Deuterium-Hydrogen Exchange During the Catalytic Deuteration of Methyl Oleate[1]

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Abstract

Extensive exchange of deuterium for carbon-bonded hydrogen takes place during the catalytic reduction of methyl oleate with gaseous deuterium. Mass spectrometric analysis of the deuterated stearate shows that it is composed as follows: a small part of one molecular species contains no deuterium; the largest contains 1 atom of deuterium; nearly as much contains 2 atoms; and progressively smaller numbers of molecules contain 3, 4, 5, and up to 11 atoms of deuterium per molecule.

No appreciable exchange occurred with the hydrogen of methyl stearate in the presence of active palladium catalyst and deuterium.

Methyl oleate, containing up to 9 atoms of deuterium in one species and 1½ moles of deuterium average per mole, has been separated from the partially deuterated ester. This octadecenoate has 60–65% of its double bonds in *trans* configuration and only 18% of its double bonds in the original 9,10-position.

Introduction

In 1959, Dinh-Nguyen and Ryhage (4) reported that extensive exchange of deuterium for carbon-bonded hydrogen took place during the catalytic deuteration of methyl oleate. They found that large portions of the stearate contained 2, 3, 4, and 5 atoms of deuterium per molecule; smaller portions contained greater numbers of deuterium atoms, and a trace of the stearate contained up to 30 atoms of deuterium.

Extensive exchange of deuterium for carbon-bonded hydrogen would be expected either from the Horiuti-Polanyi theory (6) used to explain the hydrogenation of ethylene, or from the half-hydrogenation-dehydrogenation theory employed by Allen and Kiess (1) to explain double bond migration during catalytic hydrogenation of fatty acid esters. Each time the fatty acid ester changes from the diadsorbed to the monoadsorbed species or from the unsaturated to the half-hydrogenated species, a deuterium atom can exchange with a hydrogen atom on the chain. Burwell et al. (2,3,9) have described hydrogen-deuterium exchange in hexane and hexene on nickel, platinum, and palladium catalysts in both gas and liquid phases. They have shown that extensive exchange takes place on hexene during its saturation in the liquid phase. They have also shown extensive exchange takes place on hexane in the gas phase at temperatures and on catalysts similar to those used in the hydrogenation of vegetable oils.

The exchange of deuterium for hydrogen during the catalytic saturation of double bonds is useful in studying the mechanism of double bond hydrogenation. Such information aids in selecting catalysts and establishing conditions for improving the hydrogenation of soybean oil. Of particular interest is comparing exchange, double-bond position migration, and *cis,trans* isomerization during the course of catalytic hydrogenation.

Preliminary experiments were conducted to determine some of the basic characteristics of deuterium-hydrogen exchange during catalytic saturation of the double bond of methyl oleate. A series of experiments was run to determine if deuterium exchanged with methyl stearate under normal hydrogenation conditions. Another series was run to determine what effect different metal catalysts had on the amount of exchange and if there was any correlation with the *cis, trans* isomerizing characteristics of the catalysts.

Experimental Procedures

High-purity methyl oleate used in these experiments was obtained from two sources. One sample prepared in this laboratory by fractional crystallization was 99.8% pure containing a 0.2% methyl palmitate impurity. The other sample of methyl oleate came from Hormel Institute and was better than 99.9% pure, with no detectable impurity. The purity of both samples was determined on a 4 ft. x ¼ in. glass column packed with 10% polyvinyl acetate on 60/80 Chromosorb W in a Pye-Argon chromatograph. The deuterium, prepared electrolytically from heavy water, was shown to be 95.5% deuterium and 4.5% hydrogen as measured by a mass spectrometer. Standard tank hydrogen was used without purification. The catalysts were commercial hydrogenation catalysts—5% platinum on carbon, 5% palladium on carbon, 47% reduced stabilized nickel on kieselguhr, and 30% nickel in reduced cottonseed oil. All catalysts were used without pretreatment. In one experiment, a sulfur-poisoned nickel isomerization catalyst was used.

The hydrogenations were performed in simple glass manometric apparatus. The reaction flask was 100 ml round-bottom, equipped with a 24/40 ₸ joint and a short side arm closed with a rubber serum cap. The flask was connected by a capillary tube to a 50 ml gas burette and a mercury leveling bulb. Temperature of the flask was maintained by immersion in a thermostatically controlled silicone oil bath. A Teflon-encased magnetic stirrer supplied agitation to the reaction mixture.

To determine if deuterium exchange would take place on saturated esters, methyl stearate was exposed to deuterium and catalyst. The procedure was to place ca. 0.05 g of catalyst in the flask with the stirrer, evacuate the air, fill the flask with hydrogen at atmospheric pressure, and allow the system to come to thermal equilibrium. Methyl oleate, 0.5 g, was then added through the serum cap with a syringe and the stirrer started. The flask was maintained at atmospheric pressure by balancing the mercury level as the hydrogen in the flask was absorbed. Stirring was continued for ½ hr after no more hydrogen was absorbed to ensure complete conversion of the oleate to stearate. Hydrogen was then pumped off and replaced by deuterium gas; the newly formed methyl

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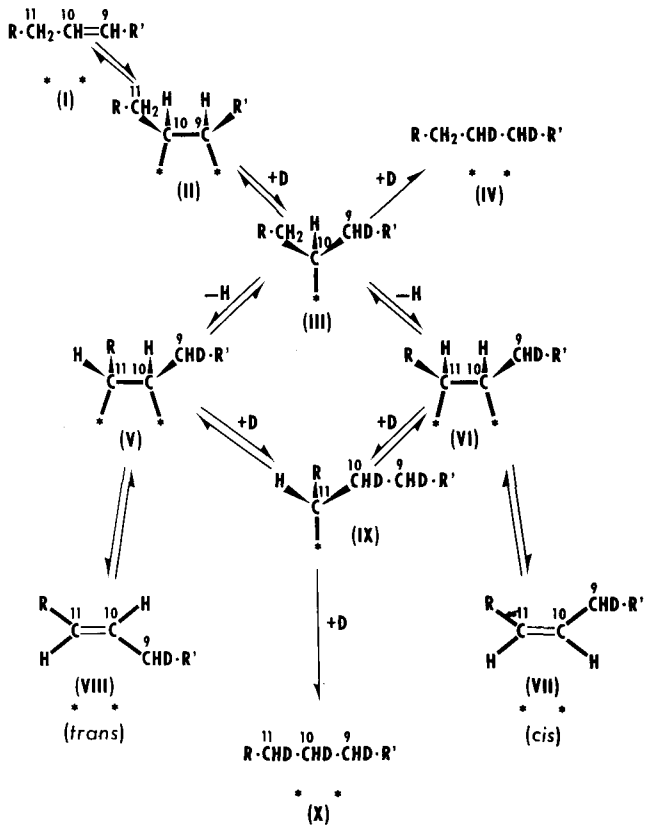

FIG. 1. Extensions of Horiuti-Polanyi mechanism applied to catalytic deuteration.

stearate was stirred for 1 hr in the presence of the "active" catalyst and deuterium at the hydrogenation temperature. A 0.1 g sample of the ester was withdrawn from the reaction mixture for analysis by mass spectrometry. Methyl oleate, 0.1 g, was then added to the remaining reaction mixture, and an absorption of deuterium was noted to prove that the catalyst was still active. This experiment was run with a nickel catalyst at 140C, and with a palladium catalyst at 40, 100, and 140C.

To determine what effect different catalysts had on the amount of exchange, deuterations were run with 4 different catalysts, and samples were taken when the reaction was 50% complete. The procedure was to place ca. 0.05 g catalyst in the reaction flask equipped with a stirrer, evacuate the air, fill the flask with deuterium at atmospheric pressure, and allow the system to come to thermal equilibrium. Methyl oleate, 0.5 g, was then added through the serum cap with a syringe and the stirrer was started. The volume of deuterium, corrected for temp and barometric pressure, necessary to reduce half of the methyl oleate, was calculated and the stirrer was stopped when this amount of deuterium was absorbed. Ca. 0.1 ml of the reaction mixture was withdrawn with a syringe for subsequent analysis, the stirrer was started again, and the reaction allowed to run to complete saturation. The sample removed at 50% completion was analyzed by infrared and gas chromatography. The saturated sample was analyzed by mass spectrometry.

To determine the percentage of *trans* configuration of the unsaturated material, infrared absorption was measured at 10.4 μ in carbon disulfide according to the technique of Shreve et al. (8). The mass spectral analysis was done on a Bendix time-of-flight instrument with an inlet and source temperature of 150C.

Two of the partially saturated deuteration mixtures were separated into octadecanoate and octadecenoate on a reversed-phase liquid chromatographic column by the method of Hirsch (5) with a rubber stationary phase. Location of the position of double bonds in the octadecenoate was determined by the oxidative cleavage method of Jones and Stolp (7), followed by dibasic acid analysis by gas chromatography.

Results and Discussion

Extension of the Horiuti-Polanyi theory to catalytic deuteration of the double bond (Fig. 1) would pre-

TABLE I

Analysis of Samples

	Methyl oleate catalytically deuterated to methyl octadecanoate				Partially deuterated and separated octadecenoate			Methyl stearate with catalyst and deuterium				Normal methyl stearate
Catalyst	Platinum on carbon	Palladium on carbon	47% Nickel	30% Nickel	Unsat. Sulfur-poisoned nickel	Unsat. Palladium on carbon	Sat. Palladium on carbon	Palladium on carbon	Palladium on carbon	Palladium on carbon	47% Nickel	
Temperature, C	40	40	140	140	140	40	40	40	100	140	140	
% *trans*	20.9	59.4	43.1	34.4	49	63.3	....	....	....	....	....	....
Avg D/molecule (MS)	2.91	2.90	3.33	3.24	3.27	1.32	1.68	....	....	....	....	0
Mass spectrum												
293	0.3	0.3	0.5	0.5	2.3	2.5	0.3	....	....	....	....	....
294	0.5	0.7	1.1	0.8	5.8	5.6	0.8	....	....	....	....	....
295	1.0	1.8	3.1	1.3	9.3	12	2.3	....	....	....	....	....
296	2.7	3.8	5.9	3.8	28	97	4.7	4.5	3.9	2.8	3.6	3.8
297	6.6	10	12	7.5	62	100	9.9	3.5	3.9	7.8	10	9.1
298	44	80	78	43	90	66	99	100	100	100	100	100
299	88	100	100	83	100	35	100	*30*	*30*	*36*	*32*	30
300	100	89	93	100	77	17	69	10	11	12	9.3	9.3
301	78	72	80	86	58	9.5	41	5.0	5.6	5.3	3.5	3.1
302	53	53	65	64	32	4.8	23	2.9	3.6	3.0	1.8	1.4
303	34	37	47	45	16.3	2.8	13	1.8	2.2	2.1	0.9	0.6
304	21	26	33	30	9.3	1.5	8.8	0.9	1.5	1.6	0.5	0.2
305	13	18	25	21	5.8	0.9	5.4	0.6	0.8	1.3	0.4	....
306	8.3	12	19	13	3.5	0.5	3.7	....	....	1.0	0.2	....
307	5.5	8.6	13	8.7	2.3	....	2.4	....	....	0.6	0.1	....
308	3.6	6.1	10	6.1	2.3	....	1.5	....	....	0.6	....	....
309	2.6	4.1	7.1	3.7	1.2	....	1.0	....	....	....	....	....
310	1.8	2.9	4.9	2.6	....	....	0.7	....	....	....	....	....
311	1.4	1.9	3.0	1.5	....	....	....	....	....	....	....	....
312	1.1	1.3	2.3	1.1	....	....	....	....	....	....	....	....
313	0.9	0.9	1.7	0.9	....	....	....	....	....	....	....	....
314	0.6	0.6	1.1	0.7	....	....	....	....	....	....	....	....
315	0.5	0.4	0.8	0.4	....	....	....	....	....	....	....	....
316	....	....	....	....	....	....	....	....	....	....	....	....
Column No.	1	2	3	4	5	6	7	8	9	10	11	12

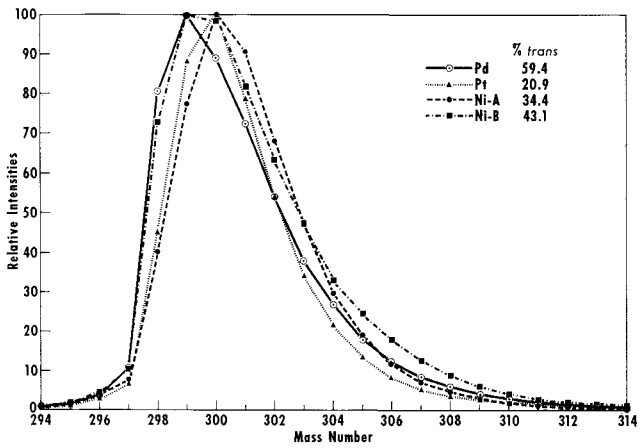

FIG. 2. Exchanged methyl stearate; Pd, 5% palladium on carbon; Pt, 5% platinum on carbon; Ni-A, 30% nickel in reduced cottonseed oil; Ni-B, 47% reduced stabilized nickel on Kieselguhr.

dict: a) an extensive exchange of deuterium for carbon-bonded hydrogen, along with b) double-bond migration, and c) *cis,trans* isomerization. Results of the mass spectrometric analysis of methyl stearate samples deuterated with four different catalysts are shown in the first four columns of Table I. Table I gives the molecular weight region of the mass spectra normalized with the highest mass number in this region given the value of 100. If there were no exchange and the reaction went directly I, II, III, to IV (Fig. 1) with no reverse or side reactions, one would expect two atoms of deuterium to add to the double bond to form methyl dideutero stearate, which would have a parent peak mass spectrum two mass units higher than normal methyl stearate, i.e., 300. It is apparent that some species of deuterated stearate are formed in which up to 11 atoms of deuterium are present. For comparison, normal methyl stearate is given in column 12 of Table I. On the other hand, if the reversible reactions between the monoabsorbed and the diabsorbed species take place, species V through X, and many more with large numbers of deuterium atoms, will appear depending on the rate of the reverse reactions compared to the rate of the simple saturation reaction. Column 12 cannot be used as a definitive description of the methyl stearate mass spectrum because of considerable peak overlap due to limited instrument resolution.

To interpret the data, one must know whether hydrogen-deuterium exchange takes place on saturated methyl esters. Methyl stearate was, therefore, exposed to deuterium under hydrogenation conditions as described above. When results in columns 8 through 11 in Table I are compared with those for natural methyl stearate, in column 12, one sees that no significant exchange has taken place.

It appears that most of the hydrogen displaced by deuterium does not freely mix with the gas phase but, instead, appears to be held close to the catalyst and competes with the deuterium during the exchange process. Evidence for this conclusion is the observation that despite the exchange of up to 10 atoms of deuterium on some molecules, the final product contains an average of only 2.5 atoms of deuterium per molecule or only slightly more than the 2 atoms per molecule which would be expected with simple saturation and no exchange. More support for this conclusion is in the spectra of deuterated stearate (columns 1–4 and 7); mass peaks 298 and 299 were among the highest in the spectrum. The only possible explanation for these peaks is that the molecules were saturated with either two atoms of hydrogen or one atom of hydrogen and one atom of deuterium. In columns 2, 3, 5, and 7, and on two curves of Figure 2, the 299 peak is the highest in the spectrum. Consequently, the hydrogen removed from the oleate to the catalyst surface actively competes with the deuterium to return to the oleate.

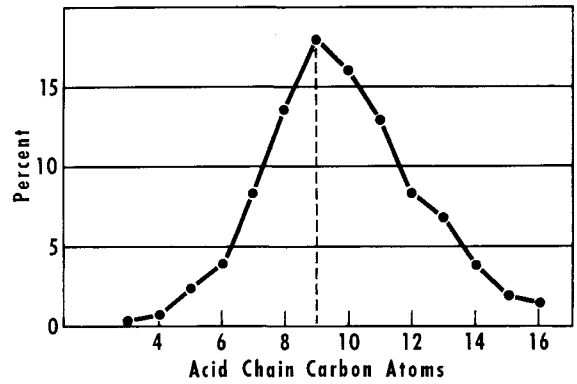

FIG. 3. Dibasic acids from oxidative cleavage of octadecenoate.

From the theory represented in Figure 1, one might find in the incompletely saturated mixtures some free methyl octadecenoate that was exchanged but not saturated. This material has been found. Two samples were prepared: one with a sulfur-poisoned nickel catalyst that went only 20% to completion in 8 hr; the other with a palladium-on-carbon catalyst which was stopped at 50% completion. In both of these samples the oleate was separated from the stearate by a reversed-phase liquid chromatographic column.

The mass spectrum of the methyl octadecenoate separated from the sulfur-poisoned nickel catalyst reaction (column 5 of Table I) is very highly exchanged. The mass spectrum of methyl octadecenoate separated from the palladium catalyst reaction is given in column 6 of Table I, and that of the deuterated methyl octadecanoate separated from the same reaction in column 7. The methyl octadecenoate and methyl octadecanoate from the palladium reduction are definitely exchanged but not as extensively as that from the slow reacting sulfur-poisoned nickel-catalyzed reaction.

The infrared spectrum of the octadecenoate isolated chromatographically from the palladium deuterogenation (column 6) shows that 63% of the esters have double bonds in the *trans* configuration when calculated as methyl elaidate. An analysis of the dibasic esters formed by oxidative cleavage of this octadecenoate (Fig. 3) shows the double bonds migrate extensively over a wide range extending from the 3- to 16-positions until only 18% are left in the original 9,10-position.

In Figure 2, the relative intensities of the mass spectra of the samples made with four different catalysts (columns 1–4) are plotted against mass number to show the differences in the amount of exchange for each catalyst. The mass spectra are of the saturated methyl stearate, whereas the percentage *trans* values were measured on the samples taken half way through the hydrogenation. No simple correlation between isomerization and migrating tendencies of catalysts for double bonds is apparent.

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Phospholipids of Tuna White Muscle

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Abstract

The composition of the lipids from the white muscle of five tuna fish has been determined. Total extractable lipid varied from 0.5%–10.3% of the tissue wet weight; phospholipid content ranged from 0.3%–0.6%. The separation of the phospholipid components was made by column chromatography with activated silicic acid and stepwise elution, with increasing concentrations of methanol in chloroform. The components were identified by chemical tests and infrared (IR) spectra. Tuna white muscle contained an average of 0.5% phospholipid on wet weight basis; 23% was cephalin, 54% lecithin, 8% sphingomyelin, 2% phosphoinositide, with small amounts of unidentified components. Ten to 30% of the lecithin and cephalin fractions were in the form of plasmalogens. The gas-liquid chromatographic analyses of the 12–22 carbon fatty acids of the lecithin, cephalin, and neutral fractions are presented. The cephalins were characteristically high in stearic acid and low in palmitic acid, in contrast to the lecithins.

Introduction

WHOLE LIPID EXTRACTS of fish muscle contain a complex mixture of neutral and phospholipids (1). The fatty acids derived from these components offer a broader mixture of chain lengths and degrees of unsaturation than do those found in the lipids from tissues of land animals. Phospholipids in lipid extracts of tuna muscle have been investigated by Katada (2), who showed that the components of light and dark meat varied in composition and complexity. Improved methods of fractionation and techniques for analysis prompted a reinvestigation of the distribution and composition of phospholipids from tuna white muscle. This paper describes the resolution of the components of whole lipid extracts by silicic acid chromatography, and the composition of the fractions obtained.

TABLE I

Identification of Tuna Samples [a]

Sample	Weight	Fat-free residue	Total lipid	Total phospholipid
	kg.	% wet weight	% wet weight	% wet weight
Albacore I	7	22.6	10.3	0.41
Albacore II	12	27.4	1.2	0.26
Albacore III	7	23.4	8.8	0.62
Albacore IV	5	23.4	9.0	0.37
Yellowfin I	11	22.3	0.5	0.31

[a] The albacore, *Thunnus alalunga*, and yellowfin, *Thunnus albacares*, samples were obtained as follows: Albacore I and III, from San Francisco area, fresh, 2 and 3 days old; Albacore IV, from Oregon area, frozen, 11 wk old; Albacore II and Yellowfin I, from Japanese area, frozen, unknown age. Data on percentage composition refer to white muscle only.

Experimental Procedure

Source of material. Five tuna were investigated. Four were albacore, *Thunnus alalunga,* and the fifth yellowfin, *Thunnus albacares.* The frozen tuna were allowed to thaw partially and then were eviscerated. White muscle was carefully separated from the red meat. The source, weight, and length of storage for each fish is included in Table I.

Extraction. The procedure for extraction of lipid from white muscle was based on the method of Bligh and Dyer (3). The tissue, in 100 g lots, was blended mechanically for 2 min with 3 vol of chloroform/methanol (2:1) solution. 100 ml chloroform and 100 ml distilled water were added to the mixture, and blending was repeated for 1 min. The homogenate was filtered and the filtrate allowed to separate into chloroform and aqueous layers. The tissue residue was re-extracted with 150 ml chloroform, and the mixture again filtered. The chloroform-lipid layers were combined. Further extraction of the residue failed to yield a significant amount of lipid. A few crystals of hydroquinone were added during homogenization, and also to the combined chloroform-lipid phase, to inhibit oxidation. The crude extracts contained 2–29 mg lipid/ml extract. They were stored under nitrogen at −13C. These extraction and storage procedures gave products which had a very low degree of oxidation. The peroxide method of Dam and Granados (4) gave values close to zero when performed on fresh extracts.

Fractionation. Whole lipid extracts were fractionated by silicic acid chromatography. The silicic acid (Mallinckrodt, No. 2847) was first washed successively with methanol, 20% methanol in chloroform, chloroform and methanol. The washed silicic acid was then dried under vacuum at 120–140C to remove residual methanol, excess moisture, and absorbed oxygen. Purified nitrogen was introduced into the flask after 2 days of drying, and the material was stored in this manner. The water content of the silicic acid preparations varied from 8–9% as determined by ignition at ca. 750C.

Trial chromatographic columns were run to determine the approximate phospholipid content of the individual samples of whole lipid. Ca. 100 mg whole lipid was applied to a 10 g silicic acid column. The neutral fraction was separated from the phospholipid components by stripping the non-phosphorus fractions off the column with chloroform followed by methanol to elute the phospholipids. The lipid load of each preparative column was determined by the percentage of phospholipid in each trial run. Large scale separations were carried out with not more than 6 mg phospholipid per g adsorbent. The preparative glass columns were 4.5 cm diam and 70 cm