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14. A rapid survey of organic chemistry teaching manuals shows this to be the method of choice for carrying out the experiment at the undergraduate level.

Inclusion Complex of Permethylated-β-Cyclodextrin with Benzaldehyde

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A stable solid new inclusion complex with benzaldehyde and permethyl-β-cyclodextrin was obtained by recrystallization method. The structure of the benzaldehyde-permethyl-β-cyclodextrin inclusion complex in the solid and solution state have been studied by UV, IR, ^{1}H-NMR, ^{13}C-NMR and FAB-mass spectroscopy.

Introduction

In the past decade, cyclodextrins (CDX) have received considerable attention in biological and pharmaceutical research[1-3]. Because of their unique physical and chemical properties, these compounds serve as complexing agents for drugs in controlled drug delivery formulation and as enzyme models, etc. Microencapsulation of drugs can lead to desirable modification of the physical and chemical properties and improvements in stability of air- or light- sensitive substances, water solubility, suppression of unpleasant taste or odor and *in vivo* bioavailability. Cyclodextrins therefore have been extensively applied to improve the physicochemical properties of various drugs[4,5].

Cyclodextrins are cyclic oligosaccharides composed of at least six α-1,4 linked α-D-glucosyl residues, which have the shape of a hollow, truncated cone with primary and secondary hydroxyl groups crowning the narrower and the wider ends, respectively. In the solid state and in solution state, they can form inclusion complexes with a variety of guest molecules. Methylated cyclodextrins can form inclusion complexs with several guest molecules, some of which are more stable than the corresponding unmodified cyclodextrin complexes.

The pharmaceutical use of benzaldehyde was first attempted in the use of fig fruit (Ficus carica L.) as a traditional carcinostatic drug. Kochi *et al.*[6] have been interested in the clinical use of fig and they had observed suppression of Ehrlich carcinoma in mice by its extracts. The active component in the fig was benzaldehyde as revealed by GC-MS, UV and IR methods. To determine the carcinostatic activity of the purified authentic benzaldehyde, 100 mg/kg dose was injected daily intraperitoneally to seven BDF mice which had been implanted with adenocarcinoma 755 (AC755) subcutaneously 24 hours prior to administration with the benzaldehyde. A 40% inhibition of the tumor cell growth compared to the untreated mice was reported.

However, benzaldehyde (BAL) is an oily liquid and has some unfavorable properties, such as instability in air and light, low water solubility, and unpleasant odor. The pharmaceutical use of benzaldehyde is therefore limited. Thus, some modification is required in order to use benzaldehyde as an orally acceptable drug. Upon complexation with cyclodextrins, benzaldehyde become powdered and can be conveniently manufactured as tablets[7].

Benzaldehyde was first used in inclusion complexation with α, β, and γ-cyclodextrin by Takeuchi *et al.*[7]. The X-ray crystal structure of the α-cyclodextrin-benzaldehyde (1 : 1) complex hexahydrate was determined[8]. The crystal structure of the hexakis (2,3,6-tri-o-methyl) α-cyclodextrin-benzaldehyde (1 : 1) complex was also investigated by X-ray method[9]. Studies of the benzaldehyde inclusion complexes with α-, β-, and permethylated-α-cyclodextrin by solid state ^{13}C-NMR spectroscopy provide further solid state information. The molecular dynamics and mobility of benzaldehyde inclusion complexes with cyclodextrins was studied by observing chemical shift changes and line-widths in solid state ^{13}C-NMR spectra of these complexes. These spectral studies were also applied to propose the benzaldehyde and cyclodextrins com-

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plexes structural orientation in the solid state. These structural results were comfirmed by X-ray results[8,9].

The solid complex of benzaldehyde with three cyclodextrins was prepared by Uekama *et al.*, with molar ratios found to be 1:1 (BAL/α-CDX), 3:2 (BAL/β-CDX) and 2:1 (BAL/γ-CDX)[11]. The stoichiometries of the complexes in solid phase were analyzed on the basis of data in the plateau region of the solubility diagrams. The shapes of the β-, γ-cyclodextrin-benzaldehyde complex solubility curves have not been explained fully, therefore the ratios of these complexes were ambiguous.

In this study a better defined recrystallization method was used to prevent presence of any artifacts from the precipitation of free permethyl-β-cyclodextrin and permethyl-β-cyclodextrin-benzaldehyde complex.

Experimental

Instrumentation

NMR Spectroscopy. All structural studies of complexes by ^{1}H-NMR were recorded on Nicolet NT-470 spectrometer with 16 K computer memory operating at 469.5 MHz. DMSO-d_6 (Aldrich Chemical Co.) was used as external reference with a signal at 3.03 ppm relative to TMS (at 0.0 ppm) for ^{1}H-NMR spectra. A 5 mm sample tube was used.

Proton decoupled ^{13}C-NMR spectra were run on a Nicolet NT-200 spectrometer operating at 50.31 MHz. DMSO-d_6 (Aldrich Chemical Co.) was used as external standard to DSS (0.0 ppm) for ^{13}C-NMR spectra. More than 5000 accumulations of interferograms, 32 K data points and a 12 mm sample tube was used. The concentration of NMR samples was 10 mM and 0.1 M deuterated phosphate buffer (pD 7.4) was used for the solvent. All 470 MHz ^{1}H-NMR spectra spins (7 spins) were calculated by the Raccoon spin simulation program.

UV/VIS Spectroscopy. UV/VIS measurements were made on a Beckman DU-7HS spectrophotometer controlled by a built-in high speed microprocessor. The UV/VIS spectra obtained were recorded on a Beckman video copier interfaced with DU-7HS spectrometer.

IR Spectroscopy. IR spectra were obtained with the Perkin-Elmer Model 1600 FT-IR spectrophotometer which included 16 K of battery-backed memory, software with extensive graphics and data processing capability, based on Perkin-Elmer's CDS-3 Infrared data system. The model 1600 can operate in single beam ratio, single beam, or interferogram mode. The IR spectral total range was from 7800 to 100 cm^{-1} with 4 cm^{-1} resolution. All samples for IR spectral analysis were prepared by the KBr (Aldrich Chemical Co.) disc method at room temperature. Samples of benzaldehyde and KBr were prepared in a similar manner except that the ingredients were mixed under nitrogens. All spectra were obtained from 4000 to 600 cm^{-1}.

Mass Spectrometry. The Fast Atom Bombardment (FAB) mass spectra were obtained with a Kratos MS-50 sector mass spectrometer utilizing 3:1 dithiothreitol/dithioerythritol as the matrix. An accelerating voltage of 8 KV and 100 sec/dec scanning rate were used for the experiments.

Chemicals

β-cyclodextrin was obtained from Chemical Dynamics Corp. South Plainfield, NJ. Permethyl-β-Cyclodextrin (PM-β-CDX) was prepared following a modification (dimethylformamide was used instead of dimethylsulfoxide) of the procedure reported by Szejtli *et al.*[12].

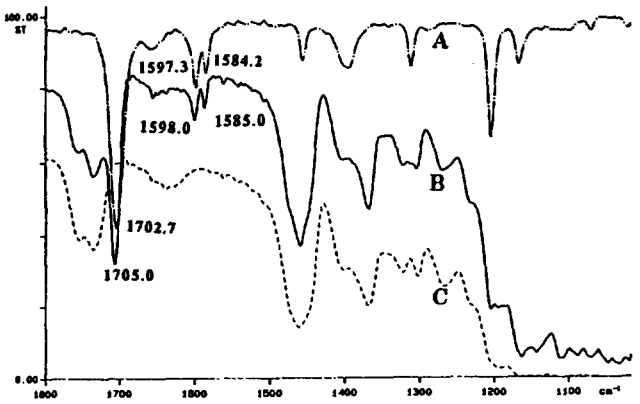


Figure 1. IR absorption spectra of (A) benzaldehyde, (B) permethyl-β-cyclodextrin-benzaldehyde complex, and (C) permethyl-β-cyclodextrin.

Methods

Preparation of Phosphate Buffer. Phosphate buffer was prepared by mixing precalculated amounts of monobasic and dibasic phosphate stock solutions.

To prepare the deuterated buffer, the regular buffer was lyophilized, exchanged once with 99.5% D_2O (Aldrich Chemical Co.) and redissolved to the original volume with 99.5% D_2O.

Preparation of the Permethyl-β-Cyclodextrin Benzaldehyde Inclusion Complex. Permethyl-β-cyclodextrin (0.715 g, 0.5 mmole) was dissolved in 20 m*l* double distilled water at room temperature. An equimolar quantity of benzaldehyde (50 μ*l*, 0.5 mmole) was added to this solution. This solution was then sealed in a flask using a rubber septum under nitrogen.

This solution was stirred at 80℃ until white precipitate appeared. This precipitate was filtered and washed with few drops of water. This complex was recrystallized again in the 80℃ distilled water, then filtered and dried in low vacuum at room temperature for 24 hours to yield 0.536 g, 69.8%; mp. 112℃. The 470 MHz ^{1}H-NMR spectral integration showed 1:1 ratio complex of permethyl-β-cyclodextrin-benzaldehyde. These spectral data will be analyzed in the results and discussion section.

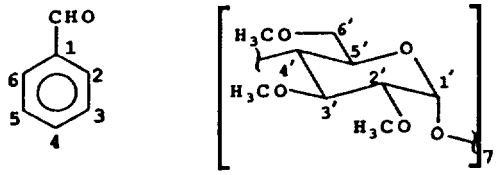
Results and Discussion

Permethyl-β-Cyclodextrin-Benzadehyde Inclusion Complex

Permethyl-β-cyclodextrin was synthesized to make a new inclusion complex with benzaldehyde. The structure of this new complex was first studied by FT-IR spectroscopy, followed by UV, ^{1}H-NMR, ^{13}C-NMR, mass spectrometry studies for the solution structure.

Infrared and Ultraviolet Spectroscopy. The IR spectrum of this solid complex obtained by the KBr disk method was analyzed. Comparisons of the absorption frequencies of benzaldehyde with those of permethyl-β-cyclodextrin inclusion complex are shown in Figure 1. The carbonyl stretching

Table 1. 470 MHz ^{1}H-NMR Chemical Shift of Benzaldehyde Before and After Complexation with Permethyl-β-Cyclodextrin in pD 7.4 0.1 M Phosphate D_2O Buffer. DMSO-d_6 was Used as an External Reference



Protons	Permethyl-β-CDX -benzaldehyde 1 : 1 Complex (ppm)	Permethyl-β-CDX (ppm)	Benzaldehyde (ppm)	Difference (Hz)
CHO	9.9810		9.9350	21.62
2,6	8.0080		7.9590	23.00
3,5	7.6760		7.6250	23.94
4	7.8040		7.7600	20.66
1′	5.2820	5.2820		0
2′	3.3480	3.3480		0
3′	3.6590	3.6840		−11.75
4′	3.7480	3.7465		0.71
5′	3.8440	3.8580		−6.58
6a′	3.8550	3.8450		4.70
6b′	3.6720	3.6485		11.05
2′-OCH_3	3.6130	3.6030		4.70
3′-OCH_3	3.5240	3.5120		5.64
6′-OCH_3	3.4000	3.3790		9.87

band and the phenyl bands were found to shift to larger wavenumbers than benzaldehyde alone. The UV molar extinction coefficient of the permethyl-β-cyclodextrin-benzaldehyde 1 : 1 complex was 46280 $cm^{-1}M^{-1}$ at 249.5 nm and it was found to be different from that of benzaldehyde alone 52180 $cm^{-1}M^{-1}$. No peak maximum shift of the complexed benzaldehyde was observed.

Proton Nuclear Magnetic Resonance Spectroscopy Analysis. Integration of the 470 MHz ^{1}H-NMR spectrum of the permethyl-β-cyclodextrin-benzaldehyde complex in neutral solution showed a 1 : 1 molar ratio. The ^{1}H-NMR chemical shift changes are summarized in Table 1. The chemical shift and coupling constants of permethyl-β-cyclodextrin and its benzaldehyde complex were calculated by the seven spin simulation computer program. These results are shown in Figures 2-4 and Table 2. The significant downfield shift of the aldehyde and all phenyl ring protons indicated that the entire molecule may be included in the hydrophobic cavity of permethyl-β-cyclodextrin. The downfield shifts of the phenyl protons are interpreted as being due to the absense of aromatic ring stacking upon complexation. Most importantly the upfield shifts of H-3′ and H-5′ of permethyl-β-cyclodextrin explained by the anisotropic effect of an aromatic ring current. These results strongly indicated that the aromatic moiety was included in the cavity.

^{13}C-NMR Analyses. The solution state structure of the permethyl-β-cyclodextrin benzaldehyde were studied by

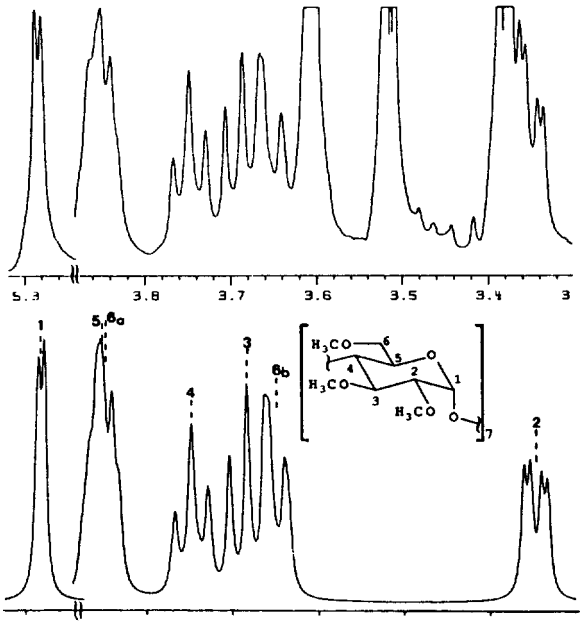


Figure 2. 470 MHz ^{1}H-NMR spectrum of permethyl-β-cyclodextrin (top) and its spin simulated spectrum (*bottom). *2,3,6-O-CH_3 protons are not included in the spin simulation.

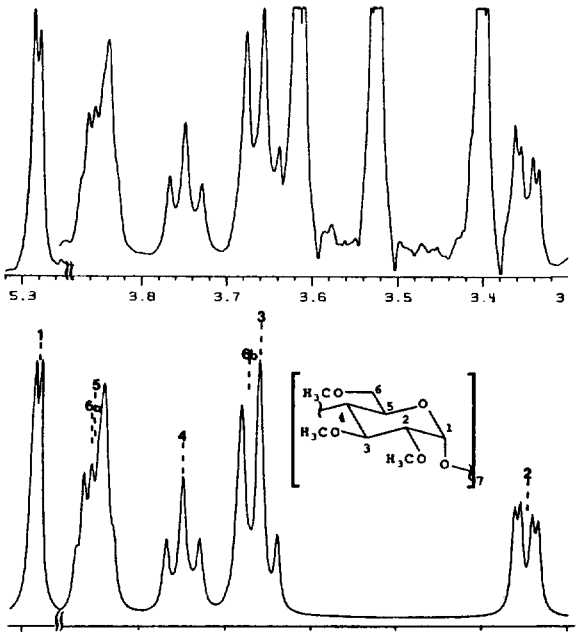


Figure 3. 470 MHz ^{1}H-NMR spectrum of permethyl-β-cyclodextrin-benzaldehyde (top) and its spin simulated spectrum (*bottom). *2,3,6-O-CH_3 protons are not included in the spin simulation.

^{13}C-NMR spectroscopy. These spectra are shown in Figure 5 and data summarized in Table 3. There are at the four parameters which could contribute to changes in the ^{13}C chemical shifts experienced by the cyclodextrins upon complexation with benzaldehyde. These parameters are; removal of water from the cavity, shielding by the guest's aromatic π cloud, conformational changes, and steric interactions. The peak assignments were based on the work published by Gerb

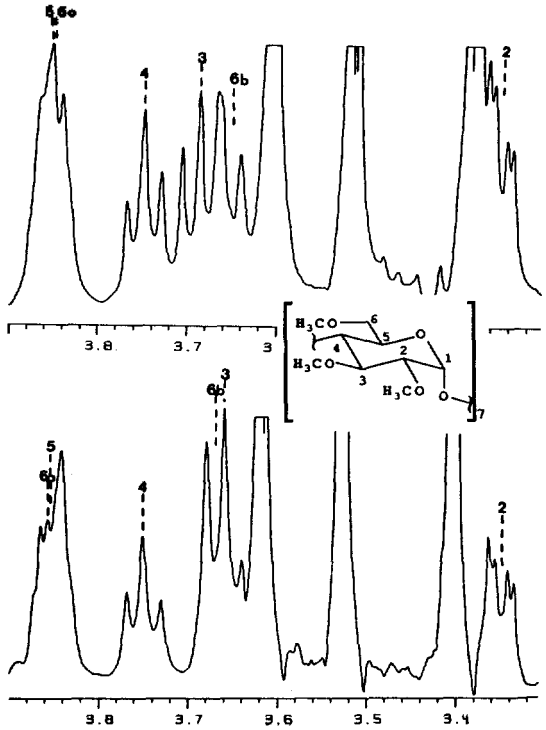
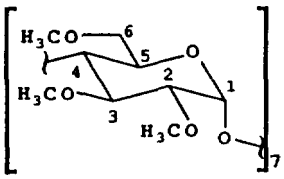


Figure 4. 470 MHz ^{1}H-NMR spectra of permethyl-β-cyclodextrin (top) and permethyl-β-cyclodextrin-benzaldehyde (bottom).

Table 2. 470 MHz ^{1}H-NMR Computer-Simulated Spectra Data for Permethyl-β-Cyclodextrin and Permethyl-β-Cyclodextrin-Benzaldehyde Complex



Protons	Permethyl-β-CDX	Permethyl-β-CDX-BAL
	Chemical shift (ppm)	
1	5.2820	5.282
2	3.3480	3.348
3	3.6840	3.659
4	3.7465	3.748
5	3.8580	3.844
6*a*	3.8450	3.855
6*b*	3.6485	3.672
	Coupling constant (Hz)	
J_{12}	3.5	3.5
J_{15}	−1.5	−0.5
J_{23}	9.7	9.7
J_{34}	9.2	9.2
J_{45}	9.5	9.5
J_{46a}	−1.0	−0.5
J_{46b}	−0.5	−0.5
J_{56a}	3.5	4.2
J_{56b}	2.0	2.0
J_{6a6b}	−10.0	−11.0

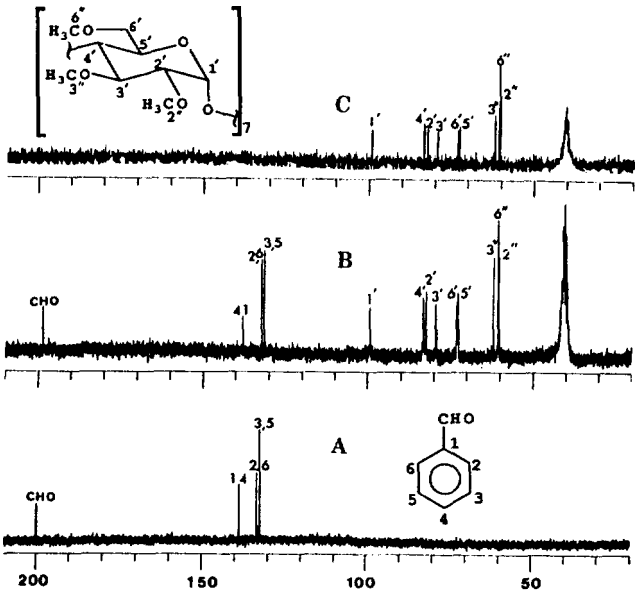
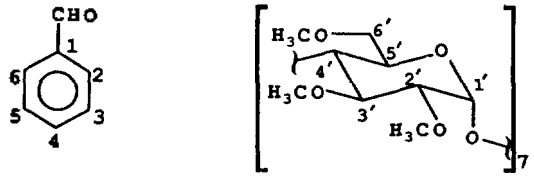


Figure 5. 50.3 MHz ^{13}C-NMR spectra of (A) benzaldehyde, (B) benzaldehyde-permethyl-β-cyclodextrin, (C) permethyl-β-cyclodextrin.

Table 3. 50.3 MHz ^{13}C-NMR Chemical Shift of Benzaldehyde Before and After Complexation with Permethyl-β-Cyclodextrin in 0.1 M Phosphate D_2O Buffer pD 7.4, DMSO-d_6 was Used as an External Reference



	Carbons	Permethyl-β-CDX -benzaldehyde 1:1 Complex (ppm)	Permethyl-β-CDX (ppm)	Benzaldehyde (ppm)	Difference (Hz)
	CHO	198.080		199.294	−61.06
	1	137.471		138.466	−50.05
	2,6	131.873		132.719	−42.55
	3,5	131.051		131.830	−39.18
	4	137.311		138.087	−39.03
	1′	98.900	99.800		−45.27
a	2′	81.819	82.788		−48.70
a	3′	78.949	79.732		−39.38
a	4′	82.719	83.722		−50.45
	5′	72.209	73.188		−49.23
	6′	72.535	73.758		−52.46
b	2′-OCH_3	59.863	60.896		−51.96
b	3′-OCH_3	61.542	62.472		−46.78
	6′-OCH_3	60.175	61.202		−51.66

[a] assignments may be reversed within set C_2', C_3', C_4'. [b] assignments may be reversed within set C_2', C_3'.

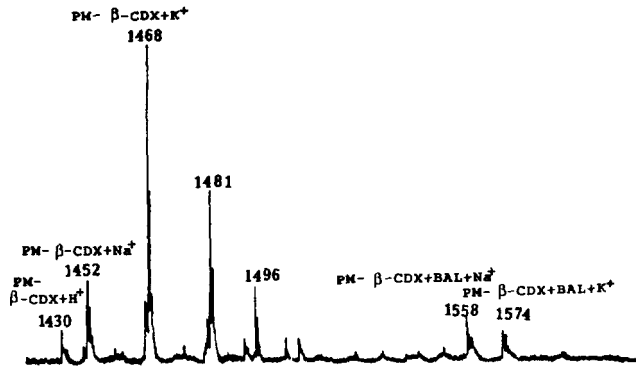


Figure 6. FAB mass spectrum of permethyl-β-cyclodextrin-benzaldehyde complex.

et al.[13]. Previous ^{13}C-NMR studies of cyclodextrin inclusion complexes with benzoic acid, p-nitro phenol, and p-nitro phenolate in aqueous solution have shown that the included lead (head) carbons are largely shielded (upfield shift) compared to the deshielding of corresponding para (tail) carbons[14,15]. Thesse upfield shifts have been attributed to the changes of the electric environment of aqueous solvent (high dielectric constant) to that of the nonpolar cavity (low dielectric constant). Similar distinctive patterns of ^{13}C displacements have been observed for benzaldehyde. The aldehyde carbon is more shielded (upfield shift) than the phenyl cabons. These shifts are induced by transference of the benzaldehyde from the free state, surrounded by polar water molecules, to the relatively nonpolar permethyl-β-cyclodextrin cavity. These results indicated that the aldehyde group was the leading group inserted in the hydrophobic cavity. The large upfield shifts of permethyl-β-cyclodextrin after complexation may be due to the presence of steric effect upon strong complexation.

Mass Spectrometry. The FAB mass spectra of permethyl-β-cyclodextrin-benzaldehyde complex and shown in Figure 6. The m/z of 1558 and 1574 are assigned to the sodium ion (permethyl-β-CDX-BAL$-$Na$^+$) and potassium ion (permethyl-β-CDX-BAL+K$^+$) adducts of permethyl-β-cyclodextrin-benzaldehyde 1 : 1 complex, respectively. The peak at m/z 1430, 1452, and 1468 are attributed to the β-cyclodextrin+H$^+$, β-cyclodextrin+Na$^+$ and β-cyclodextrin+K$^+$, respectively. The appearance of the molecular ion peaks for permethyl-β-cyclodextrin-benzaldehyde adducts with sodium and potassium ions clearly indicates the formation of a stable solid complex between permethyl-β-cyclodextrin and benzaldehyde.

In conclusion, this research clearly shows the formation of a stable solid 1:1 complex between permethyl-β-cyclodextrin and benzaldehyde.

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