



Study of the Supramolecular Inclusion of β -Cyclodextrin with Andrographolide

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Abstract

An inclusion complex of β -cyclodextrin with andrographolide (Andro) was prepared by using a convenient new method of microwave irradiation. The structure of the inclusion complex was determined by UV and IR analyses as well as ^1H NMR, ^{13}C NMR and two dimensional NOE spectroscopic measurements. The results indicated that the possible stoichiometry of complex formation is 1:1 (guest:host ratio) and the two isomeric 1:1 inclusion complexes are present simultaneously in solution. Thermal studies proved the thermal stability of the inclusion complex.

Introduction

Andrographolide (Andro) (Figure 1), a diterpene lactone isolated from *Andrographis paniculate* Nees [1], was shown to have several biological activities including analgesic, antipyretic and anti-inflammatory effects [2, 3] and has been used in the treatment of dysentery. However, it could not be used as a drug of oral administration due to its poor water solubility, which prevents it from being absorbed well in the body. In addition, it is unstable towards oxygen. These disadvantages limit the application of Andro. β -Cyclodextrin (β -CD) (Figure 2) is known to contain a hydrophobic cavity suitable for the inclusion of various organic molecules [4]. In pharmaceuticals, β -CD is used to increase solubility and stability and to reduce the toxicity of drugs [5]. Currently the widely used methods to prepare inclusion compounds are: coprecipitation, kneading, freeze-drying and cogrinding [6]. Recently, microwave irradiation has been developed for rapid organic synthesis [7]. According to a very recent report, carrying out the inclusion reactions using microwave heating, as opposed to conventional methods, has the major advantages of shorter reaction times and higher yield of products [8]. In the present work, we prepared the inclusion compound of Andro/ β -CD under microwave irradiation. It has been found that the high temperature so rapidly obtained in the reaction vessels significantly reduced the reaction times. The product was characterized by UV and FTIR spectroscopy. Furthermore, since the structural characterization is of particular significance for supramolecular host-guest complexes [9], we also investigated the structure of Andro/ β -CD by using ^1H NMR, ^{13}C NMR and 2D NOE spectroscopy. In addition,

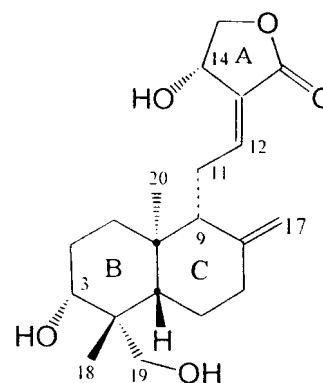


Figure 1. Structural formula of Andrographolide [1].

the thermal stability of the inclusion complex was studied by DTA and TGA measurements.

Experimental

Materials

β -CD (99.5%, Suzhou weijing Plant, China) was purified by recrystallization from distilled water. Andrographolide was extracted from *Andrographis paniculata* Nees in our laboratory. The ^1H NMR, ^{13}C NMR and 2D NOE measurements were carried out in D_2O and DMSO-d_6 (Aldrich Co.) solutions. Other chemicals were of analytical reagent grade purity.

Instrumental methods

Infrared spectra were recorded with a Nicolet AVATAR 360 FT-IR using the KBr disc method. Microwave irradiation was carried out with a Galanz WP 700L20 Microwave Oven

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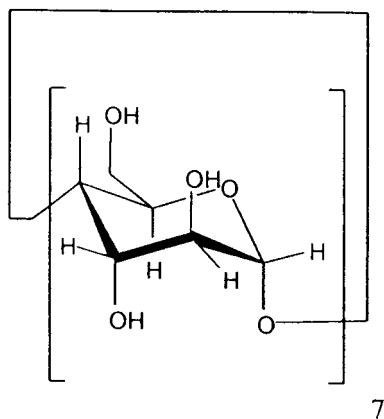


Figure 2. Structural formula of β -cyclodextrin.

(Guangdong, China) under atmospheric pressure. Differential thermal analysis (DTA) and thermogravimetric analysis (TGA) measurements were obtained using a Shangping ZRY-2P Simultaneous Thermal Analyzer (Shanghai, China). The experiments were performed at a linear heating rate of $5^\circ\text{C}/\text{min}$ in the temperature range from 30°C to 400°C . Elemental analysis was conducted with a Carlo Erba 1106 vario EL Elementar (Germany). ^1H NMR, ^{13}C NMR and 2D NOE were recorded on a Bruker AM-400 NMR spectrometer in D_2O - DMSO-d_6 (1:1 v/v).

Preparation of the inclusion complex of β -CD with Andro

A mixture of 0.04 mmol β -CD and 0.02 mmol Andro was ground in a glass container. Minimum amounts of solvents (ethanol/water = 1:1 v/v) were added. The mixture was reacted for 90s at 60°C in the microwave oven. After the reaction was complete, adequate amounts of solvents were added to remove the residual β -CD and Andro, then the precipitate was filtered and the inclusion complex was thus obtained.

Solubility studies

The phase-solubility diagram was recorded according to Xueyi Ma *et al.* [10, 11]. For that purpose, aqueous solutions of β -CD with concentrations of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and $4.0 \times 10^{-4}\text{ M}$ were prepared. Excess amounts of Andro were added to each solution of β -CD. The solutions were then placed in the microwave oven for 90s at 60°C , then centrifuged and carefully filtered. The 5 mL samples of filtrates were diluted to 10 mL with ethanol. Their absorption was measured by UV spectrophotometry (226 nm). The phase-solubility diagram was therefore obtained (Figure 3).

Results and discussion

FT-IR Study

Figure 4 shows the infrared spectra of β -CD, Andro, a physical mixture of Andro and β -CD at a 1:2 molar ratio as well as the complex obtained under microwave irradiation. Characteristic bands arising from $-\text{C}=\text{O}$ (1726 cm^{-1}) and $-\text{C}=\text{C}$

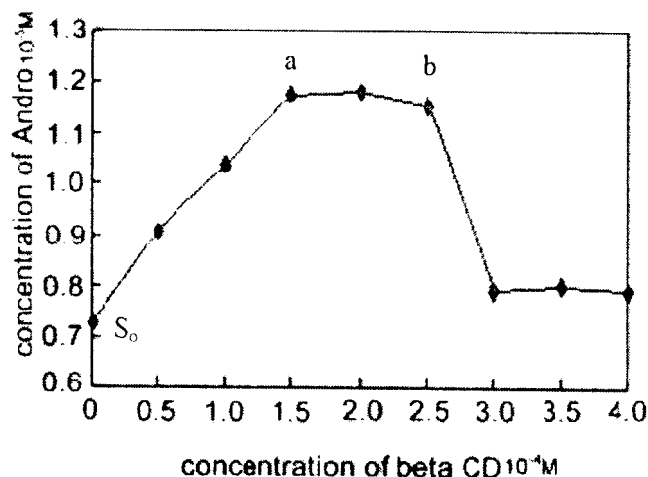


Figure 3. Solubility curve of Andro in aqueous solutions of β -CD.

Table 1. ^1H NMR chemical shift values for β -CD in the absence and the presence of Andro (molar ratio 1:2)

Proton	β -CD (δ_0)	β -CD-Andro (δ)	$\Delta\delta$ ($\delta - \delta_0$)
H-1	4.924	4.825	-0.099
H-2	3.515	3.398	-0.117
H-3	3.771	3.671	-0.100
H-4	3.470	3.361	-0.109
H-5	3.641	3.597	-0.044
H-6	3.739	3.643	-0.096

(1674 cm^{-1}) observed for pure Andro and the physical mixture, shift to 1745 and 1668 cm^{-1} respectively in the case of the complex. Furthermore, the intensity and shape of these two bands change dramatically for the inclusion compound as compared to those for Andro and the physical mixture. These indicated that the vibrating and bending of the guest molecule (Andro) was restricted due to the formation of an inclusion complex [12], and very likely the lactone ring in Andro was inserted into the cavity of β -CD.

NMR studies

NMR spectroscopy has been previously used to establish inclusion modes and stoichiometries [13]. In the present work, ^1H NMR, ^{13}C NMR as well as the 2D NOE measurements were performed to elucidate the structure of Andro/ β -CD. The ^1H NMR chemical shift values of β -CD in the free and complexed state are shown in Table 1. All of the six β -CD protons show noteworthy up-field shifts. These observations prove the reality of the inclusion and show that the driving forces for the formation of the inclusion complex are hydrophobic interactions [13].

Since the Andro molecule contains two parts, ring A and the connected rings B&C, this may lead to two isomeric 1:1 complexes and a 1:2 complex. To ascertain the structure of the inclusion complex, ^1H NMR and ^{13}C NMR spectroscopy studies of Andro were therefore undertaken. The difference in chemical shift values between Andro in the free and complexed state are presented in Tables 2 and 3.

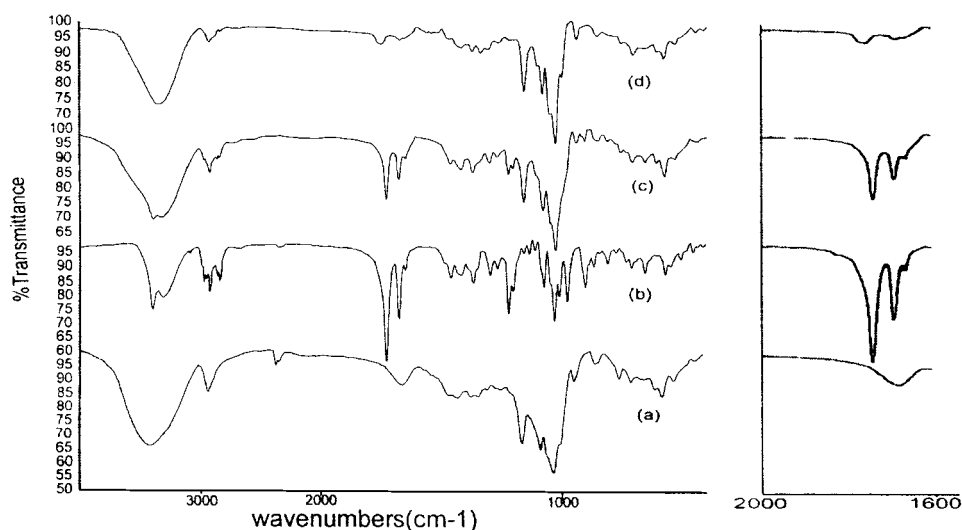


Figure 4. FT-IR Spectra of (a) β -CD (b) Andro (c) physical mixture and (d) the inclusion complex of Andro/ β -CD.

Table 2. ^1H NMR chemical shifts corresponding to Andro in the absence and presence of β -CD

Andro proton	Andro(δ_0)	β -CD-Andro(δ)	$\Delta\delta$ ($\delta - \delta_0$)
H-11	2.493	2.500	0.007
H-12	6.731	6.697	-0.034
H-14	4.935	4.920	-0.015
H-15a	4.344	4.341	-0.003
H-15b	4.098	4.084	-0.014
H-17	4.759	4.771	0.012
H-18	0.997	1.011	0.014
H-19a	3.857	3.860	0.003
H-19b	3.283	3.267	-0.016
H-20	0.559	0.571	0.012

Table 3. ^{13}C NMR chemical shifts corresponding to Andro in the absence and presence of β -CD

Andro carbon	Andro(δ_0)	β -CD-Andro(δ)	$\Delta\delta$ ($\delta - \delta_0$)
C-1	37.60	37.69	0.09
C-2	28.41	28.46	0.05
C-3	80.21	80.27	0.06
C-5	55.37	55.47	0.10
C-6	24.99	25.03	0.04
C-7	38.56	38.62	0.06
C-8	149.09	149.10	0.01
C-9	56.69	56.78	0.09
C-11	25.67	25.72	0.05
C-12	150.51	150.47	-0.04
C-13	129.04	129.10	0.06
C-15	76.72	75.39	-1.33
C-17	109.52	109.57	0.05
C-18	23.61	23.67	0.06
C-19	64.22	68.73	4.51
C-20	15.83	15.87	0.04

The proton and carbon assignments were taken from Fujita *et al.* [1]. As shown in Table 2, the proton signals of Andro's three rings all showed up or down-field shifts between the free and complexed state, indicating they are all affected as a result of complexation. It can be deduced from this information that both parts of Andro probably entered the inner cavity of β -CD.

Table 3 shows that all Andro's carbons except C-12 and C-15 undergo a positive shift due to being deshielded by complexation, which indicated they are close to the wide side of β -CD rather than deep in the cavity [14, 15]. Inoue *et al.* have interpreted this phenomenon on the basis of reaction field theory [14]. In addition, it was noticed that in ring C, the carbon signal of C-8 showed almost no shift while the signal of C-17 shifts 0.05 ppm. Coupling this with the chemical shift change of H-17, we judged that the methylene group of C-17 was inserted into one β -CD molecule together with ring A while ring B and ring C were inside another, with ring C partially exposed. As with the result of the ^1H NMR, the possible inclusion complexes inferred from ^{13}C NMR are two isomeric 1:1 complexes and the 1:2 complex. The proposed structure of Andro/ β -CD judging from the ^{13}C NMR is shown in Figure 5. However, all of the considerations about the inclusion model inferred from the NMR chemical shift data are merely speculative and need to be confirmed by NOE's and elemental analysis. The result of the 2D NOE experiment (Figure 6) is compatible with the aforementioned inclusion mode. Table 4 shows the condition of the cross-peaks between the protons of β -CD and Andro in Andro/ β -CD. There is a set of cross-peaks connecting the H-1~H-6 resonances of β -CD to the hydrogen signals of the protons in the two parts of Andro. The 2D NOE cross-peaks and a few intermolecular connectivities observed may be due to a very weak complexation between Andro and β -CD.

Elemental analysis experiment

The final stoichiometry is determined by elemental analysis. The result is: C%: Found 50.27, Calculated (1:1 complex) 50.13; H%, Found 6.88, Calculated (1:1 complex) 6.74.

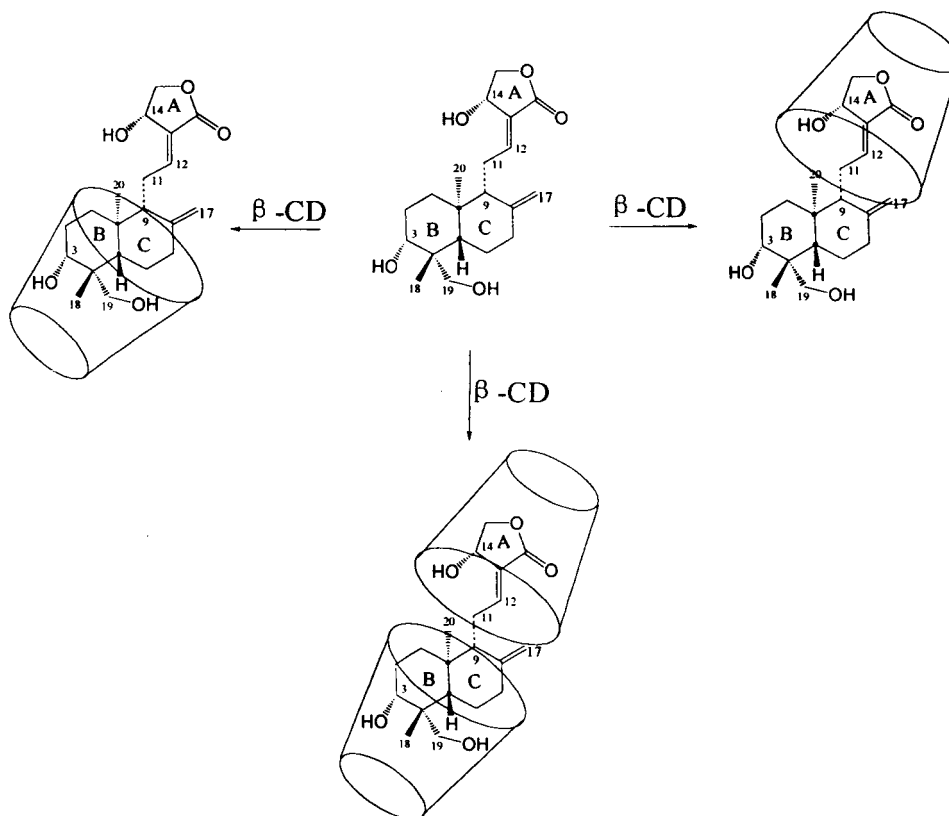


Figure 5. Proposed models for the Andro/ β -CD inclusion complex.

Table 4. 2D NOE effects in the inclusion complex of β -CD with Andro

β -CD proton	4-CH ₃	10-CH ₃	H-19	Andro H-17	proton H-11	H-14	H-15
H-1	+ ^a	-	++	+	++	-	+
H-2	+	-	++	+	+	-	++
H-3	-	++	+	+	++	-	++
H-4	-	-	++	+	-	-	++
H-5	+	-	+	-	++	++	+
H-6	+	++	+	+	++	+	++

^a ++ strong; + medium; - no NOE effects.

It indicates that Andro formed two isomeric 1:1 inclusion complexes with β -CD and the 1:2 inclusion complex is not formed

Solubility studies

The phase-solubility diagram for the complex formation between Andro and β -CD is presented in Figure 3 [16]. Considering this curve, it can be seen that the apparent solubility of Andro increases due to the formation of a soluble inclusion complex between Andro and β -CD. When the solubility limit of this complex is reached (point a), the ascending linear portion starts levelling-off and further addition of β -CD results in the precipitation of a microcrystalline complex. The concentration of uncomplexed Andro is maintained constant by dissolution of solid Andro. The complex formation continues in the plateau region and it precipitates from saturated solution as the concentration of β -CD increases. At

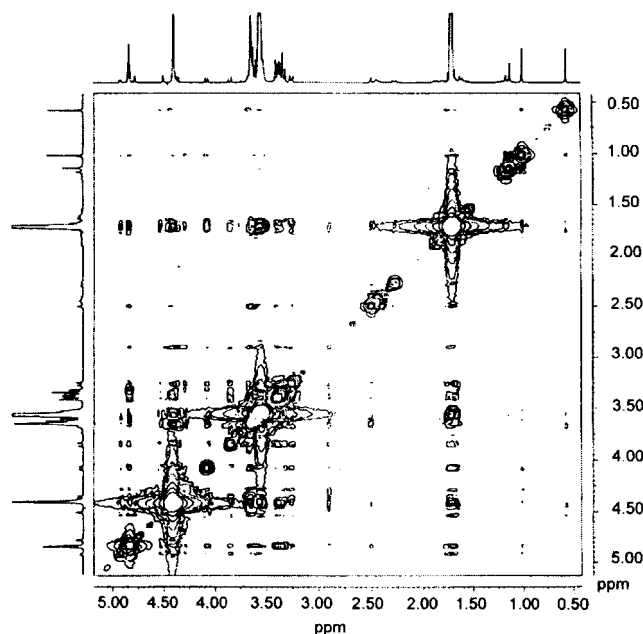


Figure 6. The 400 MHz 2D NOE spectrum of Andro/ β -CD in D₂O-DMSO-d₆. Intermolecular connectivities are shown in the diagram.

point b, all solid Andro has been consumed, and further addition of β -CD resulted in depletion of Andro in solution by complex formation and precipitation of an insoluble complex. It is clear that the solubility diagram of Andro in the presence of β -CD belongs to a Bs type.

The stoichiometric ratio evaluated from the diagram is a little more than 1:1 (host:guest ratio). Coupled with the

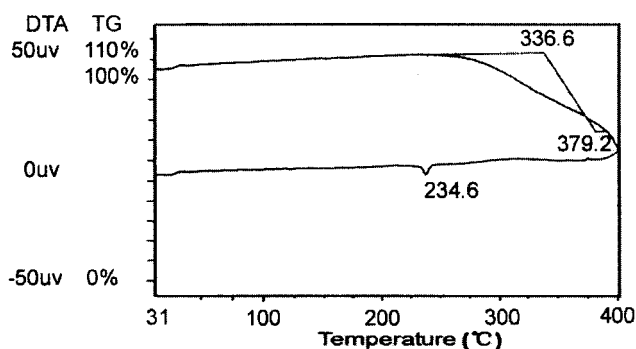


Figure 7. DTA and TGA thermograms for Andro.

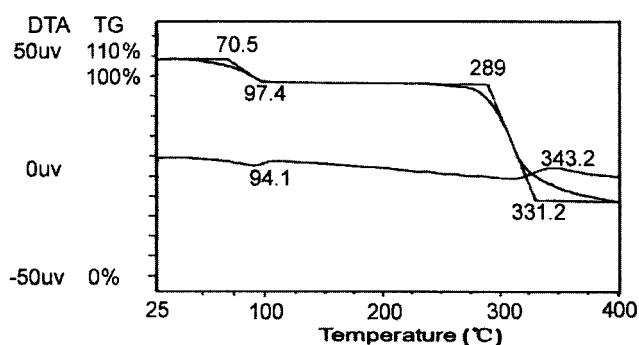


Figure 8. DTA and TGA thermograms for β -CD.

above information, the 1:1 stoichiometry is correct for the inclusion complex of Andro with β -CD.

DTA and TGA measurements

Differential thermal analysis (DTA) and thermogravimetric analysis (TGA) were performed to investigate the thermal stability of Andro/ β -CD (Figures 7–9). Figure 7 shows the DTA and TGA curves of Andro. In the DTA curve, an endothermic behavior was observed at 234.6°C, while no weight loss corresponding to this temperature in the TGA curve was found. This indicated that it was a physical change. Coupled with the melting point of Andro (232°C), we can conclude that this point represents a phase change of Andro from the solid state to the liquid state.

The DTA and TGA curves of β -CD are shown in Figure 8. Undoubtedly, water was evolved beginning at about 94°C because it is an endothermic behavior in the DTA curve and associated with a loss in weight in the TGA curve. The exothermic behavior at about 343°C in the DTA curve is indicative of decomposition of β -CD because a great loss in weight can be seen in the TGA curve at this temperature.

In the DTA thermogram of Andro/ β -CD (Figure 9), there were three processes. The endotherm peak at 67.4°C was due to water evolving in comparison with the weight-loss data (TGA curve). The exothermic behavior at 240.6°C indicated recrystallization of the Andro/ β -CD, because the thermogravimetric data did not show any loss associated with this peak, which means it was a chemical rather than a physical transition. The second exothermic feature on the DTA confirmed the above deduction. The weight-loss temperature was 289.7°C, which was just the same as that of

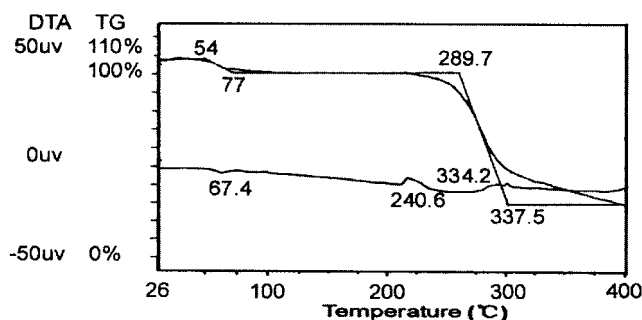
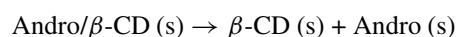
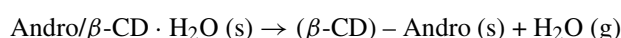


Figure 9. DTA and TGA thermograms for Andro/ β -CD.

β -CD (Figure 8), indicating decomposition of β -CD. With this information the thermal decomposition reaction and recrystallization changes were:



From the above information, it can be seen that before being included in β -CD, Andro melted at 234°C while in the case of the inclusion complex, it began to recrystallize until 240°C. These findings show that the thermal stability of Andro was improved when it was included in β -CD.

Conclusions

The inclusion complex of Andro with β -CD prepared under microwave irradiation was studied in this work. The structure of the inclusion complex was inferred from the NMR and the elemental analysis data. However, since the X-ray crystal diffraction has not been carried out, the structure interpretation based on the NMR experiments is tentative and awaits confirmation.

Acknowledgement

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