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Solvent Effects on an Enzymatic Ester Synthesis in Supercritical Carbon Dioxide

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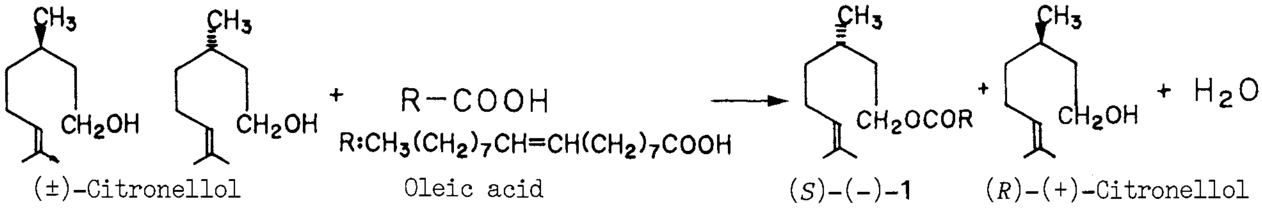
The ester synthesis from oleic acid and (±)-citronellol by lipase was carried out in supercritical carbon dioxide(SC-CO_2). In the near-critical region, the rate of reaction was considerably enhanced and (*S*)-(-)-oleic acid 3,7-dimethyl-6-octenyl ester was stereoselectively formed. The results were discussed with taking account of solvent properties of SC-CO_2 and aggregation phenomenon about reacting species at high pressures.

Recently supercritical carbon dioxide (SC-CO_2) has been recognized as an attractive medium for biocatalytic reactions and others because of its interesting solvent properties.$^{1)}$ However, the consequence of its solvent effects on biocatalysis has not been explored in microscopic level. In the present work, the lipase-catalyzed esterification of oleic acid and (±)-citronellol has been made in SC-CO_2 medium and high pressure effects on reaction rate and stereoselectivity have experimentally been examined. The effects have been discussed with solvent properties of SC-CO_2 as studied previously$^{2)}$ and with aggregation phenomenon about reacting species using apparent activation volume and aggregation cluster size.



The enzyme used was *Candida cylindracea* lipase MY (30 units per mg powder) from Meito Sangyo Co., Ltd. and immobilized on aminopropyl glass beads activated with glutaraldehyde by dissolving 3000 units of the lipase in 10 mL phosphate buffer at pH 7.5. Oleic acid, (±)-citronellol, (*R*)-(+)-citronellol, (*S*)-(−)-citronellol, and aminopropyl glass beads were obtained from Sigma Chemical Co., Ltd. The reaction was performed by a continuous flow operation system as described previously.$^{3)}$ The CO_2 was delivered into a packed bed reactor(130 mm long and 4.6 mm i.d.) at a flow rate of 0.17 NL/min(1 NL= 1 L of gas at 293 K and 101.3 kPa). Oleic acid and (±)-citronellol were fed into a mixing cell at flow rates of 2.44 and 3.15 μmol/min, respectively, and then dissolved in SC-CO_2. The initial concentrations of the substrates were below 0.1% and never exceeded their solubility limit. The reaction was carried out at 7.58-19.30 MPa and 304.1-313.1 K. The effluent solution was flashed to atmospheric pressure and the reaction mixture was dissolved in ethanol and collected in a receiver. The reacting species were analyzed by a gas chromatograph after they were completely separated from the ethanol. The product(**1**), oleic acid 3,7-dimethyl-6-octenyl ester, was isolated from the reaction mixture by column chromatography (Silica gel, 60-240 mesh; solvent, hexane/ethyl acetate=20:1, v/v) The optical rotation of the product(**1**) was measured in ethanol with a

Table 1. Reaction rate, optical purity, and yield of product for the esterification of (±)-citronellol and oleic acid in SC-CO_2

Run	Pressure MPa	Temperature K	Reaction rate$^{a)}$ μmol h^{-1} g-cat^{-1}	$[\alpha]_D$	Optical purity$^{b)}$ %	Yield %
1	7.58	308.1	3.08	-0.45	23.4	1.2
2	8.41	308.1	8.58	-0.08	4.1	3.4
3	10.15	308.1	10.18	-0.04	2.1	4.0
4	15.03	308.1	13.94	-0.04	2.1	5.4
5	19.03	308.1	15.81	0.00	0.0	5.8
6	8.41	304.1	9.27	-1.90	98.9	3.6
7	8.41	313.1	6.27	-0.04	2.1	2.5

a) μmol of oleic acid 3,7-dimethyl-6-octenyl ester formed.

b) Determined by dividing $[\alpha]_D$ observed by $[\alpha]_D$ -1.92 for the ester formed from (*S*)-(−)-citronellol and oleic acid at 353 K in the presence of concentrated surfuric acid.

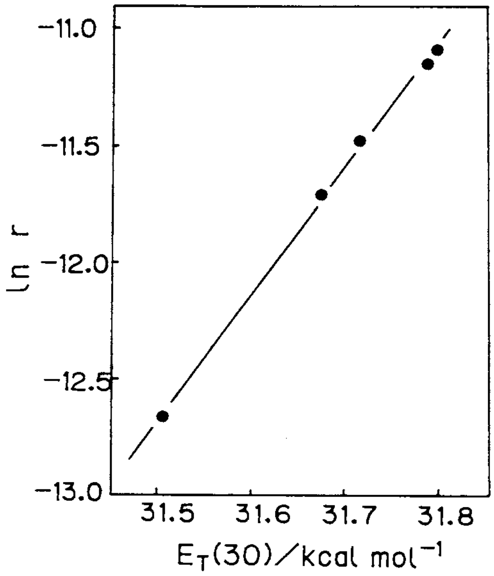


Fig. 1. Relationship between the reaction rate and $E_T(30)$ value of SC-CO_2.

polarimeter. For comparison, the esterification was also carried out by the immobilized lipase (corresponding to 3000 units) in 50 ml of water-saturated cyclohexane containing 10 mmol oleic acid and 13 mmol (±)-citronellol at 304.1 K with shaking.

It was found that the amount of the product(**1**) formed increased with time on stream and reached the steady value in about 1 h under conditions used. The steady rate of reaction continued throughout an experimental period of 20 h. Table 1 gives the steady rate of reaction, μmol of the product(**1**) formed at different pressures and temperatures. The reaction rate was enhanced by increasing pressure and this is pronounced near the critical region; the rate at 8.41 MPa is larger by a factor of 3 than that at 7.58 MPa.

In Fig. 1 the observed steady rate of reaction is plotted against the $E_T(30)$ value for the dye-organized cybotactic region of the SC-CO_2 at each reaction condition. The $E_T(30)$ was previously determined by an IR method and can characterize the polarity of the solvent in the cybotactic region about reacting species.[2) Figure 1 shows a good linear relation,

$$\ln r = 5.508E_T(30) - 185.220 \qquad (1)$$

with a correlation coefficient of 0.9959, suggesting that the activated complex involved in the esterification is more polar than the reactants, and it is more stabilized in the solvent at more polar state, resulting in larger rate of reaction.

Table 1 also shows the optical purity of the product calculated from the specific rotation $[\alpha]_D$, which was determined as $[\alpha]_D=\alpha/(C\times\ell)$, where α is the rotation observed, C is the concentration of the product(**1**), and ℓ is cell length. The optical purity was found to be sensitive to reaction conditions; it is nearly 100% at 8.41 MPa and 304.1 K, indicating that (*S*)-(-)-(**1**) is stereoselectively formed, while it is much smaller at higher pressures and temperature in spite of small deviations from the unity-stereoselectivity condition. Furthermore, in water-saturated cyclohexane solution the stereoselective esterification of (±)-citronellol was not observed at all. Although Sonomoto et al.[4) carried out the esterification from 5-phenylvaleic acid and (±)-citronellol by *C. cylindracea* lipase in water-saturated cyclohexane solution, the stereoselective esterification of (±)-citronellol has not been effected.

For the optical purity of the product(**1**), no good correlation was found with the $E_T(30)$ value. Although the mechanism and kinetics are not yet clear for the present binary-substrate enzymatic reaction in SC-CO_2, a simplified treatment is attempted to discuss the observed high pressure effect on the stereoselectivity in terms of apparent activation volume for the reaction and aggregated cluster size of reacting species. According to the transition state theory, the volume of activation for a reaction is given by

$$\Delta V^{\dagger}=-RT(d\ln k/dp)_T \quad (2)$$

where k is the rate constant. In the present treatment, the reaction rate is used instead of the rate constant in analogy with the simplified evaluation of apparent activation energy $E_a=-Rd\ln r/d(1/T)$. Then, $\Delta V^{\dagger}$ is evaluated by

$$\Delta V^{\dagger}=-RT(d\ln r/dP)_T \quad (3).$$

From equations (1) and (3),

$$\Delta V^{\dagger}=-5.508RT(dE_T(30)/d\rho)_T\rho\beta \quad (4)$$

is obtained. ρ is the density of SC-CO_2 and β is the isothermal compressibility ($-(dV/dP)_T/V$). Using $\Delta V^{\dagger}$ and the fluctuation theory of Debenedetti,$^{5)}$ the cluster size ξ_c at infinite dilution can be calculated as described previously$^{6)}$ by

$$\xi_c=\rho N_{av}k_BT\beta-\Delta V^{\dagger}\beta \quad (5)$$

where N_{av} is Avogadro's number and k_B is Boltzmann's constant. It was found that the cluster size increased as the pressure decreased. The size changes sensitively with pressure in the near-critical region. Figure 2 represents the relationship between the cluster size and the optical purity of the product(**1**) formed. The stereoselective esterification of (±)-citronellol to (*S*)-(-)-(**1**) significantly sets in beyond $\xi_c=20$ and the greater cluster size, the higher the optical purity. This result strongly suggests that the aggregation influences the reaction mechanism and (*S*)-(-)-(**1**) is stereoselectively formed when the extent of aggregation about the activated complex exceeds a certain limit in the near-critical region. Probably this could be because the great aggregation of CO_2 is allowed to deform the enzyme into a steric structure suited for the attack by (*S*)-(-)-citronellol upon the activated complex, and consequently (*S*)-(-)-(**1**) would be stereoselectively produced.

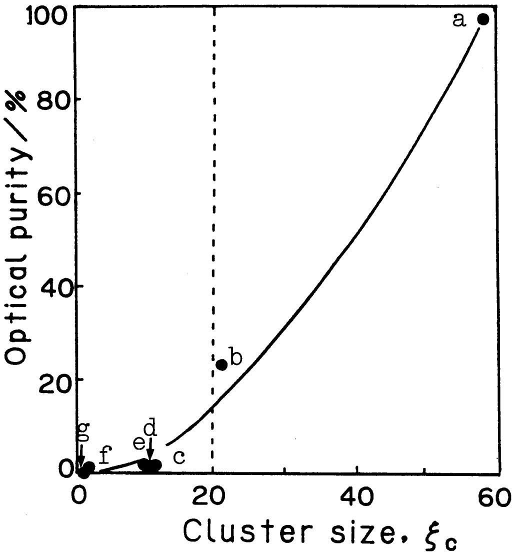


Fig. 2. Relationship between the optical purity of the product(**1**) and the cluster size for the esterification in SC-CO_2. Temperature(K) and pressure(MPa): (a)304.1, 8.41 (b)308.1, 7.58 (c)313.1, 8.41 (d) 308.1, 8.41 (e)308.1, 10.18 (f) 308.1, 15.03 (g)308.1, 19.03.

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