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Kinetics of base catalyzed racemization of ibuprofen enantiomers

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Abstract

The kinetics of base catalyzed racemization of ibuprofen enantiomers has been studied in DMSO-water mixed medium. The dynamic equilibrium rate of keto-enol tautomerism leading to racemization of ibuprofen enantiomers, is proportional to the concentrations of base catalyst and substrate. A kinetic model capable of predicting the time course of racemization, under different base and substrate concentrations, is established and experimentally verified.

Keywords: Ibuprofen enantiomer; Resolution; Racemization kinetics; Base catalysis

1. Introduction

There is today, an increasing trend towards the use of optically pure enantiomers for chiral drugs, because they are more target-specific and have fewer side effects than racemic mixtures (Stinson, 1995). Ibuprofen, *R*,*S*-2-(4-isobutylphenyl) propionic acid, is an important member of the non steroidal anti-inflammatory drugs (NSAIDS) consisting of 2-arylpropionic acids (profens), its racemic mixture of the two enantiomers R-(−) and S-(+) is being used, but its biological activity is mainly from the *S*-enantiomer (Hutt and Caldwell, 1984). In recent years, the large scale preparation of biologically active *S*-ibuprofen has been intensively studied, with the methods including asymmetric synthesis (Sonawane, et al., 1992), chemical resolution (Geroffen, 1990) and enzyme catalyzed kinetic resolution (Roure, et al., 1997; Tsai, et al., 1997). An advantage of using resolution method is that the well developed technology to produce racemate, can be utilized.

For any resolution process of a racemic mixture, the maximum theoretical yield of the target enantiomer is 50%. If the other undesired enantiomer can be racemized and then re-resolved, the racemate can be transformed completely to the desired enantiomer, by cyclic resolution. Therefore, racemization of the undesired enantiomer is

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very important to the economic feasibility of a resolution process. In our previous paper, it is reported that ibuprofen enantiomers are highly stable in acidic medium and racemize through a proposed keto-enol dynamic equilibrium in basic medium and medium composition has a strong effect on the racemization rate (Xie, et al., 1998a). In the present paper, the racemization kinetics of ibuprofen enantiomers, through base catalyzed keto-enol tautomerism has been studied in DMSO-water mixed medium, a kinetic model is established to predict the time course of racemization under different base and *R*- or S-ibuprofen concentrations.

2. Materials and methods

2.1. Materials

Racemic ibuprofen and *Candida rugosa* lipase (type VII, 835 units mg^{-1} solid) were purchased from Sigma, St Louis, MO. Celite was purchased from Beijing Chemical Reagent Company. Hexane, 2-propanol and acetic acid were of HPLC grade. Butanol, DMSO (dimethylsulfoxide), triethylamine, isooctane and other solvents as well as reagents used were of analytical grade, all were obtained commercially.

2.2. Chiral HPLC analysis

The enantiomeric excess of ibuprofen enantiomers and lipase catalyzed esterification conversion, were analyzed respectively by HPLC with a chiral column (Regis (*S*,*S*) Whelk-01, Morton Grove, IL 60053) capable of separating the *R*- and *S*-enantiomers of ibuprofen, without previous derivatization, with the ester enantiomers as the third peak. The mobile phase was composed of *n*-hexane:2-propanol:acetic acid:triethylamine (85:15:0.2:0.05, v/v) at a flow rate of 0.4 ml min^{-1}. The HPLC system is the HP 1090 liquid chromatograph, equipped with an automatic injector and a diode array detector (DAD). Ultraviolet detection at 254 nm, was used for quantification at ambient temperature.

2.3. Preparation of R- and S-ibuprofen

The *R*- and *S*-ibuprofen were prepared with lipase catalyzed enantioselective esterification, of racemic ibuprofen in organic solvent. The enzymatic reaction mixture was composed of isooctane (200 ml), racemic ibuprofen (200 mM), *n*-butanol (200 mM) and 1200 mg crude lipase with addition of 0.2 ml water for enzyme activation and 10 g celite for the dispersion of lipase particles (Xie, et al., 1998b). The reaction was carried out at 40°C by shaking at 180 rpm and stopped at a conversion of $c = 44.1\%$ and unreacted substrate enantiomeric excess $ee_S = 75.4\%$. According to $c = ee_S/(ee_S + ee_P)$, the enantiomeric excess of the produced *S*-ester $ee_P = 95.6\%$. The unreacted *R*-enantiomer excessive ibuprofen, was extracted with 200 ml 0.5 M NaOH aqueous solution. After acidified to pH < 2, the *R*-ibuprofen was extracted into 200 ml hexane. By removing the solvent under reduced pressure, 4.53 g *R*-ibuprofen (75.4% *ee*) was obtained.

The formed *S*-ester was separated by evaporating the isooctane under reduced pressure and dissolved in 100 ml DMSO. Forty milliliters of aqueous solution of 2 M H_2SO_4 was added to catalyze the chemical hydrolysis of the ester to produce *S*-ibuprofen (Xie, et al., 1998a). After a 5 h reaction at 100°C, the *S*-ester was completely hydrolyzed to *S*-ibuprofen with enantiomeric excess of 95.4%. The *S*-ibuprofen was extracted into 200 ml hexane and 3.52 g *S*-ibuprofen was obtained by removing the hexane under reduced pressure.

2.4. Racemization of ibuprofen enantiomers

All racemization reactions were carried out at 100°C in a thermostatic water bath. 10 mM *R*-ibuprofen was added to 5 ml different DMSO-water mixtures, respectively containing 100, 80, 60, 40 and 20% (v/v) DMSO for 6 h incubation, to investigate the enantiomeric stability in these media. 10 mM *R*-ibuprofen and 0.5 M NaOH were added to 5 ml different DMSO-water mixed media, respectively containing 80, 60, 40 and 20% (v/v) and no DMSO for 4 h incubation, to investigate the effect of DMSO content on the racemiza-

tion rate. At constant *R*-ibuprofen (10 mM) or NaOH (0.5 M) concentration in DMSO-water (4:1, v/v) mixed medium, racemization was, respectively carried out at varied NaOH concentration 0.1, 0.2, 0.3, 0.4, 0.5 and 0.7 M or varied *R*-ibuprofen concentration 1, 5, 10, 20, 30 and 50 mM, to, respectively investigate the effect of base or substrate concentration on the racemization rate. Samples were taken at different time intervals. After acidification the racemized *R*-ibuprofen was extracted with hexane for chiral HPLC analysis for the residual enantiomeric excess. The 24 h time courses of different concentration combinations of base-*R*-ibuprofen 0.2 M to 5 mM, 0.4 M to 10 mM, 0.6 M to 20 mM and base-*S*-ibuprofen 0.2 M to 10 mM, 0.4 M to 30 mM, 0.6 M to 50 mM were detected at different time intervals, to verify the predicting results of the kinetic model.

3. Results and discussion

3.1. Kinetic model of racemization

In basic medium, the dynamic equilibrium between ibuprofen enantiomers and the enolate may be shown in Fig. 1 (March, 1992).

Because the enolate is instable and its concentration is generally very low, the tautomerism can be thought to reach a steady state quickly, with a dynamic equilibrium rate V_{eq}. Because the catalyst OH^- is achiral, it can not discriminate between the two enantiomers. Therefore, the dynamic equilibrium rate after racemization time t devoted, respectively by the *R*- and *S*-enantiomer V_t^S and V_t^R is proportional to its concentration, as described by Eq. (1) and Eq. (2).

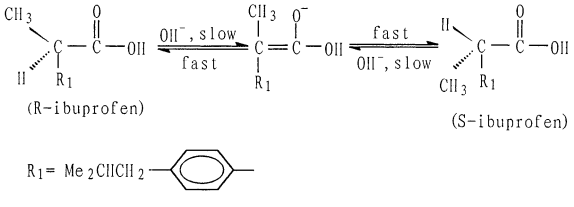

Fig. 1. Racemization of ibuprofen enantiomers by base catalyzed keto-enol tautomerism.

$$V_t^R = \frac{[A^R]_t}{[A^R]_t + [A^S]_t} V_{eq} \quad (1)$$

$$V_t^S = \frac{[A^S]_t}{[A^R]_t + [A^S]_t} V_{eq} \quad (2)$$

During steady state racemization,

$$V_t^R + V_t^S = V_{eq}$$

and $[A^R]_t + [A^S]_t = [A]_0$ (the initial substrate concentration), they remain unchanged. In time Δt, the amount of *R*- and *S*-ibuprofen transformed to enolate is $V_t^R \cdot \Delta t$ and $V_t^S \cdot \Delta t$, respectively, while the amount of the two enantiomers formed from the enolate is both $\frac{1}{2}V_t^R + \frac{1}{2}V_t^S$, because the probabilities of the achiral enolate tautomerizing to the two enantiomers, are equal. Then, the concentration of the two enantiomers at time $t + \Delta t$ can be, respectively written as Eq. (3) and Eq. (4).

$$[A^R]_{t+\Delta t} = [A^R]_t + \left(\frac{1}{2}V_t^R + \frac{1}{2}V_t^S\right)\Delta t - V_t^R \Delta t$$

$$= [A^R]_t + \frac{1}{2}(V_t^S - V_t^R)\Delta t \quad (3)$$

$$[A^S]_{t+\Delta t} = [A^S]_t + \left(\frac{1}{2}V_t^R + \frac{1}{2}V_t^S\right)\Delta t - V_t^S \Delta t$$

$$= [A^S]_t + \frac{1}{2}(V_t^R - V_t^S)\Delta t \quad (4)$$

when $\Delta t \to 0$, the equations describing the change of the enantiomer concentrations can be obtained as Eq. (5) and Eq. (6).

$$\frac{d[A_t^R]}{dt} = \frac{1}{2}(V_t^S - V_t^R) \quad (5)$$

$$\frac{d[A_t^S]}{dt} = \frac{1}{2}(V_t^R - V_t^S) \quad (6)$$

Because the enantiomeric excess $ee_t = ([A^R]_t - [A^S]_t)/([A^R]_t + [A^S]_t)$, from Eqs. (5) and (6), $d[A^R]_t/dt = -(V_{eq}/2)ee_t$ and $d[A^S]_t/dt = (V_{eq}/2)ee_t$ can be obtained. By bringing these two expressions into the differentiated form of ee_t, Eq. (7) can be obtained.

$$\frac{d\,ee_t}{dt} = \frac{1}{[A^R]_0 + [A^S]_0}\left(\frac{d[A^R]_t}{dt} - \frac{d[A^S]_t}{dt}\right)$$

$$= \frac{V_{eq}}{[A^R]_0 + [A^S]_0} ee_t \quad (7)$$

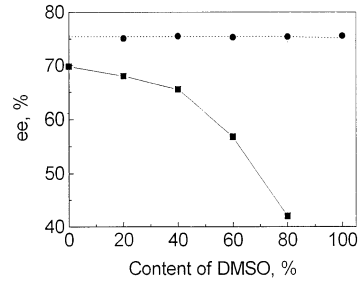

Fig. 2. Effect of DMSO content on the enantiomeric stability of R-ibuprofen (10 mM) in aqueous phase at 100°C with 0.5 M NaOH added and 4 h reaction time (■) and with no NaOH added and 6 h reaction time (●).

Integrate Eq. (7) between $t{:}0 \rightarrow t$ and $\mathrm{ee}{:}\mathrm{ee}_0 \rightarrow \mathrm{ee}_t$, the variation of enantiomeric excess during racemization can be described by Eq. (8).

$$\mathrm{ee}_t = \mathrm{ee}_0 \exp\left(-\frac{V_{\mathrm{eq}}}{[A^R]_0 + [A^S]_0} t \right) \tag{8}$$

In general, the keto-enol tautomerism rate is difficult to be detected. But in the case of tautomerism leading to enantiomer racemization, the V_{eq} can be measured by the variation of ee_t with time according to Eq. (8), the effects of base and substrate concentration can therefore be investigated.

3.2. Effect of DMSO content

The *R*- (75.4% ee) and *S*- (95.4% ee) ibuprofen used for racemization experiments, are prepared by *Candida rugosa* lipase catalyzed resolution in isooctane, as described in the experimental section. Because solvent has a strong effect on keto-enol isomerization equilibrium (Mills and Beak, 1985) and the racemization of ibuprofen enantiomers can proceed faster in basic DMSO-water mixed medium (Xie, et al., 1998a), this medium is chosen for further study. The enantiomeric stability of *R*-ibuprofen in DMSO-water mixed medium containing different amount of DMSO with and without addition of sodium hydroxide, is shown in Fig. 1. It can be seen that under the same sodium hydroxide concentration, the racemization rate (drop of ee) increases with the content of DMSO. As sodium hydroxide can not be dissolved in pure DMSO, water is needed to make the base dissolvable. The higher the DMSO content, the lower the sodium hydroxide concentration allowed to form a homogeneous phase. Although the addition of DMSO in basic aqueous medium can accelerate the racemization rate of ibuprofen enantiomer, the enantiomer is highly stable in pure DMSO and DMSO-water mixed phase if no NaOH being added. As shown in Fig. 2, with a reaction time of 6 h at 100°C in these media, the enantiomeric excess of the *R*-ibuprofen has no drop compared with the value before reaction. This indicates that there is only base catalyzed keto-enol tautomerism occurred for ibuprofen, although the uncatalyzed and acid catalyzed ketonization of some enols can take place (Kresge, 1986; Chiang et al., 1988).

3.3. Effect of base concentration

To obtain a higher racemization rate at lower base concentration, the investigation of racemization kinetics is carried out in DMSO-water mixed medium containing 80% DMSO. The 6 h racemization courses, of the prepared *R*-ibuprofen (10 mM), with an intial enantiomeric excess of 75.4% under different base concentration at 100°C are shown in Fig. 3a. The racemization rate increases with the sodium hydroxide concentration. The V_{eq} under each base concentration is obtained by fitting the corresponding time course to Eq. (8) and plotted in Fig. 3b. It can be seen that the time courses can be quite well fitted with Eq. (8) and there is a good linear relationship between V_{eq} and base concentration. This means that the dynamic equilibrium rate V_{eq} of the keto-enol tautomerism for ibuprofen with constant concentration is proportional to the sodium hydroxide concentration, as described by Eq. (9).

$$V_{\mathrm{eq}} \propto [\mathrm{NaOH}] \tag{9}$$

3.4. Effect of substrate concentration

The 6 h racemization courses of varied concentrations of the prepared *R*-enantiomer excessive ibuprofen, under the same sodium hydroxide concentration (0.5 M) at 100°C in DMSO-water (4:1,

v/v) mixed medium are shown in Fig. 4a. It can be seen that the racemization courses with different R-ibuprofen concentrations are practically overlapped and not varying with substrate concentration, the racemization rate is only dependent upon the concentration of the base catalyst. The V_{eq} with different substrate concentrations, can also be obtained by fitting the racemization courses to Eq. (8) and plotted in Fig. 4b. Again, a good linear relationship is found between V_{eq} and substrate concentration. It is to say that the dynamic equilibrium rate is also proportional to the substrate concentration. Because the enolate concentration under the keto-enol tautomerism equilibrium is very low, the total concentration of the R- and S-enantiomer of ibuprofen during racemization can be considered to be equal to the initial substrate concentration added $[A^R]_0 + [A^S]_0$. As shown in Fig. 1, the rate determining step of the keto-enol tautomerism is the enolization of ibuprofen, so at steady state the dynamic equilibrium rate is equal to the enolization rate and can be written as Eq. (10) according to Fig. 3b and Fig. 4b.

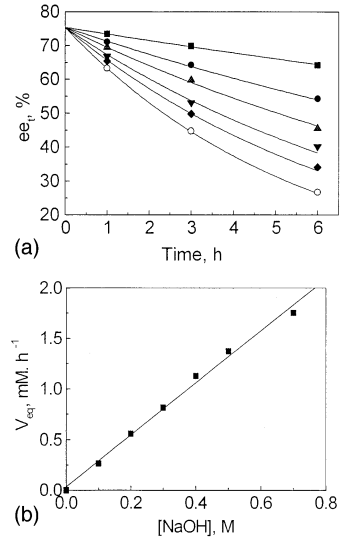

Fig. 3. Effect of sodium hydroxide concentration (■) 0.1 M; (●) 0.2 M; (▲) 0.3 M; (▼) 0.4 M; (◆) 0.5 M; (○) 0.7 M on the racemization course of *R*-ibuprofen (10 mM) (a) and variation of the keto-enol dynamic equilibrium rate V_{eq} with the base concentration (b) in DMSO-water (4:1, v/v) mixed medium at 100°C.

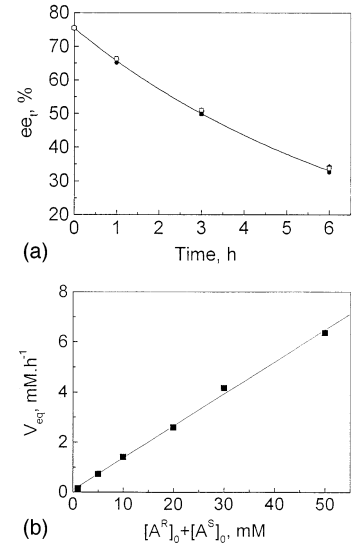

Fig. 4. Effect of *R*-enantiomer excessive ibuprofen concentration (■) 1 mM; (●) 5 mM; (▲) 10 mM; (▼) 20 mM; (◆) 30 mM; (○) 50 mM on the racemization course, solid line is the fitted result of the time course at 10 mM *R*-ibuprofen (a) and variation of the keto-enol dynamic equilibrium rate V_{eq} with the substrate concentration (b) in DMSO-water (4:1, v/v) mixed medium at 100°C under 0.5 M sodium hydroxide concentration.

$$V_{eq} = k([A^R]_0 + [A^S]_0)[OH^-] \quad (10)$$

The kinetic parameter k is obtained to be 2.57 $mM^{-1}\ h^{-1}$ by a linear analysis.

Taking Eq. (10) into Eq. (8) yields:

$$ee_t = ee_0 \exp(-k[OH^-]t) \quad (11)$$

The substrate concentation is eliminated in Eq. (11), this explains, why the variation of enantiomeric excess during racemization is related only to the base concentration and independent on the substrate concentration, as shown in Figs 3a and 4a.

3.5. Prediction of racemization time course

To verify the predicting ability of Eq. (11), the 24 h racemization courses for the prepared *R*- (75.4% ee) and S- (95.4% ee) enantiomer excessive ibuprofen are measured under different combination of base catalyst and substrate concentrations, as shown, respectively in Fig. 5a and b. It can be

seen that for both enantiomers, the predicted results are fitted well with the experimental data, in spite the enantiomeric excess of the *S*-ibuprofen is much higher than that of the *R*-ibuprofen used for kinetic study. Therefore, the enolization rate (then the dynamic equilibrium rate) can be described by Eq. (10) and the racemization caused by the keto-enol tautomerism can be predicted by the steady state model Eq. (11), which is unconcerned with the substrate concentration.

4. Conclusions

The racemization of ibuprofen enantiomers can take place when base is used as the catalyst. When DMSO-water mixture is used as the reaction medium, the racemization rate increases with the DMSO content. The proposed kinetic model of racemization can well predict the variation of enantiomeric excess during racemization with different base and substrate concentrations. The rate of the dynamic equilibrium of the tautomerism leading to racemization is proportional to the base and substrate concentration and the kinetic parameter in DMSO-water (4:1, v/v) medium is measured. The model indicates why the racemization course of ee detected only depends on the concentration of base catalyst and is not related to the substrate concentration. These results are useful for developing a racemization process of the undesired enantiomer, accompanying a resolution process of racemic ibuprofen.

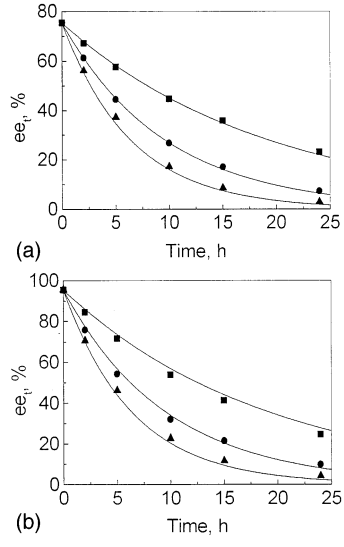

Fig. 5. Racemization courses of (a) *R*-ibuprofen with respective base and substrate concentrations (■) 0.2 M, 5 mM; (●) 0.4 M, 10 mM; (▲) 0.6 M, 20 mM and (b) *S*-ibuprofen with concentration combinations of base and substrate (■) 0.2 M, 10 mM; (●) 0.4 M, 20 mM; (▲) 0.6 M, 50 mM at 100°C in DMSO-water (4:1, v/v). Solid lines are the calculated results by Eq. (11).

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