Sucrose Solubility in Mixtures of Water, Alcohol, Ester, and Acid

P. Tsavas, S. Polydorou, E. C. Voutsas, and K. G. Magoulas*

Thermodynamics and Transport Phenomena Laboratory, Department of Chemical Engineering, National Technical University of Athens, 9, Heroon Polytechniou Str., Zographou Campus, 15780 Athens, Greece

K. Naraghi and P. J. Halling

Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Grahan Building 295, Cathedral Street, Glasgow, G1 1XL, Scotland, UK

Sucrose solubility was measured in binary and ternary mixtures of alcohol, acid, ester, and water at different temperatures. New data were measured for the solubility of sucrose in mixtures of 2-methyl-2-butanol (*tert*-pentanol) with water at 30 °C and 60 °C, propionic acid with water at 30 °C, methyl and ethyl acetate with water at 40 °C and 50 °C, and octanoic acid with *tert*-pentanol at 60 °C and in diacetone alcohol at 60 °C. Solubility measurements were also performed in multicomponent mixtures similar to those encountered in the enzymatic synthesis of fatty acid sucrose esters. Sucrose solubility was measured in mixtures of water with *tert*-pentanol and sucrose palmitate and in mixtures of water with *tert*-pentanol, sucrose palmitate and palmitic acid at constant water activity ($a_w = 0.1$) at 60 °C. Little solubilization of sucrose in small chain esters and *tert*-pentanol was observed. The solubility of sucrose in *tert*-pentanol increases in the presence of sucrose ester and the opposite effect in the presence of palmitic acid.

Introduction

Fatty acid sugar esters are used as detergents or emulsifiers in food, cosmetics, and pharmaceutical industries.[1] Their synthesis through enzymatic reactions in nonaqueous media[2−4] has received a growing interest because it operates with higher specificity and under milder conditions compared with classical chemical synthesis. As a result the products contain fewer, completely biodegradable, components.

To design bioreactors for such lipase-catalyzed sugar esterification, we need to understand effects on the kinetics and equilibrium of the reaction. These effects will be influenced by the thermodynamics of the reaction mixture, which contains sugar, fatty acid, ester product, water, and the organic solvent. Solvation interactions in this mixture are also important for the design of separation processes for the purification of products.

Modeling of these mixtures to predict solvation behavior is not easy, because of the presence of the sugar moieties. These contain many hydroxyl groups close to each other, with the possibility of intramolecular interactions, which must be considered in any model. A further complication is the possibility that the sugar ester molecules will show surfactant behavior in the organic reaction mixture. The accurate modeling of these sugar esterification reaction mixtures requires a considerable amount of experimental phase equilibrium data. Although there is a satisfactory amount of experimental phase equilibrium data[5−9] and thermodynamic models[10,11] applicable in aqueous solutions of sugars, very little is available for nonaqueous media.[12−13]

* Corresponding author. Telephone: +301 07723152. Fax: +301 07723155. E-mail: mag@chemeng.ntua.gr.

For the extension of the database, in this work new data were measured for the solubility of sucrose in mixtures with the following:

(a) *tert*-pentanol with water at 30 °C and 60 °C;

(b) propionic acid with water at 30 °C;

(c) methyl and ethyl acetate with water at 40 °C and 50 °C;

(d) octanoic acid with *tert*-pentanol at 60 °C;

(e) diacetone alcohol at 60 °C;

(f) sucrose palmitate, palmitic acid, water. and *tert*-pentanol at 60 °C;

Experimental Section

Materials. Sucrose, extra pure, and diacetone alcohol (99.5% purity) were purchased from Riedel de Haen, and 2-methyl-2-butanol (*tert*-pentanol) (99% GC grade), sucrose (99+%, used for the measurements on the full reaction mixture), and palmitic acid (99%), from Aldrich. Propionic acid (99%) and methyl acetate (99.5%) were purchased from Fluka, octanoic acid (99%) was purchased from Sigma, and ethyl acetate (99.8%) and water (HPLC grade) were purchased from LabScan. The sucrose palmitate was product P-1670 from Ryoto, specified as 80% monoester and 20% higher esters.

Analytical Methods and Equipment. Determination of sucrose solubility in mixtures of *tert*-pentanol with water and in diacetone alcohol was performed by HPLC. The system used consisted of an ICI-LC1110 pump, a GBC-LC1240 refractive index detector, and a Lichrosorb NH_2 250 × 4.0 mm column (Bischoff). The eluent used was a mixture of acetonitrile and water (80/20 v/v). The conditions used were 1 mL/min flow rate, temperature 30 °C, and the injection volume was 50 μL.

10.1021/je010280d CCC: $22.00
Published on Web 03/27/2002

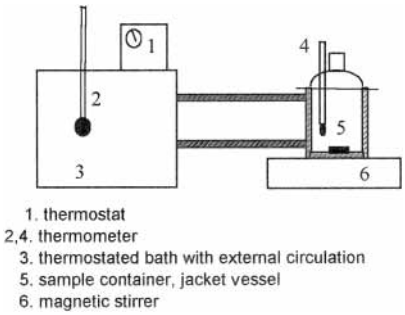

Figure 1. Solubility measurements experimental setup.

Dissolved sucrose concentrations in the complete reaction mixtures and in methyl acetate were determined by a similar HPLC method using the same column. The instrument had a Thermo-separation LC pump and a Spectra system RI-150 detector. The mobile phase was a mixture of ethanol and water (90/10 v/v), with column temperature 40 °C and injection volume 15 μL. Sucrose was dissolved in the eluent mixture, and the solutions were used as standards for the calibration curves.

The solubility of sucrose in mixtures of propionic acid or ester with water and octanoic acid with *tert*-pentanol was determined enzymically.[14] Sucrose was hydrolyzed in 1.2 M HCl for 1 h at 50 °C and neutralized with 1.2 M NaOH. The solubility of sucrose was determined by measuring glucose with a glucose oxidase-chromogen reagent (Sigma Chemical).

Solubility Measurements. The experimental setup used for the solubility measurements is presented in Figure 1. One to four jacketed vessels of about 150 cm^3 were loaded with the solvent mixtures. Each mixture was prepared by weighting the desired amount of each solvent with a 0.1 mg precision balance. The accuracy in mixture composition was 0.1% in mass fraction. The temperature was set at the desired level and the solvent was added to the vessels. Once the desired temperature was reached, excess sucrose over the expected solubility was added to the solution. At constant temperature, monitored by the thermometer (± 0.1 °C), the solution was stirred (600−800 rpm) with a magnetic stirrer, until equilibrium was reached. Samples of the solution were withdrawn, from the top of the thermostated bath, at 24 h intervals using pipets with a slightly higher temperature than the solution temperature in order to avoid any precipitation. The samples are filtered with prewarmed 0.22 μm nylon filters (polypropylene housing). When the difference in the value of sucrose solubility (g/L) in 24 h intervals was less than 2% (uncertainty of the analytical method) equilibrium was considered to have been reached. Then the solution was allowed to stand at constant temperature for about 24 h to enable any finely dispersed solids to settle down and samples were taken with and without filtering. For the measurements on the complete reaction mixture, the following procedure was used. All components were first dried over molecular sieves (4 Å) for at least 1 week. A solution of known concentrations of palmitic acid and sucrose ester in *tert*-pentanol was placed in a vial with excess solid sucrose and a magnetic stirrer bar. The vial (with an open top) was then placed inside a large jar containing an agent to control humidity. The large jar was then sealed with a lid, and the whole apparatus placed in a glovebox with controlled air temperature of 60 °C. A magnetic stirrer underneath drives the bar in the test mixture. The experimental setup used is presented in Figure 2. Materials used to control humidity were molecular sieves (water activity, a_w, close to 0), and saturated solutions (solid-rich slush) of LiCl (a_w = 0.11) or K_2CO_3 (a_w = 0.43).[15]

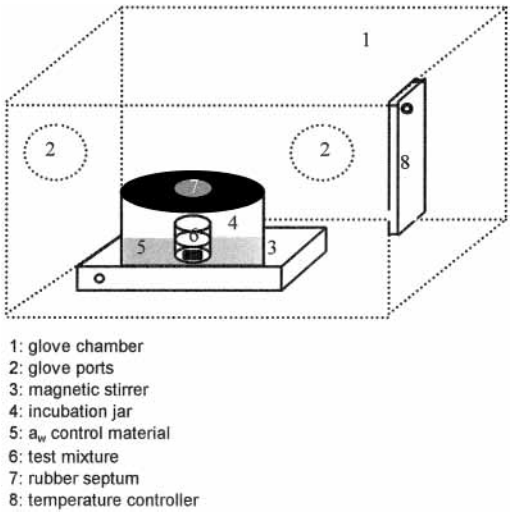

Figure 2. Solubility measurements experimental setup at controlled water activity.

Table 1. Solubility of Sucrose (1) in *tert*-Pentanol (2) + Water (3) Mixtures[a]

t / °C	$100w_3$	$100w_2$	S / g·L^{-1}	σ
30	0.20	99.80	0.21	0.030
30	1.98	98.02	0.96	0.011
30	5.13	94.87	1.85	0.040
60	0.20	99.80	0.42	0.009
60	1.99	98.01	1.78	0.029
60	5.12	94.88	2.90	0.050

[a] w_i is the mass fraction of component i in sugar free base, S is the solubility of sucrose in the mixture (mass of sucrose/volume of solution), and σ is the standard deviation of the measurement.

To avoid temperature changes, sampling was done inside the glovebox, with all apparatus preequilibrated at 60 °C. Samples were filtered through 0.22 μm nylon filters. For water measurements, a syringe was used to inject 50−100 μL directly and immediately into a coulometric Karl Fischer instrument. Samples of 100 μL for HPLC measurement were transferred to vials, which could then be allowed to cool. Additional solvent was added, keeping all components in solution. Analysis of samples at various times showed that 24 h was normally necessary for equilibration.

Results and Discussion

Solubility in tert-pentanol + water mixtures. Sucrose solubility in *tert*-pentanol is a bottleneck for the scale-up of the enzymatic synthesis of sucrose esters, since it affects the equilibrium conversion and the reaction rate.[16,17] The solubility of sucrose in *tert*-pentanol at 30 °C and 60 °C is presented in Table 1. To ensure that equilibrium was reached the dissolution rate in *tert*-pentanol was measured at both temperatures (Figure 3). It can be seen that equilibrium was reached within about 24 h of stirring. The effect of water on sucrose solubility in *tert*-pentanol is also presented in Table 1.

The experimental measurements of sucrose solubility in mixtures of water with *tert*-pentanol show that the addition of water at relative low concentrations increases signifi-

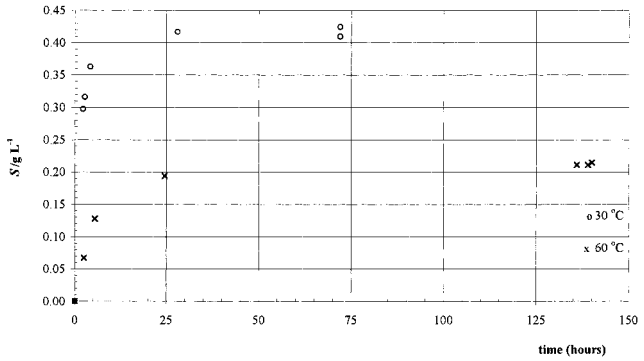

Figure 3. Dissolution rate of sucrose in *tert*-pentanol.

Table 2. Solubility of Sucrose (1) in Propionic Acid (2) + Water (3) Mixtures at 30 °C[a]

$100w_2$	$100w_3$	S / g·L^{-1}	σ
100.00	0.00	0.3	0.003
80.04	19.96	135.2	2.569
69.97	30.03	196.8	2.165

[a] w_i is the mass fraction of component i in sugar free base, S is the solubility of sucrose in the mixture (mass of sucrose/volume of solution), and σ is the standard deviation of the measurement.

Table 3. Solubility of Sucrose (1) in Octanoic Acid (2) + *tert*-Pentanol (3) Mixtures at 60 °C[a]

$100w_2$	$100w_3$	S / g·L^{-1}	σ
40.02	59.98	0.27	0.006
30.06	69.94	0.31	0.006
10.08	89.92	0.39	0.004

[a] w_i is the mass fraction of component i in sugar free base, S is the solubility of sucrose in the mixture (mass of sucrose/volume of solution), and σ is the standard deviation of the measurement.

cantly the solubility of sucrose. For example with the addition of 2 mass % water to *tert*-pentanol, sucrose solubility is four times higher than the solubility in pure *tert*-pentanol.

Solubility in acid + water and acid + alcohol mixtures. The solubility of sucrose in mixtures of propionic acid at 30 °C with water and octanoic acid with *tert*-pentanol at 60 °C was measured. The results are presented in Tables 2 and 3. In these mixtures, where sucrose hydrolysis can take place, sucrose analysis was performed by the enzymatic method. For the analysis of sucrose with the enzymatic method, an aliquot (0.1 mL) of the sample (1 mL) was first hydrolyzed in 1.2 M HCl for 1 h at 50 °C and then neutralized with 1.2 M NaOH, and the initial glucose content of the sample was analyzed. If glucose content in the sample was lower than 0.1 mass %, which is the percentage of glucose impurity in sucrose, then it is considered that hydrolysis did not take place and sucrose solubility was measured.

In the mixtures examined, with the experimental methodology used for the solubility measurements, hydrolysis took place only in those mixtures containing acetic acid, and therefore, solubility measurements in these mixtures were not possible with the procedure used.

From the experimental results it is shown that the addition of water increases the solubility of sucrose in propionic acid and the presence of octanoic acid in *tert*-pentanol decreases sucrose solubility.

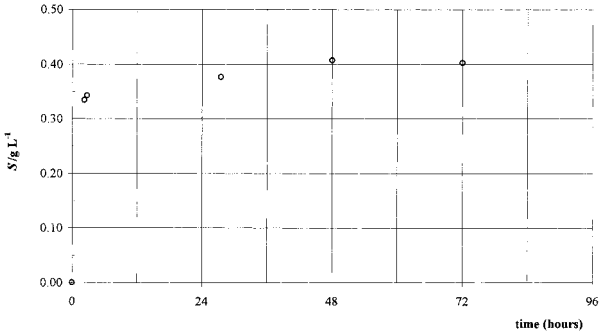

Figure 4. Sucrose dissolution in diacetone alcohol at 60 °C.

Table 4. Solubility of Sucrose (1) in Methyl Acetate (2) + Water (3) Mixtures[a]

t / °C	$100w_2$	$100w_3$	S / mg·L^{-1}	σ	anal. method
40	100.0	0.0	3.40	0.340	HPLC
40	100.0	0.0	3.57	0.093	enzymatic
40	98.0	2.0	4.89	0.112	enzymatic
50	100.0	0.0	9.17	0.174	enzymatic
59	100.0	0.0	17.10	0.855	HPLC
65	97.5	2.5	78.70	3.935	HPLC

[a] w_i is the mass fraction of component i in sugar free base, S is the solubility of sucrose in the mixture (mass of sucrose/volume of solution), and σ is the standard deviation of the measurement.

Table 5. Solubility of Sucrose (1) in Ethyl Acetate (2) + Water (3) Mixtures[a]

t / °C	$100w_2$	$100w_3$	S / mg·L^{-1}	σ
40	98.75	1.25	3.63	0.062
50	100.00	0.00	3.21	0.074
50	98.50	1.50	9.87	0.247

[a] w_i is the mass fraction of component i in sugar free base, S is the solubility of sucrose in the mixture (mass of sucrose/volume of solution), and σ is the standard deviation of the measurement.

Solubility in ester + water mixtures. Sucrose solubility in mixtures of methyl acetate and ethyl acetate with water at 40 °C and 50 °C was measured. The results are presented in Tables 4 and 5.

As shown, sucrose is more soluble in methyl acetate than in ethyl acetate. It should be noted that the solubilities of sucrose in mixtures of methyl and ethyl acetate are reported in milligrams/liter.

Solubility in Diacetone Alcohol. Diacetone alcohol is a potential solvent for the synthesis of fatty acid sucrose esters.[17] For that reason the dissolution rate and the solubility of sucrose in diacetone alcohol at 60 °C was measured (Figure 4). The solubility of sucrose in diacetone alcohol is 0.4 g/L, which is very close to the solubility of sucrose in *tert*-pentanol.

Solubility in Multicomponent Mixtures. Sucrose solubility measurements were performed in mixtures similar to those encountered in enzymatic synthesis of fatty acid sucrose esters. Mixtures of defined composition in solvent, fatty acid and sugar ester were equilibrated at 60 °C with excess solid sugar at controlled water activity (a_w), equal to 0.1. The water activity of the system was controlled by equilibration with a vapor phase of known relative humidity. Two sets of experiments were performed. In the first set no acid (palmitic acid) was present in the mixture. The results are presented in Table 6 and Figure 5. The

Table 6. Solubility of Sucrose (1) in Water (2) + *tert*-Pentanol (3) + Sucrose Palmitate (4) Mixtures (a_w = 0.1, T = 60 °C)[a]

$100w_2$	$100w_3$	$100w_4$	S / g·L^{-1}
0.55	99.45	0.00	0.39
0.53	92.50	6.96	0.62
0.60	86.40	13.00	0.85
0.61	81.08	18.31	0.86
0.58	76.42	23.00	0.97
0.57	72.24	27.19	0.90
0.60	68.48	30.92	1.27
0.54	62.08	37.38	1.43
0.58	56.72	42.70	1.85

[a] w_i is the mass fraction of component i in sugar free base, and S is the solubility of sucrose in the mixture (mass of sucrose/volume of solution).

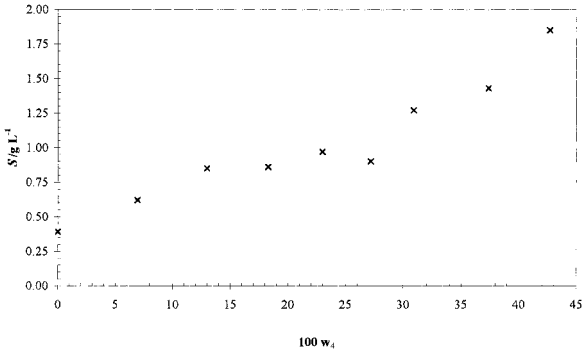

Figure 5. Solubility of sucrose (1) in the following reaction mixture (excluding acid): water (2) + *tert*-pentanol (3) + sucrose palmitate (4), a_w = 0.1, t = 60 °C.

Table 7. Solubility of Sucrose (1) in Water (2) + *tert*-Pentanol (3) + Sucrose Palmitate (4) + Palmitic Acid (5) Mixtures (a_w = 0.1, T = 60 °C)[a]

$100w_2$	$100w_3$	$100w_4$	$100w_5$	S / g·L^{-1}
0.46	85.36	0.00	14.18	0.16
0.45	80.19	6.03	13.32	0.39
0.46	75.60	11.38	12.55	0.47
0.45	71.53	16.15	11.87	0.46
0.49	67.83	20.42	11.27	0.54
0.46	64.54	24.28	10.71	0.66
0.41	61.53	27.79	10.22	0.63
0.40	56.29	33.89	9.35	0.88
0.42	51.85	39.03	8.61	1.16

[a] w_i is the mass fraction of component i in sugar free base, S is the solubility of sucrose in the mixture (mass of sucrose/volume of solution).

effect of the addition of palmitic acid on the solubility of sucrose in mixtures of *tert*-pentanol, sucrose palmitate, and water at fixed water activity is presented in Table 7 and Figure 6.

From the results obtained, it is shown that a decrease about 35% in sucrose solubility is observed with the addition of palmitic acid. This result agrees with the experimental results of sucrose solubility in mixtures of octanoic acid with *tert*-pentanol. The presence of sucrose palmitate favors the solubilization of sucrose and water. With increasing sucrose palmitate concentration the solubility of sucrose increases (Figures 5 and 6). It was also observed that sucrose is more soluble in *tert*-pentanol with the addition of esters of sucrose with long chain acids but very little solubilization of sucrose in methyl and ethyl acetate was observed.

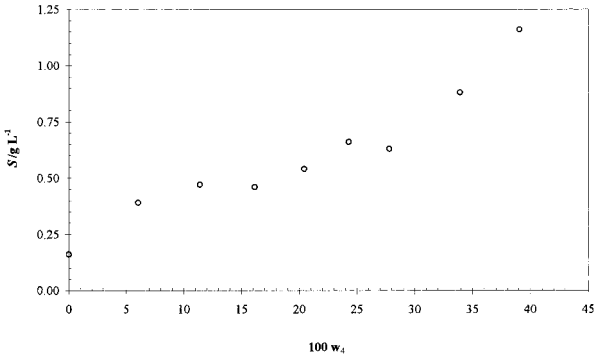

Figure 6. Solubility of sucrose (1) in the following reaction mixture: water (2) + *tert*-pentanol (3) + sucrose palmitate (4) + palmitic acid (5), a_w = 0.1, t = 60 °C.

Conclusions

In this work new data were measured for the solubility of sucrose in mixtures of *tert*-pentanol with water at 30 °C and 60 °C, propionic acid with water at 30 °C, methyl and ethyl acetate with water at 40 °C and 50 °C, octanoic acid with *tert*-pentanol at 60 °C, diacetone alcohol at 60 °C, and sucrose fatty acid esters, palmitic acid, water and *tert*-pentanol at 60 °C.

Sucrose solubility in mixtures of *tert*-pentanol with water increases significantly at relative low water concentrations (2 mass %). The addition of water increases also, the solubility of sucrose in propionic acid and the presence of octanoic acid in *tert*-pentanol decreases sucrose solubility. Addition of sucrose palmitate in *tert*-pentanol favors the solubilization of sucrose and water. With increasing sucrose palmitate concentration the solubility of sucrose increases. Sucrose is more soluble in *tert*-pentanol with the addition of esters of sucrose with long chain acids but very little solubilization of sucrose in methyl and ethyl acetate was observed.

Literature Cited

(1) Akoh, C. C.; Swanson, B. G. *Carbohydrate polyesters as fat substitutes*; Marcel Dekker: New York, 1994.

(2) Tsuzuki, W.; Kitamura, Y.; Suzuki, T.; Kobayashi, S. Synthesis of Sugar Fatty Acid Esters by Modified Lipases. *Biotechnol. Bioeng.* **1999**, *64*, 267−271.

(3) Degn, P.; Pedersen, L.; Zimmermann, W. Lipase-catalyzed synthesis of glucose fatty acid esters in *tert*-butyl alcohol. *Biotechnol. Lett.* **1999**, *21*, 275−280.

(4) Acros, J.; A., Bernabe, M.; Otero, C. Quantitative Enzymatic Production of 6-*O*-Acylglucose Esters. *Biotechnol. Bioeng.* **1998,** *57*, 505−509.

(5) Stephen, H.; Stephen, T. *Solubility of Inorganic and Organic Compounds, Parts1 and 2. Binary systems;* Pergamon Press: Oxford, England, 1963.

(6) Mullin, J. W. *Crystallization*; Butterworth: London, 2nd ed., 1972.

(7) Peres, A. M.; Macedo, E. A. Phase equilibria of D-glucose and sucrose in mixed solvent mixtures: Comparison of UNIQUAC-based models. *Carbohydr. Res.* **1997**, *303*, 135−151,

(8) Young, F. E.; Jones, F. T. Sucrose Hydrates. *J. Phys. Chem.* **1949**, *53*, 1334−1350.

(9) Haeseler, Von G.; Luca, S. F.; Misselhorn, K. *Branntweinwirtschaft* **1968**, *108*, 405−408,

(10) Achard, C.; Gros, J. B.; Dussap, C. G. *Cah. Sci.* **1992**, *10*, 93−101.

(11) Catte, M.; Dussap, C. G.; Gros, J. B. A physical chemical UNIFAC model for aqueous solutions of sugars. *Fluid Phase Equilib.* **1995**, *96*, 1−25.

(12) Peres, A. M.; Macedo, E. A. A modified UNIFAC model for the calculation of thermodynamic properties of aqueous and non-aqueous solutions containing sugars. *Fluid Phase Equilib.* **1997**, *139*, 47−74.

(13) Spiliotis, N.; Tassios, D. A UNIFAC model for phase equilibrium calculations in aqueous and nonaqueous sugar solutions. *Fluid Phase Equilib.* **2000**, *173*, 39−55.

(14) Christakopoulos, P.; Li, L. W.; Kekos, D.; Macris, B. J. Direct conversion of sorghum carbohydrate to ethanol by a mixed microbial culture. *Bioresour. Technol.* **1993**, *45*, 89−92

(15) Greenspan, L. Humidity fixed point of binary saturated aqueous solutions. *J. Res. Natl. Bur. Stand.* **1977**, *81A*, 89−95.

(16) Woudenberg van Oosterom, M.; van Rantwijk, F.; Sheldon, R. A. Regioselective acylation of disaccharides in *tert*-butyl alcohol catalyzed by *Candida antarctica* lipase. *Biotechnol. Bioeng.* **1996**, *49*, 328−333.

(17) Ferrer, M.; Cruces, M. A.; Bernabe, M.; Ballesteros, A.; Plou, F. J. Lipase-catalyzed regioselective acylation of sucrose in two-solvent mixtures. *Biotechnol. Bioeng.* **1999**, *65*, 10−16.

Received for review October 12, 2001. Accepted February 8, 2002. This research was supported by EC Biotechnology Project No. PL98-0363.

JE010280D