89. *Chemical Actions of Ionising Radiations on Aqueous Solutions. Part VIII. Hydroxylation of Benzoic Acid by Free Radicals produced by X-Rays.*

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The hydroxylation of benzoic acid by free hydroxyl radicals produced by *X*-rays has been investigated. All three isomeric hydroxybenzoic acids were formed and their relative proportions have been estimated. The formation of salicylic acid has been investigated quantitatively, and its dependence on pH determined.

In previous papers it was shown that the action of ionising radiations, such as *X*-rays, on dilute aqueous solutions arises from the formation of free radicals (Weiss, *Nature*, 1944, **153**, 748; *Brit. J. Radiol.*, Suppl. 1, 1947; Stein and Weiss, *J.*, 1949, 3245). Reactions due to hydrogen atoms and hydroxyl radicals could be demonstrated and it was shown, in particular in the case of nitrobenzene (*J.*, 1949, 2074; 1950, 2704), that when oxygen-containing solutions of aromatic compounds are irradiated with comparatively small doses of *X*-rays the products are not only qualitatively, but also quantitatively, identical with those formed by the action of the hydrogen peroxide–ferrous salt (Fenton's) reagent, which is well known to produce free radicals. The use of penetrating radiations enables one to work quantitatively and within a pH range wider than is possible with Fenton's reagent. The present investigation is concerned with the action of free hydroxyl (and HO_2) radicals on dilute aqueous solutions of benzoic acid. In earlier, qualitative accounts (Stein and Weiss, *Nature*, 1948, **161**, 650; *J.*, 1949, 3245) we reported the isolation of *o*- and *p*-hydroxybenzoic acids from the products of the reaction in the approximate ratio of 1 : 2·5. The presence of the *m*-isomer could not be established then with certainty.

The action of γ-rays on aqueous solutions of benzoic acid has been investigated by Kailan (*Monatsh.*, 1920, **41**, 312) who isolated formic and oxalic acid, evidently the products of a far-reaching oxidation. The action of hydrogen peroxide on ammonium benzoate has been studied by Dakin and Herter (*J. Biol. Chem.*, 1907, **3**, 419) who have found the formation of approximately equal amounts of *o*-, *m*-, and *p*-hydroxybenzoic acid. Merz and Waters (*J.*, 1949, 2427), using Fenton's reagent, showed the formation of salicylic acid. Dakin considered the hydroxylation of aromatic compounds a typically biological reaction, which he attempted to reproduce *in vitro* by the use of hydrogen peroxide. However, of the metabolic products of benzoic acid, only the products of conjugation, *e.g.*, hippuric and benzoylglucuronic acids, were known until recently (cf. R. T. Williams, "Detoxication Mechanisms," Chapman and Hall, 1947), when Lederer (*J.*, 1949, 2115) identified *o*-, *m*-, and *p*-hydroxybenzoic acids in the urine of pregnant mares in the ratio 7 : 1 : 13. In the somewhat related case of the action of phenyl radicals on ethyl benzoate, Hey (*J.*, 1934, 1967) found that all three isomers are formed, the *p*-isomer predominating.

Our aim was to provide further quantitative experimental evidence on the problem of free-radical substitution in aromatic systems, and accordingly particular attention was paid to establishing which of the isomeric hydroxybenzoic acids was formed and their relative proportions. The mechanism of the reaction involved when ionising radiations act upon dilute aqueous solutions of aromatic compounds has been discussed in detail in previous papers (*loc. cit.*). The method of irradiation was the same as in the case of nitrobenzene (*loc. cit.*). Attempts to use the same analytical technique, *i.e.*, infra-red analysis, failed because in the present case chemical similarity made the quantitative separation of the relatively small amount of the products from the starting material impossible, whilst in the presence of a large excess of benzoic acid the bands of the products were masked in the infra-red spectrogram. Paper chromatography, according to Thorpe's method (Bray, Thorpe, and White, *Biochem. J.*, 1950, **46**, 271; personal communication from Dr. W. V. Thorpe) was therefore used for the separation and semi-quantitative estimation of the isomers. In this way it was shown that all three possible isomers were formed in the reaction, and in experiments carried out at pH $\sim$ 3, *i.e.*, in a solution of benzoic acid in water, the ratio of *o* : *m* : *p* was approximately 5 : 2 : 10.

By using colorimetric methods, the formation of the *o*-isomer, salicylic acid, has been investigated in greater detail. Fig. 1 shows that up to a dose of 5×10^4 E.U. the formation of

salicylic acid, in the presence of oxygen, is a linear function of the dose. Fig. 2 shows the dependence of the yield of salicylic acid on the pH; this curve is very similar to that obtained previously for the formation of phenol from benzene (*loc. cit.*). A solution of sodium benzoate has been used as the substrate in a method of dosimetry of ionising radiations (Day and Stein, *Nature*, 1949, **164**, 671), and Fig. 2 confirms that variations in pH around a mean value of pH ~8 will not affect the yield materially.

The results show that, as in the case of nitrobenzene, in the presence of the *meta*-directing carboxyl group hydroxylation takes place in all three possible positions, whilst in the case of the hydroxylation of phenol, carrying the powerfully *ortho-para*-directing hydroxyl group, the *m*-isomer is apparently not formed at all (to be published). In the present case the preponderance of the *p*- and the *o*- over the *m*-isomer is even more pronounced than for nitrobenzene, and the proportion of the *p*-isomer far exceeds that to be expected statistically. We hope to discuss the implications of our experimental results on completion of this series of papers on the free-radical hydroxylation of aromatic compounds.

Diphenyl was also produced, probably by a decarboxylation, which we have not investigated

FIG. 1.

Dependence of the yield of salicylic acid on the total dose (200 *ml. of* 0·2% *benzoic acid solution*; pH ~3).

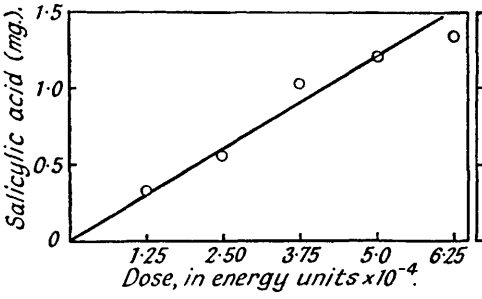
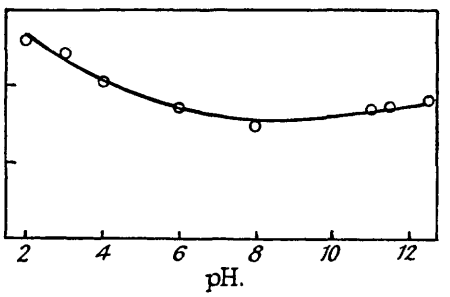


FIG. 2.

Dependence of the yield of salicylic acid on pH (200 *ml. of* 0·2% *benzoic acid solution; constant dose of* 5 × 10^4 E.U.).



in detail. No attempt was made to identify any diphenyldicarboxylic acids that may have been formed in accordance with the reaction scheme which we have proposed previously (*loc. cit.*) for this type of reactions.

EXPERIMENTAL.

Irradiations.—These were carried out as described previously (Farmer, Stein, and Weiss, *J.*, 1949, 3241). A Victor Maximar *X*-ray set was used at 200 kv. and 15 ma., without filtration. The dose, determined according to Day and Stein's method (*loc. cit.*), was 2350 E.U./min., when 200 ml. of the solution were irradiated.

Benzoic Acid.—AnalaR material was used without further purification. Solutions were 0·2% with regard to benzoic acid, and the pH was adjusted by the addition of dilute sulphuric acid or sodium hydroxide as required. Unless otherwise stated, experiments were carried out with the solution of pure benzoic acid without further additions, at a pH ~3.

Isolation of diphenyl. Extraction of the neutralised solution yielded a neutral fraction, from which, after chromatography on alumina, crystals of diphenyl were obtained having m. p. 66° (uncorr.), not depressed on admixture with an authentic specimen.

Determination of salicylic acid. This was carried out in the irradiated solution directly, using Jorissen's reagent, or the ferric chloride method (cf. Snell, "Colorimetric Methods of Analysis," Vol. II) (see Figs. 1 and 2). The two methods gave identical results, when suitable allowances were made for blanks. A Spekker colorimeter was used for the analysis.

Separation and estimation of isomers. Purely chemical and infra-red analytical methods have proved unsuitable. To separate the *o*-isomer alone, the method of Bray, Thorpe, and White (*loc. cit.*) or that due to Evans, Parr, and Evans (*Nature*, 1949, **164**, 674) can be used. It is impossible in this manner to separate the *m*- from the *p*-isomer satisfactorily. For this purpose an improved method due to Thorpe (personal communication) was employed, using as solvent a mixture of butanol (4 vols.), pyridine (8 vols.), saturated aqueous sodium chloride (5 vols.), and aqueous ammonia (*d* 0·880. 3 vols.). The paper chromatogram was run upwards with this mixture on Whatman filter paper No. 1. On development with diazotised *p*-nitroaniline and sodium carbonate solutions, excellent separation of the three isomers was obtained and no other distinct spots were observable on the chromatogram, so that apparently, in agreement with our expectation, dihydroxybenzoic acids were not formed under the experimental conditions chosen. In order to obtain sufficient products for the chromatogram, six solutions (each containing 200 ml. of 0·2% benzoic acid solution at pH 3) were irradiated with a dose of

5×10^4 E.U., and the solutions were united and evaporated, after neutralisation, at a low temperature, in a vacuum, to the smallest possible volume at which precipitation did not take place. This volume was measured accurately, and an aliquot part run as described, in parallel with synthetic mixtures containing various amounts and relative proportions of the isomers. By a process of bracketing, the ratio of *o* : *m* : *p* approximately 5 : 2 : 10 was obtained, from a series of 5×6 irradiations. The yield of salicylic acid in one irradiation of 5×10^4 E.U. was 1·2 mg., that of the *p*- 2·5 mg., and of the *m*-isomer 0·5 mg., approximately. Other experiments were carried out in which, instead of several short irradiations, one solution was given a dose of the order of $\sim 10^6$ E.U. Under these conditions the oxygen available in the solution is insufficient and the greater part of the experiment occurs virtually in its absence. The resulting solution was treated as described above, and yielded a ratio of *o* : *m* : *p* approximately 6 : 4 : 10.

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