ESR Study of the Alkaline Degradation of Carbohydrates in DMSO. Part 1. Glucose

Paloma Calle, Angela Sanchez, and Carlos Sieiro *
Departamento de Química Física Aplicada, Fac. de Ciencias, Universidad Autónoma, 28049-Madrid, Spain

A mechanism for glucose degradation in alkaline media has been proposed based upon ESR spectroscopy studies. This degradative process has been carried out with aqueous Bu_4NOH in dimethyl sulphoxide (DMSO) at room temperature. Several radicals with semidione-like structures are generated during this process, the presence of an α-hydroxycarbonyl group in the molecule being a crucial requisite for the formation of such radicals. Cleavage of the C(3)–C(4) bond, due to a base catalysed retroaldolization is the first step of the degradation. The important role of DMSO in this process as a methylating agent has also been demonstrated. The corresponding hyperfine splittings (hfs) and *g*-factors of the radicals detected are given.

Many features regarding the degradation of carbohydrates have been researched. The cleavage and rearrangement processes able to effect the fragmentation of the carbon chain have been studied previously,[1,2] and this cleavage plays an important role in the alkaline degradation pattern. Some kinetic aspects have also been investigated[3] in order to clarify the mechanism of these transformations. The degradation products from sugars have been described as a cause of important physical changes in food qualities such as browning.[4] These investigations are described in the known 'Maillard reaction'.[5] Some authors have found an *in vitro* development of free radicals from sugars, which can cause DNA strand scission.[6] It has been reported that this interaction of a radical from sugar with DNA inhibits viral infections[7] and could also be a plausible cause of ageing.[8] Due to the biological and technological importance of the study of the reactions of monosaccharides in alkaline media, which entail the formation of free radicals, we have analysed these intermediates by means of ESR spectroscopy.

The aim of this work is to propose and to justify the reaction pathway of the degradation of sugars in alkaline media using DMSO as solvent. The degradation process was carried out by means of an aqueous solution of Bu_4NOH in DMSO at room temperature. In this first paper we present the results obtained for D-glucose. All monosaccharides and disaccharides will be analysed in further papers. The structural elucidation of the radicals generated is described and their corresponding magnetic parameters are given and analysed.

Results and Discussion

The conditions for the generation of long lived radical species in the alkaline degradation of D-glucose have been analysed by using different solvents and bases, see Table 1. Based upon the above results, the best conditions encountered, namely DMSO as solvent and Bu_4NOH as base, have been used in this study.

When a mixture of D-glucose and Bu_4NOH in DMSO at room temperature was placed in the cavity of the ESR spectrometer the development of an intense ESR signal was observed at the minimum operating time, *i.e.*, about three minutes after the samples had been mixed. The ESR spectrum exhibits a complex hyperfine structure of twenty asymmetrical lines due to the presence of more than one radical anion each with different *g*-factors. The hyperfine structure consists of three radical components, R1, R2, and R3 (Figure 1). This pattern changes with time because of nonidentical evolution of the three species involved. The ESR parameters of these radicals and their assignments are shown in Table 2.

Table 1. Radical generation conditions.[a]

Solvent	Base	Temp./°C	Signal detection time/min
Water	NaOH, KOH	100	1–3
Water–ethanol	NaOH, KOH	70	1–3
Water–ethanol	R_4NOH	50	1–3
DMSO	R_4NOH	25	>60

[a] [D-glucose] 1–6 × 10^{-2} mol dm^{-3}; [Base] 1–3 × 10^{-1} mol dm^{-3}.

In order to determine certain properties of the radicals generated, we have studied the effect of several reaction conditions on the ESR signal development. Thus, we established the optimal experimental requirements for signal detection and we gained further insight into the understanding of the D-glucose degradation mechanism.

Effect of Reaction Time.—The time-dependence of the relative intensities of R1, R2, and R3 was studied. Radical R1 is the major component in practically all ranges. The intensities of R1 and R2 increase in an early step of the reaction and then remain at a constant level. However, the intensity of radical R3 increases through all the intervals considered (from 3 to 70 min). It was also observed that the R1:R2 ratio is *ca.* 6.5.

Effect of Tetrabutylammonium Concentration.—Different amounts of base were added to a fixed concentration of D-glucose and the mixtures were scanned fifteen minutes later. The results obtained indicate an important sensitivity of the ESR signal to the concentration of the base. Moreover, the three radicals show a similar behaviour, reaching a maximum intensity at 150 mmol dm^{-3} concentration of Bu_4NOH.

Effect of D-Glucose Concentration.—When different amounts of D-glucose were added to a constant base concentration and the spectra were registered fifteen minutes later, the maximum signal intensity of the radicals was reached at a concentration around 70 mmol dm^{-3}. Therefore, all experiments were carried out at 70 mmol dm^{-3} D-glucose and 150 mmol dm^{-3} Bu_4NOH.

Effect of the Solvent.—When D-glucose was treated in the same manner in different solvents (DMF, water, ethanol–water, methanol–water, and acetonitrile) at room temperature,

Table 2. ESR parameters of radicals analysed (see Scheme 1).

Radical	Hyperfine splitting[a] (G) a_1	a_2	a_3	g-value[b]	a_H/a_D
R1	7.90 (1 H)	5.25 (3 H)	—	2.004 96	—
R2	8.70 (1 H)	7.60 (3 H)	—	2.004 84	—
R3	5.90 (6 H)	—	—	2.004 72	—
R4[c]	2.65 (Ha)	2.35 (Hb)	0.8 (3 H)	2.004 13	—
R5	2.23 (6 H)	1.95 (2 H)	—	2.004 50	—
R6	8.00 (1 H)	0.80 (3 D)	—	2.004 97	6.50
R7	8.70 (1 H)	1.15 (3 D)	—	2.004 73	6.50
R8	0.85 (6 D)	—	—	2.004 71	6.60
R9	1.20 (1 D)	0.80 (3 D)	—	2.004 99	6.90, 6.50

[a] ±0.01G, unless otherwise stated. [b] ±0.000 10. [c] Speculative assignment based on canonical structures.

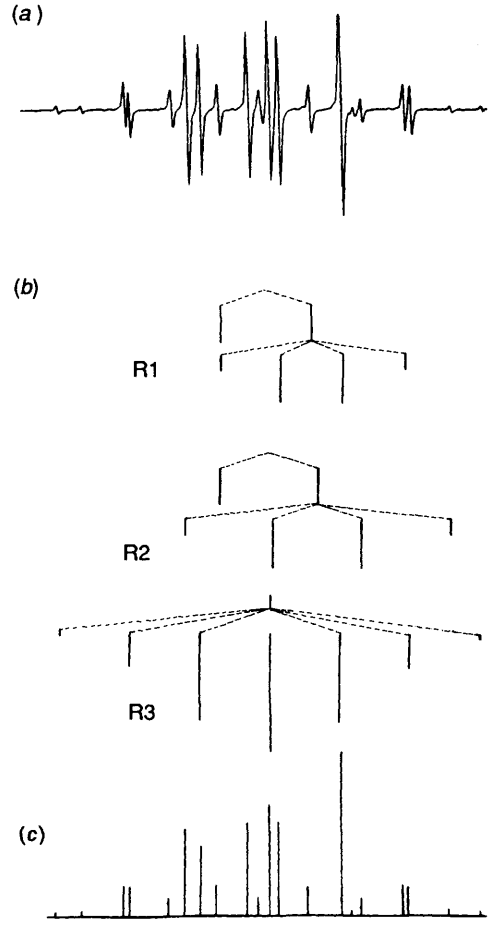


Figure 1. (*a*) ESR Spectrum of radicals generated by reaction of D-glucose with Bu_4NOH in DMSO: ([D-glucose] 20 mmol dm^{-3}; [Bu_4NOH] 200 mmol dm^{-3}; reaction time, 30 min); (*b*) subspectrum of R1, R2, and R3; (*c*) stick plot simulation.

radicals were not observed. This result suggests that DMSO plays a more important role than just as a simple solvent.

Effect of Sugar Configuration.—In order to elucidate what kind of compounds are able to produce the same radical anions obtained in the case of D-glucose, further ESR investigations of a variety of sugars and related carbonyl compounds were carried out. The sugars used for these studies were: D-mannose, D-fructose, D-ribose, D-erythrose, and D-glyceraldehyde. The ESR spectra of D-mannose and D-fructose exhibited hyperfine structures which are in good agreement with those obtained for D-glucose. Therefore, this degradative process does not depend on the C(2) configuration of the aldose or ketose monosaccharides. These experimental results are in good agreement with previous reports,[9] where early steps in sugar degradation were studied.

The initial processes undergone by carbohydrates in alkaline media, including the ionization of cyclic monosaccharides, followed by the transformation of the sugar anion into an ene–diol anion, are assumed to proceed through open chain intermediates. The ene–diol anion, Scheme 1, is considered to

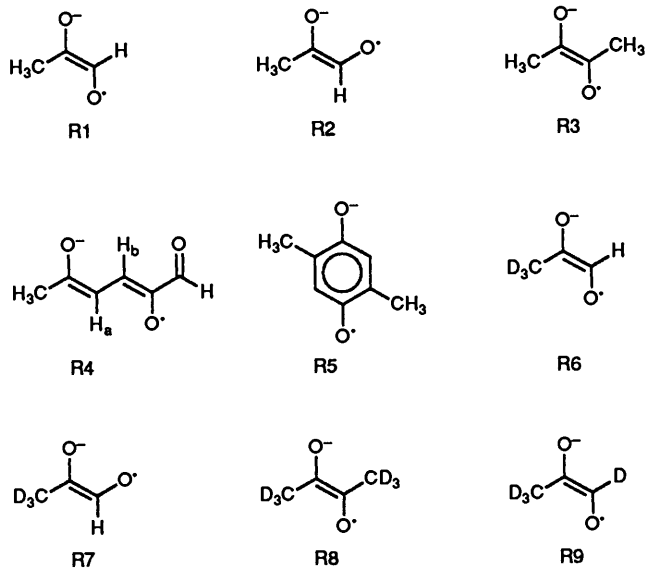
Scheme 1.

exist predominantly in the *Z*-configuration[10] with rapid exchange of the hydrogen atom between the two oxygens. This ene–diolate species, an early step in the formation of radicals, is a common intermediate for the three hexoses studied: D-glucose, D-mannose, and D-fructose. The spectra obtained for shorter chain monosaccharides such as D-ribose, D-erythrose, and glycolaldehyde presented a more complicated structure; however, the radicals R1, R2, and R3 were also observed. Analogously, D-glyceraldehyde displayed identical spectral features to the hexoses previously studied.

The ability to give the characteristic hyperfine spectrum was only observed in compounds possessing a carbonyl group. When inositol, D-glucitol, glycerine or ethylene glycol were submitted to the standard conditions, no ESR signals were detected. However, the presence of a carbonyl group was not sufficient to obtain radical species in these processes. Indeed, compounds lacking an α-hydroxycarbonyl functionality, such as 2-deoxy-D-glucose, acetone, acetaldehyde or formaldehyde failed to produce radicals.

Due to the fact that R1, R2, and R3 were obtained from the smallest molecule studied, glycolaldehyde, it can be assumed that the presence of an α-hydroxycarbonyl group in the molecule is a requisite for the formation of such radicals.

Radical Structure.—The formation of fragments of lower molecular weight than D-glucose is clearly demonstrated by HPLC and ^1H NMR analysis of the reaction mixtures. From ESR hyperfine splittings, it is deduced that radicals R1, R2, and R3 have methyl groups. Moreover, the ^1H NMR spectrum of the degradation products of D-glucose clearly shows butanedione to be present in the mixture. Therefore, it seems reasonable to assume that these radicals have a semidione like



Scheme 2.

structure: $R\dot{C}O{=}\bar{C}OR'$, where R = CH_3 and R' = H for R1 (*trans*) and R2 (*cis*) and R = R' = CH_3 for R3, Scheme 2. The *cis* and *trans* isomers were readily differentiated on the basis of hfs published data.[11]

As mentioned above, the R1:R2 ratio did not change significantly with the experimental conditions, although the total semidione concentration increased or decreased considerably. This indicates that both isomers are in thermodynamic equilibrium. With the aim of proving the structure and origin of radicals R1, R2, and R3, the appropriate α-dicarbonyl and α-hydroxycarbonyl model substrates were studied:

$$X^1COCOX^2 \text{ (Reduction)} \longrightarrow X^1\bar{C}O{=}\dot{C}OX^2 \longleftarrow \text{(Oxidation) } X^1COCHOHX^2$$

In the case of R1 and R2, methylglyoxal and hydroxyacetone were chosen whereas biacetyl and acetoin were selected for R3.

(a) *Methylglyoxal and hydroxyacetone.* Under standard conditions, methylglyoxal gives rise to a complex ESR spectrum, shown in Figure 2, in which radicals R1, R2 and a new radical denoted as R4 were detected. The corresponding hfs and *g*-factor are shown in Table 2. To determine the structure of R4, the effect of the reaction time and substrate concentrations on the development of the radicals were studied. It was observed that R1 is the major species at the beginning of the reaction but its intensity decreases suddenly, whereas R2 and R4 show a much smaller intensity decrease. Additionally, after fifteen minutes of reaction, radical R4 shows the most intense ESR signal. The presence of R4 can be explained[12] as an aldol condensation product from two methylglyoxal molecules and corresponds to radical $H_3C\bar{C}O{=}CHCH{=}\dot{C}OCHO$. On the other hand, when hydroxyacetone and dihydroxyacetone were submitted to the standard conditions, the ESR spectrum obtained was composed of radicals R1, R2, and R3. These facts confirm the proposed structures for R1 and R2.

(b) *Biacetyl and acetoin.* By degradation of biacetyl in the same conditions, radical R3 could be expected, but the spectrum obtained was completely different, and a new radical R5 with two hfs was observed, see Table 2. These parameters and its *g*-factor agree with the hfs values of the 2,5-dimethyl *p*-benzosemiquinone radical anion,[13] generated by a double aldol condensation of two molecules of biacetyl.[12]

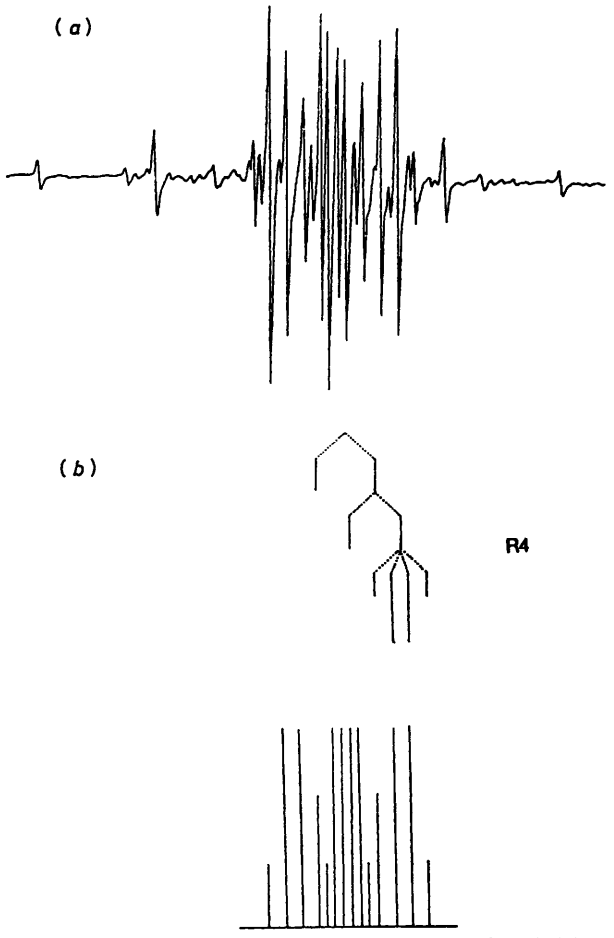


Figure 2. (*a*) ESR spectrum obtained by treatment of methylglyoxal with Bu_4NOH in DMSO: ([methylglyoxal] 15 mmol dm^{-3}; [Bu_4NOH] 150 mmol dm^{-3}; time reaction 15 min); (*b*) R4 radical anion: sub-spectrum analysis and stick plot simulation.

The *trans*-2,3-butane semidione isomer was identified as the R3 radical when 3-hydroxybutanone (acetoin) was treated with Bu_4NOH in DMSO. In fact, the ESR spectrum contained seven intense lines assigned to radical anion $CH_3\bar{C}O{=}\dot{C}OCH_3$. The hfs and *g*-factors comply with species R3 and the *trans* conformation was confirmed by comparison with literature data.[11]

Mechanism of the Alkaline Degradation of Glucose in DMSO.—To propose a mechanism for the degradation of D-glucose in alkaline DMSO, it is necessary to know the origin of the methyl groups of radicals R1, R2, and R3. The methylating character of DMSO under basic conditions has already been reported.[14] Therefore, if the methyl groups arise from the solvent, a significant change in the ESR spectrum should be observed in [$^2H_6$]DMSO. Indeed, when the reaction of D-glucose with Bu_4NOH was carried out in [$^2H_6$]DMSO, a quite different and more complicated ESR spectrum than the one obtained with DMSO was detected, see Figure 3. Since species R1, R2, and R3 were not observed, it is fair to suppose that the formal substitution of CH_3 by CD_3 takes place in radicals R1, R2, and R3, this fact being the origin of an increased number of lines and a shorter spectrum (from 36 to 16 G). The radicals present in this spectrum were denoted as R6, R7, R8, and R9, where the three former ones arise from formal subsitution of CH_3 by CD_3 in R1, R2 and R3 respectively. On the other hand, the origin of R9 is explained in Scheme 3.

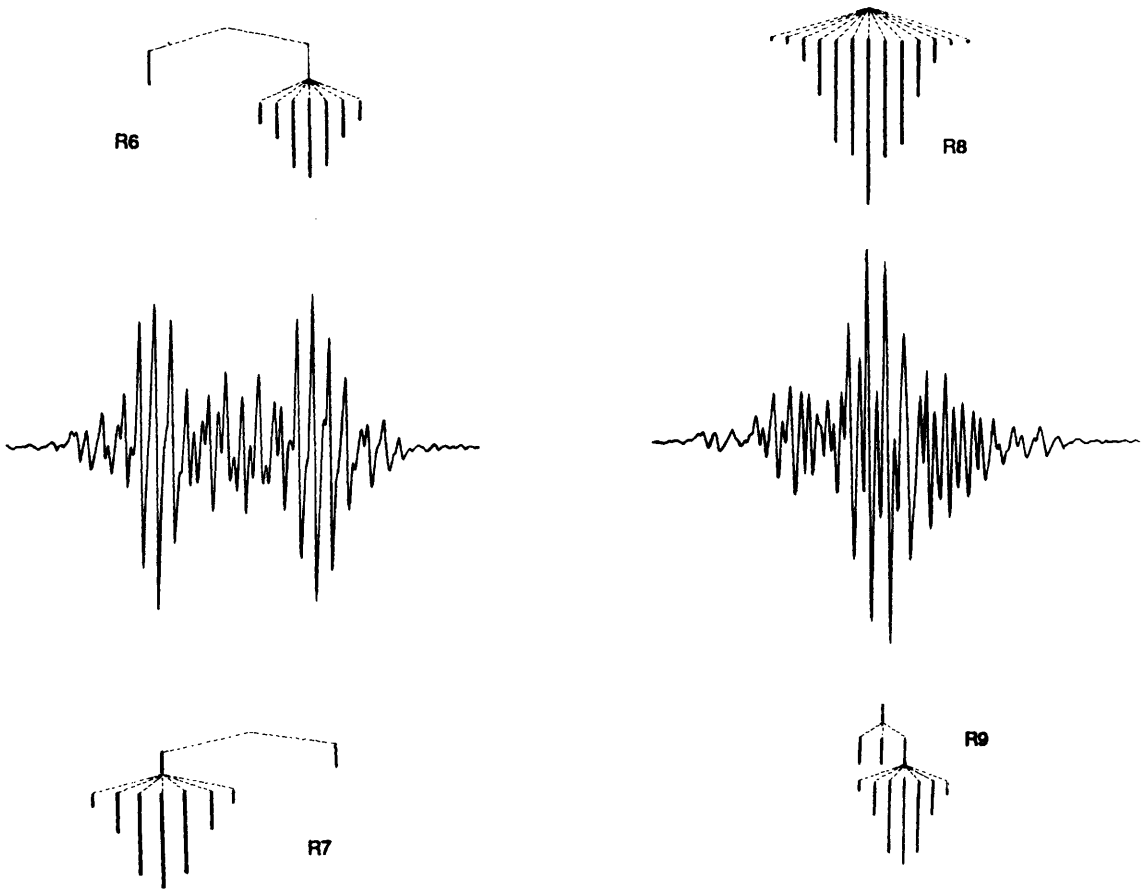
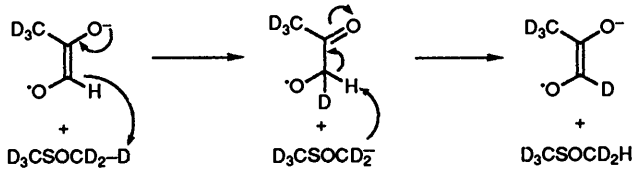


Figure 3. ESR spectra obtained in [$^2H_6$]DMSO; ([D-glucose] 70 mmol dm^{-3}; [Bu_4NOH] 150 mmol dm^{-3}); sub-spectrum analysis and stick plot simulation.



Scheme 3.

In all cases, the splitting constants due to deuterium are in satisfactory agreement with the theoretical ratio $a_H:a_D$ of 6.5.[15]

In order to account for the formation of radicals R1, R2, and R3, we propose the degradation mechanism outlined in Scheme 4. The above reaction pathway was derived from the following experimental evidence. (*a*) The precursors of the R1, R2, and R3 radicals are the corresponding α-hydroxycarbonyl compounds, which are generated in the degradative process. (*b*) The methyl groups of these radical anions proceed from DMSO. (*c*) D-Glucose and glyceraldehyde give rise to the same radicals under similar conditions which allows us to propose as a first step of the degradation, the cleavage of D-glucose at the C(3)–C(4) bond, due to a base catalysed retroaldolization process. This is in agreement with previously postulated mechanisms.[1,16]

The cleavage of D-glucose gives rise to glyceraldehyde and its corresponding anion A1. Next, A1 is attacked by the DMSO anion, generating A2, HCHO, and CH_3SO^-. The species A2 is the anion of hydroxyacetone which gives rise directly to R1 and R2. On the other hand, if A2 incorporates a new molecule of DMSO, by loss of CH_3SO^- dianion A3, which subsequently gives rise to radical R3, is obtained.

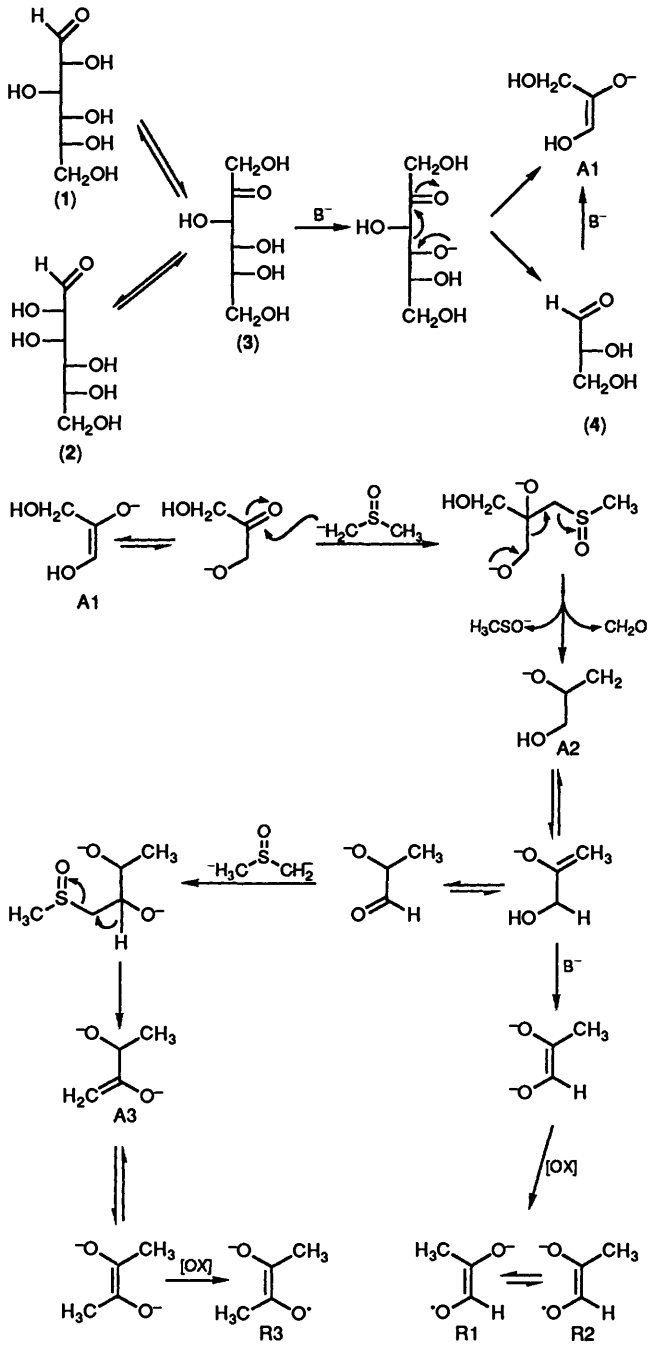
Experimental

Materials.—The monosaccharides and related substances used were commercial products: D-glucose, D-mannose, D-fructose, D-ribose (for biochemistry, Merck); D-erythrose (60%), D-glyceraldehyde (80%), dihydroxyacetone, hydroxyacetone, methylglyoxal (40%), glycolaldehyde, 2-deoxy-D-glucose, acetoin (Aldrich); biacetyl (Fluka); tetrabutylammonium hydroxide solution (40%) (BDH); DMSO (for analysis, Merck); [$^2H_6$]DMSO (for NMR spectroscopy, Merck).

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). An optimum modulation width of 0.05–0.12 G was used. The *g*-factor was determined by means of a high precision frequencymeter and gaussmeter. In the case of complex spectra the splitting constants were obtained by comparing the experimental spectra with those simulated by means of the program EPRISM.[17] Relative intensities of ESR signals were calculated from the following relation:[18]

$$I = C\frac{Y\Delta H_{pp}^2 R}{GMg^2(\text{scan})^2} \qquad R = \frac{\sum_j D_j}{D_k}$$

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



Scheme 4. Compounds: (**1**) D-glucose; (**2**) D-mannose; (**3**) D-fructose; (**4**) D-glyceraldehyde.

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 NMR spectra were recorded in the Fourier transform mode with a Varian XL-300 spectrometer.

Chromatography.—The analysis of glucose fragmentation was carried out by HPLC using a Waters 510 chromatograph equipped with a Waters 420 differential refractometer as detector. The reaction mixture (glucose, 70 mmol dm^{-3} and Bu_4NOH, 150 mmol dm^{-3}) was chromatographed on a column (0.40 × 30 cm) of Sugar Park 1 with water as eluant (0.5 cm^3 min^{-1}).

Acknowledgements

This work was supported by a grant of the Comisión Asesora de Investigación Científica y Técnica, Madrid.

References

1 (*a*) C. Lagercrantz, *Acta Chem. Scand.*, 1964, **18**, 1321; (*b*) H. G. J. de Wilt and B. F. M. Kuster, *Carbohydr. Res.*, 1972, **23**, 343.

2 H. S. Isbell, H. L. Frush, and E. T. Martin, *Carbohydr. Res.*, 1973, **26**, 287. (*a*) H. G. J. de Wilt and B. F. M. Kuster, *ibid.*, 1971, **19**, 5; (*b*) H. G. J. de Wilt and I. Lindhout, *ibid.*, 1972, **23**, 333; (*c*) S. P. Monliek, *ibid.*, 1978, **63**, 165; (*d*) J. M. de Bruijn, A. P. G. Kieboom and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 1987, **106**, 35.

4 (*a*) M. Namiki, T. Hayashi, and S. Kawakishi, *Agric. Biol. Chem.*, 1973, **37**, 2935; (*b*) T. Hayashi and M. Namiki, *ibid.*, 1980, **44**, 2575; *ibid.*, 1981, **45**, 933; (*c*) M. Namiki and T. Hayashi, *J. Agric. Food Chem.*, 1975, **23**, 487; *ibid.*, 1980, **44**, 2575.

5 (*a*) J. E. Hodge, *J. Agric. Food Chem.*, 1953, **1**, 928; (*b*) M. Namiki and T. Hayashi, *Am. Chem. Soc. Symp. Ser.*, 1983, **215**, 21; M. Namiki and T. Hayashi, *Prog. Food. Nutr. Sci.*, 1981, **5**, 81.

6 (*a*) N. Kashimura, J. Morita, I. Sato, Z. Kumazawa, S. Nishikawa, S. Ito, Y. Koma, and M. Komada, *Third Int. Symp. Maillard Reaction*, 1985, 41; (*b*) A. Murata and K. Kitagawa, *Agric. Biol. Chem.*, 1973, **37**, 1145.

7 J. Morita, N. Kashimura, and T. Komano, *Agric. Biol. Chem.*, 1980, **44**, 883.

8 T. Komano, S. Naujou, K. Ueda, and S. Fujii, *Third Int. Symp. Maillard Reaction*, 1985, 40.

9 (*a*) J. F. Harris, *Carbohydr. Res.*, 1972, **23**, 207; (*b*) H. G. J. de Wilt and B. F. M. Kuster, *ibid.*, p. 1972, **23**, 343; (*c*) T. M. de Bruijn, A. P. G. Kieboom, and H. van Bekkum, *Recl. Trav. Chim. Pays-Bas*, 1986, **105**, 176.

10 H. G. K. de Wilt, A. P. G. Kieboom, and H. van Bekkum, *Carbohydr. Res.*, 1979, **74**, 157.

11 (*a*) H. Zeldes and R. Livingston, *J. Chem. Phys.*, 1967, **47**, 1465; (*b*) A. L. Buley, R. O. C. Norman, and R. J. Pritchett, *J. Chem. Soc. B*, 1966, 849; (*c*) G. A. Russell, D. F. Lawson, H. L. Malkus, R. D. Stephens, G. R. Underwood, T. Takano, and V. Malatesta, *J. Am. Chem. Soc.*, 1974, **96**, 5830; (*d*) G. A. Russell and R. D. Stephens, *J. Phys. Chem.*, 1966, **70**, 1320.

12 P. Calle, Ph.D. Thesis, 'ESR Study of the Alkaline Degradation of Carbohydrate in DMSO Medium,' Universidad Autónoma, Madrid, 1989.

13 P. D. Sullivan, *J. Am. Chem. Soc.*, 1975, **97**, 3992.

14 (*a*) E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, 1962, **84**, 866; (*b*) G. A. Russell, E. G. Janzen, H. Becker, and F. J. Snentovsky, *ibid., 1962*, **84**, 2652; (*c*) M. Chayovsky and E. J. Corey, *J. Org. Chem.*, 1963, **28**, 254; (*d*) C. Walling and L. Bollyky, *ibid.* p. 1963, **28**, 256.

15 R. G. Lawler, J. R. Bolton, M. Karplus, and G. K. Fraenkel, *J. Am. Chem. Soc.*, 1971, **47**, 2149.

16 (*a*) T. Hayashi, Y. Ohta, and M. Namiki, *J. Agric. Food Chem.*, 1977, **25**, 1282; (*b*) T. Hayashi and M. Mamiki, *Am. Chem. Soc. Symp. Ser.*, 1985, **3**, 29; (*c*) T. Hayashi and M. Mamiki, *Agric. Biol. Chem.*, 1980, **44**, 2575; (*d*) *ibid.*, 1981, **45**, 933; (*e*) *ibid.*, 1986, **50**, 1965; (*f*) T. Hayashi, S. Mase, and M. Mamiki, *ibid.*, 1985, **49**, 3131.

17 M. A. Martinez-Jimeno and C. Sieiro, Applied Physical Chemistry Dept., University Autónoma of Madrid; EPRISM is a sophisticated program for ESR spectra simulation, implemented for PC (XT/AT or compatible) provided with graphic card (CGA or similar). This program permits the simulation of complex spectra composed of several radicals with different g-factors. Software available upon request.

18 J. E. Wertz and J. R. Bolton, 'Electron Spin Resonance. Elementary Theory and Practical Applications,' McGraw-Hill, New York, 1972.

Paper 9/04564C
Received 24*th October* 1989
Accepted 22*nd February* 1990