Molecular Modeling (MM2 and PM3) and Experimental (NMR and Thermal Analysis) Studies on the Inclusion Complex of Salbutamol and β-Cyclodextrin

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The inclusion complex of salbutamol and β-cyclodextrin (β-CD) is studied by computational (MM2 and PM3) and experimental techniques. Molecular modeling calculations predict two different orientations of salbutamol in the β-CD cavity in vacuo and in aqueous solution. In vacuo calculations show that the introduction of the aromatic ring of salbutamol is preferred to the introduction of the *tert*-butyl group into the β-CD cavity. However, in aqueous solution both computational methods predict the introduction of the alkyl chain instead of the aromatic ring in the β-CD cavity contrary to experimental results published previously. These quantitative predictions were experimentally confirmed here by studying the inclusion complex in solution by NMR. A 1:1 stoichiometry was found by ^{1}H NMR studies for this complex. A 2D ROESY (rotating-frame Overhauser enhancement spectroscopy) experiment shows that there are no cross-peaks between the aromatic protons of salbutamol and any of the protons of β-CD. Cross-peaks for the protons of the *tert*-butyl group and protons inside the cavity of β-CD demonstrate the full involvement of this group in the complexation process and confirm the orientation of the complex predicted by molecular modeling. The solid-state complex was prepared and its stoichiometry (β-CD·$C_{13}H_{21}NO_3$·$8H_2O$) and dissociation process studied by thermogravimetric analysis.

Introduction

The study of inclusion complexes of organic molecules with cyclodextrins has attracted the interest of both experimental and theoretical chemists during the past decades.[1,2] These macromolecules are torus-shaped cyclic oligosaccharides commonly consisting of six to eight α-D-glucopyranose units bound by α(1−4) linkages that are named α-, β-, and γ-cyclodextrins.[3] In particular, β-cyclodextrin (β-CD) has an internal cavity shaped like a truncated cone of about 8 Å deep and 6.0−6.4 Å in diameter. This cavity possesses a relatively low polarity that can accommodate guest organic molecules inside.[4] By this means, the physical properties, such as solubility, stability, volatility, sublimation, etc., of the guest molecules are modified, and the resulting inclusion complexes have found numerous practical applications in pharmaceutical sciences and in different fields of chemistry ranging from analytical to synthetic chemistry.[5,6]

The studies of cyclodextrin inclusion complexes are mainly addressed to clarify the following aspects: to prove that the inclusion takes place, to determine the stoichiometry of the complex, and to determine the three-dimensional structure of the supermolecule.[7−12] The thermodynamic and kinetic aspects of the complex formation are also of interest in such kinds of studies.[13−15] These studies have been carried out by different experimental techniques such as X-ray diffraction, NMR spectroscopy, thermal analysis, electrochemical methods, and so forth.

The use of molecular modeling techniques for the study of cyclodextrins was somewhat limited until recently due to the size and flexibility of such molecules, which represent a real challenge for computational methods.[16] On the other hand, the fact that these molecules are frequently studied experimentally in aqueous media imposes some restrictions on the use of theoretical approaches to the study of cyclodextrin inclusion complexes. However, in recent years the number of studies reporting the use of quantum mechanics and molecular mechanics calculations for investigating the 3D structure

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(1) Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743.
(2) Uekama, K.; Hirama, F.; Irie, T. *Chem. Rev.* **1998**, *98*, 2045.
(3) Bender, N. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer: New York, 1978.
(4) Vögtle, F. *Supramolecular Chemistry*: *An Introduction*; John Wiley and Sons, Ltd: New York, 1991; p 135.
(5) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344.
(6) Szejtli, J. *Cyclodextrins Technology*; Kluwer Academic Publisher: Dordrecht, The Netherlands, 1988; Chapters 2 and 3.
(7) Ivanov, P. M.; Salvatierra, D.; Jaime, C. *J. Org. Chem.* **1996**, *61*, 7012.
(8) Salvatierra, D.; Jaime, C.; Virgili, A.; Sánchez-Ferrando, F. *J. Org. Chem.* **1996**, *61*, 9578.
(9) Fronza, G.; Mele, A.; Redenti, E.; Ventura, P. *J. Org. Chem.* **1996**, *61*, 909.
(10) Entrena, A.; Jaime, C. *J. Org. Chem.* **1997**, *62*, 5923.
(11) Ishizu, T.; Kintsu, K.; Yamamoto, H. *J. Phys. Chem. B* **1999**, *103*, 8992.
(12) Hada, S.; Ishikawa, S.; Neya, S.; Funasaki, N. *J. Phys. Chem. B* **1999**, *103*, 2579.
(13) Yim, C. T.; Zhu, X. X.; Brown, G. R. *J. Phys. Chem. B* **1999**, *103*, 597.
(14) Madrid, J. M.; Mendicuti, F.; Mattice, W. L. *J. Phys. Chem. B* **1998**, *102*, 2037.
(15) Gosh, M.; Zhang, R.; Lawler, R. G.; Seto, C. T. *J. Org. Chem.* **2000**, *65*, 735.
(16) Lipkowitz, K. B. *Chem. Rev.* **1998**, *98*, 1829.

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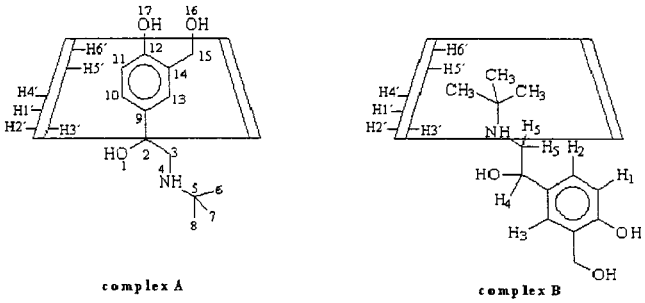

Figure 1. The two possible orientations of the salbutamol in the β-CD cavity and numbering of atoms used in the present work.

and properties of such complexes has increased dramatically.[16] Accordingly, the combination of experimental and computational studies has been recognized as a powerful tool for the study of the geometry of complexation.[17−19]

In the current work we study the inclusion complex of salbutamol and β-CD. Salbutamol (albuterol) is a β_2-selective adrenoceptor agonist with pronounced bronchodilatory, cardiac, uterine, and metabolic effects.[20] Previous studies reported the characterization of the stoichiometry and some insights on the 3D geometry of this inclusion complex by using phase solubility, differential scanning calorimetry, and ^{1}H NMR.[21,22] However, a detailed description of the geometry of this complex in solution remains uninvestigated. There are no unequivocal conclusions in the literature on the mode of inclusion and the conformation of the drug inside the host cavity. Here we make an intensive investigation of the 3D geometry of this complex in vacuo and in the presence of water molecules by using molecular mechanics and semiempirical quantum chemical calculations. We find that both MM2 and PM3 calculations predict the same mode of inclusion of the drug molecule in the cyclodextrin. This inclusion mode, computed in the presence of water molecules, differs from that previously reported in the literature. Our theoretical findings are unequivocally confirmed by using 2D-ROESY (rotating-frame Overhauser enhancement spectroscopy) experiments.

Results and Discussion

The following nomenclature will be used in order to interpret the computational and experimental results obtained in the present work. The two different orientations in which salbutamol can be introduced into β-CD, i.e., by introducing the aromatic ring or the *tert*-butyl group, will be referred to as complex A or complex B, respectively (Figure 1). Inner protons in β-CD are named H3′, H5′, and H6′ while outer protons are H1′, H2′, and H4′. Aromatic protons in salbutamol are named H_1, H_2, and H_3, and the protons in the alkyl chain are designed as H_4, H_5, and CH_3 as shown in Figure 1.

Molecular Modeling Studies. The geometry of β-CD was obtained from its crystallographic structure as determined by X-ray diffraction and deposited in the Cambridge databank. The structure of the salbutamol was built by using Hyperchem,[23] and its geometry was then minimized to a maximum energy gradient of 0.1 kcal/(Å mol) with the MM2 force field by using the Polak−Ribiere (conjugate gradient) minimizer. This structure was used as input for semiempirical SCF-MO calculations using the PM3 Hamiltonian.[25] The geometry optimization was carried out by using the Polak−Ribiere algorithm to a maximum energy gradient of 0.05 kcal/(Å mol). This geometry was always used in all calculations of host/guest complexes.

The first calculations of the host/guest complexes were conducted to determine which of the two orientations of salbutamol (complex A or B in Figure 1) is preferred in the complex with β-CD in absence of solvent, i.e., in vacuo. These two orientations were built by docking the structure of salbutamol into the cavity of β-CD and then minimizing the energy of the complex with molecular mechanics. The molecular mechanics MM2 calculations[24] show that the complex A is preferred by 6.89 kcal/mol with respect to complex B. This means that the preferred orientation of the complex is that in which the aromatic ring of salbutamol is introduced inside the CD cavity leaving the alkyl chain in an external position. Similar results were reported by Cabral Marques et al. by using the COSMIC molecular docking routine and supported by NMR experimental observations.[21b]

The experimental results obtained by Cabral Marques et al. as well as those that will be obtained here are considering the salbutamol β-CD complex in aqueous solution. Consequently, we decided to carry out computational studies of this complex by explicitly considering the water molecules in the calculations. We included the salbutamol β-CD complex into a box of 40 molecules of water (for details see Experimental Section). Full-geometry optimizations of the host/guest complexes into the water boxes were then carried out by using the MM2 force field. The TIP3P water potential, equilibrated at 300 K and 1 atm, was used in these calculations.

In this case, MM2 calculations show that the preferred orientation of the complex is that in which the *tert*-butyl group of salbutamol is inside the cavity of the β-CD. Accordingly, complex B is favored by 3.01 kcal/mol with respect to complex A. This different orientation of the salbutamol molecule in the cavity of the β-CD with respect to the in vacuo results pointed out the necessity of conducting more precise calculations for the study of this complex. Consequently, we selected the semiempirical quantum chemical method PM3.[25] The application of these semiempirical calculations to the system under study represents a real computational challenge. In the presence of 40 molecules of water, this system is composed of 713 orbitals, 436 double-occupied levels, and 872 electrons. In fact, in the review paper of Lipkowitz on computational studies of cyclodextrins, only 20 papers reporting semiempirical calculations were compiled.[16] However, it is necessary here to use a more precise

(17) Parrot-López, H.; Galons, H.; Coleman, A. W.; Djedaïni, F.; Keller, N.; Perly, B. *Tetrahedron: Asymmetry* **1990**, *1*, 367.
(18) Amato, M. E.; Lombardo, G. M.; Pappalardo, G. C.; Scarlatta, G. *J. Mol. Struct.* **1995**, *350*, 71.
(19) Ivanov, P. M.; Jaime, C. *J. Mol. Struct.* **1996**, *377*, 137.
(20) Hoffman, B. B.; Lefkowitz, R. J. In *Godman & Gilman's The Pharmacological Basis of Therapeutics*; Hardman, J. G., Limbird, L. E., Eds.; McGraw-Hill: New York, 1996; p 214.
(21) (a) Cabral Marques, H. M.; Hadgraft, J.; Kellaway, I. W. *Int. J. Pharm.* **1990**, *63*, 259. (b) Cabral Marques, H. M.; Hadgraft, J.; Kellaway, I.; Pugh, W. J. *Int. J. Pharm.* **1990**, *63*, 267.
(22) Lemesle-Lamache, V.; Wouessidjewe, D.; Chéron, M.; Duchêne, D. *Int. J. Pharm.* **1996**, *141*, 117.
(23) *Hyperchem 3.0 for Windows*; Hypercube, Inc., 1993.
(24) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127.
(25) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *209*, 221.

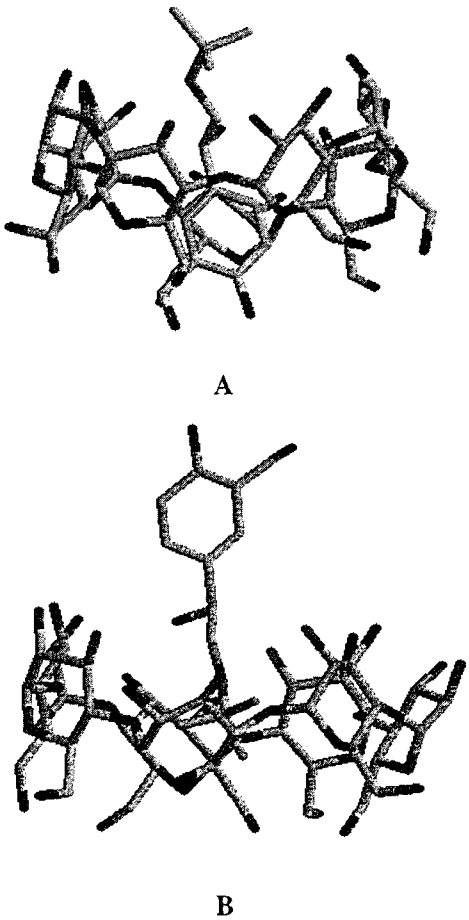

Figure 2. Energy-minimized (PM3) structures of salbutamol β-CD complex in the two different orientations studied in vacuo: (A) orientation A; (B) orientation B.

approximation for understanding the nature of the differences in the orientation of salbutamol in β-CD in aqueous solution.

The PM3 calculations of the two different orientations in vacuo show a coincidence with the results of MM2 giving a preference of only 1.59 kcal/mol to complex A with respect to complex B. The molecular graphics of both inclusion complexes in vacuo calculated by the PM3 method are illustrated in Figure 2. The dipole moment of the complex in orientation A is 7.74 D while that in orientation B is only 5.43 D, which is comparable to that of β-CD (5.96 D).

This semiempirical method also shows coincidence with MM2 in identifying the preferred orientation of the complex in the presence of water molecules. In this case, the energy difference between both orientations is 17.15 kcal/mol, showing a significant preference for complex B instead of complex A. The dipole moments of orientations A and B in the presence of water are raised to 11.13 and 7.77 D, respectively. The computational results concerning the orientation of the salbutamol β-CD complex in aqueous solution do not coincide with the experimental findings of Cabral Marques et al. for the orientation of this complex studied by one-dimensional NMR spectroscopy. We will return to this problem in a further section where a detailed NMR study of this complex is carried out to determine its preferred orientation.

An inspection of the results of both MM2 and PM3 calculations for the complex permits us to identify some structural features that help in understanding the energetic preference of orientation B with respect to orientation A in aqueous solution. In the orientation in which the aromatic ring is introduced into the β-CD cavity, the water molecules are distributed in a slightly asymmetric way with respect to the plane bisecting the glucose rings of the cyclodextrin. That is, 60% of the water molecules are situated on the narrower side of β-CD while 40% are on the wider side. The salbutamol molecule is introduced from the wider side of β-CD, leaving both hydroxyl groups of the aromatic ring outside the narrower side of the cavity. Thus, water molecules are distributed so as to avoid interaction with the hydrophobic *tert*-butyl group, which is on the wider side. A total of 60% of the water molecules are situated on the narrower side interacting with the hydroxyl groups bonded to the phenyl ring.

This asymmetry in the distribution of water molecules is dramatically increased when orientation B of the complex is analyzed. In this case, when the *tert*-butyl group is introduced into the β-CD cavity, almost three-quarters (72.5%) of the water molecules are situated on the wider side of the cyclodextrin where the hydroxyl groups of the guest's phenyl ring are placed. This way, the water molecules avoid the interaction with the hydrophobic *tert*-butyl groups that are situated inside the cavity of β-CD, which is translated into enhanced complex stability. Accordingly, β-CD acts as a cage in which the hydrophobic part of salbutamol is "hidden" leaving the hydrophilic part of the molecule free to interact with the solvent giving a greater stability to the complex. Illustrations of the molecular graphics of both orientations of salbutamol in the cavity of β-CD in aqueous solution are given in Figure 3.

After the complexation with β-CD, most of the atomic charges on the salbutamol atoms are changed as can be seen in Table 1. The carbon atom charges on the aromatic ring, which is inside the cyclodextrin cavity, become more negative in the complex A compared to the free salbutamol molecule. This is especially true for atoms 11, 13, and 14 (see Figure 1). Significant alteration of the atomic charge values is also observed for the oxygen atoms of the hydroxyl groups bonded to the aromatic ring in complex A. The charges for these atoms in complex B are almost unaltered with respect to the free salbutamol. A similar landscape is observed for both complexes in aqueous solution. As expected, the main charge variation in complex B is observed for atoms of the *tert*-butyl group. However, these atom charges (6, 7, and 8) also suffer some alteration in complex A probably due to their interaction with the external groups of β-CD. A great variation is also observed in the charge of the nitrogen atom (4) in all complexes indicating the interaction of the amino group with the cyclodextrin.

The atomic charges on hydrogen atoms are given in Table 2. The charges of aromatic protons do not suffer significant variations in complexes A and B with respect to the free salbutamol molecule. However, a small increase of the positive charge is observed for both complexes, especially for protons H_2 and H_3 in complex A where the aromatic ring is introduced into the cavity of β-CD. In complex B, the protons of the CH and CH_2 groups (H_4 and H_5) of the alkyl chain suffer a significant increment of the positive charge due probably to their interaction with the protons of the external part of β-CD. Increments in the charges of these protons are also observed for complex A indicating that when the aromatic

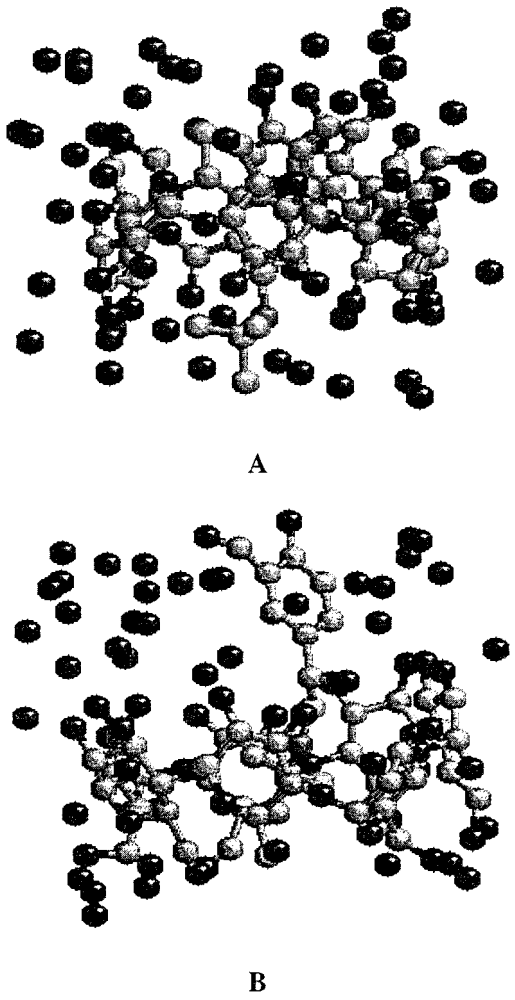

Figure 3. Energy-minimized (PM3) structures of salbutamol β-CD complex in the two different orientations studied in aqueous solution: (A) orientation A; (B) orientation B.

ring penetrates the β-CD cavity these protons are interacting with the groups in the entrance of the cyclodextrin cavity. These findings will be useful in order to understand some of the results discussed in the following section devoted to NMR experiments.

NMR Studies. Seven samples with different salbutamol/β-CD ratios ranging from 0.25 to 5 were prepared and their ^{1}H NMR spectra were recorded according to the procedures described in the Experimental Section. Each sample spectrum shows induced chemical shifts for the protons of β-CD (Figure 4) and salbutamol, which denoted the formation of the inclusion complex in solution. To determine the stoichiometry of the complex in solution, we plot the Job's diagrams[26] based on the induced chemical shifts of selected protons in salbutamol and β-CD (Figure 5). The induced chemical shift of proton H_5 and the protons of the *tert*-butyl group, $H(CH_3)$, of salbutamol and that corresponding to proton H5′ of β-CD are used to plot these diagrams. The Job's diagram for the protons of salbutamol shows a maximum at guest/(host + guest) ratio (r) of 0.5 indicating that there is one molecule of salbutamol for each molecule of β-CD. The corresponding diagram based on the induced chemical shift of protons inside the cavity of β-CD shows $r = 0.5$ confirming the 1:1 stoichiometry of the complex. Therefore the most probable salbutamol/β-CD ratio is about 1:1.

Table 1. Atomic Charges Calculated by PM3 Method for Salbutamol in the Different Inclusion Complexes Studied[a]

atom[b]	salbutamol	complex A	complex B	complex A (H_2O)	complex B (H_2O)
1	−0.315	−0.323	−0.339	−0.339	−0.371
2	0.138	0.131	0.139	0.140	0.139
3	−0.108	−0.115	−0.122	−0.118	−0.126
4	−0.067	−0.081	−0.075	−0.071	−0.082
5	−0.033	−0.029	−0.024	−0.024	−0.026
6	−0.115	−0.119	−0.131	−0.125	−0.130
7	−0.117	−0.132	−0.136	−0.147	−0.143
8	−0.144	−0.152	−0.178	−0.169	−0.164
9	−0.187	−0.196	−0.191	−0.196	−0.189
10	−0.040	−0.044	−0.032	−0.049	−0.039
11	−0.156	−0.171	−0.153	−0.168	−0.193
12	0.143	0.156	0.150	0.151	0.149
13	−0.032	−0.044	−0.034	−0.047	−0.045
14	−0.196	−0.244	−0.245	−0.201	−0.230
15	0.129	0.108	0.125	0.101	0.115
16	−0.316	−0.322	−0.319	−0.337	−0.316
17	−0.231	−0.252	−0.226	−0.266	−0.230

[a] Charges for free salbutamol molecule are given for comparison. [b] See Figure 1 for numbering of atoms in salbutamol.

Table 2. Atomic Charges Calculated by PM3 Method for Hydrogen Atoms in Salbutamol in the Different Inclusion Complexes Studied

atom[a]	salbutamol	complex A	complex B	complex A (H_2O)	complex B (H_2O)
H_1	0.115	0.119	0.119	0.116	0.126
H_2	0.124	0.130	0.129	0.123	0.133
H_3	0.107	0.117	0.104	0.125	0.125
H_4	0.057	0.063	0.066	0.066	0.063
H_5	0.059	0.064	0.067	0.061	0.069
$(CH_3)_3$	0.046	0.0503	0.054	0.055	0.054

[a] See Figure 1 for numbering of atoms in salbutamol.

It is observed that the protons of the β-CD in the complex are shifted upfield and those of salbutamol are shifted downfield as similarly reported by Cabral Marques et al. The induced chemical shifts ($\Delta\delta$) observed in the current work, which are significantly smaller than those reported by these authors in ref 21, are given in Table 3. It is clear that these small induced chemical shifts offer poor information about the orientation of salbutamol in the cavity of β-CD. To clarify this important point, we will study the 2D NMR spectra for this complex.

Among the several techniques that exist to predict the geometry of β-CD inclusion complexes, we select the 2D rotating frame ^{1}H−^{1}H nuclear Overhauser effect (2D ROESY) because of its well-known reliable results in these kinds of studies.[27] The observation of the 2D ROESY spectrum given in Figure 6 shows a complete lack of cross-peaks for the aromatic protons of the salbutamol with any proton of β-CD. This observation confirms our prediction that the aromatic ring of salbutamol is not involved in the complexation with cyclodextrin. More interesting is the observation of a cross-peak of the protons of the *tert*-butyl group with protons H5′ and H6′ of cyclodextrin. This indicates that the *tert*-butyl group is fully involved in the formation of the inclusion complex with β-CD as predicted by molecular modeling and that orientation B is the preferred one in

(26) Job, P. *Ann. Chim.* **1928**, *9*, 113.

(27) Schneider, H.-J.; Hacket, F.; Rüdiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 1755.

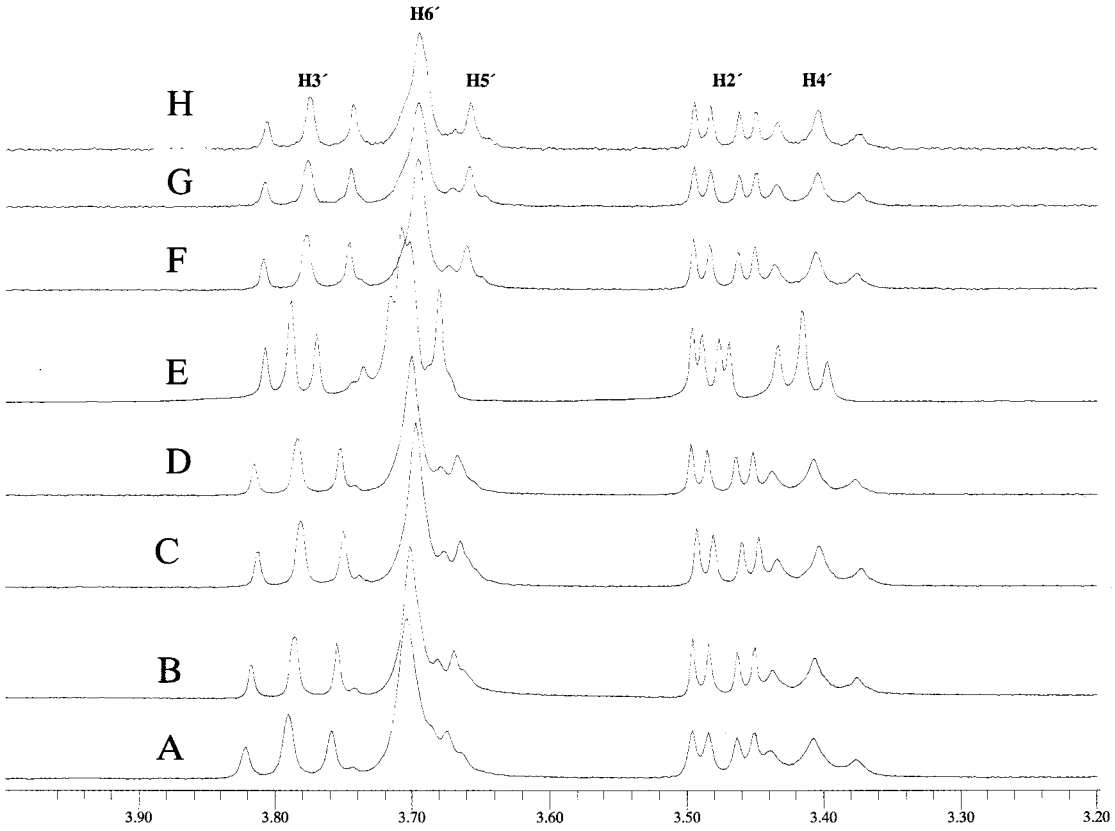

Figure 4. Partial 500 MHz ^{1}H NMR spectra of host protons for solutions of salbutamol/β-CD complexes: (A) β-CD; [salbutamol]/[β-CD] ratios, (B) $^1/_4$, (C) $^1/_3$, (D) $^1/_2$, (E) $^1/_1$, (F) $^1/_{0.7}$, (G) $^1/_{0.5}$, (H) $^1/_{0.2}$.

aqueous solution. According to PM3 calculations, the atomic charges of the protons of the CH and CH_2 (H_4 and H_5) groups of the alkyl chain suffer a significant alteration that we interpreted as being due to the possible interaction with the external side of the β-CD cavity. Protons of the CH_2 group give rise to intense cross-peaks with protons H2′ and H4′ of the β-CD confirming the interaction of this part of salbutamol with the external groups of cyclodextrin. This interaction is not observed in the ROESY spectra for protons of the CH group, which indicates that the introduction of salbutamol into the cavity is not deep enough for this group to interact with the external part of the cavity. The *tert*-butyl group is introduced only to the middle of the cavity, giving rise to a cross-peak with the protons H5′ and H6′ of the cyclodextrin as previously observed. This geometric picture of the salbutamol β-CD inclusion complex coincides with that predicted by MM2 and PM3 calculations in which the *tert*-butyl group is introduced from the wider side to the middle part of the cavity leaving the aromatic ring far from the cyclodextrin interior.

Thermal Analysis. The main objectives of this part of the study are to investigate the presence or lack of water molecules in the solid-state complex as well as to gain insights about the possible mechanism of dissociation of such a complex. With these objectives, we selected thermogravimetric (TG) analysis[28] to study the solid-state complex of salbutamol and β-CD. The solid-state inclusion complex of salbutamol and β-CD was prepared as described in the Experimental Section. The thermogravimetric (TG) curve of this complex is given in Figure 7. It is observed that the complex has a 10.9% mass loss between 55 and 102 °C followed by a 17.45% mass loss between 205 and 260 °C and a large mass loss over 280 °C. The first stage of this process corresponds to the dehydration of the complex, and the analysis of the TG permits us to calculate the number of water molecules in the complex. According to this estimation, the stoichiometry of the salbutamol β-CD inclusion complex in the solid state is as follows: β-CD·$C_{13}H_{21}NO_3$·$8H_2O$.

The second endothermic peak corresponds to the evaporation of the salbutamol molecule from the β-CD cavity, corresponding to the breaking of the weak intermolecular forces that are acting between salbutamol and β-CD in the complex. The final endothermic peak over 280 °C is consistent with the decomposition of β-CD. Taking into account the information provided by the thermal analysis, the dissociation of β-CD·$C_{13}H_{21}NO_3$·$8H_2O$ takes place by the following three-step process:

$$\beta\text{-CD}\cdot C_{13}H_{21}NO_3\cdot 8H_2O(s) \rightarrow \beta\text{-CD}\cdot C_{13}H_{21}NO_3(s) + 8H_2O(g)$$

$$\beta\text{-CD}\cdot C_{13}H_{21}NO_3(s) \rightarrow \beta\text{-CD}(s) + C_{13}H_{21}NO_3(g)$$

$$\beta\text{-CD}(s) \rightarrow \text{decomposition}$$

Conclusions

The inclusion complex of salbutamol and β-CD has been studied by computational and experimental techniques. Molecular modeling calculations at two different

(28) (a) Li, X.-T.; Li, J.-H.; Zhang, G.-E.; Xi, G.-X.; Lou, X.-D. *Thermochim. Acta* **1995**, *262*, 165. (b) Li, J.-H.; Zhang, N.; Li, X.-T.; Wang, J.-Y. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1997**, *28*, 95. (c) Yu, S.-Z.; Li, X.-T.; Li, J.-H.; Wang, J.-Y.; Tiang, S.-J. *J. Therm. Anal.* **1997**, *49*, 1517.

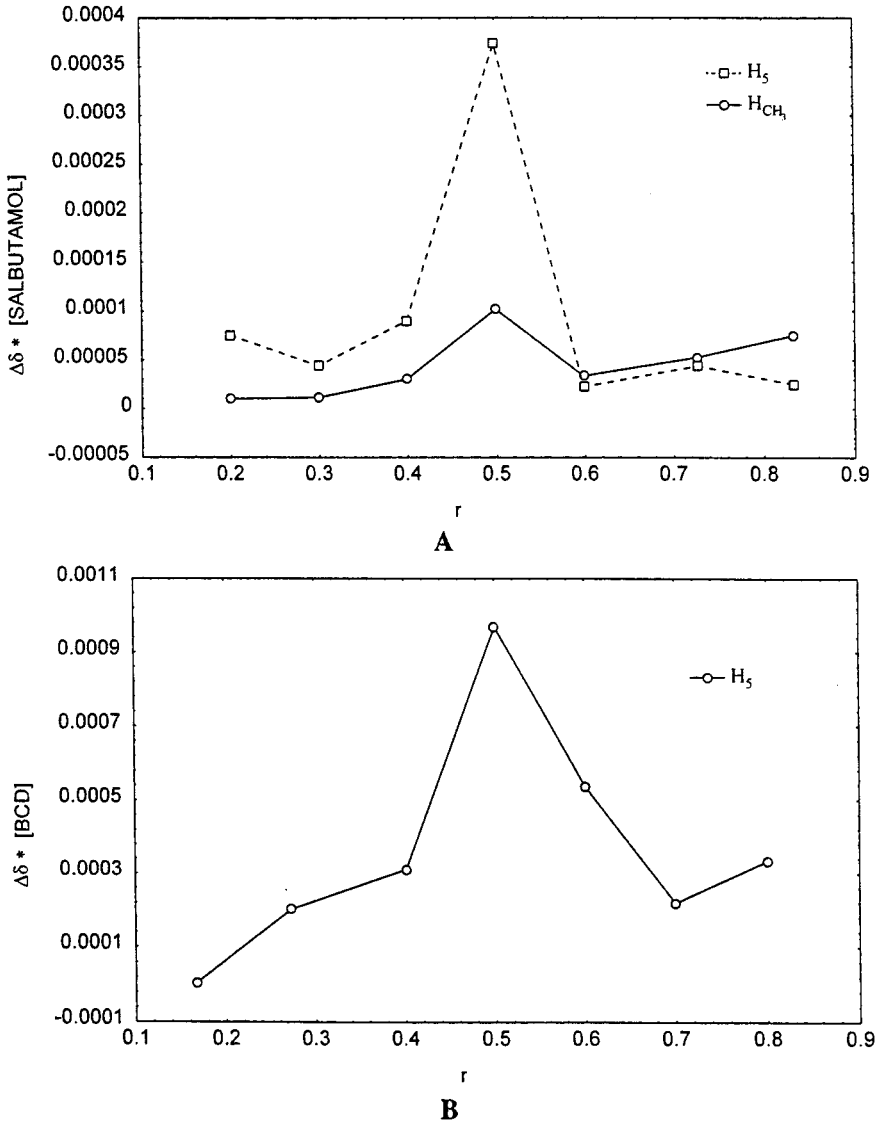

Figure 5. Job's diagram for the salbutamol/β-CD complex: (A) plot of data corresponding to protons H_5 and $H(CH_3)$ of salbutamol; (B) plot of data corresponding to H5′ of β-CD. The values on the *x*-axis correspond to guest/(host + guest) ratio (*r*).

Table 3. Chemical Shifts of Protons in β-CD and Salbutamol Free and in the Inclusion Complex

β-CD			salbutamol		
proton	free	complex	proton	free	complex
H1′	4.893	4.891	H_1	7.172	7.179
H2′	3.475	3.462	H_2	7.106	7.114
H3′	3.786	3.780	H_3	6.775	6.782
H4′	3.422	3.413	H_4	4.734	4.686
H5′	3.675	3.649	H_5	3.066	3.068
H6′	3.702	3.697	$H(CH_3)$	1.208	1.221

levels of approximation (MM2 and PM3) predict a different orientation of salbutamol in the β-CD cavity in vacuo vs in an aqueous solution. In the absence of solvent, both methods predict that the introduction of the aromatic ring of salbutamol (complex A) is preferred to the introduction of the *tert*-butyl group (complex B) into the β-CD cavity. However, in aqueous solution, MM2 and PM3 quantitatively predict complex B as more stable than complex A. The energy difference between both orientations of salbutamol in the cavity is especially significant from the PM3 results. These quantitative predictions were confirmed experimentally by studying this inclusion complex in solution. The ^1H NMR allowed us to deduce a 1:1 stoichiometry for this inclusion complex. A 2D ROESY experiment confirmed the orientation of the complex predicted by molecular modeling by showing that there are no cross-peaks between the aromatic protons of salbutamol and any of the protons of β-CD. Cross-peaks were observed, however, for the protons of the *tert*-butyl group and for protons inside the cavity of β-CD, which demonstrate the full involvement of this group in the complexation process. Finally, the stoichiometry (β-CD·$C_{13}H_{21}NO_3$·$8H_2O$) and the dissociation process of the complex in the solid state were studied by using thermogravimetric analysis.

The most important conclusion of the current work is addressed to the use of molecular modeling techniques in predicting the orientation geometry of β-CD inclusion complexes. Taking into account that most of these complexes are studied by NMR techniques in solution, the importance of explicitly or implicitly considering the solvent in these calculations is obvious. This is not, however, the first time that this observation has been carried out. Amato et al.[29] has studied several inclusion

(29) Amato, M. E.; Lipkowitz, K. B.; Lombardo, G. M.; Pappalardo, G. C. *J. Chem. Soc., Perkin Trans. 2* **1996**, 321.

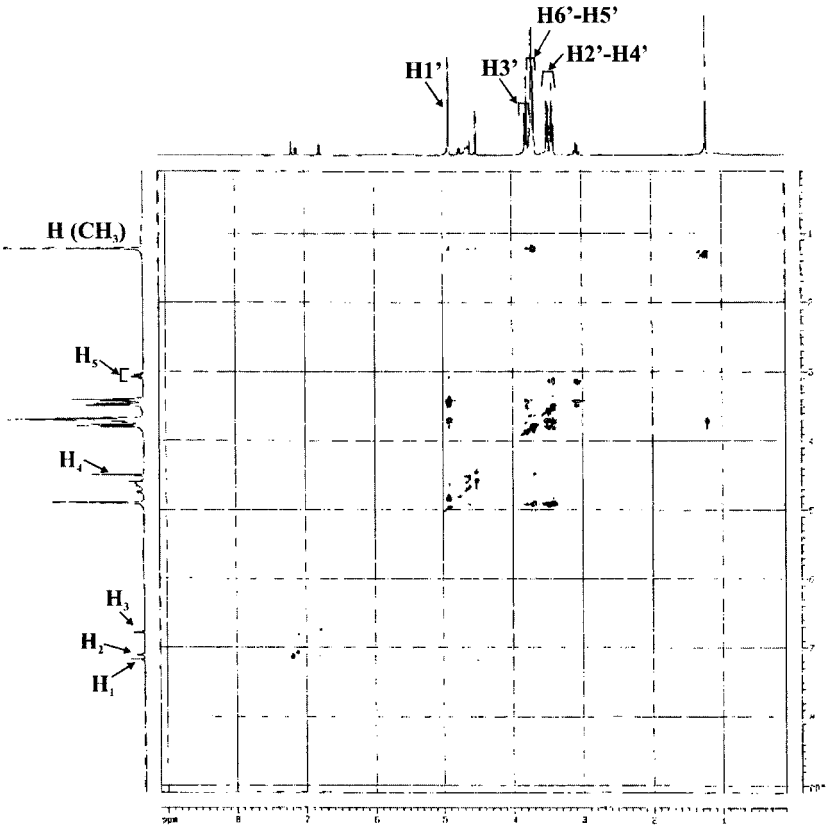

Figure 6. NMR spectrum corresponding to the 2D ROESY experiment carried out on the salbutamol/β-CD complex in solution.

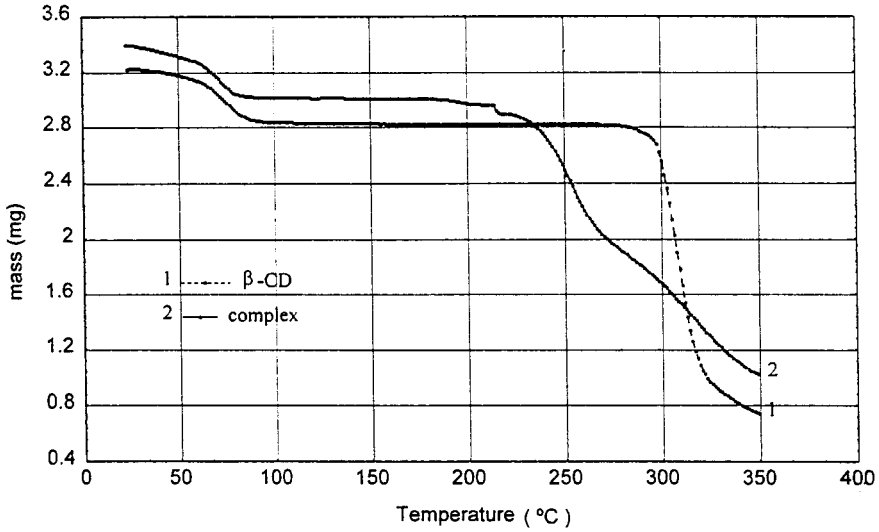

Figure 7. Thermogravimetric curves of β-CD (1) and salbutamol/β-CD complex in solid state (β-CD·$C_{13}H_{21}NO_3$·$8H_2O$) (2).

complexes by NMR and molecular dynamics by concluding that calculations performed for molecules in vacuo gave uncertain results that evidenced failure of this model to reproduce the experimental results. The explicit inclusion of water molecules in the model overcomes this failure. At present, several treatments of solvent in computational techniques are available at different levels of theory, and the use of them in studying β-CD inclusion complexes in solution is strongly recommended.

Experimental Section

Molecular modeling was carried out on an IBM Pentium III 700 MHz personal computer. The MM2 force field[24] and the semiempirical PM3 method[25] implemented in Hyperchem software[23] were used for molecular modeling calculations. The geometry parameters of β-CD were taken from X-ray diffraction as deposited in the Cambridge Crystallographic Databank. The calculations were carried out by docking the optimized structure of salbutamol into the β-CD cavity and allowing for full geometry optimization at the corresponding level of calculation. The consideration of aqueous solvent in the calculations was done by considering a box of water molecules with the following dimensions: $x = 15.0$ Å, $y = 14.0$ Å, and $z = 16.0$ Å. The minimum distance between solvent and solute atoms was fixed at 2.3 Å. Molecular graphics shown in this work were built by RasWin Molecular Graphics software.

β-CD was generously donated by Roquette-Laisa, Spain, and salbutamol and D_2O were purchased from INFA, no. 187103, Italy and Aldrich, respectively.

A solution of salbutamol in D_2O was prepared by dissolving

2.72 mg of salbutamol in 1 mL of D_2O. A solution of β-CD was prepared by dissolving 13.0 mg of β-CD in 1 mL of D_2O. Fixed volumes of salbutamol and β-CD were mixed to give predetermined final volume of 1 mL. The relative salbutamol/β-CD proportions were confirmed from direct integration of their NMR spectra. The solid-state complex was prepared by kneading. Salbutamol and β-CD at a 1:1 proportion were mixed in solid state, and an equivalent amount of water was added to make a dense paste. The resulting mixture was dried at 40 °C and then milled and sieved.

NMR spectra were recorded in a 500 MHz spectrometer. The experiments were carried out in unbuffered D_2O solution. ROESY experiments were performed for the solution of molar ratio 1:1 using a mixing time of 227 ms and a spectral width of 4504.5 Hz.

Thermogravimetric analysis was carried out under the following experimental conditions: sample mass, around 3.4 mg; atmosphere, air; sample holder, aluminum capsule; temperature range, 30−350 °C; heating speed, 5 °C/min.

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