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Nuclear Magnetic Resonance (NMR) Spectroscopy of Inclusion Compounds of Tolbutamide and Chlorpropamide with β-Cyclodextrin in Aqueous Solution[1,2]

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A structural study of the inclusion compounds of tolbutamide and chlorpropamide with β-cyclodextrin in aqueous solution was attempted by means of proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) experiments. The changes in chemical shift (^{1}H, ^{13}C) and in relaxation times (^{1}H-$T_1\rho$, ^{13}C-T_1) suggested that the drug phenyl moiety was included in the cavity of β-CD mainly by hydrophobic interaction, and that the primary hydroxy side of β-CD was tightly associated with each drug. The binding mechanism and binding sites between the drug molecules and β-CD are discussed in detail.

Keywords——^{1}H-NMR; ^{13}C-NMR; inclusion compounds; β-cyclodextrin; tolbutamide; chlorpropamide; chemical shift; relaxation time; hydrophobic interaction

Extensive studies[4–11] on inclusion compounds of various medicinally useful molecules with β-cyclodextrin (β-CD) have been reported.

Previous studies have shown that the inclusion compounds of β-CD with antiinflammatory drugs can be prepared by the freeze-drying method,[7] and also that the dissolution rate[8] and bioavailability[9] of such inclusion compounds were enhanced compared with those of intact drugs.

The inclusion compounds of sulfonylureas with β-CD have been studied by circular dichroism spectroscopy, by high performance liquid chromatography and by the solubility method.[6d,10] Further, the molecular motions in the inclusion compound of β-CD with tolbutamide (TBA) in aqueous solution have been examined by means of ^{13}C nuclear relaxation experiments.[11] However, the dynamic properties and mechanism of formation of the β-CD/TBA inclusion compound in aqueous solution are still not underestood in detail. In the present work, the NMR technique was used to elucidate in detail the structures of the inclusion compounds of TBA and chlorpropamide (CLP) with β-CD in aqueous solution.

1) This paper forms Part XXXIII of "Physico-chemical Approach to Biopharmaceutical Phenomena." The preceding paper, Part XXXII: H. Ueda and T. Nagai, *Chem. Pharm. Bull.*, **28**, 1016 (1980).
2) A part of this work was presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August 1979.
3) Location: *Ebara-2-4-41, Shinagawa-ku, Tokyo, 142, Japan.*
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Experimental

Materials——The following materials were used; 99.8% deuterium oxide (Merck), sodium hydroxide-d_1 solution (about 40% sodium deuterium oxide in D_2O, Merck). Highly purified sulfonylureas and β-CD were obtained as follows: tolbutamide from Hoechst Japan Co., Ltd., mp 128.5—129.5°; chlorpropamide from Taito Pfizer Co., Ltd., mp 127—129°; β-CD from Teijin Ltd. All chemicals were used after recrystallization followed by removal of the solvent *in vacuo*.

Methods——The ^1H spectra and ^{13}C spectra were observed with a JNM-FX 100 spectrometer operating at ^1H-99.65 MHz and ^{13}C-25.01 MHz in the pulsed Fourier transform mode. All spectra were obtained at $24.5 \pm 0.5°$. The ^1H-chemical shifts are given relative to external tetramethylsilane within ± 0.002 ppm. The ^{13}C-chemical shifts are given relative to external tetramethylsilane within ± 0.039 ppm. The values of $1/T_{1\rho}$ for the various protons were obtained from the spin-locking sequence[12] according to equation 1:

$$M_\rho(t) = M_0 \exp(-t/T_{1\rho}) \qquad \text{(eq. 1)}$$

where $M_\rho(t)$ is the macroscopic magnetization at t, M_0 is th equilibrium magnetization at $t=0$, t is the spin-locking time, and $T_{1\rho}$ is the spin-lattice relaxation time in the rotating frame. The operating conditions were as follows: $\pi/2$ pulse at 15 μsec; locking pulse($H_{1\rho}$) at 2.0 gauss; spin-locking times changing several times (at least 9 points) between 0—5 sec. The ^{13}C spin-lattice relaxation time(T_1) measurement were carried out without degassing by the inversion recovery method[13] using a ($-180°$-τ-$90°$-T-) sequence, where T is greater than 5 T_1 for carbons being measured. The T_1 values were obtained by least-squares analysis of a plot of $\ln(A_\infty - A_t)$ *vs.* t, where A_∞, A_t, and t are the intensity at time ∞(after a single 90° pulse), the intensity at time t, and the pulse interval time in seconds, respectively. The slope of the line was taken as $-1/T_1$, with an accuracy of $\pm 10\%$.

Results and Discussion

1) ^1H-NMR

Figure 1 shows the effects of TBA and CLP on the ^1H-NMR spectrum of β-CD in aqueous solution. The H-5 signal of β-CD could not be directly observed by 100 MHz-NMR, because it overlapped with the H-6 and H-3 signals in the spectral region of 4.1—3.8 ppm. It was clear that a new sharp signal assigned to H-5 progressively shifted to higher field, becoming separated, in the presence of increasing amounts of TBA and CLP.

Figure 2 shows the TBA- and CLP-induced chemical shifts of β-CD at various molar ratios of each drug to β-CD. It was evident that the H-3, H-6, and H-5 signals shifted to higher field from their initial positions.

It was considered possible that the phenyl moiety of each drug molecule was included in the cavity of β-CD; H-3, H-5, and possibly H-6 (located within the cavity of β-CD) would then be affected by anisotropic shielding due to the phenyl moiety, whereas H-1, H-2, and H-4 (located outside the cavity) were relatively unaffected. Considering the difference in relative magnitude of $\Delta\delta$ between H-5 and H-3, it can be assumed that the association between a drug and β-CD may take place by approach of the drug molecule to the primary hydroxy side of β-CD, by analogy with barbiturates/β-CD.[14] However, this assumption was not consistent with the results of ^{13}C-measurement, *i.e.*, that the ^{13}C-shifts of C-2 and C-3 were greater than that of C-5, as will be mentioned later. In any case, it appeared that the primary hydroxy side of β-CD was tightly associated with each drug, based on the results of ^{13}C-measurement. Therefore, the shifts of all the signals in β-CD to higher field suggested that a hydrophobic interaction was predominant between the drugs and β-CD.

Figure 3a,b shows the effect of β-CD on the ^1H-NMR spectra of TBA and CLP in aqueous solution. All of the proton signals of TBA and CLP shifted to lower field with increasing amount of β-CD. Similar chemical shift changes have been observed for other drug systems.[6b,15] Except for the alkyl side chains of both molecules, the shift to low field of all

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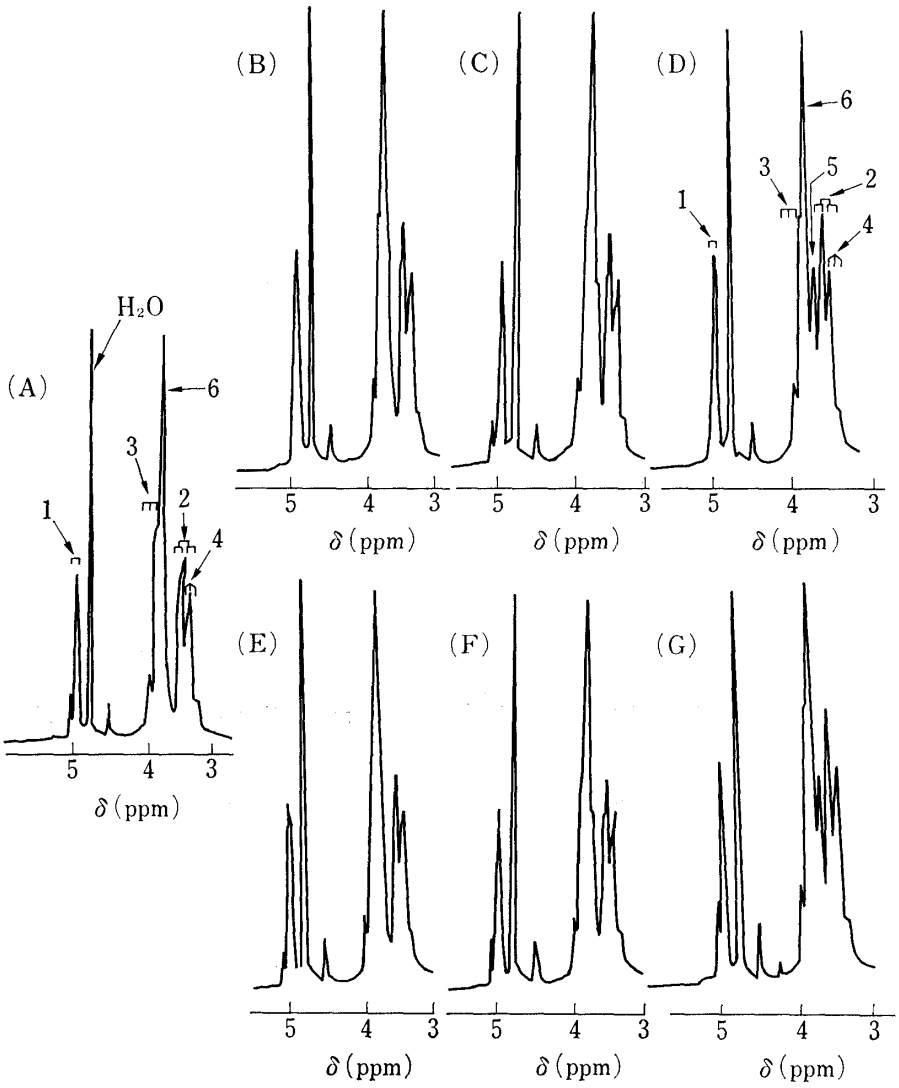

Fig. 1. ^{1}H-NMR Spectra of β-Cyclodextrin[a] (1×10^{-1} M) containing Various Amounts of Tolbutamide and Chlorpropamide

Molar ratio of tolbutamide/β-cyclodextrin: (A) 0.00, (B) 0.25, (C) 0.50, (D) 1.00.
Molar ratio of chlorpropamide/β-cyclodextrin: (A) 0.00, (E) 0.25, (F) 0.50, (G) 1.00.
Solvent: 0.2 N NaOD in D_2O.
a) Assigned following refs. 6-b, 23 and 24.

other signals might be induced by diamagnetic anisotropy of particular bonds of β-CD[16] and van der Waals shifts.[17] The low-field shift of the alkyl side chain might be induced by steric perturbation,[18] as will be discussed in more detail later in connection with the ^{13}C-NMR data. Plots of the molar ratio of β-CD: TBA or β-CD: CLP *vs.* the change in chemical shifts of each drug indicated that a 1: 1 complex was formed, though some ambiguity remains, as no further substantial changes of the chemical shifts took place above a molar ratio of 1: 1 (β-CD: drug) with both drugs. This speculation is consistent with the results obtained by the solubility method.[11]

The molecular motions in the inclusion complex of β-CD with TBA and CLP were examined in further detail by means of ^1H-$T_{1\rho}$ measurement. The effects of β-CD on the ^1H-relaxation

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times of TBA and CLP are summarized in Table I a, b. Table I a, b shows that the addition of β-CD to solutions of TBA and CLP decreased the relaxation times of all peaks of both molecules. However, the effect was more pronounced in the case of the phenyl protons of both molecules. The above results suggest a specific interaction between the drug phenyl moiety

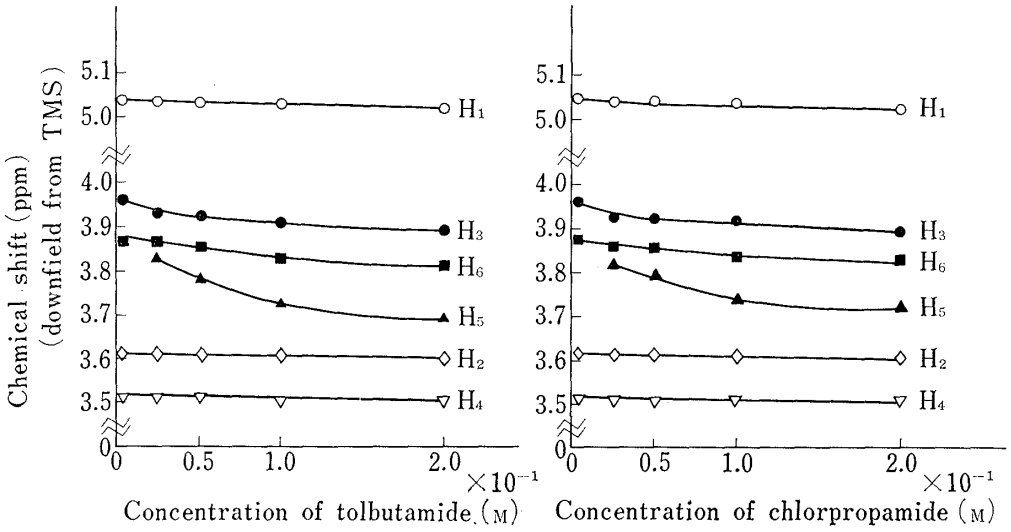


Fig. 2. Variation of ^{1}H Chemical Shifts of 0.1 M β-Cyclodextrin with Concentration of Tolbutamide and Chlorpropamide

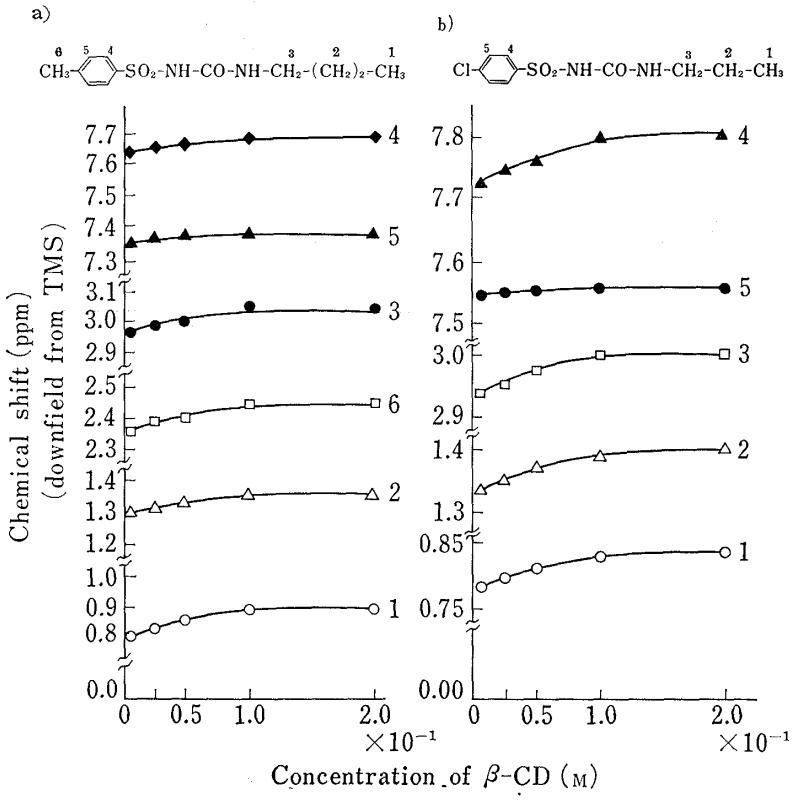
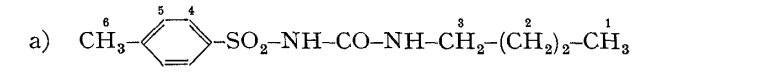
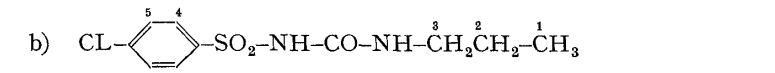


Fig. 3a, b. Variation of ^{1}H Chemical Shifts of 0.1 M Tolbutamide and 0.1 M Chlorpropamide with Concentration of β-Cyclodextrin

Table Ia, b. ^{1}H-Relaxation Times ($T_1\rho$) of Tolbutamide and Chlorpropamide in the Presence or Absence of β-Cyclodextrin (β-CD)

a) 

Proton	$T_1\rho$(sec) without β-CD(I_0)	$T_1\rho$(sec) with β-CD(I)	(I_0/I)
1	1.88	0.61	3.08
2	1.50	0.39	3.84
3	1.07	0.24	4.45
4	2.23	0.40	5.56
5	2.27	0.41	5.53
6	1.09	0.26	4.19

b) 

Proton	$T_1\rho$(sec) without β-CD(I_0)	$T_1\rho$(sec) with β-CD(I)	(I_0/I)
1	1.76	0.73	2.41
2	1.60	0.57	2.80
3	1.23	0.38	3.20
4	2.76	0.42	6.57
5	2.71	0.41	6.60

Tolbutamide, 0.1 M; chlorpropamide, 0.1 M; β-cyclodextrin, 0.1 M; solvent, 0.2 N NaOD in D_2O.

and β-CD.[19-21] These ^{1}H-relaxation data provide further support for the view that the phenyl moiety of each drug molecule is included in the cavity of β-CD in solution.

2) ^{13}C-NMR

Hundred and fifty mg/ml of TBA, 150 mg/ml of CLP, 200 mg/ml of β-CD and mixtures of these compounds in 2N NaOH solution (containing 30% D_2O) were prepared to study the effect of β-CD on the ^{13}C-spectra and relaxation times (^{13}C-T_1) of TBA and CLP. The results are shown in Table II a, b. Except for C_6 of TBA in the TBA/β-CD system, all phenyl signals showed shifts to higher field, indicating a predominant hydrophobic interaction.[22] Except for C_5 and C_7 of CLP in the CLP/β-CD system, the phenyl signals were shifted to higher field. In the TBA/β-CD and CLP/β-CD systems, the shifts to higher field suggested that the phenyl moiety of each molecule is included in the cavity of β-CD, as expected from the ^{1}H-measurements.

The low-field shifts observed for C_6 of TBA and C_5 of CLP can be considered to reflect hydrogen bonding between the sulfonamide moiety(-SO_2NH-) and β-CD. Such a speculation dose not conflict with the suggested hydrophobic interaction. The reason for the low-field shift of C_7 on CLP is not clear.

The alkyl side chain of TBA in the presence of β-CD showed shifts to higher field, in the order $C_4>C_3>C_2>C_1$. In the CLP/β-CD system, the shifts to higher field on the alkyl side

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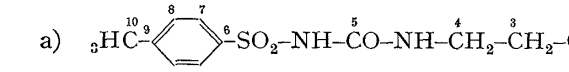
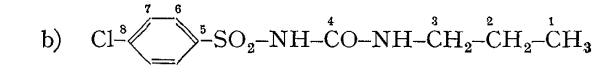
TABLE IIa, b. ^{13}C Chemical Shifts and Relaxation Times (T_1) of Tolbutamide and Chlorpropamide in the Presence or Absence of β-Cyclodextrin (β-CD)

a) 

Carbon	Chemical shift (ppm)			T_1(sec)		
	without β-CD(δ_0)	with β-CD(δ)	$\delta-\delta_0$	without β-CD(I_0)	with β-CD(I)	I_0/I
1	13.596	13.596	0.000	2.17	1.69	1.28
2	19.687	19.638	−0.047	0.95	0.74	1.28
3	31.772	31.625	−0.147	0.54	0.42	1.28
4	40.300	40.104	−0.196	0.39	0.20	1.96
5	162.120	162.175	0.055	5.21	3.46	1.51
6	141.997	142.238	0.241	3.90	2.57	1.51
7	129.280	129.233	−0.047	0.47	0.27	1.75
8	126.453	126.356	−0.097	0.47	0.29	1.45
9	140.780	140.585	−0.195	7.25	4.45	1.64
10	20.905	21.002	0.097	1.16	0.08	1.45

Tolbutamide, 150 mg/ml; β-cyclodextrin, 200 mg/ml; solvent, 2N NaOH in 30% D_2O.

b) 

Carbon	Chemical shift (ppm)			T_1(sec)		
	without β-CD(δ_0)	with β-CD(δ)	$\delta-\delta_0$	without β-CD(I_0)	with β-CD(I)	I_0/I
1	11.012	11.061	0.049	2.34	1.69	1.54
2	22.952	22.952	0.00	0.92	0.60	1.54
3	42.395	42.298	−0.097	0.43	0.26	1.67
4	162.315	162.370	0.055	6.36	3.70	1.72
5	142.192	142.293	0.101	7.70	4.61	1.67
6	128.937	128.789	−0.148	0.54	0.23	2.33
7	128.110	128.157	0.047	0.52	0.28	1.85
8	137.225	137.170	−0.055	6.91	4.34	1.59

Chlorpropamide, 150 mg/ml; β-cyclodextrin, 200 mg/ml; solvent, 2N NaOH in 30% D_2O.

chain were not clear except in the case of C_3, but the overall tendency was the same as that of TBA. This higher field shift in the ^{13}C-spectra of the alkyl side chain and the low-field shift in the ^1H-spectra of the alkyl side chain might be induced by a steric compression effect.[17] It is possible that when the phenyl moiety of the drug molecule is included in the cavity of β-CD, the alkyl side chain may extrude from the cavity and may be in a sterically different situation from that in aqueous solution.

The T_1 values of TBA and CLP in the presence and absence of β-CD are also listed in Table II a, b. The values obtained are a little smaller than those of a previous report,[11] possibly due to differences in measuring conditions. In the presence of β-CD, all T_1 values decreased by a factor of 1.4—1.5, with a slightly larger decrease in T_1 of the phenyl moiety. Usually, ^{13}C-NMR is not much affected by intermolecular interactions, as in ^1H-NMR, but the above results indicate that the molecular motion of TBA and CLP is reduced as a consequence of inclusion within β-CD.

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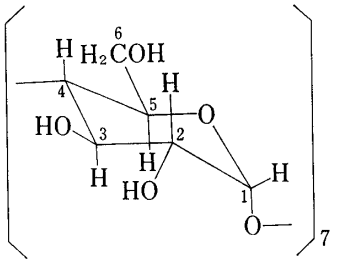
Table IIIa, b. ^{13}C Chemical Shifts of β-Cyclodextrin[a] in the Presence or Absence of Tolbutamide (TBA) and Chlorpropamide (CLP)



β-cyclodextrin

a) Tolbutamide

Carbon	Chemical shift (ppm)		
	without TBA(δ_0)	with TBA(δ)	$\delta-\delta_0$
1	103.483	103.160	−0.323
2	73.778	73.485	−0.303
3	74.635	74.460	−0.175
4	82.446	81.816	−0.630
5	72.411	72.362	−0.049
6	61.031	60.620	−0.411

b) Chlorpropamide

Carbon	Chemical shift (ppm)		
	without CLP(δ_0)	with CLP(δ)	$\delta-\delta_0$
1	103.483	103.211	−0.272
2	73.778	73.532	−0.256
3	74.635	74.460	−0.175
4	82.446	81.867	−0.579
5	72.411	72.316	−0.095
6	61.031	60.669	−0.362

β-Cyclodextrin, 200 mg/ml; tolbutamide 150 mg/ml; chlorpropamide, 150 mg/ml; solvent, 2 N NaOH in 30% D_2O.
a) Assigned following refs. 25 and 26.

Table III a,b shows the effects of TBA and CLP on the ^{13}C-NMR spectrum of β-CD in aqueous solution. All signals of β-CD showed shifts to higher field in the presence of TBA or CLP. C_1 and C_4 of β-CD were affected more than other carbons. This effect may have resulted from a small conformational change of β-CD, as the C_1 and C_4 positions are mobile because β-CD is α-(1,4) linked. The primary alcohol group (C_6) of β-CD was also affected; its internal rotation may decrease with the inclusion of TBA and CLP.

Measurements of the ^{13}C-relaxation time of β-CD in aqueous solution were carried out to investigate the effects of TBA and CLP in detail. However the relaxation times of β-CD upon addition of TBA and CLP were so short that accurate values could not be obtained under the present experimental conditions. Consequently, we cannot discuss the molecular dynamics of β-CD here.

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