Production of Propylene Glycol Fatty Acid Monoesters by Lipase-Catalyzed Reactions in Organic Solvents

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Fatty acid monoesters of propylene glycol (1 ,2-propanediol) are good water-in-oil emulsifiers. These esters were synthesized enzymatically to overcome the problems associated with chemical processes. A *Pseudomonas* lipase was added to reaction mixtures containing propylene glycol and various acyl donors (fatty acids, fatty acid ethyl esters, fatty acid anhydrides and triglycerides) in organic solvents, and the mixtures were shaken at 30°C. The products were analyzed by gas chromatography. The yield of monoesters was affected by the acyl donors, organic solvents, temperature, water content, pH memory and reaction time. The anhydrous (lyophilized) enzyme and fatty acid anhydrides were best for monoester production. The optimum pH ranges were 4–5 and 8–10. The yields of propylene glycol monolaurate, monomyristate, monopalmitate, monostearate and monooleate with 50 mM fatty acid anhydrides as acyl donors were 97.2, 79.6, 83.7, 89.7 and 93.4 mM, respectively; those with 50 mM fatty acids as acyl donors were 37.3, 28.7, 28.7, 35.3 and 36.2 mM, respectively. The yields of propylene glycol monopalmitate, monostearate and monooleate with 50 mM triglycerides as acyl donors were 87.4, 65.1 and 83.2 mM, respectively.

KEY WORDS: Emulsifier, enzyme reaction in organic solvent, lipase, propylene glycol fatty acid monoester.

Propylene glycol (1,2-propanediol) monoesters are good water-in-oil emulsifiers with low hydrophilic–lipophilic balance values (1). They have been approved by the U.S. Food and Drug Administration (FDA) for use in foods (2) and are most often used in cakes, cake mixes, whipped toppings and bread (3). They can be used in combination with monoglycerides to obtain excellent cake batter behavior, resulting in increased cake volume and uniform structure. They are also good for whipped toppings due to their aerating and foam-stabilizing properties. Synthesis of propylene glycol monoesters by chemical methods, e.g., esterification of propylene glycol with fatty acids in the presence of acid or alkaline catalysts usually results in a complex mixture (4–6). Enzyme-catalyzed conversion may be more efficient and selective.

In the present work, we have discovered that a *Pseudomonas* lipase can catalyze the acylation of propylene glycol with fatty acids, fatty acid ethyl esters, anhydrides and triglycerides as acyl donors in anhydrous organic solvents for facile synthesis of propylene glycol monoesters. The effects of acyl donors, organic solvents, temperature, water content and pH memory were also investigated.

EXPERIMENTAL PROCEDURES

Materials. Lipase from *Pseudomonas* sp. (Amano "PS," specific activity 34 IU/mg solid) was obtained from Amano Pharmaceutical Co. (Nagoya, Japan). Standard propylene glycol monolaurate (PGML), dilaurate (PGDL), monomyristate (PGMM), dimyristate (PGDM), monopalmitate (PGMP), dipalmitate (PGDP), monostearate (PGMS), distearate (PGDS), monooleate (PGMO) and dioleate (PGDO) were prepared by refluxing an equal amount of propylene glycol and the respective fatty acid anhydride at 160–180°C for 10 h, and they were purified by silica gel 60 chromatography. These standards were identified and quantitated by gas chromatography as described in the Methods section. The retention times for PGML, PGDL, PGMM, PGDM, PGMP, PGDP, PGMS, PGDS, PGMO and PGDO were 3.06, 7.19, 3.25, 7.53, 4.01, 7.59, 3.17, 10.11, 3.64 and 10.69 min, respectively. Silica gel 60, palmitic acid, stearic acid, oleic acid, ethyl palmitate, hexane, toluene, chloroform, dibutyl ether, cyclohexane, *n*-heptane, octane and molecular sieve (0.3 nm) were obtained from Merck (Darmstadt, Germany). Propylene glycol, lauric acid, myristic acid, lauric anhydride, myristic anhydride, palmitic anhydride, stearic anhydride, oleic anhydride, ethyl laurate, ethyl stearate, triolein and olive oil were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Methods. For standard reaction, the commercial lipase powder (0.2 g) was added to a reaction mixture (4 mL) containing 500 mM propylene glycol and 50 mM acyl donor (fatty acids, acid anhydrides, fatty acid ethyl esters or triglycerides) in organic solvent. The reaction mixture was incubated in an orbital shaker with a speed of 250 rpm at 30°C. At various time intervals, 1 μL of the reaction mixture was withdrawn and analyzed by gas chromatography (Hitachi model G-3000; Hitachi, Tokyo, Japan) in a 3-m glass column packed with Supelco's (Bellefonte, PA) 3% OV101 on 80/100 chromosorb WHP (carrier 1.4 kg/cm^2 nitrogen; injector temperature 400°C; detector flame-ionization detector, temperature 350°C). The temperature program (20°C/min) was: 180 to 340°C for lauroyl esters; 200 to 340°C for myristoyl and palmitoyl esters; 220 to 340°C for oleoyl esters; 240 to 340°C for stearoyl esters. The product compositions (monoesters and diesters) were determined by comparing the peak area with calibration curves of the respective standards.

To study the effect of water content on the lipase-catalyzed synthesis, the enzyme was lyophilized by Savant Speed Vac Concentrator (Savant Instruments, Inc., Farmingdale, NY) under 50 millitorr for 24 h. Water was removed from organic media by 3 Å molecular sieves (Merck). To study the pH effect on synthesis, the lipase was dissolved in 10 mM mixed Good's buffer solution of different pH (10 mM each of BICINE, CAPS, sodium acetate and BIS-TRIS propane were mixed and adjusted to various pH values by either concentrated HCl or NaOH) and then lyophilized as described earlier.

RESULTS AND DISCUSSION

Among nine commercial lipases tested [Amano lipases AP-6, LP-101-S, MAP-10, PS, FAP-15 and N-Conc were obtained from Amano Pharmaceutical Co.; *Candida cylindracea* lipase Type VII and porcine pancreatic lipase were

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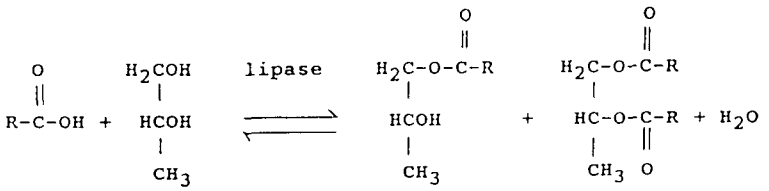
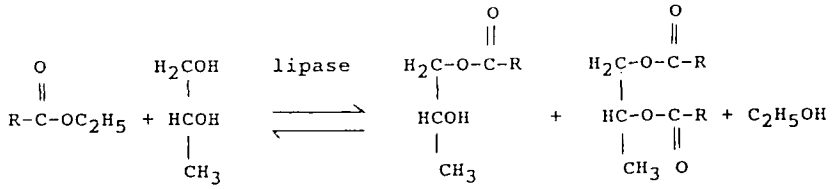
purchased from Sigma; Lipozyme was supplied by Novo Co., (Bagsvaerd, Denmark)], Amano "PS" (*Pseudomonas* sp.) lipase showed the best catalytic efficiency and specificity for enzymatic synthesis of propylene glycol monoester. Scheme 1 illustrates the routes whereby the *Pseudomonas* lipase can catalyze sequential acylation of propylene glycol with various acyl donors, including fatty acids, fatty acid ethyl esters, fatty acid anhydrides and triglycerides by esterification, transesterification and acyl transfer reactions. The yield of monoesters was affected by the acyl donors, organic solvents, temperature, water content, pH memory and reaction time. Fatty acid ethyl esters appeared to be poor substrates for lipase-catalyzed synthesis of propylene glycol monoesters. As shown in Table 1, the yields of PGML, PGMM, PGMP, PGMS and PGMO were only 9.7, 11.8, 13.0, 14.4 and 9.0 mM, respectively, from 50 mM of fatty acid ethyl ester and 500 mM propylene glycol in hexane at 30°C. Perhaps esters in general are not good donors.

Fatty acids appeared to be better acyl donors than fatty acid esters for the lipase-catalyzed synthesis of propylene glycol monoesters. As shown in Table 2, the yields of PGML, PGMM, PGMP, PGMS and PGMO were 37.3, 28.7, 28.7, 35.3 and 36.2 mM, respectively, with 50 mM fatty acid as acyl donor. The reaction rates were also faster. The carbon number of fatty acid and the degree of unsaturation apparently affected the yield of propylene glycol monoester (Fig. 1 and Table 2). The polarity of organic solvents also greatly affected the yield. As shown in Figure 2, the optimum log *P* (the logarithm of the

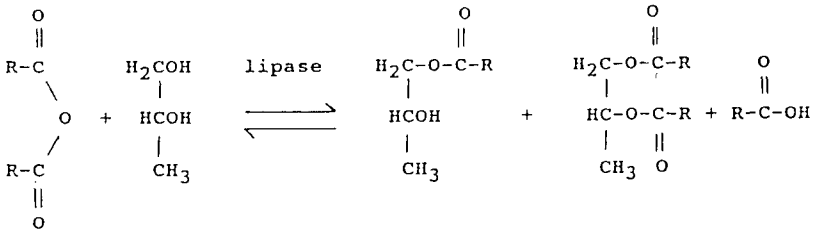
(1) Esterification from 1,2-propanediol and fatty acid



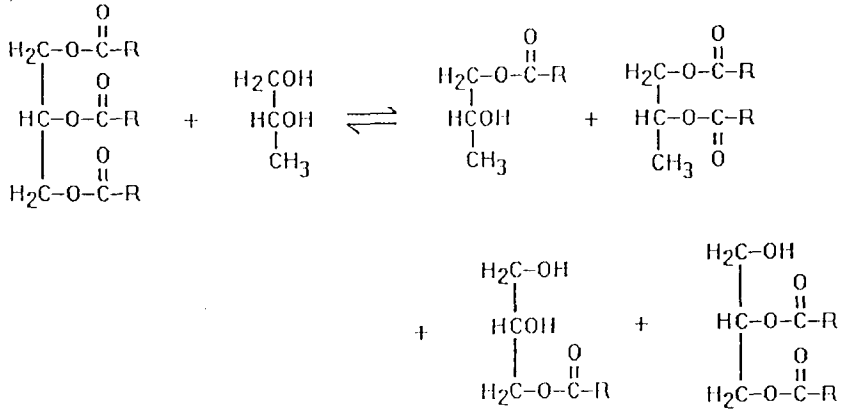
(2) Transesterification from fatty acid ethyl ester and 1,2-propanediol



(3) Acyl transfer from fatty acid anhydride and 1,2-propanediol



(4) Transesterification from triglyceride and 1,2-propanediol



SCHEME 1

TABLE 1

Lipase-Catalyzed Synthesis of Propylene Glycol Monoesters from Propylene Glycol and Various Fatty Acid Ethyl Esters[a]

System	Reaction time (h)	Conversion (%)	Product composition (mM)	
			Monoester	Diester
Ethyl laurate	6	29.0	9.7	2.4
Ethyl myristate	22	64.6	11.8	9.8
Ethyl palmitate	22	33.4	13.0	1.9
Ethyl stearate	12	40.2	14.4	2.8
Ethyl oleate	48	18.0	9.0	0.1

[a]The lipase (0.2 g) was added to a reaction mixture (4 mL) containing 500 mM propylene glycol and 50 mM fatty acid ethyl ester in hexane at 30°C.

TABLE 2

Lipase-Catalyzed Synthesis of Propylene Glycol Monoesters Propylene Glycol and Various Fatty Acids[a]

System	Reaction time (h)	Conversion (%)	Product composition (mM)	
			Monoester	Diester
Lauric acid	3	98.8	37.3	6.0
Myristic acid	6	99.7	28.7	10.3
Palmitic acid	24	97.3	28.7	20.5
Stearic acid	6	93.0	35.3	7.1
Oleic acid	6	79.3	36.2	1.7

[a]The lipase (0.2 g) was added to a reaction mixture (4 mL) containing 500 mM propylene glycol and 50 mM fatty acids in hexane at 30°C.

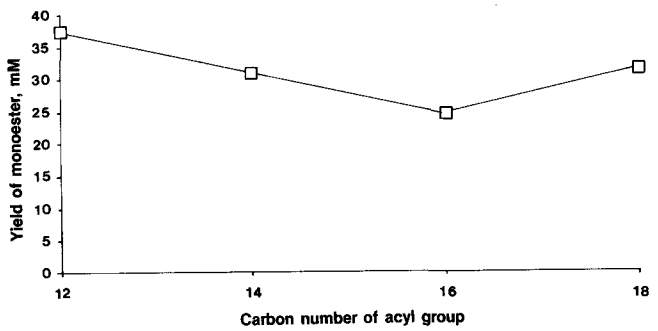


FIG. 1. Effect of acyl group carbon number of saturated fatty acid on the synthesis of propylene glycol monoesters by lipase in organic solvent. The lipase (0.2 g) was incubated in a reaction mixture (4 mL) containing 500 mM propylene glycol and 50 mM fatty acid (lauric, myristic, palmitic and stearic acid) in hexane at 30°C for 3 h.

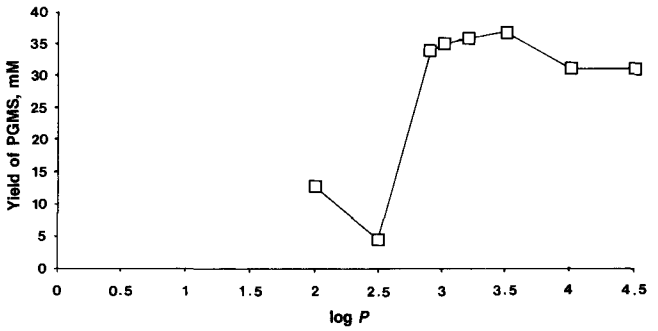


FIG. 2. Effect of log *P* value of solvents on the synthesis of propylene glycol monostearate (PGMS) by lipase. The lipase (0.2 g) was added to a reaction mixture (4 mL) containing 500 mM propylene glycol and 50 mM stearic acid in various organic solvents at 30°C for 4 h. The log *P* values of chloroform, toluene, dibutyl ether, pentane, cyclohexane, hexane and *n*-heptane, octane are 2.0, 2.5, 2.9, 3.0, 3.2, 3.5, 4.0 and 4.5, respectively. The log *P* is defined as the logarithm of the partition coefficient in a standard octanol–water two-phase system (Ref. 9).

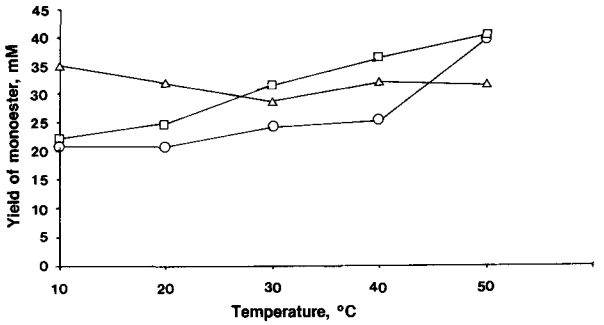


FIG. 3. Effect of temperature on the synthesis of propylene glycol monoester by lipase in hexane. The lipase (0.2 g) was added to a reaction mixture (4 mL) containing 500 mM propylene glycol and 50 mM fatty acid in hexane at various temperature for 3 h. The reaction temperatures were 10, 20, 30, 40 and 50°C. (○) Propylene glycol *monopalmitate*, (□) propylene glycol monostearate, (△) propylene glycol monostearate.

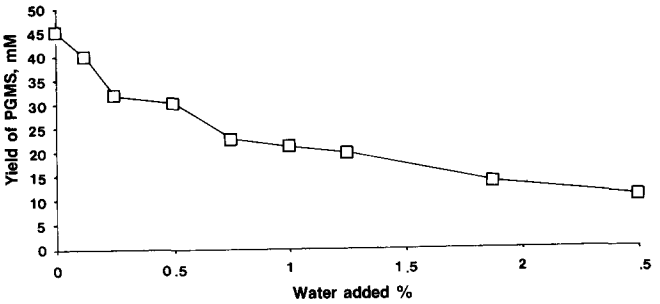


FIG. 4. Effect of water on the synthesis of PGMS by lipase. The lipase (0.2 g) was added to a reaction mixture (4 mL containing 500 mM propylene glycol and 50 mM stearic acid in hexane with various amounts of added water at 30°C for 1 h. Abbreviation as in Figure 2.

partition coefficient in a standard octanol–water two-phase system) (7) was 2.9–3.5 for the production of PGMS at 30°C for 4 h. Organic solvents with high log *P* values are also reported to be well-suited for the esterification of ethylene glycol with vinyl laurate in the presence of Lipozyme (8). The temperature effect is shown in Figure 3. The yields of PGMP and PGMS increased slightly as the temperature was increased up to 50°C. In contrast, the yield of PGMO slightly decreased as the temperature was increased. This is possibly due to the degree of fatty acid unsaturation.

The effect of water content on the synthesis of PGMS by lipase is shown in Figure 4. The *Pseudomonas* lipase

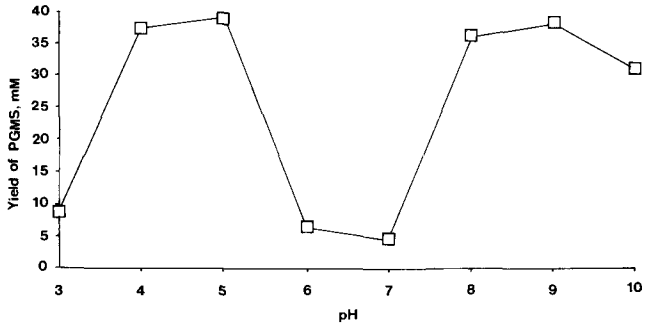


FIG. 5. Dependence of PGMS formation on the pH of the aqueous solution from which the lipase was lyophilized. Experimental conditions: 0.2 g of the lipase was added to a reaction mixture (4 mL) containing 500 mM propylene glycol and 50 mM stearic acid in *n*-hexane; the suspension was shaken at 30°C and 250 rpm for 1 h. Abbreviation as in Figure 2.

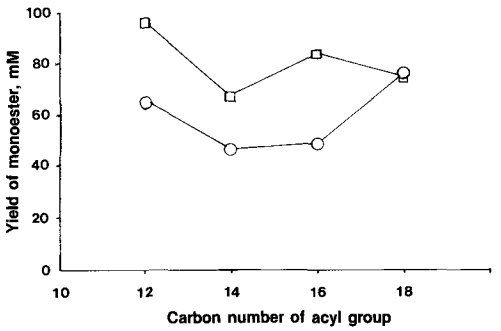


FIG. 6. Effect of acyl group carbon number of saturated fatty acid anhydrides on the synthesis of propylene glycol monoesters by lipase in organic solvents. The lipase (0.2 g) was added to a reaction mixture (4 mL) containing 500 mM fatty acid anhydride in toluene at 30°C for 3 h. The organic solvents were (□) toluene and (○) hexane.

TABLE 3

Lipase-Catalyzed Synthesis of Propylene Glycol Monoesters from Propylene Glycol and Various Fatty Acid Anhydrides[a]

System	Reaction time (h)	Conversion (%)	Product composition (mM)	
			Monoester	Diester
Lauric anhydride				
Hexane	3	99.7	65.0	17.1
Toluene	6	97.4	94.2	2.6
Myristic anhydride				
Hexane	3	97.1	46.2	25.7
Toluene	6	96.2	79.6	8.3
Palmitic anhydride				
Hexane	3	99.2	48.6	25.6
Toluene	3	89.9	83.7	3.1
Stearic anhydride				
Hexane	3	94.9	76.4	11.4
Toluene	24	98.3	89.7	4.3
Oleic anhydride				
Hexane	3	96.8	72.2	12.2
Toluene	6	99.3	93.4	2.9

[a]The lipase (0.2 g) was added to a reaction mixture (4 mL) containing 500 mM propylene glycol and 50 mM fatty acid anhydrides in hexane at 30°C.

performs best under lyophilized conditions. One-hour incubation under the anhydrous conditions gave high yield of PGMS (45 mM), which is higher than that reported in a six-hour incubation with nonlyophilized commercial enzyme (Table 2). The yield of PGMS decreased as the water content increased. The low yield (0–48%) of lipase-catalyzed synthesis of propylene glycol oleate in phosphate buffer, previously observed by Okumura *et al.* (9), is possibly due to the effect of high water content in their reaction system. Berger *et al.* (8) also reported that high yield of diol esters can be obtained from lipase-catalyzed esterification of silica gel-adsorbed diols with vinyl esters in nearly anhydrous (water content <0.6%) nonpolar organic solvent. The yield of PGMS was also affected by the pH of the aqueous solution from which the lipase was lyophilized, a phenomenon named "pH memory" by Klibanov (10). As shown in Figure 5, there were two optimum pH ranges, 4–5 and 8–10, for the lyophilized lipase-catalyzed synthesis of PGMS. We speculate that this is due to the presence of multiple enzyme forms in the commercial lipase preparations (11).

Fatty acid anhydrides appeared to be the best substrates for the lipase-catalyzed synthesis of propylene glycol monoesters. As shown in Table 3, the yields of PGML, PGMM, PGMP, PGMS and PGMO were 65.0, 46.2, 48.6, 76.4 and 72.2 mM, respectively, from 50 mM fatty acid anhydrides and 500 mM propylene glycol in hexane at 30°C. They were 94.2, 79.6, 83.7, 89.7 and 93.4 mM, respectively, in toluene. The acylation of propylene glycol with fatty acid anhydrides has two reaction steps, acyl transfer from the fatty acid anhydride to propylene glycol being the first, and the second step being the esterification of glycol with fatty acid produced from the first step reaction. The effect of acyl group carbon number of fatty acid anhydride on the synthesis of propylene glycol monoester is shown in Figure 6. The best substrates in toluene and hexane were lauric anhydride and stearic anhydride, respectively. This suggests that the *Pseudomonas* lipase specificity can be changed by solvent engineering.

To investigate the feasibility of using lipase-catalyzed transesterification for the production of propylene glycol monoesters from natural fat and vegetable oils, which are cheap sources of acyl donors, triglycerides were used as substrates for the acylation of propylene glycol. As shown in Table 4, the yields of PGMP, PGMS and PGMO were 87.4, 63.6 and 83.2 mM, respectively, with respective triglycerides as acyl donors in hexane at 30°C for 96 h. Toluene appeared to be a poor solvent in this case. In previous papers, we reported that lipases were able to catalyze the alcoholysis of triglycerides and peracylated carbohydrate in pure alcohols, such as ethanol and isopropanol, which served both as substrate and co-solvent (12–14). However, so far we have not found a lipase that was active for the alcoholysis of triglyceride in pure propylene glycol. Because the yields of propylene glycol monoesters were larger than 50 mM and less than 100 mM with 50 mM triglyceride as acyl donors, the products must be a mixture of monoglycerides and diglycerides, which are also useful emulsifiers. The lipase-catalyzed synthesis of propylene glycol esters is a kinetic process as follows:

TABLE 4

Kinetics of Lipase-Catalyzed Transesterification of Propylene Glycol with Various Triglycerides[a]

	Product composition (mM)					
	3 h		24 h		96 h	
System	Monoester	Diester	Monoester	Diester	Monoester	Diester
Triplamitin						
Hexane	38.9	2.7	63.0	8.2	87.4	18.6
Toluene	6.9	0.9	18.9	1.2	35.7	2.5
Tristearin						
Hexane	10.6	2.9	65.1	3.8	63.6	42.0
Toluene	3.3	<0.1	16.4	<0.1	28.8	<0.1
Triolein						
Hexane	37.2	2.8	57.8	6.5	83.2	17.7
Toluene	4.2	<0.1	46.5	2.1	43.2	3.5

[a]The lipase (0.2 g) was added to a reaction mixture (4 mL) containing 500 mM propylene glycol and 50 mM triglyceride in toluene or hexane at 30°C.

propylene glycol $\overset{k1}{\rightarrow}$ propylene glycol monoester $\overset{k2}{\rightarrow}$ propylene glycol diester.

Various reaction conditions, including acyl donors, temperature, organic solvents and water content, could change the enzyme specificity, which can lead to an increased k1/k2 ratio and selective accumulation of monoesters.

ACKNOWLEDGMENT

This work was supported in part by an NSC grant to JFS from National Science Councils, R.O.C. (NSC 81-0418-B001-01).

REFERENCES

1. Stutz, R.L., A.J. Del Vecchio and R.J. Tenney, *Food Prod. Develop. 7*:52 (1973).
2. Artman, N.R., *J. Am. Oil Chem. Soc. 52*:49 (1975).
3. Nash, N.H., and L.M. Brinkman, *Ibid. 49*:457 (1972).
4. Goldsmith, H.A., *Chem. Rev. 33*:257 (1943).
5. Mattson, F.H., and R.A. Volpenhaim, *J. Lipid Res. 3*:281 (1962).
6. Buchnea, D., in *Fatty Acids and Glycerides*, edited by A. Kusis, Vol. 1, Plenum Press, New York, 1978, Chapter 5.
7. Laane, C., S. Baeren, R. Hilhorst and C. Veeger, in *Biocatalysis in Organic Media*, edited by C. Laane, J. Tramper and M.D. Lilly, Elsevier, Amsterdam, 1987, p. 65.
8. Berger, M., K. Laumen and M.P. Schneider, *Biotechnol. Lett. 14*:553 (1992).
9. Okumura, S., M. Iwai and Y. Tsujisaka, *Biochim. Biophys. Acta 575*:156 (1979).
10. Klibanov, A.M., *Trends Biochem. Sci. 14*:141 (1989).
11. Shaw, J.F., C.H. Chang and Y.J. Wang, *Biotechnol. Lett. 11*:779 (1989).
12. Shaw, J.F., D.L. Wang and Y.J. Wang, *Enzyme Microb. Technol. 13*:544 (1991).
13. Shaw, J.F., and E.T. Liaw, in *Biocatalysis in Organic Media*, edited by C. Laane, J. Tramper and M.D. Lilly, Elsevier, Amsterdam, 1987, p. 233.
14. Shaw, J.F., and E.T. Liaw, *J. Chinese Biochem. Soc. 18*:73 (1989).

[Received April 15, 1993; accepted April 11, 1994]