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ENZYMES IN ASYMMETRIC SYNTHESIS: EFFECT OF REACTION MEDIA ON THE PLE CATALYSED HYDROLYSIS OF DIESTERS[1]

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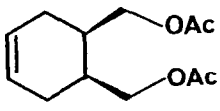
Summary: *Organic cosolvents and other addenda can influence the rate and the enantioselectivity of pig liver esterase catalysed hydrolysis of meso diacetates and diesters, the magnitude of the effect being strongly dependent on the nature of substrate.*

Dissymmetrization of meso diesters and diacetates through enzyme catalysed hydrolysis is a powerful tool for preparation of chiral "building blocks" to be used in the synthesis of natural products.[3] In particular, pig liver esterase (PLE) has been extensively employed due to its wide specificity. Unfortunately, however, enantiomeric excesses are not always high, thus limiting the enzyme's potential utility.

SCHEME



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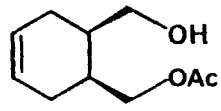

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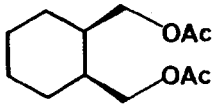

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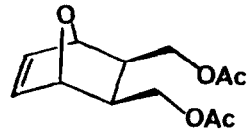

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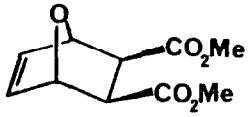

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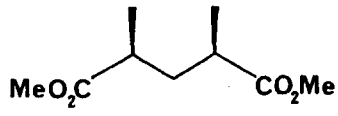

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Quite recently better results have been achieved with pig pancreatic lipase (PPL).[4,5] However this method is limited to the hydrolysis of acylated meso diols, and the enantiomer obtained is often opposite to that formed with PLE.

Generally reactions employing PLE are carried out in water with, occasionally, some amount

of organic solvent added with the purpose of dissolving the substrate.[6-8] However, it is known that PLE is a mixture of five isoenzymes[9] which, in principle, could respond differently to changes in reaction conditions. Starting from this consideration and with the purpose to collect some information on the problem of the relationship between solvent composition and enzyme's stereospecificity[10] we have started a study on the effect of various addenda on the rate and enantioselectivity of PLE catalysed hydrolysis of some representative meso diesters, chosen for their importance with respect to synthetic applications.

First we performed our experiments on the meso diacetate **1**,[11] whose PLE catalysed hydrolysis was known to give (1S,2R) monoacetate **2** with moderate enantioselection (40%) and yield (43%).[4] We were particularly interested in the production of (1S,2R) **2** because it is a possible starting material for the synthesis of (-)-*allo*-yohimbane.[13]

The results of hydrolysis of **1** with PLE under various conditions are reported in Table 1. In all cases the reaction was stopped after consumption of 1 eq. of NaOH. The data show that the addition of organic solvents to water provoked a decrease in the rate of hydrolysis, accompanied by an increase of chemical yields and asymmetric induction. The most striking result was obtained with *t*-butyl alcohol: in the presence of 10% of this cosolvent we were able to prepare **2** on a gram scale in 75% isolated yield and with an enantiomeric excess of 96%.

Table 1. Enantioselective hydrolysis of meso diacetate 1 with PLE[a]

Entry	Conditions	Reaction time (h)	Relative rate	Yield[b]	e.e.[c,d]
1	H_2O	0.98	1	60%	55%[e]
2	H_2O : DMSO 8:2	1.40	0.70	62%	59%
3	H_2O : DMSO 6:4	3.45	0.28	72%	72%
4	H_2O : DMF 8:2	2.78	0.35	74%	83.5%
5	H_2O : DMF 6:4	f	-	-	-
6	H_2O : DMEU[g] 8:2	9.16	0.11	63%	61%
7	H_2O : *t*-BuOH 95:5	1.40	0.70	76%	93.5%
8	H_2O : *t*-BuOH 9:1	2.23	0.44	78%	96%
9	0.5 M LiCl in H_2O	1.42	0.69	66%	65%
10	albumine[h] in H_2O	0.75	1.31	63%	78%
11	albumine[h] in H_2O : *t*-BuOH 9:1	1.62	0.61	88%	94%

a: All reactions were performed at 32.0°C, pH 7 (maintained by an automatic burette), on 50 mg of substrate using 7 ml of solvent and 110 units of PLE (Sigma)(2.2 units/mg substrate), and were stopped after consumption of 1 eq. of NaOH. b: Determined by g.l.c. as ratio (monoacetate)/(diacetate + monoacetate + diol) using a RSL 150 Capillary column (T = = 180°C); isolated yields are usually 5% lower. c: Determined by ^1H n.m.r. in the presence of $Eu(hfc)_3$ on the chromatographed product. d: (1S,2R) monoacetate was always preferentially formed (see ref. 4, 5). e: The difference between this value and that reported by Schneider (ref. 4) may be due to the different enzyme employed (Boehringer). f: Extremely slow reaction. g: DMEU = dimethyl ethylene urea (1,3-dimethyl-2-imidazolidinone). h: 210 mg of bovine serum albumine were used.

We also examined the effect of additives of different natures. The addition of an inorganic salt (LiCl) caused a slight increase of e.e. (entry 9), while albumine[14] was responsible for a remarkable enhancement of both rate and asymmetric induction.

Since the most impressive effect was shown by *t*-butyl alcohol, we examined the effect of this cosolvent on PLE catalysed hydrolysis of other substrates. Table 2 reports the relative rates and the stereoselectivities in pure water and water : *t*-butyl alcohol 9:1, for diesters **3** - **6**.[15] In every case a decrease of reactivity was observed, while the effect on the enantioselectivity does not appear to follow a general trend. Although a moderate gain in enantioselectivity occurred for **4**, no significant change was observed for **5** and **6**, while the e.e. dropped from 12% to nearly 0 in the case of **3**.

Table 2. Enantioselective hydrolysis with PLE of a series of meso diacetates and diesters[a]

Entry	Substrate	Conditions	Reaction time (h)	Enzyme/substrate ratio (units/mg)	Relative rate	e.e. (%)
1	**3**	H_2O	3.9	10	1	12[b, c]
2	**3**	H_2O : *t*-BuOH 9:1	5.53	10	0.70	0[b]
3	**4**	H_2O	0.40	0.6	1	57[b, d]
4	**4**	H_2O : *t*-BuOH 9:1	3.16	0.6	0.06	68[b, d]
5	**5**	H_2O	9	0.8	1	78[e, f]
6	**5**	H_2O : *t*-BuOH 9:1	11	0.8	0.80	82[e, f]
7	**6**	H_2O	0.72	0.65	1	60[e, g]
8	**6**	H_2O : *t*-BuOH 9:1	1.42	0.65	0.73	54[e, g]

a: All reactions were performed at 32°C and pH 7 with an automatic burette; in the case of **3** and **4** they were stopped after consumption of 1 eq. of NaOH. **b**: Determined by ^1H n.m.r. in the presence of $Eu(hfc)_3$. **c**: (1R,2S) monoacetate was preferentially obtained (ref. 4, 5). **d**: Absolute configuration was not established. **e**: Determined by ^1H n.m.r. in the presence of $Eu(hfc)_3$ of the lactone obtained through $LiBH_4$ reduction. **f**: (1R,2S,3R,4S) monoester was preferantially obtained (ref. 16). **g**: (2S,4R) monoester was preferentially formed (ref. 19).

The collected results suggest that organic cosolvents as well as other addenda can affect the enantioselectivity and the rate of PLE catalysed hydrolysis of meso diesters, this effect being strongly dependent on the nature of substrate. Studies for gaining more insight into this effect are in progress in our laboratory.

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References and Notes

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15. **3** was synthesized by acetylation (Ac_2O, Et_3N, DMAP, CH_2Cl_2) of the commercially available diol. **5** was prepared, according to ref. 16, from the corresponding anhydride (ref. 17). $LiAlH_4$ reduction of **5** in THF, followed by acetylation (Ac_2O, Et_3N, DMAP, CH_2Cl_2), gave **4**. Finally **6** was prepared from the corresponding anhydride (ref. 18) by treatment with MeONa and Me_2SO_4 in MeOH.
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