Metabolism of 9-Ketodec-2-enoic Acid by Worker Honeybees (*Apis mellifera* L.)*

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ABSTRACT: 9-Keto-*trans*-dec-2-enoic acid, a compound which has a specific physiological role in the inhibition of queen-rearing behavior in worker honeybees, was prepared in radioactive form and fed to worker bees. The major metabolites were found in the gut or whole abdomen. These were identified as 9-ketodecanoic acid, 9-hydroxydecanoic acid, and 9-hydroxydec-2-enoic acid. The conversion into these compounds was sufficiently rapid to account for the manifestation of queenless behavior in worker bees which have been separated from the queen for short periods of time.

9-Keto-*trans*-dec-2-enoic acid is a physiologically active component of the "queen substance" elaborated by queen honeybees (*Apis mellifera* L.). The isolation and identification of this compound (Butler *et al.*, 1961) was the result of a long series of biological experiments (Butler, 1959). This pheromone[1] was characterized simultaneously in England (Callow and Johnston, 1960) and in France (Barbier and Lederer, 1960), and it has been synthesized by a variety of methods (see Fieser and Fieser, 1963).

The behavior of worker honeybees is markedly altered by removal of the single queen from a colony. A most striking manifestation of the "queenless" behavior is queen rearing. Within 24 hours after the queen is removed from the colony, workers enlarge several cells which contain young worker larvae and extend the cells outward and downward. The larvae in these cells are fed copiously on royal jelly, which causes them to develop into queens. Butler *et al.* (1961) have shown that this behavior is inhibited by the administration of 9-ketodec-2-enoic acid to queenless worker bees. The "queen substance" consists of 9-ketodecenoic acid and other unidentified active and synergistic compounds that provide the queen with a subtle method for controlling the activities of her workers and limiting competition by young queens. If the queen dies or is unable to produce sufficient queen substance, the workers respond by rearing young queens. One of the young queens may replace the old queen, or the colony may divide by swarming.

In addition to inhibition of queen rearing, 9-ketodec-2-enoic acid has two other important functions in organizing the social behavior of honeybees. It inhibits the development of the ovaries of worker honeybees (Butler *et al.*, 1961; Butler and Fairey, 1963) and serves to attract male bees (drones) to virgin queens for the purpose of mating (Gary, 1962; Pain and Ruttner, 1963; Butler and Fairey, 1964). It is not yet clear whether this substance is acting in some cases as a true hormone (e.g., effect on oogenesis) and in others as a purely sensory stimulant (e.g., in drone attraction), or if its action is by a similar mechanism in all cases.

The fact that queenless behavior begins within a few hours of separation of the workers from a queen indicates that workers quickly deplete their supply of queen substance. In order to study this process [2-^{14}C]-9-ketodec-2-enoic acid has been prepared and administered to worker bees. The results suggest that 9-ketodec-2-enoic acid is readily converted into inactive compounds.

Materials

[2-^{14}C]9-Ketodec-2-enoic Acid. Several variations of the procedure of Jaeger and Robinson (1961) were used for the synthesis of this material, and the following procedure was found to be the most convenient. 9-Ketodec-2-enoic acid (Glaxo, Ltd., kindly supplied by Dr. R. K. Callow) was esterified by refluxing with methanolic HCl. The keto ester (900 mg) was converted into the ketal by treatment with ethylene glycol and *p*-toluenesulfonic acid in benzene (Migrdichian, 1957). The pure ketal (787 mg) was separated from unreacted keto ester by chromatography on silicic acid in 30% ether in hexane. The pure ketal was eluted with about 1.25 column volumes of solvent, after which the keto ester emerged. On gas-liquid chromatography with a liquid phase of polydiethylene glycol succinate, the ketal ester gave one peak with a retention

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† Holder of a National Institutes of Health Career Program Award (GM 8521).

[1] The term *pheromone* was coined by Karlson and Butenandt (1959) and has come into wide use in entomology and the behavioral sciences. It refers to a compound produced and excreted by one individual and evoking a characteristic behavioral response in a second animal of the same species, *e.g.*, sex attractants, food markers, etc. (see Karlson and Butenandt, 1959; Wilson, 1963).

time of 1.98 relative to that of dimethyl sebacate (cf. retention time for methyl 9-ketodec-2-enoate, 1.50 relative to dimethyl sebacate). The infrared spectrum of the ketal ester showed no ketone absorption, but several peaks at 1100–1200 cm^{-1}.

The ketal ester (280 mg) in dichloromethane (3 ml) was ozonized for 3 min. at −65°. After flushing with oxygen and nitrogen, 958 mg of triphenylphosphine was added in 2 ml dichloromethane (Stein and Nicolaides, 1962), and the solution was allowed to come to room temperature. A concentrated solution of $NaHSO_3$ was added and the dichloromethane was evaporated under a stream of nitrogen. The white precipitate was collected by filtration and washed with ether. Treatment of the bisulfite complex with saturated Na_2CO_3 freed the ketal aldehyde (29 mg). The aldehyde had a characteristic infrared spectrum (2780 and 1702 cm^{-1}) and it had a retention time of 0.51 relative to dimethyl sebacate on gas chromatography.

The aldehyde was condensed with [2-^{14}C]malonic acid by the method of Jaeger and Robinson (1961). The crude product was treated with HCl in acetone to cleave the ketal and regenerate the keto group. The resulting keto acid mixture was examined by converting a small aliquot into the methyl esters by treatment with diazomethane. Gas chromatography showed that the mixture consisted of approximately 45% of the desired 9-ketodec-2-enoic acid, 52% of 9-ketodec-3-enoic acid, and 2% of an unidentified impurity. The specific activity of the mixture was approximately 3×10^6 dpm/mg.

The crude acid mixture was subjected to a selective esterification procedure (Weaver *et al.*, 1964), in which α,β-unsaturated acids are converted into esters at a much slower rate than saturated or other unsaturated acids. The acids were dissolved in 0.5 ml of methanol and 1 drop of 12 N HCl was added. After 40 minutes at room temperature, the reaction mixture was poured into ice-cold, saturated $NaHCO_3$ solution. After extraction of the esters (12.7 mg) with ether, the solution was acidified, and the free acids (10.7 mg) were recovered with ether. The free acid fraction had a specific activity of approximately 3.6×10^6 dpm/mg. This material was purified further by chromatography on buffered silicic acid (Weaver *et al.*, 1964) and by chromatography on thin layers of silicic acid to yield 1.6 mg of material that was homogeneous, as judged by gas chromatography and thin-layer chromatography with radioautography. The specific activity was approximately 9.6×10^6 dpm/mg.

Experimental Procedure

Feeding. Four feeding experiments were performed.

EXPERIMENT 1. The bees (574) received one experimental meal of 365 μg of [^{14}C]9-ketodecenoic acid (1650 cpm/μg), and were killed after 24 hours.

EXPERIMENT 2. The bees (610) received one experimental meal of 500 μg of [^{14}C]9-ketodecenoic acid (1650 cpm/μg), and were killed after 72 hours.

EXPERIMENT 3. The bees (171) received 3 experimental meals of 480, 550, and 265 μg of [^{14}C]9-ketodecenoic acid (1650 cpm/μg) at 0, 24, and 48 hours, and were killed after 72 hours.

EXPERIMENT 4. The bees (315) received one experimental meal of 500 μg of [^{14}C]9-ketodecenoic acid (9310 cpm/μg), and were killed after 72 hours.

The worker bees were removed from a hive and caged in a small wooden box having a glass front plate, screens for ventilation, and a small piece of honeycomb. The labeled acid was neutralized with $NaHCO_3$ and dissolved in honey, and the bees were allowed to feed *ad libitum*. After the test compound had been consumed the bees were fed honey and given water until they were killed.

Extraction and Analysis. EXPERIMENTS 1 AND 2. The bees were anaesthetized with CO_2 and dissected into head, thorax, gut, and remainder of abdomen. The tissues were ground with 20 ml of chloroform-methanol (2:1) per gram of tissue in a Waring Blendor with glass beads. After filtration, the residue was extracted twice more with chloroform-methanol. The extracts were washed with 0.05 volume of 0.1 N NaCl, combined, and evaporated to dryness in a rotary evaporator. The free fatty acid fraction was obtained by using the method of Weiss *et al.* (1960). For each gram of total lipid 50 ml of carbon tetrachloride and 200 ml of a 0.2 M solution of potassium chloride in 40% aqueous ethanol that contained 0.5% ammonia was added. The phases were equilibrated by shaking, and the aqueous layer was removed. The carbon tetrachloride layer was extracted three additional times with the aqueous ethanol solution, the combined aqueous layers were neutralized with dilute HCl, and the solution was concentrated in a rotary evaporator. The free fatty acids were extracted with ether. The neutral fraction which remained in the carbon tetrachloride phase usually contained labeled materials. These were examined by chromatography on silicic acid (Barron and Hanahan, 1958).

EXPERIMENT 3. A column of Dowex 1 OH^-, 30 ml, was washed with wet ether, and the lipids (21.3 mg) were added in wet ether (Lakshminarayana *et al.*, 1960). Neutral lipids were eluted with 300 ml of wet ether and the free fatty acids were eluted with 100 ml of 0.25 N NaOH in 25% aqueous methanol. The acid fraction was chromatographed on a silicic acid column to give two radioactive fractions: fraction 8 was eluted with 50% ether in hexane and fraction 10 was eluted with ether. Gas chromatography of the esters (see Table V) showed that each fraction contained only one labeled compound.

EXPERIMENT 4. The total lipids were dissolved in ether, and the ether solution was extracted three times with a saturated solution of sodium bicarbonate. The bicarbonate solution was neutralized with acid and the free fatty acids were extracted into ether solution. The ether solutions of the neutral materials and of the free fatty acids were dried over $MgSO_4$ and evaporated to dryness.

The free fatty acid fraction, obtained by one of the above methods, was analyzed by adsorption or partition

TABLE I: Distribution of ^{14}C in Tissues.[a]

	Expt 1		Expt 2		Expt 3		Expt 4	
	Total cpm ($\times 10^{-3}$)	cpm/g ($\times 10^{-3}$)	Total cpm ($\times 10^{-4}$)	cpm/g ($\times 10^{-4}$)	Total cpm ($\times 10^{-4}$)	cpm/g ($\times 10^{-4}$)	Total cpm ($\times 10^{-4}$)	cpm/g ($\times 10^{-4}$)
Head	1.83	4.6	0.8	2.88	1.08	1.17	1.5	6.6
Thorax	2.77	4.4	1.55	2.08	1.32		2.15	5.0
Abdomen	8.30	18.8	5.61	5.3	5.09	3.03	9.6	25.4
Gut	8.42	20.0	11.8	22.8	12.1	2.74	22.1	2.57

[a] Counts in total lipid.

TABLE II: Distribution of ^{14}C between Lipid Fractions.[a]

	Total Lipid		Neutral Lipid		FFA[b]	
	Total cpm ($\times 10^{-4}$)	cpm/mg ($\times 10^{-2}$)	Total cpm ($\times 10^{-4}$)	cpm/mg ($\times 10^{-2}$)	Total cpm ($\times 10^{-4}$)	cpm/mg ($\times 10^{-2}$)
Head	1.5	0.66	0.91	0.97	0.98	2.04
Thorax	2.15	0.54	3.8	0.88	0.06	0.51
Abdomen	9.6	2.54	5.5	1.83	2.7	22.0
Gut	22.1	2.57	9.6	1.16	10.0	34.0

[a] These data are from experiment 4. [b] FFA, free fatty acid.

chromatography on silicic acid (Weaver *et al.*, 1964), followed by gas-liquid chromatography.

Partition columns were prepared from 10 g of silicic acid mixed with 8.5 ml of 1 N citrate buffer at *p*H 5.75 and packed in hexane. The mixture of acids was added as a slurry in hexane and eluted with a linear gradient of 1-butanol in hexane (the total volume was 400 ml and the final butanol concentration was 20%). The radioactivity of each 5-ml fraction was determined by counting an aliquot with a Packard Tri-Carb liquid scintillation counter. Adsorption chromatography was carried out on columns of silicic acid (Unisil, Clarkson Chemical Co., Williamsport, Pa.), employing 1 g of adsorbant for 10 mg of acid mixture. The fractions were eluted with increasing concentrations of ether in hexane, followed by pure ether and methanol.

Fractions from the partition columns were usually further fractionated by thin-layer chromatography on silica gel G (Brinkmann Instruments, Great Neck, N.Y.) with the solvent systems hexane–ether–acetic acid (60:40:2 or 50:50:2). The labeled compounds were located by radioautography (Zalkin *et al.*, 1963), the silica gel in the areas of radioactive compounds on the plates was loosened from the plate and sucked into funnels with sintered glass disks (Baumann *et al.*, 1965), and the compounds were recovered by elution with ether.

The fractions from chromatography on silicic acid or thin-layer plates were converted into the methyl esters by treatment with diazomethane, and the esters were subjected to gas-liquid chromatography, as described previously (Weaver *et al.*, 1964). The esters emerging from the column were trapped at the exit in glass U-tubes cooled in acetone–dry ice and washed into scintillation sample bottles with scintillation fluid. The radioactive esters were identified by comparison of relative retention times with authentic samples of hydroxy and ketodecanoic acid esters (Weaver *et al.*, 1964). In addition, the labeled esters were subjected to chemical reactions and then chromatographed again and compared to reference compounds. These chemical reactions included the following.

Hydrogenation. An aliquot of the ester solution was hydrogenated in ethanol over Adams catalyst at room temperature and atmospheric pressure for 3 hours. The solution was filtered, the solvent was removed under a stream of nitrogen, and the sample was dissolved in fresh solvent for injection.

Acetylation. An aliquot of the ester solution was treated with a few μl of pyridine and acetic anhydride at room temperature for an hour. The solution was injected into the chromatographic apparatus without further treatment.

Reduction. An aliquot was reduced with $NaBH_4$ in 2-propanol at room temperature overnight. The solution was acidified and extracted with ether. The ether was removed in a stream of nitrogen and the residue was taken up in solvent for injection.

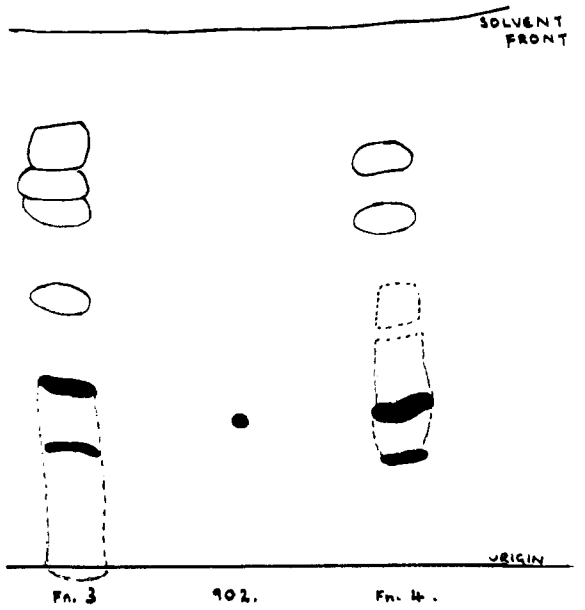


FIGURE 1: Thin-layer chromatography of fractions obtained by partition chromatography of the free fatty acids of abdomens (expt 2). The solvent mixture was hexane–ether–acetic acid (60:40:2). The radioactive compounds are shown as black spots. Other compounds (nonradioactive) were detected by spraying with a mixture of indicators and $KMnO_4$ (Pásková and Munk, 1960). Solid lines represent a strong color reaction while dotted lines represent a weak reaction; 902 is a laboratory abbreviation for 9-ketodec-2-enoic acid.

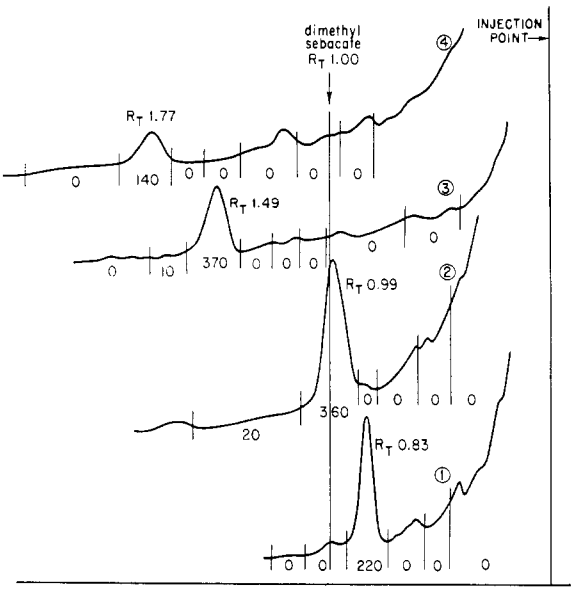


FIGURE 2: Gas-liquid chromatography of the methyl esters of the fatty acids removed from thin-layer plates (Figure 1). The conditions are those described by Weaver *et al.* (1964). Fractions were collected between the vertical marks shown along each chromatogram. The numbers shown between these marks refer to the level of radioactivity in the collected fractions, in cpm (see Table IV).

Oxidation. An aliquot of the ester was oxidized by a solution of CrO_3 in pyridine (Poos *et al.*, 1953) at room temperature overnight. The solution was neutralized with dilute acid and extracted with ether. The residue from the ethereal solution was dissolved in solvent for injection.

Results and Discussion

Total lipids from tissues of dissected bees were assayed for radioactivity. Table I summarizes the distribution of radioactive compounds derived from labeled 9-ketodecenoic acid in several tissues. While there is some variation from one experiment to another, it is clear that the greatest proportion of labeled metabolites is found in the gut and abdomen, even after three days.

Crude lipids from various tissues were partitioned (see experimental section) into neutral and acid fractions. As shown in Table II, only the free fatty acid fraction contained substances of high specific radioactivity, and most of the radioactive free fatty acids were found in the gut and abdomen.

The free fatty acids from the gut or abdomen were fractionated by partition chromatography. Four labeled fractions were obtained as shown in Table III. The two major fractions were purified further by thin-layer chromatography and individual acids were located by radioautography and by means of spray reagents. Four acids accounted for nearly all of the radioactivity of the free fatty acid fraction (Figure 1). Two of these were found in fraction 3 from the partition

TABLE III: Partition Chromatography of the Free Fatty Acid Fraction.

Fraction No.	Tubes Combined	Weight (mg)	Radioactivity (cpm)	Specific Activity (cpm/mg)
1, 1[a]	1–14	2.5	>100	
1, 2	15–21	5.3	3,000	560
1, 3	23–33	4.3	11,000	2,550
1, 4	35–44	1.5	20,000	1,300
4, 1[b]	1–4	1.77	740	420
4, 2	5–24	4.3	3,400	790
4, 3	25–34	3.4	69,000	20,300
4, 4	40–50	3.15	7,600	2,400

[a] 1, data of experiment 1, free fatty acids from abdomen. [b] 4, data of experiment 4, free fatty acids from the gut.

TABLE IV: Gas-Liquid Chromatographic Behavior of Labeled Acid Methyl Esters.[a]

Partition Column Fraction (Table III)	R_F of Acid on Thin-Layer Plate	Radio-activity (cpm)	Relative Retention Time[b]	Authentic Compound
1, 3	0.228[c]	470	0.99[d]	9-Hydroxydecanoate
	0.335	520	0.83	9-Ketodecanoate
1, 4	0.206	560	1.77	9-Hydroxydec-2-enoate
	0.294	2,220	1.49	9-Ketodec-2-enoate
4, 3	0.3	35,000	0.83	9-Ketodecanoate
	0.2	10,000	0.99	9-Hydroxydecanoate
4, 4[e]	0.3	500	0.83	9-Ketodecanoate
	0.2	300	0.99	9-Hydroxydecanoate

[a] The acids eluted from thin-layer plates were esterified with diazomethane and chromatographed. The effluent esters were collected and assayed for radioactivity. The positions of the peaks which contained most of the radioactive material are recorded and compared with the positions of peaks given by authentic compounds (see Weaver *et al.*, 1964). [b] Gas-liquid chromatography of methyl esters, relative to dimethyl sebacate. Only one radioactive compound was detected in each fraction. [c] Figure 1. [d] Figure 2. [e] This fraction contained only small amounts of the saturated acids; no unsaturated acids were found in experiment 4.

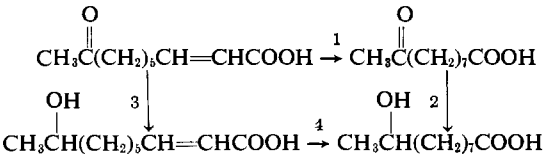
TABLE V: Identification of Labeled Compounds.

Relative Retention Time of Fraction	Reaction	Retention Time Shifted to:	Corresponding Compound
0.82	Hydrogenation	0.82	Methyl 9-ketodecanoate
	Acetylation	0.82	
	Reduction	0.99	Methyl 9-hydroxydecanoate
0.99	Hydrogenation	0.99	Methyl 9-hydroxydecanoate
	Acetylation	0.77	Methyl 9-acetoxydecanoate
	Oxidation	0.82	Methyl 9-ketodecanoate

column and had mobilities on thin-layer plates comparable to 9-ketodecanoic acid and 9-hydroxydecanoic acid. The other two acids, in fraction 4, corresponded to 9-ketodecenoic acid and 9-hydroxydecenoic acid.

The individual acids were eluted from thin-layer plates and esterified by treatment with diazomethane. The esters collected from the effluent stream of the gas chromatography column showed only one radioactive compound in each case (Figure 2 and Table IV). The compounds had appropriate relative retention times corresponding to those of authentic acids as shown in Table IV. In another experiment (expt 3) the isolated labeled acids were esterified and subjected to simple chemical manipulations followed by gas chromatography, thus confirming the identification of these compounds (Table V).

The major free fatty acid metabolites of 9-ketodec-2-enoic acid in the honeybee are therefore 9-ketodecanoic acid, 9-hydroxydecanoic acid, and 9-hydroxydec-2-enoic acid. The saturated compounds constitute a much larger proportion of the labeled metabolites than 9-hydroxydecenoic acid (see Table IV), indicating that the route involving reduction of the double bond as a first step (1 and 2, below) is the major one:



In the experiments of longer than 24 hours, the amount of labeled 9-ketodec-2-enoic acid remaining is very small, usually less than 5% of the total labeled free fatty acids. This is true even in experiment 3, where a small number of bees received doses greatly exceeding what one could expect that they would receive under

normal hive conditions in a similar time period. This demonstrates that the worker bees have a large capacity for converting 9-ketodecenoic acid into metabolites.

9-Hydroxydec-2-enoic acid and 9-ketodecanoic acid were shown by Pain *et al.* (1962) to be devoid of activity in the queen-rearing inhibition assay. 9-Hydroxydecanoic acid has not been tested for this biological activity as far as we know, but it is probably inactive. The conversion of the physiologically active 9-ketodec-2-enoic acid into these inactive substances could account for the rapid manifestation of queenless behavior by worker bees. Unlike hormones, which act on target tissues in the organism, the pheromones are thought usually to act on sense receptors to trigger a behavioral pattern (Wilson, 1963); at least such is the case when rapid alterations in behavior are involved. Thus in the honeybee worker 9-ketodec-2-enoic acid inhibits queen-rearing behavior when presented in liquid or vapor form, but it does not have this effect when injected directly into the body cavity (Butler and Fairey, 1963). It would be expected that, if this is true, some mechanism should be present for inactivating the pheromone after the behavior pattern has been released, so that the sense receptors can be prepared for a subsequent event. On the other hand, Butler and Fairey (1963) have shown that 9-ketodec-2-enoic acid inhibits oogenesis when it is injected into the hemolymph. This would indicate that the active substance must remain active until it passes into the hemolymph from the gut. The results of the experiments with labeled 9-ketodec-2-enoic acid are in harmony with both of these requirements. While most of the active molecules are converted into metabolites, a small amount of the active acid remains, even after rather long periods of time.[2] This may indicate inactivation of most of the pheromone so that regurgitated material will not trigger sense receptors, but a small amount of active material will remain to be absorbed into the hemolymph for inhibition of oogenesis.

In all experiments radioactive compounds were found in tissues other than those of the gut or abdomen, and in fractions other than the free fatty acids. These compounds have not been identified and their significance cannot be judged at present.

These experiments do not permit one to conclude that the ketodecenoic acid is actually metabolized in the cells of the honeybee gut, for no attempt was made to remove or inactivate the gut flora. This point does not seem to be a critical one, however, for the results show clearly that the normal honeybee worker has a high capacity for the metabolism of 9-ketodec-2-enoic acid.

While 9-hydroxydec-2-enoic acid has no activity in the control of queen-rearing behavior, it is present in queen mandibular glands, and Butler *et al.* (1964) have reported that it has another role in controlling the behavior of worker bees during swarming. Although this acid is produced by workers from 9-ketodec-2-enoic acid, it is formed in much smaller amounts than the saturated acids.

These experiments were begun in the hope of testing a hypothesis that a pheromone cycle might exist in honeybees. That is, worker bees could receive the 9-ketodec-2-enoic acid from the queen and convert it into an inactive form which had the same carbon chain. The inactive form could then be transported to the worker mandibular glands where it could be passed back to the queen in the form of a queen food. The queen could then convert it back into an active form by a very simple enzymatic reaction. This scheme would have the advantage of saving the relatively enormous amount of energy required for the complete synthesis of the fatty acid chain. Recently, Callow *et al.* (1964) have established the presence of 9-hydroxydec-2-enoic, 9-hydroxydecanoic, and 9-ketodecanoic acids in the mandibular glands of queen bees. It is, therefore, distinctly possible that similar compounds are involved in the synthesis of 9-ketodec-2-enoic acid by queen bees and the metabolites produced by worker bees. Further work will be necessary in order to test this pheromone cycle hypothesis, however.

[2] Labeled 9-ketodec-2-enoic acid was recovered in experiments 1, 2, and 3, but not in 4 (see Table IV).

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Decenoic, Dodecenoic, and Tetradecenoic Acids in the *Lactobacteriaceae**

William M. O'Leary

ABSTRACT: Gas-chromatographic analyses of the fatty acids of several species of the *Lactobacteriaceae* demonstrated the presence of C_{10}, C_{12}, and C_{14} monoenoic acids. Further chemical studies of such compounds recovered by preparative scale gas chromatography indicated that they were, respectively, *cis*-3-decenoic, *cis*-5-dodecenoic, and *cis*-7-tetradecenoic acids, members of the *cis*-11-octadecenoic acid series (i.e., $CH_3(CH_2)_3CH{=}CH(CH_2)_nCOOH$). There were also evidences of small amounts of Δ^3-dodecenoic and Δ^5-tetradecenoic acids in two species of streptococcus. This demonstration of the presence of shorter members of the *cis*-11-octadecenoic acid series in these organisms is particularly significant. Several years ago, it was postulated, even though it was not possible with the methodology then available to detect these compounds, that they should be intermediates in the lengthening mode of monoenoic fatty acid biosynthesis which occurs in these, and other, microorganisms. The evidence presented in this paper of the occurrence of these compounds supports the validity of earlier concepts.

As a result of studies of fatty acid requirements and metabolism in lactobacilli and other bacteria, it was suggested several years ago that at least one mechanism for the biosynthesis of long-chain monounsaturated fatty acids in microorganisms was the progressive lengthening of already unsaturated short chain fatty acids (O'Leary and Hofmann, 1957; Hofmann *et al.*, 1959). Further studies and expansions of this concept have been presented by Bloch and his co-workers (Bloch *et al.*, 1961; Scheuerbrandt and Bloch, 1962; Erwin and Bloch, 1964) who have shown that two major pathways for unsaturated fatty acid biosynthesis occur in microorganisms, aerobic dehydrogenation of long-chain saturated acids and anaerobic elongation of short-chain monoenoic acids. This subject has been extensively reviewed by O'Leary (1962a) and Kates (1964).

Investigations of the lengthening process have been done mostly with bacteria containing *cis*-11-octadecenoic acid as their sole or principal octadecenoic acid and palmitoleic acid as their hexadecenoic acid. These acids have a similar structure from the terminal methyl group through the double bond and differ from there to the carboxyl group only in the number of intervening methylene groups. It has been found that in these organisms members of a homologous series of such acids down to at least ten carbons in chain length satisfy exogenous fatty acid requirements and are converted ultimately into the longer chain intracellular acids (O'Leary, 1962a). However, the hypothesis that the lengthening pathway of fatty acid biosynthesis involves a series of monoenoic acids as intermediate steps has suffered from the failure actually to detect the shorter members of the series in bacterial cells. Aside from the report of Bloch *et al.* (1961) of the occurrence of tetradecenoic acid in certain clostridia and the report of a similar acid in corynebacteria by Asano and Takahashi in 1945, the presence in bacteria of unsaturated fatty acids shorter than hexadecenoic acid has been doubtful. This paper reports the occurrence of

* From the Department of Microbiology, Cornell University Medical College, New York City. *Received March 22, 1965.* This investigation was supported by a research grant (AI-03326) from the National Institutes of Health, U.S. Public Health Service.