Resolution of a Chiral Ester by Lipase-Catalyzed Transesterification with Poly(ethylene glycol) in Organic Media

J. Shield Wallace, Kristin B. Reda, Mark E. Williams, and Cary J. Morrow*

Department of Chemistry, University of New Mexico, Albuquerque, New Mexico 87131

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Enantioselective transesterification of the *S* enantiomer from racemic 2,2,2-trichloroethyl 3,4-epoxybutanoate by poly(ethylene glycol) in warm diisopropyl ether media using porcine pancreas lipase as the catalyst is described. The two ester enantiomers were separated by cooling and filtering. The unchanged *R* enantiomer was shown to have an enantiomeric excess (ee) of ≥96% by its conversion to (*R*)-(−)-carnitine chloride. The *S* enantiomer of the ester was removed from the PEG by enzyme-catalyzed transesterification with methanol and the resulting methyl ester estimated to have ≥89% ee.

Introduction

The resolution of enantiomers by enantioselective hydrolysis with an enzyme has been exploited extensively for at least 15 years.[1-3] However, the recent discovery that many hydrolytic enzymes work well in low polarity organic solvents[4-8] has allowed enzymatic resolution to be extended to new reactions such as esterification[9-14] and transesterification.[4,9,10,12,15] Resolution by enzymatic transesterification solves some of the problems associated with the enzymatic hydrolyses such as the low solubility of many organic compounds in water, the difficulty of recovering the enzyme for reuse, and the requirement for adjusting the pH as the reaction progresses.[16] Moreover, an increase in enzyme stability has been reported when it is used in an organic solvent and higher reaction temperatures are tolerated.[5] The resolution may be carried out by having one enantiomer of a racemic ester be transesterified with an achiral alcohol,[9] or, more commonly, by having one enantiomer of a racemic alcohol transesterify an achiral ester.[4,9,10,12,15] While the latter case, which is useful for resolving alcohols, leads to the necessity for separating an ester from an alcohol, the former case, which is useful for resolving esters, leaves both enantiomers in the form of esters, and separation may require a difficult chromatography or a careful distillation.[9] This is probably a major reason for the very limited application of the method in the resolution of esters.

In attempting to improve the ease of separating the two esters, while retaining the advantages associated with resolution by enzyme-catalyzed transesterification in organic media, we sought to transesterify with an alcohol that would impart very different properties to the modified enantiomer. Our recent success in carrying out polymerizations using lipase-catalyzed transesterification of diesters by diols[17,18] suggested that the enzyme should be able to use a polymeric alcohol as the transesterifying substrate. The best candidate appeared to be a readily available, achiral, solid alcohol having low solubility in the reaction solvent, for the properties of the new ester arising from transesterification of one enantiomer of the racemic ester should be dominated by those of the polymeric alcohol moiety. The ester product, therefore, should also be insoluble and easily separable from the unchanged ester enantiomer by filtration. Isolation of the soluble unmodified enantiomer would then only require evaporation of the solvent while isolation of the polymer-bound enantiomer and recovery of the enzyme could be achieved by extracting the polymer into a solvent that is not detrimental to the enzyme and filtering. The ester could be cleaved from the polymer by enzymatic or chemical transesterification with a low molecular weight alcohol or by hydrolysis.

Results and Discussion

We have now shown that the *S* enantiomer of racemic 2,2,2-trichloroethyl (*R*,*S*)-3,4-epoxybutanoate (**1**) can be selectively transesterified by low molecular weight (∼1500) poly(ethylene glycols) (PEG) using porcine pancreas lipase (PPL) as the catalyst. The reaction requires about 5 h to consume one-half of the racemic ester at the 45 °C temperature required to keep the PEG as a molten phase in the diisopropyl ether solvent. An identical reaction mixture with the enzyme left out was allowed to stand for 5 h at 45 °C to prove that the transesterification requires the enzyme to be present. No trichloroethanol was observed to form by VPC analysis. Separation of the PEG ester **2** from the unchanged (*R*)-**1** is achieved by cooling the reaction mixture to 0 °C and filtering off the enzyme and the solidified **2**. As expected, the *R* enantiomer can be isolated in high yield and high enantiomeric excess by evaporating the solvent from the filtrate. The PEG ester **2** of the *S* enantiomer is recovered by extracting it into methylene chloride and filtering off the catalyst. The *S* ester could be removed from the PEG in 92% yield by converting it to the corresponding methyl ester, (*S*)-(−)-**3**, using PPL-catalyzed transesterification with methanol.

The structure and enantiomeric purity of the (*R*)-**1** was proven through its conversion to the well-known (−)-carnitine chloride (**4**) by enzymatic hydrolysis of the trichloroethyl ester with Amano P, a nonspecific lipoprotein

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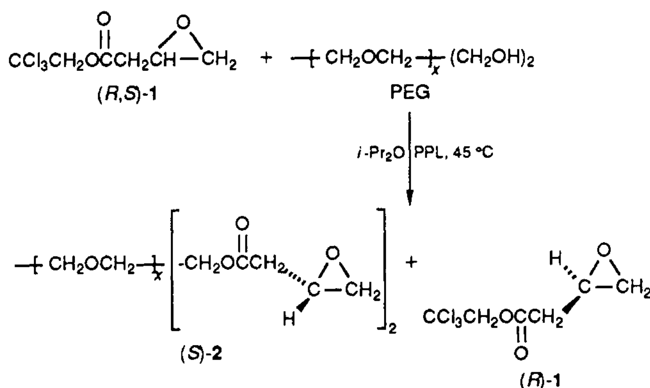
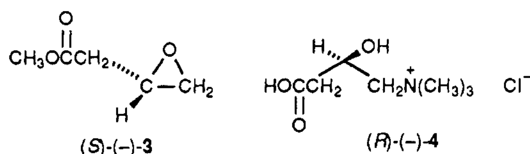


lipase from *Pseudomonas* species, followed by treatment with trimethylamine and acidification with HCl as described by Bianchi et al.[19] The carnitine chloride displayed $[\alpha]_D$ −22.9° corresponding to an enantiomeric excess of >96% by comparison with the literature[20] value of $[\alpha]_D$ −23.7° for the natural product.



It is noteworthy that resolution of 3,4-epoxybutyrate by enantioselective enzymatic hydrolysis of the methyl or other alkyl ester has recently been reported by Mohr, Rösslein, and Tamm[21,22] and by Bianchi et al.[19] In the former report, the methyl ester was hydrolyzed with pig liver esterase to give 40% recovery of unchanged *R* ester and a 30% yield of the *S* acid. An enantiomeric excess of 97% was reported for the *S* acid based on its conversion to (+)-γ-amino-β-hydroxybutyric acid. The authors established an ee of 82% for the unchanged methyl (*R*)-3,4-epoxybutyrate ($[\alpha]^{22}_D$ +10.67° (c = 1.8, $CHCl_3$)) by converting it to methyl (*S*)-3-hydroxypentanoate and comparing the specific rotation found with that reported for a sample of the enantiomer of the same material that had been shown to be optically pure by HPLC analysis of the (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetate derivative.[23] On the basis of a specific rotation of −10.67° indicating an ee of 82% for methyl (*S*)-3,4-epoxybutyrate ((*S*)-(−)-4), the rotation of −11.61° we observed for the (*S*)-(−)-4 that was isolated from the PEG by transesterification corresponds to an ee of 89% for that compound.

A survey of some 13 different enzyme preparations by Bianchi et al. led to the conclusion that PPL (called steapsin in the paper) provided the best enantioselectivity when alkyl (particularly butyl, isobutyl, and octyl) esters were hydrolyzed.[19] To achieve an ee of >95% for the unchanged *R* ester, it was necessary to hydrolyze 60–70% of the starting material. Of greater significance for the present work, hydrolysis of isobutyl 3,4-epoxybutanoate using Amano P as the catalyst occurs without stereoselectivity. We have confirmed that hydrolysis of 2,2,2-trichloroethyl (*R*,*S*)-3,4-epoxybutanoate ((*R*,*S*)-1) in the presence of Amano P also leads to the complete consumption of both enantiomers of the substrate after only 4 h, the same period as was required to hydrolyze (*R*)-1 during its conversion to carnitine chloride. Thus, the high enantiomeric purity of (*R*)-1 is attributable entirely to the selectivity of the PPL during the transesterification by PEG and not to a double resolution process involving the Amano P. Unfortunately, we have been unsuccessful in achieving hydrolysis or transesterification of (*S*)-2 except under enzymatic catalysis, a problem encountered previously by others.[19] Thus, the stereochemical purity reported here for (*S*)-3 may reflect the effect of a double resolution.

Extension of this method of resolution to other esters requires only (1) that the enzyme be selectively acylated by one enantiomer of a racemic ester, (2) that the acyl enzyme be able to transfer the acyl group to PEG, and (3) that acylation of the PEG not modify its solubility properties substantially. Previous reports on the resolution of chiral esters by enzyme-catalyzed hydrolysis (which should proceed through the same acyl enzyme intermediate as the transesterification) suggest that hydrolytic enzymes are able to select one enantiomer of a racemic ester, at least in aqueous media. However, these studies may be of limited use for predicting enantioselectivity in organic solvents. Moreover, much of the work using enzymes for enantioselective hydrolysis that has been reported to date has been carried out with pig liver esterase for which there is only limited experience working in nonaqueous systems. Exploration of the scope and limitations of the method introduced here requires that points 2 and 3 be addressed as well. The results of such studies will be the subject of future reports. A polymer suitable for the resolution of alcohols in an analogous process is also under development.

Experimental Section

2,2,2-Trichloroethyl 3-Butenoate. Following the general method of Hassner and Alexanian,[24] 8.130 g (95.0 mmol) of vinylacetic acid was dissolved in 50 mL of methylene chloride and treated successively with 14.34 g (96.0 mmol) of 2,2,2-trichloroethanol and 0.40 g (3.3 mmol) of 4-(dimethylamino)pyridine (DMAP). The mixture was then cooled to 0 °C and, after 15 min, 19.81 g (96.0 mmol) of dicyclohexylcarbodiimide (DCC) and 25 mL of methylene chloride were added with stirring. A white precipitate was observed to form immediately. When the DCC addition was complete, the mixture was allowed to warm to ambient temperature and remain there for 2 days. The precipitate was then filtered off and the filtrate was washed successively with three portions of a saturated solution of citric acid, two portions of saturated aqueous sodium bicarbonate, and one portion of saturated brine. After being dried over magnesium sulfate, the solvent was evaporated to give a light yellow oil. The oil was passed through a 4-in. column of silica (Merck Grade 60, 60 Å) eluting with methylene chloride to obtain 18.45 g (89.3%) of a colorless liquid that was shown by VPC to be >95% pure. ^1H NMR ($CDCl_3$): δ 3.25 (dt, J = 6.9, 1.4 Hz, 2 H), 4.76 (s, 2 H), 5.25 (m, 2 H), 5.95 (m, 1 H). ^{13}C NMR ($CDCl_3$): δ_C 169.8, 129.0, 119.3, 95.0, 73.9, 38.5.

2,2,2-Trichloroethyl (*R*,*S*)-3,4-Epoxybutanoate [(*R*,*S*)-1]. Following the general method of Dahill, Dorsky, and Easter,[25] 10.0 g of 2,2,2-trichloroethyl 3-butenoate was dissolved in 50 mL of methylene chloride and treated with 11.1 g (~1.4 equiv) of *m*-chloroperoxybenzoic acid in 100 mL of methylene chloride. The reaction was allowed to continue for 3 days at ambient temperature after which time VPC analysis showed the starting alkene to have been consumed. The mixture was then cooled in an ice bath and saturated sodium sulfite solution added with stirring until a KI/starch test was negative. The *m*-chlorobenzoic acid that precipitated during the reaction was filtered off and the filtrate was washed successively with three portions of the aqueous sodium sulfite solution, two portions of saturated aqueous sodium

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bicarbonate, and one portion of saturated brine. After being dried over magnesium sulfate, the solvent was evaporated and the oily product purified by distillation in vacuo, bp 65-70 °C/0.1 mm. ^{1}H NMR ($CDCl_3$): δ 2.59 (dd, J = 4.8, 2.6 Hz, 1 H), 2.72 (d, J = 5.79 Hz, 2 H), 2.86 (t, J = 4.4 Hz, 1 H), 3.35 (m, 1 H), 4.78 (s, 2 H). ^{13}C NMR ($CDCl_3$): δ_C 168.4, 94.6, 73.9, 47.3, 46.2, 37.5. Anal. Calcd for $C_6H_7Cl_3O_3$: C, 30.87; H, 3.02. Found: C, 31.53; H, 3.15.

Preparation of the Poly(ethylene glycol) Substrate. PEG of molecular weight 1300-1600 (Aldrich) was chosen because of its solubility and melting point characteristics. Very low molecular weight oligomers were removed by the following procedure. To 200 mL of anhydrous isopropyl ether under a dry nitrogen atmosphere was added 25 g of PEG. The mixture was stirred and warmed to ~45 °C. During the 2 h the mixture was held at this temperature, the polymer melted and formed a separate phase at the bottom of the reaction vessel. Upon cooling to 0 °C in an ice/water bath, the PEG solidified and some dissolved polymer precipitated. The solid PEG was fractured into a relatively fine powder by rapid stirring. The powder was recovered by filtration, washed with additional cold isopropyl ether, and dried under vacuum at room temperature. The procedure yielded 24.2 g of PEG.

Resolution of 2,2,2-Trichloroethyl (*R*,*S*)-3,4-Epoxybutanoate [(*R*,*S*)-1]. To 100 mL of anhydrous isopropyl ether in a 300-mL three-necked round-bottom flask equipped with a magnetic stirrer and a dry nitrogen inlet was added 16 g (11.0 mmol) of the previously prepared PEG. The mixture was heated to ~45 °C with stirring and then 4.0 g (17.1 mmol) of (*R*,*S*)-1 and 4.3 g of PPL (35% protein, activity = 35-70 units per mg, Sigma), which had been dried for 3 days in vacuo over phosphorus pentoxide as we have described elsewhere,[17] were added in rapid succession. After 4.5 h, VPC analysis indicated that 50% of the starting ester had been consumed and the reaction was stopped by rapidly cooling the reaction vessel in an ice/water bath. The mixture of PPL and esterified PEG was filtered from the cold mixture and washed with cold isopropyl ether. The filtrate was concentrated by evaporation to yield 1.72 g (86%) of 2,2,2-trichloroethyl (*R*)-(+)-3,4-epoxybutanoate [(*R*)-1], $[\alpha]^{25}_D$ +5.05° (c = 4, $CHCl_3$); the ^{1}H and ^{13}C NMR spectra were identical with those described above for the racemic material.

The recovered PEG was dissolved in CH_2Cl_2 and freed of the insoluble PPL by suction filtration using a fritted glass funnel. The recovery was 15.82 g (94.6%) of a solid. (The theoretical yield of acylated polymer was estimated as 16.73 g by assuming that the acyl portion from one half the starting (*R*,*S*)-1 becomes bound to the polymer. Alternatively, it may be estimated as 16.83 g by assuming the acyl portion of all unrecovered 1 became bound to the polymer. In this case, the recovery would be 94.0%.) ^{1}H NMR ($CDCl_3$): δ 3.62 (s) and 2.6 (br s) were strong absorptions arising from the PEG; δ 2.54 (ddd), 2.80 (t), 3.25 (m) comprise a weak set of absorptions from the 3,4-epoxybutyrate. There is no absorption near δ 4.8 for the methylene of a trichloroethyl ester.

Isolation of (*S*)-3,4-Epoxybutanoate as the Methyl Ester. To 100 mL of anhydrous isopropyl ether was added 14.5 g (9.4 mmol) of the recovered PEG and the mixture was stirred and heated to 50 °C. In rapid succession 8.25 g (250 mmol) of anhydrous methanol and 2.2 g of PPL were added to the mixture. After approximately 30 h, the reaction was stopped by cooling the reaction vessel in an ice/water bath; then the solidified PEG was collected by filtration and washed with cold isopropyl ether. The filtrate was concentrated by evaporation to yield 0.91 g (92%) of methyl (*S*)-(-)-3,4-epoxybutanoate; $[\alpha]^{20}_D$ -11.61° (c = 1.8, $CHCl_3$) [lit.[22] $[\alpha]^{r.t.}_D$ +10.67° (c = 1.8 $CHCl_3$) for the unchanged enantiomer from the enzymatic hydrolysis]; the proton NMR spectrum was identical with that described previously[21] and the TLC and VPC behaviors were identical with those of a racemic, authentic sample.

Conversion of 2,2,2-Trichloroethyl (*R*)-3,4-Epoxybutanoate [(*R*)-1] to (*R*)-(-)-Carnitine Chloride (3). 2,2,2-Trichloroethyl (*R*)-3,4-epoxybutanoate [(*R*)-1] (1.5 g, 6.42 mmol) was suspended in 15 mL of 0.1 M phosphate buffer that had been adjusted to pH 7.8. To this mixture was added 200 mg of the lipoprotein lipase Amano P from *Pseudomonas* sp. (AMANO Int'l Enzyme Co. Troy, VA), and the mixture stirred at ambient temperature while maintaining the pH near 7.5 by slow addition of 1 M aqueous NaOH. After ~4 h, the consumption of base ceased and the reaction mixture was extracted with 2 × 10 mL of methylene chloride to remove the trichloroethanol that had been freed. Following the method of Bianchi et al.,[19] the aqueous solution of (*R*)-3,4-epoxybutanoate was converted to (*R*)-(-)-carnitine chloride (4) in 72% yield, $[\alpha]^{25}_D$ -22.9° (c = 1, H_2O) (lit.[20] $[\alpha]^{25}_D$ -23.7°); mp 146 °C dec (lit.[20] 142 °C dec). ^{1}H NMR (D_2O): δ 2.51 (two dd, 2 H), 3.06 (s, 9 H), 3.34 (m, 2 H), 4.52 (m, 1 H). The enantiomeric excess as determined by comparison of the optical rotation with the literature value is 96.6%.

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Lipase-Catalyzed Preparation of Optically Active γ-Butyrolactones in Organic Solvents

Arie L. Gutman,* Kheir Zuobi, and Tamar Bravdo

Department of Chemistry, Technion-Israel Institute of Technology, Haifa, 32000, Israel

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Lipases in anhydrous organic solvents catalyze the lactonization of esters of γ-hydroxy carboxylic acids with a high degree of stereospecificity. Under these conditions the lipases exhibit both enantioselectivity and prochiral selectivity. We exploited the enzymes' enantioselectivity for synthesis of chiral lactones from racemic γ-hydroxy esters and their prochiral stereospecificity, i.e. the ability to discriminate between enantiotopic groups of a prochiral molecule, for the enantioconvergent lactonization of symmetrical γ-hydroxy diesters. This approach was used to develop a convenient, high-yielding, and stereoselective route to several optically active γ-substituted γ-butyrolactones.

Enzymes are widely exploited as catalysts in asymmetric synthesis and resolution.[1] It is now well established that hydrolytic enzymes such as lipases, esterases, and proteases can function also in organic solvents and can be used for certain types of transformations which are difficult or impossible to do in water.[2] The most common reactions

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