

Studies on the Pyridine Analogues of Malachite Green

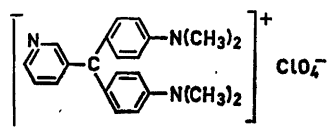
II. Protolytic Equilibria and Reaction Rates of 3-Pyridine Green in Aqueous Solution

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The studies of the protolytic equilibria and reaction rates in aqueous solution of a pyridine analogue of Malachite Green, called 4-Pyridine Green, described in a previous paper, have been continued by the corresponding studies of another pyridine analogue of Malachite Green, called 3-Pyridine Green. Five protolytic constants, three hydration constants and eight rate constants have been determined.

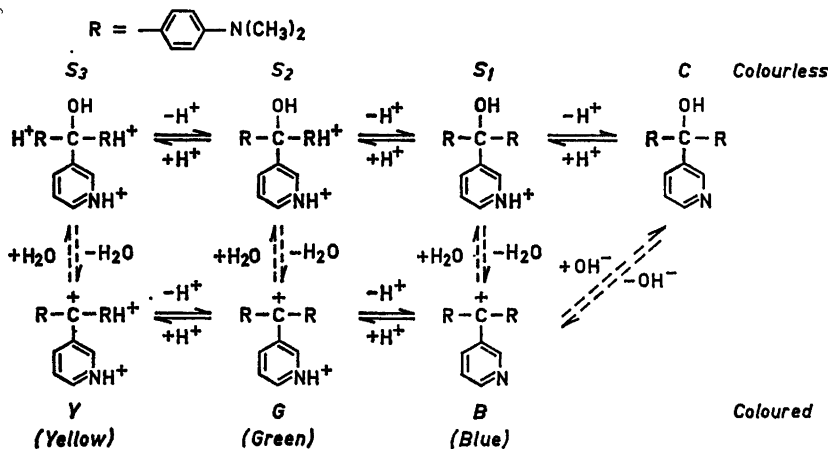
In part I of this investigation¹ the protolytic equilibria and the reaction rates of a pyridine analogue of Malachite Green, called 4-Pyridine Green, were studied spectrophotometrically in aqueous solutions. These studies have been continued by the corresponding studies of another pyridine analogue of Malachite Green, called 3-Pyridine Green, with the structural formula



3-Pyridine Green, like 4-Pyridine Green, follows a reaction-equilibrium scheme analogous to the one found by Cigén for Crystal Violet;² see p. 448. The equilibrium constants and the rate constants are defined as the corresponding constants of 4-Pyridine Green and Crystal Violet. The used symbols and notations are also analogous to those used in Refs.¹⁻²

EQUATIONS

The following, non-approximated, equations can be derived (*cf.* Refs.¹⁻²) for the absorbancy, e_0 , immediately after mixing a dye stock solution with



a buffer solution, the equilibrium absorbancy, e_∞ , and the over-all rate constant, k :

$$e = \frac{K_1 K_2 e_B + h K_1 e_G + h^2 e_Y}{K_1 K_2 + h K_1 + h^2} \quad (1)$$

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

$$k = \frac{1}{t} \ln \frac{e_0 - e_\infty}{e_t - e_\infty} = \frac{h^3 k_2 + h^2 k_4 K_1' + h k_6 K_1' K_2' + k_8 K_1' K_2' K_3'}{h^3 + h^2 K_1' + h K_1' K_2 + K_1' K_2' K_3'} + \frac{h^2 k_1 + h k_3 K_1 + k_5 K_1 K_2 + k_7 oh K_1 K_2}{h^2 + h K_1 + K_1 K_2} \quad (3)$$

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

EXPERIMENTAL

3-Pyridine Green Perchlorate. The leuco base and a dyestuff perchlorate were prepared by the method described for 4-Pyridine Green in Ref.¹ from N,N-dimethylaniline and 3-pyridine aldehyde (from Light & Co, Colnbrook, England). Concentrated hydrochloric acid was used as a condensing agent and the oxidization was carried out with lead dioxide. The leuco base was obtained as a white powder, m.p. 111–113°C. The dyestuff perchlorate consisted of very small, blue-green crystals. (Found: C 60.0; H 5.76; N 9.23; O 15.0; Cl 9.44. Calc. for $C_{22}H_{24}N_3O_4Cl$: C 61.5; H 5.63; N 9.77; O 14.9; Cl 8.25). The carbinol base of the dyestuff was also prepared and was obtained as a slightly brownish powder. (Found: C 75.5; H 7.07; N 12.1; O 4.55. Calc. for $C_{22}H_{26}N_3O$: C 76.0; H 7.25; N 12.1; O 4.61). The absorption curves of the perchlorate and the carbinol base in an acetate buffer with $pH = 4.68$ were so nearly identical that they could not be drawn apart in a graph. The perchlorate was used throughout the measurements.

A dyestuff stock solution, containing all of the dyestuff as coloured species, was prepared by dissolving a weighed amount of the dyestuff perchlorate in acetone. The chemicals used for the buffer solutions and the apparatus used for the measurements were the same as¹ in Refs.^{1,2} The measurements were carried out in essentially the same manner as was described for Crystal Violet and 4-Pyridine Green.

Table 1. 3-Pyridine Green. e_0 - and e_∞ -values in phosphate buffers. $C_M = 1 \times 10^{-5}$ M; $d = 1$ cm; $T = 20.0^\circ\text{C}$; $\lambda = 630$ m μ and 660 m μ .

[H ⁺] $\times 10^7$ M	$\lambda = 630$ m μ		$\lambda = 660$ m μ
	e_0	e_∞	e_0
0.173	0.885	0.0319	0.365
0.262	0.887	0.0476	0.367
0.355	0.886	0.0628	0.364
0.548	0.887	0.0934	0.366
0.775	0.892	0.1242	0.367
1.324	0.884	0.1945	0.365
2.056		0.266	
3.119		0.344	
4.69		0.426	
Mean	0.887		0.366

MEASUREMENTS AND RESULTS

Absorption curves. The absorption curves of the three coloured species B, G, and Y are shown in Fig. 1. The curve of B was obtained from the equilibrium absorbancies in a phosphate buffer, pH = 6.61. At this pH B is the only coloured species present in appreciable concentration. Only a fraction of the dyestuff is present, however, as the species B; the remainder is distributed among the colourless species C, S₁, and S₂. The molar absorptancy coefficients were therefore determined in the following manner: 1 ml of the dye stock solution in acetone, which contained all of the dyestuff as coloured species, was mixed with suitable phosphate buffers, pH = 6.9–7.8, and the e_0 -values were determined. Within this pH-range $e_0 \approx e_B$. Table 1 shows the results obtained at two wave lengths, $\lambda = 630$ m μ and 660 m μ . The mean values $e_B = 0.887$ and 0.366 , respectively, were obtained ($C_M = 1 \times 10^{-5}$ M; $d = 1$ cm).

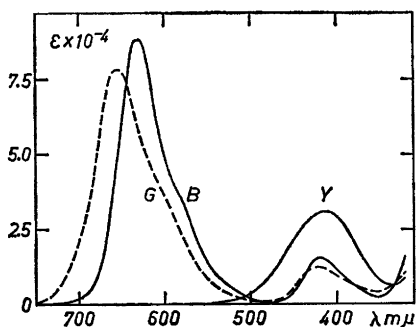


Fig. 1. Absorption curves of 3-Pyridine Green in aqueous solutions. The curves B, G, and Y are the curves of the blue species B, the green species G, and the yellow species Y, respectively.

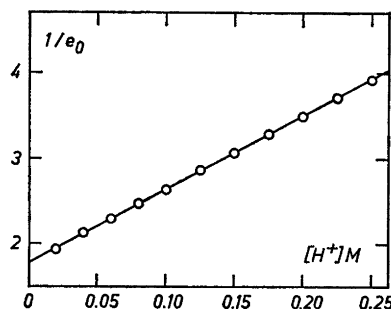


Fig. 2. $1/e_0$ versus $[\text{H}^+]$ for the determination of K_1 .

The curve of G was calculated from the e_0 -values at different wave lengths in an acetate buffer, pH = 3.26, by the use of eqn. (1). K_2 was obtained from measurements described below and e_B was obtained from the absorption curve of B. The concentration of Y was negligible.

The curve of Y was calculated in essentially the same manner as the curve of G from the e_0 -values in 0.500 M HCl. At this hydrogen ion concentration [B] is negligible. K_1 was obtained from measurements described below and e_G was obtained from the absorption curve of G.

The results can be summarized as follows:

	λ_{\max}	$\epsilon_{\max} \times 10^{-4}$
B	632 and 419 m μ	8.9 and 1.6
G	655 and 425 m μ	7.7 and 1.2
Y	413 m μ	3.1

Determination of K_1 . In strongly acid solutions ($[H^+] \gg K_2$) eqn. (1) can be approximated to

$$\frac{1}{e_0} = \frac{1}{e_G} + \frac{1}{e_G K_1} h \quad (4)$$

From Fig. 2 ($C_M = 1 \times 10^{-5}$ M; $d = 1$ cm; $\lambda = 630$ m μ) we obtain the intercept on the ordinate-axis $y = 1.784$ and the slope $l = 8.58$ M $^{-1}$. Hence $K_1 = 0.208 \pm 0.005$ M and $e_G = 0.561$.

Determination of K_2 . Within a pH-range, where [Y] can be neglected, eqn. (1) can be approximated and transformed to

$$\frac{h}{e_0 - e_B} = \frac{K_2}{e_G - e_B} + \frac{h}{e_G - e_B} \quad (5)$$

Table 2. 3-Pyridine Green. e_0 -values in HCl + KCl-buffers for the determination of K_1 . $C_M = 1 \times 10^{-5}$ M; $d = 1$ cm; $T = 20.0^\circ\text{C}$; $\lambda = 630$ m μ .

[H $^+$] M	e_0	[H $^+$] M	e_0
0.040	0.470	0.150	0.326
0.060	0.435	0.175	0.304
0.080	0.403	0.200	0.286
0.100	0.379	0.225	0.269
0.125	0.349	0.250	0.255

Table 3. 3-Pyridine Green. e_0 -values in acetate buffers for the determination of K_2 . $C_M = 1 \times 10^{-5}$ M; $d = 1$ cm; $T = 20.0^\circ\text{C}$; $\lambda = 660$ m μ ; $e_B = 0.366$.

[H $^+$] $\times 10^4$ M	e_0	[H $^+$] $\times 10^4$ M	e_0
1.291	0.466	2.838	0.533
1.799	0.492	3.785	0.562
2.275	0.513	5.585	0.599

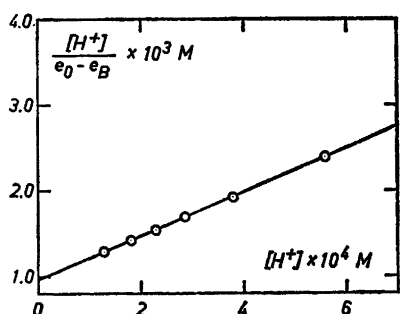


Fig. 3. $[H^+]/(e_0 - e_B)$ versus $[H^+]$ for the determination of K_2 .

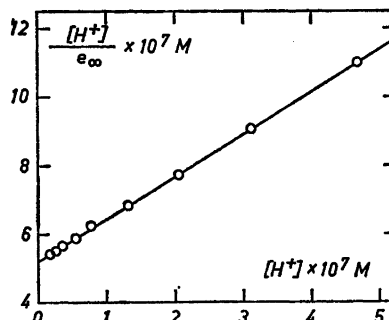


Fig. 4. $[H^+]/e_\infty$ versus $[H^+]$ for the determination of $1 + K_6$ and $K_3'K_6$.

The measurements were carried out in acetate buffers, $\text{pH} = 3.25\text{--}3.75$, at the wave length $\lambda = 660 \text{ m}\mu$. $e_B = 0.366$ was obtained from e_0 in phosphate buffers, $\text{pH} = 6.9\text{--}7.8$ (Table 1). $C_M = 1 \times 10^{-5} \text{ M}$; $d = 1 \text{ cm}$; $T = 20.0^\circ\text{C}$. From Fig. 3 the intercept on the ordinate-axis $y = 0.000967 \text{ M}$ and the slope $l = 2.57$ are obtained and thus $K_2 = (3.76 \pm 0.08) \times 10^{-4} \text{ M}$ and $e_G = 0.755$.

Determination of $K_3'K_6$ and $1 + K_6$. Within a pH-range, where B, S_1 , and C are the only species to be considered, eqn. (2) is approximated to

$$\frac{h}{e_\infty} = \frac{K_3'K_6}{e_B} + \frac{1 + K_6}{e_B} h \quad (6)$$

Fig. 4 shows h/e_∞ versus h within the pH-range 6.3–7.8. ($C_M = 1 \times 10^{-5} \text{ M}$; $d = 1 \text{ cm}$; $\lambda = 630 \text{ m}\mu$; $e_B = 0.887$). The straight line has the intercept on the ordinate-axis $y = 5.19 \times 10^{-7} \text{ M}$ and the slope $l = 1.238$. $K_3'K_6 = 4.60 \times 10^{-7} \text{ M}$ and $1 + K_6 = 1.098$.

Determination of K_5 and K_4 . Eqn. (2) can be approximated and rewritten as

$$K_5 + \frac{K_4}{K_1} h = \frac{e_G}{e_\infty} + \frac{K_2 e_B}{h e_\infty} - \frac{K_2(1 + K_6)}{h} - 1 \quad (7)$$

within the pH-range 3.0–3.7, where [Y] and [C] can be neglected. If the right member of eqn. (7), Q , is plotted versus h , a straight line is obtained. The intercept of this line on the ordinate-axis gives K_5 , and the slope gives K_4/K_1 . The known values $K_2 = 3.76 \times 10^{-4} \text{ M}$; $1 + K_6 = 1.098$ were used in the calculation of Q . The measurements of e_∞ were carried out at two different wave lengths, $\lambda = 630 \text{ m}\mu$ ($e_B = 0.887$; $e_G = 0.561$) and $\lambda = 660 \text{ m}\mu$ ($e_B = 0.366$; $e_G = 0.755$). The following results were obtained: $\lambda = 630 \text{ m}\mu$: $K_5 = 1.38$; $K_5 = 1.56 \times 10^3$. $\lambda = 660 \text{ m}\mu$: $K_5 = 1.38$; $K_4 = 1.47 \times 10^3$, since $K_1 = 0.208 \text{ M}$. Hence $K_5 = 1.4 \pm 0.1$.

The hydration-equilibrium constant K_4 was also calculated from the equilibrium absorbancies in HCl + KCl-buffers ($[H^+] = 0.0020\text{--}0.0080 \text{ M}$) by using the expression

Table 4. 3-Pyridine Green. e_{∞} -values in acetate buffers and HCl + KCl-buffers for the determination of K_5 and K_4 .

Acetate buffers $C_M = 1 \times 10^{-5} \text{ M}; d = 1 \text{ cm}$			HCl + KCl-buffers $C_M = 2 \times 10^{-5} \text{ M}; d = 5 \text{ cm}; \lambda = 630 \text{ m}\mu$		
$[\text{H}^+] \times 10^4 \text{ M}$	$\lambda = 630 \text{ m}\mu$	e_{∞} $\lambda = 660 \text{ m}\mu$	$[\text{H}^+] \text{ M}$	$E_{\infty} = 5e_{\infty}$	$K_4 \times 10^3$
1.291	0.474		0.002	0.382	1.70
1.799	0.406	0.254	0.003	0.246	1.71
2.275	0.351	0.236	0.004	0.181	1.72
2.838	0.295	0.213	0.005	0.144	1.71
3.785	0.230	0.181	0.006	0.120	1.70
5.585	0.159	0.141	0.008	0.090	1.69
9.12	0.095	0.097			
				Mean	1.71
K_5 graph.	1.38	1.38			
K_4 graph.	1.57×10^3	1.47×10^3			

$$K_4 = \frac{K_1 K_2 e_B + h K_1 e_G}{h e_{\infty}} - \frac{K_1 K_2 (1 + K_6)}{h^2} - \frac{h + K_1 (1 + K_5)}{h} \quad (8)$$

Table 4 shows the obtained results. $C_M = 2 \times 10^{-5} \text{ M}; d = 5 \text{ cm}; \lambda = 630 \text{ m}\mu; E_B = 10 \times 0.887; E_G = 10 \times 0.561; K_1 = 0.208 \text{ M}; K_2 = 3.76 \times 10^{-4} \text{ M}; 1 + K_6 = 1.098; K_5 = 1.4$. The mean value $K_4 = (1.7 \pm 0.2) \times 10^3$ was obtained. This value is more reliable than the values obtained within the pH-range 3.2–3.7, since the equilibrium $Y + \text{H}_2\text{O} \rightleftharpoons S_3$ is more dominating at the higher hydrogen ion concentrations, and the absorbancies are yet sufficiently high to make accurate measurements possible. The reproducibility was good in HCl + KCl-buffers, whereas it was less good in acetate buffers.

Calculation of K_1' . This constant could not be determined directly but was calculated from the relation

$$K_1' = \frac{K_1 K_5}{K_4} \quad (9)$$

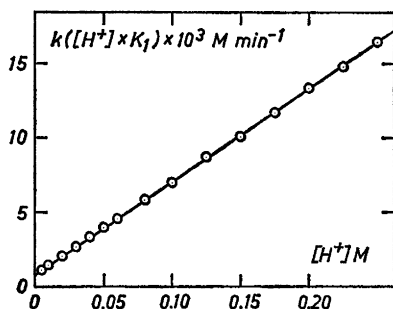


Fig. 5. $k([\text{H}^+] + K_1)$ versus $[\text{H}^+]$ for the determination of k_1 . $[\text{H}^+] = 5\text{--}250 \text{ mM}$.

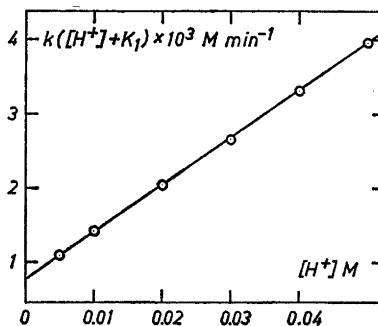


Fig. 6. $k([\text{H}^+] + K_1)$ versus $[\text{H}^+]$ for the determination of k_3 . $[\text{H}^+] = 5\text{--}50 \text{ mM}$.

Thus $K_1' = (1.7 \pm 0.4) \times 10^{-4} \text{ M}$, since $K_1 = 0.208 \text{ M}$, $K_5 = 1.4$ and $K_4 = 1.7 \times 10^3$.

Determination of k_1 , k_2 , k_3 and k_4 . Figs. 5 and 6 show $k(h + K_1)$ versus h ($K_1 = 0.208 \text{ M}$) within the pH-range 0.6–2.3. A straight line is obtained according to the expression

$$k(h + K_1) = k_1 h + k_3 K_1 \quad (10)$$

which is valid in strongly acid solutions. Fig. 5 gives the slope $l = 0.0625 \text{ min}^{-1}$ and Fig. 6, which is an enlargement of the lower, left part of Fig. 5, gives the intercept on the ordinate-axis $y = 8.78 \times 10^{-4} \text{ M min}^{-1}$. $k_1 = 0.063 \pm 0.001 \text{ min}^{-1}$ and $k_3 = (3.7 \pm 0.1) \times 10^{-3} \text{ min}^{-1}$. Since $k_2 = k_1/K_4$ and $k_4 = k_3/K_5$, we also obtain $k_2 = (3.7 \pm 0.5) \times 10^{-5} \text{ min}^{-1}$ and $k_4 = (2.7 \pm 0.4) \times 10^{-3} \text{ min}^{-1}$.

Determination of k_7 and k_8 . In strongly alkaline solutions the overall rate constant, k , increases linearly with $[\text{OH}^-]$ according to the equation

$$k = k_5 + k_8 + k_7 \text{oh} \quad (11)$$

$k_5 + k_8 \ll k_7 \text{oh}$ within the pH-range 11.2–12.0 as can be seen from Fig. 7, where the straight line runs through the origin. The slope of the line $l = 93.0 \text{ min}^{-1} \text{ M}^{-1}$ gives $k_7 = 93 \pm 2 \text{ min}^{-1} \text{ M}^{-1}$. $k_8 = k_7 K_w / K_3' K_6$ gives $k_8 = (2.5 \pm 0.1) \times 10^{-6} \text{ min}^{-1}$, since $K_w = 1.24 \times 10^{-14} \text{ M}^2$ and $K_3' K_6 = 4.60 \times 10^{-7} \text{ M}$.

Determination of k_5 . In phosphate buffers (pH = 6.7–7.8) eqn. (3) can be approximated to

$$k - k_7 \text{oh} = k_5 + \frac{h k_6 + k_8 K_3'}{h + K_3'} \quad (12)$$

Fig. 8 shows $k - k_7 \text{oh}$ as a function of h . An extrapolation of the almost straight line to $[\text{H}^+] = 0$ gives $k_5 + k_8 = 5.23 \times 10^{-3} \text{ min}^{-1}$. $k_8 \ll k_5$ and thus $k_5 = (5.2 \pm 0.1) \times 10^{-3} \text{ min}^{-1}$.

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

$$k'' = \frac{h k_6 K_2'}{h^2 + h K_2' + K_2' K_3'} \quad (13)$$

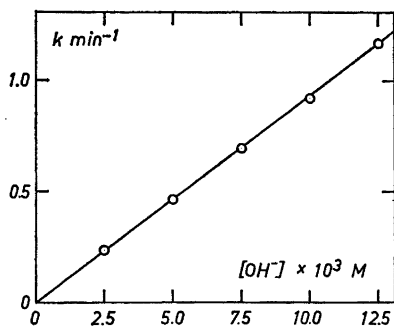


Fig. 7. The over-all rate constant, k , versus $[\text{OH}^-]$ for the determination of k_7 .

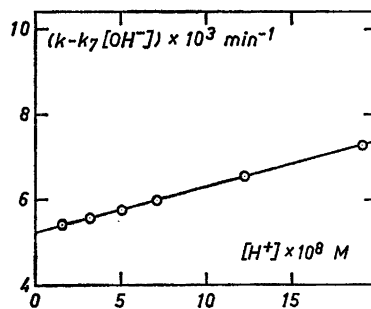


Fig. 8. $k - k_7[\text{OH}^-]$ as a function of $[\text{H}^+]$ for the determination of k_5 .

where

$$k'' = k' / \left[1 - \frac{k'h^2}{k_6 K_1' K_2'} \right] \quad (14)$$

and

$$k' = k - \frac{h^3 k_2 + h^2 k_4 K_1' + k_8 K_1' K_2' K_3'}{h^3 + h^2 K_1' + h K_1' K_2' + K_1' K_2' K_3'} - \frac{h^2 k_1 + h k_3 K_1 + k_5 K_1 K_2 + k_7 \text{oh} K_1 K_2}{h^2 + h K_1 + K_1 K_2} \quad (15)$$

The differentiation of eqn. (13) with regard to h shows that k'' has a maximum when $h = \sqrt{K_2' K_3'}$ and hence

$$k''_{\max} = \frac{k_5 \sqrt{K_2' K_3'}}{2 K_3' K_6 + K_6 \sqrt{K_2' K_3'}} \quad (16)$$

The third term of the right member of eqn. (15) can be calculated by using the constants already determined. In the second term all the constants except K_2' and K_3' are known. These two constants are determined by a series of successive approximations. As a first approximation k'' is put equal to k and from Fig. 9 $\text{pH}_{k_{\max}} = 4.96$ and $k_{\max} = 0.0353 \text{ min}^{-1}$ are obtained and thus $K_2' K_3' \approx 1.2 \times 10^{-10} \text{ M}^2$. These values are used in eqn. (16) together with the known values of $k_5 = 5.2 \times 10^{-3} \text{ min}^{-1}$ and $K_3' K_6 = 4.60 \times 10^{-7} \text{ M}$. Then approximate values of K_6 , K_3' , K_2' and $k_6 = k_5/K_6$ are obtained, which are used for the calculation of k' and k'' . A new set of values can then be calculated from eqn. (16). The calculations are repeated until further calculations do not appreciably change the obtained results. In Fig. 9 the dashed curve represents the final values of k'' as a function of pH within the pH-range 3.9–6.0. The following results are obtained: $\text{pH}_{k''_{\max}} = 4.958 \pm 0.005$; $k''_{\max} = 0.0300 \text{ min}^{-1}$

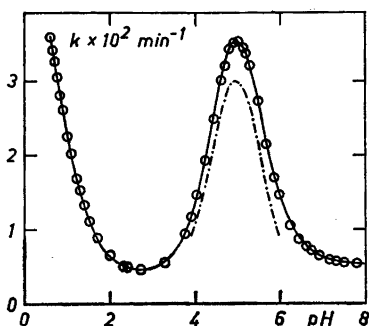


Fig. 9. The over-all rate constant, k , as a function of pH within the pH-range 0.6–8. The symbols \circ represent experimental k -values, the dashed curve represents the k'' -values and the full-drawn curve has been calculated from eqn. (3).

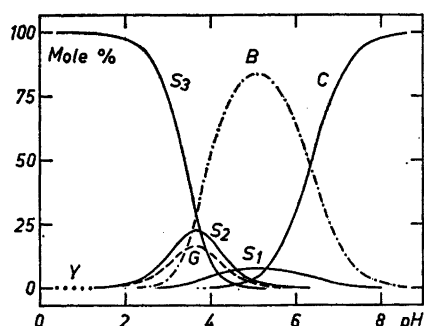


Fig. 10. The distribution of 3-Pyridine Green between the different species at equilibrium as a function of pH.

Table 5. Comparison between the corresponding constants of Crystal Violet, 4-Pyridine Green and 3-Pyridine Green.

	Crystal Violet	4-Pyridine Green	3-Pyridine Green		Crystal Violet	4-Pyridine Green	3-Pyridine Green
K_1	0.122	0.146	0.208	k_1	0.117	0.056	0.063
$K_2 \times 10^4$	40.6	1.46	3.76	$k_2 \times 10^5$	148	1.8	3.7
$K_1' \times 10^4$	0.49	1.7	1.7	$k_3 \times 10^3$	6.6	3.9	3.7
$K_2' \times 10^5$	1.8	5.0	2.4	k_4	0.207	0.0011	0.0027
$K_3' \times 10^6$	2.5	1.8	5.1	$k_5 \times 10^3$	0.37	8.3	5.2
				k_6	2.620	0.0069	0.058
K_4	79	3100	1700	k_7	4.12	107	93
K_5	0.032	3.5	1.4	$k_8 \times 10^6$	148	0.6	2.5
K_6	0.00015	1.2	0.091				

and hence $K_6 = 0.091 \pm 0.002$; $K_3' = (5.1 \pm 0.2) \times 10^{-6}$ M; $K_2' = (2.4 \pm 0.2) \times 10^{-5}$ M and $k_6 = 0.058 \pm 0.004$ min⁻¹.

The constants k_6 , K_2' , K_3' and K_1' were also calculated from the kinetic measurements reported above by means of a high-speed electronic computer. The following values were obtained: $k_6 = 0.058$ min⁻¹; $K_2' = 2.4 \times 10^{-5}$ M; $K_3' = 5.2 \times 10^{-6}$ M and $K_1' = 1.8 \times 10^{-4}$ M.

In Fig. 9 the circles represent experimental k -values, the dashed curve represents the final k'' -values and the full-drawn curve is calculated from the non-approximated eqn. (3) by using the determined values of the constants. Fig. 10 shows the distribution of the dyestuff between the species B, G, Y, S₃, S₂, S₁, and C at equilibrium.

Table 5 shows a comparison between the corresponding constants of Crystal Violet, 4-Pyridine Green and 3-Pyridine Green. A discussion of the results will be reserved until the studies of the remaining Pyridine analogue, 2-Pyridine Green, which are now proceeding, have been completed.

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