Chemoenzymatic Epoxidation of Unsaturated Fatty Acid Esters and Plant Oils[1]

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ABSTRACT: In the presence of an immobilized lipase from *Candida antacrtica* (Novozym 435[R]) fatty acids are converted to peroxy acids by the reaction with hydrogen peroxide. In a similar reaction, fatty acid esters are perhydrolyzed to peroxy acids. Unsaturated fatty acid esters subsequently epoxidize themselves, and in this way epoxidized plant oils can be prepared with good yields (rapeseed oil 91%, sunflower oil 88%, linseed oil 80%). The hydrolysis of the plant oil to mono- and diglycerides can be suppressed by the addition of a small amount of free fatty acids. Rapeseed oil methyl ester can also be epoxidized; the conversion of C=C-bonds is 95%, and the composition of the epoxy fatty acid methyl esters corresponds to the composition of the unsaturated methyl esters in the substrate.

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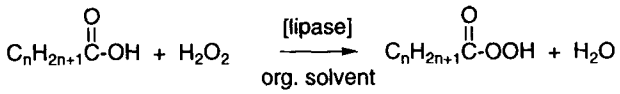
KEY WORDS: Epoxidation of plant oils, epoxidation of rapeseed oil methyl ester, hydrogen peroxide, lipase, perhydrolysis, peroxy acids.

The catalytic activity of lipases for esterification and ester hydrolysis has found various applications in oleochemistry. A novel lipase-catalyzed reaction of fatty acids was first described by a research group from Novo Nordisk A/S (Copenhagen, Denmark) (1–4). Some immobilized lipases—especially Novozym 435[R] (EC 3.1.1.3; an immobilized form of *Candida antarctica* on polyacrylate resin) catalyze the formation of peroxy fatty acids from fatty acids and hydrogen peroxide (Scheme 1). Normally this reaction can only be achieved using strong mineral acids like concentrated sulfuric acid as catalysts and—in the case of longer chain fatty acids—acids serve even as solvent (5). Catalyzed by the immobilized lipase, the reaction takes place under very mild conditions; therefore sensitive acids can also be converted to peracids. Björkling *et al.* (2,3) have carried out *in situ* Prileshajev-epoxidations of simple olefins by peroxy acids, which were prepared this way. Natural product synthesis (6), peracid formation in a membrane reactor (7) and Baeyer-Villiger-oxidations (8) have been additional achievements in this area.

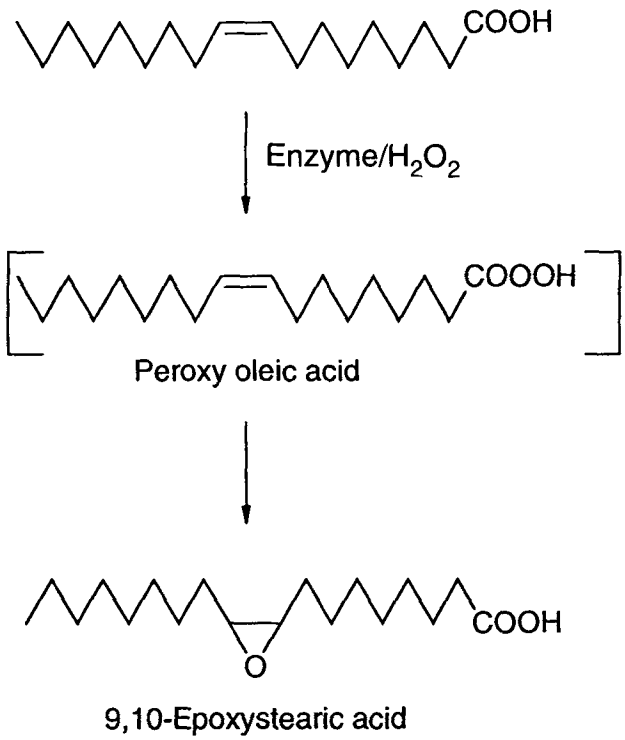
Catalytic reactions involving the C=C-bond in unsaturated



SCHEME 1

fatty acids and their esters have been our major field of research in the last few years (9–11). This also included C=C-oxidations by peracetic acid, hydrogen peroxide, and other single oxygen donors (12–15). The starting point of our own research concerning lipase-catalyzed oxidations was the chemoenzymatic "self-" epoxidation of unsaturated fatty acids (16).

If the lipase-catalyzed preparation of peroxy fatty acids is applied to unsaturated fatty acids, a two-step reaction will take place (Scheme 2). In the first step, the unsaturated fatty acid (oleic acid is shown here as an example) is converted to



SCHEME 2

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an unsaturated peroxy fatty acid. This unsaturated peracid is only an intermediate; it epoxidizes itself (Prileshajev-epoxidation) in the second step, and the final product of the reaction is the epoxy acid; the unsaturated fatty acid provides both a C=C-bond and a percarboxyl-group for a "self-"epoxidation. By this method, several natural, internal unsaturated fatty acids were epoxidized. Most notably all common constituents of unsaturated plant oils (oleic-, linoleic-, linolenic-, and erucic acid) were epoxidized smoothly. Now we report the use of a similar technique for the epoxidation of unsaturated fatty acid esters.

EXPERIMENTAL PROCEDURES

Materials. Novozym 435^R was kindly supplied by Novo Nordisk AS; 60% H_2O_2 (concentrations of hydrogen peroxide are all given as weight percentages in water) was supplied by Peroxid-Chemie/Interox (Pullach, Germany). 35% H_2O_2 was purchased from Merck (Darmstadt, Germany). Rapeseed oil (Brökelmann; Hamm), sunflower oil and linseed oil (Caesar & Loretz, Hilden) were standard products of German agriculture.

Methyl esters were converted to free fatty acids by a 1 N solution of KOH in ethanol/H_2O (9:1, vol/vol) afterward. Oleic acid (90%) was supplied by Nippon Oils & Fats Inc. (Tokyo, Japan). All other chemicals were purchased from Sigma (Deisenhofen, Germany), Fluka (Neu-Ulm, Germany), or Aldrich (Steinheim, Germany). Samples of epoxidized fatty acids for comparison were made by Prileshajev-Epoxidation with buffered peracetic acid and/or by epoxidation with dimethyldioxirane, prepared as described by Adam *et al.* (17).

Analysis. Gas chromatography (GC) was performed on a Hewlett-Packard model 5890 Series II instrument equipped with a flame-ionization detector and a Chromatography Service SE-54 capillary column (25 m × 0.2 mm; 0.25 μm) (Palo Alto, CA). The temperature program used was: 70°C, 5 min *iso*; 12°C/min to 240°C; 16 min *iso*). The identity of the products was confirmed by comparison with authentic samples and/or GC–mass spectrometry (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 methyl esters by CH_2N_2 before GC analysis. Yield and conversions were measured with the help of an internal standard (phthalic acid diethyl ester). A correction factor was determined for 9,10-epoxystearic acid methyl ester.

Oxidation reactions. In a typical chemoenzymatic epoxidation of an unsaturated carboxylic acid, the 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 (18)] 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 10–66 h. The reaction mixture was heated to 40°C in the case of the epoxidation of terminal C=C-bonds. Afterward 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.

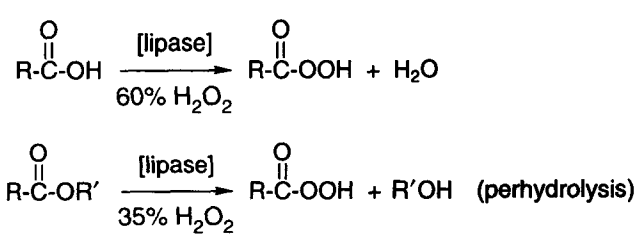
The procedure for the epoxidation of unsaturated plant oils is very similar. Rapeseed oil (10 mmol, *ca.* 40 mmol C=C) and free rapeseed oil fatty acids (2 mmol, *ca.* 2 mmol C=C) are dissolved in 100 mL toluene. After addition of 800 mg lipase, 48 portions (each 54 μL) of H_2O_2 were added (35%, 60 mmol in total). The work-up procedure is analogous to the epoxidation of free fatty acids. In addition, the product was washed with a 5% $NaHCO_3$-solution to remove the epoxidized free fatty acids.

RESULTS AND DISCUSSION

Peroxy fatty acids can not only be obtained by the lipase-catalyzed reaction of free fatty acids with hydrogen peroxide, but also by lipase-catalyzed perhydrolysis of their esters (Scheme 3). All advantages of lipase-catalyzed peracid formation over the conventional synthesis using mineralic acids as catalysts are also valid for the perhydrolysis. For synthetic applications the perhydrolysis of fatty acid esters is more convenient than the reaction of the free acids: whereas the perhydroxylation of free acids to peroxy acids proceeds—according to Björkling *et al.* (3) and to our own results—best with 60% hydrogen peroxide, 35% H_2O_2 is favorably applied for perhydrolysis. Hydrogen peroxide in high concentration is not easily accessible and should be handled—at least in larger quantities—only with some knowledge about the appropriate precautions, but on the other hand 35% H_2O_2 is a standard oxidant.

Consequently peroxy fatty acids could also be made by perhydrolysis of plant oils (Scheme 4). If an unsaturated plant oil is treated that way, the peroxy acids generated will epoxidize the C=C-bonds, and the reaction product will be a mixture of epoxidized tri-, di- and monoglycerides, glycerol, and epoxy fatty acids. To our surprise, the analysis of the reaction product showed that—although >95% of the C=C-bonds had been epoxidized—the amount of free fatty acids was <5% (acid value around 7).

The amount of free fatty acids in the reaction mixture is determined by three interconnected equilibria (Scheme 5). Therefore it is difficult to interpret these results. However,



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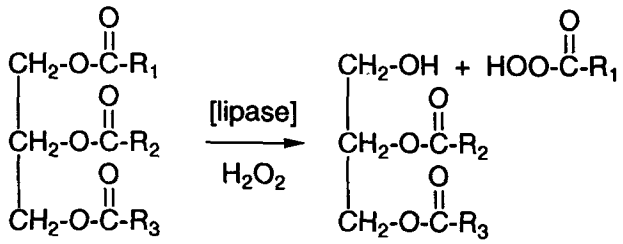

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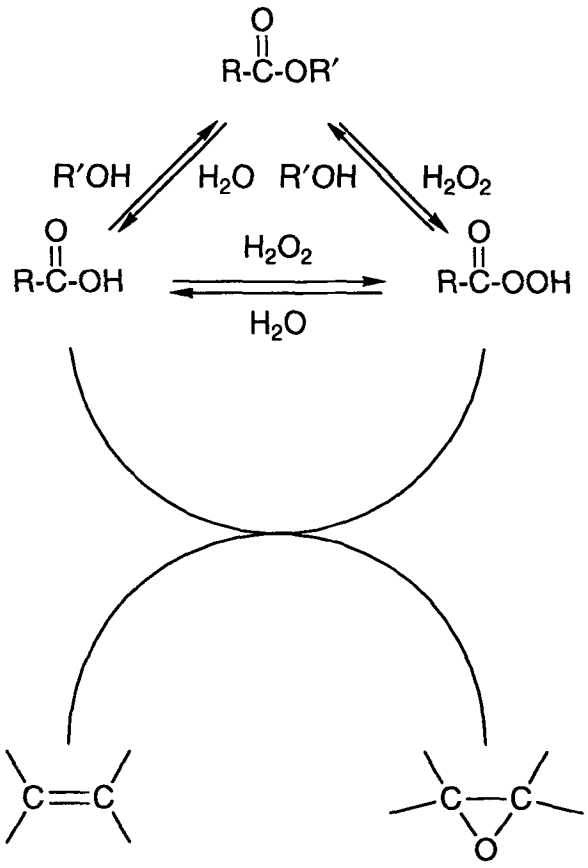


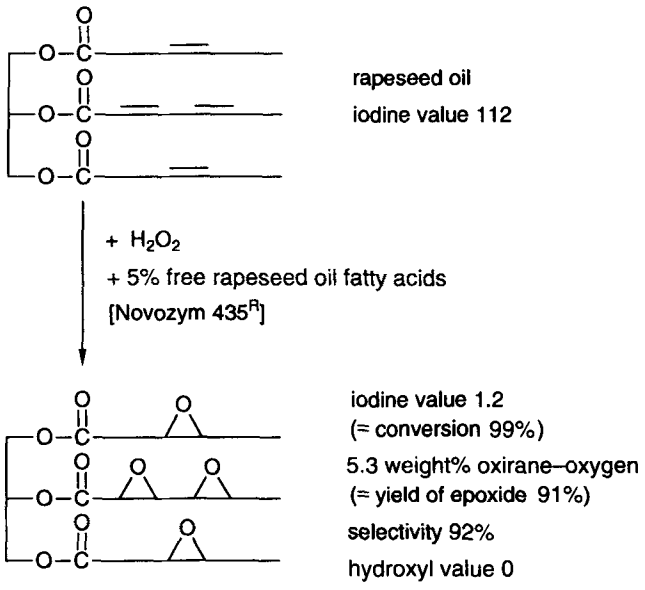
SCHEME 5

our tentative explanation is the following: The reaction takes place in an organic solvent (toluene), which is saturated by both water and hydrogen peroxide. Because the solubility of hydrogen peroxide in apolar solvents is higher than that of water, the equilibria disfavor the formation of carboxylic acids.

The formation of free acids in the reaction product is not a major drawback of this epoxidation method, because free acids can be separated easily afterward. Unfortunately the formation of free acids is accompanied by a formation of di- and monoglycerides. The problem can be solved by simply adding a small amount of free fatty acids before the reaction. Five mol% are sufficient to suppress the formation of di- and monoglycerides completely, obviously because then there is practically no more hydrolysis. First rapeseed oil was epoxidized chemoenzymatically in the following way (Scheme 6). To rapeseed oil (*ca.* 63% $C_{18:1}$, 21% $C_{18:2}$, 7% $C_{18:0}$, and 7% $C_{18:3}$; iodine value 112), 5 mol% free fatty acids (of the same composition and origin) were added. After reaction with hydrogen peroxide catalyzed by Novozym 435^R, only 1% of the double bonds remained (iodine value 1.2). The oxirane–oxygen content was 5.3%, which is 91% of the theoretical value. The hydroxylvalue of the product is 0; hence there is positively no oxirane-ring opening. The acid value is the same before and after the reaction (acid value 24–25), indicating that no hydrolysis occurs. The free fatty acids—of course in their epoxidized form after the reaction—can be removed by washing with an $NaHCO_3$ solution. Because the rapeseed oil and the free fatty acids were of the same composition, it can not be said whether transesterification has occurred.

The same procedure has been carried out with sunflower oil (and 5 mol% sunflower oil fatty acids), and an epoxide yield of 88% was achieved. The epoxidations of rapeseed and sunflower oil, which were achieved nearly quantitatively by Novozym 435^R/H_2O_2, are not very demanding tasks and they can be carried out by chemical methods as well. The epoxidation of highly unsaturated plant oils like linseed oil is more difficult. Such oils are prone to allylic oxidation if traces of impurities are present.

We performed the epoxidation of linseed oil in the same way as described above (Scheme 7). The epoxidation of linseed oil (*ca.* 15% $C_{18:1}$, 15% $C_{18:2}$, and 60% $C_{18:3}$; iodine value 203) with addition of 5% free linseed oil fatty acids by Novozym 435^R/H_2O_2 leads to a product with a iodine value of 18 (91% conversion) and a oxirane–oxygen content of 8.1 weight % (88% selectivity). Again the hydroxyl value is 0.



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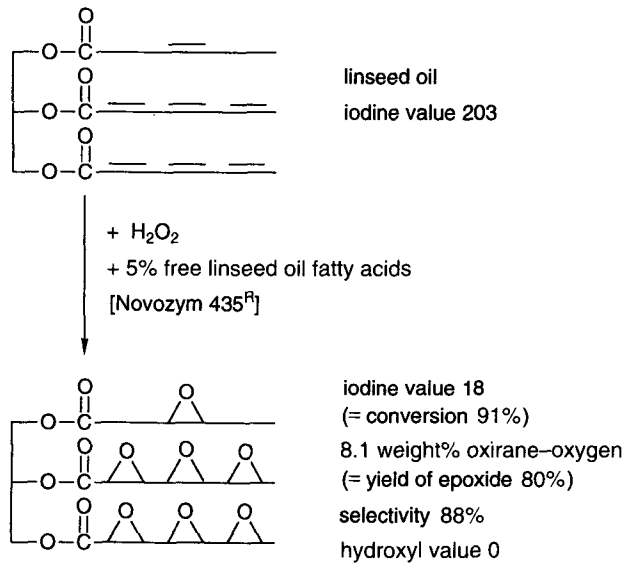

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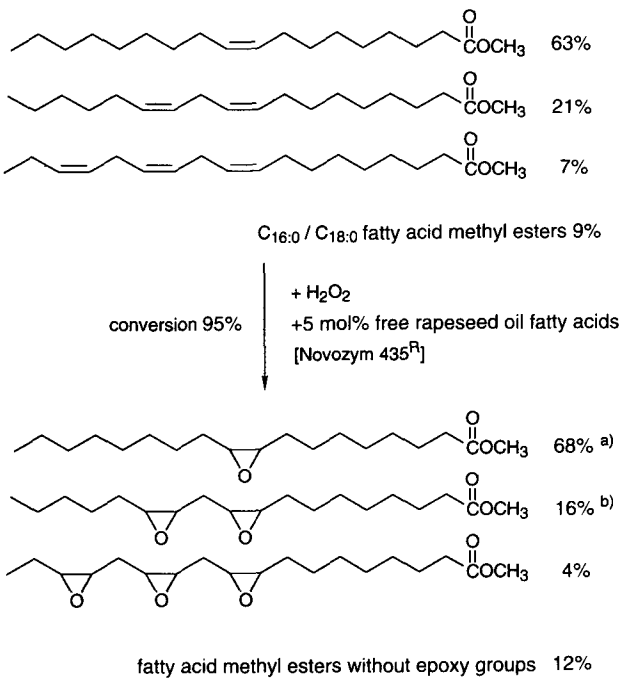

a) includes monoepoxides with one or two C=C-bonds
b) includes diepoxides with one C=C-bond

SCHEME 8

Especially these results show the selectivity of the chemoenzymatic epoxidation of unsaturated fatty compounds.

Epoxidation by lipase-catalyzed generation of peroxy acids can be carried out not only with plant oils but with other unsaturated esters as well. As an example, we chose the epoxidation of rapeseed oil methyl ester (RME). RME is sold in Germany and other European countries at gas stations as a diesel substitute and at roughly the same price as diesel fuel. The chemoenzymatic epoxidation of RME provided the following results (Scheme 8). The reaction products, which has been analyzed by GC are of a similar distribution (mono-, di, and triepoxides vs. mono-, di-, and triunsaturates) as the substrate, and an overall conversion related to C=C-bonds of 95% was achieved. In our view epoxidized RME is a product from a cheap basic material with a high potential for further applications.

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REFERENCES

1. Godtfredsen, S.E., O. Kirk, and F. Björkling, A Process for Preparing Peroxocarboxylic Acids Using an Enzyme Catalyst, WO 91/04333 (1991).
2. Björkling, F., S.E. Godtfredsen, and O. Kirk, Lipase-Mediated Formation of Peroxycarboxylic Acids Used in Catalytic Epoxidation of Alkenes, *J. Chem. Soc., Chem Commun.*:1301–1303 (1990).
3. Björkling, F., H. Frykman, S.E. Godtfredsen, and O. Kirk, Lipase-Catalyzed Synthesis of Peroxycarboxylic Acids and Lipase-Mediated Oxidations, *Tetrahedron 48*:4587–4592 (1992).
4. Kirk, O., M.W. Christensen, T. Damhus, and S.E. Godtfredsen, Enzyme-Catalyzed Degradation and Formation of Percarboxylic Acids, *Biocatalysis 11*:65–77 (1994).
5. Weigert, W.M. (ed.), Wasserstoffperoxid und seine Derivate, Hüthig, Heidelberg, 1978, pp. 73–110.
6. de Zoete, M.C., F. van Rantwijk, L. Maat, and R.A. Sheldon, Selective Oxidation of Penicillin G with Hydrogen Peroxide and with Enzymatically Generated Peroxyoctanoic Acid, *Recl. Trav. Chim. Pays-Bas 112*:462-463 (1993).
7. Cuperus, F.P., S.T. Bouwer, G.F.H. Kramer, and J.T.P. Derksen, Lipases Used for the Production of Peroxycarboxylic Acids, *Biocatalysis 9*:89 (1994).
8. Lemoult, S.C., P.F. Richardson and S.M. Roberts, Lipase-Catalyzed Baeyer-Villiger Reactions, *J. Chem. Soc., Perkin Trans. I*: 89 (1995).
9. Warwel, S., Industriechemikalien durch Olefin-Metathese natürlicher Fettsäureester, *Nachr. Chem. Tech. Lab. 40*:314–320 (1992).
10. Warwel, S., P. Bavaj, B. Ercklentz, M. Harperscheid, M. Rüsch gen. Klaas, and S. Thomas, Industriechemikalien durch Metathese und Oxidation ungesättigter Fettstoffe, edited by M. Eggersdorfer, S. Warwel and G. Wulff, in *Nachwachsende Rohstoffe—Perspektiven für die Chemie*, VCH-Verlagsgesellschaft, Weinheim, Germany, 1993, pp. 69–96.
11. Warwel, S., P. Bavaj, M. Rüsch gen. Klaas, and B. Wolff, Polymerbausteine aus Pflanzenölen durch katalytische Reaktionen edited by H. Eierdanz in *Perspektiven nachwachsender Rohstoffe in der Chemie*, VCH-Verlagsgesellschaft, Weinheim, Germany, 1996, pp. 119–135.
12. Warwel, S., M. Rüsch gen. Klaas, and M. Sojka, Formation of Vicinal Diols by Re_2O_7-Catalyzed Hydroxylation of Alkenes with Hydrogen Peroxide, *J. Chem. Soc., Chem Commun.*: 1578–1579 (1991).

13. Warwel, S. , M. Sojka, and M. Rüsch gen. Klaas, Synthesis of Dicarboxylic Acids by Transition-metal Catalyzed Oxidative Cleavage of Terminal Unsaturated Fatty Acids, *Top. Curr. Chem. 164*:79–98 (1993).
14. Warwel, S., and M. Rüsch gen. Klaas, Production of Carboxylic Acids, U.S. Patent 54344946 (1994).
15. Rüsch gen. Klaas, M., P. Bavaj, and S. Warwel, Transition-Metal Catalyzed-Oxidative Cleavage of Unsaturated Fatty Acids, *Fat Sci. Technol. 97*:359–369 (1995).
16. Warwel, S., and M. Rüsch gen. Klaas, Chemoenzymatic Epoxidation of Unsaturated Carboxylic Acids, *J. Mol. Catal. B 1*:29 (1995).
17. Adam, W., J. Bialas and L. Hadjiarapoglou, A Convenient Preparation of Acetone Solutions of Dimethyldioxirane, *Chem. Ber. 124*:2377 (1991).
18. Novo Nordisk A/S Enzyme Process Division, Organic Syntheses, product information sheet, 1992.

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