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Lipase Catalyzed Synthesis of Peroxycarboxylic Acids and Lipase Mediated Oxidations.

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Abstract: Lipase catalyzed synthesis of long chain peroxycarboxylic acids from hydrogen peroxide and free carboxylic acid was investigated. A 51% yield of peroxytetradecanoic acid was achieved when using a two phase system of toluene and water. The peroxy acids thus formed were applied for *in situ* oxidation of alkenes, in general leading to high yields of the corresponding epoxide. For example, a quantitative yield of cyclohexene oxide and a 94% yield of 1-hexadecene oxide was achieved in a solvent-free process.

The possible use of lipases as catalysts in organic synthesis has been extensively explored in recent years. In particular, the ability of these enzymes to catalyse hydrolysis, transesterification and esterification has been investigated.[1,2] In spite of the fact that these reactions are usually carried out under "unnatural" conditions it has appered that the high degree of selectivity and efficacy of lipases exhibited in their natural environment is also expressed in synthesis. Lipases have even been reported to catalyse reactions with substrates such as amines which can be transformed into amides.[3]

The broad substrate selectivity of most lipases has been found to apply also for hydrogen peroxide which is accepted as a nucleophile in the catalyticformation of peroxycarboxylic acids (Scheme 1).[4]

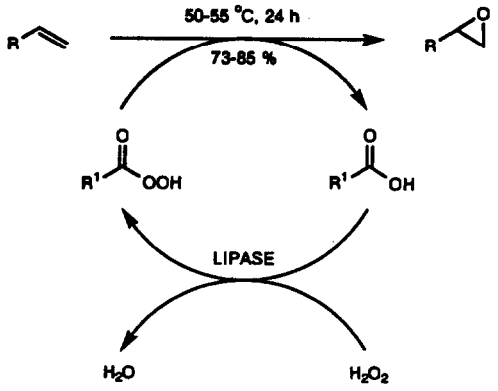
$$RCOOH + H_2O_2 \xrightarrow{\text{LIPASE}} RCOOOH + H_2O$$

$R = C_5H_{11}$- $C_{15}H_{31}$

Scheme 1

As demonstrated in the following this process can be carried out under conditions where peroxycarboxylic acids are applied *in situ* for oxidation of organic substances such as alkenes and sulphides.[5] Today alkenes are oxidized industrially by formic or acetic acid/hydrogen peroxide where the corresponding peracids are formed *in situ*.[6] The oxidised products, alkene oxides and sulphoxides, find extensive use as synthetic intermediates, for example in the pharmaceutical industry or in polymer manufacturing.[7]

In this context, the further development and improved use of enzymatically synthesized peroxycarboxylic acids for *in situ* oxidations was explored (Scheme 2).



R= C_6H_{13}-$C_{14}H_{29}$, R^1= $C_{13}H_{27}$

Scheme 2

PEROXY ACID FORMATION

As shown previously, peroxy acids are formed from carboxylic acid and hydrogen peroxide in the presence of lipases in a two-phase system of hexane and water.[4]

Lipases

Using octanoic acid as a model substrate the immobilized lipase derived from *Candida antarctica* was found to be the most efficient enzyme for peroxyoctanoic acid synthesis (Table 1).

Table 1: Synthesis of peroxyoctanoic acid using different lipases.[a]

Lipase	Activity (kLU/g)	Peroxyoctanoic acid formed in 120 min
Candida antarctica comp. A+B [b]	22,5	151
Pseudomonas sp. [c]	100,8	87
Humicola sp. [c]	459,6	82
Mucor miehei [b]	85,6	61
Candida Cylindracea [b]	34,2	<1
Candida antarctica comp. A [b]	26,7	<1
Candida antarctica comp. B [b]	24,3	152

a) **Typical procedure:** Octanoic acid (0.5ml, 3.15mmol) was dissolved in hexane (5ml) in the presence of immobilized lipase (100mg) at room temperature.Hydrogen peroxide (0.25ml, 4.45mmol, 17.8M) was then added. The peroxyoctanoic acid yield was determined by HPLC as compared with an autentic sample prepared as previusly described.[11]

b) immobilised on polyacrylate resin

c) immobilised on polypropylene resin.

Other immobilized lipases as those derived from *Pseudomonas sp.*, *Humicola sp.* and *Mucor miehei* also showed good activities in the process while, in contrast, the lipase derived from *Candida cylindracea* was found not to catalyse the transformation under the reaction conditions tested. Accordingly, the lipase derived from *Candida antarctica* was used throughout our further investigations.

Among the lipase carriers tested, polyacrylate and polypropylene were found to be superior. The enzymes were noncovalently adsorbed onto the hydrophobic materials as previously reported.[8] Ionic resins of polystyrene were not effective under the reaction conditions tested.

Solvents

The influence of different solvents on the process was also investigated. The best yields were obtained in toluene, xylene and nitromethane. Lower yields of peroxy acids were generally found using water miscible solvents. Accordingly, toluene or no solvents was used in our further investigations.

Substrates

Straight chain carboxylic acids with 6 to 16 carbon atoms were tested as substrates. The yields of the peroxy acids formed in toluene using 17.5 M hydrogen peroxide varied from 36 % - 44 %. Branched fatty acids were converted in much lower yields.

Hydrogen peroxide concentration

The concentration of the hydrogen peroxide in the water phase of the reaction mixture was found to be a significant parameter. The best yields of peroxycarboxylic acids were obtained when using a 17.5 M to 22 M concentration of the peroxide doner (Table 2).

Table 2: Yield of peroxyteteadecanoic acid using different concentration of hydrogenperoxide. [a]

H_2O_2 Concentration (M)	Yield of peracid after 30 min. (%)	60 min. (%)	180 min. (%)	24 h. (%)
6	8,8	10,4	11,2	12,1
12	14,2	21,2	23,8	25,9
17,5	27,6	36,2	43,8	45,6
20	27,6	36,2	43,8	45,6
22	23,3	31,3	47,5	50,8
23,4	9,6	9,7	9,6	9,6
24,6	6,8	6,2	7,8	-

[a] **General procedure:** Tetradecanoic acid (719 mg, 3.15 mmol) is dissolved in toluene (5 ml) in the presence of immobilized *C. antarctica* lipase (100 mg, component A and B) at room temperature followed by hydrogen peroxide (4.45 mmol). Peroxytetradecanoic acid yields were determined by HPLC as compared with an authentic sample.[11]

CATALYTIC OXIDATION

The catalytically formed peroxy acids were used for *in situ* oxidation of organic compounds. The oxidation of olefins to the corresponding epoxides was found to be particulary effective. In the case of liquid alkenes, epoxidations were best carried out in a solvent-free system using the carboxylic acid in amounts of 10-25 mol-%.

Cyclic alkeneoxides

Cyclic alkenes were found to be converted smoothly into the corresponding epoxides by the enzyme mediated oxidation. Pure products were obtained in almost quantitative yields after 24 hours (Table 3).

Substituted cyclic alkenes were quickly epoxidized but were also unstable, forming byproducts. More sensitive epoxides like methylenecyclohexane oxide were preferably prepared using toluene as a co-solvent.

Table 3: Epoxidation of substituted alkenes

Alkenes	Yield of epoxide after 6h. (%)	Yield of epoxide after 24h. (%)
1,2-dimethyl-cyclohexene	91	- [c]
1-methyl-cyclohexene	62 [a]	- [c]
cyclooctene	52	96
cyclohexene	69	98
cyclopentene	66	> 99
methylenecyclohexane [b]	49	97

[a] Forming byproducts.
[b] Toluene is used as a co-solvent.
[c] The epoxidation is not run further as a full turnover was reached in less than 6 hours.

Terminal alkene oxides
Terminal long chain alkenes were found to be amenable to the lipase mediated oxidation (Table 4). For example, a 94% yield of 1-hexadecene oxide was achieved after 48 hours at 50 °C when 1.5 eq.of 10M (30% w/w) hydrogen peroxide and 0.25 eq. of tetradecanoic acid was used.

Table 4: Epoxidation of terminal unbranched alkenes

Olefins	Yield of epoxide after 6h (%)	Yield of epoxide after 24h. (%)
1-Octene	51	73
1-Decene	54	82
1-Dodecene	51	79
1-Tetradecene	54	85
1-Hexadecene	50	80

The epoxidation step, and not formation of peroxycarboxylic acid, was found to be the rate limiting step in the enzyme mediated oxidation of terminal alkenes.

Other oxidations
Sulphur containing compounds (especially sulphide) were oxidized easily. For example, dibutylsulphide was oxidized to a mixture of the corresponding sulphoxide and sulphone. Furthermore, cyclopentanone was oxidized to δ-valerolactone in a Baeyer-Villiger oxidation,[9] however in poor yield (16.5%).

Slow addition of hydrogen peroxide
The yields of alkene oxides could be increased by adding hydrogen peroxide gradually to the reaction mixtures rather than in one portion as was the case in the standard procedure. For example, the yield of cyclohexene oxide yield rose from 17% to 95% when hydrogen peroxide was added over 4.5 hours (Table 5).

Similarly the yield of 1-octene oxide rose from 50% to 73% when hydrogen peroxide was added over 12 h. instead of over 4.5 h.

Due to their basic nature, nitrogen containing compounds are difficult to oxidize with peroxy acids and, using the enzymatic procedure, only negative results have been obtained. It has been reported that yields can be increased in chemical peroxidation of amines by using an exess of peracid, why the herein described catalytic method do not seem well suited.[10]

Table 5: Yields of alkene oxides under gradual addition of Hydrogen peroxide

Rate of hydrogen peroxide addition	Yield of cyclohexene oxide after 24h (%)	Yield of 1-octene oxide after 24h (%)
All at once	17	-
Addition over 3 hours	55	-
Addition over 4 hours	91	-
Addition over 4.5 hours	95	50
Addition over 6 hours	-	63
Addition over 12 hours	98	73

In comparison with traditional acid catalyzed oxidation of carboxylic acids to peroxycarboxylic acids, the enzyme catalyzed procedure is a mild and safe alternative. These caracteristics of the enzym mediated process is particularly important due to the possibility of using the percarboxylic acids generated for *in situ* oxidations. For example, it becomes possible to oxidize acid sensitive substances not amenable to oxidation by classical means.

EXPERIMENTAL:

^{1}H-NMR spectra were recorded on a Brucker VM 400 spectrometer using TMS as internal standard. HPLC analyses were performed using a Shimadzu LC-4A and a Shimadzu LC-6A chromatograph both equipped with UV detectors. Lichrosorb RP-18 and RP-8 columns were used with a MeOH/H_2O 70:30 with 0.1% formic acid, EtOH (96%)/phosphate buffer (0.05M, pH=4) gradient or a MeCN/phosphate buffer (0.05M, pH=6.5) gradient as eluents, Milli Q water was used throughout. Short path distillation was performed with a Büchi GKR-50. Capillary gaschromatography was run on a Hewlett Packard 5890A equipped with filtered off, the water phase was separated and the organic phase was washed with water several times (3x30 ml). The remaining tetradecanoic acid wchrompack SPB-1, CFS-5626, 60m, 0.25mm ID column. Concentration of hydrogen peroxide was determined by Cerimetric titration. The oxygen content of the peroxycarboxylic acids references was determined by Iodometric titration.

Microorganisms were deposited at Deutsche Sammlung für Microorganismen (DSM) under the following deposit numbers: *Candida Antarctica:* DSM 3855, DSM 3908 and DSM 3909, *Humicola sp.:* DSM 3819, DSM 4109, *Pseudomonas sp.* :DSM 3959. *Candida cylindracea* lipase was obtained from Sigma chemical company. The lipases were used as immobilized preparations on macroporous polyacrylic, polypropylic or polyphenolic resins.[8] The lipase activity was measured in KLU = kilo lipase unit, a lipase unit based on hydrolysis of tribututyrin (mol fatty acid liberated per minute). A detailed description of this method is available from Novo Nordisk A/S (AF 95/5).

Enzyme mediated epoxidations:

Cyclohexeneoxide: Tetradecanoic acid (13.5g, 59.2 mmol) was dissolved in cyclohexene (60ml, 586mmol) and immobilised *C. antarctica* lipase (4.86g), was added. The reaction was initiated with hydrogen peroxide (51.6ml, 17.5M, 896mmol) which was added over 12 hours at 25 °C. The yield of cyclohexene oxide was determined after 24 hours (98% yield) by GC by comparison with an authentic sample. The reaction wasstopped and pentane (300ml+100ml) was added. The lipase was filtered off and the water phase was separated. The organic phase was washed twice with water and dried over $MgSO_4$ followed by distillation of the solvent at atmospheric pressure. The product was then distilled at reduced pressure. bp_{760}=131 °C. (bp_{760}=131.5 °C)[12] Yield 44.9g (78%). GC (99.5% pure). Spectroscopic data were in accordance with previous published.[13]

1,2-Deceneoxide: Tetradecanoic acid (4.52g, 19.8 mmol) was dissolved in 1-decene (15 ml, 79.2 mmol) and immobilised *C. antarctica* lipase (1g) was added followed by hydrogen peroxide (6.96 ml, 122 mmol, 17.5M) added in four equal portions after 0, 1.5, 3 and 4.5 hours. The yield of decene oxide was determined after 6 (54% yield) and 24 hours (81.7% yield) by GC. The reaction was stopped and the reaction mixture was kept in the freezer overnight. Then, 70 ml ice cold hexane was added and the crystalline tetradecanoic acid was washed with water several times (3 x 30 ml). The remaining tetradecanoic acid was extracted with $NaHCO_3$ (75ml, sat'd, aq), the water phase was separated and the organic phase was filtered and washed with NaCl (50 ml, sat'd, aq) and water (50 ml). Drying ($MgSO_4$), evaporation and distillation bp_2= 67-68 °C (bp_{10}= 89 °C)[14] gave a 6.2 g (50%) yield. ^{1}H-NMR ($CDCl_3$) = 0.88 (t,3H), 1.27-1.56 (m,14H), 2.46 (dd,1H), 2.75 (dd,1H), 2.89-2.94 (m,1H).

Other oxidations

Dibutylsulfoxide: Tetradecanoic acid (1.63g, 7.15 mmol) was dissolved in dibutylsulfide (5 ml, 28.5 mmol) and immobilised C. antarctica lipase (419 mg) was added. During mechanical stirring hydrogen peroxide (2.49 ml, 43.6 mmol, 17.5 M) was added in six equal portions after 0, 0.5, 1, 1.5, 2 and 3 hours. A yield of 56% sulphoxide and 44% sulphone was obtained after 4 hours, as determined by GC analysis, as compared with authentic samples.

δ-Valerolactone: Tetradecanoic acid (228mg, 1mmol) was dissolved in cyclopentanone (0.265ml, 3mmol) and toluene (5ml) in the presence of immobilized C. antarctica lipase (100mg, component A and B). Then, hydrogen peroxide (0.5ml, 17.5M, 8.75mmol) is added. A 17% yield of the product is reached in 24 h as determined by GC analysis, as compared with an authentic sample.

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