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Chemical and Biological Synthesis of Chiral Epoxides

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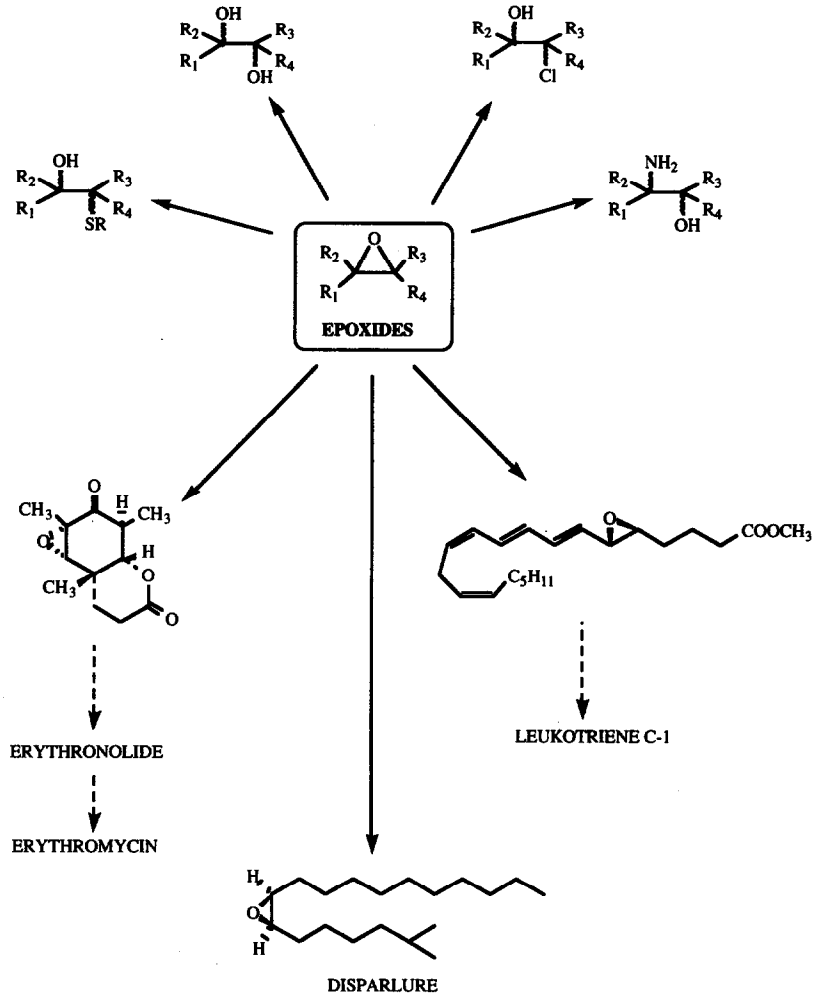
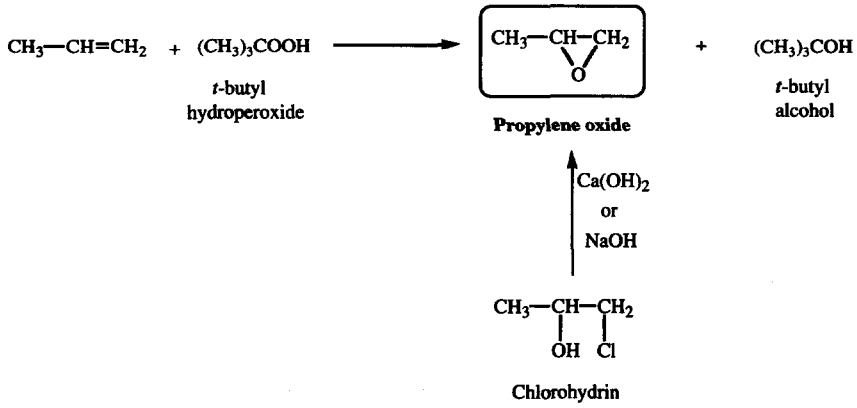
I - INTRODUCTION

The epoxide functional group is one of the most useful intermediates in organic synthesis. Epoxides are versatile products and they easily undergo stereospecific ring-opening reactions to form bifunctional compounds. This explains the great interest in the development of methods for the synthesis of optically pure epoxides. Epoxides are very important chiral buildings blocks in organic synthesis and can be used either as key intermediates in the preparation of more complex optically pure bioactive compounds such as leukotriene, erythromycin, GABOB (γ-amino-β-hydroxybutyric acid) or as end products which also have biological activities such as the gipsy moth pheromone : (+)-disparlure.



Two major processes, which correspond to the two classical methods for epoxide synthesis, are used industrially to produce propylene oxide : peroxidation of an alkene or reaction of a base on a chlorohydrin.



In the above two cases, the epoxide obtained is racemic. However today many papers emphasize the need to prepare epoxides with a high state of enantiomeric purity.

This paper deals with the main methods of synthetizing chiral epoxides. These methods fall into two categories :

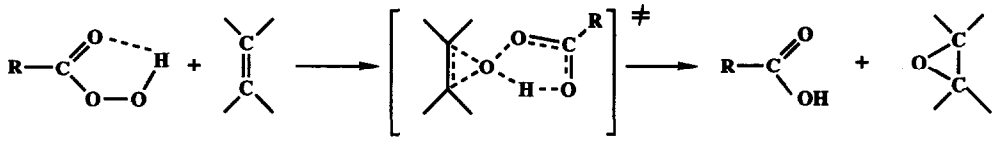
- Chemical synthesis of chiral epoxides
- Biological synthesis of chiral epoxides.

II - CHEMICAL SYNTHESIS OF CHIRAL EPOXIDES

1 EPOXIDATION OF OLEFINS WITH PERACIDS

1.1 Enantioselective epoxidation of alkenes

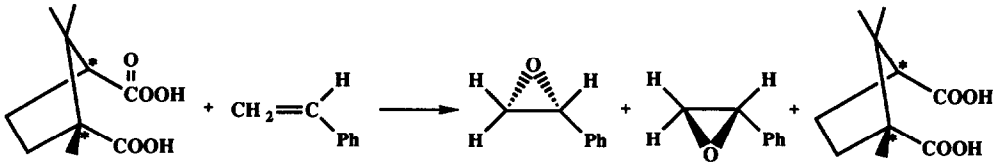
In 1909, PRILESCHAJEW[1] was the first to report epoxidation by organic peracids : the reaction involves the attack of the intramolecularly H-bonded peracid (the electrophilic reagent) on the olefin (the nucleophilic entity).



The yields were very low (about 10 %).

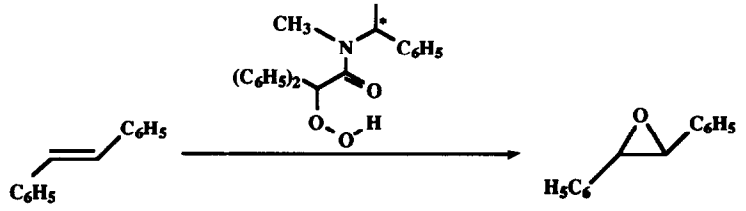
m-Chloroperbenzoic has been the peracid the most often used to epoxidize olefins. The addition of oxygen on the double bond is stereospecific[2], *i.e.* a *trans* olefin gives a *trans* epoxide and a *cis* olefin a *cis* epoxide. But as none of the reagents was chiral, the epoxides formed were racemic.

The first publications about asymmetric epoxidation reactions only appeared sixty years later. HENBEST[3,4] used a chiral reagent : the (+)-monoperoxycamphoric acid. Assays were carried out with various terminal olefins.



Whatever the alkene used, the enantiomeric excesses were never higher than 5%. PIRKLE and RINALDI[5] obtained, for the styrene oxide, a 9.2% enantiomeric excess by using a highly purified and freshly distilled (+)-monoperoxycamphoric acid. This low diastereoselectivity is due to the long distance between the inductive chiral center and the induced chiral center[6].

More recently, the use of complex optically active hydroperoxides, prepared by oxidation of 2,3-unsaturated glycosides with hydrogen peroxide[7] or by the reaction of peroxides such as H_2O_2 with isocyanates or nitriles[8], has given excellent yields (> 85%) of 1,2-epoxides and 2,3-epoxides but again with enantiomeric excesses not exceeding 35%.



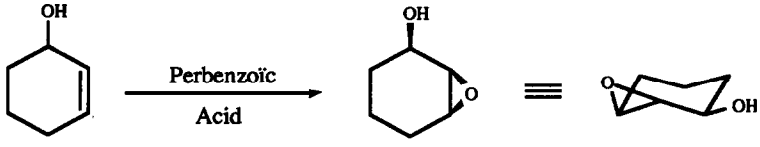
trans

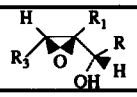
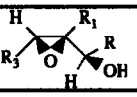
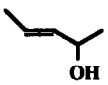
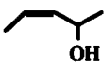
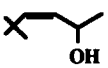
ee = 31 % ; Yield = 95 %

1.2 Enantioselective epoxidation of allylic alcohols

As asymmetric epoxidation from unfunctionalized alkenes with peracids does not give good results, a hydroxyl group is added to the α carbon atom of the double bond. This group participates in the reaction, regulating the stereochemical course of the reaction[9].

HENBEST and WILSON[10] were the first to establish that peracid epoxidation of allylic alcohols occurs principally *cis* to the alcohol. They showed that in the absence of steric hindrance, cyclohexenols are epoxidized stereoselectively by organic peracids (perbenzoïc acid was used) to preferentially yield *cis* epoxyalcohols. By testing different allylic alcohols, CHAUTEMPS and PIERRE[11] obtained the same results. However, for larger ring sizes (e.g. cyclooct-2-en-1-ol), epoxidation selectively gives *trans* epoxyalcohols[12].



Ethylenic alcohol		
	Epoxyalcohols obtained	
	% trans	*% cis*
	37	63
	10	90
	< 2	> 98
	5	95

1.3 Conclusion

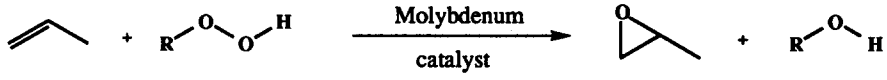
The use of organic peracids, as epoxidizing reagents, gives products with very low optical purity. Therefore it cannot be considered as a good method of synthesis.

2 EPOXIDATION CATALYZED BY METALS : SHARPLESS METHOD

2.1 Use of transition metals

The use of derivatives from transition metals (molybdenum, vanadium or titanium) as catalysts in the epoxidation reactions of alkenes in the presence of hydroperoxides dates from the sixties[13]. KOLLAR[14] described a process for the production of epoxides in the presence of soluble compounds of these metals.

This reaction was rapidly developed into a commercial process. 500 million lb of propylene oxide are produced annually in the world by the epoxidation of propylene catalyzed by a molybdenum compound with alkyl hydroperoxide according to the HALCON process[15]. The alkyl peroxide is available from the autooxidation of an appropriate hydrocarbon. The molybdenum increases the selectivity and accelerates the reaction[16].



Selective epoxidation of olefins with this kind of reagent (hydroperoxide / catalyst : group 5B or 6B transition metals) is characterized by an electrophilic addition of the activated oxygen to the less hindered side of the double bond.

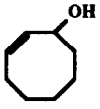
2.2 Precursors of the SHARPLESS reagent

The entity "transition metal-ligands" has been used for the epoxidation of allylic alcohols since 1975. While molybdenum is used for the epoxidation of alkenes, vanadium is preferred for that of allylic alcohols

because the reaction proceeds more quickly. Various kinds of ligands have been tested and shown to favour either a *cis* or *trans* conformation.

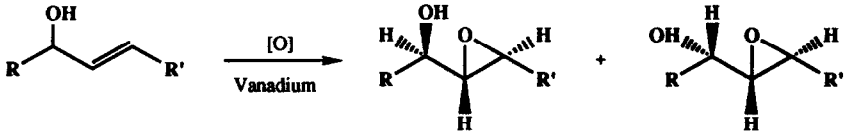
2.2.1 Cyclic allylic alcohols

One of the first systems used was [*t*-BuOOH-($VO(acac)_2$)] (acac = acetylacetonate CH_3-CO-CH = C (O-) CH_3). Many authors[17-21] have demonstrated that excellent isomeric purities (> 98 %) can be obtained with such a system. Indeed, in contrast to peracids[22], the hydroxyl group exerts a *cis* directive effect irrespective of ring size.

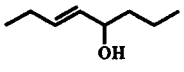
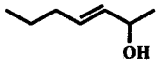
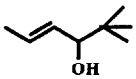
Substrate	Yield	Isomeric purity of epoxyalcohol (% *cis*)
Cyclohex-2-enol	89 %	> 99 %
trans-5-*t*-butyl-cyclohex-2-enol	92 %	> 99 %
cis-5-*t*-butyl-cyclohex-2-enol	9 %	> 99 %
	83 %	95 %

2.2.2 Acyclic allylic alcohols

The behavior of acyclic allylic alcohols parallels that observed for medium ring allylic alcohols. The results, presented in the table below, show that (contrary to peracid epoxidation[23,24]) the *cis* isomer is predominantly formed during epoxidation with *tert*-butyl hydroperoxide and the vanadium derivative.



Trans *Cis*

Olefin	$VO(acac)_2$ / *t*-BuOOH		MCPBA	
	cis	*trans*	*cis*	*trans*
	63	37	34	66
	64	36	30	70
	79	21	44	56

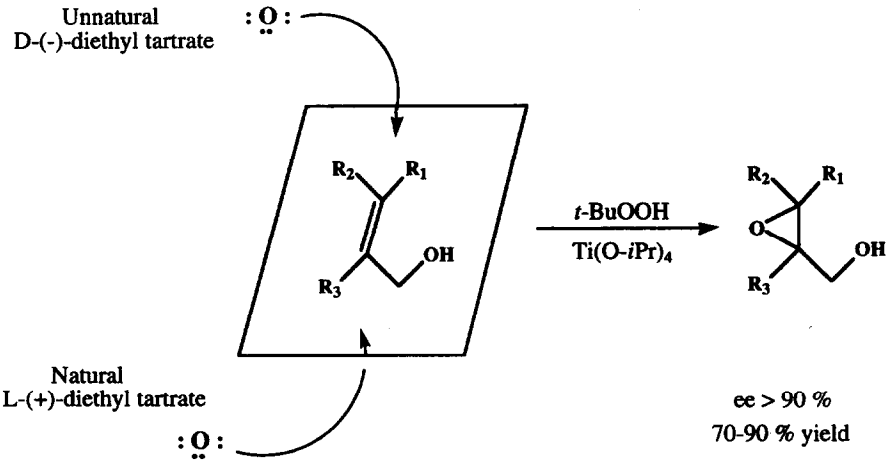
Yields of these reactions are not mentioned.

2.3 The SHARPLESS reaction

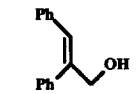
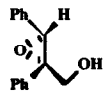
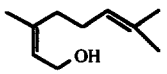
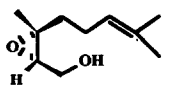
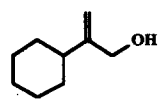
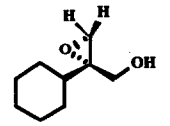
SHARPLESS described his goal as creating "man-made catalysts that are at least as good as, or probably even better than enzymes, very specific catalysts"[25]. His greatest contribution to progress was to exploit organometallic chemistry in which the central metal atom is coordinated with chiral ligands to guide and closely orientate the stereochemical course of the reaction. Since a metal atom has no intrinsic chirality, the key to catalytic chiral synthesis is the use of ligands that are chiral.

2.3.1 Mechanism

In 1980, KATSUKI and SHARPLESS[26] reported a new process of asymmetric epoxidation, catalyzed by a metal, from allylic alcohols. They used a system - known now as the SHARPLESS reagent - containing [(+) or (-)-diethyl tartrate, titanium tetraisopropoxide and *tert*-butyl hydroperoxide] in a non polar solvent such as dichloromethane. All these products are commercially available at a low to moderate cost. The direction of the attacks depends on the diethyl tartrate used in the reaction. Therefore the stereochemistry of the epoxidation can be correctly predicted.

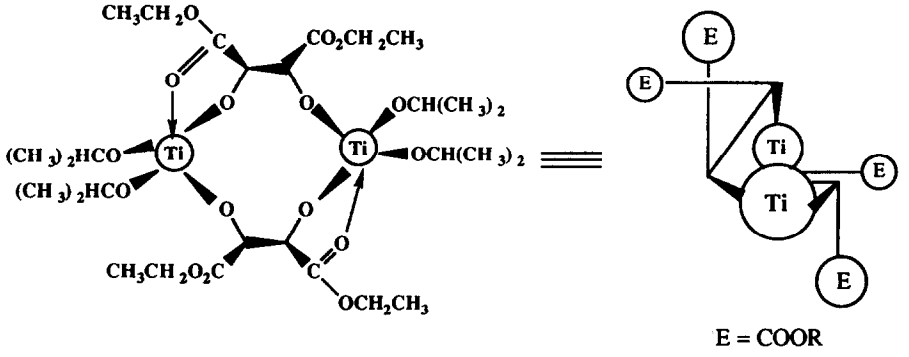


The table below gives some examples of the direct applications of this reaction.

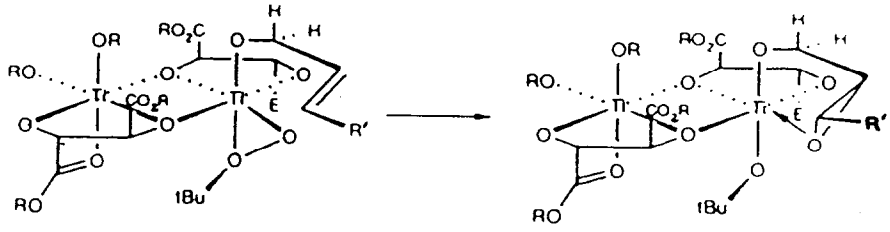
Allylic alcohol	Epoxyalcohol	Yield	ee	Conf.
		87 %	> 95 %	(2S,3S)
		79 %	94 %	(2S,3R)
		81 %	> 95 %	(2S)

These results show that, whatever the substitution pattern around the starting allylic alcohol is, very good yields (> 70%) and excellent enantiomeric excesses (> 90%) are obtained. In 1986, the addition of activated molecular sieves to the asymmetric epoxidation process permitted virtually all reactions to be carried out with only 5-10 % of the titanium-tartrate catalyst[27].

SHARPLESS and coworkers[28] proved that selectivity of the reaction is due to a catalytic species : a dimer in which two metal atoms are connected by two tartrate molecules to form a ten-membered ring. The tartrate ester groups extend outward from this ring "like the vanes of a windmill", thereby limiting the manner in which the allylic alcohol and hydroperoxide can bind to the metal.

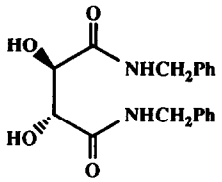


In recent studies, new ligands have been used in an effort to elucidate the mechanism of the enantioselection. However these studies have only resulted in the proposal of certain hypotheses : it seems that both steric and electronic effects play a role and influence the possible conformations of the allylic alkoxide. The proposed mechanism of the reaction can be schematized as follow[29] :



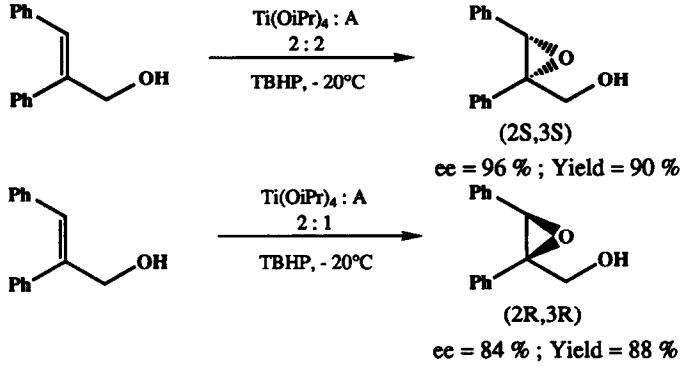
2.3.2 Modification of the Sharpless reagent

Many modifications to ligands have been proposed for enhancing enantiomeric excesses. Only one example, studied by LU and coworkers[30], is of interest : replacement of diethyl tartrate ligands by diamide derived from tartaric acid such as :



A

By varying the titanium tetraisopropoxyde / diamide ratio, enantiofacial selection can be either in the usual sense or the inverse. The two isomers of the epoxide can also be obtained from the appropriate derivative of the inexpensive and natural L-(+)-tartaric acid.



2.3.3 Limitations

The SHARPLESS method, although it may seem universal, only results in large enantiomeric excesses with primary or secondary allylic alcohols. This reaction is very sensitive to preexisting chirality[31] : the (R) or (S) absolute configuration of a starting secondary allylic alcohol can affect the *cis* or the *trans* character of the epoxide formed. If the carbon which bears the hydroxyl group also carries a sterically hindered group, a decrease or even a complete loss of enantioselectivity is observed.

Some cases are known in which the reaction does not work at all :

- either the substrate reacts very slowly and is converted into epoxides with very low enantiomeric excesses[32]

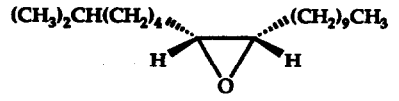
- or the substrate is epoxidized very quickly and with a very high enantioselectivity but the epoxyalcohol is unstable to the reaction conditions. The Lewis acidic titanium IV reageant is also a catalyst to open the cycle. The catalyst then acts as a poison or according to SHARPLESS the substrate is a "suicide substrate". The catalyst opens the epoxide, leading to a diol, which binds with the chiral complex and inactivates it[33,34].

A comprehensive review of titanium catalyzed asymmetric reaction according to the substrate structure was reported by JOHNSON and SHARPLESS himself[35].

2.3.4 Examples

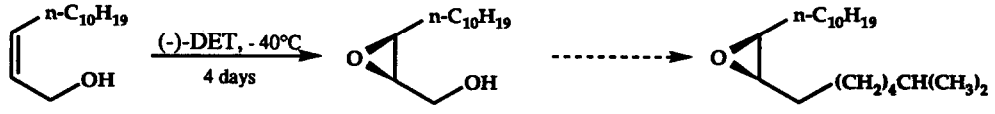
Examples of epoxides as end products are rare. But there is one which does demonstrate the great importance of chirality. This is disparlure, the sex attractant of the gipsy moth.

This insect is a serious pest in hardwood forests and orchards. The larva, which hatch during the spring, are particularly voracious and able to denude a tree in a few weeks. The extremity of the abdomen of the female contains a sex attractant. The pheromone was isolated from an extraction of 70,000 abdomens and identified (Disparlure = (7R,8S)-(+)-7,8-epoxy-2-methyloctadecane). The active isomer has the (7R,8S) configuration. A concentration as low as 10^{-10} g/mL of this isomer can be detected by a male. In contrast its enantiomer, even in a solution 10^6 times more concentrated, is inactive[36].



(7R,8S)-(+)-7,8 epoxy-2-methyloctadecane
(= Disparlure)

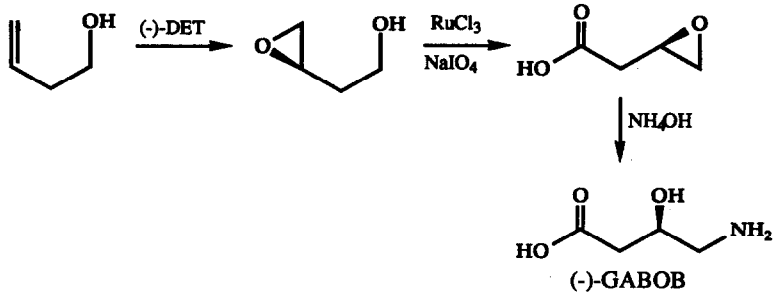
The synthesis of this substance, using the Sharpless reagent, is very important[37].



ee = 91 %
Yield = 4,8 %

Unfortunately, the epoxidation yield is very low because the epoxyalcohol is highly water-soluble making extraction of the product difficult.

ROSSITER and SHARPLESS[38], in synthetizing (-)-γ-amino-β-(R)-hydroxybutyric acid (GABOB), an antiepileptic and hypotensive drug, have highlighted the particularities of reactions with homoallylic alcohols in which the selectivity rules are inversed compared with those known for allylic alcohols.



(-)-GABOB
ee = 49 % ; Yield = 66 %

They have improved the synthesis of (-)-GABOB from ascorbic acid which required ten steps and gave a 10 % overall yield (ee = 20 %)[39]. The enantiomeric excess obtained by the SHARPLESS method is not particularly good because the starting alcohol is homoallylic.

2.4 Conclusion

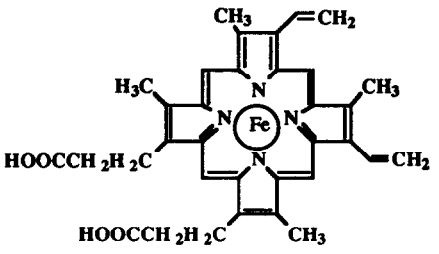
The Sharpless method, although only suitable for allylic alcohols, stimulated interest in asymmetric epoxidation techniques. Epoxides are obtained, in the most cases, with very good yields and with excellent enantiomeric excesses (> 90 %).

3 EPOXIDATION CATALYZED BY METALLOPORPHYRINS

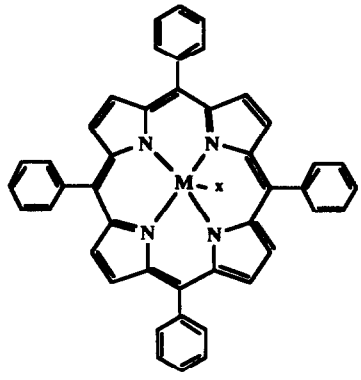
For the last decade, transition metal (metalloporphyrin) complexes have been used to catalyze epoxidations. These entities can reproduce and mimic all reactions catalyzed by heme-enzymes (cytochromes P-450, peroxidases...). Synthetic metalloporphyrins are analogous to the prosthetic group of heme-containing enzymes which selectively catalyze various oxidation reactions, with the same transition metal (iron) and the

same macrocyclic ligand (protoporphyrin IX). Chemoselectivity is achieved through careful selection from all the possible different metals and ligand combinations.

There has been a continual increase in the number of publications on metalloporphyrins since the beginning of the 80s[40]. The major difficulty in the selective epoxidation of unfunctionalized olefins lies in controlling olefin approach to the active oxidant. Several transition metals have been used (M = Fe, Mn, Mo, Cr) as well as various macrocycle substitutions.

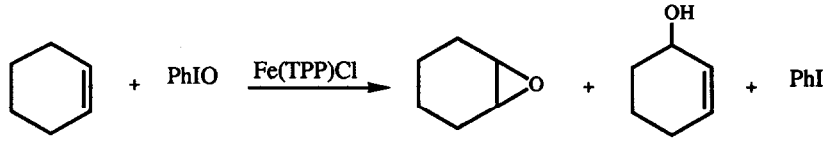


Protoporphyrin IX



Synthetic tetraarylporphyrin
Fe(TPP)

In 1979, GROVES and coworkers[41] used iodosylbenzene as the oxidant and catalyzed the epoxidation of olefins by Fe(TPP)Cl.

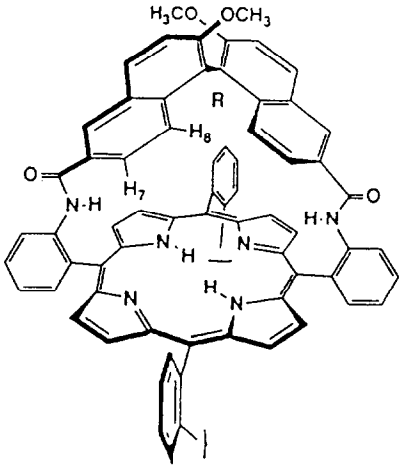


Cis olefins are more reactive than *trans* olefins toward porphyrin-catalyzed epoxidation by iodosylbenzene. For example, *cis* stilbene is epoxidized by Fe(TPP)Cl with a 77% yield whereas *trans* stilbene does not react under the same conditions[42]. In contrast, the use of a Mn porphyrin yields a mixture of *cis* and *trans* isomers[43]. High selectivity is induced by sufficient noncovalent interactions between the substrate and the metalloporphyrin. It is increased when the phenyl groups are substituted by methyl[44].

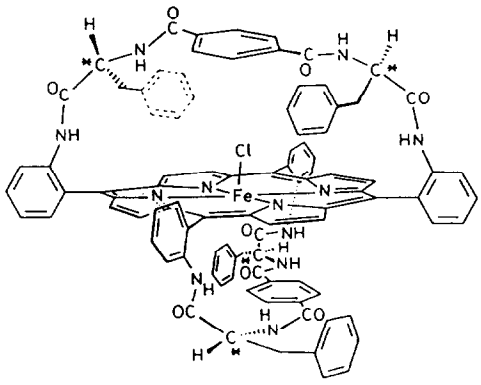
Use of NaOCl as oxidant promotes epoxidation with good yields and high regio and stereoselectivities (especially by adding pyridine derivates) for terpenes and steroïds[45,46] as well as for dienes which give rise to monoepoxides[47,48].

Chiral porphyrins, prepared in different ways (chiral units attached to preformed porphyrins[49], chiral substituents introduced during the synthesis of the porphyrins[50] or chiral porphyrins synthesized without introduction of chiral groups[51]) have been as effective as asymmetric epoxidation catalysts. Epoxidation of cyclic olefins with the ([binaphthylcarboxyamido]-phenyl)porphyrin[49] gives very average enantiomeric excesses (20% < ee < 50%).

Binaphthyl metalloporphyrin (BINAP)



"Basket handle" metalloporphyrin



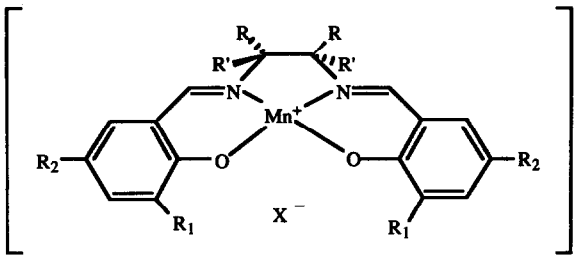
Prochiral olefins, such as β-methylstyrene, can be epoxidized with a 72% enantiomeric excess, either with "picket" metalloporphyrins ("Basket handle" iron-porphyrin)[52] or with bulby ligands porphyrins[53,54]. Chiral binaphthyl bridges give a rigid conformation and a chiral environment to the metallic center, which recognises the substrate.

Olefin	Porphyrin	Yield	ee	Conf.	Ref.
cis Methylstyrene	D_4 Symmetric	91 %	76 %	(1R,2S)	55
p-chlorostyrene	Basket handle	35 %	50 %	R	52
Styrene	BINAP	67 %	48 %	R	49
1-octene	BINAP	31 %	20 %	R	49

The enantiomeric excesses are rather low. The problem is that the chiral groups surrounding the macrocycle must be far enough from the central metal to permit easy entry of the substrate but must be close enough to generate high enantioselectivities. While the epoxidation of *cis* disubstituted olefins with these porphyrins generally exhibits good level of enantioselectivity, the epoxidation of *trans* olefins shows poor selectivity.

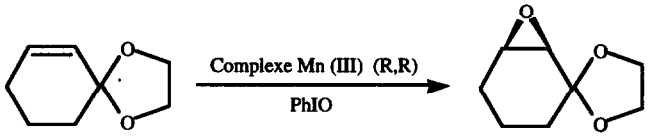
At the present time, the best enantiomeric excesses with different olefins are based on chiral salen ligands[56]. Contrary to the porphyrin systems, salen complexes bear tetravalent and thus potentially stereogenic carbon centers in the vicinity of the metal binding site. The proximity of the reaction site to the ligand dissymmetry improves the enantioselectivity of the reaction[57].

The following scheme shows the generalized structure for chiral salen complexes :

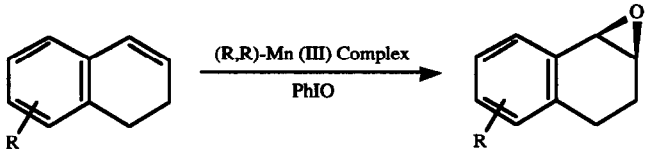


JACOBSEN and coworkers[57] were the first to report asymmetric catalysis with chiral Mn(III) salen complexes. These systems are generally derived from chiral 1,2-diamino-1,2-diphenylethane. Systematic variations of the steric and electronic nature of the different substitutents led to the discovery of catalysts that are particularly effective for the epoxidation[58]. Recently, KATSUKI[59] showed the relative importance of each substitutent (R, R', R_1, R_2) on the enantiofacial selection of *cis* or *trans* olefins.

Trans olefin did not show so good enantioselectivity as *cis* olefin. The enantiomeric excess is substrate dependent but can reach 93%[60,61]. The most selective catalyst for the epoxidation of a wide range of unfunctionalized olefins is the Mn salen complexe where R = phenyle, R' = R_2 = H and R_1 = *t*-butyle.

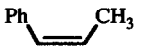
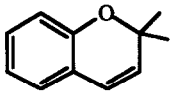
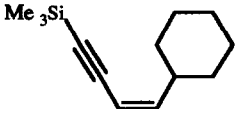


ee = 93 % ; Yield = 63 %



ee = 60-77 % ; Yield = 50-80 %

Another salen complex (R_1 = R_2 = *t*-Bu, R' = H and R = cyclohexane) displays high enantioselectivity with various *cis*-disubstituted alkenes[58]. Some examples are collected in the table :

Olefin	Isolated Yield (%)	% ee of major epoxide
	84	92
	87	98
	65	98

The use of this catalyst was also reported for the enantioselective epoxidation of cinnamate esters[62]. Epoxidation of *cis*-ethyl cinnamate in the presence of 4-phenylpyridine N-oxide yielded a mixture of the corresponding *cis* and *trans* epoxides in a ratio 4-5/1. The *cis* epoxide was obtained with 98 % yield and 93 % enantiomeric excess.

The mechanisms operating in such systems are very complex and depend on various factors such as the oxidant, the metallic center, the nature of the porphyrin or of the ligands and the substrate itself. More details about the role of each of these factors are given in the reports of COLLMAN[63,64] and MEUNIER[40] for the porphyrins and in that of JACOBSEN[58] for the salen complexes.

Conclusion

The interest of epoxidation catalyzed by metalloporphyrins and salen complexes lies in:

- the possibility of using various oxidants (iodosylbenzene, sodium hypochlorite, peroxides)
- the choice in ligands which yield different selectivities for olefin epoxidation.

Asymmetric induction catalyzed by porphyrins is very promising. Preliminary epoxidation reactions with cyclic or acyclic alkenes having a terminal double bond give very satisfactory results (good yields and quite high enantiomeric excesses (70% < ee < 80%)). Assays to improve the enantiomeric excesses were carried out by immobilizing porphyrins on inorganic supports such as silica[65] or clay materials[66]. Although this method is, at present, just at its beginning, results are very encouraging.

The best understanding in the role that each substitutent (R, R', R_1, R_2) of the salen complex plays should permit improvements in the enantioselectivity of the reaction. This method of epoxidation is also very interesting and very promising.

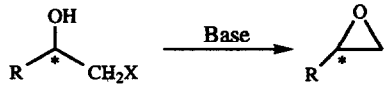
4 OTHER METHODS

Many other methods of asymmetric chemical epoxidation exist but they are often eclipsed by those methods described above. However, particular cases can be of interest even though the enantiomeric excesses are quite low.

4.1 From chiral precursors

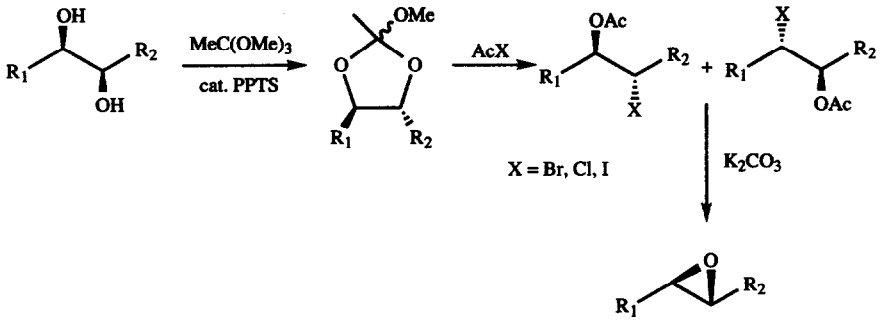
Organic optically pure products, isolated from natural compounds, synthesized or available commercially, can serve as precursors for the synthesis of chiral epoxides.

An alcohol with a good leaving group adjacent to the hydroxyl group can be easily converted into an epoxide by treatment with an appropriate base. The reaction involves an inversion of the configuration on the carbon atom bearing the leaving group[67].



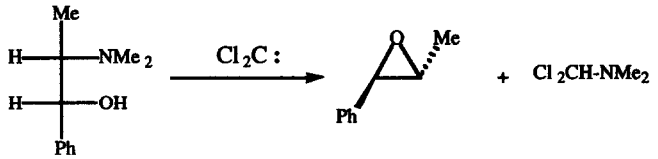
X = Br, Cl, OTs, OH...

SHARPLESS and coworkers[68] have reported the stereospecific conversion of enantiomerically enriched vicinal diols into the corresponding epoxides in a high yielding, two or three steps, "one-pot" procedure. The enantiomeric excesses of epoxides are as good as those of the starting vicinal diols[69].



Such a synthetic strategy shifts the emphasis of the problem to one of preparing optically active chiral compounds.

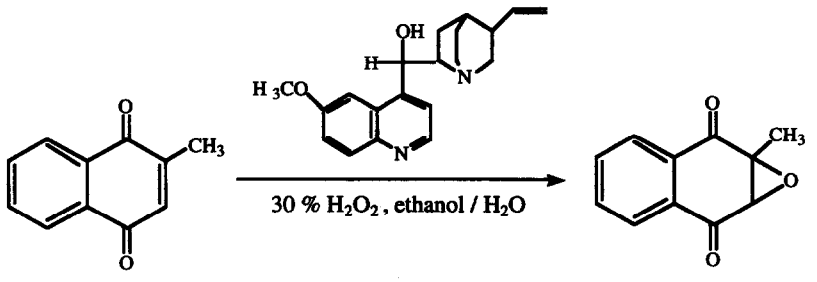
CASTEDO and coworkers[70] used as reagent, a phase transfer catalyst. The reaction of dichlorocarbene (Cl_2C :) with optically active tertiary β-ethanolamines gives rise to a high yield of epoxides with complete stereospecificity (ee ≥ 95%).



ee ≥ 95 % ; Yield = 80 %

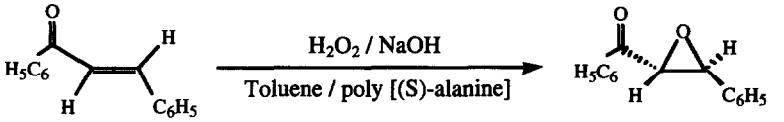
4.2 Use of phase-transfer catalysts

Epoxidation of electron-poor olefins, such as chalcones and quinones, was carried out using quaternary ammonium salts derived from alkaloids or quinine-derived under phase-transfer conditions[71]. Chemical yields are excellent while enantiomeric excesses are low and vary according to the nature of the solvent : the higher the dielectric constant, the lower the enantiomeric excess[72].



Yield = 90 %
ee (dichloromethane) = 28 %
ee (benzene) = 64 %

JULIA and coworkers[73] used synthetic chiral peptides in a triphase catalysis.



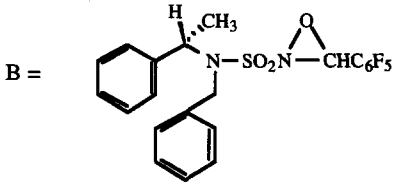
ee = 93 % ; Yield = 85 %

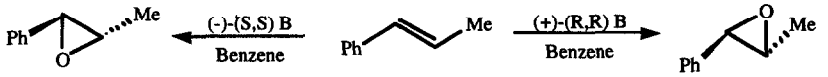
Polypeptides are used as "synthetic enzymes". Assays with different kinds of polypeptides (polyvaline, polyglutamate...)[74,75] have shown that poly-(L)-valine is the best catalyst. Its replacement by poly-(D)-valine results in the epoxide optical inverse.

4.3 Use of chiral reagents

4.3.1 Chiral oxaziridines

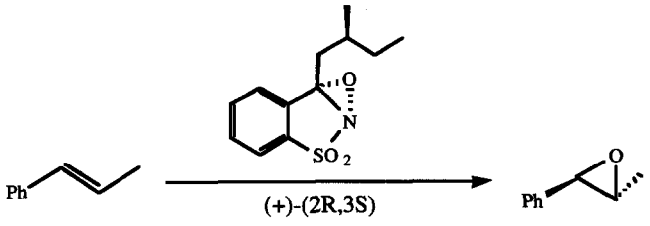
DAVIS and coworkers[76] have experimented with an asymmetric epoxidation method for unfunctionalized alkenes by chiral camphoroxaziridines. Yields are very good (80-90%) but the optical purities are low (12-35%). The stereochemistry of the product is controlled by the configuration of the oxaziridines. The best enantiomeric excesses (13-65%) are observed with the 3-pentafluorophenyl-2-sulfamyloxaziridine (B)[77] or with the 3-substituted-1,2-benzisothiazole-1,1-dioxide-N-sulfoxaziridine[78]. A planar orientation of the oxaziridine with reacting olefin in the stereodetermining transition state was proposed based on the observed sense and degree of enantioselectivity. A more detailed report has been written by DAVIS himself[79] .





(1S,2S)
ee = 64.7 %

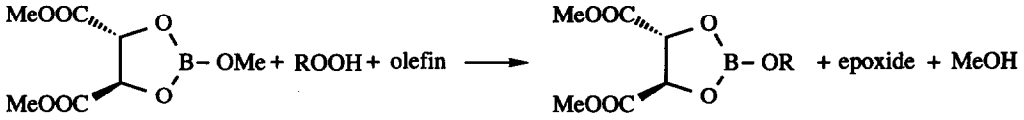
(1R,2R)
ee = 52.0 %



(1R,2R)
ee = 51 %; Yield = 20 %

4.3.2 Chiral borates

MANOURY and coworkers[80] have tested chiral alcohol borates for the asymmetric epoxidation of prochiral unfunctionalized alkenes.



Yields and enantiomeric excesses have not yet proven particularly satisfactory (Yield = 35-75%; ee = 6-51%).

5 CONCLUSION

This summary of chemical asymmetric epoxidation methods is not exhaustive and only attempts to highlight the advantages and disadvantages of three major synthetic pathways:

- Use of peracids : this method is easy but only results in low enantiomeric excsses.

- Use of the Sharpless reagent : the enantiomeric excesses obtained are excellent but the presence of an allylic alcohol in the starting material is obligatory. The reaction products are epoxyalcohols which are nevertheless very useful and flexible building blocks.

- Use of metalloporphyrins and related salen complexes: this is undoubtedly the method of the future. However at present, the enantiomeric excesses are, in most cases, low. The major drawback consists in synthetizing the metalloporphyrins (fixed carbon skeleton but various ligands), which is not always easy.

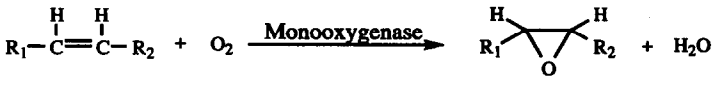
All these methods were and are still subject to further variations (e.g. different solvents, ligands) aimed at optimizing chemical and / or optical yields. However the field of organic chemistry has not yet been able to provide the answers to all the problems posed by asymmetric syntheses and many enantiomeric excesses remain to be improved. With this scenario as an established fact, except in regard to the synthesis of epoxyalcohols, attention has also turned towards the use of biological catalysts.

III - BIOLOGICAL SYNTHESIS OF CHIRAL EPOXIDES

Epoxides are involved in the metabolism of many aliphatic and aromatic compounds in plants as well as in mammals. Biological epoxidation is classed as an oxygenation reaction, differing from an oxidoreduction reaction in that it leads to the incorporation of one or several oxygen atoms in the molecule of substrate. Enzyme potentiality allows both regio and stereospecific oxygenation reactions, which are very difficult to carry out chemically. The use of enzymes for such reactions is one of the most fascinating applications in bioconversion.

1 DIRECT STEREOSPECIFIC EPOXIDATION

Monooxygenases can activate molecular oxygen incorporating one oxygen atom into the substrate and reducing the other atom to water. In this way alkenes can be converted into epoxides.

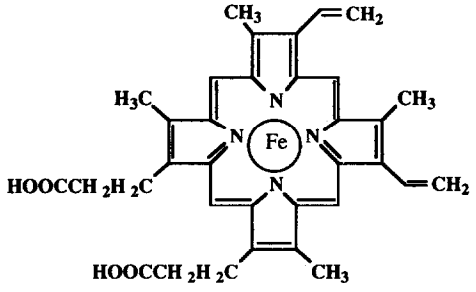


Monooxygenases are found in many living organisms : bacteria, yeasts, insects, plants, mammal tissues. They are used for organic asymmetric reactions either in a more or less purified enzymatic form (cytochromes P-450) or in whole-cell microorganisms (bacteria, fungi).

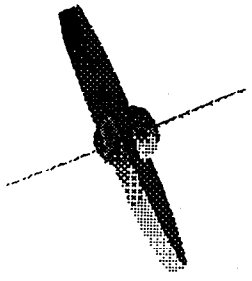
1.1 Isolated monooxygenases : the cytochromes P-450

1.1.1 Definition

Cytochromes are chromoproteins (= heteroproteins in which the prosthetic group is a pigment) and act as enzymatic electron carriers. The prosthetic group to which the cytochromes P-450 are covalently bound (binding thiolate-cysteine) is heme, an iron porphyrin[81,82].



The molecule is planar.



About thirty isozymes of cytochrome P-450 have been isolated, from insects, plants, yeasts, fungi as well as from various mammal tissues[83].

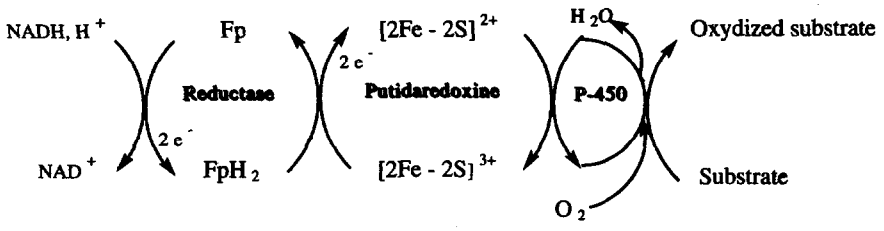
These enzymes are involved in various biosyntheses and biodegradations of endogen molecules such as steroïds and fatty acids. For example, some isoforms found in liver protect the body from attacks by foreign chemical compounds through oxidizing toxic products. However some of the metabolic transformations mediated by cytochrome P-450 produce toxic or carcinogenic products[84]. The epoxide produced from vinyl chloride by liver cytochrome P-450 is known to induce tumors in that organ.

Such observations explain the fascination of this type of reaction and the reason for the studying its mechanism. We will highlight some results and more important points. For detailed information, refer to the books of OMURA[85] and ORTIZ de MONTELLANO[86].

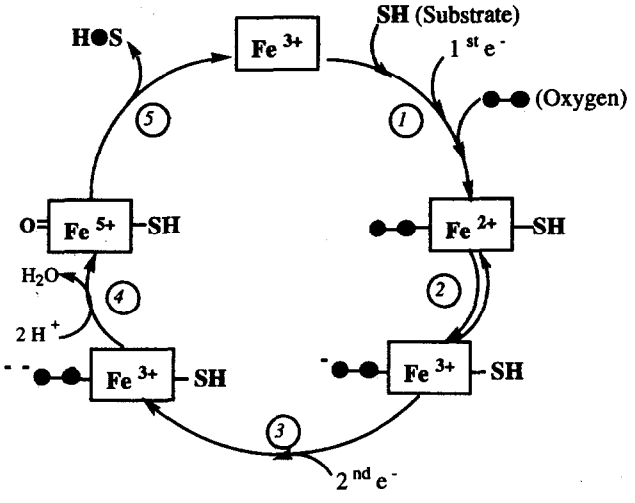
1.1.2 Mechanism

The first monooxygenase of the cytochrome P-450 family to be isolated and characterized by crystallography, came from *Pseudomonas putida*. It catalyzes the first step in the degradation of camphor by hydroxylating it[87] explaining why it is referred to as cytochrome P-450 $_{CAM}$. Cytochrome P-450 receives the

electrons from NADH *via* two proteins : a ferredoxin called putidaredoxine (an iron sulphur protein [2 Fe-2S]) and a flavinic enzyme which acts as a dehydrogenase and is called putidaredoxine reductase[88].



The enzymatic catalytic cycle of cytochrome P-450 is carried out in 5 steps [89]:



1. At the beginning, cytochrome P-450 in an oxidized state, receives the substrate first, then one electron and finally oxygen after the reduction of iron.

2. The second step is in equilibrium with the third one. Oxygen passes to the superoxide state (O_2^-).

4. The arrival of the second electron involves the transformation of the superoxide to the peroxide state. The binding between the oxygen atoms is broken, excluding a water molecule and producing a highly oxidative entity called oxene $Fe^{V} = O$, which oxidizes the substrate placed in the active site.

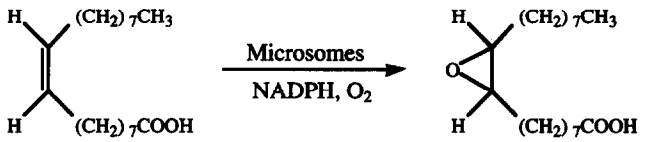
5. The last step is not fully understood. Breaking the peroxide bond results in an unstable and very reactive oxygenated product : the hydroxylated product is liberated and then the cycle can start again.

Study of the epoxidation mechanism of a double bond catalyzed by cytochromes P-450 has been approached from both a theoretical[90] and stereochemical standpoint; both CO bonds seem to be formed simultaneously. Indeed, the stereochemistry of 4-octene[91] and *cis* stilbene[92] remain unchanged during the epoxidation.

1.1.3 Epoxidation by rat liver microsomes

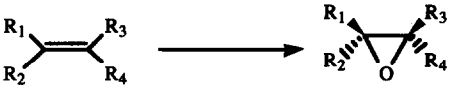
Epoxides are very reactive molecules capable of binding to components of mammal tissues by acting on proteins, DNA or RNA, a characteristic which accounts for their high toxicity. Generally epoxides are metabolized either by epoxide hydrolases (conversion into diols) or by glutathione-S-transferases.

The epoxidation of foreign olefins and aromatic compounds by rat liver microsomes has been studied. Microsomes are very small particles associated with the endoplasmic reticulum. They can be isolated by ultra-centrifugation and contain P-450 cytochromes[93].



This assay was carried out on an analytical scale. Neither the reaction yield nor the enantiomeric excess were determined.

The monooxygenases of such systems can recognize the different enantiotopic faces of the double bond of small, aliphatic, unfunctionalized prochiral olefins[94].



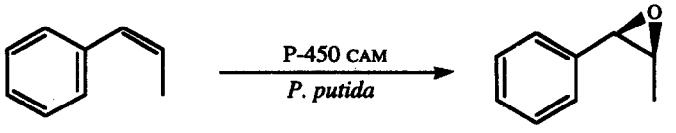
R_1	R_2	R_3	R_4	Enantiomers ratio	ee
H	H	Me	H	30 (R) : 70 (S)	40 %
H	H	$HC{=}CH_2$	H	30 (R) : 70 (S)	40 %
H	Me	Me	Me	0	0
H	H	Et	Me	39 (R) : 61 (S)	22 %

The observed enantiomeric excess depends on the structure and in particular on the degree of the olefinic double bond substitution.

Polycyclic hydrocarbons[95] or aromatic olefins[96] have been epoxidized by such systems. In such reactions, the epoxide is only a short lived intermediate often difficult to isolate, the final product being a diol[97].

1.1.4 Applications of cytochromes P-450

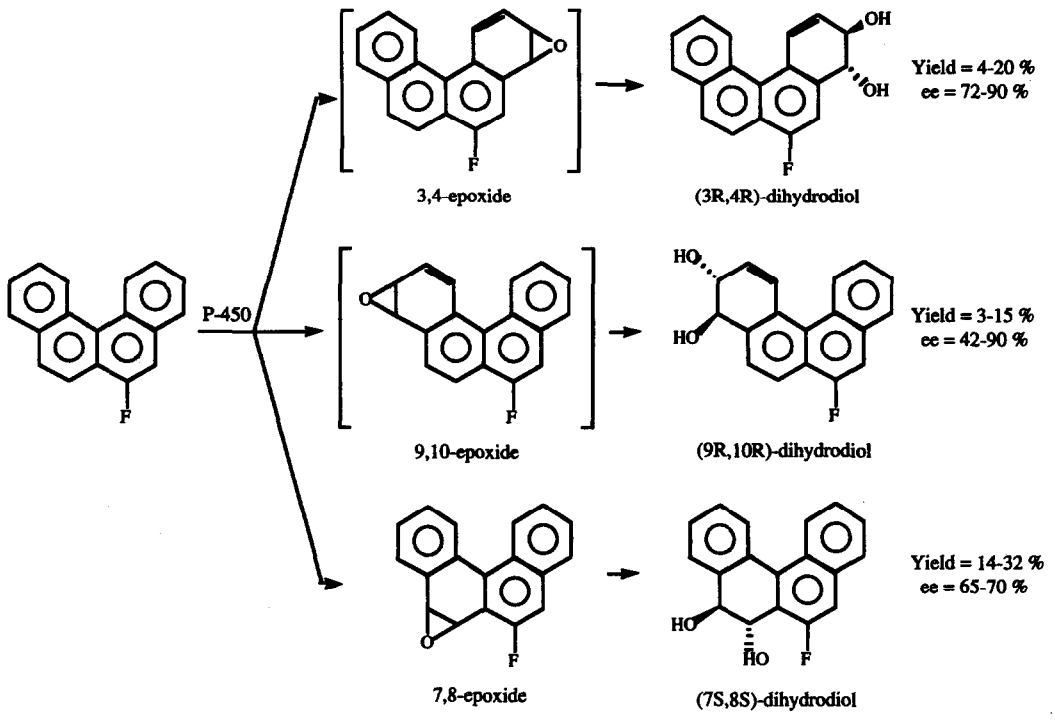
. Epoxidation of *cis* β-methylstyrene[98]



(1R,2S)

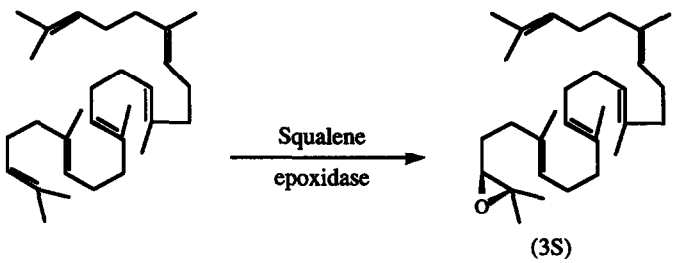
Yield = 85 % ; ee = 10 %

. Oxidative metabolism of carcinogenis 6-fluorobenzo [c] phenanthrene[99]



The P-450 cytochromes used are isolated from rat liver microsomes. The binding mode of the substate at the active site of the P-450 cytochrome dictates the type of epoxide obtained.

. Epoxidation of squalene by squalene epoxidase[100-102]



(3S)

Squalene is an important compound because it is the precursor of the steroïds. Squalene epoxidase is an enzyme which requires FAD, NADPH, oxygen and NADPH-P-450 cytochrome reductase. Unfortunately, assays were carried out in an analytical scale. Neither reaction yield, nor enantiomeric excess were reported. (3S)-2,3-oxidosqualene is then converted to lanosterol (animals, fungi) or to cycloartenol (higher plants) by one or several oxidosqualene cyclase enzymes.

1.2 Bacterial monooxygenases

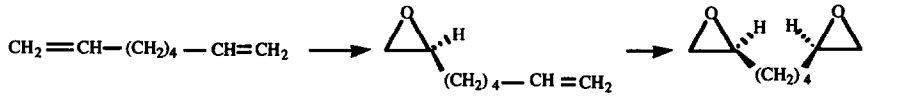
Bacteria are microorganisms rich in monooxygenases and epoxidation reactions by bacteria have been extensively studied. Assays have been mainly based on the difference between monooxygenase activity on the various growth media used to cultivate bacteria.

1.2.1 Bacteria grown on alkanes

1.2.1.1 Bacteria of *Pseudomonas species*

The formation of terminal epoxides from alkenes was first reported by Van der LINDEN[103] who described the epoxidation of 1-octene by *Pseudomonas aeruginosa* cells grown on heptane. He postulated that epoxides were formed by enzymes already present in the cells which were closely related or identical to the alkane oxidizing system, which normally produces the primary alcohol at the terminal methyl group. Epoxide formation seems to occur when the "activated oxygen" species is confronted with the reactive olefinic π system. However, this epoxidation reaction only takes place when the double bond is terminal since 2-octene does not give rise to the corresponding epoxide[104].

MAY and SCHWARTZ[105] have shown that 1,7-octadiene, which does not contain a terminal methyl group, is entirely converted by *Pseudomonas oleovorans* to (R)-(+)-7,8-epoxy-1-octene with an enantiomeric excess greater than 80%. However the reaction does not stop at this point. Since the starting product possesses two terminal double bonds, diepoxide formation is possible. Cells of *Pseudomonas oleovorans* grown in the presence of octane and then put with a racemic mixture of 7,8-epoxy-1-octene gives rise to the formation of the diepoxide product from the racemic monoepoxide with a very low enantiomeric excess (20%) of R sites. In contrast, diepoxide is produced from octadiene *via* the obligate monoepoxide intermediate with an enatiomeric excess of R sites of nearly 80%[106]. These findings show that the configuration of the preformed epoxide group influences the stereochemistry of the second epoxide. In this reaction, the opposite ends of the substrate do not function independently.

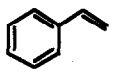
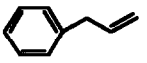
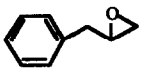
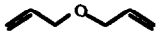
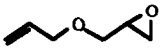
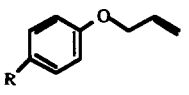
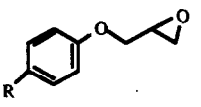
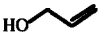


(R)-(+)-7,8-epoxy-1-octene ee > 80 %

(R,R)-(+)-1,2-7,8 diepoxyoctane ee > 80 %

Results show that structural limiting factors exist and that the enzymatic system does not accept any substrate. The monooxygenases of *Pseudomonas oleovorans* can produce epoxides from six to twelve carbon atoms containing terminal alkenes[107] (if the number of carbon atoms is smaller than six, the hydroxylated compound is produced) but not from cyclic or non terminal alkenes[108]. They accept allylbenzenes[109], *O*-alkylated derivatives[110] but not allylic alcohols[111] The epoxide products are generally of the (R) configuration.

Various substrates have been tested as shown in the next Table.

Substrates	Products	ee	Conf.	Ref.
n-C_8H_{17}-CH=CH_2	n-C_8H_{17} 	62 %	R	109
	-	-	-	108
		not determined	-	108
		81 %	R	109
 R = MeO R = $CH_3OCH_2CH_2$		99 % 100 %	R S	110 110
	-	-	-	111

Mechanism

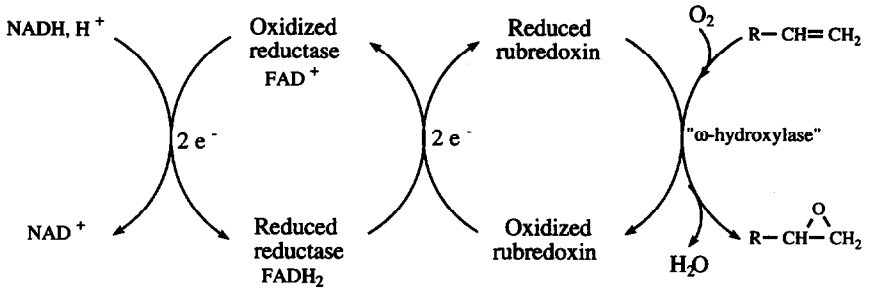
The enzymatic system responsable for these reactions is called the ω-hydroxylation system and was discovered by COON[112]. It catalyzes the hydroxylation of the terminal methyl groups of alkanes and fatty acids but also, as shown above, the epoxidation of alkenes with one or two terminal double bonds. Whereas 1-octene is either epoxidized to 1,2-epoxyoctane or hydroxylated to 7-octen-1-ol, 1,7-octadiene is only epoxidized to 7,8-epoxy-1-octene : the epoxidation reaction is easier than the hydroxylation reaction.

The enzymatic system of *Pseudomonas sp.*, involved in the epoxidation reaction, contains three components:

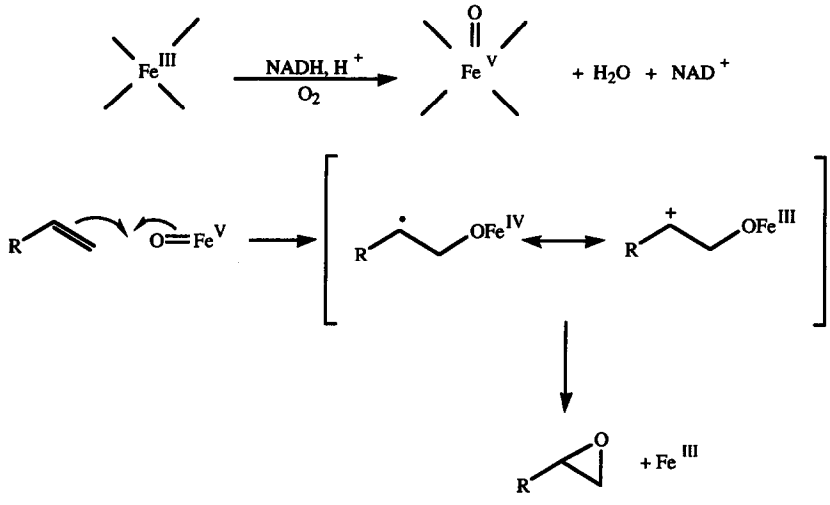
• Rubredoxin-reductase, a flavoprotein, transports two electrons from NADH to rubredoxin.

• Rubredoxin, a non-haem protein, functions as an electron carrier : it accepts (one by one) two electrons from the reduced reductase and gives them to the hydroxylase.

• "ω-hydroxylase" binds the substrate and molecular oxygen, activates oxygen and inserts one of the oxygen atoms into the substrate[113,114].



Other mechanistic studies about oxygen activation using *trans, trans* 1,8-dideutero-1,7-octadiene[115] have shown that a straightforward "oxenoid" mechanism, comparable to that operative in peracid, does not work. Attack of the oxo-iron group on the double bond gives a radical or a cationic intermediate, which closes preferentially by the *si* face to yield to the (R) epoxide[111]. Epoxidation stereospecificity is strictly controlled by the steric environment of the enzyme's active site, which prevents the free rotation of the radical intermediate[112].



Improvement of epoxide yield

Enantiomeric excesses in these reactions are very high but improving chemical yields has been the subject of much research. One of the limiting factors is the inhibition observed by the product (e.g. when the 7,8-epoxy-1-octene concentration reaches 0,8 g /L [116]).

The addition of a non aqueous and apolar solvent, such as cyclohexane, eliminates the epoxide by transferring it through to the organic layer[117] and avoids the problem of inhibition. The yield can reach 90%. However the presence of organic compounds changes cell morphology and can affect the activity of the enzyme[118].

To improve the yield, *Pseudomonas oleovorans* is grown on a medium containing a high concentration in 1-octene. The quantity of 1,2-epoxide produced in these conditions is tenfold that produced by resting-cells

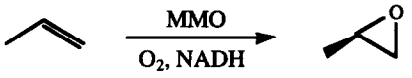
grown on a medium which does not contain octene[119]. Many studies have focused on the effect alkenes exert on cell growth and morphology and which hence influence the optimal conditions for epoxide preparation[120].

Conclusion

By varying the solvent, reaction time and growth-medium, the enzymatic system of *Pseudomonas oleovorans* can produce chiral epoxides at the terminal position, mainly in the (R) form. Yields are good and enantiomeric excesses from linear alkenes of six to twelve carbon atoms are high.

1.2.1.2 Methylotrophic bacteria

Methylotrophic bacteria possess a non specific NAD(P)H-dependent methane monooxygenase (MMO), which hydroxylates alkanes to alcohols but which can also epoxidize alkenes.



(R)-1,2-epoxypropane
ee = 10 %

Methane-consuming bacteria only produce epoxypropane from propene and not an alcohol[121]. HOU and coworkers[122] discovered that resting cell suspensions of methane-grown bacteria (*Methylococcus capsulatus*) only epoxidize C2 to C4 gaseous alkenes. Strongest activity is observed with propylene and ethylene. Liquid alkenes are not epoxidized. Cell-free extracts of these cultures can also epoxidize gaseous alkenes from C2 to C4 to 1,2-epoxyalkanes[123,124].

Novel methane-utilizing bacteria can epoxidize liquid alkenes. The thermophilic methylotrophic bacterium H-2[125] exhibits broad substrate specificity (C2 to C6 alkenes with terminal or subterminal double bonds) but enantiomeric excesses have not been determined.

The advantage of using such methane-users (such as *Methylosinus trichosporium* [126]) lies in the fact that the epoxyalkane is not further metabolized and that it accumulates extracellularly[127]. However, MMO generally gives rise terminal epoxides with very low enantiomeric excesses (< 10%) mainly in the (R) form[128].

1.2.2 Bacteria grown on alkenes

1.2.2.1 Bacteria grown on gaseous alkenes

Several gaseous alkene-utilizing microorganisms have also been described in the literature. Bacteria of the genera *Rhodococcus*, *Micrococcus*, *Mycobacterium* and *Xanthobacter* contain NADH-dependent monooxygenases that are able to form epoxyalkanes.

HOU and coworkers[129] have described several bacteria that grow on gaseous alkenes as their sole source of carbon. Short chain alkenes are epoxidized by all the microorganisms tested in the presence of NADH. WOODS and MURRELL[130] have studied the epoxidation by *Rhodococcus rhodochrous* and shown that the epoxidation rate of gaseous alkenes is very high.

MAHMOUDIAN and MICHAEL[131,132] have tested different genera of bacteria utilizing ethene or propene. They showed that reactions are stereoselective. They obtained, with 18 strains of bacteria, the (R)-

1,2-epoxypropane (ee = 90-96%), the (R)-1,2-epoxybutane (ee = 90-98%) and the *trans* (2R,3R)-epoxybutane (ee = 64-88%). 1,2-epoxides and 2,3-epoxides are stereospecifically formed.

Mycobacterium

Bacteria of the species *Mycobacterium*[133] are of special interest. Their enzymatic system contains two different monooxygenases[134] An "alkene monooxygenase" induced by growth on alkenes is specific for alkenes. This alkene monooxygenase epoxidizes alkenes stereospecifically and does not hydroxylate alkanes or alkenes. An alkane monooxygenase also present epoxidizes alkenes non stereospecifically and hydroxylates alkanes as well as alkenes. The bacteria can oxidize propene to (R)-1,2-epoxypropane with an 80% enantiomeric excess. Cell immobilization of the various mycobacteria increases reaction yield while conserving the same specificity[135].

However, in both cases, the rate of the epoxidation reaction is very slow. Moreover, a high concentration in epoxide inhibits the alkene monooxygenase[136].

Xanthobacter

Due to the above mentioned drawbacks to using *Mycobacterium*, a strain of *Xanthobacter* species has been tested[137]. The rate of the epoxidation is higher than with *Mycobacterium* and the specificity of the substrate broader. However it also contains 1,2-epoxyalkane-degrading enzymes and so epoxides only accumulate when non-growth alkenes are oxidized. Nevertheless 2,3-epoxybutane is detected during the epoxidation of *cis* and *trans* butene. Enantiomeric excesses are not mentioned.

1.2.2.2 Bacteria grown on longer chain terminal alkenes

Alkene-utilizing bacteria have two advantages compared with alkane-utilizing bacteria:

- they form epoxides with very high enantiomeric excesses whereas alkane-utilizing bacteria tend to form racemic epoxides[138]
- they can only epoxidize and do not hydroxylate olefins.

However, they have a very limited substrate specificity and contain epoxide-degrading enzymes that hydrolyse the product at a much faster rate than epoxidation.

Corynebacterium equi

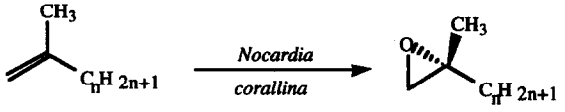
This microorganism was grown on an inorganic medium containing 1-hexadecene as the sole source of carbon. The epoxidation of linear alkenes with carbon chains longer than 14[139] and with a terminal double bond proceeds stereospecifically to give (R)-(+)-1,2-epoxyhexadecane with a 100 % enantiomeric excess[140].

(R)

ee = 100 %; Yield = 40 %

Nocardia corallina

Cells of *Nocardia corallina* when grown on C3-C4 and C-13 to C-18 alkenes produce the corresponding 1,2-epoxyalkanes[141].



n	Yield	% ee	Conf.
3	32 %	76	R
4	55 %	90	R
5	56 %	88	R

A very wide range of substrates can be epoxidized[142,143].

Epoxides	Absolute conf.	ee
1,2-epoxyheptane	R	94 %
1,2-epoxy-2-methylhexane	R	90 %
Epichlorhydrin	S	81 %
Styrene oxide	R	69 %
Octylglycidil ether	R	92 %
2,3-epoxyoctane	no determination of the enantiomeric excess or of the absolute configuration	

In all cases, enantiomeric excesses are very high or even excellent. The chemical yields are quite good (30-70%).

Other genera of bacteria

Production of epoxyalkanes is also possible with the bacterium *Nitrosomonas europaea* which contains an ammonium monooxygenase involved in the conversion of ammonium to hydroxylamine. HYMAN and WOOD[144] report the formation of epoxyethane with this microorganism demonstrating that the ammonium monooxygenase is responsible for the epoxidation reaction and that there is competition between NH_3 and C_2H_4 for the active site. Unfortunately, this study was only on an analytical scale and neither enantiomeric excesses, nor yields are given.

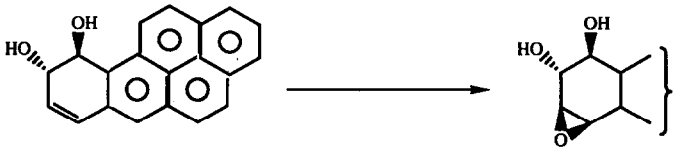
Conclusion

All these bacteria, growing on alkanes or alkenes, produce with average yields but good enantiomeric excesses, 1,2-epoxides or sometimes, 2,3-epoxides. There are two drawbacks :

- Almost all the epoxides have (R) configuration
- Bacteria are highly substrate specific : a strain of bacterium can yield an optically pure epoxide from one particular substrate but a racemic epoxide from another.

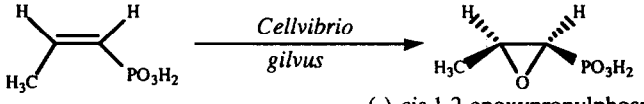
1.3 Monooxygenases from other microorganisms

Fungi can also produce epoxides. *Cunninghamella elegans* gives rise to 9,10 benzo [c] pyrenediol-7,8-epoxides[145].



Other substrates studied (biphenyl, dibenzofuran, naphthalene) show that the enzymes involved are NADPH-dependent monooxygenases, which exhibit stereoselective and regioselective properties different from those of mammal monooxygenases[146].

An interesting fungal epoxidation was described by WHITE and coworkers[147] who report the transformation by a number of *Penicillium* strains of *cis* propenylphosphonic acid into fosfomycin ((-)-*cis*-1,2-epoxypropenylphosphonic acid), a broad-spectrum antibiotic. AISAKA and coworkers[148] also used a bacterium, *Cellvibrio gilvus*, for this synthesis. In this case, the yield (9 µg / mL) of product is greater and the incubation time shorter.



(-)-*cis* 1,2-epoxypropylphosphonate
= fosfomycin

1.4 Conclusion

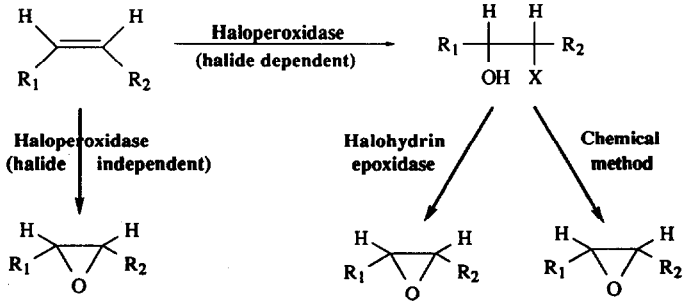
Direct epoxidation by monooxygenases generally yields chiral epoxides at the end of the carbon chain and in the (R) configuration (except in the case of the microsomes). The yields are average with good enantiomeric excesses. Although bacterial monooxygenases are the bacteria most frequently used for chiral epoxide production, monooxygenases of cytochromes P-450 isolated from rat liver microsomes are much studied for elucidating the enzymatic mechanism of the epoxidation.

2 INDIRECT EPOXIDATION

2.1 Use of peroxidases

2.1.1 Haloperoxidases

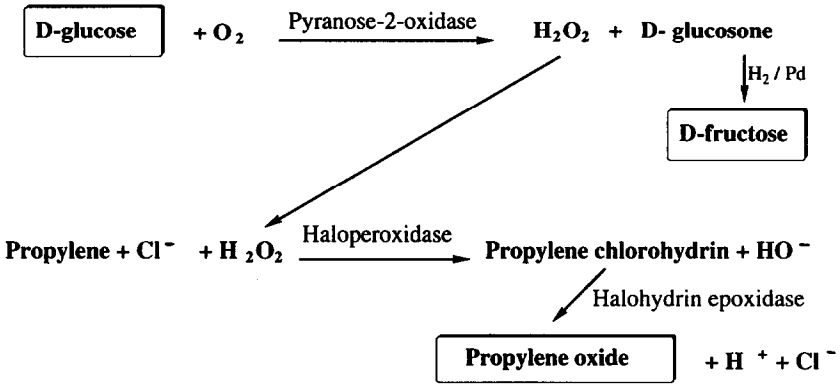
Haloperoxidases (depending on their type) can catalyze the formation of epoxides in two ways, schematized below:



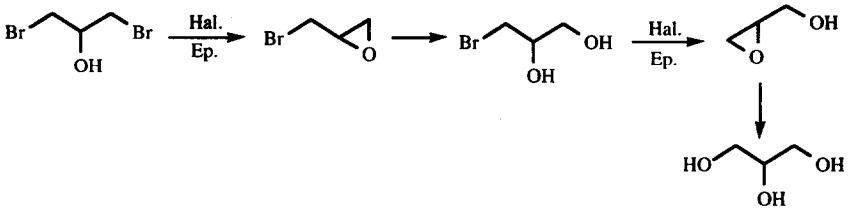
α,β-halohydrins can be obtained either chemically or biologically using haloperoxidases or by microbiologically reducing α-halogenated ketones.

2.1.1.1 Halohydrin epoxidases

Combining haloperoxidase and halohydrin epoxidases is the first method of producing epoxides. At the end of the seventies, great interest was taken in enzymatic methods of producing propylene oxide. The Cetus process[149], shown below, involves three reactions each catalyzed by a specific enzyme[150].



This halohydrin epoxidase[151] was isolated from *Flavobacterium sp.* by CASTRO and BARTNICKI[152], who studied 2,3-dibromopropanol detoxification into glycol *via* the formation of various epoxides.



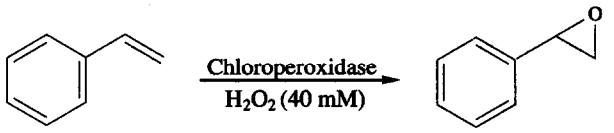
To establish the enantioselectivity of this enzyme, GRIENGL[153] investigated different halohydrin structures. The results were disappointing : the best result was obtained with racemic 1-bromo-2-octanol with a 58% enantiomeric excess. The epoxide with the (S) configuration was formed.

Recently, another halohydrin epoxidase has been isolated from a strain of *Arthrobacter sp.*[154]. This enzyme catalyzes the conversion of vicinal C2 or C3 haloalcohols to the corresponding epoxides.

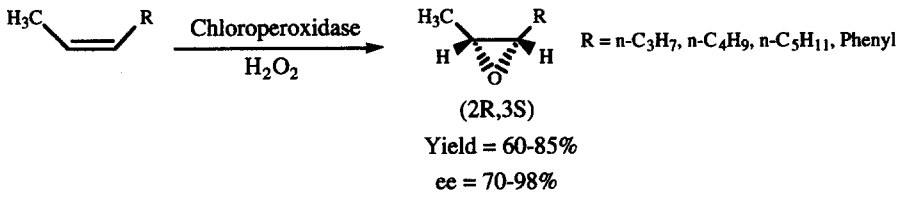
In both cases, the epoxides obtained are not degraded. However haloperoxidases generally give racemic products[155] and epoxides produced by this method exhibit very low enantiomeric excesses.

2.1.1.2 Chloroperoxidases

Chloroperoxidase from *Caldaryomyces fumago* catalyzes halide independent epoxidation of alkenes in the presence of hydrogen peroxide[156]. Enantiomeric excesses are not indicated.



Recently, ALLAIN and coworkers[157] have described that under controlled conditions, chloroperoxidase is highly effective for the enantioselective catalytic epoxidation of a variety of simple olefins by hydrogen peroxide.

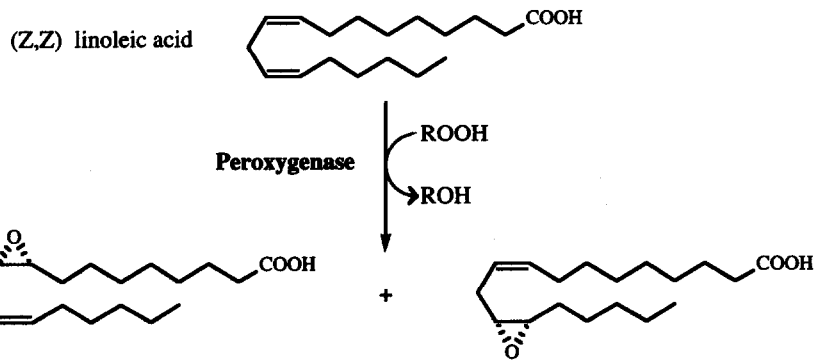


Good results are obtained with *cis* alkenes, which have a subterminal double bond. *Trans* olefins are unreactive substrates.

2.1.2 Other peroxidases

2.1.2.1 Soyabean peroxygenases

BLEE and SCHUBERT[158] isolated a peroxygenase from the microsomes of cotyledons and seeds of soyabean. This unique oxygenase can catalyze the epoxidation of mono and polyunsatured fatty acids such as oleic and linoleic acids[159].



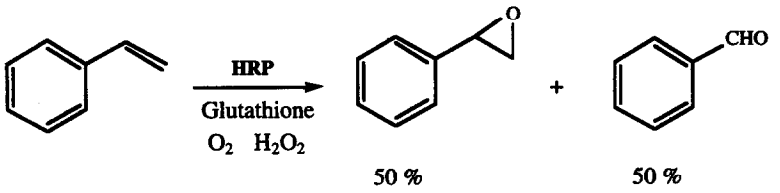
(9R,10S)-epoxy-12-(Z)-octadecenoic acid
ee = 90 %

(12R,13S)-epoxy-9-(Z)-octadecenoic acid
ee = 80 %

The soyabean peroxygenase exhibits high stereoselectivity since no epoxidation can be observed with unsaturated acids having the double bond in the *trans* configuration[160]. For the double bond in a *cis* configuration however, enantiomeric excesses are high.

2.1.2.2 Horseradish peroxidase

Horseradish peroxidase (HRP) can epoxidize styrene in the presence of glutathione[161]. The epoxidation reaction yields a racemic compound.



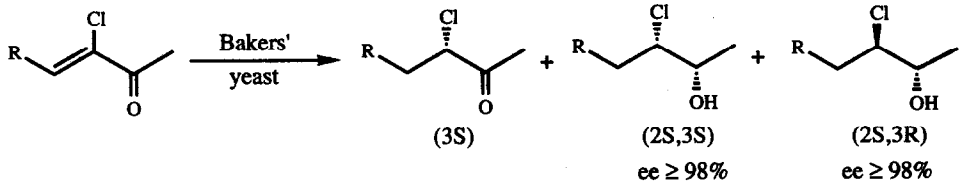
2.2 Other methods

Various "α-bichiral" synthons such as α-halohydrins or α-diols can give rise to chiral epoxides.

2.2.1 From optically active α-halohydrins

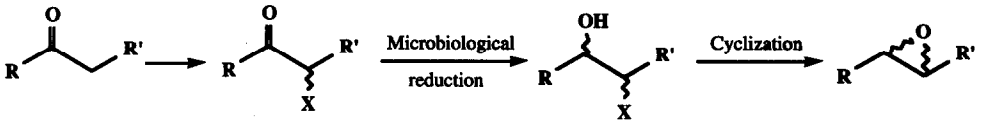
Optically active α-halohydrins can be produced by either microbiological reduction of α-haloketones or enzymatic hydrolysis of chloroacetate[162]. The epoxides are then chemically obtained by a treating with a base (KOH, NaOH).

Microbial reduction of α-haloketones has only been reported for a very limited number of substrates and microorganisms. UTAKA and coworkers[163] reduce linear unsaturated α-choroketones using bakers' yeast. Two isomers of chlorohydrin are obtained with very high enantiomeric excesses.



A more detailed study about the effects of the halide used and bioconversion conditions is reported by de CARVALHO and OKAMOTO[164]. Best results are obtained by using bakers' yeast under non-fermenting conditions to reduce chloroacetophenone. There is no conversion of the α-halohydrin to the corresponding epoxides. CABON and coworkers[165] have reported stereospecific reduction of α-halogeno-β-ketoesters, key intermediates in the synthesis of biologically active products such as Diltiazem and the side chain of taxol using various microorganisms. The corresponding halogenoesters are produced in very high enantiomeric excesses and variable yields since competitive dechlorination reaction takes place. The halogenoesters are converted to 2,3-epoxyesters using various alkaline reagents. The enantiomeric excesses of the epoxides are the same as those of the starting alcohol.

BESSE and VESCHAMBRE[166,167] succeeded in preparing the four isomers of various halohydrins (bromohydrins and chlorohydrins) by appropriate microbiological reduction (yeasts, fungi or bacteria) of the corresponding haloketones.



X = Br, Cl

R = CH_3

R' = C_5H_{11}, Benzyle or Phenyle

Chiral halohydrins obtained by microbiological reduction of α-haloketones

	Microorganism	Halohydrin		Yield
		Conf.	e.e.	
R = CH_3, R' = C_5H_{11} X = Br	Freeze-dried Bakers' yeast	(2S,3S)	≥ 98 %	25 %
	Freeze-dried Bakers' yeast	(2S,3R)	≥ 98 %	25 %
	Aspergillus niger	(2R,3R)	≥ 98 %	48 %
	Lactobacillus kefir	(2R,3S)	92 %	24 %
R = CH_3, R' = Benzyle X = Br	Freeze-dried Bakers' yeast	(2S,3S)	≥ 95 %	56 %
	Freeze-dried Bakers' yeast	(2S,3R)	≥ 95 %	37 %
	Aspergillus niger	(2R,3R)	≥ 95 %	48 %
	Cunninghamella elegans	(2R,3S)	92 %	16 %
R = CH_3, R' = Phenyle X = Cl	Freze-dried Bakers' yeast	(1S,2S)	≥ 98 %	21 %
	Rhodotorula glutinis	(1S,2R)	≥ 98 %	11 %

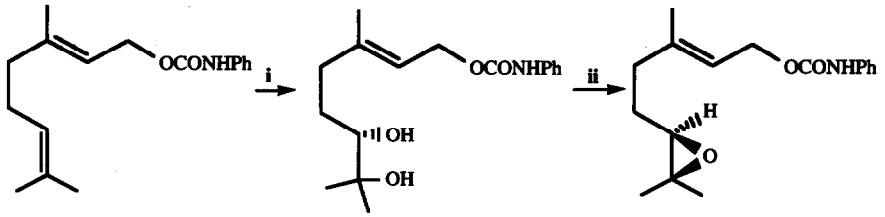
The optically pure halohydrins were then converted to epoxides by different alkaline treatments. The four isomers of 2,3-epoxyoctane, 4-phenyl-2,3-epoxybutane and oxide of β-methylstyrene were obtained with very high enantiomeric excesses and good yields[168].

WEIJERS and coworkers[169] screened a number of yeasts and bacteria for their ability to reduce α-halogenated ketones. The halohydrins obtained are directly converted in the bioconversion medium into epoxides. The enantiomeric excess and absolute configuration for a given 1,2-epoxyalkane varies with the microorganism used. No organism was found to achieve enantioselective reduction of 2,3-haloketones.

2.2.2 From optically active diols

Optically active α-diols can be produced biologically from either α-diketones[170] or ethylenic compounds *via* an epoxide which is not isolated[171]. Depending on the microorganism used and the bioconversion conditions, different diol enantiomers are obtained.

FOURNERON and coworkers[172] have developed a method for diol cyclization when one of the hydroxyl functions is attached to a quaternary carbon atom.



(2S) (2R)

ee ≥ 95 %

i- *A. niger* ii- *p*-toluenesulfonylchloride / dry benzene then NaH

2.3 Conclusion

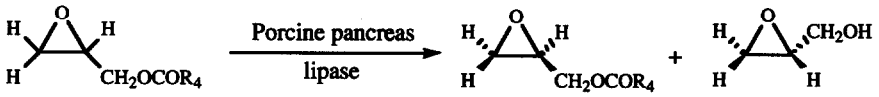
The use of peroxidases, although it has the advantage of being a natural method, does not resolve all the problems posed. Indeed, epoxides are often in a racemic form. The solution lies in synthetizing an optically pure chiral precursor such as a halohydrin or a diol which can then be cyclized to obtain the chiral epoxide.

3 ENZYMATIC RESOLUTION

Enantiomerically enriched epoxides can be prepared by enzymatic resolution. Either an enantiomer of a racemic mixture is metabolized faster than the other, or enzymatic resolution is due to another function on the epoxide (e.g. ester hydrolysis).

3.1 Use of lipases

Racemic epoxides are cheap and easily available *via* various chemical processes. Chiral epoxyalcohols can be obtained indirectly from a racemic mixture by the selective hydrolysis of glycidol esters by lipases[173].

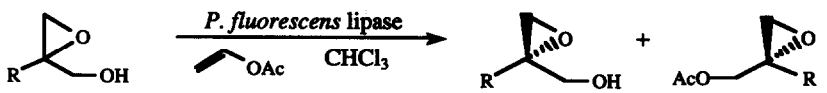


$R_4 = CH_3$ ee = 55 %

R_4 = n-butyl ee = 98 %

The enantioselectivity depends on the R_4 structure. The longer the carbon chain is, the higher the enantiomeric excess.

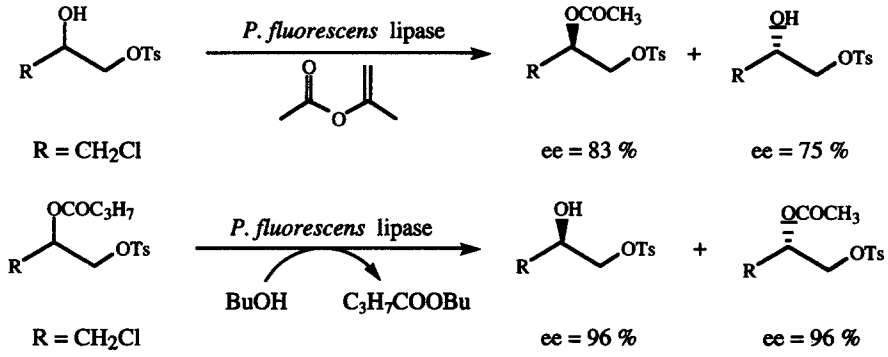
Epoxyalcohols can also be obtained with very high enantiomeric excesses if hydrolysis is stopped at a low conversion rate. This method has been used by many people[174,175]. Minor variations in the method have appeared : the transesterification process, discovered by DEGUEIL-CASTAING and coworkers[176] and applied, for example, to the synthesis of 2-substituted epoxyalkanols[177] gives excellent results.



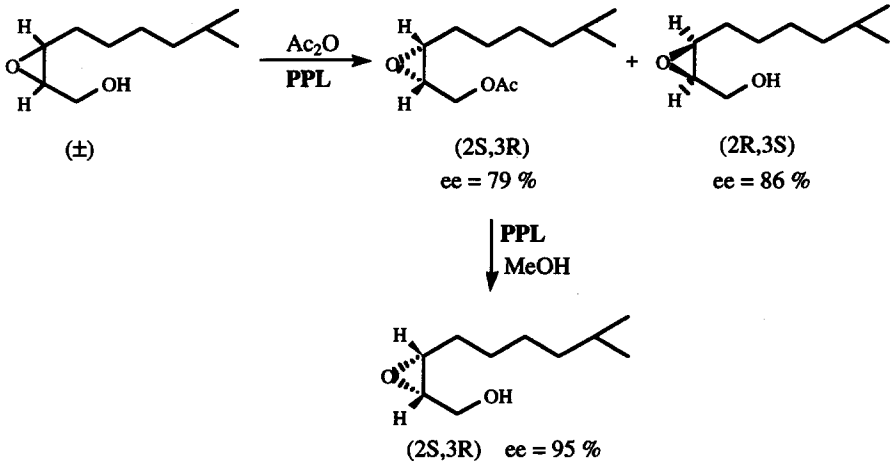
(R,S) (S) (R)

ee ≥ 98 %

Various optically active 1,2-epoxyalkanes, which do not bear other functional groups, can also be obtained : the lipase mediates either the esterification or the hydrolysis and an optically pure diol monotosylate is synthetized[178].



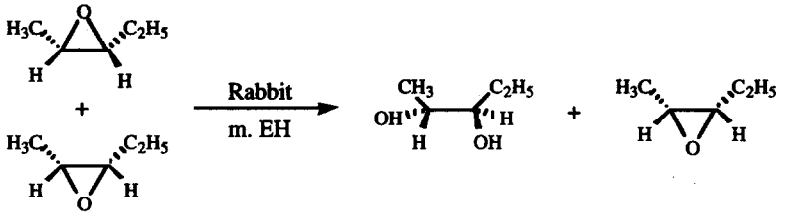
The optically pure alcohols are then converted by alkaline treatment into high yields of the corresponding epoxides. This method has been applied to the synthesis of (2S,3R)-2,3-epoxy-8-methyl-1-nonanol, a key intermediate in the synthesis of the sex pheromone disparlure, using pig pancreatic lipase (PPL)[179].



3.2 Enantioselective degradation of epoxides

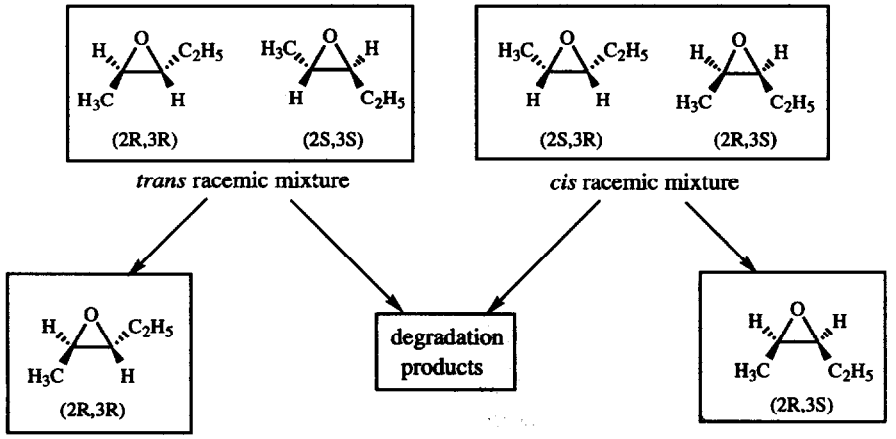
Another way to obtain optically active pure epoxides is to use enzymes which degrade epoxides enantioselectively. Epoxides are biologically toxic compounds which are rapidly enzymatically metabolized into less toxic products, generally diols. Many papers report studies about detoxifying enzymes (epoxide hydrolases, both microsomal (m EH) and cytosolic (c EH) from mammal liver[180]). This method implies the direct degradation of one stereoisomer from a racemic mixture. The reaction is schematized on the next page[181].

Many studies have demonstrated that depending on the epoxide type and the epoxide hydrolase (cytosolic or microsomal), enantiomeric excesses can vary between 20 and 98%[182]. However, this method is difficult on an industrial scale.



cis racemic mixture (2R,3R) (2S,3R) ee ≥ 98 %

Another approach is the use of whole-cell microorganisms. Very few reports refer to microbial enzymes. WEIJERS and coworkers[183,184] report that *Xanthobacter* PY_2 degrades epoxides stereoselectively: this microorganism only metabolizes (2S)-enantiomers, resulting in optically pure (2R,3S) and (2R,3R) isomers respectively from the racemic mixture of *cis* and *trans* epoxides.



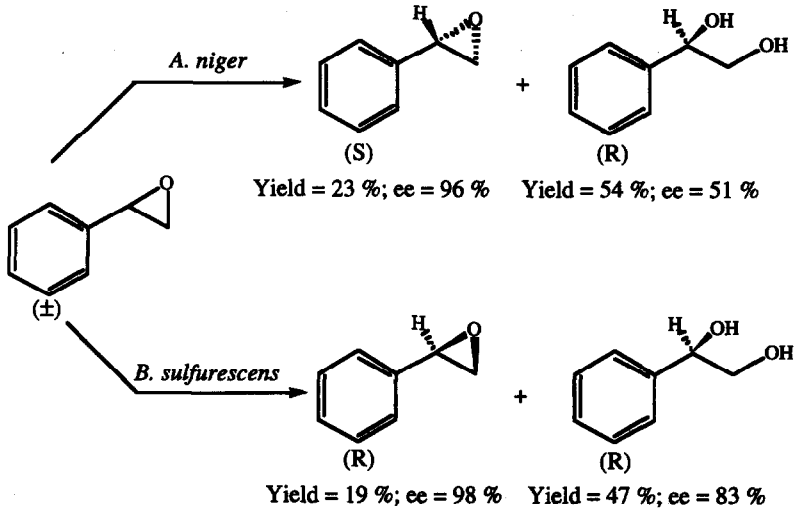
It is also possible to resolve racemic mixtures.

The same team[185] has recently found another strain (*Nocardia* H8) which is able to discriminate between enantiomeric forms of several 1,2-epoxyalkanes. Indeed, both enantiomers of 1,2-epoxides are fully degraded but at different rates and as in the previous case, optically pure (2R) epoxides can be obtained. For 2,3-epoxides, degradation with *Nocardia* gives the same results as those with *Xanthobacter*. The (2S) enantiomer is not metabolized and is obtained with an enantiomeric excess greater than 98%.

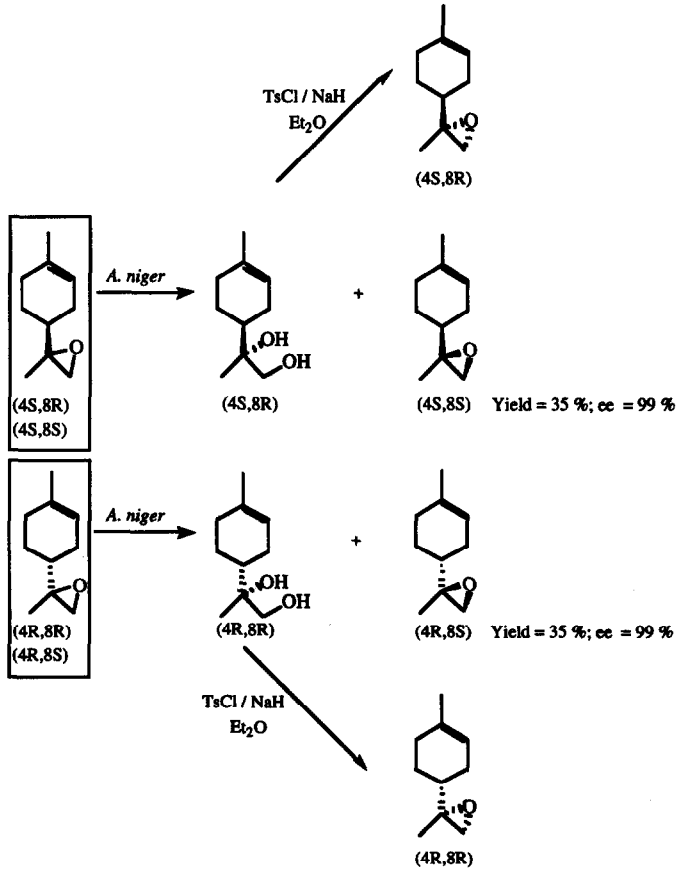
Other work has shown that epoxide hydrolases are present in many microorganisms: *Rhodococcus* sp.[186], *Aspergillus niger*[187], *Beauveria sulfurescens*[188]. Microbial enantioselective hydrolysis is a very interesting method for producing epoxides with very high enantiomeric excesses and on a multigram preparative scale. Two approach strategies have been exploited by the FURSTOSS team:

- either enantioselective discrimination of a racemic mixture[188].

Two microorganisms -fungi- can perform highly enantioselective hydrolysis of styrene oxide.



• or diastereoselective hydrolysis of a mixture of diastereoisomers[187]



This method yields the four stereoisomers of 8,9-epoxylimonene in optically pure form.

In both cases, all the isomers of the epoxide can be produced on an industrial scale with high optical purity.

3.3 Conclusion

All these enzymatic resolution methods can convert inexpensive and readily available racemic mixtures of epoxides into optically active epoxides with excellent enantiomeric excesses. The procedure is easy. The major disadvantage of these methods is that the theoretical maximum yield of the chiral product (based on the racemic starting material) is usually 50% which is fairly low. However, resolution is a good method of preparing optically pure 2,3-epoxides.

4 Conclusion

Although an initial broad screening of microorganisms is necessary, enzymatic methods seem well adapted to the production of asymmetric epoxides. Epoxidation conditions are gentle, the catalytic effect and stereospecificity are very good and the starting product does not need to have a particular structure.

Microbial epoxidations of alkenes (C6 to C20) have been tested in the preparation of chiral (R) epoxides but only at a terminal position. Generally, these biological conversions yield products having a 70 to 100% enantiomeric excess with average yields.

Microbial reduction of haloketones or diketones can yield all the isomers of the corresponding halohydrins or diols with very high yields and excellent enantiomeric excesses. These precursors can then be converted into optically pure epoxides.

Optically pure linear epoxides can be produced using enzymatic resolution but the yield is limited to 50 % and often only one configuration of the epoxide is obtained.

IV - CONCLUSION

This report about chiral epoxide synthesis illustrates the broad diversity of chemical and biological methods available.

Chemical methods, especially the Sharpless reagent, result in the majority of cases in epoxides having enantiomeric excesses greater than 90 % in most of the cases. Organometallic catalysts can "work under more flexible conditions than biological systems. Also, they don't need to work in water and don't have complicated cofactors and all these things around that has to be gotten rid of when the product is purified" (KB SHARPLESS)[25]. However the substrates which can be used are limited because the method only works with allylic alcohols, although recent studies using porphyrin and salen metal complexes are highly promising.

Microbiological studies result in the formation of terminal epoxides of the (R) configuration. Yields are average but the enantiomeric excesses are excellent. Substrate specificity, the necessity to work in water and enzyme inhibition by the product are the major disadvantages to these methods.

In the final analysis, any method is universal. Perhaps the easiest way is to start from a chiral precursor and to convert it into an epoxide. However the problem then exists as how to synthetize the optically pure precursor !!

Remarks : Chiral epoxides are useful synthons in organic synthesis and the different ways to synthesize them have been extensively studied. This report does not claim to be exhaustive and we may have omitted some work that should have been included. For any such oversights that may have occured, we extend our apologies.

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