

Solvent-free Lipase-Catalyzed Preparation of Long-Chain Alkyl Phenylpropanoates and Phenylpropyl Alkanoates

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An enzymatic method was developed for the preparation of medium- or long-chain alkyl 3-phenylpropanoates (alkyl cinnamates), particularly alkyl hydroxy- and methoxy-substituted cinnamates such as oleyl *p*-coumarate and oleyl ferulate. The various alkyl cinnamates were formed in high to moderate yield by lipase-catalyzed esterification of cinnamic acid and its analogues with fatty alcohols in vacuo at moderate temperatures in the absence of drying agents and solvents. Immobilized *Candida antarctica* lipase B was the most effective biocatalyst for the various esterification reactions. The relative esterification activities were of the following order: dihydrocinnamic > cinnamic > 3-methoxycinnamic > dihydrocaffeic \approx 3-hydroxycinnamic > 4-methoxycinnamic > 2-methoxycinnamic > 4-hydroxycinnamic > ferulic \approx 3,4-dimethoxycinnamic > 2-hydroxycinnamic acid. With respect to the position of the substituents at the phenyl moiety, the esterification activity increased in the order meta > para > ortho. *Rhizomucor miehei* lipase demonstrated moderate esterification activity. Compounds with inverse chemical structure, that is, 3-phenylpropyl alkanoates such as 3-(4-hydroxyphenyl)propyl oleate, were also obtained in high yield by esterification of fatty acids with the corresponding 3-phenylpropan-1-ols.

KEYWORDS: Alkyl cinnamates; alkyl 3-(hydroxyphenyl)propanoates; oleyl coumarates; alkyl 3-(methoxyphenyl)propanoates; oleyl ferulate; palmityl ferulate; 3-phenylpropyl alkanoates; 3-(4-hydroxyphenyl)oleate; immobilized *Candida antarctica* lipase B; lipase-catalyzed esterification

INTRODUCTION

Minor constituents of plants are gaining importance due to their proposed health benefits (1, 2). For example, epidemiological and nutritional studies suggest that the consumption of foods containing high proportions of phenolic antioxidants may prevent chronic diseases such as coronary heart disease and colorectal cancer (3–5). The beneficial effects of phenolic antioxidants, such as 3-(hydroxyphenyl)propanoates (hydroxy cinnamic acids), on health have been attributed to their antioxidant capacity, particularly their ability to protect low-density lipoproteins from oxidative attack (6). The antioxidant capacity and biological availability of several of these compounds may be further improved by increasing their lipophilicity (7–12), and the range of applications of such lipophilic antioxidants may be extended by their possible use as additives for food and nutraceutical (12, 13) as well as cosmetic and technical applications (14–18).

Hydroxy cinnamic acids and their methoxy derivatives such as 4-hydroxycinnamic (*p*-coumaric), ferulic, caffeic and sinapic acids are common constituents of plants, particularly of cereals such as rice, rye, and wheat, in which they are predominantly esterified to cell wall polysaccharides (19–24). Recently,

Mendez (25) demonstrated the occurrence of dihydrocinnamic acid derivatives, for example, dihydro-*p*-coumaric and dihydroferulic acids, in bracken fern (*Pteridium aquilinum*). Although hydroxy cinnamic acids esters of polysaccharides occur most commonly in plants, various other esters of cinnamic acid and its analogues including medium- or long-chain alkyl esters have also been detected, for example, alkyl ferulates (Figure 1), which are predominantly found in the cork layers of several plant species (26–32). In addition, similar compounds with inverse chemical structure, that is, 3-(4-hydroxyphenyl)propenyl (*p*-coumaryl) alkanoates, were isolated from apple (*Malus domestica*) fruits (33, 34).

Various methods are known for the chemical esterification of cinnamic acid and its analogues, for example, esterification of hydroxy cinnamic acids or their tetrahydropyranyl derivatives with various alcohols in the presence of *N,N*-dicyclohexylcarbodiimide in tetrahydrofuran or pyridine (9, 35). Moreover, esterification of hydroxy cinnamic acids including ferulic acid with alkanols in the presence of acid catalysts such as sulfuric acid or *p*-toluenesulfonic acid has been described using benzene, toluene, or chlorobenzene as the solvents (18). Sitostanyl ferulate was prepared by esterification of acetylated ferulic acid with sitostanol in the presence of *N,N*-dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine (36). Enzymatic esterification is preferred, however, over chemical esterification for the prepara-

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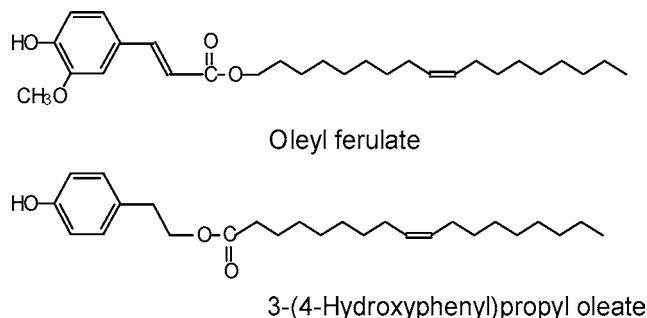


Figure 1. Chemical structures of the alkyl cinnamate (alkyl 3-phenylpropanoate) derivative oleyl ferulate and the structurally similar inverse dihydrocinnamyl (3-phenylpropyl) alkanooate derivative 3-(4-hydroxyphenyl)propyl oleate.

tion of hydroxy cinnamic acids esters that may be used in foods. Little is known about lipase-catalyzed esterification of cinnamic acid derivatives, particularly hydroxy cinnamic acids (12). Enzymatic esterification procedures requiring organic solvents or using the alcohol component as the solvent have been reported for the preparation of short- and medium-chain alkyl esters of hydroxy cinnamic acids including alkyl ferulates (37–43). Similarly, the corresponding compounds with inverse chemical structure, that is, 3-phenylpropyl and 3-phenylpropenyl alkanooates, may be formed by esterification of fatty acids with various dihydrocinnamyl and cinnamyl alcohol derivatives (3-phenylpropan-1-ols and 3-phenylpropen-1-ols). Such “retro” compounds may also be of interest because they are expected to have antioxidant properties equivalent to those shown for alkyl hydroxycinnamates (33, 34).

The aim of the present work was to develop a simple and environmentally friendly method for the preparation of various lipophilic medium- and long-chain alkyl 3-phenylpropanoates, particularly hydroxy- and methoxycinnamates, by esterification of 3-phenylpropanoic and 3-phenylpropenoic acids and their analogues with medium- or long-chain fatty alcohols as well as of the structurally inverse 3-phenylpropyl alkanooates by esterification of medium- or long-chain fatty acids with 3-phenylpropan-1-ols using lipases as biocatalysts. The esterification reactions were performed at moderate temperatures without solvents or drying agents using reduced pressure to remove reaction water.

MATERIALS AND METHODS

Materials. 1-Dodecanol, 1-hexadecanol, and *cis*-9-octadecen-1-ol (oleyl alcohol) as well as *trans*-cinnamic acid [(*E*)-3-phenylpropenoic acid], dihydrocinnamic acid (3-phenylpropanoic acid), 2- and 3-hydroxy- as well as 2-, 3-, and 4-methoxycinnamic acids, 4-hydroxy-3-methoxycinnamic acid (ferulic acid), 3,4-dihydroxycinnamic acid (caffeic acid), 3,4-dihydroxydihydrocinnamic acid (dihydrocaffeic acid), 4-hydroxy-3,5-dimethoxycinnamic acid (sinapic acid), methyl *trans*-cinnamate, cinnamyl alcohol (3-phenylpropen-1-ol), dihydrocinnamyl alcohol (3-phenylpropan-1-ol), 3-(4-hydroxyphenyl)propan-1-ol, and 3-(3,4-dimethoxy)propan-1-ol were obtained from Sigma-Aldrich-Fluka (Deisenhofen, Germany). 4-Hydroxycinnamic acid (*p*-coumaric acid) was a product of Alfa Aesar (Karlsruhe, Germany). For comparison, *trans*-ferulic acid was partially isomerized to *cis*-ferulic acid by UV irradiation in methanolic solution (44).

Immobilized lipase preparations from *Candida antarctica* (lipase B, Novozym 435), *Rhizomucor miehei* (Lipozyme RM IM), and *Thermomyces lanuginosus* (Lipozyme TL IM) were kindly provided by Novozymes, Bagsvaerd, Denmark. Various other enzymes were also checked for esterification activity using cinnamic acid derivatives and *cis*-9-octadecen-1-ol as the substrates, including lipases from *Candida rugosa* (Sigma), *Candida lipolytica* (Fluka), *Chromobacterium viscosum*

(Biocatalysts, Pontypridd, Mid Glamorgan, U.K.), *Rhizopus arrhizus*, *Rhizopus niveus*, *Rhizopus javanicus* (all from Fluka), and *Fusarium oxysporum* (Lipopan F BG, Novozymes), and pectinase from *Aspergillus niger* (Serva, Heidelberg, Germany) as well as plant proteases/esterases including bromelain from *Ananassa comosus*, papain from *Carica papaya*, and ficin from *Ficus carica* (all from Sigma).

Lipase-Catalyzed Reactions. As a typical example, 4-methoxycinnamic acid (53.4 mg, 0.3 mmol) was esterified with *cis*-9-octadecen-1-ol (80.4 mg, 0.3 mmol) in the presence of 50 mg of the various lipase preparations by magnetic stirring in a screw-capped tube in vacuo at 80 °C for periods of up to 144 h with water-trapping in the gas phase using potassium hydroxide pellets. A moderate vacuum (80 kPa) was used to prevent substantial loss of substrates. Samples of the reaction products were withdrawn at various intervals, extracted with diethyl ether, and filtered through a 0.45 μ m syringe filter to remove the biocatalyst. An aliquot of the filtrate was analyzed as given below.

Similar reaction conditions were used for the preparation of phenylpropyl alkanooates with inverse chemical structure. As a typical example, oleic acid (84.8 mg, 0.3 mmol) was esterified with cinnamyl alcohol (40.2 mg, 0.3 mmol) under identical conditions as described above for the esterification of cinnamic acid derivatives using Novozym 435 lipase preparation as biocatalyst.

Enzyme units were calculated from the initial rates (4 or 24 h) of esterification of the various 3-phenylpropanoic and 3-phenylpropenoic acids with alkanols as well as the corresponding esterification of fatty acids with various 3-phenylpropanol and 3-phenylpropenol derivatives. One unit of enzyme activity was defined as the amount of enzyme (grams) that produced 1 μ mol of the respective alkyl or phenylpropyl ester per minute.

Thin-Layer Chromatography (TLC). Aliquots were withdrawn from the reaction mixtures, and free carboxy groups of the various compounds of the reaction mixture were methylated using a solution of diazomethane in diethyl ether. The conversion was checked by TLC on 0.3 mm layers of silica gel H (E. Merck, Darmstadt, Germany), and spots were located by iodine staining and charring by spraying with 30% sulfuric acid followed by heating (200 °C).

The different methods of development (A and B) used led to the following R_f values of the various compounds: (A) isohexane–diethyl ether (1:1), one run to the top, long-chain alkyl cinnamates and dihydrocinnamates, 0.55–0.6; long-chain methoxy-substituted 3-phenylpropyl alkanooates, 0.5–0.55; long-chain 3-(3,4-dimethoxy-phenyl)propyl alkanooates, 0.4; fatty acid methyl esters, 0.75; saturated and unsaturated long-chain fatty alcohols, 0.3; unesterified fatty acids and 3-phenylpropanoic acids, 0.4–0.45; (B) isohexane–diethyl ether (1:1), developed up to half of the plate, followed by the same solvent system to the top, long-chain alkyl 3-(hydroxyphenyl)propenoates, 0.6–0.65; medium- and long-chain alkyl ferulates, 0.5; long-chain 3-(4-hydroxyphenyl)propyl oleate, 0.6; 3-phenylpropan-1-ol (dihydrocinnamyl alcohol) and 3-phenylpropen-1-ol (cinnamyl alcohol) 0.3; 3-(4-hydroxyphenyl)propan-1-ol and 3-(3,4-dimethoxyphenyl)propan-1-ol, 0.1–0.15; unesterified fatty acids and 3-phenylpropanoic acids, 0.45–0.55.

Similarly, 0.5 mm layers of silica gel H were used for the separation of reaction products by preparative TLC. The various fractions were scraped off the plates and extracted from silica gel using water-saturated diethyl ether. Reaction mixtures containing phenolic hydroxy compounds were separated under similar conditions as described above using method B.

Purification by Crystallization. Alkyl 3-phenylpropanoates and 3-phenylpropyl alkanooates were extracted from the immobilized biocatalysts with diethyl ether and purified by column chromatography and/or crystallization from isohexane or mixtures of isohexane–diethyl ether (1:1). The following melting points were determined using a Kofler heating block: oleyl 2-hydroxycinnamate, 37–39 °C; palmityl 2-hydroxycinnamate, 84–85 °C; palmityl 3-hydroxycinnamate, 78–79 °C; oleyl 4-hydroxycinnamate, 38–39 °C; palmityl 4-hydroxycinnamate, 89–90 °C; palmityl 2-methoxycinnamate, 46–47 °C; palmityl 3-methoxycinnamate, 47–48 °C; palmityl 4-methoxycinnamate, 59–60 °C; lauryl ferulate, 45–48 °C; palmityl ferulate, 61–63 °C; oleyl ferulate, 30–33 °C; oleyl dihydrocaffeate, 21–23 °C; oleyl caffeate, 78–79 °C; cinnamyl palmitate (3-phenylprop-2-en-1-yl palmitate), 45–

46 °C; 3-(4-hydroxyphenyl)propyl palmitate, 60–61 °C; 3-(3,4-dimethoxyphenyl)propyl palmitate, 35–36 °C. Other long-chain alkyl cinnamates or cinnamyl alkanooates such as oleyl cinnamate, oleyl dihydrocinnamate, oleyl 3-hydroxycinnamate, oleyl 2-methoxycinnamate, oleyl 3-methoxycinnamate, oleyl 4-methoxycinnamate, oleyl 3,4-dimethoxycinnamate, 2-ethylhexyl ferulate, oleyl sinapate, cinnamyl oleate, 3-phenylpropyl palmitate, 3-(4-hydroxyphenyl)propyl oleate are liquids at room temperature.

Column Chromatography. As a typical example, the reaction mixture resulting from the esterification of ferulic acid with oleyl alcohol (around 400 mg) was dissolved in 2 mL of diethyl ether and applied to a 25 × 2 cm i.d. silica gel 60 column (Merck). The column was eluted first with 30 mL of isohexane and then with 30 mL portions of various isohexane–diethyl ether mixtures (95:5, 9:1, 8:2, 7:3). Elution with isohexane–diethyl ether (1:1) yielded around 300 mg of oleyl ferulate, which was crystallized from isohexane–diethyl ether (1:1).

Gas Chromatography (GC). Aliquots of esterification products were removed from the reaction mixture, dissolved in dichloromethane, and filtered through a 0.45 μm PTFE syringe filter to remove the lipase catalysts. The filtrate was concentrated, dissolved in diethyl ether, and treated with an ethereal solution of diazomethane to convert the unreacted carboxy groups to the corresponding methyl esters. The resulting mixture of methyl esters, unreacted alkanols or 3-phenylpropanols, and alkyl cinnamates or 3-phenylpropyl alkanooates was analyzed by GC. The phenolic hydroxy group of 2-hydroxycinnamic acid derivatives was methylated to the 2-methoxy compounds by treatment with an ethereal solution of diazomethane in the presence of catalytic amounts of silica gel (45) to avoid coumarin formation during high-temperature GC. 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 15 m × 0.25 mm i.d., 0.1 μm 400-1HT fused silica capillary column (Quadrex Corp., New Haven, CT) using hydrogen as the carrier gas (column pressure of 50 kPa).

Three different temperature programs were used to separate the various compounds of reaction mixtures: (method A) initially at 150 °C for 2 min, followed by linear programming from 150 to 180 °C at 2 °C/min, then from 180 to 360 °C at 20 °C/min, and finally kept at 360 °C for 2 min; (method B) initially at 140 °C for 2 min, followed by linear programming from 140 to 180 °C at 5 °C/min, then from 180 to 250 °C at 10 °C/min (5 min isothermally), then from 250 to 410 °C at 20 °C/min, and finally kept at 410 °C for 2 min; and (method C) initially at 80 °C for 2 min, followed by linear programming from 80 to 280 °C at 10 °C/min, and finally kept at 280 °C for 5 min. Injector and detector temperatures were maintained at 380 °C. Retention times of the various reaction products and starting materials are given in **Table 1**.

Peaks in gas chromatograms were assigned by comparison of their retention times with those of peaks from TLC fractions that had been identified by GC-MS. Response factors of FID were determined using purified compounds, and percentages of peak areas were calculated using a Hewlett-Packard GC ChemStation software. For the determination of enzyme activities, small proportions of methoxy compounds that had been formed during carboxymethylation of hydroxycinnamic acids with diazomethane were calculated as the original hydroxy compounds.

Reversed Phase High-Performance Liquid Chromatography (RP-HPLC). Aliquots of purified reaction products were dissolved in diethyl ether, filtered as described for GC, concentrated, and dissolved in acetonitrile. Samples were analyzed for their composition by RP-HPLC as follows: the HPLC system consisted of a Merck-Hitachi pump L-6200 (E. Merck) equipped with a column oven (VDS Optilab, Berlin, Germany) set at 30 °C, a Knauer K 2501 UV–vis detector (Knauer, Berlin, Germany) set to 220 nm, and a PL-ELS 2100 (Polymer Laboratories, Darmstadt, Germany) ELSD detector (thermostated to 30 °C for acetonitrile), which were used in series. UV and mass traces were monitored and evaluated in a Knauer Eurochrom for Windows, Preparative Version, data acquisition unit (Knauer).

Long-chain alkyl 3-phenylpropanoates, alkyl 3-phenylpropenoates, and 3-phenylpropyl alkanooates were separated on a 250 mm × 4 mm i.d., 5 μm LiChrospher RP-18e column (Phenomenex, Aschaffenburg,

Table 1. GC Retention Times of Various Medium- and Long-Chain Alkyl 3-Phenylpropanoates, Alkyl 3-Phenylpropenoates, and 3-Phenylpropyl Alkanooates and Starting Materials

alkyl 3-phenylpropanoates, alkyl 3-phenylpropenoates, and 3-phenylpropyl alkanooates as well as starting materials ^a	retention time (min)
Method A ^b	
methyl dihydrocinnamate	1.0
methyl cinnamate	1.2
methyl 2-methoxycinnamate	1.6
methyl 3-methoxycinnamate	1.6
methyl 4-methoxycinnamate	1.8
methyl 3-hydroxycinnamate	1.8
methyl 4-hydroxycinnamate	1.9
oleyl alcohol	6.6
oleyl dihydrocinnamate	12.5
oleyl cinnamate	13.8
oleyl 2-methoxycinnamate	13.6
oleyl 3-methoxycinnamate	13.7
oleyl 4-methoxycinnamate	13.8
oleyl 3-hydroxycinnamate	13.3
oleyl 4-hydroxycinnamate	13.4
Method B ^b	
methyl ferulate	0.9
lauryl alcohol	1.0
palmityl alcohol	3.6
oleyl alcohol	5.5
lauryl ferulate	15.3
palmityl ferulate	18.6
oleyl <i>cis</i> -ferulate	19.7
oleyl <i>trans</i> -ferulate	20.3
oleyl sinapate	19.0
Method C ^b	
3-phenylpropan-1-ol	1.6
cinnamyl alcohol	2.1
3-(4-hydroxyphenyl)propan-1-ol	6.2
3-(3,4-dimethoxyphenyl)propan-1-ol	6.8
methyl oleate	10.7
3-phenylpropyl oleate	17.4
3-phenylpropenyl oleate (cinnamyl oleate)	17.7
3-(4-hydroxyphenyl)propyl oleate	19.2
3-(3,4-dimethoxyphenyl)propyl oleate	19.9

^a Carboxylic acids were converted to the methyl esters by reaction with diazomethane. ^b For details see Materials and Methods.

Germany) and precolumn using (method A) acetonitrile isocratically at a flow rate of 1.0 mL/min, (method B) acetonitrile isocratically at a flow rate of 0.6 mL/min, and (method C) methanol–water–acetic acid (50:50:0.5) isocratically at a flow rate of 1 mL/min and a UV detector set at 320 nm. Injections (around 20–60 μg) of the reaction products were carried out with a Rheodyne 7161 sample injector (Cotati, CA) equipped with a 20-μL sample loop. Peak areas and percentages were calculated using the Eurochrom software. Retention times of the various reaction products and starting materials are given in **Table 2**.

GC-MS Analyses. The fragmentation of the various alkyl 3-phenylpropanoates or 3-phenylpropyl alkanooates formed by lipase-catalyzed esterification reactions was studied by GC-MS (EI mode). Compounds containing carboxy groups were analyzed after derivatization to the corresponding methyl esters using an ethereal solution of diazomethane and purification by preparative TLC or column chromatography. GC-MS analyses were carried out on a Hewlett-Packard model 5890 series II/5989A apparatus equipped with a 15 m × 0.25 mm i.d., 0.25 μm Rtx-5MS fused silica capillary column (Restek Germany, Bad Homburg, Germany), using the electron impact ionization (EI, 70 eV) mode. Alternatively, separations were carried out on a 15 m × 0.25 mm i.d., 0.1 μm 400-1HT (Quadrex Corp.) fused silica capillary column. The separation conditions were identical for both columns. The carrier gas was He at a flow rate of 1.0 mL/min. The column temperature was initially kept at 140 °C for 2 min and then programmed from 140 to 180 °C at 5 °C/min, held there for 5 min, and then programmed from 180 to 360 °C at 20 °C/min; the final temperature was held for 9 min. Other operating conditions were split/splitless injector in split mode

Table 2. RP-HPLC Retention Times of Various Medium- and Long-Chain Alkyl 3-Phenylpropanoates, Alkyl 3-Phenylpropanoates, and 3-Phenylpropyl Alkanoates and Starting Materials

alkyl 3-phenylpropanoates, alkyl 3-phenylpropanoates, 3-phenylpropyl alkanoates, and starting materials ^a	retention time (min)
Method A ^b	
oleyl dihydrocinnamate	14.9
oleyl cinnamate	16.6
oleyl 2-methoxycinnamate	15.7
oleyl 3-methoxycinnamate	15.0
oleyl 4-methoxycinnamate	14.2
oleyl 3,4-dimethoxycinnamate	11.7
oleyl ferulate	4.7
methyl ferulate	2.7
oleyl alcohol	5.9
3-(4-hydroxyphenyl)propyl oleate	8.7
3-(4-hydroxyphenyl)propyl palmitate	9.0
3-phenylpropyl (dihydrocinnamyl) palmitate	15.0
3-phenylpropyl (cinnamyl) palmitate	13.2
3-(3,4-dimethoxyphenyl)propyl palmitate	10.7
Method B ^b	
oleyl 2-hydroxycinnamate	18.6
oleyl 3-hydroxycinnamate	17.5
oleyl 4-hydroxycinnamate	17.4
2-ethylhexyl ferulate	5.4
dodecyl ferulate	9.3
palmityl ferulate	18.3
oleyl ferulate	17.3
oleyl dihydrocaffeate	12.5
oleyl caffeate	14.2
oleyl sinapate	15.4
Method C ^b	
<i>cis</i> -ferulic acid ^c	6.0
<i>trans</i> -ferulic acid ^c	5.6

^a Carboxylic acids were converted to the methyl esters by reaction with diazomethane. ^b For details see Materials and Methods. ^c Separated as free carboxylic acids.

(split 1:10, temperature 360 °C), interface temperature of 360 °C, and ion source temperature of 200 °C.

For example, the following molecular ions and important mass fragments were observed: *m/z* oleyl cinnamate 398 [M]⁺, 149 [M - C₁₈H₃₅O]⁺, 131 [M - C₁₈H₃₅O₂]⁺; oleyl dihydrocinnamate 400 [M]⁺, 149 [M - C₁₈H₃₅]⁺, 133 [M - C₁₈H₃₅O]⁺; oleyl 2-hydroxycinnamate, 414 [M]⁺, 165 [M - C₁₈H₃₃]⁺, 146 [M - C₁₈H₃₆O]⁺; 3-hydroxy- and 4-hydroxycinnamates 414 [M]⁺, 164 [M - C₁₈H₃₄]⁺, 147 [M - C₁₈H₃₅O]⁺; palmityl 2-hydroxy-, 3-hydroxy-, and 4-hydroxycinnamates 388 [M]⁺, 164 [M - C₁₆H₃₂]⁺, 147 [M - C₁₆H₃₃O]⁺; palmityl 2-methoxy-, 3-methoxy-, and 4-methoxycinnamates 402 [M]⁺, 178 [M - C₁₆H₃₂]⁺, 161 [M - C₁₆H₃₃O]⁺; oleyl 2-methoxy-, 3-methoxy-, and 4-methoxycinnamates 428 [M]⁺, 178 [M - C₁₈H₃₄]⁺, 161 [M - C₁₈H₃₅O]⁺; oleyl 3,4-dimethoxycinnamate 458 [M]⁺, 208 [M - C₁₈H₃₄]⁺, 191 [M - C₁₈H₃₅O]⁺; oleyl sinapate 474 [M]⁺, 224 [M - C₁₈H₃₄]⁺, 207 [M - C₁₈H₃₅O]⁺, 167 [M - C₂₀H₃₅O₂]⁺; 2-ethylhexyl ferulate 306 [M]⁺, 194 [M - C₈H₁₆]⁺, 177 [M - C₈H₁₆O]⁺; dodecyl ferulate 362 [M]⁺, 194 [M - C₁₂H₂₄]⁺, 177 [M - C₁₂H₂₅O]⁺; palmityl ferulate 418 [M]⁺, 194 [M - C₁₆H₃₂]⁺, 177 [M - C₁₆H₃₃O]⁺; oleyl ferulate 444 [M]⁺, 194 [M - C₁₈H₃₄]⁺, 177 [M - C₁₈H₃₅O]⁺; oleyl dihydrocaffeate 432 [M]⁺, 182 [M - C₁₈H₃₄]⁺, 165 [M - C₁₈H₃₅O]⁺; oleyl caffeate 430 [M]⁺, 180 [M - C₁₈H₃₄]⁺, 163 [M - C₁₈H₃₅O]⁺; 3-phenylpropyl oleate 400 [M]⁺, 118 [M - C₁₈H₃₄O₂]⁺; 3-phenylpropyl palmitate 374 [M]⁺, 118 [M - C₁₆H₃₂O₂]⁺; 3-phenylprop-2-enyl palmitate (cinnamyl palmitate) 372 [M]⁺, 239 [M - C₉H₉O]⁺, 133 [M - C₁₆H₃₁O]⁺, 117 [M - C₁₆H₃₁O₂]⁺; 3-(3,4-dimethoxyphenyl)propyl palmitate 434 [M]⁺, 196 [M - C₁₆H₃₀O]⁺, 178 [M - C₁₆H₃₁O₂]⁺; 3-(4-hydroxyphenyl)propyl oleate 416 [M]⁺, 134 [M - C₁₈H₃₄O₂]⁺; 3-(4-hydroxyphenyl)propyl palmitate 390 [M]⁺, 134 [M - C₁₆H₃₂O₂]⁺.

RESULTS AND DISCUSSION

3-Phenylpropenoic (cinnamic) acid analogues such as 4-hydroxycinnamic (*p*-coumaric), ferulic, and sinapic acids are

natural phenolic acids occurring ubiquitously in all parts of plants. These hydroxylated cinnamic acids gain importance because of their antioxidant capacity and their beneficial effects on human health. Phenolics including hydroxylated cinnamic acids predominantly occur as hydrophilic compounds. Their use as food additives is limited, therefore, to hydrophilic phases in foods. Lipophilization of hydroxy cinnamic acids derivatives is of great importance in extending their field of applications to fatty food phases (12).

Lipase-catalyzed lipophilization of phenolic antioxidants, particularly hydroxycinnamic acids and similar compounds with analogous or inverse chemical structure (**Figure 1**), by esterification of various 3-phenylpropanoic acids with long-chain fatty alcohols or of fatty acids with various 3-phenylpropanols is of special interest for their application as lipophilic antioxidants in oil-based food and feed as well as in cosmetic and technical products. Recently, we have shown that plant steryl and stanyl esters can be efficiently prepared from sterols and stanols via lipase-catalyzed esterification with fatty acids under environmentally friendly conditions, particularly in the absence of organic solvents and drying reagents such as molecular sieves or sodium sulfate (46, 47). In continuation of the above work, we have developed an enzymatic method for the preparation of lipophilic alkyl esters of 3-phenylpropanoic (dihydrocinnamic) and 3-phenylpropenoic (cinnamic) acid derivatives by esterification with medium- or long-chain fatty alcohols. Similarly, compounds with inverse chemical structure, that is, 3-phenylpropyl alkanoates, are prepared by lipase-catalyzed esterification of long-chain fatty acids with various 3-phenylpropan-1-ols. The reactions proceed in vacuo at moderate temperature in the absence of organic solvents and drying reagents using immobilized lipases as biocatalysts.

Enzyme Screening. Various esterases, particularly lipases, were checked for the esterification reaction of 4-methoxycinnamic acid with oleyl alcohol (*cis*-9-octadecen-1-ol) as the screening system. The conversions were performed for 24 h at 50 and/or 80 °C in vacuo (80 kPa) without solvent using 50 mg of the respective enzyme preparation. The results of these experiments demonstrated that immobilized lipase B from *Candida antarctica* (Novozym 435) was the biocatalyst with highest enzyme activity for the above esterification reaction (data not shown). A similar esterification activity was determined for immobilized lipase from *Rhizomucor miehei* (Lipozyme RM IM), whereas all other enzymes of the screening, including immobilized lipase from *Thermomyces lanuginosus* (Lipozyme TL IM), lipases from *Candida rugosa*, *Candida lipolytica*, *Chromobacterium viscosum*, *Rh. arrhizus*, *Rh. niveus*, *Rh. javanicus*, *F. oxysporum*, and pectinase from *Aspergillus niger* as well as plant esterases/proteases including papaya (*Carica papaya*) latex, bromelain from *Ananassa comosus*, and ficin from *F. carica*, demonstrated very low esterification activity, if any, under the screening conditions described above (data not shown).

Lipase Specificities. The time course of the esterification of cinnamic acid with oleyl alcohol at 80 °C using three commercial immobilized lipases, Novozym 435, Lipozyme RM IM, and Lipozyme TL IM, is shown in **Figure 2**. It is obvious from these results that Novozym 435 shows the highest conversion rates, whereas moderate and very low esterification rates were obtained for Lipozyme RM IM and Lipozyme TL IM, respectively, as biocatalysts.

Figure 3 shows the time course of the esterification of various dihydrocinnamic (3-phenylpropanoic) and cinnamic (3-phenylpropenoic) acids with oleyl alcohol at 80 °C in vacuo using

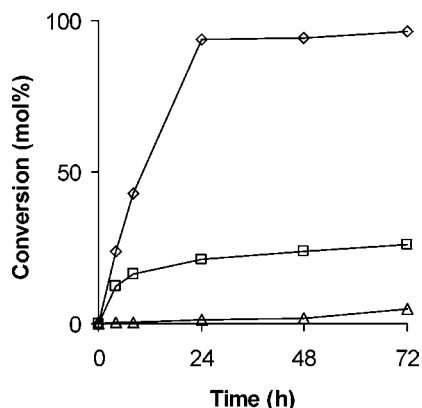


Figure 2. Time course of the formation of oleyl cinnamate by esterification of cinnamic acid with oleyl alcohol catalyzed by various immobilized microbial lipases (\diamond , Novozym 435; \square , Lipozyme RM IM; \triangle , Lipozyme TL IM). Reaction conditions: molar ratio of cinnamic acid to oleyl alcohol, 1:1; 50 mg of immobilized lipase; 80 °C. Values are the mean of two determinations. For details see Materials and Methods.

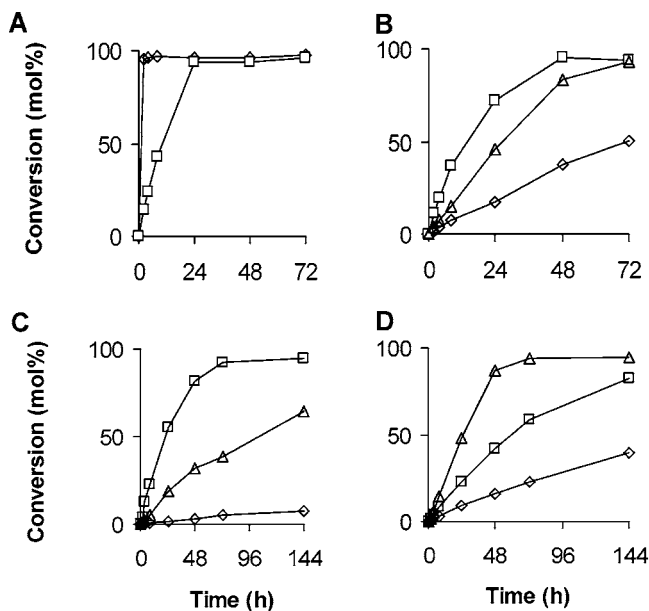


Figure 3. Time course of the formation of oleyl esters of cinnamic acid and its analogues including \diamond , oleyl dihydrocinnamate, and \square , oleyl cinnamate (A); \diamond , 2-methoxy-, \square , 3-methoxy-, and \triangle , 4-methoxycinnamate (B); and \diamond , 2-hydroxy-, \square , 3-hydroxy-, and \triangle , 4-hydroxycinnamate (C), by Novozym 435-catalyzed esterification of dihydrocinnamic, cinnamic, methoxycinnamic, and hydroxycinnamic acids with oleyl alcohol. Time course of the formation of oleyl ferulate (D) by esterification of ferulic acid with oleyl alcohol using different amounts of Novozym 435 lipase (\diamond , 50 mg; \square , 100 mg; \triangle , 250 mg). Values are the mean of two determinations. For details see Materials and Methods.

Novozym 435 as biocatalyst. In particular, the formation of oleyl dihydrocinnamate and oleyl cinnamate (Figure 3A), various oleyl methoxy- and hydroxycinnamates, for example, 2-methoxy-, 3-methoxy-, and 4-methoxycinnamates (Figure 3B), 2-hydroxy-, 3-hydroxy-, and 4-hydroxycinnamates (Figure 3C), and oleyl ferulate (4-hydroxy-3-methoxycinnamate) (Figure 3D), by Novozym 435-catalyzed esterification of the corresponding cinnamic acids with oleyl alcohol is demonstrated over a period of 72 or 144 h. It is obvious from these results that (a) highest esterification activity is observed with dihydrocinnamic acid (3-phenylpropanoic acid) as the substrate (Figure 3A); (b) introduction of a $>C=C<$ double bond to form cinnamic acid

(3-phenylprop-2-enoic acid) leads to a large decrease of lipase activity (Figure 3A); (c) introduction of a methoxy group (Figure 3B) or a hydroxy group (Figure 3C) on the phenyl ring leads to further loss of lipase activity; and (d) the presence of more than one methoxy and/or hydroxy group in the substrate molecule (e.g., ferulic, caffeic, and sinapic acids) results in an additional reduction of enzyme activity as demonstrated in Figure 3D and Table 3.

Table 3 shows the enzyme activities of three commercial immobilized lipases and maximum conversions for the esterification of various dihydrocinnamic and cinnamic acids with medium- or long-chain fatty alcohols and of fatty acids with various 3-phenylpropanols and 3-phenylpropenols under different reaction conditions. These data demonstrate that immobilized lipase B from *C. antarctica* (Novozym 435) is generally superior to immobilized lipase preparations from *Rh. miehei* (Lipozyme RM IM) and, particularly, *Th. lanuginosus* (Lipozyme TL IM) in the production of alkyl dihydrocinnamates and cinnamates. Together with the results shown in Figure 3 the following relative esterification activities are found for Novozym 435: dihydrocinnamic > cinnamic > 3-methoxycinnamic > dihydrocaffeic \approx 3-hydroxycinnamic > 4-methoxycinnamic > 2-methoxycinnamic > 4-hydroxycinnamic > ferulic \approx 3,4-dimethoxycinnamic > 2-hydroxycinnamic acid. With respect to the position of substituents on the phenyl moiety, the esterification activity of Novozym 435 increases in the order 3- > 4- > 2-position (meta > para > ortho) (Figure 3B,C). It is worth noting that the enzyme activity of Lipozyme RM IM is similar to or even higher than that of Novozym 435 for the esterification of meta-substituted cinnamic acids including 3-hydroxy-, 3-methoxy-, and 3,4-dimethoxycinnamic acids with oleyl alcohol. However, conversions found for Novozym 435-catalyzed esterifications of cinnamic acid analogues are clearly higher than those observed for Lipozyme RM IM-catalyzed ones (Table 3). The Novozym 435-catalyzed esterification of long-chain fatty acids with various 3-phenylpropanols generally produces the corresponding 3-phenylpropyl alkanooates in high yield (Table 3).

Reaction Conditions. Generally, enzyme activity for the esterification of cinnamic acid and its analogues with oleyl alcohol was highest using immobilized lipase B from *C. antarctica* (Novozym 435) at 80 °C in vacuo. Moderate esterification activity is observed for immobilized lipase from *Rh. miehei* (Lipozyme RM IM) and rather low activity for immobilized lipase from *Th. lanuginosus* (Lipozyme TL IM) (Table 3 and Figure 2). All other lipases and esterases screened as described above show far lower esterification activities, if any (data not shown).

Depending on the reaction conditions, conversions to alkyl dihydrocinnamates and cinnamates as well as 3-phenylpropyl alkanooates of up to 98 mol % were obtained by esterification of various dihydrocinnamic and cinnamic acids with medium- and long-chain fatty alcohols such as lauryl, palmityl, and oleyl alcohol or of 3-phenylpropanols with long-chain fatty acids such as palmitic and oleic acids catalyzed by Novozym 435 lipase in vacuo. Increasing the reaction temperature from 60 to 80 °C increased the enzyme activity for the formation of various oleyl 3-phenylpropanoates by 2–6-fold as shown for the esterification of cinnamic and dihydro-, methoxy-, and hydroxycinnamic acids with oleyl alcohol (Table 3).

Under the reaction conditions described, equimolar mixtures of substrates or relatively low excess of one of the substrates is necessary to obtain acceptable yields of methoxy- and/or hydroxy-substituted alkyl cinnamates, for example, oleyl 4-hy-

Table 3. Enzyme Activities of Various Lipases for the Esterification of 3-Phenylpropenoic (Cinnamic) Acid and Its Analogues with Medium- or Long-Chain Saturated and Unsaturated Fatty Alcohols and for the Esterification of Alkanoic Acids with 3-Phenylpropanol Analogues

3-phenylpropenoic (cinnamic) acid and its analogues and fatty acids	medium- and long-chain fatty alcohols and 3-phenylpropanols	lipase	max conversion ^a (mol %) after [h]	enzyme activity ^a (units/g)	±SEM (n = x)
dihydrocinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	98 [4]	98 ^b	±0.28 (n = 2)
dihydrocinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	96 [72] ^c	22.9 ^c	±1.6 (n = 2)
dihydrocinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	91 [4]	22.8 ^d	±0.03 (n = 2)
dihydrocinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme TL IM	57 [72]	2.8	±0.05 (n = 2)
cinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	94 [24]	6.0	±0.48 (n = 3)
cinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	96 [72] ^c	2.7 ^c	±0.79 (n = 2)
cinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	26 [72]	3.1	±0.64 (n = 2)
cinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme TL IM	5 [72]	nd ⁱ	– (n = 2)
2-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	51 [72]	0.9	±0.15 (n = 3)
2-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	12 [72] ^c	nd ^c	– (n = 2)
2-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	7 [72]	nd	– (n = 2)
2-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme TL IM	<1 [72]	nd	– (n = 2)
3-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	96 [48]	4.9	±0.56 (n = 4)
3-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	95 [72] ^c	1.8 ^c	±0.15 (n = 2)
3-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	58 [72]	5.6	±0.39 (n = 3)
3-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme TL IM	4 [72]	nd	– (n = 2)
4-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	94 [72]	1.8	±0.25 (n = 3)
4-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	22 [72] ^c	0.3 ^c	±0.01 (n = 2)
4-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	35 [72]	0.9	±0.14 (n = 2)
4-methoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme TL IM	2 [72]	nd	– (n = 2)
2-hydroxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	8 [144]	nd	– (n = 2)
2-hydroxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	<1 [144]	nd	– (n = 2)
2-hydroxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme TL IM	<1 [144]	nd	– (n = 2)
3-hydroxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	94 [144]	3.3	±0.40 (n = 2)
3-hydroxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	28 [144]	1.9	±0.26 (n = 4)
3-hydroxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme TL IM	<1 [144]	nd	– (n = 2)
4-hydroxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	65 [144]	0.6	±0.01 (n = 2)
4-hydroxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	10 [144]	0.6	±0.03 (n = 2)
4-hydroxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme TL IM	<1 [144]	nd	– (n = 2)
3,4-dimethoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	25 [72]	0.3	±0.06 (n = 4)
3,4-dimethoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	4 [72] ^c	nd ^c	– (n = 2)
3,4-dimethoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	27 [72]	1.3	±0.26 (n = 3)
3,4-dimethoxycinnamic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme TL IM	2 [72]	nd	– (n = 2)
ferulic acid	2-ethylhexan-1-ol	Novozym 435	73 [144]	2.0	±0.17 (n = 3)
ferulic acid	dodecan-1-ol	Novozym 435	27 [72]	0.3 ^e	±0.03 (n = 5)
ferulic acid	hexadecan-1-ol	Novozym 435	15 [72]	0.3 ^e	±0.04 (n = 3)
ferulic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	25 [72]	0.4 ^e	±0.03 (n = 2)
ferulic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	82 [144] ^f	0.5 ^{e,f}	±0.03 (n = 4)
ferulic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	94 [144]; ^g 98 [48] ^h	0.4 ^{e,g}	±0.02 (n = 2)
ferulic acid	<i>cis</i> -9-octadecen-1-ol	Lipozyme RM IM	27 [72] ^f	0.3 ^{e,f}	±0.01 (n = 2)
dihydrocaffeic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	30 [72]	3.4	±0.15 (n = 2)
caffeic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	33 [144]	0.1 ^g	0.01 (n = 2)
sinapic acid	<i>cis</i> -9-octadecen-1-ol	Novozym 435	23 [144] ^g	nd	– (n = 2)
oleic acid	cinnamic alcohol	Novozym 435	79 [4]	19.7	±0.21 (n = 2)
palmitic acid	cinnamic alcohol	Novozym 435	85 [24]	11.5	±2.3 (n = 2)
oleic acid	3-phenylpropan-1-ol	Novozym 435	93 [4]	23.3	±0.03 (n = 2)
palmitic acid	3-phenylpropan-1-ol	Novozym 435	93 [4]	23.3	±0.19 (n = 2)
oleic acid	3-(4-hydroxyphenyl)propan-1-ol	Novozym 435	90 [4]	22.4	±0.61 (n = 2)
palmitic acid	3-(4-hydroxyphenyl)propan-1-ol	Novozym 435	96 [8]	22.0	±0.25 (n = 2)
oleic acid	3-(3,4-dimethoxyphenyl)propan-1-ol	Novozym 435	91 [8]	21.8	±2.6 (n = 2)
palmitic acid	3-(3,4-dimethoxyphenyl)propan-1-ol	Novozym 435	85 [8]	19.4	±3.2 (n = 2)

^a Standard assay conditions, if not otherwise indicated: 0.3 mmol of carboxylic acid + 0.3 mmol of alcohol; immobilized lipase/assay, 50 mg; 80 °C; 80 kPa; 4 h.

^b Twelve and a half milligrams of Novozym 435. ^c Maximum conversion and enzyme activity at 60 °C. ^d Enzyme activity after 2 h: 45 units/g. ^e Enzyme activity after 24 h. ^f One hundred milligrams of immobilized lipase preparation. ^g Two hundred and fifty milligrams of Novozym 435. ^h Molar ratio ferulic acid to oleyl alcohol, 1:2 or 2:1; 250 mg of Novozym 435. ⁱ nd, not determined.

droxycinnamate and oleyl ferulate (**Table 3**), which differs from the enzyme-catalyzed esterification methods developed by others (12, 37–43). Moreover, the procedure described above for the Novozym 435-catalyzed esterification of various substituted cinnamates including hydroxycinnamates requires much less time as compared to those reported by others (37, 39–42). Large amounts of immobilized lipase B from *C. antarctica* (Novozym 435) are necessary, however, to obtain satisfactory yields, particularly of alkyl cinnamates bearing more than one methoxy or hydroxy group (**Table 3** and **Figure 3**). This was found in previous studies as well (12, 37, 39, 40). Further research will deal with experiments reducing the enzyme concentration for

the preparation of methoxy- and hydroxy-substituted alkyl dihydrocinnamates and cinnamates as well as 3-phenylalkyl alkanooates.

The molecular ions and typical mass fragments of the esterification products, that is, alkyl cinnamates and cinnamyl alkanooates, obtained by GC-MS confirmed the chemical structures of these compounds as given above. These data agree well with those given in the literature (32).

In conclusion, we have developed a lipase-catalyzed esterification procedure for the preparation of lipophilic alkyl cinnamates and similar compounds with inverse chemical structure, particularly hydroxy- and/or methoxy-substituted alkyl

3-phenylpropanoates and alkyl 3-phenylpropanoates as well as 3-phenylpropyl alkanates, in high to moderate yield. Immobilized lipase B from *C. antarctica* (Novozym 435) and, to a lesser degree, immobilized lipase from *Rh. miehei* (Lipozyme RM IM) were found to be the most effective biocatalysts for these reactions, which were performed at moderate temperatures in vacuo in the absence of solvents and drying agents. The reaction products, particularly hydroxy- and/or methoxy-substituted medium- and long-chain alkyl 3-phenylpropanoates and 3-phenylpropyl alkanates such as oleyl ferulate and 3-(4-hydroxyphenyl) palmitate, may be useful lipophilic antioxidants for food and feed as well as cosmetic and technical applications.

LITERATURE CITED

- Cox, B. D.; Whichelow, M. J.; Prevost, A. T. Seasonal consumption of salad vegetables and fresh fruit in relation to the development of cardiovascular disease and cancer. *Public Health Nutr.* **2000**, *3*, 19–29.
- van't Veer, P.; Jansen, M. C. J. F.; Klerk, M.; Kok, F. J. Fruits and vegetables in the prevention of cancer and cardiovascular disease. *Public Health Nutr.* **2000**, *3*, 103–107.
- Lee, J.; Koo, N.; Min, D. B. Reactive oxygen species, aging, and antioxidative nutraceuticals. *Compr. Rev. Food Sci. Food Saf.* **2004**, *3*, 21–33.
- Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.
- Ou, S.; Kwok, K.-C. Ferulic acid: pharmaceutical functions, preparation and applications in foods. *J. Sci. Food Agric.* **2004**, *84*, 1261–1269.
- Andreasen, M. F.; Landbo, A.-K.; Christensen, L. P.; Hansen, A.; Meyer, A. S. Antioxidant effects of phenolic rye (*Secale cereale* L.) extracts, monomeric hydroxycinnamates, and ferulic acid dehydromers on human low-density lipoproteins. *J. Agric. Food Chem.* **2001**, *49*, 4090–4096.
- Anselmi, C.; Centini, M.; Granata, P.; Sega, A.; Buonocore, A.; Bernini, A.; Facino, R. M. Antioxidant activity of ferulic acid alkyl esters in a heterophasic system: a mechanistic insight. *J. Agric. Food Chem.* **2004**, *52*, 6425–6432.
- Chen, J.-H.; Shao, Y.; Huang, M.-T.; Chin, C.-K.; Ho, C.-T. Inhibitory effect of caffeic acid phenethyl ester on human leukemia HL-60 cells. *Cancer Lett.* **1996**, *108*, 211–214.
- Chen, J.-H.; Ho, C.-T. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. *J. Agric. Food Chem.* **1997**, *45*, 2374–2378.
- Kikuzaki, H.; Hisamoto, M.; Hirose, K.; Akiyama, K.; Taniguchi, H. Antioxidant properties of ferulic acid and its related compounds. *J. Agric. Food Chem.* **2002**, *50*, 2161–2168.
- Murakami, A.; Nakamura, Y.; Koshimizu, K.; Takahashi, D.; Matsumoto, K.; Hagihara, K.; Taniguchi, H.; Nomura, E.; Hosoda, A.; Tsuno, T.; Maruta, Y.; Kim, H. W.; Kawabata, K.; Ohigashi, H. FA15, a hydrophobic derivative of ferulic acid, suppresses inflammatory responses and skin tumor promotion: comparison with ferulic acid. *Cancer Lett.* **2002**, *180*, 121–129.
- Figueroa-Espinoza, M.-C.; Villeneuve, P. Phenolic acids enzymatic lipophilization. *J. Agric. Food Chem.* **2005**, *53*, 2779–2787.
- Sultana, R.; Ravagna, A.; Mohmmad-Abdul, H.; Calabrese, V.; Butterfield, D. A. Ferulic acid ethyl ester protects neurons against amyloid β -peptide(1–42)-induced oxidative stress and neurotoxicity: relationship to antioxidant activity. *J. Neurochem.* **2005**, *92*, 749–758.
- Adams, T. B.; Cohen, S. M.; Doull, J.; Feron, V. J.; Goodman, J. I.; Marnett, L. J.; Munro, I. C.; Portoghese, P. S.; Smith, R. L.; Waddell, W. J.; Wagner, B. M. The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. *Food Chem. Toxicol.* **2004**, *42*, 157–185.
- Compton, D. L.; Laszlo, J. A.; Isbell, T. A. Cinnamoyl esters of lesquerella and castor oil: novel sunscreen active ingredients. *J. Am. Oil Chem. Soc.* **2004**, *81*, 945–951.
- Compton, D. L.; Isbell, T. A.; Harry-O'kuru, R. E. Novel sunscreen reagents from hydroxy-substituted acylglycerides. U.S. Patent Appl. 20040258743 A1, Dec 23, 2004.
- Schneider, S.; Deckhardt, K.; Hellwig, J.; Küttler, K.; Mellert, W.; Schulte, S.; van Ravenzwaay, B. Octyl methoxycinnamate: Two generation reproduction toxicity in Wistar rats by dietary administration. *Food Chem. Toxicol.* **2005**, *43*, 1083–1092.
- Taniguchi, H.; Nomura, E.; Tsuno, T.; Minami, S. Ferulic acid ester antioxidant/UV absorbent. Eur. Patent Appl. 0681825 A2, Nov 15, 1991.
- Krygier, K.; Sosulski, F.; Hogge, L. 2. Composition of phenolic acids in rapeseed flour and hulls. *J. Agric. Food Chem.* **1982**, *30*, 334–336.
- Krygier, K.; Sosulski, F.; Hogge, L. 3. Composition of phenolic acids in cereal and potato flours. *J. Agric. Food Chem.* **1982**, *30*, 337–340.
- Ralph, J.; Grabber, J. H.; Hatfield, R. D. Lignin-ferulate cross-links in grasses: active incorporation of ferulate polysaccharide esters into ryegrass lignins. *Carbohydr. Res.* **1995**, *275*, 167–178.
- Faulds, C. B.; Williamson, G. The role of hydroxycinnamates in the plant cell wall. *J. Sci. Food Agric.* **1999**, *79*, 393–395.
- Sun, R. C.; Sun, X.-F.; Zhang, S.-H. Quantitative determination of hydroxycinnamic acids in wheat, rice, rye, and barley straws, maize stems, oil palm frond fiber, and fast-growing poplar wood. *J. Agric. Food Chem.* **2001**, *49*, 5122–5129.
- Zhou, Z.; Robards, K.; Helliwell, S.; Blanchard, C. The distribution of phenolic acids in rice. *Food Chem.* **2004**, *87*, 401–406.
- Mendez, J. Dihydrocinnamic acids in *Pteridium aquilinum*. *Food Chem.* **2005**, *93*, 251–252.
- Adamovics, J. A.; Johnson, G.; Stermitz, F. R. Ferulates from cork layers of *Solanum tuberosum* and *Pseudotsuga menziesii*. *Phytochemistry* **1977**, *16*, 1089–1090.
- Baldé, A. M.; Claeys, M.; Pieters, L. A.; Wray, V.; Vlietinck, A. J. Ferulic acid esters from stem bark of *Pavetta owariensis*. *Phytochemistry* **1991**, *30*, 1024–1026.
- Laver, M. L.; Fang, H. H.-L. Ferulic acid esters from bark of *Pseudotsuga menziesii*. *J. Agric. Food Chem.* **1989**, *37*, 114–116.
- Chatterjee, A.; Dhara, K. P.; Rej, R. N.; Ghosh, P. C. Hexacosylferulate, a phenolic constituent of *Pinus roxburghii*. *Phytochemistry* **1977**, *16*, 397–398.
- Kawanishi, K.; Yasufuku, J.; Ishikawa, A.; Hashimoto, Y. Long-chain alkyl ferulates in three varieties of *Ipomoea batatas* (L.) Lam. *J. Agric. Food Chem.* **1990**, *38*, 105–108.
- Sarker, S. D.; Shaheen, E. M.; Eynon, E.; Nahar, L. Caffeic acid decyl ester: an antioxidant principle from *Phleum pratense*. *Chem. Nat. Compd.* **2005**, *41*, 293–296.
- Yunoki, K.; Musa, R.; Kinoshita, M.; Tazaki, H.; Oda, Y.; Ohnishi, M. Presence of higher alcohols as ferulates in potato pulp and its radical-scavenging activity. *Biosci., Biotechnol., Biochem.* **2004**, *68*, 2619–2622.
- Whitaker, B. D.; Schmidt, W. F.; Kirk, M. C.; Barnes, S. Novel fatty acid esters of *p*-coumaryl alcohol in epicuticular wax of apple fruit. *J. Agric. Food Chem.* **2001**, *49*, 3787–3792.
- Cefarelli, G.; Abrosca, B. D.; Fiorentino, A.; Izzo, A.; Monaco, P. Isolation, characterization, and antioxidant activity of *E*- and *Z*-*p*-coumaryl fatty acid esters from cv. annurca apple fruits. *J. Agric. Food Chem.* **2005**, *53*, 3525–3529.
- Bernards, M. A.; Lewis, N. G. Alkyl ferulates in wound healing potato tubers. *Phytochemistry* **1992**, *31*, 3409–3412.
- Condo, A. M., Jr.; Baker, D. C.; Moreau, R. A.; Hicks, K. B. Improved method for the synthesis of *trans*-feruloyl- β -sitostanol. *J. Agric. Food Chem.* **2001**, *49*, 4961–4964.
- Compton, D. L.; Laszlo, J. A.; Berhow, M. A. Lipase-catalyzed synthesis of ferulate esters. *J. Am. Oil Chem. Soc.* **2000**, *77*, 513–519.

- (38) Giuliani, S.; Piana, C.; Setti, L.; Hochkoeppler, A.; Pifferi, P. G.; Williamson, G.; Faulds, C. B. Synthesis of pentylferulate by a feruloyl esterase from *Aspergillus niger* using water-in-oil microemulsions. *Biotechnol. Lett.* **2001**, *23*, 325–330.
- (39) Guyot, B.; Bosquette, B.; Pina, M.; Graille, J. Esterification of phenolic acids from green coffee with an immobilized lipase from *Candida antarctica* in solvent-free medium. *Biotechnol. Lett.* **1997**, *19*, 529–532.
- (40) Stamatis, H.; Sereti, V.; Kolisis, F. N. Studies on the enzymatic synthesis of lipophilic derivatives of natural antioxidants. *J. Am. Oil Chem. Soc.* **1999**, *12*, 1505–1510.
- (41) Stamatis, H.; Sereti, V.; Kolisis, F. N. Enzymatic synthesis of hydrophilic and hydrophobic derivatives of natural phenolic acids in organic media. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 323–328.
- (42) Topakas, E.; Stamatis, H.; Biely, P.; Kekos, D.; Macris, B. J.; Christakopoulos, P. Purification and characterization of a feruloyl esterase from *Fusarium oxysporum* catalyzing esterification of phenolic acids in ternary water-organic solvent mixtures. *J. Biotechnol.* **2003**, *102*, 33–44.
- (43) Twu, Y.-K.; Shih, I.-L.; Yen, Y.-H.; Ling, Y.-F.; Shieh, C.-J. Optimization of lipase-catalyzed synthesis of octyl hydroxyphenylpropionate by response surface methodology. *J. Agric. Food Chem.* **2005**, *53*, 1012–1016.
- (44) Jung, M. Y.; Jeon, B. S.; Bock, J. Y. Free, esterified, and insoluble-bound phenolic acids in white and red Korean ginsengs (*Panax ginseng* C.A. Meyer). *Food Chem.* **2002**, *79*, 105–111.
- (45) Lutz, A.; Knörr, W.; Spiteller, G. Produkte des reduktiven Abbaus von α -(Acyloxy)plasmalogenen aus Rindergewebe-Lipiden mit LiAlH_4 . *Liebigs Ann. Chem.* **1991**, 1151–1155.
- (46) Weber, N.; Weitkamp, P.; Mukherjee, K. D. Fatty acid steryl, stanyl and steroid esters by esterification and transesterification in vacuo using *Candida rugosa* lipase as catalyst. *J. Agric. Food Chem.* **2001**, *49*, 67–71.
- (47) Weber, N.; Weitkamp, P.; Mukherjee, K. D. Steryl and stanyl esters of fatty acids by solvent-free esterification and transesterification in vacuo using lipases from *Rhizomucor miehei*, *Candida antarctica*, and *Carica papaya*. *J. Agric. Food Chem.* **2001**, *49*, 5210–5216.

Received for review January 6, 2006. Revised manuscript received February 23, 2006. Accepted February 23, 2006.

JF060052T