FORMATION OF MECHANO-RADICALS IN D-GLUCOSE AND LACTOSE

TETSU KUDO AND HELMUT HEUSINGER
Institut für Radiochemie der Technischen Universität, München 8046 Garching (West Germany)

ABSTRACT

Radical formation in ball-milled mono- and di-saccharides was found to be especially effective for α-lactose monohydrate and α-D-glucose monohydrate. For these two sugars, spin-trapping experiments were performed by using pentamethyl-nitrosobenzene (PMNB) and tri-*tert*-butylnitrosobenzene (BNB). The sugar radicals could not be trapped directly, but they transferred a hydrogen atom to BNB and an electron to PMNB. Based on the e.s.r. spectra in the solid state and the results of the spin-trapping experiments, a mechanism for radical formation is proposed.

INTRODUCTION

Formation of mechano-radicals is a general feature of polymers[1]. They originate from fractures of the polymer chain. It is proposed that application of mechanical strain leads to a fracture of the chain if the energy necessary to break the van der Waals forces between the monomer units of adjacent chains becomes larger than the energy necessary to break a bond in the polymer chain[2,3,4]. By a rough estimate, Steurer and Hess[2] concluded that, for carbohydrates, mechanical fracture takes place if the number of monomer units is > 12.

Stranski *et al.*[5] observed that some organic compounds show triboluminescence during grinding. This triboluminescence, which is especially marked for sugars, is attributed to a recombination of positive and negative charges, which gives rise to light emission. In this respect, the primary processes of mechanical degradation show similarities to radiation chemistry, where excited states, formed by charge recombination, are important intermediates. For the decomposition of excited states, radical formation is in competition with light emission. These considerations led us to investigate by e.s.r. spectroscopy the formation of mechano-radicals in mono- and di-saccharides, and to attempt to trap the radicals.

Hasegawa and Tsuchida[6] detected radical species by e.s.r. spectroscopy during the grinding of sucrose, D-glucose, and lactose. We have investigated α-D-glucose, α-D-glucose monohydrate, D-galactose, D-mannose, and D-fructose, and the di-saccharides sucrose, maltose, α-lactose and its monohydrate, and β-lactose. The formation of mechano-radicals was most effective in α-lactose monohydrate and in α-D-glucose monohydrate.

EXPERIMENTAL

BNB was a gift from Dr. M. Tabata (Hokkaido University), and PMNB was prepared in our laboratory[7]. Lactose monohydrate was purified by recrystallisation, and pyridine was dried by molecular sieves before use. Other chemicals were commercial materials and were used without further purification.

Ball-milling was carried out either at 77 K or room temperature in the vessel shown in Fig. 1, connected to a commercial vibrator. For the trapping experiments, a spin-trapping solution was placed in an e.s.r. sample-tube, which, after three degassing cycles (freeze–pump–thaw method), was joined to the milling vessel. The whole vessel-sample tube system was filled with helium gas after degassing. In the presence of a spin-trapping solution, the part of the e.s.r. sample-tube which contained the solution was kept at 77 K during the milling process before e.s.r. measurements.
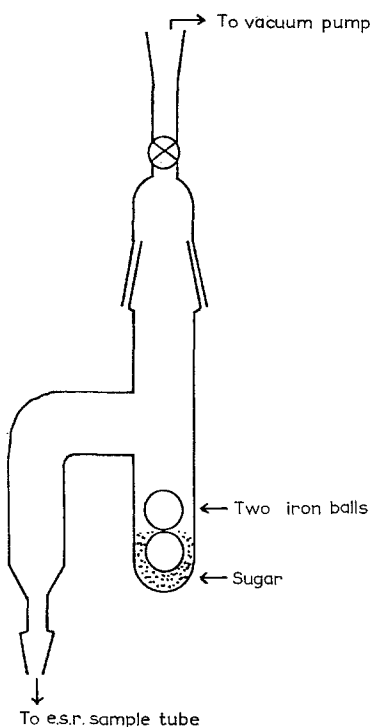

Fig. 1. The vessel for ball-milling.

The ball-milled sugar was promptly transferred to the sample tube after cooling the whole system to 77 K. Spectra were recorded on a Varian Century Line ESR spectrometer, using a field-frequency lock. Temperature control of the samples was achieved by a modified, variable-temperature system (Varian).

RESULTS AND DISCUSSION

Solid-state e.s.r. patterns of the ball-milled sugars are shown in Figs. 2 (lactose monohydrate) and 3 (D-glucose monohydrate). Both sugars were ball-milled at 77 K followed by storage at various temperatures. Only two spectra for each sugar are shown: directly after ball-milling and after storage at room temperature; in both cases, the e.s.r. spectra were recorded at 77 K, and the amplification of recording of the radicals after storage at room temperature was twice as large as that before storage. For lactose monohydrate, the effect of milling at room temperature was investigated. The spectra showed the same triplet e.s.r. pattern as that obtained, after storage at room temperature, from lactose monohydrate ball-milled at 77 K.

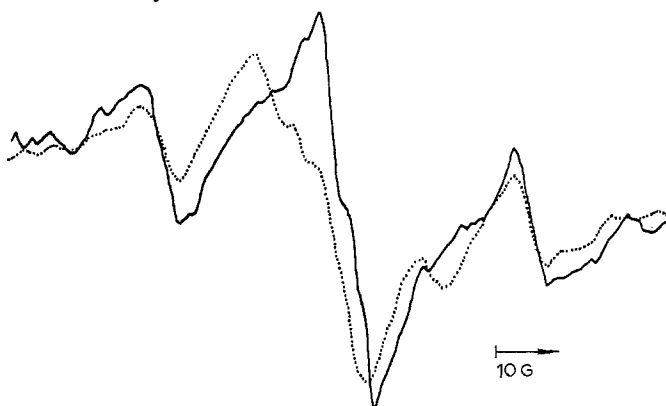Oxygen had no effect on the generation of, or on the generated, mechano-

Lactose monohydrate

10 G

Fig. 2. E.s.r. spectra observed at 77 K after the ball-milling of lactose monohydrate: ——, directly after milling at 77 K; ····, after storage at room temperature.
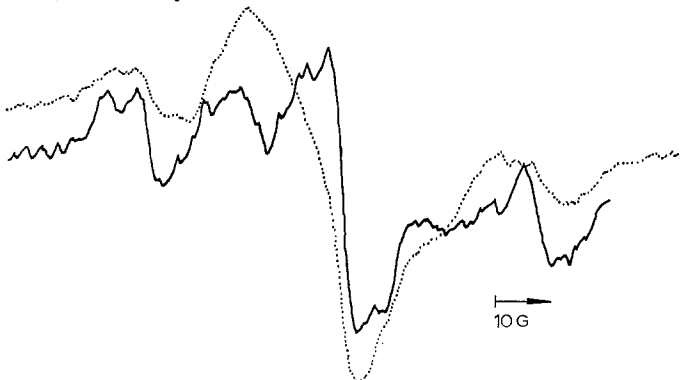
D-Glucose monohydrate

10 G

Fig. 3. E.s.r. spectra observed at 77 K after the ball-milling of D-glucose monohydrate: ——, directly after milling at 77 K; ····, after storage at room temperature.
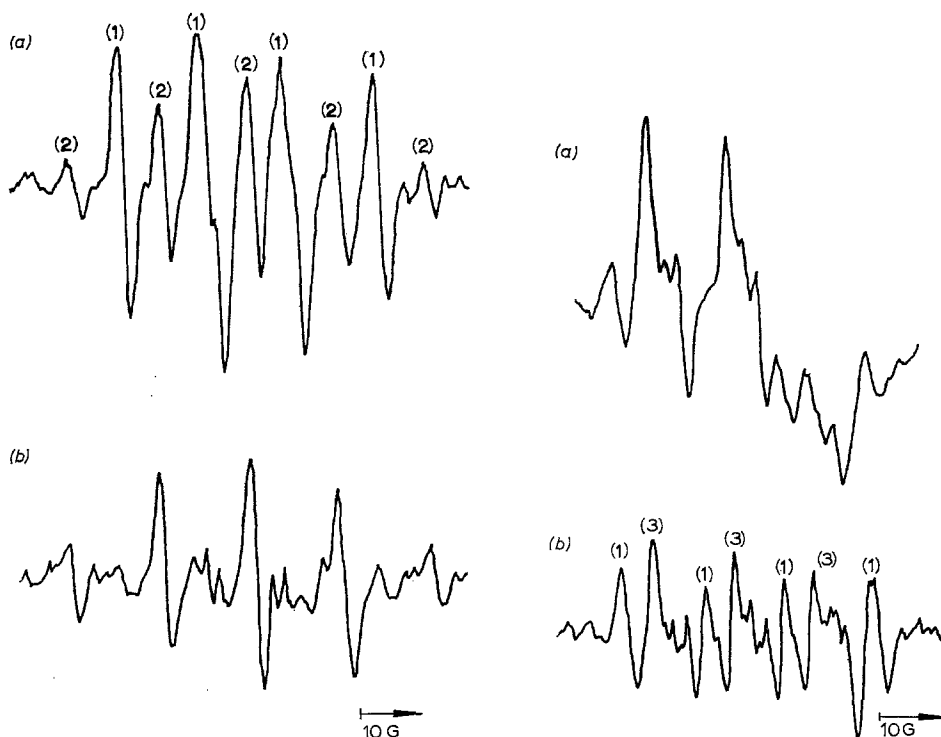
Fig. 4. E.s.r. spectra observed at 248 K: (a) after contact between ball-milled D-glucose monohydrate and the methanol solution of BNB; (b) after storage of the sample in (a) for 85 min at room temperature.

Fig. 5. E.s.r. spectra observed (a) at 213 K after contact between ball-milled D-glucose monohydrate and the CH₃OD solution of BNB; (b) at 273 K after contact between ball-milled D-glucose monohydrate and the CD₃OD solution of BNB.

radicals. Figs. 4 and 5 are the results of spin-trapping experiments for ball-milled D-glucose monohydrate with BNB in various solvents. Fig. 4 shows the spin-adducts (**1** and **2**) obtained in methanol, one (**1**) of which decayed on storage, while the other (**2**) survived. Fig. 5a shows the spin-adducts in CH_3OD, and Fig. 5b those in CD_3OD. In both cases, adduct **3** was found; in the former case, adducts **1** and **2** were also found (not shown here). In the latter case, adduct **1** was also identified, as shown in Fig. 5. In this trapping experiment, D-glucose was ball-milled at 77 K and then brought into contact with the BNB–solvent system at elevated temperatures. With pyridine as solvent, only adduct **1** was formed. The results of trapping experiments for lactose monohydrate and D-glucose were identical.

For lactose monohydrate, chemical reactions between BNB and the stored mechano-radical system were examined. Lactose was ball-milled at 77 K, heated at room temperature for 30 min, and then brought into contact with the BNB–MeOH system. The resulting e.s.r. spectra were also composed of adducts **1** and **2**.

The adducts **1–3** have been assigned[8] as BNBH·, BNB·̄ CH_2OH, and BNBD·,
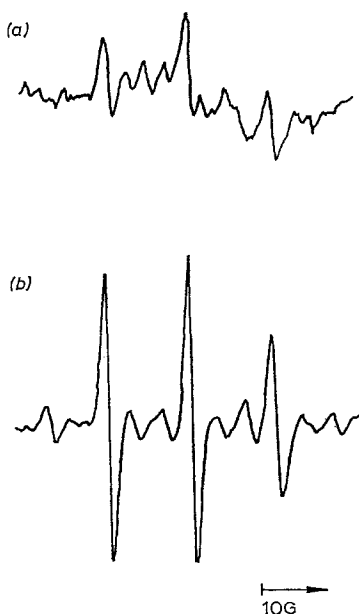
Fig. 6. E.s.r. spectra observed (*a*) at 248 K after contact between ball-milled lactose monohydrate and the pyridine solution of PMNB; (*b*) at 243 K after γ-irradiation of the pyridine solution of PMNB at 77 K.

respectively. Fig. 6*a* shows the spectra from the trapping experiment of lactose mechano-radicals, using PMNB in pyridine. The results were the same regardless of whether or not the ball-milled lactose monohydrate was warmed up to room temperature prior to contact with the PMNB–pyridine system.

Fig. 6*b* is the spectrum obtained after irradiation of PMNB–pyridine solution at 77 K as reference. A comparison of Figs. 6*a* and 6*b* shows that both triplets have the same h.f. coupling constant (13.5×10^{-3}T) and g-factor (2.0055). Based on this fact, we attribute this spectrum to PMNB$\dot{}$. This assignment is supported by the fact that the same e.s.r. spectrum was also found for γ-irradiated solutions of PMNB in MeOH and CD_3CD_2OD.

Based on these experimental results, the properties of the mechano-radicals can be summarised as follows. (*1*) The spectra of the mechano-radicals of α-D-glucose monohydrate and α-lactose monohydrate in the solid phase are very similar. They are not well resolved and no unambiguous interpretation can be given. For lactose, the spectrum may be interpreted as a triplet ($a_1 = a_2 = \sim3 \times 10^{-3}$T) superimposed on a quartet ($a_1 \sim2 \times 10^{-3}$T, $a_2 \sim4 \times 10^{-3}$T) or an asymmetric triplet ($a_1 = a_2 = \sim2 \times 10^{-3}$T). Only the triplet with splitting 3×10^{-3}T remains after warming the samples to room temperature. The behaviour of the D-glucose radicals is similar.

(*2*) The reaction of the mechano-radicals with BNB in methanol and pyridine results in addition of hydrogen to BNB both for D-glucose and lactose.
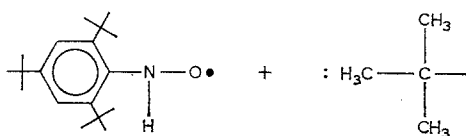
(*3*) They react with PMNB forming PMNB⁻·.

(*4*) The formation of the spin adducts is not influenced by storage of the samples at room temperature before contact with the spin-trapping system.

(*5*) The mechano-radicals are able to abstract hydrogen atoms from alcohols. This can be deduced from the formation of the CH_2OH-adduct with BNB in methanol as solvent.

These properties give some information on the structure of the radicals. Two radicals are generated by ball milling at 77 K. The structure of the radicals in D-glucose and lactose should be identical.

The radical site transfers a hydrogen atom to BNB. This adduct is observed when methanol or pyridine is used as solvent. In the case of CH_3OD, where exchange of the hydroxyl hydrogens of the sugar with the alcohol takes place, the D-adduct is formed. From these results it can be deduced that the hydrogen of the H-adduct originates from the HO group of the sugar.
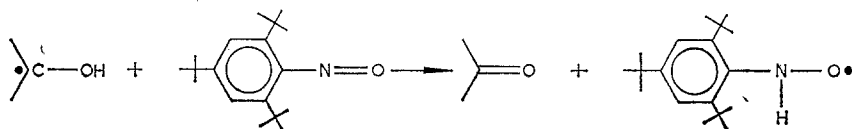
Another possibility for BNBH-formation could be proton transfer from an HO group to BNB⁻·, as observed[8] in the radiolysis of CH_3OH in the presence of BNB. However, for the mechano-radicals, no evidence for formation of BNB⁻· was obtained.

In order to explain the formation of PMNB⁻·, the radical species must contain an electron-releasing group. This may be either a trapped electron or a radical anion. According to Box *et al.*[9,10], trapped electrons are observed in various sugar systems by γ-irradiation at liquid helium temperature, but they decompose at temperatures ⩾ 50 K. Therefore, electrons could be the precursors of the radical species in our experiments. In this respect, the greater tendency for radical formation in D-glucose monohydrate and lactose monohydrate, in comparison with the corresponding anhydrous sugars, may be due to a better stabilisation of the negative charge in the presence of crystal water.

In the radical anion produced by electron addition to D-glucose or lactose, the negative charge should be localised at an oxygen atom. The e.s.r. spectra obtained for lactose and D-glucose are almost identical. This indicates that radical formation is not due to scission of the glycosidic bond in lactose. Therefore, the radical anions (**4** and **5**) should be formed by opening of the lactol bridge. In both cases, the negative

CH$_2$OH CH$_2$OH

4 5 6

R = H (D-glucose)
R = D-galactosyl (lactose)
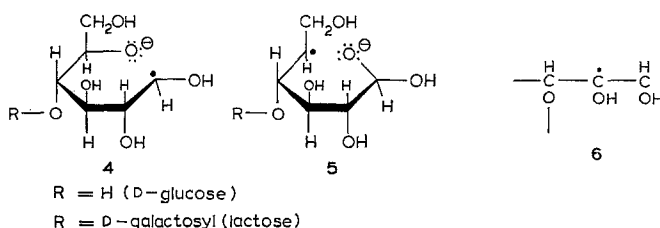
charge is localised at the oxygen, but only the radical anion **4** contains the structural unit $>\dot{C}$-OH, which should be responsible for the hydrogen transfer to BNB.

A definite correlation between the e.s.r. spectra and the radical structure is difficult, because the splitting of the β-protons strongly depends on steric factors. If the glucose ring retains its structure in the radical anion **4**, due to hydrogen bonds with adjacent molecules, the β-hydrogen is axial and therefore shows strong interaction with the unpaired electron, resulting in large splitting. In this case, a correlation with the quartet having splitting 4×10^{-3}T for the β-hydrogen and 2×10^{-3}T for the α-hydrogen would be reasonable. If the ring structure is not retained, the α- and β-hydrogens may both have a splitting of $\sim 2 \times 10^{-3}$T, resulting in a triplet. The triplet with splitting $\sim 3 \times 10^{-3}$T probably corresponds to a radical in D-glucose and lactose containing two β-hydrogens.

For the radiolysis of lactose monohydrate crystals, Ueda et al.[11] postulated a radical of type **6**. The β-proton of this radical shows a splitting of 2.5×10^{-3}T directly after irradiation at 77 K and 3.1×10^{-3}T after storage at 193 K. In our studies, a radical of this type could be formed by hydrogen abstraction from an adjacent sugar molecule by the radical anion **5**. This assumption is supported by the fact that the mechano-radicals are able to abstract hydrogen from methanol, producing the CH$_2$OH radical.

ACKNOWLEDGMENTS

REFERENCES

1 H. H. KAUSCH, *Polymer Fractures*, Springer, Berlin, 1978.
2 E. STEUER AND K. HESS, *Z. Phys. Chem.*, 193 (1943) 248–257.
3 J. SOHMA AND H. SAKAGUCHI, *Adv. Polym. Sci.*, 20 (1975) 109–158.
4 J. F. JANSSON AND B. TERSELIUS, *Appl. Polym. Symp.*, 35 (1979) 455–468.
5 G. GROSS, J. N. STRANSKI, AND G. WOLFF, *Z. Elektrochem.*, 59 (1955) 346–350.
6 H. HASEGAWA AND H. TSUCHIDA, *Abstr. Debates ESR, 16th, Sendai, 1977*, pp. 122–123.

7 T. DOBA, I. ICHIKAWA, AND H. YOSHIDA, *Bull. Chem. Soc. Jpn.*, 50 (1977) 3124–3126.
8 M. SHIOTANI, S. MURABAYASHI, AND J. SOHMA, *Int. J. Radiat. Biol.*, 8 (1976) 483–495.
9 H. C. BOX, E. E. BUDZINSKI, H. G. FREUND, AND W. R. POTTER, *J. Chem. Phys.*, 70 (1979) 1320–1325.
10 E. E. BUDZINSKI, W. R. POTTER, G. POTIENKO, AND H. C. BOX, *J. Chem. Phys.*, 70 (1979) 5040–5044.
11 H. UEDA, *J. Phys. Chem.*, 67 (1963) 2185–2190.