(Fig. 6). With the antibody to hog kidney mutarotase type II, similar spur formation was observed between precipitin lines formed by the crude extracts from hog tissues and those from bovine tissues (not shown in the figure). The spur formation in these case may be due to partially altered antigenic properties of the enzymes from closely related but different species, *i.e.* rat *vs.* mouse and hog *vs.* beef. The antibody to rat or hog kidney mutarotase type II, however, clearly inhibited the enzyme activity of the crude extract from mouse or bovine tissues, respectively.

The antibody against rat kidney mutarotase type II neither reacted with nor inhibited the enzymes extracted from the tissues of hog, beef, and rabbit. A similar situation was also observed with the antibody against hog kidney mutarotase type II and the tissues of rat (Fig. 5), mouse, and rabbit. These results indicate that the enzyme in rat tissues is quite different from those contained in hog, beef, and rabbit, and that the enzyme in hog tissues is different from those in rat, mouse, and rabbit.

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Solid-State Nuclear Magnetic Resonance Spectroscopy and Raman Spectroscopy of the Inclusion Compound of Tolbutamide with β-Cyclodextrin[1,2]

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A structural study of the inclusion compound of tolbutamide with β-cyclodextrin (β-CD) in the solid state was attempted by means of carbon-13 high resolution solid-state nuclear magnetic resonance (NMR) experiments. The change in chemical shift of tolbutamide suggested that the phenyl moiety of the drug molecule was included in the cavity of β-CD in the solid state. This view was also supported by the change of the C–H out- of -plane vibration of the phenyl moiety of tolbutamide in the Raman spectrum. The solid-state NMR technique appears to be useful for studies on the nature of inclusion compounds of drug molecules with β-CD.

Keywords——^{13}C high resolution solid-state NMR; magic-angle spinning method; cross-polarization; inclusion compounds; β-cyclodextrin; tolbutamide; chemical shift; Raman spectroscopy

As part of a series of physicochemical studies of sulfonylureas,[3] the molecular motions of tolbutamide included with β-cyclodextrin (β-CD) in aqueous solution have been investigated by means of a nuclear magnetic resonance (NMR) technique.[3c] The inclusion compounds of tolbutamide and other sulfonylureas such as acetohexamide with β-CD can be easily obtained as microcrystalline powders, and enhancement of the dissolution rate[4] and bioavailability[5] of such inclusion compounds have been reported.

On the other hand, inclusion compounds of drugs with β-CD have scarcely been investigated by X-ray analysis[6] because of the difficulty in obtaining large single crystals. Therefore, the mechanism of formation of guest molecule/β-CD inclusion compounds in the solid state is still not understood in detail. In recent years, the application of ^{13}C-NMR techniques for the analysis of solid samples has developed dramatically.[7–11]

In the present work, the solid-state NMR technique (cross-polarization/magic-angle spinning method (CP/MAS)) and Raman spectroscopy were used to elucidate in detail the structure of the inclusion compound of tolbutamide with β-CD in the solid state.

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 tolbutamide (mp 128.5—129.5°C) and β-CD were obtained from Hoechst Japan Co., Ltd., and Teijin Ltd., respectively. Preparation of inclusion compound: 2 g of tolbutamide and 15 g of β-CD in 1000 ml of water were sealed in a flask and stirred gently at room temperature for 2 weeks. The inclusion compound precipitated as a microcrystalline powder, which was filtered off, washed with a little water, and then dried under a vacuum at room temperature for 24 h. This powder corresponded to 1:1 tolbutamide/β-CD reported by Uekama.[12]

Methods——^{13}C spectra in solution were observed on a JEOL FX-100 spectrometer operating at 25.01 MHz at 24.5±0.5°C. The ^{13}C chemical shifts were measured relative to external tetramethylsilane (TMS). ^{13}C spectra in the solid state were determined at 15 MHz on a JEOL FX-60Q spectrometer equipped with a magic angle solid-state accessory.[13] Dipolar decoupling and magic angle spinning were employed. The sample spinning rate was 2.5 kHz. With a 0.7 msec cross-polarization contact time and 3~5 s recycle time, ~10000 transients were collected for each sample. All spectra in the solid state were obtained at 30°C. Adamantane, having peaks at 38.3 ppm and 29.3 ppm from TMS, was used as an external reference. The Raman spectra were recorded with a JRS-U1-S laser Raman spectrophotometer, using the 514.5 nm line of an Ar^+ laser.

Results and Discussion

Figure 1 shows ^{13}C-NMR spectra of tolbutamide and tolbutamide/β-CD in aqueous solution; the latter was also discussed in detail in the previous paper.[3c]

The ^{13}C high resolution NMR spectrum of solid samples could not be obtained by using the ordinary pulse Fourier transform technique and spectrometers employed routinely for liquid samples. The following three phenomena seemed to cause this failure: 1) ^{1}H-^{13}C dipolar broadening, 2) long ^{13}C spin-lattice relaxation times, 3) chemical shift anisotropy. However, these problems can now be overcome by means of solid-state NMR techniques. These techniques involve high-power ^{1}H decoupling,[7–11] cross-polarization (CP),[8,9,14,15] and the magic-angle spinning method (MAS).[8,9,16–18]

Figure 2 shows the ^{13}C-NMR spectra of the solid samples obtained by CP/MAS. The spectral lines of the solid samples are not resolved clearly compared with those in solution spectra, but can be assigned by comparison with the spectra in aqueous solution. As shown in Fig. 2-a, the peaks of C_4 and C_5 (adjacent to the nitrogen atom of tolbutamide) were not observed in the solid-state owing to broadening produced by nuclear quadrupole interaction.[19,20] Further, the quaternary carbon atoms (C_6, C_9) of the phenyl moiety show a large separation (*ca.* 7.5 ppm) compared with that in solution (*ca.* 1.2 ppm).

As shown in Fig. 2-b, the spectrum of β-CD is not resolved adequately, and hence the structure of the inclusion compound of tolbutamide with β-CD cannot be discussed on the basis

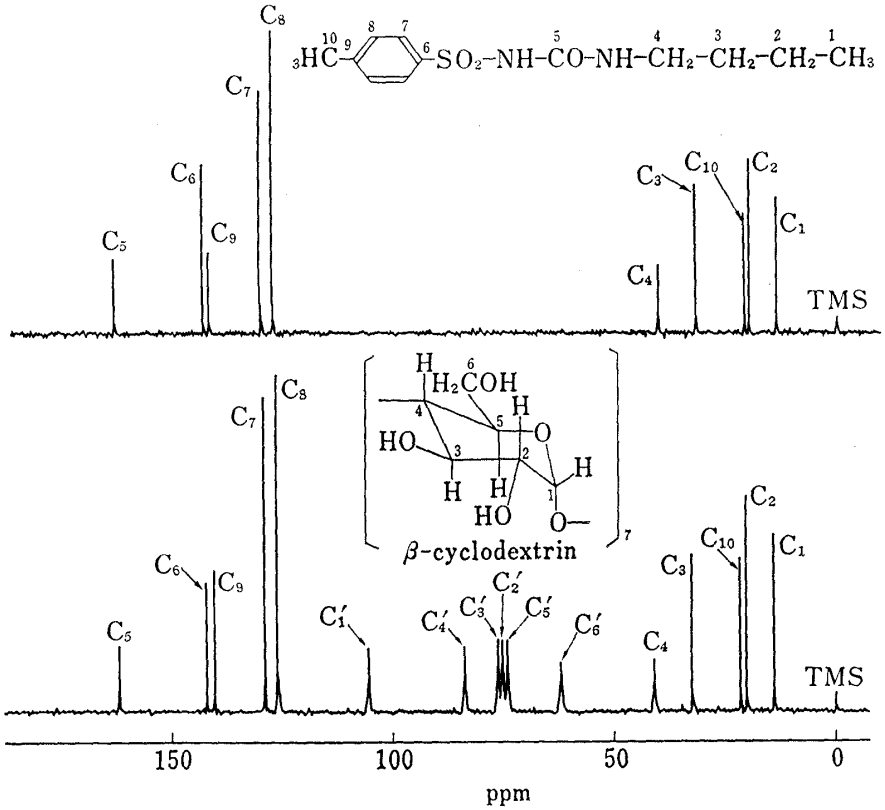

Fig. 1. ^{13}C NMR Spectra of Tolbutamide (Top) and Tolbutamide/β-Cyclodextrin (Bottom) in Solution

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

of the change in the spectrum of β-CD in this study. The C_6' peak of β-CD in Fig. 2-b is weak compared with the case shown in Fig. 2-c. This phenomenon may be a result of differences in conformation, hydrogen bonding, or crystal packing.[21,22] However, more experimental and theoretical investigations are necessary before a difinite explanation can be provided.

As shown in Fig. 2-c the spectrum of the inclusion compound of tolbutamide with β-CD can be approximately regarded as the sum of the spectra of tolbutamide and β-CD. However, only two peaks can be seen for tolbutamide in the low-field range, while four peaks were observed for the drug alone in Fig. 2-a. Considering the location of their chemical shifts, it seems likely that the peaks of C_6 and C_7 in the phenyl moiety shift to overlap with C_9 and C_8 and/or cannot be detected due to line broadening. This result seems to be due to the sterically different situation following the formation of the inclusion compound. Therefore, this result suggested that the phenyl moiety of tolbutamide is included in the cavity of β-CD in the solid state as well as in the liquid state. This view is consistent with the results obtained by Raman spectroscopy, as described later.

Thus, CP/MAS ^{13}C-NMR experiments can give useful information about the conformation of pharmaceutical complexes in the solid state, for which X-ray analysis is not applicable because of the difficulty in obtaining single crystals.

A part of the Raman spectrum of tolbutamide is shown in Fig. 3. In a tolbutamide/β-cyclodextrin (molar ratio 1: 1) physical mixture system, no marked changes in intensity and shift were observed in the overall wave number range (204—1767 cm^{-1}) measured. In the inclusion compound, a marked increase of intensity is observed for the peak at 801 cm^{-1}, which is assigned to the C–H out-of-plane vibration of the phenyl moiety of the tolbutamide molecule. Such a change in intensity at 801 cm^{-1} was not observed on going from tolbutamide alone to a tolbutamide/β-cyclodextrin (1: 1) physical mixture system. The other peaks of

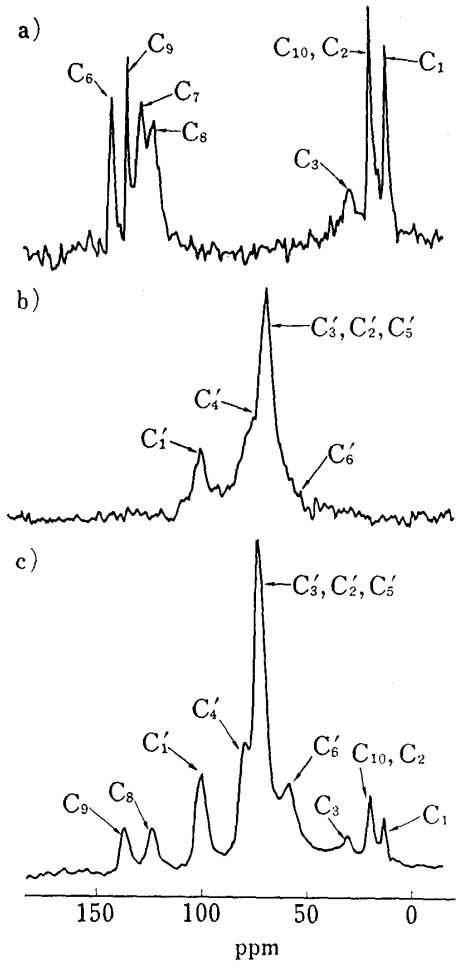

Fig. 2. Magic-Angle Cross-Polarization ^{13}C NMR Spectra of Tolbutamide (Top), β-Cyclodextrin (Middle), and Tolbutamide/β-Cyclodextrin (1 : 1) Inclusion Compound (Bottom)

a) Number of transients(N)=500, b) N=681, c) N=10000.

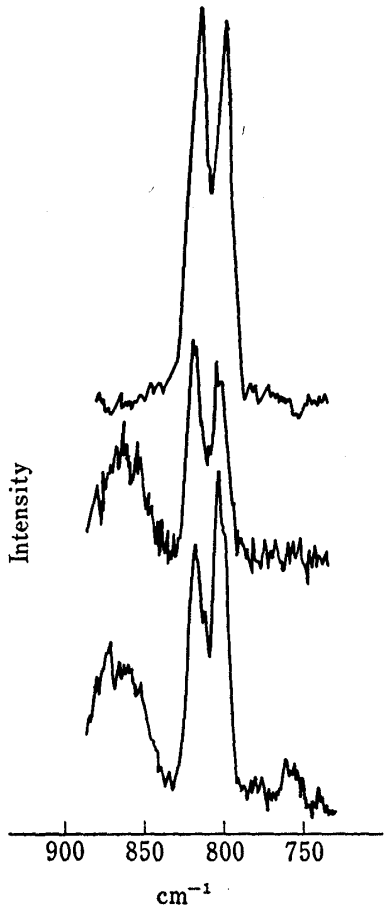

Fig. 3. Raman Spectra of Tolbutamide (Top), Tolbutamide/β-Cyclodextrin (1 : 1) Physical Mixture (Middle), and Tolbutamide/β-Cyclodextrin (1 : 1) Inclusion Compound (Bottom)

the inclusion compound do not show any appreciable changes. It is well known that changes of relative intensity in the Raman spectrum are attributable to intramolecular changes of the polarizability component.[23,24] Consequently, it seems that the phenyl moiety of tolbutamide is involved in the formation of the inclusion compound.

The structure of tolbutamide/β-CD inclusion compound in the solid state is being investigated in more detail by improving the measurement conditions.

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Controlled Release of Prednisolone from Ethylene-Vinyl Acetate Copolymer Matrix[1)]

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Ethylene-vinyl acetate (EVA) copolymer was evaluated as a carrier for controlled release of prednisolone. The vinyl acetate content of EVA copolymer varied from 8 to 33% w/w. Increase in vinyl acetate comonomer content of EVA copolymer matrix brought about an increase in the release rate. The release rate could be controlled by modifying the ethylene-vinyl acetate ratio in the polymer matrix. Fabrication parameters such as matrix coating and drug content also significantly affected the release kinetics. Matrices composed of EVA copolymer could be useful vehicles for the controlled release of the corticosteroid.

Keywords——ethylene-vinyl acetate copolymer; comonomer ratio; biomaterial; controlled release; drug delivery; prednisolone

Many techniques have been utilized to develop controlled or sustained release drug delivery systems. The release rate of a drug from a polymer matrix may be controlled by variations of the dimensional parameters, the drug concentration, and the polymer system. A wide range