Evidence of the Juvenile Hormone Methyl(2E,6E)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate(JH-3) in Insects of Four Orders

K. H. Trautmann, P. Masner, A. Schuler

Dr. R. Maag AG, Dielsdorf, Schweiz

and

M. Suchý and H.-K. Wipf

Socar AG, Dübendorf, Schweiz

(Z. Naturforsch. **29 c**, 757–759 [1974]; received August 5, 1974)

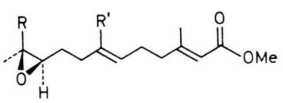
Quantitative Determination, Insects, Juvenile Hormones

With the help of radioactive dilution analysis allowing the qualitative and quantitative determination of all three presently known juvenile hormones (JH-1 to 3) the following seven species of four orders were investigated in the adult stage: Coleoptera: *Tenebrio molitor, Leptinotarsa decemlineata;* Orthoptera: *Schistocerca gregaria;* Blattodea: *Blatta orientalis, Leucophaea maderae, Nauphoeta cinerea;* Hymenoptera: *Apis mellifera.*

In all these species of the 3 known juvenile hormones only methyl (2E,6E)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate (JH-3) was found, in an amount of 0.5 to 11 ng per gram of body weight. The results of the chemical analyses were confirmed biologically by the *Galleria* wax test.

The results demonstrate the wide spread occurrence of JH-3 in insects of different orders.

So far three naturally occurring juvenile hormones are known (JH-1, JH-2 and JH-3). From the extracts of three species of undissected giant silk moths, *Hyalophora cecropia* [1, 2], *Hyalophora gloveri* [3] and *Samia cynthia* [4] methyl (2E,6E)-(10R, 11S)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (JH-1) and the 7-methyl-analog methyl (2E, 6E)-(10R)-10,11-epoxy-3,7,11-trimethyl-2,6-tridecadienoate (JH-2) have been isolated and identified. Recently Judy *et al.* [5] extracted methyl (2E,6E)-(10 R)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate (JH-3) in addition to the known JH-2 from the culture medium of corpora allata of sphingid moth, *Manduca sexta.* The JH-3 was also found by the same group [6] in an orthopterous insect, *Schistocerca vaga,* and by Müller *et al.* [7] in *Periplaneta americana* by using *in vitro* techniques.



JH-1: R=R′=ethyl
JH-2: R=ethyl, R′=methyl
JH-3: R=R′=methyl

In order to answer the question whether these hormones also occur in other insects, particularly from other orders, we developed an analytical method allowing the simultaneous qualitative and quantitative determination of all three compounds. In a first communication [8] we reported the presence of JH-3 in the coleopterous species *Melolontha melolontha.* Our detection method makes use of radioactive dilution analysis. In comparison to the endogenous hormone content a very small amount of tritium labelled JH-1 is added to the ether extract. The addition of only one radioactive tracer hormone permits the isolation of all three compounds because of their very similar behaviour during the purification procedure chosen.

Here we report the presence of JH-3 in seven other species from four different orders.

Requests for reprints should be sent to Dr. K. H. Trautmann, Dr. R. Maag AG, Chemische Fakrif, *Dielsdorf,* Switzerland.

Materials and Methods

Animals

Colorado beetles, *Leptinotarsa decemlineata,* were reared on potato plants under long day conditions (25 °C, 60–70% r h, 18 hours photoperiod). The beetles were in the reproduction phase.

Mealworm beetles, *Tenebrio molitor* L., were kept in crowded conditions (25 °C, 60–70% r h) and fed on bran. For the extraction reproducing animals were taken only when they were 8 to 16 days old.

Desert locusts, *Schistocerca gregaria,* were maintained in mass colonies (30 °C, 50–60% r h) on young wheat plants. For the analysis only older adults were used.

Three roach species, *Blatta orientalis, Leucophaea maderae* and *Nauphoeta cinerea* were mass reared (25 °C, 60 – 70% r h) on dog food pellets and water. Only adult roaches in the reproduction phase were taken for our investigations.

Worker honey bees, *Apis mellifera*, of various ages were received from the Swiss Federal Dairy Research Station, Bee Section, Liebefeld, CH-3097.

Purification, isolation and structure confirmation

The purification and isolation work and the structure confirmation were carried out in the same manner as described in detail for *Melolontha melolontha* [8].

Results and Discussion

When ever possible animals in the reproductive phase were chosen for work-up since it is known that the level of juvenile hormone is very high during egg maturation [9] and that the presence of the hormone is also necessary for the proper function of the genital accessory glands of the male [10]. The results obtained by radioactive dilution technique [8] are summarized in Table I. In all cases only JH-3 was found. If present JH-1 and JH-2 would have been detected with equal sensitivity. The amount of JH-3 is expressed in nanograms per gram of body weight.

From the order of Coleoptera *Leptinotarsa decemlineata* (Chrysomelidae) was investigated. 11.1 ng of JH-3 per gram of body weight were detected. Besides *Melolontha melolontha* [8] this is the second beetle species where the same hormone has been found. We therefore reinvestigated *Tenebrio molitor* (Tenebrionidae), in particular since our earlier negative results (detection limit 0.1 ng/g) with this species [8] had been obtained with extracts of young, three to six days old adults and of larvae. In the present investigation eight to sixteen days old reproducing beetles were used. JH-3 was found in a concentration of 7.4 ng per gram. These two results demonstrate the great importance of the age of an investigated insect species. The fact that the hormone concentration in larvae is below the detection limit of our method (0.1 ng/g) parallels the situation observed in larvae of the grasshopper, *Locusta migratoria* [11]. These findings were further supported by *in vitro* experiments with the corpora allata of *Periplaneta americana* [7] where glands of reproducing females synthesized much more hormone than those of larvae.

Form the order Blattodea three roach species were chosen, *Blatta orientalis* (Blattidae), *Leucophaea maderae* (Blaberidae) and *Nauphoeta cinerea* (Blaberidae). Again only JH-3 could be detected in each of the three species in a concentration not markedly different from that found in beetles. These results are consistent with those found recently for *Periplaneta americana* (Blattidae) [7].

From the order of Orthoptera the desert locust, *Schistocerca gregaria* (Acrididae), was investigated and only JH-3 was found. Its occurrence in *Schisto-*

Table I. Content of JH-3 and the biological activity of the GC-solution corresponding to JH-3 in insect species of various orders.

Insect species	Total animal weight [g]	Average weight per animal [g]	JH-3 content [ng/g body weight]	Biological activity [GU/g body weigth]
Tenebrio molitor L. reproductive adults 8 to 16 days old	1022	0.15	7.4	30
Leptinotarsa decemlineata reproductive adults	500	0.14	11.1	286
Blatta orientalis reproductive adults	842	0.39	3.1	333
Leucophaea maderae reproductive adults	820	2.09	3.5	161
Nauphoeta cinerea mainly reproductive adults	1642	0.5	6.1	83
Schistocerca gregaria older adults	409	2.76	0.5	10
Apis mellifera workers of various ages	740	0.12	2.8	62

cerca vaga has already been reported [6]. The JH-3 content of 0.5 ng per gram is very low. Either the inherent biosynthetic activity of corpora allata is low in this species as observed by Pratt and Tobe [12] or part of the animals analysed had already passed the reproductive stage.

As a representative of Hymenoptera the worker honey bee, *Apis mellifera* (Apidae) was chosen. JH-3 was detected in a concentration of 2.8 ng per gram.

For all insect species investigated the evidence of JH-3 was substantiated by glass capillary GC. With the exception of *Schistocerca gregaria*, where the amount of JH-3 was very low, the structure was further confirmed by GC-MS analysis, as already described [8]. Due to its relatively low intensity, the molecular ion was only observed in the spectrum of the extract from *Leptinotarsa decemlineata*. However, in the upper mass range the following characteristic fragments generally occurred: *m/e* 235 ($M^+ - .OCH_3$), 234 ($M^+ - CH_3OH$), 219 ($M^+ - CH_3OH$, $.CH_3$) and 206 ($M^+ - CH_3OH$, CO). In the intermediate mass range *m/e* 163, 153, 149, 135, 121 and 114 are the most intense ions. Of these *m/e* 114 () is a particularly characteristic rearrangement ion.

An aliquot of two GC-injection solutions which correspond to the purified fractions of JH-1/JH-2 and JH-3 respectively, was diluted with olive oil and tested biologically in the *Galleria mellonella* wax test [13] for each of the species investigated. The results were compared with the corresponding standards. Under our conditions 1.4 pg JH-1 or 62 pg JH-3 correspond to one *Galleria* unit (GU). As the solution corresponding to the JH-1/JH-2 fraction contained labelled JH-1 at a concentration of 0.6 pg/μl (0.3 pg per *Galleria* pupa) we would expect about 25% activity in the wax test if no endogenous JH-1 or JH-2 is present in the extract. For the three holometabolous species *(Apis mellifera, Leptinotarsa decemlineata* and *Tenebrio molitor)* the activity of 30 – 33% was found, for the four more primitive species 0 – 4%. In no case was the presence of endogenous JH-1 or JH-2 indicated either by GC (detection limit 0.1 ng/g) or biological activity (upper limit 0.2 ng JH-1 per gram). In the solutions corresponding to the JH-3 fraction the presence of JH-3 was confirmed approximately at the level indicated by quantitative GC for all species investigated (Table I); only in the case of *Blatta orientalis* is the biological activity higher than expected from GC analysis by a factor of six.

Evidence of JH-3 in four diverse insect orders and in the case of Coleoptera and Blattodea in two different families suggests an universal function for this hormone which is biosynthetically much simpler than JH-1 and JH-2. Although the JH-3 content in six of seven insects investigated is higher than thirty fold the detection limit (0.1 ng per gram) of our method we could not detect the presence of the two other hormones. Judy *et al.* [5] have shown that JH-2 and JH-3 are produced in about equal quantities by culturing porpora allata of *Manduca sexta* and we found a ratio of 4:1 for JH-1 and JH-2 in young adults of *Hyalophora cecropia* [8]. Under these circumstances it seems probable that JH-1 and JH-2 do not occur in the insects of the present investigation. This is further supported by the fact that by cultivating the endocrine glands of *Periplaneta americana* [7] and *Schistocerca vaga* [6] only JH-3 is synthesized. The synthesis of the other two hormones was never observed.

Further investigations are under way in order to clarify whether the three known hormones occur in insects from other orders.

We thank Dr. Gerig, Swiss Federal Dairy Research Station, CH-Liebefeld, for providing the honey bees. The authors are also grateful to Dr. Neuner and Mr. Zarske, Givaudan AG, CH-Dübendorf, for their valuable help performing the GC-MS analyses. We are also grateful to Dr. Vonder Mühll, Dr. R. Maag AG, CH-Dielsdorf, for stimulating discussions.

[1] H. Röller, K. H. Dahm, C. C. Sweely, and B. M. Trost, Angew. Chem. Int. Ed. **6**, 179 [1967].
[2] A. S. Meyer, H. A. Schneiderman, E. Hanzmann, and H. I. Ko, Proc. Nat. Acad. Sci. U.S. **60**, 853 [1968].
[3] K. H. Dahm and H. Röller, Life Sci. **9**, 1397 [1970].
[4] H. Röller and K. H. Dahm, Proc. Conf. Workshop on Hormonal Heterophyly, in press.
[5] K. J. Judy, D. A. Schooley, L. L. Dunham, M. S. Hall, B. J. Bergot, and J. B. Siddall, Proc. Nat. Acad. Sci. U.S. **70**, 1509 [1973].
[6] K. J. Judy, D. A. Schooley, M. S. Hall, B. J. Bergot, and J. B. Siddall, Life Sci. **13**, 1511 [1973].
[7] P. J. Müller, P. Masner, K. H. Trautmann, M. Suchý, and H.-K. Wipf, Life Sci., in press.
[8] K. H. Trautmann, A. Schuler, M. Suchý, and H.-K. Wipf, Z. Naturforsch. **29 c**, 161 [1974].
[9] R. H. Johnson and L. Hill, 6th Conference of European Comparative Endocrinologists, Montpellier, 101 [1971].
[10] V. B. Wigglesworth, Quart. J. Microscop. Sci. **79**, 91 [1936].
[11] R. A. Johnson and L. Hill, J. Insect Physiol. **19**, 1921 [1973].
[12] G. E. Pratt and S. S. Tobe, Life Sci. **14**, 575 [1974].
[13] J. De Wilde, G. B. Staal, C. A. D. De Kort, A. De Loof, and G. Baard, Proc. Kon, Nederl. Akad. Wetensch., Ser. C **71**, 321 [1968].