The Rhodium-Catalyzed Deuteration of Unsaturated Triglycerides

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Tripalmitolein, triolein, trilinolein and trilinolenin were deuterated with deuterium gas and Wilkinson's catalyst [$(Ph_3P)_3RhCl(I)$]. Recrystallization of the products from acetone yielded highly pure deuterium-labelled triglycerides (TG). The deuterated TG were converted to methyl esters and analyzed by gas chromatography/mass spectroscopy to determine the deuterium distribution. Methyl palmitate-9,10-d_2 (from tripalmitolein) and methyl stearate-9,10-d_2 (from triolein) yielded isotopic distributions of 93-97% d_2, methyl stearate-9,10,12,13-d_4 (from trilinolein) of 74% d_4 and methyl stearate-9,10,12,13,15,16-d_6 (from trilinolenin) of only 58% d_6. Because the deuterium-labelled TG were to be used in human metabolism studies, atomic absorption spectroscopy was used to determine if any residual rhodium was present. No rhodium was detected at 70 ppb (the minimum detection limit).

INTRODUCTION

This paper describes the preparation of deuterium-labelled, saturated triglycerides (TG) in a one-step synthesis from commercially available unsaturated TG. These compounds were required for our studies concerning the metabolism of fatty acid isomers in humans (1). Because 2-3 g of TG were required for each feeding study, a procedure for preparing multi-gram quantities of these compounds, in high yield and good isotopic purity (>90%), was needed. In this manuscript, isotopic purity will be defined as the appropriate percent d_2, d_4 or d_6 (determined by mass spectrometry) as tabularized in Table 1. The reduction of unsaturated TG with deuterium gas and Wilkinson's catalyst [$(Ph_3P)_3RhCl(I)$] (2) was found to provide a quick route to these compounds. Emken (3) had found deuterium scatter to occur during the Rh-catalyzed, partial deuteration of polyunsaturated fatty acid (PUFA) methyl esters. However, no deuterium distributions for the completely saturated compounds were given. In this study TG composed of either mono-, di- and trienoic fatty acids were reduced with deuterium gas, converted to the fatty acid methyl esters (FAME), and analyzed by gas chromatography/mass spectroscopy (GC/MS) to determine the isotopic purity. To minimize confusion, all deuterium distributions will be given in terms of the FAME, not the TG, although the latter is assumed to have the same distribution.

EXPERIMENTAL

Instruments. Average deuterium content and deuterium distribution were determined on a Finnigan 4500 Mass Spectrometer interfaced with a gas chromatograph (GC-MS) equipped with a 30 m Supelcowax 10 capillary column (.32 mm ID ×x 0.5 micron film thickness). The GC was programmed from 150°C to 235°C at 5°C/min. Helium was used as carrier gas. Samples were analyzed by chemical ionization (conditions: isobutane reagent gas, 70 ev). Rh catalyst concentrations were measured with a Perkin-Elmer atomic absorption spectrophotometer Model 303 (AA) (70 ppb lower detection limit).

Reagents. The following reagents were used as received: tripalmitolein, triolein, trilinolein, trilinolenin (with purities of >99%; Nu-Chek-Prep, Elysian Fields, MN), chloro-

TABLE 1

Mass Analysis for Deuterium

Methyl esters	Number of deuterium atoms (%)										Avg. no. deut. atoms per molecule[a]
	0	1	2	3	4	5	6	7	8	9	
Palmitate-d_2 (9,10)	0.1	2.1	97.2	0.6	-	-	-				1.98 (99%)
Stearate-d_2 (9,10)	-	3.7	93.7	2.5	-	0.1	-	-			1.99 (99%)
Stearate-d_4 (9,10,12,13)	0.2	-	1.8	11.2	76.0	9.3	1.4	-	0.1		3.96 (99%)
I[b]	-	3.5	42.8	40.4	10.9	-	2.4				2.68 (134%)
Stearate-d_6 (9,10,12,13,15,16)	-	-	0.2	0.7	3.6	17.2	58.9	15.9	3.1	0.6	5.98 (99%)
II[c]	-	0.7	-	5.8	40.2	36.0	17.2	-	-	0.1	4.63 (115%)
III[c]	-	1.1	-	10.0	38.9	36.9	12.8	0.3	-	0.1	4.51 (113%)

[a]The number in parentheses is the avg. no. deut. atoms per molecule divided by the number of deuterium atoms that theoretically should be present.

[b]Monoenoic *trans* precursor to stearate-d_4.

[c]Both II and III are precursors to stearate-d_6. They are both monoenes and *trans* in configuration.

tris(triphenylphosphine)rhodium (Strem Chemicals, Newburyport, MA) and deuterium gas, 98% (Matheson Gas Products, Secaucus, NJ).

Methods. Benzene (20 ml) was added to a 50 ml, one-necked, round-bottomed flask (heat-dried), and was degassed three times with nitrogen (N_2) gas/vacuum (28 in. Hg), and three times with deuterium D_2 gas/vacuum. Rh catalyst (100 mg) was added and the system was degassed another three times with D_2/vacuum. The TG (1.1 g) was added by pipette and the system was degassed twice with D_2/vacuum and the homogenous solution was stirred magnetically. A pressure transducer was used to maintain a pressure of 780 mm Hg in the closed system. Deuterium gas was added in 250 ml portions via a 250-ml, piston-controlled gas cylinder. Gas uptake was measured (via piston movement) by a linear displacement potentiometer and was recorded on a strip- chart recorder (4,5).

After 3.5 hr, stirring was stopped and the flask was flushed with nitrogen and placed in a -20°C freezer for two hr. The crystals formed were removed by cold filtration, washed with cold benzene and redissolved in 100 ml acetone with heat. The solution was filtered hot and placed in a -20°C freezer for three hr. The crystals were isolated by cold vacuum filtration and again crystallized from acetone. The crystals were dried in a vacuum oven at 45°C. Reaction yields varied from 80-90%. Larger samples (10 g) of tripalmitolein and triolein were also reduced. For these reactions, the amount of each reagent was increased by a factor of nine.

Samples of the final deuterated products were converted to methyl esters for analysis by both GC and GC/MS (6). For preparation of the methyl esters, ca. 20 mg of the sample were dissolved in 1.5 ml benzene, 1.5 ml of 10% HCL in methanol was added and the solution was heated in a sealed tube at 65°C for two hr. After cooling, the solution was extracted with hexane and the organic layer was dried over sodium sulfate. After filtration and solvent removal, the FAME were ready for analysis. AA was used to assure that the Rh levels of the final TG were below our limits of detectability (<70 ppb) (7).

RESULTS

The results of rhodium-catalyzed reduction reactions are tabulated in Table 2. The yields are highest for deuteration of TG composed of monounsaturated FA, and tend to decrease with increasing unsaturation per FA. All melting points were consistent with the literature. Tripalmitolein and triolein were reduced more rapidly, while the last 5-10% trilinolein and trilinolenin intermediates were reduced very slowly. *Trans* isomers are more slowly reduced by Wilkinson's catalyst than are the *cis* isomers. The monoenoic intermediate (ca. 5%) from the trilinolein reduction [see Compound (I), Table 1] was shown to be *trans* in configuration (by GC), as were the ca. 3% intermediates still remaining in the trilinolenin reduction [see Compounds (II) and (III), Table 1]; all showed a large degree of deuterium scatter. Deuterium uptake was consistent with theory.

Table 1 summarizes the deuterium distribution of the final products plus various intermediates. The average number of deuterium atoms (avg. no. deut. atoms) per molecule is a summation of the number of deuterium atoms (percents) times the number of deuterium atoms; the percent in parentheses is the avg. no. deut. atoms per molecule divided by the number of deuterium atoms that theoretically should be present. The results were obtained by GC/MS of the methyl esters prepared by the transesterification of the reduced TG.

DISCUSSION

Data presented in Tables 1 and 2 show that this catalytic deuteration procedure is useful only for preparation of dideutero species from tripalmitolein and triolein of sufficient isotopic purity (>85%) for our human metabolism studies. However, if high isotopic purity is not required, TG composed of PUFA may be used. The reduction does go to completion, although the *trans* isomers are reduced more slowly. The reduction of TG composed of polyunsaturated FA resulted in H/D exchange with subsequent isotope scattering. As shown by Emken (3), and confirmed by the GC/MS data, *trans* isomers are formed as intermediates (perhaps from conjugated diene precursors) in the Rh-catalyzed reduction of methylene-interrupted polyolefins. The *trans* isomers are reduced more slowly and show more H/D exchange. This may be noted in the high percentage of deuterium incorporation in Compounds (I), (II) and (III). Not all data from this study are consistent with Emken's work—other reduction pathways may exist, especially in the case of trilinolenin. A note of caution: If one looks only at "isotopic purity" (last column, Table 1), then all of the results look good. However, a glance at the actual deuterium distributions shows that this is not the case. Therefore, caution should be exercised when such data are presented in the literature. Unless carefully defined, "isotopic purity" is largely a worthless term.

TABLE 2

Analyses of Rhodium-Catalyzed Reduction Products

Starting material	product yield (%)	Melting point(C) Obs	Lit (8)	TG purity (%)	D_2 uptake (ml) Obs	Theo
Tripalmitolein[a]	98	65.5-67.0	65.8	98	867	823
Triolein[b]	90	71.0-72.5	71.0	99	89	84
Trilinolein[b]	87	70.5-71.5	71.0	95	168	177
Trilinolenin[b]	81	71.0-72.0	71.0	97	264	249

[a]Sample size 10 g.

[b]Sample size 1.1 g.

Since these compounds were to be used in human metabolism studies, careful purification was required. Crystallization was found to be the best way to remove any traces of the rhodium catalyst. Actually, only one crystallization was required to reduce the rhodium concentration to less than 70 ppb, our lower limit of rhodium detectability by AA. Utilizing this procedure, 10 g batches of deuterated tripalmitin and tristearin can be quickly prepared in good yield and of good isotopic purity.

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