Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 1821–1826

TETRAHEDRON: *ASYMMETRY*

Candida rugosa lipase as an enantioselective catalyst in the esterification of methyl branched carboxylic acids: resolution of *rac*-3,7-dimethyl-6-octenoic acid (citronellic acid)

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Received 27 April 1999; accepted 10 May 1999

Abstract

Some chiral methyl branched alkanoic and alkenoic acids were used as substrates in esterifications with 1-hexadecanol in cyclohexane at water activity a_w=0.8 catalysed by immobilised *Candida rugosa* lipase (CRL). Citronellic acid was one of several chiral 2- and 3-methyl branched acids that were successfully resolved by the catalyst. With 3-methyl-branched substrates the *R*-enantiomers reacted fastest, whereas with 2-methyl acids the lipase generally displayed *S*-preference except in one case where it displayed *R*-preference. When a double bond was present in the chain, the selectivity of CRL (*E*=24–51) was greater compared with the corresponding saturated acid (*E*=17–23). Attempted transesterifications of vinyl acetate with some chiral 2- or 3-methyl branched primary alcohols using crude CRL gave very low *E*-values (*E*=1–2).

1. Introduction

The number of articles published on the use of lipases in organic chemistry has increased enormously during recent years.[1] Among them, some deal with esterification of chiral methyl branched alkanoic acids catalysed by *Candida rugosa* lipase (CRL).[2–8] Recently, we described the synthesis of 3,7,11-trimethyl-2-tridecyl esters, the sex pheromone of the pine sawfly *Microdiprion pallipes* using racemic citronellol as a building block.[9] In order to synthesise stereoisomerically pure isomers of the *M. pallipes* pheromone, building blocks of very high stereoisomeric purities are required. Citronellene, citronellal, citronellol and citronellic acid in enantiomeric excess of ~98% are available from the chiral pool, but do not satisfy our needs. Recently, a transesterification reaction was described in which vinyl acetate reacted with 3,7-dimethyl-1-octanol catalysed by crude CRL giving the ester and the remaining alcohol in enantiomeric excess of over 98%.[10] However, in our hands, all attempts to repeat this resolution

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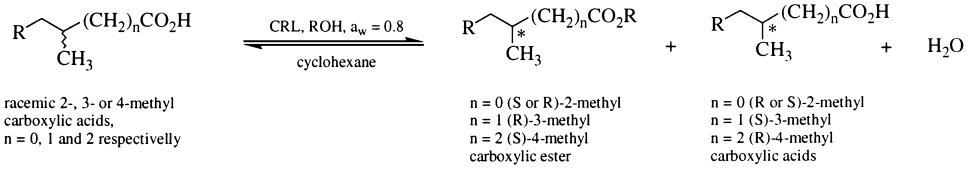
0957-4166/99/$ - see front matter
PII: S0957-4166(99)00178-0

were fruitless, the reaction was slow and the calculated enantiomeric ratio was as low as 1. Low *E*-values (1–2, *S*-enantiomers preferred by the lipase) were also observed when 3,7-dimethyl-6-octen-1-ol (citronellol), 2,6-dimethyl-5-hepten-1-ol and 2-methyl-1-butanol were used in similar transesterifications of vinyl acetate.

Because we have earlier described successful CRL-catalysed resolutions by esterification of 2-methylalkanoic[2–6] and 4-methylalkanoic acids[7] we decided to apply this strategy for racemic 3-methyl acids. We now describe what constitutes to our knowledge the first successful resolution of racemic citronellic acid using CRL in an esterification reaction in an organic solvent. In addition we describe resolutions of some other synthetically useful chiral methyl branched carboxylic acids.

2. Results and discussion

The results are summarised in Table 1. A carboxylic acid was reacted at a constant water activity (a_w=0.8) in cyclohexane with hexadecan-1-ol catalysed by CRL (Scheme 1). With a 2- or 4-methylalkanoic acid the enantiopreference of CRL is the *S*-enantiomer.[2–8] However, when we used 3-methyl acids as substrates, an unexpected *R*-preference was registered. Thus, 3,7-dimethyl-6-octenoic acid[11] (entry 1) gave the product *R*-ester as 89.6% ee and the remaining substrate as 30.0% ee, which gave a calculated[12] conversion of 25% and the *E*-value[12] as *E*=24. Recently, Berglund et al. described a CRL-catalysed hydrolysis of 2-methyl-6-(2-thienyl)hexanoic acid ethyl ester and found that the lipase shows an *R*-enantiopreference in this case.[13] Molecular modelling suggested[14] that the acyl-binding tunnel of CRL is too narrow to harbour the thienyl group. Therefore, the lipase must have two different productive modes for the binding of enantiomers of chiral acyl donors. The resolution of 3,7-dimethyl-6-octenoic acid was slow, 6 days to reach 25% of conversion. The explanation for this might be that the *S*-enantiomer does not fit into the active site tunnel of CRL. Instead, the (*R*)-3,7-dimethyl-6-octenoic acid might react via an alternative productive mode, similar to the case of 2-methyldecanoic acid ester.[14] The slow reaction rate of the 3-methyl substrate might also be the consequence of conformational changes required in order to make it mimic a 2*S*-substrate. Such changes might demand more energy for the 3*S*-enantiomer than for the 3*R*-enantiomer.

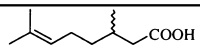
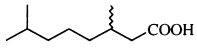
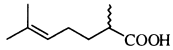
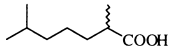
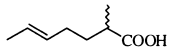
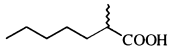
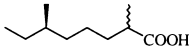
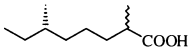
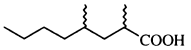
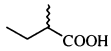
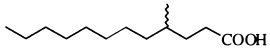


Scheme 1. Resolution of racemic 2-, 3- and 4-methyl carboxylic acids in esterification reactions with long chained 1-alcohols in cyclohexane at water activity a_w=0.8 using immobilised *Candida rugosa* lipase (CRL) as a chiral catalyst

3,7-Dimethyloctanoic acid[15] gave a lower *E*-value (*E*=17, entry 2), and reacted slower than its unsaturated analogue, citronellic acid. However, the *R*-enantiomer was again the faster reacting one.

A structure similar to citronellic acid but with the methyl branching at carbon 2 was represented by the 2,6-dimethyl-5-heptenoic acid.[23] The expected 2*S*-enantiopreference (*E*=41, entry 3) was now shown by CRL. The hydrophobic tunnel of CRL is evidently wide enough to accommodate the methyl branch five carbons away from the carboxyl group. This was not surprising, because even 2-arylpropionic acids are accepted as substrates by CRL. More surprising is that even though the rates are slow, the enantiopreference is 2*S* for 2-arylpropionic acids.[1]

Table 1
Candida rugosa lipase as chiral catalyst in esterification of methyl branched carboxylic acids

Entry[a]	Substrate	The faster reacting isomer			ee_s[b]	Conversion[c]	Time	E[d]
		ee_p[b] (%)	Sign of optical Rotation	Configuration	(%)	(%)	(h)	
1		89.6	+	3R[16]	30.0	25.0	144.0	24
2		81.7	+	3R[17]	51.6	38.7	508.8	17
3		92.6	+	2S[e,18]	45.0	32.7	3.8	41
4		84.8	+	2S[18]	41.1	32.6	14.6	18
5		93.3	+	2S[e,19]	56.8	37.8	13.3	51
6		90.1	+	2S[19]	16.1	15.1	5.4	23
7		96.2	+	(2S6R)[g,20]	2.4	2.4	2.4	53[f]
8		86.5	+	(2S6S)[g,20]	8.7	9.1	5.0	15[f]
9		35	+	(2R4S)[21]	10	22	71	1.6[f]
10		47.2	+	2S[22]	27.4	36.7	6.0	3.6
11[h]		84	+	4S[7]	53	38	58	19

a) A cyclohexane solution (1 vol) containing a methyl branched carboxylic acid (0.15M), hexadecan-1-ol (0.15M), icosane (25 mg / ml solution) as an internal standard and Na_2SO_4 / Na_2SO_4 x 10 H_2O (a_w = 0.8) was stirred for 0.25 h in a sealed flask at room temperature. The immobilised lipase (20.3 mg particles / ml solution) was added and the mixture was stirred with a magnetic stirring bar at 700 rpm. **b)** Determination of the enantiomeric excess of the product and substrate were made by GC as the corresponding 1-phenethyl amides obtained as described.[3] **c)** The conversion ξ at each point was obtained from ee_s and ee_p according to $\xi = ee_s / (ee_s+ee_p)$. **d)** The E-values were calculated from the ee_p and the conversion ξ using the equation deduced by Chen *et al.*[12] **e)** Hydrogenation to acids known in the literature. **f)** The "E-values" were calculated as the CRL:s preference for 2R-isomers over 2S-isomers or vice versa. **g)** The acids were reduced (LAH) and the sign of optical rotation of the product alcohols were compared to enantiomeric alcohols known in the literature. **h)** See ref.[7] for experimental details and analysis methods.

When comparing 2,6-dimethyl-5-heptenoic acid (*E*=41, entry 3) with 2,6-dimethylheptanoic acid[24] (*E*=18, entry 4) the expected *S*-enantiopreference was found and, similar to entries 1 and 2, a higher *E*-value was registered for the unsaturated substrate. This was also the case for 2-methyl-5-heptenoic acid[25] (*E*=51, entry 5) compared with 2-methylheptanoic acid[26] (*E*=23, entry 6). The lower enantioselectivities of CRL for saturated acids might be due to the higher steric demands of the sp^3-hybridised substrates compared with the sp^2 ones. The unsaturated substrates might also be favoured by higher electron densities in the carbon chain, i.e. an electronic effect. Our results indicated that the enantioselectivity of CRL increased when a double bond was situated five or six bonds away from the carboxyl group. Thus, when the resolution is performed it should be beneficial to have unsaturation in the carbon chain. If desired, the double bond can later be removed by hydrogenation.

(6*R*)-2,6-Dimethyloctanoic acid[27] (*E*=53, rel. to the 2-pos., entry 7) gave a high *E*-value compared to the diastereomeric (6*S*)-2,6-dimethyloctanoic acid[27] (*E*=15, rel. to the 2-pos., entry 8) which gave a similar but somewhat lower *E*-value than entry 4, 2,6-dimethylheptanoic acid (*E*=18, entry 4). This diastereoselectivity of CRL indicates that it should be possible to enrich the (2*S*,6*R*) isomer from a mixture with the (2*S*,6*S*) isomer, although this minor structural difference is situated five bonds from the carboxyl group.

Although 2- and 4-monomethylalkanoic acids show *S*-preference,[2–7] we found that a diastereomixture of 2,4-dimethyloctanoic acid[28] (*syn*:*anti*, 56:44, entry 9) reacted slowly to give mainly a mixture of the

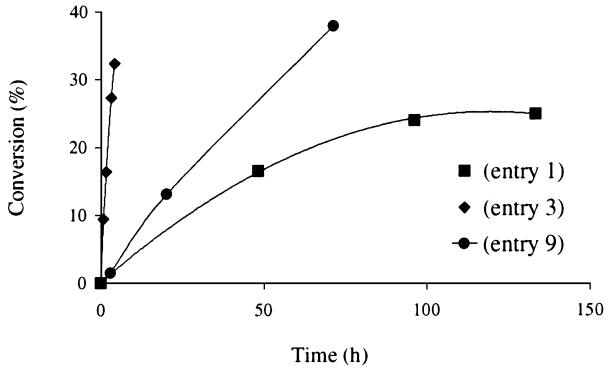


Figure 1. Progress curves (monitored by GC) for the esterification of 3,7-dimethyl-6-octenoic acid (entry 1), 2,6-dimethyl-5-heptenoic acid (entry 3) and a mixture of the four isomers of 2,4-dimethyloctanoic acid (entry 9)

(2*R*,4*S*)- and (2*R*,4*R*)-isomers, with the former as the preferred one. The '*E*-value' (*E*=1.6, rel. to the 2-pos.) was very low with the *syn*:*anti*-relationship reversed in the product (44:56).

The reaction in entry 9 gave a slower production of ester (with low 2*R*-preference) compared to that of entry 3 (with high 2*S*-preference) but a faster production of ester than in entry 1 which had a high 3*R*-preference (Fig. 1). The results presented in Table 2 show that fast average reaction rates were obtained when the enzyme showed a 2*S*-preference and slow rates indicated that the preference is either 2*R* or 3*R*.

Table 2
The average conversion rate (monitored by GC) of the substrate acids used in CRL catalysed esterifications

Substrate	Conversion rate (% h^{-1})
3,7-Dimethyl-6-octenoic acid (entry 1)	0.17
3,7-Dimethyloctanoic acid (entry 2)	0.08
2,6-Dimethyl-5-heptenoic acid (entry 3)	8.61
2,6-Dimethylheptanoic acid (entry 4)	2.23
2-Methyl-5-heptenoic acid (entry 5)	2.84
2-Methylheptanoic acid (entry 6)	2.80
(6R)-2,6-Dimethyloctanoic acid (entry 7)	1.00
(6S)-2,6-Dimethyloctanoic acid (entry 8)	1.82
2,4-Dimethyloctanoic acid (entry 9)	0.31
2-Methylbutanoic acid (entry 10)	6.12

As pointed out earlier,[29] 2-methylbutanoic acid gave a very low *E*-value (*E*=3.6, entry 10) probably due to the small difference between the methyl and the ethyl groups.

The hydrolysis of olive oil catalysed by CRL has been shown to be sensitive to the presence of cations and to the nature of the buffer present.[30] In order to investigate if the presence of salt ions (e.g. Na^+ and SO_4^{2-}) influenced the activity and enantioselectivity of the lipase, the reaction in entry 6 was started after 24 h of pre-equilibration in a dessicator at a_w=0.8 in an open vessel. The *E*-value was lower (*E*=19) and the activity observed was half of that obtained when using the standard conditions of entry 6 (*E*=23) where water activity was regulated by a salt buffer. Thus, it is beneficial to have the sodium sulphate water buffer present in the reaction vessel. However, other salt buffers might have other effects.

In summary, with the *E*=24 obtained with citronellic acid, it should, in theory,[12] be possible to obtain enantiomerically pure product and substrate using a two-step kinetic resolution. Our results indicated that the enantioselectivity of CRL increases when a double bond is situated five or six bonds away from the

carboxyl group and also give some support to the existence of two different productive binding modes[13] of the enantiomers of a chiral acyl donor to the active site of CRL.

Presently, we are trying to enhance the enantioselectivity of CRL in the resolutions of 3,7-dimethyl-6-octenoic acid and 2,6-dimethyl-5-heptenoic acid and the results will be published in the near future.

Acknowledgements

We thank Dr. Per Berglund for fruitful discussions and for valuable comments on the manuscript. For financial support we thank the Swedish Natural Science Research Council (NFR) and the Commission of the European Communities, Agriculture and Fisheries (FAIR), specific RTD program, contract no. FAIR1-CT95-0339, 'Pine sawfly pheromones for sustainable management of European forests'. This study does not necessarily reflect the commission's view and in no way anticipates its future policies in this area.

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