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Host−Guest System of Nimbin and β-Cyclodextrin or Its Derivatives: Preparation, Characterization, Inclusion Mode, and Solubilization

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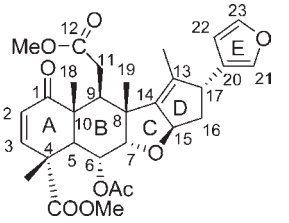
The inclusion complexation behavior, characterization, and binding ability of nimbin with β-cyclodextrin (β-CD) and its derivatives were investigated in both solution and the solid state by means of XRD, DSC, ^{1}H and 2D NMR, and UV−vis spectroscopy. The results showed that the water solubility and thermal stability of nimbin were obviously increased in the inclusion complex with cyclodextrins. This satisfactory water solubility and high thermal stability of the nimbin/CD complexes will be potentially useful for their application as herbal medicines or healthcare products.

KEYWORDS: Nimbin; β-cyclodextrin; inclusion complex; binding ability; characterization; inclusion mode

INTRODUCTION

The neem tree, *Azadirachta indica* A. Juss, is a tropical plant that has long been used in agriculture and medicine. According to folklore, the bark, leaves, and fruit have been used as medicines for the treatment of a variety of human ailments, particularly against diseases of bacterial and fungal origin (*1*−*3*). Many studies have demonstrated that its seed contains abundant limonoids and simple terpenoids that are responsible for its biological activity (*4*−*8*). Among these limonoids, nimbin (**Scheme 1**), a representative of ring C seco-tetranortriterpenoids, is considered to be one of the most significant active ingredients due to its various effects on insects and is a potential molecule for improving the pesticidal properties of the products and a good chemomarker for evaluation of germplasm (*9*). Furthermore, nimbin is widely used in herbal medicine/healthcare products such as anti-inflammatories, antipyretics, antifungals, antihistamines, antiseptics, and antioxidants (*4*, *10*). For example, nimbin has been shown to be 4 times as effective as the steroid hydrocortisone in the treatment of eczema and psoriasis, but without side effects such as thinning of the skin (*11*). Additionally, docking studies carried out with different herbal ligands suggest that nimbin is one of the best herbal candidates to replace the synthetic drugs thiolactomycin and cerulenin (*12*). However, the use of nimbin and other limonoids as biopesticides or herbal medicines is greatly limited by its low water solubility and bioavailability (*13*, *14*). Although much effort has been made to improve its water solubility and stability by introducing some nontoxic solubilizers (*15*, *16*), nimbin and other limonoids still cannot be sufficiently dissolved in water. Therefore, the search for an efficient and nontoxic carrier for nimbin has become important to further its clinical application.

Scheme 1. Structure of Nimbin



It is well-known that cyclodextrins (CDs) are truncated-cone polysaccharides mainly composed of six to eight D-glucose monomers linked by α-1,4-glucose bonds. They have a hydrophobic central cavity and a 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 drug in the pharmaceutical industry (*17*−*19*). This fascinating property enables CDs to be successfully utilized as drug carriers (*20*−*22*), separation reagents (*23*), enzyme mimics (*24*), and photochemical sensors (*25*), etc. The same effect has been demonstrated in the agriculture industry (*26*−*33*). Recently, some studies and patents of inclusion complex formation between CDs and limonoids have been reported (*34*).

In this paper, we aim to report the preparation and characterization of some water-soluble inclusion complexes formed by nimbin and β-cyclodextrin (β-CD) and its derivatives: 2-hydroxypropyl-β-cyclodextrin (HPβCD), heptakis-(2,6-di-*O*-methyl)-β-CD (DMβCD), and heptakis(2,3,6-tri-*O*-methyl)-β-CD (TMβCD) (**Scheme 2**). We were particularly

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Scheme 2. Structure of β-CD and Its Derivatives

β-CD $R^1=R^2=R^3=H$
HPβCD $R^1=R^3=H$, $R^2=CH_2CH(CH_3)OH$
DMβCD $R^1=R^2=Me$, $R^3=H$
TMβCD $R^1=R^2=R^3=Me$

interested in exploring the solubilization effect of CDs on nimbin and the binding ability of the resulting inclusion complexes, which would provide a useful approach for obtaining novel nimbin-based healthcare products with high water solubility, high bioavailability, and low toxicity.

MATERIALS AND METHODS

Materials. Nimbin (FW = 540, PC > 95%) was obtained by microwave-assisted extraction from dry neem leaf in Yunnan Province, China. β-CD (average substitution degree = 1135), 2-hydroxypropyl-β-cyclodextrin (HPβCD, average substitution degree = 1380), heptakis(2,6-di-*O*-methyl)-β-CD (DMβCD, average substitution degree = 1331), and heptakis(2,3,6-tri-*O*-methyl)-β-CD (TMβCD, average substitution degree = 1429) were 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.

Preparation of Nimbin/β-CD, Nimbin/HPβCD, Nimbin/DMβCD, and Nimbin/TMβCD Complexes. Nimbin (0.03 mM, 16.2 mg) and CD (0.01 mM) were completely dissolved in a mixed solution of ethanol and water (ca. 7 mL, v/v = 1:5, given the poor water solubility of nimbin, ethanol was used), and the mixture was stirred for 5 days at room temperature. After evaporation of the ethanol from the reaction mixture, the uncomplexed nimbin was removed by filtration. The filtrate was evaporated under reduced pressure to remove the solvent and dried in vacuum to give the nimbin/CD complexes. Nimbin/β-CD complex (yield 91%): ^{1}H NMR (500 MHz, D_2O, TMS) δ 0.6−2.42 (m, 18H, nimbin protons), 3.40−3.90 (m, >50H, H-2−6 of β-CD and some protons of nimbin), 4.97−4.99 (s, 7H, H-1 of β-CD). Nimbin/HPβCD complex (yield 90%): ^{1}H NMR (500 MHz, D_2O, TMS) δ 0.6−2.4 (m, 39H, 18H nimbin protons and 7 × CH_3 of HPβCD), 3.15−3.95 (m, >110H, H-2−6 and CH_2- and CH_3-2,3,6 of HPβCD and some protons of nimbin), 5.10−5.12 (s, 7H, H-1 of HPβCD). Nimbin/DMβCD complex (yield 82%): ^{1}H NMR (500 MHz, D_2O, TMS) δ 0.6−2.4 (m, ca. 18H nimbin protons), 3.00−3.75 (m, >90H, H-2−6 and OCH_3-2,6 of DMβCD and some protons of nimbin), 5.03−5.04 (s, 7H, H-1 of DMβCD). Nimbin/TMβCD complex (yield 80%): ^{1}H NMR (500 MHz, D_2O, TMS) δ 0.6−2.4 (m, ca. 18H nimbin protons), 3.15−3.95 (m, >110H, H-2−6 and OCH_3-2,3,6 of TMβCD and some protons of nimbin), 5.21−5.23 (s, 7H, H-1 of TMβCD).

Determination by UV Spectra. Absorption spectra measurements were carried out with a Shimadzu UV 2401 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 nimbin, a water/ethanol (v/v = 4:1) solution was used in the spectral measurements. The concentration of nimbin was held constant at 0.05 mM. Then, an appropriate amount of CDs was added with the final concentrations varied from 0 to 2.80−6.00 mM (β-CD, 0, 0.18, 0.36, 0.71, 1.01, 1.44, 2.94, 6.00 mM; HPβCD, 0, 0.24, 0.47, 0.67, 1.37, 2.80 mM; DMβCD, 0, 0.47, 0.67, 0.96, 1.37, 1.96, 2.80 mM; TMβCD, 0, 0.24, 0.48, 0.96, 1.37, 1.96, 4.00 mM). The absorption spectra measurement was taken after 1 h. The measurements were done in the 220−400 nm spectral range. All experiments were carried out in triplicate.

^{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. ^{1}H 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.

Powder X-ray Diffraction. 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 2θ = 0.02° between 2θ = 3° and 50°.

Thermal Analyses. Differential scanning calorimetry (DSC) and thermogravimetric (TG) measurements were performed with a 2960 SDT V3.0F instrument and a Netzsch STA 449F3, respectively, at a heating rate of 10 °C/min from room temperature to 400 °C in a dynamic nitrogen atmosphere (flow rate = 70 mL/min).

Solubilization Studies. 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 20 ± 2 °C. The solution was then filtered on a 0.45 μm cellulose acetate membrane. The filtrate was evaporated under reduced pressure to dryness, and the residue was dosed by the weighing method.

RESULTS AND DISCUSSION

Spectral Titration. Quantitative investigation of the inclusion complexation behavior of β-CD and its derivatives with nimbin 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 nimbin. As illustrated in **Figure 1**, the absorbance intensity of nimbin gradually increased with the stepwise addition of β-CD, HPβCD, DMβCD, and TMβCD. 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 (*17*), these results indicate that the binding behavior is mainly dependent on the individual structural features of the host and guest. Assuming a 1:1 stoichiometry for the nimbin/β-CD inclusion complex, the inclusion complexation of nimbin with β-CD could be expressed by eq 1, and the stability constant (K_S) can be calculated from eq 2, where [nimbin/β-CD], [nimbin], [β-CD], $[\text{nimbin}]_0$, and $[\beta\text{-CD}]_0$ refer to the equilibrium concentration of the nimbin/β-CD inclusion complex, the equilibrium concentration of nimbin, the equilibrium concentration of β-CD, the original concentration of nimbin, and the original concentration of β-CD, respectively, and $\Delta\varepsilon$ is the differential molar extinction coefficient of nimbin in the absence and presence of β-CD. According to Lambert−Beer law, we can obtain that the concentration of the nimbin/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.

$$\text{nimbin} + \text{CD} \overset{K_S}{\rightleftharpoons} \text{nimbin}\cdot\text{CD} \tag{1}$$

$$K_S = \frac{[\text{nimbin}\cdot\text{CD}]}{[\text{nimbin}][\text{CD}]} = \frac{\Delta A/\Delta\varepsilon}{([\text{nimbin}]_0 - \Delta A/\Delta\varepsilon)([\text{CD}]_0 - \Delta A/\Delta\varepsilon)} \tag{2}$$

$$\Delta A = \left(\Delta\varepsilon([\text{nimbin}]_0 + [\text{CD}]_0 + 1/K_S) \pm \sqrt{\Delta\varepsilon^2([\text{nimbin}]_0 + [\text{CD}]_0 + 1/K_S)^2 - 4\Delta\varepsilon^2[\text{nimbin}]_0[\text{CD}]_0}\right)\Big/2 \tag{3}$$

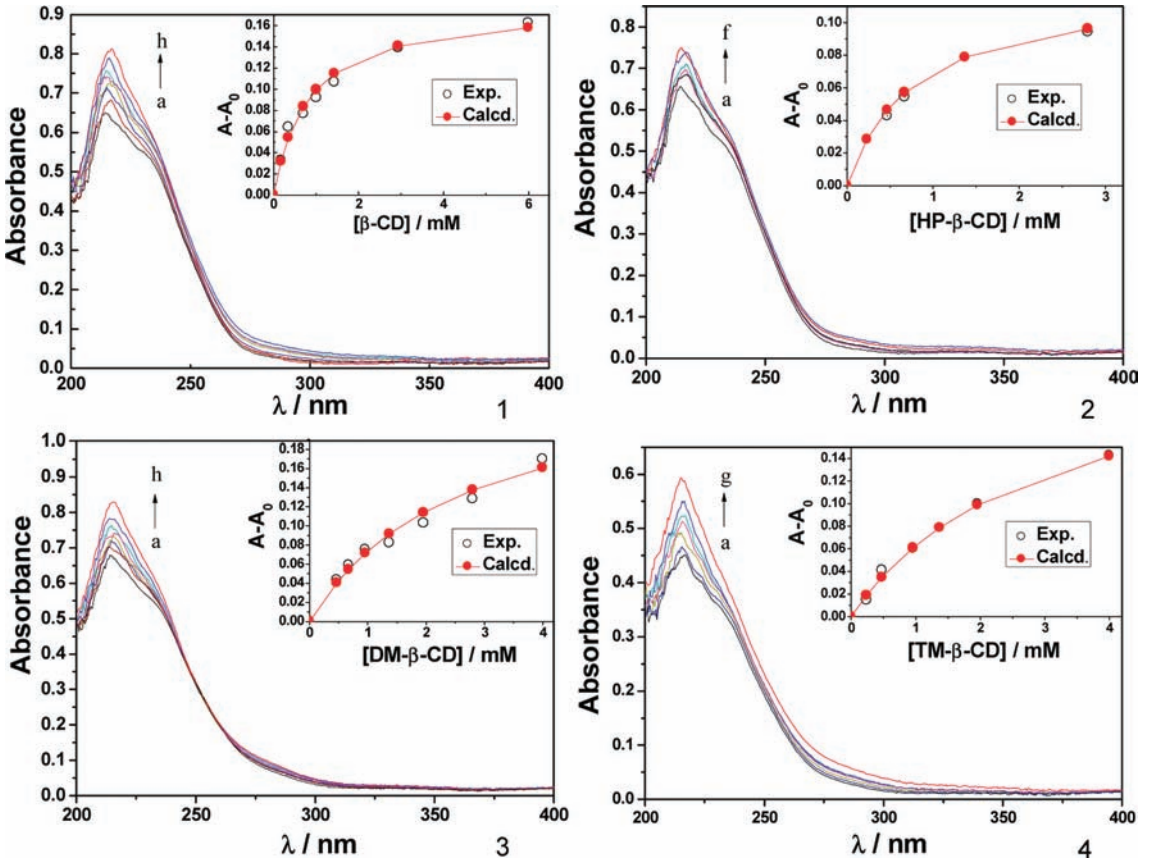

Figure 1. UV−vis spectral changes in nimbin (0.05 mM) and the nonlinear least-squares analysis (inset) of the differential intensity (ΔA at 216 nm) used to calculate the complex stability constant (K_S) upon addition of β-CD (**1**:, 0−6.00 mM, from a to h), HPβCD (**2**, 0−2.80 mM, from a to f), DMβCD (**3**, 0−4.00 mM, from a to h), and TMβCD (**4**, 0−4.00 mM, from a to g) in a water/ethanol (v/v = 4:1, ca. pH 10.5) mixed solution.

Table 1. Stability Constant (K_S) and Gibbs Free Energy Change ($-\Delta G°$) for the Inclusion Complexation of CDs with Nimbin Guest in a Water/Ethanol (v/v = 4:1, ca. pH 10.5) Mixed Solution

host	K_S (M^{-1})	log K_S	$-\Delta G°$ (kJ mol^{-1})
β-CD	1279	3.10	17.73
HPβCD	1360	3.13	17.88
DMβCD	390	2.59	14.79
TMβCD	351	2.54	14.53

Using a nonlinear least-squares curve-fitting method (*35*), we obtained the complex stability constant for each host−guest combination. **Figure 1** (inset) illustrates a typical curve-fitting plot for the titration of nimbin with β-CD, HPβCD, DMβCD, and TMβCD, which shows the excellent fit between the experimental and calculated data and the 1:1 stoichiometry of the nimbin/CDs inclusion complexes. In repeated measurements, the K_S values were reproducible within an error of ±5%. The stability constant (K_S) and Gibbs free energy change ($-\Delta G°$) for the inclusion complexation of CDs with nimbin are listed in **Table 1**.

Binding Ability. Extensive studies have revealed that the size/shape-fit concept plays a crucial role in the formation of inclusion complexes of host CDs 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. CDs possess a cyclic truncated cone cavity with a height of 0.79 nm, an inner diameter of 0.62−0.78 nm, and a cavity volume of 0.262 nm^3 for β-CD (*34a*, *36*−*38*). The host−guest size match may dominate the stability of the complexes formed between CDs and nimbin. From **Table 1**, we can see that the binding constants for the complexation of nimbin with β-CD, HPβCD, DMβCD, and TMβCD were in the following order: HPβCD > β-CD > DMβCD > TMβCD. By comparing the enhancement effect of all kinds of β-CDs for nimbin, HPβCD and β-CD gave a stronger K_S value than DMβCD and TMβCD, which demonstrated that HPβCD and β-CD can complex better with the guest nimbin than the methylated CDs. Considering the structural features of the host and guest, we deduced that the hydrogen bond between the hydroxyl arms of HPβCD or the hydrogen atoms of β-CD and the oxygen atoms of nimbin may strengthen the host−guest association. In contrast, the methylated CDs (DMβCD and TMβCD) showed a weaker K_S value due to the larger and deeper CD cavity, which caused the intermolecular hydrogen bond to become weaker (*39*, *40*).

^{1}H and 2D NMR Analysis. To explore the possible inclusion mode of the nimbin/CD complex, we compared the ^{1}H NMR spectra of nimbin in the presence of host CDs (**Figure 2**). Owing to its poor water solubility, nimbin is transparent to ^{1}H NMR under most conditions when D_2O is used as a solvent. Assessment of the nimbin complex by ^{1}H NMR clearly demonstrated the presence of the framework protons of the nimbin molecule, consistent with the significant solubilization. As illustrated in **Figure 2**, the majority of nimbin protons displayed chemical shifts at δ 1.0−2.5, which were distinct from the CD protons (usually at δ 3.0−5.0). By comparing the integration area of these protons with that of the CD's H-1 protons, we calculated the inclusion stoichiometry of the nimbin/CD complexes, that is, 1:1 for the nimbin/β-CD and nimbin/TMβCD complexes. The 1:1 inclusion stoichiometry was also observed in the nimbin/HPβCD and nimbin/DMβCD complexes.

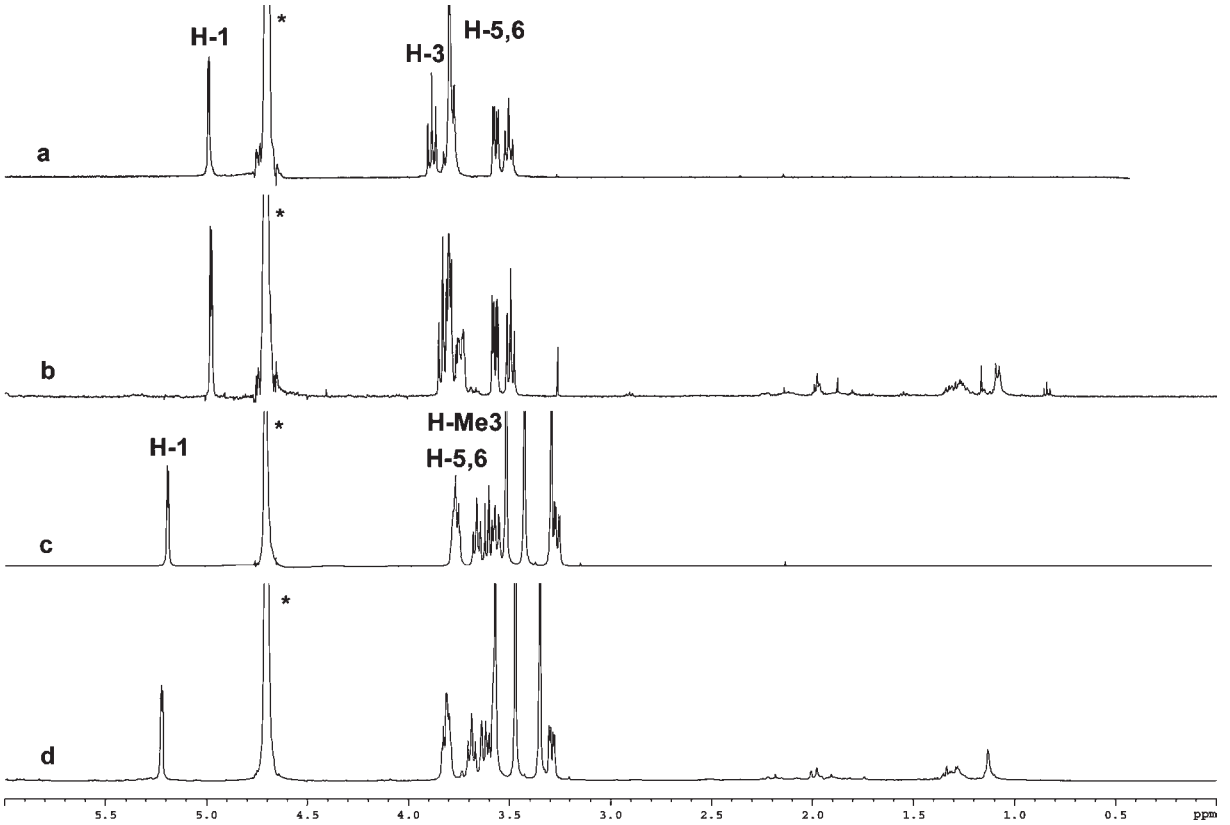

Figure 2. ^{1}H NMR spectra of nimbin in the absence and presence of β-CD and TMβCD in D_2O at 25 °C, respectively: (**a**) β-CD; (**b**) nimbin/β-CD complex; (**c**) TMβCD; (**d**) nimbin/TMβCD complex (asterisk highlights the water peak).

Table 2. Chemical Shifts (δ) of the β-CD, TMβCD, Nimbin/β-CD, and Nimbin/TMβCD Complexes

		δ			
		β-CD	nimbin/β-CD complex	TMβCD	nimbin/TMβCD complex
H-1	d	4.99	4.98	5.19	5.22
H-2	dd	3.57	3.57	3.25	3.29
H-3	dd	3.88	3.83	3.61	3.64
H-4	dd	3.50	3.50	3.66	3.71
H-5	m	3.80	3.80	3.76	3.81
H-6	dd	3.78	3.75	3.55	3.60
H-Me2	s			3.42	3.47
H-Me3	s			3.51	3.57
H-Me6	s			3.28	3.35

To further explore the inclusion mode, the chemical shifts of β-CD protons in the absence and presence of nimbin were examined (**Table 2**). Inclusion complexation with nimbin had a negligible effect on the δ values of the H-1, H-2, H-4, and H-5 protons of β-CD (< 0.01 ppm). In contrast, those values of the H-3 and H-6 protons exhibited relatively weak but significant changes (0.03−0.05 ppm), which could be caused by the hydrogen bond between the hydroxyl arms of β-CD and the oxygen atoms of nimbin. It is noteworthy that the H-3 protons shifted ca. 0.05 ppm but that the H-5 protons showed no appreciable shifts after inclusion complexation. Because both the H-3 and H-5 protons are located in the interior of the β-CD cavity and the H-3 protons are near the wide side of the cavity whereas the H-5 protons are near the narrow side, this phenomenon may indicate that nimbin should penetrate into the β-CD cavity from the wide side. In contrast, all of the TMβCD protons showed appreciable shifts after inclusion complexation with nimbin (0.03−0.07 ppm). By comparing these chemical shifts, we found that the shifts of the H-5 (0.06 ppm) and H-6 (0.05 ppm) protons were larger than those of the H-3 protons (0.03 ppm), indicating that nimbin may enter the cavity of TMβCD from the narrow side. It was also revealed that nimbin should penetrate into the HPβCD cavity from the wide side but enter the cavity of DMβCD from the narrow side (see the Supporting Information).

Two-dimensional (2D) NMR spectroscopy provides important information about the spatial proximity between host and guest atoms by observation of intermolecular dipolar cross-correlations (*41*). Two protons that are 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 (*42*). To gain more conformational information, we obtained 2D ROESY of the inclusion complexes of nimbin with CDs. The ROESY spectrum of the nimbin/CD complex (**Figure 3**) showed appreciable correlation of the H-22 proton of nimbin with the H-3 protons of β-CD (peak a), as well as key correlations between the H-19 protons of nimbin and the H-3 and H-4 protons of β-CD (peak b). These results indicate that the E ring (furan ring) and C/D rings of nimbin are included in the β-CD cavity. The ROESY spectrum of the nimbin/TMβCD complex (**Figure 4**) also showed significant correlations between the H-23 proton of nimbin and the H-3 protons of TMβCD and between the H-22 proton of nimbin and the H-3, H-4, and H-5 protons of TMβCD (peak c), as well as key correlations between the H-19 protons of nimbin and the H-3, H-4, and H-5 protons of TMβCD (peak d). These results indicate that the E ring (furan ring) and C/D rings of nimbin are also included in the TM-β-CD cavity. It can also be shown that nimbin should be included in the HPβCD and DMβCD cavity in similar ways (see the Supporting Information).

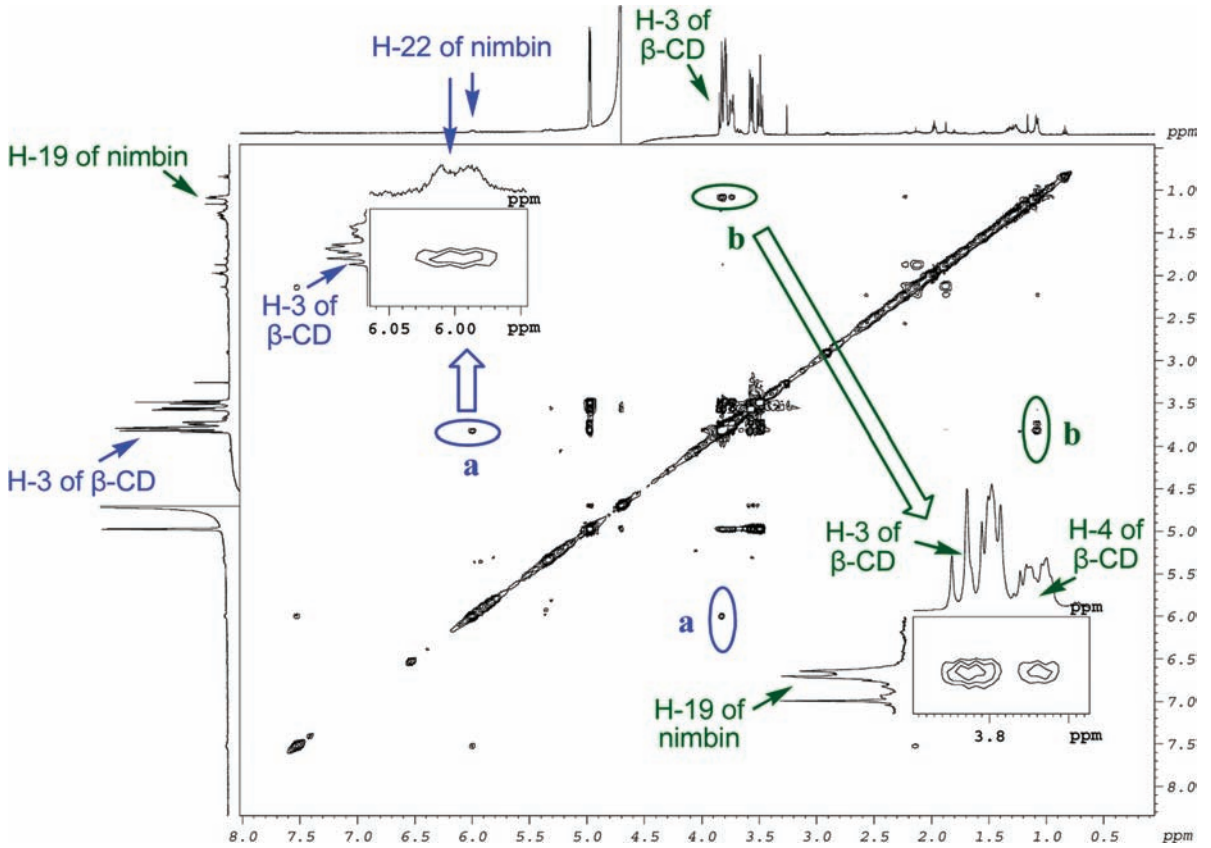

Figure 3. ROESY spectrum of the nimbin/β-CD complex in D_2O.

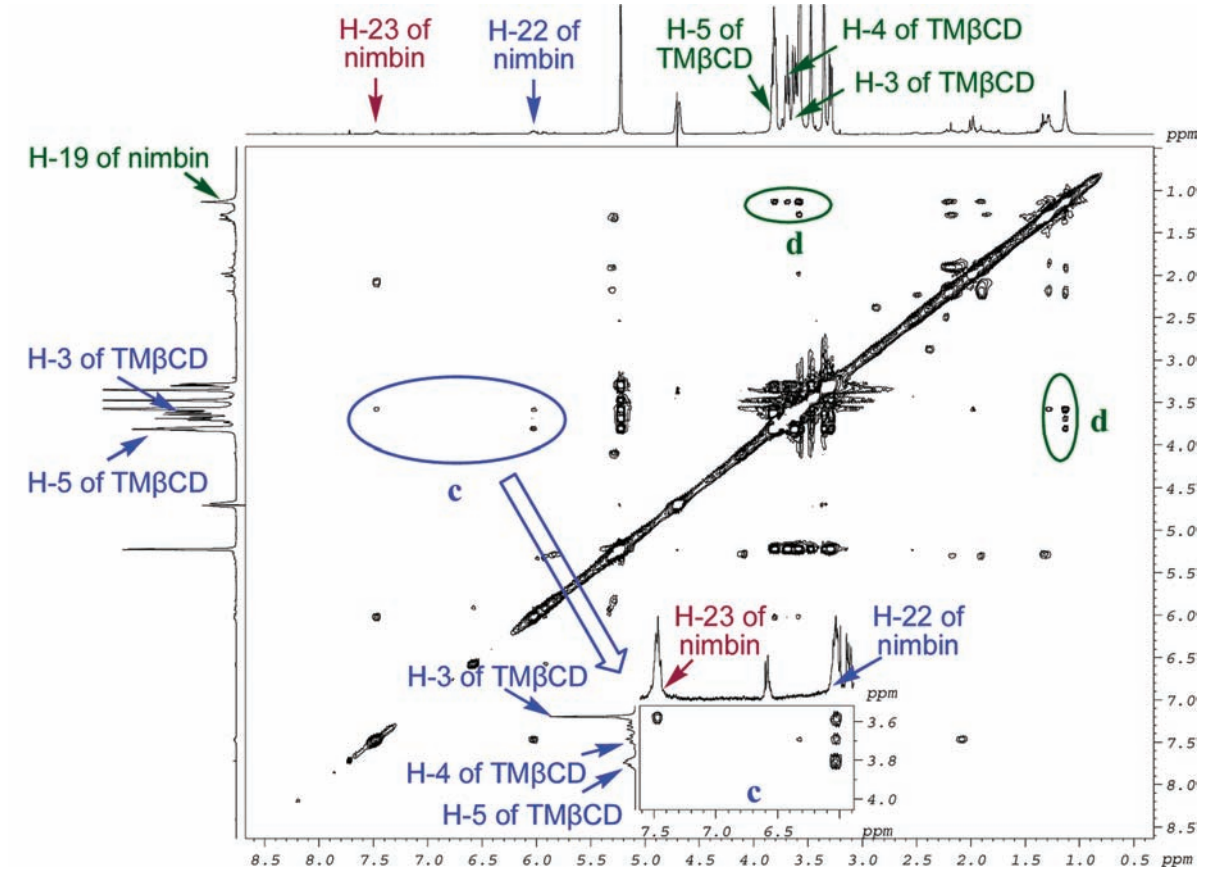

Figure 4. ROESY spectrum of the nimbin/TMβCD complex in D_2O.

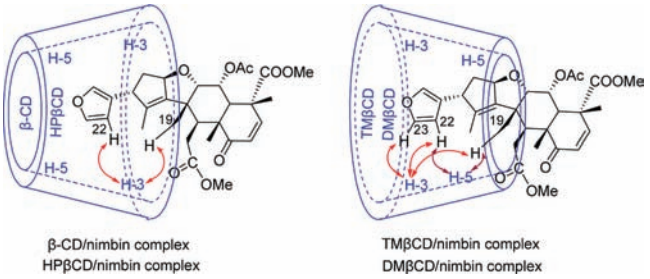

Figure 5. Possible inclusion mode and significant NOESY (↔) correlations of the nimbin/β-CD, nimbin/HPβCD, nimbin/DMβCD, and nimbin/TMβCD inclusion complexes.

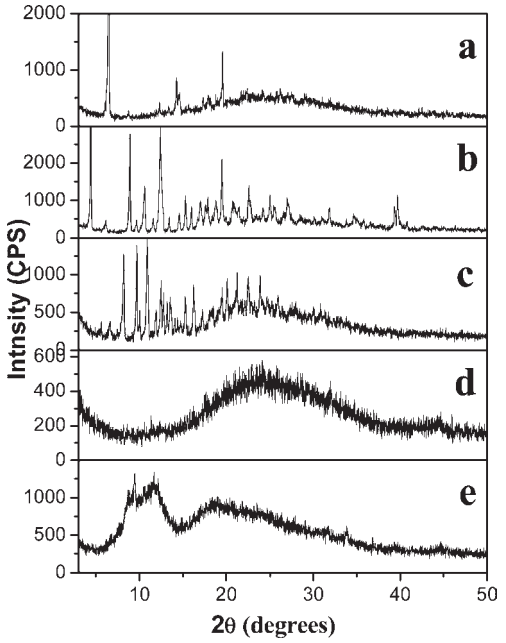

Figure 6. XRD patterns of (**a**) nimbin, (**b**) β-CD, (**c**) TMβCD, (**d**) nimbin/β-CD inclusion complex, and (**e**) nimbin/TMβCD inclusion complex.

On the basis of these observations, together with the 1:1 stoichiometry, we deduced the possible inclusion modes of nimbin with CDs as illustrated in **Figure 5**.

XRD Analysis. The XRD patterns of nimbin, β-CD, and TMβCD as well as their inclusion complexes are illustrated in **Figure 6** (the XRD of HPβCD and DMβCD and their inclusion complexes with nimbin can be seen in the Supporting Information). As indicated in **Figure 6**, nimbin is amorphous (**Figure 6a**), but β-CD (**Figure 6b**) and TMβCD (**Figure 6c**) are in crystalline form. In contrast, the XRD of the nimbin/β-CD and nimbin/TMβCD complexes (**Figure 6d**,**e**) showed halo patterns, which were quite different from the superimposition of crystalline β-CD (or TMβCD) and the amorphous nimbin, indicating the formation of the inclusion complex between β-CD (or TMβCD) and nimbin. The similar phenomenon for HPβCD and DMβCD and their inclusion complexes can be found (see the Supporting Information). In addition, most of the crystalline diffraction peaks of β-CD, TMβCD, HPβCD, or DMβCD disappeared after complexation with nimbin, indicating that the complexation of nimbin reoriented the CD molecules to some extent. Furthermore, the less sharp peaks in the XRD of the nimbin/β-CD complex may imply that nimbin/β-CD, nimbin/HPβ-CD, and nimbin/DMβ-CD possess a more amorphous structure than the nimbin/TMβCD complex.

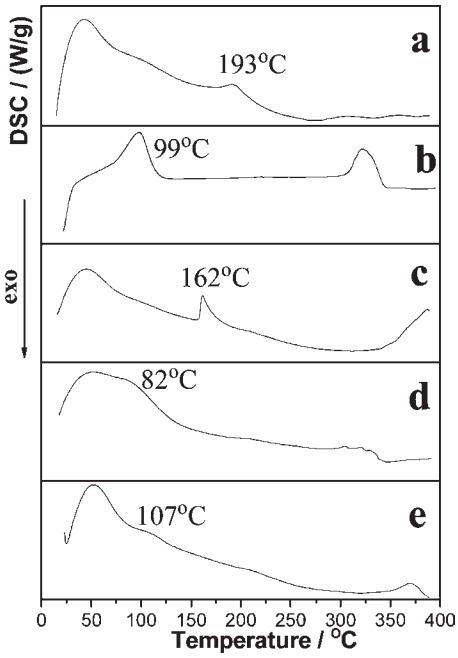

Figure 7. DSC thermograms of (**a**) nimbin, (**b**) β-CD, (**c**) TM-β-CD, (**d**) nimbin/β-CD inclusion complex, and (**e**) nimbin/TM-β-CD inclusion complex.

Thermal Analysis. The thermal properties of the nimbin/β-CD and nimbin/TMβCD complexes were investigated by TG methods (see the Supporting Information). A systemic analysis of the TG curves showed that nimbin decomposes at ca. 210 °C, β-CD at ca. 200 °C, and TMβCD at ca. 170 °C. However, the thermal stability of their inclusion complexes differed; that is, the decomposition temperatures were ca. 230 and 250 °C for the nimbin/β-CD and nimbin/TMβCD complexes, respectively. These results indicate that nimbin's usual thermal properties were altered after inclusion complexation. In similar tests, the nimbin/HPβCD or nimbin/DMβCD complex also shows a high decomposition temperature up to 220 or 250 °C, respectively.

The DSC thermogram gave further information about the thermal properties of the nimbin/β-CD and nimbin/TMβCD complexes. As shown in **Figure 7**, the DSC curve of nimbin contained an endothermic peak at 193 °C. In contrast, the DSC curve of pristine β-CD or TMβCD had an endothermic peak at 99 or 162 °C, respectively. However, in the DSC curves of the CD/nimbin complexes, the endothermic peaks at about 193 °C corresponding to the free nimbin disappeared, coinciding with the appearance of a new exothermic peak at 82 °C (or 107 °C) in the case of the nimbin/β-CD (or nimbin/TMβCD) system. In similar tests, the nimbin/HPβCD or nimbin/DMβCD complex also shows the appearance of a new exothermic peak at 51 or 73 °C, and the endothermic peak at about 193 °C disappears (see the Supporting Information). This suggested that the nimbin/TMβCD complex is more stable than the nimbin/β-CD, nimbin/HPCD, or nimbin/DMCD complex.

These results not only further confirm the formation of nimbin/CDs complexes but also indicate that the four resultant nimbin/CDs complexes start to decompose only at a temperature above 220 °C, which means that these complexes are fairly stable from a thermal viewpoint.

Solubilization. The water solubility of the nimbin/CD complex was assessed by the preparation of its saturated solution (*43*). An excess amount of complex was placed in 2 mL of water (ca. pH 6.0), and the mixture was stirred for 1 h. After removal of the insoluble substance by filtration, the filtrate was evaporated

under reduced pressure to dryness and the residue was dosed by the weighing method. The results show that the water solubility of the nimbin, compared with that of native nimbin (ca. 50 μg/mL), was remarkably increased to approximately 4.7, 3.8, 1.3, and 2.4 mg/mL by the solubilizing effects of β-CD, HPβCD, DMβCD, and TMβCD, respectively. In the control experiment, a clear solution was obtained after dissolution of the nimbin/β-CD (19.7 mg), nimbin/HPβCD (15 mg), nimbin/DMβCD (10.6 mg), or nimbin/TMβCD (14.8 mg) complex, respectively, which was equivalent to 4.7, 3.8, 1.3, or 2.4 mg of nimbin, in 1 mL of water at room temperature. This confirmed the reliability of the obtained satisfactory water solubility of the nimbin/CD complex, which will be beneficial for the medical utilization of this compound.

Conclusions. The inclusion complexation behavior, characterization, and binding ability of nimbin with β-CD and its derivatives (HPβCD, DMβCD, TMβCD) were investigated. The results showed that β-CD and its derivatives can enhance the water solubility of nimbin. Given the shortage of applications of nimbin and the easy and environmentally friendly preparation of the nimbin/β-CD complex, this inclusion complexation should be regarded as an important step in the design of a novel formulation of nimbin for biopesticide and herbal medicine or healthcare products.

Supporting Information Available: ^{1}H NMR and ROESY spectrum, XRD patterns, and TG and DSC thermograms of the nimbin/CDs complexes. This information is available free of charge via the Internet at http://pubs.acs.org.

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