The Effect of Inclusion in β-Cyclodextrin on the Chemistry of Peroxides: Reactions of Radicals with β-Cyclodextrin

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Abstract. Studies by electron paramagnetic resonance (EPR), differential scanning calorimetry, thermogravimetric analysis, HPLC and NMR showed that radicals produced by thermolysis and photolysis of benzoyl peroxide, t-butyl peroxide and cumene hydroperoxide included in β-cyclodextrin (β-CD), undergo significant reaction with the β-CD. The formation of β-CD radicals was observed by EPR. Products formed by addition of radicals to β-CD were also observed. Such host:guest radical reactions explain the reported stabilization of peroxides, found with β-CD inclusion, as being primarily due to the interruption of chain reactions by trapping of the chain carriers. A small increase in activation barrier for cleavage of the included peroxide in β-CD was also observed.

Key words: β-cyclodextrin radicals, benzoyl peroxide, cumene hydroperoxide, t-butyl peroxide, peroxide stabilization, electron paramagnetic resonance (EPR).

1. Introduction

Over the last decade many studies have been conducted on the effect of inclusion within cyclodextrin (CD) on the rates, products and yields of various reactions. Because CD can be used to impart significant water solubility to otherwise insoluble molecules, the push towards environmentally benign synthesis is likely to see increased interest in CDs as a reaction medium. CDs have the potential to change the chemistry of their guests, stabilizing some [1–4] and altering the reaction products of others [5–13]. In such reports of free radical reactions, the CD hosts are treated as inert and considered not to take any part in the free radical chemistry. Given that the CD is simply a carbohydrate, it might be expected to undergo reactions with many of the free radicals generated in the course of these reactions. Only recently has this aspect of CD chemistry begun to be seriously considered [7, 9, 10, 14, 15]. UV spectra attributed to CD radicals have been reported in flash photolysis of benzophenone/CD complexes [10] and pulse radiolysis of C_{60}/γ-CD complexes [16]. Concurrently reports have appeared in which inclusion within CD

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is claimed to stabilize important industrial free radical precursors such as benzoyl peroxide (BP), azoisobutyronitrile (AIBN), t-butyl peroxide (t-BuP) and cumene hydroperoxide (CHP) against thermal and photochemical decomposition [1, 2, 17–19].

Consideration of these two bodies of literature suggested that free radical reaction with CDs might be an important process in a number of the studies of reactions in CD, and might also produce the reported stabilization of peroxides in CD. We have studied the chemistry of BP, t-BuP and CHP in β-CD. Radicals formed from these compounds were chosen as being the most likely to react with CDs. If no reaction occurs between such radicals and CD, then this pathway is unlikely to be important for other reactions that occur in CD, through free-radical mechanisms. This study has focused particularly on the chemistry of BP which is known to photolytically and thermally undergo cleavage to form the benzoyloxy radical [20–22]. This species can then undergo further radical reactions.

2. Experimental

2.1. MATERIALS

Natural β-CD (American Maize Products Company) was recrystallized from aqueous solution. BP from Aldrich was recrystallized from chloroform and methanol. Benzoic acid, from Aldrich, was recrystallized from methanol. t-BuP and CHP were used as received, from Aldrich.

2.2. DIFFERENTIAL SCANNING CALORIMETRY (DSC) AND THERMOGRAVIMETRIC ANALYSIS (TGA)

DSC and TGA were conducted using a Dupont 951 Thermogravimetric Analyzer. Samples in each case were ramped up to 400 °C at variable ramp rates in the range of 1-10 °C/min. For DSC measurements, the apparatus was calibrated for temperature and enthalpy by melting high purity indium metal. Dried samples (3–9 mg), placed in capped aluminum pans, were then put into a DSC module, and purged with nitrogen. DSC runs were referenced to empty sample pans. TGA measurements were conducted in a similar fashion, except uncapped samples were placed in a TGA module and purged with nitrogen.

2.3. POWDER XRD

Powder XRD on the included and mechanical mixtures of BP and β-CD were done on a Phillips 3600 diffractometer using a K_α copper source.

2.4. NMR MEASUREMENTS

^{1}H NMR spectra were recorded on a 360 MHz Bruker NMR Spectrometer.

2.5. EPR MEASUREMENTS

EPR spectra were recorded on a hybrid EPR spectrometer consisting of a Varian E-109 bridge with an IBM ER 073, 10 in. magnet. Data was collected on a Macintosh II microcomputer. The CD complex was put in a 4 mm o.d. Suprasil sample tube which was evacuated to 50 mTorr pressure. The sample tube was then placed in a Varian 4531 rectangular cavity, fitted with an Oxford Products liquid helium cryogenics system. Irradiations were carried out *in situ* at 10 K, using an unfiltered 1000 W Hg/Xe arc lamp. Temperature dependence studies were carried out on samples which had been irradiated for a total of 30 minutes. Tests incorporating a thermometer in the sample tube confirmed that no significant temperature rise occurred due to photolysis. All spectra were run with 0.2 mW microwave power, 1 Gauss, 100 kHz modulation, 8 minute scans and 0.128 second time constant.

2.6. PREPARATION OF INCLUSION COMPOUNDS AND MECHANICAL MIXES

Known amounts of β-CD were dissolved in 200 mL of water. The solution was stirred rapidly and heated until the CD dissolved. After cooling, known amounts of t-BuP, CHP, and BP dissolved in diethyl ether were added to the solution. The resulting solutions were stirred rapidly for a minimum of 6 h. Formation of a milky white precipitate was observed. The precipitate was collected by filtration, dried in a vacuum desiccator containing P_2O_5, and found to be the desired complexes.

Mechanical mixes of BP and β-CD in a 1 : 1 molar ratio were made by adding the peroxide to the β-CD and grinding with a mortar and pestle. (Since BP is unstable, **extreme caution** is advised in such procedures.) Powder XRD was used initially to establish complex formation. The most apparent difference between the XRD of the mechanical mixture and the complex of BP with β-CD is the presence of additional reflections at $2\theta = 13°$ in the complex spectrum, which are absent for the mechanical mixture.

^{1}H NMR spectra of the complexes were run in deuterated dimethyl sulfoxide (DMSO-d_6) as both the CD and peroxide components of the complexes were soluble in this solvent. It was found that BP decomposed in DMSO-d_6, following near first order kinetics, to benzoic acid. Detectable decomposition took place within half a day. Similar behavior had been found by Pryor and Bickley [23], who found accelerated decomposition of BP in the presence of sulfides and disulfides. Accordingly, the DMSO solutions were observed immediately after the complexes were dissolved so that the amount of decomposition was not significant.

The stoichiometry of the complex formed was determined from the ratio of the peak areas for the ortho aromatic protons of the BP and the H-1 glucoside protons of the CD, and indicated that BP formed a 1 : 1 complex with β-CD when evaporated from diethyl ether.

Complexes of CHP and t-BuP included in β-CD were prepared similarly by adding the liquid peroxides directly to aqueous solutions of β-CD. The stoichiom-

etry of these complexes was determined in a similar manner to that described for BP and likewise found to be 1 : 1.

2.7. STUDIES OF β-CD DERIVATIVES

Two BP/β-CD complex samples were respectively thermolyzed at a temperature of 95 °C for 6 h and photolyzed for 18 h., after which the complexes were dissolved in water and repeatedly extracted with chloroform. The dried aqueous extract from each sample was then dissolved in HPLC grade methanol and analyzed by HPLC. NMR spectra of the dried organic and aqueous extracts dissolved in DMSO-d_6, with dioxin added as an internal standard for quantitative determination, were also collected.

2.8. HPLC STUDIES

HPLC was conducted on a liquid chromatographic system composed of a Shimadzu Model LC 10AD pump connected to a Rheodyne Model 2392 sample injector with a 10 mL sample loop, and a Shimadzu Model SPD 10A variable wavelength UV absorbance detector in line with a Varex Model ELSD IIA evaporative light scattering detector. The column used was a commercially obtained Millipore 10 μm C$_{18}$Radial-Pak cartridge pressurized in a Waters RCM (8 × 10) cartridge holder. All separations were done using a 10% methanol and 90% water eluent mixture. A range of varying sugar types at equimolar concentrations showed that the light scattering detector response was linear with molecular weight.

2.9. THERMAL AND PHOTOLYTIC DECOMPOSITION OF BP

Samples (0.06 g) of the dried complex, and an equivalent mechanical mixture were weighed into quartz cells (for photolysis) and glass test tubes (for thermolysis), and either photolyzed in a Rayonet irradiator at 360 nm or placed in an oil bath at 90 °C for varying lengths of time. The irradiated and thermolyzed samples were either dissolved directly into DMSO-d_6 for NMR analysis, or weighed amounts were dissolved in water and then extracted with five 20-mL portions of HPLC grade chloroform, to be later made up to 125 mL. It was determined by HPLC that this procedure was adequate to extract more than 99% of the BP and photoproducts.

Product formation was monitored by ^{1}H NMR by following the growth of the integrated *ortho* peaks of benzoic acid.

3. Results and Discussion

3.1. THERMAL ANALYSIS

Figure 1 shows the derivative TGA plots and superposed DSC scans for BP and β-CD uncomplexed (1A), in a physical mixture (1B) and complexed (1C). The TGA
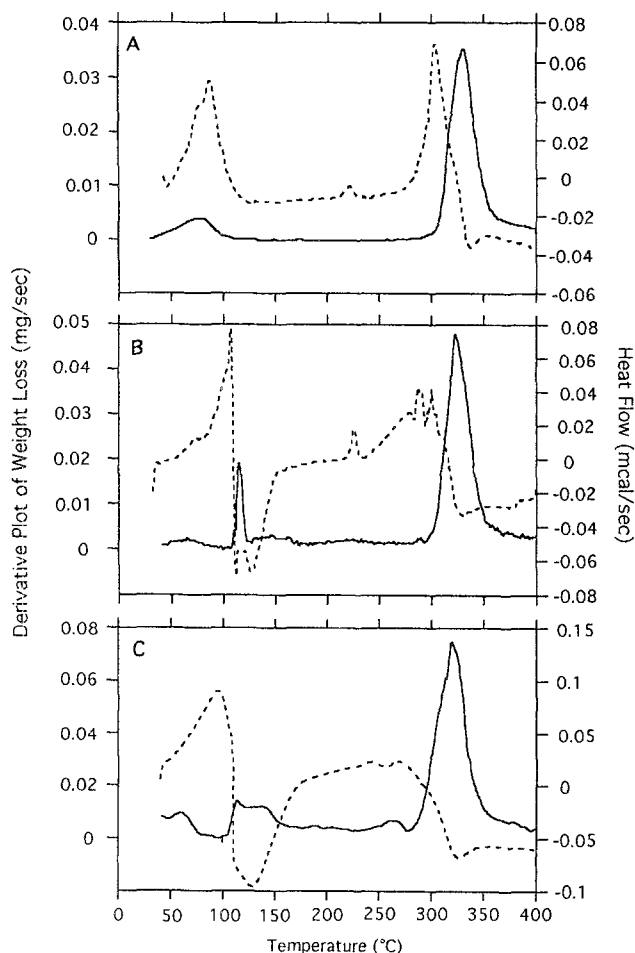
Figure 1. TGA (——) and DSC (- - -) curves for (A) recrystallized β-CD, and (B) BP physically mixed with β-CD (C) BP complexed with β-CD.

thermogram of β-CD alone (Figure 1A) shows two major decomposition peaks centered at 70 °C and 330 °C. These correspond to the loss of water of association and to the degradation of the CD. Both these features occur in two stages which are resolved by the observed minima of the corresponding peaks in the derivative form of the DSC. β-CD has 11 molecules of water of association. The six water molecules which reside in the cavity are lost below 64 °C and the other more strongly bound ones are liberated between 64–100 °C [24]. The DSC thermogram of β-CD also shows a peak at 210 °C which has been previously assigned to a reversible transformation of β-CD [24]. This peak is not found when the CD is complexed and thus structurally rigid. This can either be due to a conformation change in the ring itself or to changes in the packing of the CD.

The uncomplexed β-CD begins to decompose at 270 °C. The decomposition occurs in at least two stages, most likely in a similar manner to that of cellulose. Initially the polysaccharide undergoes depolymerization by *trans*-glycosylation to produce a variety of monosaccharide derivatives, to form a tar fraction. Water loss by dehydration of the sugar units and fission of the sugar units also proceeds concurrently to give a variety of unsaturated compounds [25].

When BP is physically mixed with β-CD (Figure 1B) additional exothermic and weight loss peaks are observed in the DSC and TGA thermograms, respectively, at 100 °C. These correspond to the decomposition of BP.

When BP is included into β-CD and compared to the physical mixture, four important differences become apparent. Firstly the endothermic peaks associated with water loss are markedly reduced in the inclusion complex. Secondly the reversible endothermic peak at 210 °C which is representative of the free β-CD is not present in the DSC. These observations are expected as the water molecules of inclusion are displaced with included peroxide, and the cyclodextrin rings become more structurally rigid. Thirdly the decomposition peaks of the peroxide, commencing at 100 °C, are considerably broader in the complex than in the mechanical mixture. The reason for this broadening will be discussed later in the context of stabilization studies.

The fourth important observation is the presence of an additional weight loss peak in the TGA at 250 °C, which coincides with a slightly endothermic process present in the DSC. This extra peak is not present in the mechanical mixture. This peak implies that decomposition of the included BP leads either to a more volatile dextrin derivative, or to a dextrin decomposition product with a lower decomposition temperature than that of β-CD.

Figures 2A and 2B show the derivative TGA plots and superposed DSC thermogram scans for cumene hydroperoxide and t-BuP, respectively. Comparison of the TGA and DSC thermograms for the peroxides shows three interesting observations. The TGAs for the two complexes show very little water of inclusion, indicating strong complexation, with a 'good fit' for inclusion. Some water of inclusion is expected to be present to act as spacer molecules to aid in the fit of the guest in the complex. Secondly the complexes show large endothermic peaks at 110 °C and 140 °C for CHP and t-BuP, respectively. These temperatures correspond to the decomposition temperatures of the peroxides. However, no corresponding weight loss is apparent in the TGA thermograms. The TGAs do however, show additional decomposition peaks at 250 °C and 220 °C for CHP and t-BuP, respectively. These results are consistent with the addition to the CD of some of the radicals produced by peroxide decomposition. The decomposition peaks at temperatures below that of β-CD are then attributed to these CD derivatives.

Studies on the decomposition of modified β-CD, whereby each of the free hydroxyls (—OH) in the sugar moiety have either been acetylated (—OCOMe) or methylated (—OMe), show increased decomposition temperatures, whilst replacement with phenylcarbamoyl groups (—OCONHPh) gives the onset of decompo-
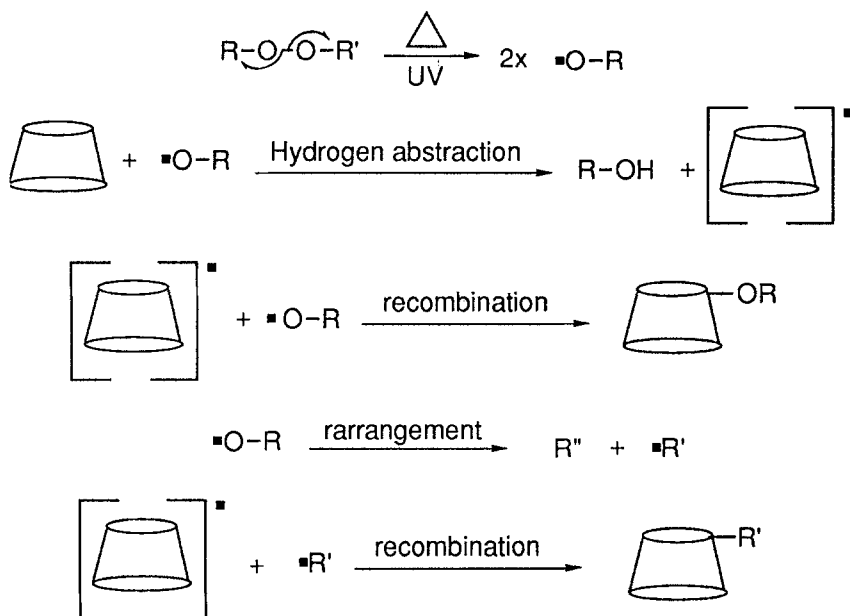
(A)

(B)

(C)
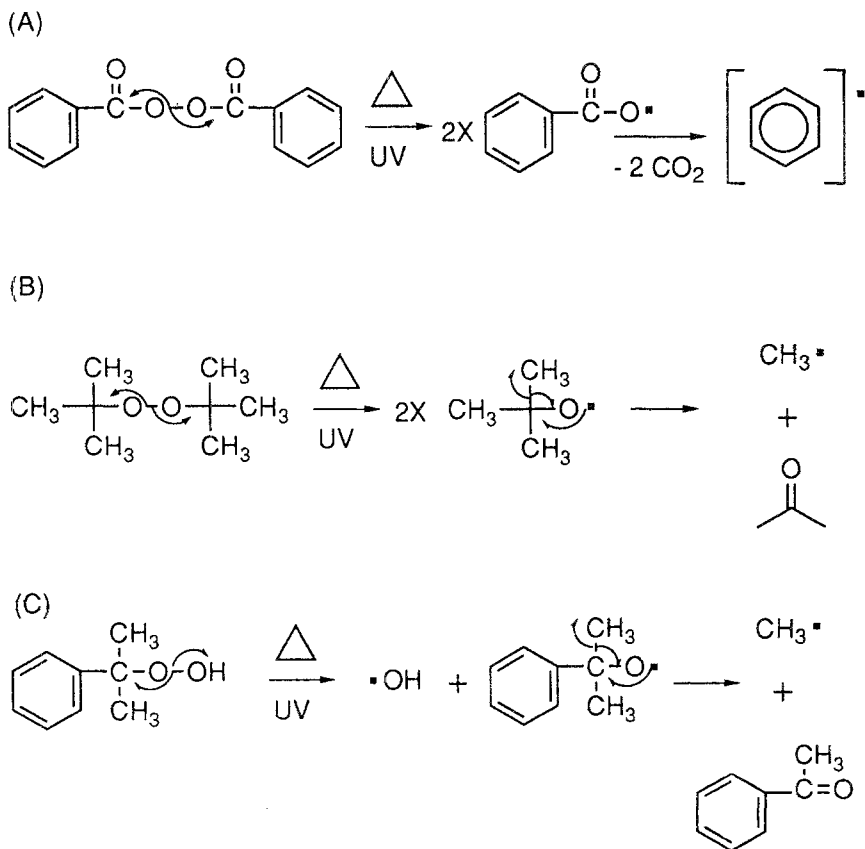
Scheme 1.

sition at lower temperature [24]. These observations suggest that the cyclodextrin may be derivatized by the benzoyloxyl radicals (PhCOO•) or phenyl radicals, that form from BP decomposition as shown in reaction Scheme 1A. Such addition of benzoyloxyl or phenyl groups to the CD would be expected to result from hydrogen abstraction to form a CD radical observed by EPR (*vide infra*), followed by radical recombination (Scheme 2). Similar derivatives may be formed between the recombination of CD radicals and oxyl radicals of CHP and *t*-BuP before these species, confined in the CD cavity, undergo rearrangement to yield methyl radicals.

For a 1:1 BP/β-CD complex, this mechanism predicts addition of only one aromatic group per CD, since one of the two radicals produced by peroxide cleavage is required to abstract hydrogen to form the CD radical.

3.2. EPR STUDIES

Figure 3 shows the EPR spectra from the 1:1 complex (Figure 3A) and the mechanical mixture (Figure 3B) of BP and β-CD, photoirradiated at 10 K, as a function of
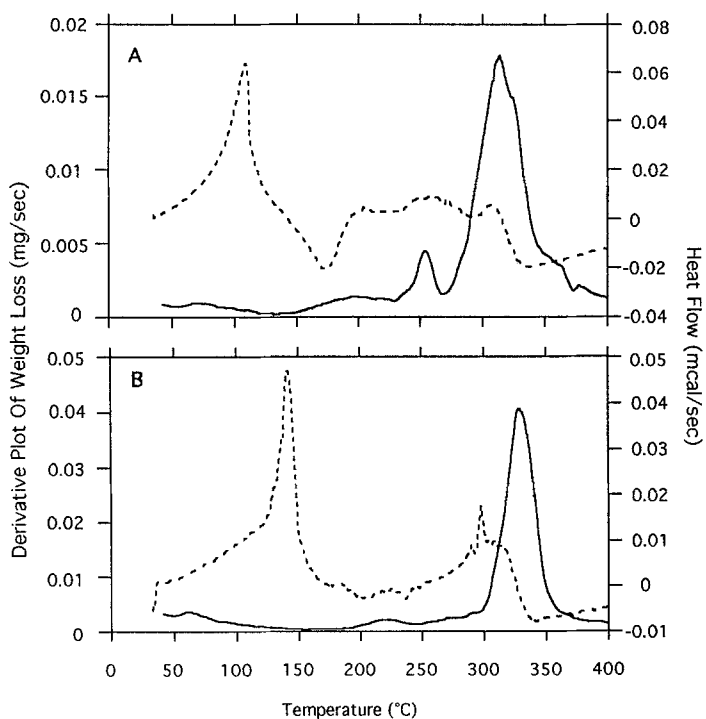
$$R-O-O-R' \xrightarrow[\text{UV}]{\triangle} 2x \ \bullet O-R$$

+ $\bullet O-R$ $\xrightarrow{\text{Hydrogen abstraction}}$ R–OH +

+ $\bullet O-R$ $\xrightarrow{\text{recombination}}$ ⌣–OR

$\bullet O-R$ $\xrightarrow{\text{rarrangement}}$ R" + $\bullet$R'

+ $\bullet$R' $\xrightarrow{\text{recombination}}$ ⌣–R'

Scheme 2.

Figure 2. TGA (—) and DSC (- - -) curves for (A) CHP complexed with β-CD, and (B) t-BuP complexed with β-CD.
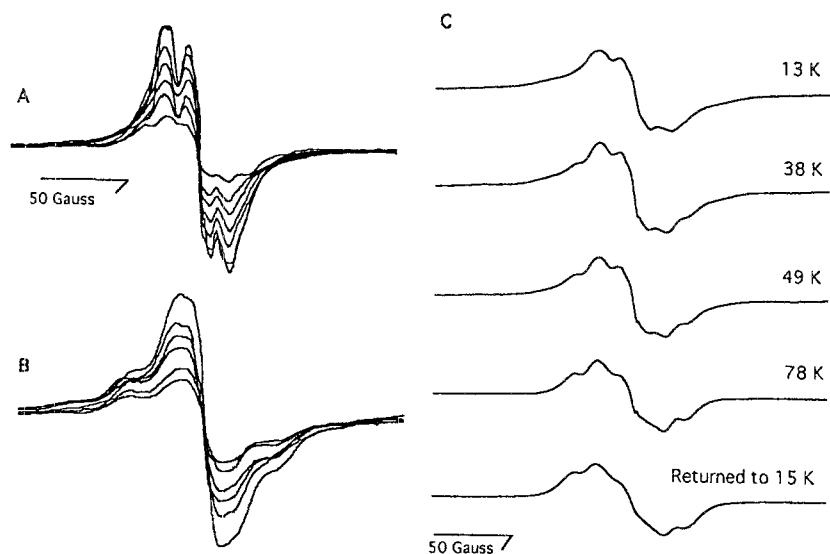
Figure 3. EPR spectrum from UV-irradiated BP in (A) complex, and (B) mechanical mix with β-CD. Spectra in each case are from the sample irradiated for 0.5, 2, 4, 8, and 16 min. with the signal growing monotonically. (C) Effect of temperature after 15 min. irradiation. The spectra were recorded on the same sample in order from top to bottom.

photoirradiation time. There are clearly significant differences in the spectra of the two samples. Samples of CD when photoirradiated do not show any EPR signals. If the radicals produced by photolysis are not freely rotating the EPR spectra will appear as a 'powder pattern'. In such spectra the anisotropic components are not averaged out, giving rise to a complex and ill-resolved EPR spectra, of the type observed. Figure 3C shows the effect of annealing the complex over a range of temperatures. It is apparent that the sample undergoes irreversible changes. However the signal is not sufficiently well resolved to make identification of the radical(s) feasible.

Other precursors were used in order to gain insight into the identity of the radical(s) formed. Figures 4A and 4B show the temperature dependence of the EPR spectra from β-CD complexes of CHP and t-BuP, photolyzed in the solid state. A 1 : 3 : 3 : 1 quartet with a 22.98 G coupling constant is present in both spectra. This spectrum can be identified as that of the methyl radical which is reported [26–28] to have a coupling constant between 22.6 and 22.9 G. The spectra of the methyl radical is well resolved because even at 22 K the methyl radical is rotating freely. Fitting of these spectra found that a Lorentzian lineshape fitted significantly better than a Gaussian lineshape. This again is consistent with relatively free rotation. Observation of isotropic EPR spectra from cyclohexadienyl radicals included in cyclodextrin at temperatures above 80 K have previously been reported by Baugh *et al*. [29]. Since the cyclohexadienyl radical is much larger than the methyl radical it is not surprising that the methyl radical is still freely rotating at 22 K.
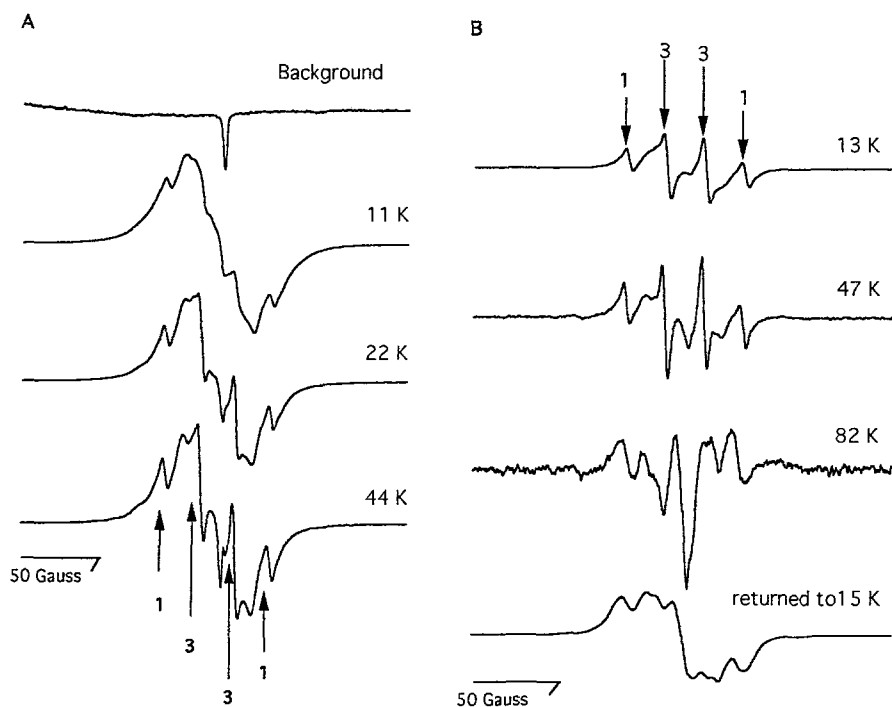
Figure 4. Temperature dependence of the EPR spectra obtained from UV-irradiation of (A) CHP, and (B) *t*-BuP complexed with β-CD, irradiated for 15 min. The spectra were recorded in order from top to bottom.

Both the *t*-BuP and the CHP show the presence of a broad component as well as the methyl spectrum. Double integration of the experimental spectrum and of the contribution from the methyl radicals showed that the methyl radical forms approximately 30% of the radicals detected from the *t*-BuP complex and only ca. 7% of the radicals detected from the CHP. This result at first appears counter-intuitive as the methyl signals are the most prominent feature of the spectra, but because EPR spectra are plotted as a first derivative, sharp peaks are emphasized over broad peaks. The methyl radicals observed are known to be formed in both cases [30–32] by rearrangement of the initially formed peroxy species, as shown in reaction Schemes 1b and 1c. The extent of formation was reported [30–32] to be solvent dependent, with methyl radicals being formed more readily in aqueous solutions. Observation of methyl radicals by EPR has previously been reported by Svedja and Volman [33] from irradiation of *t*-BuP at 77 K. As well as methyl radicals, these authors reported that two other signals were observed, attributed to *t*-butoxy radical and to methylene radicals.

When the temperature of the photoirradiated *t*-BuP complex is raised the methyl radical signal disappears, leaving only the broad EPR signal. Only slight changes in this broad signal resulted from the annealing at 80 K. The broad signal did

not match either of the signals observed by Svedja and Volman [33] and so is unlikely to be the EPR spectrum of either the t-butoxy radical or radicals produced by hydrogen abstraction from acetone. The broad spectrum is similar to those observed from the photoirradiated BP/β-CD complexes. Raising the temperature of the photoirradiated CHP complex to 44 K resulted in a growth of the methyl radical signal. This is presumably due to the activation barrier to intramolecular rearrangement being higher in the CHP complex than in the t-BuP complex.

The lower proportion of methyl radical signal observed in the case of the CHP is consistent with formation of a hydroxide radical and a methyl radical, rather than the two methyl radicals formed from t-BuP.

3.2.1. *Identity of the Broad Signal*

In the case of the CHP both hydroxide radicals and methyl radicals are expected to be formed (reaction Scheme 1c). The only likely reactions of both methyl radicals and hydroxide radicals are hydrogen abstraction and radical recombination. Likewise in the case of t-BuP we observe significant formation of radicals other than methyl or t-butoxy. Since these signals are not consistent with those of methylene radicals derived from acetone the only remaining possibility is that they are formed by hydrogen abstraction from the CD. The broad signal observed in the case of the CHP is not consistent with the spectra expected for the hydroxide radical which, given its small size, would be expected to be isotropic. Presumably the hydroxide radicals produced are both more mobile and more reactive than the methyl radicals and so even at 10 K they abstract hydrogen immediately, giving rise to a broad CD signal.

Decomposition of BP occurs through a number of steps (reaction Scheme 1a) forming a number of different radical pairs in the crystalline state. In the case of the polycrystalline complexes studied it is uncertain whether well defined radical pairs are formed. Benzoyloxy radicals are expected to have large anisotropy in g-factor, resulting in broad EPR lines. It is possible that some part of the broad signal we are observing may be due to benzoyloxy radicals in CD; however we believe that the weight of evidence supports identification of the broad EPR signal observed as being due to CD radicals.

To the best of our knowledge there has not been any previous report of EPR spectra from CD radicals in the solid state which we can use to determine which radicals are formed. The observation of CD radicals in solution by EPR has been reported by Komiya *et al.* [34]. They report observation of a species with $g = 2.0038$, and coupling constants of 11.76 G (2H) and 5.49 G (1H) which they assign to a C-5 CD radical. More recent work by Gilbert *et al.* [35] gives the g factor of the C-5 D-glucose radical as 2.0031 with coupling constants of 33.3 G (1H), 9.9 G (1H) and 7.1 G (1H). Madden and Fessenden have reported [36] that the C5 radical in α-methyl-D-glucopyranoside has a g-factor of 2.0038 and coupling constants of 26.8 G (2H), 18.7 G(1H) and 1.8 G (1H). Although there is clearly a discrepancy
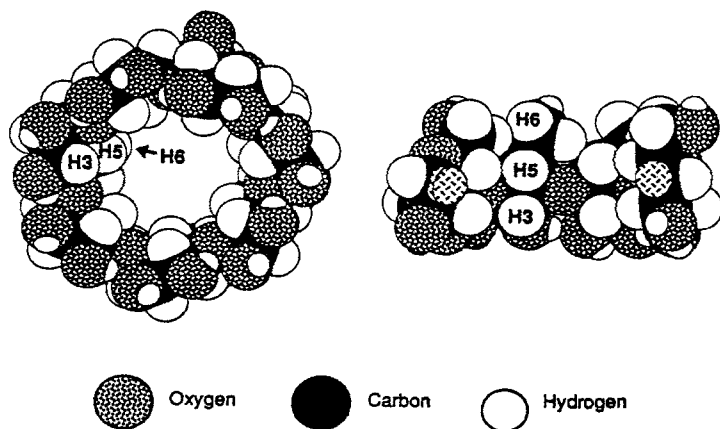
Figure 5. 'Space filling models' of β-CD showing the positions of the C-3, C-5 and C-6 hydrogens. Left is a view from the primary hydroxyl side. A cross-sectional view showing the inside of the cavity is displayed on the right.

between these and later reports, the signal-to-noise ratios are substantially better. We have been unable to find any set of reported g-factor and coupling constants which match the reported CD coupling constants. It is possible that the constrained conformation of the CD substantially alters the coupling constants. However it appears to us more likely that the EPR peaks assigned to a single CD radical result from a number of different radicals. The TiO_2/H_2O_2 oxidation system used has generally given rise to a variety of sugar radicals [35, 37, 38].

In the solid state, bombardment of single crystals of various glucopyranosides with X-rays produces stable alkoxy radicals at O-6 and O-2 of the glucose ring, as well as a deprotonated hydroxyalkyl radical at C-6, and a radical due to hydrogen abstraction at C-5 [39–42]. In the complexes, however, steric factors would be expected to control which radicals are formed by H-abstraction. If the peroxides are assumed to have an extended structure then the free radicals are produced inside the CD cavity. It is then likely that the hydrogens inside the cavity will be abstracted. As can be seen from Figure 5, the H-5, H-6 and H-3 protons would be the most likely to be abstracted. The formation of these carbon centered cyclodextrin radicals is also consistent with preliminary molecular orbital calculations, which indicate that carbon-centered CD radicals are thermodynamically more stable [43] than oxygen-centered radicals. Because a number of different radicals could be formed, all of which will have different EPR spectra, we would expect some variation between the spectra observed from the different complexes, as we indeed observe.

3.3. STABILIZATION STUDIES

A recent patent by Osa *et al.* [17] reports that the inclusion of peroxides into β-CD offers a means of stabilizing the peroxides thermally and photolytically. No suggestion as to the mechanism of stabilization has been reported. The DSC and TGA thermograms of BP complexed with β-CD show much broader peaks for the peroxide decomposition than the mechanical mixture. One can envisage two possible stabilization mechanisms resulting from inclusion in the CD: the CD can either impose thermodynamic or kinetic stability. Since the time taken for weight loss to occur at any given temperature ramp is indicative of the rate of the reaction occurring, the broader decomposition peak in the complex can be explained by the fact that inclusion inhibits the rate of peroxide decomposition in the solid phase. However, since the temperature at which BP decomposes, in complex form or mechanically mixed with β-CD, is not shifted from that expected for free BP, the stability resulting from inclusion must be due to slowing of radical chain reactions which propagate peroxide decomposition.

The CD molecule can act to inhibit peroxide chain reactions in two ways. It could slow down peroxide cleavage by creating a contained environment, such that greater radical recombination occurs, effectively decreasing the rate of radical formation. Alternatively, the CD host may itself react with the primary radicals to form less reactive secondary radicals and derivatives, thus quenching the reaction.

To investigate the effects of inclusion on the kinetic parameters of thermal decomposition of peroxides, a TGA multiheating rate method [44] was employed to determine activation energies and frequency factors for BP complexed, and mechanically mixed, with β-CD. This procedure involves recording the decomposition over a variety of temperature ramp rates and determining the shift in the temperature at which a particular weight loss occurs. This shift can be used to determine the activation energy and frequency factor for the reaction. The method used in this investigation is applicable only to first order kinetic reactions such as the initiation of BP decomposition. It has been used successfully to predict polymer lifetimes [44], and the activation energy and reaction order of water loss from β-CD [45].

The decomposition temperature for the peroxide in the included complex and for the mechanical mix, was monitored at 11% weight loss. At this decomposition point weight loss due to water liberation is minimal, but the extent of peroxide decomposition is still low. The activation energy for decomposition of BP in the included complex was found to be qualitatively around 10% higher than that in the mechanical mix. This increase can be thought of as the extra energy input required to break the peroxide bond and allow the benzoyloxyl radicals to move out of the cyclodextrin cavity. Higher internal energy must be possessed by a complexed BP than a free peroxide molecule if it is to cleave and overcome recombination. This represents one method by which cyclodextrins stabilize energetic materials, but
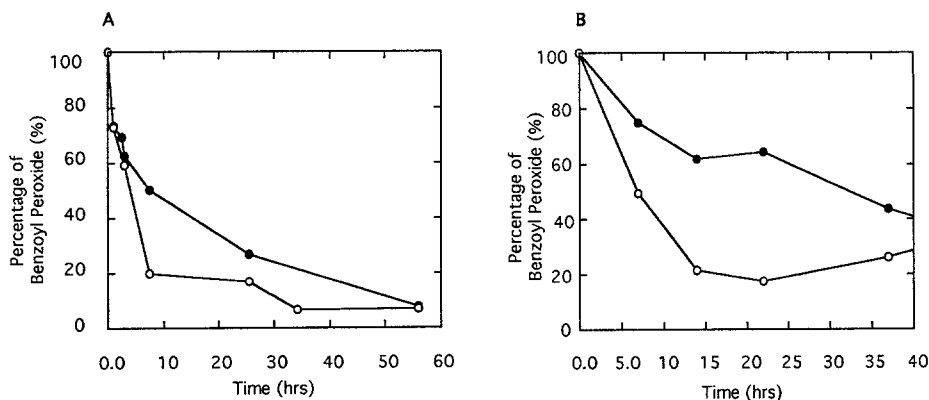
Figure 6. Percentage of BP remaining after (A) thermolysis at 90 °C, and (B) photolysis of a complex of BP and β-CD (●), and a mechanical mix (○).

is likely to be a smaller factor than the inhibition of chain reactions produced by the reaction of the radicals with cyclodextrin.

3.4. PRODUCT ANALYSIS

To determine whether the products of decomposition are altered by inclusion into β-CD, and to what extent, complexes and physical mixtures of BP and β-CD in the solid state, were photolyzed and thermolyzed for varying lengths of time.

After 45 h UV irradiation at 40 °C the complex had turned a pale yellow color, while the simultaneously irradiated mechanical mixture turned an intense yellow color. Control samples kept in aluminum foil showed no color change. Therefore the color change is the result of a photochemical and not a thermal reaction. Similar color changes were observed in samples irradiated at 77 K and 10 K. Likewise, mechanical mixture samples thermolyzed at 90 °C displayed a more dramatic color change than samples of the BP/β-CD complex.

The fact that complexation significantly decreases the rate at which BP decomposes can be seen by the lower conversions of BP during thermolysis and photolysis of the complex, as compared to thermolysis of the mechanical mixture (Figure 6). Although conversions are lower, thermolysis of the complex shows higher yields of benzoic acid (37.8% at 90 °C) than for the mechanical mixture (22.6% at 90 °C) or for BP alone (7.3% at 70 °C). This is consistent with hydrogen abstraction being a major reaction pathway for the decomposition of BP included in β-CD. The extent of benzoic acid formation also offers an explanation for the reported increase in stability of BP reported in CD. Decomposition of BP is generally believed to occur through a chain reaction. Hydrogen abstraction therefore results in chain termination since the cyclodextrin radicals are expected to be much more stable than the other intermediate radicals.
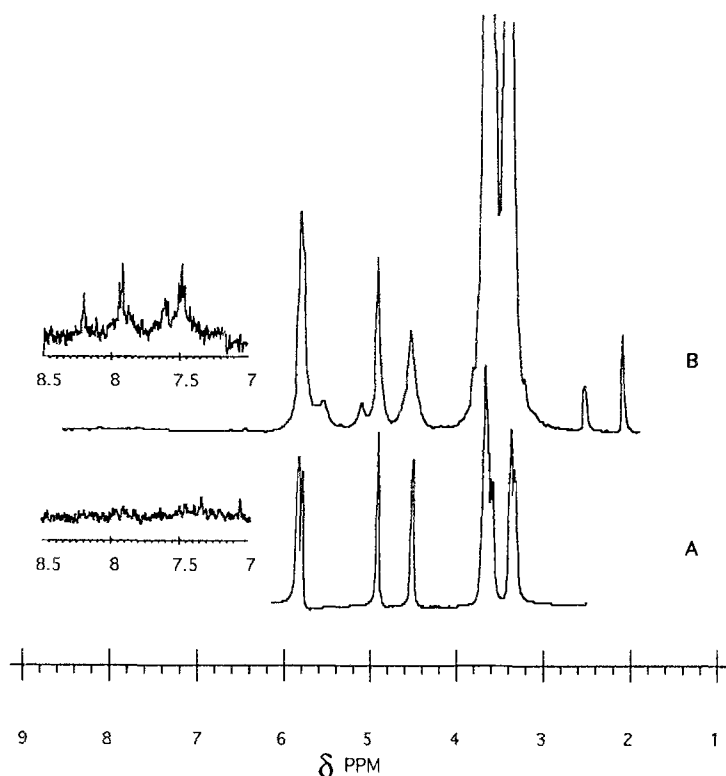
Figure 7. NMR of (A) β-CD, and (B) the thermolysis products of BP/β-CD complex obtained in the aqueous portion of a water/chloroform extraction. Inset is the aromatic region which has been magnified by × 10.

Evidence for the formation of CD derivatives is found in Figure 7, which shows the ^{1}HNMR spectra of β-CD (Figure 7A), and of the dried aqueous extract of a BP/β-CD complex thermolyzed for 24 h at 95 °C (Figure 7B). The assignment of the ^{1}H peaks for CD is well known [46]. The spectrum of the complex shows significant differences from that of β-CD. As well as the growth of additional peaks in the sugar part of the spectrum, which indicates the formation of other sugar products, the presence of aromatic protons in the spectra shows that addition of phenyl groups to the sugars has occurred. Any aromatic material not covalently bound to sugar molecules would have been extracted into the organic phase. More importantly, these peaks are not observed for the products of reaction in the mechanical mix analogs, indicating that these products are only formed when the BP is included. Integration of the aromatic portion of the NMR spectrum, and assuming incorporation of only one phenyl group per CD, showed that the extent of addition was approximately 15%.

If it is assumed that the mass loss, observed in the BP/β-CD complex TGA at 270 °C is predominantly due to loss of the added aromatic group, and that the mass
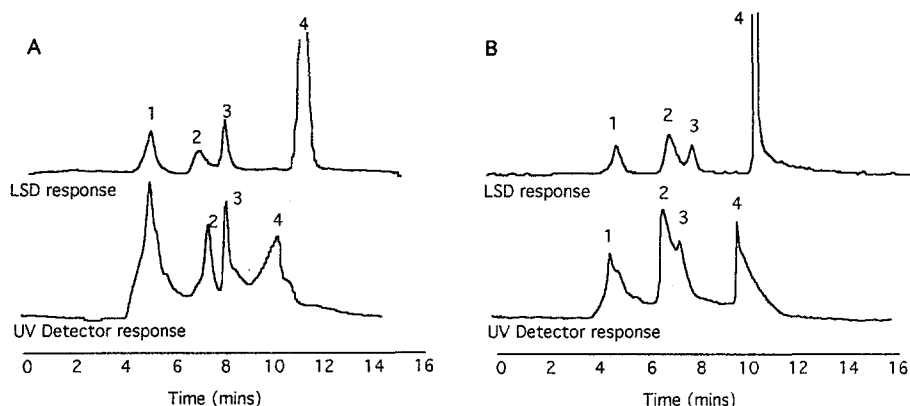
Figure 8. HPLC chromatographs of thermolyzed and extracted complexes of (A) BP/β-CD, and (B) CHP/β-CD.

loss at 300 °C is due to CD decomposition, it can be estimated that 20% of CD molecules have aromatic addition. This compares favorably with the NMR results and HPLC results (following).

3.5. HPLC OF THE WATER SOLUBLE FRACTION

Samples of BP/β-CD, CHP/β-CD and *t*-BuP/β-CD were thermolyzed for 12 h and the organic and sugar products were separated by dissolving the complex in water and then extracting with chloroform. The water soluble portion was dried, dissolved in methanol, and then injected onto the HPLC column. The chromatographs from BP/β-CD and CHP/β-CD using both the light scattering detector (LSD) and a UV detector set at 254 nm are shown in (Figure 8). The LSD gives a linear response for mono- and polysaccharides and so gives quantitative information. Four peaks are resolved in each case, although there is evidence for a number of unresolved peaks.

   Based on retention time, peak 4 was identified as unreacted β-CD, while peaks 1, 2 and 3 are products of reaction. This is confirmed as these peaks grow with increasing thermolysis time. From comparison of the relative concentrations of the product peaks detected by LSD and that detected by UV, peaks 1, 2 and 3 all represent species which have chromophores with extinction coefficients much greater than that of the alchoholic groups of non-derivatized saccharides. Furthermore these chromophores all have absorption maxima around 250 nm, which is indicative of aromatic functionality. This is conclusive evidence that during thermolysis the BP and CHP have reacted with the CD to yield CD derivatives. The extent of formation of water soluble CD derivatives is 20–30%, in good agreement with the NMR and TGA results.

When a mechanical mixture of BP and β-CD is prepared in the same manner as the complex, only peaks 2, 3, and 4, are observed, in somewhat smaller yields. Thus, peak 1 of both the BP/β-CD and CHP/β-CD complex must be a saccharride product(s) of reaction, which is only produced during thermolysis of the complexed peroxide. The retention times of peaks 1, 2 and 3 derived from both the BP/β-CD and CHP/β-CD complexes have almost identical retention times, indicating that the product(s) formed from thermolysis of the respective complexes have a very similar physical nature in terms of how they are separated chromotographically. The retention time of peak 3 for both the BP and CHP complex products was comparable to that for a range of linear saccharides: D-maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose, that were run as standards, and whose retention times were all identical. This strongly suggests that peak 3 results from a ring-opened CD. The presence of significant absorbance at 254 nm suggests that this ring opening may be accompanied by addition of an aromatic ring.

Generally C_{18} columns have not been used in a reversed phase mode with monosaccharides as they have very little interaction with them. However this column can be used to separate oligosaccharides such as maltodextrins and CDs, principally because of host–guest interactions, and by size exclusion [47]. In such instances the longer the retention time the greater the host–guest interactions; conversely, shorter retention times indicate less host–guest interaction. This suggests that peak 1, the retention time of which is much less than that of β-CD or any linear sugar, may result from a species such as a reacted CD molecule whose internal cavity had been blocked with a rigid functional group. This suggestion is consistent with the absence of peak 1 from the mechanical mixtures. (It might be suggested that some of the peaks observed might be due to organic impurities. However our extraction tests showed better than 99% separation.)

That significant amounts of aromatic CD products were formed from the mechanical mixtures is at first sight surprising. However the grinding process we use produces finely divided particles, so that the peroxide and CD are in intimate contact over a large surface. The rate of reaction of the peroxides is higher in the mechanical mixture than in the complex so the amount of products will also be proportionately higher.

HPLC of the water soluble fraction of the products from the thermolysis of t-BuP/β-CD complex showed only the peak for unreacted β-CD. This is surprising given the expectation of reactions similar to those of BP and CHP. However it is likely that if the β-CD was derivatized by methyl radicals produced from t-BuP decomposition, the product CDs would not have very large differences in size and hence might not be separable from β-CD under the conditions used. If peak 3 is due to linear sugars then the absence of this peak in the t-BuP chromatographs indicates that t-BuP is not causing significant opening of the CD ring.

4. Conclusions

We have formed 1 : 1 inclusion complexes between β-CD and BP, t-BuP, and CHP. The thermal and photochemistry of these complexes is summarized in Scheme 2, and consists of cleavage of the peroxide bond to form peroxyl radicals, rearrangement (in the case of the t-BuP and CHP), followed by abstraction of hydrogen from the cyclodextrin to form a cyclodextrin radical. This cyclodextrin radical can then undergo radical recombination to give the compounds observed in TGA, NMR and HPLC. It seems likely that the cyclodextrin radical also undergoes rearrangements typical of sugar radicals giving ring-opening and other products.

The formation of cyclodextrin radicals is important as it explains some of the stabilization reported to occur when thermally or photolytically unstable compounds form cyclodextrin inclusion complexes. This stabilization occurs because cyclodextrins trap the intermediate radicals involved in chain reactions, thereby slowing the chain reaction. This may also be the mechanism of stabilization in many other systems such as benoxaprofen/β-CD, where stabilization against photodecomposition is observed [4] and radicals are formed as reaction intermediates. Although the stability of the radical precursors used in this study was by design less than that of other studies, their reactivity was comparable to radicals present in other studies. It is therefore clear from our results that such free radical–CD reactions must be considered in analyzing the results of a number of other studies reported in the literature.

Acknowledgements

References

1. Y. Matsui, H. Naruse, K. Mochida and Y. Date: *Bull. Chem. Soc. Jpn.* **43**, 1910 (1970).
2. Y. Matsui, H. Naruse, K. Mochida and Y. Date: *Bull. Chem. Soc. Jpn.* **43**, 1909 (1970).
3. J. Szejtli, in: *Inclusion Compounds* (Eds. J.L. Atwood, J.E.D. Davies and D.D. MacNicol), p. 331. Academic Press (1984).
4. T. Hoshino, K. Ishida, T. Irie, F. Hirayama and K. Uekama: *J. Incl. Phenom.* **6**, 415 (1988).
5. V. Ramamurthy, in: *Photochemistry in Organized and Constrained Media* (Ed. V. Ramamurthy), p. 303. VCH Publishers Inc. (1991).
6. V. Ramamurthy and D.F. Eaton: *Acc. Chem. Res.* **21**, 300 (1988).
7. C.J. Abelt, J.S. Lokey and S.H. Smith: *Carbohydr. Res.* **192**, 119 (1989).
8. C.A. Chesta and D.G. Whitten: *J. Am. Chem. Soc.* **114**, 2188 (1992).
9. Y.L. Chow, J. Michon, P. Michon, C. Morat and A. Rassat: *Tetrahedron Lett.* **33**, 3315 (1992).
10. S. Monti, L. Flamigni, A. Martelli and P. Bortolus: *J. Phys. Chem.* **92**, 4447 (1988).
11. K. Pitchumani, M.C.D. Manickam and C. Srinivasan: *Tetrahedron Lett.* **32**, 2975 (1991).
12. V.P. Rao, M.B. Zimmt and N.J. Turro: *J. Photochem. Photobiol. A: Chem.* **60**, 335 (1991).

13. G.D. Reddy, G. Usha, K.V. Ramanathan and V. Ramamurthy: *J. Org. Chem.* **51**, 3085 (1986).
14. A.M. Aquino, C.J. Abelt, K.L. Berger, C.M. Darragh, S.E. Kelley and M.V. Cossette: *J. Am. Chem. Soc.* **112**, 5819 (1990).
15. K.L. Berger, A.L. Nemecek and C.J. Abelt: *J. Org. Chem.* **56**, 3514 (1991).
16. K.I. Priyadarsini, H. Mohan, J.P. Mittal, D.M. Guldi and K.-D. Asmus: *J. Phys. Chem.* **98**, 9565 (1994).
17. T. Osa, A. Ueno, A. Oskakada and M. Nakoji: *E. Patent Appl.* 0411951A1, (1991).
18. I. Tabushi, in: *Inclusion Compounds* (Eds. J.L. Atwood, J.E.D. Davies and D.D. MacNicol), p. 445. Academic Press (1984).
19. H. Taguchi, N. Kunieda and M. Kinoshita: *Makromol. Chem.* **184**, 925 (1983).
20. G. Yamauchi and N. Hirota: *J. Am. Chem. Soc.* **107**, 5021 (1985).
21. H. Misawa, K. Sawabe and S. Takahara: *J. Chem. Soc. Chem. Commun.* 357 (1988).
22. K. Nozaki and P.D. Bartlett: *J. Am. Chem. Soc.* **68**, 1686 (1946).
23. W.A. Pryor and H.T. Bickley: *J. Org Chem.* **37**, 2885 (1972).
24. S. Kohata, K. Jyodoi and A. Ohyoshi: *Thermochim. Acta* **217**, 187 (1993).
25. F. Shaizadeh and W.F. Degroot, in: *Thermal Uses and Properties of Carbohydrates and Lignins* (Ed. F. Shaizadeh, K.V. Sarkanen and D.A. Tillman), p. 1. Academic Press (1976)
26. I.A. Zlochower, W.R. Miller and G.K. Fraenkel: *J. Chem. Phys.* **42**, 3339 (1965).
27. H. Fischer and H. Hefter: *Z. Naturforsch* **23a**, 1763 (1968).
28. A.L.J. Beckwith and R.O.C. Norman: *J. Chem. Soc. (B)* 400 (1969).
29. P.J. Baugh, J.I. Goodall and J. Bardsley: *J. Chem. Soc. Perkin Trans. 2,* 700 (1977).
30. M.J. Davies and T.F. Slater: *Biochem. J.* **245**, 167 (1987).
31. M.J. Davies and T.F. Slater: *Biochem. J.* **240**, 789 (1986).
32. W. Bors, D. Tait, C. Michel, M. Saran and M. Erben-Russ: *Isr. J. Chem.* **24**, 17 (1984).
33. J. Svejda and D.H. Volman: *J. Phys. Chem.* **73**, 4417 (1969).
34. T. Komiya, T. Yamada and S. Nara: *Agric. Biol. Chem.* **40**, 1917 (1976).
35. B.C. Gilbert, D.M. King and C.B. Thomas: *J. Chem. Soc. Perkin Trans. 2,* 1186 (1981).
36. K.P. Madden and R.W. Fessenden: *J. Am. Chem. Soc.* **104**, 2578 (1982).
37. B.C. Gilbert, D.M. King and C.B. Thomas: *J. Chem. Soc. Perkin Trans. 2,* 675 (1983).
38. B.C. Gilbert, D.M. King and C.B. Thomas: *Carbohydr. Res.* **125**, 217 (1984).
39. K.P. Madden and W.A. Bernhard: *J. Phys. Chem.* **83**, 2643 (1979).
40. K.P. Madden and W.A. Bernhard: *J. Phys. Chem.* **84**, 1712 (1980).
41. K.P. Madden and W.A. Bernhard: *J. Chem. Phys.* **72**, 31 (1980).
42. K.P. Madden and W.A. Bernhard: *J. Phys. Chem.* **86**, 4033 (1982).
43. K. Phelps, M.N. Lehmann and M.G. Bakker: Unpublished results (1995).
44. L. Krizanovsky and V. Mentlik: *J. Therm. Anal.* **13**, 571 (1978).
45. A. Szafranek: *J. Therm. Anal.* **34**, 917 (1988).
46. S. Bulusu, T. Axenrod, B. Liang, Y. He and L. Yuan: *Magn. Reson. Chem.* **29**, 1018 (1991).
47. G.D. Mcginnis, S. Prince and J. Lowrimore: *J. Carbohydr. Chem.* **5**, 83 (1986).