Chemoenzymatic Preparation of Atrolactic and Mosher's Acid using *Aspergillus oryzae* Protease

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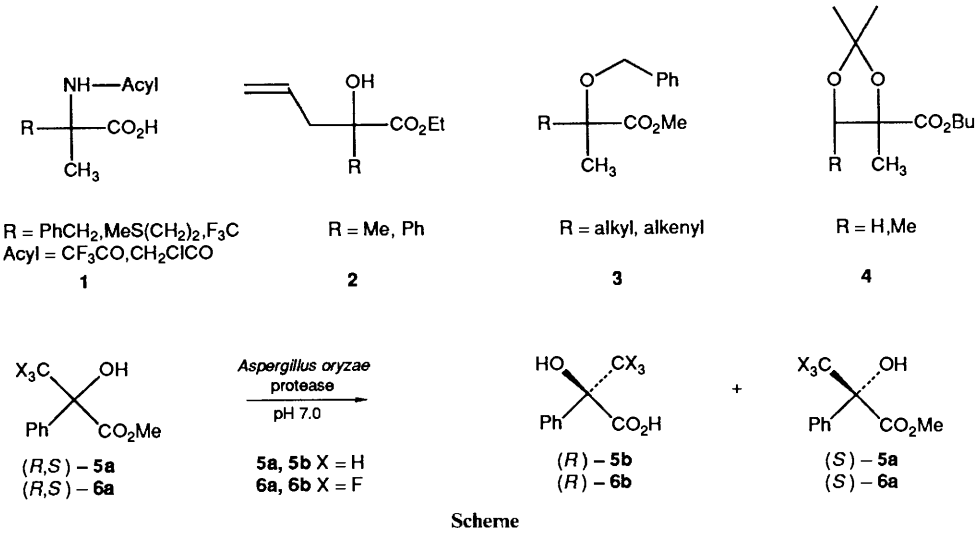
Sterically demanding α-methyl- and α-trifluoromethyl-mandelic esters **5a** and **6a** were conveniently resolved on a large scale by a protease from *Aspergillus oryzae* leading to both enantiomers of atrolactic acid **5b** and a precursor of Mosher's acid **6b** in 75–88% e.e. Single recrystallisation led to optically pure material.

Biocatalytic resolution of esters using hydrolytic enzymes has emerged as a powerful tool for the preparation of optically active alcohols and carboxylic acids.[1] While the majority of lipases, esterases and proteases seem to be almost unlimited in scope as long as the centre of chirality still bears a hydrogen atom as in the case of esters of secondary alcohols or α-monosubstituted carboxylates, the resolution of their higher substituted analogues such as esters of tertiary alcohols and α,α-disubstituted carboxylates remains a challenge. In general, hydrolases do not easily accept such sterically demanding substrates. Thus, in contrast to the former types of substrates,[1] only few examples of enzymatic resolution are reported for the latter. For instance, α-methyl-α-amino acids have been resolved using acylase I from hog kidney *via* their *N*-acyl derivatives **1**.[2] For α-hydroxy-α-methyl carboxylates **2**[3] or derivatives thereof such as **3**[4] and **4**[5] pig liver esterase and *Candida cylindracea* (CC) lipase were used, respectively. Prochiral α,α-disubstituted malonates were asymmetrically hydrolysed by a number of enzymes such as PLE,[6] CC lipase[7] and α-chymotrypsin.[8] Some of these methods, however, are impeded by slow reaction rates[2,4,5] or insufficient selectivity of the enzyme[3] depending on the type of substrate structure.

During a screening for novel enzymes possessing unusual properties for the biotransformation of non-natural organic compounds we found that a protease from *Aspergillus oryzae** is a useful biocatalyst for the resolution of α-substituted mandelic esters such as atrolactic[9] **5b** and a precursor of Mosher's acid **6b**.[10,11] The *O*-methyl derivative of **6b** is a powerful chiral auxiliary for the determination of the optical purity of alcohols and amines.[10,12]

Among the hydrolytic enzymes tested, methyl atrolactate (*RS*)-**5a** was hydrolysed by crude porcine pancreatic lipase,† α-chymotrypsin‡ and *Aspergillus oryzae* protease† with moderate selectivity leading to acid (*R*)-**5b** and ester (*S*)-**5a** in fair optical purity. In contrast, the sterically closely related trifluoromethyl ester (*RS*)-**6a** was only accepted by proteases from *Aspergillus oryzae*, *Aspergillus sojae*§ and subtilisin,§ while the former exhibited good selectivity (E = 26). By this means, multigram batches of (*RS*)-**6a** were conveniently resolved using a known two-step technique[13] in order to obtain an optimum in chemical and optical yield. Both (*R*)-**6b** and (*S*)-**6a** were

* Sigma Chem. Co., type XXIII.
† Sigma Chem. Co., type II.
‡ Protease N, Amano Pharm. Co.
§ Sigma Chem. Co., type XIX.



Scheme

Table 1 Enzymatic hydrolysis of α-substituted mandelic esters

Substrate	Enzyme	Conversion (%)	Acid	E.e. (%)	Ester	E.e. (%)	E[15]
(*RS*)-**5a**	Porcine pancreatic lipase †	55	(*R*)-**5b**	63	(*S*)-**5a**	82	11
(*RS*)-**5a**	α-Chymotrypsin †	52	(*R*)-**5b**	58	(*S*)-**5a**	63	7
(*RS*)-**5a**	*Aspergillus oryzae* protease *	48	(*R*)-**5b**	75	(*S*)-**5a**	70	14
(*RS*)-**6a**	Subtilisin ‡	49	(*R*)-**6b**	25	(*S*)-**6a**	25	2
(*RS*)-**6a**	*Aspergillus sojae* protease §	57	(*R*)-**6b**	17	(*S*)-**6a**	20	1.5
(*RS*)-**6a**	*Aspergillus oryzae* protease *	40	(*R*)-**6b**	88			26
		60			(*S*)-**6a**	88	
(*RS*)-**6a**	*Aspergillus oryzae* protease[a]	44	(*R*)-**6b**	73	(*S*)-**6a**	58	11

[a] Immobilized on VA-Epoxy Biosynth.[14]

obtained in 88% e.e. Single recrystallization of the highly optically enriched acids **5b** and **6b** led to enantiomerically pure material. Chemical transformation of (*S*)-**6a** and (*R*)-**6b** following known procedures[10] gave both enantiomers of Mosher's acid in good yield. When *Aspergillus oryzae* protease was covalently immobilized onto an epoxy resin[14] in order to facilitate reusability of the enzyme and extractive work-up, the selectivity dropped considerably.

The results presented here show that the protease from *Aspergillus oryzae* is a biocatalyst particularly useful for the resolution of sterically demanding α,α-disubstituted carboxylates. Further studies on the unusual properties of this enzyme are in progress.

Experimental

Synthesis of Substrates.—Acids (*RS*)-**5b** and (*RS*)-**6b** were obtained in a one-pot reaction from acetophenone or its α,α,α-trifluoro derivative, respectively, *via* the corresponding cyanohydrins which, in turn, were hydrolysed without purification.[9,10] Standard esterification (excess of MeOH, saturated with gaseous HCl, 12 h) gave substrates (*RS*)-**5a** (51%), b.p. 124–126 °C/14 mmHg, 83–85 °C/1.3 mmHg[16] and (*RS*)-**6a** (82%), b.p. 85 °C/2 mmHg.[10]

Enzymatic Hydrolysis.—Substrate ester (*RS*)-**5a**/(*RS*)-**6a** (6 g) was added to a vigorously stirred solution of *Aspergillus oryzae* protease † (3 g) in phosphate buffer (0.1 mol dm^{-3}, pH 7.0; 150 ml) while the pH was kept constant by autotitration. At the appropriate conversion which was reached after 6–8 h ester (*S*)-**5a**/(*S*)-**6a** was extracted with CH_2Cl_2 (2 × 100 ml, recovery rate 90–95%). The aqueous phase was then acidified to pH < 2 and acid (*R*)-**5b**/(*R*)-**6b** was extracted with ether (3 × 100 ml, recovery rate 70–80%). Single recrystallization of acids **5b** and **6b** from toluene–hexane gave optically pure material.

Optical purities were determined by ^1H and/or ^{19}F NMR spectroscopy of methyl esters **5a** and **6a** using [Eu(hfc)$_3$]. Analytical samples of acids **5b** and **6b** were esterified (CH_2N_2–ether) prior to analysis.

Compound (*R*)-**5b**: m.p. 111 °C, $[\alpha]_D^{20}$ −28.0° (*c* 1.13, EtOH), 75% e.e. {lit.,[17] m.p. 115 °C, $[\alpha]_D^{25}$ −35.4° (*c* 3.5, EtOH)}.

Compound (*S*)-**5a**: $[\alpha]_D^{20}$ +4.57° (*c* 7.9, EtOH), 82% e.e. {lit.,[17] $[\alpha]_D^{25}$ +5.0° (*c* 5.2, EtOH)}.

Compound (*R*)-**6b**: m.p. 117–23 °C, $[\alpha]_D^{20}$ +27.3° (*c* 0.80, MeOH), $[\alpha]_D^{20}$ −21.8 (*c* 0.65, $CHCl_3$), >99% e.e. {lit.,[10] m.p. 123–124 °C, $[\alpha]_D^{20}$ −22.5 (*c* 2.70, $CHCl_3$)}.

Compound (*S*)-**6a**: $[\alpha]_D^{20}$ +26.3° (*c* 0.7, $CHCl_3$), e.e. 88% {lit.,[10] $[\alpha]_D^{19}$ +6.90° (neat, *l* = 1) e.e. 40%}.

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