

# Fatty Acid Steryl, Stanyl, and Steroid Esters by Esterification and Transesterification in Vacuo Using *Candida rugosa* Lipase as Catalyst

Nikolaus Weber,\* Petra Weitkamp, and Kumar D. Mukherjee

Institute for Biochemistry and Technology of Lipids, H. P. Kaufmann-Institute, Federal Centre for Cereal, Potato and Lipid Research, Piusallee 68, D-48147 Münster, Germany

Sterols (sitosterol, cholesterol, stigmasterol, ergosterol, and 7-dehydrocholesterol) and sitostanol have been converted in high to near-quantitative yields to the corresponding long-chain acyl esters via esterification with fatty acids or transesterification with methyl esters of fatty acids or triacylglycerols using lipase from *Candida rugosa* as biocatalyst in vacuo (20–40 mbar) at 40 °C. Neither organic solvent nor water is added in these reactions. Under similar conditions, cholesterol has been converted to cholesteryl butyrate and steroids (5 $\alpha$ -pregnan-3 $\beta$ -ol-20-one or 5-pregnen-3 $\beta$ -ol-20-one) have been converted to their propionic acid esters, both in moderate to high yields, via transesterification with tributyrin and tripropionin, respectively. Reaction parameters studied in esterification include the temperature and the molar ratio of the substrates as well as the amount and reuse properties of the *C. rugosa* lipase. Lipases from porcine pancreas, *Rhizopus arrhizus*, and *Chromobacterium viscosum* are quite ineffective as biocatalysts for the esterification of cholesterol with oleic acid under the above conditions.

**Keywords:** *Steryl esters; stanyl esters; steroid esters; fatty acids; Candida rugosa lipase; solvent-free esterification; esterification in vacuo; transesterification in vacuo*

## INTRODUCTION

Steryl esters, particularly cholesteryl esters, are widely used for technical applications such as liquid crystal display devices. Moreover, fatty acid esters of sterols and steroids are well-known ingredients of cosmetic, nutraceutical, and pharmaceutical formulations (Koshiro, 1987). Recently, plant steryl and stanyl esters have been found to be effective in lowering plasma cholesterol concentration by inhibiting the absorption of cholesterol from the small intestine (Miettinen et al., 1995; Sierksma et al., 1999; Weststrate et al., 1999). They are, therefore, added to special margarines that are commercially available as functional foods with the ability to reduce both total and low-density lipoprotein (LDL) cholesterol levels (Wester, 2000).

Usually, fatty acid esters of sterols, stanols, and steroids are prepared by chemical esterification of the corresponding sterol compounds with fatty acids or interesterification with fatty acid methyl esters as well as by their reaction with fatty acid halogenides or anhydrides (Spener, 1979). Enzymatic procedures for the preparation of steryl esters requiring organic solvents and molecular sieves or other drying agents are well-known (Faber and Riva, 1992; Haraldsson, 1992; Koshiro, 1987; Kosugi et al., 1989; Myojo and Matsufune, 1995; Riva and Klivanov, 1988; Riva et al., 1989). Very recently, a method has been described for the preparation of steryl esters of polyunsaturated fatty acids in an aqueous system (Shimada et al., 1999). In

this paper we report the enzymatic preparation of carboxylic acid esters, particularly fatty acid esters, of sterols, stanols, and steroids in high yield by esterification and transesterification of fatty acids and other carboxylic acid esters, respectively, with the 3-hydroxy group of sterols, stanols, or steroids in vacuo at moderate temperature using immobilized lipase from *Candida rugosa* as the catalyst. Neither organic solvent nor water is required. Moreover, a drying reagent such as a molecular sieve is not used.

## EXPERIMENTAL PROCEDURES

**Chemicals.** Sitostanol (stigmastanol), ergosterol, 7-dehydrocholesterol, 5-pregnen-3 $\beta$ -ol-20-one, 5 $\alpha$ -pregnan-3 $\beta$ -ol-20-one, thiocholesterol, palmitic acid, stearic acid, oleic acid, linoleic acid, methyl laurate, methyl oleate, tributyrin, triolein, 2-ethylhexanecarboxylic acid, porcine pancreas type II lipase (700000–1500000 units·g<sup>-1</sup>) and *C. rugosa* type VII lipase (850000 units·g<sup>-1</sup>) were obtained from Sigma-Aldrich-Fluka (Deisenhofen, Germany). Cholesterol, cholesteryl acetate, sitosterol (LAB grade), and stigmasterol were purchased from Merck (Darmstadt, Germany). Lipase from *Chromobacterium viscosum* (144000 units·g<sup>-1</sup>) was a product of Biocatalysts (Pontypridd, Mid Glamorgan, U.K.). Lipase from *Rhizopus arrhizus* (50000 units·mL<sup>-1</sup>) was obtained from Boehringer-Mannheim (Mannheim, Germany). Tripropionin was a product of Acros Organics/Fisher Scientific (Schwerte, Germany). Sunflower oil (Deutsche Thomy GmbH, Karlsruhe, Germany) was purchased from a local supermarket.

**Lipase-Catalyzed Reactions.** Unless stated otherwise, fatty acids or fatty acid methyl esters, 300  $\mu$ mol each, or triacylglycerols (200  $\mu$ mol) were esterified or transesterified with sterols and stanols, 100  $\mu$ mol each, in the presence of 50 mg of *C. rugosa* lipase preparation by magnetic stirring in a screw-capped tube in vacuo at 40 °C for various periods with water- (or methanol)-trapping in the gas phase using KOH

\* Author to whom correspondence should be addressed (telephone +49-251-43510; fax +49-251-519 275; e-mail ibtfett@uni-muenster.de).

pellets. 5-Pregnen-3 $\beta$ -ol-20-one and 5 $\alpha$ -pregnan-3 $\beta$ -ol-20-one, 50  $\mu$ mol each, were transesterified under similar experimental conditions with 500  $\mu$ mol of tripropionin in the presence of 100 mg of *C. rugosa* lipase preparation in vacuo at 35 °C. The vacuum used was 20–40 mbar, measured at room temperature.

Esterification reactions with lipases from *Ch. viscosum* and porcine pancreas were performed using 100  $\mu$ mol of cholesterol, 300  $\mu$ mol of oleic acid, and 50 mg of the respective enzyme preparation for 24 h in vacuo at 40 °C. In the case of *R. arrhizus* lipase, 50  $\mu$ L of the enzyme preparation was dried in vacuo at 40 °C before use.

**Thin-Layer Chromatography (TLC).** Aliquots were withdrawn from the reaction mixtures, and the conversion was checked by TLC on 0.3 mm layers of silica gel H (Merck) using isohexane/diethyl ether (95:5, v/v); spots were located by iodine staining. Alternatively, the TLC plates were sprayed with a 30% aqueous sulfuric acid and heated in an oven kept at 120 °C;  $\Delta^5$ -sterols and their esters appeared as red spots and  $\Delta^{5,7}$ -sterols and their esters as blue spots (Liebermann–Burchard test). The  $R_f$  values of the various compounds were as follows: fatty acid steryl and stanyl esters, 0.6–0.7; fatty acid methyl esters, 0.4–0.5; triacylglycerols, 0.3–0.35; sterols and stanols, 0.1–0.15; unesterified fatty acids, <0.1.

For the separation of mixtures containing 5-pregnen-20-one-3 $\beta$ -yl propionate, 5 $\alpha$ -pregnan-20-one-3 $\beta$ -yl propionate, and tripropionin, the TLC plates were developed with isohexane/diethyl ether (80:20, v/v);  $R_f$  values were as follows: 5-pregnen-20-one-3 $\beta$ -yl propionate and 5 $\alpha$ -pregnan-20-one-3 $\beta$ -yl propionate, 0.2–0.25; tripropionin, 0.15–0.2; 5-pregnen-3 $\beta$ -ol-20-one and 5 $\alpha$ -pregnan-3 $\beta$ -ol-20-one, <0.05.

**Gas Chromatography (GC).** In esterification reactions aliquots of reaction mixtures, ~15 mg, were extracted twice with 2 mL of diethyl ether, each. The ether extract was concentrated and treated with a solution of diazomethane in diethyl ether to convert the unreacted fatty acids to methyl esters. The resulting mixture of methyl esters, unreacted sterols, or stanols and fatty acid steryl or stanyl esters was analyzed by GC. In transesterification reactions aliquots of products consisting of fatty acid methyl esters or triacylglycerols, unreacted sterols, stanols, or steroids as well as carboxylic acid steryl, stanyl, or steroid esters were analyzed without derivatization by GC. All GC samples, dissolved in dichloromethane, were filtered through a 0.45  $\mu$ m syringe filter before injection into the gas chromatograph. A Hewlett-Packard (Böblingen, Germany) HP-5890 series II gas chromatograph equipped with a flame ionization detector was used. Separations were carried out on a 0.1  $\mu$ m Quadrex 400-1HT (Quadrex Corp., New Haven, CT) fused silica capillary column, 15 m  $\times$  0.25 mm i.d., using hydrogen as the carrier gas (column pressure = 50 kPa) initially at 160 °C for 2 min, followed by linear programming from 160 to 180 °C at 5 °C $\cdot$ min $^{-1}$  and from 180 to 410 °C at 20 °C $\cdot$ min $^{-1}$ ; the final temperature of 410 °C was held for 10 min. The split ratio was 1:10, and the injector as well as flame ionization detector temperature was 350 °C. Peaks in the gas chromatograms were assigned by comparison of their retention times with those of commercially available standards or those prepared by chemical or enzymatic synthesis. Peak areas and percentages were calculated using Hewlett-Packard 3365 series GC ChemStation software.

**Purification of Steryl and Stanyl Esters by Column Chromatography.** In a typical example, the reaction mixtures resulting from lipase-catalyzed esterification of oleic acid with sitostanol were taken up in 3 mL of diethyl ether, and the enzyme catalyst was separated by centrifugation. The supernatant was removed, the solvent was evaporated, and the reaction products (~120 mg), dissolved in 1 mL of isohexane/diethyl ether (9:1, v/v), were applied to a column (20 cm  $\times$  1 cm i.d.) packed with silica gel as a slurry in isohexane. Elution with 20 mL of isohexane/diethyl ether (95:5, v/v) yielded 60.5 mg of sitostanyl oleate (88% of theory; purity >95%). Subsequent elution with 20 mL of isohexane/diethyl ether (1:1, v/v) yielded unreacted oleic acid together with sitostanol.

**Purification of Steryl and Stanyl Esters by Deacidification.** In a typical example, the reaction products (~65 mg) resulting from lipase-catalyzed esterification of oleic acid with a stoichiometric amount of sitostanol were taken up in 6 mL of diethyl ether and the enzyme catalyst was separated by centrifugation. The supernatant was removed and extracted three times with 3 mL, each, of 2% aqueous sodium carbonate solution, followed by repeated extractions with water, to remove the unesterified oleic acid as sodium salt. The diethyl ether phase was dried over anhydrous sodium sulfate to yield 60 mg of a product containing 94% sitostanyl oleate, 5% sitostanol, and <1% oleic acid.

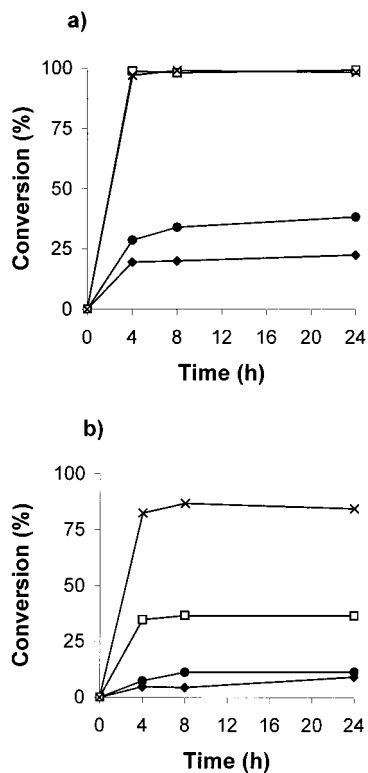
**Purification of 5-Pregnen-20-one-3 $\beta$ -yl Propionate and 5 $\alpha$ -Pregnan-20-one-3 $\beta$ -yl Propionate.** The reaction mixtures resulting from lipase-catalyzed transesterification of tripropionin with 5-pregnen-3 $\beta$ -ol-20-one were taken up in 3 mL of diethyl ether, and the enzyme catalyst was separated by centrifugation. The supernatant was removed, the solvent was evaporated, and the reaction products (~140 mg), dissolved in 6 mL of isohexane, were extracted three times with 3 mL, each, of methanol/water (95:5, v/v) to remove most of the tripropionin. The isohexane phase was dried over anhydrous sodium sulfate, concentrated to a small volume, and purified via silica gel chromatography, as described above, by elution with 30 mL of isohexane/diethyl ether (4:1, v/v) to yield 8.4 mg of 5-pregnen-20-one-3 $\beta$ -yl propionate (45% of theory; purity = 93%). Similarly, 5 $\alpha$ -pregnan-20-one-3 $\beta$ -yl propionate was purified via extraction and silica gel chromatography as described above.

**Melting Points.** Melting points of steryl esters determined with a Thermovar heating block (Reichert, Vienna, Austria) were as follows: sitostanyl oleate, 44–45 °C; sitostanyl linoleate, <20 °C; cholesteryl stearate, 83–84 °C; cholesteryl oleate, 48–50 °C; cholesteryl laurate, 92–93 °C; cholesteryl butyrate, 110–111 °C; stigmasteryl oleate, 43–44 °C; ergosteryl oleate, 76–77 °C; 7-dehydrocholesteryl oleate, 69–70 °C; 5-pregnen-20-one-3 $\beta$ -yl propionate, 122–123 °C.

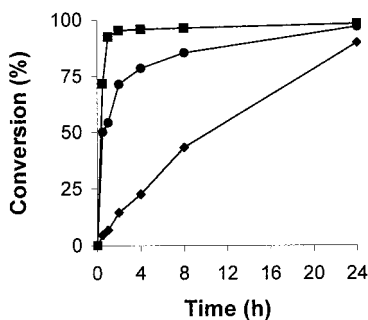
## RESULTS AND DISCUSSION

Lipase-catalyzed esterification and interesterification reactions have been widely used for bioorganic synthesis and biotransformation of fats and other lipids; however, little is known so far on the application of such reactions in the preparation of short- and long-chain acyl esters of sterols, stanols, and steroids (Haraldsson, 1992; Kazlauskas and Bornscheuer, 1998). We show here that esterification and interesterification reactions, catalyzed by *C. rugosa* lipase under vacuum, provide fatty acyl esters of sterols and stanols in near-quantitative yields. Similarly, short-chain acyl esters of sterols and steroids are obtained in high yields by transesterification with short-chain triacylglycerols, catalyzed by *C. rugosa* lipase in vacuo at moderate temperature. Neither organic solvent nor water is added to the reaction mixtures, and no drying agent such as a molecular sieve is used.

Figure 1 shows the time course of esterification of sitostanol with oleic acid, catalyzed by various proportions of *C. rugosa* lipase in vacuo at 40 and 60 °C. It is evident that a near-quantitative conversion is achieved at 40 °C. Figure 1 also shows that the extent of conversions is lower at 60 °C than at 40 °C, which we attribute to a partial deactivation of the lipase at the higher temperature. It is also evident from Figure 1 that at both temperatures the extent of conversion increases with increasing proportion of the lipase; however, at 40 °C increasing the amount of lipase from 25 to 50 mg does not lead to any further increase in the extent of esterification. In all of the following enzymatic reactions, if not stated otherwise, 50 mg of immobilized *C. rugosa* lipase was used.



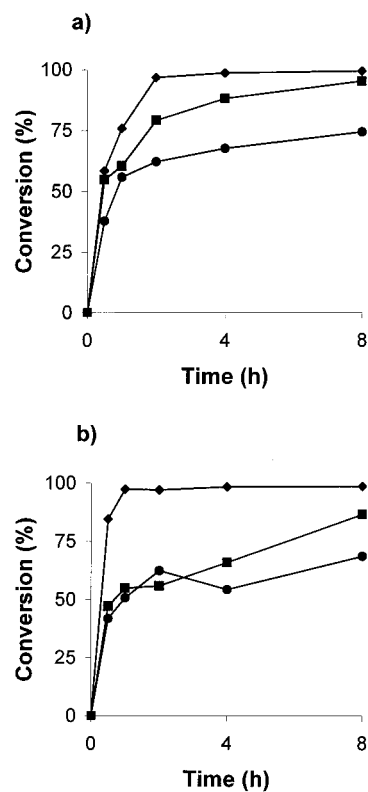
**Figure 1.** Time course of the formation of sitostanyl oleate by esterification of 300  $\mu\text{mol}$  of oleic acid with 100  $\mu\text{mol}$  of sitostanol, catalyzed by different amounts of *C. rugosa* lipase [( $\blacklozenge$ ) 6.25 mg; ( $\bullet$ ) 12.5 mg; ( $\square$ ) 25 mg; ( $\times$ ) 50 mg] in vacuo at (a) 40 and (b) 60  $^{\circ}\text{C}$ .



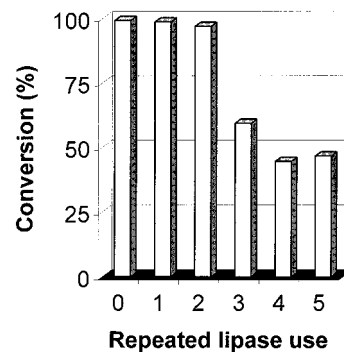
**Figure 2.** Time course of the formation of sitostanyl oleate by esterification of sitostanol with oleic acid with various molar ratios [( $\blacklozenge$ ) 1:1; ( $\bullet$ ) 1:3; ( $\blacksquare$ ) 1:5], catalyzed by *C. rugosa* lipase (25 mg/100  $\mu\text{mol}$  of sitostanol) in vacuo at 40  $^{\circ}\text{C}$ .

Blank experiments carried out at 40  $^{\circ}\text{C}$  in vacuo by reacting sitostanol with oleic acid in the absence of *C. rugosa* lipase for 24 h did not produce any sitostanyl oleate. Control experiments carried out at 40  $^{\circ}\text{C}$  under atmospheric pressure by reacting sitostanol with oleic acid in the presence of *C. rugosa* lipase for 24 h led to a conversion of only 49.9% of sitostanol into sitostanyl oleate as compared to 93.9% conversion in the assay in vacuo, which is consistent with the findings of Shimada et al. (1999) on the low rate of esterification of cholesterol by *C. rugosa* lipase in the presence of water.

Figure 2 shows the effect of varying the molar ratios of the substrates on the time course of esterification of sitostanol with oleic acid, catalyzed by *C. rugosa* lipase in vacuo. It is obvious that increasing the molar ratio of sitostanol to oleic acid from 1:1 to 1:3 and finally to 1:5 leads to a steep increase in the extent of esterification.



**Figure 3.** Time course of the formation of (a) sitostanyl oleate and (b) cholesteryl oleate via ( $\blacklozenge$ ) esterification of 300  $\mu\text{mol}$  of oleic acid, ( $\bullet$ ) transesterification of 300  $\mu\text{mol}$  of methyl oleate, and ( $\blacksquare$ ) transesterification of 150  $\mu\text{mol}$  of triolein with 100  $\mu\text{mol}$  of either sitostanol or cholesterol (experimental conditions: 50 mg of *C. rugosa* lipase; 30 mbar; 40  $^{\circ}\text{C}$ ).



**Figure 4.** Formation of sitostanyl oleate by esterification of 400  $\mu\text{mol}$  of oleic acid with 200  $\mu\text{mol}$  of sitostanol, catalyzed by *C. rugosa* lipase (100 mg/incubation) in vacuo at 40  $^{\circ}\text{C}$  after repeated use of the lipase.

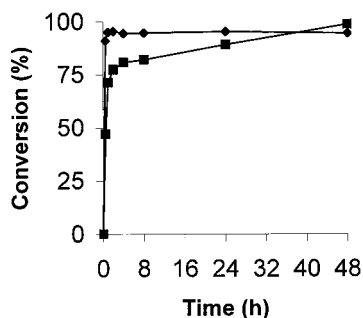
Figure 3 shows the time course of formation of sitostanyl oleate and cholesteryl oleate during esterification of the corresponding sterols with oleic acid and their transesterification with methyl oleate or triolein, both catalyzed by *C. rugosa* lipase in vacuo. With both sterols the esterification with oleic acid is almost complete well within 2 h of reaction, whereas the rates of transesterification of both sitostanol and cholesterol with either methyl oleate or triolein are distinctly lower (Figure 3 and Table 1). Blank experiments carried out at 40  $^{\circ}\text{C}$  in vacuo by reacting sitostanol with either methyl oleate or triolein in the absence of *C. rugosa* lipase for 24 h did not produce any sitostanyl oleate.

Figure 4 shows the effect of repeated lipase use on the extent of esterification of sitostanol with oleic acid, catalyzed by *C. rugosa* lipase in vacuo. It is evident that

**Table 1. Esterification and Transesterification of Sterols and Stanols in Vacuo Catalyzed by *C. rugosa* Lipase<sup>a</sup>**

sterol or stanol	fatty acid or fatty acid ester	time (h)	maximum conversion <sup>b</sup> (%)	enzyme activity <sup>c</sup> (units·g <sup>-1</sup> )
sitostanol	oleic acid	2	96.8	25.3
sitostanol	methyl oleate	8	74.3	18.7
sitostanol	triolein	8	95.1	20.3
sitostanol	sunflower oil	4	97.8	8.2 <sup>d</sup>
sitostanyl oleate	myristic acid	24	1.1	0.3
sitosterol <sup>e</sup>	oleic acid	48	73.0	15.7
sitosterol <sup>e</sup>	methyl oleate	48	52.9	12.3
sitosterol <sup>e</sup>	triolein	48	95.9	14.3
cholesterol	oleic acid	1	97.4	32.3
cholesterol	methyl oleate	48	78.8	17.0
cholesterol	triolein	24	94.0	18.3
cholesterol	myristic acid	48	1.2	0.2
cholesterol	stearic acid	48	1.4	0.1
cholesterol	methyl laurate	8	49.0	11.3
cholesterol	tributyrin	24	96.0	6.6 <sup>d</sup>
cholesterol	2-ethylhexanoic acid	24	0	
cholesteryl acetate	oleic acid	72	2.6	<0.1
cholesteryl acetate	methyl oleate	72	4.0	<0.1
thiocholesterol	oleic acid	24	0	
stigmasterol	oleic acid	16	98.8	4.0 <sup>f</sup>
ergosterol	oleic acid	16	98.6	4.3 <sup>f</sup>
7-dehydrocholesterol	oleic acid	16	89.9	3.6 <sup>f</sup>

<sup>a</sup> Experimental conditions: 100  $\mu\text{mol}$  of sterol; molar ratio of sterol:fatty acid and sterol:fatty acid methyl ester, 1:3, sterol:triacylglycerol including tributyrin, 2:3; amount of *C. rugosa* lipase, 50 mg; 20–40 mbar; 40 °C. <sup>b</sup> Determined by GC. <sup>c</sup> Enzyme units were calculated as 1  $\mu\text{mol}$  of steryl ester formed·min<sup>-1</sup>·g<sup>-1</sup> *C. rugosa* lipase from the initial rates (1 h) of esterification or transesterification as described under Experimental Procedures. <sup>d</sup> Four hour value was used for calculation of enzyme activity. <sup>e</sup> Commercial sitosterol preparation contained around 55% sitosterol, 40% campesterol and 5% stigmasterol. The conversions were calculated as total amount of steryl esters formed. <sup>f</sup> Three hour value was used for calculation of enzyme activity.



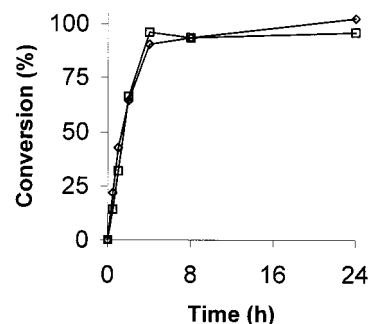
**Figure 5.** Time course of the formation of cholesteryl oleate (◆) and sitostanyl oleate (■) under competitive conditions by esterification of 100  $\mu\text{mol}$  of oleic acid with 100  $\mu\text{mol}$  of an equimolar mixture of cholesterol and sitostanol in vacuo at 40 °C, catalyzed by 50 mg of *C. rugosa* lipase.

the activity of the lipase is fully retained after two repeated uses. From the third repeated use onward a distinct reduction in the extent of esterification is observed (Figure 4).

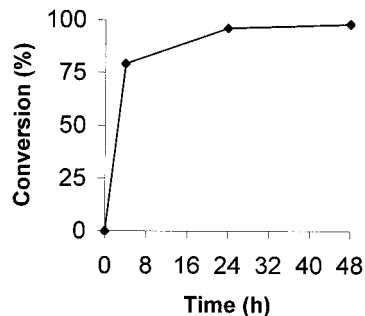
The time course of formation of steryl esters by esterification of an equimolar mixture of cholesterol and sitostanol with oleic acid, catalyzed by *C. rugosa* lipase in vacuo, is shown in Figure 5. The data show that, under competitive conditions, initially cholesteryl oleate is formed at a higher rate than sitostanyl oleate; however, after prolonged reaction (>24 h) both sterols are almost quantitatively esterified. This is consistent with our data shown in Figure 3 and Table 1 for the formation of cholesteryl and sitostanyl oleate.

Figure 6 shows the time course of formation of steryl esters by esterification of an equimolar mixture of oleic and linoleic acids with sitostanol, catalyzed by *C. rugosa* lipase in vacuo. It is evident that both acids react at the same rate and both oleic and linoleic acids are almost quantitatively esterified in ~4 h.

The time course of formation of cholesteryl butyrate by transesterification of cholesterol with tributyrin is



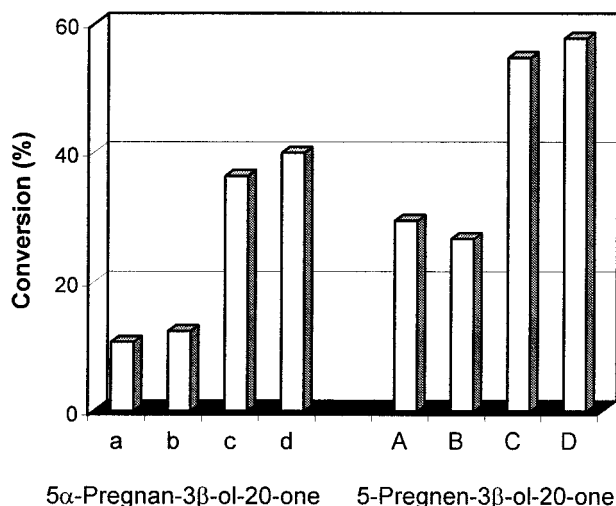
**Figure 6.** Time course of the formation of sitostanyl oleate and sitostanyl linoleate under competitive conditions by esterification of 100  $\mu\text{mol}$  of an equimolar mixture of oleic acid (◇) and linoleic acid (□) with 100  $\mu\text{mol}$  of sitostanol, catalyzed by 50 mg of *C. rugosa* lipase in vacuo at 40 °C.



**Figure 7.** Time course of the formation of cholesteryl butyrate by transesterification of 150  $\mu\text{mol}$  of tributyrin with 100  $\mu\text{mol}$  of cholesterol, catalyzed by 50 mg of *C. rugosa* lipase in vacuo at 40 °C.

shown in Figure 7. These data show that near-quantitative conversion of cholesterol to cholesteryl butyrate is attained after 24 h, which is similar to the data on maximum conversion (96%) of cholesterol to cholesteryl oleate by transesterification with triolein (Table 1).





**Figure 8.** Time course of the formation of 5 $\alpha$ -pregnan-20-one-3 $\beta$ -yl propionate and 5-pregnen-20-one-3 $\beta$ -yl propionate by transesterification of 50  $\mu$ mol of either 5 $\alpha$ -pregnan-3 $\beta$ -ol-20-one or 5-pregnen-3 $\beta$ -ol-20-one with tripropionin (molar ratio 1:10), catalyzed by 50 (a, b, A, B) and 100 mg (c, d, C, D) of *C. rugosa* lipase in vacuo at 35 °C for 16 (a, c, A, C) and 40 h (b, d, B, D).

Figure 8 shows the conversion of two steroids, that is, 5 $\alpha$ -pregnan-3 $\beta$ -ol-20-one and 5-pregnen-3 $\beta$ -ol-20-one, to their propionic acid esters via transesterification with tripropionin, catalyzed by *C. rugosa* lipase in vacuo. Moderate conversion (~35–55%) of both steroids to their propionates occurs after 16 h of reaction using 100 mg of *C. rugosa* lipase; however, the conversions (Figure 8) are not as high as in the transesterification of cholesterol with tributyrin (Figure 7 and Table 1).

Table 1 summarizes the data on maximum conversion and enzyme activity in the esterification and transesterification of various sterols and stanols with different acyl donors, catalyzed by *C. rugosa* lipase in vacuo. With sitostanol, sitosterol, and cholesterol most of the acyl donors lead to near-quantitative conversion to the corresponding steryl and stanyl esters at high rates, as is evident from the data on enzyme activity (Table 1). The esterification of the heat-sensitive diunsaturated sterols such as stigmaterol, ergosterol, and, particularly, 7-dehydrocholesterol with oleic acid also approaches completion after 16 h of reaction at 40 °C. On the other hand, esterification of cholesterol with myristic and stearic acids leads to very little formation of fatty acyl esters (Table 1), which we attribute to the high melting points of the substrates. Transesterifications of sitostanyl oleate with myristic acid and of cholesteryl acetate with either oleic acid or methyl oleate yield only small proportions (2–4%) of cholesteryl oleate. Esterification of cholesterol with 2-ethylhexanoic acid and thioesterification of thiocholesterol with oleic acid do not yield any steryl esters at all, which we attribute to steric hindrance.

Lipases from porcine pancreas, *R. arrhizus*, and *Ch. viscosum* were also tested as biocatalysts for the esterification of cholesterol with oleic acid in vacuo at 40 °C. With all three of these lipases the extent of conversion to cholesteryl oleate does not exceed 2–4% even after a reaction period of 24 h (data not shown).

Our data show that a wide variety of sterols, stanols, and steroids can be efficiently converted in near-

quantitative yields to the corresponding long-chain acyl esters via esterification or transesterification using various acyl donors, such as fatty acids, their methyl esters, or triacylglycerols, and lipase from *C. rugosa* as biocatalyst in vacuo at moderate temperature. Similarly, sterols and steroids are converted to their short-chain acyl esters in high yields via transesterification using short-chain triacylglycerols as acyl donors and lipase from *C. rugosa* as biocatalyst in vacuo. These findings should stimulate the application of such lipase-catalyzed reactions for the preparation of steryl, stanyl, and steroid esters.

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