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Lipase-Catalysed Transesterifications using 2,2,2-Trifluoroethyl Butyrate: Effect of Temperature on Rate of Reaction and Enantioselectivity+

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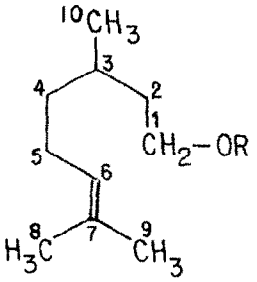
Abstract : Temperature of the reaction and the solvent used markedly influenced the enantioselectivity and rate of transesterification reaction catalysed by *Candida cylindracea* between 2,2,2-trifluoroethyl butyrate (TFEB) and 3,7-dimethyl-6-octenol, and that between TFEB and 2,2-dimethyl-1,3-dioxolane -4-methanol.

Biocatalysis in organic media[1] has led to the introduction of a new fundamental variable into studies of enzyme-substrate interactions and several systems (biphasic systems, reverse micelles and monophasic systems) and classes of enzymes (oxidoreductases, hydrolases and isomerases) have been successfully employed for synthetic purposes.[2] In this context, especially widespread are the lipase-catalysed esterifications and transesterifications in anhydrous organic solvents for regioselective acylation of polyhydroxylated natural compounds[3] and for resolution of racemic alcohols and acids.[4] However, it is only recently that the effect of the nature of organic solvents on the regio-[5] and enantioselectivity[6] of lipases has been investigated by Klibanov and coworkers and others. In some cases it has been established that the nature of solvent has a profound effect on substrate specificity, regioselectivity and enantioselectivity of the enzyme. In the present study, we have investigated the enantioselectivity of *Candida cylindracea* lipase as an interesterification catalyst with (±)-3,7-dimethyl-6-octenol (**1**) and (±)-2,2-dimethyl-1,3-dioxolane -4-methanol (**3**) in anhydrous organic solvents (*n*-hexane, *n*-decane, isooctane and carbon tetrachloride) for the following reasons: (i) this enzyme is catalytically active in a number of organic solvents and its behaviour in such media has been thoroughly investigated, (ii) enantioselectivity of these enzymes increases in organic solvents and (iii) this enzyme requires no added co-factors.[7] We have also studied the effect of temperature on the enantioselectivity of *Candida cylindracea* lipase with (±)-**1** and (±)-**3** over the range 2-60°C.

We have selected 3,7-dimethyl-6-octenol and 2,2-dimethyl-1,3-dioxolane-4-methanol as target molecules for resolution in different organic solvents with 2,2,2-trifluoroethyl butyrate as active ester for transesterification because of their potential use in perfumery industry and as a chiron, respectively. The preliminary investigation of CCL with both these substrates in different organic

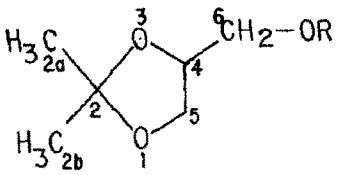
+ A part of this work was presented at the IUPAC-NOST International Symposium on Enzymes in Organic Synthesis held in New Delhi (India) on 6-9 January 1992.

solvents revealed that isooctane is best suited for realisation of maximum conversion. The general procedure used was : a weighed amount of 3,7-dimethyl-6-octenol or 2,2-dimethyl-1,3-dioxolane-4-methanol (2-3 mmol) was dissolved in dry isooctane (20-30 ml) and 2,2,2-trifluoroethyl butyrate (2.5 equivalent) was added, followed by CCL (100-150 mg). The suspension was shaken at 250 rpm and progress of the reaction was monitored on TLC. After completion, the reaction was quenched by filtering off the enzyme. The solvent was removed to dryness *in vacuo* and the product ester as well as the unreacted starting alcohol were isolated by column or thin layer chromatography. The product esters were unambiguously identified from their spectral data[8] and specific rotations of unreacted starting alcohols were measured and the optical purities determined by comparison of our rotation values with the corresponding literature values.[9]



1 R = H

2 R = $CO\text{-}CH_2\text{-}CH_2\text{-}CH_3$



3 R = H

4 R = $CO\text{-}CH_2\text{-}CH_2\text{-}CH_3$

The optical purities of esters obtained by transesterification of 3,7-dimethyl-6-octenol (**1**) and 2,2-dimethyl-1,3-dioxolane-4-methanol (**3**) by CCL in anhydrous isooctane at different temperatures are tabulated below:

Entry	Temp(°C)	3,7-dimethyl-6-octenol (1)			2,2-dimethyl-1,3- dioxolane-4-methanol (3)		
		Time/h	Conv.(%)	Optical Purity(%)	Time/h	Conv.(%)	Optical purity %
1	2	13	50	0	25	0	No reaction
2	15	11	50	27.4	15	40	22.8
3	25	8	50	61.7	10	40	16.6
4	38	8	50	23.4	8	40	6.7
5	60	23	50	72.3	45	0	No reaction

Salient features of the reactions are:

a) The rate of interesterification between both the alcohols **1** and **3** and 2,2,2- trifluoroethyl butyrate was found to be maximum in isooctane and hence we studied the effect of temperature in isooctane only.

b) The rate as well as enantioselectivity in case of 3,7-dimethyl-6-octenol was found to be dependent on temperature of the reaction. The rate of interesterification was maximum at 38°C and it slightly decreased when the temperature was increased to 60°C or decreased to 2°C. The enantioselectivity of CCL was found to be maximum at 60°C and the enzyme preferentially catalysed the esterification of (-)-isomer. No appreciable selectivty was observed at 2°C or 38°C.

c) Interesterification with 2,2-dimethyl-1,3-dioxolane-4-methanol was also dependent on temperature. The maximum rate was observed at 38°C and no appreciable reaction was observed at 2°C or 60°C. In case of 2,2-dimethyl-1,3-dioxolane-4-methanol, the lipase does not show any marked preference for either of the two enantiomers.

d) The reactions performed on 3,7-dimethyl-6-octenol and 2,2-dimethyl-1,3-dioxolane-4-methanol under the same conditions, but without adding the enzyme did not indicate any interesterification.

The present study unambiguously demonstrates that the temperature of the reaction influences the enatioselectivity of *Candida cylindracea* lipase towards transesterifications on (±) -3,7-dimethyl-6-octenol and (±) -2,2-dimethyl-1,3-dioxolane-4-methanol. Since it is likely that such a behaviour also occurs with other compounds, these results are of notable practical interest because *Candida cylindracea* lipase is widely used in organic solvents for racemate resolutions. In general, the selectivity of enzyme is inversely proportional to temperature,[10,11] because the flexibility of enzyme increases with the rise in temperature and hence selectivity decreases. However, we have observed that the selectivity of CCL increases with rise in temperature in case of 3,7-dimethyl-6-octenol, whereas the results on 2,2-dimethyl-1,3-dioxolane-4-methanol (**3**) are in conformity with the earlier observations. In the course of these studies, we have isolated 3,7-dimethyl-6-octenyl butyrate (**2**) and 2,2-dimethyl-1,3-dioxolane-4-methyl butyrate (**4**) in the pure form for the first time and they have been fully characterised from their spectral data.[8]

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8. **^{1}H NMR (200 MHz, Bruker, $CDCl_3$), ^{13}C NMR (50 MHz, Bruker, $CDCl_3$), IR (nujol) and CIMS spectral data of esters 2 and 4.**

3,7-Dimethyl-6-octenyl butyrate (2): ^{1}H NMR, δ 0.89 (3H,d, J = 7 Hz, H - 10), 0.95 (3H,t,J = 8Hz, H-4'), 1.22-1.42 (3H,m,H-3 and H-4), 1.60-1.85 (10H,m,H-5,H-8,H-9 and H-3'), 1.92-2.12 (2H,m,H-2), 2.24 (2H,t,J = 8Hz,H-2'), 4.12 (2H,t,J =6Hz, H-1) and 5.09 (1H,t,J = 7Hz,H-6); ^{13}C NMR, δ 13.69 (q,C-4'), 18.54 (q,C-10), 19.47 (t,C-4), 25.47 (m,C-8 and C-9), 25.70 (m,C-3'), 29.59 (d,C-3), 35.63 (t,C-2), 36.35 (t,C-5), 37.06 (t, C-2'), 62.77 (t,C-1), 124.69 (d,C-6), 131.25 (s,C-7), 173.73 (s,C-1'); IR ν_{max} (cm^{-1}), 1730, 1460, 1380, 1250 and 1180; MS (CI) m/z (rel. int.), 227 ([M+1]$^+$,100), 155 (45), 137 (62), 95 (7), 83 (16), 71 (5). *2,2 - Dimethyl-1,3-dioxolane-4-methyl butyrate (4):* ^{1}H-NMR, δ 0.93 (3H,t,J = 8Hz, H-4'), 1.35 and 1.40 (6H,2s, gem dimethyl), 1.65 (2H, sextet, J=8Hz ,H-3'), 2.35 (2H,t,J = 8Hz,H-2'), 3.71-3.80 (1H,m,H-5), 4.08-4.19 (3H,m,H-5 and H-6), 4.27-4.35 (1H,m,H-4); ^{13}C NMR, δ 13.41 (q, C-4'), 18.20 (t,C-3'), 25.21 (q,C-2a), 26.49 (q,C-2b), 35.79 (t,C-2'), 64.29 (t,C-5), 66.22 (t,C-6), 73.53 (d,C-4), 109.58 (s,C-2), 173.14 (s,C-1'); IR ν_{max} (cm^{-1}), 1738, 1448, 1360, 1240 and 1165; MS (CI) m/z (rel. int.), 203 ([M+1]$^+$,65),187 (33), 145 (100), 101 (14), 71 (5), 43 (2).

9. **Specific rotations of alcohols 1 and 3.**

$[\alpha]_D^{19}$ + 5.3 (neat) of (R) -(+) -3,7-Dimethyl 6-octenol (**1**) : *Aldrich Catalog Handbook of Fine Chemicals,* 1990-1991, Cat No. 30,346-1, p.339. $[\alpha]_D^{25}$ +15.2 (neat) of (S) -(+)- 2,2-Dimethyl-1,3-dioxolane-4-methanol (**3**) : *Aldrich Catalog Handbook of Fine Chemicals,* 1990-1991, Cat. No. 23,774-4, p.516.

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