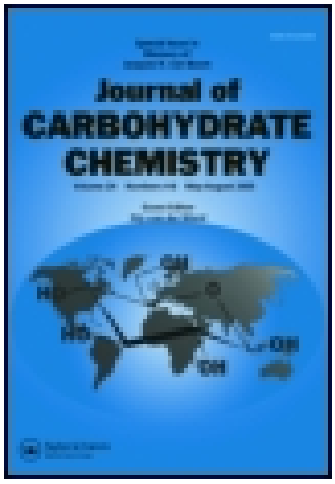

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

Enzymic Hydrolysis of Methyl 2,3-O-Acetyl-5-Deoxy-α and β-D-Xylofuranosides - An Active-Site Model of Pig Liver Esterase

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Published online: 23 Aug 2006.

To cite this article: Jitka Moravcová , Zita Vanclová , Jindra Čapková , Karel Kefurt & Jan Staněk (1997) Enzymic Hydrolysis of Methyl 2,3-O-Acetyl-5-Deoxy-α and β-D-Xylofuranosides - An Active-Site Model of Pig Liver Esterase, Journal of Carbohydrate Chemistry, 16:7, 1011-1028, DOI: 10.1080/07328309708005734

To link to this article: http://dx.doi.org/10.1080/07328309708005734

J. CARBOHYDRATE CHEMISTRY, 16(7), 1011-1028 (1997)

ENZYMIC HYDROLYSIS OF METHYL 2,3-DI-*O*-ACETYL-5-DEOXY-α AND β-D-XYLOFURANOSIDES - AN ACTIVE-SITE MODEL OF PIG LIVER ESTERASE

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Received September 6, 1996 - Final Form April 23, 1997

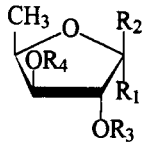
ABSTRACT

The regioselective enzymic hydrolysis of methyl 2,3-di-*O*-acetyl-5-deoxy-α-D-xylofuranoside (**1**) and methyl 2,3-di-*O*-acetyl-5-deoxy-β-D-xylofuranoside (**2**) in the presence of pig liver esterase (PLE) was studied by GLC. Diacetate **2** gave exclusively methyl 3-*O*-acetyl-5-deoxy-β-D-xylofuranoside (**6**) while diacetate **1** produced both methyl 2-*O*-acetyl-5-deoxy-α-D-xylofuranoside (**3**) and methyl 3-*O*-acetyl-5-deoxy-α-D-xylofuranoside (**4**) in low yield. At high conversion, methyl 5-deoxy-α-D-xylofuranoside (**7**) was the only product. The first-order rate constants, Michaelis constants, and maximal velocities were determined for **1**, **2**, and the monoacetates **3** - **6**. The results were interpreted on the basis of a recent active-site model for PLE.

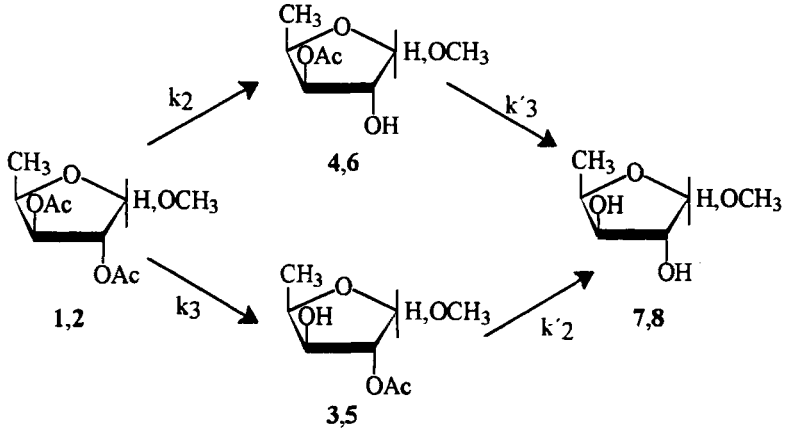
INTRODUCTION

Enzymes have now been widely accepted as valuable catalysts in the efficient synthesis of chiral synthons.[1] Especially esterases, such as pig liver esterase (PLE, E.C. 3.1.1.1.), a serine hydrolase, are attractive[2] due to their broad substrate specifities and high stereoselectivities. Stability and low cost represent additional advantages of these enzymes which operate without the need for coenzymes. Commercially available PLE is a mixture of six isoenzymes which possess the same specifity.[3] Several models have been proposed to rationalize the stereochemical behaviour of PLE,[4] and recently a generally applicable active site model for PLE was developed[5] that allows the prediction of both the stereochemistry of PLE-catalyzed hydrolyses, as well as the enantiomeric excess. This model was designed based on the results obtained in the hydrolyses of approximately 100 prochiral or racemic methyl esters of cyclic or acyclic dicarboxylic acids, for which the achiral methoxyl leaving group is the simplest possible. Much less attention was focused towards the interpretation of the stereoselectivity observed in the hydrolysis of acetate esters of chiral alcohols, although the previous results suggested that acetates of (±)-*trans*-cycloalkane-1,2-diols,[6] *meso*-2,5-bis(acetoxymethyl)-3,4-(isopropylidenedioxy)tetrahydrofuran,[7] (±)-*trans*-1,2-bis(acetoxymethyl)cyclohexane and related compounds,[8] or (±)-*trans*-2-aryl-1,2-cyclohexanediol-1-acetate[9] are suitable substrates for PLE. Among monosaccharide acetates, methyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside,[10] methyl 2,3-di-*O*-acetyl-α- and β-D-threofuranosides,[11] and 2,3,5-tri-*O*-acetyl-1,2-*O*-isopropylidene-α-D-hexofuranoses[12] were studied previously. In contrast, very low chemical and optical yields were obtained for PLE hydrolysis of (±)-*cis*-1,2-diacetoxymethylcycloalkanes[13] in which case the migration of acetyl group cannot be omitted during work up as had been described previously.[10,12]

The title acetates were chosen as model compounds having a flexible furanose ring with two *trans*-oriented vicinal ester functions, and the regioselectivity of PLE hydrolysis was correlated with an active-site model[5] of PLE.



1 $R_1 = OCH_3$, $R_2 = H$, $R_3 = R_4 = Ac$
2 $R_2 = OCH_3$, $R_1 = H$, $R_3 = R_4 = Ac$
3 $R_1 = OCH_3$, $R_2 = R_4 = H$, $R_3 = Ac$
4 $R_1 = OCH_3$, $R_2 = R_3 = H$, $R_4 = Ac$
5 $R_2 = OCH_3$, $R_4 = R_1 = H$, $R_3 = Ac$
6 $R_2 = OCH_3$, $R_3 = R_1 = H$, $R_4 = Ac$
7 $R_1 = OCH_3$, $R_2 = R_3 = R_4 = H$
8 $R_2 = OCH_3$, $R_1 = R_2 = R_4 = H$
9 $R_1 = OCH_3$, $R_2 = R_3 = H$, $R_4 = Ts$
10 $R_2 = OCH_3$, $R_3 = R_1 = H$, $R_4 = Ts$
11 $R_1 = OCH_3$, $R_2 = H$, $R_3 = Ac$, $R_4 = Ts$
12 $R_2 = OCH_3$, $R_1 = H$, $R_3 = Ac$, $R_4 = Ts$



Scheme 1

RESULTS AND DISCUSSION

Methyl 2,3-di-*O*-acetyl-5-deoxy-α-D-xylofuranoside (**1**) and methyl 2,3-di-*O*-acetyl-5-deoxy-β-D-xylofuranoside (**2**)[14] and all new mono-*O*-acetyl derivatives **3** - **6** were prepared by acetylation of diols **7** and **8**, respectively. Acetylation was performed with a 10 fold molar excess of acetic anhydride in pyridine under kinetic control. The new compounds **3** - **6** were identified in the usual way (^{1}H NMR, ^{13}C NMR, MS) and the structures of both 2-*O*-acetates **3** and **5** were also confirmed by correlation with

methyl 2-*O*-acetyl-5-deoxy-3-*O*-(*p*-toluenesulfonyl)-α- and β-D-xylofuranosides **11** and **12** prepared independently from the known[15] derivatives **9** and **10**.

The hydrolysis of both **1** and **2** can be expressed by four different competing reactions as outlined in Scheme 1. Thus, the regioselectivity is determined by the individual ratios of the corresponding rate constants.

The kinetics of PLE hydrolysis was monitored in such a way that samples of the reaction mixture were withdrawn in regular intervals over a period of several hours. The solvent was evaporated, the residue was diluted with methanol, and the supernatant was directly analyzed by GLC (Figures 1 and 2). A linear time-dependence of the logarithm of the molar concentration of each substrate **1** - **6** was found, thus being evidence of the first-order reaction. The first-order rate constants were computed as the slopes of these relationships [10^4 s^{-1}]: **1**, (k_2 + k_3) = 0.85, k_2'= 2.56, k_3'= 0.16; **2**, (k_2 + k_3) = 1.46, k_2'= 0.97, k_3'= 0.04. The results clearly show that the high regioselectivity of PLE hydrolysis of **2** is determined[16] by $k_2 >> k_3'$ and $k_3 << k_2'$. Thus the 3-*O*-acetate **6** is accumulated during reaction and it can be isolated in high yield nearly to 90 % (Figure 2). The PLE hydrolysis of diacetate **1** produces both monoacetates **3** and **4** in low yield (Figure 1). The monoacetate **3** is formed more rapidly than **4** ($k_3 > k_2$)[17] and even the rate of its further

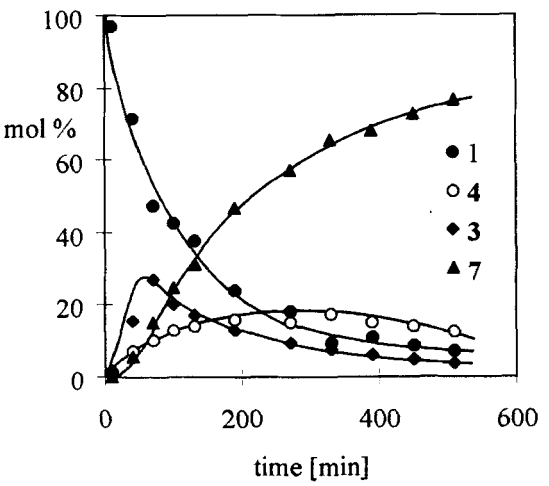


Figure 1. Enzymic hydrolysis of diacetate **1**.

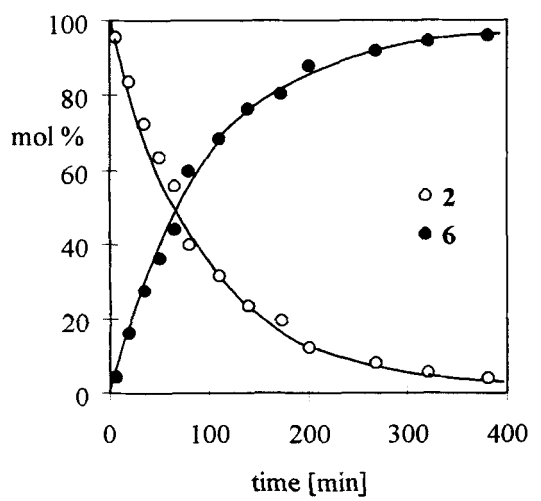


Figure 2. Enzymic hydrolysis of diacetate **2**.

Table 1. The K_M and V_{max} values for the substrates **1 - 6**

Substrate	ρ	$K_M 10^2$ [moldm^{-3}]	$V_{max} 10^5$ [moldm^{-3}s^{-1}]
1	0.9869	3.09	0.255
2	0.9837	2.21	0.206
3	0.9766	1.29	1.312
4	0.9939	2.13	0.578
5	0.9812	1.21	1.558
6	0.9308	2.05	0.110

deacetylation increases. The monoacetate **4** is hydrolyzed to the diol **7** by a slower reaction ($k_3'/k_2 = 0.5$). At high conversions the diol **7** is the only product. According to the kinetic study, it can be concluded that deacetylation of both **1** and **2** at the position 3 accelerates the subsequent hydrolysis of 2-*O*-acetate (α-series: $k_2'/k_3 = 5$, β-series: $k_2'/k_3 > 100$) and therefore the free hydroxyl at the position 3 must stabilize a complex PLE-substrate. In contrast, the free hydroxy group at position 2 results in a decrease of the subsequent hydrolysis rate (α-series: $k_3'/k_2 = 0.5$, β-series: $k_3'/k_2 = 0.03$) indicating the destabilization of a corresponding PLE-substrate complexes. Further, the two possible orientations of **1** in the active site of PLE appear essentially equivalent ($k_3/k_2 = 1.8$).[17] In the case of **2**, one of two binding modes is greatly favored over the other ($k_2/k_3 > 100$)[16] reflecting the stabilization of a PLE-**1** complex by the *trans*-oriented vicinal methoxyl at C-1 and *O*-acetyl at C-2. These conclusions are also supported by the observations in the threofuranoside series.[11]

A convenient method for the study of an enzyme-substrate complex seems to be the determination of the Michaelis constant (K_M) and the maximal velocity (V_{max}). These parameters had not been applied to the PLE hydrolysis of sugar acetates at all, although they could be helpful in the most stable complex recognition.

Thus, the PLE hydrolyses of **1 - 6** under a variety of different initial concentrations ($[S]_o$) were followed by measuring the consumption of the sodium hydroxide solution necessary for the neutralization of the resulting acetic acid. Corresponding initial

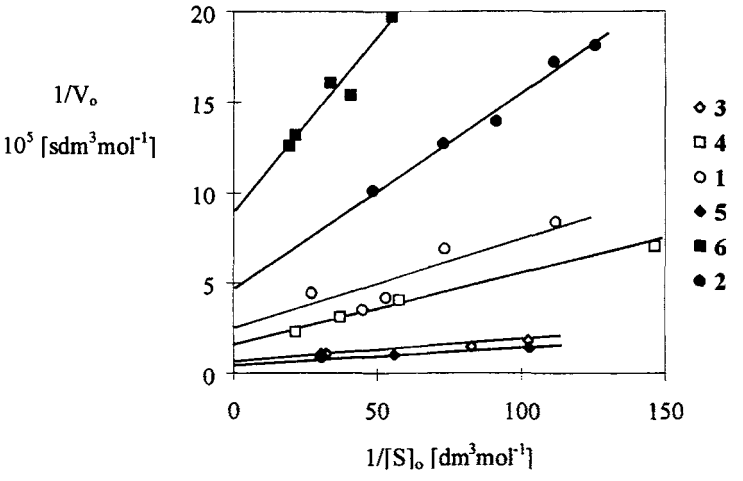


Figure 3. Double-reciprocal Linweaver-Burke plot.

velocities (V_o) were taken off as the slopes of these titration curves in the first minute of the reaction. A double-reciprocal Lineweaver-Burk plot (Figure 3) was found to be linear according to the correlation coefficient (ρ) and then it was used to calculate both K_M and V_{max} (Table 1). For most enzymes, K_M lies between 10^{-1} and 10^{-6} M, a high K_M suggests weak binding, a low K_M suggests strong binding of a substrate into active site of enzyme. The K_M values found for the substrate **1** - **6** range from 1.10^{-2} to 3.10^{-2} M (Table 1) and therefore these substrates are rather weakly bound in the enzyme-substrate (ES) complex. Among them, the 2-*O*-acetyl derivatives **3** and **5** with the lowest K_M values are the best ones giving also the highest V_{max} values. This observation agrees well with the kinetic results discussed above and it shows once more the importance of the free hydroxyl group at position 3. The K_M values of substrates **2**, **4**, and **6** are comparable; they indicate small differences in the stability of the ES complexes. However, the V_{max} value of **6** is extremely low and this finding is in agreement with the kinetic study in which the PLE hydrolysis of **6** represents the slowest reaction. In the case of **1**, the K_M value reflects the formation of two ES complexes and therefore it appears not to be useful.

Finally, we attempted to interpret the regioselectivity of PLE hydrolyses of model substrates using a generally applicable active site model[5] for PLE (Figure 4) and

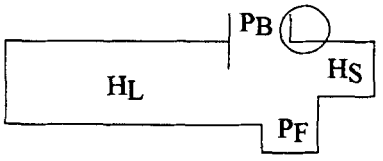
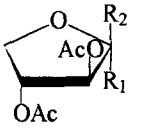
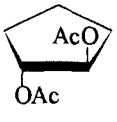


Figure 4. Top perspective view of the active site model of PLE. The model consists[5] of four binding regions - two hydrophobic pockets (H_L, H_S) and two more polar cavities (P_F, P_B). The ester group to be hydrolyzed must locate in the serine region (circle) and the remainder of the substrate simultaneously fits into the appropriate binding regions. Hydrophobic moieties of substrates bind preferentially in the smaller H_S binding site. The unhydrolyzed ester function of 1,2-diesters is located in the P_F pocket and other groups, including hydrophobic moieties, can also extend into P_F. The P_B site interacts well with hydrogen-bond donors, and alcohol, ether, and carbonyl functions locate comfortably here. The rear boundary of P_B pocket and the area above the model are open.

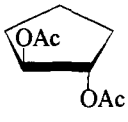
we applied the described rules[5] also to the known examples of a PLE hydrolysis of diol diacetates.[6,11] For the structurally similar acetates of methyl α- and β-threofuranosides **13** and **14**, the regioselectivity of PLE hydrolysis has been described previously.[11] Diacetate **14** gave a mixture of both mono-*O*-acetyl derivatives which were stable under conditions used, and the PLE hydrolysis of **13** led exclusively to the 3-*O*-acetyl derivative. The presence of methyl at C-4 in **1** and **2** resulted in a lower reaction rate[18] as compared to **13** and **14**. In addition, the racemic *trans*-1,2-diacetoxycyclopentanes **15**, **16** represent the most simple structures related to furanosides **1**, **2**, **13**, and **14**. Interestingly, (*R*,*R*)-**15** was readily converted into its monoacetate while (*S*,*S*)-**16** was completely hydrolyzed to the diol.[6]



13 $R_1 = OCH_3$, $R_2 = H$
14 $R_2 = OCH_3$, $R_1 = H$



15



16

With respect to the kinetic scheme 1 the following analyses must be applied to both starting acetates and all intermediates. The results described[6] for PLE hydrolysis of

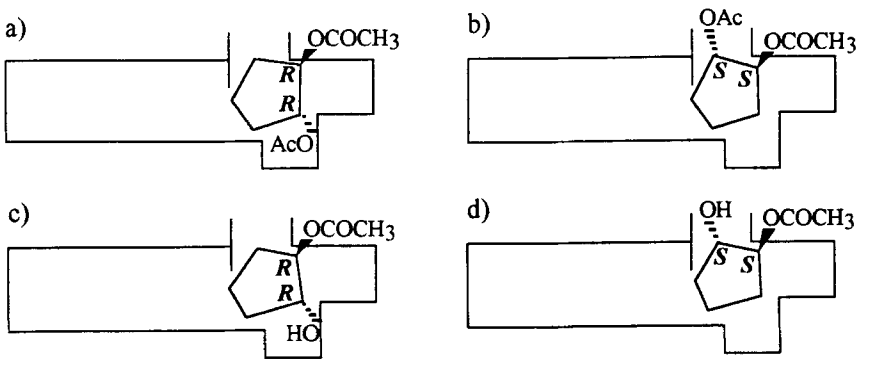


Figure 5. Binding orientation of *trans*-cyclopentandiol diacetates **15** (a) or **16** (b) and their corresponding monoacetates (c, d).

15 and **16** are in agreement with the Jones's model of PLE active site[5] (Figure 5a, b). If the ester function of **15** is located[19] in the serine sphere, the remaining *O*-acetyl group is directed towards the P_F pocket. In this orientation, the methyl group of the non-hydrolyzed ester moiety can fit efficiently into the smaller H_S pocket (Figure 5a). In the case of **16**, the second *O*-acetyl moiety is placed into the P_B pocket allowing a hydrogen-bonding interaction (Figure 5b). The strength of this hydrogen bond is probably lowered by the hydrophobic character of the terminal methyl group and therefore the rate of hydrolysis of **15** is higher than that of substrate **16**, as confirmed experimentally.[6] Removal of one *O*-acetyl group from **15** terminates the subsequent PLE hydrolysis[6] because the corresponding ES complex with the free hydroxyl group oriented into the P_F area is probably prohibited[5] due to the very polar character of the substituent (Figure 5c). In contrast, the ES complex leading to the product of total hydrolysis of **16** is highly favourable (Figure 5d). The free hydroxy group fully accommodates in the P_B site due to a strong hydrogen-bonding, the ES complex is stabilized and thus the rate of hydrolysis increases.

Sugar acetates **1**, **2**, **13**, and **14** represent more complex substrates and their behaviour towards PLE attack is determined unambiguously by the C-1 and C-2 relative configuration. The substrates **1** and **14** with *cis* orientation of methoxyl at C-1 and *O*-acetyl group at C-2, are hydrolyzed in accordance with the Jones's PLE active site

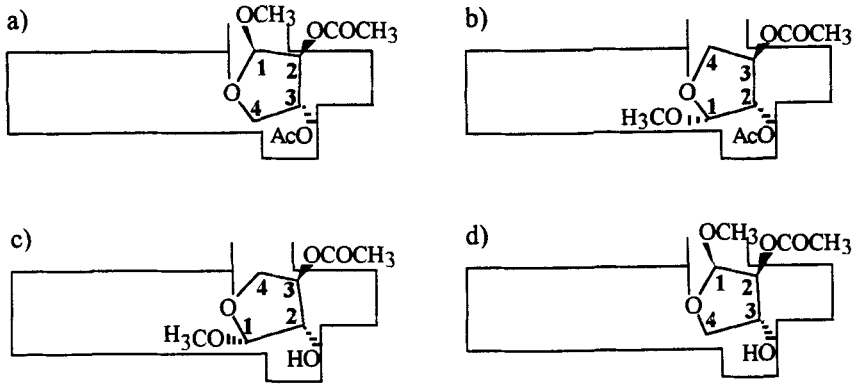


Figure 6. Binding orientations of methyl di-*O*-acetyl-β-D-threofuranoside (**14**) for the PLE hydrolysis at position 2 (a) and 3 (b). ES complexes for the hydrolyses of corresponding monoacetates (c, d).

model as well. For diacetate **14**, the hydrolysis of the 2-*O*-acetyl and 3-*O*-acetyl group occurs with almost equal facility[11] because the two competing ES complexes appear essentially equivalent (Figure 6a, b). It is important to note that the proposed orientation of **14** into the PLE active site seems to be the same as this one suggested for (*R,R*)-**15** (Figure 5a). The subsequent hydrolysis of the corresponding monoacetates does not proceed at all[11] since the required complexes are not allowed (Figure 6c, d) for identical reasons as discussed above for (*R,R*)-**15** (Figure 5c).

Similarly, two potential ES complexes leading to the hydrolysis of the 2-*O*- or 3-*O*-acetyl group can be considered for **1** (Figure 7a, b). Both complexes are characterized by the position of non-hydrolyzed ester group which is directed towards the polar P_B site as in the case of (*S,S*)-**16** (Figure 5b). The resulting monoacetates **3** and **4** give the thermodynamically stable ES complexes with strong interaction of the free hydroxyl group with the P_B site (Figure 7c, d). Therefore the rate of their hydrolysis to the diol **7** increases. This fact is in total agreement with the results observed for the hydrolysis of (*S,S*)-**16**, as mentioned above.

When the relative orientation between C-1 and C-2 is *trans* (substrate **2** and **13**), PLE exclusively attacks the 2-*O*-acetyl group and leaves the 3-*O*-acetyl group intact. It is important to note that PLE does not distinguish between the absolute configurations

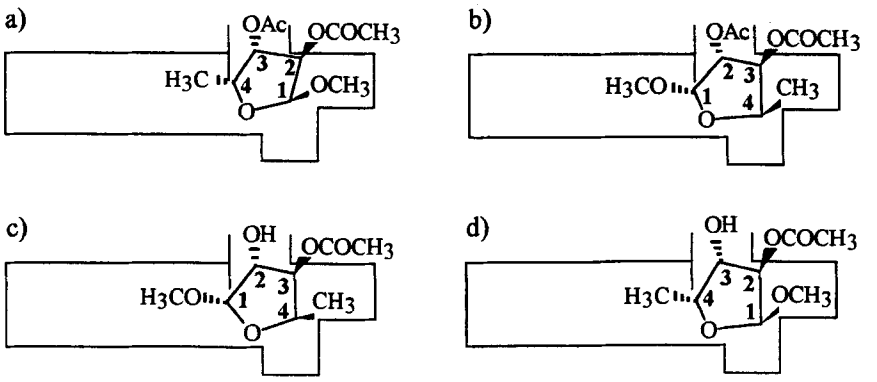


Figure 7. Binding orientations of diacetate **1** for the PLE hydrolysis at position 2 (a) and 3 (b). ES complexes for the hydrolyses of monoacetate **3** (c) and **4** (d).

of C-1, C-2 and C-3. These results are not in a full agreement with the Jones's active-site model. According to the rules,[5] the corresponding ES complexes for the hydrolysis of **2** (Figure 8a, b) and **13** (Figure 8c, d) are all allowed but those ES complexes considered for the hydrolysis at position 3 (Figure 8b, d) cannot actually exist.

The situation becomes more evident if the stereochemistry of the complexes of PLE with monoacetate **5** and **6** is also taken into account. 2-*O*-Acetate **5** is the best substrate for PLE giving the most stable ES complex stabilized by the strong hydrogen bond between free hydroxyl at position 3 and the P_B site (Figure 9a). In contrast, 3-*O*-acetate **6** is hydrolyzed with the lowest reaction rate although its ES complex is characterized by the orientation of free hydroxyl at position 2 into the P_B site as well (Figure 9b). It can be formulated that hydrogen bonding interactions between PLE and substrate play no primary role in the determining rates of hydrolysis. In such a case, the hydrophobic interactions appear to be the limiting factor because the binding orientations of those substrates which are not hydrolyzed with PLE (Figure 8b, d, and 9b) are characterized by the empty space in the H_L pocket.[19] The existence of at least one hydrophobic interaction is undoubtedly required for the ES complex formation. Additional hydrogen bonding interaction can only increase the stability of the corresponding ES complex. The free hydroxyl group is evidently too polar to extend

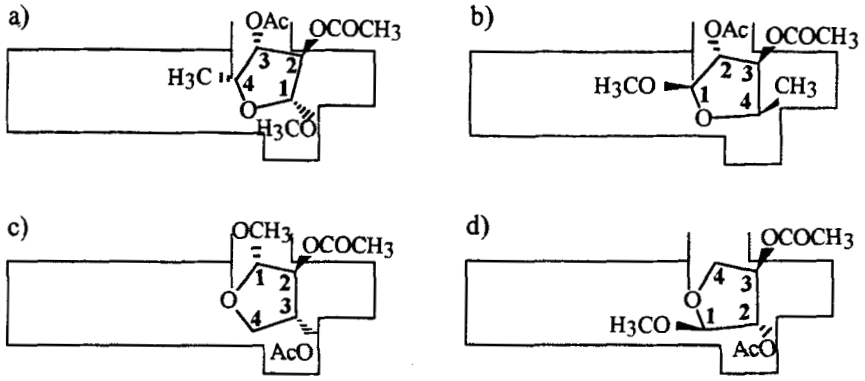


Figure 8. ES complexes for the hydrolysis of **2** at position 2 (a) and 3 (b). ES complexes for the hydrolysis of **13** at position 2 (c) and 3 (d).

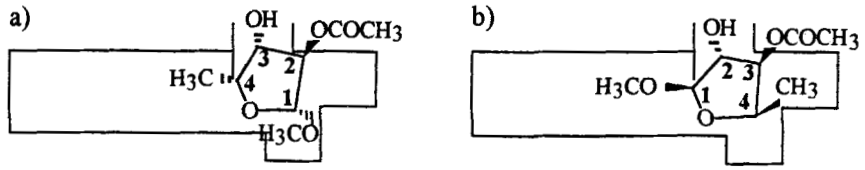


Figure 9. ES complexes for the hydrolysis of **5** (a) and **6** (b).

into the P_F pocket and such ES complexes (Figure 5c, 6c, and 6d) are not stable enough. No regioselectivity-reversing influence of ring heteroatom of the model compounds was found in contrast to the results described previously.[7]

CONCLUSIONS

The regioselectivity observed in the PLE-catalyzed hydrolysis of acetate esters derived from simple cyclic chiral diols can be interpreted by the Jones's active-site model originally proposed[5] for the prochiral or racemic methyl diesters. Furthermore, this model allows the elucidation of the kinetics observed in complex enzymic hydrolyses of polyfunctional substrates and in cases where the rate-limiting step is the formation of an enzyme-substrate complex.

EXPERIMENTAL

General Methods. Melting points were determined with a Kofler hot block and are uncorrected. Optical rotations were measured on an Opton Photoelectric Precision Polarimeter 0.005. NMR data were extracted from spectra measured in solution of $CDCl_3$ (TMS as an internal standard) with a BRUKER AM-400 spectrometer. Carbon-signal shifts were made by HETCOR experiment and proton-signal shifts were obtained by first order analysis of the spectra using COSY experiments and a selective decoupling. Mass spectra were recorded on a JEOL DX 303 instrument using an EI technique at 70 eV. The enzyme catalyzed hydrolyses were carried out under nitrogen in a pH-stat RTS 822 (Radiometer, Denmark) using thermostated vessels. Porcine liver esterase (PLE, 25.9 Umg^{-1} of protein, based on ethyl acetate as a substrate) was obtained from Sigma (USA) as a suspension in 3.2 M $(NH_4)_2SO_4$, containing 8 mg of protein per mL. All solvents were dried prior to distillation and stored under molecular sieves. Solvents were removed under reduced pressure below 40 °C. Column chromatography was performed on Silica Gel Lachema (Brno, Czech Republic), 100 - 160 μm, and TLC on Silica Gel G according to Stahl, 10 - 40 μm (Merck, Darmstadt, Germany) on using the elution systems (v/v): benzene - ethanol (A, 100 : 1; B, 100 : 5; C, 100 : 20) and benzene - acetone (D, 8 : 1; E, 1 : 1). Compounds on TLC plates were visualized by spraying with 1 % cerium(IV) sulfate in 10 % sulfuric acid and subsequent mineralization.

GLC Analysis. Analyses were performed on a Hewlett-Packard 5890 A instrument equipped with flame-ionization detector. Fused silica capillary column (50 m x 0.31 mm I.D.) coated with crosslinked phenyl methyl silicone (5%, film thickness 0.52 μm) was used with nitrogen as a carrier gas at a flow rate 91.8 $mLmin^{-1}$ (split 1 : 50). Temperature was programmed: 100 °C (5 min), 4 °C/min up to 160 °C, detector 230 °C, injector 200 °C. The following retention times (in min) were obtained: **1**, 16.36; **2**, 16.92; **3**, 13.14; **4**, 12.35; **5**, 11.78; **6**, 13.60; **7**, 7.97; **8**, 8.92.

The Kinetics of the Enzyme Catalyzed Reaction. To a 0.035 M solution (2 mL) of each substrate **1** - **6** in 0.5 M KCl at 25 °C, after adjustment of the pH to 8.0 and

stirring for 10 min, PLE (10 μL of original concentrate) was added. By titration with 0.103 M NaOH the pH of solution was kept at 8.0 and aliqouts of the reaction mixture (100 μL) were withdrawn during several hours. The reaction was stopped by addition of toluene (100 μL), the mixture was concentrated to dryness, and then diluted with methanol (100 μL). The supernatant was directly analyzed by GLC. The first-order rate constant was computed as a slope of a linear dependence between the logarithm of the molar concentration of the starting compound and reaction time. The reported value of each rate constant is an average of two determinations.

The Determination of K_M and V_{max}. To a solution of each substrate **1** - **6** (0.004 - 0.08 mmol) in 0.5 M KCl (2 mL) at 25 °C and pH 8.0 was added a suspension of PLE (10 μL of original concentrate). An initial time-dependence of consumption of 0.103 M NaOH necessary to maintain pH 8.0 was measured during 10 min. The initial velocity was then calculated as a slope of linear dependence of molar concentration of originating CH_3COOH and reaction time during first minute of rection.

Methyl 2-*O*-acetyl-5-deoxy-α-D-xylofuranoside (3). Methyl 3-*O*-acetyl-5-deoxy-α-D-xylofuranoside (4). Diol **7** (370 mg, 2.5 mmol) was acetylated with acetic anhydride (2.3 mL, 25 mmol) in pyridine (20 mL) at ambient temperature for 30 min. The excess of acetic anhydride was decomposed by water and the reaction mixture was concentrated to dryness. The residue (430 mg) was separated on a silica gel (100 g, system D) to give diacetate **1** (98 mg, 23 %, R_F 0.69, system D) followed by monoacetate with R_F 0.41 (system D) which was identified as 3-*O*-acetate **4**, syrup, 85 mg, 21% yield, $[\alpha]_D^{21}$ +177.8° (*c* 1.1, $CHCl_3$). ^1H NMR, δ[ppm]: 1.16 (d, 3H, $J_{4,5}$ = 6.5 Hz, H-5), 2.11 (s, 3H, CH_3CO), 2.90 (d, 1H, $J_{2,OH}$ = 5.1 Hz, OH), 3.49 (s, 3H, CH_3O), 4.20 (ddd, 1H, H-2), 4.43 (dq, 1H, H-4), 4.95 (d, 1H, $J_{1,2}$ = 4.7 Hz, H-1), 5.07 (dd, 1H, $J_{3,4}$ = 5.4 Hz, $J_{2,3}$ = 3.7 Hz, H-3), ^{13}C NMR, δ[ppm]: 15.12 (C-5), 21.36 (CH_3CO), 56.15 (CH_3O), 74.15 (C-4), 77.68 (C-2), 80.68 (C-3), 101.71 (C-1), 171.42 (CO). MS is given in Table 2.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.53; H, 7.37. Found: C, 50.67; H, 7.32.

Further elution gave crystalline 2-*O*-acetate **3** (100 mg, 24% yield, R_F 0.36, system D), mp 75 - 76 °C (diethyl ether - petroleum ether), $[\alpha]_D^{21}$ +154.6° (*c* 2.6,

$CHCl_3$). ^1H NMR, δ[ppm]: 1.28 (d, 3H, $J_{4,5}$ = 6.3 Hz, H-5), 2.15 (s, 3H, CH_3CO), 2.90 (bs, 1H, OH), 3.40 (s, 3H, CH_3O), 4.32 (ddd, 1H, $J_{3,4}$ = 6.7 Hz, $J_{3,OH}$ = 7.2 Hz, H-3), 4.36 (dt, 1H, H-4), 4.82 (dd, 1H, $J_{2,3}$ = 4.3 Hz, H-2), 5.03 (d, 1H, $J_{1,2}$ = 4.6 Hz, H-1), ^{13}C NMR, δ[ppm]: 15.13 (C-5), 21.39 (CH_3CO), 55.91 (CH_3O), 74.88 (C-4), 75.30 (C-3), 82.49 (C-2), 100.68 (C-1), 172.28 (CO). MS is given in Table 2.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.53; H, 7.37. Found: C, 50.61; H, 7.35.

Finally, the unreacted diol **7** (140 mg) was eluated with system E.

Methyl 2-*O*-acetyl-5-deoxy-β-D-xylofuranoside (5). Diol **8** (190 mg, 1.3 mmol) was acetylated with acetic anhydride (1.2 mL, 13 mmol) in pyridine (10 mL) at ambient temperature for 80 min. The reaction mixture was worked up as described above, and chromatography of a residue (250 mg) on silica gel (75 g, system A) afforded diacetate **2** (100 mg, 34% yield). Further elution gave a major monoacetate (120 mg, 49% yield) identified as 2-*O*-acetate **5**, syrup, $[\alpha]_D^{24}$ -47.0° (*c* 1.2, $CHCl_3$). ^1H NMR, δ[ppm]: 1.35 (d, 3H, $J_{4,5}$ = 6.6 Hz, H-5), 2.10 (s, 3H, CH_3CO), 3.40 (s, 3H, CH_3O), 3.40 (bs, 1H, 3-OH), 3.99 (bd, 1H, $J_{3,4}$ = 4.4 Hz, $J_{2,3} \cong$ 0.8 Hz, H-3), 4.39 (dq, 1H, H-4), 4.86 (s, 1H, $J_{1,2} \cong$ 0 Hz, H-1), 5.01 (bs, 1H, H-2). ^{13}C NMR, δ[ppm]: 15.12 (C-5), 20.60 (CH_3CO), 54.93 (CH_3O), 74.74 (C-3), 79.22 (C-4), 81.74 (C-2), 105.93 (C-1), 169.89 (CO). MS is given in Table 2.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.53; H, 7.37. Found: C, 50.58; H, 7.39.

The elution with system C gave diol **8** (24 mg).

Methyl 3-*O*-acetyl-5-deoxy-β-D-xylofuranoside (6). To a solution of diacetate **2** (190 mg, 0.8 mmol) in 0.5 M KCl (5 mL) at 25 °C and pH 8.0 a suspension of PLE (100 mL of original concentrate) was added. The hydrolysis was continued till the consumption of 0.103 M NaOH reached the theoretical value for one ester group hydrolysis (6 - 7 h). The reaction was stopped by toluene (0.5 mL) and the mixture was concentrated to dryness. The residue was extracted with $CHCl_3$ (3 x 50 mL), combined extracts were dried with $MgSO_4$, and then concentrated. The following composition of the product mixture was found by GLC: **2**, 8%; **6**, 83.5 %; **8**, 7 %; **5**, 1 %. Chromatography on silica gel (75 g, system A) recovered diacetate **2** (20 mg), and gave 3-*O*-acetate **6** (135 mg, 87% yield) as a syrup, $[\alpha]_D^{19}$ -101.1° (*c* 0.8, $CHCl_3$). ^1H NMR,

Table 2. Mass spectra of monoacetates **3** - **6**

	Relative Abundance								
m/z	**3**	**4**	**5**	**6**	*m/z*	**3**	**4**	**5**	**6**
189	-	-	-	2.3	87	39.9	20.7	44.1	18.6
160	5.9	0.3	-	-	86	19.9	25.0	19.9	7.1
159	7.4	2.2	11.6	13.6	85	8.5	4.3	14.2	4.3
131	-	0.3	-	2.3	75	3.5	7.8	2.9	4.3
130	1.9	1.2	8.1	16.7	74	4.3	1.4	4.3	2.1
129	0.9	0.6	3.1	4.5	73	7.1	7.1	7.8	7.1
121	-	0.4	-	2.8	71	7.1	14.3	7.8	17.1
118	2.5	0.4	5.2	2.3	70	25.6	41.4	32.8	48.6
117	-	-	3.1	2.3	69	5.7	10.0	5.0	10.7
116	0.9	0.6	7.8	4.0	68	4.3	2.8	2.1	2.8
115	3.1	0.7	11.6	9.7	61	23.5	34.2	25.6	31.4
113	0.8	-	5.2	-	59	5.7	5.0	4.3	5.7
103	2.6	1.7	8.7	13.6	58	20.7	10.0	14.3	7.1
101	1.2	0.7	4.9	6.8	57	19.2	10.0	17.1	9.3
100	1.3	1.0	3.1	5.7	55	2.1	2.1	1.4	2.8
99	11.2	6.4	28.4	27.8	45	5.7	5.7	4.3	5.7
98	1.2	0.5	5.2	5.1	43	100	100	100	100
97	0.9	0.3	3.4	3.4	42	3.5	5.7	2.9	7.1
88	1.0	11.4	12.8	12.9	41	5.0	7.8	4.3	8.6

δ[ppm]: 1.29 (d, 3H, $J_{4,5}$ = 6.61 Hz, H-5), 2.15 (s, 3H, CH_3CO), 3.45 (s, 3H, CH_3O), 4.16 (dd, 1H, H-2), 4.53 (ddd, 1H, H-4), 4.83 (d, 1H, $J_{1,2}$ = 1.53 Hz H-1), 4.93 (dd, 1H, $J_{2,3}$ = 1.95 Hz, $J_{3,4}$ = 5.34 Hz, H-3). ^{13}C NMR, δ[ppm]: 15.40 (C-5), 20.70 (CH_3CO), 55.53 (CH_3O),75.82 (C-4), 79.74 (C-3), 80.42 (C-2), 108.96 (C-1), 171.34 (CO). MS is given in Table 2.

Anal. Calcd for $C_8H_{14}O_5$: C, 50.53; H, 7.37. Found: C, 50.60; H, 7.38.

The elution with system C afforded diol **2** (14 mg).

Methyl 5-deoxy-3-*O*-(*p*-toluenesulfonyl)-α-D-xylofuranoside (9). Methyl 5-deoxy-3-*O*-(*p*-toluenesulfonyl)-β-D-xylofuranoside (10). 5-Deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose[20] (2.3 g, 0.013 mol) was dissolved in pyridine (15 mL) and solid *p*-toluenesulfonyl chloride (5.1 g, 0.025 mol) was added at 5 °C. The mixture was kept for 48 h and after decomposition with water, it was extracted with $CHCl_3$ (3 x 100 mL). Combined organic layers were washed with water, diluted H_2SO_4, $NaHCO_3$ and finally with water. The solution was dried with $MgSO_4$ and concentrated to dryness. The residue without any purification was treated with a strong cation exchanger Dowex 50Wx8 (in H^+ form, 2 mL) in methanol (25 mL) under reflux at 80 °C. After 4 h, no starting compound was present according to TLC and two spots with R_F 0.33 and 0.28 (system B) in a ratio 1 : 1 were visible. The cation exchanger was filtered off, the solution concentrated, and the residue was separated on silica gel (100 g, system B). The crystalline compounds **9**, mp 68 - 70 °C (ethyl acetate - petroleum ether), and **10**, mp 70 - 72 °C (ethyl acetate - petroleum ether), were obtained in overall yields of 39 and 47 %, respectively. Specific rotations as well as ^{1}H NMR data were in accord with those published previously.[15]

Methyl 2-*O*-acetyl-5-deoxy-3-*O*-(*p*-toluenesulfonyl)-α-D-xylofuranoside (11).

A. Compound **9** (250 mg, 0.833 mmol) was acetylated with acetic anhydride (2.5 mL) in pyridine (5 mL), and the reaction mixture was worked up as described above. Flash chromatography (silica gel, 30 g, system A) yielded **11** (257 mg, 85% yield), $[\alpha]_D^{19}$ +156.4° (*c* 1.6, $CHCl_3$). ^{1}H NMR, δ[ppm]: 1.28 (d, 3H, $J_{4,5}$ = 6.5 Hz, H-5), 1.91 (s, 3H, CH_3O), 2.45 (s, 3H, CH_3Ph), 3.22 (s, 3H, CH_3CO), 4.38 (dq, 1H, $J_{3,4}$ = 6.3 Hz, H-4), 4.89 (dd, 1H, H-2), 5.00 (dd, 1H, $J_{1,2}$ = 4.6 Hz, H-1), 5.18 (dd, 1H, $J_{2,3}$ = 4.6 Hz, H-3), 7.35 - 7.80 (4H, arom.).

B. To a solution of **3** (50 mg, 0.29 mmol) in pyridine (2 mL) was added solid *p*-toluenesulfonyl chloride (100 mg, 0.52 mmol) at 5 °C. The mixture was stirred, and the reaction was stopped by addition of water when no starting **3** was visible on TLC (**3**, R_F 0.21; **11**, R_F 0.54; system B). The mixture was concentrated and **11** was purified by

flash chromatography (silica gel, 10 g, system B). Pure **11** was obtained as a syrup in the 85% yield identical with that prepared in procedure A (optical rotation, ^{1}H NMR).

Methyl 2-*O*-acetyl-5-deoxy-3-*O*-(*p*-toluenesulfonyl)-β-D-xylofuranoside (12).

A. Compound **10** (250 mg, 0,833 mmol) was acetylated in the same way as described above. Flash chromatography afforded crystalline **12** (270 mg, 90% yield), mp 74 - 75 °C (ethyl acetate - petroleum ether), $[\alpha]_D^{17}$ -42.5° (*c* 1.1, $CHCl_3$). ^{1}H NMR, δ[ppm]: 1.33 (d, 3H, $J_{4,5}$ = 6.6 Hz, H-5), 2.00 (s, 3H, CH_3CO), 2.43 (s, 3H, CH_3Ph), 3.32 (s, 3H, CH_3O), 4.46 (dq, 1H, H-4), 4.75 (d, 1H, $J_{1,2}$ = 0.9 Hz H-1), 4.84 (dd, 1H, $J_{3,4}$ = 5.4 Hz, $J_{2,3}$ = 1.9 Hz , H-3), 5.01 (dd, 1H, H-2), 7.34 - 7.83 (4H, arom.).

B. Starting from **5** (50 mg, 0.20 mmol) and using the same procedure as for **11**, crystalline **12** was obtained in the 88% yield. Optical rotation, melting point as well as ^{1}H NMR were identical with those described by procedure A.

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16. The rate constant k_3 can be neglected against k_2 because only traces of diol **8** were present in a reaction mixture. Thus, the sum ($k_2 + k_3$) is actually k_2.
17. According to the initial rates of the formation of both **3** and **4** in the early stage of **1** deacetylation, the ratio k_3/k_2 can be estimated as 1.8. It means that $k_3 = 0.55 \times 10^{-4}$ s^{-1} and $k_2 = 0.30 \times 10^{-4}$ s^{-1}.
18. Although no rate constants are given in ref.,[11] the half-time of the hydrolysis can be roughly estimated.
19. The binding of hydrophobic groups must occur in the H_S area rather than in the H_L site, but the cyclopentyl ring is marginally too large for optimum fit into H_S. It may extend partially into H_S pocket.[5]
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