Radiation Chemistry of Carbohydrates. Part V.† γ-Radiolysis of *scyllo*-Inositol in Deoxygenated and Oxygenated Aqueous Solution

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scyllo-Inositol has been γ-irradiated in deoxygenated and oxygenated N_2O-saturated aqueous solutions. The products have been identified and their *G* values determined. In oxygen free solutions at 0° the following products have been found: (*G* values in parentheses): *scyllo-myo*-inosose (3.0), *myo*-inositol (2.1), deoxyinosose (1.1), and H_2O_2 (0.45). Except for H_2O_2, the *G* values depend on dose rate and temperature. A reaction scheme is given which accounts for these dependences. In oxygenated solution only *scyllo-myo*-inosose (6.15) and H_2O_2 (3.9) were observed. The *G* values did not vary much with temperature.

THE mechanism of the free radical chemistry and of the free-radical-induced autoxidation of simple organic compounds in aqueous solutions has attracted much attention recently. In the present paper we publish results obtained with *scyllo*-inositol, which is a cyclic polyalcohol. This compound has been chosen because it is completely symmetric in the sense that attack by OH radicals produces only one primary free radical. In some ways *scyllo*-inositol can serve as a model compound for the structurally more complicated monosaccharides. The inositols are of some biological importance [1-6] and hence their radiation-induced degradation has already received some attention.[7]

EXPERIMENTAL

Preparation of scyllo-*Inositol.*—*scyllo-myo*-Inosose was prepared according to Posternak [8] from *myo*-inositol (Merck) by oxidation with the aid of the bacterium *Acetobacter suboxydans.*‡ After 10 days incubation these solutions were centrifuged, decolourised with charcoal, and evaporated to dryness. The residue was washed several times with water and once with methanol. *myo*-Inositol (24 g) yielded crude *scyllo-myo*-inosose (19 g), crystals, m.p. 191—196° (decomp.). Crude *scyllo-myo*-inosose did not show a C=O absorption at 1 600—1 800 cm^{-1}. A trimethylsilylated sample gave *scyllo-myo*-inosose only in very small quantities on g.l.c. Hydroxy-group determination with activated triethylborane [9] gives 7 mol. equiv. of ethane § indicating that in the crude material *scyllo-myo*-inosose is present as its hydrate.

Pure *scyllo-myo*-inosose was obtained by precipitation with methanol. Crude material (1 g) was dissolved in hot water (80°; 30 ml). After partial concentration *in vacuo* (to *ca.* 10 ml) methanol (50 ml) was added. After refrigeration for several days *scyllo-myo*-inosose was precipitated as crystals. The first fraction had m.p. 189—190° (decomp.), later fractions m.p. 185—186° (altogether 700 mg recovered). After a second recrystallization including decolourisation with charcoal pure *scyllo-myo*-inosose (500 mg) was obtained, crystals, m.p. 179—180° (decomp.), $\nu_{max.}$ 1, 730 cm^{-1}.

It should have been possible to purify the inosose *via* its

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phenylhydrazone.[10] With our crude material the phenylhydrazone yield was too poor for this procedure to be used. Furthermore, when inosose was freed from the hydrazone a series of products was formed (among them probably dehydrated inososes).

Prior to this study Carter *et al.*[11] noted that when precipitating *scyllo-myo*-inosose from methanol several fractions with different decomposition points were obtained.

Penta-*O*-acetyl-*scyllo-myo*-inosose was prepared according to Posternak [8] and Stanacev and Kates [12] by acetylation of *scyllo-myo*-inosose. After recrystallization from acetic acid pure penta-acetate, m.p. 212° (lit., 211°,[8] 147°,[8] 212—213° [12]), was obtained. After a second recrystallization the m.p. decreased (199—201°) and reached 150° after several days in a desiccator. No difference in the penta-acetates (m.p. 212 or 150°) can be observed by n.m.r., i.r., or mass spectroscopy, in the agreement with previous results.[13]

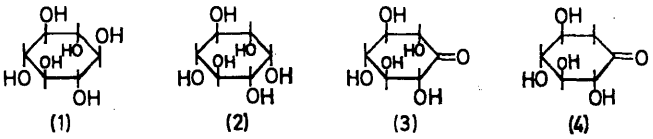
scyllo-Inositol was prepared by reducing the penta-acetate (1 g) with $NaBH_4$ (200 mg) in methanol (25 ml) according to Stanacev and Kates [12] and hydrolysing the residue according to Posternak.[8] To remove the borate the concentrated solution was passed through an ion exchange column (Merck; strongly acidic) and dried in a rotary evaporator. Boric acid was removed as its methyl ester by adding methanol to the residue and distilling off the methanol. This procedure was repeated several times. The residue was suspended in methanol and the crystals (360 mg) were filtered off; m.p. 330° (decomp.). After recrystallization from water pure material (180 mg), m.p. 342—343° (lit. 355—356°,[12] 352° [8]), was obtained. The purity was checked by n.m.r. and g.l.c. for all samples of *scyllo*-inositol used in the irradiation experiments.

Irradiation and Analysis.—Irradiations were carried out in a Nuclear Engineering Ltd. panorama ^{60}Co γ-source and the dose rate was changed by placing samples at different positions. Aqueous solutions of *scyllo*-inositol were scrubbed either with O_2-free N_2O for 30 min prior to irradiation, or with 80 : 20 (v/v) N_2O–O_2 which was kept bubbling during irradiation to avoid O_2 depletion.

Irradiated samples were dried in a rotary evaporator at 20° and either silylated directly [14] [2 mg sample; 60 μl pyridine, 100 μl *NN*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), and 40 μl chlorotrimethylsilane (TMCS) left overnight prior to injection], or methoximated [15] (9 mg sample, 180 μl pyridine, 8 mg methoxyamine hydrochloride shaken overnight at 40°) and subsequently silylated (350 μl BSTFA; 140 μl TMCS). Reduction with $NaBH_4$ or $NaBD_4$ was carried out as described previously.[16] For a 12 mg sample, $NaBH_4$ or $NaBD_4$ (54 mg) in water (0.7 ml) was used. These samples were trimethylsilylated using pyridine (120 μl), BSTFA (200 μl), and TMCS (80 μl). Separations were carried out on packed (2% SE-52, 2 m; 1% SE-30, 3 m) or on glass capillary (DC 560, 24 m, i.d. 0.35 mm) columns. Separations of *scyllo*- and *myo*-inositol are poor on the packed columns and a quantitative determination of *myo*-inositol was not possible. Although the method of the 'internal standard' (methyl glucoside) was used for quantitative measurements the yields for *scyllo-myo*- and deoxy-inosose were *ca.* 15% higher when measured in the packed than in the glass capillary column. The difference could not be explained. Values given in this paper are based on measurements in the glass capillary column. In the glass capillary column the temperature must be kept below 160° in order to prevent decomposition of the trimethylsilyl ether of the *scyllo-myo*-inosose. Using a temperature program (1° min^{-1} starting at 140°) the trimethylsilyl ether of deoxy-inosose was eluted after 14, that of *scyllo-myo*-inosose after 20.5, that of *scyllo*-inositol after 29, and that of *myo*-inositol after 34 min.

RESULTS

γ-Radiolysis of scyllo-*Inositol in Deoxygenated* N_2O-*Saturated Aqueous Solutions.*—When N_2O-saturated solutions of *scyllo*-inositol (1) (10^{-2}M) are irradiated at natural pH three major products are observed: deoxyinosose (4), *scyllo-myo*-inosose (3), and *myo*-inositol (2). The products



were identified by g.l.c. using trimethylsilylation and methoximation combined with trimethylsilylation to produce suitable derivatives. The identification of the products was by combined g.l.c.–mass spectrometry. *scyllo-myo*-Inosose and *myo*-inositol were available as reference compounds and could be compared straightforwardly. The identification of deoxyinosose can be made on the basis of the mass spectrum of its trimethylsilyl ether. The molecular ion (*m/e* 450) was not observed under our conditions, but *m/e* 360 (*M* − 90), 270 (*M* − 90 − 90), 255 (*M* − 15 − 90 − 90), and 246 ($COCH_2CHOSiMe_3$-$CHOSiMe_3$), and other indicative ions were observed. This assignment is backed by the interpretation of the mass spectra from samples reduced with $NaBH_4$ and $NaBD_4$ respectively (trimethylsilylated) and from the methoximated trimethylsilyl derivative. The mass spectra of the trimethylsilyl ethers of deuteriated open chain deoxypolyalcohols show very pronounced decomposition patterns which allow firm assignments.[16] In the cyclitol series, however, no such marked difference in the mass spectra of trimethylsilyl ethers of cyclitols and deoxycyclitol was observed. β-Fragmentation, one of the pronounced fragmentation processes, however, introduced an increase in the intensity of the ion (A), *m/e* 217, from 21—28% in trimethylsilyl ethers of *scyllo*- and *myo*-inositol to 31 and 46% in the two trimethylsilyldeoxyinositols. On deuteriation this and other peaks were shifted by one unit.

The methoximated trimethylsilyl derivatives are more suitable for identification in the series of cyclitoses. Although the identification is not possible by means of prominent fragments, inosose and deoxyinosose can be distinguished on the basis of ion (B), *m/e* 172, in the case of deoxyinosose, and (C), *m/e* 170, in the case of inosose. Similarly the pair *m/e* 244 and 242 may be explained in terms of the ions (D) and (E). The lower retention time of the

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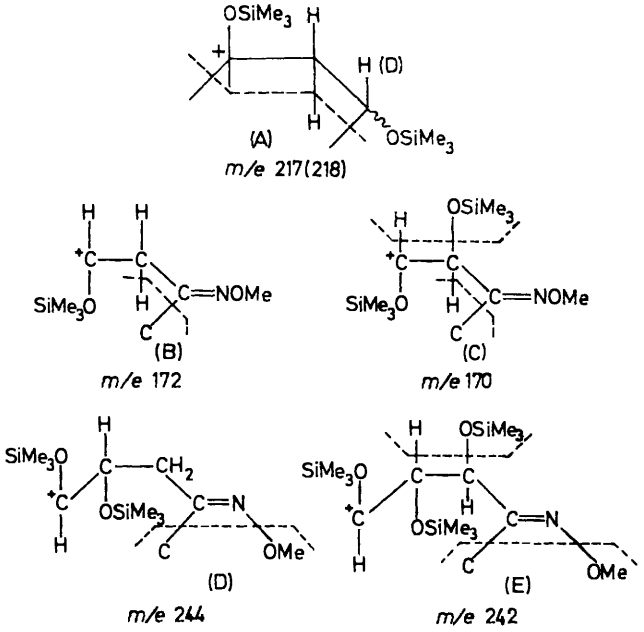
compound in question compared with that of inosose is in good agreement with its assignment to deoxyinosose.



The yields of deoxyinosose (4), *scyllo-myo*-inosose (3), and *myo*-inositol (2) are linear with dose up to 8×10^{18} eV g^{-1}. From the slope of these lines G values have been calculated and are tabulated in Table 1. For *myo*-inositol in particular the scatter in the measured values becomes considerable if G values are <1, and an error of the order of ± 0.2 was estimated. At doses $>10^{19}$ eV g^{-1} the yield–dose curves decline. The yield of H_2O_2 has been found to be

TABLE 1

G Values of products from 10^{-2}M-*scyllo*-inositol irradiated at pH7 in deoxygenated, N_2O-saturated aqueous solution

10^{-18} Dose rate (eV g^{-1} h^{-1})	G(*scyllo-myo*-Inosose) 0°	25°	G(Deoxy-inosose) 0°	25°	G(*myo*-Inositol) 0°	25°
135		2.5		1.6		1.1
65	3.0	2.4	1.1	1.7	2.1	1.2
18		2.0		1.3		0.7
2.5		1.7		1.3		0.5

linear with dose and a value of $G(H_2O_2) = 0.45$ was calculated.

As seen from Table 1 G values vary with both temperature and dose rate. At 0° a rather complete redox and material balance was obtained (see Discussion section) whereas at 25°, especially at low dose rates there is an appreciable lack of products on the basis of the yield of the *scyllo*-inositolyl radicals formed by the water radicals [G(OH + H) = 6]. We therefore investigated the formation of other products. Although g.l.c. revealed only one further minor product, they can be made visible by t.l.c. with a sample irradiated at the lowest dose rate (X_1 and X_2 in Table 2). These products are not observed at the highest dose rate used, nor in the 0° experiment. Samples irradiated in acidic media (pH 2) show a spot (X_3 in Table 2) on t.l.c. plates.

These products have not been sufficiently characterized. However, new bands at 1 600—1 630 cm^{-1} appear in the i.r. in a neutralized sample irradiated at pH 2. These absorption bands may be due to products containing the structural unit CH(OH)=CH–CO– (ν 1 650 and 1 615 cm^{-1} are reported for such a structural unit if hydrogen-bonding occurs; ν 1 605 cm^{-1} is given for C=C). At 780 cm^{-1} (C=C–H) a new absorption also occurs. Dehydrated inososes and dehydrated deoxyinososes may give rise to these absorptions and in fact apart from *scyllo-myo*- and deoxy-inosose there are some new compounds detected by g.l.c. when the products were methoximated and trimethylsilylated. However, their concentration was too low for mass spectra to be obtained.

TABLE 2

R_F Values of products from γ-irradiated *scyllo*-inositol (10^{-2}M) in deoxygenated N_2O-saturated aqueous solutions

Compound	R_F Values	
X_1	0 [a,c]	0.16 [b,c]
X_2	0	0.30
scyllo-Inositol	0	0.50
myo-Inositol	0.03	0.50
scyllo-myo-Inosose	0.06	0.50
Deoxyinosose	0.32	0.79
X_3	0.50	0.86

[a] Solvent acetone–water (85 : 15); silica gel plates (Merck). [b] Solvent acetone–water (60 : 40); silica gel plates (Merck), streaking up to R_F 0.3. [c] Spray: $AgNO_3$–NaOH.

At pH 8 and room temperature (dose rate 18×10^{18} eV g^{-1} h^{-1}) the yield of (2)—(4) was lower than at pH 7 [G(*scyllo-myo*-inosose) = 0.7, G(deoxyinosose) = 0.7, G(*myo*-inositol) = 0.3]. At pH 11 no products could be identified.

γ-Radiolysis of scyllo-*Inositol in* N_2O–O_2 *saturated Aqueous Solutions.*—Solutions of *scyllo*-inositol (10^{-2}M) were saturated with a mixture of 80% N_2O and 20% O_2([N_2O] in water = 2×10^{-2}M, [O_2] in water = 3.2×10^{-4}M at 20°). In this way all e_{aq}^- are converted into OH radicals, and all *scyllo*-inositolyl radicals into the corresponding peroxyl radicals. Under these conditions only *scyllo-myo*-inosose and H_2O_2 are formed as stable products. As in deoxygenated solutions the yield–dose plots deviate from linearity and only the straight section up to 6×10^{18} eV g^{-1} has been used to calculate the G values. At a dose rate of 18×10^{18} eV g^{-1} h^{-1} G(*scyllo-myo*-inosose) was 6.15 at 0° and 6.6 at 25°. $G(H_2O_2)$ has been found to be 3.85 at 0° and 3.9 at 25°.

If a solution of *scyllo*-inositol (10^{-2}M) which is kept at 0° is given a series of pulses from a van de Graaff generator to a total dose of 1.1×10^{18} eV g^{-1} an intermediate acid can be titrated. The G value after 1 min is $\leqslant 1.5$. The acid decays rapidly and after 2—3 min no acid can be detected.

If irradiations are carried out at pH 4 further products are formed. These are believed to be dehydrated inososes. Evidence for this again comes from i.r. data (as above) and new peaks in g.l.c. No quantitative measurements have been carried out.

DISCUSSION

On γ-radiolysis of water, OH radicals, solvated electrons (e_{aq}^-), and H atoms are formed as reactive intermediates [reaction (1)]. In N_2O saturated solutions

$$H_2O \rightsquigarrow {}^{\bullet}OH,\ e_{aq}^-,\ H^{\bullet} \qquad (1)$$

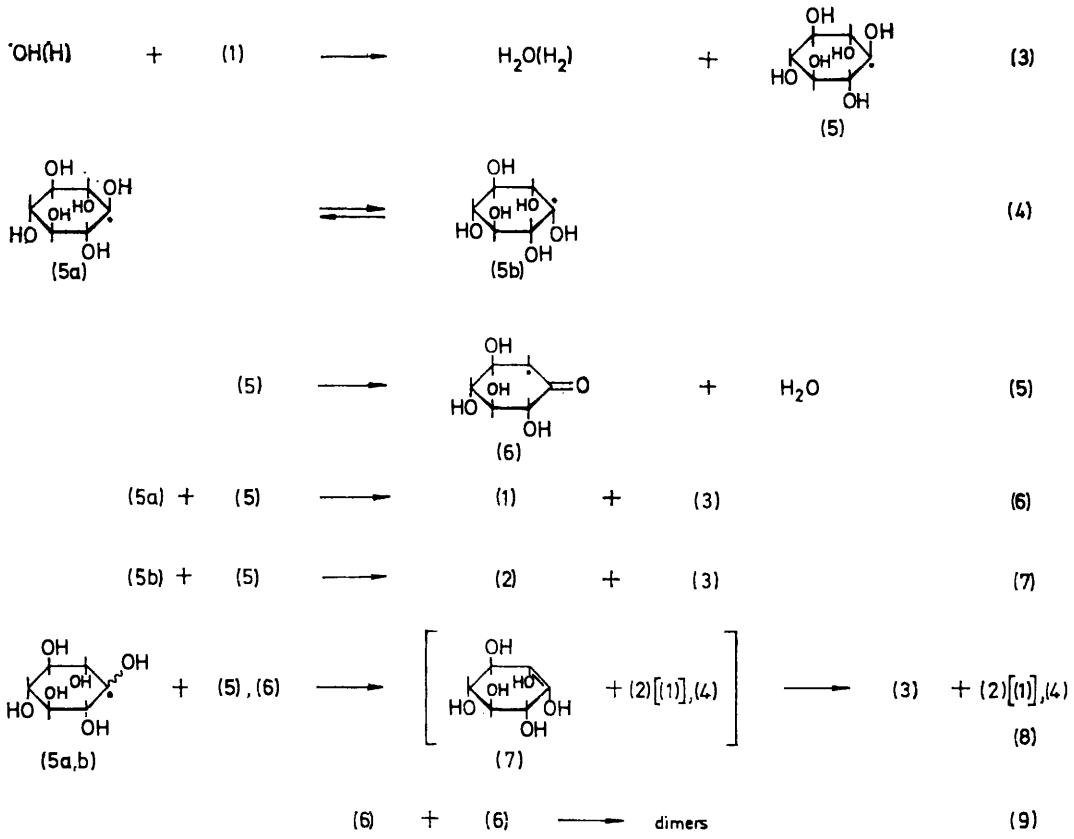
the solvated electrons are converted into OH radicals

$$e_{aq}^- + N_2O \longrightarrow OH + OH^- + N_2 \qquad (2)$$

reaction (2)]. OH Radicals and H atoms abstract hydrogen atoms bound to carbon from the solute giving rise to radical (5) [reaction (3)]. Hydrogen abstraction from the OH groups is rather unlikely.[17]

It is probable that radical (5) can exist in two isomeric forms (a and b) since α-hydroxyalkyl radicals are not planar.[18] However, deviation from planarity may be only small.

Radical (5) can eliminate water to give (6) [reaction (5)]. This water elimination process is typical for αβ-dihydroxyalkyl radicals and has been extensively studied on a series of compounds by e.s.r.,[19-22] product analysis,[23-29] and pulse radiolysis.[30] Radicals (5) and (6) may disproportionate to give the observed products (2)—(4) [reactions (7) and (8)]. The disproportionation of (5) leads to *myo*-inositol (2) [reaction (7)]. Material balance considerations [G(ox) = G (red); G(inosose) ≈ G(deoxy-inosose) + G(*myo*-inositol)] indicate that *scyllo*-inositol is not or only to a small extent re-formed in the disproportionation reaction [reaction (6)]. The preferential formation of *myo*-inositol is expected to be due to steric reasons. In the formation of *scyllo-myo*-inosose (3) by dehydrogenation of radical (5) [reaction (8)] an enol may be the intermediate as has been found for the disproportionation reactions of other hydroxyalkyl radicals.[31-34] Radicals (6) can be reduced to give deoxyinosose (4) [reaction (8)]. The dimerization of (6) [reaction (9)] competes with this reaction. The dimerization product could not be isolated under our experimental conditions; however, in similar systems, *e.g.* ethylene glycol [24] and *meso*-erythritol, [29] the analogous dimers have been characterized and measured quantitatively. In these systems it has been shown that the



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radicals comparable with (5) and (6) give the dimers (5 + 5) and 5 + 6) only in negligible amounts.

With increasing temperature reaction (5) is believed to become faster, *i.e.* more of the primary radicals (5) are converted into (6) before the radicals disappear by disproportionation. The same holds for a change in dose rate. Its decrease favours the first-order reaction (5) over the second-order reactions (6)—(8).

Therefore, at higher temperatures, especially at low dose rate the relative concentration of radicals (5) will decrease and that of radicals (6) will increase. At the first stage this will affect the *G* value of *myo*-inositol more than that of *myo-scyllo*-inosose because the latter still originates from the dehydrogenation of radicals (5) by (6). With no (5) for dehydrogenation available (6) disappears by dimerization [reaction (9)]. Therefore, with decreasing dose rate the *G* values of all three products [(2)—(4)] must decrease, that of *myo*-inositol (2) most strongly (Table 1). As stated above, an equivalent of dimers could not be measured although their formation is indicated by t.l.c. in low dose rate experiments.

Oxygen-containing Solutions.—In O_2- and N_2O-containing solutions only two products, *scyllo-myo*-inosose (3) and H_2O_2, are obtained. G(3) Equals G(OH). This means that every OH radical leads to (3). $G(H_2O_2)$ Amounts to a value about half G(3) if the so called 'molecular' $G(H_2O_2)$ (0.7) is substracted from the H_2O_2 yield. The simplest explanation for the formation of these products is the assumption of a dissociation of the peroxyl radicals into inosose and HO_2 radicals followed by a disproportionation of the HO_2 and O_2^- radicals, respectively.

At the lowest dose rates used this mechanism may operate predominantly as was discussed in the case of ethanol oxidation in aqueous solution.[35] However, under pulse conditions where the acidic transient has been observed there is certainly a bimolecular decay of the peroxyl radical followed by a much more complicated mechanism which will be discussed elsewhere.[36]

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