Synthesis of Polyfunctional Glycerol Esters: Lipase-Catalyzed Esterification of Glycerol with Diesters[1]

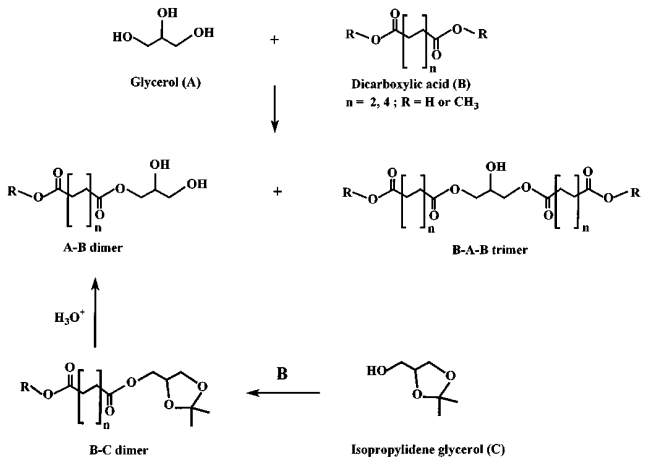
P. Villeneuve, T. A. Foglia*, T. J. Mangos, and A. Nuñez

U.S. Department of Agriculture, ARS, Eastern Regional Research Center, Wyndmoor, PA 19038

ABSTRACT: The enzymatic esterification of glycerol with dicarboxylic acids or esters was studied to produce mono- and/or diesterified glycerol adducts. Such materials are useful synthons in the synthesis of biodegradable polymers and surfactants. In this work two strategies were studied for preparing these prepolymeric glycerol derivatives: the lipase-catalyzed esterification of free glycerol with diacids or esters and the reaction of supported or protected glycerol with diesters. For example, reaction of isopropylidene glycerol with dimethyl sebacate gave a >95% yield of isopropylidene glycerol-monomethyl sebacate ester. Reaction of glycerol supported on silica with dimethyl adipate gave a 40% yield of glycerol-monomethyl adipate ester. Best yields of glycerol-mono- and diesters (70% and 10%, respectively) were obtained by direct esterification of free glycerol with a diester in a solvent-free system containing small amounts of water (<4%).

JAOCS 75, 1545–1549 (1998).

KEY WORDS: Dicarboxylic acids, dimethyl esters, glycerolysis, partial glycerides, polyesters, prepolymers.



SCHEME 1.

The lipase-catalyzed esterification of aliphatic dicarboxylic acids with diols has been studied extensively for the production of bifunctional precursors in the synthesis of biodegradable polymers (1–3). In this regard, the enzymatic esterification of glycerol with diacids also would be attractive (Scheme 1). The latter reaction should result in the formation of mono- and/or diesterified glycerol products that can be used as trifunctional synthons in the synthesis of glycerol-derived biopolymers or surfactants. The synthesis of such partial glycerides can be achieved using either chemical or enzymatic methods (4). Chemical methods typically are carried out with inorganic catalysts at high pressure and temperature and suffer several drawbacks such as low yields, unwanted side reactions, and decomposition products that result in discolored reaction products. Enzymes (lipases), on the other hand, operate under milder conditions and hence offer advantages over chemical methods. Basically, three strategies have been used in the lipase-catalyzed synthesis of partial glycerides: (i) partial hydrolysis of triacylglycerols; (ii) glycerolysis of triacylglycerols; and (iii) direct esterification of glycerol with acyl donors. The latter strategy, however, deals with substrates that often have very different solubility characteristics, which often inhibits their reaction with each other. A number of publications have addressed this problem by devising different methods to modify the solubility of glycerol, for example by adsorption onto inert supports (5–7), use of the isopropylidene protecting group (8–10), or complexation with phenyl boronic acid (PBA) (11,12). Other approaches to overcoming the glycerol solubility limitation include carrying out the reaction with substrates in biphasic systems (13), by microemulsion (14), or by addition of water to the reaction media (15). One objective of our present study is the synthesis of polyfunctional glycerol esters of dicarboxylic acids for use as prepolymeric synthons. Attempts to prepare these synthons using the above enzymatic methods, however, gave either modest yields of product or required multistep procedures. In this paper we report these results, along with those obtained using a newly developed procedure that provided good yields of the desired glycerol mono-(or di-)adipate (or sebacate) esters.

MATERIAL AND METHODS

Materials. Glycerol (99%), isopropylidene glycerol (98%), adipic and sebacic acids and their dimethyl ester derivatives (>97%) were obtained from Sigma Chemical (St. Louis,

[1]Presented in part at the 89th Annual Meeting of the American Oil Chemists' Society, Chicago, Illinois, May 1998.

*To whom correspondences should be addressed at the Eastern Regional Research Center, USDA, ARS, 600 East Mermaid Lane, Wyndmoor, PA 19038. E-mail: tfoglia@arserrc.gov.

MO). *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Pierce (Rockford, IL). Lipozyme™ IM60 was a generous gift from Novo Nordisk (Franklinton, NC). All solvents were high-performance liquid chromatography (HPLC) grade and were purchased from Burdick and Jackson (Muskegon, MI).

Gas chromatographic (GC) analysis. Silylated samples were analyzed on a Hewlett-Packard (HP, Wilmington, DE) gas chromatograph model 5890 Series II equipped with a DB1-HT (J&W Scientific, Folsom, CA) methyl-silicone capillary column, 15 m × 0.32 mm i.d., film thickness 0.1 μm. The chromatographic conditions were cold on-column capillary injection, flame-ionization detection at 370°C, and helium carrier gas at 5.5 mL min^{-1}. Separations were made using the following oven temperature profile: initial temperature 70 to 350°C at 20°C/ min^{-1}, final time 4 min.

Gas chromatography–mass spectroscopy (GC–MS) analysis. Samples as trimethylsilyl (TMS) derivatives were diluted with methylene chloride and injected (1 μL) into an HP gas chromatograph model 5890 Series II Plus equipped with a capillary inlet and a HP Mass Selective Detector (MSD) model 5972 Series set to scan from *m/z* 50 to *m/z* 600 at 1.5 scans/s at 70 eV. The capillary column was 30 m × 0.25 mm coated with 0.25 μm of 5% cross-linked phenyl methyl silicone (HP-5MS). The oven temperature was programmed from 80 to 230°C at 10°C/min. The injector port temperature was 230 and the detector transfer line was 240°C. The carrier gas was helium at a flow of 1 mL min^{-1} at split ratio 50:1.

Esterification of isopropylidene glycerol. Dimethyl sebacate was mixed with isopropylidene glycerol (ester/isopropylidene glycerol molar ratio 2:1) in the presence of Lipozyme™ (10% w/w of diester) at 60°C under reduced pressure (160 mm Hg). At the end of the reaction, the enzyme was removed by filtration. The isopropylidene protective group was removed from the glycerol-diester adduct (Scheme 1) by placing the crude reaction mixture into acetonitrile/1 M aqueous HCl (20 mL, 4:1 vol/vol) and stirring at room temperature for 6 h. The mixture was diluted with water and the glycerol monomethyl sebacate ester (A-B dimer, Scheme 1) was isolated by extraction with ether.

Esterification of glycerol supported on silica. Glycerol was adsorbed onto silica gel (230–400 mesh, Sigma) following the procedure described by Berger *et al.* (5). Briefly, equal weights of glycerol and silica were mechanically mixed until the liquid glycerol was completely adsorbed onto the silica to give a free-flowing powder. Sebacic acid dimethyl ester and adsorbed glycerol (ester/glycerol molar ratio 2:1) were then mixed in the presence of Lipozyme™ (10% w/w of the diester) at 60°C under reduced pressure (160 mm Hg).

Direct esterification of glycerol. Sebacic acid or dimethyl sebacate (50 mmol) and glycerol (25 mmol) were mixed in the presence of Lipozyme™ (10% w/w of glycerol). The mixture was stirred magnetically at 60°C under reduced pressure (160 mm Hg) to continuously remove the water or methanol co-products produced during esterification.

Addition of PBA to the reaction. Dimethyl sebacate (or adipate) was mixed with an appropriate amount of glycerol and PBA (ester/glycerol molar ratio, 2:1; glycerol/PBA molar ratio, 1:1) in the presence of Lipozyme™ (10% w/w of glycerol) at 60°C under reduced pressure (160 mm Hg).

Esterification reactions in biphasic medium. The experimental procedure used for the biphasic reactions paralleled that described by El Zant *et al.* (13) for the synthesis of monoacylglycerols. Typically, 5 g (20 mmol of glycerol) of a glycerol/water mixture (75:25 vol/vol) was mixed with 5 mL of chloroform containing 40 mmol of sebacic acid or dimethyl sebacate. Lipozyme™ (50 mg) was added and the mixture was stirred magnetically at 60°C.

Addition of small quantities of water. Dimethyl sebacate (50 mmol) was reacted with the appropriate amount of glycerol (25 mmol) and a predetermined amount of water (wt% of reactants) in the presence of 10% w/w of enzyme. The reaction was stirred under reduced pressure (160 mm Hg) at 40°C, a temperature at which methanol produced during the reaction was removed continuously but not the water added to the reaction medium.

Analysis of reaction mixtures. Reactions were monitored by GC and product characterization was made by GC–MS. For biphasic reactions both the organic and glycerol/water phase were sampled. Aliquots at selected time intervals and unreacted glycerol and the mono- and diester glycerol adducts converted to TMS derivatives before analysis. Typically 250 μL of the sample was taken, to which was added 200 μL of pyridine and 25–50 μL of BSTFA silylating reagent, and the samples were heated at 40°C for 20-30 min.

RESULTS AND DISCUSSION

Esterification of isopropylidene glycerol. Acylation of the isopropylidene derivative of glycerol has been widely used for the synthesis of monoacylglycerols (8–10). Our initial studies with isopropylidene glycerol were carried out in hexane solvent at 60°C using Lipozyme™ as the catalyst and dimethyl sebacate as the acyl donor. Under these conditions, the conversion of isopropylidene glycerol to its monomethyl sebacate ester (B-C dimer, Scheme 1) derivative was 50% after 12 h reaction. When the same reaction was repeated in a solventfree system under reduced pressure, the yield of B-C dimer was nearly quantitative after 7 h reaction (Fig. 1). This ester was converted to the A-B dimer, glycerol-1-monomethyl sebacate ester, by controlled partial acid hydrolysis of the B-C dimer (Scheme 1). The structural assignment for the A-B dimer was made from interpretation of its mass spectral fragmentation pattern (Scheme 2).

Besides the good solubility of isopropylidene glycerol in organic solvents, a main advantage of the method is that no diacylglycerol products are formed as when using free glycerol. A disadvantage of the procedure is that it requires additional processing steps, namely, the placement and removal of the isopropylidene-protecting group. Because of this, we turned our attention to other glycerol-protecting techniques.

Reaction with glycerol supported on silica. Prior adsorption of glycerol onto a solid support as a way of overcoming

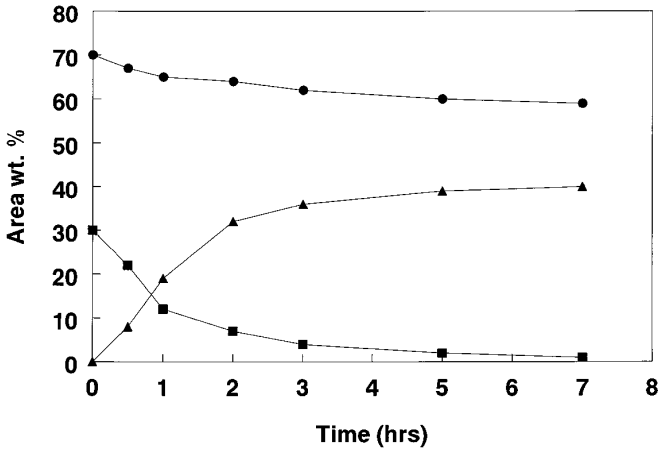
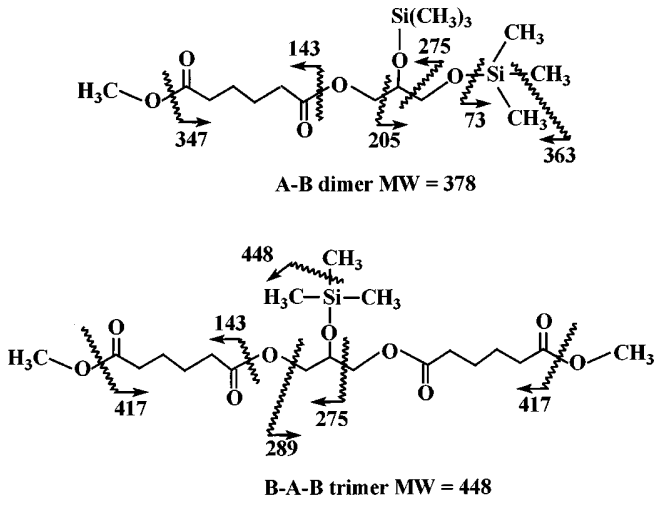


FIG. 1. Esterification of isopropylidene glycerol with dimethyl sebacate as followed by gas chromatography. Diester/glycerol molar ratio 2:1; *T* = 60°C; Lipozyme™ (10% w/w; Novo Nordisk, Franklinton, NC); reduced pressure (160 mm Hg). (■), Isopropylidene glycerol; (●), dimethyl sebacate; (▲), Isopropylidene glycerol monomethyl sebacate ester (B-C dimer).



SCHEME 2.

the low solubility of glycerol in nonpolar organic media was first described by Berger and Schneider for the lipase-catalyzed synthesis of monoacylglycerols (5–7). In those studies, the glycerol/silica preparation was suspended in an organic solvent, thus creating an artificial liquid–liquid interface which was thought to favor the enzymatic acylation of glycerol. In this way, a supported glycerol preparation was obtained as a free-flowing powder. This preparation was then suspended in hexane containing dimethyl sebacate (molar ratio ester/glycerol 2:1) in the presence of the enzyme at 60°C. After 24 h reaction only a minor amount of glycerol-1-monomethyl sebacate ester (A-B dimer) (Table 1) was formed. We repeated the same experiment in a solventfree system under reduced pressure with the expectation of driving the reaction to completion. Under these conditions, the yield of glycerol-1-monomethyl sebacate ester was only about 33% (Table 1) with no glycerol-1,3-di(monomethyl sebacate) ester (B-A-B trimer, Scheme 1) being detected.

Addition of PBA in the reaction medium. Organoboronic acids are known to form boronate complexes with diols and sugars, resulting in enhanced solubility of these polyols in organic solvents (11–12). Accordingly, compounds such as PBA that are successfully used in chemical processes might advantageously be used as both solubilizing and protecting agents for glycerol in lipase-catalyzed esterification reactions. Several reactions were carried out in hexane solvent at 60°C with Lipozyme™ and dimethyl esters as acyl donors at varying glycerol/PBA molar ratios (from 1:3 to 1:1). In all cases, however, the formation of the desired glycerol-ester adducts did not exceed 3% (Table 1). The lack of success using this approach could be attributed to the known inhibitory effect of boronic acid on lipase activity when this reagent is used in excess. Because of the limited success obtained with the supported or complexed glycerol reactions, we turned our attention to direct acylation of free glycerol.

Direct esterification of free glycerol. The most direct method for the synthesis of partial glycerol esters is the esterification of free glycerol with an acyl donor in the absence of solvent. In our attempts to esterify glycerol with bifunctional acyl donors, the first approach studied was the reaction of free glycerol with dicarboxylic acids (adipic or sebacic acid) in a solvent-free system (Scheme 1). The enzyme catalyst used in this reaction was Lipozyme™, a 1,3-regioselective lipase preparation. An excess of the diacid was used to limit oligomerization of the initial ester products, since we were interested in obtaining the mono- or diesterified glycerol adducts (A-B dimer and B-A-B trimer, Scheme 1). Reactions initially were carried out between adipic acid and glycerol at varying diacid/glycerol molar ratios, from 2:1 to 10:1, at 60°C. Under these conditions, however, GC detected no ester products after 96 h reaction. Similar experiments were repeated with dimethyl adipate, but under reduced pressure in order to remove the methanol. Even under these conditions, only a minor amount of the A-B dimer product (Scheme I) was detected by GC (<4%, Table 1). The poor results obtained above were attributed to the poor interfacial contact of the two reactants due to their disparate polarity.

Another approach to acylation of glycerol is to conduct the reaction in a chloroform/water system, as described by El Zant *et al.* (13), for the synthesis of 1(3)-monoacylglycerols from glycerol and monocarboxylates. Using dimethyl sebacate as the acyl donor under these conditions, however, only a negligible yield of glycerol-1-monomethyl sebacate ester (<3%) was obtained. The major reaction noted was the hydrolysis of the starting diester to a mixture of monomethyl sebacate and sebacic acid. Using sebacic acid as acyl donor gave a slightly better yield of the desired monoester product (7%, Table 1).

Our attention next turned to a study of the reaction of glycerol with diesters to which small amounts of water were added. Under these conditions, enough glycerol was solubilized in the aqueous phase to create a liquid–liquid interface, which we thought would improve contact between the two substrates. The reaction was carried out in hexane solvent containing water (4% w/w of the total substrates) between

TABLE 1
Esterification of Glycerol with Diacids or Diesters Using 1,3-Selective Lipase

Method	Acyl donor and reaction conditions	Results
Isopropylidene glycerol	Dimethyl adipate or sebacate; 60°C; reduced pressure	Quantitative yield of isopropylidene glycerol monomethyl sebacate ester (B-C dimer)
Glycerol adsorbed onto silica	Dimethyl sebacate; 60°C; reduced pressure	Formation of glycerol-1-monomethyl sebacate ester (A-B dimer, 33% yield)
Phenyl boronic acid complexation of glycerol	Dimethyl sebacate or adipate; hexane solvent; 60°C	Formation of glycerol-1-monomethyl sebacate ester (A-B dimer, 4% yield)
Direct esterification of glycerol	Dimethyl sebacate or sebacic acid; 60°C; reduced pressure	Formation of glycerol-1-monomethyl sebacate ester (A-B dimer, 4% yield)
Reaction in biphasic media	Sebacic acid or dimethyl sebacate; chloroform/water solvent, 60°C	Formation of glycerol-1-monomethyl sebacate ester (A-B dimer, 7% yield)
Esterification of glycerol with added water	Dimethyl sebacate; 40°C; reduced pressure	Formation of glycerol-1-monomethyl sebacate ester (A-B dimer, 70%) and glycerol-1,3- di-(monomethyl sebacate) ester (B-A-B trimer, 10%)

glycerol and dimethyl sebacate (diester/glycerol molar ratio 2:1) using 10 wt% of enzyme at 60°C. In this case, the formation of both glycerol-1-monomethyl sebacate ester (A-B dimer) and glycerol-1,3 di(monomethylsebacate) ester (B-A-B trimer, Scheme 1) were produced in an overall yield of 49% after 72 h reaction. The assignment for the trimer structure was made from its MS fragmentation pattern (Scheme 2). The reaction was repeated without solvent under reduced pressure (160 mm Hg) at 40°C, which allowed removal of the methanol co-product but not the water added to the medium. In this instance, the overall yield of products improved to 80% (Table 1) after 24 h with the major product being glycerol-1-monomethyl sebacate ester (Fig. 2). The influence of the amount of water added also was studied (Fig. 3). This study showed that a 4 wt% addition of water gave the best yields of glycerol-monomethyl and dimonomethyl sebacate esters. With larger amounts of water the yields of ester products decreased, and hydrolysis of the newly formed products and starting diesters increased.

The results obtained in this study are summarized in Table 1. In this work we have shown that the lipase-catalyzed esterification of glycerol with diacids is limited because of the differing polarity and, hence, solubility properties of these reactants. Therefore, the direct esterification of nonmodified glycerol with diacids or diesters in solventfree systems resulted in poor conversion to glycerol ester adducts. Modification of glycerol by adsorption onto a solid support (silica) or use of the isopropylidene-protecting group gave better results but required additional processing steps. Addition of a boronic acid complexing reagent (PBA) to the system to solubilize glycerol did not improve the yields of the desired ester products, probably because of an inhibitory effect of PBA on the enzyme. Reactions in biphasic systems also were studied, but without significant improvement in the conversion of glycerol to the desired ester products. In contrast, good yields of the A-B dimer and B-A-B trimer adducts (Scheme 1) could

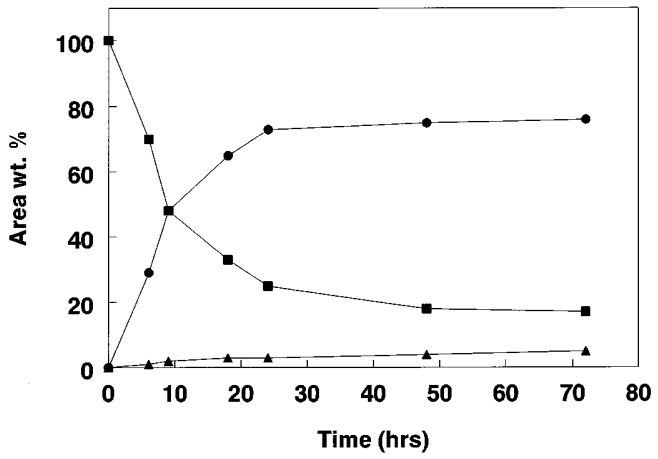


FIG. 2. Esterification of glycerol with dimethyl sebecate in the presence of water (4% w/w); diester/glycerol molar ratio 2:1; $T = 40°C$; Lipozyme™ (10% w/w); reduced pressure (160 mm Hg). (■), Glycerol; (●), glycerol monomethyl sebacate ester (A-B dimer); (▲), glycerol dimonomethyl sebacate ester (B-A-B trimer). For manufacturer see Figure 1.

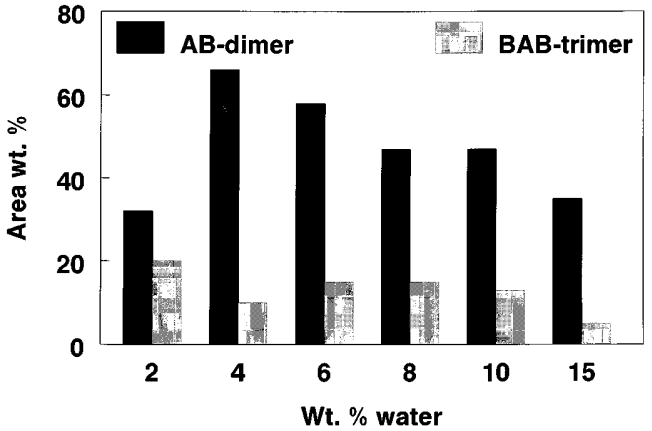


FIG. 3. Effect of water content on yield of products (A-B and B-A-B adducts) from esterification of glycerol with dimethyl sebacate. Diester/glycerol molar ratio 2:1; $T = 40°C$; Lipozyme™ (10% w/w); reduced pressure (160 mm Hg). For manufacturer see Figure 1.

be obtained by the simple addition of a small amount of water (4% w/w) to the reaction in solvent-free medium. Under these conditions, the combined yield of the glycerol mono- and dimethyl sebacate esters (A-B and B-A-B adducts) was approximately 80%. The obtained mixture of glycerol mono- and dimonomethyl sebacate (or adipate) esters will next be evaluated as starting materials for the synthesis of glycerol-based polymers and surfactants.

ACKNOWLEDGMENT

The authors thank Robert DiCiccio for his assistance in carrying out portions of the work reported herein.

REFERENCES

1. Binns, F., S.M. Roberts, A. Taylor, and C.F. Williams, Enzymatic Polymerization of an Unactivated Diol/Diacid System, *Chem. Soc. Perkin Trans. 1*:899–903 (1993).
2. Wang, Z.L., K. Hiltunen, P. Orava, J. Seppala, and Y.Y. Linko, Lipase-Polyester Synthesis, *Macro. Sci. Appl. Chem. A33*:599–612 (1996).
3. Linko, Y.Y., Z.L. Wang, and J. Seppala, Lipase-Catalyzed Synthesis of Poly (1,4-Butyl Sebacate) from Sebacic Acid or Its Derivatives with 1,4-Butanediol, *Biotechnology 40*:133–138 (1995).
4. Bornscheuer, U.T., Lipase-Catalyzed Syntheses of Monoacylglycerols, *Enz. Microb. Tech. 17*:578–586 (1995).
5. Berger, M., K. Laumen, and M.P. Schneider, Enzymatic Esterification of Glycerol I. Lipase-Catalyzed Synthesis of Regioisomerically Pure 1,3-*sn*-Diacylglycerols, *J. Am. Oil Chem. Soc. 69*:955–960 (1992).
6. Berger, M., and M.P. Schneider, Enzymatic Esterification of Glycerol II. Lipase-Catalyzed Synthesis of Regioisomerically Pure 1(3)-*rac*-Monoacylglycerols, *J. Am. Oil Chem. Soc. 69*:961–965 (1992).
7. Waldinger, C., and M.P. Schneider, Enzymatic Esterification of Glycerol I. Lipase-Catalyzed Synthesis of Regioisomerically Pure 1,3-*rac*-Diacylglycerols Derived from Unsaturated Fatty Acids, *Ibid. 73*:1513–1519 (1996).
8. Akoh, C.C., Lipase-Catalyzed Synthesis of Partial Glycerides, *Biotech. Lett. 15*:949–954 (1993).
9. Hess, R., U.T. Bornscheuer, A. Capewell, and T. Scheper, Lipase-Catalyzed Synthesis of Monostearoylglycerol in Organic Solvents from 1,2-*O*-Isopropylidene Glycerol, *Enz. Microb. Tech. 17*:725–728 (1995).
10. Bornscheuer, U.T., and T. Yamane, Fatty Acid Vinyl Esters as Acylating Agents: A New Method for the Enzymatic Synthesis of Monacylglycerols, *J. Am. Oil Chem. Soc. 72*:193–197 (1995).
11. Steffen, B., A. Ziemann, S. Lang, and F. Wagner, Enzymatic Monoacylation of Trihydroxy Compounds, *Biotech. Lett. 14*:773–778 (1992).
12. Castillo, E., A. Marty, D. Combes, and J. S. Condoret, Polar Substrates for Enzymatic Reactions in Supercritical CO_2: How to Overcome the Solubility Limitation, *Ibid. 16*:169–174 (1994).
13. El Zant, A., M. Pina, J. Graille, J. Grimaud, and G. Renard, Synthese enzymatique d'alpha monoglycerides en milieu biphasique, *Oleagineux 43*:355–358 (1988).
14. Bornscheuer, U.T., H. Stamatis, A. Xenakis, T. Yamane, and F.N. Kolisis, A Comparison of Different Strategies for Lipase-Catalyzed Synthesis of Partial Glycerides, *Biotech. Lett. 16*:697–702 (1994).
15. Fureby, A. M., P. Adlercreutz, and B. Mattiasson, Glyceride Synthesis in a Solvent Free System, *J. Am. Oil Chem. Soc. 73*:1489–1495 (1996).

[Received July 6, 1998; accepted August 18, 1998]