The Esterase Catalysed Resolution of Lactones and Spirodilactone

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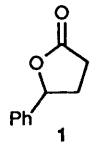
The pig liver esterase catalysed hydrolysis of spirodilactone and γ-phenyl-γ-butyrolactone gives optically active products (>90% e.e., after 50% reaction). However, the similarly substituted acyclic ester analogues of the lactone do not exhibit enantioselectivity suggesting that the acylation step shows the selectivity. Racemic spirodilactone can be converted, in principle, entirely into one of its enantiomers.

Although pig liver esterase does not readily catalyse the hydrolysis of simple unsubstituted lactones, *e.g.* β-propiolactone, γ-butyrolactone, δ-valerolactone and ε-caprolactone, we have shown that this enzyme does hydrolyse dihydrocoumarin and substituted cyclic carbonates[1] and β-lactams.[2] A comparison of the selectivity of enzymes formally labelled as esterases is of interest because of the different ground state conformations of acyclic esters—usually *Z*—compared with that of lactones—usually *E*. There is therefore the possibility of differential binding of the alcohol and carboxylic acid residues in esters and lactones.

Pig liver esterase catalyses the hydrolysis of γ-phenyl-γ-butyrolactone, **1**, with a k_{cat}/K_m of 1.94×10^3 mol^{-1} dm^3 s^{-1} at pH 7.4 and 25 °C. The enzyme is highly enantioselective as judged by NMR, the kinetics effectively terminating after 50% reaction. When the racemic mixture is hydrolysed, the unreacted lactone $[\alpha]_D^{20}$ −6.1 (*c* 1, $CHCl_3$) can be recovered after 50% reaction (90% yield, >94% e.e.). The product hydroxy acid of the hydrolysed *S*-lactone can be relactonised at pH 2 and recovered with retention of configuration $[\alpha]_D^{20}$ +6.2 (*c* 1, $CHCl_3$) (80% yield, 94% e.e.). The absolute configuration of the lactone **1** has not been reported but a comparison with other systems would suggest that it is the *S*-lactone which is the substrate.[3]

Interestingly, acyclic esters with similar chiral centres in the alcohol and carboxylic acid residues are not enantioselectively discriminated by pig liver esterase. For example, the racemic γ-substituted methyl butanoate ester **2** is a good substrate



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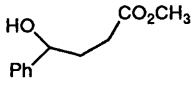

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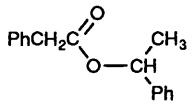

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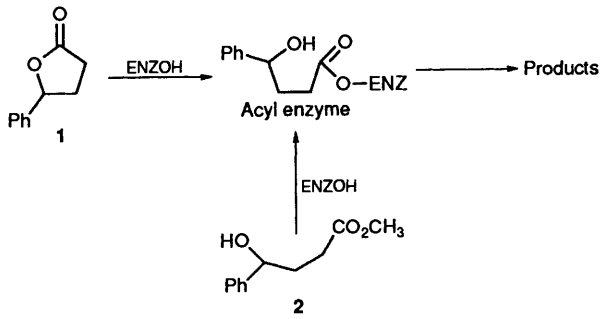
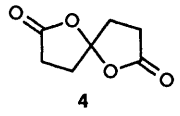
(k_{cat}/K_m = 3.5×10^4 mol^{-1} dm^3 s^{-1} at pH 7.4) as is the ester **3** with a chiral centre at the α-position of the alcohol residue (k_{cat}/K_m = 8.2×10^4 mol^{-1} dm^3 s^{-1}). However, there is no selectivity shown between the enantiomers. Furthermore, lactone **1** and ester **2** are expected to generate similar acyl enzyme intermediates (Scheme 1), suggesting that discrimination occurs at the acylation stage of the hydrolysis of the lactone. There is thus no differential enantioselectivity for the deacylation step whilst that seen for acylation probably results from the different *E*/*Z* conformations of lactones and esters.

The pig liver esterase catalysed hydrolysis of the racemic spirodilactone **4** at pH 7.4 terminates after 50% hydrolysis. The isolated spirodilactone has a $[\alpha]_D^{20}$ 31.9. The spirodilactone has not apparently been previously resolved, but NMR suggests



Scheme 1



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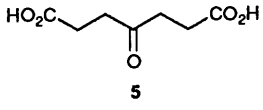

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>90% e.e. The achiral hydrolysis product, ketopimelic acid, **5**, may be relactonised to generate the racemic lactone[4] which may be resubjected to further esterase catalysed hydrolysis. Three cycles of this procedure led to 75% conversion (w/w) of the racemic spirolactone **4** into one of its enantiomers.

Experimental

Kinetics.—The reaction was monitored by isocratic HPLC using Lichrosorb C18-RP and eluting with 50% acetonitrile–water for the γ-phenyl-γ-butyrolactone but with 35% acetonitrile–water for the spirodilactone. Reaction conditions gave a good first-order disappearance of reactant and appearance of product, and the second-order rate constant k_{cat}/K_m was obtained from k_{obs}/[enzyme].

Resolution.—Racemic γ-phenyl-γ-butyrolactone (1 g, 6.17 mmol) was dissolved in 50 cm^3 of acetonitrile and made up to 1 dm^3 with pH 7.4 phosphate buffer. 1.0 cm^3 of pig liver esterase solution (100 mg protein/9.1 cm^3) was then added and the reaction monitored until 50% hydrolysis by HPLC. The unreacted lactone was then extracted with ether (3 times) and dried over magnesium sulfate. The ether was then removed by rotary evaporation to give a white solid which was recrystallised from toluene. The aqueous layer containing the hydroxy acid was adjusted to pH 2.0 and left to stand for 48 h. The lactone formed was then extracted as described above. The enantiomeric excess was determined from the NMR (270 MHz) using tris[3-(heptafluoropropylhydroxymethylene)(+)-camphorato] europium(III).

References

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