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

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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: 
\[
R + 2H^+ \rightleftharpoons B + 2H^+ \rightleftharpoons G; \quad C' + 3H^+ \rightleftharpoons C + 2H^+ \rightleftharpoons S' + H^+ \rightleftharpoons S^*; \quad G + H_2O \rightleftharpoons S^*; \quad B + H_2O \rightleftharpoons S'; \quad R + H_2O \rightleftharpoons C; \quad R + OH^- \rightleftharpoons C'.
\]
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 \textit{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 derivatives \( ^{1-4} \) cannot 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 \textit{ortho}-quinoid structure then becomes possible giving an additional coloured form. The corresponding \textit{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. \( ^{3} \). The remaining rate constants will be reported in this paper.

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MALACHITE GREEN DERIVATIVES

The reaction-equilibrium scheme which was applied to Crystal Violet $^5$ can also be applied to $\omega$-hydroxy MG and the same non-approximated equations for equilibria and reaction rates can be used. The differences in magnitude of the corresponding constants in the dyes mentioned requires, however, quite different approximations of the equations.

The changes in colour nuances and colour intensities that take place when the pH of a water solution of $\omega$-hydroxy MG is changed depend on a series of reactions according to the following simplified scheme. \((R = C_6H_4NMe_2)\).

\[
\begin{align*}
S'' & \overset{\text{OH}}{\underset{\text{OH}}{\rightleftharpoons}} S' \overset{-H^+}{\underset{+H^+}{\rightleftharpoons}} C' \overset{-H^+}{\underset{+H^+}{\rightleftharpoons}} C \\
\overset{+H_2O}{\underset{-H_2O}{\rightleftharpoons}} \overset{+H_2O}{\underset{-H_2O}{\rightleftharpoons}} \overset{+H_2O}{\underset{-H_2O}{\rightleftharpoons}} \overset{+OH^-}{\underset{-OH^-}{\rightleftharpoons}}
\end{align*}
\]

Whole arrows indicate that the reactions proceed with a non-measurable velocity — that is, instantaneously — whereas dashed arrows indicate that the reactions take place with a measurable velocity.

The following definitions and symbols are used $^*$

a) Dissociation constants: $K_1 = \frac{[G]}{[B][H^+]}$; $K_2 = \frac{[R][H^+]}{[B]}$; $K_3 = \frac{[S'][H^+]}{[S'']}$;

$$K_4 = \frac{[C][H^+]}{[S']}$$; $K_5 = \frac{[C'][H^+]}{[C']}$

b) Hydration constants: $K_2 = \frac{[S'']_\infty}{[G]_\infty}$; $K_6 = \frac{[B]_\infty}{[S']_\infty}$; $K_7 = \frac{[R]_\infty}{[C]_\infty}$

c) Rate constants:

\[
\begin{align*}
k_1 &= \text{rate constant for the reaction } G + H_2O \rightarrow S'' \\
k_2 &= \text{rate constant for the reaction } S'' \rightarrow G + H_2O \\
k_3 &= \text{rate constant for the reaction } B + H_2O \rightarrow S' \\
k_4 &= \text{rate constant for the reaction } S' \rightarrow B + H_2O
\end{align*}
\]

*$^*$ The constants $K_1 - K_7$ are defined in the same manner as in Ref.$^1$ but correspond to the following constants within brackets in Ref.$^4$: $K_1(1/K_2)$, $K_2(K_3)$, $K_3(1/K_4)$, $K_4(K_5)$, $K_5(1/K_6)$, $K_6(1/K_7)$, $K_7(1/K_8)$, $K_8(1/K_9)$.  

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\[
\begin{align*}
&k_5 = \text{R + H}_2\text{O} \rightarrow \text{C} \\
k_6 = \text{C} \rightarrow \text{R + H}_2\text{O} \\
k_7 = \text{R + OH}^- \rightarrow \text{C'} \\
k_8 = \text{C'} \rightarrow \text{R + OH}^- \\
k &= \text{rate constant for the total reaction}
\end{align*}
\]

d) \(e_R, e_B, e_G\) = the molar extinction coefficient of R, B, and G.

\(e_0, e_t, e_\infty\) = the absorbance per cm of a solution at the times 0 and \(t\) and at equilibrium (thickness of absorbing layer \(d = 1\) cm).

\(E_0, E_t, E_\infty\) = the absorbance when \(d \neq 1\) cm.

\(e_R, e_B, e_G\) = the absorbance per cm of a solution containing only R, B or G.

\(C_M\) = the total concentration of dyestuff.

\(h = [\text{H}^+]\); \(\text{oh} = [\text{OH}^-]\); \(K_w = [\text{H}^+] [\text{OH}^-]\).

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, \(e_\infty\), 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.

\[
e_0 = \frac{K_7e_G + \text{he}_B + h^2K_1e_G}{K_7 + h + h^2K_1}
\]

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, pH ≈ 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, pH < 5, [R] can be omitted in comparison to [B] + [G]. Eqn. (1) is then approximated and transformed to

\[
\frac{h}{e_B - e_0} = \frac{h}{e_B - e_G} + \frac{1}{K_1(e_B - e_G)}
\]

(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

\[
\frac{1}{e_B - e_0} = \frac{1}{e_B - e_R} + \frac{h}{K_7(e_B - e_R)}
\]

(3)

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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, $e_\infty$

The following equation for $e_\infty$ can be derived

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

Determinant 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

$$K_2 = \frac{e_0}{e_\infty} + \frac{e_B - e_\infty (1 + h K_1 + 1/k_8)}{e_\infty h K_1} \quad (5)$$

The constant $K_2$ can be calculated from eqn. (5) when $K_1$ and $K_8$ are known. The term $1/K_8$ has the character of a correction term since $K_8 > 1$.

Determinant of $K_8$. The hydration constant $K_9$ can not be determined solely through equilibrium measurements. These have to be completed with kinetic measurements.

Determinant 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 $K_9 > 1$ eqn. (4) is approximated and transformed to

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

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 $K_9$ are tried in eqn. (6) until a constant value of $K_9$ is obtained in a pH-range about $pK_8$.

Determinant of $K_9$, $K_4$, and $K_4$. The constants $K_3$, $K_4$, and $K_4$ 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 $R + OH^- \Leftrightarrow C'$, 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):

$$k = \frac{1}{t} \ln \frac{e_0 - e_\infty}{e_0 - e_t} = \frac{h^2 k_2 + h^2 k_3 K_2 + k_3 K_4 K_8 + k_3 K_4 K_8 + k_3 K_9 K_8}{h^3 + h^2 K_3 + h K_3 K_4 + K_3 K_4 K_8} + \frac{h^2 k_1 K_1 + h k_3 + k_7 K_7 + k_7 K_7 h}{h^3 K_1 + h + K_7} \quad (7)$$

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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

$$k = k_1 \frac{hK_1}{1 + hK_1} + k_2$$  \hspace{1cm} (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

$$k = k_5 + k_7\text{oh} + k_8$$ \hspace{1cm} (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:

$$k_7\text{oh}[\text{R}]_\infty = k_8[\text{C}]_\infty, K_8[\text{C}]_\infty = [\text{R}]_\infty, h[\text{C}]_\infty = K_8[\text{C}]_\infty, \text{ and } h \cdot \text{oh} = K$$

which give

$$k_8 = k_7K_wK_8/K_8$$ \hspace{1cm} (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 $k_8$ is obtained from the expressions $k_5[\text{R}]_\infty = k_8[\text{C}]_\infty$ and $K_8[\text{C}]_\infty = [\text{R}]_\infty$ which gives $k_8 = k_5K_8$.

The determination of the remaining constants $k_3$, $k_4$, $K_3$, $K_4$, and $K_8$ 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), dimethylamline (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 $\text{C}_15\text{H}_14\text{O}_5\text{N}_4\text{Cl}$: 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 acetic acid which was then diluted with a potassium chloride solution giving a final solution with ionic strength 0.5 M and containing 4% of acetic acid. The stock solution, with $C_M = 2 \times 10^{-4}$ M, was buffered very weakly with an acetate buffer, pH = 4.5, and was diluted, when necessary, with 0.5 M potassium chloride containing 4% of acetic acid. 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 ≥ 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 ≤ 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 Roughton and modified by Dalziel 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 ≈ 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: λ_{max} = 624

\[\text{MeHNC}_4\text{H}_4\]
\[\text{C} - \text{C}_4\text{H}_4\text{OH}\]
\[\text{MeNC}_4\text{H}_4\text{H}^+\]

in dilute hydrochloric acid.

**Fig. 1.** Absorption curves of o-hydroxy Malachite Green. Curve R is the molecule \((\text{MeNC}_4\text{H}_4)_3\text{CC}_4\text{H}_4\text{O}\) in ammonium-ammonium chloride buffer. Curve B is the ion \([\text{MeNC}_4\text{H}_4)_3\text{CC}_4\text{H}_4\text{OH}]^+\) in acetate buffer. Curve G is the ion \([\text{MeNC}_4\text{H}_4)_3\text{CC}_4\text{H}_4\text{OH}]^+\) in dilute hydrochloric acid.

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\( m_u \) and 436 \( m_u \), \( \epsilon_{\text{max}} = 1.03 \times 10^6 \) and \( 1.23 \times 10^4 \). The curve G was obtained in dilute hydrochloric acid: \( \lambda_{\text{Gmax}} = 455 \ m_u \), \( \epsilon_{\text{Gmax}} = 2.6 \times 10^4 \). It was calculated for 20 different wavelengths from eqn. (5) when \( K_1 \), \( K_2 \), and \( \epsilon_0 \) were determined. The curve R is obtained in an ammonia—ammonium chloride buffer, \( \text{pH} = 10.5 \), by extrapolation of the absorbance to \( t = 0 \) at 47 different wavelengths: \( \lambda_{\text{Rmax}} = 609 \ m_u \) and 408 \( m_u \), \( \epsilon_{\text{Rmax}} = 7.8 \times 10^4 \) and 1.2 \( \times 10^4 \).

**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 \( B + \text{H}^+ \leftrightarrow G \) is reached instantaneously during the mixing, where upon the solution fades owing to the reaction \( G + \text{H}_2\text{O} \rightarrow S^* \). By determining the fading rate and extrapolating the absorbance to \( t = 0 \), \( \epsilon_0 \) was obtained. The measurements were carried out at the wavelength 624 \( m_u \), where the absorption curve for the blue-green ion B has a sharp maximum and \( \epsilon_0 \ll \epsilon_B \). In these acid solutions we need not consider the component R.

In Fig. 2 \([\text{H}^+]/(\epsilon_B - \epsilon_0)\) is plotted against \([\text{H}^+]\). According to eqn. (2) a straight line is obtained; the slope, \( l \), and the intercept, \( y \), on the y-axis give \( \epsilon_B - \epsilon_0 = 1/l \) and \( K_1 = l/y \). From the figure the following values are obtained: \( l = 1.003 \), \( y = 0.0343 \ M \), which gives \( C_M = 1 \times 10^{-5} \ M \), \( e_B = 1.015 \) \( \epsilon_G = 0.018 \) 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 \( B \leftrightarrow R + \text{H}^+ \) is reached instantaneously where upon the solution quickly fades owing to the reaction \( R + \text{H}_2\text{O} \rightarrow C \). 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 \( m_u \) where the difference between \( \epsilon_B \) and \( \epsilon_R \) is sufficient to permit eqn. (3) to be used. According to eqn. (3) a straight line is obtained when \( 1/(\epsilon_B - \epsilon_0) \) is plotted as a function of \( [\text{H}^+] \).

**Fig. 2.** \([\text{H}^+]/(\epsilon_B - \epsilon_0)\) as a function of \([\text{H}^+]\) for the determination of \( K_1 \).

**Fig. 3.** \( 1/(\epsilon_B - \epsilon_0) \) as a function of \([\text{H}^+]\) for the determination of \( K_1/(K_4 + 1) \).

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Table 1. Determination of $K_1$ and $k_1$ and $e_0$-values for the determination of $K_1$. $\lambda = 624$ m\textmu; $C_M = 1 \times 10^{-4}$ M; $T = 20.0^\circ$C; $d = 1$ and 5 cm; $e_B = 1.015$.

<table>
<thead>
<tr>
<th>[H$^+$] M</th>
<th>$e_0$</th>
<th>$e_{\infty}$</th>
<th>$K_1$</th>
<th>$k$ min$^{-1}$</th>
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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, $e_B = 0.250$); $y = 9.80$, $l = 3.60 \times 10^6$ M$^{-1}$ which gives $e_R = 0.148$, $e_R = 5.92 \times 10^4$, and $K_1 = (2.72 \pm 0.15) \times 10^{-9}$ M.

Determination of $K_2$. The dye stock solution was mixed with appropriate HCl + KCl-buffers. The solutions were thermostated and the equilibrium absorbances, $E_{\infty}$, were read off after 6 h. $C_M = 1 \times 10^{-6}$ 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. $e_{\infty}$- and $e_0$-values for the determination of $K_2$ and $K_5$; $T = 20.0^\circ$C.

<table>
<thead>
<tr>
<th>$\lambda = 624$ m\textmu</th>
<th>$C_M = 2.5 \times 10^{-6}$ M</th>
<th>$C_M = 5 \times 10^{-6}$ M</th>
<th>$\lambda = 625$ m\textmu</th>
<th>$e_R = 0.275$</th>
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<td>$E_{\infty} = 5$ $e_{\infty}$</td>
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<td>$e_R$</td>
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<td>0.153</td>
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<tr>
<td>0.551</td>
<td>0.166</td>
<td>0.127</td>
<td>0.156</td>
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</tr>
<tr>
<td>0.332</td>
<td>0.159</td>
<td>0.085</td>
<td>0.153</td>
<td></td>
</tr>
<tr>
<td>0.242</td>
<td>0.154</td>
<td>0.065</td>
<td>0.151</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>0.153</td>
</tr>
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</table>

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Determination of $K_8$ and $K_9$. The dye stock solution was mixed with ammonia-ammonium chloride buffers and the equilibrium absorbancies, $E_{\infty}$, were read off after 10—20 min. $C_M = 5 \times 10^{-6} \text{ M}$, $d = 5 \text{ cm}$, $\lambda = 624 \text{ nm}$, $e_8 = 0.496$, $e_R = 0.274$. 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 $K_8 = 6.1 \times 10^{-10} \text{ M}$ is chosen. The results are found in Table 2. As a mean value $K_9 = 0.153 \pm 0.005$ was obtained when $K_8 = (6.1 \pm 0.2) \times 10^{-10} \text{ M}$.

Note. Borate buffers cannot be used for the determination of $K_7$, $K_8$, and $K_9$ since borate ions react which $o$-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 [NH$_4$] was kept $\leq 0.05 \text{ M}$ in the ammonia-ammonium chloride buffers.

Kinetic measurements

Determination of $k_1$ and $k_2$. The total fading rate, $k$, was determined in the measurements from which $e_9$ for the determination of $K_9$ 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 $k_1 = 0.0448 \pm 0.0010 \text{ min}^{-1}$ was obtained. Then $k_2$ is obtained from the expression $k_1 = k_2K_2$ which gives $k_2 = 0.0114 \pm 0.0003 \text{ min}^{-1}$.

Determination of $k_3$, $k_6$, $k_7$ and $k_8$. The kinetic measurements were carried out in strongly alkaline solutions. The dye stock solution was mixed with solutions of the type $x \text{ M } \text{NaOH} + (0.5 - x) \text{ M KCl}$ where $x = 0.025, 0.100, 0.150, 0.200$, and $0.250$. In Fig. 4 $k$ is plotted as a function of [OH$^-$]. According to eqn. (9) a straight line is obtained with the slope, $l = 0.967 \text{ min}^{-1} \text{ M}^{-1}$ and the intercept on the $y$-axis, $y = 0.735 \text{ min}^{-1}$. This gives $k_7 = 0.97 \pm 0.02 \text{ min}^{-1} \text{ M}^{-1}$ and $k_5 + k_8 = 0.735 \text{ min}^{-1}$. From eqn. (10) $K_8 = (3.0 \pm 0.2) \times 10^{-6} \text{ min}^{-1}$ is obtained ($K_w = 1.24 \times 10^{-14} \text{ M}^2$). Thus $k_8$$<$$k_5$, and therefore Fig. 4 gives $k_8 = 0.735 \pm 0.010 \text{ min}^{-1}$. The rate constant $k_8$ can be calculated from the expression $k_8 = k_3K_9$ which gives $k_9 = 0.113 \pm 0.05 \text{ min}^{-1}$.

The remaining constants $k_3$, $k_4$, $K_3$, $K_4$, and $K_8$ 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 [H$^+$] by the following approximation procedure. In the pH-range 4—6 eqn. (7) can be approximated and written as

$$k - A = \frac{hk_4K_3}{h^2 + hK_3 + K_3K_4} + k_3$$

(11)

where

$$A = \frac{h^2k_2 + k_6K_3K_4}{h^2 + hK_3 + K_3K_4} - \frac{hk_1K_4}{h} - \frac{k_5K_7}{h}$$

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 $K_4$.

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Previously investigated para-hydroxy MG and para-methoxy MG (Ref.1) and meta-hydroxy MG and meta-methoxy MG (Ref.2) 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 $K_4 = 3.7 \times 10^{-4}$ M. A comparison between the constants, already determined, for o-hydroxy MG and o-methoxy MG

<table>
<thead>
<tr>
<th></th>
<th>$K_3$</th>
<th>$k_1$</th>
<th>$k_2$</th>
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</thead>
<tbody>
<tr>
<td>o-methoxy</td>
<td>29.2</td>
<td>4.65</td>
<td>0.0530</td>
</tr>
<tr>
<td>o-hydroxy</td>
<td>30.3</td>
<td>3.92</td>
<td>0.0449</td>
</tr>
</tbody>
</table>

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 [H+] it is found that $k - A$ has a maximum when $[H^+] = \sqrt{K_3K_4}$. From Fig. 5 $(k - A)_{\text{max}} = 0.199$ is obtained when pH = 4.93 which gives $K_3K_4 = 1.40 \times 10^{-10}$ M². Eqn. (11) can be transformed to

$$k_3 = \frac{k - A}{hK_3K_4} \left( \frac{1}{h^2 + hK_3 + K_2K_4} + 1 \right) \quad (12)$$

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since $k_4 = k_3 K_4$. Different values of $K_3$ were tried until the best possible constant value of $k_3$ was obtained from eqn. (12). The term $K_4$ was obtained from the expression $K_4 K_8 K_6 = 1$, 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, $K_6 = 263 \pm 10$, $k_9 = 0.00129 \pm 0.00005$ min$^{-1}$, $k_4 = 0.341 \pm 0.015$ min$^{-1}$.

By definition $K_1 K_2 K_3 K_6 = 1$ and $K_8 K_1 / K_4 K_9 = 1$. The constant $K_4$ was determined from the former expression. If this value of $K_4$ is used in the latter expression, $K_8 K_1 / K_4 K_9 = 1.10$ 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, $\Delta$, represents the experimentally obtained $k$-values minus $A$. The full drawn curve I is calculated from the non-approximated eqn. (7) and the symbol, $\circ$, 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.

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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

\[
\begin{align*}
&\text{\text{C} + \text{H}_2\text{O} \rightarrow \text{C} - \text{R}} \\
&\text{\text{O}}
\end{align*}
\]

proceeds 3000 times as fast as the reaction

\[
\begin{align*}
&\text{\text{C} + \text{H}_2\text{O} \rightarrow \text{C} - \text{R}} \\
&\text{\text{O}}
\end{align*}
\]

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 = C₆H₄NMe₂) that proceed about

<table>
<thead>
<tr>
<th>(K_1)</th>
<th>(K_2)</th>
<th>(K_3\times 10^4)</th>
<th>(K_4\times 10^4)</th>
<th>(K_5\times 10^4)</th>
<th>(K_6\times 10^4)</th>
<th>(K_7\times 10^4)</th>
<th>(K_8\times 10^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-hydroxy MG</td>
<td>28.2</td>
<td>2.38</td>
<td>2.5</td>
<td>5.2</td>
<td>600</td>
<td>1.08</td>
<td>1.20</td>
</tr>
<tr>
<td>o-hydroxy MG</td>
<td>29.2</td>
<td>3.93</td>
<td>3.3</td>
<td>4.2</td>
<td>263</td>
<td>0.027</td>
<td>6.1</td>
</tr>
</tbody>
</table>

\[k_1\] \[k_2\] \[k_3\] \[k_4\] \[k_5\] \[k_6\] \[k_7\] \[k_8\]

<table>
<thead>
<tr>
<th>(k_1)</th>
<th>(k_2)</th>
<th>(k_3)</th>
<th>(k_4)</th>
<th>(k_5)</th>
<th>(k_6)</th>
<th>(k_7)</th>
<th>(k_8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-hydroxy MG</td>
<td>0.111</td>
<td>0.0461</td>
<td>0.00304</td>
<td>1.82</td>
<td>0.00024</td>
<td>0.0020</td>
<td>2.06</td>
</tr>
<tr>
<td>o-hydroxy MG</td>
<td>0.0448</td>
<td>0.0114</td>
<td>0.00129</td>
<td>0.341</td>
<td>0.735</td>
<td>0.113</td>
<td>0.97</td>
</tr>
</tbody>
</table>

The rate constants \(k_4\), \(k_5\), \(k_7\) and \(k_8\) for p-hydroxy MG were not published in Ref. This is because they have been calculated now from earlier measurements.

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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.

REFERENCES


Received August 2, 1961.