JUVENILE HORMONE ACTIVITY IN THE HAEMOLYMPH AND THE ANAL SECRETION OF THE QUEEN OF MACROTERMES SUBHYALINUS (RAMBUR) (ISOPTERA, TERMITIDAE)*

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INTRODUCTION

Lüscher (these proceedings) has demonstrated that the caste determining influence of reproductives in a group of Kalotermes or Zootermopsis workers or nymphs resembles closely the effects of juvenile hormone (JH).

With Kalotermes, experiments have been devised to show that the determining principle of reproductives is given off by the anus; plugging the anus with shellac abolishes the inhibiting effect of female reproductives on replacement reproductive production (Lüscher, 1960). It is furthermore well known that faecal material of reproductives and other members of the termite colony are taken up orally and distributed to other individuals (trophallaxis) (see McMahan, 1969).

We address ourselves to the question of whether JH-active substances are distributed by the reproductives to the larvae and nymphs by way of trophallaxis.

MATERIALS AND METHODS

Mounds of Macrotermes subhyalinus near Olorgesailie and Sultan Hamud (Kenya) were opened; the anal secretion of the queen was directly collected with capillaries in the half-opened royal chamber, and transferred into diethyl ether; ethanol (6:1 v/v); a few crystals of phenylthiourea were added. The material collected was shaken and cooled with dry ice. The queen was sectioned in loco, and the haemolymph and other organs treated the same way. In the laboratory each sample was extracted three times with ether (anhydrous, peroxide free, Mallinckrodt Chemical Works, St. Louis, Missouri, USA), the ether fractions were combined, dried with Na_2SO_4, evaporated to dryness at 10^{-1} Torr, taken up with ether and sealed under nitrogen for transport and storage in dry ice.

The method of vapour application of ZR-512 is described by Wanyonyi and Lüscher (These proceedings).

The Galleria wax test, originally developed by Schneiderman and Gilbert (1958), was used as described by de Wilde et al. (1968); 50 pupae were treated with each dilution, a dose response curve was established for each sample, and the Galleria Unit (= G.U.) determined graphically. 1 G.U. is the amount of material necessary per test pupa

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to evoke a positive response in 50% of them.

EXPERIMENTS AND RESULTS

1) Transmission of JH- active substances from one individual to another. 15 orphaned, full-grown larvae of Zootermopsis angusticollis (Hodotermitidae), collected in Del Monte Park, Pacific Grove, California, were subjected to the vapours of 1 mg of ZR- 512 (6) for 6 days. The treated larvae were then placed together with 6 untreated last instar nymphs. 20 days later 3 of the untreated nymphs had moulted into pre-soldier- nymph intermediates, forms that have otherwise only been observed in colonies treated with JH- mimics (Wanyonyi and Lüscher; Lüscher, these proceedings).

The fact that the JH- mimic is transmitted from one member of a colony to another does not prove that the same will be the case with the termite's own hormone. The stability of ZR- 512 is much higher than that of natural Cecropia- JH. But the experiment nevertheless demonstrates that lipids are distributed in a colony as well as water-soluble material (see McMahan, 1969).

2) JH- activity in the anal secretion of the queen. To extract JH- activity from the anal secretion of female reproductives we chose the large species, Macrotermes subhyalinus, from East Africa. For comparison, the activity in the blood of the queen was also determined; the huge size of her corpora allata (see Noirot, 1969a) and the large number of eggs produced each day (an estimated 40,000) led us to expect a high JH-titre. As a negative control we chose extracts of soldiers; they possess small corpora allata, their female reproductive organs are rudimentary (see Noirot, 1969a, b), and they are not the target organisms for the hypothetical pheromone with JH-activity.

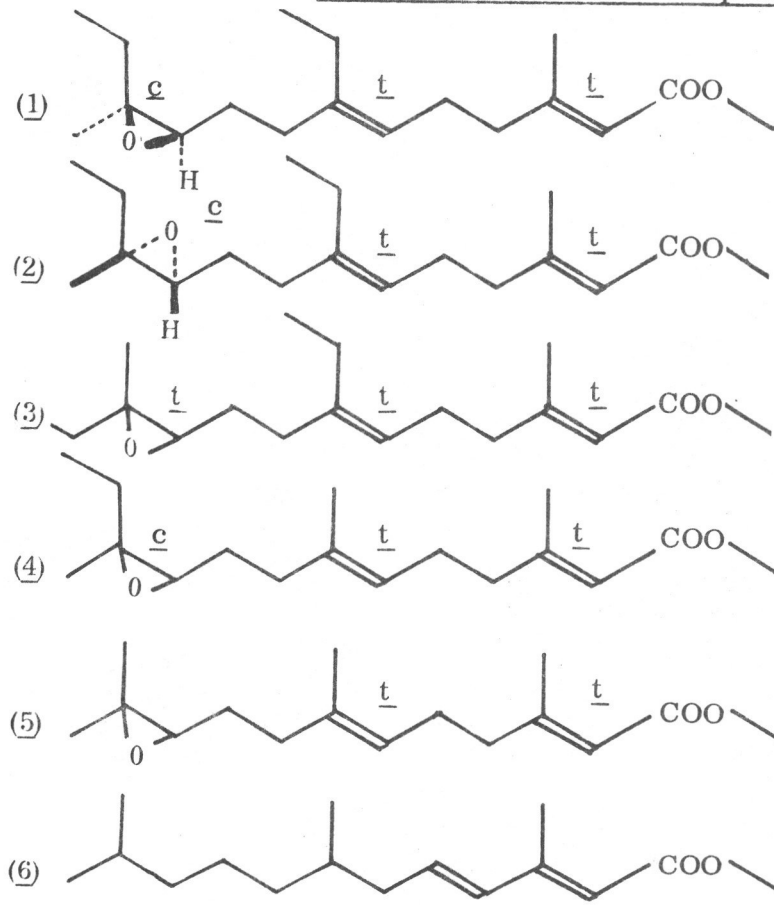
The JH bioassay used was the Galleria wax test. A termite or cockroach test would have been preferable for the detection of possible termite-specific activity. But their sensitivity is too low: 1 µg of (±)-JH-1 per test animal is needed to induce soldier formation in Kalotermes (Lüscher, 1969), and 100 ng per adult Nauphoeta cinerea to induce a vitellogenin titre that shows up in an Ouchterlony immune-test (G. Bühlmann and D. Meyer, unpublished). The specificity and sensitivity of the Galleria test is higher (Table 1).

Table 1. Biological activity of JH and some related compounds in the Galleria wax test (D. Meyer, H. Röller; W.S. Johnson, unpublished)

Compound		Specific Activity (= G.U. per µg Material) x 10^3	Source of the Compound
(+)-JH-1	(1)	1000	Loew and Johnson, 1971
(−)-JH-1	(2)	50	Loew and Johnson, 1971
(±)-all-trans isomer of JH-1	(3)	8	Dahm, Roller and Trost, 1968
(±)-JH-2	(4)	560	J. Siddall, Zoecon Corp.
(±)-methyl 10,11-epoxy-farnesoate	(5)	0.5[a]	Bowers et al. 1965

[a] A. Meyer et al., 1970. Their value is transformed to be in proper relation to our measurements with JH-1.

Structure of JH- active compounds:



JH-titre measurements with this test in Hyalophora cecropia larvae (table 2) resulted in surprisingly low values, although the structure of the JH's of this species is known to be JH-1 (1) and JH-2 (2).

The JH-titre determined in a few other lepidopteran and non-lepidopteran species were also very low.

Table 3 gives the JH-activity found in Macrotermes.

The JH-activity of the queen's anal secretion is not very high if compared with an estimated 1.5 ml secretion given off per day. It remains to be investigated whether the few eggs suspended in the anal secretion and extracted together with it are responsible for the activity. In Hyalophora cecropia JH-activity in the eggs has indeed been demonstrated (Gilbert and Schneiderman, 1961). The intestine tissue carrying the secretion is practically inactive as are the accessory glands (the only glands known in the abdominal tip). Sannasi et al. (1972) claim to have found JH-activity in the anal secretion of queens as well as in soldiers of 5 Indian species of Termitidae with the help of a systemic Tenebrio assay. But since neither quantitative data nor the criteria for the recognition of specific JH-activity are given, the comparison of our data with theirs is impossible.

3) Identification of Termite JH. So far only the structures of JH for 4 lepidopteran species have been established, for Hyalophora cecropia, H. gloveri, Samia cynthia, and, tentatively, Manduca sexta, as JH-1 (1),

Table 2. JH- activity in extracts of larval haemolymph of Hyalophora cecropia. Galleria wax test (D. Meyer and H. Röller, unpublished)

Age of Larvae	G.U. per g Haemolymph[a)]	Haemolymph Collected per Larva in mg
3rd instar, 0- 1 day-old	< 900	8
2- 3 day-old	1760	27
4- 5 day-old	960	26
4th instar, 0- 1 day-old	183	85
2- 3 day-old	375	148
5- 6 day-old	92	264
5th (= last) instar, 0- 4 day-old	2.5	154
5- 8 day-old	4.4	500
9-13 day-old	0.8	670

[a)] 1 G.U. of (±)-JH-1 determined during these measurements varied from 0.7 to 2 pg/pupa.

Table 3. JH-activity of different extracts of Macrotermes subhyalinus, as determined with the Galleria wax test

Body Parts	G.U. per g Fresh weight[a)]	g Fresh weight of Organ or Animal
queen, anal secretion	25,000	0.021 (collected in 20 min.)
whole intestine	100 G.U./ animal	not determined
ovarian accessory gland	310 G.U./ animal	not determined
haemolymph	47,600	3.870
major soldiers, whole	3,240	0.114
minor soldiers, whole	350	0.065

[a)] 1 G.U. of (±)-JH-1 was determined as 2.8 pg/pupa during these measurements.

JH-2 (4) and methyl 10,11-epoxy-farnesoate (5) (Röller et al. 1967; A. Meyer et al. 1968; Dahm and Röller, 1970; D. Meyer, K. Dahm and H. Röller, unpublished; K. Judy and J. Siddal, unpublished). Low doses of (+)-JH-1 can substitute in all tested insect orders for the species' own corpora allata. This, however, constitutes no proof that JH is structurally identical with JH-1 in those insects. An effort is being made to isolate termite JH; only detailed knowledge of its chemical properties will make it possible to study the JH-activity in the anal secretion comparatively.

The JH-active fraction of queen haemolymph extracts shows the same R_f- values on the two thin layer systems described by Dahm and Röller (1970) as JH-1; with benzene: 5% ethyl acetate all activity was found between R_f 0.27 and 0.41, with chloroform: ethyl acetate 2:1 v/v between R_f 0.68 and 0.85. JH-1, JH-2 and methyl 10,11-epoxy-farnesoate will not separate under these conditions. On gas chromatography on OV-1 at 185°, no peak of the expected size was found at the time when JH-1 emerges.

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