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Structural Study of Inclusion Complex of Andrographolide with β-Cyclodextrin Prepared under Microwave Irradiation

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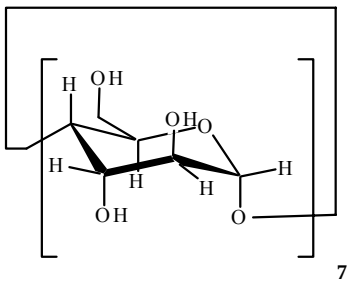
Abstract: An inclusion complex of β-cyclodextrin with andrographolide (Andro) was prepared by using a convenient method of microwave irradiation. The structure of the inclusion complex was determined by the ^{1}H NMR, 2D NMR spectroscopy as well as the elemental analysis.

Keywords: *Andrographolide*, β-cyclodextrin, inclusion complex, NMR, microwave irradiation.

Andrographolide (Andro) (**Figure 1**) is a diterpene lactone isolated from *Andrographis paniculate Nees*[1]. It has showed several biological activities including analgesic, antipyretic and anti-inflammatory effects[2]. However, its poor water solubility and unstability towards oxygen restrained its application. In pharmaceutics, β-CD is used to increase solubility and stability[3]. We have prepared the inclusion compound of Andro/β-CD (**Figure 2**) under microwave irradiation[4]. It has been found that the high temperature so rapidly obtained in the reaction vessels significantly reduced the reaction times. The structure of Andro/β-CD has been studied by the methods of NMR spectroscopy as well as elemental analysis.

Figure 1 Structural formulae of β-cyclodextrin and andrographolide



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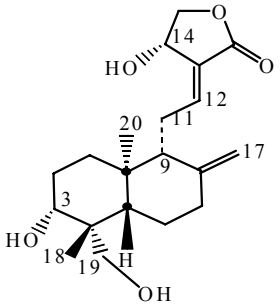

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Microwave irradiation was carried out with a Galanz WP 700L20 Microwave Oven (Guangdong, China) under atmospheric pressure. Elemental analysis was conducted with a Carlo Erba 1106 vario EL Elementar (Germany). All NMR spectra were recorded with a Bruker AM-400 NMR spectrometer in D_2O-DMSO-d_6 (1:1 v/v).

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 Andro/β-CD complexes were prepared under microwave irradiation. A mixture of 0.04 mmol β-CD and 0.02 mmol Andro was ground in a glass container. Minimum amounts of solvents were added into it. 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.

The ^{1}H 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[5].

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

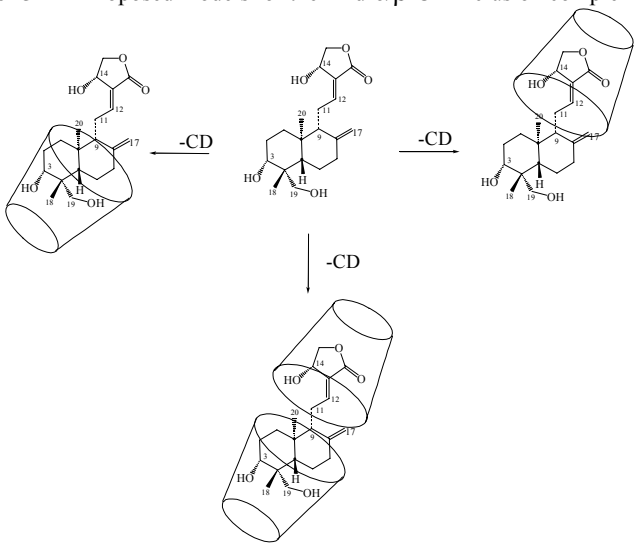
Proton	β-CD (δ_0)	β-CD-Andro (δ)	$\Delta\delta$ (δ-δ_0)
H-1	4.924	4.825	-0.099
H-2	3.515	3.398	-0.117
H-3	3.771	3.671	-0.100
H-4	3.470	3.361	-0.109
H-5	3.641	3.597	-0.044
H-6	3.739	3.643	-0.096

Table 2 shows that 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. As a result, two isomeric 1:1 complexes and a 1:2 complex may be at present in solution simultaneously. The possible structure formulae of β-CD/Andro inclusion complex is shown in **Figure 2.**

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

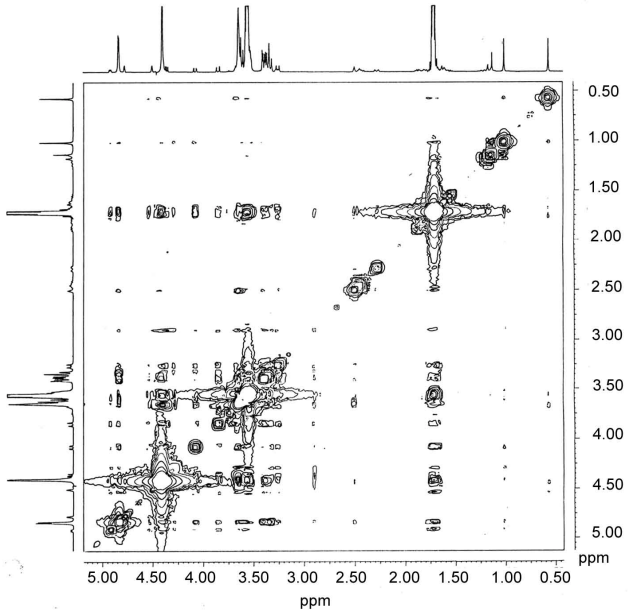
Andro proton	Andro(δ_0)	β-CD-Andro(δ)	$\Delta\delta$ (δ-δ_0)
H-11	2.493	2.500	0.007
H-12	6.731	6.697	-0.034
H-14	4.935	4.920	-0.015
H-15	4.098	4.084	-0.014
H-17	4.759	4.771	0.012
H-18	0.997	1.011	0.014
H-19	3.283	3.267	-0.016
H-20	0.559	0.571	0.012

Figure 2 Proposed models for the Andro/β-CD inclusion complex



The result of the 2D NOE experiment (**Figure 3**) is coincident with the aforementioned inclusion mode. There is a set of crossing peaks connecting the H-1~H-6 resonances of β-CD to the hydrogen signals of the protons in the two parts of Andro.

Figure 3 The 2D NOE spectrum of Andro/ β -CD (400 MHz) in D_2O-DMSO-d_6



The final stoichiometry is determined by elemental analysis. C, H (%): Calcd. 50.13, 6.74; Found 50.27, 6.88. It indicated that Andro formed two isomeric 1:1 inclusion complexes with β-CD and the 1:2 inclusion complex did not exist.

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