

Studies on Derivatives of Malachite Green. Protolytic Equilibria and Reaction Rate Constants of *o*-Hydroxy Malachite Green

RUNE CIGÉN

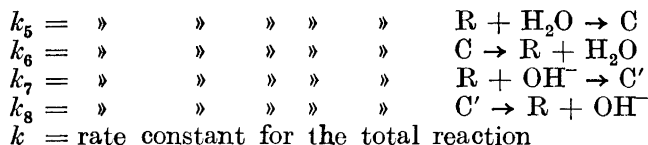
*Department of Inorganic and Physical Chemistry, Chemical Institute, University of Lund,
Lund, Sweden*

In aqueous solutions *o*-hydroxy Malachite Green gives seven species, three coloured (R, B, and G) and four colourless (C', C, S', and S'') between which the following reversible reactions take place; $R + 2H^+ \rightleftharpoons B + H^+ \rightleftharpoons G$; $C' + 3H^+ \rightleftharpoons C + 2H^+ \rightleftharpoons S' + H^+ \rightleftharpoons S''$; $G + H_2O \rightleftharpoons S''$; $B + H_2O \rightleftharpoons S'$; $R + H_2O \rightleftharpoons C$; $R + OH^- \rightleftharpoons C'$. The equilibria between G, B, and R and between C', C, S', and S'' are reached instantaneously whereas the hydration equilibria are reached only after a certain time.

Five dissociation, three hydration, and eight reaction rate constants for the above indicated reactions have been determined by spectrophotometric measurements in aqueous solutions having a constant ionic strength 0.5 M at 20.0°C.

The *ortho*-hydroxy group has a very strong influence on the hydration reactions in the pH-range, 8–10, where the proton of the –OH group is released. The hydration reaction $R + H_2O \rightarrow C$, for example, proceeds 3000 times faster than the corresponding reaction for *para*-hydroxy Malachite Green. The rate constants of the reactions in acid solutions, on the contrary, are 2–4 times smaller for *o*-hydroxy than for *p*-hydroxy Malachite Green.

The reaction scheme which has been proposed and used in the investigations on Malachite Green (MG) and a number of its derivatives¹⁻⁴ can not be used without extension for *o*-hydroxy MG, because the *o*-hydroxy-derivative gives, in alkaline solutions, two additional forms, one coloured and one colourless. The reason for this is that the –OH group reacts like an acid and releases its proton at pH 8–9. An *ortho*-quinoid structure then becomes possible giving an additional coloured form. The corresponding *para*-derivative reacts in the same manner and for this dye the dissociation, hydration and some of the reaction rate constants were determined in Ref.¹. The remaining rate constants will be reported in this paper.



- d) $\varepsilon_{\text{R}}, \varepsilon_{\text{B}}, \varepsilon_{\text{G}}$ = the molar extinction coefficient of R, B, and G.
 e_0, e_t, e_{∞} = the absorbance per cm of a solution at the times 0 and t and at equilibrium (thickness of absorbing layer $d = 1$ cm).
 E_0, E_t, E_{∞} = the absorbance when $d \neq 1$ cm.
 $e_{\text{R}}, e_{\text{B}}, e_{\text{G}}$ = the absorbance per cm of a solution containing only R, B or G.
 C_{M} = the total concentration of dyestuff.
 $h = [\text{H}^+]$; $oh = [\text{OH}^-]$; $K_w = [\text{H}^+][\text{OH}^-]$.

The constants in the equilibrium scheme can be calculated if the following data are determined for a large number of solutions with varying pH: 1) The absorbance, e_0 , at the moment of mixing a dye stock solution with the appropriate buffers. 2) The absorbance, e_{∞} , when equilibrium is reached. 3) The pH of the solutions. 4) The total rate constant, k , for the reactions that change the intensity of the colour of the solutions either through hydration of the coloured forms (R, B, and G) to the colourless (C', C, S', and S'') or *vice versa*.

EQUATIONS

1. The equation for the initial absorbance, e_0

The following equation for e_0 can be derived.

$$e_0 = \frac{K_7 e_{\text{R}} + h e_{\text{B}} + h^2 K_1 e_{\text{G}}}{K_7 + h + h^2 K_1} \quad (1)$$

Eqn. (1) requires that the dyestuff is present only as the coloured forms R, B, and G when it is mixed with the buffer solution. This can be realized in a dye stock solution which is buffered very weakly with an acetate buffer, $\text{pH} \approx 4.5$. In such a stock solution the dye is present to an extent of about 99.5 % as the coloured ion B.

Determination of K_1 . In acid solutions, $\text{pH} < 5$, [R] can be omitted in comparison to [B] + [G]. Eqn. (1) is then approximated and transformed to

$$\frac{h}{e_{\text{B}} - e_0} = \frac{h}{e_{\text{B}} - e_{\text{G}}} + \frac{1}{K_1(e_{\text{B}} - e_{\text{G}})} \quad (2)$$

When h , e_0 and e_{B} have been determined K_1 and e_{G} can be obtained from eqn. (2) by a graphical method.

Determination of K_7 . In alkaline solutions [G] can be omitted in comparison to [B] + [R]. Eqn. (1) is approximated and transformed to

$$\frac{1}{e_{\text{B}} - e_0} = \frac{1}{e_{\text{B}} - e_{\text{R}}} + \frac{h}{K_7(e_{\text{B}} - e_{\text{R}})} \quad (3)$$

If e_B and h are known K_7 and e_R can be determined from eqn. (3) by a graphical method.

2. The equation for the equilibrium absorbance, e_∞

The following equation for e_∞ can be derived

$$e_\infty = \frac{K_7 e_R + h e_B + h^2 K_1 e_G}{K_7 + K_7/K_9 + K_7 K_8/h K_9 + h(1 + 1/K_6) + h^2 K_1(1 + K_2)} \quad (4)$$

Determination of K_2 . In acid solutions $[R]_\infty$, $[C]_\infty$, and $[C']_\infty$ can be omitted in comparison to $[B]_\infty + [G]_\infty + [S']_\infty + [S'']_\infty$. Eqn. (4) can be approximated and transformed to

$$K_2 = \frac{e_G}{e_\infty} + \frac{e_B - e_\infty(1 + hK_1 + 1/K_6)}{e_\infty h K_1} \quad (5)$$

The constant K_2 can be calculated from eqn. (5) when K_1 and K_6 are known. The term $1/K_6$ has the character of a correction term since $K_6 \gg 1$.

Determination of K_6 . The hydration constant K_6 can not be determined solely through equilibrium measurements. These have to be completed with kinetic measurements.

Determination of K_8 and K_9 . In alkaline solutions the only species of the dye to be regarded are B, R, S', C, and C'. In view of this and the fact that $K_6 \gg 1$ eqn. (4) is approximated and transformed to

$$K_9 = \frac{e_\infty(1 + K_8/h)}{e_R + h(e_B - e_\infty)/K_7 - e_\infty} \quad (6)$$

when $h \approx K_7$. From eqn. (6) the hydration constant K_9 and the dissociation constant K_8 are calculated by the following method. Different values of K_8 are tried in eqn. (6) until a constant value of K_9 is obtained in a pH-range about pK_8 .

Determination of K_3 , K_4 , and K_6 . The constants K_3 , K_4 , and K_6 can be determined only by a combination of equilibrium and kinetic measurements.

3. The equation for the total rate constant k

For dyes of this type the proton equilibria between the coloured species R, B, and G as well as between the colourless species C', C, S', and S'' are reached instantaneously. The hydration equilibria between a coloured and a corresponding colourless form as well as the equilibrium $R + OH^- \rightleftharpoons C'$, on the other hand, are reached with a measurable velocity.

The following equation can be derived for the total rate constant, k (cf. Ref.⁵):

$$k = \frac{1}{t} \ln \frac{e_0 - e_\infty}{e_0 - e_t} = \frac{h^3 k_2 + h^2 k_4 K_3 + h k_6 K_3 K_4 + k_8 K_3 K_4 K_8}{h^3 + h^2 K_3 + h K_3 K_4 + K_3 K_4 K_8} + \frac{h^2 k_1 K_1 + h k_3 + k_5 K_7 + k_7 K_7 h}{h^2 K_1 + h + K_7} \quad (7)$$

Determination of k_1 and k_2 . Approximate values of k_1 and k_2 can be obtained by kinetic measurements in strongly acid solutions, $\text{pH} \geq 2$. Eqn. (7) is then approximated to

$$k = k_1 \frac{hK_1}{1 + hK_1} + k_2 \quad (8)$$

From eqn. (8) the rate constants k_1 and k_2 can be calculated when K_1 and K_2 have been determined since $k_1[\text{G}]_\infty = k_2[\text{B}]_\infty$ at equilibrium and therefore $k_1 = k_2K_2$. More refined values of k_1 and k_2 are calculated later from an equation which is not approximated as much as eqn. (8), when at least approximate values of k_3 and k_4 have been determined.

Determination of k_5 , k_6 , k_7 , and k_8 . In strongly alkaline solutions, $\text{pH} > 11$, the following approximations of eqn. (7) proved to be applicable

$$k = k_5 + k_7\text{oh} + k_8 \quad (9)$$

If k is plotted as a function of $[\text{OH}^-]$ a straight line is obtained. The slope of this line gives k_7 and the intercept on the y -axis gives $k_5 + k_8$.

An expression for k_8 can be obtained from the following equations: $k_7\text{oh}[\text{R}]_\infty = k_8[\text{C}']_\infty$, $K_9[\text{C}]_\infty = [\text{R}]_\infty$, $h[\text{C}']_\infty = K_8[\text{C}]_\infty$, and $h \cdot \text{oh} = K_w$ which give

$$k_8 = k_7K_wK_9/K_8 \quad (10)$$

However, $k_8 \ll k_5$ and therefore the intercept mentioned above giving $k_5 + k_8$ in reality gives only k_5 . Then the rate constant k_6 is obtained from the expressions $k_5[\text{R}]_\infty = k_6[\text{C}]_\infty$ and $K_9[\text{C}]_\infty = [\text{R}]_\infty$ which gives $k_6 = k_5K_9$.

The determination of the remaining constants k_3 , k_4 , K_3 , K_4 , and K_6 are performed in a very special way which is described in the "Measurements and Results" part.

EXPERIMENTAL

Chemicals and solutions. *o-Hydroxy Malachite Green.* The leuco compound was prepared in a nitrogen atmosphere by refluxing a mixture of *o*-hydroxy-benzaldehyde (0.1 mole), dimethylaniline (0.3 mole), conc. hydrochloric acid (0.3 mole) and urea (0.1 mole) for 18 h. The leuco compound was recrystallized from benzene-ethanol. White needles were obtained, m.p. 132°C. The dyestuff was prepared by oxidizing 3.6 g of the leuco compound dissolved in 5 ml of 5 M hydrochloric acid and diluted with 500 ml of 10 % acetic acid. For an oxidizing agent 3.0 g of lead dioxide was used. From the oxidized product a perchlorate was prepared that was obtained as small shining green balls of needle-like crystals after repeated recrystallizations from 1 % acetic acid. (Found: C 62.0; H 5.96; O 17.91; N 6.39; Cl 8.09. Calc. for $\text{C}_{23}\text{H}_{25}\text{O}_7\text{N}_2\text{Cl}$: C 62.1; H 5.66; O 17.98; N 6.30; Cl 7.97).

A stock solution of the dye was prepared by weighing and dissolving the perchlorate in acetone which was then diluted with a potassium chloride solution giving a final solution with ionic strength 0.5 M and containing 4 % of acetone. The stock solution, with $C_M = 2 \times 10^{-5}$ M, was buffered very weakly with an acetate buffer, $\text{pH} \approx 4.5$, and was diluted, when necessary, with 0.5 M potassium chloride containing 4 % of acetone. The perchlorate is so slightly soluble in water that it is almost impossible to prepare reproducible stock solutions with a fixed C_M by dissolving the perchlorate directly in 0.5 M potassium chloride.

Hydrochloric acid, sodium acetate, potassium dihydrogen phosphate, disodium hydrogen phosphate, ammonium chloride, ammonia, and sodium hydroxide, all of analytical grade,

were used in the buffer solutions. *Potassium chloride* of analytical grade was used to keep the ionic strength constant (0.5 M).

Apparatus. The determinations of $[H^+]$ were carried out titrimetrically ($pH < 2$ and $pH \geq 12$) and potentiometrically ($2 < pH < 12$). For the potentiometric measurements a reference solution ($pH = 2$) having the composition 0.01 M HCl + 0.49 M KCl was used. In this paper pH always means $-\log[H^+]$ and not $-\log a_{H^+}$. For the electrodes quinhydrone and platinum-plate were used for $pH \leq 8$ and platinum-black and hydrogen gas for $pH > 8$. The temperature was kept at 20.0°C.

For the spectrophotometric measurements a Beckman DU quartz spectrophotometer with photomultiplier was used. The spectrophotometer was furnished with arrangements, used for some measurements in alkaline solutions, for rapid kinetic studies according to the principles for a rapid flow apparatus worked out by Hartridge and Roughton⁶ and modified by Dalziel⁷ for a Beckman DU spectrophotometer. Our arrangement differs from that of Dalziel's in some respects. The two solutions which are to react are fed to the reacting chamber by two synchronously driven 100 ml all-glass syringes. Dalziel used a pressure chamber for this purpose. A carefully selected 8 mm Pyrex-capillary with 2 mm bore is used as an absorption cell. The capillary is placed vertically and a slit, 2 mm high and 0.2 mm wide, is placed close to the capillary in the path of the beam from the spectrophotometer. This slit can be centered, by means of a micrometer screw, before the capillary, thus making the refraction in the curved surfaces of the capillary very small. Further, the optical length of the part of the solution in the capillary through which light is passed can be considered as constant.

MEASUREMENTS AND RESULTS

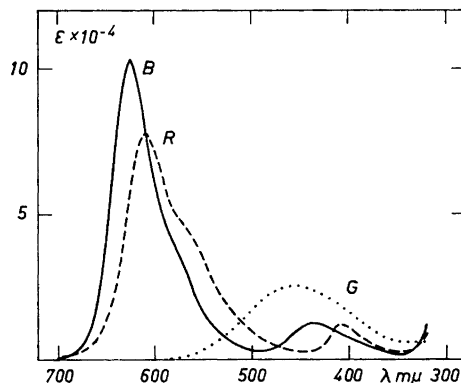
Preliminary measurements showed that for *o*-hydroxy Malachite Green, as in the previously investigated *p*-hydroxy MG, the equilibrium $B + H_2O \rightleftharpoons S'$ is strongly displaced towards the blue-green ion B. By mixing the dye stock solution with a very small amount of an acetate buffer, giving $pH \approx 4.5$, a stock solution is obtained in which the dyestuff is present to an extent of >99.5 % as the coloured form B. If such a stock solution is mixed with appropriate buffer solutions the equilibria $B + H^+ \rightleftharpoons G$ and $B \rightleftharpoons R + H^+$ are reached instantaneously and e_0 in eqn. (1) can be determined. The initial absorbance, e_0 , is obtained through extrapolation of the absorbance to the time $t = 0$ since the solutions fade owing to the formation of the colourless species by hydration of the coloured species.

In Fig. 1 absorption curves of the three coloured species R, B, and G are seen. The curve B was obtained in acetate buffer, $pH = 4.5$: $\lambda_{Bmax} = 624$

Fig. 1. Absorption curves of *o*-hydroxy Malachite Green. Curve R is the molecule $(Me_2NC_6H_4)_2CC_6H_4O$ in ammonia-ammonium chloride buffer. Curve B is the ion $[(MeNC_6H_4)_2CC_6H_4OH]^+$ in acetate buffer. Curve G is the ion

$$\begin{array}{l} \text{MeHNC}_6\text{H}_4 \\ \quad \quad \quad \diagdown \\ \quad \quad \quad \text{C}-\text{C}_6\text{H}_4\text{OH} \\ \quad \quad \quad \diagup \\ \text{MeNC}_6\text{H}_4 \end{array}$$

$^{2+}$ in dilute hydrochloric acid.



$m\mu$ and $436 m\mu$, $\epsilon_{\max} = 1.03 \times 10^5$ and 1.23×10^4 . The curve G was obtained in dilute hydrochloric acid: $\lambda_{G\max} = 455 m\mu$, $\epsilon_{G\max} = 2.6 \times 10^4$. ϵ_G was calculated for 20 different wavelengths from eqn. (5) when K_1 , K_2 , and e_B were determined. The curve R is obtained in an ammonia—ammonium chloride buffer, pH = 10.5, by extrapolation of the absorbance to $t = 0$ at 47 different wavelengths: $\lambda_{R\max} = 609 m\mu$ and $408 m\mu$, $\epsilon_{R\max} = 7.8 \times 10^4$ and 1.2×10^4 .

Determination of K_1 . A series of solutions having the composition x M HCl + $(0.5 - x)$ M KCl were quickly mixed with an equivalent volume of the dye stock solution. The mixing was carried out by injecting the thermostated buffer directly into the thermostated absorption cell in the absorption cell house. The equilibrium $B + H^+ \rightleftharpoons G$ is reached instantaneously during the mixing, where upon the solution fades owing to the reaction $G + H_2O \rightarrow S''$. By determining the fading rate and extrapolating the absorbance to $t = 0$, e_0 was obtained. The measurements were carried out at the wavelength $624 m\mu$, where the absorption curve for the blue-green ion B has a sharp maximum and $\epsilon_G \ll \epsilon_B$. In these acid solutions we need not consider the component R.

In Fig. 2 $[H^+]/(e_B - e_0)$ is plotted against $[H^+]$. According to eqn. (2) a straight line is obtained; the slope, l , and the intercept, y , on the y -axis give $e_B - e_G = 1/l$ and $K_1 = l/y$. From the figure the following values are obtained: $l = 1.003$, $y = 0.0343$ M, which gives ($C_M = 1 \times 10^{-5}$ M, $e_B = 1.015$) $e_G = 0.018$ and $K_1 = 29.2 \pm 0.3$ M $^{-1}$.

Determination of K_7 . The dissociation constant K_7 was determined in the same way as K_1 . Equivalent volumes of a dye stock solution and ammonia—ammonium chloride buffers were mixed. The equilibrium $B \rightleftharpoons R + H^+$ is reached instantaneously where upon the solution quickly fades owing to the reaction $R + H_2O \rightarrow C$. The concentration of the dye was kept very low, $C_M = 2.5 \times 10^{-6}$ M, since there was a risk of a precipitation of the almost insoluble carbinol, C.

The measurements were carried out at the wavelength $624 m\mu$ where the difference between e_B and e_R is sufficient to permit eqn. (3) to be used. According to eqn. (3) a straight line is obtained when $1/(e_B - e_0)$ is plotted as a function

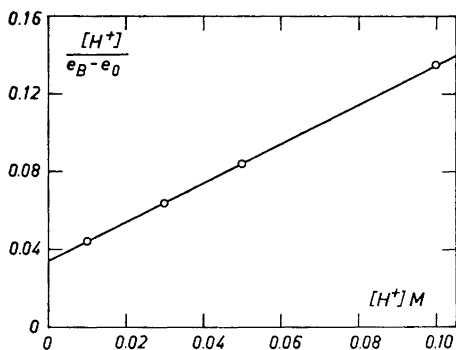


Fig. 2. $[H^+]/(e_B - e_0)$ as a function of $[H^+]$ for the determination of K_1 .

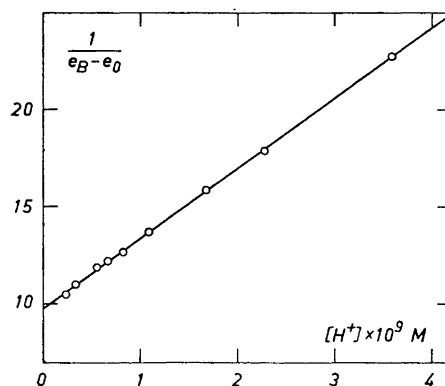


Fig. 3. $1/(e_B - e_0)$ as a function of $[H^+]$ for the determination of $K_7/(K_6 + 1)$.

Table 1. Determination of K_2 and k_1 and e_0 -values for the determination of K_1 . $\lambda = 624$ m μ ; $C_M = 1 \times 10^{-5}$ M; $T = 20.0^\circ\text{C}$; $d = 1$ and 5 cm; $e_B = 1.015$.

[H ⁺] M	e_0	e_∞	K_2	$k \text{ min}^{-1}$	$k_1 \text{ min}^{-1}$
0.010	0.789	0.419	3.92	0.0229	0.0435
0.020		0.266	3.89		
0.030	0.546	0.194	3.92	0.0329	0.0440
0.040		0.1526	3.96		
0.050	0.421	0.1268	3.94	0.0383	0.0444
0.060		0.1092	3.89		
0.080	0.311	0.0846	3.92	0.0443	0.0458
0.100	0.273	0.0688	3.97	0.0465	0.0461
Mean			3.93		0.0448

of [H⁺] (Fig. 3). The solutions fade, however, much more rapidly than the solutions used for the determination of K_1 . Therefore, the extrapolated e_0 -values (Table 2) are not so reliable. The difference between e_B and e_0 is rather small which also increases the uncertainty. From Fig. 3 the following values are obtained ($C_M = 2.5 \times 10^{-6}$ M, $e_B = 0.250$): $y = 9.80$, $l = 3.60 \times 10^9 \text{ M}^{-1}$ which gives $e_R = 0.148$, $\varepsilon_R = 5.92 \times 10^4$, and $K_7 = (2.72 \pm 0.15) \times 10^{-9}$ M.

Determination of K_2 . The dye stock solution was mixed with appropriate HCl + KCl-buffers. The solutions were thermostated and the equilibrium absorbancies, E_∞ , were read off after 6 h. $C_M = 1 \times 10^{-5}$ M, $d = 1$ and 5 cm. The hydration constant K_2 was calculated from eqn. (5) ($e_B = 1.015$, $e_G = 0.018$). The results are found in Table 1 from which the mean value $K_2 = 3.93 \pm 0.04$ is obtained.

Table 2. e_∞ - and e_0 -values for the determination of K_7 and K_9 ; $T = 20.0^\circ\text{C}$.

$\lambda = 624 \text{ m}\mu$	$C_M = 2.5 \times 10^{-6} \text{ M}$ $d = 1 \text{ cm}$	$C_M = 5 \times 10^{-6} \text{ M}$ $d = 5 \text{ cm}$	$\lambda = 625 \text{ m}\mu$ $e_R = 0.275$
[H ⁺] $\times 10^9 \text{ M}$	e_0	$E_\infty = 5 e_\infty$	K_9
3.60	0.206	0.470	0.154
2.28	0.194	0.338	0.152
1.68	0.187	0.275	0.152
1.09	0.177	0.205	0.154
0.824	0.171	0.168	0.154
0.668	0.168	0.144	0.153
0.551	0.166	0.127	0.156
0.332	0.159	0.085	0.153
0.242	0.154	0.065	0.151
Mean			0.153

Determination of K_8 and K_9 . The dye stock solution was mixed with ammonia-ammonium chloride buffers and the equilibrium absorbancies, E_∞ , were read off after 10–20 min. $C_M = 5 \times 10^{-6}$ M, $d = 5$ cm, $\lambda = 624$ m μ , $e_B = 0.496$, $e_R = 0.274$. The constant K_9 was calculated from eqn. (6) by trying different values for K_8 . A reasonable constancy for K_9 is obtained within the rather broad pH-range 8.4–9.6 if the value $K_8 = 6.1 \times 10^{-10}$ M is chosen. The results are found in Table 2. As a mean value $K_9 = 0.153 \pm 0.005$ was obtained when $K_8 = (6.1 \pm 0.2) \times 10^{-10}$ M.

Note. Borate buffers cannot be used for the determination of K_7 , K_8 , and K_9 since borate ions react with *o*-hydroxy MG giving complex species. This complex formation has been investigated and the results will be published. The dye also seems to react with ammonia when the concentration of ammonia is high. Therefore $[\text{NH}_3]$ was kept ≤ 0.05 M in the ammonia-ammonium chloride buffers.

K i n e t i c m e a s u r e m e n t s

Determination of k_1 and k_2 . The total fading rate, k , was determined in the measurements from which e_0 for the determination of K_1 was obtained. From eqn. (8) an approximate value of k_1 was initially calculated. A refined value for k_1 was obtained from eqn. (7) when k_3 and k_4 were approximately determined. Table 1 contains the k -values and the refined k_1 -values. The mean value $k_1 = 0.0448 \pm 0.0010$ min $^{-1}$ was obtained. Then k_2 is obtained from the expression $k_1 = k_2 K_2$ which gives $k_2 = 0.0114 \pm 0.0003$ min $^{-1}$.

Determination of k_5 , k_6 , k_7 and k_8 . The kinetic measurements were carried out in strongly alkaline solutions. The dye stock solution was mixed with solutions of the type x M NaOH + $(0.5 - x)$ M KCl where $x = 0.025, 0.100, 0.150, 0.200,$ and 0.250 . In Fig. 4 k is plotted as a function of $[\text{OH}^-]$. According to eqn. (9) a straight line is obtained with the slope, $l = 0.967$ min $^{-1}$ M $^{-1}$ and the intercept on the y -axis, $y = 0.735$ min $^{-1}$. This gives $k_7 = 0.97 \pm 0.02$ min $^{-1}$ M $^{-1}$ and $k_5 + k_8 = 0.735$ min $^{-1}$. From eqn. (10) $k_8 = (3.0 \pm 0.2) \times 10^{-6}$ min $^{-1}$ is obtained ($K_w = 1.24 \times 10^{-14}$ M 2). Thus $k_8 \ll k_5$, and therefore Fig. 4 gives $k_5 = 0.735 \pm 0.010$ min $^{-1}$. The rate constant k_6 can be calculated from the expression $k_6 = k_5 K_9$ which gives $k_6 = 0.113 \pm 0.05$ min $^{-1}$.

The remaining constants k_3 , k_4 , K_3 , K_4 , and K_6 were determined by a large number of kinetic measurements in the pH-range 3.9–11. It has been possible to determine the constants from the experimentally obtained connection between k and $[\text{H}^+]$ by the following approximation procedure. In the pH-range 4–6 eqn. (7) can be approximated and written as

$$k - A = \frac{hk_4 K_3}{h^2 + hK_3 + K_3 K_4} + k_3 \quad (11)$$

where

$$A = \frac{h^2 k_2 + k_6 K_3 K_4}{h^2 + hK_3 + K_3 K_4} - hk_1 K_1 - \frac{k_5 K_7}{h}$$

In the actual pH-range A is a correction term that can be calculated with sufficient accuracy using rather highly approximated values of K_3 and K_4 .

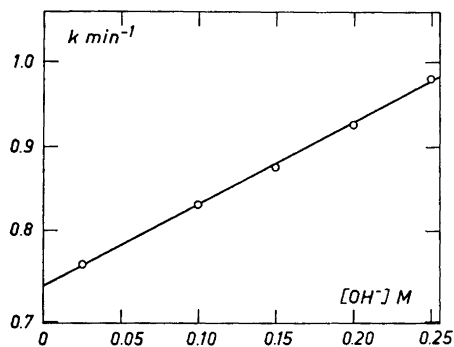


Fig. 4. The total rate constant k plotted against $[\text{OH}^-]$ for the determination of k_6 and k_7 .

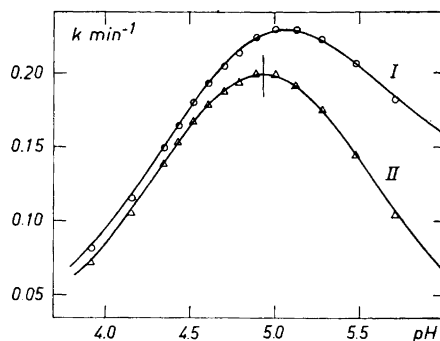


Fig. 5. Curve I. The total rate constant k plotted against pH. The points represented by the symbol \circ are experimentally obtained; the full-drawn curve is calculated from eqn. (7). Curve II. The total rate constant k minus the term A in eqn. (11) plotted against pH. The full-drawn curve is calculated from eqn. (11).

Previously investigated *para*-hydroxy MG and *para*-methoxy MG (Ref.¹) and *meta*-hydroxy MG and *meta*-methoxy MG (Ref.³) show that there is a comparatively small difference between corresponding constants of *p*-hydroxy MG and *p*-methoxy MG and between corresponding constants of *m*-hydroxy MG and *m*-methoxy MG. Therefore it seems reasonable to assume that K_3 and K_4 for *ortho*-hydroxy MG have about the same values as K_3 and K_4 for *ortho*-methoxy MG. The constants of the latter dye have been determined (Ref.⁴): $K_3 = 2.8 \times 10^{-5}$ M and $K_4 = 3.7 \times 10^{-6}$ M. A comparison between the constants, already determined, for *o*-hydroxy MG and *o*-methoxy MG

	K_1	K_2	k_1	k_2
<i>o</i> -methoxy MG	29.2	3.92	0.0449	0.0114
<i>o</i> -hydroxy MG	30.3	4.65	0.0530	0.0108

gives further support for using the K_3 - and K_4 -values of *o*-methoxy MG in the correction term A in eqn. (11) as a first approximation. In Fig. 5, curve I, the experimentally obtained k -values (circles) are plotted as a function of pH whereas curve II shows $k - A$ as a function of pH.

If $k - A$ in eqn. (11) is differentiated with respect to $[\text{H}^+]$ it is found that $k - A$ has a maximum when $[\text{H}^+] = \sqrt{K_3 K_4}$. From Fig. 5 $(k - A)_{\text{max}} = 0.199$ is obtained when $\text{pH} = 4.93$ which gives $K_3 K_4 = 1.40 \times 10^{-10}$ M². Eqn. (11) can be transformed to

$$k_3 = \frac{k - A}{\frac{hK_3K_6}{h^2 + hK_3 + K_3K_4} + 1} \quad (12)$$

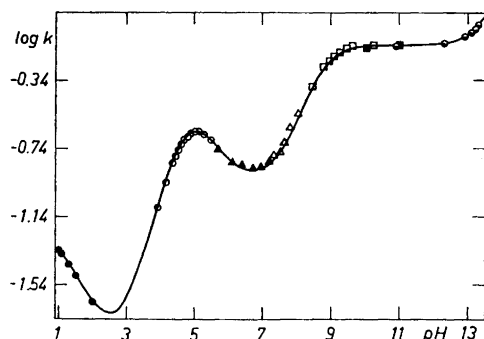


Fig. 6. $\log k$ for the total rate constant plotted against pH. The full drawn curve is calculated from eqn. (7). The points represented by the different symbols are experimentally obtained in the following solutions: ●, "fading" in HCl + KCl-buffers; ○, "restoring of colour" in acetate buffers; ▲, "restoring of colour" in primary phosphate buffers; △, "fading" in primary phosphate buffers; □, "fading" in ammonia-ammonium chloride buffers; ■, "fading" in secondary phosphate buffers; ●, "fading" in NaOH + KCl-buffers.

since $k_4 = k_3 K_6$. Different values of K_3 were tried until the best possible constant value of k_3 was obtained from eqn. (12). The term K_6 was obtained from the expression $K_1 K_2 K_3 K_6 = 1$, by definition, when K_3 was fixed and K_1 and K_2 were known.

When $K_3 = 3.3 \times 10^{-5}$ M a constant value of k_3 is obtained; $k_3 = 0.00129$. The remaining constants can now be calculated. The following values were obtained: $K_3 = (3.30 \pm 0.10) \times 10^{-5}$ M, $K_4 = (4.24 \pm 0.15) \times 10^{-6}$ M, $K_6 = 263 \pm 10$, $k_3 = 0.00129 \pm 0.00005$ min⁻¹, $k_4 = 0.341 \pm 0.015$ min⁻¹.

By definition $K_1 K_2 K_3 K_6 = 1$ and $K_6 K_7 / K_4 K_9 = 1$. The constant K_6 was determined from the former expression. If this value of K_6 is used in the latter expression, $K_6 K_7 / K_4 K_9 = 1.10$ is obtained. The difference of 10 % must be considered as tolerable if we think of the uncertainty in K_7 and K_9 and the method used to calculate K_6 and K_4 .

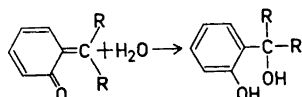
In Fig. 5 the full drawn curve II is calculated from eqn. (11) using the values of the constants, listed above, also in the correction term A . The symbol, △, represents the experimentally obtained k -values minus A . The full drawn curve I is calculated from the non-approximated eqn. (7) and the symbol, ○, represents the experimentally obtained k -values. The agreement between the k -values, calculated from eqn. (7), and the experimentally obtained k -values is very good as can be seen in the diagram.

In Fig. 6 $\log k$ is plotted as a function of pH for the pH-range 1–13.5. Eqn. (7) was used in calculating the $\log k$ values. The symbols are experimentally obtained $\log k$ -values. The agreement between the calculated and the obtained k -values is very good except in the pH-range 6–8. In this range the error limits are relatively large owing to experimental difficulties. The kinetic measurements in this pH-range, however, have not been used to calculate any constants in the equilibrium scheme.

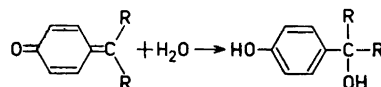
The rate constant k is the rate constant of the total reaction which is manifested as a more or less complete fading of the solution for measurement or a more or less complete restoring of the colour. In the cases when the colour is restored the dye stock solution initially has been more or less decolourized by adding a small amount of a suitable acid or alkaline buffer. When equilibrium has been reached the decolourized solution is mixed with an appropriate buffer and the kinetic measurements can be started.

DISCUSSION

A comparison between the values of the constants for *para*-hydroxy MG and *ortho*-hydroxy MG shows some interesting facts (Table 3). Whether the —OH group is situated *ortho*- or *para* to the central carbon atom of the dye is a matter that does not influence the values of the constants for the reactions in acid solution very much. On the other hand, some of the reactions in alkaline solutions are strongly influenced by the position of the —OH group. The reaction



proceeds 3000 times as fast as the reaction



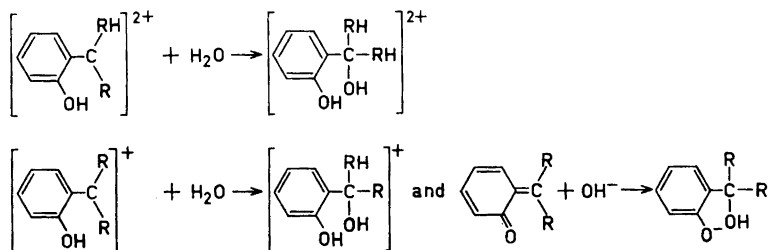
in spite of the fact that the electron-donating —OH group in the *ortho*-position increases the electron density on the central carbon atom more than in the *para*-position and should make a bonding of the negatively polarized oxygen of a water molecule more difficult. The former reaction is facilitated by the favorable conditions for hydrogen bonding and proton transfer between the *ortho*-hydroxy group and the —OH group on the methane carbon atom.

However, the stronger electron repelling property of the *ortho*-hydroxy group is seen in the following reactions ($R = C_6H_4NMe_2$) that proceed about

Table 3. A comparison between the values of the constants for *p*-hydroxy MG and *o*-hydroxy MG.

	K_1	K_2	K_3 $\times 10^5$	K_4 $\times 10^6$	K_5	K_7 $\times 10^7$	K_8 $\times 10^{10}$	K_9
<i>p</i> -hydroxy MG	28.2	2.38	2.5	5.2	600	1.08	1.20	8.4
<i>o</i> -hydroxy MG	29.2	3.93	3.3	4.2	263	0.027	6.1	0.153
	k_1	k_2	k_3	k_4	k_5	k_6	k_7	k_8
<i>p</i> -hydroxy MG	0.111	0.0461	0.00304	1.82	0.00024	0.0020	2.06	1.79 $\times 10^{-3}$
<i>o</i> -hydroxy MG	0.0448	0.0114	0.00129	0.341	0.735	0.113	0.97	3.0 $\times 10^{-6}$

The rate constants k_5 , k_6 , k_7 and k_8 for *p*-hydroxy MG were not published in Ref.¹ They have been calculated now from earlier measurements.



2 times as slowly for *o*-hydroxy MG as the corresponding reactions for *p*-hydroxy MG. But this effect is not seen in the hydration equilibria which are more strongly displaced towards the non-hydrated coloured forms of *p*-hydroxy MG than of *o*-hydroxy MG.

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