532. *Effects of γ-Radiation. Part II.*[1] *Characterisation of the Products from Glycollic Acid.*

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The non-volatile products formed by γ-irradiation of glycollic acid solutions *in vacuo* have been isolated and identified as glyoxylic, oxalic, and tartaric acid. They are explained by formation and reactions of the carboxyhydroxymethyl radical, $\cdot CH(OH)\cdot CO_2H$. The effect of oxygen on the reactions of the radical is discussed. Preliminary studies of the effect of γ-radiation on the products have been made.

Preliminary chromatographic and ionophoretic studies of glycollic acid solutions which had been irradiated *in vacuo* showed the formation of glyoxylic (characteristic yellow colour with aniline hydrogen phthalate[2]), tartaric, and oxalic acid, and prompted a more detailed study. In the present work, the initial glycollic acid concentration was 0·1% (0·0132M), and the dose-rate $3{\cdot}8 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$ (determined by the ferrous ammonium sulphate actinometer[3]) by which, in 24 hr., approximately a quarter of the glycollic acid had been degraded. After irradiation for 24 hr. under these conditions *in vacuo*, the solutions contained essentially glycollic, glyoxylic, tartaric, and oxalic acid, but at least three other minor components were detected which were believed to be compounds akin to dihydroxytartaric acid and further radical-addition products from tartaric and glycollic acid. Much longer irradiation gives[1] considerable quantities of non-diffusible polymeric materials, and some such polymer is therefore to be expected after 24 hr.; however, after 12 hr. only glycollic, glyoxylic, tartaric, and oxalic acid could be detected.

After irradiation for 24 hr. *in vacuo*, glycollic and oxalic acid were isolated as such

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[1] Part I, Barker, Grant, Ward, and Stacey, preceding paper.

[2] Partridge, *Nature*, 1949, **164**, 443.

[3] Hardwick, *Canad. J. Chem.*, 1952, **30**, 17; Donaldson and Miller, *J. Chim. phys.*, 1955, **52**, 578.

(characterised as *S*-benzylthiuronium salts), glyoxylic acid as its 2 : 4-dinitrophenylhydrazone, and tartaric acid as its *p*-nitrobenzyl ester.

The mechanism of formation of these compounds is probably as follows,[4] hydrogen abstraction being by the radicals directly formed from the solvent:

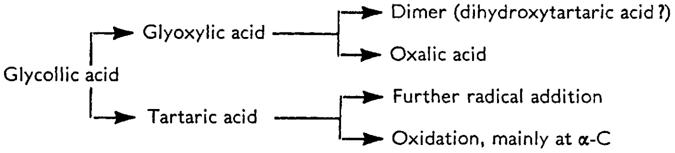
$$\begin{gathered}
H_2O \rightsquigarrow H\cdot + \cdot OH \\
HO\cdot CH_2\cdot CO_2H \xrightarrow[-H]{} \cdot CH(OH)\cdot CO_2H \\
2\cdot CH(OH)\cdot CO_2H \longrightarrow HO_2C\cdot CH(OH)\cdot CH(OH)\cdot CO_2H \\
\cdot CH(OH)\cdot CO_2H \xrightarrow[\cdot OH]{} (HO)_2CH\cdot CO_2H\ [\longrightarrow CHO\cdot CO_2H + H_2O] \\
(HO)_2CH\cdot CO_2H \xrightarrow[-H]{} \cdot C(OH)_2\cdot CO_2H \\
\cdot C(OH)_2\cdot CO_2H \xrightarrow[\cdot OH]{} (HO)_3C\cdot CO_2H\ [\longrightarrow HO_2C\cdot CO_2H + H_2O]
\end{gathered}$$

Dehydrogenation of glycollic acid by radicals from the solvent may be expected because of the activation of the α-hydrogen atom by the carboxyl group. In fact, the direct effect of γ-radiation on polycrystalline glycollic acid *in vacuo* results in almost exclusive formation of the carboxyhydroxymethyl radical,[5] and detection of this radical by its electron spin resonance spectrum shows the readiness with which the process occurs. Subsequent work [6] has shown that the rates of formation of glyoxylic and tartaric acid are those expected for primary products, and that of oxalic acid is as expected for a later product.

Brief chromatographic and ionophoretic studies have been made of the γ-irradiation of solutions of glyoxylic, tartaric, and oxalic acid *in vacuo*. Glyoxylic acid yielded oxalic acid, but also at least two other acidic compounds which had R_T (*i.e.*, *R* relative to tartaric acid) 1·0 and 0·6, corresponding to C_4 and C_6 molecules. Ionophoretic mobilities showed the equivalent weight to remain approximately constant, suggesting the formation of a dibasic " dimer " and a tribasic " trimer." These products reduced aniline hydrogen phthalate,[1] and the former behaves similarly to dihydroxytartaric acid which would be the expected product if condensation of two carboxydihydroxymethyl radicals $\cdot C(OH)_2\cdot CO_2H$ had occurred.

Oxalic acid yielded only traces of non-volatile products, mainly one component having R_T 0·6, which suggests that under certain circumstances reductive coupling can occur.

Tartaric acid produced only a small amount of one faster-moving component, tentatively postulated as a C_3 aldehyde. This component was non-acidic and in mobility equalled mesoxalic acid. Aniline hydrogen phthalate disclosed this component and differentiated it by its colour (brown) from mesoxalic acid (yellow). The main component from tartaric acid was acidic and had R_T 0·3 and the ionophoretic mobility expected for a C_8 tetrabasic dimer. Also, on chromatography, components in the tartaric acid region became detectable with aniline hydrogen phthalate, suggesting some oxidation of the tartaric acid without C–C scission. No glyoxylic or glycollic acid, however, could be detected in the products from tartaric acid. These observations are compatible with the above mechanism, which may be extended, thus:



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[4] Cf. Johnson, Scholes, and Weiss, *J.*, 1953, 3091; Hart, *Radiation Res.*, 1954, **1**, 53; Garrison, Haymond, and Weeks, *ibid.*, p. 97.

[5] Grant, Ward, and Whiffen, *J.*, 1958, 4635.

[6] Grant and Ward, Part III, following paper.

We next studied degradation by γ-radiation of aqueous glycollic acid solutions continuously aerated with oxygen. Glyoxylic and oxalic acid were found as before, and in higher yields, with tartaric acid in very much smaller quantities.

It appears unlikely that the initial abstraction of hydrogen would be modified by the presence of oxygen, but the oxygen would readily yield peroxy-radicals from organic radicals once these had been formed:

$$\mathrm{HO{\cdot}CH_2{\cdot}CO_2H} \xrightarrow[-\mathrm{H}]{} \mathrm{{\cdot}CH(OH){\cdot}CO_2H} \xrightarrow[\mathrm{O_2}]{} \mathrm{{\cdot}O{\cdot}O{\cdot}CH(OH){\cdot}CO_2H}$$

$$\mathrm{{\cdot}O{\cdot}O{\cdot}CH(OH){\cdot}CO_2H + {\cdot}HO_2} \longrightarrow \mathrm{CHO{\cdot}CO_2H + H_2O_2 + O_2}$$

Many workers[4] have established this type of reaction and shown that such peroxy-radicals react, almost exclusively, to yield a molecule where, ultimately, a hydroxyl group is substituted on the peroxy-radical site, in this case yielding glyoxylic acid. The reaction of the initial radical with oxygen would thus much reduce the formation of tartaric acid, since the life of the peroxy-radical is likely to be much longer than that of the parent radical in the presence of excess of oxygen.

This concept is substantiated by observations on the irradiation of glyoxylic, oxalic, and tartaric acid separately in the presence of oxygen, where the formation of components by C–C linkage virtually ceased. Glyoxylic acid appeared to yield solely oxalic acid, which, in turn, yielded no detectable non-volatile products. Tartaric acid, also, showed no components with $R_{\mathrm{T}} < 1$, but there was more formation of compounds sensitive to aniline hydrogen phthalate in the tartaric acid region than there was *in vacuo*, also of components behaving as mesoxalic acid and glyoxylic acid (the latter in traces).

The reaction of the radicals with oxygen, giving ultimately glyoxylic acid, explains why polymers are not formed in such cases: for it is addition of these radicals to one another that is believed to cause polymer formation from, *e.g.*, glycollic acid *in vacuo*.[1,7]

It should be emphasised that both in the presence and in the absence of oxygen some degradation occurs to volatile products such as formaldehyde, formic acid, and carbon dioxide. This aspect is considered elsewhere.[6]

EXPERIMENTAL

Irradiation Procedure.—Solutions were placed round a 200 c ^{60}Co γ-radiation source similar to that described by Gibson and Pearce.[8] The solutions were stirred continuously during irradiation. The dose-rate throughout was $3{\cdot}8 \times 10^{16}$ ev. min.$^{-1}$ ml.$^{-1}$, determined by the ferrous ammonium sulphate actinometer, [$G(\mathrm{Fe^{++}} \longrightarrow \mathrm{Fe^{+++}}) = 15{\cdot}6$].[3] The volume irradiated was 4 l. Alternatively smaller volumes in units of 20 ml. were irradiated at a dose-rate of $14{\cdot}2 \times 10^{16}$ ev min.$^{-1}$ ml.$^{-1}$.

Identification of the Products formed by Irradiation of Glycollic Acid in vacuo.—(i) *Fractionation of the non-volatile solute.* A solution of glycollic acid (4 g.) in water (4 l.) was boiled *in vacuo* for 2 hr. with frequent shaking. The vessel was sealed under a vacuum and irradiated for 24 hr. (total dose, $5{\cdot}5 \times 10^{19}$ ev ml.$^{-1}$) with gentle stirring. The solution was then freeze-dried[9] and the solid (*ca.* 4·1 g.) dissolved in water (100 ml.) and neutralised with excess of calcium carbonate with vigorous stirring. The precipitates A″ (immediate) and B″ (after 48 hr.) were removed. The filtrate was freeze-dried, dissolved in water, filtered from calcium carbonate, concentrated, and allowed to crystallise repeatedly, to give crystals (C′) and mother-liquor, which was freeze-dried (D′). A″ and B″ were extracted with cold 2N-acetic acid to yield residues A′ (0·27 g.) and B′ (0·74 g.) and extracts E′ and F′ respectively.

Fractions C′, D′, E′, and F′ were separately passed in dilute aqueous solution through an excess of Zeo-Karb 225 (H$^+$ form) to remove calcium. Freeze-drying the eluates gave materials C (3·5 g.), D (0·25 g.), E (7 mg.) and F (12 mg.). A′ and B′ were treated with an insufficiency of 2N-sulphuric acid, stirred, and filtered, yielding solutions A and B, which were freeze-dried.

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[7] Barker, Grant, Ward, and Stacey, *Nature*, 1959, **183**, 376.

[8] Gibson and Pearce, *Chem. and Ind.*, 1957, 613.

[9] Grant and Ward, *J. Sci. Instr.*, 1959, **36**, 133.

(ii) *Chromatography and ionophoresis of fractions A—D.* Each fraction was analysed by chromatography in propionic acid–butan-1-ol–water (6 : 3 : 4 v/v) and ionophoresis in 0·75M-formate buffer (pH 2·0) and in 0·2M-acetate buffer (pH 5·0). Components were detected with aniline hydrogen phthalate,[2] alkaline silver nitrate,[10] and Chlorophenol Red.[11] These analyses indicated that fraction A contained oxalic and tartaric acid (trace), B contained tartaric and oxalic acid (trace), C contained glycollic and glyoxylic acid (trace), and D contained glyoxylic and glycollic acid.

A similar fractionation was carried out on a 0·1% solution of glycollic acid (4 l.) which had been irradiated for 12 hr. (total dose $2{\cdot}3 \times 10^{19}$ ev ml.$^{-1}$). No compound other than glycollic, glyoxylic, tartaric, and oxalic acid was detected.

(iii) *Identification of major products.* Material A′ was dissolved in 2N-hydrochloric acid and reprecipitated at 100° in the presence of acetic acid by the addition of urea to the boiling solution. This process was repeated. Then the precipitate was suspended in excess of dilute sulphuric acid and extracted continuously with diethyl ether for 48 hr. The ethereal extract was evaporated and the residual oxalic acid recrystallised from a small volume of water (yield 60 mg.). It had m. p. 101°, raised to 188° (m. p. and mixed m. p.) after drying (P_2O_5; 60°/vac.; 3 hr.). It was heated with diphenylamine: the cooled melt, on extraction with ethanol, gave a blue solution which had an absorption spectrum ($\lambda_{\text{max.}}$ 604 mμ) identical with that of ethanolic Aniline Blue prepared from authentic oxalic acid in the same way.[12] 12·11 mg. of the acid neutralised 26·65 ml. of 0·01N-potassium hydroxide (phenolphthalein) (equiv., 45·5. Calc. for $H_2C_2O_4$: equiv., 45·0). The neutralised solution was concentrated until almost saturated, and to the hot solution was added hot ethanolic *S*-benzylthiuronium chloride (0·4 ml.; 1·5 g. of reagent in 10 ml. of ethanol). The solution was allowed to cool, and the crystalline product washed with a little water and ethanol. The bis-*S*-benzylthiuronium oxalate had m. p. and mixed m. p. 193° (Found: C, 51·1; H, 5·2; N, 13·5; S, 15·1. Calc. for $C_{18}H_{22}O_4N_4S_2$: C, 51·2; H, 5·3; N, 13·3; S, 15·2%).

A suspension of solid B′ (0·6 g.) in 2N-sulphuric acid (3·0 ml.) was stirred for 2 hr., freeze-dried, dried (P_2O_5) at 60° *in vacuo*, and boiled in ethanol. The ethanol solution was filtered and slowly evaporated, depositing crystals which on recrystallisation from ethanol and drying (P_2O_5) at 60° *in vacuo* gave tartaric acid (35 mg.), m. p. 202° [Found: equiv. (titration), 74·1. Calc. for $(HO{\cdot}CH{\cdot}CO_2H)_2$: 75·04]. The neutralised solution was concentrated until almost saturated, mixed with ethanolic *p*-nitrobenzyl bromide (0·1 g. in 1 ml.) and refluxed for 2 hr. Crystals which separated on cooling were recrystallised from aqueous ethanol (m. p. 146°) (Found: C, 51·5; H, 3·9; N, 6·8. Calc. for $C_{18}H_{16}O_{10}N_2$: C, 51·4; H, 3·8; N, 6·7%).

A solution of solid C′ (1·0 g.) was passed through a column of Zeo-Karb 225 (H^+ form) and freeze-dried. The residue was twice sublimed at 35° *in vacuo*, to yield glycollic acid (320 mg.), m. p. and mixed m. p. 79° (Found: equiv., 76·2. Calc. for $HO{\cdot}CH_2{\cdot}CO_2H$: equiv., 76·05). The *S*-benzylthiuronium salt had m. p. and mixed m. p. 145° (Found: C, 49·3; H, 5·6; N, 11·9; S, 13·2. Calc. for $C_{10}H_{14}O_3N_2S$: C, 49·6; H, 5·8; N, 11·6; S, 13·2%). The acid gave the characteristic violet colour[13] with 2 : 7-dihydroxynaphthalene in concentrated sulphuric acid at 100°.

Material D′ (0·2 g.) was treated as C′, giving the free acids which gave a strongly positive test with Schiff's reagent and the blue colour reaction[14] for glyoxylic acid with 2 : 3 : 4-trihydroxybenzoic acid in concentrated sulphuric acid at 50°. The acids (20 mg.) derived from D′ were dissolved in a solution of 2 : 4-dinitrophenylhydrazine (0·1 g.) in concentrated hydrochloric acid (18·2 ml.), diluted to 100 ml.; this gave glyoxylic acid 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 195° (Found: C, 38·0; H, 2·5; N, 22·1. Calc. for $C_8H_6O_6N_4$: C, 37·8; H, 2·4; N, 22·1%).

The small fractions E and F were not examined.

Irradiation of Glycollic Acid in the Presence of Oxygen.—(i) *Fractionation of the non-volatile products.* Glycollic acid (4 g.) in water (4 l.) was degassed as for the irradiation *in vacuo*. The solution was then saturated with oxygen for 2 hr., irradiated, with a continuous stream of

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[10] Trevelyan, Procter, and Harrison, *Nature*, 1950, **166**, 444.

[11] Block, Durrum, and Zweig, " A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press Inc., New York, 1955, p. 169.

[12] Feigl and Fehden, *Mikrochemie*, 1935, **18**, 272.

[13] Eegriwe, *Z. analyt. Chem.*, 1932, **89**, 123.

[14] *Idem, ibid.*, 1934, **100**, 325.

oxygen passing through it, for 24 hr. (total dose 5·5 × 10^{19} ev ml.$^{-1}$), freeze-dried, and fractionated as above, giving fractions A_O' (1·02 g.), B_O' (41 mg.), C_O' (3·4 g.), D_O' (0·75 g.), E_O' (35 mg.), and F_O' (10 mg.), the subscript o indicating irradiation in oxygen.

(ii) *Chromatography and ionophoresis.* These analyses gave similar results to those above, except that A_O contained no minor ingredients, and B_O was a much smaller fraction.

A similar analysis after 12 hours' irradiation showed glycollic, glyoxylic, and oxalic acid only.

(iii) *Identification of the major products.* Material A_O (oxalic acid) was prepared and identified as before, giving a positive test with diphenylamine,[12] equiv. 45·2, and m. p. and mixed m. p. 101° (189° anhydrous). The bis-*S*-benzylthiuronium salt had m. p. and mixed m. p. 194° (Found: C, 51·3; H, 5·1; N, 13·0. Calc. for $C_{18}H_{22}O_4N_4S_2$: C, 51·2; H, 5·3; N, 13·3%).

Material B_O was not present in sufficient quantity for characterisation.

Material C_O (glycollic acid), prepared as above, gave a positive test with 2 : 7-dihydroxynaphthalene,[13] equiv. 76·3, m. p. and mixed m. p. 78°, and a *S*-benzyl thiuronium salt, m. p. and mixed m. p. 145° (Found: C, 49·5; H, 5·8; N, 11·4. Calc. for $C_{10}H_{14}O_3N_2S$: C, 49·6; H, 5·8; N, 11·6%).

Material D_O (glyoxylic acid) gave positive reactions with Schiff's reagent and with 2 : 3 : 4-trihydroxybenzoic acid,[14] and a 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 195° (Found: C, 37·6; H, 2·5; N, 22·1. Calc. for $C_8H_6O_6N_4$: C, 37·8; H, 2·4; N, 22·1%).

Chromatography and Ionophoresis of the Non-volatile Products from Irradiated Solutions of Glyoxylic, Oxalic, and Tartaric Acids.—0·1% Solutions of glyoxylic acid, oxalic acid, and (+)-tartaric acid were irradiated in 20 ml. portions, each degassed as described above, (*a*) *in vacuo* or (*b*) in a stream of oxygen (dose-rate, 14·2 × 10^{16} ev min.$^{-1}$ ml.$^{-1}$ for 7 hr.; total, 5·9 × 10^{19} ev ml.$^{-1}$). After storage for 1 hr. the solutions were freeze-dried. Appreciable weight losses were observed with oxalic acid during irradiation both in a vacuum and in oxygen, and with glyoxylic acid during irradiation in the presence of oxygen.

The products were redissolved in water and subjected to paper chromatography and ionophoresis as described above.

In the presence of oxygen, glyoxylic acid gave only oxalic acid; in the absence of oxygen, a smaller amount of oxalic acid was formed, with slower-moving components having R_T 1·0 and 0·6. These last two components were detected by aniline hydrogen phthalate, Chlorophenol Red, and silver nitrate. Ionophoresis showed oxalic acid in both systems and a component with M_T 1 in the absence of oxygen.

Oxalic acid gave no products in the presence of oxygen, but, *in vacuo*, small amounts of a material having R_T 0·6 and M_T 1·0 were detected by all three reagents.

Tartaric acid in oxygenated solution showed colour development at R_T 1·0 with aniline hydrogen phthalate, and there were components behaving as mesoxalic and glyoxylic acid. In the absence of oxygen, colour development occurred at R_T 1·0 with aniline hydrogen phthalate and 0·3 with all three reagents. In addition, a component reacting with aniline hydrogen phthalate and silver nitrate, but not with Chlorophenol Red, was evident at R_T 1·5.

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