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Investigation of (β-Cyclodextrin)-Lappaconitine Inclusion Complex

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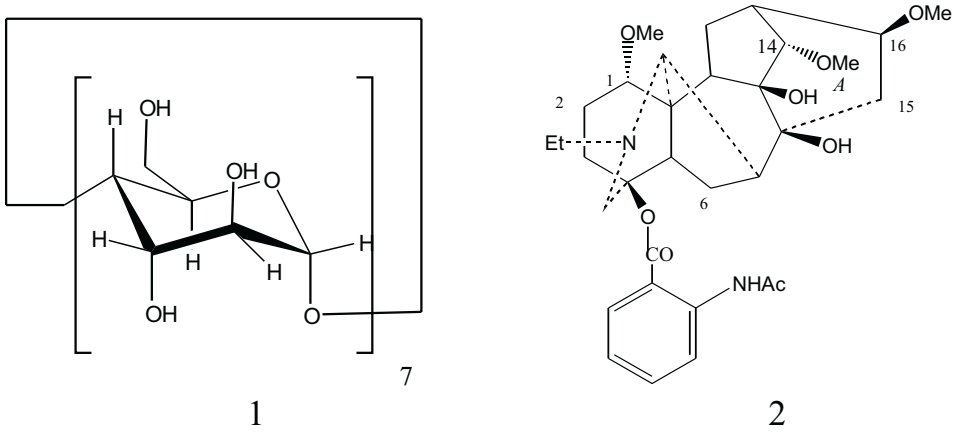
The structure of the inclusion complex of Lappaconitine (Lap) and β-cyclodextrin (β-CD) was studied by the UV, infrared, and NMR spectroscopy, as well as X-ray powder diffractometry. The stability constant of the complex in water is 275 M^{-1}, determined from the straight line portion of the phase-solubility diagram.

Key words: *Aconitum delphinium*, lappaconitine, β-cyclodextrin, inclusion complex

Lappaconitine (Lap) (Fig. 1 part 2) is a diterpenoid akaliamide, naturally occurring in roots and rhizomes of *Aconitum* and *delphinium* [1,2]. Lap reveals bradycardic, hypotensive, antinocieptive activity [3–5]. This compound can be used as nonarcotic analgesic medicine because of its peripheral and central analgesic activity



1 2

Figure 1. Structural formulae of β-cyclodextrin (**1**) and lappaconitine (**2**).

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[6–7]. Moreover, it has the remarkable effect of restoring the protective activity against infections and is useful for treating and preventing viral, fugal and opportunistic infections [8]. But its application is restrained owing to its poor water solubility, toxicity and side effects on humans.

In a number of pharmaceutical studies, CDs have been reported to interact with many drug molecules to form inclusion complexes. These inclusion complexes have been extensively used to improve water solubility of poorly soluble drugs, to reduce their toxicity [9], and to increase the dissolution rate [10–12]. The aim of the present work was to study the inclusion complex of Lap and β-CD (Fig. 1 part 1) in order to increase water solubility of Lap and hopefully reduce its toxicity. The (β-CD)-Lap complexes were prepared by either co-grinding or kneading. The products have been characterized by the solubility measurement, as well as UV, FTIR, NMR spectroscopy and X-ray powder diffractometry.

EXPERIMENTAL

Chemicals: β-CD (99.5%, Suzhou Weijing Plant, China) was purified by recrystallization from distilled water. Lap was purchased from Lanzhou Hechengyao Co. (Lanzhou,China). The ^{1}H NMR and 2D-NMR measurements were carried out by using a D_2O and DMSO-d_6 solvent (Aldrich). Other chemicals were of the analytical reagent grade purity.

Preparation of the (β-CD)-Lap complex: The (β-CD)-Lap complexes were prepared by either co-grinding or kneading of a mixture of solid host and guest compounds. The mixture of Lap and β-CD (molar ratio 1:1) was prepared by mixing of both compounds in a glass mortar. In the co-grinding procedure, the mixture of Lap and β-CD (1:1 molar ratio) was ground in a glass ball mill for 30 min at room temperature. In the kneading procedure, a 20 ml water suspension of 1.134g (1 mmol) of β-CD and 0.584 g (1 mmol) of Lap were vigorously stirred for 15 h. Then the resulting solution was dried with a rotary evaporator at 45°C.

Characterization: Infrared spectroscopy was conducted with a Nicolet 170SX Infrared Spectrometer, using the KBr disc method. Powder X-ray diffraction patterns were obtained by using a Rigaku D/max-2400 diffractometer (Japan), with Ni-filtered 2 CuKα radiation, voltage 40 kV, current 40 mA, DS/ss 10, RS 0.15 mm at a scanning speed of 8°/min. The ^{1}H-NMR and 2D-NMR spectra were recorded with a Bruker AM 400 NMR spectrometer. In order to increase the complex solubility, 0.1 ml DMSO-d_6 was added to a 0.4 ml D_2O test solution. Chemical shifts were measured with respect to the residual water signal at 4.69 ppm. All measurements were carried out at 25°C.

Solubility measurements: The phase-solubility diagram was recorded according to Higuchi *et al*. [13]. For that purpose, aqueous solutions of β-CD with concentrations of 0, 1, 3, 4, 5, 7 mM were prepared. Excess amounts of Lap were added to each solution of β-CD. Next, the solutions were agitated for 15 h at 30°C, then centrifuged and carefully filtered. The 5 ml samples of filtrates were diluted to 10 ml with ethanol. Their absorbances were measured by UV spectrophotometry after appropriate dilution with ethanol (308 nm). Figure 2 shows the equilibrium phase-solubility diagram for the (β-CD)-Lap complex in water. The isotherm is a Bs type solubility curve and shows an initial linear section up to 5 mM^{-1} β-CD. This indicates that a soluble complex (1:1) is formed rapidly and the lower solubility limit of the complex is at about 0.34 mM Lap. The method is employed for the determination of the complex stability constant K by using the relation [14]: $K = \mathrm{tg}\,\Phi/S_0(1 - \mathrm{tg}\,\Phi)$. tgΦ is the slope of the initial section of the curve; S_0 is the aqueous solubility of Lap.

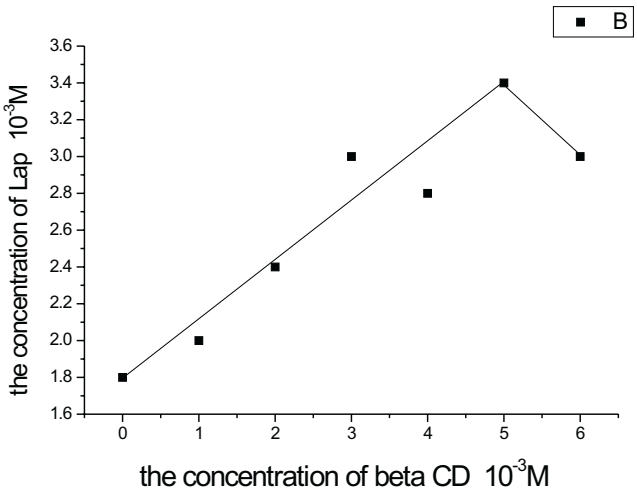

Figure 2. Solubility curve of Lap in the aqueous solutions of β-CD.

RESULTS AND DISCUSSION

Properties of the (β-CD)-Lap complex: Infrared spectroscopy analysis provides some structural information about the (β-CD)-Lap inclusion complex. The band at 1027 cm^{-1} ascribed to hydroxyl group of β-CD shifts to 1033 cm^{-1} for the complex. The intensity and shape of the bands, corresponding to hydroxyl in β-CD, change sharply for the inclusion complex as compared to those for genuine β-CD, indicating that the β-CD molecules are in different environments in these two compounds. In addition, decreases in the intensities of many bands are observed in (β-CD)-Lap complex, compared to a solid mixture of Lap and β-CD. The bands ascribed to carboxyl group, and phenyl group are not changed as expected, which indicates that the benzene ring is not inserted into the cavity of β-CD (see Figure 3).

The X-ray powder diffraction patterns for β-CD, Lap, (β-CD)-Lap and the solid mixture of the host and guest compounds are shown in Figure 4. The X-ray diffraction peaks in the 2θ region of 12.74°,13.4°,18.16° for β-CD (Curve 1 in Fig. 4) and 5.9°, 13.62° for Lap (Curve 2 in Fig. 4) are absent in the complex pattern (Curve 3 in Fig. 4). But these peaks are observed in the solid mixture of Lap and β-CD (Curve 4 in Fig. 4). This behavior can be interpreted as an approximate superposition of the components, namely β-CD and Lap. Importantly, the complex pattern is markedly different. These observations reinforce the evidence from the IR spectral analysis that the solid precipitated from the β-CD and Lap solution is a microcrystalline inclusion complex of β-CD and Lap.

When the size of the two parts of Lap, which may enter into the cavity of β-CD is considered, the following inference is supported reasonably (Table 1). As the diame-

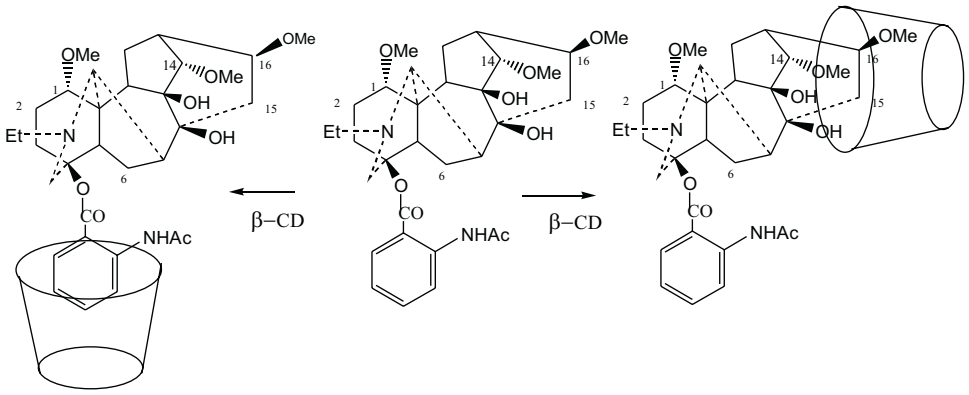

Figure 3. Structural formulae of proposed (β-CD)-Lap inclusion complex.

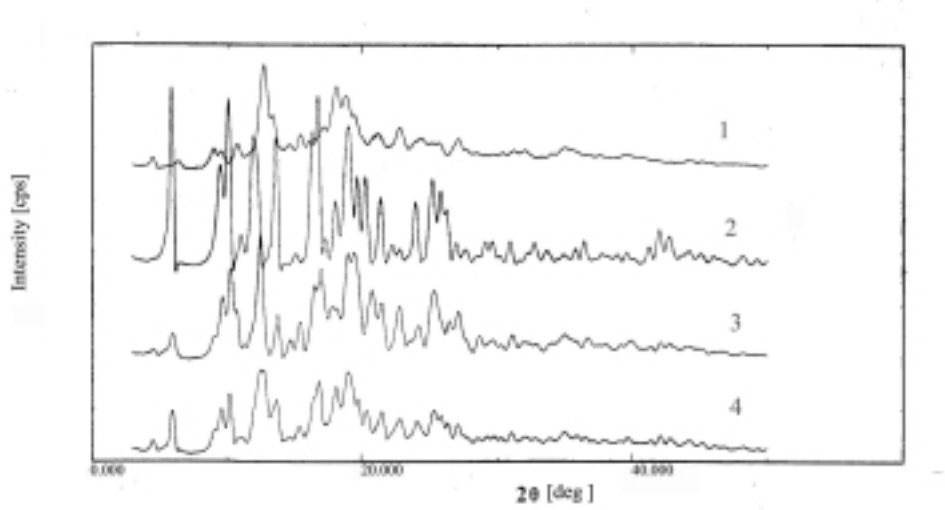

Figure 4. The X-ray powder diffractogram patterns of β-CD (Curve 1), Lap (Curve 2), the (β-CD)-Lap complex (Curve 3), and solid mixture (Curve 4).

ter of the β-CD cavity is 6.5 Å [15], it is ring *A* that matches the cavity well. The NMR spectroscopy provides information on the location of the guest within the CD cavity [16,17]. In the presence of Lap, the chemical shifts of β-CD protons show noteworthy up-field shifts of the resonances of protons H-3, H-5 which are oriented towards the interior of β-CD cavity (Table 2). The proton H-6 chemical shift moves down field, while the chemical shifts of H-1, H-2 and H-4 protons are unaffected as a result of complexation. These observations clearly prove the inclusion complex formation

[18,19] and are consistent with the reasoning of DeMarco and Thakker [20] that the screening enviroment should be sensed only by the hydrogens on the inner surface (H-3 and H-5), but not by the hydrogens on the outer surface if inclusion occurs.

Table 3 provides the chemical shift values for Lap in the inclusion complex. The chemical shifts of the hydrogen of the $–OCH_3$ group, $-^{15}CH_2$ group, and $-^{16}CH$ group of ring *A* of Lap shift down-field in the inclusion complex as compared to those of Lap (Fig. 5). In contrast, the chemical shifts of H belonging to the benzene ring do not change. These observations indicate that inclusion of Lap occurs by insertion of ring *A*.

Table 1. The dimension of two parts of Lap .

Part	Ring A	Benzene
Size (Å)	2.768 Å	5 Å

The values are estimated by the authors based on bond lengths [21].

Table 2. The ^{1}H NMR spectroscopy chemical shifts (ppm) for β-CD in the absence and the presence of Lap (molar ratio 1:1).

Proton	β-CD (δ_0)	(β-CD)-Lap (δ)	$\Delta\delta(\delta - \delta_0)$
H-1	4.92438	4.92433	–0.00005
H-2	3.48968	3.48667	–0.00301
H-3	3.7706	3.73257	–0.03803
H-4	3.044593	3.43837	–0.00756
H-5	3.66531	3.62922	–0.03609
H-6	3.69676	3.70994	0.01318

Table 3. The ^{1}H NMR chemical shifts (ppm) of the Lap in the absence and the presence of β-CD (molar ratio 1:1).

Proton	Lap (δ_0)	(β-CD)-Lap (δ)	Δδ ($\delta - \delta_0$)
$-OCH_3$	3.20689	3.36801	0.16121
$-^{15}CH_2-$	1.71917	1.76217	0.043
$-^{16}CHOCH_3-$	3.82498	3.89770	0.07272

Two-dimensional NOE measurement also proved the inference on the manner of inclusion. That is a set of crossing peaks connects both H-3 and H-5 resonances of β-CD to the hydrogen signals of the $–OCH_3$ group, $-^{15}CH_2$ group, $-^{16}CH$ group of Lap (Fig. 5). The 2D-NOE cross-peaks and a few intermolecular connectivities observed may be due to very weak complexation between Lap and β-CD. All these results indicate that ring *A* of the guest is inserted into the β-CD cavity.

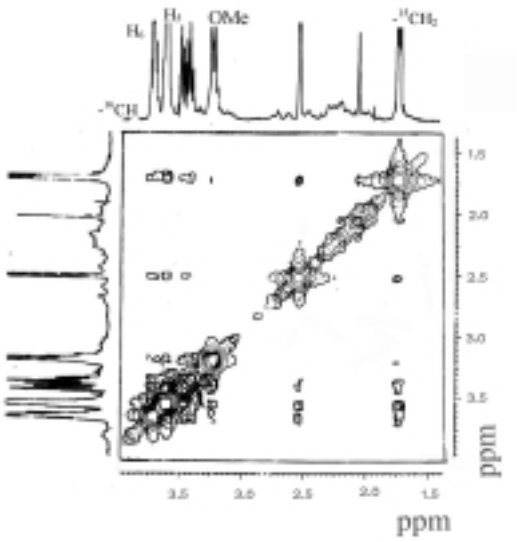

Figure 5. The 400 MHz 2D-NOESY spectrum of the (β-CD)-Lap complex in D_2O-DMSO-d_6. Intermolecular connectivites are shown in the diagram.

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