Enzymatic Esterification of Glycerol I. Lipase-Catalyzed Synthesis of Regioisomerically Pure 1,3-*sn*-Diacylglycerols

Matthias Berger, Kurt Laumen and **Manfred P. Schneider***
FB 9, Organische Chemie, Bergische Universität—GH—Wuppertal, D-5600 Wuppertal 1, Germany

Regioisomerically pure 1,3-*sn*-diacylglycerols are conveniently prepared in high yields (>80%) and in large quantities by enzymatic esterification of glycerol in the presence of various 1,3-selective lipases *(Chromobacterium viscosum, Rhizopus delemar, Rhizomucor miehei)* and a variety of different acyl donors like free fatty acids, fatty acid alkyl esters and vinyl esters. All reactions are carried out in aprotic organic solvents of low water content, namely *n*-hexane, diethyl ether or tBuOMe. The creation of an artificial interphase between the solvent-immiscible hydrophilic glycerol and the hydrophobic reaction media by the adsorption of glycerol onto a solid support prior to use was essential for the success of these transformations. The effects of reaction conditions and the regioselectivities of the lipases on the product yields are described in detail.

KEY WORDS: 1,3-*sn*-Diacylglycerols, direct esterification, irreversible transesterification, lipase, regioselectivity, reversible transesterification, solid support, synthesis.

Regioisomerically pure 1,3-*sn*-diacylglycerols are potentially attractive starting materials for numerous synthetic applications, such as the preparation of phospholipids, glycolipids and lipoproteins (1,2). They are useful as drug carriers and have been employed for the preparation of conjugates derived from various drugs, including anti-inflammatory compounds (3,4), γ-aminobutyric acid (GABA) (5,6), chlorambucil (7,8), levo-dihydroxy phenylalanine (L-DOPA) (9), bupranolol (10) and many others, to produce drugs with improved bioavailability and reduced side effects. They also have been reported to be potent activators of enzymes and may take part in a variety of biological processes (11,12). Their use as emulsifiers for the preparation of processed foods is well documented (13,14), although in these cases impure product mixtures are often employed.

The considerable pharmaceutical and synthetic potential of these molecules has not been exploited to any great extent due to the inaccessibility of these molecules in synthetically useful quantities. Several chemical and biotechnological methods for the synthesis of regioisomerically pure 1,3-*sn*-diacylglycerols have been reported (15–21). Unfortunately, however, all of these methods either involve multistep reaction sequences and/or tedious isolation and purification steps to obtain the desired 1,3-*sn*-diacylglycerols in isomerically pure form.

No facile and direct method for the preparation of the title compounds in synthetically useful quantities (>100 g) is presently available. This also is reflected in the high prices of these compounds. In the present paper we describe a simple, one-step procedure by which regioisomerically pure 1,3-*sn*-diacylglycerols can be obtained by direct esterification of glycerol with a variety of acyl donors in the presence of 1,3-selective lipases.

*To whom correspondence should be addressed at FB 9, Organische Chemie, Bergische Universität—GH—Wuppertal, Gaußstraße 20, D-5600 Wuppertal 1, Germany.

EXPERIMENTAL PROCEDURES

All solvents were of technical grade and distilled before use. Lipase from *Rhizomucor miehei* (Lipozyme) was a gift from Novo Industri S/A (Bagsvaard, Denmark); lipases from *Rhizopus delemar* and *Pseudomonas* sp. were gifts from Amano Pharmaceutical Co. (Nagoya, Japan); and lipase from *Chromobacterium viscosum* was purchased from Toyo Jozo Co. Ltd. (Shizuoka, Japan). Regioselectivity of the lipases was determined as described previously (22). Other materials were purchased from Fluka Chemie AG (Buchs, Switzerland).

Product composition and purity was checked by gas-liquid chromatography (GLC) of the corresponding trimethylsilylethers (TMS-ethers) on a Carlo Erba (Milan, Italy) Fractovap 2150 series capillary gas chromatograph equipped with a 25 m × 15 mm SE-30-fs-column (Macherey-Nagel, Düren, Germany). Helium was used as the carrier gas with a pressure of 0.6 bar; cold split injection (1:25) was used. The detector temperature was 350°C, and the following temperature program was used—200°C (1 min isothermal) to 350°C (3°C/min; 1 min isothermal).

Adsorption of glycerol onto solid supports: typical procedure. Equal amounts of water-free glycerol and the desired support were mechanically mixed until the glycerol liquid was completely adsorbed and a free-flowing "dry" powder was obtained. The preparations so obtained can be stored under anhydrous conditions for several months.

*Synthesis of solid 1,3-*sn*-diacylglycerols via irreversible acyl transfer: typical procedure.* Glycerol (46 g, 0.5 mol) adsorbed onto 46 g of silica gel (70–230 mesh) was suspended in 1 L of tBuOMe. To the suspension was added 226 g (1 mol) of vinyl laurate and 2.0 g of lipase from *Rhizomucor miehei* (Lipozyme). The mixture was stirred at room temperature for 24 h, and the reaction progress was monitored by thin-layer chromatography (TLC). After removal of the solid components (immobilized biocatalyst and silica gel) by filtration and evaporation of the solvent, a crude reaction mixture was obtained containing about 85% of 1,3-*sn*-dilaurin (GLC). The solid mixture was recrystallized from dry methanol to yield 190 g (84%) regioisomerically pure (>99.5%) 1,3-*sn*-dilaurin as a white powder (m.p. 56.5°C). The purity of the product was determined by ^{1}H-nuclear magnetic resonance (NMR) spectroscopy, TLC and GLC.

*Synthesis of liquid 1,3-*sn*-diacylglycerols via irreversible acyl transfer: typical procedure.* Glycerol (0.92 g, 10 mmol) adsorbed onto 1 g of silica gel (70–230 mesh) was suspended in 20 mL of tBuOMe. Vinyl caprylate (3.4 g, 20 mmol) and 50 mg of lipase from *Rhizopus delemar* were added to the suspension. The mixture was stirred at room temperature for 96 h, and the reaction progress was monitored by means of TLC. After removal of the solid components (immobilized biocatalyst and silica gel) by filtration and evaporation of the solvent, a crude reaction mixture was obtained which contained about 91% of 1,3-*sn*-dicaprylin (GLC). The colorless liquid was dissolved in a 1:1 mixture of hexane and diethyl ether and filtered over a short silica gel column (5 cm) to yield 3.2

g (83%) 1,3-*sn*-dicaprylin, a colorless liquid that contained about 3% of the 1,2-isomer. This was further purified by column chromatography on silica gel with the same solvent mixture to yield 3.0 g (80%) of regioisomerically pure (>98%) 1,3-*sn*-dicaprylin, the purity of which was determined by ^{1}H-NMR spectroscopy and GLC analysis.

*Synthesis of solid 1,3-*sn*-diacylglycerols via reversible acyl transfer or direct esterification: typical procedure.* Glycerol (4.6 g, 0.05 mol) adsorbed onto 4.6 g silica gel (70–230 mesh) was suspended in 100 mL of tBuOMe. To the suspension was added 20 g (0.1 mol) or lauric acid [alternatively 22 g (0.1 mol) of methyl laurate], 2.0 g of lipase from *Rhizomucor miehei* (Lipozyme) and 5 g of molecular sieves (3Å, alternatively 4Å). The mixture was stirred at room temperature for 48 h, and the reaction progress was monitored by TLC. After removal of the solid components (immobilized biocatalyst and silica gel) by filtration and evaporation of the solvent, a crude reaction mixture was obtained containing about 88% of 1,3-*sn*-dilaurin (GLC). The solid mixture was recrystallized from dry methanol to yield 18.2 g (80%) regioisomerically pure (>99.5%) 1,3-*sn*-dilaurin as a white powder (m.p. 56°C). The purity of the product was determined by ^{1}H-NMR spectroscopy, TLC and GLC.

Preparation of trimethylsilyl derivatives for GLC analysis: typical procedure. The corresponding anhydrous glyceride (1 mg) and 0.2 mL of a mixture consisting of *bis*(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilan (TMCS) in a ratio of 10:1 were mixed and heated at 70°C for three hours. This mixture (0.2 μL) was analyzed by GLC. When samples from liquid reaction mixtures were analyzed, the solvent was evaporated under a stream of dry nitrogen prior to their conversion.

RESULTS AND DISCUSSION

In view of the high regioselectivities displayed by many lipases for the 1,3-positions of glycerides, and based on our own previous experience (23), the enzyme-catalyzed esterification of glycerol itself seemed to provide a highly attractive route to regioisomerically pure 1,3-*sn*-diacylglycerols.

Unfortunately, glycerol is immiscible with hydrophobic organic solvents, and all attempts for its enzymatic esterification in these media have proven to be unsuccessful (24). We have found that this problem can be overcome easily by adsorbing glycerol onto a solid support. Presumably this process creates an artificial liquid-liquid interphase generally assumed to be involved in lipase-catalyzed transformations of glycerides, *e.g.*, natural fats and oils. The reaction scheme is outlined in Figure 1.

In typical experiments, glycerol and the corresponding solid support (*e.g.*, silica gel) were first mixed mechanically until free flowing "dry" powders were obtained, which could be used for the following enzymatic esterifications. These were carried out in nearly anhydrous organic solvents (preferably tBuOMe) with a variety of acyl donors (free fatty acids, fatty acid methyl esters and fatty acid vinyl esters) in the presence of different lipases. For this, the glycerol preparations were suspended in tBuOMe, and the acyl donor and the biocatalyst were added. The mixtures were then stirred at room temperature while the esterification reactions were monitored by TLC. After completion of the esterification, both the enzyme and the solid support were removed simultaneously by simple filtration, and the esterification products remained in solution. After evaporation of the solvent, crude reaction products were obtained that already contained high proportions of the desired 1,3-isomers (85–90%), together with small quantities of monoacylglycerols (3–8%) and the 1,2-isomer (2–7%) (Table 1).

The purity of all 1,3-*sn*-diacylglycerols was determined by means of ^{1}H- and ^{13}C-NMR spectroscopy (25), and alternatively by TLC (26) and GLC (27). Because solid 1,3-*sn*-diacylglycerols can be recrystallized without detectable acyl group migrations, these materials easily can be obtained as isomerically pure (>99%) compounds and in practically unlimited quantities (Table 1). Liquid 1,3-*sn*-diacylglycerols are more difficult to purify. Distillation under reduced pressure leads to a 60:40 mixture of 1,2(2,3)- and 1,3-*sn*-diacylglycerols (28). Although purification of these compounds can be achieved by conventional column chromatography, synthetically useful quantities of products are difficult to prepare this way. For a rapid and convenient purification of multigram quantities, the crude reaction products are simply dissolved in a 1:1 mixture of *n*-hexane and diethyl ether, followed by "filtration" of the solution through a short column of silica gel. By this method, all monoacylglycerols are substantially removed, and the desired 1,3-*sn*-diacylglycerols are obtained with only traces of 1,2-diacylglycerols (Table 1).

Both the yield and isomeric purities of the diacylglycerols depend on several factors, such as nature

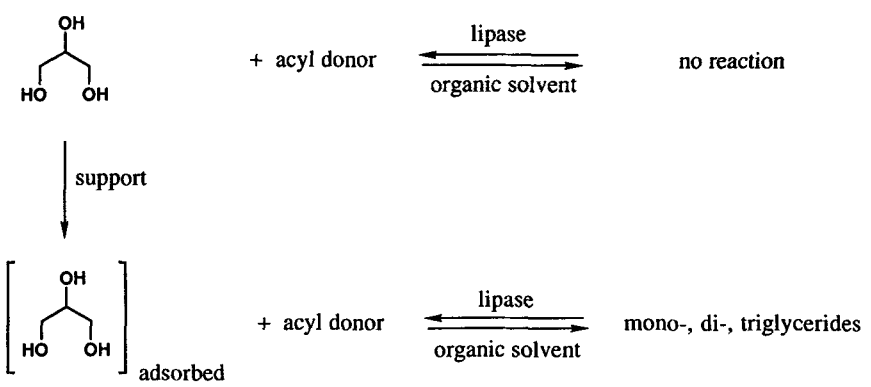


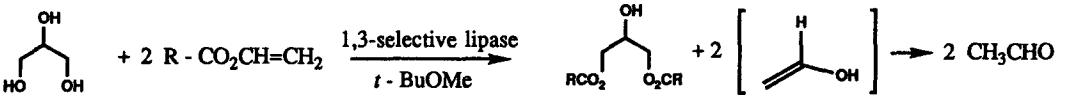
FIG. 1. Suitable pathway for the esterification of glycerol in organic nonpolar solvents.

TABLE 1

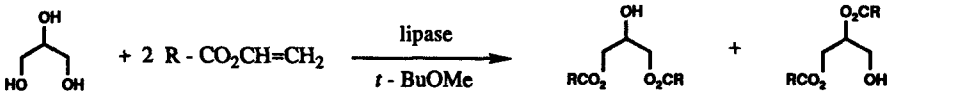
Synthesis of Regioisomerically Pure 1,3-*sn*-Diacylglycerols

Product	Enzyme[a]	Acyl donor	Time (h)	Conv.[b] (%)	Composition of crude reaction mixture (%)[c] 1,3-DAG	1,2-DAG	MG	Yield[d] (%)	Purity[e] (%)
1,3-*sn*-Divalerin	A	Valeric acid	96	95	85	2	8	74	98.5[f]
1,3-*sn*-Dicaprylin	A	Vinyl caprylate	96	>98	91	2	6	80	98[f]
1,3-*sn*-Dicaprin	B	Vinyl caprinate	24	>98	90	5	4	84	>99.5[g]
1,3-*sn*-Dilaurin	B	Vinyl laurate	24	>98	93	4	3	85	>99.5[g]
1,3-*sn*-Dimyristin	B	Vinyl myristate	24	>98	91	5	4	82	>99.5[b]
1,3-*sn*-Dipentadecanoin	B	Pentadecanoic acid	48	95	88	4	3	79	>99.5[g]
1,3-*sn*-Dipalmitin	B	Vinyl palmitate	24	>98	93	4	3	80	>99.5[g]
1,3-*sn*-Distearin	B	Vinyl stearate	24	>98	88	4	7	81	>99.5[g]
1,3-*sn*-Diolein	C	Oleic acid (>99%)	120	95	82	4	9	70	98[f]

[a]Enzyme A, lipase from *Rhizopus delemar*; B, lipase from *Rhizomucor miehei*; and C, lipase from *Chromobacterium viscosum*.
[b]Based on acyl donor (thin-layer chromatography).
[c]Determined by gas-liquid chromatography (GLC).
[d]Isolated yield, after purification.
[e]Determined by GLC.
[f]Liquid, after column chromatography over boric acid-impregnated silica gel, eluent diethyl ether/*n*-hexane (1:1).
[g]Solid, after recrystallization from dry methanol.



[1]



[2]

of the solid support, ratio of glycerol and solid support, regioselectivity of the lipases, nature of the acyl donor, and reusability of support and biocatalyst. These factors had to be studied in detail to develop an optimized synthetic procedure.

Nature of the solid support. As outlined above, the successful enzymatic esterification of glycerol depends on its adsorption onto a solid support prior to the enzymatic transformation. After the initial discovery of this basic principle, numerous substances were tested for their suitability as carriers for glycerol. The synthesis of 1,3-*sn*-dilaurin from glycerol and vinyl laurate by irreversible acyl transfer was chosen as a model reaction (Eq. [1]). The different supports (Table 2) were combined with equal amounts of glycerol and mixed mechanically until free-flowing powders were obtained. The solids thus obtained were suspended in tBuOMe, after which vinyl laurate and the biocatalyst were added. The reaction mixtures were stirred at room temperature. For comparison and as a control reaction, the enzymatic esterification of glycerol was studied under exactly the same conditions, but without the solid support.

From Table 2, it is evident that all of the solid supports employed are clearly accelerating the esterification reactions, although there are considerable quantitative differences. In general, the highest rates were observed with inorganic supports. Silica gel (230–400 mesh), florisil and porous glass beads gave the best results. In contrast, the control reaction—without the employment of a solid support—remained biphasic, and only 5% conversion of acyl donor was observed, even after an extended reaction time of up to seven days.

TABLE 2

Esterification of Glycerol: Dependence on the Nature of the Solid Support

Solid support	Rel. reaction rate[a]	Complete conversion after (days)
Silica gel, 230–400 mesh	1	1
Florisil	0.99	1
Porous glass beads (345 Å)	0.99	1
Silica gel, 70–230 mesh	0.98	2
Act. charcoal	0.97	2
Celite 535	0.94	2
Chromosorb WAW	0.85	3
Volaspher	0.85	3
Aluminium oxide (neutral)	0.82	4
Triacetyl cellulose	0.32	70% after 7 days
Starch	0.17	50% after 7 days
No support	<0.01	5% after 7 days

[a]Determination *via* initial conversion rate, obtained by gas-liquid chromatography.

Ratio of glycerol and solid support. As demonstrated above, practically no reaction is observed if the enzymatic esterification of glycerol is attempted without prior adsorption onto a solid support. Clearly, to develop an optimized procedure for the preparation of glycerides, the dependence of reaction rates and product yields on the ratio of solid support and glycerol had to be studied. As a model reaction we chose the synthesis of 1,3-*sn*-dilaurin

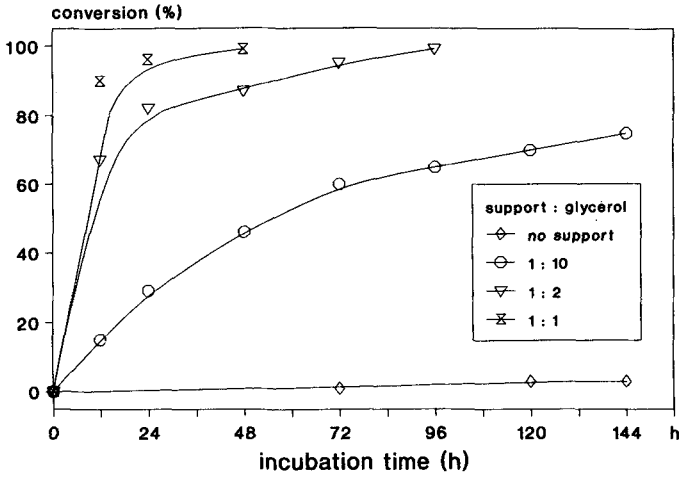


FIG. 2. Lipase-catalyzed esterification of glycerol: dependence of reaction rate on the ratio between glycerol and solid support (silica gel).

from glycerol and vinyl laurate (Eq. [1]) once again. Because of the excellent results obtained with silica gel as solid support, this material was chosen for this dependence study.

It is clear from Figure 2 that reaction can already be observed, even with a ratio of support/glycerol as low as 1:10. The observed rates, however, are still too slow to be practical. Furthermore, no complete conversions can be achieved, even after prolonged reaction times. The observed rates of transformation seem to be somewhat proportional to the amount of support employed. Optimal results were achieved with a 1:1 ratio of glycerol and silica gel, which produced a nearly quantitative conversion within 24 h.

Regioselectivity of lipases. Two classes of mono-acid diacylglycerols are possible—achiral 1,3-*sn*- and racemic 1,2(2,3)-diacylglycerols. The lipase-catalyzed esterification of glycerol under the conditions described above generally leads to a mixture of the two regioisomers (Eq. [2]). The biocatalysts employed (lipases) are usually classified into two groups according to their positional specificity observed in enzymatic transformations of glycerides. They are distinguished as 1,3-specific and unspecific ("random") lipases (29). Recent results have shown, however, that such a distinct classification is incorrect. In fact, lipases display a broad spectrum of regioselectivities ranging from virtually 1,3-specific to entirely unspecific (22,30).

Consequently, the observed ratios between the two regioisomers obtained in the above esterification will be directly dependent on the regioselectivity of the lipase employed. To develop an optimized synthetic procedure, detailed knowledge of the regioselectivity displayed by different lipases is therefore of considerable importance. The regioselectivities of lipases, determined in protic or biphasic aqueous reaction media, are inherently unreliable due to uncontrollable acyl group migrations (17). We found, however, that these acyl group migrations do not occur if glycerides are synthesized in aprotic, organic media like those employed in the synthetic reactions described above. This observation provided the basis for a recent study in which we were able to develop a method for determination of the regioselectivities displayed by lipases during transformations of glycerides in organic media (22). We defined a value for the regioisomeric excess of a given lipase as:

$$\text{RE} = \%\ \text{r.e.} = \%\ (1,3\text{-isomer}) - \%\ (1,2\text{-isomer}) \qquad [3]$$

which was termed the RE-value of the investigated lipase. Clearly, a high RE-value indicates a 1,3-specific lipase, whereas lower values indicate an increasing loss of 1,3-regioselectivity. The RE-value is quite useful for the comparison of different lipases regarding their ability to synthesize regioisomerically pure 1,3-*sn*-diacylglycerols; so we used this RE-value as a basis for choice of biocatalyst for an optimized synthetic procedure. The results are shown in Table 3.

The lipase from *Pseudomonas* sp. is nonselective, whereas the lipases from *Rhizomucor miehei, Chromobacterium viscosum* and *Rhizopus delemar* show good to excellent selectivity toward the primary hydroxyl functions in glycerol. With this information on hand, the amount of produced 1,3-diacylglycerol in a given reaction mixture can be predicted with rather high accuracy.

The calculated yields of 1,3-*sn*-dilaurin obtained in the model reaction (Eq. [1]) in the presence of four different lipases are in good agreement with the experimental data. Regarding the regioselectivity, the best results are obtained with the lipase from *Rhizopus delemar*, which produces almost exclusively 1,3-*sn*-dilaurin. Unfortunately, however, the relative specific activity of this enzyme is rather low compared to the lipase from *Pseudomonas* sp., which has a much lower regioselectivity. For a practical synthesis of 1,3-*sn*-diacylglycerols on a synthetically useful scale, a reasonable compromise between these two factors—specific activity and regioselectivity—must be found. For laboratory-scale preparations, the lipase from *Rhizomucor miehei* proved to be the best choice due to the relatively high specific activity and a satisfactory regioselectivity.

Nature of the acyl donor. The synthesis of numerous 1,3-*sn*-diacylglycerols was performed with different acyl donors, including free fatty acids, fatty acid methyl esters and fatty acid vinyl esters. The results, summarized in Table 4 and exemplified in Figure 3, clearly show that the highest reaction rates and product yields are achieved when vinyl esters are used as acyl donors. Due to the irreversibility of these esterification reactions (Eq. [1]), and in contrast to direct esterification (Eq. [4]) or reversible acyl transfer reactions (Eq. [5]), no problems are encountered regarding the complete conversion of the acyl donor.

If readily available methyl esters of fatty acids are employed as acyl donors under the conditions of reversible acyl transfer (Eq. [5]), equilibria are created that proved difficult to shift into the direction of the desired 1,3-*sn*-diacylglycerols. Even if a large excess of the acyl donor is employed and molecular sieves (4Å) are used to remove the produced MeOH, lower reaction rates and product yields are observed compared to other acyl donors (Fig. 3). In contrast, the direct esterification of glycerol with free fatty acids as acyl donors (Eq. [4]) proved to be quite successful. With the addition of 3Å molecular sieves, it was possible to remove the product water with high efficiency, allowing a nearly quantitative conversion of the fatty acid employed. Excellent yields of the desired products were achieved in this manner.

TABLE 3

Esterification of Glycerol: Predicted and Observed Products

Enzyme	RE-value[a]	Rel. activity[b] (h)	Time (h)	Conv.[c] (%)	DG content[d] (%)	1,3-Isomer (%)		1,2-Isomer (%)	
						Predicted[e]	Found	Predicted[e]	Found
Rhizopus delemar	97.4	0.23	96	95	90	98.7	97.7	1.3	2.3
Rhizomucor miehei	83.7	0.87	24	>98	97	92.0	93.9	8.0	6.1
Chromobacterium viscosum	92.6	0.65	48	>98	93	96.3	94.1	3.7	5.9
Pseudomonas fluorescens	16.8	1	24	>98	97	58.4	63.3	41.6	36.7

[a]See ref. 22.
[b]Determination *via* initial conversion rate, gas-liquid chromatography (GLC).
[c]Conversion (thin-layer chromatography).
[d]Content (GLC).
[e]Calculated from Eq. [3].

TABLE 4

Esterification of Glycerol: Dependence on the Nature of the Acyl Donor

Product	Acyl donor	Rel. activity[a]	Time (h)	Conv.[b] (%)	Yield[c] (%)	Purity[d] (%)
1,3-*sn*-dilaurin	Vinyl laurate	1	24	>98	85	>99.5
dto.	Lauric acid	0.75	48	95	79	>99.5
dto.	Methyl laurate	0.32	96	90	71	>99.5
1,3-*sn*-dipalmitin	Vinyl palmitate	0.97	24	>98	80	>99.5
dto.	Palmitic acid	0.83	48	93	77	>99.5
dto.	Methyl palmitate	0.25	96	88	69	>99.5
1,3-*sn*-distearin	Vinyl stearate	0.99	24	>98	81	>99.5
dto.	Stearic acid	0.79	48	>90	76	>99.5
dto.	Methyl stearate	0.27	96	90	68	>99.5

[a]Determination *via* the initial conversion rate (gas-liquid chromatography, GLC).
[b]Conversion of acyl donor (thin-layer chromatography).
[c]Isolated yield after recrystallization from dry methanol.
[d]Purity (GLC).

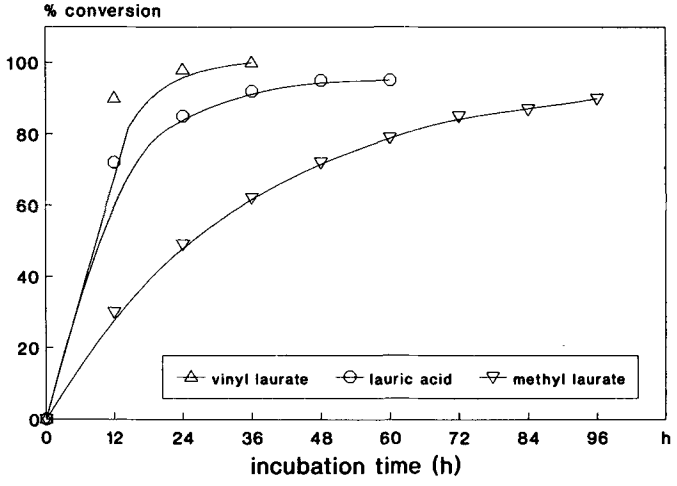
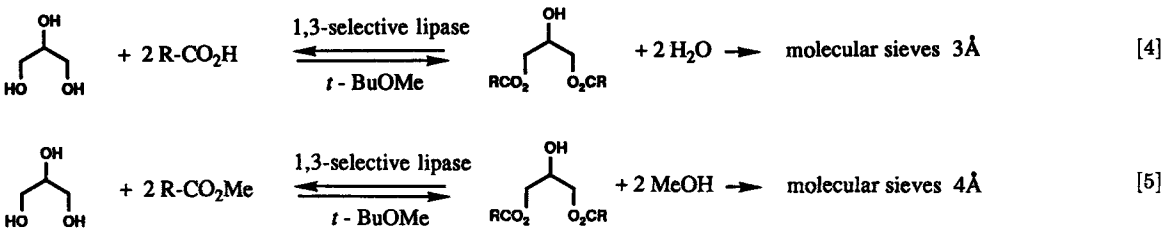


FIG. 3. Lipase-catalyzed esterification of glycerol: dependence of reaction rate on the nature of the acyl donor.

Reusability of support and biocatalyst. Important for the application of the described method on a larger scale is, of course, the reusability of both the solid support and the employed biocatalyst. With the immobililzed lipase from *Rhizomucor miehei* (Liopzyme) and silica gel as a solid support, this problem was studied by using the esterification of glycerol with vinyl laurate as a model reaction. After complete conversion of the reactants, both the enzyme (immobilized on Duolite) and the solid support were removed from the reaction mixture by filtration. The isolated mixture of solids was dried *in vacuo* and reused as a support for glycerol, without adding any additional enzyme. The glycerol preparation thus obtained was again esterified with vinyl laurate under otherwise identical con ditions. As shown in Figure 4, the recovered mixture of immobilized enzyme and solid support can be reused several times without any dramatic loss of activity. Even after five consecutive applications, 60% of the original catalytic activity remains. Of course, further optimization would still be required for a technical application of the process.



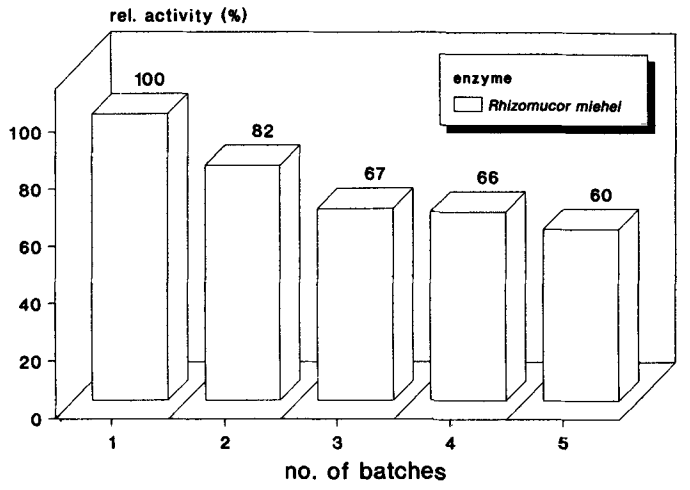


FIG. 4. Lipase-catalyzed synthesis of 1,3-*sn*-dilaurin: reusability of biocatalyst (lipase from *Rhizomucor miehei*, immobilized on anion exchange resin Duolite, Lipozyme).

The above procedure for the synthesis of regioisomerically pure 1,3-*sn*-diacylglycerols provides ready access to these interesting compounds on a synthetically useful scale. Good to excellent yields were obtained in the transformations, which are largely dependent on the regioselectivity of the employed biocatalyst and on the nature of the acyl donor.

Because both the catalyst and the solid support can be reused several times, the scale-up of the described method into an industrial process is greatly facilitated. The availability of regioisomerically pure 1,3-*sn*-diacylglycerols now allows a thorough study of their potential for numerous technical, pharmaceutical and synthetic applications.

ACKNOWLEDGMENT

This work was financially supported by the BMFT (Bundesministerium für Forschung und Technologie, Germany; project no. 0319195A).

REFERENCES

1. Wehrli, H.P., and Y. Pomeranz, *Chem. Phys. Lipids 3*:357 (1969).
2. Van Deenen, L.L.M., and G.H. De Haas, *Biochim. Biophys. Acta 70*:538 (1963).
3. Kumar, R., and J.D. Billimoria, *J. Pharm. Pharmacol. 30*:754 (1978).
4. Paris, G.Y., D.G. Cimon, D.L. Garmaise, L. Swett, J.W. Carter and P.Y. Young, *Eur. J. Med. Chem. 17*:193 (1980).
5. Jacob, J.N., G.W. Hesse and V.E. Shashoua, *J. Med. Chem. 30*:1573 (1987).
6. Jacob, J.N., G.W. Hesse and V.E. Shashoua, *Ibid. 33*:733 (1990).
7. Garzon-Aburbeh, A., J.H. Poupaert, M. Claesen, P. Dumont and G. Atassi, *Ibid. 26*:1200 (1983).
8. Saraiva Goncalves, J.C., C. Razzouk, J.H. Poupaert and P. Dumont, *J. Chromatogr. 494*:389 (1989).
9. Garzon-Aburbeh, A., J.H. Poupaert, M. Claesen and P. Dumont, *J. Med. Chem. 29*:687 (1986).
10. Mantelli, S., P. Speiser and H. Hauser, *Chem. Phys. Lipids 37*:329 (1985).
11. Kido, H., N. Fukusen, K. Ishidoh and N. Katunuma, *Biochem. Biophys. Res. Commun. 138*:275 (1986).
12. Siegel, D.P., J. Banschbach, D. Alford, H. Ellens, L.J. Lis, P.J. Quinn, P.L. Yeagle and J. Bentz, *Biochemistry 28*:3703 (1989).
13. Swern, D., in *Bailey's Industrial Oil and Fat Products*, Vol. 1, Wiley Interscience, New York, 1979, p. 3.
14. Pratt, C.D., and W.W. Hays, *Food Eng. 24*:109 (1952).
15. Ikeda, I., X.P. Gu, I. Mijamoto and M. Okahara, *J. Am. Oil Chem. Soc. 66*:822 (1989).
16. Bentley, P.H., and W. McCrae, *J. Org. Chem. 35*:2082 (1970).
17. Mattson, F.H., and R.A. Volpenheim, *J. Lipid Res. 3*:281 (1962).
18. Howe, R.J., and T. Malkin, *J. Chem. Soc.*, 2663 (1951).
19. Osada, K., K. Takahashi and M. Hatano, *J. Am. Oil Chem. Soc. 67*:921 (1990).
20. Zaks, A., *Ibid. 66*:484 (1989).
21. Holmberg, K., and E. Osterberg, *Ibid. 65*:1544 (1988).
22. Berger, M., and M.P. Schneider, *Biotechnol. Lett. 13*:333 (1991).
23. Schneider, M., K. Laumen, D. Breitgoff, D. Wullbrandt, M. Schlingmann and R. Keller, European Patent No. 254,243 (1987).
24. Eigtved, P., T.T. Hansen and C.A. Miller, in *Proceedings of the World Conference on Biotechnology for the Fats and Oils Industry*, edited by T. Applewhite, American Oil Chemists' Society, Champaign, 1987, p. 134.
25. Rabiller, C., and F. Maze, *Magnetic Resonance in Chemistry 27*:582 (1989).
26. Schuch, R., and K. Mukherjee, *Appl. Microbiol. Biotechnol. 30*:332 (1989).
27. Riva, M., A. Daghetta and M. Galli, *Riv. Ital. Sost. Grasse 58*:432 (1981).
28. Kodali, D.R., A. Tercyak, D.A. Fahey and D.M. Small, *Chem. Phys. Lipids 52*:163 (1990).
29. Iwai, M., in *Lipases*, edited by B. Borgström and H.L. Brockmann, Elsevier Science Publishers, Amsterdam, 1984, p. 416.
30. Yamane, T., in *Proceedings of the World Conference on Biotechnology for the Fats and Oils Industry*, edited by T. Applewhite, American Oil Chemists' Society, Champaign, 1987, p. 17.

[Received January 24, 1992; accepted July 3, 1992]