The Synthesis and Photochemistry of Anthraquinone-2,6-disulfonyl-beta-cyclodextrin

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THE SYNTHESIS AND PHOTOCHEMISTRY OF ANTHRAQUINONE-2,6-DISULFONYL-β-CYCLODEXTRIN

A Thesis
Presented to
The Faculty of the Department of Chemistry
The College of William and Mary in Virginia

In Partial Fulfillment
Of the Requirements for the Degree of
Master of Arts

by
Angela M. Aquino
1989
APPROVAL SHEET

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

Master of Arts

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Approved, August 25, 1989

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>2</td>
</tr>
<tr>
<td>BACKGROUND</td>
<td>4</td>
</tr>
<tr>
<td>(\beta)-Cyclodextrin</td>
<td>4</td>
</tr>
<tr>
<td>Inclusion Compounds</td>
<td>6</td>
</tr>
<tr>
<td>Capping</td>
<td>7</td>
</tr>
<tr>
<td>Cyclodextrin Influence in Photochemical Reactions</td>
<td>11</td>
</tr>
<tr>
<td>Modified Cyclodextrins in Photochemical Reactions</td>
<td>15</td>
</tr>
<tr>
<td>Photochemistry of Anthraquinones</td>
<td>17</td>
</tr>
<tr>
<td>Electron Transfer</td>
<td>19</td>
</tr>
<tr>
<td>EXPERIMENTET</td>
<td>21</td>
</tr>
<tr>
<td>Chlorinations</td>
<td>21</td>
</tr>
<tr>
<td>Capping Reactions</td>
<td>22</td>
</tr>
<tr>
<td>Warm Capping</td>
<td>22</td>
</tr>
<tr>
<td>Cold Capping</td>
<td>24</td>
</tr>
<tr>
<td>Photolysis</td>
<td>27</td>
</tr>
<tr>
<td>Regioisomeric Determination</td>
<td>28</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>30</td>
</tr>
<tr>
<td>Synthesis and Purification</td>
<td>30</td>
</tr>
<tr>
<td>Characterization</td>
<td>33</td>
</tr>
<tr>
<td>Nuclear Magnetic Resonance</td>
<td>34</td>
</tr>
<tr>
<td>Proton</td>
<td>34</td>
</tr>
<tr>
<td>Carbon</td>
<td>38</td>
</tr>
<tr>
<td>Infrared Spectroscopy</td>
<td>41</td>
</tr>
<tr>
<td>Ultraviolet Spectroscopy</td>
<td>41</td>
</tr>
<tr>
<td>Regioisomeric Determination</td>
<td>44</td>
</tr>
<tr>
<td>Photolysis</td>
<td>48</td>
</tr>
<tr>
<td>Molecular Modeling</td>
<td>52</td>
</tr>
</tbody>
</table>
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LIST OF FIGURES

Figure 1. $\beta$-Cyclodextrin ............................................ 5
Figure 2. Enhanced Binding ............................................ 7
Figure 3. Looper’s Walk ............................................. 8
Figure 4. Regioisomeric Cyclodextrin Capping ...................... 9
Figure 5. Influence of R Group on Regiochemistry ................. 9
Figure 6. Capping Reaction Impurities ................................ 11
Figure 7. Cyclodextrin Sensitizers .................................... 16
Figure 8. Positional Isomer Distribution ......................... 32
Figure 9. Anthraquinone Numbering Scheme .................... 34
Figure 10. Proton NMR A. HPLC Pure B. After 60°C .............. 36
Figure 11. Carbon NMR A. HPLC Pure B. After 60°C ............. 39
Figure 12. Attached Proton Test .................................... 40
Figure 13. IR .......................................................... 42
Figure 14. UV .......................................................... 43
Figure 15. Regioisomer Derivatization ............................ 45
Figure 16. HPLC of Regioisomer Separation ..................... 47
Figure 17. Photolysis in Isopropanol ............................... 49
Figure 18. Photolysis A. In CH₃CN B. In iPrOH ................ 50
Figure 19. Photolysis in Acetonitrile ............................... 51
Figure 20. AC Capped Cyclodextrin - Top View ................... 54
Figure 21. AC Capped Cyclodextrin - Side View .................. 55
Figure 22. AD Capped Cyclodextrin - Top View .................. 56
Figure 23. AD Capped Cyclodextrin - Side View ............... 57
Abstract

β-Cyclodextrin was rigidly capped using anthraquinone-2,6-disulfonyl chloride. The crude $6^{A,C(D)}-(anthraquinone-2,6-disulfonyl) -β-$ cyclodextrin was purified by flash reverse-phase chromatography, followed by high pressure liquid chromatography. Characterization by $^1H$ NMR, $^{13}C$ NMR, IR, UV and TLC supported a monocapped structure.

The capping reaction was carried out at $0^oC$ and $60^oC$ to determine the effect of temperature on regioisomer distribution. The product mixture from each was converted to the corresponding $A,C$- and $A,D$-bis(4-$t$-butylthiophenoxides) by nucleophilic displacement with NaSPh-$t$-Bu. The $A,C/A,D$ regioisomer ratio was determined by HPLC on a carbohydrate analysis column.

Preliminary photolysis experiments were carried out using NMR and HPLC to monitor the reaction. Irradiation of the anthraquinone-2,6-disulfonyl β-cyclodextrin gave rise to oxidation of the cyclodextrin by hydrogen abstraction from the excited quinone moiety.
SYNTHESIS AND PHOTOCHEMISTRY OF
ANTHRAQUINONE-2,6-DISULFONYL-β-CYCLODEXTRIN
INTRODUCTION

Cyclodextrins have recently become a well known and widely used commodity in both science and industry. Although initially isolated in 1891, they were not studied much for the next three quarters of a century. Cyclodextrin research remained relatively slow as late as the mid 1970s but has grown exponentially since 1975. Currently, several hundred papers pertaining to cyclodextrin chemistry are published each year. Why this sudden surge of interest? The answer lies in the potential applications that have been found in research and industry alike. Cyclodextrins are interesting mainly because of their inclusion properties, both in the solid state and in aqueous solution. They possess a cavity of 5-7Å which enables them to include molecules, or parts of molecules, with dimensions similar to those of the cavity. When these inclusion complexes are formed, the geometry and chemical composition of the cyclodextrin determine the selectivity of any processes which may take place on the included molecule. Before the 1970s the definitive structure remained unclear, and the properties of cyclodextrins, such as their ability to act as "host" molecules and thus their usefulness as enzyme models, were just beginning to be understood. As soon as researchers caught a glimpse of the unique properties of the environment provided by the cyclodextrin cavity, the rapid expansion of research began.

As a result of intense research during the past decade, approval by the Food and Drug Administration and the realization of β-cyclodextrin production on an industrial scale, cyclodextrin is now a
multi-million dollar industry, being used in pharmaceuticals, foods, cosmetics, toiletries, pesticides, and more. Cyclodextrin serves a variety of purposes, ranging from stabilization of flavors and odors to providing a medium in which to encapsulate drugs. ¹, ²

The cyclodextrin being utilized in industry today is effective in its unaltered form because the environment provided by the cyclodextrin cavity inherently has an extraordinary degree of influence on the reactions of bound "guest" molecules. Modified cyclodextrins have recently been the subject of great interest because of the increased degree of selectivity that can be achieved. Much of the early research has gone into "biomimetic chemistry", ³ where chemists have attempted to imitate the selectivity of enzymatic processes by incorporating appropriate geometric control into normally random chemical reactions. Modifications have produced some powerful catalysts, and the similarity of these hosts to enzymes has increased since a catalytic functionality is introduced in close proximity to the pocket containing the substrate. In these studies cyclodextrin has shown the ability not only to direct the course of the reaction to produce a single product, but also to accelerate the reaction, acting as a catalyst. With endless possibilities for further variation, great potential remains to design modified cyclodextrins not only for improving enzyme models, but also for a variety of other chemical applications. The challenge lies in altering the cyclodextrin to increase further the remarkably high degree of selectivity of the reaction, while simultaneously maximizing the already large accelerations. As a result, the majority of current research involves chemical modification of the cyclodextrin to fashion a
novel structure, whether it is monosubstituted, disubstituted, capped or joined with another to form a duplex.

Very recently, cyclodextrins have been utilized not only in thermal chemistry, but also in photochemical reactions in which their basis of influence lies in restricting and organizing the environment provided by the cyclodextrin cavity. The way the included guest is bound within the cavity has been shown to dominate its photochemistry, especially if the cyclodextrin has been appended with a molecule that has already been proven to influence or take part in photochemical reactions, such as anthraquinone. The research in this paper focused on forming a β-cyclodextrin capped with an anthraquinone to serve as a photosensitizer in photochemical reactions. Although a limited number of similar compounds have been synthesized and used advantageously in chemical reactions, research in this area to date has been very limited.

**BACKGROUND**

**β-Cyclodextrin**

Cyclodextrins are cyclic oligosaccharides containing 6 to 12 α-1,4 linked D glucopyranose units in the C1 (chair) conformation. The three homologues of general interest are α-, β-, and γ-cyclodextrin, consisting of 6, 7 and 8 glucose units respectively. The most important structural features of all cyclodextrins are their hydrophobic interior cavity, their hydrophilic faces, and their toroidal shape. All of the secondary hydroxyls are located on the wider face, and all of the primary hydroxyls on the other, which is somewhat narrower as a result
of the free rotation of the primary hydroxyls effectively reducing the diameter of the cavity on that face (See Figure 1).  

Figure 1. β-Cyclodextrin

The cavity, therefore, is lined by hydrogen atoms and glycosidic oxygen bridges. The non-bonding electron pairs of these glycosidic oxygen bridges are directed toward the inside of the cavity, producing high electron density within a hydrophobic cavity.

A secondary belt is formed inside the cavity by the hydrogen bonding of the C2-OH of one glucopyranose unit to the C3-OH of the neighboring glucopyranose unit. Measurements of the H/D exchange for α-, β-, and γ-cyclodextrins have proven β-cyclodextrin to have the strongest hydrogen-bonding system. It is unique because the ring of hydrogen bonds inside the cavity is complete, unlike α-cyclodextrin which has 1 glucopyranose unit in a distorted position and, therefore, has only 4 out of 6 possible hydrogen bonds or γ-cyclodextrin which is slightly larger and more flexible.

This hydrogen-bond network makes β-cyclodextrin the least soluble in water, but it is also responsible for giving strength to the rigid cyclodextrin structure and is possibly the reason that selectivity of
primary vs. secondary substitution is greater for β-cyclodextrin than
for α-cyclodextrin by a factor of 3. 8 This network of hydrogen-bonding
has been shown to persist even in solution. In experiments conducted in
dimethyl sulfoxide, a strong competitor for intramolecular hydrogen
bonding, the 3-hydroxyl proton NMR signal at 5.2 δ is unchanged. 9 For
this reason, β-cyclodextrin is the most useful structure when selec­
tively modifying the primary face to produce a rigid structure or
environment in which to carry out a controlled chemical reaction.

Inclusion Compounds

As stated earlier, the value of cyclodextrins lies in their ex­
traordinary ability to form inclusion compounds. This means that as a
"host" molecule they can admit and bind an appropriately sized "guest"
in the cavity without covalent bonds. The major forces to which the
binding of a nonpolar guest molecule is commonly attributed are
hydrophobic interactions 10 and van der Waals forces. 1

The theory of hydrophobic interactions is based on the concept
that guest molecules, being less hydrophilic than water, can be included
in the cyclodextrin cavity in aqueous solution because occupation of the
slightly apolar cavity by water molecules is an energetically un­
favorable process (polar-apolar interactions). Water molecules are
readily substituted by less polar "guest" molecules. The driving force
for complexation then is the substitution of high-enthalpy water
molecules by an appropriate guest, thereby also reducing the entropi­
cally unfavorable orientation of water molecules on the surface of the
hydrophobic guest dissolved in water. 10,11 X-ray studies on inclusion
complexes have been done to confirm the absence of water molecules from
the cavity and the location of the guest within van der Waals contact with the cavity wall.\textsuperscript{12}

Despite this unique hydrophobic recognition, cyclodextrin's binding ability is restricted partly because of the limited hydrophobic surface area with which it can contact the surface of a given guest molecule. The interior of the cavity, containing ether oxygens, is slightly polar, and both ends of the cavity are open to the solvent. Consequently, the guest is not immobilized, so the geometry and spatial arrangements are not as well defined as they could and must be to achieve the high degree of specificity desired.

\textbf{Capping}

Increased binding was first accomplished by Emert and Breslow in 1975\textsuperscript{13} by introducing hydrophobic moieties onto the rim of the cyclodextrin which enhances binding by increasing the hydrophobic area participating in the process. Since the primary hydroxyl groups are less hindered and react more quickly than the secondary hydroxyls, cyclodextrin was modified at all primary hydroxyl groups to form a hydrophobic floor on one side of the torus.

\textbf{Figure 2. Enhanced Binding}
The above compounds are now known as flexibly capped compounds. In 1982 Tabushi et al. developed a series of disulfonyl and dicarbonyl compounds in which a single appendage was attached to the primary face in two positions. Reaction at one center ensured the second point of attachment to be on the same face and in a position determined by the limited range a tethered cap can swing. Such compounds are now referred to as rigidly capped cyclodextrins.

Tabushi's model for the mechanism attributed to this reaction is called the Looper's Walk (See Figure 3). Initially, one of the acid chloride groups attaches by addition-elimination to a primary hydroxyl of the cyclodextrin, enforcing a looper's walk on the second acid chloride group. It has three distinct possibilities for the second point of attachment, resulting in an AB, AC or AD capped structure (See Figure 4). The position of the glucose monomer to which the second
attachment is made depends primarily on distance since it will attach at the point where the acid chloride can encounter a hydroxyl group with minimal conformational strain.

The nature of the R group is also very important in the sense that the more rigid R is, the stricter the regiochemical restriction becomes. For satisfactorily rigid R's, the site of the second attachment is mainly determined by the average distance between the two sulfur atoms.
In the case of the planar caps, such as anthraquinones, the intramolecular distance between the two reactive centers is the most important variable. An estimate of the distance between reactive centers can be used to predict the regioisomers which will result from a given capping reaction.

For example, m-benzene disulfonyl chloride$^{15}$ reacts to attach only AB, whereas compounds which can span a longer reactive distance, such as 1,1'-methylenebis-(benzene-4 sulfonyl chloride),$^{12}$ are less selective in their reactions. The range of spans which can give rise to AC or AD structures overlaps sufficiently that many capped compounds are formed as a mixture of AC/AD regioisomers. Only in compounds that are extremely short or long can any selectivity in regioisomers be expected. A few examples of regiospecific AD capped compounds have been synthesized, such as trans-stilbene-4,4'-disulfonyl chloride which cannot cap AC due to its exceptionally long distance between sulfonyl groups.$^{16}$

Another possibility to consider with AC capping is the dicapped structure, consisting of two capping agents attached at the AC and A'C' positions. One possible driving force for this occurrence is the entropically favorable decrease in hydrophobic surface area which both caps maintain in contact with water. AD capping, on the other hand, does not have this competitive mechanism because in the absence of severe steric contortions by the cyclodextrin or the cap this would require two molecules to occupy virtually the same space.

Other possible products from capping reactions may be the result of two pathways suggested by Tabushi to compete with the looper's walk. Although it remains unclear how efficient they are, both intermolecular condensation and monodentate capping are prime candidates for mechanisms.
to form unwanted products. Intermolecular condensation results when the second sulfonyl chloride reacts with and attaches itself to a primary hydroxyl group on another cyclodextrin molecule. When multiplied, this leads to higher molecular weight oligomers. Monodentate capping, in contrast, singly links more than one cap to each cyclodextrin.

Figure 6. Capping Reaction Impurities

Cyclodextrin Influence in Photochemical Reactions

Organized media of many varieties are known to influence both thermal and photochemical reactions because the environment provided by the host can effectively control, modify, and enhance chemical reactivity.  

Cyclodextrin inclusion provides a relatively new type of
organic medium which has already been proven to affect many photochemical and photophysical properties. Among the many studies reported are emission intensity enhancement, \(^{19}\) intramolecular excimer or exciplex formation, \(^{20}\) fluorescence quenching due to inclusion of a fluorophore or quencher, \(^{21}\) and excimer fluorescence due to dimers included in the cavity. \(^{22}\)

Chemical reactions are influenced by restriction and organization of the environment provided by the cyclodextrin cavity, which result in many different modes of influence in both unimolecular and bimolecular reactions. The interior of the cyclodextrin cavity constitutes an isolated environment that can geometrically constrain the guest by limiting the space available for molecular motions and by stabilizing conformations that are less favorable than in free solution. \(^{4}\) Reactive sites of the entrapped species which are encircled by the cyclodextrin are also protected from incoming chemical reagents. Thus, the photochemistry is limited to intramolecular events, except in cases of multiple occupation of cavities. The hydrophobic nature of the cavity can also affect photoprocesses that are sensitive to solvent polarity or dielectric properties. \(^{5}\) These features allow cyclodextrins to be used as a microvessel to carry out selective phototransformations by taking advantage of conformational control, site selectivity and restriction of motion. Several examples have been reported where stereoselective inclusion of organic molecules has led to dramatic differences in photochemical reactivity, selectivity and product distribution.

Cyclodextrin's ability to enforce certain conformations on an included guest and its ability to alter its excited state chemistry were found in the conformationally dependent photoreactions of benzoin alkyl
ethers and α-alkyl dibenzylketones. The photolysis of benzoin alkyl ethers in aqueous solution gives photoproducts resulting mainly from the Norrish Type I pathway. Even though the benzoin alkyl ethers contain γ-hydrogens, this pathway does not involve γ-hydrogen abstraction. Upon complexation with β-cyclodextrin, Norrish Type II photoproducts were formed in nearly quantitative yield. Since Type II products result from γ-hydrogen abstraction, these results can be explained by a conformation suitable to γ-hydrogen abstraction being enforced upon the transition state. A conformation consistent with these results would be one with the benzene ring inside the cavity and the alkyl group outside where it would be available for γ-hydrogen abstraction. Similar results were found in the closely analogous system of α-alkyl dibenzyl ketones. In aqueous solution photolysis results in products from both the Norrish Type I and the Norrish Type II pathways; however, upon complexation with β-cyclodextrin, only Norrish Type I photoproducts were formed. Since the transition state for the Type II pathway requires a six membered transition state, it is conformationally dependent, whereas the transition state for the Type I pathway is not. These results can be explained by the inclusion of the alkyl group with the benzene ring inside the cavity. This would prevent γ-hydrogen abstraction by the carbonyl oxygen and promote a Type I reaction pathway. In both of the above examples, the restrictions enforced upon the transition states bring about selectivity that was not present in aqueous solution.

The cis-trans photoisomerization of stilbene is another unimolecular reaction selectively restricted by the cyclodextrin cavity. Upon excitation in isotropic solution, the geometric isomerization favors the cis-isomer. When included in solution with
cycloexdextrin, however, the trans-isomer is predominant in the photo-
stationary state. This result is an example of limited space available
inside the cavity for molecular motions. The formation of the cis-
isomer becomes energetically unfavorable due to greater steric
interaction with the cycloexdextrin rim.25

Selectivity was also found by Ueno et.al.26 when using azo dyes
to regulate the hydrolysis of p-nitrophenylacetate with β-cycloexdextrin
as a catalyst. Stronger bonding of the trans azo dye was reported, and
photolysis of trans-p-phenylazobenzoate β-cycloexdextrin complex resulted
in conversion to the cis form of the azo dye.

In other cases, the cycloexdextrin sterically blocks certain poten-
tial sites of the included species from intermolecular attack. This
feature has been exploited in the photo-Fries, photo-Claisen and related
rearrangements.4 These rearrangements involve an initial cleavage fol-
lowed by a reorganization of the fragments and give one specific isomer
when carried out with cycloexdextrin. For example, the photo-Fries rear-
rangement of phenyl esters in organic solvents typically results in a
mixture of o- and p-hydroxy acetophenones. Irradiation as a solid
complex with cycloexdextrin or in an aqueous solution with cycloexdextrin
yields only the o-isomer due to the restricted mobility the cavity
imposes on the radical intermediates of this reaction.27

Aggregation effects can be seen in complexes formed with more than
one guest such as in the regioselective and stereoselective
photodimerization of anthracene-2-sulfonate.28 In water, all four pos-
sible regioisomers are formed. However, upon cycloexdextrin complexation
the reaction proceeds via a 2:2 complex with the sulfonate groups
directed away from each other, and only one regioisomer is produced.
The products formed are significantly affected by the substituent groups attached to the aromatic ring because they limited the possible complexes formed with the cyclodextrin. This example is one of dimerization accelerated and regiochemically controlled by constraints imparted by unmodified cyclodextrins.

Modified Cyclodextrins in Photochemical Reactions

Chemical modification of cyclodextrin has been used to create even more powerful systems for ground state and photochemical reactions.\textsuperscript{3,4} As mentioned above capping not only increases binding by 11-24 times,\textsuperscript{9} but also affords special recognition between the host and guest due to their especially close proximity. The effects of few other types of structural modifications have been examined in the light of their photochemical properties. Three examples of photochemically-active, derivatized cyclodextrins have been reported. Attaching a benzophenone,\textsuperscript{29} a rose bengal,\textsuperscript{30} and a porphyrin\textsuperscript{31} moiety to \(\beta\)-cyclodextrin resulted in host sensitizer systems for triplet energy transfer, singlet oxygen generation and photoreduction, respectively.

Inclusion complexes of capped cyclodextrins afford special recognition between the host and guest due to their especially close proximity.\textsuperscript{10} Tabushi, et al. capped \(\beta\)-cyclodextrin with benzophenone, and succeeded in producing a system capable of structurally specific and efficient triplet energy transfer from the benzophenone-\(p,p'\)-dicarboxylate capped \(\beta\)-cyclodextrin to the included naphthalene guests (1-naphthylbromide or 1-naphthylethylbromide). Even at low
concentrations of bromonaphthalene, irradiation of benzophenone afforded complex phosphorescence from both the benzophenone and bromonaphthalene moieties. The energy transfer was estimated at 60%. This novel type of energy transfer is allowed only for host-guest combinations of exact structural recognition.  

Modified β-cyclodextrins have also been used as a means to chemically reorganize a photochemical system, resulting in a significant effect on the rate of a photosensitized process. Neckers et al. appended cyclodextrin with rose bengal, a singlet oxygen photosensitizer, and observed enhanced quenching of the singlet oxygen by included anthracene and β-carotene guests. The modified cyclodextrin in this example micro-organized the system in such a way that caused the singlet oxygen to be generated directly in the quenching sphere of the included quencher. Since the interacting species were much closer than
they would be in fluid solution, the problem of diffusion was partially
eagnated, artificially enhancing the quenching rate.

A third example is taken from the research of Bolton, Weedon and
Gonzalez who appended an electron donating porphyrin moeity to
cyclodextrin in an attempt to model photosynthesis. Upon excitation of
the porphyrin chromophore, they observed electron transfer reduction of
included p-benzoquinone. This study also examined the dependence of
electron transfer efficiency upon the reduction potential of the ac­
ceptor. Anthraquinone-2-sulfonate was successfully used as an acceptor,
indicating the viability of an anthraquinone capped cyclodextrin as a
potential photoinduced electron transfer catalyst.

While research has not previously been done on quinone capped
cyclodextrins, the success of experiments involving other photosen­s­itizers as capping agents suggests that the micro-environment of a
capped cyclodextrin does not inhibit such photoprocesses. Because this
paper describes the synthesis and photochemical properties of β-CD
capped with an anthraquinone and examines its viability as a photooxida­tion sensitizer, it is important to address the basics of anthraquinone
photochemistry and electron transfer.

Photochemistry of Anthraquinones

The photochemistry of anthraquinones has been well documented. The
chemistry of the water-soluble anthraquinone sulfonates and disul­
fonates is most relevant to this study since the anthraquinones are
attached to β-CD via a sulfonate ester bond. Anthraquinone sulfonate
(AQS) photochemistry is dominated by triplet state reactions due to
rapid and efficient intersystem crossing from the singlet state. The excited triplet AQS then reacts via one of two mechanisms depending on the available substrates: electron-transfer or hydrogen abstraction from the solvent or other hydrogen donor. For example, photolysis of 2-AQS in aqueous NaBr produces Br₂ by photooxidation of the bromide anion to atomic bromine. This simple and clean reaction can be used for solar energy storage. Much of the interest in water-soluble anthraquinones derives from the desire to perfect solar energy conversion schemes.

Hydrogen abstraction predominates with certain substrates; for example, 2-propanol is readily oxidized to acetone with anthraquinone-2-sulfonate. The photooxidation mechanism is initiated by an hydrogen-abstraction rather than an electron transfer as shown by Wells and coworkers. In the absence of substrates with either low oxidation potentials or abstractable hydrogens, the anthraquinone sulfonates will react with water to generate hydroxyanthraquinone sulfonates. This side reaction destroys their utility in solar energy storage schemes.

Many studies have investigated the involvement of electron transfer in the excited state chemistry of quinones. Wubbels and coworkers concluded that quinone photosubstitution reactions followed electron transfer routes in which intermediate radical ions were involved. Other studies showed that triplet 9,10-anthraquinone-2,6-disulfonic acid, in aqueous media, will accept electrons from a variety of anions (Xⁿ⁻), such as halide, carbonate, sulfate, and hydroxide, according to the scheme:

\[ Q + X^{n-} \rightarrow Q^- + X^{(n-1)-} \]

These studies imply that once attached to a cyclodextrin, a photochemically excited quinone would lead to electron transfer if in
the presence of a bound guest which has a lower oxidation potential than
the excited state reduction potential of the quinone.

**Electron Transfer**

The intrigue of photochemical reactions stems from the ability of
photoexcited molecules to undergo processes that are impossible in the
ground state. Excited states have a high energy content along with
different nuclear coordinates and electron distributions when compared
to the ground state. Often the energy minimum exists on excited state
potential energy surfaces at nuclear coordinates corresponding to energy
maxima for ground state surfaces.

Following the initial absorption of light, a molecule is raised to
an electronically excited singlet state. If intersystem crossing is
rapid, photochemical reaction will occur via the triplet state, which
among other possibilities has the ability to undergo electron transfer
processes. Photosensitization usually guarantees reactions via triplet
excitation states. A sensitizer must be present in sufficient con-
centration and absorb more strongly than the reactants. For electron
transfer to occur a close proximity is required for effective orbital
overlap. The reaction range is usually limited to distances of less
than 10 Å.40,41

The energetics of electron transfer, succinctly described by the
Rehm-Weller equation, are important in determining both the viability
and the rate of a given reaction.42

\[ \Delta G_{et} = - E_{0,0} + E_{ox} - E_{red} - \frac{e^2}{\varepsilon a} \]
In order for a reaction to be feasible, the energy supplied by the light and stabilization of the ions by the solvent must exceed the energy required to oxidize the donor and reduce the acceptor. If in contact with one another, and the energetics are favorable according to the Weller equation, electron transfer will occur. Since the excited state reduction potential of anthraquinone is about 1.8 V (\(E_{o,o} + E_{red}\)), donors whose oxidation potential is less than 1.8 volts will be photooxidized. Following electron transfer, a few possible means for deactivation exist. These include separation of the ions formed or back electron transfer if the ions do not separate.
EXPERIMENTAL

Commercially available β-cyclodextrin (Aldrich or Amaizo) was used after vacuum drying (0.05mm) at 100°C for 12 hrs with a liquid N₂ trap. Pyridine was fractionally distilled; the fraction boiling between 114°C and 115°C was collected and stored over activated 4Å molecular sieves. ¹H and ¹³C NMR spectra were obtained with a GE QE-300 spectrometer. UV/VIS spectra were measured on a Beckman DU-70. TLC was carried out on 0.25 mm (60F-254) precoated silica plates (Baker); spot detection was done with UV and staining with vanillin (Fisher). Flash reverse-phase column chromatography was done with Baker RP-18 silica gel. High performance liquid chromatography was performed on a Waters 660 system equipped with a variable wavelength absorption detector using a Whatman ODS-3 analytical column. Preparative HPLC was performed on a Waters 244 system equipped with a UV absorption detector (254 nm) using a Whatman Magnum 20 column packed with ODS. Regioisomer determination was performed on a Waters 660 system using a Waters carbohydrate analysis column 84038. Melting points were taken on a Thomas-Hoover capillary m.p. apparatus and are uncorrected. Irradiations were carried out with a Hanovia 450W medium pressure lamp using glass filter sleeves (Pyrex or uranium glass). Molecular modeling was done using Alchemy II from Tripos Associates.

Chlorinations

Anthraquinone-2,6-disulfonyl chloride:
Anthraquinone-2,6-disulfonic acid disodium salt (3.00g, 7.28 mmol) was covered with SOCl\(_2\) (9.00g, 75.7 mmol) and stirred at room temperature under a CaSO\(_4\) drying tube until SO\(_2\) evolution had subsided.\(^{44}\) Dimethylformamide (0.09g, 123 mmol) was added dropwise. The mixture was heated to reflux (79°C) for a minimum of 6 hours, cooled to room temperature and then quenched with ice. The resulting yellow solid was collected by vacuum filtration, washed with water, and dried in vacuo affording the disulfonyl chloride (2.92g, 7.20 mmol, 97%) The crude disulfonyl chloride was recrystallized from benzene (250 mL), affording (1.54g, 5.92mmol, 66%) pure anthraquinone-2,6-disulfonyl chloride (m.p. 248-250°C).

\(^1\)H NMR (DMSO-d\(_6\)) δ 8.1 (dd, 4H), 7.32 (dd, 4H)

Diphenylether -4,4'-disulfonyl chloride:

Diphenyl ether (5.0 g, 0.29 mmol) was added dropwise to chlorosulfonic acid (10 mL, 150 mmol), under a CaSO\(_4\) drying tube, and let stir 2 hours at room temperature.\(^{45}\) After quenching with H\(_2\)O, the reaction mixture was filtered on a Buchner funnel and recrystallized in 3:1 heptane/benzene, yielding 2.74 g (7.46 mmol, 55%, m.p.124-127°C).

Capping Reactions

Warm Capping

\(6^\text{A}_{\text{C(D)}}\)-anthraquinone-2,6-disulfonyl)-\(\beta\)-cyclodextrin (1):
β-Cyclodextrin (1.02 g, 0.90 mmol) was added slowly to pyridine (300 mL), and the mixture was sonicated until the β-cyclodextrin dissolved completely. The solution was heated under N₂ and 25 mL of pyridine was distilled off to remove any H₂O present. After cooling to room temperature, anthraquinone-2,6-disulfonyl chloride (0.37 g, 0.90 mol) was slowly added and stirred until completely dissolved. The reaction mixture was then heated to 60°C for three hours. Following decantation from viscous oligomeric precipitates, the pyridine was removed under high vacuum with warming (T < 40°C). The residue was washed with acetone, filtered, and dried in vacuo overnight. Traces of pyridine hydrochloride and anthraquinone-2-sulfonic acid were removed by flash reverse phase using a gradient elution of 0 to 30% aqueous CH₃CN. The fractions were analyzed by TLC, and those containing the desired compound (R_f=0.65, 5:4:3 n-BuOH, EtOH, H₂O) were concentrated in vacuo, affording the β-Cyclodextrin derivative (0.21 g, 0.14 mmol, 15%). Final purification was accomplished through preparative HPLC using an acetonitrile-water gradient elution (20% to 29%). Approximately 50% of the injected material was recovered on this step, affording an overall yield of 10% HPLC pure product.

¹H NMR (CD₃CN/D₂O) δ 8.83 (br s, 1H), 8.81 (br s, 1H), 8.67 (d, J=8.2 Hz 1H), 8.62 (br d, J=8.2 Hz, 1H), 8.55 (d, J=8.2 Hz, 1H), 8.51 (br d, J=8.3 Hz, 1H), 5.19 (m, 3H), 5.07 (m, 2H), 4.95 (m, 1H), 4.82 (m, 1H), β-CD resonances;

¹³C NMR (DMSO-d₆) δ 181.0, 180.5, 180.3, 141.5, 140.5, 140.4, 139.3, 137.2, 136.7, 136.3, 134.7, 134.1, 132.3, 124.9, 102.5, 101.9, 101.6, 82.0, 81.3, 73.2, 72.7, 72.4, 71.1, 70.7, 68.8, 60.0, 59.6

UV (1:1 CH₃CN/H₂O) λ (log ε) 211 nm (4.5), 255 nm (4.7), 323 nm (3.7).
Cold Capping

\[ \text{\(6^6\text{C(D)}(\text{anthraquinone-2,6-disulfonvl})-\beta-\text{cyclodextrin (1)}: \)} \]

\(\beta\)-Cyclodextrin (6.0 g, 5.29 mmol) was slowly added to pyridine (300 mL), and the mixture was sonicated until the \(\beta\)-CD dissolved completely. The solution was heated under \(\text{N}_2\) and 35 mL pyridine was distilled off to remove any \(\text{H}_2\text{O}\) present. After cooling to room temperature, anthraquinone-2,6-disulfonvl chloride (2.2 g, 5.42 mmol) was slowly added and stirred until completely dissolved. The reaction mixture was then cooled to \(0^\circ\text{C}\) for three hours, followed by the removal of pyridine under high vacuum with warming (\(T < 40^\circ\text{C}\)). The residue was washed with acetone, filtered, and dried in vacuo overnight.

Traces of pyridine hydrochloride and anthraquinone-2,6-sulfonic acid were removed by flash reverse phase using a gradient elution of 0 to 30% aqueous CH\(_3\)CN. The fractions were analyzed by TLC, and those containing the desired compound (\(R_f = 0.65, 5:4:3\) n-BuOH, EtOH, \(\text{H}_2\text{O}\)) were concentrated in vacuo affording the \(\beta\)-cyclodextrin derivative (1.87 g, 1.27 mmol, 23%). Final purification was accomplished through preparative HPLC using an acetonitrile-water gradient elution (20% to 29%). Approximately 50% of the injected material was recovered on this step, affording an overall yield of 9% HPLC pure product.

\(^1\text{H NMR (DMSO-\text{d}_6/\text{D}_2\text{O}) \(\delta 8.61 \text{ (br s, 2H)}, 8.52 \text{ (m, 2H)}, 8.36 \text{ (br d, J=8.3 Hz, 1H)}, 8.29 \text{ (br d, J=8.3 Hz, 1H)}, 5.02 \text{ (m, 3H)}, 4.86 \text{ (m,2H)}, 4.72 \text{ (m, 2H)}, \) \beta\text{-CD resonances;}}\)

\(^{13}\text{C NMR (DMSO-\text{d}_6/\text{H}_2\text{O}) \(\delta 180.3, 181.0, 134.6, 137.2, 140.4, 141.14, 133.1, 132.4, 131.3, 130.6, 127.9, 127.4, 127.2, 128.7, 128.1, 125.6, 124.9, 124.0, 123.8, 102.5, 101.9, 101.6, 101.1, 82.0, 81.3, 80.5, 78.8, 73.4, 73.1, 72.7, 72.4, 72.1, 69.7, 68.8, 60.0, 59.6, 59.2, 59.0}}\)
UV (1:1 CH$_3$CN/H$_2$O) $\lambda$ (log $\epsilon$) 211 nm (4.5), 255 nm (4.7), 323 nm (3.7).

Anal. Calc. for C$_{56}$H$_{84}$O$_{46}$S$_2$·5H$_2$O: C, 43.19; H, 5.44; S, 4.12.

Found: C, 43.08; H, 5.28; S, 4.03 (ave. of two determinations).

6$_A$6C(D)diphenyl ether 4,4'-disulfonyl-$\beta$-cyclodextrin (2):

$\beta$-Cyclodextrin (9.52 g, 8.39 mmol) was covered with pyridine (480 mL), and the mixture was sonicated until the $\beta$-cyclodextrin dissolved completely. The solution was heated under N$_2$ and 50 mL pyridine was distilled off to remove any H$_2$O present. After cooling to room temperature, diphenyl ether-4,4'-disulfonyl chloride (2.68 g, 7.30 mmol) was slowly added and stirred for 7 hours at room temperature, followed by the removal of pyridine under high vacuum with warming (T < 40°C). The residue was washed with acetone, filtered, and dried in vacuo overnight.

Traces of pyridinium hydrochloride and diphenyl ether-4,4'-sulfonic acid were removed by flash reverse phase using a gradient elution of 0 to 30% aqueous CH$_3$CN. The fractions were analyzed by TLC, and those containing the desired compound ($R_f=0.67$, 5:4:3 n-BuOH, EtOH, H$_2$O) were concentrated in vacuo affording the $\beta$-cyclodextrin derivative (1.95 g, 1.36 mmol, 16%). Final purification was accomplished through preparative HPLC using an acetonitrile-water gradient elution (20% to 29%). Approximately 50% of the injected material was recovered on this step, affording an overall yield of 8% HPLC pure product.

$^1$H NMR (DMSO-$d_6$/D$_2$O) $\delta$ 8.00 (q, 4H), 7.42 (d, 2H), 7.20 (d, 2H), 4.0-5.10 (br m, 7H), $\beta$-CD resonances;
$^{13}$C NMR (DMSO-d$_6$/D$_2$O) $\delta$ 160.9, 159.8, 130.9, 130.4, 130.0, 121.4, 119.1, 102.4, 102.2, 101.6, 101.2, 82.0, 81.7, 81.5, 81.2, 80.9, 72.5, 72.3, 72.0, 71.8, 70.3, 60.1, 59.9, 59.2

UV (1:1 CH$_3$CN/H$_2$O) $\lambda$ (log $\epsilon$) 249 nm (8.4)
**Photolysis**

**Photolysis of 1 - Method A:**

Compound 1 (4.2 mg, 2.86 μmol) was dissolved in D$_2$O/CD$_3$CN (0.5 and 0.4 mL, respectively), and the solution was transferred to an NMR tube and degassed with N$_2$ for 10 min. The tube was placed into an ice-bath which was situated next to a Hanovia 450W medium pressure mercury arc lamp in a Pyrex immersion well. Aluminum foil was placed around the immersion well, and an elliptical hole was cut out of the wrap. The solution in the capped NMR tube was irradiated, and the photolysis was monitored periodically by NMR. The solution was irradiated for 65 min. After the lamp was extinguished, air was bubbled through the solution for 15 min.

**Photolysis of 1 - Method B:**

Compound 1 (4.2 mg, 2.86 μmol) was dissolved in D$_2$O/CD$_3$CN (0.8 and 0.2 mL, respectively), the solution was transferred to a round bottom flask and degassed with N$_2$ for 10 min. Irradiation was carried out as above, and the reaction was monitored periodically by HPLC (starting material showed a retention time of 25.0 min when eluted with 20% CH$_3$CN. The solution was irradiated for 10 hrs. After the lamp was extinguished, air was bubbled through the solution for 10 min. The HPLC of the air-sparged product shows three major new peaks with retention times of 13, 17 and 19 min with the latter two being the largest (detection at 320 nm).
**Regioisomeric Determination**

**AB, AC, and AD-Bis(4-t-butylphenyl-sulfenyl)-β-cyclodextrin from Diphenyl Ether β-cyclodextrin:**

4-t-Butylthiophenol (46.3 mg, 0.278 mmol) and NaOMe (15.0 mg, 0.278 mmol) was dissolved in absolute MeOH (0.46 mL) under nitrogen. The MeOH was removed *in vacuo* leaving a white powder of sodium 4-t-butylthiophenolate. To the sodium salt was added the diphenyl ether-capped β-cyclodextrin (2) (0.025 g, 0.0175 mmol) and dry DMF (0.90 mL). The resultant solution was heated at 80°C for 12 hours under nitrogen. After cooling, the precipitated disulfonic acid disodium salt was filtered off and the filtrate was concentrated to dryness *in vacuo*. H₂O (1.5 mL) and aq. HCl (1 drop) were added to the pale brown residue to bring the pH below 2. The mixture was then extracted with Et₂O (1.6 mL x 4), and the aqueous layer was combined with the insoluble product. The resulting A,C(D)-bis(4-t-butylphenyl-sulfenyl)-β-cyclodextrin, obtained after concentrating the ethereal solution *in vacuo*, was dissolved in a 3.5:1, CH₃CN:H₂O solution to analyze by HPLC using a Water's carbohydrate analysis column.

**AC & AD Bis(4-t-butylphenyl-sulfenyl)-β-cyclodextrin from anthraquinone-2,6-disulfonyl-β-cyclodextrin:**

4-t-Butylthiophenol (45.3 mg, 0.272 mmol) and NaOMe (15.0 mg, 0.278 mmol) were dissolved in absolute MeOH (0.463 mL) under nitrogen. The MeOH was removed *in vacuo* leaving a white powder of sodium 4-t-butylthiophenolate. To the sodium salt was added the anthraquinone-2,6-disulfonyl-β-cyclodextrin (0.025 g, 0.0175 mmol) and dry DMF (0.865 mL).
The resultant solution was heated at 80°C for 12 hours under nitrogen. After cooling, the precipitated disulfonic acid disodium salt was filtered off and the filtrate was concentrated to dryness in vacuo. H₂O (1.5 mL) and aq. HCl (1 drop) were added to the pale brown residue to bring the pH below 2. The mixture was then extracted with Et₂O (1.6 mL x 4), and the aqueous layer was combined with the insoluble product. The resulting A,C(D)-bis(4-t-butyphenyl-sulfenyl)-β-cyclodextrin, obtained after concentrating the ethereal solution in vacuo, was dissolved in a 3.5:1, CH₃CN:H₂O solution to analyze by HPLC using a Water’s carbohydrate analysis column.
RESULTS AND DISCUSSION

Synthesis and Purification

Since no detailed reasons were given for conditions used in previous studies of cyclodextrin capping, anthraquinone capping reactions seemed to fall within the parameters reported by Tabushi, and the same general procedure was followed. Extreme care was taken to carry out the reaction under very dry conditions. Cyclodextrin was dried at 100°C under high vacuum for at least 12 hours. Pyridine was distilled by fractional distillation. The fraction boiling at 114-115°C was collected and stored over activated sieves to insure that it remain extremely dry. After dissolving the cyclodextrin in the dry pyridine, a second distillation was necessary to remove the H₂O/pyridine azeotrope, which came off at 94°C. Cyclodextrin is not soluble at temperatures above 45°C and the capped compound cannot withstand the high temperatures. Therefore, the cap must be added after the cyclodextrin solution has cooled to room temperature. The capping agent was also dried under a high vacuum for at least 12 hours immediately prior to addition to remove any water that might have been present. The concentration of cyclodextrin was kept constant at 1.76 x 10⁻³ M. The concentration of capping agent also remained constant because attempts to increase the concentration of the cap failed due to its limited solubility in pyridine.

Following the given reaction period it is important to promptly remove the pyridine under vacuum at a temperature below 40°C. Extremely low yields were observed when the reaction mixture was allowed to sit for longer than one hour or warmed above 40°C. For similar reasons it
is equally important to dry the solid product on the vacuum line after removing the pyridine, especially if storing the product overnight. Before separation on a reverse-phase column, a large amount of pyridinium hydrochloride is present. This hygroscopic substance will draw water into the reaction mixture, promoting hydrolysis of the capped product and making any separation by reverse-phase difficult. Washing with acetone aided in removing pyridinium hydrochloride, but the majority must be separated by reverse-phase chromatography. Problems with eluent flow could be avoided by grinding the crude reaction mixture through a 100 mesh sieve prior to adding it to the reverse-phase column. At every stage in the workup care must be taken to minimize time in aqueous solution, keep the temperature below 40°C and keep the product under a nitrogen atmosphere whenever possible. Due to the extremely fast rate of hydrolysis, these precautions must be strictly adhered to in order to obtain the maximum possible yield.

Temperature was the only variable altered in the capping reaction. The two conditions used were 0°C and 60°C. After separation on a reverse phase column, the average yield for the warm capping reactions was 10-15%, and the highest yield was 18%. For the capping reaction carried out at 0°C, the average yield was about the same (18%) and the highest yield increased to 23%. Although a slightly greater yield was obtained in the cold (0°C) capping reaction, no appreciable differences in yield were found due to temperature variations. The only observed difference was that the cold capping reaction remained clear until the removal of pyridine, whereas the warm (60°C) capping reaction was clouded by oligomeric precipitates within one hour of heating. Tabushi et. al. observed the same effect and attributed it to the slow buildup of
polymeric materials from intermolecular condensation, which becomes thermodynamically more favorable with higher temperatures and longer reaction periods. Since overall yield of singly capped β-cyclodextrin does not seem to be decreased by this mechanism, intramolecular capping appears to be more effective than intermolecular oligomerization.

The low yield and extreme difficulty in purification of capped cyclodextrins are due in part to the multiplicity of products obtained. Reverse-phase chromatography successfully partitions off the unreacted cyclodextrin, diacid chlorides, diacids and pyridinium hydrochloride, but the degree of separation of other products is unknown. Each fraction was analyzed by TLC. The spots which demonstrated UV activity contained the anthraquinone in some form, and the spots which charred darkly when stained with vanillin contained cyclodextrin. Cyclodextrin derivatives possessed both characteristics. Fractions which eluted in 10-20% CH$_3$CN indicated by TLC (UV activity and charring) the presence of
at least two other cyclodextrin derivatives in substantial crude yield. At times the mass isolated in these fractions was almost as great as the mass of capped cyclodextrin recovered in later fractions. However, HPLC separation proved them to be a mixture of many products, a large portion of which was the diacid. The monocapped derivative was identified by intense UV activity and dark charring of vanilla on TLC plates. This compound came through the column in 30% CH$_3$CN and displayed a 25 minute retention time by analytical HPLC in aqueous 20% CH$_3$CN. The crude yield of capped $\beta$-cyclodextrin after the reverse-phase column averaged 15-20%, of which 50% could be isolated as HPLC pure anthraquinone-2,6-disulfonyl $\beta$-cyclodextrin.

Preparative HPLC clearly showed the monocapped derivative to be the primary product. However, many other products were also indicated. Attempts were made to identify them, but the relative mass recovered was so small and characterization so difficult that definite conclusions could not be drawn. Two peaks, in particular, were very strongly UV active, but the mass recovered was less than 25% of the mass of pure capped cyclodextrin isolated. Possible explanations include tethered cyclodextrin, multifunctionalized compounds and dicapped structures.

**Characterization**

Methods of characterization of organic molecules, such as TLC, IR and UV spectroscopy, cannot cope with the multiplicity of products, especially positional isomers, expected from capping reactions. Therefore, characterization of monocapped cyclodextrin is complex and often uncertain.
Nuclear Magnetic Resonance

Proton

$^1$H NMR spectroscopy proved to be of only limited utility in characterizing the capped product. The stoichiometry of cap to cyclodextrin was established by a ratio of integrated areas of aromatic quinone protons to the anomeric cyclodextrin protons. This ratio can easily distinguish between capped and dicapped structures. Figure 10A shows a 1:1 ratio, supporting a monocapped structure.

The first peak is the unique proton located at the C1 and C5 positions, followed by a doublet representing the protons at the C3 and C4 positions, and another doublet representing the protons at C6 and C7. The numbering scheme is as shown below:

![Figure 9. Anthraquinone Numbering Scheme](image)

A well-defined $^1$H NMR of a capped cyclodextrin shows that these doublets have shifted due to the slightly different environments encountered by the sets of protons that were in identical environments on the free anthraquinone. Another indication of capping is the formation of new peaks in the anomeric region of cyclodextrin. In unmodified
cycloextrin, this region contains only one singlet at 4.8 ppm to represent the anomeric protons in identical environments. The integrated ratio of the aromatic to the anomeric region for a pure capped compound should be six to seven. The ratio found for the sample of pure anthraquinone-2,6-disulfonyl-β-cycloextrin isolated here was found to be 6 to 6.5.

$^1$H NMR of another main fraction isolated by preparative HPLC indicated the presence of the diacid and cycloextrin in a 1:1 ratio. The presence of diacid may indicate an inclusion complex, but this is difficult to confirm due to the extremely fast rate of hydrolysis. A fair amount of acid is always isolated with each sample. Although
Figure 10. Proton NMR  A. HPLC Pure  B. After 60°C
inclusion compounds should not be formed in the initial capping reaction due to the anhydrous environment of pyridine, subsequent workup leaves room for hydrolysis, creating conditions favorable to inclusion formation. If an anthraquinone is included in the cyclodextrin cavity, the C$_3$-H and C$_5$-H protons should show a significant upfield shift because they are directed into the cavity. The C$_1$-H, C$_2$-H, C$_4$-H and C$_6$-H proton shifts should remain unaffected because they are directed toward the exterior of the cavity. Penetration of the benzenoid rings of the quinone into the cyclodextrin would create anisotropic deshielding of the C$_3$ and C$_5$ protons. Also, the presence of the ring carbonyls on the included guest could introduce shift changes for interior protons that are in close proximity to the electronegative oxygens, depending on the degree of intrusion into the cavity. Notice must also be given to the solvent used when interpreting such spectra. If done in D$_2$O, conclusions concerning the shift will reflect the preference of the guest for the hydrophobic environment of the cyclodextrin cavity. However, if done in DMSO, the hydrophobic binding is destroyed, and the guest may not necessarily prefer the environment of the cavity to the non-aqueous environment of the solvent.

Theoretically, $^1$H NMR should be useful in qualitatively distinguishing between singly and doubly linked caps by nature of the aromatic signals. The freely rotating tethered cap should exhibit sharp resonances and splitting, rather than those of aromatic protons that are motionally hindered. This provides another interpretation of the $^1$H NMR of one of the other products isolated. The aromatic region should strongly resemble the diacid because the environment of the protons has not been drastically changed. The aromatic region of the tethered cap
does indicate covalent bonding of some sort, although not to the extent shown by the capped structure. In this case spectral data should only be used to supplement inferences based on results of separation procedures which have been shown to be effective in similar situations.

Interesting information can be obtained from the $^1$H NMR of the capped compound taken after a carbon NMR at 60°C. Both the aromatic and the anomeric regions have changed from the original NMR of the pure capped compound. The aromatic region more closely resembles the acid, and the aromatic region shows less disturbance but still shows evidence of some covalent bonding. This seems to indicate that the higher temperature promoted partial hydrolysis. It is also consistent with HPLC retention times of tethered cyclodextrins and the observation that the peak in question increased when the capped cyclodextrin remained in aqueous solution for long periods of time. Anthraquinone-1-sulfonyl and 2-sulfonyl-$\beta$-cyclodextrin have demonstrated the ability to remain in aqueous solution for longer periods of time without suffering complete hydrolysis.

Carbon

$^{13}$C NMR can show more precise information regarding the quinone cap attachment. Breslow$^{45}$ found that the resonance from a substituted carbon will be shifted downfield, and those for the neighboring carbons on either side will be shifted upfield. Figure 11 shows the characteristic downfield shift of glucose resonances from 60 to 70 ppm confirming derivatization at the C6 position. The attached proton test shown in Figure 12 can be used to distinguish between carbons with an odd or even number of attached hydrogens, by a peak in the up or down direction. The C6 appearing at 70 ppm is directed upward, providing
Figure 11. Carbon NMR  A. HPLC Pure  B. After 60°C
Figure 12. Attached Proton Test
further confirmation of attachment exclusively at the C6 position.

The carbon NMR taken of the pure capped cyclodextrin after being heated to 60°C can be as informative as the corresponding proton NMR. It shows the presence of acid in the aromatic region and the growth of another carbonyl peak at 180 ppm. This implies hydrolysis at one of the two previous points of attachment.

**Infrared Spectroscopy**

IR is of limited value except for establishing the presence of some basic units. A few identifiable features of quinones are the asymmetric carbonyl stretching vibration at 1720 cm$^{-1}$ and the nuclear carbonyl quinone peak at 1680 cm$^{-1}$. The presence of the sulfonyl linking groups is shown by the asymmetric and symmetric sulfonyl stretches at 1370 and 1160 cm$^{-1}$, respectively (See Figure 13). The broad, rolling band in the hydroxyl area between 3500 and 3300 cm$^{-1}$ is characteristic of cyclodextrin.

**Ultraviolet Spectroscopy**

Assuming the applicability of the Beer-Lambert relationship, the presence of a dicapped cyclodextrin or an excess quantity of starting quinone should result in enhanced absorption in the UV region, where cyclodextrin is inactive. Figure 14 shows the strongly allowed quinoid $\pi-\pi^*$ transition at 256 nm and the allowed $\pi-\pi^*$ transition at 333 nm. Anthraquinone in methanol has a $\lambda_{max}(\log \epsilon)$ = 325 nm(4.02), which shifts to 330nm(3.70) for 2-anthraquinone sulfonic acid in water. The corresponding transition for the capped cyclodextrin appears at 323 nm.
Figure 13. IR
Figure 14. UV
with a similar log ε of 3.7. This figure effectively rules out the possibility of a doubly capped structure because the observed enhancement would be at least double.

**Regioisomeric Determination**

Regioisomeric determination poses a major problem. It is extremely difficult to distinguish one from another, and even more difficult to successfully separate them without irreversible derivatization. Many different methods have been reported to accomplish both separation and identification. Most of these studies have involved a combination of $^{13}$C NMR, double capping, derivatization, and degradation by Taka-amylase.

Breslow reported separation by HPLC using Microporasil but was unable to identify the different regioisomers. No other report has been made of regioisomer separation by HPLC without derivatization. Tabushi and co-workers developed a method to convert capped cyclodextrin into corresponding bis(4-t-butylthiophenoxide) derivatives $^{13,14}$ by the reaction shown in Figure 14. Conversion of diphenyl ether capped β-cyclodextrin yielded three bifunctionalized cyclodextrin derivatives. Following separation, the isomers were identified individually using $^{13}$C NMR and attempts at dicapping. The A,B functionalized β-cyclodextrin was easily distinguished from the A,C and A,D because the $^{13}$C NMR chemical shifts of C-1', C-4' and C-6' on the glucose ring bearing the substituent X(A) are strongly affected by a substituent X(B) on the adjacent ring, while the C-1, C-4 and C-6 $^{13}$C shifts on the other rings are only slightly affected by X(A). Consequently, a clear difference in chemical shift of 0.54 ppm between the C-4' on rings A and B was used to
distinguish between A, B and either A, C or A, D regioisomers; the latter two exhibited practically nonexistent splitting of the C-4' absorption. This assignment was supported by MS on Taka-amylose-catalyzed hydrolysis products. Since A, C regiosomers exhibit $^{13}\text{C}$ NMR spectra very similar to

Figure 15. Regioisomer Derivatization

A, D isomers, a different approach was needed to identify them. A two-fold excess of capping agent was used to elicit dicapping on each of the unidentified compounds, followed by conversion to their respective bis(sulfide) derivatives. The one which displayed a markedly different retention time from previous runs was identified as the tetrasubstituted dicap derivative and therefore must have been a product of the A, C regioisomer, because A, D capped derivatives do not lead to dicapping.

Diphenyl ether 4,4'-disulfonyl-β-cyclodextrin was synthesized as reported and derivatized to replicate the separation by HPLC of AB, AC and AD regioisomers. The bis(4-τ-butylthiophenoxide) derivatives could not be separated on a reverse phase column. The above experiment could only be duplicated using a carbohydrate analysis column as reported. Acceptable results were obtained. The peaks corresponding to the AB, AC
and AD derivatives were reported to have retention times of 10.40, 14.03 and 15.35 minutes respectively. Peaks were obtained at 9.7, 12.8 and 14.0 minutes and used as a standard for subsequent attempts at regioisomer determination for anthraquinone-2,6-disulfonyl-β-cyclodextrin. The reported percentages of AC and AD regioisomers for diphenyl ether capped β-cyclodextrin were 38% AD/51% AC at 25°C and 6% AC/94% AC at 60°C. At higher temperatures one isomer was favored. Similarly, we found the distribution of regioisomers in anthraquinone-2,6-β-cyclodextrin to vary with temperature. At 0°C 58%AD/42%AC was found. At 60°C AD capping prevailed with 79%AD and 21%AC. The percentage was found to be the same on the crude reaction mixture and the sample purified by reverse-phase indicating that the sample need not be purified before regioisomer distribution can be determined. Additional confirmation of the presence of AC capping was found by reacting the β-cyclodextrin with a two-fold excess of capping agent. The resulting product showed a distinct spot by TLC and when converted to the bis(sulfide) derivative a different retention time was observed for the tetrasubstituted dicap derivative.
Figure 16. HPLC of Regioisomer Separation

A. Standard

B. Derivative from 0°C Capping Reaction

C. Derivative from 60°C Capping Reaction
51 and 38%. We found 48 and 47% by integration of the corresponding HPLC peaks.

Regioisomeric determination of anthraquinone capped cyclodextrin proved to be more complex. Preliminary results indicate that a mixture of AC and AD regioisomers is obtained in the cold capping reaction, but warm capping results in the predominance of a single regioisomer. Retention time alone would identify this with the AC regioisomer. This would parallel the behavior of the diphenyl ether capped cyclodextrin, which was reported to have 94% AC and only 6% AD when run at 60°C.

Photolysis

Irradiation in H$_2$O/CH$_3$CN and H$_2$O/iPrOH solutions under deaerated conditions showed loss of starting material coincidental with the appearance of new aromatic NMR signals which could be almost completely converted back to starting material by passing air through the sample (See Figure 16). Since photolysis of anthraquinones in i-PrOH solutions is known to generate the hydroquinone, we conclude that the same reaction is taking place in the CH$_3$CN solution. HPLC monitoring showed that isopropanol solutions suppressed formation of the photoproducts with retention times of 13, 17, and 19 minutes.

These results indicate that the capped cyclodextrin has suffered oxidation via intramolecular hydrogen-abstraction from the photoexcited quinone. This well known quinone reaction generated the hydroquinone.
Figure 17. Photolysis in Isopropanol
Figure 18. Photolysis A. In CH$_2$CN B. In iPrOH
Figure 19. Photolysis in Acetonitrile
The quinone is regenerated by the reaction of the hydroquinone with O₂ which also results in the reduction of O₂ to H₂O₂. In the presence of i-PrOH the starting material can be regenerated, whereas in other solvents new products are formed. The source of hydrogen in the latter case must be the cyclodextrin because the same products appear in the aqueous t-BuOH, and the photoreactions of sulfonated anthraquinones in aqueous solutions result in hydroxyanthraquinone.

After approximately six hours of irradiation the starting material was no longer depleted. This can be explained by internal filtering. At this point photolysis effectively stops because the hydroquinone absorbs so much of the incoming light that a negligible amount reaches the starting material.

**Molecular Modeling**

Computer modeling was done with Alchemy II. Although most results obtained were very near reported values obtained by other means, the potential functions of this software are unknown. Thus, data must be interpreted in relative rather than absolute terms.

The reactive distances were measured for the quinone cap and β-cyclodextrin. Crystal coordinates were used to create the anthraquinone structure which was then minimized prior to attachment to cyclodextrin. With the SO₂ group at the 2 and 6 positions, the sulfur to sulfur distance is 9.9 Å. The range of spans for AC capping of the cyclodextrin was found to be 4.31-11.57 Å, and 5.60-13.63 Å for AD capping. This is in general agreement with reported values obtained from crystallographic data.
Capped structures were created and minimized. The relative energies are only valid for comparison between different minimized structures and for comparing the relative factors contributing to strain. The quinone appears bent on both the AC and AD capped structures as a result of strain. The AC structure appears more strained than the AD structure. Figures 20-23 show views of AC and AD capped cyclodextrin. This strain may be a factor in the rate of hydrolysis of the capped compound.

Distances were also measured between the nuclear carbonyl and the C6 hydroxyls on neighboring glucose rings. One C6 hydrogen on each side is within 2 Å. Although they were not well within the limits needed to allow hydrogen abstraction, given motion hydrogen abstraction is indeed possible. This reinforces the hypothesis of intramolecular hydrogen abstraction upon photolysis.
Figure 20. AC Capped Cyclodextrin - Top View
Figure 21. AC Capped Cyclodextrin - Side View
Figure 22. AD Capped Cyclodextrin - Top View
Figure 23. AD Capped Cyclodextrin - Side View
Notes


[Notes to pages 10-16]


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