Studies on the Pyridine Analogues of Malachite Green

III. Protolytic Equilibria and Reaction Rate Constants of 2-Pyridine Green in Aqueous Solutions

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In two previous papers [1,2] the protolytic equilibria and reaction rates of two pyridine analogues of Malachite Green, called 4-Pyridine Green and 3-Pyridine Green, have been studied spectrophotometrically at 20°C in aqueous solutions with constant ionic strength 0.5 M. These investigations have been completed by the corresponding studies of the remaining pyridine analogue, called 2-Pyridine Green.

The results of the measurements are compared with the results of the corresponding measurements of Crystal Violet,[3] 3-Pyridine Green, and 4-Pyridine Green. All these dyes have three basic groups and the reaction-equilibrium scheme proposed by Cigén for Crystal Violet can be used for all of them. This scheme includes three coloured species and four colourless species. The former are in momentaneously reached protolytic equilibria with each other ($Y \rightleftharpoons G + H^+ \rightleftharpoons B + 2 H^+$), and so are the latter ($S_3 \rightleftharpoons S_2 + H^+ \rightleftharpoons S_1 + 2 H^+ \rightleftharpoons C + 3 H^+$). Transitions between coloured and colourless species occur by reactions with water molecules ($Y + H_2O \rightleftharpoons S_3$; $G + H_2O \rightleftharpoons S_2$; $B + H_2O \rightleftharpoons S_1$) or hydroxide ions ($B + OH^- \rightleftharpoons C$) proceeding at a measurable rate. The values of the hydration-equilibrium constants K_4, K_5 and K_6 for the reactions $Y + H_2O \rightleftharpoons S_3$, $G + H_2O \rightleftharpoons S_2$ and $B + H_2O \rightleftharpoons S_1$, respectively, are correlated with the electronegativity of the pyridine groups and the dimethylaniline group, and so are the values of the dissociation constant K_1, corresponding to the equilibrium $Y \rightleftharpoons G + H^+$.

The kinetic measurements support the opinion that the reaction mechanisms are complex. Two other possible reaction schemes are discussed.

I n parts I and II of this investigation [1,2] the protolytic equilibria and the reaction rates of two pyridine analogues of Malachite Green, called 4-Pyridine Green (4-PG) and 3-Pyridine Green (3-PG), respectively, were studied spectrophotometrically in aqueous solutions with the constant ionic strength 0.49 M. The present paper is devoted to a corresponding study of the remaining pyridine analogue, called 2-Pyridine Green (2-PG).
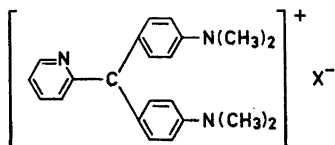
The dyestuff 2-Pyridine Green, which has the schematic structural formula was first prepared by Harries and Lénart in 1915.[4] Its behaviour in aqueous solutions is analogous to the behaviour of the other two pyridine analogues and Crystal Violet (CV).[3] The reactions can be visualized by the following reaction-equilibrium scheme:
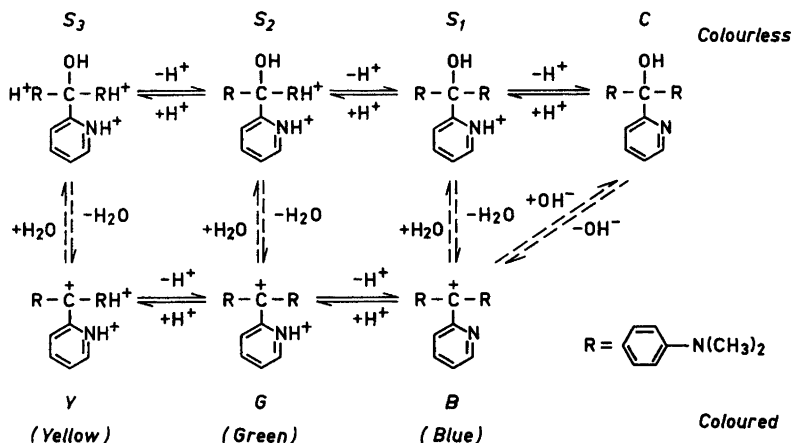
Dashed arrows indicate reactions proceeding at a measurable rate and full drawn arrows indicate reactions proceeding at a non-measurable rate. This scheme is schematic and does not imply any reaction mechanisms, which most probably are complex.

The following constants and notations are defined:

(a) Equilibrium constants: $K_1 = [G][H^+]/[Y]$; $K_2 = [B][H^+]/[G]$; $K_1' = [S_2][H^+]/[S_3]$; $K_2' = [S_1][H^+]/[S_2]$; $K_3' = [C][H^+]/[S_1]$; $K_4 = [S_3]_\infty/[Y]_\infty$; $K_5 = [S_2]_\infty/[G]_\infty$; $K_6 = [S_1]_\infty/[B]_\infty$.

(b) Rate constants:

k_1 rate constant for the reaction $Y + H_2O \rightarrow S_3$
k_2 »   »   »   »   » $S_3 \rightarrow Y + H_2O$
k_3 »   »   »   »   » $G + H_2O \rightarrow S_2$
k_4 »   »   »   »   » $S_2 \rightarrow G + H_2O$
k_5 »   »   »   »   » $B + H_2O \rightarrow S_1$
k_6 »   »   »   »   » $S_1 \rightarrow B + H_2O$
k_7 »   »   »   »   » $B + OH^- \rightarrow C$
k_8 »   »   »   »   » $C \rightarrow B + OH^-$
k »   »   »   »   over-all reaction.

(c) Notations: h $= [H^+]$; oh $= [OH^-]$; $K_W = [H^+][OH^-]$.

ε_B, ε_G, ε_Y molar absorbancy coefficients of B, G, and Y, respectively.

e_0, e_t, e_∞ absorbancy per cm of a solution at the times 0 and t and at equilibrium.

E_0, E_t, E_∞ total absorbancies.

e_B, e_G, e_Y absorbancy of a solution containing only B, G or Y.

C_M total concentration of the dyestuff.

The constants were determined in essentially the same manner as was described in Refs.[1-3] The dyestuff was not, however, quite stable in aqueous solutions but the absorbancy of an aqueous dye solution decreased slowly after the equilibria, indicated in the scheme above, had been reached. Thus, when an acetone solution of the carbinol was mixed with a buffer solution, the absorbancy of the resulting solution increased, reached a value which remained constant for some hours, and then slowly decreased. The decrease of the absorbancy was within 24 h after equilibrium would have been reached noticeable only within the pH-range 3.6—6.3 (0—2.4 % within 24 h). After another 24 h the decrease was noticeable also at 3.6 >pH > 6.3. The decrease of the absorbancy was most rapid around pH $= 5$. The instability of the dyestuff decreased the reliability of the measurements. The kinetic measurements were, however, but little affected, for measurements of the reactions "colourless → coloured" and "coloured → colourless" gave for a given hydrogen ion concentration the same results within the experimental error. The equilibrium absorbancies were determined within 8—10 half-lives after the mixing of the dye solution and the buffer solution to make the errors due to the instability as small as possible.

In neutral and alkaline solutions the carbinol was precipitated. Therefore, the rate constants k_7 and k_8 could not be determined.

EXPERIMENTAL

2-Pyridine Green Carbinol. The leuco base was prepared from N,N-dimethylaniline (0.3 mole) and 2-pyridine aldehyde (0.1 mole) (from Light & Co, Colnbrook, England). It was obtained as a slightly brownish powder, m.p. 109—110°C.

The leuco base was dissolved in dilute hydrochloric acid and oxidized to dyestuff with lead dioxide. Attempts to prepare a pure dyestuff perchlorate were unsuccessful. The carbinol was prepared by slow addition of sodium hydroxide to the dyestuff solution, which was cooled and vigorously stirred. The carbinol became dark brown on heating and therefore could not be recrystallized. It was dissolved in cold absolute ethanol and reprecipitated by the addition of water. This procedure was repeated until optical constancy was attained. The carbinol base was obtained as a slightly brownish powder. (Found: C 75.7; H 6.91; N 12.1; O 4.56. Calc. for $C_{22}H_{25}N_3O$: C 76.1; H 7.25; N 12.1; O 4.61).

A carbinol stock solution was prepared by dissolving a weighed amount of the carbinol in acetone. The solution was colourless and stable.

The chemicals used for the buffer solutions were hydrochloric acid, sodium acetate, disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium hydroxide, all of analytical grade. Potassium chloride of analytical grade was used to keep the ionic strength constant (0.49 M). The apparatus used for the measurements was the same Beckman DU spectrophotometer as was used for the measurements in Refs.[1-3] The temperature was 20.0°C.

EQUATIONS

The following three fundamental equations for the initial absorbancy, e_0, the equilibrium absorbancy, e_∞, and the over-all rate constant, k, can be derived (*cf.* Ref.[3]):

$$e_0 = \frac{K_1 K_2 e_B + h K_1 e_G + h^2 e_Y}{K_1 K_2 + h K_1 + h^2} \tag{1}$$

$$e_\infty = \frac{K_1 K_2 e_B + h K_1 e_G + h^2 e_Y}{K_1 K_2 (1 + K_6 + K_3' K_6/h) + h K_1 (1 + K_5) + h^2 (1 + K_4)} \tag{2}$$

$$k = \frac{1}{t} \ln \frac{e_0 - e_\infty}{e_t - e_\infty} = \frac{h^3 k_2 + h^2 k_4 K_1' + h k_6 K_1' K_2' + k_8 K_1' K_2' K_3'}{h^3 + h^2 K_1' + h K_1' K_2' + K_1' K_2' K_3'} +$$

$$\frac{h^2 k_1 + h k_3 K_1 + k_5 K_1 K_2 + k_7 o h K_1 K_2}{h^2 + h K_1 + K_1 K_2} \tag{3}$$

The approximate equations used for the determination of the constants will be considered in connection with the determination of the constants.

It was not possible to prepare a dyestuff solution containing all of the dyestuff as coloured species. Eqn. (1) should therefore be written

$$e_0 = \frac{K_1 K_2 e_B' + h K_1 e_G' + h^2 e_Y'}{K_1 K_2 + h K_1 + h^2} \tag{4}$$

where

$$\frac{e_B'}{e_B} = \frac{e_G'}{e_G} = \frac{e_Y'}{e_Y} = \frac{[B]_0 + [G]_0 + [Y]_0}{C_M} \tag{5}$$

MEASUREMENTS AND RESULTS

Absorption curves. The absorption curves of the species B, G, and Y are shown in Fig. 1. The curve of B was determined from the equilibrium absorbancies at pH = 5.89. At this pH-value B is the only coloured species to be considered. The molar absorbancy coefficients of B (and G) could not, however, be determined from the e_0-values in phosphate buffers, since there was no dyestuff solution available, containing all of the dyestuff as coloured species. ε_B (and ε_G) had to be determined in an indirect way, described below.

The curves of G and Y were calculated from the e_0-values at $[H^+] = 0.005$ M and 0.490 M, respectively. The same aqueous dye stock solution was used for the determination of both curves, and the measurements were carried out within 1—2 h. The absorbancy of the dye stock solution did not change during this time. The fraction of coloured species present in the aqueous dye stock solution was evaluated from the experimental value of e_0 at the wave length $\lambda = 640$ mμ and $[H^+] = 0.005$ M, and the value of e_0 at the same wave length and hydrogen ion concentration, calculated from eqn. (1). The indirectly determined $\varepsilon_B = 8.82 \times 10^4$ and $\varepsilon_G = 4.28 \times 10^4$, and the known values of K_1 and K_2 were then used. $\varepsilon_G = e_G/C_M$ and $\varepsilon_Y = e_Y/C_M$ were calculated
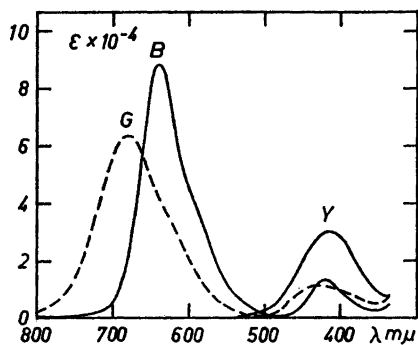
Fig. 1. Absorption curves of the blue ion $[NH_4C_5 \cdot C \cdot (C_6H_4NMe_2)_2]^+$, the green ion $[HNH_4C_5 \cdot C \cdot (C_6H_4NMe_2)_2]^{2+}$ and the yellow ion

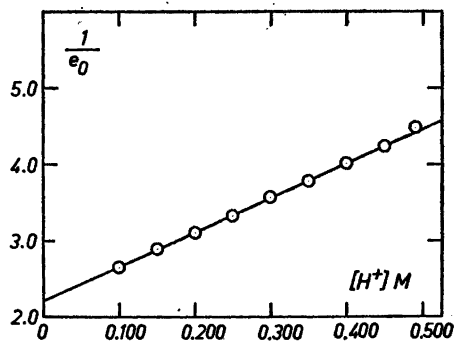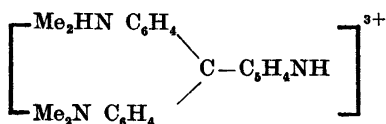$$\begin{bmatrix} Me_2HN\ C_6H_4 \\ \diagdown \\ C-C_6H_4NH \\ \diagup \\ Me_2N\ C_6H_4 \end{bmatrix}^{3+}$$

Fig 2. $1/e_0$ versus $[H^+]$ for the determination of K_1.

from eqn. (1). [Y] and [B] were neglected at $[H^+] = 0.005$ M and 0.490 M, respectively. The following results were obtained:

	λ_{max}	$\varepsilon_{max} \times 10^{-4}$
B	639 mμ and 419 mμ	8.8 and 1.3
G	680 mμ and 426 mμ	6.3 and 1.1
Y	411 mμ	3.0

Determination of K_1. Fig. 2 shows $1/e_0$ versus h in HCl + KCl-buffers, $[H^+] = 0.100-0.490$ M, a straight line according to the equation

$$\frac{1}{e_0} = \frac{1}{e_G{}'} + \frac{1}{e_G{}'K_1}\,h \tag{6}$$

which is valid within a pH-range, where $[H^+] \gg K_2$ ($e_Y{}'$ is negligible at the used wave length, $\lambda = 640$ mμ). The line has the intercept on the ordinate-axis $y = 2.21$ and the slope $l = 4.50$ ($C_M = 2 \times 10^{-5}$ M; $d = 1$ cm; $\lambda = 640$ mμ; $T = 20.0°C$). $K_1 = 0.49 \pm 0.01$ M. An aqueous dye solution was prepared by mixing 10 ml of the carbinol stock solution in acetone with 240 ml of 0.50 M KCl. After 27—30 h samples of this solution were mixed with equal volumes of suitable HCl + KCl-buffers. The initial absorbancies, e_0, were determined.

Determination of K_2. This constant was determined from the e_0-values in HCl + KCl-buffers, $[H^+] = 0.002-0.012$ M. The measurements were carried out as was described for the determination of K_1. Eqn. (1) can be rearranged to

$$\frac{h}{e_B{}'-e_0}\,(1+X) = \frac{K_2}{e_B{}'-e_G{}'} + \frac{1}{e_B{}'-e_G{}'}\,h \tag{7}$$

Table 1. 2-Pyridine Green. e_0-values for the determination of K_1. $C_M = 2 \times 10^{-5}$ M; $d = 1$ cm; $\lambda = 640$ mμ; $T = 20.0$°C.

[H$^+$] M	e_0	[H$^+$] M	e_0
0.100	0.378	0.350	0.264
0.150	0.345	0.400	0.249
0.200	0.322	0.450	0.236
0.250	0.300	0.490	0.223
0.300	0.280		

where

$$X = \frac{he_0}{K_1(e_B{}' - e_G{}')} \tag{8}$$

The term X is a correction term amounting to 0.005—0.024 within the used pH-range. In eqn. (8) e_0 and K_1 are known. An approximate value of $1/(e_B{}' - e_G{}') \approx 5$ is obtained from a plot of $h/(e_B{}'-e_0)$ *versus* h. $h(1 + X)/(e_B{}'-e_0)$ *versus* h can be seen in Fig. 3 ($C_M = 1 \times 10^{-5}$ M; $d = 1$ cm; $\lambda = 640$ mμ; $T = 20.0$ °C; $e_B{}' = 0.379$). The intercrept on the ordinate-axis $y = 0.82 \times 10^{-2}$ M and the slope $l = 5.13$ give $\mathbf{K_2} = \mathbf{(1.60 \pm 0.05)} \times \mathbf{10^{-3}}$ **M** and $e_G{}' = 0.184$.

Table 2. 2-Pyridine Green. e_0-values for the determination of K_2 and $e_G{}'$. $C_M = 1 \times 10^{-5}$ M; $d = 1$ cm; $\lambda = 640$ mμ; $T = 20.0$°C; $e_B{}' = 0.379$.

[H$^+$] M	e_0	[H$^+$] M	e_0
0.0020	0.270	0.0080	0.213
0.0040	0.239	0.0100	0.207
0.0060	0.222	0.0120	0.203
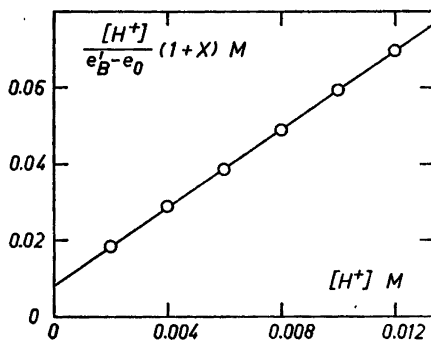
Fig. 3. [H$^+$]$(1 + X)/(e_B{}'-e_0)$ *versus* [H$^+$] for the determination of K_2 and $e_G{}'$.
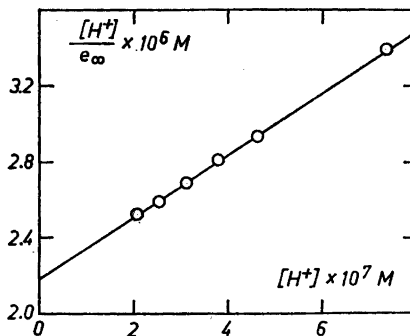
Fig. 4. [H$^+$]/e_∞ *versus* [H$^+$] for the determination of $(1 + K_6)/e_B$ and $K_3{}'K_6/e_B$.

Determination of $(1 + K_6)/e_B$ *and* $K_3'K_6/e_B$. 1 ml of the dye stock solution in acetone was mixed with 49 ml of suitable phosphate buffers, pH = 6.1—6.7. The absorbancies increased, reached the equilibrium values, which remained constant for 10—20 h, and then decreased very slowly. The e_∞-values were read 12—16 h after the mixing of the dye stock solution with the buffer solutions. The following equation is valid within the used pH-range:

$$\frac{h}{e_\infty} = \frac{K_3'K_6}{e_B} + \frac{1+K_6}{e_B} h \qquad (9)$$

The straight line in Fig. 4 has the intercept on the ordinate-axis $y = 2.18 \times 10^{-6}$ M and the slope $l = 1.636$ ($C_M = 1 \times 10^{-5}$ M; $d = 1$ cm; $\lambda = 640$ mμ; $T = 20.0$ °C). Hence $K_3'K_6/e_B = 2.18 \times 10^{-6}$ M and $(1 + K_6)/e_B = 1.64$ are obtained.

Table 3. 2-Pyridine Green. e_0-values and k-values in phosphate buffers and acetate buffers. $C_M = 1 \times 10^{-5}$ M; $d = 1$ cm; $\lambda = 640$ mμ; $T = 20.0$°C.

pH	e_∞	k min^{-1}	pH	e_∞	k min^{-1}
3.263	0.0725	0.00604	5.272	0.461	0.01320
3.426	0.110	0.00650	5.471	0.422	0.01169
3.556	0.147	0.00690	5.545	0.403	0.01120
3.657	0.180	0.00709	5.743	0.344	0.00997
3.752	0.215	0.00788	5.843	0.314	0.00927
3.900	0.269	0.00852	5.894	0.296	0.00897
4.132	0.353	0.00994	5.985	0.251	0.00857
4.321	0.416	0.01104	6.135	0.216	0.00795
4.501	0.453	0.01266	6.334	0.158	0.00731
4.682	0.479	0.01367	6.420	0.135	0.00710
4.768	0.485	0.01400	6.506	0.116	0.00696
4.880	0.490	0.01423	6.596	0.0978	0.00691
4.990	0.487	0.01424	6.683	0.0822	0.00676
5.113	0.480	0.01382			

Determination of $(1 + K_5)/e_B$ *and* K_4/e_B. These quantities were obtained from the equilibrium absorbancies in acetate buffers (pH = 3.3—4.1), determined in the manner described above. The e_∞-values were read 12—18 h after the mixing of the dye stock solution in acetone with the buffer solutions. The expression

$$\frac{Q}{e_B} = \frac{K_2}{he_\infty} + \frac{e_G}{e_B} \times \frac{1}{e_\infty} - \frac{K_2}{he_B}\left(1 + K_6 + \frac{K_3'K_6}{h}\right) =$$
$$\frac{1+K_5}{e_B} + \frac{1+K_4}{K_1e_B} h \qquad (10)$$

can be derived. The determined values $K_2 = 1.60 \times 10^{-3}$ M; $e_G/e_B = e_G'/e_B' = 0.486$ (*cf.* above, $e_B' = 0.379$ and $e_G' = 0.184$); $(1 + K_6)/e_B = 1.64$, $K_3'K_6/e_B = 2.18 \times 10^{-6}$ M were used for the calculation of Q/e_B. Plotting Q/e_B *versus* h gives a straight line with the intercept on the ordinate-axis $y =$

23.8 and the slope $l = 3.33 \times 10^4$. Thus we obtain $(1 + K_5)/e_B = 23.8$ and $(1 + K_4)/e_B = 1.64 \times 10^4$, since $K_1 = 0.49$ M. It can be seen that $K_4 \gg 1$, and therefore $K_4/e_B = 1.64 \times 10^4$.

Estimation of k_7. Since the carbinol was precipitated in alkaline solutions the rate constants k_7 and k_8 could not be determined. Measurements within the pH-range $11.3-12.0$ showed that the absorbancy rapidly decreased to zero when an aqueous dye solution was mixed with NaOH + KCl-solutions. From the maximum k-values obtained the limit $k_7 \leq 80$ min^{-1}M^{-1} could be estimated.

Determination of k_5. In phosphate buffers, pH $= 5.7-6.7$, eqn. (3) can be approximated to

$$k = \frac{h^2 k_4 + h k_6 K_2'}{h^2 + hK_2' + K_2'K_3'} + k_5 \tag{11}$$

The slightly curved line, which is obtained when k is plotted as a function of h, is shown in Fig. 5. An extrapolation of this line to h $= 0$ gives $\mathbf{k_5 = 0.0062 \pm 0.0001\ min^{-1}}$. Reliable measurements at pH ≥ 6.7 were made difficult by the precipitation of the carbinol.
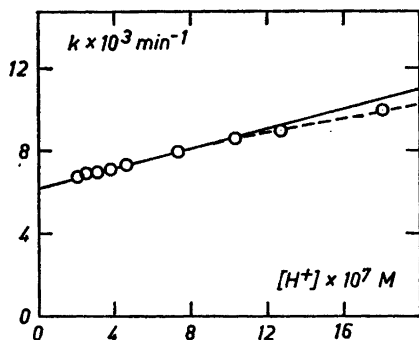

Fig. 5. The over-all rate constant, k, as a function of [H$^+$] for the determination of k_5.
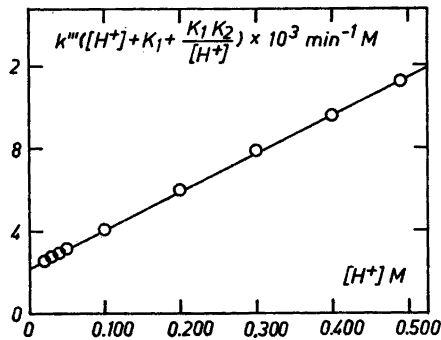
Fig. 6. $k'''(\text{[H}^+] + K_1 + K_1K_2/\text{[H}^+])$ versus [H$^+$] for the determination of k_1 and k_3.

Determination of k_1 and k_3. In strongly acid solutions, pH $= 0.3-1.7$, eqn. (3) can be approximated and written

$$k'''(h + K_1 + K_1K_2/h) = k_3K_1 + k_1h \tag{12}$$

where

$$k''' = k - \frac{k_5K_1K_2}{h^2 + hK_1 + K_1K_2} \tag{13}$$

Fig. 6 shows $k'''(h + K_1 + K_1K_2/h)$ *versus* h. The straight line has the intercept on the ordinate-axis $y = 0.00216$ min^{-1} M and the slope $l = 0.0184$ min^{-1}. Hence $\mathbf{k_1 = 0.0184 \pm 0.0004\ min^{-1}}$ and $\mathbf{k_3 = 0.0044 \pm 0.0002\ min^{-1}}$, since $K_1 = 0.49$ M.

Determination of e_B, K_6, K_3', K_2' *and* k_6. Eqn. (3) is written

$$k'' = \frac{h k_6 K_2'}{h^2 + h K_2' + K_2' K_3'} \tag{14}$$

where

$$k'' = \frac{k'}{1 - \dfrac{k' h^2}{k_6 K_1' K_2'}} \tag{15}$$

and

$$k' = k - \frac{h^2 k_1 + h k_3 K_1 + k_5 K_1 K_2 + k_7 \text{oh} K_1 K_2}{h^2 + h K_1 + K_1 K_2} - \tag{16}$$

$$\frac{h^3 k_2 + h^2 k_4 K_1' + k_8 K_1' K_2' K_3'}{h^3 + h^2 K_1' + h K_1' K_2' + K_1' K_2' K_3'} = \frac{h k_6 K_1' K_2'}{h^3 + h^2 K_1' + h K_1' K_2' + K_1' K_2' K_3'}$$

Eqn. (14) is differentiated with regard to h, and it is found that k'' has a maximum for $h = \sqrt{K_2' K_3'}$, and thus

$$k_{\text{max}}'' = \frac{k_5 \sqrt{K_2' K_3'}}{2 K_3' K_6 + K_6 \sqrt{K_2' K_3'}} \tag{17}$$

The term $(h^2 k_1 + h k_3 K_1 + k_5 K_1 K_2)/(h^2 + h K_1 + K_1 K_2)$ in eqn. (16) can be calculated by using the constants already determined (the reaction paths $B + OH^- \rightleftharpoons C$ are neglected within the used pH-range, 3.4—6.4). The term $(h^3 k_2 + h^2 k_4 K_1' + k_8 K_1' K_2' K_3')/(h^3 + h^2 K_1' + h K_1' K_2' + K_1' K_2' K_3')$ contains only unknown constants, which were determined by a series of successive approximations. As a first approximation we put

$$k'' \approx k - \frac{h^2 k_1 + h k_3 K_1 + k_5 K_1 K_2}{h^2 + h K_1 + K_1 K_2} = k - A$$

pH at $(k-A)_{\text{max}} = 4.92$; $(k-A)_{\text{max}} = 0.00808$ min^{-1} and thus $K_2' K_3' \approx 1.2 \times 10^{-5}$ M, were obtained. We further made the assumption that $K_3' K_6 \approx K_3' K_6 / e_B = 2.18 \times 10^{-6}$ M. These values were inserted into eqn. (17) together with $k_5 = 0.0062$ min^{-1}. $K_6 = 0.40$ was calculated and, since $(1 + K_6)/e_B = 1.64$, $e_B = 0.86$ is obtained and thus $K_3' K_6 = 1.9 \times 10^{-6}$ M. This value was again inserted into eqn. (17) and the calculations were repeated until further recalculations did not appreciably change the values of K_6 and e_B. The following values were obtained: $K_6 = 0.44$; $e_B = 0.88$; $K_3' = 4.3 \times 10^{-6}$ M; $K_2' = 3.3 \times 10^{-5}$ M; $K_4 = 1.4 \times 10^4$; $K_5 = 20$; $k_2 = k_1/K_4 = 1.3 \times 10^{-6}$ min^{-1}; $k_4 = k_3/K_5 = 2.2 \times 10^{-4}$ min^{-1}; $k_6 = k_5/K_6 = 1.4 \times 10^{-2}$ min^{-1} and $K_1' = K_1 K_5/K_4 = 6.8 \times 10^{-4}$ M.

The obtained values were used for the calculation of k' and k''. pH$_{k''\text{max}} = 4.922$ and $k'' = 0.00808$ min^{-1} (*i.e.* $k_{\text{max}}'' = (k-A)_{\text{max}}$). The new values were used in eqn. (17) and the calculations were repeated until further calculations did not change the values of K_6 and e_B. The following values were finally obtained: $\mathbf{K_6 = 0.44 \pm 0.02}$; $\mathbf{e_B = 0.88 \pm 0.04}$; $\mathbf{K_3' = (4.3 \pm 0.3) \times 10^{-6}}$ M; $\mathbf{K_2' = (3.3 \pm 0.3) \times 10^{-5}}$ M; $\mathbf{K_4 = (1.4 \pm 0.3) \times 10^4}$; $\mathbf{K_5 = 20 \pm 3}$; $\mathbf{k_2 =}$

(1.3 ± 0.3) × 10⁻⁶ min⁻¹; $k_4 =$ **(2.2 ± 0.3) × 10⁻⁴ min⁻¹;** $k_6 =$ **0.014 ± 0.001 min⁻¹ and** $K_1' =$ **(7 ± 1) × 10⁻⁴ M.** Since $e_G/e_B = 0.486$ we also obtain $\mathbf{e}_G =$ **0.43 ± 0.03.** The value of K_2' can be compared with $K_6 K_2/K_5$ $(=K_2'$ from the definitions) $= 3.5 \times 10^{-5}$ M.

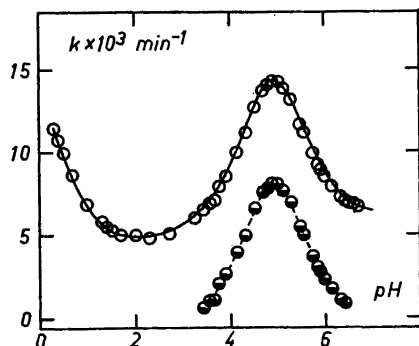*Fig. 7.* The over-all rate constant, k, as a function of pH. The symbols ○ represent experimental k-values, the symbols ◑ k''-values and the full drawn curve has been calculated from eqn. (3).

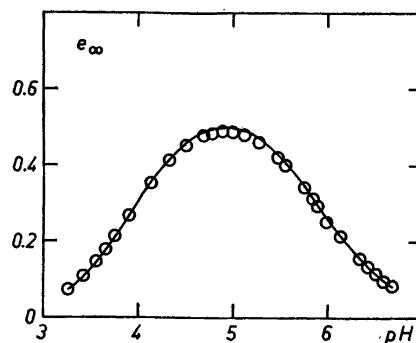*Fig. 8.* The equilibrium absorbancies, e_∞, as a function of pH. The symbols ○ represent experimental e_∞-values and the full drawn curve has been calculated from eqn. (2).

Fig. 7 shows the over-all rate constant, k, as a function of pH within the pH-range 0—7. The empty circles represent experimentally determined k-values, the half-filled circles represent k''-values calculated from eqns. (15) and (16) by the method described above. The full drawn curve has been calculated from eqn. (3) (the contribution from the reactions B + OH⁻ ⇌ C is neglected). The determined values of the constants have then been used.

Fig. 8 shows the equilibrium absorbancies, e_∞, as a function of pH within the pH-range 3—7. The circles are experimentally found values and the full drawn curve is calculated from eqn. (2) by using the determined values of e_B, e_G and the equilibrium constants K_1, K_2, K_4, K_5, K_6, and K_3'.
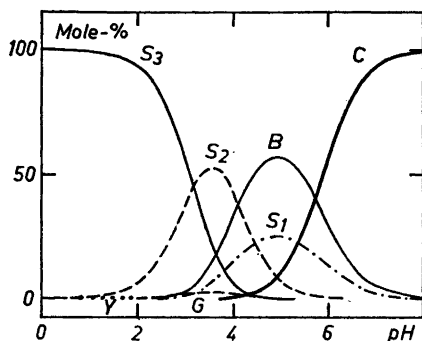

Fig. 9. The distribution of the dyestuff between the species B, G, Y, S_3, S_2, S_1, and C at equilibrium.

The distribution of the dyestuff between the species B, G, Y, S_1, S_2, S_3, and C at equilibrium is shown as a function of pH in Fig. 9.

DISCUSSION

Table 4 shows a comparison between the corresponding constants of Crystal Violet, 2-Pyridine Green, 3-Pyridine Green and 4-Pyridine Green. All these dyes have three basic groups and follow analogous reaction-equilibrium schemes. CV has three identical basic groups (dimethylamino groups), whereas the pyridine analogues (PG) have only two dimethylamino groups, the third basic group being the pyridine nitrogen atom.

Table 4. Comparison between the corresponding constants of Crystal Violet, 2-Pyridine Green, 3-Pyridine Green, and 4-Pyridine Green.

	Crystal Violet	2-Pyridine Green	3-Pyridine Green	4-Pyridine Green
K_1	0.122	0.49	0.21	0.146
$K_2 \times 10^4$	40.6	16.0	3.76	1.46
$K_1' \times 10^4$	0.49	7	1.7	1.7
$K_2' \times 10^5$	1.8	3.3	2.4	5.0
$K_3' \times 10^6$	2.5	4.3	5.1	1.8
K_4	79	14 000	1700	3100
K_5	0.032	20	1.4	3.5
K_6	0.00015	0.44	0.091	1.2
$k_1 \times 10^2$	11.7	1.8	6.3	5.6
$k_2 \times 10^5$	148	0.13	3.7	1.8
$k_3 \times 10^3$	6.6	4.4	3.7	3.9
$k_4 \times 10^3$	207	0.22	2.7	1.1
$k_5 \times 10^3$	0.37	6.2	5.2	8.3
$k_6 \times 10^2$	262	1.4	5.8	0.69
k_7	4.12	—	93	107
$k_8 \times 10^5$	14.8	—	0.25	6.1

The hydration equilibria $B + H_2O \rightleftharpoons S_1$, $G + H_2O \rightleftharpoons S_2$ and $Y + H_2O \rightleftharpoons S_3$ of PG differ markedly from the corresponding equilibria of CV in being displaced towards the colourless species S_1, S_2 and S_3. The electro-negative pyridine groups cause a decrease of the electron density of the central methane carbon atom as compared with CV, and the hydrated, carbinolic species are stabilized. The trend among the pyridine analogues is rather complicated. K_6 increases in the order 3-PG $<$ 2-PG $<$ 4-PG, whereas K_4 and K_5 increase in the order 3-PG $<$ 4-PG $<$ 2-PG. The species G and Y have a proton attached to the pyridine nitrogen and the unexpectedly great values of $K_{4(2\text{-PG})}$ and $K_{5(2\text{-PG})}$ may be due to the close proximity of the protonated pyridine nitrogen and the reaction centre, which makes the 2-pyridinium group more strongly electron-attracting than the 3-pyridinium and 4-pyridinium groups. The un-

protonated 4-pyridine group, however, seems to be more electronegative than the unprotonated 2-pyridine group.

The dissociation constant K_1 increases in the order CV < 4-PG < 3-PG < 2-PG. The increase of K_1 as one passes from CV to PG is also due to the electronegativity of the pyridinium groups. The order between the pyridine analogues is different from the order found for the hydration-equilibrium constants and suggests some correlation between the value of K_1 and the distance between the amino nitrogen and the pyridine nitrogen.

The proton from the reaction B + H$^+$ ⇌ G of PG is attached to the pyridine nitrogen atom (cf. Discussion in Ref.[1]) and therefore $K_{2(CV)}$ is not comparable to $K_{2(PG)}$. The pyridine analogues can be regarded as pyridine derivatives with the electron-attracting bis-(4-dimethylaniline)-methylidene cation substituted in *ortho*-, *meta*- and *para*-position. The great value of $K_{2(2\text{-PG})}$ as compared to $K_{2(3\text{-PG})}$ and $K_{2(4\text{-PG})}$ may be partly due to an *ortho*-effect of the bulky substituent.

The absorption curves of the species B and G of unsymmetrical triarylmethane dyes have the same general shape within the visible region of the spectrum.[6] There is one intense band at $\lambda = 600-700$ mμ (the x-band), and one weak band at $\lambda = 400-500$ mμ (the y-band). The former corresponds to a polarization along an axis through the dimethylamino groups, and the latter corresponds to a polarization along an axis perpendicular to this axis. It has been theoretically predicted [7] and found in practice [8] that the substitution of the phenyl group of Malachite Green for a more electronegative group should cause a bathochromic shift of the x-band. Table 5 shows that the absorption curves of the species B and G of CV and PG agree with this prediction. The species B of CV is, however, symmetrical and its absorption curve has only one band, the x-band, at $\lambda = 590$ mμ. The great value of λ_{Gmax} of 2-PG supports the opinion that the 2-pyridinium group is

Table 5. Comparison between the absorption maxima of Malachite Green, Crystal Violet, 2-Pyridine Green, 3-Pyridine Green, and 4-Pyridine Green.

	Species	λ_{max} mμ		$\varepsilon_{\text{max}} \times 10^{-4}$	
Malachite Green [5]	B	618	430	8.6	1.8
Crystal Violet	B	590	—	10.0	—
	G	631	424	8.3	1.4
2-Pyridine Green	B	639	419	8.8	1.3
	G	680	426	6.3	1.1
3-Pyridine Green	B	632	419	8.9	1.6
	G	655	425	7.8	1.2
4-Pyridine Green	B	636	420	7.9	1.3
	G	660	(395)	5.1	1.4

very strongly electron-attracting, since the wave length of the x-band increases with the electro-negativity of the third aryl group (*cf.* Ref.[8]).

The fact that $k_{3(PG)} < k_{5(PG)}$ is not quite consistent with a simple reaction mechanism involving the addition of a polarized water molecule to the dye cation as a rate-determining step. The higher positive charge of G would make it probable that $k_3 > k_5$ for such a mechanism (as it is with CV). The reaction-equilibrium scheme on page 1534 (scheme I) shows only the over-all reactions $B + OH^- \rightleftharpoons C$, $B + H_2O \rightleftharpoons S_1$, $G + H_2O \rightleftharpoons S_2$ and $Y + H_2O \rightleftharpoons S_3$, and does not consider the reaction mechanisms, which most probably are complex. Therefore, it seems difficult to draw any conclusions from the values of the experimental rate constants as long as the reaction mechanisms have not been established.

In addition to scheme I two other possible reaction-equilibrium schemes may be considered: $(R = Ar \cdot C \cdot (C_6H_4NMe_2)_2$

Scheme II.

and
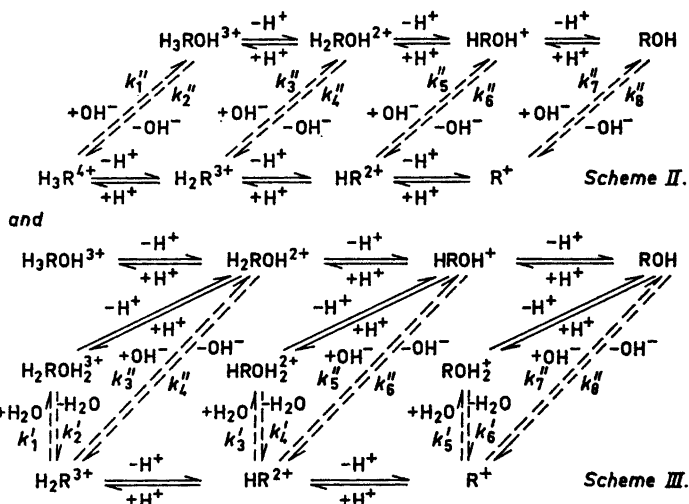
Scheme III.

From scheme II an expression similar to eqn. (3) can be derived. (The dissociation constant $K_3 = [H_2R^{3+}][H^+]/[H_3R^{4+}]$ is defined).

$$k = \frac{h^2 k_1'' K_W + hk_3'' K_W K_3 + k_5'' K_W K_1 K_3 + k_7'' oh K_1 K_2 K_3}{h^3 + h^2 K_3 + h K_1 K_3 + K_1 K_2 K_3} + \frac{h^3 k_2'' + h^2 k_4'' K_1' + hk_6'' K_1' K_2' + k_8'' K_1' K_2' K_3'}{h^3 + h^2 K_1' + h K_1' K_2' + K_1' K_2' K_3'}$$

(18)

This equation becomes analogous to eqn. (3) for $h \ll K_3$. Scheme III is identical with the "more probable" reaction scheme suggested by Cigén [3] for Crystal Violet. It is a combination of schemes I and II, in which the reactions $H_2R^{3+} + H^+ \rightleftharpoons H_3R^{4+}$ and $H_3R^{4+} + OH^- \rightleftharpoons H_3ROH^{3+}$ have been omitted. This reaction scheme gives an expression for the over-all rate constant, k, that is quite analogous to eqn. (3).

$$k = \frac{h^2 k_1' + h(k_3' + k_3'' K_W / K_1) K_1 + (k_5' + k_5'' K_W / K_2) K_1 K_2 + k_7'' oh K_1 K_2}{h^2 + h K_1 + K_1 K_2} +$$

$$\frac{h^3 k_2' + h^2 (k_4' + k_4'') K_1' + h(k_6' + k_6'') K_1' K_2' + k_8'' K_1' K_2' K_3'}{h^3 + h^2 K_1' + h K_1' K_2' + K_1' K_2' K_3'} \quad (19)$$

Thus these three reaction schemes seem indistinguishable and schemes II and III can eliminate the difficulty that $k_3 < k_5$. There are, however, two serious objections to scheme II: (1) The absorbancy of a strongly acid dye solution ($[H^+] = 5-18$ M) increases with increasing hydrogen ion concentration.[9] Such a behaviour is not consistent with scheme II, for the species H_3R^{4+} should be colourless. (2) The experimental values of k_1, k_3 and k_5 would give unreasonably great values to the rate constants $k_1'' = k_1 K_3 / K_W$, $k_3'' = k_3 K_1 / K_W$ and $k_5'' = k_5 K_2 / K_W$. This objection can also be made to scheme III unless the contributions from $k_3'' K_W / K_1$ and $k_5'' K_W / K_2$ to the experimental rate constants k_3 and k_5, respectively, are too small to be significant. Consequently, of the proposed reaction-equilibrium schemes, only scheme I is in accordance with the obtained results, although it offers no explanation of the fact that $k_3 < k_5$.

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