Radiation Chemistry of Carbohydrates, XV
OH Radical Induced Scission of the Glycosidic Bond in Disaccharides*

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In the γ-radiolysis of deoxygenated, N_2O-saturated aqueous solutions of maltose, lactose, gentiobiose, melibiose, trehalose and sucrose carbohydrate products containing five or six carbon atoms have been identified and their G-values measured. These products originate from the OH radical induced scission of the glycosidic bond of the disaccharides. The nature of the products is in agreement with a reaction scheme proposed previously for the radical induced scission of the glycosidic bond of cellobiose. It involves hydrolysis and the rearrangements of radicals with the free spin next to the glycosidic bond and to the lactol bridge. The nature the glycosidic bond (α, β, 1–4, 1–6, 1–1, 1, 2) has only little influence on the G-values of its scission which range between about 1.9 and 3.5.

Introduction

OH radicals are very reactive species and react at nearly diffusion controlled rates with carbohydrates[1] by abstracting carbon bound hydrogen atoms. In a recent study on cellobiose[2,3] it has been shown that the radicals at the positions C-4, C-1′ and C-5′ undergo a number of reactions which eventually lead to the scission of the glycosidic linkage. A detailed reaction scheme has been postulated[3] to account for $> 98\%$ of the material found in the C_5 and C_6 product fraction. The present study makes use of the structural differences of various disaccharides in order to test the proposed scheme, e.g. in lactose, products from the galactose moiety can be distinguised from those of the glucose moiety. Gentiobiose and melibiose have the 1.6-linkage, in contrast to the 1.4-linkage in cellobiose, maltose, and lactose. The differences in the effects of α- and β-glycosidic linkages can be tested by comparing cellobiose and maltose, or gentiobiose and melibiose. Sucrose (fructose, a keto sugar as sub-unit) and trehalose (1.1-linkage) are further cases in point.

The OH radicals are most conveniently generated by γ-irradiating deoxygenated N_2O saturated aqueous solutions of the disaccharides (10^{-2} mol · l^{-1}). Under these conditions the solvent water is radiolysed according to reaction (1).

$$H_2O \text{ — } \gamma \rightarrow OH\cdot, e^-_{aq}, H\cdot, H^+, OH^-, H_2O_2, H_2 \quad (1)$$

$$e^-_{aq} + N_2O \rightarrow \cdot OH + OH^- + N_2 \quad (2)$$

The solvated electron (e^-_{aq}) is converted by N_2O into OH radicals (reaction (2)). The reactive species of the system then consist of $\sim 90\%$ OH radicals and $\sim 10\%$ H atoms. Both abstract carbon bound hydrogen atoms, the latter with a somewhat slower rate.

The radiolysis of aqueous solutions of some disaccharides has already been investigated by the groups of KOCHETKOV[4–8] and PHILLIPS[9,10], although largely under somewhat different conditions. Product studies on the radiation chemistry of disaccharides and polysaccharides in the solid state[11–16] may also, at least in part, be considered relevant to the present investigation.

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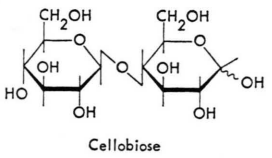
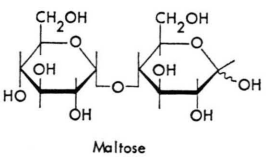

Cellobiose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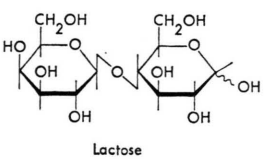
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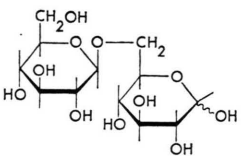
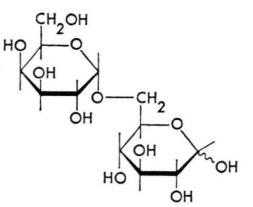
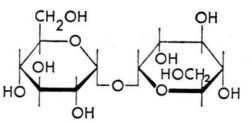
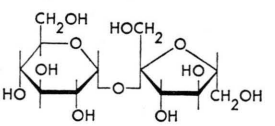

Gentiobiose


Melibiose


Trehalose


Sucrose

Results and Discussion

The disaccharides were γ-irradiated in deaerated N_2O saturated aqueous solutions (10^{-2} mol · l^{-1}). Products were analysed by GC–MS [2] after derivatisation of the products by direct trimethylsilylation, by $NaBD_4$ reduction followed by trimethylsilylation or by methoximation followed by trimethylsilylation as described previously [17].

In the present study only the products with six and five carbon atoms have been analyzed. They originate from the radical induced scission of the glycosidic bond. Most of the products identified have already been described in the preceding studies on cellobiose [2, 3], glucose [17] and fructose [18]. Only in the few cases where new products are encountered will the interpretation of the mass spectra be given. In general, the identification of the products has not been as detailed as in the cellobiose study [2].

Table I. γ-Radiolysis of dissacchrides in deoxygenated N_2O saturated aqueous solutions. G-values of products as TMS derivatives, $NaBH_4$–TMS derivatives and methoxime-TMS derivatives.

	Cellobiose [3]	Matlose	Lactose	Gentiobiose	Melibiose	Trehalose	Sucrose
TMS-derivatised samples							
Glucose	2.1	2.0	1.2	1.6	1.15	3.5	1.6[a]
Galactose	–	–	0.6	–	0.50	–	–
Fructose	–	–	–	–	–	–	1.1[a]
Gluconic acid	0.70	0.15	–	0.10	–	0.4	0.2
Galactonic acid	–	–	0.35	–	0.10	–	–
4-Deoxyglucose	0.27	0.05	0.32	–	–	–	–
$NaBH_4$–TMS-derivatised samples							
Glucitol	2.9	3.0	1.7	2.6	2.05	5.5	3.85
Galactitol	0.03	0.10	1.7	0.02	0.80	traces	–
Mannitol	traces	0.05	traces	0.03	traces	traces	1.1
1-Deoxyhexitols	0.02	traces	0.01	0.30	0.30	traces	0.10
2- and 3-Deoxyhexitols	1.3	1.5	1.5	0.38	0.31	1.4	0.15
Arabinitol	0.07	0.12	0.15	0.03	0.03	traces	0.25
Ribitol	traces	traces	–	–	–	traces	–
Xylitol	–	–	–	0.01	0.01	traces	traces
2-Deoxyribitol	0.17	0.11	–	traces	traces	traces	traces
2-Deoxyxylitol	–	–	0.10	traces	traces	traces	traces

[a] Methoxime-TMS derivatives.

Thus, the yields of the various deoxyhexitols (obtained through reduction of deoxy compounds) largely appear as two sums and no attempt has been made to differentiate in detail. Table I collects most of the data. Each value given in this table is based on at least five measurements in the dose range $1.5 \cdot 10^{18}$ to $20 \cdot 10^{18}$ eV g^{-1}. The G-values given are initial G-values (extrapolated to zero dose). Since glucose and galactose (glucitol and galactitol after reduction) are most strongly affected because of their marked dose dependence (*cf.* ref[3]) the accuracy of these values will suffer from this extrapolation. At low enough pH, maltose and sucrose hydrolyze to a small extent even without irradiation. A provision to preclude this consisted in bringing the pH up to ≈ 7.5 with $Ba(OH)_2$. Under these conditions the disaccharides were stable. On irradiation the pH only fell to 6.5, and for the short work-up period corrections were not necessary. Acids are reducible with $NaBH_4$ only as lactones, and incomplete lactonisation prior to, or ring-opening during, reduction may give somewhat too low G-values for the deoxyhexonic acids. The reduction method does not allow a full determination of the hexosuloses and hexodialdoses. There, the methoximation technique is superior. Again, except for the identification of key products, a detailed analysis (which is possible) has not been sought. In favourable cases the G-values are thought to be accurate within $\pm$ 10%, but in cases as described above a higher limit of errors has to be considered.

In the preceding paper[3], a detailed scheme for the radical induced scission of the glycosidic bond of cellobiose has been given. Scheme 1 repeats this in a condensed form with the emphasis being laid upon the key reactions, omitting the subsequent free radical reactions of the subunit radicals. For the latter see ref.[3,17].

Maltose and lactose

Maltose largely follows the route of cellobiose. However, there is a noticeable reduction in G(4-deoxyglucose) which is also reflected in G(gluconic acid). One might interprete this result as a competition between reactions (3) and (5) (reaction (11) does not play a significant role in the formation of 4-deoxyglucose[3]). Maltose (an α-glucoside) is known to hydrolyse much more easily than cellobiose (a β-glucoside). Hence, the enhanced rate of hydrolysis may also persist in the radical although one would expect that the conformational differences are rapidly lost once the radical is formed. However, the high yield of 4-deoxyglucose in the case of lactose (β-glucoside) (Table I) which is comparable to that from cellobiose tends to support this interpretation.

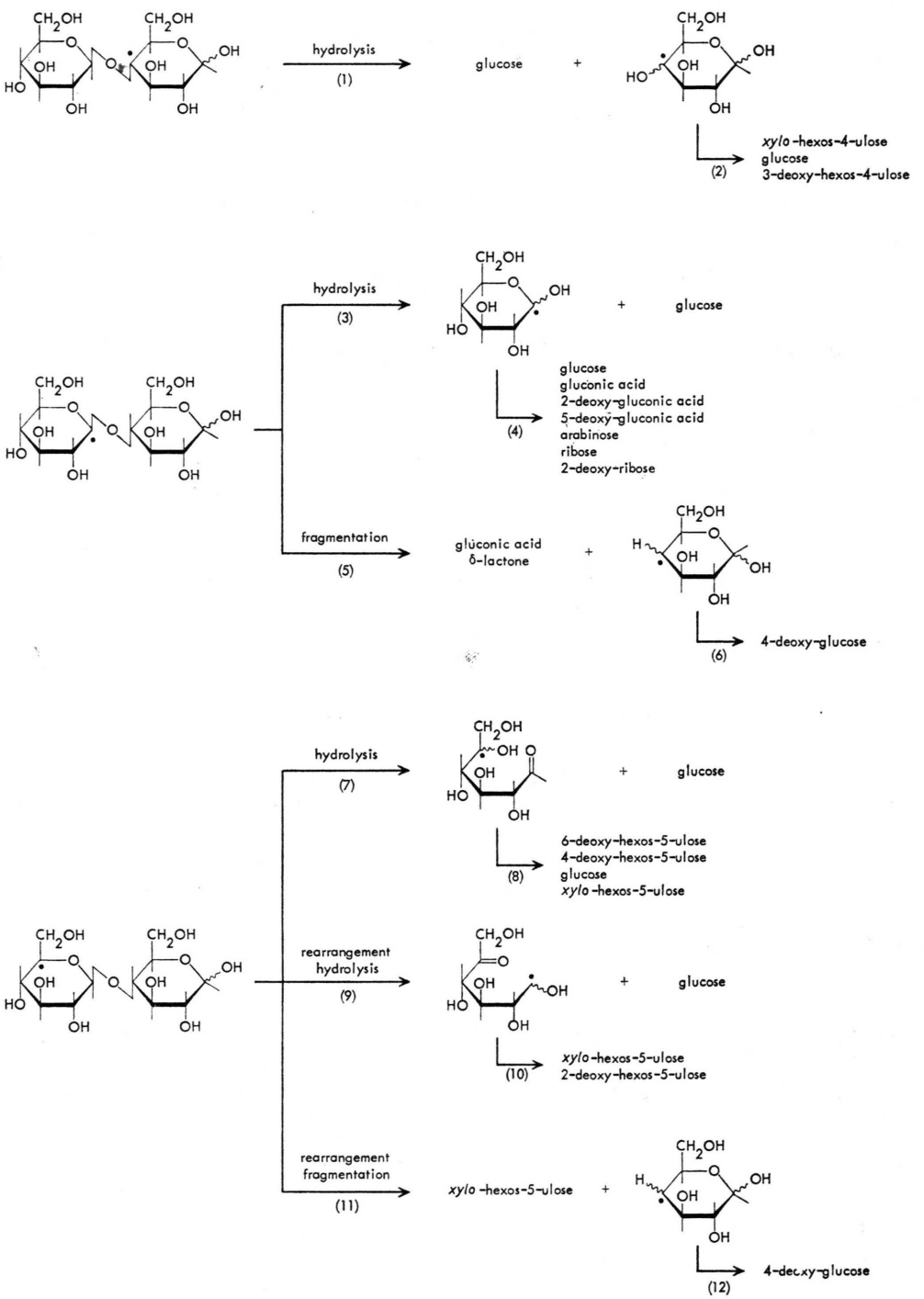
Lactose has been used to test some of the mechanistic suggestions of the Scheme since this compound contains one galactose unit, instead of only glucose units in the case of cellobiose and maltose. In agreement with the Scheme all the hexonic acid appears as galactonic acid, and 2-deoxyxylose (instead of 2-deoxyribose) are found.

Gentiobiose and melibiose

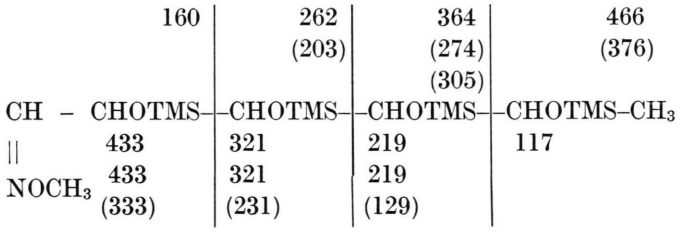
Gentiobiose and melibiose introduce a new aspect by the 1.6-linkage. This may lead to a scission of the glycosidic linkage *via* the radical at C-6 (similar to reaction (1) in the Scheme). In accordance with this, we identified *gluco*-hexodialdose (methoxime-TMS derivative; compared with an authentic sample[17]). The equivalent process to process (5) of the Scheme leads in these two disaccharides to radicals centered at C-6 which would give rise to the formation of 6-deoxyglucose. 6-Deoxyglucose might further be formed in processes similar to (11) and (12) in the Scheme (*xylo*-hexos-5-ulose has been identified as a product, *see* below). 6-Deoxyulose has been identified both as $NaBD_4$–TMS[19] and methoximation-TMS derivative. Prominent fragment ions in the mass spectrum of the $NaBD_4$–TMS sample are: *m/e* 73 (100%), 103 (10%), 104 (15%), 117 (95%), 118 (30%), 129 (8%), 147 (35%), 206 (30%), 207 (7%), 218 (9%), 219 (15%), 231 (5%), 308 (3%), 320 (18%), 333 (0.3%).

117	219 (129)	321 (231)	423 (333)	
CH_3–CHOTMS–	–CHOTMS–	–CHOTMS–	–CHOTMS–	–CHDOTMS
	410 (320)	308 (218)	206	104

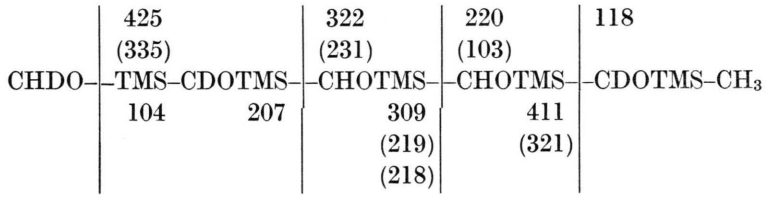
Scheme proposed for the radical induced scission of the glycosidic linkage of cellobiose[3]



There is only a small interference by the material of an adjacent somewhat overlapping GC-peak as is obvious from *m/e* 118 and 207. (The most relevant fragment ions of the mass spectrum of this product are given below). The methoxime-TMS derivative also shows an indicative mass spectrum with typical fragment ions at *m/e* 73 (70%), 117 (100%), 129 (4%), 147 (10%), 160 (30%), 203 (1.5%), 204 (1.5%), 219 (5%), 231 (1.5%), 262 (< 1%), 274 (< 1%), 277 (7%), 305 (2%), 321 (2%), 333, 336, 364, 376 (< 1%).



However, 6-deoxyglucose is not the only 6-deoxy compound as is evidenced by a number of further GC-peaks in the 6-deoxyhexitol-TMS region of a $NaBD_4$–TMS treated sample. The mass spectrum of the isomer which suffers least from interference shows the prominent fragment ions at *m/e* 73 (100%), 104 (12%), 117 (5%), 118 (76%), 130 (6%), 147 (18%), 206 (5%), 207 (7%), 218 (4%), 219 (3%), 220 (5%), 231 (1%), 308 (< 1%), 309 (1%), 320 (3%), 321 (4%), 335 (< 1%).



The precursor of this derivative is thought to be 6-deoxyhexos-2,5-diulose, underlain to some extent by 6-deoxyhexos-5-ulose. Both compounds are thought to arise from radical reactions after attack at C-5. A conclusive mechanism is not seen at present.

The detection of these products may indicate new possible routes for the radical induced scission of the glycosidic linkage not encountered in the previous study.

In accordance with the Scheme is the formation of galactonic acid and the absence of gluconic acid in melibiose, and the identification of *xylo*-hexos-5-ulose (methoxime-TMS, authentic material available[20]).

Trehalose and sucrose

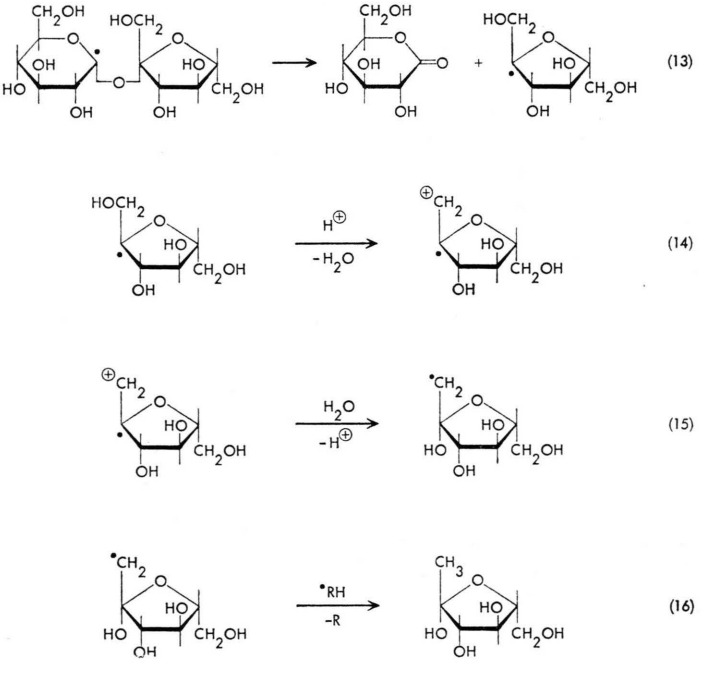
In trehalose, key products are D-glucose, D-gluconic acid, 5-deoxy-D-xylo-hexonic acid, and 2-deoxyhexos-5-ulose. The formation of *xylo*-hexos-5-ulose is evidenced by a prominent iditol peak in a reduced sample. There are also traces of arabinitol, ribitol and 2-deoxyribitol. These products are in agreement with routes from C-1, C-1′ and C-5, C-5′ (*cf.* Scheme).

The processes in sucrose are less well documented. No mass spectra were available and product identification is based on GC retention times (peak matching). D-Glucose, D-fructose and D-gluconic acid are the major products. There are substantial amounts of arabinose but only traces of 2-deoxyribose. It is possible that this prominence of arabinose is connected with the fructose moiety, but at the present time no mechanistic proposal can be put forward to account for this. An interesting product is 1-deoxyfructose. Its assignment is based on the fact that in the 1-deoxyhexitol TMS ether region two peaks with approximately equal intensity appear. The well-known compound 6-deoxy-D-*threo*-2,5-hexodiulose[18] gives under the same conditions (reduction, trimethylsilylation) four GC peaks, two of them coinciding with the peaks in question. The mechanistic proposal for the formation of this compound is, for the primary

step, the same as route (5) in the Scheme. Reaction (13) gives rise to gluconic acid lactone (identified) and a radical which will eliminate the OH groups, at C-1 with the help of a proton (reaction (14)). Water addition at C-2 and loss of a proton gives the radical centered at C-1, (reaction (15)). The reaction as written in three steps migth also proceed in one step after proton addition at C-1 of the fructose moiety. In a disproportionation reaction with other radicals present in the system the radical will centered at C-1 give 1-deoxyfructose, (reaction (16)).

The above sequence is well established with phosphate as the leaving group [21]. Similar reactions lead to 6-deoxy-D-*threo*-2,5-hexodiulose in crystalline fructose [18] and to 6-deoxyhexos-5-ulose in cellobiose (*cf.* reaction (8) in the Scheme).

There is also some material found in the deoxyhexitol series with the deoxy-group in the interior of the molecule. The rentention times indicate that it is largely due to 2- and 5-deoxyglucitols. Therefore, 2-deoxygluconic acid, 2-deoxyhexos-5-ulose and 5-deoxygluconic acid are likely precursors.



Quantitative aspects

The radical induced scission of the glycosidic linkage is largely governed by hydrolytic processes of the radicals involved. Radical rearrangements appear to play a minor role and can be monitored in the 1,4-linked disaccharides as 4-deoxyglucose (reactions (5) and (11) in the Scheme), and in the 1,6-disaccharides as 6-deoxyglucose. In this respect the 1,1-linked trehalose and the 1,2-linked sucrose are less well understood. Some gluconic acid could be due to such a process.

All the hydrolytic processes eliminate one of the subunits unaltered. G(glucose) is 2.1 in cellobiose, 2.0 in maltose and the combined yield of G(glucose) and G(galactose) is 1.8 in lactose. These values differ by only 10% which suggests that the influence of steric differences on this process is small. In the study on cellobiose [3] it was shown that the attack at C-4 is small compared to the combined attack at C-1′ and C-5′. This is also indicated by the results obtained with lactose, where the galactose yield is only half of the glucose yield. The galactose yield is somewhat higher than one would expect from the data on cellobiose [3] but small contributions from other radicals (*e.g.* C-5 and C-3) could account for this.

Again, G(glucose) from gentiobiose is the same as the combined yield of G(glucose) and G(galactose) from melibiose. G(glucose) is twice as much as G(galactose), in agreement with a preferential scission from the radicals at C-1′ and C-5′ compared

to that from C-6 and C-5. The high glucose yield in the case of trehalose may be due to the fact that in this compound four very efficient sites for the radical induced scission are available, both at C-1 and C-1′ as well as at C-5 and C-5′. In the 1,4-linked disaccharides most of the scission orginated from C-1′ and C-5′. In sucrose there is more glucose eliminated than fructose. The radical induced scission of this compound is not yet well enough understood to draw any conclusions, and warrants a detailed study.

The approximate G-value of the scission of the glycosidic bond can be calculated without too large an error by summing up the non-radical fragments: always glucose in the case of the reactions (1), (3), (7), (9) of the Scheme. The importance of reaction (5) of the Scheme can be evaluated by the yield of the deoxysugar from reaction (6). Reaction (11) appears to be of little importance and is neglected, as is glucose from free radical precursors. With these simplifying assumptions (extended to the other disaccharides) G(scission of the glycosidic bond) in cellobiose is about 2.4, in maltose 2.05, in lactose 2.1, in gentiobiose 1.9, in melibiose 1.95, in trehalose 3.5, and in sucrose 2.8.

Apart from cellobiose[3] (where there were difficulties with the gluconic acid determinations) the yield of the hexitols are markedly higher than the combined yield of the aldoses (in sucrose: glucose and fructose), hexosuloses and the hexonic acids. This difference is thought to exceed the limits of error. It is therefore suggested that in competition to hydrolytic steps, esters are formed in disproportionation reactions of the radicals involved. On reduction these esters would also give rise to hexitols.

Conclusions

In conclusion it can be said that the Scheme appears to give a reasonable prediction of the products formed in the free radical induced scission of the glycosidic linkage of disaccharides. Even in the case of sucrose [which is different from the other disaccharides because of the keto-sugar subunit] some of the routes have been verified. The wealth of the present data appears to justify estimates of the radiation induced degradation of starch and other carbohydrate polymers. This question is of interest to food sterilization and radiation grafting of cotton material.

Experimental

The disaccharides maltose, lactose, gentiobiose, melibiose (all Merck), trehalose (Roth), and sucrose (Mallinckrodt) were used without further purification. The analytical procedures were described previously[3,17,18] except for a minor variation of the GC (53 m glass capillary column OV 101, temperature programmed 2 K min^{-1} 120–260 °C) and methoximation conditions (80 °C for 1 h).

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