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Stereochemistry and dynamics of the inclusion complex of (*S*)-(+)-fenoprofen with cyclomaltoheptaose (β-cyclodextrin)

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ABSTRACT

The inclusion complex of the calcium salt of (*S*)-2-(3-phenoxyphenyl)propionic acid with cyclomaltoheptaose (β-cyclodextrin) was investigated by ^{1}H NMR spectroscopy. The stoichiometry of the complex was determined by the continous variation method and the association constant by the Benesi–Hildebrand procedure. Measurements of proton selective relaxation rates allowed the dynamics of the complex to be investigated and the determination of intermolecular ^{1}H{^{1}H}-NOE enhancements revealed the stereochemistry in solution.

INTRODUCTION

Cyclomalto-oligosaccharides (cyclodextrins, CDs) each possesses a toroidal "cup-shaped" cavity which can accommodate hydrophobic guests in aqueous solution[1]. These inclusion complexes are of interest as models for the study of enzyme catalysis[2] and for use in chromatography for separating structural isomers, diastereomers, and enantiomers[3]. CDs are used extensively to increase the bioavailability and solubility of drugs[1].

An understanding of the relationship between the structure of the guest and its chemical and biological behaviour in the inclusion complex requires a detailed knowledge of the dynamics and host–guest orientation. In this context, the interaction of a guest molecule with a CD is reflected in changes of various NMR parameters which provide information on these aspects.

We now report an NMR investigation of an aqueous solution of the inclusion complex formed by cyclomaltoheptaose (βCD, **1**) and the calcium salt of the non-steroidal anti-inflammatory drug (*S*)-(+)-2-(3-phenoxyphenyl)propionic acid[4,5]

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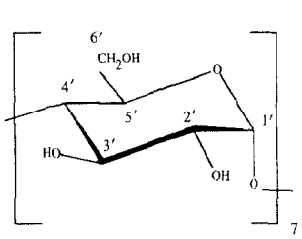
(**2**). The stoichiometry, association constant, and the geometry of the complex in solution have been determined.



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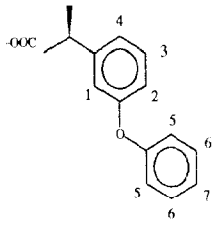

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RESULTS AND DISCUSSION

^{1}H NMR Chemical shift data.—The ^{1}H NMR spectra (D_2O, 25°C, 8 mM, 300 MHz) of mixtures of βCD and **2** (Fig. 1) contained only one set of resonances for each proton or group of equivalent protons, indicating that the reversible exchange between free and complexed ligand is fast on the NMR time-scale.

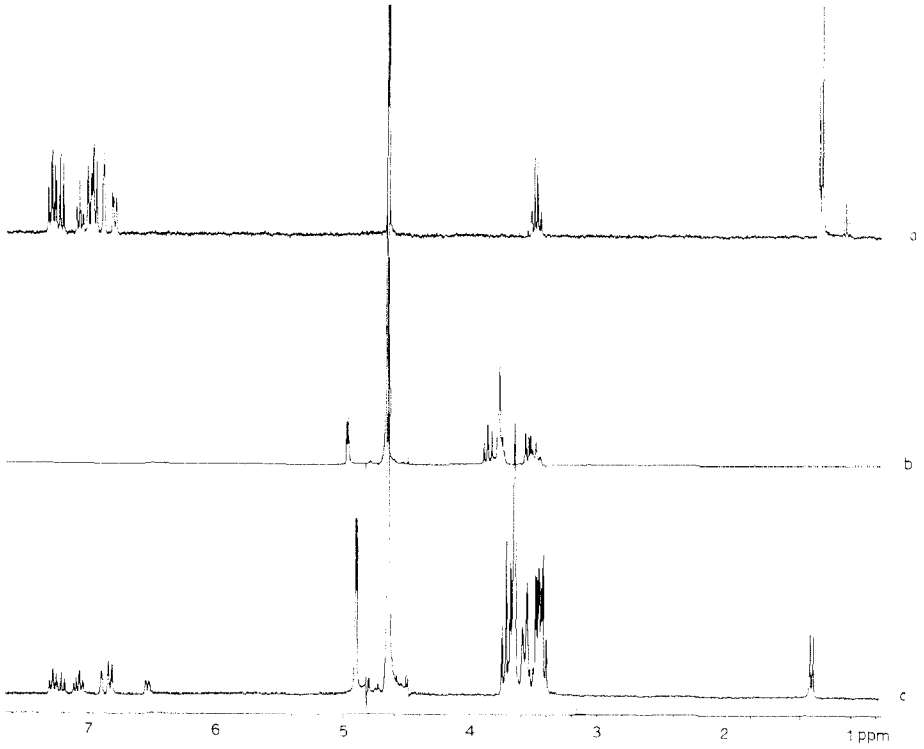

Fig. 1. ^{1}H NMR spectra (300 MHz, 25°C, D_2O, internal standard HDO) of (a) **2** (8 mM), (b) βCD (8 mM), and (c) an equimolar βCD–**2** mixture (8 mM).

TABLE I

^{1}H NMR chemical shift data (300 MHz, D_2O, 25°C, internal HDO) of **2** (8 mM) and βCD (8 mM) free and in the mixture (1:1 molar ratio), and the corresponding complexation shifts ($\Delta\delta = \delta_{compl} - \delta_{free}$, Hz)

	Proton	δ_{free}	δ_{compl}	$\Delta\delta$
2	H-1	6.890	6.891	+0.3
	H-2	6.804	6.538	−79.8
	H-3	7.235	7.211	−7.2
	H-4	6.997	7.048	+15.3
	H-5	6.957	6.822	−40.4
	H-6	7.302	7.278	−7.2
	H-7	7.074	7.087	+3.9
	CH	3.482	3.546	+19.2
	CH_3	1.243	1.304	+18.2
βCD	H-1′	4.964	4.893	−21.3
	H-2′	3.544	3.458	−25.8
	H-3′	3.860	3.711	−44.7
	H-4′	3.478	3.424	−16.2
	H-5′	3.760	3.562	−59.4
	H-6′,6′	3.775	3.649	−37.8

The chemical shift data for the mixture were different from those for the free compounds (Fig. 1, Table I). In particular, the resonances of the protons of βCD, located within or near the cavity (H-3′, 5′, 6′, 6′) showed remarkably large upfield shifts in the mixture. A minor shift was observed for the resonances of H-1′,2′,4′ located on the exterior of βCD. Thus, the association involves the cavity of βCD[6]. Indeed, Johnson–Bovey calculations[7–9] show that the shielding component normal to the plane of one aromatic nucleus is greater in magnitude than the deshielding component in the plane of the ring for any given distance from the centre of the ring. Thus, unless the aromatic substrate has a preferred fixed orientation within the βCD cavity, the resonances of all protons of βCD within the cavity will be shifted upfield when the inclusion complex is formed.

Since the resonances of H-3′ and H-5′, which are located in the wider and narrower side, respectively, are shielded markedly in the complex, the aromatic substrate must penetrate deeply into the cavity.

Remarkable upfield shifts of the resonances of H-2 and H-5 (−79.8 and −40.4 Hz, respectively) of **2** in the complex and less deshielding of H-4,7 and CH and CH_3 of the side chain were observed. The upfield shift of the resonances of H-2,5 can be attributed to the shielding effect of the hydrophobic cavity and also reflects variation of the dihedral angle between the two aromatic rings due to conformational changes produced by the inclusion. The downfield shift of the resonances of the alkyl protons could be attributed to the interaction with the hydrophilic external part of the βCD. The same effect was observed for the H-4 resonance, whereas that of H-1 was little affected.

Stoichiometry, association constant, and thermodynamic parameters.—The stoichiometry of the βCD–**2** complex was determined by using the continous variation

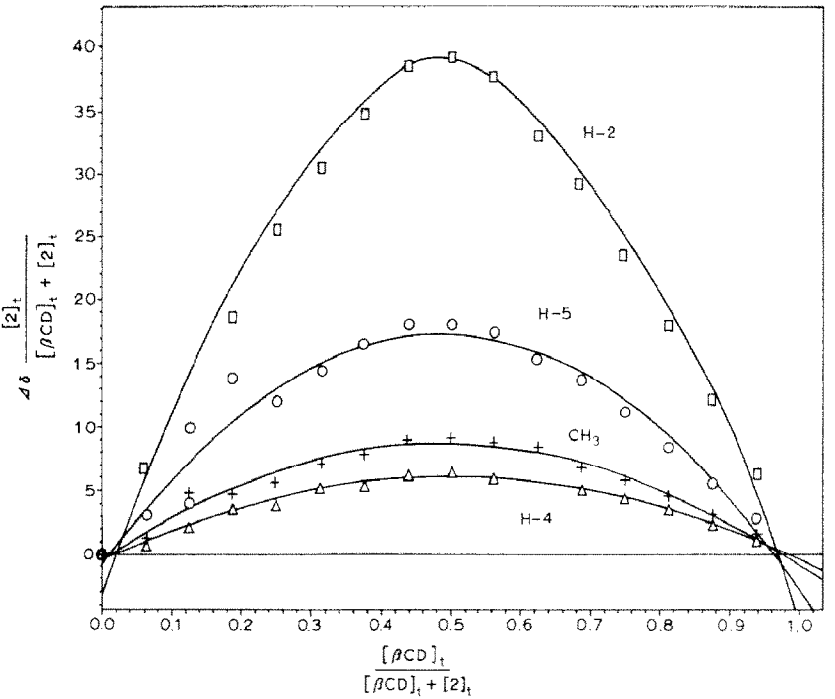

Fig. 2. Continous-variation plot (Job plot) for protons of **2** in βCD–**2** mixtures (total concentration, 8 mM); $\Delta\delta$ is the variation in chemical shift with respect to the free compound.

method[10]. The ^{1}H chemical shift data were determined for βCD–**2** mixtures where the total concentration was kept constant at 8 mM and the initial molar fraction (r) of **2** was varied from 0 to 1. The corresponding Job plots[10] for the protons of **2** are reported in Fig. 2. Each curve shows a maximum corresponding to a molar fraction of 0.5 and a highly symmetrical shape, indicating that the complex has solely 1 : 1 stoichiometry.

The association constant K was evaluated by the Foster–Fyfe[11] graphical method, equivalent to that used by Benesi and Hildebrand[12] in the evaluation of K from optical data for 1 : 1 complexes. By this method, one component is observed in the presence of a large excess of the other. Under these conditions, the difference between the chemical shift ($\Delta\delta_{obs}$) of one proton resonance of the minor component (A) in the presence of the other (B) and in the free state, is correlated to the association constant by the equation

$$\Delta\delta_{obs}/[B]_t = K\Delta\delta_c - K\Delta\delta_{obs}$$

where $[B]_t$ is the total concentration of the species B used in each solution, and $\Delta\delta_c$ is the difference between the chemical shift of a resonance for the free molecule and that in the complex.

Thus, the ^{1}H NMR spectra were recorded for solutions where the concentration of **2** was kept at 0.1 mM and that of βCD ranged from 2 to 15 mM. The plot of

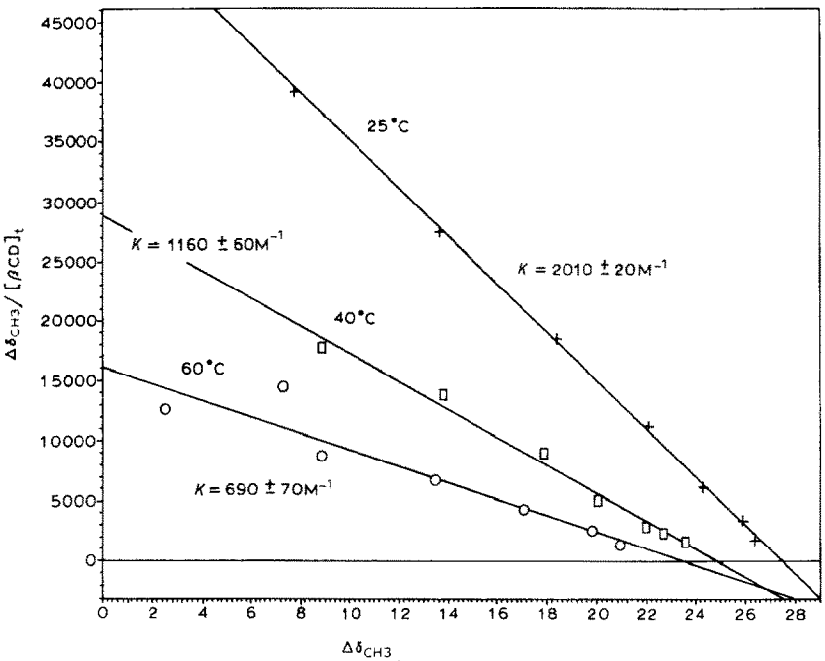

Fig. 3. Determination of the association constant of the βCD–2 complex at 25, 40 and 60°C.

$\Delta\delta_{obs}/[\beta CD]_t$ against $\Delta\delta_{obs}$ gave straight lines, the negative gradient of which afforded K. The plots relative to measurements of chemical shifts of the methyl-proton resonances of **2** at 25, 40, and 60°C are reported in Fig. 3. At 25°C, the value of the association constant obtained was 2010 M^{-1}.

The enthalpy (ΔH) and entropy (ΔS) of complexation were obtained by the classical method based on the analysis of the temperature dependence of the complexation constant. The association constant of the βCD–**2** complex (Fig. 3) rapidly decreased from 2010 to 690 M^{-1} on increasing the temperature from 25 to 60°C. The values of ΔH (-3.0 ± 0.2 kcal/mole) and ΔS (-2.5 ± 0.3 cal · $mole^{-1}$ · deg^{-1}) were calculated by plotting ln K against $1/T$. The negative value of the entropy factor could indicate minor conformational freedom of **2** because of its deep penetration into the cavity. The enthalpy of complexation was negative, indicating that the complex dissociates when the temperature is increased.

Dynamics of the inclusion complex.—The degree of perturbation of the motional freedom of **2** caused by the inclusion process was evaluated by means of proton selective relaxation methods[13].

The rate of relaxation of a proton i can be measured[13] non-, mono-, and bi-selectively. The nonselective relaxation rate (R_{ns}^{i}) is measured by following the recovery of the inverted signal of a proton i under simultaneous inversion of all protons in the spin system. The monoselective relaxation rate (R_{s}^{i}) is measured by selectively inverting the signal of proton i and the biselective one (R_{ij}^{i}) by

simultaneous inversion of the spin pair *ij*. These rates of relaxation contain different contributions from intramolecular dipolar cross-relaxation terms (σ_{ij}) and direct terms (ρ_{ij}) and each includes a term ρ^* which takes into account other sources of relaxation:

$$R_{ns}^{i} = \Sigma_j \rho_{ij} + \Sigma_j \sigma_{ij} + \rho^*$$

$$R_{s}^{i} = \Sigma_j \rho_{ij} + \rho^*$$

$$R_{ij}^{i} = \Sigma_j \rho_{ij} + \sigma_{ij} + \rho^*$$

The difference between the bi- and mono-selective relaxation rates permits the extraction of the cross-relaxation term σ_{ij} for the proton pair *ij*:

$$R_{ij}^{i} - R_{s}^{i} = \sigma_{ij}$$

In the initial rate approximation[14], this term, which describes the magnetisation transfer between the spins *i* and *j*, depends on the internuclear distance r_{ij} and on the reorientational correlation time τ_c of the vector *ij*:

$$\sigma_{ij} = 0.1\gamma^4\hbar^2 < r_{ij} >^{-6} \left[6\tau_c/\left(1 + 4\omega^2\tau_c^2\right) - \tau_c\right]$$

where γ is the gyromagnetic ratio, ω is the proton Larmor frequency, and $\hbar$ is the reduced Planck's constant. In the fast-motion (extreme-narrowing) regime, $\omega^2\tau_c^2 \ll 1$, the cross-relaxation term assumes the simplified form

$$\sigma_{ij} = (1/2)\gamma^4\hbar^2 r_{ij}^{-6}\tau_c \qquad (1)$$

Dynamic information (τ_c) can be obtained using the above equation by determining σ_{ij} of a proton pair *ij*, the internuclear distance (r_{ij}) of which is known.

The mono (R^2) and bi-selective (R^2_{2-3}) relaxation rates of H-2 of 4 mM **2** were measured in the free state and in the presence of a two-fold molar excess of βCD (Table II). By subtracting these two quantities, the cross-relaxation term for the proton pair H-2,3 was calculated for the free state and in the βCD–**2** mixture. The latter value is the weighted average of the corresponding terms for the free (σ_f) and complexed (σ_b) compounds

$$\sigma_{obs} = X_b\sigma_b + X_f\sigma_f \qquad (2)$$

where X_b and X_f are the molar fractions of the complexed and free **2**, respectively.

TABLE II

Monoselective relaxation rates (R^2, s^{-1}) of H-2, biselective relaxation rates (R^2_{2-3}, s^{-1}), observed cross-relaxation terms (σ_{2-3}, s^{-1}), and correlation times (τ_{c2-3}, ps) of H-2,3 in **2** (4 mM) and in a βCD–**2** mixture (molar ratio 2:1)

	R^2_2	R^2_{2-3}	σ_{2-3}	τ_{c2-3}
2	0.181 ± 0.01	0.248 ± 0.01	0.067	47.5
βCD–**2**	0.910 ± 0.01	1.029 ± 0.01	0.119	88.0

In the solution containing only free **2**, the cross-relaxation term σ^2_{2-3} was 0.067 s^{-1}. By introducing this value and that of the known distance r_{2-3} (2.43 Å) into eq *1*, the value of the correlation time of the vector 2–3 in the free **2** was obtained as $4.75 \cdot 10^{-11}$ s (Table II). By the hypothesis of isotropic motion, this value can be used to describe the overall motion of the molecule.

In the βCD–**2** mixture (molar ratio 2:1), the cross-relaxation term, calculated from the observed bi- and mono-selective relaxation rates ($\sigma^2_{2-3} = R^2_{2-3} - R^2$), increased to 0.119 s^{-1}. By introducing the values of σ_{obs} (0.119 s^{-1}) and σ_f(0.067 s^{-1}) and the calculated molar fractions X_b and X_f (0.898 and 0.102, respectively, from the association constant), in eq *2*, the value 0.125 s^{-1} was determined for the cross-relaxation term of proton pair H-2,3 of **2** in the bound state. The correlation time of $8.8 \cdot 10^{-11}$ s was then obtained by means of eq *1*.

The correlation times for free and bound **2** were compared with that of free βCD (obtained by measurements of the rates of spin–lattice relaxation of the ^{13}C nuclei).

For a ^{13}C nucleus directly linked to at least one proton, the major relaxation process comes usually from the ^{13}C,H dipolar interaction, and the determination of the ^{13}C T_1's can be related to the correlation time for the isotropic overall molecular reorientation using eq *3*.

$$1/NT_1 = \hbar^2 \gamma_H^2 \gamma_C^2 r_{CH}^{-6} \tau \qquad (3)$$

where N is the number of linked protons at distance r_{CH} (1.10 Å), and γ_H and γ_C are the gyromagnetic ratios of ^1H and ^{13}C nuclei, respectively.

The ring ^{13}C T_1's (C-1′/5′) of free 2 mM βCD were determined, which were similar to each other, and the mean value $\langle T_1 \rangle_{1'-5'}$ was 0.22 s which was used to calculate (using eq *3*) the value $2.2 \cdot 10^{-10}$ s of the correlation time of free βCD.

On the basis of the above results, it was concluded that an independent motion of the included **2** exists with respect to the cavity. Indeed, the motion of **2** slowed down by a factor of ~2 on inclusion into βCD, but the correlation time of the included molecule was 2–3 times shorther than that calculated for βCD. This independent motion, which probably takes place around the axis of symmetry of the βCD, could be determined by the non-directive and weak forces involved in the complexation and by the symmetry of the cavity.

NOE data.—A deeper insight into the stereochemical features of the inclusion complex was obtained by means of $^1H\{^1H\}$-NOE measurements in βCD–**2** mixtures. The observability of intermolecular NOEs between H-3′ and H-5′ of βCD and the protons of **2** confirms that the guest is inside the cavity of the host.

As shown in Fig. 4a, the irradiation of the methyl protons of **2** produced the expected intramolecular NOEs on the resonances of MeC*H*, H-4, and H-1 of **2**, but also an intense intermolecular NOE on the resonance of H-3′ of βCD. The irradiation of H-1 of **2** produced intramolecular NOE on the CH_3CH proton resonances and a strong intermolecular NOE on the resonance of H-3′ of βCD (Fig. 4b). The absence of intramolecular NOEs between H-1 and H-5 indicated

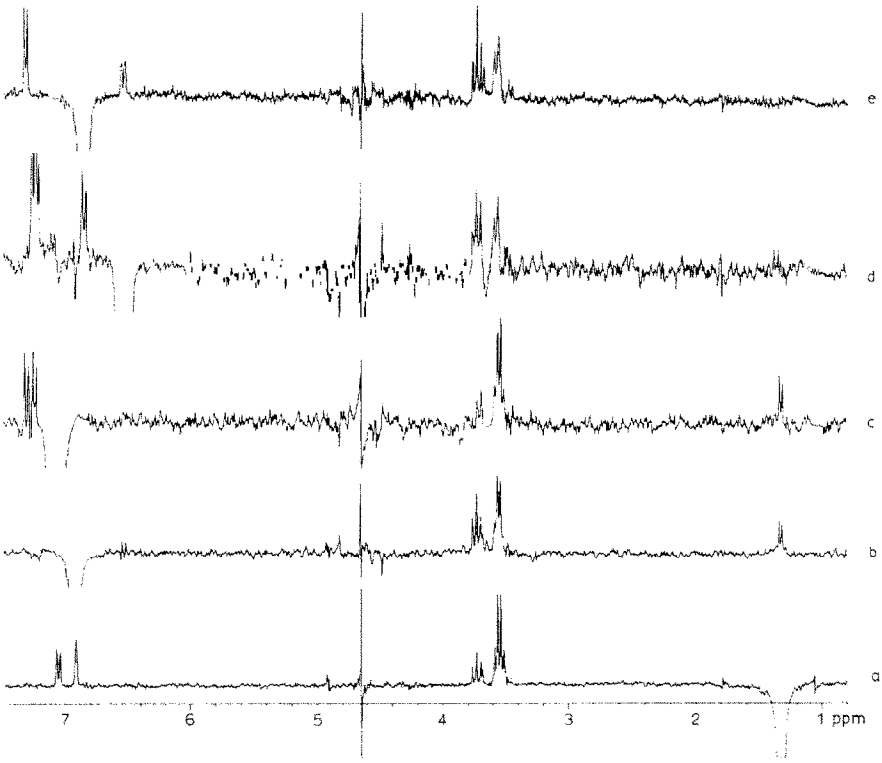

Fig. 4. $^{1}H\{^{1}H\}$-NOE difference spectra (300 MHz, 25°C, D_2O, internal HDO) of the equimolar βCD–**2** mixture corresponding to saturation of the following protons of **2**: (a) CH_3, (b) H-1, (c) H-4, (d) H-2, and (e) H-5.

that **2** assumes the preferred conformation where H-1 is remote from any proton of the other aromatic nucleus. The irradiation of H-4 (Fig. 4c) gave a less important NOE on the resonance of H-3′ of βCD. The saturation of the H-2 (Fig. 4d) and H-5 (Fig. 4e) gave significant intermolecular NOEs on the resonances of both internal protons of βCD (H-3 and H-5). In the contrast, irradiation of H-7 gave no significant intermolecular NOEs.

The above results provide a detailed picture of the 1:1 βCD–**2** inclusion complex in solution. Thus, **2** penetrates deeply into the cavity from the larger-diameter side with H-7 extruded from the smaller-diameter side. The alkyl group remains external to the larger side of the βCD (Fig. 5).

The inclusion is probably favoured by interaction of the aromatic nuclei and the hydrophobic cavity of βCD, and by the interaction of the protruding carboxylate group of **2** and the hydrophilic external part of the cavity. The deep insertion of **2** is reflected in the slowing down of its molecular motion. However, an independent motion of the substrate with respect to the cavity also occurs, which is probably due to the weak nature of the forces involved in the complexation and to the symmetry of the system.

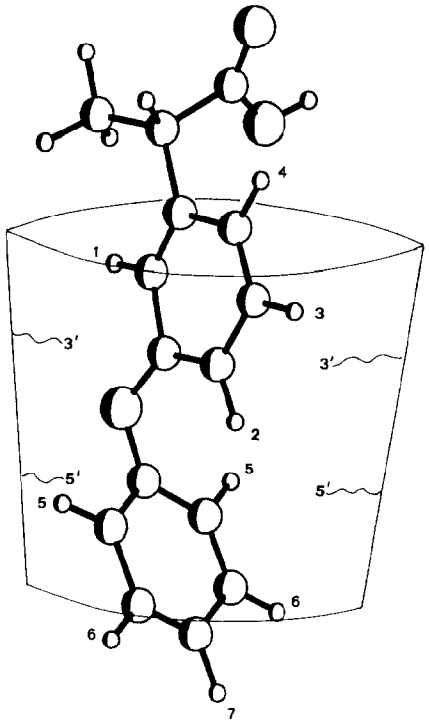

Fig. 5. Suggested model of the βCD–2 inclusion complex on the basis of the solution NMR data.

The structure of the complex in solution is different from that in the solid state[15] where the existence of a head-to-tail dimeric form has been revealed. This possibility is not supported by the NOE data for solutions, where there is no evidence of the proximity of the alkyl group, and hence of the carboxylate, to the smaller side of the βCD. Indeed, irradiation of H-7 does not produce intermolecular NOEs.

EXPERIMENTAL

Compound **2** was kindly provided by the Eli Lilly Company.

The NMR measurements were performed on solutions in D_2O with a Varian VXR spectrometer (300 MHz for ^1H, 75 MHz for ^{13}C) and the temperature was controlled to ± 0.1°C. The $^1H\{^1H\}$-NOE experiments were performed on degassed samples in the difference mode. The decoupler power used was the minimum required to saturate the spin of interest. A waiting time of 5–10 s was used to allow the system to reach the equilibrium. Each NOE experiment was repeated at least four times.

The selective relaxation rates were measured in the initial rate approximation[14] by giving a selective π pulse with the proton decoupler at the selected frequency

for 25 ms. After the delay, τ, a nonselective $\pi/2$ pulse was given to detect the longitudinal magnetisation. The biselective measurements were carried out by alternating the decoupler offset between the two desired frequencies for a total decoupling time of 40 ms. Each proton selective relaxation rate measurement was repeated at least three times. The ^{13}C spin–lattice relaxation times were measured by the inversion–recovery method.

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