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The Biochemistry of Long-Chain, Nonisoprenoid Hydrocarbons. III. The Metabolic Relationship of Long-Chain Fatty Acids and Hydrocarbons and Other Aspects of Hydrocarbon Metabolism in *Sarcina lutea**

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ABSTRACT: Fatty acids added to cultures of *Sarcina lutea* caused changes in the hydrocarbon composition of the cells.

These changes were consistent with a mechanism of synthesis in which the major fatty acid of the cells condensed with the added fatty acid or with fatty acids that increased in response to the added fatty acid and in which one of the fatty acids participating in the condensation was decarboxylated. In the presence of acetate in the medium, exogenous palmitate was incorporated into the hydrocarbon by a mode of entry in which it was specifically not decarboxylated. In media with low acetate, 60–70% of exogenous palmitate incorporated into the hydrocarbon was decarboxylated. Under conditions of incorporation in which the palmitate was not decarboxylated, the carboxyl carbon of the palmitate occurred in monounsaturated hydrocarbons specifically on the side of the double bond opposite that in which the remainder of the aliphatic chain from palmitate was located. Evidence for the direct conversion of monounsaturated hydrocarbons into saturated derivatives and for the failure of ketones to serve as intermediates in the incorporation of fatty acids into hydrocarbons is presented. Alternative mechanisms for the intermediary conversion of fatty acids into hydrocarbons by the condensation of the carboxyl carbon and α-carbon of acids with decarboxylation of one of the acids (head-to-head condensation) that bypasses the requirement for ketones or secondary alcohols are presented.

The large proportion of monounsaturated hydrocarbons in *Sarcina lutea* that have branched methyl groups at both ends of the molecule and the high proportion of branched-chain fatty acids in the lipids of this organism suggested that the hydrocarbons were synthesized by head-to-head condensation of two molecules of fatty acids. Oxidation of the double bond of the C-29 monounsaturated hydrocarbons gave rise to equal amounts of *anteiso*-C-15 and -C-14 fatty acids (each approximately 41% of the total), and this coupled with the fact that no *anteiso*-C-14 fatty acid occurred in the cell lipids further suggested that decarboxylation of one of the fatty acids occurred in the condensation mechanism (Albro and Dittmer, 1969a). Subsequent studies on the incorporation of the ^{14}C-labeled aliphatic chains of isoleucine and valine and of acetate into fatty acids and hydrocarbons of *S. lutea* were fully consistent with this mechanism for hydrocarbon biosynthesis (Albro and Dittmer, 1969b). We present here data from *in vivo* studies on the incorporation of ^{14}C-labeled fatty acids into hydrocarbons that further support the fatty acid head-to-head condensation mechanism and give some insight into the details of the mechanism. Preliminary studies of the role of aliphatic ketones in the biosynthesis and the interconversion of alkenes and alkanes are also reported.

Methods

General culture conditions for *S. lutea*, isolation procedures for lipids, details of gas–liquid partition and thin-layer chromatographic systems, and methods used for assaying radioactivity in lipid samples are described in the first two papers of this series (Albro and Dittmer, 1969a,b). Palmitic-16-^{14}C and -1-^{14}C acids (Calbiochem) with radiopurities of 98%, myristic-1-^{14}C

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acid (Nuclear-Chicago) with a radiopurity of 99%, and octadecane-1-^{14}C (Nuclear-Chicago) with a radiopurity of 99.4% were used in this study. Radiopurity was established by chromatography on silica gel thin-layer plates and by gas–liquid partition chromatography with Apiezon L liquid phase.

In addition to the use of broth cultures, some experiments were carried out with uniformily spread cultures or "lawns" on trypticase soy agar plates. Cultures were usually grown for 24 hr after inoculation, the labeled compound was added, and incubation was continued for the specified time. Cells were harvested by scraping them from the agar and the lipids were isolated and fractionated as usual.

Heptacosanone-^{14}C was synthesized by the method of Weygand (1945). Myristate-1-^{14}C (20 mg; μCi/mg) was converted into the barium salt and heated at 200° for 5 hr under slight vacuum. The condensation products were then distilled at 1 mm and 350°. The distillate was purified by two successive chromatographic fractionations on Florisil and again on silicic acid after saponification. The final product gave only one spot after silica gel thin-layer chromatography with benzene–chloroform (9:4) as visualized with both acidic 2,4-dinitrophenylhydrazine and Rhodamine 6G. All of the radioactivity was localized in this spot. No label was found with hydrocarbons, fatty acid esters, alcohols, or free fatty acids when the product was cochromatographed with these compounds. When samples were fractionated on a preparative W-98 gas–liquid partition chromatography column, 90% of the injected activity was collected in the region where heptacosanone should have chromatographed. Undecan-6-one and tricosan-12-one were used as reference standards. Both infrared spectra (CCl_4 solutions and dried films) of the compound and ultraviolet–visible spectra of $CHCl_3$ and ethanol solutions of the dinitrophenylhydrazine derivative were consistent with the identification as a long chain ketone. The final recovery of ketone was 33% of the initial mass of the myristic acid and 16.9% of the initial radioactivity. The fact that the specific activity (disintegrations per minute/mg) was one-half that of the myristic acid-1-^{14}C precursor indicated that the product, as expected from the reported reaction mechanism (Neunhoeffer and Paschke, 1939; Bamdas and Shemyakin, 1948), was probably hetacosan-14-one-14-^{14}C.

Results

Effect of Media Fatty Acids on the Composition of Total Lipid Fatty Acids and Hydrocarbons. If fatty acids added to culture media are taken up by *S. lutea* and incorporated into the hydrocarbons, then, depending upon the mechanism of biosynthesis, the amount of hydrocarbon of certain chain lengths may change accordingly. For example, Kolattukudy (1967) has pointed out that the elongation–decarboxylation mechanism of hydrocarbon biosynthesis in plants would be expected to produce only odd-carbon hydrocarbons from even-carbon fatty acids. In examining this experimental approach, the effect of oleate and palmitate when added to cultures of *S. lutea* was determined.

Oleic acid (15 mg/l.) when added to trypticase soy broth cultures completely inhibited further growth; but cells grown in the media described by Ikawa (1963) supplemented with oleic acid did show some growth. Identical innocula of *S. lutea* were transferred to flasks of this medium or the same medium plus 15 mg of sodium oleate/l. and the cells were harvested after 48 hr. The yield of cells grown in the presence of oleate was 75% of that found with the control. No detectable effect of the oleate on the yield of hydrocarbon was observed but the control cells had one-third the fatty acid content of those grown with oleate. The data are given in Table I. No oleate was detected in the lipid extract or the media from which the cells were harvested. Although the standard deviations for the fatty acid composition of duplicate experiments were large, significantly higher amounts of palmitate (1.5-fold, $p < 0.05$) and myristate (2-fold, $p < 0.02$) and lower amounts of C-16:1 (3-fold, $p < 0.05$) and branched C-16 acids (2-fold, $p < 0.1$) were found in the cells exposed to oleate. The only significant differences in the hydrocarbons were higher amounts of one of the two C-28 and one of the two C-30 fractions separated by gas–liquid partition chromatography. The increases, 2- and 1.5-fold ($p < 0.05$ and 0.01), respectively, were comparable with that observed for the increases in the normal C-14 and C-16 fatty acids. There were no changes in the relative concentrations of odd-carbon hydrocarbons that approached statistical significance.

TABLE I: Yield of Cells, Total Lipid Fatty Acids, and Hydrocarbons from 48-hr *S. lutea* Cultures Grown in Synthetic Media with and without Added Oleic Acid.

Media	Cell Dry Wt (g/l. of culture)	Total Lipid Fatty Acids[b]	Hydrocarbons[b]
Control[a]	3.63	25.4	3.18
Control + 15 mg of sodium oleate/l.	2.72	57.3	2.44

[a] Synthetic media described by Ikawa (1963). [b] In milligrams per liter of culture.

When cells were grown for 48 hr in trypticase soy broth medium supplemented with 20 mg/l. of sodium palmitate, no significant change in the concentration of any of the lipid fatty acids, including palmitate, occurred. Analyses of extracts of the medium indicated that 80% of the palmitic acid had been taken up from the media after 48 hr. One of the two C-30 hydrocarbon fractions and the C-31 hydrocarbons increased in concentration 2-fold.

The Fate of C-1 and -16 of Palmitic Acid in Hydrocarbon Biosynthesis. Comparison of the degree to which the radioactivity of fatty acids labeled in the carboxyl

TABLE II: Fate of C-1 and -16 of Exogenous Palmitate in the Synthesis of Hydrocarbons.[a]

Form of Labeled Substrate Added to Media	Media	Specific Activity of Hydrocarbons: Pamitate-1-^{14}C Precursor (dpm/mg)	Specific Activity of Hydrocarbons: Palmitate-16-^{14}C Precursor (dpm/mg)	Activity Ratio of 1-^{14}C Labeled/16-^{14}C Labeled
Sodium palmitate	M73b	226	241	0.94 ± 0.14
Sodium palmitate	M73b	201	220	0.91 ± 0.14
Potassium palmitate	Trypticase soy broth	4010	11210	0.36 ± 0.07
Free palmitic acid	Trypticase soy broth	880	2510	0.35 ± 0.06
Free palmitic acid	Trypticase soy broth + 2 g/l. of $CH_3COONa \cdot 3H_2O$	324	315	1.00 ± 0.06

[a] Equal amounts of palmitate-1-^{14}C and -16-^{14}C were added to separate 200-ml aliquots of freshly inoculated cultures prepared with the media indicated. The cultures were incubated under identical conditions for 24 hr, the cells were harvested, and the hydrocarbons were isolated and assayed for radioactivity. The ratios of the specific activities of the hydrocarbons labeled with palmitate-1-^{14}C to that labeled with palmitate-16-^{14}C were calculated as a measure of the proportion of the palmitate that was decarboxylated in hydrocarbon biosynthesis. The variability in the amount of activity incorporated in the various experiments was in part due to the differences in specific activities of the precursor acids. The sodium salts had 1.17×10^5 dpm/mg, potassium salts had 6.69×10^6 dpm/mg, and the free acids in the two experiments had 1.48×10^6 and 1.33×10^5 dpm/mg, respectively. The 1-^{14}C and 16-^{14}C compounds were adjusted in concentration with unlabeled acid to give the same specific activity. Aliquots of approximately 2 mg of hydrocarbon were counted.

carbon as compared with the remaining carbons of the molecule has been used to help elucidate the biosynthetic pathway for hydrocarbons in plants (*e.g.*, Kolattukudy, 1966–1968b). In general, the fact that C-1 was found to be incorporated equally as well as the remainder of the molecule has been cited as evidence in support of an elongation–decarboxylation pathway.

In a series of experiments, 50 ml of a 24-hr culture of *S. lutea* was added to 1 l. of fresh medium and mixed well. Equal portions were transferred to sterile flasks to provide matched cultures and palmitate labeled in either C-1 or -16 was added to the flask in the form of free fatty acids or sodium or potassium salts in equivalent masses (15 mg/l.) and specific activities. Incubation was continued to mid-log phase, the cells were harvested, and the hydrocarbons were isolated and assayed for radioactivity. The ratios of the specific activities of the hydrocarbons labeled from palmitate-1-^{14}C to that from -16-^{14}C are given in Table II. They varied from 0.94 in M73b medium to 0.35 in trypticase soy broth medium. The difference for the two media appears to be due to the acetate content of the M73b medium since supplementation of trypticase soy broth media with acetate caused the ratio to increase to 1.0.

Location of C-1 of Palmitate in Monounsaturated Hydrocarbons Relative to the Double Bond. When *S. lutea* was grown for 19 hr on trypticase soy agar plates with palmitate-16-^{14}C, only 3.8 ± 0.4% of the label (total of 18,300 dpm) was released by Schmidt degradation (decarboxylation) of the fatty acids produced by oxidizing the double bond of the unsaturated hydrocarbons. After labeling with palmitate-1-^{14}C, 91.0 ± 0.6% of the label (total of 13,680 dpm) was released as CO_2 during Schmidt degradation of the fatty acid oxidation products.

This experiment showed that the double bond evidently marked the point of condensation of the fatty acids in the biosynthesis of the hydrocarbons. In the head-to-head condensation mechanism for hydrocarbon biosynthesis proposed by Chibnall and Piper (1934), a secondary alcohol was dehydrated to form a monounsaturated intermediate. In the dehydration step, the double bond could be formed to either side of the carbon bearing the hydroxyl group as follows:

$$RCH_2COOH + R'CH_2COOH \longrightarrow \longrightarrow$$

$$RCH_2\underset{\displaystyle OH}{\underset{|}{C}}HCH_2R' \longrightarrow RCH{=}CHCH_2R' \text{ and/or } RCH_2CH{=}CHR'$$

The potential for such specificity exists whatever the intermediates of condensation, and the use of a secondary alcohol here should be considered as being for illustrative purposes only. The observation reported above that *S. lutea* will incorporate exogenous palmitate into its hydrocarbons only without decarboxylation when acetate is added to the media made it possible to check the specificity of this step of the biosynthesis.

S. lutea was cultured for 24 hr in trypticase soy broth medium supplemented with 2 g/l. of sodium acetate trihydrate and 20 mg/l. of potassium palmitate-1-^{14}C

(4 μCi/l.). The fatty acids of the total lipids and oxidation products of the unsaturated hydrocarbons were methylated and fractionated by gas–liquid partition chromatography. The distribution of activity in various fractions is given in Table III.

TABLE III: Specificity in the Formation of the Double Bond in the Synthesis of Monounsaturated Hydrocarbons.[a]

Fatty Acid Fraction Assayed	% of Label in Fraction
Total lipid	
C-16 acids	96.4 ± 0.1
Oxidized hydrocarbons	
C-13, C-14, C-15 + C-17	94.0 ± 0.2
C-16	6.0 ± 0.3

[a] After being cultured in 1 l. of trypticase soy broth medium supplemented with 2 g of sodium acetate and 4 μCi of potassium palmitate-1-^{14}C for 24 hr, the cells were harvested and the total lipid fatty acids and hydrocarbons were isolated. The hydrocarbons were oxidized and the fatty acids produced were methylated. These methyl esters and the methyl esters of the fatty acids from the total lipids were separated by gas–liquid partition chromatography on a preparative cyclohexanedimethanol succinate column (Albro and Dittmer, 1969a). A total of 80 mg of fatty acids from the total lipids was obtained and this had 190,920 cpm of ^{14}C. A 8-mg sample was fractionated. A total of 9 mg of acids from the hydrocarbons with 5410 cpm of ^{14}C was obtained and the total sample was fractionated. As determined by the area under the gas–liquid partition fractionation pattern, 6% of the acids derived from the hydrocarbon was palmitate and 4% of the total lipid fatty acids.

The recovery of 96.4% of the label in the lipid fatty acids in palmitic acid showed that little redistribution of the label of the acid fed occurred. On the other hand, the fact that most of the label recovered in the fatty acids derived from the monounsaturated hydrocarbons was in acids other than palmitic acid meant that C-1 of palmitic acid ended up across the double bond from the remainder of the carbon chain derived from palmitate.

Interconversion of Unsaturated and Saturated Hydrocarbons. Early stationary phase *S. lutea* has approximately 90% monounsaturated hydrocarbons, As the cells continue into stationary phase the percentage decreases (with a corresponding increase in saturated hydrocarbons) at an average rate of 0.35%/hr (Albro and Dittmer, 1969a). The ^{14}C incorporated into hydrocarbons from isoleucine-^{14}C accumulates in alkanes at approximately this same rate. These increases would be most logically accounted for by a conversion of the alkenes into alkanes although the net increase in hydrocarbon content during stationary phase is sufficiently large that saturated hydrocarbon biosynthesis independent of alkene formation is possible.

To test this, hydrocarbons were isolated from *S. lutea* after incubation with acetate-2-^{14}C or isoleucine-U-^{14}C. The unsaturated hydrocarbons were purified by the mercuric acetate procedure and supplied to 24-hr uniformly spread cultures on trypticase soy agar. Incubation was continued at room temperature for 3 or 7 hr. The cells were scraped from the plates and the hydrocarbons were isolated and fractionated into saturated and unsaturated fractions on silica gel thin-layer plates impregnated with silver nitrate. In 3 hr with acetate-labeled hydrocarbons the percentage of label in the saturated hydrocarbons increased from 0.3% of the total in the hydrocarbons fed to 2.2% in the hydrocarbons isolated after incubation. This represents an average of conversion of 0.63%/hr. Isoleucine-labeled hydrocarbons over a 7-hr period gave a conversion rate of 0.37%/hr. In either case the rate of conversion of alkenes into alkanes is as great or greater than that needed to account for the increased proportion of alkanes.

In an attempt to establish whether alkanes are converted into alkenes, octadecane-1-^{14}C (0.5 μCi) was supplied to agar cultures as described above, and the plates were incubated for 7 hr. The recovery of ^{14}C in the hydrocarbons after incubation was 100%, and no label was found associated with octadecene when the alkene fraction obtained by the mercuric acetate procedure was fractionated by preparative gas–liquid partition chromatography. When *S. lutea* was grown up in trypticase soy broth with 10 mg/l. of either *n*-tetracosane, *n*-octadecane, or hexamethyltetracosane, the hydrocarbons were readily taken up and could be extracted from the harvested cells by extraction with neutral solvents. No *n*-tetracosene, *n*-octadecene, or hexamethyltetracosene could be detected in the extracts even after 48-hr incubation. These experiments are consistent with the conversion of alkenes into alkanes by *S. lutea;* but if the reverse process occurs, it is either extremely slow or occurs under conditions which were not tested.

Long-Chain Ketones as Hydrocarbon Precursors. Chibnall and Piper (1934) proposed that long-chain aliphatic ketones and secondary alcohols may serve as intermediates in the conversion of fatty acids into hydrocarbons by head-to-head condensation. We previously reported that both ketones and secondary alcohols occur as components of the nonsaponifiable lipids of *S. lutea* (Albro and Dittmer, 1969a). Using the methods described in that report for the isolation and purification of these compounds, we have further established that label from acetate-2-^{14}C, palmitate-16-^{14}C, and ^{14}C-labeled, branched-chain C-15 fatty acids isolated from *S. lutea* grown in the presence of isoleucine-U-^{14}C was incorporated into these ketones and secondary alcohols as well as other nonsaponifible lipids including aldehydes and primary alcohols.

However, when heptacosanone-^{14}C (total of 7.4×10^4 dpm) was added to uniformly spread cultures of *S. lutea* on trypticase soy agar and the cultures were incubated for an additional 5 or 7 hr, only 0.032 ± 0.007 or

0.092 ± 0.005%, respectively, of the activity was found in the hydrocarbons isolated from the cells. Under the same conditions, label from palmitate-16-^{14}C was incorporated into the hydrocarbons to the extent of 0.70 ± 0.01% in 7 hr. Under these conditions it is apparent that exogenous ketones were not as good precursors of hydrocarbons as exogenous fatty acids. Various reasons for the poor conversion of exogenous ketones may be evoked including the question of their availability to the biosynthetic system in intact cells. Because of the development of an *in vitro* system which would help obviate these difficulties, further *in vivo* studies with this approach were not carried out. The results of studies with the *in vitro* system will be described in subsequent papers of this series.

Discussion

Although there would not appear to be any obvious reason why oleic acid should produce the changes observed in the fatty acid composition reported here, the changes in fatty acids and hydrocarbons appear to be related. The major fatty acid in the cells remains the branched C-15 acid. The observed increase in C-28 hydrocarbon may have resulted from an increase in the extent to which the increased amount of C-14 fatty acid condensed with the C-15 fatty acid (with decarboxylation), and similarly the increase in the C-30 hydrocarbon would be related to the increase in the C-16 fatty acid. In either case, elongation–decarboxylation of neither C-14 nor C-16 fatty acids would give rise to C-28 or C-30 hydrocarbons. Our interpretation of the results is supported by the findings with palmitic acid. The increases in C-30 and C-31 hydrocarbons would arise due to an increase in the head-to-head condensation with decarboxylation of the exogenous palmitate with the major endogenous fatty acid, branched C-15, and with itself. Obviously, the C-31 hydrocarbon could also arise by elongation–decarboxylation, but this would not apply to the C-30 component. Further support for this proposed explanation comes from the fact that the equivalent chain lengths of the C-28 and C-30 hydrocarbons calculated from the gas–liquid partition chromatograph are consistent with compounds having one normal and one branched aliphatic terminal.

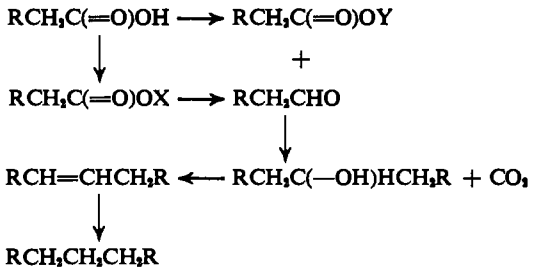
If palmitic acid is incorporated into hydrocarbons through an elongation–decarboxylation pathway, the specific activity of hydrocarbons labeled from palmitate-1-^{14}C should be equal to that of those labeled from palmitate-16-^{14}C under identical growth conditions. If label from palmitate-^{14}C was incorporated into hydrocarbon *via* acetate derived from palmitate by β-oxidation, the hydrocarbon labeled from palmitate-1-^{14}C should have the same or higher specific activity than that labeled from palmitate-16-^{14}C. Partial degradation of the palmitate to shorter chain acids followed by incorporation of these acids would give specific activities of hydrocarbons labeled from palmitate-1-^{14}C less than that labeled from -16-^{14}C.

If biosynthesis is by head-to-head condensation, a variety of possibilities also present themselves. The simplest case would be the one in which fatty acids enter into hydrocarbon biosynthesis at random with an equal probability of condensing with or without decarboxylation. That is, if the palmitate condensed with fatty acid "A" either palmitate or fatty acid "A" would have an equal chance of being decarboxylated. Under such circumstances, the ratio of the specific activities of the hydrocarbons labeled from palmitate-1-^{14}C to that labeled from -16-^{14}C should equal 0.5. Previous experiments reported by us (1969b) indicate that fatty acids do not necessarily enter into the biosynthesis of hydrocarbon in a manner proportional to their concentration in the total lipids, and there may be differences in the extent to which given fatty acids may undergo decarboxylation. The consequence of this would be that the ratio of specific activities of hydrocarbons labeled from palmitate-1-^{14}C to that from 16-^{14}C may be 1.0 or less but not necessarily 0.5.

Within the context of some of the possible interpretations of data obtained by this type of experiment, Kollatukudy (1966, 1967) cited the fact that the carboxyl carbon of exogenous fatty acids supplied to plant systems is not lost in hydrocarbon biosynthesis as primary evidence in support of an elongation–decarboxylation pathway and to rule out the participation of head-to-head condensation. In a more recent report of experiments of this type on a wider range of species (Kollatukudy, 1968b), the question of the validity of the interpretation of the data was raised relative to the possibility that exogenous fatty acids may be incorporated specifically without decarboxylation. We have now shown that this can be the case with *S. lutea*. Exogenous palmitate supplied to cultures in the presence of acetate in the medium was incorporated only by a route in which C-1 was not lost. In the absence of acetate, from 60 to 70% of the carboxyl carbons were not incorporated. It might logically be concluded that the presence of the acetate causes a shift from a predominantly head-to-head condensation pathway to elongation–decarboxylation or a shift between alternate fates of palmitic acid prior to incorporation which would give rise to differences in the degree to which the carboxyl carbon would be incorporated by an elongation–decarboxylation mechanism. However, experiments carried out on the incorporation of acetate-^{14}C and isoleucine-^{14}C reported earlier (Albro and Dittmer, 1969b) which showed incorporation patterns inconsistent with an elongation–decarboxylation pathway were carried out in media with high acetate (M73b), and it appears that the acetate causes a shift in the mode of entry by which the exogenous palmitate enters into a head-to-head condensation. In the absence of direct evidence for the elongation–decarboxylation pathway in plants and in the light of the report by Kaneda (1968) that the head-to-head condensation mechanism does occur in tobacco, the possibility that exogenous fatty acids supplied to plants may be specifically incorporated without decarboxylation in a condensation mechanism must be seriously considered.

The selective handling of exogenous palmitate by *S. lutea* implies that there are two distinct fatty acid pools or derivatives that contribute to hydrocarbon biosynthesis, and these pools or derivatives represent two

Scheme I



different modes of entry. The greatest distinction between the two modes of entry is that one involves obligatory decarboxylation of the fatty acid. In the past, the condensation has always been postulated to occur between fatty acids or derivatives at the oxidative level of acids (Chibnall and Piper, 1934; Wanless *et al.*, 1955; Kaneda, 1967). A consequence of this is that long-chain ketones and secondary alcohols would be obligatory intermediates of biosynthesis. The occurrence of ketones and alcohols in the nonsaponifiable lipid fractions of plants and bacteria tends to lend support to this hypothesis just as the occurrence of very long-chain fatty acids (>C-20) in plants has been cited as evidence for a elongation–decarboxylation pathway (Kolattukudy, 1967, 1968a). The idea of ketones and secondary alcohols as intermediates of the head-to-head condensation persisted in spite of evidence that these compounds are not converted into hydrocarbons (Kollatukudy, 1966; Kaneda, 1967).

Our failure to show that ketones could be converted into hydrocarbons more effectively than fatty acids and the concept of two modes of entry of fatty acids into hydrocarbons led us to consider a radical modification of the original head-to-head condensation mechanism as shown in Scheme I.

Early in the biosynthetic process the fatty acid would thus be converted into two different derivatives designated here by "X" and "Y." The "X" derivative would then be reduced to the level of an aldehyde which then would condense with the "Y" derivative. A secondary alcohol would be formed directly, thus bypassing the ketone as an intermediate. The ketones that occur as cell constituents could be synthesized from the alcohols.

Detailed investigation of the *S. lutea* system carried out primarily with cell-free preparations described in the next paper of this series shows that instead of the condensation occurring with an aldehyde, the alk-1-enyl aliphatic group of a neutral plasmalogen is incorporated into the hydrocarbon. As a consequence of this the secondary alcohol does not serve as an intermediate, and an alkene is the product of the condensation. The finding reported here that the carboxyl carbon of the fatty acid that does not undergo decarboxylation is located in the hydrocarbon across the double bond from the remainder of the aliphatic group derived from this fatty acid is particularly significant to this mechanism.

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