ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 1 (1995) 29–35

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Chemo-enzymatic epoxidation of unsaturated carboxylic acids

Siegfried Warwel *, Mark Rüsch gen. Klaas

Institute for Biochemistry and Technology of Lipids, H.P. Kaufmann-Institute, Federal Centre for Cereal, Potato and Lipid Research, Piusallee 68, D-48147 Münster, Germany

Received 9 May 1995; revised 6 June 1995; accepted 27 June 1995

Abstract

Unsaturated carboxylic acids are converted to percarboxylic acids catalyzed by an immobilized lipase from *Candida antarctica* (Novozym 435^R). These unsaturated percarboxylic acids are only intermediates and epoxidize themselves in good yields and almost without consecutive reactions. The mechanism of the oxygen-transfer is found to be predominantly intermolecular and the formation of the percarboxylic acids proceeds via two different catalytic reactions. The lipase is surprisingly stable under the reaction conditions; it is recovered and reused fifteen times to produce epoxy-stearic acid on a multi-gram scale.

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

1. Introduction

More and more lipases are applied in organic synthesis for various kinds of esterification and ester hydrolysis. A novel application of lipases was described by Björkling and co-workers [1–3]. They have shown that some immobilized lipases – and in particular one from *Candida antarctica* on polyacrylate resin – are able to catalyze the conversion of carboxylic acids with hydrogen peroxide to percarboxylic acids. In absence of a strong mineral acid, which is normally required as a catalyst for this conversion, Björkling and co-workers used the prepared percarboxylic acids in situ for oxidations, mainly for the Prileshajev epoxidation of simple olefins. In the meantime de Zoete et al. [4] applied this method in natural product synthesis and Cuperus et al. [5] have shown that this peracid formation is also possible in a membrane reactor.

In connection with our previous studies concerning C=C oxidations of olefins and unsaturated fatty acids to the corresponding epoxides [6], ketones [6,7], vicinal diols [6,8] and to mono- and dicarboxylic acids by oxidative C=C cleavage [6,9,10] we now applied the chemo-enzymatic epoxidation to such materials, which contain the carboxyl group as well as the C=C double bond in the same molecule, i.e., unsaturated carboxylic acids.

2. Experimental

2.1. Materials

Novozym 435^R was kindly supplied by Novo Nordisk AS. 6-Heptenoic acid, 9-decenoic acid and 13-tetradecenoic acid were prepared by met-

* Corresponding author.

SSDI 1381-1177(95)00004-6

athetic ethenolysis of petroselinic, oleic and erucic acid methyl ester as described before [6,11,12]. Ricinoleic acid was supplied by Unichema. Pure petroselinic acid methylester was made by preparative MPLC of coriander oil fatty acid methylester. These methyl esters were converted to free fatty acids by a 1 n solution of KOH in ethanol–H_2O (9:1) afterwards. Oleic acid (90%) was supplied by Nippon Oils & Fats. All other chemicals were purchased from Sigma or Aldrich. 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. [13].

2.2. *Analysis*

Gas chromatography was performed on a Hewlett Packard Model 5890 Series II instrument equipped with a flame ionisation 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 gas chromatography–mass spectrometry (GC–MS) spectra. GC–MS spectra were obtained on a Hewlett Packard HP 5989A mass spectrometer coupled to a HP 5890 Series II gas chromatograph. 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). Correction factors were determined for 10,11-epoxyundecanoic acid methyl ester and 9,10-epoxystearic acid methyl ester.

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 an unsaturated carboxylic acid, the acid (5 mmol) was dissolved in 10 ml toluene and the lipase (100 mg $\hat{=}$ 700 U; 1 $\hat{=}$ U1 mmol lauric acid propyl ester formed in 15 min [14]) was added. After stirring for 15 min 15 μl of 60% H_2O_2 were added by a Metrohm 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. 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.

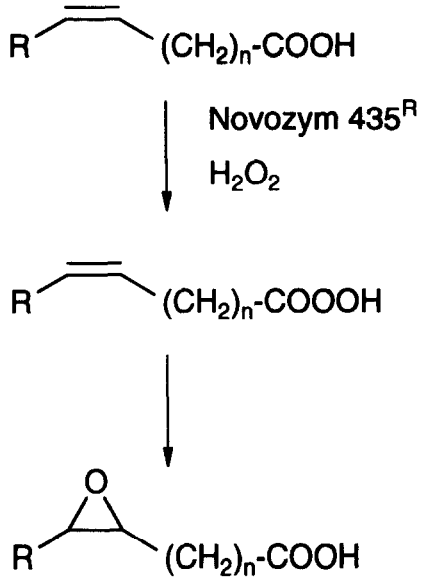
In the same way the chemo-enzymatic epoxidation of 1-octene was carried out; an equimolar mixture of 1-octene and a saturated (or an epoxidized) carboxylic acid was used as the starting material.

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.

If a reuse of the lipase was intended, it would be stored immediately after filtration in toluene. For an enzyme recycling an excess of unsaturated compound (not of H_2O_2 as in all other cases) was applied; this was found to be essential for the stability of the lipase.

3. Results and discussion

For this chemo-enzymatic 'self-'epoxidation (an enzymatically prepared unsaturated peroxy acid epoxidizes itself) (Scheme 1) we used the same immobilized lipase as Björkling et al. (Novozym 435^R from Novo Nordisk) and oxidized not only natural, internal unsaturated fatty acids but also terminal unsaturated ones, which are either commercially available (10-undecenoic



Scheme 1.

acid) or which we obtained by rhenium-catalyzed metathesis of natural fatty acid methyl esters with ethylene [6,11,12].

Table 1 shows the results of the self-epoxidation of unsaturated carboxylic acids. Oleic acid yields 72% epoxide with an excellent selectivity. There is no formation of byproducts like dihydroxycarboxylic acids (by epoxide ring-opening with water) or estolides (by epoxide ring-opening with another molecule of carboxylic acid). The only byproduct is a small amount of peracid (<2%). The position of the double bond (Δ9 in (Table 1: 1/1) and Δ11 in (Table 1: 1/2)) does not effect the yield, neither does a hydroxyl group near the double bond (Table 1: 1/3).

Beside mono-unsaturated carboxylic acids also dienoic compounds like linoleic acid can be used as starting material. Linoleic acid can be converted to diepoxystearic acid (Table 1: 1/4) or monoepoxyoctadecenoic acid (Table 1: 1/5) depending on the ratio $C{=}C/H_2O_2$; both products are obtained selectively. However, the two possible isomers of the monoepoxy derivative are formed in nearly equal amounts.

Whereas internal unsaturated acids yield about 70–90% epoxy acids, the yields for ω-unsaturated carboxylic acids are about 10% lower. They also need a threefold longer reaction time and a higher temperature.

The low rate for the epoxidation of an α,β-unsaturated acid (Table 1: 1/9) is in full agreement with results of the usual Prileshajev

Table 1
Chemo-enzymatic self-epoxidation of unsaturated carboxylic acids by H_2O_2–Novozym 435[R]

No.	Educt	Product	Yield (%, GC)	Time/temp.
1/1	Oleic acid [a]	9,10-Epoxystearic acid	72	16 h/rt
1/2	Petroselinic acid [b]	6,7-Epoxystearic acid	77	16 h/rt
1/3	Ricinoleic acid [c]	9,10-Epoxy-12-hydroxystearic acid	78	16 h/rt
1/4	Linoleic acid [d,e]	9,10-12,13-Diepoxy-stearic acid	83	16 h/rt
1/5	Linoleic acid [d,f]	9,10-(12,13)-Epoxy-12-(9)-octadecenoic acid	91 [g]	16 h/rt
1/6	9-Decenoic acid	9,10-Epoxydecanoic acid	67	72 h/40°C
1/7	10-Undecenoic acid	10,11-Epoxyundecanoic acid	61	72 h/40°C
1/8	13-Tetradecenoic acid	13,14-Epoxytetradecanoic acid	68	72 h/40°C
1/9	2-Octenoic acid	2,3-Epoxyoctanoic acid	9	16 h/rt
1/10	6-Heptenoic acid	(6,7-Epoxyheptanoic acid) [h]	0	72 h/40°C

5 mmol unsaturated carboxylic acid, 100 mg Novozym 435[R] (*Candida antarctica* on polyacrylic resin), 10 ml toluene, 24×15 μl H_2O_2 (7.5 mmol, 60%), each after 15 min, $C{=}C{-}COOH/H_2O_2 = 1{:}1.5$ (molar), enzyme concentration: 0.05 mol C=C/g enzyme.

[a] *cis*-9-Octadecenoic acid.

[b] *cis*-6-Octadecenoic acid.

[c] 12-Hydroxy-*cis*-9-octadecenoic acid.

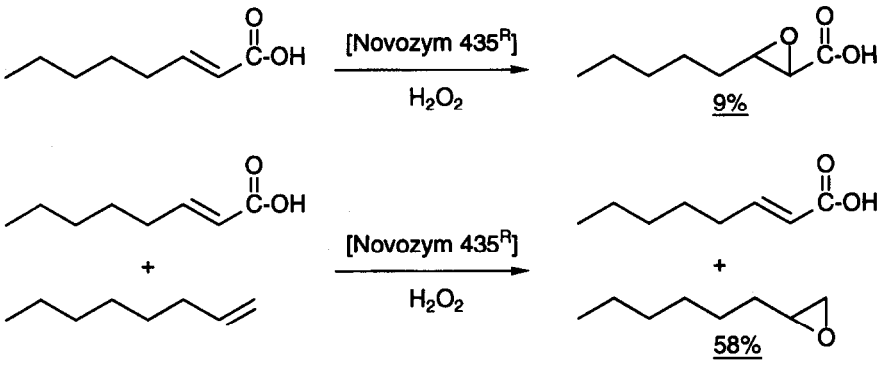
[d] *cis,cis*-9,12-octadecadienoic acid.

[e] 2.5 mmol linoleic acid; $C{=}C/COOH/H_2O_2 = 2{:}1{:}3$ (molar).

[f] 24×10 μl H_2O_2 (5 mmol, 60%) each after 15 min; $C{=}C/COOH/H_2O_2 = 2{:}1{:}1$ (molar).

[g] Related to H_2O_2.

[h] Expected.



Scheme 2.

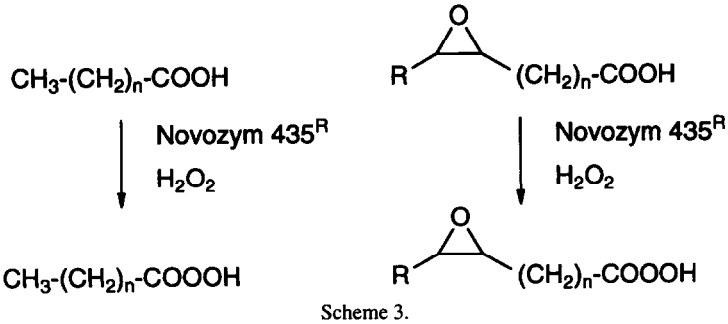
epoxidation, which is also very slow for α,β-unsaturated carboxylic acids [15]. If another unsaturated compound like 1-octene or 7-tetradecene is added during the reaction, it will be smoothly epoxidized here; therefore we conclude that 2-octenoic peroxy acid is formed, but the 'self-'epoxidation is retarded (Scheme 2).

Only 6-heptenoic (Table 1: 1/10) acid is not epoxidized at all; although the educt has completely vanished after the reaction, no oxidation product is detectable by GC. Here, too, the peracid is formed, but the loss of the product cannot be explained yet. So in conclusion, this chemo-enzymatic epoxidation is an extraordinary facile and selective method to produce unprotected epoxycarboxylic acids; high selectivity is much more difficult to obtain with conventional epoxidation methods because especially omega-epoxy-carboxylic acids will form di- and oligomers by epoxide ring opening with other molecules of carboxylic acid, if traces of a strong acid are present.

The different reactivity of terminal and internal unsaturated carboxylic acids in the chemo-enzymatic epoxidation cannot be explained only by the position of the double bond as we established by comparison experiments with preformed peracetic acid. To examine the reaction in detail we first converted various saturated acids and their terminal or internal epoxidized counterparts to the corresponding peroxy acids (Scheme 3). The amount of peroxy resp. peroxy-epoxy compounds was estimated by a combination of iodometric and cerimetric titration (see Table 2).

Afterwards we epoxidized 1-octene in situ with these peracids; so the yield of 1,2-epoxyoctane is an indirect measurement for the peracid formed (Scheme 4).

Table 2 and Table 3 summarize the results of these experiments. Whereas 9,10-epoxy octadecanoic acid is converted just as well as octadecanoic acid, 10,11-epoxyundecanoic acid is only reluctantly converted to the corresponding peracid. The difference between saturated/internal epoxidized acids and, on the other hand, terminal epoxidized acids is even more pronounced, if 1-octene is epoxidized by these acids. Using 10,11-epoxyundecanoic acid a yield of only 20%



Scheme 3.

Table 2
Enzymatic formation of peroxy- and peroxy-epoxy carboxylic acids by H_2O_2–Novozym 435^R

No.	Carboxylic acid	Yield of peroxy acid (%, by titr.)
2/1	Undecanoic acid	100
2/2	10,11-Epoxyundecanoic acid	64
2/3	Octadecanoic acid	92
2/4	9,10-Epoxyoctadecanoic acid	90

100 mg Novozym 435^R (*Candida antarctica* on polyacrylic resin). 1 mmol carboxylic acid, 10 ml toluene, 24 × 10 μl H_2O_2 (5 mmol. 60%), each after 15 min, acid/H_2O_2 = 1/5 (molar). Enzyme concentration: 0.01 mol COOH/g enzyme.

Table 3
Chemo-enzymatic epoxidation of 1-octene by C_{11}- and C_{18}-carboxylic acids and H_2O_2/Novozym 435^R

No.	Carboxylic acid	Yield of 1,2-epoxyoctane (%, GC)
3/1	Undecanoic acid	55
3/2	10,11-Epoxyundecanoic acid	20
3/3	Octadecanoic acid	60
3/4	9,10-Epoxyoctadecanoic acid	59

1 mmol 1-octene (112.2 mg), 100 mg Novozym 435^R (*Candida antarctica* on polyacrylic resin), 1 mmol carboxylic acid, 10 ml toluene, 24 × 10 μl H_2O_2 (5 mmol, 60%), each after 15 min, octene/acid/H_2O_2 = 1:1:5 (molar). Enzyme concentration: 0.01 mol C=C/g enzyme.

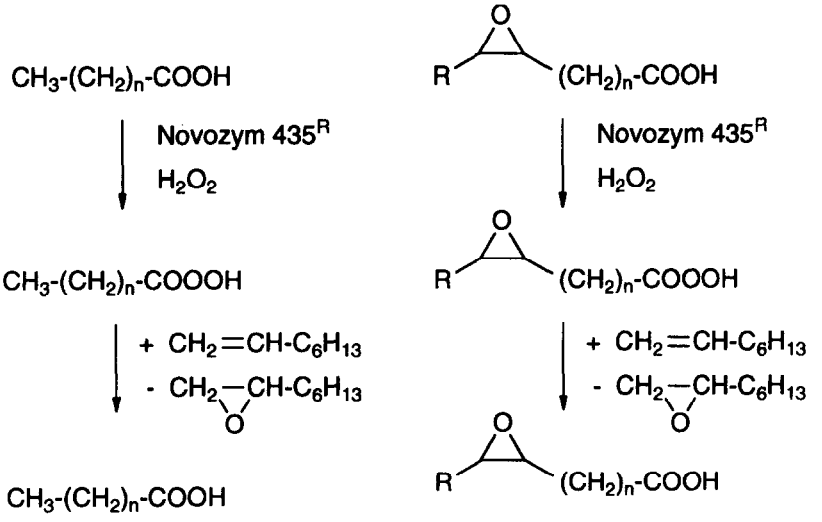
1,2-epoxyoctane is achieved, whereas the yield, which were achieved using the other acids, are three times as high.

To understand the impact of these results on the chemo-enzymatic epoxidation of internal and terminal unsaturated carboxylic acids it must be considered, that 'self-'epoxidation does not necessarily imply an intramolecular oxygen transfer from the percarboxyl group to the double bond. Intramolecular and intermolecular oxygen transfer are both possible; because the reaction is slowed down by dilution, we conclude that it is predominantly intermolecular.

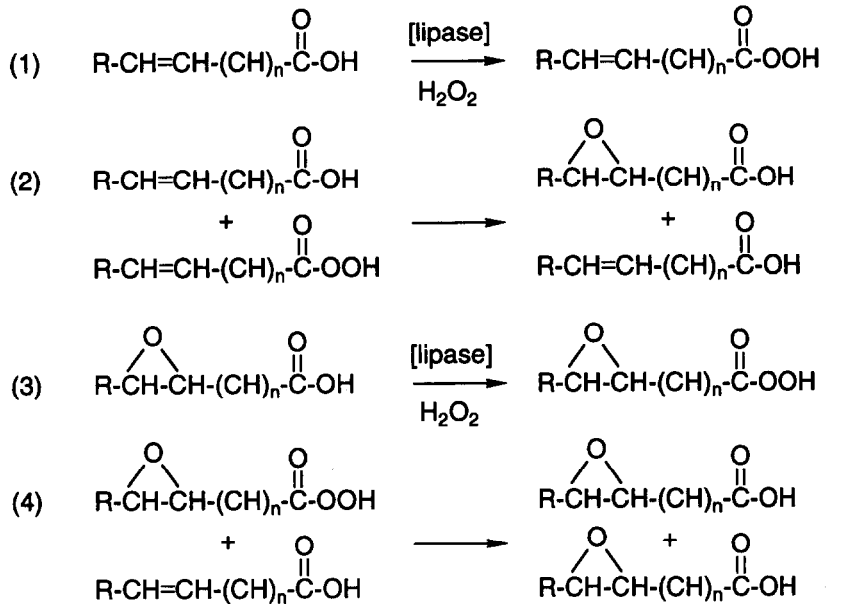
These results explain the longer reaction time and the lower yields in the chemo-enzymatic epoxidation of ω-unsaturated acids, because the reaction has to proceed via the percarboxylation of the epoxyacids after most of the unsaturated acid is consumed (see Scheme 5).

Initially in both cases (epoxidation of 10-undecenoic acid and oleic acid) the reaction proceeds only via Eqs. (1) and (2). While the reaction advances, Eqs. (3) and (4) assume more significance and, because reaction (3) is much slower for 10-undecenoic acid, retard the whole epoxidation. The oxidation of oleic acid proceeds fast through both Eqs. (1) and (3) and therefore the reaction is fast and nearly complete conversion is possible.

For a preparative application on a larger scale the stability and reuse of the immobilized enzyme is of the greatest importance. Generally lipases



Scheme 4.



Scheme 5.

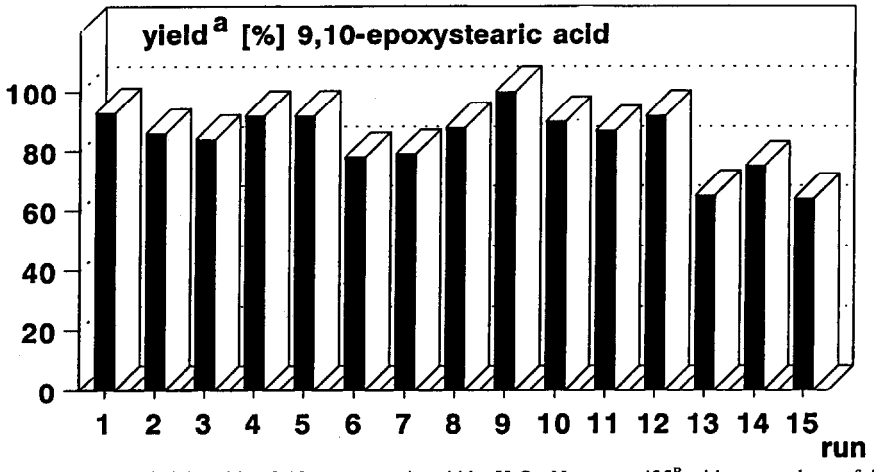


Fig. 1. Preparative self-epoxidation of oleic acid to 9,10-epoxystearic acid by H_2O_2–Novozym 435^R with repeated use of the bio-catalyst. 8 ml oleic acid/8 ml toluene, 200 mg Novozym 435^R, 24×20 μl H_2O_2 (10 mmol, 60%) each after 15 min, reaction time 16 h at room temperature. [a]mol-% related to H_2O_2.

will be deactivated in non-polar solvents, if these solvents contain larger amounts of polar substances like water. Furthermore the 60% H_2O_2 used is a very strong oxidant and is therefore expected to reduce the live-span of the enzyme even more. Nevertheless, performing the epoxidation using an excess of oleic acid, we were able to recover the enzyme by filtration and to use it again fifteen times; after that it is still active (see Fig. 1).

In total about 40 g 9,10-epoxystearic acid were produced with 200 mg Novozym 435^R. Hence the preparative usefulness of this chemo-enzymatic reaction is obvious.

Acknowledgements

We thank Dr. K.D. Mukherjee for discussions on lipase chemistry, Dr. K. Vosmann for GC–MS

spectra, Novo Nordisk A/S for Novozym 435[R] and Solvay-Interox/Peroxid-Chemie for H_2O_2. We gratefully acknowledge support of this work by the Deutsche Forschungsgemeinschaft (Schwerpunktprogramm Peroxidchemie/Sauerstofftransfer).

References

[1] S.E. Godtfredsen, O. Kirk, F. Björkling and L.B. Christensen, WO 91/06574 (31.10.90).
[2] F. Björkling, S.E. Godtfredsen and O. Kirk, J. Chem. Soc., Chem Commun., (1990) 1301.
[3] F. Björkling, H. Frykman, S.E. Godtfredsen and O. Kirk, Tetrahedron, 48 (1992) 4587.
[4] M.C. de Zoete, F. van Rantwijk, L. Maat and R.A. Sheldon, Recl. Trav. Chim. Pays-Bas, 112 (1993) 462.
[5] F.P. Cuperus, S. T. Bouwer, G.F.H. Kramer and J.T.P. Derksen, Biocatalysis, 9 (1994) 89.
[6] S. Warwel, P. Bavaj, B. Ercklentz, M. Harperscheid, M. Rüsch gen. Klaas and S. Thomas, in M. Eggersdorfer, S. Warwel, G. Wulff (Eds.), Nachwachsende Rohstoffe – Perspektiven für die Chemie, VCH Verlag, Weinheim, 1993, p. 69
[7] S. Warwel, W. Pompetzki and E.A. Deckwirth, Fat Sci. Technol., 93 (1991) 210.
[8] S. Warwel, M. Rüsch gen. Klaas and M. Sojka, J. Chem. Soc., Chem. Commun., (1991) 1578.
[9] S. Warwel, M. Sojka and M. Rüsch gen. Klaas, Top. Curr. Chem., 164 (1993) 79.
[10] S. Warwel and M. Rüsch gen. Klaas, US 54344946 (6.9.94).
[11] S. Warwel, H.-G. Jägers and A. Deckers, EP 444264 (12.12.90).
[12] S. Warwel, H.-G. Jägers and S. Thomas, Fat Sci. Technol., 94 (1992) 323.
[13] W. Adam, J. Bialas and L. Hadjiarapoglou, Chem . Ber., 124 (1991) 2377.
[14] Novo Nordisk A/S, Enzyme Process Division, Organic Syntheses, Product Information Sheet, 1992.
[15] A.S. Rao, in B.M. Trost, I. Fleming and S.V. Ley (Eds.), Oxidation, Comprehensive Organic Synthesis, Vol. 7, Pergamon Press, Oxford, 1991, p. 359.