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Chemoenzymatic preparation of (2*S*,3*S*)- and (2*R*,3*R*)-2,3-butanediols and their esters from mixtures of *d*,*l*- and *meso*-diols

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Abstract—An efficient method of preparing the pure enantiomers of 2,3-butanediol from commercially available mixtures of the *d*,*l*- and *meso*-isomers was developed. It furnished (2*S*,3*S*)-2,3-butanediol with >99% e.e. and a >99.5/0.5 diastereomeric ratio and (2*R*,3*R*)-2,3-butanediol in 95% e.e. and >95/<5 diastereomeric ratio.

1. Introduction

The pure enantiomers of 2,3-butanediol are useful as auxiliaries and can serve as excellent building blocks in the asymmetric syntheses of chiral compounds containing two vicinal stereogenic centres. Both enantiomers are commercially available but rather expensive. In contrast, mixtures containing both the *meso*- and the *d*,*l*-isomers are inexpensive and readily available. However, there is a lack of efficient synthetic methodology for the synthesis of pure enantiomers of 2,3-butanediol from these mixtures.

One established method of preparing enantiopure 2.3-butanediols involves a multistep synthesis starting from the readily available enantiomers of tartaric acid.[1] The 2,3-butanediol enantiomers can also be obtained using various other routes. Chiral transition metal complexes have been used successfully as asymmetric catalysts in the reduction of acyloins to many enantiopure 1,2-diols, but they have not been used successfully to obtain (2*R*,3*R*)- or (2*S*,3*S*)-2,3-butanediol and the best e.e.s reported are about 70 and 62–89%, respectively.[2a] Asymmetric dihydroxylation of *trans*-2-butene gives (2*R*,3*R*)-2,3-butanediol with an e.e. of 72%.[2b]

When successful, biocatalytic routes are very attractive for the preparation of pure enantiomers. Fermentations of a number of carbohydrates provide one of the 2,3-butanediol enantiomers in excess, most usually (2*R*,3*R*)-butanediol is formed preferentially,[3] which is probably why this is less expensive than its (2*S*,3*S*)-enantiomer. A biocatalysed single step reductive procedure for the preparation of (2*S*,3*S*)-butanediol has recently been described, in which a butanedione cetyl reductase from *Bacillus stearothermophilus* is employed for the reduction of 2,3-butanedione, to provide the enantiopure diol in 40% yield.[4] However, this process has major drawbacks in that the reductase is not readily available and that it is a co-factor-dependent (NADH) enzyme.[4] The prescribed co-substrate used for recycling of the co-factor is *endo*-bicyclo[3.2.0]hept-2-en-6-ol, which is not commercially available but has to be prepared through a multistep procedure.[4]

The enzymatic resolution via esterification of C_2-symmetric diols such as *d*,*l*-2,3-butanediol with vinyl esters has been studied by several groups.[5–7] Thus, the three lipases, Amano PS (*Pseudomonas cepacia* lipase),[5,7] Novozym 435 (*Candida antarctica* lipase B)[6] and porcine pancreatic lipase (PPL),[5] all efficiently resolve the racemic diol. All react with (*R*)-enantiopreference. The *meso*-diol usually predominates in commercially available mixtures of *meso*- and *d*,*l*-2,3-butanediols. Unfortunately, because the *meso*-diol reacts at a similar rate as the preferred enantiomer of the *d*,*l*-diol in the first esterification step, obtaining the remaining (*S*,*S*)-diol enantiomer diastereomerically pure is difficult. However, at low conversions it is possible to obtain the pure (*R*,*R*)-diester.[5] A recently described hydrolytic reaction of the carbonate of the *d*,*l*-diol, catalysed by a whole-cell system (*Pseudomonas diminuta*), furnishes

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the remaining (*S*,*S*)-carbonate (45%, 95% e.e.).[8] However, this method has the drawback that the microorganism is not readily available but has to be grown directly before the reaction.

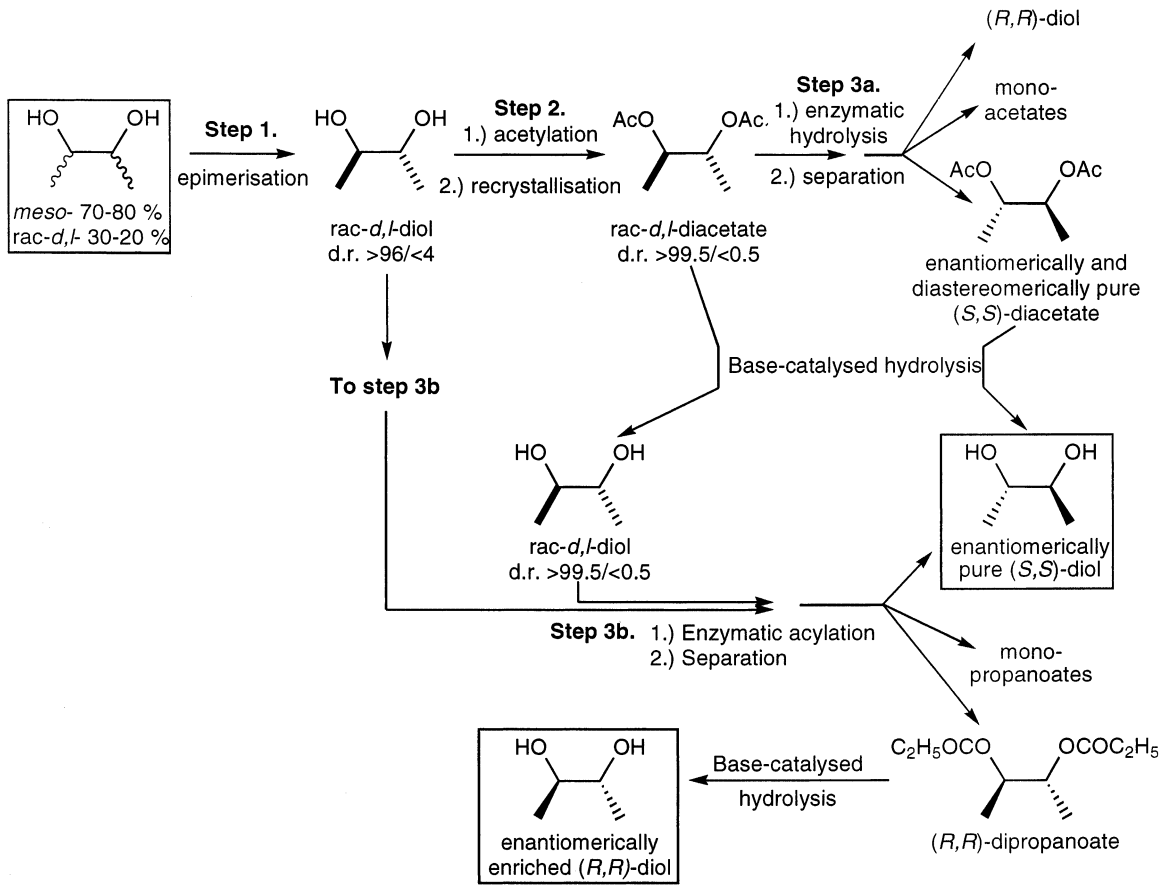
In view of the need for improvements in this area, we investigated the enantiosynthesis of 2,3-butanediols and report herein an efficient preparation of the highly pure 2,3-butanediol enantiomers. This route involved three key steps (Scheme 1) starting from mixtures containing both the *meso*- and *d*,*l*-diastereomers. When commercial mixtures of diastereomers of 2,3-butanediol were used as starting materials for preparing highly enantiomerically enriched isomers, the *meso*-isomer had to be either removed or epimerised. The latter option was preferred because a higher yield of diastereomerically pure *d*,*l*-diol would then be possible. Indeed, such an epimerisation has been described in the literature,[9] but has not found frequent use. As enantiomerically pure (2*S*,3*S*)-2,3-butanediol was more difficult to obtain than the other enantiomer, we concentrated on its preparation.

2. Results and discussion

The *meso*-isomer present in the starting commercial 2,3-butanediol mixtures was epimerised[9] to give a *d*,*l*-mixture (step 1, Scheme 1), which was then acetylated. Crystallisation of the resulting diacetate easily removed the residual *meso*-isomer (step 2, Scheme 1). The highly pure *d*,*l*-diacetate [>99.5/<0.5 diastereomeric ratio (d.r.)], or the equally pure *d*,*l*-diol derived from it, was subjected to enzymatic kinetic resolution by hydrolysis or esterification, respectively (steps 3a and 3b, Scheme 1). Both (2*R*,3*R*)- and (2*S*,3*S*)-butanediols were obtained in high e.e. (>99.5/0.5 *d*,*l*/*meso* d.r.). Furthermore, both *d*,*l*-2,3-butanediol of 99.5/0.5 d.r. and *meso*-2,3-butanediol with d.r. of 99/1 were obtained on a preparative scale.

2.1. Epimerisation of *meso*- to *d*,*l*-2,3-butanediol

A note in the literature describes the epimerisation of *meso*-2,3-butanediol to *d*,*l*-2,3-butanediol.[9] When we applied this method to a mixture of 2,3-butanediol containing a 37/63 d.r. of *d*,*l*/*meso*-forms, using one equivalent of sodium in refluxing toluene over 24 hours, the d.r. was reversed to >96/<4. In order to see whether the d.r. could be improved in the epimerisation reaction, we studied that reaction further, using various reagents and solvents. In the light of the proposed mechanism,[10] the addition of benzophenone could be expected to facilitate the reaction. However, no improvement in the yield or d.r. was observed. Neither the use of potassium nor that of *n*-butyllithium led to any improvement in the yield or d.r. The results are shown in Table 1.



Scheme 1. The strategy used for obtaining the enantiomers of 2,3-butanediol from a mixture of *meso*- and *d*,*l*-diols.

Table 1. Epimerisation of *meso*-2,3-butanediol

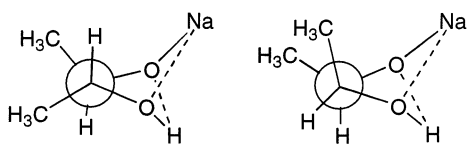
Reagent	Solvent	Time (h)	D.r.[a] (*d*,*l*/*meso*)
Na	Xylene	6	94/6
Na	Toluene	24	96/4
Na + 1% PhCOPh	Toluene	24	90/10
Na + 5% PhCOPh	Toluene	24	88/12
Butyllithium	Toluene	24	87/13
K	Toluene	48	46/54

[a] Diastereomeric ratios (d.r.) were determined by GC (see Section 4.1).

All reagent systems studied, except that with potassium, caused high degrees of epimerisation. The best reagent was sodium in the absence of additives. Thus the sodium in toluene system used in the original report[9] consistently gave the best d.r. of >94/<6. However, because of the higher boiling point of xylene, reaction in that solvent was found to be advantageous, giving considerably shorter reaction times with virtually no loss in the d.r. of the product.

Assuming that the diastereomeric ratios obtained in the reactions in toluene and xylene represented the equilibrium ratios between the *d*,*l*- and *meso*-monosodium salts, the energy difference between them should be around 2 kcal/mol in favour of the *d*,*l*-salt. We tentatively suggested the simplified chelated structures shown in Fig. 1 for these salts. An MM2 calculation using Chem3D Pro™ showed that such a *d*,*l*-salt should be more stable than the *meso*-salt by 0.8 kcal/mol (Fig. 1). Similar calculations on various model compounds showed that cyclic derivatives of the *d*,*l*-form were generally more stable than those derived from the *meso*-form. Thus the energy differences were, for example, 1.9 kcal/mol between the cyclic carbonates of 2,3-butanediol, 1 kcal/mol between the formaldehyde acetals and 1.8 kcal/mol between the acetone ketals.

Measurements of gas phase basicity by mass spectrometry has shown that mono-protonated *d*,*l*-2,3-butanediol is more stable than the mono-protonated *meso*-diol by approximately 0.3 kcal/mol.[11]



(**a**) 0 + 0.8 kcal / mol
(**b**) 0 + 2 kcal / mol
dl-salt *meso*-salt

Figure 1. Total energy difference between the monosodium salts: (**a**) derived from MM2 calculations and (**b**) estimated from the *d*,*l*- and *meso*-diol ratio obtained in the epimerisation reaction.

Table 2. Melting points of *meso*- and *d*,*l*-2,3-butanediols and of some of their derivatives

Entry	M.p. (°C)		Reference
	meso-	*d*,*l*-	
2,3-Butanediol	34.4	7.6	12
Diacetate	2.5–3.0	41.0–41.5	12
Dibenzoate	75.5–76.2	53.0–54.0	12
Benzophenone ketal	141–142	120–122	13
Dipropanoate	Oil	Oil	14
Dibutyrate	Oil	Oil	14

2.2. Improvement of the diastereomeric purity of *d*,*l*-2,3-butanediol obtained from epimerisation

Epimerisation reactions of commercial diol mixtures repeatedly furnished the *d*,*l*-diol in a *d*,*l*/*meso* diastereomeric ratio of >94/<6. This was clearly unsatisfactory, because highly diastereomerically and enantiomerically pure (*R*,*R*)- or (*S*,*S*)-diol was desired. Therefore we looked for a simple procedure that would eliminate the residual *meso*-diol from the *d*,*l*-2,3-butanediol samples. Because the *d*,*l*-2,3-butanediol had a lower melting point than its *meso*-isomer, the use of any crystallisation strategy involving the *d*,*l*-diol was precluded (see Table 2). However, this melting point difference could be utilised in the preparation of pure *meso*-2,3-butanediol by subjecting the mixture of *meso*- and *d*,*l*-2,3-butanediols (with d.r. of 85/15) to a freeze and filter sequence at 5°C. From this simple process *meso*-diol with d.r. of >99/<1 (*meso*/*d*,*l*) was obtained.

Among most of the known derivatives of *d*,*l*- and *meso*-2,3-butanediols, the latter have the higher melting points. Alternatively, one or both of the diastereomeric derivatives are liquids (see Table 2 for examples). One notable exception is the *d*,*l*-diacetate, which has a higher melting point than the *meso*-diacetate.

Acetylation of our epimerised *d*,*l*-diol samples of ≈95/5 d.r. with pyridine and acetic anhydride furnished the diacetate, which was recrystallised two or three times from pentane or light petroleum yielding the *d*,*l*-diacetate of >99.5/<0.5 d.r. The purified *d*,*l*-diacetate was hydrolysed in methanol with potassium carbonate to give the *d*,*l*-diol with a d.r. of >99.5/<0.5 in 78% yield. All of our attempts to further purify for example the *d*,*l*-dibenzoate (with d.r. of ≈95/5) by crystallisation failed.

2.3. Enzymatic resolution by acylation with vinyl propanoate

The results of earlier studies of enzyme-catalysed kinetic resolutions by transesterification of esters with 2,3-butanediol[5–7] and other 1,2-diols[15] or hydrolysis of their esters[5b,15] led us to study the effects of similar reactions on the pure *d*,*l*-diacetate (d.r. 99.5/0.5) as well as the pure *d*,*l*-diol samples prepared as above. In both the hydrolysis and esterification reactions, all of the enzymes examined catalysed the reactions of the (*R*,*R*)-

enantiomers more strongly than those of the (*S*,*S*)-enantiomers. The reactions studied were all sequential kinetic resolutions (Schemes 2 and 3). If the final product was the desired one, a major advantage was that the enantioselectivity of the two steps reinforced each other. The total E value, E_T, was usually high, because $E_T = 0.5 \cdot E_1 \cdot E_2$ (where E_1 and E_2 are the enantioselectivities in each sequential step).[16] Simultaneously, the remaining trace of achiral *meso*-diol or *meso*-diester should be converted to its chiral monoester. Thus, provided that the conversion of the starting material was higher than 50%, the diastereomeric purity of the slow-reacting substrate, either the (*S*,*S*)-diol or (*S*,*S*)-diester, would not become lower than that of the starting material.

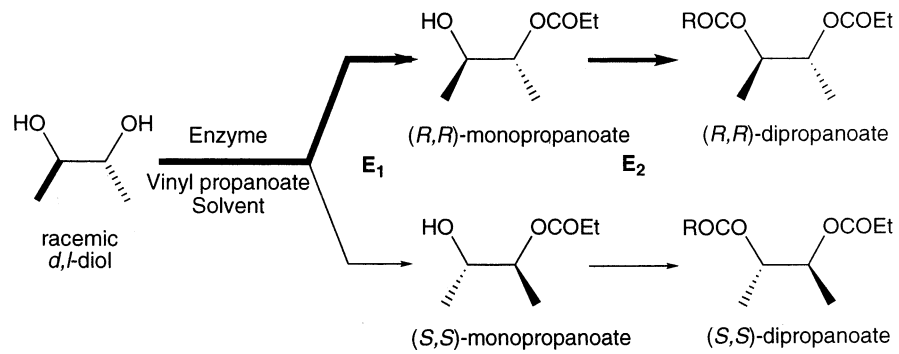
When working on the esterification reaction, we wanted to employ a simple work-up procedure without chromatographic separation. As both the mono- and diacetates of 2,3-butanediol are moderately water-soluble, we decided to use a more lipophilic acyl donor than vinyl acetate. As long chain vinyl esters were rather expensive, we chose vinyl propanoate as a compromise between price and lipophilicity. The results of earlier studies[5–7] led us to select three enzymes as catalysts for the transesterification, PCL, CALB and PPL.

The results of the transesterifications of vinyl propanoate with the racemic diol are summarised in Table 3. The progress curves of PCL- and PPL-catalysed reactions are shown in Fig. 2. The symbols represent experimental data and the lines represent theoretical curves calculated using the SeKiRe program.[17]

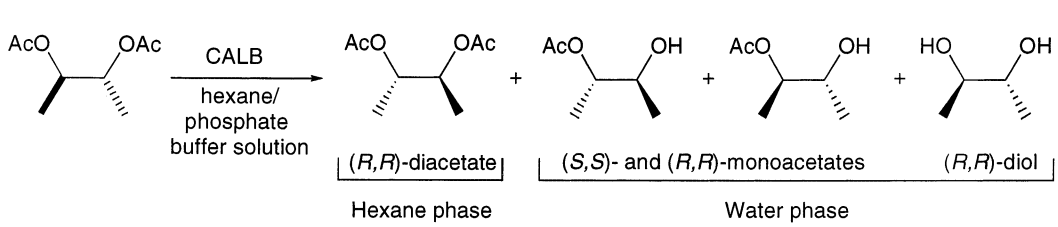
Judging the data collected from small-scale experiments, we found PPL to be the best catalyst when the remaining substrate, the (*S*,*S*)-diol, was the desired product. The second step of the reaction with this catalyst was much slower than the first one (Fig. 2b). Therefore we considered this two-step reaction as being a one-step reaction, i.e. $E \approx E_1$. We found that $E_1 \approx 40$. An advantage of using vinyl propanoate instead of vinyl acetate was that the second step seemed to be considerably slower than when vinyl acetate was used.[5] A conversion of >55% of the substrate was thought to leave the remaining substrate in high enantiomeric and diastereomeric purity. In order to obtain the pure remaining (*S*,*S*)-diol when using PPL, PCL and CALB, the reactions were stopped at 57, 75 and 85% conversion, respectively (based on the conversion of the substrate, the *d*,*l*-diol). In a reaction performed on a large scale, one could expect to obtain the highest isolated yield (≈35–40%) in that catalysed by PPL. However, in this case, it was difficult to push the conversion of the diol to more than 52–53% due to the extremely long reaction times. The e.e. of the remaining diol in the large-scale PPL reactions was ≈96% and the d.r. was unchanged in relation to the starting material. However, when starting from *d*,*l*-diol with a d.r. of 96/4 and using lipase PS on a large scale, highly pure remaining diol (≈20%, >99.5% e.e., >98/<2 d.r. from *d*,*l*-diol, ≈96/4 d.r.) was obtained (see Fig. 2a).

After removal of the enzyme by filtration, the remaining substrate was isolated by aqueous extraction of the TBME solution, followed by back extraction of the aqueous extract with dichloromethane to remove trace monopropanoate, allowing isolation of the pure (*S*,*S*)-diol.

Because of the slow rate of reaction of PPL in the second step, the (*R*,*R*)-diester was also most conveniently prepared using PCL as the catalyst (Fig. 2a). At 75% conversion of the starting *d*,*l*-diol and after removal of the enzyme, aqueous extraction of the product mixture removed the remaining diol. The (*R*,*R*)-dipropanoate with e.e. of 95% was obtained in



Scheme 2. Sequential enzyme-catalysed kinetic resolution of racemic *d*,*l*-2,3-butanediol by transesterification of vinyl propanoate.



Scheme 3. Lipase-catalysed hydrolysis of *d*,*l*-2,3-diacetoxybutane.

Table 3. Results of small-scale screening experiments[a] with sequential enzyme-catalysed kinetic resolution of *d,l*-2,3-butanediol by transesterification of vinyl propanoate in TBME at ambient temperature

Enzyme	Time (h)	c_S (%)[b]	c_P (%)[c]	E.e.[d] (%)	Yield[e] (%)	E_1[f]	E_2[f]	$E_{T(max)}$	D.r.[g]
PCL	50	75	38	>99	≈20	13	26	166	>99/<1
PPL	150	57	7	>98	≈35	≈40	≈200	>1000	>98/<2
CALB	7.5	85	22	97	n.d.	≈4	≈10	21	n.d.

[a] *d,l*-2,3-Butanediol (1–10 mmol, ≈95/5 d.r.) was acylated with vinyl propanoate in *tert*-butyl methyl ether as described in Section 4.8, scaled down to fit the appropriate amount of starting diol. Work-up was by chromatography as described in Section 4.8.
[b] Conversion of starting material: c_S=(converted diol)/(initial diol).
[c] Conversion to final product: c_P=(dipropanoate)/[(remaining diol)+(monoprop.)+(diprop.)].
[d] Refers to remaining substrate.
[e] Yield of remaining substrate based on the starting *d,l*-diol.
[f] *E* values were estimated fitting the experimental data with those generated by the program SeKiRe.[17]
[g] Diastereomeric ratio of the remaining diol substrate.

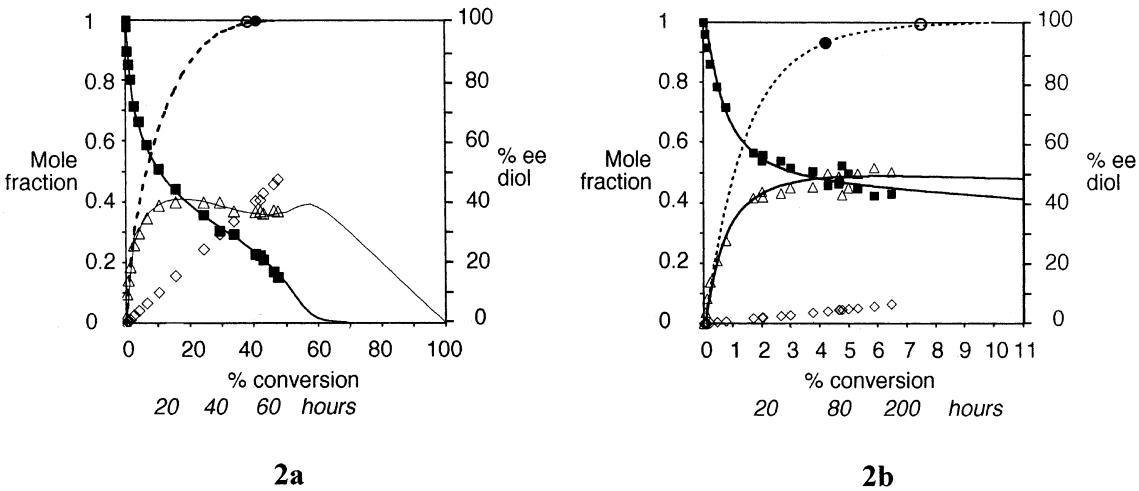


Figure 2. Progress curves of enzyme-catalysed esterification of *d,l*-2,3-butanediol with vinyl propanoate. Conversion c=[dipropanoate]/([diol]+[monopropanoate]+[dipropionate]). Symbols: ■, mole fraction of diol; △, mole fraction of monopropanoate; ◇, mole fraction of dipropionate. Solid lines represent calculated theoretical mole fraction progress curves for the two former compounds. Symbols ○ and ●: e.e. of diol from small- and large-scale experiments, respectively; - - - calculated, theoretical e.e. curve of (*S,S*)-diol. (**a**) PCL reactions. (**b**) PPL reactions.

approximately 40% yield after column chromatography. Product with higher e.e. could be obtained by stopping the esterification at a lower conversion.

2.4. Enzymatic resolution by hydrolysis

An alternative to kinetic resolution by acylation of the *d,l*-diol was kinetic resolution by hydrolysis of the diacetate. As described above, the diastereomerically pure diacetate of *d,l*-2,3-butanediol was easily obtained. The *d,l*-diacetate being crystalline at room temperature, we decided to study the hydrolytic reaction in two solvent systems (Scheme 3).

Kinetic resolution of some 3,4-unsaturated 1,2-diacetates by CALB-catalysed hydrolysis has been investigated previously.[18] The best results were obtained using water containing 25% methanol, which gave both the diacetates, and the corresponding monoacetates in high e.e.

We decided to study the enzyme-catalysed hydrolysis of the diacetate of *d,l*-2,3-butanediol in both a 25% aqueous methanol system and a biphasic solvent system consisting of hexane/water 2/1. The results of the hydrolytic reactions are shown in Table 4. When comparing 25% methanol with the hexane/water two-phase system, we found the latter to be more efficient in terms of reaction time and work-up procedure (see Scheme 3). Although PPL gave a good E_1 value in the reaction of vinyl propanoate with pure *d,l*-diol (vide supra), it was only moderately enantioselective in the hydrolysis of the *d,l*-diacetate (E=7). When CALB or PCL was used, higher enantioselectivity was obtained: E=40 and E=39, respectively (in the first hydrolysis step of the diacetate). However, CALB reacted faster than PCL. When CALB was used, the reaction was stopped at 57% conversion of the diacetate. GC analysis showed that neither the diol nor the monoacetate was present in the hexane phase. Thus the work-up procedure was very simple, consisting of only two or three hexane extractions of the water phase. This extraction leaves the diacetate as the sole solute present in the hexane phase and evaporation, followed by recrystallisation, furnished the diacetate in approximately 34% yield based on the racemic substrate.

Table 4. Lipase-catalysed hydrolysis of *d*,*l*-2,3-diacetoxybutane

Entry	Lipase	Solvent	Time (h)	Conversion[a] (%)	E.e.[b] (%)	E_1[c]
1	CALB	25% MeOH (aq.)	6	57	99.9	≈100
2	CALB	Hexane/H_2O	4	57.7	99.8	40
3	PCL	Hexane/H_2O	24	56.1	98.95	39
4	PPL	Hexane/H_2O	200	67	47.5	7

[a] Conversion = converted starting material/initial starting material.
[b] Enantiomeric excess of the remaining (*S*,*S*)-diacetate.
[c] Enantiomeric ratio for the first step calculated from the e.e. of the remaining substrate.

3. Summary

Provided the content of the *meso*-isomer was high enough in the commercially available mixtures of *meso*- and *d*,*l*-2,3-butanediols, crystallisation of the crude mixtures furnished pure *meso*-diol. The commercially available *meso*/*d*,*l*-mixtures were transformed into crude *d*,*l*-diol (>96/<4 d.r.) by means of an epimerisation reaction.

The diastereomeric ratio of the product could be easily enhanced by recrystallisation of the corresponding *d*,*l*-diacetate. The enzyme-catalysed hydrolysis of the racemic *d*,*l*-diacetate as well as acylation of racemic *d*,*l*-diol gave both (2*S*,3*S*)- and (2*R*,3*R*)-butanediols with very high d.r. and e.e. (in some cases after alkaline hydrolysis of the esters obtained). The overall yield of (2*S*,3*S*)-2,3-butanediol was 13–18% based on the starting *d*,*l*/*meso*-mixture. The simple work up procedures used here should make this approach attractive for the large-scale preparation of the various stereoisomers studied.

4. Experimental

4.1. General

Mixtures of the diastereomers of 2,3-butanediol were purchased from several suppliers, such as Sigma–Aldrich, Sweden AB (*meso*: ≈63%, *d*,*l*: ≈37%) and Merck Eurolab Norden (*meso*: ≈80%, *d*,*l*: ≈20%). Unless otherwise stated, the commercially available chemicals were used as delivered. Specific optical rotations were measured using a Perkin–Elmer polarimeter 241. Silica gel 60, 230–400 mesh, was used for preparative liquid chromatography (MPLC), using an increasing amount of ethyl acetate in cyclohexane as eluent. GC analysis was performed on a Varian 374000-01 gas chromatograph equipped with a CPWAX 52CB capillary column [30 m, 0.32 mm i.d., d_f = 0.25 μm, carrier gas He (15 psi), 110°C isothermal] to determine diastereomeric ratios (d.r.) and conversions. The conversion of the enzyme-catalysed propanoylation reactions was determined by GC analysis when this column was used. The individual components in the reaction mixture were calibrated against each other to give the corresponding response factors *R* (calculated on a molar basis related to *d*,*l*-2,3-butanediol) and relative retention times (the rel. ret. time of the *d*,*l*-diol was set at 1.0) for butanediol: [*R* (rel. ret. time) *d*,*l*: 1 (1; actual time, 8.12 min); *meso*-butanediol: 1 (1.14); its monopropanoate: *d*,*l*: 1.90 (1.36); *R*,*S*/*S*,*R*: 1.90 (1.44) and its dipropanoate: *meso*: 2.84 (1.26); *d*,*l*: 2.84 (1.51)]. The corresponding acetates were analysed in a similar way, using the same column and conditions (compound, rel. ret. time): monoacetate of *meso*-diol, 1.09; *d*,*l*-monoacetate, 1.16; *meso*-diacetate, 0.79, *d*,*l*-diacetate, 0.93. The enantiomeric purity of the various samples of butanediol was best determined after conversion to the diacetate via acylation of a small sample. [A small-scale version of the procedure described in Section 4.4 was used with the following modification: after the acylation reaction, ice-water was added and the mixture was stirred for 1 h. The aqueous mixture was extracted with pentane and the pentane extract was washed with 2 M HCl (aq.) and aqueous $NaHCO_3$ (sat) and then subjected to GC analysis.] The enantiomeric purity was determined by means of a Varian 3300 GC equipped with a capillary column coated with β-DEX 120 [2,6-dimethyl-3-pentyl-β-cyclodextrin, 30 m, 0.32 mm i.d., d_f = 0.25 μm, carrier gas He (15 psi), 80°C (8 min), 1°C/min→100°C (30 min); compound, ret. times (min); (*R*,*R*)-diol, 16.49; (*S*,*S*)-diol, 17.21; *meso*-diol, 19.13; (*S*,*S*)-monoacetate, 25.34; (*R*,*R*)-monoacetate, 25.79; *meso*-diacetate, 24.17; (*S*,*S*)-diacetate, 26.38; (*R*,*R*)-diacetate. 29.53]. Medium pressure preparative liquid chromatography (MPLC) was performed on straight phase silica gel (Merck 60, 230–400 mesh) using an increasing gradient of ethyl acetate in hexane (0→100%). The enzymes were stored at 4°C in a desiccator over dried silica gel. Immobilised *C. antarctica* lipase B (CALB, Novozyme 435, LCC 0013-1) was a gift from Novo Nordisk A/S. The specific activity was 7400 PLU/g. Lipase from *P. cepacia* (PCL, Amano PS) was a gift from Amano Pharmaceutical Co., Nagoya, Japan. The specific activity was 30.0 units/mg. Crude lipase from Porcine Pancreas, PPL, Type II, was purchased from Sigma Chemical Co., USA. The specific activity was 39 units/mg at pH 7.4 or 190 units/mg at pH 7.7.

4.2. *meso*-2,3-Butanediol

2,3-Butanediol (800 g, *d*,*l*/*meso*, 15/85, Merck) was kept at 5°C for 24 h in a cold room. The colourless oil partly crystallised to give a white creamy mixture. This was poured into a wide 1 L sintered glass funnel on top of

a beaker. The set-up was stored in the cold room at 5°C for 10 days letting the liquid drain into the beaker. The resulting solid was collected, furnishing sticky colourless crystals (480 g, 60%), 1/99 d.r. (*d*,*l*/*meso*).

4.3. *d*,*l*-2,3-Butanediol: Procedure 1

Dry xylene (2 L) was introduced in a pre-dried three-necked flask equipped with a mechanical stirrer, a dropping funnel and a condenser with a calcium chloride guard-tube. Sodium (88.18 g, 3.84 mol; large pieces, which were cleaned under dry xylene by cutting away the oxide layer) was added. A mixture of *meso*- and *d*,*l*-2,3-butanediols (346 g, 3.84 mol) was added slowly through a dropping funnel (**CAUTION**: H_2 evolution). The mixture was then refluxed gently until all of the sodium had been consumed (usually 4–6 h) and then cooled to room temperature. Water (450 mL) was added cautiously under vigorous stirring. The mixture was transferred to a separation funnel and shaken intensely for 5 min. The water phase was collected and the organic phase was shaken vigorously with water (2×75 mL). The combined aqueous phase was extracted with pentane (300 mL) to remove residual xylene, neutralised (HCl, aq., conc.) to pH ≈ 7, and then continuously extracted with diethyl ether for 80 h. The ether was evaporated under reduced pressure, furnishing an oil, which was distilled. The fraction boiling at 75–77°C/10 mmHg was collected to give a colourless oil (289 g, 83%). GC showed a *d*,*l*/*meso*-ratio (d.r.) of 94.6/5.4.

4.4. *d*,*l*-2,3-Diacetoxybutane

d,*l*-2,3-Butanediol (200 g, 2.22 mol, 94.6/5.4 d.r.) was slowly added to a solution of acetic anhydride (838 mL, 8.88 mol) and pyridine (718 mL, 8.88 mol). The mixture was stirred at room temperature for 1 h (**CAUTION**: exothermal reaction) and subsequently heated for 0.5 h until it refluxed gently. After cooling, the solvent was removed in vacuo (10 mmHg) through a short column. The fraction boiling below 75°C was discarded. After cooling to room temperature overnight, the residue gave the crude *d*,*l*-diacetate as a crystalline mass with a yellow oil as a contamination. The oil (≈110 g) was removed by filtration and put into a freezer, where an additional crop of the diacetate crystallised, which was collected by filtration at −30°C. The combined solid material was recrystallised by dissolving it in a minimum amount of pentane at ≈30°C. The solution was cooled to −30°C in a stoppered round-bottomed flask. At this temperature, filtration by suction through a glass tube with a fritted tip, which was inserted in the flask, furnished colourless crystals of the *d*,*l*-diacetate. Four recrystallisations gave colourless crystals (290 g, 75%); >99.5/0.5 d.r. by GC; mp 42–43°C (Lit. 41.0–41.5°C[12] and 42.6–43°C[19b]).

4.5. *d*,*l*-2,3-Butanediol: Procedure 2

The recrystallised *d*,*l*-diacetate described in Section 4.4 (46 g, 0.264 mol) was dissolved in methanol (500 mL). Powdered and dried K_2CO_3 (55 g, 0.45 mol) was added. The mixture was stirred under reflux (1 h). After cooling, the mixture was filtered. The solid collected was stirred with diethyl ether (400 mL), filtered and the solid was washed once more with ether (400 mL). The methanol solution from above was evaporated to leave a volume of 120 mL and combined with the ether washings from above and the resulting mixture was dried over $MgSO_4$. After filtration and washing of the solid with ether (400 mL), the solvent was evaporated and the residue was distilled without using a column. The fraction with bp 76–77°C/10 mmHg was collected. The *d*,*l*-diol was obtained as a colourless oil (18.6 g, 78%); 99.5/0.5 d.r.

4.6. (2*S*,3*S*)-2,3-Diacetoxybutane

A mixture of *d*,*l*-2,3-diacetoxybutane (200 g, 1.15 mol, >99.5/<0.5 d.r.) from Section 4.5 above, hexane (1800 mL), 1 M phosphate buffer solution [1100 mL, pH 7.6 (0.17 M KH_2PO_4, 22.65 g/L and 0.83 M K_2HPO_4, 145 g/L)] and decane (20 mL) was stirred vigorously with a mechanical stirrer for 15 min at room temperature to bring about equilibrium partition of the diacetate between the resulting phases. A GC sample from the upper layer (hexane) was withdrawn and analysed in relation to the internal standard (decane) as a zero-point reference for conversion monitoring by GC. Immobilised CALB (33 g) was added to the mixture, which was then stirred until a conversion of approximately 57% was reached (usually 3–5 h). If the e.e. of the remaining diacetate was <99.5% at this point, stirring was continued for a short time, until the e.e. was >99.5%. The enzyme was removed by filtration and the liquid phases were separated. The organic phase was collected and the water phase was extracted with hexane (4×200 mL). The combined hexane solution was concentrated by evaporation through a long column packed with glass helices. The fraction boiling below 75°C was discarded, including a small amount of water that followed azeotropically. The residue was cooled in a stoppered round-bottomed flask in a freezer overnight to give crystals together with a liquid phase, which was removed by suction at −25°C through a Pasteur pipette pressed to the bottom of the flask. The crystals were recrystallised twice in the same way from pentane. After drying under vacuum (10 mmHg, 0°C for 5 min) colourless crystals were obtained (68.3 g, 68% based on half the amount of the racemic diacetate used), >99.5% e.e.; >99.5/<0.5 d.r., mp 24.0–24.5°C (Lit.[19b] 25.7–25.9°C), $[\alpha]_D^{25} = -13.7$ (neat), −13.7 (*c* 2.0, hexane) (Lit.[19] $[\alpha]_D^{20} = +13.7$ for the enantiomer (neat)).

4.7. (2*S*,3*S*)-2,3-Butanediol via Procedure 2

(2*S*,3*S*)-2,3-Diacetoxybutane (23 g) was hydrolysed as described in Section 4.5. The same work-up procedure furnished the diol (9.5 g, 81%). $[\alpha]_D^{25} = +14$ (neat). >99.5% e.e.; >99.5/<0.5 d.r.

4.8. (2*S*,3*S*)-Butanediol via enzymatic acylation

A mixture of *d*,*l*-2,3-butanediol (10 g, 11.1 mmol,

>99.5/<0.5 d.r.), vinyl propanoate (60 mL, 555 mmol) and *tert*-butyl methyl ether (80 mL) was equilibrated by stirring at ambient temperature for 10 min. Enzyme from *P. cepacia* (Lipase Amano PS, 1.4 g) was added to the mixture. The esterification reaction was followed by GC. Samples were withdrawn continuously from the start up to the point when the conversion of the diol was 75%, which usually required approximately 50 h. The enzyme was then isolated by filtration and washed with a small amount of solvent. The combined solvent plus the excess vinyl propanoate was concentrated to a small volume, using distillation through a column packed with glass helices, to give an oil ≈30 g, which was subjected to MPLC. The dipropanoate eluted first (see below), followed by a mixture of monopropanoates, which was discarded, and finally the diol. The diol containing fractions were concentrated to yield an oil, which was distilled (75–78°C/10 mmHg) to give a colourless oil (2.3 g, 23%). >99.5% e.e.; >99.5/<0.5 d.r., $[\alpha]_D^{20}=+14$ (neat).

The enzyme-catalysed reaction was repeated on a 10 times larger scale, starting from *d,l*-diol of 94/6 d.r. The enzyme was then collected by filtration and washed with solvent and the solution obtained was extracted with water (4×100 mL). The combined water phase was extracted with equal volumes of dichloromethane several times until the organic solvent failed to extract any further monopropanoate. The water phase was then continuously extracted with ether for 80 h. The ether extract was evaporated under reduced pressure furnishing an oil, which was distilled. The fractions boiling at 75–77°C/10 mmHg were collected to give a colourless oil (18–25 g, 18–25%), >99% e.e., >95/<5 d.r., $[\alpha]_D^{20}=+14$ (neat).

The small-scale procedure above was also used for the propanoylation of *d,l*-diol (10 g, 99.5/0.5 d.r.) with vinyl propanoate but using PPL instead of lipase PS. The reaction was stopped at 52–54% conversion of the diol, which usually required a reaction time exceeding 10 days. Work-up using chromatography as described above furnished the diol (≈3.5 g, ≈35%), ≈96% e.e.; >99/<1 d.r., $[\alpha]_D^{20}=+14$ (neat). In this procedure, the monopropanoate mixture obtained was not isolated, neither was the dipropanoate, the yield of which was very low due to the slow second acylation step in this reaction.

4.9. (2*R*,3*R*)-2,3-Dipropionyloxybutane

Evaporation of the solvent from the first eluting fraction in chromatography (described in Section 4.8, first paragraph) gave the title compound as a colourless oil (9 g, 40%). 95% e.e.; >95/<5 d.r., $[\alpha]_D^{20}=+9.25$ (neat).

4.10. (2*R*,3*R*)-2,3-Butanediol

The (2*R*,3*R*)-dipropionyloxybutane sample (8 g) described in Section 4.9 was hydrolysed and worked up according to Procedure 2 (Section 4.5) to give an oil (2.7 g, 75%), 95% e.e.; >95/<5 d.r., $[\alpha]_D^{20}=-13.5$ (neat).

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