370. *The Ionization of Basic Triphenylmethane Dyes.*

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The basic triphenylmethane dyes, at a given pH, reach equilibrium between kations [*e.g.*, (I)] and carbinols [*e.g.*, (II)] at rates which vary considerably for different members. Potentiometric titration being unsuitable for the determination of the basic ionization constants, a photometric method has been devised which is of general applicability to the study of kations like these, which are capable of isomerism to carbinols.

In this way, the rates of carbinol formation, and the degree of ionization *at equilibrium*, have been determined at various pH values. The ionization constants of these substances are independent of the pH values at which they were obtained and thus may be compared with those derived from a simple ionization such as that of an aromatic amine.

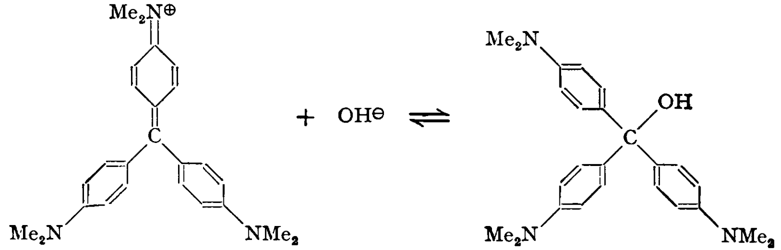
The significance of carbinol formation in relation to the biological action of these substances is discussed.

The aminotriphenylmethane dyes are of interest from both chemical and biological angles. Though their use as dyes has diminished owing to the production of more stable substances, they occupy an important position in the theory of colour and structure; the low concentration in which some of them inhibit the growth of certain bacteria compares favourably with all other known substances (Kligler, *J. Exp. Med.*, 1918, **27**, 463; Professor S. D. Rubbo, private communication; see also Addendum, p. 1731).

It has recently been shown that, in a series of acridine bases, bacteriostatic power is proportional to the degree of ionization at pH 7 (Albert, Rubbo, Goldacre, Davey, and Stone, *Brit. J. Exp. Path.*, 1945, **26**, 160). A similar relation for the basic triphenylmethanes was suggested by Stearn and Stearn (*J. Bact.*, 1924, **9**, 491), who, because of the lack of ionization constants in the literature, made rough estimates of comparative basic strength on the basis of the inductive constants of substituent groups. Accurate measurements of the ionization constants of these substances are now reported, most of them for the first time.

Although acceptable measurements of several second and third ionization constants have been reported (Adams and Rosenstein, *J. Amer. Chem. Soc.*, 1914, **36**, 1452; Schwarzenbach, *Helv. Chim. Acta*, 1938, **21**, 1636), the position regarding the more interesting first constants has long been unsatisfactory. Sidgwick and Moore (*J.*, 1909, **95**, 889) and Sidgwick and Rivett (*ibid.*, p. 899) examined the effect of acid and alkali on the carbinols and salts (respectively) of brilliant-green, malachite-green, and Doebner's violet and concluded that the bases must be very strong. Rumpf (*Ann. Chim.*, 1935, [xi], **3**, 404) reported first ionization constants for *p*-aminotriphenylcarbinol (pK_a, *ca.* 4·6), Doebner's violet (*ca.* 5·8), and parafuchsin (*ca.* 7) by potentiometric titration in dilute alcohol, although this solvent complicates the picture because of ether formation (cf. Fischer, *Ber.*, 1900, **33**, 3356). Rumpf (*loc. cit.*) also roughly confirmed these figures colorimetrically. Henriquez (*Rec. Trav. chim.*, 1933, **52**, 991) obtained data from which the first ionization constant of malachite-green may be calculated.

Hantzsch and Osswald (*Ber.*, 1900, **33**, 274, 752) showed that the conductivity of the aminotriphenylmethane bases, after liberation from their salts with alkali, decreases with time. A parallel phenomenon was encountered when potentiometric titration of crystal-violet and malachite-green was attempted, the pH continuing to fall slowly after each addition of alkali. This drop in pH is brought about by the steady transformation of the dye ion (I) into the colourless carbinol (II), an isomerism caused by a hydroxyl ion attacking the central carbon atom.



(I: crystal-violet, ion.) (II; crystal-violet, carbinol.)

An experimental method, which attempts to overcome the defects of previous methods, is described below. Water has been chosen as solvent so that the results can be used to interpret biological data. Colorimetry has been preferred to potentiometry because it permits of more accurate measurement in the dilutions (10^{-6}M.) at which it is necessary to work in order to retain the carbinol in aqueous solution. The speed at which the equilibrium (I $\longleftrightarrow$ II) was attained was measured at various pH values, and the degree of ionization, at each pH value, was calculated only after equilibrium had been established. Thus the method differs from those of Hantzsch, of Sidgwick and co-workers (*loc. cit.*) and of Hochberg and LaMer (*J. Amer. Chem. Soc.*, 1941, **63**, 3110), who took no steps to control the ever-changing pH of their solutions. It is not possible to use their results to calculate equilibrium ionization constants because the speed of the reaction and the position of the equilibrium depend on the pH.

In the present method, ionization constants were calculated from the degrees of ionization of the bases at equilibrium at various pH values, by means of the law of mass action:

$$pK = pH + \log \alpha/(1 - \alpha) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where α = degree of ionization.

Typical results for malachite-green and 4-dimethylaminotriphenylcarbinol are given in Table I.

The constancy of the p*K* over a wide pH range indicates that the simple law of mass action, equation (1), is being obeyed, in spite of the complexity of the reaction, and of the possibility of the co-existence of various forms of ion (coloured and uncoloured) and of base as suggested by Hantzsch, by Henriquez, and by Schwarzenbach, Mohler, and Sorge (*Helv. Chim. Acta*, 1938, **21**, 1636).

TABLE I.

Effect of pH on degree of ionization.

(*a*) Malachite-green, 2·7 × 10^{-6}M. at 25°.

pH.	Extinction coefficient (*E*) (red filter).	Degree of ionization (α).	log $\alpha/(1 - \alpha)$.	p*K*.
4·00	0·113	1·00	—	—
6·09	0·097	0·86	0·80	6·89
6·60	0·075	0·66	0·30	6·90
6·78	0·060	0·53	0·06	6·84
6·97	0·052	0·46	−0·07	6·90
7·10	0·044	0·39	−0·20	6·90
7·42	0·026	0·23	−0·52	6·90
10·1	0·000	0·00	—	—

(*b*) 4-Dimethylaminotriphenylcarbinol, 4 × 10^{-5}M. at 25°.

pH.	*E* (violet filter).	α.	log $\alpha/(1 - \alpha)$.	p*K*.
1·0	0·555	1·00	—	—
3·96	0·467	0·85	0·76	4·72
4·27	0·417	0·76	0·50	4·77
4·49	0·360	0·66	0·29	4·78
4·61	0·322	0·59	0·15	4·76
4·80	0·261	0·48	−0·05	4·75
4·98	0·201	0·37	−0·25	4·73
4·99	0·197	0·36	−0·26	4·73
9·20	0·000	0·00	—	—

TABLE II.

Ionization constants in water at 25° ± 2° and half-equilibrium times.

$T\frac{1}{2}$ = time required for degree of ionization to fall half-way from 100% to its equilibrium value, at a pH value equal to the p*K*.

Substance (dye and/or relevant carbinol).	Conc. × 10^6 (molar).	p*K*.	$T\frac{1}{2}$, hours.
Crystal-violet (4 : 4′ : 4″-trisdimethylaminotriphenylcarbinol)	1·8	9·36	7·5
Malachite-green (4 : 4′-bisdimethylaminotriphenylcarbinol)	2·7	6·90	2·1
Brilliant-green (4 : 4′-bisdiethylaminotriphenylcarbinol)	2·0	7·90	2·9
4-Dimethylaminotriphenylcarbinol	40·0	4·75	—
Pararosaniline (parafuchsin; 4 : 4′ : 4″-triaminotriphenylcarbinol)	3·0	7·57 carbinol >13 imine	0·85
Doebner's violet (4 : 4′-diaminotriphenylcarbinol)	42·0	5·38	—
Michler's hydrol (4 : 4′-bisdimethylaminodiphenylcarbinol)	34·0	5·61	<0·01
Auramine (4 : 4′-bisdimethylaminodiphenylcarbinolamine)	3·9	9·78 carbinol 10·71 imine	18·5
Fast acid green N [light green SF yellowish; Lissamine green SFS; di-sodium salt of 4 : 4′-di-(*N*-ethyl-*p*-sulphobenzylamino)triphenylcarbinol-4″-sulphonic acid]	4·5	11·0 (approx.)	—

TABLE III.

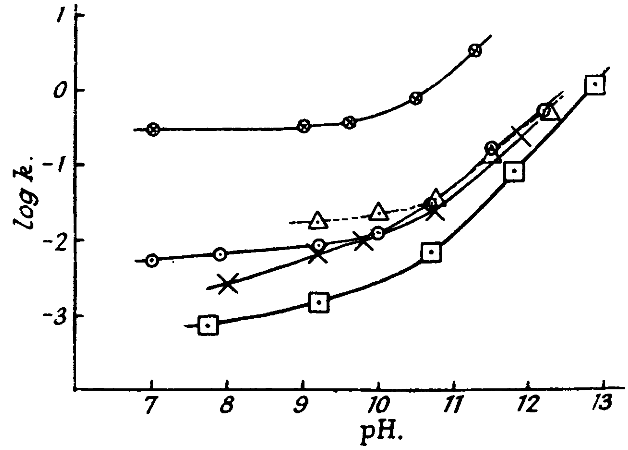
Rate of disappearance of the coloured ion of malachite-green.

Concentration : 1·3 × 10^{-5}M. at pH 7·90 (20°).

Time, mins.	Extinction coefficient (red filter).	*k* × 10^3.	Time, mins.	Extinction coefficient (red filter).	*k* × 10^3.
2	0·424	—	42	0·320	6·4
12	0·386	—	52	0·300	6·4
22	0·362	6·4	∞	0·100	—
32	0·341	6·0			

Ionization constants determined in this way for a number of compounds are given in Tables II and V. The attainment of equilibrium with many of the triphenylmethanes required one or two days. A rough indication of the speed is given in the fourth column (Table II), which is the time required to proceed half-way to equilibrium at a pH equal to the p*K*. Velocity constants for the initial rate of disappearance of the coloured ion at various pH values are plotted in the annexed Figure. These represent apparent first-order reactions between the dye ion and the hydroxyl ion, for the latter is in large excess and is not consumed, the buffer strength being 10^{-2}M. compared with a dye concentration of about 10^{-6}M. A typical result is given in Table III. *K* was found to be independent of the dye concentration over a six-fold range.

Crystal-violet, malachite-green, brilliant-green, and Michler's hydrol are tertiary amines, the ions of which exist in equilibrium with the corresponding carbinol form. However, in the primary amines (pararosaniline, auramine), an additional basic form appears, the dehydrated carbinol, variously known as anhydro-base, imine or Homolka base [*e.g.*, $(NH_2\cdot C_6H_4)_2C{:}C_6H_4{:}NH$ for pararosaniline]. Having a different structure from the carbinol, it should have a different p*K*. The equilibrium p*K* as determined for these primary amines is the resultant of the simultaneous ionization of the imine and the carbinol. It was possible to obtain separate ionization constants by observing at each pH the degree of ionization (*a*) at the beginning, after a primary fast equilibrium (equation 2), and (*b*) after the system had finally reached equilibrium due to a subsequent slow reaction (equation 3).



× *Brilliant-green* 25°. ⊙ *Malachite-green* 20°. ⊡ *Crystal-violet* 25°.
△ *Parafuchsin* 18°. ⊗ *Doebner's violet* 25°.

Fast reaction : $X'{=}NH_2^{\oplus} + OH^{\ominus} \longrightarrow X{=}NH$ (imine) $+ H_2O$ . . (2)

Slow reaction : $X'{=}NH + H_2O \longrightarrow OH{-}X{-}NH_2$ (carbinol) . . . (3)

Reaction (2), which involves only ions, is almost instantaneous. It yields a p*K* for the imine (constant, *e.g.*, for auramine within ±0·1 p*K* unit over a range of 20—83% ionization) which is higher than that found when the system has finally reached equilibrium (Table IV).

Table IV.

Instantaneous and equilibrium ionization constants of triphenylmethane dyes having a primary amino-group.

	Auramine.	Pararosaniline.
Instantaneous p*K* of imine	10·71	about 13—14
Equilibrium p*K*	9·84	7·57
Carbinol p*K* (calc.)	9·78	7·57
[Carbinol]/[imine] at equilibrium	8·5	10^6

Auramine, though not a triphenylmethane dye, was included because of its close relation to pararosaniline. Only a rough estimate for the imine p*K* of the latter was possible because it was necessary to work at pH 13. This is above the range of accurate measurement by the glass electrode, and the pH was calculated from the concentration of potassium hydroxide in the solution. In aqueous solution, the imine changed much more rapidly into the carbinol than was the case with auramine. The amount of imine present was determined by taking advantage of the fact that it is yellow and soluble in ether, whereas the salt and the carbinol do not dissolve in ether. In wet ether the change to the carbinol (by hydration) is slow. The pH giving half the maximum depth of colour in the ether, when the solution was shaken with a given ratio of ether immediately after mixing the buffer and the dye, was taken as the p*K* of the imine (Table IV).

In auramine the imine and carbinol are colourless, but the slow change of the former into the latter was followed in the ultra-violet region at 3650 A. where the difference in light absorption was great. A velocity constant at pH 10 of approximately 10^{-2} was obtained. The change is ascribed to the transformation of imine (strong base) into carbinol (weaker base), both eventually reaching equilibrium with the coloured ion.

Results.—These will be discussed under five headings.

(i) *Basic strength.* The basic triphenylmethane dyes might be expected to be strong bases, for some, such as crystal-violet, are quaternary and all are (vinylogous) amidines and guanidines and hence should have a far greater degree of resonance in the ions than exists in the non-ionized bases. For example, guanidine has a p*K* of 13·6 (Hall and Sprinkle, *J. Amer. Chem. Soc.*, 1932, **54**, 3469) and benzamidine of 11·6 (0·1M., cf. Albert, Mills, and Royer, *J.*, 1948, 1452). It is seen from Table II that the common basic triphenylmethane dyes have comparatively low p*K* values. These differences may be ascribed to (*a*) the great tendency of the hydroxyl ion to become attached to the central carbon atom and form a carbinol, and (*b*), in some cases, the effect of substitution on the symmetry of the resonance; when substitution diminishes the symmetry, the ionic resonance, and so the basic strength, may be diminished (Pauling, "The Nature of the Chemical Bond," Cornell, N.Y., 1940, p. 215). The difference in strength between benzamidine and guanidine (2·0 p*K* units) is comparable with that between corresponding pairs of vinylogues, *e.g.*, malachite-green and crystal-violet (2·5 units), and Doebner's violet and parafuchsin (2·2 units).

Basic strength in this series is augmented by alkylation, *e.g.*, crystal-violet is 1·8 units stronger than pararosaniline; brilliant-green, in which ethyl groups are substituted in the parent Doebner's violet, is 2·5 units stronger than the latter and 1·0 unit stronger than the methyl-substituted malachite-green.

The p*K* of Michler's hydrol is surprisingly low, when compared with that of malachite-green, in which a phenyl group replaces the central hydrogen atom of the hydrol. The phenyl group is usually base-weakening, through its electron-attracting effect, and this is one of the rare examples in which it brings about the opposite result.

In Fast acid green N the three negatively-charged sulpho-groups repel hydroxyl ions from the dye ion, and this effect augments the usual inductive effect, so that the basic strength is increased by three p*K* units above that of the parent, brilliant-green.

The value of the p*K* found for malachite-green (6·90 at 25°) agrees well with a value of 6·96 at 20°, calculated from data reported by Henriquez (*loc. cit.*).

(ii) *Velocity constants.* In the Figure (which shows the dependence of the initial velocity constant on pH) the slope of the curves above pH 10 is approximately 45°, indicating that the speed of the reaction is proportional to the hydroxyl-ion concentration. This is consistent with the view that the dominating reaction is the combination of the dye cation with the hydroxyl ion, until the rate of formation of the carbinol equals its rate of dissociation.

The curves become more nearly horizontal at about pH 10, below which the rate of carbinol-formation is faster than that corresponding to the concentration of hydroxyl ions present. A similar effect was observed by Schwarzenbach, Mohler, and Sorge (*loc. cit.*) at pH 1 for phenolindophenol, and was ascribed to a back reaction, becoming great at and below a pH equal to the p*K*, which was 0·95. However, the deviation from linearity occurs at the p*K* only for crystal-violet, and at a much higher pH for the other substances. The reason for the deviation is obscure. It is unlikely to be caused by a precipitation of the carbinol, unseen because of the low concentration of dye used (about 1—10 mg./l.) because the constancy of the p*K* with change in pH (Table I), and of the velocity constant with change in concentration, indicates that precipitation does not occur.

(iii) *Imines.* From the instantaneous p*K* (due to the imine) and the equilibrium p*K*, the carbinol p*K* of auramine and rosaniline can be calculated (see Table IV) as follows:

$$\begin{aligned} K_{\text{equilib.}} &= \frac{[\mathrm{H}^+]\{[\mathrm{B}_{\text{carbinol}}] + [\mathrm{B}_{\text{imine}}]\}}{[\mathrm{BH}^+]} \\ &= \frac{[\mathrm{H}^+][\mathrm{B}_{\text{carbinol}}]}{[\mathrm{BH}^+]} + \frac{[\mathrm{H}^+][\mathrm{B}_{\text{imine}}]}{[\mathrm{BH}^+]} \\ &= K_{\text{carbinol}} + K_{\text{imine}} \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (4) \end{aligned}$$

where $K_{\text{equilib.}}$ = equilibrium K, B_{carbinol} = carbinol base, B_{imine} = imine base, BH^+ = ion, K_{imine} = imine K, and K_{carbinol} = carbinol K.

That there should be an equilibrium between the imine base and carbinol base is reasonable, as both are in equilibrium with the ion (equation 5). The statement that the change of imine to carbinol is " irreversible " (Sidgwick, " Organic Chemistry of Nitrogen," Oxford, 1937, 91) is probably intended to convey that the equilibrium is displaced largely to one side.

$$[B_{\text{imine}}] + [H_2O] \rightleftharpoons [B_{\text{carbinol}}] \quad . \; . \; . \; . \; . \; . \; . \; . \quad (5)$$

Since the concentration of water is constant, the ratio of imine to carbinol is constant. This ratio can be calculated from the K_{carbinol} and K_{imine} from the law of mass action for each reaction :

$$\text{pH} - \text{p}K_{\text{imine}} = \log([B_{\text{imine}}]/[BH^+]$$

$$\text{pH} - \text{p}K_{\text{carbinol}} = \log([B_{\text{carbinol}}]/[BH^+]$$

Therefore, $$\log([B_{\text{carbinol}}]/[B_{\text{imine}}] = \text{p}K_{\text{imine}} - \text{p}K_{\text{carbinol}} \quad . \; . \; . \; . \; . \quad (6)$$

Thus, of the total non-ionized auramine base in equilibrium with its ion, 92% is in the form of the carbinol and 8% in the form of the imine (Table IV), whereas in rosaniline only one part in a million is in the form of the imine. Thus, the " unstable intermediate carbinol," $Me_2N{\cdot}C_6H_4{\cdot}C(OH)(NH_2){\cdot}C_6H_4{\cdot}NMe_2$ postulated by Hantzsch (*loc. cit.*) is really the main form in which non-ionized auramine exists in solution. It is unlikely that the assignment of the pK's to the imine and the carbinol should be reversed, for experiment shows that in those compounds which can form only a carbinol (*e.g.*, crystal-violet) the reaction is very slow (Table II), whereas the reaction assigned to the imine pK is instantaneous (Table IV). For example, it was found experimentally, for a given dye, that the initial extinction coefficients of a series of solutions differing only in pH were different for primary amines such as auramine, but identical for tertiary amines such as crystal-violet.

The ratio, $[B_{\text{carbinol}}] : [B_{\text{imine}}]$, may be regarded as a hydration constant (equation 5), and in the two compounds examined the hydration (addition of one molecule of water across the double bond) goes almost to completion.

(iv) *The interpretation of the p*K. The pK values reported here have been calculated from the observed degrees of ionization. In these calculations the quantity measured has always been the amount of coloured ion (at equilibrium) under given conditions compared with the total amount of coloured ion under those less alkaline conditions which ensure the presence of the maximum amount of it.

No regard has been paid to the various forms of colourless bases that may exist (cf. Schwarzenbach; Henriquez, *locc. cit.*), apart from the imine and carbinol, or to the colourless (hydrated) ions which appear to exist in the so-called " white salts " (Hantzsch and Osswald, *loc. cit.*). There is an equilibrium between all these forms in solution; the pK values reported are based on the observed degrees of ionization, which will be n% of the maximum for the coloured ion when it is n% of the maximum for any colourless ion, provided that equilibrium has been reached. For any one substance, the pK value for each type of monoacidic cation may be different, but the degree of ionization at a given pH, expressed as a fraction of the total amount capable of existing in the one particular ionic form at equilibrium, is the same for each. The concentration of colourless ions seems to be very small. No evidence has been found for the existence of the " ammonium bases " postulated by Hantzsch.

The pK values reported above facilitate calculations of changes in the concentration of the coloured ion with changing pH. Such calculations, which were not available to Stearn and Stearn (*loc. cit.*), will be of value in testing their hypothesis that the coloured cations were the species responsible for anti-bacterial activity in this series. The predicted order of basic strength (brilliant-green > crystal-violet > fuchsin) * derived by these authors from a consideration of inductive constants, is not supported by the values in Table II. It is to be expected, from considerations of the distribution of lipophobic and lipophilic groups in the various ions, that differences in adsorbability on the bacteria will also contribute to differences in biological activity.

The method used here for the triphenylmethanes is equally applicable to other series where equilibrium involving carbinols occurs. Table V gives the results of applying it to some quaternary acridines.

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* Fuchsin is a mixture of parafuchsin and a homologue which is *C*-methylated *ortho* to the central carbon atom.

TABLE V.

Ionization constants of some quaternary acridines in water at 25° ± 1°.

Substance.	pK_a.	Conc. × 10^{-5} (molar).
10-Methylacridinium bromide	9·75	3·0
5-Amino-10-methylacridinium bromide	11·01	2·0
2-Amino-10-methylacridinium bromide	11·85	2·0

(v) *Interpretations of miscellaneous results obtained with basic triphenylmethane dyes.* Hantzsch and Osswald (*loc. cit.*) found that when one equivalent of potassium hydroxide was added to an M/250-crystal-violet solution, the colour of the last portion of the crystal-violet persisted with great tenacity no matter how long the two substances were left in contact; he ascribed this to the accidental entry of CO_2. However, calculation from the pK reveals that the reaction could never go to completion under these conditions; the pH would have fallen to 10·7 (corresponding to 5% ionization) when 5% of the alkali was left. A further fall in pH would cause increased ionization of crystal-violet (pK 9·36). This alone would suffice to explain the persistent colour.

Sidgwick and Rivett (*loc. cit.*) found a pK_a of 5·8 for Doebner's violet, when hydrochloric acid was added to a solution of the carbinol, and of 10·6 when alkali was added to the salt. Evidently not sufficient time had elapsed to allow equilibrium to be reached, but the former figure may be compared with the constant pK (Table II) of 5·38 from measurements made over the whole of the pH scale.

It has also been stated that brilliant-green has $K = 0·00531$ (Sidgwick and Moore, *loc. cit.*), which would make it stronger than ammonia, but the present results show that it is weaker.

The Figure indicates that, even in neutral solutions of the dyes, slow reaction with hydroxyl ions takes place. For example, at pH 7·3, the degree of ionization of malachite-green slowly falls from 100% at zero time to 28·5% at infinite time. The time factor must be borne in mind when using the pK_a to calculate the degree of ionization in a solution of known pH. If stock solutions are required to be kept for some time, the slow precipitation of carbinol can be avoided by the addition of hydrochloric acid to bring the solution to about pH 4.

The dyeing of wool by crystal-violet was investigated by Elöd (*Trans. Faraday Soc.*, 1933, **29**, 327) over a pH range of 0—12. The amount of dye taken up increased with rising pH to a maximum at pH 8·5, above which it decreased sharply. The author ascribed this to an " enlargement of the particles " of the dye, but it is evident that, as the solution proceeds to equilibrium above this pH, the concentration of coloured ion will fall, especially if carbinol is precipitated.

When a slight excess of alkali was added to solutions of various members of this series, the carbinols which were precipitated were usually strongly dyed with the ion from which they were produced. This suggests a strong tendency to complex formation between the ion and carbinol which is also exhibited at the oil–water interface. When pure carbon tetrachloride or chloroform was shaken with a neutral aqueous solution of crystal-violet (or other member of the series), the colour remained in the water, but after several hours it appeared in the organic solvent. The time required for this to happen was shorter at higher pH values. If the carbinol was first added to the chloroform (by extracting some of the dye solution to which excess of alkali had been added), the chloroform immediately extracted the colour from the aqueous phase. A similar result was obtained when a trace of oleic acid was added to the solvent. Evidently an oil-soluble complex of ion and carbinol was formed.

Heymann and Joffe (*Trans. Faraday Soc.*, 1942, **38**, 408) drew attention to an unusual property of methyl-violet and malachite-green. Although many substances which are active at the air–water interface are comparably active at the oil–water interface, crystal-violet and brilliant-green exerted a much greater effect at the oil–water interface. Schulman and Cockbain (*Trans. Faraday Soc.*, 1940, **36**, 651) have shown that, when a complex can be formed between an oil-soluble and a water-soluble substance, an exceptionally great lowering of the oil–water interfacial tension occurs when the substances are present in the appropriate phases. A likely interpretation of the results of Heymann and Joffe is that a small quantity of carbinol formed by reaction with hydroxyl ions enters the oil, and it is immediately fixed at the interface by complex formation with coloured ions in the water. This could not happen at the air–water interface, so that the tension lowering there is slight.

Deviation from Beer's law in the cations of the compounds studied was found to be appreciable down to concentrations of the order of 10^{-5}M. In general, the non-alkylated amines deviated 10% at about one-tenth the concentration of those having tertiary amino-groups. The deviation suggests that a tendency to micelle formation in the ions is facilitated by primary amino-groups, probably through hydrogen bonding (cf. Rabinowitch and Epstein, *J. Amer. Chem. Soc.*, 1941, **63**, 69; Michaelis and Granick, *ibid.*, 1945, **67**, 1212).

EXPERIMENTAL.

Care was taken to free the distilled water from dust by filtration, since dust was observed to adsorb the dye readily and to remove an appreciable proportion of it at the high dilution used. Equal amounts of a solution of the dye salt were added to a series of 0·01M-borate, -phosphate, or -acetate buffers of various pH values; potassium hydroxide was used for high pH values. The pH was measured with a glass electrode before and after photometry, and the depth of colour estimated on a Hilger absorptiometer, using the filter which was found by experiment to give a maximum change in extinction coefficient with change in pH. If a turbidity was found in the upper pH range (by observing any scattering at right angles to a beam of light), a lower concentration of dye was used until no precipitation occurred. Readings were taken at intervals until equilibrium was established.

The ionization constants were determined by finding the degree of ionization at various pH values by optical means. The degree of ionization of the triphenylmethane dyes was taken as proportional to the extinction coefficient of the solution, corrected for deviation from Beer's law where necessary. The extinction coefficients corresponding to 0% and 100% ionization were taken as those which were constant over a range of several pH units at the upper and lower end, respectively, of the range. In some cases a small correction for the second ionization constant, obtained from the literature or determined experimentally, was applied in the calculation of the extinction value for 100% ionization. At low degrees of ionization the correction was usually negligible.

Reaction velocities at various pH values were calculated from the rate of disappearance of the coloured ion. Readings were taken every few minutes in a room in which the temperature was approximately constant.

With the quaternary acridines (Table V), there was only a small difference between the light absorption of the ion and that of the carbinol, whereas there was a large difference in fluorescence. The degree of ionization was measured on the Klett photoelectric fluorimeter.

Some potentiometric titrations were attempted in 50% acetone, which, unlike alcohol, does not react chemically with the carbinols. At a glass electrode, 10-methylacridinium bromide gave a p*K* of 8·71 (cf. 9·75 in water, Table V), equilibrium being reached almost instantly after each addition of alkali. Equilibration was much slower with crystal-violet: the pH of a half-neutralized M./200 solution in 50% aqueous acetone (kept stoppered in the dark) fell steadily from an initial value of 12·2 (glass electrode, uncorrected) to 9·0 in 5 hours, 8·7 in 2 days, and 8·2 in 30 days (cf. p*K* 9·36 in water, Table II). Thus, acetone has lowered these two equilibrium values by approximately 1 p*K* unit, and it was found to depress the p*K* of 5-aminoacridine, which cannot form a carbinol, by the same amount.

Materials.—Crystal-violet was purified as the carbinol, m. p. 219° (corr.), by the method of Kovache (*Ann. Chim.*, 1918, [ix], **10**, 247). Malachite-green, brilliant-green, and parafuchsin were purified by the partition method of Lewis, Magel, and Lipkin (*J. Amer. Chem. Soc.*, 1942, **64**, 1782). 4-Dimethylaminotriphenylcarbinol, m. p. 92—93°, and 4 : 4′-diaminotriphenylcarbinol, m. p. 173—175° (decomp.), were prepared and purified according to Baeyer and Villiger (*Ber.*, 1904, **37**, 2857). Michler's hydrol, m. p. 102—103°, was prepared by the method of Nathanson and Miller (*Ber.*, 1889, **22**, 1879). Auramine base, m. p. 136°, was liberated from a pure specimen of auramine and recrystallized from benzene. Fast acid green N (required for approximate results only) was used in the form of Lissamine green SFS (I.C.I.). The preparation of the acridinium compounds is described in Albert, Rubbo, Goldacre, Davey, and Stone (*loc. cit.*).

ADDENDUM (by S. D. RUBBO; June 10th, 1949).

Bacteriostatic Action of Aminotriphenylmethanes.

Highest dilutions of triphenylmethanes completely preventing visible growth in 48 *hours at* 37° (*pH* 7·2—7·4).

Medium: A, plain broth; B, 10% serum broth.

		Organisms.				
-triphenylcarbinol.	Medium.	*Cl. welchii.* 1 in.	*Strep. pyogenes.* 1 in.	*Staph. aureus.* 1 in.	*B. coli.* 1 in.	*Proteus.* 1 in.
4-Dimethylamino-	A	10,000	*	*	*	*
	B	*	*	*	*	*
4 : 4′-Diamino- (Doebner's violet)	A	10,000	80,000	20,000	*	*
	B	*	20,000	20,000	*	*
4 : 4′-Bisdimethylamino- (malachite-green)	A	1,280,000	320,000	80,000	*	*
	B	320,000	80,000	40,000	*	*
4 : 4′ : 4″-Triamino- (parafuchsin; pararosaniline)	A	160,000	160,000	160,000	*	*
	B	10,000	80,000	160,000	5,000	*
4 : 4′-Bisdiethylamino- (brilliant-green)	A	640,000	1,280,000	1,280,000	*	*
	B	160,000	320,000	40,000	*	*
4 : 4′ : 4″-Trisdimethylamino- (crystal-violet)	A	160,000	320,000	2,560,000	10,000	20,000
	B	80,000	80,000	640,000	5,000	20,000

* Growth not prevented by 1 : 5,000 dilution.

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