J Am Oil Chem Soc (2012) 89:2077–2089
DOI 10.1007/s11746-012-2106-y

ORIGINAL PAPER

Controlling Product Composition of Metathesized Triolein by Reaction Concentrations

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Received: 20 March 2012 / Revised: 13 June 2012 / Accepted: 19 June 2012 / Published online: 18 July 2012

Abstract Triolein was used as a model material to investigate the effect of concentration on self metathesis of vegetable oils. The metathesis reaction using Grubbs' second generation catalyst (used at a level of 2.5 mol % of triolein) was carried out at 38 °C using dichloromethane as the solvent. The products from three reaction concentrations were investigated: neat, 10 and 20 mmol/L. The products from the reactions were separated by column chromatography and the fractions were characterized by ^{1}H-NMR, ^{13}C-NMR, MS and FTIR. Mono-cyclic and multi-cyclic triacylglycerol-based compounds and different level aliphatic triacylglycerol-like oligomers were produced, but the compositions of the products were found to be significantly controlled by the reaction concentrations. Cyclic compounds were favorably produced at lower reaction concentrations, whereas, linear oligomers were favorably produced at higher reaction concentrations. Cyclic compounds were formed mainly from adjacent fatty acid chains on the glycerol backbone. In the neat reactions, only linear oligomers were produced. The *trans*/*cis* ratios increased as concentration was increased.

Keywords Metathesis · Triolein · Grubbs catalysts · Reaction concentration · Cyclic compounds · Oligomers

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Introduction

The metathesis reaction of triacylglycerols and unsaturated fatty acid derivatives has been known since van Dam et al. [1] applied metathesis reactions to fatty acid derivatives in 1972. The olefin metathesis reaction is a process of the substituent's exchange between two reacting olefins in an equilibrium reaction: a transalkylidenation [2]. It involves two stepwise reactions. First, a catalyst (a transition metal carbene complex) reacts with an olefin in a [2 + 2] fashion to generate an unstable metallacyclobutane intermediate. Following this, the intermediate either opens to give a new metal carbene and a new olefin, or it reverts to the starting material.

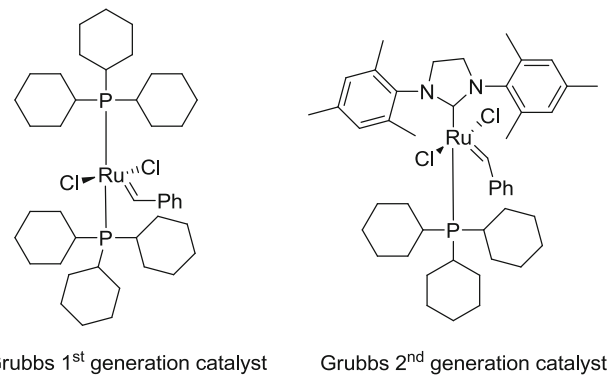
The metathesis reaction of renewable raw materials [3, 4], such as fats and oils, is rapidly becoming an important reaction to produce safer, less toxic fine chemicals [5] and monomers for the polymer industry [6]. This approach to petrochemical replacements can contribute to the development of a sustainable chemistry industry with concomitant reductions in CO_2 emissions. It also can contribute to the reduction of toxic chemicals by avoiding by-products and waste [4] through the application of an efficient catalytic approach in metathesis [7].

In the past, a tungsten-tin catalyst system [1, 8] was widely used for the metathesis of vegetable oils and unsaturated fatty acids. However, this catalyst system presents a number of disadvantages, including the disposal of solvent and used catalysts, as well as sensitivity to moisture and oxygen. These complicate the reaction process and affect reproducibility [9]. Among the numerous attempts made to develop improved active and stable catalytic systems, the work of the Grubbs group [7, 10] stands out; they discovered a series of well-defined, active and stable ruthenium-based catalysts, in particular the Grubbs'

first and second generation catalysts (see Scheme 1). These catalysts have been significantly employed in studying the metathesis of unsaturated fatty acids and vegetable oils [9, 11].

Metathesized vegetable oils, such as high-oleic sunflower oil [12], soybean oil [8] and the triacylglycerol triolein [9, 11] have been studied. Metathesized soybean oil and its modified products have been explored as waxes [13] and additives to improve the drying properties of soybean oil [8]. Elevance renewable sciences [13] brought these materials to the forefront on the commercial market. The metathesis of neat triolein was used as a model system to study the metathesis reaction of more complicated vegetable oils [9, 11]. These studies have demonstrated that the products of metathesized triolein include oligomers, mono cyclic structures, as well as their *cis*/*trans*-configurations. The predominance of one or more of these classes of products is important to the final physical properties of the produced material, and therefore very important in determining end uses such as lubricants, waxes, or additives.

The reaction conditions (such as catalyst, starting materials, temperature, etc.) have been shown to have significant effects on the products of metathesis. For example, Tian et al. [11] have reported that the metathesis of triolein and soybean oil performed at relatively high temperature (55 °C) using Grubbs' second generation catalyst yield not only linear oligomers and mono cyclic structures (in both the *cis*- and *trans*-configurations) but also cross-linked rubber materials. The production of these undesirable cross-linked rubber materials have been avoided when a lower temperature (room temperature in Tian et al. report) is used with however, a longer reaction temperature (i.e., 240 h). Biermann et al. [12] reported that at a temperature of 70 °C, when a Hoveyda–Grubbs' second generation catalyst and a chain stopper were used, the reaction yielded a series of highly branched polyesters (triacylglycerol-like linear oligomers) and no cross-linked materials. When Grubbs' first generation catalyst was used, even without a chain stopper, the cross-linked materials have not been detected. In our present study we have used as a model system (pure triolein) and examined the role of reaction concentration on the products of metathesis reaction. The metathesis reaction was performed using Grubbs' second generation catalyst and CH_2Cl_2 as solvent. The reaction was performed at the highest possible temperature of 38 ± 1 °C, just below the boiling point of the solvent (39.6 °C). This study extends the database relating to the parameters of metathesis and furthers our understanding of the metathesis of vegetable oils and their derivatives.



Scheme 1 Grubbs' catalysts

Experimental

Materials

Triolein (99 %), Grubbs' second generation catalyst and ethyl vinyl ether were purchased from Sigma-Aldrich. Dichloromethane was dried by distillation over calcium hydride.

Instruments

Nuclear Magnetic Resonance Spectroscopy (^{1}H- and ^{13}C-NMR)

1D ^{1}H-NMR 1D ^{1}H spectra were acquired on a Bruker Avance III 400 spectrometer ($\nu(^1\text{H})$ 400.22 MHz; Bruker BioSpin MRI GmbH, Karlsruhe, Germany) equipped with a 5 mm BBO probe. Spectra were acquired at 25 °C over a 16 ppm spectral window with a 1-s recycle delay and 32 transients.

1D ^{13}C-NMR 1D ^{13}C-spectra were acquired on a Bruker Avance III 400 spectrometer ($\nu(^{13}\text{C}) = 100.65$ MHz; Bruker BioSpin MRI GmbH, Karlsruhe, Germany) equipped with a 5 mm BBO probe. Spectra were acquired at 25 °C over a 240 ppm spectral window with a 0.2-s recycle delay and 2,048 transients.

Mass Spectroscopy (MS)

Electrospray ionization mass spectrometry (ESI–MS) analyses were performed using a QStar XL quadrupole time-of-flight mass spectrometer (AB Sciex, Concord, ON, Canada) equipped with an ionspray source and a modified HSID interface (Ionics, Bolton, ON, Canada). The ion source and interface conditions were adjusted as follows: ionspray voltage (IS = 4,500 V), nebulising gas (GS1 = 45), curtain gas (GS2 = 45), declustering potential (DP = 60 V) and HSID temperature (T = 200 °C). Multiple-charged ion signals were reconstructed using the

BioTools 1.1.5 software package (AB Sciex, Concord, ON).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra were acquired using a Thermo Scientific Nicolet 380 FTIR spectrometer (Thermo Electron Scientific Instruments LLC, Fitchburg, WI) fitted with a PIKE MIRacleTM attenuated total reflectance (ATR) system (PIKE Technologies, Madison, WI, USA). Solid samples were placed onto the ATR crystal area and held in place by a pressure arm. Liquid samples were poured onto the ATR crystal area for signal acquisition. The signal was acquired with the following parameters: scanning number = 32; resolution = 4.000; sample gain = 8.0; mirror velocity = 0.6329; and aperture = 100.

High Performance Liquid Chromatography (HPLC)

HPLC was carried on a Waters Alliance (Milford, MA, USA) e2695 HPLC system fitted with a Waters ELSD 2424 evaporative light scattering detector. The HPLC system includes an inline degasser, a pump, and an auto-sampler. The temperature of the column (C18, 150 × 4.6 mm, 5.0 μm, X-Bridge column, Waters Corporation, MA, USA) was maintained at 35 °C by a Waters Alliance column oven. The ELSD nitrogen flow was set at 25 psi with nebulization and drifting tube maintained at 12 and 55 °C, respectively. Gain was set at 500. The mobile phase was chloroform : acetonitrile (50:50)v run for 30 min at a flow rate of 0.2 mL/min. 1 mg/mL (w/v) solution of sample in chloroform was filtered through single step filter vial (Thomson Instrument Company, CA, USA) and 0.5 μL of sample was passed through the C18 column by reversed-phase in isocratic mode. All solvents were HPLC grade and obtained from VWR International (Mississauga, ON, Canada).

Gas Chromatography (GC)

The GC analysis was carried out on an Agilent 6890 N Network System (Palo Alto, CA, USA) equipped with a flame ionization detector (FID), using a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm, 0.2 μm, Varian, Middelburg, The Netherlands). The carrier gas was H_2. The following column temperature conditions were employed: initial hold at 45 °C for 4 min, thereafter rising to 175 °C at a rate of 13 °C/min, followed by a final hold at 175 °C for 27 min. The injector temperature was 250 °C with a purge flow of 40 mL/min at 0.1 min.

Metathesis of Triolein

A 6-g sample (99 % pure) of triolein dissolved in CH_2Cl_2 with a concentration of 10 mmol/L (R1), 20 mmol/L (R2), or neat (R3) was heated to 38 ± 1 °C under a protective N_2 atmosphere. Then, 144 mg Grubbs' second generation catalyst was added. The reaction mixture was stirred at 38 °C for 6 h. The reaction was quenched with 20 mL ethyl vinyl ether. This reaction mixture was concentrated on a rotary evaporator and the residue was re-dissolved in 200 mL hexane. The hexane solution was filtered through a Celite layer to give a clear yellow solution. After concentration with a rotary evaporator, the samples were purified by a column chromatography with ethyl acetate and hexane as the eluent. The structure of these fractions is presented in Schemes 2 to 4. The following compounds contain *trans*- and *cis*-configurations and positional isomers. The yield presented is the weight percentage of pure fractions in the mixtures. The yields of the produced olefins in the 10 mmol/L reaction (R1), 20 mmol/L reaction (R2) and neat reaction (R3) are 28.3, 26.7 and 21.4 %, respectively.

The Structure of Fractions From the Reaction with 10 mmol/L (R1)

Triolein and its isomers (R1–2)

Yield: 8.1 %

HPLC peak retention time t_R(min): 12.6

MS: $C_{57}H_{104}O_6$, Cal. 885, found 603.5, 902.8 $[M + NH_4]^+$

^{1}H-NMR (in $CDCl_3$, ppm), δ = 5.45–5.35 (4H, m,–C<u>H</u>=C<u>H</u>–), 5.30–5.28 (1H, m,–CH_2C<u>H</u>(O)CH_2–), 4.34–4.30 (2H, dd,–C<u>H_2</u>CH(O)C<u>H_2</u>–), 4.19–4.15 (2H, dd, –C<u>H_2</u>CH(O)C<u>H_2</u>–), 2.35–2.31 (6H, t, –C(=O)C<u>H_2</u>CH_2–), 2.04–1.96 (12H, m,=CHC<u>H_2</u>CH_2–), 1.65–1.62 (6H, m, –C(=O)CH_2C<u>H_2</u>–), 1.32–1.29 (62H, m,–C<u>H_2</u>–), 0.92–0.89 (9H, t, –C<u>H_3</u>);

^{13}C-NMR (in $CDCl_3$, ppm), δ = 173.48, 173.06, 130.70, 130.39, 130.22, 129.92, 69.09, 62.31, 34.41, 34.25, 32.80, 32.12, 29.87-29.18, 27.40, 25.10, 25.06, 22.90, 14.32;

6, 23-dioxo-1,5-dioxacyclotricos-14-en-3-yl octadec-9-enoate and/or 5,22-dioxo-1,4-dioxacyclodocos-13-en-2-yl-methyl octadec-9-enoate and its isomers (R1–3)

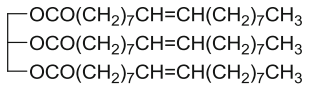
Yield: 35.0 %

t_R(min): 10.0

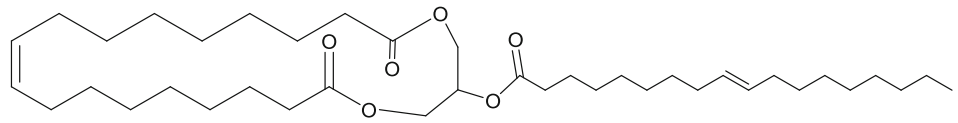
MS: $C_{39}H_{68}O_6$, Cal.632.95, found 633.5 $[M + H]^+$, 650.5 $[M + NH_4]^+$

^{1}H-NMR (in $CDCl_3$, ppm), δ = 5.41–5.24 (5H, m,–C<u>H</u>=C<u>H</u>– and, –CH_2C<u>H</u>(O)CH_2–), 4.47–4.09 (4H, dd, –C<u>H_2</u>CH(O)C<u>H_2</u>–), 2.37–2.31 (6H, m, –C(=O)C<u>H_2</u>CH_2–), 2.07–1.96 (8H, m, =CHC<u>H_2</u>CH_2–), 1.65–1.62 (6H, br,

Scheme 2 Structures of compounds from the reaction with 10 mmol/L (**R1**). The compounds present contain *cis*-, *trans*-configurations, and positional isomers on glycerol skeletons



R1-2



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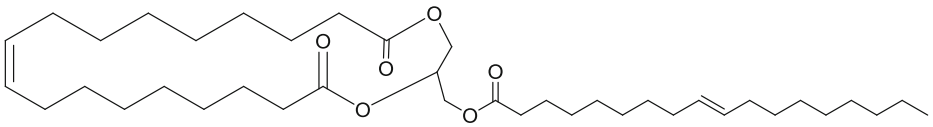
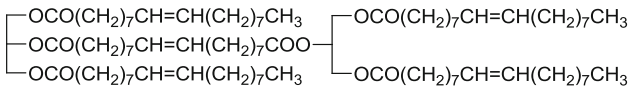
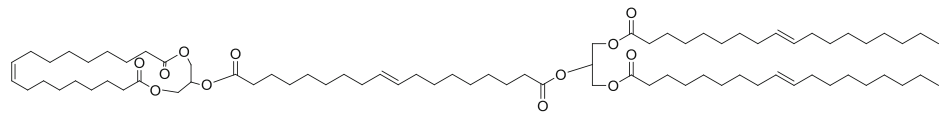


R1-3



R1-4



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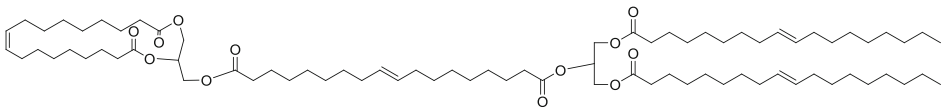
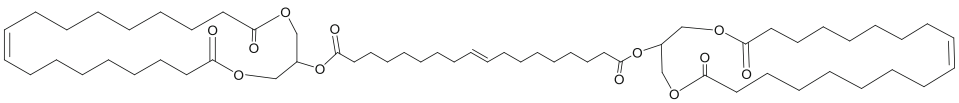
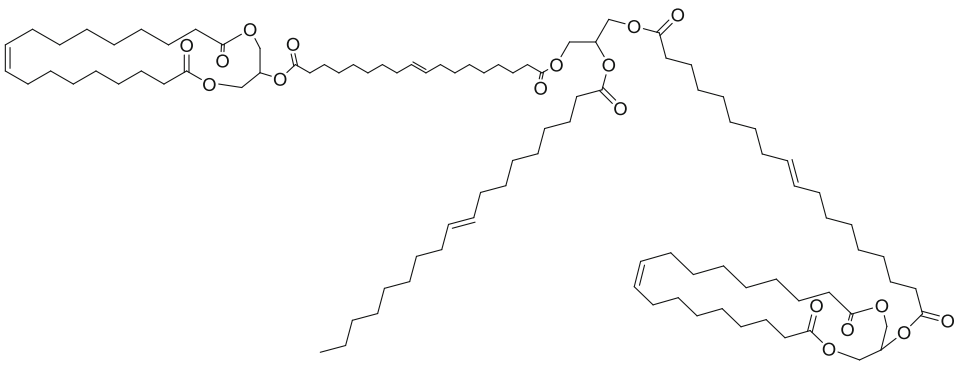


R1-5



R1-6



R1-7

–C(=O)CH_2CH_2), 1.37–1.29 (36H, m, –CH_2–), 0.92–0.88(3H, t,–CH_3);

^{13}C-NMR (in $CDCl_3$, ppm), $\delta = 173.48$, 173.06, 130.97, 130.80, 130.70, 130.38, 130.23, 129.93, 69.11, 68.95, 62.54, 62.47, 62.31, 61.72, 34.55, 34.43,34.43, 34.38, 34.30, 34.25, 32.83, 32.78, 32.36, 32.11, 31.14, 29.99-28.59; 28.24, 27.95, 27.89,27.45, 27.39, 27.07, 26.87,26.78, 25.19, 25.07, 24.95, 24.85,22.90, 14.33;

Bis (1,3-bis(octadec-9-enoyloxy)propan-2-yl) octadec-9-enedioate and its isomers (R1–4)

Yield: 2.8 %

t_R(min): 14.2

MS: $C_{96}H_{172}O_{12}$, Cal.1518.39, found 351.3, 597.4, 613.4, 1535.4 $[M + NH_4]^+$

^{1}H-NMR (in $CDCl_3$, ppm), $\delta = 5.32$-5.26 (10H, m, –CH=CH–), 5.22–5.17 (2H, m, –CH_2CH(O)CH_2–), 4.24–4.20(4H, dd, –CH_2CH(O)CH_2–), 4.09–4.03 (4H, dd, –CH_2CH(O)CH_2–), 2.26–2.22 (12H, m, –C(=O)C$H_2$$CH_2$–), 1.94–1.87 (20H, m, =CHC$H_2$$CH_2$–), 1.54 (12H, br, –C(=O)CH_2CH_2–), 1.22–1.19 (96H, m, –CH_2–), 0.83–0.79 (12, t, –CH_3);

^{13}C-NMR (in $CDCl_3$, ppm), $\delta = 173.40$, 172.99, 130.63, 130.30, 69.11, 62.33, 34.43, 34.26, 32.80, 32.13, 29.89-29.20, 27.41, 25.08, 22.91, 14.34

1-(2,3-bis(octadec-9-enoyloxy)propyl) 18-(5,22-dioxo-1,4-dioxacyclodocos-13-en-2-yl)methyl octadec-9-enedioate and/or 1-(1,3-bis(octadec-9-enoyloxy)propan-2-yl) 18-(6,23-dioxo-1,5-dioxacyclotricos-14-en-3-yl) octadec-9-enedioate and its isomers (R1–5)

Yield: 7.5 %

t_R(min): 11.2

MS: $C_{78}H_{136}O_{12}$, Cal.1265.91, found 351.2,603.5, 983.8 1283.1 $[M + NH_4]^+$

^{1}H-NMR (in $CDCl_3$, ppm), $\delta = 5.41$–5.28 (10H, m, –CH=CH–), 4.48–4.15 (8H, m, –CH_2CH(O)CH_2–), 2.36–2.32 (12H, t, –C(=O)C$H_2$$CH_2$–), 2.04–1.98 (16H, m, =CHC$H_2$$CH_2$–), 1.64–1.60 (12H, br, –C(=O)CH_2CH_2–), 1.32–1.29 (72H, m, –CH_2–), 0.92–0.89 (6H, t, –CH_3)

^{13}C-NMR (in $CDCl_3$, ppm), $\delta = 173.48$, 173.07, 130.98-130.24, 69.20, 69.11, 62.55, 62.33, 34.43-34.26, 32.80, 32.12, 29.94-28.85, 28.28, 27.90, 27.46, 25.10, 22.91, 14.34

Bis (6,23-dioxo-1,5-dioxacyclotricos-14-en-3-yl) octadec-9-enedioate and its isomers (R1–6)

Yield: 8.8 %,

t_R(min): 9.2

MS: $C_{60}H_{100}O_{12}$, Cal.1013.43, found 351.2, 365.2, 1030.8 $[M + NH_4]^+$

^{1}H-NMR (in $CDCl_3$, ppm), $\delta = 5.40$–5.30 (8H, m, –CH=CH– and –CH_2CH(O)CH_2-), 4.46-4.10 (8H, m, -CH_2 CH(O)CH_2-), 2.35-2.31 (12H, m, -C(=O)C$H_2$$CH_2$–), 2.06–1.98 (12H, m, =CHC$H_2$$CH_2$–), 1.64 (12H, br, –C(=O) CH_2CH_2–), 1.31 (48H, br, –CH_2–);

^{13}C-NMR (in $CDCl_3$, ppm), $\delta = 173.46$, 173.08, 130.96, 130.80, 130.10, 129.92, 69.18, 62.61, 62.38, 61.80, 34.54-34.24, 32.82, 32.35, 32.10, 31.13, 29.91-28.58, 28.23, 27.90, 27.40, 26.77, 25.19-24.83,

18-bis (6,23-dioxo-1,5-dioxacyclotricos-14-en-3-yl) '1,1-2(octadec-9-enoyloxy)propane-1,3-diyl dioctadec-9-enedioate and its isomers (R1–7)

Yield: 5.0 %

t_R(min): 10.1

MS: $C_{99}H_{168}O_{18}$, Cal.1646.38, found 351.2, 645.5, 840.7$[M + 2NH_4]^{2+}$, 1663.3 $[M + NH_4]^+$

^{1}H-NMR (in $CDCl_3$, ppm), $\delta = 5.40$-5.29 (13H, m, –CH=CH– and –CH_2CH(O)CH_2–), 4.47–4.14 (12H, m, –CH_2CH(O)CH_2–), 2.35–2.31 (18H, m, –C(=O)C$H_2$$CH_2$–), 2.06–1.98 (20H, m, =CHC$H_2$$CH_2$–), 1.63 (18H, br, –C(=O)CH_2CH_2–), 1.31–1.26 (84H, m, –CH_2–), 0.92–0.88 (3H, t, –CH_3);

^{13}C-NMR (in $CDCl_3$, ppm), $\delta = 173.50$, 173.05, 130.96-130.03, 129.92, 69.19, 69.09, 62.61, 62.32, 61.79, 34.54-34.24, 32.82, 32.12, 31.13, 29.98-28.58, 25.10, 24.84, 22.90, 14.33

The Structure of Fractions from the Reaction with 20 mmol/L (R2)

Triolein and its isomers (R2–2)

Yield: 12.8 %

t_R(min): 12.6

MS, ^{1}H– and ^{13}C-NMR refer to **R1–2**

6, 23-dioxo-1,5-dioxacyclotricos-14-en-3-yl octadec-9-enoate and/or 5,22-dioxo-1,4-dioxacyclodocos-13-en-2-yl-methyl octadec-9-enoate and its isomers (R2–3)

Yield: 31.3 %

t_R(min): 10.0

MS, ^{1}H– and ^{13}C-NMR refer to **R1–3**

Bis (1,3-bis(octadec-9-enoyloxy)propan-2-yl) octadec-9-enedioate and its isomers (R2–4)

Yield: 13.1 %

t_R(min): 14.2

MS, ^{1}H– and ^{13}C-NMR refer to **R1–4**

1-(2,3-bis(octadec-9-enoyloxy)propyl) 18-(5,22-dioxo-1,4-dioxacyclodocos-13-en-2-yl)methyl octadec-9-enedioate and/or 1-(1,3-bis(octadec-9-enoyloxy)propan-2-yl) 18-(6,23-dioxo-1,5-dioxacyclotricos-14-en-3-yl) octadec-9-enedioate and its isomers (R2–5)

Yield: 9.5 %

t_R(min): 11.2

MS, ^{1}H– and ^{13}C-NMR refer to **R1–5**

18-bis (1, 3-bis(octadec-9-enoyloxy)propan-2-yl) '1,1-3-(octadec-9-enoyloxy)propane-1,2-diyl dioctadec-9-enedioate and its isomers (R2–6)

Yield: 2.2 %

t_R(min): 15.9

MS: $C_{135}H_{240}O_{18}$, Cal.2151.34, found 603.5, 1093.5$[M + 2NH_4]^{2+}$, 1283.1, 2167.9 $[M + NH_4]^+$

^{1}H-NMR (in $CDCl_3$, ppm), $\delta = 5.41–5.36$ (14H, m, –CH=CH–), 5.28 (3H, t, $–CH_2CH(O)CH_2–$), 4.33–4.29 (6H, dd, $–CH_2CH(O)CH_2–$), 4.19–4.14 (6H, dd, $–CH_2CH(O)CH_2–$), 2.35–2.31 (18H, t, $–C(=O)CH_2CH_2–$), 1.99–1.96 (28H, m, $=CHCH_2CH_2–$), 1.63 (18H, br, $–C(=O)CH_2CH_2–$), 1.31–1.29 (132H, m, $–CH_2–$), 0.92–0.88 (15H, t, $–CH_3$)

^{13}C-NMR (in $CDCl_3$, ppm), $\delta = 173.40$, 172.99, 130.63, 130.30, 69.11, 26.33, 34.43, 34.26, 32.82, 32.13, 31.15, 29.89-29.20, 25.10, 22.91, 14.34

The structure of fractions from the neat reaction (R3)

Triolein and its isomers (R3–2)

Yield: 21.1 %

t_R(min): 12.6

MS, ^{1}H– and ^{13}C-NMR refer to **R1–2**

Bis (1,3-bis(octadec-9-enoyloxy)propan-2-yl) octadec-9-enedioate and its isomers (R3–3)

Yield: 12.4 %

t_R(min): 14.2

MS, ^{1}H– and ^{13}C-NMR refer to **R1–4**

18-bis (1, 3-bis (octadec-9-enoyloxy)propan-2-yl) '1,1-3-(octadec-9-enoyloxy)propane-1,2-diyl dioctadec-9-enedioate and its isomers (R3–4)

Yield: 16.3 %

t_R(min): 15.9

MS, ^{1}H– and ^{13}C-NMR refer to **R2–6**

1-(21,43-bis((octadec-9-enoyloxy)methyl)-18,23,40,45,62,68-hexaoxo-19,22,41,44,63,67-hexaoxapentaoctaconta-9,31,53,76-tetraen-65-yl) 18-(1,3-bis(octadec-9-enoyloxy)propan-2-yl) octadec-9-enedioate and its isomers (R3–5)

Yield: 11.2 %

t_R(min): 19.4

MS: $C_{174}H_{308}O_{24}$, Cal. 2784.29 found 603.5, 1409.2 $[M + 2NH_4]^{2+}$, 2801.4 $[M + NH_4]^+$

^{1}H-NMR (in $CDCl_3$, ppm), $\delta = 5.37–5.24$ (22H, m, –CH=CH– and $–CH_2CH(O)CH_2–$), 4.28–4.26 (8H, dd, $–CH_2CH(O)CH_2–$), 4.13–4.07 (8H, dd, $–CH_2CH(O)CH_2–$), 2.29–2.26 (24H, t, $–C(=O)CH_2CH_2–$), 2.02–1.94 (36H, m, $=CHCH_2CH_2–$), 1.85–1.55 (24H, m, $–C(=O)CH_2CH_2–$), 1.27–1.24 (168H, m, $–CH_2–$), 0. 84–0.82 (18H, t, $–CH_3$)

^{13}C-NMR (in $CDCl_3$, ppm), $\delta = 173.48$, 173.07, 130.72, 130.40, 129.94, 69.11, 62.33, 34.43, 34.27, 32.85, 32.80, 32.13, 29.94-29.21, 25.10, 22.92, 14.35

1-(21,42-bis((octadec-9-enoyloxy)methyl)-18,23,40,45,62,68-hexaoxo-19,22,41,44,63,67-hexaoxapentaoctaconta-9,31,53,76-tetraen-65-yl)18-(2-(18-(1,3-bis(octadec-9-enoyloxy)propan-2-yloxy)-18-oxooctadec-9-enoyloxy)-3-(octadec-9-enoyloxy)propyl) octadec-9-enedioate and its isomers (R3–6)

Yield: 8.9 %

t_R(min): 24.4

MS: $C_{213}H_{376}O_{30}$, Cal. 3417.25, found 1725.5$[M + 2NH_4]^{2+}$, 3433.9 $[M + NH_4]^+$

^{1}H-NMR: (in CD_3Cl, ppm): $\delta = 5.36–5.30$ (22H, m, –CH=CH–), 5.25–5.23 (5H, m, $–CH_2CH(O)CH_2–$), 4.29–4.24 (10H,dd, $–CH_2CH(O)CH_2–$), 4.14–4.10 (10H, dd, $–CH_2CH(O)CH_2–$), 2.30–2.26 (30H, t, $–C(=O)CH_2CH_2–$), 1.99–1.93 (44H, m, $=CHCH_2CH_2–$), 1.59 (30H, br, $–C(=O)CH_2CH_2–$), 1.28–1.24 (204H, m, $–CH_2–$), 0.88–0.84 (21H, t, $–CH_3$);

^{13}C-NMR(in CD_3Cl, ppm): $\delta = 173.47$, 173.06, 130.52, 130.23, 129.93, 69.09, 62.31, 34.41, 34.25, 32.81, 32.13, 29.99-29.28, 27.44, 27.39, 25.11, 25.06, 22.90, 14.33;

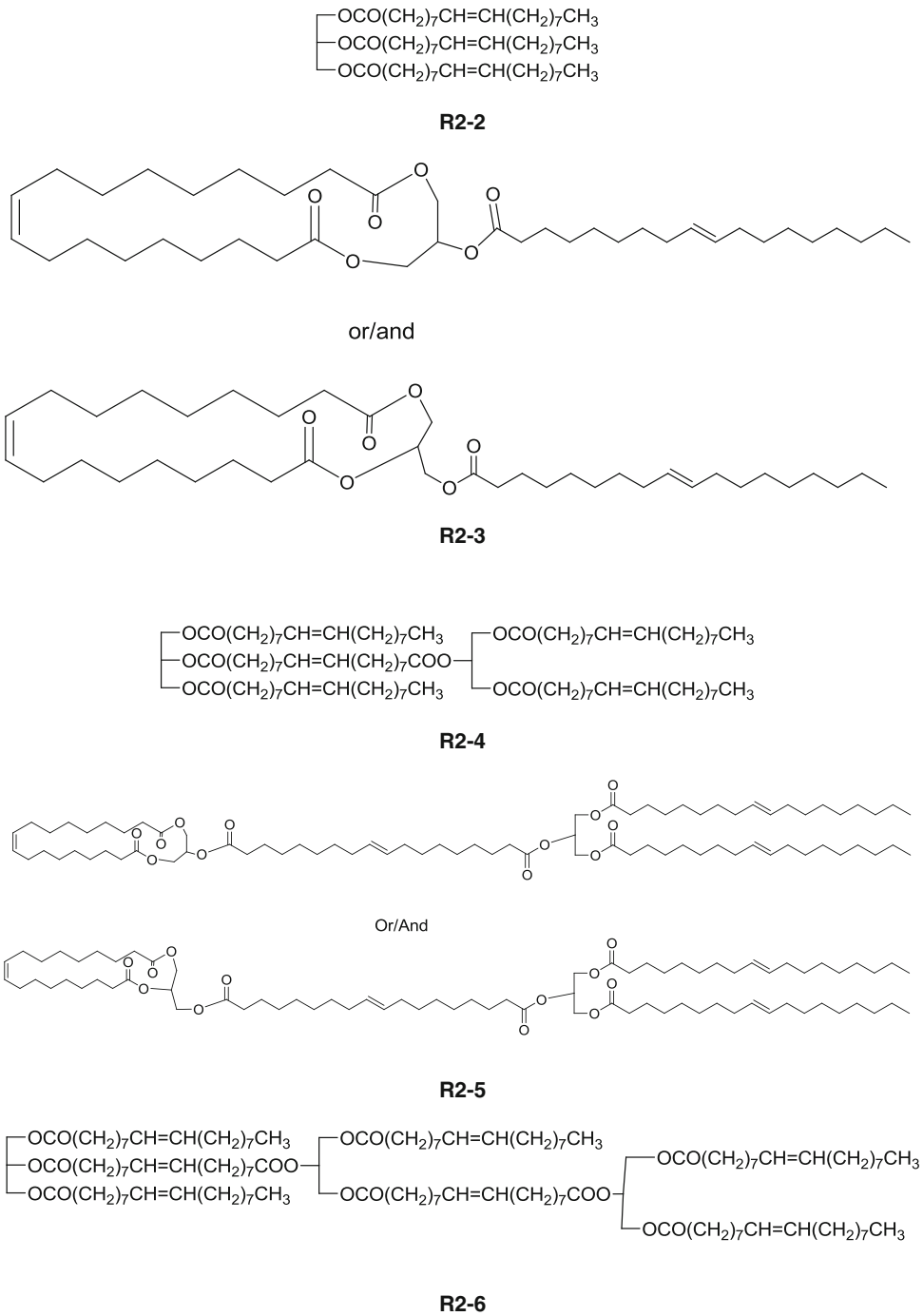
Results and Discussion

Metathesis of Triolein

In the metathesis reaction of triolein, there are two reaction types: intramolecular and intermolecular. Intramolecular reactions produce macrocyclic structures, whilst intermolecular reactions produce aliphatic oligomers. In theory, intermolecular or intramolecular reactions can be favoured by varying reaction conditions, such as the catalyst system, reaction temperature and reaction concentration. This work focused only on variations in the reaction concentration.

The structures of the achieved compounds from the metathesis reaction with different reaction concentrations [10 mmol/L (R1), 20 mmol/L and a neat reaction (R3)] are presented in Schemes 2, 3 and 4, respectively. Note that the olefin, octadec-9-ene, which was also produced in the three reactions carried out for this study, has been presented [11] and is therefore, not presented or discussed further here. Four different cyclic compounds (R1–3, R1–4, R1–6 and R1–7 in Scheme 2) and two linear compounds (R1–2 and R1–5 in Scheme 2) were produced by **R1**. Two cyclic compounds (R2–3 and R2–5 in Scheme 3) and three linear oligomer (R2–2, R2–4 and R2–6 in Scheme 3) were found in the products of **R2** (Scheme 3). Only linear compounds (see Scheme 4) were found in the neat reaction **R3**. The weight percentages of pure fractions obtained from column chromatography with elute of mixture of hexanes and ethyl acetate are presented in Sect. 2.3. The total weight percentages of cyclic compounds in R1, R2 and R3 are 56, 41 and 0 %, respectively, comparing to the percentages of linear compounds (excluding the olefin) with 11, 28 and 70 %, respectively. Clearly, lower reaction concentration of triolein favours intramolecular reactions of substrate and leads to a greater production of cyclic compounds. As the reaction concentration is increased, the tendency for intermolecular reactions increases leading to increased production of linear compounds

Scheme 3 Structures of compounds from the reaction with 20 mmol/L (**R2**). The compounds present contain *cis*-, *trans*-configurations, and positional isomers on glycerol skeletons



Characterization

Structures shown in Schemes 2–4 were fully confirmed by NMR and mass spectra.

NMR

^{1}H-NMR In all ^{1}H-NMR spectra, –C**H**=C**H**– is present at δ 5.38–5.27 ppm, –C(=O)C**H**$_2$CH$_2$– at δ 2.35–2.31 ppm, =CHC**H**2– at δ 2.04-1-94 ppm, –C(=O)CH$_2$C**H**$_2$– at δ 1.65 ppm, –CH$_2$– at δ 1.32–1.29 ppm, –CH$_3$ at δ 0.88 ppm. The ratios of protons corresponding to –OC$\underline{H}_2$CHC$\underline{H}_2$O– and -C$\underline{H}_3$, as well as the proton of O=CC$\underline{H}_2$–, were used to identify the structures of those compounds and are listed in Table 1. The experimental values were fully consistent with the theoretical ones.

The differences detected in ^{1}H-NMR between linear oligomers and cyclic compounds are mainly on the protons of the glycerol skeleton and double bonds, i.e., –CH$_2$C**H**CH$_2$–, –C**H**$_2$CHC**H**$_2$– and –C**H**=C**H**–.

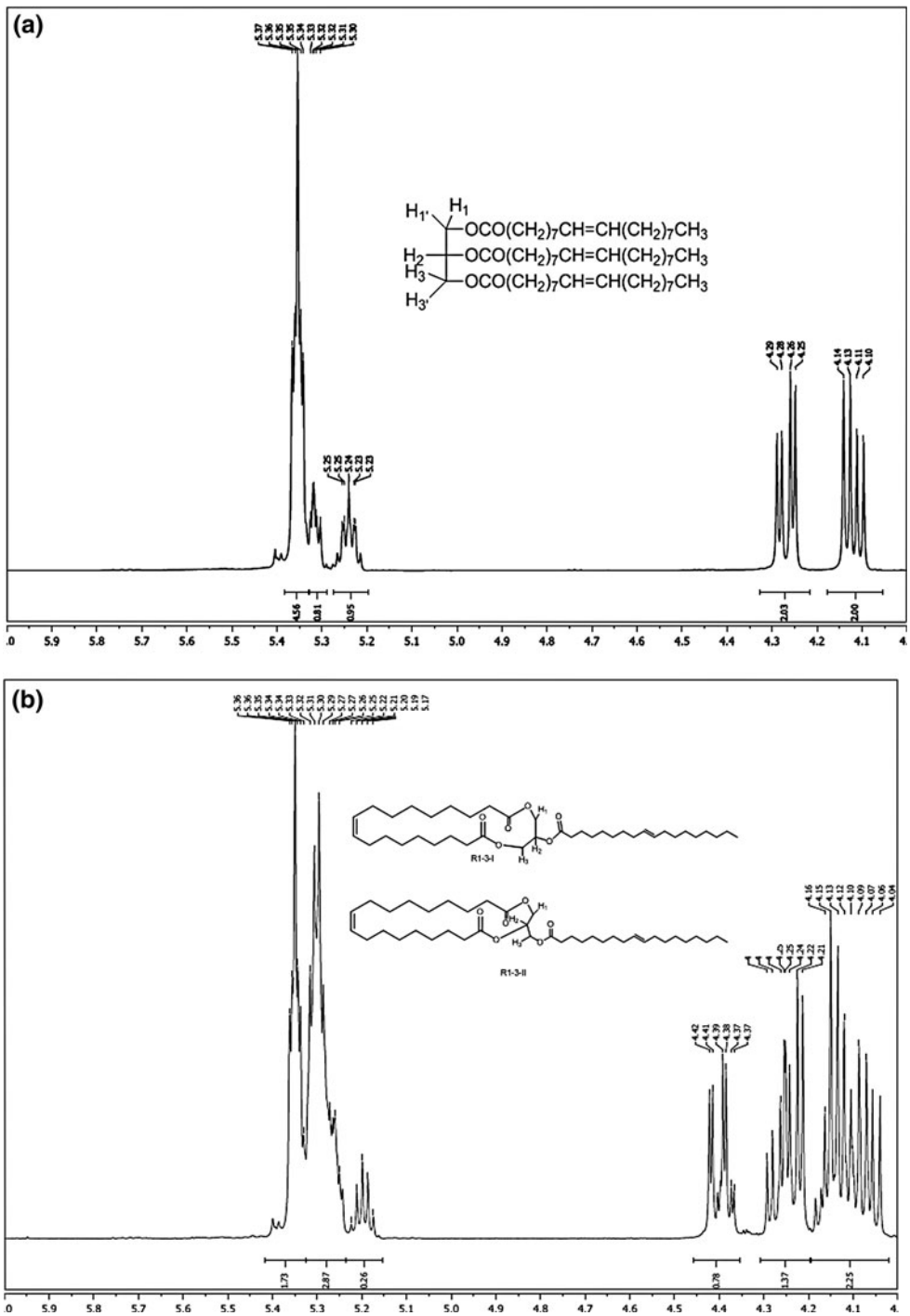


Fig. 1 ^{1}H-NMR spectra **a R1–2** and **b R1–3**

Typical ^{1}H-NMR spectra of linear and cyclic compounds are shown in Fig. 1a, b, respectively. As can be seen, the chemical shift of –$CH_2C\mathbf{H}CH_2$– in the linear compounds is presented at 5.25–5.23 ppm and that of –$C\mathbf{H}_2CHC\mathbf{H}_2$– at 4.29–4.25 ppm and 4.14–4.10 ppm. The cyclic compounds are mixtures of two positional isomers, one with *sn*-1 and *sn*-3 (**I**) ring structure, a so-called symmetric compound, and the other with a *sn*-1 and *sn*-2 (**II**) ring, a so-called asymmetric compound, (i.e., R1–3-I and R1–3-II Fig. 1b, respectively). The chemical environment of the cyclic compounds is more complex than that of linear oligomers, as illustrated by the obvious differences in their ^{1}H-NMR spectra (see Fig. 1a, b). The chemical shifts at 5.25–5.20 ppm, which originated from the *β*-proton located at the *sn*-2 position (**H_2** in Fig. 1b), were observed in the symmetrical cyclic compounds as well as the linear compounds.

The chemical shifts due to –$C\mathbf{H}_2CHC\mathbf{H}_2$– at positions *sn*-1 and *sn*-3 (**$H_{1(3)}$**) in Fig. 1b) in the symmetrical cyclic

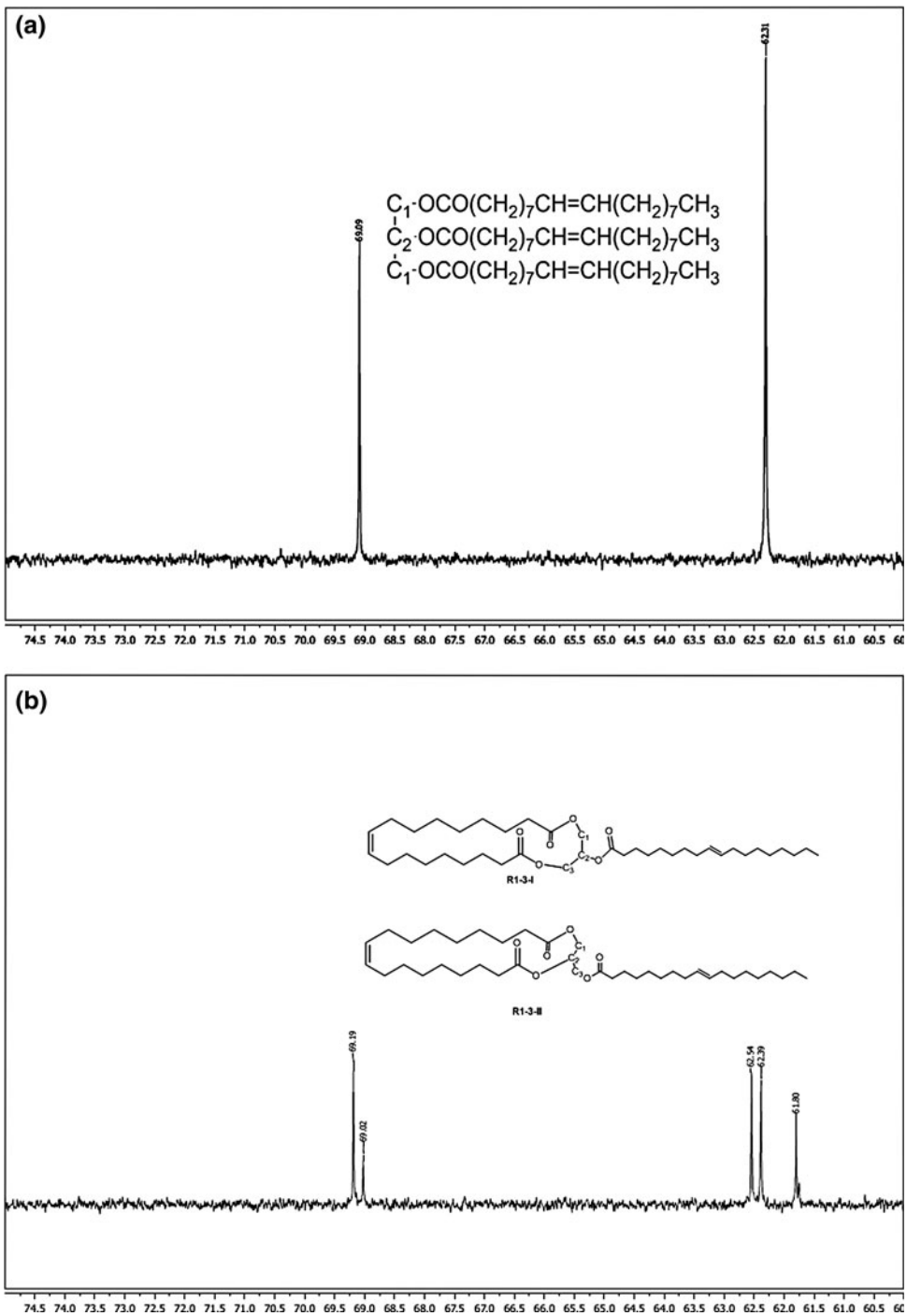


Fig. 2 ^{13}C-NMR spectra of **a R1–2** and **b R1–3**

compounds are also present at the same range as in the linear compounds. The chemical shifts are not affected by the ring structure in the symmetrical cyclic compounds. On the other hand, the chemical shifts of the β-proton at the *sn*-2 position in the asymmetric cyclic compounds ($\mathbf{H_2}$ in Fig. 1b), move to the δ 5.37–5.30 ppm range and is partially overlapped by the peaks of the double bond. The relative amount of asymmetric: symmetric β-protons in the cyclic compound are found to be close to 3:1. The chemical shifts in the asymmetric compounds due to the proton at the position *sn*-3 which is out of the ring ($\mathbf{H_3}$, Fig. 1b) are presented at 4.42–4.37 ppm and 4.16–4.04 ppm. Note that they are different from those observed in their linear counterparts. The proton in the ring at the position *sn*-1 presented a chemical shift at 4.28–4.10 ppm. The ratio of the amount of protons at *sn*-3 in the asymmetric cyclic compound to the total amount of protons at all the other *sn*-1 and *sn*-3 positions as determined by their respective

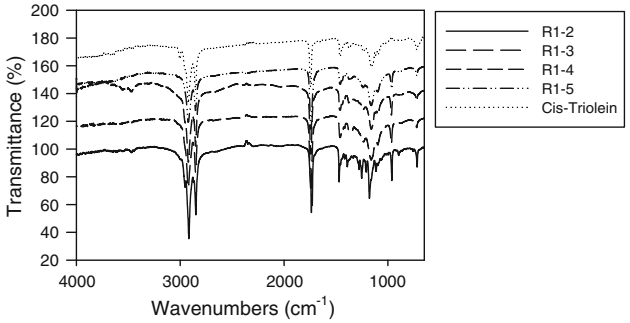


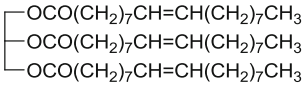
Fig. 3 FTIR of metathesized triolein products. The structures of samples are shown in Scheme 3

integrals was found to be 0.57, a value practically equal to the theoretical value of 0.60 which is consistent with a ratio of asymmetrical:symmetrical values of 3–1.

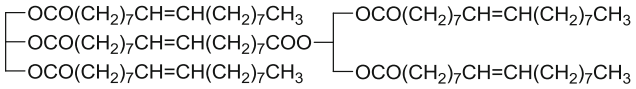
The double bonds presented chemical shifts in the range of 5.38–5.27 ppm. Two groups of chemical shifts are observed for the linear compounds at 5.38–5.34 ppm and 5.33–5.30 ppm (Fig. 1a). Based on the ^{1}H-NMR of triolein (100 % *cis*) and trielaidin (100 % *trans*) reference materials, the above chemical shifts are assigned to the double bonds in the *trans*-geometry and *cis*-geometry, respectively. The relative amount of double bonds in the *trans*- to *cis*-configuration as estimated by the integrals of their respective chemical shifts is about 4–6. These values have been confirmed by GC (see Sect. 3.3 below).

The protons of double bond in the cyclic compounds presented three overlapping groups of chemical shifts spanning from 5.38 to 5.25 ppm. The chemical shifts at 5.38–5.33 ppm of the first group are similar to those observed for double bonds of the linear compounds in the *trans*-configuration. They are therefore assigned to the double bonds in the *trans*-configuration which are located in segments out of the ring (proton I). The second group with chemical shifts at 5.32–5.29 ppm includes the range of chemical shifts which were observed for the *cis*-configuration in the linear compounds. Therefore, it probably represents the chemical shifts from the out of ring double bonds in the *cis*-configuration. The very high intensity of the signal in this range, suggests that the group also includes the signal from the protons at the ring double bonds in the *trans*-configuration (proton II). This assignment is likely because such ringed structures are reported to contain mainly *trans*-configuration double bonds [14]. The higher values of chemical shifts observed for the

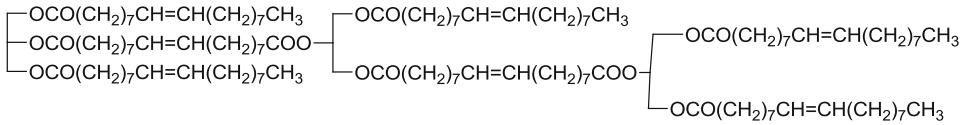
Scheme 4 Structures of compounds from neat reaction (**R3**). The compounds present contain *cis*-, *trans*-configurations, and positional isomers on glycerol skeletons



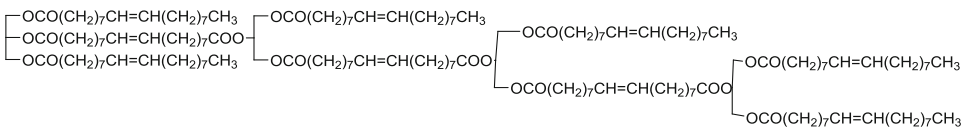
R3-2



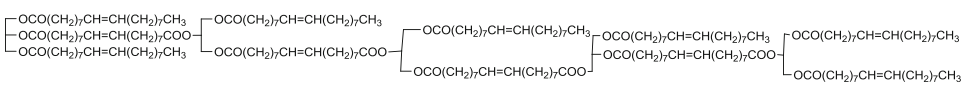
R3-3



R3-4



R3-5



R3-6

proton II compared to proton I is probably due to ring-strain in the cyclic compounds. The third group of shifts at 5.27–5.25 is probably due to proton II with the *cis*-configuration and β-proton in the asymmetric compounds (**H_2** in Fig. 1b).

^{13}C-NMR The main structural differences between the different compounds manifested in the ^{13}C-NMR chemical shifts of double bonds and the glycerol skeleton similarly to ^{1}H-NMR. –**C**H=**C**H– is present at δ 130.7–129.9 ppm. The chemical shift at $\delta < 130.0$ ppm is assigned to a *cis*-geometry, and at $\delta > 130.0$ ppm to a *trans*-geometry [11]. The carbon of glycerol skeleton (–CH_2CH(O)CH_2–) is present at the range of 69.2–61.6 ppm depending on their structures. There are two chemical shifts present at $\delta \sim 69.09$ ppm (–CH_2**C**H(O)CH_2–) and ~62.31 ppm (–**C**H_2CH(O)**C**H_2–) in ^{13}C-NMR spectra of linear oligomers, as shown in Fig. 2a. Three more peaks, $\delta = 69.19$, 62.54 and 61.80 ppm, are present in^{13}C-NMR spectra of cyclic compounds (Fig. 2b) are originated from C2 in **R1–3-II**, C1in R1–3-II at $\delta \sim 62.54$ ppm, and C3 in R1–3-II respectively.

FTIR

The ester carbonyl stretching band (C=O) in the glyceride skeleton is present at 1744 cm^{-1} and O–C–C and C–C(=O)–O stretching bands are present at 1165 and 1110 cm^{-1}, respectively. The stretching bands of CH groups are present in the region of 3000–2800 cm^{-1}. The

Table 1 Ratio of selected protons in ^{1}H-NMR spectra of fractions

Compounds		–OC$\underline{H_2}$CHC$\underline{H_2}$O–	O=CC$\underline{H_2}$–	–C$\underline{H_3}$
R1–2	Theoretical	1	1.5	2.25
	Experimental	1	1.52	2.19
R1–3	Theoretical	1	1.5	0.75
	Experimental	1	1.47	0.67
R1–4	Theoretical	1	1.5	1.5
	Experimental	1	1.46	1.42
R1–5	Theoretical	1	1.5	0.75
	Experimental	1	1.55	0.88
R1–6	Theoretical	1	1.5	0
	Experimental	1	1.46	0.08
R1–7	Theoretical	1	1.5	0.25
	Experimental	1	1.48	0.33
R3–3	Theoretical	1	1.5	1.25
	Experimental	1	1.49	1.23
R3–4	Theoretical	1	1.5	1.125
	Experimental	1	1.50	1.129

The detailed ^{1}H-NMR analyses are presented in the experimental section. The structures of samples are shown in Schemes 2 and 4

Table 2 Results of ESI–MS. FW represents the formula weight; ESI–MS were recorded using the method presented in the experimental section

Compounds	R1–2	R1–3	R1–4	R1–5	R1–6	R1–7	R2–6	R3–5	R3–6
FW	885	632.95	1518.39	1265.91	1013.43	1646.38	2151.34	2784.29	3417.25
ESI–MS (m/z)									
Product ions	902.8 $[M + NH_4]^+$	633.5 $[M + H]^+$	1535.4 $[M + NH_4]^+$	1283.1 $[M + NH_4]^+$	1030.8 $[M + NH_4]^+$	1663.3 $[M + NH_4]^+$	2167.9 $[M + NH_4]^+$	1409.2 $[M + 2NH_4]^{2+}$	1725.5 $[M + 2NH_4]^{2+}$, 3433.9 $[M + NH_4]^+$
		650.5 $[M + NH_4]^+$					1093.5 $[M + 2NH_4]^{2+}$	2801.4 $[M + NH_4]^+$	
Fragment ions	603.5	351.2	338.3, 351.3, 597.4, 613.4	351.2, 637.3, 983.8	351.2, 365.2	351.2, 645.5, 830.6	338.3, 603.5	338.3, 505.4, 603.5	338.3,

infrared absorption at 967 cm^{-1} in FTIR spectra (see Fig. 3) is assigned to *trans*-geometry [15]

MS

The mass spectra of the triglyceride compounds were recorded by electrospray ionization mass spectrometrometry (ESI–MS). The presented compounds are readily ionized by ammonium. For both cyclic compounds (i.e., R1–3, R1–5 and R1–6) and the linear oligomers (i.e., R1–2 and R1–4) with the molecular weight lower than 1600, and the strong ion signals were observed readily from singly-ammoniated molecules; whilst, the oligomers with higher molecular weight in the range ~1600–4000 Da (i.e., R1–7 and R2–6, R3–5 and R3–6) were observed as both singly- and multiply-ammoniated molecules. As seen in Table 2, the MS data of presented compounds match their corresponding formula weights very well.

trans-/*cis*-Configuration

Similar to other carbon–carbon coupling reactions, such as Wittig olefination reaction [16], the metathesis of olefin with a transition metal catalyst gives a mixture of the two possible products (*trans*-isomers and *cis*-isomers). The *trans*-isomer (in both cyclic and linear compounds) is the dominant one [11, 17] in these reactions. The *cis*-/*trans*-configuration produced from olefin metathesis reaction has been studied widely. *trans*-/*cis*-selectivity can be influenced by reaction temperature, solvent or substitutions of the substrate, but mainly by the catalyst system [17, 18] and substrates [14, 19]. Lee and Grubbs [14] found that the ratio of *trans*/*cis* was about 3.6–4.8:1 depending on the substrate when Grubbs' second generation catalyst was used. Compounds here contain the *cis*-/*trans*-configuration. This was confirmed by FTIR, ^{1}H- and ^{13}C-NMR spectra, as mentioned above.

To investigating the effect of reaction concentration on the ratio of *trans*-/cis-configuration, the composition of **R1–2**, **R2–2** and **R3–2**, which are representative of linear compounds produced in R1, R2 and R3 respectively, were determined by GC. The samples were first methylated using the method described by Cantellops et al. [20]. The resultant methyl fatty acids were measured by GC, with the pure methyl oleate and methyl elaidate as standards. The ratio of *trans*/*cis* in **R1–2**, **R2–2** and **R3–2** are 4.6:1, 4.8:1 and 5.8: 1, respectively. This suggests that higher concentration produces higher *trans*-content.

Conclusion

In the metathesis reaction of triolein, there are two readily apparent trends related to intramolecular and intermolecular reactions. The former produces macrocyclic structures; the latter aliphatic oligomers. Products of the metathesis have been shown to be affected by reaction concentration, with increased cyclic compounds produced at lower concentrations, and increased aliphatic oligomers at higher concentrations. Only aliphatic oligomers were found in the neat reaction. The cyclic compounds produced contain positional isomers in the glycerol skeletons: those with a cyclic structure between positions 1 and 3 in glycerol skeleton (**I**), and those between positions 1 and 2 (**II**). Based on the ^{1}H-NMR spectra, the ratio between **I** and **II** is about 1:3, which demonstrates that intramolecular reactions are three times as likely to occur between the neighbouring chains than between the *sn*-1 and *sn*-3 positions. Reaction concentration also has an impact on the *trans*/*cis* ratios of double bonds in the products. Our results show that higher concentration reactions result in an increase in *trans*-configurations.

Acknowledgments We express our thanks to the Ontario Soybean Growers/Grain Farmers of Ontario, Elevance Renewable Sciences, NSERC, GPA-EDC, Industry Canada, and Trent University for their financial support for the research. We also give our thanks to our group members, Jiaqing Zuo, Ali Mahdevari, Carolyn Virginia Payne, and Latifeh Ahmadi for their kind assistance.

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