Structural Effects in the Enzymatic Resolution of 2-Octanol

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The effect of the alkyl group in various butyric esters on the resolution of racemic 2-octanol, catalysed by porcine pancreatic lipase, has been studied in ether containing 0.5 wt % of water. (*R*)-2-Octyl butyrate was obtained in high optical purity, the enantiomeric excess varying from 100 % for the 2,2,2-trifluoroethyl ester to 87 % for the 2-chloroethyl ester. Reaction rates depend strongly on the polar and steric effects of the alkyl group.

Enzymes have become important tools in the preparation of asymmetric organic compounds and in the resolution of racemic mixtures, especially since it has been observed that several enzymes not only retain their activity but often function more effectively when the water used as a solvent is replaced by organic solvents.[1] Porcine pancreatic lipase has been found to catalyse esterification and transesterification reactions in nearly anhydrous organic solvents. Because of the high stereospecifity found in these reactions, the method has been succesfully employed for the resolution of racemic alcohols and carboxylic acids. In the resolution of secondary alcohols only the (*R*)-isomer of the alcohol was found to be reactive.[2]

The purpose of this work was to study the structural effects of the alkyl component of the ester employed in the enzymatic resolution of 2-octanol according to eqn. (1).

$$PrCO_2CH_2R + 2\ (\pm)\text{-2-octanol} \rightarrow (R)\text{-}PrCO_2\text{-2-octyl} + (S)\text{-2-octanol} + RCH_2OH \quad (1)$$

Experimental

Materials. The enzyme used was a crude porcine pancreatic lipase (E.C.3.1.1.3) purchased from Sigma Chemical Co. (Product L 3126).

The esters were prepared from the corresponding alcohols and butyryl chloride at room temperature or with slight warming when necessary. The reaction was followed by ^{1}H NMR spectroscopy. Chloromethyl butyrate was prepared from butyryl chloride and paraformaldehyde.[3] The esters were purified by distillation.

The following alkyl butyrates, $PrCO_2CH_2R$, were prepared [R, b.p./°C/(*p*/Torr), yield (% of theory)]: CF_3, 112–114/760, 87; CCl_3, 95/15, 92; $CHCl_2$, 93–94/24, 95; CH_2Cl, 85/22, 90; CH_2CH_3, 143/760; CBr_3, 93/1.0, 96; CMe_3, 168/760, 87; $CHMe(CH_2)_4CH_3$ 94–95/4.1, 88; Cl, 79/43, 23.

The measured NMR data were consistent with the structures. For tribromoethyl butyrate (no literature data available) the NMR data are as follows: ^{1}H NMR (400 MHz, $CDCl_3$): 1.02 (3 H, t), 1.76 (2 H, m), 2.48 (2 H, t), 4.94 (2 H, s); ^{13}C NMR (100 MHz, $CDCl_3$): 13.8 (CH_3), 18.4 (CH_2), 36.0 (CH_2), 76.5 (CBr_3), 76.8 (CH_2), 171.9(CO).

Resolution experiments. The resolution of 2-octanol was performed by the procedure of Kirchner *et al.*[2] Lipase (8.00 g) was weighed into a conical flask and 10.4 g (0.08 mol) of racemic 2-octanol, 0.096 mol (20 % excess) of the butyric ester, 55 ml of dried diethyl ether and an amount of water to give a total water concentration of ca. 3.3 % of the amount of the enzyme (0.5 % of total) were added. The mixture was shaken at room temperature and the reaction was stopped by filtering off the enzyme after ca. 45 % conversion, except in the case of the most unreactive esters. The mixture was dried with magnesium sulfate. 2-Octanol and 2-octyl butyrate were separated by distillation. The specific rotations of the compounds prepared were determined with a JASCO Model DIP-360 digital polarimeter. (*R*)-2-Octanol was not isolated from the ester formed, but it can easily be obtained in pure form, without racemisation by alkaline alcoholysis or hydrolysis[2] taking place, by acyl–oxygen fission.

The initial rates and the progress of the reactions were determined by taking samples from the reaction mixture at intervals and analysing them by GLC by measuring the ratio of the starting and formed esters. A Perkin Elmer gas chromatograph equipped with a 25 m OV-225 capillary column was used. The column temperature was usually 80 °C for 4 min, then increased by 30 °C min^{-1} initially to 120 °C for 1 min and finally to 150 °C. The results are given in Table 1. Similar results were also obtained when toluene was used as the solvent instead of ether.

The yield was calculated by assuming that only a half of the racemic ester reacts via the enzyme-catalysed reaction. No uncatalysed reaction with 2-octanol was found even in the case of the most highly activated ester, 2,2,2-trifluoroethyl butyrate. No reaction took place between neopentyl butyrate and 2-octanol even in the presence of the enzyme.

Table 1. The formation of (*R*)-2-octyl butyrate from racemic 2-octanol and the esters $PrCO_2CH_2R$, catalysed by porcine pancreatic lipase in ether at room temperature.

R	v_0/μmol min^{-1} g^{-1}	Reaction time/h	Degree of conversion (%)	Yield (%)	e.e. (%)
CF_3	13	70	46	79	100
CCl_3	3.9	134	43	65	97
$CHCl_2$	2.4	340	45	80	90
CH_2Cl	0.23	1750	36	31	87
CH_2CH_3	0.07	1750	26	26	93
$C(CH_3)_3$	–	–	–	–	–

In addition to the esters presented in Table 1, the reaction of 2,2,2-tribromoethyl butyrate was studied, but it was found to be practically unreactive, evidently because of inactivation of the enzyme by hydrogen bromide formed from tribromoethanol[4] according to eqn. (2).

$$CBr_3CH_2OH \rightarrow HBr + [CBr_2{=}CHOH] \rightarrow HBr + CHBr_2CHO \quad (2)$$

Discussion

As shown in Table 1, the products obtained from the reaction of several butyrate esters with racemic 2-octanol catalysed by commercial porcine pancreatic lipase were of very high enantiomeric purity. When 2,2,2-trichloroethyl butyrate was the substrate, the yield (65 %), enantiomeric excess (e.e. 97 %) and specific rotation (−7.13°, *c* 5, diethyl ether, 25 °C) obtained after 43 % conversion were in good agreement with the corresponding values 70 %, 95 % and −7.0°, respectively, obtained by Kirchner *et al.*[2] under similar reaction conditions.

The initial rates of the enzymatic 2-octanolyses of the butyrates studied strongly depend on the structure of the substrate (Table 1). Different linear free-energy relationships are widely used to study organic reactivity in non-enzymatic reactions. Enzymatic ester hydrolysis has a marked resemblance to basic ester hydrolysis, and a linear Hammett relationship is observed, e.g., for the acylation or deacylation of serine proteases, subtilisin and chymotrypsin, by phenyl acetates in water.[5,6] Furthermore, a Hammett analysis of the subtilisin-catalysed hexanolysis of *para*-substituted phenyl acetates in several organic solvents has revealed marked similarities in the transition-state structure of the enzymatic reaction in non-aqueous and aqueous media.[6]

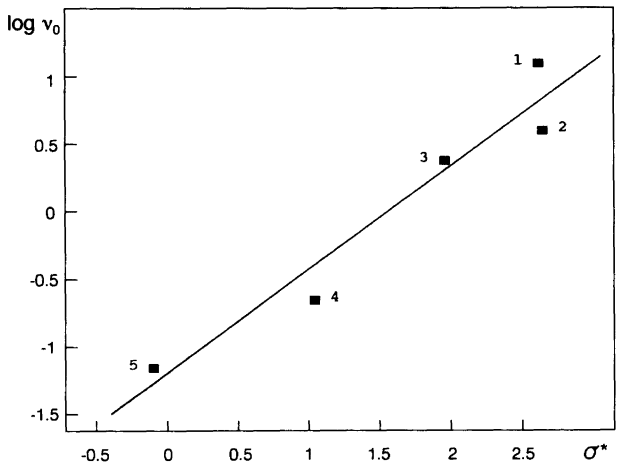

Fig. 1. Taft correlation for v_0 of porcine pancreatic lipase-catalysed 2-octanolyses of substituted alkyl butyrates, $PrCO_2CH_2R$, in ether: R = CF_3 (1), CCl_3 (2), $CHCl_2$ (3), CH_2Cl (4) and CH_2CH_3 (5).

In the case of aliphatic esters the Taft equation has been widely used to interpret structure–reactivity correlations.[7] The dependence of the initial rates of the esters studied as a function of Taft's σ* constants is presented in Fig. 1. Although the lipase was used without purification and the rates include at least the acylation and deacylation steps of the enzymatic reaction, a positive and relatively good correlation between the polar substituent constants and the rates of the enzyme-catalysed alcoholysis is observed. The unexpectedly high rate difference between 2,2,2-trifluoroethyl and 2,2,2-trichloroethyl butyrates with almost the same σ* values (2.60 and 2.65, respectively) is, without doubt, an indication of the role of steric factors of the alkyl group of the ester on the enzyme-catalysed reaction. Unfortunately, steric reaction parameters for the substituted alkyl groups are not available, and the rates of the alkaline and acid ester hydrolyses of the corresponding esters are not known. However, on the basis of the present results, it can be concluded that similar structural effects are of importance both in normal basic hydrolysis and in enzyme-catalysed alcoholysis reactions; in the last-mentioned reaction steric effects evidently have a more important role.

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