J Amer Oil Chem Soc (2007) 84:343–348
DOI 10.1007/s11746-007-1049-1

ORIGINAL PAPER

Acyl Migration Kinetics of 2-Monoacylglycerols from Soybean Oil via ^{1}H NMR

David L. Compton · Karl E. Vermillion · Joseph A. Laszlo

Received: 21 November 2006 / Accepted: 13 February 2007 / Published online: 17 March 2007

Abstract The acyl migration kinetics of neat 2-monoacylglycerol (2-MAG) to form 1-MAG was determined using ^{1}H NMR spectroscopy to monitor the β-proton integration ratios of the two species over time. 2-MAG was synthesized by the Novozym 435-catalyzed alcoholysis of soybean oil and isolated by solvent extraction or molecular distillation at a mole fraction ($X_{2\text{-MAG}}$) of 0.94 relative to total MAG. The kinetics parameters of the neat 2-MAG acyl migration were investigated over the temperature range of 23–80 °C. The 2-MAG mol fraction remained unchanged at 23 °C over the course of 168 h and reached an equilibrium of $X_{2\text{-MAG}} = 0.09$ at only 80 °C. Modeling of the kinetics data revealed a 2-MAG half life ($t_{1/2}$) of 3,500 and 22.8 h at 23 and 80 °C, respectively, with an activation energy of 79.0 ± 6.5 kJ mol^{-1}. The use of ^{1}H NMR spectroscopy proved an expedient method for monitoring the acyl migration in 2-MAG compared to other reported methods (e.g. GC, HPLC, and ^{13}C-NMR spectroscopy), requiring no sample manipulation and minimizing the deleterious effects of high temperatures and solvent exposure.

Product names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may be suitable.

D. L. Compton (✉) · K. E. Vermillion · J. A. Laszlo
New Crops and Processing Technology Research Unit,
USDA, Agricultural Research Service,
National Center for Agricultural Utilization Research,
1815 N. University St., Peoria, IL 61604, USA
e-mail: comptond@ncaur.usda.gov

Keywords Acyl migration · Ethanolysis · Kinetics · 2-Monoacylglycerol · ^{1}H NMR

Introduction

Monoacylglycerols (MAG) are used in food, pharmaceuticals, and cosmetics as emulsifying agents [1, 2]. Additionally, 2-monoacylglycerols (2-MAG) are convenient reagents for the synthesis of structured lipids [3] although acyl migration often hampers the formation of specific positional isomers [4, 5]. 2-MAG acyl migration is also a cornerstone of understanding the chemistry of lipid metabolism [6].

The synthesis of 2-MAG is complicated by the spontaneous acyl migration of the fatty acid (FA) moiety from the *sn*-2 position to the *sn*-1(3) of the glycerol backbone to form 1-MAG. Irimescu et al. [7] demonstrates that 2-MAG can be synthesized and isolated in high 2-MAG:1-MAG ratios (>9:1) through the regiospecific ethanolysis of TAG catalyzed by Novozym 435 (immobilized *Candida antartica* lipase B) using large excesses of ethanol. The rate of acyl migration of 2-MAG to1-MAG, and thus the purity of 2-MAG, is affected by temperature, solvent, and acid and base impurities, which catalyze the migration [4]. Previous studies indicate that the acyl migration of MAG reaches an equilibrium resulting in a 2-MAG:1-MAG ratio of ~1:9 [8, 9].

To date, few studies have addressed the direct measurement of spontaneous 2-MAG acyl migration. Previous studies have detailed the use of GC or HPLC to resolve the concentrations 2-MAG and1-MAG in samples as monitored over time in solution. The acyl migration of long chain 2-MAG has been described in chylomicra and hexane solutions [6, 9]. The GC analysis requires prolonged

exposure of the MAG to solvents and high temperatures, which accelerates acyl migration. Thus, the MAG are chemically modified to prevent further acyl migration during analysis. Similarly, the use of HPLC analysis requires prolonged exposure of the MAG to solvents and elevated temperatures [7]. A mild, rapid, and convenient method to determine 2-MAG:1-MAG ratios was needed that reduced the solvent and temperature effects on the acyl migration of MAG during analysis.

We recently described a solventless, biocatalytic process that esterifies the *sn*-1(3) positions of 2-MAG (from soybean oil, SBO) with feruloyl moieties [10]. These feruloylated lipids have garnered much interest as cosmeceutical ingredients. The purity of 2-MAG in relation to 1-MAG is a concern for production of regiospecific feruloylated lipids. The previously described GC and HPLC methods proved too inconvenient and led us to search for a more rapid and mild analytical technique for determining 2-MAG:1-MAG ratios. Herein, we report the acyl migration kinetics of neat 2-MAG (not in solvent) using ^{1}H NMR spectroscopy to determine 2-MAG:1-MAG ratios via the integration of the β protons on the glycerol backbones of the respective species.

Materials and Methods

Materials

Wesson brand SBO was purchased from a local grocery. Novozym 435, *Candida antartica* lipase B immobilized on a macroporous acrylic resin, was purchased from Brenntag Great Lakes (Chicago, IL, USA). Fatty acid methyl esters (FAME) standards were obtained from Alltech Associates (Deerfield, IL, USA). Solvents were reagent grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

2-MAG Synthesis

The 2-MAG synthesis was adapted from that of Irimescu et al. [7] Ethanol (200 g, 4.34 mol) and SBO (50 g, 5.65×10^{-2} mol) were combined at room temperature with stirring until emulsified. Novozym 435 (25 g) was added and the emulsion was stirred for 4 h at room temperature. The reaction mixture was filtered to remove the Novozym 435 and the excess ethanol was removed under vacuum at 25 °C to yield an oil concentrate. Fatty acid ethyl esters were removed by dissolving the oil concentrate in 200 mL of 95:5 acetonitrile/water (v:v). The solution was extracted with three 200 mL portions of hexane. The hexane washes were discarded and the acetonitrile was then removed under vacuum at 25 °C. The resultant concentrate was then dissolved in 200 mL of chloroform. The chloroform solution was washed with 200 mL of 9:1 water/ethanol (v:v). The aqueous/ethanol layer was washed with two 200 mL portions of chloroform. The three chloroform phases were combined, and the chloroform was removed under vacuum at 25 °C to yield 21.1 g of a light yellow oil that was determined by ^{1}H NMR spectroscopy (see below) to be a >9:1 ratio of 2-MAG:1-MAG. The 2-MAG was stored neat at –25 °C. Alternatively, the fatty acid ethyl esters were removed from the oil concentrate by molecular distillation (120 °C, 16 mTorr) to yield 18.4 g of the identical product. The 2-MAG/1-MAG mixture immediately after purification, 2-MAG ($CDCl_3$, 500 MHz): δ 5.36 (4.13 H, m), 4.95 (1.00 H, m, H-b), 3.85 ppm (4.11 H, pseudo dd, H-a), 2.79 (1.96 H, m), 2.39 (2.30 H, m), 2.17 (2.39 H, broad s), 2.06 (4.44 H, m), 1.66 (2.34 H, m), 1.35 (17.32 H, m), 1.00 (0.32 H, t), and 0.92 ppm (3.14 H, m). 1-MAG glycerol protons ($CDCl_3$, 500 MHz): δ 4.21 (0.18 H, dd, H-a′), 3.95 (0.10 H, m, H-b′), and 3.66 ppm (0.21 H, dd, H-c′). Proton frequency assignments are detailed below in the Results and Discussion section and in Figs. 1 and 2.

NMR

^{1}H- and ^{13}C- NMR spectra were obtained on a Bruker Avance 500 spectrometer (500 MHz ^{1}H/125.77 MHz ^{13}C) using a 5 mm BBI probe. All samples were dissolved in $CDCl_3$, and all spectra were acquired at 27 °C. Chemical shifts are reported as ppm from tetramethylsilane calculated from the lock signal (Ξ_D = 15.350609%).

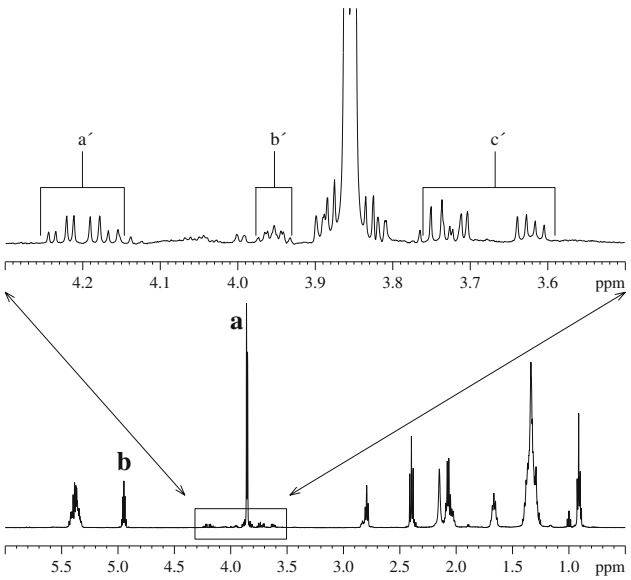


Fig. 1 ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of 2-MAG immediately after purification. The upper spectrum is an expansion of the area in the *box*. *Letters* denote glycerol proton assignments as illustrated in Scheme 1

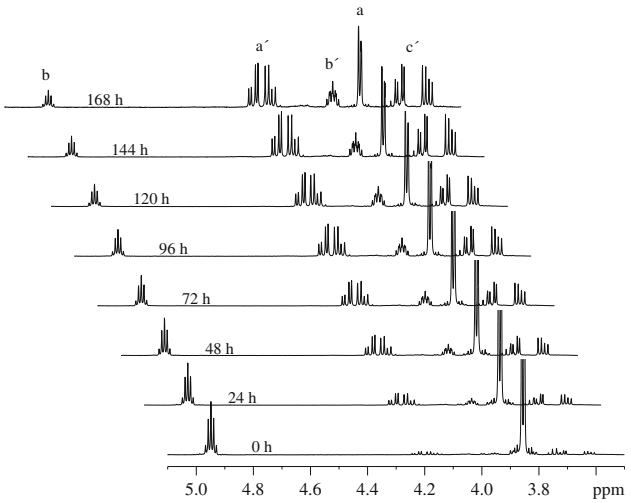


Fig. 2 ^{1}H NMR spectra ($CDCl_3$, 500 MHz) of the glycerol protons illustrating the acyl migration in 2-MAG to form 1-MAG at 60 °C over 168 h. Spectra were acquired within 2 min of the 2-MAG aliquots (~20 μL) being dissolved in $CDCl_3$ (~0.75 mL). *Letters* denote glycerol proton assignments as illustrated in Scheme 1

2-MAG (1.0 mL) was sealed in HPLC vials and heated in a sand bath inside a Cole-Parmer Laboratory Oven (Chicago, IL, USA) at 23, 40, 60, and 80 °C. Aliquots (~20 μL) were taken periodically and dispensed into NMR tubes. The tubes were capped and stored at –25 °C. The aliquots were dissolved in ~0.75 mL of $CDCl_3$ at room temperature, and the ^{1}H NMR spectra were obtained within 2 min of dilution. Experiments were performed in triplicate at each temperature.

GC

Analyses were performed using a Hewlett–Packard 5890 Series II gas chromatograph (Palo Alto, CA, USA), equipped with a flame ionization detector and autosampler/injector. Analyses were conducted on a SP 2380 30 m × 0.32 mm i.d. (Supelco, Bellefonte, PA, USA) column. SP 2380 analysis was adapted from that of Isbell et al. [11] with a 1.4 mL/min column flow with a helium head pressure of 138 kPa; split ratio 10:1; programmed ramp 120–135 °C at 10 °C/min, 135–175 °C at 3 °C/min, and 175–265 °C at 10 °C/min; injector and detector temperatures set at 250 °C. Saturated and unsaturated C_8 to C_{30} FAME standards were used for assignment of fatty acid methyl ester obtained from SBO and 2-MAG.

Methyl esters of SBO and 2-MAG were prepared for GC analysis by treating 10 mg samples with 0.5 mL of 0.5 M KOH/methanol in a sealed vial for 1 h at 100 °C in a heating block. After cooling to ambient temperature, 1.5 mL of 1 M H_2SO_4/methanol was added, the vial re-sealed, and the vial heated to 100 °C for 15 min. The mixture was transferred to a 2 dram vial and 1 mL of water added. The solution was extracted with 1 mL of hexane. The hexane extract was dried over sodium sulfate and injected into the GC for FAME analysis.

FFA Analysis

Percent FFA in the 2-MAG was determined using a Metrohm Ltd. (Herisau, Switzerland) 751 GPD Titrino, following the AOCS Method Te 2a-64 [12] with ethanol substituted for methanol. Acid values were run in duplicate and the average values reported.

Results and Discussion

2-MAG Synthesis and Characterization

The Novozym 435-catalyzed ethanolysis of SBO was adapted from the methods of Irimescu et al. [7] Although under certain conditions Novozym 435 is known to exhibit low regiospecificity towards the glycerol backbone, Irimescu et al. demonstrates that high alcohol/TAG ratios results in high Novozym 435 1,3-regiospecificity. Therefore, we conducted the 2-MAG synthesis using the optimized ethanol/SBO molar ratio of 78:1. The 2-MAG synthesis and subsequent purification by solvent extraction were conducted at 25 °C or below to suppress acyl migration rates. The resultant 2-MAG was isolated in excess of 90% relative to 1-MAG as determined by ^{1}H NMR (see below). The fatty acid composition of the SBO and the 2-MAG were determined by GC (Table 1) and were in good agreement with values previously reported [13]. The high *sn*-1(3) regiospecificity of the Novozym 435 under the reported reaction conditions was confirmed by the similarity of the FA composition of the 2-MAG (Table 1) and the FA composition at the *sn*-2 position of SBO reported in

Table 1 Fatty acid composition of SBO and 2-MAG (determined by GC analysis of the FAME obtained from SBO and 2-MAG)

Fatty acid	Composition (mol%)[a]	
	SBO	2-MAG
16:0	10.7 ± 0.0	ND
18:0	4.5 ± 0.1	ND
18:1	24.9 ± 0.1	20.3 ± 0.1
18:2	51.5 ± 0.1	69.2 ± 0.2
18:3	7.1 ± 0.1	7.8 ± 0.1
Other	1.4 ± 0.2	3.0 ± 0.3

ND none detected

[a] Values are relative to total fatty acids in each sample and the data are the means of $n = 3$ samples, reported with standard deviations

the literature [13]. The FFA content of the isolated 2-MAG was determined to be 1.23 ± 0.36% using AOCS standard methods [12]. The low FFA content was confirmed by quantitative ^{13}C NMR using the combined integration value of the 2-MAG and 1-MAG C1 carbonyl peaks (174.3–174.1 ppm) relative to the integration of the FFA C1 carbonyl peak (176.0 ppm) and was found to be <2% (data not shown) [14]. Low FFA content was desired since acids can catalyze the acyl migration.

Determining 2-MAG:1-MAG Ratios via ^{1}H NMR Spectroscopy

Figure 1 shows the ^{1}H NMR spectrum of 2-MAG obtained immediately after purification and isolation. The peaks at 5.36 and 2.79 ppm and farther up field were attributed to protons of the 2- and 1-MAG FA moieties and are consistent with the shifts obtained for the FA moieties of SBO [15]. The exception was the broad singlet at 2.17 ppm assigned to the glycerol –OH protons.

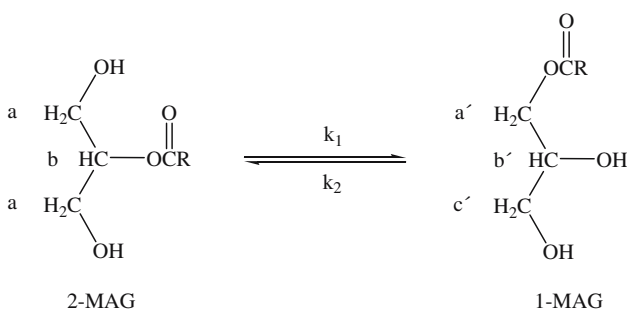
The region from 5.0 to 3.5 ppm (Fig. 1) included the peaks attributed to the glycerol backbone protons. From the chemical structure of 2-MAG (Scheme 1) it was expected that glycerol protons would produce two sets of multiple peaks in a 4:1 ratio. The integration of the peaks at 3.85 and 4.95 ppm was 4.1:1 and were assigned as the 2-MAG glycerol backbone protons, labeled a and b, respectively (Scheme 1). Three sets of peaks in a 2:1:2 ratio were expected for the 1-MAG glycerol backbone protons (Scheme 1). The expanded region of the ^{1}H NMR spectra from 4.3 to 3.5 ppm (Fig. 1) shows the peaks attributed to the 1-MAG glycerol protons, labeled a′, b′ and c′. The integration of the peaks resulted in a 1.8:1:2.1 ratio. The peak shifts and assignments of the 1-MAG glycerol protons correlated well to those found for the glycerol protons of 1(3)-stearoyl-*sn*-glycerol [16].

The b- and b′-protons (Scheme 1) are unique in the fact that each was equimolar to the moles of 2-MAG and 1-MAG, respectively. Therefore, the relative ratio of the integration values of the b- and b′-protons could be used to determine the relative molar ratio of 2-MAG to 1-MAG. Figure 2 illustrates the change of the b:b′ proton ratio at 60 °C over 168 h. The mole fraction of 2-MAG ($X_{2\text{-MAG}}$) for each time point was calculated using Eq. 1 where H_b is the integration value for the b-proton and $H_{b'}$ is the integration value for the b′-proton. The mole percent of 2-MAG was obtained by multiplying $X_{2\text{-MAG}}$ by 100.

$$X_{2-\text{MAG}} = H_b/(H_b + H_{b'}) \quad (1)$$

We were interested in determining the susceptibility of neat 2-MAG to undergo acyl migration to form 1-MAG, and the use of ^{1}H NMR spectroscopy to determine the mol% of neat 2-MAG was a more rapid and convenient method of analysis compared to those previously reported. For example, the acyl migration of 2-MAG to 1-MAG is monitored by analyzing aliquots of 2-MAG/hexane solutions using GC [9]. Because higher temperatures increase the rate of acyl migration [6, 7], the length of time spent in solution at high injector, oven, and detector temperatures (up to 400 °C) requires that the 2-MAG samples be silylated to prevent further chemical rearrangement of the MAG during the analysis. Likewise, the use of HPLC to elucidate 2-MAG:1-MAG ratios requires that the samples be subjected to prolonged solvent exposure (15 min) at temperatures up to 40 °C [7]. GC and HPLC methods also require the use of internal standards to determine the 2- and 1-MAG concentrations.

It is conceivable that the integration of the b- and b′-carbons (Scheme 1) could be used to determine the $X_{2\text{-MAG}}$ [14, 17, 18]; however, the ^{13}C-NMR spectrum tends to be convoluted, inhibiting integration, and the prolonged solvent exposure needed to obtain the number of acquisitions necessary for accurate integrations accelerates acyl migration (discussed below). Alternatively, the use of ^{1}H NMR required that the MAG sample be in solution for <2 min at 27 °C to acquire the spectra, minimizing any acyl migration that may have occurred during the analysis. Analysis of ^{1}H NMR spectra of 2-MAG left in the NMR probe at 27 °C obtained at 1 min intervals over 10 min showed the loss of <2.0 mol% of 2-MAG (data not shown) within the first 4 min of acquisitions. The use of ^{1}H NMR spectroscopy provided rapid results while eliminating many of the inconveniences of GC, HPLC, and ^{13}C-NMR analyses for determining 2-MAG:1-MAG ratios, requiring no chemical modification of the sample before analysis, minimal solvent exposure, and ambient temperatures.



Scheme 1 MAG FA acyl migration

2-MAG Acyl Migration Kinetics

The FA group of 2-MAG spontaneously isomerizes from the *sn*-2 position on the glycerol backbone to the *sn*-1 position and results in an equilibrium containing more than

90% 1-MAG. Although the rate of acyl migration has been examined in solution [4], we were interested in determining the rate of acyl migration of neat 2-MAG. ^{1}H NMR provided a convenient analytical method for determining acyl migration rates while minimizing solvent and temperature effects during analysis. Figure 3 shows the change in 2-MAG mol% over the course of a week at various temperatures. The 2-MAG mol% remained unchanged at –15 °C (data note shown) and 23 °C. The acyl migration of neat 2-MAG increased with increasing temperature but only reached equilibrium within the time course of the experiment at 80 °C. Compared to the lack of acyl migration found in the neat 2-MAG at 23 °C (2-MAG mol% 94), solvent has a profound effect on acyl migration rates as evidenced by the mol% of 2-monoolein and 2-monopalmitin obtained in hexane solutions and model chylomicra emulsions (12 and 35%, respectively) after 30 h at 25 °C [5, 6, 9].

Acyl migration kinetics were modeled employing a reversible first-order reaction scheme in which [2-MAG] and [1-MAG] are the concentration of 2-MAG and 1-MAG, respectively, and k_1 and k_2 are the respective forward and reverse rate constants:

$$[\text{2-MAG}] \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [\text{1-MAG}] \tag{2}$$

The rate law is

$$\mathrm{d}[\text{2-MAG}]/\mathrm{d}t = -k_1[\text{2-MAG}] + k_2[\text{1-MAG}] \tag{3}$$

At any given time, t, with $[\text{2-MAG}]_0$ representing the initial 2-MAG concentration, mass balance provides

$$[\text{1-MAG}] = [\text{2-MAG}]_0 - [\text{2-MAG}] \tag{4}$$

Substituting Eq. 4 into Eq. 3 gives

$$d[\text{2-MAG}]/\mathrm{d}t = -k_1[\text{2-MAG}] + k_2[\text{2-MAG}]_0 - [\text{2-MAG}] \tag{5}$$

The reaction rate goes to zero at equilibrium. By defining $[\text{2-MAG}]_e$ as the concentration of 2-MAG at equilibrium, Eq. 5 becomes

$$0 = -k_1[\text{2-MAG}]_e + k_2([\text{2-MAG}]_0 - [\text{2-MAG}]_e) \tag{6}$$

Incorporating Eq. 6 into Eq. 5 and integrating leads to the solution [19]

$$t = (k_1 + k_2)^{-1} \ln(([\text{2-MAG}]_0 - [\text{2-MAG}]_e])/([\text{2-MAG}] - [\text{2-MAG}]_e)) \tag{7}$$

The equilibrium constant K for the reaction, determined from the measured equilibrium concentrations of [2-MAG] and [1-MAG], may be define as

$$K = [\text{2-MAG}]/[\text{1-MAG}] = k_1/k_2 \tag{8}$$

and, therefore

$$k_1 + k_2 = (K + 1)k_1/K = Ck_1 \tag{9}$$

Substituting Ck_1 for the $k_1 + k_2$ term in Eq. 7 and rearranging provides an explicit expression of [2-MAG] as a function of time

$$[\text{2-MAG}] = (([\text{2-MAG}]_0 - [\text{2-MAG}]_e)/\exp(Ck_1t)) + [\text{2-MAG}]_e \tag{10}$$

The 2-MAG mol% was found to be 9.1 after 168 h at 80 °C. Based on this value the equilibrium constant K was set to 10.11 for all reactions (i.e., assumed to be independent of temperature). This value is somewhat higher than those of various MAGs (K values ranging from 4.6 to 8.5) reported by Boswinkel [9]. The initial 2-MAG mol% was 94.4 (i.e., $[\text{2-MAG}]_0$) for all reactions. For each temperature, k_1 was determined by minimizing the sum of squared residuals (the difference between observed and calculated [2-MAG] using Eq. 9). Table 2 summarizes the k_1 values determined for solventless acyl migration in the temperature range 23–80 °C. The determined k_1 values were used to construct the [2-MAG] decay curves in Fig. 3. Half-life

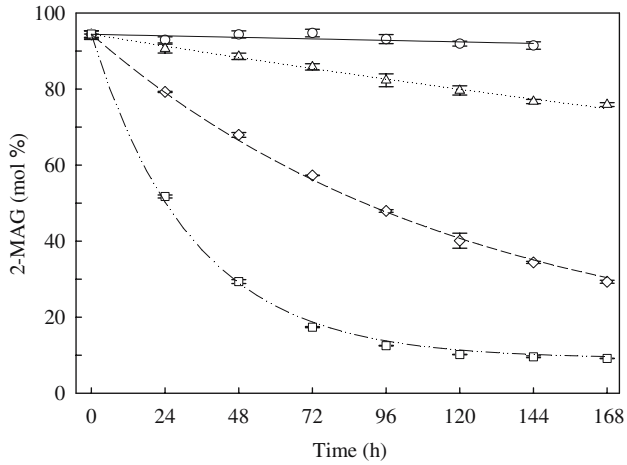


Fig. 3 The influence of temperature on the acyl migration in 2-MAG to form 1-MAG: 23 °C (*open circles*), 40 °C (*open triangles*), 60 °C (*open diamonds*), 80 °C (*open squares*). Data are the mean of $n = 3$ measurements, and *error bars* denote one standard deviation from the mean. The *lines* represent values derived from the kinetics model (Eq. 10) and fitted parameters (Table 2)

Table 2 First-order reaction constants for solventless acyl migration in 2-MAG

Temperature (°C)	k_1 (h^{-1})	$t^a_{1/2}$ (h)
23	0.000180	3500
40	0.00141	447
60	0.00752	83.8
80	0.0276	22.8

[a] $t_{1/2} = (k_1 + k_2)^{-1} \ln(2)$

estimates also are given in Table 2. The rate constant observed here at 23 °C for the chemical rearrangement of 2-MAG to 1-MAG in the absence of solvent was two-orders of magnitude slower than that found for monobutyrin at 30 °C in water-saturated hexane Boswinkel [9], reflecting the importance of solvent catalysis in acyl migration. The rate constants from Table 2 followed an Arrhenius relationship (ln k_1 vs. 1/RT), resulting in a correlation factor of r^2 0.986. The activation energy of the neat 2-MAG acyl migration calculated from the slope of this Arrhenius relationship was 79.0 ± 6.5 kJ mol^{-1}, twofold higher than the activation energy of the acyl migration of 2-monoolein determined in isooctane (40 kJ mol^{-1}) [4].

Acknowledgments The authors thank Kendra Brandon and Leslie Smith for their excellent technical assistance.

References

1. Van Haften JL (1976) Fat-based food emulsifiers. J Am Oil Chem Soc 53:831A–835A
2. Henry C (1995) Monoacylglycerides: the universal emulsifiers. Cereal Foods World 40:734–738
3. Schid U, Bornscheuer UT, Soumanou MM, McNeill GP, Schid RD (1998) Optimization of the reaction conditions in the lipase-catalyzed synthesis of structured triacylglycerides. J Am Oil Chem Soc 75:1527–1531
4. Fureby AM, Virto C, Adlercreutz P, Mattiasson B (1996) Acyl migration in 2-monoolein. Biocatal Biotransform 14:89–111
5. Xu X, Skands ARH, Hoy S-E, Mu H, Balchen S, Adler-Nissen J (1998) Production of specific-structured lipids by enzymatic interesterification: elucidation of acyl migration by response surface design. J Am Oil Chem Soc 75:1179–1185
6. Lyubachevskaya G, Boyle-Roden E (2000) Kinetics of 2-monoacylglycerol acyl migration in model chylomicra. Lipids 35:1353–1358
7. Irimescu R, Iwasaki Y, Hou CT (2002) Study of TAG ethanolysis to 2-MAG by immobilized *Candida antarctica* lipase and synthesis of symmetrically structured TAG. J Am Oil Chem Soc 79:879–883
8. Mattson FH, Volpenhein RA (1962) Synthesis and properties of glycerides. J Lipid Res 3:281–295
9. Boswinkel G, Derksen JTP, van't Riet K, Cuperus FP (1996) Kinetics of acyl migration in monoglycerides and dependence on acyl chain length. J Am Oil Chem Soc 73:707–711
10. Laszlo JA, Compton DL (2006) Enzymatic glycerolysis and transesterification of soybean oil for enhanced production of feruloylated mono- and diacylglycerols. J Am Oil Chem Soc 83:765–770
11. Isbell TA, Lowery BA, DeKeyser SS, Winchell ML, Cermak SC (2006) Physical Properties of triglyceride estolides from lesquerella and castor oils. Ind Crop Prod 23:256–263
12. Firestone D (ed) (1994) Official and tentative methods of the American Oil Chemists' Society, 4th edn. AOCS, Champaign
13. The Lipid Library, Triacylglycerols: Part 1. Composition, http://www.lipidlibrary.co.uk/Lipids/tag1/index.htm (accessed July 2006)
14. Hidalgo FJ, Zamora R (2003) Edible oil analysis by high-resolution nuclear magnetic resonance spectroscopy: recent advances and future perspectives. Trends Food Sci Technol 14:499–506
15. The Lipid Library, ^{1}H NMR spectroscopy of fatty acids and their derivatives: quantification by NMR, http://www.lipidlibrary.co.uk/nmr/1NMRquan/index.htm (accessed July 2006)
16. The Lipid Library, ^{1}H NMR spectroscopy of fatty acids and their derivatives: glycerol esters, http://www.lipidlibrary.co.uk/nmr/1NMRglyc/index.htm (accessed July 2006)
17. Gunstone FD (1994) High resolution ^{13}C NMR. A technique for the study of lipid structure and composition. Prog Lipid Res 33:19–28
18. Gunstone FD (1991) ^{13}C-NMR studies of mono-, di- and triacylglycerols leading to qualitative and semiquantitative information about mixtures of these glycerol esters. Chem Phys Lipids 58:219–224
19. Masel RI (2001) Chemical kinetics and catalysis. Wiley, New York, pp 80–81