Pergamon

Tetrahedron Vol. 50, No. 48, pp. 13753-13764, 1994

0040-4020/94 $7.00+0.00

0040-4020(94)00898-1

Correlation of the Absolute Conformation of *sec* Alcohols Derived from Macrocyclic Lactones of Resorcylic Acid and Their Stereoselective Transacetylation in Organic Solvents by *Pseudomonas fluorescens* Lipase

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Abstract: Transacetylation of diastereomeric pairs of *sec*. alcohols, derived from macrocyclic lactones of resorcylic acid; 7α,β-*trans*-zearalenols (**1**,**2**, full names; (*3S,7R* and *3S,7S*) *trans*-3,4,5,6,9,10-octahydro-7,14,16-trihydroxy-3-methyl-1H-2-benzoxacyclotetradecine-1-ones), 7α,β-*cis*-zearalenols (**5**,**6**), and 7α,β-zearanols (**9**,**10**, full names; (*3S,7R* and *3S,7S*) 3,4,5,6,9,10,11,12-decahydro-714,16-trihydroxy-3-methyl-1H-2-benzoxacyclotetradecine-1-ones) by vinylacetate, catalyzed by *Pseudomonas sp.* and *Pseudomonas fluorescens* lipase in n-heptane and acetonitrile has been studied. It is highly stereoselective; diastereomeric excess of 7β-O-acetates was usually near 100%. K_M, V_{max} and specificity constant V_{max}/K_M do not significantly differ for 7β-stereoisomers **2**, **6** and **10**. However, they notably change on going from n-heptane to acetonitrile, and the specificity constant drops by factor *ca* 10-100. The enzyme-bound water is assumed to be partly released in the latter solvent, thus diminishing the interaction with the hydrophilic region of the substrates, and affecting the activity, but not the stereoselectivity of lipases. To correlate the structural and conformational properties of these substrates with unexpectedly high diastereoselectivity of enzymatic transacetylation, the solid state structures of 7β-isomers **2**, **4**, **6**, **10** and **12** have been determined by X-ray analysis. The X-ray structure analysis has revealed that the 7α and 7β isomers possess notably different and in some cases almost mirror-image related absolute conformations around the reactive center. The importance of conformational chirality in the hydrophobic region of these substrates for stereoselection in transacylation by microbial lipases is discussed.

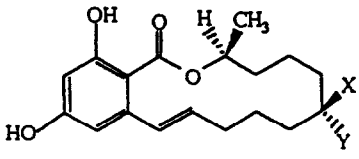
INTRODUCTION

The lipolytic enzyme activity in organic solvents has been already well documented, and the synthetic exploration of this biocatalytic feature has become of an ever growing research interest[1,2]. This technique has enjoyed particularly rapid development due to the enhanced solubility of the substrates in organic solvents, as compared to aqueous media. The number of organic molecules prepared *via* enzymatic catalysis in organic solvents, in particular those obtained by transacylation of various alcohols by lipases, has blossomed during the past few years. This usually unfavourable process is driven by both the lack of water and the use of the vinylesters functioning as a carrier of the acyl group, since upon the formation of the acyl-enzyme intermediate the liberated vinylalcohol isomerizes to acetaldehyde. The microbial lipases accept a broad number of racemic and prochiral substrates, imposing not too restricted structural requirements upon them. The enantioselective acylation of prochiral diols is well documented; it ranges from transacylation of enantiotopic hydroxy groups by internal comparison[3] in 1,3-diols[4] and 1,4-diols[5], to more remote groups in 1,n-diols[6]. Racemic alcohols are successfully resolved regardless of the hydroxy group position, either

attached to the asymmetric carbon[7], or remote from the chiral center[8]. Chiral 1,2 diols with C_2 symmetry have also been resolved[9a,b]. In these latter cases the lipolytic enzymes recognize the hydroxy groups that are enantiotopic through external comparison[3]. Diols from the sugar series, without internal symmetry, could be (dia)stereoselectively acylated[9c].

Growing interest in the synthetic potential of lipases from some microbial strains[10], *e. g. Pseudomonas sp. Pseudomonas fluorescens, Mucor michiei, Candida cylindracea, Rhyzopus, etc.*, has led to several models in predicting their stereoselectivity. Bjorkling *et al.*[11] have analyzed the three dimensional active site interactions of some lipases, Faber[12] and Poppe and Novak[13] have included this topic in their recent monographs.

We have recently investigated some commercially available lipases for stereoselective hydrolysis of 7α,β–acetates **3,4** and **11,12** in phosphate buffer[14]. A very high (up to 100%) 7β-diastereoselectivity has been found for both the C(11)=C(12) *trans*-unsaturated (**3,4**) and the saturated substrates (**11,12**). Such high stereoselectivity was unexpected, considering the distance from the perturbing groups to the reactive group on the chiral center. There are two chains with at least three methylenic groups separating C(7) from the nearest functionality; similar open-chain substrates are converted with low stereoselectivity in an analogous



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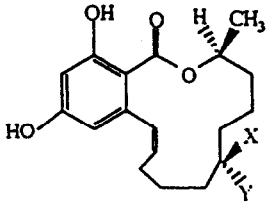

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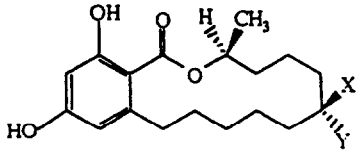

9-12

	X	Y		X	Y		X	Y
1	H	OH	5	H	OH	9	H	OH
2	OH	H	6	OH	H	10	OH	H
3	H	$OCOCH_3$	7	H	$OCOCH_3$	11	H	$OCOCH_3$
4	$OCOCH_3$	H	8	$OCOCH_3$	H	12	$OCOCH_3$	H

enzymatic reaction[15,16]. This result has suggested that the high enantioselectivity of lipases can be related to their recognition of *absolute conformation* around the chiral center of the reactive stereoisomer. This stereochemical aspect of the interaction of lipases with a broad range of substrates seems not to be properly recognized. The terms *absolute conformation* and the *sense of helicity*, defined by Snatzke[17,18] as a sign of the torsional angle between two chromophores, and broadly used in the exciton coupling treatment of the relation between CD spectra of chiral molecules and conformation of the coupled chromophores that rise specific electronic transitions, seem to remain limited to this chiral spectroscopic method[19,20].

Herewith we report on the stereoselectivity of the reverse reaction, *i.e.* transacetylation in organic solvents of macrocyclic *sec* alcohols **1,2 5,6**, and **9,10**, catalyzed by some microbial lipases, and we analyse the results in view of the conformational properties of stereoisomers, based on the CD and X-ray data.

RESULTS AND DISCUSSION

The stereoselectivity of the lipase catalyzed reaction has been correlated with the conformational properties of the specific substrates, macrolactones of resorcylic acid (**1-12**) -determined in both solid state and acetonitrile solution. In the exploratory experiments the relative reactivity and stereoselectivity of *Pseudomonas sp.* lipase were determined for the series of solvents with a broad range of the log P values, following the approach suggested by Klibanov *et al.*[28], Table 1. In all solvents this enzyme exhibited high or even complete stereoselectivity for the 7β-isomers of diastereomeric pairs, **1,2**, **5,6** and **9,10**. Their conversion and initial rate have generally varied from very low in hydrophilic solvents to rather high in hydrophobic solvents.

Interestingly, the unsaturated 7β-*trans* isomer **2** exhibited rather consistent enhancement of reactivity with increasing log P value of the solvent, whereas for its saturated congener **10** such correlation was not observed; in diisopropylether and acetonitrile the high conversion and initial rate were accompanied by a 10-15% decrease in stereoselectivity.

Then our attention has focused on the reactivity of pure 7β isomers **2,6**, and **10** in the two solvents: n-heptane being representative of hydrophobic media, and acetonitrile being representative of hydrophilic ones. The reactivity of lipases with the investigated substrates in acetonitrile was particularly fortuitous.. All CD measurements were performed in this solvent, since high solvating properties, the absence of hydrogen bonding and other highly polar interactions with the solute, have ranked acetonitrile as a preferable solvent for CD spectroscopic studies. It has been repeatedly reported[28b,c], however, that in acetonitrile as well as in the other water-miscible solvents, lipases exhibited significantly lower reactivity and enantioselectivity than in the water-immiscible ones, mainly due to the deteriorative effect of the free and enzyme-bound water in the latter.

The kinetic parameters for transesterification of 7β-isomers **2**, **6**, and **10** are given in Table 2. The variation for two solvents of notably different hydrophobicity is particularly significant for the specificity constant V_{max}/K_M, which drops by a factor of *ca* 10-100 when changing from hydrophobic to hydrophilic solvent. The difference in values of K_M and V_{max}/K_M between the three substrates is rather small, however. This indicates that binding is being controlled by the hydrophilic resorcylic region in these substrates. The stereoselectivity remains almoust unaffected, however, since the absolute conformation of hydrophobic, "non-chromophoric" region, remains unaffected in all the solvents examined.

Correlation between conformational properties of macrocyclic substrates and reactivity in the lipase catalyzed transesterification.- High stereoselectivity in enzymatic hydrolysis and acylation of stereoisomeric resorcylic lactones indicated that some characteristic conformational properties of the bound substrate have been maintained, both in aqueous medium and in organic solvents. In order to better understand this result, we have analyzed the structure of macrocyclic lactones as comprised of a hydrophilic "chromophoric" region and a lipophilic "non-chromophoric" region, Fig. 1, Formulae A-C.

Table 1: *Pseudomonas sp.* Lipase Catalyzed Transesterification of *trans*-7α,β-Zearalenols **1,2**, *cis*-7β-Zearalenol **6** and 7α,β-Zearanols **9,10** in Organic Solvents.

Substrate	Solvent	logP[a]	t (h)	Conversion (%)	v_0 (nMs^{-1})	Product configuration	d.e. (%)
1,2	n-heptane	4.0	30	42.9	144.9	3S,7S (7β)	100
	n-hexane	3.5	30	25.7	41.6		
	cyclohexane	3.2	48	21.4	18.5		
	diisopropyl ether	2.8	30	37.0	70.1		
	dichlormethan	1.25	48	10.3	8.08		
	tetrahydrofuran	0.49	48	6.7	6.08		
	acetone	-0.23	30	6.4	7.54		
	acetonitrile	-0.33	30	11.5	24.3		
	1,4-dioxane	-1.1	48	0	0		
6	n-heptane	4.0	30	97.2[b]	821.8	3S,7S (7β)	
	acetonitrile	-0.33	30	32.0[b]	71.49		
9,10	n-heptane	4.0	28	21.0	88.2	3S,7S (7β)	100
	diisopropyl ether	2.8	24	49.9	283.0		86.1
	chloroform	2.0	30	0	0		---
	acetone	-0.23	29	32.1	65.4		100
	acetonitrile	-0.33	30	45.9	192.0		90.6
	1,4-dioxane	-1.1	29	9.52	6.7		100

[a] Values cited in ref. 38. [b] Transesterification of the mixtures of *cis/trans*-7α- (75% of *cis*-7α, **5** and 25% of *trans*-7α, **1**) and *cis/trans*-7β-zearalenols (75% of *cis*-7β, **6** and 25% of *trans*-7β, **2**) have shown the complete inertness of 7α-diastereomers.

The absolute conformation of the "chromophoric" region can be defined by torsional angles formed by the [C11-C12-C121-C161] and [C16-C161-C1-O1] bonds. The conformation of this hydrophilic part of the macrocyclic lactones in solution can be principally determined by the CD spectra analysis. Our previous analysis of the chirally perturbed resorcylic acid chromophore in similar compounds[20] reveals the strong influence of the nature of C11-C12 bond and [C11-C12-C121-C161] torsional angle on the transition moment of the B_{2u} band. Since the resorcylic acid lactones **1-12** possess the *E*-configuration of the lactone group, and the C11-C12 bond may adopt various conformations determined by the torsional angle [C11-C12-

Table 2. Kinetic Parameters for Transesterification of *trans*-7β-Zearalenol (**2**), *cis*-7β-Zearalenol (**6**) and 7β-Zearanol (**10**) in n-Heptane and Acetonitrile

Substrate	Solvent	K_M (mM)	V_{max} (μM s^{-1})	V_{max}/K_M (ms^{-1})
2	n-heptane	3.1±0.2	0.57±0.04	0.184
	acetonitrile	12.5±0.8	0.12±0.01	0.001
6	n-heptane	15.5±0.6	2.23±0.1	0.144
	acetonitrile	10.4±0.4	0.12±0.02	0.012
10	n-heptane	7.6±0.03	0.98±0.01	0.129
	acetonitrile	46.5±1.2	0.85±0.01	0.018

121-161], the correlation of the CD data with the absolute conformation of the "chromophoric region" of macrocyclic lactones is not straghtforward. However, our previous CD studies revealed the high conformational stability of these macrocycles in solution[20a], and thus justified a systematic crystallographic study related to their reactivity with enzymes.

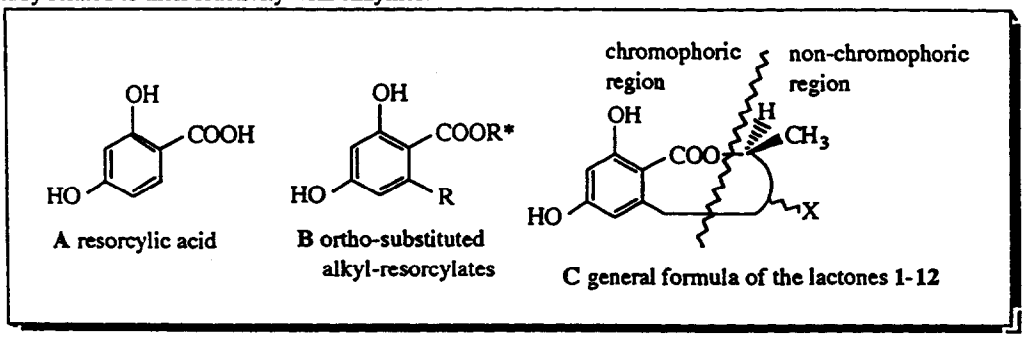

Figure 1. Two regions in macrocyclic lactones, resorcylic acid derivatives.

We have therefore completed the preparation of good quality single crystals of most 7β-isomers. The ORTEP drawings of the solid state structures of these compounds are presented in Fig. 2. The absolute configuration at the C(7) chiral center has been assigned using a known chirality at C3(*S*) as an internal standard. For all the 7β-isomers the absolute configuration at C7 is *S*, whereas for 7α-it is *R*. It was interesting to observe that *in both groups of stereoisomers the C(7) substituent (hydroxy or acetoxy) adopted a quasi-equatorial position*, seemingly well exposed to the enzymatic active site. One would therefore expect their availability to the enzymes in both isomers. However, only the 7β isomers have proven reactive with most microbial lipases examined. To account for such an unexpectedly high diastereoselectivity we have examined the difference between 7α and 7β isomers in more detail. Notable differences between the solid state conformations of the pairs of isomers have been observed. They are nearly mirror-image for the helical segments in the region between C(121)-C(8) for **1,2** (structural data for **1** are taken from ref 29) and also in the region C(4)-C(7) for **11,12** Fig. 3. The computer designed projections of the resolved structures, maintaining the best overlap in the aromatic region, also reveal notable conformational differences in the

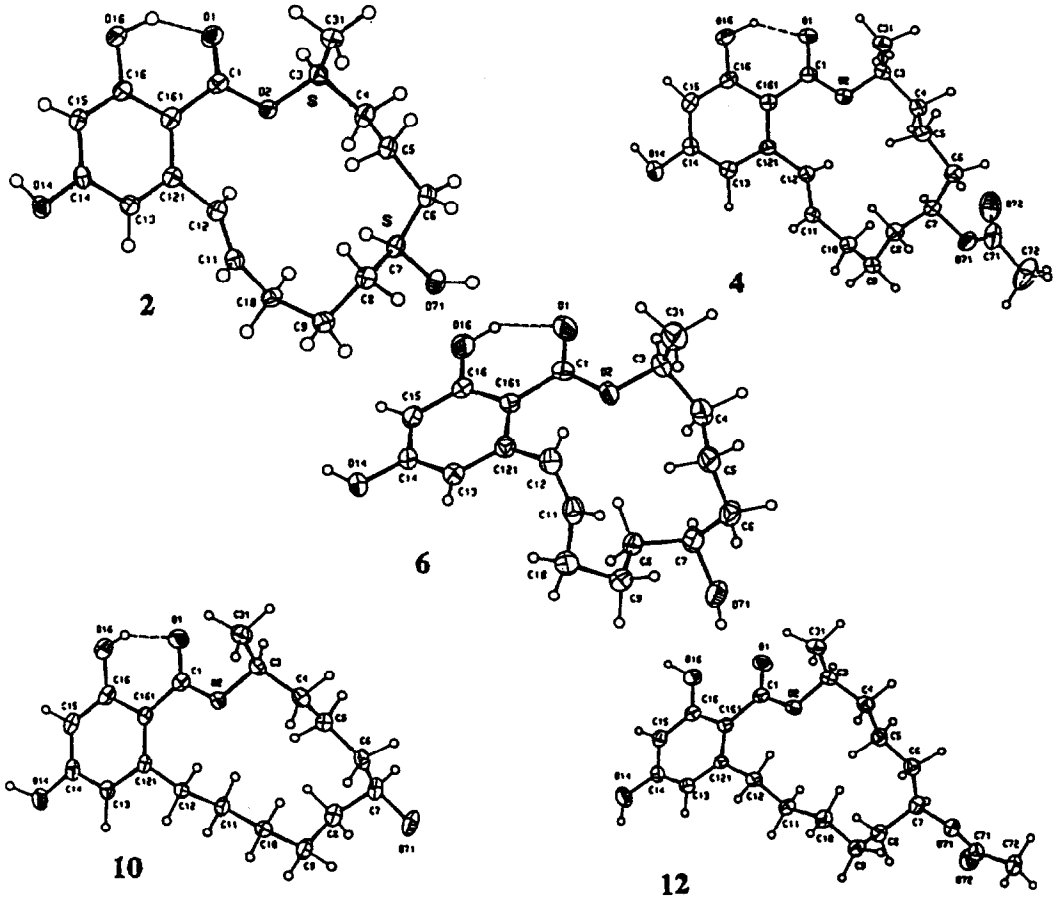

Figure 2. ORTEP plots of the structures **2**,**4**,**6**,**10** and **12**.

lipophilic regions of all investigated 7α and 7β isomers; most torsional angles in this region exhibit opposite signs for the two diastereomers. All these conformational data converge to the conclusion that the *absolute conformation of the hydrophobic region determines binding preference for 3S,7S stereoisomers*. This relation is schematically presented in Fig. 4.

In the formerly proposed models[12,13,30], the L and M groups are defined in the traditional manner, based on their steric requirements, as in Fig. 4A. We broaden this concept in the sense of helicity, represented by the "M" and "L" helical segments. The helical structure 4B can match, while that of 4C mismatches, the hydrophobic lid near to the active site of the lipase. The 7β-isomers either possess a stable conformation matching chirality, or they can be induced to adopt a required absolute conformation on binding. Thus, different reactivity of the studied macrocyclic stereoisomers in transacetylation by lipases is largely due to their different absolute conformations adopted in the solution or induced on binding.

The separation of the hydrophilic chromophoric region from the hydrophobic region in macrocyclic lactones **1-12** seems to play an important role in their stereoselective transformations by lipases. While the

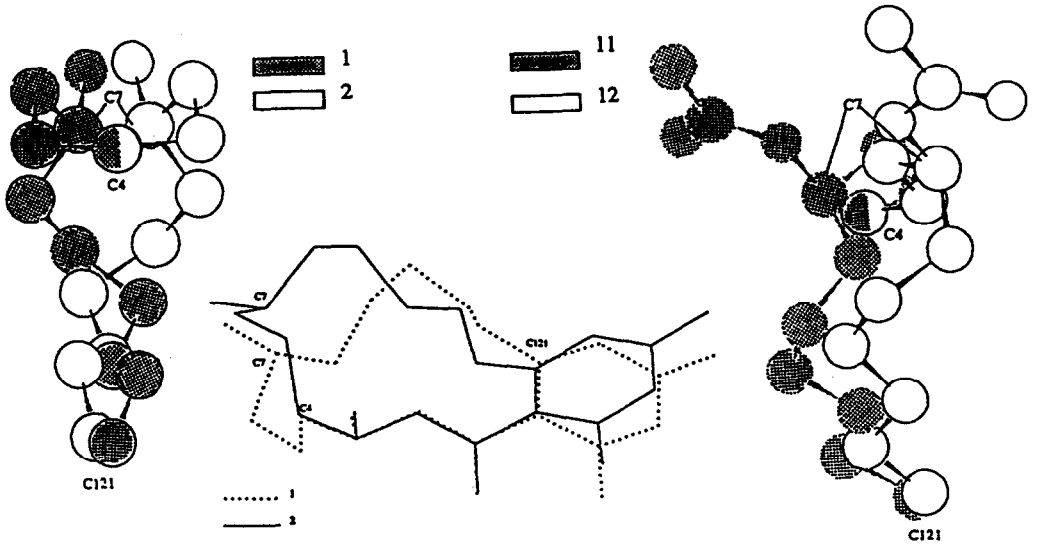

Figure 3. Conformational differences in the "hydrophobic region" of macrocyclic ring in diastereomeric pairs **1,2** and **11,12**.

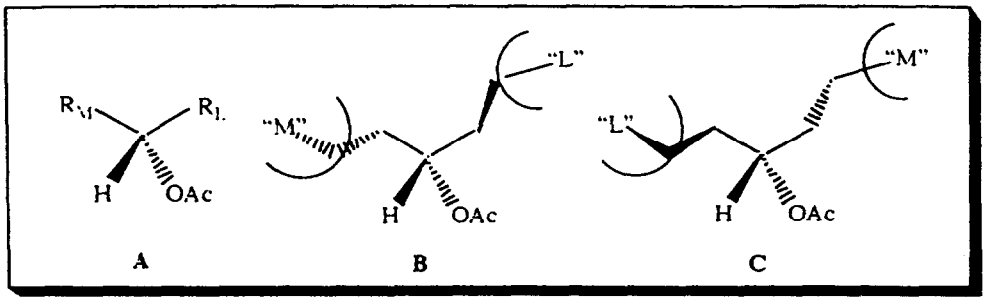

Figure 4. Traditional model (A) and helical model (B,C); schematic presentation of the matching (B) and mismatching (C) absolute conformation of chiral substrates.

hydrophobic region tends to enter into the "open lid", that liberates an important hydrophobic surface around the active site[30,36], the presumably hydrated hydrophilic (resorcylic) region orients to the enzyme-bound water interface. The mode of interaction proposed herein explains why the resorcylic lactones are good substrates in acetonitrile, as well; the enzyme-bound water promotes the interaction with the hydrophilic region, leaving the conformationally unperturbed hydrophobic "non-chromophoric" region to match the hydrophobic lid. Presumably, the reactive conformations of the bound 7α and 7β stereoisomers are of nearly opposite helicity. Following the Curtin-Hammett principle, according to which the most stable conformation might not be the reactive one, we assume that on binding only the 7β isomers can easily adopt the reactive absolute conformation.

A detailed structure of the active site based on crystallographic data is presently available for *Mucor miehei*[31], *Candida rugosa*[32], *Geotrichum candidum*[33], and *Pseudomonas glumae* lipase[34], whereas only preliminary crystallographic data are available for the *Pseudomonas sp.*[33] and *Pseudomonas putida*[35] lipases.

The amino acid sequences of microbial lipases build up a highly conserved region around a Ser residue on the active site. This active site is sheltered by a lid which is formed of one or more helices or surface loops[36]. The catalytic center of lipases is formed of a triad of amino acids wherein Ser seems to be obligatory,and the other two amino acids, His and Asp, take part in the so called "charge relay system" mechanism[37]. In the three-dimensional structures of *Streptomyces griseus* and *Geotrichum candidum* lipases the catalytic Ser is located at a tight turn between the C terminus of the β-strand and N terminus of an α-helix[32,33]. This helical conformation at the active site presumably represents an important element for discrimination of stereoisomeric substrates.

In conclusion, we have shown that there exists correlation between conformational properties of resorcylic acid macrocyclic lactone derivatives and the stereoselectivity of lipase catalyzed transacylation in organic solvents. It has the origin in the conformationally restricted mobility and marked differences in the absolute conformations within "lipophilic region" of the stereoisomeric pairs, whereas "hydrophilic region" influences the reactivity in the organic solvents of different polarity.

EXPERIMENTAL SECTION

General considerations. The HPLC separations were performed on a HP 1050 instrument, with UV detector at 254 nm and HP 3396A integrator, using reverse phase column NovaPak C18 (Waters, 3.9x150 mm). Elution was performed with water:methanol:acetonitrile mixture (40:30:30), at a flow-rate of 1mL/min and a pressure of 160-165 bar. The retention times (min) were as follows: **1**, 3.25; **2**, 2.78; **3**, 14.65: **4**, 10.01: **5**, 5.40; **6**, 4.35; **7**, 16.12; **8**, 14.33; **9**, 3.51; **10**, 2.56; **11**, 13.72; **12**, 9.63.

Materials. The lipases (EC 3.1.1.3, from *Penicillium camemberti, Candida cylindracea, Rhyzopus oryzae, Pseudomonas sp., Aspergillus niger, Humicola lanuginosa, Geotrichum candidum, Candida lipolytica, Rhyzopus niveus, Mucor javanicus,* and *Rhizopus delemar*) were obtained from Amano Pharmaceutical Co., Nagoya, Japan, as liophylized extracellular enzymes of unknown specific activity. The *Pseudomonas fluorescens* lipase (activity 31.5 U/mg, molecular weight 36 kDal) was purchased from Fluka BioChimica.

Compounds **1** and **2** were prepared as a *ca* 45:55 7α,β-diastereomeric mixture, by $NaBH_4$ reduction of zearalenone(x,y=O in **1**) according to the described procedure[20a]. Diastereomers were separated by crystallization from methanol. For the X-ray analysis crystals of **2** were obtained by slow evaporation from methanol-water (12.5:1), compounds **3**,**4** were obtained by acetylation of **1**,**2** according to the literature method[14]. Compounds **5** and **6** were obtained by photochemical isomerization of **1** and **2** according to the described procedure[21], and the separation of pure **5** and **6** was performed either by HPLC (with water-methanol-acetonitrile 40:30:30 as eluant) or by chromatography on silicagel (dichloromethane-ethylacetate 2:1 as eluant). Compounds **9**,**10** were obtained by the RaNi catalyzed hydrogenation of zearalenone, and their separation was performed by crystallization from acetonitrile-water. The 7α,β-O-acetates were prepared by acylation of the corresponding alcohols according to the standard procedure.

Enzymatic transacetylation. Some exploratory experiments were performed with *Pseudomonas sp.* lipase (6mg) slurried in 5mL of the solvent. To this slurry, diastereomeric 7α,β-mixtures (0.03 mmol, 5mM, diastereomeric composition 1:1) and vinylacetate (1 mL) were added. The reaction was performed in a

thermostated shaker at 225 rpm and 25±1°C. The samples (200 μL) were taken at regular time intervals, filtered through a teflon filter, and analyzed by HPLC.

Determination of the kinetic parameters with *Pseudomonas fluorescens* lipase (10-10.5 mg, 4.6-4.8 x 10^{-5} mM) in acetonitrile and n-heptane (5 mL) was performed with **2** (7.2-57 mg, 3.8-29.7 mmol), **6** (4.0-53.4 mg, 2.1-27.7mM) and **10** (9.4-70.8 mg, 4.9-36.6 mM), i.e within the concentration range of the calculated value of Michaelis-Menten constant K_M.

Calculations. The initial rates were calculated from Stinchoff equation (1)[22], using for P the percentage of the acylated product (HPLC) at the time intervals (*t*);

$$y=(t/P)=1/v_0 + t/P_{eq} \qquad (1)$$

The initial rates were obtained from the reciprocal values of the increment in Eq. (1). Other kinetic parameters, maximal rate (V_{max}) and (K_M), are calculated by inserting the reciprocal initial rates and substrate concentrations into the Lineweaver-Burk equation (2);

$$1/v_0 = (K_M/V_{max})\, s + 1/V_{max} \qquad (2)$$

The maximal reaction rate was obtained from the increment, whereas K_M/V_{max} was calculated from the slope of the plot. The kinetic parameters were also calculated from the Michaelis-Menten Eq. (3), using nonlinear least square method (NLLSQ program).

$$v_0 = (V_{max}\, s)/(K_M + s) \qquad (3)$$

The figures with Lineweaver-Burk plots for **2**,**6**, and **10** in n-hexane and acetonitrile are collected in the Supplementary material and the most relevant numerical data are collected in Table 3.

X-ray Structure Determination and Refinement. The crystal data and details of the structure determination are listed in Table 3. The intensity data were collected on an Enraf-Nonius CAD4 diffractometer with graphite monochromatized Cu radiation. The measurements were carried out at room temperature for the compounds **4**, **6**, **10** and **12**, whereas the ones for **2** at liquid nitrogen temperature (100 K). The reference reflections showed a loss of intensity: 0.5% for **6**, 0.8% for **4**, 2% for **10** and 3% for **2**. No significant intensity reductions were observed for **12**. Data were corrected for a decay, Lorentz and polarization effects using SDP[23]. An absorption correction was not applied. The structures were solved by SHELX86[24]. Refinement was performed by full-matrix least squares with the SHELX77[25] system of programs using the *F* values. The H-atoms were generated on stereochemical grounds and refined under restricted conditions according to the pivot atoms. The O-bonded hydrogen atoms were located by the difference Fourier maps and the O-H distances were adjusted to the theoretical value; the hydrogen atoms of three disorder water molecules in **2**, one of water molecules in **4**, and hydroxyl hydrogen atom at C(7) in **10** were not located. The crystal structure of **2** has included the crystalline solvents: methanol with population 75%, crystallographically determined water molecules with populations of 82%, 38%, and 18%. In order to get more precise coordinates of disordered water molecules, the low temperature data were used. The crystal structure of **4** contained a crystalline water molecule, as well. Scattering factors were those included in SHELX77. Molecular geometry was calculated by the EUCLID program package[26]. The ORTEP plots were prepared by the ORTEP II program[27].

Supplementary Material: Figures with Lineweaver-Plots for **2**,**6**, and **10** in n-heptane and acetonitrile, ORTEP drawings, and the list of refined coordinates and e.s.d's for **2**, **4**, **6**, **10**, and **12**.

Table 3. Crystal Data and Details of the Structure Determination

compound	**2**	**4**	**6**	**10**	**12**
a) Crystal data					
Molecular formula	$C_{18}H_{24}O_5$x1.4H_2Ox0.75CH_3OH	$C_{20}H_{26}O_6$ x 0.5H_2O	$C_{18}H_{24}O_5$	$C_{18}H_{26}O_5$	$C_{20}H_{28}O_6$
M_r	369.63	371.28	320.37	322.39	364.43
a [Å]	8.302(4)	17.8096(9)	5.750(2)	5.130(1)	5.2492(2)
b [Å]	18.585(8)	8.2102(4)	11.322(2)	11.669(1)	16.9970(5)
c [Å]	24.071(6)	15.9087(9)	26.658(3)	28.006(6)	21.2127(5)
ß [°]		122.096(4)			
V [$Å^3$]	3713.9(8)	1970.6(2)	1735.1(7)	1676.3(4)	1892.6(1)
Dc [gcm^{-3}]	1.326	1.256	1.226	1.282	1.283
Z	8	4	4	4	4
Crystal system	orthorhombic	monoclinic	orthorhombic	orthorhombic	orthorhombic
Space group	$P2_12_12_1$	$C2$	$P2_12_12_1$	$P2_12_12$	$P2_12_12_1$
Crystal size [mm]	0.30 x 0.20 x 0.35	0.31 x 0.07 x 0.37	0.28 x 0.11 x 0.31	0.14x 0.07 x 0.32	0.09 x 0.07 x 0.36
μ Cu*K* α [cm^{-1}]	7.97	7.34	6.97	7.19	7.28
F (000)	1596	796	688	696	784
b) Data Collection					
Temperature [K]	100	295	295	295	295
θ_{min} , θ_{max} [°] for cell det.	22.5, 74.33	40, 45	20, 42	13.97, 37	32,47
No. of reflections used for cell det.	25	25	25	25	25
θ_{min} , θ_{max}	2.5, 74	2.6, 74	2.5, 70	2.5, 74	2.6, 74
ω/2θ scan [°]	Δω=1.07 + 0.26 tanθ	Δω=0.76 + 0.20 tanθ	Δω=1.03 + 0.36 tanθ	Δω=0.64+ 0.26 tanθ	Δω=0.64 + 0.21 tanθ
hkl limits	0, 10; 0, 23; -30, 0	-22, 0; -10, 0; -19, 19	0, 7; -13, 0; 0, 32	-6, 0; -14, 0; -34, 0	0, 6; -21, 0; -26 ,0
Reflections measured	4289	2235	1951	2050	2288
c)Refinement					
Independent reflections observed with $I > 2\sigma(I)$	3366	2026	1527	1634	1733
No. of parameters	569	289	256	248	278
Quantity minimized, $\Sigma w \, \|\|F_o\| - \|F_c\|\|^2$					
$w = k / (\sigma^2(F_o) + gF_o^2)$, g	0.0023	0.041	0.0057	0.012	0.0036
R, wR	0.050, 0.054	0.045, 0.049	0.046, 0.054	0.08, 0.081	0.039, 0.047
Goodness of fit, S	1.22	0.34	0.48	0.64	0.46
Max. shift /error $(\Delta/\sigma)_{max}$	0.032(O20,x)	-0.029(C16,z)	0.002(C16,z)	-0.007(C5,y)	0.008(O2, y)
Residual electron density, $(\Delta\rho)_{max}$, $(\Delta\rho)_{min}$ [$eÅ^{-3}$]	0.57, -0.25	0.16, -0.26	0.17, -0.23	0.32, -0.44	0.13, -0.18

ACKNOWLEDGEMENT

We acknowledge the financial support of Ministry of Science and Technology of Republic Croatia (Projects 1-07-179, and 1-07-257), and of NIH, USA (Project JFP 965). Most of the enzymes used were generous gift of the Amano Pharmaceutical Co.

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(*Received in UK* 5 *September* 1994; *revised* 10 *October* 1994; *accepted* 14 *October* 1994)