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ARTICLE TYPE

Occurrence of spontaneous resolution of ketoprofen with a racemic crystal structure by simple crystallization under nonequilibrium preferential enrichment conditions

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Nearly racemic ketoprofen, which satisfies the requirements for the occurrence of preferential enrichment, was spontaneously resolved into the two enantiomers by simple crystallization under nonequilibrium conditions using high concentrations.

In connection with the industrial and pharmaceutical needs of chiral organic substances, exploitation of economically and environmentally acceptable enantiomeric resolution methods along with catalytic asymmetric syntheses has been the subject of considerable recent development. In this context, spontaneous resolution of racemic compound crystals, which are composed of a regular packing of a pair of *R* and *S* enantiomers in the crystal, by simple crystallization from solvents had been believed to never occur for more than a century.[1] However, recently we reported that preferential enrichment, a spontaneous resolution phenomenon observed for a certain kind of racemic mixed crystals (or solid solutions) consisting of a random arrangement of two enantiomers in the crystal,[2,3] was applicable to two amino acids (alanine and leucine) and the cocrystal of (DL)-phenylalanine with fumaric acid,[4,5] which are classified as a racemic compound with a high eutectic *ee* value[6] and at the same time display an appropriate polymorphic transition during crystallization under nonequilibrium conditions using high concentrations.

To extend the scope of preferential enrichment to pharmaceutically relevant compounds, we have carried out an extensive search in the Cambridge Structural Database (CSD) for chiral drugs with an appropriate crystal structure for exhibiting preferential enrichment. Consequently, we have found that nonsteroidal anti-inflammatory drugs (NSAID) such as ibuprofen (space group, $P2_1/c$),[7] ketoprofen (*P*-1),[8] and naproxen (*P*bca),[9] which are popular therapeutic agents for the treatment of various pathophysiological conditions, have the desired racemic crystal structure containing heterochiral *RS* dimer chains as well as homochiral 1D *R* and *S* chains. Among them, only ketoprofen satisfied all the five requirements for the occurrence of preferential enrichment which we have clarified thus far; (1) high eutectic *ee* value, (2) sufficient solubility difference (pure enantiomer >> racemate), (3) unique crystal structure and polymorphic transition, (4) partial crystal disintegration, and (5) deposition of nonracemic mixed crystals.[2-5] No polymorphic transition was observed during crystallization for ibuprofen and naproxen. Indeed, only ketoprofen showed a good preferential enrichment phenomenon, despite a single-component crystal.[2] Ketoprofen is currently marketed as the racemic drug. However,

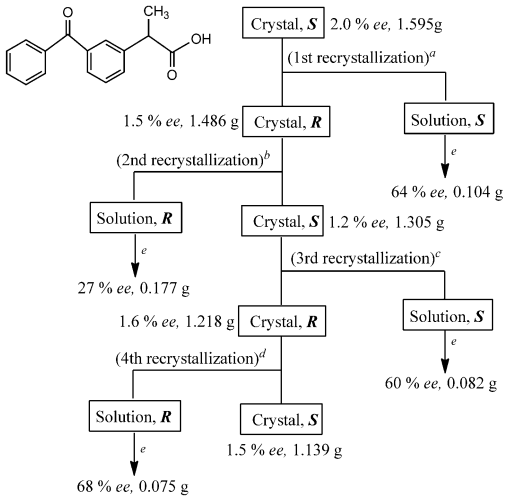

Fig. 1 Preferential enrichment of ketoprofen. Conditions: [a]H_2O-EtOH (v/v 1:1, 3.7 mL) at –16°C for 4 days; [b]H_2O-EtOH (v/v 1:1, 2.8 mL) at –16°C for 4 days; [c]H_2O-EtOH, (v/v 1:1, 2.4 mL) at –16°C for 4 days; [d]H_2O-EtOH (v/v 1:1, 2.2 mL) at –16°C for 4days; [e]removal of the solvent by evaporation. The *ee* values were determined by HPLC analysis (see Figure S1).

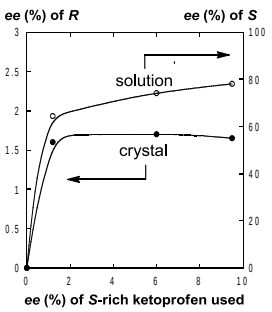

Fig. 2 Relation between the *ee* values of *S*-rich ketoprofen in the ca. 10-fold supersaturated solutions (horizontal axis) before crystallization and the chirality and reached *ee* values in the deposited crystals (left vertical axis) and in solution (right vertical axis) after crystallization. Conditions: –16°C for 4 days.

the biological activity resides in the *S* enantiomer, while the *R* enantiomer is therapeutically inactive.[10] Furthermore, it is known that there is no significant enantiomeric inversion of *R* to *S* in humans.[11] Here we report the preferential enrichment phenomenon of nearly racemic ketoprofen which can behave like the desired racemic mixed crystal showing an appropriate polymorphism and allowing the deposition of nonracemic crystals under nonequilibrium preferential enrichment conditions.

By considering the physicochemical properties of ketoprofen (solubility: 57.8 and 31.1 mg/mL for the *S* enantiomer and racemate, respectively, at 25°C in H_2O-EtOH (v/v 1:0.9)) and its binary and ternary phase diagrams (eutectic point: 82% *ee*),[10] we determined the optimum preferential enrichment conditions. Namely, when crystallization was carried out from the 10-fold supersaturated H_2O-EtOH (v/v 1:1) solution of nonracemic ketoprofen of more than 1% *ee* (e.g., *S*-rich) at –16°C, the excess *S* enantiomer was enriched in the mother liquor until the deposited crystals were slightly enriched with the opposite *R* enantiomer (Figure 1), which is characteristic of the preferential enrichment phenomenon.[2] The *ee* of *S* enantiomer in the mother liquor after crystallization rose nonlinearly with increasing *ee* of *S* enantiomer in the initial supersaturated solutions, while the *ee* of *R* enantiomer in the deposited crystals reached a plateau at around 1.5 % *ee* (Figure 2). Similar results were obtained when crystallization was performed by using *R*-rich crystals of more than 1 % *ee*. Thus, repetition of recrystallization of each crop of resulting deposited crystals successively from the supersaturated solution without stirring led regularly to not only an alternating enrichment of the two enantiomers up to 68% *ee* in the mother liquors but also a slight enrichment (ca. 1.5% *ee*) of the opposite enantiomer in the deposited crystals (Figure 1).

As a practical enantiomeric resolution technique of ketoprofen, the subsequent recrystallization of the resulting enantioenriched crystals (~68% *ee*) from ether under the conditions for depositing racemic compound crystals improved the *ee* values of *R* and *S* enantiomers up to more than 90 % in the mother liquors.

The crystal structure of (*RS*)-ketoprofen analyzed by us was identical to that retrieved from CSD (Figure 3 and S2).[8] Molecules of ketoprofen form homochiral 1D *R* and *S* chains along the *c*-axis through (i) an intermolecular C(sp^2)-H···O=C (2.66 Å) hydrogen bond [Graph set: C(6)][11] between a phenyl hydrogen atom and a carboxyl oxygen atom and (ii) another intermolecular C(sp^2)-H···O=C (2.71 Å) hydrogen bond [C(5)] between a hydrogen atom on another benzene ring and the neighboring keto carbonyl oxygen atom. Such adjacent antiparallel homochiral 1D *R* and *S* chains constitute a heterochiral *RS* dimer chain structure along the *c*-axis by the formation of a cyclic dimer structure through conventional O-H···O=C (O···O: 2.63 Å) hydrogen bonds [$R^2_2(8)$] between neighboring two carboxyl groups. These heterochiral *RS* dimer chains interact with each other by off-centered centrosymmetric C-H---π contact between the hydrogen atom on the chiral carbon atom and the neighboring phenyl ring along the *b*-axis (Figure 3b), creating a loosely packed crystal structure which can allow the partial crystal disintegration to occur in case irregular molecular alignment areas are formed in the crystal lattice under kinetic crystallization conditions (see Figure 5).

The powder X-ray diffraction pattern simulated from the X-ray crystallographic data of the single crystal of (*RS*)-ketoprofen was identical with the experimental pattern of the fine

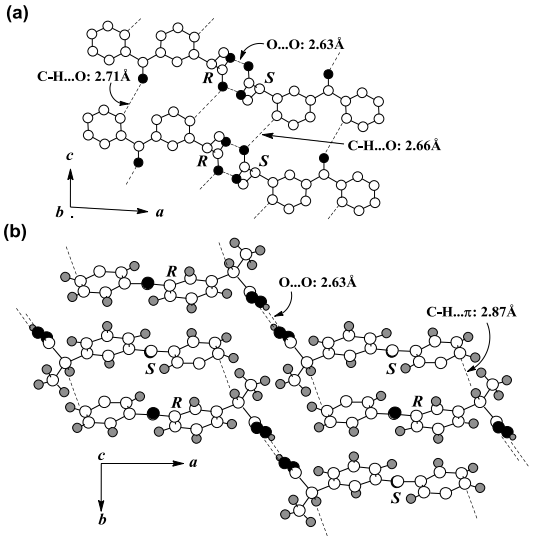

Fig. 3 Crystal structure of (*RS*)-ketoprofen. (a) A view down the *b*-axis. (b) A view down the *c*-axis. See Figure S2 for a view down the *a*-axis.

polycrystalline powder sample of the deposited nonracemic crystals of 1.2% *ee* obtained by the preferential enrichment experiment (Figure 4a). Furthermore, DSC analyses of the *RS* crystals and the deposited crystals of 1.2% *ee* showed a sharp endothermic peak at 96.7 and 96.1 °C, respectively, which were higher than that (77.6°C) of the pure *S* enantiomer (Figure 4b). These experimental results strongly suggest that (i) the deposited crystals of 1.2% *ee* are monophasic and (ii) they are neither a conglomerate nor a chiral lamellar twin of opposite handedness but either a racemic mixed crystal of two enantiomers or an achiral lamellar twin of racemic compound type.[5,6] By SEM observation of the deposited crystals, no thin plate form crystal characteristic of an achiral lamellar twin crystal was detected, but instead very fine polycrystals with irregular bumpy surfaces which were reminiscent of considerable crystal disintegration were observed (Figure S3).[4,5] Thus, from these results and X-ray crystallographic analyses, the deposition of nonracemic mixed crystals under the preferential enrichment conditions was strongly supported.

To propose the mechanism of preferential enrichment of ketoprofen, the association modes of enantiomers in the supersaturated solution just before crystallization and in the deposited crystals after crystallization were compared. The distinct difference in the in situ ATR-IR spectra between the supersaturated solution and the deposited crystals (Figure S4) suggested the occurrence of polymorphic transition during crystallization.[2-5] Based on this observation and the crystal structure of deposited crystals, together with the thus far proposed mechanism of preferential enrichment,[2-5] the observed preferential enrichment phenomenon of ketoprofen can be interpreted in terms of the following successive processes (in case of using a slightly *S*-rich sample) (Figure 5a) : (i) preferential formation of homochiral 1D *R* and *S* chains in the slightly *S*-rich solution, (ii) a solid-to-solid polymorphic transition of the metastable, slightly *S*-rich mixed crystals composed of the same homochiral *R* and *S* chains into the stable, slightly *S*-rich mixed crystals mainly composed of heterochiral cyclic dimer chains, and (iii) partial crystal disintegration in the irregular molecular alignment area, where an even number of

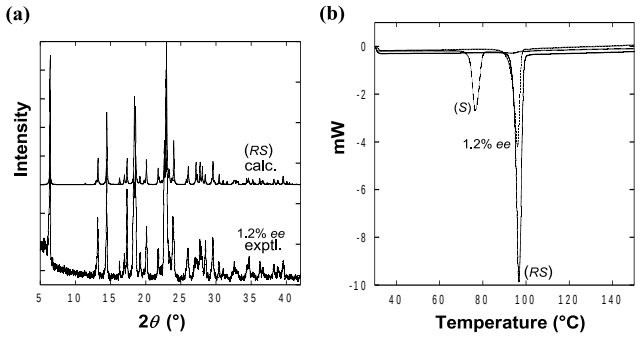

Fig. 4 Comparison of crystal properties between (*RS*)-ketoprofen and deposited crystals (1.2% *ee*, *S*-rich) after recrystallization. (a) X-ray powder diffraction pattern simulated from the X-ray crystallographic data of (*RS*)-ketoprofen (up) and experimental pattern of the deposited (*S*)-rich crystals (down). (b) DSC profiles of (*RS*)- and (*S*)-ketoprofen, and the deposited (*S*)-rich crystals.

homochiral *S* chains (four in this case) are surrounded by two *R* chains before polymorphic transition inside the transformed crystal lattice to selectively release the excess *S* enantiomer into solution until the deposited crystals are slightly enriched with the opposite *R* enantiomer (ca.1% *ee*), resulting in the generation of crystal defects incapable of undergoing crystal growth any longer (Figure S3). This crystal disintegration should occur at the incomplete dimer sites due to the presence of a weakened hydrogen bond between two carboxyl groups (O···O: 4.33 and 7.24 Å for *S'-S* and *S'-R'* dimers, respectively, vs 2.63 Å for *R-S* cyclic dimer), which arises from the positional disorder of the two enantiomers (Figure 5b).

Conclusions

We have demonstrated that nearly racemic ketoprofen can behave like a racemic mixed crystal showing polymorphic transition and allowing the deposition of nonracemic crystals under nonequilibrium crystallization conditions, eventually leading to the occurrence of preferential enrichment, despite a single-component crystal. This result strongly supports the propriety of our proposed mechanism and five requirements with respect to preferential enrichment. We believe that preferential enrichment should become a potent enantiomeric resolution method for a lot of racemic compound crystals, together with the further development of nonequilibirum crystallization technologies.

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Notes and references

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† Electronic Supplementary Information (ESI) available: HPLC chromatograms of *RS* sample and *R*- and *S*-rich ones; racemic crystal structure viewd down the *a*-axis; SEM images of deposited *S*-rich crystals; comparison of in situ ATR-IR spectra. See DOI: 10.1039/b000000x/

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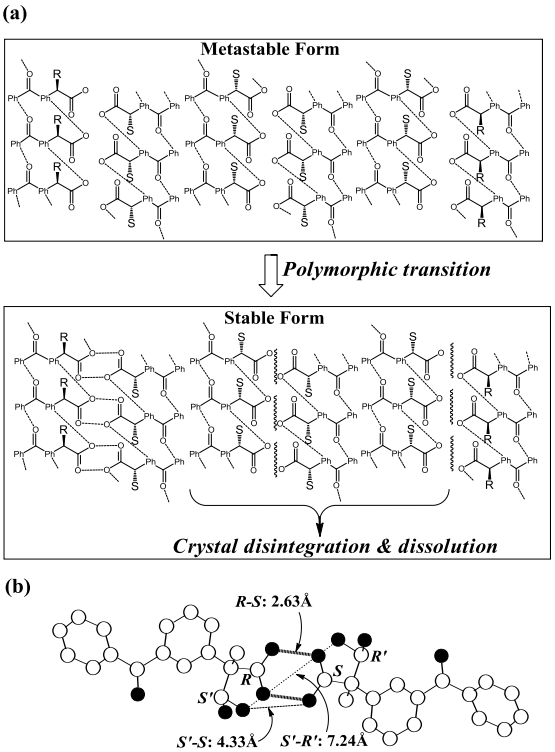

Fig. 5 The proposed mode of polymorphic transition of nonracemic ketoprofen. (a) Transformation of a metastable form into a stable one. (b) Hypothetical cyclic dimer structure in the crystal with orientational disorder on an asymmetric carbon atom.

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