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Lipase-catalyzed preparation of peroxy acids and their use for epoxidation [1]

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Abstract

The lipase-catalyzed reactions of carboxylic acids and carboxylic acid esters with hydrogen peroxide are used to generate various peroxy acids at room temperature and without mineral acids. Whereas Novozym 435^{R} will only be able to catalyze the conversion of free unbranched carboxylic acids to peroxy acids, α-methylsubstituted carboxylic acid ethylesters can be converted to peroxy acids by perhydrolysis using the same enzyme. These peroxy acids are used in-situ for the epoxidation of unsaturated compounds and thus a new versatile epoxidation method for organic synthesis is proposed.

Keywords: Lipase; Hydrogen peroxide; Peroxy acids; Perhydrolysis; Epoxidation

1. Introduction

Peroxy acids are important oxidants in organic synthesis as well as in chemical industry. The major oxidation by peracids is the Prileshajev-epoxidation of C=C-bonds, but there are also the Baeyer–Villiger oxidation and numerous transition-metal catalyzed oxidations [1]. In spite of their importance as oxidants the number of peroxy acids available for organic synthesis is rather limited: peroxy formic acid and peroxy trifluoroacetic acid are formed readily in-situ from the acids and hydrogen peroxide, whereas peracetic acid, *m*-chloro-peroxy benzoic acid (mcpba) and the magnesium-salt of monoperoxy phtalic acid are commercial products.

Generally peroxy acids can be made by the reaction of a carboxylic acid with hydrogen peroxide:

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[1] Based on an oral presentation at the 6th International Symposium on the Activation of Dioxygen and Homogeneous Catalytic Oxidation, Noordwijkerhout (NL), April 14–19, 1996.

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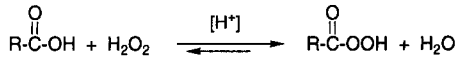
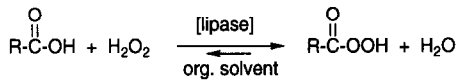


However, the need of a mineral acid and the severe reaction conditions for long chain acids are restrictions of this synthetic method.

The conversion of carboxylic acids and hydrogen peroxide to the peroxy acid (and water) can also be catalyzed by a lipase:



This reaction has first been carried out intentionally by Björkling et al. [2–7] and the same group has also performed in-situ epoxidations using these peracids. Although the reaction is catalyzed by almost any lipase or esterase, the activity and stability of Novozym 435^R (*Candida antarctica lipase* on polyacrylic resin) for peroxy acid formation is outstanding. Other applications have been natural product synthesis [8], peroxy acid formation in a membrane reactor [9] and Baeyer–Villiger oxidations [10].

The starting point of our own work in this field has been the 'self'-epoxidation of unsaturated fatty acids [11]. In this reaction unsaturated carboxylic acids were converted to unsaturated peroxy acids; in the second step these unsaturated peroxy acids epoxidize themselves with 60–90% yield and 98% selectivity. However, we found some ($< 2\%$) peroxy epoxy acids in the reaction product. That observation raised the question of the substrate range of the Novozym 435^R-catalyzed reaction of carboxylic acids with hydrogen peroxide, which now was investigated in detail.

2. Experimental

2.1. Materials

Novozym 435^R was kindly supplied by Novo Nordisk AS. 60% Hydrogen peroxide (percentages of hydrogen peroxide are always given as wt% H_2O_2 in water) was supplied by Peroxide Chemie/Interox. 35% hydrogen peroxide was purchased from Merck. 1,2-epoxyoctane was purchased from Lancaster. All other chemicals were purchased from Sigma, Merck or Aldrich. Ethylesters were made from the corresponding free acids by esterification in 1,2-dichlorethane catalyzed by *p*-toluylsulfonic acid and purified by destillation (2-*p*-chlorphenoxypropanoic acid ethylester: 87–95°C/0.8 mbar; 2-phenoxypropanoic acid ethylester: 102–103°C/6 mbar; 2-phenylpropanoic acid ethylester 82–90°C/7 mbar).

2.2. Analysis

Gas chromatography was performed on a Hewlett Packard model 5890 Series II instrument equiped with a flame ionization detector and a Chromatography Service SE-54 capillary column of 25 m length. The identity of the products was confirmed by comparison with authentic samples and/or GC-MS spectra. GC-MS spectra were obtained on a Hewlett Packard HP 5989A mass spectrometer

coupled to a HP 5890 Series II GC. All free carboxylic and peroxy acids were converted to their methylesters by CH_2N_2 before GC-analysis. Yield and conversions were measured with the help of an internal standard (heptanoic acid ethylester). A correction factor was determined for 1,2-epoxyoctane.

The amount of peracids was measured by a combination of titrations. First a iodometric titration (0.1 N $Na_2S_2O_3$) was carried out to determine the total active oxygen content, followed by a cerimetric titration (0.1 N $Ce(SO_4)_2$) to determine the H_2O_2-content. In the absence of organic hydroperoxides the difference of these two titrations is the peracid content. To obtain a correct mass balance, organic and water phase have both to be analyzed.

2.3. Oxidation reactions

In a typical chemo-enzymatic epoxidation of 1-octene (5 mmol ≙ 560 mg), a carboxylic acid (5 mmol) was dissolved in 10 ml toluene and the lipase (100 mg ≙ 700 U; 1 U ≙ 1 mmol lauric acid propylester formed in 15 min [12]) was added. After stirring for 15 min 15 μl of 60% H_2O_2 were added by a Methrom 665 Dosimat, which has been modified to function automatically and time dependently. Every 15 min, the addition was repeated until all H_2O_2 (7.5 mmol, 360 μl) was added and stirring was continued for a further 16 h at 40°C. Afterwards the lipase was removed by filtration, the mixture was washed with water to remove the excess H_2O_2 and the organic phase was dried over Na_2SO_4. The reaction mixture was analyzed by GC.

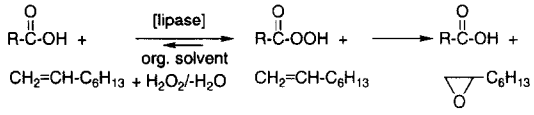
Epoxidation by perhydrolyis was carried out in a similar way, except that a carboxylic acid ester (10 ml) was used as solvent as well as for peroxy acid generation. Furthermore 35% H_2O_2 was used (7.5 mmol, 648 μl).

To obtain peroxy carboxylic acids, the same procedure was used, but no unsaturated compound was added and after addition of all the hydrogen peroxide stirring was continued for 15 min. Afterwards the yield of peroxy acid was determined by titration.

3. Results and discussion

3.1. Various peroxy acids by reaction of carboxylic acids with hydrogen peroxide

With Novozym 435^R as the biocatalyst forty carboxylic acids were used as substrates for the reaction with hydrogen peroxide in toluene. Two different methods were applied to estimate the amount of peroxy acids generated. The peroxy acid content can be measured by titration or the peroxy acid can be used in-situ to epoxidize 1-octene:



1-octene was chosen because it is more difficult to epoxidize than e.g. cycohexene, that is often used as a test substrate for new epoxidation methods; epoxidation of cyclohexene is about 25 times faster than that of 1-octene [13]. Table 1 shows the results of these experiments.

Table 1
Peroxy acids by reaction of carboxylic acids with 60% H_2O_2/Novozym 435[R]

No.	Carboxylic acid	Yield of RCO_3H (by titration) [a]	Yield of 1,2-epoxy-octane (by GC) [b]
1	propanoic acid	n.e.	41%
2	butanoic acid	n.e.	50%
3	pentanoic acid	n.e.	46%
4	hexanoic acid	n.e.	56%
5	heptanoic acid	n.e.	60%
6	octanoic acid	> 98%	63%
7	nonanoic acid	n.e.	52%
8	decanoic acid	n.e.	62%
9	undecanoic acid	> 98%	55%
10	dodecanoic acid	> 98%	63%
11	tetradecanoic acid	n.e.	64%
12	octadecanoic acid	92%	66%
13	docosanoic acid	n.e.	64%
14	12-hydroxy-octadecanoic acid	n.e.	68%
15	9,10-epoxy-octadecanoic acid	89%	59%
16	9,10-dihydroxy-octadecanoic acid	n.e.	64%
17	10,11-epoxy-undecanoic acid	64%	20%
18	dodecandioic acid	n.e.	9%
19	dodecandioic acid mono-2-butylester	n.e.	60%
20	succinic acid mono-dodecylester	n.e.	12%
21	maleic acid	0% [c]	19%
22	2-phenylacetic acid	> 98%	62%
23	3-phenylpropionic acid	40%	15%
24	4-phenylbutanoic acid	> 98%	58%
25	2-phenylpropanoic acid	0%	3%
26	2-methylpropanoic acid	58%	25%
27	2-ethylhexanoic acid	0%	0%
28	2-hexyldodecanoic acid	n.e.	0%
29	3-ketopentanoic acid	n.e.	12%
30	2,2-dimethylpropanoic acid	0%	0%
31	10-aminodecanoic acid	0%	0%
32	2-hydroxypropanoic acid	[d]	0% [d]
33	2-hydroxy-2-phenylacetic acid	[d]	0%[d]
34	δ-valerolactone	[d]	4% [d]
35	acrylic acid	0% [c]	0%
36	methacrylic acid	0% [c]	0%
37	benzoic acid	0%	0%
38	phtalic acid	n.e.	0%
39	homophtalic acid	n.e.	0%
40	ferrocenyl-acetic acid	n.e.	0%

[a] 5 mmol carboxylic acid, 100 mg Novozym 435[R] (*Candida antarctica lipase* on polyacrylic resin), 10 ml toluene, 24 × 15 μl H_2O_2 (7.5 mmol, 60%), each after 15 min; $COOH:H_2O_2$ = 1:1.5 (M); 6 h at RT.
[b] 5 mmol 1-octene, 5 mmol carboxylic acid, 100 mg Novozym 435[R] (*Candida antarctica lipase* on polyacrylic resin), 10 ml toluene, 24 × 15 μl H_2O_2 (7.5 mmol, 60%), each after 15 min; $C = C:COOH:H_2O_2$ = 1:1:1.5 (M); 16 h at 40°C.
[c] Traces of 'self'-epoxidation in the absence of 1-octene.
[d] Oligomerization.

Generally we found, that the epoxidation of 1-octene is a very sensitive method to estimate the amount of peroxy acid. In all cases a higher peroxy acid content leads to a higher yield of 1,2-epoxyoctane, but a small difference in peroxy acid concentration makes a bigger difference in epoxide yield. The correspondence of peroxy acid formation and epoxidation indicates, that the kind

Table 2
Perhydrolysis of dodecanoic acid esters by H_2O_2/Novozym 435[R]

No.	Dodecanoic acid ester	Solvent	H_2O_2 (conc. w/w)	Peroxy acid (by titration)
1	free acid	toluene	60%	> 98%
2	ethylester	toluene	60%	41%
3	ethylester	toluene	35%	63%
4	trifluorethylester	toluene	35%	> 98%
5	ethylester	ethylester	35%	> 98% [a]

100 mg Novozym 435[R] (*Candida antarctica* on polyacrylic resin), 1 mmol carboxylic acid ester, 10 ml solvent, H_2O_2 (5 mmol, 24 parts each after 15 min.
[a] Related to H_2O_2.

of peroxy acids does not have any influence on the epoxidation step under these conditions. Because the epoxidation of 1-octene was such a sensitive probe, it was carried out routinely, whereas the peroxy acid concentration was not measured in all cases. To ensure that no uncatalyzed reaction did occur, 'blank' experiments without enzyme were carried out; no peracids (respectively no epoxidation) were found. The same was done with deactivated Novozym 435[R] (boiling in methanol for some hours) and again, no reaction occurred.

Good substrates are converted almost quantitatively to the peroxy acids. The experimental error in the peroxy acid measurement in the presence of H_2O_2 was estimated as 2%; hence the conversion is > 98% — it cannot be quantitative in the strong sense of the word, because it is an equilibrium reaction. All fatty acids C_4 to C_{22} are good substrates as well as 2-phenylacetic acid, 4-phenyl butanoic acid, dodecandioic acid monobutylester and fatty acids with internal hydroxy- or epoxy-groups (Table 1, Nos. 14–16). The reaction of dodecandioic acid may be hindered by its low solubility in toluene. Acids that are prone to radical polymerization (Table 1, Nos. 35 and 36) are not suitable; however the oligomerization may indicate that peroxy acids are formed, because 'blank' experiments in the absence of the lipase but with H_2O_2 have shown very little oligomerization. The suspected formation of peroxy acrylic acid will be examined in more detail in the future. Acids with α-hydroxy-groups and lactones (Table 1, Nos. 32–34) form oligoesters because of the esterification activity of Novozym 435[R].

Unfortunately with the exception of isobutyric acid (Table 1, No. 26) neither α-substituted carboxylic acids (Table 1, Nos. 25, 27, 28 and 30) nor aromatic acids (Table 1, Nos. 37–40) can be

Table 3
Chemo-enzymatic epoxidation of 1-octene by peroxy acids from branched and aromatic acids and their ethylesters

No.	Carboxylic acid/ester	Yield of 1,2-epoxyoctane (%, GC) free acids [a]/ethylesters [b]
1	2-methylpropanoic acid/ethylester	25%/70%
2	*p*-chlorophenoxypropanoic acid/ethylester	6%/68%
3	2-chloropropanoic acid/ethylester	n.e/65%
4	2-phenylpropanoic acid/ethylester	3%/66%
5	2-phenoxypropanoic acid/ethylester	0%/62%
6	2-ethylhexanoic acid/ethylester	0%/0%
7	phtalic acid/ethylester	0%/0%

[a] 1 mmol 1-octene (112.2 mg), 100 mg Novozym 435[R] (*Candida antarctica lipase* on polyacrylic resin), 1 mmol carboxylic acid, 10 ml toluene, 24 × 10 μl H_2O_2 (5 mmol, 60%), each after 15 min.
[b] 1 mmol 1-octene (112.2 mg), 100 mg Novozym 435[R] (*Candida antarcticalipase* on polyacrylic resin), 10 ml carboxylic acid ester, 24 × 18 μl H_2O_2 (5 mmol, 35%), each after 15 min.

converted at all; that totally excludes the synthesis of peroxy acids with a chiral center near the carboxylic group by Novozym 435^R-catalyzed conversion of free carboxylic acids.

3.2. Peroxy acids by reaction of carboxylic acid esters with hydrogen peroxide (perhydrolysis)

To overcome these difficulties we were looking for an alternative and began to study the perhydrolysis of carboxylic acid esters by Novozym 435^R, a reaction, that has never been used preparatively before. According to the following scheme a carboxylic acid ester reacts with hydrogen peroxide to the peroxy acid and an alcohol:

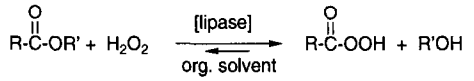
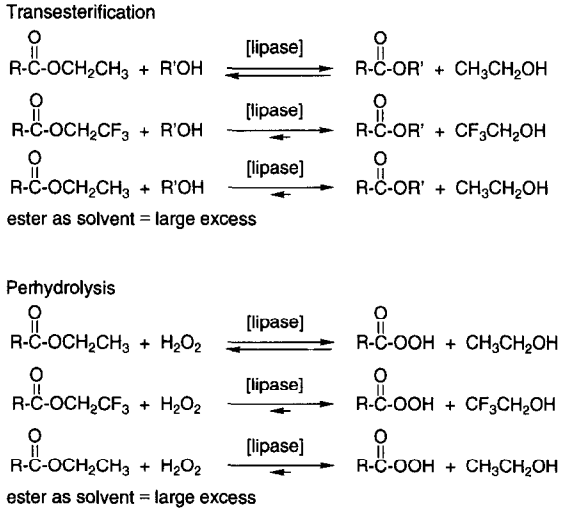


Table 2 shows the results of the biocatalytic perhydrolysis of lauric acid (dodecanoic acid) esters in comparison to the reaction of the free acid.

If the enzymatic perhydrolysis of lauric acid ethylester was carried out under the same conditions as the conversion of lauric acid, the peroxy acid yield would decrease from > 98% to 41% (Table 2, Nos. 1 and 2). Fortunately, a higher yield of peroxy acid can be achieved from the ethylester, if 35% H_2O_2 is used instead of 60% H_2O_2 (Table 2, Nos. 2 and 3). This is an important advantage of perhydrolysis over the conversion of the free acids, because 60% H_2O_2 is not easily available and requires some knowledge in handling.

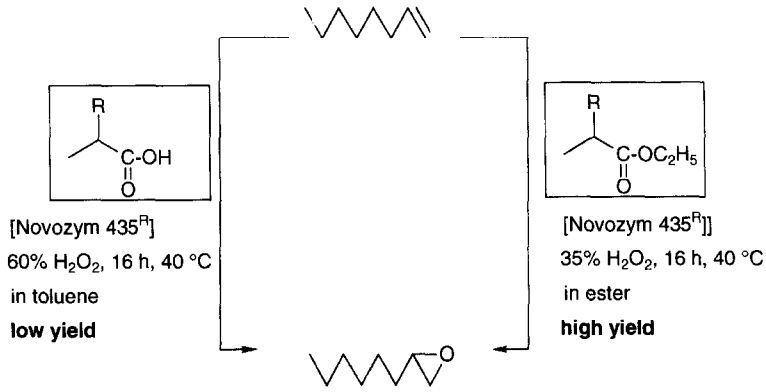
A quantitative yield of peroxy lauric acid can be achieved by perhydrolysis of the trifluorethylester (Table 2, No. 4). The use of trifluorethylesters as quasi non-reversible substrates for lipase-catalyzed transesterification has been described before [14]; the lipase-catalyzed reaction of trifluorethanol (reverse reaction) is slow and the same seems to be true for peroxy acids:



However, it is most advantageous to carry out perhydrolysis not in an inert solvent but in the esters itself (Table 2, No. 5). This possibility to change the position of the equilibrium is again derived from

lipase-catalyzed transesterification [15,16]. Now the hydrogen peroxide is converted quantitatively to the peroxy acids and the driving force of the reaction is the large excess of the ester.

The improved method of perhydrolysis in the ester as the solvent and with 35% hydrogen peroxide was now used to make those peroxy acids, for which the direct conversion of the carboxylic acids had more or less failed. Again the epoxidation of 1-octene served as a test reaction to estimate the suitability of the substrates for peroxy acid generation:

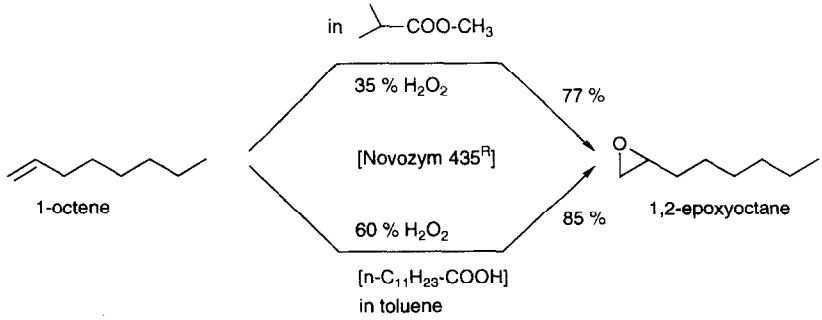


As it can be seen in Table 3, some interesting peroxy acids can only be made by perhydrolysis. Whereas free α-methylsubstituted carboxylic acids are unsuitable for peroxy acid generation, their methylesters are readily converted to peroxy acids (Table 3, Nos. 1–5). The yield of 60–70% 1,2-epoxyoctane indicates a nearly complete conversion to peroxy acids (compare Table 1). These substrates contain an asymmetric C-atom; hence the preparation of achiral peroxy acids may be thus achieved. Further investigation in this area is under way.

In conclusion Novozym 435^R seems to be quite limited to slender molecules, because even perhydrolysis does not proceed with branched carboxylic acids esters with a bigger side-chain or with aromatic acid esters (Table 3, Nos. 6 and 7).

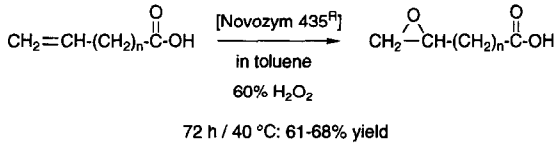
3.3. Epoxidation by lipase-catalyzed peroxy acid generation

Epoxidation of C=C-unsaturated compounds by peroxy acids, which are generated by catalysis with Novozym 435^R, can be performed in two ways, as described in the following scheme:

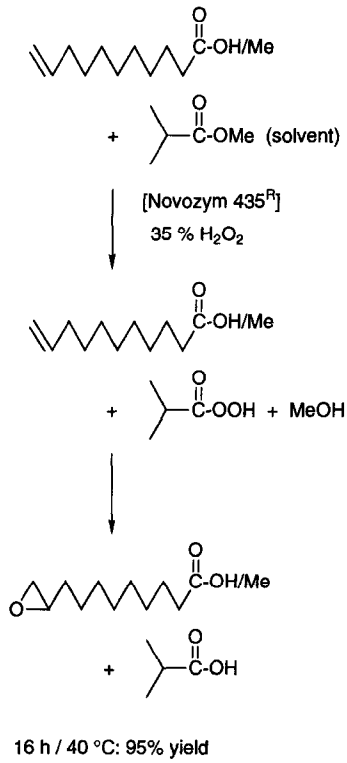


The lower way has been proposed by Novo [17]. Alternatively the epoxidation can be performed in isobutyric acid methylester as solvent and carboxyl-source — any other fatty acid ester will do, but we prefer isobutyric acid methylester, because it is quite volatile and therefore easy to remove — and using 35% hydrogen peroxide (concentrations down to 20% can be used without any effect on the epoxide yield). Only this method is attractive for organic synthesis and even more, it is cheap: if the enzyme is used only one time and discarded afterwards it is less expensive than buying peracetic acid or mcpba. We have already been able to show that the lipase can be recovered by a simple filtration and reused at least fifteen times [11], so that this epoxidation method is even more competitive.

The large excess of the carboxyl function in the perhydrolysis makes the reaction reasonably fast; this may be demonstrated by the following example. The chemoenzymatic 'self'-epoxidation of ω-unsaturated carboxylic acids is very selective, but due to the mechanism of the reaction [11], 60–70% yield are reached only after 72 h at 40°C:



The same epoxidation can be achieved by perhydrolysis using the same enzyme, but 35% hydrogen peroxide. Now the yield of 10,11-epoxyundecanoic acid is 95% after 16 h at room temperature.



The most promising application of this technique is the 'self'-epoxidation of plant oils. Because plant oils are fatty acids esters of glycerol, they can be epoxidized without any additional carboxyl-source by Novozym $435^R/H_2O_2$ [18]; epoxidized soybean oil is an important PVC-stabilizer and extender [19].

Beyond this particular application, the epoxidation of C=C-bonds by perhydrolysis should be considered as a new alternative for fine chemical synthesis.

Acknowledgements

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