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E.s.r. study of the alkaline degradation of disaccharides in methyl sulfoxide*

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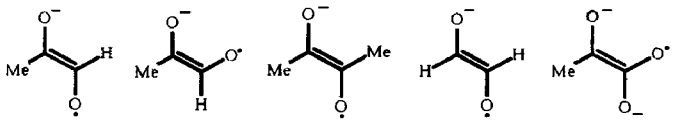
ABSTRACT

The degradation of a series of deoxy sugars and disaccharides in methyl sulfoxide–tetrabutylammonium hydroxide has been studied by e.s.r. spectroscopy. The generation of radicals and the types of radical formed are dependent on the anomeric configuration of non-reducing moieties and on the positions of the linkages and deoxy groups. Mechanisms for the pathways of degradation are proposed.

INTRODUCTION

Previous work[1,2] has shown that radicals are formed during the alkaline degradation of monosaccharides in methyl sulfoxide. Based on e.s.r. data, the radicals were assigned the semi-dione structures **1–5.** These findings allowed postulation[3–5] of the formation of C_2, C_3, and C_4 fragments by a retroaldolisation reaction[6]. These fragments can react with the methyl sulfoxide anion[7–9] to produce α-hydroxycarbonyl compounds that are the immediate precursors of the above radical species[10]. These pathways compete with other non-radical processes.



1 2 3 4 5

The fragmentation process is dependent on the structure of the monosaccharide (chain length and configuration). Thus, hexoses-I (D-glucose, D-fructose, D-mannose, D-idose, and D-sorbose) yielded the semi-diones **1–3** by cleavage of the C-3–C-4 bond in retroaldol reactions[11], as shown in the following sequence.

* Studies of the Alkaline Degradation of Carbohydrates in Methyl Sulfoxide by E.s.r. Spectroscopy, Part 3. For Part 2, see ref. 2.
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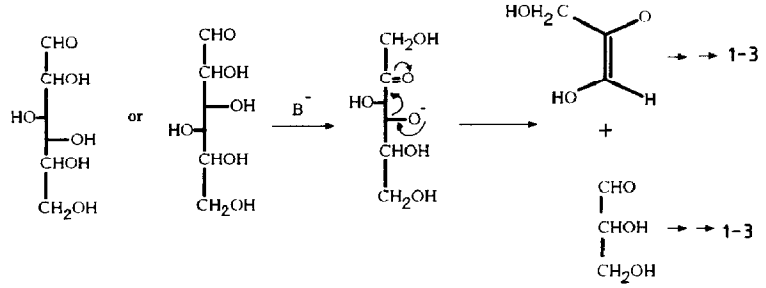
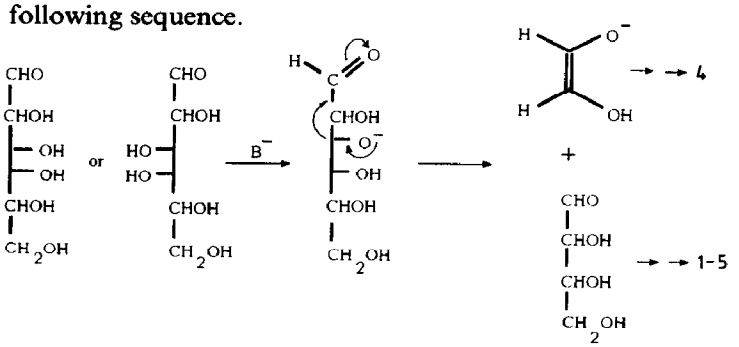


In contrast, hexoses-II (D-allose, D-altrose, D-galactose, D-talose, and D-tagatose) yielded the semi-diones **1–5** by cleavage[12] of the C-2–C-3 bond, as shown in the following sequence.



We now report an extension of this study to disaccharides and deoxy sugars.

EXPERIMENTAL

Spectroscopic data. — A Varian E-12, X-band spectrometer operating at 100 kHz was employed. Measurements were carried out using low microwave power (2–5 mW). An optimum modulation width of 0.05–0.12 G was used. The *g* factor was determined by means of high-precision frequency and gauss meters. The splitting constants in complex spectra were obtained by comparing the experimental spectra with those simulated by EPRSIM[13*].

The relative intensities of the e.s.r. signals were calculated from eqn. *1* (ref. 14).

$$I = \frac{Y \Delta H_{pp}^2 R}{G M_g^2 (\text{scan})^2} \, , \; R = \frac{\sum_j D_j}{D_k} \, , \qquad (1)$$

where I is proportional to the spin concentration of the paramagnetic species, Y is half the peak-to-peak amplitude of the first derivative line, ΔH_{pp} is the peak-to-peak line width, D_k is the degeneracy of the most intense line, D_j is the sum of the degeneracies of all the lines in the spectrum, G is the gain of the signal amplifier, and M is the modulation amplitude in Gauss.

* EPRSIM is a sophisticated program for the simulation of e.s.r. spectra, implemented for PC (XT/AT or compatible) provided with a graphic card (CGA or similar). This program permits the simulation of complex spectra generated by several radicals with different *g* factors. Software is available upon request.

The n.m.r. spectra were recorded in the F.t. mode with a Varian XL-300 spectrometer.

Chromatography. — H.p.l.c. was performed with a Waters 510 chromatograph equipped with a 410 differential refractometer. Each reaction mixture (70mM disaccharide and 150mM tetrabutylammonium hydroxide) was analysed at regular intervals on a column (0.40 × 30 cm) of Sugar Pak 1 by elution with water at 0.5 mL/min.

Generation of radicals. — Solutions of disaccharides (70mM) and tetrabutylammonium hydroxide (150mM) in methyl sulfoxide were mixed and transferred immediately to a measuring e.s.r. cell. Spectra were recorded at room temperature and the evolution of the radical anions was checked at regular intervals.

RESULTS AND DISCUSSION

Methyl α- and β-D-glucopyranoside were studied in order to establish the effect of the configuration at the anomeric centre, and 2-deoxy-D-*arabino*-hexose (**6**, 2-deoxy-D-glucose), 3-deoxy-D-*ribo*-hexose (**7**, 3-deoxy-D-glucose), 5-deoxy-D-*threo*-hexulose (**8**, 5-deoxy-D-sorbose), L-rhamnose (**9**), and D-fucose (**10**) were chosen in order to study the effect of the absence of particular hydroxyl groups. The disaccharides studied were trehalose (**12**), maltose (**13**), cellobiose (**15**), sucrose (**16**), turanose (**17**), and lactose (**18**).

Methyl α- and β-D-glucopyranoside. — On degradation of these glycosides with alkali under the standard conditions (see Experimental), the α anomer gave no radicals, but the β anomer yielded radicals **1–3** that were obtained also from D-glucose[1] but with much lower signal intensities.

Thus, the degradative pathway is markedly affected by the configuration at the anomeric centre and it can be predicted that radicals will be formed only from the appropriate β-linked disaccharides. The differences in the behaviour of the methyl glucosides could reflect differences in their rates of hydrolysis in the basic medium. Thus, methyl[15,16] and phenyl[17,18] β-D-glucopyranosides are much more alkali-labile than the corresponding α anomers, which has been attributed to electronic and steric factors[15,19]. The delay observed in the formation of radicals from β-D-glucopyranosides, relative to D-glucose, can be explained on the basis of the need for prior hydrolysis.

Deoxyhexoses. — The results for the deoxy sugars **6–10** are summarised in Table I.

No radical species were formed from 2-deoxy-D-glucose (**6**), since the compound does not contain the necessary α-hydroxycarbonyl group. 3-Deoxy-D-glucose (**7**) yielded the radicals **1–3**, in contrast to D-allose, from which radicals **1–5** were formed. Thus, the reaction of **7** involves cleavage of the C-3–C-4 bond (Scheme 1) to yield **A2** and glyceraldehyde (**11**); cleavage of the C-2–C-3 bond is not possible for **7**.

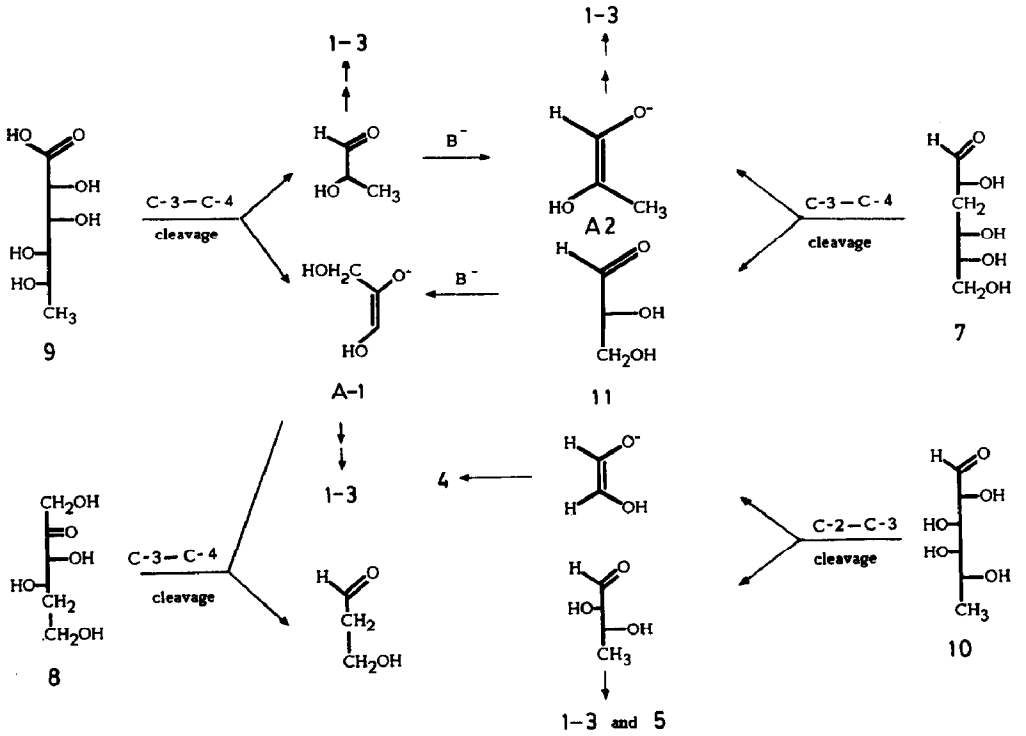
The e.s.r. spectrum obtained from 5-deoxy-D-sorbose (**8**) is similar to that from D-sorbose, but the signal intensities were significantly lower (Table I). No signal attributed to the radicals **4** and **5**, formed by cleavage of the C-2–C-3 bond, could be detected. Cleavage of the C-3–C-4 bond in **8** (Scheme 1) yields two fragments, only one of which (**A1**) can yield the radicals **1–3**.

TABLE I

Intensities of the e.s.r. signals[a,b]

Compound	*Radical*				
	1	**2**	**3**	**4**	**5**
6	——(44.00)[c]	——(8.00)	——(13.33)	——(——)	——(——)
7	23.33(4.67)	4.50(1.13)	13.87(0.80)	——(1.80)	——(0.80)
8	10.00(28.33)	2.13(4.80)	4.20(12.33)	——(——)	——(——)
9	27.67(33.33)	5.13(6.32)	7.87(24.32)	——(——)	——(——)
10	25.32(8.34)	5.13(1.27)	13.60(6.00)	1.87(1.61)	2.00(1.60)

[a]After reaction for 40 min. [b]Calculated from eqn. *1*; all the values are normalised with respect to the radical **1** obtained from 5-deoxy-D-sorbose (**8**). [c]The values in parenthesis correspond to the hydroxy derivative.

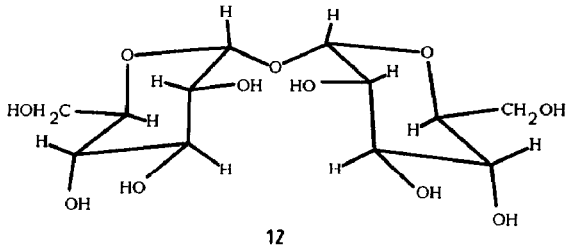


Scheme 1. Reaction pathways for **7–10**.

The radicals formed from L-rhamnose (**9**) and D-fucose (**10**) were the same as those formed from D-mannose and D-galactose, and involved cleavage of the C-3–C-4 bond (Scheme 1). The more extensive formation of the radicals **1–3** from D-fucose than from D-galactose is due to the fact that cleavage of the C-2–C-3 bond in the former gives a fragment that is the immediate precursor of the radicals **1–3**. The formation of these radicals from D-galactose must involve double methylation of glycolaldehyde.

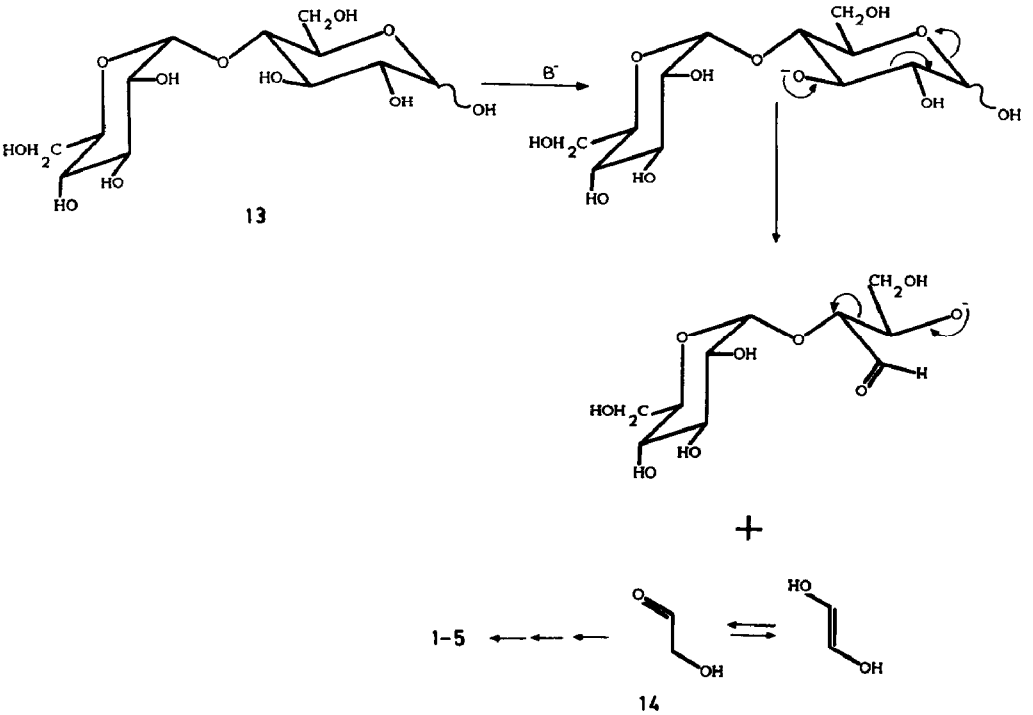
Thus, if the deoxy group is at position 5 or 6, the cleavage will involve the same bond as in the corresponding hydroxy compound. For a 3-deoxyhexose, the cleavage will involve the C-3–C-4 bond to yield the radicals **1–3**; for a 4-deoxyhexose, the cleavage would probably involve the C-2–C-3 bond, giving rise to radicals **1–5**. This process is impossible for a 2-deoxy derivative.

α,α-Trehalose. — Under the standard conditions, no radical species were generated from α,α-trehalose (**12**) during 1 h. This result accords with the finding for methyl α-D-glucopyranoside.



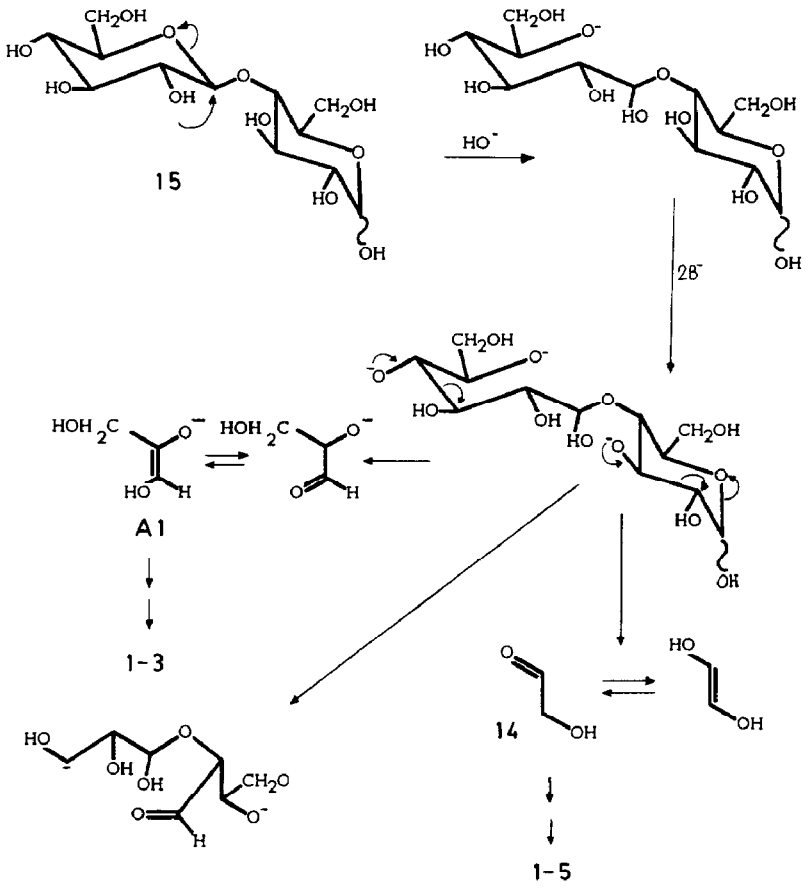
12

Maltose. — The e.s.r. spectrum obtained from maltose (**13**) indicated the formation of the radicals **1–5**. Since the α-D-glucopyranosyl group cannot yield any radical species, it is assumed that **1–5** arise by cleavage of the C-2–C-3 bond in the reducing moiety. Cleavage of the C-3–C-4 bond is not possible since this would require a



13 14

Scheme 2. Reaction pathway for maltose (**13**).

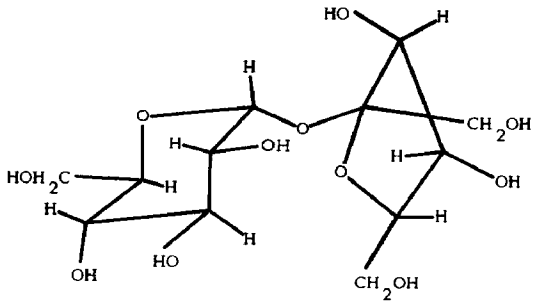


Scheme 3. Reaction pathway for cellobiose (**15**).

hydroxyl group at position 4. The radicals obtained probably arise from glycolaldehyde (**14**) (*cf.* ref. 20), as shown in the mechanism proposed in Scheme 2.

Cellobiose. — Degradation of this disaccharide (**15**) gave radicals **1–5**, as did maltose. However, the radicals **1–3** were significantly more intense from cellobiose than from maltose, possibly by cleavage of the non-reducing unit by analogy with methyl β-D-glucopyranoside. Thus, radicals **1–3** could be generated from both the reducing and non-reducing units, whereas radicals **4** and **5** would be generated only from the former unit, as shown in Scheme 3. An intermolecular displacement at the glycosidic linkage by a hydroxyl ion, a mechanism previously suggested at low concentrations of base[21] or low temperatures[22], could account for the reactivities of maltose (**13**) and cellobiose (**15**), and for the stability of α,α-trehalose (**12**).

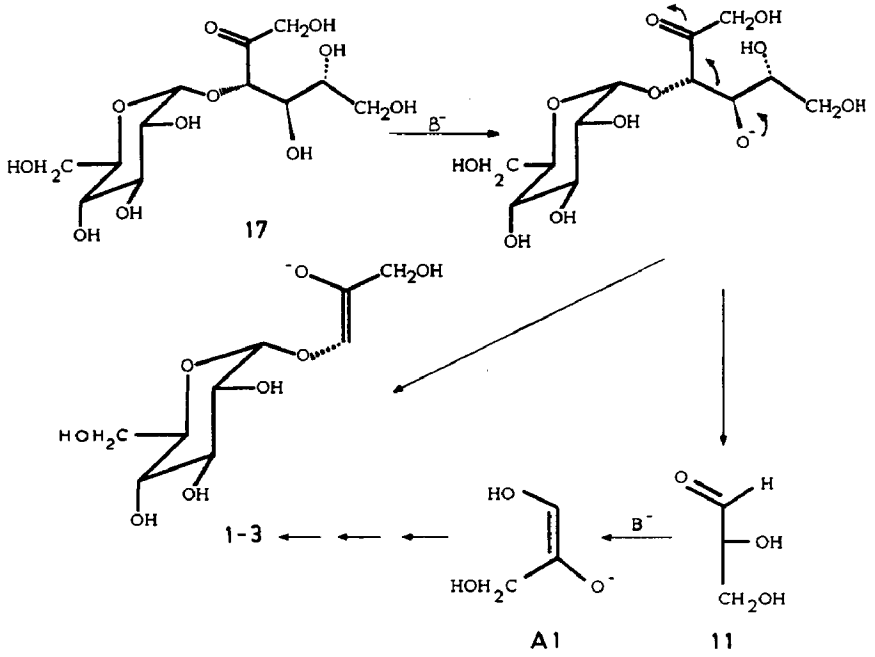
Sucrose. — No radical species were generated from sucrose (**16**) during 1 h. Thus, the β-D-fructopyranosyl group, like the α-D-glucopyranosyl group, does not give rise to radicals, and h.p.l.c. showed that both α,α-trehalose (**12**) and sucrose (**16**) were stable under the alkaline conditions used in this work.



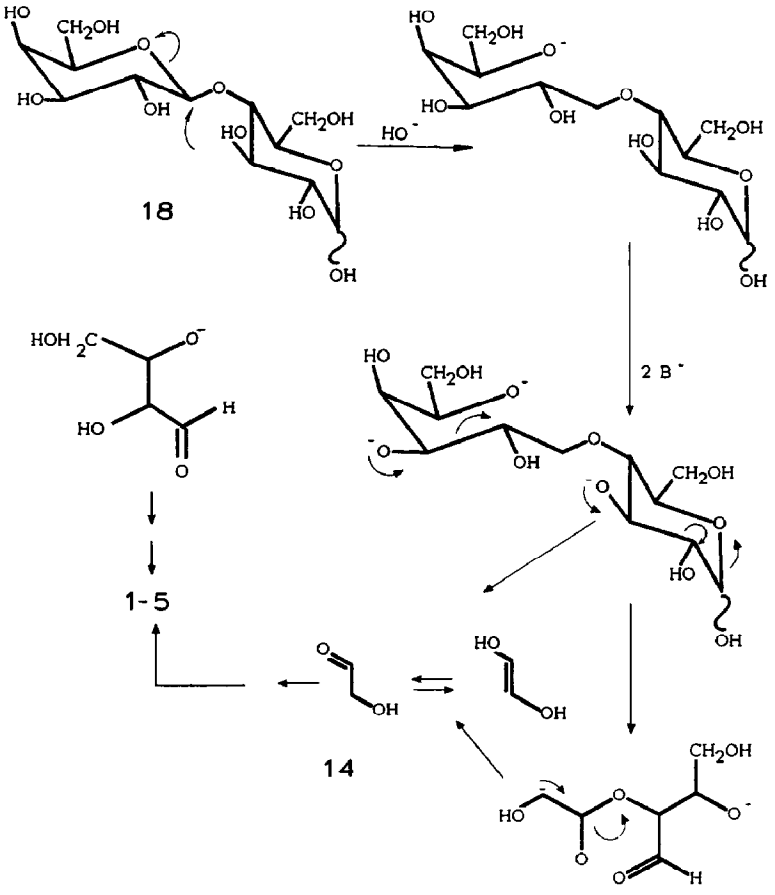
16

Previous studies[23,24] have shown that sucrose had unusual alkali lability under conditions (1.0–5.7M base at 100–170°) where methyl α-D-glucopyranoside and methyl β-D-fructofuranoside were not affected significantly. A co-operative process involving both the glucose and fructose moieties was suggested[24] as the main pathway of degradation. This process required the ionisation of more than one hydroxyl group in the sucrose molecule, which did not occur under the conditions used here.

Turanose. — The e.s.r. spectrum obtained from turanose (**17**) indicated the generation of the radicals **1–3**, which can be produced only from the fructose residue since the glucosyl group is α. From the results found for 3-deoxy-D-glucose (**7**), it is assumed that the C-3–C-4 bond in the fructose moiety is cleaved to give **11** from which the radicals **1–3** can be generated[1]. The reaction pathway proposed is shown in Scheme 4.



Scheme 4. Reaction pathway for turanose (**17**).



Scheme 5. Reaction pathway for lactose (**18**).

Lactose. — The e.s.r. spectrum obtained from lactose (**18**) reflects the formation of the radicals **1–5**, and the reducing glucose residue probably reacts as in maltose and cellobiose. However, the signals for the radicals **4** and **5** were more intense than those from maltose (**13**). This finding suggests that the galactosyl group in **18** yields the radicals **1–5** by cleavage of the C-2–C-3 bond, as proposed[2] for galactose. The susceptibility of β-galactosides to basic hydrolysis, in contrast to the α anomers, has been recorded[15,17]. The reaction pathway is shown in Scheme 5.

The foregoing results show that the degradation of disaccharides in methyl sulfoxide–tetrabutylammonium hydroxide is highly dependent on the anomeric configuration of the non-reducing unit. The position of the linkage in the disaccharides and that of a deoxy group in the monosaccharides are also crucial. If the non-reducing unit is D-glucosyl, only the β anomer will yield radical species (**1–3**, as obtained from D-glucose). An α-D-glucopyranosyl or a β-D-fructofuranosyl moiety will not react. The reducing hexose residue always reacted under the conditions studied. A 3-linked unit gives the radicals **1–3** by cleavage of the C-3–C-4 bond. A 4-linkage promotes cleavage

of the C-2–C-3 bond, yielding radicals **1–5**. For **5**- or **6**-linkages, the bond cleaved is the same as that in the corresponding monosaccharide. However, the pathways concerned are probably only minor routes, because weak e.s.r. signals of relatively stable radicals do not always reveal major fragmentation pathways.

ACKNOWLEDGMENT

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