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BIOCHEMISTRY AND REGULATION OF PHEROMONE PRODUCTION IN *BLATTELLA GERMANICA* (L.) (DICTYOPTERA, BLATTELLIDAE)

COBY SCHAL[1,*] EDINA L. BURNS,[1] MICHAL GADOT,[1] JODY CHASE[2] and GARY J. BLOMQUIST[2]

[1]Department of Entomology, Cook College, Rutgers University, New Brunswick, NJ 08903 and [2]Department of Biochemistry, University of Nevada, Reno, NV 89557-0014, U.S.A.

Abstract—Females of the German cockroach, *Blattella germanica*, produce a contact sex pheromone consisting of the methyl ketones 3,11-dimethyl-2-heptacosanone, 3,11-dimethyl-2-nonacosanone, 29-hydroxy- and 29-oxo-3,11-dimethyl-2-nonacosanone. We review evidence in support of the hypothesis that in adult females the hydrocarbon 3,11-dimethylnonacosane is oxidized to the corresponding methyl ketone pheromone. Recent studies on the precursors and directionality of synthesis of the methyl-branched alkane indicate that it is formed by the insertion of methylmalonyl units derived from propionate, isoleucine, valine, methionine and succinate early in chain elongation. The hydrocarbon is then hydroxylated and oxidized at the 2-position to form the methyl ketone pheromone. The *in vivo* synthesis of pheromone and its accumulation on the cuticle are correlated to the synthesis of juvenile hormone (JH) by the corpora allata (CA) *in vitro* and to oocyte development, suggesting common regulation by JH of pheromone production as well as other reproductive events. The patterns of pheromone and hydrocarbon production in starved, allatectomized and head-ligated females, as well as in females rescued with hormone-replacement therapy, suggest two mechanisms of regulation of pheromone production: a JH-induced conversion of hydrocarbon to pheromone is related to the CA cycle and to oocyte development, while a JH-independent mechanism, which is probably related to feeding, supplies precursors for hydrocarbon biosynthesis.

Key Word Index: German cockroach; sex pheromone; methyl ketone; methyl-branched alkane; juvenile hormone; corpora allata; *Blattella germanica*

INTRODUCTION

Cockroaches have long served as useful models for studies of the neuro-hormonal regulation of vitellogenesis and reproduction (reviews in Huber *et al.*, 1990). The endocrine regulation of sex pheromone production has been studied extensively with sensitive but time-consuming and only semi-quantitative behavioral assays (Barth and Lester, 1973; Schal and Smith, 1990). However, until recently, studies of sex pheromone biosynthesis in cockroaches were precluded because their structures and sites of synthesis had not been elucidated. Herein we review reçent work on the biosynthesis and regulation of the contact sex pheromone produced by adult female *Blattella germanica*. Blomquist *et al.* (1987), Schal *et al.* (1990a) and Schal and Smith (1990) have discussed comparative aspects of the regulation of production of contact and volatile pheromones in cockroaches and other insects.

From behavioral observations of courtship in the German cockroach, Roth and Willis (1952) concluded that a non-volatile contact pheromone, contained in the cuticular wax of females, elicited the wing-raising courtship response in males. Nishida and co-workers (review: Nishida and Fukami, 1983) isolated and identified three components from a hexane wash of 224,000 females. All three components possess a 3,11-dimethyl-2-nonacosanone skeleton with methyl, hydroxyl or carbonyl groups at the C_{29} position. The 3,11-branching pattern appears to be essential for activity (Sato *et al.*, 1976) and although both the methyl- and 29-hydroxy-components have been shown to have (3S,11S) configurations (Nishida *et al.*, 1979), all combinations of stereochemical isomers of the 3,11-positions yield similar wing-raising responses in males (Mori *et al.*, 1978). Biological activity of the pheromone is proportional to its polarity at the C_{29} end; thus, 3,11-dimethyl-2-nonacosanone is less active by an order of magnitude than the 29-hydroxy-analog, and the 29-oxo analog exhibits intermediate activity. Also, reduction of the C_2 carbonyl to a hydroxyl group increases activity by about 10-fold, while a methylene in place of the carbonyl group eliminates activity (Nishida and Fukami, 1983). A fourth pheromone component, 3,11-dimethyl-2-heptacosanone, recently identified from adult females, was found to be less effective than the C29 homolog (Jurneka *et al.*, 1989; Schal *et al.*, 1990b), confirming the results reporting that the length of the alkyl chain is important for activity (Sato *et al.*, 1976).

Interestingly, each of the 4 components elicits the full range of behavioral responses in males, and there appears to be no synergism either between the C27 and C29 methyl ketones or between the C29 methyl ketone pheromone and cuticular hydrocarbons

*Author to whom all correspondence should be addressed.

(Schal *et al.*, 1990b). Thus, the female sex pheromone complex of the German cockroach differs from most lepidopteran and dipteran pheromones, where omission of even minor components affects male response qualitatively and quantitatively. Also, the German cockroach contact pheromone system can accommodate greater changes in pheromone specificity than most lepidopteran systems, which may explain, at least in part, frequent observations of interspecific courtship in cockroaches.

Below we review some of our recent work on the biosynthesis and endocrine regulation of production of cuticular hydrocarbons and the contact sex pheromone of the female German cockroach. We also present new evidence on the relationship between juvenile hormone and pheromone biosynthesis.

MATERIALS AND METHODS

Insects and dissections

Cockroach nymphs that hatched within 4 days of each other were reared in 2 liter glass jars and fed pelleted Purina dog chow and water *ad libitum*. Newly ecdysed (day 0) adult males and females were separated daily and isolated individually or in groups as stated in the results. Both nymphs and adults were kept at 27°C under a 12:12 light:dark photoperiodic regime. Isolated females were allowed to mate on day 8 and females that did not mate were discarded.

Allatectomies (denoted as -CA), head-ligations, decapitations, egg-case implants and hormone therapy experiments were performed as detailed in Schal *et al.* (1990a). Allatectomies were performed on day-0 and some females were exposed to hydroprene replacement therapy (100 μg on a 9 cm dia filter paper lining the dish housing the female) from day-8 to day-15. Starvation was initiated on day-0, as was hydroprene replacement therapy (100 μg on filter paper). Head ligation and decapitation were performed on day-2, as was hydroprene therapy (10 μg on filter paper), and females were extracted on day 8; they were compared to females starved from day 2 to day 8, and to allatectomized females.

Lipid extraction and quantification

Cuticular lipids of individual females were extracted with two 5 min washes, each in 2 ml hexane. Two internal standards, *n*-hexacosane (15 μg) and 14-heptacosanone (0.4 μg), were included during extraction for quantification of the hydrocarbons and the methyl ketone pheromone components, respectively, by gas–liquid chromatography (GLC). The hexane extracts were separated on Biosil-A mini-columns: hydrocarbons were eluted with 7–8 ml hexane, and the oxygenated compounds were eluted with 7–8 ml diethyl ether. Samples were analysed on a HP 5890 GLC equipped with a flame-ionization detector and interfaced with a HP 3390A integrator. Splitless injection was made into a 15 m × 0.53 mm ID SPB-1 column, programmed from 80 to 270 at 20°C per min and then to 320 at 3°C per min. The injector and detector were maintained at 330°C. Data are presented as mean ± SEM.

Details on NMR experiments are in Chase *et al.* (1990).

In vivo synthesis of pheromone

Sodium [1-^{14}C]propionate (56mCi/mmol from ARC, St Louis, Mo.) was injected in 3 μl saline into CO_2-anesthetized females (0.56 μCi per female). After a 10 h incubation in the scotophase at 27°C, 5 groups of 3 females per age were extracted with hexane as described above. The radiolabeled cuticular lipids were separated by TLC (silica gel 60F, EM Science) developed twice in hexane–diethyl ether (93:7 v/v). The hydrocarbon and methyl ketone fractions were counted in a Beckman 3801 liquid scintillation spectrometer.

In vitro synthesis of juvenile hormone

The radiochemical assay for juvenile hormone biosynthesis was adapted from Pratt and Tobe (1974) as described in Gadot *et al.* (1989b).

RESULTS AND DISCUSSION

Biochemistry of hydrocarbon and sex pheromone synthesis

The cuticular extracts of males, females and nymphs of *B. germanica* differ significantly: only adult females accumulate large amounts of the C_{27} and C_{29} methyl ketones and only the 3,11-isomer is present (Jurenka *et al.*, 1989; Schal *et al.*, 1990b). The cuticular hydrocarbons, on the other hand, are qualitatively identical in the three groups, and the major hydrocarbon component is an isomeric mixture of 3,7- 3,9- and 3,11-dimethylnonacosane (Augustynowicz *et al.*, 1987; Carlson and Brenner, 1988; Jurenka *et al.*, 1989). Jurenka *et al.* (1989) suggested, based on the structural similarities between the hydrocarbon and the methyl ketone pheromone components, that the production of the pheromone results from the sex-specific oxidation of its hydrocarbon analog. We conducted experiments designed to examine the biosynthesis of methyl-branched alkanes, to determine whether the methyl branching units were added during the early or late stages of chain elongation, and to test the hypothesis that the branched hydrocarbons serve as precursors to the pheromone components.

To determine whether the methyl groups of the methyl-branched alkanes are incorporated early or toward the end of chain elongation, adult female *B. germanica* were injected with [1-^{13}C]acetate, [2-^{13}C]acetate or [1-^{13}C]propionate (Chase *et al.*, 1990). Carbon-13 NMR of the extracted cuticular hydrocarbons, compared with the natural abundance ^{13}C-NMR spectrum, clearly showed that carbon-1 is enriched by [2-^{13}C]acetate, and in 3-methyl and 3,χ-dimethylalkanes, carbon-2 is enriched by [1-^{13}C]-acetate. Carbon-4, but not carbon-2, is enriched by [1-^{13}C]propionate, and neither [1-^{13}C]acetate nor [2-^{13}C]acetate enrich carbon-4 of 3,χ-dimethylalkanes (Chase *et al.*, 1990). These results clearly demonstrated that carbons 1 and 2 of acetate are incorporated as the chain initiator, that the carbon skeleton of propionate serves as the methyl branch donor, and that the methyl branch unit was added as the second group in methyl-branched alkane biosynthesis (Fig. 1). The apparent labeling of methyl-branched fatty acids of 16–20 carbons by [1-^{14}C]-propionate and of only straight chain saturated and monounsaturated fatty acids by [1-^{14}C]acetate argues that methyl-branched fatty acids are intermediates in branched alkane biosynthesis (Chase *et al.*, 1990).

In the housefly (Dillwith *et al.*, 1982), American cockroach (Halarnkar *et al.*, 1985) and the cabbage looper moth (de Renobales and Blomquist, 1983), radiolabeled valine is incorporated as the methyl branch of branched alkanes, whereas in the termite *Zootermopsis* sp., succinate is the precursor to the methyl branch unit (Chu and Blomquist, 1980). In the German cockroach, the amino acids [G-^{3}H]valine, [4,5-^{3}H]isoleucine and [3,4-$^{14}C_2$]methionine (all of which can be metabolized to propionate),

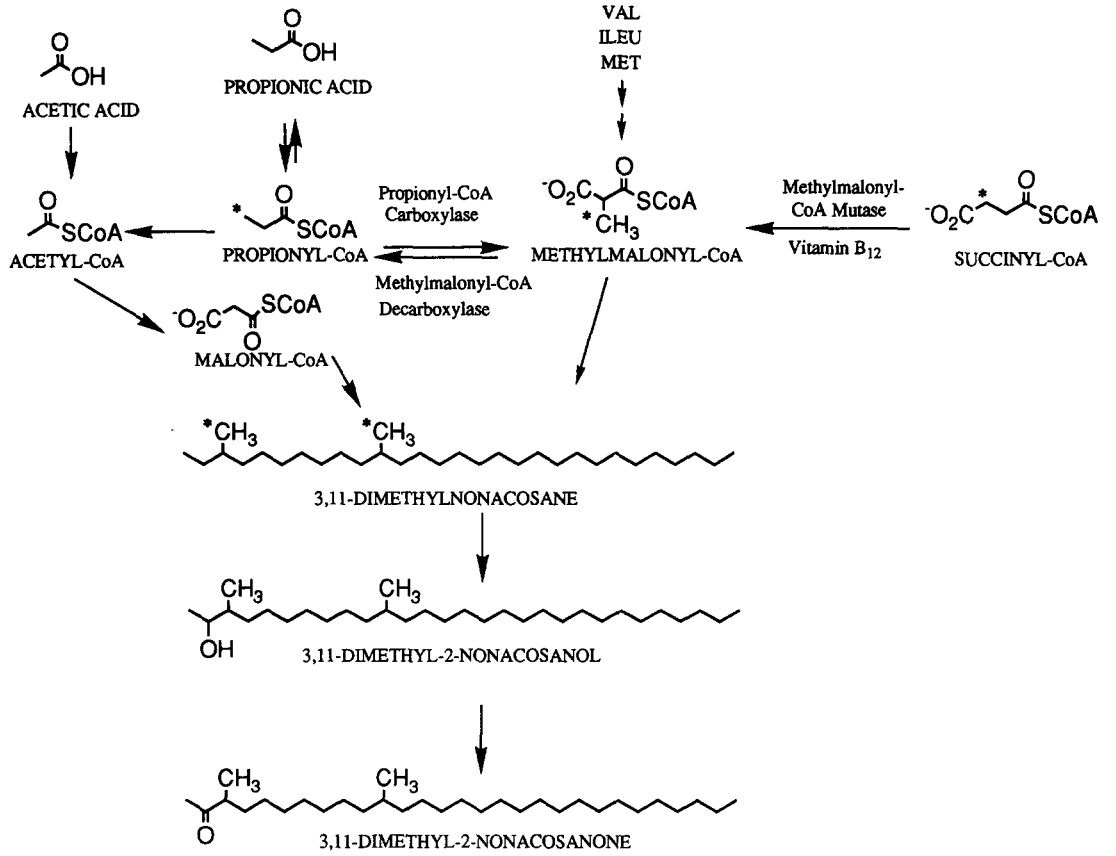

Fig. 1. Proposed precursors and metabolic pathways for the biosynthesis of 3,11-dimethyl-2-nonacosanone, the major female sex pheromone component of the German cockroach, *B. germanica*.

[1-^{14}C]propionate, and succinate label the hydrocarbon and methyl ketone fractions (Chase *et al.*, 1990). NMR studies with [3,4,5-$^{13}C_3$]valine confirmed that its carbon skeleton becomes the methyl branch unit for hydrocarbon biosynthesis (Fig. 1); carbons 3, 4 and 5 of valine become the tertiary, the methyl branch and the internal secondary carbons, respectively, of the hydrocarbon molecule (Chase *et al.*, 1990). The more efficient incorporation into hydrocarbons of [2,3-$^{14}C_2$]succinate rather than [1,4-$^{14}C_2$]-succinate also suggests that succinate is metabolized to methylmalonyl-CoA for incorporation into branched hydrocarbons (Fig. 1), as occurs in the termite. If succinate were metabolized to acetate through malate and pyruvate prior to incorporation into hydrocarbon, both labeled carbons from [1,4-$^{14}C_2$] succinate would be lost as CO_2. However, both labeled carbons from [2,3-$^{14}C_2$]succinate were incorporated into hydrocarbon, suggesting that succinate is metabolized to methylmalonyl-CoA, as occurs in the housefly (Halarnkar *et al.*, 1987).

The hypothesis that the sex pheromone arises from the insertion of an oxygen into the preformed hydrocarbon chain was recently examined by topical application of synthetic [11,12-$^3H_2$]3,11-dimethylnonacosane and [11,12-$^3H_2$]3,11-dimethyl-2-nonacosanol to vitellogenic *B. germanica* females (Chase, Schal, Touhara, Prestwich and Blomquist, unpublished). Radioactivity from the methyl-branched alkane was detected in the two fractions containing 3,11-dimethyl-2-nonacosanol and 3,11-dimethyl-2-nonacosanone. Interestingly, the conversion of [11,12-$^3H_2$]3,11-dimethyl-2-nonacosanol to the corresponding methyl ketone pheromone was very efficient, indicating that the sex pheromone of the German cockroach arises via the hydroxylation and subsequent oxidation of 3,11-dimethylnonacosane (Fig. 1). It is possible that a similar hydroxylation and subsequent oxidation at the 29-position of 3,11-dimethyl-2-nonacosanone gives rise to 29-hydroxy- and 29-oxo-3,11-dimethyl-2-nonacosanone, and studies are being performed to examine this possibility. Since both male and female German cockroaches possess 3,11-dimethylnonacosane, and the production of pheromone is related to juvenile hormone biosynthesis (below), Chase *et al.* (1990) suggested that the sex-specific and 3,11-dimethyl branching pattern-specific conversion of the hydrocarbon to the methylketone pheromone may be regulated by juvenile hormone.

Regulation of pheromone production

(1) Relation between pheromone and juvenile hormone biosynthesis. With a few exceptions, most oviparous and ovoviviparous cockroaches undergo a sexual maturation period preceeding the first vitellogenic cycle. Upon mating, females cease pheromone production and become sexually unreceptive to courting males, but both pheromone production and receptivity may reappear after several oviposition

cycles in oviparous species, or after each protracted period of gestation is ovoviviparous species. The temporal pattern of the onset of receptivity and pheromone production, oocyte development, and JH biosynthesis in *B. germanica* suggests a common neuro-endocrine regulation.

The oviparous German cockroach exhibits a reproductive pattern functionally intermediate between oviparity and ovoviviparity. The vitellogenic phase of oocyte maturation starts on days 4–5 in most individually isolated females, and the oocytes grow most rapidly between days 7 and 10 [Fig. 2(A)]. Females that mate on day 8 generally ovulate and oviposit on days 12–13 and they carry the egg-case externally for 22 days during which time the basal oocytes do not grow. The gestation period is thus functionally similar to "pregnancy" in ovoviviparous and vivparous cockroaches (Roth and Stay, 1962; Tobe and Stay, 1985). The amounts of the most abundant pheromone components (C27 and C29 methyl ketones) increase during oocyte maturation prior to ovulation (Schal *et al.*, 1990b), and the amount of cuticular 3,11-dimethyl-2-nonacosanone has been shown to be related to specific physiological stages in both virgin and mated females, with little change during the protracted pregnancy (Schal *et al.*, 1990a).

We examined one complete gonotrophic cycle of the German cockroach to relate changes in the synthesis and accumulation of pheromone to JH biosynthesis and oocyte development. Cuticular methyl ketone pheromone synthesis, as assayed by the *in vivo* incorporation of radiolabel from sodium-[1-^{14}C]propionate, is related to the activity of the corpora allata (CA) *in vitro*, and both are related to oocyte development in individually isolated females

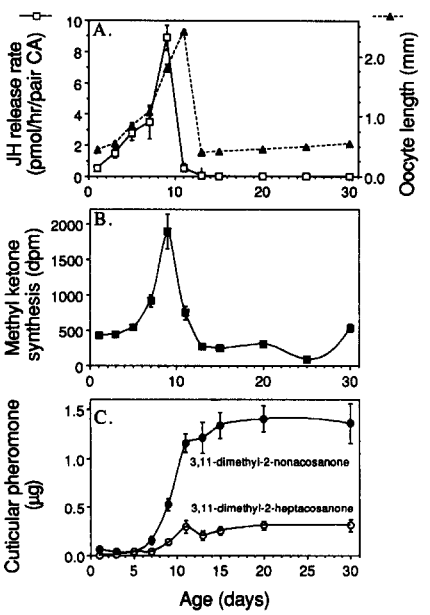

Fig. 2. Relation among (A) juvenile hormone biosynthesis *in vitro* (n = 4–20 per mean), basal oocyte length (n = 4–17 per mean), (B) pheromone biosynthesis *in vivo* (n = 5 per mean), and (C) the accumulation of the two methyl ketone pheromone components on the cuticle throughout one complete gonadotrophic cycle (n = 10–20 per mean). Data in (A) are recalculated from Gadot *et al.* (1989b).

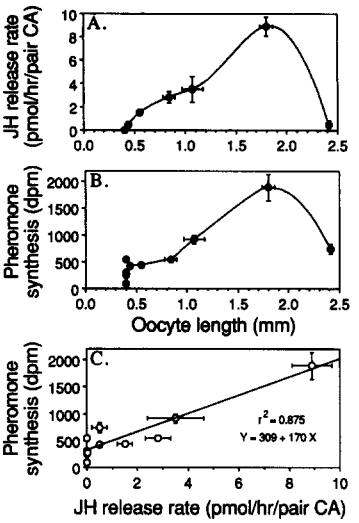

Fig. 3. Relation of (A) juvenile hormone biosynthesis *in vitro* and (B) methyl ketone pheromone biosynthesis *in vivo* to basal oocyte in *Blattella germanica*. (C) Relation between juvenile hormone biosynthesis *in vitro* and methyl ketone pheromone biosynthesis *in vivo*.

(Fig. 2). Females with small basal oocytes synthesize JH and pheromone at low rates. The JH biosynthetic rates increase in a precise relation to oocyte growth [Fig. 2(A)] (Gadot *et al.*, 1989b) and pheromone synthesis [Fig. 2(B)]. Corresponding with chorion formation (oocytes longer than 2.3 mm) and ovulation, JH and pheromone synthesis decline, and in gravid females both are low and growth of the basal oocytes is arrested (Fig. 2).

The amounts of 3,11-dimethyl-2-nonacosanone and 3,11-dimethyl-2-heptacosanone remain relatively unchanged from emergence until day 5, and increase dramatically between 5 and 11 days after emergence [Fig. 2(C)], corresponding to the pattern of synthesis. The amounts of both components remain relatively constant after oviposition and during "pregnancy", followed by a second rapid increase in relation to the second oocyte maturation cycle (Schal, Burns, Jurenka, Chase and Blomquist, unpublished). Pheromone accumulation on the cuticle thus appears to be related to JH biosynthesis (and probably JH titer) much as is basal oocyte length. As both the *in vivo* incorporation of [1-^{14}C]propionate into pheromone and the *in vitro* incorporation of L-[methyl-^{3}H]-methionine into JH-III are similarly related to the size of the basal oocytes [Fig. 3(A), (B)], a significant linear relation is evident between the two biosynthetic parameters [Fig. 3(C)].

(2) Effects of modulators of JH biosynthesis on pheromone production. Since pheromone production in the German cockroach is related to JH biosynthesis, factors that modulate the activity of the CA also affect pheromone production. Thus, the adult female CA are activated more rapidly in grouped females than in isolated females, and the amount of cuticular pheromone by day-6 is greater in grouped females [compare Fig. 2(C) and Schal *et al.*, 1990b].

Because ovarian development and pheromone production are similarly related to CA activity, it is possible that the ovaries might affect pheromone

production either directly by producing specific modulators, or indirectly by affecting CA activity. For example, in the house fly, where pheromone production is also related to ovarian maturation, the ovaries play a key direct role in pheromone biosynthesis: removal of the ovaries shortly after the female emerges inhibits the production of pheromone, while reimplantations of previtellogenic oocytes or treatment with 20-hydroxyecdysone restores pheromone production (review: Blomquist *et al.*, 1987).

In the cockroaches *Diploptera punctata* and *Nauphoeta cinerea* ovariectomy abolishes the activation of the CA (review: Tobe and Stay, 1985). However, removal of the ovaries did not suppress pheromone production in the cockroaches *Byrsotria fumigata* (Barth, 1962) and *Supella longipalpa* (Smith and Schal, 1990), suggesting that, in these species, either the CA become active in ovariectomized females, or alternatively, that the relatively low CA activity in ovariectomized females is necessary and sufficient for pheromone production. In support of the latter hypothesis, intact *S. longipalpa* females initiate pheromone production and calling (pheromone release) when JH biosynthesis is very low (<5% of peak rates) in both the first and second gonadotrophic cycles (Smith *et al.*, 1989; Smith and Schal, 1990). As in other cockroach species, ovariectomy also fails to abolish pheromone production in *B. germanica* (Schal, 1988). However, in contrast to other species, the CA of ovariectomized *B. germanica* are activated to peak JH biosynthetic rates that are not significantly different from those in intact females (Gadot *et al.*, 1991).

Virgin *B. germanica* females usually abort their egg-cases prematurely within 3 days of ovulation (Gadot *et al.*, 1989a), and as with artificial premature removal of the egg-case in mated females, the CA are activated prematurely (for the second ovarian cycle), as evidenced by an acceleration of oocyte development (Roth and Stay, 1962). Virgin females, and mated females with manually removed egg-cases, thus experience a more rapid activation of the CA and greater accumulation of pheromone on the cuticle (Schal *et al.*, 1990a). Conversely, implantation of a wax-filled egg capsule into the vestibulum of newly ecdysed females inhibits the growth of the terminal oocytes, presumably through inhibition of CA activity via the ventral nerve cord (Roth and Stay, 1959), and it significantly inhibits the production of pheromone, as assayed 15 days after treatment (Fig. 4; Schal *et al.*, 1990a).

The anti-allatin precocene II also inhibits or delays both pheromone production and oocyte growth in a dose-dependent manner. Since both parameters can be rescued by treatment with the JH analog (JHA) hydroprene (Fig. 4; Schal *et al.*, 1990a), precocene appears to influence pheromone production indirectly by inhibiting JH production. Similarly, starvation, which inhibits CA activity in newly ecdysed *B. germanica* (Schal, unpublished), also results in a significantly reduced accumulation of cuticular pheromone by day 15. Here too, hydroprene rescues pheromone production in a dose-dependent manner, but to significantly lower levels than in normally fed JHA-induced females or in allatectomized JHA-induced females (Fig. 4; Schal *et al.*, 1990a; note

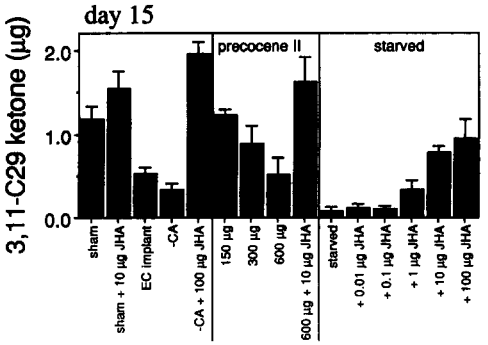

Fig. 4. Accumulation of 3,11-dimethyl-2-nonacosanone, the major sex pheromone component of *Blattella germanica*, on the cuticle of 15-day-old females after various experimental treatments applied to newly emerged females (n = 7–13 per treatment). Data from Schal *et al.* (1990a).

that Table 3 in Schal *et al.*, 1990a should read µg, not ng).

Pheromone production in the German cockroach can be induced with an exogenous JHA in the absence of the head, but hydroprene rescues pheromone production in allatectomized females more effectively than in either intact starved females or in head-ligated or decapitated females (Figs 4 and 5; Schal *et al.*, 1990a). In Fig. 5 the head-ligations and decapitations were conducted on day 2, some insects were exposed to hydroprene and all females were extracted on day 8. These data suggest a dependence of pheromone production on feeding, which may be related to two independent mechanisms: (1) starvation in intact females directly inhibits CA activity in *B. germanica* (Schal, unpublished), and (2) it may limit the availability of precursors for hydrocarbon and pheromone biosynthesis in starved as well as in head-ligated and decapitated females. The importance of JH in pheromone production is clearly evident from the inducibility of pheromone production by hydroprene in starved females by days 8 and 15 (Figs 4 and 5).

The relationship between the precursor hydrocarbon, 3,11-dimethylnonacosane, and its pheromone analog is evident in starved females, where the hydrocarbon supply is limited, probably due to the lack of feeding. The amount of the GLC peak corresponding to the 3,11-(major isomer), 3,9- and 3,7-dimethylnonacosane in 15-day-old starved

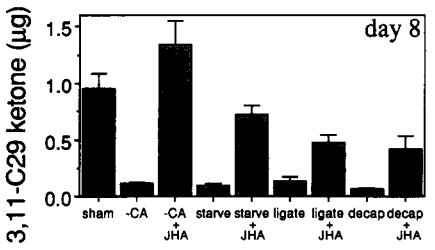

Fig. 5. Accumulation of 3,11-dimethyl-2-nonacosanone on the cuticle of 8-day-old females after various experimental treatments (n = 5–10 per treatment). All hydroprene (JHA) treatments used 10 µg applied in acetone to filter papers to which individual females were exposed. See Materials and Methods and Schal *et al.* (1990a) for experimental conditions.

females is only $10.1 \pm 4.1\ \mu g$ compared with $39.2 \pm 2.5\ \mu g$ in fed females ($P < 0.01$, t-test). By contrast, allatectomized females can feed and, in the absence of endogenous JH, the cuticular (and internal—unpublished) hydrocarbon increases to $50.0 \pm 5.8\ \mu g$, well beyond the amount found by day-15 in sham-treated intact females ($39.2 \pm 2.5\ \mu g$, $P < 0.01$, t-test). This is likely due to both the lack of conversion of the hydrocarbon to methyl ketone as well as the lack of uptake of hydrocarbons by the oocytes. Furthermore, stimulation of pheromone production with hydroprene in either head-ligated or decapitated females reduces the cuticular C29 dimethyl alkane by 16 and 27%, respectively, by day 8 (unpublished). We therefore hypothersize, based on these results and the apparent conversion of the dimethyl alkane to the methyl ketone pheromone, that JH regulates pheromone production by increasing the activity of the enzyme system (presumably involving a polysubstrate mono-oxygenase) that converts, the 3,11-dimethylalkane to the corresponding methyl ketone.

The incomplete suppression of pheromone production in allatectomized females (Figs 4 and 5), increasing amounts of pheromone in older allatectomized females (Schal, unpublished), and increased pheromone synthesis *in vivo* before any detectable increases in JH synthesis (Fig. 2, day 30), together argue for a JH-independent mechanism for pheromone production that is probably related to feeding. For instance, if females resume feeding before hatching of the egg-case on days 32–33, hydrocarbon precursors may accumulate, resulting in increased pheromone production on day 30. Our preliminary results indicate that both the feeding pattern in isolated females (Hamilton and Schal, 1988) and the pattern of hydrocarbon synthesis are similarly related to the ovarian cycle, with substantial feeding, and synthesis and accumulation of hydrocarbons before ovulation. A relationship between feeding and hydrocarbon synthesis was also shown during larval development in *Trichoplusia ni* (Dwyer *et al.*, 1986) and *Spodoptera eridania* (Guo and Blomquist, unpublished). Thus, accumulation of cuticular pheromone may result from a combination of feeding-stimulated synthesis of hydrocarbons (precursor accumulation internally) and their JH-induced conversion to pheromone.

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