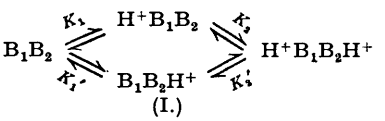
106. *Synthetic Antimalarials. Part XXXVI. Physicochemical Studies on Pyrimidine Derivatives.*

By J. C. Gage.

The ultraviolet absorption spectra and electrolytic dissociation constants of certain pyrimidine derivatives containing a 2-diethylaminoethylamino-substituent have been measured and are discussed in relation to their structure and antimalarial activity. It is suggested that in this type of compound both the basic side chain and the heterocyclic nucleus must be ionised for the specific antimalarial activity to be exhibited.

In Part XXXIV of this series (Gage, this vol., p. 221) an attempt has been made to correlate certain physical properties of the substituted diguanides with their structure and antimalarial activity. The discovery of high specific activity in members of this chemical type was preceded by the synthesis of active pyrimidine derivatives, and it was as a result of the formal similarities which can be demonstrated between the two types that the investigation into the antimalarial potentialities of the diguanides was undertaken (Curd and Rose, Part I, *J.*, 1946, 343; Part X, *ibid.*, p. 729). It is possible, therefore, that a more complete knowledge of the structure of the pyrimidines may increase our understanding of the antimalarial action of the diguanides.

Calculation of Dissociation Constants.—The four-component equilibria of an unsymmetrical diacid base B_1B_2, where B_1 is the more basic centre, may be represented by (I) (Irvin and Irvin, *J. Amer. Chem. Soc.*, 1947, **69**, 1091), where K_1, K_1', K_2, and K_2' are the equilibrium constants of the reaction of protons with the various proton acceptors, and K_1/K_2' and K_1'/K_2 are both equal to E, the electrostatic interaction between the two basic centres. In this communication K_1 and K_2 are regarded as the first and the second dissociation constant of the base B_1B_2; K_1 is therefore the equilibrium constant of the reaction of a proton with the more basic centre of B_1B_2, and K_2 is the constant of the reaction of the ion thus formed with a second proton. Irvin and Irvin (*loc. cit.*) have written the equilibrium in the reverse direction to that given in (I); their K_1 is concerned with the first-stage dissociation of $H^+B_1B_2H^+$. The definitions adopted here are preferred since they maintain the convention used by Christophers (*Ann. Trop. Med. Parasitol.*, 1937, **31**, 43; 1940, **34**, 1) in his study of the basicity of certain antimalarial drugs and which has been followed in Part XXXIV (Gage, *loc. cit.*). Irvin and Irvin, moreover, for calculating their potentiometric titration data, have followed Adam's definition (*J. Amer. Chem. Soc.*, 1916, **38**, 1503) of the two constants; for the spectrometric determination of the dissociation constant of $H^+B_1B_2H^+$ they have employed a constant K_s with a different definition. When K_1 is very much less than K_1', the potentiometric titration constants of these authors approach the constants K_1 and K_2 which are used in this communication; and when $[H^+]$ is very much less than K_2', K_s will also approach



(I.)

K_2. With a large group of compounds, which includes the pyrimidine derivatives studied in this investigation, the different definitions will lead to the same values for the dissociation constants; when compounds such as the alkylenediamines are investigated, which have K_1 and K_1' of the same order, the preferred definitions have the advantage of providing a simpler physical chemical interpretation, and they can be used for both the titrimetric and the spectrometric investigations.

Barton (*Nature*, 1947, **160**, 752) has developed an equation for determining the constants K_1 and K_1' and the electrostatic term E from potentiometric titration data provided that one of them is known; for the equilibrium (I), the activity coefficients being neglected, this may be written in the form (1), where α, β, γ, and $[H^+]$ are measured values from the titration. If

$$\alpha/\gamma[H^+]^2 = \beta(1/K_1 + 1/K_1')/\gamma[H^+] + E/K_1K_1' \quad . \quad . \quad . \quad . \quad . \quad (1)$$

K_1/K_1' is of the order 0·2 or less, equation (1) does not yield accurate results, and it is not, therefore, suitable for the pyrimidine derivatives with which this communication is concerned. It may be used for the aliphatic diamines provided that K_1 is equal to K_1'; since the dissociation of an amino-group appears to be affected by the proximity of a second uncharged amino-group in the molecule (Table II), the value of K_1 or K_1' cannot be predicted from measurements on the alkylamines. Irvin and Irvin (*loc. cit.*) have used Reed and Berkson's analysis (*J. Physical Chem.*, 1929, **33**, 760) as developed by Clark and Perkins (*J. Amer. Chem. Soc.*, 1932, **54**, 1228) for redox titrations. The following treatment is simpler; it enables K_1 and K_2 as defined above to be calculated from potentiometric titration curves, and K_2 from spectrometric observations.

From a consideration of the principles of electroneutrality and the conservation of mass, equation (2) may readily be deduced, where C is the total molar concentration of base, and A is the number of moles neutralised. When K_2 is very much greater than $2[H^+]$, this reduces to (3), whence the dissociation exponent pK_1 is given by (4); similarly, when K_1 is very much less than $[H^+]$ the value of pK_2 is given by (5).

$$\frac{1 + K_1/K_1' + 2[H^+]/K_2}{1 + K_1/K_1' + K_1/[H^+] + [H^+]/K_2} = \frac{A + [OH^-] - [H^+]}{C} = y \quad . \quad . \quad . \quad (2)$$

$$K_1/[H^+] = (1 - y)/y(1 + K_1/K_1') \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

$$pK_1 = pH - \log_{10}[(1 - y)/y] - \log_{10}(1 + K_1/K_1') \quad . \quad . \quad . \quad . \quad (4)$$

$$pK_2 = pH - \log_{10}[(2 - y)/(y - 1)] + \log_{10}(1 + K_1/K_1') \quad . \quad . \quad . \quad (5)$$

The relative magnitudes of the dissociation exponents of the aliphatic tertiary amines and the aminopyrimidines given in Tables I and II, from which an approximation to K_1/K_1' can be

TABLE I.

With the exception of the new compound (IX), the antimalarial activities have been taken from previous communications in this series; the tests were performed against *P. gallinaceum* in chicks.

	Potentiometric titration.		Absorption spectra.		Antimalarial activity.	
Ref. in text.	pK_1.	pK_2.	pK_1.	pK_2.	Dose, mg./kg.	Activity
(II)	—	4·95	—	5·0	—	—
(III)	—	7·7	—	7·6	—	—
(V)	9·55	7·5	—	7·7	—	—
(VI)	9·8	6·85	—	7·1	—	—
(XIII)	9·7	7·9	—	—	80	++
					40	±
(VII)	—	—	—	6·6	—	—
(XVI)	9·1	6·2	—	—	160	+ to ±
					80	—
(VIII)	9·05	6·6	—	6·7	80	++
					40	+
(IX)	—	6·8	—	6·65	—	—
(X)	9·6	6·6	—	6·8	80	+
					40	—
(XI)	9·3	5·5	—	5·35	—	—
(XIV)	9·3	4·05	—	4·0	—	—
(XII)	9·8	8·33	—	(pK_2 = 1·9)	40	++
(XV; R = Cl)	—	—	10·0 *	—	—	—
(XV; R = NO_2)	—	—	9·4 *	—	—	—

* Refers to acid dissociation constant of hydroxyl group.

TABLE II.

Compound.		pK_1.	pK_2.	$\Delta pK = 10g_{10}\ 1/E$.
Triethylamine		10·8	—	—
Tetraethylethylenediamine:	0·01M	9·55	6·75	2·8
	4×10^{-4}M	9·3	6·65	2·65
Ethylenediamine:	0·01M	9·7	7·3	2·4

derived, indicate that for the dialkylaminoalkylaminopyrimidines the second logarithmic term in (4) and (5) can be neglected. From this approximate value of K_1K_1' it may be deduced that the concentration of the ion $B_1B_2H^+$ in the equilibrium (I) is negligible, but that it may not be possible to obtain a solution of the ion $H^+B_1B_2$ which is free from appreciable amounts of B_1B_2 or $H^+B_1B_2H^+$. It may not, therefore, be possible to determine the absorption spectrum of this ion with accuracy. The equation used in Part XXXIV (Gage, *loc. cit.*) may be modified so that the term signifying the extinction coefficient of the singly charged ion is eliminated. K_1 can be determined by measuring the optical density at two or more pH values selected so that the concentration of $H^+B_1B_2H^+$ is negligible; similarly, the measurements for K_2 are taken when the concentration of B_1B_2 is negligible. The constants are given by equations (6) and (7), where $\varepsilon_{B_1B_2}$ and $\varepsilon_{H^+B_1B_2H^+}$ are the molar extinction coefficients of B_1B_2 and $H^+B_1B_2H^+$, and ε' and ε'' are the extinction coefficients of solutions at hydrogen-ion concentrations $[H^+]'$ and $[H^+]''$. This method has been found more convenient than the graphical method of Vlés and Gex

$$K_1 = (\varepsilon' - \varepsilon'')/\{(\varepsilon_{B_1B_2} - \varepsilon')/[H^+]' - (\varepsilon_{B_1B_2} - \varepsilon'')/[H^+]''\} \quad . \quad . \quad . \quad . \quad (6)$$

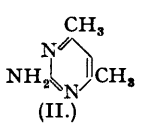
$$K_2 = \{[H^+]'(\varepsilon_{H^+B_1B_2H^+} - \varepsilon') - [H^+]''(\varepsilon_{H^+B_1B_2H^+} - \varepsilon'')\}/(\varepsilon - \varepsilon'') \quad . \quad . \quad . \quad (7)$$

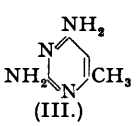
(*Compt. rend.*, 1925, **180**, 1342). From the constants determined by equations (6) and (7) the optical density of $H^+B_1B_2$ at selected wave-lengths may be calculated.

The dissociation exponents obtained with the titrimetric and spectrometric methods are, in general, in good agreement. The experiments do not permit a statistical calculation of error, but it is probable that the great majority of the results by potentiometric titration are subject to an error of less than $\pm 0{\cdot}05$ unit. The precision of the spectrometric method depends on the magnitude of the difference between $\varepsilon_{B_1B_2}$ and $\varepsilon_{H^+B_1B_2H^+}$ at the chosen wave-length; as mentioned in Part XXXIV, the error can be minimised by replacing the extinction coefficients in (6) and (7) by the difference between the coefficients at two selected wave-lengths, and it is unlikely that any of the results are subject to an error of more than $\pm 0{\cdot}1$ unit.

Discussion of Results.—Formulæ (VIII), (XIII), and (XIV) are examples of pyrimidine derivatives showing specific antimalarial activity. All are characterised by a 2-diethylaminoethyl group attached to a substituted 2 : 4-diaminopyrimidine group through one of the amino-nitrogen atoms. These two basic centres give rise to two dissociation constants, and a comparison of the two dissociation exponents with those of the two isolated component groups (Tables I and II) indicates that the first (pK_1) may be attributed to the terminal nitrogen atom of the basic side chain, and the second (pK_2) associated with the heterocyclic group; to facilitate comparisons the dissociation exponents of the aminopyrimidines without the dialkylaminoalkyl group have been classified as pK_2 values

The basicity of 2-amino-4 : 6-dimethylpyrimidine (II) is appreciably increased by the replacement of a methyl by an amino-group (III), but there is no evidence from absorption spectra of the existence of a second basic centre in (III) in 0·1N-hydrochloric acid (Fig. 1). It may be reasoned, therefore, that the ion of (III) exists as a hybrid between the various resonance forms (IV).


(II.)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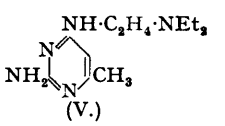
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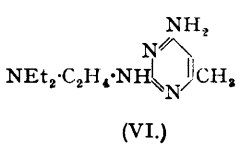
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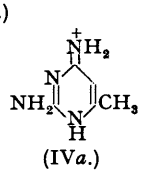
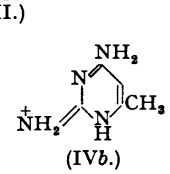
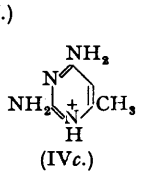
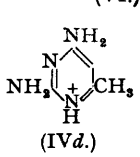

(IV*a*.)


(IV*b*.)

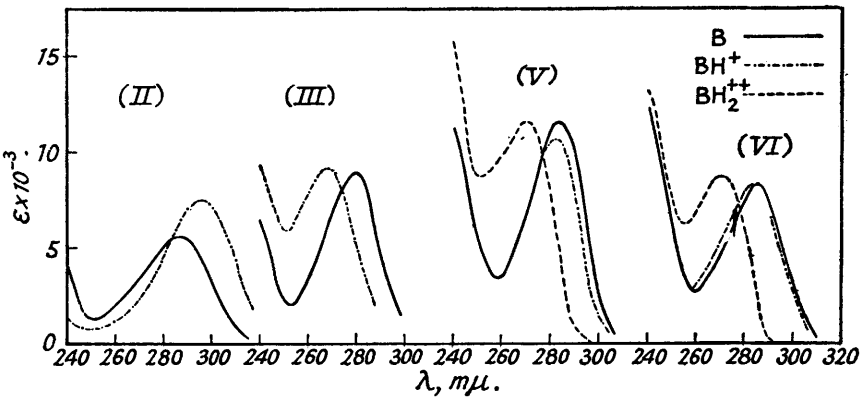

(IV*c*.)


(IV*d*.)

It is possible to locate the proton on the N_1 or the N_3 heterocyclic nitrogen, but it is reasonable to assume that the ion will be more stable when the charge concentration on the protonised

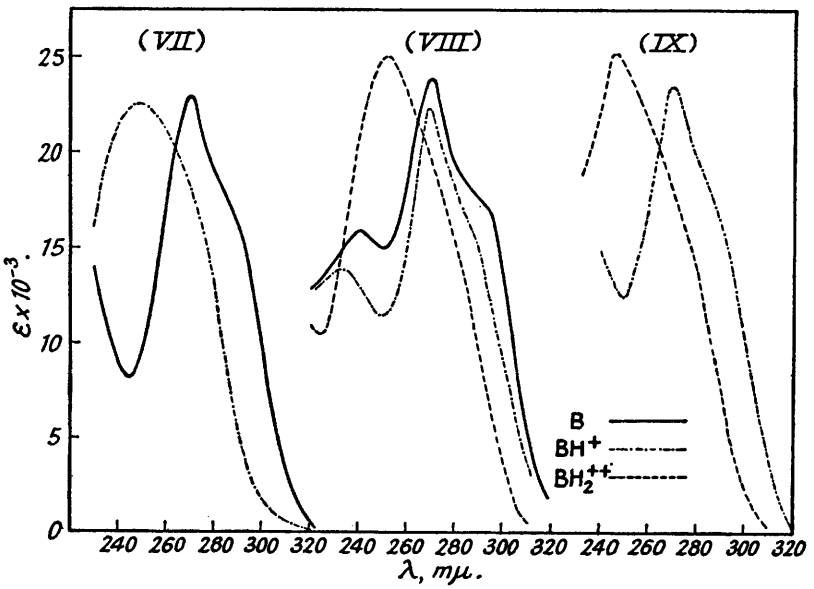
nitrogen is more remote from the charge distributed between the two amino-groups; that is, the ion with the proton on the N_1 nitrogen as in (IV) will have the lower energy. Moreover, (IV) permits the existence of a *p*-quinonoid form (IV*a*) in the ion; Albert and Goldacre (*Nature*, 1944, **153**, 467) have discussed the greater stability of this form over the corresponding *o*-quinonoid structure. Derivatives of (III) with an amino-group replaced by a dialkylaminoalkylamino-group have been prepared (Hull, Lovell, Openshaw, Payman, and Todd, Part III, *J.*, 1946,

FIG. 1.



357; Hull, Lovell, Openshaw, and Todd, Part XI, *J.*, 1947, 41); when a 2-diethylaminoethylamino-group replaces the 4-amino-group (V) there is no appreciable change in the dissociation of the heterocyclic system, but when this substituent replaces the 2-amino-group (VI) there is a significant decrease in basicity. It will be seen in Table II that the electro-negative inductive effect of the positive charge along the chain in the tetraethylethylenediamine ion is about

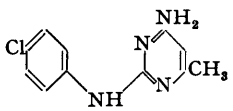
FIG. 2.

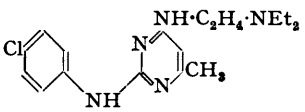


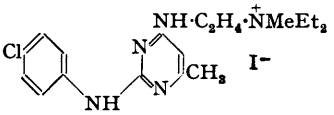
2·8 pK units. The absence of depression of the heterocyclic dissociation in (V) by the presence of this electro-negative substituent may be attributed to an attendant increase in the resonance energy of the ion which offsets the inductive effect of the charged basic side chain. This will obtain if the resonance form (IV*a*) is of a lower energy than (IV*b*), that is, if a greater proportion of the cationic charge is located on the 4- than on the 2-amino-group; the addition of the charged basic side chain to the former group will increase the energy of (IV*a*) and may make it

approach more closely to that of (IV*b*), thus increasing the resonance energy and the stability of the ion. In (VI) the electronegative effect of the charged side chain would be reinforced by a decrease in resonance energy resulting from a further separation of the energy levels.

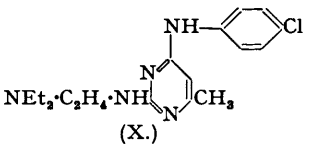
When the electronegative *p*-chloroanilino-group replaces the 2-amino-group (VII) there is also a decrease in basicity, but there is no further decrease when a charged basic side chain replaces the 4-amino-group (VIII); this is in accord with the above argument. Moreover, it is to be expected that the effect of changing the diethylaminoethyl group from the 2- to the 4-amino-group will diminish when a second electronegative group is attached to the unsubstituted amino-group; the more nearly the electron affinities of the two groups approach, the smaller will be the effect of interchanging them. No significant difference has been found between the pK_2 values of the two isomers (VIII) and (X). The absorption spectra of (VII) and (VIII) (Fig. 2) bear no obvious relation to those of (III) and (V), which suggests that the aryl and pyrimidine nuclei are not isolated by the bridging imino-group and migration of electrons is possible between them. In (VIII) the addition of a proton to the terminal nitrogen atom of the basic side chain is attended by a small change in absorption spectrum; this is sufficient only for an approximation of the pK_1 vlue. A more accurate pK_1 value can be obtained from potentiometric titration and, like those of mepacrine and pamaquin (Christophers, *Ann. Trop. Med. Parasitol.*, 1937, **31**, 43; 1940, **34**, 1), two active antimalarials which carry the same type of basic side chain, it is found to approach the pK_1 value of the tertiary amino-group in the corresponding aliphatic diamine. A comparison of the spectra of (VII), (VIII), and (IX) indicates that the absorption of the un-ionised heterocyclic system is slightly affected by the uncharged chain, but hardly at all by the charged or quaternised chain. There is no evidence from this of an interaction between the terminal nitrogen atom of the chain and the heterocyclic system.

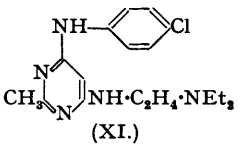

(VII.)

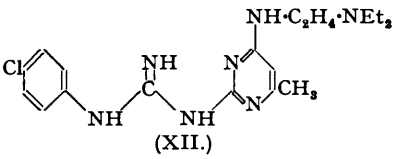

(VIII.)


(IX.)

Formulæ (X) and (XI) represent isomers of (VIII) (Curd, Davis, Owen, Rose, and Tuey, Part VI, *J.*, 1946, 370; Basford, Curd, and Rose, Part VIII, *ibid.*, p. 713); the former, like (VIII), is an active antimalarial and has a similar pK_2 value, while the latter has a significantly lower pK_2 value and is without specific antimalarial activity. This observation suggests there may be some correlation between antimalarial activity and dissociation constants, a point which has previously been made by Christophers (*Trans. Faraday Soc.*, 1943, **39**, 333). Additional support for this hypothesis is to be found in the antimalarials typified by the formulæ (XII) (Curd and Rose, Part IV, *J.*, 1946, 362) and (XIII) (Hull, Lovell, Openshaw, Payman, and Todd, Part III, *ibid.*, p. 357), which show a higher antimalarial activity than (VIII) and also possess a higher pK_2 value. On the other hand, the triazine derivative (XIV) (Curd, Landquist, and Rose, Part XII, *J.*, 1947, 154) shows a lower tendency to accept a proton on the heterocyclic system and is biologically inactive. It may tentatively be suggested that in this type of compound the heterocyclic system must exist to a significant extent in the ionised form at physiological pH values for the specific antimalarial activity to be apparent.


(X.)


(XI.)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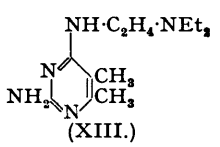
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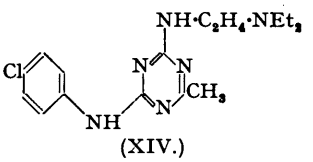
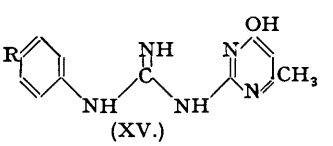
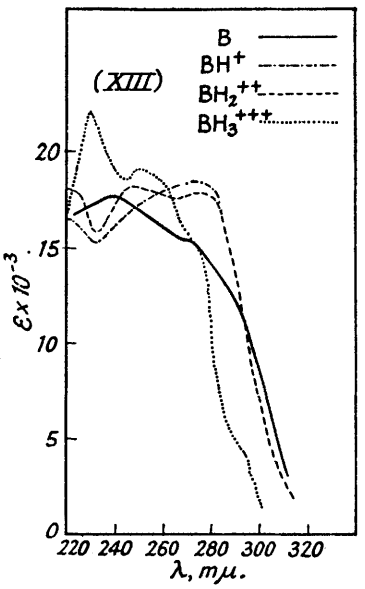

(XIV.)


(XV.)

A high specific antimalarial activity has been obtained with 2-*p*-chlorophenylguanidino-4-2′-diethylamino-6-methylpyrimidine (XII). This compound has three dissociation constants; the pK_1 and pK_2 values are similar to those of (VIII), while the pK_3 value may be attributed to the guanidino-group linking the two rings. Two hydroxypyrimidine intermediates of (XIII) have been described by Curd and Rose (*loc. cit.*) (XV; R = Cl or NO_2); absorption spectra measurements show there to be a change in ionisation of these over the pH range 9—11. It is reasonable to ascribe this to the acidic dissociation of the hydroxyl group, as the values for (XII) indicate that a basic centre of the required strength is very improbable in this compound; and the calculated pK values show a significant difference which is in harmony with the observed difference in carbonate solubility. The suggestion advanced by Curd, Landquist, and Rose (*loc. cit.*) to account for this observation, *viz.*, that the molecule might exist to an appreciable extent in a fully conjugated form, is not supported by absorption-spectra measurements, which give no indication of an increased $\lambda_{max.}$ in (XII) over (VIII) (Fig. 3).

FIG. 3.



It has been suggested by Curd, Davey, and Rose (*Ann. Trop. Med. Parasitol.*, 1945, **39**, 157) that the specific antimalarial activity of pyrimidine derivatives such as (VIII) and (XII) is due to the antagonism of these compounds towards riboflavine, which they attribute to the formal resemblance between the drugs and the growth factor. Hull, Lovell, Openshaw, Payman, and Todd (*loc. cit.*) have suggested an analogous explanation for the activity of pyrimidine derivatives such as (XIII), with the drug-antagonising enzyme systems containing adenosine. This investigation is not designed to prove or disprove either of these hypotheses, which may well both be correct, but it does throw emphasis on the possibility that the pharmacodynamic group in both of these types may be the same and that the function of the substituents may be to achieve a favourable electron distribution. The effect of alkyl substituents in the pyrimidine nucleus on antimalarial activity provides the greatest challenge to this hypothesis; it is possible that the inactivity of (XV), a homologue of (VIII) without the 6-methyl group, may be connected with its lower pK_2 value, but this argument cannot be applied to the difference in activity between (V) and (XIII), since the pK_2 value of (V) is within the range of those compounds showing antimalarial activity.

EXPERIMENTAL.

A 0·01M-solution of tetraethylethylenediamine was titrated with 0·02N-hydrochloric acid, and a 4×10^{-4}M-solution in excess of hydrochloric acid was titrated with 0·01N-sodium hydroxide. The pyrimidine derivatives were obtained as the hydrochlorides; the non-aryl members were titrated at 0·01M-concentration with 0·02N-sodium hydroxide, while the lower solubility of the aryl derivatives necessitated a 4×10^{-4}M-solution titrated with 0·01N-alkali. The temperature was controlled at 25° ± 0·5°.

The dissociation exponents of the tetraethylethylenediamine were calculated by equations (9) and (10), $K/_1K_1'$ being assumed equal to unity, and are given in Table II. The exponents of the basic-side-chain pyrimidines were calculated by these equations on the assumption that the logarithmic term is negligible; this is not entirely justifiable for compound (XII), and its exponents may be subject to a slight error.

The method of determining the absorption spectra was the same as in Part XXXIV, with the exception that a chloride–phosphate–borate buffer, $8{\cdot}33 \times 10^{-3}$M to each anion, and with suitable addition of hydrochloric acid or sodium hydroxide, was used to control the hydrogen-ion concentrations.

The following *methiodide* was synthesised for this investigation.

2-p-*Chloranilino*-4-2′-*diethylaminoethylamino*-6-*methylpyrimidine* 2′-*Methiodide* (IX).—2-*p*-Chloranilino-4-2′-diethylaminoethylamino-6-methylpyrimidine (VIII) (8·4 g.) was dissolved in benzene (10 c.c.), and methyl iodide (4 g.) added. After 24 hours an insoluble resin was separated and recrystallised twice from alcohol–acetone to give a colourless solid, m. p. 165—166° (Found: C, 45·5; H, 5·85; N, 14·9; Cl, 7·75; I′, 26·1. $C_{18}H_{27}N_5ClI$ requires C, 45·4; H, 5·7; N, 14·7; Cl, 7·5; I′, 26·7%).

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