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Studies of cyclodextrin inclusion complexes. II. Molecular modelling and ^{1}H-NMR evidence for the salbutamol-β-cyclodextrin complex

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Summary

β-Cyclodextrin (β-CYD) has a hydrophobic cavity in which salbutamol may be clathrated. The ability of a guest molecule to penetrate the CYD host is determined by its size, stereochemistry and polarity. Intermolecular interactions such as the hydrophobic interaction, van der Waals forces, H bonding and other physical forces are involved in the inclusion complexation process. Proton-NMR was employed to assess the mode of inclusion of salbutamol within the β-CYD cavity. The technique is based on the shielding of the CYD and drug protons. If inclusion does occur, protons located within or near the CYD cavity should be strongly shielded. The spectra showed upfield shifts of the CYD protons in the presence of salbutamol and the salbutamol protons shifted downfield in the presence of β-CYD. The downfield shifts of the aromatic protons were greater than those of the aliphatic protons, suggesting that the aromatic ring of salbutamol interacts more strongly with the β-CYD. The interior protons of the CYD molecule were shielded as a result of the anisotropy of the guest aromatic moiety. The highest shifts of β-CYD protons occurred for a molar ratio of 1:1 (salbutamol:β-CYD) indicating the probable stoichiometry of the complex. Molecular graphical computation showed that the minimum van der Waals energy positioning of salbutamol relative to β-CYD occurs when the aromatic ring of salbutamol penetrates the cavity leaving the aliphatic chain externalised.

Introduction

In cyclodextrins nuclear magnetic resonance (NMR) and X-ray diffraction studies indicate the C-1 chair conformation for the constituent glucose molecules (Rao and Foster, 1963). CYDs (Figs 1 and 2) are toroidal molecules with a truncated cone shape having secondary hydroxyl groups on the C-2 and C-3 atoms located on one side of the torus (wider side), while the primary hydroxyl groups on C-6 are positioned on the opposite, narrower side of the torus. The CH groups carrying the protons H-1, H-2 and H-4 are located on the exterior of the molecule, consequently the external faces of CYDs are hydrophilic. The interior of the torus offers an environment of much lower polarity than is present in water so it can be considered as a 'hydrophobic cavity', which is

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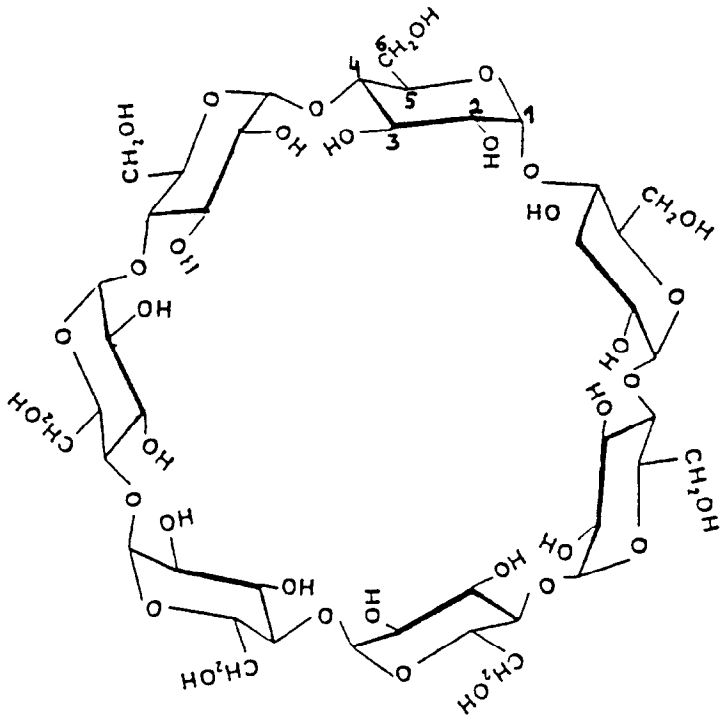

Fig. 1. Complete structure of β-CYD.

lined by two rings of CH groups (H-3 and H-5) and by a ring of glucosidic 'ether oxygens' (O-4) (Jones et al., 1984; Uekama and Otagiri, 1987), with H-6 located near the cavity.

These characteristics allow various types of drugs ('guests') to be clathrated (encased) forming non-covalently bonded inclusion complexes either in the solid phase or in aqueous solutions (Saenger,

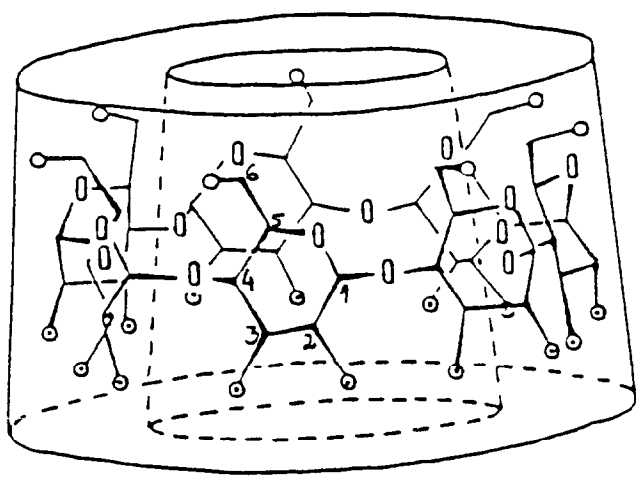

Fig. 2. Side view of β-CYD.

1980; Uekama, 1981; Szejtli, 1982). Analysis of the binding parameters indicated that van der Waals-London dispersion forces are responsible for the differences in binding energy between the various CYDs-substrate complexes and must have a suitable shape and size to be physically accommodated in the CYD cavity (Bergeron et al., 1978). In spite of technical difficulties due to the low solubility of CYD in D_2O (Jones et al., 1984), proton-NMR can provide an indication of the orientation of the guest molecule within the torus of the CYD.

Using the solubility method of Higuchi and Connors (1965) Cabral Marques et al. (1990) measured the stability constants of the complexes formed between α-, β-, Me-β- and γ-CYD and salbutamol. The variation in stability of the complexes suggested that the salbutamol was entering the toroidal cavity, fitting most snugly into that of Me-β- and β-CYD.

The aim of this paper, therefore, is to gain insight into the mode of inclusion of salbutamol in the β-CYD cavity and to employ computer modelling in support of the experimental data.

Materials and Methods

Materials

Salbutamol B.P. (base) was a generous gift from Lilly Research Centre Ltd, Surrey, U.K, Forum (batch no. 73267023). β-CYD was obtained from Chinoin (Budapest) (Ref. No. 850436). Freshly prepared distilled water was used throughout. Salbutamol purity was established by comparison of its melting point (154–155 °C) with literature values (155 °C) (British Pharmacopoeia, 1988), and was used without further purification. CYDs were used as received. Complexes were prepared employing molar ratios of 1 : 1, 1 : 2 and 2 : 1 using the freeze-drying method as reported in the previous article (Cabral Marques et al., 1990).

Apparatus

The NMR spectra were obtained using a Perkin Elmer R 32 (90 MHz) and a Bruker W M 360 (360 MHz) spectrometer. A high vacuum freeze drier (Modulyo Edwards, Crawley, U.K.), CHEM-X * molecular modelling program (Chemical Design, Oxford) and COSMIC * molecular modelling program (Smith, Kline and French Research) were from the indicated sources.

Preparation of the solid complex

The solid salbutamol-β-CYD complexes were prepared by dissolving appropriate amounts of the salbutamol (0.3590 g) and the β-CYD (1.7025 g) in water (100 ml) in a molar ratio of 1 : 1, or 1.7025 g of β-CYD and 0.7180 or 0.1795 g of salbutamol in order to achieve molar ratios of 1 : 2 and 2 : 1, respectively. These solutions were freeze-dried for 48 h (protected from light).

NMR studies

^{1}H-NMR spectroscopy has been employed to examine the mode of interaction of β-CYD with a variety of aromatic substrates (substituted benzoic acids, substituted phenols, acetylsalicylic acid and tetracycline) (Demarco and Thakkar, 1970; Thakkar and Demarco, 1971). Large molecules were shown to form inclusion compounds if groups of a suitable size and shape could fit into the β-CYD cavity. Similar results for the inclusion of aromatic compounds with α-CYD were obtained by Wood et al. (1977).

β-CYD (Figs. 1 and 2) has primary and secondary OH groups crowning opposite ends of its torus; H-3 and H-5 directed towards the interior, H6 on the rim and H-1, H-2 and H-4 located to the exterior. It is expected that if inclusion does occur, protons located within or near the cavity (e.g. H-3, H-5 or H-6) should be strongly shielded due to the anisotropy of the aromatic moiety, whereas protons located on the exterior of the torus (H1, H2 and H4) should be relatively unaffected. Alternatively, if association takes place on the exterior of the torus, H-1, H-2 and H-4 should be more strongly affected. It is well recognised that upfield shifts are observed in the CYD pro-

* CHEM-X was developed and distributed by Chemical Design Ltd., Oxford, U.K. COSMIC was developed and distributed by Dr A. Vinter, Smith, Kline and French Research Ltd.

tons when hydrophobic interactions between drug and CYD occur (Thakkar and Demarco, 1971; Ueda and Nagai, 1980) with downfield shifts observed for the drug protons.

Methods

Complete assignment of CYD protons was not possible for spectra obtained from the 90 MHz instrument: the 360 MHz spectrometer permitted the required degree of spectral resolution.

The technique employed in these studies (Demarco and Thakkar, 1970; Thakkar and Demarco, 1971) was based on the shielding of the interior protons of the CYD ring as a result of the anisotropy of the guest aromatic moiety (Frank and Cho, 1978). The peak values assigned for β-CYD and salbutamol are in agreement with those of Ueda and Nagai (1980) and Aboul-Enein et al. (1981), respectively. The large peak at 4.8 ppm, due to small amounts of DHO and H_2O as impurities, was considered as an internal standard in the measurement of the chemical shifts of the peaks of β-CYD in the presence and absence of the salbutamol.

Results and Discussion

As expected for inclusion in the presence of salbutamol, the β-CYD spectrum is shifted upfield and the salbutamol spectrum is shifted downfield in the presence of β-CYD. The anomeric hydrogen atoms, H1, appear at the farthest downfield position and are equatorial, whereas the rest of the β-CYD protons are axial (Frank and Cho, 1978).

TABLE 1

Changes in chemical shifts (δ) of β-CYD protons in the presence of increasing concentrations of salbutamol.

Salb./β-CYD ratio (M)	Proton (δ ppm)					
	H_1 (5.080)	H_2 (3.655)	H_3 (3.975)	H_4 (3.595)	H_5 (3.862)	H_6 (3.880)
0.5	0.010	0.015	0.025	0.015	0.037	0.020
1.0	0.030	0.030	0.055	0.035	0.052	0.040
2.0	0.025	0.015	0.035	0.023	0.043	0.025

Change in chemical shift = $\delta_{CYD} - \delta_{Salb./\beta\text{-}CYD}$.

The expected anisotropic shielding of the H5 signal of β-CYD could not be clearly observed in the absence of salbutamol because it overlapped the H6 signal, appearing as a shoulder in the region of 3.85–3.87 ppm. A sharp signal assigned to H5 progressively shifting to higher field became apparent in the presence of increasing amounts of salbutamol. This has also been described by Otagiri et al. (1975) for phenothiazines and Ueda and Nagai (1980) with tolbutamide and chlorpropamide.

The effect of the magnitudes of the chemical shifts induced by salbutamol in the β-CYD proton signals at various molar ratios of salbutamol to β-CYD can be seen in Table 1. It was evident that all the protons experienced a shielding effect, moving to higher fields from their initial position, suggesting that salbutamol may interact both on the exterior surface of the CYD as well as in the interior. It was therefore considered possible that the phenyl moiety of the salbutamol was included

TABLE 2

Changes in chemical shifts (δ) of some salbutamol protons in the presence of increasing concentrations of β-CYD

Salb./β-CYD ratio (M)	Change in chemical shift of proton:						
	Aromatics			$CH\text{-}C\underline{H}_2\text{-}N$		$C\underline{H}\text{-}CH_2\text{-}N$	$(C\underline{H}_3)_3$
(δppm):	(6.697)	(7.131)	(7.210)	(3.08)	(3.110)	(4.605)	(1.240)
0.5	0.064	0.040	0.032	0.02	0.020	0.035	0.035
1.0	0.071	0.043	0.030	0.04	0.045	0.025	0.010
2.0	0.032	0.025	0.025	0.00	0.004	0.020	0.017

Change in chemical shift = $\delta_{Salb./\beta\text{-}CYD} - \delta_{CYD}$.

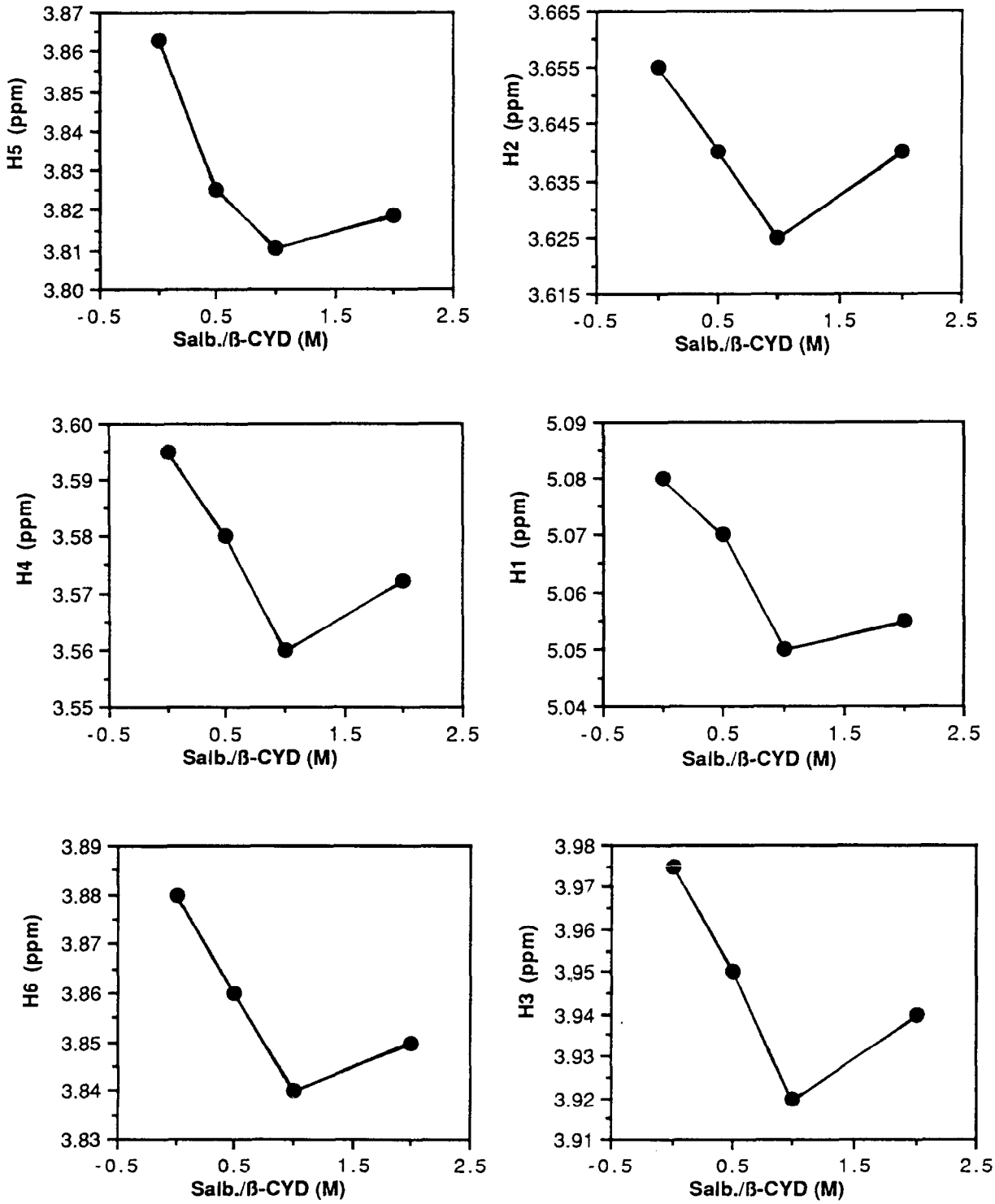

Fig. 3. Variation of H1, H2, H3, H4, H5 and H6 chemical shifts of 1M β-CYD with concentration of salbutamol.

in the cavity of β-CYD, as H3, H5 and H6 (located within the cavity of β-CYD) were affected by anisotropic shielding, although H1, H2 and H4 (located outside the cavity) were also affected (perhaps by interaction with other, non-penetrating salbutamol molecules). Since the magnitudes of $\Delta\delta$ for H5 and H3 are very similar, it can be deduced that the molecule is included very deeply into the cavity.

The shifts of all the signals in β-CYD to higher field suggested that a hydrophobic interaction was predominant between the drug and the β-CYD (Thakkar and Demarco, 1971; Ueda and Nagai, 1980).

Table 2 summarizes the effects of the β-CYD on some of the proton chemical shifts in salbutamol. Unfortunately, some proton signals were too weak to be quantitatively analysed under the present experimental conditions. With increasing amounts of β-CYD all the signals moved downfield, probably due to steric perturbation through inclusion complexation formation (Otagiri et al., 1975; Suzuki and Sasaki, 1979; Ueda and Nagai, 1980; Uekama et al., 1982). Other authors consider that the low field shift might be induced by diamagnetic anisotropy of particular bonds or regions of the host, van der Waals shifts or steric perturbation (Suzuki and Sasaki, 1979).

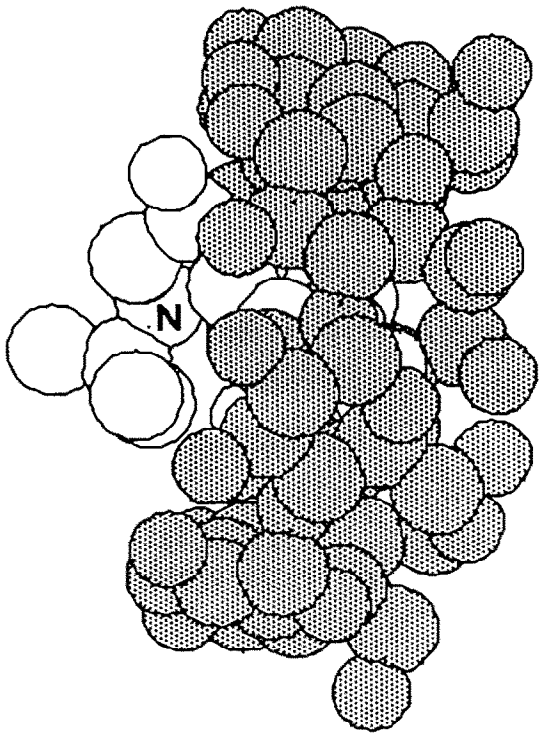

Fig. 5. Relative position of salbutamol/β-CYD at minimum van der Waals energy.

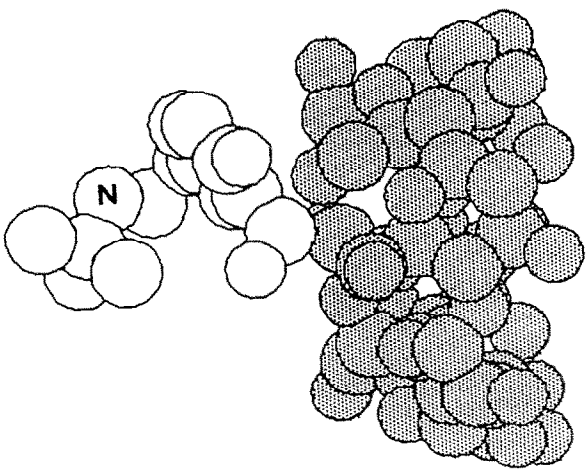

Fig. 4. Relative position of salbutamol/α-CYD at minimum van der Waals energy. Key (in this figure and in Figs 5 and 6): shaded molecule, cyclodextrin; unshaded molecule, salbutamol; larger atoms, carbon; smaller atoms, oxygen; nitrogen of salbutamol labelled separately; hydrogen atoms not shown.

The downfield shifts of the aromatic protons were greater than those of the aliphatic protons, suggesting that the aromatic ring of salbutamol is strongly interacting with the β-CYD. Individual β-CYD proton shifts were plotted against molar ratio (salbutamol:β-CYD) (Fig. 3), where similar relationships were observed for all protons. The highest shifts of the β-CYD protons took place for a molar ratio of 1 : 1 (salbutamol/β-CYD) which indicates that a 1 : 1 complex is formed. This is consistent with the results obtained by the solubility method (Cabral Marques et al., 1990).

Molecular graphics evidence for salbutamol inclusion within β-CYD

The COSMIC molecular graphics system was used to find the minimum energy conformation of salbutamol. The minimum energy positioning of salbutamol relative to α- and β-CYDs was found

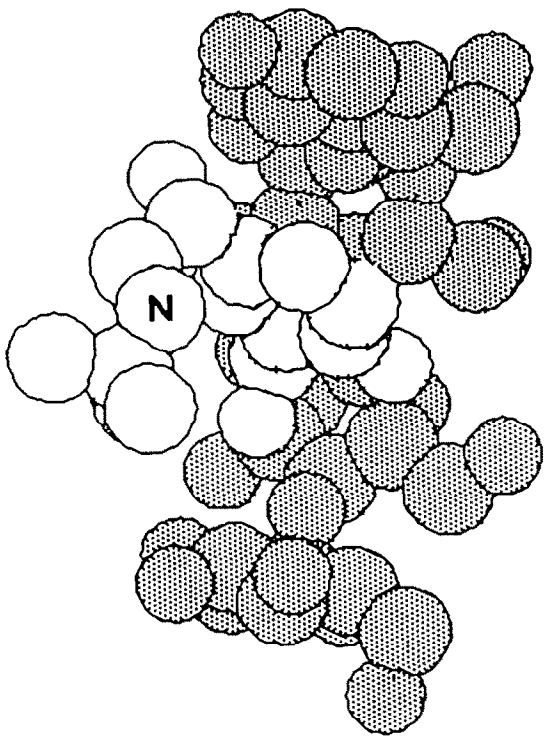

Fig. 6. Relative position of salbutamol/β-CYD at minimum van der Waals energy (section).

using the COSMIC molecular docking routine. The COSMIC files were converted into CHEM-X files for printing Figs 4–6. This showed that salbutamol cannot penetrate the cavity of the α-CYD molecule from either side (aromatic ring or *tert*-butyl grouping) (Fig. 4) which is in agreement with the K_s obtained for this complex (the smallest) in the preceding article (Cabral Marques et al., 1990). However, the aromatic ring of salbutamol may penetrate the cavity of the β-CYD molecule, leaving the amine grouping externalised (Figs 5 and 6). This supports the K_s values obtained for this complex (the largest) and the present ^{1}H-NMR spectroscopy results. These indicate a high degree of interaction between the aromatic protons of salbutamol and the protons lining the β-CYD torus. The relatively small chemical shift changes in the aliphatic protons of salbutamol suggest that this part of the molecule lies outside the torus. The idea of a 1 : 1 salbutamol:β-CYD complex is supported by computer graphics (Fig. 6), which would indicate that only one salbutamol molecule can enter the β-CYD cavity.

Conclusions

According to the theoretical data from the COSMIC molecular graphics program and the experimental data from ^{1}H-NMR we can conclude that β-CYD forms an inclusion complex with salbutamol, where only the salbutamol aromatic ring penetrates the β-CYD cavity and the aliphatic chain rests outside the cavity. From a consideration of these results it may be concluded that the probable stoichiometric ratio of the complex is 1 : 1 (salbutamol : β-CYD).

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