Given these successes in these two series of compounds, it would seem reasonable that the method might be applied to other series of benzoannulenes or fused annulenes which have suitable internal NMR probes and for which enough examples exist to provide the necessary calibrations.

Conclusion

Our goal of showing a simple relationship between bond localization in a series of benzannulenes and strength of the ring current as measured by chemical shift shielding has been achieved. The linear relationship, though derived empirically by using examples of the series as calibrants, can be used predictively for other members of the series and in the bulk of the cases examined gives very good results (<0.5 ppm error).

As a result, the ring current shielding effect on the internal protons of many benzannulenes can now be reasonably estimated and depends primarily on the degree of bond localization in the macroring caused by the annelating ring. The relative positions of fusion by more than one annelating ring, as a consequence of their effect on bond localization, is of considerable importance and determines whether the macrocyclic ring will be highly diatropic or not.

Registry No. 1, 956-84-3; **2**, 66093-76-3; **3**, 65649-31-2; **4**, 66093-77-4; **5**, 66093-78-5; **7**, 80697-00-3; **8**, 80697-01-4; **9**, 80697-02-5; **10**, 80697-03-6; **11**, 80697-04-7; **12**, 80697-05-8; **13**, 80697-06-9; **14**, 80697-07-0; **15**, 80697-08-1; **16**, 80697-09-2; **17**, 80697-10-5; **18**, 80697-11-6; **19**, 80697-12-7; **20**, 80697-13-8; **21**, 80718-99-6; **22**, 80697-14-9; **23**, 80697-15-0; **24**, 80697-16-1; **25**, 80697-17-2; **26**, 65504-39-4; **27**, 54811-14-2; **28**, 80697-18-3; **29**, 54811-12-0; **30**, 54811-08-4; **31**, 80697-19-4; **32**, 80697-20-7; **33**, 80697-21-8; **34**, 80697-22-9; **35**, 80697-23-0; **36**, 80697-24-1; **37**, 80697-25-2; **38**, 65649-32-3; **39**, 80697-26-3; **40**, 80697-27-4.

ESR Study of the Attack of Photolytically Produced Hydroxyl Radicals on α-Methyl-D-glucopyranoside in Aqueous Solution[1]

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Contribution from the Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556. Received August 28, 1981

Abstract: Six free radicals were observed when an aqueous solution of H_2O_2 and α-methyl-D-glucopyranoside was photolyzed using the in situ photolysis ESR method: a C6 primary hydroxyalkyl radical, a C2 secondary hydroxyalkyl radical, a C7 primary oxyalkyl radical, a Cl primary oxyalkyl radical, a C3 secondary hydroxyalkyl radical, and a C5 secondary oxyalkyl radical. The pH dependence of the concentration of these species, their reactions, stereochemical factors influencing radical stability, and comparison with product analysis studies are discussed.

Introduction

Carbohydrates are ubiquitous in nature, serving as energy sources, structural members, and components of nucleic acids in biological systems. Although the ionic chemistry of carbohydrates is well established,[2] the study of carbohydrate radiation and free-radical chemistry is a recent development.[3] Glucose (αGlu) is one of the most abundant monosaccharides in nature. Its derivative, α-methyl-D-glucopyranoside (αMeGlu), has the major structural features of αGlu, plus stability against anomerization in solution (see Figure 1). Single-crystal ESR and ENDOR studies of αGlu and αMeGlu have identified the free radicals generated by X-irradiation at 12 and 77 K and the thermally induced reactions of these transient intermediates.[4-7] In this report, these studies are extended to radicals generated by photolysis of αMeGlu/H_2O_2 aqueous solutions.

Experimental Section

Solutions in this study were freshly prepared with Millipore reagent-grade water, 40 mM αMeGlu (Aldrich), 0.678 M H_2O_2 (Fisher ACS), and 25 or 100 mM phosphate buffer (Fisher or Baker) with H_2SO_4 (Fisher ACS) or KOH (Baker) used to adjust pH as measured with a Sensorel glass electrode.

First-derivative ESR spectra were recorded with a steady-state in situ photolysis system using a Varian V-4502 ESR spectrometer with dual rectangular cavity, circulator, bias arm, and GaAs FET microwave amplifier. Microwave frequency was measured with a Hewlett-Packard 5245L counter with 5255A plug-in frequency converter. Microwave power was measured with an HP 431B power meter. Magnetic field measurements were made using a Varian Fieldial sweep unit, calibrated against the $(CH_3)_2\dot{C}OH$ radical. Relative signal intensities and g factors were calibrated against a Varian strong pitch sample (assumed $g = 2.0028$). Intensities were calculated as the product of first-derivative peak-to-peak line amplitudes multiplied by the square of peak-to-peak line width.

Nitrogen-saturated solutions were passed in a continuous flow system through a quartz ESR flat cell of 0.4 mm spacing and were photolyzed with unfiltered light from a 1-W Hanovia 977B-1 lamp. The temperature of the photolyzed sample was controlled by flowing thermostated N_2 gas past the flat cell, and was measured using a Doric DS-100-T3 thermocouple thermometer, with the sensor located at the flat cell outlet. The flow rate was typically 10 mL/min.

Results and Free-Radical Assignments

The ESR spectrum of 40 mM αMeGlu photolyzed at pH 5.5 and 8 °C is shown in Figure 2. Three major and three minor species, as indicated by stick figures, are present.

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[1] The research described herein was supported by the Office of Basic Energy Sciences of the Department of Energy. This is Document No. NDRL-2274 from the Notre Dame Radiation Laboratory.

[2] See, e.g., Ferrier, R. J.; Collins, P. M. "Monosaccharide Chemistry"; Penguin Books: Middlesex, England, 1972, and references therein.

[3] See, e.g., von Sonntag, C. In "Advances in Carbohydrate Chemistry and Biochemistry", Vol. 37; Tipson, R. S., and Horton, D., (Eds.); Academic Press: New York, 1980, and references therein.

[4] Madden, K. P.; Bernhard, W. A. *J. Chem. Phys.* **1979**, *70*, 2431-37.

[5] Madden, K. P.; Bernhard, W. A. *J. Chem. Phys.* **1980**, *72*, 31-33.

[6] Madden, K. P.; Bernhard, W. A. *J. Phys. Chem.* **1979**, *83*, 2643-49.

[7] Madden, K. P.; Bernhard, W. A. *J. Phys. Chem.* **1980**, *84*, 1712-17.

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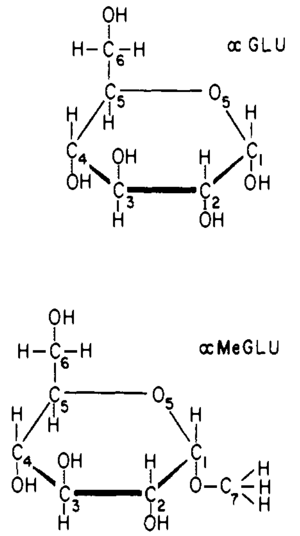


Figure 1. The molecular structures and numbering systems for α-D-glucose and α-methyl-D-glucopyranoside.

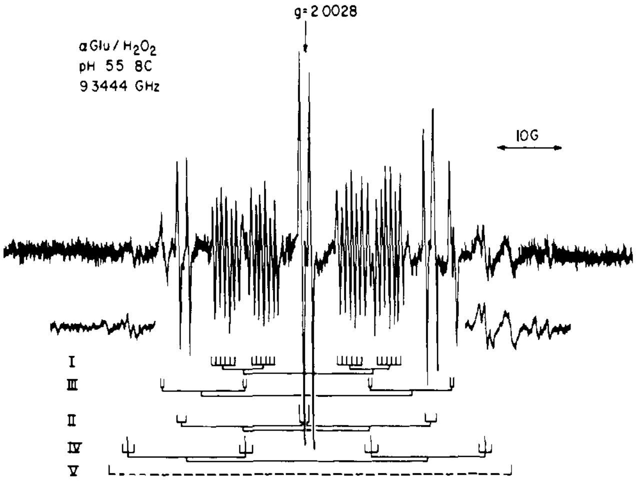
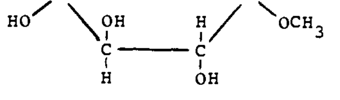


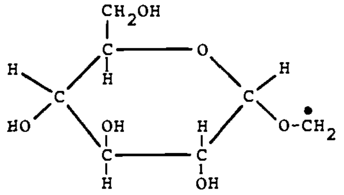
Figure 2. X-band first-derivative ESR spectrum of an aqueous N_2 saturated αMeGlu/H_2O_2 solution photolyzed at pH 5.5 and 8 °C. Stick strucures refer to radical species listed in the text. Intensities shown in stick figures are for unpolarized radical populations.

Species I has four groups of six lines, with a *g* factor of 2.0032 and major hyperfine splittings of 18.53 and 5.70 G. The 18-G splitting is temperature insensitive, indicative of an α proton, while the latter shows a temperature variation typical of β protons over the 8–27 °C range. The 0.72-, 1.28-, and 1.42-G splittings are contained within each sextet. The two smaller splittings are unaffected by temperature or deuteration of labile protons, while the larger splitting increases to 1.56 G at 27 °C, and collapses to a 0.23 triplet upon deuteration of hydroxyl protons, identifying this as a hydroxyl splitting. These characteristics lead us to an assignment of species I as a C6 primary hydroxyalkyl radical (C6PHA).



C6PHA

Species II shows a 1:2:1 triplet of doublets, with a *g* factor of 2.0033 and hyperfine splittings of 18.30 G (two equivalent protons) and 1.43 G that are temperature invariant from 8 to 27 °C, and unaffected by proton–deuteron exchange in the solvent. The assignment of this species as a C7 centered primary oxyalkyl radical (C7POA) is straightforward.

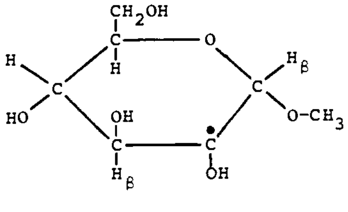


C7POA

Species III has four doublets, with a *g* factor of 2.0031, two β proton splittings of 30.84 and 12.2 G, and a 0.61-G splitting (from a hydroxyl proton) which is affected by exchange in D_2O. The hyperfine coupling constant for β proton is approximated by[8]

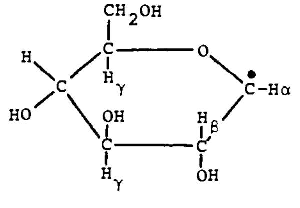
$$a_{\text{iso}}^{H_\beta} = \rho(B_0 + B_2 \cos^2 \theta)$$

where ρ is the spin population on the central carbon atom (~0.9), $B_0 \simeq 0$ G, $B_2 \sim 45$ G, and θ is the torsion angle between the unpaired electron orbital symmetry axis, the C_α–C_β bond direction, and the C_β–H_β bond direction. The ESR data thus give θ values of 29 and 57° for the 30.84- and 12.2-G couplings. Examination of αMeGlu molecular models shows that all secondary hydroxyalkyl radicals formed by H atom abstraction would have both θ values near 0 °C, except for a C2 centered species, which would give values of 23 and 60°, in good agreement with the ESR data.



C2SHA

Species IV has a set of 1:2:1 triplets on the wings of the ESR spectrum, with a *g* factor of 2.0030 and hyperfine splittings of 38.9, 17.65, and 0.83 G (two equivalent small couplings), all of which are insensitive to temperature variation or hydroxyl group deuteration. This spectrum is assigned to a C1 primary oxyalkyl radical (C1POA), with the small couplings due to γ protons H(5) and H(3).



C1POA

Species V shows two small, broad absorptions between the outer lines of the C2SHA radical and species VI. The other features of this species are obscured by other absorption lines. The spectral extent of the radical is 59.6 G, with an uncorrected *g* factor of ~2.0028. These peaks narrow upon deuteration of labile protons, revealing a barely resolved 0.49-G 1:2:1 triplet. At pH 4 this species reaches maximum intensity and diminishes in intensity in more acidic or basic solutions, disappearing completely below pH 2.5 or above pH 8. These characteristics are expected for a C3 or C4 centered secondary hydroxyalkyl radical, which would have two β proton splittings of ~30 G, and an unresolved OH splitting (~0.1 G) manifest by the narrowing of the triplet lines in D_2O solution. Similar behavior was seen for the myoinositol secondary hydroxyalkyl radical.[9] The equivalence of the small

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[8] Heller, C.; McConnell, H. M. *J. Chem. Phys.* **1960**, *32*, 1535–39.
[9] Gilbert, B. C.; King, D. M.; Thomas, C. B. *J. Chem. Soc., Perkin Trans. 2* **1980**, 1821–27.

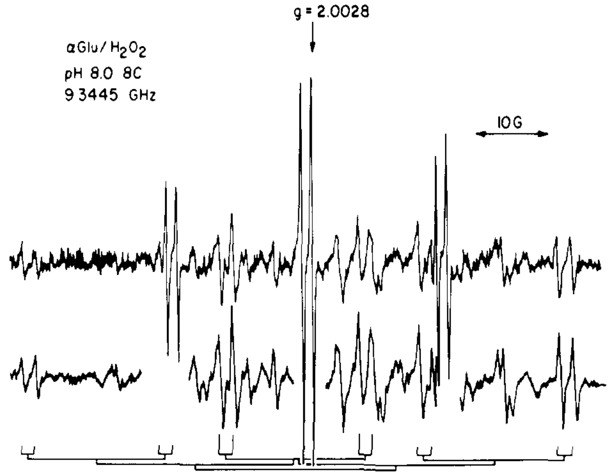
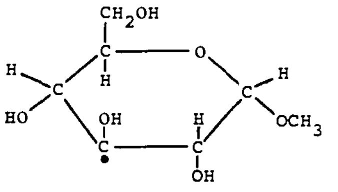


Figure 3. X-band derivative ESR spectrum of an aqueous N_2 saturated solution of αMeGlu/H_2O_2 photolyzed at 8 °C, with pH 8.0. Stick figure is shown for species VI, with intensities shown for an unpolarized radical population.

couplings favors an assignment as a C3SHA radical, the more symmetric of the two choices.

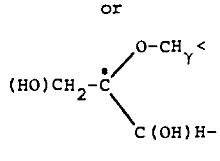


C3SHA

Species VI has a *g* factor of 2.0038 and hyperfine splittings of 26.8 (two equivalent protons), 18.7, and 1.8 G. Raising the pH of the solution from 5.5 to 8.0 causes this signal to grow in intensity as C6PHA ESR signal intensity decreases (see Figure 3). The two 26.8-G splittings, on the basis of magnitude, can be assigned unequivocally as β protons. The 1.8-G coupling which is unaffected by exchange can also be assigned with certainty to a γ proton. The 18.7-G coupling could be either an α or β-proton, leading to consideration of two possible radical structures. The

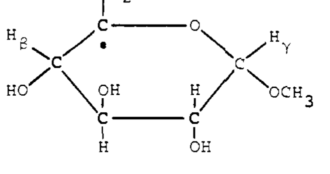
$\mathrm{(HO)CH_2\text{-}\dot{C}H\text{-}C(O)\text{-}CH<}$ VI A

or



VI B

magnitude of the *g* factor tends to support VIB as the proper assignment. Camaioni et al.[10] studied the $CH_3\text{-}\dot{C}H\text{-}C(O)\text{-}CH(CH_3)_2$ radical in an adamantane matrix at 328 K, finding a *g* factor 2.0047. Structure VIB has been observed in αMeGlu single-crystal studies[4] with $g_{iso} \simeq 2.0036$, in good agreement with the observed values. We assign species VI as the C5 secondary oxyalkyl radical (C5SOA).



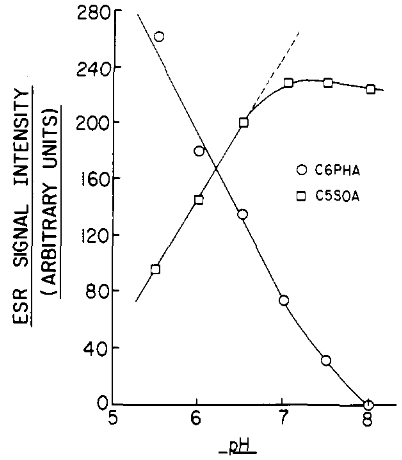


Figure 4. Plot of ESR signal intensity [(height × (width)²), summed over all peaks] as a function of pH for the C6PHA (○) and C5SOA (□) radicals.

Discussion

I. Relative Yield of Products. In the literature of free-radical carbohydrate chemistry, there have been a number of conflicting reports concerning whether or not the ·OH radical will show specificity in hydrogen atom abstraction at various positions in sugars and cyclic polyalcohol molecules. Norman and Pritchett[11] have stated that the C(1)–H(1) bond in D-glucose is specifically susceptible to ·OH attack, and Dizdaroglu et al.[12] have implied such specificity in their report of a total G value of 1.17 for Cl derived products in the radiolysis of N_2O saturated aqueous glucose solutions. Our results are in accord with the recent work on myoinositol of Gilbert and colleagues[9] as we found no great specificity in ·OH radical attack on a given C–H bond in the αMeGlu system. If the probability of hydroxyl radical formation at a site is proportional to the number of C–H bonds surrounding a specific carbon atom, one would expect (assuming no fast reactions, and similar bimolecular termination kinetics for the various radicals) an ESR intensity ratio of 3:2:1 for the C7POA, C6PHA, and each of the ring carbon centered radicals , respectively. At pH 2.5 and 8 °C, this ratio is 3.4:1.9:1 for the C6PHA, C7POA, and C2SHA species. The relative yield of C3SHA (C4SHA) could not be accurately determined, but is estimated at 0.25. Missing completely are the ESR absorptions observed in the single-crystal study[4] from the C1 and C5 hydrogen abstraction products, which, if formed, should be quite stable owing to extensive delocalization of the unpaired electron spin via hyperconjugation with neighboring bonds. Malatesta and Ingold[13] have assessed the relative reactivity of CH bonds adjacent to ring oxygen atoms in cyclic ethers, $\rangle$C(H)–O–R, and found the reactivity of these bonds toward *tert*-butoxyl attack was related to the dihedral angle θ between the HCO plane and normal to the COR plane, with hydrogen abstraction probable when θ is near 0°, and unlikely for $\theta = 90°$. Molecular models of αMeGlu give 90 and 25° for the θ angles relevant to the C1 and C5 hydrogen abstraction products, respectively. In 2-methoxy- (axial), 6-methyl- (equatorial) tetrahydropyran, this configuration resulted in a relative reactivity per equivalent hydrogen value (ρ) of 0.16 and $\sim$0 for the radicals analogous to the C1 and C5 radicals ($\rho \equiv 1$ for the C–H bond adjacent to the ring oxygen in tetrahydropyran). Thus, stereoelectronic factors destabilizing incipient oxyalkyl radical formation seem operative in this system, suppressing formation of these species.

The observed ratio of ESR signal intensities indicates that there is only a small perturbation upon the statistically expected radical distribution at all other sites, in accord with the results of Gilbert et al.[9]

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(10) Camaioni, D. M.; Walter, H. F.; Jordan, J. E.; Pratt, D. W. *J. Am. Chem. Soc.* **1973**, *95*, 7978–92.

(11) Norman, R. O. C.; Prichett, R. J. *J. Chem. Soc. B* **1967**, 1329–32.

(12) Dizdaroglu, M.; Henneberg, D.; Schomberg, G.; von Sonntag, C. *Z. Naturforsch Teil B* **1975**, *30*, 416–25.

(13) Malatesta, V.; Ingold, K. U. *J. Am. Chem. Soc.* **1980**, *103*, 609–14.

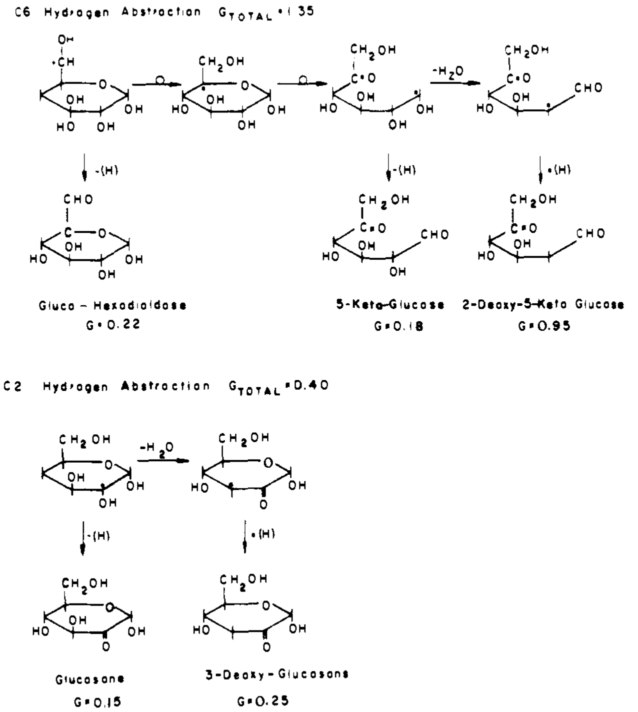


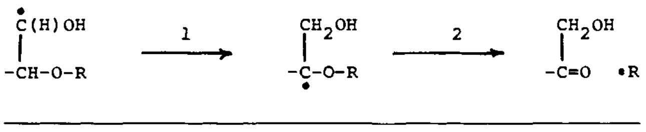
Figure 5. Scheme for reactions of C6 and C2 radicals in αGlu. (H) designates hydrogen atom lost or gained in disproportionation reactions.

II. Reactions of the C6PHA and C5SOA Radicals. In single-crystal studies of α-D-glucose[4] and αMeGlu,[6] the fate of the C6PHA radical depends upon its protonation state. The deprotonated αMeGlu radical rearranges at 150 K to a C5SOA radical via a 1,2 hydrogen shift, while the protonated αGlu radical abstracts an adjacent H atom in the crystal structure. The conversion of C6PHA to C5SOA as a function of pH is shown in Figure 4, and shows that near-neutral pH is necessary for efficient conversion of these radicals.

Possible explanations for this conversion are a change in ·OH attack with decreasing pH, an alkoxy radical mechanism producing either C6PHA or C5SOA as a function of pH or a rearrangement of the type observed in the αMeGlu single-crystal study. A study of the free-radical products of ·OH radical attack in aqueous solutions of glycerol[14] revealed no change in primary or secondary hydroxyalkyl radical yield over the pH range 8.5–3.8, making this an unlikely explanation for the C6PHA → C5SOA conversion. An alkoxy radical precursor is also unlikely, as alkoxy radical yield in polyhydroxyl compounds attacked by ·OH is less than 0.1% of the total radical yield.[15] A rearrangement of the type observed in the single-crystal study is thus suggested.

The primary hydroxyalkyl radical formed from ethanol has a p*K* of 11.51, as measured by ESR.[16] The αMeGlu C6PHA radical would be expected to have a similar value, so a small, but highly reactive population of deprotonated primary hydroxyalkyl radicals must be responsible for this transformation.

This conversion can be added to the decay mechanism suggested by Dizdaroglu et al.,[12] for the C6PHA radical:

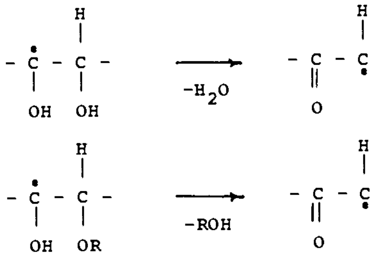


$$\begin{matrix}\dot{C}(H)OH\\ |\\ -CH-O-R\end{matrix}\ \xrightarrow{1}\ \begin{matrix}CH_2OH\\ |\\ -\underset{\bullet}{C}-O-R\end{matrix}\ \xrightarrow{2}\ \begin{matrix}CH_2OH\\ |\\ -C{=}O\end{matrix}\ \ \bullet R$$

Reaction 1 appears facile at neutral pH, but reaction 2 is much slower, as its product is not seen in the ESR spectrum, although it should be quite stable if formed. This ether to keto rearrangement is also slow in the case of the C7POA radical; in spite of the increased spin delocalization afforded by a C7POA to C1POA radical rearrangement, C1POA has only a small fraction of the intensity of C7POA over a pH range of 1.5–10, and a temperature range of 8–50 °C.

III. Reactions of Secondary Hydroxyalkyl Radicals. Gilbert found that myoinositol secondary hydroxyalkyl radicals can be grouped as those with two large (ca. 30 G) β proton couplings due to two axial protons, or those with one large and one small (~6.2 G) β proton coupling. The radicals in the latter group eliminated water in an acid-catalyzed reaction with a rate constant (3×10^6 dm^3 mol^{-1} s) considerably greater than the former group, owing to the overlap between the half-filled π orbital and the adjacent C-O bond.

Dizdaroglu et al.[17] have proposed an ether function elimination reaction, analogous to β hydroxyl elimination, to explain a radiation-induced chain reaction in crystalline fructose.



$$-\underset{OH}{\overset{\bullet}{C}}-\underset{OH}{\overset{H}{C}}-\ \xrightarrow{-H_2O}\ -\underset{O}{\overset{}{\underset{\|}{C}}}-\overset{H}{\underset{\bullet}{C}}-$$

$$-\underset{OH}{\overset{\bullet}{C}}-\underset{OR}{\overset{H}{C}}-\ \xrightarrow{-ROH}\ -\underset{O}{\overset{}{\underset{\|}{C}}}-\overset{H}{\underset{\bullet}{C}}-$$

This reaction does not occur in our solution studies. Both the C2SHA and C6PHA radicals satisfy the stereoelectronic requirement of maximal π orbital overlap with the C_β–O bond, yet the C6 center undergoes the conversion to C5SOA, and the C2SHA radical decays (most likely via disproportionation reactions) to a diamagnetic species, as no product ESR signal is seen.

IV. Comparison of ESR Results with Product Analysis of Irradiated N_2O Saturated α-Glucose Aqueous Solutions. Since αMeGlu and αGlu are so similar, it is instructive to compare the reactions of αMeGlu seen in the ESR study to those deduced by end-product analysis of irradiated N_2O saturated aqueous αGlu solutions, particularly those involving the C6 and C2 centers, since these centers contribute most of the intensity of the αMeGlu system and are directly relevant to αGlu. Our modifications of the C2 and C6 radical reaction schemes in αGlu are shown below in Figure 5. All steps are consistent with the paths suggested by Dizdaroglu,[12] but we have added the C6 → C5 rearrangement and deleted ether group eliminations. The *G* values shown in the scheme are those of Table III of ref 12, with the exception of that for 5-keto-2-deoxyglucose. The compound determined for this G value is 2-deoxyglucitol which would have 2-deoxygluconic acid and 2-deoxy-5-ketoglucose as precursors. Because of the negligible concentration of C1 radicals observed in αMeGlu (and expected in αGlu for the same reasons), we consider the 0.95-G value to be due to the latter compound. Given this modification of the scheme, the ratio of the *G* values for these two products (3.37:1) is in good agreement with the observed ratio of ESR signal intensities.

Registry No. α-MeGln, 97-30-3.

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[14] Madden, K. P., unpublished data.

[15] Asmus, K.-D.; Möckel, H.; Henglein, A. *J. Phys. Chem.* **1973**, *77*, 1218–21.

[16] Laroff, G. P.; Fessenden, R. W. *J. Phys. Chem.* **1973**, *77*, 1283–88.

[17] Dizdaroglu, M.; Leitich, J.; von Sonntag, C. *Carbohydr. Res.* **1976**, *47*, 15–23.