

## Radiation Effects on the Glycosidic Bond in Some Crystalline Disaccharides

GÖRAN LÖFROTH and TORBJÖRN GEJVALL

Radiobiology Department, University of Stockholm, Wallenberg Laboratory, Lilla Frescati, S-104 05 Stockholm 50, Sweden

Irradiated crystalline disaccharides have been analyzed by gas chromatography of the trimethylsilyl derivatives for the presence of constituent monosaccharides after dissolving in pyridine or water. Sucrose yields D-fructose and  $\alpha$ -D-glucose in equal amounts, with initial G-values of 0.8. Maltose monohydrate yields equal amounts of  $\alpha$ - and  $\beta$ -D-glucose with  $G \approx 0.15$ , and trehalose dihydrate yields  $\alpha$ -D-glucose with  $G = 0.8$ . By means of these results and previously reported degradation yields, it is demonstrated that the major part of the radiation damaged molecules in sucrose, maltose monohydrate, and trehalose dihydrate has intact glycosidic bonds. No detectable amounts of D-glucose or D-galactose were found in irradiated lactose monohydrate.

The susceptibility of the glycosidic bond in crystalline disaccharides to ionizing radiations has been mentioned in the early literature,<sup>1-4</sup> but no conclusive data have yet been reported.

The total degradation yields for four crystalline disaccharides have been reported in a previous communication<sup>5</sup> together with degradation yields, measured as degradation of their constituent monosaccharides, for three of these disaccharides (sucrose, maltose monohydrate, and trehalose dihydrate). The results showed that the damage to these disaccharides is mainly associated with only one of the monosaccharide moieties of any degraded disaccharide molecule. These results give rise to two possible interpretations. The glycosidic bond may be either intact, joining the damaged monosaccharide moiety (or part of it) with the undamaged one, or broken, in which case a constituent monosaccharide is released. We have now measured the radiation induced release of constituent

monosaccharides for sucrose, maltose monohydrate, trehalose dihydrate, and lactose monohydrate, and the present results give information about the radiation sensitivity of the glycosidic bonds.

### EXPERIMENTAL

The <sup>60</sup>Co  $\gamma$ -irradiation and the disaccharides, sucrose,  $\beta$ -maltose monohydrate,  $\alpha,\alpha$ -trehalose dihydrate, and  $\alpha$ -lactose monohydrate, have previously been described.<sup>5</sup>

For the determination of released constituent monosaccharides in the irradiated crystalline disaccharides, a known amount of anhydrous pyridine was added to a weighed amount of the irradiated or unirradiated sample to give a concentration of approximately 10 mg carbohydrate/ml pyridine. The samples were either directly dissolved in pyridine or first dissolved in water and subsequently freeze-dried.

The trimethylsilyl derivatives were prepared by addition of hexamethyldisilazane and trimethylchlorosilane to the pyridine solution, as described by Sweeley *et al.*<sup>6</sup> As reference samples unirradiated disaccharides, and occasionally irradiated disaccharides, were used to which were added known small amounts of the appropriate monosaccharides dissolved in pyridine or water.

The pyridine solutions of the derivatives were analyzed by gas chromatography on a Perkin Elmer F11 with FID detection.<sup>7</sup> Columns were: 1.4 m  $\times$  2.2 mm of 2% SE 30 on Chromosorb W 60–80 mesh at 160 °C and 1.2 m  $\times$  2.2 mm of a 2:1 mixture of 8% QF 1 and 4% SF 96 on Gas Chrom P 100–120 mesh at 140 °C.

### RESULTS

For the investigated disaccharides, with the exception of lactose monohydrate, irradiation gave rise to small amounts of the constituent

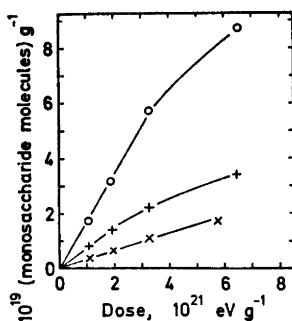


Fig. 1.  $\gamma$ -Radiation induced release of monosaccharides from crystalline disaccharides vs. dose: O, D-fructose +  $\alpha$ -D-glucose from sucrose; +,  $\alpha$ -D-glucose from trehalose dihydrate; x,  $\alpha$ - +  $\beta$ -D-glucose from maltose monohydrate.

monosaccharides which did not appear in unirradiated samples. Sucrose yielded D-fructose and  $\alpha$ -D-glucose in equal amounts. Maltose monohydrate yielded  $\alpha$ - and  $\beta$ -D-glucose in equal amounts, whereas trehalose dihydrate only gave rise to  $\alpha$ -D-glucose. The yield-dose curves for the formation of these free constituent monosaccharides are given in Fig. 1 and the initial G-values are given in Table 1. Neither  $\alpha$ - and  $\beta$ -D-glucose nor  $\alpha$ - and  $\beta$ -D-galactose were present in detectable amounts in irradiated lactose monohydrate (Table 1).

There were no detectable differences in the gas chromatograms or yields of monosaccharides between samples analyzed directly after irradiation, and samples analyzed after dissolving in water and subsequent freeze-drying, with the exception that mutarotation occurred for D-glucose when the aqueous solutions were not frozen immediately after dissolving the sample.

Table 1. Initial G-values for the release of constituent monosaccharides from crystalline disaccharides.

	G-value			
	D-Fruc-tose	$\alpha$ -D-Glucose	$\beta$ -D-Glucose	$\beta$ -D-Galac-tose
Sucrose	0.8	0.8		
Maltose.H <sub>2</sub> O		$\approx 0.15$	$\approx 0.15$	
Trehalose.2H <sub>2</sub> O		0.8		
Lactose.H <sub>2</sub> O		< 0.1		< 0.1

## DISCUSSION

The fact that D-glucose released from sucrose, maltose monohydrate, and trehalose dihydrate are the anomers which would be expected from the spatial configuration of the disaccharide, shows that the breakage of the glycosidic bond is concomitant with retention of the geometry of the carbon atom involved in the bond. An inversion, e.g. from  $\alpha$ - to  $\beta$ -D-glucose in sucrose, of a detectable magnitude ( $G \approx 0.1$ ) would have been noticed. The monosaccharides are probably already present as such in the solid state, as their formation is independent of the method used to dissolve the irradiated sample. The pyranoside and furanoside forms of D-fructose have been resolved by GLC of their trimethylsilyl derivatives.<sup>8</sup> They are, however, not separated by the experimental conditions used in the present investigation (cf. Ref. 6) which were employed to separate and quantitate the small amounts of released constituent monosaccharides.

Lactose monohydrate behaves differently from the three other investigated disaccharides. The initial total degradation yield for lactose monohydrate with  $G \approx 50$  is also exceptionally high compared with the G-values of 6–8 for the other disaccharides.<sup>5</sup> The absence of released glucose and galactose in irradiated lactose monohydrate is in agreement with data given by von Sonntag and Dizdaroglu.<sup>9</sup>

The G-values for the radiation induced degradation of sucrose, trehalose dihydrate, and maltose monohydrate have been determined to be 6, 7, and 8, respectively and the G-values for the degradation of their constituent monosaccharides have been determined to be the same or slightly higher,<sup>5</sup> i.e. for sucrose:  $G(-\text{glucose}) + G(-\text{fructose}) = 6$ , for trehalose dihydrate:  $G(-\text{glucose}) = 7$ , and for maltose monohydrate:  $G(-\text{glucose}) = 10$ .

From these data and the present results on the release of constituent monosaccharides (Table 1), it can be calculated that radiation damaged molecules of the type S–O–S\*, in which one monosaccharide moiety (S) is damaged but has an intact glycosidic bond, account for more than 70 % of the degradation in sucrose and maltose monohydrate and for about 90 % in trehalose dihydrate. It can also be calculated that processes leading to breakage

of the glycosidic bond have  $G \leq 1.6$  in sucrose,  $G \leq 2.3$  in maltose monohydrate, and  $G \leq 0.8$  in trehalose dihydrate. This result makes it difficult to assign the glycosidic bond as origin and/or trapping site of the free radicals as these are formed with G-values of 3 – 5.<sup>10,11</sup>

*Acknowledgement.* This investigation has been supported by grants from the Swedish Atomic Research Council.

#### REFERENCES

1. Wolfrom, M. L., Binkley, W. W. and McCabe, L. J. *J. Amer. Chem. Soc.* 81 (1959) 1442.
2. Moody, G. J. and Phillips, G. O. *Chem. Ind. (London)* (1959) 1247.
3. Khenokh, M. A., Kuzicheva, E. A. and Evdokimov, V. F. *Tr. 2-go Vses. Soveshch. po. Radiats. Khim., Akad. Nauk SSSR, Otd. Khim. Nauk, Moscow* (1960) 409.
4. Dilli, S. and Garnett, J. L. *Chem. Ind. (London)* (1963) 409.
5. Löfroth, G. *Intern. J. Radiation Phys. Chem.* 4 (1972) 277.
6. Sweeley, C. C., Bentley, R., Makita, M. and Wells, W. W. *J. Amer. Chem. Soc.* 85 (1963) 2497.
7. Gejvall, T. and Löfroth, G. *Acta Chem. Scand.* 27 (1973) 1108.
8. Curtius, H.-C., Müller, M. and Völlmin, J. *A. J. Chromatogr.* 37 (1968) 216.
9. von Sonntag, C. and Dizdaroglu, M. *Z. Naturforsch. B* 28 (1973) 367.
10. Löfroth, G. *Acta Chem. Scand.* 21 (1967) 1997.
11. Baugh, P. J., Kershaw, K. and Phillips, G. *O. J. Chem. Soc. B* (1970) 1482.

Received April 19, 1974.