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MACROCYCLIC LACTONES VIA BIOCATALYSIS IN NON-AQUEOUS MEDIA

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Summary: The enantiospecificity of lipase-catalyzed lactonization of chiral (w-1)-hydroxy acids to form diolides in non-aqueous medium was investigated.

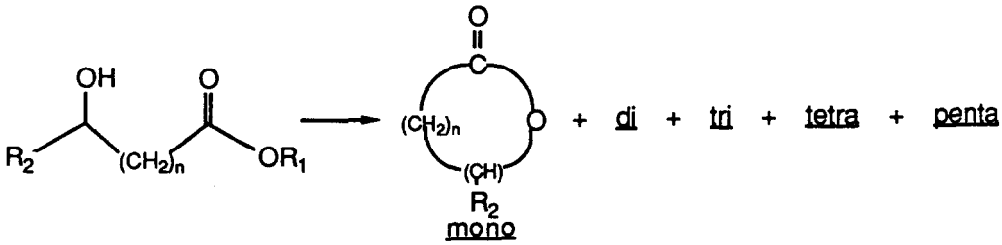
The lipases[1] (triacylglycerol hydrolases EC 3.1.1.3) are uniquely suited for catalyzing preparative enantiospecific esterification and transesterification reactions in non-aqueous media, because they are relatively stable in non-polar organic solvents at moderate temperatures.[2] This methodology has now been successfully employed for the resolution of many chiral acids and alcohols.[3]

Recently, lipases have also been used for the preparative synthesis of macrocyclic lactones via the lactonization of hydroxy acids and esters[4]; or via the direct condensation of diacids with diols[5] in nearly anhydrous organic media. Our interest in exploring the potential synthetic utility of this biocatalytic transformation prompted us to further examine the relationship of substrate structure to the product profile, and to determine whether the intramolecular lactonization reaction proceeds with high degrees of enantiospecificity. The results of these studies constitute the subject of this report.

When 10-hydroxydecanoic acid was exposed to the lipases[6] in anhydrous isooctane, no decanolide was detected; instead, a complex mixture of di-, tri-, tetra- and penta-lactones were formed in different ratios depending on the lipase used. For example, the lipases of *Pseudomonas sp.* (AK and K-10) and porcine pancreas (PPL) afforded di- and trilactones as the major products whereas the lipases of *Candida cylindracea* and *Mucor meihei* gave mostly tri- and tetralactones (Table 1). On the other hand, hexadecanolide was found to be the dominant product after exposure of 16-hydroxyhexadecanoic acid to the lipases, except the lipase of *Mucor meihei* which formed hexadecanodiolide predominantly. These results clearly show that the product profile varies with the length of the hydroxy acid, the lipase, and is considerably more complex than those reported previously by other workers[7].

We then turned our attention to the question of enantiospecificity of the intramolecular esterification reaction. In the case of chiral racemic (w-1)-hydroxy acids, enzymatic lactonization afforded a complex mixture of diastereomeric lactones of varying ring sizes. For example, when (±)-7-hydroxyoctanoic acid was exposed to the lipase of *Pseudomonas sp.* (AK) in anhydrous isooctane at 25°C[10] for 144 hr, a mixture of di- (17%), tri- (7%) and tetra- (2%) lactones were obtained. Careful chromatographic separation and analysis of the dilactone fraction revealed the presence of two diastereomeric lactones, $\widehat{R\text{-}S}$ and $\widehat{R\text{-}R/S\text{-}S}$ in a ratio of 1:2.4 (Table 2).

Table 1. Lipase-catalyzed lactonization of w-hydroxy acids



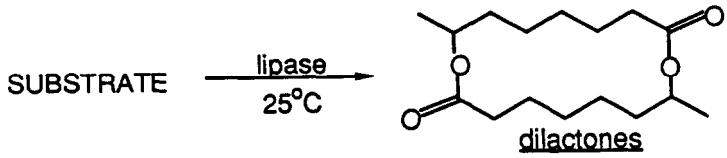
				Isolated yields of lactones (%)				
R_1	R_2	n	Lipase	mono	di	tri	tetra	penta
H	H	8	*Pseudomonas sp.* AK	0	53	16	4	*
			Pseudomonas sp. K-10	0	33	14	15	7
			Porcine pancreas	0	57	20	6	2
			Candida cylindracea	0	4	26	11	*
			Mucor meihei	0	2	20	15	*
H	H	14	*Pseudomonas sp.* AK	66	26	*		
			Pseudomonas sp. K-10	62	30	*		
			Porcine pancreas	46	21	12		
			Candida cylindracea	19	15	9		
			Mucor meihei	3	43	15		

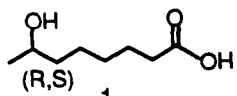
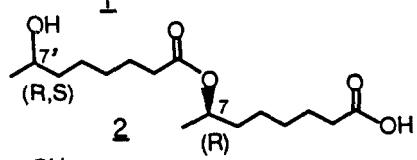
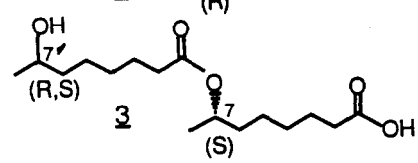
*Trace quantity (by TLC)

To gain a better understanding of the enantiospecificity of the inter- and intramolecular esterification steps, we synthesized[8,9] the pair of diastereomeric acyclic dimeric esters **2** and **3** (optically active at C-7 and racemic at C-7').

The results of the incubation studies after exposure of the diastereomeric dimers to the *Pseudomonas sp.* AK lipase are tabulated in Table 2. The diastereomeric diolides ($\widehat{R\text{-}S}$ vs. $\widehat{R\text{-}R}$ and $\widehat{S\text{-}S}$) were separated by column chromatography[10] and the diolide ($\widehat{R\text{-}R}$ and $\widehat{S\text{-}S}$) was then hydrolyzed with 2N KOH/MeOH (1:1), and converted into methyl-7-hydroxyoctanoate (CH_2N_2/ether); its enantiomeric excess was determined by PMR spectroscopy in the presence of $Eu(hfc)_3$.[10] The relative rates of lactonization of the diastereomeric dimers appear to follow the order: $\widehat{R\text{-}R}$ > $\widehat{R\text{-}S}$ (C-7'-C-7) > $\widehat{S\text{-}S}$ and no cyclization was noted with the $\widehat{S\text{-}R}$ (C-7'-C-7) diastereomer of **2**. While these observations suggest that the configuration of the hydroxyl at the C-7' position prefers to be R, the stereochemistry of the hydroxyl at both chiral centers

Table 2. Enantiospecificity of lipase-catalyzed intramolecular esterification



Substrate	Reaction time (hr)	Isolated yields (%) R-S	Isolated yields (%) R-R & S-S	Ratio of R-R/S-S
 1	144	5	12	96/4
 2	90	0	19	100/0
 3	90	8	2	0/100

appears to influence the lactonization reaction because the S-S diastereomer of **3** was lactonized but the S-R (C-7'-C-7) diastereomer of **2** was not.

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References and Notes

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6. The lipases were purchased from commercial sources: *Pseudomonas sp.* (AK and K-10) and *Mucor meihei* were products of Amano; *Candida cylindracea* OF-360 (Meito Sangyo); porcine pancreatic lipase (PPL) was a product of Sigma. After an extensive investigation of the experimental conditions, the following were found to be optimal: To 500 mg of substrate was added 2 g of lipase in 200 ml of anhydrous isooctane. The reaction mixture was stirred at 65°C for 48 hours. At 45°C, a similar product profile was obtained but the yield of lactonic products was found to be lower. The lactones were isolated by elution of a silica gel (MN Kieselgel 60; 70-270 mesh) column (2x40 cm) with hexane-ethyl acetate (50:1 to 20:1). The PMR spectra of all the lactones in Table 1 exhibited a triplet at 82.3 ppm ($-CH_2-COO-$) and a triplet centered at δ 4.1 ppm ($R_2=H$) (J=6 Hz; $-COO-CH_2-$) or ($R_2=CH_3$) a sextet centered at δ 4.9 ppm (J=6 Hz; $-COOCH(CH_3)-$). All the lactones (mono, di, tri, tetra and penta) exhibited (M+1) and (M-18) peaks. [See: Biemann, K. "Mass Spectrometry Organic Chemical Applications," McGraw-Hill, New York, 1962; pp. 55-56.]

7. It is noteworthy that ref. 4b reported the formation of only mono- and dilactones and that the lipase of *Candida cylindracea* was unable to catalyze lactonization.

8. A. Scilimati, T. Ngooi and C. J. Sih. *Tetrahedron Lett.* (1988) in press.

9. R-ter-Butyl-7-hydroxyoctanoate ($[\alpha]_D^{23}$ -7.8°, c, 0.65 $CHCl_3$; ee >99%) was condensed with (±)-7-tetrahydropyranoxyoctanoate (DCC; DMAP/ethyl ether; 25°C); the protecting groups were removed using HCO_2H at 25°C for 6 hours to yield the diastereomeric dimers **2**. S-ter-Butyl-7-hydroxyoctanoate ($[\alpha]_D^{22}$ +7.8°; ee >99%) was subjected to a similar sequence of reactions for the preparation of the dimers, **3**. The optically-active R- and S-ter-butyl-7-hydroxyoctanoates were prepared using an enzymatic resolution procedure.[8]

10. Several reaction temperatures (25°C, 45°C and 65°C) were examined. The best yield of the dilactones was obtained at 25°C. At higher temperatures, trilactones became the dominant products. The dilactones were separated via flash chromatography over a silica gel (40 μ, J. T. Baker Chemical Co.) column (2x40 cm) using hexane-ethyl acetate (40:1 to 10:1). The $\widehat{R\text{-}S}$ lactone resided in fractions 78-106 and the $\widehat{R\text{-}R}/\widehat{S\text{-}S}$ lactone resided in fractions 114-156. Addition of $Eu(hfc)_3$ to a $CDCl_3$ solution of methyl-7-hydroxyoctanoate resulted in the resolution of the methoxy singlet at δ 3.7; ee values were then determined based on integral ratio.

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