winter.

Summary

Green larvae of *Cephonoded hylas* turn to deep dark red in colour 5 days after the last larval molt, 24 hours later vanishment of red pigments from the larval epidermal cells of the integument, and further 12 hours later pale green prepupae are raised.

Such depigmentation as mentioned above was induced by molting hormone and inhibited by juvenile hormone. It was also shown that the critical period during which the production of molting hormone necessary for depigmentation occurred at the middle stage of cocoon formation.

Acknowledgement: The author is grateful to the late Prof. M. Fukaya, Messrs. S. Yagi, and N. Agui, Tokyo University of Education for their interest and advice in this investigation.

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A Stimulant of the American Cockroach, *Periplaneta americana* L. (Orthoptera: Blattidae), Occurring in *Solidago altissima* L. (Compositae). Chikao Nishino and Keiko Tsuzuki (Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo, Japan) Received Jan. 27, 1975. *Botyu-Kagaku*, 40, 62, 1975.

11. セイタカアワダチソウ (*Solidago altissima* L.) に含まれるワモンゴキブリ (*Periplaneta americana* L.) の興奮物質

西野親生，都筑啓子（三菱化成生命科学研究所） 50. 1. 27 受理

セイタカアワダチソウ (*Solidago altissima* L.) よりワモンゴキブリ (*Periplaneta americana* L.) の興奮物質を単離した．この物質 0.05mg を与えると，雄雌ともに活性試験を受けたゴキブリの10～20%が，5分以内に特徴ある行動で興奮した．この物質の構造は，化学的あるいはスペクトルによる知見からセスキテルペンアルコールである α-カジノールまたは T-カジノールのいずれかであることが判明した．

Since the insect moulting hormone, ecdysones, were isolated from some plants[1,2], many chemists have given their attentions to plant products possessing pheromonal or hormonal activity for insects.

Recently, Bowers and Bodenstein[3] reported that few plant-derived compounds, D-bornyl acetate, α-santalol and β-santalol, caused sexual excitement in the male American cockroach (*Periplaneta americana* L.). They also found that the male American cockroach shows typical sexual behaviour to the hexane or methanol extracts of 18 flowering plants. However, the structure of sex pheromone mimics still remains undetermined.

We also have been interested in active constituents occurring in plants for noxious insects, especially, cockroaches. As the first target, our investigations were directed to stimulants for the American cockroach in plants of the *Compositae* family found widely in Japan. By examining 12 species of them, the methanol extracts of 6 species produced strong response from the male cockroach (Table 1). Particularly, the cockroach exposured to the methanol extract of the leaf of *S. altissima* L. (*Seitaka-awadachi-so*) displayed an interesting behaviour which is different from the characteristic sexual excitment.

We have been carried out studies on the isolation and the structure of the active constituent of *S. altissima* which is the most heinous weed and distributed throughout Japan. The spectral and chemical evidences born out that the stimulant, $C_{15}H_{26}O$, should be α-cadinol or T-cadinol.

Cockroach used in bioassay and bioassay

Table 1. Plants of the *Compositae* Family Examined for Excitability with the Male American Cockroach.

	Name of plant	Part examined	Activity
1	*Petasites japonicus* Miq.	l	+
		s	−
2	*Aster tataricus* L.	l	−
		s	−
3	*A. japonicus* Franch *et* Sav.	l	+
4	*Taraxacum platycarpum* Dahlst.	l	+
		s	+
5	*Artemisia vulgaris* L. *var. indica* Maxim.	l	+
6	*Cirsium* sp.	l	+
7	*Solidage altissima* L.	l	+
		s	+
		r	−
8	*Chrysanthemum coronarium* L. *var. spatiosum* Bailey.	l	+
		s	+
9	*Youngia japonica* DC.	w p	−
10	*Sonchus oleraceus* L.	l	−
11	*Ambrosia elatior* L.	l	−
		s	−
12	*Lactuca* sp.	l	−
		s	−

l = leaf s = stem r = root w p = whole plant

procedure[4]: The cockroaches used in the bioassays were obtained from the laboratory colony and reared at 27°C on rat food and water soaked into absorbent cotton. Adults were collected each day during July and August (1974) as soon as they emerged. They were held together in a container (23×33×24cm) with wood shelters, the food and water at 27°C. Rearing of the insects carried out in the dark for 9 hrs. (9.00 AM–6.00 PM) and under illumination for 15 hrs. (6.00 PM–9.00 AM on next day). Assays were performed in the afternoon using the container reared about 80 adult males at 27°C. The diet and water were taken away just before the assay. A certain amount of acetone solution of each fraction obtained in the course of isolation of the stimulant was applied on a glass plate (2.5 × 2.5cm), in the case of pure stimulant, a filter paper (1×1 cm) absorbed a certain amount was placed on the glass plate. After removal of the solvent at room temperature, the glass plate located 3 cm apart from the shelter (Fig. 1) and then covered the container with stainless-steel wire-netting. We evaluated the activity within 5 min. after exposure to the

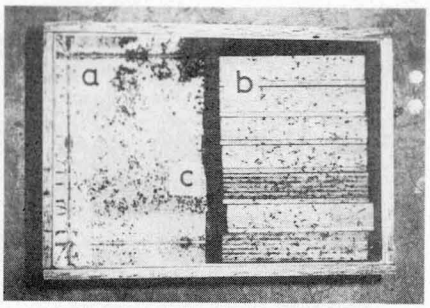


Fig. 1. Container used in bioassay
a : Container, b : wood shelters,
c : glass plate

sample under an electric torch in the following criteria:

(+) at least a few* cockroaches gather in front of shelters with characteristic restless behaviour (Fig. 2-b).

(−) all cockroaches remain unchanged in their behaviour (Fig. 2-a).

* The number varies according as the purity of sample.

Isolation of the stimulant: Travelling of the stimulant during isolation process was monitored by the bioassay described above with the male cockroach.

The leaves (13 kg) of *S. altissima* L. harvested at Tokyo in October (1974) were extracted with methanol. The ethyl acetate soluble portion (146 g) of the methanol extract was chromatographed over silicic acid and eluted stepwise with *n*-hexane containing an increasing ratio of benzene. The activity was found in the 40–100% benzene eluates (24 g). This active fractions purified by chromatography on a column of silicic acid with a solution of chloroform in benzene. The obtained 60–100% chloroform eluates (4.5 g) were acetylated with a mixture of acetic anhydride and pyridine at 50°C. The activity was observed in the non-acetate portions (2.6 g) collected by chromatography over silicic acid eluted with benzene. The non-acetate was chromatographed twice over silicic acid (ethyl acetate in *n*-hexane) and afforded active fractions (29–36% ethyl acetate eluates) (470 mg). The fractions (140 mg) was purified finally by preparative gas chromatography (GC) (25% DEGS) to give the stimulant (**1**) (20.6 mg) as a colorless oil. Sixty nine mg of **1** are expected to be included in 13 kg of the leaves.

Homogeneity was established by thin-layer chromatography (TLC) on silica gel G (Merck) with benzene (Rf 0.33) and chloroform (Rf 0.63). The GC (5% DEGS on Shimalite W, 150°C) of **1** showed a single peak at 16.23 min.

Response of cockroach to the stimulant (**1**): The stimulant (**1**) was dissolved in acetone in the proportion of 1 mg to 50 μl. From this solution, 25 μl (corresponding to 0.5 mg of **1**) were taken up to a filter paper (1×1cm) with micro syringe and then were completely absorbed into the filter paper removing the solvent at room temperature.

The test samples, 0.1, 0.05 and 0.01 mg, were prepared in the same manner. The preparation placed on the glass plate, and assayed by the bioassay procedure mentioned above. Tests for the female performed in the same way with about 70 adult cockroaches. Fig. 2 showed the behaviours caused by 0.05 mg of **1**. None of the insects was observed in front of the shelters

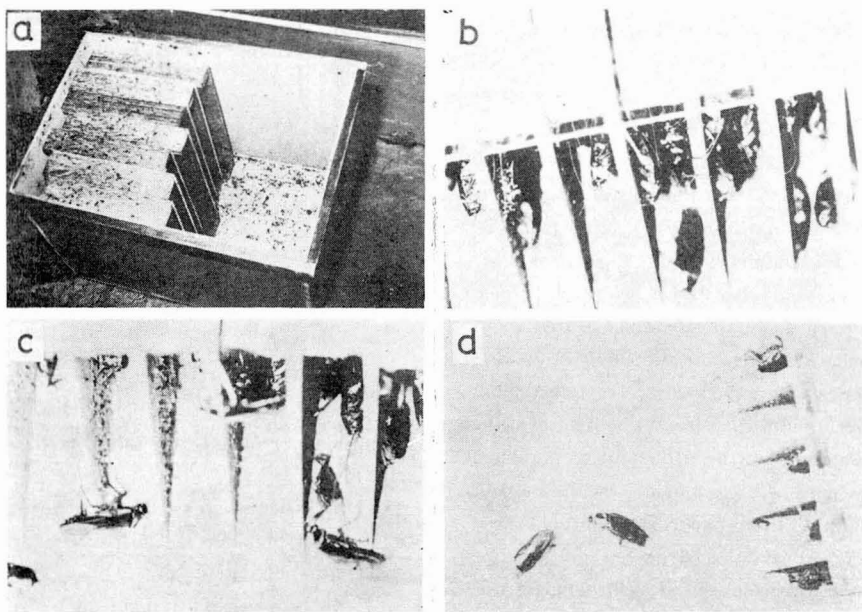


Fig. 2. Typical behaviours of the male American cockroach caused by 0.05mg of the stimulant within 5 min. a : without the stimulant, b : the insects gather in front of the shelters (2–3 min. later), c : transit the shelters, d : excited insects run about.

Table 2. Number of Cockroach Gathered in Front of Shelters with Characteristic Restless Behaviour within 5 min.

Exp.[a]	Weight (mg) of the stimulant	Males responded[b]	Cockroaches assayed	Females responded	Cockroaches assayed
A[c]	0.05 0.1 0.5 1.0	3 7 7 9	77	5 7 6 6	66
B	0.05 0.1 0.5	12 10 12	77	8 9 10	66
C	0.05 0.1 0.5	8 14 15	77	13 12 7	66
D	0.01 0.05 0.1 0.5	7 12 3 11	69	8 10 4 5	64
E	0.01 0.05 0.1 0.5	7 13 10 7	68	7 7 9 4	64

a : The experiment was performed at 2 days interval.
b : The assay for the male was prior to the female.
c : Assayed at 24°C.

without 1, or exposuring to non-active principles (Fig. 2-a). By giving of 0.05 mg of 1, as shown in Fig. 2-b, 10-20% of cockroaches gather in front of the shelters after 2-3 min. with restless behaviours. The behaviours are characterized in the following ; 1) they shake antennae nervously, 2) hurriedly walk in shelters and 3) several insects exhibit exercises in bending and stretching their legs. A half of the congregated insects move violently among the shelters (Fig. 2-c), and finally several run about inner wall and bottom of the container in extreme excitement (Fig. 2-d). It seems that intensity of these responses have no relation to the distance from the sample.

Contrary to our expectations, the female cockroaches also elicit by the stimulant with the behaviours similar to those of the male. However, both sexes were not attracted to the sample, and, the male did not show the typical sexual response, consisting of wing flutter, extended abdomen and attempted copulation.

What kind of the excitement due to the stimulant will become apparent from results of the following ecological and physiological studies.

Table 2 indicates the number of the insect displaying the response as seen in Fig. 2-b, wihin 5 min. The largest number were observed at 0.05 mg in some experiments and the number reduced, on the whole, in concentration higher than 0.05 mg. Because of short interval (10 min.) in giving each sample, saturation of an atmosphere with the stimulant may contribute to weakening effect of cnocentration. As the result, no significant variance was recognized between the male and the female, although, the male somewhat acted promptly.

Structure of the stimulant (1) : The compound (1) showed no UV (EtOH) absorption maximum above 210 nm (ε_{210}=1500). From the IR [(KBr*): 3160, 1150 cm^{-1}] spectrum of 1 and the behaviour of 1 to acetylation, the presence of tertiary hydroxyl group was indicated. In the high resolution mass spectrum (Hi-MS) of 1, the M^+ peak did not appear, but the largest mass number was observed at m/e 204.1867 (calcd. value for $C_{15}H_{24}$ 204.1878). Catalytic hydrogenation of 1 (1 mg) in ethanol afforded compound (2) (0.96

* The oil was triturated with KBr powder.

mg). Its Hi-MS showed the M^+ peak at m/e 224.21380 (calcd. value for $C_{15}H_{28}O$ 224.21416). Therefor the molecular formula, $C_{15}H_{26}O$ (MW= 222), was assigned to **1**.

The PMR* spectrum of **1** exhibited one tertiary methyl (1.08 ppm, 3H, singlet), two secondary methyls (0.74, 0.85 ppm, each doublet, J=6.5 Hz), one vinyl methyl (1.56 ppm, 3H, broad singlet) and ond olefinic proton (5.38 ppm, 1H, broad singlet).

The IR (1380 and 1395 cm^{-1}), mass [m/e 161 (222-18-43), base peak] and PMR [two doublets (J=6.5 Hz) at 0.74 and 0.85 ppm] spectra of **1** suggested the presence of an isopropyl group.

Compound (2) means that **1** has one double bond, i.e., the methyl and the olefinic proton must be situated on the same double bond. This was supported by decoupling experiments. By mutual irradiation of the broad singlet signals at 1.56 and 5.38 ppm in the PMR spectrum of **1**, each signal changed to a sharp singlet and a singlet with 3.0 Hz in half width, respectively. This half width value shows that a methine group attaches to the carbon atom bearing the vinyl proton (=CH-CH). In the PMR spectrum measured with 1.6 mg of **1**, tertiary methyl signal at 1.08 ppm shifted to 11.23 ppm by addition of 3.0 mg of shift reagent, Eu $(DPM)_3$. This evidence pointed out that the tertiary methyl group and the tertiary hydroxyl group are present as a grouping CH_3-C-OH[5].

The functional groups revealed are shown in Fig. 3. Calculation of the degree of hydrogen

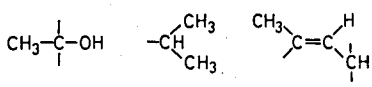
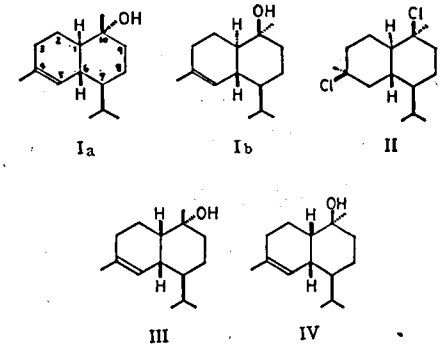


Fig. 3.

deficiency shows that **1** is a bicarbocyclic sesqiterpene alcohol, especially, cadinols. On treatment with dry hydrogen chloride, **1** (2.2 mg) yielded compound (3) (1.2 mg) whose PMR ($CDCl_3$) indicated signals at 0.81 (3H, doublet, J = 6.5), 0.92 (3H, doublet, J = 6.5 Hz), 1.57 (3H, singlet) and 1.61 ppm (3H, singlet). In the mass spectrum of **3**, the prominent peaks appeared at m/e 204, 161, 133, 119, 105 (base peak) 93, 91, 77 and 41. These PMR and mass spectra adding to TLC and GC coincided with those of authentic cadinene dihydrochloride (II). The relative configurations on C-1, C-6 and C-7 were determined by the formation of **3** (II). On the shapes of olefinic proton in the PMR spectrum,



Ia Ib II

III IV

it has been reported that *trans*-fused ring cadinols, α-cadinols (Ia)[6] and T-cadinol (Ib)[7], appeared as a broad singlet whereas *cis*-fused isomers, δ-cadinol (III)[8] and T-muurolol (IV)[9], as a doublet with J = 5-6.5 Hz. The broad singlet signal due to olefinic proton of **1** also supported *trans*-ring fusion in **1**.

All the data described above born out that the stimulant (1) should be α-cadinol (Ia) or T-cadinol (Ib). Investigations on the orientation of the hydroxyl group of **1** are in progress.

Summary

A stimulant of the American cockroach was isolated from the leaf of *Solidago altissima* L. From 10 to 20% of both sexes showed characteristic restless behaviour by 0.05 mg of the stimulant within 5 min. On the basis of the spectral and chemical studies, we have deduced that the stimulant is a sesquiterpene alcohol, α-cadinol or T-cadinol.

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* Unless otherwise stated, the spectra were taken in carbon tetrachloride at 60 MHz.

dustries Co., Ltd., for GC-High-MS spectra measurements.

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Studies on Pyrethroidal Compounds* Part V. Synthesis and Toxicity of Thermal Decomposition Products of Furamethrin. Yasuo Abe, Nobushige Itaya, Haruka Oouchi and Yoshio Fujita (Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd., 4-2-1 Takatsukasa, Takarazuka City, Hyogo, Japan) Received March 5, 1975. *Botyu-Kagaku*, 40, 67, 1975.

12. ピレスロイド系化合物の研究（第5報）フラメスリン熱分解生成物の合成と毒性 安部八洲男，板谷信重，大内 晴，藤田義雄（住友化学工業株式会社生物科学研究所農薬事業部研究部）
50. 3. 5 受理

次の5種類のフラメスリン熱分解生成物を調製した：(±)-*trans*-chrysanthemic acid (I), (±)-pyrocin (II), (±)-dihydrochrysanthemolactone (III), 5-formyl-2-furylmethyl (±)-*cis, trans*-chrysanthemate (V) および 5-(2′-formylethenyl)-2-furylmethyl (±)-*cis, trans*-chrysanthemate (VIII). VIII の合成には V をグリニアル反応によって 5-(1′-Hydroxy-2′-propenyl)-2-furylmethyl (±)-*cis, trans*-chrysanthemate (VI) に導き，次いでハロゲンによって転位反応を併って 5-(3′-Halogeno-1′-propenyl)-2-furylmethyl (±)-*cis, trans*-chrysanthemate (VII a, b) に導き，これを二酸化マンガンで酸化する方法がうまくいった．

調製した分解生成物および2種類の条件で加熱したフラメスリンの殺虫効力を，イエバエ，アカイエカについて油剤及び微量滴下法で評価した．分解生成物5種についてはいずれも，ノックダウン効力，致死効力ともに何ら示さず，加熱フラメスリンの殺虫力はその中に含まれるフラメスリン含量に比例していた．従って，加熱フラメスリンの殺虫力は，その中に含まれるフラメスリンによるものであると推定した．

In a previous paper[1] the authors identified five thermal decomposition products from (±)-*cis, trans*-furamethrin, which is a synthetic pyrethroid and also known as prothrin, and proposed the pathways of the pyrolysis. The decomposition products are: (±)-*trans*-chrysanthemic acid (I), (±)-pyrocin (II), (±)-dihydrochrysanthemolactone (III), 5-formyl-2-furylmethyl (±)-*cis, trans*-chrysanthemate (V) and 5-(2′-formylethenyl)-2-furylmethyl (±)-*cis, trans*-chrysanthemate (VIII). This paper deals with syntheses of the thermal decomposition products. And also their toxicities to houseflies (*Musca domestica*) and mosquitoes (*Culex pipiens*) are examined in order to clarify the influence of heat on effectiveness of furamethrin.

Furamethrin purified by distillation was heated under two different conditions, either at 130°C for 10 hr or at 200°C for 7 hr. The temperature of the former condition is near to that of domestic electric vaporizer for anti-mosquito mat, and gave a brown viscous liquid, containing 21.5% of furamethrin. The latter condition gave a solid (*ca.* 30%) insoluble in organic solvent ($CHCl_3$, AcOEt, MeOH, DMSO and acetone) and a soluble

* Part IV; see reference (1).