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BIOFORMATION OF OPTICALLY PURE EPOXIDES

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Abstract: Optically pure epoxides are valuable building blocks in organic synthesis which may be produced either chemically or biologically. In this review an overview is presented on the various biological methods available and some strong and weak points of these systems are considered.

Epoxides are versatile intermediates in organic synthesis because they are reactive molecules. Their ring may easily be opened by a variety of nucleophiles[1] yielding a broad range of valuable products. Several simple epoxides as for instance ethylene oxide and racemic propylene oxide have a long history as bulk chemicals while more complex, optically pure epoxides are attracting more and more attention as high-value chiral intermediates. Optically pure propylene oxides (1,2-epoxypropanes), glycidols and styrene oxides are examples of compounds now commercially available as chiral building blocks.

In recent years, a great deal of research energy has been devoted to the development of catalytic methods in the production of optically pure epoxides. The Katsuki-Sharpless method[2] reported in 1980 is a very well known catalytic procedure for the asymmetric epoxidation of allylic alcohols which still inspires to further research[3]. An interesting new development from the last years is metal-catalyzed asymmetric epoxidation of unfunctionalized olefins[4,5]. These chemical methods will not be considered here. They have been dealt with very recently in a special volume of Tetrahedron Asymmetry[6].

Biocatalysis in the production of optically pure epoxides has also been studied in recent years, but with rather limited research efforts. Not only in comparison with the research input in catalytic asymmetric synthesis of epoxides, but also when compared with several other biocatalytic processes. The interest in the biocatalytic production of epoxides most likely will increase in the coming years not only

because of the growing importance of epoxides as chiral building blocks, but also because many biological systems are now at hand in the production of these compounds.

In this article an overview is presented over the various biological methods available and some strong and weak points of each system will be considered. Other short reviews on biological epoxide formation have appeared[7,8] and it will not be attempted here to cover all aspects in detail. Omissions in this review are amongst others a discussion of eukaryotic systems, while lipase-catalyzed reactions are discussed only very briefly.

Biosynthesis or biotransformation ?

Microbial reactions leading to valuable products are conveniently divided into *de novo* biosynthetic reactions and into biotransformation reactions. Compounds ordinarily produced by organisms to sustain their metabolic functioning (primary metabolites) as well as more complex molecules not having a direct role in the central metabolism of organisms (secondary metabolites) may be formed and excreted by microorganisms in a series of reactions from simple, readily available carbon sources. Some of the more successful biotechnological processes rely on these biosynthetic routes as for instance the production of amino acids or antibiotics. However, epoxides usually are not natural intermediates in biodegradative or biosynthetic routes. Only in isolated cases, when organisms grow at the expense of alkenes, may epoxides show up as metabolites. And even in these cases the metabolism leading from an alkene or aromatic compound to the epoxide involves only one single enzyme-catalyzed step. Consequently, epoxide formation in all cases fully relies on biotransformation reactions. Such reactions require preformed substrate molecules to allow a single catalytic reaction step in the production of the required optically pure epoxide. As will be seen later, several preformed molecules are candidates as starting materials for biocatalytic epoxide production e.g. alkenes, haloketones, racemic halohydrins, or racemic epoxides. Depending on the type of reaction under consideration, either whole cells or purified enzymes are to be used.

Lipases in the production of optically pure epoxides

Lipase-catalyzed reactions are relatively familiar to the organic chemist since it is not necessary to deal with a living organism. The biocatalyst is mostly commercially available and the reaction conditions as well as the subsequent product isolation procedures fit well in the routine of a chemical laboratory.

Lipases are used in the resolution of racemic esters of epoxyalcohols[9] to produce both forms of the epoxyalcohol. The procedures are straight forward and several companies carry out optimized processes in the production of optically pure (R)- and (S)-glycidol[10,11].

Similarly, lipases have been applied in the asymmetric hydrolysis of racemic halohydrin esters[12] e.g. in the production of optically pure styrene oxides.

Lipases may also be successfully used as a transesterification catalyst especially when vinyl or

isopropenyl esters are employed as the acylating agent[13]. Using this procedure it for instance was also possible to obtain optically pure 2-chloro-1-phenylethanol (97% ee) and hence optically pure styrene oxide.

Surprisingly, lipase also generated peracids from an acid and hydrogen peroxide. The peracid thus obtained was used in a cyclic process in the formation of an epoxide from an alkene[14]. The process, however, is not likely to result in optically pure epoxides.

Asymmetric reduction of haloketones by whole cells

Microbial reduction of haloketones has been reported for only a very limited number of substrates and organisms. The asymmetric reduction of bromo- and chloroacetophenone resulted in (R)-2-halohydrins from which optically pure styrene oxide (95% ee) was formed[15]. Recently, we studied the microbial reduction of chloroacetone into chiral 1-chloro-2-propanol in order to produce optically pure 1,2-epoxypropanes[16]. It was observed that optically pure (S)-1,2-epoxypropane could be formed by using different organisms, including a lactic acid bacterium and two yeasts. This method to obtain pure (S)-1,2-epoxypropane is relatively simple since the whole cells used were not very susceptible to the halohydrin produced.

Enantioselective dehalogenation of halohydrins

Optically pure halohydrins may also be obtained enzymatically by kinetic resolution of racemic halohydrins. As opposed to the previous method, now subterminal halogen compounds should serve as starting materials. But the information on dehalogenases acting on halohydrins is limited. Already in 1969 an enzyme from a *Flavobacterium* sp. was described[17] to be involved in the dehalogenation of halohydrins and resulting in epoxide formation. The enzyme later on was advocated by Cetus Corporation[18] as a useful biocatalyst in combination with haloperoxidases for the production of epoxides[19]. Advantages of the process would be the use of halide ion rather than free halogen and a recycling of the halide ion was anticipated. However, the process apparently yielded racemic epoxides. Dehalogenases reacting with halohydrins and forming epoxides in crude extracts were detected in a *Pseudomonas* sp. and in an *Arthrobacter* sp.[20]. The enzyme from the *Arthrobacter* sp. was later purified and characterized[21]. It dehalogenated various halohydrins including 3-chloro-1,2-propanediol and 1,3-dichloro-2-propanol resulting in glycidol and epichlorohydrin, respectively. No information was given on the enantioselectivity of the reaction with 3-chloro-1,2-propanediol. Very recently a *Pseudomonas* sp. was described growing at the expense of racemic 2,3-dichloro-1-propanol[22]. Various halohydrins were dehalogenated to give diols. Interestingly, the enzyme was enantioselective and Kasai *et al*[23] were able to use immobilized cells containing the enzyme for the production of optically pure (R)-epichlorohydrin (99.3% ee) with (S)-2,3-dichloro-1-propanol remaining (100% ee) from the racemate. The process was very stable since it was possible to run the reaction for 50 days without an appreciable loss of activity.

Optically pure halohydrin from a prochiral substrate

The above described method of enantioselective dehalogenation of halohydrins gives a yield of 50% or less of the optically pure compound based on the amount of starting material. In the special case of prochiral 1,3-dichloro-2-propanol it is theoretically possible to obtain a yield of 100 % optically pure 3-chloro-1,2-propanediol and hence optically pure glycidol. This method has been studied in detail[24,25] and a maximal optical purity of 3-chloro-1,2-propanediol of 74.8 % ee was obtained. The enzyme described by the Japanese group was similar to the dehalogenase described by the Dutch researchers[21].

Direct epoxidation of alkenes

Various attempts have been undertaken to use whole cells in the bioconversion of alkenes into epoxides. These attempts wisely have been generally restricted to alkenes not having other functional groups. Whole cells are likely to also attack such functions resulting in undefined product mixtures. In some organisms, even the simple alkenes may not only be epoxidized but also be hydroxylated to the corresponding alcohol. Direct epoxidation of alkenes is restricted to whole cells since cofactor regeneration is required[26].

It has been clear for a long time that microbial processes would not be competitive with chemical methods if racemic epoxides are produced[27]. Interestingly, it has been observed that the enantiomeric excess of a particular epoxide produced is very much dependent on the organism employed. For instance several strains have been screened for the optical purity of 1,2-epoxypropane produced from propene[28-30]. Some organisms produced the S-enantiomer in excess, others gave the racemic epoxide, while one strain gave almost pure (R)-1,2-epoxypropane (Table 1). Obviously, an initial screening program is of utmost importance in selecting a suitable epoxide-producing strain.

Table 1. Enantiomeric composition of 1,2-epoxypropane produced by various bacteria.

Bacterial strain	% (R)-1,2-epoxypropane
Nitrosomonas europaea ATCC 19178[28]	32
Pseudomonas sp. P9y[28]	45
Methylosinus trichosporium OB3b[28]	54
Mycobacterium sp. P2y[28]	78
Mycobacterium sp. E20[29]	88
Mycobacterium sp. E3 [29]	93
Aerococcus sp. M186[30]	97
Mycobacterium sp. L1[28]	99

Unfortunately, a strain will not do equally well in the production of several optically pure epoxides. To illustrate this point, three organisms are shown in Table 2 which are known to transform various alkenes to the corresponding epoxides. An optical purity of 98% may be satisfactory for an application but a purity of 90 % is generally not of great use. Consequently, various organisms would have to be used as biocatalysts for the production of the respective epoxides. Economically, this is a serious drawback because it requires much research and subsequent technological efforts to develop an appropriate organism for each product. Production processes therefore never will be very flexible in switching from one epoxide to another.

To appreciate the research efforts needed in understanding the epoxide-producing bacteria and their mono-oxygenases one may consult articles on methane-oxidizing bacteria[38], on *Pseudomonas oleovorans*[39,40], on *Nocardia corallina*[35] and on alkene-utilizing bacteria[37,41] and references given in those papers.

Other problems also arise in the production of optically pure epoxides by direct epoxidation of alkenes. Especially when scaling up the system, product toxicity and product isolation deserve attention. Epoxides are of course not only reactive in organic chemistry but also in biological systems where they inhibit the producing organisms. Consequently, only low concentrations (10-100 mM) of the epoxide can be accumulated in the aqueous environment[42]. Furthermore, product isolation causes problems. Both of these difficulties have been dealt with in detail by using organic solvents as a second phase not only in the production of lower epoxides[43], but also in the production of epoxides from long chain alkenes[35,44].

Table 2. Enantiomeric composition of various epoxides produced by three bacteria.

Bacterial strain	epoxide produced	optical purity
Mycobacterium sp. L1	1,2-epoxypropane[28]	99% R
	1-chloro-2,3-epoxypropane[28]	98% S
	1,2-epoxybutane[28]	90% R
	trans-2,3-epoxybutane[28]	87% R,R
Pseudomonas oleovorans	3-(4-methoxyphenoxy)-1,2-epoxypropane[31]	96% R
	7,8-epoxy-1-octene[32]	92% R
	1,2-epoxyoctane[33]	85% R
	1,2-epoxydecane[34]	80% R
	3-butoxy-1,2-epoxypropane[31]	70% R
Nocardia corallina B-276	1,2-epoxytridecane[35]	96% R
	1,2-epoxydecane[35]	93% R
	1,2-epoxy-2-methylpentane[36]	88% R
	styrene oxide[35]	85% R
	1,2-epoxyhexane[35]	83% R

Enantioselective degradation of epoxides

Another biotransformation method to obtain optically pure epoxides is using whole cells or enzymes that degrade epoxides stereoselectively. Such a procedure at best has a yield of 50% but may nevertheless be interesting since racemic epoxides are relatively cheap.

The enantioselective hydrolysis of epoxyalkanes by liver epoxide hydrolase and glutathion S-transferase have been studied by Wistuba and Schurig[45] and several aspects of these enzymes, including regio- and stereoselectivity towards epoxides, have been described[46-49]. Epoxide hydrolases from liver in biotransformation reactions have been dealt with recently[50]. Very recently it was also shown liver microsomes may be used in the stereoselective ring opening of epoxides with amines[51], and quite remarkably, also lipase catalyzed the selective ring opening of epoxides with 2-propylamine[52].

Microbial enantioselective degradation of epoxides has received only very limited attention. Most likely because epoxides are not usual intermediates in bacterial metabolism. An epoxide hydrolase from a *Pseudomonas* sp. was characterized by Jacobs *et al.*[53]. The enzyme did not require cofactors and catalyzed the hydrolysis of several epoxides, including epichlorohydrin, propylene oxide, and also styrene oxide. The enzyme, unlike mammalian epoxide hydrolase, unfortunately did not show stereoselective conversion of the chiral substrates.

We have studied bacterial metabolism of epoxides in several organisms isolated on various substrates. A *Xanthobacter* sp. isolated on styrene degraded styrene oxide not by a hydrolase as observed by Jacobs *et al*[53], but by an isomerization of the epoxide to phenylacetaldehyde[54]. The range of epoxides degraded

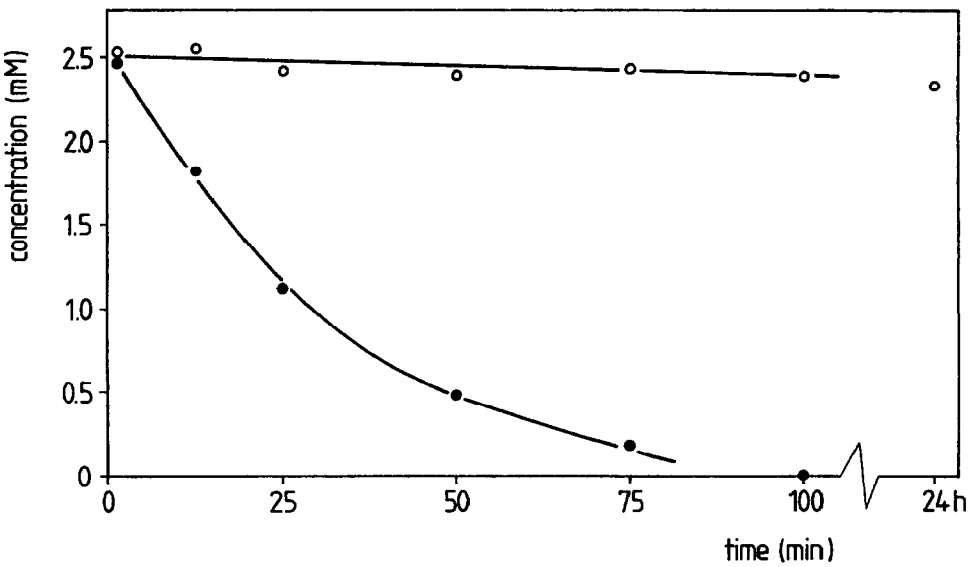

Fig. 1 Enantioselective degradation of racemic *trans*-epoxybutane by *Xanthobacter* Py2. (2S,3S)-Epoxybutane (●); remaining optically pure (2R,3R)-epoxybutane (O).

by this enzyme was very narrow, but it degraded both isomers of styrene oxide. Many other bacteria were isolated on alkenes or on epoxides[55,56]. Surprisingly, no epoxide-degrading enzymes resembling mammalian enzymes were detected. The most interesting organism eventually chosen for further studies was *Xanthobacter* Py2[55]. The organism was isolated on propene and was able to degrade several 1,2- and 2,3-epoxyalkanes. The enantioselectivity of the epoxide-degrading capacity of the organism was tested in whole cells since it was difficult to obtain any enzyme activity in extracts. The organism degraded both enantiomers of 1,2-epoxyalkanes tested but interestingly kinetic resolution was obtained with 2,3-epoxyalkanes. Only (2S)-forms were metabolized by the bacterium, resulting in pure (2R)-2,3-epoxyalkanes (Fig. 1).

Presently, we are studying the enzyme responsible for this reaction. It has been assessed the enzyme requires a so far unknown cofactor for activity (C.A.G.M. Weijers and J. Swaving, personal communication).

Enantioselectivity in 1,2-epoxyalkane degradation was detected in a few cases only[56]. In a specific example it was possible to obtain optically pure (R)-epichlorohydrin (>98 % ee) from a racemic mixture, but the yield was only 19 %.

Concluding remarks

Many chemical and biological approaches are available in the preparation of epoxides in high enantiomeric purity. The procedures of choice for preparative purposes obviously will depend very much on the compound under consideration and also on the expertise available in a research group.

Similarly, it is generally also not clear which method should be followed when larger quantities of an epoxide are to be produced. No general, leading method has evolved so far, possibly because no real and competitive market has been developed for optically pure epoxides. Presently, 1,2-epoxypropane, glycidol and styrene oxide are available from many companies, but it appears that the quantities needed of these products are rather limited. Fluka Chemie AG for instance produces epoxypropanes chemically, glycidols via the Sharpless method, and styrene oxides using a lipase acting on racemic chloroacetyl-2-chloro-1-phenylethanol (W. Ganci, personal communication). This choice for the Sharpless method is striking in view of the claims by others on lipase-catalyzed processes for glycidols[10,11]. Equally striking is the use of lipases in the production of styrene oxides since chemical alternatives involving catalytic reduction of chloromethylketone[57,58] or conversion of an amine into a halide[59] are available. The use of whole cells in the production of optically pure epoxides on a commercial scale has till now only been reported by Nippon Mining Co. which sells different 1,2-epoxyalkanes produced by *Nocardia corallina*[35]. Other companies have had similar interests, but apparently have not commercialized their processes.

In the long run, if one or more optically pure epoxides would be required as bulk product, it seems whole cells would stand a good chance to be used in production processes. Consider for example styrene oxide as a product to be produced on a large scale. Chemical methods available are metal-catalyzed

asymmetric epoxidation[4,60], procedures involving halohydrins[57-59] and kinetic resolution[61,62]. Enzymatic routes known do start from halohydrins and involve lipases[12,13] or reductases[15]. Therefore, apart from the presently problematic metal-catalyzed reactions, all methods involve halohydrins. As elegantly described by Sheldon[63], catalytic and salt-free technologies should be applied in the long run. The classical chlorohydrin route in the production of ethylene oxide in his opinion should be regarded as a process to manufacture $CaCl_2$ with ethylene oxide as a by-product. Such considerations clearly point at the importance of organisms directly converting styrene into optically pure styrene oxide in one single step. Undoubtly it is possible to find organisms producing either 100% (R)- or (S)-styrene oxide (S. Hartmans, personal communication). In principle it should then be possible to develop a multiphase production process according to the lines described by Furuhashi[35], and by Witholt[44] who estimated that the price of such type of chemicals produced on a large scale should be in the range of 3-10 $ per kilogram. Such type of process now based on the presence of an organic phase might become even more attractive since we recently obtained styrene-degrading organisms growing in a two phase water-styrene medium (F. Weber, personel communication). A second organic phase serving as a substrate/product reservoir would eventually not be required any more provided the product would easily be separable from the substrate.

A weak point of a monooxygenase-catalyzed reaction remains the very restricted range of optically pure epoxides produced by a single organism (Table 2). In this respect the application of enzymes degrading epoxides enantioselectively is still of interest since these enzymes seem to act on a homologous range of epoxides[55]. The scope for these enzymes will further expand if not only water but also other nucleophiles would be involved in ring opening. A remarkable example involving cyanide was presented recently by Nakamura *et al.*[64]. At present, however, knowledge on epoxide-degrading enzymes is too limited to assess their potential.

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