c), 80.7 (t), 98.6 (c), 99.5 (t), 148.4 (c), 148.9 (t); multiplicity of completely decoupled CMR signals was due to the coexistence of $C_{2,3\text{-}cis}$-(c) and $C_{2,3\text{-}trans}$-(t) isomers.

Treatment of pure anhydroserricornin (II, 54 g) with iso-PrOH (600 ml) and water (200 ml) in the presence of TsOH (20 g) at 35°C for 6 hr gave 4,6-dimethyl-7-hydroxy-nonan-3-one (serricornin, I) (51.9 g) with anhydroserricornin (II, 7.1 g). GC analysis showed that this stereoisomeric mixture of serricornin was composed of (4*S**, 6*S**, 7*S**)[1] isomer (33%), (*S**, *S**, *R**)[2] isomer (9%), (*S**, *R**, *S**) isomer (48%), and (*S**, *R**, *R**) isomer (10%). The assignments of the stereochemistries were established by the complete decoupled CMR data of each acetate of serricornin stereoisomers (Table 1).

Column chromatography of the stereoisomeric mixture of serricornin on silica gel furnished pure (*S** *R** *S**)- and (*S** *R** *R**) serricornins. Pure (*S** *S** *S**) and (*S** *S** *R**) serricornins could be obtained by the $C_4$ epimerization of (*S** *R** *R**) and (*S** *R** *S**) serricornins, respectively, because the chromatographic behaviors of (*S** *S** *S**) and (*S** *S** *R**) serricornins were very close and the direct separation on the column failed.

[1] A stereochemical sign with asterisks such as (4*S**,6*S**,7*S**) means the racemate of the respective enantiomers.

[2] Stereochemical signs such as (*SSS*), (*SSR*), (*SRS*), and (*SRR*) mean (4*S*,6*S*,7*S*), (4*S*,6*S*,7*R*), (4*S*,6*R*,7*S*) and (4*S*,6*R*,7R), respectively.

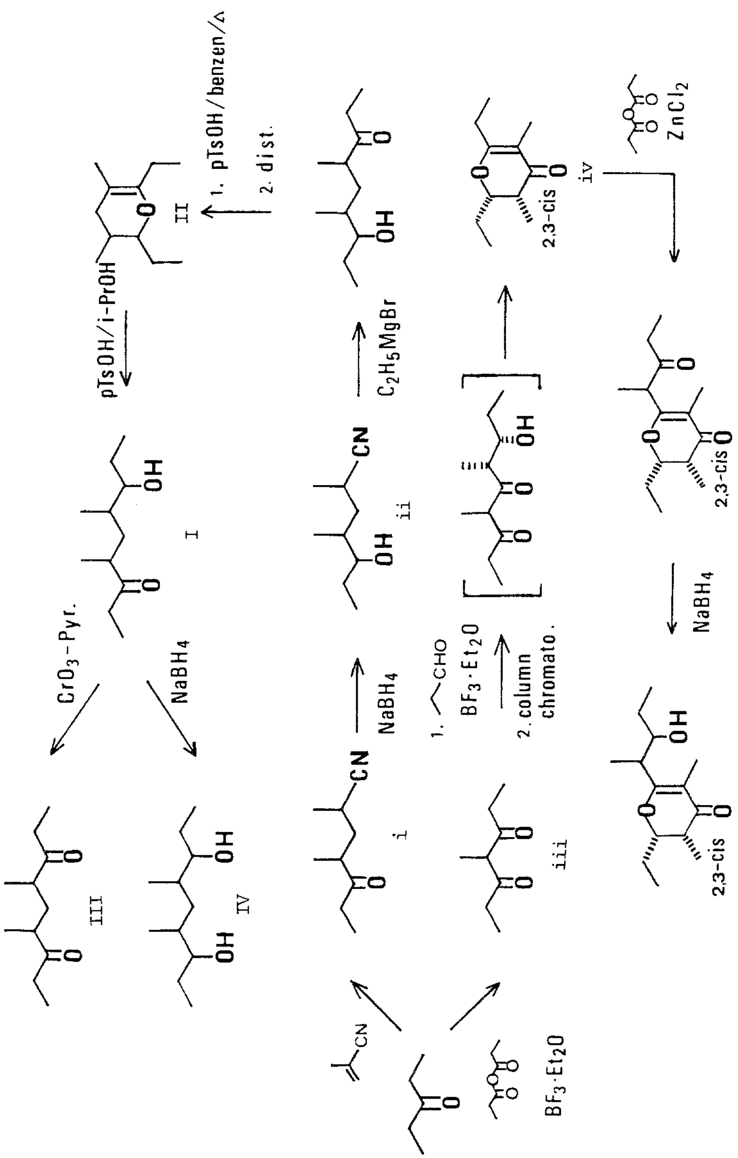
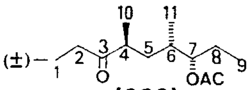
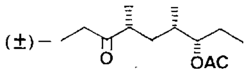
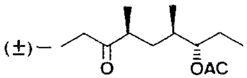
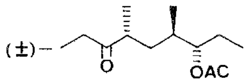



FIG. 2. Synthesis of the sex pheromone components.

TABLE 1. CMR DATA OF ACETATES OF SERRICORNIN STEREOISOMERS[a]

| Structure | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | C-7 | C-8 | C-9 | C-10 | C-11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  (SSS) | 7.84 | 34.22 | 214.88 | 43.53 | 24.22 | 33.70 | 78.04 | 35.94 | 10.18 | 16.67 | 14.45 |
|  (RSS) | 7.84 | 34.28 | 214.83 | 43.35 | 24.22 | 33.70 | 77.75 | 36.39 | 10.18 | 17.32 | 14.63 |
|  (SRS) | 7.78 | 34.11 | 214.88 | 43.88 | 23.34 | 34.11 | 78.92 | 35.51 | 10.00 | 18.08 | 15.85 |
|  (RRS) | 7.84 | 34.34 | 214.94 | 43.58 | 23.28 | 33.81 | 79.09 | 35.01 | 10.06 | 16.21 | 15.33 |

[a] The previous stereochemical assignments of (*S*, *R*, *S*)- and (*R*, *R*, *S*)-serricornin described in Chuman et al. (1981a) were revised. The assignment in this article was reversed.

Among these four enantiomeric mixtures of serricornin, only the ($S^*R^*S^*$) isomer yielded a cubic crystalline material (mp 46.5–47.5°C). X-ray analysis of the crystalline material established the structure to be ($2R^*$, $3S^*$, $5R^*$, $6S^*$)-2,6-diethyl-3,5-dimethyl-2-hydroxy-tetrahydropyran, which corresponded to the hemiketal form of ($S^*R^*S^*$)-ketoalcohol form of serricornin (M. Mori et al., 1984). IR ($cm^{-1}$, KBr): 3400(s), 2970(s), 2930(s), 2870(s), 1460(s), 1400(s), 1375(s), 1265(m), 1180(s), 1150(s), 1090(s), 1070(s), 1040(m), 1000(s), 970(s), 920(s), 900(m); PMR ($CDCl_3$, $\delta$ TMS): 0.7–1.0 (12H,m), 1.2–1.8(8H,m), 3.34 (1H,ddd, $J$ = 3, 7.5, 10 Hz); CMR ($CDCl_3$, $\delta$ TMS): 7.1, 9.6, 16.4, 17.6, 25.6, 32.6, 35.0, 36.7, 37.1, 76.0, 98.2.

*4,6-Dimethylnonan-3,7-dione (III).* The mixture of serricornin (I, 3.7 g) and $CrO_3$–pyridine complex in pyridine (150 ml) was stirred for 48 hr at 10°C and poured into water. The mixture was extracted with benzene and the benzene layer was washed with 10% aq HCl to eliminate pyridine. After washing with water and drying over $Na_2SO_4$, the mixture was concentrated in vacuo to give 4,6-dimethylnonan-3,7-dione (III, 3.5 g). MS ($m/z$), 184($M^+$, 1): 41(10), 57(100), 86(40), 99(4), 127(4), 142(2), 155(1); IR ($cm^{-1}$, film): 2970(s), 2940(s), 2880(s), 1710(s), 1460(s), 1410(m), 1375(s), 1100(s), 1020(m), 975(s); PMR ($CDCl_3$, $\delta$ TMS): 1.04(6H, t, $J$ = 7 Hz), 1.07 (6H, d, $J$ = 7 Hz), 1.6 (2H, m), 2.4 (6H, m).

*4,6-Dimethylnonan-3,7-diol (IV).* A solution of serricornin (I, 5.6 g) is iso-PrOH (10 ml) was added dropwise to a stirred and ice-cooled suspension of $NaBH_4$ (0.38 g) in iso-PrOH (20 ml) and the mixture was stirred at room temperature overnight. The mixture was poured into ice-water, treated with 10% aq acetic acid, and extracted with ether. The ether layer was washed with water and dried over $Na_2SO_4$ to give 4,6-dimethylnonan-3,7-diol, (IV, 5.3 g). MS ($M/z$): 41(40), 43(40), 55(40), 57(40), 59(60), 69(40), 83(16), 86(8), 123(16), 141(4); IR ($cm^{-1}$, film): 3300(s), 2960(s), 2930(s), 2875(s), 1455(s), 1370(s), 1340(s), 1250(m), 1240(m), 1140(m), 1100(m), 1060(m), 975(s), 950(s); PMR ($CDCl_3$, $\delta$ TMS): 0.8–1.0 (12H, m), 1.2–1.7 (8H, m), 3.4 (2H, m); 3,7-Diacetoxy-4,6-dimethylnonane (diacetate of IV), MS ($m/z$): 43(100), 55(16), 69(20), 70(24), 83(10), 86(6), 103(11), 109(5), 123(11), 141(7), 152(2), 154(3).

*2,3-*cis*-2,3-Dihydro-3,5-dimethyl-2-ethyl-6-(1-methyl-2-oxobutyl)-4H-pyran-4-one (Serricorone, VI).* A solution of $BF_3$–$Et_2O$ (28.4 g) in ether was added dropwise to a stirred and ice-cooled solution of 4-methylheptan-3,5-dione (iii, 28.4 g) in dry ether (100 ml) at 5–10°C. To the mixture, propionaldehyde (18.5 g) was added with stirring. After addition, the mixture was continuously stirred for 10–16 hr, subsequently poured into ice-water and extracted with ether. The ether layer was washed with $Na_2SO_3$, water, and dried over $Na_2SO_4$. The ether layer was concentrated *in vacuo,* and the residue was distilled to furnish 2,3-dihydro-3,5-dimethyl-2,6-diethyl-4H-pyran-4-one (iv) (18.8 g) in 56% yield, bp 73–82°C/3 mm Hg. Capillary GC analysis revealed that the ratio of the $C_{2,3\text{-}cis}$

and $C_{2,3\text{-}trans}$ isomers was 3:1. Pure 2,3-*cis* pyranone was obtained by column chromatography. MS ($m/z$): 182($M^+$, 33), 55(67), 57(93), 83(89), 113(100), 153(1); PMR ($CDCl_3$, $\delta$ TMS): 0.98 (3H, t, $J$ = 7 Hz), 1.03 (3H, d, $J$ = 7 Hz), 1.14 (3H, t, $J$ = 7 Hz), 1.73 (3H, s), 2.2–2.4 (3H, m), 4.12 (1H, ddd, $J$ = 8.2, 5.8, 3.1 Hz); CMR ($CDCl_3$, $\delta$ TMS): 9.1, 9.5, 9.8, 10.9, 23.6, 25.6, 42.5, 82.0 (cf. $C_{2,3\text{-}trans}$: 83.7); signals due to the carbons belonging to the carbonyls and the fully substituted double bond were not observed because of the short pulse interval used for the CMR measurement.

The mixture of the $C_{2,3\text{-}cis}$ pyranone (iv) (33.6 g) and propionic anhydride (78 g) was stirred in the presence of $ZnCl_2$ (24.9 g) for 48 hr at 40°c. After cooling, the mixture was poured into ice-water and the ether layer was separated. The ether layer was washed with saturated $NaHCO_3$ to remove excess propionic anhydride and with 10% aq $Na_2CO_3$, water and dried over $Na_2SO_4$. After concentration, the residue was dissolved in 40% aq dimethylamine–methanol (30 ml) and allowed to stand for 24 hr. After filtration, the filtrate was poured into ice-water and extracted with ether. The ether layer was washed with water and dried over $Na_2SO_4$. The ether solution was concentrated, and the residue was chromatographed on silica gel to give 2,3-*cis*-2,3-dihydro-3,5-dimethyl-2-ethyl-6-(1-methyl-2-oxobutyl)-4H-pyran-4-one (2,3-*cis* serricornone) (VI, 26.8 g) in 60% yield. MS ($m/z$): 238 ($M^+$, 5), 43(13), 55(27), 57(100), 69(7), 83(28), 97(8), 109(15), 112(9), 113(30), 124(18), 139(4), 153(7), 182(53), 183(7); IR ($cm^{-1}$, film): 2950(s), 2930(s), 2860(s), 1710(s), 1660(s), 1605(s), 1455(m), 1370(m), 1340(m), 1200(m), 1135(w), 1120(m), 1045(m); PMR ($CDCl_3$, $\delta$ TMS): 0.99 (3H, t, $J$ = 7.8 Hz), 1.03 (3H, d, $J$ = 7.8 Hz), 1.07 (3H, d, $J$ = 7.1 Hz), 1.30 (3H, d, $J$ = 6.9 Hz), 1.33 (3H, d, $J$ = 6.9 Hz), 1.80 (3H, s), 1.4–1.8 (2H, m), 2.4 (3H, m), 3.70 (1H, q, $J$ = 6.6 Hz), 4.15 (1H, m); CMR ($CDCl_3$ $\delta$, TMS): 7.8, 9.5, 9.7, 12.7, 12.9, 23.3, 23.6, 33.9, 42.7, 49.1, 49.5, 8.27, 83.0 (cf. $C_{2,3\text{-}trans}$ isomer: 84.3); signals due to the carbons belonging to the carbonyls and the double bond were not observed.

*2,3-cis-2,3-Dihydro-3,5-dimethyl-2-ethyl-6-(1-methyl-2-hydroxybutyl)-4H-pyran-4-one (serricorole, VII).* To an ice-cooled suspension of $NaBH_4$ (0.38 g) in EtOH (20 ml), a solution of 2,3-*cis*-serricorone (VI, 2.38 g) in EtOH (5 ml) was added without stirring, and the resulting solution was allowed to stand for 5–6 hr. The solution was added to ice-water and extracted with ether. The ether layer was washed with water and dried over $Na_2SO_4$. Concentration in vacuo gave 2,3-*cis*-2,3-dihydro-3,5-dimethyl-2-ethyl-6-(1-methyl-2-hydroxybutyl)-4H-pyran-4-one (serricorole VII, 1.6 g). MS ($m/z$): 43(16), 55(16), 57(49), 59(67), 69(23), 70(30), 83(46), 96(15), 97(18), 109(38), 111(13), 112(99), 113(100), 117(15), 124(37), 141(17), 153(28), 182(80); IR ($cm^{-1}$, film): 3400(bds), 2950(s), 2920(s), 2870(s), 1650(s), 1640(s), 1600(s), 1455(s), 1370(s), 1190(m), 1150(m), 1130(m), 1060(m), 1030(m), 970(m); PMR ($CDCl_3$, $\delta$ TMS): 0.9–1.2 (12H, m), 1.4–1.6 (4H, m), 1.75 (3H, s), 2.21 (2H, m), 2.82 (1H, m), 3.5–4.2

(2H, m). 2,3-*cis*-2,3-Dihydro-3,5-dimethyl-2-ethyl-6-(1-methyl-2-acetoxybutyl)-4H-pyran-4-one (acetate of 7), MS ($m/z$): 282($M^+$, 4), 43(67), 57(15), 69(10), 70(12), 83(52), 97(58), 101(26), 109(10), 113(16), 124(24), 125(11), 152(53), 153(100), 171(22), 182(76), 193(66), 222(59).

## RESULTS AND DISCUSSION

### *Structural Elucidation of the Components*

*(4S, 6S, 7S)-4,6-Dimethyl-7-hydroxynonan-3-one (Serricornin, I).* Compound I was isolated from the 2–5% ether/hexane fraction of column chromatography and purified by preparative GC after acetylation. $[\alpha]_D^{23}$-17.7, $[\alpha]_{546}$-19.7, $[\alpha]_{435}$-36.8, $[\alpha]_{365}$-70.3 (c = 0.155, hexane), MS ($m/z$): 168 ($M^+$-$CH_3COOH$, 8), 43(100), 55(24), 57(71), 69(39), 70(20), 83(27), 86(64), 99(13), 111(27), 127(4), 128(5), 139(20), 153(1), 157(20); IR ($cm^{-1}$, film): 2960(s), 2940(s), 2870(s), 1735(s), 1715(s), 1460(m), 1370(m), 1240(s), 1100(m), 1020(m), 960(m), 890(m); PMR ($CDCl_3$, $\delta$ TMS): 0.86 (3H, t, $J$ = 7 Hz), 0.89 (3H, d, $J$ = 7 Hz), 1.05 (3H, t, $J$ = 7 Hz), 1.08 (3H, d, $J$ = 7 Hz), 1.43 (2H, m), 1.60 (1H, m), 1.66 (2H, m), 2.06 (3H, s), 2.44 (2H, q, $J$ = 7 Hz), 2.63 (1H, m), 4.78 (1H, m), irradiation at 1.43, 1.60, 2.44, and 2.63 simplified the t at 0.86 to s, the t at 0.89 to s, the t at 1.05 to s, and the d at 1.08 to s, respectively; CMR ($CDCl_3$, $\delta$ TMS): 7.84, 10.18, 14.45, 16.67, 21.06, 24.22, 33.70, 34.22, 35.98, 43.53, 78.04, 170.88, 214.88.

Structural elucidation of serricornin (compound I) as its acetate form by spectroscopic evidence and its synthesis has already been reported (Chuman et al., 1979a,b). The absolute configurations of the three chiral centers at $C_4$, $C_6$, and $C_7$ in the serricornin molecule were established to be (4*S*,6*S*,7*S*) by several synthetic studies of serricornin stereoisomers (Chuman et al., 1981a; K. Mori et al., 1981; M. Mori et al., 1982a) and (4*S*,6*S*,7*S*)-serricornin, the natural isomer (K. Mori et al., 1982; M. Mori et al., 1982b; Mori and Watanabe, 1984).

In addition to the stereochemistry, the serricornin molecule is also of interest because it can exist in both the acyclic chain and cyclic hemiketal forms. This assumption was strongly supported by the actual occurrence of the hemiketal form of ($S^*R^*S^*$)-serricornin as a crystalline material. Recently, detailed assignments of high-field 500-MHz PMR spectral data revealed that natural (*SSS*)-serricornin existed at equilibrium between the acyclic keto alcohol form and cyclic hemiketal form with a ratio of 1:2.5 in $C_6D_6$ solution. The 500-MHz PMR studies of the serricornin stereoisomers also indicated that nonnatural isomers, ($S^*R^*S^*$)- and ($S^*R^*R^*$)-serricornins existed predominantly in the hemiketal form and the keto alcohol form, respectively, at equilibrium, but ($S^*S^*R^*$)-serricornin existed at equilibrium in the same ratio as that of

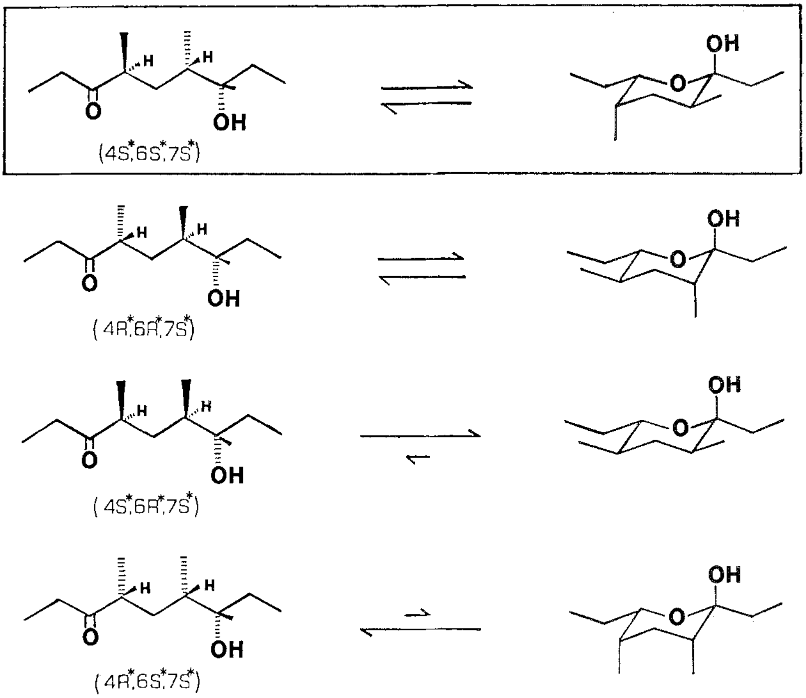



FIG. 3. Equilibrium between the acyclic keto alcohol and the cyclic hemiketal forms of serricornin.

(*S* S* S**)-serricornin (Figure 3). These differences of the equilibrium relationships among serricornin stereoisomers were considered to be due to the stereochemical relationship between $C_4$ and $C_6$ methyls in the hemiketal molecule. Although this equilibrium between the keto alcohol and the hemiketal forms seems to be closely related to the occurrence of the sex pheromone activity of serricornin, it remained unknown whether the active form of serricornin was the acyclic keto alcohol form or the cyclic hemiketal form and whether both were required with some ratio in the copulatory communication of the cigarette beetle. Judging from the inactivity of anhydroserricornin (II), which formed easily by dehydration of serricornin, it is possible that the structural conversion between keto alcohol–hemiketal and anhydroserricornin has a regulatory function of the occurrence and disappearance of the pheromone activity (Figure 4).

*4,6-Dimethylnonan-3,7-dione (III).* Compound III was isolated from the acetylated 2–5% ether/hexane fraction and purified by preparative GC. $[\alpha]_D^{23}$ +6.0, $[\alpha]_{545}$ +0, $[\alpha]_{436}$ +0, $[\alpha]_{365}$-34.0 (c = 0.05, hexane); MS (m/z): 184 ($M^+$, 1), 41(10), 57(100), 86(49), 99(6), 127(11), 142(5), 155(3); IR($cm^{-1}$, film): 2790(s), 2925(S), 2870(s), 1710(s), 1460(m), 1375(m), 1100(m),

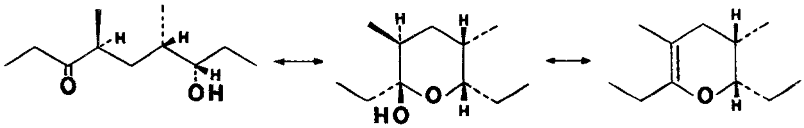



FIG. 4. Structural conversion of the serricornin molecule.

1020(w), 965(m); PMR ($CDCl_3$, $\delta$ TMS): 1.04 (6H, t, $J$ = 7 Hz), 1.08 (6H, d, $J$ = 7 Hz), 1.66 (2H, m), 2.44 (4H, q, $J$ = 7 Hz), 2.55 (2H, m), irradiation at 2.55 simplified at t at 1.04 and the d at 1.08 to bds and the m at 1.66 to s, respectively.

Structural elucidation of 4,6-dimethyl-nonan-3,7-dione by spectroscopic evidence has already been reported (Chuman et al., 1979a).

*4,6-Dimethyl-7-hydroxy-4-nonen-3-one (V).* Compound V was isolated from the acetylated 2–5% ether/hexane fraction and purified by preparative GC. $[\alpha]_D^{23}$-24.8, $[\alpha]_{546}$-41.1, $[\alpha]_{435}$-56.7, $[\alpha]_{365}$-114.4 (c = 0.09, hexane); MS (m/z): 168($M^+$-$C_2H_5COH$, 2), 43(100), 57(15), 67(8), 69(8), 97(33), 109(7), 126(93), 137(24), 155(12), 166(2); IR ($cm^{-1}$, film): 2960(s), 2920(s), 2870(s), 1730(s), 1665(s), 1455(m), 1370(m), 1240(s), 1085(m), 1040(m), 1015(m), 960(m), 900(m); PMR ($CDCl_3$, $\delta$ TMS): 0.87 (3H, t, $J$ = 7 Hz), 1.02 (3H, d, $J$ = 7 Hz), 1.09 (3H, t, $J$ = 7 Hz), 1.4–2.0 (3H, m), 1.79 (3H, d, $J$ = 1.5 Hz), 2.66 (2H, q, $J$ = 7 Hz), 2.08 (3H, s), 4.78 (1H, m), 6.38 (1H, d, $J$ = 7 Hz), irradiation at 1.6, 2.66, and 6.38 simplified the t at 0.87 to s, the t at 1.09 to s, and the d at 1.79 to s, respectively.

Precise mass determination of the ions at $m/z$ 166 and 168, which were caused by a loss of $CH_3COOH$ and $C_2H_5COH$ from molecular ion, respectively, in EI-MS (found 166.1386; calcd. 166.1358; found 168.1160; calcd 168.1150), established the molecular formula to be $C_{13}H_{22}O_3$ which was also confirmed by the presence of an ion at $m/z$ 244 ($M^+ + NH_4$) in the CI-MS spectrum.

Three oxygen atoms in the molecule were attributable to an $\alpha,\beta$-unsaturated carbonyl (1665 $cm^{-1}$) and an acetoxyl group (1730 $cm^{-1}$). Therefore, three unsaturations were due to these two carbonyls and the double bond.

By decoupled PMR experiments, five methyls were assigned to be a methyl (0.87) attached to a methylene, a methyl (1.02) attached to a methine, a methyl (1.09) attached to an $\alpha$-carbonylmethine, a methyl (1.79) attached to the double bond, and a methyl (2.08) in the acetoxyl group. These spectroscopic data indicated that compound C was a dehydro derivative of serricornin acetate. Judging from the presence of the conjugated double bond with carbonyl group in the molecule, 7-acetoxyl-4,6-dimethyl-4-nonen-3-one was assigned for the acetate of V.

*(2S,3R) - 2,3 - Dihydro - 3,5 - dimethyl - 2 - ethyl - 6 - (1 - methyl - 2 - oxobutyl)-4H- pyran-4-one (Serricorone, VI).* Compound VI was isolated from the 100% ether fraction and purified by preparative GC. MS (*m*/*z*): 238 ($M^+$, 15), 43(10),55(10), 57(77), 69(7), 83(18), 97(11), 109(15), 112(10), 113(31), 124(19), 139(7), 153(10), 182(100), 183(12); IR($cm^{-1}$, film); 2970(s), 2940(s), 2850(s), 1715(s), 1660(s), 1605(s), 1460(m), 1380(m), 1360(m), 1350(m), 1240(m), 1210(m), 1160(w), 1140(m), 1120(m), 1090(m), 1050(m), 960(m); PMR ($CDCl_3$, $\delta$ TMS, 400 MHz): 0.59, 0.60 (3H, t, $J$ = 7.3 Hz), 0.83, 0.85 (3H, d, $J$ = 7.3 Hz), 0.95, 0.97 (3H, t, $J$ = 7.3 Hz), 1.16, 1.18 (3H, d, $J$ = 6.8 Hz), 1.36 (2H, m), 1.768, 1.773 (3H, s), 2.10 (2H, m), 2.22 (1H, m), 3.11 (1H, q, $J$ = 6.8 Hz), 3.50 (1H, m); irradiation at 1.36, 2.10, 2.22, and 3.10 simplified the ts at 0.59, 0.60 to bds, the ts at 0.95, 0.97 to ss, the ds at 0.83, 0.85 to ss, and the ds at 1.16, 1.18 to ss, respectively; CMR ($CDCl_3$, $\delta$ TMS): 7.78, 9.5, 9.7, 12.7, 23.3, 23.6, 33.9, 42.7, 49.1, 49.5, 82.6, 83.3; signals of the carbons belonging to the carbonyls and the double bond were not observed.

Structural elucidation of serricorone (VI) by spectroscopic evidence and its synthesis has already been reported (Chuman et al., 1983).

*(2S,3R) - 2,3 - Dihydro - 3,5 - dimethyl - 2 - ethyl - 6 - (1 - methyl - 2 - hydroxybutyl) - 4H- pyran-4-one (Serricorole, VII).* Compound VII was isolated from the 100% ether fraction and purified by preparative GC. MS (*m*/*z*): 240($M^+$, 28), 57(10), 59(8), 70(8), 82(10), 83(35), 112(37), 113(100), 133(28), 145(11), 153(8), 182(43), 183(21); PMR ($CDCl_3$, $\delta$ TMS): 0.9–1.1 (12H, m), 1.4–1.8 (4H, m), 1.76(3H, s), 2.35 (1H, m), 2.88 (1H, m), 3.75 (1H, m), 4.06 (1H, m). Acetate of compound G was also obtained from the 100% ether fraction after acetylation. MS (*m*/*z*); 282 ($M^+$, 4), 43(83), 57(16), 69(18), 70(12), 83(52), 97(50), 101(23), 109(13), 113(16), 124(23), 125(15), 137(10), 152(25), 153(100), 171(29), 182(73), 193(51), 222(54); PMR ($CDCl_3$, $\delta$ TMS): 0.83–1.3 (12H, m), 1.4–1.8 (4H, m), 1.76 (3H, s), 1.97 (3H, s), 2.3–2.4 (1H, m), 2.95 (1H, m), 3.97 (1H, m), 5.02 (1H, m).

Structural elucidation of serricorole (VII) by spectroscopic evidence and its synthesis has already been reported (Chuman et al., 1983). Recently, the absolute configurations of $C_2$ and $C_3$ of serricorone (VI) and serricorole (VII) were established to be (2*S*,3*R*) by comparative study on the ORD curves of natural ones with that of stegobinone (XX), the sex pheromone of the drugstore beetle.[3]

*2,6-Diethyl-3,5-dimethyl-3,4-dihydro-2H-pyrane (Anhydroserricornin, II) and 4,6-Dimethylnonan-3,7-diol (IV).* Compound II and compound IV were detected in the 2–5% ether/hexane fraction. Mass spectra of compounds II and IV were identical to those of the synthetic anhydroserricornin (II) and 4,6-dimethylnonan-3,7-diol (IV), respectively.

[3] Private communication from Dr. Ebata and Prof. K. Mori, University of Tokyo, Japan.

*The Pheromone Activity of the Components*

The hexane extract of the female could elicit the pheromone activity, which was observed as sequential behavior of the males; an antennal elevation with mesothoracic legs, rapid zig-zag locomotion toward the pheromone source, and copulation attempts with other test males. To investigate the contribution of each component to the biology activity, the sex pheromone activity of each component was estimated by the behavioral bioassay and EAG experiments using synthetic materials. Three parameters were employed in our bioassay: (1) attractiveness—the number of males that climbed up the filter impregnated with test chemicals (total number of times males climbed up filter paper exceeds 10 because some males climbed up filter paper several times); (2) sex stimulation—the number of homosexual couples induced; and (3) reactivity—number of males responding among 10 test males (Figure 5).

Results of our bioassay indicated that ($S^*S^*S^*$)-serricornin[4] (I) could elicit the strongest sex pheromone activity on all parameters of this bioassay among the test chemicals. It showed that serricornin was the main component of the sex pheromone of this insect which could evoke strong attractiveness and sex stimulation for the males. Serricorone (VI) and serricorole (VII) could also stimulate strong sexual activity which induced the mass-mounting behavior among test males, but these components showed somewhat weak attractiveness. These components were considered to contribute to the supplementary factors of sex stimulation by cooperation with serricornin in the copulation of this insect. 4,6-Dimethylnonan-3,7-dione (III) and 4,6-dimethylnonan-3,7-diol (IV) had slight sex pheromone activity, but anhydroserricornin (II) elicited almost no typical activity. These results were entirely opposite to those reported by Levinson et al. (1981). Judging from the small amount of components II and IV in the female extract, these were not considered to play an important role in the occurrence of sex pheromone activity.

These observations were consistent with the results of EAG experiments except that of 4,6-dimethylnonan-3,7-dion III, the EAG intensity of which cannot be explained at present.

Consequently, it was demonstrated that sex pheromone activity of this insect was evoked mainly by serricornin, although it remained uncertain whether the active form was the acyclic keto alcohol or cyclic hemiacetal. Serricorone (VI) and serricorole (VII) might act with serricornin (I) as supplementary factors to enhance the sex stimulation in the copulation.

[4]In this bioassay ($S^*S^*S^*$)-serricornin, which was purified from stereoisomeric mixture of serricornin, was used as testing material. A recent study on the stereochemistry–pheromone activity of serricornin revealed that only ($S,S,S$)-serricornin, a naturally occurring isomer, had the strong sex pheromone activity among eight stereoisomers (Mochizuki et al., 1984).

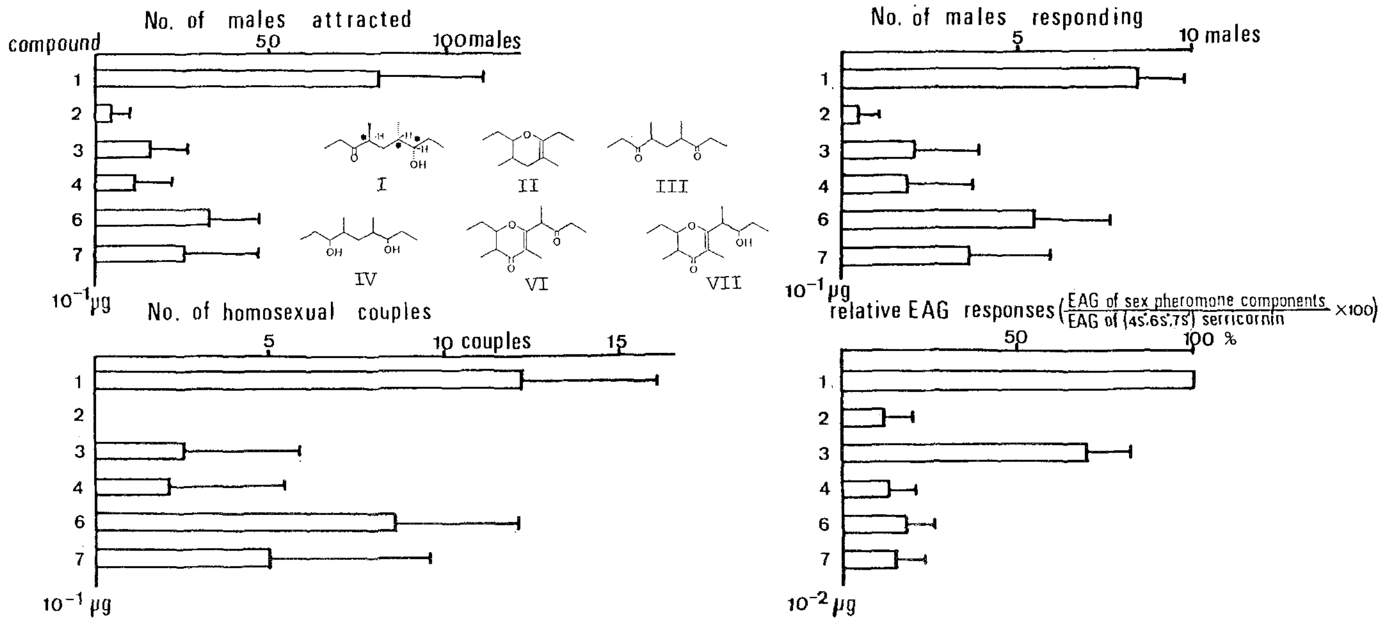



FIG. 5. Results of bioassay and EAG experiments with synthetic material. Ten unmated males were used in the bioassay with the observation time for 10 min. EAG responses of each component were normalized with the respect to those of ($S^*$,$S^*$,$S^*$)-serricornin.

*Relationship between Sex Pheromone Components and Polyketide Biosyntheses*

Seven components isolated in our study on the sex pheromone of cigarette beetle could be divided into two groups according to the carbon skeletons of their gross structures. One of the groups was made up by C-11 series components, serricornin (I), anhydroserricornin (II), 4,6-dimethyl-nonan-3-one (III), 4,6-dimethylnonan-3,7-diol (IV), 4,6-dimethyl-7-hydroxy-4-nonen-3-one (V); and another was comprised of C-14 series components, serricorone (VI) and serricorole (VII). These components had structural similarities regarding the position of the functional groups and methyl branches in their structure. The 3,7-bifunctional and 4,6-dimethyl relationship in C-11 series could be also expanded to the 3,5,7,9 multifunctional and 4,6,8 trimethyl relationship in C-14 series. These structural features suggested that these C-11 and C-14 components might be derived from the corresponding C-11 and C-14 polyketide precursors which were formed by the condensation of four propionate units and five propionate units, respectively (Chuman, 1981b) (Figure 6).

It could be assumed that C-11 precursor 3,5-dioxo-7-ol (XXIV), was converted to 3-oxo-5,7-diol by regioselective hydrogenation of $C_3$-carbonyl group, followed by dehydration to give 4,6-dimethyl-7-hydroxy-4-nonen-3-one (V). Stereoselective hydrogenation of the double bond in V furnished serricornin (I),

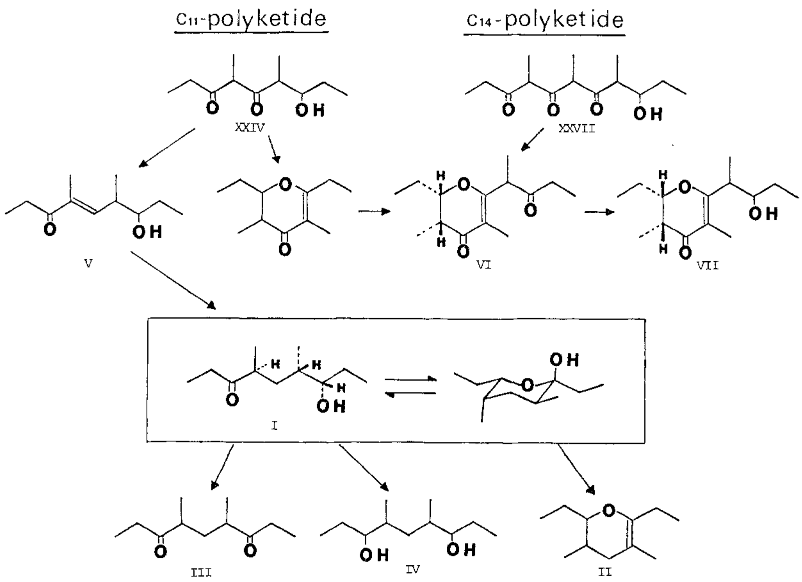



FIG. 6. Possible polyketide biosynthesis of the sex pheromone components of the cigarette beetle.

which formed the equilibrium between the acyclic keto alcohol and cyclic hemiacetal structures.

This equilibrium of serricornin (I) gave anhydroserricornin (II) by dehydration, 4,6-dimethyl-nonan-3,7-dione (III) by oxidation of the $C_7$-hydroxyl, and 4,6-dimethyl-nonan-3,7-diol (IV) by hydrogenation of the $C_3$ carbonyl. It was also shown that the C-14 precursor, 3,5,7-trioxo-9-ol (XXVII), might furnish serricorone (VI) by dehydrative cyclization between $C_5$ and $C_9$, followed by the selective hydrogenation of the carbonyl in the side chain to give serricorole 7. Another route for the formation of serricorone (VI) from $C_{11}$ pyranone by an aldol condensation with a propionate unit could be considered.

This assumption of polyketide biosynthesis was supported by the biomimetic syntheses of serricorone (VI) and stegobinone (XX), which were synthesized via polyketide precursors (Ansell et al., 1979; Sakakibara and Mori, 1979; Chuman et al., 1983; Ono et al., 1983).

Many biologically active substances of insects that seemed to be related to the polyketide biosynthesis were reported as defensive substances of opilionids, (VIII-XII, XV, XXIII) (Jones et al., 1976, 1977), and of mutilid wasps, (XII, XVI, XVII) (Fales et al., 1980), alarm pheromone of ants (XII-XIV) (Fales et al., 1972; Riley et al., 1974), an aggregation pheromone of bark beetle (XII,

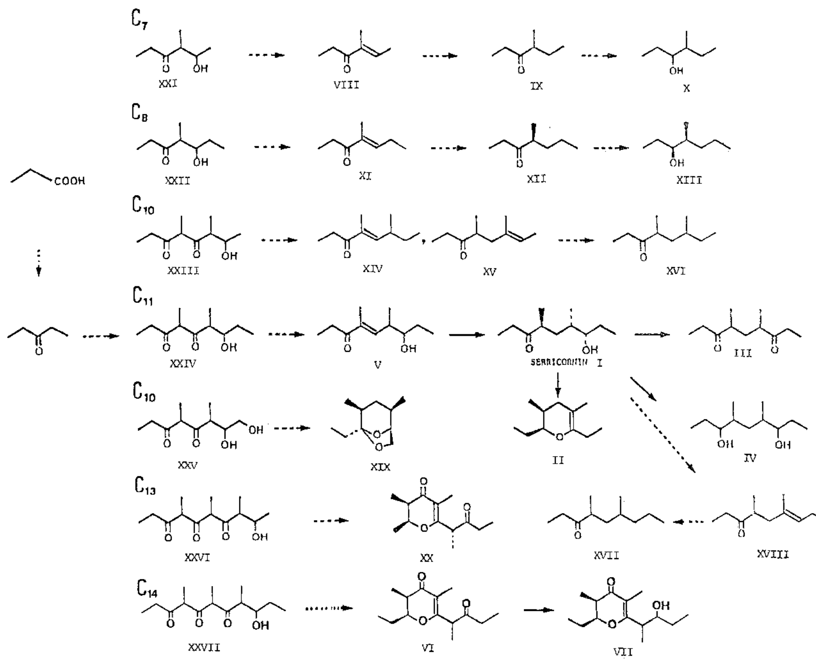



FIG. 7. Biologically active substances of insects related to polyketide biosynthesis.

XIX) (Pearce et al., 1975), an aggregation pheromone of rice and maize weevil (XXII) (Schmuff et al., 1984), and a sex pheromone of drugstore beetle (XX) (Kuwahara et al., 1978) (Figure 7).

*Acknowledgments*—We would like to thank Prof. Kenji Mori, University of Tokyo, for his kind encouragement and the great interest for our study. We also wish to thank Dr. Mikio Ono, Fuji Flavour Co., Ltd., for his cooperative work on the synthetic parts of this study. We are grateful to Dr. J. H. Tumlinson, Insect Attractants, Behavior, and Basic Biology Research Laboratory of the USDA, Gainesville, Florida, for his valuable advice on this article.

## REFERENCES

ANSELL, J.M., HASSNER, A., and BURKHOLDER, W. E. 1979. The synthesis of the drugstore beetle pheromone (stegobinone). *Tetrahedron Lett.* 27:2497–2498.

BURKHOLDER, W.E. 1970. Pheromone research with stored-product coleoptera, pp. 1–20, *in* D. Wood, R. Silverstein, and M. Nakajima (eds.). Control of Insect Behavior by Natural Products. Academic Press, New York.

CHUMAN, T., KOHNO, M., KATO, K., and NOGUCHI, M. 1979a. 4,6-Dimethyl-7-hydroxynonan-3-one, a sex pheromone of cigarette beetle (*Lasioderma serricorne* F.). *Tetrahedron Lett.* 25:2361–2364.

CHUMAN, T., KATO, K., and NOGUCHI, M. 1979b. Synthesis of (±)-serricornin, 4,6-dimethyl-7-hydroxynonan-3-one, a sex pheromone of cigarette beetle (*Lasioderma serricorne* F.). *Agric. Biol. Chem.* 43:2005–2006.

CHUMAN, T., KOHNO, M., KOTO, K., NOGUCHI, M., NOMI, H., and MORI, K. 1981a. Stereoselective synthesis of *erythro*-serricornin, (4*R*,6*R*,7*S*)- and (4*S*,6*R*,7*S*)-4,6-dimethyl-7-hydroxynonan-3-one, stereoisomers of the sex pheromone of cigarette beetle. *Agric. Biol. Chem.* 45:2019–2023.

CHUMAN, T. 1981b. Studies on biologically active substances and these applications, aroma constituents of Turkish tobacco and the sex pheromone of cigarette beetle (*Lasioderma serricorne* F.). *Yuki Gosei Kagaku Kyokaishi* 39:1183–1199.

CHUMAN, T., MOCHIZUKI, K., MORI, M., KOHNO, M., ONO, M., OHNISHI, T., and KATO, K. 1982. The pheromone activity of (±)-serricornins for male cigarette beetle (*Lasioderma serricorne* F.). *Agric. Biol. Chem.* 46:593–595.

CHUMAN, T., MOCHIZUKI, K., MORI, M., KOHNO, M., KATO, K., NOMI, H., and MORI, K. 1982b. Behavioral and electroantennogram responses of male cigarette beetle (*Lasioderma serricorne* F.) to optical active serricornins. *Agric. Biol. Chem.* 46:3109-3112.

CHUMAN, T., MOCHIZUKI, K., KATO, K., ONO, M., and OKUBO, A. 1983. Serricorone and serricorole, new sex pheromone components of cigarette beetle. *Agric. Biol. Chem.* 47:1413–1415.

COFFELT, J.A., and BURKHOLDER, W.E. 1972. Reproductive biology of the cigarette beetle, *Lasioderma serricorne.* 1. Quantitative laboratory bioassay of the female sex pheromone from females of different ages. *Ann. Entomol. Soc. Am.* 65:447–450.

FALES, H.M., BLUM, M.S., CREWE, R.M., and BRAND, J.M. 1972. Alarm pheromones in the genus *Munica* derived from the mandibular gland. *J. Insect Physiol.* 18:1077–1088.

FALES, H.M., JAOUNI, T.M., SCHMIDT, J.O., and BLUM, M.S. 1980. Mandibular gland allomones of *Dasymutilla occidentalis* and other mutillid wasps. *J. Chem. Ecol.* 6:895–903.

JONES, T.H., CONNER, W.E., KLUGE, A.F., EISNER, T., and MEINWALD, J. 1976. Defensive substances of opilionids. *Experientia* 32:1234–1235.

JONES, T.H., MEINWALD, J., HICK, K., and EISNER, T. 1977. Characterization and synthesis of volatile compounds from defensive secretions of some "daddy longlegs" (Arachnidae: Opiliones: *Leiobunum* spp.). *Proc. Natl. Acad. Sci. U.S.A.* 74:419–422.

KUWAHARA, Y., FUKAMI, H., HOWARD, R., ISHI, I., MATSUMURA, F., and BURKHOLDER, W.E. 1978. Chemical studies on the Anobiidae: sex pheromone of the drugstore beetle, *Stegobium paniceum* (L.) (Coleoptera). *Tetrahedron* 34:1769–1974.

LEVINSON, H.Z., LEVINSON, A.R., FRANCKE, W., MACKENROTH, W., and HEEMANN, V. 1981. The pheromone activity of anhydroserricornin and serricornin for male cigarette beetles (*Lasioderma serricorne* F.) *Naturwissenschaften* 68:148.

MOCHIZUKI, K., CHUMAN, T., MORI, M., KOHNO, M., and KATO, K. 1984. Activity of stereoisomers of serricornin, sex pheromone of the cigarette beetle (*Lasioderma serricorne* F.). *Agric. Biol. Chem.* 48:2833.

MORI, K., and WATANABE, H. 1984. A new synthesis of serricornin [(4*S*,6*S*,7*S*)-4,6-dimethyl-7-hydroxy-3-nonanone], the sex pheromone of the cigarette beetle. *Tetrahedron* In press.

MORI, K., NOMI, H., CHUMAN, T., KOHNO, M., KATO, K., and NOGUCHI, M. 1981. Determination of the absolute configuration at C-6 and C-7 of serricornin (4,6-dimethyl-7-hydroxy-3-nonanone), the sex pheromone of the cigarette beetle. *Tetrahedron Lett.* 22:1127–1130.

MORI, K., NOMI, H., CHUMAN, T., KOHNO, M., KATO, K., and NOGUCHI, M. 1982. Synthesis and absolute stereochemistry of serricornin [(4*S*,6*S*,7*S*)-4,6-dimethyl-7-hydroxy-3-nonanone], the sex pheromone of the cigarette beetle. *Tetrahedron* 38:3705–3711.

MORI, M., CHUMAN, T., KOHNO, M., KATO, K., NOGUCHI, M., NOMI, H., and MORI, K. 1982a. Absolute stereochemistry of serricornin, the sex pheromone of cigarette beetle, as determined by the synthesis of its (4*S*,6*R*,7*R*)-isomer. *Tetrahedron Lett.* 23:667–670.

MORI, M., CHUMAN, T., KATO, K., and MORI, K. 1982b. A stereoselective synthesis of "natural" (4S,6S,7S)-serricornin, the sex pheromone of cigarette beetle, from levoglucosenone. *Tetrahedron Lett.* 23:4593–4596.

MORI, M., CHUMAN, T., and KATO, K. 1984. Cyclic hemiacetal and acyclic chain—the two forms of serricornin. *Tetrahedron Lett.* 25:2553–2556.

ONO, M., OHNISHI, I., CHUMAN, T., KOHNO, M., KATO, K., and NOGUCHI, M. 1980. A novel synthesis of (±)-serricornin, 4,6-dimethyl-7-hydroxy-nonan-3-one, the sex pheromone of cigarette beetle. *Agric. Biol. Chem.* 44:2259–2260.

ONO, M., OHNISHI, I., KUWAHARA, Y., CHUMAN, T., and KATO, K. 1983. Synthesis of (±)-2,3-*cis*-stegobione, (±)-2,3,-*cis*-2,3-dihydro-2,3,5-trimethyl-6(1-methyl-2-oxobutyl)-4H-pyran-4-one, the sex pheromone of drugstore beetle. *Agric. Biol. Chem.* 47:1933–1934.

PEARCE, G.T., GORE, W.E., SILVERSTEIN, R.M., PEACOCK, J.W., CUTHBERT, P.A., LANIER, G.N., and SIMEONE, J.B. 1975. Chemical attractants for the smaller European elm bark beetle *Scolytus multistriatus* (Coleoptera: Scolytidae). *J. Chem. Ecol.* 1:115–124.

RILEY, R.G., SILVERSTEIN, R.M., and MOSER, J.C. 1974. Isolation, identification, synthesis and biological activity on volatile compound from the heads of *Atta* ant. *J. Insect Physiol.* 20:1629–1637.

SAKAKIBARA, M., and MORI, K. 1979. Synthesis of a stereoisomeric mixture of 2,3-dihydro-2,3,5-trimethyl-6-(1-methyl-2-oxobutyl)-4H-pyran-4-one, the sex pheromone of the drugstore beetle. *Tetrahedron Lett.* 27:2401–2402.

SCHMUFF, N.R., PHILIPS, J.K., BURKHOLDER, W.E., FALES, H.M., CHEW, C.-W., ROLLER, P.P., and MA, M. 1984. The chemical identification of the rice weevil and maize weevil aggregation pheromone. *Tetrahedron Lett.* 25:1533–1534.