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Study of the Podophyllotoxin/β-Cyclodextrin Inclusion Complex

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Abstract. The structure of the podophyllotoxin (P)/β-cyclodextrin (β-CD) inclusion complex has been studied by infrared spectroscopy, UV spectroscopy, NMR spectroscopy and X-ray diffractometry. The association constant is 128 M^{-1} in water, calculated from the straight portion of the phase-solubility diagram.

Key words: *Podophyllum bexan drum*, podophyllotoxin, β-cyclodextrin, inclusion complex.

1. Introduction

Podophyllotoxin (P) is a lignan, naturally occurring in roots and rhizomes of *Podophyllum emodi* Wall var. Chineusis sprague [1]. This compound is valuable because it is used for the preparation of semi-synthetic derivatives, such as etoposide and teniposide, which are clinically applied as cytostatics for the treatment of several types of cancer [2, 3]. Podophyllotoxin (Figure 1) itself is used in the treatment of some types of cancer [4–6], but its application is restrained due to its high toxicity and side effect on the human body.

Cyclodextrins have been reported in a number of studies in the pharmaceutical field to interact with many drug molecules to form inclusion complexes. These inclusion complexes have been extensively used to improve the water solubility of poorly soluble drugs, to reduce the toxicity [7] and increase the dissolution rate [8–10]. The aim of the present work was to study the inclusion complex of podophyllotoxin with β-cyclodextrin in order to increase podophyllotoxin's water solubility and hopefully reduce its toxicity. The podophyllotoxin/β-cyclodextrin complexes were prepared by either co-grinding or kneading. Characterization of the products has been carried out by solubility measurement, NMR, FTIR, and UV spectroscopy and X-ray diffractometry.

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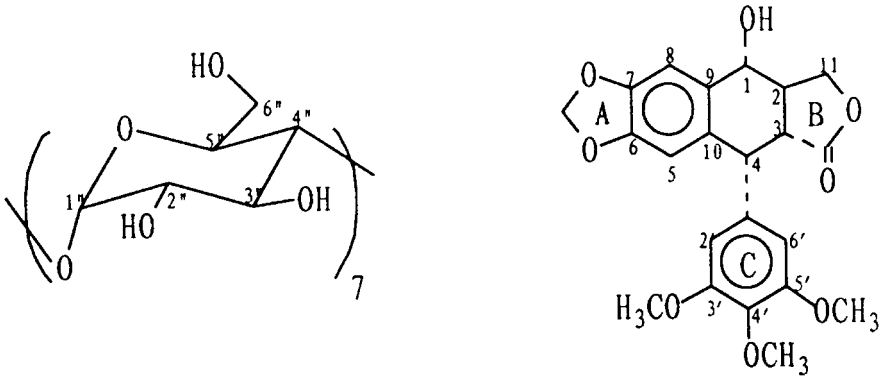

Figure 1. The structure of β-cyclodextrin and podophyllotoxin.

2. Experimental

2.1. MATERIALS

β-CD (99.5% Suzhou Weijing Plant, China) was purified by recrystallization from distilled water. Podophyllotoxin was purchased from Lanzhou Hechengyao Company. ^{1}H-NMR and 2D-NMR measurements were carried out using D_2O solvent (TOKYO KASEI). Other materials were of analytical reagent grade.

2.2. PREPARATION OF SAMPLES

2.2.1. *Physical Mixture*

A physical mixture of Podophyllotoxin and β-CD (molar ratio 1 : 1) was prepared by simple mixing in a ceramic mortar.

2.2.2. *Co-grinding*

A mixture of podophyllotoxin and β-CD (1 : 1 molar ratio) was ground in a ceramic ball mill for thirty minutes at room temperature.

2.2.3. *Kneading*

(a) Podophyllotoxin (0.083 g) was added to aqueous β-CD solution (0.227 g/100 ml) at room temperature. The resulting solution at a molar ratio of 1 : 2 was thereafter stirred for 15 h at 45 °C, and then put in a refrigerator at 0 °C for 30 h. The complex products were filtered with suction.

(b) 1.134 g (1 mmole) of β-CD and 0.414 g (1 mmole) of Podophyllotoxin were vigorously stirred in 20 ml of water for 15 h. The solution was dried with a rotary evaporator apparatus at 45 °C.

2.3. CHARACTERIZATION

2.3.1. *Infrared Spectroscopy*

Infrared spectrometry was conducted with a Nicolet 170SX Infrared Spectrometer, using the KBr disc method.

2.3.2. *X-ray Diffractometry*

Power X-ray patterns were obtained using a Rigaku D/max-2400 diffractometer (Japan), with CuKα radiation, voltage 40 kV, current 40 mA, DS/ss 10, RS 0.15 mm at a scanning speed of 8°/min.

2.3.3. *NMR Measurements*

^{1}H-NMR and 2D-NMR Spectra were recorded with a Bruker AM-400 NMR Spectrometer in D_2O. All measurements were carried out at 25 °C.

2.4. SOLUBILITY MEASUREMENT

The phase-solubility diagram was recorded according to Higuchi et al. Aqueous solutions of β-CD with concentrations of 0, 1, 2, 3, 4 and 5.0×10^{-3} M were prepared. An excess amount of Podophyllotoxin was added to the solution of β-cyclodextrin respectively, agitated for 15 h at 30 °C, then centrifuged and carefully filtered. 5 ml of filtrates were measured out and diluted to 10 ml with pure EtOH. Their absorbances were measured by UV spectrophometry after appropriate dilution with EtOH (291 nm).

Figure 2 shows the equilibrium phase, solubility diagram obtained for the P/β-CD system in water. The isotherm is a Bs type solubility curve and shows an initial linear section up to 3–4 $\times 10^{-3}$ M^{-1} β-CD. This indicates that a soluble complex (1 : 1) is formed by a fast process and the solubility limit of the complex is at about 1.1×10^{-3} M^{-1} of podophyllodoxin. The method is employed for determining the association constant value using the relation [11]:

$$K = \frac{\mathrm{tg}\phi}{S_0(1 - \mathrm{tg}\phi)},$$

where K = association constant value for the complex; tgϕ = slope of the initial section of the curve; S_0 = aqueous solubility of P.

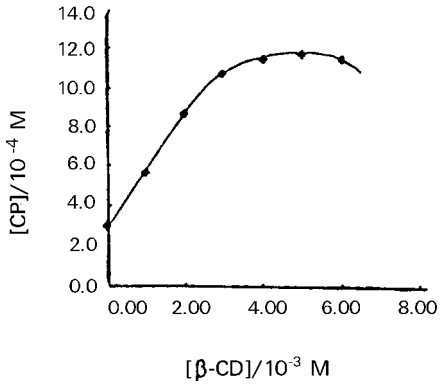

Figure 2. Solubility curve of podophyllotoxin in aqueous solution of β-CD.

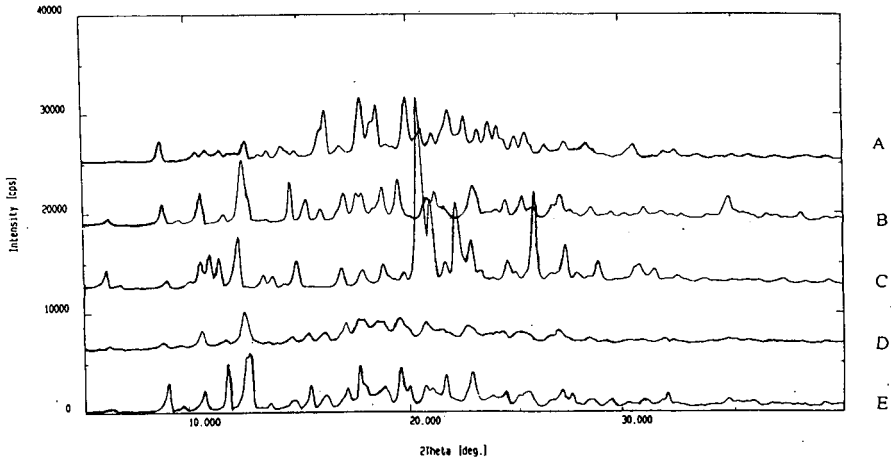

Figure 3. X-ray diffractograms of: (A) podophyllotoxin; (B) physical mixture; (C) co-grinding; (D) kneading; (E) β-CD.

3. Results and Discussion

3.1. CHARACTERIZATION OF THE COMPLEX

Figure 3 shows the X-ray diffraction patterns of podophyllotoxin, β-CD, the physical mixture and inclusion complexes. The diffraction pattern of the physical mixture is simply the superposition of signals of the two components, while those of the inclusion complexes are different and their positions are different from that of podophyllotoxin and β-CD. These are attributed to the co-existence of the complex [12] and free *P* and β-CD [13]. Many new peaks appear, indicating the formation of an inclusion complex.

When the dimensions of the three parts of *P* which may insert into the cavity of β-CD are considered, the inference is supported reasonably (Table I). As the

Table I. The dimension of the three parts of podophyllotoxin

Part	*A*	*B*	*C*
Dimension (Å)	2.276	4.344	6.498

The values are estimated by the authors referring to data from CRC Hand Book of Chemistry and Physics 73rd Edition 1992–1993 Bond Lengths in Organic Compounds.

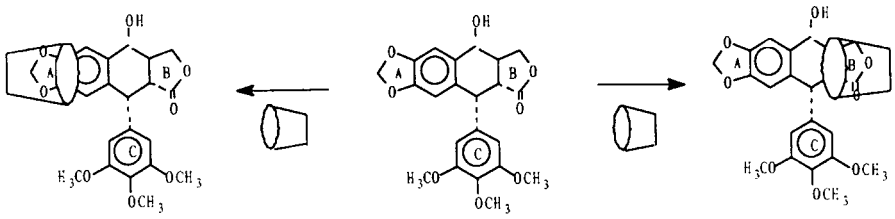

Figure 4. Proposed models for the inclusion complexes.

diameter of the cavity of β-CD is 6.5 Å [14], it is ring *A* or *B* that matches the cavity well, ring *C* is too large to insert into it (Figure 4).

Infrared analysis provides much information about the inclusion complex. The characteristic band belonging to carbonyl at 1765 cm^{-1} is observed for pure podophyllotoxin and the physical mixture. In the case of the complexes obtained by co-grinding and kneading, the band shifts to 1768 cm^{-1} and 1755 cm^{-1}, respectively. The band belonging to the hydroxyl group at 3466 cm^{-1} is prominent in the IR spectra of pure podophyllotoxin and the inclusion complex but is hidden by bands of hydroxyl groups in spectra of the physical mixture. The intensity and shape of the bands belonging to hydroxyl in β-CD change sharply in the inclu-

Table II. ^{1}HNMR chemical shifts (ppm) of β-CD in the absence and the presence of *P* (molar ratio 1 : 1)

Proton	(Free) δ_0	With *P* δ	$\delta - \delta_0$
1	4.934	4.919	−0.015
2	3.512	3.513	0.001
3	3.832	3.686	−0.146
4	3.449	3.435	−0.014
5	3.720	3.632	−0.088
6	3.744	3.661	−0.083

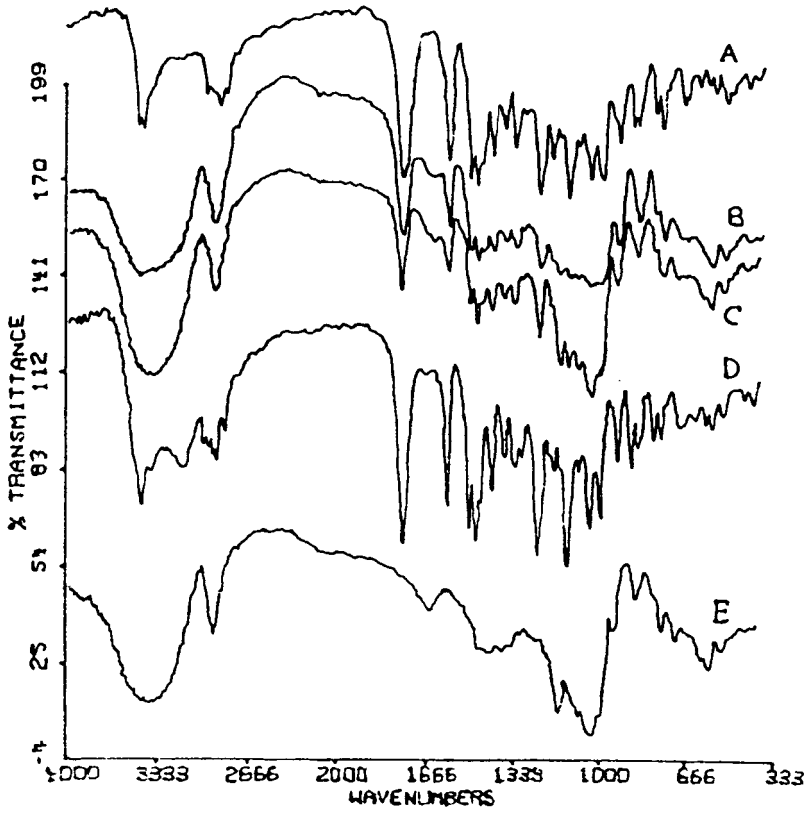

Figure 5. IR spectra of: (A) podophyllotoxin; (B) physical mixture; (C) co-grinding; (D) kneading; (E) β-CD.

Table III. ^{1}HNMR chemical shifts (ppm) of podophyllotoxin in the absence and the presence of β-CD (molar ratio 1 : 1)

Proton		Free	With β-CD	
		δ_0	δ	$\delta - \delta_0$
1		4.754	4.755	0.001
4		4.538	4.545	0.007
5		4.428	4.432	0.004
8		7.026	7.275	0.249
11	α	4.538	4.537	−0.001
	β	4.160	4.162	0.002
—OCH_2O—	α	5.841	5.732	−0.109
	β	5.837	5.679	−0.158
2′6′		6.378	6.380	0.002

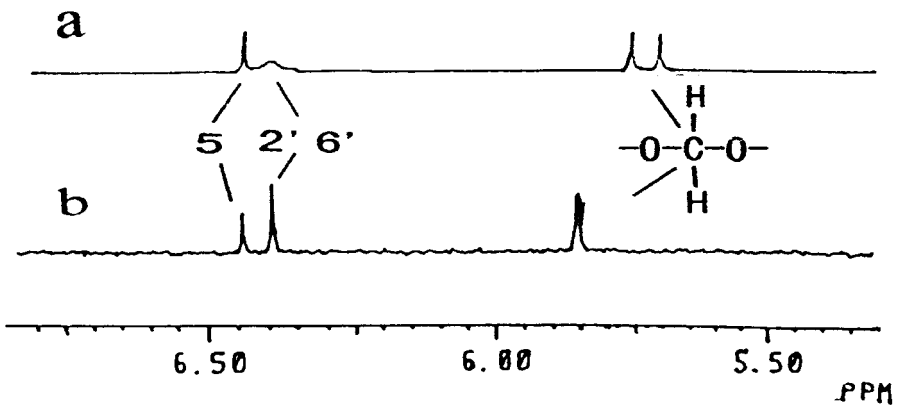

Figure 6. Partial 400 MHz ^{1} HNMR spectra of the —OCH_2O— group of podophyllotoxin in (a) the inclusion complex (molar ratio 1 : 1) (b) pure.

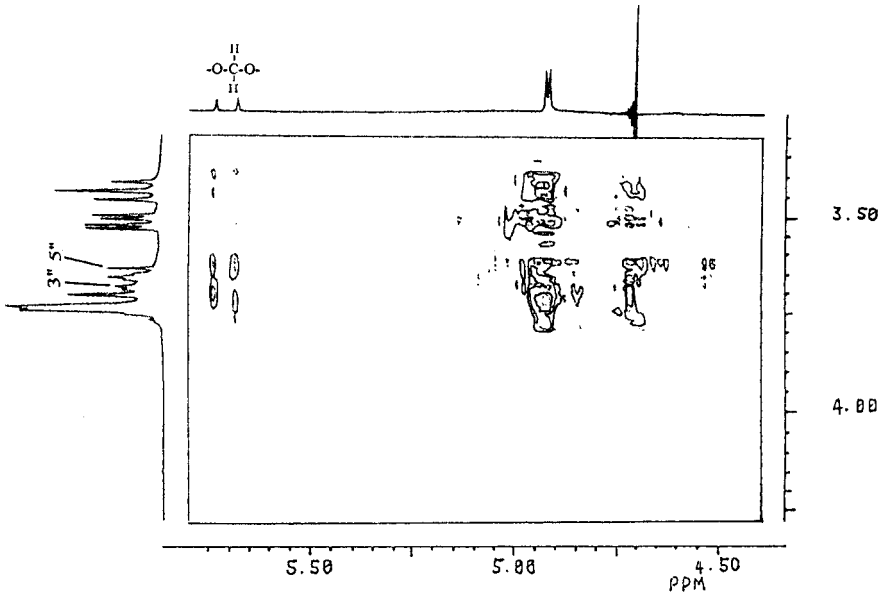

Figure 7. Expanded region of the NOESY spectrum of the Podophyllotoxin/β-CD inclusion complex.

sion complex compared with pure β-CD, indicating that the β-CD molecules are in different environments in the two states. (Figure 5). From the phase-solubility diagram, it results that P and β-CD form an inclusion complex in aqueous solution with a low association constant (K = 128 M^{-1}) . The structure of the complex was investigated by NMR spectroscopy.

Table II provides the NMR chemical shift values of β-CD in the free state and in the inclusion complex, showing that the presence of podophyllotoxin results in noteworthy upper field shifts of the resonances of protons H-3″, H-5″ of β-CD which are located on the inner surface of the β-CD cavity and clearly prove the presence of the inclusion complex [15–17].

Table III provides the chemical shift values of podophyllotoxin in the inclusion complex, showing that the chemical shifts of the two hydrogens of the —OCH_2O— group belonging to ring *A* of podophyllotoxin shift up field in the inclusion complex compared with pure podophyllotoxin, and the two hydrogen signals are separated from each other in the inclusion complex (Figure 6). In contrast, the chemical shifts of H-11α and H-11β belonging to ring *B* do not change. These facts indicate that inclusion of podophyllotoxin occurs by involving ring *A*.

Two dimensional NOE measurement also proved the facts, a set of cross peaks connects both the H-3″ and H-5″ resonances of β-CD to the hydrogen signals of the —OCH_2O— group of podophyllotoxin (Figure 7). All these facts indicate that ring A of the guest is inserted into the β-CD cavity.

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