Enzymes in Organic Synthesis. Part 3.[1] Synthesis of Enantiomerically Pure Prostaglandin Intermediates by Enzyme-catalyzed Transesterification of (1*SR*,2*RS*,5*SR*,6*RS*)-Bicyclo[3.3.0]octane-2,6-diol with Trichloroethyl Acetate in an Organic Solvent

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Since the pancreatin-catalyzed esterification of the diol (**1**) with 2,2,2-trichloroethyl acetate in tetrahydrofuran is significantly slower than the esterification of the enantiomer *ent*-(**1**), the diacetate *ent*-(**3**) can be obtained from the racemic diol *rac*-(**1**) in a chemical yield of 30—35% and an enantiomeric excess (e.e.) of 97—>99%. When the remaining enantiomerically enriched diol (**1**) with an e.e. in the order of 55—68% was subjected once more to the pancreatin-catalyzed esterification its e.e. could be enhanced to 90%. Then acetylation and recrystallization afforded the diacetate (**3**) in a chemical overall yield of 37% and an e.e. of >99%.

The racemic diacetate *rac*-(**3**)[2] has proved to be a versatile starting material for the synthesis of racemic prostaglandins, such as 11-deoxy $PGF_{2\alpha}$, 11-deoxy PGE_2, PGA_2, PGE_2, and $PGF_{2\alpha}$.[3,4] Both enantiomers (**3**) and *ent*-(**3**) can be transformed by enantioconvergent routes into enantiomerically pure prostaglandins with natural configuration.[5] Therefore, the resolution of the diacetate *rac*-(**3**) or its precursor *rac*-(**1**) evoked our interest. First attempts in this direction were based on the enzyme-catalyzed hydrolysis of the diacetate *rac*-(**3**) with esterases of the coral *Plexaura homomalla* Esper.[6] In this way the diacetate (**3**) was obtained in a chemical yield of 36% and an e.e. of 88%, the monoacetate *ent*-(**2**) in a chemical yield of 41% and an e.e. of 41%, and the diol *ent*-(**1**) in a chemical yield of 15% and an e.e. of 82%. The low substrate concentration and the isolation problems prompted us to use recent experience in enzyme-catalyzed transesterification in organic solvents for the development of a more efficient resolution procedure.[7–10]

Results and Discussion

When the racemic diol *rac*-(**1**) was allowed to react at 23 °C for *ca.* 15 h in a mixture of dry tetrahydrofuran and triethylamine with an excess of 2,2,2-trichloroethyl acetate in the presence of pancreatin, the conversion into the diacetate *ent*-(**3**) reached *ca.* 35% as indicated by quantitative t.l.c. After removal of the enzyme by filtration and evaporation of solvents and reagents under reduced pressure the reaction product was separated by flash chromatography and subjected to Kugelrohr distillation. Thus the diacetate *ent*-(**3**) was obtained in a chemical yield of 30—35%† with an e.e. of 97—>99% and the unconverted diol (**1**) in a chemical yield of 50—60% with an e.e. in the order of 55—68%. The monoacetate *ent*-(**2**) was obtained only as by-product in a chemical yield of 2—4% with an e.e. of *ca.* 40%.

Performing the enzyme-catalyzed transesterification at 40 °C raised the reaction rate, but did not influence the chemical yield and the e.e. of the products.

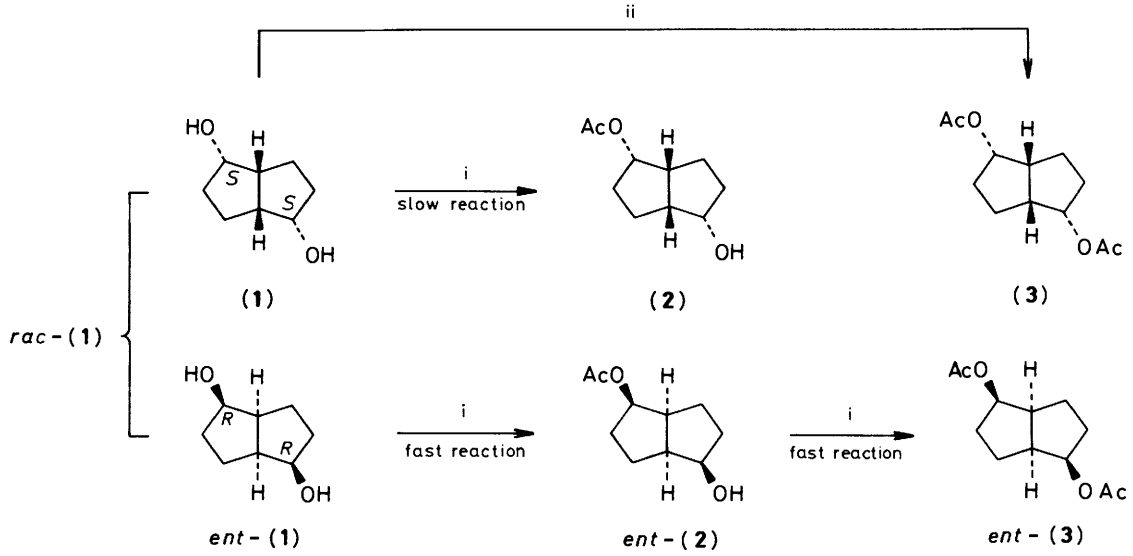
When the enantiomerically enriched diol (**1**) with an e.e. of 55—68% was subjected once more to the transesterification with 2,2,2-trichloroethyl acetate for 24 h at 23 °C it could be recovered in 75% chemical yield with an e.e. of 90%, together with 7% of the diacetate *ent*-(**3**) with an e.e. of 97%. Acetylation of the diol (**1**) with acetic anhydride in pyridine and recrystallization from hexane afforded the diacetate (**3**) with an e.e. of >99%. In this way the pancreatin-catalyzed transesterification of the racemic diol *rac*-(**1**) with 2,2,2-trichloroethyl acetate gives both diacetates (**3**) and *ent*-(**3**) in good chemical yield and very high enantiomeric purity. The enzyme catalyzes, with high enantioselectivity, the esterification of the (2*R*)- and (6*R*)-hydroxy group of the diol *ent*-(**1**), affecting the (2*S*)- and (6*S*)-hydroxy group of the diol (**1**) only to a minor extent. The rate for the esterification of the second (*R*)-hydroxy group of *ent*-(**1**) proved to be higher than for the first, only a trace of the monoacetate *ent*-(**2**) being detected during t.l.c. monitoring and isolated from the reaction mixture.

Compared with the previously described hydrolysis of *rac*-(**3**) with esterases from *Plexaura homomalla* Esper[6] the pancreatin-catalyzed transesterification of the diol *rac*-(**1**) with trichloroethyl acetate in an organic solvent has several advantages. Already without optimization of the reaction conditions the substrate concentration is much higher and isolation of the products as well as their separation are easier. Furthermore, both enantiomeric diacetates (**3**) and *ent*-(**3**) can be obtained in high enantiomeric purity.

Experimental

Tetrahydrofuran was dried with sodium wire. Triethylamine was distilled from, and stored over, potassium hydroxide. Pancreatin, qualified as 6 × NF, is a mixture of crude porcine pancreatic enzymes with protease, amylase, and lipase activities. The product purchased from Fa. Belger, Kleinmachnow, G.D.R., had a water content of 5.4% (Karl-Fischer-titration) and a lipase activity of 820 U/g (triolein as substrate). Quantitative t.l.c. was carried out on HPTLC plates (E. Merck) precoated with silica gel 60 using ethyl acetate–hexane (1:1) as solvent. For visualization the plates were treated with 5% sulphuric acid in ethanol and heated to *ca.* 150 °C. For the quantification of the spots the TLC-scanner Shimadzu CS-930 was used. Optical rotation was measured with the photoelectric

† All yields are related to the racemic or enantiomerically enriched starting material.



Scheme. Enantioselective enzyme-catalyzed esterification of the diol *rac*-(**1**). *Reagents and conditions:* i, $AcOCH_2CCl_3$, pancreatin, THF, NEt_3, 23 °C, 15.5 h and 40 °C, 10 h, respectively; ii, Ac_2O, Py, 23 °C, 12 h

polarimeter Polamat A (Carl Zeiss Jena) at 546 and 578 nm and extrapolated to 589 nm. The determination of the enantiomeric excess was further carried out on the basis of g.l.c. analysis of the diesters prepared from the diols (**1**) and *ent*-(**1**) with (+)-α-methoxy-α-trifluoromethylphenylacetic acid [(+)-Mosher-acid]. This analysis was performed on a fused silica capillary column (50 m × 0.25 mm) coated with OV-1. Differential scanning microcalorimetry (d.s.c.) was carried out on a DSC-1B (Perkin-Elmer). Flash chromatography was performed on silica gel 60 (0.063—0.04 mm) using hexane–ethyl acetate (9.5:0.5→2:3).

(1R,2S,5R,6S)-*Bicyclo*[3.3.0]*octane*-2,6-*diyl Diacetate* (**3**).—Triethylamine (0.1 ml, 0.7 mmol), 2,2,2-trichloroethyl acetate (1 ml, 7 mmol), and pancreatin (0.5 g) were added to a solution of the diol (**1**) (142 mg, 1 mmol) with an e.e. of 68% in tetrahydrofuran (2.5 ml). This enantiomerically enriched diol (**1**) was obtained as a co-product in the preparation of *ent*-(**3**) (see below). After being stirred at 23 °C for 24 h the suspension was filtered. The filter cake was washed with ethyl acetate (3 × 5 ml). The solvents and the excess of 2,2,2-trichloroethyl acetate were distilled off under reduced pressure and the residue was separated into two t.l.c. homogeneous fractions by flash chromatography followed by Kugelrohr distillation. The less polar fraction was the diacetate *ent*-(**3**) (16 mg, 7%), b.p. 100—110 °C (bath temp.)/27 Pa, m.p. 37.5—39 °C; $[\alpha]_D^{25}$ +110.2° (*c* 1.143 in $CHCl_3$); e.e. 97% (based on optical rotation).

The more polar fraction was the diol (**1**) (106 mg, 75%), b.p. 130—144 °C (bath temp.)/2.7 Pa; $[\alpha]_D^{25}$ +44.0° (*c* 2.122 in $CHCl_3$); e.e. 90% (based on optical rotation and g.l.c.).

The diacetate (**3**) (150 mg, 88%) was obtained by reaction of this product with acetic anhydride in pyridine at 23 °C for 12 h and recrystallization from hexane; m.p. 37.5—39 °C; $[\alpha]_D^{25}$ −113.1° (*c* 2.260 in $CHCl_3$); e.e. > 99% [based on g.l.c., lit.,[6] $[\alpha]_D^{25}$ −114° (*c* 2.0—2.6 in $CHCl_3$); e.e. 100%].

(1S,2R,5S,6R)-*Bicyclo*[3.3.0]*octane*-2,6-*diyl Diacetate* ent-(**3**).—(a) *Transesterification at* 23 °C. Triethylamine (1 ml, 7 mmol), 2,2,2-trichloroethyl acetate (10 ml, 70 mmol), and pancreatin (5 g) were added to a solution of *rac*-(**1**) (1.42 g, 10 mmol) in tetrahydrofuran (25 ml). After being stirred at 23 °C for 15.5 h the reaction mixture was worked up as described in the foregoing procedure. Flash chromatography afforded three t.l.c. homogeneous fractions, which were subjected to Kugelrohr distillation. The first eluated fraction was *ent*-(**3**) (723 mg, 32%), b.p. 100—110 °C (bath temp.)/27 Pa, m.p. 37.5—39°C (after spontaneous crystallization of the distillate); $[\alpha]_D^{25}$ +113.1° (*c* 2.065 in $CHCl_3$); e.e. >99% (based on g.l.c.) or 99.2% (based on differential scanning calorimetry). The fraction of medium polarity was the monoacetate *ent*-(**2**) (64 mg, 3.5%), b.p. 90—100 °C (bath temp.)/51 Pa; $[\alpha]_D^{25}$ +23.6° (*c* 2.036 in $CHCl_3$); e.e. 42% (based on optical rotation). The most polar fraction was the diol (**1**) (809 mg, 57%), b.p. 130—144 °C (bath temp.)/2.7 Pa; $[\alpha]_D^{25}$ +27.0° (*c* 2.087 in $CHCl_3$); e.e. 55% (based on optical rotation).

(b) *Transesterification at* 40 °C. Reaction of the diol *rac*-(**1**) (142 mg, 1 mmol) according to procedure (a) at 40 °C afforded after a reaction time of 10.5 h and work-up the diacetate *ent*-(**3**) (68 mg, 30%), m.p. 37.5—39 °C, $[\alpha]_D^{25}$ +113.1° (*c* 2.260 in $CHCl_3$), e.e. >99% (based on g.l.c.) and the diol (**1**) (78 mg, 55%), $[\alpha]_D^{25}$ +28.8° (*c* 2.005 in $CHCl_3$), e.e. 59% (based on optical rotation).

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