Radiation Chemistry of Carbohydrates. Part XVIII.† The Extreme and Variable Susceptibility of Crystalline Lactose to Ionising Radiations

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Acid production (G = *ca.* 45) and overall decomposition ($-G$ = *ca.* 55) of α-lactose monohydrate after γ-irradiation is considerably higher than for the anhydrous α- and β-anomers, which behave similarly (G_{acid} = *ca.* 3·2; $-G$ = *ca.* 22). Production of stable radicals is the same for all the anomers ($G_{radical}$ = *ca.* 3·9). Diffuse reflectance and i.r. spectroscopy confirm that the enhanced yield of acid products for the monohydrate occurs directly in the solid state. It is suggested that the remarkable radiation susceptibility of the monohydrate could be a function of the crystal structure in which one water molecule is hydrogen-bonded to four lactose molecules. The presence of water promotes acid production and this is manifested in a high radiation decomposition. When water is absent the acid yield is low and irradiation leads to an enhanced neutral product formation but an overall lower degree of destruction.

THE extent of decomposition ($-G$ value) of crystalline D(+)-glucose on γ-irradiation is dependent on the physical state of the solid [1,2] and on the presence of water of hydration.[1-3] Acid formation parallels the overall decomposition and is a reliable indication of the degradation of this monosaccharide, when irradiated in various physical states. Introduction of imperfections into the crystalline lattice results in greater radiation stability and it is the presence of extensive defects which is responsible for the low $-G$ value for freeze-dried D(+)-glucose.[1,2]

Chemical traps capable of accepting electronic excitation energy, such as aromatic molecules, greatly influence the $-G$ value of α- and β-dextrins,[4-6] and maximum protection occurs when the first singlet level (E_1) for the associated aromatic molecule is *ca.* 4—5 eV, indicating that exciton states within this energy range can be selectively transferred from the crystalline carbohydrate to the aromatic molecule.[6] For solid carbohydrates generally radical yields in no way provide an indication of the overall radiation damage.[2,3,5]

Some evidence, based on acid yields, indicates that α-lactose monohydrate behaves differently on γ-irradiation.[3] Here, we have examined the overall degradation of lactose on γ-irradiation in relation to the crystalline form and hydration of this disaccharide. The results establish that crystalline α-lactose monohydrate is more susceptible to damage by ionising radiations than any other carbohydrate yet examined.

EXPERIMENTAL

Materials.—AnalaR α-lactose monohydrate and anhydrous β-lactose were commercial. Anhydrous α-lactose was prepared by heating the α-lactose monohydrate *in vacuo* according to Bell's method [7] and sealed under vacuum. α-Lactose monohydrate was also prepared [8] by recrystallisation from water below 90 °C, and it was established that the α-lactose monohydrate purified in this way had identical $[\alpha]_D = 90°$ [7] to that obtained commercially.

Irradiations.—Samples of the various anomers were irradiated in glass tubes in air or *in vacuo*, and also in Polythene tubes for selected irradiation in air. For e.s.r. measurements the samples were irradiated in Spectrosil quartz tubes (3 mm i.d.) in air and *in vacuo*. The anhydrous α-lactose was prepared by the method [7] indicated above in Spectrosil quartz tubes to which Pyrex–quartz graded seals had been attached and then sealed under vacuum before irradiation and e.s.r. measurement. Dose rates were determined by use of the Fricke dosimeter and were in the range 2—9 × 10^{17} eV g^{-1} min^{-1}. A correction (4%) was applied to obtain the dose absorbed by the solid samples owing to the difference in electron density between the solid carbohydrates and the dosimeter solution.

Acid Determination.—Acid formation was estimated after the irradiated samples had been dissolved in triply-distilled water by potentiometric titration with standard NaOH. Acid production is given here on the assumption that the acid products are monobasic.

Radiation Decomposition.—The radiation decomposition of each anomer was determined by the direct isotope dilution method. The irradiated samples (1 g) were dissolved in triply-distilled water (10 ml) [9] and [^{14}C]-α-lactose monohydrate (10 μCi) in water (5 ml) added. The solution was freeze-dried *in vacuo* and additionally dried over P_2O_5 *in vacuo*. Octa-*O*-acetyl-lactose was prepared [10a,b] and recrystallised from ethanol to constant m.p. and specific activity. The radiochemically pure derivative (10 mg) was dissolved in a POPOP liquid scintillator solution (10 ml) and the radioactivity measured with a Nuclear Chicago liquid scintillation counter (Series 720). The activities of the derivatives from γ-irradiated samples were related to comparably treated unirradiated samples to determine the % radiation-induced decomposition. The degradation was measured progressively over a dose range of 0—100 × 10^{19} eV g^{-1}.

Electron Spin Resonance.—E.s.r. spectra of the radiation-induced free radicals in lactose (7—8 mg/mm in Spectrosil quartz tubes) were recorded on a Varian V4502 e.s.r.

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spectrometer operating at 100 kcycles field modulation with use of the lowest possible microwave power level (<1 mW). The static (first) moment of the derivative absorption curve was calculated with an Amsler integrator, type 2001/380, and compared with the first moment of e.s.r. spectra of radicals in γ-irradiated glycine. The spin concentration per g in the dose range 7×10^{18}—$1{\cdot}2 \times 10^{20}$ eV g^{-1} was calculated on the basis of γ-irradiated glycine, G(radical) = 5.[11]

Reflectance Spectra.—Diffuse reflectance spectra of the irradiated polycrystalline lactose samples were recorded on a Unicam SP 700 u.v. and visible spectrophotometer fitted with the SP 735 diffuse reflectance attachment. Unirradiated lactose was found to be a suitable non-absorbing reference as compared with magnesium oxide. The remission function at a particular wavelength was calculated by use of the Kubelka–Munk expression[12] (1) where r_∞ is

$$f(r_\infty) = \frac{(1 - r_\infty)^2}{2r_\infty} = \frac{2{\cdot}303\varepsilon c}{s} \qquad (1)$$

the percentage reflectivity, ε is the molar extinction coefficient, c is the molar concentration, and s is the scatter constant. Care was taken to ensure that the range of particle size and packing of the various samples was identical. The dose range employed was 0—75×10^{19} eV g^{-1}.

I.r. Spectra.—I.r. spectra of the irradiated solid samples prepared in potassium bromide discs were recorded in the region 1400—2500 cm^{-1} on a Perkin-Elmer 221 spectrophotometer.

RESULTS

Acid Production.—Yield–dose curves for acid production were determined for γ-irradiated anhydrous α- and β-anomers of lactose and α-lactose monohydrate over the dose range 0—28×10^{19} eV g^{-1}. The G(acid) values calculated from the results are summarised in Table 1.

TABLE 1

G-Values for γ-irradiated lactose

Anomer	G(acid)	$-G$ max.[d]	$-G$ min.[e]	G(radical)[f]
Anhydrous α-	3·2 ± 0·6	24 ± 3	13	3·9 ± 0·2
Anhydrous β-	3·2 ± 0·6	21 ± 3	10	
Monohydrate α-	47 ± 3[a]	60 ± 5	52	
	42 ± 2[b]	53 ± 5		
	47 ± 5[c]			

[a] Recrystallised. [b] Commercial (AnalaR) at 293 K. [c] Commercial (AnalaR) at 77 K. [d] $-G$ Calculated from initial slope. [e] $-G$ Determined for highest dose region investigated. [f] Based on G(radical) = 5 for glycine.

Radiation Decomposition.—The degradation of the various anomers with dose was determined by use of the direct isotope dilution analysis method and typical decomposition dose curves are shown in Figure 1. In all instances the rate of degradation decreases with increasing dose. From the initial slopes of the curves $-G$ values were calculated and the values summarised in Table 1 are based on several individual experiments. Maximum, *i.e.*, initial, and minimum $-G$ values are given since the decomposition–dose curves deviate markedly from linearity. The minimum $-G$ value is for the highest dose region which has been investigated.

Free-radical Production.—The increase in spin concentration with dose was determined for the various forms of

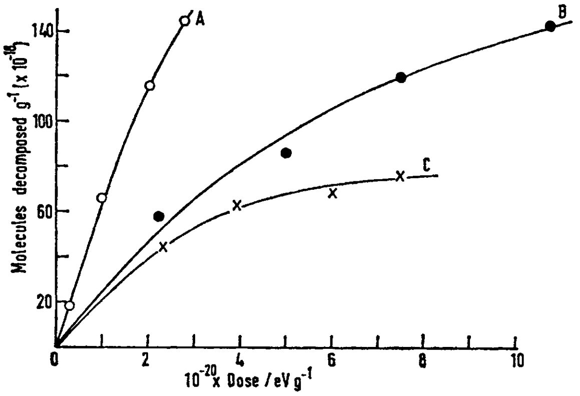

FIGURE 1 Degradation of lactose during γ-irradiation as measured by isotope dilution analysis; A, α-lactose monohydrate; B, anhydrous α-lactose; C, anhydrous β-lactose

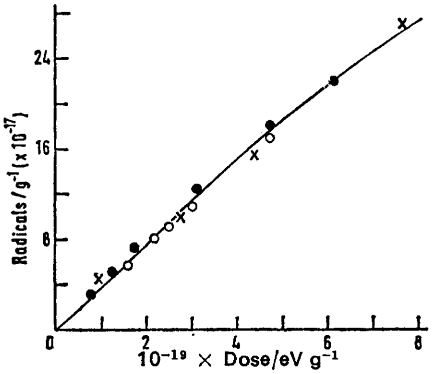

FIGURE 2 Free-radical formation during the γ-irradiation of lactose; ×, anhydrous α-lactose; ●, anhydrous β-lactose; ○, α-lactose monohydrate

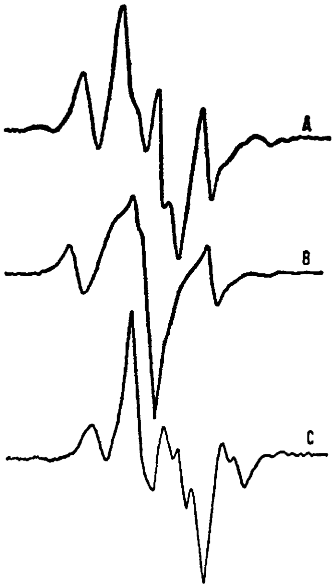

FIGURE 3 E.s.r. spectra of radiation-induced radicals in γ-irradiated lactose; A, α-lactose monohydrate; B, anhydrous β-lactose; C, anhydrous α-lactose

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[11] Based on information compiled by Dr. A. Muller, Institut fur Strahlenbiologie, Kernforschungszentum, Karlsruhe; K. Kershaw, Ph.D. Thesis, Salford, 1971.

lactose as described previously.[2] The yield–dose curve (Figure 2) is linear up to a dose of *ca.* 7 × 10^{19} eV g^{-1} corresponding to a radical concentration of 3 × 10^{18} spins g^{-1}. Initial *G*(*radical*) values were determined from the linear portion of the curve (Table 1). Typical e.s.r. spectra are shown in Figure 3.

Diffuse Reflectance Spectra.—After γ-irradiation α-lactose monohydrate and anhydrous γ-lactose contain a product, or products, which shows a broad u.v. and visible solid-state absorption in the region 200—450 nm, with maxima in the 250—260, 300—330, and 400—450 nm regions. The increase in concentration of these products with dose is given by the increase in the function, $f(r_\infty)$, with dose (Figures 4 and 5). When *G*(absorbing products) is adjusted to unity for anhydrous β-lactose, the relative initial *G*-values for the formation of the absorbing products at the various wavelengths for the irradiated anomers studied can be determined from the initial slope of the yield–dose curves (Figures 4 and 5); these are summarised in Table 2.

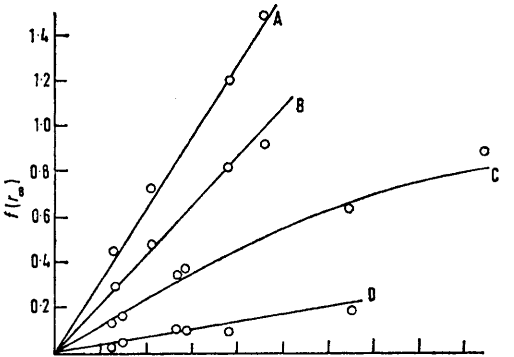

FIGURE 4 Formation of irradiation products which absorb at 256 nm and 322 nm measured from diffuse reflectance spectra of γ-irradiated lactose; Kubelka–Munk function $f(r_\infty)$ at 256 nm: A, α-lactose monohydrate; B, anhydrous β-lactose; $f(r_\infty)$ at 322 nm: C, α-lactose monohydrate; D, anhydrous β-lactose

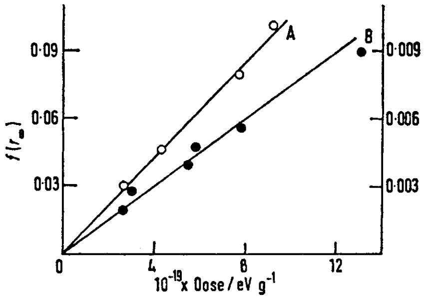

FIGURE 5 Formation of irradiation products which absorb at 400 nm measured from diffuse reflectance spectra of γ-irradiated lactose; A, α-lactose monohydrate (left hand ordinate); B, anhydrous β-lactose (right hand ordinate)

I.r. Spectra.—The increase in i.r. absorption at 1725 cm^{-1} in recrystallised α-lactose monohydrate was determined after irradiation. The area of the small band obtained by integration was taken as a measure of the intensity of absorption. The increase in this band was linear up to a dose *ca.* 3 × 10^{20} eV g^{-1}. Over the same dose range the increase in absorption was extremely small for anhydrous β-lactose. Relative *G*(COO) are summarised in Table 2.

TABLE 2

Comparison of *G*-values [a]

Anomer	Absorption [b] λ/nm 256	322·5	400	−*G* [c]	*G*(COO) [d]	*G*(acid) [e]
Anhydrous β-	1	1	1	1	1	1
Monohydrate α-	2·7	6·3	14	3·0	>10	13—15

[a] Based on anhydrous β-lactose; *G*-values adjusted to unity. [b] Diffuse reflectance. [c] Isotope dilution. [d] Infrared; *G*-value in arbitrary units. [e] Direct titration.

DISCUSSION

On the basis of acid production and overall decomposition (−*G*), α-lactose monohydrate is considerably more susceptible to degradation on γ-irradiation than the anhydrous α- and β-anomers. Acid production can be measured with greater accuracy than overall degradation and is, therefore, a better indication of small differences in the behaviours of the samples. No difference can be observed in this respect between the anhydrous α- and β-anomers. However, it is significant that *G*(acid) for the recrystallised α-lactose monohydrate ($G = 47 \pm 3$) is consistently greater than for the AnalaR sample ($G = 42 \pm 2$). Such a relatively small difference cannot with certainty be established by the isotope dilution method (initial $-G = 60 \pm 5$ and 53 ± 5 respectively) although our values do show a similar difference. The error is significant since meaningful −*G* values must be measured over small % decompositions (0—6%). Moreover, the degradation–dose curves rapidly deviate from linearity, probably owing to the build-up of irradiation products within the crystal lattice. Crystallographic evidence [13] indicates that as much as 7% of the lactose is present in α-lactose monohydrate as the β-anomer, and the presence of this impurity could reduce the overall decomposition, as occurred with freeze-dried D(+)-glucose.[1,2] The cross-sections for the interaction of excitation states with defects or impurities should be large.[14] On a mass basis, 7% β-lactose would reduce *G*(acid) from 47 to 43, since *G*(acid) for β-lactose is only 3·2.

The behaviours of the anhydrous α- and β-lactose are not very markedly different from each other. The initial −*G* values (24 ± 3 and 21 ± 3, respectively) and initial *G*(acid) (3·2 ± 0·6) are similar and the main observable difference is the decreased rate of decomposition with increasing dose found for the β-anomer (Figure 2). Their decomposition, although considerably greater than for other organic systems examined,[15] is significantly less than for α-lactose monohydrate, when −*G* values

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and G(acid) are the criteria. When radical production is considered, however, their behaviour is not markedly different and G(radical) is *ca.* 4 for the monohydrate and anhydrous anomers. A similar distinction could be made with D(+)-glucose also [1,2] for which gas and radical production were unchanged with varying physical state of the solid. Acid production and $-G$ values, however, varied and when these parameters were used the degradation was considerably reduced if imperfections were introduced into the polycrystalline α-D-glucose by freeze-drying or when water is present as in the monohydrate.

The actual e.s.r. spectra show overall features which are comparable with other mono- and di-saccharides which have been examined and considered in detail elsewhere.[16]

Thus, it is clear, as for D(+)-glucose, that a process appears to be in operation which leads to acid production and decomposition and is dependent on the crystal form of the carbohydrate. The yield of stable radicals, however, is not influenced by the crystal form or the presence of water in the solid.

Since our analytical methods for acid and overall decomposition measurements are based on dissolving the irradiated carbohydrate in water and subsequent analysis, it is necessary to consider whether the influence of water during dissolution does not in some way contribute to the anomalous behaviour of α-lactose monohydrate. Measurement of carboxy-production by i.r. spectroscopy directly in the solid state, however, confirms that G(carboxy) for the α-lactose monohydrate is greater by more than a factor of 10 than for anhydrous α- and β-lactose; G(acid) from direct acid measurements is greater by a factor of 13—15 (Table 2). Diffuse reflectance spectroscopy also confirms that absorbing products are formed in the solid state in greater quantity in the irradiated monohydrate than in the anhydrous lactose. The differential, as given by the ratio of the Kubelka–Munk functions, is not the same over the whole range of wavelengths studied, as would be expected if only one type of product having the same extinction coefficient in each anomer were responsible for the entire absorption in the solid state (Table 2). Between 322 and 400 nm the ratio of $f(r_\infty)$ varies between 6 and 14 for the monohydrate compared with anhydrous β-lactose, and approaches the ratios of 13—15 for the G(acid) yields at the longer wavelength. Acids, therefore, predominate in the products absorbing at 400 nm and, moreover, contribute mainly to the enhanced $-G$ value of the monohydrate. An additional absorbing product (or products) is certainly contributing to the absorption near 256 nm, since the ratio of $f(r_\infty)$ values here is only 2·6. This difference must be due to the presence of certain neutral products. Radicals of the type RCHOH have been observed during pulse radiolysis of concentrated aqueous carbohydrate solutions, with maximum absorption near 260 nm.[17] Another ever-present product in all carbohydrate systems induced by irradiation is a product which absorbs at 267 nm and has been identified as malonaldehyde.[18]

The extinction coefficient for carbohydrate radicals is certainly not greater than 800,[17] whereas malonaldehyde has an extinction coefficient of *ca.* 27,900.[19] If both were present a dialdehydic compound would contribute more to the absorption, even though it could be present in lower yield. The absorption of this type of compound falls off rapidly with increasing wavelength, and may account for the fact that the $f(r_\infty)$ ratio approaches that of G(acid) measured after dissolution at longer wavelengths if it is assumed that the extinction coefficients of the solid-state acid products in the two forms are similar.

Thus, to account for the absorptions differing by a factor of only 2·6 in the 260 nm region the solid neutral products must contribute considerably more to the absorption here than the acid products, since the acid products are formed in much lower yield in the anhydrous β-lactose. G(neutral products) calculated from actual values of $-G$ and G(acid products) and the equation: $-G = G(\text{acid products}) + G(\text{neutral products})$ is a factor of 1·5—2 greater for anhydrous β-lactose than for α-lactose monohydrate. This is in agreement with the reflectance evidence that the yield of neutral products is greater in solid anhydrous β-lactose, provided these products are identical to those produced in α-lactose monohydrate.

The extreme radiation susceptibility of α-lactose monohydrate is, therefore, not due to any secondary influence of water outside the crystal; the products are already present in the solid state after irradiation. We are unable to postulate any satisfactory mechanism which can account for the high initial $-G$ value of *ca.* 55. Although we cannot, owing to the accuracy of the method, be certain that we are truly observing the initial $-G$ it is certain that the true value, if different, would be higher and not lower than the initial $-G$ we observe. Certain solid-state isomerisations with comparably high G-values have been reported,[20] but reactions necessitating bond scission appear to be energetically unlikely, unless secondary chain reactions can be identified. However, it is apparent that the primary and secondary radiation processes which lead ultimately to the destruction of a lactose molecule are dependent on whether or not water is present in the crystal. In the case of α-lactose monohydrate almost one acidic molecule is formed for every lactose molecule decomposed. The high acid yield must be directly dependent on the presence of water and to a certain extent its environment in the three-dimensional hydrogen-bonded lattice since, when water is absent, the acid yield is low. Further, in the

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anhydrous forms the radiation processes are considerably less efficient in promoting fragmentation of a lactose molecule despite the fact that the neutral product yield is enhanced. With the monohydrate it is unlikely that ionisation and excitation could result in the reaction of a water molecule with the four closest lactose molecules to which it is hydrogen bonded[13] to make the enhanced $-G$ value in relation to D(+)-glucose energetically more acceptable. However, this must be a consideration if some type of chain reaction is postulated.

Glycosidic scission as a major process can be eliminated as no free D-glucose or galactose could be detected by t.l.c. in the dose range investigated.[10b, 21] The behaviour is now similar to the more extensive ring fragmentation found during mass-spectral breakdown of certain glucosides[22] rather than simpler glucosidic bond scission which occurs with γ-irradiated glucosides.[23]

[1/2427 *Received, 20th December,* 1971]

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