



Regioselective hydrolysis of diesters of (*Z*)- and (*E*)-2-methyl-butenedioic acids by PLE

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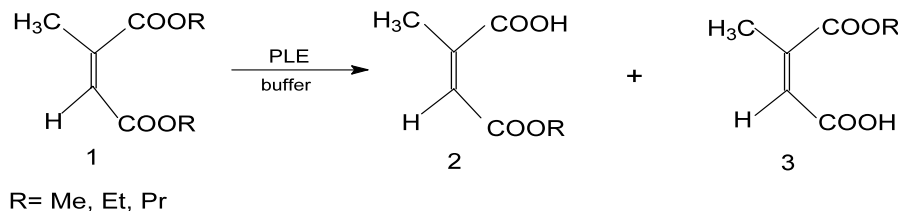
Abstract—Dimethyl, diethyl and dipropyl esters of (*Z*)- and (*E*)-2-methyl-butenedioic acids (citraconic and mesaconic acid) were hydrolysed by pig liver esterase, PLE. For the diethyl esters the enzyme showed low regioselectivity for both the (*Z*)- and the (*E*)-isomers. However, the hydrolysis of dimethyl and dipropyl (*Z*)-2-methyl-butenedioic acid gave a ratio of the monoesters of 95:5 and 85:15. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

During recent decades, enzymes and in particular hydrolases have been used frequently in organic synthesis. The enzymes are mainly from microbial sources and lipases have often been utilised. However, the esterase pig liver esterase (PLE) is also extensively used due to its unique substrate selectivity as well as long tradition. In the 1970s and 1980s several reports were published on the use of PLE in chiral synthesis, for example in the preparation of 3-substituted glutaric acid diesters^{1,2} and various cyclic diesters.^{3–5} In the 1990s Jones' active site model⁶ appeared, further rationalising the use of PLE with respect to choice of suitable substrate and steric outcome of the hydrolysis. PLE has been applied in organic solvents in transesterifications to kinetically resolve alcohols,^{7,8} and recently the enzyme was used in the kinetic resolution of sulphoximinocarboxylates.⁹ A major focus of enzymatic catalysis in general (and not surprisingly) is enantioselective reactions and this also

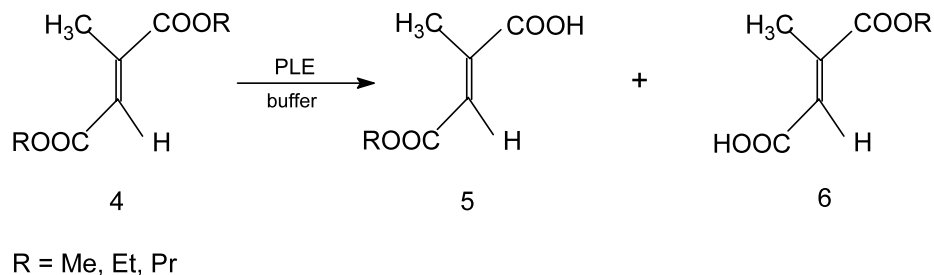
applies to PLE, for which Zhu and Tedford published a review on this aspect in 1990.¹⁰ Less attention has been given to regioselective reactions, with the exception of glyceride modification where lipases have been utilised. There are, however, also some reports on regioselective reactions with the use of PLE, for example in carbohydrate chemistry,¹¹ in the study of media effects on the hydrolysis of different types of (*E*)/(*Z*)-diastereotopic diesters and (*E*)/(*Z*)-diastereomeric monoesters¹² and in the hydrolysis of saturated and α,β -unsaturated cyclopropanecarboxylic esters.¹³

Geometric isomers have different physical properties, so in principle they may be isolated by traditional methods, although in practice this is not necessarily straightforward. As enzymes are gradually incorporated into the organic chemists' tool-kit, these catalysts should now also be considered when a regioselective reaction is desired.



Scheme 1.

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Scheme 2.

2. Results and discussion

In this paper we describe a biocatalytic method to obtain monoesters of (*Z*)-2-methyl-butenedioic acid (citraconic acid) by using pig liver esterase (PLE).[†] In addition the esterase's (PLE) selectivity towards diesters of the (*E*)-isomer, mesaconic acid is determined. The substrates and reactions are shown in Schemes 1 and 2. There was, as expected, a considerable variation in reaction rates with respect to the length of the alcohol moiety. For both isomers the reaction rate decreases as the alcohol moiety increases. Generally the (*E*)-isomers hydrolyse faster (5–15 times) than the (*Z*)-isomers, thus making PLE appropriate for separation of (*Z*)- and (*E*)-diesters in a mixture. Under the present reaction conditions the relative reaction rate for the (*E*)-isomers varies by a factor of 2 (methyl:ethyl:propyl=3:2:1.5) whereas for the *Z*-isomers this factor reaches 10 (methyl:ethyl:propyl=1:0.2:0.1). The rate of the enzymatic hydrolysis of the butyl esters was too low to be measured here. Although PLE has previously been shown not to hydrolyse a dipropyl ester of 3,3-dimethyl-1,2-carboxylic acid,¹⁴ the 'similar' dipropyl esters of **1** and **4** were hydrolysed in our experiment.

Table 1 summarises the regioselectivity of PLE with respect to the (*Z*)- and (*E*)-diesters.[‡] In the hydrolysis of the (*E*)-diesters, regioselectivity was relatively low and did not vary with the alcohol part. However, for the (*Z*)-isomers the ester group in the least substituted environment was preferred in the enzymatic hydrolysis giving mainly compound **3** as product. It is also striking how the selectivity of the enzyme varies with the alco-

[†] Enzymatic hydrolysis was conducted by addition of PLE in (NH₄)₂SO₄ (25–200 μl) to the diesters of (*E*)- and (*Z*)-2-methyl-butenedioic acid (1.2–2.4 mmol) in K₂HPO₄ (0.1 M, 100 mL) at rt. The extent of hydrolysis was monitored by either titration with 1.0 M NaOH or by GC analysis (after acidification to pH 2 and extraction of mono- and diesters into Et₂O).

[‡] Regioselectivity was determined by ¹H NMR (400 MHz) by integration of the olefinic proton signals for the methyl, ethyl and propyl esters of **5** and **6** and for the ethyl and propyl esters of **2** and **3**. This method was not conclusive for the methyl esters of **2** and **3**, nor was HMBC NMR due to too close chemical shifts of the carbonyl-Cs (169.21 and 169.23 ppm). The methyl ester of compound **3** was selectively reduced according to microscale adaptations of procedures in Ref. 15. The reduction products were identified (as the thermally ring-closed lactone) by GC/MS analysis and confirmed by comparison with the authentic lactone, 2-methyl-2(5*H*)-furanone. This confirmed the methyl ester of compound **3** as the main enzymatic hydrolysis product.

Table 1. Regioselectivity of the hydrolysis of diesters of (*Z*)- and (*E*)-2-methyl-butenedioic acids (citraconate and mesaconate) by PLE measured at 80–90% conversion (mol monoester/mol diester initially)

	Me	Et	Pr
(<i>Z</i>)-ester (2:3)	5:95	50:50	15:85
(<i>E</i>)-ester (5:6)	65:35	60:40	60:40

hol moiety for the (*Z*)-diesters. The methyl and propyl diesters are hydrolysed with very high selectivity (95:5, 85:5), whilst for the ethyl diester the enzyme shows practically no discrimination between the ester groups.

3. Conclusion

Diesters of (*Z*)- and (*E*)-2-methyl-butenedioic acid were hydrolysed with PLE. The enzyme showed little regioselectivity for the (*E*)-diesters. The (*Z*)-isomers were, however, hydrolysed with high regioselectivity for the dimethyl and dipropyl diesters.

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