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ESR SPIN TRAPPING ANALYSIS OF GAMMA INDUCED RADICALS IN SUCROSE: II[1]

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Radicals induced by gamma-irradiation of sucrose, in the solid state at different temperatures and in aqueous solution, have been investigated by the spin trapping method. Electron spin resonance (ESR) combined with high performance liquid chromatography (HPLC), followed by spectral analysis with a simulation program (Voyons) revealed seven main radical species. A comparative study of the ESR signals from spin trapped gamma-induced radicals in some glycosides, disaccharides, ^{13}C specifically labelled carbohydrates, as well as in several deoxysucroses and fructans, led to the assignment of a chemical structure to five out of the seven sucrose-nitroxide adducts previously evidenced. Sucrose is shown to be a conceivable model for the study of fructans gamma-radiolysis mechanism in aqueous solution.

KEY WORDS: Sucrose, deoxysucroses, fructans, gamma-irradiation, spin-trapping, ESR spectra simulation.

INTRODUCTION

In order to understand the gamma-radiolysis of sucrose, experiments have been carried out using the spin trapping method.[2] 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 tBu–N=O).

In these conditions, numerous nitroxide radicals are produced.

$$S^{\cdot} + tBu{-}N{=}O \rightarrow tBu{-}\underset{\displaystyle O^{\cdot}}{\overset{\displaystyle |}{N}}{-}S$$

Their ESR signals, which show only slightly different a_N and a_H hyperfine coupling constants and very similar g-factors, are all superimposed. The resulting ESR spectra appear highly complex. High Performance Liquid Chromatography (HPLC) has been used to separate this mixture of sugar-nitroxide radicals.[3–5]

Glycosides, disaccharides, fructans, ^{13}C specifically labelled carbohydrates and several deoxysucroses are used to assign a chemical structure for five out of the seven sucrose-nitroxide evidenced radicals.[6] The trapped species in several fructans irradiated in aqueous solution are found to be identical to those in sucrose. Sucrose, therefore, can be considered as a conceivable model for the radiolysis mechanism study of these important plants constituents.[6,7]

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MATERIALS AND METHODS

Commercial sugars were purchased from Aldrich, Boehringer Mannheim, Fluka, Merck, Prolabo, Sigma and TCI Companies. The deoxysucroses were provided by Béghin-Say Company as well as the fructans GF_2, GF_3 and GF_4 (generously given as a "Profeed®" mixture). The methyl-fructosides and the ^{13}C specifically labelled sugars were synthesized in the 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 solid state at − 196°C, 25°C, 100°C, or 2 kGy at 0°C for aqueous phase irradiation) were chosen in order to obtain suitably intense ESR signals.

One minute before trapping, 0.8 ml of a 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 the preceding water–ethanol solution of MNP (2.4 ml). In the case of irradiation in liquid phase, to prevent the formation of radicals derived from ethanol, the sugar was dissolved in a saturated aqueous solution of MNP (stirred for 12 h), irradiated, and then added to ethanol.

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 ESR spectrometer was used first as a detector. Collected fractions (one per minute) were cooled in an ice bath until the spectra were recorded.[5,8] Each fraction contained no more than four radicals and the tBu–$NO^{\cdot}$–tBu radical was readily separated. Simulations of ESR spectra were performed on a Deskpro Compaq 386/25 computer with the "Voyons" program,[9,10] a general interactive simulation program, written in Turbo Pascal for IBM compatible 16–32 bit microcomputers, to study spectroscopic data (quantitative analysis of multicomponent ESR spectra in liquid phase, non-linear curve fitting, Fourier Transform, curve convolution–deconvolution, etc.). As an illustration , a typical ESR spectrum of sucrose with its simulation, in experimental conditions such that three nitroxide–sugar radicals are spin trapped, is displayed on Figure 1.

RESULTS AND DISCUSSION

Seven radical species (S1 to S7) have been previously evidenced (Table I). A chemical structure has been assigned to two of them. S1 (Figure 2) was identified by means of specifically ^{13}C labelled methyl-α-D-glucopyranoside and S3 (Figure 3) by comparison with the spin trapped radicals in irradiated polyols.[1]

The assignment of three other radicals, S2, S4 and S5, results from a comparison of hyperfine coupling constants and stabilities of spin trapped radicals in numerous sugars (sucrose, 4-deoxysucrose, 6-deoxysucrose, 4,6-dideoxysucrose, 6,6′-dideoxysucrose, fructans (Figure 4)) (Tables I to VI resp.), and in specifically labelled derivatives, correlated with the study of some oligosaccharides.

Assignment of Radical S2[11]

S2 radical (a_N = 1.55 mT, a_H = 0.06 mT) is obtained in all irradiation conditions. A similar radical is trapped in methyl-β-D-fructofuranoside, O-α-D-glucopyranosyl-(1 → 3)-β-D-fructofuranosyl-(2 → 1)-α-D-glucopyranose (melezitose) (Figure 5) and

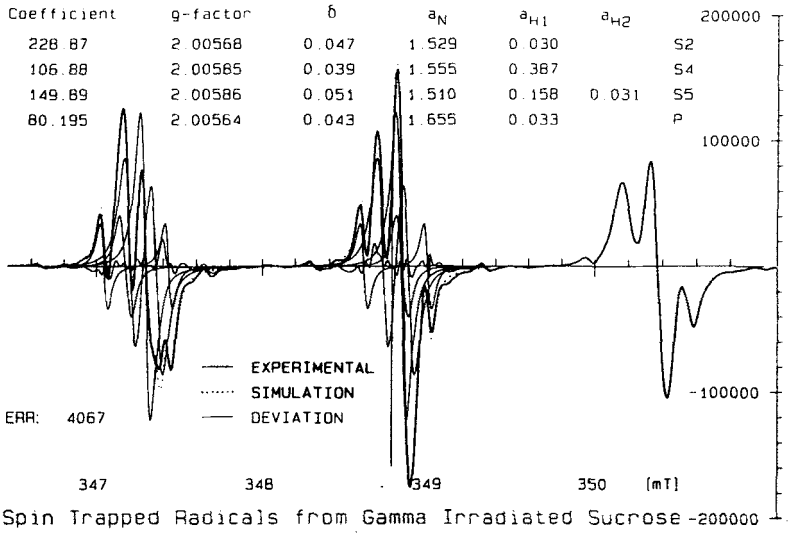


FIGURE 1 ESR spectrum of the nitroxide sugar radicals from sucrose, gamma irradiated (~2 kGy) at ~0°C in an aqueous solution of 2-methyl-2-nitroso-propane (MNP); ESR recording is performed at room temperature, in water–ethanol [2 : 1] solvent; sweep width: 6.915 mT; central field: 348.77 mT; frequency: 9.796 GHz; g = 2.0060 position is indicated by a vertical line. Experimental, Simulation and Deviation spectra are displayed as well as the individual components; ERR: value of the standard deviation. On top, the parameters (coefficient, *g* factor, linewidth and splitting constants a_N, a_{H1} and a_{H2}, expressed in mT) are printed for the nitroxide sugar radicals (S2), (S4) and (S5); the P component is due to the spin trap.

in fructans O-β-D-fructofuranosyl-((2 → 1)-β-D-fructofuranosyl)$_n$-(2 → 1)-α-D-glucopyranose (n = 1(kestose or GF_2), n = 2 (nystose or GF_3), n = 3 (GF_4), n = 36 (inulin)).

However, S2 radical is lacking in ionized commercial β-D-fructose or in methyl-β-D-fructopyranoside irradiated in aqueous solution. We should note that crystalline β-D-fructose has the β-D-fructopyranose configuration,[12] and that its aqueous solution at 0°C contains 12% of β-D-fructofuranose and 88% of β-D-fructopyranose.[13] Thus, experiments on the β-D-fructose can be considered like experiments on the β-D-fructopyranose (Figure 6).

These facts show that trapping of radical S2 requires the presence of a β-D-fructofuranose cycle in the irradiated molecule. A study of the S2 contribution in global spectra of spin trapped fructans confirms this localization (Figure 7). Actually, the S2 contribution increases with the number of fructofuranose cycles. In inulin, which can be considered as exclusively constituted by β-D-fructofuranose cycles, S2 is the single trapped species.

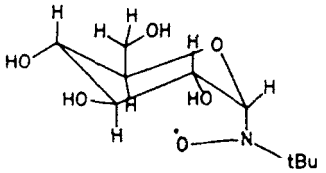


FIGURE 2

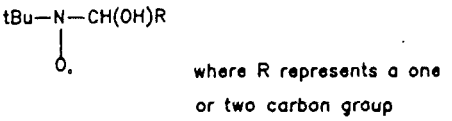
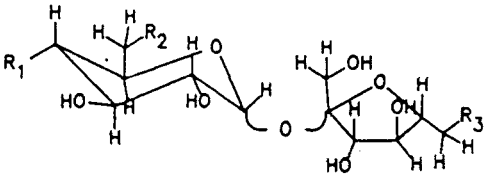


FIGURE 3

A first hypothesis was that a radical, similar to S1, but located on the carbon C-2 of the β-D-fructofuranose cycle, should also be generated.[1] However, the ESR spectrum of S2 radical is not modified in spin trapped ionized methyl-[2-^{13}C]-β-D-fructofuranoside. Consequently, the glycosidic linkage does not lead to S2 by cleavage, but locks the fructose cycle in the β-D-fructofuranose conformation. This allows the S2 radical formation.

The transition from a pyranosidic cycle (6 carbons) to a furanosidic cycle (5 carbons) involves the formation of a CH_2OH group on the carbon C-5. The particular gamma sensitivity of such a group is well known and has been evidenced



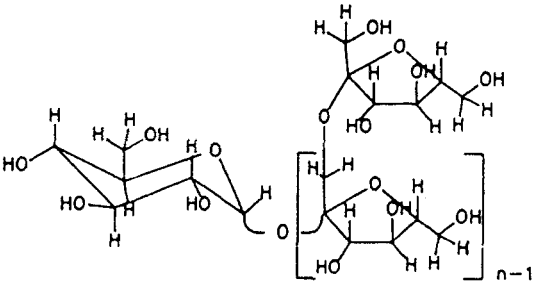
R_1=R_2=R_3=OH: sucrose

R_1, R_2=R_3=OH: 4-deoxysucrose

R_2=H, R_1=R_3=OH: 6-deoxysucrose

R_1=R_2=H, R_3=OH: 4,6-dideoxysucrose

R_2=R_3=H, R_1=OH: 6,6'-dideoxysucrose



fructans: GFn

FIGURE 4

TABLE I
ESR parameters of the sucrose nitroxide adducts

	a_N(mT)	a_{H1}(mT)	a_{H2}(mT)	a_{H3}(mT)	Irradiation conditions: H_2O 0°C	Powder −196°C	Powder 25°C	Powder 100°C
S1	1.43	0.24	0.20	0.13	–	–	+	+
S2	1.55	0.06	–	–	+	+	+	+
S3	1.54	0.19	–	–	–	–	–	+
S4	1.54	0.39	–	–	+	–	–	–
S5	1.48	0.18	0.03	–	+	+	+	+
S6	1.48	0.03	–	–	–	–	–	+
S7	1.42	0.10	–	–	–	+	–	–

in the case of α-D-glucopyranose, β-D-glucopyranose, α-D-mannopyranose, methyl-α-D-glucopyranoside and G_n (defined as O-α-D-glucopyranosyl-$(1 \rightarrow 4)_{n-1}$-α-D-glucopyranose) with $n = 2$ to $n = 7$.[14-17] A radical with similar stability and splitting constants ($a_N = 1.44$ mT, $a_{H1} = 0.05$ mT) was localized in the vicinity of the carbon C-6 of maltose (G_2) irradiated in aqueous solution. Moreover, this species and S2 radical were both trapped in the case of melezitose irradiated in aqueous phase.

Accordingly, the structure shown in Figure 8 is assigned to S2 radical.

We note that radical S2 is spin trapped in every conditions for sucrose and all its studied deoxyderivatives, with one exception: S2 is found in aqueous solution only, for the 6,6′-dideoxysucrose. It has been shown[18] that, in the crystalline state, an intramolecular H-bond exists between $O_{6'}$--H--O_5, in sucrose and its derivatives, except, obviously, when $O_{6'}H$ is missing, like in 6,6′-dideoxysucrose. This H-bond vanishes in aqueous solution.[19] Consequently, we propose[6] that two different mechanisms can lead to S2 radical (or two close S2 and S2′ radicals). In the crystalline state, a direct radiolysis mechanism, involving the $O_{6'}H$ may occur for sucrose and all its deoxyderivatives experimented in this report, except for the 6,6′-dideoxysucrose. In aqueous solution, an indirect radiolysis mechanism, by $OH^{\cdot}$ attack, is expected for sucrose and its deoxyderivatives, including the 6,6′-dideoxysucrose.

Assignment of Radical S4

S4 radical ($a_N = 1.54$ mT, $a_H = 0.39$ mT) is trapped when irradiation is performed in aqueous solution only. It is also trapped in gamma-irradiated fructans (GF_2, GF_3 and

TABLE II
ESR parameters of the 4-deoxysucrose nitroxide adducts

	a_N(mT)	a_{H1}(mT)	a_{H2}(mT)	a_{H3}(mT)	Irradiation conditions: H_2O 0°C	Powder −196°C	Powder 25°C	Powder 100°C
S1	1.43	0.24	0.20	0.13	–	–	+	+
S2	1.53	0.04	–	–	+	+	+	+
S3	1.54	0.19	–	–	–	–	–	+
S5	1.51	0.17	0.04	–	+	+	+	+

TABLE III
ESR parameters of the 6-deoxysucrose nitroxide adducts

	a_N(mT)	a_{H1}(mT)	a_{H2}(mT)	a_{H3}(mT)	Irradiation conditions			
					H_2O	Powder		
					0°C	−196°C	25°C	100°C
S1	1.43	0.24	0.20	0.13	−	−	+	+
S2	1.53	0.04	−	−	+	+	+	+
S3	1.54	0.19	−	−	−	−	−	+
S5	1.50	0.19	0.03	−	+	+	+	+
D6	1.38	0.06	−	−	−	+	−	−

GF_4), glucose oligomers G_n ($n = 2$ to $n = 7$), trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) and cellobiose (4-O-β-D-glucopyranosyl-β-D-glucopyranose) (Figure 9).

It is not trapped when experiments are carried out with α-D-glucopyranose, β-D-fructose, methyl-α-D-glucopyranoside, phenyl-α-D-glucopyranoside, melibiose (6-O-α-D-glucopyranosyl-β-D-glucopyranose) and lactose (4-O-β-D-galactopyranosyl-α-D-glucopyranose) in aqueous solution (Figure 10).

S4 radical appears to be localized on the glucose unit of sucrose molecule. The presence of this glucopyranose cycle is not sufficient and a glycosidic linkage seems to be necessary too. A study of the S4 contribution in global spectra of spin trapped fructans confirms this localization (Figure 7): the S4 contribution decreases with the number of fructofuranose cycles. In inulin (which can be considered as exclusively composed of β-D-fructofuranose cycles) S4 is not observed.

Radiolytic studies of aqueous D-mannitol and D-sorbitol,[20] using the spin trapping method, led to a general S4 chemical structure (Figure 11).[16] But the exact localization of the unpaired electron was not yet determined.

S4 radical is not observed when spin trapping experiments are carried out with 4-deoxysucrose, 6-deoxysucrose, 4,6-dideoxysucrose, 6,6′-deoxysucrose gamma-irradiated in aqueous solution. Accordingly, the hydroxyl groups on carbon C-4 and on carbon C-6 seem to be necessary for the formation and/or stabilization of S4 radical. Thus, the unpaired electron must be localized on the carbon C-5 of the α-D-glucopyranose cycle.

An experiment with GF_2, ^{13}C specifically labelled on the carbon C-6 of the α-D-glucopyranose unit, supports this localization. The spectra analysis shows, instead of radical S4, a radical species with a small further splitting ($a_N = 1.54$ mT,

TABLE IV
ESR parameters of the 4,6-deoxysucrose nitroxide adducts

	a_N(mT)	a_{H1}(mT)	a_{H2}(mT)	a_{H3}(mT)	Irradiation conditions			
					H_2O	Powder		
					0°C	−196°C	25°C	100°C
S1	1.43	0.24	0.20	0.13	−	−	−	+
S2	1.54	0.03	−	−	+	+	+	+
S3	1.54	0.19	−	−	−	−	−	+
S5	1.52	0.16	0.03	−	+	+	−	−

TABLE V
ESR parameters of the 6,6′-dideoxysucrose nitroxide adducts

	a_N(mT)	a_{H1}(mT)	a_{H2}(mT)	a_{H3}(mT)	Irradiation conditions: H_2O 0°C	Powder −196°C	Powder 25°C	Powder 100°C
S1	1.43	0.24	0.20	0.13	−	−	−	+
S2	1.56	0.05	−	−	+	−	−	−
S5	1.51	0.16	0.03	−	+	+	+	+
S6	1.48	0.03	−	−	−	−	−	+
S7	1.44	0.10	−	−	−	+	−	−

a_H = 0.39 mT, a_{13C} = 0.05 mT) which is fully consistent with a radical on the C-5 of the α-D-glucopyranose unit.

In order to obtain this type of radical, two mechanisms can be considered. A hydrogen abstraction from the carbon C-4 (a) or from the carbon C-6 (b), followed by a rearrangement,[21] can lead to species (c) and (d). S4 radical is trapped in the case of maltose, but not in the case of lactose. So, the configuration of the hydrogen on carbon C-4 does influence the S4 formation.

The structure (e) shown in Figure 12 is, therefore, assigned to S4 radical.

Assignment of Radical S5

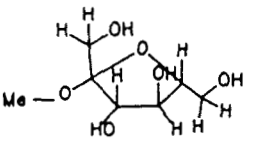
S5 radical (a_N = 1.48–1.52 mT, a_{H1} = 0.15–0.19 mT, a_{H2} = 0.03–0.04 mT) is trapped under all irradiation conditions, in spin trapping experiments carried out with sucrose, 4-deoxysucrose, 6-deoxysucrose and 6,6′-dideoxysucrose. This species is observed by spin trapping of 4,6-dideoxysucrose gamma-irradiated at −196°C or in aqueous solution. It is also trapped with gamma irradiation of fructans GF_2, GF_3 and GF_4 in aqueous solution (only conditions where fructans spin trapping is feasible).

A study of the S5 contribution in global spectra of spin trapped fructans shows its localization on the α-D-glucopyranose cycle (Figure 7). Indeed, the S5 contribution decreases with the number of fructofuranose units. In inulin, S5 is not observed.

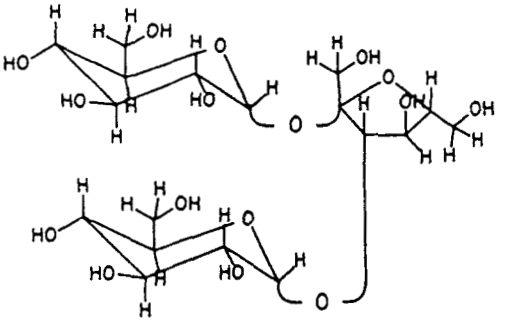
The main way of radical species formation, during the irradiation of crystalline carbohydrates, is by ionization of the oxygen atoms of the molecule.[22,23] S5 radical is obtained by trapping of sucrose, 4-deoxysucrose, 6-deoxysucrose 4,6-dideoxysucrose

TABLE VI
ESR parameters of the fructans nitroxide adducts

	a_N(mT)	a_{H1}(mT)	a_{H2}(mT)	Irradiation conditions: H_2O 0°C	Powder −196°C	Powder 25°C
S2	1.52	0.04	−	+	+	−
S3	1.54	0.19	−	−	−	+
S4	1.56	0.39	−	+	−	−
S5	1.49	0.15	0.03	+	−	−
S7	1.40	0.11	−	−	+	−



methyl– β –D–fructofuranoside

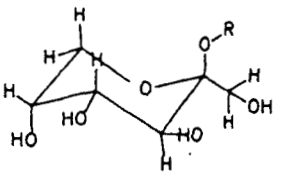


melezitose

FIGURE 5

and 6,6′-dideoxysucrose gamma-irradiated in the solid state. Thus, the oxygen atom O-5 is likely responsible for S5 formation.

To check this hypothesis, GF_2, ^{13}C specifically labelled on the carbon C-6 of the glucose unit, was synthesized and irradiated in aqueous solution. ESR spectra of the corresponding spin trapped radicals were recorded under identical experimental conditions. Spectra analysis shows, instead of radical S5, a radical species with a further splitting (a_N = 1.51 mT, a_{H1} = 0.15 mT, a_{H2} = 0.03 mT, a_{13C} =



R=H: β –D–fructopyranose

R=CH_3: methyl–β–D–fructopyranose

FIGURE 6

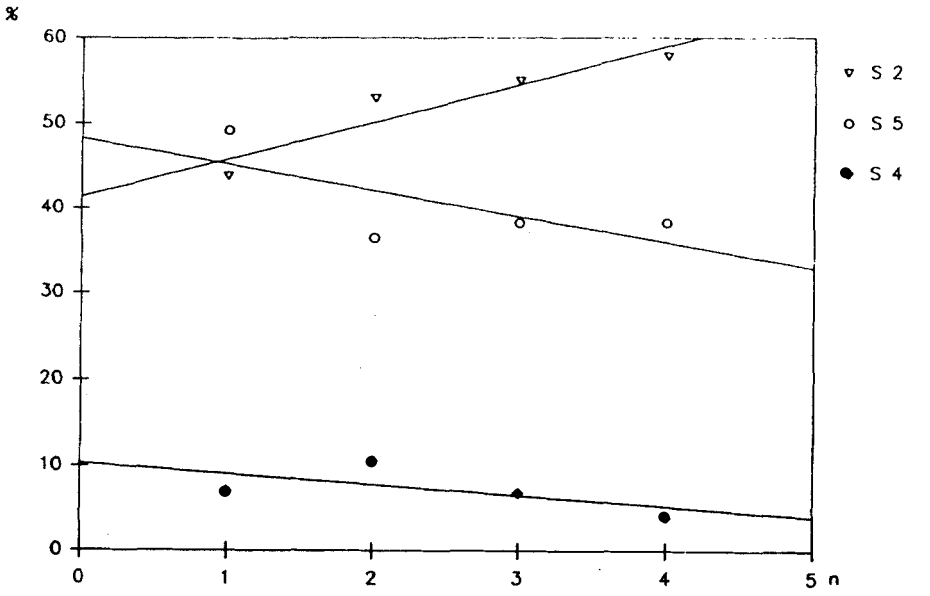


FIGURE 7 S2, S4 and S5 contributions = $f(n)$ in spin trapped GF_n.

0.10 mT) which is consistent with a radical on the C-5 of the α-D-glucopyranose unit.

The great similitude between the spectra obtained with sucrose, 4-deoxysucrose and 6-deoxysucrose indicates that the hydrogen atoms on carbons C-4 or C-6 are not responsible for the a_{H1} splitting, but, more probably, for the small a_{H2} splitting (when coupling constants and linewidths have close values, the hyperfine structure, if any, vanishes). Thus, the a_{H1} splitting might be due to an hydrogen atom located next to the unpaired electron (closer than the carbon C-6) and not linked to the carbons C-6 or C-4.

Accordingly, the structure shown in Figure 13 is assigned to S5 radical. The main splitting (a_{H1} = 0.15–0.19 mT) might be due to the axial hydrogen on carbon C-3, geographically close to the O˙ of the trapped species. This explanation is in agreement with the variability of the a_{H1} coupling constant (corresponding with geometric fluctuations due, for example, to the varying chain size in fructans).

The lack of spin trapped S5 radical in 4,6-dideoxysucrose, irradiated in solid state at 25°C and at 100°C (S5 is recorded in every conditions for all the other derivatives) seems to indicate a lower efficiency of spin trapping for this particular deoxyderivative: S5 is recorded only when the sugar has been irradiated in presence

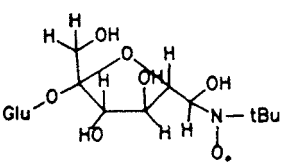
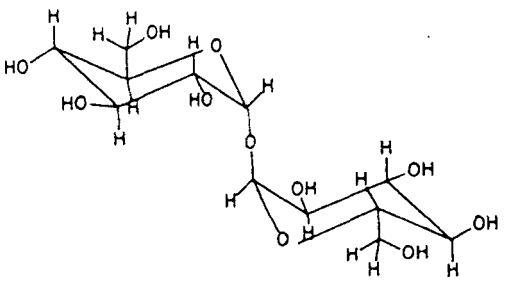
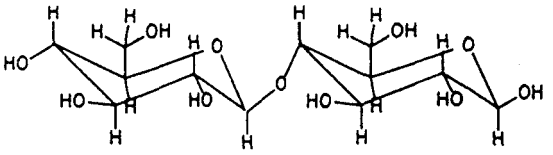


FIGURE 8



trehalose



cellobiose

FIGURE 9

of the spin trap (in aqueous solution at 0°C) or when stabilized by low temperature (at −196°C in solid state). The non polar area found for this derivative around C4–C5–C6, not attractive for the polar end of the spin trap tBuNO, might lower the spin trapping yield.

Case of Radical S6

S6 radical (a_N = 1.48 mT, a_{H1} = 0.03 mT) is trapped after gamma-irradiation of polycrystalline sucrose at 100°C only. Its contribution to the global spectrum is lower than 10%. It is also observed, in the same experimental conditions, with 6,6′-dideoxysucrose. In other deoxysucroses, simulations are not precise enough to determine if S6 is present in small quantities (lower than 5%), or not.

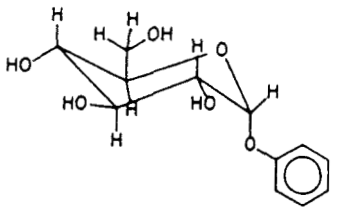
In these experimental conditions, spin trapping of fructans is not feasible. So, we cannot determine on which unit (α-D-glucopyranose of β-D-fructofuranose) is localized the unpaired electron.

Case of Radical S7

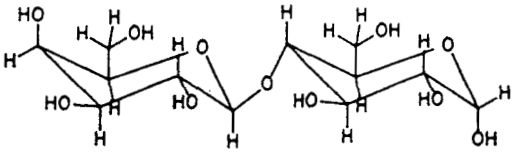
S7 radical (a_N = 1.42 mT, a_{H1} = 0.10 mT) is trapped after gamma-irradiation of polycrystalline sucrose at −196°C only. It is also trapped, in the same experimental conditions, with fructans (GF_2, GF_3 and GF_4) and with the 6,6′-dideoxysucrose.

In the case of the 6-deoxysucrose, a species with close spectroscopic characteristics is observed (D6, a_N = 1.38 mT, a_{H1} = 0.06 mT). For the other deoxysucroses, simulations cannot determine if S7 is present in small quantities (lower than 5%), or not.

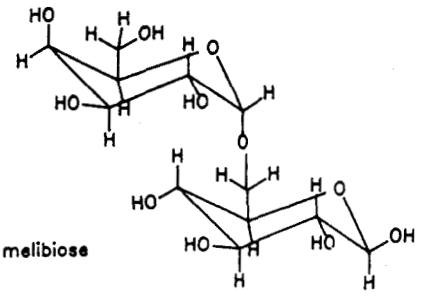
In these experimental conditions, spin trapping of fructans is difficult. S7 is observed, S5 is lacking, and trapping of S3 radical is favoured, due to the slow dissolution of oligomers in the trapping solution. Study of inulin in these experimental



phenyl–α–D–glucopyranoside



lactose



melibiose

FIGURE 10

conditions is impossible. We, therefore, cannot determine on which cycle (α-D-glucopyranose or β-D-fructofuranose) is localized the unpaired electron.

This paper deals with gamma radiolysis of sucrose, and reports the identification of five out of the seven sucrose-nitroxide radicals, detected by means of the spin trapping technique. These radical species are identified through a combined use of the ESR spectral data, extended to oligomers (fructans) and to studies of the effects of chemical modifications (deoxysucroses) or isotopic substitutions.

In aqueous phase (only condition where fructans spin trapping is feasible) trapped radicals in fructans and in sucrose are the same. Consequently, this study shows that,

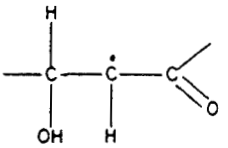
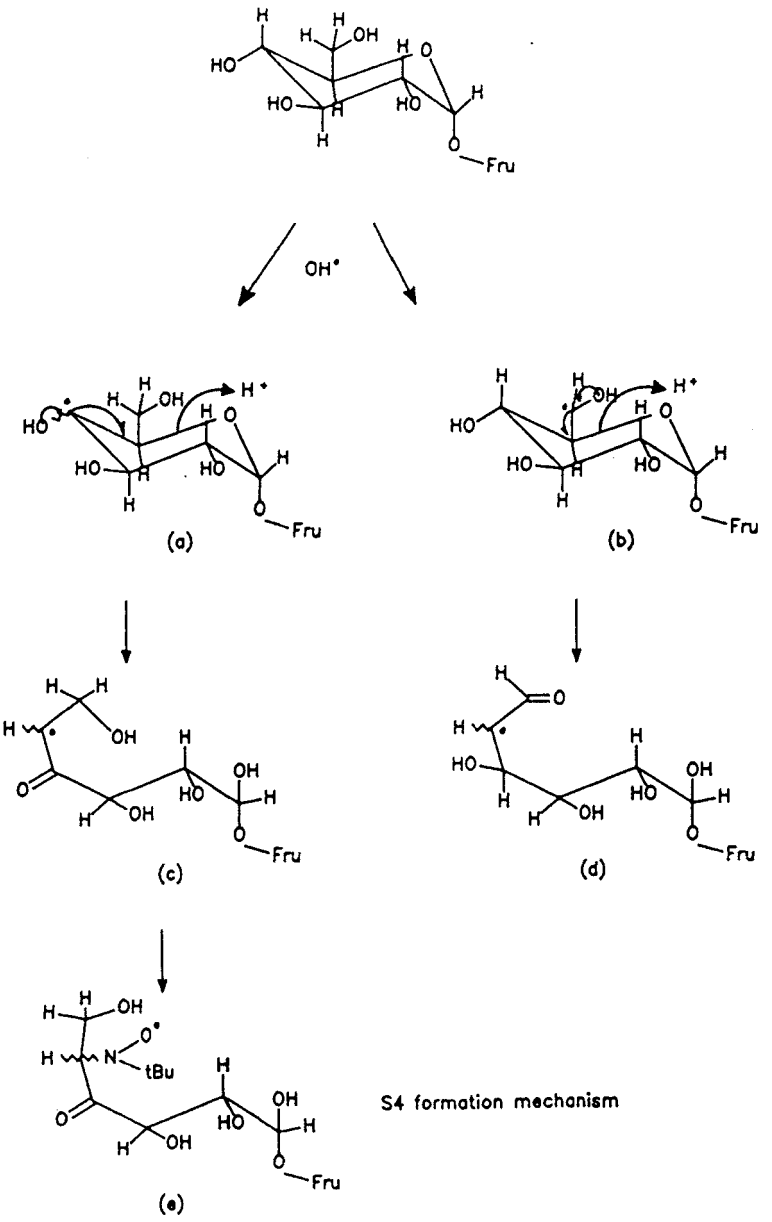


FIGURE 11



S4 formation mechanism

FIGURE 12

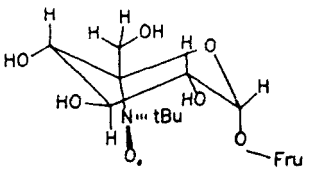


FIGURE 13

in aqueous solution, sucrose can be considered as a conceivable model for a fructans radiolysis approach.

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