CHEMISTRY LETTERS, pp. 1393-1396, 1977. Published by the Chemical Society of Japan

CARBON 13 SPIN-LATTICE RELAXATION TIMES OF THE INCLUSION COMPLEX OF β-CYCLODEXTRIN WITH SULFATHIAZOLE IN AQUEOUS SOLUTION

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Carbon 13 spin-lattice relaxation times for inclusion complex of β-cyclodextrin with sulfothiazole in aqueous solution have been measured by an inversion-recovery method. The reduction of molecular motions and changes in carbon 13 chemical shifts in both species suggest a predominantly hydrophobic interaction.

Extensive studies on inclusion complexes of β-cyclodextrin (β-CyD) with various organic molecules have been reported.[1-4] However, the dynamic properties of the guest molecule embedded in hydrophobic cavity of β-CyD as well as a definite mode of inclusion in solution still remained speculative. Since carbon 13 nuclear magnetic resonance (^{13}C NMR) spectroscopy is particularly well-suited for this kind of investigations,[5] we have examined the molecular motions in the inclusion complex of β-CyD with sulfathiazole (ST) in aqueous solution by means of ^{13}C nuclear relaxation. ST was chosen as an adequate guest molecule toward β-CyD, because the sulfur-containing drugs such as phenothiazine,[6] thiobarbiturates,[7] and sulfonamides[8] showed the significantly large binding constants. The NMR spectra were taken on a Jeol PFT-100 spectrometer operating at 25.03 MHz, interfaced with Jeol EC-100 Fourier transform computer with 16 K memory. The NMR spectra of degassed samples (150 mg/ml ST, 200 mg/ml of β-CyD, and their mixture in 2 N NaOD solution) were obtained in 10-mm spinning tube at ambient temperature (about 38°) using a deuterium lock. The ^{13}C chemical shifts were referenced to external tetramethylsilane with accuracy of ± 0.025 ppm. The ^{13}C spin-lattice relaxation time (T_1) measurements were carried out by the

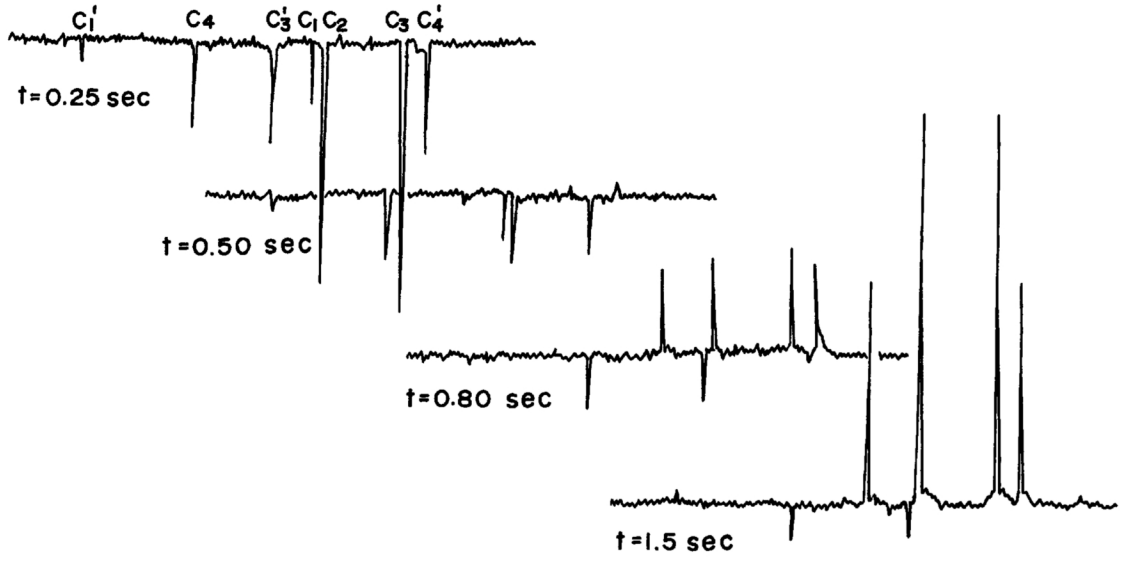
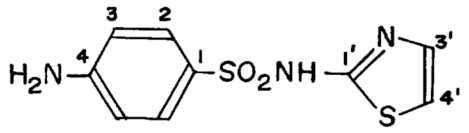


Fig. 1. The frequency-domain ^{13}C NMR spectra of sulfathiazole (150 mg/ml in 2 N NaOD) by the inversion-recovery method. Only four spectra out of eight are shown for clarity. Conditions were: sweep width, 4000 Hz; pulse width, 24.8 μsec (90°); T, 6.0 sec; 500 scans. C_1, C_4, and C_1' relaxed too slowly for T_1 determination by this procedure and in some cases separate experiments with a different set of t values were applied. Assignment of chemical shifts following ref. 11.

inversion-recovery-technique[9] ($-180°-t-90°-T-$) with $T > 5T_1$ for carbons being measured: The T_1 values were obtained by least squares analyses of $\ln (A_\infty - A_t)$ vs. t, where A_∞, A_t, and t are the intensity at time ∞ (after single 90° pulse), the intensity at time t, and the pulse interval time in seconds, respectively.[10] The slope of the line was taken as $-1/T_1$, with accuracy of ± 10 %.

Figure 1 shows some partially relaxed ^{13}C NMR spectra of ST taken for the measurements of T_1. The T_1 value of ST in the presence and absence of β-CyD are listed in Table I. Upon inclusion in β-CyD, all the T_1 values decreased by a factor of 3, where significant decrease in T_1 of C_1 and C_4 was noted. This indicates that the molecular motion of ST, particularly the phenyl moiety, reduced as a consequence of the coupling of its motion to that of β-CyD. A large decrease in T_1 values observed for the carbons along a long molecular axis may be ascribed to the anisotropic tumbling of the guest molecule within β-CyD cavity.[5] The T_1 values of β-CyD in the presence and absence of ST are shown in Table II. For β-CyD itself, the primary alcohol group (C_6) showed smaller T_1 value compared to the rigid skeleton (C_{1-5}), which may be due to internal rotation about the C_5 - C_6 bond. After inclusion of ST, T_1 values of all the β-CyD carbons decreased slightly but significantly, where the reduction of internal motion of

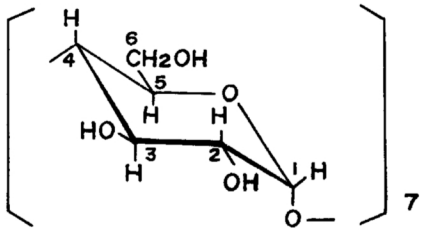
Table I. ^{13}C Relaxation Times (T_1) and Chemical Shifts of Sulfathiazole[a] in the Presence and Absence of β-Cyclodextrin (β-CyD)



Carbon	T_1 (sec) without β-CyD (I_o)	T_1 (sec) with β-CyD (I)	I_o/I	Chemical Shift (ppm) without β-CyD (δ_o)	Chemical Shift (ppm) with β-CyD (δ)	$\delta - \delta_o$
1	20.22	4.48	4.51	131.33	131.48	+0.15
2	0.97	0.36	2.69	129.87	129.73	-0.14
3	1.03	0.35	2.94	116.31	116.07	-0.24
4	15.80	3.47	4.55	151.77	151.72	-0.05
1'	28.95	9.08	3.18	171.47	171.42	-0.05
3'	0.86	0.27	3.19	138.51	138.40	-0.11
4'	0.73	0.25	2.92	111.88	111.58	-0.30

a) Assignment following ref. 11.

Table II. ^{13}C Relaxation Times (T_1) and Chemical Shifts of β-Cyclodextrin (β-CyD)[a] in the Presence and Absence of Sulfathiazole (ST)



Carbon	T_1 (sec) without ST (I_o)	T_1 (sec) with ST (I)	I_o/I	Chemical Shift (ppm) without ST (δ_o)	Chemical Shift (ppm) with ST (δ)	$\delta - \delta_o$
1	0.077	0.064	1.20	104.85	104.51	-0.34
2	0.079	0.072	1.10	75.83	75.74	-0.09
3	0.082	0.065	1.26	75.01	74.91	-0.10
4	0.072	0.063	1.14	83.69	83.10	-0.59
5	0.077	0.054	1.43	73.59	73.25	-0.34
6	0.054	0.033	1.64	62.13	61.79	-0.34

a) Assignment following ref. 12.

CH_2OH group was relatively large compared to that in the cyclic framework. These results indicate that ST molecule may participate in the interaction at interior and exterior of the β-CyD cavity to form rigid complex, which may result in the highest binding constant among the sulfonamides.[8]

Chemical shift changes in both ST and β-CyD were also monitored and the results are shown in Table I and II, respectively. Except for the C_1 of ST, all other signals in both species showed the shifts to higher-field, indicating a predominantly hydrophobic interaction.[13] The down-field shift observed for C_1 signal may be the reflection of the hydrogen bonds involved between amino group of ST and some parts of β-CyD. Such a possibility would not be precluded by the suggested hydrophobic interaction.

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(Received September 7, 1977)