E.S.R. Studies for Chemical Modification of Biologically Active Nucleic Acids by the Sulphite Radical Anion, SO_3^-

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The addition of the sulphite radical anion, SO_3^-, [generated from the system Ti^{3+}–ethylenediaminetetra-acetic acid–hydrogen peroxide–sodium sulphite in aqueous alkaline solution at pH 9] to uracil derivatives such as 2-thiouracil and 6-methyl-2-thiouracil was observed by use of an e.s.r. method coupled with a rapid-mixing flow technique, indicating that the SO_3^- radical is one of the active agents for the chemical modification of biologically active nucleic acids by the hydrogen sulphite ion.

Since the discovery of 4-thiouridine as a constituent of certain transfer RNAs,[1] chemical reactions of thiouracils have received considerable attention.[2] Hayatsu *et al.* reported that the reaction of 4-thiouridine or 2-thiouracil with the hydrogen sulphite–oxygen system at pH 7 and room temperature gives uridine-4-sulphonate[3,4] or uracil-2-sulphonate,[5] respectively. These reactions proceed *via* intermediates which can be isolated and characterized in certain cases, and their formation involves a reaction between the free radicals generated from oxygen and the hydrogen sulphite ion (one of which is assumed to be the sulphite radical anion, SO_3^-).[4,5]

We have already reported that the SO_3^- radical is formed during the reaction of $NaHSO_3$ with Ce^{4+} in an acidic aqueous solution.[6] A rapid-mixing flow technique coupled with e.s.r. measurements[7,8] enabled us to detect the SO_3^- radical and secondary radicals formed if suitable reactants are present in the solution with the primary radicals (*e.g.* compounds having a C=C,[9] C=S,[10] or C≡C bond[11]). Since then, we have investigated further the reaction of the SO_3^- radical with thiouracils in aqueous alkaline solution by use of the same method: some new intermediate radicals are formed during the reactions and the present paper reports these results.

E.s.r. measurements were carried out on a JEOL-PE-1X e.s.r. spectrometer (X-band) with 100 kHz field modulation in conjunction with a JEOL mixing chamber and T-shaped cell which enabled three reagents to mix almost simultaneously. This apparatus enabled us to detect radicals having life-times of 5—100 ms. The hyperfine coupling constants and *g*-values were calibrated by comparison with an aqueous solution of Fremy's salt ($g = 2.0055$, $a^N = 1.30$ mT)[12] in a capillary tube attached to the sample.

For the generation of the SO_3^- radical in alkaline aqueous solution,[13] commercial samples of $TiCl_3$ (20% v/v, Wako), H_2O_2 (30%, Mitsubishi Gas Chemical), and Na_2SO_3 (Wako) were used without further purification. The conditions for oxidation with and without an added organic compound were the same except that, in the latter case, only two of the three entry ports into the mixing chamber were employed and the third solution referred to below was omitted. Three solutions were prepared: the first solution contained 0.01 M Ti^{3+}, 0.01 M ethylenediaminetetra-acetic acid (EDTA), and 0.1 M Na_2SO_3; the second contained 0.05 M H_2O_2; and the third contained the organic compound (0.1 M). Aqueous ammonia was added to the first and third solutions to give a pH of 9. The thiouracils used in the present study were commercial 2-thiouracil and 6-methyl-2-thiouracil.

When solutions of Ti^{3+} and EDTA, H_2O_2, and Na_2SO_3 were mixed in the cavity of the e.s.r. spectrometer, the pH after mixing being 9, a singlet absorption line was observed at $g = 2.0028$ (Figure 1). The *g*-value of this absorption line is almost identical with the g_{av} for the SO_3^- radical generated by the irradiation of K_2SO_4 single crystals.[14] Therefore, we assigned this radical species as the SO_3^- radical.

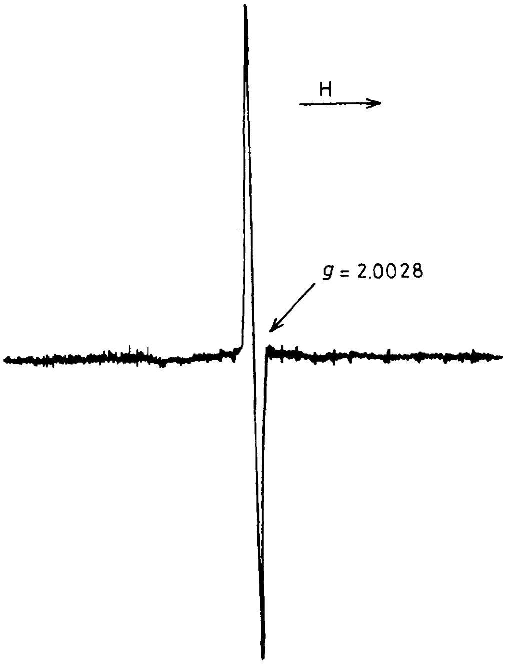

Figure 1. E.s.r. spectrum of the SO_3^- radical generated from the Ti^{3+}–EDTA–H_2O_2–Na_2SO_3 system at pH 9 in an aqueous solution.

Since, when either Ti^{3+} or H_2O_2 was omitted, no e.s.r. spectrum could be observed, the SO_3^- radical may be formed by one-electron oxidation of the sulphite ion by the hydroxyl radical which is generated by the reaction of Ti^{3+} ion with H_2O_2[7] [equations (1)—(3)], taking into consideration the p*K* of HSO_3^- of 7.2.[15]

$$Ti^{3+} + H_2O_2 \rightarrow Ti^{4+} + HO^- + HO\cdot \qquad (1)$$

$$HSO_3^- \rightleftharpoons SO_3^{2-} + H^+ \qquad (2)$$

$$HO\cdot + SO_3^{2-} \rightarrow OH^- + SO_3^{\cdot-} \qquad (3)$$

When 2-thiouracil was added as the third solution and allowed to react with the solution mentioned above, the absorption line due to the SO_3^- radical was reduced considerably and another singlet line appeared at $g = 2.0092$. Similarly, when 6-methyl-2-thiouracil was added to the system, a new e.s.r. absorption line was observed at $g = 2.0093$. These spectra are quite different from those observed

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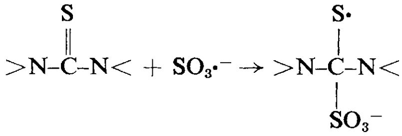
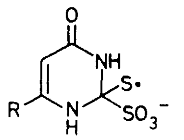
in the reaction of the hydroxyl radical with thiouracils.[16] Further, these spectra were not obtained in the absence of any one of Ti^{3+}, H_2O_2, or sodium sulphite. These results suggest that the SO_3^- radical participates in the formation of new radical species. The *g*-values (2.0092—2.0093) of the newly formed radicals are higher than those of carbon radicals and are near to those of an aromatic thiyl radical or radical anion.[17] Also, no hyperfine structures due to an N nucleus and/or protons were observed. These indications imply that the spectra are not due to carbon radicals, but instead to sulphur radicals which are known to show a fairly large spin–orbit coupling constant.[18]

Since the SO_3^- radical is inactive towards hydrogen abstraction,[9] it seems likely that this radical adds to the carbon site of the C=S bond of thiouracils leading to the formation of a new sulphur radical [equation (4)], in analogy with the addition of the SO_3^- radical to thioureas.[10] Therefore, we assigned the structures (**1**; R = H) and (**1**; R = Me) to the radicals formed during the reaction of the SO_3^- radical with 2-thiouracil and 6-methyl-2-thiouracil, respectively.



$$>\text{N}-\overset{\overset{\displaystyle \text{S}}{\|}}{\text{C}}-\text{N}< + \text{SO}_3^{\cdot -} \rightarrow >\text{N}-\underset{\underset{\displaystyle \text{SO}_3^-}{|}}{\overset{\overset{\displaystyle \text{S}\cdot}{|}}{\text{C}}}-\text{N}< \qquad (4)$$

When 2-thiouracil derivatives were treated with HSO_3^- in the presence of oxygen, uracil-2-sulphonates were obtained.[5] Assuming that the reacting species is the free radical generated from oxygen and HSO_3^- and since it is known that the SO_3^- radical is formed during the autoxidation of HSO_3^-,[19] the SO_3^- radical is assumed to be one of the active agents for the chemical modification of uracils.[5] Therefore, we believe that our findings give direct evidence that the SO_3^- radical is the active species for the chemical modification of biologically active nucleic acids by the hydrogen sulphite ion.



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