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DSC and NMR Study on the Inclusion Complex of Lappaconitine with β-Cyclodextrin

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Abstract: The inclusion complex of lappaconitine (Lap) with β-cyclodextrin (β-CD) has been studied by the differential scanning calorimetry (DSC) and ^{1}H-NMR, 2D-NMR spectroscopy. The structure of the complex has been deduced.

Keywords: *Aconitum delphinium*, lappaconitine, β-cyclodextrin, inclusion complex, DSC, NMR.

Lappaconitine (**Figure 1**) is a diterpenoid alkaloid, naturally occurring in roots and rhizomes of *Aconitum* and *delphinium*[1]. Lap reveals bradycardic and hypotensive activity[2]. But its application is restrained owing to its poor water solubility, toxicity and side effects on humans. In a number of pharmaceutical studies, β-CD has been reported to interact with many drug molecules to form inclusion complexes for improving the water solubility of drugs, and reducing their toxicity[3]. We have prepared the β-CD/Lap complex (**Figure 2**) in order to decrease the toxicity and increase the solubility of Lap. The structure of β-CD/Lap complex has been studied by the methods of differential scanning calorimetry (DSC) and NMR spectroscopy.

Figure 1 Structural formulae of -cyclodextrin and lappaconotin

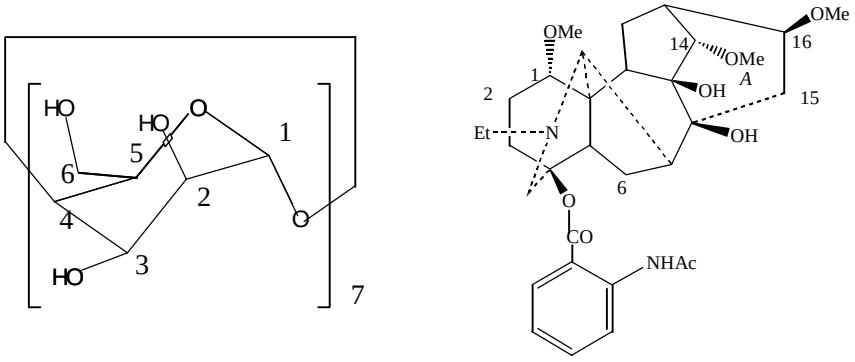

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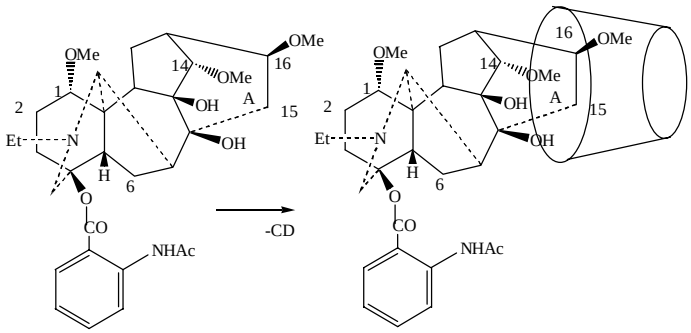
Figure 2 The possible structural formulae of -CD/Lap inclusion complex

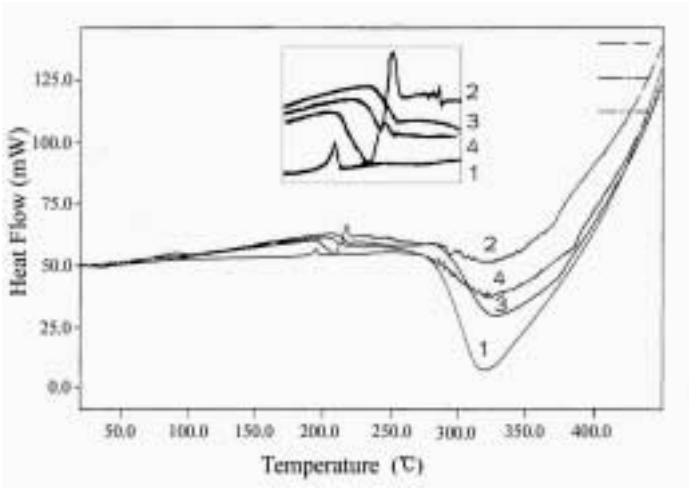


Differential scanning calorimetry of β-CD/Lap was obtained using a Perkin-Elmer series 7 differential scanning calorimeter (DSC). DSC analysis was performed using a temperature scan rate of 5ºC/min in the temperature range from 0ºC to 450ºC. All NMR spectra were recorded with a Brucker AM-400 NMR spectrometer in D_2O-DMSO-d_6 (4:1).

β-CD (99.5%, Suzhou Weijing Plant, China) was purified by recrystallallization from distilled water. Lap was purchased from Lanzhou Hechengyao Co. (Lanzhou, China). The β-CD/Lap complexes were prepared by kneading a mixture of solid host and guest compounds. 20 mL water suspension of 1.134 g (1 mmol) of β-CD and 0.584 g (1 mmol) of Lap were vigorously stirred for 15 h. Then water was distilled off under reduced pressure at 45ºC. The residue of Lap and β-CD was ground in a glass mortar.

The result of DSC of β-CD, Lap, the solid mixture, β-CD/Lap complex are shown in the **Figure 3**. The β-CD/Lap (curve 4 in **Figure 3**) complex shows an endothermic peak at 210ºC. But the peak of Lap (curve 2 in **Figure 3**) is at 215ºC. At the same temperature range, the residue (curve 3 in **Figure 3**) and β-CD (Curve 1 in **Figure 3**) did not show the endothermic peak. This is attributed to the coexisting of the crystalline.

Figure 3 The DSC curves of (1) β-CD; (2) Lap; (3) solid residue; (4) β-CD/Lap



In the presence of Lap, the chemical shifts of β-CD protons show significant up-field shifts of the resonances of protons H-3', H-5' which are oriented towards the interior of β-CD cavity (**Table 1**). The chemical shift of proton H-6' moves down field while the chemical shifts of H-1', H-2' and H-4' protons are unaffected. These observations clearly proved the inclusion complex formation[4] as Demarco and Thakkar[5] pointed out that the screening enviroment should be sensed only for the hydrogens on the inner surface (H-3' and H-5'), but not by the hydrogens on the outer surface if inclusion occured.

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

Proton in β-CD	β-CD (δ_0)	β-CD/Lap (δ)	$\Delta\delta$ (δ-δ_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'	3044593	3.43837	-0.00756
H-5'	3.66531	3.62922	-0.03609
H-6'	3.69676	3.70994	0.01318

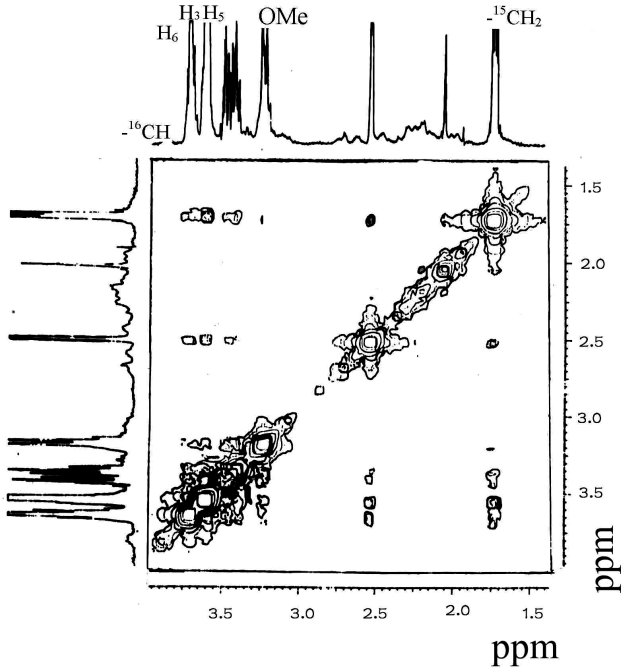
Table 2 provides the chemical shift values for Lap in the inclusion complex. The chemical shifts of the hydrogen of the $-^{16}COCH_3$ group, $-^{15}CH_2$ group, and $-^{16}CH$ group of ring *A* of Lap shift down-field in the inclusion complex as compared with those of Lap itself. In contrast, the chemical shifts of protons belonging to the benzene ring do not change. These observations indicate that inclusion of Lap occurs by insertion of ring *A*.

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

Proton in Lap	Lap (δ_0)	β-CD/Lap (δ)	$\Delta\delta$ (δ-δ_0)
$-^{16}COCH_3$	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. There were the correlations between the resonances of H-3', H-5' in β-CD and signals of the $-^{16}COCH_3$ group, $-^{15}CH_2$ group, $-^{16}CH$ group of Lap (**Figure 4**). 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 4 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



References

1. M. Shamma, P. Chinnasamy, G. A. Miana, *et al.*, *J. Nat. Prod.*, **1979**, *42*, 615.
2. M. Ono, T. Satoh, *Jpn. J. Pharmacol*., **1991**, *55*, 523.
3. K. Uekama, F. Hirayama, T. Lvie, *Chem. Rev*., **1998**, *98*, 2045.
4. S. Senel, U. Cakaglu, M. Sumnu, D. Duchene, A. A. Hincal, *J. Inclusion Phenom*., **1992**, *14*, 171.
5. P. V. DeMarco, A. L. Thakkar, *J. Chem. Soc*., *Chem. Commun*., **1970**, 2.

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