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Enantioselective Transesterifications of 2-Methyl-1-alcohols Catalysed by Lipases from *Pseudomonas*

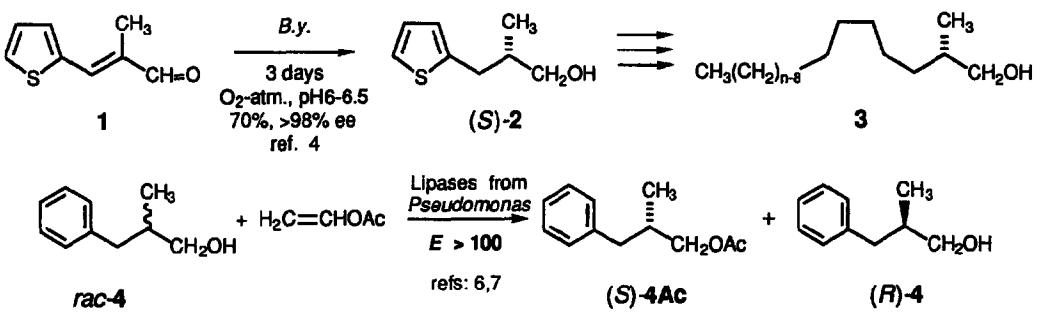
Ove Nordin, Erik Hedenström and Hans-Erik Högberg*

Chemistry, Department of Science and Engineering, Mid Sweden University,
S-851 70 Sundsvall, Sweden.

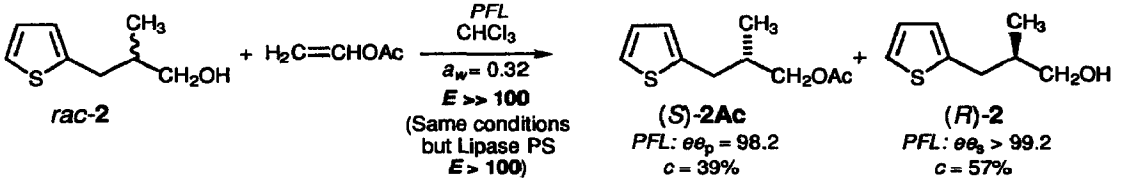
Abstract: Racemic β-methyl-2-thiophenepropanol was resolved ($E \approx 200$) *via* transesterification catalysed by lipase from *Pseudomonas fluorescens* using an excess of vinyl acetate in chloroform at an initial water activity: $a_w = 0.32$. When trying to resolve *rac*-2-methyl-1-alkanols more modest *E*-values were obtained ($E \approx 10$) and were of the same order of magnitude irrespective of substrate chainlength, water activity, immobilization, acyl donor or other *Pseudomonas* derived lipases. However, the reaction rates are affected by variations of these parameters. Both the rates and *E*-values were influenced by the nature of the solvent.

Enantiomerically pure 2-methyl-1-alcohols and their derivatives are valuable synthetic intermediates for the preparation of stereochemically pure insect pheromones and other natural products containing methyl branched alkyl chains.[1,2] Many enantioselective methods leading to such alcohols have been developed both by using asymmetric chemical synthesis and various bioorganic methods.[3]

We have recently described the Baker's yeast (*B.y.*) reduction of the unsaturated aldehyde **1** which leads to virtually enantiomerically pure *(S)*-β-methyl-2-thiophenepropanol *(S)*-**2** (≈70%, >98% *ee*), which was subsequently transformed into various 2-methyl-1-alkanols **3** of unchanged *ee via* a short and efficient reaction sequence.[4] However, since this *B.y.*-reduction furnishes only one enantiomer, *(S)*-**2**, alternative routes[5] must be explored to prepare enantiomerically pure *(R)*-**2**.



Lipase P (Amano)[6] and *PFL*[7,8] from *Pseudomonas* is known to react with high enantioselectivities in irreversible transesterifications of some 2-methyl-1-alcohols, notably with *rac*-β-methylbenzenepropanol. The *PFL*-catalyzed resolution of *rac*-2-methyl-1-decanol, *rac*-**3b**, by transesterification with vinyl acetate was recently reported to readily give the (*S*)-acetate (*S*)-**3bAc** (at 40% conversion) and the remaining substrate, (*R*)-alcohol *(R)*-**3b** (at 60% conversion), both claimed to be of >98% *ee*.[8,9] These *ee*-values should correspond to *E*-values well over 100. Using other *Pseudomonas* derived lipases (Amano P and Amano PS) lower enantioselectivities for similar substrates in transesterifications with vinyl acetate have been reported.[10] In the latter publication a study of the effect of variations of the chain length of the acyl donor and the effects of solvent changes were briefly described and vinyl acetate in dichloromethane was found to give the best *E*-values. On the other hand, in the Amano PS catalysed resolution transesterification with vinyl acetate of the allylic alcohol group in *rac-trans*-sobrerol, an allylic monoterpene diol, *tert*-amylalcohol as solvent gave by far the best *E*-value.[11]



We have recently reported that variation of the water activity has a great influence on the *E*-values when using lipase from *Candida rugosa* as the catalyst for the esterification of 2-methylalkanoic acids in cyclohexane.[12] However, using lipase PS and lipoprotein lipase, both from *Pseudomonas sp.*, as the catalyst for transesterifications of the racemic secondary alcohol, (±)-sulcatol (6-methyl-5-hexene-2-ol), enantioselectivity seemed to be independent of water activity.[13]

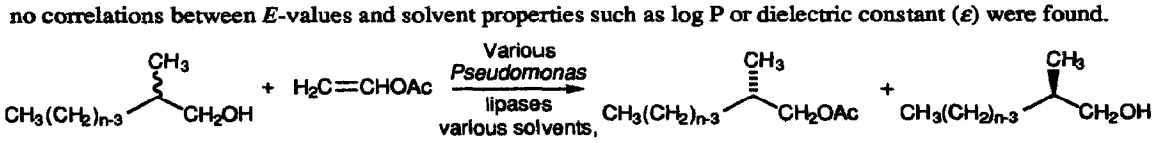
In order to be able to prepare efficiently both antipodes of 2-methyl-1-alkanols enantiomerically pure we have now studied different *Pseudomonas* derived enzymes as catalysts for the resolution of various 2-methyl-1-alcohols by transesterification. Using racemic β-methyl-2-thiophenepropanol *rac*-**2** as the substrate in a *PFL*-catalysed transesterification with vinyl acetate as the acyl donor in chloroform with an initial water activity of $a_w = 0.32$, we found, to our satisfaction, that this reaction was highly enantioselective ($E \approx 200$). Thus at 39 % conversion the acetate of *(S)*-**2** was produced in >98% *ee* and at 57% conversion enantiomerically pure remaining substrate *(R)*-**2** was produced{>99 % *ee* by GC: method described below; $[\alpha]_D^{25}$ +21.1 (c 0.8 MeOH), Lit.[3] *(S)*-**2**: $[\alpha]_D^{25}$ -19.3 (neat)}. Using Amano PS lipase under the same conditions a slightly lower enantioselectivity was observed ($E \approx 130$).

Although, as mentioned above, the preparation of the individual enantiomers of 2-methyl-1-alkanols *(S)*-**3** and *(R)*-**3** from either of the now readily accessible enantiomerically pure antipodes of β-methyl-2-thiophenepropanol **2** should be straightforward,[4] we were of course also interested in improving the preparation of the desired enantiomerically pure 2-methyl-1-alkanols directly *via* the already described *PFL*-catalysed transesterification mentioned above.[8,9] However, we were unable to reproduce the high *ee*:s claimed[9] for this resolution of *rac*-2-methyldecanol (*rac*-**3b**). Since our reaction conditions could have been different from those previously described[8,9] and therefore responsible for the low enantioselectivities we observed, we have now studied the effects of variations of the water activity of the reaction medium, of the solvent and of the acyl donor in the *PFL*-catalysed transesterifications of some racemic 2-methyl-1-alkanols *rac*-**3**.

In all experiments described below the conversions were monitored by GC and, for the determinations of the *E*-values, the reactions were stopped at ≈ 40 % conversion. The *E*-values were calculated by measuring the *ee*:s of the *(S)*-acetates (details: see experimental section).

Since no water is produced in the transesterification with vinyl acetate, the water activity (a_w) should remain roughly constant during the course of the reaction. The initial water activity was adjusted as described below. The results from the transesterifications of *rac*-2-methyl-1-decanol (*rac*-**3b**) at different water activities at 25 °C are presented in Figures 1 and 2. Although the reaction rates increased with increasing water activity, the *E*-values decreased although probably not significantly.

The influence of the solvent on the *E*-values in the transesterification of *rac*-2-methyl-1-decanol (*rac*-**3b**) is shown in Figure 3. These reactions were all performed at an initial $a_w = 0.32$, and the concentrations of substrate and vinyl acetate were the same in all experiments. The rate increased in the order dichloromethane, chloroform, tetrahydrofuran (THF) and 1,1-dimethyl-1-propanol (*t*-AmOH). The E-values for chloroform and dichloromethane were the same, whereas for THF and especially for *t*-AmOH they were lower. However no correlations between *E*-values and solvent properties such as log P or dielectric constant (ε) were found.



3a: $n = 8$; **3b**: $n = 10$; **3c**: $n = 12$; **3d**: $n = 14$

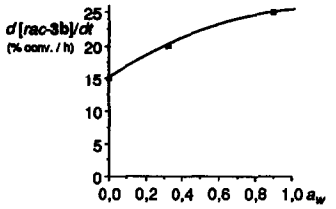


Figure 1. Initial rates of *PFL*-mediated transesterification of 2-methyl-1-decanol *rac*-**3b** with vinyl acetate in chloroform at 25 °C at different initial water activities.

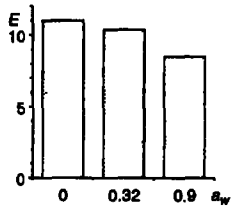


Figure 2. *E*-values in *PFL*-mediated transesterification of 2-methyl-1-decanol *rac*-**3b** with vinyl acetate in chloroform at 25 °C at different initial water activities.

The chain length of the alcohol had little effect on *E*-values except in the case of 2-methyloctanol (*rac*-**3a**), which gave a significantly lower value (see Figure 4.). These experiments were all performed at $a_w = 0.32$ in chloroform at 25 °C.

Different *Pseudomonas* derived lipases were used with 2-methyl-1-decanol (*rac*-**3b**). The Amano lipases PS and AK gave the *E*-values 10.7 and 9.9 under standard conditions ($CHCl_3$, $a_w = 0.32$, 25 °C) as compared with 10.4 for *PFL*. Lowering the temperature gave an *E*-value of 11.8 at 1 °C (*PFL*). Two additional acyl donors were also tested. Vinyl laurate gave unchanged *E*-value (10.6) but reduced rate. Another acyl donor which has been used for irreversible transesterifications is ethyl thiooctanoate.[14] The reactions become irreversible through evaporation of the produced volatile thioethanol. In our case we tested ethyl thioacetate in a closed vessel (reversible conditions) and interrupted the reactions at low conversions. The reaction was very slow but the *E*-value was essentially unchanged (10.7).

Immobilization of *PFL* on celite[13] increased the reaction rate of the standard reaction (vinyl acetate, *rac*-2-methyldecan-1-ol, $CHCl_3$) but no effect was noticed on the *E*-value.

Thus we have shown that despite efforts to optimise the resolution by transesterification of 2-methyl-1-alkanols **3** by variation of the reaction conditions the maximum *E*-value that can be achieved lies slightly above 10. This value is not high enough for simple preparation of the 2-methyl-1-alkanols of the high enantiomeric purities needed for use in *e.g.* the synthesis of pheromones of some pine sawflies. However, multistep procedures[10,15,16] can be used if low yields can be accepted or, if enantiomerically pure 2-methyl-1-alkanols (**3**) with $n \geq 7$ are needed, the transesterification of racemic β-methyl-2-thiophenepropanol (*rac*-**2**) can be utilised.

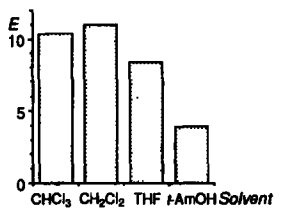


Figure 3. *E*-values in *PFL*-mediated transesterification of 2-methyl-1-decanol *rac*-**3b** with vinyl acetate in various solvents at 25 °C and $a_w = 0.32$.

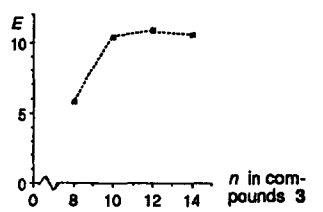


Figure 4. *E*-values in *PFL*-mediated transesterification of 2-methyl-1-alcanols *rac*-**3** of various chainlengths *n* with vinyl acetate in chloroform at 25 °C and $a_w = 0.32$.

Experimental

PFL (E.C.3.1.1.3), lipase from *Pseudomonas fluorescens* was obtained from Fluka. The specific activity was 31.5 U / mg. Lipase PS (30.0 U / mg) and lipase AK (25.2 U / mg) from *Pseudomonas sp.* were obtained from Amano. The enzymes were stored at 4°C in a desiccator over dried silica gel. The acyldonors vinyl acetate and vinyl laurate are commercially available and were used without further purification.

Transesterification reactions at preequilibrated water activities. General procedure: A slight modification of the method described earlier[9] was used. Enzyme (PFL: 13.7 mg, Lipase PS: 14.4 mg, Lipase AK: 17.1 mg) and a *rac*-2-methylalcohol (*rac*-**3**) or *rac*-β-methyl-2-thiophenepropanol (*rac*-**2**) (1.0 mmol) was mixed with solvent (1.8 ml) in the reaction flask containing a magnetic stirring bar. The mixture in the open flask was stirred for 24 h in a sealed container over saturated salt solutions of known water activity [Valivety et al.[17]: $MgCl_2 \cdot 6H_2O$ ($a_w = 0.32$) and $ZnSO_4 \cdot 7H_2O$ ($a_w = 0.90$)]. The acyl donor and the solvent were equilibrated to the same water activity in another sealed container. After 24 h the equilibrated solvent was added to the reaction flask to com-

pensate for loss by evaporation. The acyl donor (3.7 mmol) was added to the reaction flask, which was immediately sealed with a septum. The mixture was stirred at 400 rpm. The conversion was followed by periodical withdrawal of samples (10 µl). When the reaction had reached the desired conversion (≈ 40 %) the mixture was filtered through a filter of low porosity and the filter was washed with n-pentane (3 ml). The components were separated by MPLC using silicagel 60 (Merck) with a gradient of increasing amount of diethylether in *n*-pentane as eluent. The esters were obtained pure (>99% by GC) without any trace of the remaining alcohol.

Determination of conversion. The conversions in the transesterification reactions were determined using a Varian 3300 gas chromatograph equipped with a 30 m x 0.32 mm *I.D.* capillary column coated with cross-linked Carbowax ® 20M, d_f = 0.25µm ; carrier gas He 15 psi, split ratio 30:1. The conversions were calculated from the areas of the ester peaks relative to the peaks from the alcohol which was calibrated against the racemic esters.

Determination of enantiomeric excess and calculation of E-*values.* The *ee*:s of the alcohols were determined by analysing the diastereomeric mixture of the corresponding 2-methylacyl-1-phenylethylamides[18]. Baseline separation was readily obtained using the same GC and conditions described above. The *E*-values were calculated using Sih's method (irreversible case).[19]

rac-2-Methyl-1-alcohols (rac-2 and rac-3). The racemic alcohols were prepared by reduction with $LiAlH_4$ in diethyl ether using the method described below but the ester was replaced by the appropriate racemic 2-methyl acid which was prepared by the methods described in the literature: 2-methyloctanoic acid, 2-methyldecanoic acid, 2-methyldodecanoic acid[20], 2-methyltetradecanoic acid[21] and α-methyl-2-thiophenepropanoic acid[22].

Ethyl thioacetate. Ethyl thioacetate was prepared from ethanthiol and acetylchloride as described for ethyl thiooctanoate[14].

(S)-2-Methyl-1-alcohols [(S)-2 or *(S)-3].* The chemically pure, enantiomerically enriched esters obtained as described above (≈ 0.3 mmol) were dissolved in anhydrous diethyl ether (1 ml) and added to a stirred solution of $LiAlH_4$ (30 mg) in anhydrous diethyl ether (3 ml) under argon. The mixture was stirred at room temperature 1 h and then quenched with water / THF (1:1, 60 µl) followed by 15% NaOH (30 µl) and water (20 µl). After refluxing for 10 min the mixture was filtered, washed with diethyl ether and dried ($MgSO_4$). After evaporation the pure alcohols [(*S*)-2 or (*S*)-3] was obtained in quantitative yield.

Acknowledgements

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