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BIOCHEMISTRY AND ENDOCRINE REGULATION OF SEX PHEROMONE PRODUCTION IN THE HOUSEFLY AND GERMAN COCKROACH

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INTRODUCTION

There has been a dramatic increase of interest in the biosynthesis of insect sex pheromones over the last decade (Prestwich and Blomquist, 1987). It now appears that many of the rather novel compounds that are components of insect sex pheromones are produced by the addition of a few ancillary enzymes to those of "normal" metabolism. For example, the carbon skeletons of many lepidopteran sex pheromones are produced from common fatty acids by a novel delta-11 desaturase and highly specific chain-shortening reactions (Bjostad et al., 1987). In the Coleoptera, sex pheromones are often produced by the use of one or two highly specific enzymes that convert dietary material, usually isopreniod, to active pheromones (Vanderwel and Oehlschlager, 1987). By using one or a few ancillary enzymes to alter the products of the usual lipid metabolic pathways or to alter dietary constituents, the insect requires much less genetic material than would be necessary to code for a complete set of enzymes that would be expressed only in the pheromone-producing tissue. This phenomenon appears to exist in the housefly, *Musca domestica,* and the German cockroach, *Blattella germanica,* as hormone control of several enzymes could account for alteration of the products of cuticular lipid biosynthesis to produce the female-specific sex pheromone components.

Studies in the early 1960's implicated juvenile hormone (JH) in the regulation of volatile sex pheromones in several cockroaches and beetles (Barth, 1965). The use of bioassays to monitor pheromone production in these studies, however, made it difficult to quantify levels of pheromones and impossible to determine whether regulation occurred at the level of biosynthesis or release. Studies using radiochemical techniques to monitor sex pheromone production in the female housefly demonstrated that sex pheromone biosynthesis was induced by ecdysteroids produced by the maturing ovary. In cockroaches, beetles, flies, and some moths, the same hormones that control vitellogenesis also regulate sex pheromone production, thus coordinating egg maturation with mating. Recent work in several species of Lepidoptera has demonstrated that a neuropeptide, called pheromone biosynthesis activating neuropeptide (PBAN), induces sex pheromone production (Raina and Menn, 1987). There is considerable interest in the hormonal control of enzymes that produce sex pheromones, and a number of laboratories are currently addressing this problem. Results from work on the housefly and the German cockroach are discussed herein.

BIOSYNTHESIS AND ENDOCRINE REGULATION OF SEX PHEROMONE PRODUCTION IN THE HOUSEFLY

A sex pheromone was first demonstrated in the housefly by Rogoff et al. (1964),

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identified as Z9-23:Hy [(Z)-9 tricosene, muscalure] (Carlson et al., 1971), and shown to attract males in an olfactometer at doses as low as 0.07 µg (Adams et al., 1984). The C_{23} epoxide (9,10-epoxytricosane), C_{23} ketone [(Z)-14-tricosen-10-one] (Uebel et al., 1978) and methylalkanes (Uebel et al., 1976; Rogoff et al., 1980) are also part of the pheromone. Adams and Holt (1987) showed that each of these pheromone components had different roles in male courtship behavior. Z9-23:Hy increased male mating strike activity toward females and other males. The epoxide and ketone decreased the number of homosexual mating strikes, thus acting as a sex recognition factor. The methylalkanes acted as arrestants and increased the amount of time that a male spent with a treated model.

Females with previtellogenic ovaries at stage 2 or 3 (0-2 days post emergence) do not have detectable amounts of any of the C_{23} sex pheromone components (Dillwith et al., 1983). The C_{23} sex pheromone is first detected at stage 4 and steadily increases to a maximum by stage 8.

The sex pheromone of the housefly consists of modified cuticular lipids. Newly emerged males and females have similar cuticular lipid profiles: the major hydrocarbon components are (Z)-9-alkenes of 27 carbons and longer. From stages 4 through 8 (vitellogenic stages), both the percentage of alkenes and the percentage of Z9-27:Hy in the alkene fraction decrease, while the percentage of Z9-23:Hy increases. As Z9-23:Hy is readily converted to both the C_{23} epoxide and ketone (Blomquist et al., 1984b; Ahmad et al., 1987), the increase in Z9-23:Hy is mirrored by a concomitant increase in the C_{23} epoxide and ketone. The incorporation of radioactivity from labeled acetate into the C_{23} sex pheromone components follows a pattern consistent with that for the amount of pheromone present and emphasizes the role that the maturing ovary plays in initiating sex pheromone production (Dillwith et al., 1983).

Because pheromone production is correlated with ovarian development, it is possible that both processes are regulated by a common factor, as has been demonstrated in some cockroaches and beetles, where JH induces vitellogenesis and sex pheromone production. Alternatively, a product of the developing ovary could initiate pheromone production. To determine which process occurs in the housefly, experiments were performed to examine the effect of removing the corpus allatum-corpus cardiacum (CA-CC) complex and of ovariectomy on sex pheromone production. While removal of the ring gland had no effect on preventing sex pheromone production, flies ovariectomized within 6 hours of emergence did not produce detectable amounts of any of the C_{23} pheromone components. If a product from the developing ovary were involved in inducing sex pheromone synthesis or the ovary mediated in regulation by other systems, then reimplanting ovaries into ovariectomized insects should restore pheromone production. When insects ovariectomized within 6 hours of emergence received ovaries on day 4 and were then maintained for 3 days, they produced C_{23} sex pheromone components at levels similar to a female with stage 5 ovaries. All of the implanted ovaries became vitellogenic with stages varying from 4-6 (Adams et al., 1984a, b). Thus, it was concluded that either the ovary mediated in pheromone production or a product from the ovary stimulated sex pheromone biosynthesis.

Because females of some insects have repeated reproductive cycles and mate only during specific periods within each cycle, it has been suggested that pheromone production might be humorally mediated. Engelmann (1960) first suggested that products from the corpora allata mediated receptivity in the female cockroach *Leucophaea maderae*. Subsequent studies with the Cuban cockroach, *Byrostria fumigata* (Barth, 1961, 1962), showed that females required functional CA to produce sex pheromone. Furthermore, JH was shown to mediate sex pheromone production in other insects, including the cockroaches *Pycnoscelus indicus* (Barth, 1965), and *Supella longipalpa* (Smith and Schal, 1990a), the moth *Pseudaletia unipuncta* (Cusson and McNeil, 1989) and various beetles (Vanderwel and Oehlschlager, 1987) including *Tenebrio molitor* (Menon, 1970, 1976) and *Ips paraconfusus* (Hughes and Renwick, 1977; Borden et al., 1969). As JH induces vitellogenesis and regulates ovarian development and other reproductive events in many insect species, the observation that JH also regulates sex pheromone production suggested that the same hormone may be used to coordinate different reproductive events (Blomquist and Dillwith, 1983).

In many Diptera, ecdysteroids have been shown to play a role in regulating reproductive processes, including vitellogenin synthesis (Adams et al., 1985; Hagedorn, 1985; Bownes, 1986). To determine if ecdysteroids can restore sex pheromone production in ovariectomized flies, a single dose of 20-hydroxyecdysone (20-HE) was injected into insects that had been ovariectomized within 6 hr of emergence. At 16 and 24 hrs after 20-

HE treatment, flies produced all of the C_{23} pheromone components. Doses as low as 50 ng injected repeatedly (20-HE has a half life of 16 minutes in the housefly [Adams et al., 1985]) at regular intervals into ovariectomized insects induced sex pheromone production.

Vitellogenin is normally produced only by females, but the injection of 20-HE into male *Drosophila melanogaster* (Bownes, 1982) and *Sarcophaga bullata* (Huybrechts and De Loof, 1977, 1981) induced vitellogenin production. Thus, experiments were performed to determine whether male houseflies, which normally do not produce any of the C_{23} sex pheromone components, could be induced to produce female-specific pheromones after ovary implants or injections of 20-HE. Implanting ovaries or injecting 20-HE into male houseflies induced sex pheromone production, including all of the C_{23} pheromone components, in a dose and time dependent manner (Blomquist et al., 1984a). Control males did not produce any of the C_{23} pheromone components. Thus, these data demonstrate that males can biosynthesize female sex pheromone, and that treatment with 20-HE alters the production of cuticular components such that Z9-23:Hy becomes a major product. Males and females of all ages readily metabolize Z9-23:Hy applied to their surfaces to the corresponding epoxide and ketone (Blomquist et al., 1984b) via a cytochrome P450 polysubstrate mono-oxygenase (Ahmad et al., 1987).

Further evidence that ecdysteroids directly induce sex pheromone production comes from studies in which isolated male housefly abdomens were injected with 20-HE, and assayed 24 hours later for their ability to produce Z9-23:Hy. The results showed that radioactivity was incorporated into Z9-23:Hy (Blomquist and Adams, unpublished data).

Female houseflies that had the CA-CC complex removed within 6 hours of eclosion still produced Z9-23:Hy, C23 epoxide and C23 ketone when assayed at 4 to 6 days postemergence (Blomquist and Adams, unpublished observations) and also developed ovaries to stage 4. This suggests that JH is not directly required for pheromone production. More recent studies were performed to determine if JH, while unable to induce sex pheromone biosynthesis by itself, might synergize the effect of ecdysteroids in inducing sex pheromone production. The results of this study (Blomquist and Adams, unpublished), in which varying doses of a JH analogue and 20-HE were administered at 18 hour intervals further indicate that JH does not play a role in inducing sex pheromone biosynthesis.

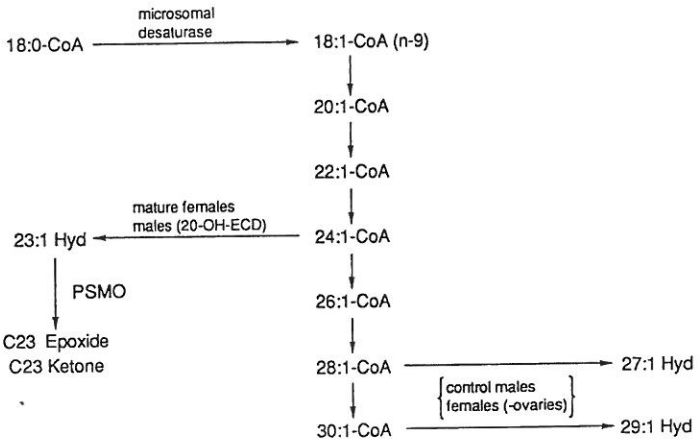

Fig. 1. Biosynthesis of alkenes by houseflies. Mature females and males treated with 20-hydroxyecdysone (20-OH-ECD) produce Z9-23:Hy whereas newly emerged females, control males and ovariectomized females produce alkenes of 27 carbons and longer.

Sex pheromone-producing tissues in insects vary in complexity from individual unicellular glands distributed throughout the integument to elaborate internal cellular glands (Percy-Cunningham and MacDonald, 1987). Schlein et al. (1980) reported that unicellular glands on the legs of female houseflies synthesized the sex pheromone. Subsequent work using isolated tissue and radio-tracer techniques demonstrated that the sex pheromone com-

ponents of the housefly were synthesized by epidermal tissue (Dillwith et al., 1981; Dillwith and Blomquist, 1982) as expected for modified components of cuticular lipid (Blomquist et al., 1987.

Biosynthesis of Z9-23:Hy in female houseflies occurs by the microsomal elongation of oleoyl-CoA to a 24 carbon fatty acyl moiety which is then converted to an alkene one carbon shorter (Fig. 1) (Blomquist et al., 1987). The elongation of oleoyl-CoA to 20-, 22- and 24-carbon monounsaturated acyl derivatives requires malonyl-CoA and either NADPH or NADH (de Renobales et al., 1986; Vaz et al., 1988). Microsomes prepared from abdomens have higher elongation activity than those prepared from either thorax or head tissue (Vaz et al., 1988), and microsomes prepared from abdominal epidermal tissue have higher activity than those prepared from fat body tissue. Preliminary data using microsomal preparations from 2 day old males and 1-day-old females (both of which produce alkenes of 27 carbons and longer) versus 3 or 4 day old females or males treated with 20-HE (in which the major hydrocarbon produced is Z9-23:Hy) indicate differences in the rates of elongation of 18:1-CoA, 20:1-CoA, 22:1-CoA and 24:1-CoA. In both situations, 18:1-CoA is readily elongated, but when the insect is producing primarily Z9-27:Hy (control males or previtellogenic females), larger amounts of 26:1 fatty acid and 28:1 fatty acid are produced than when the insect is producing Z9-23:Hy. This indicates that the elongation reactions play a role in determining the chain length of the hydrocarbon product. Furthermore, when 24:1-CoA is used as the substrate, it is much more efficiently elongated by microsomes prepared from insects which are synthesizing alkenes of 27 carbons and longer (males and previtellogenic females) than those which produce Z9-23:Hy (Vaz et al., 1989). A possible explanation for the differences in chain length specificity of the housefly elongation system under conditions where ecdysteroids are present (mature females) or absent (males, previtellogenic females) is that there are two or more chain-elongation systems, each with a different chain length specificity. There could be one enzyme or set of enzymes that elongates fatty acyl CoAs of 18:1 to 24:1, and then a different elongation enzyme or set of enzymes that elongate 24:1 to 28:1 and 30:1. This possibility is currently being examined.

BIOSYNTHESIS AND ENDOCRINE REGULATION OF SEX PHEROMONE PRODUCTION IN THE GERMAN COCKROACH

Roth and Willis (1952) demonstrated that *B. germanica* females possess a substance in the cuticular wax that could elicit courtship wing-raising displays in males. Unlike the American cockroach, *Periplaneta americana*, in which the pheromone could be absorbed to filter paper or feces and would elicit wing- raising in an olfactometer, the pheromone of *B. germanica* stimulated male courtship only upon contact (Roth and Willis, 1952). Four components have been identified, all of which possess a 3,11-dimethyl ketone skeleton: 3,11-dimethyl-2-nonacosanone (Nishida et al., 1974), 3,11-dimethyl-2-heptacosanone (Schal et al., 1990b), 29-hydroxy-3-11-dimethyl-2-nonacosanone (Nishida et al., 1976), and 29-oxo-3,11-dimethyl-2-nonacosanone (Nishida and Fukami, 1983). The methyl ketone fraction of male extracts did not contain either the C_{27} or C_{29} component and did not elicit sexual responses in males (Nishida and Fukami, 1983; Schal et al., unpublished).

The ratio of 3,11-dimethyl-2-nonacosanone to 3,11-dimethyl-2-hepta-cosanone in surface washes of sexually receptive females was approximately 60:40, while the other two components comprised only minor fractions of the mass (Schal et al., 1990b). The alcohol was more active, however, and the 29-oxo methyl ketone was less active than 3,11-dimethyl-2-nonacosanone (Nishida and Fukami, 1983), and the C27 methyl ketone was at least an order of magnitude less effective at eliciting male wing-raising behavior (Schal et al., 1990b). Fifty and 75 percent of males responded to 1 and 3.5 ng of 3,11- dimethyl-2-nonacosanone, respectively. There are also various unknowns in the TLC methyl ketone fraction which may comprise other pheromone components. Moreover, presence of 3,11-dimethylhentriacontane in cuticular extracts of females (Carlson and Brenner, 1988), together with the suggestion that the methyl ketones are formed through oxidation of the respective alkanes by a 3,11-dimethyl specific enzyme (Jurenka et al., 1989), would predict that 3,11-dimethyl-2-hentriacontanone will be found as another pheromone component.

Activity of the sex pheromone blend on behavior of *B. germanica* was strikingly different from blends in other insects (e.g., Lepidoptera) including the housefly. Each component alone could elicit the complete courtship wing-raising response in male *B. ger-*

manica (Nishida and Fukami, 1983; Schal et al., 1990b), whereas in most insects deletion of components reduces sexual responses (review: Tamaki, 1985). Combining the two methyl ketones did not enhance their activity, but rather their combined effectiveness was intermediate between the activity of each component alone (Schal et al., 1990b). Likewise, cuticular hydrocarbons, which are qualitatively identical in nymphs and adult males and females (Jurenka et al., 1989), did not enhance the activity of the pheromone.

Behavioral assays of males, using excised female antennae from various age insects, showed that the antennae from newly emerged females and vitellogenic females elicited wing-raising in most males, but male response was low to antennae of previtellogenic females (Nishida and Fukami, 1983; Schal et al., 1990b). Gas-chromatographic analyses of female cuticular washes showed, however, that the amounts of both C_{27} and C_{29} methyl ketones, and the C_{27} and C_{29} methyl alcohol gradually increased after emergence, in relation to oocyte maturation, until ovulation and oviposition (day 9 grouped females or day 12 isolated females) (Schal, 1988; Schal et al., 1990a, 1990b). This discrepancy between behav-ioral and analytical results prompted Schal et al. (1990b) to hypothesize that newly emerged females might contain other factors that can induce wing-raising in males. Following ovipo-sition in mated females, the mass of 3,11-dimethyl-2-nonacosanone remained relatively unchanged through approximately 20 days of "pregnancy" (the female retains the egg case externally, attached to the vestibulum), during which time oocyte development is arrested. Pheromone production and oocyte maturation resume after the egg case hatches (Schal et al., 1990a; unpublished). In virgin females, the first infertile egg case is aborted prematurely (Gadot et al., 1989b), and both oocyte maturation and pheromone production resume earlier than in mated females (Schal et al., 1990a). Thus, a correlation between pheromone production and oocyte maturation was established.

The *in vivo* incorporation of radioactivity from labeled propionate into the methyl ketone fraction followed a pattern, as in the housefly, that was consistent with that for mass (Schal, Burns, Gadot, Jurenka, Blomquist, unpublished). The greatest incorporation of radioactivity corresponded to the most rapid oocyte development, while during pregnancy little ^{14}C was incorporated into pheromone components. The patterns of pheromone biosynthesis over time was identical to that of JH biosynthesis by the CA *in vitro* (Gadot et al., 1989a). As a function of basal oocyte length, the pattern of pheromone synthesis (Schal, Burns, Gadot, Jurenka, Blomquist, unpublished) corresponded well with that of JH biosynthesis in both virgin and mated females (Gadot et al., 1989a). Thus it appeared that JH, which plays a key role in reproduction in B. germanica (see Kunkel, 1981; Gadot et al., 1989a), also controls pheromone production.

In the cockroach *Byrsotria fumigata*, allatectomy inhibited both pheromone production and mating (Barth, 1962), but mating occurred in allatectomized females treated with pheromone (Roth and Barth, 1964). In *Supella longipalpa* both volatile sex pheromone production and calling behavior were suppressed by allatectomies (Smith and Schal, 1990a). In both species, treatment with JH or a JH analog induced pheromone production (Bell and Barth, 1970; Smith and Schal, 1990a). In *B. germanica*, allatectomy, decapitation, head-ligation, or inhibition of the CA with precocene or with egg case implants into the vestibulum, all reduced, but did not eliminate, pheromone production (Schal et al., 1989a). Reimplantations of CA and treatment of CA-deprived- or CA- inhibited-females with a JH analog induced pheromone production in a dose-dependent relationship (Schal et al., 1990a). Thus, it appears that pheromone production in *B. germanica* is related to JH, and it proceeds, albeit at low rates, in CA-deprived females. Long-term allatectomy studies revealed that the rates of pheromone synthesis (radio-tracer studies) and accumulation (gas chromatographic studies) were low in allatectomized females. By day 30, however, they contained equal amounts of pheromone to sham-operated females (Schal, Burns, Blomquist, unpublished). Differences between head-ligated and allatectomized females (Schal et al., 1990a) suggest that this non-JH-regulated process might be related to feeding.

Feeding appeared to affect pheromone production in two ways. First, by affecting CA activity, feeding or starvation could influence pheromone production. Weaver (1984) showed that food and water were essential for stimulation of CA activity and oocyte growth in *P. americana*. Protein-deprived *Diploptera punctata* females had lower rates of JH synthesis in vitro and they oviposited later than control females (Woodhead and Stay, 1989). In *B. germanica,* starvation completely suppressed JH synthesis (Schal, unpublished). Females starved from day 0, with access to water only, accumulated less 3,11-dimethyl-2-nonacosanone by day 15 than allatectomized females (Schal et al., 1990a). Starved females,

induced by treatment with a juvenile hormone analog, produced significantly less pheromone than either induced or control fed females, indicating that, in addition to its effect on JH, feeding might directly influence pheromone production. Because allatectomized females accumulated greater amounts of 3, 11-dimethylnonacosane (presumed pheromone precursor) than sham-operated females, and starved females do not, our working hypothesis is that starvation removes or redirects essential substrates for pheromone synthesis.

Thus, two mechanisms appear to regulate sex pheromone production in *B. germanica*: a JH-dependent mechanism which may induce conversion of a late precursor (possibly hydrocarbon) to pheromone (see below), and a feeding-related mechanism which provides substrates for the synthesis of hydrocarbons.

Regulation of the contact sex pheromone of *B. germanica* appears to differ significantly from regulation of volatile sex pheromones in other cockroach species. Using behavioral assays, allatectomy was shown to completely suppress pheromone production in *B. fumigata* (Barth, 1962), *P. americana* and *Pycnoscelus indicus* (cited in Barth and Lester, 1973). In *Supella longipalpa,* males normally respond in an olfactometer to 0.001 of a female equivalent (Liang and Schal, 1990), but males did not respond to extracts of whole allatectomized females and female calling behavior was also suppressed (Smith and Schal, 1990a).

B. germanica also respond differently to induction by JH or JH analog than do species with volatile pheromones. Both low and high doses of JHA induced pheromone production in allatectomized and intact *B. germanica* females (Schal et al., 1990a). Allatectomized *B. fumigata, P. americana,* and *S. longipalpa* females treated with low dosages of JH analog produced pheromone (Bell and Barth, 1970; Barth and Lester, 1973; Smith and Schal, 1990a), but exposure of both *B. fumigata* (Bell and Barth, 1970) and *S. longipalpa* (Smith and Schal, 1990a) to high doses of JH analog delayed or suppressed pheromone production and calling behavior (in *S. longipalpa*). The hypothesis that high titers of JH turn off pheromone production because they are normally associated with mating (Bell and Barth, 1970) does not explain this phenomenon, as CA from virgin and mated *S. longipalpa* females attained the same levels of JH synthesis *in vitro* (Smith et al., 1989), and pheromone production is not suppressed in virgins when the CA are maximally active (Smith and Schal, 1990a).

Also, in *B. germanica*, unlike *B. fumigata* and *S. longipalpa*, mating does not inhibit pheromone production; in fact, CA activity increased after mating (Gadot et al., 1989a) and both mass of the pheromone (Schal, 1988; unpublished) and its synthesis (Schal, Burns, Gadot, Jurenka, Blomquist, unpublished) increase after mating. Pheromone is also produced in mated *B. germanica* females in the second ovarian cycle (Schal et al., 1990a), whereas in *S. longipalpa,* mating suppresses pheromone production and calling for at least 12 ovarian cycles (Smith and Schal, 1990b). Thus, as with JHA applications, a direct positive relationship appears to exist between endogenous JH and pheromone production in *B. germanica:* as JH and JHA increase, so does pheromone production.

Regulation of contact pheromones in *B. germanica* thus appears to be more similar to regulation of pheromone production in the housefly (above) than to other cockroaches (see Schal and Smith, 1990). As in *B. germanica*, both mass and synthetic rate of the sex pheromone increased in vitellogenic and post-vitellogenic female houseflies (Dillwith et al., 1983) and accumulation of pheromone on the cuticle continued after mating. As in *B. germanica,* pheromone production in the housefly was induced by both low and high doses of the regulatory hormone (JH and 20-HE, respectively).

It is possible, as in the housefly, that ovarian factors mediate the regulation of pheromone production. Ovariectomy did not eliminate pheromone production in the ovoviviparous *B. fumigata* (Barth, 1962) and in the oviparous *S. longipalpa* (Smith and Schal, 1990a). However, because neither study measured both pheromone production and JH synthesis in ovariectomized females, it is not known whether the CA were activated or whether pheromone production occurred in the absence of JH. For example, in the cockroaches *Nauphoeta cinerea* and *D. punctata* removal of the ovaries abolished both the activation and the cyclical activity of the CA (Lanzrein et al., 1981; Stay et al., 1983). In ovariectomized *B. germanica* the C_{29} methyl ketone pheromone accumulated on the cuticle (Schal, 1988) and the CA were activated and exhibited at least one cycle of activity, as established by the *in vitro* radiochemical assay (Gadot et al., 1990). Thus, in all cock-

roaches examined to date, ovariectomy did not suppress pheromone production, indicating either that the CA can be activated to normal levels or that very low titers of JH can induce pheromone production. The latter was shown to be true for *S. longipalpa* in which CA activity in vitro was approximately 5% of peak activity when volatile pheromone production was first detected in behavioral assays (Smith et al., 1989; Smith and Schal, 1990a).

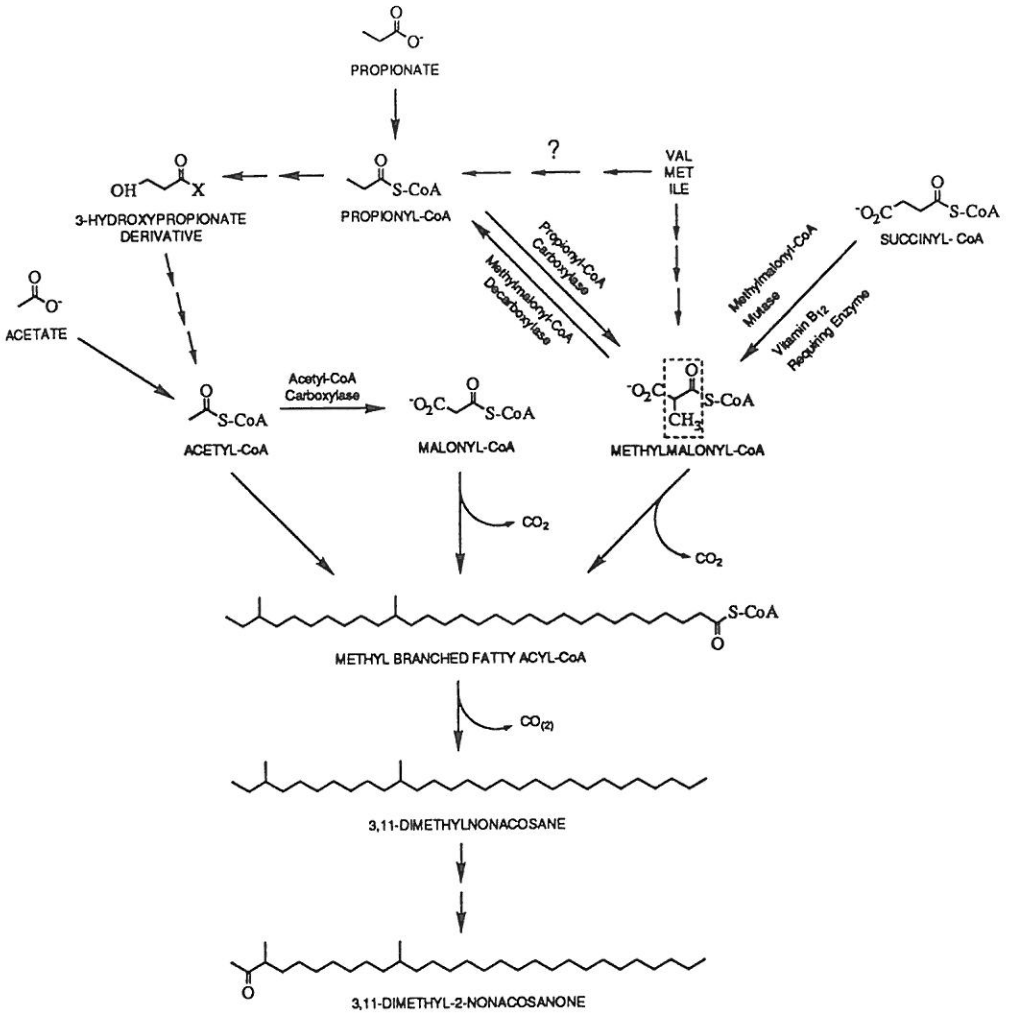

Fig. 2. Proposed biosynthetic pathway for the major female sex pheromone component of the German cockroach, *Blattella germanica*.

Whole females and isolated body parts of sexually mature females elicited the complete courtship wing-raising response in males (Roth and Willis, 1952; Nishida and Fukami, 1983). As in the housefly, determination of the site of pheromone synthesis by behavioral assays or by GC may be misleading. Preliminary data showed that 33% of extractable cuticular pheromone occurred on the wings of *B. germanica* females (24% on the abdomen), but it is likely that radiotracer techniques will show that pheromone is synthesized elsewhere (most probably by abdominal epidermal cells) and concentrated on the wings.

The major hydrocarbon component of *B. germanica* females 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) and Chase et al. (1990) suggest that the production of the 3,11-dimethyl ketone pheromone could result from the oxidation of its hydrocarbon analog by a sex-specific polysubstrate mono-oxygenase (Fig. 2). Preliminary studies indicate that radiolabel from [10,11-^{3}H]dimethylnonacosane applied to females at times when they are actively producing sex pheromone is recovered in the methyl ketone pheromone fraction (Chase, Schal, Touhara, Prestwich and Blomquist, unpublished). Also, the cuticular mass of both the dimethylnonacosane and dimethylheptacosane, the presumed precursors of 3,11-dimethyl-2-nonacosanone and 3,11-dimethyl-2-heptacosanone, respectively, remained relatively unchanged during the ovarian cycle, except for slight increases before the onset of pheromone synthesis, and sharp declines during the most active phase of pheromone synthesis. Other hydrocarbons (e.g., n-nonacosane) increased gradually over time with no apparent relation to the gonotrophic cycle (Schal, unpublished). Finally, in allatectomized females the mass of dimethylalkanes increased over time (Schal, Burns, and Blomquist, unpublished) and presumably contributed to greater synthesis and accumulation of pheromone (see above). These data lend support to the above hypothesis.

The precursors and directionality of biosynthesis of the methyl branched cuticular hydrocarbons were examined by Chase et al. (1990). The amino acids [G-^{3}H]valine, [4,5-^{3}H]isoleucine, and [3,4-$^{14}C_2$]methionine labeled the hydrocarbon fraction in a manner indicating that the carbon skeletons of all three amino acids serve as precursors to methylmalonyl-CoA, the methyl branch group donor (Fig. 2). The incorporation of [1,4-$^{14}C_2$]- and [2,3-$^{14}C_2$]succinates into the hydrocarbon and acylglycerol plus polar lipid fractions indicates that succinate also serves as a precursor to methylmalonyl-CoA. Carbon-13 NMR analyses showed that [1-^{13}C]propionate labeled the carbon adjacent to the tertiary carbon, and, for the 3,x-dimethylalkanes, that carbon-4 but not carbon-2 was enriched. This indicates that the methyl branching groups of the 3, x-dimethylalkanes are inserted early in the chain elongation process. [3,4,5-$^{13}C_3$]Valine labeled the methyl and tertiary carbons as well as the carbons adjacent to the tertiary carbon of the methyl branched alkanes. These data, taken together, indicate that the methyl branch is formed early in chain elongation by the insertion of methylmalonyl-CoA units derived from either branched chain amino acids or succinate. Subsequent acetate units are added, the methyl branched fatty acyl group is converted to hydrocarbon, and finally the 3,11-dimethylalkane oxidized to the corresponding methyl ketone (Fig. 2) (Chase et al., 1990).

SUMMARY

The sex pheromones of the female housefly and German cockroach consist of modified cuticular lipid components and are regulated by the same hormones that are involved in other reproductive events. In the female housefly, the sex pheromone components Z9-23:Hy, C_{23} epoxide, and C_{23} ketone first appear when the female becomes vitellogenic and are induced by ecdysteroids which are produced in the maturing ovary. Ovariectomy prevents sex pheromone production whereas reimplantation of ovaries or treatment with 20-HE restores sex pheromone production. Apparently 20-HE induces changes in the chain length specificities of the enzymes that are involved in elongating fatty acyl-CoAs and the step that reductively converts long chain fatty acyl-CoAs to hydrocarbon. The Z9-23:Hy formed under the influence of ecdysteroids is then converted to the corresponding epoxide and ketone via polysubstrate monooxygenase (PSMO) activity, which does not appear to be under endocrine regulation. In the German cockroach, males and females of all ages produce 3,11-dimethylnonacosane as a major cuticular lipid component, which is then apparently converted to the methyl ketone pheromone components in females under the influence of juvenile hormone (JH). Ovariectomy of German cockroaches does not inhibit sex pheromone production, but allatectomy partially depresses the synthesis and accumulation of sex pheromone. Juvenile hormone analogs induce pheromone production in both allatectomized and intact females.

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