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BIOSYNTHESIS OF METHYL BRANCHED HYDROCARBONS OF THE GERMAN COCKROACH *BLATTELLA GERMANICA* (L.) (ORTHOPTERA, BLATTELLIDAE)

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Abstract—The precursors and directionality of synthesis of the methyl branched cuticular hydrocarbons and the female contact sex pheromone, 3,11-dimethyl-2-nonacosanone, of the German cockroach, *Blattella germanica*, were investigated by radiotracer and carbon-13 NMR techniques. 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 the methyl branch group donor. The incorporation of [1,4-$^{14}C_2$]- and [2,3-$^{14}C_2$]succinates into the hydrocarbon and acylglycerol/polar lipid fractions indicated that succinate also served 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 and not carbon-2 was enriched. [1-^{13}C]Acetate labeled carbon-2 of these hydrocarbons. This indicates that the methyl branching groups of the 3,*x*-dimethylalkanes were inserted early in the chain elongation process. [3,4,5-$^{13}C_3$]Valine labeled the methyl, tertiary and carbon adjacent to the tertiary carbon of the methyl branched alkanes. Thus, the methyl branched hydrocarbon was formed by the insertion of methylmalonyl units derived from propionate, isoleucine, valine, methionine and succinate early in chain elongation.

Key Word Index: German cockroach, sex pheromone, hydrocarbons, methyl ketone, carbon-13 NMR, methylalkanes, *Blattella germanica*

INTRODUCTION

Methyl branched hydrocarbons are present in a variety of organisms where they have diverse functions. In insects they are present on the surface of the exoskeleton where they play a major role in preventing water loss, and in many species, serve in chemical communication (Howard and Blomquist, 1982; Blomquist *et al.*, 1987). In the German cockroach, *Blattella germanica*, mono- and dimethyl branched alkanes make up the majority of the cuticular lipids present on the insect's surface (Augustynowicz *et al.*, 1987; Carlson and Brenner, 1988; Jurenka *et al.*, 1989). The major hydrocarbons on adult female German cockroaches are 3,*x*-dimethylnonacosane where *x* is 11, 9 or 7 (Jurenka *et al.*, 1989).

The female contact sex pheromone of the German cockroach is present on the cuticular surface and elicits in males the wing raising courtship response that precedes mating. This pheromone has been identified as a series of three oxygenated derivatives of 3,11-dimethylnonacosane: 3,11-dimethyl-2-nonacosanone, 29-hydroxy-3,11-dimethyl-2-nonacosanone and 29-oxo-3,11-dimethyl-2-nonacosanone, with the major component being the methyl ketone (Nishida and Fukami, 1983).

The biosynthesis of methyl-branched hydrocarbons in insects has been investigated in a number of species (Blomquist *et al.*, 1987). Propionate, after conversion to methylmalonyl-CoA, has been shown to be the immediate source of the methyl branch unit of 3-methyl and internally branched alkanes. In the housefly (Dillwith *et al.*, 1982), American cockroach (Halarnkar *et al.*, 1985; Dwyer *et al.*, 1981) and the cabbage looper (de Renobales and Blomquist, 1983), the amino acid valine serves as the precursor to the methyl branch group, whereas in the termite *Zootermopsis species*, succinate is converted to methylmalonyl-CoA and then incorporated into methyl branched alkanes (Chu and Blomquist, 1980; Blomquist *et al.*, 1980).

Experiments were performed to determine the precursors of the methyl branching unit in the hydrocarbons and methyl ketone of the German cockroach, to determine whether the methyl branching units were added during the early or late stages of chain elongation, and to determine if methyl branched fatty acyl intermediates were present. The results of these experiments are reported herein.

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MATERIALS AND METHODS

Insects

German cockroaches were reared in glass jars and fed dog chow and water *ad libitum*. They were kept at 27°C with a 12:12 light:dark cycle. Males and females were separated on the day of adult emergence (day 0) and females were reared in groups until day 8, at which time they were utilized for the radiotracer experiments. Experiments involving ^{13}C-labeled substrates began on day 0.

Labeled substrates

Sodium [1-^{14}C]acetate (55 mCi/mmol) and L-[3,4-$^{14}C_2$]methionine (59 mCi/mmol) were obtained from Research Products International Corp., Mount Prospect, Ill. Sodium [1-^{14}C]propionate (4.58 mCi/mmol) was obtained from Pathfinder Lab. Inc., St Louis, Mo. [2,3-$^{14}C_2$]Succinate (15 mCi/mmol), [1,4-$^{14}C_2$]succinate (20.5 mCi/mmol), [G-^{3}H]valine (10 Ci/mmol) and [4,5-^{3}H]isoleucine (18 Ci/mmol) were obtained from ICN Radiochemicals, Irvine, Calif.

DL-[3,4,5-$^{13}C_3$]Valine was supplied by the Los Alamos Scientific Laboratory, Los Alamos, N.M., and was prepared as described by Whaley *et al.* (1979). Potassium [1-^{13}C]acetate and sodium [2-^{13}C]acetate were obtained from MSD Isotopes, Rahway, N.J. Sodium [1-^{13}C]propionate was obtained from Sigma Chemical Company, St Louis, Mo.

In vivo radiotracer experiments

Labeled substrates (2 μCi for [1-^{14}C]acetate, 1 μCi for the other substrates) in 1–2 μl of aqueous solution were injected into the abdomen of 8-day-old virgin adult female cockroaches (triplicate groups of three). After specified times, the insects were placed in a −20°C freezer. The cuticular hydrocarbon and methyl ketone fractions were extracted and separated as described by Jurenka *et al.* (1989). The internal lipids were then extracted from the whole insects by the procedure of Bligh and Dyer (1959). To separate internal hydrocarbons and methyl ketones from other lipids, the lipids were first hydrolyzed to free fatty acids by refluxing with 5% KOH in 95% ethanol (w/v) at 60°C for 40 min and then extracted with diethyl ether. The ether extract was then dried under a stream of N_2 and the hydrocarbon and methyl ketone fractions separated by thin-layer chromatography (TLC) on silica gel Type H developed in hexane:diethyl ether (90:10 v/v) and compared to standards. After separation by TLC the hydrocarbon and methyl ketone fractions were scraped into test tubes and extracted with diethyl ether. These fractions were then assayed for radioactivity by liquid scintillation counting.

The free fatty acids were extracted after acidifying the hydrolysis mixture and methylated (Blomquist *et al.*, 1982). The methyl esters were then analyzed by radio-high performance-liquid chromatography (HPLC) (Jurenka *et al.*, 1987). A C8 reverse-phase column (particle size 5 μm, 25 cm × 4.6 mm) was coupled to a Spectra Physics SP8700 solvent delivery system set at a flow rate of 1 ml/min with the mobile phase isocratic (acetonitrile:water, 85:15 v/v). Radioactivity was detected using a Radiomatic Instruments Flo-one Beta radioactive flow detector.

The internal acylglycerol/polar lipid fraction was isolated as one band by thin-layer chromatography (TLC) in hexane:ether (95:5 v/v), extracted from the silica gel with chloroform:methanol:water:acetic acid (50:39:1:10 v/v) and aliquots assayed for radioactivity by liquid scintillation counting.

Carbon-13 NMR experiments

Two-hundred and fifty μg of sodium [1-^{13}C]propionate (90 + %) in 1 μl of Ringer's solution were injected daily or on alternate days into adult female insects beginning on day 0 after emergence to the adult. Insects were removed and stored at −20°C when they died. At the end of 18 days, all remaining insects were killed by freezing at −20°C and pooled. An identical procedure was utilized for incorporation of [1-^{13}C]acetate and [2-^{13}C]acetate. [3,4,5-$^{13}C_3$]Valine was incorporated by mixing the substrate with crushed dog food in a 1:3 w/w ratio. The fourteen insects ingested 23.5 mg labeled valine in 9 days, after which the insects were killed, cuticular lipids and internal lipids extracted and the hydrocarbons isolated as described above. The [1-^{13}C]propionate-labeled hydrocarbon fractions were analyzed on a GE QE-300 NMR at 75 MHz. The total hydrocarbon fractions labeled by [3,4,5-$^{13}C_3$]valine, [2,3-$^{13}C_2$]succinate, [1-^{13}C]acetate and [2-^{13}C]acetate were analyzed on a Brucker AC-300 at 75 MHz. Reported chemical shifts are relative to internal $CDCl_3$ at 77 ppm. Resonance assignments were made based on calculated values (Levy and Nelson, 1972) and the values obtained for mono- and dimethylalkane standards (Pomonis *et al.*, 1990).

RESULTS

[1-^{14}C]Propionate labels both the internal and cuticular hydrocarbon and the methyl ketone fractions to a greater extent than does [1-^{14}C]acetate, as is shown in Table 1. There is an almost linear increase in the percent incorporation of both substrates into the cuticular hydrocarbons for up to 8 h whereas there is little increase in the percent incorporation of radioactivity into the internal hydrocarbon after the 2 h time period. This suggests that the labeled acetate

Table 1. Incorporation of [1-^{14}C]propionate and [1-^{14}C]acetate into cuticular and internal hydrocarbon and methyl ketone fractions after 2, 4, 8 and 24 h incubation time periods. $n = 3$. Data are means ± SD

	% Incorporation			
	2 h	4 h	8 h	24 h
		[1-^{14}C]Propionate		
Cuticular				
Hydrocarbon	0.23 ± 0.11	0.51 ± 0.11	0.95 ± 0.27	1.5 ± 0.14
Methyl ketone	0.010 ± 0.008	0.013 ± 0.003	0.016 ± 0.008	0.021 ± 0.003
Internal lipids				
Hydrocarbon	5.16 ± 1.1	5.49 ± 1.5	8.92 ± 1.1	2.94 ± 0.8
Methyl ketone	0.004 ± 0.002	0.018 ± 0.013	0.019 ± 0.004	0.025 ± 0.019
Acylglycerols	—	—	0.11 ± 0.014	—
		[1-^{14}C]Acetate		
Cuticular				
Hydrocarbon	0.07 ± 0.01	0.14 ± 0.06	0.26 ± 0.02	0.63 ± 0.18
Methyl ketone	0.006 ± 0.002	0.009 ± 0.004	0.008 ± 0.003	0.012 ± 0.010
Internal lipids				
Hydrocarbon	1.97 ± 0.35	1.75 ± 0.16	2.14 ± 0.36	1.95 ± 0.29
Methyl ketone	0.010 ± 0.002	0.006 ± 0.003	0.004 ± 0.001	0.005 ± 0.001
Acylglycerols	—	—	2.32 ± 1.24	—

Table 2. Assignment of resonances

Chemical shift	Assigned carbons
14.1	C_1, C_n of normal and internally* branched alkanes
19.2	Methyl branch carbon of 3-methylalkanes
19.7	Methyl branch carbon of internally* branched alkanes
22.7	C_2 and C_{n-1} of *n*- and internally* branched alkanes
26.8	Carbon x† + 2
29.4	C_2 of 3-methyl- and 3,*x*-dimethylalkanes
29.7	All isolated methylene carbons
30.0	Carbon x† + 3 and carbon 6 of 3-methylalkanes
31.9	C_3, C_{n-2} of *n*- and internally branched methylalkanes
32.8	Tertiary carbon of internally branched alkanes (carbon *x*)
34.4	Tertiary carbon of 3-methyl- and 3,*x*-dimethylalkanes
36.6	C_4 of 3-methyl- and 3,9- and 3,11-dimethylalkanes
37.1	Carbon x† + 1
37.4	Carbon y‡ + 1 and y + 3

*Internally branched alkane is defined as having the methyl branch carbon 5 or more carbons from the end of the chain.
†Carbon *x* is the tertiary carbon of internally branched alkanes.
‡Carbon *y* is the tertiary carbon of dimethylalkanes in which there are 3 methylene groups between the methyl branch carbons.

and propionate are incorporated into lipid within 2 h after injection, and that the increased amount of radioactivity in the cuticular hydrocarbons reflects the transport over time of internal hydrocarbon to the surface of the insect.

Radioactivity from [1-^{14}C]propionate and [1-^{14}C]acetate is incorporated into the methyl ketone fraction at much lower levels than into hydrocarbon. In addition, for [1-^{14}C]propionate, there is a time-dependent increase in the percent incorporation of radioactivity into both the cuticular and internal methyl ketone fraction for up to 24 h. [1-^{14}C]Acetate was readily incorporated into the acylglycerol/polar lipid fraction, but [1-^{14}C]propionate did not efficiently label the acylglycerol/polar lipid fraction.

To determine whether the methyl groups of the methylalkanes were incorporated early or toward the end of the elongation process, groups of adult female insects were injected with [1-^{13}C]acetate, with [2-^{13}C]acetate or with [1-^{13}C]propionate. The hydrocarbons from these insects were extracted, isolated and analyzed by carbon-13 NMR, and compared with the natural abundance spectrum of these hydrocarbons. The methyl ketone fraction was also analyzed but did not contain enough material to produce interpretable spectra. Table 2 lists the assignment of each resonance to the appropriate carbons in the mixture of alkanes. These assignments were made based on the method described by Levy and Nelson (1972) and from the resonances of known mono- and dimethylalkane standards (Pomonis *et al.*, 1990).

Figure 1 presents the natural abundance ^{13}C-NMR spectrum of the total hydrocarbon fraction from *B. germanica*. The spectra of the hydrocarbon fraction after enrichment with [1-^{13}C]acetate [Fig. 2(A) and (B)] clearly show that certain carbons are enriched over the natural abundance spectrum (Fig. 1). Of particular interest are the resonances of carbon 1, 14.1 ppm, which is enriched by [2-^{13}C]acetate; of carbon 2 of 3-methyl- and 3,*x*-dimethylalkanes, 29.4 ppm, which is enriched by [1-^{13}C]acetate; and of carbon 4 of 3-methyl and 3,*x*-dimethylalkanes, 36.6 ppm, which is not enriched by either [1-^{13}C]- or [2-^{13}C]acetate. These results would be predicted if carbons 1 and 2 are incorporated as the acetate unit during the initial chain synthesis step.

The ^{13}C-NMR spectrum of the hydrocarbons from the [1-^{13}C]propionate treated insects (Fig. 3) clearly shows that the resonances at 36.6, 37.1 and 37.4 ppm are enriched. The resonance at 36.6 corresponds (Table 2) to carbon 4 of the 3,*x*-dimethylalkanes. This resonance is not detectable in the natural abundance sample and was not enriched from [2-^{13}C]acetate. The resonances at 37.1 and 37.4 correspond to the carbon adjacent to the tertiary carbon of internally branched alkanes and the carbons adjacent and internal to the tertiary carbons of the dimethylalkanes which have their branch points separated by 3 methylene units. In addition to confirming that propionate serves as the methyl branching donor, the enrichment of carbon 4 but not carbon 2 (resonance at 29.4) of the 3,*x*-dimethylalkanes, together with the enrichment of carbon 2 by acetate, provides conclusive evidence that the propionate unit was added as the second group in alkane biosynthesis.

The percent incorporation of radiolabeled amino acids and of succinates labeled in the 2,3- and 1,4-positions into both cuticular and internal

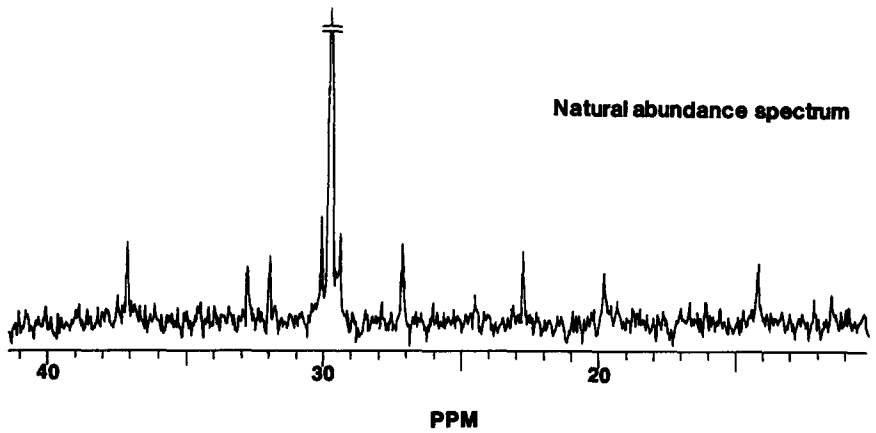

Fig. 1. Carbon-13 NMR spectrum of natural abundance hydrocarbons from the German cockroach. Hydrocarbon was extracted and isolated and NMR analysis was performed as described in Materials and Methods.

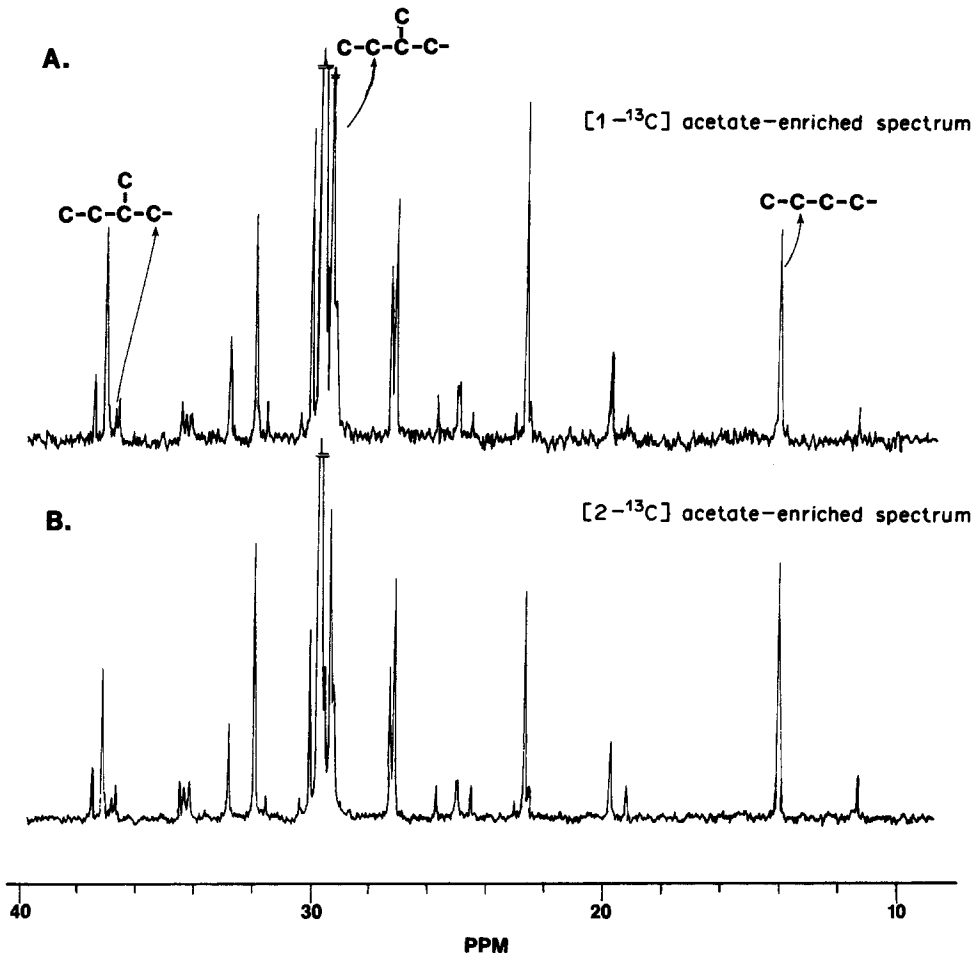

Fig. 2. Carbon-13 NMR spectra of [1-^{13}C]acetate (A) and [2-^{13}C]acetate (B) enriched hydrocarbons from the German cockroach. Labeled acetates were administered, hydrocarbon extracted and isolated and NMR analyses were performed as described in Materials and Methods.

hydrocarbon and internal acylglycerols is presented in Table 3. The amino acids valine, isoleucine and methionine can be oxidatively metabolized to methylmalonyl-CoA which could then be used in methyl branched hydrocarbon biosynthesis. These amino acids labeled both internal and cuticular hydrocarbons at relatively high rates, with isoleucine being incorporated to the highest extent. The differences in the specific activities of the amino acids, our lack of knowledge of pool sizes of endogenous amino acids, and the expectation that isoleucine would be metabolized to acetyl-CoA and methylmalonyl-CoA make comparisons between the incorporation of the three amino acids difficult. Nevertheless, the relatively high incorporation of each of these amino acids into hydrocarbon strongly suggests that this insect uses all three as precursors to the methyl branching unit. In contrast to the incorporation of acetate and propionate into hydrocarbon, there was an increase in the percent of the radioactivity incorporated into hydrocarbon (Table 3) from the three amino acids for up to 24 h.

Incorporation of these substrates into acylglycerols plus more polar lipids for 8 h time periods is also presented in Table 3. The relatively low levels of incorporation of amino acids and succinate into this fraction as compared to incorporation into hydrocarbon for the same time period further suggests that the carbon skeletons of these substrates are metabolized to propionyl-CoA. The higher percent incorporation of isoleucine into acylglycerol is probably accounted for by the fact that the carbon skeleton of isoleucine can also be converted to acetate which readily labels all lipid classes.

The interpretation that the carbon skeleton of these amino acids becomes the methyl branch unit for hydrocarbon biosynthesis is confirmed by studies which utilized [3,4,5-$^{13}C_3$]valine. The NMR spectrum of the total hydrocarbon fraction from insects fed labeled valine is shown in Fig. 4. Based on the resonance assignments from Table 2 and the splitting pattern observed from this tri-labeled substrate, it is apparent that carbons 3, 4 and 5 of valine become the tertiary, the methyl branch and the internal secondary carbons, respectively, of the hydrocarbon molecule. The signals from the methyl branch carbon and the carbon adjacent to the tertiary carbon are each split by one carbon-13, consistent with the incorporation of the labeled carbons of [3,4,5-$^{13}C_3$]valine as an intact unit. The splitting pattern for

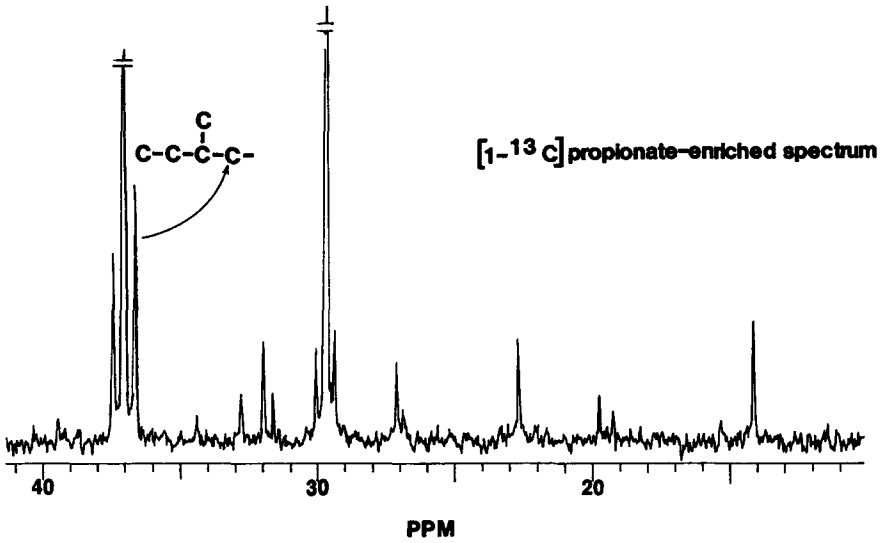

Fig. 3. Carbon-13 NMR spectrum of [1-^{13}C]propionate enriched hydrocarbons from the German cockroach. Labeled propionate was administered, hydrocarbon extracted and isolated and NMR analyses were performed as described in Materials and Methods.

the tertiary carbon is very complex and is interpreted as arising from the signal for this carbon split by the two adjacent labeled carbons (methyl branch carbon and carbon adjacent to the tertiary carbon) and is made yet more complex by the slight differences in the chemical shift for the tertiary carbons near the end of the carbon chain versus those more internal.

In a termite, succinate was shown to be a precursor to the methyl branch unit after conversion to a methylmalonyl-CoA unit (Chu and Blomquist, 1980). In the studies reported here, both [2,3-$^{14}C_2$]- and [1,4-$^{14}C_2$]succinates were incorporated into hydrocarbon, with the [2,3-$^{14}C_2$]succinate incorporated at about twice the rate of the [1,4-$^{14}C_2$]succinate at the 8 and 24 h intervals (Table 3). If succinate were converted to methylmalonyl-CoA and then incorporated into hydrocarbon, both carbons from the [2,3-$^{14}C_2$]succinate would label hydrocarbon, whereas only one of the carbons from the [1,4-$^{14}C_2$]succinate would label hydrocarbon. On the other hand, if succinate were metabolized to malate, then through pyruvate to acetate prior to incorporation into hydrocarbon, both carbons from the [1,4-$^{14}C_2$]succinate would be lost as CO_2 whereas both carbons from the [2,3-$^{14}C_2$]succinate would be incorporated into hydrocarbon. The approximate 2:1 ratio of incorporation of the succinate labeled in the 2,3-positions compared to succinate labeled in the 1,4-positions at the 8 and 24 h time periods is consistent with succinate first being metabolized to methylmalonyl-CoA

Table 3. Percent incorporation of [G-^{3}H]valine, [4,5-^{3}H]isoleucine, [4,5-$^{14}C_2$]methionine, [2,3-$^{14}C_2$]succinate and [1,4-$^{14}C_2$]succinate into cuticular and internal hydrocarbon and internal acylglycerol/polar lipid fractions after 2, 4, 8 and 24 h incubation time periods. $n = 3$. Data are means ± SD

Hydrocarbon source	% Incorporation 2 h	4 h	8 h	24 h
		[G-^{3}H]Valine		
Cuticular	0.02 ± 0.001	0.05 ± 0.01	0.09 ± 0.01	0.25 ± 0.04
Internal	0.19 ± 0.03	0.28 ± 0.05	0.41 ± 0.05	0.43 ± 0.05
Acylglycerol/polar lipid	—	—	0.08 ± 0.01	—
		[4,5-^{3}H]Isoleucine		
Cuticular	0.08 ± 0.01	0.20 ± 0.02	0.50 ± 0.13	0.95 ± 0.15
Internal	1.04 ± 0.14	1.60 ± 0.14	1.94 ± 0.51	2.05 ± 0.32
Acylglycerol/polar lipid	—	—	0.29 ± 0.04	—
		[3,4-$^{14}C_2$]Methionine		
Cuticular	0.03 ± 0.012	0.08 ± 0.03	0.30 ± 0.46	0.50 ± 0.10
Internal	0.43 ± 0.16	0.37 ± 0.11	1.80 ± 0.03	0.83 ± 0.25
Acylglycerol/polar lipid	—	—	0.03 ± 0.02	—
		[2,3-$^{14}C_2$]Succinate		
Cuticular	0.05 ± 0.01	0.11 ± 0.03	0.16 ± 0.01	0.18 ± 0.06
Internal	0.30 ± 0.09	0.61 ± 0.05	0.50 ± 0.21	0.42 ± 0.01
Acylglycerol/polar lipid	—	—	0.54 ± 0.10	—
		[1,4-$^{14}C_2$]Succinate		
Cuticular	0.01 ± 0.003	0.01 ± 0.0	0.06 ± 0.01	0.09 ± 0.03
Internal	0.10 ± 0.009	0.14 ± 0.01	0.27 ± 0.02	0.25 ± 0.03
Acylglycerol/polar lipid	—	—	0.03 ± 0.01	—

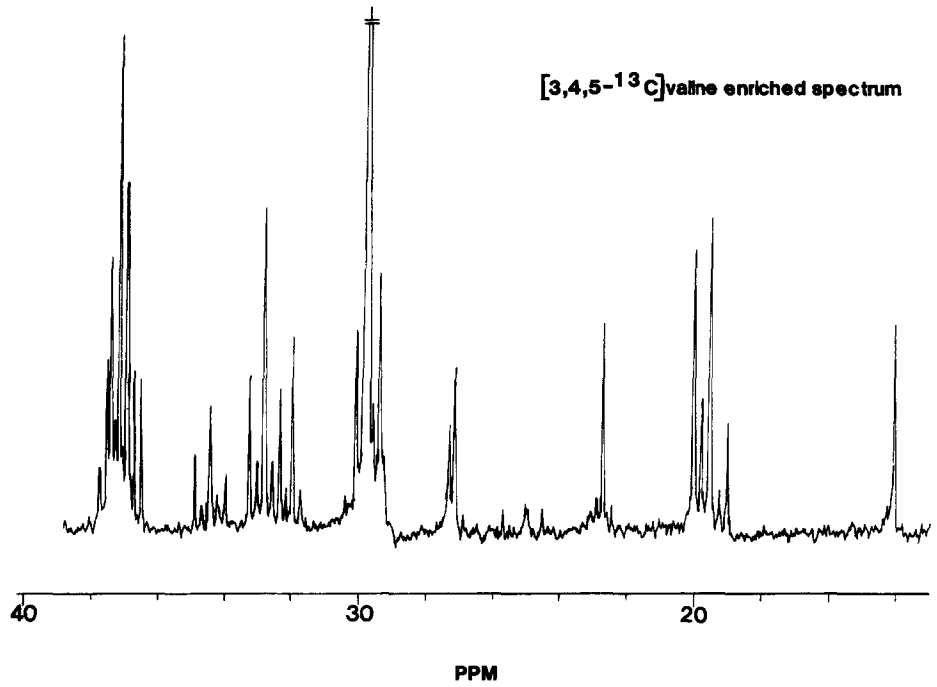

Fig. 4. Carbon-13 NMR spectrum of [3,4,5-$^{13}C_3$]valine enriched hydrocarbons from the German cockroach. Labeled valine was administered, hydrocarbon extracted and isolated and NMR analyses were performed as described in Materials and Methods.

and then incorporated into hydrocarbon as the methyl branch donor. The greater incorporation of [2,3-$^{14}C_2$]succinate into acylglycerol/polar lipid fraction compared to [1,4-$^{14}C_2$]succinate also indicated conversion of succinate into acetate. However, any labeling of hydrocarbons from [1,4-$^{14}C_2$]succinate shows conversion of succinate to a methylmalonyl unit. Therefore we conclude that the labeling of hydrocarbon from [1,4-$^{14}C_2$]succinate must be through conversion of succinate to methylmalonyl-CoA.

Proposed intermediates in the biosynthesis of branched hydrocarbons are methyl branched fatty acids. Evidence for the presence of these intermediates was sought by injecting [1-^{14}C]propionate into 8-day-old adult females for 3 days. After 3 injections of 1 μCi/injection, the internal fatty acids were isolated and analyzed as their methyl ester derivatives by radio-HPLC. The retention times of the fatty acid methyl esters (FAMEs) obtained after labeling with [1-^{14}C]propionate were compared to retention times obtained from FAMEs labeled with [1-^{14}C]acetate (Fig. 5). Acetate labeled the straight chain saturated and monounsaturated fatty acids 14:0, 16:0, 18:0, 16:1 and 18:1. In contrast, the fatty acids labeled with [1-^{14}C]propionate had retention times that were related, but not identical, to those of straight chain even or odd carbon number fatty acids (Fig. 5 insert), indicating that propionate labels fatty acids which are methyl branched and of ordinary chain length. Analysis of the total fatty acids of the German cockroach by gas–liquid chromatography showed no mass for compounds other than the straight chain saturated and unsaturated components. This indicates that the fatty acids labeled by [1-^{14}C]propionate are present in very small quantities, presumably as transient intermediates, and suggests that they are intermediates in branched alkane biosynthesis. The lack of detectable mass precluded the use of gas chromatography–mass spectrometry to positively identify these putative intermediates in branched chain alkane synthesis.

DISCUSSION

The structural similarities between the hydrocarbon and the pheromone components in the German cockroach suggest that they are generated from the same carbon skeletons (Jurenka *et al.*, 1989). The precursors to the methyl branch unit of the methyl branched hydrocarbon and methyl ketone include both the amino acids that can be metabolized to propionate (valine, isoleucine and methionine) and succinate. In the housefly, *Musca domestica*, succinate did not serve as a methyl branch donor (Halarnkar *et al.*, 1987) whereas valine and isoleucine did (Dillwith *et al.*, 1982). Methionine was not tested in the housefly, but it did not serve as an efficient precursor to propionate for use in juvenile hormone synthesis in *Manduca sexta* (Brindle *et al.*, 1987).

The conversion of succinyl-CoA to methylmalonyl-CoA in insects is dictated in part by the levels of vitamin B_{12}. The termite *Zootermopsis species* contains relatively high amounts of vitamin B_{12} (Wakayama *et al.*, 1984) and readily converts succinate to methylmalonyl-CoA which is incorporated into methyl branched alkanes (Blomquist *et al.*, 1980; Chu and Blomquist, 1980). The housefly, which has no detectable levels of B_{12}, is unable to perform this conversion, but instead converts succinate to acetate

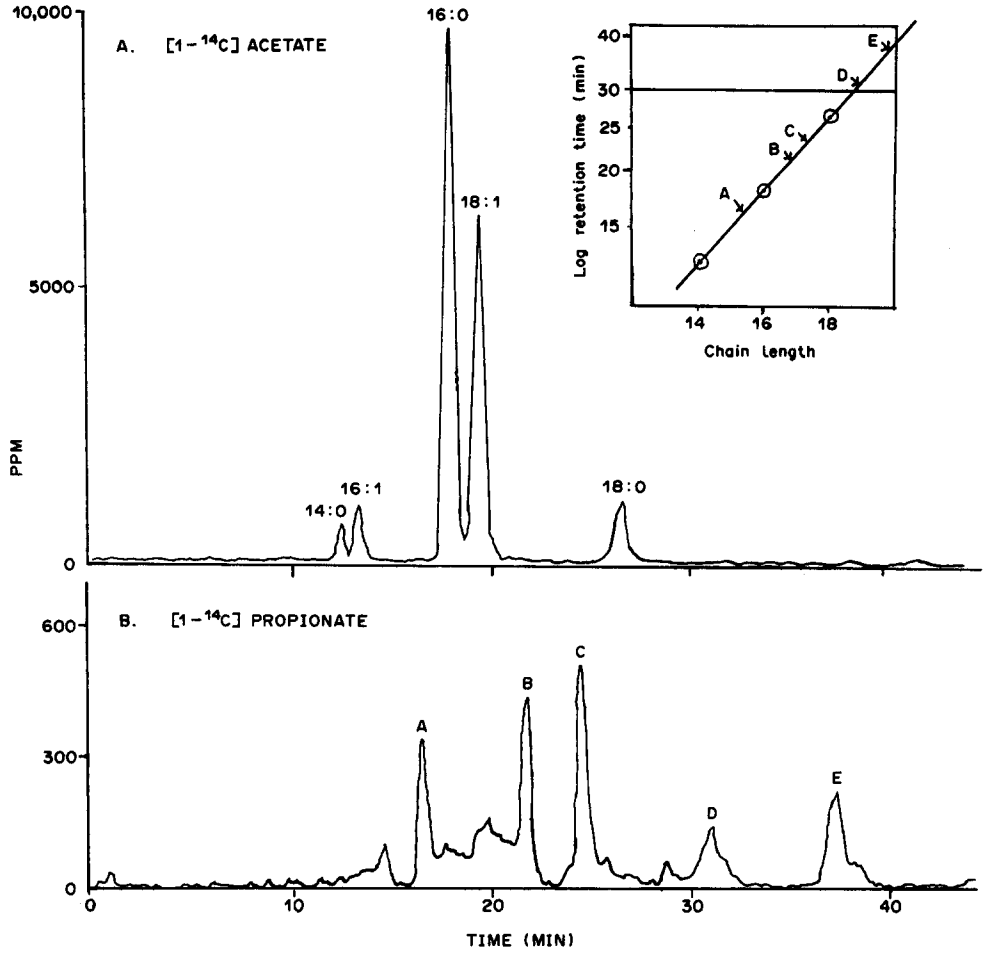

Fig. 5. Radio-HPLC analyses of the fatty acid methyl esters of the fatty acids of the German cockroach isolated after treatment with [1-^{14}C]acetate (A) and [1-^{14}C]propionate (B). Labeled material was injected, the fatty acids extracted, isolated and derivatized and radio-HPLC performed as described in Materials and Methods. The insert shows the retention times of the propionate-labeled fatty acids superimposed on a standard curve of known straight-chain fatty acids.

via malate and pyruvate (Halarnkar *et al.*, 1987). The German cockroach, which is known to have appreciable levels of B_{12} (Blomquist and Chase, unpublished), seems to be able to utilize both of these pathways for metabolism of succinyl-CoA. Succinate is apparently converted to acetate as indicated by the labeling of the acylglycerol/polar lipid fraction with [2,3-$^{14}C_2$]succinate but not with [1,4-$^{14}C_2$]succinate. In addition, the lower but significant labeling of the hydrocarbon fractions by [1,4-$^{14}C_2$]succinate indicates that some succinate is converted to methylmalonyl-CoA for branched hydrocarbon biosynthesis.

The methyl branch groups of dimethylalkanes are inserted early in the chain elongation process in the German cockroach, as demonstrated by the enrichment of carbon-4 but not carbon-2 from [1-^{13}C]propionate. This is consistent with the observations from both NMR and mass spectral studies in the American cockroach (Dwyer *et al.*, 1981) and the housefly (Dillwith *et al.*, 1982). The observation that, in the German cockroach, propionate labeled fatty acids of 16–20 carbons that appeared to be methyl branched would also be consistent with the insertion of the methyl groups early in chain elongation.

The formation of 9,10-epoxytricosene and 14-tricosen-10-one in the housefly (Ahmad *et al.*, 1987) and the epoxide pheromone of the Gypsy moth (Kasang *et al.*, 1974) occur via insertion of an oxygen into a preformed hydrocarbon chain. It is conceivable that the methyl ketone sex pheromone of the German cockroach arises via the hydroxylation and subsequent oxidation of the 3,11-dimethylnonacosane to the corresponding ketone. Further work is underway in our laboratory to explore this process.

The contact sex pheromones of the housefly and tsetse fly are associated with the cuticular lipids. In the tsetse fly, the female produces very long chain methyl branched alkanes that are present on the female but essentially absent from the male (Nelson and Carlson, 1986). In the housefly, (Z)-9-tricosene is not produced until the female initiates vitellogenesis (Dillwith *et al.*, 1983) and its production appears to be initiated by ecdysteroids (Adams *et al.*, 1984; Blomquist *et al.*, 1984a, 1987). The polysubstrate monooxygenase (Ahmad *et al.*, 1987) activity that

converts the alkene to the corresponding epoxide and ketone is present in both males and females of all ages (Blomquist *et al.*, 1984b). These results, taken together, suggest that the process that controls pheromone production in the housefly is the step which converts fatty acyl groups to the hydrocarbon. In contrast, both the male and female German cockroach possess relatively high amounts of the parent hydrocarbon, 3,11-dimethylnonacosane, both in the nymphal stages and as adults (Jurenka *et al.*, 1990), and apparently the step that regulates pheromone production is the conversion of the hydrocarbon to the methyl ketone. The production of the methyl ketone pheromone appears to correlate with both juvenile hormone levels and vitellogenesis, thus indicating a common endocrine regulation of both processes (Schal *et al.*, 1990).

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