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ORIGINAL PAPER

Enantioselective synthesis of 3-hydroxytetradecanoic acid and its methyl ester enantiomers as new antioxidants and enzyme inhibitors

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Abstract Optically pure *R* and *S* enantiomers of 3-hydroxytetradecanoic acid and its methyl esters were synthesised by porcine pancreas lipase catalysed hydrolysis of racemic methyl 3-hydroxytetradecanoate in aqueous medium, with the aim of determining their antioxidant, antielastase and antiurease activities. The effects of the weight ratio of substrate/lipase and the reaction time were investigated. Optimum reaction conditions were determined. The resolution reaction with porcine pancreas lipase afforded (*R*)-3-hydroxytetradecanoic acid, which is a component of bacterially important lipid A, with greater than 99 % ee in excellent enantiomeric ratio (>900) after 7 h incubation with a substrate/lipase weight ratio of 3:1 and 43 % conversion of the substrate. Methyl (*S*)-3-hydroxytetradecanoate, which is the unreacted enantiomer of the racemic substrate, could be recovered with 98 % ee after 7 h resolution with a substrate/lipase weight ratio of 1:1 and 60 % conversion. (*R*)-3-Hydroxytetradecanoic acid was converted to its ester and the *S* methyl ester to its acid. This biocatalytic enantioselective resolution in aqueous medium presents an environmentally friendly and green chemistry method for the synthesis of *R* and *S* enantiomers of 3-hydroxytetradecanoic acid and its methyl esters.

Keywords Enzymes · Enantiomeric resolution · Lipid A · Enzymatic hydrolysis

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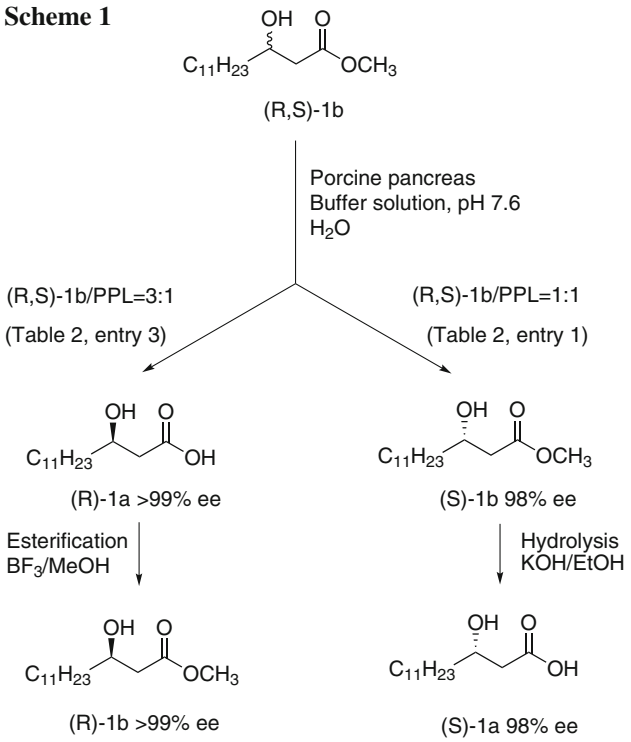
Introduction

Bacterial lipopolysaccharides (LPS) are major constituents of the outer membranes of cell walls in Gram-negative bacteria, and these molecules play an important role in the development of septic shock in humans [1, 2]. Lipid A bound to a polysaccharide portion is a glycolipid component of LPS. The lipid A is responsible for most of the endotoxic activities of LPS. Optically active (*R*)-3-hydroxytetradecanoic acid ((*R*)-**1a**) is the most common fatty acid constituent of lipid A. Fukase et al. [3] reported that the lipid A analogue with the *S* configuration was more strongly bioactive than the natural *R* type. The methyl esters of (*R*)- and (*S*)-3-hydroxytetradecanoic acids ((*S*)-**1b** and (*R*)-**1b**) are also valuable compounds in the synthesis of biologically active natural compounds [4, 5]. Therefore, many synthetic methods have been described for the synthesis of (*R*)-**1a**, (*S*)-**1a**, (*S*)-**1b** and (*R*)-**1b**, including synthesis from (*S*)-3-hydroxy-γ-butyrolactone [6] and (*S*)-epichlorohydrin [7] and asymmetric reduction by fermentation with baker's yeast [8] and use of modified nickel catalysts [9–11]. In our previous study [12], (*S*)-**1a** with 57 % ee was obtained by asymmetric reduction with chirally modified $NaBH_4$. One of the effective ways to produce these enantiomerically pure compounds consists of the enzymatic kinetic resolution of racemic mixtures. Bornscheur et al. [5] reported that the kinetic resolution of racemic 3-hydroxyesters by lipase-catalysed transesterification was achieved in different organic solvents. Optically pure 3-hydroxyalkanoates were obtained by enzymatic hydrolysis and enzymatic transesterification [4]. On the other hand, lipase-catalysed resolution of 3-hydroxytetradecanoic acid methyl ester in tetrahydrofuran using vinyl acetate gave the enantiomerically pure *R* acid, *R* ester and *S* acid [13].

This present study has two aims. One aim is to investigate the resolution of racemic methyl 3-hydroxytetradecanoate ((*R,S*)-**1b**) by porcine pancreas lipase (PPL) in the water phase (Scheme 1), because in the literature survey, no report was found describing the use of PPL in aqueous buffer solution for the synthesis of 3-hydroxytetradecanoic acid and its methyl ester enantiomers. The effects of reaction parameters such as concentration of substrate and enzyme, reaction time, conversion and enantioselectivity were considered. The optimised reaction conditions, including the most effective weight ratio of the substrate to enzyme and the reaction time for the lipase-catalysed hydrolysis of (*R,S*)-**1b** in aqueous medium, are reported.

The other aim of this study is to analyse the antioxidant, elastase and urease activities of the optically pure *R* and *S* enantiomers of 3-hydroxytetradecanoic acid and its methyl ester via synthesised in this work. According to the literature, no other study has examined the antioxidant, elastase and urease activities of *β*-hydroxy fatty acid isomers except our published study which reports the excellent antielastase, antiurease and antioxidant activities of racemic 3–13 monohydroxyeicosanoic acid isomers [14]. In recent years elastase, urease and antioxidant activities have gained great importance. Elastase inhibition is a protective tool against skin diseases and aging. Urease inhibition is of medical, agricultural and environmental significance. New antioxidants for foods, cosmetics and medicines are increasing too. No report exists on the antioxidant and enzyme inhibition activities of chirally pure *β*-hydroxytetradecanoic acid and its methyl ester isomers.

Scheme 1



Results and discussion

*Preparation of racemic methyl 3-hydroxytetradecanoate ((**R,S**)-1b)*

The starting material, racemic methyl 3-hydroxytetradecanoate, was synthesised using the acetoacetic ester and $NaBH_4$ methods [15] and characterised by FT-IR, ^1H NMR and elemental analyses. The spectroscopic data were identical to those of (*S*)-**1b** and (*R*)-(**1b**).

Choice of lipase species

Lipase is a useful catalyst for fatty acid derivatives, especially in enantioselective reactions [16]. In kinetic resolution of racemic compounds, the enzyme distinguishes between the two enantiomers of a racemic mixture, so that one enantiomer is readily transferred to the product faster than the other. PPL is an economic, commercially available and non-microbial enzyme that has high enantioselectivity. Many lipases react selectively with the *R* enantiomer [17–19]. The enzyme PPL chosen in this study hydrolysed selectively the *R* enantiomer making the formation of (*R*)-**1a** possible. The unreacted (*S*)-**1b** was recovered after purification by column chromatography with good to excellent optical purities. (*R*)-**1a** isolated (Table 1, entry 3) was esterified easily to (*R*)-**1b** by treatment with BF_3/MeOH. (*S*)-**1a** was obtained via chemical hydrolysis of the unreacted (*S*)-**1b** (Table 1, entry 1) (Scheme 1). The conditions in entry 3 of Table 2 were the best conditions to synthesize (*R*)-**1a** with greater than 99 % ee. The conditions in entry 1 of Table 2 can be used for the synthesis of enantiomerically pure (*S*)-**1b** (Scheme 1).

Effects of the substrate/lipase weight ratio and reaction time on the enantioselective enzymatic hydrolysis

(*R,S*)-**1b** was exposed to kinetic resolution using PPL (300 units/mg) as biocatalyst with various reaction times. pH was kept constant at 7.6 using citric acid/Na_2HPO_4 buffer solutions (0.02 M). The optimum temperature was set to 36 °C. Experiments were performed as described in Table 2 (entries 1–8) to determine the optimum weight ratio of the substrate to lipase and the reaction time. The resolution reaction with PPL was stopped via dichloromethane after the indicated reaction times shown Table 2. In entry 3, the reaction time was selected as 7 h. In order to optimize the reaction time, 7 h was halved to 3.5 h in entries 6 and 8 and then doubled to 14 h in entries 5 and 7. The lipase amount was held constant and the substrate/PPL weight ratio was changed.

Table 1 shows the results of the resolution entries, conversion (*X*) and enantiomeric ratio (*E*) in the course of hydrolysis of (*R,S*)-**1b** by PPL.

Table 1 Enantioselective resolution of (*R*,*S*)-**1b** with PPL

Entry	(*R*)-**1a** produced				(*S*)-**1b** recovered	
	$[\alpha]_D^{20}$ / ° dm^{-1} g^{-1} cm^3 (c = 1, $CHCl_3$)[a]	ee_p^b /%	Conversion (X)/% [20]	E [20]	$[\alpha]_D^{20}$ / ° dm^{-1} g^{-1} cm^3 (c = 1, $CHCl_3$)[c]	ee_s^b/%
1	−10	63	60	19	+18.1	98
2	−12	75	54	20	+16.3	88
3	−16	>99	43	>900	+14	76
4	−14.2	89	45	38	+13.5	73
5	−14.5	91	45	48	+13.8	75
6	−12.5	78	53	24	+16.5	89
7	−12.2	76	54	22	+16.4	89
8	−14.7	92	55	54	+13.9	75

E enantiomeric ratio

[a] $[\alpha]_D^{25} = -16.2$ (c = 1, $CHCl_3$) [8], $[\alpha]_D^{25} = -15.1$ (c = 1.06, $CHCl_3$) [13]

[b] Enantiomeric excess values (ee, %) were determined by chiral HPLC

[c] For methyl (*R*)-3-hydroxytetradecanoate $[\alpha]_D = -18.5$ (c = 1.05, $CHCl_3$) [8]

Table 2 PPL-catalysed hydrolysis of (*R*,*S*)-**1b** with varying weight ratios of substrate/PPL and reaction times

Entry[a]	Substrate/mg/ suspension/cm^3	PPL/mg/sus-pension/cm^3	Substrate/PPL weight ratio	Reaction time/h
1	1,000[b]:56[b]	1,000:56	1:1	7
2	1,000:28	500:28	2:1	7
3	1,000:18.67	333:18.67	3:1	7
4	1,000:9.33	166.6:9.33	6:1	7
5	1,000:18.67	333:18.67	3:1	14
6	1,000:18.67	333:18.67	3:1	3.5
7	1,000:9.33	166.6:9.33	6:1	14
8	1,000:9.33	166.6:9.33	6:1	3.5

[a] Methyl (*R*,*S*)-3-hydroxytetradecanoate (1,000 mg, 3.88 mmol) was used in these experiments (pH 7.6, temperature 36 °C)

[b] 1 cm^3 of lipase suspension, prepared from 1 g of lipase and 15 cm^3 of water, hydrolysed 50 mg trimyristine [21]

The best resolution for (*R*)-**1a** was entry 3. (*R*)-**1a** was synthesised with greater than 99 % ee in excellent enantioselectivity ($E > 900$) after 7 h incubation with a substrate/PPL weight ratio 3:1 and 43 % conversion of (*R*,*S*)-**1b** (entry 3). (*S*)-**1b** could be obtained in optically pure ratio only according to entry 1.

Conclusion

We have carried out an efficient enantioselective strategy to prepare pharmacologically valuable enantiomers of 3-hydroxytetradecanoic acid and its methyl esters via PPL-catalysed kinetic resolution of the corresponding racemic (*R*,*S*)-**1b** in aqueous medium. (*R*)-**1a** was synthesised with greater than 99 % ee in excellent enantioselectivity ($E > 900$). (*S*)-(**1b**) was obtained with 98 % ee.

The described process in this study is enantioselective, economical and convenient requiring only a facile separation by column chromatography. The desired four enantiomers (*R*)-**1a**, (*S*)-**1a**, (*S*)-**1b** and (*R*)-**1b** could be obtained without the need for additional crystallisations. This enzymatic route reduces the number of operations to two simple procedures: hydrolysis and separation. The use of PPL for this enzymatic resolution in an aqueous environment proved to be an excellent methodology for the preparation of these enantiopure bioactive products which will be analysed for their antioxidant, elastase and urease activities.

Experimental

PPL (porcine pancreas lipase (type II crude)) was supplied from Sigma Chemical Co. (St. Louis, USA). All reagents and solvents were of analytical grade and used as provided without further purification. The reactions were monitored using TLC plates (Merck 60 F-254). Column chromatography was performed on silica gel 60 (70–230 mesh). ^1H and ^{13}C NMR spectroscopy were carried out in $CDCl_3$ and recorded on a Varian 400 MHz spectrometer using TMS as an internal standard. Mass spectroscopy (ESI) was performed on a Thermo Finnigan spectrometer. A Mattson 1000 series spectrometer was used for IR spectroscopy. Optical rotations were measured on an Optical Activity AA-55 digital polarimeter and the results were reported from the averages of at least five measurements. Enantiomeric excesses (ee) of products were determined with a

Shimatsu\DGU-20A5 HPLC apparatus fitted with a Chiralcel OD Chiral column.

Chiral analysis

The enantiospecifity of the enzymatic reaction was determined after purification of the products (*R*)-**1a** and (*S*)-**1b** by column chromatography. The enantiomeric excesses of the products (*R*)-**1a** and (*S*)-**1b** were determined by chiral HPLC using a Chiralcel OD column. (*R*)-**1a** and (*S*)-**1a** did not give any signals on the Chiralcel OD column. On the other hand, their corresponding methyl esters (*R*)-**1b** and (*S*)-**1b** could be detected on the Chiralcel OD column. In order to determine the ee values by chiral HPLC the racemic substrate (*R,S*)-**1b** was separated on Chiralcel OD column into its enantiomers (*R*)-**1b** and (*S*)-**1b**. (*R*)-**1b** elutes before (*S*)-**1b**. (*R*)-**1a** was transformed to its corresponding methyl ester (*R*)-**1b** for measurement of its ee. Conversion (*X*) and enantiomeric ratio (enantioselectivity, *E*) were calculated according to Chen et al.'s equation [20]. The absolute configurations of the acid and ester were assigned by comparison of their optical rotations with the literature data. The results are summarised in Table 1.

General procedure for the enantioselective synthesis of 3-hydroxytetradecanoic acid and its methyl ester

Enzymatic hydrolyses were carried out using the conditions described in Table 2 varying the substrate/PPL weight ratios and reaction times (entries 1–8). Different suspension amounts of the racemic ester (*R,S*)-**1b** (1,000 mg, 3.88 mmol) were incubated with PPL (Sigma L-3126) in water at pH 7.6 (citric acid/Na_2HPO_4 buffer solutions (0.02 M)) and 36 °C. The enzyme was settled, unless the reaction mixture was stirred or shaken. Thus, the resulting suspension was shaken in an ultrasonic bath. The progress of the reaction was monitored by TLC (developing solvent $CHCl_3/CH_3OH/CH_3COOH = 100:10:1$). After the time shown in Table 2, the reaction was stopped with the addition of CH_2Cl_2. The enzyme was removed by vacuum filtration over Celite and washed with CH_2Cl_2. The organic phase was separated and washed with distilled water. After drying over anhydrous Na_2SO_4, the solvent was removed in vacuo to give a mixture of (*R*)-**1a** and (*S*)-**1b** that was separated via column chromatography (flash SiO_2, developing solvent $CHCl_3/CH_3OH/CH_3COOH = 100:10:1$).

*(R)-3-Hydroxytetradecanoic acid ((R)-***1a***, $C_{14}H_{28}O_3$)*
This compound was obtained as a white powder in greater than 99 % ee (Table 1, entry 3). M.p.: 71–72 °C (Ref. [13] 72.0–72.5 °C); $[\alpha]_D^{20} = -16/^\circ\ dm^{-1}\ g^{-1}\ cm^3$ ($c = 1$, $CHCl_3$). HPLC analysis: (*R*)-**1a** was converted to its corresponding methyl ester (*R*)-**1b**, and analysed on the Chiralcel OD column.

*Methyl (R)-3-hydroxytetradecanoate ((R)-***1b***, $C_{15}H_{30}O_3$)*
(*R*)-**1a** (100 mg, 0.407 mmol) was dissolved in 3 cm^3 methanol. Then 3 cm^3 BF_3/MeOH reagent was added, and the solutions were refluxed at 100 °C for 2 min. After the reaction was completed, excess alcohol was removed under vacuum at room temperature. Then 30 cm^3 petroleum ether and 20 cm^3 water were added, and the solution was shaken vigorously for 5 min before the layers were allowed to separate. The bottom layer was dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo to give (*R*)-**1b** as white crystals in 100 % yield (105 mg) and greater than 99 % ee. $[\alpha]_D^{20} = -18.4/^\circ\ dm^{-1}\ g^{-1}\ cm^3$ ($c = 1$, $CHCl_3$). HPLC analysis: Chiralcel OD chiral column, mobile phase *n*-hexane/2-propanol 98:2, flow rate 0.8 cm^3/min, wavelength 220 nm; t_R: 8.05 min for the (*R*)-isomer, not observed for the (*S*)-isomer. The spectroscopic data were identical to those of (*S*)-**1b**.

*Methyl (S)-3-hydroxytetradecanoate ((S)-***1b***, $C_{15}H_{30}O_3$)*
This compound was obtained as white crystals in 98 % ee (Table 1, entry 1). M.p.: 30–31 °C (Ref. [8] 28–30 °C); $[\alpha]_D^{20} = +18.1/^\circ\ dm^{-1}\ g^{-1}\ cm^3$ ($c = 1$, $CHCl_3$). HPLC analysis: Chiralcel OD chiral column, mobile phase *n*-hexane/2-propanol 98:2, flow rate 0.8 cm^3/min, wavelength 220 nm; t_R: 8.05 min for the (*R*)-isomer, 10.70 min for the (*S*)-isomer.

*(S)-3-Hydroxytetradecanoic acid ((S)-***1a***, $C_{14}H_{28}O_3$)*
(*S*)-**1b** (100 mg, 0.387 mmol), 0.09 g KOH in 1 cm^3 water and 1 cm^3 methanol were placed in a round-bottomed flask which was mounted over a magnetic stirrer and refluxed for 60 min. Unreacted ester, if any, was removed by ether extraction and the carboxylic acid was recovered by the methods given below. The aqueous portion was acidified to pH 2 with 6 N hydrochloric acid and extracted with ether (3×5 cm^3). The combined ether extracts were dried over Na_2SO_4 and concentrated on a rotary evaporator. The acid (*S*)-**1a** was obtained after drying under vacuum as a white powder in 80 % yield (76 mg) and 98 % ee (Table 1, entry 1). $[\alpha]_D^{20} = +16.1/^\circ\ dm^{-1}\ g^{-1}\ cm^3$ ($c = 1$, $CHCl_3$). It was analysed on Chiralcel OD column as its methyl ester (*S*)-**1b**. The spectroscopic data were identical to those of (*R*)-**1a**.

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