Remarkable acceleration of a lipase-catalyzed reaction by a thiacrown ether additive: buffer-free highly regioselective partial hydrolysis of 4-acetoxy-2-methylbut-2-enyl acetate

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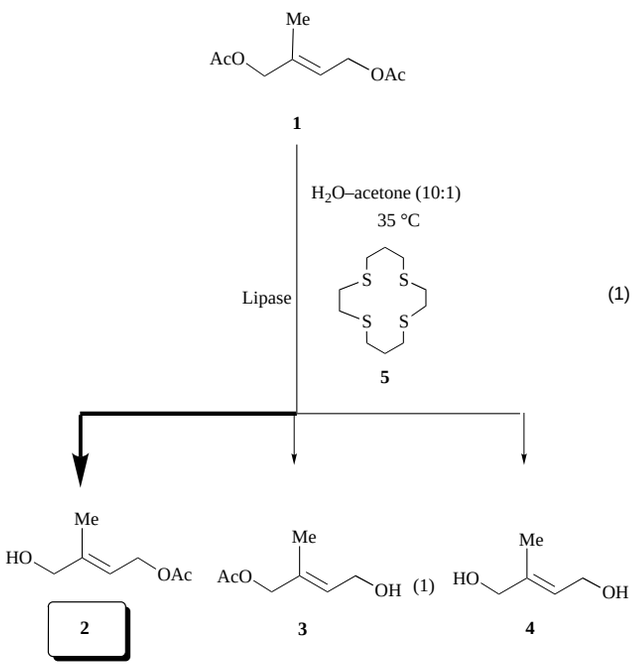
The reaction efficiency of the lipase-catalyzed partial hydrolysis of 4-acetoxy-2-methylbut-2-enyl acetate is greatly enhanced by the presence of 5 mol% of a thiacrown ether, particularly remarkable improvement being observed in CRL- and PCL-catalyzed reactions. 4-Hydroxy-2-methylbut-2-enyl acetate has thus been simply prepared by buffer-free lipase-catalyzed hydrolysis.

Lipase-catalyzed regioselective acetylation or deacetylation are known to be useful for the preparation of partially acetylated compounds.[1,2] We recently reported a simple preparation of 4-hydroxy-3-alkylbut-2-enyl acetate **2** by such a reaction, although an extended reaction time was necessary at 0 °C to achieve good regioselectivity.[3] In addition, lipase-catalyzed reactions in a buffer solution proceeded so rapidly that they formed a diol; this caused a significant drop in the chemical yield of the desired monoacetate **2**.

Reinhoudt *et. al.* reported that serine proteases were activated by crown ethers, especially by 18-crown-6.[4] We also found that some crown ethers had the potential to enhance both the enantioselectivity and reaction rate in the lipase-catalyzed hydrolysis of 2-cyano-1-methylethyl acetate.[5] In particular, 1,4,8,11-tetrathiacyclotetradecane **5** was confirmed as the best additive of 35 types of crown ethers and their acyclic analogues tested.[6] In this paper, we describe how the reaction rate for the partial hydrolysis of the diacetate **1** catalyzed by *Candida rugosa* lipase (CRL) or *Pseudomonas cepacia* (PCL) was greatly improved by addition of catalytic amounts of the thiacrown ether **5**; the reaction efficiency of the PCL- and CRL-catalyzed reaction was thus greatly improved [eqn. (1) and Fig. 1)].

Five types of lipases were chosen and their activity was tested in the presence of the thiacrown ether **5** in the hydrolysis of the diacetate **1**[7] as a model compound. The hydrolysis was carried out in a non-buffered aqueous solution to exclude any effect of complexation between the crown ether and the metal cation.[8] The resulting monoacetates, a mixture of **2** and **3**, were converted into the corresponding *tert*-butyldimethylsilyl ether and the regioselectivity of the reaction was determined by capillary GLC analysis. The enzymes employed catalyzed hydrolysis of **1** at the sterically bulky position to afford the monoacetate **2** preferentially, although their reactivity was not satisfactory in the absence of additive. Addition of the thiacrown ether **5** greatly enhanced the reaction rate in most cases especially in CRL- or PCL-catalyzed reactions (Table 1 and Fig. 1).

It should be emphasized that there were clearly differences in the additive effect depending on the origin of the enzymes (Fig. 1). When the CRL-catalyzed reaction was carried out in the presence of 5 mol% of thiacrown ether (see Fig. 1), the rate was



accelerated more than 13-fold, with the regioselectivity being slightly enhanced (up to 87%) and with a high (81%) reproducible yield (entry 3 in Table 1). An even greater acceleration of the reaction rate was achieved in the presence of 33 mol% of thiacrown, reaching a 27-fold increase over that observed in the absence of thiacrown ether (entry 4 in Table 1). Addition of thiacrown ether also greatly improved the rate of PCL-catalyzed reactions (entries 7 and 8). Although like CRL-catalyzed reactions, an increased amount of thiacrown ether further accelerated the PCL-catalyzed reaction (entry 8), the regioselectivity remained at the level achieved with a 5 mol% additive concentration. The presence of a large amount of thiacrown ether did not inhibit the enzymatic reaction, while an excessive amount caused acceleration of further hydrolysis of the monoacetate to a diol; a significant drop in the yield of monoacetate was thus observed (entry 9).[9]

Because substrate **1** is easily hydrolyzable, the diacetate **1** was

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Table 1 Results of lipase-catalyzed partial hydrolysis of the diacetate **1**[a]

Entry	Enzyme[b]	Thiacrown **5**	Time (h)	Yield of **2** + **3**	Ratio (**2**:**3**)	Yield of **1** (recovered)	Yield of **4**[c]	Relative rate[d]
1	CRL	0 (pH 7.2)	0.5	61	80:20	0	6	122
2	CRL	0	20	87	74:26	4	1	4
3	CRL	5 mol%	1.5	81	87:13	8	0	54
4	CRL	33 mol%	0.8	85	86:14	0	0	106
5	PCL	0 (pH 7.2)	0.5	65	80:20	0	5	130
6	PCL	0	2.0	74	96:4	3	0	37
7	PCL	5 mol%	1.0	87	98:2	6	0	87
8	PCL	33 mol%	0.7	89	95:5	0	0	127
9	PCL	100 mol%	0.7	75[e]	86:14	0	0 (9)[e]	130
10	PPL	0	144	51[f]	94:6	49	0	0.4
11	PPL	5 mol%	120	14[f]	94:6	73	0	0.1
12	PPL	33 mol%	144	40[f]	91:9	39	0	0.3
13	AL	0	10	20	88:12	50	0	2
14	AL	5 mol%	12	61	94:6	6	4	5
15	F	0	144	33[f]	81:19	47	0	0.02
16	F	5 mol%	24	50	86:14	50	0	2

[a] The reaction was conducted in a mixed solvent of deionized water and acetone (water–acetone, 10:1). [b] PCL (Amano: Lipase PS); CRL (Meito: lipase OF); AL: *Acromobacter* sp. lipase (Meito); F: porcine pancreatic lipase (Amano); PPL: porcine pancreatic lipase (Sigma Type II). [c] Because of the hydrophilicity of the diol produced, significant loss was observed during the isolation process. [d] The rate was calculated from percentage conversion per reaction time. [e] The value in parenthesis is a GLC yield. Significant loss was observed during the isolation process, because the presence of a large amount of thiacrown ether makes it difficult to isolate the product from the reaction mixture by silica gel flash column chromatography. [f] Enzymes lose their reactivity under the conditions employed, hence the reaction stopped at the indicated hydrolysis ratio.

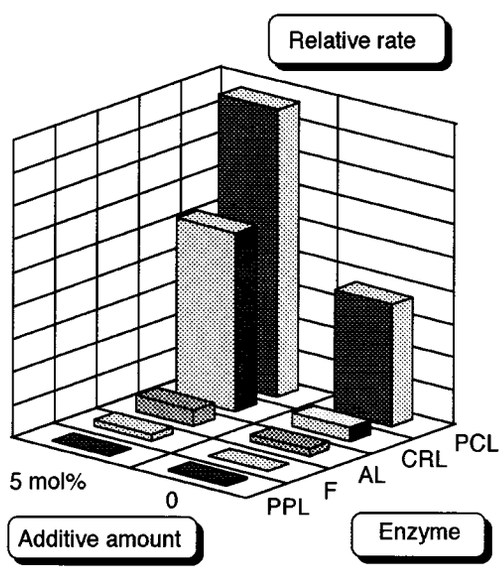

Fig. 1 Relative rate change by addition of the thiacrown ether **5**

naturally hydrolyzed to give **2** and **3** non-regioselectively in a buffer solution even at pH 6.5–7.0 at room temperature. Lipase-catalyzed reactions in a buffer solution proceeded so rapidly that they formed the diol **4**; this caused a significant drop in the chemical yield of the monoacetates (entries 1 and 5). The efficiency of the PCL- and CRL-catalyzed reaction was thus greatly improved by addition of the thiacrown ether **5**; this makes the present reaction particularly important for the practical preparation of the monoacetate **2**.

A PPL-catalyzed reaction, in contrast, was not modified by thiacrown ether additive even in the presence of 33 mol% of **5** (entry 12). The regioselectivity was not changed in lipase F or AL-catalyzed reactions, although the reaction rate was similarly improved (entries 14 and 16). Our employment of this crown ether additive cannot change the original stereochemistry of the product, but it does enhance its potential ability to a level at which the reaction can be used practically.

Why does the crown ether modify lipase performance? We currently assume that two factors are involved in the reaction.[6a] One is the interaction between the thiacrown ether and the enzyme. The employed crown ether may interact with certain sites of the lipase, thereby modifying its local conformation, activating it, and causing a change in the stereoselectivity of the enzymatic reaction.[6a] That this occurs seems to be very probable since the additive activity of the thiacrown **5** depends on the origin of the enzymes employed and a great change was observed in CRL-catalyzed reaction. Crystal structures of CRL show that it has two conformations that differ mainly in the orientation of a helical surface loop.[10] The thiacrown ether additive may convert the closed form of CRL to the open form which can enhance regioselectivity, as suggested by the results of CRL-catalyzed reaction after treatment of propan-2-ol[11] or sodium deoxycholate.[12] The origin suggested by Reinhoudt *et al.* for 18-crown-6 activation was that the crown ether modifies the conformational change of the enzyme by trapping water molecules at the active site or enzyme surface.[4f] We assume that the mechanistic details of 18-crown-6 and thiacrown **5** are different, the former being hydrophilic and the latter very hydrophobic. In view of the thiacrown **5** being unable to capture water, it may interact with the enzyme directly.

The second factor is complexation of the crown ether **5** with substrate **1** or products, **2** and **3**. Crown ethers bind neutral organic molecules in the crystal states and non-polar media.[13] The thiacrown ether may bind alcohol or ester molecules in the course of the reaction, so that chemical equilibrium of the hydrolysis occurring near the active site could be modified. When the thiacrown ether was added to a $CDCl_3$ solution of the product alcohol, **2** or **3**, large ^{13}C NMR spectral changes were observed for the carbonyl carbon signals of both products (Fig. 2),[14] while no significant induced chemical shift was observed for substrate **1**. The induced chemical shift of a compound by a thiacrown ether seems to be a good guideline for considering the additive effect in the present reaction. It is suggested that the thiacrown ether makes the product unsuitable for the reverse reaction by trapping it, thus accelerating diffusion of the alcohol into the bulk water phase.

In summary, the presence of a catalytic amount of thiacrown ether gave rise to a highly regioselective and efficient lipase-catalyzed reaction in a buffer-free medium. This type of regioselective partial hydrolysis of the diacetate **1** is impossible under conditions of alkaline hydrolysis. The amount of thiacrown ether employed was only 5 mol% of that of the substrate; this corresponds to a 250-fold amount of crown **5** compared with the lipase molecule.[6a] This work represents not only a significant advance in the preparation of partially acetylated

Table 2 ^{13}C NMR spectra of the monoacetates **2** and **3** in the presence of the thiacrown ether **5** (in 0.05 M $CDCl_3$)

		δ_C						
Compound	Amount of **5**	C-5	C-7	C-4	C-1	C-2	C-3	C-6
2	0	13.79	20.96	60.89	67.57	118.52	140.84	171.13
2	2.0 equiv.	13.79	20.94	60.80	67.60	118.56	140.75	170.97
	balance	0	+0.02	−0.09	+0.03	+0.04	−0.09	−0.16
		C-5	C-7	C-4	C-1	C-3	C-2	C-6
3	0	14.00	20.88	58.84	69.04	127.09	133.12	170.92
3	2.0 equiv.	14.00	20.86	58.90	68.91	126.91	133.27	170.73
	balance	0	−0.02	+0.06	−0.13	−0.18	+0.15	−0.19

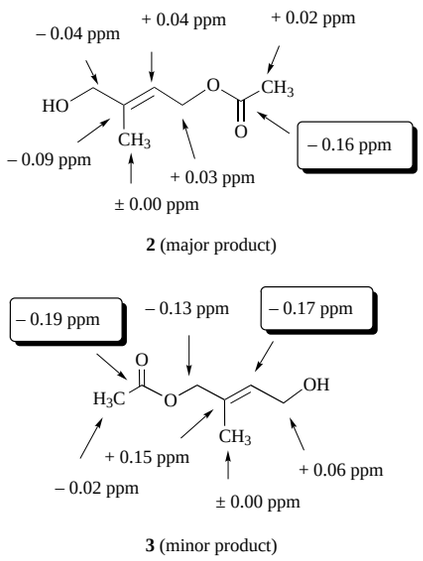

Fig. 2 Induced ^{13}C NMR (50 MHz) spectral change. The values induced mean differences in ^{13}C NMR chemical shift in the absence and in the presence of 2.0 equiv. of **5** in 0.05 M $CDCl_3$.

compounds but also provides a new aspect in application of enzymatic reaction to organic synthesis.

Experimental

Reagents and solvents were purchased from common commercial sources and were used as received or purified by distillation from appropriate drying agents. Reactions requiring anhydrous conditions were run under an atmosphere of dry argon. Silica gel (Wako gel C-300,300E) was used for column chromatography and silica gel (Wako gel B-5F) for thin layer chromatography. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Varian VXR-200 (200 MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) in $CDCl_3$ as an internal reference; *J* values are given in Hz. IR spectra were obtained on JASCO A-102 and FT/IR-230 spectrometers. The regioselectivity was determined by capillary gas chromatography (Chiraldex G-Ta, diam. 0.25 mm × 20 m, 100–150 °C, He).

Lipase-catalyzed partial hydrolysis of the diacetate 1

PCL powder (75 mg) was added to deionized water (2.4 ml) and this suspension was vigorously shaken for 5 min. The mixture was then centrifuged at 3000 rpm for 5 min after which the supernatant (1.2 ml) was used for the enzymatic reaction. The lipase solution (1.2 ml) was added to the diacetate **1** (75 mg, 0.403 mmol) together with the thiacrown **5** (5.4 mg, 5 mol% compared with substrate) in acetone (0.12 ml). The mixture was stirred at 35 °C for 0.7 h, the progress of the reaction being monitored by GLC analysis using a Quadlex MS (diam. 0.25 × 25 m). After consumption of the starting material **1**, the reaction was quenched by the addition of crushed ice and NaCl to the mixture which was then immediately extracted with CH_2Cl_2. The extract was dried ($MgSO_4$) and evaporated. The crude product was purified by silica gel flash chromatography (hexane–AcOEt, 7:1) to give a mixture of **2** and **3** (51.50 mg, 0.357 mmol) in 89% yield. The ratio of **2** and **3** was determined as 95:5 by capillary GLC analysis using Chiraldex G-Ta (diam. 0.25 × 20 m, He) as *tert*-butyldimethylsilyl ether **5** and **6**. The monoacetates **2** and **3** were isolated by silica gel thin layer chromatography (TLC), although triplicate TLC separation was needed.

4-Acetoxy-2-methylbut-2-enyl acetate 1.[7] Bp 105 °C/2 Torr (Kugelrohr); R_f 0.2 (hexane–ethyl acetate, 6:1); δ_H(200 MHz, $CDCl_3$) 1.69 (3H, s), 2.02 (3H, s), 2.05 (3H, s), 4.45 (2 H, s), 4.59 (2H, d, *J* 6.8), 5.57 (1H, tq, *J* 6.92, 1.32); δ_C(50 MHz, $CDCl_3$) 14.04, 20.82, 60.49, 68.43, 121.58, 135.68, 170.55 and 170.80; ν_{max}(neat on NaCl)/cm^{-1} 2930, 1730, 1430, 1220 and 950.

4-Hydroxy-3-methylbut-2-enyl acetate 2. Bp 122 °C/15 Torr (Kugelrohr); R_f 0.5 (hexane–ethyl acetate, 1:1); δ_H(200 MHz, $CDCl_3$) 1.67 (3H, s), 2.00 (3H, s), 2.16 (1H, br s, OH), 3.98 (2H, s), 4.58 (2H, d, *J* 6.8) and 5.52–5.57 (1H, m); δ_C(50 MHz, $CDCl_3$) 13.59, 20.78, 60.80, 67.10, 118.02, 140.79 and 171.12; ν_{max}(neat on NaCl)/cm^{-1} 3400, 2900, 2850, 1720, 1240 and 950 (Found: C, 57.82; H, 8.40. Calc. for $C_7H_{12}O_3$: C, 58.32; H, 8.39%).

4-Hydroxy-2-methylbut-2-enyl acetate 3. Bp 125 °C/15 Torr (Kugelrohr); R_f 0.5 (hexane–ethyl acetate, 1:1); δ_H(200 MHz, $CDCl_3$) 1.60 (1H, br s, OH), 1.67 (3H, s), 2.06 (3H, s), 4.17 (2H, d, *J* 6.8), 4.45 (2H, s) and 5.64 (1H, t, *J* 6.6); δ_C(50 MHz, $CDCl_3$) 13.97, 20.87, 58.83, 68.98, 121.01, 133.12 and 170.86; ν_{max}(neat on NaCl)/cm^{-1} 3400, 2950, 2850, 1730, 1230 and 780 (Found: C, 58.02, H, 8.56. Calc. for $C_7H_{12}O_3$: C, 58.32; H, 8.39%).

2-Methylbut-2-ene-1,4-diol 4.[15] Bp 86 °C/5 Torr (Kugelrohr); R_f 0.3 (CH_2Cl_2–methanol, 10:1); δ_H(200 MHz, $CDCl_3$) 1.65 (3H, s), 3.23 (2H, br s, OH), 3.97 (2H, s), 4.16 (2H, d, *J* 6.8) and 5.61 (2H, dq, *J* 6.8, 1.4); δ_C(50 MHz, $CDCl_3$) 13.63, 58.69, 67.38, 123.26 and 137.99; ν_{max}(neat on NaCl)/cm^{-1} 3369, 2919, 1675, 1458 and 997.

4-*tert*-Butyldimethylsilyloxy-3-methylbut-2-enyl acetate 6. Bp 90 °C/2.5 Torr (Kugelrohr); R_f 0.7 (hexane–ethyl acetate, 3:1); δ_H(200 MHz, $CDCl_3$) 0.054 (6H, s), 0.90 (9H, s), 1.65 (3H, s), 2.04 (3H, s), 4.02 (2H, s), 4.62 (2H, d, *J* 7.3) and 5.60 (1H, tq, *J* 7.1, 1.5); δ_C(50 MHz, $CDCl_3$) 13.53, 18.34, 20.96, 25.86, 60.88, 67.37, 117.35, 140.66 and 171.03; ν_{max}(neat on NaCl)/cm^{-1} 2950, 2850, 1740, 1230 and 960 (Found: C, 61.04; H, 10.54. Calc. for $C_{13}H_{26}SiO_3$: C, 60.42; H, 10.14%).

4-*tert*-Butyldimethylsilyloxy-2-methylbut-2-enyl acetate 7. Bp 90 °C/2.5 Torr (Kugelrohr); R_f 0.7 (hexane–ethyl acetate, 1:1); δ_H(200 MHz, $CDCl_3$) 0.057 (6H, s), 0.89 (9H, s), 1.65 (3H, s), 2.06 (3H, s), 4.22 (2H, d, *J* 6.0), 4.45 (2H, s) and 5.58 (1H, t, *J* 6.2); δ_C(50 MHz, $CDCl_3$) 14.11, 18.34, 20.90, 25.92, 59.77, 69.28, 128.53, 131.05 and 170.80; ν_{max}(neat on NaCl)/cm^{-1}

2950, 2850, 1740, 1240 and 840 (Found: C, 60.64; H, 10.44. Calc. for $C_{13}H_{26}SiO_3$: C, 60.42; H, 10.14%).

Results of GLC analysis. Chiraldex G-Ta, diam. 0.25 nm × 20 m, carrier gas: He 40 ml min^{-1}, temp: 100 °C, inlet pressure 1.35 kg cm^{-2}, amount 400 ng, detection: FID; Bu^tMe_2Si ether **6**: R_t 22.8 min, Bu^tMe_2Si ether **7**: $R_{t(s)}$ 25.8 min.

NMR binding experiments

^{13}C NMR studies were carried out with a Varian VXR-200 spectrometer. The thiacrown ether **5** was dissolved in $CDCl_3$ at a concentration of 0.05 mol l^{-1} and the results are summarized in Table 2.

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8 Crown ethers bind metal cations with varying strength depending on their structure. The lipase solution employed was confirmed to contain the following alkali and alkaline-earth metal cations: Na^+, 6.5×10^{-4} mol l^{-1}; K^+, 4.6×10^{-4} mol l^{-1}; Mg^{2+}, 6×10^{-4} mol l^{-1}; Ca^{2+}, 6.3×10^{-4} mol l^{-1}.

9 Marked formation of the diol was observed (18% after 1.0 h reaction) when the reaction was carried out in the presence of 300 mol% of thiacrown ether compared with the substrate while the starting diacetate still remained (2.3%) at that time. Because the thiacrown ether is very hydrophobic, the reaction mixture became very thick as a result of the thiacrown ether being insoluble.

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14 Because the enzymatic reaction was performed in water, a ^{13}C NMR experiment was also carried out in D_2O as solvent. Due to the very low solubility of the thiacrown ether **5** in D_2O, however, no spectral change was detected in the ^{13}C NMR signal of the monoacetate.

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