BIOSYNTHESIS OF 3-METHYLPENTACOSANE IN THE COCKROACH PERIPLANETA AMERICANA

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Received March 3, 1975

Summary: The biosynthesis of 3-methylalkanes was investigated in the cockroach Periplaneta americana. Between 0.2 and 0.3 percent of the labelled acetate and propionate injected into the insect was incorporated into the cuticular hydrocarbons, compared to 0.01 percent for labelled isoleucine. Twenty-three ± four percent of the [2-^{14}C]acetate, 42 ± 3 and 44 ± 4 percent of the [2-^{14}C] and [3-^{14}C]propionate, and 75 ± 5 percent of the [1-^{14}C]propionate incorporated into the cuticular hydrocarbons was found in 3-methylpentacosane. These results indicate that propionate serves as the source of the branching methyl group, suggesting a pathway in which this precursor is incorporated during the penultimate step in 3-methylalkane biosynthesis in insects.

Normal and methyl branched hydrocarbons are common constituents of the cuticular lipids of plants and insects (1,2). The biosynthesis of these cuticular components has received considerable attention in plants, with convincing evidence on the pathways leading to the major components reported (1,3,4). In contrast, the biosynthetic pathways for insect cuticular hydrocarbons have not received extensive study. Acetate has been shown to be readily incorporated into the hydrocarbons of live insects (2,5,6), excised cuticles (2,6), and oenocyte rich fat body preparations (7). Indirect evidence on n-alkane biosynthesis obtained from studying the formation of secondary alcohols (8,9,10) in insects suggests that an elongation-decarboxylation pathway is operative. However, direct evidence on the biosynthetic pathways for either the normal or methyl branched hydrocarbons in insects is not available.

The pathways leading to the formation of the 3-methylalkanes are especially controversial. In plants, isoleucine has been shown to be oxidized to 2-methylbutyric acid, which is incorporated into 3-methylalkanes during the initial stages of chain elongation (1,3,11). Propionate incorporated during the penultimate step has been suggested to give rise to the branching methyl group of the 3-methylalkanes in insects (6). In the present paper, the source of the methyl

branching group has been investigated by comparing the incorporation of labelled substrates into 3-methylpentacosane in the cockroach Periplaneta americana.

P. americana was chosen for this study because of its simple hydrocarbon composition. The major hydrocarbons are n-pentacosane (10 percent), 3-methylpentacosane (16 percent), and cis-cis-6,9-heptacosadiene (73 percent) (12,13).

EXPERIMENTAL PROCEDURES

Sodium[2-^{14}C]acetate(55 mCi/mM), [2-^{14}C]D,L-mevalonic acid lactone(10.3 mCi/mM), sodium[methyl-^{14}C]methylmalonate(10.5 mCi/mM)., sodium[1-^{14}C]propionate(0.55 mCi/mM), sodium[2-^{14}C]propionate(27 mCi/mM) and sodium[3-^{14}C]propionate(0.8 mCi/mM) were purchased from New England Nuclear. [G-^{3}H]L-Isoleucine(2.7 Ci/mM), [G-^{3}H]L-valine (6.8 Ci/mM) and [methyl-^{14}C]L-methionine(50 mCi/mM) were purchased from ICN. [R-^{3}H]n-Tricosane(14 mCi/mM) was obtained as described earlier (8).

A starter colony of P. americana was obtained from Dr. Mary Ross. Colonies of P. americana were reared in metal containers on a diet of ground dry dog food and an agar-water (1:99) gel fed ad lib. Late instar nymphs were used in all studies.

Labelled substrates were injected between the second and third abdominal segments in 2 µl solvent. Acetate, isoleucine, valine, mevalonate, and methylmalonate were injected in a water solution, propionate in ethanol, and palmitic acid in hexane. The insects were kept at room temperature and sacrificed after 6 hours. In some cases larger amounts of labelled substrates and longer incubation times were used where greater amounts of labelled hydrocarbons were needed for radio-gas-liquid-chromatography (radio-GLC).

The cuticular lipids were extracted by immersion of the insects in hexane for 10 min. After the volume was reduced by evaporation under nitrogen, the residue was transferred to a Pasteur pipette containing 50 mg Bio Sil A in 1 ml hexane. The hydrocarbons were eluted into a vial with 8 ml hexane. The sample was divided into two equal fractions and taken to dryness under nitrogen. One fraction contained one-half of the total hydrocarbons. The branched hydrocarbons were isolated by adding molecular sieve 5 Å (14) to the other fraction, followed

by agitation for six hours in 1 ml iso-octane. The branched hydrocarbons in iso-octane were transferred to another vial, the molecular sieve washed twice with an additional 1 ml of iso-octane and the washes combined and taken to dryness under nitrogen.

Samples were transferred to counting vials in hexane, the solvent evaporated to dryness under nitrogen, and 10 ml of a fluor solution (0.4% PPO in toluene) added. Samples were counted for 10 min on a Packard Tri Carb liquid scintillation counter. All data points are the average of 3 to 6 experiments and 3 insects were used in each experiment.

The same procedure described above was used to isolate total and branched hydrocarbon fractions from insects not injected with labelled substrates. GLC of both the total and branched samples was performed on a 6 ft x 1/8 in column containing 3% SE-30 on gas chrom Q programmed from 150 to 280° C at 8° C per min. Integration was obtained by disc integration. Radio-GLC of the hydrocarbons isolated after incubation with sodium[2-^{14}C]acetate and sodium[1-^{14}C]propionate was run under identical conditions. A 9:1 stream splitter was used and each peak was collected in a Pasteur pipette, the sample washed into a scintillation vial with fluor solution and counted.

Branched alkanes isolated from experiments using labelled [2-^{14}C]acetate and samples of [R-^{3}H]n-tricosane were each divided into two equal fractions. One fraction was evaporated to dryness under nitrogen and taken through the molecular sieve procedure described above. The control and molecular sieved fractions were then counted by scintillation counting to determine the percent recovery of branched and normal alkanes from molecular sieve 5 Å under the conditions used in this study.

RESULTS

Labelled acetate, propionate, and methylmalonate were readily incorporated into the cuticular hydrocarbons of P. americana (Table I). Acetate and propionate were incorporated most readily, with 0.2 to 0.3 percent of the injected label appearing in the hydrocarbon fraction, compared to 0.13 percent for methylmalonate

Table I. Incorporation of labelled acetate, propionate, and methylmalonate into the cuticular hydrocarbon of P. americana.

Substrate	Percent of label incorporated into hydrocarbon	Label Incorporated into Hydrocarbon: Percent in branched[a]	Label Incorporated into Hydrocarbon: Percent in straight chain[a]
[2-^{14}C]acetate	0.25 ± 0.10	23 ± 4	77 ± 4
[3-^{14}C]propionate	0.26 ± 0.14	44 ± 4	56 ± 4
[2-^{14}C]propionate	0.23 ± 0.08	42 ± 3	58 ± 3
[1-^{14}C]propionate	0.2 ± 0.05	75 ± 5	25 ± 5
[Methyl-^{14}C]methylmalonate	0.13 ± 0.05	45 ± 4	55 ± 4

[a]Corrected for loss of branched alkane into 5 Å molecular sieve. Eighty-eight ± two percent of the branched alkane is recovered.

Twenty-three ± four percent of the acetate incorporated into hydrocarbon was found in the branched fraction, compared to 42 ± 3 and 44 ± 4 percent for [2-^{14}C] and [3-^{14}C]propionate and 45 ± 4 percent for [methyl-^{14}C]methylmalonate. However, 75 ± 5 percent of the [1-^{14}C]propionate incorporated into hydrocarbon was found in the branched chain components (Table I).

GLC of the total and branched hydrocarbons from P. americana (Figure I) shows that the only major component not included in Molecular Sieve 5 Å is 3-methylpentacosane. To confirm the removal of n-alkanes and determine the percent of recovery of branched alkanes after inclusion in molecular sieve 5 Å, labelled normal and branched alkanes were included in molecular sieve 5 Å. Eighty-eight ± two percent of 3-methylpentacosane was recovered, compared to less than 1 percent of n-tricosane.

To verify that [1-^{14}C]propionate was selectively incorporated into 3-methylpentacosane, radio-GLC of the hydrocarbon fractions isolated after incubation with [2-^{14}C]acetate and [1-^{14}C]propionate was run. The results showed that acetate was incorporated into each component in about the same proportion as the percent composition of hydrocarbon. [1-^{14}C]Propionate, however, was incor-

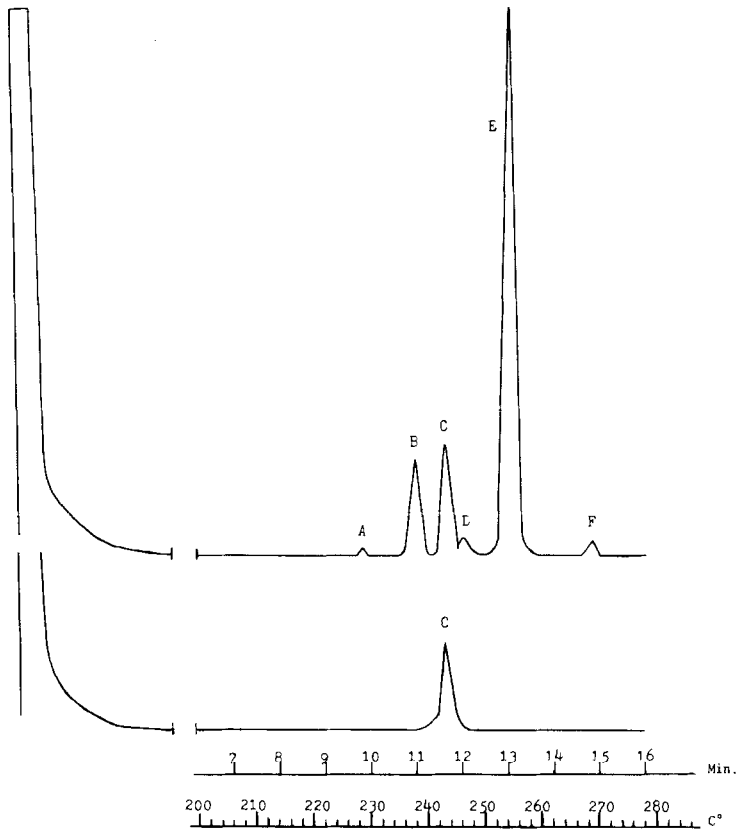

Figure I. GLC traces of total (top) and branched (bottom) hydrocarbon of P. americana. Peaks are identified as (A) n-tricosane, (B) n-pentacosane, (C) 3-methylpentacosane, (D) n-hexacosane, (E) cis-cis-6,9-heptacosadiene, and (F) n-nonacosane (6).

porated preferentially into 3-methylpentacosane, with about a 3-fold increase in the specific activity in this branched alkane over that from the same peak in the experiment using labelled acetate.

Labelled L-isoleucine, L-valine, and L-methionine were not readily incorporated into the hydrocarbons of P. americana, and mevalonic acid lactone does not appear to be selectively incorporated into the branched fraction (Table II). Only 0.01 and 0.03 percent of injected labelled L-isoleucine and L-valine are incorporated into the hydrocarbon fraction, although 99 ± 1 and 90 ± 3 percent of the label incorporated is found in the branched components. No incorporation of the methyl group from L-methionine into hydrocarbons was detected. Mevalonic

Table II. Incorporation of labelled isoleucine, valine, methionine, and mevalonic acid lactone into the cuticular hydrocarbon of P. americana.

Substrate	Percent of label incorporated into hydrocarbon	Label Incorporated into Hydrocarbon: Percent in branched[a]	Label Incorporated into Hydrocarbon: Percent in straight chain[a]
[G-^{3}H]isoleucine	0.01 ± 0.01	99 ± 1	1 ± 1
[G-^{3}H]valine	0.03 ± 0.01	90 ± 3	10 ± 3
[Methyl-^{14}C]methionine	<0.01	-	-
D,L[2-^{14}C]mevalonic acid lactone	0.10 ± 0.03	29 ± 5	71 ± 5

[a]Data corrected for loss of branched alkane into 5 Å molecular sieve. Eighty-eight ± two percent of branched alkane is recovered.

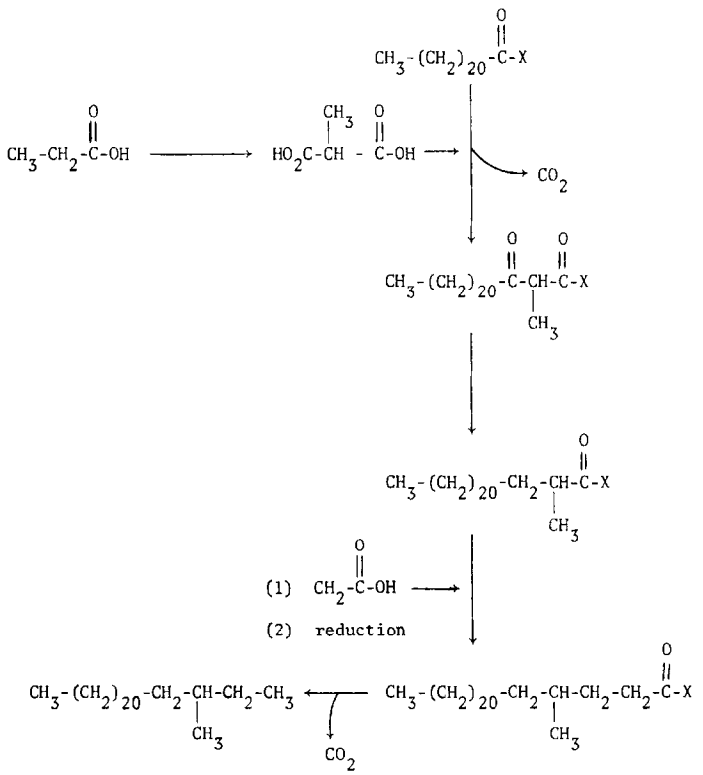

Figure II. Suggested pathway for the biosynthesis of 3-methylalkanes in insects.

acid lactone was incorporated into the hydrocarbon fraction at low levels, but the isoprenoid unit is apparently not incorporated intact, since only 29 ± 4 percent of the label incorporated is found in the branched fraction.

DISCUSSION

Results of these experiments suggest that insects utilize a different pathway for the biosynthesis of 3-methylalkanes than that demonstrated in plants. L-Isoleucine, which serves as the branching methyl group donor for 3-methylalkanes in plants (1,3,11), is poorly incorporated into the hydrocarbon fraction in P. americana. Although essentially all of the label from L-isoleucine incorporated into hydrocarbons is found in the branched fraction, similar data was obtained for L-valine, which is not a likely precursor for the 3-methylalkanes. This suggests a random incorporation of these branched chain substrates at very low levels via a minor pathway.

Figure II shows a proposed pathway for the biosynthesis of 3-methylalkanes in insects. Propionate is converted to methylmalonate, and incorporated as the penultimate unit into 3-methylpentacosane. An acetyl group is then added and decarboxylated. The preferential incorporation of labelled propionate and methylmalonate into 3-methylpentacosane compared to acetate supports this hypothesis

A comparison of the data on the incorporation of [1-^{14}C], [2-^{14}C], and [3-^{14}C] propionate suggests that propionate can be metabolized to other precursors, and that these precursors are incorporated into both branched and straight chain hydrocarbons. The number of metabolic pathways involving propionate is sufficiently great to preclude any simple resolution of this problem. However, the data suggest that a significant portion of the propionate incorporated into hydrocarbon is metabolized to an acetyl unit with loss of the carboxyl carbon (15). This would account for the preferential incorporation of the [1-^{14}C]propionate and the lower, but nearly equal incorporation of the [2-^{14}C] and [3-^{14}C]propionate into 3-methylpentacosane, as the labelled carboxyl carbon would be lost during conversion to an acetyl unit.

This data appears to substantiate the suggestion made by Conrad and Jackson that the branching methyl group in the 3-methylalkanes arises from propionate (6). It also suggests the possibility of a similar incorporation of propionate into internally branched monomethylalkanes in insects, in which additional acetyl units could be added prior to decarboxylation.

ACKNOWLEDGEMENTS

We are grateful to Dr. Mary Ross for supplying us with a starter colony of P. americana. This work was supported in part by a Cottrell grant from Research Corporation and a Sigma Xi research grant to Gary J. Blomquist.

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