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The Kinetics of the Racemization of Oxazepam in Aqueous Solution

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The kinetics of racemization of oxazepam was examined by an high-performance liquid chromatographic (HPLC) method modified for simultaneous determination of each enantiomer of oxazepam and oxazepam acetate by using a chiral stationary phase in order to clarify the factors affecting the racemization rate. The racemization of oxazepam is too fast to allow the isolation of optically active oxazepam. Optically active oxazepam acetate, which was stable enough in configuration to be isolated by HPLC, could be utilized to determine the racemization rate of oxazepam as follows. In the strongly alkaline pH region, the hydrolysis rate of oxazepam acetate was larger than the racemization rate of oxazepam, and more than 93% enantiomeric purity of oxazepam in solution was obtained on hydrolysis of optically active oxazepam acetate at pH 14 and 0 °C. The racemization rates of oxazepam were determined over the pH range from 0.5 to 13.5 at a constant ionic strength of 0.5. The dependence of the racemization rates on the pH, ionic strength and dielectric constant of the reaction mixture suggests that the racemization of oxazepam can be ascribed to spontaneous reaction of neutral oxazepam species in the neutral pH region and to hydroxide ion-catalyzed reaction of neutral oxazepam or to spontaneous reaction of dissociated oxazepam species in the basic pH region.

Keywords——racemization; kinetics; oxazepam; chiral stationary phase; optically active oxazepam acetate

Introduction

Many drug products containing an optically active drug are on the market. It is well known that the enantiomers of a certain drug often differ from each other in biological activities. Therefore it is very important to clarify the kinetics of epimerization and racemization of optically active drugs in order to certify the safety and effectiveness of drug preparations containing optically active drugs.

Oxazepam (7-chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one) is a sedative, which has been used as a racemate. To our knowledge, the kinetics of racemization of oxazepam have not yet been followed successfully. Šunjić *et al.* have attempted to isolate optically active oxazepam by diastereomeric esterification of oxazepam with camphanic acid, followed by hydrolysis of optically active oxazepam camphanate. They failed because rapid racemization occurred during the hydrolysis.[1] On the other hand, epimerization of 1-α-(*S*)-phenylethyl oxazepam, which could be resolved by silica gel column chromatography because of its relatively slow epimerization, was examined kinetically.[2]

Isolation of optically active esters of oxazepam has been reported, for oxazepam camphanate[1] and oxazepam hemisuccinate.[3] These reports suggest that the configuration of the chiral center of oxazepam in the form of oxazepam ester is stable enough to allow the isolation of optically active oxazepam ester. Thus, we considered that optically active oxazepam acetate, for example, may be easily isolated and utilized to study the racemization of oxazepam by simultaneous determination of each enantiomer of oxazepam acetate and

oxazepam during the hydrolysis of optically active oxazepam acetate.

Pirkle and Tsipouras have reported enantiomeric separation of oxazepam and oxazepam acetate by high-performance liquid chromatography (HPLC) on a chiral stationary phase.[4] Their HPLC method seems to be applicable for simultaneous determination of each enantiomer of oxazepam and oxazepam acetate with some modifications.

In the present paper the factors affecting the hydrolysis rate of oxazepam acetate and the racemization rate of oxazepam were examined to establish conditions where optically active oxazepam acetate is hydrolyzed to optically active oxazepam completely without racemization of oxazepam. The kinetics of racemization of oxazepam was studied by using optically active oxazepam obtained under the established conditions, and the effects of pH, isopropanol concentration and ionic strength on the racemization rate of oxazepam were clarified.

Experimental

Materials——Oxazepam was kindly supplied by Banyu Pharmaceutical Co., Ltd., Tokyo, Japan. Oxazepam acetate was prepared from oxazepam and acetyl chloride with pyridine as a catalyst.[5] Oxazepam acetate was resolved semi-preparatively by HPLC on a chiral stationary phase. The optically active oxazepam acetate was stable in isopropanol solution for more than one month at 25 °C. All other chemicals were of reagent grade.

HPLC——Simultaneous determination of each enantiomer of oxazepam acetate and oxazepam was carried out by HPLC using a chiral stationary phase. The chromatographic system consisted of a model 655 solvent delivery system equipped with a model 655—11 detector operating at 254 nm (Hitachi Co., Ltd., Tokyo, Japan). A chiral stationary phase column was purchased (Sumipax OA-2000, 25 cm × 4 mm, Sumika Chemical Analysis Service Ltd., Osaka, Japan), and eluted with hexane–CH_2Cl_2–isopropanol–ethanol (10 : 10 : 1 : 3) for the determination of each enantiomer of oxazepam and oxazepam acetate, or with hexane–CH_2Cl_2–isopropanol (20 : 20 : 3) for the semi-preparative resolution of oxazepam acetate (flow rate: 1.0 ml/min).

Kinetic Study——For kinetic studies, the following aqueous buffer solutions were used: pH < 2, hydrochloric acid–potassium chloride; pH 2.4, citrate (0.09—0.18 M); pH 4.4, acetate (0.1—0.2 M); pH 6—7, 11.5—12, phosphate (0.05—0.1 M); pH 8—10, borate (0.12—0.24 M); pH 11, carbonate (0.08—0.15 M); pH > 12, sodium hydroxide. The ionic strength of the solution was adjusted to 0.5 by adding potassium chloride, unless otherwise stated.

Hydrolysis of oxazepam acetate was examined at 40 °C (pH 2—10) or 0 °C (pH 0.5, pH > 11). One milliliter of the optically active oxazepam acetate solution was added to 10 ml (pH 2—10) or 17 ml (pH 0.5, pH > 11) of buffer solution pre-equilibrated at 40 or 0 °C. One milliliter of the reaction mixture was withdrawn at appropriate intervals and added to 10 ml of the ice-cold mobile phase solvent for HPLC (containing 5 mM nitrazepam as an internal standard) and the mixture was vigorously shaken. The organic layer was injected into the chromatograph within 3 min after sampling to avoid the racemization of oxazepam in the extraction solvent.

Racemization of oxazepam was studied at 0 °C (pH > 2.4) or 20 °C (pH < 2.4). One milliliter of the optically active oxazepam acetate solution was added to 1 ml of ice-cold 1 M NaOH and the solution was kept for 30 min at 0 °C, then 1 ml of ice-cold 1 M HCl was added to neutralize the excess alkali. The enantiomeric purity of oxazepam in the resulting solution was more than 93%. To the optically active oxazepam solution was added 15 ml of buffer solution of appropriate pH, and *d*- and *l*-oxazepam were assayed as described above.

Results

Hydrolysis of Oxazepam Acetate at Various pHs

Figure 1 shows typical high-performance liquid chromatograms of *l*-oxazepam acetate solution, indicating that enantiomers of oxazepam and oxazepam acetate were successfully separated from one another.

Figure 2 shows the time course of hydrolysis of *l*-oxazepam acetate at pH 10 and 40 °C. *l*-Oxazepam acetate was hydrolyzed to oxazepam and formed equal amounts of *d*- and *l*-oxazepam. A small amount of *d*-oxazepam acetate was detected, indicating that racemization of oxazepam acetate took place. Oxazepam acetate was hydrolyzed to oxazepam according to pseudo first-order reaction kinetics in the pH range studied. In the pH range from 2 to 10, oxazepam was formed as a racemate from optically active oxazepam acetate, which reveals that the racemization rate of oxazepam is much larger than the hydrolysis rate of oxazepam

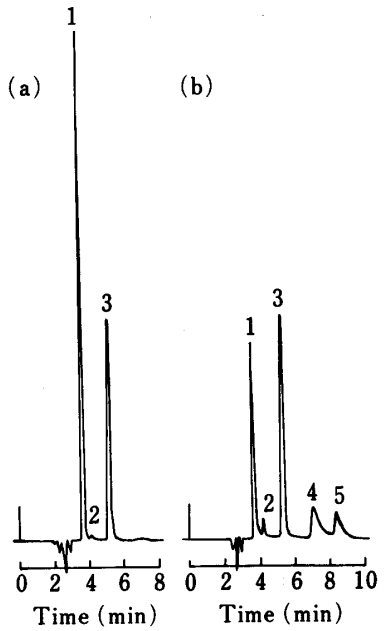

Fig. 1. Chromatograms of the Reaction Mixture of *l*-Oxazepam Acetate Immediately (a) and 40 min (b) after Dissolution at pH 10 and 40 °C

1, *l*-oxazepam acetate; 2, *d*-oxazepam acetate; 3, nitrazepam (internal standard); 4, *l*-oxazepam; 5, *d*-oxazepam.

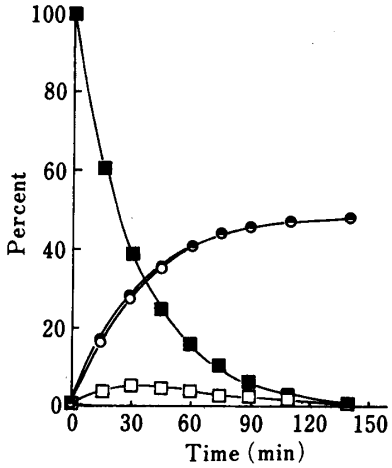

Fig. 2. Hydrolysis of *l*-Oxazepam Acetate (■) and Formation of *d*-Oxazepam Acetate (□), *l*-Oxazepam (●) and *d*-Oxazepam (○) at pH 10 and 40 °C

Solid lines represent regression lines according to Chart 2. Rate constants estimated to be 0.00346 min^{-1} for k'_{rac} and k'_{-rac}, 0.0285 min^{-1} for k_{hyd} and k'_{hyd} and 0.786 min^{-1} for k_{rac} and k_{-rac}.

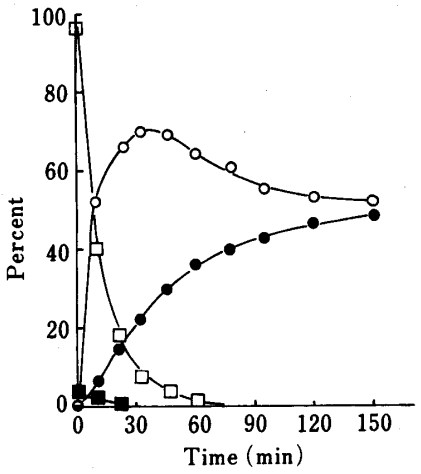

Fig. 3. Hydrolysis of *d*-Oxazepam Acetate (□) and *l*-Oxazepam Acetate (■) and Formation of *d*-Oxazepam (○) and *l*-Oxazepam (●) at pH 13 and 0 °C

Solid lines represent regression lines according to Chart 2. Rate constants estimated to be 0.0037 min^{-1} for k'_{rac} and k'_{-rac}, 0.0775 min^{-1} for k_{hyd} and k'_{hyd} and 0.0123 min^{-1} for k_{rac} and k_{-rac}.

acetate. The racemization of oxazepam acetate *per se* was observed in the pH range from 8 to 10.

Figure 3 shows the time course of hydrolysis of *d*-oxazepam acetate at pH 13, where hydrolysis of oxazepam acetate was carried out at 0 °C because the hydrolysis was rapid. At this pH, *d*-oxazepam acetate was hydrolyzed to *d*-oxazepam, which inverted to *l*-oxazepam successively. The hydrolysis rate of oxazepam acetate was larger than the racemization rate of oxazepam. This suggests that when optically active oxazepam acetate is hydrolyzed completely to optically active oxazepam under strongly alkaline conditions, the oxazepam solution may be of high enantiomeric purity, and that the racemization of oxazepam at an appropriate pH and temperature can be followed by using this optically active oxazepam solution.

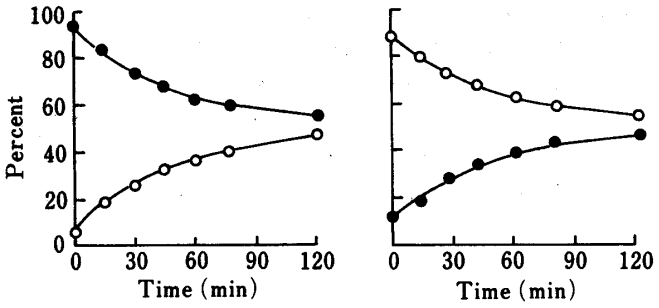

Fig. 4. Racemization of *l*-Oxazepam (●) and *d*-Oxazepam (○) at pH 12 and 0 °C

Solid lines represent regression lines according to Chart 1. Rate constants were estimated to be 0.0108 and 0.00999 min^{-1} (a) and 0.0104 and 0.0112 min^{-1} (b) for k_{rac} and k_{-rac}, respectively.

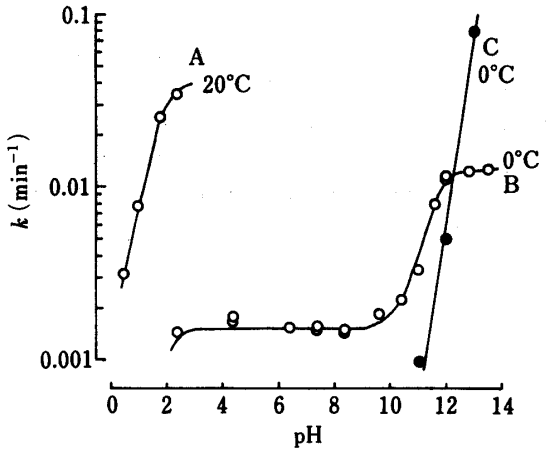

Fig. 5. The pH-Rate Profiles for Racemization of Oxazepam (○) and Hydrolysis of Oxazepam Acetate (●)

Curves A and B represent regression lines according to Eq. 2. Rate constants were estimated to be 0.00161 min^{-1} and 2.69×10^{-16} $mol^{-2}\,l^2\,min^{-1}$ for k_0 and k_2, respectively, at 0 °C and 0.0438 min^{-1} for k_0 at 20 °C. Curve C represents the linear relationship between log*k* and pH with a slope of +1.

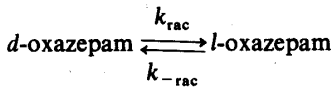

Chart 1

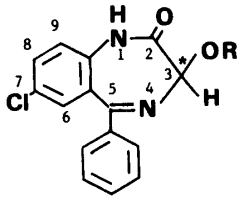

Fig. 6. Structures of Oxazepam and Its Acetate

R = H, oxazepam; R = CH_3CO, oxazepam acetate.
*: Chiral center.

At pH 0.5, *d*-oxazepam acetate was hydrolyzed to oxazepam rapidly. The concentration of *d*-oxazepam, however, was slightly larger than that of *l*-oxazepam.

Racemization of Oxazepam

Figure 4 shows the typical time course of racemization of oxazepam. A reversible racemization was found to occur between *d*- and *l*-oxazepam, as represented in Chart 1. where k_{rac} and k_{-rac} are the racemization rate constant from *d*- to *l*-oxazepam and that from *l*- to *d*-oxazepam, respectively. The experimental data shown in Fig. 4 were fitted to Chart 1 by nonlinear regression analysis (MULTI[6]) and excellent fits between the experimental data and the theoretical values were observed. General acid-base catalysis was not observed in the pH range studied.

The pH-rate profile of apparent first-order rate constant of oxazepam racemization (k_{rac}) and that of oxazepam acetate hydrolysis (k_{hyd}) are shown in Fig. 5. The racemization rate observed increased with increasing pH above pH 10. It became constant at pH higher than 13, indicating that the dissociation of oxazepam reduced the racemization rate. In the neutral region the racemization rate was independant of pH. In the acidic region, the racemization rate was determined at 20 °C because the racemization was slow. The racemization rate decreased with decreasing pH, indicating that the protonation of oxazepam reduced the racemization rate.

In the pH range studied, oxazepam exists in three different forms: as the protonated

species, the neutral form and the deprotonated species reported by Barrett *et al.*[7] They suggested that sites of protonation and deprotonation are the position 4 nitrogen and the hydroxyl group, respectively (see Fig. 6). The apparent dissociation constants of oxazepam at 0 and 20 °C were determined to be 1.7 and 11.5 at 20 °C and 2.1 and 11.6 at 0 °C, respectively, by the spectrophotometric method of Barrett *et al.*[7] These values agree with the reported values.[7] The rate constant of racemization can be expressed by the following equation

$$k_{rac}=(k_0^+ +k_H^+[H^+])f^+ +(k_0+k_H[H^+] +k_{OH}[OH^-])f+(k_0^- +k_{OH}^-[OH^-])f^- \quad (1)$$

where k_H^+ and k_H refer to the proton-catalyzed racemization of protonated and neutral oxazepam species, respectively, k_0^+, k_0 and k_0^- refer to the spontaneous racemization of protonated, neutral and dissociated oxazepam species, respectively, and k_{OH} and k_{OH}^- refer to the hydroxide ion-catalyzed racemization of neutral and dissociated oxazepam species, respectively. The f^+, f and f^- are mole fractions of protonated, neutral and dissociated oxazepam species, respectively. The respective mole fractions can be described in terms of the proton activity and the dissociation constants of oxazepam, K_{a1} and K_{a2}, and thus Eq. 1 can be rewritten as follows

$$k_{rac}=\frac{1}{([H^+]^2+[H^+]K_{a1}+K_{a1}K_{a2})}\left\{k_H^+[H^+]^3+k_1[H^+]^2 +k_0K_{a1}[H^+]+k_2+k_{OH}^-K_{a1}K_{a2}K_W\frac{1}{[H^+]}\right\} \quad (2)$$

where

$$k_1=k_0^+ +k_HK_{a1}$$

$$k_2=k_{OH}K_{a1}K_W+k_0^-K_{a1}K_{a2}$$

K_W: autoprotolysis constant of water.

Rate constants k_H^+, k_{OH}^-, k_0, k_1 and k_2 were estimated by using a nonlinear curve-fitting program. The estimated values were 1.61×10^{-3} min^{-1} and 2.69×10^{-16} mol^{-2}l^2 min^{-1} for k_0

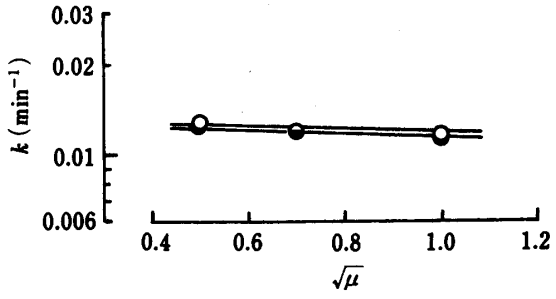

Fig. 7. Semilogarithmic Plots of k_{rac} (○) and k_{-rac} (●) as a Function of the Square Root of Ionic Strength ($\sqrt{\mu}$) for Racemization of Oxazepam at pH 13 and 0 °C

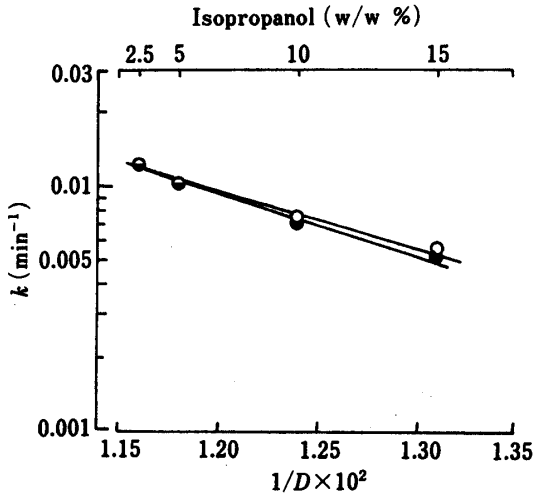

Fig. 8. Semilogarithmic Plots of k_{rac} (○) and k_{-rac} (●) as a Function of the Reciprocal of Dielectric Constant (1/D) for Racemization of Oxazepam at pH 13.5 and 0 °C

and k_2 at 0 °C, respectively, and 0.0438 min^{-1} for k_0 at 20 °C. Values of k_H^+, k_1 and k_{OH}^- were found to be much smaller than those of k_0 and k_2 and could be taken as zero for our purposes. The experimental data fitted well the calculated values as shown in Fig. 5.

In order to obtain information on the reacting species in the basic region, the effects of ionic strength and dielectric constant on the racemization rate were studied.

The effects of ionic strength of the reaction mixture on the racemization rate were studied at pH 13 and 0 °C. The ionic strength was varied from 0.25 to 1.0 by the addition of potassium chloride. As shown in Fig. 7, a linear relationship with a slope of almost zero was observed between the logarithm of k_{rac} or k_{-rac} and the square root of ionic strength ($\sqrt{\mu}$), suggesting the absence of charge on one reactant at least.[8]

The effects of the dielectric constant of the reaction mixture on the racemization rate were studied by changing the isopropanol concentration at pH 13.5. The dielectric constant was calculated from the data reported by Åkerlöf.[9] As shown in Fig. 8, a linear relationship with a negative slope was observed in the plots of the logarithm of k_{rac} and k_{-rac} against the reciprocal of the dielectric constant of the medium, suggesting that the racemization of oxazepam is a reaction between a negatively charged ion and a neutral molecule with a dipole moment.[10]

Discussion

As shown in Fig. 1a, oxazepam acetate can be successfully isolated by HPLC using the chiral stationary phase, and optically active oxazepam acetate obtained is stable in isopropanol solution. Figure 1b shows that the concentrations of the enantiomers of oxazepam acetate and oxazepam can be determined simultaneously by HPLC as described in this paper and suggests that the hydrolysis rate of optically active oxazepam acetate and the racemization rate of oxazepam can be determined simultaneously.

At pH above 11, the difference between the hydrolysis rate of oxazepam acetate and the racemization rate of oxazepam is small enough to determine each rate simultaneously. The reaction of optically active oxazepam acetate in this pH range is represented by Chart 2 (see also Fig. 6).

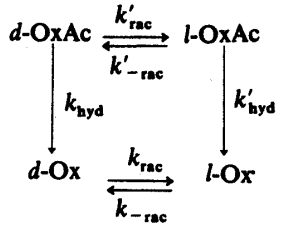

Chart 2

The experimental data shown in Fig. 3 were fitted to the model represented in Chart 2 by nonlinear regression analysis. Rate equations were integrated numerically by the Runge-Kutta-Gill method[11] and the rate constants k'_{rac}, k_{hyd} and k_{rac} were assumed to be equal to k'_{-rac}, k'_{hyd} and k_{-rac}, respectively. The estimated value for k_{rac} was 0.0123 min^{-1} and agreed with the racemization rate constant observed at pH 13, which was determined separately by using optically active oxazepam.

In the pH range below 10, the racemization rate of oxazepam was much larger than the hydrolysis rate of oxazepam acetate, so that simultaneous determination of the racemization rate of oxazepam and the hydrolysis rate of oxazepam acetate is impossible without application of rapid analytical methods (Fig. 2). However, at pH 14, complete hydrolysis of oxazepam acetate without racemization of oxazepam was observed, and a solution containing more than 93% of oxazepam enantiomer can be obtained. This can be interpreted in terms of

the different pH dependencies of the hydrolysis rate and the racemization rate (Fig. 5). By adding buffer solution to the optically active oxazepam obtained, the racemization rate of oxazepam at an appropriate pH could be determined easily, as shown in Fig. 4.

At pH 0.5, where slow racemization of oxazepam was observed, low enantiomeric purity of oxazepam was observed on the hydrolysis of *d*-oxazepam acetate, and at this pH the hydrolysis of oxazepam acetate is not represented by Chart 2. This may be interpreted in terms of an *SN*1-type mechanism, which includes intramolecular proton transfer from the N(4) atom to the ester carbonyl group, leading to a flat boat conformation, as suggested in the case of oxazepam hemisuccinate.[12] Further experimental work, however, will be needed to clarify the mechanism, and will be the subject of a future paper.

The pH dependence of the racemization rate (Fig. 5) suggests that the hydroxide ion-catalyzed racemization of dissociated oxazepam species does not occur in the pH range studied. It may be ascribed to the inhibition of the attack of hydroxide ion by the negative charge present near the reaction center. The effects of ionic strength and dielectric constant on the racemization rate (Figs. 7 and 8) support this speculation. The racemization of oxazepam can be regarded as the spontaneous reaction of neutral oxazepam species in the neutral pH region and the hydroxide ion-catalyzed reaction of neutral oxazepam species or the spontaneous reaction of deprotonated oxazepam species in the basic pH region.

The kinetic data described above suggest rapid racemization of oxazepam in the body, and it is unlikely that orally administrered dosage forms of optically active oxazepam are more effective than those of racemic forms. Hydroxide ion was found to be one of the significant factors affecting the racemization rate of oxazepam, and it should be noted that base-catalyzed racemization or epimerization of various optically active drugs could be accelerated by some drug additives.

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