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Enzymatic Preparation of Optically Active α- and β-Hydroxy Carboxylic Acid Derivatives

Liisa T. Kanerva* and Oskari Sundholm

Department of Chemistry, University of Turku, SF-20500 Turku, Finland

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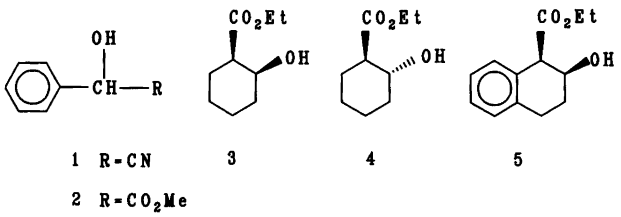
The lipase PS-catalysed resolutions of mandelonitrile (**1**) and methyl and ethyl hydroxy carboxylates **2–5** have been performed using $(PrCO)_2O$, $PrCO_2CH=CH_2$ and $PrCO_2CH_2CF_3$ as achiral reagents in THF and toluene. High enantioselectivity (e.e. close to 100%) was observed, especially when butyric anhydride was used as an acylating reagent for the secondary HO group of **2–5**. The acylation using $PrCO_2CH_2CF_3$ was impractical owing to the slow reaction rate. In the acylation of **1** enantioselectivity was only moderate.

Optically active α- and β-hydroxy carboxylic acid derivatives are chiral derivatizing agents and important building blocks of many biologically active compounds.[1,2] In the synthesis of optically active compounds, the value of biocatalytic methods has become increasingly recognized.[3,4] Thus, hydrolytic enzymes, especially lipases, are recommended for use in the kinetic resolution of racemic alcohols or carboxylic acids owing to their ability to catalyse many reactions with high enantioselectivity both in aqueous and non-aqueous solutions.

In organic solvents, the biocatalytic resolution of bifunctional α- and β-hydroxy carboxylic acids is, at least theoretically, possible using the hydroxy carboxylic acid itself or its alkyl ester as an acyl donor to an achiral nucleophile. However, the lipase-mediated esterification of a free carboxylic acid with alcohols produces water which has an unfavourable effect on the equilibrium of the reaction. Moreover, e.g., free mandelic acid seems to be an inhibitor of many lipases.[2] On the other hand, lipase-mediated transesterifications, such as the alcoholysis of alkyl α- or β-hydroxy carboxylates, proceed slowly and usually the resolution result is unsatisfactory owing to the reversibility of enzymatic reactions. Accordingly, for the lipase PS-catalysed transesterification between **3** or **4** and 1-hexanol in 2-methyl-2-butanol the enantiomeric excesses (e.e.) of the unchanged substrates and the corresponding hexyl esters obtained are of the order of 50% when the reactions stop within 7 days in an equilibrium position corresponding to approximately 50% conversion.[5] In this paper, we describe a gram-scale resolution of racemic α- and β-hydroxy carboxylic acids by the lipase-mediated acylation of the secondary hydroxy group of the methyl and ethyl carboxylates **2–5** and of mandelonitrile **1** using an activated ester ($PrCO_2CH_2CF_3$), enol ester ($PrCO_2CH=CH_2$) and carboxylic acid anhydride [$(PrCO)_2O$)] as acyl donors. Lipase PS from *Pseudomonas cepacia* was used as a catalyst throughout this work owing to its general usefulness in the preparation of optically active secondary alcohols and to its good stability in a wide variety of organic solvents.[6] THF and toluene were used as the solvents throughout this work.



Scheme 1.

Experimental

Carboxylic acid anhydrides, the enantiomers of methyl lactate and **2** as well as racemic **1** and **2** were products from Aldrich. Methyl mandelate (**2**) was recrystallized from cyclohexane before use. The compounds **3-5** and 2,2,2-trifluoroethyl butyrate were prepared as previously described.[5] Vinyl butyrate, which was distilled before use, was purchased from Tokyo Kasei Kogyo Co. The solvents were of the best analytical grade and were stored over molecular sieves (3 Å). Lipase PS (*Pseudomonas cepacia*) was from Amano Pharmaceuticals. It was used as such, except when the effect of immobilization on the enzymatic activity was tested. For that purpose, the enzyme was adsorbed on Celite (Sigma) as previously described.[7]

A solution of the acylating agent [0.2 M in the cases of $PrCO_2CH_2CF_3$ and $PrCO_2CH=CH_2$; 0.3 M in the case of $(PrCO)_2O$] and one of the nucleophiles **1–5** (0.1 M) in an organic solvent were added to a known amount of lipase PS (100 mg ml^{-1}). After sonication the mixture was shaken at room temperature throughout the course of the reaction. The reactions were stopped by filtering off the enzyme followed by the evaporation of the solvent. The products, the unchanged alcohol (**1–5**) and

the butyrate formed (**1a–5a**), were separated by flash chromatography.[8]

The progress of the acylations of **2–5** was followed by GLC equipped with a 25 m NB-30 capillary column. In the case of **1**, the unchanged enantiomer in the reaction sample was first derivatized with acetic anhydride before being injected into a gas chromatograph equipped with a Chiral Chromback CP-Cyclodextrin-β-2,3,6-M-19 column.[9] This method allowed us to obtain the extent of conversion and the e.e. values for the two enantiomers of **1** (one as an acetate and the other as a butyrate) simultaneously from one sample.

The enantiomeric excesses of the enantiomers of **2** were obtained by eqn. (1), where $[\alpha]_D^{20}$ (lit.)[10] for the (*R*)- and

$$\text{e.e.} = [\alpha]_D(\text{obs.})/[\alpha]_D(\text{lit.}) \quad (1)$$

(*S*)-enantiomers are −144 and 144 (*c* 1, MeOH), respectively. For that purpose the (*S*)-butyrate (**2a**) was first debutyrylated by acid-catalysed methanolysis. In the cases of **3** and **4**, both the recovered hydroxy carboxylic acid ethyl esters (**3** and **4**) and the corresponding butyrylated products (**3a** and **4a**) were subjected to acid-catalysed methanolysis prior to the e.e. determination. The enantiomeric methyl protons of the methyl esters thus obtained were determined by ^{1}H NMR spectroscopy (80 MHz) in the presence of Eu(hfc)$_3$ in $CDCl_3$ [δ 5.50 and 5.85 (3 H, singlets, CO_2Me, *cis*) and δ 4.90 and 5.25 (3 H, singlets, CO_2Me, *trans*)]. To check the validity of the ^{1}H NMR method, the specific rotations observed for the enantiomers of **3** and **4** (Table 1) were compared with the corresponding values of $[\alpha]_D$(lit.).[11–14] The acid-catalysed methanolyses of **5** and **5a** were not successful. In these cases, the enantiomeric methyl protons of ethyl carboxylate of **5** [δ 1.86–2.16 (3 H, triplets, $CO_2CH_2CH_3$)] and both the methylenic and methyl protons of **5a** [δ 4.67–4.97 (2 H, quartets, $CO_2CH_2CH_3$) and 1.50–1.72 (3 H, triplets, $OCOCH_2CH_2CH_3$] were determined by the ^{1}H NMR method.

Discussion

The lipase PS-catalysed acylation of the secondary HO group of α- and β-hydroxy carboxylic acid esters (**2–5**) is the method of choice to resolve racemic hydroxy carboxylic acids in organic solvents (Table 1). Thus, our results for the resolution of **2** in Table 1 are in a good agreement with the previous results for the lipase PS-catalysed acetylation of butyl mandelate with vinyl acetate in diisopropyl ether.[2] In that case, the e.e. value of >99% for the recovered butyl mandelate was obtained at 57% conversion (12 h, 40°C).

The lipase PS-catalysed deacylation of acylated **2–5** with 1-alcohols is another possible method of resolving **2–5**.[6] In that case, the alcohol product (**2–5**) formed does not work as a nucleophile in the reverse reaction with alkyl carboxylate mainly because of steric reasons. However, these deacylations are considerably slower than the corresponding acylation reactions. Thus, e.g., less than 10% of acetylated **2** is converted into methyl mandelate by 1-hexanolysis in toluene within 3 days. A similarly slow rate of the reaction was also observed for the PPL- or CCL-catalysed deacylation of *O*-acetylated butyl mandelate in diisopropyl ether.[15] Furthermore, the enantioselectivity in these deacylations is lower than in the corresponding acylation reactions.

From Table 1 it is evident that the lipase PS catalysed esterifications of **2–5** with butyric anhydride in anhydrous organic solvents is a good method of resolving racemic secondary alcohols with very high enantioselectivity; the acylation of **1** was achieved only with moderate selectivity. The non-enzymatic acylation is observed only in the reaction of **2** with butyric anhydride in THF. Butyric rather than acetic anhydride was used to suppress the rate of the non-enzymatic reactions. Moreover, the rate of enzymatic acylation is considerably higher for esterification with acid anhydrides than for transesterification with carboxylic acid esters. In particular 2,2,2-trifluoroethyl butyrate is a poor substrate for the present acylations. As is the case with most enzymes in organic solvents, the

Table 1. The lipase PS-catalysed resolution of **1–5** with various acylating agents in organic solvents.

					Recovered alcohol (**1–5**)				Produced ester (**1a–5a**)			
Substrate	Acylating agent	Solvent	*t*/h	Conversion(%)	Yield[a](%)	Isomer	e.e.(%)	$[\alpha]_D$	Yield[a](%)	Isomer	e.e.(%)	$[\alpha]_D$
1	$(PrCO)_2O$	THF	48	60	72	*R*	80	+33[c]	67	*S*	63	−4.9[c]
1	$(PrCO)_2O$	THF	48	51	80	*R*	70	+27[c]	93	*S*	68	−4.7[c]
1	$PrCO_2CH{=}CH_2$	THF	72	61	—	*R*	50	—	—	*S*	33	—
2	$(PrCO)_2O$	THF[b]	67	49	77	*R*	82	−118[d]	76	*S*	85	+101[d]
2	$(PrCO)_2O$	Toluene	30	54	56	*R*	98	−141[d]	87	*S*	87	+97[d]
2	$PrCO_2CH{=}CH_2$	Toluene	44	47	91	*R*	68	−97[d]	87	*S*	83	+94[d]
2	$PrCO_2CH_2CF_3$	Toluene	72	8	—	—	—	—	—	—	—	—
3	$(PrCO)_2O$	Toluene	21	49	79	1*R*,2*S*	87	+20[e]	84	1*S*,2*R*	>>95	−14[e]
3	$(PrCO)_2O$	THF	74	50	51	1*R*,2*S*	>>95	+23[e]	67	1*S*,2*R*	>>95	−16[e]
3	$PrCO_2CH{=}CH_2$	THF	92	50	—	1*R*,2*S*	>>95	+22[e]	88	1*S*,2*R*	>>95	−14[e]
3	$PrCO_2CH_2CF_3$	THF	168	43	66	1*R*,2*S*	68	+16[e]	65	1*S*,2*R*	>>95	−16[e]
4	$(PrCO)_2O$	THF	48	50	95	1*S*,2*S*	94	+43[e]	67	1*R*,2*R*	98	−34[e]
5	$(PrCO)_2O$	THF	48	50	70	1*R*,2*S*	>>95	−56[e]	75	1*S*,2*R*	>>95	+150[e]

[a]Yields of the isolated products calculated using the degree of conversion. [b]Non-enzymatic reaction observed. [c](*c* 5, $CHCl_3$, 20°C). [d](*c* 1, MeOH, 20°C). [e](*c* 1, $CHCl_3$, 25°C).

lipase PS-catalysed reactions take place faster in the more hydrophobic toluene than in THF.[6]

Bianchi *et al.*[7] were the first to use acid anhydrides as acyl donors and lipase PS immobilized on Celite as a catalyst for highly enantioselective esterifications of primary and secondary alcohols in benzene. For the resolution of **2** with butyric anhydride, the effect of immobilization was found to suppress the reaction rate considerably. In the case of *Candida cylindracea* lipase, on the other hand, the removal of the co-produced acid or the adsorption of the enzyme onto Celite seem to be essential for high selectivity when acid anhydrides are used whereas complete stereospecificity is obtained when vinyl acetate is the acyl donor.[16] However, in the case of lipase PS catalysis vinyl butyrate is not a significantly better acylating agent than butyric anhydride in that respect (Table 1). To support this, the values of the enantioselectivity ratio, v_o^R/v_o^S, were determined to be 8, 5 and 5 for the reactions of (*R*)- and (*S*)-methyl lactate and 270, 45 and 13 for those of (*R*)- and (*S*)-**2** with 2,2,2-trifluoroethyl and vinyl butyrates and butyric anhydride in toluene, respectively. According to these results, lipase PS is less sensitive to the acylating agent than is CCL. That is why lipase PS was used as received to obtain the results of Table 1.

Under basic conditions, cyanohydrins decompose easily to aldehydes and HCN whereas the corresponding acylated compounds are relatively stable. The acid-catalysed hydrolysis of an optically active cyanohydrin, on the other hand, affords the corresponding optically active α-hydroxy carboxylic acid.[17] Thus, it is possible, at least in theory, to prepare the stable acylated (*S*)-**1** in almost 100% yield through the lipase-catalysed acylation of **1** followed by *in-situ* racemization and re-use of the unacylated (*R*)-enantiomer.[18–20] The hydrolysis of (*S*)-**1** under acidic conditions should then result in **2**. However, the enantioselectivity results of the present work as well as the other available data[18–21] for the enzymatic acylation of **1** show the superiority of the direct enzymatic resolution of **2** over the chemoenzymatic route. On the other hand, the lipase-catalysed hydrolysis or alcoholysis of acylated **1** as well as the (*R*)- or (*S*)-oxynitrilase catalysed condensation of HCN with benzaldehyde in organic solvents is the method of choice for the preparation of optically pure (*R*)- or (*S*)-**1**.[17,21–23]

The acylations of **3–5** by lipase PS tend to stop at 50% conversion. At this stage the two enantiomers can be obtained with practically 100% optical purity (e.e. >>95% in Table 1 means that only one enantiomer was detected by the ^{1}H NMR method used). The previous resolutions using the lipase (*Pseudomonas fluorescence*)-catalysed hydrolysis of the acetylated hydroxy esters of **3** and **4** proceed slightly faster than the present acylation reactions, but only one of the enantiomers, the hydrolysis product, can be obtained optically pure.[24]

A rule based on the sizes of the substituents at the stereocentre has previously been formulated for the enantioselectivity of lipase PS.[25] According to this rule the enzyme should show preferred (*S*)-specificity in the acylations of **1** and **2** and (*R*)-specifity in the case of **3–5**. The results of Table 1 are in an excellent accordance with this proposal for the enantiomers of **1–4**. The application of this rule to **5** results in (1*R*,2*S*)- and (1*S*,2*R*)-configurations by lipase PS catalysis for the unchanged (−)-**5** and for its acylation product (+)-**5a**, respectively.

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