

γ -IRRADIATION-INDUCED RING-OPENING OF POLYCRYSTALLINE CYCLOAMYLOSE HYDRATES*

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ABSTRACT

The chemical modifications induced in polycrystalline cycloamylose hydrates during γ -irradiation have been investigated by using g.l.c.-m.s. to analyse the mono-saccharide mixtures formed on hydrolysis. Unchanged substrate and material retaining the original cyclic structure were removed by precipitation prior to hydrolysis, and the products therefore reflect the effect of the radical-induced opening of the cycloamylose ring structure. The following products were identified: glucose and glucono-1,5-lactone (1), 4-deoxy-xylo-hexose (2), arabinose (3), ribose (4), 2-deoxy-erythro-pentose (5), 3-deoxy-erythro-hexos-4-ulose (6), xylo-hexos-5-ulose (7), 6-deoxy-xylo-hexos-5-ulose (8), 5-deoxy-xylo-hexodialdose (9), 2,6-dideoxyhexos-5-ulose (10), xylose (11), 5-deoxypentose (12), 3-deoxypentulose (13), erythrose (14), and threose (15). Products 1-9 appear to be terminals of the "anhydroglucose" chain. Established free-radical reactions, typical for carbohydrates, are invoked to account for these products.

INTRODUCTION

The γ -irradiation of cycloamyloses¹ and the radiation protection of α - and β -cycloamyloses in complexes with organic substrates²⁻⁴ has already found wide attention. Analytical techniques used were thin-layer and paper chromatography, and e.s.r. spectroscopy. The extent of radiation damage in complexed and uncomplexed cycloamyloses was monitored by determination of the increase in acidic and reducing terminal groups and the total open-chain products, gravimetrically. However, the precise nature of the chemical modifications and the locations of the initial sites of

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attack in the molecule have not been ascertained. The high molecular weights of the cycloamyloses prevent direct application of g.l.c. to the radiation products and preclude identification by g.l.c.-m.s. Recently, these techniques have been used to identify the majority of the sugar products of up to six carbon atoms formed during the γ -irradiation of 2-deoxy-D-erythro-pentose⁵, cellobiose^{6,7}, and D-glucose⁸ in N₂O-saturated, aqueous solution, and α -lactose monohydrate⁹, 2-deoxy-D-erythro-pentose¹⁰, D-fructose^{11,12}, and D-glucose¹² in the solid state. In addition, unbranched and branched polyols, resulting from radical reactions occurring on γ -irradiation of ethylene glycol¹³, glycerol¹⁴, and erythritol¹⁵ in aqueous solution, have been identified by g.l.c.-m.s. As a result, many of the radical reaction processes occurring both in solution and in the solid state, hitherto unknown, have been elucidated.

We now report an extension of these studies to cycloamyloses, as a preliminary to an investigation of the irradiation of biologically important polysaccharides of connective tissue and the extracellular matrix.

EXPERIMENTAL

Materials. — The α - and β -cycloamyloses (Schardinger dextrans¹⁶) were prepared in hydrated form according to the method described by French¹⁶ and the modified separation procedure of Cramer and Henglein¹⁷. They were also obtained from Corn Products Corporation, Belgium.

Glucamylase (from *Aspergillus niger*) and alpha-amylase (from hog pancreas) were obtained from Merck. Further references materials were synthesised according to the methods cited by Dizdaroglu *et al.*⁸.

Irradiation and work-up. — The α - and β -cycloamyloses were γ -irradiated in hydrated forms under ambient conditions to doses up to 1.17×10^{21} eV.g⁻¹ on a ⁶⁰CO γ -source⁸ (dose rate, 1.3×10^{18} eV.g⁻¹.min⁻¹). Each irradiated material was dissolved in the minimum volume of triply-distilled water, and the unmodified cycloamylose was removed with a selective organic precipitant (toluene or tetrachloroethane^{16,17}). The precipitated cycloamylose was filtered-off and the filtrate concentrated and re-treated with the precipitant. The filtrate was boiled to remove the organic-water azeotrope, and then concentrated by rotary evaporation. The residue was hydrolysed to monosaccharide components using Dowex 50W x8 (H⁺) ion-exchange resin, glucamylase¹⁸, and alpha-amylase. The hydrolysis with the acidic resin required a rigorous 72-h reflux at 100° to effect ~90% conversion into monosaccharides. Enzymic hydrolysis with glucamylase (1% w/w sugar) was milder but effected only partial conversion into monosaccharides as indicated by t.l.c. Inhibition of enzyme action is due probably to the presence of lactones¹⁹ in the hydrolysate mixture. Alpha-amylase was less effective than glucamylase. Further hydrolysis using both enzymes, sequentially or simultaneously, was attempted. However, the most effective hydrolysis procedure involved the sequence of alpha-amylase, glucamylase, and the acidic resin. T.l.c. (Kieselguhr; acetone-ethyl acetate-water 5:4:1)²⁰ was

used to analyse the monosaccharides in the hydrolysate mixture. To monitor oligosaccharide products prior to hydrolysis, the same support phase was used with 2-propanol-ethyl acetate-water (1:7:2)²¹. Control hydrolyses were performed on unirradiated cycloamylose in order to monitor chemical changes induced hydrolytically; t.l.c. revealed only glucose and partially hydrolysed oligosaccharide material.

A large proportion of the hydrolysate mixture was unmodified D-glucose, which was removed by chromatography on a column (2 m \times 2.5 cm) of Kieselgel 40 (Merck), with acetone-ethyl acetate-water (5:1:4). Acidic monosaccharide components were also separated from neutral components by ion-exchange chromatography (Merck, strongly basic resin in the acetate form) and recovered by elution with dilute formic acid¹⁰.

Reduction of the hydrolysates to the respective polyols, after freeze-drying, was performed with NaBH₄ or NaBD₄²². The procedure for trimethylsilylation was that described by Sweeley *et al.*²³.

G.l.c. and mass spectrometry. — The glass-spiral capillary columns, the g.l.c. and g.l.c.-m.s. instrumentation used in this investigation, and the preparation of samples for g.l.c.-m.s. analysis have been described elsewhere^{6,24,25}. Quantitative data on the yields of products were not achieved as for mono- and disaccharides^{5,7,8,12}. This study is thus qualitative, and only an indication of the predominating radical reactions is obtainable from the chemical modifications identified.

RESULTS

Samples of the cycloamyloses in hydrated form were irradiated to doses of $\sim 10^{21}$ eV.g⁻¹ sufficient to effect $\sim 4\%$ conversion into products based² on *G*(ring opening) = 6.6. The hydrolysate mixture, obtained from the irradiated cycloamylose as described in the Experimental, was fractionated by column chromatography, reduced (NaBH₄ or NaBD₄), trimethylsilylated, and identified by g.l.c.-m.s. Many of the monosaccharides identified are also produced on irradiation of cellobiose^{6,7} and D-glucose⁸. The chemical modifications induced in the cycloamylose molecule on γ -irradiation are listed in Table 1. Proof of structures by g.l.c. and g.l.c.-m.s. analysis is given in this paper only for those products that have not been described previously.

6-Deoxy-D-xylo-hexos-5-ulose (8) had been shown, *via* the Me₃Si ethers of 6-deoxyhexitols-1,5-*d*₂ to be a product from D-glucose⁸ and cellobiose⁷, but only by g.l.c. assignment using authentic materials. This deoxyhexosulose has been identified here by g.l.c.-m.s. analysis of the Me₃Si ethers of the 6-deoxyhexitols-1,5-*d*₂, which have typical fragment ions with *m/e* 103 (12%), 104 (18), 118 (95), 206 (25), 218 (10), 220 (15), 308 (8), 320 (13), and 334 (1).

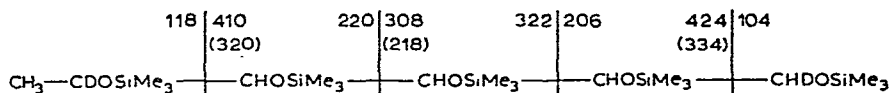
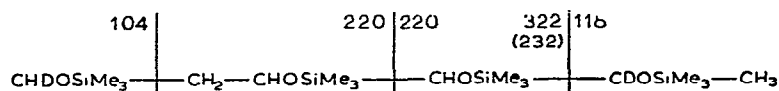


TABLE I
CHEMICAL MODIFICATIONS DEDUCED FROM MONOSACCHARIDES IDENTIFIED IN HYDROLYSATE MIXTURES FROM
 γ -IRRADIATED α - AND β -CYCLOAMYLOSE HYDRATES

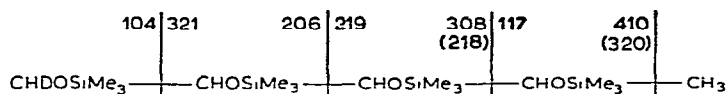
No.	Chemical modification to D-glucose residue	Site attacked ^a	Compound determined ^b	Ref.	Precursor in hydrolysate mixture
1	1,5-Lactone	C-1	Glucono-1,5-lactone	6, 8	Same
2	4-Deoxy-	C-1 \rightarrow C-4' (C-5 \rightarrow C-1)	4-Deoxy-xyllo-hexose	6	Same
3	Five-carbon	C-1	Arabinitol-1-d	6, 8	Arabinose
4	Five-carbon	C-1	Ribitol-1-d	6, 8	Ribose
5	Five-carbon	C-1	2-Deoxy-erythro-pentitol-1-d	6, 8	2-Deoxy-erythro-pentose
6	3-Deoxy-4-keto-	C-4	3-Deoxyhexitol-1,4-d ₂	6, 8	3-Deoxy-erythro-hexos-4-ulose
7	5-Keto-	C-5 \rightarrow C-1	Iditol-1,5-d ₂	6, 8	xyllo-Hexos-5-ulose
8	6-Deoxy-5-keto-	C-5	6-Deoxyhexitol-1,5-d ₂	(7, 8)	6-Deoxy-xyllo-hexos-5-ulose
9	5-Deoxy-aldo-	C-6	5-Deoxyhexitol-1,6-d ₂	8	5-Deoxy-xyllo-hexodialdose
10	2,6-Dideoxy-5-keto-	Unknown	2,6-Dideoxyhexitol-1,5-d ₂	P.w. ^c	2,6-Dideoxyhexos-5-ulose
11	Five-carbon	Unknown	Xylitol-1-d	8	Xylose
12	Five-carbon	Unknown	5-Deoxypentitol-1-d	P.w. ^c	5-Deoxypentose
13	Five-carbon	Unknown	3-Deoxypentitol-2-d	6, 8	3-Deoxypentulose
14	Four-carbon	Unknown	Erythritol-1-d	6, 8	Erythrose
15	Four-carbon	Unknown	Threitol-1-d	6, 8	Threose

^aC-1 \rightarrow C-4' refers to initial attack at C-1 followed by rearrangement of radical site to C-4'. ^bDetermined after reduction with NaBD₄ (g.l.c.-m.s.). ^cP.w. = present work.

2,6-Dideoxyhexos-5-ulose (10) is reduced by NaBD_4 to 2,6-dideoxyhexitol-1,5- d_2 . Its Me_3Si ether shows the typical fragment ions at m/e 103 (18%), 104 (45), 118 (50), 220 (10), 232 (3), 244 (2), 245 (2), 246 (2), and 322 (1%).



5-Deoxypentose (12) is reduced by NaBD_4 to 5-deoxypentitol-1- d . Its Me_3Si ether shows the typical fragment ions at m/e 103 (24%), 104 (22), 117 (70), 206 (10), 218 (14), 219 (19), 308 (13), 320 (1), 321 (<1), and 335 (<1).



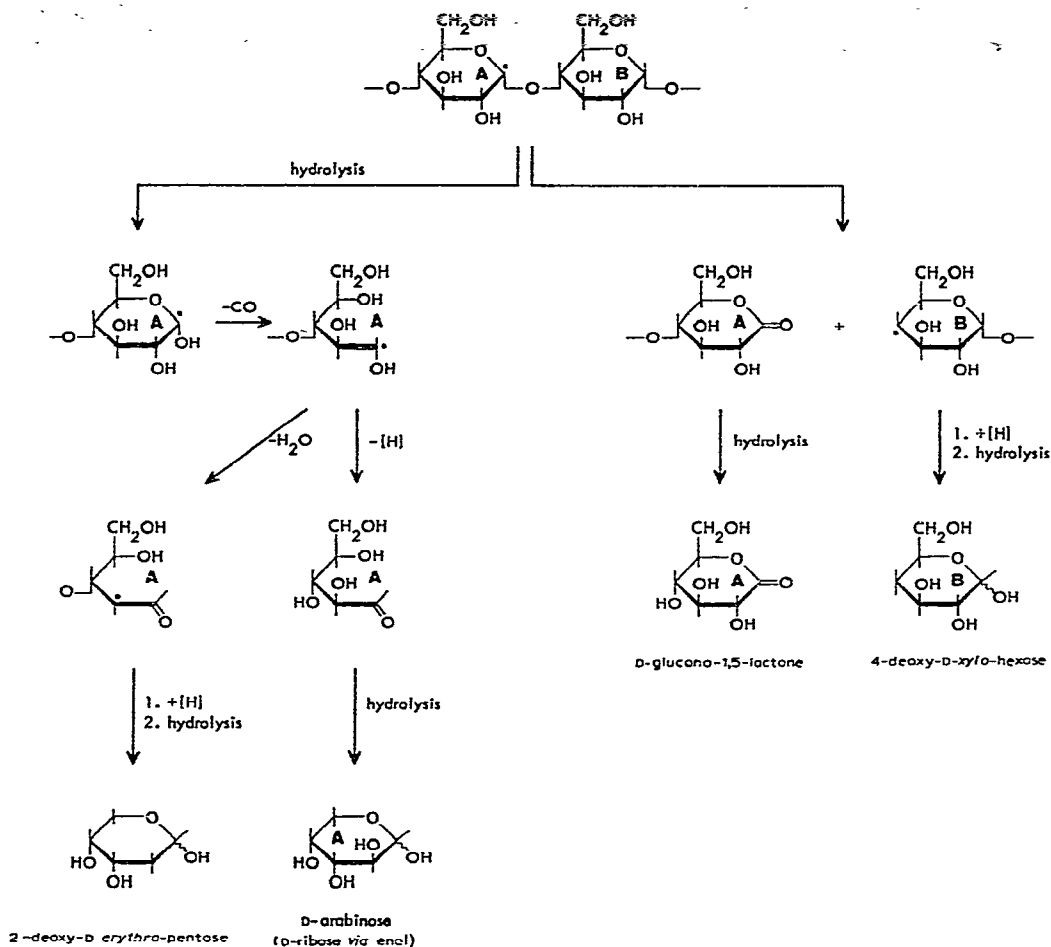
DISCUSSION

The emphasis in this investigation is on the identification of the chemical modifications induced in the cycloamyloses on ring opening caused by irradiation. With this knowledge, an attempt can be made to locate the initial sites of attack and to propose radical-reaction mechanisms that account for the ring opening. The results will be discussed in the light of mechanisms proposed for the radical-induced scission of the glycosidic linkage of cellobiose in aqueous solution⁷. The results obtained with the present system support and, in part, supplement favourably this mechanistic concept.

By analogy with previous work^{5-15,26}, it can be assumed that the locations of radiation-induced keto groups in the D-glucose residues coincide with the sites of initial attack, *i.e.*, the primary-radical sites. These keto groups arise in irradiated carbohydrates by reaction steps involving (a) disproportionation and, possibly, hydrogen-abstraction reactions of primary and secondary radicals; (b) radical rearrangement; (c) eliminations of water, HOR, and carbon monoxide; and (d) hydrolysis of the acetal bond if the radical site is at a neighbouring carbon atom. These processes have been amply discussed^{7,8}.

The radiation-induced, radical-reaction processes leading to the chemical modifications identified, summarised in Table I, are illustrated in Schemes 1-4. The last step in each reaction pathway is hydrolysis to the monosaccharide identified in the hydrolysate mixture.

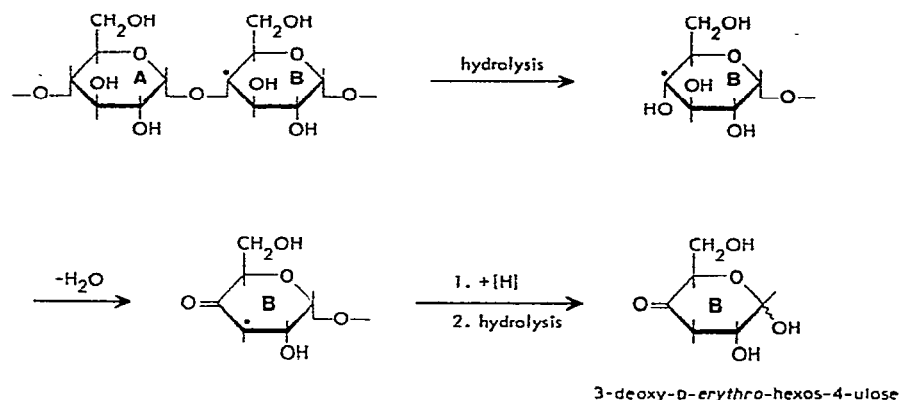
The radical-reaction processes, leading to functional group changes and resulting from initial attack at C-1, are outlined in Scheme 1. A glucono-1,5-lactone function is formed either after hydrolysis of the glycosidic bond next to the radical position followed by removal of a hydrogen atom or, preferably, radical rearrange-



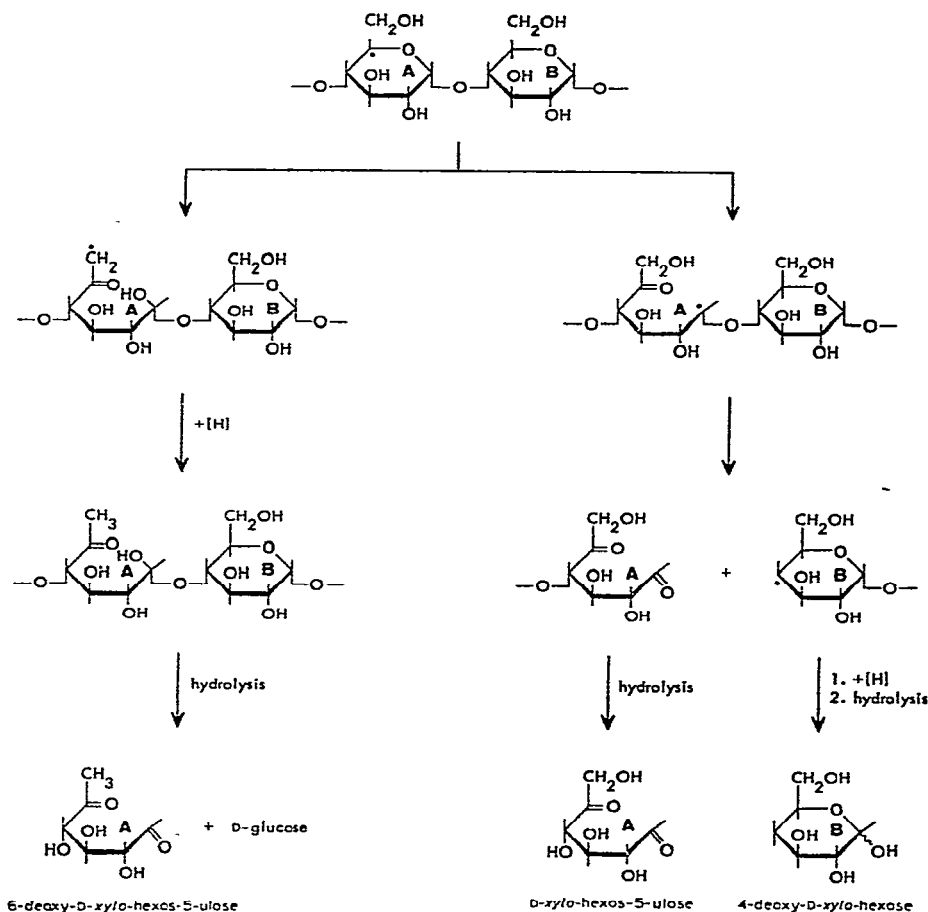
Scheme 1. Chemical modifications following radical formation at C-1 of a cycloamylose D-glucose residue. [H] denotes a hydrogen atom transferred in a disproportionation or hydrogen-abstraction reaction.

ment, C-1 \rightarrow C-4. In the latter instance, the transfer of the radical site to C-4 yields a 4-deoxy function after hydrogen transfer by disproportionation. The modified, unbranched oligosaccharide would thus contain these two functions on terminal residues and be derived from malto-hexa- or -heptaose. Other reactions, involving loss of CO and H₂O from C-1 radicals, are proposed to account for the formation of five-carbon sugars, namely D-arabinose and 2-deoxy-D-erythro-pentose. D-Ribose is thought to be formed *via* an enol in the disproportionation reaction^{7,8}. D-Arabinose is the major product (see also Scheme 1).

The 3-deoxy-4-keto function is formed *via* a radical hydrolysis step followed by elimination of water and addition of hydrogen (Scheme 2).



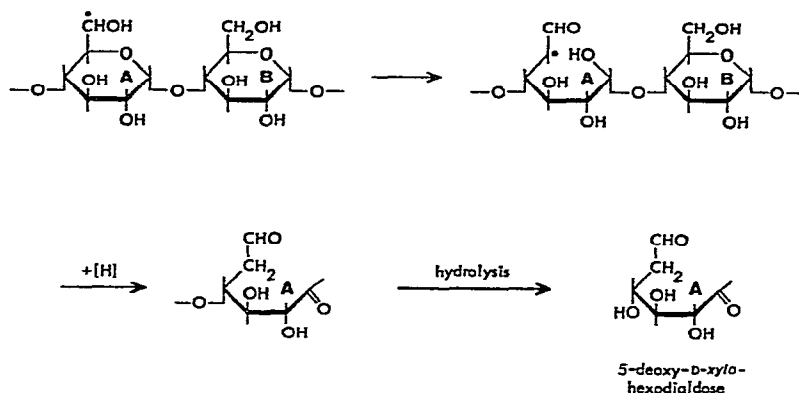
Scheme 2. Chemical modifications following radical formation at C-4 of a cycloamylose D-glucose residue. $[H]$ denotes a hydrogen atom transferred in a disproportionation or hydrogen-abstraction reaction.



Scheme 3. Chemical modifications following radical formation at C-5 of a cycloamylose D-glucose residue. $[H]$ denotes a hydrogen atom transferred in a disproportionation or hydrogen-abstraction reaction.

Scheme 3 illustrates the radical reactions leading to chemical modifications identified following initial attack at C-5. A radical rearrangement, C-5 \rightarrow C-1, accounts for the formation of a 5-keto function. A 4-deoxy function is produced after further rearrangement of the radical site, C-1 \rightarrow to C-4', followed by hydrogen transfer by disproportionation. Thus, formation of a 4-deoxy function is possible *via* initial attack at C-1 or C-5. The unbranched, doubly modified oligosaccharides of the maltohexa- or hepta-ose type can also possess 4-deoxy and 5-keto functions on terminal residues. A 6-deoxy-5-keto function has also been identified, and the radical-reaction process leading to this modification is considered to commence at C-5, as the keto group is located at this position.

As shown in Scheme 4, the 5-deoxy-xylo-hexodialdose function is probably formed *via* initial radical attack at C-6 followed by a rearrangement, C-6 \rightarrow C-5, and hydrogen transfer by disproportionation. A similar reaction for crystalline D-fructose leads to a chain reaction giving rise to 6-deoxy-D-threo-2,5-hexodiulose¹¹. No satisfactory mechanisms can be proposed for the formation of the chemical modifications 10–15. Dideoxy sugars and sugars having fewer carbon atoms than the starting material are typical for solid-state irradiations¹².



Scheme 4. Chemical modifications following radical formation at C-6 of a cycloamylose D-glucose residue. [H] denotes a hydrogen atom transferred in a disproportionation or hydrogen-abstraction reaction.

It is evident from the wide range of products formed from carbohydrates during irradiation that the radicals initially produced are able to undergo facile reactions in the solid state. Chain reactions^{9–11} have even been observed. It has been proposed that radicals that are reactive and participate in the chain reaction during irradiation of 2-deoxy-D-erythro-pentose¹⁰ are structurally different from the trapped radicals observed by e.s.r.²⁷. Also, Gräslund and Löfroth²⁸ have concluded that their data for γ -irradiated, single crystals of trehalose dihydrate and sucrose monohydrate are consistent with the formation of secondary radicals *via* elimination of water from primary radicals.

In irradiated cycloamylose hydrates, the radicals are considerably less stable than in other carbohydrate systems. In the cycloamylose hydrates²⁹, the radicals produced by ionizing radiation decay rapidly above -30° . They are unstable at room temperature³ and thus will react rapidly *via* the reaction steps considered in Schemes 1–4. In addition, since the cycloamyloses are loosely hydrated³⁰, hydrolysis of the radicals may also occur, as proposed recently for cellobiose⁷ and D-glucose⁸ when irradiated in aqueous solution.

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