E.S.R. STUDY OF ALKALINE, OXIDATIVE DEGRADATION OF SAC-CHARIDES. IDENTIFICATION OF 2,5-DIHYDROXY-*p*-BENZO-SEMIQUINONE

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ABSTRACT

The formation of radical intermediates during alkaline, oxidative degradation of saccharides and α -hydroxycarbonyl compounds has been studied by e.s.r. spectroscopy. Quantum chemical calculation and experiments in alkaline D₂O solution showed that the dominant component of the overall spectrum corresponds to 2,5-dihydro-*p*-benzosemiquinone. Formation of this radical was also observed in the alkaline-degradation products of cellulose, starch, and (4-Omethylglucurono)xylan in the presence of air.

INTRODUCTION

Investigations on the chromophores generated during delignification of wood in alkaline medium without additives (peroxides, quinones, etc.), have thus far paid little attention to the formation of radical structures from the saccharide portion¹. These products are of interest from the viewpoint of possible resorption of the low-molecular weight degradation products on the saccharide component, which causes a decrease of brightness of pulp.

It is well known that free radicals can be generated by the effect of aqueous, alkaline solutions on saccharides in the presence of air². Oxidation with low oxygen concentration in alkaline solutions of α -hydroxyketones is a very effective method of forming anion radicals of the semidone type; but in an inert atmosphere (helium), no e.s.r. spectra were observed³. According to Lagercrantz², several types of radical structures are formed simultaneously during alkaline degradation of saccharides. Their concentration depends on the starting material, the concentration of the aqueous solution, and the temperature and time of reaction in the presence of oxygen. This work set out to demonstrate that saccharides—microcrystal-line cellulose, starch, (4-O-methylglucurono)xylan, and monosaccharides—if subjected to alkaline degradation, all yield the same aromatic semidone radical, point-

ing to a common fragmentation and recombination (via aldol condensation) pathway.

EXPERIMENTAL

Materials. — These were from the sources noted: glycolaldehyde, DLglyceraldehyde (Fluka AG); "dihydroxyacetone" dimer (Koch-Light Lab. Ltd.); D-mannose, D-galacturonic acid (B.D.H. Chem. Lab.); D-glucuronic acid (Sigma Chem. Co.); L-rhamnose (Calbiochem); microcrystalline cellulose (Avicel); 2,5-dihydroxy-p-benzoquinone (Aldrich); tetrahydrofuran, D-xylose. D-arabinose, Dribose, D-glucose, D-galactose, potassium hydroxide (Lachema. ČSSR, analytical grade); and deuterium oxide (Merck AG). (4-O-Methylglucurono)xylan was prepared according to Ebringerová *et al.*⁴. The distilled water used was not deoxygenated before the experiments.

General methods. — The e.s.r. spectra were recorded with an X-band spectrometer (Varian E-4). The measurements were taken at 1 mW microwave power and $5\,\mu\text{T}$ modulation, when no deformation of the spectra was observed. A sample cell for aqueous solutions was used for the measurements. In determination of the concentrations of spins, flame-sealed glass tubes were used (inner diameter 2.5 mm) and the measurements were performed at -100° in comparison with a standard concentration of Strong Pitch. The g-factors were determined by using as standards Strong Pitch and nitroxide radical.

Formation of radicals. — Solutions (M) of substrate in 6–12M solutions of potassium hydroxide were prepared in the presence of air and immediately poured into the cell, which was kept at 100°. After cooling to room temperature, the e.s.r. signals were measured. 2.5-Dihydroxy-*p*-benzoquinone was dissolved in tetrahydrofuran and extracted with 12M aqueous potassium hydroxide. The aqueous phase was transferred into the cell, which was kept at 100° Measurement of e.s.r. signals was made after cooling to room temperature.

In the experiments with D_2O , M solutions of substrate in D_2O were also prepared. To this solution was added granulated potassium hydroxide in such quantity as to give concentrations in the range 6–12M. After complete dissolution of all components, the solution was put into the measuring cell, kept at 100°, and then measured at the room temperature. Some samples were measured immediately without keeping at the higher temperature.

In the experiments in glass tubes, 0.5 mL of M D-glucose solution in 6M potassium hydroxide was quantitatively introduced (syringe) into the tube, which was immediately flame-sealed. The sample was kept at 100° and then cooled in liquid nitrogen and measured at -100° .

RESULTS AND DISCUSSION

Typical forms of e.s.r. spectra observed for alkali-degraded saccharides and



Fig. 1. The e.s r. spectra of radicals formed on alkaline, oxidative degradation of saccharides A: DL-Glyceraldehyde in 12M potassium hydroxide kept for 3 25 min at 100°. B L-rhamnose in 12M potassium hydroxide kept for 3.25 min at 100°. C: Water-soluble fraction of starch (mol. wt. = 5900) in 6M potassium hydroxide kept for 10 min at 100°.

 α -hydroxycarbonyl compounds are shown in Fig. 1. Detailed analysis shows that the spectra are multicomponent (Fig. 2). Component D is the most prevalent in all spectra presented here; three other components (see Fig. 2) were also found to contribute to the overall spectrum. As a result of the different g-factors of the individual single components, the resulting spectra are asymmetrical. The spectrum of D has the same structure in all cases studied. It is a superposition of three further single-component spectra: a main component J (triplet, 0.08 mT) and two satellites E (doublet, 0.68 mT) of a triplet (0.08 mT), and F (doublet, 0.28 mT) of a triplet (0.08 mT). All have the same g-factor and their intensities are in the ratios J:E:F = 1:0.016:0.038 in all examples studied.

In the experiments with D_2O , partial substitution of protons by deuterium takes place, causing a change in the structure of the spectrum (Fig. 3). Complete substitution by deuterium changes the basic triplet (E) into a five-line spectrum (C) having the splitting constant 0.0123 mT, which corresponds to deuterium. Partial substitution by deuterium changes the triplet E into spectrum D. Addition of these two components to component E without deuterium gives the final spectrum. The ratios of three components E:D:C = 1:4.8:7.5 was determined by simulation of the spectra.



Fig. 2 Theoretical e.s.r. spectra of radicals formed on alkaline, oxidative degradation of saccharides T¹ Overall theoretical spectrum, corresponding to spectrum B in Fig. 1. D. Theoretical spectrum of radical 2 (g = 2.0045) F. Theoretical spectrum of radical 2 with ¹³C splitting in position 1 or 4 (g = 2.0045) F. Theoretical spectrum of radical 2 with ¹³C splitting in one of the following positions 2.3.5, or 6 (g = 2.0045) G. Theoretical spectrum of component G (g = 2.0046) a = 0.1175 mT, doublet) H. Theoretical spectrum of component H (g = 2.0046, a = 0.0825 mT, doublet) I. Theoretical spectrum of component H (g = 2.0046, a = 0.1235 mT quartet) *T*. Theoretical spectrum of radical 2 without ¹³C splitting (g = 2.0045). (Components G, H. and J will be discussed in a future paper.)

Fig. 4 shows the amplified satellite parts (spectra E and F) of the samples substituted by deuterium as originally given in Fig. 2. The splitting of the doublets appears to originate in the hydrogen atoms.



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Fig. 3. The e.s.r spectrum of radicals formed on alkaline degradation of D-glucose in a D₂O solution of potassium hydroxide. A: Experimental spectrum (gain = 4×10^3). B: Overall theoretical spectrum. C: The component of the theoretical spectrum caused by interaction of two equivalent deuterium atoms with an unpaired electron. D: The component of the theoretical spectrum caused by interaction of two equivalent protons with an unpaired electron. E: The component of the theoretical spectrum caused by interaction of two equivalent protons with an unpaired electron.



Fig. 4. The e.s.r. spectrum of radicals formed on alkaline degradation of 1-rhamnose in a D_2O solution of potassium hydroxide (gain = 3.2×10^3). The asymmetry of the spectrum is caused by mutually shifted components having different g-factors (see Fig. 2). The interpretation of the central part of the spectrum is shown in Fig. 3.

TABLE I

Posttion	Atom	$\mathbf{a}_{\mathbf{t}}^{\exp}\left[mT ight]^{a}$	$x_i^{\rm AP} [mT]^h$	$a_{i}^{T}[mT]$
1.4	н	0.079	0.08	0.24
	¹ '(0.662	0.68	() 79
2,3,5,6	130	0.282	0.28	11 31

COMPARISON OF THE EXPERIMENTAL AND THFORE IICAE VALUES OF HYPERFINE SPEEDING-CONSTANTS OF 2,5-DIHYDROXY-*p*-BENZOSEMJQUNONE

"Results by Reitz et al ⁵ ^bOur results ' Results by the INDO method"

Lagercrantz² ascribes the spectrum J (Fig. 2) to the radical 1. On the basis of e.s.r. spectra measured by Reitz et al.⁶, Russell et al.⁵, corrected the proposed structure of the radical 1. Reitz did not specify the substrate used. We have used 2.5-dihydroxy-*p*-benzoquinone as the substrate to prove the structure of radical **2**. The hypothesis that 2,5-dihydroxy-*p*-benzosemiquinone (2) is formed in alkaline degradation of saccharides in the presence of air was thus confirmed. This verification is shown in Fig. 2, which combines three simple components E. F. and J into a single component D. The hyperfine splitting constants determined experimentally by Reitz et al.⁶ are: $a_{\rm H} = 0.079 \,{\rm mT}$, $a_{\rm C}^{1/4} = 0.662 \,{\rm mT}$, and $a_{\rm C}^{2/5+6} = 0.282 \,{\rm mT}$ Interestingly, the hyperfine structure of the spectrum also shows lines for ¹⁵C, although the structure was not enriched with this nucleus. It may thus be assumed that interaction of an unpaired electron with two protons and/or the nucleus of only one ¹³C atom will be present in the observed spectrum as a result of the rare occurrence of this interaction. In structure 2, two different groups of equivalent positions of ¹³C atoms exist with regard to the interaction of the unpaired electron. namely, positions 1.4 and 2,3,5,6.

Quantum chemical calculation by the INDO method with standard parametrization⁷ gives for ¹³C the following splitting constants: $a_c^{1/4} = 0.79$ mT and $a_c^{5/3/5,6} = 0.31$ mT. It follows that, in the overall spectrum, three different spectra are present with respect to a position that has a sufficient concentration of ¹³C atoms.

The natural abundance of ¹³C in admixture with ¹²C is 1.11% With regard to the number of equivalent positions in radical 2 for the contribution of individual components in spectrum D (Fig. 2), the ratios $J_*E:F = 1.0.022(0.044)$ are found. These ratios are in good qualitative agreement with that obtained from the experimentally determined spectrum.

The hyperfine splitting-constants of two equivalent protons according to Reitz *et al.*⁶ is 0.079 mT. Calculation by the INDO method gave the higher value of $a_{\rm H} = 0.24$ mT (Table I). However, this value falls into the general range of results obtained by the INDO method for benzosemidones⁷. McLachlan's method⁸ ($h_{\rm O} = 1.5$; $k_{\rm C-O} = 1.6$; $\lambda = 1$; Q = 2.7 mT) gave $a_{\rm H}^{\rm T} = 0.117$ mT.

When a limited amount of air is present the radical intermediates of alkaline degradation of saccharides are stabile. For instance, the concentration of spins, generated in the flame-sealed tube for 20 h at 100° from a M solution of D-glucose

in 6M potassium hydroxide, was 26.22×10^{16} spin/g of substrate. The original solution before thermal treatment did not contain any measurable quantity of free radicals, whereas after 10 s of generation, under the same conditions as before, the concentration was 6.93×10^{16} spin/g. The highest spin-concentration (49.64 $\times 10^{16}$ spin/g) was found after 3 min of generation.

When the radicals were generated in an aqueous-solution sample-cell at 100° in the presence of air, their lifetime was shorter than one h. These different results may be explained on the basis of formation of an anion radical of the semidone type. The formation and loss of this radical may be assumed to result from interaction with an oxygen molecule in the triplet state³. The formation of aromatic compounds in the alkaline degradation of saccharides under an inert atmosphere is well known⁹. The fact that similar spectra arise from all starting materials used in our experiments confirms the assumption that, first of all, fragmentation to two-, three-, and four-carbon fragments takes place. These fragments recombine and are cyclized in base-catalyzed aldol condensations. The phenols formed give phenolates in the alkaline medium, and are converted by the action of oxygen (air) into semiquinones or semidones; further loss of an electron caused by oxygen of air results in formation of benzoquinones¹⁰.

Fig. 1 shows that alkaline degradation of water-soluble starch (mol. wt. 5900) also gives approximately the same spectrum as that from monosaccharides. Similar spectra were also found when cellulose and (4-*O*-methylglucurono)xylan were used. It may therefore be concluded that alkaline degradation of polysaccharides in the presence of air gives a mixture of aromatic semidone radicals, resulting from an identical fragmentation and recombination (*via* aldol condensation) reaction-pathway. This observation is an important contribution to our general understanding of the mechanism of alkaline degradation of monosaccharides and polysaccharides in the presence of air.

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