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E.S.R. SPIN TRAPPING ANALYSIS OF GAMMA INDUCED RADICALS IN SUCROSE

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Radicals induced by gamma-irradiation in sucrose, in the solid state at different temperatures and in aqueous solution, have been studied by the spin-trapping method. Electron spin resonance (ESR) combined with high performance liquid chromatography (HPLC) then spectra analysis with a simulation program (VOYONS), revealed seven main radical species. Their nitrogen and hydrogen splitting constants were compared with those obtained from fructose and glucose units. Assignments of chemical structures are discussed for three radical species.

KEY WORDS: E.S.R., HPLC, simulation, spin-trapping, sucrose, gamma-irradiation, labelled sugars.

INTRODUCTION

Sucrose contains α-D-glucopyranose and β-D-fructofuranose residues bound by a glycosidic linkage. In order to understand the gamma-radiolysis of sucrose, experiments have been carried out using the spin trapping method.[1] The sugar radicals are converted into long-lived nitroxide spin adducts in the liquid phase (water-ethanol ratio 2:1) by reaction with 2-methyl-2-nitrosopropane (MNP or ^{t}BU-N = O):

In these conditions numerous nitroxides (Fig. 1) are produced: their ESR signals which show only slightly different a_N and a_H hyperfine coupling constants and have very similar g-factors are all superimposed, making highly complex spectra. High performance liquid chromatography (HPLC) has been used to separate this mixture of sugar-nitroxide radicals.[2-4]

MATERIALS AND METHODS

Sugars were purchased from Fluka, Merck, Aldrich and Prolabo Companies, and MNP from Aldrich. Labelled sugars were synthesized in our Laboratory.

The sugars were irradiated in a ^{137}Cs cell, supplying a dose rate of 3.4 kGy h^{-1}. The doses (20 kGy for irradiation in the solid state at − 196°C, 25°C, 100°C or 2 kGy at 25°C for liquid-phase irradiation) were chosen in order to obtain suitably intense E.S.R. signals.

One minute before trapping, 0.8 ml of 12 mg per ml solution of MNP in deoxygenated ethanol, was added to 1.6 ml of deoxygenated water. The sugar (200 mg) was dissolved in water-ethanol solution of MNP (2.4 ml). In the case of irradiation in the liquid phase, to prevent the formation of radicals derived from ethanol, the sugar was dissolved in an aqueous solution of MNP (stirred overnight), irradiated, and then added to ethanol.

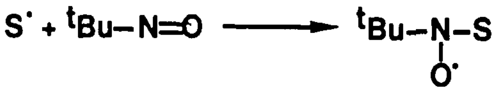


FIGURE 1

The nitroxide solution was immediately injected onto a Waters semi-preparative C18 micro-bondapak column, cooled to 5°C, in order to increase the radicals stability. The water-ethanol eluent was delivered at a flow rate of 1.ml. min^{-1} and a Bruker 200 D 10 E.S.R. spectrometer was used first as a detector. Collected fractions (one per minute) were cooled in an ice bath until the spectra were recorded.[4,5] Each fraction contained no more than four radicals and the t-Bu-NO°-tBu radical was readily separated.

The simulations of the E.S.R. spectra were performed on a Deskpro Compaq 386/25 computer with the "VOYONS" program,[6] a general interactive simulation program, written in Turbo Pascal for IBM compatible 16–32 bit microcomputers, which allows the quantitative study of spectroscopic data (circular dichroism, ESR, NMR, etc) with different algorithms (non-linear curve fitting, Fourier Transform, curve convolution-deconvolution, etc).

RESULTS AND DISCUSSION

Assignment of a common radical in sucrose, fructose and glucose

The comparison of hyperfine coupling constants and stabilities of spin trapped radicals in sucrose, β-D-fructose and α-D-glucose, reported in Tables 1 to 3 resp., shows one common radical only. This radical, with a_N = 15.4 G, a_H = 1.9 G is trapped under all irradiation conditions, but mainly at high temperature, where it is rather persistent. The same radical has been observed with several other experimented sugars, such as methyl-1-α-glucose or maltose, and with polyols, like xylitol, dulcitol, sorbitol and mannitol, or in irradiated starches and dextrins. Consequently it has been assigned the following structure:[4,5] Fig. 2.

where R represents a one or two carbon group.

Assignment of radical S1

The S1 radical, spin trapped in sucrose, has also been found in irradiated methyl-1-α-

TABLE 1
Specific parameters of the sucrose nitroxide adducts

					Irradiation conditions			
					H_2O	Powder		
	a_N	a_{H_1}	a_{H_2}	a_{H_3}	25°	−196°	25°	100°
S1	14.3	2.4	2	1.3	.	.	+	+
S2	15.5	0.6	–	–	+	+	+	+
S3	15.4	1.9	–	–	.	.	.	+
S4	15.4	3.9	–	–	+	.	.	.
S5	14.8	1.8	0.3	–	+	+	+	+
S6	14.8	0.3	0.2	–	.	.	.	+
S7	14.2	1	–	–	.	+	.	.

TABLE 2
Specific parameters of the β-fructose nitroxide adducts

				Irradiation conditions			
				H_2O	Powder		
	a_N	a_{H_1}	a_{H_2}	25°	– 196°	25°	100°
F1	15	1	–	+	+	+	.
F2	15.3	2.1	0,3	+	+	+	.
F3	15.4	1.9	–	.	.	.	+

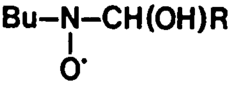
TABLE 3
Specific parameters of the α-glucose nitroxide adducts*

				Irradiation conditions			
				H_2O	Powder		
	a_N	a_{H_1}	a_{H_2}	25°	– 196°	25°	100°
G1	15.4	4.7	–	+	+	+	.
G2	15.4	1.9	–	+	+	+	+
G3	15	1	–	+	+	+	.

*Partial list, including only radicals G1, G2, G3, with a_N splitting constants comparable to those derived in sucrose and fructose.

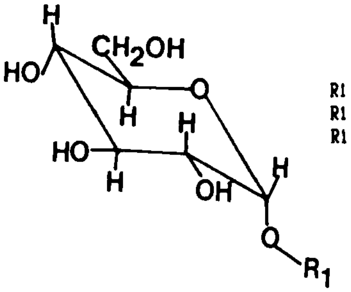
glucose, and in maltose[4] but not in glucose or in methyl-1-β-glucose. This strongly suggests, first, that the glycosidic linkage is necessary for radical S1 to be formed, and, secondly, that S1 is independent on the nature of the R1 group: it can be a methyl group as well as a fructose or another glucose unit (Figure 3).

Thus, the S1 radical should be localized on the glucose unit, possibly on the carbon C1. To check this hypothesis, methyl-1-α-glucose-1-13C was synthesized, and the ESR spectra of the corresponding spin trapped radicals were recorded under identical experimental conditions. The spectra analysis shows, instead of radical S1, a radical species with a further splitting (a_N = 14.3 G, a_{H_1} = 2.4 G, a_{H_2} = 2 G, a_{H_3} = 1.3 G, a_{13C} = 4.3 G) which is fully consistent with a radical on the C1 of the glucose unit.



$$\mathrm{Bu{-}\underset{\substack{|\\ O^{\bullet}}}{N}{-}CH(OH)R}$$

FIGURE 2



R1 = Methyl
R1 = β-D-Fructofuranosyle
R1 = α-D-Glucopyranosyle

FIGURE 3

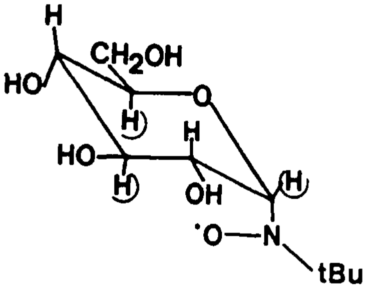


FIGURE 4

The failure to detect radical S1 with methyl-1-β-glucose and the anomeric effect[7] suggest that radical S1 be assigned the structure shown in Figure 4.

The observed hydrogen coupling constants arise from the interaction of the unpaired electron with the axial C1 hydrogen, and from two long range couplings with the C3 and C5 hydrogens.[8]

Assignment of radical S2

The radical S2 (a_N = 15.5 G, a_H = 0.6 G) was observed both in sucrose (Table 1) and in methyl-2-β-fructofuranoside, but not in fructose. This again suggests a radical localized near the glycosidic linkage, but on the fructose unit. We have now undertaken the synthesis of methyl-2-β-fructofuranoside, labelled on the carbon C2, in order to test this hypothesis.

CONCLUSION

This paper deals with gamma radiolysis of sucrose and reports the ESR detection of seven different radicals by means of the spin trapping technique. One of the observed species was positively identified through a combined use of the ESR spectral data and of isotopic substitution. A conceivable structure is proposed for a second species, but its identification is not definite.

Acknowledgments

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