increased, but the tolerance varied to the lower levels during 20 generations of selection.

3) The F_{12} generation selected with BPMC showed a 5.5-fold resistance to BPMC, but the resistance did not increase to further selection until 20 generations.

4) No change in the LD_{50} values to NAC was found during first five generations of selection with NAC. Thereafter the tolerance to NAC was slightly increased by successive selections.

5) The LD_{50} values to DN, DB and NB did not change significantly during selection for 15 generations with DN, for 18 ones with DB and for 21 ones with NB, respectively. When selected for 10 generations with DB, the tolerance to diazinon and BPMC caused about 2-and 4-fold increse respectively. The LD_{50} values to NAC did not change significantly during selection for 21 generations with NB. While smaller increase in the tolerance to BPMC was found, but degree of the increase of tolerance to BPMC was lower in the selection with NB than that in the case of successive selection with BPMC.

Electroantennograms and Behavioral Response of the Termite *Coptotermes formosanus* (Shiraki) to the Extracts of Fungus Infected Wood and Fungus Mycelium. Minoru YAMADA (Fishery Laboratory, Nagoya University, Nagoya) and Haruo MATSUO* (Wood Research Institute, Kyoto University, Uji, Kyoto) Received May 31, 1976. *Botyu-Kagaku*, 41, 185, 1976.

29. 腐朽材ならびに菌体抽出液などに対するイエシロアリの行動と EAG

山田 稔（名古屋大学農学部水産学教室）松尾治夫（京都大学木材研究所木材生物部門） 51. 5. 31 受理

今までシロアリの EAG に関する報文はなかった．本報では，イエシロアリの珠数状触角に電極をさし込んで，腐朽材ならびに菌体抽出液などに対する触角の感応を電気生理学的に捉え，これと行動（とくに，道しるべ反応）との関連性を調べた．EAG における脱分極の大きさは，道しるべ反応の大きさと相関性はなかったようだが，忌避反応との相関性はかなりあった．

The currect trend toward minimizing the use of insecticides has led to an increased interest in the use of insect attractants and repellents for pest control. Contributions toward the elucidation of the olfactory mechanism of insects are needed to facilitate the finding and effective use of these behavior-manipulating odorous chemicals. Recently, the trail-following activity of extracts from fungus-infected wood and fungus mycelium was examined[1]. Results of these studies showed that some extracts were attractive to the termite *Coptotermes formosanus* (Shiraki), and the other extracts were repellent to the termite.

Accordingly, the work to be reported here is an attempt to compare the effect of extract fractions on antennal responsiveness with whole organism (behavioral) responsiveness.

Material and Methods

The worker termites *Coptotermes formosanus*, bred in the laboratory, were used.

Electrophysiological preparation:

The antenna (Fig. 1) of the living insect was fixed mechanically with wax so that recording was possible with circulatory and respiratory systems as intact as possible. The EAG recordings were essentially the same as in earlier

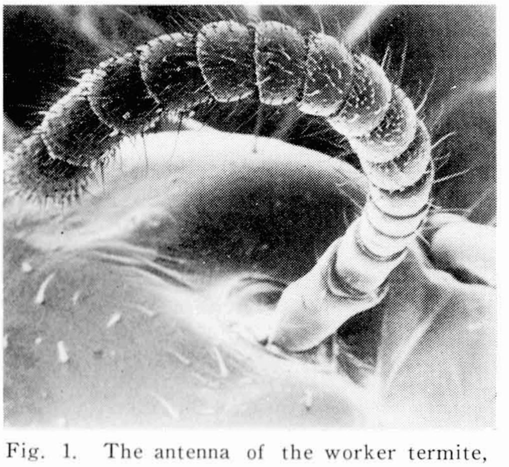

Fig. 1. The antenna of the worker termite, *Coptotermes formosanus*, observed by scanning electron microscope (×100).

* Please adress correspondence to H. Matsuo

investigations by Schneider[3,4]. The antenna was stimulated by the direct injection of the saturated odor vapor into a stream of purified air.

Behavioral (whole organism) preparation:

The behavioral test was the same as those described earlier[1]. A worker termite was placed at the center of a circle (10cm diameter) drawn on a glass plate with a glass capillary tube containing a test extract solution. After reaching the circumference of the circle, the aggregate time (in seconds) for which the termite followed the circumference within 60 seconds was recorded. This test was repeated three times, and each time a termite which had not yet been subjected to the test was used. The evaluation and judgement standards on the trail-following activity are shown in the following description.

Mark	Judgement standards
0	We judged the extract had a trail-following activity in the following cases: i), ii) or iii). i) One or more of 3 termites walked on the circumference for a long time (30-60 sec.). ii) Two or more of 3 termites walked on the circumference (15-30 sec.). iii) Only one of 3 termites walked on the circumference (15-30 sec.), and moreover the total time of the other two was 15sec. or more.
X	We judged the extract had no trail-following activity in cases other than the above.
XX	We judged the extract had a repellent activity especially in the case that all 3 termites turned back in a flurry as soon as they reached the circumference of the circle and repeated this action several times, finally went quickly across the circumference.

Chemical preparation:

The extracts of the 30 days-decayed pine woods (AS, BS, CS, DS, ES, FS and GS) were prepared in the same way as described by Matsuo and Nishimoto[1]. Seven species of brown rot fungi used were *Tyromyces palustris* Murr. (AS), *Daedalea dickinsii* Yasuda (BS), *Lenzites trabea* Pers. ex Fr. (CS), *Gloeophyllum saepiarium* Karst. (DS), *Coniophora puteana* Karst. (ES), *Serpula lacrymans* S. F. Gray (FS) and *Lentinus lepideus* Fr. (GS). The extracts of the fungi themselves (Let, Sel, Leb, Gal, Cov, Pyc and Pop) were also prepared as described by Matsuo and Nishimoto[1]. Seven species of the fungi were *Lenzites trabea* Pers. ex Fr. (Let), *Serpula lacrymans* S. F. Gray (Sel), *Lenzites betulina* Fr. (Leb), *Ganoderma lucidum* Karst. (Gal), *Coriolus versicolor* Quél. (Cov), *Pycnoporus coccineus* Bond. et Sing. (Pyc) and *Porodaedalea pirni* (Pop). The latter five are white rot fungi. Letn is the neutral fraction of the extract (Let).

Results and Discussion

Table 1 shows the behavioral responses to the extracts. The extracts of the pine wood blocks (AS, BS, CS, FS) and the extract of the fungi themselves (Let, Sel, Letn) had a trail-following activity (attractant) at a relatively high concentration. On the other hand, the wood extracts (DS, ES, GS) and the fungi extracts (Leb, Gal, Cov, Pyc, Pop) showed repellent activity at a high concentration. Formic acid which is contained in Dufour's gland of the ant *Acanthomyops claviger*[2] and propionic acid showed a repellent activity only at the highest concentration (10^{-3}g). There were no compounds which showed a repellent activity at a low concentration. It is of interest that some extracts (Letn, Let, CS, AS) and cinnamyl alcohol had a trail-following activity only at some limited range of concentration. For example, Letn had a trail-following activity at the concentrations 10^{-7} and 10^{-8}g, but the activity disappeared at concentrations above 10^{-6}g. However, no repellent extracts showed such a tendency as seen in attractant extracts, e. g., Letn.

Fig. 2. shows recordings made with one antenna during the series of one experiment without change of the electrode position.

All extracts and the control air current (which is compressed from a empty polyethylen syringe) elicited a slow negative deflection in the antenna. This negative deflection (so-called electroantennogram=EAG) means here that the periphery of the antenna becomes negative in relation to its base, and could be interpreted as a simultaneous expression of receptor potentials in the sensory cells located near the different electrode[4,5].

Table 1. Responses of the termite to the extracts and the chemical compounds. This was made on the basis of "Judgement standers" given in the text.

Tested compound	The mass of the solute (order) gained with a capillary tube from the extracts (original solutions) and their dilutions (g) and the results of the responses.					
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
AS	X	0	X	X	X	X
BS	0	0	0	X	X	X
CS	X	0	0	0	X	X
DS	XX	XX	X	X	X	X
ES	XX	XX	XX	X	X	X
FS	0	X	X	X	X	X
GS	XX	XX	X	X	X	X
Letn	X	0	0	X	X	X
Let	X	X	X	X	0	0
SeI	0	0	X	X	X	X
Leb	XX	XX	X	X	X	X
Gal	XX	XX	XX	X	X	X
Cov	XX	XX	X	X	X	X
Pyc	XX	XX	X	X	X	X
Pop	XX	XX	X	X	X	X
Formic acid	XX	X	X	X	X	X
Propionic acid	XX	X	X	X	X	X
Cinnamyl alcohol	X	X	X	X	0	0
Ether (control)	X	X	X	X	X	X

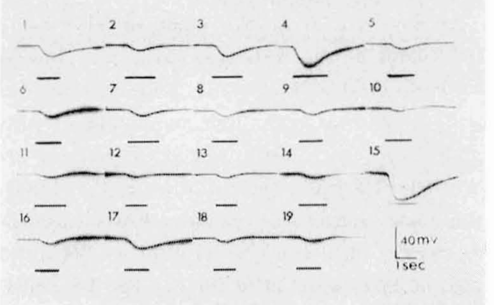

Fig. 2. Comparison of the shape and amplitude of EAGs due to differences in the stimulus extracts. Black bars below the antennograms indicate the duration of the stimulus air current. The stimulus concentrations were the original solution (10^{-3}g) of Table 1 for the BS, DS and FS and 1/10 dilution (10^{-4}g) for the others.

The amplitudes of the EAGs vary considerably with the compounds tested. For example, Formic acid No. 15 in Fig. 2) elicited a very large EAG (30mV), and the DS (No. 4) produced the highest amplitude of EAG (23mV) among the extracts. Many extracts of the fungi themselves elicited the small EAGs which were not different from the control-EAG (3mV).

On the other hand, it is of interest to note that the shapes of the EAGs did not depend upon the substances tested : the antenna of the termite gave very similar EAG shapes to the repellent (such as the DS or the ES) and the attractant (substance with the trail-following activity such as the AS or the CS) of this species.

This observation agrees well with the results obtained from the fruit-piercing moth, *Oraesia excavata*[6]. The greatest problem arising from this observation lies in the fact that the termite responds behaviorally in perfectly opposite ways to the repellent and the attractant. There could be several alternatives by which this somewhat confusing picture could be explained. The behavioral responses are the consequence of complicated integrations in the brain which receives the odor messages from all antennal receptors. While the EAG is composed of the receptor potentials elicited simultaneously in many functionary different types of receptors around the electrode. As with taste receptors of insects[7], when an olfactory receptor of the insect is stimulated excitatorily, it produces an "excitatory" receptor potential (a slow negative potential which is recorded extracellularily from a single sensillum on the antenna). When it is stimulated inhibitorily, a slow positive potential (an "inhibitory" receptor potential) could be recorded from the same receptor[8,9].

Therefore, for instance, if the repellent-specific receptors which were specialized for the detection of the repellent were excited with the repellent, the antenna would produce a slow negative deflection-EAG; on the other hand, if the attractant-specific receptors were excited with the attractant, the antenna would also produce a slow negative deflection-EAG. Consequently, the repellent-EAG and the attractant-EAG could not be distinguished by appearance. However, so far there have been no neurophysiological results to show such specific receptors in the antenna of the worker termite of this species, although many kinds of insects, if not all, are thought to have the specialized receptors to biologically important odorants, such as food odor or pheromone[5,9,10,11]. Even if there were no specific receptors for the repellent and the attractant in the antenna, the so-called "generalist"[5] could also produce visually a similar shape of EAG, for the "generalists" could respond excitatorily or inhibitorily both to the repellent and the attractant. For example, if the repellent had an inhibitory effect on some olfactory receptors and at the same time had an excitatory effect on many other olfactory receptors around the region of the different electrode, the shape of EAG thus recorded could be a slow negative deflection, for the excitatory receptor potentials could be more dominant than the inhibitory receptor potentials around the different electrode. A similar assumption could also hold true for the slow negative deflection-EAG to the attractant.

For explanations of these complicated phenomena we must wait for the detailed investigation of olfactory receptors on the antenna as been done by many investigators[5,9], and also of the olfactory center which receives odor messages from the olfactory receptors of the antenna[12].

In Table 2 EAG-amplitudes can only be compared with one another if they are given in the same column. It is therefore possible to state that the response to the AS in the column (I) is larger than those to the BS and the CS in the column (I); but it cannot be said that the response to the AS in the column (III) is smaller than those to the BS and the CS in the column (I). As can be seen from Table 2 the amplitude of EAGs to one extract (for example, the AS) varies considerably from antenna to antenna, whereas the relative amplitudes of the AS-EAG to other various extracts in each column does not change so remarkably. Such variations in the EAG-amplitude may derive from the following reasons: (1) the physiological conditions of the antenna (the periphery of the different electrode on the antenna could be damaged at the time when the antenna is fixed mechanically with wax); (2) the recording conditions of the EAG (resistance between the recording electrode and the olfactory sensory receptors with which the electrode is in electrical contact).

At any rate, from the point of view of the amplitudes of the EAG-responses, the following becomes clear: (1) Formic acid and propionic acid elicited a very high amplitude of EAG, sometimes, over 100mV and 90mV respectively; (2) The DS-EAG showed always the highest amplitude among the extracts-EAGs, although we need to discount it because the concentrations used at EAG were 10^{-3}g for the BS, DS and FS and 10^{-4}g for the others. The AS-EAG followed the DS-EAG; (3) The EAGs of the extracts of the decayed wood were larger than those of the extract of fungi themselves.

When comparing the behavioral data (Table 1) with the electrophysiological (EAG) data (Table 2), it is difficult to find a definit relationship between them. For example, (1) in behavioral responses (Table 1) some extracts (such as the AS or the BS) act on the termite as the attractant and the other extracts (the DS or the Gal) serve as the repellent. While, in the EAG

Table 2. A summary of the EAGs of five individuals. Each value of the EAGs in vertical series (column) represents the amplitude (mV) of slow negative deflection recorded from one antenna without change of the electrode position. The stimulus concentrations were the original solution (10^{-3}g) of the Table 1 for the BS, DS and FS and 1/10 dilution (10^{-4}g) for the others. $\bar{x}$ is the mean value of five or four measurements.

Tested compound	Tested antenna					
	I	II	III	IV	V	$\bar{x}$
AS	55	66	13	6	13	29
BS	20	15	5	2	5	9
CS	25	25	13	4	6	15
DS	55	65	35	14	18	37
ES	25	25	6	2	5	13
FS			15			15
GS	25	25	8	3	7	14
Letn	25	17	8	2	6	12
Let	20	25	6	2	5	12
Sel	20	15	6	2	5	10
Leb	10	13	3	1		7
Gal	10	13	3	2		7
Cov	10	13	3	2		7
Pyc	10	13	3	2		7
Pop	10	13	5	2		8
Formic acid	>100	>100	29	29		
Propionic acid	90	93	16		20	55
Cinnamyl alcohol	60	57	8	5		33
Ether	10		3	2	5	5
Control	10	10	3	2	4	6

responses, all extracts produced the same shape of the EAG as shown in Fig. 2, that is, the slow negative deflections; (2) the EAG-amplitudes of the AS are always much larger than those of the BS, while behaviorally the BS extract acts as the attractant at a higher concentration than 10^{-5}g and the AS at the concentration of 10^{-4}g (Table 1).

Fig. 3 shows the EAG-amplitudes at different concentrations of the AS, CS and DS. In the lower concentration range (between 10^{-6} and 10^{-10}g), the EAG of these three extracts are small and seem to be about equal to each other. Above the concentration of 10^{-5}g, however, the DS-EAG rises steeply, while the CS-EAG remains constant for the whole concentration range. The AS-EAG falls between the DS-EAG and the CS-EAG.

Table 3 shows each response of ten termites at each condition of the AS, CS and DS. No termites showed any response at less than a concentration of 10^{-6}g of the AS, 10^{-7}g of the CS or 10^{-5}g of the DS respectively. However, some termites showed a little trail-following response at the concentration of 10^{-5}g of the AS and 10^{-3}g of the CS, and showed a clear trail-following response at the cocentration of 10^{-4}g of the AS and 10^{-4} to 10^{-6}g of the CS. On the other hand, some termites showed a repellent response at the concentration of 10^{-3}g of the AS, and 10^{-3} and 10^{-4}g of the DS.

Fig. 4 shows the number of the termites which showed a trail-following response at different concentrations of the AS, CS and DS on the basis of Table 3.

Fig. 5 shows the number of the termites which

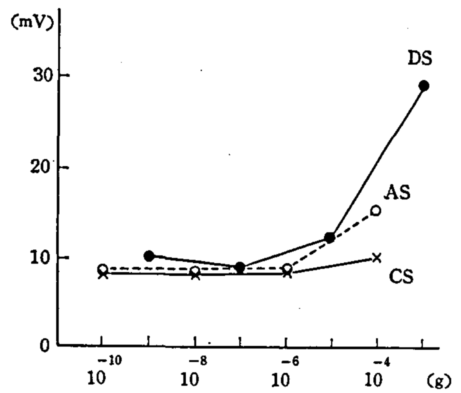

Fig. 3. The electrophysiological (EAG) stimulus-response curve.
Ordinate: EAG-amplitude (mV).
Abscissa: The concentration (g) of the extract and its dilutions.
Illustrated is a typical experiment performed with one animal and one electrode position. Amplitude values are averages of two mesurements of one the individual.

showed the repellent response at different concentrations of the AS, CS and DS on the basis of Table 3. In the lower concentration range (between 10^{-5} and 10^{-8}g), the number of the termites responding to these three extracts is 0. However, above 10^{-4}g concentration the number of those responding to the DS rises steeply, while the number of those responding to the CS remains 0 for the whole concentration range. The number of those responding to the AS falls between the number of those responding to the CS and the DS. When comparing the EAG-stimulus response curve (Fig. 3) with the stimulus-response curve of behavior (Fig. 4 and 5), it is apparent that there is no correlation between the EAG-amplitude in Fig. 3 and the trail-following responses in Fig. 4. However, Fig. 5 seems to show a positive correlation between the magnitude of the EAGs and the repellent responses of behavior.

Table 3. Results of the bioassay of the AS, CS and DS. 10 termites were tested in each condition. The aggregate time (in second) during which the termite followed the circumference within 60 seconds after reaching the circumference of the circle was recorded.
R: Repellent activity
*: Trail-following activity (walked more than 15 sec).
The others were no activity (walked only 1-14 sec).

Tested compound	The mass of the solute (order) gained with a capillary tube from the extracts (original solutions) and their dilutions (g) and the results of the responses.					
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
AS	1, 1	1, 10	9, 1	1, 1	1, 1	1, 1
	1, 2	21*, 3	1, 3	1, 2	1, 1	1, 1
	1, 1	1, 1	1, 1	1, 1	1, 1	1, 1
	1, 1	2, 1	1, 1	1, 1	1, 1	1, 1
	R, 1	1, 2	1, 2	1, 1	1, 1	1, 1
CS	1, 1	10, 3	2, 15*	47*, 1	1, 1	1, 1
	6, 2	1, 26*	21*, 25*	1, 40*	1, 1	1, 1
	2, 5	15*, 10	22*, 45*	35*, 30*	1, 1	1, 1
	1, 3	18*, 41*	3, 18*	1, 1	1, 1	1, 1
	1, 2	10, 25*	20*, 21*	12, 7	1, 1	1, 1
DS	R, R	1, 1	1, 1	1, 1	1, 1	1, 1
	R, 1	R, 1	1, 1	1, 1	1, 1	1, 1
	R, R	1, 2	1, 1	1, 1	1, 1	1, 1
	R, 1	1, R	1, 1	1, 1	1, 1	1, 1
	1, R	R, 1	1, 1	1, 1	1, 1	1, 1

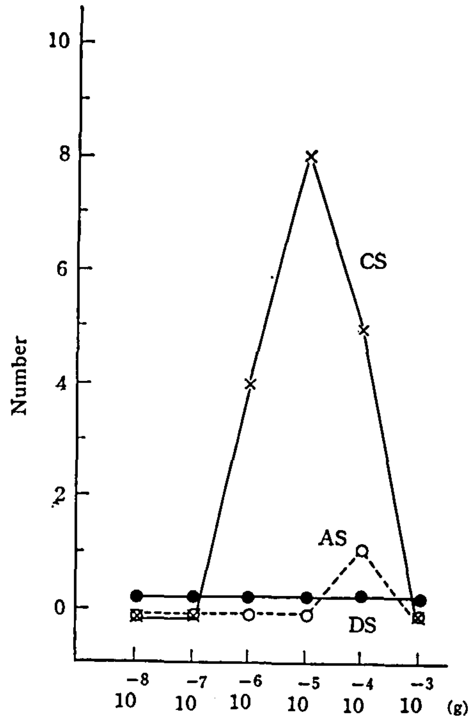

Fig. 4. The trail-following response curve.
Ordinate: Number of the termites which exhibited the trail-following activity (*) as shown in Table 3.
Abscissa: See Fig. 3.

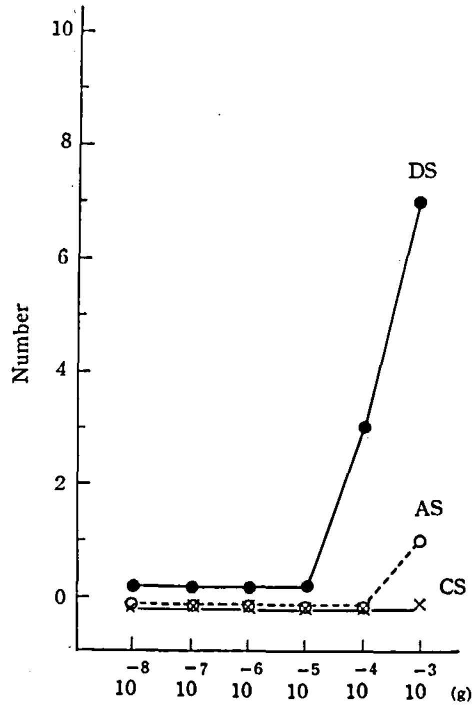

Fig. 5. The repellent response curve
Ordinate: Number of the termites which demonstrated repellent activity (R) as shown in Table 3.
Abscissa: See Fig. 3.

From the results described above the following general conclusions could be derived: (1) EAG technique has limitations for relating the EAG responses to the behavior responses of the termite, especially to the trail-following response. Therefore, it seems essential that a more intricate technique, such as the single cell recordings of the olfactory nervous system, be employed to get information as to how attractant or repellent is accepted at an individual olfactory receptor and integrated in the central nervous system and eventually ends in a given behavioral response; (2) Figs. 3 and 5, together with the responses to formic acid and propionic acid in Tables 1 and 2, indicate the possibility of some correlation between the EAG-amplitude and the repellent activity of behavior, although unfortunately sufficient quantitative data are not yet available to allow us to draw a clear conclusion.

Acknowledgement: We wish to thank Prof. T. Tamura and Dr. K. Nishimoto for their encouragement and valuable advice, Mr. Mose Matsuba for his correction of the english manuscript, and Mrs. K. Koga for her secretarial assistance.

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Hormonal Control of the Body-colour Change in Larvae of the Larger Pellucid Hawk Moth, *Cephonodes hylas* L. (2). Hajime Ikemoto (Tokyo Prefectural Isotope Research Station, Fukasawa, Setagaya, Tokyo 158) Received June 4, 1976. *Botyu-Kagaku*, 41, 192, 1976.

30. オオスカシバの体色変化に関するホルモン的制御(2) 池本 始（東京都立アイソトープ総合研究所，東京都世田谷区深沢） 51. 6. 4 受理

オオスカシバの幼虫からアラタ体を摘出すると終令末期にみられる暗赤色化を少しばかりはやめる．おなじような現象はアビエチン酸誘導体（methyl-6, 7-dioxo-5α, 10α-podocarpa-8, 11, 13-trien-15-oate）の経口投与によってもみとめられる．

The green larvae of *Cerura vinula* (Lep. Notodontidae) turn to dark red prior to cocoon formation. Bückmann[1] showed that decapitation is capable of evoking to accelerate reddening of the epidermis in *Cerura* larvae. Probably reddening of the epidermis in *Cerura* larvae may be prevented by juvenile hormone (JH) secreted from corpora allata. The larvae of *Cephonodes hylas* exhibit chromatic change similar to that of *Cerura* larvae. Author demonstrated that JH and its analogue ZR 512 suppress the dark reddening which occurs at the end of the last instar of *Cephonodes* larvae[2].

In this report, it was shown that extirpation of corpora allata accelerate the dark reddening in *Cephonodes* larvae. Furthermore, the effect of A-11, a derivative of abietic acid and kojic acid which have been known as anti JH agent on the dark reddening in *Cephonodes* larvae was reported.

Materials and Methods

Animals

The last instar larvae of *Cephonodes hylas* were used. The larvae were reared as described previously[3].

Oral administration of two chemicals

Kojic acid was obtained from Wako pure chemical Ind. Ltd., Osaka and A-11 (Methyl-6, 7-dioxo-5α, 10α-podocarpa-8, 11, 13-trien-15-oate)[5] was supplied by the courtesy of the Laboratory of Applied Zoology, Tokyo University of Education. A-11 had been donated to the laboratory from Mr. S. Murakoshi, Kanagawa Sericultural Experiment Center.

The appropriate amount of kojic acid and A-11 was dissolved in distilled water and acetone (Wako, special grade), respectively. And the leaves of Cape jusmine were dipped in these solutions for a few minutes. After dipping, the excess water containing kojic acid was shaked out from leaves and leaves dipped in A-11 acetone solution were dried in air to remove acetone, then these leaves were fed to *Cephonodes* larvae throughout the last instar in order to examine whether two test chemicals, kojic acid and A-11, accerelate the dark reddening which occurs at the end of the last instar or not. As control, the leaves dipped with distilled water and acetone, respectively, were fed to the larvae.

Results

Extirpation of corpora allata

Corpora allata were removed from the larvae 2 or 3 days after the last larval ecdysis. Sham operated larvae were not fed on the food grass, because allatectomized larvae did not take the food grass, being different from silkworm, *Bombyx*