Coloured Species formed from the Titanium(IV)–4-(2′-Pyridylazo)resorcinol Reagent in the Spectrophotometric Determination of Trace Amounts of Hydrogen Peroxide

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A study was made of the formation of a ternary complex in a mixture of Ti^{IV}, 4-(2′-pyridylazo)resorcinol (par), and H_2O_2 in aqueous solution at pH 8.6 to determine the usefulness of the Ti–par reagent in the trace analysis of H_2O_2. The absorption spectrum of this complex at pH 8.6 shows a sharp peak at 508 nm with $\varepsilon = 3.6 \times 10^4$ dm^3 mol^{-1} cm^{-1}. The 1 : 1 : 1 composition of the complex, Ti^{IV} : par : H_2O_2, and the conditional stability constant $K' = 6.6 \times 10^{18}$ dm^3 mol^{-1} were determined by spectrophotometry. The ^{1}H n.m.r. spectrum of the Ti^{IV}–par–H_2O_2 solution and elemental analysis of the complex showed par to be co-ordinated to Ti^{IV} as a terdentate ligand without any detectable cleavage, and H_2O_2 as a unidentate ligand. The absorption (λ = 508 nm) of the ternary complex is attributable to the conjugated double bond system of par with a charged quinone structure. The structure of the complex is considered to be stabilized by bridging of the pyridine N atom and the Ti through hydrogen bonding involving the H_2O_2.

During the course of our studies on the formation of complexes in ternary systems such as M^{m+}–Y–H_2O_2 (M^{m+} and Y denote metal ion and chelating reagent respectively) it was found that certain mixtures containing M^{m+} and Y could be employed to advantage as spectrophotometric reagents for the determination of H_2O_2.[1–5] Of such solutions, a mixture of Ti^{IV} and 4-(2′-pyridylazo)resorcinol (denoted as Ti–par) was particularly useful, having high sensitivity and selectivity.[1] The Ti–par reagent was thus applied with success to the determination of trace biological substances in serum using appropriate enzyme combinations to produce H_2O_2 through enzymatic oxidation.[6,7]

A few spectrophotometric methods employing M^{m+}–Y–H_2O_2 systems have been examined for determining trace amounts of H_2O_2 or metal ions.[8–13] However, there is little definitive evidence for the formation of ternary complexes involving M^{m+} , Y, and H_2O_2 and their actual structure in solution.[14] Consequently the present study on the structure of the coloured species formed in the Ti^{IV}–par–H_2O_2 system was undertaken.

Experimental

Materials and Apparatus.—All chemicals used were of analytical reagent grade and used without further purification. The solution of Ti^{IV} was prepared by dissolving titanium tetrachloride (24 cm^3) in 4 mol dm^{-3} HCl (500 cm^3). The solution thus obtained was standardized by titration with ethylenediaminetetra-acetate (edta), followed by dilution with water to give 10 mmol dm^{-3} of Ti^{IV}. The solution of par (10 mmol dm^{-3}) was prepared by dissolving par (0.1075 g; Dojindo Co. Ltd., Kumamoto, Japan) in 0.2 mol dm^{-3} NaOH (5 cm^3) and diluting to 50 cm^3 with water. The solution of H_2O_2 (0.1 mol dm^{-3}) was prepared as follows: 30% H_2O_2 (5.5 cm^3) was diluted with water to 500 cm^3 and standardized by titration with potassium permanganate. The above three solutions were further diluted with water in order to obtain test solutions of the required concentrations for each experiment.

Absorption spectra were measured with a Hitachi double-beam spectrophotometer, model 210, and a 10-mm quartz cell. The pH of the solutions was measured with a Toshiba-Beckman pH meter, model SS-2. Mass spectra were measured with a Hitachi double-focusing mass spectrometer M-80.

Proton n.m.r. measurements were made on a JEOL FT NMR spectrometer, FX 200, at ambient temperature. As an external reference, sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate–D_2O solution was used. The ^{1}H signals of water and H_2O_2 in the sample were eliminated by presaturation, irradiating at the appropriate resonance frequency.

Sample Preparation for Elemental Analysis.—A solid sample for elemental analysis was prepared as follows. The complex was prepared by adding 10 mmol dm^{-3} par and 10 mmol dm^{-3} H_2O_2 solutions (500 cm^3) to the 10 mmol dm^{-3} titanium(IV) solution (500 cm^3). After adjusting the pH of the mixture to *ca.* 9 by the addition of NaOH solution, the solution was allowed to flow through a column (120 x 4 cm) of Dowex 2X8 anion-exchange resin. Sodium chloride solution (0.1 mol dm^{-3}) was used as the eluant, and the reddish coloured eluate was collected and concentrated under reduced pressure. After removal of NaCl using a Sephadex G-15 resin, the eluate was evaporated to dryness and the resulting dark reddish precipitate was recrystallized from water as a powdery solid (Found: C, 32.75; H, 3.00; N, 10.20%).

Results and Discussion

Absorption Spectrum.—The absorption spectrum of free par at pH 8.6 in aqueous solution displays a band with $\lambda_{max.}$ 415 nm [curve (*a*) in Figure 1] and ε_{par} of 2.6×10^4 dm^3 mol^{-1} cm^{-1}. In aqueous solution, par dissociates in three steps (Scheme 1) and pK_1, pK_2, and pK_3 are 2.3, 6.9, and 12.4 respectively.[15] Therefore the absorption band at 415 nm obtained at pH 8.6 is related to the species of HL^- in Scheme 1.

In the presence of Ti^{IV} at pH 8.6 the absorption band at 415 nm showed a bathochromic shift to $\lambda_{max.}$ *ca.* 523 nm due to the formation of a Ti^{IV}–par complex [Figure 1, curve (*b*)]. However, this peak gradually diminished at ambient tempera-

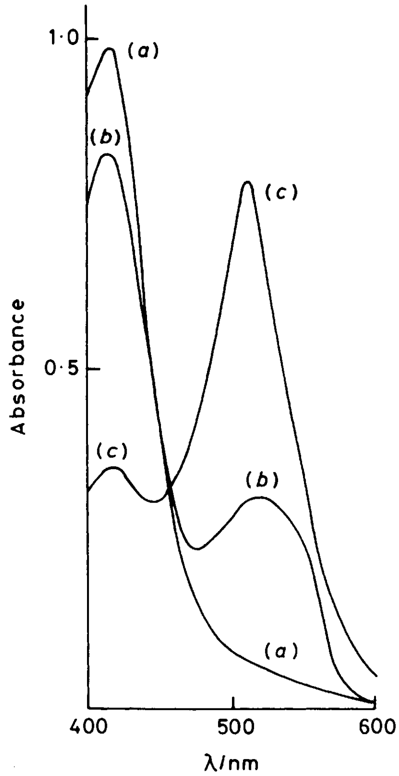
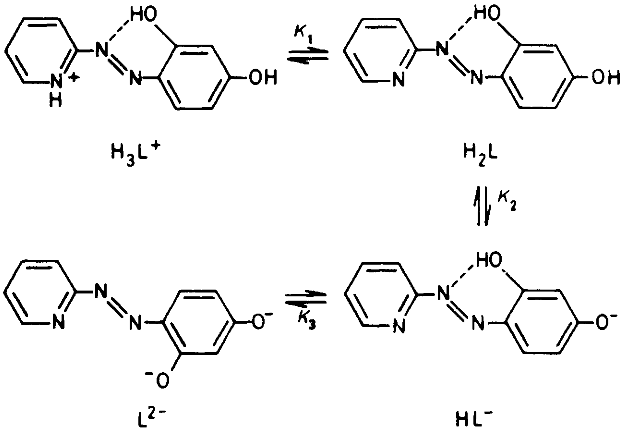


Figure 1. Absorption spectra at pH 8.6 : (*a*) par; (*b*) Ti^{IV}-par; and (*c*) Ti^{IV}-par-H_2O_2. Concentrations: $[Ti^{IV}],[par] = 3 \times 10^{-5}$; $[H_2O_2] = 1.9 \times 10^{-5}$ mol dm^{-3}



Scheme 1. Dissociation steps of par

ture and rapidly above 30 °C. In the latter case, the peak had disappeared completely within a few minutes. At the same time, a peak appeared at 415 nm, indicating that the formation of the stable hydroxo-titanyl polymer {approximate formula: $TiO_2(OH)_4[TiO_2(OH)_2]_n\ Ti(OH)_4$}) may possibly result in the elimination of Ti^{IV} from the Ti^{IV}-par complex.

When H_2O_2 was added to the solution containing par and Ti^{IV} at pH *ca.* 2 and the pH of the solution was raised to 7.6—13 a new sharp peak appeared at $\lambda_{max.}$ 508 nm due to the formation of the Ti^{IV}-par-H_2O_2 complex [Figure 1, curve (*c*)]. Constant absorbance values were obtained within a few minutes, and remained virtually unchanged over a period of 24 h at room temperature. The absorbance at 508 nm was proportional to the concentration of H_2O_2, and the spectra exhibit only one isosbestic point, indicating the formation of a complex with a single composition. The composition of the complex was determined at pH 8.6 by continuous variation and mole-ratio methods. The results indicated the presence of a 1:1:1 Ti^{IV}-par-H_2O_2 complex, in accord with the formula proposed by Li and Cheng.[16]

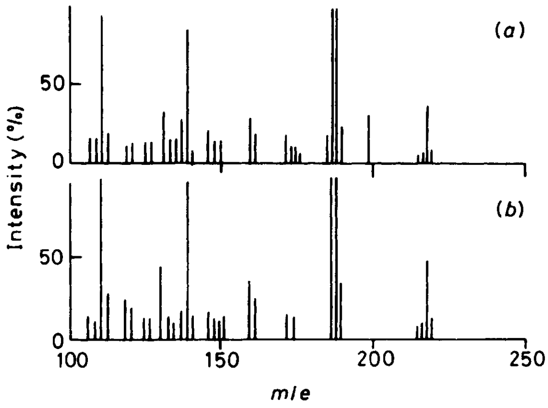


Figure 2. Electron ionization mass spectra: (*a*) par extracted from the Ti^{IV}-par-H_2O_2 complex with chloroform; (*b*) untreated par

Stability Constant.—A conditional stability constant *K*′ for the Ti^{IV}-par-H_2O_2 complex at pH 8.6 was determined for the complex in aqueous solution, where c_{par} is the concentration of

$$Ti^{IV}\text{-}H_2O_2 + par \rightleftharpoons Ti^{IV}\text{-par-}H_2O_2 \qquad (1)$$

$$K' = [Ti^{IV}\text{-par-}H_2O_2]/[Ti^{IV}\text{-}H_2O_2]c_{par}$$

free par. Since the value of *K*′ was large, it was calculated using the ligand substitution reaction between edta and par complex of peroxotitanium(IV) with the aid of the conditional stability constant, $K'_{edta} = 5 \times 10^{18}$ dm^3 mol^{-1} for the Ti^{IV}-edta-H_2O_2 complex.[11] Each species concentration at equilibrium could be calculated from the absorbance of the solution containing Ti^{IV}, par, edta, and H_2O_2. The value for *K*′ was found to be 6.6×10^{18} dm^3 mol^{-1} and indicates that the Ti^{IV}-par-H_2O_2 complex was very stable in aqueous solution at pH 8.6.

Since the Ti^{IV}-par complex decomposed rapidly on raising the pH from *ca.* 2 to 8.6, the stability constant for the Ti^{IV}-par-H_2O_2 complex could not be calculated *via* addition of H_2O_2 to the Ti^{IV}-par complex.

Characteristics of par and Titanium in the Complex.—On the basis of the experimental results described below, par was found to form a complex without undergoing any detectable cleavage. The co-ordinated par was first released from Ti^{IV} by the addition of 0.1 mol dm^{-3} HCl, and then extracted with chloroform. The absence of Ti^{IV} in the chloroform phase was confirmed by atomic absorption analysis. After evaporation of the chloroform and drying under reduced pressure, the dark reddish residue was studied by mass spectrometry. As shown in Figure 2, the molecular ion peak in the electron ionization mass spectrum of par released from the complex was in accord with that of par itself, *m*/*e* 216.

The quadrivalent state of titanium in the complex was confirmed by polarography. The polarogram obtained for a solution containing titanium salt along with par and H_2O_2 at pH 8.6 showed rather complicated features with some cathodic waves, but there were no anodic waves due to the oxidation of Ti^{III}. Confirmation was also obtained from the e.s.r. spectral data for the same solution, but no signal of Ti^{III} was detected.

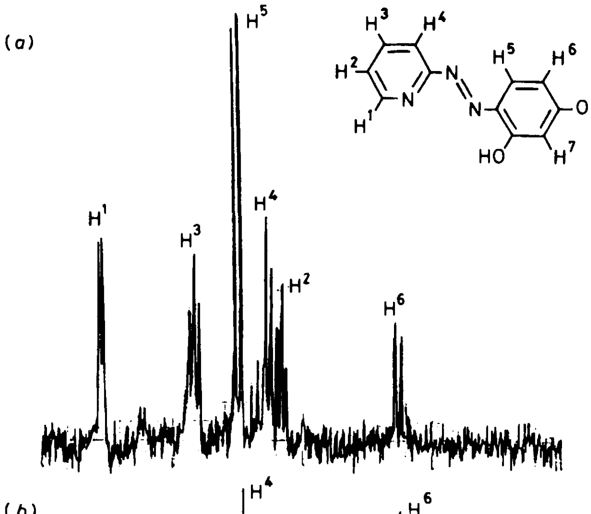
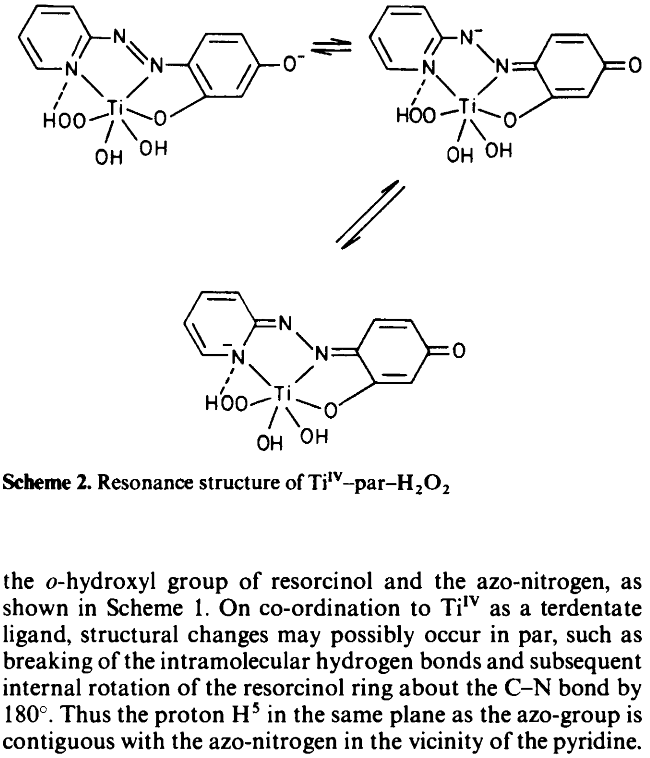


Figure 3. Proton n.m.r. spectra at pD 8: (*a*) Ti^{IV}–par–H_2O_2; (*b*) par; (*c*) Ti^{IV}–par

Table. Proton n.m.r. shifts of par[a]

	pD	H^1	H^2	H^3	H^4	H^5	H^6	H^7
L^{2-} [b]	13	8.36	7.20	7.87	7.65	7.50	6.05	5.78
HL$^-$ [b]	8	8.18	7.09	7.80	7.42	6.96	6.20	5.54
Ti-par-H_2O_2	8	8.54	7.16	7.80	7.25	7.50	6.32	—
Ti–par	8	8.28	7.10	7.88	7.56	7.10	6.30	5.16
Co–par–dien[c]	10	8.14	7.16	7.92	7.48	7.64	6.23	5.78

[a] In D_2O. Chemical shifts in p.p.m. referred to sodium 3-trimethylsilyl[2,2,3,3-$^2H_4$]propionate. [b] par = H_2L. [c] Ref. 17.



Scheme 2. Resonance structure of Ti^{IV}–par–H_2O_2

Proton N.M.R. Spectrum of Co-ordinated par in the Ti^{IV}–par–H_2O_2 *System*.—A comparison of 200-MHz ^1H n.m.r. spectra of par, Ti^{IV}–par, and Ti^{IV}–par–H_2O_2 in solution was made to elucidate the structure of co-ordinated par in the Ti^{IV}–par–H_2O_2 complex. The observed signals are well separated (Figure 3). In the Table are listed the chemical shifts of the protons of free par at pD 8 and 13, and of co-ordinated par in Ti^{IV}–par and in Ti^{IV}–par–H_2O_2 at pD 8 (for numbering of the protons see Figure 3).

A comparison of the spectrum of Ti^{IV}–par–H_2O_2 in solution with that of par in solution at pD 8 revealed the spin–spin coupling in par to remain essentially unchanged upon co-ordination; however, the ^1H n.m.r. spectrum of the Ti^{IV}–par–H_2O_2 solution was characterized by chemical shifts of the H^5 and H^1 protons. The H^5 signal appeared at 6.96 p.p.m. for free par, but at 7.5 p.p.m. for Ti^{IV}–par–H_2O_2. Fujimoto and co-workers[17] have pointed out the intramolecular hydrogen-bonded structure of free par at pD < 11, between the proton of the *o*-hydroxyl group of resorcinol and the azo-nitrogen, as shown in Scheme 1. On co-ordination to Ti^{IV} as a terdentate ligand, structural changes may possibly occur in par, such as breaking of the intramolecular hydrogen bonds and subsequent internal rotation of the resorcinol ring about the C–N bond by 180°. Thus the proton H^5 in the same plane as the azo-group is contiguous with the azo-nitrogen in the vicinity of the pyridine. It should be susceptible to the effects of both magnetic anisotropy due to the π-electron system of the azo-group and the ring current of resorcinol. Such an environment may cause a downfield shift in this signal when the complex is placed in a magnetic field. Similar downfield shifts have been observed for this proton in Co^{III}–par–dien (dien = diethylenetriamine) and Co^{III}–tan [tan = 1-(thiazol-2′-ylazo)-2-naphthol], in which either par or tan is co-ordinated as a terdentate ligand.[17]

To explain the downfield shift of H^1 a careful inspection was made of the molecular model for the Ti^{IV}–par–H_2O_2 complex. It was found that a proton of H_2O_2 co-ordinated to Ti^{IV} was close to the nitrogen of the pyridine ring (as shown in Scheme 2), so that interaction between the nitrogen and this proton facilitated an increase in the electropositivity of H^1 and a carbon adjacent to the nitrogen. The bridging of Ti^{IV} and nitrogen by H_2O_2 to form a five-membered ring may contribute to the stabilization of the Ti^{IV}–par–H_2O_2 complex.

Further consideration of the molecular model of the complex suggests that when OH^-, H_2O, and bulky organic peroxo-compounds occupy the site of H_2O_2, such ring formation becomes impossible because of the smaller molecular size of OH^- and H_2O, and the larger size of organic peroxo-compounds compared to H_2O_2. On the other hand, HCO_3^-, which is very similar to H_2O_2 in molecular size, may bridge Ti^{IV} and the pyridine nitrogen thus stabilizing the coloured Ti^{IV}–par complex. In fact, the absorption spectrum of a solution containing Ti^{IV}, par, and HCO_3^- (5 000-fold excess) showed a peak at 523 nm and the ^1H n.m.r. spectrum showed a downfield shift of H^1 and H^5. These findings support the assumption that the presence of a molecule of suitable size for bridging between par and Ti^{IV} may result in a stable coloured species.

Structure of the Complex.—On the basis of the ^1H n.m.r. data described above it may be concluded that both par and H_2O_2 are co-ordinated to Ti^{IV} as terdentate and unidentate ligands, respectively, the colour of the Ti^{IV}–par–H_2O_2 complex is attributable to the resonance structure of co-ordinated par, and H_2O_2 may contribute to stabilizing the linkage of par and Ti^{IV} through bridging. The most probable structure of the Ti^{IV}–par–H_2O_2 complex is thus as shown in Scheme 2.

This was supported by elemental analysis data (*cf.* Experimental section) on the coloured solid product isolated from the Ti^{IV}–par–H_2O_2 solution. Since the coloured species in the solution was adsorbed on the column of anion-exchange resin (Dowex 2X8) and then eluted by 0.1 mol dm^{-3} NaCl it is negatively charged. Then, $Na[Ti^{IV}(C_{11}H_{10}N_3O_6)]\cdot H_2O$ can be considered as the composition of the isolated coloured solid product. The elemental compositions corresponding to this formula are 35.7% C, 3.3% H, and 11.3% N, but these values are not in fair agreement with those from elemental analysis, 32.75% C, 3.0% H, and 10.2% N. However, the ratio C:H:N of 11:12:3 required by the formula was in accord with that from elemental analysis. Thus the skeleton of par molecule remains essentially unchanged in the complex.

Conclusions

The resonance structure shown in Scheme 2 is proposed as the most probable for the Ti^{IV}–par–H_2O_2 ternary complex formed in a solution containing Ti^{IV}, par, and H_2O_2. The large molar absorption coefficient of the complex arises from the extensively conjugated double bond system of par having a charged quinone structure. The higher value, $\varepsilon = 36\,000$ dm^3 mol^{-1} cm^{-1}, compared with that of the yellow peroxo-complex ($\varepsilon = 700$ dm^3 mol^{-1} cm^{-1}) formed with Ti^{IV} and H_2O_2, renders Ti–par useful as a spectrophotometric reagent for the determination of H_2O_2 with high sensitivity.

In this study the features of the complex were not completely clarified but it is evident that H_2O_2 does not participate in the redox reaction but rather in the co-ordination with Ti^{IV} in the formation of the coloured species. The compatible molecular size of H_2O_2 for bridging par and Ti^{IV} may be the reason for the high stability of the ternary complex and on this basis the selectivity of the Ti–par reagent for H_2O_2 can be explained.

The present investigation provides a basis for further research on M^{m+}–Y–H_2O_2 type systems; the data obtained may lead to the discovery of more effective reagents for use in the trace analysis of H_2O_2.

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