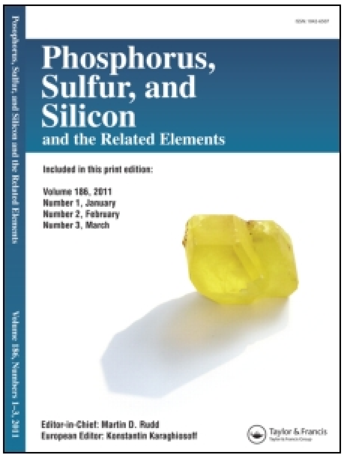

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# STRUCTURAL INFLUENCE ON THE OXIDATION OF THIOL ACIDS BY 2,6-DICHLOROPHENOLINDOPHENOL (A COENZYME Q MODEL)

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# STRUCTURAL INFLUENCE ON THE OXIDATION OF THIOL ACIDS BY 2,6-DICHLOROPHENOLINDOPHENOL (A COENZYME Q MODEL)

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The oxidative behaviour of 2,6-dichlorophenolindophenol towards thiol acids has been described with particular reference to steric isomers of mercaptopropionic acid (MPA). The reactions were studied in presence of hydroxyl ion. The rates of oxidation of 2-MPA and 3-MPA have been compared *vis-a-vis* the influence of [OH$^-$]. The kinetic orders in both cases are two in indophenol and one in thiol acid. Hydroxyl ion retards the rate of oxidation of 2-MPA while accelerating behaviour is shown towards 3-MPA. Mechanisms consistent with the experimental observations have been laid down postulating the formation of an active dimeric intermediate in aqueous medium and adducts of indophenol in non-aqueous medium.

2,6-Dichlorophenolindophenol (hereafter referred to as indophenol and denoted as In) finds useful application in determination of SH group in membranes.[1] Its reactions with thiols were first reported by Basford and Huennekens[2] and later by Hadler *et al.*[3] These studies which were mainly assay-oriented revealed the highly complex nature of these reactions. Mechanistic studies employing indophenol as an oxidant were, perhaps for the first time, made by Leach *et al.*[4] using N-methylacridan (a coenzyme I model) as the substrate. The various analytical aspects of indophenol and related compounds have been reported recently by Svehla and coworkers[5] revealing their involvement in medicine and biochemistry. In a recent publication,[6] the kinetics of the oxidation of ascorbic acid by indophenol, a well known reaction of biochemical importance, has been described. The studies on this oxidant become even more significant when one considers its functional part which is a quinone moiety. In this respect, it resembles coenzyme Q. Thus, systematic kinetic studies on its reactions with various sulfhydryl compounds,[7–12] which play a very prominent role in human metabolism, were taken up in these laboratories in order to get an insight into the complex mechanism of these reactions. The present communication is a part thereof.

## EXPERIMENTAL

### *Reagents*

*A*) The sample of 2-mercapto propionic acid was used as received from Evans Chemetics, New York. Solutions were prepared by diluting the sample and subsequent standardisation with a known iodine solution.[13] Double-distilled water was used throughout these investigations. The solution of 2,6-dichlorophenol-indophenol was prepared by an exact weighing of a B.D.H. grade sample. This was standardised against a known solution of ascorbic acid.[14] The solution of indophenol was stored in a cool dark place because its stability is affected by light and temperature.[15] Other reagents such as sodium hydroxide, electrolytes used to study the effect of ionic strength, etc., were either of B.D.H. AnalaR or E. Merck's G.R. grade.

*B*) The solution of 3-MPA (Evans Chemetics, New York) was prepared by dissolving an exactly weighed quantity in distilled methanol (B.D.H., b.p. 63–64°C). The solution of indophenol was also prepared in methanol. Thiol solutions were stored in nitrogen atmosphere to avoid the atmospheric oxidation.

The oxidation products, i.e. the corresponding disulfides were prepared by oxidising thiol acid with ferric alum.[16] The product was recrystallised from ether. Dihydroindophenol (the leuco-dye) was prepared by bleaching a known volume of the dye solution with $SO_2$ gas.[7]

### *Procedure*

The progress of the reaction was followed by measuring the depletion in indophenol concentration with time. A Klett–Summerson Photoelectric Colorimeter fitted with filter no. 59 spectral range (565–630 nm) was used for this purpose. The choice of this light filter appears appropriate since the dye shows maximum absorbance at 620 and 630 nm in methanol and water respectively ($\varepsilon_{max} = 2.0 \cdot 10^4$ and $2.5 \cdot 10^4$ cm$^2$ mole$^{-1}$).

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Moreover the spectral interference due to reaction products is overruled because the disulphide and the leuco-dye absorb in the uv region of spectrum. These spectra were recorded on a Unicam Spectrophotometer SP-500 using silica cells with a light path of 1 cm.

The reaction vessel was made of pyrex glass coated with Black Japan. The flask was thermostatted with a variation of ±0.1°C. An aliquot of 5 ml was withdrawn and the concentration was computed from optical density measurements.

### *Stoichiometry*

The thiol acids interact with indophenol in a molar ratio of 2:1. Thus,

$$2\text{RSH} + \text{In} \longrightarrow \text{RSSR} + \text{H}_2\text{In}$$

The stoichiometry was confirmed analytically as well as polarographically as described by Kapoor and coworkers.[17]

## RESULTS

### *Part I. Oxidation of 2-Mercaptopropionic Acid*

The reaction between 2-MPA and indophenol follows second-order kinetics in indophenol. The runs were made with ten- to fifteen-fold excess of thiol acid over indophenol keeping other variables fixed. Two typical runs are given in Tables I and II to establish second order in indophenol by Ostwald's isolation method.

Second-order plots were drawn for the runs obtained with excess of thiol (Figure 1). These plots give straight lines confirming second order in indophenol. The graphical values of "$k_2$" agree

TABLE I

[2-MPA] = $5.0 \cdot 10^{-4}$ M; [In] = $5.0 \cdot 10^{-5}$ M; [NaOH] = 0.25 M;
Temp. = 30°C; $\mu$ = 0.25 M

| Time sec. | Optical density | $[\text{In}] \cdot 10^5$ M | $k_1 \cdot 10^4$ sec$^{-1}$ | $k_2 \cdot$ l $\cdot$ mole$^{-1}$ sec$^{-1}$ |
|---|---|---|---|---|
| 00 | 0.736 | 5.00 | — | — |
| 53 | 0.664 | 4.50 | 19.90 | 41.93* |
| 173 | 0.592 | 4.00 | 12.90 | 28.90 |
| 278 | 0.532 | 3.60 | 11.82 | 27.98 |
| 403 | 0.464 | 3.15 | 11.47 | 29.15 |
| 518 | 0.420 | 2.85 | 10.86 | 29.13 |
| 638 | 0.384 | 2.60 | 10.25 | 28.94 |
| 742 | 0.352 | 2.40 | 9.89 | 29.20 |
| 916 | 0.316 | 2.15 | 9.21 | 28.94 |
| 1215 | 0.280 | 1.90 | 7.96 | 26.86 |
| 1487 | 0.236 | 1.60 | 7.66 | 28.58 |

Av. value of $k_2$ (except*) = 28.63 l $\cdot$ mole$^{-1}$ sec$^{-1}$.
Graphical value of $k_2$ = 28.55 l $\cdot$ mole$^{-1}$ sec$^{-1}$.
Standard deviation = 2.04%.

TABLE II

[2-MPA] = $7.5 \cdot 10^{-4}$ M; [In] = $5.0 \cdot 10^{-5}$ M; [NaOH] = 0.25 M;
Temp. = 30°C; $\mu$ = 0.25 M

| Time sec. | Optical density | $[\text{In}] \cdot 10^5$ M | $k_1 \cdot 10^4$ sec$^{-1}$ | $k_2 \cdot$ l $\cdot$ mole$^{-1}$ sec$^{-1}$ |
|---|---|---|---|---|
| 00 | 0.736 | 5.00 | — | — |
| 51 | 0.620 | 4.20 | 21.19 | 74.69* |
| 158 | 0.540 | 3.75 | 19.92 | 46.80 |
| 279 | 0.452 | 3.05 | 17.72 | 45.81 |
| 552 | 0.324 | 2.20 | 14.88 | 46.13 |
| 652 | 0.296 | 2.00 | 14.06 | 46.02 |
| 761 | 0.264 | 1.80 | 13.43 | 46.71 |
| 930 | 0.236 | 1.60 | 12.26 | 45.69 |
| 1220 | 0.208 | 1.40 | 10.43 | 42.15* |

Av. value of $k_2$ (except*) = 46.18 l $\cdot$ mole$^{-1}$ sec$^{-1}$.
Graphical value of $k_2$ = 44.44 l $\cdot$ mole$^{-1}$ sec$^{-1}$.
Standard deviation = 0.46%.

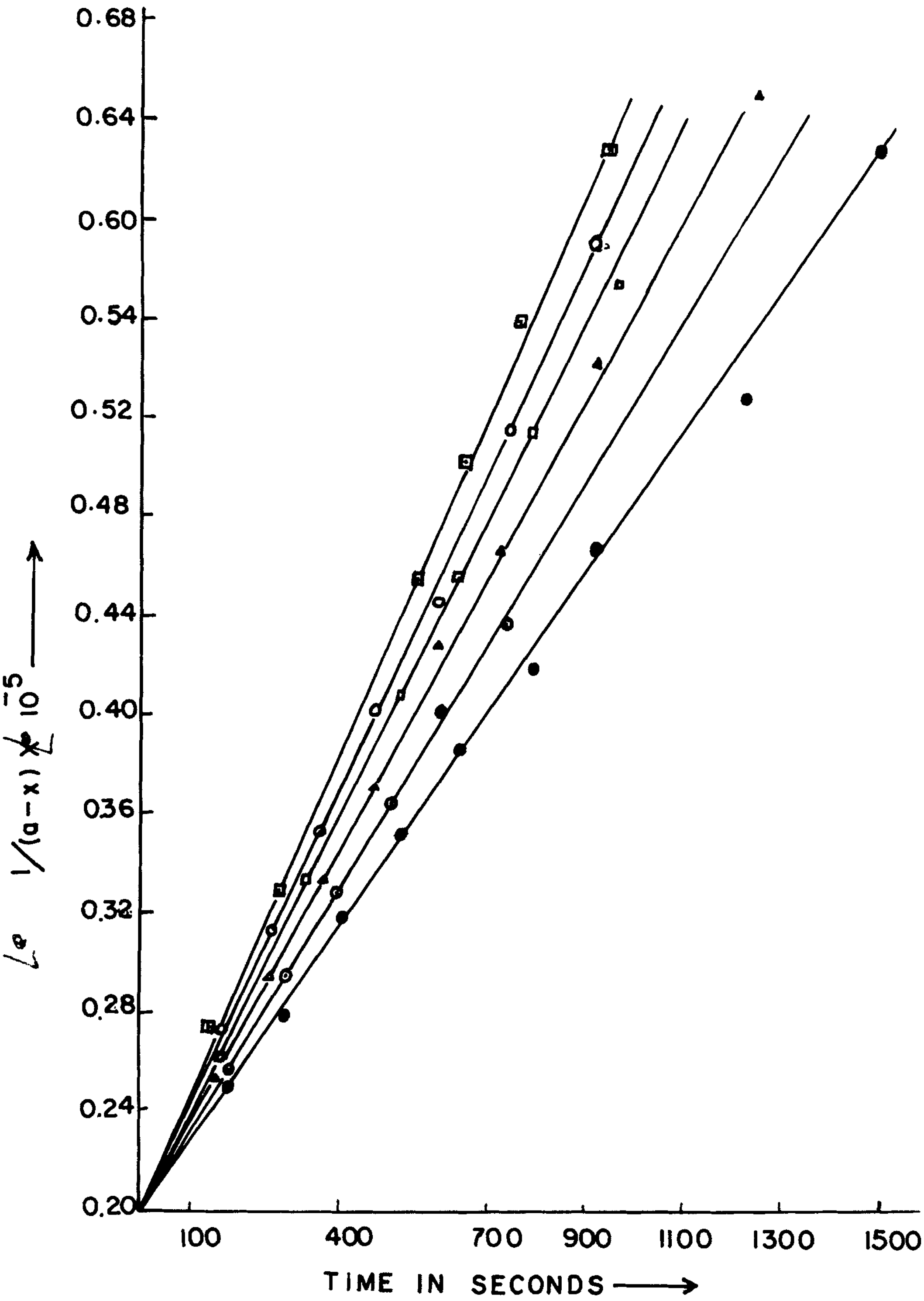


FIGURE 1 [In] = 5.0 × $10^{-5}$ M; [NaOH] = 0.25 M; [RSH] ● = 5.0 × $10^{-4}$ M, ⊙ = 5.5 × $10^{-4}$ M, 4 = 6.0 × $10^{-4}$ M; ☐ = 6.5 × $10^{-4}$ M; O = 7.0 × $10^{-4}$ M; ⊡ = 7.5 × $10^{-4}$ M.

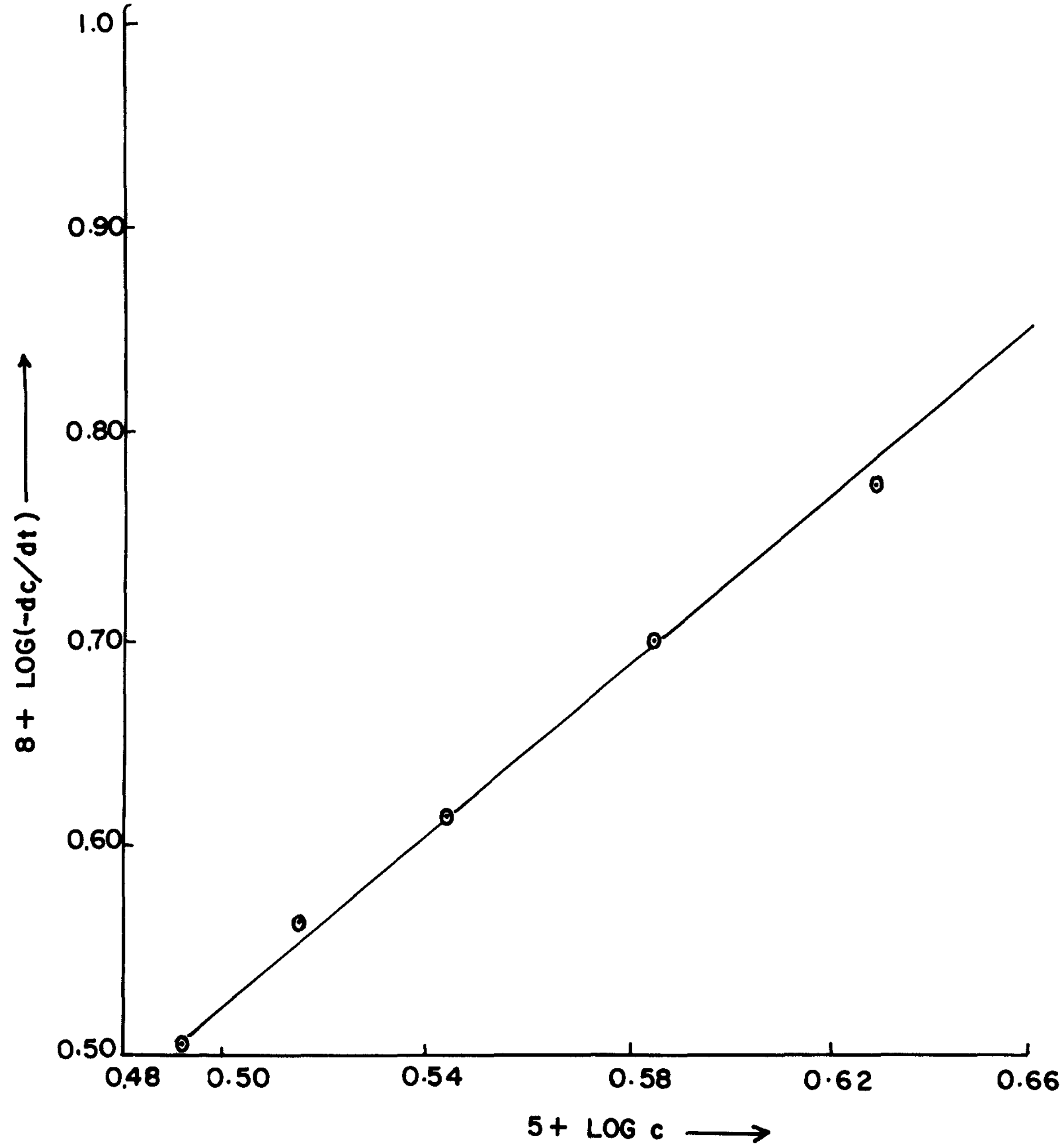


FIGURE 2

fairly well with the calculated values. The order of reaction was further confirmed by the differential method. A plot of (log $-dc/dt$) against "log $c$" for the run with ten-fold excess of thiol gives a straight line with a slope of 2.1 (Figure 2).

The rate constants obtained for the runs depicted in Figure 1 are summarised in Table III.

It is evident from the above Table that the values obtained for the relation "$k_2$/[2-MPA]" are fairly constant and this shows that the order in thiol acid is unity. This was again confirmed by initial rate measurement method. The initial rates were measured by mirror technique at 10% consumption of the dye for the runs given in Table III and the

TABLE III

Dependence of rate on the concentration of 2-MPA

[In] = $5.0 \cdot 10^{-5}$ M; [NaOH] = 0.25 M; Temp. = 30°C; $\mu = 0.25$ M

| [2-MPA] $\cdot 10^4$ M | $\frac{k_2}{1 \cdot \text{mole}^{-1}\,\text{sec}^{-1}}$ | $\frac{k_2}{[\text{2-MPA}] \cdot 10^{-3}}$ |
|---|---|---|
| 5.0 | 28.63 | 57.26 |
| 5.5 | 33.18 | 60.30 |
| 6.0 | 36.48 | 60.80 |
| 6.5 | 39.44 | 60.67 |
| 7.0 | 42.63 | 60.85 |
| 7.5 | 45.61 | 60.81 |

residual concentration of the acid was calculated from the stoichiometric ratio (2:1). The values are given in Table IV.

The order of reaction in thiol acid was calculated using the relation:

$$n = \frac{\log(-\mathrm{d}c/\mathrm{d}t)_2 - \log(-\mathrm{d}c/\mathrm{d}t)_1}{\log C_2 - \log C_1}$$

The average value of "n," i.e. order of reaction in thiol for different pairs of "C" comes out to be 1.2. It may be mentioned here that the variations in thiol concentration could be performed in a limited range only because above a particular concentration the initial part of the reaction becomes very fast and the kinetic studies become difficult. Moreover, in such variations the reproducibility is also found in jeopardy.

The rate is retarded on increasing [OH$^-$] (Table V). No simple quantitative relation was, however, in evidence between the two.

TABLE IV

Initial rate measurement for the oxidation of 2-MPA

$[\text{In}]_0 = 5.0 \cdot 10^{-5}$ M; [NaOH] = 0.25 M; Temp. = 30°C; $\mu = 0.25$ M

| S. no. | [In] $\cdot 10^5$ M | $[\text{2-MPA}]_{\text{residual}} \cdot 10^4$ M | $(-\mathrm{d}c/\mathrm{d}t) \cdot 10^8$ |
|---|---|---|---|
| 1 | 4.5 | 4.9 | 6.282 |
| 2 | 4.5 | 5.4 | 6.950 |
| 3 | 4.5 | 5.9 | 7.750 |
| 4 | 4.5 | 6.4 | 8.772 |
| 5 | 4.5 | 6.9 | 9.173 |
| 6 | 4.5 | 7.4 | 10.480 |

TABLE V

Dependence of rate on sodium hydroxide concentration

[2-MPA] = $5.0 \cdot 10^{-4}$ M; [In] = $5.0 \cdot 10^{-5}$ M; Temp. = 30°C

| [NaOH] M | $\frac{k_2}{1 \cdot \text{mole}^{-1}\,\text{sec}^{-1}}$ |
|---|---|
| 0.10 | 49.69 |
| 0.15 | 37.84 |
| 0.20 | 32.82 |
| 0.25 | 28.63 |
| 0.30 | 26.69 |
| 0.35 | 24.38 |
| 0.40 | 20.74 |

The rate decreases on increasing the initial concentration of indophenol in a linear manner. Table VI shows the variation in second-order rate constant at different [In]. This can be explained *vis-a-vis* the conjugate addition to the quinone ring (discussed later).

The rate is not influenced on increasing the ionic strength of the reaction system as well as on the addition of reaction products. The rate increases on increasing the dielectric constant of the medium (Table VII). These results indicate that the reaction takes place between two dipolar molecules forming a polar product.[18] The dielectric constant was varied by adding different volumes of twice distilled ethanol in the reaction system.

The activation parameters of the reaction were determined by studying the effect of temperature on rate. These studies were made in aqueous as

TABLE VI

Dependence of rate on initial concentration of indophenol

[2-MPA = $5.0 \cdot 10^{-4}$ M; [NaOH] = 0.25 M; Temp. = 30°C $\mu = 0.25$ M

| [In] $\cdot 10^5$ M | $\frac{k_2}{1 \cdot \text{mole}^{-1}\,\text{sec}^{-1}}$ | $(k_2 \cdot [\text{In}]) \cdot 10^3$ |
|---|---|---|
| 5.0 | 28.63 | 1.432 |
| 4.5 | 32.82 | 1.477 |
| 4.0 | 36.17 | 1.446 |
| 3.5 | 42.89 | 1.501 |
| 3.0 | 47.47 | 1.424 |
| 2.5 | 64.59 | 1.614 |

well as in aqueous-ethanol medium. The results are summarised in Table VIII.

TABLE VII

Dependence of rate on dielectric constant of the medium

[2-MPA] = $5.0 \cdot 10^{-4}$ M; [In] = $5.0 \cdot 10^{-5}$ M; [NaOH] = 0.25 M; Temp. = 30°C; $\mu$ = 0.25 M

| Dielectric constant (D) | $k_2$ $\mathrm{l \cdot mole^{-1}\,sec^{-1}}$ |
|---|---|
| 75.25 | 28.58 |
| 75.15 | 26.35 |
| 72.83 | 21.06 |
| 70.95 | 17.66 |
| 68.44 | 13.22 |

## *Part II. Oxidation of 3-Mercaptopropionic Acid*

The order of reaction in indophenol in this case, was also determined by Ostwald's isolation method. For this, a number of runs with 10 to 20 fold excess of 3-MPA were made keeping other constituents unchanged (Figure 3). The run obtained for 20-fold excess of 3-MPA has been described in Table IX. The composition of the solvent [aqueous methanol 99% (v/v)] was maintained constant in these studies.

TABLE VIII

Activation parameters of the oxidation of 2-MPA

[2-MPA] = $5.0 \cdot 10^{-4}$ M; [In] = $5.0 \cdot 10^{-5}$ M; [NaOH] = 0.25 M; $\mu$ = 0.25 M

| Solvent | °T | $k_2$ $\mathrm{l \cdot mole^{-1}\,sec^{-1}}$ | ΔH* $\mathrm{k = cal\ mole^{-1}}$ | ΔS* e.u. | ΔF* $\mathrm{k = cal\ mole^{-1}}$ |
|---|---|---|---|---|---|
| Water | 293 | 16.74 | 8.70 | −24.09 | 15.00 |
| | 298 | 20.90 | | | |
| | 303 | 28.58 | | | |
| | 308 | 34.44 | | | |
| 10% Ethanol | 293 | 10.94 | 9.98 | −20.48 | 16.18 |
| | 298 | 14.70 | | | |
| | 303 | 21.06 | | | |
| | 308 | 25.45 | | | |

TABLE IX

Second-order kinetic analysis of the reaction between 3-MPA and indophenol

[3-MPA] = $1.0 \cdot 10^{-3}$ M; [In] = $5.0 \cdot 10^{-5}$ M; [NaOH] = 0.02 M; $CH_3OH$ = 99% (v/v); Temp. = 25°C; $\mu$ = 0.02 M

| Time in sec. | Optical density | [In] $\cdot 10^5$ M | $k_1 \times 10^5$ $\mathrm{sec^{-1}}$ | $k_2$ $\mathrm{l \cdot mole^{-1}\,sec^{-1}}$ |
|---|---|---|---|---|
| 00 | 0.640 | 5.00 | — | — |
| 50 | 0.600 | 4.65 | 145.10 | 30.10* |
| 140 | 0.545 | 4.10 | 141.80 | 31.35 |
| 250 | 0.460 | 3.50 | 142.80 | 34.28 |
| 338 | 0.425 | 3.20 | 125.30 | 33.28 |
| 450 | 0.365 | 2.80 | 128.90 | 39.92 |
| 570 | 0.350 | 2.60 | 114.80 | 32.38 |
| 742 | 0.315 | 2.30 | 104.80 | 31.64 |
| 920 | 0.280 | 2.00 | 99.73 | 32.60 |
| 1200 | 0.240 | 1.70 | 89.93 | 32.35 |

Average value of $k_2$ (except *) = 32.85 $\mathrm{l \cdot mole^{-1}\,sec^{-1}}$.
Graphical value of $k_2$ = 32.38 $\mathrm{l \cdot mole^{-1}\,sec^{-1}}$.
Standard deviation = 4.67%.

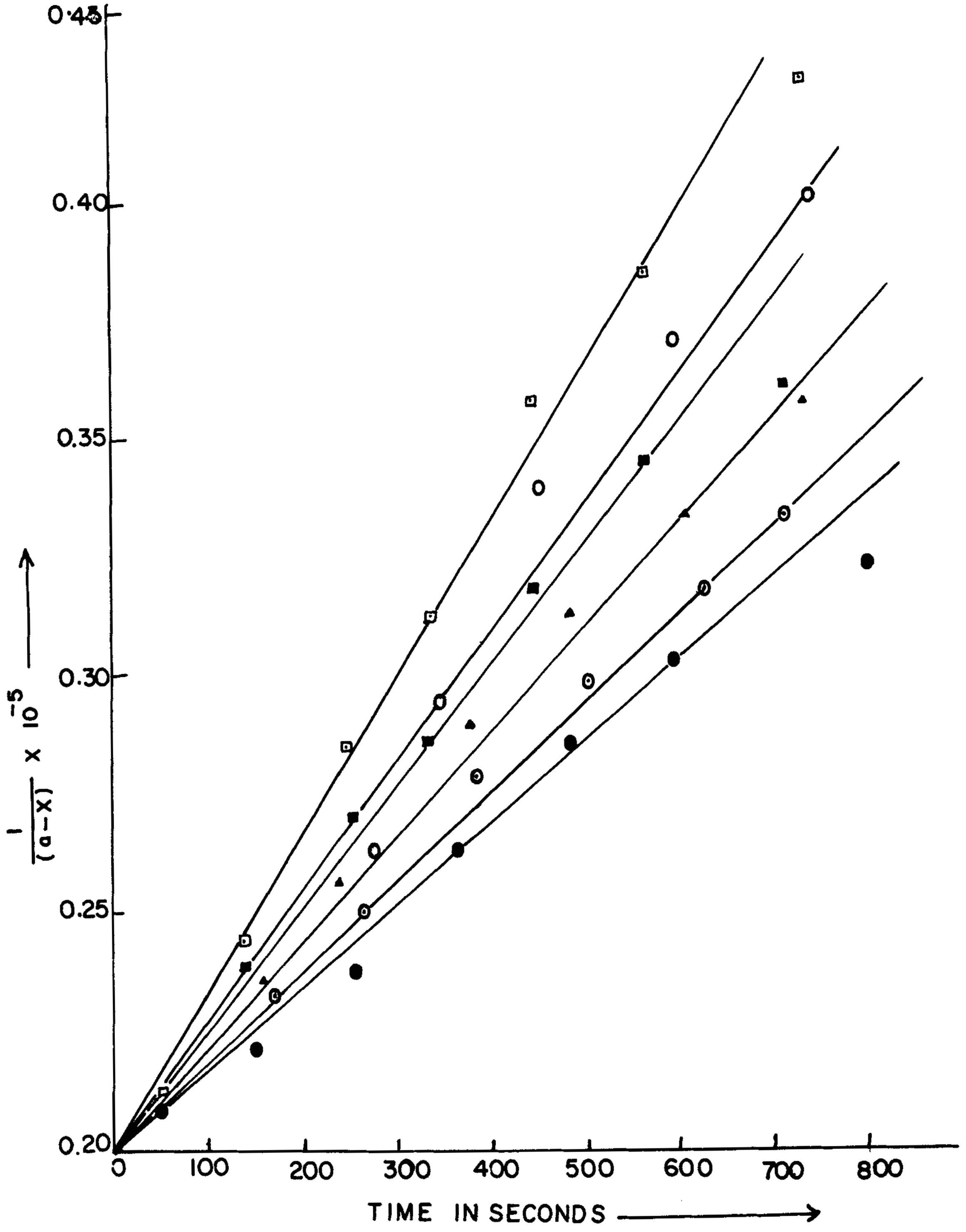


FIGURE 3 [In] = 5.0 × $10^{-5}$ M; [NaOH] = 0.02 M; [MPA] ● = 5.0 × $10^{-4}$ M, ⊙ = 6.0 × $10^{-4}$ M; ▲=7.0 × $10^{-4}$ M, □ = 8.0 × $10^{-4}$ M, O = 9.0 × $10^{-4}$ M, ⊡ = 10.0 × $10^{-4}$ M.

TABLE X

Dependence of rate on the concentration of 3-MPA $[In] = 5.0 \cdot 10^{-5}$ M; [NaOH] = 0.02 M; $CH_3OH$ = 99% (v/v); Temp. = 25°C; $\mu$ = 0.02 M

| $[3\text{-MPA}] \cdot 10^4$ M | $\frac{k_2}{l \cdot mole^{-1} sec^{-1}}$ | $\frac{k_2}{[3\text{-MPA}] \cdot 10^{-3}}$ |
|---|---|---|
| 5.0 | 15.50 | 31.04 |
| 6.0 | 18.53 | 30.88 |
| 7.0 | 21.88 | 31.99 |
| 8.0 | 23.80 | 29.75 |
| 9.0 | 26.64 | 29.61 |
| 10.0 | 32.43 | 32.43 |

TABLE XII

Determination of order of reaction in 3-MPA from initial rate measurement method

$[In]_0 = 5.0 \cdot 10^{-5}$ M; [NaOH] = 0.02 M; $CH_3OH$ = 99% (v/v) Temp. = 25°C; $\mu$ = 0.02 M

| S. no. | $[In] \cdot 10^5$ M | $[RSH]_{residual} \cdot 10^4$ M | $(-dc/dt) \cdot 10^8$ |
|---|---|---|---|
| 1 | 4.5 | 4.9 | 5.291 |
| 2 | 4.5 | 5.9 | 6.037 |
| 3 | 4.5 | 6.9 | 7.202 |
| 4 | 4.5 | 7.9 | 7.615 |
| 5 | 4.5 | 8.9 | 8.664 |
| 6 | 4.5 | 9.9 | 9.658 |

The order of reaction in indophenol was again confirmed by van't Hoff's differential and initial rate measurement methods. The rate constants at different concentrations of thiol and indophenol are given in Tables X and XI. Table X implies that the order of reaction in thiol acid is again unity. The same conclusion is drawn from the initial rate measurement also (Table XII).

As mentioned earlier, the initial rates for the above runs were measured at 10% consumption of the dye. From the stoichiometric ratio, the residual concentration of thiol acid was calculated. In contrast with the observations on the oxidation of 2-MPA, the rate of oxidation in the present case increases on increasing $[OH^-]$ (Table XIII). A linear relation is found to exist between the rate and $[OH^-]$.

The reaction products, again, are not found to influence the rate. A similar behaviour is observed on varying the ionic strength of the reaction system. Bivalent and trivalent cations could not be studied in these variations due to the presence of alkali. The activation parameters were evaluated as described earlier. The values of ΔH*, ΔS* and ΔF* are found to be 5.55 kcal mole$^{-1}$, −35.39 e.u. and 15.59 kcal mole$^{-1}$ respectively.

TABLE XI

Dependence of rate on the initial concentration of indophenol

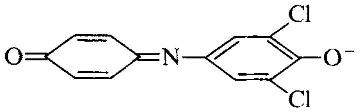
$[3\text{-MPA}] = 5.0 \cdot 10^{-4}$ M; [NaOH] = 0.02 M; $CH_3OH$ = 99% (v/v); Temp. = 25°C; $\mu$ = 0.02 M

| $[In] \cdot 15^5$ M | $\frac{k_2}{l \cdot mole^{-1} sec^{-1}}$ | $\frac{k_2}{k_2[In] \cdot 10^4}$ |
|---|---|---|
| 5.0 | 15.50 | 7.750 |
| 4.5 | 18.57 | 7.447 |
| 4.0 | 18.51 | 7.404 |
| 3.5 | 20.95 | 7.333 |
| 3.0 | 23.35 | 7.004 |

## DISCUSSION

The kinetic orders obtained in the principal reactants suggest common pathways for these reactions but a diversity is indicated by the influence of hydroxyl ion. The results of the oxidation of 2-mercaptopropionic acid are best explained if indophenol dimer is assumed to be the active species. It appears that the solvent and hydroxyl ion influence the formation of this dimer as shown below.

The sodium salt of indophenol will dissociate to give the anion (I):



(I)

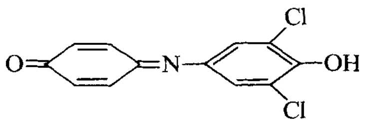
TABLE XIII

Dependence of rate on hydroxyl ion concentration $[3\text{-MPA}] = 5.0 \cdot 10^{-4}$ M; $[In] = 5.0 \cdot 10^{-4}$ M; $CH_3OH$ = 99% (v/v); Temp. = 25°C

| [NaOH]M | $\frac{k_2}{l \cdot mole^{-1} sec^{-1}}$ | $\frac{k_2}{[OH^-] \cdot 10^{-2}}$ |
|---|---|---|
| 0.010 | 7.10 | 7.1 |
| 0.015 | 11.11 | 7.4 |
| 0.020 | 15.50 | 7.7 |
| 0.025 | 18.95 | 7.5 |
| 0.030 | 22.28 | 7.6 |

I (being a salt of weak acid and strong base) will hydrolyse in aqueous medium to produce the phenolic compound (II). Thus,

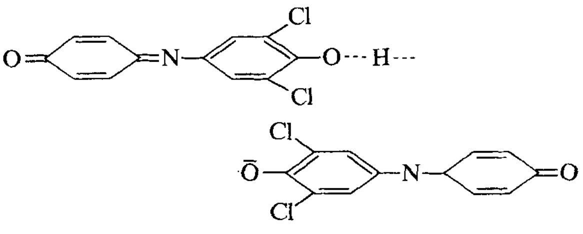
$\mathrm{I} + \mathrm{H_2O} \rightleftharpoons$



$+ \mathrm{OH^-}$ (1)

(II)

There is thus a likelihood that II and I may combine through hydrogen bond forming a dimer (D) as shown below.



(D)

The formation of the dimer (D) and its subsequent participation can be schematically shown in Scheme I.

$$2\mathrm{In} + \mathrm{H_2O} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{D} + \mathrm{OH^-} \quad (2)$$

$$\mathrm{D} + \mathrm{RSH} \xrightarrow{k_2} \mathrm{RSI\dot{n}} + \mathrm{HI\dot{n}} \quad (3)$$

$$\mathrm{RSI\dot{n}} + \mathrm{RSH} \xrightarrow{\text{fast}} \mathrm{RSSR} + \mathrm{HI\dot{n}} \quad (4)$$

$$\mathrm{HI\dot{n}} + \mathrm{RSH} \xrightarrow{\text{fast}} \mathrm{RS\cdot} + \mathrm{H_2In} \quad (5)$$

$$2\mathrm{RS\cdot} \xrightarrow{\text{fast}} \mathrm{RSSR} \quad (6)$$

SCHEME I

(Here RSH denotes 2-MPA).

The formation of half-reduced indophenol (HIṅ) has been suggested earlier by Leach *et al.*[4] while species like "RSIṅ" has been proposed in case of quinones.[19] The participation of free radicals in this case has been qualitatively demonstrated by the positive polymerisation test with vinyl acetate.

The rate of reaction for the above scheme is given as:

$$-\frac{\mathrm{d[In]}}{\mathrm{d}t} = k'_1[\mathrm{In}]^2 - k_{-1}[\mathrm{D}][\mathrm{OH^-}] \quad (7)$$

(where $k'_1 = k_1[\mathrm{H_2O}]$).

On assuming steady state for "D"

$$[\mathrm{D}] = \frac{k'_1[\mathrm{In}]^2}{k_{-1}[\mathrm{OH^-}] + k_2[\mathrm{RSH}]} \quad (8)$$

On substituting the value of [D] in Eq. (7) and assuming $k_{-1}[\mathrm{OH^-}] \gg k_2[\mathrm{RSH}]$,

$$-\frac{\mathrm{d[In]}}{\mathrm{d}t} = \frac{k'_1 k_2[\mathrm{RSH}][\mathrm{In}]^2}{k_{-1}[\mathrm{OH^-}]} \quad (9)$$

Equation (9) demands an inverse linear relation between the rate and $[\mathrm{OH^-}]$ which has not been quantitatively observed. This can be attributed to the resonance stabilization of indophenol anion[20] in presence of hydroxyl ion.

On the other hand in a non-aqueous medium, the formation of dimer (D) does not seem probable. In this case, indophenol molecule is shown to form an adduct with $\mathrm{OH^-}$ ion ($\mathrm{InOH^-}$), a species reported in case of quinones.[21] Thus, Scheme II accounts for the participation of the adduct and its reaction with 3-MPA.

$$\mathrm{In} + \mathrm{OH^-} \overset{K}{\rightleftharpoons} \mathrm{InOH^-} \quad (10)$$

$$\mathrm{In} + \mathrm{RSH} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{RSHIn} \quad (11)$$

$$\mathrm{RSHIn} + \mathrm{InOH^-} \xrightarrow{k_4} \mathrm{C^*} + \mathrm{OH^-} \quad (12)$$

$$\mathrm{C^*} + 3\mathrm{RSH} \xrightarrow{\text{fast}} \underset{\text{(one or more steps)}}{\text{Reaction products}} \quad (13)$$

SCHEME II

(C* stands for "RSIṅ" and "HIṅ" free radicals, while "RSHIn" is reported to be an addition product).[3]

On the basis of the above scheme, the rate of reaction is given by the expression:

$$-\frac{\mathrm{d[In]}}{\mathrm{d}t} = k_3[\mathrm{In}][\mathrm{RSH}] - k_{-3}[\mathrm{RSHIn}] \quad (14)$$

If steady-state is assumed for "RSHIn," then

$$[\mathrm{RSHIn}] = \frac{k_3[\mathrm{In}][\mathrm{RSH}]}{k_{-3} + k_4[\mathrm{InOH^-}]} \quad (15)$$

On substituting $[\mathrm{InOH^-}] = \mathrm{K}[\mathrm{In}][\mathrm{OH^-}]$ and inserting the value of [RSHIn] in Eq. (14):

$$-\frac{\mathrm{d[In]}}{\mathrm{d}t} = \frac{k_3 k_4 \mathrm{K}[\mathrm{RSH}][\mathrm{In}]^2[\mathrm{OH^-}]}{k_3 + k_4 \mathrm{K}[\mathrm{In}][\mathrm{OH^-}]} \quad (16)$$

If $k_{-3} \gg k_4K[In][OH^-]$ which is supported by a large negative entropy, Eq. (16) is simplified to the following form:

$$-\frac{d[In]}{dt} = \frac{k_3k_4K[RSH][In]^2[OH^-]}{k_{-3}} \qquad (17)$$

The simplified rate Eq. (17) is obtained if step (12) in the above scheme is considered as the rate determining step. Then

$$-\frac{d[In]}{dt} = k_4[RSHIn][InOH^-] \qquad (18)$$

On substituting

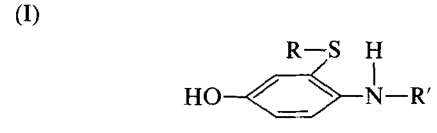
$$[RSHIn] = \frac{k_3}{k_{-3}}[RSH][In] \text{ and } [InOH^-]$$

the above equation can be written as:

$$-\frac{d[In]}{dt} = \frac{k_3k_4K}{k_{-3}}[RSH][In]^2[OH^-] \qquad (19)$$

The arguments given in favour of the two alternatives could be tested by studying the influence of variations in [In] and $[OH^-]$. At sufficiently large concentrations of indophenol and alkali, the reaction should show a first-order behaviour in "In" and the rate should be indifferent to $[OH^-]$ according to first alternative. If second alternative is correct, there should not be a transition in the order of reaction with respect to these species. The exact nature of the rate-determining step(s), however, could not be established because Beer's law does not permit the variations in [In] in the higher concentration range and secondly, on increasing $[OH^-]$ sufficiently, the rate is extremely fast and the runs do not yield reproducible results.

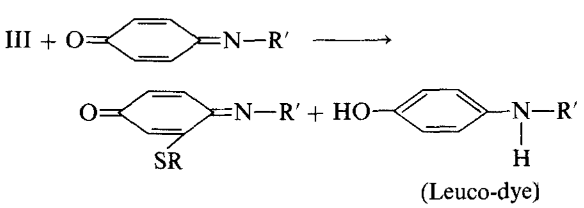
Schemes I and II explain the princiapl kinetic features of the reaction except the retarding influence of the initial concentration of indophenol. This can be explained by considering the addition reactions[2,3] taking place between thiol and indophenol:



(I)

(III)

Species (III) is reported to be a 1,4-addition product and is the precursor of a dye-conjugate (IV).



(Leuco-dye)

(IV)

The dye-conjugate is reported to be relatively less reactive[3] and it seems likely that at higher concentrations of indophenol this side reaction becomes prominent producing a retardation in rate.[7] Further, it appears that due to this side reaction the simple second-order dependence of rate on [In] is obscured (Tables VI and XI). Incidentally, the transition in the order of reaction on varying indophenol concentration is similar to the observations of Laidler and Hoare[22] in the urease-catalysed hydrolysis of urea. In this case, the change in the order in urea from one at lower concentrations to zero (and negative) at higher concentrations has been attributed to the interference of the substrate caused by a change in the binding site.[23] In the light of this and looking to the configuration of the dye-oxidant, the explanation given above seems logical.

The effect of alkali on the rate of oxidation of 3-MPA becomes more important looking to the position of SH group in the molecule which is $\beta$ to carboxyl group. Danehy[24] has reported the over-oxidation of these compounds by iodine through the formation of unstable sulphenic acids (RSOH) and the extent of over-oxidation increases on increasing pH from 3 to 6. Should this happen, a simplified Scheme III would explain the kinetic behaviour of the two thiol acids and indophenol as shown below.

$$\mathrm{In} + \mathrm{RSH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{RSHIn} \qquad (20)$$

$$\mathrm{RSHIn} + \mathrm{In} \xrightarrow{k_2} \mathrm{RSI\dot{n}} + \mathrm{HI\dot{n}} \qquad (21)$$

SCHEME III

The rate of oxidation for the above steps would be given by

$$-\frac{d[In]}{dt} = k_1[In][RSH] - k_{-1}[RSHIn] + k_2[RSHIn][In] \qquad (22)$$

On assuming steady state for "RSHIn" and substituting the value of [RSHIn] in Eq. (22), the rate would be given by

$$-\frac{d[\mathrm{In}]}{dt} = \frac{2k_1k_2[\mathrm{RSH}][\mathrm{In}]^2}{k_{-1} + k_2[\mathrm{In}]} \tag{23}$$

If $k_{-1} \gg k_2[\mathrm{In}]$; then

$$-\frac{d[\mathrm{In}]}{dt} = \frac{2k_1k_2[\mathrm{RSH}][\mathrm{In}]^2}{k_{-1}} \tag{24}$$

The steric influence of thiol acid molecule was further proved by the authors by studying the behaviour of *o*-mercaptobenzoic acid[10] and thiobenzoic acid[9] towards indophenol in methanol medium. While the rate of oxidation of the former was enhanced on increasing [$OH^-$], the latter resembled 2-mercaptopropionic acid. It seems, therefore, that the interaction of alkali with indophenol in non-aqueous medium and its attack on SH group present in $\beta$ position (susceptible to over-oxidation on steric grounds) are responsible for increasing the rate of oxidation.

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