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КРАТКИЕ СООБЩЕНИЯ

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NMR INVESTIGATION ON THE INTERACTION OF β-CYCLODEXTRIN AND AMPICILLIN

© 2010 **S.K. Upadhyay*, S.M. Ali**

Department of Chemistry, Aligarh Muslim University, Aligarh – 202 002, UP, India

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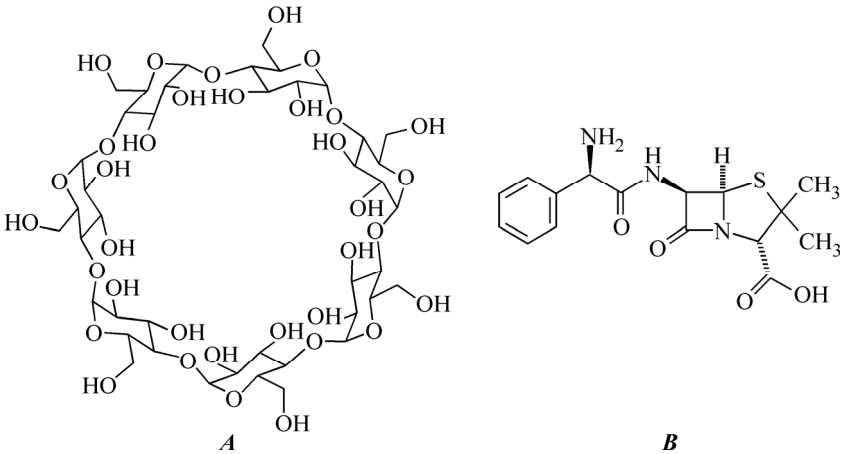
The penetration of ampicillin molecule into the β-cyclodextrin cavity in aqueous solution is studied by ^{1}H NMR, 2D COSY and ROESY spectroscopy. Obtained data suggest that the phenyl moiety of ampicillin is included inside the hydrophobic cavity of β-cyclodextrin molecule in the solution, and the mode and depth of this inclusion is confirmed on the basis of 2D ROESY spectral data.

K e y w o r d s: NMR spectroscopy, COSY, ROESY, inclusion complex.

Abbreviations: CDs, cyclodextrins; β-CD, β-cyclodextrin; AMP, ampicillin; ^{1}H NMR, proton nuclear magnetic resonance spectroscopy; 2D, two-dimensional; COSY, COrrelation SpectroscopY; ROESY, Rotational nuclear Overhauser Effect SpectroscopY; NOEs, Nuclear Overhauser Effects.

Ampicillin (AMP), 6-[D(–)-α-Aminophenylacetamido]penicillanic acid, is a D-Phg-based commercial antibiotic which is widely used as a medication against a variety of gram-positive and gram-negative bacteria (Fig. 1). In particular, this penicillin-like, broad-spectrum β-lactam antibacterial agent is currently used to treat such infections as gonorrhea and meningitis.

Cyclodextrins (CDs) were discovered in 1891 by Villiers by digesting starch with *Bacillus amylobacter* [1]. CDs are chiral, truncated cone shape, cyclic oligosaccharides built up from glucopyranose units; they have been studied extensively as host molecules in supramolecular chemistry [2]. The three major types of CDs are crystalline, homogeneous, non-hygroscopic substances, consisting of six (α-), seven (β-), or eight (γ-) D-glucose units attached by α-(1→4) glycosidic linkages (Fig. 1). CDs are widely applied in pharmaceutical formulations to enhance the stability, solubility and bioavailability of drug molecules [3, 4]. These host-guest complexes are considered as new entities and so they are required to be characterized for their approval as a drug.



A *B*

Fig. 1. Structural representation of (*A*) host β-CD and (*B*) guest AMP molecules

* E-mail: skupadhyay@msn.com

NMR spectroscopy is unique among the methods available for structure determination of host-guest complexes at atomic resolution, as the NMR data can be recorded in solution [4, 5]. The importance of the NMR spectroscopy in CD field is due to its ability to study complexation systems and to determine their stoichiometry, association constants and conformations of involved molecules as well as to give information on their symmetry and dynamics. NMR is also an effective tool in chiral recognition studies. ROESY is a two-dimensional technique based on NOE, in which cross-peaks may be observed between protons if the corresponding internuclear distance is smaller than 3—4 Å. It gives information about the part of the guest included inside the CD cavity, the mode of penetration, *i.e.* either from narrower or wider side of the rim, the depth of penetration and orientation of the guest.

The formation of an inclusion complex between a guest and CD results in the chemical shift changes ($\Delta\delta$) in both the host and guest protons. Inclusion of a guest molecule inside the host CD cavity is mainly characterized by the upfield shift variation of the CD protons located inside the cavity (H-3′ and H-5′), while other CD protons (H-1′, H-2′, H-4′ and H-6′) are less affected. The guest protons generally show downfield shift changes upon complexation but sometimes highfield shift changes in the guest proton signals are also observed.

We are interested in the NMR spectroscopic study of complexation of pharmaceutical compounds with CDs [6, 7] and report herein our results on the interaction of β-cyclodextrin (β-CD) and ampicillin (AMP) in aqueous solution.

Experimental. Materials. Ampicillin (AMP) was kindly donated by a local drug company while β-CD was obtained from Geertrui Haest, Cerestar Application Centre, Food & Pharma Specialities, France, and these were used as received. All other reagents were of analytical reagent grade.

Methods. The total molar concentration of host and guest was kept constant at 10 mM ([β-CD]/[AMP] = 10mM). β-CD and AMP solutions were mixed in 1:1 molar ratio in 5 mm WILMAD NMR tube (Sigma-Aldrich). All the ^{1}H NMR and 2D NMR (COSY, ROESY) spectra of pure β-CD, pure AMP and β-CD — AMP mixtures were obtained at 300 K on a Bruker 600 MHz spectrometer using a 5 mm CPTCI 1H-detection NMR probe. The chemical shift values reported in δ (ppm) were calculated with reference to residual solvent (D_2O) resonance at 4.800 ppm. The 2D ROESY spectrum was collected using a standard ROESY pulse sequences with mixing time of 500 ms under spin lock condition. Chemical shifts changes ($\Delta\delta$) were calculated according to the formula: $\Delta\delta = \delta_{(complex)} - \delta_{(free)}$.

Results and Discussion. The assignment of β-CD protons was done with the help of their specific shapes, 2D COSY and ^{1}H NMR spectral data. Our previous results show upfield shift of host β-CD cavity protons (H-3′ and H-5′) and downfield shift of guest protons, implying formation of inclusion complexes [6, 7]. The present study involving β-CD — AMP mixture also shows similar results. The H-3′ and H-5′ protons of β-CD in the β-CD — AMP mixture shows upfield shift while the other β-CD protons (H-1′, 2′, 4′ and 6′) show negligible shift changes. These upfield shifts have been attributed to magnetic anisotropy effects in the β-CD cavity, arising due to the inclusion of a π-electron-rich group. Such a group in AMP molecule is a phenyl ring and the shifts indicate its inclusion in the β-CD cavity. The cavity protons of β-CD (H-3′ and H-5′) show almost similar upfield chemical shift changes, suggesting that the inclusion of guest molecule into the β-CD hydrophobic cavity occurs from the H-3′ and/or H-5′ side. The chemical shifts data of β-CD protons in the presence as well as in the absence of AMP are given in Table 1.

Since the ^{1}H NMR data were not conclusive of inclusion of guest molecule inside the β-CD cavity, we performed the 2D ROESY spectral studies to confirm our results. The assignment of AMP protons was done on the basis of the ^{1}H NMR and 2D COSY spectral data. AMP ^{1}H NMR (600 MHz, D_2O, 300 K) δ (ppm): 1.378 (s, 3H, C**H**$_3$), 1.385 (s, 3H, C**H**$_3$), 4.186 (s, 1H, N—C**H**—COOH), 5.188 (s, 1H, C**H**—NH_2), 5.465 (dd, 1H, NH—CH—C**H**—S), 5.489 (dd, 1H, NH—C**H**—CH—S), 7.500 (m, 5H, C_6**H**$_5$).

All the aromatic protons of AMP in the β-CD – AMP mixture shifted downfield but other AMP protons showed insignificant shift changes. The chemical shifts for the protons of AMP both in the

Table 1

^{1}H *NMR* (600 MHz) *chemical shift data* (ppm) *of* β-CD *protons in the presence and absence of* AMP*

β-CD protons	δ_{free}	$\delta_{complex}$	$\Delta\delta = \delta_{(complex)} - \delta_{(free)}$
H-1′	5.051	5.048	–0.003
H-2′	3.623	3.619	–0.004
H-3′	3.945	3.921	–0.024
H-4′	3.565	3.560	–0.005
H-5′	3.835	3.816	–0.019
H-6′	3.866	3.861	–0.005

* Negative value indicates upfield shift changes.

Table 2

^{1}H *NMR* (600 MHz) *chemical shift data* (ppm) *of* AMP *protons in the presence and absence of* β-CD*

AMP proton	δ_{free}	$\delta_{complex}$	$\Delta\delta = \delta_{(complex)} - \delta_{(free)}$
H-1	1.378	1.358	–0.020
H-2	1.385	1.393	+0.008
H-3	4.186	4.189	+0.003
H-4	5.465	5.455	–0.010
H-5	5.489	5.495	+0.006
H-6	5.188	5.179	–0.009
H-7 to H-11	7.500	7.62	+0.120

* Negative value indicates upfield shift changes.

absence and presence of β-CD are given in Table 2. The downfield displacement of the aromatic protons indicates that they are close to an electronegative atom, like oxygen, which imparts to the β-CD cavity its hydrophobicity and the aromatic ring is very close to form an inclusion complex of host-guest type. The 2D ROESY spectrum of β-CD — AMP mixture shows the existence of intermolecular NOEs between the cavity protons (H-3′ and H-5′) of β-CD and aromatic protons of AMP (Fig. 2). These results suggest that the inclusion of phenyl ring of AMP into β-CD cavity is from wider as well as narrower rim sides of the β-CD macrocycle. On the basis of ^{1}H NMR data for the free β-CD, free AMP and the β-CD – AMP mixture, as well as 2D ROESY spectral data, the inclusion complex has two possible structures as shown in Fig. 3.

The penetration of AMP molecule into the β-CD cavity was clearly demonstrated by changes in the ^{1}H NMR data. ^{1}H NMR and 2D ROESY spectral data suggest that the phenyl moiety of AMP is deeply included inside the hydrophobic cavity of β-CD molecule in the solution. 2D ROESY spectral data for β-CD — AMP mixture (1:1) confirms the existence of two complexes in which phenyl ring penetrates into β-CD cavity from wider as well as narrower rim sides.

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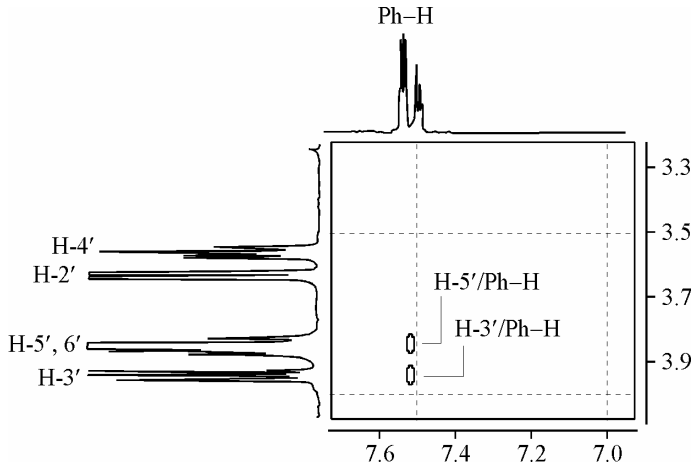

Fig. 2. 600 MHz 2D ROESY spectrum (τ_m = 500 ms) of 1:1 β-CD — AMP mixture showing the intermolecular NOEs between β-CD cavity protons (H-3′ and H-5′) and phenyl ring protons of AMP

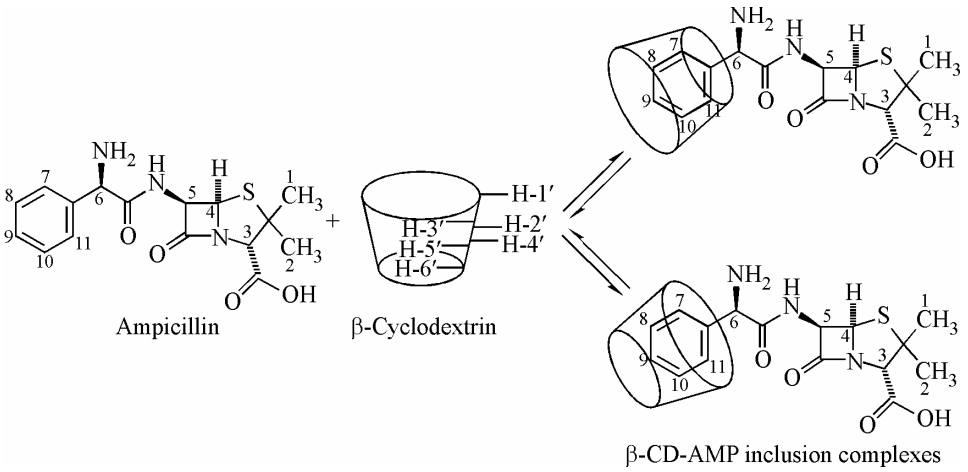

Fig. 3. Possible structures of β-CD — AMP inclusion complexes

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