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Structure, Thermal Stability and Water Solubility of (Tanshinone II-A)/(β-Cyclodextrin) Inclusion Complex

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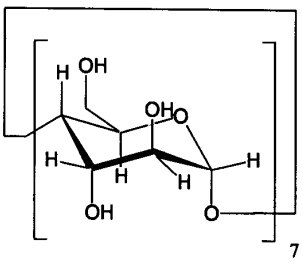
The structure of the inclusion complex of tanshinone II-A and β-cyclodextrin formed under microwave irradiation was studied by the UV, IR, and NMR spectroscopy. The association constant of the complex in water is 210 M^{-1}, as determined from the double reciprocal curve by UV spectroscopy. The enhanced water solubility of the complex was found. Thermal studies proved the increased thermal stability of the inclusion complex.

Key words: tanshinone II-A, β-cyclodextrin, inclusion complex, microwave irradiation

Tanshinone II-A (Tan II-A) (Fig. 1 formula 2) is a diterpene quinone naturally occurring in roots of *Salvia miltiorrhiza*. It reveals antibacterial, inhibition of platelet adhesion and aggregation activity. This compound can be used as a drug for treatment of coronary heart disease [1]. Due to its poor solubility in water, Tan II-A cannot be administered by injection and, therefore, its clinical use is limited. β-Cyclodextrin (β-CD) (Fig. 1 formula 1) is a macrocyclic oligosaccharide built up by 7 glucose units, possessing a hydrophobic central cavity suitable for inclusion of various organic molecules. In pharmaceutical applications, β-CD has been extensively used to enhance stability, solubility and dissolution rate [2–4] of drugs as well as to reduce their toxicity [5].



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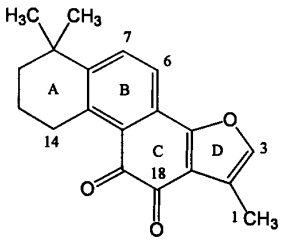

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Figure 1. Structural formulae of β-cyclodextrin (1) and tanshinone II-A (2).

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The aim of the present work is to check if the inclusion of Tan II-A by β-CD increases its water solubility. Currently, the widely used preparation methods of inclusion compounds are: coprecipitation, kneading, freeze-drying and cogrinding. Recently, microwave irradiation has been introduced for rapid organic synthesis [6]. Major advantages of inclusion reactions carried out under microwave heating, as compared to conventional methods, is shorter reaction time and higher yield of products [7]. Therefore, we prepared the inclusion compound of (Tan II-A)/(β-CD) under microwave irradiation. It appeared that the high temperature so rapidly attained significantly reduced the reaction time. The structure of the product was characterized by the UV, FTIR and NMR spectroscopy. In addition, the thermal stability of the inclusion complex was studied by DTA and TGA, while solubility of the complex was tested by the UV spectroscopy.

EXPERIMENTAL

Materials: β-CD (99.5% purity, Suzhou Weijing Plant, China) was purified by recrystallization from distilled water. Tan II-A was extracted from *Salvia miltiorrhiza Nees* in our laboratory. The DMSO-d_6 (Aldrich) solutions were used for the ^{1}H NMR and ^{13}C NMR spectroscopy measurements. Other chemicals used were of analytical reagent grade purity.

Preparation of the inclusion complex of (Tan II-A)/(β-CD): A mixture of 0.06 mmol β-CD and 0.03 mmol Tan II-A was ground in a glass container for 2 minutes. Minimum amounts of solvents (methanol to water was 1:1, *v/v*) were added. The mixture was allowed to react for 90 s at 60°C in a microwave oven. After the reaction was completed, adequate amounts of solvents were added to remove the residual β-CD and Tan II-A, then the precipitate was filtered out and the inclusion complex was obtained from the precipitate.

Instrumentation and methods: The UV spectra were recorded with a SHIMADZU UV-260 spectrophotometer (Japan). IR spectra of the KBr pellets were recorded with a Nicolet AVATAR 360 FT-IR (American). Microwave irradiation was carried out with a Galanz WP 700L20 Microwave Oven (Guangdong, China) under atmospheric pressure. The differential thermal analysis (DTA) and thermogravimetric analysis (TGA) measurements were performed by using a Shangping ZRY-2P Simultaneous Thermal Analyzer (Shanghai, China). A linear heating rate of 10°C/min in the range 20°C to 650°C was applied. Elemental analysis was conducted with a Carlo Erba 1106 vario EL Elementar (Germany). The NMR spectra were recorded on a Bruker AM-400 NMR spectrometer (Germany) at 25°C.

RESULTS AND DISCUSSION

Inclusion complex stoichiometry: The double reciprocal curves of inclusion complex of (Tan II-A)/(β-CD) was recorded according to the Benesi-Hildebrand method [8]. For that purpose, 1.0×10^{-4} M Tan II-A aqueous solution was prepared. The 0–7.5 ml β-CD solution samples with concentrations of 1.0×10^{-2} M were added to each 1 ml solution sample of Tan II-A. Then, the samples were diluted to l0 ml with water. Their UV absorption was measured at 272 nm. The method was employed for the determination of the complex association constant, K_a, by using the relation:

$$1/\Delta A = 1/\Delta\varepsilon[G] + 1/\Delta\varepsilon[G][CD]K_a \quad (1)$$

Where ΔA is the difference in absorption of the Tan II-A aqueous solutions in the presence and absence of β-CD. $\Delta\varepsilon$ stands for difference in molar absorption coefficients of (Tan II-A)/(β-CD) and Tan II-A, while [G] and [CD] are concentrations of Tan II-A and β-CD, respectively. From a linear relationship of $1/\Delta A$ *versus* 1/[CD], we conclude that Tan II-A and β-CD form a 1:1 inclusion complex (Fig. 2) [9]. All the experiments were carried out at 25°C, pH = 7.

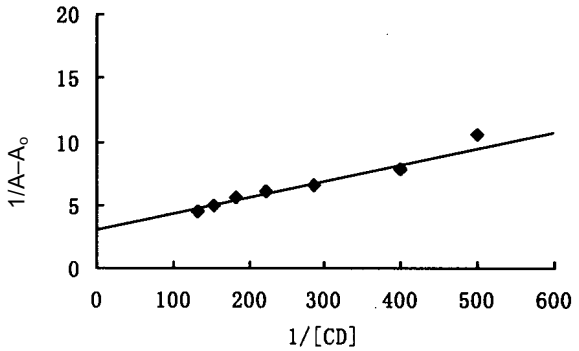

Figure 2. Double reciprocal curve, according to eq. (1), for inclusion complex of (Tan II-A)/(β-CD).

Solubility studies: The solubility diagram was constructed according to Ma *et al*. [10]. For that purpose, 0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mM β-CD aqueous solutions were prepared. Excess amounts of Tan II-A were added to each solution of β-CD. The solutions were placed in the microwave oven for 90 s at 60°C, then centrifuged and filtered out. The 5 ml samples of the filtrate were diluted with methanol to 10 ml. The UV absorption of the resulting solutions was measured at 272 nm and used for constructing a solubility diagram (Fig. 3). Apparently, solubility of Tan II-A increased due to formation of an inclusion complex of Tan II-A and β-CD. The solubility curve reached maximum at 4.0×10^{-3} M β-CD. For higher concentrations of β-CD the solubility slightly decreased as a microcrystalline complex precipitated.

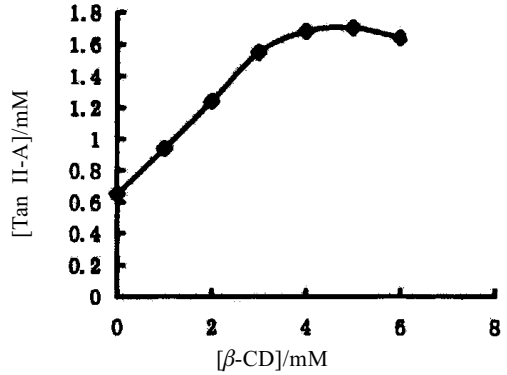

Figure 3. Solubility curve for Tan II-A in aqueous solutions of β-CD.

FT-IR spectroscopy: Figure 5 shows the IR spectra for β-CD, Tan II-A, the inclusion complex of (Tan II-A)/(β-CD) and a mixture of β-CD to Tan II-A in a mole ratio of 2:1. Characteristic bands corresponding to –OH (3383 cm^{-1}), observed for pristine β-CD, shifted to 3396 cm^{-1} for the complex. Then, –C=O (1670 cm^{-1}), –C=C (1535 cm^{-1}) bands, observed for genuine Tan II-A, shifted to 1668 cm^{-1}, 1530 cm^{-1} for the complex, respectively. Furthermore, the intensity and shape of the two latter bands changed dramatically for the inclusion compound, as compared to those for Tan II-A and the physical mixture. These results indicate that the vibrating and bending of a Tan II-A molecule was restricted due to the formation of an inclusion complex [11] and, very likely, the lactone ring and benzene ring were inserted into the cavity of the β-CD molecule.

NMR spectroscopy: The NMR spectroscopy has been previously used to establish inclusion modes and complex stoichiometry. In the present work, the ^{1}H NMR and ^{13}C NMR spectroscopy measurements were performed to elucidate the structure of (Tan II-A)/(β-CD). The ^{1}H NMR chemical shift values of β-CD in the free and complexed form are shown in Table 1. In the presence of Tan II-A, the β-CD signals showed shifts of *ca*. 0.015–0.190 ppm. The chemical shifts of β-CD protons revealed, noteworthy, up-field changes of proton H-3 (0.190 ppm) and H-5 (0.046 ppm), which are located on the inner surface of the β-CD cavity. These two latter shifts clearly prove formation of the inclusion complex [12,13].

Since the Tan II-A molecule contains ring A, B, C and D (Formula 2 in Fig. 1), this may lead to two isomeric 1:1 complexes and one 1:2 complex. In order to ascertain the structure of the inclusion complex, ^{1}H NMR and ^{13}C NMR spectroscopy studies of Tan II-A were, therefore, undertaken. The difference in chemical shift values between Tan II-A in the free and complexed form are presented in Tables 2 and 3. As shown in Table 2, all proton signals of the Tan II-A three rings A, B and D (ring C have no proton signal) of the free form were down-field shifted (by *ca*. 0.01–0.06 ppm) as compared to complexed form, indicating that they are all affected by complexation. Hence, both parts of the Tan II-A molecule entered, presumably, the inner cavity of the β-CD molecule. Table 3 displays that all Tan II-A carbon signals, except those for C-4 and C-7 (showing up-field shift changes by 0.045 ppm and 0.153 ppm, respectively), undergo a positive shifts due to deshielding caused by complexation. These shifts indicate that these carbon atoms interact with the wide rim of the β-CD molecule rather than penetrate deeply its cavity [14,15]. Inoue *et al.* [15] have interpreted this phenomenon on the basis of a reaction field theory. In addition, it was noticed that C-14 and C-16 showed small shift changes equal to 0.015 ppm and 0.010 ppm in ring A and ring B, respectively, while the signal of C-6 changes by 0.031 ppm. From the above data, we inferred that the ring A and ring B were inserted in one β-CD molecule, while ring C and ring D in another one, forming two different isomers. The proposed structure of (Tan II-A)/(β-CD), based on the NMR spectra analysis, is shown in Figure 4.

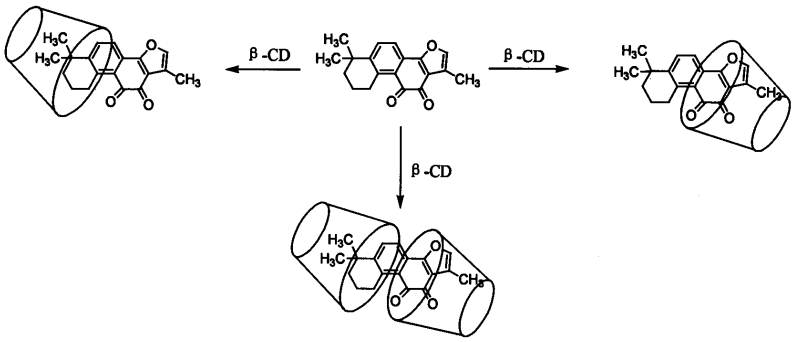

Figure 4. Proposed scheme for the (Tan II-A)/(β-CD) inclusion complex formation.

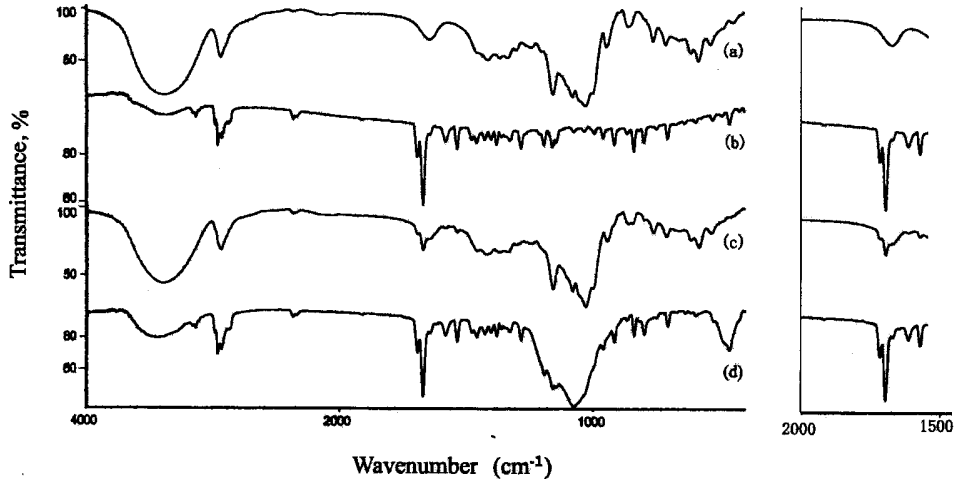

Figure 5. The FT-IR spectra for (a) β-CD, (b) Tan II-A, (c) inclusion complex of (Tan II-A)/(β-CD), (d) mixture of Tan II-A and β-CD, mole ratio 1:2.

Table 1. The ^{1}H NMR chemical shifts (ppm) for β-CD in the absence and presence of Tan II-A.

β-CD Proton	β-CD (δ_0)	Tan II-A/β-CD (δ)	$\Delta\delta(\delta - \delta_0)$
H-1	4.820	4.835	0.015
H-2	3.296	3.312	0.016
H-3	3.644	3.454	–0.190
H-4	3.338	3.399	0.061
H-5	3.580	3.534	–0.046
H-6	3.644	3.620	–0.024

Table 2. The ^{1}H NMR chemical shifts (ppm) for Tan II-A in the absence and presence of β-CD.

Tan II-A Proton	Tan II-A (δ_0)	Tan II-A/β-CD (δ)	$\Delta\delta(\delta - \delta_0)$
H-3	7.564	7.589	0.025
H-6	7.798	7.822	0.024
H-7	7.718	7.722	0.004
H-10,11	1.230	1.281	0.051
H-12	1.611	1.627	0.016
H-13	1.696	1.729	0.033

Table 3. The ^{13}C NMR chemical shifts (ppm) corresponding to Tan II-A in the absence and presence of β-CD.

Tan II-A Carbon	Tan II-A (δ_0)	Tan II-A/β-CD (δ)	$\Delta\delta(\delta - \delta_0)$
C-1	8.261	8.276	0.015
C-2	119.592	119.638	0.046
C-3	142.045	142.060	0.015
C-4	160.208	160.163	–0.045
C-5	133.146	133.192	0.046
C-6	126.415	126.446	0.031
C-7	119.913	119.760	–0.153
C-8	148.974	149.020	0.046
C-9	34.026	34.056	0.030
C-10,11	31.171	31.202	0.031
C-12	37.094	37.109	0.015
C-13	18.426	18.457	0.031
C-14	29.218	29.233	0.015
C-15	142.533	142.576	0.046
C-16	126.222	126.232	0.010
C-17	182.188	182.233	0.045
C-18	174.739	174.785	0.046
C-19	119.189	119.226	0.037

DTA and TGA measurements: Thermal studies of macromolecule compounds can result in determination of properties, such as their stiffness, toughness or stability. Moreover, melting-point, phase-transition, pyrolysis, and curing temperatures can be accurately determined. In the present work, DTA and TGA measurements were performed in order to investigate thermal stability of (Tan II-A)/(β-CD) (Figs. 6–8). Figure 6 shows the DTA (solid) and TGA (dash) curves for Tan II-A. In the DTA cu-

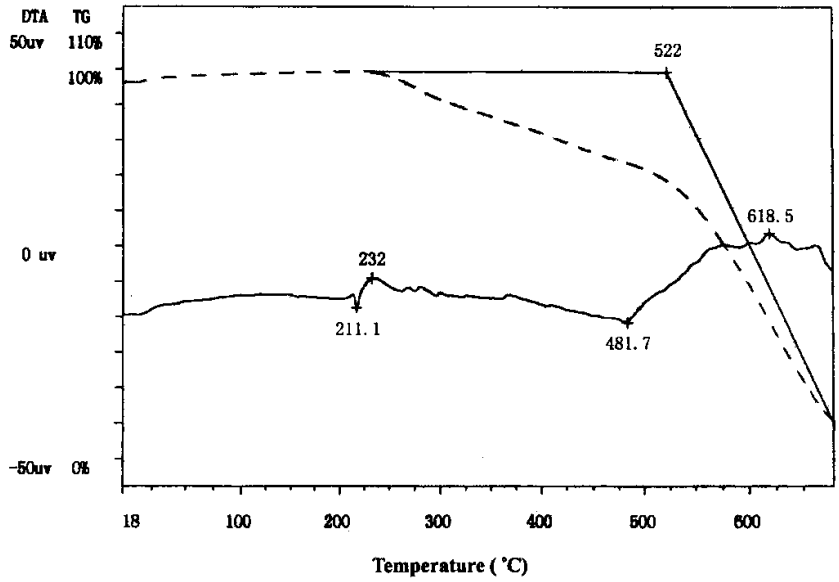

Figure 6. DTA (solid) and TGA (dash) thermograms for Tan II-A.

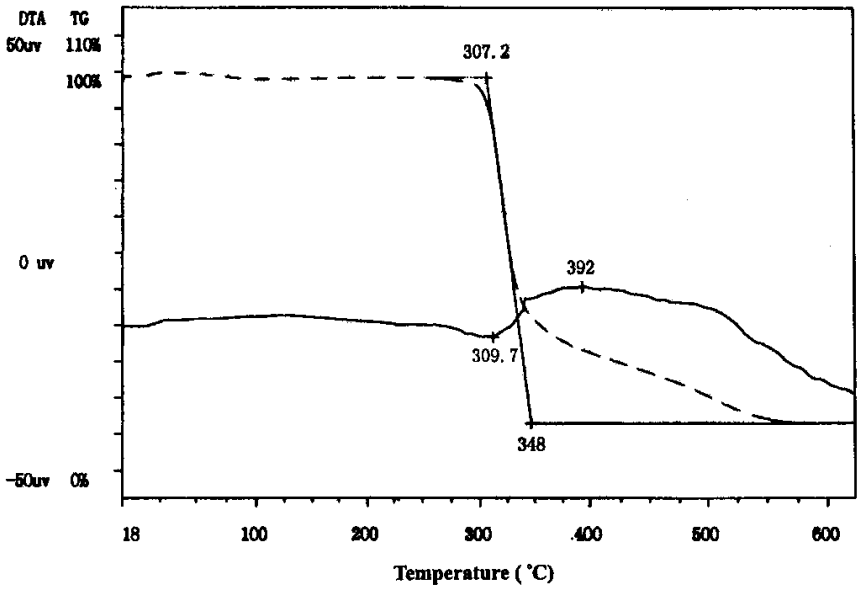

Figure 7. DTA (solid) and TGA (dash) thermograms for β-CD.

rve, an endothermic behavior was observed at 211.1°C, while no mass loss at this temperature was found in the DTA curve. This behavior indicated a physical transformation. Coupling this datum with the melting point of Tan II-A (209°C), we can infer that the point of 211.1°C represents a phase transition of Tan II-A from the solid to liquid state. Figure 7 reveals the DTA (solid) and TGA (dash) curves for

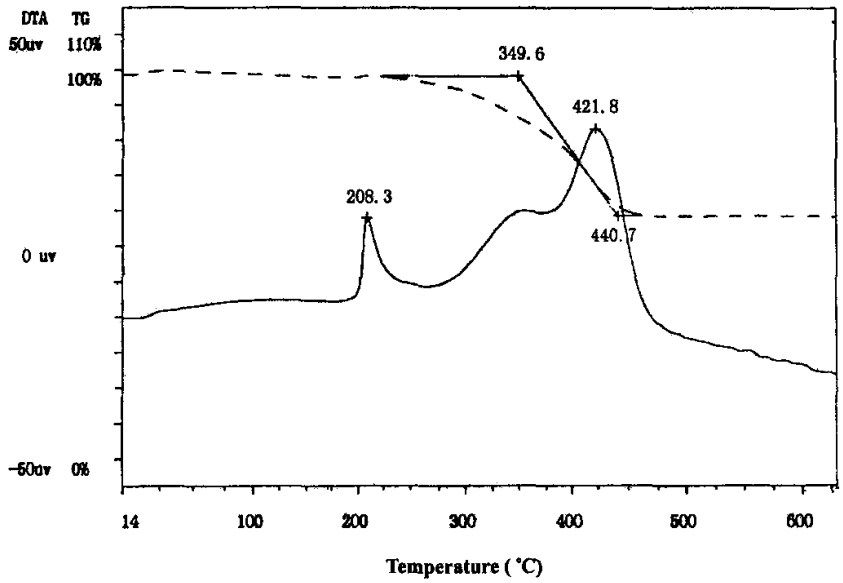

Figure 8. DTA (solid) and TGA (dash) thermograms for (Tan II-A)/(β-CD).

β-CD. The exothermic behavior at 392°C in the DTA curve is indicative for decomposition of β-CD because a great loss of mass can be seen in the TGA curve at this temperature. The DTA (solid) and TGA (dash) curves for (Tan II-A)/(β-CD) are shown in Figure 8. The exothermic behavior at 208.3℃ may correspond to recrystallization of the (Tan II-A)/(β-CD) complex, because no mass loss associated with this peak is shown by the thermogravimetic data. This result indicates a chemical rather than physical transition. The mass started to decreased at 349.6℃. Therefore, recrystallization and thermal decomposition were:

(Tan II-A)/(β-CD) (s) → Tan II-A (s) + β-CD (s) (2)

β-CD (s) → CO_2 (g) + H_2O (g) (3)

So, with included Tan II-A, the mass loss for (Tan II-A)/(β-CD) (349.6℃) occurs at higher temperature than that for the free β-CD (307.2℃). Comparing with genuine β-CD, we found that the thermal stability of (Tan II-A)/(β-CD) was increased.

Elemental analysis: The complex stoichiometry was also validated by elemental analysis. The result was: C %, found 51.14, calculated (1:1 complex) 51.26; H %, found 6.06, calculated (1:1 complex) 6.16. This result indicated that Tan II-A and β-CD formed 1:1 inclusion complexes and that the 1:2 inclusion complex was not formed.

CONCLUSIONS

The inclusion complex of Tan II-A with β-CD, prepared under microwave irradiation, was studied in this work. The apparent solubility of Tan II-A increases due to the formation of the inclusion complex of Tan II-A and β-CD. The structure of the inclusion complex was inferred from the UV and NMR spectroscopy results as well as the elemental analysis data. We proposed two isomeric 1:1 complex structures (Fig. 4).

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