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X-Ray Diffraction and NMR Study of Inclusion Complex of Podophyllotoxin with β-Cyclodextrin

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Abstract: The inclusion complex of podophyllotoxin (P) with β-cyclodextrin (β-CD) has been studied by X-ray diffractometry and ^{1}HNMR、^{13}CNMR spectroscopy. The complex structure is deduced.

Keywords: *Podophyllum bexan drum*; podophyllotoxin; β-cyclodextrin; inclusion complex;.X-Ray; NMR.

Podophyllotoxin (**Figure 1**) is a lignan, naturally occurring in roots and rhizomes of *Podophyllum emodi* Wall. var. Chinese Sprague[1]. This compound is clinically used to treat several types of cancer[2]. However, as a drug, it is restrainted due to its high toxicity and side effect to human body. We have prepared the inclusion complex of podophyllotoxin with β-cyclodextrin (**Figure 2**) in order to decrease toxicity and increase solubility of podophyllotoxin. The structure of the inclusion complex of the podophyllotoxin with β-cyclodextrin have been studied by the methods of X-ray diffraction and ^{1}HNMR,^{13} CNMR spectroscopy.

Powder 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. All NMR spectra were recorded with a Bruker AM-400 NMR spectrometer in DMSO-d_6

Figure 1. The structure of β-cyclodextrin and podophyllotoxin

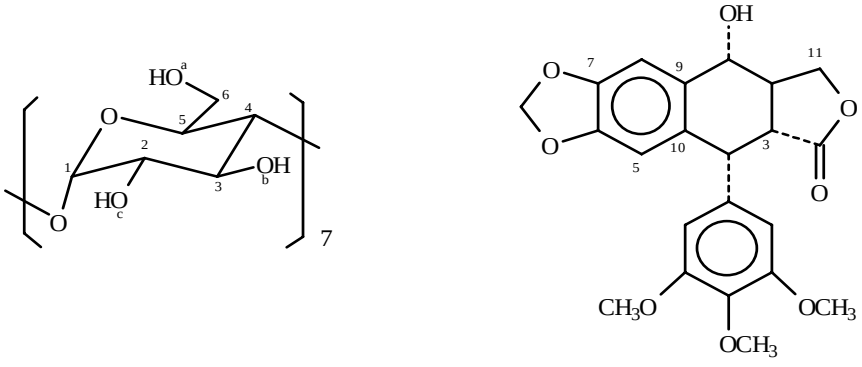


Figure 3 shows the X-ray diffraction patterns of podophyllotoxin, the physical mixture, inclusion complex, rotate-drying and β-CD. The diffraction pattern of physical mixture is simply the superposition of signals of the two components, while that of inclusion mixture is different and its peaks change apparently contrasted with those of podophyllotoxin and β-CD. This is attributed to the co-existing of the crystalline non-included components and the inclusion complex[3]. The pattern of inclusion complex exhibits obvious difference in crystalline characteristics from those of the two components. Many new peaks appear, indicating the formation of inclusion complex.

Table 1 provides the ^{1}HNMR chemical shift values of β-CD in free state and in inclusion complex, showing that the presence of podophyllotoxin results in dramatic low 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[4].

Table 1. ^{1}HNMR chemical shifts (ppm) of β-CD in absence andin presence of P

Proton	Free	With P	
	δ_0	δ	$\delta - \delta_0$
1	4.84	4.83	-0.01
2	3.37	3.45	0.08
3	3.67	3.91	0.24
4	3.30	3.32	0.02
5	3.61	3.89	0.28

Table 2 provided the chemical shift values of podophyllotoxin in inclusion complex, showing that the chemical shifts of H-11 and H-13 in inclusion complex obviously shift up field compared with protons with in pure podophyllotoxin, while chemical shifts of H-2, and H-5, and protons belonging to three –OCH_3 groups almost do not change between the two states. These facts indicate that podophyllotoxin is probably bounded as pattern A and pattern B, but not pattern C. (**Figure 2**)

The results of the ^{13}CNMR measurements are shown in **Table 3**. The C-8, C-10 and C-2′, 6′ signals show remarkable low field shift, whereas the C-1 signal shows remarkable high field shift. In the inclusion complex, these carbons are nearer to the secondary hydroxyl rim of β-CD than other ones in podophyllotoxin, so the interaction between the hydroxyl rim and the five carbons are more intense than that between the rim and other carbons in podophyllotoxin. The secondary hydroxyls repel OH-1 in podophyllotoxin, the enhancing the density of electron cloud around C-1, and attract the electron cloud of C-8, C-10 and C-2′, 6′ and causing low field or high field shift respectively.

The dimension of the guest molecular in volume in pattern B is 4.3 Å[5] and the diameter of the cavity of β-CD is 6.5Å[6]. It is the pattern B that matches the cavity more suitable than pattern A or C. Based on the data of X-ray diffraction ^{1}HNMR and ^{13}CNMR the formation of inclusion complex of podophyllotoxin with β-CD is deduced as pattern B (**Figure 2**)

Table 2. ^{1}HNMR chemical shifts (ppm) of podophyllotoxinin the absence and in the presence of β-CD

Proton	Free δ_0	With β -CD δ	$\delta - \delta_0$
3	3.13724	3.14075	0.00351
4	4.08525	4.08000	-0.00525
5	6.46009	6.46422	0.00413
8	7.09671	7.08930	-0.00741
11	4.46649	4.37349	-0.09300
13	5.97306	5.91121	-0.06185
2'6'	6.32559	6.31853	-0.00706
1-OH	5.78994	5.78436	-0.00558

Table 3. The changes (Δδ) of ^{13}CNMR chemical shifts (ppm) of podophyllotoxin between in the absence (δ_0) and in the presence of β-CD (δ)

Carbon	Δδ	Carbon	Δδ	Carbon	Δδ	Carbon	Δδ
1	-0.017	6	-0.007	10	0.026	1'	-0.010
3	-0.010	7	-0.005	11	-0.009	2',6'	0.023
4	-0.006	8	0.016	12	-0.007	3',5'	-0.004
5	-0.005	9	0.003	13	-0.005	4'	0.002

Note: $\Delta\delta = \delta - \delta_0$

Figure 2. Three inclusion patterns

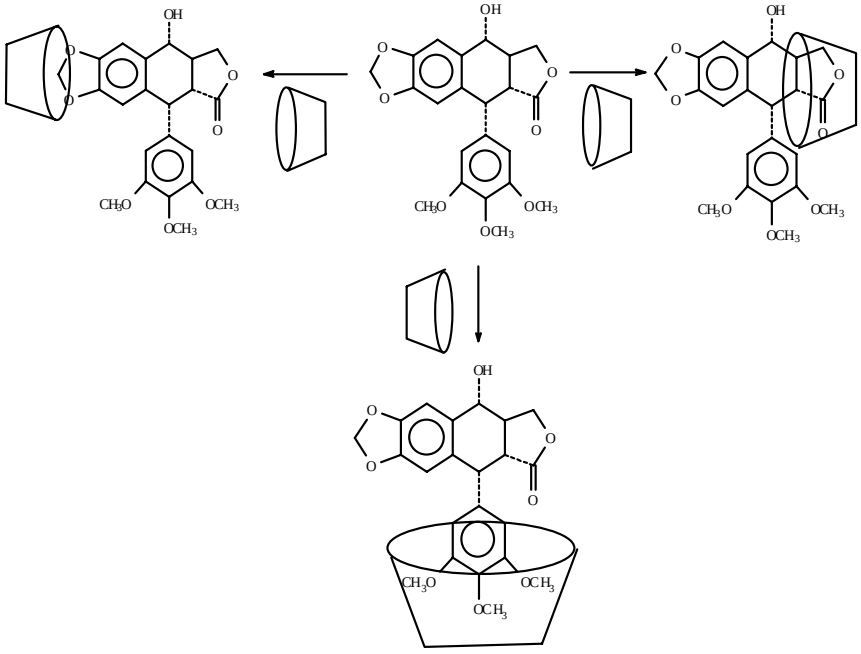
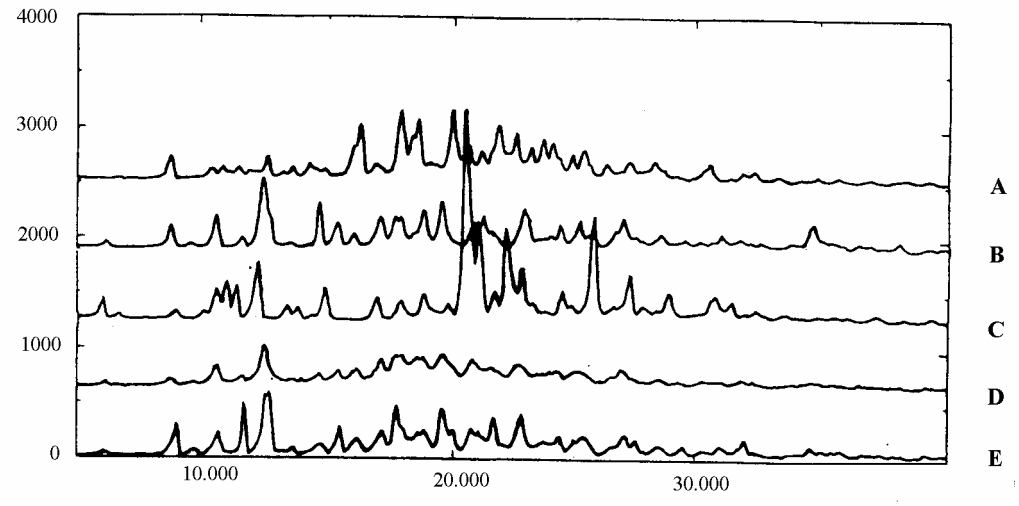


Figure 3 X-ray diffractograms of (A) podophyllotoxin (B) physical Mixture (C) inclusion complex



(D) rotate-drying (E) β-CD

References and Notes

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