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Lipase-catalyzed Transesterification in organic Solvents: Preparation and Enantiodifferentiation of optically enriched 4(5)-alkylated 1,4(1,5)-olides

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Abstract: Porcine pancreatic lipase (PPL) catalyzed intramolecular transesterification of n-propyl esters of 4(5)-hydroxyalkanoic acids (C_5-C_{12}) in diethyl ether (20°C) yielded (S)-4-alkylated 1,4-olides of high optical purity (ee > 80%) and optically pure (R)-4-hydroxyalkanoic-n-propylesters, but exhibited low enantioselectivity for (S)-5-alkylated 1,5-olides (ee=10-20%). The chiral analysis of 4(5)-hydroxyalkanoic esters was performed by HRGC and HPLC after their derivatization with (R)-(+)-1-phenylethylisocyanate, (S)-O-acetyllactic acid chloride, and (R)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid chloride. The enantiodifferentiation of 1,4(1,5)-olides was achieved by HPLC on a chiral phase (ChiraSpher) using an on-line optical rotation detector (ChiraMonitor).

Introduction

4(5)-Alkylated 1,4(1,5)-olides are widely used as intermediates in the synthesis of natural products and are important, widespread flavour compounds[1-3]. Most of these substances are chiral compounds and their potential physiological activity, such as, e.g., odour or taste, depends on their absolute configuration[4,5]. Due to the importance of this class of chemicals, a large number of publications dealing with their stereoselective synthesis have been provided[6-8]. As an alternative, microbial reduction of 4(5)-keto acids with subsequent lactonization has been described[9-11]. Consequently, the use of enzymes in organic medium has also been reported. Recently, the intramolecular transesterification of 4-hydroxyalkanoic acid methyl esters using porcine pancreatic lipase (PPL) has been studied and found to be a suitable method to prepare 1,4-olides of high ee values[12]. This paper concerns the PPL catalyzed transesterification of n-propyl esters of 4(5)-hydroxyalkanoic acids ($C_{5,6}$-$C_{12,13}$) in organic solvents with respect to reactivity and enantioselectivity of lactone formation. In addition, the methods of chiral analysis of the hydroxyesters and lactones are described.

Experimental

Chemicals: The lipase from porcine pancreas (PPL, type II) was obtained from Sigma (Deisenhofen, Germany). The reactions were performed with the crude powder without further purification. The 4(5)-alkylated 1,4(1,5)-olides and all other commercial chemicals including solvents (redistilled before use) were purchased from Aldrich (Steinheim, Germany) and Roth (Karlsruhe, Germany). The 4- and 5-hydroxyalkanoic acid esters were prepared from the corresponding lactones (i) by alkylation of their silver salts[13], (ii) acidic alcoholysis[14], and (iii) alkylation of their potassium salts[5].

Transesterification: To a solution of 2.5 mmol of each (R,S)-4(5)-hydroxyalkanoic ester in 10 ml diethyl ether (reactions at room temperature) or n-hexane (reactions at 60 °C) 0.5 g PPL was added and the mixture continuously stirred. After defined reaction times, the enzyme was filtered off and the mixture analyzed by HRGC and HPLC.

Separation of Products: The solution obtained after filtration of the enzyme was evaporated under vacuum. The residue was subjected to preparative thin-layer-chromatography (glass plates coated with 1.25 mm silica gel 60 PF_{254}) using chloroform as eluent. In all cases the hydroxyesters (R_f = 0.2) and the lactones (R_f = 0.5) were recovered in highly pure forms (> 95% by HRGC).

Chiral analysis of Hydroxyalkanoic Acid Esters: (i) In a conical vial 1,5 µl hydroxyester was mixed with 3 µl R-1-phenylethylisocyanate (PEIC). After 2h incubation at 110°C, 0.5 ml methanol was added and the solution analyzed by HRGC. Temperature program: 140-300°C at 6°C/min; for 4-hydroxynonanoic ester: 180-300°C at 1°C/min. (ii) In a conical vial 1 µl hydroxyester was mixed with 10 µl CCl_4, 5 µl pyridin and 4 µl S-O-acetyllactateacide chloride (ALAC)[11,15]. After 3 h incubation at r.t., the solution was subjected for analysis by HRGC. Temperature program: 140-300°C at 2°C/min. (iii) In a conical vial 2 µl reduced 5-hydroxyester was mixed with 6 µl (R)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid chloride (MTPA-Cl) and 12 µl pyridine. After 1h incubation at 100°C, 0.5 ml methanol was added and the solution analyzed by HPLC. Reduction of the isolated 5-hydroxyester (50-150 mg) with $LiAlH_4$ (300 mg) was carried out in 20 ml diethyl ether. After refluxing for 2h, hydrolysis was performed by addition of 1 ml dist. water and 300 µl NaOH (15%). The precipitate was filtered off and the solvent evaporated under vacuum. The residue contained pure chiral 1,5-diols and n-propanol as confirmed by HRGC.

Capillary Gas Chromatography (HRGC): A Hewlett-Packard 5710A gas chromatograph with a Gerstel capillary injection (T=200°C)/detection (T=300°C) system was used. Split injection (1:50) was employed. The flow rates were 2.5 ml/min helium (carrier gas), 30 ml/min nitrogen (make-up gas), 30 ml/min hydrogen and 300 ml/min air (detector gases). A J&W fused silica DB-5 WCOT capillary column (30 m x 0.25 mm i.d.; d_f = 0.25 µm) was employed. The above-mentioned temperature programs were used. Product formation rate was determined using 2-octyl dodecanoate as internal standard. The temperature program for this was 4 min isothermal at 140°C, then 140-300°C at 10°C/min.

High Performance Liquid Chromatography (HPLC): A Knauer pump model 64 (Berlin, Germany) with an injection valve 7125 (Rheodyne; sample loop = 20 µl) and an UV-detector (Knauer, Berlin, Germany) with variable wavelength was employed. The analysis of the diastereomeric di-MTPA esters of the diols (cf. above) was performed at 254 nm by use of a silica gel column (250*4 mm, Knauer, Berlin, Germany) and n-hexane-diethyl ether (96+4) (3ml/min) as eluent. For the enantioseparation of the 1,4(1,5)-olides a ChiraSpher column (Merck, Darmstadt, Germany) and n-hexane - tert. butyl methyl ether (95+5) as eluent (1.2 ml/min) were

used[16,17]. The determination of the order of elution was performed employing an on-line optical rotation detector (ChiraMonitor, Zinsser, Frankfurt, Germany), which connected in series with the UV-detector. Injected amounts were 50 ng/enantiomer.

Results and discussion

Preparation of 4-alkylated 1,4-olides

Since 4-hydroxyalkanoic acids spontaneously lactonize, the PPL catalyzed intramolecular transesterification was carried out as outlined in Figure 1. The n-propyl esters were selected due to the following reasons: (i) with methyl and ethyl esters non-enzymatic relactonization was observed leading to a 10-20 % decrease of

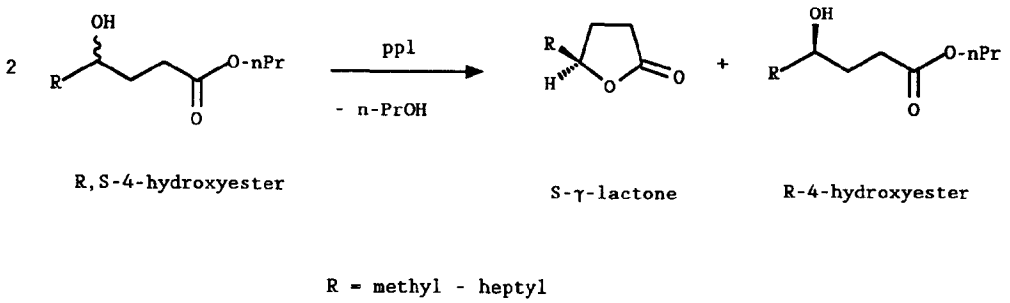

Figure 1. PPL catalyzed intramolecular transesterification of 4-hydroxyalkanoic acid n-propyl esters (scheme).

optical yield; (ii) the i-propyl esters were not accepted as substrates by the enzyme (controlled period, 10 days of reaction).

Among the methods described for the preparation of 4-hydroxyalkanoic acid n-propyl esters[5,13,14] the nucleophilic substitution of the potassium salts of the hydroxy acids on n-bromopropane in DMF was found to be most suitable; the homologues from C_5-C_{12} were obtained in 82-96 % yields. Using these esters the PPL catalyzed transesterification was carried out in diethyl ether at 20°C. The results obtained for the series of C_5-C_{12} hydroxy esters are summarized in Table 1. As a typical example, in Figure 2 the reaction profile of 4-hy-

Table 1. Results of the PPL catalyzed transesterification of the 4-hydroxyalkanoic acid n-propyl esters (4-C_5-4-C_{12}). (diethyl ether; T = 20°C; reaction time: 20 h)

ester	yield(%)[1]	%ee S-lactone	%ee R-ester
4-C_5	25.0	55.1	70.3
4-C_6	43.7	78.6	90.1
4-C_7	55.8	80.3	93.5
4-C_8	60.1	77.5	>98.0

continuation of table 1

4-C_9	71.2	60.8	>98.0
4-C_{10}	73.9	60.9	>98.0
4-C_{11}	76.5	58.3	>98.0
4-C_{12}	77.1	55.4	>98.0

[1] conversion yield

droxynonanoic acid n-propyl ester is outlined. As shown from Table 1 and Figure 2 the following conclusions can be drawn: (i) high optical yields of (S)-4-alkylated 1,4-olides (ee=80%) and 4-hydroxyalkanoic acid

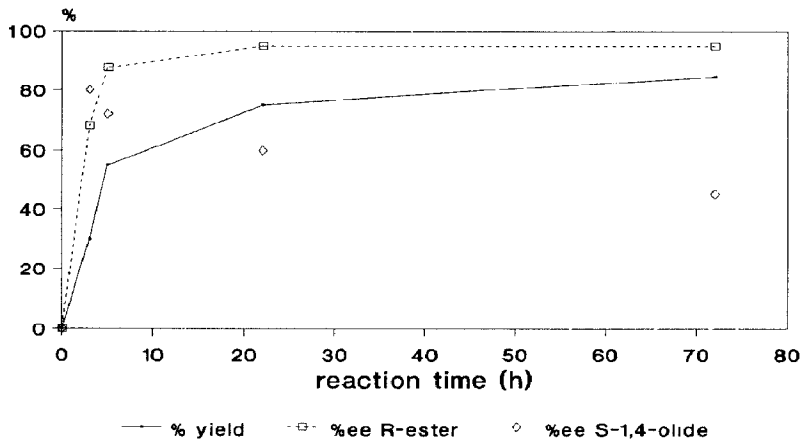

Figure 2. Reaction profile of the PPL catalyzed transesterification of 4-hydroxynonanoic acid n-propyl ester. (diethyl ether; T = 20°C).

propyl esters (ee > 98%) were obtained; (ii) fast reaction rates, in particular with the long-chain homologues, were observed; (iii) a decrease of ee value was observed after reaching the reaction maximum. In comparison with other PPL catalyzed reactions[18-20] the high reaction rates might be caused by the fact that the acyl acceptor (hydroxy function) does not need to diffuse to the acylated enzyme[21], but is *a priori* available. By fixation of the hydroxy function in the substrate and a potential formation of an enantioselective hydrogen bond in the active center of the acylated enzyme the extents of the mobilities are limited giving rise to the high optical yields of products. The above-mentioned decrease of ee values can be explained by non-enzymatic relactonization of 4-hydroxyesters. This effect was observed to be more pronounced on increasing the reaction temperature to 60 °C (experiments carried out in n-hexane).

Preparation of 5-alkylated 1,5-olides

The homologous series of 5-hydroxyalkanoic acid n-propyl esters of C_6-C_{13} was subjected to PPL catalyzed transesterification as outlined in Figure 3. The preparation of esters was achieved as mentioned above. In

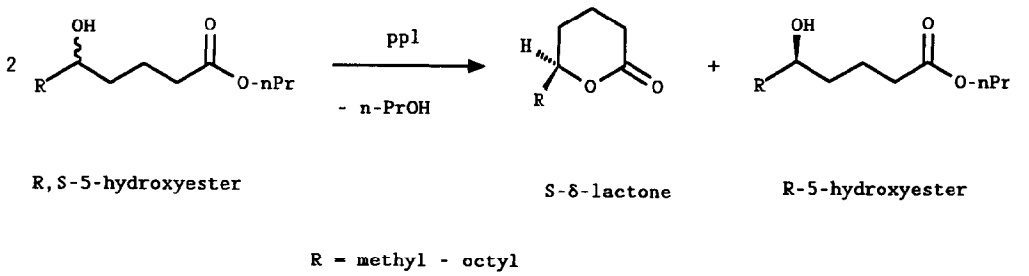

Figure 3. PPL catalyzed intramolecular transesterification of 5-hydroxyalkanoic acid n-propyl esters (scheme).

Table 2. Results of the PPL catalyzed transesterification of the 5-hydroxyalkanoic acid n-propyl esters (5-C_6 - 5-C_{13}). (diethyl ether; T = 20°C; reaction time: 5 days)

ester	yield(%)[1]	%ee S-lactone	%ee R-ester
5-C_6	12.0	10.1	10.3
5-C_7	12.7	10.6	11.1
5-C_8	13.8	10.3	13.5
5-C_9	13.1	11.5	13.0
5-C_{10}	15.2	13.8	13.1
5-C_{11}	15.9	13.9	14.1
5-C_{12}	17.5	15.3	14.8
5-C_{13}	18.1	17.4	14.4

[1] conversion yield

comparison to the 4-hydroxyalkanoic acid esters the reaction rates determined for the 5-hydroxyesters were four to five times lower. In addition, low enantioselectivity was observed (Table 2). As a typical example, in Figure 4 the reaction profile of 5-hydroxynonanoic acid n-propyl ester is outlined. Increase of temperature up to 60°C (experiments carried out in n-hexane) did not improve, therefore, the preparation of optically enriched 1,5-olides via transesterification of 5-hydroxyalkanoic acid n-propyl esters is strongly limited by the substrate specificity of PPL.

Chiral analysis

The chromatographic chiral analysis of the 4- and 5-hydroxyalkanoic acid esters was performed after their derivatization with optically pure reagents to diastereomers using an achiral stationary phase. The 4-hydroxyesters with chain lengths from C_5 to C_9 were separated as their 1-phenylethyl urethanes by HRGC (Table 3). The HRGC separation of long-chain homologues as well as the first three homologues of 5-hydroxyesters

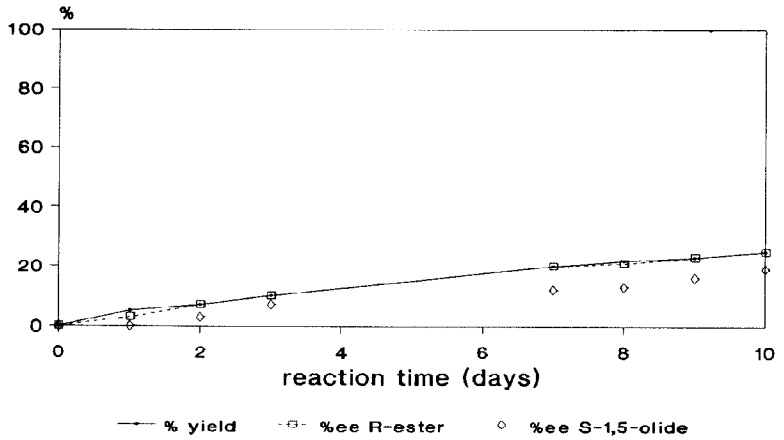

Figure 4. Reaction profile of the PPL catalyzed transesterification of 5-hydroxynonanoic acid n-propyl ester. (diethyl ether; T = 20°C)

Table 3. Chromatographic results of the diastereomeric (S)-(-)-1-phenylethyl urethanes (PEU) and the S-ALAC esters of the 4- and 5-hydroxyalkanoic acid n-propyl esters (4-C_5-4-C_{12} and 5-C_6-5-C_8) (cf. Experimental).

derivatives	t_1 (min)[1]	t_2 (min)	α[2]
4-C_5-PEU	18.49 (S)	18.73 (R)	1.013
4-C_6-PEU	19.46 (S)	19.65 (R)	1.010
4-C_7-PEU	20.60 (S)	20.71 (R)	1.005
4-C_8-PEU	21.81 (S)	21.90 (R)	1.004
4-C_9-PEU	48.08 (S)	48.39 (R)	1.006
4-C_{10}-ALAC	35.78 (S)	36.03 (R)	1.006
4-C_{11}-ALAC	39.86 (S)	40.06 (R)	1.005
4-C_{12}-ALAC	43.91 (S)	44.07 (R)	1.004
5-C_6-ALAC	21.92 (R)	22.33 (S)	1.022
5-C_7-ALAC	25.44 (R)	25.67 (S)	1.010
5-C_8-ALAC	28.73 (R)	28.90 (S)	1.007

[1] The order of elution was determined polarimetrically

[2] α = selectivity (= t_2/t_1)

(C_6 to C_8) was achieved using their diastereomeric ALAC esters (Table 3). In order to separate the long-chain 5-hydroxyalkanoic acid n-propyl esters (C_9-C_{13}) reduction to the 1,5-diols and their derivatization to diastereomeric di-MTPA esters were performed. As shown from Table 4, separation of these high-boiling

Table 4. Chromatographic results of the diastereomeric di-MTPA esters of the 1,5-diols (C_9-C_{13}) (cf. Experimental).

derivatives	t_1 (min)[1]	t_2 (min)	α[2]	R[3]
C_9	11.23 (S)	13.48 (R)	1.200	2.3
C_{10}	10.12 (S)	12.39 (R)	1.224	2.1
C_{11}	9.46 (S)	11.61 (R)	1.227	2.0
C_{12}	8.91 (S)	10.89 (R)	1.222	2.0
C_{13}	8.48 (S)	10.29 (R)	1.213	1.9

[1] The order of elution was determined polarimetrically

[2] α = selectivity (= t_2/t_1)

[3] R = resolution (= $(2(t_2-t_1))/(w_1+w_2)$; w = peak with)

derivatives was achieved by HPLC on silica gel. However, due to similar retention data, homologues could not be differentiated. The chiral analysis of 4(5)-alkylated 1,4(1,5)-olides was carried out by HPLC using a chiral polyacrylamide phase (ChiraSpher)[16,17]. The order of elution was determined using an on-line optical rotation detector (ChiraMonitor) connected in series to an UV-detector. In Table 5, the results of separation are summarized.

Table 5. Chromatographic results of the enantiodifferentiation of the 1,4(1,5)-olides by using ChiraSpher (cf. Experimental).

X,Y-olides	t_S(min)[1]	t_R(min)	k_S[2]	k_R	α[3]
1,4-C_5	19.86	22.30	7.27	8.29	1.140
1,4-C_6	13.77	16.12	4.74	5.72	1.206
1,4-C_7	10.66	12.59	3.48	4.29	1.233
1,4-C_8	9.52	11.71	2.97	3.88	1.230
1,4-C_9	9.01	11.48	2.75	3.78	1.275
1,4-C_{10}	8.56	10.58	2.58	3.43	1.328
1,4-C_{11}	8.21	10.30	2.47	3.35	1.355
1,4-C_{12}	7.97	10.06	2.33	3.21	1.377
1,5-C_6	22.35	26.67	8.72	10.60	1.215

continuation of table 5

1,5-C_7	14.93	17.90	5.44	6.72	1.234
1,5-C_8	13.16	15.31	4.68	5.61	1.200
1,5-C_9	11.54	13.42	3.96	4.77	1.205
1,5-C_{10}	10.33	12.06	3.44	4.17	1.214
1,5-C_{11}	9.53	11.18	3.13	3.84	1.227
1,5-C_{12}	9.22	10.93	2.96	3.70	1.250
1,5-C_{13}	8.86	10.57	2.81	3.54	1.261

[1] The order of the elution was determined polarimetrically

[2] k = capacity factor (= $(t-t_0)/t_0$)

[3] α = selectivity (= t_2/t_1)

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