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The Oxidation of beta-Cyclodextrin Via the Photolysis of 6-beta-Cyclodextrin Benzoyl Formate

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In Partial Fulfillment

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Masters of Arts

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by

Heather Alison Creswick

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

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ABSTRACT

The oxidation of β-cyclodextrin via the photolysis of 6-β-cyclodextrin benzoyl formate was attempted. Oxidation at the C-6 carbon of one glucose residue, followed by reaction with diamines is a method for bridging cyclodextrin. Reaction of the oxidized cyclodextrin with 1,1-diphenylhydrazine failed to produce an imine; therefore, it was concluded that the photolysis of the ester did not yield a mono-aldehyde derivative of cyclodextrin. Also, analysis of the ester by ^{13}C-NMR and analytical HPLC indicated that the ester was never formed.

THE OXIDATION OF β-CYCLODEXTRIN VIA THE PHOTOLYSIS OF 6-β-CYCLODEXTRIN BENZOYL FORMATE

INTRODUCTION

The unique nature of cyclodextrin molecules has captured the interest of scientists since they were discovered by Villiers in 1891.[1] Research in this area was uneventful until Schardinger produced cyclodextrin from the action of the amylase of Bacillus macerans on starch and later isolated the various types via selective precipitation with appropriate organic compounds.[2] This finding was significant because it revealed the origin of cyclodextrin and offered a method of preparation that is still used today. The knowledge of cyclodextrin's vast capablilties was not fully appreciated until its structure was determined by Freudenberg many years later.[3]

A unique characteristic of cyclodextrins is their relatively hydrophilic exterior and hydrophobic interior which makes them analogous to micelles. The ability to incorporate guest molecules into their cavity via hydrophobic interactions is an important property that is the basis for their enzyme-like ability to bind substrates and/or catalyze reactions. Catalyses by cyclodextrin are categorized as covalent and non-covalent. The latter utilizes the cavity as a sterically

restricted reaction medium without the formation of a covalent intermediate.

Cyclodextrin has been proven to catalyze a number of reactions including the hydrolysis of phenyl esters, amides, and organophosphates.[4] In addition to accelerating reactions, cyclodextrin is used to increase the solubility of certain compounds or to stabilize volatile substances. One benefit of cyclodextrin's solubility in water can be seen in the fluorescent labeling of proteins and plasma membranes. Dansyl chloride is used to label proteins and plasma membranes; however, it is only soluble in organic solvents. The dansyl chloride-β-cyclodextrin complex allows fluorescent labeling to occur in aqueous solution, thus avoiding the use of organic solvents.[5] Cyclodextrin has important commercial applications in the pharmacuetical, flavors and fragrances, and agricultural chemical industries. It has been found that cyclodextrin can complex with certain drugs, insecticides, and coenzymes and improve their effectiveness.

Although cyclodextrin can act as enzymes in model studies, it does not possess enzyme-like specificity for binding substrates. This is largely because its cavity is open at both ends. Modifications to cyclodextrin such as capping, the closing off of one end, and bridging, the linking of two cyclodextrins, enable it to bind substrates more effectively, thus approximating the binding capabilities of some enzymes (figs. 4a, 4b). One area of cyclodextrin

research focuses on the bridging of derivatized cyclodextrin molecules. These dimers provide a more rigid structure containing two hydrophobic binding sites which gives rise to binding constants analogous to antigen-antibody binding constants.[6] In addition to the enhanced binding capacity of duplex cyclodextrins, the bridge can assume a catalytic role. For example, a cyclodextrin dimer containing a metal-binding group in the bridge has catalyzed the hydrolysis of bound substrates.[7] Recent studies have demonstrated that doubly bridged cyclodextrins at adjacent sugar residues are much more geometrically well-defined than the singly bridged versions. The "loveseat" model (fig. 5b) has a poor geometry for binding substrates because the two cyclodextrin rings are pointed away from each other. On the other hand, the "clamshell" (fig. 5a) model acts like a hinge to firmly bind guest molecules and since the cyclodextrins are at right angles to each other, bent substrates are more readily bound.[6]

The oxidation of cyclodextrin to a mono-aldehyde could be one of the many ways to prepare cyclodextrin for bridging. A method of oxidation currently being studied is the photooxidation of carbonyl compounds containg γ-hydrogens. Excitation via photolysis triggers a Norrish II reaction (scheme 5) resulting in γ-hydrogen abstraction followed by α-bond cleavage of a carbon-carbon bond. It is proposed that photolysis of a cyclodextrin-containing carbonyl compound will follow the Norrish II mechanism and yield oxidized

cyclodextrin. The resulting aldehyde should then readily react with various diamines to create bridges. Fluorescent molecules can act as substrates to test the binding capacity of the duplex cyclodextrin.

The photooxidation of cyclodextrin could provide an alternative to other methods of oxidation, thus introducing a simpler pathway to bridging cyclodextrin molecules.

BACKGROUND

Cyclodextrins, sometimes referred to as Schardinger dextrins, are a ring of D(+)-glucose units in C-1 (chair) conformations connected by α-(1,4)-linkages.[4] They consist of six, seven, and eight glucose units and are designated α-, β-, and γ-cyclodextrin respectively (fig. 1). Molecules with fewer than six units do not exist because of mechanical strain.

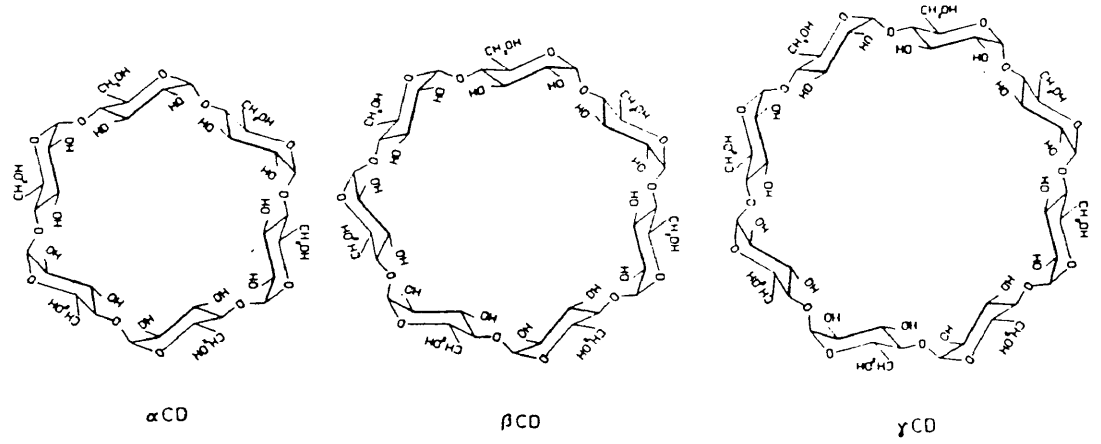


Fig. 1 α-, β-, and γ-Cyclodextrins

The cyclization of a chain of glucose residues causes the enthalpy to increase +6.60, +4.41, and +4.40 kcal/mol for α-,

β-, and γ-cyclodextrin respectively.[8] The unfavorable energy suggests that linearity is the preferred structure. However, the entropy factors, +14.4, +9.1, and +8.1 kcal/mol, favor cyclization and override the enthalpy considerations.[10b] The increased entropy is due to the disorganization and reorganization of surrounding water molecules as the chain of glucose residues forms a ring.

Cyclodextrin is shaped like a torus with the C-2 and C-3 hydroxyl groups on one face (secondary side) and the C-6 hydroxyl group on the other face (primary side). The primary hydroxyls are mobile and can rotate to block the cavity, while the secondary hydroxyls remain relatively fixed, allowing intramolecular hydrogen-bonding between consecutive units to occur. This secondary hydrogen-bonding is absent in α-cyclodextrin because one of the six glucose units is rotated out of the plane of the others. Cyclodextrins containing more than seven glucose units have rings of secondary hydrogen-bonding, but are too flexible to maintain a rigid geometry. Therefore, β-cyclodextrin has the most defined conformation. This rigidity of β-cyclodextrin allows for the selective functionalization of the primary over the secondary side. Thus, β-cyclodextrin is more easily manipulated and hence, preferrable to study.

The cyclodextrin interior consists of two rings of C-H bonds which, in combination with the ring of glucosidic oxygens, creates a relatively apolar, hydrophobic interior.[4]

In aqueous media, hydrophobic interactions between apolar substrates and the cyclodextrin cavity drive the formation of inclusion complexes.

Inclusion Complexes:

Since, cyclodextrin's can bind substrates in a similar way to enzymes, they might possess enzyme-like functions. The cavity dimensions are important to effectively bind a substrate. α-, β-, and γ-Cyclodextrins have internal diameters of 5.7, 7.8 and 9.5 Å respectively (fig.2),[9] and again, β-cyclodextrin seems to surpass the other homologues in binding capacity.

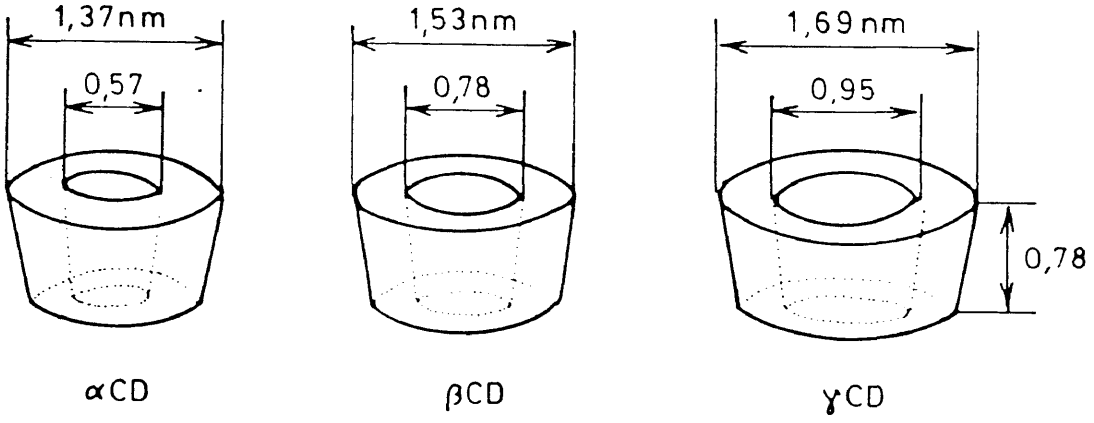


Fig. 2 Dimensions of α-, β-, γ-Cyclodextrins

The formation of inclusion complexes is not only driven by hydrophobic interactions, but it is also characterized by a favorable enthalpy change and a slightly favorable entropy

change. Although cyclodextrin can bind substrates in various solvents, binding occurs most effectively in water. This is because water molecules are trapped in the cavity and are considered high in potential energy since they cannot fully hydrogen bond with adjacent water molecules. When guest molecules are encorporated, they displace these high enthalpy molecules and the result is a favorable enthalpy change.[10]

A hydrophobic molecule in an aqueous environment induces its neighboring water molecules to form more hydrogen bonds with each other. This results in a highly ordered cage around the hydrophobic molecule. Consequently, there is a decrease in entropy due to the ordering of the water molecules around the apolar substrate. Therefore, when a hydrophobic molecule displaces the water molecules from the cavity of cyclodextrin there is a slight increase in entropy. This hydrophobic interaction is the main driving force for complexation (fig. 3).

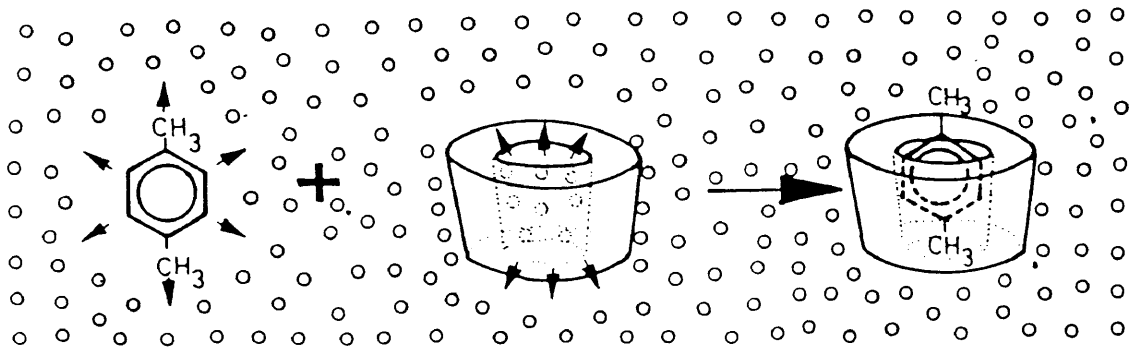


Fig. 3 Inclusion complex formation

Van der Waals interactions, including dipole-induced dipole interactions and London dispersion forces, also play a role in substrate binding.[4] These forces contribute significantly to the closeness of the guest molecule to the wall of the cyclodextrin. In this way, van der Waals forces can influence the geometry of the guest molecule. Another governing force in substrate binding is hydrogen-bonding between the guest and the hydroxyl groups of the cyclodextrin.[11]

The ability of cyclodextrin to form inclusion complexes enables it to serve as an enzyme model. The host-guest relationship is sufficient in cyclodextrin alone; however, increased binding capacities have been noted for modified cyclodextrin molecules.

Bridging:

Simple cyclodextrin has been known to bind guest molecules and to catalyze some reactions within its cavity. However, the open ends of the molecule prevent the inclusion complex from assuming a well-defined geometry and, therefore, lower cyclodextrin's binding capacity.[12]

To alleviate this problem, modifications to cyclodextrin have been carried out. Capping cyclodextrin with certain molecules on the primary side provides a "hydrophobic floor"[12]

which strengthens cyclodextrin's ability to bind a substrate (fig. 4a). Fluorescence studies have revealed that substrate binding by capped cyclodextrin is 11-24 times stronger than binding by the cyclodextrin alone.[13]

The formation of a duplex cyclodextrin linked at the C-6 carbon provides even stronger binding (fig. 4b). These dimers have two hydrophobic binding sites and a bridge that can sometimes catalyze reactions with the substrate. The two cyclodextrins in conjunction with the bridge serve as multiple recogition sites which make the geometry of the cyclodextrin-substrate complex even more well-defined.

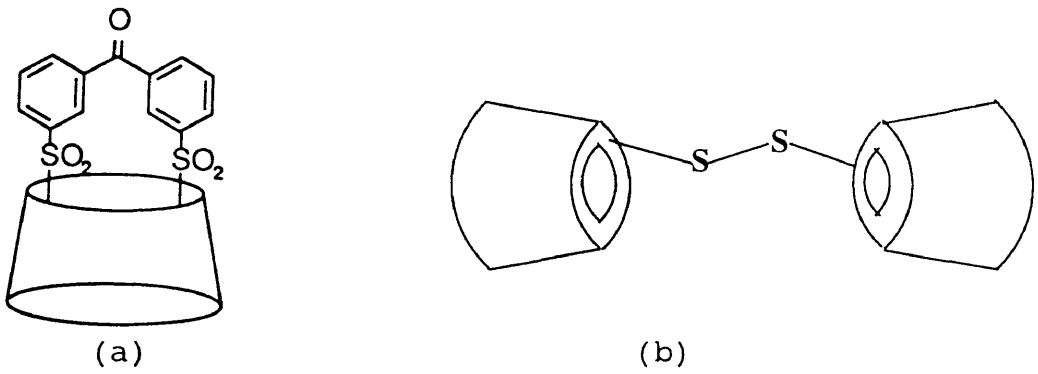
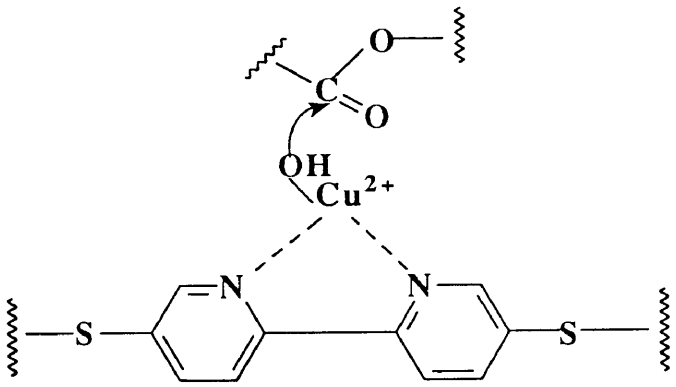


Fig. 4 Capped cyclodextrin (a), Duplex cyclodextrin (b)

Binding constants for the duplex cyclodextrin-guest complex can be determined by circular dichromism or fluorescence studies. More rigid links between the cyclodextrins result in binding constants that approximate those of medium-affinity antibodies.[14] The additive contribution of the second cyclodextrin was illustrated by

Tabushi *et al.* The association constant for methyl orange, a guest dye molecule with two hydrophobic recognition sites, was 3160 M^{-1} with duplex cyclodextrin, a value much greater than with a single cyclodextrin (520 M^{-1}).[15]

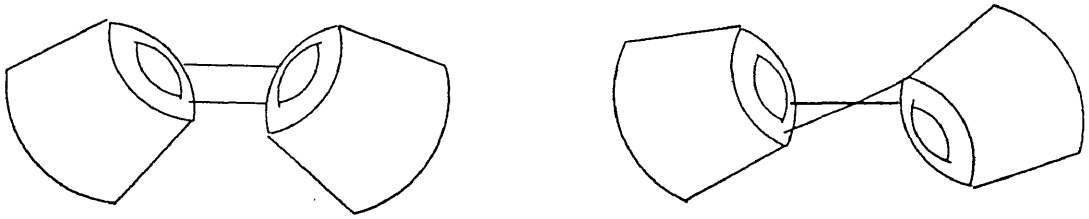
In addition to the increased binding capacity of duplex cyclodextrin, the link between the two can assume a catalytic role. The most commonly catalyzed reaction is hydrolysis, a process that is abundant in biological systems. Breslow and Zhang have prepared a cyclodextrin dimer with a metal-binding group in the link. Certain metal complexes with this group can catalyze the hydrolysis of bound substrates (scheme 1).[7]



Scheme 1 Hydrolysis via a catalytic link

Doubly bridged cyclodextrins at adjacent C-6 carbons exist in two forms. The "loveseat" model (fig. 5a) has aversive geometry because it directs the two cyclodextrins away from each other; hence, it cannot bind substrates with much success. On the other hand, the "clamshell" model (fig.

5b) acts as a hinge to firmly bind guest molecules. This additional bridge results in binding constants as large as 7 x 10^8 M^{-1},[6] which exceeds those for singly bridged dimers (1 x 10^8).[14] Although the "clamshell" dimer binds all guest molecules more tightly, it is most suited for binding bent substrates because the bridges are situated at right angles to each other.



(a) (b)

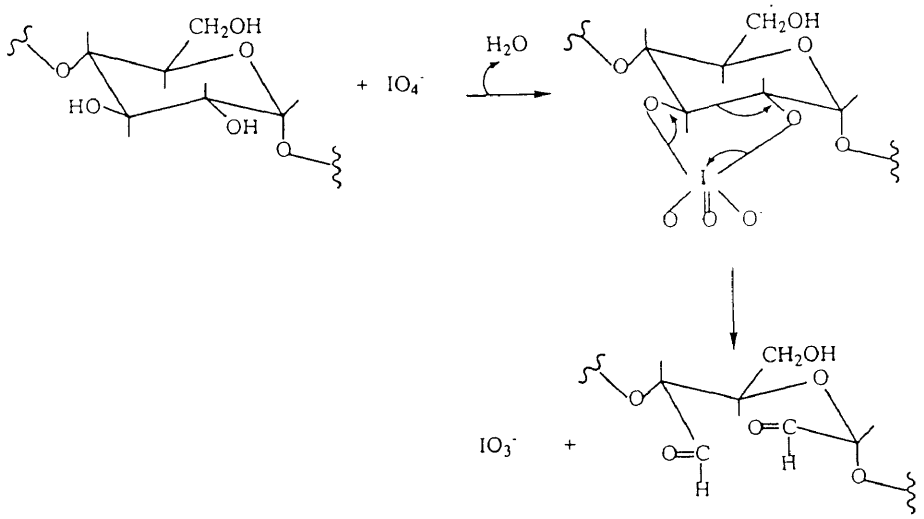
Fig. 5 Clamshell model (a) and loveseat model (b) of duplex cyclodextrin

The ability of duplex cyclodextrins to approximate the binding capacity of certain enzymes and to catalyze reactions of bound substrates is still being studied. Whether singly or doubly bridged, these dimers are paving the way for more routine use of cyclodextrins as enzyme models.

Oxidation:

The synthesis of cyclodextrin derivatives depends on the selective funtionalization of cyclodextrins. One possible functionalization is simple oxidation. For example, oxidation at a C-6 carbon followed by reaction with diamines would provide a means for bridging cyclodextrins.

Dialdehyde derivatives of α-, β-, and γ-cyclodextrin can be produced by oxidation with sodium metaperiodate (sheme 2). Periodate oxidation is selective for vicinal hydroxyls and results in the cleavage of the bond between the C-2 and C-3 carbons of the cyclic glucose residues, introducing a dialdehyde. Kobayashi *et al.* demonstrated this process in α-cyclodextrin and concluded that less than half of the cyclodextrin was oxidized even with total consumption of the periodate.[16] Since the dialdehyde readily forms hydrates, its structure could not be deciphered from the native α-cyclodextrin. Therefore, the dicarboxylate derivative of the dialdehyde was analyzed by ^{13}C-NMR and IR data and the formation of the dialdehyde was confirmed. The main interest of these dial-cyclodextrins is their reaction with nitrogen containing groups such as amino acids. Interactions of this sort allow the dial-cyclodextrin to function as enzyme inhibitors and reagent labelers.[16]



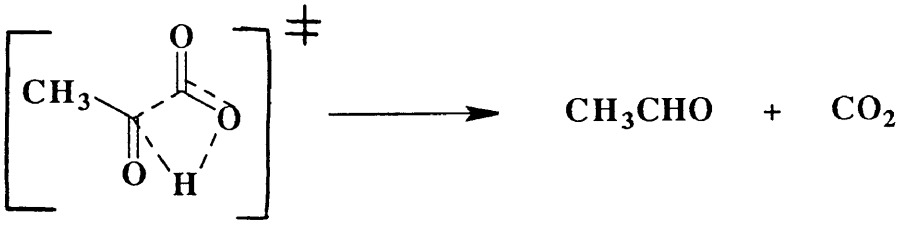
Scheme 2 Mechanism of dialdehyde formation

The chromium trioxide-pyridine complex was discovered as an oxidizing agent for the conversion of alcohols to ketones and aldehydes in 1953 by Poos *et al.*[17] The percent yields reported for this method are not high, but exceed those obtained with the use of chromic acid alone. Experiments revealed that ammonium dichromate in aqueous pyridine oxidized benzyl alcohol to benzaldehyde in yields of 33%; whereas, a yield of 63% was noted when the chromium(VI) oxide-pyridine complex was used.[17] It was concluded that the pyridine complex was selective for alcohol oxidation, leaving olefins and thiol ether linkages untouched. Studies on the CrO_3-pyridine oxidation of cyclodextrin have not been reported to date. However, it is known that this oxidation is not selective for a particular carbon center and can result in uncontrolled oxidation of the cyclodextrin molecule. In this research, cyclodextrin oxidized by CrO_3-pyridine was used as model to

test analytical methods.

An important finding which led to the development of this project involves the thermal decomposition of pyruvic acid and pyruvate esters as a means of oxidation. Information about the behavior of these compounds can be applied to similar compounds containing cyclodextrin; thus, providing another possible method for selectively oxidizing cyclodextrin.

The thermal decomposition of pyruvic, oxalic, and glyoxylic acids leads to an aldehyde through a unimolecular, internal hydrogen-atom transfer via a four-membered transition state (scheme 3). Cleavage of the carbon-carbon bond occurs, yielding carbon dioxide and an aldehyde.[18] The same products were produced when these acids were photolyzed; therefore, photolysis is a viable means of oxidation.



Scheme 3 Four-membered transition state

Similar results were obtained for the thermal decomposition of α-keto esters such as pyruvate. Decarboxylation was observed as the mode of cleavage followed by hydrogen abstraction which produced an aldehyde and carbon dioxide.

Since thermolysis and photolysis of pyruvic acids produced the same products, it was assumed that these esters would also produce the same products when photolyzed. However, Leermakers *et al*. discovered that carbon monoxide was the initially formed fragment when α-keto esters were photolyzed, suggesting that decarbonylation occurred instead.[19] Thus, there is little correlation between the photochemical and pyrolytic behavior of these esters.

The formation of α-keto esters containing β-cyclodextrin is currently being studied. It is hypothesized that photolysis of this compound will yield an aldehyde derivative of cyclodextrin.

Photochemistry:

In a photochemical reaction, a molecule is excited by light to a high energy state, and then undergoes a reaction. This state involves the promotion of electrons to an anti-bonding orbital. In this process, electrons are reorganized, but retain their original spin state. The result is the achievement of a non-minimum energy geometry in the excited state which can be relaxed thermally through vibration. The excited state can also undergo intersystem crossing. This process involves the conversion of a singlet to a triplet state by inverting the spin of the excited electron to match

that of the other unpaired electron. The triplet state can also relax to acheive a minimum energy geometry. The singlet state relaxes rapidly, so reactions of photoexcited molecules usually involve the longer lived triplet state.

Competitive processes limit the longevity of the excited state. The excited state can decay back to the ground state by radiation. This process involves the emission of light either through a transition between states of the same multiplicity, fluorescence, or between states of different multiplicity, phosphorescence. The rate of fluorescence emission is 10^5-10^9 s^{-1}, a rate much higher than that of phosphorescence (10^3-10^5 s^{-1}).[20] Non-radiative decay allows the excited molecule to transfer its energy to surrounding molecules thermally without the emission of light.

Lastly, quenching causes the return of excited molecules to their ground state. In this case, the energy of the excited molecule of interest is transferred to the quencher. Since the reactant is no longer excited, a photochemical reaction cannot occur.

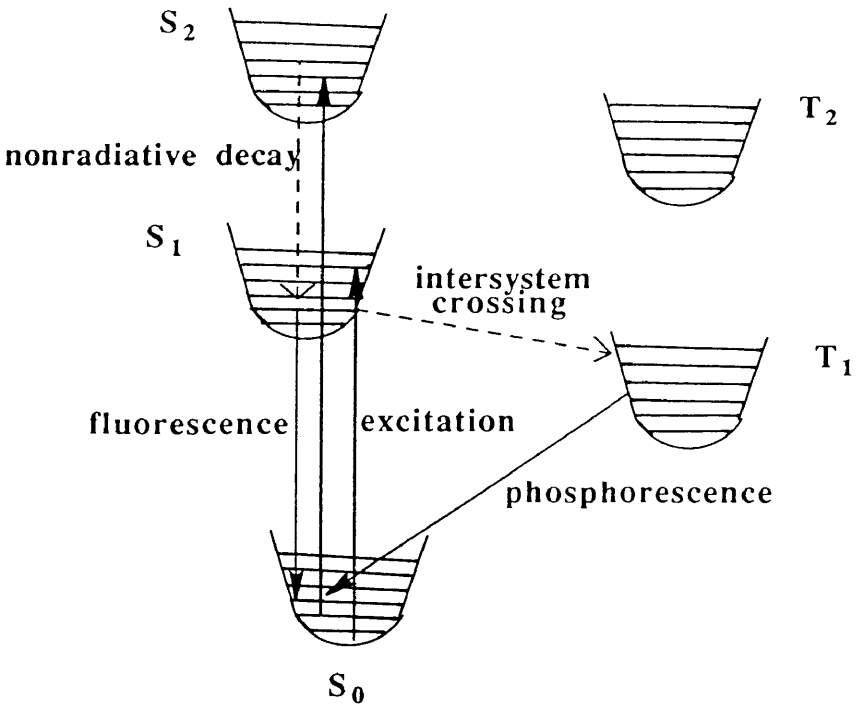


Fig. 6 Photochemical processes

Competing processes prevent the complete participation of every excited molecule in photochemical reactions. The quantum yield is a measure of the fraction of excited molecules that are actually involved in the reaction. It varies for reactants and reaction conditions. The highest yields are obtained in chain reactions where the excitation of one molecule initiates a series of reactions that render product and regenerate reactive intermediates.

The importance of reactions involving photoexcited molecules is three-fold. First, the high energy of the

excited states can initiate reactions that would otherwise be energetically unfavorable if ground states were involved. Also, the occupation of antibonding orbitals by excited electrons allows a correlation of orbital symmetry that could not be achieved in the ground state. Therefore, reactions that were not permitted due to orbital symmetry are now allowed when an excited state is involved. Finally, photochemical reactions can occur in a singlet or triplet state, unlike most thermal processes which involve only singlet states.

Photochemistry of Carbonyl Compounds:

As previously mentioned, the photolysis of α-keto esters containing β-cyclodextrin is a possible method for oxidizing cyclodextrin; therefore, understanding the photochemistry of carbonyl compounds is important.

The excited state for most ketones is the n-π^* state. When excitation occurs, an electron from an oxygen non-bonding orbital is transferred to the antibonding orbital of the carbonyl. Initially, the singlet state is formed; however, intersystem crossing to the triplet state takes place rapidly. In cases where the carbonyl is conjugated, a π-π^* excited state is usually involved. Here, a bonding π electron is promoted to the antibonding π^* orbital (fig 7).

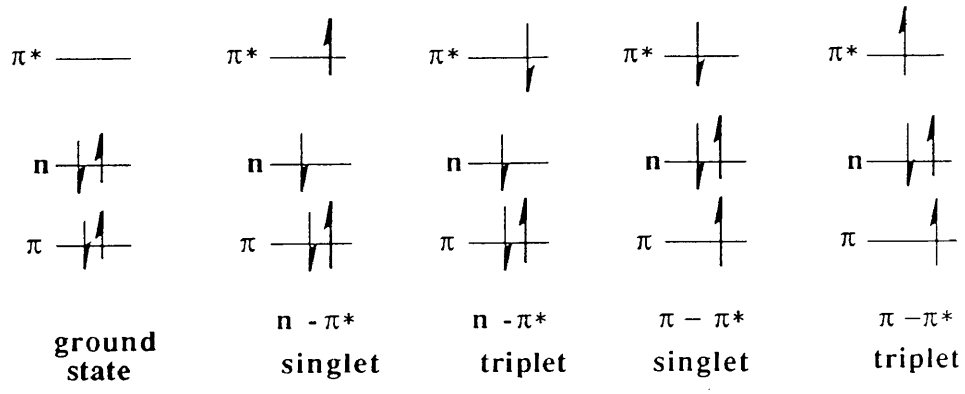
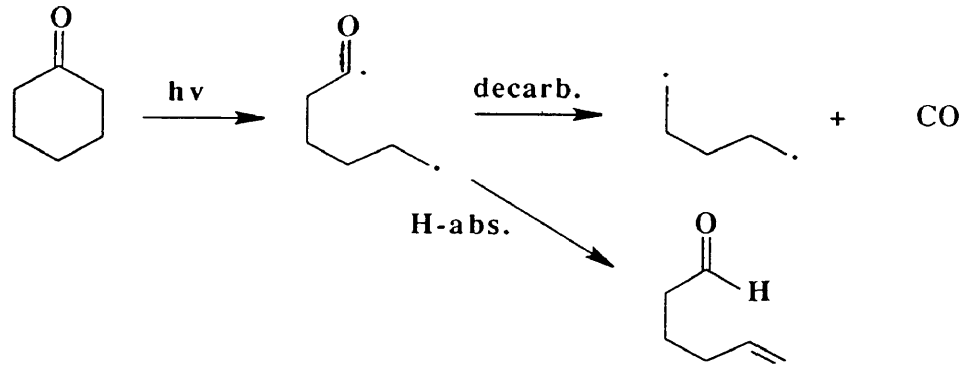


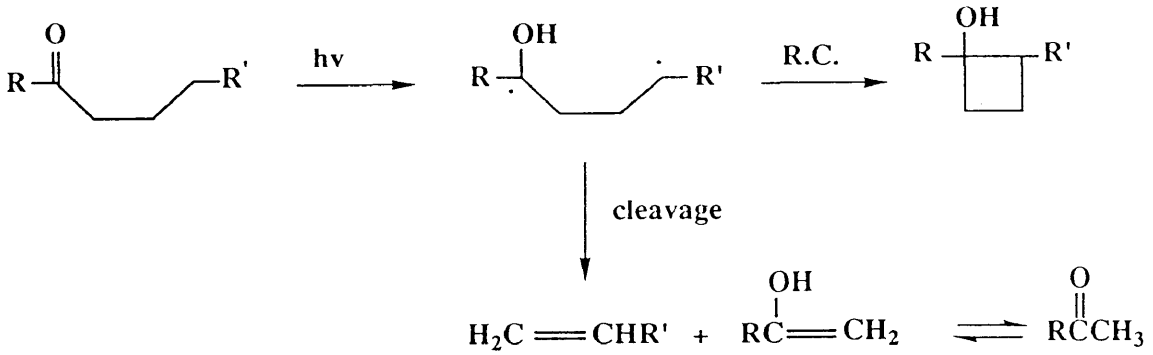
Fig. 7 MO diagrams for excitation of carbonyl compounds

The main photochemical reaction of carbonyl compounds is the Norrish type I reaction, where α-bond cleavage occurs and is often followed by decarbonylation or intramolecular hydrogen abstraction (scheme 4).



Scheme 4 Norrish I mechanism

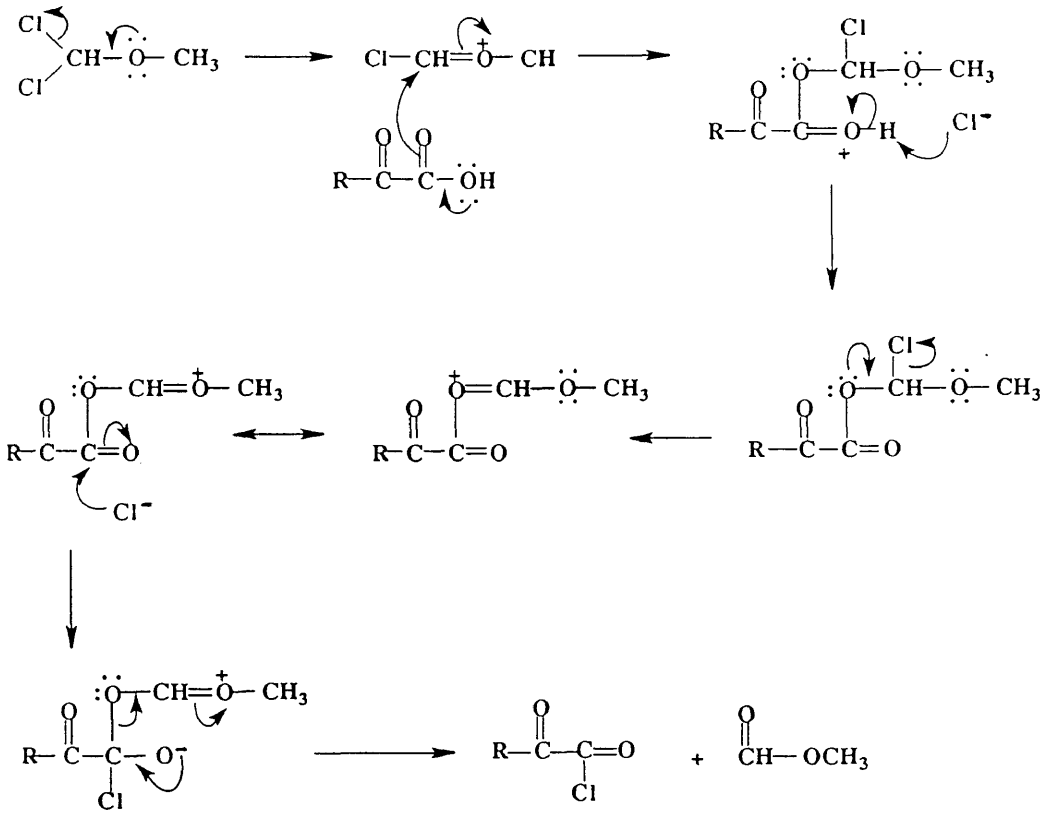
The Norrish type II mechanism is less common and involves γ-hydrogen abstraction followed by α-bond cleavage or ring closure (scheme 5).



Scheme 5 Norrish II mechanism

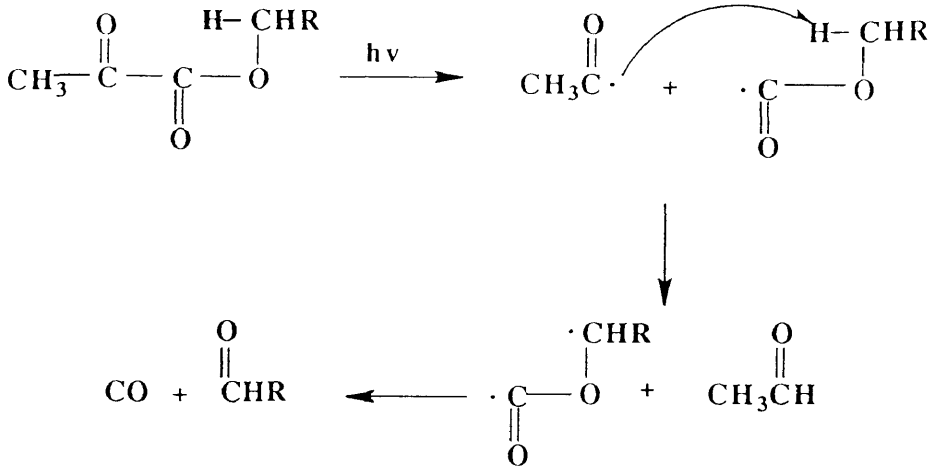
Photooxidation:

The photooxidation reactions of interest here involve the selective oxidation of hydroxyl groups to carbonyls using α-keto esters. This process is considered mild because it targets only primary and secondary hydroxyls.[23] The α-keto esters are prepared by reacting the alcohol of interest with the α-keto acid chloride. The acid chloride is prepared by reacting an acid, such as pyruvic acid, with α,α-dichloromethyl methy ether (scheme 6). Ottenheijm and De Man found that α,α-dichloromethyl methyl ether was a better chlorinating species than thionyl chloride or oxalyl chloride.[24]



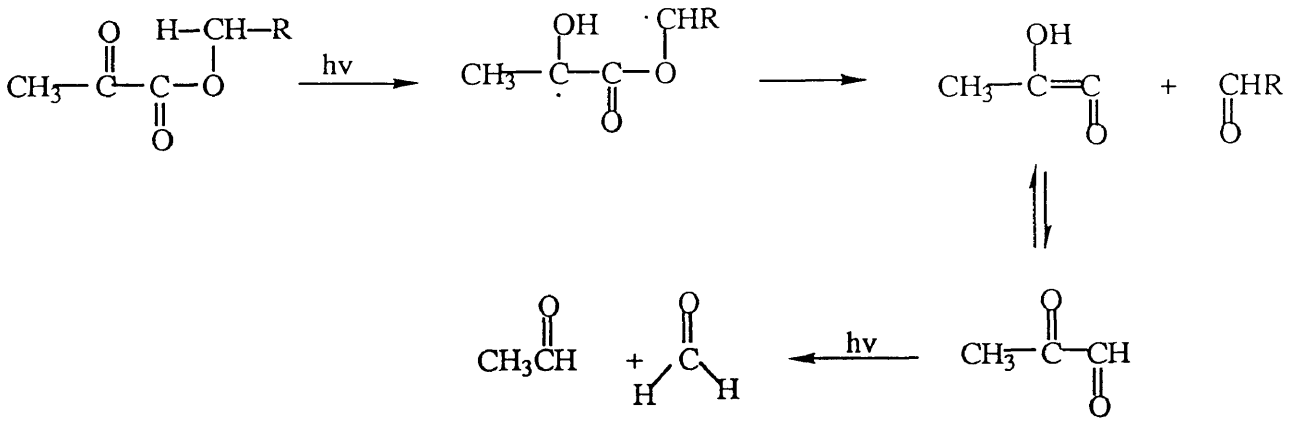
Scheme 6 Formation of an α-keto chloride

Esterification of this acid chloride with the alcohol to be oxidized is followed by the photolysis of the resulting ester. Photolysis of α-keto esters in solution result in the formation of carbon monoxide, a small amount of carbon dioxide, and acetaldehyde; seemingly indicating a Norrish I pathway (scheme 7).



Scheme 7 Possible pathway for photolysis of an α-keto ester

However, acetaldehyde also could be produced if a Norrish II mechanism was followed (scheme 8).



Scheme 8 Alternate pathway for photolysis of an α-keto ester

The excited state responsible for the photochemistry of carbonyl compounds, such as α-keto esters, is the triplet state as shown by quenching studies with naphthalene. Since the singlet excited state of naphthalene is of higher energy

than that of the ester, quenching cannot occur. However, napthalene can quench α-keto esters in the triplet state. Studies have shown that in the presence of napthalene oxidation yields decrease, hence the triplet state is involved in photochemical reactions.[25]

The n-π^* triplet state, rather than the π-π^* triplet state, is believed to be involved in the photochemistry of carbonyl compounds. Evidence for this is seen by a large change in dipole moment when the excited state is formed. Also, the higher energy of the non-bonding orbital makes removal of an electron more probable.

It is likely that the reaction follows the Norrish II mechanism since Encinas *et al.* demonstrated that the triplet lifetime was dependent on the type of hydrogen in the γ-position.[26] If the Norrish I mechanism were operating, the lifetime would not be dependent on the γ-hydrogen; therefore, a Norrish II mechanism is probable.

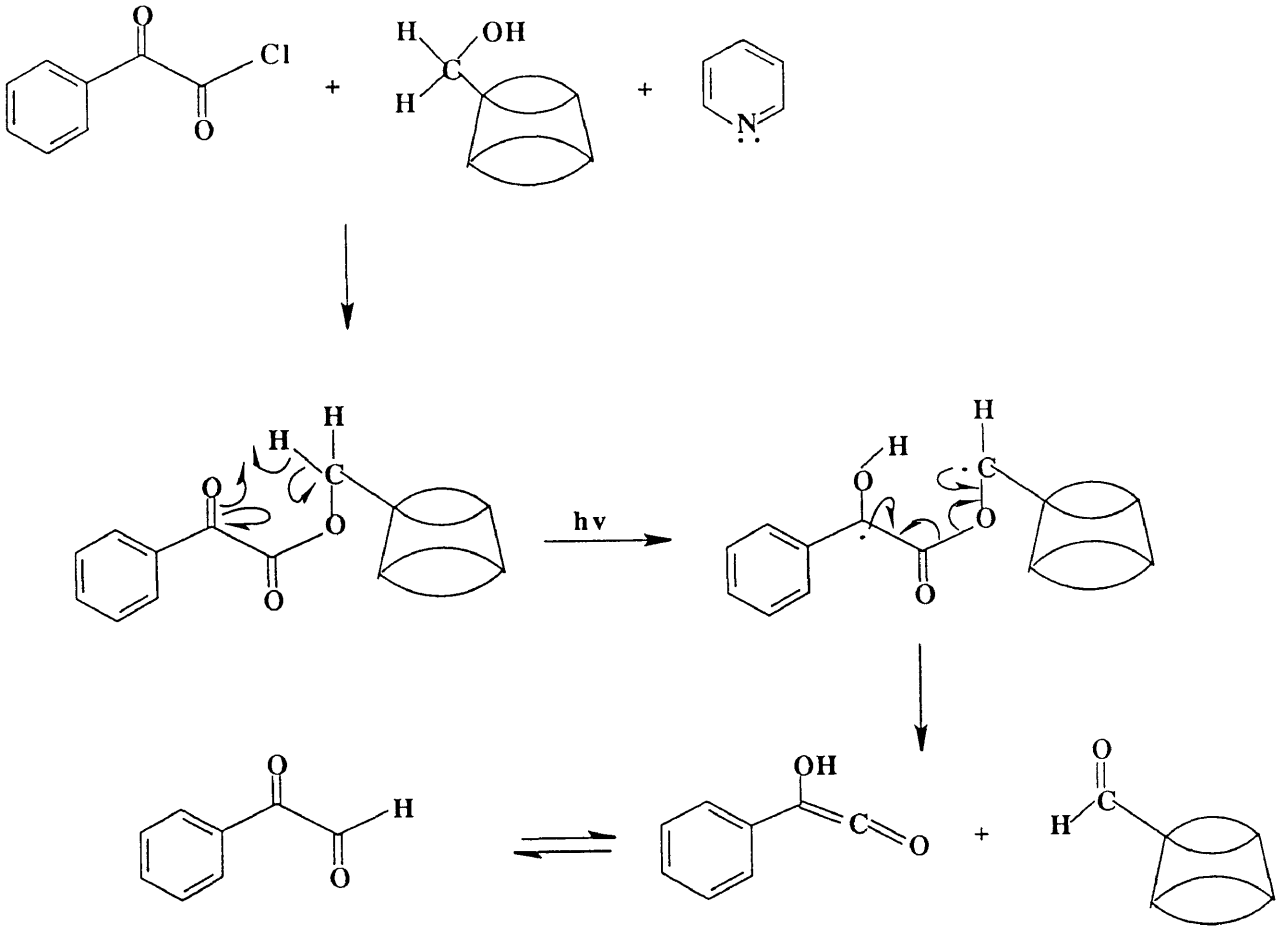
The goal of this project was to use the photolysis of α-keto esters to selectively oxidize β-cyclodextrin.

Photooxidation of β-cyclodextrin:

The photooxidation of carbohydrates via the esterification process described above has been studied. R. W. Binkley reported that photochemical oxidation is more

versatile than oxidation with chromium trioxide/pyridine and more consistent than dimethyl sulfoxide-based oxidations.[27] The latter suggests that solvent considerations are of great importance. Studies by Binkley revealed that oxidation yields decreased in the presence of increasingly polar solvents and experiments with benzene pyruvates show that photoreduction occurred in the presence of hydrogen donating solvents.[28]

Since photooxidation of carbohydrates resulted in good yields of the corresponding carbonyl compounds, it was assumed that a complex carbohydrate like cyclodextrin could be oxidized in this fashion. Current research is focused on the oxidation of β-cyclodextrin via the photolysis of 6-β-cyclodextrin benzoyl formate (scheme 9). Upon photolysis, the ester proceeds through a Norrish II mechanism to yield an aldehyde derivative of β-cyclodextrin. The hydrogens on the C-6 carbon are diastereotopic; therefore, abstraction should be selective for one over the other. The ester is formed at the C-6 carbon of one glucose unit of cyclodextrin by reacting an α-keto chloride with cyclodextrin (scheme 10). The α-keto chloride is formed by reacting benzoylformic acid with an α,α-dichloromethyl methyl ether as previously illustrated (scheme 6).



Scheme 9 Oxidation of β-CD via photolysis of 6-β-Cyclodextrin Benzoyl Formate

Once the cyclodextrin has been oxidized, reaction with various amines to form imines should be simple. This could provide a significant new route to the bridging of cyclodextrin molecules. As discussed earlier, duplex cyclodextrins approximate the binding capacity of medium-affinity anitbodies, making them more efficient than simple cyclodextrins as host molecules. Like enzymes, they can also catalyze reactions of bound substrates. The success of

cyclodextrins as enzyme models is dependent on their ability to tightly bind guest molecules. Research to date has demonstrated that duplex cyclodextrins serve as the best hosts and that they can function as efficient catalysts.

EXPERIMENTAL

Commerically available β-cyclodextrin was vacuum dried (0.05 mm, liquid N_2 trap) at 100°C for 12 h. Pyridine was fractionally distilled, and the fraction between 114° and 115°C was collected. ^1H and ^{13}C NMR spectra were obtained with a GE QE-300 spectrometer. Thin layer chromatography was done on Baker 0.25 mm (60F-254) precoated silica plates, and spots were detected by staining with vanillin. High-pressure liquid chromatography was performed on a Waters 600E system equipped with a variable wavelength absorption dectector (254 nm) and a Varex evaporative light scattering detector. A Whatman ODS-3 analytical column was used and a linear gradient was applied (gradient 1: 10% to 100% aq CH_3CN, 90 min.; gradient 2: 5% to 95% aq CH_3CN, 90 min; gradient 3: 5% to 15% aq CH_3CN, 50 min.). Preparative HPLC was performed on a Waters 224 system equipped with a UV absorption detector (254 nm) using a Whatman Magnum 20 column packed with ODS-3. Irradiations were carried out either in a Pyrex glass filter sleeve with a Hanovia 450 W medium-pressure lamp or a Rayonet equipped with 350 nm bulbs.

Benzoyl Formic Acid Chloride:

Approximately 5 grams of benzoylformic acid was recrystallized in 90 mL of carbon tetrachloride and dried *in vacuo* overnight. The recovered benzoylformic acid (4.13 g, 0.027 mol) was heated in an oil bath to 50° C while adding an equimolar amount of α,α-dichloromethyl methyl ether (3.16 g, 0.027 mol) dropwise. The mixture was heated at 50° C for 30 minutes. The resulting α-keto chloride (2.84 g, 0.019 mol) was isolated by distillation and stored in the freezer. ^{1}H-NMR (chloroform) δ 8.1 (d, 2H), δ 7.6 (t, 1H), δ 7.4 (t, 2H)

6-β-Cyclodextrin Benzoyl Formate:

Approximately 10 grams of β-cyclodextrin was added to 200 mL of pyridine, and pyridine was distilled using a short path column until the distillate boiled at 114° C. The solution was cooled to room temperature, an equimolar amount of α-keto chloride (1.34 g, 0.0081 mol) was added, and the mixture was stirred for 1 hr. The pyridine was removed by vacuum distillation, and the resulting material (9.35 g, 0.0075 mol)

was dried *in vacuo* overnight.

The ester was purified by several methods. First, the solid was ground with a mortar and pestle, rinsed with acetone, and filtered on a buchner funnel. This process was repeated three times. The solid was then extracted via a Soxhlet extractor with 500 mL of acetone overnight. Finally, the ester was dissolved in 1000 mL of water, precipitated in 1000 mL of acetone, and filtered on a buchner funnel. The filtrate which contained the ester was concentrated *in vacuo*, and the residue was dried *in vacuo* for a few hours. The ester was then washed with acetone, collected on a buchner funnel, and dried *in vacuo* overnight.

The ester (3.8 g, 0.003 mol) was analyzed by ^{1}H-NMR, prep and analytical HPLC (gradient 2). Isolation by preparative HPLC was not successful.

^{1}H-NMR (DMSO-d_6) δ 8.9 (d, 2H), δ 8.6 (t, 1H), δ 8,1 (t, 2H), δ 4.7 (s, 7H), and β-cyclodextrin resonances;

^{13}C-NMR δ 210, 207, 146, 142, 130, 127, 102, 82, 72, 60.

Oxidation of 6-β-CD Benzoyl Formate:

3.8 g of ester was dissolved in 250 mL of DMSO, degassed with N_2 for 1 hr., and photolyzed overnight. DMSO was removed by vacuum distillation, leaving 3.2 g of material. The residue was dissolved in 100 mL of water, and washed three

times with 100 mL portions of ether. The water layer was concentrated *in vacuo*, and the solid residue was collected on a buchner funnel with acetone and dried *in vacuo*. The ether layer was concentrated *in vacuo* and then dried *in vacuo*. The residue was analyzed via ^{1}H-NMR.

Recrystallization of 1,1-Diphenylhydrazine Hydrochloride:

10.0 grams of phenylhydrazine hydrocholride was dissolved in 30 mL of water and heated until boiling. The solution was neutralized with an equimolar amount of sodium bicarbonate (5.81 g, 0.069 mol). Norite was added to the hot solution and removed by gravity filtration. The resulting crystals (9.83 g, 0.068 mol) were washed with acetone, collected on a buchner funnel, and dried *in vacuo*.

1,1-Diphenylhydrazone:

A mixture consisting of 2.0 grams of aldehyde, 1.5 mL of acetic acid, and 0.5 mL of water was heated to dissolution, then cooled to room temperature.

Another mixture containing 1.4 g of recrystallized 1,1-diphenylhydrazine, 16 mL absolute alcohol, an equimolar amount of sodium bicarbonate (0.813 g, 0.009 mol), and 13 mL of water was heated until the solution was clear. The two solutions were combined and stirred overnight.

The phenylhydrazone (0.9 g, 0.0007 mol) was purified by dilution with water to a total volume of 150 mL, followed by five washings each with 100 mL of ether. The ether layers were discarded. The water layer was diluted to a total volume of 1000 mL with acetone. The resulting precipitate was collected on a buchner funnel, and the filtrate was concentrated *in vacuo*. The filtrate residue was washed with acetone, filtered on a buchner funnel and dried *in vacuo* overnight. It was analyzed by ^{1}H-NMR and analytical HPLC (gradient 1).

Reduction of 6-β-cyclodextrin Benzoyl Formate:

1 g ester was dissolved in 75 mL of water and 75 mL of isopropanol. In a separate flask, 0.88 g (0.023 mol) of sodium borohydride was dissolved in 14 mL of water. The two solutions were mixed together and stirred overnight. The solution was then concentrated *in vacuo* after 5 mL of acetone and 2 mL of acetic acid were added. The residue was dried in vacuo overnight and analyzed by ^{1}H-NMR and HPLC (gradient 2).

Recrystallization of Anthraquinone-2-sulfonic acid:

5.0 g of anthraquinone-2-sulfonic acid, sodium salt monohydrate was dissolved in 150 mL of water and heated to boiling. Gravity filtration of the hot solution was followed by the addition of 30.0 g of sodium chloride and 0.4 grams of sodium chlorate. The solution was then heated and water was added until it was clear. The solution was cooled and crystals were collected on a buchner funnel. The recovered anthraquinone-2-sulfonic acid (3.4 g, 0.01 mol) was dried *in vacuo*.

Oxidation of β-CD with Anthraquinone-2-sulfonic acid:

The absorbance for 10 mg of anthraquinone-2-sulfonic acid was determined on the DU-70 spectrophotometer. The relationship between mass and absorption was the basis for calculating the mass of anthraquinone required to yield an absorption of one. 61.8 mg of anthraquinone-2-sulfonic acid and an equimolar amount of cyclodextrin (213.4 mg, 0.00018 mol) was dissolved in 1000 mL of water. The solution was purged with nitrogen for 1 hr. and photolyzed overnight. The solution was concentrated *in vacuo*, dissolved in a minimum amount of water, purged with air, and anaylzed by HPLC (gradient 2). The solid was dissolved again in 1000 mL of water for an overnight liquid-liquid extraction with ether.

The oxidized cyclodextrin was concentrated *in vacuo* and analyzed by HPLC.

Photolysis of 6-(Anthraquinone-2-sulfonyl)-β-cyclodextrin:

75 mg fractions of CD-6-2SO_2AQ were purified on preparatory HPLC (20%-30% CH_3CN, 40 min.) The proper fractions were concentrated *in vacuo*, washed with acetone and allowed to dry at room temperature overnight. The purified CD-6-2SO_2AQ was dissolved in a 10 mL solution of 50% deionized water and 50% aq. CH_3CN and purged with N_2 for 5 min. The solution was photolyzed in a Rayonet for 1 h under a balloon of N_2 and monitored by HPLC (gradient 2). The photolyzed solution was purged with air for 1 hr. followed by the addition of 100 mg Na_2CO_3 and analyzed by HPLC.

Oxidation of β-CD with Poly 4-Vinylpridinium Dichromate:

0.50 g of β-CD was dissolved in 30 mL of water and 3 equivalents of PVPDC (0.285 g, 0.0013 mol) was added. The solution was heated to 70° C while stirring and analyzed on HPLC (gradient 3).

Oxidation of β-CD with Chromium Trioxide:

1.0 g of β-CD was dissolved in 10 mL of pyridine. 0.273 g (0.0027 mol) of CrO_3 was added to 70 mL of pyridine slowly and the mixture was stirred vigorously. The two solutions were mixed together, stirred for 30 min., and left undisturbed overnight. The pyridine was removed by distillation *in vacuo*. The residue was dissolved in 50 mL of water, precipitated in 750 mL of acetone, and buchner filtered. This process was repeated. The crystals were analyzed by HPLC (gradient 3).

Pyrolysis of 6-β-CD Benzoyl Formate:

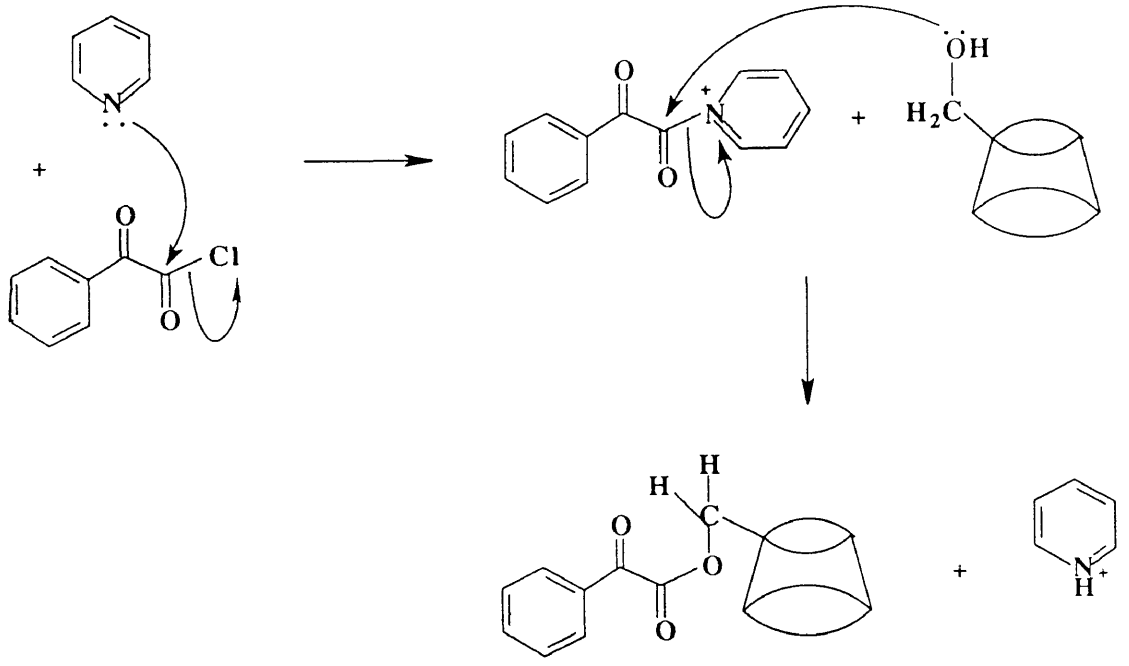
1.0 g of ester was heated in a test tube to 200° C in an oil bath for 1 hr. The pyrolyzed ester was dissolved in water and analyzed by HPLC (gradient 3).

RESULTS AND DISCUSSION

Synthesis:

The preparation of 6-β-cyclodextrin benzoyl formate is essential for the oxidation of β-cyclodextrin via the Norrish II mechanism. The first step in its synthesis was to prepare an α-keto chloride by reacting α,α-dichloromethyl methyl ether with benzoylformic acid. This reaction was carried out in high yield and good purity as reflected in the ^{1}H-NMR data (Fig. 8).

Next, the α-keto chloride was slowly added to a solution of β-cyclodextrin and pyridine. Pyridine reacts with the α-keto chloride to facilitate the formation of the ester via a displacement reaction (scheme 10). Since water would have reacted preferentially with the acid chloride, pyridine was used instead of water as the solvent.



Scheme 10 Mechanism for formation of the ester

Evidence for the existence of an ester was provided by ^{1}H-NMR and thin layer chromatography (TLC). The ^{1}H-NMR of the purified ester showed an aromatic region and cyclodextrin. Integration of the aromatic peaks and the glucose C-1 hydrogen peak resulted in the proper 2:1:2:7 ratio (Fig. 9, 10).

^{13}C-NMR revealed two carbonyl groups, a benzene ring, and the six carbons of cyclodextrin. However, the downfield shift of the C-6 carbon of the glucose residue that forms the ester linkage and the upfield shift of the corresponding C-5 carbon were not observed (Fig. 11). Although this finding suggested that an ester may not have been formed, experimentation continued assuming that it had based on the ^{1}H-

NMR and TLC evidence.

Photolysis:

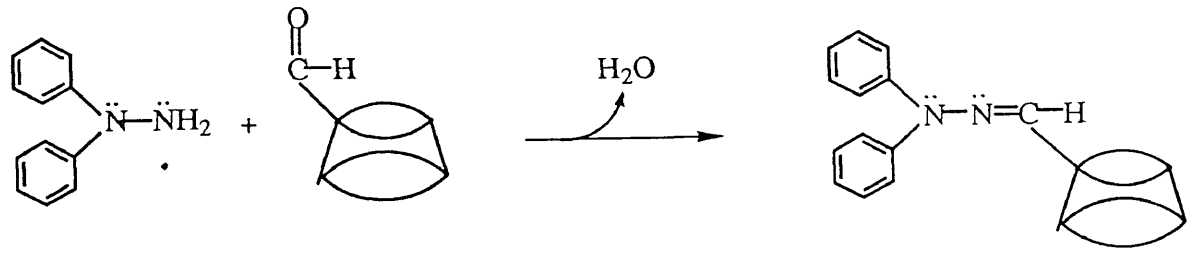
The choice of solvent was of great importance to insure proper γ-hydrogen abstraction. The presumed α-keto ester was soluble in both DMSO and pyridine. Since excited carbonyls are known to undergo electron-transfer with amines, DMSO was selected over pyridine because the electron pair on the nitrogen of pyridine might interfere with hydrogen abstraction by the carbonyl group.

The photolyzed ester was extracted with ether to isolate the hoped for oxidized cyclodextrin from the benzaldehyde. This purification technique was sufficient to produce the separate products as shown by the ^{1}H-NMRs. The NMR of the ether portion reveals an aromatic region similar to that for benzoyl formic acid; however, benzaldehyde was not observed (Fig. 12). The aromatic region appears slightly upfield from the aromatic region of the starting material, suggesting the cleavage of the ester. The C-1 peak of cyclodextrin is apparent, suggesting that some cyclodextrin remained in this layer. The ^{1}H-NMR and ^{13}C-NMR of the aqueous layer showed the disappearance of the aromatic region and the presence of cyclodextrin (Fig. 13, 14). The oxidation of cyclodextrin at the C-6 carbon is not evident by ^{1}H-NMR because a signal for an aldehydic hydrogen could not be observed. Cyclodextrin

aldehydes exist as hydrates; thus, the aldehydic hydrogen could be obscurred by the resonances of the C-1 hydrogens. Loss of the aromatic portion is consistent with a γ-hydrogen abstraction and α-cleavage producing an oxidized cyclodextrin.

1,1-Diphenylhydrazone:

Oxidized cyclodextrin can be connected by reaction with a bis primary amine. However, 1,1-diphenylhydrazine was used instead of a bis primary amine to show the existence of an oxidized cyclodextrin (scheme 11). The diphenyl moiety should facilitate purification by preparative HPLC.



Scheme 11 Formation of 1,1-diphenylhydrazone

The purified product presumed to be 1,1-diphenylhydrazone did not show aromatic protons by ^{1}H-NMR, which suggests that the reaction did not occur (Fig. 15). In addition, results obtained by analytical HPLC showed a multitude of peaks, none of which corresponded to the expected hydrazone peak which should elute after cyclodextrin and before 1,1-diphenylhydrazine (Fig. 16). The large number of peaks

suggest that the reaction was not clean. The inability to produce a phenylhydrazone was attributed to the absence of an aldehyde. There are several possibilities that might explain the unsuccessful oxidization of cyclodextrin to an aldehyde.

Characterization of 6-β-Cyclodextrin Benzoyl Formate:

Based on the ^{13}C-NMR of the ester, it was hypothesized that the ester may not have been formed at all. If this was the case, photolysis would not follow the Norrish II mechanism to yield an aldehyde of cyclodextrin.

To investigate this hypothesis, the presumed ester was purified further on preparatory HPLC and fractions were collected. This additional purification should have provided evidence for the existence or absence of an ester. However, ^{1}H-NMR analysis of these fractions showed a different aromatic region than the original ester or the absence of one altogether (Fig. 17, 18). Therefore, the ester could not be successfully purified via HPLC and proof for its formation could not be obtained.

An analytical HPLC equipped with a light scattering device showed peaks with retention times of 30.90 min. for the ester spiked with cyclodextrin and 31.79 min. for cyclodextrin alone. Since a single peak appeared for the spiked mixture with a retention time similar to that of cyclodextrin, it

could be concluded that the peak represents cyclodextrin and that the ester did not exist.

To confirm this finding, a reduction of the presumed ester was performed. The conversion of the α-keto moiety to its corresponding alcohol would suggest the presence of an ester and also test for a "mobile" ester. A "mobile" ester refers to the possibility that hydrolysis and/or transesterification between adjacent hydroxy groups or other cyclodextrin molecules was occurring, thus preventing detection and isolation of the ester. Reduction of the ester should result in a less electron withdrawing species, hence, a more stable ester which could be seen on HPLC. ^{1}H-NMR data showed an aromatic region inconsistent with a mono-substituted phenyl group and analysis by HPLC resulted in a single peak around the region of cyclodextrin. This is clear evidence against the formation of the ester.

Another approach to determine the presence of an ester was pyrolysis. Thermal cleavage of the bonds of the ester followed by hydrogen abstraction was the anticipated reaction. Results from HPLC showed no peaks; therefore, it was concluded that the pyrolysis destroyed the presumed ester and information about the ester could not be obtained.

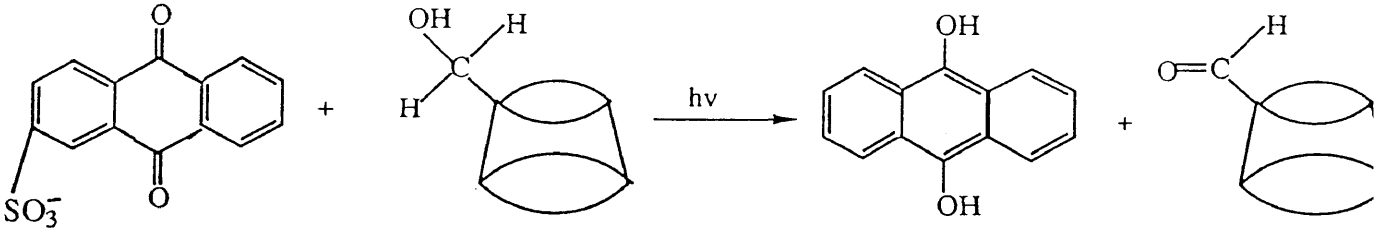
Oxidation of β-Cyclodextrin:

Different oxidation methods were employed to demonstrate that cyclodextrin could indeed be oxidized. The oxidation of cyclodextrin with a chromium trioxide-pyridine complex seemed to be successful. Analysis by analytical HPLC showed two peaks with retention times of 27.28 min. and 31.22 min. (Fig. 19). After spiking the oxidized solution with cyclodextrin, the second peak was enhanced, providing evidence that the first peak was oxidized cyclodextrin. It was expected that the aldehyde would elute before native cyclodextrin because it tends to form a hydrate, making it more hydrophilic. This demonstrated that cyclodextrin could be oxidized and that it could be distinguished from native cyclodextrin by HPLC. The exact site of oxidation; however, could not be determined because CrO_3-pyridine is not selective for a particular carbon center.

Oxidation with Poly 4-vinylpyridinium dichromate did not produce an aldehyde (Fig. 20) because in aqueous solution, the aldehyde readily forms a hydrate which is then oxidized to an acid. Not only is an acid undesirable, but it is inconvenient because an acid would elute as its conjugate base.

Oxidation of cyclodextrin with anthraquinone-2-sulfonic acid was tested to confirm that an aldehyde could be formed via photolysis. Upon photolysis, the anthraquinone abstracts hydrogens from the C-6 carbon of cyclodextrin to yield a hydraquinone and an aldehyde (scheme 12). The solution was

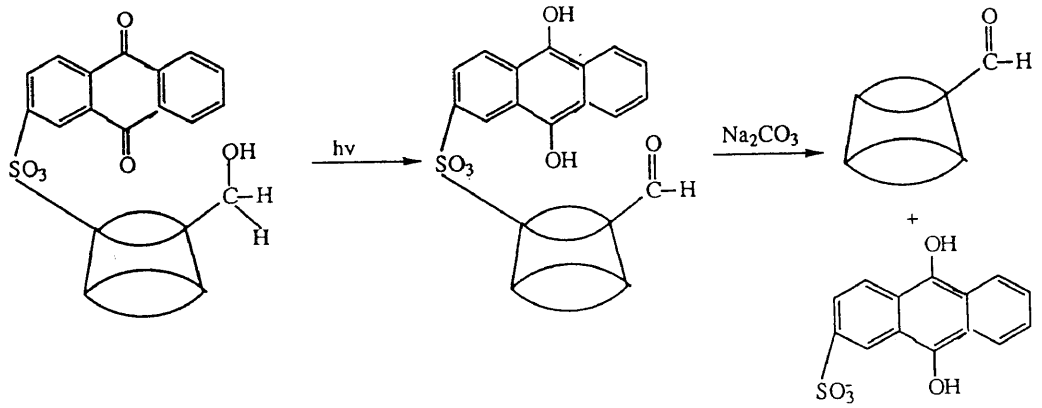
purged with air to remove the hydraquinone.



Scheme 12 Oxidation of β-CD with Anthraquinone-2-Sulfonic Acid

Results from HPLC showed a peak for cyclodextrin at 19.53 min., but did not show one for oxidized cyclodextrin. The yellow color of the solution suggested that the photoproducts absorbed the light before the reactant could; therefore, the photolysis could not proceed to completion and the cyclodextrin was oxidized very little if at all.

On the other hand, photolysis of 6-(anthraquinone-2-sulfonyl)-β-cyclodextrin yielded what appeared to be oxidized cyclodextrin (scheme 13).



Scheme 13 Photooxidation via 6-(Anthraquinone-2-Sulfonyl)-β-Cyclodextrin

Two peaks appeared on HPLC with retention times of 21.66 min.

and 35.17 min. (Fig. 21). The first peak might be oxidized cyclodextrin while the second peak represents the native form. The discrepancy between the retention times for oxidized cyclodextrin via the CrO_3 oxidation and the above photolysis was not resolved.

CONCLUSIONS

The photolysis of α-keto esters gives rise to oxidation of the γ-carbon. It was hypothesized that the photolysis of an α-keto cyclodextrin ester would follow the same mechanism and result in an oxidized cyclodextrin. The aldehyde derivatives could then react easily with bis-primary amines to form duplex cyclodextrin.

Attempts to oxidize β-cyclodextrin via the photolysis of 6-β-cyclodextrin benzoyl formate were unsuccessful. Thin layer chromatography and ^{1}H-NMR spectra of the ester provide evidence for the formation of a cyclodextrin derivative. However, analysis of the ester by ^{13}C-NMR and HPLC suggest that an ester was never formed. The latter finding in association with the successful photooxidation of β-cyclodextrin via the 6-(anthraquinone-2-sulfonyl)-β-cyclodextrin complex led to the conclusion that the ester never existed. As a result, an aldehyde could not be formed by the Norrish II mechanism. Since the reaction of amines with aldehydes is the simplest method for bridging cyclodextrin, it is advised that other methods of oxidation be tried in the future.

An alternative method of oxidation involves the photolysis of an ether formed by reacting α-bromoacetone with

β-cyclodextrin.[27] The photolysis follows a Norrish II mechanism, resulting in a mono-aldehyde derivative of β-cyclodextrin. The use of an ether instead of an ester eliminates the possibility of a mobile species because of the stronger bonding involved.

Another possibility for ensuring proper oxidation via photolysis is to protect the cyclodextrin. Among the many modifications to cyclodextrin described by Croft and Bartsch, the benzoylated analog of a 6-tetratrityl derivative of β-cyclodextrin prepared by Bergeron *et al*.[28] seems to provide the best protection. The derivative contains four tritylated primary hydroxyls, two benzoylated primary hydroxyls, and seven benzoylated secondary hydroxyls; thus, leaving one primary hydroxyl free for reaction. This modification is reversible by treatment with dilute acid and sodium methoxide in methanol.[29]

The main goal of cyclodextrin research is to maximize the ability of cyclodextrin to bind substrates. Duplex cyclodextrin most closely approximates the binding capacity of some enzymes; therefore, the study of these dimers is of great importance. To date, singly and doubly bridged cyclodextrins have been isolated; however, much research still remains to determine the best method for their synthesis.

Fig. 8 ^{1}H-NMR of Benzoyl Formic Acid Chloride

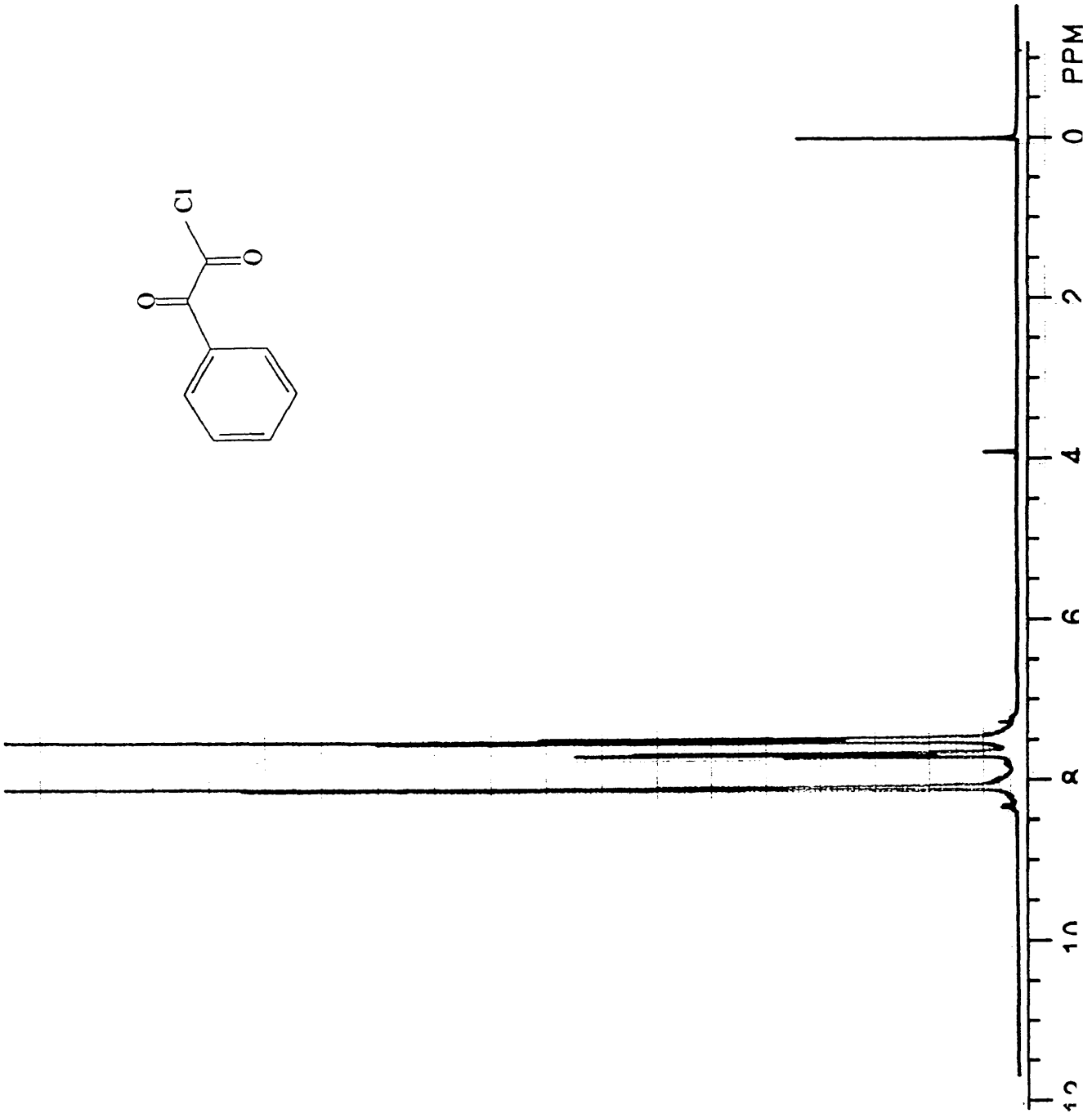


Fig. 9 ^{1}H-NMR of 6-β-Cyclodextrin Benzoyl Formate

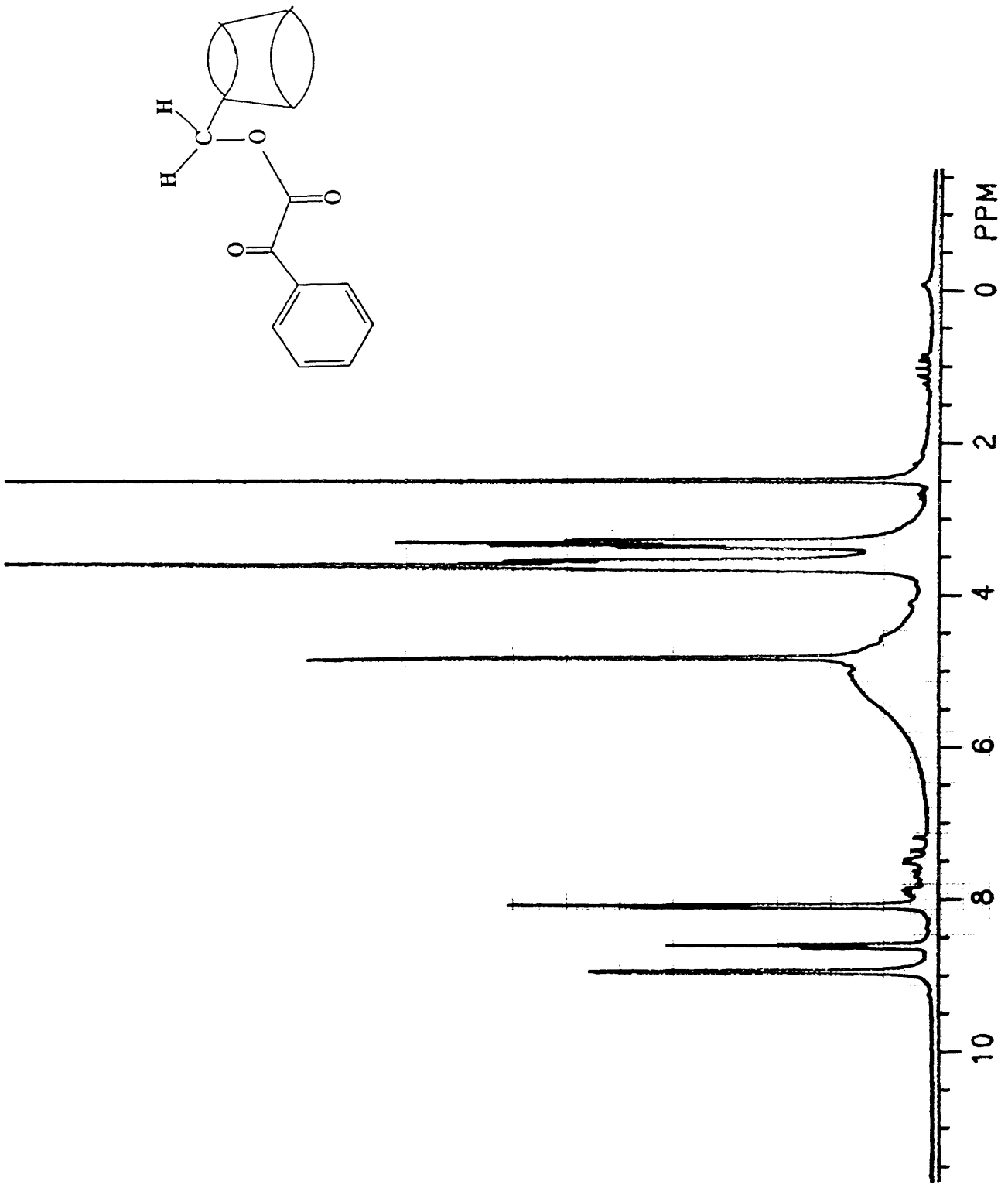


Fig. 10 ^{1}H-NMR of 6-β-Cyclodextrin Benzoyl Formate

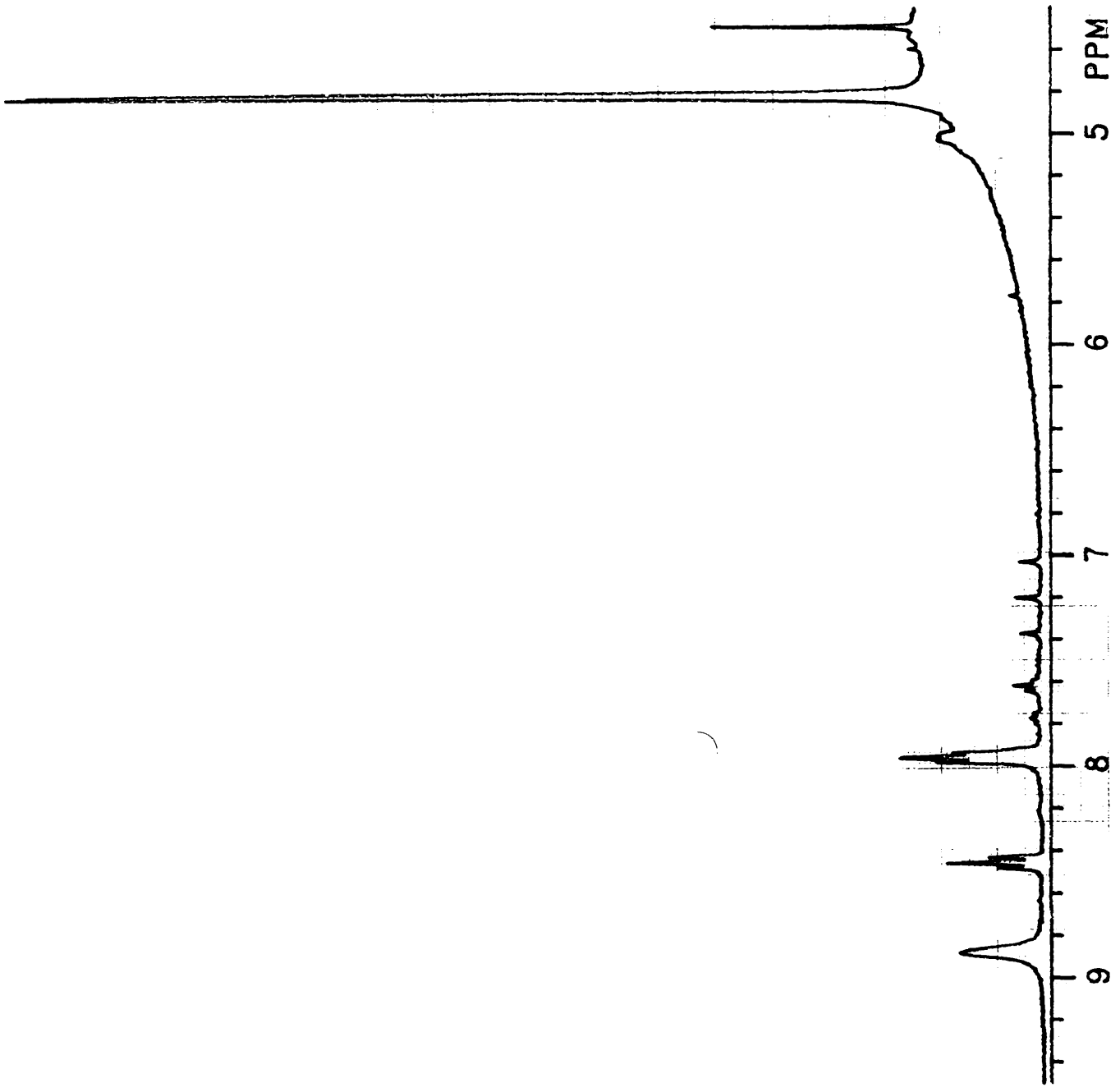


Fig. 11 ^{13}C-NMR of 6-β-Cyclodextrin Benzoyl Formate

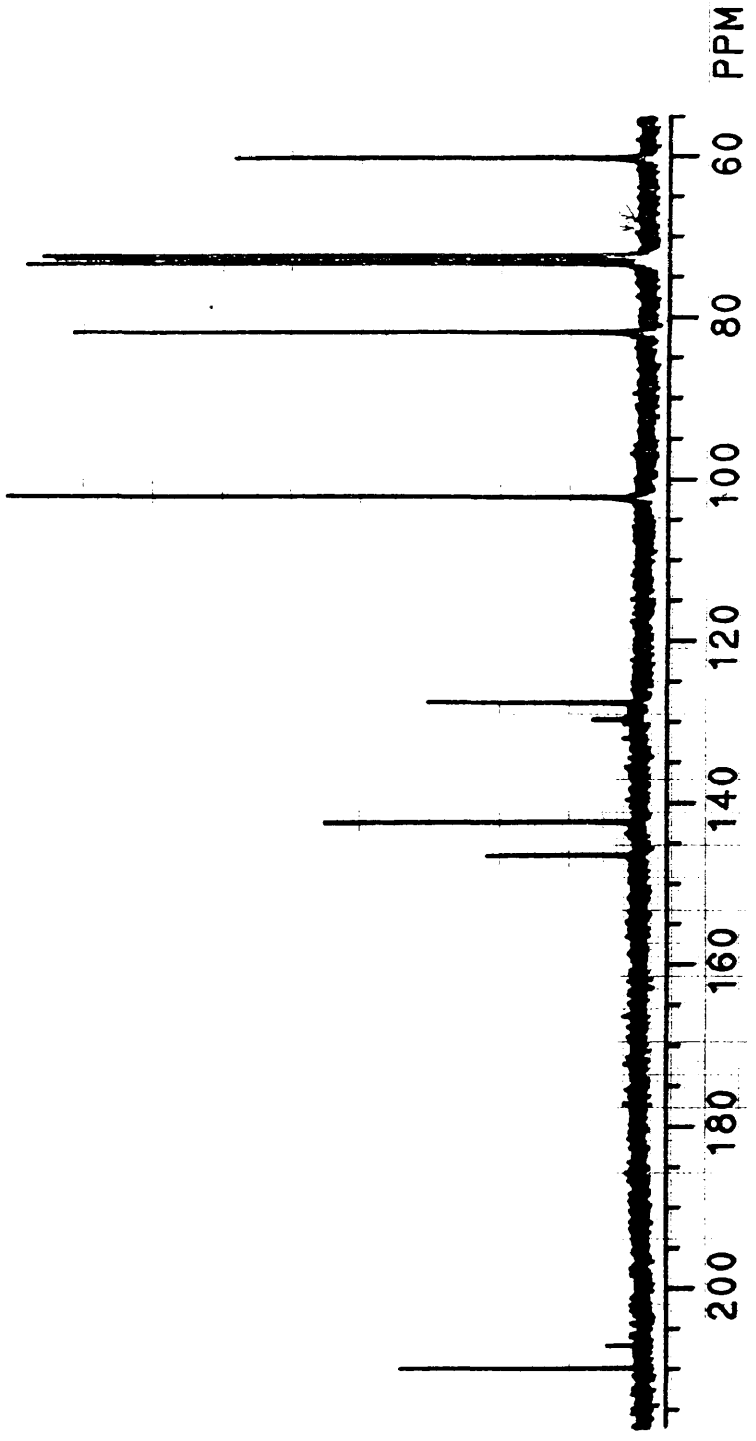


Fig. 12 ^{1}H-NMR of Ether Layer From Ether Extraction

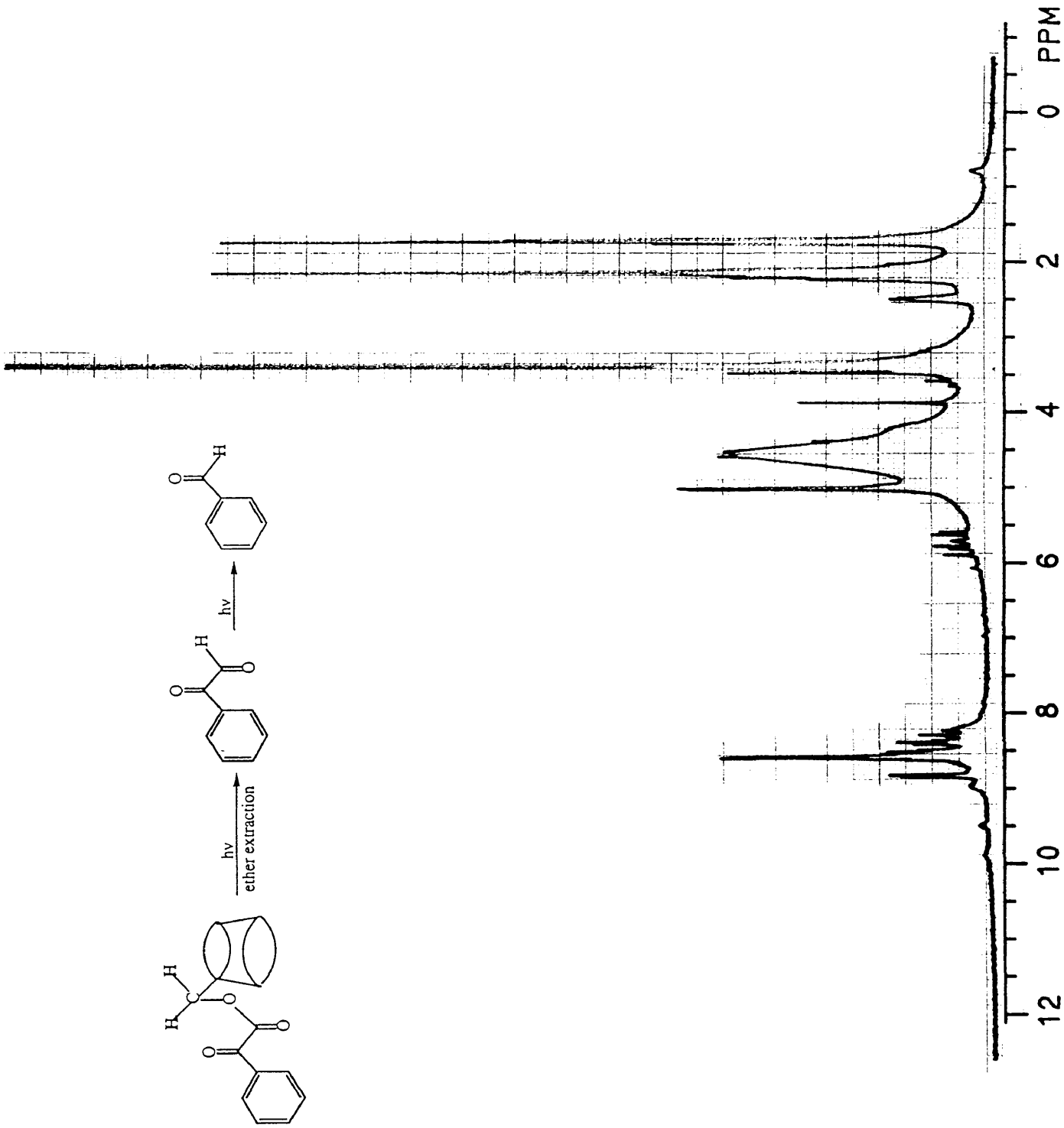


Fig. 13 ^{1}H-NMR of Aqueous Layer From Ether Extraction - Aldehyde Derivative of β-Cyclodextrin

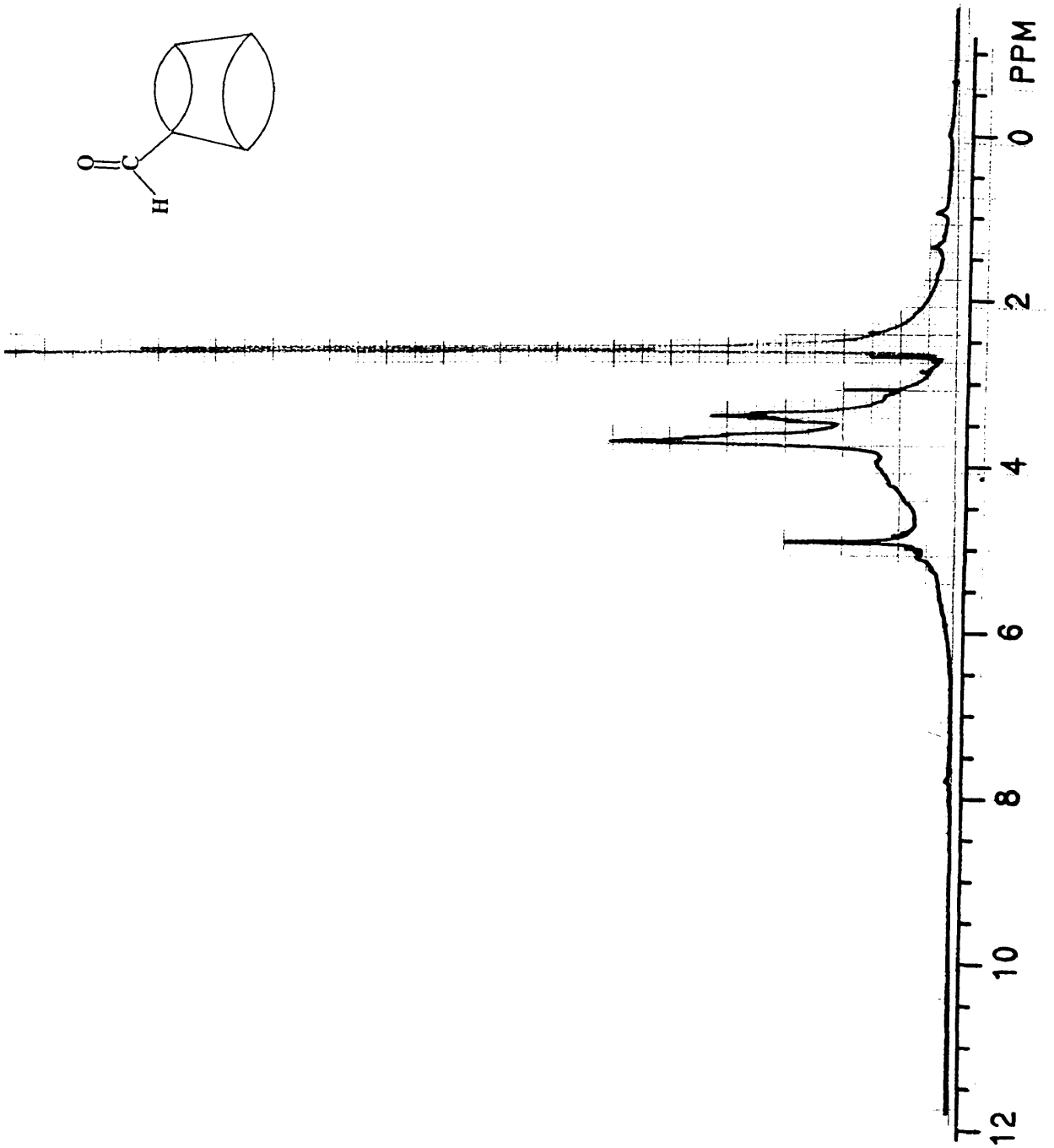


Fig. 14 ^{13}C-NMR of the Aldehyde Derivative of β-Cyclodextrin

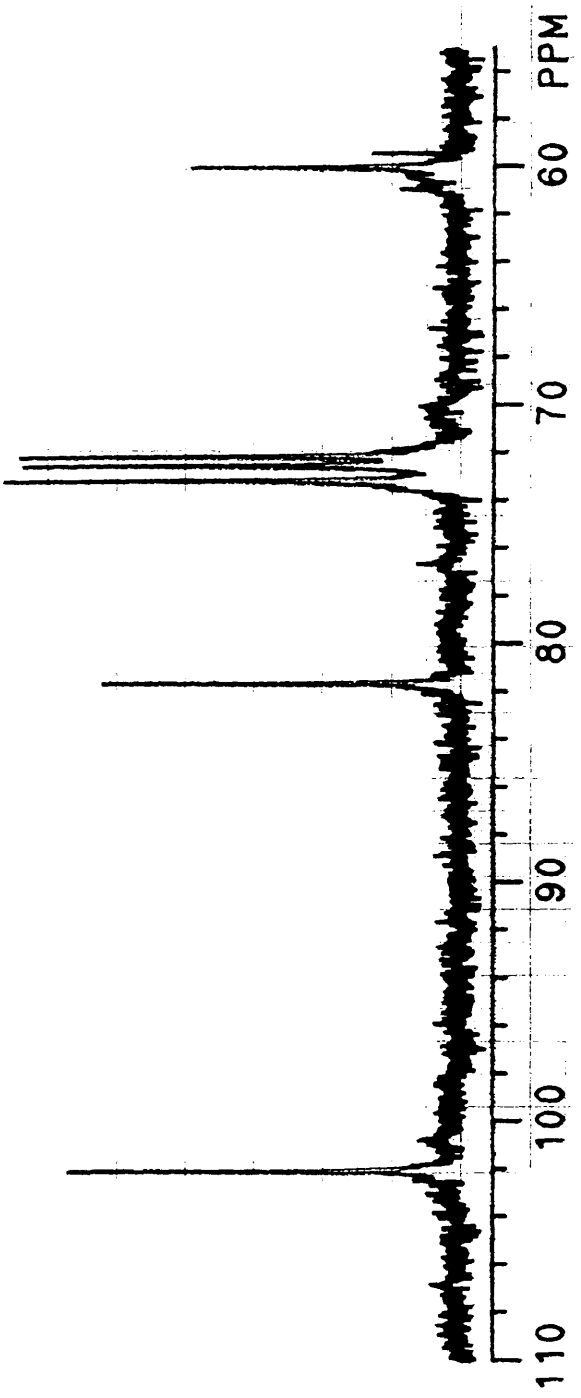


Fig. 15 ^{1}H-NMR of 1,1-Diphenylhydrazone

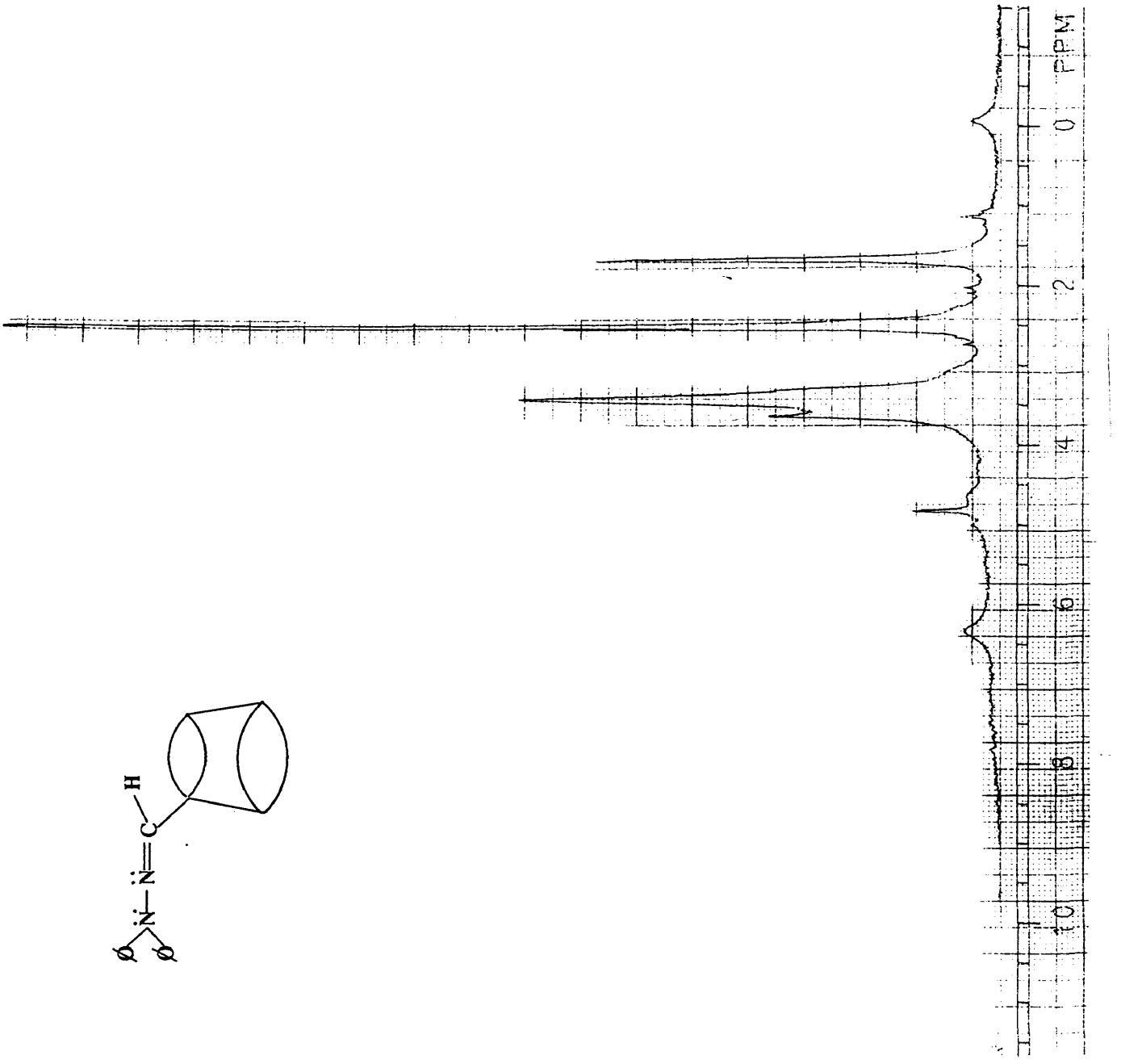


Fig. 16 Analytical HPLC of 1,1-Diphenylhydrazone

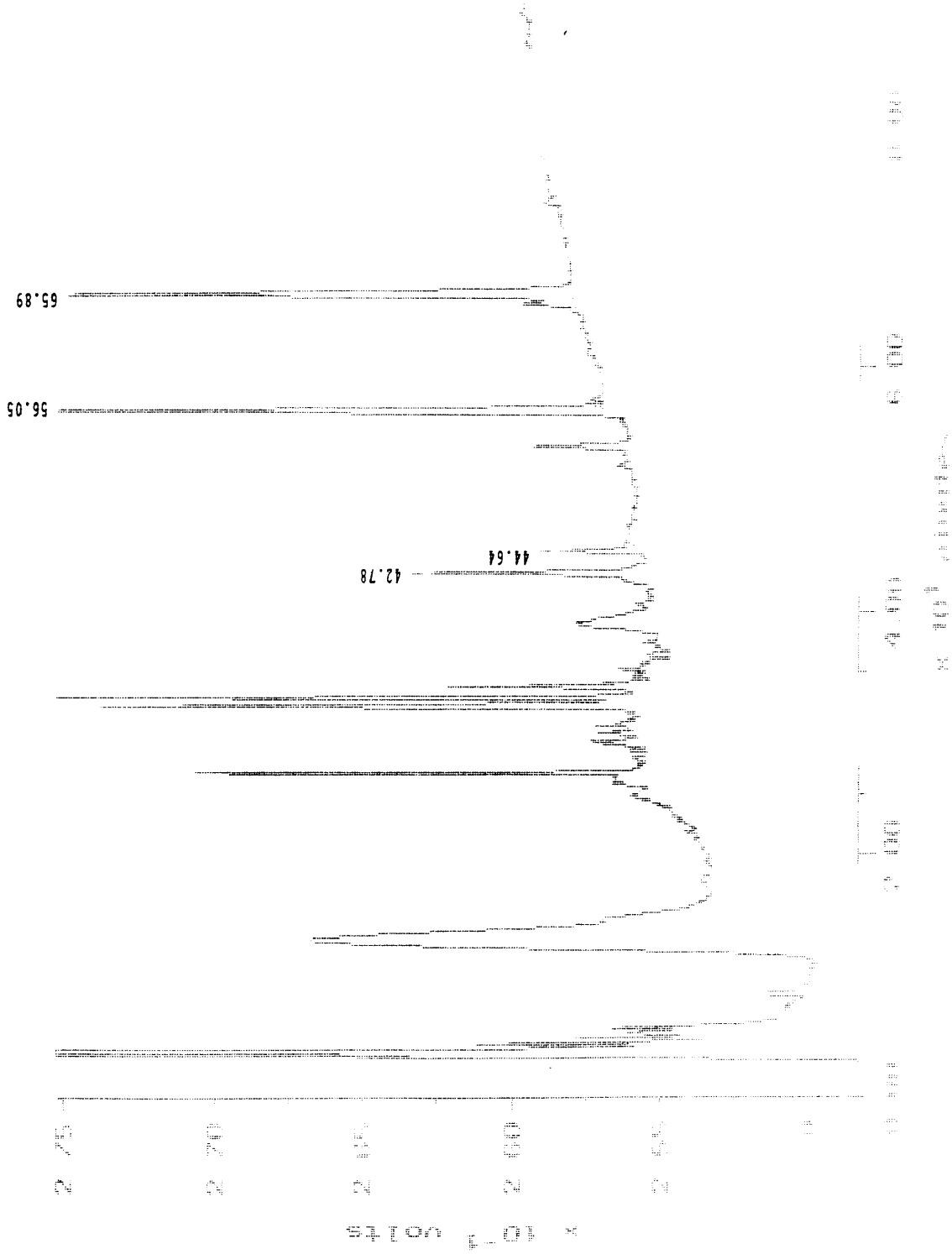


Fig. 17 ^{1}H-NMR of Ester Fractions Collected on Preparative HPLC

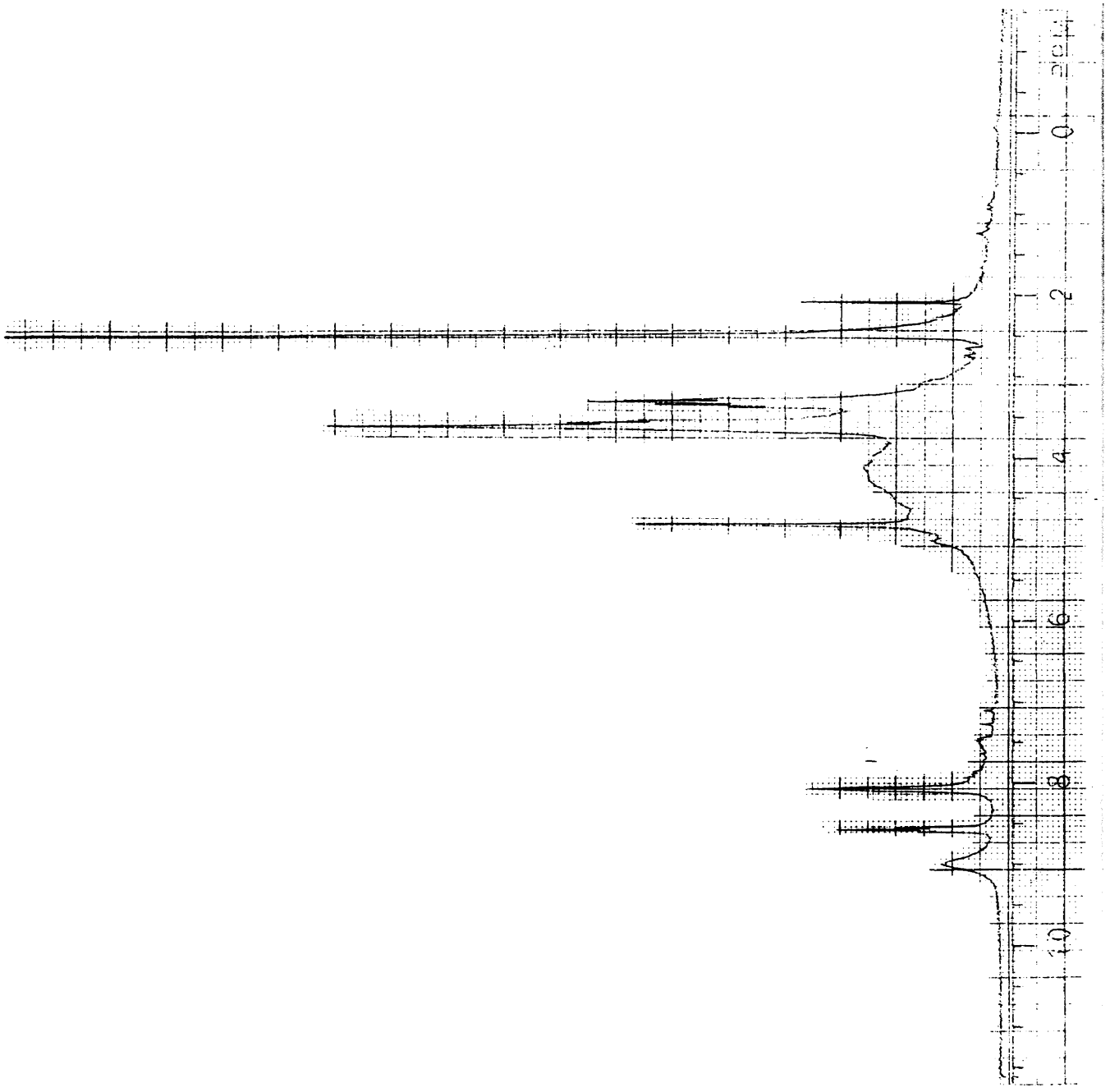


Fig. 18 ^{1}H-NMR of Ester Fractions Collected on Preparative HPLC

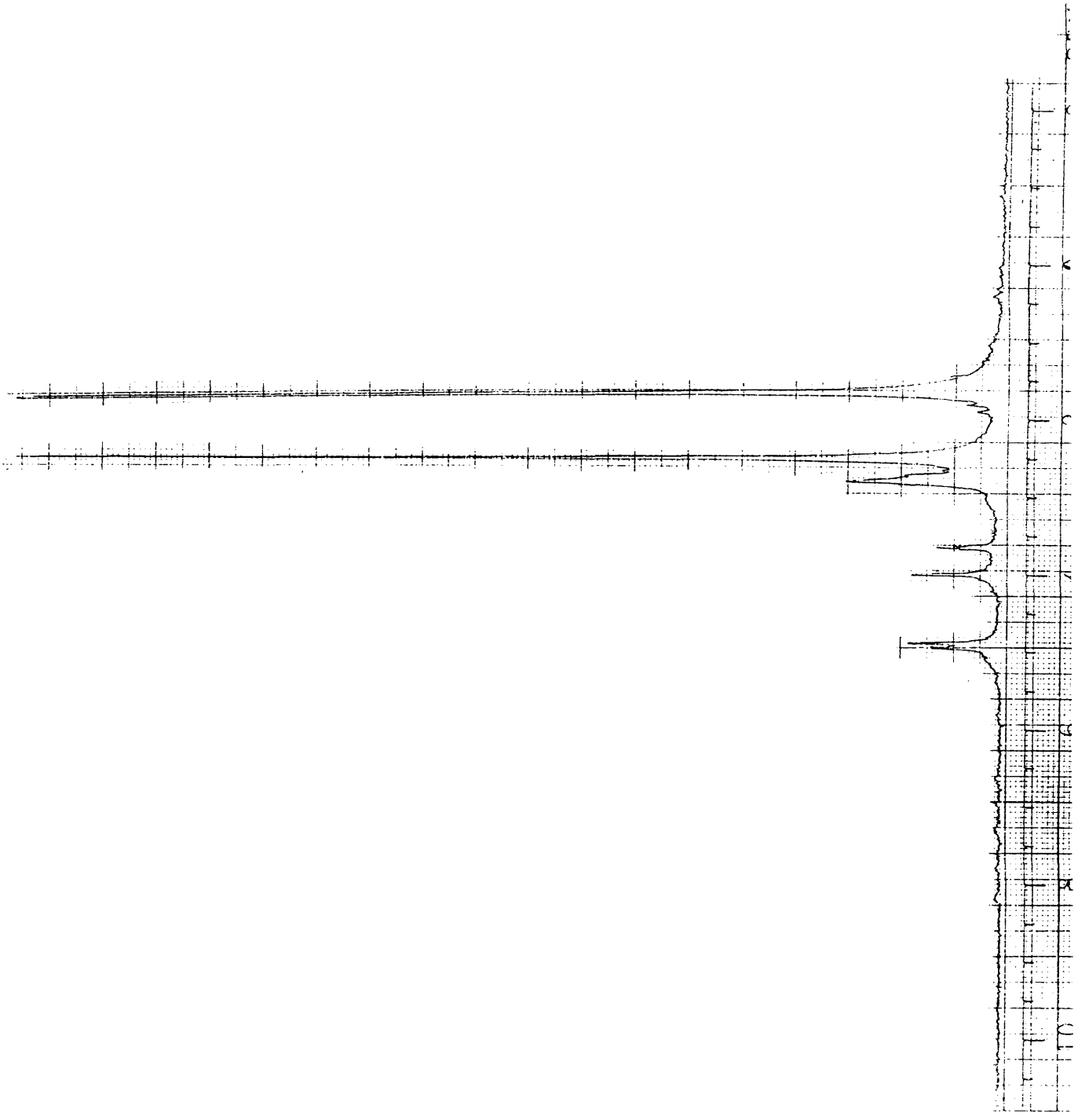


Fig. 19 Analytical HPLC of Chromium Trioxide-Pyridine Oxidation of β-Cyclodextrin

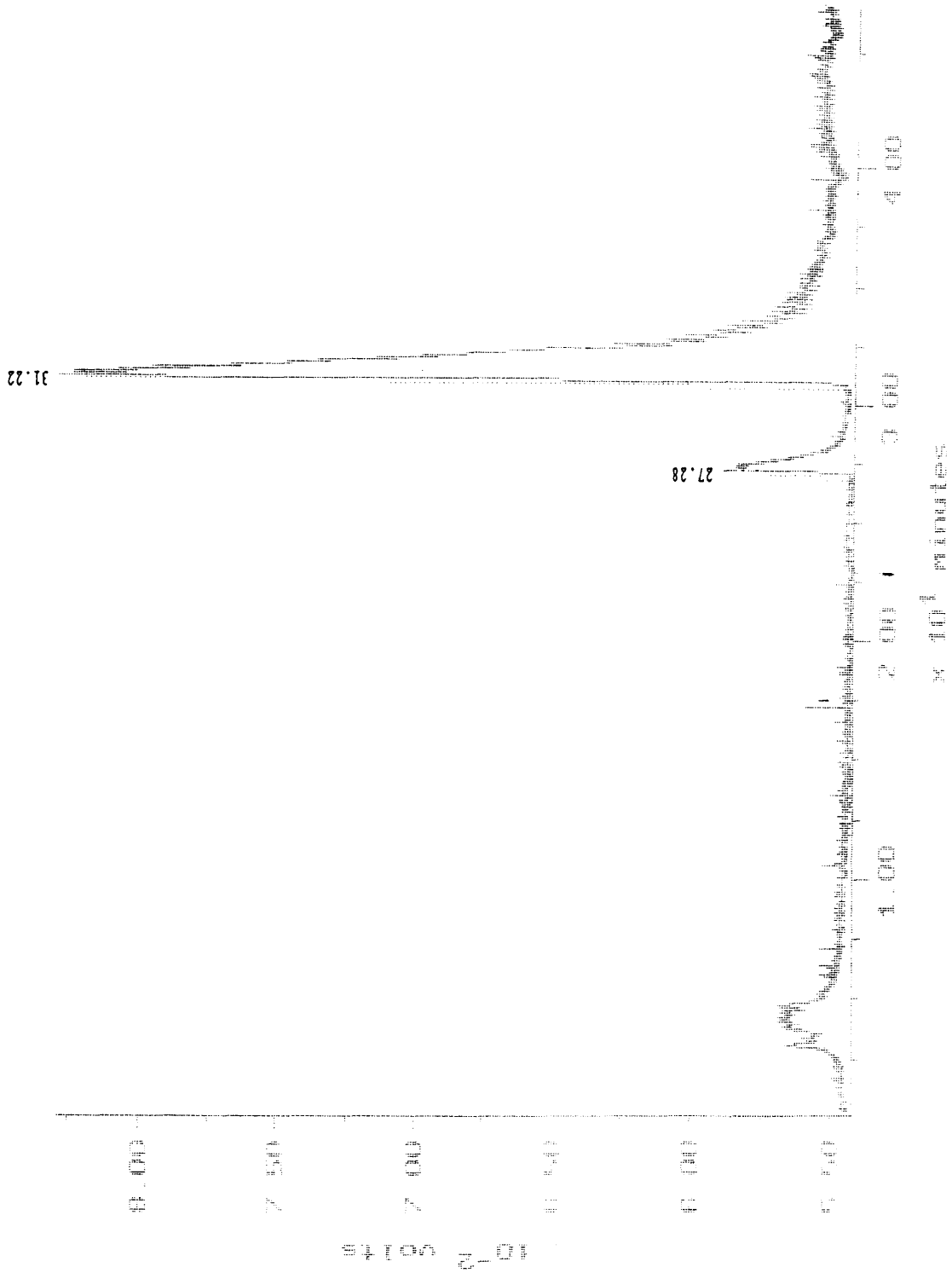


Fig. 20 ^{1}H-NMR of Poly 4-Vinylpyridinium Dichromate Oxidation of β-Cyclodextrin

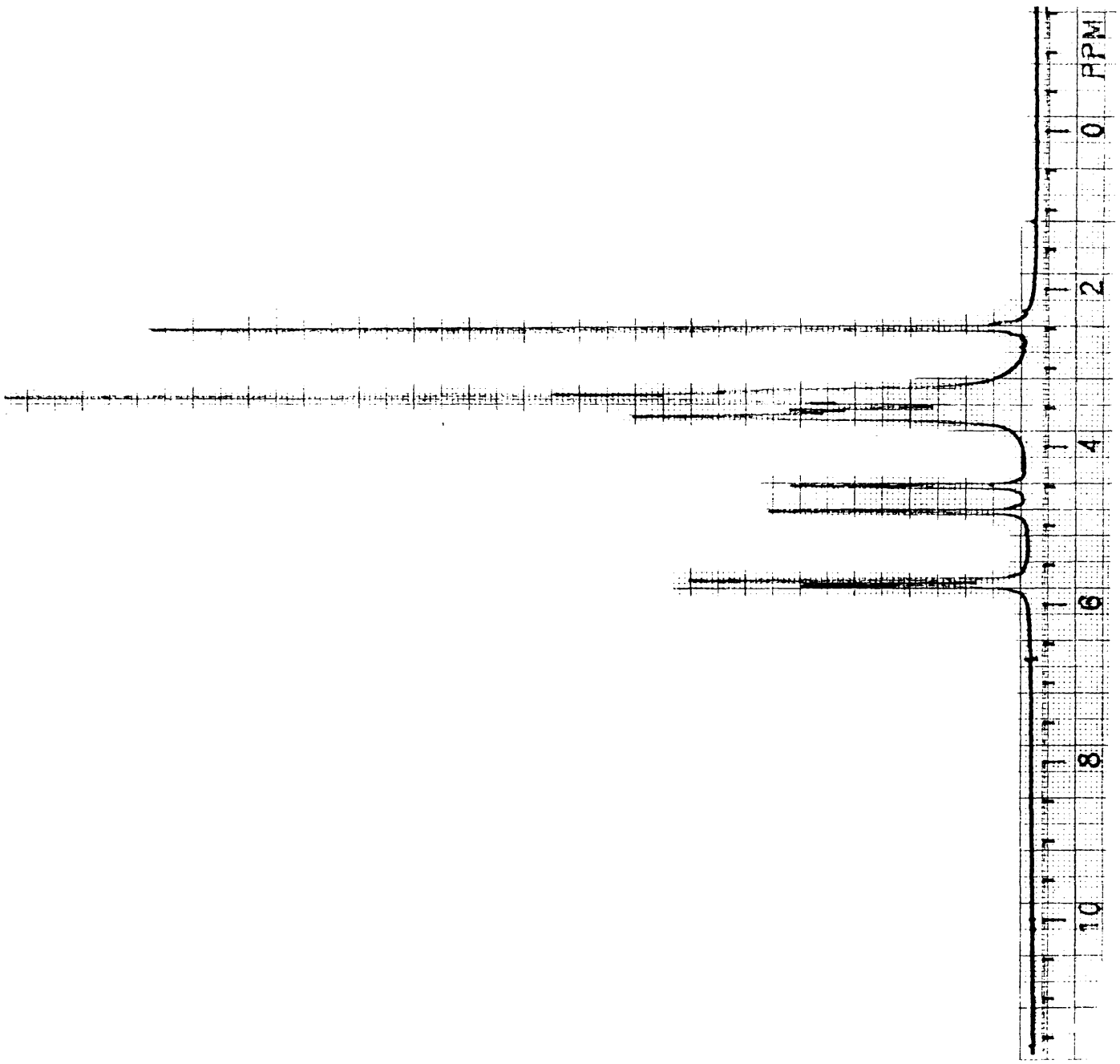
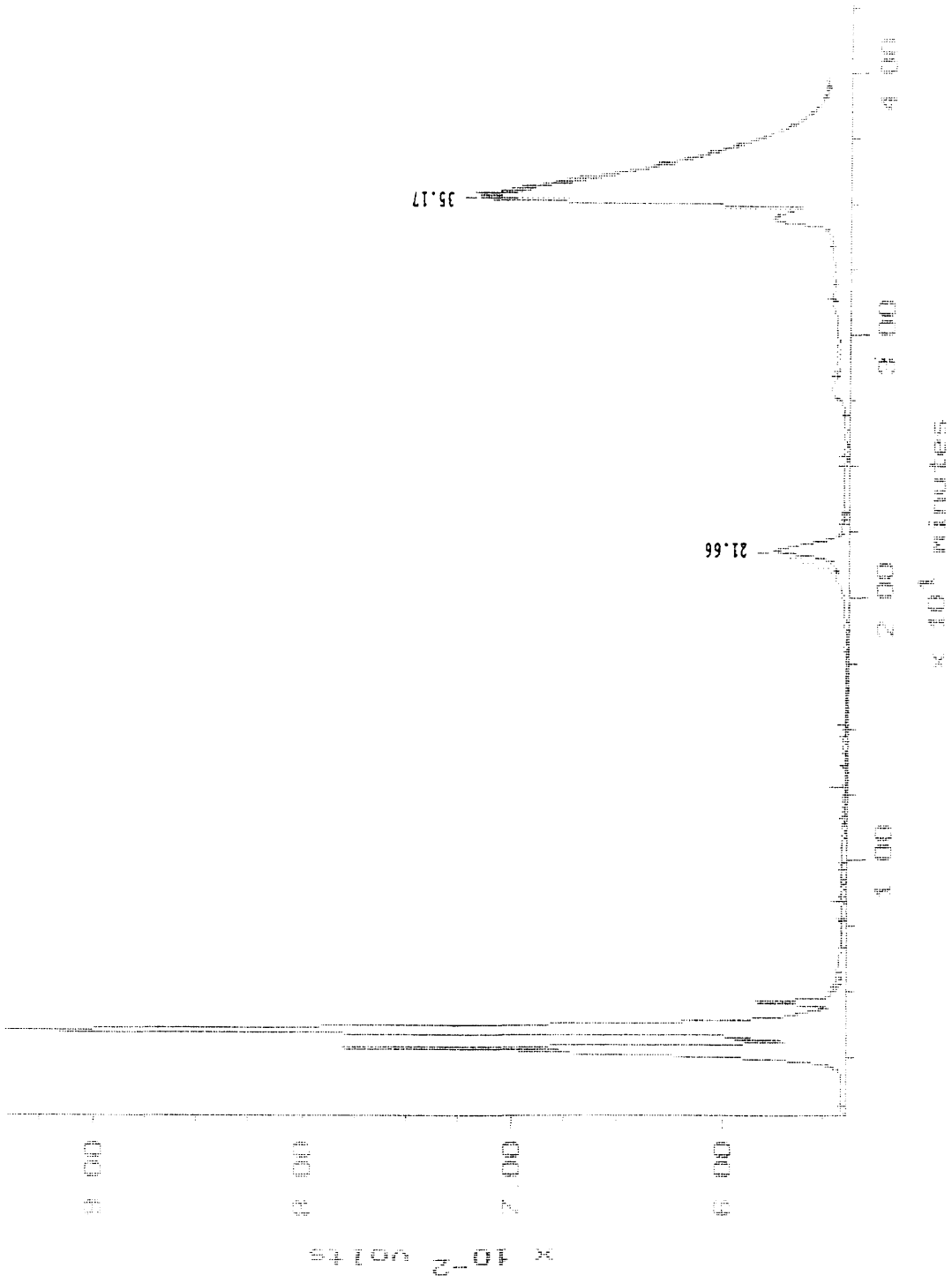


Fig. 21 Analytical HPLC of Photolyzed 6-(Anthraquinone-2-Sulfonyl)-β-Cyclodextrin



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