

**519.** *Chemical Actions of Ionising Radiations on Aqueous Solutions. Part V. Hydroxylation of Nitrobenzene by Free Radicals produced by X-Rays.*

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Following the quantitative investigation of the hydroxylation of nitrobenzene by free hydroxyl radicals produced by the hydrogen peroxide-ferrous salt reagent (*J.*, 1949, 2074), it is now shown that the action of X-rays on dilute aqueous solutions of nitrobenzene leads to the same products. The ratio of the *o*-, *m*-, and *p*-isomers is essentially the same as in the previous case, thus confirming again the assumption of free-radical formation by ionising radiations. The use of this method enables one to employ more refined analytical techniques and to study the effect of pH on the relative ratio of the various products. It is shown that the position occupied by the nitro-group is also attacked, resulting in a denitration with the formation of nitric acid. The quantitative results are in agreement with a reaction mechanism involving two distinct steps (*J.*, 1949, 3245).

In a previous paper (*J.*, 1949, 2074) we discussed the action of Fenton's hydrogen peroxide-ferrous salt reagent on dilute aqueous solutions of nitrobenzene. This reagent is well known to yield free hydroxyl radicals (Haber and Weiss, *Proc. Roy. Soc., A*, 1934, **147**, 332) and our main

interest was in the quantitative relationship between the three isomeric nitrophenols which can be formed in the attack of these radicals on nitrobenzene. However, this reagent does not lend itself readily to quantitative work, especially when the resulting products are phenolic. The decomposition of hydrogen peroxide makes reproducibility difficult, and the ferric salt formed in the reaction interacts with the phenolic product forming complex products, and in certain cases it is capable of oxidising them further to yield quinonoid products. Moreover, the pH of the solution must be kept within rather narrow limits to prevent the precipitation of the iron salts.

Using Fenton's reagent, we have shown that all three isomers are formed, and the rather crude estimate possible gave their proportions approximately as *o*- 25–30%, *p*- 50–55%, *m*- 20–25% of the total monohydroxy-nitrobenzenes produced. In addition we have demonstrated the formation of 3:3'-dinitrodiphenyl. This indicated the intermediate formation of free nitrophenyl radicals in the reaction. Subsequently, Merz and Waters (*J.*, 1949, 2427) also isolated *o*-nitrophenol in a similar reaction, but not the *m*- or the *p*-isomer.

Recently, we have shown (Stein and Weiss, *Nature*, 1948, 161, 650; *J.*, 1949, 3245) that the action of ionising radiations on dilute aqueous solutions results in formation of free radicals according to :



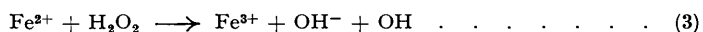
(cf. Weiss, *Nature*, 1944, 153, 748; *Brit. J. Radiol.*, Suppl. 1, 1947), and we have isolated products from the irradiations of various substrates in aqueous systems, which were identical with those obtained previously by the use of the ferrous salt-hydrogen peroxide reagent. The use of the ionising radiations for the production of the free radicals has several important advantages : there is no external reagent introduced or present after completion of the reaction, which can be carried out at any desired pH. We have already shown that this method can be applied to the hydroxylation of aromatic rings (Stein and Weiss, *loc. cit.*; Stein, Ph.D. Thesis, Durham, 1949), as in benzene, benzoic acid, nitrobenzene, and phenol.

The aim of the present work was twofold : first, to ascertain whether the products of the reaction are in fact identical with those obtained by the use of Fenton's reagent, and thus to show whether it is permissible to draw conclusions from the use of this method with regard to free-radical hydroxylations in general; and, secondly, to use this method to obtain more accurate results.

The irradiations were carried out in the manner previously described (Farmer, Stein, and Weiss, *J.*, 1949, 3241), 200 ml. of a saturated solution of nitrobenzene in water being used. It has been shown (Stein and Weiss, *loc. cit.*) that both hydrogen atoms and hydroxyl radicals formed according to reaction (1) are capable of reacting under suitable conditions. It is possible, however, as shown previously, to remove the H atoms to a very large extent, *e.g.* by means of molecular oxygen :



Although the hydrogen atoms themselves tend to reduce the yield of phenols, the HO<sub>2</sub> radicals formed according to reaction (2), on the other hand, increase these yields. Thus irradiation in the presence of oxygen is to some extent equivalent to the use of Fenton's reagent, which also generates both the OH and the HO<sub>2</sub> radicals (Haber and Weiss, *loc. cit.*) :



All our irradiations were carried out with air-saturated solutions, which contain sufficient oxygen to maintain essentially these conditions up to doses of X-rays of the order of  $5 \times 10^4$  E.U. (for the discussion of these considerations see Parts I and II, *J.*, 1949, 3241, 3245); with benzene (Day and Stein, *Nature*, 1949, 164, 671; and Part II, *loc. cit.*) the conditions under which OH and HO<sub>2</sub> are the active radicals could be clearly determined. In the present work we have therefore limited our doses to  $3.5 \times 10^4$  E.U. (except where stated otherwise), *i.e.*, well within the above limit. The yield of phenols in 200 ml. of solution at this dose was of the order of  $10^{-6}$  mol., and to obtain sufficient material for analysis the products from several irradiations (up to ten) had to be united. In addition to maintaining the conditions necessary for the effective removal of the hydrogen atoms, these low doses and low yields of the products ensured that competition between the primary products of the reaction and nitrobenzene for the available radicals should be negligible, and thus in fact the products finally found should correspond to the primary reactions alone. Since the concentration of nitrobenzene in a saturated aqueous solution is approximately  $10^{-2}$ M., this is always likely to be the case.

The analytical procedure employed involved the separation of the *o*-nitrophenol (and the unchanged nitrobenzene) from the other (non-volatile) products of the reaction by steam-distillation *in vacuo* at a temperature of about 40°. The *o*-nitrophenol in the distillate was determined colorimetrically. The residue from the steam-distillation was further separated into neutral and phenolic fractions, and the resulting mixture of *m*- and *p*-nitrophenol analysed by infra-red absorption spectroscopy.

The yield of *o*-nitrophenol (at pH ~6) is shown in Fig. 1 and is seen to be linear with the dose as long as sufficient oxygen is present to react according to equation (2). Fig. 1 should be compared with the similar figures given for benzene (*loc. cit.*).

The results obtained for the ratio of the three isomeric nitrophenols at different pH values are given in Table I. Each of the results is the mean of at least three independent experiments

TABLE I.

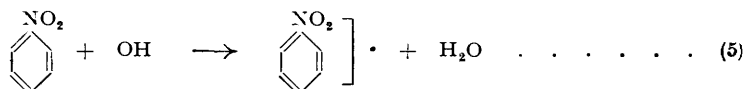
Yield of nitrophenols at different pH values in 200 ml. of saturated solution of nitrobenzene in water. (Dose:  $3.5 \times 10^4$  E.U., given in the presence of air.)

pH.	Total nitrophenols (mg.).	Percentage of total,		
		<i>o</i> .	<i>m</i> .	<i>p</i> .
2	1.23	34.5	31	34.5
6	1.07	35.5	29	35.5
12	0.90	27.5	27	45.5

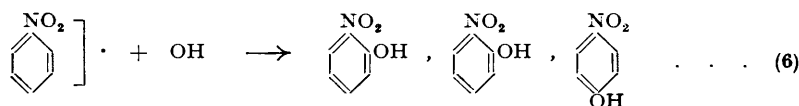
(see Experimental). The values for *o*-nitrophenol were within  $\pm 5\%$ ; those for the *m*- and the *p*-isomer, which were obtained by infra-red analysis, were within  $\pm 10\%$  of the mean values given in Table I. These results show once again that from nitrobenzene all three possible isomers are formed, unlike the case of phenol, where only the *o*- and the *p*-isomer are apparently formed (Stein, Thesis, *loc. cit.*; Stein and Weiss, in preparation). The relative ratios depend on the pH of the solution, as does the total amount of nitrophenols formed. The ratio obtained when using Fenton's reagent agrees well with the more accurate determinations now recorded. One can now claim with some confidence that the use of ionising radiations as a means of investigating the reactions of the free OH (and HO<sub>2</sub>) radicals is justified.

The monohydroxynitrobenzenes thus estimated comprise, however, only 40—50% of the total reaction products. The remainder can be separated into different parts, and it was thus shown that the amount of the neutral fraction, consisting mainly of dinitrodiphenyl, was, in experiments carried out at pH ~6, only slightly less by weight than that of the total nitrophenols; hence, both together account for about 80—85% of the total reaction products. The residue had a phenolic character and consisted largely of phenol, and to a less extent of nitrophenols (probably dinitrodiphenols and dihydroxynitrobenzenes, giving a red colour in alkaline solution). The mode of formation of the phenol will be discussed below.

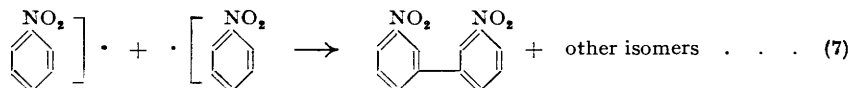
The results once again support the hypothesis of a two-step attack according to:



followed by either



or



Reaction (7), affording dinitrodiphenyl, which has been isolated, presumes the occurrence of reaction (5), since in no other way can even the qualitative results (Loebl, Stein, and Weiss, *loc. cit.*; Merz and Waters, *loc. cit.*) be explained. In addition, it has been shown in the similar case of benzene (*loc. cit.*) that the quantitative relationships obtained are not compatible with any other mechanism. The ratio of the isomers found here, together with our preliminary results on the hydroxylation of phenol, showing the absence of the *m*-isomer, resorcinol, indicate

the operation of a directive effect in these free-radical substitutions. The detailed discussion will be postponed till the completion of our work on some other monosubstituted benzene derivatives.

FIG. 1.

Dependence of the yield of *o*-nitrophenol on the X-ray dose (air-saturated solutions at pH ~ 6).

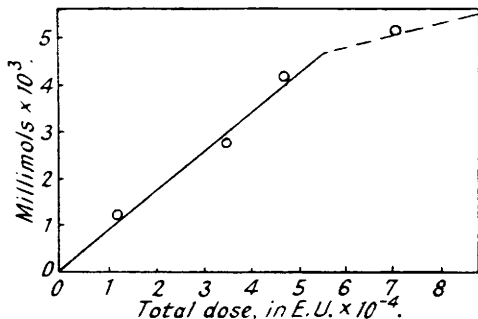
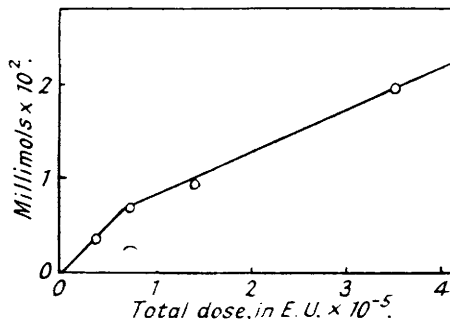
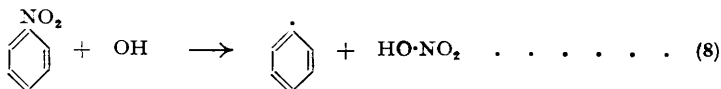


FIG. 2.

Dependence of the yield of nitrate ion on the X-ray dose (air-saturated solutions at pH ~ 6).



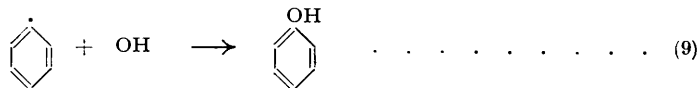
The substitution reactions discussed above accounted for the attack at the 2-, 3-, 4-, 5-, and 6-positions of the nitrobenzene ring. There was, however, reason to suppose that position 1 might also be susceptible to attack by the radicals, for it has been shown (Fichter and Bonhôte, *Helv. Chim. Acta*, 1920, **3**, 395) that in electrolytic oxidations, which presumably involve free hydroxyl radicals as intermediates, the nitro-group attached to an aromatic ring can be removed. We have therefore investigated the possibility of the formation of nitrate in our reaction, *e.g.*, according to :



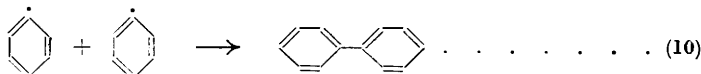
which assumes that the position occupied by the nitro-group is in some way analogous to the other positions. In fact we have been able to demonstrate the formation of nitrate in the irradiated solution. Fig. 2 shows the dependence of the yield of nitrate ion on the dose at pH ~6. The decline of the yield per unit dose after exhaustion of the dissolved oxygen points to the formation of nitrate ion being in fact due to hydroxyl radicals, and not to hydrogen atoms. We have not found any nitrite in our experiments.

Experiments on the influence of pH on the formation of the nitrate ion have yielded identical amounts at pH values of approximately 2, 6, and 12. Since, however, the determination of nitrate in the very small amounts present was open to an error of not less than ±10%, all we can say is that the amount formed is apparently not appreciably influenced by the pH, and probably less so than the ratio of hydroxylated isomers. The amount of nitrate ion formed was about equivalent to one of the phenolic isomers formed at pH 6.

Reaction (8) should result in the formation of free phenyl radicals, which may further react according to :



or



as well as with the formation of products containing the nitro-group in one ring only. Reaction (9) will then be the source of the phenol which we have found.

EXPERIMENTAL.

*Irradiation Arrangements.*—These were identical with those described in Parts I and II (*loc. cit.*). A Victor Maximar set was used at 200 kv. and 15 ma., without filtration. The dose was determined by the method of Day and Stein (*loc. cit.*), and found to be 2350 E.U./min.

*Nitrobenzene Solution.*—Redistilled nitrobenzene was used to saturate distilled water. Further purification of the water does not influence our results. 200-Ml. portions were irradiated at a time. All irradiations were carried out in the presence of air. Acid pH values were maintained with sulphuric acid, alkaline values by sodium hydroxide.

*Separation of the Reaction Mixture.*—Several batches of irradiated solutions were united and acidified, and the excess of nitrobenzene, with all the *o*-nitrophenol, steam-distilled in a vacuum at approx. 40° into dilute alkali. Experiments on synthetic mixtures of the three isomeric nitrophenols showed that it is possible to determine these separately as follows. The *o*-nitrophenol as above, the yellow colour in the alkaline solution being measured directly on a Spekker colorimeter by use of Ilford filter No. 602. In the residue the *p*-nitrophenol can be determined similarly in a buffered solution at pH 7.0, where the *m*-isomer does not give any colour. The *m*-isomer can then be determined by using Folin's reagent, this being the only isomer which reacts with it. It has been impossible, however, to employ this procedure, which gave very satisfactory results on synthetic mixtures, since in irradiated solutions the presence of the dinitrodiphenols and the dihydroxynitrobenzenes interfered with the determination of the *p*-isomer, and the phenol, which does not distil over quantitatively under the conditions described, interfered with the determination of the *m*-isomer. However the *o*-isomer could be determined as described above, by exhaustive steam-distillation, which affords quantitative separation from the isomers (Arnall, *J.*, 1924, **125**, 811). Our experience on the determination of the *o*-nitrophenol in the distillate by measuring the yellow colour developed in alkaline solution has shown that reproducible results are obtained, and the calibration curve, obtained by using synthetic material (recryst., *m. p.* 44°), gave a linear dependence.

*Detection of phenol.* The distillate containing the *o*-nitrophenol gives the Folin test characteristic of phenol but not given by *o*-nitrophenol. On the other hand, the presence of phenol does not interfere with the colorimetric determination of the *o*-nitrophenol as described: only part of the phenol distills over under these conditions. The phenol in the residue does not interfere with the determination of *m*- and *p*-nitrophenols by the infra-red method.

The residue after distillation was extracted exhaustively with ether. The ethereal extract was either evaporated to dryness and weighed, giving the sum of all products except the *o*-nitrophenol, or extracted with alkali and then evaporated, giving the neutral fraction. The original ethereal extract, or in the second case the ethereal extract of the acidified phenol fraction, was then evaporated and submitted to infra-red analysis. Table II shows in greater detail some of the results obtained and the reproducibility of our experiments.

TABLE II.

*Yields of the isomeric nitrophenols in 200 ml. of a saturated aqueous solution of nitrobenzene, in the presence of air. (Dose: approx.  $3.5 \times 10^4$  E.U.)*

pH.	Yield, mg.			% of isomers.			pH.	Yield, mg.			% of isomers.		
	<i>o</i> -	<i>m</i> -	<i>p</i> -	<i>o</i> -	<i>m</i> -	<i>p</i> -		<i>o</i> -	<i>m</i> -	<i>p</i> -	<i>o</i> -	<i>m</i> -	<i>p</i> -
2	0.41	0.36	0.47	33.5	29	37.5	6	0.35	0.28	0.36	35.5	28	36.5
2	0.43	0.40	0.38	35	33.5	31.5	6	0.40	0.34	0.40	35	30	35
2	0.42	0.35	0.42	35.5	29	35.5	12	0.24	0.21	0.44	28	23	49
2	0.42	0.43	0.42	33	34	33	12	0.24	0.28	0.39	27	30	43
6	0.38	0.31	0.38	35.5	29	35.5	12	0.27	0.26	0.38	28	29	43

*Determination of Nitrate.*—The presence of nitrate ion was proved by means of brucine, diphenylbenzidine, and pyrogallol (see Snell, "Colorimetric Methods of Analysis," Vol. I., pp. 635, 638, 641), and we used Haase's brucine reaction (see Snell, *loc. cit.*) for the quantitative determination of nitrate. Calibration curves were obtained by using "AnalaR" potassium nitrate; blank tests were carried out using un-irradiated solutions and synthetic mixtures containing the other products of the reaction. Even this method, however, does not give very consistent results. It involves the destruction of all nitrophenols in the sample with concentrated sulphuric acid and potassium permanganate before addition of the reagent, and we found that, although different experiments carried out on the same day with the same batch of reagents agreed very well among themselves, yet they did not agree with experiments carried out on other days. Table III shows some results obtained by this colorimetric method, those in each vertical column being obtained on the same day. It is clear that the yield of nitrate is but little affected by pH.

TABLE III.

pH.	Extinction (arbitrary units measured on Spekker photometer).				
2	14	9	8	10	
6	14	11	8	9	
12	13	9	8	8	

*Tests for Nitrite.*—To ascertain whether nitrite ion is formed in the reaction, the Griess test (cf. Feigl, "Qualitative Analysis by Spot Tests," 1939, p. 215) and that due to Parker (*Analyst*, 1949, **74**, 112), using naphthylamine hydrochloride in alcohol, have been employed. These were very sensitive with synthetic mixtures containing potassium nitrite, and gave negative results on irradiated solutions.

Infra-red analyses were very kindly carried out by the Physical Chemistry Division, Imperial Chemical Industries Limited, Billingham. Nitromethane was used as solvent, calibration curves being taken with authentic samples of the single components and their mixtures.

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