Production of glycidyl ethers by chemo-enzymatic epoxidation of allyl ethers

Pär Tufvesson [a,*], Dietlind Adlercreutz [a], Stefan Lundmark [b],
Mircea Manea [b], Rajni Hatti-Kaul [a]

[a] *Department of Biotechnology, Center for Chemistry and Chemical Engineering,*
Lund University, P.O. Box 124, SE-221 00 Lund, Sweden
[b] *Perstorp Specialty Chemicals AB, R&D, SE-284 80 Perstorp, Sweden*

Abstract

Production of glycidyl ethers is industrially carried out by reacting alcohols with epichlorhydrin, a potentially carcinogenic compound. This paper investigates a less hazardous alternative—that of a chemo-enzymatic process in which *Candida antarctica* lipase B catalysed generation of peracid from a carboxylic acid is followed by a Prileshajev epoxidation of the corresponding allyl ether. Trimethylolpropane monoallyl ether (TMPME) was used as a model substrate. A maximal epoxide product yield of 77% was achieved through the optimization of temperature, acid concentration and hydrogen peroxide concentration. Peracid formation was considerably faster than the subsequent epoxidation step, and accumulation of the peracid was found to be important to drive the epoxidation forward.
© 2008 Elsevier B.V. All rights reserved.

Keywords: Epoxidation; Glycidyl ether; Allyl ether; Lipase; Peracid

1. Introduction

Glycidyl ethers constitute a group of industrially important epoxides used in several applications such as cross-linkers in surface coatings. By far the most commonly used glycidyl ethers are the ones derived from bisphenol A (BPA) and aliphatic polyols, respectively [1]. The polyol-based products are often used to improve the properties, such as viscosity, of the BPA-based glycidyl ether resins [2]. The industrial process for the production of glycidyl ethers uses epichlorhydrin where the epoxy ring is generated by addition of hypochloric acid to a double bond followed by ring closure and elimination under basic conditions [1]. Epichlorhydrin is a highly reactive compound and is widely used in the industry. Recently a process for the production of epichlorhydrin from glycerol, a by-product of biodiesel production, has been developed by Solvay Chemicals [3]. Nevertheless, since epichlorhydrin is a mutagenic substance [4], phasing out the use of this chemical should be highly prioritised in the indus-

try. Hence it would be useful to investigate alternative synthetic routes for glycidyl ether production.

One possible alternative is through epoxidation of the corresponding allyl ether by a Prileshajev epoxidation process in which a peracid is used to transfer oxygen to a double bond through a nucleophilic attack [5]. The peracid is used either preformed or produced *in situ* from a carboxylic acid and hydrogen peroxide using a strong acid or ion-exchange resin as catalyst [6]. The reaction conditions employed, however, make the reaction prone to side reactions, like the acid-catalysed ring opening of the oxirane group.

A more gentle approach was presented by Björkling et al. [7] who have earlier shown that lipases such as *Candida antarctica* lipase B catalyse the formation of peroxy carboxylic acids from hydrogen peroxide and the corresponding carboxylic acid. As the oxygen from the peracid is utilized for epoxidation of the double bond in the unsaturated compound, the acid is regenerated and becomes available to the enzyme for further reaction. Such a method has mostly been used for epoxidation of fatty acids having internal double bonds [8], but has also been reported for epoxidation of various other organic compounds ranging from cyclohexene to alpha-pinene and styrene

---

* Corresponding author. Tel.: +46 46 222 73 63; fax: +46 46 222 47 13.
E-mail address: par.tufvesson@biotek.lu.se (P. Tufvesson).
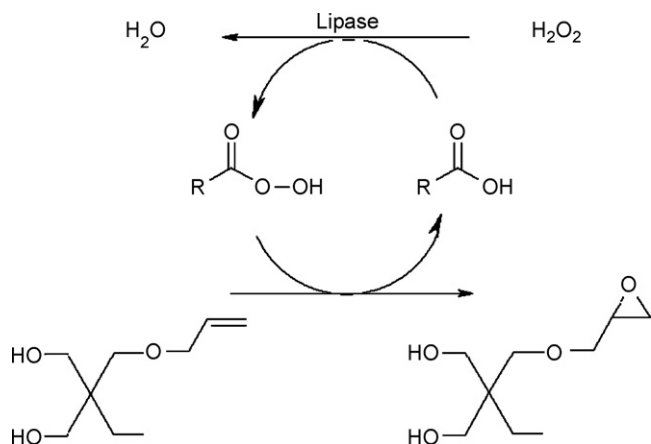
Scheme 1. Schematic representation of chemo-enzymatic epoxidation of TMPME.

[9–12]. In this context it should be stressed that the choice of alkene substrate has an enormous impact on the results, as different molecules vary greatly in their readiness to be epoxidised. Electron-donating groups in the vicinity of the double bond increase the reaction rate while electron-withdrawing groups, such as allylic oxygen, have the opposite effect [13]. As an example, cyclohexene reacts 600 times faster than allyl acetate with peracetic acid at 25 °C [14].

Unfortunately many reports have studied alkenes that are easy to epoxidise (e.g. cyclo-octene), while industrial interest would be for epoxidation of compounds on the other end of the reactivity spectra such as terminal olefins and allylic compounds [15].

In the present work we have evaluated the potential of this chemo-enzymatic approach to epoxidise terminally unsaturated allyl ether using trimethylolpropane monoallyl ether (TMPME) as a model substance (see Scheme 1). We have analysed the parameters having the predominant effect on the reaction rate and product yield while trying to understand the underlying reason for the effects observed.

2. Experimental

2.1. Materials

Novozym®435 (immobilised *Candida antarctica* lipase B) was kindly supplied by Novozymes A/S, Bagsvaerd, Denmark; trimethylolpropane monoallyl ether was from Perstorp Speciality Chemicals AB (Perstorp, Sweden). Caprylic acid, Grade II (ca. 95%), aqueous hydrogen peroxide (50% w/w), and urea–hydrogen peroxide complex (98%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other solvents and chemicals used were of analytical grade.

2.2. Methods

2.2.1. Epoxidation of TMPME

The general procedure was as follows: immobilised lipase B from *C. antarctica* (Novozym®435) was weighed (50 mg) in to a reaction vial, followed by addition of 2 ml of a solu-

tion of 1 M TMPME and caprylic acid (0.3–5.2 M) in toluene. Hexadecane was present as an internal standard for analysis. The reaction was started by adding aqueous hydrogen peroxide solution or urea–hydrogen peroxide complex in a 20% molar excess compared to the allyl ether. The contents were mixed vigorously under temperature-controlled conditions in a thermo-mixer (HLC Biotech, Bovenden, Germany).

For monitoring the progress of the epoxidation the whole reaction volume was used for analysis thus avoiding errors due to partitioning of the compounds in the different phases.

The reaction yield was defined as the maximum concentration of product obtained during the course of the reaction (C_{max}) compared to the initial concentration of the starting material in percent.

$$\text{Yield} (\%) = \frac{C_{max} \text{ epoxide}}{C_{initial} \text{ allyl ether}} \times 100.$$

Conversion of TMPME was calculated as the percent decrease in the allyl ether concentration ($C_{initial}$ allyl ether $- C_{min}$ allyl ether) as compared to its initial concentration at the start of the reaction.

$$\text{Conversion} (\%) = \frac{C_{initial} \text{ allyl ether} - C_{min} \text{ allyl ether}}{C_{initial} \text{ allyl ether}} \times 100.$$

Reaction rates were calculated as the molar change in concentration per hour using 50 mg of enzyme preparation and 2 ml of reaction volume.

$$\text{Rate} (M/h) = \frac{C_{t_2} - C_{t_1}}{\text{time}}.$$

2.2.2. Analysis by gas chromatography

Gas chromatography (GC) was used to monitor the conversion of TMPME to the glycidyl ether, using a Shimadzu model GC-14A (Kyoto, Japan) instrument equipped with a flame ionisation detector and a fused silica capillary column from Supelco (SPB™-5, 15 m × 0.32 mm × 0.25 μm film thickness). The sample injection volume was 1 μl. The oven was programmed to start holding at 80 °C for 2 min, thereafter increasing the temperature to 250 °C in 8.5 min and holding at this temperature for 5 min. The retention times of the analytes were: 3.5 min for caprylic acid, 4.9 min for TMPME, 6.4 min for the glycidyl ether, and 6.6 min for hexadecane (internal standard). Injector and detector temperatures were set at 275 °C.

The peak identities in the gas chromatogram were confirmed by GC–MS spectra on a Thermo Finnigan Trace GC122000 series (Thermo Electron Corporation, MA) using a HP-5MS column (30 m × 0.25 mm × 0.5 μm film thickness).

To establish the GC response factor of the glycidyl ether, oxirane content was also measured potentiometrically by titration according to Jay [16].

2.2.3. Enzymatic formation of peracid in the absence of allyl ether

The reaction was performed in the same way as described in Section 2.2.1, but without the addition of TMPME. Aliquots were withdrawn from the organic phase after letting the liquid

stand to allow phase separation. The concentration of peracid over time was measured by titration of 100 μl of sample, diluted in 10 ml of acetic acid/isopropylalcohol (3/2) containing 1 ml of saturated potassium iodide, with 0.01 M sodium thiosulphate [17].

Hydrogen peroxide was found to be only marginally dissolved in the organic phase and hence did not have an effect on the analysis of the peracid.

2.2.4. Elucidation of by-products by mass spectrometry

The side-products formed during the epoxidation reaction were analyzed by nanospray mass spectrometry on an API QSTAR® Pulsar quadrupole TOF mass spectrometer (MDS, Ontario, Canada), equipped with a turbo-ion spray ion source. Aliquots were diluted 1:1 with acetonitrile. MS and MS/MS spectra were recorded in the mass range of m/z 50–700 using positive ionisation mode. Argon was used as the collision gas.

3. Results and discussion

3.1. Choice of acyl donor

Chemo-enzymatic epoxidation of TMPME was investigated using toluene as the reaction medium, based on the earlier reports with other substrates showing the reaction performance to be superior in aromatic solvents [13]. Preliminary experiments were done using different carboxylic acids (C8, C10, C12 and C16) for peracid generation. Though similar results were obtained with either of these acids at concentration of 0.3 M, caprylic acid (C8) provided the best reaction conditions because of its high solubility in toluene allowing it to be used at a wider range of concentrations and temperatures as compared to the longer chain acids which are insoluble at higher concentrations (~0.3 M) at room temperature.

As done in other reports, epoxidation was also studied using ethyl acetate and dimethyl carbonate, respectively, as acyl donors (in place of carboxylic acids) with the idea of facilitating purification of the product downstream of the synthesis as well as minimising the acid related epoxide degradation [18]. However, ethyl acetate resulted in slow epoxidation rates, while no conversion could be seen using dimethyl carbonate. Based on these observations, caprylic acid was used for further studies.

3.2. Effect of acid concentration

Caprylic acid concentration was varied between 0.3 and 5.2 M for the chemo-enzymatic epoxidation of 1 M TMPME at 40 °C. The conversion of TMPME to the glycidyl ether was followed using GC-FID. As shown in Fig. 1, the reaction rate and TMPME conversion to the glycidyl ether was highly dependent on the acid concentration, with a significant increase being observed up to 3 M caprylic acid. The conversion of TMPME went hand in hand with epoxide formation up to an acid concentration of 1.5 M after which the relative amount of product formed decreased. At caprylic acid concentrations higher than 3 M, the product yield was in fact decreased while there was only a slight increase in the reaction rate and conversion. The decrease
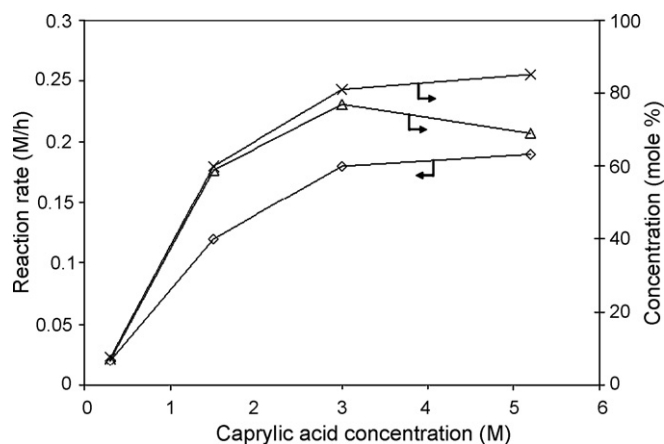

Fig. 1. Effect of caprylic acid concentration on initial rate of epoxide formation (◇), final substrate conversion (×) and product yield (△) in the chemo-enzymatic epoxidation of 2 ml of 1 M TMPME using 250 μl of 30% (w/w) hydrogen peroxide solution (20% molar excess compared to TMPME) and 50 mg Novozym®435 at 40 °C.

in product yield was attributed to increased acid related degradation of the product. Maximal TMPME conversion of 85% was achieved at 5.2 M caprylic acid, while the highest product yield was 77% at 3 M caprylic acid, corresponding to a productivity of 0.1 mol glycidyl ether/h/L.

3.3. Effect of hydrogen peroxide concentration and temperature

Using 3 M caprylic acid, the effect of temperature and hydrogen peroxide concentration on the epoxidation of TMPME was investigated. These factors were studied together as they were found in our preliminary studies to exert a synergistic influence on the reaction. Temperature was varied between 30 °C and 50 °C, and hydrogen peroxide concentration was 20–50% (w/w). As expected, TMPME conversion rate was increased with increase in temperature and H_2O_2 concentration (Fig. 2). How-
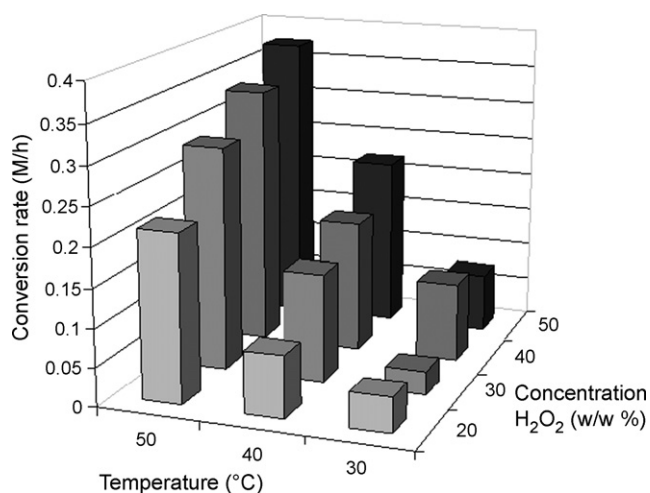

Fig. 2. Effect of temperature and hydrogen peroxide concentration on the rate of conversion of TMPME in the chemo-enzymatic epoxidation process. The reaction mixture contained 2 ml of 1 M TMPME and 3 M caprylic acid to which H_2O_2 was added in 20% molar excess (compared to TMPME).
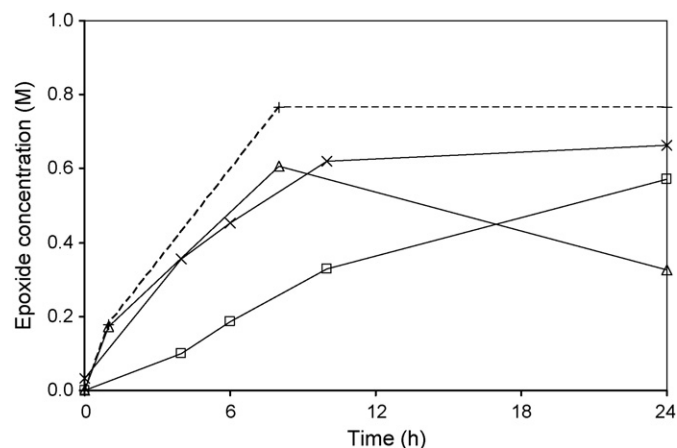
Fig. 3. Comparison of the chemo-enzymatic epoxidation of 2 ml of 1 M TMPME using as source of oxygen, 50% (w/w) aqueous hydrogen peroxide at 40 °C (+), and urea–hydrogen peroxide at 30 °C (□), 40 °C (×) and 50 °C (△), respectively. Caprylic acid at a concentration of 3 M was used for peracid generation and the hydrogen peroxide was added in a 20% molar excess.

ever, the product yield was drastically reduced at 50 °C, possibly due to product degradation and/or occurrence of side reactions leading to by-product formation. Increasing the concentration of H_2O_2 from 20% (w/w) to 50% (w/w) had the opposite effect and was seen to reduce the extent of product degradation. A temperature of 40 °C seemed to give the best balance between reaction rates and stability of the epoxide product; a yield of 77% was achieved in less than 8 h using 50% (w/w) hydrogen peroxide (Fig. 3).

Possible routes of by-product formation could be the lipase catalysed esterification between the alcohol groups on the allyl ether and caprylic acid or ring opening of the epoxide group and subsequent reactions. The former route was ruled out since no ester product was formed by running the reaction in the presence of pure water instead of hydrogen peroxide. Furthermore, nanospray-MS analysis of the by-products indicated the presence of a diol (m/z 209) and a dimer (m/z 399), suggesting ring opening to be the predominant side reaction taking place. Acid catalysed hydrolysis of the epoxide in aqueous solution is the most probable cause of ring opening reactions [20].

Using urea–hydrogen peroxide complex instead of aqueous hydrogen peroxide has been suggested to eliminate degradation related to water [21], and was thus evaluated for TMPME epoxidation at 30–50 °C. The results were compared to the best results achieved using aqueous hydrogen peroxide. As shown in Fig. 3, using urea–hydrogen peroxide did not improve the epoxidation, and as in the case of aqueous hydrogen peroxide significant degradation of the product could be seen at 50 °C.

3.4. Impact of peracid formation on the epoxidation reaction

Chemo-enzymatic epoxidation is typically believed to occur according to Scheme 1, where the peracid formed is continuously consumed without a substantial build up in peracid concentration [9]. Allyl ethers, owing to the vicinity of the ether oxygen, are considerably less prone to epoxidation than
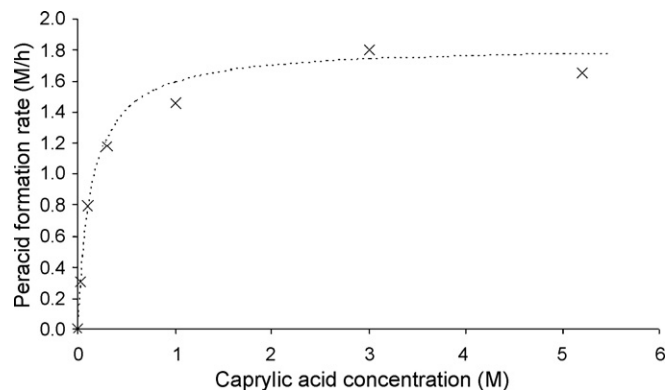

Fig. 4. Initial rate of formation of peracid using 2 ml of caprylic acid solution at different concentrations, 250 μl of 30% (w/w) hydrogen peroxide and 50 mg Novozym®435 at 40 °C. The dotted line shows the Michaelis–Menten equation with a K_m and V_{max} of 0.14 M and 1.8 M/h, respectively. K_m and V_{max} were obtained using a Lineweaver–Burk plot.

other unsaturated compounds. It was thus considered essential to investigate the enzyme catalysed peracid formation separately from the subsequent epoxidation step (i.e. by performing the lipase-catalysed peracid formation in the absence of the allyl ether).

Peracid formation is a reversible two-substrate reaction reaching an equilibrium determined by the following equation [22]:

$$K = \frac{[\text{peracid}][\text{water}]}{[\text{acid}][\text{H}_2\text{O}_2]} \tag{1}$$

While the rate of peracid formation did not considerably increase above 0.3 M caprylic acid as seen in Fig. 4, equilibrium peracid concentration was found to increase up to 0.7 M with increase in caprylic acid concentration to 3 M in the absence of TMPME (see Fig. 5). Due to the high reaction rate in the peroxidation reaction maximal peracid concentrations were reached already in 30–60 min. The amount of peracid produced in the peroxidation reaction was then correlated to its utilization in the corresponding chemo-enzymatic epoxidation reaction. Inter-
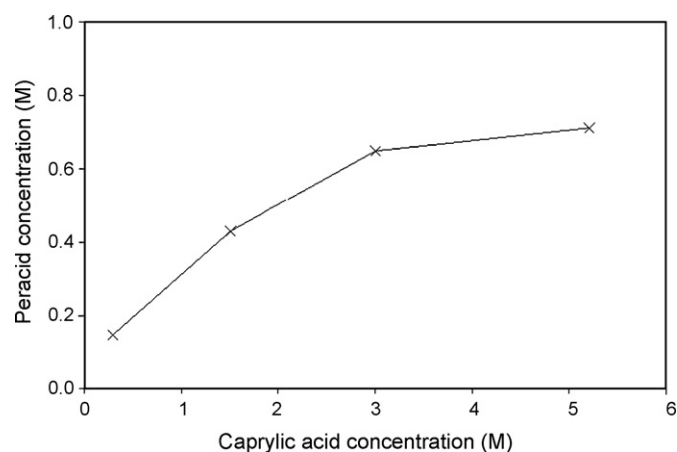

Fig. 5. Maximum concentration of peracid obtained using 2 ml of caprylic acid solution at different concentrations, 150 μl of 50% (w/w) hydrogen peroxide and 50 mg Novozym®435 at 40 °C.
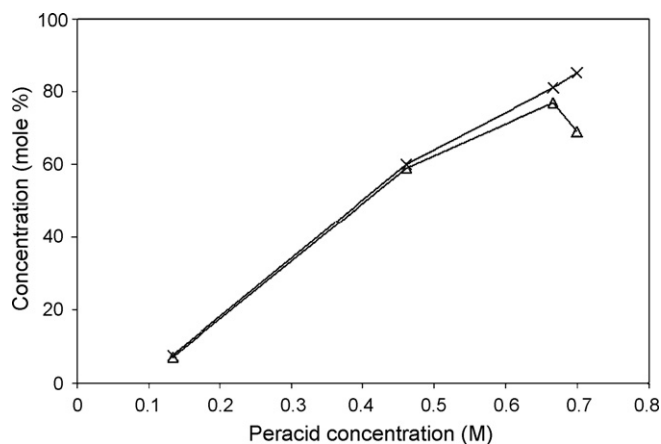
Fig. 6. Effect of maximum peracid concentration on final TMPME conversion ($\times$) and glycidyl ether yield ($\triangle$) during chemo-enzymatic epoxidation of 1 M TMPME using 50% (w/w) aqueous H_2O_2 at 40 °C. The caprylic acid concentrations corresponding to the respective peracid concentrations can be seen in Fig. 5.

estingly, it was found that the amount of TMPME converted was practically identical to the amount of peracid produced in the absence of TMPME. The same was true for the amount of epoxide produced, except at the highest peracid concentration (obtained at an acid concentration of 5.2 M), where the epoxide yield was decreased due to acid related degradation as discussed in Section 3.2 (Fig. 6).

The equilibrium in Eq. (1) would be favoured towards peracid formation as the peracid gets utilized during epoxidation reaction. However, since epoxidation of the allyl ether is relatively slow (as indicated above), the continued formation of peracid is severely affected. Furthermore, the prolonged exposure of the lipase to the oxidizing reaction conditions results in its loss of activity and hence its capacity to catalyse the formation of the peracid. Investigations on lipase stability during chemo-enzymatic epoxidation performed in our laboratory have shown that the enzyme is totally deactivated in 5 h on exposure to 35% hydrogen peroxide at 40 °C [19].
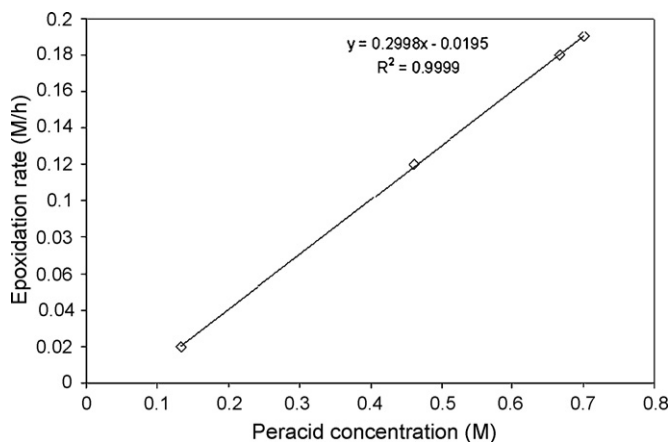

Fig. 7. Effect of maximum peracid concentration on initial rate of epoxidation of 1 M TMPME using 50% (w/w) aqueous H_2O_2 at 40 °C. The caprylic acid concentrations corresponding to the respective peracid concentrations can be seen in Fig. 5.

It thus seems that peracid is in fact first accumulated before being consumed through epoxidation of the allyl ether double bond, making it more like two consecutive reactions than two simultaneous reactions. A plot of the epoxidation rate against the amount of peracid produced in the peroxidation reaction showed a linear relationship (Fig. 7), confirming that peracid concentration is the important parameter for epoxidation of an allyl ether.

4. Conclusion

This paper demonstrates the chemo-enzymatic epoxidation of an allyl ether to be a possible alternative route for the production of a corresponding glycidyl ether. However, the allyl ethers with a terminal double bond in proximity to an ether bond are much less reactive as compared to other raw materials with internal double bonds that have been used in earlier reports for chemo-enzymatic epoxidation. This was evident in the requirement of a much higher concentration of carboxylic acid (and thereby peracid) for enabling efficient epoxidation of the allyl ether double bond. Maximum product yield of 77% was obtained from 1 M TMPME at 40 °C and 20% molar excess of 50% (w/w) H_2O_2. Higher temperature resulted in by-product formation as a result of ring opening of the epoxide.

Although this study shows that good yields of the glycidyl ether can be obtained under relatively moderate reaction conditions, a major limitation for the industrial implementation of the chemo-enzymatic epoxidation process would be the development of a lipase preparation with good operational stability at high concentration of hydrogen peroxide used for epoxidation.

References

[1] H.Q. Pham, M.J. Marks, Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley, Hoboken, NJ, 2004.
[2] S.-J. Park, T.-J. Kim, J.-R. Lee, J. Polym. Sci., Part B: Polym. Phys. 38 (2000) 2114–2123.
[3] M. McCoy, Chem. Eng. News 84 (2006).
[4] A.K. Giri, Mutat. Res. 386 (1997) 25–38.
[5] D. Swern, JACS 69 (1947) 1692–1698.
[6] R.L. Musante, R.J. Grau, M.A. Baltanas, Appl. Catal. A 197 (2000) 165–173.
[7] F. Björkling, S.E. Godtfredsen, O. Kirk, J. Chem. Soc., Chem. Commun. (1990) 1301–1303.
[8] S. Warwel, M.R.G. Klaas, J. Mol. Catal. B: Enzym. 1 (1995) 29–35.
[9] G.D. Yadav, I.V. Borkar, AIChE J. 52 (2006) 1235–1247.
[10] M.A. Moreira, T.B. Bitencourt, M.D.G. Nascimento, Synth. Commun. 35 (2005) 2107–2114.
[11] M.R.G. Klaas, S. Warwel, J. Mol. Catal. A: Chem. 117 (1997) 311–319.
[12] V. Skouridou, H. Stamatis, F.N. Kolisis, J. Mol. Catal. B: Enzym. 21 (2003) 67–69.

[13] R.R. Sienel, R. Rieth, Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH Verlag GmbH, Weinheim, Germany, 2007, pp. 534–536.

[14] Höllriegelskreuth, Epoxidation (1981) 11–17, Chapter 6.

[15] W. Sandersson, Pure Appl. Chem. 72 (2000) 1289–1304.

[16] R.R. Jay, Anal. Chem. 36 (1964) 667–678.

[17] Metrohm-Bulletin, Determination of the peroxide number.

[18] M.R.G. Klaas, S. Warwel, Org. Lett. 1 (1999) 1025–1026.

[19] U. Törnvall, C. Orellana-Coca, R. Hatti-Kaul, D. Adlercreutz, Enzyme Microb. Technol. 40 (2007) 447–451.

[20] T.W.G. Solomons, Organic Chemistry, 6th ed., John Wiley, Hoboken, NJ, 1996.

[21] E.G. Ankudey, H.F. Olivio, T.L. Peeples, Green Chem. 8 (2006) 923–926.

[22] I. Hilker, D. Bothe, J. Pruss, H.-J. Warnecke, Chem. Eng. Sci. 56 (2001) 427–432.