

An *In situ* Photolysis–E.S.R. Investigation of 5-Bromo- and 5-Nitro-2-furoic Acid

P. Maruthamuthu and S. Steenken*

Max-Planck Institute für Strahlenchemie, Mülheim a.d. Ruhr 1, West Germany

The radicals generated by the u.v. photolysis of 5-bromo- and 5-nitro-2-furoic acid are identified as those generated by the corresponding reactions of hydroxyl radicals in radiation chemical studies.

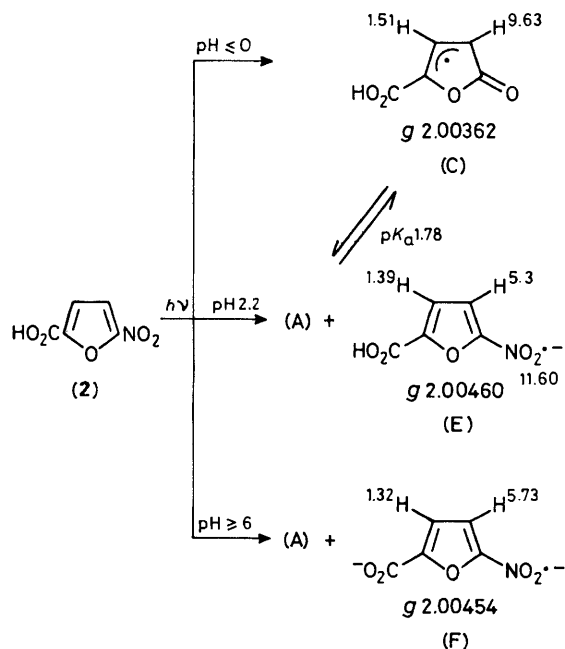
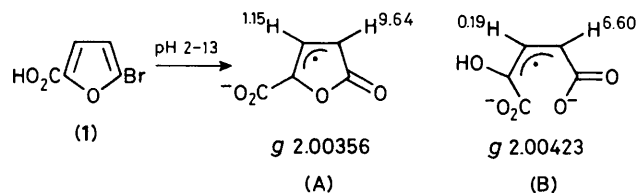
There have been a number of investigations on the mechanism of enhanced radiation and photochemical sensitivity of biological molecules in the presence of sensitizers.^{1,2} In radiation chemical studies, electron transfer to the sensitizer from ion-pairs and radicals present in the cellular target molecule or addition of the sensitizer to the radical generated

by ionising radiation or both have been suggested as the most probable mechanism. However, in photochemical sensitization processes, since the primary action of the light is to affect the sensitizer, it is, in part, essential to know which reactive intermediates are formed from the sensitizer after light absorption. We have therefore undertaken an *in situ* photoly-

sis-e.s.r. investigation of 5-bromo- and 5-nitro-2-furoic acids in aqueous solution containing no other additive. These compounds were chosen because, in many model systems of chemical radiosensitization, nitro-furans proved to be excellent sensitizers, their effectiveness increasing with increasing electron affinity.³⁻⁵

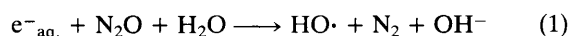
Aqueous solutions containing the substrate (2–4 mM) adjusted to the desired pH by addition of phosphate buffer KOH, or HClO₄ were photolysed in the cavity of an e.s.r. spectrometer and *g* factors and coupling constants of the radicals (accurate to 30 mG and 5×10^{-5} , respectively) were determined as described earlier.⁶ The e.s.r. parameters of all the radicals observed are presented in Scheme 1, together with the assigned structures. On photolysis of 5-bromo-2-furoic acid (1) in the pH range 2–13, the radicals (A) and (B) are produced with no 5-bromo substituent. From the intensity of the e.s.r. signals, the concentrations of (A) and (B) at pH 11.5 are in the ratio 4:1. Radicals (A) and (B) are also produced⁷ in the reaction of the acid (1) with HO·, but in a 1:3 ratio at pH 11.5.⁷ The formation of (A) and (B) may be explained by HO· addition at C-5 followed by two competitive processes (Scheme 2): (a), fast elimination of HBr to form (A), and (b), ring opening and hydrolysis to form (B).

Since these radicals are generated simply by photolysing the parent compounds in the present investigation, the mechanism of formation of (A) and (B) may be similar to that of the HO· reaction *via* photoionisation, or intermolecular electron transfer forming a cation radical may be the primary step as shown in Scheme 3. We suggest that reaction (ii) for the formation of the cation radical is more favourable than (i) because there was no change in the intensity of the signals due

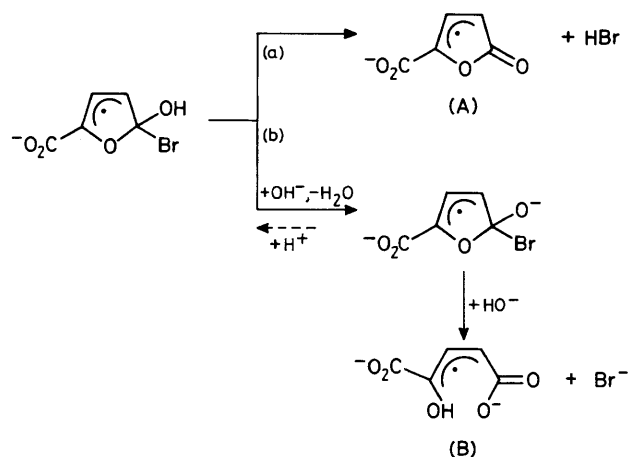


Scheme 1

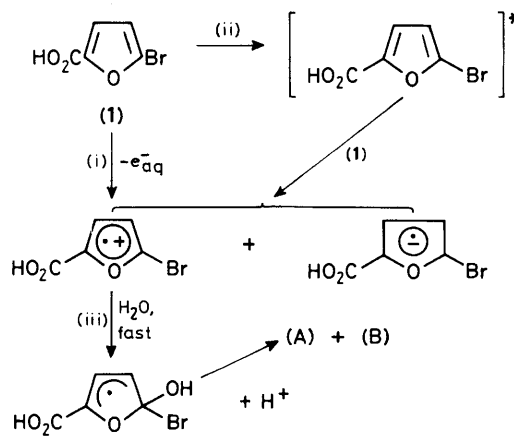
to (A) and (B) when the photolysis was carried out with N₂O-saturated solution. If reaction (i) is operative then the conversion of e⁻_{aq.} in HO· according to reaction (1) should increase the concentrations of (A) and (B) since these radicals are also generated by HO· reaction with the substrate as mentioned earlier. Reaction (iii) in Scheme 3 shows that only the cation radical is responsible for the formation of (A) and (B) and not the anion radical. A plot of [(A)]/[(B)] vs. pH shows a steady increase from 2.1 at pH 4.5 to 5.0 at pH 13.5 implying that reaction (a) in Scheme 2 occurs more efficiently under alkaline conditions. To discover if detectable radical species resulting from the anion radical are formed *in situ* radiolysis-e.s.r. experiments were carried out with argon-saturated solutions containing 1–2 mM of substrate and 0.1 M Bu^tOH or HCO₂Na, under which conditions only e⁻_{aq.} would react with the substrate. E.s.r. signals were not detected in these experiments proving that the anion radical formed disappears by a mechanism which does not contribute the formation of (A) and (B).



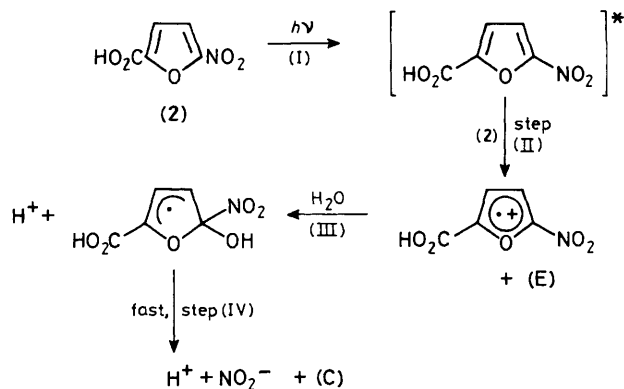
The radicals generated by the photolysis of 5-nitro-2-furoic acid (2) at various pH values are also shown in Scheme 1. At pH ≤ 0, only the oxidised species (C) is observed, possibly because under photolytic conditions at pH ≤ 0, the reduced species (E) undergoes fast proton exchange involving the NO₂⁻ group (pK_a 1.22).⁷ At pH 2.2 the two radicals (A) and



Scheme 2



Scheme 3



(E) are observed in an estimated ratio of 3:1. The former is deprotonated (C) and the latter, the nitro anion radical. The hyperfine coupling constants for 3-H in (C) and (A) are quite different from those for 4-H. A plot of $a(3\text{-H})$ vs. pH is a good pH titration curve from which the pK_a of (C) is estimated to be 1.78. At $\text{pH} \geq 6$, radical (A) is formed in a similar concentration to that at $\text{pH} 2.2$, along with (F), the carboxy group-deprotonated analogue of (E). The pK_a value of (E) is reported⁷ as 3.77. A plot of $[(A)]/[(F)]$ vs. pH shows a steady increase with pH ($[(A)]/[(F)] = 0.4$ and 1 respectively at $\text{pH} 5.7$ and 11).

The formation of the denitrated and nitroanion radicals which are protonated or deprotonated depending upon the pH of the medium and also the observation that there was no change in the intensity of any of these radicals when the experiments were conducted in N_2O -saturated solution strongly suggest that the photolytic reaction occurs via intermolecular electron transfer between the excited and unexcited molecules as shown in Scheme 4.

The occurrence of intermolecular electron transfer between the excited and the unexcited molecule [step (II)] is demonstrated by conducting the photolysis experiments with solutions containing 2 mM of substrate and 5–100 mM of Pr^iOH under neutral and alkaline conditions. The concentration of the denitrated radical (A) decreases by > 50% for a solution containing 0.1 M of Pr^iOH . This accords with the expectation that Pr^iOH also competes for the excited molecule and consequently reduces the concentration of (A). However, the corresponding increase in the concentration of the nitroanion radical (F) is less than half of its original concentration in the blank experiment. The reaction of the cation radical with water to form the hydroxyl radical adduct followed by the elimination of the nitro group should occur in a very fast step as in the case of elimination of Br^- from (1).

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