Tetrahedron: Asymmetry Vol. 1, No. 11, pp. 783-792, 1990
Printed in Great Britain

0957-4166/90 $3.00+.00
Pergamon Press plc

Esterification in Non-Aqueous Media: Activity and Selectivity of Porcine Pancreas Carboxylesterase Depending on the Structure of the Alcoholic Substrate

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(*Received* 8 *October* 1990)

Abstract. Enzymatic esterification in n-hexane was carried out using various unsaturated and aromatic (R,S)-alcohols, dodecanoic acid and a crude pancreatic extract. A "model" was developed describing reactivity and selectivity of the enzymatic catalysis. These parameters were found to depend on the structural features of the side chains at the reactive chiral center. In several homologous series of substrates reversal of configuration of the preferred enantiomer was observed. The evaluated "model" will be a helpful tool to establish future synthetic strategies.

Introduction

By reaction of a racemic alcohol with an achiral fatty acid under lipase catalysis the alcohol enantiomers are converted at different reaction rates, resulting in optical enrichment of the produced ester and the remaining alcohol (kinetic resolution)[1-4]. In spite of the importance of this system, in which mostly the crude enzyme extract from porcine pancreas has been used, systematic studies enabling to develop synthetic strategies on the basis of an enzymatic "model", e.g. for the enantioselectivity, are rather scarce. First attempts have been made by Ohno et al. (1986)[5] using porcine liver esterase (PLE) and, recently, by Ehrler and Seebach (1990)[6] with the "model" for the enantioselectivity of porcine pancreas lipase (PPL). This paper concerns the effect of structural features of the alcoholic substrate on both the activity and enantioselectivity of the enzyme extract from porcine pancreas, with the aim to establish a "model" for the enzymatically catalyzed esterification in organic media.

Experimental

Educts. Commercially available and synthesized (R,S)-alcohols were used. Their sources and the modes of analysis are summarized in Table 1.

Table 1. Sources of alcohols and their modes of analysis

Substrate	Source[a]	Determination of the configuration	Determination of the ee value
1-Buten-3-ol	A	Polarimetry	MTPA
1-Penten-3-ol	A	Polarimetry	MTPA
1-Hexen-3-ol	A	Polarimetry	MTPA
1-Hepten-3-ol	L	Polarimetry	MTPA
1-Octen-3-ol	A	Polarimetry	MTPA
1-Nonen-3-ol	L	Polarimetry	MTPA
1-Penten-4-ol	A	Polarimetry	PEIC
1-Hexen-4-ol	B	Polarimetry	PEIC
1-Hepten-4-ol	B	NMR	HTA
1-Octen-4-ol	A	NMR, HRGC	PEIC
1-Hexen-5-ol	S[7]	Polarimetry	MTPA
E-3-Penten-2-ol	A	Hydrogenation	PEIC
E-4-Hexen-3-ol	F	Polarimetry, HRGC	PEIC
E-2-Hepten-4-ol	B	HRGC	MTPA
E-2-Octen-4-ol	S[7]	NMR	Lipodex C
E-3-Hepten-2-ol	S[7]	Hydrogenation	PEIC
E-3-Octen-2-ol	L	Hydrogenation	PEIC
Z-3-Hepten-2-ol	S[8]	Hydrogenation	MTPA
Z-3-Octen-2-ol	S[8]	Hydrogenation	PEIC
1-Phenyl-2-propen-1-ol	S[7]	Polarimetry	PEIC
4-Phenyl-E-3-buten-2-ol	S[7]	Hydrogenation	PEIC
4-Phenyl-2-butanol	S[7]	NMR	PEIC
3-Octanol	F	Polarimetry	PEIC
1-Octin-3-ol	A	HRGC	MTPA

[a] A = Aldrich; B = Bader; F = Fluka; L = Lancaster; S = Synthesis.

Esterification. To a solution of 2.5 mmol of each (R,S)-alcohol and dodecanoic acid in 25 ml n-hexane (dist.) one g porcine pancreas extract (Sigma) was added and the mixture continuously stirred at 70°C for 24 h under reflux. After cooling, the enzyme was filtered off and the volume adjusted to 50 ml using diethyl ether. From this solution 1 ml was taken and 0.2 ml of a standard solution (25.1 mg/ml n-butyldodecanoate for C_4- and C_5-alcohols; 19.1 mg/ml n-heptyldodecanoate for C_6- to C_9- and aromatic alcohols) was added. The mixture was analyzed by high resolution gas chromatography (HRGC).

HRGC. A Hewlett-Packard 5710 A gas chromatograph with a Gerstel capillary injection/detection system was used. Split injection (1:50) was employed. The flow rates were 2.5 ml/min He (carrier gas), 30 ml/min N_2 (make-up gas), 30 ml/min H_2 and 300 ml/min air (detector gases). The injector and detector temperatures were 200°C and 300°C. A J & W fused

silica WCOT DB-5 column (30 m, 0.259 mm i.d.; df = 0.25 μm) was employed. The temperature program was 4 min. isothermal at 80°C, then 80-300°C at 10°C/min. The reaction rate was calculated by % ester = $Area_{Ester} \times m_S \times D \times 100 / Area_{Stand.} \times MW_{Ester} \times M$ (m_S = mass of the standard in the sample; D = dilution factor; MW = molecular weight; M = Quantity of the alcohol, mmol).

Separation of products. The n-hexane-diethyl ether solution obtained after filtration of the enzyme was evaporated under vacuum. The residue was subjected to preparative TLC (silica gel 60 PF_{254}, df = 1.25 mm) using pentane + diethyl ether (9+1) as solvent. In all cases the ester was recovered in a highly pure form (> 95 % by HRGC), whereas the alcohol fraction was slightly contaminated with dodecanoic acid (Rf data: alcohol, 0.08-0.13; dodecanoic acid, 0.17-0.20; ester, 0.4-0.6). Reduction of the isolated ester with $LiAlH_4$ (300 mg) to release the esterified alcohol was carried out in 20 ml diethyl ether. After refluxing for 20 hrs, hydrolysis was performed by addition of 1 ml dist. water and 300 μl NaOH (15 %). The precipitate was filtered off and the solvent evaporated under vacuum. The residue contained pure chiral alcohol and 1-dodecanol as confirmed by HRGC.

Chiral analysis. Different HRGC techniques (cf. Table 1) were used after derivatization with (i) (R)-(-) α-methoxy-α-trifluoromethylphenylacetylchloride (MTPA-Cl, Mosher's reagent), (ii) (R)-(+)-phenylethylisocyanate ((R)-(+)-PEIC) and (iii) (R)-α-phenylpropionylchloride (HTA-Cl) as well as (iv) without derivatization (Lipodex C). (i) In a conical vial 1.5 μl alcohol was mixed with 4 μl pyridine and 2.5 μl MTPA-Cl. After 1 h incubation at 105°C, 0.5 ml methanol was added and the solution analyzed by HRGC (1 μl injection; temperature program, 100-200°C at 2°C/min). (ii) and (iii) In a conical vial 1.5 μl alcohol was mixed with 2.5 μl (R)-(+)-PEIC (HTA-Cl). After 2 h incubation at 105°C, 0.5 ml methanol was added and the solution analyzed by HRGC (cf. (i)). (iv) Direct HRGC enantioseparation was performed on a 30 m x 0.25 mm i.d. Lipodex C column (Macherey & Nagel)[9] (isothermal at 60°C).

Determination of the configurations of the enriched enantiomers. A. Polarimetry. This technique was used in cases where the correlation between the configuration and the sense of rotation was known from the literature. The sample (0.05 mmol) was dissolved in 1 ml ethanol and measured in a Perkin Elmer Polarimeter 241 MC. B. Hydrogenation. In cases where this correlation was not known, the products were hydrogenated to saturated alcohols whose configurations were well known: To 1 ml

ethanol 0.05 mmol of the sample and 100 mg of the catalyst (10 % Pd on active coal) were added. The reaction was carried out over 3 hrs in a hydrogenation bomb under H_2 atmosphere (30-50 bar). The catalyst was filtered off and polarimetry of the solution was performed. C. HRGC. Assuming that the order of elution of two neighbours in a series of homologues does not change in HRGC, the configuration of a compound can be derived by the order of elution of its homologue. In most cases HRGC was used to assure the results obtained by other methods. Only for E-2-heptene-4-ol and 1-octine-3-ol the configuration was evaluated by HRGC alone. D. NMR. According to Helmchen et al.[10,11] the alcohols were converted into diastereomeric esters of HTA. Diastereotopic protons gave distinct signals in the NMR spectra. The relative positions and intensities of these signals were used for the evaluation of configuration. In order to release the optically enriched alcohols from the dodecanoic acid esters hydrolysis was performed using methanolic KOH (3.4 g; 10 % KOH; 24 hrs). After the addition of 1 ml of dist. water, the alcohol was extracted with 2 x 20 ml of diethyl ether. The organic phase was dried over anhydrous Na_2SO_4 and evaporated. Optically pure HTA-Cl was obtained by reaction of 3 mmol (R)-(-)-HTA with 2 ml of oxalylchloride during 5 min at room temperature. In 5 ml $CHCl_3$ 1 mmol alcohol and 3 mmol HTA-Cl were mixed and the mixture kept at 60°C for 3-5 d until complete reaction was indicated by HRGC. The ester was isolated by prep. TLC as mentioned above and dissolved in 1.5 ml $CDCl_3$/TMS. NMR analysis was performed at 200 MHz using a Bruker AC 200 apparatus.

Results and discussion

First of all, standard experimental conditions were developed enabling us to efficiently esterify chiral (R,S)-alcohols (cf. Experimental). In a series of experiments the influence of structural features of the alcohols used on the activity and the selectivity of the enzymatic catalysis was studied.

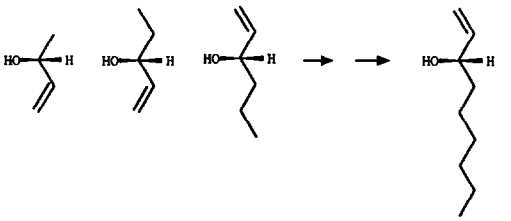
A. (R,S)-1-Alken-3-ols

The results obtained in the homologous series of (R,S)-1-alken-3-ols are outlined in Table 2. In this series the configuration of the preferably converted enantiomer changed between the C_5- and C_6-alkenol. Such a reversal of configuration has been previously observed in PLE catalyzed hydrolyses.[12] For better understanding this phenomenon, the structures

Table 2. Enzyme catalyzed esterification of homologous 1-alken-3-ols

Substrate	Ester formation (%)	Enantiomeric excess (%) Ester	Alcohol	Preferred enantiomer
1-Buten-3-ol	33.2	50.7	n.d.	R
1-Penten-3-ol	28.0	35.0	17.6.	R
1-Hexen-3-ol	26.1	46.7	12.9	S
1-Hepten-3-ol	13.6	52.2	13.5	S
1-Octen-3-ol	24.3	59.1	24.1	S
1-Nonen-3-ol	28.4	58.5	22.3	S

of these enantiomers are outlined as follows:



In this projection, the shorter, less bulky side chains are in the top position. In the homologous series of 1-alken-3-ols, the reversal of configuration occurred in the case when both side chains were equal, i.e. 1-penten-3-ol. At the same time, the ee value reached a minimum.

B. (R,S)-1-Alken-4-ols

The data obtained in this series are summarized in Table 3. The ester formation rates were found to be similar to those determined for the

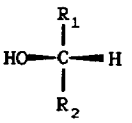
Table 3. Enzyme catalyzed esterification of homologous 1-alken-4-ols

Substrate	Ester formation (%)	Enantiomeric excess (%) Ester	Alcohol	Preferred enantiomer
1-Penten-4-ol	35.9	55.3	55.9	R
1-Hexen-4-ol	26.4	40.3	21.5	R
1-Hepten-4-ol	19.9	63.5	17.0	S
1-Octen-4-ol	20.5	81.1	28.2	S

1-alken-3-ols. However, in the case of 1-alken-4-ols, a continuous decrease of ester formation was observed. With increasing chain length, the reactive OH group is more and more shifted into the center of the molecule and less accessible by the enzyme. Again reversal of configuration, i.e. between the C_6- and C_7-alkenol was observed. The ee values of the esters reached higher values in the series of 1-alken-4-ols than in that

of 1-alken-3-ols. This indicates the influence of an unsaturated bonding relative to the chiral reactive group on the selectivity of the enzymatic catalysis.

As a first conclusion, it can be stressed that the preferably esterified enantiomer exhibits the following configuration:



(I)

R_1 is the shorter, less bulky, R_2 the longer (larger) side chain.

C. (R,S)-E-3-Alken-2-ols

These compounds containing the reactive OH group at a peripheral position of the molecule were esterified fast, with nearly constant rates and high selectivity (Table 4). They did not differ in their reactivity

Table 4. Enzyme catalyzed esterification of homologous E-3-alken-2-ols

Substrate	Ester formation (%)	Enantiomeric excess (%) Ester	Alcohol	Preferred enantiomer
E-3-Penten-2-ol	43.0	79.1	77.6	R
E-3-Hepten-2-ol	40.3	86.1	97.0	R
E-3-Octen-2-ol	42.6	93.8	97.7	R

from the corresponding alkanols.[2] Obviously, the E-configurated double bond did not exhibit any influence on the enantioselectivity that was dependent, on the other hand, on the structure of the side chains at the chiral C-atom. With increasing chain length, i.e. increasing discrepancy between R_1 and R_2, the enantioselectivity also increased.

D. (R,S)-E-2-alken-4-ols

In this series a drastic decrease of the ester formation rate with increasing chain length was observed (Table 5). The reduced accessibility of the reactive group for the enzyme in the higher homologues may be responsible for this effect. In analogy to the 1-alken-3-ols and 1-alken-4-ols reversal of the configuration of the preferably esterified enantiomer was found, again at the position where both side chains were equal.

Table 5. Enzyme catalyzed esterification of homologous E-2-alken-4-ols

Substrate	Ester formation (%)	Enantiomeric excess (%) Ester	Alcohol	Preferred enantiomer
E-2-Penten-4-ol	43.0	79.1	77.6	R
E-2-Hexen-4-ol	33.7	90.0	60.8	R
E-2-Hepten-4-ol	12.0	71.9	11.1	R
E-2-Octen-4-ol	5.7	80.0	1.1	S

E. Isomeric hexenols

Due to the above-mentioned reasons both the increased reactivity of 1-hexen-5-ol (OH at C_2) and the reversal of the configuration between 1-hexen-3-ol and 1-hexen-4-ol ("model" of the proportions of the side chains) seemed to be plausible (Table 6).

Table 6. Enzyme catalyzed esterification of isomeric hexenols

Substrate	Ester formation (%)	Enantiomeric excess (%) Ester	Alcohol	Preferred enantiomer
1-Hexen-3-ol	26.1	46.7	12.9	S
1-Hexen-4-ol	26.4	40.3	21.5	R
1-Hexen-5-ol	43.3	71.1	72.9	R

F. Z-/E-isomeric alkenols

The ester formation rates of Z-isomers were found to be considerably lower than those of the analogous E-isomers (Table 7). Obviously, the

Table 7. Enzyme catalyzed esterification of Z/E-isomeric alkenols

Substrate	Ester formation (%)	Enantiomeric excess (%) Ester	Alcohol	Preferred enantiomer
Z-3-Hepten-2-ol	6.9	53.3	1.2	R
E-3-Hepten-2-ol	40.3	86.1	97.0	R
Z-3-Octen-2-ol	14.3	40.6	9.1	R
E-3-Octen-2-ol	42.3	93.8	97.7	R

reactive center is protected by the particular geometry of the allylic Z-double bond. In all cases, the (R)-enantiomer was the preferred substrate. However, the esterification of E-isomers exhibited much higher selectivity. At the moment we are not able to explain this effect.

G. Influence of saturation degree

In the study of C_8-alcohols that differed in their saturation degree variations in the activity and the selectivity of the enzymatic catalysis were found (Table 8). Both the ester formation rate and the enan-

Table 8. Enzyme catalyzed esterification of C_8alcohols: Influence of the degree of saturation

Substrate	Ester formation (%)	Enantiomeric excess (%) Ester	Alcohol	Preferred enantiomer
3-Octanol	28.6	80.7	41.1	R
1-Octen-3-ol	24.3	59.1	24.1	S
1-Octen-3-ol	8.3	10.3	2.8	S

tioselectivity decreased with higher saturation degree. (Note: The change from (R)- to (S)-ester is not a real reversal of the configuration, but only caused by applying the rules of *Cahn-Ingold-Prelog*.

H. Aromatic alcohols

In this series nearly constant ee values, but decreasing reactivity of saturated alcohols in comparison to the unsaturated members were observed (Table 9). Obviously, the aromatic ring in the substrate is the main

Table 9. Enzyme catalyzed esterification of aromatic alcohols

Substrate	Ester formation (%)	Enantiomeric excess (%) Ester	Alcohol	Preferred enantiomer
1-Phenyl-2-propen-1-ol	19.3	85.7	30.0	R
1-Phenyl-1-propanol[a]	18.0	86.9	19.6	R
4-Phenyl-E-3-buten-2-ol	43.8	88.5	73.1	R
4-Phenyl-2-butanol	28.9	83.3	44.4	R

[a]Data taken from (X).

factor determining the selectivity, whereas other influences, such as a double bond, are less important. In the stereochemical projection of the converted enantiomers the large benzene ring is at the bottom position, i.e. the aromatic substrates are in accordance with the above postulated "model".

Conclusions

The structural presumptions outlined in formula I for the preferably esterified enantiomer were confirmed by a number of experiments (Tables 2-9). As to quantify the enantioselectivity, various influences have to be discussed. Concerning the effect of a double bond, the following conclusions can be drawn:

(i) The presence of a double bond at the end of the substrate leads to a distinct decrease in selectivity, i.e. approximately 20-30 % in comparison to the corresponding alkanols (Tables 2,3,6,8). With increasing distance between the double bond and the reactive group its influence decreases (cf. Tables 2,3). (ii) Double bonds inside linear molecules do not influence the selectivity in a significant way (Tables 4,5). However, an important exception consists in the occurrence of an allylic Z-double bond that tremendously influences the selectivity (Table 7).

As to aromatic systems (Table 9), the phenyl group is the dominating structural element that effects considerably the selectivity of the catalysis. Other stereochemical effects are less important.

Regarding the reactivity in the enzymatic catalysis, two criterions have to be mentioned, i.e. (i) the length of R_1 (cf. formula I): the shorter R_1, the better accessible is the reactive OH group for the enzyme; (ii) the absence of an allylic double bond. Sterical increase of R_1 results in a loss of reactivity of an enantiomer (Tables 3,5). Generally it can be ruled out: The enantioselectivity is the higher the more the side chains of the substrate differ. However, some exceptions were found, i.e. 1-hepten-4-ol and 1-octen-4-ol. But it should be considered that the studies were carried out with a crude pancreatic extract. In order to characterize the active (iso)enzyme according to its kinetic and structural features, enzyme purification is needed. Actually, there are some hints that lipase is not responsible for the catalysis in organic media[13-15]. This hypothesis was confirmed by our recent preliminary studies; work to characterize the effective enzyme fraction is under progress.

Acknowledgment. The financial support of this work by the Deutsche Forschungsgemeinschaft, Bonn, is gratefully acknowledged. The authors thank W. Murtiashaw, Pfizer Inc., Groton, USA, for providing several polarimetric data.

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