COMMUNICATION

Chemoenzymatic autocatalytic Baeyer–Villiger oxidation

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Autocatalytic Baeyer–Villiger oxidation of cyclic ketones to lactones mediated by lipases has been achieved under extremely mild conditions in dry media using urea–hydrogen peroxide as the primary oxidant. Lipase catalysed perhydrolysis is enantiospecific and gives 6-substituted caprolactones or the corresponding hydroxy acids in enantiomerically enriched form.

Introduction

The ability of lipases to catalyse perhydrolysis of acyl substrates is well documented.[1] The preference of enzymes for H_2O_2 relative to H_2O is ten- to a hundred-fold. However, perhydrolysis is reversible in the presence of all lipases. Therefore effective accumulation of peroxycarboxylic acid is only feasible when water concentration is low. Nevertheless, the lipase catalysed preparation of long chain peroxycarboxylic acids has been found useful in related epoxidations of alkenes[2] and conversion of sulfides into sulfoxides.[3]

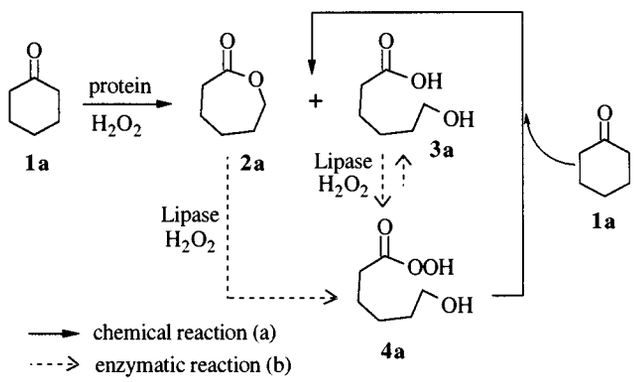
Recently, Roberts and co-workers[4] published a lipase mediated Baeyer–Villiger lactone preparation starting from several cyclic ketones with myristic acid and 30% H_2O_2 as the peroxide donor in organic solvents. Such a method avoids the use of aggressive and poorly storage-stable peroxycarboxylic acid and gives Baeyer–Villiger reactions with yields comparable with those obtained in *m*-chloroperoxybenzoic acid (MCPBA)[5] or magnesium monoperoxyphthalate (MMPP)[6] oxidations. However, long reaction times (about one week) and difficulties in the elimination of excess myristic acid need to be improved. Moreover enzymatic stereopreferences cannot be expected in this reaction.

At the present time, only monooxygenases in whole cells, or isolated[7] and genetically modified *Saccharomyces cerevisiae* cells[8] are useful for this purpose but this otherwise very promising method remains too expensive for pratical synthetic use.

We felt that use of lipases could represent an interesting alternative and we decided to investigate the possibility of autocatalytic Baeyer–Villiger oxidation of cyclic ketones to lactones, mediated by lipases. In the study of enzymatic acetylcholine peroxyhydrolysis, Kirk *et al.*[1] observed, in control experiments carried without lipase, the formation of traces of peroxy acid. This led us to expect that H_2O_2 assisted by protein side chain carboxylic groups would start Baeyer–Villiger oxidations. The lactone thus formed would then undergo lipase mediated perhydrolysis to peroxy acids which would in turn bring about Baeyer–Villiger oxidation of the ketones, thereby achieving an autocatalytic process (Scheme 1).

Results and discussion

In the light of previous experiments, we conducted the lipase-catalyzed perhydrolysis, followed by chemical Baeyer–Villiger oxidation, in a dry medium without organic solvent. In a typical experiment the immobilized lipase was impregnated with a diethyl ether solution of ketone **1a–g**. The ether was removed and the peroxide donor was subsequently added to this solid mixture in one portion. Reaction were performed in a



Scheme 1 Lipase-catalysed perhydrolysis, followed by chemical Baeyer–Villiger oxidation

thermostated oil bath under different conditions of time and temperature.

In keeping with Kirk's[1] investigation, Novozym SP 435 (supported *Candida antarctica* lipase) was found to be the best choice of enzyme preparation. An 80% conversion of 2-methylcyclohexanone **1b** was observed in two days, using H_2O_2–H_2O as hydroperoxide donor, while lipase PS (*Pseudomonas* species lipase) immobilized on Hyflo Super Cel (HSC) exhibited a 10 times lower activity. Bovine albumin BSA, or Lipozyme (supported *Mucor miehei* lipase), were found to lead to less than 1% conversion.

With the aim of raising the peroxy acid concentration, relative to that obtained in the usual H_2O_2–H_2O (35%) system, we tested urea–hydrogen peroxide (UHP) as the oxidant. UHP is described as a mild and safe alternative to anhydrous hydrogen peroxide, relatively stable, easy to handle and commercially available.[6,9] It has already been employed as primary oxidant in metal or carbodiimide mediated epoxidation.[10] Indeed, lipase catalyzed perhydrolysis under anhydrous conditions (UHP) followed by chemical Baeyer–Villiger oxidation on 2-hexylcyclohexanone **1f** was found to give 11% of conversion after 24 h and 65% after 48 h, while only 11% and 20% conversion after respectively 24 h and 48 h were observed in H_2O_2–H_2O (Fig. 1).

The transformation of ε-caprolactone (hexano-6-lactone) by Novozym is very fast.[11] The rate of autocatalytic Baeyer–Villiger oxidation of ketone **1a** reported in Fig. 2 is therefore that of the Baeyer–Villiger process itself (k_a). This rate is highly dependent upon the size and the position of substituents in the lactone. k_a is superior to k_b (rate of enzymatic reaction) for **1b–1f** (Fig. 2).

In our earlier experiments, we had found that Novozym mediated lactone hydrolysis, esterification and perhydrolysis are enantiospecific. For instance, hydrolysis of 7-methyloxepan-2-one **2b** to the corresponding hydroxy acid provides the (*S*)-lactone with enantiomeric excess of 85% at 53% conversion and butanolysis of **2b** gives (*S*)-lactone with enantiomeric excess of 56% at 37% conversion. We therefore explored the possibility of connecting enzyme mediated perhydrolysis with chemical Baeyer–Villiger oxidations, with the aim of achieving enantio-

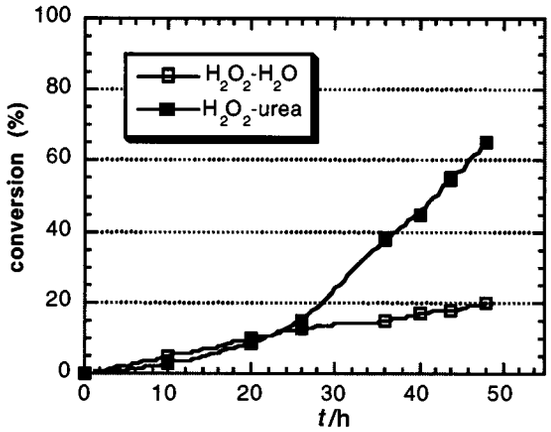


Fig. 1 Time-course of the Baeyer–Villiger reaction of **1f** in the presence of Novozym, and UHP or H_2O_2–H_2O as primary oxidants at 25 °C

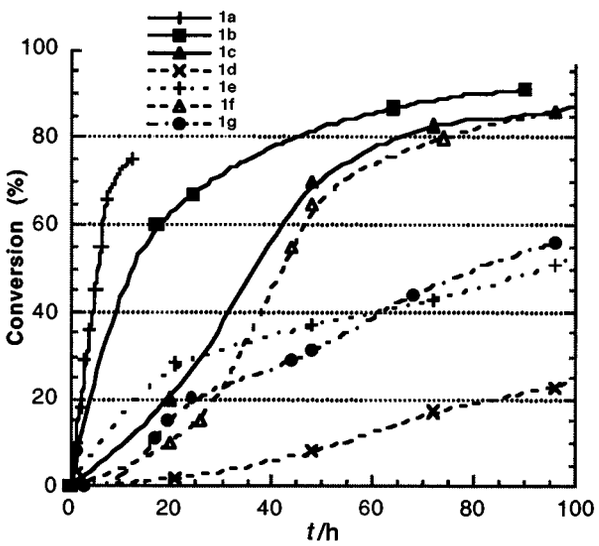
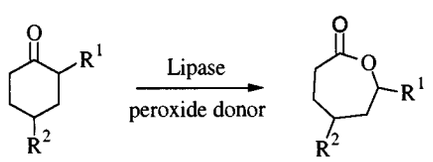


Fig. 2 Time-course of Baeyer–Villiger oxidation of **1a–1f** at 25 °C mediated by SP 435–UHP



1a-g **2a-g**

a: $R^1 = H$ $R^2 = H$ **e:** $R^1 = H$ $R^2 = C_4H_9^t$

b: $R^1 = CH_3$ $R^2 = H$ **f:** $R^1 = C_6H_{13}$ $R^2 = H$

c: $R^1 = C_4H_9^n$ $R^2 = H$ **g:** $R^1 = C_6H_5$ $R^2 = H$

d: $R^1 = C_4H_9^t$ $R^2 = H$

Scheme 2 Baeyer–Villiger oxidations of α- or γ-substituted cyclohexanones **1a–g** to lactones **2a–g**

selective synthesis of lactones and hydroxy acids. As may be seen from Table 1, best results are obtained with 2-alkylcyclohexanones. In Fig. 3 are reported values for substrate conversion, lactone accumulation and lactone ee as a function of time during 2-methylcyclohexanone oxidation. The relative rates of ketone oxidation and lactone perhydrolysis differ with temperature. For instance for 2-hexylcyclohexane (Fig. 4), the highest lactone accumulation corresponds to Baeyer–Villiger at 40 °C ($k_a \gg k_b$). At 80 °C, $k_a \geqslant k_b$ and the yield drops to 20%. However, the best ee values were obtained at 25 °C or 80 °C. Finally, the optimal conditions for (*S*)-lactone **1f** preparation are room temp. and 78 h.

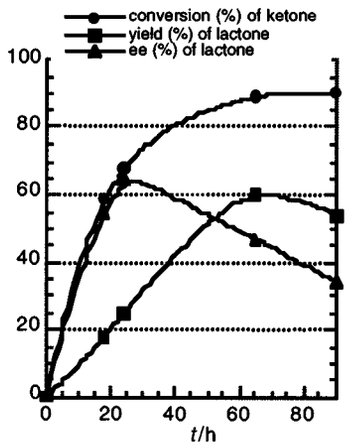


Fig. 3 Time-course of Baeyer–Villiger oxidation of **1b** (conversion, yield of **2b** and ee of **2b**)

Conclusion

The present method provides a very mild and simple alternative to the other methods previously used for *in situ* generation of peroxycarboxylic acids. Moreover, the lipase-mediated autocatalytic Baeyer–Villiger oxidation reaction should prove useful for stereospecific preparation of lactones or hydroxy acids from ketones.

Experimental

All reactions were carried out under standard conditions and were not optimized for particular substrates.

Materials and methods

Novozym SP 435 (immobilized *Candida antarctica* lipase) and Lipozyme (immobilized *Mucor miehei* lipase) were kindly gifted by Novo Nordisk. *Pseudomonas cepacia* lipase (LP) was provided by Amano Co. and bovine albumin (BSA), Hyflo Super Cel (HSC) and hydrogen peroxide (35%) were supplied by Fluka, while urea–hydrogen peroxide (UPH 35 wt% H_2O_2), cyclohexanone **1a**, 2-methylcyclohexanone **1b** and 2-phenylcyclohexanone **1g** were obtained from Acros. Cyclohexanones: **1c** (2-butyl-), **1d** (2-*tert*-butyl-), **1e** (4-*tert*-butyl-), **1f** (2-hexyl-) were synthesized in 44–60% yield from cyclohexanone by C-alkylation, according to a literature method.[12]

Analytical methods

NMR Spectra were recorded on Bruker equipment at 250 or 400 MHz in $CDCl_3$ solution with tetramethylsilane as an internal standard. Reactions were followed by GC on a 6000 Vega Series with FID detector, a Spectra-Physics SP 4290 integrator and an OV1 column (12 m). The detector and the injector temperatures were set at 300 °C and 290 °C respectively. Column temperature was programmed in the range 50–280 °C in the case of cyclohexanones: **1a–f**, and 100–280 °C for **1g** (10 °C min^{-1}). The following retention times (min) were observed: **1b**: 4.48; **2b**: 8.01; **1c**: 6.94; **2c**: 9.66, **1d**: 5.40; **2d**: 7.90, **1e**: 6.02; **2e**: 9.22, **1f**: 7.95; **2f**: 10.56, **1g**: 4.35; **2g**: 7.50.

The ee values of lactones were directly determined by chiral GC analysis on Lipodex D column (25 m) with racemic compounds as standard references. The conditions were: detector 280 °C, injector 280 °C. Column 70–180 °C (0.7 bar) 5 °C min^{-1} in the case of **2b**, 130 °C (0.8 bar) for **2c**, 130 °C (1 bar) for **2d–f** and 130 °C (1.2 bar) for **2g**. The following retention times (min) were observed: **2b**: 31.38 (*R*), 31.90 (*S*); **2c**: 37.57 (*R*), 38.30 (*S*); **2d**: 35.97 (*R*), 37.35 (*S*); **2e**: 29.71 (*R*), 32.30 (*S*); **2f**: 37.85 (*R*), 38.41 (*S*); **2g**: 74.18 (*R*), 73.45 (*S*).

Table 1 Baeyer–Villiger oxidation of cyclohexanones **1b**–**g** mediated by UHP/SP435

Ketone	*T*/°C	*t*/h	Conversion (%)	Yield **2**[a] (%)	ee **2**[b] (%)	Abs. conf.[c]
1b	25	40	80	44	60	*S*
	40	15	97	14	57	*S*
	70	1	30	17	18	*S*
1c	25	96	86	43	72	*S*
	40	19	61	34	14	*S*
	70	14	79	36	46	*S*
1d	25	120	34	21	20	*R*
1e	25	96	51	31	23	*S*
	40	48	74	28	17	*S*
	70	48	85	38	20	*S*
1f	25	74	80	35	57	*S*
	40	48	60	36	14	*S*
	70	24	60	30	51	*S*
	80	26	70	15	47	*S*
1g	25	96	56	39	10	*R*
	40	72	95	55	20	*R*
	70	25	96	40	10	*R*

[a] Yields of lactones **2b**–**g** were determined by GC using internal standards. [b] Values for enantiomeric excess were obtained by chiral GC. [c] The absolute configurations were determined on the basis of ref. 7.

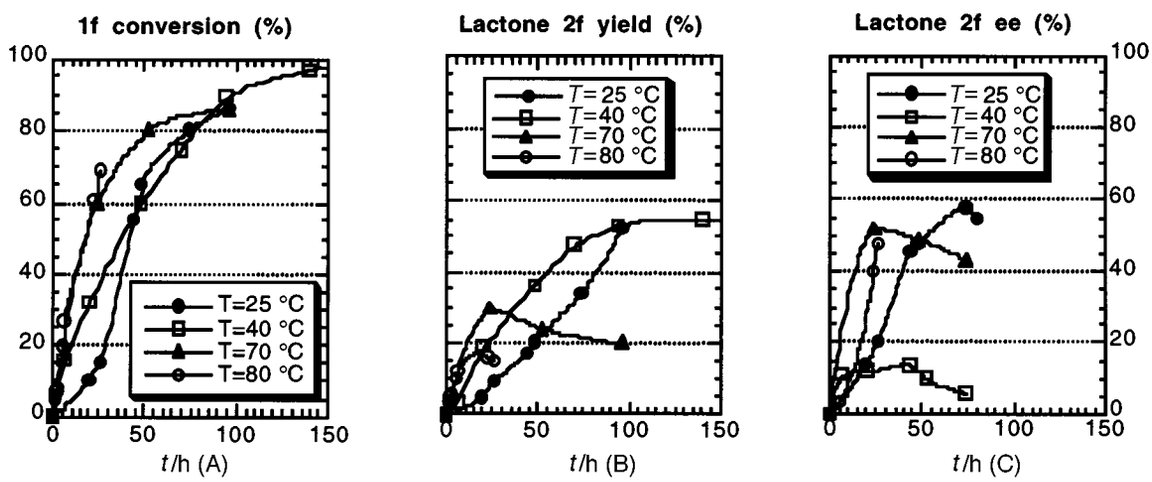


Fig. 4 Rates of Baeyer–Villiger oxidation at different temperatures for 2-hexylcyclohexanone **1f**: (A) conversion of **1f**, (B) yields of **2f**, (C) ee of **2f**

Typical synthesis procedure

The substrate **1a**–**g** (1 mmol) was dissolved in diethyl ether (0.5–1 ml) and impregnated on Novozym SP 435 (0.5 g). Ether was removed. Then 1 g of urea–hydrogen peroxide (UHP) was added in one portion. The reaction mixture was thermostated in a oil bath and stirred in time and temperature conditions indicated in Table 1. After cooling, the mixture was washed on sintered glass with diethyl ether (10 ml) (ketone and lactone fraction) and with chloroform (10 ml) (hydroxy acid). For analytical purposes, the organic phases were concentrated; the residues were dissolved in dry diethyl ether and methylated with (trimethylsilyl)diazomethane. GC retention times and ^{1}H NMR data of enantiomeric lactones and esters were identical with those of the racemic compounds prepared by chemical Baeyer–Villiger oxidation (3 equiv. $NaHCO_3$, 1.1 equiv. MCPBA in CH_2Cl_2). Enantiomeric excess of the lactones was determined on chiral GC column.[7]

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