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Thermodynamics of the lipase-catalyzed esterification of glycerol and *n*-octanoic acid in organic solvents and in the neat reaction mixture☆

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Abstract

The thermodynamics of the lipase-catalyzed esterification of glycerol with *n*-octanoic acid have been investigated with acetonitrile, benzene, and toluene as solvents and in the neat reaction mixture (no organic solvent added). This esterification reaction leads to five products: 1-monooctanoyl glycerol, 2-monooctanoyl glycerol, 1,2-dioctanoyl glycerol, 1,3-dioctanoyl glycerol and 1,2,3-trioctanoyl glycerol. This, in turn leads to a total of 12 reactions. Values of the equilibrium constants for these reactions have been measured (HPLC, GC, and LC/MS) at 37°C in the above mentioned media. The equilibrium constants range from 0.9 to 20.7, 0.20 to 8.0, 0.23 to 10.0, and 0.57 to 2.2 in acetonitrile, benzene, toluene, and neat media, respectively. Relative standard molar Gibbs free energies of formation $\Delta_f G^0_m$ of 1-monooctanoyl glycerol, 2-monooctanoyl glycerol, 1,2-dioctanoyl glycerol, 1,3-dioctanoyl glycerol and 1,2,3-trioctanoyl glycerol in the organic solvents and in the neat reaction mixture have been calculated and used to compactly summarize the thermodynamics of these reactions. The results show an approximate correlation with the permittivities of the solvents. Published by Elsevier Science B.V.

Keywords: Dioctanoyl glycerol; Equilibrium constants; Esterification; Glycerol; Lipase; Monooctanoyl glycerol; *n*-Octanoic acid; Trioctanoyl glycerol

1. Introduction

There has been significant interest in recent years in biocatalysis carried out in non-aqueous media [1–3]. There are several reasons for this interest. Most importantly, organic solvents overcome the difficulty of dissolving hydrophobic substances. Also, reactions carried out in organic solvents can often be used to shift the position of equilibrium to the formation of desired products. Finally, bacterial contamination of fermentors, a major problem in aqueous media, can be avoided.

Lipases (EC 3.1.1.3) have been one of the commonly used biocatalysts for the acylation of the hydroxyl group(s) in glycerol [4–9], the stereoselective esterification of racemic mixtures [10,11], and for transesterification reactions [9,12] in organic solvents. Consequently, the lipase-catalyzed modification of fats and oils in organic media has seen significant industrial development. Some selected references related to this area are cited [13–15]. A quantitative understanding of the energetics of these systems must

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Table 1
Reactions involved in the esterification of glycerol and *n*-octanoic acid[a]

glycerol(sln) + *n*-octanoic acid(sln) = 1-monooctanoyl glycerol(sln) + H_2O(sln)	(1)
glycerol(sln) + *n*-octanoic acid(sln) = 2-monooctanoyl glycerol(sln) + H_2O(sln)	(2)
glycerol(sln) + 2 *n*-octanoic acid(sln) = 1,2-dioctanoyl glycerol(sln) + 2 H_2O(sln)	(3)
glycerol(sln) + 2 *n*-octanoic acid(sln) = 1,3-dioctanoyl glycerol(sln) + 2 H_2O(sln)	(4)
glycerol(sln) + 3 *n*-octanoic acid(sln) = 1,2,3-trioctanoyl glycerol(sln) + 3 H_2O(sln)	(5)
1-monooctanoyl glycerol(sln) + *n*-octanoic acid(sln) = 1,2-dioctanoyl glycerol(sln) + H_2O(sln)	(6)
1-monooctanoyl glycerol(sln) + *n*-octanoic acid(sln) = 1,3-dioctanoyl glycerol(sln) + H_2O(sln)	(7)
2-monooctanoyl glycerol(sln) + *n*-octanoic acid(sln) = 1,2-dioctanoyl glycerol(sln) + H_2O(sln)	(8)
1-monooctanoyl glycerol(sln) + 2 *n*-octanoic acid(sln) = 1,2,3-trioctanoyl glycerol(sln) + 2 H_2O(sln)	(9)
2-monooctanoyl glycerol(sln) + 2 *n*-octanoic acid(sln) = 1,2,3-trioctanoyl glycerol(sln) + 2 H_2O(sln)	(10)
1,2-dioctanoyl glycerol(sln) + *n*-octanoic acid(sln) = 1,2,3-trioctanoyl glycerol(sln) + H_2O(sln)	(11)
1,3-dioctanoyl glycerol(sln) + *n*-octanoic acid(sln) = 1,2,3-trioctanoyl glycerol(sln) + H_2O(sln)	(12)

[a] In these reactions, the term "sln" denotes the solution in which the reaction has occurred, i.e. either an organic solvent or the neat mixture of the reactants and products.

rely on a knowledge of the thermodynamics and kinetics of the reactions of interest. In fact, several thermodynamic studies of enzyme-catalyzed reactions in organic solvents have been performed [11,16–22]. However, only the studies of Janssen et al. [17,18] deal with the biochemically important esterification and transesterification reactions of glycerol. Consequently, we have carried out a thermodynamic investigation of the lipase-catalyzed esterification of glycerol and *n*-octanoic acid with organic solvents.

For the aforementioned esterification reaction, there are five possible products (see Fig. 1): 1-monooctanoyl glycerol, 2-monooctanoyl glycerol, 1,2-dioctanoyl glycerol, 1,3-dioctanoyl glycerol, and 1,2,3-trioctanoyl glycerol. This leads to the 12 reactions given in Table 1. In these reactions the term "sln" denotes the solution in which the reaction has occurred, i.e. either an organic solvent or the neat mixture of the reactants and products. In this study, equilibrium constants for these reactions have been measured at 37°C with the solvents acetonitrile, benzene, and toluene, and also in the neat mixture (no solvent). This study provides additional insight into the distribution of the various species formed in these reactions than has been obtained to date. Additionally, the equilibrium constants for the first five reactions in Table 1 have been used to calculate the relative standard molar Gibbs free energies of formation $\Delta_f G^0_m$ of 1-monooctanoyl glycerol, 2-monooctanoyl glycerol, 1,2-dioctanoyl glycerol, 1,3-dioctanoyl glycerol and 1,2,3-trioctanoyl glycerol in these solvents. Some general trends in the results have also been observed.

Monogylcerides are widely used as emulsifiers in the food, pharmaceutical, and cosmetic industries [8] and medium-chain triglycerides of octanoic and decanoic acids are used as a dense form of calories for patients with pancreatic insufficiency [23]. Also there is industrial interest in these reactions because of the use of the product esters in drug-delivery systems [24].

2. Experimental

2.1. Materials

The substances used in this study, their chemical abstract service (CAS) numbers, empirical formulas, molar masses, sources, [1] and purities as determined by gas chromatography (GC) are given in Table 2. Immobilized Lipozyme IM (lipase from *Rhizomucor miehei*) was kindly supplied by Novo Nordisk BioChem North America, Inc.

2.2. Chromatography

The analysis of glycerol, *n*-octanoic acid, monooctanoyl glycerol, dioctanoyl glycerol, and 1,2,3-trioctanoyl glycerol was carried out with an Agilent

[1] Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedures adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

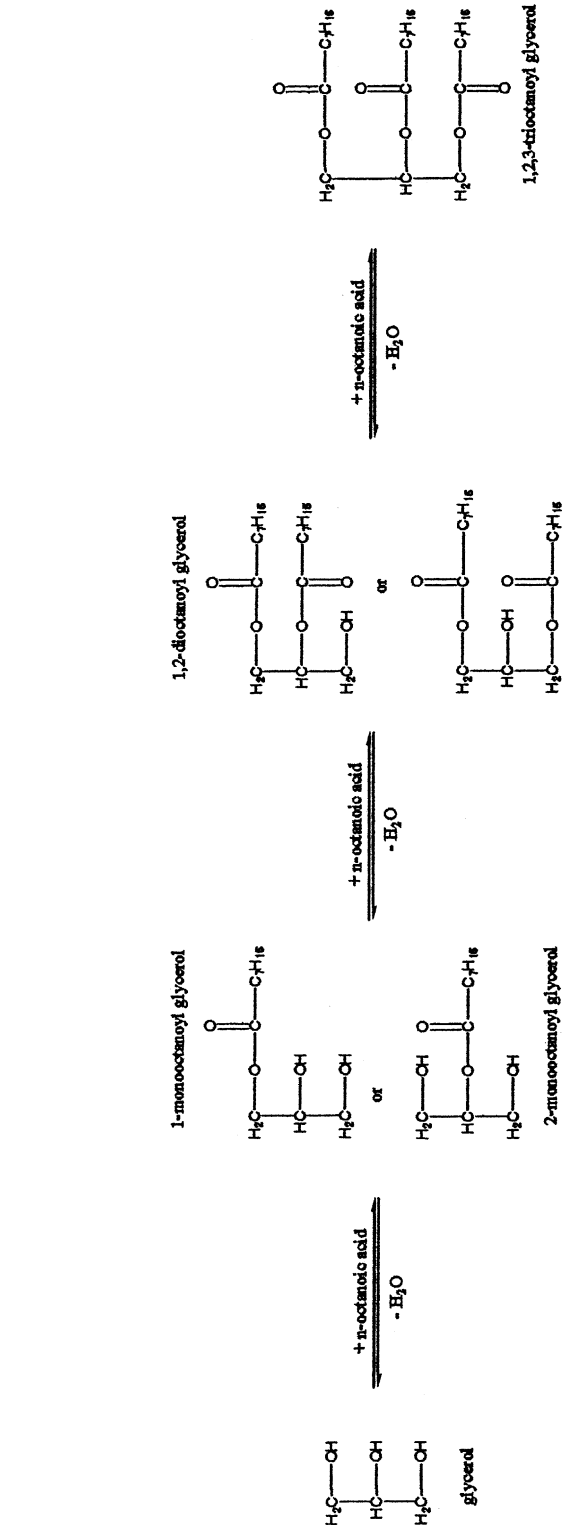

Fig. 1. The esterification reactions of glycerol and *n*-octanoic acid.

5890 gas chromatograph (GC) equipped with a flame ionization detector. A fused silica Phenomenex ZB-FFAP column (30 m long, 0.53 mm i.d.) was used. The head pressure of the helium carrier gas was 283 kPa. The injector and detector temperatures were 250 and 270°C, respectively. The initial column temperature of 100°C was held for 1 min and then raised to 240°C at a rate of 20°C min^{-1} and then held at 240°C for 20 min. The substance *n*-dodecanoic acid was used as an internal standard for the analysis of glycerol, *n*-octanoic acid, monooctanoyl glycerol, dioctanoyl glycerol and 1,2,3-trioctanoyl glycerol. The GC analysis showed a single peak for the pair (1,2-dioctanoyl glycerol and 1,3-dioctanoyl glycerol) and also for the pair (1-monooctanoyl glycerol and 2-monooctanoyl glycerol). Separations of these pairs were attempted by varying the temperature of the column but were not successful. The retention times of *n*-octanoic acid, glycerol, *n*-dodecanoic acid, monooctanoyl glycerol (total), dioctanoyl glycerol (total) and 1,2,3-trioctanoyl glycerol were, respectively, 6.12, 7.50, 8.05, 10.1, 15.5 and 22.6 min.

Response factor ratios (concentration/area) were determined with a standard solution of glycerol, *n*-octanoic acid, 1-monooctanoyl glycerol, *n*-dodecanoic acid, 1,3-dioctanoyl glycerol and 1,2,3-trioctanoyl glycerol that was gravimetrically prepared with 2-methyl-2-butanol as a solvent. By using this solution, the response factor ratios with respect to *n*-dodecanoic acid were determined for glycerol, *n*-octanoic acid, 1-monooctanoyl glycerol, 1,3-dioctanoyl glycerol, and 1,2,3-trioctanoyl glycerol.

An Agilent Model 1100 high-performance liquid chromatograph (HPLC) was used to separate and quantitatively measure concentrations of 1,2-dioctanoyl glycerol and 1,3-dioctanoyl glycerol. This analysis used a variable UV detector (wavelength $\lambda = 225$ nm) and a Zorbax Extend-C18 Column (4.6 mm i.d., 250 mm long) thermostatted at 35°C. The mobile phase consisted of (A) acetonitrile with 0.1% (v/v) acetic acid and (B) isopropyl alcohol with 0.1% (v/v) acetic acid. The smooth gradient used for the analysis was: A = 90% and B = 10% at $t = 0$ min; A = 80% and B = 20% at $t = 10$ min; and A = 70% and B = 30% at $t = 20$ min. The flow rate was 0.50 cm^3 min^{-1}. Under these conditions, typical retention times for 1-monooctanoyl glycerol, *n*-octanoic acid, 1,3-dioctanoyl glycerol, 1,2-dioctanoyl glycerol and 1,2,3-trioctanoyl glycerol were 6.4, 6.8, 8.8, 9.1 and 19.4 min, respectively. Since a sample of 2-monooctanoyl glycerol was not available and could not be synthesized without extensive efforts, the separation between 1-monooctanoyl glycerol and 2-monooctanoyl glycerol was not attempted.

Table 2
Principal substances used in this study with their chemical abstracts service (CAS) numbers, empirical formulas, molecular masses M_r, vendors (S = Sigma, A = Aldrich, F = Fisher, M = Mallinckrodt and N = Novo Nordisk), mole fraction purity x as stated by the vendor, and methods used to determine the mole fraction purity

Substance	CAS no.	Formula	M_r	Supplier	x	Method[a]
Acetonotrile	75-05-8	C_2H_3N	41.05	F	0.99	GC
Benzene	71-43-2	C_6H_6	78.11	F	0.99	GC
Glycerol	56-81-5	$C_3H_8O_3$	92.09	S	0.99	GC
n-Hexane	110-54-3	C_6H_{14}	86.18	S	0.99	GC
1,2-Dioctanoyl glycerol	104195-35-9	$C_{19}H_{36}O_5$	344.49	S	0.98	TLC
1,3-Dioctanoyl glycerol	1429-66-9	$C_{19}H_{36}O_5$	344.49	S	0.99	GC
n-Dodecanoic acid	143-07-7	$C_{12}H_{24}O_2$	200.32	S	0.99	GC
Lipozyme IM	9001-62-1			N		
1-Monooctanoyl glycerol	19670-49-6	$C_{11}H_{22}O_4$	218.29	S	0.99	GC and TLC
2-Monooctanoyl glycerol	4228-48-2	$C_{11}H_{22}O_4$	218.29	[b]		
2-Methy-2-butanol	75-85-4	$C_5H_{12}O$	88.15	A	0.99	GC
n-Octanoic acid	124-07-2	$C_8H_{16}O_2$	144.21	S	0.99	GC
Toluene	108-88-5	C_7H_8	92.14	M	0.99	GC
1,2,3-Trioctanonoyl glycerol	538-23-8	$C_{27}H_{50}O_6$	470.68	S	0.99	GC and TLC

[a] These are the methods used by the vendors to determine the purity of these compounds.
[b] Made in situ.

2.3. Karl–Fischer analysis

The concentrations of water in the reaction mixtures were measured with a Metrohm model 633 Karl–Fischer titrator and a 665 Dosimat as described previously [21]. The instrument was calibrated with 1-octanol saturated with water [25]. In the calibration experiments, ≈0.03 g of 1-octanol saturated with water was withdrawn from a stock solution with a 50 μl syringe and injected into the solvent (methanol + hydranal composite 2) in the Karl–Fischer apparatus. Reaction mixture samples (0.02–0.09 g) were withdrawn with a 100 μl syringe and similarly injected into the (methanol + hydranal composite 2) solution.

2.4. Equilibrium measurements

Equilibrium measurements were carried out by approaching the position of equilibrium from two opposite starting positions: (glycerol + *n*-octanoic acid) were used for one position (forward direction) and (1,2,3-trioctanoyl glycerol + H_2O) was used for the second position (reverse direction). Typical starting solutions for the reaction carried out with an organic solvent were (glycerol (0.013 g) + octanoic acid (0.048 g) + Lipozyme IM (1.0 g) + solvent (10.0 g)) for the forward direction and (1,2,3-trioctanoyl glycerol (0.053 g) + Lipozyme IM (1.0 g) + solvent (10.0 g)) for the reverse direction. For the neat reaction, starting solutions were (glycerol (1.9 g) + octanoic acid (9.4 g) + Lipozyme IM (1.8 g)) for the forward direction and (1,2,3-trioctanoyl glycerol (9.3 g) + water (0.020 g) + Lipozyme IM (1.7 g)) for the reverse direction. The vials containing these reaction mixtures were placed in a water bath set at (37.0 ± 0.05)°C. These solutions were shaken laterally (≈25 shakes min^{-1}) and allowed to equilibrate. The solutions were periodically analyzed with the GC to determine the extent of reaction. Following 3–4 weeks equilibration time, the respective concentration ratios (c(1,2,3-trioctanoyl glycerol)/(c(total dioctanoyl glycerol)) and (c(total dioctanoyl glycerol)/c(total monooctanoyl glycerol)) obtained from both starting positions were found to be very nearly equal. The mixtures were then analyzed for water by using the Karl–Fischer method and for the remaining reactants by using the HPLC and GC methods described above. Additional details concerning the analytical procedures follow.

Equilibrium measurements using other solvents (*n*-hexane, dimethyl formamide, and *tert*-amyl alcohol) were also attempted. Due to the very low solubility of glycerol in *n*-hexane, it was not possible to study this reaction using this solvent. Clearly, higher alkanes will not be useful for this study since the solubility of glycerol is even lower than in *n*-hexane. Also, since it was found that the rate of reaction was too slow (equilibration times of 30 days were attempted) when dimethyl formamide and *tert*-amyl alcohol were used as solvents, it was not possible to use these solvents in this study.

2.5. Analytical procedures

The analysis of the reaction mixtures was carried out by using a procedure, which is now described. An internal standard solution (100 μl of *n*-dodecanoic acid (c = 24 mM) in 2-methyl-2-butanol) was added to 1 cm^3 of the organic-phase containing the reactants and products. However, for analysis of the neat reaction mixture(s), 100 μl of this reaction mixture and 100 μl of an internal standard solution (*n*-dodecanoic acid (c = 24 mM) in *n*-hexane) were added to a vial and then diluted with 0.8 cm^3 of *n*-hexane. In this case, *n*-hexane was used as a solvent because of the limited solubility of the various esters in 2-methyl-2-butanol. Approximately 0.6 μl of the (reaction mixture + internal standard) solutions were injected into the GC and analyzed as described above. Tightly capped vials and air-tight syringes were used throughout this analysis. Also, all dilutions were done gravimetrically. The concentrations of glycerol, *n*-octanoic acid, monooctanoyl glycerol (total), dioctanoyl glycerol (total), and 1,2,3-trioctanoyl glycerol were determined from their respective chromatographic areas, the response factor ratios, and the known concentrations and chromatographic areas of the *n*-dodecanoic acid internal standard. As mentioned above, separations of the pairs of substances (1-monooctanoyl glycerol and 2-monooctanoyl glycerol) and (1,2-dioctanoyl glycerol and 1,3-dioctanoyl glycerol) could not be achieved on the GC. Therefore, the GC analysis for monooctanoyl glycerol and for dioctanoyl glycerol yield total combined concentrations for these respective pairs of substances.

In order to determine the ratio of the amounts of 1,3-dioctanoyl glycerol and 1,2-dioctanoyl glycerol,

the following procedure was used. First 100 μl of neat reaction mixture was diluted with 1 cm^3 of hexane and analyzed with the HPLC as described above. This analysis showed that the average ratio of the peak areas of 1,3-dioctanoyl glycerol to 1,2-dioctanoyl glycerol was 1.47 ± 0.03 ($n = 8$), where n is the number of measurements. Analysis of the reaction mixture in acetonitrile was done by direct injection into the HPLC (no dilution). In this case, the average ratio of the aforementioned peak areas was 1.52 ± 0.15 ($n = 8$). The reason for this larger uncertainty is that, for the acetonitrile reaction mixture, the peak areas corresponding to 1,3-dioctanoyl glycerol and 1,2-dioctanoyl glycerol were ≈20 times smaller than the corresponding peak areas obtained using the diluted solution from the neat reaction mixture. While the peak areas could have been increased by using a larger sample loop, doing this would have led to overlapping peaks that would have not have allowed for the measurement. The important result is that the peak area ratios of 1,3-dioctanoyl glycerol to 1,2-dioctanoyl glycerol in the neat solvent and in acetonitrile are in agreement. The ratio of the peak areas of 1,3-dioctanoyl glycerol and 1,2-dioctanoyl glycerol in toluene and in benzene could not be determined due to interference with the respective solvent peaks. However, based on the values obtained for the neat reaction mixture and for the reaction carried out in acetonitrile, the ratio of 1.47 was, in all cases, used to calculate the concentrations of 1,3-dioctanoyl glycerol and 1,2-dioctanoyl glycerol.

2.6. LC/MS analysis

An LC/MS analysis of the reaction mixture was carried out to identify the 1,3-dioctanoyl glycerol and 1,2-dioctanoyl glycerol peaks in the HPLC chromatograms. An Agilent Model 1100 LC/MS (in-line vacuum degasser, binary gradient pump, autosampler, variable-wavelength UV–VIS absorbance detector, and an atmospheric pressure chemical ionization (APCI) source on a single-quadrupole mass spectrometer) was used for this analysis. The absorbance detector and mass spectrometer were in series, and splitting of the mobile phase flow was not used. Data were recorded and analyzed using Agilent ChemStation software. Samples were introduced into the APCI source by using reversed-phase HPLC under the same HPLC conditions as described above. Pertinent operating details are: sample injection volume = 20 μl; absorbance was monitored at $\lambda = 225$ nm; flow rate of N_2(g) ($t = 200^\circ$C and $p = 344$ kPa) was 4000 $cm^3\ min^{-1}$; vaporizer temperature = 400°C; capillary voltage = 3500 V; corona current = 10 μA; and fragmentor voltage = 50 V. The electrospray ionization (ESI) source was operated to generate positive ions. The quadrupole mass analyzer scanned a mass to charge (m/z) range from $m/z = 100$ to $m/z = 600$. Using this procedure, the LC/MS analysis of standard samples of 1,3-dioctanoyl glycerol and 1,2-dioctanoyl glycerol were carried out. The spectra obtained were then compared with the spectra of the peaks with the same retention times in the reaction mixture. The identities of the 1,3-dioctanoyl glycerol and of the 1,2-dioctanoyl glycerol peaks were then established by means of the observed differences in their ion fragment intensities.

3. Results and discussion

Results of analyses of the reaction mixtures are given in Table 3. As mentioned earlier (see Section 2), the ratio (c(1,3-dioctanoyl glycerol)/c(1,2-dioctanoyl glycerol)) = 1.47 was used to calculate the concentrations of 1,3-dioctanoyl glycerol and of 1,2-dioctanoyl glycerol from the measured values of c(total dioctanoyl glycerol). This value is based upon very precise measurements performed on the neat reaction mixture and somewhat less precise measurements performed on the reaction mixture with acetonitrile as the solvent. It should also be noted that the concentration of monooctanoyl glycerol given in this table is equal to the sum of the concentrations of 1-monooctanoyl glycerol and of 2-monooctanoyl glycerol. In the absence of a sample of 2-monooctanoyl glycerol, for purposes of calculating reaction quotients and equilibrium constants, it was assumed that esterification of the hydroxyl groups at position 1 is twice as likely as that of the OH group at position 2. Thus, the concentration of 1-monooctanoyl glycerol was taken equal to (2/3)c(total monooctanoyl glycerol) and the concentration of 2-monooctanoyl glycerol was assumed to be equal to (1/3)c(total monooctanoyl glycerol). Should, this matter be resolved in the future, the results given in Table 3 can easily be used to recalculate values of

Table 3
Measured concentrations of water (H_2O), glycerol ($C_3H_8O_3$), *n*-octanoic acid ($C_8H_{18}O_2$), total monooctanoyl glycerol ($C_{11}H_{22}O_4$), 1,2-dioctanoyl glycerol ($C_{19}H_{36}O_5$), 1,3-dioctanoyl glycerol ($C_{19}H_{36}O_5$), and 1,2,3-trioctanoyl glycerol ($C_{27}H_{50}O_8$) in acetonitrile, toluene, benzene, and in the neat reaction mixture (no solvent) at 37°C[a]

Direction	$c(H_2O)$[b] (mol (kg sln)$^{-1}$)	$c(C_3H_8O_3)$ (mol (kg sln)$^{-1}$)	$c(C_8H_{18}O_2)$ (mol (kg sln)$^{-1}$)	$c(C_{11}H_{22}O_4)$[c] (mol (kg sln)$^{-1}$)	$c(C_{19}H_{36}O_5)$[d,e] (mol (kg sln)$^{-1}$)	$c(C_{19}H_{36}O_5)$[e,f] (mol (kg sln)$^{-1}$)	$c(C_{27}H_{50}O_8)$ (mol (kg sln)$^{-1}$)
Acetonitrile							
Forward	0.393	1.75×10^{-2}	4.52×10^{-2}	1.08×10^{-2}	3.90×10^{-3}	5.74×10^{-3}	4.80×10^{-4}
Forward	0.393	1.70×10^{-2}	4.24×10^{-2}	1.33×10^{-2}	2.74×10^{-3}	4.02×10^{-3}	3.40×10^{-4}
Forward	0.393	1.92×10^{-2}	4.44×10^{-2}	1.22×10^{-2}	3.89×10^{-3}	5.71×10^{-3}	4.54×10^{-4}
Forward	0.393	1.66×10^{-2}	4.31×10^{-2}	8.36×10^{-3}	1.51×10^{-3}	2.21×10^{-3}	3.51×10^{-4}
Forward	0.393	1.82×10^{-2}	4.62×10^{-2}	1.27×10^{-2}	3.54×10^{-3}	5.20×10^{-3}	3.95×10^{-4}
Reverse	0.539	8.48×10^{-3}	5.97×10^{-2}	4.70×10^{-3}	8.70×10^{-4}	1.28×10^{-3}	1.97×10^{-4}
Reverse	0.539	8.16×10^{-3}	5.94×10^{-2}	5.37×10^{-3}	1.95×10^{-3}	2.86×10^{-3}	2.45×10^{-4}
Reverse	0.539	1.01×10^{-2}	6.70×10^{-2}	7.57×10^{-3}	2.24×10^{-3}	3.29×10^{-3}	2.69×10^{-4}
Reverse	0.539	1.55×10^{-2}	6.24×10^{-2}	1.21×10^{-2}	2.08×10^{-3}	3.16×10^{-3}	3.50×10^{-4}
Neat reaction mixture (no solvent)							
Forward	2.31	0.169	2.51	0.317	0.189	0.278	0.088
Forward	2.31	0.175	2.51	0.351	0.266	0.389	0.201
Forward	2.31	0.171	2.69	0.277	0.147	0.217	0.067
Forward	2.31	0.168	2.60	0.290	0.170	0.250	0.125
Forward	2.31	0.178	2.40	0.310	0.183	0.269	0.127
Reverse	0.943	3.91×10^{-2}	2.11	0.217	0.387	0.568	0.416
Reverse	0.943	3.08×10^{-2}	2.11	0.210	0.345	0.508	0.545
Reverse	0.943	3.52×10^{-2}	2.13	0.201	0.348	0.512	0.469
Reverse	0.943	3.87×10^{-2}	2.07	0.210	0.335	0.493	0.423
Reverse	0.943	3.07×10^{-2}	2.10	0.212	0.342	0.503	0.387
Toluene							
Forward	1.34×10^{-2}	1.48×10^{-4}	1.19×10^{-2}	1.19×10^{-4}	1.36×10^{-4}	2.01×10^{-4}	2.29×10^{-4}
Forward	1.34×10^{-2}	1.40×10^{-4}	1.19×10^{-2}	9.33×10^{-5}	1.32×10^{-4}	1.94×10^{-4}	2.48×10^{-4}
Forward	1.34×10^{-2}	1.46×10^{-4}	1.19×10^{-2}	1.08×10^{-4}	1.34×10^{-4}	1.97×10^{-4}	2.40×10^{-4}
Forward	1.34×10^{-2}	1.43×10^{-4}	1.20×10^{-2}	8.40×10^{-5}	1.40×10^{-4}	2.07×10^{-4}	2.40×10^{-4}
Reverse	1.16×10^{-2}	1.48×10^{-4}	1.34×10^{-2}	9.69×10^{-5}	1.94×10^{-4}	2.84×10^{-4}	4.22×10^{-4}
Reverse	1.16×10^{-2}	1.56×10^{-4}	1.31×10^{-2}	6.13×10^{-5}	1.94×10^{-4}	2.86×10^{-4}	4.44×10^{-4}
Reverse	1.16×10^{-2}	1.40×10^{-4}	1.29×10^{-2}	8.66×10^{-5}	1.99×10^{-4}	2.93×10^{-4}	4.56×10^{-4}
Reverse	1.16×10^{-2}	1.41×10^{-4}	1.28×10^{-2}	1.12×10^{-4}	1.99×10^{-4}	2.92×10^{-4}	4.45×10^{-4}
Benzene							
Forward	1.45×10^{-2}	2.26×10^{-4}	1.43×10^{-2}	1.28×10^{-4}	2.46×10^{-4}	3.61×10^{-4}	3.72×10^{-4}
Forward	1.45×10^{-2}	1.88×10^{-4}	1.39×10^{-2}	1.12×10^{-4}	2.27×10^{-4}	3.34×10^{-4}	3.46×10^{-4}
Forward	1.45×10^{-2}	2.16×10^{-4}	1.38×10^{-2}	1.75×10^{-4}	2.17×10^{-4}	3.19×10^{-4}	3.17×10^{-4}
Forward	1.45×10^{-2}	2.05×10^{-4}	1.40×10^{-2}	1.05×10^{-4}	2.17×10^{-4}	3.19×10^{-4}	3.21×10^{-4}
Forward	1.45×10^{-2}	2.03×10^{-4}	1.43×10^{-2}	1.09×10^{-4}	1.87×10^{-4}	2.74×10^{-4}	3.47×10^{-4}
Forward	1.45×10^{-2}	2.07×10^{-4}	1.39×10^{-2}	1.01×10^{-4}	2.13×10^{-4}	3.13×10^{-4}	3.67×10^{-4}
Reverse	1.18×10^{-2}	1.60×10^{-4}	1.45×10^{-2}	1.07×10^{-4}	1.90×10^{-4}	2.79×10^{-4}	2.80×10^{-4}
Reverse	1.18×10^{-2}	1.93×10^{-4}	1.36×10^{-2}	1.47×10^{-4}	2.03×10^{-4}	2.98×10^{-4}	3.85×10^{-4}
Reverse	1.18×10^{-2}	2.08×10^{-4}	1.36×10^{-2}	1.50×10^{-4}	2.02×10^{-4}	2.97×10^{-4}	3.80×10^{-4}
Reverse	1.18×10^{-2}	2.10×10^{-4}	1.43×10^{-2}	1.23×10^{-4}	2.10×10^{-4}	3.08×10^{-4}	4.18×10^{-4}
Reverse	1.18×10^{-2}	1.89×10^{-4}	1.31×10^{-2}	1.28×10^{-4}	1.82×10^{-4}	2.68×10^{-4}	3.75×10^{-4}

[a] These concentrations *c* are expressed as amount of substance divided by mass of solution and are given in columns 2–8. The term "forward" states that the reaction was started with (glycerol + *n*-octanoic acid) and similarly "reverse" states that the reaction was started with 1,2,3-trioctanoyl glycerol.

[b] The concentrations of water reported in column two are the averages of three to five measurements.

[c] The concentration of monooctanoyl glycerol is the sum of the concentrations of 1-monooctanoyl glycerol and of 2-monooctanoyl glycerol. The individual concentrations of 1-monooctanoyl glycerol and of 2-monooctanoyl glycerol are later calculated from this total concentration by assuming a concentration ratio of 2:1 (see Section 3).

[d] 1,2-Dioctanoyl glycerol ($C_{19}H_{36}O_5$).

[e] In all cases, the ratio (c(1,3-dioctanoyl glycerol)/c(1,2-dioctanoyl glycerol)) = 1.47 was used to calculate the concentrations of 1,3-dioctanoyl glycerol and of 1,2-dioctanoyl glycerol from the measured values of c(total dioctanoyl glycerol) (see Section 2).

[f] 1,3-Dioctanoyl glycerol ($C_{19}H_{36}O_5$).

the reaction quotients and equilibrium constants for the reactions that are affected by this assumption.

The general form for the reactions studied herein (see Table 1) is

$$aA(\text{sln}) + bB(\text{sln}) = cC(\text{sln}) + dD(\text{sln}), \quad (1)$$

where a, b, c, and d are the stoichiometric numbers of the reactants and products. The equilibrium constants K corresponding to reactions 1–12 are defined according to the general relationship

$$K = \frac{[\text{C}]^c[\text{D}]^d}{[\text{A}]^a[\text{B}]^b} \quad (2)$$

where [ ] denotes concentrations. It is important to note that the water is a reactant in all of the reactions studied and that its concentration is included in Eq. (2). In this study, we have used concentrations expressed as mol (kg solution)$^{-1}$. This is a convenient measure since all solutions were prepared and analyzed on a gravimetric basis. Also, if the densities of the solutions are known, it is easy to convert these values to concentrations on a molarity basis (mol dm^{-3}). Most importantly, since all of these reactions are symmetrical, the values of the reaction quotients and equilibrium constants are independent of the units used to express the concentrations of the reactants and products. Table 4 contains the values of the reaction quotients and equilibrium constants that are calculated from the concentrations given in Table 3. The uncertainties in the values of Q are equal to two estimated standard deviations of the mean. The values of the equilibrium constants were calculated by pooling all of the reaction quotient results for a given reaction from both directions. In the majority of instances, the values of the reaction quotients obtained from both directions of reaction were found to be in statistical agreement. However, in some cases the respective reaction quotients are not in agreement and equilibrium may not have been achieved. This lack of agreement is considered to be a possible source of systematic error in the determination of the equilibrium constants. Additional possible systematic errors are estimated to be $0.05Q$ in the measurements of the concentrations of the reactants and products and $0.01Q$ due to sample impurities. Therefore, in all cases, the final error estimates in the equilibrium constants were obtained by first calculating the statistical error expressed as one estimated standard deviation of the mean and then combining it in quadrature with the possible systematic errors in the measurements of concentrations and sample impurities. This gave values of combined standard uncertainties [26]. These combined standard uncertainties were then multiplied by two to obtain expanded uncertainties. These values were judged to be satisfactory estimates of overall error in the values of the equilibrium constants except in those cases where they did not fully cover the possible set of values of the reaction quotients obtained from both directions of reaction. In these cases, the uncertainties were further expanded so as to also cover these values.

A more compact way of summarizing the results of this study uses relative standard molar Gibbs free energies of formation $\Delta_f G^0_m$. This calculation is based upon the arbitrary assignment of values of zero to a few substances in the system of reactions. This convention has been used in biochemical thermodynamics when dealing with the ATP series of compounds [27,28] and is a compact way of summarizing information. Accordingly, values of $\Delta_f G^0_m = 0$ have been assigned to H_2O, *n*-octanoic acid, and glycerol for each of the respective solvents as well as for the neat reaction mixture. Then, values of $\Delta_f G^0_m$ of 1-monooctanoyl glycerol, 2-monooctanoyl glycerol, 1,2-dioctanoyl glycerol, 1,3-dioctanoyl glycerol, and 1,2,3-trioctanoyl glycerol were calculated from the equilibrium constants given in Table 4. These values, given in Table 5, can be used to calculate standard molar Gibbs free energies of reaction $\Delta_r G^0_m$ and the equilibrium constants (K_1 to K_{12}) for the entire system of reactions.

Janssen et al. [20] studied the reaction of glycerol with *n*-octanoic acid by using the neat reaction mixture. They [20] reported equilibrium constants at 35°C for the formation of monoester K_m, diester K_d, and triester K_t which they defined as

$$K_m = \frac{c(\text{total monoester})c(\text{H}_2\text{O})}{c(\text{glycerol})c(n\text{-octanoic acid})} \quad (3)$$

$$K_d = \frac{c(\text{total diester})c(\text{H}_2\text{O})}{c(\text{total monoester})c(n\text{-octanoic acid})} \quad (4)$$

$$K_t = \frac{c(\text{triester})c(\text{H}_2\text{O})}{c(\text{total diester})c(n\text{-octanoic acid})} \quad (5)$$

Thus, these reactions pertain to total amounts of the various isomeric forms of the monoester and diester. Their reported equilibrium constants are $K_m = 1.4$,

Table 4
Reaction quotients Q and equilibrium constants K for the esterification reactions of glycerol and *n*-octanoic acid at 37°C in acetonitrile, benzene, toluene, and without any solvent (the neat mixture of reactants and products)[a]

	Acetonitrile	Benzene	Toluene	Without solvent
Q_1(for)[b]	3.83 ± 0.51	0.40 ± 0.08	0.52 ± 0.06	1.09 ± 0.11
Q_1(rev)[b]	3.96 ± 0.41	0.39 ± 0.04	0.40 ± 0.05	1.82 ± 0.19
K_1[b]	3.89 ± 0.65	0.40 ± 0.08	0.46 ± 0.12	1.45 ± 0.58
Q_2(for)[b]	1.92 ± 0.29	0.20 ± 0.04	0.26 ± 0.03	0.55 ± 0.05
Q_2(rev)[b]	1.98 ± 0.20	0.20 ± 0.02	0.20 ± 0.03	0.91 ± 0.10
K_2[b]	1.94 ± 0.33	0.20 ± 0.04	0.23 ± 0.06	0.73 ± 0.28
Q(for)	15.2 ± 1.3	1.12 ± 0.11	1.19 ± 0.02	0.93 ± 0.22
Q(rev)	13.1 ± 4.4	0.75 ± 0.04	1.07 ± 0.09	2.04 ± 0.18
K_3	14.1 ± 5.4	0.96 ± 0.25	1.13 ± 0.15	1.48 ± 0.78
Q(for)	22.8 ± 1.6	1.65 ± 0.16	1.75 ± 0.04	1.36 ± 0.31
Q(rev)	19.4 ± 6.3	1.11 ± 0.06	1.57 ± 0.13	3.00 ± 0.26
K_4	20.7 ± 7.9	1.40 ± 0.33	1.66 ± 0.24	2.2 ± 1.1
Q(for)	16.0 ± 1.5	1.84 ± 0.15	2.36 ± 0.11	0.54 ± 0.22
Q(rev)	17.0 ± 3.4	1.20 ± 0.17	2.14 ± 0.23	1.17 ± 0.22
K_5	16.4 ± 4.2	1.55 ± 0.52	2.25 ± 0.35	0.86 ± 0.55
Q(rev)[b]	3.6 ± 0.9	2.86 ± 0.47	2.30 ± 0.33	0.84 ± 0.12
Q(rev)[b]	3.3 ± 1.1	1.95 ± 0.17	2.73 ± 0.26	1.16 ± 0.04
K_6[b]	3.5 ± 1.3	2.45 ± 0.86	2.52 ± 0.58	0.98 ± 0.26
Q_7(for)[b]	5.2 ± 1.2	4.21 ± 0.69	3.42 ± 0.52	1.23 ± 0.17
Q_7(rev)[b]	4.9 ± 1.5	2.86 ± 0.24	4.01 ± 0.39	1.65 ± 0.06
K_7[b]	5.1 ± 1.7	3.6 ± 1.3	3.70 ± 0.81	1.44 ± 0.38
Q_8(for)[b]	7.1 ± 1.7	5.73 ± 0.93	4.61 ± 0.65	1.68 ± 0.24
Q_8(rev)[b]	6.6 ± 2.1	3.90 ± 0.33	5.45 ± 0.51	2.25 ± 0.09
K_8[b]	6.9 ± 2.4	4.9 ± 1.8	5.0 ± 1.1	1.96 ± 0.52
Q_9(for)[b]	4.3 ± 0.8	4.72 ± 0.89	4.57 ± 0.67	0.49 ± 0.16
Q_9(rev)[b]	4.4 ± 1.0	3.10 ± 0.37	5.46 ± 0.63	0.64 ± 0.08
K_9[b]	4.3 ± 1.1	4.0 ± 1.6	5.0 ± 1.1	0.57 ± 0.25
Q_{10}(for)[b]	8.5 ± 1.6	9.44 ± 1.8	9.2 ± 1.3	0.97 ± 0.33
Q_{10}(rev)[b]	8.7 ± 2.1	6.17 ± 0.7	10.9 ± 1.3	1.29 ± 0.16
K_{10}[b]	8.6 ± 2.0	8.0 ± 2.5	10.0 ± 2.2	1.13 ± 0.50
Q_{11}(for)	1.26 ± 0.43	1.65 ± 0.14	1.99 ± 0.08	0.57 ± 0.13
Q_{11}(rev)	1.40 ± 0.41	1.60 ± 0.22	2.00 ± 0.07	0.57 ± 0.08
K_{11}	1.33 ± 0.50	1.62 ± 0.24	1.99 ± 0.22	0.57 ± 0.13
Q_{12}(for)	0.86 ± 0.30	1.12 ± 0.10	1.35 ± 0.06	0.39 ± 0.10
Q_{12}(rev)	0.95 ± 0.28	1.09 ± 0.15	1.36 ± 0.04	0.39 ± 0.05
K_{12}	0.90 ± 0.34	1.10 ± 0.16	1.35 ± 0.15	0.39 ± 0.10

[a] The reaction quotients Q are the average of 4–6 measurements. The equilibrium constants K are the averages of the reaction quotients obtained from two different initial positions of reaction. The uncertainties in the values of Q are equal to two estimated standard deviations of the mean; the uncertainties in the equilibrium constants are discussed in the text (see Section 3).

[b] The calculations of the values of Q and K for reactions 1, 2, 6, 7, 8, 9, and 10 were done on the assumption that the ratio of the concentrations of 1-monooctanoyl glycerol and 2-monooctanoyl glycerol is 2:1.

Table 5
Relative standard molar Gibbs free energies of formation $\Delta_f G^0_m$ of species involved in the esterification of glycerol and *n*-octanoic acid in organic solvents at 37°C ($T = 310.15$ K)[a]

$\Delta_f G^0_m$ (kJ mol^{-1})				
Substance	Acetonitrile	Benzene	Toluene	Without solvent
Water	0.0	0.0	0.0	0.0
Glycerol	0.0	0.0	0.0	0.0
n-Octanoic acid	0.0	0.0	0.0	0.0
1-Monooctanoyl glycerol	−3.50 ± 0.40	2.36 ± 0.42	2.01 ± 0.50	−0.96 ± 0.66
2-Monooctanoyl glycerol	−1.71 ± 0.41	4.15 ± 0.47	3.79 ± 0.51	0.81 ± 0.67
1,2-Dioctanoyl glycerol	−6.82 ± 0.60	0.11 ± 0.53	−0.32 ± 0.32	−1.01 ± 0.89
1,3-Dioctanoyl glycerol	−7.81 ± 0.64	−0.87 ± 0.55	−1.31 ± 0.31	−2.01 ± 0.89
1,2,3-Trioctanoyl glycerol	−7.21 ± 0.45	−1.13 ± 0.58	−2.09 ± 0.33	0.39 ± 0.96

[a] The values of $\Delta_f G^0_m$ of water, glycerol, and *n*-octanoic acid in each of these organic media have been arbitrarily assigned to be zero. These relative Gibbs free energies of formation should not be confused with standard Gibbs free energies of formation.

$K_d = 1.1$, and $K_t = 0.6$. The values of these equilibrium constants that are calculated from the results of our study are $K_m = 2.18 \pm 0.40$, $K_d = 1.62 \pm 0.20$, and $K_t = 0.23 \pm 0.03$. Since uncertainties were not assigned to the values of K_m, K_d, and K_t in the study of Janssen et al. [20], it is not possible to state whether or not the values are in agreement. However, it is seen that the respective results are not too different and that they also display the same general trend in values, namely $K_m > K_d > K_t$.

Some trends in the results are apparent. For the addition of the first octanoic acid to glycerol (K_1 and K_2) the order of the values of the equilibrium constants with the various solvents is K(acetonitrile) > K(neat) > K(benzene and toluene). However, for the addition of the second octanoic acid (K_6, K_7, and K_8) to monooctanoic acid, the order is K(acetonitrile) > K(benzene and toluene) > K(neat). For the addition of the third octanoic acid (K_{11} and K_{12}) the order is K(benzene and toluene) ≈ K(acetonitrile) > K(neat). Earlier studies [16,21] have attempted correlations of results of equilibrium measurements in organic solvents with permittivities and activity coefficients. The results obtained in this study show a correlation with the permittivities ε of the solvents [29] which are ε(acetonitrile) = 36.64, ε(toluene) = 2.38, and ε(benzene) = 2.28. However, this ordering is judged to be somewhat inexact since the permittivity of the neat reaction mixture is not known and is difficult to estimate from the relative permittivities of the substances present and which are not all known. Also, a correlation based on only a few data points must necessarily be considered to be very approximate. The necessary activity coefficients are not available and it is not possible to attempt even a limited correlation with this quantity.

Examination of Table 5 allows one to order the relative stabilities of the monooctanoyl, dioctanoyl, and trioctanoyl species. Thus, one sees the following order for the stabilities of the species: di-1,3 > tri > di-1,2 > mono-1 > mono-2 for acetonitrile; tri > di-1,3 > di-1,2 > mono-1 > mono-2 for benzene and for toluene; and di-1,3 > di-1,2 ≈ mono-1 > tri > mono-2 for the neat system. This stability order is the same for both benzene and for toluene which are very nearly the same as for acetonitrile. Interestingly, the order of the stabilities is somewhat different with the neat system. In summary, the information obtained in this and similar studies provide the essential data on representative systems which can be used to gain insight into the energetics of this important class of enzyme-catalyzed reactions. The results can also be used to calculate the amounts of the various species present at equilibrium in a reaction mixture and is needed for the optimization of product yields.

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