Radical Reactions of Carbohydrates. Part 3.[1,2] An Electron Spin Resonance Investigation of Base-catalysed Rearrangements of Radicals derived from D-Glucose and Related Compounds

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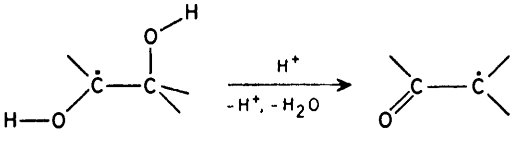
E.s.r. observations have been made on the rearrangements of radicals formed from the reaction between ·OH and a series of simple sugars at pH > 7. Two types of semidione have been identified [for example D-glucose gives geometric isomers of both $HOCH_2{\cdot}CHOH{\cdot}CHOH{\cdot}CHOH{\cdot}C(O{\cdot})=C(O^-)H$ and $HOCH_2{\cdot}C(O{\cdot})=C(O^-)H$]; their formation provides two routes to radical-induced base-catalysed degradation of carbohydrates, involving ring-opening at the O–C(1) bond and, in the second case, C–C cleavage. We suggest that the latter reaction involves a Grob-type fragmentation and that this may account for the formation of malondialdehyde in these systems.

IT has previously been shown that when the hydroxyl radical (generated from the radiomimetic Ti^{III}–H_2O_2 couple in an aqueous flow system [3]) is allowed to react with some relatively simple carbohydrates (*e.g.* myoinositol [1] and D-glucose [2,4]) in the cavity of an e.s.r. spectrometer all possible radicals which can result from C–H abstraction are detected. The lack of selectivity in these reactions is in marked contrast to the stereoselectivity exhibited in the subsequent acid-catalysed rearrangement [reaction (1)] of some of the first-formed radicals; this proceeds much more readily when the departing β-hydroxy-group occupies an axial position, eclipsing the orbital of the unpaired electron.[1,2]

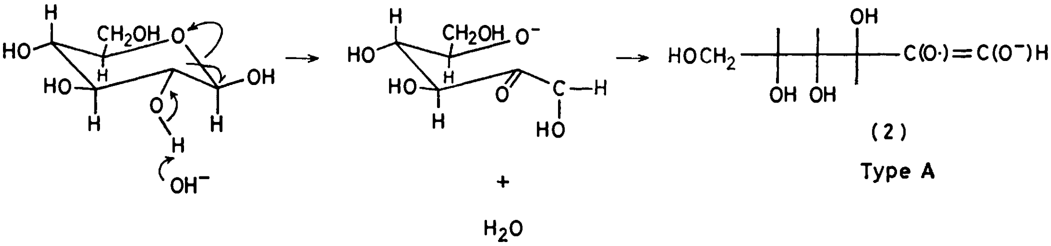


(1)

(1)

The analogous base-catalysed rearrangements of αβ-dihydroxyalkyl radicals are known to occur [5] and mechanistically similar reactions have been proposed [4] for radicals formed from D-glucose and related substances at high pH. Although signals from the rearranged carbonyl-conjugated radicals (1) were not detected in this study [4] (these radicals are known [6] to be readily reduced by Ti^{III}–EDTA complexes) the spectra of two types of semidione (1,2-diketone radical-anion) were observed. These semidiones, hereinafter referred to as Types A and B, were proposed to result from a ring-opening process analogous to reaction (1), for example the fragmentation of the C(2) abstraction radical as shown in reaction (2) for β-D-glucose, and the retro-aldol sequence illustrated by reaction (3).

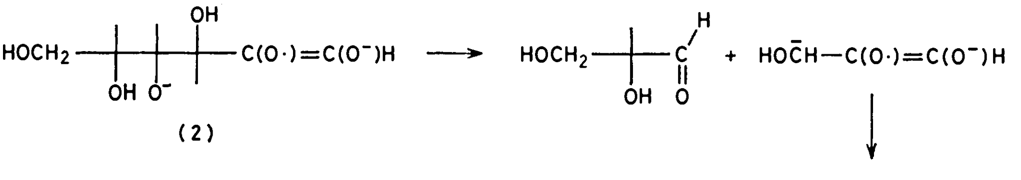
As a continuation of our investigations into the selectivity of ·OH in its reactions with carbohydrates and into possible radical routes for the degradation of carbohydrates, we have extended our earlier studies [1,2] to the pH region 5—10. We obtained preliminary evidence to suggest that Type B radicals do not derive solely (if at all) *via* reaction (3); for example, our results indicate that as the pH is raised, the concentration of (3) in-



(2)

(2)

Type A



$HOCH_2C(O{\cdot})=C(O^-)H$ (3)

(2)

(3)

Type B

creases, but *not* at the expense of that of (**2**). With this in mind we have paid particular attention to both the structural assignments and mechanistic interpretations previously proposed,[4] and have considered other means by which semidiones may result (for example, it has also previously been shown [7] that αβ-dihydroxyalkyl radicals may be oxidised to semidiones by hydrogen peroxide at high pH).

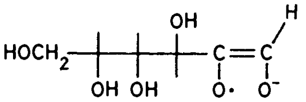
RESULTS AND DISCUSSION

E.s.r. spectra were generally obtained by mixing in a three-way flow system (with a dead-time of *ca.* 60 ms) solutions containing the carbohydrate (*ca.* 0.035 mol dm^{-3}), hydrogen peroxide, and titanium(III) ions, the last solution containing the disodium salt of ethylenediaminetetra-acetic acid as a sequestering agent; ammonia was also added to this stream so that the mixed (effluent) stream had the required pH (typically in the range 5—10). In some experiments in which the presence of metal ions was to be avoided, dilute aqueous solutions containing hydrogen peroxide and a carbohydrate were subjected to direct u.v.-irradiation as they flowed slowly through the e.s.r. cavity.

The Structure and Formation of Type A Semidiones.—When the pH was raised from *ca.* 4 during the oxidation of D-glucose with the Ti^{III}–H_2O_2 couple, the spectra of all radicals formed by C–H abstraction were reduced in intensity and virtually removed by pH *ca.* 7. Semidione radicals first became detectable at pH *ca.* 6; radicals of this type would, when formed, be expected to give relatively prominent e.s.r. spectra on account of the slower rates of their dimerization and reduction (a result of their overall negative charge) [8] than the corresponding reactions of simple carbonyl-conjugated radicals produced by reaction (1).

From a commercial sample of D-glucose (a mixture of the α- and β-anomers) the spectrum detected at pH *ca.* 7 appeared to be a simple doublet of doublets, but under conditions of higher resolution could be seen to comprise almost overlapping resonances from two radicals with closely similar parameters (see Table 1). We agree with the earlier assertion [4] that these are from the *cis*- and *trans*-isomers of a semidione. The distinction between *cis*- and *trans*-forms is made on the basis of adding Mg^{2+} to the system, since this is known [9] to lead to the formation of Mg^{2+} chelates of the *cis*, but not the *trans*, semidiones. As expected, the spectrum of one of the semidiones [assigned the *cis*-structure (4a)] was removed and replaced by another similar signal [with *a*(1H) 0.955, *a*(1H) 0.675 mT, *g* 2.0046] assigned to the appropriate magnesium complex. Evidence for the assignment of the structure of the side-chain in the *cis*- and *trans*-isomers, (4a) and (4b), respectively, derives from the behaviour of other substrates (see later).

In order to probe further the mechanistic implications of these findings, we studied separately the behaviour of the individual anomers α- and β-D-glucose (using freshly prepared ice-cooled solutions to inhibit mutarotation). Two striking differences between the behaviour of the two anomers were observed. First, whereas reaction of α-glucose yielded both (4a) *and* (4b), with the former in slight excess, reaction of β-glucose led to the detection solely of the *cis*-isomer (4a). Secondly, the concentration of semidione (4a) derived from β-glucose was considerably greater than the total concentration of (4a) and (4b) from α-glucose under otherwise identical conditions. We also established that, for these and other substrates (see later), Type A semidiones are formed by rapid transformations involving first-formed radicals; that they are not derived from subsequent oxidation by •OH of molecular products formed from these radicals is indicated by the finding that the intensity of the signals was reduced for slower flow rates.


(4a)

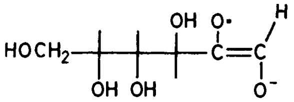

(4b)

Table 1 contains details of the Type A semidiones obtained from other substrates at pH 7. For some compounds two resolvable signals were detected; assignment to individual isomers (*cis* and *trans*) was then made on the basis of observations made when Mg^{2+} was added to the system. In other cases only a single spectrum (which may of course comprise two overlapping signals from different isomers) was detected; although in most of these examples the addition of Mg^{2+} led to the diminution of the signal (suggesting that some *cis*-isomer has thus been complexed) the observations could not be interpreted unambiguously.

α-D-Mannose reacted to give the same radicals, (4a) and (4b), and in the same ratio, as its epimer α-D-glucose [as would be expected if reaction (2) represents the appropriate pathway]. Two similar radicals (5a) and (5b), but with the *cis*-isomer predominating, were also detected from D-glucuronic acid (present as a mixture of α- and β-forms). With a mixture of the anomers of D-galactose the width of the peaks suggested that the two isomers (6a) and (6b) are formed (and the addition of Mg^{2+} led to reduced signal intensity but not to the appearance of any further signals); no distinction between these two can be made. D-Galacturonic acid behaved similarly (see Table 1). For the deoxyhexose α-L-rhamnose the signal comprised that of the *cis*-isomer (8a) [with Mg^{2+} the appropriate chelate had *a*(1 H) 0.940, *a*(1 H) 0.570 mT, *g* 2.0046], together with a weak signal from the *trans*-isomer (8b). D-Xylose reacted similarly, to give (9a) and (9b) with the former predominant (though the parameters for the Mg^{2+} chelate of the former could not be determined unambiguously). D-Ribose and L-arabinose produced spectra which were identical to each other and which evidently contained (unresolved) signals from the geometrical isomers (10a) and (10b).

The detection of semidiones from the substrates so far mentioned is in keeping with the suggestion (*cf.* ref. 4) that the mechanism of formation requires the presence

TABLE 1

E.s.r. spectra of Type A semidiones derived from the reaction of ·OH and some carbohydrates at pH *ca.* 7

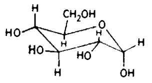
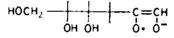
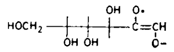
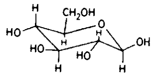
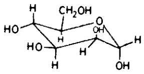
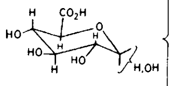
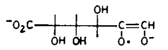
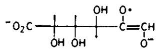
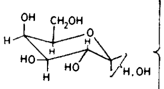
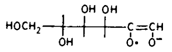
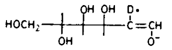
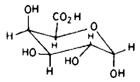
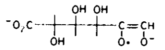
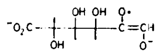
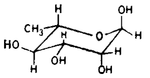
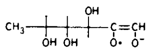
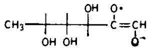
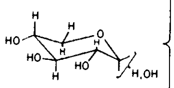
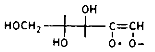
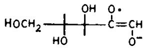
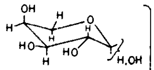
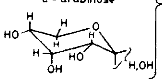
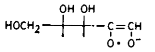
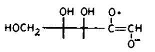
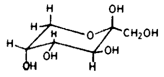
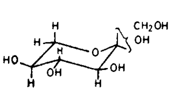
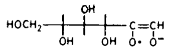
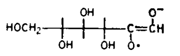
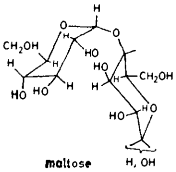
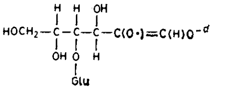
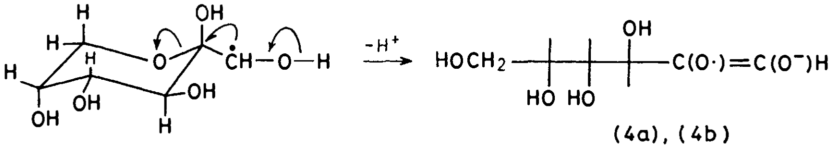
Substrate	Radicals detected	Hyperfine splittings [a]		
		$a(\alpha\text{-H})$	$a(\beta\text{-H})$	g [b]
 α-D-glucose	 (4a)	0.900	0.485	2.0049
	 (4b)	0.840	0.450	2.0050
 β-D-glucose	(4a)			
 α-D-mannose [c]	(4a) (4b)			
 D-glucuronic acid	 (5a)	0.900	0.485	2.0049
	 (5b)	0.840	0.450	2.0050
 D-galactose	 (6a) [d]	0.890	0.205	2.0049
	 (6b)			
 α-D-galacturonic acid [c]	 (7a) [d]	0.890	0.155	2.0049
	 (7b)			
 α-L-rhamnose	 (8a)	0.890	0.355	2.0049
	 (8b)	0.815	0.370	2.0050
 D-xylose	 (9a)	0.875	0.315	2.0049
	 (9b)	0.810	0.335	2.0050
 L-arabinose  D-ribose [e]	 (10a)[e]	0.910	0.310	2.0050
	 (10b)[e]			
 β-D-fructose	(4a) (4b)			
 L-sorbose	 (12a)	0.870	0.250	2.0049
	 (12b)	0.835	0.310	2.0050

Table 1 (*continued*)

Substrate	Radicals detected	Hyperfine spittings[a] a(α-H)	Hyperfine spittings[a] a(β-H)	g[b]
 maltose	 $\mathrm{HOCH_2{-}C(H)(OH){-}C(H)(O{-}Glu){-}C(OH)(H){-}C(O\cdot){=}C(H)O^{-}}$[d] (15)	0.875	0.425	2.0049

[a] ±0.005 mT. [b] ±0.0001. [c] Sample largely (>80%) this anomer. [d] Signals from the two isomeric forms were indistinguishable. [e] The radicals from D-ribose are the mirror images of (10a) and (10b).

in the pyranose ring skeleton of both 1- and 2-hydroxy-substituents [*cf.* reaction (2)]. As would be expected on this basis, we were unable to detect Type A semidiones from the substituted analogues 1-*O*-methyl-α-D-glucose, 1-*O*-methyl-β-D-glucose, 2-aminoglucose, and 2-deoxy-D-ribose. In contrast, a very weak spectrum, evidently from the appropriate Type A semidione(s), was obtained from 3-*O*-methyl-α-D-glucose, though an unambiguous analysis was not possible; the weakness of this is thought to reflect both the weakness of the corresponding spectrum from α-D-glucose itself and the low concentration of the precursor radical observed for this substrate.[2]

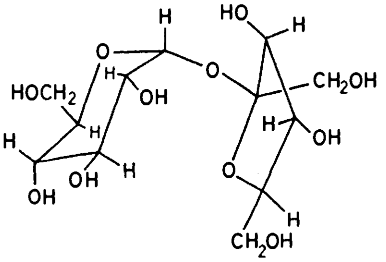


(11) (4a), (4b) (4)

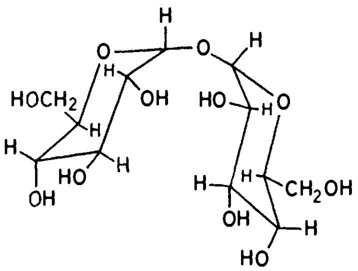
We should note here that the larger of the two doublet splittings (*ca.* 0.9 mT) for the Type A semidiones is characteristic of an α-proton in the structure $-C(O\cdot)=C(O^-)H$; the second splitting is associated with a β-proton interaction and the greater variation in its magnitude with structure [compare, for example, the isomers of (6) with those of (4) and (5)] is due to the slightly different conformational preferences of the attached chains.[4]

The spectrum obtained from β-D-fructose is, as found earlier,[4] analysed in terms of signals from the radicals (4a) and (4b) as detected from α-D-glucose (but with an enhanced signal-to-noise ratio and [(4a)] : [(4b)] *ca.* 2.5 : 1). It has been suggested that these arise *via* a mechanism, closely resembling reaction (2), which involves ring-opening of the C(1)-derived radical (11) [reaction (4)]; the increased intensity of the signals from (4a) and (4b) from this substrate compared with those from glucose probably reflects the statistical factor of 2 involved in the initial formation of (11). *cis* and *trans* Type A semidiones [(12a) and (12b)] were also obtained from the ketose L-sorbose (see Table 1).

As would be expected on the basis of the mechanisms for Type A semidione formation which have so far been proposed, no such radical could be detected from sucrose [α-D-glucopyranosyl-β-D-fructofuranoside (13)] or the 1,1-linked glucose derivative trehalose [α-D-glucopyranosyl-α-D-glucopyranoside (14)]; on the other hand the detection of a weak Type A signal from maltose can be rationalized in terms of the formation of (15) *via* attack at 2-H in the right-hand ring, which possesses the free 1-hydroxy-group necessary for semidione formation.

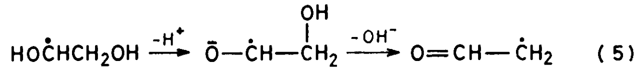


(13)



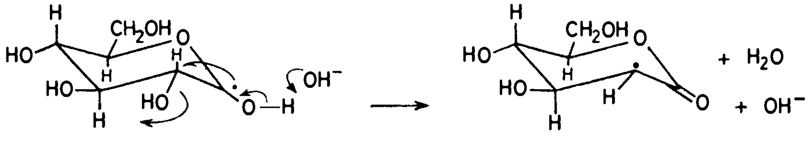
(14)

Further Mechanistic Considerations.—The base-catalysed ring-opening process [*e.g.* reaction (2)] which has been proposed for the formation of Type A semidiones from aldo-hexoses and -pentoses appears to be formally analogous not only to the equivalent acid-catalysed process involving loss of the β-hydroxy-group [reaction (1)] but also to the corresponding base-catalysed elimination [see *e.g.* reaction (5)] in which the departure of the (β) leaving group is evidently assisted by the prior ionization of the α-hydroxy-group.[5]

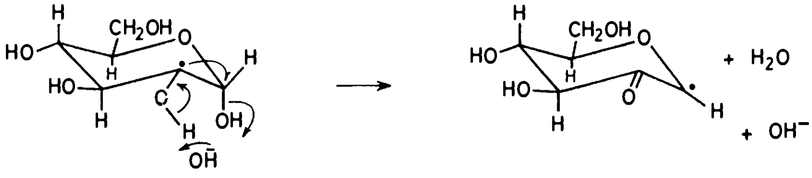


$$\mathrm{HO\dot{C}HCH_2OH} \xrightarrow{-\mathrm{H}^+} \bar{\mathrm{O}}\text{—}\dot{\mathrm{C}}\mathrm{H}\text{—}\overset{\mathrm{OH}}{\mathrm{C}}\mathrm{H_2} \xrightarrow{-\mathrm{OH}^-} \mathrm{O{=}CH\text{—}\dot{C}H_2} \qquad (5)$$

We would expect that, by analogy with the rearrangement of many αβ-dioxygen-substituted radicals from carbohydrates in acid,[2] similar loss of β-OH$^-$ groups would occur under basic conditions [*e.g.* reactions (6) and (7) for radicals (16) and (17) respectively, derived from α-D-glucose and •OH]. We would further anticipate that reaction (7) would be particularly rapid, and faster than both the corresponding reaction from the analogous β-D-glucose-derived radical and also the alternative ring-opening reaction (2) since, as previously noted for acid conditions, the elimination reaction occurs most readily for an axial β-C–O bond (and with loss of OH$^-$ faster than OR$^-$).[2]



(16) (18) (6)



(17) (19) (7)

Neither (18) nor (19) was detected in experiments involving $\mathrm{Ti^{III}}$–$\mathrm{H_2O_2}$ and α-D-glucose; these and other carbonyl-conjugated radicals formed by similar routes would be expected to be susceptible to destruction *via* reduction by $\mathrm{Ti^{III}}$–EDTA complexes.[4,6] For this reason we studied the reactions of some monosaccharides with •OH when this was generated by *in situ* photolytic decomposition of hydrogen peroxide and found that signals could be detected as long as relatively slow flow rates were employed (<2 ml min^{-1} *). The reaction of •OH generated in this way with both α- and β-D-glucose at pH *ca.* 7 led to the detection of similar weak spectra (in contrast, significant differences were found between the behaviour of the anomers in the $\mathrm{Ti^{III}}$–$\mathrm{H_2O_2}$ system): these comprised signals from the *cis*-semidione (4a), together with traces from (4b), and the carboxy-conjugated radical (18) [with *a*(1 H) 2.020, *a*(1 H) 3.840, and *a*(1 H) 0.075 mT; *g* 2.0035]; no trace of (19) could be discerned. At low pH (*ca.* 1.5) both (18) and (19) were clearly detectable in both $\mathrm{Ti^{III}}$–$\mathrm{H_2O_2}$ and photolytic experiments. We suggest that the observation that at high pH the ring-opening pathway for (17) [*cf.* reaction (2)] occurs rather than the loss of the β-OH group [which is ideally suited for elimination, *via* reaction (7)], reflects the prior ionization at these pH values of 1- rather than 2-OH.† This ionization would be expected not only to suppress elimination of OH$^-$ but also to encourage ring-cleavage by providing stabilization of the resulting radical.

The observation that α- and β-glucose do not yield identical amounts of the two isomers (4a) and (4b) under the $\mathrm{Ti^{III}}$–$\mathrm{H_2O_2}$ rapid-flow conditions clearly indicates that the reaction which produces the semidiones does not proceed from a radical obtained by reaction of •OH with the open-chain form of the substrate. Since it also implies that the ratio of isomers detected from a given substrate is not governed by thermodynamic control, we believe that in the rapid-flow experiment the product-radical ratios are governed by *kinetic* control of rearrangement of first-formed cyclic radicals (as also noted previously [13] for the formation of semidiones in hydroxy-ketone oxidation).‡ The apparent exclusive formation of the *cis*-isomer (4a) from β-D-glucose [reaction (8)] becomes immediately understandable in view of the proposed geometry of the transition state. For the corresponding radical from α-D-glucose (17) the dihedral angle between the C(1)–O and C(2)–O bonds is *ca.* 90°, so the production of both geometric isomers (4a) and (4b) is understandable. The lower concentration of semidiones produced from (17) than from (20) may be due to the expected lower acidity of the 1-OH in the former (*cf.* pK_a values for α- and β-glucose [11]).

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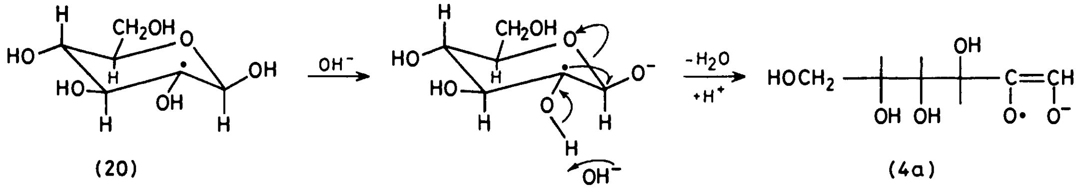
* As Czapski has pointed out,[10] for optimum signals the flow rate has to be such that the flow-time of the solution through the cavity is longer than the radical lifetime.

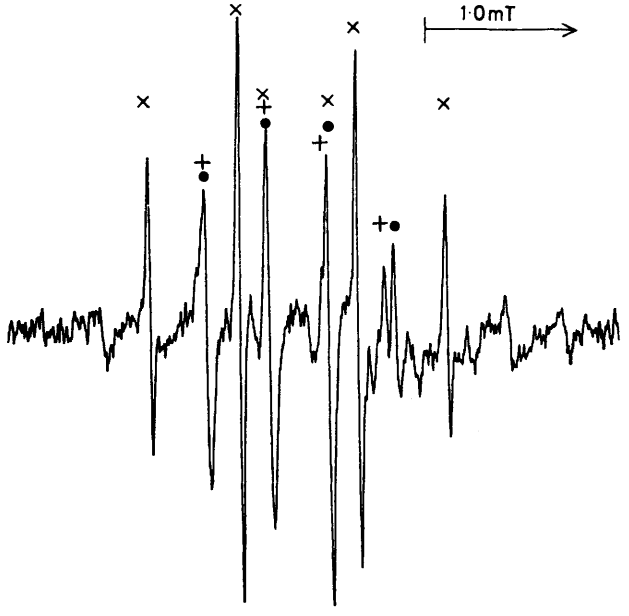
† This is as expected on the basis of the much greater acidity of the $\mathrm{C^1}$-OH group (pK_a *ca.* 12) compared with the other hydroxy-groups in the parent compound; [11] the fact that the presence of a radical centre lowers pK_a values for α- more than β-OH groups (typically 4.5 *versus* 1.5 units [12]) would not be expected to outweigh this effect.

‡ This is not necessarily expected to be true for the *slow*-flow photolysis experiments where the residence time of the relatively long-lived semidiones in the cavity is considerably longer.



(20) (4a) (8)

The Structure and Formation of Type B Semidiones.—(a) *Results.* When the pH was raised further in experiments involving α- or β-D-glucose and ·OH (generated from the Ti^{III}–H_2O_2 reaction under conditions of rapid flow) the signals from (4a) [and from (4b) in the case of the α-anomer] were joined by those from further semidione-type radicals at pH > 6.5; these were much more intense at pH 9 (see Figure). These signals are from the two isomers (21a) and (21b) of the semidione which also results from the oxidation of 1,3-dihydroxyacetone under similar conditions [reaction (9)]; assignments to *cis*- and *trans*-isomers (see Table 2) are made on the basis of experiments with this substrate in the presence and absence of Mg^{2+} (as outlined earlier). The *trans*-isomer (21a) dominated the spectra from both α- and β-glucose, with a ratio of [(21a)] : [(21b)] of *ca.* 5 : 1 in each case. In contrast, the corresponding ratio was *ca.* 1 : 3 when the species were generated by the oxidation of dihydroxyacetone; when the oxidation of α- and β-glucose was carried out with ·OH generated by photolysis of hydrogen peroxide under slow-flow conditions at pH *ca.* 9, the ratio was 2.5 : 1.



E.s.r. spectrum from the reaction of ·OH and α-D-glucose at pH *ca.* 9: ● (4a); + (4b); × (21a), (21b). The presence of (21b) as the minor component of the mixture responsible for the peaks marked × is revealed under conditions of higher resolution. Other minor peaks are from the radical formed in the reaction of ·OH with EDTA

The following substrates also gave rise to the same two semidiones in rapid-flow experiments under comparable conditions to those described for glucose: α-D-mannose, D-galactose, 3-*O*-methyl-α-D-glucose (at low intensity), 2-aminoglucose, D-fructose, L-sorbose, and sucrose. Other Type B semidiones were obtained from α-L-rhamnose {identified as the isomers of[14] MeC(O·)=C(O⁻)H, with [(22a)] : [(22b)] *ca.* 4 : 1}, and L-arabinose, D-xylose, and D-ribose [identified as the isomers of[7] HC(O·)=C(O⁻)H, with the *trans*-form present in only trace quantities: see Table 2], but no analogous radicals could be detected from either α- or β-1-*O*-methylglucosides, 2-deoxyribose, or the disaccharides trehalose and maltose. The spectra from D-glucuronic acid and D-galacturonic acid could not be satisfactorily analysed.

It is particularly significant that the spectra from α-L-rhamnose are from the isomers of MeC(O·)=C(O⁻)H, which indicates that these radicals are derived from the C(6)—C(4) fragment of the sugar, ruling out a mechanism of formation akin to reaction (3), which would require the production of the semidiones from C(3)—C(1). Further, the pentoses all gave the spectra of the isomeric *two*-carbon semidiones (23a and b) and not a three-carbon radical as would be required by the previously proposed mechanism.[4,*]

Several other observations have particular mechanistic significance. First, when the oxidation of α-D-glucose with ·OH (in the rapid-flow system) at pH 9 was carried out in ^2H_2O, the spectra from (21a) and (21b) were identical to those obtained in ^1H_2O, indicating that no deuterium had been incorporated. Similar results were obtained for β-D-fructose. This clearly rules out the reverse-aldol reaction shown in reaction (3). Secondly, radicals (21a) and (21b) also result from reaction of 3-*O*-methyl-α-D-glucose, which again suggests that it is carbon atoms C(6)—C(4) [rather than C(3)—C(1)] in glucose and its derivatives which are the source of the B Type semidiones. Thirdly, we found that as the pH was raised and the Type B semidiones were formed, there was no significant diminution of the signals from the Type A species, which rules out the possibility that they are the precursors of the Type B species. We also found that the signals from the Type B radicals do not increase in intensity at slower flow rates: weaker signals are obtained. This implies that the Type B radicals are derived by rapid reaction of one of the first-formed radicals with base and that an alternative mechanism (*e.g.* involving subsequent oxidation with ·OH of a product from the radical oxidation) is not operating.

(b) *Mechanisms of reaction.* (i) Aldoses. The formation of semidiones from the C(6)–C(4) portion of the

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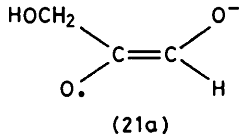
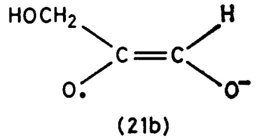
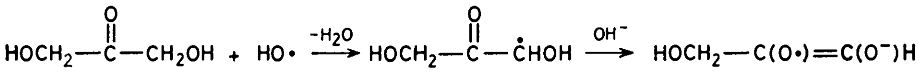
[*] A similar structural reinterpretation is now agreed by the authors of ref. 4 (P. R. West, personal communication).



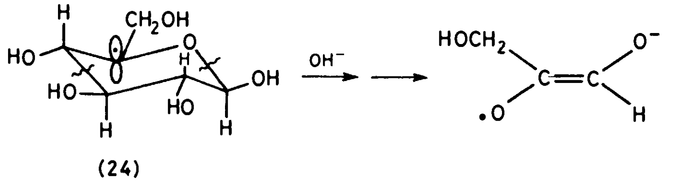
(21a)



(21b)



$$\mathrm{HOCH_2{-}\overset{O}{\overset{\|}{C}}{-}CH_2OH + HO\cdot \xrightarrow{-H_2O} HOCH_2{-}\overset{O}{\overset{\|}{C}}{-}\dot{C}HOH \xrightarrow{OH^-} HOCH_2{-}C(O\cdot){=}C(O^-)H} \quad (9)$$

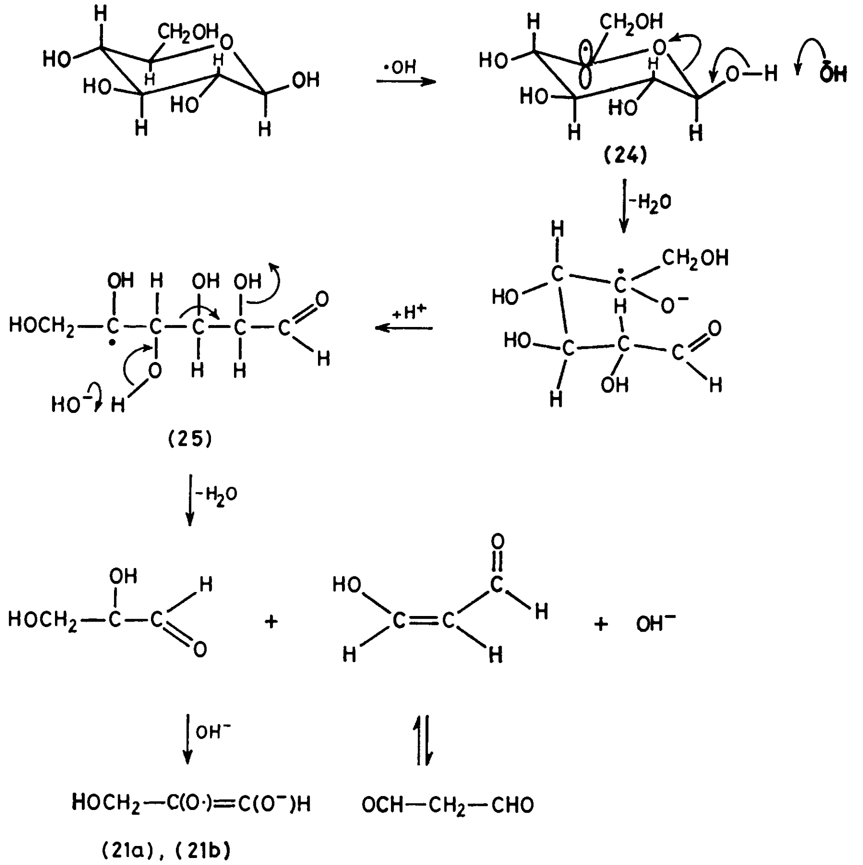


(10)

aldohexoses [*e.g.* $HOCH_2C(O\cdot){=}C(O^-)H$ from glucose and related compounds] indicates that oxidation has occurred at C(5); this suggests that the C(5) abstraction radical (24) is the appropriate precursor. We therefore envisage that the overall process involved may be represented by the fragmentation (10), which bears a formal similarity to typical β-elimination reactions.

Additionally we must explain why the reaction does not proceed when either the 1- or the 4-hydroxy-groups in the reacting ring is replaced by an alkoxy substituent (*e.g.* as in trehalose, maltose, 1-*O*-methyl-α- and 1-*O*-methyl-β-D-glucose), though a 3-methoxy-group does not prevent reaction. This suggests that the base-catalysis necessary involves the ionization of both 1- and 4-OH. We also note that the epimers D-glucose (both anomers) and D-galactose behave identically, which suggests that the ring geometry has been lost at the crucial stage in the reaction.

We suggest that the formation of Type B semidiones from aldoses can be accounted for in terms of the mechanism illustrated for β-D-glucose in Scheme 1

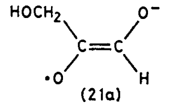
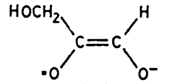
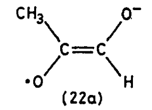
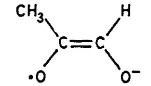
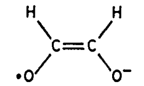
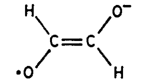


SCHEME 1 Proposed mechanism of formation of Type B semidiones from aldoses (illustrated for β-D-glucose)

(analogous schemes may be written for the other substrates listed in Table 2). The important intermediate (25) is formed by base-catalysed ring opening of the radical (24) [(25) could also result from attack of •OH at C(5) in the open-chain form, but we consider this order of reaction less likely than that proposed in view of the low percentage of the open-chain form of the parent under these conditions[15]. Now ionization of (25) at 4-OH, as evidently required, either concerted with or followed by C–C fission and loss of OH^-, leads to the production of the semidione and malondialdehyde. This type of fragmentation reaction, for which the driving force is the production of a stabilized radical and neutral molecule (as well as the favourable entropy factor), has several important precedents in base-catalysed degradations of carbohydrates: thus, in many cases C(3)–C(4) cleavage is preceded by ionization of 4-OH.[15,16] The fragmentation in which the C–C bond is broken also clearly resembles elimination reactions of the Grob type (see *e.g.* ref. 17). Of particular note is that in the •OH-induced base-catalysed degradation of a large number of simple sugars, including aldohexoses and aldopentoses, malondialdehyde has been identified in the products (see *e.g.* ref. 18) and, further, that we have identified this compound in the products of the oxidation of D-glucose and other substrates with the Ti^{III}–H_2O_2 couple at pH 9 (see later) (*n.b.* it has been proposed that this compound is responsible for the observed cytotoxic and carcinogenic properties of irradiated sugars[19]).*

TABLE 2

E.s.r. spectra of Type B semidiones derived from reaction of •OH with some carbohydrates at pH *ca.* 9

Substrates	Radical(s) detected[a]	Hyperfine splittings[b] *a*(α-H)	Hyperfine splittings[b] *a*(β-H)	*g*[c]
α-D-Glucose β-D-Glucose α-D-Mannose D-Galactose 3-*O*-Methyl-α-D-glucose 2-Aminoglucose β-D-Fructose L-Sorbose Sucrose	 (21a)	0.850 (1 H)	0.650 (2 H)	2.0050
	 (21b)	0.885 (1 H)	0.650 (2 H)	2.0049
α-L-Rhamnose	 (22a)	0.900 (1 H)	0.620 (3 H)	2.0049
	 (22b)	0.975 (1 H)	0.860 (3 H)	2.0047
D-Xylose D-Ribose L-Arabinose	 (23a)	0.860 (2 H)		2.0050
	 (23b)	0.78 (2 H)[d]		2.0048

[a] For ratios, see text. [b] ±0.005 mT. [c] ±0.0001. [d] ±0.01 mT.

If Scheme 1 does indeed represent the mechanism for Type B semidione formation from aldohexoses then the stereochemistry of the semidiones obtained becomes immediately understandable. Thus under the conditions of *kinetic* control of the reaction, which are believed to be appropriate for the rapid-flow experiments (see earlier), the C–C bond to be broken in the hexoses [*i.e.* C(3)–C(4)] would be expected to avoid the same side of the molecule as the CH_2OH group [conformation (26)]; in this way the formation of *trans*-semidiones from glucose, rhamnose, *etc.* can be rationalised. In contrast, for the pentose-derived radicals, the preferred conformation is expected to resemble (27), with resultant formation of the *cis*-semidione. These views are reinforced by the inspection of models constructed on the basis of attributing to the chain being broken the antiperiplanar arrangement of bonds so often favoured in the Grob fragment-

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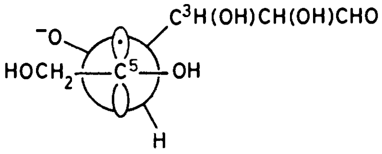
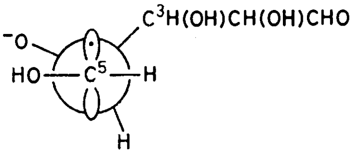
* Various routes to the formation of malondialdehyde in such systems have been previously discussed, though none was felt to be satisfactory.[20] We suggest that Scheme 1 offers one pathway to malondialdehyde for sugars of certain types.

ation [see *e.g.* reaction (11) for conformation (28) of the hexose-derived radical (25)]; this leads to increased rigidity and, consequently, enhanced steric repulsion.

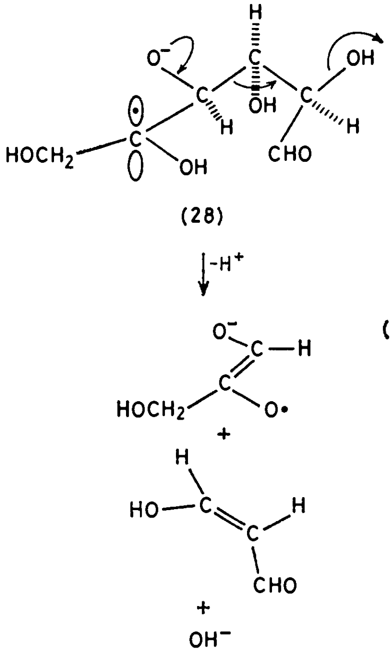
We also suggest that the relatively weak signals obtained from the 3-methoxy- and 2-amino-analogues reflect the poorer leaving-group abilities of, for the former case, MeOCH=CHCHO and OH^-, and for the latter case, HOCH=CHCHO and NH_2^-.



(26)



(27)



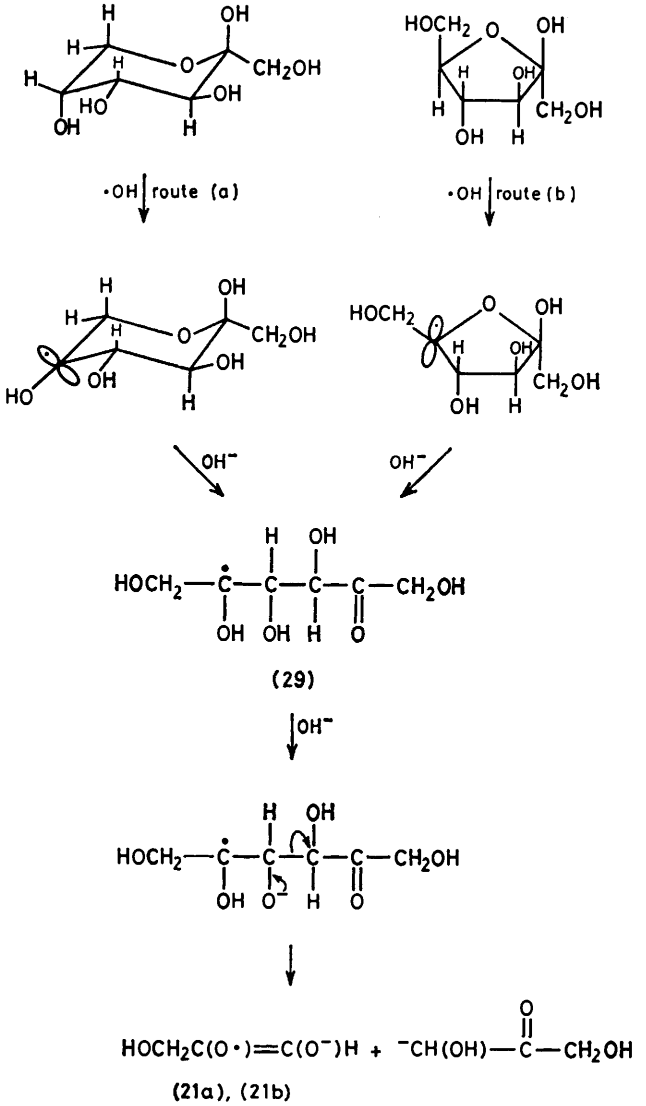
(11)

(ii) Ketoses. In order to account for the formation of the *cis*- and *trans*-isomers of $HOCH_2C(O\cdot)=C(O^-)H$ from fructose and sorbose we require, by analogy with arguments presented above for aldoses, that the semidiones are derived from carbon atoms C(4)–C(6) of the substrate and that the mechanism involves reaction of the C(5)-precursor radical *via* elimination of a good anionic leaving group. These essential features are all possessed by the two mechanisms outlined in Scheme 2 for β-D-fructose and which differ in that route (a) proceeds from radical oxidation of the pyranose form of the sugar whereas route (b) involves the furanose form [which is known to be present to a significant extent [21]]. In both routes the open-chain radical (29), produced *via* ionization of C(1) (as described for β-D-glucose in Scheme 1), reacts *via* a base-catalysed retro-aldol reaction (of a type well known [15] in the base-catalysed degradation of sugars); the leaving group is the relatively stabilized anion $^-CH(OH)C(O)CH_2OH$. The preference for the *trans*-semidione (21a) would again be understandable if steric requirements govern the adoption of a preferred conformation akin to (26) in the transition state.

An alternative mode of formation of (21a) and (21b) is set out in Scheme 3 (with R = H); the key features of this mechanism are as follows. In the radical (30), formed by hydrogen-atom abstraction from C(5) in the furanose form, ionization of the adjacent hydroxy-group occurs (*cf.* Schemes 1 and 2). This is followed by a concerted anionic fragmentation of the Grob type [*cf. e.g.* Scheme 1] in which the negative charge is accommodated on the oxygen adjacent to the radical centre, *i.e.* the incipient semidione itself. A mechanism of this type is, of course, not possible for the pyranose analogues.

We are unable to distinguish between the closely related mechanisms set out in Schemes 2 and 3 for fructose; indeed, they not only all produce the same set of products but also proceed from the radical formed by attack at C(5). Further, since both pyranose and furanose forms of the sugar exist together in equilibrium it is quite possible that the mechanisms operate together to form the Type B semidiones.

Lastly we note that Scheme 3 (with R = OGlu) would also account for the formation of the semidiones (21a) and (21b) from sucrose, the only disaccharide studied with a furanose ring; the exclusively pyranose disaccharides trehalose and maltose, for which such a concerted β-type fragmentation cannot occur, did not give Type B semidiones.



Scheme 2 Possible mechanisms for the formation of Type B semidiones from the C(5) (pyranose) and C(5) (furanose) radicals from β-D-fructose

Analysis for the Presence of Malondialdehyde.—We have employed three tests for the detection of malondialdehyde in mixtures of products. Two of these involve the formation of characteristic coloured complexes with 2-methylindole [22] and with thiobarbituric acid.[23] Positive reactions were obtained from the 2-methylindole systems when the substrate, titanium(III), and hydrogen peroxide were present; control experiments, in which one of the reagents was omitted, proved negative.

The most intense colours were obtained at high pH from those substrates, *e.g.* glucose and xylose, which we would predict, on the basis of Scheme 1, to lead to the formation of malondialdehyde. Positive, though much weaker, responses were shown by the same substrates at low pH and also by those substrates, *e.g.* maltose and 1-*O*-methyl-α-glucose, which we would not expect to produce the dialdehyde; this suggests that there may be other routes to this compound which remain to be elucidated. The thiobarbituric acid test was less satisfactory but the results were consistent with the generation of malondialdehyde under similar circumstances.

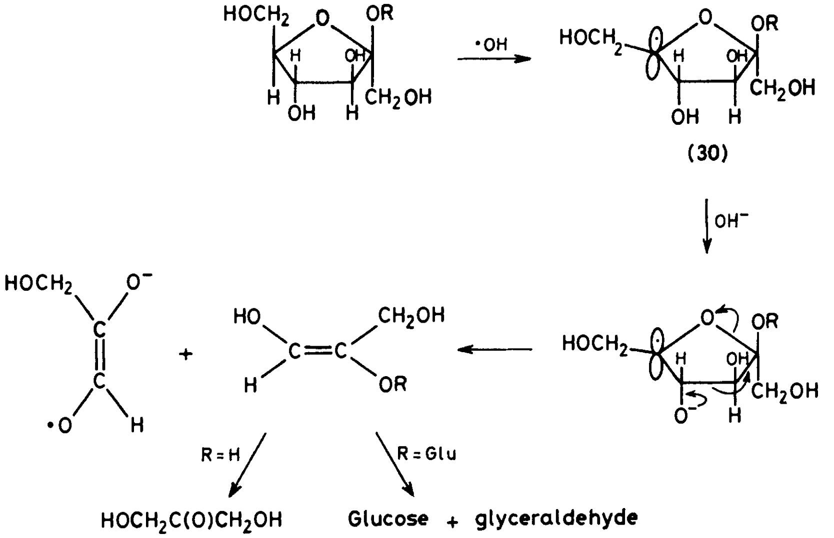
The third test is the demonstration that, on t.l.c., the coloured product formed from 2-methylindole and the products of degradation had the same R_F value as that from the indole and authentic malondialdehyde.[18b] Our experiments suggest that this test is not reliable: 2-methylindole alone has an R_F value identical to the product of the reaction of the indole with malondialdehyde. It also gives a positive colour reaction with acetic acid, one of the co-solvents in the system, although this is too weak to be responsible for the absorption seen in the carbohydrate degradation mixtures.

EXPERIMENTAL

Details of the spectrometers, flow system, and spectrum measurements have been given previously.[1,2] For the three-way flow experiments conducted here the solutions contained (i) 0.007 mol dm^{-3} titanium(III) [added as 12.5% (w/v) $TiCl_3$ (Fisons technical grade)] and 3 g dm^{-3} of the disodium salt of ethylenediaminetetra-acetic acid, (ii) 0.035 mol dm^{-3} hydrogen peroxide [added as 100 volume hydrogen peroxide (Fisons Ltd.)], (iii) the substrate (*ca.* 0.035 mol dm^{-3}). The pH was adjusted by the addition of ammonia solution (*d* 0.880) to the Ti^{III} stream and all solutions were purged of oxygen before and during the experiments with a stream of nitrogen. For experiments in which it was desirable to keep mutarotation to a minimum, the substrate solution was ice-cooled. Spectra of the magnesium chelates of *cis*-semidiones were recorded as above with 0.1 mol dm^{-3} $MgSO_4$ (Fisons) added to the substrate stream. Experiments with ^2H_2O were carried out with a two-way flow system, the glucose being added to the titanium-containing stream. Spectra were also obtained by the photolysis of 5% H_2O_2 solutions containing the substrate (*ca.* 1 mol dm^{-3}) as these were slowly flowed through the cavity of the spectrometer [with flow rate *ca.* 2 ml min^{-1}; the pH was adjusted with ammonia (*d* 0.880)]; the lamp employed was an Hanovia 1 kW Hg–Xe source (977B-1).

All sugars were obtained commercially (from Sigma Ltd. except for galactose and deoxyribose, which were obtained from B.D.H. Ltd.) and were used without further purification.

The presence of malondialdehyde in the products of degradation was demonstrated by standard colour tests.[22,23] For a solution of the appropriate sugar (1%), EDTA (8 g dm^{-3}) and titanium(III) chloride (0.016 mol dm^{-3}), the pH was adjusted by the addition of ammonia solution (*d* 0.880) and sufficient hydrogen peroxide was added to give a concentration of 0.085 mol dm^{-3}. The spectra of the resultant solutions were recorded on a Pye-Unicam SP800 spectrometer and the absorbances measured on an SP6 spectrometer.



SCHEME 3 Possible mechanism for the formation of Type B semidiones from β-D-fructose (R = H) and sucrose (R = Glu)

We thank the S.R.C. for a studentship (for D. M. K.), Unilever Research for financial support, Professor P. R. West for communicating his results to us prior to publication, and Dr. P. D. Collier and Mr. J. Madden for valuable discussions.

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