

Effect of Water on Enzymatic Activity and Stereoselectivity in Organic Solvents. Transesterification of a Disubstituted Malonate Diester

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The reaction rate and stereoselectivity of the preparatively important enzymatic transesterification of a prochiral symmetrical malonate diester in organic media was shown to be considerably increased by small quantities of added water.

It is now well established that hydrolytic enzymes suspended in organic solvents can be used for synthesis of chiral synthons.¹ An important advantage of working in organic solvents rather than in water is the possibility of altering the enzyme's properties by variations in the reaction medium. It has been shown that the nature of solvent may have a

profound effect on substrate specificity, as well as on the activity and enantioselectivity of enzymes.² Water content is another important variable of the reaction medium, and its influence on enzyme activity and enantioselectivity in organic solvents has also been recently investigated. Stokes³ and Klivanov⁴ found that an increased water content resulted in a

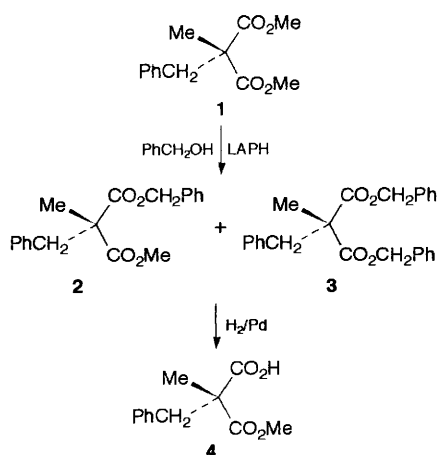


Table 1 Effect of added water on reaction rate and prochiral stereoselectivity of the enzyme catalysed reaction

Added water (%)	Initial rate ^a	Conversion 2 (%) ^b	Conversion 3 (%) ^b	E.e. (%) ^c
0	56.3	47.2	4.5	39.1
1	140.5	76.3	3.8	51.0
5	162.1	81.6	2.1	61.8

^a In $\mu\text{mol h}^{-1} (\text{g enzyme})^{-1}$. ^b % Formed after 45 h. Reaction progress was monitored by integration of ¹H NMR signals, which enabled quantitative determination of the remaining dimethyl ester **1**, the mixed benzyl methyl diester **2** and the byproduct dibenzyl ester **3**. ^c Enantiomeric excess of the (*S*)-enantiomer, determined by HPLC on a chiral column (Chiralcel OJ, Daicel) with hexane-propan-2-ol (95:5) as the mobile phase at a flow rate of 0.9 ml min⁻¹ using detection at 258 nm. The (*R*)-enantiomer of **2** had $t_R = 21$ min, and the (*S*)-enantiomer had $t_R = 31$ min.

lowering of enantioselectivity and an increase in activity, while Kitaguchi⁵ reported that addition of water is accompanied by an increase in both enzyme activity and enantioselectivity. In this communication we report for the first time that enzymes' prochiral selectivity as well as the reaction rate is markedly effected by variations in water content.

As an extension of our previous studies⁶ on exploiting enzymes' prochiral selectivity in organic solvents, we have examined the transesterification of prochiral symmetrical disubstituted malonate diesters, such as **1**, with benzyl alcohol in organic solvents (eqn. 1). Benzyl alcohol was chosen in order to enable the preparatively useful optically active half ester **4** to be prepared *via* catalytic hydrogenolysis of **2**. Screening of six commercially available lipase and three protease preparations in several organic solvents revealed that the lipase from liver acetone powder horse (LAPH), and to a lesser extent the lipase from liver acetone powder pig (LAPP), catalysed the reaction in cyclohexane at a modest rate. However, the enantiomeric excess (e.e.) of the chiral diester **2** was only around 37–40%. Furthermore, formation of **3**, which was apparent from the ¹H NMR spectrum during the early stages of the reaction (Fig. 1), served as another indication of the low chemical and stereochemical yield of the desired product **2**.

To improve the stereoselectivity of this preparatively important reaction, we attempted to alter the catalytic properties of the enzyme by changes in the reaction medium and examined the influence of various amounts of added water. As can be seen from Table 1 and Fig. 1, reaction rate and stereoselectivity were considerably improved by adding small quantities of water, with the optimum at 5% water in

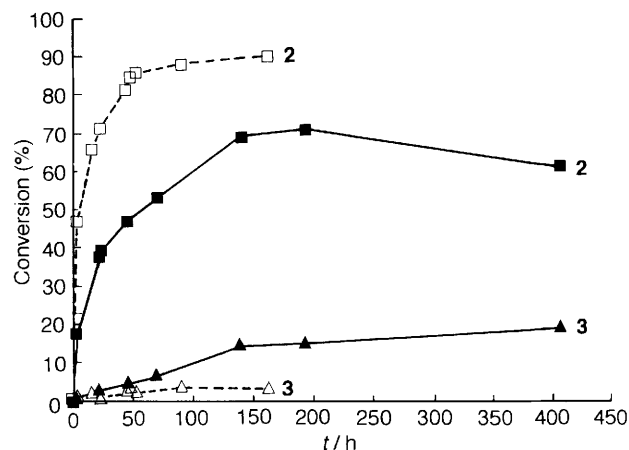


Fig. 1 Formation of **2** (squares) and **3** (triangles); ■, ▲, in cyclohexane; □, △, in cyclohexane + 5% water

cyclohexane.† Under these conditions the desired mixed diester **2** was obtained in high chemical yield and had an e.e. of 61.8%.

In a representative experiment powdered crude LAPH preparation (1.8 g) was added to a solution of the dimethyl ester **1** (500 mg) and benzyl alcohol (1.8 ml) in cyclohexane (18 ml) containing distilled water (0.9 ml), and the suspension was shaken at 40 °C at 200 rpm. The reaction was terminated by filtering off the enzyme, followed by evaporation of the solvent and fractional distillation to separate the remaining dimethyl ester **1**, the optically enriched (*S*)-enantiomer of **2** and the byproduct dibenzyl ester **3**. Palladium catalysed hydrogenolysis of **2** in dry ethanol afforded in quantitative yield the corresponding (*R*) half ester possessing two readily distinguishable functional groups and thus providing a versatile chiral building block.⁷

In conclusion we demonstrated that a three-fold enhancement in reaction rate and a 50% increase in prochiral selectivity of the lipase from LAPH could be achieved simply by adding small quantities of water. This suggests that the amount of water included in a reaction system, in addition to the nature of solvents, should be closely examined in order to optimise results for enzymatic reactions in organic media.

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- The absolute configuration of **4** was confirmed by comparing the optical rotation with that reported in: F. Bjorkling, J. Boutelje, S. Gatenbeck, K. Hult, T. Norin and P. Szmulik, *Tetrahedron*, 1985, **41**, 1347. The absolute configuration of **2** follows from that of **4**.

† Carl Fischer measurements showed that the commercial LAPH preparation contained ca. 7% (w/w) of water. The added water also seemed to absorb on the enzyme powder. Addition of more than 5% of water did not lead to a further improvement in reaction rate or stereoselectivity.