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CONVERSION OF D-FRUCTOSE INTO 6-DEOXY-D-*threo*-2,5-HEXODIULOSE BY γ-IRRADIATION: A CHAIN REACTION IN THE CRYSTALLINE STATE*

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ABSTRACT

γ-Irradiation of crystalline β-D-fructose yields 6-deoxy-D-*threo*-2,5-hexodiulose (**2**) *via* a chain reaction. The initial *G*-value at 25° is 38. With decreasing temperature, *G*(**2**) decreases strongly; however, no change in *G*(**2**) is observed on going to higher temperatures. *G*(**2**) is independent of dose rate, but decreases with increasing dose. A mechanism for the formation of **2** is proposed. Although *G*(**2**) decreases with increasing dose, γ-irradiation of D-fructose is a convenient method for obtaining **2** on a preparative scale. At a dose of 10^{21} eV.g^{-1}, D-fructose is converted into ~6% of **2**.

INTRODUCTION

The radiation chemistry of crystalline carbohydrates has found wide interest over the last decade, as some of these compounds have shown unexpectedly high *G*-values** of decomposition[1–11]. But it has only recently been possible to analyze the products[12] and to propose, in the case of α-lactose hydrate[9,10] and 2-deoxy-D-*erythro*-pentose[11], chain mechanisms which can explain the unexpectedly high radiation sensitivity. These chain reactions often lead to one main product which can be readily isolated, thereby opening up new preparative routes[10,11]. Apparently, crystalline D-fructose belongs to the group of radiation-sensitive carbohydrates, although the *G*(decomposition) values cited in the literature vary considerably. The *G*-values[13] of self-decomposition for D-fructose-^{14}C depend strongly on assumptions concerning the amount of energy absorbed by the labelled material, and vary from 4 (100% self-absorption) to 40 (10% self-absorption). Jacobs[14] reported *G*(decomposition) values of 5–6, whereas Löfroth[6,8] gave a value of 40 for low conversions. In dilute, aqueous solutions[15], *G*(decomposition) is ~4.

We now report that, on γ-irradiation, crystalline D-fructose (**1**) is mainly

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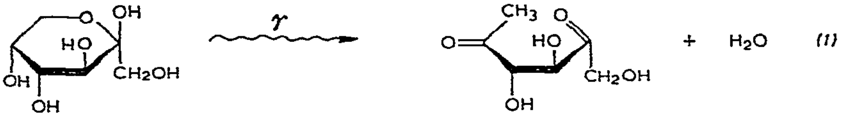
**The *G*-value is defined as the number of molecules formed or destroyed per 100 eV of energy absorbed.

converted into 6-deoxy-D-*threo*-2,5-hexodiulose (2) and water (overall reaction *1*). The yields of other products are smaller by an order of magnitude[16]. Compound **2**



1 2

was obtained by dissolving the irradiated crystals in oxygen-free water and column-chromatographic separation. The structure of **2** was proved by (*a*) mass spectrometry of its methoximated and trimethylsilylated derivatives and of the derivatives obtained after reduction with $NaBD_4$ followed by trimethylsilylation, and (*b*) n.m.r. spectroscopy of its methyl glycoside dimethyl acetal (see Experimental).

RESULTS AND DISCUSSION

The initial *G*-value of the formation of 6-deoxy-D-*threo*-2,5-hexodiulose (**2**) is 40 ±4 at 25°. With increasing dose, *G*(**2**) decreases, as can be seen from the curvature in the dose–yield plot (Fig. 1), and is largely independent of dose rate between 6.7×10^{17} and 1.6×10^{19} eV.g^{-1}.h^{-1} (Fig. 2). Between 25 and 60°, *G*(**2**) is also independent of temperature, but below 25°, it decreases strongly with decreasing

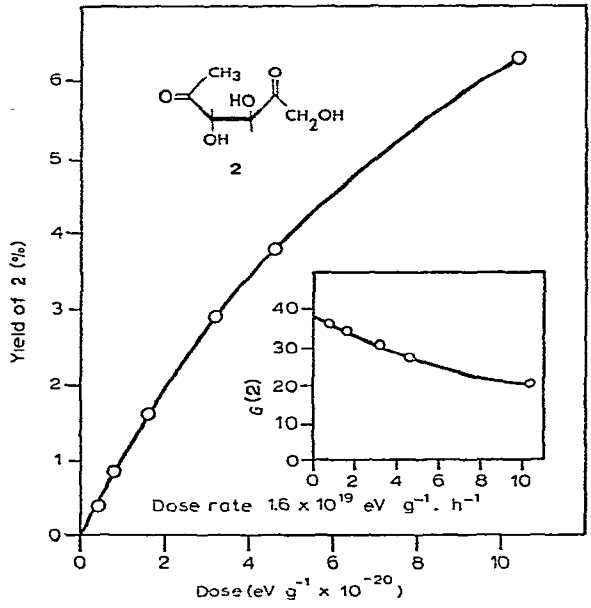


Fig. 1. Yield of 6-deoxy-D-*threo*-2,5-hexodiulose from γ-irradiated D-fructose at 25° as a function of dose. Points are mean values of several measurements. Dose rates from 6.7×10^{17} to 1.6×10^{19} eV. g^{-1}.h^{-1}. Inset: plot of *G* values versus dose.

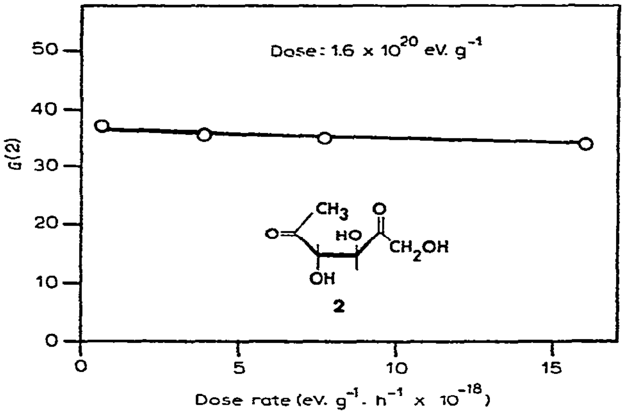


Fig. 2. *G*(6-deoxy-D-*threo*-2,5-hexodiulose) at a given dose of 1.6 × 10^{20} eV.g^{-1} as a function of dose rate.

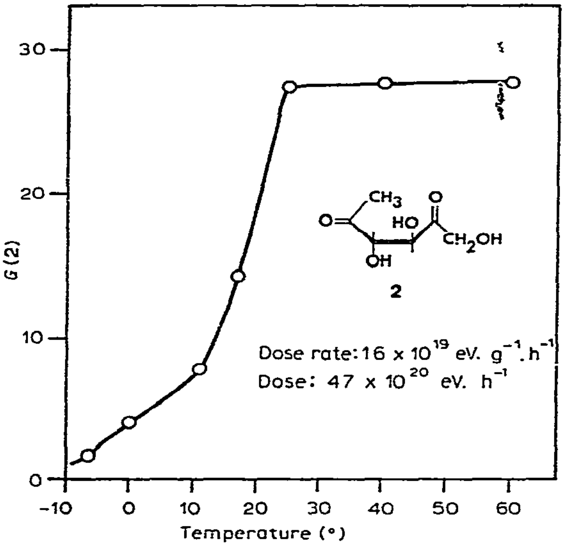
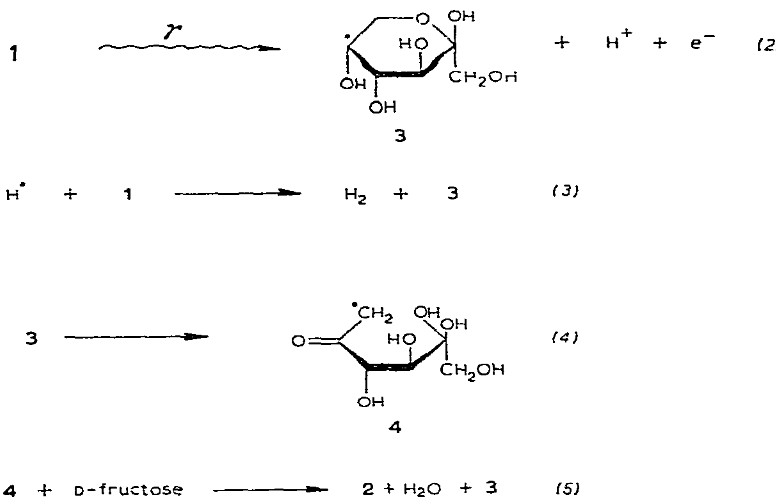


Fig. 3. *G*(6-deoxy-D-*threo*-2,5-hexodiulose) as a function of irradiation temperature. Dose: 4.7 × 10^{20} eV.g^{-1}. Dose rate: 1.6 × 10^{19} eV.g^{-1}.h^{-1}.

temperature (Fig. 3). If samples were irradiated at −78° (followed by work-up at room temperature), no **2** was found. The *G*(**2**)-value is in accord with the *G*(decomposition) of D-fructose as determined by Löfroth[6] (at a dose of 4 × 10^{20} eV.g^{-1} and a dose rate of 3 × 10^{19} eV.g^{-1}.h^{-1}). Thus, the isolated product is obviously the only major product at 25° and the large series of other products[16] play only a minor role. The high yield of **2** demands a chain reaction; chain reactions[17] have to be postulated if *G*(product) > 10. That the chain reaction does not occur during the work-up, *i.e.*, during dissolution of the irradiated crystals in oxygen-free water, is shown by their strong, radiation-induced absorption at 1730 cm^{-1}, as well as by their direct silylation in pyridine (a radical scavenger). Gejvall and Löfroth[8] have shown corresponding results for *G*(decomposition).

A mechanism for this chain reaction can be proposed as follows. γ-Irradiation will cause ionizations and electronic excitations within the crystals leading, among other things, to D-fructosyl radical-cations (*i.e.*, D-fructosyl radicals and protons), electrons (reaction *2*), and fragment radicals, *e.g.*, H atoms. Among the D-fructosyl radicals, radical 3 will be formed by direct action (reaction *2*) and/or by hydrogen abstraction caused by a radiolytically formed H atom (reaction *3*). Radical 3 may



cleave the hemiacetal bond β to the radical site, leading to radical 4 (reaction *4*). This mechanism is parallelled by the elimination of methanol[18] from $\bullet CHOH–CH_2OCH_3$. Radicals of type 4 are able to abstract hydrogen. A chain is induced if, from a neighbouring D-fructose molecule, the H-4 is abstracted (reaction *5*). Similar chains have been observed for ethylene glycol[19–22] and α-lactose hydrate[9]. If one assumes that the crystal structure, as given by the unperturbed crystal, determines the physical path of this chain reaction through the crystal, one may speculate on the nature of this path on the basis of the crystal structure of β-D-fructose. Fig. 4 shows a sequence of the crystal structure which has been generated from Rosenstein's crystallographic data[23]; the distance between C-6 and H-5 of a neighbouring molecule is only ~3.4 Å. Except for H-1, H-5 is the nearest available hydrogen atom. Since, in general, a tertiary hydrogen atom (H-5) is more easily abstracted than secondary hydrogen atoms (H-1), a preference towards an abstraction from C-5 is feasible. The route the radical chain would then take through the crystal is zig-zag along the c-axis, as depicted in Fig. 4. Judging from the crystal structure, other routes seem less likely.

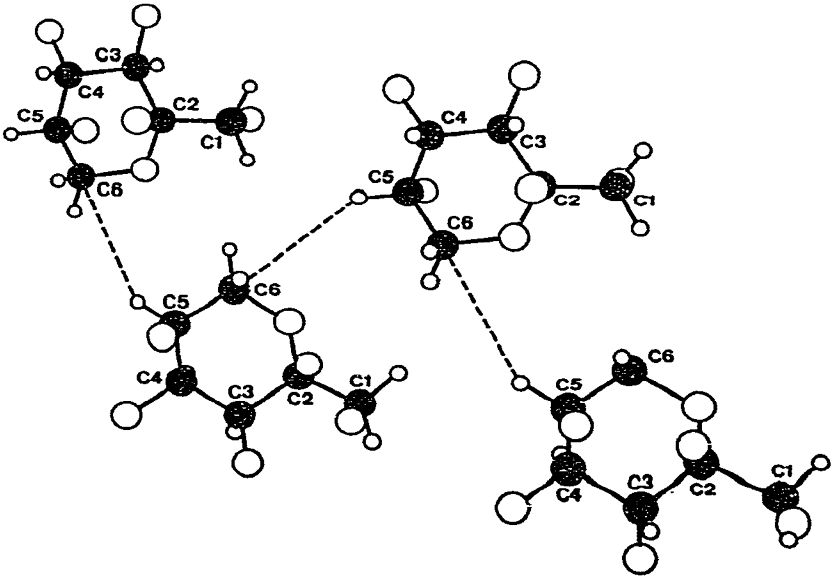


Fig. 4. Display of D-fructose molecules along the c-axis of the crystal. Dashed line denotes the expected route followed by the radiation-induced chain-reaction leading to 6-deoxy-D-*threo*-2,5-hexodiulose.

The strong temperature-dependence of the formation of **2** is not yet fully understood. At low temperatures, the chain-propagation process is suppressed; even when warming-up the samples to room temperature, no chain reaction of any importance is initiated by the radicals which are trapped in the matrix. At low temperature, a process with a lower activation energy, but probably higher pre-exponential factor, successfully competes. This process could be the abstraction of H-1. According to the crystal structure, H-1 is nearer to the radical site at C-6, but abstraction would require a higher activation energy. Such a process could fulfill the requirements. The radical at C-1 formed in this process is not expected to propagate the chain. For crystalline 2-deoxy-D-*erythro*-pentose, a chain reaction has also been observed, and it was possible to shown that the termination of the chain reaction occurs by a hydrogen-abstraction reaction not leading to a propagating radical. In the case of D-fructose, e.s.r. data[24] are not indicative enough to draw such firm conclusions. A chain termination by diffusing radicals, *e.g.*, hydrogen atoms, is unlikely because no dose-rate effect on *G*(**2**) has been found. On irradiating at temperatures >25°, no further increase in *G*(**2**) was observed. The reason for this might be that defect sites within the crystal lattice limit the chain length. The fact that defect sites (product molecules) are generated during radiolysis, and that *G*(**2**) decreases with dose, accords with such an interpretation but does not prove this hypothesis.

Chain reactions in irradiated crystalline carbohydrates, although restricted by the spatial conditions of the crystal lattice and only encountered in a few systems so far, may be used even for preparative purposes, as in the case of α-lactose hydrate where 5-deoxylactobionic acid has been isolated on a gram-scale[10]. Another example is the radiation-induced conversion of 2-deoxy-D-*erythro*-pentose into 2,5-dideoxy-D-*erythro*-pentonic acid[11] (initial *G*-value >650). Recently, 6-deoxy-D-*threo*-2,5-hexodiulose (**2**) has been identified as a product from the γ-radiolysis of D-fructose in aqueous solution[25]; however, it was not isolated prior to this study. Its stereoisomer, 6-deoxy-D-*erythro*-2,5-hexodiulose is a component of the nucleoside antibiotic decoyinine (angustmycin A)[26,27]. The radiation-induced conversion of fructose may be by far the easiest and cheapest way to obtain 6-deoxy-D-*threo*-2,5-hexodiulose (**2**), since at a dose of 4.4×10^{21} eV.g^{-1} (dose rate, 1.6×10^{19} eV.g^{-1}.h^{-1}), D-fructose is converted to ~10% into **2** and its isolation is simple.

EXPERIMENTAL

Irradiation of D-*fructose*. — Crystalline D-fructose (Merck) was subjected to γ-irradiation in a ^{60}Co-γ-source with dose rates of 6.7×10^{17}, 3.9×10^{18}, 7.7×10^{18}, and 1.6×10^{19} eV.g^{-1}.h^{-1}. The irradiated crystals were dissolved in oxygen-free water and the solution was concentrated at 35°.

T.l.c. [silica gel G (Merck); ethyl acetate–acetone–water, 4:5:1[28]] revealed two spots, R_F 0.1 (fructose) and 0.46 (detection with aniline–diphenylamine–phosphoric acid[29]). The product was isolated by chromatography on a column (200 × 10 cm) of silica gel (Woelm), using the above solvent system; 50 g of irradiated material (4.4×10^{21} eV.g^{-1}; dose rate, 1.6×10^{19} eV.g^{-1}.h^{-1}) yielded **2** as a slightly coloured syrup, $[\alpha]_D^{20}$ −60° (*c* 0.3, water).

When **2** was treated with 1% methanolic HCl, the resulting methyl glycoside of the dimethyl acetal was obtained as colourless syrup which was purified by column chromatography $[\alpha]_D^{25}$ +4° (*c* 1, methanol) (Found: C, 48.34; H, 8.20. $C_9H_{18}O_6$ calc.: C, 48.64; H, 8.16%).

Characterization of **2**. — It was not possible by standard methods[30] to convert **2** directly into a trimethylsilyl derivative suitable for g.l.c. However, prior treatment of 10 mg of **2** with 10 mg of methoxyamine hydrochloride (Serva) in 1 ml of pyridine with stirring (1 h) followed by trimethylsilylation (0.2 ml of hexamethyldisilazane and 0.1 ml of chlorotrimethylsilane) yielded a suitable derivative. G.l.c. (SF 96; 70-m glass capillary column, i.d. 0.35 mm; 175° isothermal; Varian 1400) gave two peaks with *T* 15.3 and 15.8 min (D-fructose gave derivatives with *T* 30.0 and 31.0 min). On g.l.c.–m.s.[31] (Varian 1400 and CH-5 Varian MAT), the substances of the two peaks gave identical mass spectra (Fig. 5). The peak for the molecular ion at *m/e* 436 indicates the presence of a deoxy group and two methoxime groups. A peak for M−15 is seen at *m/e* 421 and there are two intense fragments at *m/e* 174 and 262 from the cleaved molecule (see inset Fig. 5) (*cf.* ref. 32).

Reduction of **2** with $NaBH_4$ (Merck), followed by trimethylsilylation and g.l.c. of the product on a 50-m glass-capillary column (i.d. 0.35 mm) coated with SP 2250 operated at 155° isothermally, gave products corresponding to 6-deoxy-

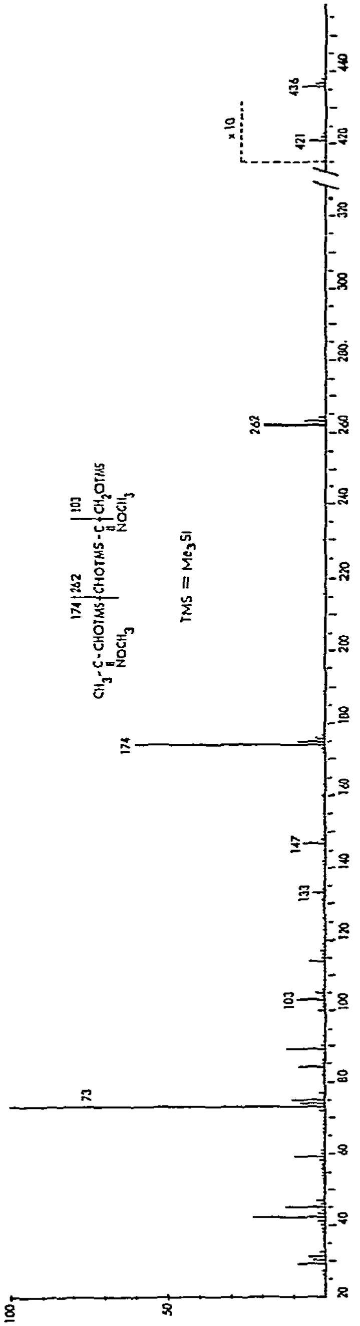


Fig. 5. Mass spectrum of the Me_3Si ether of methoximated 6-deoxy-D-*threo*-2,5-hexodiulose.

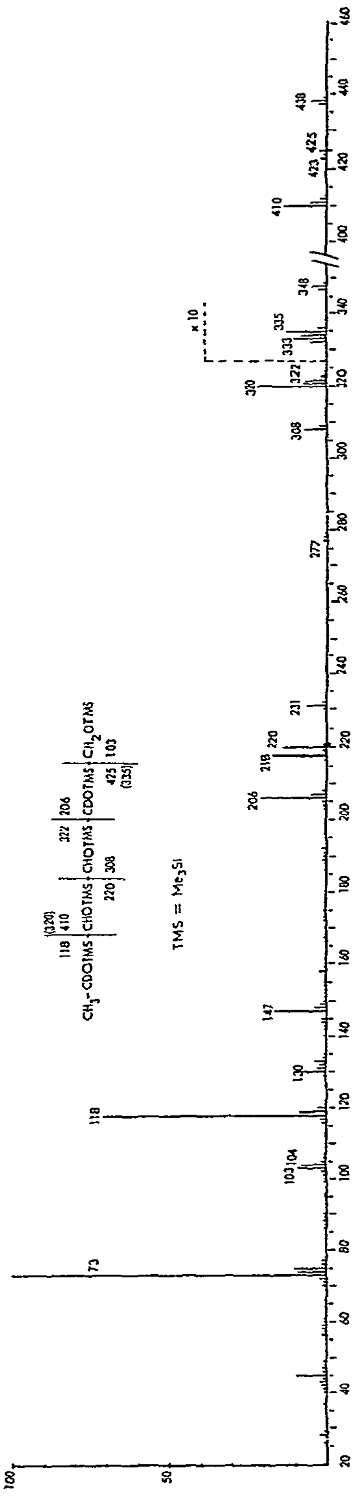


Fig. 6. Mass spectrum of the Me_3Si ether of the 6-deoxyhexitols-2,5-d_2 obtained by reduction of 6 deoxy-D-*threo*-2,5-hexodiulose with $NaBD_4$.

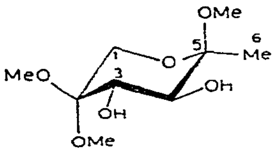
mannitol (T 12.8 min) and 6-deoxyglucitol (T 13.3 min); no reference material was available for the other two stereoisomers (T 13.6 and 14.8 min). The mass spectra corresponded to those of Me_3Si ethers of 6-deoxyhexitols[12].

Reduction of **2** with $NaBD_4$ incorporated deuterium at the positions of the C=O groups[12]. The mass spectra of the Me_3Si ethers of all the four deuterated products were virtually identical and are shown in Fig. 6.

The mass spectrum of the Me_3Si derivative of the methyl glycoside dimethyl acetal of **2** showed prominent ions at m/e 335 ($M-31$), 245 ($M-31-90$), 334 ($M-32$), 319 ($M-15-32$), 247 ($M-30-89$), 157 ($M-30-89-90$), 277 ($M-89$), 187 ($M-89-90$), and rearrangement ions at m/e 277, 261, 189, 173, 89, and 59.

The n.m.r. spectrum (D_2O) of **2** showed the following signals: δ 3–4 (complex m, CH_2, CH, OH), 1.45 and 2.30 (2 s, Me in open-chain and lactol forms, ratio ~1:1). The presence of a third form involving a cyclic enol ether of the methyl ketone group cannot be excluded.

The n.m.r. spectrum (acetone-d_6) of the methyl glycoside dimethyl acetal contained the following signals: δ 1.30 (s, 3 H, quaternary CMe), 3.14, 3.23, 3.38 (3 s, 9 H, 3 MeO), 3.17 and 3.54 (ABq, 2 H, J 12.6 Hz), 3.28 and 3.72 (ABq, 2 H, J 9.3 Hz). Two lines of the AB quartets were hidden under the MeO singlet at 3.23 but were detected unambiguously by spin-tickling experiments. The first AB-quartet originates from a $-CH_2OX$ group attached to a quaternary carbon atom, and the second to a –CHOX–CHOX– moiety flanked by quaternary carbon atoms. These data are consistent with structure **5**, which allows the derivation of structure **2**.



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The parameter J 9.3 Hz shows the antiplanar arrangement of H-3 and H-4, *i.e.*, the same stereochemistry as in fructose. Since the C-Me group shows no long-range coupling with H-4 and no nuclear Overhauser effect with H-1 and H-3, MeO-5 is probably axial.

Quantitative measurements. — Quantitative determinations were carried out by reducing **2** with $NaBH_4$ followed by trimethylsilylation and quantitative g.l.c., using ribitol as an internal standard and 6-deoxyglucitol for calibration.

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