Resolution of Mandelic Acids by Lipase-catalysed Transesterifications in Organic Media: Inversion of Enantioselectivity mediated by the Acyl Donor

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The lipase-catalysed highly enantioselective deacylation and acylation of mandelic acid derivatives and the inversion of enantioselectivity mediated by the acyl donor are described.

Mandelic acids are useful chiral reagents, and their enzymatic resolution has been attempted *via* the carbonic anhydrase-catalysed enantioselective hydrolysis of their esters.[1] However, the enantioselectivities displayed by this enzyme were only moderate. The interest of mandelic acids as substrates for lipase-catalysed enantioselective reactions arises from the presence of a hydroxy and a carboxy group at the chiral centre. Here we restrict our attention to two types of transesterifications in organic media, *i.e.*, the deacylation of the acylated hydroxy group with alcohols and the acylation of the free hydroxy group with enol esters. Of these two strategies, the latter has been considered inapplicable to mandelates[2,3] because they are sterically hindered secondary alcohols which do not react with enol and trihalogenoalkyl esters usually employed in the lipase-catalysed transesterification. Here we report that this very strategy is fully effective for the resolution of mandelic acids and, moreover, that the acyl donor can invert the enantioselectivity of the lipase-catalysed reaction.

Initially, attempted deacylation of methyl *O*-acylated mandelates with such primary alcohols as methanol and butanol in isopropyl ether using lipases from a variety of sources resulted in rather slow reactions. The combination of the *O*-acyl group of the mandelate and the alcohol as a nucleophile had a profound effect on the conversion rate, but a limited effect on the enantioselectivity of the reaction. Of the enzymes tested, lipases from *Pseudomonas cepacia* (Amano P) and *Penicillium roqueforti* (Amano R) showed high to excellent enantioselectivities ($E^4 > 30$). The enantiodiscrimination displayed by lipases from *Candida cylindracea* (Sigma Type VII) and *Candida rugosa* (Amano AY) was generally only moderate with *p*-substituted mandelic acid derivatives.† Moreover, some of the lipases were found to catalyse the transesterification not only at the acyloxy group but also at the alkoxycarbonyl group, which made the reaction complicated. Table 1 summarises the results of the deacylation of methyl *O*-butyrylmandelates **1a–e** with butanol catalysed by *P. roqueforti* lipase [eqn. (1)]. The free (*S*)-mandelates **2a–e**‡ with ⩾97% e.e. (enantiomeric excess) were obtained in all the cases examined ($E > 100$). Especially noteworthy is the formation of the mandelates **2b–d** carrying a halogen substituent in an optically pure form.

Table 1 *Penicillium roqueforti* lipase[a]-catalysed deacylation of **1** with butanol in isopropyl ether[b]

Substrate **1**	% Convn.	Time (h)	% ee of **2**
a	30	178	97 (*S*)
b	40	114	>99 (*S*)
c	42	111.5	>99 (*S*)
d	44	181.5	>99 (*S*)
e	28	155	98 (*S*)

[a] Amano R. [b] Reaction conditions: see Experimental section.

Next, we turned our attention to the acylation of mandelates carrying a free OH group with enol esters.[5] Some 10 lipases from microbial and pancreatic origins were screened for the acylation of methyl mandelate **2a** with vinyl acetate in isopropyl ether. At last we found out some lipases catalytically active in the acylation. The conversion rate and the enantioselectivity, including even the stereochemical preference (*R* or *S*), varied largely with the enzyme used. Of the enzymes tested, lipases from *Pseudomonas sp.* (Amano AK), *P. cepacia* (Amano P), and *Chromobacterium viscosum* (Toyo Jozo LP) showed rather high activities and enantioselectivities. Both of them were affected by the solvent and the ester moiety of the mandelate. The enantioselectivity was high in such solvents as isopropyl ether and benzene with moderate polarity. Of the esters tried, the methyl ester, *i.e.* methyl mandelate as a substrate, gave the best result. Based on these results, the acylation of methyl *p*-substituted mandelates **2b–e** with vinyl acetate in isopropyl ether was examined in the presence of *Pseudomonas sp.* lipase [eqn. (2)]. The results are summarised in Table 2. In this strategy, the free (*R*)-mandelates **2a–e** with ⩾98% ee were obtained as recovered substrates at *ca.* 60% conversion in all the cases (entries 1, 8, 10, 12 and 14). In the *Pseudomonas sp.* lipase-catalysed deacylation of *O*-acylated mandelates with primary alcohols, the free (*S*)-mandelates were preferentially obtained as in the *P. roqueforti* lipase-catalysed deacylation mentioned above. This means that the (*S*)-form of the mandelates reacted preferentially in both types of transesterifications, *i.e.* deacylation and acylation, when the same enzyme was used. In this case also, the halogen-substituted mandelates **2b–d** were obtained in an optically pure form. Furthermore, we succeeded in obtaining the (*R*)-mandelates with >99% ee by the gram-scale reactions in isopropyl ether using the *Pseudomonas sp.* lipase (see Experimental section for an example).

During the course of the investigation, we examined also the effect of acyl donors on the enantioselectivity and the reaction rate of the acylation. This kind of work in which a variety of acyl donors are compared systematically has scarcely been tried so far in the transesterification,[6] though the acyl donors employed must play an important role in the reaction. In particular, little

† According to ref. 3*a*, *C. cyclindracea* lipase showed a high enantioselectivity ($E = 54$) in the deacylation of the non-substituted substrate, *i.e.* butyl *O*-acetylmandelate, with butanol in isopropyl ether (7 days for 45% conversion). We found that the combination of an *O*-butyryl derivative and butanol enhanced significantly the rate of *C. cylindracea* lipase-catalysed deacylation. The conversion was further accelerated by the use of *C. rugosa* lipase (40% conversion in a few hours).

‡ The absolute configuration was determined by comparison of the observed specific rotation of the isolated product with the reported value in the literature.[1]

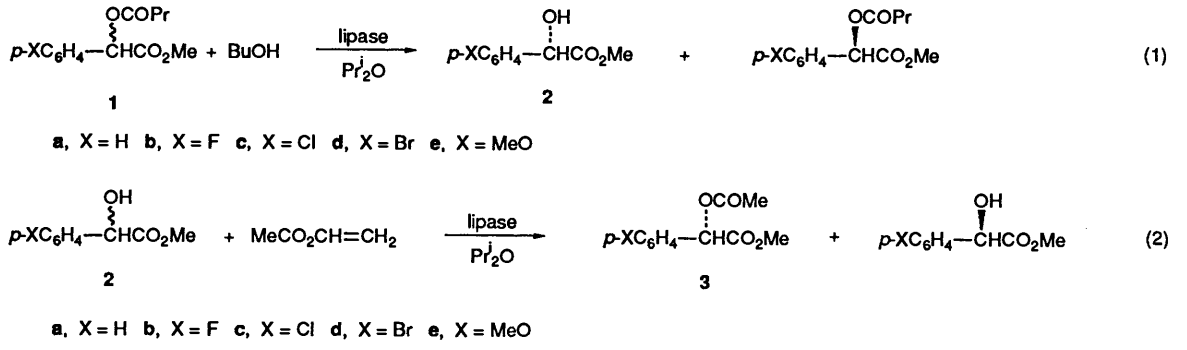

Table 2 *Pseudomonas sp.* lipase[a]-catalysed acylation of **2** in isopropyl ether[b]

Entry	Substrate	Vinyl ester as acyl donor	% Convn.	Time (h)	% ee of **2**[c]
1	**2a**	Acetate	61	192[d,e]	>99 (*R*)
2	**2a**	Propionate	57	216[d]	>99 (*R*)
3	**2a**	Butyrate	53	15 days[d]	>99 (*R*)
4	**2a**	Laurate	55	20 days[d]	>99 (*R*)
5	**2a**	Palmitate	56	25 days[d]	97 (*R*)
6	**2a**	Chloroacetate	57	48	33 (*S*)
7	**2a**	Isopropenyl acetate	64	192[d]	>99 (*R*)
8	**2b**	Acetate	60	90	>99 (*R*)
9	**2b**	Chloroacetate	59	96	11 (*S*)
10	**2c**	Acetate	58	72	>99 (*R*)
11	**2c**	Chloroacetate	59	120	13 (*S*)
12	**2d**	Acetate	56	90	>99 (*R*)
13	**2d**	Chloroacetate	55	106	7 (*S*)
14	**2e**	Acetate	63	90	98 (*R*)
15	**2e**	Chloroacetate	56	48	23 (*S*)

[a] Amano AK. [b] Reaction conditions: see Experimental section. [c] Enantiomeric excess of the recovered substrate. [d] Using 10 mg of enzyme. [e] 72 h for 60% conversion with 30 mg of enzyme.

is known about their effects on the enantioselectivity. The results in Table 2 obtained using different vinyl esters (entries 1–5) indicate that the chain length of the fatty acid moiety of the enol esters produces a marked effect on the conversion rate, but a marginal effect on the enantioselectivity of the acylation.* The vinyl esters carrying a shorter alkyl chain served as better acyl donors than those carrying a longer alkyl chain. Almost no difference was found in the conversion rate and the enantioselectivity between the different enol esters as acyl donors, *i.e.* vinyl acetate *vs.* isopropenyl acetate (entries 1 and 7). On the other hand, the result obtained using vinyl chloroacetate (entry 6) was surprising: the stereochemical preference of the enzyme was inverted from *S* to *R*, though the ee value became rather low. The inversion of enantioselectivity from *S* to *R* was also observed in the acylations of other mandelates **2b–e** by switching the vinyl ester from acetate to chloroacetate (entries 8–15). This must be the first example which shows that the acyl donor can invert the enantioselectivity of the lipase-catalysed transesterification and is of interest in connection with the elucidation of the factors controlling the lipase's enantioselectivity.

Experimental

Lipase-catalysed Deacylation of Methyl O-*Butyrylmandelates* **1** *with Butanol.*—In a typical experiment, a solution of **1** (0.25 mmol) and butanol (0.75 mmol) in isopropyl ether (0.8 cm^3) was stirred with a lipase preparation (80 mg) at 25 °C. The progress of the reaction was monitored by HPLC using an ODS column (mobile phase, MeOH aq.). After *ca.* 40% conversion, the reaction was stopped by filtering off the enzyme. The ee value of the resulting methyl mandelates **2** was determined by chiral HPLC analysis using Chiralcel OD (Daicel Chemical Co.) (mobile phase, hexane–propan-2-ol).

Lipase-catalysed Acylation of Methyl Mandelates **2**.—In a typical experiment, a solution of **2** (0.3 mmol) and an enol ester (0.9 mmol) in isopropyl ether (0.7 cm^3) was stirred with a lipase preparation (30 mg) at 25 °C. After *ca.* 60% conversion, the reaction was stopped and the ee value of the recovered mandelates **2** was determined as above.

Gram-scale Preparation of Methyl (R)-*Mandelate* [(R)-**2a**].—To a solution of methyl mandelate **2a** (3.0 g, 18 mmol) and vinyl acetate (4.7 g, 55 mmol) in isopropyl ether (50 cm^3) was added lipase Amano AK (1.5 g). The resulting mixture was stirred at 25 °C for 90 h (59% conversion). The enzyme was filtered off and the filtrate was concentrated. The residual oil was subjected to flash chromatography (hexane–ethyl acetate, 10:1, v/v) to give (*R*)-**2a** as white crystals (1.1 g, 37%;† >99% ee), $[\alpha]_D^{25}$ −115.4 (*c* 1.0 in acetone).‡

Acknowledgements

We thank Amano Pharmaceutical Co. and Toyo Jozo Co. for

* The effect of the chain length of fatty acids used as acyl donors on the esterification of menthol catalysed by immobilised lipase from *C. cylindracea* (Meito OF 360) has been investigated.[7] The reported results are different from ours in that shorter-chain fatty acids were poor substrates and the enantioselectivity was affected markedly by the chain length of the fatty acid, a high enantioselectivity being observed with shorter-chain acids. The discrepancy may be attributable to the difference of the type of reactions (transesterification *vs.* esterification) and the nature of the enzymes used.

† The theoretical maximum yield is 41%.

‡ A value of −110.5 was obtained for a sample prepared from (*R*)-mandelic acid purchased from Tokyo Chemical Industry Co.

the generous gift of lipases. We also thank Mr. T. Ishihara for his technical assistance.

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Paper 2/03583I
Received 7*th July* 1992
Accepted 16*th July* 1992