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Crassicauline A/β-cyclodextrin host–guest system: Preparation, characterization, inclusion mode, solubilization and stability

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ABSTRACT

The inclusion complexation behavior, characterization and binding ability of crassicauline A (CLA) with β-cyclodextrin (β-CD) has been investigated in both solution and the solid state by means of UV–vis spectroscopy, FT-IR, ^{1}H and 2D NMR, XRD, SEM and DSC. The results showed that the water solubility and thermal stability of CLA were obviously increased in the inclusion complex with β-CD. This satisfactory water solubility and high stability of the CLA/β-CD complex will be potentially useful for its application as herbal medicine or healthcare products.

1. Introduction

The genus *Aconitum*, mostly growing on the mountainous parts of the northern hemisphere, produce highly toxic norditerpenoid alkaloids that have attracted considerable interest because of their complex structures, interesting chemistry, and noteworthy physiological effects (Pelletier, 1984). Among these norditerpenoid alkaloids, crassicauline A (CLA, also named as bulleyaconitine A, Scheme 1) is considered to be one of the most important active ingredients due to its various effects. CLA was isolated from the traditional Chinese medicine *Aconitum crassicaule* for the first time (Wang & Fang, 1981). From then on, CLA was isolated widely from the *Aconitum* species growing on the Yunnan-Tibet Plateau (Jing et al., 2009; Yang, Chen, et al., 2008; Yang, Yang, Zhao, Zhang, & Li, 2007; Zheng, Gao, Hao, Wang, & Shen, 1997). CLA is an excellent analgesic and antiinflammatory, and can be used for treating osteoarthritis, periarthritis humeroscapularis, lumbar muscle strain and sprain (Sha & Mao, 1993). The analgesic effect of CLA was found to be 65 times as potent as morphine, without any physical dependence (Tang, Liu, Lu, Wang, & Li, 1986). To date, CLA has been approved for the treatment of chronic pain and rheumatoid arthritis in China (Wang, Gerner, Wang, & Wang, 2007). However, the use of CLA as biopesticides or herbal medicines is greatly limited by its low water solubility and bioavailability. Although much effort has been made to improve its water solubility and stability, such as microencapsule (Zhao, 2005), microemulsion (Feng, Shang, & Xu, 2007) and multivesicular liposome (Weng et al., 2003), CLA still cannot be sufficiently dissolved in water. Therefore, the search for an efficient and nontoxic carrier for CLA has become important in order to further its clinical application.

It is well known that β-cyclodextrin (β-CD) is truncated-cone polysaccharides mainly composed of seven D-glucose monomers linked by α-1,4-glucose bonds (Scheme 1). It has a hydrophobic central cavity and hydrophilic outer surface and can encapsulate various inorganic/organic molecules to form host–guest complexes or supramolecular species. This usually enhances drug solubility in aqueous solution and affects the chemical characteristics of the encapsulated this drug in pharmaceutical industry (Liu & Chen, 2006; Misiuk & Zalewska, 2009; Wu, Liang, Yuan, Wang, & Yan, 2010). This fascinating property enables them to be successfully utilized as drug carriers (Bian et al., 2009; Uekama, Hirayama, & Irie, 1998; Wang & Cai, 2008), separation reagents (Szejtli, 1998), enzyme mimics (Breslow & Dong, 1998), photochemical sensors (Ueno, 1996), etc.

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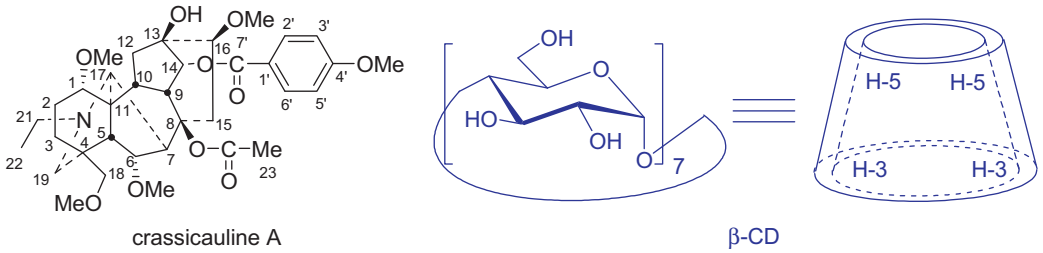

Scheme 1. The structures of crassicauline A (CLA) and β-CD.

More recently, we reported that the inclusion complexation of CDs with natural medicines such as nimbin (Yang et al., 2010), artemether (Yang, Lin, Chen, & Liu, 2009), scutellarin (Yang, Yang, Lin, Chen, & Liu, 2009) and azadirachtin B (Yang, Chen, Lin, & Liu, 2008; Yang & Lin, 2009), significantly enhanced the water solubility and bioavailability of the medicines. For example, CDs increased the water solubility of nimbin from 50 μg/mL to 1.3–4.7 mg/mL, and increased the bioavailability of artemether by 1.81-fold (Yang, Lin, et al., 2009; Yang et al., 2010). As a continuation of our studies on natural medicines/cyclodextrin inclusion complex, an inclusion complex of CLA with β-CD was investigated. To the best of our knowledge, no scientific study on this inclusion complex has hitherto been reported.

In this paper, we aim to report the preparation and characterization of a water-soluble inclusion complex formed by CLA and β-CD. We applied UV–vis spectral titration techniques to calculate the inclusion stoichiometry and stability constant of CLA/β-CD complex, and characterized the complex by FT-IR, ^{1}H NMR and 2D NMR, powder X-ray diffraction (XRD), differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). We also focused on the solubilization and stability effect of β-CD on CLA and the binding ability of the resulting inclusion complexes, which would provide a useful approach for obtaining novel CLA-based healthcare products with high water solubility, stability and bioavailability.

2. Materials and methods

2.1. Materials

CLA (FW = 643, PC > 95%) used in this work was obtained from Kunming Pharmaceutical Corporation or isolated from the roots of *Aconitum macrorhynchum* in Yunnan Province, PR China. β-CD was purchased from ABCR GmbH & Co. KG and used without further purification. Other reagents and chemicals were of analytical reagent grade. All experiments were carried out using ultrapure water.

2.2. Methods

2.2.1. Preparation of CLA/β-CD complex

The inclusion complex was prepared by a solution-ultrasonic method (Miecznik & Kaczmarek, 2007). CLA (0.03 mM, 19.3 mg) and β-CD (0.01 mM, 12.6 mg) were completely dissolved in a mixed solution of alcohol and water (ca. 10 ml, V:V = 1:4), and the mixture was reacted in a ultrasonic wave equipment for 1 h at room temperature in the dark. After evaporating the reaction mixture, the surplus CLA was removed by a 0.45 μm millipore membrane. The filtrate was evaporated under reduced pressure to isolate the solvent and dried in a vacuum dryer at 60 °C for 12 h to give β-CD/CLA inclusion complex (15.6 mg, yield 82%).

2.2.2. Preparation of CLA/β-CD physical mixture

The physical mixture was prepared by our reported method (Yang, Lin, et al., 2009). A physical mixture, to test for possible inclusion, was prepared by grinding together a 1:1 molar mixture of CLA and β-CD for 5 min with a small amount of water (the minimum amount to form a slurry) in an agate mortar.

2.2.3. Determination by UV spectra

Absorption spectra measurements were carried out with a Shimadzu UV 2401 (Japan) using a conventional 1 cm path (1 cm × 1 cm × 4 cm) quartz cell in a thermostated compartment, which was kept at 25 °C through a Shimadzu TB-85 Thermo Bath unit. Given the poor water solubility of CLA, a water/ethanol (V:V = 4:1) solution was used in the spectral measurements. The concentration of CLA was held constant at 0.021 mM. Then, an appropriate amount of β-CD was added with the final concentrations varied from 0 to 6.00 mM (0, 0.24, 0.50, 1.01, 1.44, 2.06, 2.94, 4.20, 6.00 mM). The absorption spectra measurement was taken after 1 h. The measurements were done in the 200–400 nm spectral range. All experiments were carried out in triplicate.

2.2.4. Standard curve of CLA

A series of CLA ethanol solutions with their concentrations ranging from 2.96 to 13.50×10^{-3} mg/mL were configured. The absorbance was recorded at 261 nm in UV at 37 °C in order to make a standard curve using concentrations (C, mg/mL) as x-coordinate and absorbance (A) as y-coordinate. We found the standard curve of CLA could be depicted by the equation: $A = 40.648C + 0.0017$ ($R = 0.9993$).

2.2.5. FT-IR spectra

FT-IR spectra were measured on a Nicolet Avatar 360 FTIR spectrometer with 4 cm^{-1} resolution and 64 scans between wave number of 4000 cm^{-1} and 400 cm^{-1}. Samples were prepared as KBr disks with 1 mg of complex in 100 mg of KBr.

2.2.6. ^{1}H and 2D NMR

All NMR experiments were carried out in D_2O. Tetramethylsilane was used as a reference. Samples were dissolved in 99.98% D_2O and filtered before use. 1H NMR spectra were acquired on a Bruker Avance DRX spectrometer at 500 MHz and 298 K. The one-dimensional spectra of both solutions were run with FID resolution of 0.18 Hz/point. The residual HDO line had a line width at half-height of 2.59 Hz. Two-dimensional (2D) ROESY spectra were acquired at 298 K with presaturation of the residual water resonance and a mixing (spin-lock) time of 350 ms at a field of ~2 kHz, using the TPPI method, using a 1024 K time domain in F2 (FID resolution 5.87 Hz) and 460 experiments in F1. Processing was carried out with zero-filling to 2K in both dimensions using sine (F2) and qsine (F1) window functions, respectively.

2.2.7. Powder X-ray diffraction (XRD)

XRD patterns were obtained using a D/Max-3B diffractometer with Cu Kα radiation (40 kV, 100 mA), at a scanning rate of 5°/min. Powder samples were mounted on a vitreous sample holder and scanned with a step size of $2\theta = 0.02°$ between $2\theta = 3°$ and 50°.

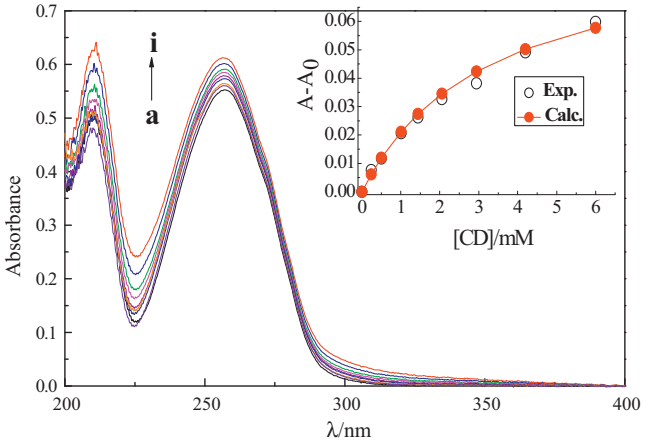

Fig. 1. UV–vis spectral changes of CLA (0.021 mM) upon addition of β-CD (from 0 to 6.00 mM, a: 0 mM, b: 0.24 mM, c: 0.50 mM, d: 1.01 mM, e: 1.44 mM, f: 2.06 mM, g: 2.94 mM, h: 4.20 mM, and i: 6.00 mM) in a water/alcohol (V:V = 4:1, ca. pH 10.5) mixed solution, and the nonlinear least-squares analysis (inset) of the differential intensity (ΔA at 261 nm) to calculate the complex stability constant (K_s).

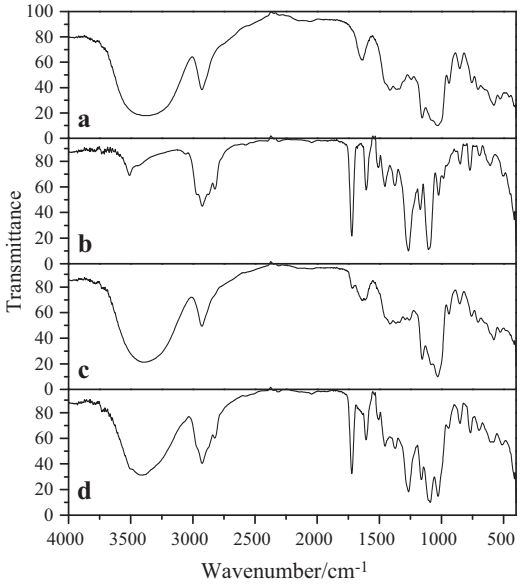

Fig. 2. FT-IR spectra of (a) β-CD, (b) CLA, (c) CLA/β-CD inclusion complex, (d) CLA and β-CD physical mixture (1:1 molar ratio).

2.2.8. Differential scanning calorimetry (DSC)

DSC measurements were performed with a 2960 SDT V3.0F instrument, at a heating rate of 10 °C/min from room temperature to 400 °C in a dynamic nitrogen atmosphere (flow rate = 70 mL/min).

2.2.9. Scanning electron microphotographs (SEM)

SEM photographs were determined on a FEI QUANTA 200. The powders were previously fixed on a brass stub using double-sided adhesive tape and then were made electrically conductive by coating, in a vacuum with a thin layer of gold (approximately 300 Å) for 30 s and at 30 W. The pictures were taken at an excitation voltage of 15, 20 or 30 kV and a magnification of 1080, 1200, 1400 or 2000×.

2.2.10. Solubilization test

An excess amount of complex was placed in 2 mL of water (ca. pH 6.0), under nitrogen, sheltered from light and the mixture was stirred for 1 h at 37 ± 2 °C. The solution is then filtered on a 0.45 μm cellulose acetate membrane. The absorbance of the filtrate was recorded at 261 nm in UV at 37 °C and the residue is dosed by the standard curve of CLA.

3. Results and discussion

3.1. Spectral titration

Quantitative investigation of the inclusion complexation behavior of β-CD with CLA was carried out in a water/ethanol (v:v = 4:1) mixed solution using a spectrophotometric titration method owing to the rather low water solubility of CLA. As illustrated in Fig. 1, the absorbance intensity of CLA gradually increased with the stepwise addition of β-CD. In all experiments, the pH of the solution did not change appreciably during the experimental procedure. As the size-fit, shape-fit, and charge-fit effects are the dominant controlling factors on the formation of inclusion complexes of β-CDs (Liu & Chen, 2006), these results indicate that the binding behavior is mainly dependent on the individual structural features of the host and guest. Assuming 1:1 stoichiometry for the CLA/β-CD inclusion complex, the inclusion complexation of CLA with β-CD could be expressed by Eq. (1), and the stability constant (K_s) can be calculated from Eq. (2), where [CLA·CD], [CLA], [CD], $[CLA]_0$ and $[CD]_0$ refer to the equilibrium concentration of the CLA/β-CD inclusion complex, the equilibrium concentration of CLA, the equilibrium concentration of β-CD, the original concentration of CLA, and the original concentration of β-CD, respectively, and $\Delta\varepsilon$ is the differential molar extinction coefficient of CLA in the absence and presence of β-CD. According to Lambert–Beer law, we can obtain that the concentration of the CLA/β-CD complex is equal to $\Delta A/\Delta\varepsilon$ (Eq. (2)). We can then derive Eq. (3) from Eq. (2). Finally, K_s can be obtained from the analysis of the sequential changes of absorption (ΔA) at various β-CD concentrations, with a nonlinear least squares method according to the curve-fitting Eq. (3).

$$\mathrm{CLA} + \mathrm{CD} \overset{K_s}{\rightleftharpoons} \mathrm{CLA \cdot CD} \tag{1}$$

$$K_s = \frac{[\mathrm{CLA \cdot CD}]}{([\mathrm{CLA}][\mathrm{CD}])} = \frac{(\Delta A/\Delta\varepsilon)}{\{([\mathrm{CLA}]_0 - \Delta A/\Delta\varepsilon)([\mathrm{CD}]_0 - \Delta A/\Delta\varepsilon)\}} \tag{2}$$

$$\Delta A = \frac{\left\{\Delta\varepsilon([\mathrm{CLA}]_0 + [\mathrm{CD}]_0 + 1/K_s) \pm \sqrt{\Delta\varepsilon^2([\mathrm{CLA}]_0 + [\mathrm{CD}]_0 + 1/K_s)^2 - 4\Delta\varepsilon^2[\mathrm{CLA}]_0[\mathrm{CD}]_0}\right\}}{2} \tag{3}$$

Using a nonlinear least squares curve-fitting method (Liu, Li, Wada, & Inoue, 1999), we obtained the complex stability constant for each host–guest combination. Fig. 1 (inset) illustrates a typical curve-fitting plot for the titration of CLA with β-CD, which shows the excellent fit between the experimental and calculated data and the 1:1 stoichiometry of the CLA/β-CD inclusion complex. In repeated measurements, the K_s values were reproducible within an error of ±5%. The stability constant (K_s) and Gibbs free energy change ($-\Delta G^\circ$) for the inclusion complexation of β-CD with CLA are listed in Table 1.

Table 1
The stability constant (K_s and $\log K_s$), molar extinction coefficient change ($\Delta\varepsilon$) and Gibbs free energy change ($-\Delta G^0$) for the inclusion complexation of β-CD with CLA guest in a water/alcohol (V:V = 4:1, ca. pH 10.5) mixed solution.

K_s/M^{-1}	$\log K_s$	$\Delta\varepsilon$/M^{-1} cm^{-1}	$-\Delta G^0$/kJ mol^{-1}
313	2.49	4210	−14.25

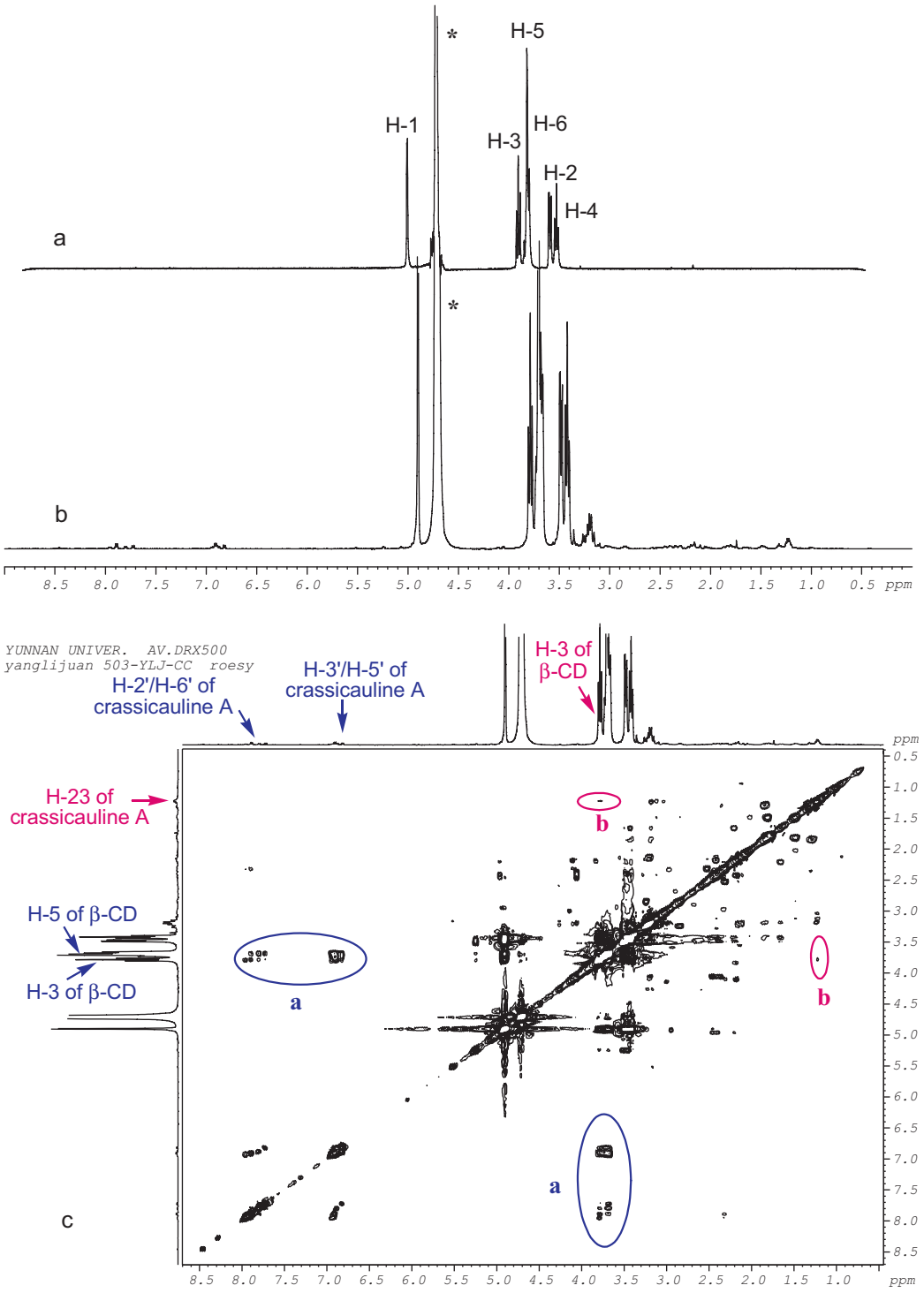

Fig. 3. ^{1}H NMR spectra of CLA in the absence and presence of β-CD in D_2O at 25 °C, respectively: (a) β-CD, (b) CLA/β-CD complex (asterisk highlights the water peak); and ROESY spectrum in D_2O at 25 °C: (c) CLA/β-CD complex.

3.2. Binding ability

Extensive studies have revealed that the size/shape-fit concept plays a crucial role in the formation of inclusion complex of host β-CD with guest molecules of various structures. On the basis of this concept, several weak intermolecular forces such as ion–dipole, dipole–dipole, van der Waals, electrostatic, hydrogen bond, and hydrophobic interactions are known to cooperatively contribute to the inclusion complexation. β-CD possess a cyclic truncated cone cavity with a height of 0.79 nm, an inner diameter of 0.60–0.65 nm, and a cavity volume of 0.262 nm^3 (Liu, Chen, Chen, & Lin, 2005; Szejtli, 1998). The host–guest size match may dominate the stability of the complex formed between β-CD and CLA. Additionally, considering the structural features of the host and guest, we deduced that the hydrogen bond between the hydrogen atoms of β-CD and the oxygen atoms of CLA may strengthen the host–guest association.

Table 2
The chemical shifts (δ) of β-CD and CLA/β-CD complex.

		δ (ppm)	
		β-CD	CLA/β-CD complex
H-1	d	4.99	4.91
H-2	dd	3.57	3.49
H-3	dd	3.88	3.79
H-4	dd	3.50	3.42
H-5	m	3.80	3.73
H-6	dd	3.78	3.69

3.3. FT-IR analysis

The FT-IR spectra of β-CD, CLA, their inclusion complex, and their physical mixture are shown in Fig. 2. The spectrum of CLA shows strong absorption bands in the range of 1840–1650, 1340–1190 and 1140–1050 cm^{-1} (Fig. 2b). Due to the absorption disturbance of β-CD, these characteristic bands can not all exist in the spectra of CLA/β-CD inclusion complex, but we can find the obvious band with a negligible rightward shift in the range of 1840–1650 cm^{-1}, which indicates the existence of CLA in the inclusion complex (Fig. 2c). In addition, the IR spectra of β-CD can be characterized by the intense band at 3780–3010 cm^{-1} corresponding to vibration of the hydrogen-bonded –OH groups as well as the band at 3000–2800 cm^{-1} assigned to absorption by the –CH and $-CH_2$ groups (Fig. 2a). After association with CLA, the spectra of the inclusion complex manifests a reduced intensity and a certain shift of the peak assigned to absorption by the hydrogen-bonded –OH groups (from 3390 to 3392 cm^{-1}). However, the spectra of the physical mixture correspond simply to the superposition of the spectra of the individual component (Fig. 2d). All these phenomena jointly indicate that some of the existing hydrogen bonds formed between –OH groups of β-CD are broken after inclusion complexation.

3.4. ^1H and 2D NMR analysis

In order to explore the possible inclusion mode of the CLA/β-CD complex, we compared the ^1H NMR spectra of CLA in the presence of host β-CD (Fig. 3). Owing to its poor water solubility, CLA is transparent to ^1H NMR under most conditions when D_2O is used as a solvent. Assessment of the CLA complex by ^1H NMR clearly demonstrated the presence of the framework protons of the CLA molecule, consistent with the significant solubilization. As illustrated in Fig. 3, the majority of CLA protons displayed chemical shifts at δ 1.0–3.4 and 6.5–8.5 ppm, which were distinct from the β-CD protons (δ 3.4–5.0 ppm). By comparing the integration area of these protons with that of the β-CD's H-1 protons, we calculated the inclusion stoichiometry of the CLA/β-CD complex, that is, 1:1 for the CLA/β-CD.

To further explore the inclusion mode, the chemical shifts of β-CD protons in the absence and presence of CLA were listed in Table 2. As can be seen from Table 2, after inclusion complexation with CLA, a relatively weak effect was observed on the δ values of H-5 protons of β-CD. In contrast, those values of H-1, H-2, H-3, H-4 and H-6 protons exhibited the significant changes (0.08–0.09 ppm). It is fairly noteworthy that H-3 protons shifted ca. 0.09 ppm, but H-5 protons showed relatively weak shifts (0.07 ppm) after resulting inclusion complex. Because both H-3 and H-5 protons are located in the interior of β-CD cavity, and H-3 protons are near the wide side of cavity while H-5 protons near the narrow side, this phenomenon may indicate that CLA should penetrate into the β-CD cavity from the wide side.

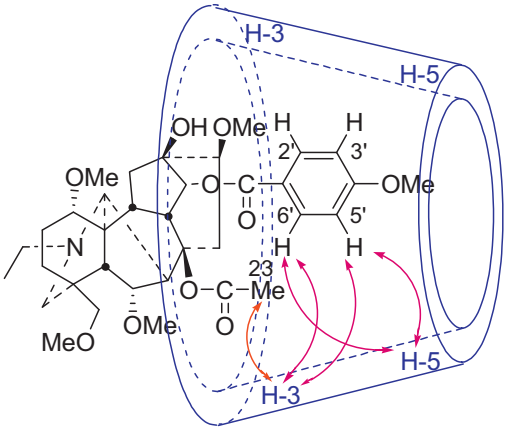

Fig. 4. Possible inclusion mode and significant NOESY (↔) correlations of the CLA/β-CD complex.

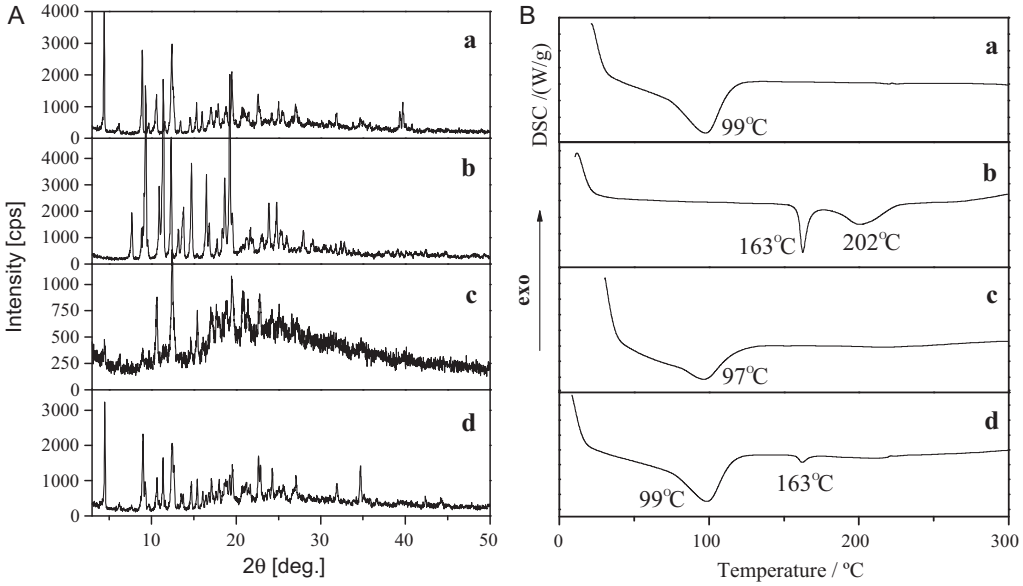

Fig. 5. (A) XRD patterns: (a) β-CD, (b) CLA, (c) CLA/β-CD inclusion complex, (d) CLA and β-CD physical mixture (1:1 molar ratio). (B) DSC thermogram: (a) β-CD, (b) CLA, (c) CLA/β-CD inclusion complex, (d) CLA and β-CD physical mixture (1:1 molar ratio).

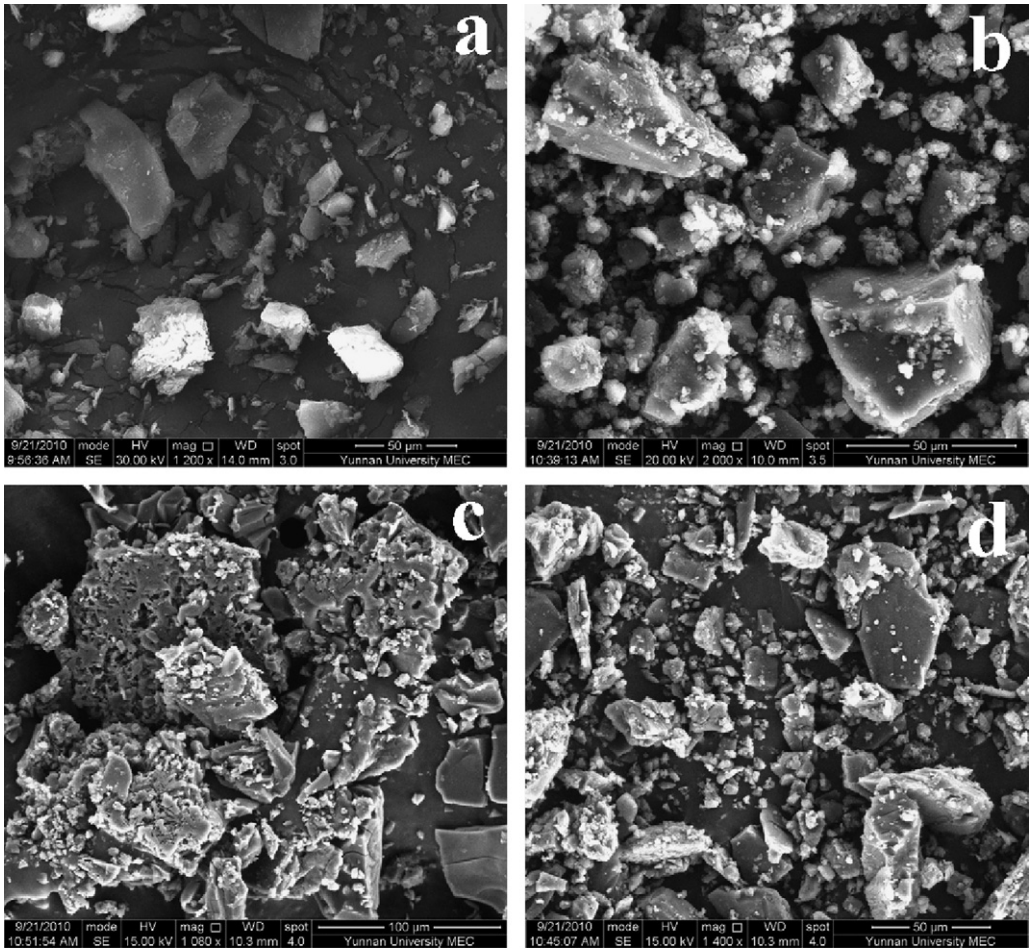

Fig. 6. Scanning electron microphotographs: (a) β-CD, (b) CLA, (c) CLA/β-CD inclusion complex, (d) CLA and β-CD physical mixture (1:1 molar ratio).

Two-dimensional (2D) NMR spectroscopy provides important information about the spatial proximity between host and guest atoms by observation of intermolecular dipolar cross-correlations (Yang, Lin, et al., 2009). Two protons closely located in space can produce a nuclear Overhauser effect (NOE) cross-correlation in NOE spectroscopy (NOESY) or ROESY. The presence of NOE cross-peaks between protons from two species indicates spatial contacts within 0.4 nm (Correia et al., 2002). To gain more conformational information, we obtained 2D ROESY of the inclusion complex of CLA with β-CD. The ROESY spectrum of the CLA/β-CD complex (Fig. 3c) showed appreciable correlations of H-2′/H-6′ and H-3′/H-5′ protons of CLA with H-3 and H-5 protons of β-CD (peaks a), as well as a key correlation of H-23 protons of CLA with H-3 protons of β-CD (peak b). These results indicate that the aryl ring and acetyl group of CLA are included in the β-CD cavity.

Based on these observations, together with the 1:1 stoichiometry deduced by ^{1}H NMR spectra and UV–vis spectrophotometric titration, we deduced the possible inclusion modes for the CLA/β-CD complex as illustrated in Fig. 4.

3.5. XRD analysis

The powder X-ray diffraction (XRD) patterns of β-CD, CLA, their inclusion complex and their physical mixture are illustrated in Fig. 6. As indicated in Fig. 5A, both β-CD (Fig. 5A(a)) and CLA (Fig. 5A(b)) are in crystalline form. The XRD of their physical mixture (Fig. 5A(d)) just is a superimposition of the XRD of β-CD and the CLA. In contrast, the XRD of their inclusion complex (Fig. 5A(c)) exhibited the amorphous halo pattern, which was quite different from a superimposition of the XRD of β-CD and the CLA, indicating the formation of the inclusion complex between β-CD and CLA. In addition, most of the crystalline diffraction peaks of β-CD disappeared after complexation with CLA, indicating that the complexation of CLA reoriented the β-CD molecule to some extent.

3.6. DSC analysis

The differential scanning calorimetry (DSC) diagram of β-CD, CLA, their inclusion complex and their physical mixture are presented in Fig. 5B. The thermogram of β-CD shows a very board endothermic band, between 50 and 120 °C, which gains a maximum at 99 °C (Fig. 5B(a)), indicating dehydration process. The trace of CLA illustrates a sharp endothermic peak at 163 °C (Fig. 5B(b)), corresponding to the melting point of CLA, followed by another endothermic peak at 202 °C. However, in the DSC curves of the CLA/β-CD complex, we can find that two endothermic peaks at about 163 and 202 °C (Fig. 5B(c)), corresponding to the free CLA disappear, and the board endothermic peak, corresponding to the free β-CD shifts from 99 °C to 97 °C. In contrast, the physical mixture of CLA and β-CD contains the melting peak at 163 °C and the board endothermic peak at 99 °C, without any shifts (Fig. 5B(d)). These fascinating results indicate the usual thermal properties were changed after inclusion complex formation between CLA and β-CD.

3.7. SEM analysis

Scanning electron microscopy (SEM) is a qualitative method used to study the structural aspects of raw materials, i.e., CDs and

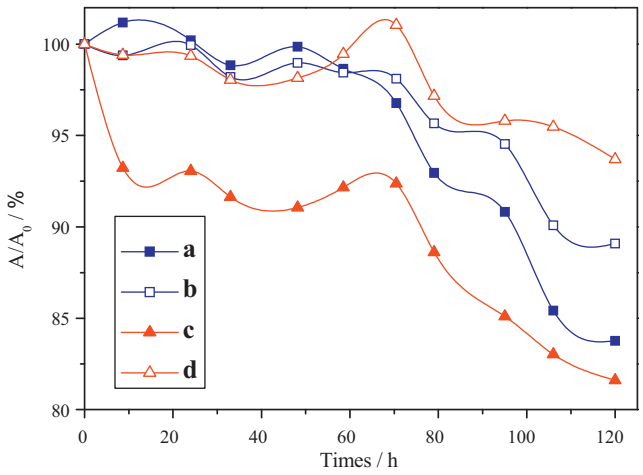

Fig. 7. The relative values (A/A_0, A is the absorbance at the recording time and A_0 is the original absorbance) of (a) CLA at pH 1.5, (b) CLA/β-CD complex at pH 1.5, (c) CLA at pH 7.6, and (d) CLA/β-CD complex at pH 7.6, with an interval of 12 ± 2 h.

drugs or the products obtained by different methods of preparation like physical mixture, solution complexation, coevaporation and others (de Araujo et al., 2008; Duchêne, 1987). SEM photographs of β-CD, CLA, their inclusion complex and their physical mixture are shown in Fig. 6. Typical crystal of β-CD and CLA are found in many different sizes. β-CD crystallizes in polyhedral form (Fig. 6a) and pure CLA appears as irregular-shaped crystal particles with large dimensions (Fig. 6b). The physical mixture CLA/β-CD reveals some similarities with the crystal of the free molecules and shows both crystalline components (Fig. 6d). However, the CLA/β-CD inclusion complex appears as compact and homogeneous plate-like structure crystal particles and is quite different from the sizes and shapes of β-CD and CLA (Fig. 6c), which confirms the formation of the inclusion complex.

3.8. Solubilization

The water solubility of CLA/β-CD complex is assessed by preparation of its saturated solution (Montassier, Duchêne, & Poelman, 1997). An excess amount of complex was placed in 2 mL of water (ca. pH 6.0), and the mixture was stirred for 24 h at 37 °C. After removing the insoluble substance by filtration, the absorbance of the filtrate was recorded at 261 nm in UV at 37 °C and the residue is dosed by the standard curve of CLA. The results show that the water solubility of the CLA, compared with that of native CLA (ca. 129 μg/mL), was remarkably increased to approximately 2.1 mg/mL by the solubilizing effects of β-CD. In the control experiment, a clear solution is obtained after dissolving CLA/β-CD complex (9.1 mg), which is equivalent to 2.1 mg of CLA, in 1 mL water at 37 °C. Additionally, similar experiments were performed in both pH 1.5 and pH 7.6 buffers. The results showed that the water solubility of β-CD/CLA complex at different pH values were similar to those in water without pH control. The water solubility of CLA/β-CD complex was found to be 1.9 mg/mL (calculated as CLA residue) at pH 7.6, while the value at pH 1.5 was 2.4 mg/mL (calculated as CLA residue). This subsequently confirmed the reliability of the obtained satisfactory water solubility of CLA/β-CD complex, which will be beneficial to the medical utilization of this compound.

3.9. Stability in biological environments

In order to evaluate the stability of CLA/β-CD in biological environments, we tracked the absorbance changes of CLA and CLA/β-CD in different buffer solutions, such as the simulated gastric acid (ca. pH 1.5) or the simulated intestinal fluid (ca. pH 7.6) (Liu, Chen, Chen, Ding, & Chen, 2005). The solid CLA or CLA/β-CD complex was quickly dissolved in the buffer solution, and the absorbance was recorded at 261 nm in UV at 37 °C with an interval of 12 ± 2 h. Fig. 7 illustrates the relative absorbance A/A_0 (A is the absorbance at the recording time and A_0 is the original absorbance) of CLA and CLA/β-CD complex at pH 1.5 and pH 7.6 with an interval of 12 ± 2 h, respectively. At pH 1.5, the relative absorbance of CLA and CLA/β-CD were similarly changed before 60 h, but the relative absorbance of CLA/β-CD declined with a slower rate than free CLA from 60 to 120 h. At pH 7.6, the relative absorbance of free CLA tapered off 7% at the first 10 h, however, the relative absorbance of CLA/β-CD dwindled only 0.5%, and the relative absorbance of free CLA diminished with a fast speed during the last 50 h. All these results indicate that CLA/β-CD is much more stable than free CLA at both pH 1.5 and 7.6.

4. Conclusions

In summary, the inclusion complexation behavior, characterization, binding ability, solubilization and stability of CLA with β-CD was investigated. The results showed that β-CD could enhance not only the water-solubility but also the stability of CLA. Given the shortage of application of CLA and the easy and environmentally friendly preparation of CLA/β-CD complex, this inclusion complexation should be regarded as an important step in the design of a novel formulation of CLA for the herbal medicine or healthcare products.

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