

Formation of free radicals in food constituents by γ -irradiation

Alexander R. Forrester and D. Bain McKay

Chemistry Department, University of Aberdeen, Old Aberdeen AB9 2UE Scotland

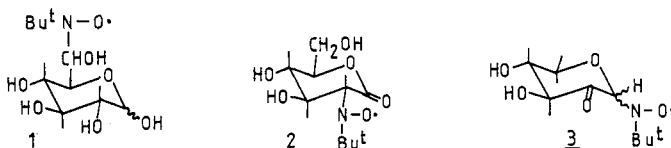
Abstract - Radicals formed on γ -irradiation of monosaccharides may be detected by spin trapping but their identification in this way is difficult. To aid identification, methods have been developed which allow generation of specific radicals from monosaccharides and also specific spin adducts derived from sugar nitrones.

INTRODUCTION

Preservation of food by γ -irradiation is an important and controversial issue worldwide. Concern has been expressed about the effect which ionising radiation has on the wholesomeness of food and especially whether toxic radiolytes are formed. Previously, we have reported on the structures and concentrations of radicals formed on γ -irradiation of spices (ref. 1) and vitamins (ref. 2). The present communication describes related work on monosaccharides.

Identification of radicals formed from monosaccharides by γ -irradiation by direct e.s.r. measurements is not feasible. Accordingly, spin trapping techniques have been used but even these have not been satisfactory mainly because of the range and number of radicals present in the solid some of which arise by rearrangement of initially formed radicals. Complex e.s.r. spectra of spin adducts are invariably produced whose full interpretation has not been achieved.

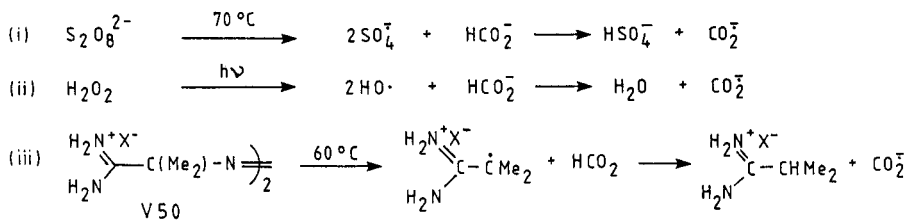
Three approaches to the resolution of this problem have been tried by the groups of Riesz (ref. 3), Raffi (ref. 4) and ourselves (ref. 5); (a) a range of structurally similar monosaccharides has been irradiated and then added to solutions of spin traps; (b) spin trapping of isotopically labelled glucose-derived radicals has been examined; (c) hplc/esr has been used in an attempt to separate and identify the components of the mixture of spin adducts. These approaches have failed to provide a satisfactory solution although the following tentative generalisations can be made which relate observed coupling constants of spin adducts to structure. Spin adducts which show a proton splitting in the range 4.8-5.6G have general structure 1; those with two doublet splittings $a_H = 1.5-2.25$ and $a_H \approx 0.5G$ have general structure 2 and those with only one doublet splitting $a_H = 1.5-2.25G$ have general structure 3. There are a number of unexplained exceptions to these



generalisations and the assignments at this stage are no better than provisional. Importantly, there have been no unambiguous structural assignments of the spin adducts of simple monosaccharides mainly because there is no method available for the specific generation of sugar-derived radicals. It was the aim of this work to develop methods for the generation of radicals of known structure derived from monosaccharides, to trap such radicals and then to assign the esr proton coupling constants, unambiguously, of their spin adducts.

RESULTS AND DISCUSSION

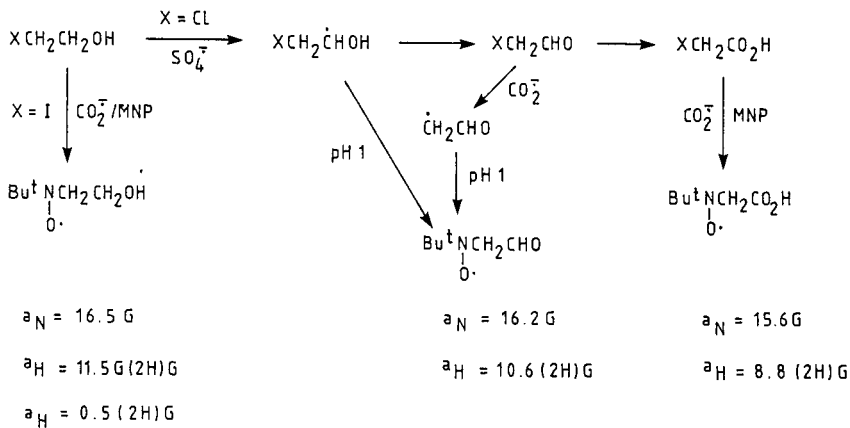
Homolytic dehalogenation of halosugars was given first consideration since Sustmann and his coworkers (ref. 6) had previously generated a number of protected sugar radicals in organic solvents by dehalogenation of the corresponding halosugars with tributyltin radicals. This method is not applicable in aqueous solution since no water soluble tin hydride is available. The carbon dioxide radical-ion ($CO_2^{\cdot-}$) also dehalogenates certain alkyl halides by electron transfer followed by halide ion loss and this was the reactant of choice in this work. It is formed by hydrogen atom removal from formate and this had to be done in a way which did not destroy the spin trap. Of the three methods examined (i-iii) that summarised in (i) was the most useful because it did not require UV photolysis which converts the trap to di-t-



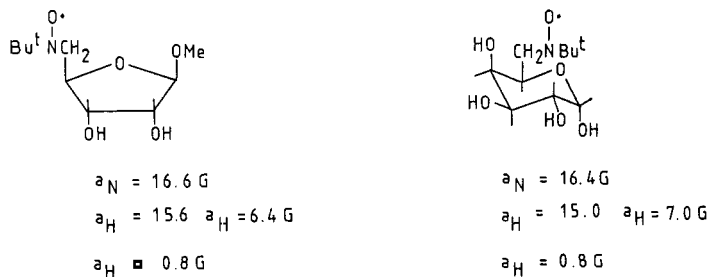
butylnitroxide and the sulphate radical-ion is an efficient hydrogen abstractor from formate ($k = 2.42 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$). Metal ions such as Ti(III) had to be avoided since they destroyed the trap. To prevent oxidation of >CHOH to >C=O (iv) in the substrate ($k = 8.7 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$) relative concentrations of substrate, $\text{S}_2\text{O}_8^{2-}$ and formate had to be optimised. For practical purposes this ratio was 0.1 : 0.005:0.5 M. Even so with model substrates $\text{XCH}_2\text{CH}_2\text{OH}$



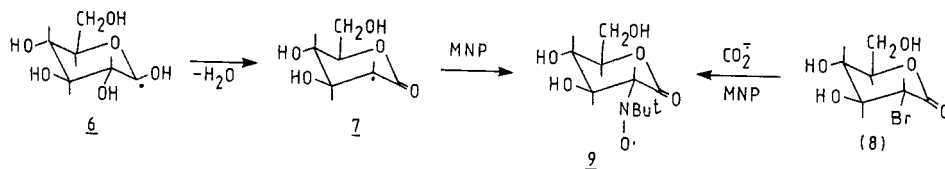
oxidative dehalogenation occurred unless $\text{X} = \text{I}$ (Scheme 1). In view of the reported $-\text{E}_{1/2}$



values for methyl chloride, bromide and iodide 2.23, 2.01 and 1.63 v, respectively and the work of Gilbert (ref. 7) who made direct esr measurements of some of these systems using $\text{Ti(III)}/\text{H}_2\text{O}_2/\text{HCO}_2^-$ as the source of $\text{CO}_2^{\cdot-}$ this result was not entirely unexpected. Application of these reaction conditions to several iodo sugars led to efficient deiodination and the trapping of the sugar radical. Some examples are given below.

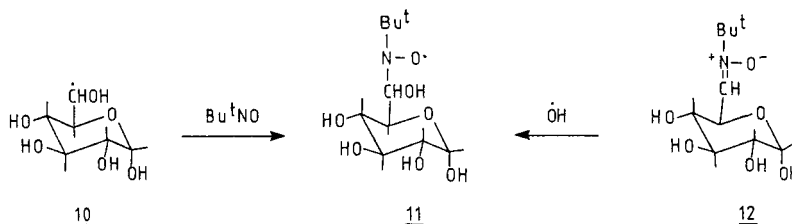


The structural requirement that a carbonyl group be adjacent to the carbon bearing the chlorine or bromine, before dehalogenation can be achieved by reaction with $\text{CO}_2^{\cdot-}$, provides a method for the specific generation of α -acylalkyl monosaccharide radicals 7. These are also thought to be present in γ -irradiated monosaccharides as a result of rearrangement of initially formed hydroxyalkyl radicals 6. Thus, treatment of the bromomonosaccharide 8 with $\text{CO}_2^{\cdot-}/\text{MNP}$ should give a spin adduct 9 which would also be formed by spin trapping the radicals present in the corresponding γ -irradiated monosaccharide. Preparation of the appropriate bromosugars is underway and the presence of these rearranged radicals 7 in the

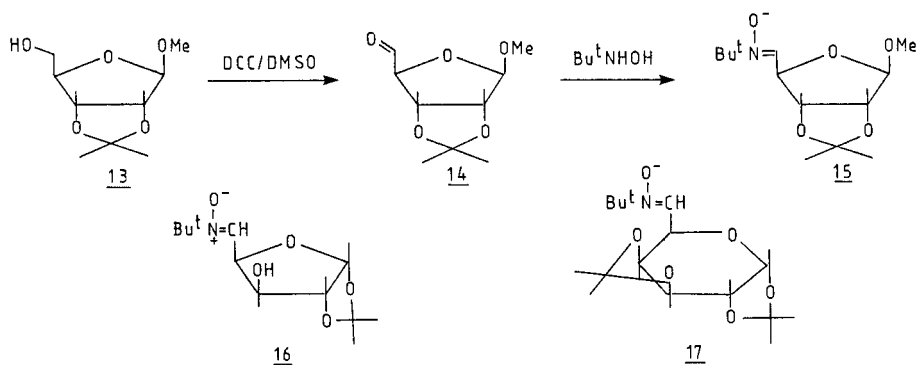


γ -irradiated sugars will be investigated in this way.

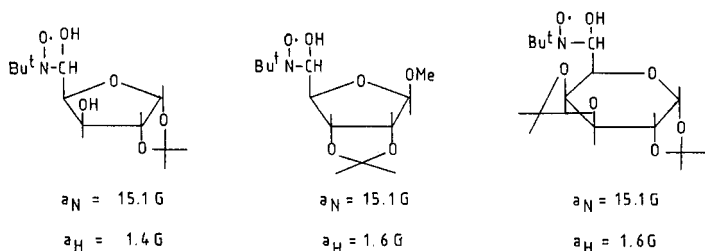
The dehalogenation route to specific monosaccharide radicals cannot be used to produce radicals derived from monosaccharides by hydrogen abstraction which are thought to be present in γ -irradiated solid monosaccharides, e.g. 10. An alternative route to the spin adducts 11



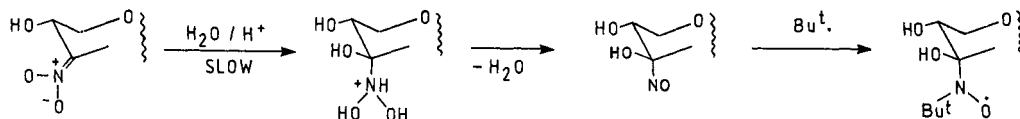
of such radicals depends on the availability of *t*-butylnitrones such as 12 and the known addition of hydroxyl radicals to them. Accordingly, a series of nitrones 15-17 was prepared by oxidation of the corresponding alcohols to aldehydes with DMSO/DCC followed by condensat-



ion with *t*-butyl hydroxylamine. When these nitrones were dissolved in an aqueous solution of hydrogen peroxide and irradiated with a U.V. lamp in an esr cavity the spectra of the corresponding hydroxy adducts were detected. The proton coupling constants of the hydroxy spin adducts of these nitrones are as indicated and clearly do not show the large proton splitting which has been associated with the spin adducts of type 11. However, the nitronium (17) has yet to be deprotected and the parent nitronium may well give the expected large proton splitting. These nitrones have also been used to trap $\text{Me}\cdot$, $\text{Bu}^t\text{O}\cdot$, $\text{PhCO}_2\cdot$, $\text{CH}_3(\text{CH}_2)_{10}\text{CO}_2\cdot$, $\text{Me}_2(\text{CN})\text{C}\cdot$ and may have general application in their own right.



A further approach to the formation of spin adducts of known structure derived from monosaccharide radicals would be to prepare nitroso monosaccharides of known structure and to use them to trap t-butyl radicals. However, there are few readily available and structurally appropriate nitrososugars and certainly none with an α -hydroxy group. The only α -hydroxy-nitroso species 18 reported are those which are alleged to mediate in the NEF reaction



(ref. 8) during the course of which an aci-nitronate is converted into a ketone under the influence of strong acid. When the NEF reaction was allowed to occur in the cavity of an esr spectrometer by mixing aqueous alkaline solutions of aci-nitronate containing the carbon radical precursor V-50 and strong acid with simultaneous U.V. irradiation of the mixing chamber no spin adducts were detected despite much variation of the reaction conditions.

In summary we have developed two different routes to spin adducts of monosaccharide radicals of known structure. These routes will be further exploited to help to identify some at least of the radicals formed on γ -irradiation of solid monosaccharides.

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