Application of Improved Methods To Assess Pathways for Biosynthesis of Long- and Very-Long-Chain Fatty Acids

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Improved microscale methods for analyzing individual carbon atoms of long- and very-long-chain fatty acids are described. These methods allow quantitation of radiolabel in at least 50% of individual carbon atoms from the carboxyl terminal of such fatty acids. These methods have been used to study metabolic pathways leading to long- and very-long-chain fatty acids of seeds. The approach begins with HPLC separation of free fatty acids from saponified, total lipid extracts. Subsequently, individual free acids are analyzed by Schmidt degradation to reveal the extent of carboxyl labeling or are subjected to α-oxidation followed by HPLC separation of free acid homologues. An improvement in the procedure for α-oxidation results in 0.2−2 times higher recovery of longer homologues. Carboxy-labeling of homologues is then determined using Schmidt degradation. For unsaturated acids, microscale hydrogenation and HPLC purification are done prior to α-oxidation. Results detail existing evidence that long- and very-long-chain fatty acids of seeds from various oilseed plants are solely formed by fatty-acid-synthase-mediated reactions. In addition, observations are consistent with the utilization of more than one pool of precursor oleic acid for synthesis of 20:1 and 22:1 in *Brassica* seeds.

Keywords: *α-Oxidation; Schmidt degradation; HPLC; hydrogenation; fatty acids*

INTRODUCTION

Previously used approaches to understanding metabolic pathways for long-chain fatty acid (lcFA) and very-long-chain fatty acid (vlcFA) formation involved radiolabeling of tissues with specifically labeled precursors, saponification to release fatty acids (FA), separation of FA species by preparative GLC or TLC as methyl esters, cleavage of esters, and ozonolysis to cleave monoenoic acids, followed by quantitation of label in products (Ohlrogge et al., 1978; Pollard and Stumpf, 1980a,b). In most of these experiments analysis was limited to determination of total label in the two halves of original FA. In certain instances specific radioactivity of products after α-oxidation of intact FA was analyzed to assess the percent of total label in $C_{(N-1)}$ to $C_{(N-4)}$, relative to the original molecule (C_N). This latter approach allowed analysis of 19−25% of individual carbon atoms (from the carboxy terminal). In addition, controlled chemical decarboxylation using the method of Dauben et al. (1953) has been used to assess labeling in the three carboxy-terminal carbons of original FA (Ohlrogge et al., 1978).

Here we have applied radiolabeling, saponification, separation of free FA by HPLC, α-oxidation, separation of free FA homologues by HPLC, and then Schmidt degradation to determine label in the carboxy-terminal carbon of homologues. Unsaturated FA were hydrogenated prior to α-oxidation and subsequent analysis. This approach allows analysis of at least 50% of individual carbon atoms of original FA (from the carboxy terminal). Using these methods we have examined in greater detail than in previous studies acetate labeling patterns of lcFA (C16−C18) and vlcFA (C20 and C22) fatty acids from developing *Arabidopsis*, *Brassica*, and soybean seeds.

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Some seed storage lipids and many plant waxes contain vlcFA as well as lcFA. The former may be up to C24 in length in plant triacylglycerides (Daun, 1984) and C40 in length in epicuticular waxes (Eigenbrode and Espelie, 1995). Previous analysis based on radiolabeling and chemical degradation as described above has provided evidence for occurrence of two metabolic pools of precursor lcFA for formation of phospholipids and another for elongation to form vlcFA of storage lipids (Pollard and Stumpf, 1980a,b) and storage waxes (Ohlrogge et al., 1978). Recently, Ajie et al. (1995), using mass spectrometric analysis of deuterium-labeled lipogenic tissues of rats and mice, found that palmitate utilized in formation of up to 24-carbon-length acids derives from two separate pools. Here we investigated the possible occurrence of multiple pools of precursor FA for elongation to vlcFA using *Brassica* embryos. We have applied the methods described above to assess this question.

MATERIALS AND METHODS

Radiolabeling and Extraction of Developing *Arabidopsis* Seeds and Developing Soybean and *Brassica* Embryos. Developing *Arabidopsis thaliana* seeds, 10 days after flowering, were isolated and incubated with 20 μL of incubation medium, containing 50 mM 2-[*N*-morpholino]-ethanesulfonic acid (MES)/NaOH, pH 5.0, 330 mM glycerol, and 10 μCi of sodium [2-^{14}C]acetate (59 μCi/mmol, Moravek Biochemicals, Brea, CA). After 6 h, seeds were washed with 300 μL of 10 mM sodium acetate, then 300 μL of 5 mM glycerol, and finally 300 μL of water to remove unincorporated isotope. They were homogenized with 1 mL of 10% KOH in methanol and then saponified in closed vials [as in Kroumova and Wagner (1995) but without the center well] for 1 h at 80 °C. Afterward, samples were cooled to 4 °C and then acidified with 200 μL of cold, concentrated HCl to form free acids. Samples were extracted three times, each with 2 mL of petroleum ether (40−60 °C bp). The combined petroleum ether extract was shaken with 1 mL of distilled water containing 50 mL of 10% (w/v) KOH in methanol to convert free fatty acids to their

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potassium salts. Aliquots of the water layer were reduced to dryness, dissolved in 65% (v/v) acetonitrile in water and 0.6 N H_3PO_4, and analyzed by HPLC (Kroumova and Wagner, 1995).

Soybean seeds cv. Elgin were kindly provided by Dr. Egli, University of Kentucky. Soybean plants were grown in the greenhouse, and maturing seeds were isolated 32 days after flowering. According to Rubel et al. (1972), the percentage of oil in seeds increases rapidly to 20% of dry weight from 24 to 40 days after flowering. Four embryos (seed coats removed) were cut in half and labeled. After 6 h, embryos were rinsed twice with 2 mL of incubation mixture without label and then extracted with 6 mL of $CHCl_3$/MeOH (2:1, v/v) for 16 h at 25 °C with shaking (Oo and Stumpf, 1979). Remaining tissue was removed and washed with 2 mL of chloroform/methanol (2:1, v/v). The combined chloroform extract and wash was shaken with 1 mL of distilled water, and the chloroform phase was evaporated to an oily residue. This residue was dissolved in 250 μL of benzene, an aliquot was counted for radioactivity, and the remainder was evaporated and then saponified in 200 μL of 1 M KOH in MeOH/H_2O (4:1, v/v) overnight at 22 °C. The saponified mixture was stored at 0 °C prior to separation of fatty acids..

Brassica napus embryos (10) were incubated in the light at 26−28 °C for 6 or 12 h in 0.1 M MES buffer at pH 5.0 and [2-^{14}C]-acetate. Lipids were extracted and saponified as described for soybean.

Radiolabeling and Extraction of Developing Fruits of *Arabidopsis thaliana*. Greenhouse-grown plants were collected 7−14 days after the first flowers opened. According to Miquel and Browse (1994) lipids begin to accumulate 5 days after flower opening. To label fruits "through the peduncle", 10−15 day postflowering fruits were detached from plants and the peduncles placed in a vial containing 20 μCi of sodium [2-^{14}C]acetate in H_2O. When the solution was absorbed, fruits were transferred to a small beaker and peduncles were immersed in distilled water and then held in a humid chamber under incandescent light at 25 °C. To label fruits "through the stem", about 20 plants were cut above the rosette leaves and stems were placed in a vial with label. After absorption of label, stems were treated as described above for fruits. After labeling through the peduncle or through the stem, fruits were collected and homogenized with chloroform/methanol (2:1, v/v) to extract lipids. The homogenate was centrifuged for 10 min at 730*g*, and the supernatant was shaken with 1 mL of water. The chloroform fraction was evaporated to dryness, and the residue was dissolved in 1 mL of benzene and analyzed for radioactivity. Saponification and subsequent analysis of fatty acids were as described above. Where high levels of radioactivity were required for degradation studies (particularly those including both Schmidt degradation and α-oxidation), we used entire fruits.

Separation of Acids by HPLC, Schmidt Degradation, and Chemical α-Oxidation. Methods for HPLC and Schmidt degradation were described previously (Kroumova and Wagner, 1995). Schmidt degradation releases the carboxy carbon of free FA as CO_2, which can be recovered and quantitated. The basic method for chemical α-oxidation was adapted from that of Harris et al. (1965) but was modified to maximize yields of fatty acids with shorter (<16:0) chains. Fractions from HPLC containing labeled, long-chain, saturated fatty acids (16:0 or 18:0) were extracted with petroleum ether (40−60 °C bp). The petroleum ether phase was transferred to a 4 mL vial with a conical interior bottom and evaporated to dryness on a heated block using an air stream. The residue was dissolved in 1 mL of acetone and ~8 μmol of standard acid was added, followed by addition of 0.5 nmol of finely divided $KMnO_4$. A stirrer was added, the vial was sealed, and the reaction was performed for 5 h at 90 °C, with continuous stirring in a heated sand bath. Vials were cooled to 4 °C, and 1 mL of 5 N H_2SO_4 was added, followed by sufficient potassium metabisulfite to decolorize residual permanganate and to dissolve precipitated manganese dioxide. Fatty acid products were extracted with petroleum ether (40−60 °C bp) and transferred to a small vial. Solvent was evaporated under an air stream, the residue was dissolved in 100−200 μL of 50−100% acetonitrile and 0.03 N H_3PO_4, and the homologous series of FA products was separated by HPLC. We found that a minimum of 60 000 dpm is needed to effectively complete α-oxidation followed by Schmidt degradation of homologues as described in this paper.

Hydrogenation of Nonsaturated Fatty Acids. The method of Taylor et al. (1981) using Adam's catalyst was reduced to a microscale and adapted for hydrogenation of the nonsaturated fatty acids, 18:1, 18:2, 18:3, 20:1, and 22:1. Hydrogen was produced by mixing a stabilized $NaBH_4$ solution and HCl. A previously described triple-well reaction vial was used (Kroumova and Wagner, 1995). The outer well contained 2.5 mL of 5 N HCl and a stir bar; the center well contained fatty acid in 1 mL of chloroform/methanol (1:1, v/v), 1 mg of Adam's catalyst, and a stir bar. The tip vial contained 0.5 mL of stabilized $NaBH_4$ solution prepared by dissolving 80 mg of NaOH in 15 mL of H_2O, addition of 0.77 mg of $NaBH_4$, dilution to 20 mL, and finally filtration through Whatman No. 1 filter paper (*Vogel's Textbook*, 1978). The vial was sealed, and the reaction was initiated by tipping the $NaBH_4$ into the HCl. Reactions were at 22 °C, with continuous stirring. After 5 h, the contents of the center well were filtered, evaporated, dissolved in acetonitrile containing 0.01 M H_3PO_4, and analyzed by HPLC.

After hydrogenation, no label was found in regions of unsaturated parent FA. Thus, hydrogenation was complete and was additionally useful for verifying the identity of parent unsaturated FA.

RESULTS AND DISCUSSION

Biosynthesis via fatty acid synthase (FAS) involves incorporation of both carbons of [2-^{14}C]acetate to yield FA lacking ^{14}C in the carboxyl carbon. We have determined that the method used for Schmidt degradation results in a precision of ±3% (Kroumova and Wagner, 1995). Therefore, recovery of ≤3% of total FA label as CO_2 after Schmidt degradation is indicative of no label in the carboxy-terminal carbon and consistent with biosynthesis via FAS. Results obtained after Schmidt degradation alone of 16- and 18-carbon species of *Arabidopsis* seeds and 16-, 18-, 20-, and 22-carbon species of *Brassica* embryos are shown in Table 1. Data obtained for all FA examined were, as expected, consistent with their formation by FAS. These results confirm the reliability of the methods used for lcFA and serve as controls for studies on vlcFA (see below).

To examine labeling patterns more thoroughly than afforded by Schmidt degradation alone, we employed a modified α-oxidation method followed by Schmidt degradation to analyze several carbons from the carboxyl ends of labeled FA. A modification of the α-oxidation method of Harris et al. (1965), together with a simple reaction vessel design and methods for isolating free FA by HPLC (Kroumova and Wagner, 1995), has allowed for independent and efficient analysis of all carbons of FA down to 9 carbons from the omega end. Shorter length products are not separated from the solvent front during HPLC of long-chain FA using the 50−100% acetonitrile (in 0.03 N H_3PO_4) gradient employed here. Modification of the gradient (Kroumova and Wagner, 1995) may make it possible to recover and analyze shorter homologues and therefore assess labeling of patterns >50% of individual FA carbon atoms. We note that while we have not examined the question directly here, recovery of FA after separation by HPLC as free acids is undoubtedly more quantitative than separation using TLC or GLC of their esters.

It was necessary to hydrogenate unsaturated FA to prevent oxidation of double bonds during α-oxidation. Figure 1 shows α-oxidation products of combined 18-carbon species (after hydrogenation) prepared from

Table 1. Analysis by Schmidt Degradation To Predict Biosynthetic Pathways for Fatty Acid Biosynthesis from [2-^{14}C]Acetate in Tissues of *Arabidopsis* and *B. napus*

tissue examined	method of labeling	labeling period (h)	% of total label in carboxyl carbon[a]					
			16:0	18:1	18:2	18:3	18:0 and 20:1	22:1
Arabidopsis								
seeds	isolated seeds	6	1.7 ± 2.1 (3)	1.2 ± 0.5 (3)	1.6 ± 0.5 (3)			
fruits	through the peduncle	20	1.6 ± 0.3 (3)	2.9 ± 0.4 (3)	3.3 ± 0.7 (3)	3.0 ± 1.5 (3)		
	through the peduncle	42	1.6 and 2.1 (2)	3.3 and 1.7 (2)	2.1 and 4.4 (2)	1.6 and 3.0 (2)	3.2 and 4.9 (2)	4.2 and 1.2 (2)
fruits	through the stem	6	1.3	1.0	1.5		1.9	
bracts plus stems	through the stem	42	1.5 ± 0.5 (6)	1.9 ± 0.7 (4)	1.6 ± 0.4 (6)	1.9 ± 0.9 (3)	2.5 and 3.8 (2)	
B. napus								
embryos	isolated embryos	6	2.8	1.6	2.0	2.0	4.0	2.0
	isolated embryos	12	3.5 and 2.2 (2)	2.8 ± 1.0 (3)	4.0 ± 0.9 (3)		3.1	1.4 ± 0.3 (2)
	predicted label in carboxyl carbon FAS		±3	±3	±3	±3	±3	±3

[a] Values represent the percent radioactivity (of total label in fatty acid) observed in the carboxyl carbon. In parentheses is the number of degradation experiments from which data were obtained. When label of a particular acid was low, repeated collections from HPLC were pooled to obtain sufficient radioactivity for degradation. Labeling "through the stem" refers to labeling of fruit-bearing stems detached above the rosette. Biosynthesis via FAS is expected to give no labeling of the carboxyl carbon of fatty acids, but because the method used for Schmidt degradation is reproducible to within ±3%, this value is listed as the predicted value for FAS at the bottom of the table.

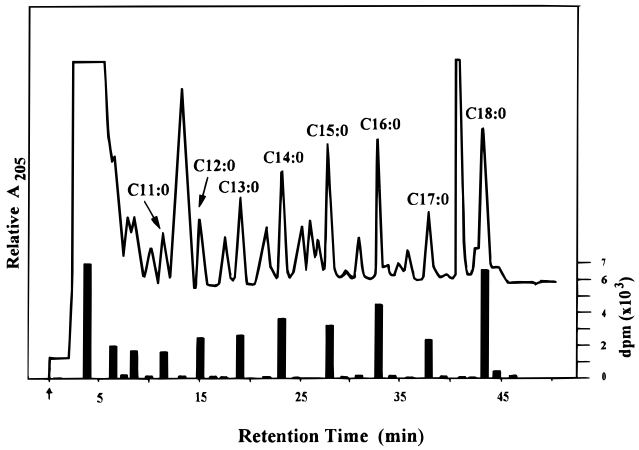

Figure 1. HPLC separation of fatty acids produced by α-oxidation of [^{14}C]-18:0. *Arabidopsis* fruit 18:0, 18:1, 18:2, and 18:3, labeled from [2-^{14}C]acetate, were pooled and hydrogenated to produce 18:0. After its separation by HPLC, addition of carrier unlabeled 18:0, and α-oxidation, HPLC was used to separate the homologous series of products shown.

fruits of [2-^{14}C]acetate-labeled *Arabidopsis*. As shown, in this case all products representing the homologous α-oxidation product series from 18:0 down to 11:0 contained label and were well separated by HPLC using a 50−100% acetonitrile gradient. Table 2 shows results of control oxidations using [U-^{14}C]-16:0, under somewhat varying oxidation conditions as well as the condition described by Harris et al. (1965). On the basis of experiments with [U-^{14}C]-16:0 standard, method 1 was most efficient and was reproducible to about ±3.0%. Compared to the method of Harris et al. (1965; designated here method 2), increasing temperature (method 1) or the amount of $KMnO_4$ (method 3) increased oxidation of initial acid and that of homologous products. Only ∼29% of standard 16:0 remained after α-oxidation using the modified method compared to ∼59% after application of the procedure of Harris. The modified method yielded from [U-^{14}C]-16:0 20% more 15:0 homologue to 95% more 10:0 homologue. Therefore, the modified method (method 1) was used for analysis of *Arabidopsis*- and soybean-derived FA. Elevation of temperature to 100 °C and above or further increasing the amount of $KMnO_4$ (above 100 mg) did not increase the efficiency of oxidation of *Arabidopsis* or soybean FA (data not shown). As shown, α-oxidation yielded similar amounts of homologous products from standard and biologically derived FA. For example, after oxidation of standard 16:0 and *Arabidopsis* 16:0, ∼29 and ∼30%, respectively, of initial acid remained unoxidized. Also shown in Table 2 is the distribution of radiolabeled homologous α-oxidation (method 1) products of 16:0 and 18:0 from *Arabidopsis* fruits and soybean embryos.

After isolation by HPLC, homologous α-oxidation products from soybean and *Arabidopsis* 16:0 and 18:0 and from *Brassica* 20:0 and 22:0 were each decarboxylated by Schmidt degradation to determine percent label in carboxyl carbons. Results are presented in Table 3. In the left column of this table α-oxidation products listed are numbered from the omega end. For example, for *Arabidopsis* seeds (6 h incubation) 12:0 is the 12-carbon α-oxidation product of 16:0 counting from the omega end. Insufficient label was present in 18:0 and, therefore, 18:0, 18:1, 18:2, and 18:3 were combined and hydrogenated, and the resulting 18:0 was isolated by HPLC and then α-oxidized. Results from α-oxidation/Schmidt degradation of labeled, isolated *Arabidopsis* seeds or fruits labeled through the peduncle for 6 or 20 h (Table 3) verify observations made after Schmidt degradation (Table 1). Clearly only even-numbered carbons were substantially labeled as expected for direct utilization of [2-^{14}C]acetate by FAS.

Detailed analysis (∼50% of carbons) of soybean embryo FA by α-oxidation/Schmidt degradation (Table 3) is entirely consistent with synthesis via FAS. As already described (Table 1), Schmidt degradation of [2-^{14}C]acetate-derived FA of *Brassica* embryos confirmed the expectation that 16−22-carbon FA produced in this tissue are synthesized by FAS. As shown in Table 3, α-oxidation/Schmidt degradation of *Brassica* 20:1 and 22:1 (hydrogenated to yield 20:0 and 22:0, respectively) gave patterns in the first 18 carbons consistent with synthesis via FAS. However, odd-numbered carbons resulting from elongation reactions (>18) contained 2−5 times the label expected if 18:0 and elongated 18:0 were derived from the same precursor pool. If only one pool was utilized, carbon, 19 and 21 of 22:0 would contain 10 and 9.1% of total label, respec-

Table 2. Distribution of Label in Homologous Series of Acids after Chemical α-Oxidation

	% of total label in each homologue									
acid oxidized	<10:0	10:0	11:0	12:0	13:0	14:0	15:0	16:0	17:0	18:0
[U-^{14}C]-16:0										
method 1[a]	12.7	5.6	7.0	10.2	9.7	18.1	8.7	28.7		
	±1.9	±0.4	±0.5	±0.3	±0.3	±0.3	±0.8	±1.7		
method 2	5.5	1.9	3.5	4.3	4.5	13.7	7.2	59.4		
	3.8	1.6	2.8	3.7	4.2	13.3	7.1	63.5		
method 3	7.7	4.7	5.8	7.3	7.0	16.6	7.5	43.4		
Arabidopsis fruits										
16:0	12.5	3.7	3.8	5.1	7.1	19.8	10.8	37.2		
	26.3	2.6	4.4	11.3	8.2	14.0	9.1	24.0		
18:0	12.2	4.3	4.7	8.1	8.1	10.3	8.8	14.6	7.4	21.4
	28.4		4.3	6.5	7.0	9.7	8.5	11.9	6.1	17.5
soybean embryos										
16:0	13.6	4.7	6.3	7.7	8.3	13.8	11.1	34.6		
	19.3	6.9	7.5	9.1	11.8	17.1	10.2	18.0		
18:0	12.4	4.4	5.6	6.9	8.5	10.1	8.7	13.6	7.1	22.6
	6.5		4.5	7.0	9.1	9.5	10.1	27.7	5.9	19.6

[a] Data for method 1 are from three independent oxidations of standard [U-14C]-16:0. Method 1 was used for *Arabidopsis* and soybean FA degradations. Two independent oxidations were made for all samples except for the oxidation of standard using method 3. Conditions for method 1 were 80−100 mg of $KMnO_4$, 5 h, 90 °C, 1−2 mL of acetone; for method 2 (Harris et al., 1965) conditions were 50 mg of $KMnO_4$, 4−6 h, 56−60 °C, 0.25−0.3 mL of acetone; and for method 3 were 60 mg of $KMnO_4$, 5 h, 56−60 °C, 0.25−0.3 mL of acetone.

Table 3. Analysis of Radiolabeling Patterns after Combined α-Oxidation and Schmidt Degradation of Principally Labeled FA from *Arabidopsis* Seeds and Fruits and *B. napus* and Soybean Embryos

	% of total label in carboxyl carbon of homologous FA								
			α-oxidized FA						
	Arabidopsis		soybean embryos (6 h)		*Brassica* embryos				predicted radioactivity in carboxyl carbon FAS
					(6 h)		(12 h)		
homologous products of α-oxidation	seeds (6 h) 16:0	fruits (20 h)[a] 16:0	16:0	18:0[b]	20:0[c]	22:0[c]	20:0[c]	22:0[d]	
9:0		24.0							20.0
10:0		2.9	5.9	2.8					0
11:0		20.5	22.0	16.8 and 22.3					16.7
12:0	0.0	1.3	3.7 and 4.1	0.0 and 3.5			6.0		0
13:0	13.8	18.5 and 17.1	17.8 and 20.2	24.1 and 15.2	7.7		9.6	9.5	14.3
14:0	1.8	1.9 and 1.9	0.8 and 1.1	1.8	4.3		5.6	3.9	0
15:0	8.9	18.7 and 20.2	18.8 and 14.1	15.9 and 16.1	8.7		16.0	7.9 and 9.6	12.5
16:0	0.8	1.6 ± 0.3	0.3 and 1.0	0.9 and 3.3	2.9	2.7	5.3	3.5 and 4.8	0
17:0				22.6 and 18.6	12.1	8.4	9.6	13.8 and 9.3	11.1
18:0				0.2 and 0.0	2.6	1.3		0.7 and 2.4	0
19:0					16.5	42.2	22.5	53.2 and 56.6	10.0
20:0					4.8	1.8	3.1	2.7 and 4.3	0
21:0						33.4		37.1 and 45.3	9.1
22:0						2.0		1.8	0

[a] Labeled through the peduncle. [b] Combined 18-carbon FA, hydrogenated. [c] 20:1 hydrogenated. [d] 22:1 hydrogenated. Values represent the percent radioactivity (of total label in homologous fatty acid products of α-oxidation) observed in the carboxyl carbon. One or two Schmidt degradations were made for each homologous acid. The right-hand column represents percentages predicted if fatty acids were derived from direct utilization of acetate by FAS. When labeling of a particular acid was low, repeated collections from HPLC were pooled to obtain sufficient radioactivity for degradation. The lengths of labeling periods are in parentheses. The method used for Schmidt degradation is reproducible to within ±3%. Data for soybean embryos were presented previously (Kroumova and Wagner, 1997).

tively, but ~50 and ~40%, respectively, were observed (Table 3). This indicates that precursor oleate is not highly labeled and most of the label in 20:0 and 22:0 resides in elongation carbons. Elongation carbons were previously shown to be highly labeled in acetate-labeled vlcFA of jojoba (Ohlrogge et al., 1978), nasturtium (Pollard and Stumpf, 1980a), and meadowfoam seeds (Pollard and Stumpf, 1980b). Apparently *Brassica* seeds elongate only oleate. In jojoba and nasturtium mainly oleate is elongated, while in meadowfoam both palmitate and oleate are substrates for elongation. Ajie et al. (1995) have suggested that the precursor (C16) pools for synthesis of 22:0 and 24:0 in animal tissues may be separate. Our results with *Brassica* are similar to those found for animal tissues (Ajie et al., 1995) and suggest not only that different pools (labeled to different extents with exogenous acetate) of precursor C18 may be used in elongation to form C20 and 22 but that these pools may contribute differentially in formation of C20 versus C22. The compartment for elongation is not clearly defined in plant or animal tissues.

CONCLUSIONS

We have used improved methods for analysis of radiolabeling patterns of lcFA and vlcFA. Improvements include the direct separation by HPLC of free lcFA and vlcFA after saponification of lipids and after α-oxidation of FA. Separation of free acids and α-oxidation obviate the need for formation and then hydrolysis of derivatives as required in TLC and GLC methods. We also describe a more efficient method for α-oxidation and microscale methods for hydrogenation of FA. These improvements allow examination of labeling patterns in at least 50% of individual carbons of lcFA and vlcFA. Using these methods we obtained more detailed confirmation than previously available that lcFA are formed by FAS-mediated reactions. Results also indicate that

elongation of oleate to form 20:1 and 22:1 in *Brassica* embryos utilizes two separate pools of oleate.

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