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Dialkyl 3,3′-Thiodipropionate and Dialkyl 2,2′-Thiodiacetate Antioxidants by Lipase-Catalyzed Esterification and Transesterification

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Medium- and long-chain dialkyl 3,3′-thiodipropionate antioxidants such as dioctyl 3,3′-thiodipropionate, didodecyl 3,3′-thiodipropionate, dihexadecyl 3,3′-thiodipropionate, and di-(*cis*-9-octadecenyl) 3,3′-thiodipropionate were prepared in high yield by lipase-catalyzed esterification and transesterification of 3,3′-thiodipropionic acid and its dimethyl ester, respectively, with the corresponding medium- or long-chain 1-alkanols, i.e., 1-octanol, 1-dodecanol, 1-hexadecanol, and *cis*-9-octadecen-1-ol, in vacuo (80 kPa) at moderate temperatures (60−80°C) without solvents. Immobilized lipase B from *Candida antarctica* (Novozym 435) was the most active biocatalyst for the preparation of medium- and long-chain dialkyl 3,3′-thiodipropionates showing enzyme activities up to 1489 units/g, whereas the immobilized lipases from *Rhizomucor miehei* (Lipozyme RM IM) and *Thermomyces lanuginosus* (Lipozyme TL IM) were by far less active (∼10 enzyme units/g). Maximum conversions to dialkyl 3,3′-thiodipropionates were as high as 92−98% after 4 h of reaction time. Similarly, dihexadecyl 2,2′-thiodiacetate was prepared in high yield using 2,2′-thiodiacetic acid or diethyl 2,2′-thiodiacetate and 1-hexadecanol as the starting materials and Novozym 435 as the biocatalyst.

KEYWORDS: Didodecyl 3,3′-thiodipropionate; dihexadecyl 2,2′-thiodiacetate; 3,3′-thiodipropionic acid (4-thiaheptane-1,7-dioic acid); 2,2′-thiodiacetic acid (3-thiapentane-1,5-dioic acid); lipase-catalyzed esterification and transesterification; immobilized lipase B from *Candida antarctica*

INTRODUCTION

Antioxidants are widely used for both food and technical applications. There is increasing interest in antioxidative additives particularly for the food and health sector. Recently, Codex Alimentarius Commission (CAC) dealt with a "Draft Standard for Fat Spreads and Blended Spreads—Request for Comments on Food Additive Provisions" where didodecyl 3,3′-thiodipropionate (dilaurylthiodipropionate, INS no. 389) and 3,3′-thiodipropionic acid (thiodipropionic acid, INS no. 388) are introduced as optional antioxidants for the food sector and grouped as "thiodipropionates" (*1*). The U.S. Department of Agriculture considers the use of both didodecyl 3,3′-thiodipropionate and 3,3′-thiodipropionic acid to be generally recognized as safe for use in foods, when the total level of antioxidant does not exceed 200 mg/kg (fat or oil basis; singly or in combination with other antioxidants) (*2*). CAC has already adopted the General Standard on Food Additives provisions (200 mg/kg) for thiodipropionates (categories 2.2.1.2 and 2.2.2). In food applications, thiodipropionates may be employed to decompose hydrogen peroxide formed during lipid oxidation. Technical applications are directed at their use as antioxidant stabilizers in various polymers including plastic films (*3*−*5*).

Antioxidative stabilizers such as medium- or long-chain dialkyl 3,3′-thiodipropionates and dialkyl 2,2′-thiodiacetates (**Figure 1**) for technical purposes are usually prepared by esterification of the corresponding thia-alkanedioic acids with medium- or long-chain alcohols using, e.g., *p*-toluenesulfonic acid as a catalyst (*6*, *7*). For food applications, however, enzymatic esterification is recommended rather than chemical esterification reactions. We have, therefore, developed a lipase-catalyzed esterification process for the preparation of medium- or long-chain dialkyl 3,3′-thiodipropionates using 3,3′-thiodipropionic acid or the corresponding short-chain alkyl esters as starting materials, which were esterified or transesterified with medium- or long-chain alcohols using immobilized microbial lipases as biocatalysts in the absence of solvents in vacuo. Under these conditions, the preparation of dialkyl 3,3′-thiodipropionates does not require any materials with deleterious effects on health and the environment. Similarly, dialkyl 2,2′-thiodiacetates were prepared by esterifying or transesterifying 2,2′-thiodiacetic acid or its short-chain alkyl esters with medium- or long-chain alcohols.

MATERIALS AND METHODS

Materials. 3,3′-Thiodipropionic acid (4-thiaheptane-1,7-dioic acid), dimethyl 3,3′-thiodipropionate, didodecyl 3,3′-thiodipropionate, dioctadecyl 3,3′-thiodipropionate, 2,2′-thiodiacetic acid (thiodiglycolic

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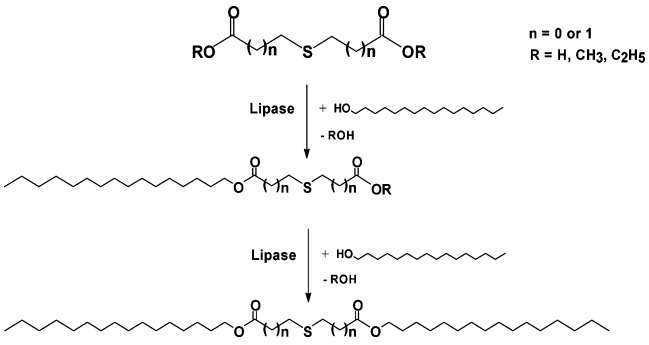

Figure 1. Reaction scheme of the successive lipase-catalyzed esterification or transesterification of thia-alkanedioates such as 3,3′-thiodipropionic acid ($n = 1$; R = H) and dimethyl 3,3′-thiodipropionate ($n = 1$; R = methyl) as well as 2,2′-thiodiacetic acid ($n = 0$; R = H) and diethyl 2,2′-thiodiacetate ($n = 0$; R = ethyl) with 1-hexadecanol yielding, e.g., dihexadecyl 3,3′-thiodipropionate and dihexadecyl 2,2′-thiodiacetate.

acid; 3-thiapentan-1,5-dioic acid), and diethyl 2,2′-thiodiacetate as well as 1-octanol, 1-dodecanol, 1-hexadecanol, and *cis*-9-octadecen-1-ol were obtained from Sigma-Aldrich-Fluka (Deisenhofen, Germany). Diethyl ether, *iso*-hexane, methyl-*tert*-butyl ether (MTBE), *tert*-butyl alcohol, benzene, acetonitrile, acetone, dichloromethane, sulfuric acid, sodium carbonate, sodium bicarbonate, sodium sulfate, and potassium hydroxide were products of VWR International (Darmstadt, Germany). A solution of diazomethane in diethyl ether was prepared by the reaction of an etherial solution of *N*-methyl-*N*-nitroso-*p*-toluolsulfonamide (Aldrich) with potassium hydroxide (*8*). Immobilized lipase preparations from *Rhizomucor miehei* [Lipozyme RM IM, 23 batch interesterification units (BIU)/g, defined as the amount of enzyme required to incorporate 1 μmol of palmitic acid into trioleoylglycerol/min from an equimolar mixture at 40 °C; 10% w/w water], *Candida antarctica* (lipase B, Novozym 435; 10500 propyl laurate units/g; 2% w/w water), and *Thermomyces lanuginosus* [Lipozyme TL IM, 170 interesterification units novo (IUN)/g] were kindly provided by Novozymes (Bagsvaerd, Denmark).

Chemical Preparation of Reaction Intermediates. Monododecyl esters of 3,3′-thiodipropionic acid were prepared by esterification of 3,3′-thiodipropionic acid (891 mg; 5 mmol) and transesterification of dimethyl 3,3′-thiodipropionate (1031 mg; 5 mmol), respectively, with 1-dodecanol (932 mg; 5 mmol) in the presence of 200 μL of concentrated sulfuric acid. The best results were obtained using *tert*-butyl alcohol as the solvent for the esterification and benzene as the solvent for the transesterification reaction. The reaction products, i.e., 3,3′-thiodipropionic acid monododecyl ester and 3,3′-thiodipropionic acid methyl dodecyl ester (methyl dodecyl 3,3′-thiodipropionate), respectively, were purified by column chromatography on Silica Gel 60 (VWR International) using mixtures of *iso*-hexane-diethyl ether and/or *iso*-hexane-diethyl ether−acetic acid−water as the eluents.

Lipase-Catalyzed Esterification and Transesterification Reactions. As a typical example, 3,3′-thiodipropionic acid (178 mg, 1 mmol) was esterified with 1-dodecanol (410 mg, 2.2 mmol) in the presence of 6−50 mg of the immobilized lipase preparation by magnetic stirring in a screw-capped tube in vacuo at 80 °C for periods up to 72 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 MTBE at 50 °C, and filtered through a 1 μm syringe filter to separate the biocatalyst. An aliquot of the filtrate was analyzed as given below. Similarly, 2,2′-thiodiacetic acid (150 mg, 1 mmol) was esterified with 1-hexadecanol (2.2 mmol). Dimethyl 3,3′-thiodipropionate (206 mg) and diethyl 2,2′-thiodiacetate (208 mg), 1 mmol each, were transesterified with 1-alkanols (2.2 mmol) under identical conditions as described above for esterification reaction using various lipases (6−50 mg, each) as biocatalysts. To determine the chain-length specificity of Novozym 435, 3,3′-thiodipropionic acid was esterified with an equimolar mixture of 1-octanol, 1-dodecanol, and 1-hexadecanol under the conditions described above using 12.5 mg of the biocatalyst and a reaction time of 15 min. Blank experiments were carried out at 80 °C in vacuo by reacting 3,3′-thiodipropionic acid, dimethyl 3,3′-thiodipropionate, 2,2′-thiodiacetic acid, or diethyl 2,2′-thiodiacetate with long-chain 1-alkanols in the absence of lipase for several hours.

Enzyme units were calculated from the initial rates (30 min) of esterification of 3,3′-thiodipropionic acid and 2,2′-thiodiacetic acid as well as transesterification of dimethyl 3,3′-thiodipropionate and diethyl 2,2′-thiodiacetate with 1-alkanols. The amounts of immobilized lipases used for the determination of enzyme units were 12.5 mg for the conversion of 3,3′-thiodipropionic acid and dimethyl 3,3′-thiodipropionate and 50 mg for the conversion of 2,2′-thiodiacetic acid and diethyl 2,2′-thiodiacetate. One unit of enzyme activity was defined as the amount of enzyme (g) that produced 1 μmol of dialkyl ester/min. Values are given as means ± SEM including the number of experiments ($n = x$).

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 (VWR International) using *iso*-hexane-diethyl ether (7:3, v/v). Free carboxy groups of the reaction products were methylated by using a solution of diazomethane in diethyl ether. Spots were located by iodine staining and charring by spraying with 30% sulfuric acid followed by heating (200 °C). The R_f values of the various compounds were as follows: medium- and long-chain dialkyl esters of 3,3′-thiodipropionic acid and 2,2′-thiodiacetic acid, 0.6−0.7; methyl-alkyl esters of 3,3′-thiodipropionic acid and 2,2′-thiodiacetic acid, 0.40−0.55; dimethyl 3,3′-thiodipropionate and dimethyl 2,2′-thiodiacetate, 0.30; diethyl 2,2′-thiodiacetate, 0.42; medium- and long-chain 1-alkanols, 0.2−0.25; and unesterified 3,3′-thiodipropionic acid and 2,2′-thiodiacetic acid, <0.1. 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.

Gas Chromatography (GC). In both esterification and transesterification reactions, aliquots of products were treated with a solution of diazomethane in diethyl ether to convert the unreacted or hydrolyzed 3,3′-thiodipropionic acid or 2,2′-thiodiacetic acid to the corresponding methyl esters. The resulting mixture of methyl esters, unreacted 1-alkanols as well as various methyl alkyl- and dialkyl esters of the above thia-alkanedioic acids, was analyzed by GC. A Hewlett-Packard (Böblingen, Germany) HP-5890 Series II gas chromatograph equipped with a flame ionization detector (FID) was used. Separations were carried out on a 0.1 μm Quadrex 400-1HT (Quadrex Corp., New Haven, CT) fused silica capillary column, 15 m × 0.25 mm i.d. using hydrogen as the carrier gas (column pressure, 50 kPa) initially at 60 °C for 2 min, followed by linear programming from 60 to 380 °C at 20 °C × min^{-1} and finally kept at 380 °C for 2 min. Reaction mixtures containing both dimethyl 3,3′-thiodipropionate and 1-dodecanol were separated using the following temperature program: 60 °C for 0.5 min, followed by linear programming from 60 to 110 °C at 8 °C × min^{-1} (5 min isothermally), then from 110 to 380 °C at 25 °C × min^{-1}, and finally kept at 380 °C for 2 min. Injector and detector temperatures were maintained at 360 and 380 °C, respectively. Reaction mixtures containing both didodecyl and octylhexadecyl 3,3′-thiodipropionates were separated on a 0.2 μm DB-23 (J&W, ASS-Chem, Bad Homburg, Germany) fused silica capillary coulmn, 40 m × 0.18 mm i.d. using hydrogen as the carrier gas (column pressure, 136 kPa) isothermally at 250 °C (injector and detector temperature, 300 °C). Peaks in gas chromatograms were assigned by comparison of their retention times with those of peaks from TLC fractions, which 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.

High-Performance Liquid Chromatography (HPLC). Aliquots of thia-alkanedioic acid dialkyl esters were analyzed for their composition by 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 25 °C, a UV/vis HPLC 332 detector (E. Merck) set to a wavelength of 210 nm, and a PL-ELS 2100 (Polymer Laboratories, Darmstadt, Germany) ELSD detector (thermostated to 30 °C for acetonitrile and 40 °C for mixtures of acetonitrile−acetone), which were used in series. Mass and UV traces were monitored and

evaluated in a KromaSystem 2000 data acquisition unit (Kontron Instruments, Milan, Italy). A Phenomenex 5 μm LiChrosphere RP-18e column (250 mm × 4 mm i.d., Phenomenex, Aschaffenburg, Germany) and precolumn were used for the separations. Medium- or long-chain methyl alkyl esters of thia-alkanedioic acids, i.e., methyl alkyl 3,3′-thiodipropionates and methyl alkyl 2,2′-thiodiacetates, were separated using acetonitrile (flow rate, 0.8 mL/min) as the eluent. Medium- or long-chain dialkyl esters of thia-alkanedioic acids, i.e., dialkyl 3,3′-thiodipropionates and dialkyl 2,2′-thiodiacetates, were separated using a mixture of acetonitrile−acetone (80:20, v/v; flow rate, 0.8 mL/min), isocratically until 3 min, then for 15 min using a gradient (80:20 to 90:10, v/v; flow rate, 1.0 mL/min). Injections (around 20−60 μg) of the reaction products in acetonitrile−acetone, 1:1, v/v, or acetone containing 2% dichloromethane) were carried out with a Rheodyne 7125 sample injector (Cotati, CA) equipped with a 20 μL sample loop. Peak areas and percentages were calculated using EuroChrom (Knauer, Berlin, Germany) software. The retention times (min) of the various compounds were as follows: dioctyl 3,3′-thiodipropionate, 5.6; didodecyl 3,3′-thiodipropionate, 13.0; dihexadecyl 3,3′-thiodipropionate, 18.7; di-(*cis*-9-octadecenyl) 3,3′-thiodipropionate, 17.9; and dihexadecyl 2,2′-thiodiacetate, 18.1. Medium- or long-chain methyl alkyl 3,3′-thiodipropionates were separated using acetonitrile as the eluent showing the following retention times (min): methyl octyl 3,3′-thiodipropionate, 4.1; methyl dodecyl 3,3′-thiodipropionate, 6.8; methyl hexadecyl 3,3′-thiodipropionate, 13.9; methyl *cis*-9-octadecenyl 3,3′-thiodipropionate, 13.0; and ethyl hexadecyl 2,2′-thiodiacetate, 13.2.

Purification of Dialkyl Esters of 3,3′-Thiodipropionic Acid and 2,2′-Thiodiacetic Acid by Deacidification and Crystallization. The medium- and long-chain dialkyl esters of 3,3′-thiodipropionic acid and 2,2′-thiodiacetic acid from various incubations were repeatedly extracted using MTBE at 50 °C. The combined extracts dissolved in *iso*-hexane [dioctyl 3,3′-thiodipropionate, didodecyl 3,3′-thiodipropionate, and di-(*cis*-9-octadecenyl) 3,3′-thiodipropionate] or diethyl ether (dihexadecyl 3,3′-thiodipropionate and dihexadecyl 2,2′-thiodiacetate) were deacidified by extracting with 2% aqueous sodium carbonate solution. The organic phase was removed and dried with sodium sulfate. After they were cooled to around 0 °C [dioctyl 3,3′-thiodipropionate and di-(*cis*-9-octadecenyl) 3,3′-thiodipropionate to −20 °C], the precipitates of the various dialkyl esters of 3,3′-thiodipropionic acid and 2,2′-thiodiacetic acid were filtered and dried in vacuo. Melting points (mp) were determined using a Kofler heating block [dioctyl 3,3′-thiodipropionate, mp 7−8 °C; didodecyl 3,3′-thiodipropionate, mp 41 °C; dihexadecyl 3,3′-thiodipropionate, mp 59−61 °C; di-(*cis*-9-octadecenyl) 3,3′-thiodipropionate, mp 16−17 °C; and dihexadecyl 2,2′-thiodiacetate, mp 45−46 °C].

In a preparative scale reaction, 3,3′-thiodipropionic acid (890 mg, 5 mmol) was esterified with 1-hexadecanol (1210 mg, 11 mmol) using 250 mg of Novozym 435. After 4 h, the reaction products were extracted with dichloromethane and deacidified by treating with solid sodium bicarbonate followed by crystallization from diethyl ether to yield dihexadecyl 3,3′-thiodipropionate with a purity of >97% (as determined by GC) and an isolated yield of ∼84% with respect to the amount of 3,3′-thiodipropionic acid used. The purity of the crystallized reaction products was determined by TLC, GC, and HPLC as described above.

GC-MS Analyses. The fragmentation of the various medium- and long-chain alkyl esters of 3,3′-thiodipropionic acid and 2,2′-thiodiacetic acid formed by lipase-catalyzed esterification or transesterification was studied by GC-MS. Monoalkyl esters were analyzed by GC-MS after derivatization to the corresponding methyl alkyl esters using an etherial solution of diazomethane. GC-MS analyses were carried out on a Hewlett-Packard model 5890 series II/5989A apparatus equipped with a 0.25 μm Rtx-5MS fused silica capillary column (Restek Germany, Bad Homburg, Germany), 15 m × 0.25 mm i.d., using the electron ionization (EI, 70 eV) mode. The carrier gas was He at a flow rate of 1.0 mL/min. The column temperature was initially kept at 60 °C for 2 min and then programmed from 60 to 360 °C at 20 °C × min^{-1}; the final temperature was held for 9 min. Other operating conditions were split/splitless injector in split mode (split, 1:10; temperature, 360 °C), interface temperature (360 °C), and ion source temperature (200 °C).

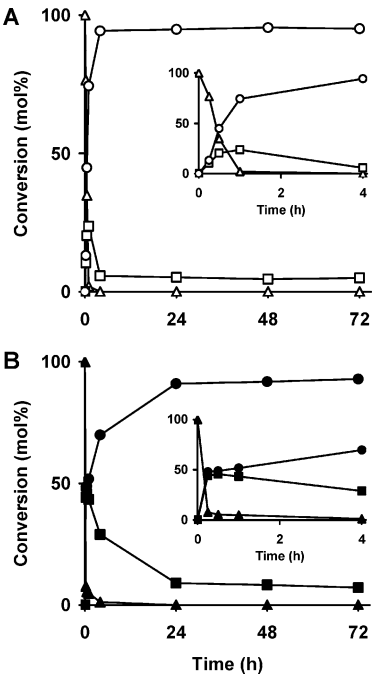

Figure 2. (**A**) Time course of the esterification of 3,3′-thiodipropionic acid with 1-dodecanol catalyzed by immobilized lipase B from *C. antarctica* (Novozym 435): △, 3,3′-thiodipropionic acid; □, 3,3′-thiodipropionic acid monododecyl ester; and ○, didodecyl 3,3′-thiodipropionate (reaction conditions: molar ratio 3,3′-thiodipropionic acid:1-dodecanol = 1:2.2; 25 mg of Novozym 435; temperature, 60 °C; and 80 kPa). Insert: esterification reaction during the first 4 h. (**B**) Time course of the transesterification of diethyl 2,2′-thiodiacetate with 1-hexadecanol at 80 °C using similar conditions as described above: ▲, diethyl 2,2′-thiodiacetate; ■, ethyl hexadecyl 2,2′-thiodiacetate; and ●, dihexadecyl 2,2′-thiodiacetate. All values are means of three determinations.

RESULTS

Lipase-catalyzed esterification of thia-alkanedioic acids as well as transesterification of their short-chain dialkyl esters with medium- or long-chain 1-alkanols lead to the corresponding medium- or long-chain monoalkyl esters of thia-alkanedioic acids and subsequently to medium- or long-chain dialkyl esters of thia-alkanedioic acids (dialkyl thia-alkanedioates) such as dihexadecyl 3,3′-thiodipropionate and dihexadecyl 2,2′-thiodiacetate as is demonstrated in **Figure 1**.

Medium- and long-chain dialkylesters of thia-alkanedioic acids such as didodecyl 3,3′-thiodipropionate, dihexadecyl 3,3′-thiodipropionate, and dihexadecyl 2,2′-thiodiacetate were prepared this way using esterification or transesterification reactions catalyzed by immobilized lipase B of *C. antarctica* (Novozym 435). The reactions were performed without solvent at moderate temparatures (60−80 °C) in vacuo (80 kPa) to remove water or short-chain alcohols under mild conditions. As an example, **Figure 2A** shows the formation of didodecyl 3,3′-thiodipropionate by Novozym 435-catalyzed esterification of 3,3′-thiodipropionic acid with 1-dodecanol over a period of 72 h. It is obvious from these results that the proportions of didodecyl 3,3′-thiodipropionate increase with time and a concomitant decrease of 3,3′-thiodipropionic acid is observed in the reaction mixture. In addition, the results of **Table 1** show that an enzyme activity of up to 3666 ± 179 equivalent esterification units/g (n = 5) is observed for the formation of total esters (mono- plus diesters) of 3,3′-thiodipropionic acid with 1-alkanols catalyzed by Novozym 435 and a conversion of 92−98% (within 4 h) to dialkyl 3,3′-thiodipropionates is achieved, if a 10%

Table 1. Maximum Conversion of Thia-Alkanedioic Acids or Their Short-Chain Dialkylesters and 1-Alkanols as Substrates to Medium- or Long-Chain Dialkyl Esters by Esterification and Transesterification as Well as Enzyme Activities of Various Immobilized Lipases as Catalysts[a]

thia-alkanedioic acid or short-chain dialkylester	1-alkanol	lipase	maximum conversion (mol %) to dialkyl esters (after h)	enzyme activity for the formation of dialkyl esters (units/g ± SEM)[b] ($n = x$)	enzyme activity for the formation of total alkyl ester equivalents (units/g ± SEM)[c] ($n = x$)
3,3′-thiodipropionic acid	1-octanol	Novozym 435	98 (4 h)[d]	1489 ± 102 (5)	3666 ± 179 (5)
	1-dodecanol	Novozym 435	94 (4 h)[d,e]	587 ± 64 (3)[e]	1503 ± 155 (3)[e]
	1-dodecanol	Novozym 435	97 (24 h)[d,f]	1330 ± 79 (3)	3336 ± 209 (3)
	1-hexadecanol	Novozym 435	94 (4 h)[d]	1373 ± 90 (3)	3436 ± 196 (3)
	cis-9-octadecen-1-ol	Novozym 435	92 (4 h)	1387 ± 146 (4)	3289 ± 290 (4)
	1-dodecanol	Lipozyme RM IM	ND[g]	4.9 ± 1.2 (3)	53 ± 7 (3)
	1-dodecanol	Lipozyme TL IM	ND	6.7 ± 2.2 (3)	64 ± 10 (3)
3,3′-thiodipropionic acid monododecyl ester	1-dodecanol	Novozym 435	ND	2228 ± 89 (2)	–
dimethyl 3,3′-thiodipropionate	1-dodecanol	Novozym 435	90 (48 h)[h]	1198 ± 39 (5)	3670 ± 29 (5)
	1-dodecanol	Lipozyme RM IM	ND	13 ± 0.5 (3)	171 ± 17 (3)
	1-dodecanol	Lipozyme TL IM	ND	11 ± 1.0 (3)	183 ± 16 (3)
3,3′-thiodipropionic acid methyl dodecyl ester	1-dodecanol	Novozym 435	ND	1517 ± 11 (2)	–
2,2′-thiodiacetic acid	1-hexadecanol	Novozym 435	92 (72 h)[i]	41 ± 3 (2)[i]	233 ± 4 (2)[i]
diethyl 2,2′-thiodiacetate	1-hexadecanol	Novozym 435	91 (24 h)[i]	326 ± 3 (2)[i]	956 ± 13 (2)[i]

[a] Standard assay conditions, if not otherwise indicated, are as follows: 1 mmol of thia-alkanedioic acid or its short-chain dialkylester + 2.2 mmol of 1-alkanol; immobilized lipase/assay, 12.5 mg; 80 °C; and 80 kPa. [b] Enzyme activities for the formation of medium- or long-chain dialkyl esters of thia-alkanedioic acids as determined by GC. [c] Enzyme activities for the formation of total medium- or long-chain alkyl ester equivalents of thia-alkanedioic acids were determined by GC, and the conversions were calculated as [μmol dialkyl ester] + [μmol monoalkyl esters × 0.5]. [d] Amount of immobilized lipase/assay used, 25 mg. [e] At 60 °C. [f] 93% after 4 h. [g] ND, not determined. [h] 85% after 4 h. [i] Amount of immobilized lipase/assay used, 50 mg.

equivalent excess of medium- or long-chain alcohol is used. After an initial increase, the proportion of the monoesters, e.g., 3,3′-thiodipropionic acid monododecyl ester, decreases as is typical of reaction intermediates (**Figure 2A**, insert).

Figure 2B demonstrates the formation of dihexadecyl 2,2′-thiodiacetate by Novozym 435-catalyzed transesterification of diethyl 2,2′-thiodiacetate with 1-hexadecanol over a period of 72 h. These results including those given in **Figure 2B** (insert) and **Table 1** show that the rate of transesterification of diethyl 2,2′-thiodiacetate with 1-hexadecanol is distinctly lower [enzyme activity 956 ± 13 equivalent units/g ($n = 2$)] for the formation of total hexadecyl 2,2′-thiodiacetates than that found for the formation of total hexadecyl 3,3′-thiodipropionates by transesterification of dimethyl 3,3′-thiodipropionate with 1-hexadecanol [enzyme activity 3670 ± 29 equivalent units/g ($n = 5$)] (**Figure 2A**). A far lower enzyme activity [233 ± 4 equivalent units/g) $n = 2$] was found for the esterification of 2,2-thiodiacetic acid with 1-hexadecanol (**Table 1**). Blank experiments carried out at 80 °C in vacuo by reacting 3,3′-thiodipropionic acid, dimethyl 3,3′-thiodipropionate, 2,2′-thiodiacetic acid, or diethyl 2,2′-thiodiacetate with long-chain 1-alkanols in the absence of lipase did not produce any long-chain ester of the above substrates.

Table 1 shows the enzyme activities of various commercial immobilized lipases as well as maximum conversions for esterification and transesterification reactions using different reaction conditions. These data demonstrate that immobilized lipase B from *C. antarctica* (Novozym 435) is by far superior to immobilized lipase preparations from *R. miehei* (Lipozyme RM IM) and *T. lanuginosus* (Lipozyme TL IM) in both the esterification and the transesterification of 3,3′-thiodipropionic acid and its dimethyl ester, respectively, with medium- or long-chain 1-alkanols. Generally, conversions to medium- or long-chain dialkyl 3,3′-thiodipropionates to an extent of 92−98% after 4 h are obtained by esterification of 3,3′-thiodipropionic acid with 1-alkanols such as 1-octanol, 1-dodecanol, 1-hexadecanol, and *cis*-9-octadecen-1-ol and a conversion of 85% (within 4 h) is attained by transesterification of its dimethyl ester with 1-dodecanol catalyzed by Novozym 435. Increasing the reaction temperature from 60 to 80 °C increases the enzyme activity for the formation of total ester equivalents by ∼140% as shown for the esterification of 3,3′-thiodipropionic acid with 1-dodecanol (**Table 1**). It is also obvious from **Table 1** and **Figure 2** that the enzyme activity of Novozym 435 is by far lower for the reaction of 1-hexadecanol with 2,2′-thiodiacetic acid and its diethyl ester as compared to the reaction of 3,3′-thiodipropionic acid and its dimethyl ester with 1-alkanols. It is worth noting, however, that the enzyme activity of the transesterification of diethyl 2,2′-thiodiacetate with 1-hexadecanol to dihexadecyl 2,2′-thiodiacetate is by far higher than the corresponding esterification of 2,2′-thiodiacetic acid. The enzyme activity of Novozym 435 for the diesterification of 3,3′-thiodipropionic acid with individual medium- and long-chain alcohols is quite similar showing no remarkable preference for any of the 1-alkanols tested. The enzyme activity of Novozym 435 is, however, slightly lower for the formation of didodecyl 3,3′-thiodipropionate by transesterification of dimethyl 3,3′-thiodipropionate (**Table 1**). Similar results are obtained for the formation of total ester equivalents (mono- plus diesters) (**Table 1**).

Incorporation of alkyl moieties with various chain-lengths into monoester, diester, and total ester fractions of 3,3′-thiodipropionates was studied by esterification of 3,3′-thiodipropionic acid with an equimolar mixture of 1-alkanols, i.e., 1-octanol, 1-dodecanol, and 1-hexadecanol, catalyzed by Novozym 435 lipase in vacuo at 80 °C for 0.25 h. As observed for the esterification of 3,3′-thiodipropionic acid with individual medium- and long-chain alcohols (**Table 1**), the enzyme activity is quite similar for the three 1-alkanols under equimolar conditions as well (data not shown).

Figure 3 shows the effects of the amount of enzyme on the rate of the esterification of 3,3′-thiodipropionic acid with 1-dodecanol at 60 (**Figure 3A**) and 80 °C (**Figure 3B**). As expected, increasing the temperature and the concentration of

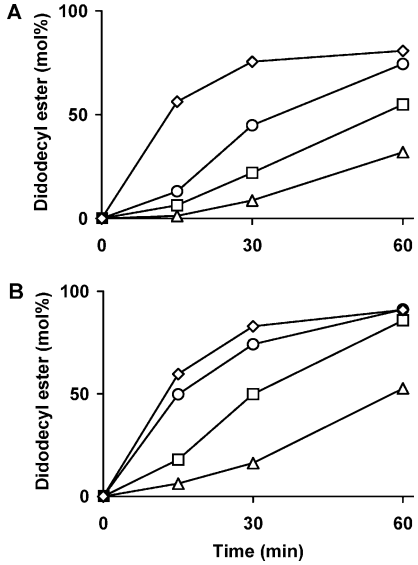

Figure 3. Time course of the formation of didodecyl 3,3′-thiodipropionate by esterification of 1 mmol of 3,3′-thiodipropionic acid with 2.2 mmol of 1-dodecanol, catalyzed by different amounts of Novozym 435 lipase preparation (△, 6.25 mg; □, 12.5 mg; ○, 25 mg; and ◇, 50 mg) in vacuo at (**A**) 60 and (**B**) 80 °C. All values are means of three determinations.

immobilized Novozym 435 lipase leads to higher esterification rates and, as a consequence, to the formation of higher proportions of didodecyl 3,3′-thiodipropionate with time. It is evident that under the reaction conditions described an amount of 25 mg of Novozym 435 yields optimum conversion of 3,3′-thiodipropionic acid at 80 °C. A higher concentration of Novozym 435 (≥50 mg/assay) is necessary to achieve satisfactory conversion of diethyl 2,2′-thiodiacetate, whereas the esterification rate is low for 2,2′-thiodiacetic acid even under these conditions (**Table 1**).

The various reaction products, i.e., mono- and dialkyl esters of thia-alkanedioic acids were analyzed by GC-MS (EI mode), and the resulting molecular and mass fragment ions are given in **Table 2**. In addition, **Figure 4** demonstrates, as an example, the fragmentation pattern of didodecyl 3,3′-thiodipropionate and

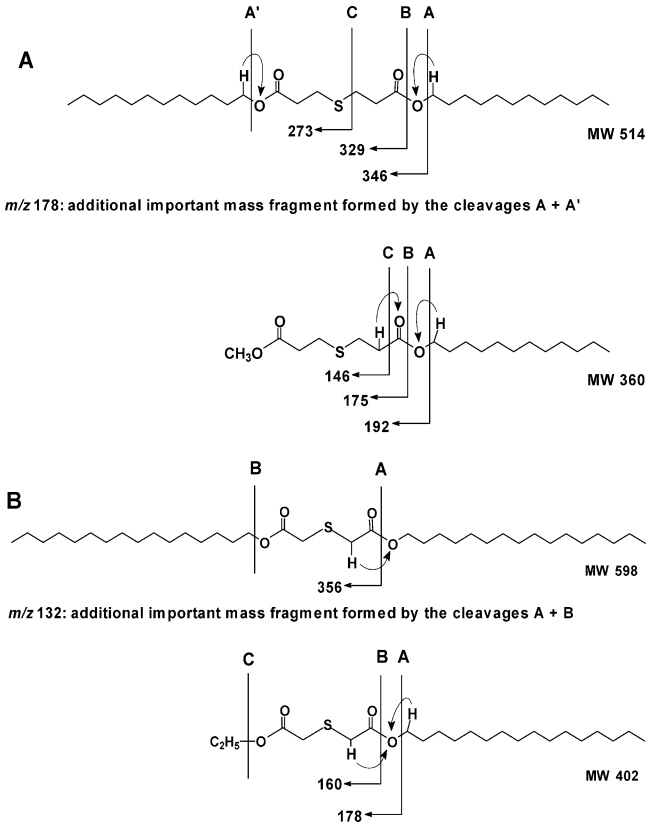

Figure 4. Mass spectrometrical fragmentation pattern of (**A**) didodecyl and methyl dodecyl 3,3′-thiodipropionate as well as (**B**) dihexadecyl and ethyl hexadecyl 2,2′-thiodiacetate.

dihexadecyl 2,2′-thiodiacetate as well as methyl dodecyl 3,3′-thiodipropionate and methyl hexadecyl 2,2′-thiodiacetate as a result of GC-MS analyses.

Table 2 shows the presence of molecular ions for both groups of thia-alkanedioic acids. Typical mass fragments detected, e.g., for methyl dodecyl 3,3′-thiodipropionate, are *m*/*z* 192 and 175 showing the loss of dodecyl and dodecyloxy groups, respec-

Table 2. Mass Fragments of Medium- and Long-Chain Mono- and Dialkyl Esters of Thia-Alkanedioic Acids

Monoalkyl Esters[a]				
methyl octyl 3,3′-thiodipropionate[b]	methyl dodecyl 3,3′-thiodipropionate[b]	methyl hexadecyl 3,3′-thiodipropionate[b] *m*/*z* (rel. %)	methyl (*cis*-9-octadecenyl) 3,3′-thiodipropionate[b]	ethyl hexadecyl 2,2′-thiodiacetate
304 (39) $[M]^+$	360 (37) $[M]^+$	416 (33) $[M]^+$	442 (<1) $[M]^+$	402 (5) $[M]^+$
273 (6) $[M - CH_3O]^+$	329 (6) $[M - CH_3O]^+$	385 (6) $[M - CH_3O]^+$	411 (<1) $[M - CH_3O]^+$	356 (3) $[M - C_2H_6O]^+$
192 (55) $[M - C_8H_{16}]^+$	192 (66) $[M - C_{12}H_{24}]^+$	192 (94) $[M - C_{16}H_{32}]^+$	192 (6) $[M - C_{16}H_{32}]^+$	178 (13) $[M - C_{16}H_{32}]^+$
175 (31) $[M - C_8H_{17}O]^+$	175 (38) $[M - C_{12}H_{25}O]^+$	175 (40) $[M - C_{16}H_{33}O]^+$	175 (9) $[M - C_{16}H_{33}O]^+$	160 (100) $[M - C_{16}H_{34}O]^+$
146 (93) $[M - C_9H_{18}O_2]^+$	146 (82) $[M - C_{13}H_{26}O_2]^+$	146 (68) $[M - C_{17}H_{34}O_2]^+$	146 (5) $[M - C_{17}H_{34}O_2]^+$	132 (53) $[M - C_{18}H_{38}O]^+$
Dialkyl Esters				
dioctyl 3,3′-thiodipropionate	didodecyl 3,3′-thiodipropionate[b]	dihexadecyl 3,3′-thiodipropionate[b] *m*/*z* (rel. %)	di-(*cis*-9-octadecenyl) 3,3′-thiodipropionate[b]	dihexadecyl 2,2′-thiodiacetate
402 (41) $[M]^+$	514 (12) $[M]^+$	626 (<1) $[M]^+$	678 (<1) $[M]^+$	598 (2) $[M]^+$
290 (69) $[M - C_8H_{16}]^+$	346 (40) $[M - C_{12}H_{24}]^+$	402 (3) $[M - C_{16}H_{32}]^+$	428 (<1) $[M - C_{18}H_{34}]^+$	375 (4) $[M - C_{16}H_{31}]^+$
273 (94) $[M - C_8H_{17}O]^+$	329 (51) $[M - C_{12}H_{25}O]^+$	385 (5) $[M - C_{16}H_{33}O]^+$	411 (1) $[M - C_{18}H_{35}O]^+$	356 (13) $[M - C_{16}H_{33}O]^+$
217 (24) $[M - C_{11}H_{21}O_2]^+$	273 (12) $[M - C_{15}H_{29}O_2]^+$	329 (2) $[M - C_{19}H_{37}O_2]^+$	355 (<1) $[M - C_{21}H_{39}O_2]^+$	
185 (11) $[M - C_{11}H_{21}O_2S]^+$	241 (18) $[M - C_{15}H_{29}O_2S]^+$	297 (5) $[M - C_{19}H_{37}O_2S]^+$	323 (<1) $[M - C_{21}H_{39}O_2S]^+$	
178 (100) $[M - C_{16}H_{32}]^+$	178 (81) $[M - C_{24}H_{48}]^+$	178 (15) $[M - C_{32}H_{64}]^+$	178 (2) $[M - C_{36}H_{68}]^+$	132 (100) $[M - C_{32}H_{66}O]^+$

[a] The various monoalkyl esters from reactions with 3,3′-thiodipropionic acid were reacted with diazomethane to form the corresponding methyl alkyl diesters for GC-MS analyses; ethyl hexadecyl 2,2′-thiodiacetate was formed in the reaction of diethyl 2,2′-thiodiacetate with 1-hexadecanol. [b] Base peak 55.

tively. The methyl hexadecyl 2,2′-thiodiacetate molecule also demonstrates the typical loss of hexadecyl and hexadecyloxy moieties at *m*/*z* 178 and 160, respectively. In addition, the various reaction products show peaks of the molecular ion and typical mass fragment ions of medium- and long-chain dialkyl esters of thia-alkanedioic acids. For example, two fragments at *m*/*z* 346 and 329 demonstrate the loss of the dodecyl and dodecyloxy moieties, respectively, from didodecyl 3,3′-thiodipropionate. The fragment *m*/*z* 178 is formed by the loss of two dodecyl moieties, and fragment *m*/*z* 241 indicates the cleavage of the thioether bond. As compared to dialkyl 3,3′-thiodipropionates, somewhat different fragmentation is found for dihexadecyl 2,2′-thiodiacetate showing the base peak at *m*/*z* 132 caused by the loss of both the hexadecyl and the hexadecyloxy moiety (**Table 2** and **Figure 4**). However, the ion at *m*/*z* 356, which is formed by the loss of a hexadecyloxy moiety, indicates similar fragmentation as found for dialkyl 3,3′-thiodipropionates.

The EI mass spectra of the medium- or long-chain mono- and dialkyl esters of both, 3,3′-thiodipropionic acid and 2,2′-thiodiacetic acid, show ions of the typical fragmentation of oxo-esters, which are formed by the loss of alkyl and alkoxy groups, respectively (**Table 2**). The cleavage of the C−O bond of the ester groups of the various mono- and dialkyl esters of 3,3′-thiodipropionic acid leads to ions that are important for the structural elucidation of these *O*-esters. The molecular ions and typical mass fragments of the reaction products of esterification and transesterification of thia-alkanedioic acids and their short-chain dialkyl esters, respectively, with 1-alkanols (**Table 2**) confirm the chemical structures of the reaction products as given in **Figure 4**. These data agree well with those given in the literature (*9*).

DISCUSSION

Recently, we have reported various enzyme-catalyzed reactions for the solvent-free preparation of food additives such as diacylglycerols and steryl esters by esterification and transesterification reactions in the absence of solvents using immobilized microbial lipases as biocatalysts and evacuation for the removal of water or short-chain alcohols (*10*−*12*). As an extension, the present work describes a simple and efficient method for the enzymatic preparation of didodecyl 3,3′-thiodipropionate and other dialkyl 3,3′-thiodipropionate antioxidants as well as dialkyl 2,2′-thiodiacetates by lipase-catalyzed esterification or transesterification as shown in **Figure 1**. Three immobilized lipase preparations were tested demonstrating the highest enzyme activity for lipase B from *C. antarctica* (Novozym 435) in both esterification and transesterification reactions (**Figure 2**), whereas lipases from *R. miehei* (Lipozyme RM IM) and *T. lanuginosus* (Lipozyme TL IM) were by far less active (**Table 1**). Particular preference for a specific 1-alkanol was not observed in the esterification of thiodipropionic acid with an equimolar mixture of 1-octanol, 1-dodecanol, and 1-hexadecanol catalyzed by Novozym 435. Concentrations of 3−6% Novozym 435 generally lead to high (92−98%) proportions of dialkyl 3,3′-thiodipropionates within 4 h (**Figure 3** and **Table 1**). Dialkyl 3,3′-thiodipropionates and dialkyl 2,2′-thiodiacetates are prepared in vacuo in the absence of solvents. The esterification and transesterification processes do not require any materials with deleterious effects on health and the environment.

GC and RP-HPLC analyses using commercial and synthetic standards for comparison as well as the data of mass spectrometry (**Table 2** and **Figure 4)** demonstrate that esterification of thia-alkanedioic acids or transesterification of their short-chain alkyl esters with medium- and long-chain alcohols proceeds as is shown in **Figure 1**. The purity of the final products, i.e., dioctyl, didodecyl, dihexadecyl, and di-(*cis*-9-octadecenyl) 3,3′-thiodipropionate as well as dihexadecyl 2,2′-thiodiacetate, was checked by RP-HPLC.

Dialkyl 3,3′-thiodipropionates are used as antioxidant stabilizers in synthetic polymers, e.g., in polyethylene films for food packaging (*4*, *13*), and migration of these antioxidants from polypropylene films into food has been observed (*5*). They may also serve as antioxidants for food applications—for example, fat spreads—as is considered by CAC and the U.S. Department of Agriculture (*1*, *2*). Similarly, dialkyl esters of 2,2′-thiodiacetic acid may serve as antioxidants for technical applications as well.

Existing data on various dialkyl 3,3′-thiodipropionates seem to adequately fulfill the Screening Information Data Set for environmental fate end points, ecotoxicity tests, and human health effects for differently substituted thiodipropionates such as didodecyl-, ditridecyl-, and dioctadecyl 3,3′-thiodipropionates (*14*). Reynolds et al. (*15*) described the fate of oral doses of didodecyl [1-^{14}C]-3,3′-thiodipropionate showing rapid elimination—mostly in the urine (≥85%)—of labeled 3,3′-thiodipropionic acid. Small proportions of radioactivity were detected in fat tissues as well, whereas radioactivity of all other organs and tissues was close to the background. Thus, metabolism and excretion of 3,3′-thiodipropionic acid seem to resemble in many respects to those of α,ω-alkanedioic acids after oral application. Similarly, 2,2′-thiodiacetic acid is known as an urinary biomarker of the cytochrome P450-catalyzed oxidation of various chemicals in the rat (*16*, *17*).

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