Journal of Chemical Ecology, Vol. 21, No. 7, 1995

WHY NOT BE A QUEEN? REGIOSELECTIVITY IN MANDIBULAR SECRETIONS OF HONEYBEE CASTES

E. PLETTNER,[1] G.R.J. SUTHERLAND,[1,3] K.N. SLESSOR,[1,*] and M.L. WINSTON[2]

[1]*Department of Chemistry*
[2]*Centre for Pest Management, Department of Biological Sciences*
Simon Fraser University
Burnaby, British Columbia, Canada V5A 1S6

(Received February 14, 1995; accepted March 10, 1995)

Abstract—Both female castes of the honeybee (*Apis mellifera* L.) synthesize hydroxylated 2(*E*)-decenoic acids in their mandibular glands. Queens produce 9-hydroxy-2(*E*)-decenoic acid as part of their primer pheromone, while workers produce the regioisomeric 10-hydroxy acid, probably as a larval food source and an antiseptic secretion. Both workers and queens are biosynthetically competent to produce the other caste's dominant hydroxylated compound, as both isomers can be detected in queens and workers. We investigated the source of the caste-determined regioselectivity of hydroxy acid biosynthesis by investigating the production and interconversion of these compounds in isolated worker honeybee mandibular glands with specifically deuterated precursors. Gas chromatographic-mass spectroscopic identification of the labeled product indicates that octadecanoic acid is converted into 10-hydroxy-2(*E*)-decenoic acid with higher efficiency than either hexadecanoic or decanoic acids. 10-Hydroxydecanoic acid is readily converted into 10-hydroxy-2(*E*)-decenoic acid as expected in the β-oxidation process. The saturated and unsaturated 10-hydroxy acids are oxidized to the corresponding ten carbon diacids.

Key Words—Hymenoptera, Apidae, *Apis mellifera* L., mandibular gland secretion, hydroxydecenoic acids, hydroxydecanoic acids, deuterated precursors, gas chromatography-mass spectroscopy, biosynthetic precursors.

INTRODUCTION

There are two female castes in a honeybee (*Apis mellifera* L.) colony: the queen and several thousand workers. The queen's main function is reproduction,

*To whom correspondence should be addressed.
[3]Present Address: Department of Chemistry, University of Utah, Logan, Utah.

0098-0331/95/0700-1017$07.50/0

although she also heads the colony by producing primer/releaser pheromones that mediate many aspects of worker behavior and partially inhibit worker reproduction. The workers perform many nonreproductive tasks, such as brood rearing, guarding, and foraging (Winston, 1987), and generally only lay eggs following two to three weeks of queenlessness and broodlessness. Beekeepers and scientists have been fascinated by the differences between queens and workers because fertilized eggs have the potential to follow different developmental pathways leading to worker or queen adults. This process of caste differentiation ultimately results in morphological and physiological differences between adult queens and workers.

One such physiological difference is the composition of the mandibular gland secretion, a blend of several compounds produced in the mandibular glands of female honeybees. Among the many compounds found in their mandibular glands, both queens and workers produce a group of oxygen functionalized 10- and 8-carbon fatty acids. The fatty acids characteristic of queens have the functional group at the penultimate ($\omega - 1$) position in the chain, while the acids typical of workers are functionalized at the last (ω) position. The major component of queen mandibular pheromone (QMP) is 9-keto-2(*E*)-decenoic acid (ODA); the second most abundant is 9-hydroxy-2(*E*)-decenoic acid (9-HDA) (Slessor et al., 1988, 1990). The most abundant component of the worker mandibular blend is 10-hydroxy-2(*E*)-decenoic acid (10-HDA), followed by 10-hydroxydecanoic acid (10-HDAA). The 8-carbon homolog, 8-hydroxyoctanoic acid (8-HOAA), is a minor worker component, as are the diacids corresponding to 10-HDA and 10-HDAA, 2(*E*)-decenedioic acid ($C_{10:1}$ DA) and decanedioic acid ($C_{10:0}$ DA), respectively.

The function of these caste-specific blends also differs. The queen-produced blend is a releaser pheromone that attracts nearby workers to the queen, giving rise to a retinue of workers around the queen (Slessor et al., 1988), among other effects (Winston and Slessor, 1992). The queen pheromone also is a primer pheromone, since it inhibits queen rearing by workers (Winston et al., 1990) and its major component, ODA, inhibits juvenile hormone III biosynthesis in workers (Kaatz et al., 1992). The worker mandibular components are not known to function as semiochemicals, but 10-HDA is found in royal jelly (Weaver et al., 1968), the worker-produced queen food and the 10-carbon diacids, $C_{10:1}$ DA and $C_{10:0}$ DA, are found in honey (Plettner, unpublished observation). Blum et al. (1959) reported antibacterial and antifungal activity for 10-HDA, and Boch et al. (1979) reported the volatile acids present as antibacterials, so this secretion may be involved in food preservation.

Even though the mandibular acids of both castes appear to have important functions in honeybee colonies, their biosynthesis has not been studied. However, the catabolism of ODA in workers was followed with radiolabeled material, in an effort to understand how the workers clear their system of the queen-

produced attractant and primer (Johnston et al., 1965). The results indicated that the ODA was reduced to 9-HDA and to the saturated analogs, 9-ketodecanoic- and 9-hydroxydecanoic acids (ODAA and 9-HDAA). This process appeared to take place mainly in the gut of the workers. The authors speculated that the catabolites of ODA might accumulate in the worker-produced royal jelly and could thus be recycled back to the queen, who would convert the catabolites back to ODA. However, analyses of royal jelly have revealed that it does not contain 9-HDA, 9-HDAA, or ODAA (Weaver et al., 1968), and the biosynthesis of mandibular acids has not been studied since. The objective of our work was to study interconversion among mandibular blend components and to find which precursor is functionalized to give the hydroxy acids, using deuterated potential intermediates and precursors and following their incorporation by gas chromatography–mass spectroscopy. We wished to understand how the caste-specific regioselective functionalization of the hydroxy acids is regulated and chose to initially study workers, since these are more readily available than queens.

The composition of queen and worker mandibular glands had been thought to be mutually exclusive, until it was found that some workers can produce ODA under queenless conditions (Ruttner et al., 1976). Further work led to the realization that this overlap does not occur under special circumstances. Even queenright workers have a small amount of 9-HDA in their mandibular glands, the ratio of 10-carbon hydroxy acids, 10-HDA–10-HDAA–9-HDA being ca. 30:10:1 (Plettner et al., 1993). We have taken advantage of this occurrence of 9-HDA in workers to study its biosynthesis simultaneously with the ω-hydroxy acids. The overlap in gland composition is not restricted to workers; mated, laying queens always have trace amounts of 10-HDAA and variable amounts of 10-HDA, along with ODA and 9-HDA, which are more abundant than the worker acids (Crewe, 1982, Plettner et al., unpublished observation). While workers retain the same ratio of mandibular components throughout their life, queens gradually change from a blend dominated by 10-HDA in newly emerged queens to a blend with mostly ODA and 9-HDA. Both castes experience an increase in the total amount of acids in the mandibular glands as they age (Slessor et al., 1990) (Figure 1). Knowledge of the mandibular gland ontogeny was useful for this project, since young individuals have low levels of endogenous material, which makes labeled products easier to detect.

METHODS AND MATERIALS

Gland Isolation and Treatment

The bees used for the assay were 1–5 days old and were kept caged in an incubator (34°C) with honey and water until required. Both glands were freshly

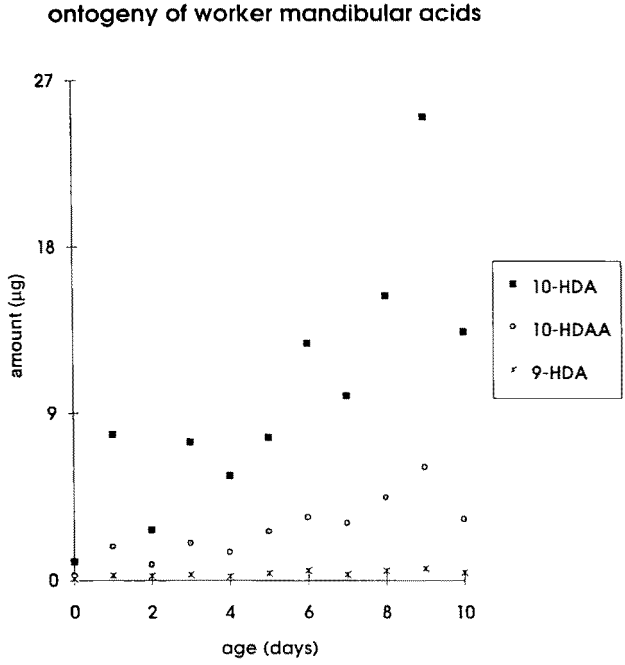

FIG. 1. Ontogeny of three hydroxy acids found in worker mandibular glands: 10-hydroxy-2(*E*)-decenoic acid (10-HDA), 10-hydroxydecanoic acid (10-HDAA), and 9-hydroxy-2(*E*)-decenoic acid (9-HDA). Data represent the total amount of compound found in a pair of mandibular glands, as determined by gas chromatography (Slessor et al. 1990). Each point is an average of three samples.

removed, washed in Tris-buffered physiological saline (pH 6.5–7.5), blotted dry with a piece of tissue, and exposed to a solution of the test substrate in DMSO. The gland was placed in an Eppendorf tube and left to perfuse at room temperature for 5–20 min, washed with physiological saline, blotted dry, and placed in methanol (13 µl) containing 10-undecenoic acid (0.2 µg/µl) as internal standard, crushed, and centrifuged at low speed. The supernatant was removed and the pellet extracted with a further portion of methanol (13 µl) containing internal standard.

Analysis

Two aliquots (2 µl) of mandibular extract were evaporated and trimethylsilyated with bistrimethylsilyltrifluoroacetamide (2 µl of neat reagent, Sigma Chemical Co., St. Louis, Missouri) for at least 40 min at room temperature (Slessor et al., 1990). One sample was diluted with hexane (to 30 µl) and

analyzed by splitless capillary gas chromatography (GC) on a Hewlett-Packard 5890A equipped with a flame ionization detector and a 30 m × 0.25 mm, (film thickness 0.25 μm) DB-1 column (program: 100°C, 1 min; 10°/min to 185°C, 4 min; 3°/min to 200°C, 0 min; 25°/min to 260°C, 20 min), head pressure 120 kPa, flow: 30 ml/min. Response was calibrated with standards of known concentration.

The second sample was diluted such that ca. 10–20 ng of the most abundant component would be injected and analyzed on a Varian 3400 GC interfaced with a Varian Saturn ion trap mass spectrometer (MS), equipped with a 30-m × 0.25-mm, (film thickness 0.25 μm) DB-5 column (program: 100°C, 1 min; 10°/min to 200°C, 3°/min to 220°C, 2.5°/min to 225°C, (15 min), head pressure 105 kPa, flow 20 ml/min. The ionization mode was EI with a filament emission current of 10 μA at 70 eV, a target value of 5500, with the automatic gain control (AGC) on and with 1 scan/sec.

Data Analysis and Statistics

Three evaluations of substrate incorporation were investigated: percentage of label incorporated, quantity of labeled product formed, and rate of incorporation. Small overlapping peaks from artifacts in GC-MS made the latter two evaluations less reliable than percentage label.

The usually dominant $(M\text{-}15)^+$ fragment, arising from loss of a methyl group from the trimethylsilyl derivative of the parent ion, was used to determine the percentage label for each compound of interest. The ion currents for the $(M\text{-}15)^+$ and $(M\text{-}15 + n)^+$ (n = number of D atoms in the compound) were recorded for each scan across the GC peak without prior background subtraction. The percentage of labeled material was obtained from a calibration curve in which the ratio of $\Sigma(M\text{-}15 + n)/\Sigma(M\text{-}15)$ had been plotted against the percentage of label, using prepared mixtures of labeled and unlabeled standard. This correction minimized the effect of overlapping ions, especially $(M\text{-}15 + nD) - n$ signal on the unlabeled (M-15) signal at higher labeling concentrations. With the AGC on, correction parameters varied only slightly with concentration of 0.1–100 ng of injected sample.

The data were analyzed by a one-way ANOVA (SAS statistical package, SAS Institute Inc., Cary, North Carolina) for differences between treatments and blanks (pairwise comparisons). Points were only rejected if a known experimental problem existed with the sample, or if they were strong outliers by the fourth spread criteria (Devore, 1991).

Labeled Substrates

General. TLC was performed on 0.25-mm-thick films of silica gel (Merck, Kieselgel 60G for TLC) on glass plates. Acidic TLC plates were prepared with 2% H_3PO_4 instead of water and were used to monitor reactions and purifications

involving free acids. Silica gel columns were prepared with Merck Kieselgel 60 (230–400 mesh). Acidic gel was prepared by suspending the silica in 2% aqueous H_3PO_4 (silica–aqueous acid 1 : 2 w/w), leaving the silica to settle, decanting the supernatant, and drying the slurry in an oven at 100°C. Acidic silica gel was used to purify compounds possessing free carboxylic acid functionalities to minimize streaking. Esters dissolved in methanol were hydrolyzed by adding 5% aqueous NaOH and stirring overnight. Hydrolyzates were acidified with 10% HCl and extracted twice with ether, the ether washed with brine, and dried over sodium sulfate. ^{1}H NMR spectra were measured in $CDCl_3$ at 400 MHz on a Bruker WM-400. Both ^{1}H NMR and GC-MS were used to confirm the structure and assess the extent of deuteration in intermediates and products. All deuterated products showed physical properties and retention times identical to the unlabeled compound, except for the differences in the ^{1}H NMR and MS fragmentation expected for the deuterium substitution pattern.

(9,9,10) D_3-10-Hydroxydecanoic Acid (D_3 10-HDAA). 10-Oxodecanoic acid was obtained from 10-undecenoic acid by OsO_4-mediated oxidation with $NaIO_4$ (Graham and Williams, 1966). This material was refluxed 4 hr under Ar at 105°C with D_2O–pyridine (6 : 7), to give (9,9) D_2-10-oxodecanoic acid (96% D_2). Reduction with $NaBD_4$ in methanol gave (9,9,10) 10-hydroxydecanoic acid (94% yield, 94% labelled).

(4,4) D_2-2(E)-Decenoic Acid [D_2 $C_{10:1}$ 2(E)]. Octanal was deuterated with D_2O–pyridine (1 : 1, 3 hr, 100°C) to give (2,2) D_2-octanal (93% D_2). Reaction with the ylide of trimethylphosphonoacetate produced by treatment with NaH (Kandil and Slessor, 1985), gave methyl (4,4) D_2-2-decenoate (ca. 86% *E* and 14% *Z*). Chromatography on silica gel (hexane–ether, 5 : 1) provided an enriched methyl ester fraction (ca. 95% *E*, 30% yield). The methyl ester was base hydrolyzed to yield the free acid (77% yield).

(4,4) D_2-10-Hydroxy-2(E)-Decenoic Acid (D_2 10-HDA). The tetrahydropyranyl derivative of 8-hydroxyoctanal (Chiron, 1982) was deuterated with pyridine–D_2O (1 : 1, 16 hr, reflux), to give (2,2) D_2-8-THPO-octanal (94% D_2). Condensation with the ylide of trimethylphosphonoacetate gave the THP derivative of methyl (4,4) D_2-10-hydroxy-2-decenoate (ca. 80% *E* and 20% *Z*). Removal of the THP group under acidic conditions (methanol–water–10% HCl, 1 : 4 : 10, room temperature for 1.5 hr) and silica gel purification resulted in methyl (4,4) D_2-10-hydroxy-2-decenoate enriched in the *E* isomer (93% *E*, 50% yield, 94% D_2). Base hydrolysis of the methyl ester gave the free acid (58% yield).

(4,4) D_2-2(E)-Decenedioic Acid (D_2 $C_{10:1}$ DA) and (2,2) D_2-Decanedioic Acid (D_2 $C_{10:0}$ DA). Small portions of D_2 10-HDA and D_2-10-oxodecanoic acid were oxidized with pyridinium dichromate (PDC) (Corey and Schmidt, 1979) to the corresponding diacids D_2 $C_{10:1}$ DA and D_2 $C_{10:0}$ DA, respectively.

(12) D_1-Octadecanoic Acid (D_1 $C_{18:0}$). Oxidation of methyl 12-hydroxy-

octadecanoate (Sigma) with pyridinium chlorochromate (PCC) gave the 12-keto compound, which was treated directly with *p*-tolylsulfonylhydrazine (2 hr reflux in MeOH) and reduced with $NaBD_4$ (2 hr 50°C in MeOH) to give methyl 12-D octadecanoate in 5% yield. The methyl ester was hydrolyzed to the free acid (83% D_1).

(7,7) D_2-8-Hydroxyoctanoic Acid (D_2 8-HOAA). Oxidation of monoacetyl 1,8-octanediol (Babler and Coghlan, 1979) with PDC gave 8-acetoxy octanoic acid. The acetate group was removed to give 8-hydroxyoctanoic acid and oxidized with PCC to the 8-oxo acid. The oxo acid was deuterated twice with pyridine–D_2O (1 : 1, 17 hr reflux, 65% D_2) and reduced with $NaBH_4$ in MeOH to give the deuterated ω-hydroxy acid.

(7,7,8,8) D_4-Decanoic Acid (D_4 $C_{10:0}$). 7-Decynoic acid was prepared by the condensation of 6-bromohexanoic acid and 1-butyne in the presence of 2.5 eq. of *n*-butyl lithium, converted to the methyl ester with BF_3–MeOH, and deuterated with deuterium and $RhCl_2[P(phenyl)_3]_2$ as a catalyst (room temperature, 1 atm) to give methyl (7,7,8,8) D_4 decanoate. Hydrolysis gave deuterated decanoic acid.

RESULTS

Treatment with labeled 10-HDAA or 10-HDA led to labeling of 8-HOAA, suggesting that both 10-carbon hydroxy acids can be shortened to 8-HOAA (Table 1). Furthermore, application of labeled 10-HDA resulted in labeling of 10-HDAA and vice versa, indicating that 10-HDA and 10-HDAA can be interconverted. Finally, treatment with labeled 8-HOAA led to no significant labeling

TABLE 1. PERCENTAGE OF LABELED PRODUCT FORMED IN INTERCONVERSION AMONG ω-HYDROXY ACIDS IN WORKER MANDIBULAR GLANDS

Treatment	Incubation time (min)	*N*	Product (means ± SE)[a] 8-HOAA	10-HDAA	10-HDA
D_2 8-HOAA	10	9	**73.4 ± 1.2 a**	1.5 ± 0.1 a	4.0 ± 1.4 a
Blank		10	6.3 ± 1.8 b	3.6 ± 0.2 b	4.1 ± 0.4 a
D_3 10-HDAA	15	9	22.6 ± 11.1 a (N = 8)	**86.0 ± 4.0 a**	15.5 ± 7.0 a
Blank		9	1.3 ± 0.7 b	0.3 ± 0.1 b	0.5 ± 0.1 b
D_2 10-HDA	20	8	48.0 ± 5.0 a	27.0 ± 4.0 a	**89.7 ± 5.1 a**
Blank		9	1.3 ± 0.7 b	0.3 ± 0.1 b	0.5 ± 0.1 b

[a]Treatments were compared to their corresponding blanks. Means within a column followed by the same letter do not differ significantly by Tukey's test ($P < 0.05$).

of the 10-carbon hydroxy acids, which suggests that 8-HOAA was not chain lengthened.

In the D_3 10-HDAA treatment, labeled 8-HOAA accumulated over time. In 1 min, 7.1 ± 3.3 ng of labeled 8-HOAA formed, while in 15 min, 120 ± 62 ng formed. In the same experiment, labeled 10-HDA did not accumulate with time; 12.6 ± 2.2 ng formed in 1 min and 9.2 ± 2.8 ng formed in 15 min. The accumulation of 8-HOAA with time and its failure to convert to other compounds suggests that it may be an end point in the hydroxy acid biosynthetic pathway. By contrast, 10-HDA did not accumulate with time and appears to be converted to other compounds. The percentages of labeled 10-HDA and 10-HDAA formed from 10-HDAA and 10-HDA, respectively, were similar (Table 1), as were the amounts formed during the 15- to 20-min treatments (data not shown). This indicates that in young workers desaturation of 10-HDAA to give 10-HDA occurs more or less at the same rate as the reverse reaction.

Apart from interconversion, the 10-carbon hydroxy acids were oxidized to their respective diacids in both queens and workers. Treatment with D_3 10-HDAA led to significant incorporation in $C_{10:0}$ DA and $C_{10:1}$ DA, while treatment with D_2 10-HDA resulted in significant labelling of $C_{10:1}$ DA, but not of $C_{10:0}$ DA.

No incorporation of label from the ω hydroxy acids into ω-1 hydroxy acids was found. The reverse incorporation of label from a labeled 9-HDA into the 10 hydroxy acids was not observed (data not shown). Both observations suggest that the ω and ω-1 hydroxy acids do not interconvert.

TABLE 2. PERCENTAGE OF LABELED MATERIAL FORMED FROM DECANOIC, 2(*E*)-DECENOIC, HEXADECANOIC, AND OCTADECANOIC ACIDS AS PRECURSORS OF WORKER MANDIBULAR GLAND COMPONENTS

Treatment	*N*	Product (mean ± SE)[a]			
		9-HDA	8-HOAA	10-HDAA	10-HDA
D_4 C10:0	9	1.0 ± 0.5	1.8 ± 1.2	0.3 ± 0.1	**0.8 ± 0.1**
Blank D_4	10	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	**0.2 ± 0.1**
D_2 $C_{10:1}$	6	5.0 ± 0.7	3.0 ± 0.4	2.3 ± 0.3	2.9 ± 0.4
Blank D_2	6	2.4 ± 1.1	3.4 ± 1.8	2.6 ± 0.3	3.9 ± 0.3
D_3 $C_{16:0}$	14	7.5 ± 2.2	**14.2 ± 3.7**	3.3 ± 0.4	3.6 ± 0.6
Blank $D_{2/3}$	10	3.3 ± 0.9	**3.3 ± 1.1**	2.6 ± 0.2	3.0 ± 0.5
D_1 $C_{18:0}$	16	**22.4 ± 2.6**	**16.9 ± 5.0**	**10.0 ± 1.4**	**13.0 ± 3.0**
Blank D_1	10	**1.5 ± 0.7**	**0.0 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**

[a] For entries in bold type, treatments and blanks differed significantly ($P < 0.05$) by Tukey's test.

TABLE 3. INTERCONVERSION AMONG POTENTIAL PRECURSORS TO HYDROXY ACIDS IN WORKER MANDIBULAR GLANDS[a]

Treatment	Product			
	$C_{10:0}$	$C_{10:1}$	$C_{16:0}$	$C_{18:0}$
D_4 $C_{10:0}$		+	n.s.	n.s.[b]
D_2 $C_{10:1}$	n.s.		n.s.	+++
D_3 $C_{16:0}$	++	++		+++
D_1 $C_{18:0}$	n.s.	++	+	

[a]Plus signs indicate significant incorporation of label into the product as compared to the blank ($P < 0.05$, Tukey's). Data corresponding to the compounds used for treatment were left blank. +, up to 20% label; ++, between 20% and 40%; +++, between 40% and 60%; n.s., not significant.
[b]The mass spectra show a small amount of label.

Of the possible precursors to the hydroxy acids tested, D_1 $C_{18:0}$ was incorporated with the greatest efficiency into both ω-1 and ω hydroxy acids (Table 2). This was followed by D_3 $C_{16:0}$, which was incorporated significantly only into 8-HOAA. D_2 $C_{10:1}$ was not incorporated significantly into any of the hydroxy acids, while D_4 $C_{10:0}$ was only weakly incorporated into 10-HDA. Furthermore, application of D_2 $C_{10:1}$ and D_3 $C_{16:0}$ resulted in significant labeling of $C_{18:0}$ (Table 3). Treatment with D_4 $C_{10:0}$ did not lead to significant labeling of $C_{18:0}$, but a small amount of the expected label was seen in the mass spectra. These results suggest that unfunctionalized fatty acids with less than 18 carbons were lengthened to $C_{18:0}$ before functionalization.

DISCUSSION

A study of the possible interconversion among ω-hydroxy 10-carbon acids demonstrated that there was ready interconversion between the saturated and the 2(*E*)-unsaturated ω functionalized species in isolated worker mandibular glands. In addition, the two 10-hydroxy 10-carbon acid substrates were readily chain shortened to the saturated 8-carbon ω acid. Both observations are in accord with the ability of these hydroxylated compounds to enter the β-oxidation pathway. 10-HDAA would first be desaturated to 10-HDA, presumably through activation as its CoA-ester. Our results indicate this to be a reversible process. Hydration of the 2–3 double bond would give a 3,10-dihydroxydecanoate. The final steps are the oxidation of the 3-hydroxy group to a keto moiety, attack of the keto group by free CoA-SH, and displacement of the acetyl-CoA head portion, giving

8-HOAA-CoA. At each step a portion could be hydrolyzed, giving rise to the corresponding free acid. Presumably, a significant proportion of the 10-HDA-CoA gets hydrolyzed, since 10-HDA is the major hydroxy acid component of the worker mandibular gland secretion. Free 3,10-dihydroxydecanoic acid appears to be a minor component of worker mandibular extracts and has been found in royal jelly, along with the other ω-hydroxy acids (Weaver et al., 1968).

In addition to β-oxidation of 8- and 10-carbon hydroxy acids, the glands have the capability to oxidize ω-hydroxy acids to diacids. While 10-HDA was oxidized only to its corresponding diacid ($C_{10:1}$ DA), 10-HDAA was converted to both its corresponding diacid ($C_{10:0}$ DA) and $C_{10:1}$ DA. The latter conversion could have occurred by desaturation of 10-HDAA to 10-HDA, followed by oxidation to the diacid, or vice versa, oxidation to $C_{10:0}$ DA preceding desaturation to $C_{10:1}$ DA. No experiment with labeled $C_{10:0}$ DA was performed to evaluate these two possibilities.

We investigated decanoic acid ($C_{10:0}$) as a potential precursor to find the fatty acid subtrate that is regioselectively oxygenated through caste-determined control. We compared incorporation of labeled straight-chain fatty acids of different chain lengths in the whole gland assay. The results indicated that D_1 $C_{18:0}$, and not D_4 $C_{10:0}$, was incorporated most efficiently into both ω and ω-1 10-carbon components. Furthermore, interconversion among precursors indicated that $C_{10:0}$, $C_{10:1}$, and $C_{16:0}$ were lengthened to $C_{18:0}$, suggesting it to be the entry point to the hydroxy acid pathway. For $C_{10:0}$ to be incorporated into mandibular acids, it must be lengthened to $C_{18:0}$ prior to functionalization, requiring the largest number of steps and experiencing considerable dilution. $C_{18:0}$ would require the smallest number of steps and would experience the smallest isotopic dilution in the final products. The somewhat low incorporation of D_3 $C_{16:0}$ may be due to a primary isotope effect, since one of the deuterium atoms would be removed during ω-functionalization. Our results are consistent with $C_{18:0}$ being the entry point to the hydroxy acid biosynthetic pathway, with either free and/or lipid-bound $C_{18:0}$ constituting a precursor reservoir that ensures continuous hydroxy acid biosynthesis by the mandibular gland.

Two routes are possible to convert $C_{18:0}$ into 10-carbon ω and ω-1 hydroxy acids, which differ only in the order in which functionalization and chain shortening take place. Functionalization may occur at the $C_{18:0}$ (or its CoA ester) stage to give 18-hydroxyoctadecanoic acid (or its CoA ester) followed by chain-shortening by β-oxidation to the 10-carbon length (Figure 2). Alternatively, after chain shortening, a bound $C_{10:0}$ intermediate is functionalized. Both routes have been observed in the biosynthesis of fatty acid-derived semiochemicals. The major component of the sex pheromone of the cabbage looper (*Trichoplusia ni*), (*Z*)-7-dodecenyl acetate, is biosynthesized from $C_{16:0}$-CoA by Δ11 desaturation to give (*Z*)-11-$C_{16:1}$-CoA followed by chain-shortening to (*Z*)-7-$C_{12:1}$-CoA, the immediate precursor (Jurenka and Roelofs, 1993). Chain-shortening

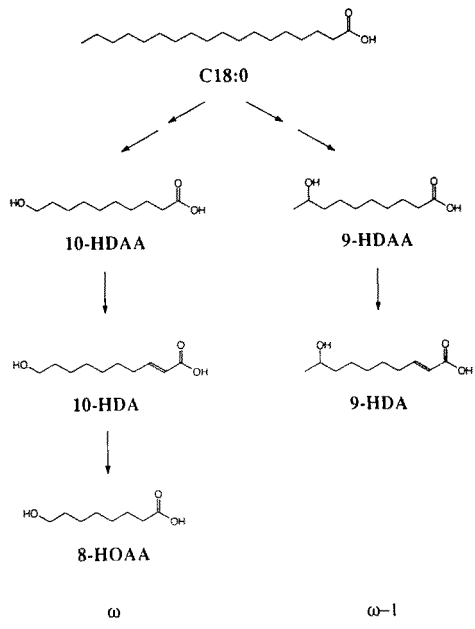

FIG. 2. Formation of ω and ω-1 hydroxy acids from octadecanoic acid ($C_{18:0}$) in worker mandibular glands. The ω hydroxy acids are: 10-hydroxydecanoic acid (10-HDAA), 10-hydroxy-2(*E*)-decenoic acid (10-HDA), and 8-hydroxyoctanoic acid (8-HOAA); the ω-1 hydroxy acids are 9-hydroxydecanoic acid (9-HDAA) and 9-hydroxy-2(*E*)-decenoic acid (9-HDA).

before functionalization is found in the red-banded leafroller moth (*Argyrotaenia velutinana*), where the major sex pheromone component, (*Z*/*E*)-11-tetradecenyl acetate, is made by $C_{18:0}$/$C_{16:0}$-CoA chain shortening to $C_{14:0}$-CoA, followed by Δ11 desaturation to give (*Z*/*E*)-11-$C_{14:1}$-CoA. (Jurenka and Roelofs, 1993). We are currently investigating which route is responsible for hydroxy acid biosynthesis in honey bee mandibular glands.

One requirement of being a queen is the regioselective functionalization of a fatty acid, or its derivative, at the ω-1 position in the production of the queen's primer pheromone. Our results indicate that the free fatty acid entry point for this caste-determined capability is $C_{18:0}$. As no evidence was found for the interconversion of ω and ω-1 hydroxy acids, two distinct functionalization reactions must be operative: ω for workers and ω-1 for queens. The positional selectivity of this hydroxylation to form hydroxy decenoic acids in the mandibular gland is one of the important developmentally derived characters that differentiate queens from workers. An understanding of the control system reg-

ulating this regioselectivity will provide a better appreciation for the caste differentiation processes in this highly social insect.

Acknowledgments—We thank Skip King, Kirsten Bray and Tracy Pickett for laboratory assistance; R.J. Cushley for the donation of D_3 16:0, and the Natural Sciences and Engineering Council of Canada for a Postgraduate Studentship to E.P. and grant support to K.N.S. and M.L.W.

REFERENCES

BABLER, J.H., and COGHLAN, M.J. 1979. A facile method for monoacetylation of symmetrical diols: Application to the total synthesis of *Z*-8-dodecenyl acetate, the sex attractant of the oriental fruit moth. *Tetrahedron Lett.* 1979:1971–1974. No. 22.

BLUM, M.S., NOVAK, A.F., and TABER, S., III. 1959. 10-Hydroxy Δ^2-decenoic acid, an antibiotic found in royal jelly. *Science* 130:452–453.

BOCH, R., SHEARER, D.A., and SHUEL, R.W. 1979. Octanoic and other volatile acids in the mandibular glands of the honeybee and in royal jelly. *J. Agric. Res.* 18:250–252.

CHIRON, R. 1982. New synthesis of royal jelly acid. *J. Chem. Ecol.* 8:709–713.

COREY, E.J., and SCHMIDT, G. 1979. Useful procedures for the oxidation of alcohols involving pyridinium dichromate in aprotic media. *Tetrahedron Lett.* 1979:399–402. No. 5.

CREWE, R.M. 1982. Compositional variability: The key to social signals produced by the honeybee mandibular glands, pp. 318–332, *in* M.D. Breed, C.D. Michener and H.E. Evans (eds.). The Biology of Social Insects. Westview Press, Boulder, Colorado.

DEVORE, J.L. 1991. Probability and Statistics for Engineering and the Sciences, 3rd ed. Brooks/Cole Pub. Co., Pacific Grove, California.

GRAHAM, S.H., and WILLIAMS, A.J.S. 1966. Alkylidenecyclobutanes. Part II. The oxidation of benzylidenecyclobutane and of bis-(*p*-methoxyphenyl)methylenecyclobutane. *J. Chem. Soc. (C)* 1966:655–660. Vol. 5.

JOHNSTON, N.C., LAW, J.H., and WEAVER, N. 1965. Metabolism of 9-ketodec-2-enoic acid by worker honeybees (*Apis mellifera* L.) *Biochemistry* 4:1615–1621.

JURENKA, R.A., and ROELOFS, W.L. 1993. Biosynthesis and endocrine regulation of fatty acid derived sex pheromones in moths, pp. 353–388, *in* D.W. Stanley-Samuelson and D.R. Nelson (eds.). Insect Lipids: Chemistry, Biochemistry and Biology. University of Nebraska Press, Lincoln and London.

KAATZ, H.H., HILDEBRANDT, H., and ENGELS, W. 1992. Primer effect of queen pheromone on juvenile hormone biosynthesis in adult worker honey bees. *J. Comp. Physiol. (B)* 162:588–592.

KANDIL, A.A., and SLESSOR, K.N. 1985. Enantiomeric synthesis of 9-hydroxy (*E*)-2-decenoic acid, a queen bee pheromone. *Can. J. Chem.* 61:1166–1168.

PLETTNER, E., SLESSOR, K.N., WINSTON, M.L., ROBINSON, G.E., and PAGE, R.E. 1993. Mandibular gland components and ovarian development as measures of caste differentiation in the honey bee (*Apis mellifera* L.) *J. Insect Physiol.* 39:235–240.

RUTTNER, F., KOENINGER, N., and VEITH, H.J. 1976. Queen substance bei eierlegenden Arbeiterinnen der Honigbiene (*Apis mellifera* L.) *Naturwissenschaften* 63:434–435.

SLESSOR, K.N., KAMINSKI, L.-A., KING, G.G.S., BORDEN, J.H., and WINSTON, M.L. 1988. The semiochemical basis of the retinue responses to the queen honey bee. *Nature* 332:354–356.

SLESSOR, K.N., KAMINSKI, L.-A., KING, G.G.S., and WINSTON, M.L. 1990. Semiochemicals of the honey bee queen mandibular glands. *J. Chem. Ecol.* 16:851–860.

WEAVER, N., JOHNSTON, N.C., BENJAMIN, R., and LAW, J.H. 1968. Novel fatty acids from the royal jelly of honeybees (*Apis mellifera* L.) *Lipids* 3:535–538.

WINSTON, M.L. 1987. The Biology of the Honey Bee, 1st ed. Harvard University Press, Cambridge, Massachusetts.

WINSTON, M.L., and SLESSOR, K.N. 1992. An essence of royalty: Honey bee queen pheromone. *Am. Sci.* 80:374–385.

WINSTON, M.L., HIGO, H.A., and SLESSOR, K.N. 1990. The effect of various dosages of queen mandibular pheromone on the inhibition of queen rearing in the honey bee (Hymenoptera: Apidea). *Ann. Entomol. Soc. Am.* 83:234–238.