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Thermodynamics of the Lipase-Catalyzed Esterification of 1-Dodecanoic Acid with (−)-Menthol in Organic Solvents

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The thermodynamics of the lipase-catalyzed stereospecific esterification of 1-dodecanoic acid with (−)-menthol in seven organic solvents have been investigated. Equilibrium measurements were performed using hexane, heptane, cyclohexane, toluene, 2,2,4-trimethylpentane, acetonitrile, and 2-methyl-2-butanol at the temperature $T = 298.15$ K. The equilibrium constants have been correlated with the (octanol + water) partition coefficients and the relative permittivities of the solvents. The saturation molalities and the (hexane + water) partition coefficients of (−)-menthol, 1-dodecanoic acid, and (−)-menthyl dodecanoate have also been determined at $T = 298.15$ K. The equilibrium constant of the esterification reaction in aqueous media has been calculated. An important finding is that the molality of water plays an important role in determining the extent of ester formation. In acetonitrile and 2-methyl-2-butanol, which have a very high molality of water, the formation of (−)-menthyl dodecanoate is considerably smaller. However, in hydrophobic solvents with lower molalities of water, the formation of (−)-menthyl dodecanoate is favored in the reaction mixture. The value of the equilibrium constant varies from 3 to 26. However, the molality of (−)-menthyl dodecanoate changes from 0.034 mmol kg^{-1} in acetonitrile to 16 mmol kg^{-1} in heptane. The change in product yield is a factor of ≈500 and is a striking example of the utility of organic solvents in biocatalysis.

Introduction

Biocatalysis in organic media has become a potentially important method for the synthesis of stereoisomers (Parida and Dordick, 1991; Hedstrom et al., 1993; Stamatis et al., 1993; Biselli et al., 1995; Kamiya et al., 1995). Practical interest in this subject arises primarily because biocatalysis in organic solvents both overcomes the difficulty of dissolving hydrophobic substances and also shifts the thermodynamic equilibrium of many enzymatic reactions to the formation of desired products. An additional advantage is that bacterial contamination of bioreactors, a major problem with the use of aqueous solutions, can be eliminated. Thus, this approach makes it possible to carry out reactions that are impractical, if not impossible, to carry out using water as the solvent. In this regard, lipase has been one of the most widely used biocatalysts for the stereoselective esterification and resolution of racemic mixtures (Kirchner et al., 1985; Lokotsch et al., 1989; Hedstrom et al., 1993; Stamatis et al., 1993; Kamiya et al., 1995) in organic media. However, none of these previous studies report equilibrium data for the reactions studied, although, in some cases, percent conversions of substances have been reported.

Presently, only a limited number of reports dealing with the thermodynamics of enzyme-catalyzed reactions in nonaqueous solvents (Janssen et al., 1993a; Janssen et al., 1993b; Valivety et al., 1991; Tewari et al., 1995; Tewari et al., 1996; Tewari, 1998) have been published. This is unfortunate, since these results are needed both for process optimization calculations and for a better understanding of the energetics of these reactions. In this study, we have determined equilibrium constants for the following esterification reaction (also see Figure 1)

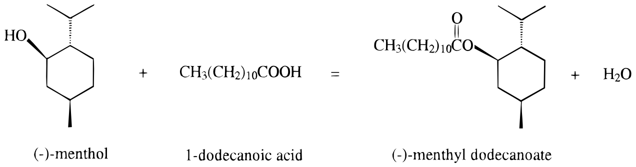

Figure 1. Structures of the substances in reaction 1.

$$\text{(−)-menthol(sln)} + \text{1-dodecanoic acid(sln)} = \text{(−)-menthyl dodecanoate(sln)} + \mathrm{H_2O(sln)} \quad (1)$$

where sln denotes any of the seven organic solvents that were used in this study. The saturation molalities and (hexane + water) partition coefficients of (−)-menthol, 1-dodecanoic acid, and (−)-menthyl dodecanoate have also been measured at the temperature $T = 298.15$ K. The equilibrium constant for the esterification reaction of (−)-menthol and 1-dodecanoic acid in aqueous media has also been calculated. The equilibrium constants are seen to correlate well with the (octanol + water) partition coefficients and the relative permittivities of the solvents.

Experimental Section

The substances used in this study, their Chemical Abstract Service (CAS) numbers, empirical formulas, molar masses, vendors,[†] and purities as determined by gas

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[†] 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.

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Table 1. Principal Substances Used in This Study with Their Chemical Abstracts Service (CAS) Registry Numbers, Empirical Formulas, Molecular Weights M_r, Suppliers (A = Aldrich, B = Baker, F = Fluka M = Mallinckrodt, S = Sigma, Y = Synthesized), Mole Fraction Purity x As Stated by the Supplier, and Method Used to Determine the Mole Fraction Purity[a]

substance	CAS no.	formula	M_r	supplier	x	method
(±)-menthol	1490-04-6	$C_{10}H_{20}O$	156.27	F	0.99	GC
(+)-menthol	15356-60-2	$C_{10}H_{20}O$	156.27	F	0.99	GC
(−)-menthol	2216-51-5	$C_{10}H_{20}O$	156.27	F	0.99	GC
1-dodecanoic acid	143-07-7	$C_{12}H_{24}O_2$	200.32	S	0.998	GC
(+)-menthyl dodecanoate	161275-87-2	$C_{22}H_{42}O_2$	338.58	Y	0.987	GC
(−)-menthyl dodecanoate	57084-14-7	$C_{22}H_{42}O_2$	338.58	Y	0.987	GC
1-dodecanol	112-53-8	$C_{12}H_{26}O$	186.32	S	0.99	GC
toluene	108-88-5	C_7H_8	92.14	M	0.99	GC
hexane	110-54-3	C_6H_{14}	86.14	B	0.99	GC
heptane	142-82-5	C_7H_{16}	100.20	M	0.997	GC
cyclohexane	110-82-7	C_6H_{12}	84.16	M	0.99	GC
2,2,4-trimethylpentane	540-84-1	C_8H_{18}	114.23	B	1.00	GC
acetonitrile	75-05-8	C_2H_3N	41.05	A	0.995	GC
2-methyl-2-butanol	75-85-4	$C_5H_{12}O$	88.15	A	0.99	GC
dipotassium phosphate	7778-77-0	K_2HPO_4	174.18	S		
phosphoric acid	7664-38-2	H_3PO_4	98.00	M		
lipase[b]	9001-62-1		5.0×10^4	S		

[a] These are the methods used by the vendor(s) to determine the purities of these substances. The chromatographic analyses of (+)-menthol, (−)-menthol, (±)-menthol, (−)-menthyl dodecanoate, and (+)-menthyl dodecanoate were carried out using the GC method described in the experimental section. [b] Type ll crude powder obtained from porcine pancreas.

chromatography (GC) are given in Table 1. The GC analysis of (±)-menthol, (−)-menthol, 1-dodecanoic acid, and (+)-menthol confirmed the purities reported by the vendors. These substances were used as received without further purification. The esters (−)-menthyl dodecanoate and (+)-menthyl dodecanoate were synthesized and purified as described below. The lipase (EC 3.1.1.3) used for biocatalysis was type II crude powder prepared from porcine pancreas.

The synthesis and purification of (+) and (−)-menthyl dodecanoate are now described. Equimolar amounts of (−)-menthol and 1-dodecanoic acid were dissolved in 50 cm^3 of tetrahydrofuran (THF). Three drops of concentrated sulfuric acid were added to the solution, which was then refluxed for 15 days. The reaction mixture was periodically analyzed by using GC to determine the extent of (−)-menthyl dodecanoate formation. After it was determined that the concentration of (−)-menthyl dodecanoate was no longer increasing, the THF was removed. The residue was dissolved in 50 cm^3 of diethyl ether and successively washed with 5% aqueous NaOH (w/v), to remove unreacted 1-dodecanoic acid, and then with distilled water. The diethyl ether layer was dried over $MgSO_4$, filtered, and concentrated to give a clear liquid product. Thin layer chromatographic analysis (5 vol % ethyl acetate in hexane) of the product indicated a mixture of (−)-menthol and (−)-menthyl dodecanoate. The ester purification was achieved by flash chromatography (40 μm silica gel, 33 cm × 3 cm, 2.5 vol % ethyl acetate in hexane). GC analysis indicated that the purified (−)-menthyl dodecanoate had a mole fraction purity > 0.995. GC/MS analysis using a Hewlett-Packard (HP) 5790 gas chromatograph coupled with a VG Analytical 70-250 mass spectrometer confirmed that the product was (−)-menthyl dodecanoate. (+)-Menthyl dodecanoate was prepared in an analogous fashion using 1-dodecanoic acid and (+)-menthol.

The chiral separation of (+)-menthol, (−)-menthol, and their esters was carried out with a Varian 3500 gas chromatograph equipped with a flame ionization detector. A cyclodextrin column (Chiraldex B-PH, Advanced Separation Technologies, 40 m × 0.25 mm i.d.) was used for the separation. The head pressure of the helium carrier gas was 2.7 bar. The injector and detector temperatures were 220 °C. The initial column temperature of 50 °C was held for 180 min and then raised to 140 °C at a rate of 5 K min^{-1} and held at 140 °C for 90 min. The retention times were 166.9 min for (−)-menthol, 170.7 min for (+)-menthol, 200.7 min for dodecanol (the internal standard), 242.1 min for dodecanoic acid, 280.7 min for (−)-menthyl dodecanoate, and 282.9 min for (+)-menthyl dodecanoate. The chromatographic analysis showed that (−)-menthol, (+)-menthol, (−)-menthyl dodecanoate, and (+)-menthyl dodecanoate had respective chiral mole fraction purities of 0.982, 0.986, 0.983, and 0.985.

The analysis of (−)-menthol, 1-dodecanoic acid, and (−)-menthyl dodecanoate was carried out with a HP 5890 gas chromatograph equipped with a flame ionization detector. The column was a fused silica HP 5 column (5% cross-linked phenylmethylsilane, 30 m long × 0.53 mm i.d. with a film thickness of 0.80 μm). The head pressure of the helium carrier gas was 2.8 bar. The injector and detector temperatures were 270 °C. The initial column temperature of 100 °C was held for 1 min and then raised to 225 °C at a rate of 20 K min^{-1} and then held at 225 °C for 20 min. 1-Dodecanol was used as an internal standard for the analysis of (−)-menthol, 1-dodecanoic acid, and (−)-menthyl dodecanoate. The retention times of (−)-menthol, 1-dodecanol, 1-dodecanoic acid, and (−)-menthyl dodecanoate were 2.3, 4.3, 4.8, and 9.0 min, respectively.

The lipase was immobilized on glutaraldehyde-treated controlled-pore glass (CPG) beads using a procedure similar to that described previously (Tewari et al., 1995). Approximately 4 g of lipase was dissolved in phosphate buffer [concentration $c(K_2HPO_4) = 0.1$ mol dm^{-3}, adjusted to pH = 7.2 with H_3PO_4]. The resulting solution was centrifuged at 2000 rpm for 10 min to remove any insoluble substances. The CPG beads were then suspended in this solution, and the resultant solution was stored overnight at 4 °C. The next day, the solution was shaken at 25 rpm for 1 h in a bath at $T = 298.15$ K. The suspension was then filtered and washed with additional phosphate buffer. The immobilized enzyme on the CPG beads was stored at 4 °C, again in the same buffer.

Equilibrium measurements were carried out by approaching equilibrium from both directions of reaction. (−)-Menthol and 1-dodecanoic acid were used for the forward direction, and (−)-menthyl dodecanoate was used for the reverse direction. Following dissolution of these substances in the organic solvents, ≈3 g of immobilized enzyme was added to each solution. Since water is essential

Table 2. Results of Equilibrium Measurements at T = 298.15 K for Esterification Reaction 1 in Hexane, Heptane, Cyclohexane, 2,2,4-Trimethylpentane, Toluene, Acetonitrile, and 2-Methyl-2-butanol[a]

direction	$10^3m(C_{12}H_{24}O_2)$/ mol (kg soln)$^{-1}$	$10^3m(C_{10}H_{20}O)$/ mol (kg soln)$^{-1}$	$10^3m(C_{22}H_{42}O_2)$/ mol (kg soln)$^{-1}$	$10^3m(H_2O)$/ mol (kg soln)$^{-1}$	K	K(combined)
			Hexane			
forward	5.55 ± 0.21	4.63 ± 0.25	15.28 ± 1.3	10.7 ± 0.6	6.4 ± 0.8	6.5 ± 0.6
reverse	4.42 ± 0.27	4.67 ± 0.19	13.07 ± 1.6	10.7 ± 0.6	6.8 ± 1.0	
			Heptane			
forward	5.00 ± 0.18	4.99 ± 0.07	16.2 ± 2.5	28.0 ± 1.2	18.2 ± 3.0	21.7 ± 2.2
reverse	2.66 ± 0.11	3.91 ± 0.12	9.6 ± 1.0	28.0 ± 1.2	25.8 ± 3.3	
			Cyclohexane			
forward	2.79 ± 0.15	4.15 ± 0.11	12.2 ± 1.2	24.6 ± 1.7	25.8 ± 3.5	23.7 ± 2.7
reverse	3.44 ± 0.14	3.43 ± 0.42	9.9 ± 1.5	24.6 ± 1.7	20.5 ± 4.3	
			2,2,4-Trimethylpentane			
forward	5.29 ± 0.26	3.59 ± 0.06	13.3 ± 0.7	24.2 ± 1.1	17.0 ± 1.5	16.2 ± 0.8
reverse	4.76 ± 0.06	4.53 ± 0.12	14.1 ± 0.3	24.2 ± 1.1	15.8 ± 0.9	
			Toluene			
forward	7.52 ± 0.11	7.53 ± 0.16	6.51 ± 0.50	92.0 ± 2.7	10.6 ± 0.9	12.0 ± 0.8
reverse	5.78 ± 0.08	6.03 ± 0.16	6.65 ± 0.60	92.0 ± 2.7	16.8 ± 1.7	
			Acetonitrile			
forward	14.86 ± 0.18	12.14 ± 0.12	0.0406 ± 0.0015	14960 ± 261	3.34 ± 0.14	3.23 ± 0.11
reverse	13.61 ± 0.17	13.42 ± 0.15	0.0362 ± 0.0021	15340 ± 78	3.04 ± 0.18	
			2-Methyl-2-butanol			
forward	16.43 ± 0.88	16.80 ± 0.66	0.1230 ± 0.0092	9649 ± 102	4.30 ± 0.43	5.82 ± 0.34
reverse	17.92 ± 0.43	17.45 ± 0.50	0.2532 ± 0.013	9806 ± 186	7.94 ± 0.52	

[a] The equilibrium constants K were calculated with eq 2. The initial molalities of (−)-menthol and 1-dodecanoic acid in the forward reaction mixtures were ≈0.013 mol kg^{-1}; for the reverse reaction mixtures, the molality of (−)-menthyl dodecanoate was ≈0.015 mol kg^{-1}. The molalities m for the solutes in solution at equilibrium are given in columns 2−5. $C_{12}H_{24}O_2$ is 1-dodecanoic acid, $C_{10}H_{20}O$ is (−)-menthol, and $C_{22}H_{42}O_2$ is (−)-menthyl dodecanoate. K(combined) was calculated as the weighted average of the values of the equilibrium constants which were measured from both directions of the reaction. The uncertainties are based on two estimated standard deviations of the mean. Final uncertainties are given in the text (see Results and Discussion).

for carrying out this reaction from the reverse direction, organic solvents saturated with water were used for all solvents except for acetonitrile and 2-methyl-2-butanol. In these two cases, sufficient water for the reverse reaction was available either from the enzyme preparation or as an impurity in the solvent itself. Teflon-stoppered glass bottles containing these solutions were then placed in a shaker bath (25 rpm) at T = 298.15 K and allowed to equilibrate. The temperature of the shaker bath was held constant to within ±0.1 K. Equilibrium or near equilibrium was reached in 5−7 days in all solvents.

The reactions starting with {(+)-menthol + 1-dodecanoic acid} for the forward direction and with (+)-menthyl dodecanoate for the reverse direction of reaction were also carried out in cyclohexane, heptane, hexane, 2,2,4-trimethylpentane, and toluene. Only trace amounts of menthyl dodecanoate were obtained from the forward direction of reaction. Similarly, only trace amounts of menthol and 1-dodecanoic acid were seen from the reverse direction of reaction. These trace amounts are attributable to the presence of (−)-menthol in the (+)-menthol and of (−)-menthyl dodecanoate in (+)-menthyl dodecanoate samples. Thus, the reaction catalyzed by lipase is considered to be completely stereospecific for (−)-menthol and for (−)-menthyl dodecanoate.

The procedure used for the quantitative analysis of the reactants is now described. A standard solution of 1-dodecanoic acid, (−)-menthyl dodecanoate, 1-dodecanol, and (−)-menthol was prepared in hexane. Response factor ratios for (−)-menthol, (−)-menthyl dodecanoate, and 1-dodecanoic acid with respect to the internal standard 1-dodecanol were determined. For the analysis of 1-dodecanoic acid, (−)-menthol, and (−)-menthyl dodecanoate, 2.0 cm^3 of equilibrated organic phase and 100 μL of an internal standard solution containing 1-dodecanol in hexane were gravimetrically added to a vial that was then capped tightly. Approximately 0.6 μL of the solution was injected into the GC and analyzed. The molalities of (−)-menthol, (−)-menthyl dodecanoate, and 1-dodecanoic acid given in Table 2 were determined from their respective chromatographic areas, the response factor ratios, and the known molality of the internal standard. The chromatograms of the reaction mixtures using 2-methyl-2-butanol as the solvent did not have any additional peak(s) that could correspond to the formation of the 2-methyl-2-butyl ester of dodecanoic acid.

The molalities of water in acetonitrile, 2-methyl-2-butanol, and toluene were determined with a Metrohm Model 633 Karl Fischer titration apparatus and a Model 665 Dosimat. The Karl Fischer titration apparatus, enclosed in a Plexiglass box, was continuously purged with dry nitrogen. The instrument was calibrated with 1-octanol saturated with water. For this calibration, approximately 0.03 g of 1-octanol saturated with water was injected with a 50 μL syringe into the solution (methanol + Hydranal Composite 2) in the Karl Fischer apparatus. Reaction mixture samples (volume = 10−30 μL) were withdrawn carefully from the organic phase of the reaction mixtures with an airtight 50 μL syringe and injected into the (methanol + Hydranal Composite 2). The volume of Hydranal Composite 2 needed to react with the water in the sample was then measured with an appropriate correction for instrument drift. The molalities of water in the reaction mixtures are based on the solubility of water in 1-octanol at T = 298.15 K reported by Leo and Hansch (1971). The previously reported saturation molalities of water (Tewari, 1998) in hexane, heptane, cyclohexane, and 2,2,4-trimethylpentane were used in the calculation of equilibrium constants.

The saturation molalities m(sat) and (hexane + water) partition coefficients $K_{h/w}$ of (−)-menthol, 1-dodecanoic acid, and (−)-menthyl dodecanoate were also measured. The

Table 3. Saturation Molalities m(sat) in Water and (Hexane + Water) and Partition Coefficients $K_{h/w}$ of (−)-Menthol, 1-Dodecanoic Acid, and (−)-Menthyl dodecanoate at T = 298.15 K[a]

substance	m(sat)/mol kg^{-1}	$K_{h/w}$[b]
(−)-menthol	$(2.34 \pm 0.12) \times 10^{-3}$	732 ± 7
1-dodecanoic acid	$(3.11 \pm 0.26) \times 10^{-5}$ [c]	$(5.78 \pm 0.33) \times 10^{4}$ [d]
(−)-menthyl dodecanoate	$(9.83 \pm 0.78) \times 10^{-6}$	$(4.18 \pm 0.52) \times 10^{5}$

[a] The uncertainties are based on two estimated standard deviations of the mean. Final uncertainties are given in the text (see Results and Discussion). [b] The molalities of the solutes in the aqueous phases were as follows: (−)-menthol, $(7.37 \pm 0.12) \times 10^{-3}$ mol kg^{-1}; 1-dodecanoic acid, $(10.03 \pm 0.63) \times 10^{-6}$ mol kg^{-1}; and (−)-menthyl dodecanoate, $(1.61 \pm 0.25) \times 10^{-6}$ mol kg^{-1}. [c] This value was determined in a previous study (Tewari, 1998). [d] The value given in this table was obtained from the molality of aqueous 1-dodecanoic acid at pH = 5.22. This measured molality is the sum of the molalities of both the ionized and nonionized forms of 1-dodecanoic acid. The value $K_{h/w} = (1.79 \pm 0.25) \times 10^{5}$ pertains to the transfer of the nonionized form of 1-dodecanoic acid to hexane (see Results and Discussion).

saturation molalities of (−)-menthol and (−)-menthyl dodecanoate were determined by approaching the position of equilibrium from two different temperatures. For each compound, approximately 0.2 g of that substance and 45 g of water were added to two 50-cm^3 Erlenmeyer flasks which were sealed with ground-glass stoppers. One of these flasks was then placed in a constant-temperature shaker bath (25 rpm) at T = 288.15 K; the other flask was placed in a similar bath set at T = 308.15 K. After 24 h both flasks were placed in a single shaker bath at T = 298.15 K and allowed to equilibrate for an additional 6 days.

Approximately 35 g of the equilibrated aqueous phase was then carefully transferred into a Teflon bottle. Then 2.0 cm^3 of hexane and 100 μL of the internal standard solution in hexane were quantitatively added to the bottle. The contents of the bottles were shaken and then centrifuged at 2000 rpm for 15 min. The molality of the substance in the hexane layer was then analyzed by GC with the procedure described above.

For the measurement of $K_{h/w}$, approximately 0.5 g of the compound was quantitatively dissolved in 2.0 cm^3 of hexane in a 50-cm^3 Erlenmeyer flask and 40 g of water was added to the flask. Using a procedure similar to that used in the measurement of the saturation molalities, one of these flasks was then placed in a constant-temperature shaker bath (25 rpm) at T = 288.15 K and the other flask was placed in a bath set at T = 308.15 K. After 48 h, both flasks were placed in a single shaker bath at T = 298.15 K and allowed to equilibrate for an additional 6 days. The molality of the solute in the aqueous phase was then determined using the same methods used for the determination of the saturation molalities. The initial molality of the solute in the organic phase and the amounts of the two phases are well-known from its preparation by gravimetric methods. Therefore, by knowing the molality of the solute in the aqueous phase, a correction can be applied in order to calculate the final molality of each compound in the hexane phase.

Results and Discussion

The equilibrium constant K for reaction 1 is

$$K = a\{(-)\text{-menthyl dodecanoate}\}\cdot a(\mathrm{H_2O})/[a\{(-)\text{-menthol}\}\cdot a(\text{1-dodecanoic acid})] \quad (2)$$

where a is the activity of the indicated substance. Since the activity is the product of the molality m and the activity coefficient γ, the equilibrium constant for reaction 1 is

$$K = (m\{(-)\text{-menthyl dodecanoate}\}\cdot m(\mathrm{H_2O})/[m\{(-)\text{-menthol}\}\cdot m(\text{1-dodecanoic acid})])\cdot(\gamma\{(-)\text{-menthyl dodecanoate}\}\cdot\gamma(\mathrm{H_2O})/[\gamma\{(-)\text{-menthol}\}\cdot\gamma(\text{1-dodecanoic acid})]) \quad (3)$$

Here, the standard state used is the hypothetical ideal solution of unit molality. Since the molalities of (−)menthol, 1-dodecanoic acid, and (−)-menthyl dodecanoate in the organic solvents are ≤0.016 mol kg^{-1} and since they are almost certainly nonionized in these solvents, it is reasonable to assume that the ratio of the activity coefficients of the products to reactants, that is, the second term on the right-hand side of eq 3, will be close to unity. Thus, the thermodynamic equilibrium constant of the reaction can be written in terms of the molalities of the products and reactants

$$K = m\{(-)\text{-menthyl dodecanoate}\}\cdot m(\mathrm{H_2O})/[m\{(-)\text{-menthol}\}\cdot m(\text{1-dodecanoic acid})] \quad (4)$$

This equilibrium constant is symmetrical and dimensionless.

The molalities of the reactants and products as determined in the equilibrium measurements are given in Table 2. The molalities of (−)-menthol, dodecanoic acid, and (−)-menthyl dodecanoate are the averages of four or five measurements. The molality of (−)-menthol from the forward direction of reaction has been corrected for the presence of the (+)-menthol impurity (mole fraction = 0.018) in the sample. Similarly, the molality of (−)-menthyl dodecanoate in the reverse reaction mixture was corrected for the (+)-menthyl dodecanoate impurity (mole fraction = 0.018). Although the mole fractions of these impurities are small, these corrections were as large as 0.07K. The molalities of water in hexane, heptane, toluene, cyclohexane, and 2,2,4-trimethylpentane were taken from a previous study (Tewari, 1998). The molalities of water in toluene, acetonitrile, and 2-methyl-2-butanol are the average of three measurements for each forward and reverse reaction mixture. The reported equilibrium constants K(combined) are the weighted averages of the results obtained from the forward and the reverse directions of the reaction.

The results of the measurements of the saturation molalities m(sat) in water and of the (hexane + water) partition coefficients $K_{h/w}$ of (−)-menthol, 1-dodecanoic acid, and (−)-menthyl dodecanoate at T = 298.15 K are given in Table 3. In all cases, the results obtained by approaching equilibrium from the two different initial temperatures were either in agreement or near agreement with each other.

The uncertainties given in Tables 2 and 3 are based only on the random errors in the measurements expressed as two estimated standard deviations of the mean. We now consider possible systematic errors in the measurements. We judge that reasonable estimates of the standard uncertainties (Taylor and Kuyatt, 1994) due to possible systematic errors in the values of the equilibrium constant K for reaction 1 are 0.03K in the moisture determinations; 0.05K in the GC measurements of the molalities of (−)-menthol, 1-dodecanoic acid, and (−)-menthyl dodecanoate; 0.01K for sample impurities; and $0.25|K(\text{forward}) - K(\text{reverse})|$ due to a possible failure to reach equilibrium. Similarly, estimates of the standard uncertainties in the saturation molalities m(sat) due to possible systematic errors are $0.03m$(sat) in the measurements of the molalities of the substances; $0.01m$(sat) for sample impurities; and $0.25|m(\text{sat, high } T) - m(\text{sat, low } T)|$ due to a possible failure to reach equilibrium. Here m(sat, high T) and m(sat, low T) refer respectively to the results obtained for each compound from the two solutions equilibrated first at T = 308.15 K and then at T = 288.15 K. Estimates of standard uncertainties in the values of the (hexane + water) partition coefficients $K_{h/w}$ are $0.03K_{h/w}$ in the measurements of the molalities of the substances; $0.01K_{h/w}$ for sample impurities; and $0.25|(K_{h/w}, \text{high } T) - K_{h/w}(\text{sat, low } T)|$ due to a possible failure to reach equilibrium.

These estimates of possible systematic error are combined in quadrature together with the statistical uncertainties in the measured values of these quantities, expressed as one estimated standard deviation of the mean, to obtain combined standard uncertainties (Taylor and Kuyatt, 1994). These combined standard uncertainties are then multiplied by 2 to arrive at a final set of results with somewhat larger estimates of total error. Thus, the final values of the equilibrium constant K for reaction 1 in the various solvents are (6.5 ± 1.0) for hexane; (21.7 ± 5.1) for heptane; (23.7 ± 4.7) for cyclohexane; (16.2 ± 2.1) for 2,2,4-trimethylpentane; (12.0 ± 3.5) for toluene; (3.2 ± 0.4) for acetonitrile; and (5.8 ± 2.0) for 2-methyl-2-butanol. The final values of the saturation molalities m(sat) in water are $(2.34 \pm 0.35) \times 10^{-3}$ mol kg^{-1} for (−)-menthol and $(9.8 \pm 1.7) \times 10^{-6}$ mol kg^{-1} for (−)-menthyl dodecanoate. The final values of the (hexane + water) partition coefficient $K_{h/w}$ are (731 ± 88) for (−)-menthol; $(5.8 \pm 0.8) \times 10^4$ for 1-dodecanoic acid; and $(4.2 \pm 0.8) \times 10^5$ for (−)-menthyl dodecanoate.

It is seen that the molality of water plays an important role in determining the extent of ester formation. In acetonitrile and 2-methyl-2-butanol, which have a very high molality of water, the formation of (−)-menthyl dodecanoate is considerably smaller. However, in hydrophobic solvents with lower molalities of water, the formation of (−)-menthyl dodecanoate is favored in the reaction mixture. The value of the equilibrium constant varies from 3 to 26. However, the molality of (−)-menthyl dodecanoate changes from 0.034 mmol kg^{-1} in acetonitrile to 16 mmol kg^{-1} in heptane. The change in product yield is a factor of ≈500 and is a striking example of the utility of organic solvents in biocatalysis.

The (−)-menthol and (−)-menthyl dodecanoate are assumed to exist only as the neutral species both in the organic solvents and in the aqueous phase. The measured molality of 1-dodecanoic acid includes both the ionized and nonionized forms of this acid. Thus, the measured value $K_{h/w} = (5.8 \pm 0.8) \times 10^4$ pertains to this mixture of species and is therefore an apparent equilibrium constant. To obtain a value of $K_{h/w}$ which pertains only to the nonionized form of 1-dodecanoic acid, we have used the pH = 5.22 at which the experiments were performed together with the value pK = 4.9 for 1-dodecanoic acid (Tewari, 1998) to calculate the fractions of these two species. The corrected value of $K_{h/w}$ which pertains to the transfer of the nonionized form of 1-dodecanoic acid is $(1.79 \pm 0.25) \times 10^5$. We now consider the following chemical reference reaction in aqueous solution

$$(-)\text{-menthol(aq)} + \text{1-dodecanoic acid(aq)} = (-)\text{-menthyl dodecanoate(aq)} + \mathrm{H_2O(l)} \quad (5)$$

The charges on all of the substances in reaction 5 are zero and therefore are omitted. The equilibrium constant K_m (molality basis) for this reaction is

$$K_m = m\{(-)\text{-menthyl dodecanoate}\}\cdot m°/[m\{(-)\text{-menthol}\}\cdot m(\text{1-dodecanoic acid})] \quad (6)$$

The standard molality ($m°$ = 1 mol kg^{-1}) has been used in the above equation to keep the equilibrium constant dimensionless. The partition coefficients $K_{h/w}$ pertain to the following equilibria:

$$(-)\text{-menthol(aq)} = (-)\text{-menthol(hex)} \quad (7)$$

$$\text{1-dodecanoic acid(aq)} = \text{1-dodecanoic acid(hex)} \quad (8)$$

$$(-)\text{-menthyl dodecanoate(aq)} = (-)\text{-menthyl dodecanoate(hex)} \quad (9)$$

Here, hex denotes hexane. Also, needed in the thermochemical cycle is the value of the standard molar Gibbs free energy change $\Delta_r G°_m$ for reaction 1 in hexane, which was determined in this study, and the solubility of water in hexane:

$$\mathrm{H_2O(l)} = \mathrm{H_2O(hex)} \quad (10)$$

$\Delta_r G^\circ_m$ for reaction 5 can then be calculated with

$$\Delta_r G^\circ_m(5) = \Delta_r G^\circ_m(1) + \Delta_r G^\circ_m(7) + \Delta_r G^\circ_m(8) - \Delta_r G^\circ_m(9) - \Delta_r G^\circ_m(10) \quad (11)$$

The values of $K_{h/w}$ obtained above were used to calculate values of $\Delta_r G^\circ_m$ for reactions 7−9. In addition, the value of the equilibrium constant for reaction 1 in hexane was used for the calculation of $\Delta_r G^\circ_m(1)$. The saturation molality of water in hexane (see above) was used to calculate $\Delta_r G^\circ_m$ for reaction 10. This thermochemical cycle calculation gives $\Delta_r G^\circ_m = -(30.1 \pm 0.8)$ kJ mol^{-1} for reaction 5 and the corresponding equilibrium constant $K_m = (1.9 \pm 0.6) \times 10^5$. These calculations show that, in aqueous solution, the formation of menthyl dodecanoate is favored over hydrolysis. Also, the equilibrium constant for reaction 5 is significantly larger than the equilibrium constants for reaction 1 in the organic solvents. Both findings are contrary to what has been previously found (Tewari et al., 1995; Tewari et al., 1996) for other esterification reactions, but the result is consistent with that recently obtained (Tewari, 1998) for the similar reaction

$$\text{1-dodecanol(aq)} + \text{1-dodecanoic acid(aq)} = \text{dodecyl dodecanoate(aq)} + \mathrm{H_2O(aq)} \quad (12)$$

We now consider some results from the literature (Kamiya et al., 1995; Lokotsch et al., 1989; Stamatis et al., 1993) that are pertinent to the present study. The aim of these previous investigations was to demonstrate both the feasibility and the extent of reaction. Thus, in these studies, the position of equilibrium was not approached from both directions and equilibrium constants were not calculated. In one investigation, Kamiya et al. (1995) studied the lipase-catalyzed formation of (−)-menthyl dodecanoate from (−)-menthol and 1-dodecanoic acid. They reported the percent formation of (−)-menthyl dodecanoate as a function of time, from which we obtain an extrapolated mole fraction conversion in 2,2,4-trimethylpentane of ≈0.92 at T = 308.15 K. Using the value of the saturation molality obtained in this study and assuming an equimolar concentration of (−)-menthol and 1-dodecanoic acid, we calculate

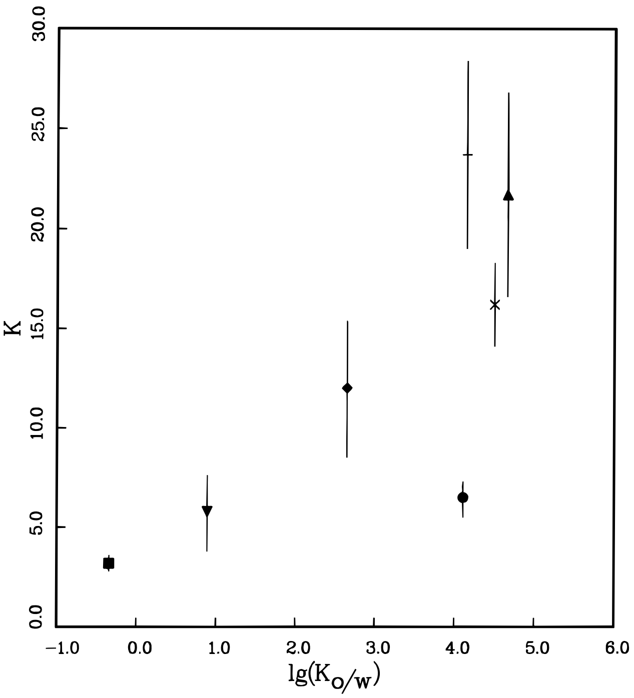

Figure 2. Equilibrium constants K for reaction 1 versus the decadic logarithm of the (octanol + water) partition coefficients $K_{o/w}$ of the seven solvents used in this study. The solvents and their respective symbols are as follows: hexane, ●; heptane, ▲; cyclohexane, +; 2,2,4-trimethylpentane, ×; toluene, ◆; acetonitrile, ■; 2-methyl-2-butanol, ▼.

the equilibrium constant for the reaction to be 3.1. This value is not too different from the result of the present study (K = 16.2). Also, the fact that it is lower than our result is consistent with equilibrium not having been obtained in their (Kamiya et al., 1995) experiments.

In the remaining two studies, the reactions are different from reaction 1, the subject of the current study, but are structurally similar esterification reactions of (−)-menthol. Thus, Lokotsch et al. (1989) studied the esterification of (−)-menthol with triacetin in 2,2,4-trimethylpentane. Using the mole fraction x of (−)-menthyl acetate that was formed ($x = 0.66$) and assuming an equimolar starting mixture of (−)-menthol and triacetin, we calculate an equilibrium constant of 3.64 at T = 303.15 K. Also, Stamatis et al. (1993) studied the esterification of (−)-menthol with several fatty acids. They plotted the mole fraction formation of (−)-menthyl oleate in 2,2,4-trimethypentane as a function of time at T = 310.15 K; the extrapolated mole fraction of (−)-menthyl oleate formed is 0.824. With the value of the saturation molality determined in the present study and again assuming equimolar concentrations of (−)-menthol and oleic acid, we obtain an equilibrium constant of ≈0.64. The values obtained from the studies of Lokotsch et al. (1989) and of Stamatis et al. (1993) are also comparable with the result of the present study, that is, K = 16.2 for reaction 1 carried out in 2,2,4-trimethypentane.

Previous investigations (Valivety et al., 1993; Janssen et al., 1993; Tewari, 1998) of enzyme-catalyzed reactions in organic solvents have sought to correlate the results as a function of some parameter that is a measure of the hydrophobicity of the solvent. Shown in Figure 2 is a plot of the equilibrium constant for reaction 1 versus the decadic logarithm of the (octanol + water) partition coefficients $K_{o/w}$ for the pertinent solvents. The values of $K_{o/w}$ for hexane, heptane, and toluene are from Tewari et al. (1982), the value of $K_{o/w}$ for cyclohexane is from Hansch and Leo (1979), the value of $K_{o/w}$ for 2,2,4-trimethylpentane is from Valivety et al. (1991), and the values of $K_{o/w}$ for acetonitrile and 2-methyl-2-butanol are from Hansch and Anderson (1967). It is seen that there is a good correlation between the equilibrium constants and the (octanol + water) partition coefficients ($K_{o/w}$) of the solvents. Indeed, a linear fit gives a good representation of the results with the exception of that obtained with hexane. Equilibrium constants for reaction 1 are plotted in Figure 3 against the relative permittivities (ϵ) of the pertinent solvents. The relative permittivities of hexane, heptane, toluene, cyclohexane, and 2,2,4-trimethylpentane are taken from Dreisbach (1955), and the values for acetonitrile and 2-methyl-2-butanol are from the *CRC Handbook of Chemistry and Physics* (Lide, 1997). Here, only a trend is demonstrated and no linear relationship is seen.

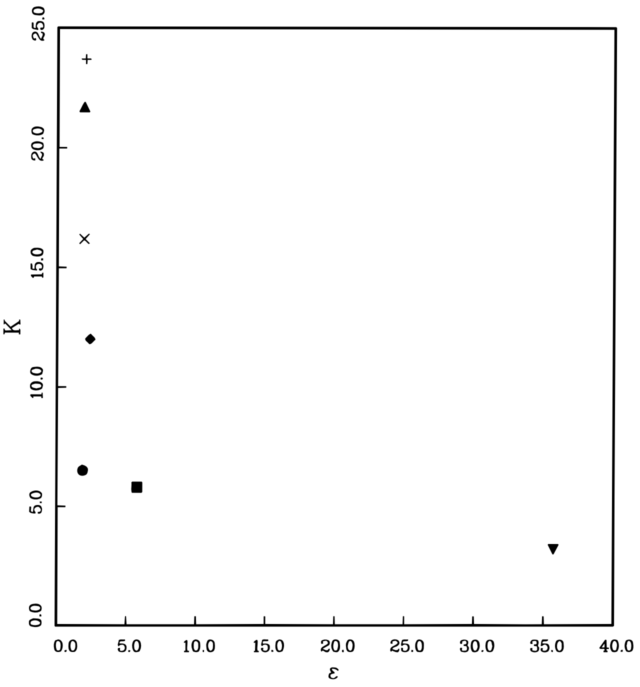

Figure 3. Equilibrium constants K for reaction 1 versus the relative permittivities (ϵ) of the pertinent solvents. The solvents and their respective symbols are as follows: hexane, ●; heptane, ▲; cyclohexane, +; 2,2,4-trimethylpentane, ×; toluene, ◆; acetonitrile, ■; 2-methyl-2-butanol, ▼. The relative permittivities of the solvents used in this study are as follows: hexane, 1.88; heptane, 1.92; cyclohexane, 2.01; toluene, 2.37; 2,2,4-trimethylpentane, 1.94; acetonitrile, 35.7; 2-methyl-2-butanol, 5.78.

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