A VERSATILE PROCEDURE FOR THE PREPARATION OF PALMITIC ACID-d_2 AND STEARIC ACID-d_6

R. O. Adlof and E. A. Emken
Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture,* Peoria, Illinois 61604

SUMMARY

Syntheses of palmitic acid-d_2 and stearic acid-d_6 are described, the latter route also having the capability of producing methyl oleate and elaidate-13,13,14,14-d_4 from the same synthetic sequence. Overall yields are in the range of 40 to 60%. These syntheses use *tris*(triphenylphosphine)-chlororhodium(I) for the incorporation of deuterium. Atomic absorption spectroscopy is used to confirm that less than 70 ppb rhodium catalyst remained in the final products.

Key Words: deuterium, fatty acids, Wittig reactions, palmitic acid, stearic acid.

INTRODUCTION

This paper describes the synthesis of palmitic acid-9,10-d_2 and stearic acid-9,10,13,13,14,14-d_6. Methyl oleate/elaidate-13,13,14,14-d_4 was also isolated in greatly improved yield (1) as an intermediate in the preparation of the stearic acid-d_6. These fatty acids were prepared from commercially available intermediates. Synthetic routes utilizing the deuteration of natural product precursors such as methyl palmitoleate (methyl *cis*-9-hexadecenoate) and methyl crepenynate (methyl *cis*-9-octadecene-12-ynoate) were not used because of problems encountered in purification and deuteration of these compounds. Synthesis via the Wittig reaction offered many advantages, such as overall yields in the range of

*The mention of firm names or trade products does not imply that they are endorsed by the U.S. Department of Agriculture over other firms or similar products not mentioned.

0362-4803/81/030419-08$01.00

Received November 2, 1979

40 to 60%, a 2- to 3-week completion time, and ease of preparation. When compared with other methods for the preparation of deuterium-labelled, saturated fatty acids, the use of tritium-free deuterium gas is much less expensive than N_2D_4 (2,3), disiamylborane/1-deuteroacetic acid (3), and the use of RCD_2CO_2H (electrolysis with $R'CH_2CO_2H$) (4) or CD_3CO_2D (5) (Kolbe reaction) precursors. Pyridine catalysed H-D exchange with aldehydes is time consuming, gives relatively poor yields, and requires large quantities of D_2O (1). Tulloch (6) used $NaBD_4$ and $LiAlD_4$ to prepare a series of gem-dideutero octadecanoates with isotopic purities of 96 to 99%. Although these isotopic purities are higher than results obtained in our laboratory, the greater expense of using $NaBD_4$ and $LiAlD_4$ plus overall yields of only 10 to 20% (vs 40 to 60%) make this procedure less attractive for large-scale preparations. If natural product precursors are not used, the deuterium atoms can be incorporated into various positions of the fatty acid, a valuable consideration for membrane interaction and conformation studies utilizing nuclear magnetic resonance spectroscopy (7-10).

RESULTS AND DISCUSSION

A number of difficulties were encountered in the use of natural product precursors in the synthesis of the deuterated palmitic and stearic acids. The isolation of methyl palmitoleate (methyl *cis*-9-hexadecenoate) from sperm whale oil (14.8% 16:1) by fractional distillation (11) was time consuming and did not give pure products. Further purification of the palmitoleate fraction by counter-current distribution using either silver nitrate/methanol (12,13) or acetonitrile/hydrocarbon (14) solvent systems would have required expensive equipment, been time consuming, and involved problems in keeping the apparatus clean while handling silver-nitrate/methanol solutions. The preparation of methyl stearate-d_6 by the $(Ph_3P)_3RhCl$-catalyzed deuteration of methyl crepenynate (methyl *cis*-9-octadecene-12-ynoate) is also not feasible. Methyl crepenynate can be obtained from *Crepis alpina* seed by grinding, extraction by petroleum ether (P.E.), transesterification, and silver resin chromatography. However, the final

material cannot be deuterated using $(Ph_3P)_3RhCl$, because impurities apparently deactivate the catalyst. Methyl linolenate (methyl *cis*-9,*cis*-12,*cis*-15-octadecatrienoate) can be deuterated with $(Ph_3P)_3RhCl$ to yield stearate-d_6, but the reaction is slow and extensive scattering of the deuterium label occurs. The alternate syntheses of palmitate-d_2 and stearate-d_6 as illustrated in Figure 1 proved easier than previously reported routes (3,15). Both preparations used the Wittig coupling reaction followed by deuteration with $(Ph_3P)_3RhCl$ catalyst. The parallel nature of the two syntheses minimizes the

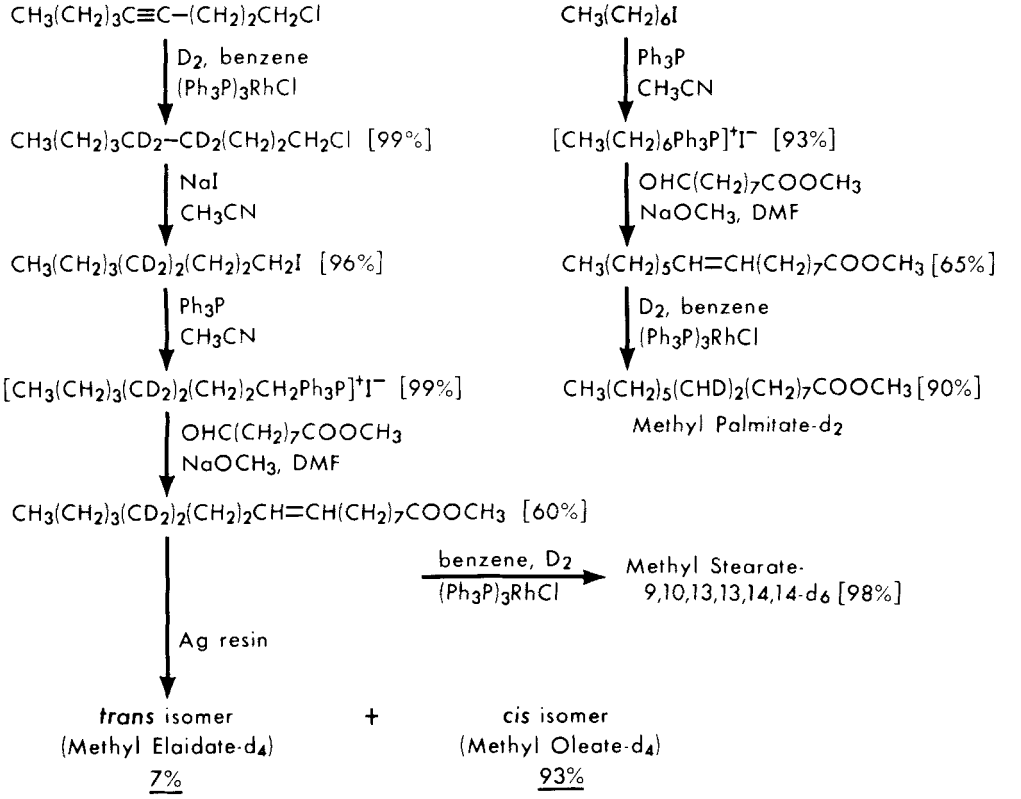

Figure 1. Synthetic schemes for Methyl Oleate/Elaidate-d_4, Methyl Palmitate-d_2, and Methyl Stearate-d_6. Yields given in brackets, isomer percents are underlined.

amount of equipment and chemicals required to prepare these compounds.

Since these fats were part of a series of compounds that were (16-18) or will be ingested by human subjects, exceptional care was necessary to ensure that no rhodium catalyst residue was present in the final product. This problem is more important than in previous preparations of deuterated fats (1,19-21), because deuteration occurs in the next to the last step. Since saturated esters are fairly insoluble in P.E., silica gel chromatography could not easily be used to remove the catalyst as described previously for monounsaturated esters. Recrystallization was found to be the best purification method. Atomic absorption spectroscopy (22) was used to confirm the number of recrystallizations sufficient to reduce the rhodium content of the final acids to safe levels (<70 ppb).

The isotopic purities of the final products range from 86 to 88% and are shown in Table I. Previously reported preparations (3,5,15,)

TABLE I

Mass Analysis for Deuterium

Methyl ester	Number of deuterium atoms (%)										Avg. No. Deut. atoms per molecule
	0	1	2	3	4	5	6	7	8	9	
Palmitate-9,10-d_2	2.0	6.8	88.8	1.6	0.4	0.4	---	---	---	---	1.93
Oleate-13,13,14,14-d_4	2.2	1.1	2.3	4.7	86.8	2.3	0.0	0.7	---	---	3,83
Stearate-9,10,13,13,14,14-d_6	0.0	0.0	0.1	0.1	0.7	7.7	87.3	3.5	0.2	0.3	5.95

[excluding Tulloch (6)] gave isotopic purities that ranged from 50 to 87%. While these methods can be used to prepare small quantities (∿1 g) of labelled fats, larger amounts would be extremely expensive.

EXPERIMENTAL

Instruments: A Nuclide 12-80G spectrometer with 70 ev impact ionization inlet, maintained at 150°C (intermediates) and 200°C (methyl stearate and methyl oleate), was used to determine isotopic purities and deuterium distribution (24). The reaction mixtures and products were analyzed on an F & M 810 Gas Chromatograph equipped with a FID and using N_2 as carrier at 35 ml/min. A 1/8 in. X 12 ft stainless steel column containing 20% OV 275, on Chromasorb WAW, 100/120 mesh was used. The final products were analyzed for rhodium with a Perkin-Elmer atomic absorption spectrophotometer Model 303 (70 ppb detection limit). Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus and are not corrected.

Reagents: The following reagents were used as received: 1-chloro-4-nonyne (Farchan Chemical Company); hexyl iodiode and triphenylphosphine (Aldrich); sodium methoxide (Harshaw Chemical Company); tris(triphenylphosphine)-chlororhodium(I) (Strem Chemicals); deuterium (98.0%) (Matheson). Other chemicals used were "ACS Certified" and, unless specifically stated, were used without further purification.

The preparation of the methyl *cis*-9- and *trans*-9-octadecenoate-13,13,14,14-d_4 isomers was similar to that used for synthesis of methyl 8-octadecenoate-17,18,-d_2 (20). Overall yields, especially in the final Wittig coupling reaction, are much higher than those previously reported for the preparation of methyl oleate-13,13,14,14-d_4 (1).

Methyl stearate-9,10,13,13,14,14-d_6. Tris(triphenylphosphine) chlororhodium (I) (3.0 g) was added to 350 ml of degassed (N_2) benzene, and the mixture was evacuated and flushed several times with deuterium (D_2). A 45 g (0.15 mol) mixture of both the *cis* and *trans* isomers of methyl 9-octadecenoate-13,13,14,14-d_4 was added, the reaction mixture was flushed with D_2 and then magnetically stirred. Deuteration was continued overnight (23.5 hr total). Benzene was removed by rotary evaporator and the residue was recrystallized three times from diethyl ether/petroleum ether (Et_2O/PE) (5 to 1-v/v) to yield 44.6 g of product (m.p. 46-48; 98% yield).

Stearic acid-9,10,13,13,14,14-d_6. Potassium hydroxide (13.6 g; 0.243 mol) was dissolved in 16 ml H_2O and added to 200 ml CH_3OH containing methyl stearate-d_6 (37.0 g; 0.122 mol). The mixture was refluxed for 1.5 hr under N_2 and cooled, and then 700 ml H_2O and 100 ml 30% HCl were added. The mixture was extracted three times with Et_2O (400,150,150 ml), the Et_2O was removed immediately by rotary evaporator, and the residue was recrystallized three times from acetone. This gave 32.4 g (92% yield) of product (m.p. = 67.5-69°C).

Methyl 8-formyloctanoate. This compound was prepared by the ozonolysis of methyl oleate and subsequent zinc/acetic acid reduction as described previously (25).

1-Heptyl triphenylphosphonium iodide. Heptyl iodide (226 g; 98% pure; 0.98 mol), triphenylphosphine (262 g; 1.0 mol) and 1.2 L of acetonitrile (CH_3CN) were combined and refluxed overnight under N_2. The homogeneous solution was cooled and CH_3CN removed with a rotary evaporator. The resulting solid was triturated with 4 L of Et_2O and dried in a vacuum dessicator to give 468.5 g (98% yield) of final product (m.p. = 128-131°C).

Methyl palmitoleate. 1-Heptyltriphenylphosphonium iodide (270.84 g; 0.555 mol) in 600 mL N,N-dimethylformamide (DMF) was added dropwise over 1.25 hr to a 2 L, 3-necked round-bottomed flask containing $NaOCH_3$ (30.0 g; 0.556 mol) and equipped with a mechanical stirrer, N_2 inlet, thermometer, and addition funnel. An ice bath was used to keep the temperature at 9°C during the addition. The orange reaction mixture was then warmed to 22°C and stirred for 45 min. The mixture was again cooled, methyl 8-formyloctanoate (117.9 g; 0.596 mol) in 300 mL DMF was added dropwise over 1 hr at 11°C, and the reaction was stirred overnight. The reaction mixture was divided into two portions, 1 L of H_2O was added to each portion, and each was extracted three times with 200-mL portions of P.E. The P.E. fractions were combined, washed three times with 150-mL portions of H_2O, and dried over magnesium sulfate ($MgSO_4$). The $MgSO_4$ was removed by vacuum filtration, and the P.E. was evaporated with a rotary evaporator. The residue was distilled

through a 6 in. Vigreux column (b.p. 126-131°C/0.1 mm Hz) to give 102 g of product (90% pure; 65% yield).

Methyl palmitate-9,10-d_2. 9.86 g of methyl palmitoleate was deuterated using 0.075 g $(Ph_3P)_3$ RhCl and 100 mL benzene. The same procedure was used as described for methyl stearate-d_6. Two recrystallizations from acetone gave 8.1 g of product (90% yield; m.p. = 29-30°C).

Palmitic Acid-9,10-d_2. Saponification of methyl palmitate-9,10-d_2 was accomplished by the same procedure described for methyl stearate-d_6. The product was recrystallized three times from acetone in a yield of 92% (m.p. = 61.5-62.5°C).

ACKNOWLEDGMENTS

To W. K. Rohwedder for MS and G. R. List for AA analyses.

REFERENCES

1. DeJarlais, W. J., and Emken, E. A., J. Labelled Compd. Radiopharm. 15:451 (1978).
2. Scholfield, C. R., Jones, E. P., Nowakowska, Janina, Selke, E., and Dutton, H. J., J. Am. Oil Chem. Soc. 39:208 (1961).
3. Rohwedder, W. K., Scholfield, C. R., Rakoff, H., Nowakowska, J., and Dutton, H. J., Anal. Chem. 39:820 (1967).
4. Klok, R., Egmond, G. J. N., and Pabon, H. J. J., Recl. Trav. Chim. Pays-Bas 93:222 (1974).
5. Dinh-Nguyen, N., Ark. Kemi 28:289 (1968).
6. Tulloch, A. P., Lipids 12:92 (1977).
7. Seelig, J., and Waespe-Sarčević, Biochemistry 17:3311 (1978).
8. Oldfield, E., Chapman, D., and Derbyshire, W., Chem. Phys. Lipids 9:69 (1972).
9. Saito, H., Schreier-Muccillo, S., and Smith, C. P., FEBS Lett. 33:281 (1973).
10. Stockton, G. W., Polnaszek, C. F., Tulloch, A. P., Hasan, F., and Smith, C. P., Biochemistry 15:954 (1976).

11. Norris, F. A., and Terry, D. E., Oil Soap 22:41 (1945).
12. Dutton, H. J., Scholfield, C. R., and Jones, E. P., Chem. Ind. 1874 (1961).
13. Scholfield, C. R., Jones, E. P., Butterfield, R. O., and Dutton, H. J., Anal. Chem. 35:1588 (1963).
14. Scholfield, C. R., Nowakowska, J., and Dutton, H. J., J. Am. Oil Chem. Soc. 37:176 (1960).
15. Dinh-Nguyen, N., Ark. Kemi 22:151 (1964).
16. Emken, E. A., Rohwedder, W. K., Dutton, H. J., Dougherty, R., Iacono, J. M., and Mackin, J., Lipids 11:135 (1976).
17. Emken, E. A., Rohwedder, W. K., Dutton, H. J., DeJarlais, W. J., Adlof, R. O., Mackin, J. F., Dougherty, R. M., and Iacono, J. M., Lipids 14:547 (1979).
18. Emken, E. A., Rohwedder, W. K., Dutton, H. J., DeJarlais, W. J., Adlof, R. O., Mackin, J. F., Dougherty, R. M., and Iacono, J. M., Metabolism 28:575 (1979).
19. Adlof, R. O., and Emken, E. A., J. Labelled Compd. Radiopharm. 15:97 (1978).
20. Adlof, R. O., Miller, W. R., and Emken, E. A., J. Labelled Compd. Radiopharm. 15:625 (1978).
21. Rakoff, H., and Emken, E. A., J. Labelled Compd. Radiopharm. 15:233 (1978).
22. Dufek, E. J., and List, G. R., J. Am. Oil Chem. Soc. 54:271 (1977).
23. Birch, A. J., and Walker, K. A. M., J. Chem. Soc. (C) 1894 (1966).
24. Rohwedder, W. K., in "Analysis of Lipids and Lipoproteins," E. G. Perkins, ed., Chap. 11, pp. 170-182 (1975).
25. Pryde, E. H., Anders, D. E., Teeter, H. M., and Cowan, J. C., J. Org. Chem. 25:618 (1960).