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CYCLODEXTRIN CHEMISTRY: SYNTHESIS OF CYCLODEXTRIN DERIVATIVES, COMPLEXATION, AND GAMMA RADIATION EFFECTS

CHIMIE DES CYCLODEXTRINES: SYNTHÈSE DES DÉRIVÉS DE CYCLODEXTRINES, DE LA COMPLEXATION, ET DES EFFETS DU RAYONNEMENT GAMMA

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RÉSUMÉ

On a étudié la chimie des cyclodextrines. L'étude a comporté la synthétisation de certains dérivés de cyclodextrines, la préparation de complexes d'inclusion sélectionnés avec des cyclodextrines et l'étude des effets d'irradiation gamma sur les cyclodextrines et certains oligosaccharides linéaires.

Dans ce rapport, on présente un bref examen de la structure et des propriétés des cyclodextrines, de la synthèse des dérivés de cyclodextrines, de leur complexation et de leurs applications. Ensuite, on décrit la synthèse de certains dérivés de cyclodextrines ainsi que la préparation des complexes d'inclusion de cyclodextrines avec certains composés organiques. Enfin, on examine les effets de l'irradiation gamma sur les cyclodextrines, certains de leurs dérivés et certains glucides liés structuralement. On a effectué les études pour deux raisons: (1) pour étudier les effets de l'irradiation gamma sur les cyclodextrines et leurs dérivés et (2) pour étudier la sélectivité au cours de l'irradiation gamma des dérivés de cyclodextrines.

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ABSTRACT

The chemistry of cyclodextrins was studied. This study included synthesising some cyclodextrin derivatives, preparing selected inclusion complexes with cyclodextrin and investigating the effects of gamma irradiation on cyclodextrins and certain linear oligosaccharides.

This report presents a brief review of the structure and properties of cyclodextrins, the synthesis of cyclodextrin derivatives, their complexation and applications. This is followed by a description of the synthesis of some cyclodextrin derivatives and the preparation of inclusion complexes of cyclodextrin with some organic compounds. Finally, the effects of gamma irradiation on cyclodextrins, some of their derivatives and certain structurally related carbohydrates are discussed. The gamma irradiation studies were carried out for two reasons: (1) to study the effects of gamma irradiation on cyclodextrins and their derivatives, and (2) to investigate selectivity during the gamma irradiation of cyclodextrin derivatives.

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CONTENTS

NOMENCLATURE

- The numbers representing chemical structures are underlined.
- CD = cyclodextrin
- CDs = cyclodextrins
- NMR or nmr = nuclear magnetic resonance
- J = value of coupling constant
- IR = infrared
- mp = melting point
- UV = ultraviolet
- HPLC = high-performance liquid chromatography
- TLC or tlc = thin-layer chromatography
- ^{1}H = proton
- h = hour
- α, β, γ, δ = alpha, beta, gamma, delta, respectively.

1. INTRODUCTION

1.1 GENERAL

1.1.1 Nomenclature

Cyclodextrins (CDs) are a class of cyclic oligosaccharides whose members consist of at least six glucopyranose units joined together by α(1→4) linkages. CDs are designated by a Greek letter to denote the number of glucose units: α- for 6, β- for 7, γ- for 8 and so on. Although CDs with up to 12 glucose residues are known, only the first three homologs have been studied extensively.

CDs are often described in the older literature as Schardinger dextrins [1]. This is attributed to the fact that Schardinger was the first to describe their preparation and properties in detail [2]. In 1904, Schardinger [2] demonstrated that these compounds could be obtained by the action of *Bacillus macerans* amylase (cyclodextrinase) on starch. However, the initial discovery of CDs is attributed to Villiers [3], who isolated them in 1891 as degradation products of starch. Comprehensive accounts of the progress towards characterizing and improving the synthesis of the parent CDs and detailed chemical and physical data for the parent CDs are found in the literature [4-8]. These compounds have occasionally been called cycloglucans. Chemical Abstracts has adopted the CD nomenclature, and their convention will be followed in this report.

1.1.2 Structure

As mentioned above, CDs consist of six, seven, eight or more D-glucose units. These units are joined through 1,4-α linkages in such a way as to form a ring - a chain bracelet where each link is a pyranose hexagon. These rings are doughnut shaped, with all the glucose units in substantially undistorted C1(D)(chair) conformations. Figure 1 shows a schematic diagram of the CDs. As can be seen in the figure, these structures possess a central cavity. This cavity is tapered slightly, so that the molecule is shaped like a truncated cone. A loop of six or more hexagons makes up the sides, each hexagon lying roughly in the plane of the sides; the depth of the cone is thus the width of the pyranose ring. Outside the cone, around the "upper," larger rim lie the secondary -OH groups of C-2 and C-3; the primary -OH groups of C-6, that is, the $-CH_2OH$ groups lie around the "lower," smaller rim. The inside of the cone consists of three bands, one on top of another: two bands of C-H's and, in between, a band of glycosidic O's.

The structures of CDs established so far have been based on X-ray crystallography of the α-CD - potassium acetate complex [9] and α-CD hexahydrate [10,11]. In addition, cyclic structures composed of α(1→4) linked glucopyranose units are consistent with other research [7].

The C1 chair conformation of the D-glucopyranose units in CDs in DMSO and D_2O has been demonstrated by ^{1}H-NMR, IR and optical rotatory dispersion spectroscopy. Woods et al. [12] recently carried out a precise

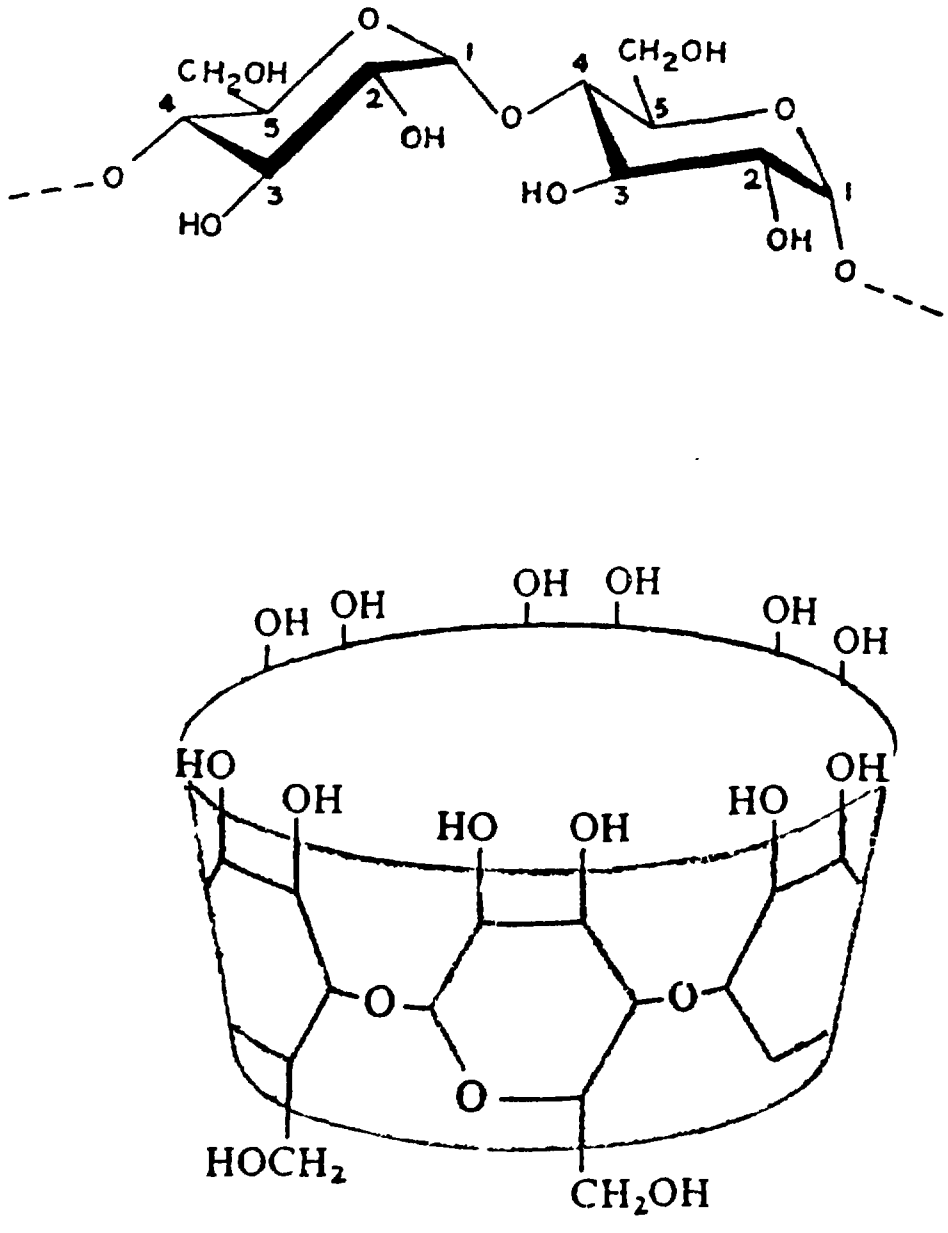


FIGURE 1: Top: Schematic Diagram of Two Glucopyranose Units of a Cyclodextrin Molecule Illustrating Details of the $\alpha(1\rightarrow4)$ Gycosidic Linkage and the Numbering system for Each Unit.
Bottom: A Cyclic Structure of α-Cyclodextrin.

study of the conformation of α-CD in solution using 220-MHz ^{1}H-NMR spectroscopy.

1.1.3 Properties

Table 1 lists the dimensional sizes of CDs and some of their important properties [7].

TABLE 1

PHYSICAL PROPERTIES OF THE CYCLODEXTRINS

Cyclodextrin	Number of glucose residues	Molecular Weight (calculated)	Water Solubility (g/100 mL)	Specific Rotation $[\alpha]_D^{25}$	Cavity Dimensions (nm)	
					Internal Diameter	Depth
α-Cyclodextrin	6	972	14.5	150.5 ± 0.5	450	670
β-Cyclodextrin	7	1135	1.85	162.5 ± 0.5	~700	~700
γ-Cyclodextrin	8	1297	23.2	177.4 ± 0.5	~850	~700
δ-Cyclodextrin	9	1459	Very Soluble	191 ± 3	-	-

The cyclization of linear glucose chains to CDs is energetically unfavorable because α-, β-, and γ-CDs have higher free energy than the corresponding linear glucose chains by +9.60, +7.17, and +8.35 kJ per mole at 25°C [4,7], respectively. However, they are fairly stable in alkaline solution. The activation energy for the scission of glycosidic bonds in β-CD is 143. kJ per mole, whereas that in the case of maltose, a linear sugar, is only 128. kJ per mole. X-ray data [10,11] indicate that the macrocyclic ring in α-CD hexahydrate is distorted. Interestingly, this distortion vanishes when α-CD forms inclusion complexes with guest molecules. The formation of inclusion complexes will be dealt with in detail later on.

CDs are not metabolized as rapidly as starch because certain α-(1,4) bonds are cleaved more slowly by α-(1,4) glucanohydrase than those in linear dextrins and are not hydrolyzed by enzymes that attack terminal groups [6]. However, the metabolism of degraded CDs and starch is comparable. All toxicity tests have shown that orally administered CDs are harmless. Enzymatically modified starch, including CDs, is also toxicologically harmless [6].

1.2 SYNTHESIS

CDs are currently prepared by the action of bacterial CGTases (cyclodextrin glycosyl transferases) on gelatized starch, which is a linear polysaccharide.

Since the enzymes do not show length specificity, the resulting CDs contain 6-12 glucose units per ring. There have been steady developments in the investigation of new enzymes for the production of CDs [13]. Some European countries and Japan have already approved the use of CDs in food products, and large-scale production has also started in the United States during the last two years [14].

β-CD is the least-expensive CD and commercially is the most-used CD at the present time. α-CD costs about 10 times as much, and γ-CD costs 100 times as much.

1.3 CYCLODEXTRIN DERIVATIVES FORMATION

Recent interest in the use of chemically modified CDs for various purposes has generated a number of papers containing information pertinent to the syntheses and reactions of these useful compounds. Although parent CDs by themselves have been used in a variety of fields, the CD derivatives may have greater scope and a broader range of applications. A principal application has been in the area of enzyme modelling, catalysis and chromatographic separations, which will be dealt with in detail later in this report. Kroft and Bartsch [8] reviewed the synthesis of chemically modified CDs in detail.

The reactive functionalities in all α-, β- and γ-CDs are the primary (position-6) hydroxyls and the secondary (positions 2 and 3) hydroxyls. So the CD derivatives were allowed to form at either all three (2,3,6) positions or selectively at any one of them. They have also been polymerized through 6 positions or threaded like beads onto a linear polymer, without forming any covalent bonds with it. Some cyclodextrin derivatives that have been prepared are listed below.

- Acylated CDs [15-17]
- Alkylated CDs [18-20]
- Tosylates, mesylates and related derivatives of CDs [21-24]
- Amino and azido derivatives of CDs [24-27]
- Halogen derivatives of CDs [25]
- Rigidly capped CDs [28-40]
- Phosphorus-containing derivatives of CDs [41]
- CD derivatives containing imidazole moieties [41-43]
- CD derivatives containing pyridine moieties [44]
- CD derivatives with alcohol, aldehyde ketone and oxime functionality [40,45]
- Carboxylic acid and related derivatives of CDs [46]
- Carbonate and carbamate derivatives of CDs [47]
- CD derivatives with silicon- boron-, or tin-containing functional groups [43,48]
- Spin-labelled CD derivatives [49-51]
- Deoxy derivatives of CD [37,52]
- CD derivatives with sulphur-containing functional groups [22,43].

The special properties of CDs are largely retained when they are linked together to form polymers [53-55]. If the degree of polymerization is sufficiently high, the CDs bound within the matrix become insoluble. If

CDs are linked to polyethers, water-soluble polymers are produced. The CDs can also be bound to polymeric resins such as Sepharose 6B.

The starting material for most of these CD derivatives is often monosubstituted 6-O-p-toluensulfonyl-CD, since the tosyl group is a good leaving group and can be replaced by many nucleophilic groups.

1.4 SYNTHESIS OF INCLUSION COMPLEXES OF CYCLODEXTRINS

The most remarkable property of the CDs is their ability to form inclusion complexes with a variety of molecules (guests). In the process of complexation, the guest compounds are included in the cavity of CDs (host) involving non-covalent bonding. Several intermolecular interactions have been proposed and discussed as being responsible for the formation of CD-inclusion complexes in an aqueous solution [6,56]: hydrophobic interactions; van der Waals interaction; hydrogen bonding; the relief of high-energy water from the CD cavity upon substrate inclusion, and the relief of conformational strain in a CD-water adduct, together with the formation of a hydrogen bonding network around the O(2), O(3) side of the CD macrocycle upon substrate inclusion [6,56].

Water plays a crucial role in the inclusion process. It has been shown that the thermodynamic stabilities of some inclusion complexes in aqueous solution decrease markedly with the addition of a dimethyl sulfoxide. Kinetic parameters determined for inclusion reactions also revealed that the rate determining step of the reaction is the breaking down of the water structure around the guest molecule and/or within the CD cavity [56]. The complexation mechanism involving some thermodynamic processes is shown in Figure 2. The process of complexation is reversible. The complex can be broken into the guest and host molecules by subjecting them into an apolar organic solvent, as illustrated by the following equilibrium:

$$\text{Cyclodextrin (host)} + \text{Organic Compound(guest)} \underset{\text{non-aqueous}}{\overset{\text{aqueous}}{\rightleftharpoons}} \text{Host: Guest-Complex}$$

The method used to synthesize the CD guest-complex depends on the properties of the guest components. Some examples of CD complexes are discussed below.

1.5 CATALYSIS BY CYCLODEXTRINS

The basic understanding of specific binding and catalysis of enzyme action is one of the significant targets of CD chemistry.

Like many enzymes, CDs have a hydrophobic binding site. CDs can strip off the unfavorable water assembly around a guest molecule by making an inclusion complex. Some X-ray studies have indicated that no water molecule will remain in an α-CD cavity [57]. CDs also have active hydroxyl groups along their rim. These hydroxyl groups are very weak acids (pKa = ca. 12) and can act as acids over a wide range of pH, and can also act as a base in a strongly alkaline solution. Therefore, CDs qualify as simplified models of certain enzymes [58,59].

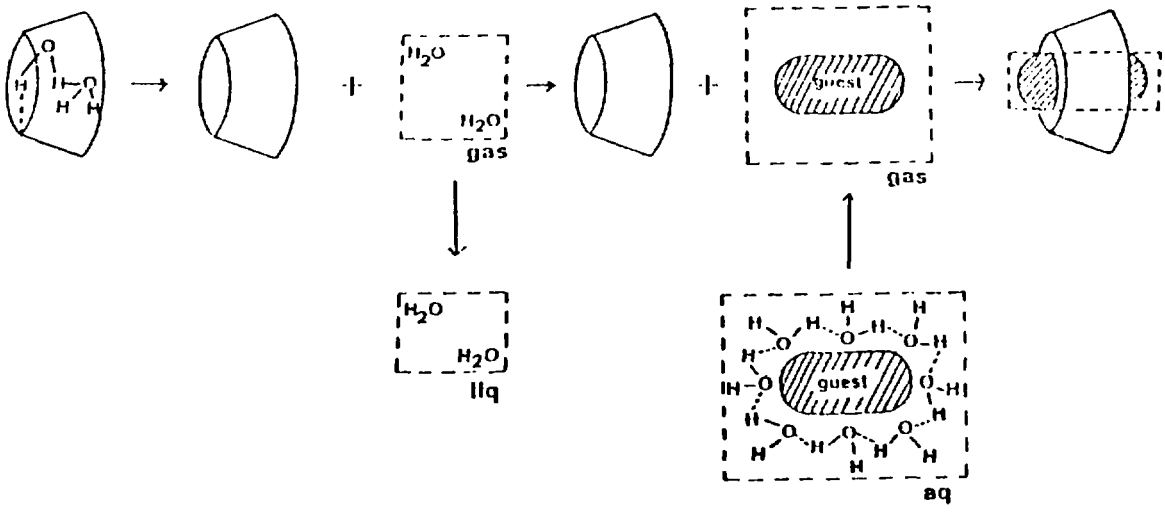


FIGURE 2: Hypothetical Termodynamic Formation Process of the Cyclodextrin Inclusion Complex

A typical example of the acid-base multiple catalysis by CDs is the cleavage of certain pyrophosphates and carboxylates [58,59]. The release of phenols from a number of meta-substituted phenyl benzoates was found to be accelerated in alkaline solutions by α- and β-CDs. The acyl portion was observed after it had attached itself to a hydroxyl group of the CD and formed a CD-benzoate, which underwent hydrolysis via a subsequent reaction at a rate independent of the nature of the phenolic group [59]. The hydrolysis of such benzoates occurred ~20 times more rapidly than in the absence of CDs. A mechanism suggested for this reaction carried out at different pH values involves nucleophilic participation by an alkoxide ion derived from the secondary hydroxyl groups of the CD. The CD pathway of binding, acylation, and deacylation is formally similar to the pathway of chymotrypsin-catalyzed hydrolysis of esters [58,59]. Several other examples of this type of multiple catalysis are known [45,60-66].

A major problem of CD models for enzyme catalyses is that the parent CD has only limited catalytic activity. Breslow's group was successful in their attempts [67-68] to improve the catalytic activity of CDs by introducing potent catalytic functional group(s) such as pyridoxamine onto CDs [67-68] to mimic transaminase enzymes for converting α-keto acids to amino acids. They also tried successfully to mimic the tryptophan synthetase and biochemical dehydroalanine formation by attaching the pyridoxal group to the primary hydroxyl groups of CDs [69].

Selectivity improves when there are two functional groups at the catalytic site. Detailed investigations of the hydrolysis activity of bisimidazolyl-β-CD prepared from rigidly capped CD for a specific substrate have demonstrated that the catalyst recognizes even a small structural change in a specific substrate [41,57,70,71]. For example, Breslow repor-

ted the catalytic cleavage of a cyclic phosphate of 4-tert-butylcatechol on complexing with a β-cyclodextrinyl-6,6'-bisimidazole. The kinetics showed a bell-shaped pH vs. rate profile, suggesting cooperative catalysis by a basic imidazole group and an acidic imidazolium group. Breslow developed other models where the enzyme mimic was very selective. For example, in the case of hydrolysis of cyclic phosphates by ribonuclease, only the P-O(1) bond of the cyclic phosphate cleaved to form the 2-phosphate of 4-tert-butylcatechol [41]. This hydrolysis mechanism is shown in Figure 3. In contrast, hydrolysis of the same cyclic phosphate in solution in the absence of the catalyst gave a 50:50 mixture of 2-phosphate- and 1-phosphate of 4-tert-butylcatechol.

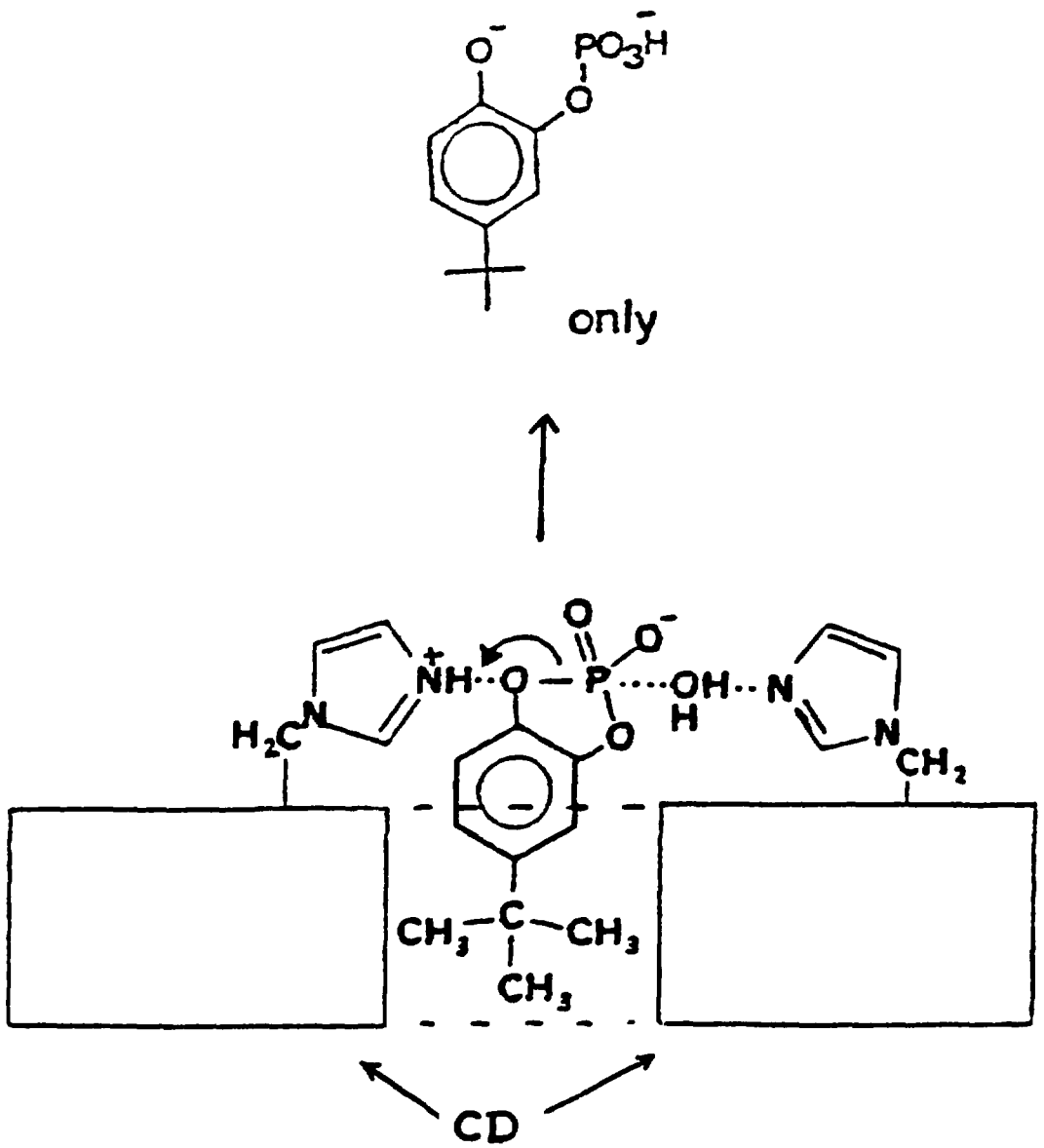


FIGURE 3: Hydrolysis Mechanism of Cyclic Phosphates by Ribonuclease

CDs also provide a chiral binding site capable of including guest ketones and induce template-directed chiral reductions; they therefore open new ways for achieving relatively difficult asymmetric synthesis. Chenevert et al. observed [72-73] that the sodium borohydride reduction of 2-cyclohexenones in an aqueous medium changes in the presence of CDs: β-CD favors the 1,4-reduction over the 1,2-reduction, whereas α-CD favors the 1,2-reduction. Other workers [74-75] observed a similar selectivity in the sodium borohydride reduction of substituted 2-cyclohexenones.

1.6 APPLICATIONS OF CYCLODEXTRINS

Although CDs have been known for nearly a century, and their ability to form inclusion complexes has been recognized for about 40 years, their practical application did not gain in importance until a decade ago. The interest in the industrial applications of CDs in the recent years is reflected especially by the increasing number of patents. The fields of interest include:

- Food preservation
- Chromatography - purification of mixture of organic compounds
- Organic synthesis; catalysis
- Pharmacology - enhancement of drug bioavailability
- Pharmaceutical industry - purification of drugs
- Biochemistry - enzyme mimics
- Agriculture - stabilization of insecticides and herbicides
- Polymers - for variety of purposes
- Spectroscopy - enhancement of luminescence.

Applications in the Food Industry The scope for using CDs in the food industry was not recognized until the 1970s when Japan and Hungary began producing them commercially. CDs can now be used in a variety of food applications [6,14] since continuing research and studies have indicated that oral consumption is not harmful. The potential for CD applications is vast, e.g.,

- There is great potential for CDs in the area of flavors.
- CDs can lessen the odors of products such as fish, mutton, garlic, yeast extracts, soybean milk, lecithin and old grains by masking the odors.
- CDs can stabilize emulsions of fats and oils (shielding the fats and oils from oxidation, and thus preventing rancidity).
- β-CD increases the volume of whipped egg white by about 30%.
- Natural pigments, such as carotenoids and flavonoids, can be stabilized with CD complexes by masking their color.
- CD complexes can protect ingredients from oxidation, light-induced reactions, thermal decomposition and evaporation loss.

Applications of Cyclodextrin-Polymers In Chromatography Specific properties of CDs are largely retained when they are linked together to form

polymers. They could be linked by many groups, e.g., through hexamethylene diisocyanate, polyethers, m-nitrophenylacrylate and many other polyfunctional groups [6]. CDs can also be bound to polymeric resins such as sepharose 6B. When compared with customary Sephadex, CD polymers have the advantage in being able to separate certain molecules of an appropriate size. Molecules with similar molecular weights or even certain isomers can also be separated.

Commercially available chromatography columns are now prepared by attaching the CDs to silica gels to provide a hydrolytically stable bonded phase. Inclusion complexing with CD phases has been found to allow unique reversed-phase separations, and is also useful in resolving certain positional, structural, and geometrical isomers as well as enantiomers [76-79]. Figure 4 shows the separation of some positional isomers of substituted naphthalene. Figure 5 shows the inclusion mechanism for β-substituted naphthalene with β-CD on a cyclobond 1 (CD) column. Many other examples have been reported in the literature [78,80-87] to illustrate the separation of organic compounds, including drugs like β-blockers, calcium-channel blockers, sedatives, antihistamines, anticonvulsants, diuretics, and synthetic opiates, by using parent and substituted CDs as immobilized stationary phases in chromatography.

Other Potential Applications of Cyclodextrins The increasing number of papers and patents in recent years indicate a stronger trend to carry out further research on CDs and apply them in industry. However, there is still a need to further develop the potential applications of CDs and their derivatives in several areas. Some of the work carried out in our laboratory is discussed in the following sections.

2. RESULTS AND DISCUSSION

The objective of this work was to study the chemistry of CDs and some possible applications in the areas of complexation, radiation effects and separations of mixtures of organic compounds. This study includes the synthesis of some CD derivatives, the complexation of a variety of organic compounds with CDs and the gamma-irradiation effects on CD derivatives.

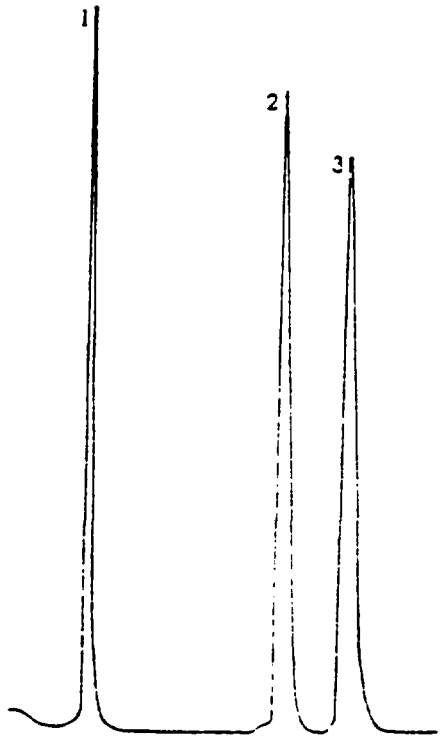
The results of this study are presented and discussed in this section.

2.1 SYNTHESIS OF CYCLODEXTRIN DERIVATIVES

CDs and some of their derivatives have already been used in research, as discussed earlier. We synthesized some CD derivatives and characterized them fully; however, we did not test their applications.

2.1.1 Preparation of Permethylated Cyclodextrins, Permethylated Maltotriose and Permethylated Maltose

Permethylated-β-CD 1 is named according to the accepted nomenclature [8,18,19,88] as heptakis-(2,3,6-tri-O-methyl)-β-CD (see Figure 6).



ANALYTES

1. 1-Naphthalene Sulfonic Acid
2. 2-Naphthalene Sulfonic Acid
3. Naphthalene

CONDITIONS

COLUMN	*CYCLOBOND I*
TYPE	*Beta*
SIZE	*250 x 4.6 mm*
MOBILE PHASE	*Methanol/TEAA* 35/65*
FLOW RATE	*1.0 mL/min*
PRESSURE	*600 psi (41 kPa)*
CHART SPEED	*0.5 cm/min*
DETECTION	*254 nm @ 0.8 AUFS*
INJECTION WT.	*5 g*

* TEAA - Triethylamine acetate (0.2%) at pH ~ 7.5

FIGURE 4: Substitution of Substituted Naphthalene

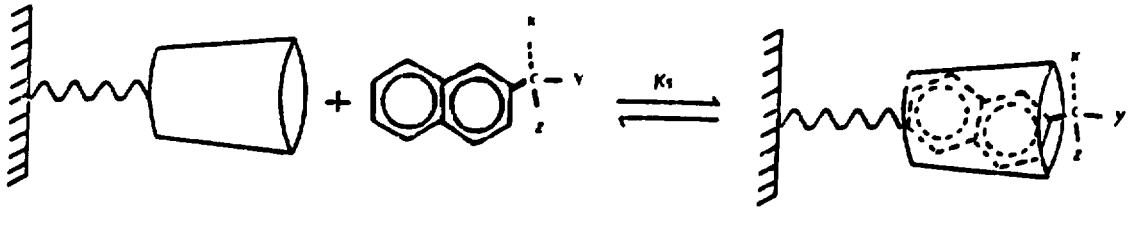
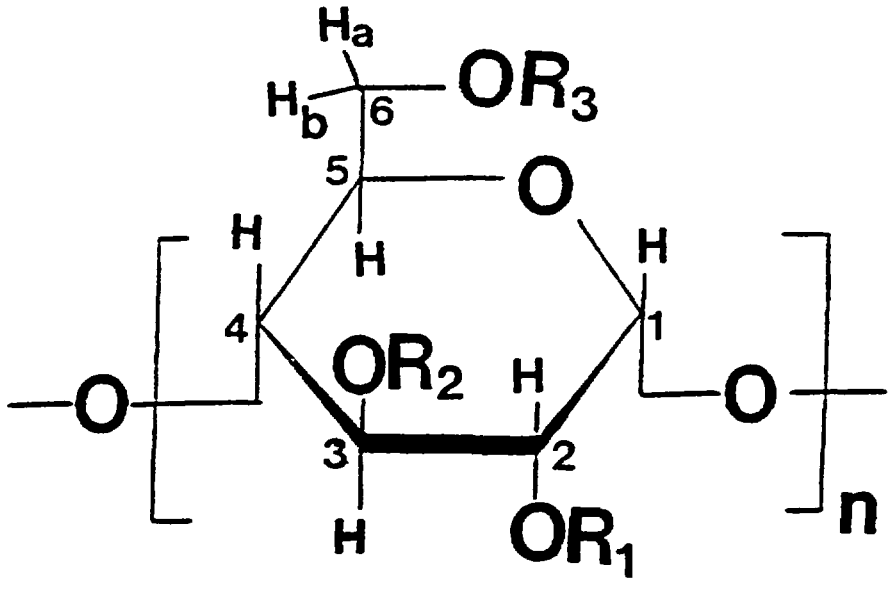


FIGURE 5: Structure of β-Cyclodextrin Showing Inclusion of β-Substituted Naphthalene



1 n = 7, $R_1 = R_2 = R_3 = CH_3$

2 n = 6, $R_1 = R_2 = R_3 = CH_3$

3 n = 8, $R_1 = R_2 = R_3 = CH_3$

4 n = 3, $R_1 = R_2 = R_3 = CH_3$, and a CH_3 group at each terminus, i.e. C_1 and C_4 positions.

5 n = 2, $R_1 = R_2 = R_3 = CH_3$, and a CH_3 group at each terminus, i.e. C_1 and C_4 positions.

6 n = 7, $R_1 = R_2 = R_3 = O{=}C{-}C_6H_5$

7 n = 7, $R_1 = R_2 = R_3 = O{=}C{-}CH_3$

8 n = 7, $R_1 = R_2 = H_2$, R_3 = Tosyl

FIGURE 6: Structures of Cyclodextrin Derivatives

It was prepared in one step in excellent yields (71.3% after crystallization) by slightly modifying Hakomori's [89] and Szejtli's [19] method using dried β-CD. Crystallization from cyclohexane (containing 1% dichloromethane) yielded crystals with the same melting point (153-154°C) reported for other solvents [19]. The ^{1}H-NMR data, obtained at 300 MHz, are similar to data obtained at lower fields [19]. It has been reported [18] that 2,6-di-O-methylated-β-CD may also be produced in the reaction. Our ^{1}H-NMR results indicate the presence of only 2,3,6-tri-O-methylated-β-CD. There are three singlet peaks at 3.494 ppm, 3.399 ppm and 3.268 ppm, each integrating for three protons relative to the H_1 integration. The assignment of the above chemical shifts to the methyls at the C_2-, C_3- and C_6- posi-

tions, respectively, was based on the analyses of ^{1}H-NMR of O-methylated glucoses [90]. The H_1 is observed as a doublet at 5.157 ppm and is coupled only to H_2 with a vicinal coupling constant of 3.48 Hz. The H_2 is cis to the H_1 and trans to the H_3. The coupling constant between the H_2 and the H_3 is 9.0 Hz. In case mono and dimethylated-β-CD were present along with the tri-methylated-β-CD, we should have observed extra peaks for the protons at the C_1- and C_2- positions and for the methyls at the C_2-, C_3- and C_6- positions. The peaks for other protons, H_3, H_4, H_5, H_{6a} and H_{6b}, overlap each other at 3.752 to 3.452 ppm. The value of coupling constant (3.48 Hz) between H_1 and H_2 in the permethylated-β-CD is slightly different than the corresponding value (3.70 Hz) in the β-CD. This is due to a slight change in the conformation of the molecule induced by the bulkier methyl groups.

Hexakis-(2,3,6-tri-O-methyl)-α-CD 2, and octakis-(2,3,6-tri-O-methyl)-γ-CD 3 were also prepared in good yields from dried α- and γ-CDs, respectively, using essentially the method described above. Their ^{1}H-NMR spectra in deuterium oxide at 300 MHz confirm their structures, which are similar to those of permethylated-β-CD. These two compounds 2 and 3 were prepared on smaller scales due to the higher price of α- and γ-CDs compared to β-CD.

The solubilities of permethylated CDs are described as being higher than the corresponding parent CDs for the purpose of complexation [7,18-20]. We found this to be true for the case of permethylated-β-CD prepared so that we could make complexes of some organic compounds with permethylated CDs and observe if these CD derivatives offered better protection to drugs during X-ray or gamma irradiation. Although complexes of permethylated CDs were not irradiated, the permethylated CDs were irradiated in a Gammacell 220. These studies will be discussed later.

In order to better understand the effects of gamma irradiation on permethylated CDs, permethylated maltotriose 4 and permethylated maltose 5 were prepared as open-chain compounds for parallel studies. These compounds were prepared from maltotriose and maltose using the same procedures that were followed for the preparation of permethylated CDs. Both products were obtained in quantitative yields and each showed one spot in thin-layer chromatography (tlc) in the solvent system ethylacetate:hexanes (1:1). These compounds were identified by the ^{1}H-NMR spectroscopy.

2.1.2 Preparation of Perbenzoylated-β-Cyclodextrin

Compound 6, heptakis-(2,3,6-tri-O-benzoyl)-β-CD, was prepared as a starting material for the selective synthesis of 6-O-methyl-β-CD, 6-O-azido-β-CD, 6-O-amino-β-CD, and 6-O-amino-2,3-di-O-methyl-β-CD [91]. These target compounds are important for their higher solubilities, different complexation mode and ease of substitution by other functional groups. The synthetic strategy was not pursued beyond the preparation of perbenzoylated-β-CD.

Compound 6 was good prepared with a slight modification to the methods of Cramer et al. [43] and Boger et al. [91]. The yield was 88%. Its structure was determined by its melting point (175-185°C, sublimation),

^{1}H-NMR at 60 MHz and IR spectra. The ^{1}H-NMR in DMSO-d_6 showed the presence of peaks due to H_1 to H_6 as well as aromatic protons. The IR spectrum showed absorption due to aromatic and aliphatic C-H at 3190-3150 cm^{-1} and 2980-2930 cm^{-1}, respectively, and to the benzoate ester group at 1722 cm^{-1}.

2.1.3 Preparation of Peracetylated-β-Cyclodextrin

Compound 7, hexakis-(2,3,6-tri-O-acetyl)-β-CD, was prepared using acetic anhydride and zinc chloride with β-CD. It was also prepared with acetic anhydride in pyridine, a procedure reported for the synthesis of 2,3-di-O-acetyl-6-bromo-6-deoxy-β-CD [52], a compound generally prepared for the synthesis of 6-deoxy analogues. The melting point and the ^{1}H-NMR were consistent with the published results. The IR spectrum also showed strong absorption at 1730 cm^{-1} due to the -O-OC-Me group. The ^{1}H-NMR (60 MHz) indicated only 70% acetylation where the compound was prepared by using acetic anhydride in pyridine.

2.1.4 Preparation of 6-O-Tosyl-β-Cyclodextrin

As the nature of the parent CDs became better known, it was recognized that these versatile compounds and their derivatives might be used in several chemical applications, including enzyme modelling studies. At the same time, it was desired to prepare CDs with a variety of functional groups such as amino, azido or halo, and they, in turn, led to the preparation of a series of CD derivatives containing tosyl (4-methyl benzenesulfonyl), mesyl (methanesulfonyl), or other related arylsulfonyl groups. These tosyl and mesyl groups are often very good leaving groups in nucleophilic substitution reactions, and are widely used in the synthesis of many substituted carbohydrates and other organic compounds. The immediate use of tosylated CDs was planned for the synthesis of amino acid substituted CDs (e.g., L-proline), which will be discussed shortly.

Moderate to low yields of monosubstituted-tosylated-β-CD 8 were obtained using the method of Lautsch et al. [21]. The structure was determined by ^{1}H-NMR at 60 MHz and IR spectra, and its melting point.

2.1.5 Preparation of N-Protected L-Proline

It was envisaged that the addition of cyclic amino acids such as L-proline to position 6 in CDs will add the following characteristics to the molecule:

(1) Since L-proline is chiral, attaching seven such molecules to one molecule of β-CD would induce more chirality into it. Also, an array of seven proline units on one surface of CD would increase the length of the left threadedness in the CD derivative. This characteristic would increase the resolving power of the modified CD to separate isomeric mixtures since one isomer would pass preferably through a left-threaded cavity compared to the other isomer because of its preferred structural orientation.

(2) Since CDs derived from amino acids would have amino or carboxylic residues at positions 6, it would be possible to prepare salts of the

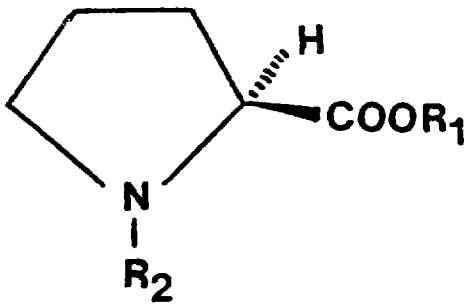
modified CDs. In such cases their solubility could be increased substantially. Also, the CD derivative could be easily bound to a stationary phase, possibly through a spacer arm, for chromatographic purposes.

The attachment of L-proline either through the amino group or through the carboxylic acid group would require prior protection of the other group. Therefore, the following N-protected-L-proline compounds were prepared for the subsequent synthetic procedures: (1) N-benzyl-L-proline 9, and (2) N-(2,4-dinitrophenylsulphenyl)-L-proline 10 (see Figure 7). Although N-(2,4-dinitrophenylsulphenyl)-L-proline was not purified and fully characterized, the structure of N-benzyl-L-proline was determined by its IR and ^{1}H-NMR (60-MHz) spectra. After protecting the N-position of L-proline, the next step would be to substitute it for the tosyl group at position 6 in 6-O-tosyl-β-CD.

2.1.6 Preparation of Biphenyl-4,4'-Disulfonyl-A,D-Capped-β-Cyclodextrin

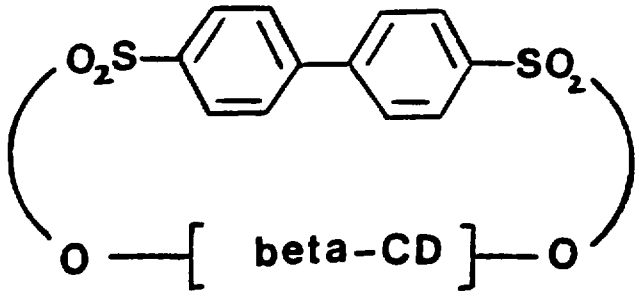
The protection of the primary hydroxyl groups (at positions 6) and the secondary hydroxyl groups (at positions 2 and 3) of the glucose moiety is a common step when synthesizing carbohydrates, and a large number of different types of protecting reagents are available for this purpose. The chemistry of selectively protecting hyroxyl groups at positions 2, 3 and 6 is well established. However, it is difficult to protect the primary hydroxyl groups selectively at two sugar units that are a certain unit length apart from each other in an open-chain oligosaccharide. For example, the selective blocking of the primary hydroxyls of two sugars, which are 3, 4 or 5 units apart, would be very difficult in maltopentaose, maltohexaose and maltoheptaose. Such an attempt would result in poor yields of the desired product because of the random blocking of any two sugars. The preparation of such compounds could be achieved by using radiation to induce the derivatized CD rings to open at two glucose units a certain distance apart, e.g., either at A,B/A,C/ or A,D positions relative to each other. Such derivatives are called capped CDs, and they have been reported in the literature [28-40]. The A,B/A,C/- and A,D-capped CDs are selectively produced in low yields using the CD and an appropriately sized bifunctional capping reagent.

Irradiation induces the CD ring to open, producing an oligosaccharide that is shorter by one sugar unit. It was envisaged that the gamma irradiation of A,D-capped β-CD would produce a maltohexaose that would be blocked at the A,D (or 1,4 sugar units) positions. Only two primary hydroxyl groups would be protected in this product. Such selectively protected sugars may have a potential demand in synthesis, and biphenyl-4,4'-disulfonyl-A,D-capped-β-CD was prepared for this purpose from the capping reagent biphenyl-4,4'-disulfonyl chloride and β-CD. This reaction requires anhydrous conditions that are described in detail in Section 3. Thin-layer chromatography of the crude product showed the presence of the desired product. The other impurities present in the crude product were small portions of unreacted CD and likely polymerized material. Due to the limitations of the solubility of the crude product in pyridine, DMSO, and the water-ethanol-acetonitrite mixture, most of the purification techniques (including chromatography) were not very successful. The yield of the



9 $R_1 = H$, $R_2 = CH_2-C_6H_5$

10 $R_1 = H$, $R_2 = S-C_6H_3[2,4-(NO_2)_2]$



11 A,D-capped-beta-CD

FIGURE 7: Structures of Cyclodextrin Derivatives

product was only about 7%. It is important to note that the product was found to partially decompose to β-CD and biphenyl-4,4'-disulfonic acid during the successive purification workup procedures. The structure determination was carried out by its melting point, thin-layer chromatography and high-resolution ^{1}H-NMR (300 MHz) and IR spectroscopy. The ^{1}H-NMR in DMSO-d_6 also indicated the presence of small amounts of an unidentified impurity.

The preparation of large quantities of this product and irradiation studies of it have been postponed indefinitely.

2.2 COMPLEXATION

We tried to complex CDs with a number of organic compounds possessing a variety of functional groups and skeletons. Some of the guest compounds did not form complexes, whereas complexes of the rest of the guests with CDs were isolated. Some of these complexes were fully characterized, but in other cases the characterization of the complexes could not be completed. A variety of analytical techniques was employed to detect the complexes. The complexation of several compounds with CDs was carried out as described in the following subsections.

2.2.1 P-Methoxyphenol-Cyclodextrin Complexes

A solid complex of p-methoxyphenol was obtained with α-CD and was characterized by HPLC, high-resolution ^{1}H-NMR and IR spectroscopy.

The HPLC analysis indicated the absence of a peak corresponding to free α-CD. The only major peak that was present had a retention time larger than that for free CD, but almost equal to that for free p-methoxyphenol. This peak is undoubtedly due to the complex since it is strongly supported by it's ^{1}H-NMR spectrum in deuterium oxide at 300 MHz. This spectrum shows the peaks, with correct integration and coupling constants, for the protons in the α-CD. Also, it shows two peaks due to p-methoxyphenol: a multiplet for aromatic protons and a singlet for the methoxy protons. The integration of all peaks confirms that p-methoxyphenol and α-CD are complexed in a ratio of 1:1. The IR spectrum of the complex obtained on a KBr pellet was compared to those of the free p-methoxyphenol and α-CD. There was only a slight change in the position of some bands due to p-methoxyphenol in the complexed form. It is difficult to say how significant the observed shift is.

Para-methoxyphenol did not complex with β- and γ-CDs. This discriminatory behavior of p-methoxyphenol with the three CDs, which possess cavities of different sizes, explains very well the condition of tight fit required for the complexation process.

2.2.2 p-Nitrophenol-Cyclodextrin Complexes

The complexation of p-nitrophenol with CDs was attempted in the preliminary work in order to develop complexation methods and analytical techniques such as IR, ^{1}H-NMR, UV and HPLC for their characterization.

When the infrared spectra of p-nitrophenol, β-CD and their suspected complex were compared, a slight shift in the position of bands for the suspected complex was evident. The ^{1}H-NMR spectrum at 60 MHz had low resolution because of the low solubility of the complex in D_2O. Only D_2O can be used as a solvent in this method since organic solvents may dissociate the complex.

The formation of an inclusion complex of p-nitrophenol with α-CD was followed by a "titration" experiment using UV spectroscopy as described in more detail in Section 3. As the complex was formed, a new peak at 400 nm appeared in addition to the p-nitrophenol molecule's existing peaks at 318 nm, 220 nm and 198 nm. The intensity of the new peak at 400 nm was found to be directly proportional to the amount of CD added. Also, the extent of complexation may be pH dependent. At lower pH values (≤ 4.0), when the guest molecule exists in the phenolic form, no complex formation was observed. At higher pH values, when the guest molecule is in the phenolate anion form, the complex is more stable. Cramer et al. [92] also observed the appearance of a peak due to the p-nitrophenolate anion (at pH 11, 20°C) at ~400 nm and a gradual increase in absorption as additional aliquots of α-CD were added.

These experiments suggest that UV spectroscopy might be a good tool to analyse the inclusion complexes, while IR spectroscopy is not generally suitable for this purpose. The CD bands in the IR spectrum change only slightly when the complex forms. On the other hand, since the ratio of the guest molecule to one glucopyranose unit in the complex is 1:6-8, the bands that could be assigned to the guest molecule are easily masked by the bands due to CD and the presence of water of crystallization.

2.2.3 Benzofuroxan-Cyclodextrin Complexes

The benzofuroxan (BFO)/α-CD complex was prepared in a single aqueous phase system as well as in a two-phase system (aqueous and diethyl ether). The yields from the two procedures were similar, 55% and 60%, respectively. But the yield was only 20% for the benzofuroxan/β-CD complex in the two-phase system, and did not change at higher temperatures (~60°C).

The purpose of preparing BFO complexes was to find if CDs offered protection to complexed organic compounds exposed to ionizing radiation. After irradiating the compounds at various doses (0 to 100 Gy) with a Siemens Stabilipan X-ray machine, the samples of BFO, CDs and their complex in aqueous solution were analysed by UV spectroscopy. The broadening and decrease in the intensities of the peaks at larger doses and absorption due to a new species indicate that CDs confer only limited protection to organic compounds upon irradiation.

2.2.4 Metoprolol-Cyclodextrin Complex

Metoprolol was obtained by hydrolysis of commercial metoprolol tartarate. It's structure was determined from IR and nmr spectra.

Metoprolol did not complex with α- and β-CDs. It's complex with γ-CDs was prepared in a two-phase system (water and diethyl ether). The

complex appeared at the interface and slowly settled to the bottom. The ether layer was separated and evaporated to verify that all the metoprolol had complexed. It was found that about 46% of the metoprolol had not complexed. Also, when the aqueous layer was evaporated, only a portion of the CD was yielded and no metoprolol was found in the aqueous layer. The formation of the complex was further confirmed by its dissociation into γ-CD and metoprolol when the solid complex was stirred vigorously in excess dichloromethane and a small amount of water followed by extraction with dichloromethane and water. This method was used to obtain CD and metoprolol separately from the water and dichloromethane layers, respectively. It is possible that the complexed portion of the metoprolol and the non-complexed portion of the metoprolol from the ether layer are the two different enantiomers, with only one isomer possessing the favored geometry to form a complex. No differentiation could be made between the two enantiomers based on their IR, nmr and HPLC data. It has been reported [76-79] that analytical HPLC columns containing CDs attached to the stationary phase were able to separate the different types of isomers. However, a separation technique such as the one being discussed has not been reported. Such a technique, if applicable, would have the advantage of separating isomers on any scale from small to large since the CDs can be isolated and reused.

2.2.5 Complexation of Other Biologically Active Organic Compounds With Cyclodextrins

Other compounds that were tried for complexation with CDs are L-proline, phenyl alanine, tryptophane, tyrosine, thalidomide, aspirin, benzylimidazole, and vitamin E. Suspected complexes of aspirin, benzylimidazole and vitamin E with γ-CD were precipitated out, but they have not be characterized yet. Thalidomide did not complex with any CD. The complexes of L-proline with γ-CD, phenylalanine with α-CD, tryptophane with γ-CD and tyrosine with α-CD were detected by HPLC using cyclobond columns. Further analyses of these suspected complexes were not pursued.

2.3 STUDIES OF GAMMA RADIATION EFFECTS ON CYCLODEXTRINS AND OTHER CARBOHYDRATES

The radiation chemistry of polysaccharides (including starch cellulose, bound saccharides, carbohydrate-containing food stuffs), oligosaccharides, disaccharides and monosaccharides has been studied and reviewed recently. Several studies on the radiolysis of low-molecular-weight saccharides were carried out with the aim of establishing material balances and the principal routes of product formation [93]. Some investigations are concerned with the influence of different parameters such as concentration, dose rate and the presence or absence of oxygen on the product distribution. Others deal with the radiolytic degradation of saccharidic materials that are of particular interest in understanding radiation-induced changes in foods and related substances [93].

Some oligosaccharides (α-, β- and γ-CDs, permethylated-α-CD, permethylated-β-CD, maltoheptaose, maltohexaose, maltopentaose, maltotriose, maltose and gentiobiose) were irradiated in a Gammacell 220 so that the radiation effects on some organic compounds complexed with CDs could be studied, and to prepare hepta-, hexa- and pentasaccharides protected at

their primary hydroxyl groups from capped-CDs. Except for gentiobiose, which possesses a β→(1,6) linkage, all other sugars possess α→(1,4) linkages between two glucose units. They were studied at different doses, at various temperatures, in aqueous solutions that were either deoxygenated or aerated.

2.3.1 Gamma Irradiation of α-, β-, and γ-Cyclodextrins

Aqueous solutions of the three CDs in triply distilled water were degassed and thoroughly saturated with nitrogen, and were irradiated at 3°C at a dose of 5000 Gy (dose rate 230.84 Gy/min). Similar samples were also irradiated at the same dose at room temperature. HPLC analysis of the two sets of samples on oligosaccharide columns showed similar results. It was found that about 40% of each of the three CDs were converted to products, which included an open-chain form of the CD less one glucose unit, e.g., γ-CD produced maltoheptaose (ca. 35%) and a 14-glucose-unit sugar (the dimerized product, ca. 40%), polymerized products (ca. 15%), and oxidized products (ca. 10%). (Since the HPLC peaks were not integrated in this case and in the following other cases, the ratio of the products was approximated from the peak heights and widths). The retention times were compared to those from standard commercial samples consisting of a mixture of saccharides. Also, the γ-CD produced slightly larger amounts of the oxidized products as well as very small amounts of maltopentaose (a 5-glucose-unit sugar that probably resulted from the oxidation of maltoheptaose) under these experimental conditions, except when the sample was prepared in ordinary undegassed distilled water.

When degassed aqueous samples of α-, β-, and γ-CDs saturated with nitrogen were irradiated at room temperature in the Gammacell 220 at a dose of 8000 Gy (dose rate 234.05 Gy/min), and analyzed by HPLC with an oligosaccharide column, it was found that more than 80 to 85% of the CDs were converted to products. The products were largely oxidized and polymerized material.

When aqueous samples of the three CDs (degassed and saturated with nitrogen) were irradiated at a lower dose of 1000 Gy (dose rate 239.81 Gy/min) at room temperature, it was found that less than 20 to 30% of the CDs were converted to products. The ratio of the products was fairly similar to the products produced at 5000 Gy.

Low-molecular-weight saccharides (1- to 4-glucose-unit sugars) were produced in very small amounts (ca. < 5%) at all three doses of 1000 to 8000 Gy and therefore are not discussed in detail.

These results indicate that a dose of 5000 Gy is best for producing maltopentaose, maltohexaose, and maltoheptaose from α-, β- and γ-CDs, respectively, i.e., the open-chain form of the CDs less one glucose unit. Less importantly, a dimerized product of these is also produced at this dose. At a dose of 1000 Gy the reaction does not proceed significantly and a large amount of the starting material is recovered. At a dose of 8000 Gy, the reaction proceeds beyond these target compounds i.e., the target compounds undergo further oxidation and polymerization. The production of these compounds agrees with the mechanism suggested by Komiya, Yamada and

Nara [94]. They proposed that the initial hydrogen abstraction at C-5 at one of the glucose moiety in the β-CD by OH radicals leads to the formation of the C-5 radical, which is easily converted into the C-1 radical along with the formation of a carbonyl group at C-5; the subsequent cleavage of a β-CD ring yields the C-4' radical of the neighboring glucose residue. Thus, the ring cleavage of β-CD proceeds mainly through this radical transfer, and subsequent combination of the C-4' radical with OH radical affords a terminal glucose unit. The degradation intermediate of β-CD thus formed is so unstable that it is easily hydrolyzed to give maltohexaose [94].

This mechanism explains the lack of maltoheptaose formation when β-CD is radiolysed. Similarly, maltohexaose and maltooctaose are not formed in the radiolysis of α- and γ-CDs, respectively. In short, the initial C-5 radical bearing glucose moiety undergoes oxidation and produces an open-chain oligosaccharide that is one glucose unit shorter than the parent CD.

2.3.2 Irradiation of Other Carbohydrates

As already discussed, one of the radiolysis products in each case is maltopentaose from α-CD, maltohexaose from β-CD and maltoheptaose from γ-CD. These three products must undergo further radiolysis. Therefore, their fate in a radiolytic process was studied independently at the same dose when they themselves were the starting materials.

Aqueous solutions of maltoheptaose, maltohexaose, and maltopentaose prepared in triply distilled water were degassed and saturated with nitrogen, and were irradiated at a dose of 5000 Gy (dose rate 229.36 Gy/min) at 3°C. HPLC analysis on the columns indicated the conversion of about 50% of the starting material to the products. All three sugars produced oxidized material, polymerized material, small amounts of dimerized material and smaller fragments of up to 3 sugar units.

Similarly, maltotriose, maltose and gentiobiose were studied under radiolysis. Another reason for the radiolysis of maltose and gentiobiose was the comparative studies between α → (1,4) linkage (in maltose) and β → (1,6) linkage (in gentiobiose). Aqueous solutions of maltotriose, maltose and gentiobiose were prepared the same way as the maltopentaose, maltohexaose and maltoheptaose, and were irradiated at a dose of 5096 Gy at 4°C (dose rate 229.96 Gy/min). HPLC analysis on oligosaccharide columns indicated that about 50% of the sugars were converted to products. In the case of maltotriose, the radiolytic products included some oxidized material, some polymerized material and a 9-sugar-unit product (maltononaose), all in about equal proportions. These results indicate that maltononaose is a trimerized product of maltotriose. The dimerized product (maltohexaose) was not detected due to the low resolution of the HPLC chromatogram. It appears that the dimer reacts with another maltotriose to produce maltononaose. In the case of maltose, there was also an 8-sugar-unit product (maltooctaose) and a 9-sugar-unit product (maltononaose) besides the oxidized and polymerized products. It is likely that maltooctaose was formed by the double dimerization of maltose. The formation mechanism of maltononaose is not certain.

In the case of gentiobiose (possessing $\beta \rightarrow (1,6)$ linkage), the main products of radiolysis were polymerized materials. In light of the mechanism proposed for the radiolysis of β-CD [94], we suggest that a free radical produced at C-5 of the glucose moiety by OH radicals has a longer lifetime and undergoes polymerization faster than oxidation to smaller products in the case of gentiobiose.

A set of aqueous solutions of maltotriose, maltose and gentiobiose, prepared in ordinary undegassed distilled water, was also irradiated along with the deoxygenated samples under the conditions described above. It was observed that irradiation at a low temperature (4°C) did not cause significant changes in the oxygenated and deoxygenated samples.

2.3.3 Irradiation of Permethylated Cyclodextrins

Aqueous solutions of permethylated-α-CD (0.008 mol/L) and permethylated-β-CD in triply distilled water were first degassed and then saturated with nitrogen before being irradiated at 5000 Gy. HPLC analysis indicated that less than 25 to 30% of the starting material was converted to the product, which was mostly polymerized product. These comparative studies indicate that permethylated CDs are more resistant to radiation than the parent CDs at a dose of 5000 Gy.

3. EXPERIMENTAL

3.1 GENERAL

High-resolution proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Bruker WH-300 (at 300 MHz) spectrometer. The spectra were measured in deuterium oxide (D_2O) with residual water as the internal standard or deuterated dimethyl sulfoxide (DMSO-d_6). Carbon-13 nmr spectra were recorded on the same spectrometer operating at 75.47 MHz. Low-resolution ^{1}H-NMR spectra were recorded on a Perkin-Elmer R12A (60 MHz) spectrometer. IR spectra were recorded on a Perkin-Elmer model-457 grating infrared spectrophotometer. HPLC data were obtained on a Beckman chromatograph model 334, Beckman pump model 110A, Beckman 421 controller, connected to Nelson-Analytical 760 series interface. The HPLC data were processed on an IBM-PC using Nelson analytical chromatography software. The melting points were determined by Fisher-Johns melting point apparatus.

Analytical thin-layer chromatography (tlc) was performed on precoated aluminum sheets with silica gel 60 F_{254} fluorescence (Whatmann) 250 μm thick. Spots were detected under ultraviolet light or visually in an iodine chamber. Silica gel kieselgel 60 (230 to 400 mesh ASTM), or Sigma type IV (325 mesh and finer) No. S-7875 containing approximately 12% alumina were used for flash chromatography [95]. All solvent evaporations were carried out on rotary evaporators. The α- and γ-CDs were obtained from Sigma Chemicals, and β-CD was obtained from the American Maize Products Co. The CDs contain 10-13% moisture, and were vacuum dried for several hours at 80-100°C at 0.15 mm (Hg) when used for synthetic procedures. Dimethyl sulfoxide was the reaction solvent in many cases, and was dried by boiling over sodium hydroxide or barium oxide followed by distillation.

Irradiations were carried out in a Gammacell 220 (cobalt 60). For irradiation purposes, the aqueous solutions were prepared in distilled or triply distilled water. In most cases these solutions were degassed and then saturated with nitrogen.

3.2 SYNTHESIS OF CYCLODEXTRIN DERIVATIVES

3.2.1 Preparation of Heptakis-(2,3,6-Tri-O-Methyl)-β-Cyclodextrin

Preparation of Sodium Hydride Sodium hydride was available as an emulsion in oil at a concentration of 57.25%. It was taken in a graduated separatory funnel with a cotton plug at the bottom. Ether (80 mL) was added to the sodium hydride and was stirred with a glass rod for 5 min. The ether layer was drained and the washing was repeated with fresh ether (20 mL). The sodium hydride was finally dried to a fine powder by blowing nitrogen slowly on top of it.

Procedure The procedure described here is essentially a modified version of that used for the synthesis of permethyl-β-cyclodextrin [96]. Dry β-CD (13.56 g, 0.0119 mol) was dissolved in dry dimethyl sulfoxide (150 mL) and was cooled to 0°C. Sodium hydride powder (12 g, 0.5 mol) was added slowly to this solution, and was stirred first for 10 min at 0°C and then at room temperature for 12 h. After cooling the resulting green solution to 0°C, methyl iodide (55.2 g, 24.71 mL) was added. The mixture was stirred at this temperature for 1 h, and then at room temperature for a further 16 h.

After cooling the reaction mixture to 0°C, the excess sodium hydride was decomposed by adding methanol (60 mL), and then the mixture was poured onto an ice-water mixture (200 mL). After 10 min this mixture was extracted with dichloromethane (3 x 100 mL). The combined dichloromethane layers were washed with water (3 x 50 mL) until the aqueous extracts were neutral to pH paper. The organic extracts were dried over anhydrous sodium sulphate and evaporated to dryness. A yellowish gummy solid was obtained and was crystallized from cyclohexane (containing 1% dischloromethane). The yield was 12.33 g (71.3%); mp 153-154°C.

^{1}H-NMR (300 MHz) in D_2O, ppm: doublet at 5.157 (^{1}H, H_1) [$^2J(H_1, H_2)$=3.48 Hz], multiplet at 3.752 - 3.452 (5Hs, H_3, H_4, H_5, H_6, H_6), singlet at 3.494 (3Hs, - OMe at C_2), singlet at 3.399 (3Hs, -oMe at C_3), singlet at 3.268 (3Hs, -oMe at C_6), double-doublet at 2.226 (^{1}H, H_2)[$^2J(H_1, H_2)$ = 3.48 Hz, $^2J(H_2, H_3)$ = 9.0 Hz].

3.2.2 Preparation of Hexakis - (2,3,6-Tri-O-Methyl)-α-Cyclodextrin 2, and Octakis - (2,3,6-Tri-O-Methyl)-γ-Cyclodextrin 3

Compounds 2 and 3 were prepared from α-CD and γ-CD, respectively, following the procedure given above for the preparation of permethylated-β-CD. The products were obtained in quantitative yields and were identified by ^{1}H-NMR. Both compounds 2 and 3 show one spot in the solvent ethylacetate-hexanes (1:1).

3.2.3 Preparation of Permethylated-Maltotriose 4 and Permethylated-Maltose 5

Compounds 4 and 5 were prepared from maltotriose and maltose, respectively, following the procedure given above for the preparation of permethylated-α-, -β- and -γ-CDs. The products were obtained in quantitative yields and were identified by proton nuclear magnetic resonance spectroscopy. Both compounds 4 and 5 show one spot in the ethylacetate and hexane (1:1) systems.

3.2.4 Preparation of Heptakis-2,3,6-Tri-O-Benzoyl)-β-Cyclodextrin 6 [91]

A drying tube and a condenser were attached to a three-neck flask. A nitrogen inlet was installed on top of the condenser and the flask was kept in an ice-salt bath. Anhydrous pyridine (29.4 mL) in chloroform (15 mL) was added to the flask. Benzoyl chloride (24.36 mL, 0.21 mol) was added in small portions to the pyridine solution over a period of approximately 2 h from a dropping funnel. After removing the funnel, β-CD (11.25 g, 0.01 mol) was added in small portions over a period of approximately 2 h so that the temperature would be maintained below 10°C. The contents of the flask were stirred for 0.5 h in the ice bath and were left in the freezer for 13 h. The mixture was then stirred again in the ice bath for 2 h.

Dichloromethane (150 mL) was added to the reaction mixture, and was extracted with aqueous sulfuric acid (2 mol/L, 2 x 50 mL). The organic layers were further extracted successively with water (100 mL), saturated sodium bicarbonate (2 x 100 mL) and finally with brine (50 mL). The dischloromethane layers were dried over anhydrous sodium sulphate and evaporated to dryness. A yellowish gummy material (29.2 g, 88%) was obtained, and was thoroughly washed with ethanol. The remaining solid, which was soluble in acetone and insoluble in water, was crystallized from a mixture of ethanol-acetone (19.6 g, 59%); mp = sublimes at 175–185°C.

IR (thin film), cm^{-1}: 3190-3150(m)[aromatic C-H stretch], 2980-2930(m) [aliphatic C-H stretch], 1822(s)[-O-OC-Ph].

^{1}H-NMR (60 MHz) in DMSO-d_6, ppm: multiplets at 6.7-8.8 (aromatic Hs), sets of multiplets at 5.8-2.8[H_1-H_6]very similar to that of β-CD Hs.

3.2.5 Preparation of Heptakis-(2,3,6-Tri-O-Acetyl)-β-Cyclodextrin 7 [15-17]

Procedure 1 Acetic anhydride (52.5 mL, 0.546 mol) was added in one portion to a 250-mL round bottom flask containing zinc chloride (2.1 g, 0.015 mol). The mixture was stirred in a boiling water bath for 10 min. After the flask was removed from the water bath, β-CD (2.5 g, 0.014 mol) was added to the flask in small amounts at a time. The mixture was then heated under reflux, under nitrogen, in the boiling-water bath for 1 h. The resulting light brown liquid was poured into an ice-water mixture (200 mL), stirred for 5 min and stored in a fridge for 2 h. A white precipitate appeared and was filtered, washed with water, and finally dried under vacuum; the yield was 1.18 g (61.6%), mp 138–144°C.

^{1}H-NMR (60 MHz) in DMSO-d_6, ppm: sets of multiplets at 5.5-3.0 (7Hs, H_1-H_6), three singlets at 2.3, 2.22 and 2.15 (9Hs, acetyl groups at C_2, C_3 and C_6).

IR (thin film), cm^{-1}: 1730 (s) [-O-OC-Me]

Procedure 2 The preparation of peracylated-β-CD by this method was carried out with acetic anhydride in pyridine [15-17].

β-CD (4.5 g) was dissolved in pyridine (90 mL) and acetic anhydride (7.6 g) was added slowly while stirring. The stirring was continued overnight at room temperature. The work-up was done in water and the product insoluble in water was collected, and was then recrystallized from ethanol-water to give a yield of 70%. The product was analyzed for the extent of acylation. Thin-layer chromatography (tlc) showed one spot of higher Rf value than that of β-CD. The IR spectrum showed a band for the ester group. The ^{1}H-NMR spectrum indicated 70% acylation.

3.2.6 Preparation of Hepta-6-O-Tosyl)-β-Cyclodextrin 8

The reaction requires anhydrous conditions. β-CD was dissolved in a dry round bottom flask and p-toluenesulfonyl chloride was added to it. After the reaction mixture was stirred for a while, it was poured into an ice-water mixture. The product, which was separated as a solid at the bottom, was filtered and washed with water. It was then washed thoroughly with acetone until a constant melting point of 170 to 175°C was obtained. The yield from various trials was 20 to 40%. The product was identified by IR and ^{1}H-NMR spectroscopy.

3.2.7 Preparation of N-Benzyl-L-Proline 9

The reaction was carried out by dissolving potassium carbonate (16.5 g, 0.10 mol) and L-proline (11.5 g, 0.1 mol) in water (120 mL). Benzyl bromide (17.1 g, 0.1 mol), which is insoluble in water, was added and the reaction mixture was heated under reflux with vigorous stirring for 8 h. The work-up was carried out by pouring the mixture into water. After being extracted with dichloromethane (2x), the aqueous layer was neutralized to pH 7 with dilute hydrochloric acid and was then extracted again with dichloromethane. The later organic extracts were first dried over anhydrous sodium carbonate and evaporated to dryness. The product was analyzed by tlc, which indicated the presence of the product and small amount of residual benzyl bromide. The benzyl bromide was removed by washing with acetone. The yield of the pure product was 30% after chromatographic separation on silica gel with acetone as the eluting solvent. The product was identified by IR and ^{1}H-NMR (60 MHz) spectroscopy.

3.2.8 Preparation of N-(2,4-Dinitrophenylsulfenyl)-L-Proline 10

Our procedure for preparing N-(2,4-Dinitrophenylsulfenyl)-L-proline 10 follows the general method for preparing the N-thiophenyl derivatives of amino acids described by Wolman [97]. Solid 2,4-dinitrophenylsulfenyl chloride (2.35 g, 10 mmol) was added in small portions over a period of ~30 min to a stirred solution of L-proline-sodium salt prepared by dis-

solving L-proline (1.15 g, 10 mmol) and sodium carbonate (1.68 g, 20 mmol) in a mixture of dioxane and water (1:1 ratio). Water (100 mL) was added after the solution was stirred for an additional 20 min. The solution was filtered and the filterate was evaporated in vacuum. The solid so obtained was taken in water (150 mL), acidified with citric acid and extracted first with ethylacetate (2 x 50 mL) and then with dichloromethane (2 x 50 mL). The combined organic extracts were washed with water, dried over anhydrous sodium sulphate and finally evaporated to dryness. A brown solid was obtained, mp 156–161°C. The product is insoluble in water, chloroform and acetone, but is slightly soluble in DMSO. Tlc in ethylacetate shows two spots: the minor spot has an R*f* value 0.9 and the major spot has a value of 0.1. No further purification was attempted.

3.2.9 Synthesis of Biphenyl-4,4'-Disulphonyl-A,D-Capped-β-Cyclodextrin 11 [98]

The target compound was needed for irradiation purposes and for use as a starting material in the preparation of selectively blocked open-chain sugars. It was prepared in very low yields during several trials. Before the actual reaction, β-CD was carefully dried in a drying pistol at 85 to 90°C at 0.15 mm (Hg) for 18 to 20 h. Commercially available anhydrous pyridine had a reported water concentration of 0.005%. The two starting materials, β-CD and biphenyl-4,4'-disulfonyl chloride, and the solvent pyridine were tested for their moisture content with a coulometer model 652 KF instrument. After treatment, the water contents of all the materials were acceptable. All glassware was dried in an oven at 130 to 140°C for several hours. Then, trial reactions were performed at small and larger scales. The reaction mixtures were purified by procedures such as column chromatography (flash chromatography), preparative thin-layer chromatography, solvent extraction, precipitation by differential solubility in different solvents, centrifugation, and final evaporation of the solvent(s).

During a typical trial, biphenyl-4,4'-disulfonyl chloride was added in four portions over a period of 1 h to a rapidly stirred solution of β-CD in pyridine at 50°C in an oil bath. Pyridine was decanted from the gummy material which separated at the bottom. The gummy material was found to consist mostly of unreacted CD and polymeric material as determined by tlc. Evaporation of the pyridine layer yielded a colorless solid. This solid indicated the presence of considerable amount of the target compound by tlc in 1-propanol:ethylacetate:water:aqueous ammonia [5:3:2:1]. The other components present in this solid were small amounts of the two starting materials and some polymeric material. The tlc R*f* values of these components the above solvent system are 0.5 for biphenyl-4,4'-disulfonyl chloride, 0.25 for capped-β-CD, 0.08 for β-CD and 0 to 0.05 for the polymeric material. Also, it was noted that the target compound partially decomposes to β-CD and biphenyl-4, 4'-disulfonic acid upon successive purification procedures. This was observed from the tlc behavior of the crude product before and after the purification workup procedures.

The structure of the target compound was confirmed by its proton nuclear magnetic resonance (300 MHz), infrared spectra, and melting point of 163 to 164°C. It's purity was checked by tlc (in the above solvent sys-

tem) and by proton-nmr, which indicate the presence of very small amounts of impurity in the final product.

^{1}H-NMR (300 MHz) in DMSO-d_6, ppm: two sets of doublet-doublet at 8.155-7.868 (aromatic protons), doublets at 4.925-4.753 (anomeric H_1), multiplets at 4.60-2.65 (H_2-H_6).

IR (KBr), cm^{-1}: Broad band centered at 3400 (s) [-OH], 3100 (w) [aromatic C-H stretch], 2920 (m) [aliphatic C-H stretch], 1620 (m) [aromatic C=C], 1360(s), 1175 (s) [o=s=o], 1050(s), 1025(s), 840(s).

3.3 INCLUSION COMPLEXES OF CYCLODEXTRINS

3.3.1 *ρ*-Methoxyphenol/*α*-Cyclodextrin Complex

This complex was prepared in a single aqueous phase because *ρ*-methoxyphenol is water soluble. The yield of the precipitated complex was 50%.

The complex was characterized by HPLC and high-resolution ^{1}H-NMR spectroscopy. The HPLC results did not indicate the presence of free CD. The only major peak had a retention time close to that for the uncomplexed *ρ*-methoxyphenol. Hence, these HPLC results do not confirm the formation of the complex. However, weight considerations and the absence of a peak due to free *α*-CD suggest that it is likely that a complex was formed.

^{1}H-NMR (300 MHz), D_2O, ppm: multiplet centered at 6.792 (4-Hs, aromatic), singlet at 3.652 (3 Hs, -OMe), doublet at 4.918 (6 Hs, C_1H protons of CD) [$^2J(H_1\text{-}H_2) \approx 3.45$ Hz], multiplets at 3.818 - 3.464 (6 Hs, C_2-C_6 protons of CD and the pattern of splitting is similar to that of CD obtained from a separate nmr analysis).

The IR spectra of *ρ*-methoxyphenol, *α*-CD and the complex were obtained by preparing KBr pellets. Some of the peaks due to *ρ*-methoxyphenol appear to be shifted slightly when the molecule is in the cyclodextrin environment.

3.3.2 *ρ*-Methoxyphenol/*β*- and *γ*-Cyclodextrin Complexes

These complexes could not be obtained possibly due to the lack of a tight fit of the guest in the cavity, or, less likely, it may be that the solubility difference between the CD and the complex was not large enough to cause precipitation.

3.3.3 *ρ*-Nitrophenol/Cyclodextrins Complexes

p-Nitrophenol was chosen as a guest molecule in order to try some complexation methods and to analyse the resulting complexes by different analytical techniques such as IR, nmr and HPLC. The compound was only slightly soluble in water and, therefore, both the single- and double-phase methods were used to crystallize the complex.

The complex was characterized by IR and nmr spectroscopy. The IR spectra of ρ-nitrophenol, β-CD and the suspected complex showed a slight shift in the position of bands in the complex. It appears that IR spectroscopy is a better technique in this case for indicating whether a complex is formed. The low-resolution ^{1}H-NMR spectra of the complex was difficult to analyse. The resolution was low due to the low solubility of the complex in D_2O. This method is limited to with D_2O as a solvent because other solvents may dissociate the complex. The following titration experiment was attempted with UV spectroscopy. Aliquots of α-CD were added to a solution of ρ-nitrophenol in water. A slight shift in the peak was observed along with the appearance of another peak, which increased as more CDs were added. It was also apparent that the complexation may be pH dependent, that is, the complex may be more stable when the phenol exists in the anion form. Similar experiments were carried out in different acetate buffers to give a pH profile. The most acidic trial (pH 4.0) showed no additional peak and very little change in the existing peaks.

3.3.4 Benzofuroxan/Cyclodextrins Complexes

The complexation of benzofuroxan (BFO) and α-CD was carried out in a two-phase system with diethyl ether. The complex precipitated at the interface with a yield of ~60%. The same complex had a 55% yield in a one-phase (aqueous) system. The complex is best identified by HPLC. The retention time of the complex was different than those of α-CD and BFO. The two complexes obtained by the above two methods looked identical with HPLC. Some residual α-CD was also present.

The benzofuroxane β-CD complex was prepared in a two-phase system at room temperature with a yield of about 20% and was identified by HPLC. This complex was also prepared with the same yield at 60°C.

A series of solutions (~0.0001 mol/L) of BFO, CD and their complex were prepared in water and were irradiated at doses of 0 to 100 Gy by a calibrated Siemens Stabilipan X-ray machine. The solutions were analyzed by UV spectroscopy. Plots of the UV absorption showed a decrease in the peak intensities and broadened at larger doses. An absorbance due to a new unidentified species formed during irradiation was also present.

3.3.5 Metoprolol/Cyclodextrin Complexes

Metoprolol was available as its tartarate salt. It was obtained from this salt using the hydrolysis method described below:

Commercial metoprolol-tartarate (127 mg) was dissolved in water (2 mL), and 50% sodium hydroxide (0.3 mL) was added. The solution was stirred for 5 min and then was extracted with ether (3 x 2 mL). The ether extracts were dried over anhydrous sodium sulphate and evaporated to dryness. A colorless solid was obtained which was different by tlc from the starting metoprolol tartarate. The IR spectrum did not show C=O absorption (for tartarate). The ^{1}H-NMR (60 MHz) in $CDCl_3$ confirmed the structure of metoprolol.

A complex of metoprolol and γ-CD was prepared by dissolving γ-CD (0.003 mol) in water (20 mL) and metoprolol (0.003 mol) in diethyl ether (5 mL). The ether solution was layered gently on top of the aqueous solution and left at room temperature without stirring. The complex was precipitated at the bottom as a colorless solid and was collected. The top ether layer was evaporated and yielded some unreacted metoprolol (46%); evaporation of the aqueous layer yielded some unreacted γ-CD. HPLC analysis of the complex showed a retention time different than the γ-CD. The complex was stirred vigorously in excess dichloromethane in the presence of a small amount of water and the dichloromethane layer was evaporated to dryness; free metoprolol was obtained as determined by tlc. IR, ^{1}H-NMR (60 MHz) and HPLC results did not differentiate between the two enantiomers.

The metoprolol did not form complexes with α-CD and β-CD when the above procedure was used.

3.3.6 Complexation of Cyclodextrins with Other Biologically Active Organic Compounds

Complexation was attempted with the following compounds and is mentioned briefly, but detailed analyses were not carried out.

(1) L-Proline with α- and γ-CDs in a one-phase system.
(2) Ph. alanine with α-CD in a one-phase system.
(3) Tryptophane with γ-CD in a two-phase system.
(4) Tyrosine with α-, β- and γ-CDs in a one-phase system.
(5) Thalidomide with α-, β- and γ-CDs in a one-phase system.
(6) Aspirin with α-, β- and γ-CDs in a one-phase system.
(7) Benzylimidazole with α-, β- and γ-CDs in a one-phase system.
(8) Vitamin E with α-, β- and γ-CDs in a one-phase system.

The following suspected solid complexes were precipitated out: aspirin with γ-CD and some β-CD but not with α-CD; benzylimidazole with γ-CD but not with α- and β-CD; Vitamin E mostly with γ-CD, little with β-CD and none with α-CD; thalidomide not with any CD. These suspected complexes could not be analyzed due to the priority given to some other projects.

The following complexes were detected only by HPLC on cyclobond columns: tryptophane with γ-CD; tyrosine with α-CD; phenylalanine with α-CD; and low yields of proline with γ-CD.

3.4 STUDIES OF THE GAMMA RADIATION EFFECTS ON CYCLODEXTRINS AND OTHER CARBOHYDRATES

The following compounds were irradiated in a Gammacell 200 (with a cobalt-60 source) under various experimental conditions to study the effects of ionizing radiation: α-, β- and γ-CDs; permethylated-α-CD and permethylated-β-CD, maltoheptaose, maltohexaose, maltopentaose, maltotriose, maltose, and gentiobiose. The samples were irradiated in sealed glass bottles in aqueous solutions prepared in triply distilled water; the solutions were then degassed and saturated with nitrogen. In some cases, the temperature was controlled inside the irradiator, while in others the at-

mosphere was not controlled for oxygen or air. Some representative cases are described in detail below.

3.4.1 Gamma Irradiation of α-, β- and γ-Cyclodextrins

Method A – Aqueous solutions (0.008 mol/L) of α-, β-, and γ-CDs were prepared in triply distilled water. The solutions were degassed thoroughly and were saturated with nitrogen. The solutions were sealed in glass bottles and irradiated in a Gammacell 220 (cobalt-60 source) at a dose of 5000 Gy (the dose rate was 230.84 Gy/min). The temperature inside the cell was controlled at 3°C. The samples were analyzed by HPLC with tw oligosaccharide columns operating at 85°C with water as the mobile phase. Detection was done by UV and refractive index detectors.

HPLC analysis indicated that about 40% of each of the three CDs were converted to products. The products consisted of the open-chain form of the CD less one glucose unit, e.g., maltoheptaose from γ-CD (approx. 35%), 14-glucose-unit sugars (the dimerized product, approx. 40%), polymerized products (approx. 15%), and oxidized products (approx. 10%). Under the same experimental conditions, one sample of γ-CD was prepared in distilled water that had neither been degassed nor saturated with nitrogen. HPLC analysis of this sample indicated the presence of a slightly larger amount of the oxidized products and also a very small amount of maltopentaose (5-glucose-unit sugar) that probably resulted from the oxidation of maltoheptaose.

Method B – Aqueous solutions (0.002 mol/L) of α-, β-, and γ-CDs were prepared in distilled water, degassed and saturated with nitrogen. They were then irradiated in a Gammacell 220 at a dose of 8000 Gy (the dose rate was 234.18 Gy/min). The temperature inside the cell was not controlled. HPLC analysis on a single oligosaccharide column operating at 85°C showed the conversion of about 80 to 85% of the CDs to the products that were largely oxidized and polymerized compounds.

Method C – Aqueous solutions of α-, β-, and γ-CDs, as prepared above, were irradiated at a dose of 1000 Gy (the dose rate was 4.17 Gy/min) and analyzed by HPLC as in Method B. It was noted that less than about 20 to 30% of the CDs were converted to products that were fairly similar to those in Method A.

3.4.2 Gamma Irradiation of Other Carbohydrates

Method D – Aqueous solutions (0.008 mol/L each) of maltose, maltotriose and gentiobiose were prepared in triply distilled water, and were then degassed and saturated with nitrogen. Another set of solutions was prepared in ordinary distilled water; these solutions were not degassed and were not saturated with nitrogen. Both sets of solutions were irradiated in a Gammacell 220 at 4°C at a dose of 5096 Gy (the dose rate was 229.64 Gy/min). All the samples were analyzed by HPLC with two oligosaccharide columns operating at 85°C with water as the mobile phase. In all cases, about 50% of the sugars were converted to products. In the case of maltotriose the products were oxidized material, polymerized material and a 9-unit sugar (maltononaose), all in roughly equal proportions as estimated

from the peaks heights and widths. In the case of maltose, there was also an 8-unit sugar (maltooctaose) and a 9-unit sugar (maltononaose) besides the oxidized and polymerized products. The formation mechanism of these products is not certain. The formation of these products was inferred from their retention times. In the case of gentiobiose (having a $\beta \rightarrow (1,6)$ linkage), the main products were polymerized materials. HPLC analysis indicated no significant difference in the results from irradiation in the presence or absence of oxygen at 4°C.

Method E – Aqueous solutions (0.008 mol/L each) of maltoheptaose, maltohexaose and maltopentaose were prepared in triply distilled water and were then degassed and saturated with nitrogen. The samples were irradiated in a Gammacell 200 at a dose of 5000 Gy (the dose time was 229.35 Gy/min) at 3°C. HPLC analysis were carried out on two oligosaccharide columns operating at 85°C where the mobile phase was water. It was found that all three sugars produced oxidized material, polymerized material, small amounts of dimerized material as well as smaller fragments up to three sugar units. In each case about 50% of the starting material remained unreacted.

3.4.3 Gamma Irradiation of Permethylated-Cyclodextrins

Aqueous solutions of permethylated-α-CD (0.008 mol/L) and permethylated-β-CD (0.004 mol/L) were prepared in triply distilled water and were then degassed and saturated with nitrogen. These samples were irradiated at a dose of 5000 Gy in a Gammacell 220 at 5°C. HPLC analysis on two oligosaccharide columns, as above, indicated that less than about 25 to 30% of the starting materials were converted to products. These results indicate that they are more stable at this dose than the corresponding parent CDs. Most of the product was polymerized material.

4. CONCLUSIONS

Three aspects of the chemistry of CDs were studied: the syntheses of some CD derivatives, preparation of CD complexes, and the effects of irradiation on CDs and some structurally related carbohydrates.

The following derivatives of CDs were synthesized: permethylated-α-, permethylated-β-, and permethylated γ-CDs; perbenzoylated-β-CD; peracetylated-β-CD; hexa-6-O-tosyl-β-CD; and biphenyl-4,4'-disulfonyl-A,D-capped-β-CD. Also, N-protected-L-proline, permethylated-maltotriose and permethylated-maltose were synthesized. The structures of these compounds were determined by spectroscopic techniques, such as IR, NMR (300 MHz, and 60 MHz), HPLC techniques and melting points. The yield of the preparations of biphenyl-4,4'-disulfonyl-A,D-capped-β-CD was low (7%), as it is generally the case of all capped-CDs.

The complexation of CDs was attempted with a number of organic compounds. The formation of complexes was detected generally by HPLC and in some cases by ^{1}H-NMR, IR, and UV spectroscopy.

When CDs in the form of deoxygenated aqueous solutions were irradiated with gamma rays, the percentage of conversion of α-, β, and γ-CDs to the products at different doses was: about 20% at 1000 Gy, about 40% at 5000 Gy and about 80% at 8000 Gy. The dose of 5000 Gy was found best for preparing open-chain CD derivatives with one less glucopyranose unit than the starting CD, and a dimerized form of this derivative. There are more oxidation and polymerization products at 8000 Gy than at lower doses. Results from experiments on the irradiation at lower temperatures (3 to 4°C) show little difference between oxygenated and deoxygenated aqueous samples of CDs. In the case of permethylated CDs, only about 25 to 30% of the starting material is converted to products at a dose of 5000 Gy. This indicates that the permethylated CDs are slightly more stable than the parent CDs at similar doses.

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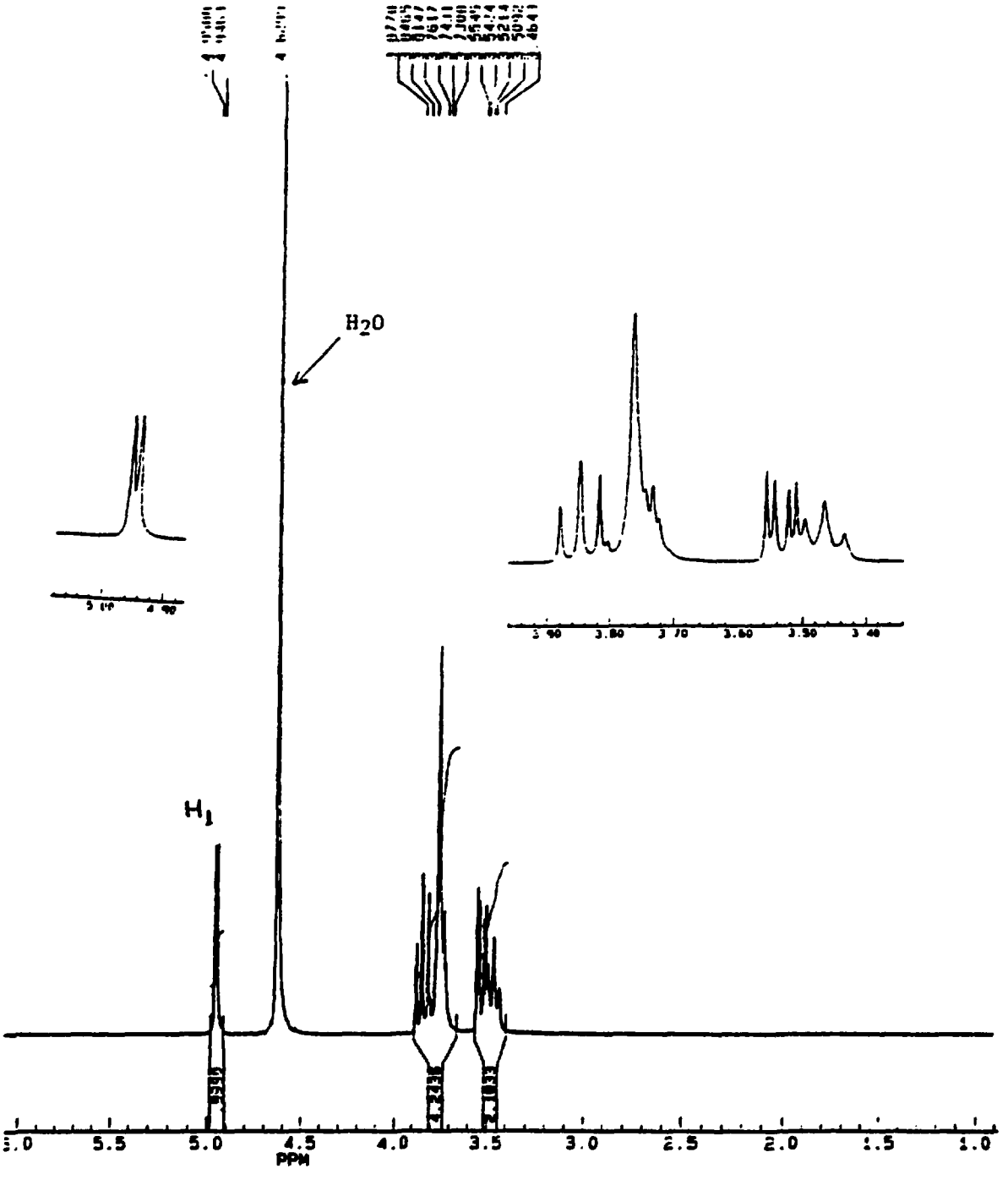
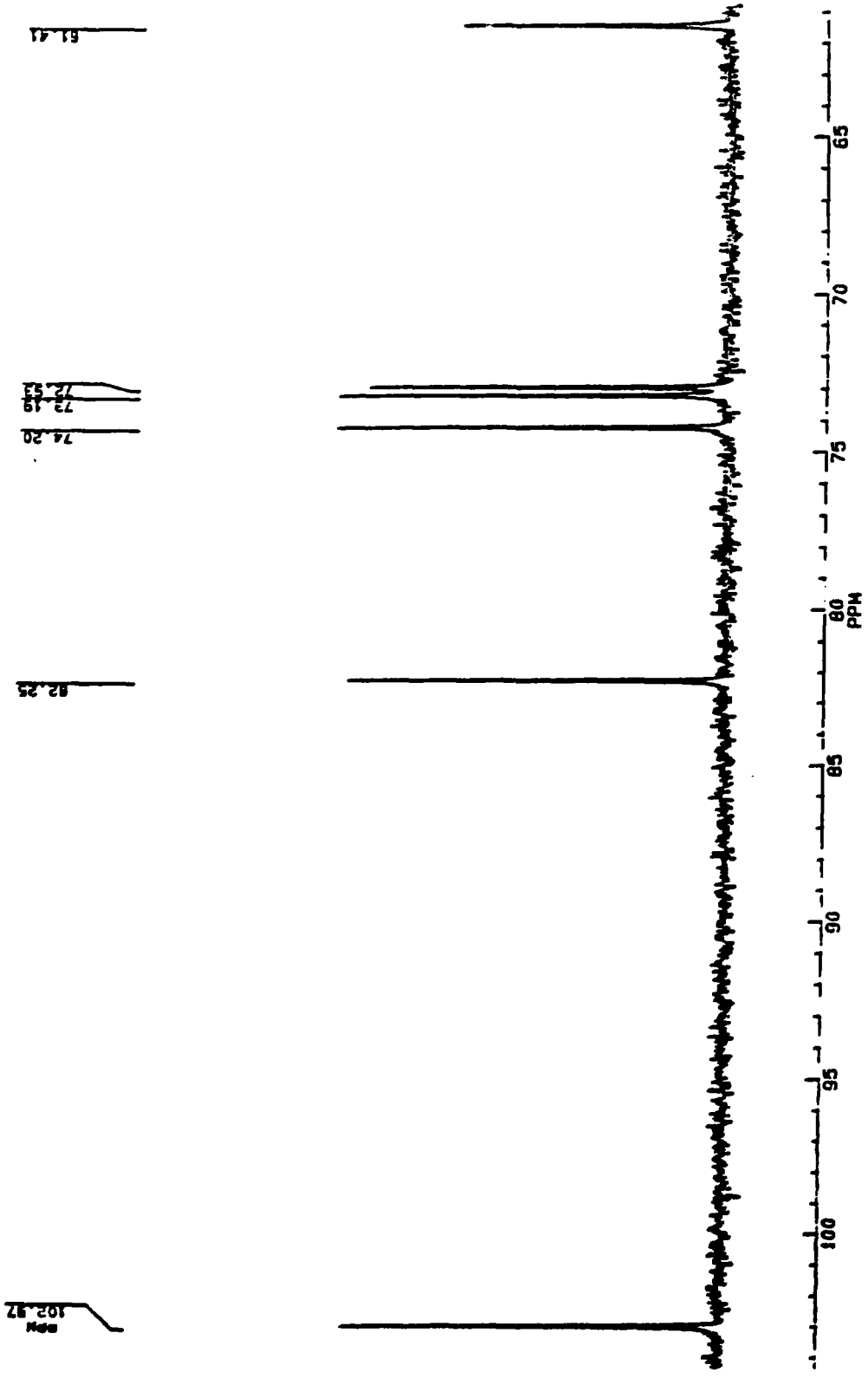
APPENDIX A

NMR SPECTRA OF CYCLODEXTRINS



1H-NMR AT 300 MHZ IN D_2O OF BETA-CD



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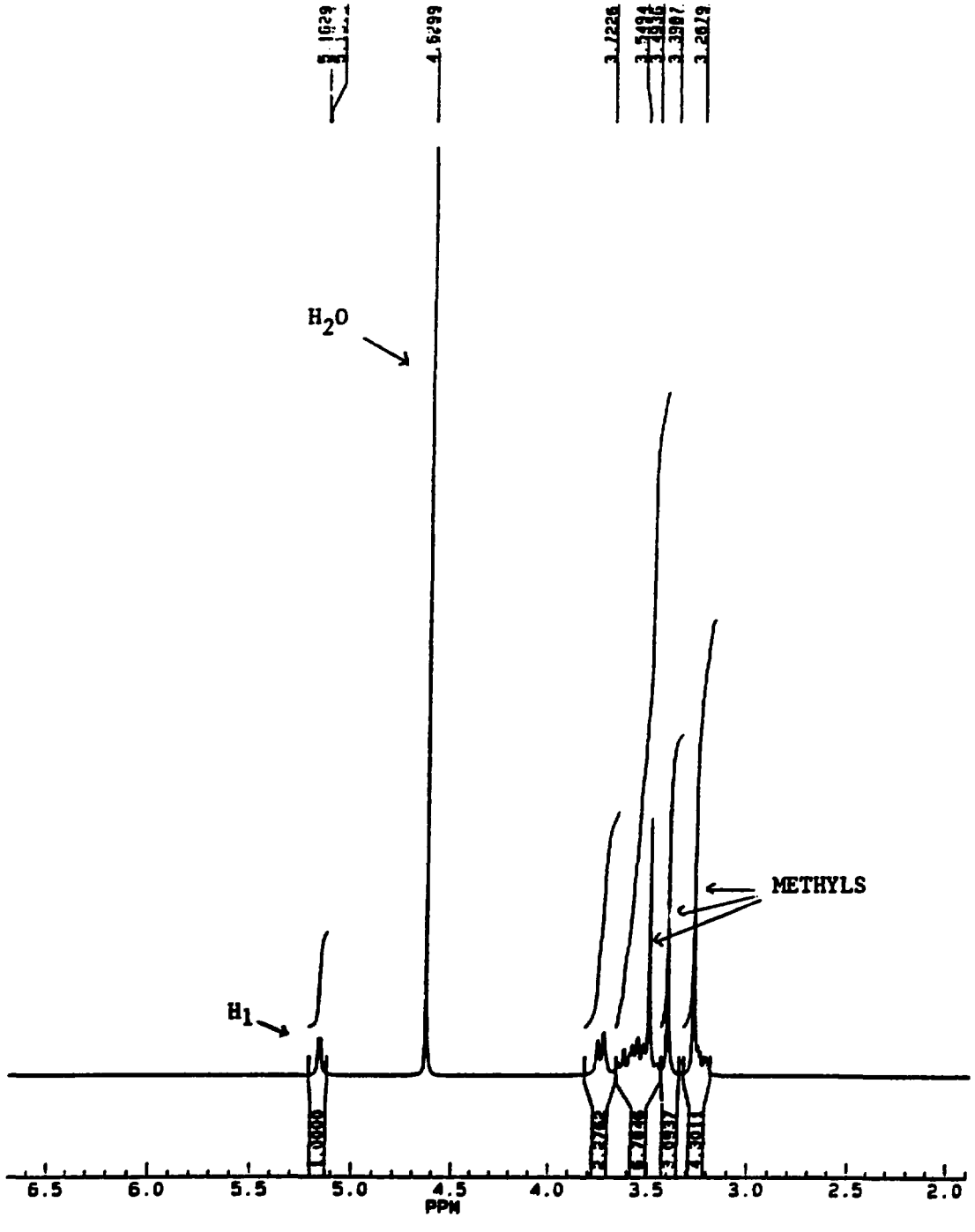
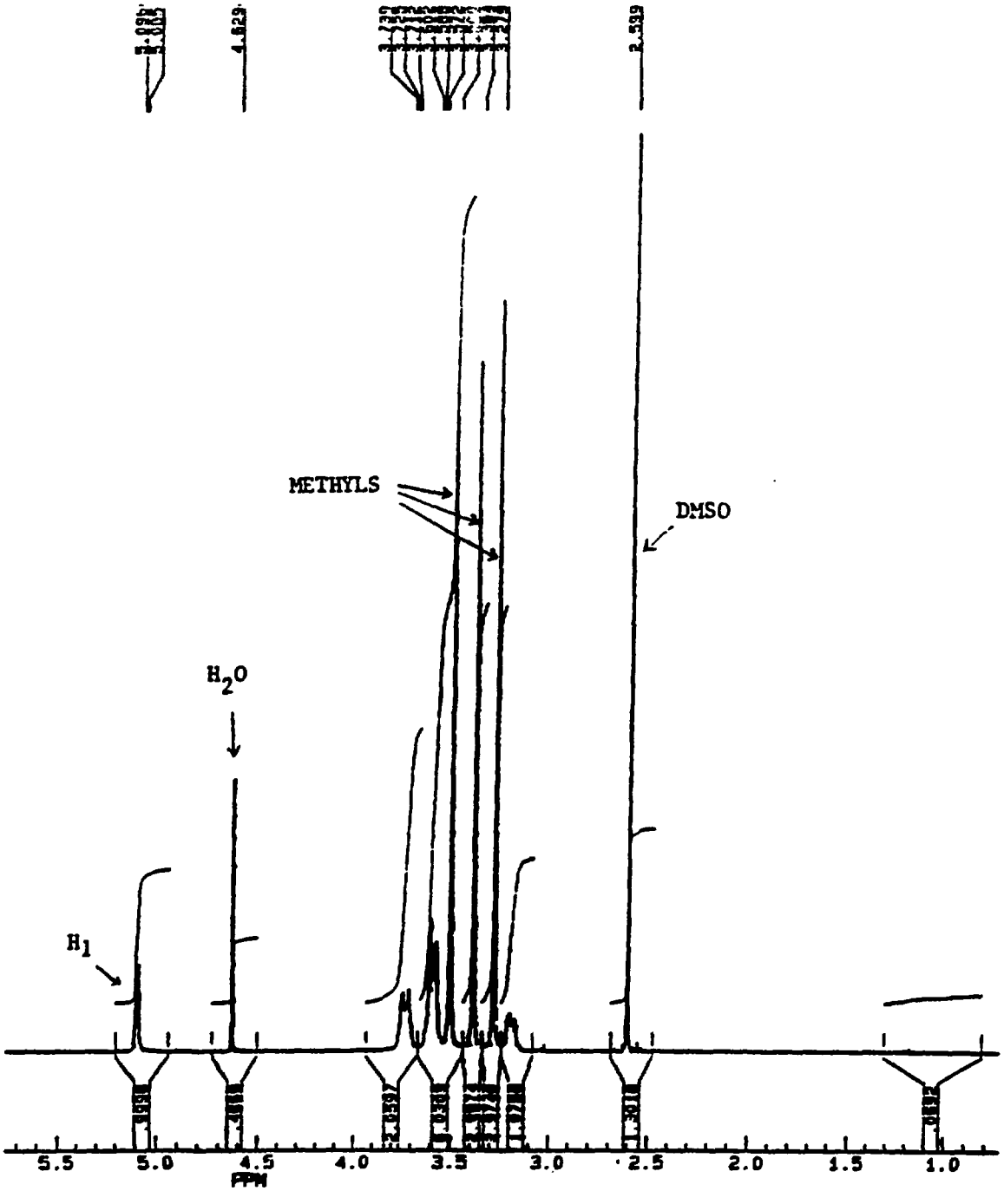


1H-NMR AT 300 MHZ IN D_2O OF

PERMETHYLATED-BETA-CD 1



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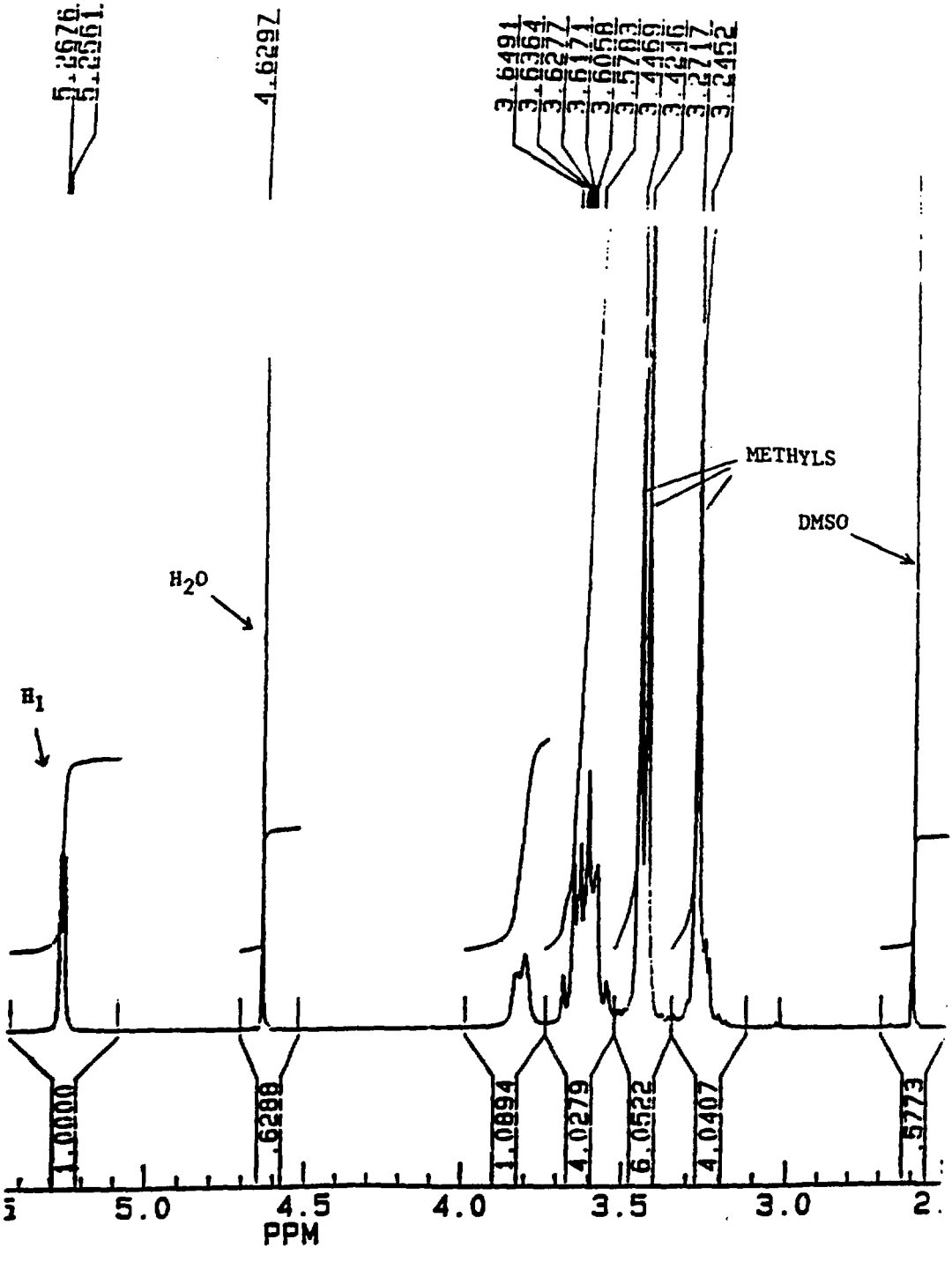
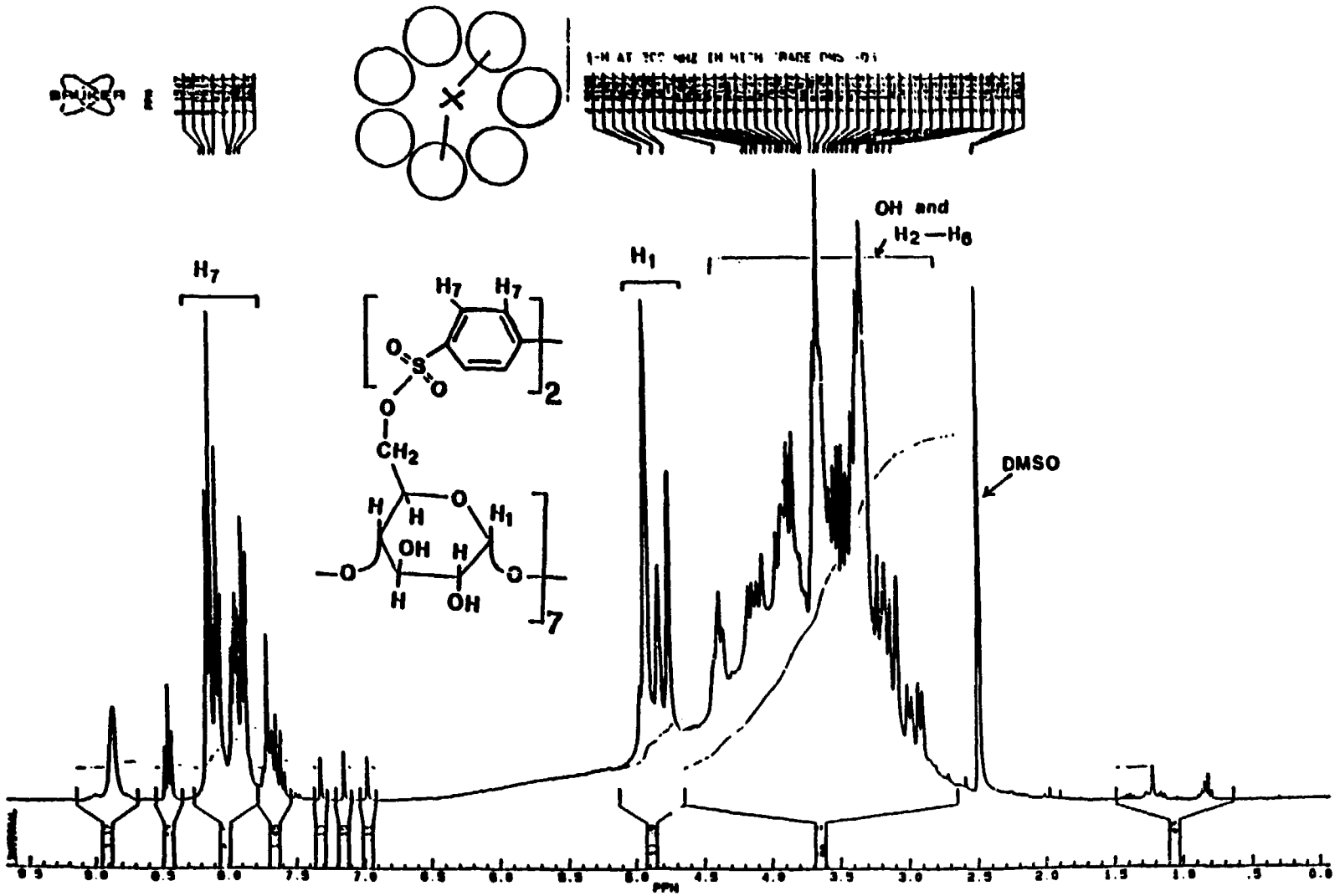
PERMETHYLATED-ALPH-CD 2

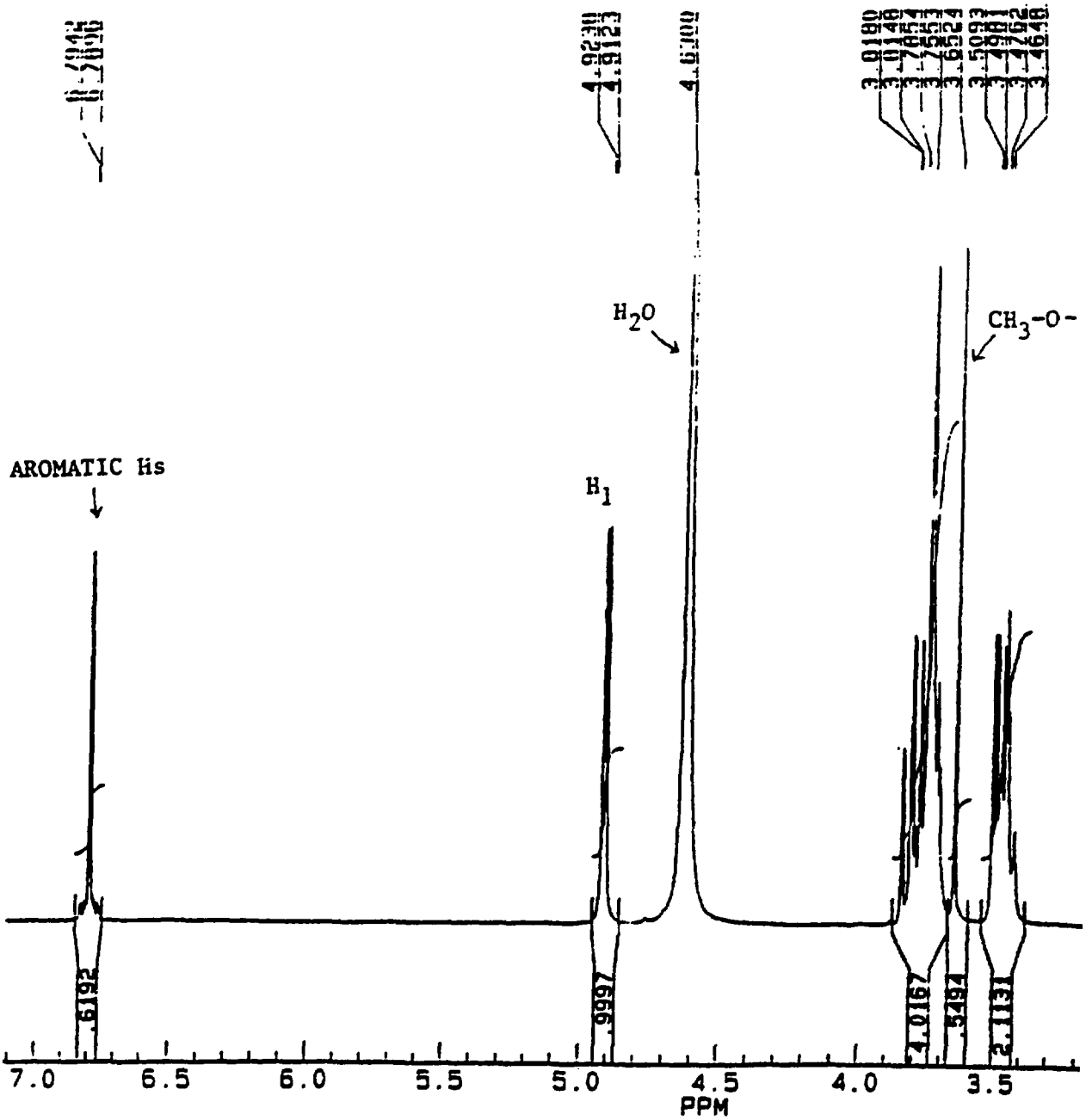


1H-NMR AT 300 MHZ IN D_2O OF

PERMETHYLATED-GAMMA-CD 3

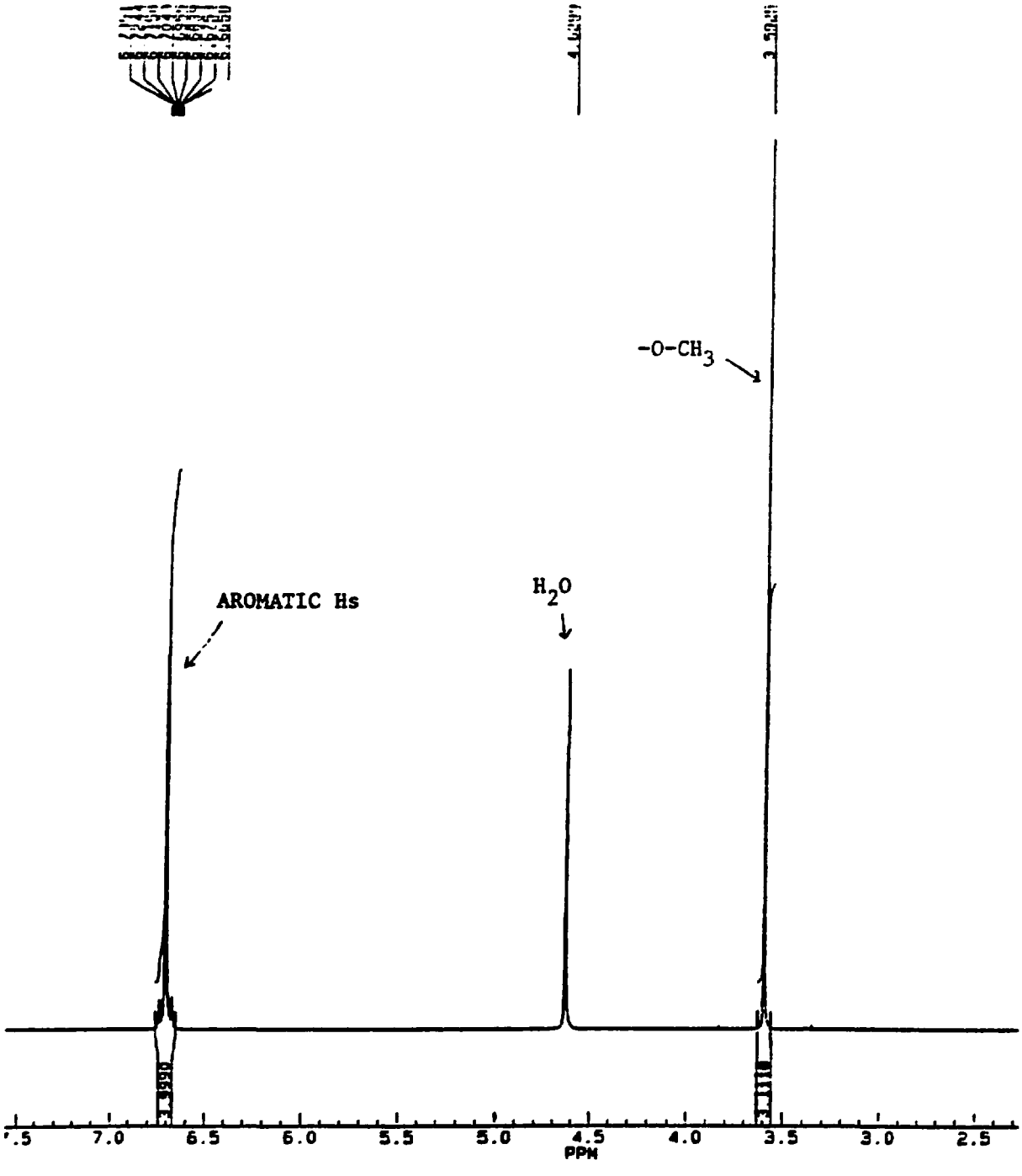


1H-NMR AT 300 MHZ IN DMSO-D_6 OF A,D-CAPPED-BETA-CD 11



1H-NMR AT 300 MHZ IN D_2O OF

COMPLEX OF ALPHA-CD + p-METHOXYPHENOL



1H-NMR AT 300 MHZ IN D_2O OF

p-METHOXYPHENOL

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