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Determination of the regioselectivity during epoxide hydrolase oxirane ring opening: a new method from racemic epoxides

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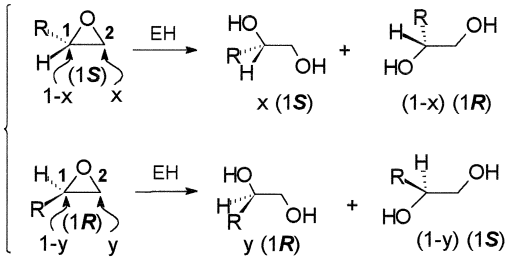
Abstract

We describe here a new method for the determination of the regioselectivity of the oxirane ring opening involved in the Epoxide Hydrolase (EH) catalysed hydrolysis of epoxides, simply by starting from the racemic epoxide as a substrate. This method also allows to simultaneously determine the *E* ratio according to Sih's equation, whose applicability in this context is discussed. This approach affords a complete characterization of the biocatalysed epoxide opening, where three different stereochemical behaviours may be distinguished.

Keywords: Epoxide ; Diol; Chiral; Epoxide hydrolase; Regioselectivity

1. Introduction

The value of epoxides and/or of their corresponding vicinal diols as synthetic intermediates for the total synthesis of optically active drugs emphasises the need to obtain these compounds in a high state of enantiomeric purity. In addition to chemo-catalytic methods [1–3] they can be obtained by using enzymes—i.e., Epoxide Hydrolases (EHs)—which catalyse the enantioselective hydrolysis of epoxides [4,5]. However, each enantiomer of the epoxide can be opened, following different kinetics, through attack at either, or both, carbon atoms of the oxirane ring. Thus, the ee of the formed diol does not depend only on the enantioselectivity of the reaction but also on its regioselectivity [6]. We describe here a new, accurate and straightforward method for the determination of this regioselectivity. This approach differs from the two main methods used up to now which consisted either in carrying out ^{18}O labelling experiments [7–11] or in performing separately the biohydrolysis of both enantiopure enantiomers [12]. Owing to the different possible stereochemical outcomes of the hydrolytic pro-



Scheme 1. General scheme of the EH catalysed hydrolysis of epoxides.

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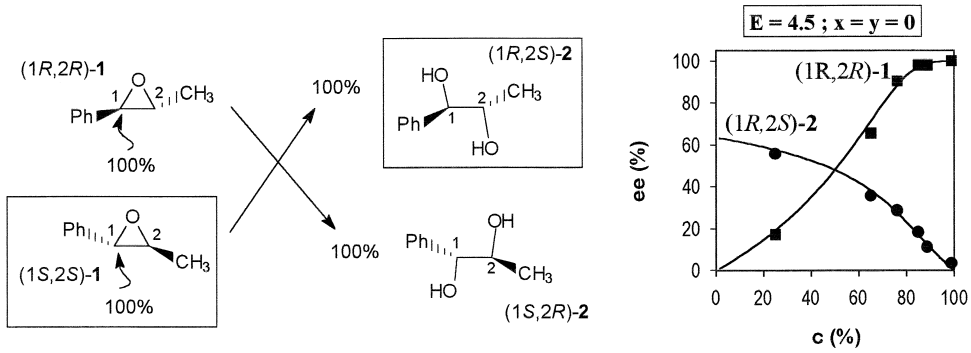

Fig. 1. Total regioselectivity on the same carbon atom ($x = y = 0$ or 1).

cess-due to the regioselectivity of the reaction-the applicability of the well-known Sih's equations [13] for the quantification of the enantioselectivity (E value) will be also discussed. This will be illustrated using results we have observed in the course of our work, aimed to study the scope and limitations of the EH catalysed hydrolyses on various substrates.

2. Results

2.1. Theoretical approach

On the basis of the mechanism of mammalian EHs [14] and of those proposed using fungal [7] and bacterial [8] EHs described in the literature, it is reasonable to postulate that an anti opening is always implied with EHs. Scheme 1 represents the general scheme of such an hydrolysis which can lead to the formation of both enantiomers of the diol from each one of the epoxide enantiomers. [1] In this scheme, the regioselectivity of the hydrolysis is defined by the x and y parameters called 'regioselectivity coefficients'. Thus, x represents the fraction of (1S)-epoxide attacked at the C-2 carbon atom, and therefore the fraction of (1S)-diol formed by hydrolysis of the (1S)-epoxide. Similarly, y represents the fraction of (1R)-epoxide attacked at the C-2 carbon atom, and therefore the fraction of (1R)-diol formed by hydrolysis of the (1R)-epoxide.

The outcome of such a reaction can be described using the following equations:

$$R_d = (R_o - R)y + (S_o - S)(1 - x), \quad (1)$$

$$S_d = (R_o - R)(1 - y) + (S_o - S)x, \quad (2)$$

$$\overline{eep} = \frac{1}{c}\left[\frac{R - S}{R_o + S_o} + \frac{2yR_o}{R_o + S_o} - \frac{2xS_o}{R_o + S_o} + \frac{2xS}{R_o + S_o} - \frac{2yR}{R_o + S_o}\right], \quad (3)$$

$$R = (1 - c)(R_o + S_o)(1 - \overline{ees})/2, \quad (4)$$

$$S = (1 - c)(R_o + S_o)(1 + \overline{ees})/2, \quad (5)$$

$$\overline{eep} = y - x + (x + y - 1)\frac{(1 - c)}{c}\overline{ees}. \quad (6)$$

In these equations, the variables are defined as follows: S_o, R_o = Initial concentration of (1S) and (1R) epoxide; S, R and S_d, R_d = Concentration of the (1S) or (1R) epoxide and diol, respectively; $\overline{ees}$ = Algebraic value of the epoxide ee = $(S - R)/(S + R)$ ($1 \geq \overline{ees} \geq -1$; $\overline{ees} > 0$ for $S > R$); $\overline{eep}$ = Algebraic value of the diol ee = $(R_d - S_d)/(S_d + R_d)$ ($1 \geq \overline{eep} \geq -1$; $\overline{eep} > 0$ for $R_d > S_d$); c = Conversion rate ($1 \geq c \geq 0$); x, y = Regioselectivity coefficients ($1 \geq x, y \geq 0$).

[1] To simplify the presentation, this diagram represents the case of the biohydrolysis of a monosubstituted epoxide, but it remains valid for any differently substituted epoxide. In order to keep homogeneity, the oxirane ring is numbered with the convention that the carbon atom C-1 is bearing the 'bigger' substituent.

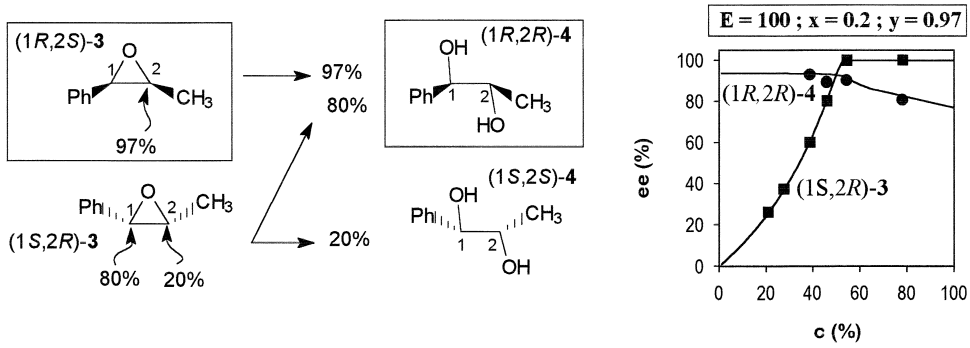

Fig. 2. Partial regioselectivity (x and $y \neq 0$ or 1): decrease of eep.

From Eqs. (1) and (2), Eq. (3) can be obtained, which does express the ee of the formed diol ($\overline{\text{eep}}$) vs. the concentration of each enantiomer of the epoxide and the regioselectivity coefficients x and y. Similarly, the concentration of each enantiomer of the residual epoxide can be expressed in Eqs. (4) and (5) as a function of their $\overline{\text{ees}}$ and of the conversion rate (c). Combination of Eqs. (3)–(5) led to Eq. (6) which represents the hydrolysis of a racemic epoxide ($R_o = S_o$). A simplification of this equation is obtained for $c = 1$ (total conversion), the ee of the 'final' diol being at this stage equal to the difference of the two regioselectivity coefficients y and x.

The use of Eq. (6) allows to determine the values of the regioselectivity coefficients x and y from experimentally determined values of c, $\overline{\text{ees}}$ and $\overline{\text{eep}}$, using a 'curve fitting' software. [2] From these values of c and $\overline{\text{ees}}$, the E value of the reaction may also be quantified by applying the well-known Sih's equation (Eq. (7)) [13]:

$$E = \frac{\ln(1-c)(1-\text{ees})}{\ln(1-c)(1+\text{ees})}. \tag{7}$$

However, because of the possible occurrence of different regioselectivities, Sih's equation involving the eep value will only be valid if the hydrolysis of one enantiomer of the epoxide only affords one enantiomer of the diol, the other one leading to the other enantiomer, i.e., if $x = y = 1$ or $x = y = 0$. To obtain an equation allowing for the determination of the E value vs. eep, we combined Eqs. (6) and (7). This led to Eqs. (8) and (9), where the x and y regioselectivity coefficients are explicited. Both these equations could thus be used to determine the E value vs. eep, using either the ees or the c values, if the regioselectivity coefficients x and y are known. If $\overline{\text{ees}} > 0$, the result obtained will be the E value, whereas if $\overline{\text{ees}} < 0$, the value obtained is $1/E$.

$$\frac{\ln\left[1 + \dfrac{c(1-2x-\overline{\text{eep}})}{x+y-1}\right]}{\ln\left[1 + \dfrac{c(1-2y+\overline{\text{eep}})}{x+y-1}\right]}, \tag{8}$$

and

$$\frac{\ln \dfrac{1-\overline{\text{ees}}}{1 + \dfrac{x+y-1}{x-y+\overline{\text{eep}}}\overline{\text{ees}}}}{\ln \dfrac{1+\overline{\text{ees}}}{1 + \dfrac{x+y-1}{x-y+\overline{\text{eep}}}\overline{\text{ees}}}}, \tag{9}$$

equal to E if $\overline{\text{ees}} > 0$; equal to $1/E$ if $\overline{\text{ees}} < 0$.

[2] The regioselectivity coefficients were calculated by nonlinear regression of the Eq. (6) using the commercial Jandel software Sigmaplot® (Marquardt–Levenberg algorithm).

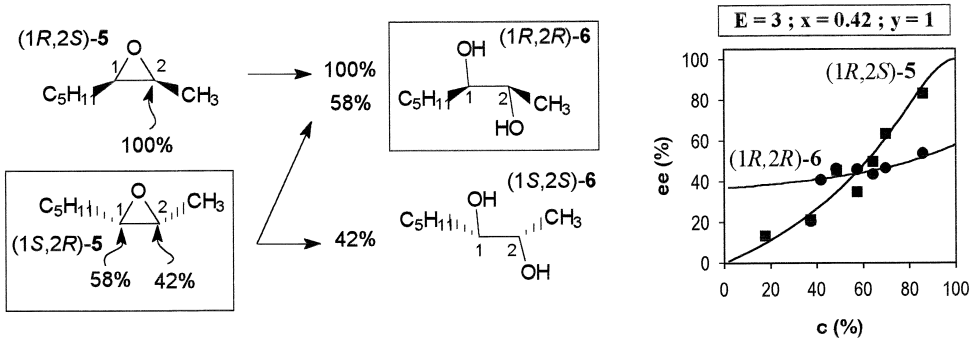

Fig. 3. Partial regioselectivity (x and $y \neq 0$ or 1): increase of eep.

2.2. Applications

From a theoretical point of view, different stereochemical behaviours can be observed starting from a racemic epoxide, since the preferential attack can occur either at the same carbon atom for both enantiomers or at one carbon atom for one enantiomer, but on the other one for its antipode. This may lead to three different figures as illustrated below through different examples of biohydrolyses carried out using an enzymatic extract from the fungus *Chaetomium globosum* LCP 679 [3] (prepared in a similar manner than the enzymatic extract of *Aspergillus niger* as described previously [15].

2.2.1. Total regioselectivity at the same carbon atom ($x = y = 0$ or 1)

Such a case has been observed, e.g., during biohydrolysis of *trans*-2-methyl-1-phenyloxirane-**1** (Fig. 1). [4] Obviously in this case, the stereochemical outcome followed the classical kinetic resolution scheme, where the ee of the product (formed diol) decreased during the reaction to reach a 0 value for $c = 1$ ($y - x = 0$), whereas the ee of the remaining epoxide increased up to 100%.

2.2.2. Partial regioselectivity (x and $y \neq 0$ or 1)

In this case, the formed diol was *not* racemic ($y - x \neq 0$) at a total conversion ratio ($c = 1$). Two different outcomes for the eep can be observed.

(a) Decrease of the eep during biohydrolysis. Such a feature was observed during biohydrolysis of racemic *cis*-2-methyl-1-phenyloxirane **3** (Fig. 2).

(b) Increase of the formed diol ee during biohydrolysis. This case was observed during biohydrolysis of *cis*-2-methyl-1-pentyloxirane **5** (Fig. 3).

2.2.3. Enantioconvergent biohydrolysis ($x = 0$, $y = 1$ or $x = 1$, $y = 0$)

Such a case has been obtained during biohydrolysis of *trans*-2-methyl-1-pentyloxirane **7** (Fig. 4). Thus, the ee of the formed diol remained high and constant during the course of the biohydrolysis.

2.2.3.1. Limitations. According to Eq. (6), it is not possible to analyse such a reaction if the ee stays equal to zero all over the reaction course (non enantioselective reaction: $E = 1$). One il-

[3] The reactions were carried out in 2 ml stirred reactors, at 27°C, in a 0.1 M potassium phosphate buffer pH 8.0 in the presence of 0.25% of DMF (v/v) and of 5–60 mg of freeze–dried enzymatic extract; the initial concentration in racemic epoxide was between 3.3 and 5.8 mM. The course of the reaction was analysed during a maximal period of 24 h.

[4] By convention, the frame enantiomer of the epoxide is the faster hydrolysedone, and the framed enantiomer of the diol is the one formed in excess.

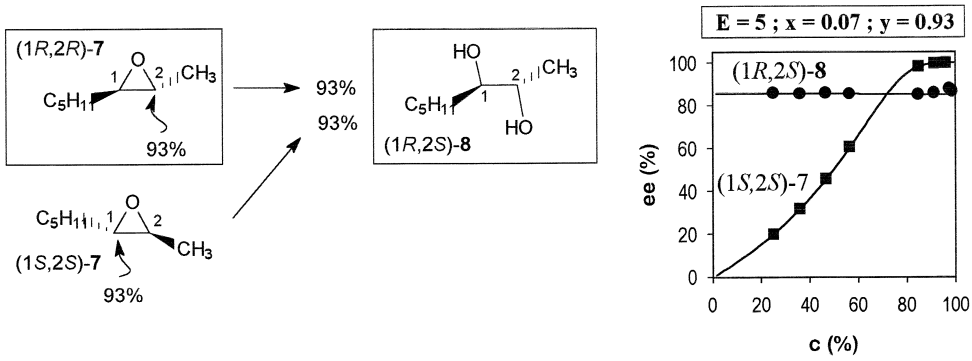

Fig. 4. Enantioconvergent biohydrolysis ($x = 0,\ y = 1$ or $x = 1,\ y = 0$).

lustration of such a case has been observed during biohydrolysis of hexyl-oxirane.

3. Conclusion

In the course of this work, we have devised a new method which allows the determination of both the regioselectivity and the enantioselectivity of an Epoxide Hydrolase-catalysed hydrolysis of an epoxide. This is achievable simply by determining, experimentally, the values of the conversion ratio, the ee of the residual epoxide and that of the formed diol, starting from the racemic epoxide. This approach makes things easier than the previously used methods. As far as regioselectivity is concerned, the results displayed in this study show the occurrence of three different behaviours: (a) a total regioselectivity on the same carbon atom, (b) a partial regioselectivity between the two carbon atoms of the oxirane ring, and (c) a total but opposite regioselectivity for each enantiomer. This last case leads to a so-called 'enantioconvergent' process which can afford an optically pure diol with a theoretical yield of 100%.

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