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LIPASE CATALYZED SYNTHESIS OF MACROCYCLIC LACTONES IN ORGANIC SOLVENTS

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Summary: A new method for the preparation of macrocyclic lactones from ω-hydroxyacid methyl esters is described. The approach utilizes intramolecular transesterification catalyzed by lipase in organic solvents. This procedure is also applicable to the synthesis of asymmetric lactones.

The potential of enzymes as practical catalysts for organic synthesis especially for asymmetric synthesis is becoming increasingly recognized[1]. Among enzymes so far investigated, lipase is one of the most advantageous, because it does not require any cofactor regeneration, and are commercially available, stable and inexpensive. Lipases have been widely used for the resolution of racemic alcohols and carboxylic acids through asymmetric hydrolyses of the corresponding esters[2]. In addition to hydrolysis, lipases should also be able to catalyze different reactions where compounds other than water serve as nucleophiles. Actually, lipases are reported to catalyze transesterification[3], esterification[4], aminolysis or oximolysis[5]. We thought that intramolecular transesterification instead of intermolecular can also be catalyzed by lipase, and stereospecific synthesis of macrocyclic lactone would become possible. Following this train of thought, we have investigated the catalytic potential of lipase toward methyl esters of ω-hydroxyacid in organic solvents. In the present paper, we report that lipase can be used for facile, preparative and efficient catalyst in synthesizing macrocyclic lactones including asymmetric one. This asymmetric lactone-synthesis have some important advantages over conventional chemical synthesis of lactones[6].

We at first surveyed several lipases for their lactone synthesizing activity using methyl 16-hydroxyhexadecanoate in benzene (Table 1). To the substrate solution (1 mM in benzene) was added a powder of each lipase (5 mg/ml), and the suspension was stirred vigorously at 40°C; samples were periodically withdrawn and analyzed by gaschromatography[7]. Of the lipases tested, only Lipase P from Pseudomonas sp. and porcine pancreatic lipase could catalyze lactonization efficiently, while other lipases showed little or no activity, indicating that lactonization activity is not the common feature of lipases but seemed a specific character of some lipases. Lactone synthesis with Lipase P in benzene proceeded linearly up to 10 hr, and reached plateau at ca. 25 hrs with 79 % conversion, probably due to the lack of the substrate.

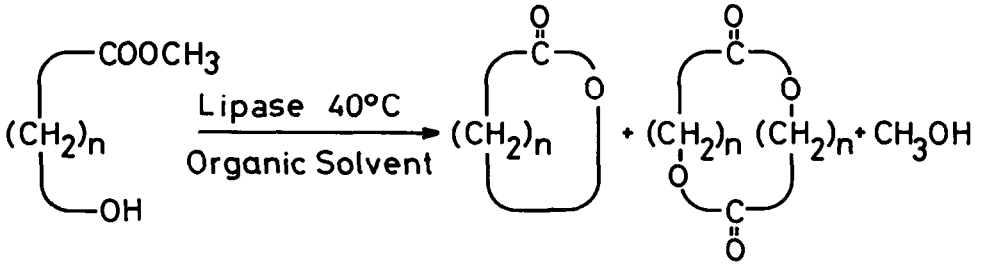


Table 1. Efficiency of Several Lipases in Lactone Synthesis from Methyl ω-Hydroxyhexadecanoate[a]

Origine of lipase	Product Y (%)[b]	Origine of lipase	Product Y (%)[b]
Pseudononas sp.[c]	78	Rhizopus delmer[e]	0
Porcine pancreas[d]	73	Candida cylindracea[d]	0
Rhizopus japonicus[c]	2	Wheat germ[d]	0

a) Reactions were performed at 40°C in 100 ml dry benzene with 500 mg of each lipase and 1 mM of methyl ω-hydroxyhexadecanoate for 72 hrs. b) Yields were determined by GLC. c) from Nagase Biochemicals Ltd., Osaka. d) from Sigma. e) from Seikagaku Kogyo Co., Ltd., Tokyo.

Because enzyme-catalyzed reactions in organic solvents are reported to be affected by solvents used[8], we also investigated the effect of solvents on the lactonization (Table 2). We observed more than 87-fold difference in the lactonization yield after 4 hr reaction. Polar solvents, such as ethyl acetate, seemed bad for the lactonization, which agreed well with the tendency in lipase catalyzed ester synthesis[9]. To know more about the effect of solvents, we selected some good solvents, and analyzed the time course of the lactone formation (Table 3). As apparent on the table, not the final yield but the initial reaction rate was different in each solvent: the initial rate in heptane was more than 7-fold higher than the rate in benzene, but the same lactone yield was obtained in both cases. The fact that lactone formation ended at ~80% yield with 1 mM substrate indicated that catalytic activity of Lipase P becomes negligible at the substrate concentration lower than 0.2 mM. Actually, Lipase P-catalyzed lactonization in benzene obeyed Michaelis-Menten kinetics with $K_{m,\ app.}$ for methyl 16-hydroxyhexadecanoate of 9.61 mM, indicating that at 0.2 mM substrate, reaction rate decreased to 1/50 of the maximal velocity. The lipase-catalyzed lactonization was also dependent on the chain length of the substrate (Table 4). When methyl 16-hydroxyhexadecanoate or 15-hydroxypentadecanoate was used, ~80 % of lactone yield was observed, while with methyl 13-hydroxytridecanoate the yield decreased to 38 %, and instead, the corresponding diolide was formed with 25 % yield. The diolide formation decreased with the increase of the chain length, and become negligible in the case of methyl 16-hydroxyhexadecanoate (3 % yield). Therefore, with Lipase P, there seems some

Table 2. Survey of Solvents for Lipase Catalyzed Lactonization[a].

Solvent used	Product Y (%)[b]	Solvent used	Product Y (%)[b]
Cyclohexane	35	iso-Octane	6
Tetrachloromethane	33	1,4-Dioxane	2
Hexane	20	Trichloroethylene	0.8
1,1,1-Trichloroethane	19	1,1,2,2-Tetrachloroethane	0.4
Heptane	16	Ethyl acetate	0
Benzene	12	Dimethylsulfoxide	0
Toluene	7		

a) All reactions were performed at 30°C on 10 ml scale with 50 mg Lipase P and 1 mM methyl ω-hydroxyhexadecanoate for 4 hrs. b) Yields were determined by GLC.

Table 3. Effect of Solvents on the Reaction Rate and the Yield in Lipase Catalyzed Lactonization[a]

Solvent used	Reaction rate[b] (nmoles mg^{-1} min^{-1})	Yield (%)[c]	Solvent used	Reaction rate[b] (nmoles mg^{-1} min^{-1})	Yield (%)[c]
n-Heptane	0.67	78	Benzene	0.09	78
n-Hexane	0.65	80	Dimethylsulfoxide	0	0
Cyclohexane	0.59	79			

a) Reactions were performed at 40°C on 300 ml scale with 1.5 g of Lipase P and 1 mM of methyl ω-hydroxyhexadecanoate. b) Reaction rate was calculated from the initial linear part of the synthesis. c) Yields were determined by GLC.

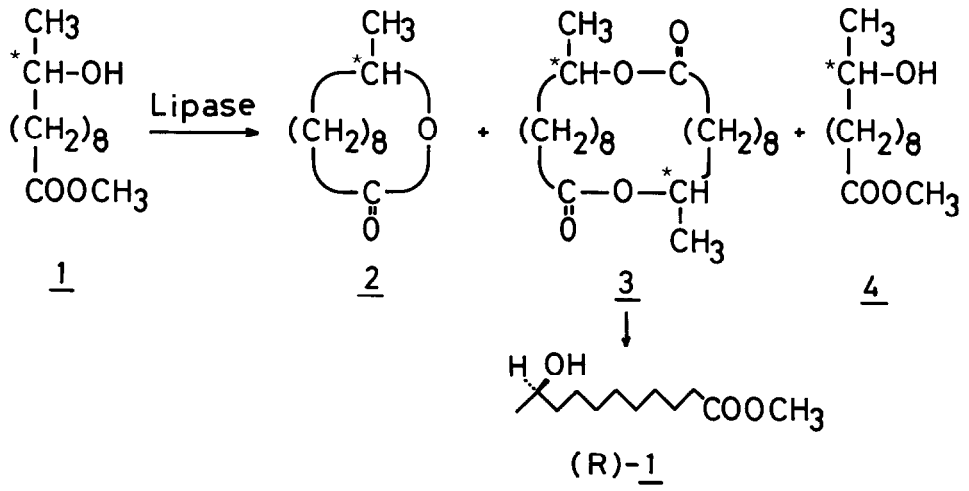
Table 4. Lactonization of Several ω-Hydroxyacid Methyl Esters with Lipase[a]

$HO-(CH_2)_n-COOCH_3$	Product[11] Y (%)[b]	$HO-(CH_2)_n-COOCH_3$	Product[11] Y (%)[b]
n=12	38	n=14	78
n=13	64	n=15	80

a) Reactions were performed at 40°C in 300 ml dry benzene with 3 g of Lipase P and 1 mM of each ester for 72 hrs. b) Isolated yield.

proper distance between ester and free OH groups for the efficient intramolecular transesterification.

To ascertain the asymmetric nature of the lipase-catalyzed lactonization, we used racemic methyl 10-hydroxyundecanoate, 1, as substrate. Because diolide formation is greater with this chain length, we isolated 45 mg of the corresponding diolide, 3[12], from 480 mg of 1. In order to estimate optical purity of the product, 2 was hydrolyzed with KOH/MeOH and methylated with diazomethane. The resulting 1 was found optically pure ($[\alpha]_D^{25}$=-6.3°, c=1.4 in MeOH; (R)-1, $[\alpha]_D^{25}$ =-5.8°, c=2.5 in MeOH[10]). Therefore, it can be concluded that Lipase P can catalyze stereospecific intramolecular transesterification leading to the formation of (R)-isomer from (ω-1)-hydroxyacid methyl ester.



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7. Lactones and diolides were determined by Hitachi 163 gaschromatogrph equipped with FID. For lactones, a glass column (5 mm x 1m) packed with Silicone OV-17 was used. Column and injection temperatures were 190 and 250°C, respectively. For diolides, a glass column (5 mm x 1 m) packed with Silicone DC-QF-1 was used. Column and injection temperatures were 250°C.
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11. All compounds gave MS, IR and NMR spectra consistent with the structures assigned.
12. EIMS, m/e 368 (M^+, $C_{22}H_{40}O_4$); NMR in $CDCl_3$ δ ppm, 4.96 (2H, m, -HC-O-), 2.26 (4H, t, $-CH_2-C=O$), 1.0-1.8 (28H, m), 1.19 (6H, d, $-CH_3$).

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