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A study of the alkaline degradation of carbohydrates in methyl sulfoxide by e.s.r. spectroscopy: Part 2, monosaccharides

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ABSTRACT

A mechanism for the degradation of monosaccharides at room temperature in methyl sulfoxide containing tetrabutylammonium hydroxide has been proposed based on e.s.r. spectroscopic data. Several radicals with semi-dione-like structures were generated during this process, and their hyperfine splittings and *g* factors have been measured. The mechanism of the reactions was dependent on the chain length and configuration of the sugar.

INTRODUCTION

We have described[1] the generation of radical species in the alkaline degradation of D-glucose in methyl sulfoxide. The radicals **1–3** detected were sufficiently stable to allow the determination of their magnetic parameters and time dependences. The structures proposed were *trans*- (**1**) and *cis*-propanesemi-dione (**2**), which were in thermodynamic equilibrium[2], and *trans*-2,3-butanesemi-dione (**3**).

Radicals **1** and **2** were generated from hydroxyacetone, and **3** from 3-hydroxybutane, by a mono-electron oxidation process, and the following mechanism was proposed: (*a*) cleavage of the D-glucose molecule by a retro-aldol reaction to yield two C_3 fragments[3], (*b*) methylation of these fragments by the methyl sulfoxide anion[4], (*c*) formation of α-hydroxycarbonyl compounds, and (*d*) mono-electron oxidation to yield the semi-diones[5].

The aims of the work now reported were to carry out an e.s.r. study of the behaviour of monosaccharides under the experimental conditions used for D-glucose[1], to establish the influence of chain length and stereochemistry on the pathways of degradation, and to propose a mechanism.

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EXPERIMENTAL

Generation of radicals. — Solutions of monosaccharides (70mM) and tetrabutylammonium hydroxide (150mM), both prepared in methyl sulfoxide, were mixed and transferred to an e.s.r. cell. The spectra were recorded at room temperature and the formation of the radical anions was monitored.

Spectroscopic data. — A Varian E-12 X-band spectrometer operating at 100-kHz field modulation was employed. Measurements were carried out using low microwave power (2–5 mW) with an optimum modulation width of 5–12 μT. The g factor was determined by means of high-precision frequency and gauss meters. For complex spectra, the splitting constants were obtained by comparing the experimental spectra with those simulated by means of the EPRSIM package. EPRSIM comprises the programs EPRSIM.EXE and COMPOSER.EXE. The first simulates a single e.s.r. spectrum and the second simulates complex spectra composed of several radicals with different g factors. These programs have been implemented for PC (XT/AT or compatible) provided with graphic card (CGA or similar). Software is available upon request.

The relative intensities of e.s.r. signals were calculated from equation *1* (ref. 6),

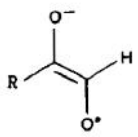
$$I = C\frac{Y\,\Delta H_{pp}^2 R}{GMg^2(\text{scan})^2}; \qquad R = \frac{\Sigma_j D_j}{D_k} \tag{1}$$

where I is proportional to the spin concentration of the paramegnetic 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.

The *in situ* electrochemical reduction involved methyl sulfoxide as the solvent and tetraethylammonium tetrafluoroborate (100mM) as the supporting electrolyte. The electrodes were Au–Au, and the solutions were degassed and maintained in an inert atmosphere during the electrolysis. A current intensity of 70 mA was applied.

RESULTS AND DISCUSSION

The degradation of several monosaccharides (Table I) has been studied by e.s.r. and the formation of long-lived radicals was detected for each sugar. Moreover, the number and intensities of the e.s.r. signals depended on the length of the sugar chain and the configuration. Thus, from D-glucose, D-mannose, D-idose, D-fructose, and D-sorbose (referred to as hexoses-I), and from trioses, the radicals **1–3** were detected (Fig. 1). On the other hand, D-allose, D-altrose, D-galactose, D-talose, and D-tagatose (hexoses-II), pentoses, and tetroses gave radicals **4** and **5** in addition to **1–3**. The formation of **1–5** in the degradation of D-threose as a function of time is represented in Fig. 2. The maximum intensities of the signals for **1–5**, calculated by using equation *1*, are shown in Table I, and the magnetic parameters are given in Table II.



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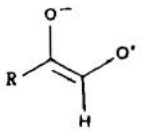

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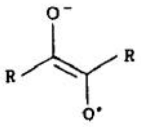

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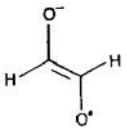

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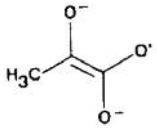

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TABLE I

Optimum values of the e.s.r. signal intensities[a,b]

Monosaccharide	*Radicals*				
	1	2	3	4	5
Hexoses-I					
D-glucose	10.00	1.88	3.98	–	–
D-mannose	8.98	1.80	7.50	–	–
D-idose	8.78	1.43	3.20	–	–
D-fructose	9.84	1.88	6.56	–	–
D-sorbose	6.71	1.10	3.20	–	–
Hexoses-II					
D-allose	0.97	0.34	0.34	0.47	0.23
D-altrose	1.88	0.31	0.31	0.50	0.14
D-galactose	1.75	0.31	1.48	0.50	0.34
D-talose	0.63	0.23	0.16	0.34	0.23
D-tagatose	1.95	0.39	1.09	0.16	0.19
Others					
D-ribose[c]	2.73	0.50	0.78	1.36	–
D-arabinose[c]	2.38	0.39	0.86	0.39	–
D-xylose	4.69	0.78	2.03	0.31	0.19
D-lyxose	3.90	0.63	1.17	0.23	0.27
D-erythrose	3.20	0.39	1.88	1.88	1.80
D-threose	2.06	0.28	2.27	2.92	3.83
D-glyceraldehyde	28.44	4.69	7.81	–	–
Dihydroxyacetone	19.53	3.59	7.81	–	–

[a] [Monosaccharide] 70mM, [Bu_4NOH] 150mM. [b] Calculated from equation *1*; all the values are normalised with respect to **1** obtained from D-glucose. [c] Radical **5** shows a low intensity, close to the experimental error.

TABLE II

E.s.r. parameters of radicals analysed

Radical	*Hyperfine splitting*[a] *(mT)*		g *value*[b]	a_H/a_D
	a_1	a_2		
1	0.790 (1 H)	0.525 (3 H)	2.00496	–
2	0.870 (1 H)	0.760 (3 H)	2.00484	–
3	0.590 (6 H)	–	2.00472	–
4	0.775 (2 H)	–	2.00519	–
5	0.620 (3 H)	–	2.00462	–
6	0.800 (1 H)	0.080 (3 D)	2.00497	6.50
7	0.870 (1 H)	0.115 (3 D)	2.00473	6.50
8	0.885 (6 D)	–	2.00471	6.60

[a] ±0.001 mT. [b] ±0.0001.

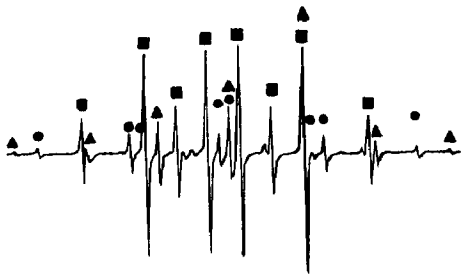


Fig. 1. E.s.r. spectrum of the reaction mixture of D-fructose (70mM) and Bu_4NOH (150mM) in Me_2SO after 15 min; ■, **1**; ●, **2**; ▲, **3**.

The structures *trans*-ethanesemi-dione and 1-alkoxy-1,2-propanesemi-dione are proposed for **4** and **5**, respectively. The structure **4** was confirmed by the fact that the magnetic parameters for the radical formed on electrochemical reduction of glyoxal (see Experimental) accorded with those of **4**. The *trans* configuration was established by comparison with literature data[7].

Treatment of glycolaldehyde with tetrabutylammonium hydroxide in methyl sulfoxide also yielded **1–5**, with the signal for **4** being the most intense (Fig. 3a). The addition of pyruvic acid increased the intensities of the signals attributed to **5**. This result, and the agreement with the published data[8] for semi-dione **5**, confirmed the structure of this radical. When the reaction of glycolaldehyde with tetrabutylammonium hydroxide was carried out in $(CD_3)_2SO$, the radicals **6–8** were formed by exchange of CH_3 groups in **1–3**, respectively, by CD_3, and the splitting constants agreed satisfactorily with the theoretical ratio[9]. This fact has been observed for D-glucose[1] and indicates that some intermediates react with methyl sulfoxide which acts as a methylating agent[10–14]. In the spectrum of a solution of glycolaldehyde in $(CD_3)_2SO$, the e.s.r. signal for **4** was the most intense (Fig. 3b). This fact suggests that the formation of **4** did not involve methyl

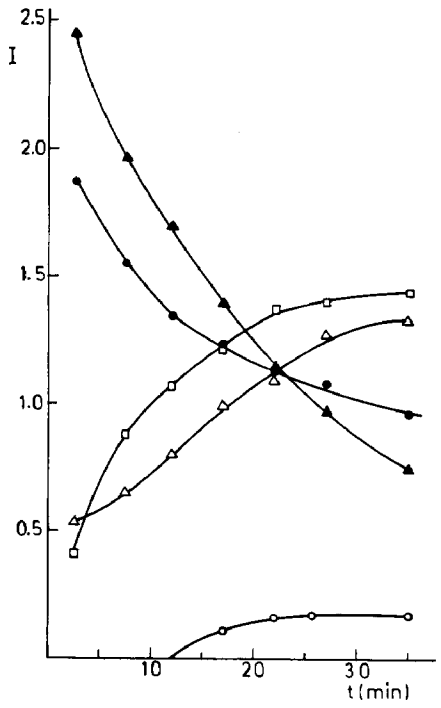


Fig. 2. E.s.r. signal intensities for radicals **1–5** from D-threose as a function of time: [D-threose] 70mM, [Bu_4NOH] 150mM; △, **1**; ○, **2**; □, **3**; ●, **4**; ■, **5**.

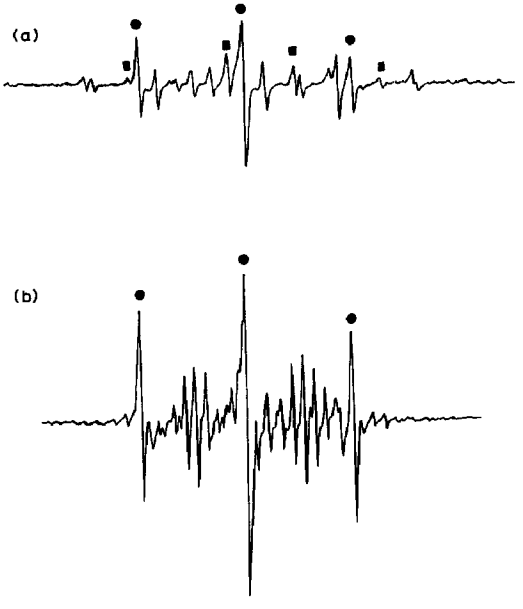
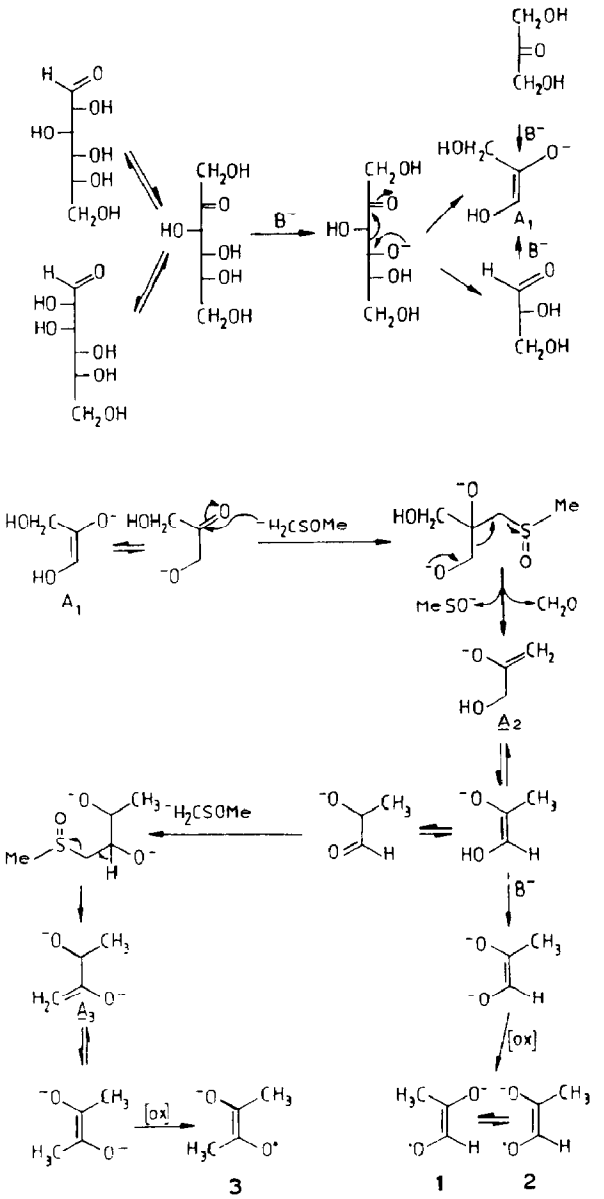


Fig. 3. E.s.r. spectra of mixtures of glycolaldehyde (70mM) and Bu_4NOH (150mM): (*a*) in Me_2SO after 3.5 min (●, **4**; ■, **5**), (*b*) in $(CD_3)_2SO$ after 3 min (●, **4**).

sulfoxide. On the other hand, the formation of **5** was not observed in $(CD_3)_2SO$, but the presence of the semi-dione $CD_3CO=COO^-$ could not be confirmed, probably due to the weakness of the signal.

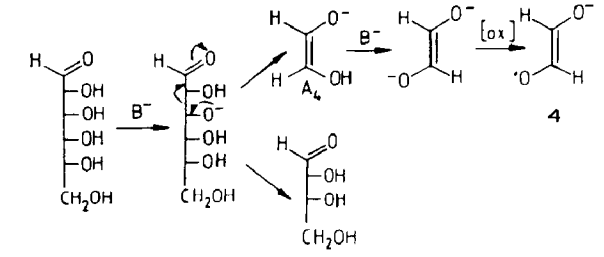
General mechanism for the degradation of monosaccharides. — In proposing a mechanism for the degradation of monosaccharides in methyl sulfoxide, it is necessary to take into account the facts that (*a*) hexoses-I and trioses give **1–3**; (*b*) hexoses-II, tetroses, pentoses, and glycolaldehyde give **1–5**; (*c*) **1** and **2** are in thermodynamic

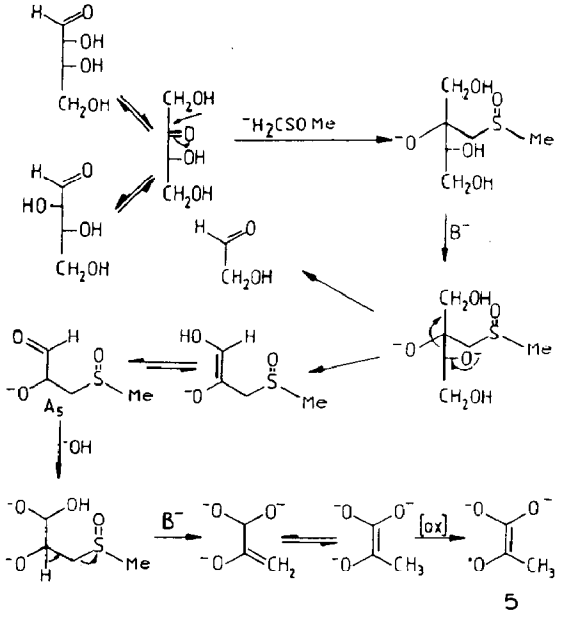


Scheme 1. Degradation pathways for hexoses-I and trioses.

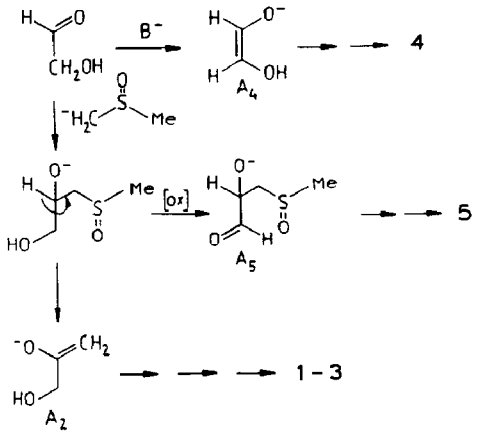
equilibrium; (*d*) **3** arises after **1** and **2**; (*e*) **4** gives the signal of maximum intensity when it is obtained from glycolaldehyde, whereas the greatest intensity of the signal for **5** is observed when it is formed from aldotetroses; (*f*) the formation of **1–3** and **5** implies the incorporation of one or two methyl groups from methyl sulfoxide; and (*g*) α-hydroxycarbonyl intermediates (RCOCHOHR′) must be generated during the degradation because they are able to incorporate one methyl group from methyl sulfoxide when R = CH_3 and R′ = H, or two methyl groups when R = R′ = H, and yield **1–4**, as has been established[15]. However, α-dicarbonyl compounds cannot yield these radicals.

Hexoses-I and trioses. — The same degradation pathway is proposed, since the radicals generated and the development of their e.s.r. signals were similar. This pathway is shown in Scheme 1. The key stage in the degradation of hexoses-I involves cleavage of the C-3–C-4 bond by a base-catalysed retro-aldol reaction of the type established[16] in order to explain the formation of the 2,5-dihydroxybenzoquinone radical anion in the degradation of D-glucose. From this cleavage, glyceraldehyde and the dihydroxyace-





Scheme 2. Degradation pathway for hexoses-II and tetroses.



Scheme 3. Degradation pathway for glycolaldehyde.

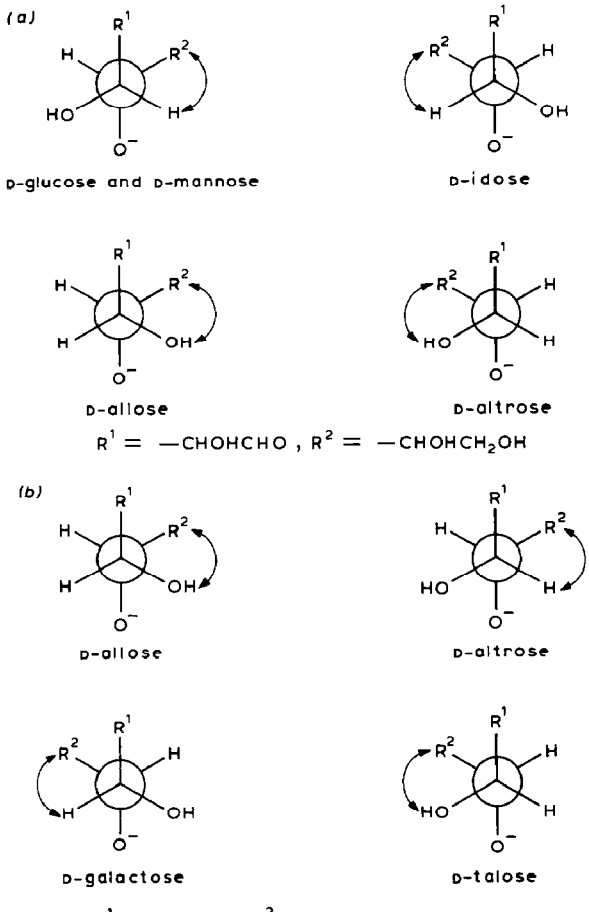


Fig. 4. Newman projections of hexoses-I and -II around (*a*) the C-3–C-4 bond, and (*b*) the C-2–C-3 bond.

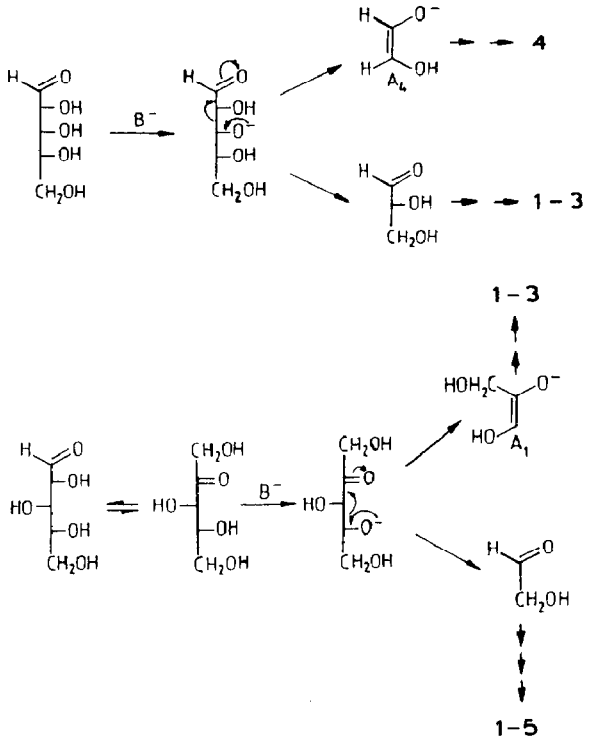
tone anion (**A1**) are obtained. The anion **A1** then reacts with $MeSOCH_2^-$ to give an intermediate that loses[17] formaldehyde and $MeSO^-$ to give the anion **A2**, which is the direct precursor of **1** and **2**. If **A2** reacts with $Me_2SOCH_2^-$, the product loses $MeSO^-$ and give **A3**, oxidation of which affords **3**.

Hexoses-II, tetroses, and glycolaldehyde. — The proposed first step in the alkaline degradation is the cleavage of the C-2–C-3 bond by a base-catalysed retro-aldol reaction. Hayashi *et al.*[17,18] proposed this mechanism in order to explain the formation of 4,4′-disubstituted pyrazine radicals in the fragmentation of sugars in the presence of amines. Thus, the glycolaldehyde anion **A4** and a tetrose are formed. The former gives **4** by proton abstraction followed by oxidation of the resulting dianion.

On the other hand, tetroses react with $Me_2SOCH_2^-$ to afford an intermediate that yields glycolaldehyde and the anion **A5**, from which **5** is formed (Scheme 2).

The formation of radicals from glycolaldehyde is shown in Scheme 3. Oxidation of **A4** gives **4**. If glycolaldehyde reacts with $Me_2SOCH_2^-$, the product yields **A5** which, in turn, may yield **5** or **A2**, and this leads to **1–3**. If the cleavage of hexoses-II involved only the C-2–C-3 bond, then higher intensities of the signals for **4** and **5**, relative to those of **1–3**, would be expected.

This fact is attributed to two competitive processes that involve cleavage of the C-3–C-4 bond, the main process for hexoses-I, and it is not possible to detect **4** and **5** and



Scheme 4. Degradation pathway for pentoses.

cleavage of the C-2–C-3 bond that competes with cleavage of the C-3–C-4 bond in hexoses-II.

The larger contribution of cleavage of the C-2–C-3 bond in hexoses-II than in hexoses-I is attributed to the interactions around the C-3–C-4 bond (see Fig. 4). Thus, when the C-2–C-3 and C-4–O^- bonds are antiperiplanar, there is an unfavourable "*gauche*" interaction (2···OH) in hexoses-II, but not in hexoses-I. For hexoses-II, if the C-1–C-2 and C-3–O^- bonds are antiperiplanar, there is no "*gauche*" interaction for D-altrose and D-galactose, whereas it is present in D-talose and D-allose, the hexoses that give the weakest signals for radicals.

Pentoses. — The anion radicals **1–5** were formed from each of the aldopentoses, but the intensity of the signals for **5** was low for D-ribose and D-arabinose. There are two possible cleavage pathways for pentoses. If the C-2–C-3 bond is cleaved, **A4** and D-glyceraldehyde are obtained; then **A4** gives **4**, and D-glyceraldehyde yields **1–3** (Scheme 4). However, if the C-3–C-4 bond is cleaved, **A1** and D-glyceraldehyde are produced. The conversion of these species into **1–5** is discussed above.

It is possible that the interactions around the C-3–C-4 bond for D-ribose and D-arabinose could promote cleavage of the C-2–C-3 bond (Fig. 5). This interpretation is in good agreement with the low intensity observed for the signal of **5** and it is the first time that radicals obtained from cleavage of both the C-2–C-3 and C-3–C-4 bonds have been observed. Moreover, the ratio of the radicals produced depends on the relative configurations at C-3 and C-4.

Thus, the alkaline degradation of monosaccharides in methyl sulfoxide affords semi-dione radicals formed by different pathways, namely, fragmentation (*via* retro-aldol reactions) and condensation with $Me_2SOCH_2^-$. These processes depend on the stereochemistry and chain length of the monosaccharide. However, the pathways

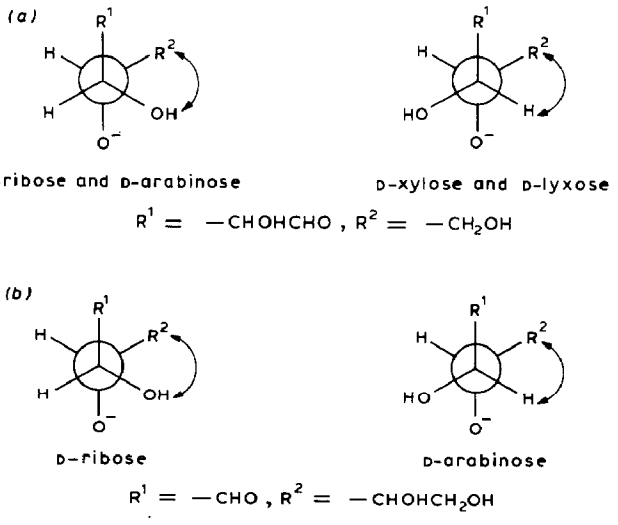


Fig. 5. Newman projections of pentoses around (*a*) the C-3–C-4 bond, and (*b*) the C-2–C-3 bond.

concerned are probably only minor routes, because weak e.s.r. signals of relatively stable radicals are unlikely to reveal major fragmentation pathways.

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