

ENZYMATIC RESOLUTION OF RACEMIC LACTONES

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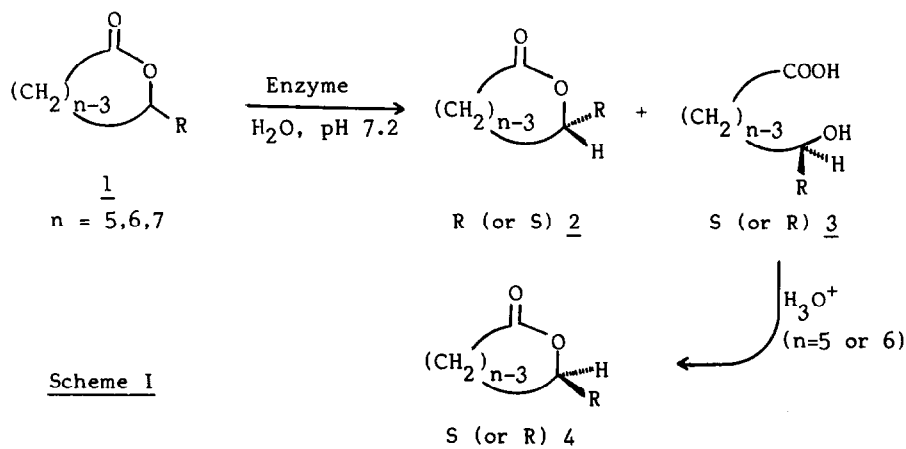
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Abstract : PPL, HLE or PLE enzymatic resolution of racemic γ, δ and ϵ -lactones gives optically active lactones (ee : 60 to 90%).

The search to methods giving chiral lactones with high enantiomeric purity is stimulated by the fact that various lactones are flavor components of fruits and wines (1), pheromones (2) or useful bifonctionnal synthons in organic chemistry (3).

Recently it has been claimed that lipases catalyze intramolecular transesterification of some hydroxyesters and led to optically active lactones (4). Since racemic lactones can be easily obtained we thought that direct enzymatic resolution of these racemates was a promising way.

In this letter we describe our findings concerning the hydrolysis of γ, δ and ϵ -chiral lactones 1 catalyzed by different hydrolases : pig pancreatic lipase (PPL), horse and pig liver acetone powder, ("crude" horse and pig liver esterase, HLE and PLE) : the use of these commercial and inexpensive enzymes allows to isolate R and S lactones 2, 4 from racemic ones 1 with a good enantioselectivity. The main features of our method are outlined in Scheme I and our results are reported in the table.



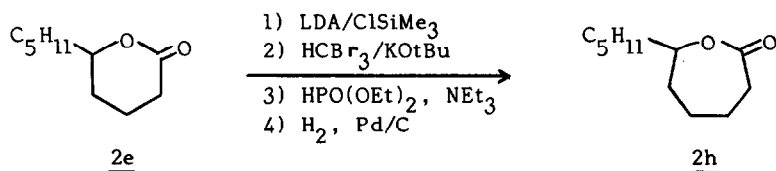
For γ -butyrolactones (entries 1-5) the best resolutions were obtained when the hydrolyses were catalyzed by PPL. The S-lactones are better substrates than their R-enantiomers and the latter are isolated with an enantiomeric excess of 60-76% after 55% conversion. For the more hydrophilic lactone 1a hydrolysis was conducted in the presence of hexane in a buffer solution (Na_2HPO_4 , 0.1 M, pH = 7.2). For the other lactones 1b-1d hydrolyses were conducted in 10% CaCl_2 solution at pH = 7.2. In fact the hydroxy acids 3 inhibit the reaction and a partial precipitation of their calcium salts allows the reaction to go to 55-60% conversion (when the hydrolysis were made in Na_2HPO_4 solution at pH = 7.2, reactions stopped after 30-40% conversion). We have observed that similarly bovine serum albumin (BSA) allows to drive the reaction up to 50% conversion (5). When the hydrolyses were catalyzed by HLE the remaining S lactones were isolated with a low enantioselectivity (entry 3 : ee \geq 15% for lactone 2b).

δ -Valerolactones (entries 6-9) were substrates of the three checked enzymes ; however only HLE gave good resolutions. Interestingly, the R-enantiomers were better substrates of these three enzymes. As for some γ -butyrolactones a better enantioselectivity was observed for the resolution of lactone 1f when the hydrolysis was made in the presence of calcium chloride. At the opposite the lactone 1e was best resolved in the absence of calcium chloride.

After separation, the hydroxyacids 3a-3f gave back the lactones 4a-4f (ee \sim 40-50%) by acidic treatment. The enantiomeric excesses of these lactones 4a-4f could be improved (for example 93% ee for 4c) by a second enzyme-catalyzed hydrolysis stopped at 40% conversion followed by a relactonization.

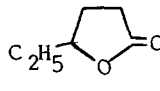
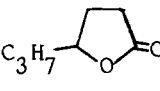
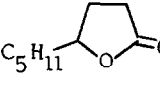
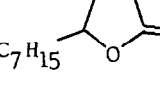
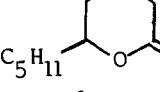
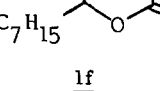
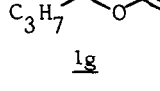
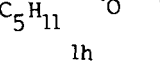
With ϵ -caprolactones (entries 10-13) two enzymes (HLE and PLE) gave satisfactory resolutions but the absolute configurations of the remaining lactones were opposite. The hydroxyacids 3g and 3h (ee \geq 50%) did not give back the ϵ -caprolactones by acidic treatment, however the cyclisation could be made using the Corey's method (8).

The absolute configurations of lactones 2a-2f - 4a-4f were deduced from their optical rotations and comparison with those of the litterature. The configurations of lactones 2h, 4h were found by chemical correlation : (-)-S- δ -decalactone 2e was transformed to (-)-lactone 2h via its silylketene acetal by addition of dibromocarbene (6), bromine reduction (7) and hydrogenation. The configurations of lactones 2g, 4g were deduced from their optical rotations and comparison with those of 2h, 4h.



Experimental part : Lactones 1 (10^{-2} mole) (9) were stirred with 20 ml of 10% CaCl_2 (\sim 2 equivalents) or 0.1M Na_2HPO_4 aqueous solution. The pH was maintained to 7.2 using a pH stat with a 2M NaOH solution. After addition of 1 g of the crude enzyme (13) the hydrolyses were carried out to 55% conversion (12-24 hours). Then the reactions were stopped by filtration on Celite and ether extraction of the remaining lactones 2. Acidification of the Celite cake and the aqueous phase (HCl, pH = 2) followed by ether extraction gave the lactones 4 (entries 1-9) or the hydroacids 3 (entries 10-13). The products were purified by liquid chromatography on silicagel and their optical rotations were

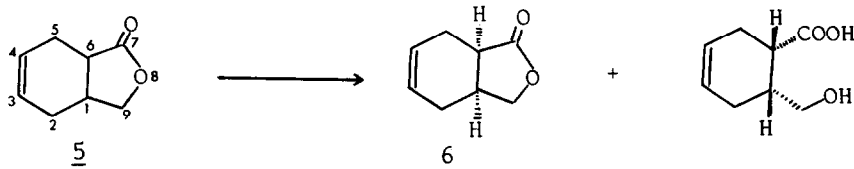
Table

Entries	Lactones <u>1</u>	Reaction conditions ^{a)}	Lactones <u>2</u>			Configurations
			$[\alpha]_D$ (Ref.)	$[\alpha]_D$ ^(b)	ee (%) ^c	
1	 <u>1a</u>	PPL, H ₂ O buffer 7.2 hexane	+47.4 (10)	+42.8	75 (90)	R
2	 <u>1b</u>	PPL, H ₂ O, 10% CaCl ₂	+56.2 (10)	+38.0	72 (67)	R
3		HLE, H ₂ O buffer 7.2	-56.2	-7.0	(15)	S
4	 <u>1c</u>	PPL, H ₂ O, 10% CaCl ₂	+48.6 (10)	+33.0	60 (68)	R
5	 <u>1d</u>	PPL, H ₂ O, 10% CaCl ₂	+42.2 (11)	+32.0	76 (76)	R
6	 <u>1e</u>	HLE, H ₂ O buffer 7.2	-60.2 (10)	-48.0	78 (80)	S
7		PPL, H ₂ O buffer 7.2		-27.0	(45)	S
8		PLE, H ₂ O buffer 7.2		-10.8	(18)	S
9	 <u>1f</u>	HLE, H ₂ O, 10% CaCl ₂	-48.8 (12)	-47.5	92 (97)	S
10	 <u>1g</u>	HLE, H ₂ O, 10% CaCl ₂		-15.0	35	(S)
11		PLE, H ₂ O 10% CaCl ₂		+26.7	62	(R)
12	 <u>1h</u>	HLE, H ₂ O, 10% CaCl ₂		-29.3	72	S
13		PLE, H ₂ O, 10% CaCl ₂		+29.3	72	R

a) The hydrolyses were stopped after 55% conversion ; b) in THF (c=2.0) ; c) from NMR spectra of the diol in presence of (+)thfc-Eu. In brackets are reported the ee calculated from the measured $[\alpha]$.

determined in THF ($c=2.0$). The overall recoveries of these reactions were about 80-90% (Yields of lactones 2 were about 35-40%). The enantiomeric excesses were determined by NMR (250 MHz), in the presence of (+)-thfc-(Eu), of the diols prepared by addition of methyllithium (14) to the lactones 2a-h, 4a-f and to the methyl hydroxyesters resulting from the reaction of CH_2N_2 with the hydroxyacids 3g and 3h.

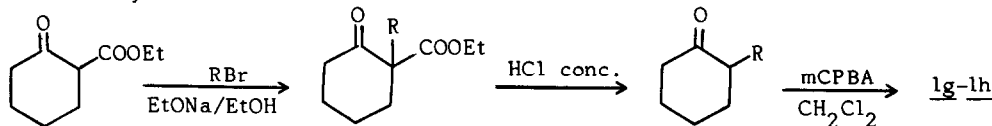
Working with chiral lactones substituted at different positions, we are now investigating the scope of this new method of resolution. For example in the case of the bicyclic lactone 5 we are able at the moment to isolate the (+)-1S-6R lactone 6 with 60% enantiomeric excess.



The progress of our results will be published in due course.

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