

979. *Effects of γ -Radiation. Part IX.* Action of γ -Radiation on Deaerated Solutions of DL-Mandelic Acid.*

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The non-volatile products formed by γ -irradiation of DL-mandelic acid in 0.1% deaerated, aqueous solution have been determined quantitatively by isotope dilution analysis. Benzaldehyde, phenylglyoxylic acid, benzyl alcohol, hydrobenzoin, and carbon dioxide are the major products of irradiation, whereas $\alpha\beta$ -diphenyltartaric acid, $\alpha\beta$ -diphenyl-DL-glyceric acid, (\pm)-benzoin, and benzoic acid are formed in lesser amounts. These products can be accounted for by reactions of the $\cdot\text{CPh}(\text{OH})\cdot\text{CO}_2\text{H}$ radical initially produced.

PREVIOUS studies¹⁻⁵ of the effects of ionising radiation on hydroxy-acids have shown that the corresponding keto-acid is formed, and that carbon dioxide is also liberated. Glycollic acid, for example, gives glyoxylic acid on γ -irradiation, both in oxygenated and deaerated solutions. Benzaldehyde has been detected on irradiation of mandelic acid both in deaerated, aqueous solution⁴ and as the solid. The decrease in the optical rotatory power of irradiated L-mandelic acid solutions^{4,5} is consistent with the initial formation of the

* Part VIII, *J.*, 1963, 1663.

¹ Grant and Ward, *J.*, 1959, 2654, 2659.

² Johnson, Scholes, and Weiss, *J.*, 1953, 3091.

³ Pratt and Putney, *Radiation Res.*, 1954, **1**, 234; 1956, **5**, 134.

⁴ Feng and Tobey, *J. Phys. Chem.*, 1959, **63**, 759.

⁵ Wright, *Discuss. Faraday Soc.*, 1952, No. 12, 60.

$\cdot\text{CPh}(\text{OH})\cdot\text{CO}_2\text{H}$ radical, and this contention is supported by recent electron spin resonance (e.s.r.) studies⁶ on polycrystalline DL-mandelic acid. Hitherto, no detailed investigation of the products of irradiated deaerated mandelic acid solutions has been reported. Isotope dilution analysis provides a convenient and accurate technique for the determination of the products from the γ -irradiation of deaerated solutions of DL-mandelic acid, labelled with carbon-14 at the asymmetric carbon atom.

RESULTS AND EXPERIMENTAL

DL- ^{14}C Mandelic Acid.— ^{14}C -carbonyl]Benzaldehyde (42 mg.; 0.5 mc) was diluted with redistilled benzaldehyde (15.8 g.) and a solution of sodium hydrogen sulphite (15 g.) in water (30 ml.) added with vigorous stirring. To the solution of the resultant bisulphite compound

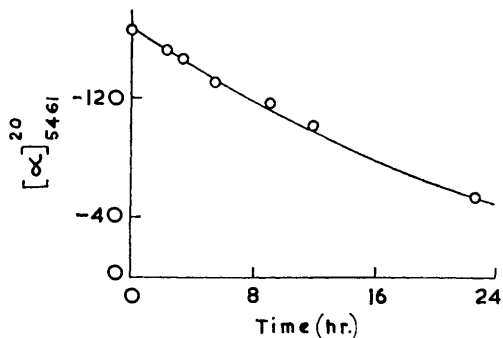


FIG. 1. Decrease in optical rotatory power of 0.1% L-mandelic acid solutions (vol. 20 ml.) at a dose rate of 0.93×10^{17} ev. $\text{ml}^{-1} \text{min}^{-1}$.

was added a solution of potassium cyanide (21 g.) in water (25 ml.), and the oily cyanohydrin which separated was extracted with ether. The residue, after removal of the solvent, was hydrolysed with boiling 9N-hydrochloric acid (100 ml.) for 18 hr. with vigorous stirring. The labelled DL-mandelic acid (16.9 g.; specific activity 3.48 $\mu\text{C}/\text{mmole}$) separated from the cooled solution and was diluted as required for use in subsequent irradiations.

$\alpha\beta$ -Diphenyl-DL-tartaric Acid.—A solution of phenylglyoxylic acid (8 g.) in propan-2-ol (80 g.) was illuminated for 18 hr. in a sealed flask with ultraviolet light from a Hanovia 220w lamp. Concentration of the irradiated solution afforded a yellow oil, from which a white precipitate was deposited on addition of benzene. The precipitate was recrystallised from methanol-benzene-light petroleum (b. p. 60–80°) to yield the product (4 g.), m. p. 152° (Found: C, 63.6; H, 4.7. $\text{C}_{16}\text{H}_{14}\text{O}_6$ requires C, 63.6; H, 4.8%) (lit.,⁷ m. p. 155°, for this compound prepared by sunlight illumination of phenylglyoxylic acid).

$\alpha\beta$ -Diphenyl-DL-glyceric Acid.—This compound, prepared according to the procedure described by Dahn, Fischer, and Loewe,⁸ had m. p. 208°.

Irradiation of L-Mandelic Acid Solutions.—The change in optical rotatory power of 0.1% L-mandelic acid solutions (20 ml.) at a dose rate of 0.93×10^{17} ev. $\text{ml}^{-1} \text{min}^{-1}$ was measured at various time intervals on a ETL-NPL (Type 143A) automatic polarimeter at 5461 Å. The results are given in Fig. 1.

Irradiation Procedure.—Solutions (4 l.) were placed round a 200-c ^{60}Co γ -radiation source similar to that described by Gibson and Pearce.⁹ The solutions were evacuated for 4 hr. at 0.3 mm. before irradiation, sealed, and stirred continuously during irradiation. The dose rate throughout was 2.18×10^{16} ev. $\text{min}^{-1} \text{ml}^{-1}$, determined by the ferrous ammonium sulphate dosimeter [$G(\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}) = 15.6$].

Determination of Products by Isotope Dilution Analysis.—A solution (4 l.) of DL- ^{14}C mandelic acid (26.32 mmoles, specific activity 1.27 $\mu\text{C}/\text{mmole}$) was irradiated *in vacuo* to a total energy input of 7.32×10^{22} ev. The individual products were determined by applying the isotope dilution method directly to aliquot parts of the untreated irradiated solution immediately after irradiation was completed. Materials containing carbon-14 were assayed by gas-counting by

⁶ Bailey, Barker, Brimacombe, Pooley, and Spence, *Nature*, 1961, **190**, 259.

⁷ Schönberg, Latif, Moubasher, and Sina, *J.*, 1951, 1364.

⁸ Dahn, Fischer, and Loewe, *Helv. Chim. Acta*, 1956, **39**, 1774.

⁹ Gibson and Pearce, *Chem. and Ind.*, 1957, 613.

the procedure previously described;¹⁰ a specific activity of 1 μC per mg. of carbon dioxide corresponded to 3.3×10^7 counts per min.

The results of a typical determination are next given.

(a) *Benzaldehyde*. To the irradiated solution (250 ml.) was added benzaldehyde (2.97 mmoles) and then ethanol until the solution was clear. Then a 0.2% solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid (300 ml.) was added and the derivative was filtered off and washed with water. Benzaldehyde 2,4-dinitrophenylhydrazone, after six recrystallisations from acetone-ethanol, had m. p. 238° and constant specific activity 1.24×10^{-2} $\mu\text{C}/\text{mmole}$.

(b) *Phenylglyoxylic acid*. To the irradiated solution (250 ml.) was added phenylglyoxylic acid (3.2 mmoles) and then a 0.2% solution of 2,4-dinitrophenylhydrazine in 2N-hydrochloric acid (400 ml.). The 2,4-dinitrophenylhydrazone was washed with water, and, after eight recrystallisations from aqueous ethanol, had m. p. 198° and constant specific activity 1.41×10^{-2} $\mu\text{C}/\text{mmole}$.

(c) $\alpha\beta$ -*Diphenyl-DL-tartaric acid*. To the irradiated solution (200 ml.) was added $\alpha\beta$ -diphenyl-DL-tartaric acid (3.43 mmoles), and the solution was gently warmed and then evaporated to dryness under reduced pressure. The residue was refluxed briefly with benzene (100 ml.), the mixture filtered, and the precipitate washed thoroughly with benzene and suspended in ether, and ethereal diazomethane added until all solids had dissolved and evolution of nitrogen had ceased. The residue, after evaporation of the solvent, was recrystallised from light petroleum (b. p. 60–80°). The dimethyl ester, after six recrystallisations, had m. p. 118–120° and constant specific activity 3.43×10^{-3} $\mu\text{C}/\text{mmole}$.

(d) *Benzyl alcohol*. To the irradiated solution (250 ml.) was added freshly distilled benzyl alcohol (4.84 mmoles); the solution was neutralised with 0.5N-sodium hydroxide and then extracted several times with ether. The dried (MgSO_4) extracts were evaporated to an oil which was treated with 1-naphthyl isocyanate (0.5 ml.) at 95–100° for 20 min. The solid 1-naphthylurethane, after eight recrystallisations from light petroleum (b. p. 60–80°), had m. p. 133–134° and constant specific activity 1.01×10^{-2} $\mu\text{C}/\text{mmole}$.

(e) *mesoHydrobenzoin*. To the irradiated solution (250 ml.) was added *meso*hydrobenzoin (3.05 mmoles); the resultant solution was neutralised with 0.5N-sodium hydroxide and extracted several times with ether. The dried (MgSO_4) extracts were evaporated to dryness; the *meso*hydrobenzoin, after six recrystallisations from aqueous ethanol, had m. p. 135° and constant specific activity 9.43×10^{-3} $\mu\text{C}/\text{mmole}$.

(f) $\alpha\beta$ -*Diphenyl-DL-glyceric acid*. To the irradiated solution (250 ml.) was added $\alpha\beta$ -diphenyl-DL-glyceric acid (3.63 mmoles) and the solution was then evaporated to dryness under reduced pressure. The residue was shaken with ether (75 ml.), and the precipitate was collected and washed with ether. The remaining material, after eight recrystallisations from methanol-benzene-light petroleum (b. p. 60–80°), had m. p. 208° and constant specific activity 1.84×10^{-3} $\mu\text{C}/\text{mmole}$.

(g) (\pm) -*Benzoin*. To the irradiated solution (250 ml.) was added DL-benzoin (2.25 mmoles) and, after neutralisation with 0.5N-sodium hydroxide, the solution was extracted with ether. The dried (MgSO_4) extract was concentrated to dryness and the residue dissolved in ethanol (50 ml.) and treated with 2,4-dinitrophenylhydrazine (300 ml.; 0.2% in 2N-hydrochloric acid). The hydrazone was filtered off and washed with water; after six recrystallisations from ethanol it had m. p. 245° and constant specific activity 4.16×10^{-3} $\mu\text{C}/\text{mmole}$.

(h) *Benzoic acid*. To the irradiated solution (250 ml.) was added benzoic acid (8.38 mmoles) and the solution was then evaporated to dryness under reduced pressure. Recrystallisation of the residue from water gave benzoic acid (0.66 g.), m. p. 121°. This material was treated with thionyl chloride (3 ml.) at 95° for 30 min. and then ammonia solution (15 ml.; d 0.88) was added to the cooled solution. The benzamide was extracted with ether, and the solvent removed; after seven recrystallisations from water, it had m. p. 127° and constant specific activity 1.04×10^{-3} $\mu\text{C}/\text{mmole}$.

(i) *Benzil*. To the irradiated solution (250 ml.) was added carrier benzil (2.33 mmoles); the resultant solution had neutralised with 0.5N-sodium hydroxide and extracted with ether. The dried (MgSO_4) extract was evaporated to dryness and the residue heated with *o*-phenylenediamine (0.25 g.) at 95–100° for 20 min. The quinoxaline, after six recrystallisations from aqueous methanol, had m. p. 125–126° and exhibited no activity.

¹⁰ Barker, Bevington, Brimacombe, and Eades, *J.*, 1962, 4508.

Determination of DL-Mandelic Acid Degraded.—The small amounts of mandelic acid degraded at low energy inputs made its direct determination inaccurate. However, at high energy inputs the mandelic acid that had not been degraded could be determined accurately by the following procedure. Solutions (4 l.) of DL- ^{14}C mandelic acid (26.32 mmoles) were irradiated *in vacuo* at energy inputs between 1.5 and 3.75×10^{23} ev. To each irradiated solution (250 ml.) was added mandelic acid, the solution evaporated to dryness, and the residual mandelic acid recrystallised from benzene to constant specific activity. A plot of the amount of mandelic acid not degraded against dose (Fig. 2) was extrapolated to zero dose, and the amount of mandelic acid not degraded at low energy inputs ($< 2 \times 10^{23}$ ev) calculated from this curve. The results of the isotope dilution analysis are tabulated.

Products from the irradiation of aqueous mandelic acid solutions *in vacuo*.

(a) Initial mandelic acid 26.32 mmoles (spec. activity 1.751 $\mu\text{C}/\text{mmole}$). Energy input 3.66×10^{22} ev (vol. 4 l.).

Product	Mandelic acid ^a	Ph·CHO	Ph·CO·CO ₂ H	$\alpha\beta$ -Diphenyl-DL-tartaric acid	Ph·CH ₂ ·OH
Carrier (mmoles)	—	2.97	2.61	2.04	4.84
Spec. activity ($\mu\text{C}/\text{mmole}$)	—	9.36×10^{-3}	1.15×10^{-2}	4.08×10^{-3}	6.36×10^{-3}
Yield (mmoles)	24.62	0.256	0.276	0.038	0.282
G Value	—2.8	0.42	0.45	0.13 ^c	0.46

Product	<i>meso</i> Hydrobenzoin	$\alpha\beta$ -Diphenyl-DL-glyceric acid ^b	(\pm)-Benzoin	Ph·CO ₂ H ^b	Ph·CO·CO·Ph
Carrier (mmoles)	2.29	2.89	1.32	7.13	2.21
Spec. activity ($\mu\text{C}/\text{mmole}$)	9.71×10^{-3}	1.12×10^{-3}	4.83×10^{-3}	6.16×10^{-4}	—
Yield (mmoles)	0.102	0.023	0.0290	0.062	—
G value	0.34 ^c	0.04	0.05	0.10	—

^a Calculated from Fig. 2. ^b Spec. activity of initial mandelic acid 1.139 $\mu\text{C}/\text{mmole}$. ^c This value includes DL- and *meso*-forms. CO₂, determined titrimetrically, 1.27 mmoles.

(b) Initial mandelic acid 26.32 mmoles (spec. activity 1.275 $\mu\text{C}/\text{mmole}$). Energy input 7.32×10^{22} ev (vol. 4 l.).

Product	Mandelic acid ^a	Ph·CHO	Ph·CO·CO ₂ H	$\alpha\beta$ -Diphenyl-DL-tartaric acid ^b	Ph·CH ₂ ·OH
Carrier (mmoles)	—	2.97	3.2	3.43	4.84
Spec. activity ($\mu\text{C}/\text{mmole}$)	—	1.24×10^{-2}	1.41×10^{-2}	3.43×10^{-3}	1.01×10^{-2}
Yield (mmoles)	23.14	0.467	0.572	0.0925	0.617

Product	<i>meso</i> Hydrobenzoin	$\alpha\beta$ -Diphenyl-DL-glyceric acid	(\pm)-Benzoin	Ph·CO ₂ H	Ph·CO·CO·Ph
Carrier (mmoles)	3.05	3.63	2.25	8.38	2.33
Spec. activity ($\mu\text{C}/\text{mmole}$)	9.43×10^{-3}	1.84×10^{-3}	4.16×10^{-3}	1.04×10^{-3}	—
Yield (mmoles)	0.181	0.042	0.059	0.109	—

^a Calculated from Fig. 2. ^b Determined on 200 ml. CO₂, determined titrimetrically, 2.36 mmoles.

(c) Initial mandelic acid 26.32 mmoles (spec. activity 0.737 $\mu\text{C}/\text{mmole}$). Energy input 1.05×10^{23} ev (vol. 4 l.).

Product	Mandelic acid ^a	Ph·CHO ^c	Ph·CO·CO ₂ H ^b	$\alpha\beta$ -Diphenyl-DL-tartaric acid ^c	Ph·CH ₂ ·OH ^c
Carrier (mmoles)	—	2.97	2.70	2.01	4.84
Spec. activity ($\mu\text{C}/\text{mmole}$)	—	1.525×10^{-2}	2.03×10^{-2}	7.44×10^{-3}	4.8×10^{-3}
Yield (mmoles)	21.94	0.837	0.766	0.136	0.423

Product	<i>meso</i> Hydrobenzoin ^c	$\alpha\beta$ -Diphenyl-DL-glyceric acid ^b	(\pm)-Benzoin ^b	Ph·CO ₂ H ^c	Ph·CO·CO·Ph
Carrier (mmoles)	2.35	3.85	2.01	8.05	2.09
Spec. activity ($\mu\text{C}/\text{mmole}$)	1.42×10^{-2}	2.62×10^{-3}	6.88×10^{-3}	1.35×10^{-3}	—
Yield (mmoles)	0.304	0.069	0.095	0.198	trace

^a Calculated from Fig. 2. ^b Initial mandelic acid spec. activity 1.166 $\mu\text{C}/\text{mmole}$. ^c Determined on 300 ml. CO₂, determined titrimetrically, 3.64 mmoles.

Determination of Carbon Dioxide.—Carbon dioxide-free nitrogen was bubbled through the irradiated solution at 60°, and the issuing gases bubbled through two absorption vessels containing N-barium hydroxide solution. After 2 hr. the absorption vessels were sealed and the barium carbonate allowed to settle overnight. Portions of the supernatant liquid were then titrated against 0.25N-hydrochloric acid, phenolphthalein being used as indicator.

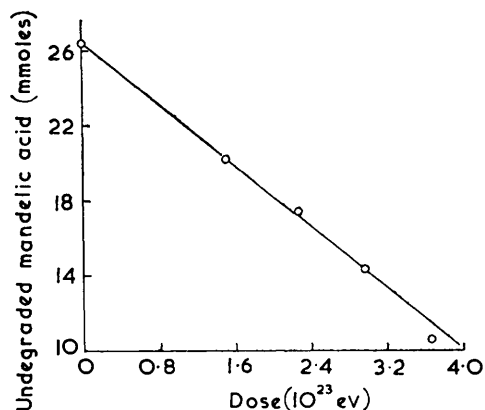


FIG. 2. Degradation of 0.1% DL-mandelic acid solution (vol. 4 l.) *in vacuo*.

Determination of Hydrogen Peroxide.—This was measured essentially by the method described by Eisenberg.¹¹ After energy inputs of 1.0×10^{23} ev the solution contained 1.7×10^{-6} g. of H_2O_2 /ml.

Irradiation of Phenylglyoxylic Acid and $\alpha\beta$ -Diphenyl-DL-Tartaric Acid.—Solutions (4 l.) of these compounds containing 5.33 and 3.31 mmoles, respectively, were irradiated to energy inputs of 1.0×10^{23} ev. The carbon dioxide produced was determined as described above. Approximately 9% and 28% of decarboxylation occurred with phenylglyoxylic acid and $\alpha\beta$ -diphenyl-DL-tartaric acid, respectively, under these conditions.

DISCUSSION

E.s.r. studies⁶ on γ -irradiated polycrystalline DL-mandelic acid have indicated that the radical $\cdot CPh(OH)\cdot CO_2H$ is formed. Studies in related fields have shown a close correlation between the radicals revealed by e.s.r. and the radicals which have been inferred from the isolation of stable end-products in deaerated, aqueous solutions. Chemical changes ensuing from the radiolysis of dilute, aqueous solutions are largely initiated by hydrogen atoms and hydroxyl radicals produced during the primary radiolysis of water. The initial formation of the $\cdot CPh(OH)\cdot CO_2H$ radical may also be inferred from the observed decrease in the optical rotatory power^{4,5} of irradiated solutions of L-mandelic acid (Fig. 1), and from preliminary investigations of the stable end-products of irradiation.⁴ Radicals of the type $\cdot CR(OH)\cdot CO_2H$ are also produced in the radiolysis of solutions of other α -hydroxy-acids.¹⁻³ The principal products resulting from the radiolysis of DL-mandelic acid solutions ($-G$ 2.8) must therefore be accounted for in terms of reaction of the $\cdot CPh(OH)\cdot CO_2H$ radical, as suggested previously by Feng and Tobey.⁴

By analogy with the radiolysis of solutions of other α -hydroxy-acids,¹⁻⁴ the $\cdot CPh(OH)\cdot CO_2H$ radicals might be expected to undergo one or more of the following reactions: (a) dimerisation; (b) disproportionation (or addition) on interaction with another radical; (c) spontaneous decarboxylation to give the $\cdot CH(OH)\cdot Ph$ radical, which would subsequently undergo reactions of type (a) and (b); and (d) degradation involving other reactive species present in solution, such as molecular hydrogen peroxide.

Dimerisation of the $\cdot CPh(OH)\cdot CO_2H$ radicals would yield a mixture of DL- and meso- $\alpha\beta$ -diphenyltartaric acid which, from stereochemical considerations, would be expected to be formed in approximately equal amounts. $\alpha\beta$ -Diphenyl-DL-tartaric acid only was

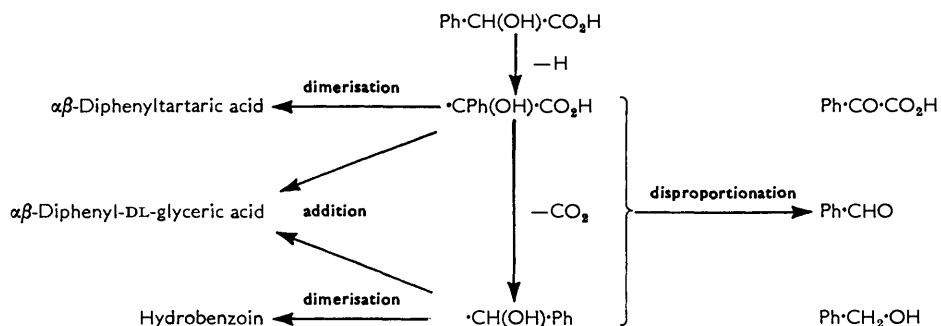
¹¹ Eisenberg, *Ind. Eng. Chem. (Analyt. Edn.)*, 1943, 327.

used as inert carrier in our assay; thus the yield of dimer is twice that shown in the Table. However, dimerisation of this type [$G(\text{dimer}) 0.13$] does not appear to predominate under these conditions. Under comparable conditions,¹ glycollic acid is principally degraded by dimerisation of the initially-formed $\cdot\text{CH}(\text{OH})\cdot\text{CO}_2\text{H}$ radical to yield tartaric acid.

Disproportionation processes of the type $2\cdot\text{CR}(\text{OH})\cdot\text{CO}_2\text{H} \rightarrow \text{R}\cdot\text{CH}(\text{OH})\cdot\text{CO}_2\text{H} + \text{R}\cdot\text{CO}\cdot\text{CO}_2\text{H}$ have been invoked by Johnson, Scholes, and Weiss² to explain the high yield of pyruvic acid formed on X -irradiation of deaerated, aqueous lactic acid solutions. Phenylglyoxylic acid ($G 0.45$) might arise from mandelic acid by a process of this type.

Spontaneous decarboxylation of the initially-formed $\cdot\text{CPh}(\text{OH})\cdot\text{CO}_2\text{H}$ radicals would furnish $\cdot\text{CH}(\text{OH})\cdot\text{Ph}$ radicals, which might either dimerise to yield hydrobenzoin (*DL*- and *meso*-forms) or disproportionate on interaction to yield benzaldehyde and benzyl alcohol. In addition, reactions between $\cdot\text{CPh}(\text{OH})\cdot\text{CO}_2\text{H}$ and $\cdot\text{CH}(\text{OH})\cdot\text{Ph}$ radicals might yield $\alpha\beta$ -diphenyl-*DL*-glyceric acid (by addition), and either phenylglyoxylic acid and benzyl alcohol, or benzaldehyde and *DL*-mandelic acid, depending on the mode of disproportionation. The possibility that benzaldehyde was largely derived by decarboxylation of phenylglyoxylic acid, as suggested⁴ for more concentrated solutions, was discounted on the grounds that only slight decarboxylation was observed on radiolysis of dilute, deaerated phenylglyoxylic acid solutions. Similarly, decarboxylation of $\alpha\beta$ -diphenyl-*DL*-tartaric acid could not account for the observed yields of hydrobenzoin and $\alpha\beta$ -diphenyl-*DL*-glyceric acid.

The initial products of γ -irradiated 1.0% *DL*-mandelic acid solutions are considered to arise by the following mechanisms.



Only benzoic acid ($G 0.1$) and benzoin ($G 0.05$), of the products assayed, are not accounted for in the foregoing reaction scheme, and these probably arise from further degradation of benzaldehyde and hydrobenzoin, respectively.

Hydroxylation of the aromatic ring, to yield phenolic compounds, is usually observed on radiolysis of solutions of compounds possessing an aromatic nucleus.¹² Compounds resulting from hydroxylation of the aromatic nucleus were not assayed in our experiments. However, the excellent agreement between the amounts of mandelic acid degraded and the amounts of the degradation products assayed indicate that such processes do not occur to any appreciable extent in the radiolysis of deaerated mandelic acid solutions

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¹² Stein and Weiss, *J.*, 1949, 3245; Phung and Burton, *Radiation Res.*, 1957, 7, 199; Baxendale and Smithies, *J.*, 1959, 779; Barelko, Kartasheva, Nouikov, and Proskurnin, Proc. 1st All-Union Conf. on Radiation Chem., 1957, p. 89; Rowbottom, *J. Biol. Chem.*, 1955, 212, 877.