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Lipophilic (Hydroxy)phenylacetates by Solvent-Free Lipase-Catalyzed Esterification and Transesterification in Vacuo

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Various long-chain alkyl (hydroxy)phenylacetates were prepared in high yield by lipase-catalyzed transesterification of the corresponding short-chain alkyl hydroxyphenylacetates and fatty alcohols in equimolar ratios. The reactions were performed in vacuo at moderate temperatures in the absence of solvents and drying agents in direct contact with the reaction mixture. Immobilized lipase B from *Candida antarctica* (Novozym 435) was the most effective biocatalyst for the various transesterification reactions. Generally, Novozym 435-catalyzed transesterifications of short-chain alkyl (hydroxy)phenylacetates with long-chain alcohols led to higher conversions and enzyme activities than the corresponding esterifications. For example, the transesterification activity was up to 4-fold higher than the esterification activity for the formation of oleyl 4-hydroxy-3-methoxyphenylacetate using Novozym 435 as a biocatalyst. The relative transesterification activities were as follows: phenylacetate > 3-methoxyphenylacetate ≈ 4-methoxyphenylacetate > 4-hydroxy-3-methoxyphenylacetate > 3-hydroxyphenylacetate ≈ 4-hydroxyphenylacetate ≫ 2-methoxyphenylacetate ≫ 3,4-dihydroxyphenylacetate. With respect to the position of methoxy and hydroxy substituents, the transesterification activity of Novozym 435 decreased in the order meta ≈ para ≫ ortho. Compounds with inverse chemical structures, for example, tyrosyl oleate, were obtained by Novozym 435-catalyzed esterification and transesterification of fatty acids and their methyl esters, respectively, with 2-phenylethan-1-ols. In contrast to the transesterifications of short-chain alkyl (hydroxy)phenylacetates with fatty alcohols, higher conversions and enzyme activities were observed for the Novozym 435-catalyzed esterifications of (hydroxy)phenylethanols with long-chain fatty acids than the corresponding transesterifications with fatty acid methyl esters.

KEYWORDS: 2-(4-Hydroxyphenyl)ethyl oleate (tyrosyl oleate); palmityl 4-hydroxyphenylacetate; oleyl 4-methoxyphenylacetate; immobilized lipase B; *Candida antarctica*; Novozym 435; solvent-free; esterification; transesterification

INTRODUCTION

Common plant-derived foods only contain low proportions of lipophilic antioxidants. Generally, the ratio of lipophilic to hydrophilic antioxidant capacity is <1:10 in fruits and vegetables (*1*). Plant antioxidants include ω-(hydroxyphenyl)alkanoic acids such as hydroxybenzoic, hydroxycinnamic, and hydroxyphenylacetic acids (*2–7*). The analogous ω-(hydroxyphenyl)alkanols, for example, 4-hydroxyphenylethanol (tyrosol) and 3-hydroxytyrosol, have also been detected in fruits and vegetables, particularly olives (*5, 8–10*). Beneficial effects on health have been attributed to the antioxidant capacity of such plant phenolic acids, particularly against oxidative attacks by radical-scavenging activity (*9, 11, 12*). Both the antioxidant capacity and the bioavailability of several of these compounds may be further improved by esterification with fatty alcohols leading to an increase of lipophilicity (*13–17*). The range of applications of such lipophilic antioxidants may be extended by their possible use as additives for food and technical applications (*14, 18, 19*). Similarly, compounds with inverse chemical structures such as fatty acid esters of hydroxyphenylethanols, for example, tyrosyl alkanoates, also may have antioxidant and biological activities as known for alkyl hydroxyphenylacetates (*13, 20*).

Enzymatic esterification of hydroxyphenylalkanoic acids may be of advantage over chemical esterification for the preparation of lipophilic alkyl hydroxyphenylalkanoates, particularly for food use, particularly because no hazardous chemicals are used for the preparation. Until now, enzymatic esterification and transesterification procedures requiring organic solvents or using the alcohol component as the solvent have been reported for the preparation of various alkyl esters of hydroxyphenylalkanoic acids including alkyl cinnamates, phenylacetates, and benzo-

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ates (*21–29*). Recently, we have described procedures for the lipase-catalyzed esterification and transesterification of (hydroxy)cinnamic acids and methyl (hydroxy)cinnamates, respectively, with medium- or long-chain alcohols, which was performed with equimolar amounts of reactants at moderate temperatures. Neither hazardous chemicals nor solvents nor drying agents were used in the reaction mixtures, and water or methanol was removed under reduced pressure (*29, 30*). This method was much more efficient than other enzymatic esterification and transesterificatioin procedures described earlier (*14*). The aim of the present work was to extend this simple and environmentally friendly lipase-catalyzed preparation of lipophilic medium- and long-chain alkyl (hydroxy)cinnamates to the preparation of the analogous (hydroxy)phenylacetates by using esterification and transesterification reactions. Additionally, compounds with inverse chemical structures such as (hydroxy)phenylethyl alkanoates were prepared by esterification and transesterification of fatty acids and their methyl esters, respectively, with (hydroxy)phenylethanols (*29–33*).

MATERIALS AND METHODS

General Section. Methyl 2-methoxyphenylacetate, methyl 3,4-dihydroxyphenylacetate, and methyl 2-(1-naphthyl)acetate were prepared from the corresponding carboxylic acids by the reaction with diazomethane (*34*). All other chemicals and substrates were commercially available. Immobilized lipase preparations from *Candida antarctica* (lipase B and Novozym 435), *Rhizomucor miehei* (Lipozyme RM IM), and *Thermomyces lanuginosus* (Lipozyme TL IM) were kindly provided by Novozymes (Bagsvaerd, Denmark).

Gas chromatography (GC) was carried out on a 15 m × 0.25 mm i.d., 0.1 μm J&W DB-5HT fused silica capillary column (Agilent Technologies, Waldbronn, Germany) using hydrogen as the carrier gas (column pressure 80 kPa). The following temperature program was used to separate the various compounds of reaction mixtures: initially at 100 °C for 1 min, followed by linear programming from 100 to 280 °C at 10 °C/min, and finally kept at 280 °C for 1 min. Injector and flame ionization detector (FID) temperatures were maintained at 380 °C. Peaks in gas chromatograms were assigned by comparison of their retention times with those of peaks from standard preparations, which were identified by GC-MS (see below). Peak areas and percentages were calculated using Hewlett-Packard GC ChemStation software. For the determination of enzyme activities, small proportions of methoxy compounds that had been formed during methoxylation of, for example, hydroxyphenylacetic acids with diazomethane were calculated as the original hydroxy compounds.

An Agilent Technologies (Böblingen, Germany) HP-6890 N gas chromatograph equipped with an autosampler and FID was used for gas chromatographic analyses. GC-MS analyses were carried out on a Hewlett-Packard model 5890 series II/5989A apparatus equipped with a 0.1 μm DB-1HT fused silica capillary column (J&W Scientific), 15 m × 0.25 mm i.d., using the electron ionization (EI or CI mode, 70 eV) mode. The carrier gas was He at a flow rate of 1.0 mL × min^{-1}. The column temperature was initially kept at 100 °C for 2 min and then programmed from 100 to 320 at 10 °C × min^{-1}, and the final temperature was held for 8 min. Other operating conditions were split/splitless injector in split mode (split 1:10; temperature, 320 °C), interface temperature (320 °C), and ion source temperature (250 °C).

Lipase-Catalyzed Reactions. As a typical example, methyl 4-hydroxyphenylacetate (49.8 mg, 0.3 mmol) was transesterified with *cis*-9-octadecen-1-ol (80.4 mg, 0.3 mmol) in the presence of 12.5−50 mg of immobilized Novozym 435 lipase by magnetic stirring in a screw-capped reaction tube. The tube was placed in a 100 mL Schlenk reaction vessel under partial vacuum (∼80 kPa) at 80 °C in the dark for periods up to 72 h with water trapping in the gas phase using potassium hydroxide pellets. This moderate vacuum (∼80 kPa) was used to prevent substantial loss of substrates. The experiments were performed in duplicate ($n = 2$). Samples of the reaction products were withdrawn at various intervals and extracted with diethyl ether. The extracts were concentrated to dryness, dissolved in 2 mL of dichloromethane, and filtered through a 0.45 μm PTFE syringe filter to separate the biocatalyst. An aliquot of the filtrate was analyzed as given below. Similar reaction conditions were used for the preparation of phenylethyl alkanoates with inverse chemical structures. As a typical example, oleic acid (84.6 mg, 0.3 mmol) was esterified with 2-(4-hydroxyphenyl)ethanol (41.4 mg, 0.3 mmol) under identical conditions as described above for the transesterification of hydroxylated phenylacetic acid derivatives using Novozym 435 lipase preparation as a biocatalyst.

Similarly, the conversions of analogous compounds were studied under competitive conditions using equimolar mixtures of, for example, methyl 4-hydroxyphenylacetate + methyl 4-hydroxyhydrocinnamate or methyl 4-hydroxybenzoate + methyl 4-hydroxyhydrocinnamate. Blanks were performed under standard assay conditions without lipase.

Enzyme units were calculated from the initial rates (usually 0.5 h) of esterification or transesterification reactions. One unit of enzyme activity was defined as the amount of enzyme (g) that produced 1 μmol/min of the respective alkyl (hydroxy)phenylacetate or fatty acid (hydroxy)phenylethyl ester.

Thin-Layer Chromatography (TLC). Aliquots were withdrawn from the reaction mixtures, and free carboxy groups of compounds were methylated (∼15 min, 20 °C) using a ∼0.2 M solution of diazomethane in diethyl ether (*34*). The conversion by esterification or transesterification was checked by TLC on 0.3 mm layers of silica gel, and spots were located by iodine staining and, if required, by charring after spraying with 30% (v/v) sulfuric acid solution followed by heating (200 °C). A mixture of *iso*-hexane-diethyl ether (1:1) was used as a solvent system as described previously (*29, 30*).

Purification. Alkyl 2-phenylacetates and 2-phenylethyl alkanoates were extracted from the immobilized biocatalysts with diethyl ether and purified by chromatography on a silica gel 60 (VWR International) column (25 cm × 2 cm i.d.) using mixtures of *i*-hexane-diethyl ether as eluents as described previously (*29, 30*). The purification of oleyl 4-hydroxyphenylacetate is given as an example. The reaction mixture (187 mg; conversion 95% as determined by GC) dissolved in 1.5 mL of diethyl ether was applied to the column and eluted first with 20 mL of *iso*-hexane and then subsequently with 20 mL portions of various *iso*-hexane-diethyl ether mixtures (97:3, 95:5, 9:1, 8:2, and 7:3). Elution with 20 mL of *iso*-hexane-diethyl ether (7:3) yielded oleyl 4-hydroxyphenylacetate (isolated yield, 148 mg and 79.1%; purity, 99%). After column chromatography, various alkyl 2-phenylacetates and 2-phenylethyl alkanoates containing saturated alkyl or acyl moieties, respectively, were crystallized from *iso*-hexane or a mixture of *iso*-hexane-diethyl ether (1:1).

GC. Aliquots of esterification and transesterification products were removed from the reaction mixture, extracted, and filtered as described above. The filtrate was concentrated in a stream of nitrogen at 40−50 °C, dissolved in diethyl ether/methanol (10:1, v/v), and treated with an ethereal solution of diazomethane to convert carboxylic acids to the corresponding methyl esters. The resulting mixture of methyl phenylacetates, unreacted 1-alkanols, 2-phenylethanols, and fatty acid methyl esters as well as medium- and long-chain alkyl phenylacetates or 2-phenylethyl alkanoates were analyzed by GC. The hydroxy groups of methyl 2-hydroxyphenylacetate and methyl 3,4-dihydroxyphenylacetate were partly methylated to the methoxy groups by treatment with an ethereal solution of diazomethane in the presence of catalytic amounts of silica gel (*35*) in order to improve FID response. The following relative retention times (RRt) were found using oleyl alcohol and methyl oleate, respectively, as reference compounds: oleyl phenylacetate, 1.81; oleyl 2-, 3-, and 4-methoxy phenylacetates, 2.00, 2.04, and 2.08, respectively; oleyl 3-hydroxy- and 4-hydroxyphenylacetates, 2.10 and 2.19, respectively; oleyl 4-hydroxy-3-methoxyphenylacetate, 2.26; oleyl 3,4-dihydroxyphenylacetate, 2.35; oleyl 4-hydroxyphenoxyacetate, 2.31; oleyl 2-(1-naphthyl)acetate, 2.38; oleyl 2-(2-naphthyl)acetate, 2.45; 2-phenylethyl oleate, 1.78; 2-(1-naphthyl)ethyl oleate, 2.33; 2-(2-naphthyl)ethyl oleate, 2.34; 2-(2-hydroxyphenyl)ethyl oleate, 2.01; 2-(3-hydroxyphenyl)ethyl oleate, 2.05; 2-(4-hydroxyphenyl)ethyl oleate, 2.06; and 2-(4-hydroxy-3-methoxyphenyl)ethyl oleate, 2.14.

Derivatization of Reaction Products by Base-Catalyzed Transmethylation. Under standard conditions, 2 mL of 0.5 M methanolic sodium methylate solution was added to 5−10 mg of the long-chain

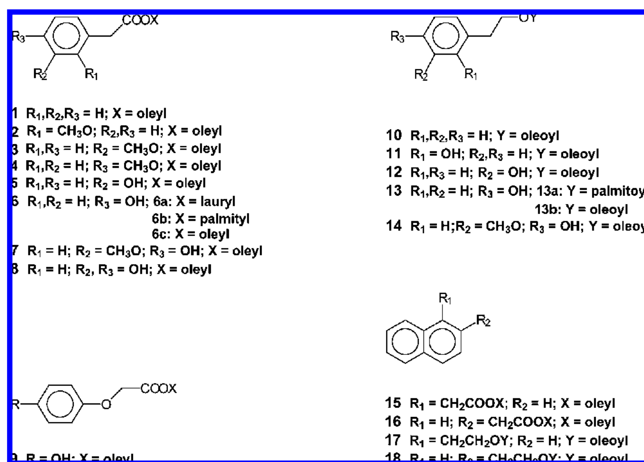

Figure 1. Chemical structures of alkyl phenylacetates (**1**−**8**), alkyl phenoxyacetates (**9**), and alkyl naphthylacetates (**15** and **16**) as well as of structurally similar inverse phenylethyl alkanoates (**10**−**14**) and naphthylethyl alkanoates (**17** and **18**) prepared by lipase-catalyzed esterification and transesterification.

alkyl (hydroxy)phenylacetates or (hydroxy)phenylethyl alkanoates and transmethylated (*34*). Derivatization mixtures of phenolic compounds were acidified before extracting. The extracts (1−2 μL) were directly injected into the gas chromatograph. GC separation of the derivatization products consisting of methyl (hydroxy)phenylacetates and long-chain alcohols or fatty acid methyl esters and (hydroxy)phenylethanols was carried out as described above. Base-catalyzed transmethylation was used to analyze the chemical structure of the lipophilic phenolic reaction products.

GC-MS Analyses. The fragmentation of the various alkyl 2-phenylacetates or 2-phenylethyl alkanoates formed by lipase-catalyzed esterification reactions was studied by GC-MS. Compounds containing carboxy groups were analyzed after derivatization to the corresponding methyl esters using an etherial solution of diazomethane and purification by preparative TLC or column chromatography. The molecular ions (EI mode) were as follows: oleyl phenylacetate, 386 $[M]^+$; oleyl 2-, 3-, and 4-methoxyphenylacetate, 416 $[M]^+$; oleyl 3-hydroxy- and 4-hydroxyphenylacetate, 402 $[M]^+$; lauryl 4-hydroxyphenylacetate, 320 $[M]^+$; palmityl 4-hydroxyphenylacetate, 376 $[M]^+$; oleyl 4-hydroxyphenylacetate, 402 $[M]^+$; oleyl 3,4-dihydroxyphenylacetate, 418 $[M]^+$; oleyl 4-hydroxyphenoxyacetate, 418 $[M]^+$; oleyl 2-(1-naphthyl)acetate and oleyl 2-(2-naphthyl)acetate, 436 $[M]^+$; 2-phenylethyl oleate, 386 $[M]^+$; and 2-(4-hydroxy-3-methoxyphenyl)ethyl oleate, 432 $[M]^+$. Mole peaks of the following compounds were determined in CI mode with methane as the reagent gas (other GC-MS conditions were identical): 2-(2-hydroxyphenyl)ethyl oleate, 402 $[M]^+$; 2-(3-hydroxyphenyl)ethyl oleate, 402 $[M]^+$; 2-(4-hydroxyphenyl)ethyl oleate (tyrosyl oleate), 402 $[M]^+$; and 2-(1-naphthyl)ethyl oleate and 2-(2-naphthyl)ethyl oleate, 436 $[M]^+$.

RESULTS

The enzyme activities of three immobilized commercial lipases, that is, Novozym 435, Lipozyme RM IM, and Lipozyme TL IM, were studied for the esterification of various phenylacetic acids as well as the transesterification of their methyl or ethyl esters with medium- and long-chain alcohols (**Figure 1** and **Table 1**). In addition, enzyme activities were determined for the esterification and transesterification of 2-(1-naphthyl)acetic and 2-(2-naphthyl)acetic acids as well as their methyl esters, respectively, with long-chain alcohols. The conversions were performed with equimolar mixtures of substrates for up to 72 h at 20−80 °C under partial vacuum (~80 kPa) without solvent and drying agent in the reaction mixture using different amounts of the respective lipase. The results given in **Table 1** show that the enzyme activity of Novozym 435 for the esterification of phenylacetic acid with oleyl alcohol is far higher than that of Lipozyme RM IM or Lipozyme TL IM. Similar results were previously obtained for the esterification and transesterification of cinnamic acid derivatives (*29, 30*). Novozym 435 was used, therefore, as a biocatalyst for all subsequent esterification and transesterification reactions.

Effects of Enzyme and Temperature. The time course of the lipase-catalyzed esterification of 4-hydroxyphenylacetic acid with oleyl alcohol (*cis*-9-octadecen-1-ol) at 80 °C using different amounts (6.3, 12.5, and 25 mg) of Novozym 435 as a biocatalyst is shown in **Figure 2**. It is obvious from these results that the conversion rate clearly increases with increasing amounts of the immobilized lipase added to the reaction mixture.

Figure 3 shows the lipase-catalyzed transesterification of methyl 3-methoxyphenylacetate with oleyl alcohol at different temperatures (20, 45, and 80 °C) using Novozym 435 as a biocatalyst. These results demonstrate that increasing the temperature from 20 to 80 °C leads to an around 6-fold increase of the conversion rate as observed at 0.5 h (**Table 1**).

Effects of Substituents. Figure 4 shows the time course of the Novozym 435-catalyzed esterification of 3-hydroxy- and 4-hydroxyphenylacetic acids as well as the Novozym 435-catalyzed transesterification of methyl 3-hydroxy- and 4-hydroxyphenylacetates both with oleyl alcohol. It is evident from these studies and further results shown in **Table 1** that the enzyme activity of the biocatalyst is far higher for the transesterification as compared to the esterification of, for example, methyl 4-hydroxyphenylacetate and 4-hydroxyphenylacetic acid (323 vs 165 units). Moreover, it is obvious from **Figure 4A** that the esterification rate of 4-hydroxyphenylacetic acid is higher than that of 3-hydroxyphenylacetic acid, whereas the transesterification rates of the methyl esters of both hydroxyphenylacetic acids are quite similar (**Figure 4B**). It is worth noting that the analogous 2-hydroxyphenylacetic acid and its methyl ester are not esterified and transesterified at all, whereas a phenoxy group as present in 4-hydroxyphenoxyacetic acid does not change the esterification activity of Novozym 435 as compared to 4-hydroxyphenylacetic acid (**Table 1**).

Figure 5 and **Table 1** demonstrate that the enzyme activities of Novozym 435 for the transesterification of the various methyl methoxyphenylacetates with oleyl alcohol depend on the position of the 2-, 3-, or 4-methoxy substituent. Under the conditions described, transesterification activity is quite similar for methyl phenylacetates with methoxy substituents at the 3- and 4-positions, whereas sterical hindrance by the bulky 2-methoxy group decreases the transesterification activity of Novozym 435 to a large extent.

The influence of the position of bulky substituents was also studied by esterification and transesterification of naphthylacetic acids and methyl naphthylacetates. **Figure 6** shows the effect of the position of the carboxymethylene group of 1-naphthyl- and 2-naphthylacetic acid as well as methyl 1-naphthyl- and methyl 2-naphthylacetate on the lipase-catalyzed esterification and transesterification with oleyl alcohol using Novozym 435 as a biocatalyst. The esterification activity of Novozym 435 is higher for 2-naphthylacetic acid than for 1-naphthylacetic acid, and similarly, the transesterification activity is higher for methyl 2-naphthylacetate than for methyl 1-naphthylacetate. It is obvious from these results that the sterical hindrance in position 1 of the naphthalene backbone decreases esterification and transesterification rates. Moreover, the data given in **Table 1** again indicate that the transesterification activity of Novozym 435 is higher for both methyl 1-naphthyl- and methyl 2-naph-

Table 1. Enzyme Activities of Immobilized *Candida antarctica* Lipase B (Novozym 435) for the Esterification and Transesterification of Various 2-Phenylacetic Acids and Their Methyl Esters, Respectively, with Medium- or Long-Chain Alcohols as Well as Fatty Acids and Their Methyl Esters with Various 2-Phenylethanols

phenylacetic acids and methyl phenylacetates or fatty acids and fatty acid methyl esters	medium- and long-chain alcohols or 2-phenylethanols	reaction products[a]	Novozym 435 (mg/assay)	temperature (°C)	maximum conversion (mol%) after (h)	enzyme activities (units/g) after (h)[b]	±SEM ($n = x$)
esterification of 2-phenylacetic acid derivatives							
phenylacetic acid[c]	*cis*-9-octadecen-1-ol	**1**	12.5	40	95 (1), 96 (4)	721	±31.0 ($n = 2$)
				20	95 (8)	439	±22.3 ($n = 2$)
2-methoxyphenylacetic acid	*cis*-9-octadecen-1-ol	**2**	12.5	80	77 (48)	21	±0.8 ($n = 2$)
3-methoxyphenylacetic acid	*cis*-9-octadecen-1-ol	**3**	12.5	80	92 (8), 97 (24)	207	±15.4 ($n = 2$)
4-methoxyphenylacetic acid	*cis*-9-octadecen-1-ol	**4**	12.5	45	94 (1)	624	±12.9 ($n = 2$)
2-hydroxyphenylacetic acid	*cis*-9-octadecen-1-ol		25	80	ND		($n = 2$)
3-hydroxyphenylacetic acid	*cis*-9-octadecen-1-ol	**5**	25	80	73 (24)	175	±4.9 ($n = 2$)
4-hydroxyphenylacetic acid[d]	*cis*-9-octadecen-1-ol	**6c**	25	80	95 (48)[b], 94 (4)[e]	165	±1.4 ($n = 2$)
4-hydroxyphenoxyacetic acid	*cis*-9-octadecen-1-ol	**9**	25	80	91 (8)[f]	151	±32 ($n = 4$)
4-hydroxy-3-methoxyphenylacetic acid	*cis*-9-octadecen-1-ol	**7**	12.5	80	88 (24), 94 (48)	145	±5.8 ($n = 2$)
1-naphthylacetic acid	*cis*-9-octadecen-1-ol	**15**	50	80	96 (48)	16	±0.9 ($n = 2$)
2-naphthylacetic acid	*cis*-9-octadecen-1-ol	**16**	50	80	94 (2), 98 (72)	155	±12.0 ($n = 2$)
transesterification of methyl or ethyl 2-phenylacetate derivatives							
methyl phenylacetate	*cis*-9-octadecen-1-ol	**1**	12.5	80	94 (4)	653	±22.2 ($n = 2$)
				20	91(8)	315	±8.9 ($n = 2$)
methyl 1-naphthylacetate	*cis*-9-octadecen-1-ol	**15**	50	80	96 (4)	39	±7.2 ($n = 2$)
methyl 2-naphthylacetate	*cis*-9-octadecen-1-ol	**16**	50	80	97 (2)	164	±14.4 ($n = 2$)
methyl 2-methoxyphenylacetate	*cis*-9-octadecen-1-ol	**2**	12.5	45	35 (48)	2.6	±0.17 ($n = 2$)
				80	79 (48)	19[g]	±1.2 ($n = 2$)
methyl 3-methoxyphenylacetate	*cis*-9-octadecen-1-ol	**3**	12.5	80	94 (4)	670	±36.8 ($n = 2$)
				45	94 (8)	430	±15.1 ($n = 2$)
				20	80 (8)	149	±14.6 ($n = 2$)
methyl 4-methoxyphenylacetate	*cis*-9-octadecen-1-ol	**4**	12.5	45	89 (8)	418	±0.2 ($n = 2$)
methyl 2-hydroxyphenylacetate	*cis*-9-octadecen-1-ol		12.5	80	ND		($n = 2$)
methyl 2-(3-hydroxyphenyl)acetate	*cis*-9-octadecen-1-ol	**5**	12.5	80	87 (4), 89 (24)	313	±3.4 ($n = 2$)
methyl 4-hydroxyphenylacetate	dodecan-1-ol	**6a**	12.5	80	94 (4), 96 (8)	439	±3.9 ($n = 2$)
methyl 4-hydroxyphenylacetate	hexadecan-1-ol	**6b**	12.5	80	93 (8), 95 (24)	297	±1.8 ($n = 2$)
methyl 4-hydroxyphenylacetate[h]	*cis*-9-octadecen-1-ol	**6c**	12.5	80	93 (8), 95 (48)	323	±0.8 ($n = 2$)
ethyl 4-hydroxyphenylacetate	*cis*-9-octadecen-1-ol	**6c**	12.5	80	92 (4), 94 (24)	485	±22.5 ($n = 2$)
ethyl 4-hydroxy-3-methoxyphenylacetate	*cis*-9-octadecen-1-ol	**7**	12.5	80	94 (4)	572	±28.7 ($n = 4$)
methyl 3,4-dihydroxyphenylacetate	*cis*-9-octadecen-1-ol	**8**	100	80	30 (48)	7	±0.1 ($n = 2$)
transesterifications under competitive conditions							
methyl 4-hydroxyphenylacetate +	*cis*-9-octadecen-1-ol		12.5	80		343	±14.0 ($n = 2$)
methyl 4-hydroxyhydrocinnamate						116	±9.1 ($n = 2$)
methyl 4-hydroxybenzoate +	*cis*-9-octadecen-1-ol		12.5	80		1.9	±0.17 ($n = 2$)
methyl 4-hydroxyhydrocinnamate						214	±40.6 ($n = 2$)
methyl 4-hydroxyphenylacetate	dodecan-1-ol		12.5	80		445	±21.8 ($n = 2$)
	+ hexadecan-1-ol					313	±47 0.5 ($n = 2$)
	+ *cis*-9-octadecen-1-ol					256	±51.1 ($n = 2$)
esterification of 2-phenylethanol derivatives							
oleic acid	2-phenylethanol	**10**	12.5	80	94 (0.5)	753	±5.8 ($n = 2$)
				20	89 (8)	462	±10.5 ($n = 2$)
oleic acid	2-(1-naphthyl)ethanol	**17**	12.5	80	94 (1)	587	±30.4 ($n = 4$)
			50		96 (0.5)	193	±0.1 ($n = 2$)
oleic acid	2-(2-naphthyl)ethanol	**18**	12.5	80	97 (8)	221	±15.7 ($n = 2$)
			50		96 (1)	169	±18.4 ($n = 2$)
oleic acid	2-(2-hydroxyphenyl)ethanol	**11**	25	80	82 (4)	194	±10.5 ($n = 2$)
			12.5	45	81 (8)	237	±9.6 ($n = 3$)
oleic acid	2-(3-hydroxyphenyl)ethanol	**12**	12.5	45	93 (4)	551	±1.6 ($n = 2$)
oleic acid	2-(4-hydroxyphenyl)ethanol	**13b**	12.5	80	95 (4)	697	±4.3 ($n = 2$)
				45	87 (24)	92	±9.9 ($n = 6$)
oleic acid	2-(4-hydroxy-3-methoxyphenyl) ethanol	**14**	12.5	80	99 (8)	286	±17.2 ($n = 2$)
transesterification of 2-phenylethanol derivatives							
methyl oleate	2-phenylethanol	**10**	12.	45	89 (4)	595	±5.0 ($n = 2$)
				20	85 (8)	263	±14.7 ($n = 2$)
triolein	2-phenylethanol	**10**	12.5	80	87 (2)	630[i]	±11.7 ($n = 2$)
methyl oleate	2-(2-hydroxyphenyl)ethanol	**11**	25	80	72 (2), 80 (48)	189	±10.6 ($n = 2$)
methyl oleate	2-(3-hydroxyphenyl)ethanol	**12**	25	80	89 (24)	160	±14.3 ($n = 4$)
methyl palmitate	2-(4-hydroxyphenyl)ethanol	**13a**	25	80	91 (8)	225	±3.8 ($n = 2$)
methyl oleate	2-(4-hydroxyphenyl)ethanol	**13b**	25	80	90 (24)	146	±3.7 ($n = 2$)
triolein	2-(4-hydroxyphenyl)ethanol	**13b**	25	80	58 (8), 63 (24)	184[i]	±5.3 ($n = 4$)
sunflower oil	2-(4-hydroxyphenyl)ethanol		25	80	68 (8), 69 (24)	180[i]	±20.8 ($n = 2$)

[a] Numbering is according to the chemical structures given in **Figure 1**. [b] Standard assay conditions, if not otherwise indicated are as follows: 0.3 mmol of carboxylic acid or methyl ester + 0.3 mmol of alcohol; immobilized lipase/assay, 12.5 mg; 80 °C; 80 kPa; and 0.5 h. [c] After subtraction of blank (=2.3 mol% oleyl 2-phenylacetate within 0.5 h at 80 °C), no conversion was detected using Lipozyme RM IM and Lipozyme TL IM, respectively. [d] After subtraction of blank (=5 mol% oleyl 4-hydroxyphenylacetate within 8 h at 80 °C or 12 mol% oleyl 4-hydroxyphenylacetate within 24 h at 80 °C). [e] Fifty milligrams of Novozym 435. [f] $n = 2$. [g] After 8 h. [h] Blank = 0.4 mol% oleyl 4-hydroxyphenylacetate within 48 h at 80 °C. [i] Molar ratio triglyceride:(hydroxy)phenylethanols (1:3). SEM, standard error of mean; ND, not detected.

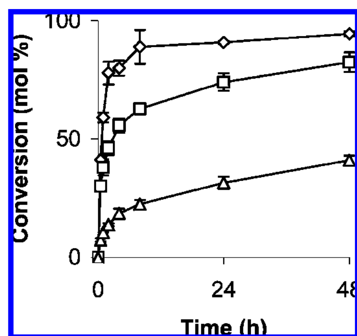

Figure 2. Lipase-catalyzed esterification of 0.3 mmol of 4-hydroxyphenylacetic acid with 0.3 mmol of oleyl alcohol at 80 °C and 80 kPa using different amounts (△, 6.3 mg; □, 12,5 mg; and ◇, 25 mg) of Novozym 435 as a biocatalyst; $n = 2$ for each.

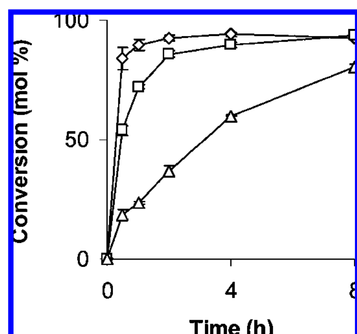

Figure 3. Lipase-catalyzed transmethylation of 0.3 mmol of methyl 3-methoxyphenylacetate with 0.3 mmol of oleyl alcohol at different temperatures (△, 20 °C; □, 45 °C; and ◇, 80 °C) and 80 kPa using Novozym 435 (12.5 mg) as a biocatalyst; $n = 2$ for each.

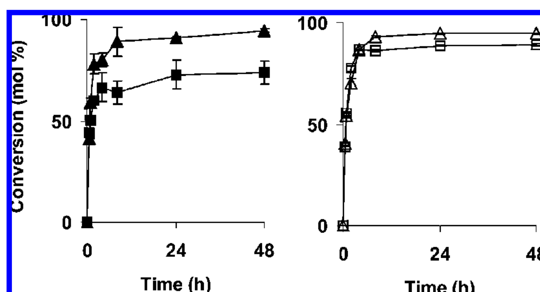

Figure 4. Time course of lipase-catalyzed (**A**) esterification of 3-hydroxy- (■) and 4-hydroxyphenylacetic acid (▲) (0.3 mmol each; 25 mg Novozym 435/assay) as well as (**B**) transesterification of methyl 3-hydoxy- (□) and 4-hydroxyphenylacetate (△) (0.3 mmol/each; 12.5 mg Novozym 435/assay) with oleyl alcohol at 80 °C and 80 kPa; $n = 2$ for each.

thylacetate as compared to the esterification activity for 2-naphthylacetic acid and, particularly, 1-naphthylacetic acid.

Very different enzyme activities were found for the Novozym 435-catalyzed transesterification of various methyl or ethyl phenylacetates with oleyl alcohol. For example, **Table 1** shows that a 4-hydroxy substituent as compared to a 4-methoxy group decreased the enzyme activity as is shown for the transesterification of methyl 4-hydroxyphenylacetate and methyl 4-methoxyphenylacetate, respectively, with oleyl alcohol. Under similar reaction conditions, introduction of a further hydoxy group into methyl 4-hydroxyphenylacetate as in methyl 3,4-dihydroxyphenylacetate again led to both substantially decreased transesterification activity (323 vs 7 units/g) and maximum conversion (94 vs 30 mol%; **Table 1** and **Figure 7**). Moreover, the time course of the Novozym 435-catalyzed transesterification of ethyl 4-hydroxyphenylacetate and ethyl 4-hydroxy-3-methoxyphenylacetate both with oleyl alcohol is shown in **Figure 7**. It is obvious from these results and the data shown in **Table 1** that—in contrast to an additional 3-hydroxy group as shown above—the presence of an additional 3-methoxy group as in 4-hydroxy-3-methoxyphenylacetate did not decrease maximum conversion of the substrate and rather increased transesterification activity of Novozym 435.

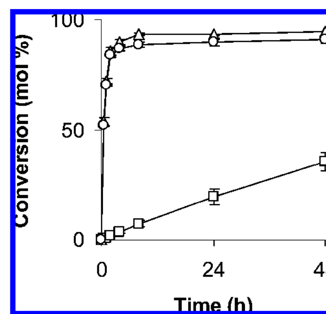

Figure 5. Effect of the position of 2-methoxy- (□), 3-methoxy- (△), and 4-methoxy-substituents (○) of methyl methoxyphenylacetates (0.3 mmol each) on the lipase-catalyzed transesterification with oleyl alcohol (0.3 mmol) at 45 °C and 80 kPa using Novozym 435 (12.5 mg) as a biocatalyst; $n = 2$ for each.

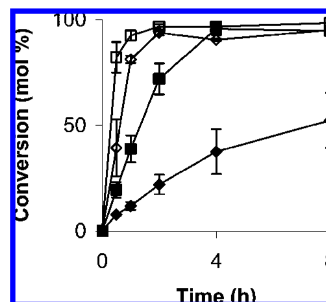

Figure 6. Effect of the position of the carboxymethylene group of 1-naphthyl- (◆) and 2-naphthylacetic acid (◇) (0.3 mmol each) as well as methyl 1-naphthyl- (■) and 2-naphthylacetate (□) (0.3 mmol each) on the lipase-catalyzed esterification and transesterification, respectively, with 0.3 mmol of oleyl alcohol at 80 °C and 80 kPa using Novozym 435 (50 mg/assay) as a biocatalyst; $n = 2$ for each.

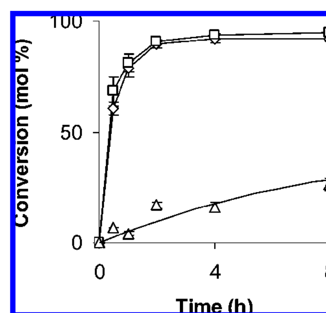

Figure 7. Effects of methoxy- and hydroxy-substituents of phenylacetates on the Novozym 435-catalyzed transesterification of 0.3 mmol of ethyl 4-hydroxyphenylacetate (◇) (12.5 mg of Novozym 435), 0.3 mmol of ethyl 4-hydroxy-3-methoxyphenylacetate (□) (12.5 mg of Novozym 435), and 0.3 mmol of methyl 3,4-dihydroxyphenylacetate (△) (100 mg of Novozym 435) with 0.3 mmol of oleyl alcohol, each at 80 °C and 80 kPa; $n = 2$ for each.

Effect of Chain-Length of the Phenylalkanoyl Moiety. **Figure 8** and **Table 1** show the enzyme activities of Novozym 435 lipase under competitive conditions for the transesterification of oleyl alcohol with methyl 4-hydroxyphenylacetate and methyl 4-hydroxyphenylpropionate (**Figure 8A**) as well as with

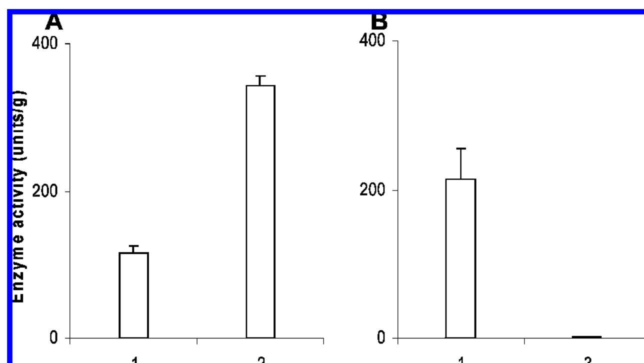

Figure 8. Transesterification activities of immobilized Novozym 435 lipase (12.5 mg) under competitive conditions for the conversions of (**A**) methyl 4-hydroxyphenylpropionate (4-hydroxyhydrocinnamate) (**1**) and methyl 4-hydroxyphenylacetate (**2**) as well as (**B**) methyl 4-hydroxyphenylpropionate (4-hydroxyhydrocinnamate) (**1**) and methyl 4-hydroxybenzoate (**3**), 0.15 mmol, each with 0.3 mmol of oleyl alcohol at 0.5 h, 80 °C, and 80 kPa; $n = 2$ for each.

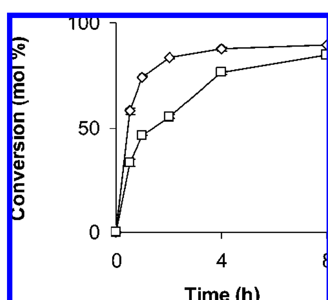

Figure 9. Esterification and transesterification of 0.3 mmol of 2-phenylethanol with 0.3 mmol of oleic acid (◇) and 0.3 mmol of methyl oleate (□), respectively, at 20 °C and 80 kPa using Novozym 435 (12.5 mg/assay) as a biocatalyst; $n = 2$ for each.

methyl 4-hydroxyphenylpropionate and methyl 4-hydroxybenzoate (**Figure 8B**). Obviously, the transesterification activity of Novozym 435 for these three compounds increases in the following order: methyl 4-hydroxyphenylacetate > methyl 4-hydroxyphenylpropionate (4-hydroxyhydrocinnamate) ≫ methyl 4-hydroxybenzoate. It is also evident from **Table 1** that lauryl alcohol (1-dodecanol) is the preferred substrate when an equimolar mixture of medium- and long-chain alcohols is transesterified with 4-hydroxyphenylacetate under competitive conditions as compared to palmityl alcohol (1-hexadecanol) and oleyl alcohol.

Phenylethyl Alkanoates with Inverse Structure. In addition to the preparation of various alkyl phenylacetates by esterification and transesterification of phenylacetic acid derivatives with long-chain alcohols, the corresponding 2-phenylethan-1-ol derivatives were reacted with fatty acids or fatty acid methyl esters to form the corresponding lipophilic fatty acid esters of, for example, 4-hydroxyphenylethanol (tyrosol) having inverse chemical structure as compared to the alkyl phenylacetates described above. **Figure 9** and **Table 1** show the esterification and transesterification of 2-phenylethanol with oleic acid and methyl oleate, respectively, at 20 °C using Novozym 435 as a biocatalyst. In contrast to our data on the esterification and transesterification of phenylacetic acid and methyl phenylacetate, respectively, these results demonstrate that esterification of 2-phenylethanol with oleic acid is preferred over transesterification of 2-phenylethanol with methyl oleate for the formation of 2-phenylethyl oleates. Similarly, the analogous 2-(1-naphthyl)ethanol or 2-(2-naphthyl)ethanol was esterified with oleic

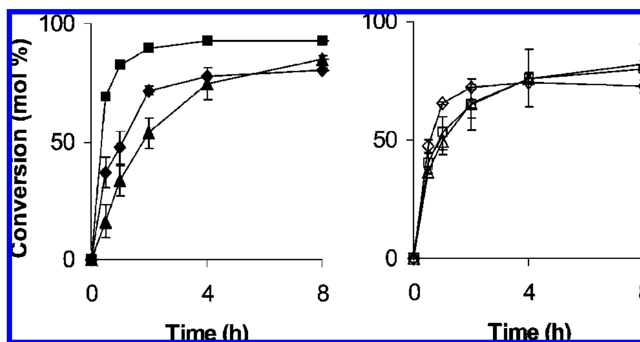

Figure 10. Effect of the position of hydroxy substituents of 2-phenylethanols on the lipase-catalyzed (**A**) esterification (12.5 mg of Novozym 435; 45 °C) of 2-hydroxy- (◆), 3-hydroxy- (■), or 4-hydroxy-phenylethanol (▲) (0.3 mmol each) with 0.3 mmol of oleic acid as well as (**B**) transesterification (25 mg of Novozym 435; 80 °C) of 2-hydroxy- (◇), 3-hydroxy- (□), or 4-hydroxy-phenylethanol (△) (0.3 mmol each) with 0.3 mmol of methyl oleate, respectively; $n = 2$ for each.

acid using Novozym 435 as a biocatalyst to study the influence of the position of bulky substituents on the conversion to the corresponding oleic acid esters. As expected, the results of these experiments show that the esterification rate was far higher for the conversion of 2-(1-naphthyl)ethanol as compared to 2-(2-naphthyl)ethanol (**Table 1**).

Figure 10 shows the effect of the position of hydroxy substituents at the phenyl moiety on the Novozym 435-catalyzed esterification of 2-hydroxy-, 3-hydroxy-, or 4-hydroxyphenylethanol with oleic acid (**Figure 10A**) as well as the corresponding transesterification with methyl oleate (**Figure 10B**). Again, esterification is preferred over transesterification, and the esterification activity of Novozym 435 increases in the following order for these three phenylethyl alcohols: 3-hydroxy- > 2-hydroxy- > 4-hydroxyphenylethanol. It is worth noting that 2-hydroxyphenylethanol was esterified and transesterified with oleic acid and methyl oleate, respectively, whereas no conversion was found for the analogous reactions of 2-hydroxyphenylacetic acid and the corresponding methyl ester with oleyl alcohol as described above. 2-(4-Hydroxy-3-methoxyphenyl)ethanol was also esterified with oleic acid in high yield using Novozym 435 as a biocatalyst (**Table 1**). The enzyme activity of Novozym 435 is distinctly lower for the transesterification of 2-hydroxy-, 3-hydroxy-, or 4-hydroxyphenylethanols with methyl oleate as compared to the esterification with oleic acid. Under similar conditions, transesterification of 4-hydroxyphenylethanol with triolein or sunflower oil led to moderate conversions only (**Table 1**), most probably because of side reactions such as transesterification/re-esterification, as was obvious from substantial concentrations of mono- and diglycerides in the reaction mixture.

DISCUSSION

Recently, we have shown that various medium- and long-chain alkyl (hydroxy)cinnamates can be efficiently prepared from hydroxycinnamic acids and their analogues via lipase-catalyzed esterification or transesterification with fatty alcohols under environmentally friendly conditions. This implies the use of nonactivated reactants in an equimolar ratio as starting materials, reduced pressure for the removal of reaction water or methanol, moderate temperatures, and avoiding organic solvents and drying reagents (*29, 30*). In continuation of the above studies, we have applied these enzymatic esterification and transesterification reactions to the preparation of lipophilic

alkyl esters of (hydroxy)phenylacetic acids such as oleyl 4-hydroxyphenylacetate. In a similar manner, the inverse fatty acid esters of (hydroxy)phenylethanols were prepared, for example, 2-(4-hydroxyphenyl)ethyl oleate (tyrosol oleate). High conversions (mostly ≥90%) were obtained within relatively short reaction periods using equimolar mixtures of acids or methyl esters and alcohols, which reduces costs and simplifies purification. In many cases, this method is superior to various other enzymatic preparations, which utilize high excess of substrates, long reaction times, drying reagents, and, in part, toxic solvents (for a review see ref *14*).

In addition, the following recommendations can be given for the preparation of alkyl phenylacetates and phenylethyl alkanoates: (i) Esterification and transesterification activities of Novozym 435 exceed by far those of Lipozyme RM IM and Lipozyme TL IM. (ii) The transesterification activity of Novozym 435 is generally higher for the preparation of alkyl phenylacetates, whereas the esterification activity is higher for the preparation of phenylethyl alkanoates. (iii) An increase of temperature and amount of Novozym 435 raise the esterification and transesterification rates. (iv) Introduction of a methoxy and, particularly, a hydroxy substituent at the phenyl moiety decreases esterification and transesterification rates. Methoxy and, particularly, hydroxy substituents in the 2-position of the phenyl moiety reduce esterification and transesterification rates far higher than in the 3- or 4-position, which is probably caused by sterical hindrance and/or hydrogen bonds (*36*). (v) In contrast to many chemical esterification procedures, the primary aliphatic hydroxy group of (hydroxy)phenylethan-1-ols is regioselectively esterified using Novozym 435 as a biocatalyst. The two specific binding sites of *C. antarctica* lipase B for alkyl and acyl moieties may explain the very different esterification and transesterification activities observed for the various analogous phenylacetate and phenylethanol substrates. These results are consistent with earlier findings on the esterification and transesterification of benzoic, phenylacetic, and, particularly, cinnamic acid derivatives (*14, 28–30, 36*).

Phenolics predominantly appear as polar antioxidants in the hydrophilic phases of foods. Lipophilization of phenolics is, therefore, of great importance to extend their field of applications to fatty phases in oil-based foods as well as multiphase food systems containing both lipophilic and hydrophilic phases (*14, 28*). Lipase-catalyzed lipophilization of antioxidative phenylalkanoic acids such as hydroxylated benzoic, phenylacetic, and cinnamic acids and their short-chain alkyl esters by esterification and transesterification, respectively, with fatty alcohols may be of special interest for food and nonfood applications. (Hydroxy)phenylalkyl fatty acid esters with inverse chemical structures obtained by lipase-catalyzed esterification and transesterification of fatty acids and fatty acid methyl esters, respectively, with (hydroxy)phenylethanols also have lipophilic properties and may be used for similar applications.

Supporting Information Available: Gas chromatograms of reaction mixtures of the transesterification of methyl 4-hydroxyphenylacetate with oleyl alcohol leading to oleyl 4-hydroxyphenylacetate and of the esterification of 2-(4-hydroxyphenyl)ethanol (tyrosol) with oleic acid leading to 2-(4-hydroxyphenyl)ethyl oleate (tyrosyl oleate). This material is available free of charge via the Internet at http://pubs.acs.org.

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