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Optical Resolution of Drugs by Cyclodextrin Complexation

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A simple method of optical resolution of racemic drugs was developed by a combination of cyclodextrin complexation and subsequent Sephadex gel chromatography. Chiral discrimination with cyclodextrins was assessed by circular dichroism measurement. The optical purities of enantiomers separated by this method were above 40% and 30% for warfarin and mandelic acid, respectively. Our results demonstrate the utility of this method for easy resolution of racemates.

Keywords——cyclodextrin; warfarin; mandelic acid; optical resolution; circular dichroism; enantiomer

It is well known that enantiomers of drugs possessing an asymmetric center show significant differences in their physiological activities. Thus, the separation of enantiomers is of primary importance for research on drug activities.

In the last few years, cyclodextrins have received growing attention from a variety of points of view.[1,2] Cyclodextrins can form inclusion complexes with so many small molecules having a diameter of 5—8 Å, and the inclusion complexes serve as models for studing many topochemical problems. Furthermore, it is known that one of the practical applications of cyclodextrin is the resolution or separation of racemates or isomeric compounds by means of complex formation.

As cyclodextrins are constructed from several number of chiral glucose moieties, they can form a diastereomeric pair with each racemic guest molecule. An application of cyclodextrins to separation of racemates was first reported by Cramer and Dietsche.[3] In a solution with 5- to 10-fold molar excess of cyclodextrin, the complex of cyclodextrin with one of the antipodes precipitated out preferentially, resulting in partial resolution of the racemate (precipitation technique). Resolution of chiral alkyl alkylphosphinates,[4] sulfoxides, sulfinates and thiosulfinate *S*-esters[5] has been reported. Uekama *et al.*[6] reported an ion-exchange liquid chromatography of the cyclodextrin complexes for the separation of E-, A-, and B-type prostaglandins. More recently, cyclodextrins were made insoluble by crosslinking with epichlorohydrin and used as the ligand for affinity chromatography (gel inclusion chromatography).[7] Racemetes of mandelic acid and its derivatives have been separated on this polymer.

In this paper, we describe a novel method for the partial resolution of chiral drugs by the formation of β-cyclodextrin inclusion complexes followed by chromatography on a Sephadex column.

Experimental

Materials——α-Cyclodextrin and β-cyclodextrin were from Wako Pure Chemical Industries, Ltd., *rac*-warfarin [3-(α-acetonylbenzyl)-4-hydroxycoumarin] (racemic warfarin) was from Sigma Chemical Co., and DL-mandelic acid was from Nakarai Chemicals Ltd. They were used without further purification.

Formation of Complexes of Cyclodextrins with Drugs——Aqueous solutions of cyclodextrin complexes were prepared by mixing cyclodextrin solution with drug solution at appropriate concentrations at room temperature.

Column Chromatographic Separation of Enantiomers——The mixed solution of cyclodextrin and enantiomers was introduced into a column of Sephadex G-15 or G-10 (2.5 × 40 cm) in water and the column was eluted with water at a flow rate of 20 ml/h. The eluate was collected in fractions of 7 ml. The elution was followed by absorbance measurements at the maximal absorption wavelengths of drugs or complexes. The fractions containing cyclodextrin-drug complex were lyophilized to get the crystalline complex and the enantiomers in the complex were separated by the addition of acetone.

Circular Dichroism and Absorption Spectra——The circular dichroism (CD) and absorption spectra of the drugs and their complexes with cyclodextrin were measured using a Jasco J-400X spectropolarimeter and Hitachi 220 spectrophotometer, respectively. The observed CD and absorption spectra were expressed in terms of molar ellipticity, $[\theta]$, and molar extinction coefficient, ε, calculated on the basis of the initial concentration of drug. In order to estimate the optical purities of the separated enantiomers, the optical rotation was measured by a Jasco digital polarimeter, model DIP-4. The values of $[\alpha]_D$ of authentic samples were taken as 100%, respectively.

Results and Discussion

Figure 1 shows the induced CD and absorption spectra of the β-cyclodextrin–*rac*-warfarin system in aqueous solution. The change of absorption spectrum indicates that the warfarin molecules interact with cyclodextrin. In the CD spectrum, two negative bands at about 320 and 290 nm with shoulders at about 282 and 270 nm and a positive band at about 240 with shoulders at about 255 and 230 nm were observed. This spectral pattern is consistent with that of (*S*)-warfarin, which has been assigned as (−)-warfarin.[8] It seems that β-cyclodextrin can discriminate the chirality of warfarins by complexing at the coumarin moiety. The cyclodextrin–warfarin complex and free warfarin should be separated by gel chromatography on Sephadex on the basis of the size difference. Furthermore, a difference of equilibrium constants between cyclodextrin complexes with each enantiomer should result in different flow rates in gel chromatography. Since the molecular weights of cyclodextrins are around 1000, Sephadex G-15 was selected for the separation. The elution profile of *rac*-warfarin with β-cyclodextrin on Sephadex G-15 is shown in Fig. 2. The ratio of the peak area of fast-eluting fractions (tube Nos. 34—43) to that of the slow-eluting fractions (tube Nos.

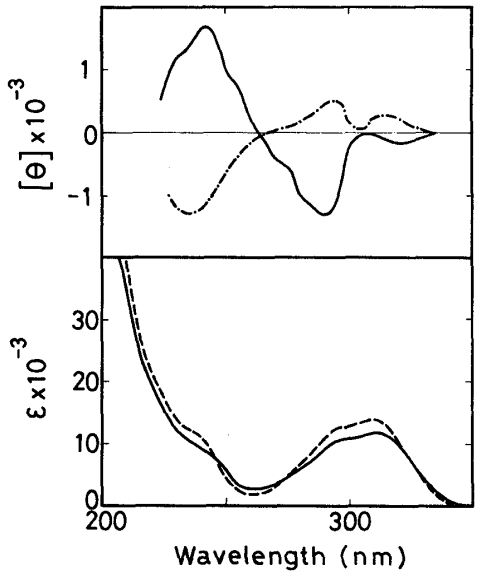

Fig. 1. The CD and Absorption Spectra of β-Cyclodextrin–Warfarin System

----, warfarin (5×10^{-4} M); ——, β-cyclodextrin–warfarin (unseparated complex) [warfarin] = 5×10^{-4} M, [β-cyclodextrin] = 5×10^{-3} M; —·—, β-cyclodextrin–warfarin complex in the fast-eluting fractions from a Sephadex G-10 column.

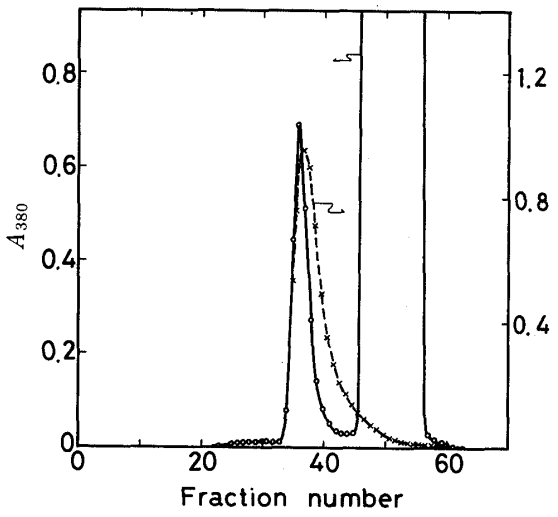

Fig. 2. Gel Filtration Chromatography of β-Cyclodextrin–Warfarin System on a Column of Sephadex G-15

(a) —○—, sample solution (50 ml) containing 2×10^{-3} M β-cyclodextrin and 1×10^{-3} M warfarin was applied to the column.

(b) —×—, sample solution (50 ml) containing 1×10^{-2} M β-cyclodextrin and 1×10^{-3} M warfarin was applied to the column.

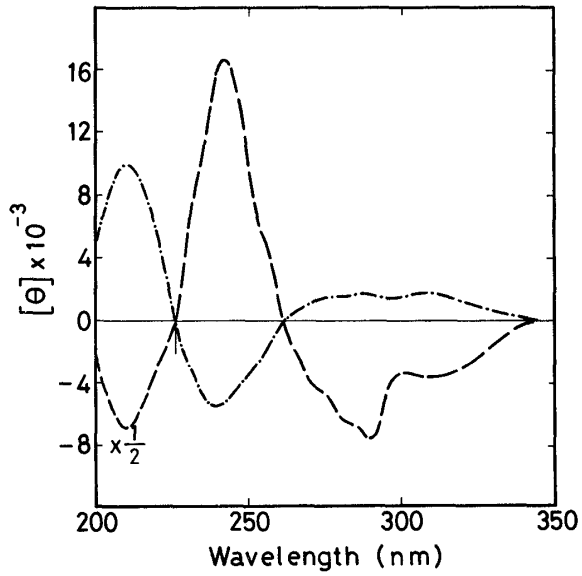

Fig. 3. The CD Spectra of Warfarins Separated by Sephadex G-15 Column

----, the fast-eluting fractions; —·—, the slow-eluting fractions.

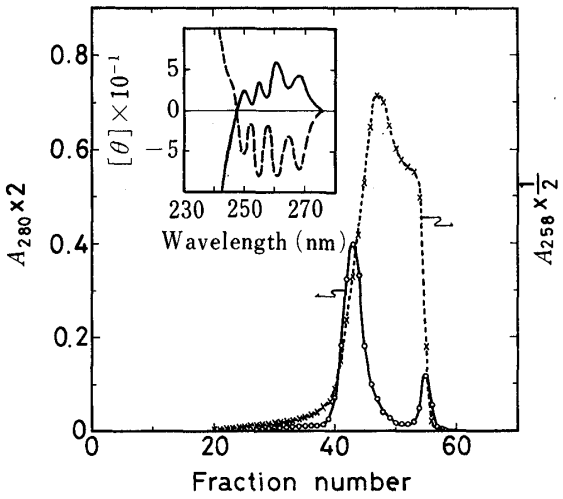

Fig. 4. Gel Filtration Chromatography of the β-Cyclodextrin–DL-Mandelic Acid System on a Column of Sephadex G-15

Sample solution (50 ml) containing 1×10^{-2} M β-cyclodextrin and 1×10^{-2} M DL-mandelic acid was applied to the column.

----, mandelic acid from fast-eluting fractions; ——, mandelic acid from slow-eluting fractions.

Inset are the CD spectra of mandelic acids separated on the Sephadex G-15 column.

46—57) was dependent on the ratio of the amounts of added cyclodextrin to warfarin. When the molar ratio of cyclodextrin to warfarin in the mixed solution was larger than about 5, it appeared that the excess cyclodextrin also formed a complex with (*R*)-warfarin, resulting in tailing in the elution diagram (Fig. 2(b)). It can be considered that (*S*)-warfarin included in cyclodextrin was eluted firstly, and that the slow-eluting fractions contained (*R*)-warfarin predominantly. By measuring the optical rotation, the optical purity of (*S*)-warfarin in the fast-eluting fractions in Fig. 2(a) could be calculated to be about 36%.[9] The optical purities of warfarins in the various fractions, of course, are different. The optical purity of the fast-eluting fractions could be increased by repeating the separation procedure. Thus, after the second separation procedure, the optical rotation of (*S*)-warfarin was increased to $[\alpha]_D = -59°$, which corresponds to an optical purity of 40%. The $[\alpha]_D$ of (*R*)-warfarin was $+45°$.

Figure 3 shows the CD spectra of β-cyclodextrin–warfarin complex from the fast-eluting fractions and warfarin from the slow-eluting fractions. It is apparent that optical resolution of *rac*-warfarin could be accomplished by the present methods.

In order to compare the present method with other methods, the resolution of mandelic acids was also examined by the use of Sephadex G-15. The elution pattern for the β-cyclodextrin–DL-mandelic acid system is shown in Fig. 4. The absorbance was also monitored at 280 nm, because an increase of absorption intensity was observed at around 280 nm. The monitoring curve at 280 nm showed two clear peaks. This may involve inclusion of the phenyl ring of mandelic acid within the cavity of cyclodextrin. In the inset of Fig. 4, the CD spectra of the fast- and slow-eluting fractions are shown. These fractions yielded D- and L-mandelic acids, respectively. The selectivity in the eluted complexes is consistent with the result the precipitation technique,[3] but not consistent with that obtained by the method of gel inclusion chromatography.[7] The optical purity of the fast-eluting fractions was 25—30%. Optical

purities of more than 30% could be obtained by repeated separation. This purity seems to be less than that obtained by gel inclusion chromatography, but higher than that by the precipitation technique.

The resolution is mainly accomplished by the formation of an inclusion complex. Nevertheless, when Sephadex G-10 was used for separation of warfarin complexes from *rac*-warfarin, the spectrum of the fast-eluting fractions showed (*R*)-warfarin-type CD (Fig. 1). The signs of the CD bands were the reverse of those observed in the β-cyclodextrin–*rac*-warfarin system on Sephadex G-15. This indicates that the order of elution on the G-10 column was the reverse of that on the G-15 column. It is likely that a conversion of selectivity occurred in the Sephadex-cyclodextrin system. Somewhat similar phenomena were reported when crosslinked β-cyclodextrin (β-cyclodextrin gel) was used for the chromatographic resolution of racemic acid and its derivatives.[7] As mentioned above, the selectivity in the present method making use of Sephadex G-15 is consistent with the results of the precipitation method.[3] The selectivity of cyclodextrin for enantiomers may be dependent on the environment, though the details are unclear.

Although the separation of racemic drugs into their enantiomers by the present method is only partial, this procedure can be easily carried out on a large scale.

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