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Resolution of isopropylidene-glycerol benzoate by sequential enzymatic hydrolysis and preferential crystallization

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Abstract: Both enantiomers of isopropylidene-glycerol are prepared from its benzoate ester with enantiomeric excess ≥95% by combined partially stereoselective lipase-catalyzed hydrolysis and preferential crystallization. © 1997 Elsevier Science Ltd. All rights reserved.

(S)- and (R)-isopropylidene-glycerol **1** are useful starting materials for the preparation of enantiomerically pure biologically active compounds, such as glycerophospholipids, β-blockers, antihypertensive and antitussive drugs.[1,2]

Although both enantiomers of isopropylidene-glycerol are accessible starting from chiral pool compounds, such as (D)-mannitol,[3] (L)-ascorbic acid[4] and (L)-serine,[5] intensive attention has been paid to the resolution of (RS)-**1** using classical or enzymatic methods. However, the transesterification of (RS)-**1** as well as the hydrolysis of the corresponding esters, catalyzed by commercially available lipases, occur with low stereoselectivity and are not suitable for a preparative purpose.[6–8]

The only possible exception of this rule is the lipase AK-catalyzed acylation of (RS)-**1** using butyric anhydride which gives (S)-**1** with e.e.=75% and acylated (R)-**1** with e.e=98%.[9]

In a previous work, we reported a procedure to improve the enantioselectivity of lipase Amano PS in the hydrolysis of the benzoate ester (RS)-**2**, by using an organic cosolvent.[10] The highest selectivity was obtained in presence of hydrophilic cosolvents (log P<0). Using as a reaction medium a mixture of aqueous buffer and dioxane (75/25 v/v), at 70% of conversion, the unreacted ester (S)-**2** (corresponding to the R-form of isopropylidene-glycerol) was obtained with an enantiomeric excess higher than 93%. Unfortunately, due to the low enantioselectivity displayed by the lipase in the initial stage of the reaction, the more useful (S)-**1** could not be isolated in enantiomerically pure form, even stopping the hydrolysis at low conversion.

In this paper we describe a new procedure for the preparation of both the enantiomers of isopropylidene-glycerol, by partially stereoselective enzymatic hydrolysis of the benzoate (R,S)-**2** followed by a preferential crystallization of the resulting partially resolved ester (Figure 1). Preferential crystallization is widely used on industrial scale for example in the manufacture of chloramphenicol and α-methyl-L-dopa.[11] Usually this method is technically feasible only with racemates belonging to the class of conglomerates,[12] that consist of mechanical mixture of crystals of the two enantiomers.

After the synthesis of (S)-**2** starting from a commercially available sample of enantiomerically pure (S)-**1**, we found that the racemic benzoate ester is a solid (m.p.=37°C), while enantiomerically pure ester is an oil. The enantiomerically pure ester does not crystallize even at temperature below −40°C, and shows a markedly higher solubility in apolar solvents (e.g. hexane) with respect to the crystalline racemic form. This is an example of a racemate belonging to the class of racemic compounds,[13] which are characterized by a crystal form in which the two enantiomers coexist in the same unit cell.

If the crystallization of a partially resolved mixture of **2** is carried out by cooling a solution in hexane, the only crystals which appear are those of the racemic compound, while the enantiomeric enrichment takes place in the mother solution. This 'a priori' unfavourable situation could lead to an

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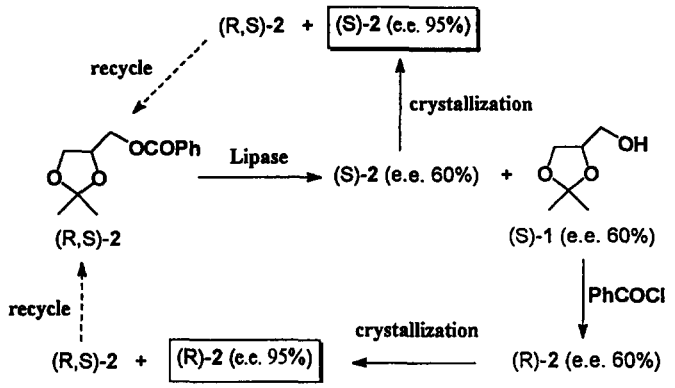
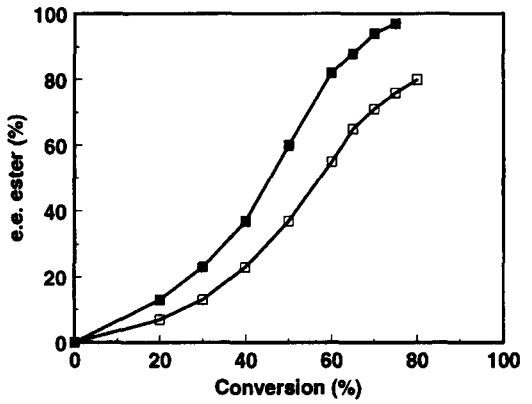


Figure 1. Resolution of (RS)-2 by combined enzymatic hydrolysis and preferential crystallization.



In a typical experiment a suspension of (RS)-2 (10 g, 42.3 mmol) and lipase Amano PS (0,7 g) in 250 ml of 10 mM phosphate buffer pH 7 (□), or phosphate buffer/dioxane 75/25 (v/v) (■) was stirred at 30°C. The pH was kept constant by continuous addition of aqueous sodium hydroxide using a pH-stat. The enantiomeric excess of (S)-2 was determined by HPLC analysis, performed on a chiral column Chiralcel OB (Daicel Chemical Industries, Ltd.) using hexane/*iso*-propanol 95/5 (v/v) as eluant.

Figure 2. Reaction profile for hydrolysis of (RS)-2 carried out in water/dioxane.

incomplete resolution, if the solubility equilibrium is reached. In the case of the ester **2** the difference in solubility is so high that an essentially quantitative separation of the racemic crystals can be achieved.

In the first step of the resolution process, the hydrolysis of the ester (RS)-**2** was carried out in phosphate buffer pH 7 additioned with dioxane (25% v/v), using as catalyst the lipase Amano PS from *Pseudomonas cepacia*. As shown in Figure 2, stopping the reaction at 50% of conversion, the enantiomeric excess of both the unreacted ester (S)-**2** and the produced alcohol (S)-**1** was 60%. It is worth noting that the hydrolysis, carried out in pure aqueous buffer, in the absence of organic cosolvent, afforded the same products with only 37% enantiomeric excess.

In the second step of the process, the partially resolved ester (S)-**2** (e.e.=60%) was dissolved in hexane and cooled at −20°C until complete crystallization of the racemic form (RS)-**2** (e.e.<4%). The remaining ester was quantitatively recovered from the mother solution with 62% yield and an enantiomeric excess higher than 95% (Table 1).

The preferential crystallization of the racemic compound was effective also starting from a poorly enriched solution of (S)-**2** (e.e.=30%) affording the enantiomerically pure ester with 30% yield. Similar results were obtained using different apolar solvents such as heptane and cyclohexane.

Table 1. Preferential crystallization of enantiomerically enriched (S)- or (R)-**2**

Crystallization solvent[a]	Starting ester e.e. (%)[b]	Soluble fraction e.e. (%)	Yield (%)	Precipitated fraction e.e. (%)	Yield (%)
n-hexane	60	95	62	4	38
n-hexane	30	95	30	3	70
n-heptane	60	93	60	7	40
cyclohexane	60	95	62	5	38

(**a**) The crystallization was carried out by cooling a solution of partially resolved (S)- or (R)-**2** (5 g) in the given solvent (30 ml) at -20°C. After 24 hours the precipitate was separated by the mother solution by fast filtration at -20°C. (**b**) (S)-(-)-**2** with e.e. = 95% corresponds to $[\alpha]_D^{25} = -8.2$ (c=1, $CHCl_3$).

In the same way, the partially resolved alcohol (S)-**1** (e.e.=60%) can be easily transformed into the corresponding ester by chemical acylation with benzoyl chloride, and subsequently recrystallized to give enantiomerically pure (R)-**2** (e.e.≥95).

The combined enzymatic hydrolysis and preferential crystallization allowed us to obtain both enantiomers of isopropylidene-glycerol with high enantiomeric excess, moreover, as shown in Figure 1, the racemic ester recovered from the precipitation (38% of the starting material) can be recycled as a substrate in the first step of the process.

In principle, the preferential crystallization of the benzoate can be used as a general method to improve the enantiomeric excess of the isopropylidene-glycerol obtained with only partial enantioselectivity also from different resolution procedures.

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