The Conjugation and Epoxidation of Fish Oil

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ABSTRACT: Wilkinson's catalyst [$RhCl(PPh_3)_3$] has been used to conjugate fish oils in high yields under very mild reaction conditions. A catalyst load of 0.35 mol% of $RhCl(PPh_3)_3$, 0.43 mol% of (o-$CH_3C_6H_4)_3P$, and 0.87 mol% of $SnCl_2 \cdot 2H_2O$ in ethanol solvent at 60°C for 2 d produces 82% conjugated Norway fish oil ethyl ester in a 91% yield. A Capelin triglyceride fish oil affords 90% conjugated fish oil in 93% yield. The Sharpless epoxidation procedure has also been employed to epoxidize fish oils. Using 0.34 mol% of CH_3ReO_3, 8.15 mol% of pyridine, and 1.03 equivalents of aq. 30% hydrogen peroxide in methylene chloride solvent at 25°C for 6 h, the Norway fish oil ethyl ester can be 100% epoxidized in an 86% yield. The Capelin fish oil gives 100% epoxidized fish oil in a 72% yield. Decreasing the amounts of CH_3ReO_3 and pyridine used in the reaction results in partially epoxidized fish oils.

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Fish oils have a triglyceride structure composed of saturated, monounsaturated, and polyunsaturated fatty acids (1). While highly unsaturated, fish oils contain no conjugated carbon-carbon double bonds. Conjugation of the polyunsaturated fatty acid chains in fish oil has been previously reported in the literature. Fish oil may be conjugated in high yields using catalytic amounts of $Ru_3(CO)_{12}$ in formic acid at 180°C in the absence of oxygen (2). When the methyl esters of fish oil fatty acids are treated with cesium *tert*-butylate in *tert*-butanol at 140°C under a nitrogen atmosphere, conjugation of the carbon-carbon double bonds occurs, but the extent of conjugation is unknown (3). Menhaden fish oil may be refluxed with NaOH in xylene for 18 h under nitrogen to produce 68% conjugation in the saponified, polyunsaturated fatty acids (4). When linseed, soybean, and fish oils are heated to 260–300°C in the presence of anthraquinone, 2–10% conjugation is observed (5). Nickel-on-carbon catalysts have also been reported to partially (30–45%) conjugate fish oils when heated for 3–6 h at 170°C under a nitrogen atmosphere (6). Most of these methods require high temperatures and harsh reaction conditions, which often result in fatty acid degradation, major side products, and low conjugation yields.

A number of other methods have been employed to conjugate other natural oils and their derivatives. For example, Wilkinson's catalyst [$RhCl(PPh_3)_3$] has been reported to conjugate linoleic acid and its methyl ester in high yields (7). The reaction also requires catalytic amounts of $SnCl_2 \cdot 2H_2O$, HCl, $FeCl_3$, $CrCl_3$, or LiCl and temperatures of 120–150°C in order for isomerization to occur. This reaction has been shown to be ligand sensitive (8). When tri-p-tolylphosphine was used as the ligand, excellent conjugation yields were obtained near room temperature (8). A rhodium-catalyzed procedure has also recently been developed in our laboratories to conjugate the polyunsaturated fatty acid esters in vegetable oils (9). Using a catalyst load of 0.1 mol% $[RhCl(C_8H_{14})_2]_2$ (C_8H_{14} = cyclooctene), 0.4 mol% (p-$CH_3C_6H_4)_3P$, and 0.8 mol% $SnCl_2 \cdot 2H_2O$ in ethanol at 60°C for 1 d, soybean oil has been conjugated in >95% yield. The same reaction conditions have been used to conjugate safflower, sunflower, walnut, corn, sesame, and peanut oil in very high yields. This methodology looked particularly promising as a very mild, but effective, procedure for the high-yield conjugation of fish oil.

Fish oils are also interesting substrates for epoxidation, because the ω-3 fatty acid chains in the fish oil triglyceride contain five or six nonconjugated double bonds. There are several reports of the epoxidation of fish oils in the literature, but the products of many of these reactions have not been fully characterized and the extent of epoxidation is unknown. Fish oils have traditionally been epoxidized by peracids (10). Monoglycerides produced by the saponification of fish oil have also been epoxidized by peracids (11). There appears to be a real need for a milder, more effective procedure for the epoxidation of fish oil, one which will not affect ester hydrolysis or epoxide ring-opening during epoxidation.

Methyltrioxorhenium (MTO) has been shown to catalyze the epoxidation of olefins in very high yields under mild conditions using 30% H_2O_2 (aq.) as the primary oxidant in alcohol solvents (12,13). The use of urea–H_2O_2 adducts as the primary oxidant in these epoxidations has broadened the scope of this chemistry (14,15). Detailed kinetic studies have been performed on the MTO-catalyzed epoxidation reactions to determine the mechanism of this reaction (16–18).

Recently, Rudolph and coworkers (19) developed a new epoxidation procedure catalyzed by MTO and pyridine. For example, 1-phenylcyclohexene may be epoxidized in a 91% yield using 0.5 mol% MTO, 12 mol% pyridine, and 1.5 equivalents of 30% aqueous hydrogen peroxide in methylene chloride at room temperature. The procedure is performed under neutral to basic reaction conditions, eliminating acid-catalyzed ring-opening reactions, which are normally a problem in epoxidation reactions. This procedure looked particularly promising as a mild effective manner to epoxidize fish oil without any of the usual hydrolysis side reactions.

We wish to report here very mild, highly effective procedures for the conjugation and epoxidation of fish oil. We have already found the conjugated fish oil quite useful in the preparation of new thermoset plastics through Lewis acid-initiated copolymerization with alkenes (20). We anticipate a number of useful industrial applications for these materials.

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EXPERIMENTAL PROCEDURES

Reagents. All reagents obtained from commercial vendors were used as received unless otherwise noted. A Norway fish oil ethyl ester was supplied by Pronova Biocare (Bergen, Norway), and a Capelin fish oil was obtained from SR-Mjol HF (Reykjavik, Iceland). Rhodium(III) chloride hydrate, triphenylphosphine, and methyltrioxorhenium(VII) were all purchased from Aldrich Chemical (Milwaukee, WI). Tin(II) chloride dihydrate and aq. 30% hydrogen peroxide were obtained from Fisher Scientific (Fair Lawn, NJ). Tri-*o*-tolylphosphine was purchased from Strem Chemicals, Inc. (Newburyport, MA). Pyridine was purchased from EM Science (Gibbstown, NJ).

*Spectroscopic analysis***.** All ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ using a Varian Unity spectrometer (Palo Alto, CA) at 300 MHz and 75.5 MHz, respectively. Infrared spectra were recorded on a Bio-Rad FTS-7 IR spectrometer (Randolph, MA). Ultraviolet-visible spectra were obtained using a Shimadzu UV-2101PC (Columbia, MD) scanning spectrophotometer.

Thin-layer chromatography (TLC). TLC was performed using commercially prepared 60-mesh silica gel plates (Whatman K6F, Clifton, NJ). The plates were visualized using UV light (254 nm) or basic $KMnO_4$ solution [3 g $KMnO_4$ + 20 g K_2CO_3 + 5 mL NaOH (5%) + 300 mL H_2O].

Rheological analysis. Viscosity measurements were performed using a double gap cylinder rheometer (Rheostress RS75; cylinder DIN 54453; Haake Corp., Karlsruhe, Germany) utilizing 65-mL samples at room temperature. The samples were subjected to increasing shear-rate sweeps from 0 to 500 s^{-1}.

Typical conjugation procedure. To 1.98 g (5.76 mmol) of Norway fish oil ethyl ester in 6 mL of EtOH were added 0.35 mol% (18.8 mg) of $RhCl(PPh_3)_3$, 0.87 mol% (11.3 mg) of $SnCl_2{\cdot}2H_2O$, and 0.43 mol% (7.5 mg) of $(o\text{-}CH_3C_6H_4)_3P$. The reaction mixture was stirred under a N_2 blanket at 60°C for 48 h. The resulting reddish-orange solution was concentrated to an oil and purified by flash chromatography on a silica gel column using a 3:1 hexane/ethyl acetate eluent system. The isomerized fish oil was obtained in 99% yield with 81% conjugation (Table 1, entry 2).

Typical epoxidation procedure. To 1.00 g (2.91 mmol) of Norway fish oil ethyl ester in 1 mL of CH_2Cl_2 was added 0.34 mol% (8.90 mg) of MTO and 8.15 mol% (67.5 mg) of pyridine. The reaction flask was then placed in a cold-water bath and was equipped with a magnetic stir bar. While stirring the reaction mixture, 1.22 mL of a 30% H_2O_2 aqueous solution (1.03 equivalents) was slowly added. After H_2O_2 addition, the reaction mixture was removed from the cold-water bath and stirred vigorously for 6 h at room temperature. Longer reaction times are necessary for larger-scale reactions. The reaction mixture was then poured into a separatory funnel and diluted with 10 mL of CH_2Cl_2. The organic layer was separated from the aqueous layer and washed with 10 mL of a saturated, aqueous NaCl solution. The CH_2Cl_2 solution was then washed with 10 mL of an aqueous 10% Na_2SO_3 solution, which removed most of the orange color from the CH_2Cl_2 layer. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to produce 0.961 g (86% yield) of 100% epoxidized Norway fish oil ethyl ester (Table 2, entry 1).

RESULTS AND DISCUSSION

The conjugation and epoxidation reactions were carried out on two different fish oils. A highly unsaturated Norway fish oil ethyl ester (EPAX 5500 EE Lot no. 60301) supplied by Pronova Biocare was examined initially. A capelin (*Mallotus villosus*) triglyceride fish oil supplied by SR-Mjol HF was also examined in this chemistry. The fatty acid compositions of the two fish oils are shown in Table 3. The capelin fish oil contains 10–15% ω-3 fatty acids, whereas the Norway fish oil ethyl ester contains approximately 60% of the ω-3 fatty acids. The ^1H NMR spectrum of the Norway fish oil indicates that each ethyl ester contains approximately 3.6 carbon–carbon double bonds. However, the ^1H NMR spectrum of the capelin fish oil indicates the presence of approximately 4.5 carbon–carbon double bonds per triglyceride.

A modified version of the conjugation procedure developed by Singer and co-workers (7,8) for linoleic acid and its methyl ester was examined as a method for conjugating the double bonds in fish oil. The Norway fish oil ethyl ester was conju-

TABLE 1
Rhodium-Catalyzed Conjugation of Fish Oils

Entry	Oil	Amount (g)	Time (h)	Temp. (°C)	Yield (%)	Conjugation (%)
1	Norway[a]	2	24	60	91	73
2		2	48	60	99	81
3		2	72	60	93	95
4		50	48	60	97	82
5		78	24	60	98	86
6		150	48	60	91	82
7	Capelin[b]	2	24	60	77	89
8		2	48	60	97	89
9		42	72	60	93	90

[a] Norway fish oil ethyl ester reactions were run using 0.35 mol% $RhCl(PPh_3)_3$, 0.43 mol% $(o\text{-}CH_3C_6H_4)_3P$, and 0.87 mol% $SnCl_2{\cdot}2H_2O$ in ethanol (1.5–2.0 mL/g fish oil).
[b] Capelin fish oil reactions were run using 1 mol% $RhCl(PPh_3)_3$, 1.25 mol% $(o\text{-}CH_3C_6H_4)_3P$, and 2.5 mol% $SnCl_2{\cdot}2H_2O$ in ethanol (1.5–2.0 mL/g fish oil).

TABLE 2
Methyltrioxorhenium (MTO)-Catalyzed Epoxidation of Fish Oils

Entry	Oil	Time (h)	MTO (mol%)	Pyridine (mol%)	Epoxidation (%)	Yield (%)
1	Norway[a]	6	0.34	8.15	100	86
2		2	0.14	1.63	81	98
3		2	0.07	0.81	41	99
4	Capelin[b]	6	0.5	12.0	100	72

[a] Norway fish oil ethyl ester.
[b] Capelin triglyceride fish oil.

gated in high yields using 0.35 mol% of $RhCl(PPh_3)_3$, 0.43 mol% of $(o\text{-}CH_3C_6H_4)_3P$, and 0.87 mol% of $SnCl_2{\cdot}2H_2O$ in ethanol solvent at 60°C. A summary of the results obtained using this procedure is shown in Table 1. The ^{1}H NMR spectra of the conjugated fish oils clearly show that some of the vinylic hydrogens (δ 5.36) move downfield (δ 5.65–6.60) upon conjugation. The integration ratio of the vinylic hydrogens (δ 5.36/δ 5.65–6.60) in the conjugated fish oil and the known fatty acid composition of the oils were used in calculating the percentage conjugation for each entry in Table 1. Increasing the reaction time for reactions performed on the same scale and at the same temperature resulted in products with higher conjugation percentages (entries 1–3). The conjugation procedure was successfully performed on 50-, 78-, and 150-g reactions in >90% yields with >80% conjugation (entries 4–6). This method produces significantly higher yields in the conjugation of fish oil than the procedures currently available in the literature. The temperature required for the reaction is much lower, and the catalyst load is comparable to other transition metal catalysts used to conjugate fatty acid oils.

The capelin triglyceride fish oil was also conjugated in high yields using reaction conditions similar to those used to conjugate the Norway fish oil ethyl ester (Table 1). Using 1 mol% of $RhCl(PPh_3)_3$, 1.25 mol% of $(o\text{-}CH_3C_6H_4)_3P$, 2.5 mol% of $SnCl_2{\cdot}2H_2O$, and 4 mL of ethanol, a 2-g sample of the capelin fish oil was conjugated in a 97% yield with 89% conjugation (entry 8). The conjugation yield does not appear to change significantly as the reaction time is increased (entries 7 and 8). Increasing the scale of the reaction to 42 g resulted in a similar percentage conjugation and overall yield (entry 9).

TABLE 3
Fish Oil Fatty Acid Composition[a]

Fatty acids (number of double bonds)	Norway fish oil ethyl ester (%)	Capelin fish oil (male) (%)	Capelin fish oil (female) (%)
0	8.9	22.6	23.5
1	18.2	63.9	58.6
2	1.1	—	—
3	1.0	—	—
4	6.0	—	—
5 (EPA)	31.7	5.8	8.6
5 (DPA)	4.3	0.5	0.9
6 (DHA)	24.7	3.6	4.8

[a] EPA, eicosa-5,8,11,14,17-pentaenoic acid; DPA, docosa-7,10,13,16,19-pentaenoic acid; DHA, docosa-4,7,10,13,16,19-hexaenoic acid.

The viscosity of the Norway and capelin fish oils increases slightly upon conjugation (Table 4). A similar increase in viscosity is seen when the rhodium-catalyzed conjugation procedure is used to conjugate Wesson soybean oil (9). The increase in viscosity of the Norway fish oil ethyl ester upon conjugation is apparent when the conjugated Norway fish oil ethyl ester is pipetted. We have not observed any evidence of polymerization in our characterization of the conjugated fish oils. The Norway fish oil ethyl ester also takes on a light yellow color after conjugation, possibly due to trace amounts of rhodium catalyst remaining in the oil after chromatography over silica gel. Attempts to remove this coloration with carbon black were not successful. The light yellow color of the conjugated fish oil does not interfere in the subsequent copolymerization of the oil with alkenes.

The Sharpless epoxidation procedure (19) was used to epoxidize the Pronova EPAX 5500 EE (Lot no. 60301) fish oil ethyl ester from Norway. A summary of the products obtained from the epoxidation reactions is shown in Table 2. The percentage epoxidation reported for each entry in Table 2 was calculated from the disappearance of the vinylic hydrogens (δ 5.36) and the appearance of the epoxide hydrogens (δ 2.79–3.11) in the ^{1}H NMR spectra of the epoxidized oils.

The Norway fish oil ethyl ester was epoxidized in an 86% yield with 100% epoxidation (entry 1) using 0.34 mol% of MTD, 8.15 mol% of pyridine, and 1.03 equivalents of aq. 30% H_2O_2 in CH_2Cl_2 at room temperature for 6 h. Epoxidation (81%) was achieved in 98% yield using 0.14 mol% of MTD, 1.63 mol% of pyridine, and greater than 1 equivalent of aq. 30% H_2O_2 in CH_2Cl_2 at room temperature for 2 h (entry 2). When 0.07 mol% of MTO, 0.81 mol% of pyridine, and 1.03 equivalents of aq. 30% H_2O_2 were reacted with the fish oil ethyl ester at room temperature for 2 h, 41% epoxidized fish oil was produced in a nearly quantitative yield (entry 3). The reaction shown in entry 1 (Table 2) was successfully scaled up to 100 g producing a 99% yield of 100% epoxidized Norway fish oil ethyl ester in 24 h. Using 0.5 mol% of MTD, 12 mol% of pyridine, and 150 mol% of aq. 30% H_2O_2 in CH_2Cl_2 at room temperature for 6 h, the capelin fish oil was 100% epoxidized in a 72% yield (entry 4). Clearly, the Sharpless epoxidation procedure is much more efficient than the peracid methods available in the literature for fish oil epoxidation. Much higher epoxidation yields are obtained, and acid-catalyzed ring-opening reactions are eliminated.

The viscosity of the Norway and capelin fish oils significantly increases after complete epoxidation (Table 4). The

TABLE 4
Viscosities of Natural and Modified Oils

Oil	Viscosity (mPa · s)
Norway fish oil ethyl ester	6.1
81% conjugated Norway fish oil ethyl ester	10.7
100% epoxidized Norway fish oil ethyl ester	87.0
Capelin fish oil	42.9
85% conjugated capelin fish oil	63.2
100% epoxidized capelin fish oil	227.5
Wesson soybean oil	59.4
82% conjugated Wesson soybean oil	76.2

Norway fish oil ethyl ester has a relatively low viscosity of 6.1 mPa · s. However, the viscosity of the 100% epoxidized Norway fish oil ethyl ester is 87.0 mPa · s. The viscosity of the epoxidized Norway fish oil ethyl ester is greater than the viscosity of the conjugated Wesson soybean oil (Table 4). The viscosity of the capelin fish oil increases from 42.9 to 227.5 mPa · s upon epoxidation. Although polymerization could explain the increase in viscosity, we did not observe evidence of polymer impurities in our characterization of the epoxidized fish oils.

The ^{1}H and ^{13}C NMR spectra of the epoxidized fish oils clearly show the disappearance of the carbon–carbon double bonds. The ^{13}C NMR spectra of the epoxidized oils also show the appearance of several sp^3-carbon atoms bound to oxygen atoms.

Several attempts were made to epoxidize the conjugated Norway fish oil ethyl ester using the Sharpless procedure. Although partial epoxidation (40–50%) was achieved in each reaction, the ^{1}H and ^{13}C NMR spectra of the products were messy and difficult to interpret. No attempt was made to epoxidize the conjugated capelin fish oil in view of these difficulties.

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