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Characterization and structural study of inclusion compounds of α-keto acids with cyclodextrins

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α-Keto acids have characteristic unpleasant smell and chemical instability, which makes the usages of the materials difficult for pharmaceutical purposes. However, the inclusion compounds of the acids with cyclodextrins have no or slight smell and are chemically stable. Especially, an inclusion compound of optically active s-α-keto-β-methylvaleric acids (S-KMV) kept its activity for at least 120 days at room temperature, unlike its salt of basic amino acid. We tried to investigate the solid state structure of the inclusion compounds S-KMV/β-CD by the use of CPMAS ^{13}C NMR spectroscopy. The comparison of the spectra of S-KMV/β-CD and KMV·1/2Ca·H_2O suggested that the S-KMV molecule is not so tightly included inside the β-CD cavity, but as a result of rotational isomerism restriction, it exists in a single conformer.

INTRODUCTION

α-Keto-acids, particularly many of those which are precursors of essential and quasi-essential amino acids, are intimately involved in the metabolism of living bodies and are hence effective for the nourishment, treatment and prevention of disease in patients suffering from renal disorders, hepatic diseases and other protein- and nitrogen-wasting diseases.[1]

However, the problems associated with the administration of an α-keto-acid to the patients are its unpleasant odor and its instability. The α-keto-acid may be used either as a free acid, or a salt of sodium, calcium, or basic amino acids. However these include the problems mentioned above or others. The free acid shows poor stability. The sodium salt adversely affects patients suffering from renal diseases. The calcium salt necessitates intake of a large amount of water due to its low solubility. A basic amino acid salt is not stable enough and storage in ordinary circumstances results in marked degradation. Its optical activity decreased steeply at room temperature and decreased gradually even at −20 °C. It is reported that an optically active sodium α-keto-acid in solution rapidly lost its activity.[2]

We obtained odorless and highly stable inclusion compounds of the acids with cyclodextrins.[3] In the present report, the methods of preparation, the stability of the products and a structural investigation by the use of CPMAS ^{13}C NMR spectroscopy are reported.

RESULTS AND DISCUSSION

Formation of inclusion compounds

The compositions of the inclusion compounds are listed in Table 1 together with their melting point data. The α-keto-acid/β-CD inclusion compounds, except for the indole-3-pyruvic acid (IPA)/β-CD, gave stoichiometric molar ratios, although in other cases it varied

Table 1 Components and melting point of inclusion compounds α-keto acids and cyclodextrins

		Components [Wt %]				
Guest	*Host*	*KA*	*CD*	*H2O*	*Molar ratio [KA/CD]*	*Melting point [°C]*
KMV	β-CD	9.8	83.2	5.4	1.0	218–223
KIV	β-CD	7.7	86.7	6.3	0.9	215–218
KIC	β-CD	10.0	88.0	3.1	1.0	220–225
	γ-CD	14.0	87.0	4.0	1.6	215–221
PPA	β-CD	10.9	87.5	5.6	0.9	220–223
	γ-CD	13.9	74.0	5.1	1.5	205–213
IPA	β-CD	10.0	80.9	7.5	0.7	264–272
PA	α-CD	7.0	88.0	4.3	0.9	218.222

KA: α-keto acid, CD: Cyclodextrin, KMV: α-keto-β-methylvaleric acid, KIV: α-keto-isovaleric acid, KIC: α-keto-isocaproic acid, PPA: β-phenylpyruvic acid, IPA: indol-3-pyruvic acid, PA: pyruvic acid.

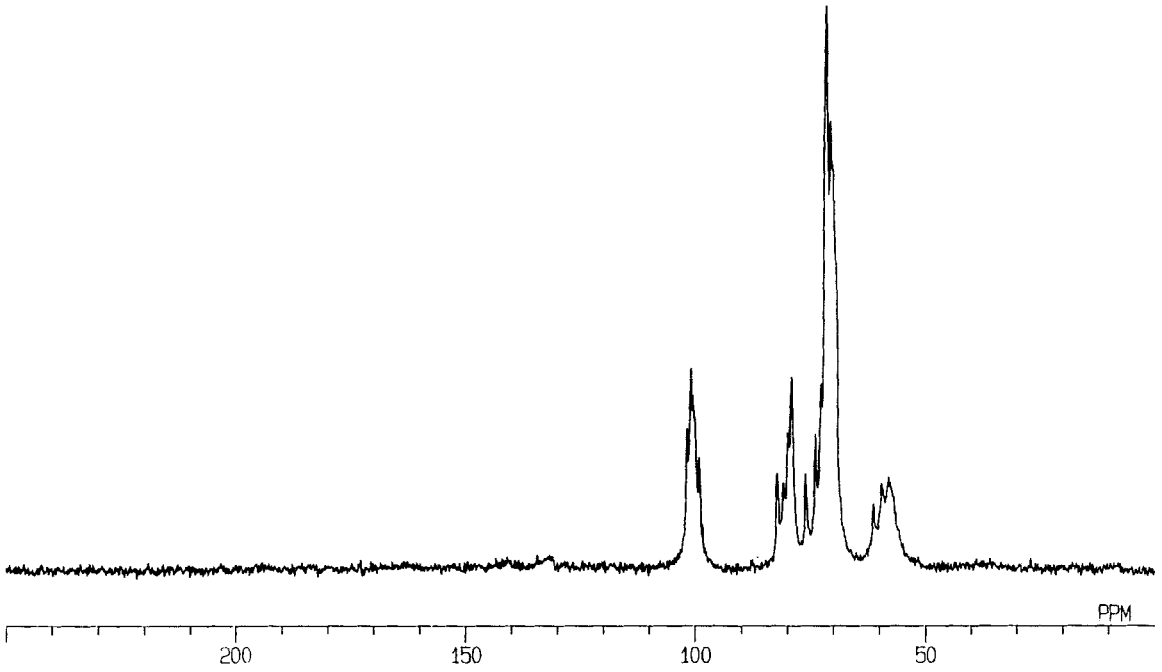

Figure 1a ^{13}C CP MAS-NMR spectrum of β-CD hydrated.

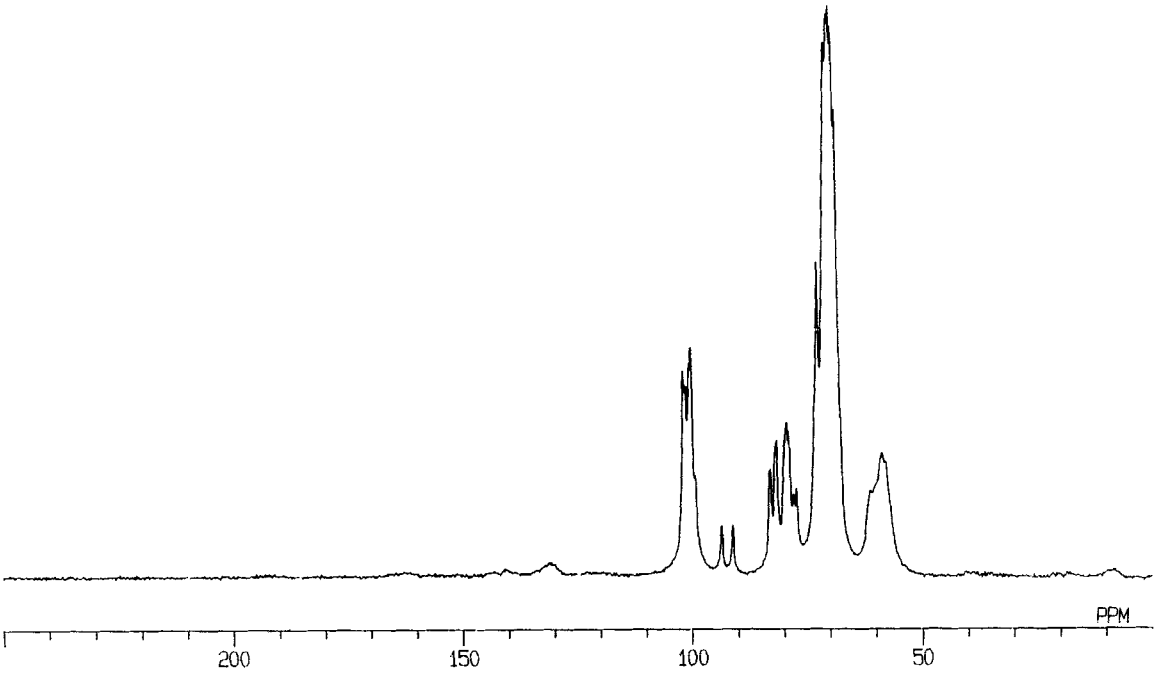

Figure 1b ^{13}C CP MAS-NMR spectrum of β-CD dehydrated.

non-stoichiometrically from 0.7 to 1.6. The products contains slight moisture even after overnight drying in a vacuum over at normal temperature and had almost no or a slight mint smell and a slight fruit taste. No compound had the unpleasant smell typical to keto acids.

Solid state NMR of β-CD

The CPMAS ^{13}C NMR spectrum of decahydrated β-CD is shown in Fig 1a. The C1 and C4 carbons of the host molecule seem to split into 7 lines of 1:2:2:1:1 and 1:1:1:3:1 patterns, respectively. In the case of dehydrated (0.9 hydrated) β-CD, as in Fig 1b, new

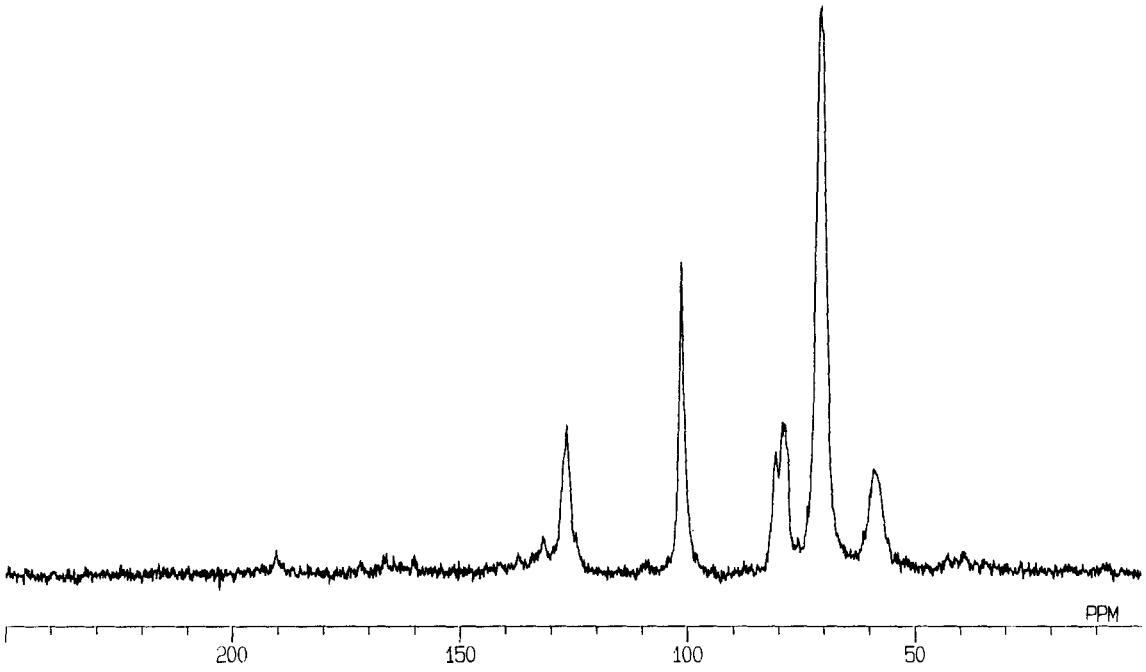

Figure 2a ^{13}C CP MAS-NMR spectrum of hydrated PPA/β-CD inclusion compound crystal.

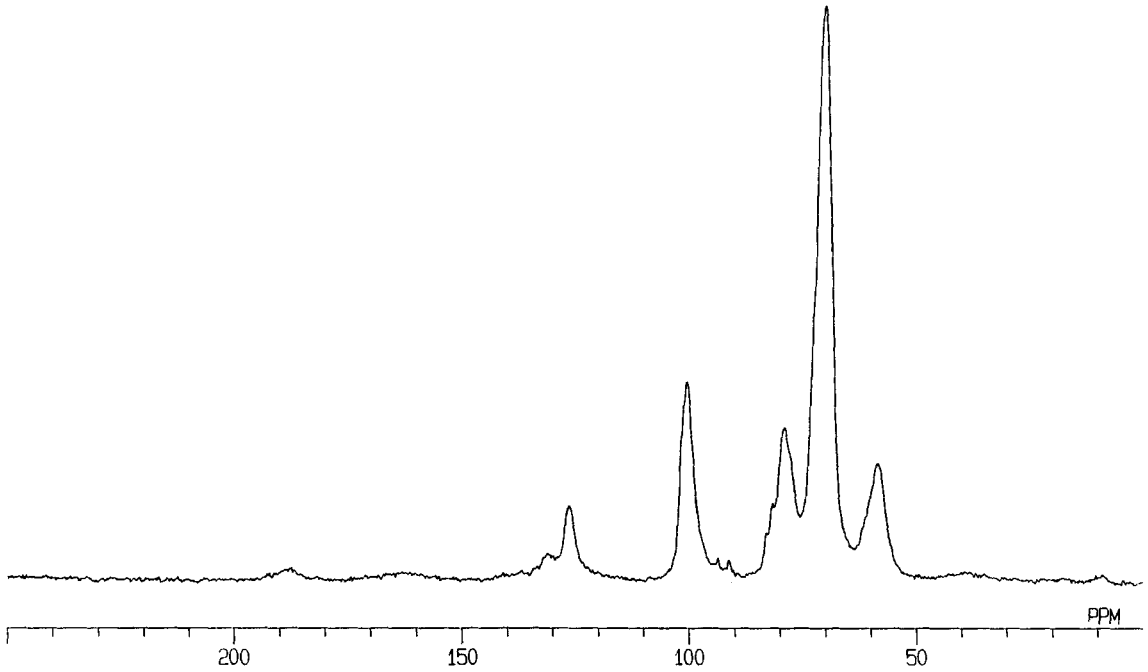

Figure 2b ^{13}C CP MAS-NMR spectrum of dehydrated PPA/β-CD inclusion compound crystal.

lines for the C1 carbons at 101.8, 93.5 and 91.1 appeared, reflecting the diversification of the state of the carbons. C4 carbons showed downfield shifts but the degree of change was less than that of C1. These results coincide with one previously reported on α-CD.[4]

Solid state NMR of aromatic inclusion compounds

Figure 2a shows a spectrum of the hydrated (9.5 hydrated) PPA (phenylpyruvic acid)/β-CD inclusion compound. The lines of the carbons assigned to β-CD changed, indicating the occurrence of PPA molecule inclusion. The C1 carbon lost is multiplet and

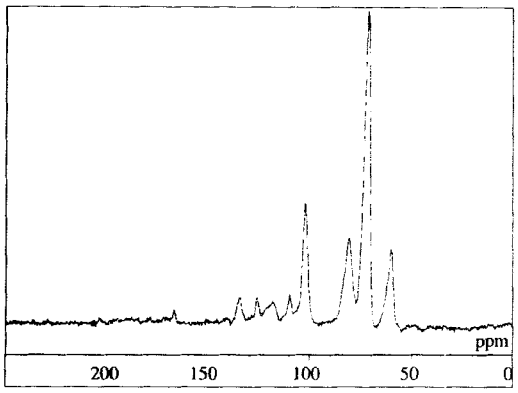

Figure 3 C CP MAS-NMR spectrum of IPA/β-CD inclusion compound crystal.

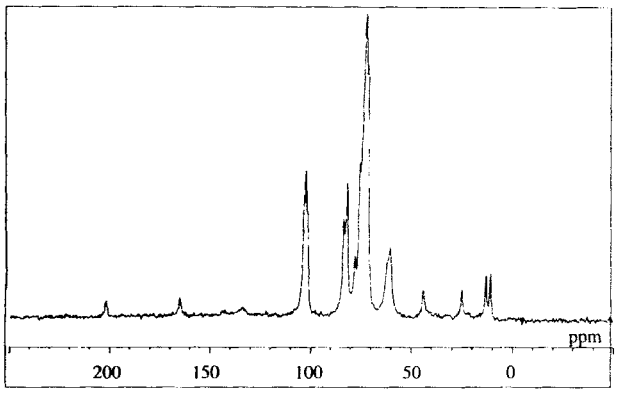

Figure 4 ^{13}C CP MAS-NMR spectrum of S-KMV/β-CD inclusion compound crystal.

converged to one line at 101.2 ppm with an upfield shoulder. The C4 carbon seems to have the same tendency. The same results were reported for the inclusion compounds of *p*-nitrophenol/β-CD[5] and hydrocortisone/β-CD.[4] These phenomena are thought to indicate the equivalency of the respective C1 and C4 host atoms of inclusion compounds suggesting the rotation of the guest molecule inside the host.

Figure 2b shows a spectrum of the dehydrated (0.8 hydrated) PPA/β-CD inclusion compound. The single line at 101.2 ppm in Fig 2a is shifted upfield (100.4 ppm) and broadened. The lines of the C4 carbons are also broadened. Comparison of the figures of dehydrated β-CD with and without guest in Fig 1b and 2b, makes it clear that the C1 and C4 atoms of β-CD had basically the same configurations whether the guest included or not. Inclusion only induced broadening. The loss of water would have decreased the mobility of the β-CD molecule. The same tendency in spectrum was also reported in the case of *p*-nitrophenol/β-CD case,[5] where dehydration caused a splitting of the lines of C1 and C4. To know the state of inclusion from the signals of the host carbons, it is preferable to use the NMR patterns of the hydrated states of β-CD and its inclusion compounds.

Figure 3 shows a spectrum of the hexahydrate IPA(indol-3-pyruvic acid)/β-CD inclusion compound with the same phenomenon as that of PPA/β-CD, although the resolution was lower.

Solid state NMR of S-KMV inclusion compounds

We investigated the state of (S)-keto-β-methylvaleric acid (S-KMV) in the inclusion compound with β-CD by CP MAS ^{13}C NMR spectroscopy. S-KMV has an asymmetric carbon at the β-CH. We had selected KMV·1/2Ca·H_2O as the reference substance for this included guest molecule, although free KMV would have been preferable, because of the intolerable smell of the free compound. The CPMAS ^{13}C NMR spectra of the S-KMV/β-CD inclusion compound and of KMV·1/2Ca·H_2O are shown in Figs 4 and 5. The peaks of KMV were readily assigned with ordinary ^{13}C NMR in deuterium oxide solution (Table 2).

The lines assigned to the β-CD carbons (Fig 4) had the same positions as those of PPA/β-CD in Fig 2a. The sharp lines assigned to S-KMV are worth noting. According to the rather greater regional mobility in the complex, there are no side bands of C=O and COOH groups of S-KMV comparable to that of the

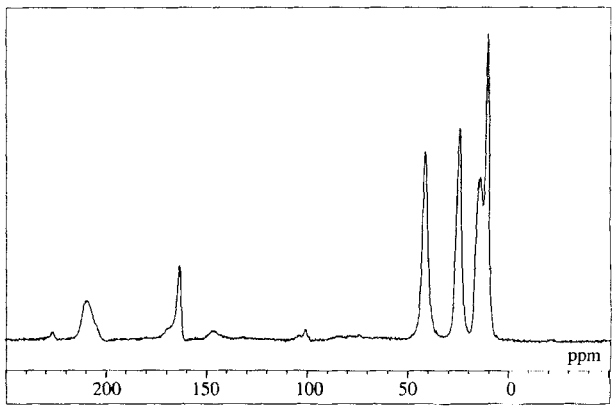

Figure 5 ^{13}C CP MAS-NMR spectrum of KMV·1/2Ca·H_2O crystal.

Table 2 Assignment of ^{13}C NMR chemical shift of KMV [ppm]

Carbon	*KMV·1/2Ca·H_2O* δo	*KMV/β-CD* δ
β-CH	42.0	43.4
γ-CH2	25.2	24.8
γ-CH3	14.7	12.7
δ-CH3	11.4	10.8
C=O	210	204
COOH	164	165

calcium salt (Fig 5). This seems to show that the S-KMV molecule is not so tightly included inside the β-CD cavity, but as a result of rotational isomerism restriction, it exists in a single conformer. The sharp γ-CH_3 line seems to support this idea. That S-KMV seems to exist in a single conformer explains the stabilization mechanism of the molecule included in CD.

EXPERIMENTAL

Materials

α-Keto-isocaproic acid (KIC), α-keto-isovaleric acid (KIV) were prepared by distillation at acidic pH of 1/2Ca salt of each acid obtained from Daiichi Pure Chemicals Co. (S)-Keto-β-methylvaleric acid (S-KMV) was prepared enzymatically from L-isoleucine. Calcium salt of α-keto-β-methylvaleric acid (KMV·1/2Ca·H_2O): Daiichi Pure Chemical Co., pyruvic acid: Wako Pure Chemical Industries, β-phenylpyruvic acid (PPA): Sigma Chemical Co., indole-3-pyruvic acid (IPA): Aldrich Chemical Co. and α-, β-, γ-cyclodextrin: Mercian Corporation.

β-CD used for NMR measurement was precipitated from water, filtered and dried at room temperature overnight for hydrated sample, or at 60 °C and 4 hours in vacuo for the dehydrated one.

Formation of the inclusion compound

To a suspension of CD in water of 250 to 330 g/l, 0.4 to 0.6 mol/l of α-keto acid was added and mixed at ambient temperature for one hour. The formed material precipitated was separated and dried overnight in a vacuum oven at room temperature.

A PPA/β-CD inclusion compound used for NMR measurement was dried at room temperature overnight for hydrated sample, or at 70 °C and 4 hours in vacuo for the dehydrated one.

Measurement of optical activity

The KMV/β-CD inclusion compound was suspended in water following pH adjustment to below 1 by hydrochloric acid. KMV was extracted with ethyl acetate and a calcium salt of KMV was precipitated by adding water and calcium chloride to the extract and adjusting pH to 4 to 6 with sodium hydroxide. The optical activity of the salt at 1% solution in 6N hydrochloric acid was measured by Perkin Elmer polarimeter model 241.

Solid state NMR

Solid state NMR, i.e. cross polarization magic angle spinning ^{13}C NMR(CPMAS ^{13}C NMR) was measured at room temperature with a Bruker AC-250 NMR spectrometer at ^{13}C Larmor frequency of 62.9 MHz for crystals of IPA/β-CD and with a JEOL EX-400 NMR spectrometer at 100 MHz for crystals of β-CD, PPA/β-CD, KMV/β-CD and KMV·1/2Ca·H_2O.

Analyses and determination

α-Keto acids were analyzed with HPLC of the resin Inertsil C8 of GL Sciences Co., and of acetonitrile/0.05 M phosphoric acid (13/87 v/v) as a mobile phase. Cyclodextrins were analyzed with HPLC of the resin GS220H of Asahi Chemical Industry Co. and water as a mobile phase.

Water content was determined by Karl Fischer's analysis.

Melting point was determined with an apparatus of model MP-1 of Ishiishoten Co.

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