53. *Synthetic Antimalarials. Part XXXIV. Physicochemical Studies on the Diguanides.*

By J. C. Gage.

The ultra-violet absorption spectra of a number of arylalkyldiguanides have been measured at a range of pH values, and the pK values calculated therefrom. Simpler molecules, including the aryl guanidines and the amidines which may be regarded as fragments of the diguanides, have been similarly studied. An attempt has been made to determine the structure of the substituted diguanide ions which show antimalarial activity, but it has not been found possible to determine which of three possible configurations is the most likely. No correlation has been observed between the measured physical properties and biological activity.

The correlation of the biological activity of chemotherapeutic agents with their molecular structure usually demands more information concerning the structure than is revealed by analytical and synthetic procedures; such factors as the spatial orientation of the molecule, the distribution of electronic charge, and the possibility of interaction between constituent groups may be of considerable importance in determining whether a compound has or has not useful pharmacological activity. Physicochemical methods can be devised to increase our knowledge of such factors, but the selection of suitable methods is limited by the consideration that it is reasonable to investigate the compounds in approximately the same environment in which they exist in the body fluids; that is to say, in dilute aqueous solution at about pH 7·4 and in the presence of electrolytes. For this reason infra-red spectroscopy and X-ray diffraction analysis may be of only limited value. A variety of experimental methods have been surveyed in the course of this investigation, and ultra-violet absorption spectra have so far been found to be of most value, since they not only provide direct information concerning structure, but also enable electrolytic dissociation constants to be calculated from which further information may be derived.

This communication deals with the structure of the substituted diguanides, and is the first of a series devoted to the physicochemical examination of antimalarial compounds. It is hoped that a more detailed knowledge of the structure of biologically active and inactive members of the various chemical types, and of the possible structural analogies which may exist between the types, may lead to an ability to recognise those pharmacodynamic groups which are responsible for antimalarial activity. The diguanides are one of the simplest chemical types containing members with a high specific antimalarial activity; a considerable number have been prepared (Curd and Rose, *J.*, 1946, 729) in an investigation which led to the highly active compound N^1-*p*-chlorophenyl-N^5-*iso*propyldiguanide (IX; R = Cl) ("Paludrine").

Discussion.—The pK values of the diguanide series listed in Tables I and IV indicate that all are di-acid bases, and at physiological pH values will exist almost entirely as the singly charged cation. It is, therefore, with the structure of this ion that this communication is mainly concerned. Diguanide itself is a very strong base, and the approximate pK_1 value recorded in Table I must be taken with some reserve. The pK_2 value, which differs by about one unit from that obtained by Sugino and Ogawa (*Rev. Phys. Chem. Japan*, 1939, **13**, 58) by electrometric methods, is lower than might be expected from formula (I) for the singly charged ion; since even if one-third of the ionic charge were carried by the central nitrogen atom the inductive effect of this charge would hardly be sufficient to depress the ionisation of the second amidine group

TABLE I.

			B.		BH^+.		BH_2^{++}.
	pK_1.	pK_2.	$\lambda_{max.}$, mμ.	$\epsilon_{max.}$	$\lambda_{max.}$, mμ.	$\epsilon_{max.}$	$\lambda_{max.}$, mμ.
$CH_3 \cdot C(:NH) \cdot NH_2$	12·1	—	224	4000	<220	—	—
$NH_2 \cdot C(:NH) \cdot NH \cdot C(:NH) \cdot NH_2$	12·8	3·1	231	9500	232	12,500	<220

TABLE II.

Compound.	pK_1.	B. $\lambda_{max.}$, mμ.	B. $\epsilon_{max.}$	BH^+. $\lambda_{max.}$, mμ.	BH^+. $\epsilon_{max.}$
p-$C_6H_4Cl\cdot NH_2$	4·7	239; 290	8,500; 1,700	<220	—
p-$C_6H_4Cl\cdot NHMe$	3·9	245; 295	10,750; 1,500	<225	—
p-$C_6H_4Cl\cdot NH\cdot C(:NH)\cdot CH_3$	9·5	236	8,100	228	7,000
p-$C_6H_4Cl\cdot NH\cdot C(:NH)\cdot NH_2$	10·6	243	9,000	234	9,750
p-$C_6H_4Cl\cdot NMe\cdot C(:NH)\cdot NH_2$	12·6	247	6,150	224	7,850
p-$C_6H_4Cl\cdot NH\cdot C(:NH)\cdot NHMe$ *...	10·85	245	9,200	236	10,250

* Compound supplied by Dr. A. F. Crowther; details of preparation to be published later in this series.

TABLE III.

p-$C_6H_4R\cdot NH_2$.

R.	pK.	B. $\lambda_{max.}$, mμ.	B. $\epsilon_{max.}$	BH^+. $\lambda_{max.}$, mμ.
H	4·8	230; 279	7,900; 1,420	<220
Cl	4·2	239; 290	8,500; 1,700	<220
Br	3·9	240; 295	10,400; 1,000	<230
I	3·8	245; 285	15,000; 1,400	<230

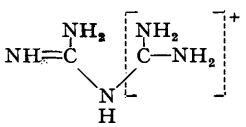
TABLE IV.

$NRR_1\cdot C(:NR_2)\cdot NH\cdot C(:NR_3)\cdot NHR_4$.

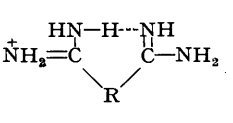
R.	R_1.	R_2.	R_3.	R_4.	pK_1.	pK_2.	B. $\lambda_{max.}$, mμ.	B. $\epsilon_{max.}$	BH^+ $\lambda_{max.}$, mμ.	BH^+ $\epsilon_{max.}$	BH_2^{++} $\lambda_{max.}$, mμ.	BH_2^{++} $\epsilon_{max.}$
C_6H_4Cl ...	H	H	H	H *	10·4	2·2	239	13,080	[235]; 253	[13,500]; 14,800	<225; 245	——; 8,600
C_6H_5 ...	H	H	H	Pr^i	11·5	1·9	236	12,700	245	13,300	<220; [245]	——; [5,500]
C_6H_4Cl ...	H	H	H	Pr^i	10·4	2·3	240	14,470	233; 253	14,000; 14,750	<225; [244]	——; [8,850]
C_6H_4Br...	H	H	H	Pr^i	11·4	2·2	246	14,400	<230; 256	——; 15,100	<230; 247	——; 10,000
C_6H_4I ...	H	H	H	Pr^i	11·0	2·2	248	15,800	*ca.* 237; 260	14,800; 16,200	<230; *ca.* 250	——; 10,150
C_6H_4Cl ...	Me	H	H	Pr^i †	12·8	2·4	243	13,500	243	15,600	<225	—
C_6H_4Cl ...	H	Me	H	Pr^i ‡	11·8	2·3	237; [260]	13,000; [10,650]	236	18,700	<225; 252	——; 9,900
C_6H_4Cl ...	H	H	Me	Pr^i ‖	12·2	2·0	240	13,600	<220; 251	——; 13,800	<220	—

* Curd and Rose, *J.*, 1946, 729.
† Curd, Hendry, Kenny, Murray, and Rose, *J.*, 1948, 1630.
‡ Crowther, Curd, Richardson, and Rose, *J.*, 1948, 1636.
‖ Birtwell, Curd, Hendry, and Rose, *J.*, 1948, 1645.

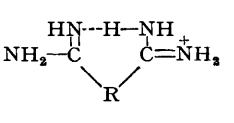
to such an extent (Branch and Calvin, " Theory of Organic Chemistry ", New York, 1941, Chap. VI). The pK_2 value of the analogous compound malondiamidine has been shown by potentiometric titration to be 9·0. It is possible that the singly charged cations of both these compounds are stabilised by hydrogen bond resonance (II*a*, *b*; R = NH or CH_2); if so the pK_2 of malondiamidine suggests that the resonance energy is not of high order. With the diguanide ion there is the possibility of further resonance forms (III*a*, *b*); it is not possible to determine whether the resonance energy associated with these forms is sufficient to account for the low pK_2 of diguanide. The diguanide ion has a further possible structure (IV); here the four terminal NH_2 groups are approximately equal; the cationic charge will be distributed among them and the ion will be stabilised by a high resonance energy.



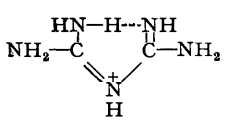
(I.)



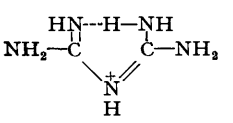
(II*a*.)



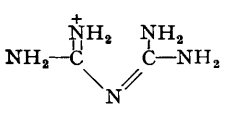
(II*b*.)



(III*a*.)



(III*b*.)



(IV.)

When a *p*-chlorophenyl group is introduced into the diguanide molecule the basicity is lowered by more than 2 pK units. The further addition of an *iso*propyl group to give (IX; R = Cl) is not attended by a rise in pK_1, but there is a marked rise in pK_1 following the introduction of a second alkyl group. These observations admit an explanation similar to that suggested by Pauling (" The Nature of the Chemical Bond ", Ithaca, New York, 2nd edit., 1944, p. 213) for the influence of substituents on the basicity of guanidine, provided that it is assumed that an alkyl group is an electron donor and not, as Pauling assumes, an electron-accepting group. The introduction of an alkyl group into the *p*-chlorophenyldiguanide ion may

FIG. 1.

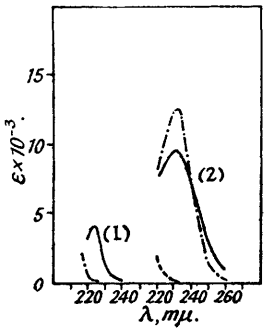
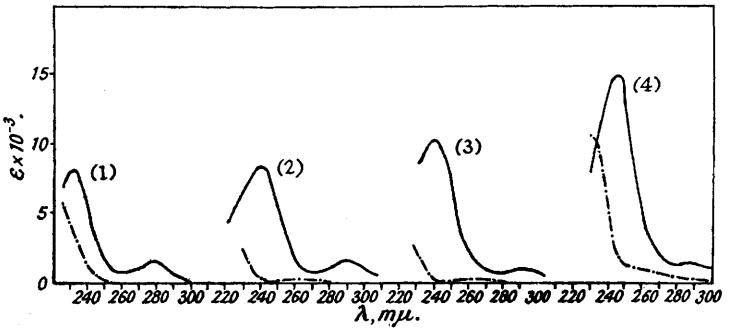
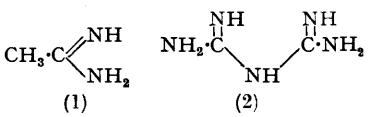


FIG. 3.





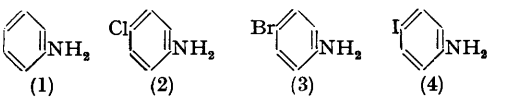
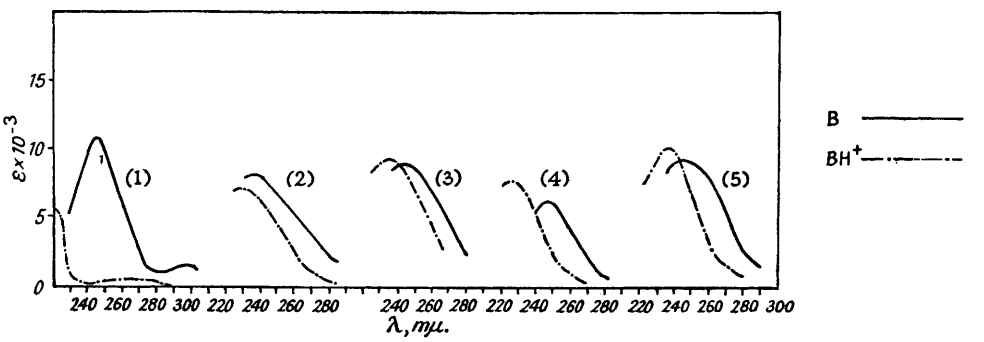
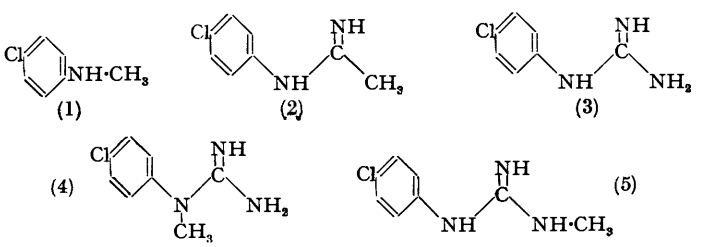


FIG. 2.

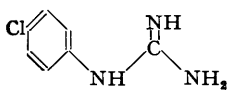


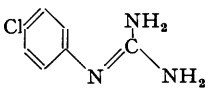


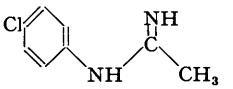
lower the resonance energy by increasing the energy difference between the resonance forms, and the attendant decrease in pK value may offset the rise to be expected from the inductive effect of the alkyl group. Further methylation may reduce this energy difference and so restore some of the resonance energy; the increase in pK value due to the alkyl group would here be reinforced by the resonance-energy contribution. The evidence derived from a consideration of dissociation constants is not sufficient to determine the structure of diguanide and its derivatives, and a search for further evidence has been made from the study of the ultra-violet absorption spectra. Since it is not possible to connect the position of the absorption maxima of these compounds with their molecular structure by *a priori* calculation, it is necessary

to consider the spectra of simpler molecules which may be regarded as component parts of the arylalkyldiguanides and whose structure is open to little doubt.

The absorption spectra of a series of *p*-chloroaniline derivatives are shown in Fig. 2. The wave-lengths of maximum absorption ($\lambda_{max.}$), the molecular extinctions ($\epsilon_{max.}$), and the calculated dissociation constants are listed in Table II. The sharp maximum of *p*-chloroaniline base is shifted to a longer wave-length by *N*-methylation, the effect to be expected from an electropositive substituent in this position. The increased contribution of the dipolar resonance form to the base is sufficient to depress the p*K* value in spite of the base-strengthening effect of the methyl group. The introduction of an amidino-group to give *p*-chlorophenylguanidine has an effect on the $\lambda_{max.}$ similar to that of a methyl group. This suggests that *p*-chlorophenylguanidine has the structure (V) rather than (VI), since in (VI) the increase in the resonance path might be expected to be associated with a shift of the maximum to a longer wave-length; moreover the $\lambda_{max.}$ is not affected by N^1-methylation of the *p*-chlorophenylguanidine. It follows also that the dipolar resonance of the amidino-group in (V) does not involve the aniline nitrogen atom, since the acquisition of a partial positive charge by this nitrogen atom would tend to decrease the tendency of electrons to migrate to the ring, and it is reasonable to suppose that this would decrease $\lambda_{max.}$ and $\epsilon_{max.}$. The basicity of this compound is lower than that of acetamidine, owing presumably to the electronegative effect of the aryl group. The $\lambda_{max.}$ of the *p*-chlorophenylguanidine ion is considerably higher than that of the *p*-chloroaniline ion; this leads to the conclusion that in the former little of the cationic charge is being carried by the aniline nitrogen. An N^1-methyl group markedly decreases the $\lambda_{max.}$ of the ion and produces a large rise in p*K* value, suggesting that a greater part of the cationic charge is now carried on the aniline nitrogen atom. On the other hand, N^2-methylation slightly increases the $\lambda_{max.}$ of the ion and slightly decreases the p*K* value in spite of its inductive effect; it therefore tends to decrease further the symmetry of charge distribution. In N-p-*chlorophenylacetamidine* (VII) the dipolar amidine resonance (VIII) does involve the aniline nitrogen atom since the $\lambda_{max.}$ is appreciably shorter than that of *p*-chloroaniline. In this compound also the form (VII) is more likely than the isomeric anil structure. The aryl group interferes with the amidine ion resonance, and the basicity is considerably less than that of acetamidine.


(V.)


(VI.)


(VII.)

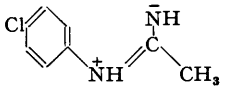
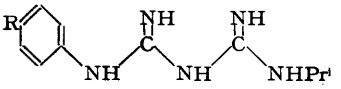
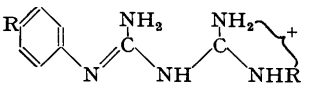

(VIII.)

Table III lists the absorption maxima and p*K* values of a series of halogenated anilines; the absorption spectra are given in Fig. 3. The p*K* values decrease, and the $\lambda_{max.}$ increase, in the order H, Cl, Br, I, which is the order of Hammett's σ values (" Physical Organic Chemistry ", New York, 1st edit., 1940, p. 188). From the spectra of substituted diguanides shown in Fig. 4 and their constants listed in Table IV it will be seen that " Paludrine " and its analogues of the type (IX; R = H, Cl, Br, I) show, as bases, an absorption maximum at approximately the same position as that of the corresponding *p*-substituted anilines, suggesting that the absorption is due to the aniline portion of the molecule, the remainder having relatively little influence.

As the $\lambda_{max.}$ of the substituted diguanide singly charged ions are greater than those of the corresponding diguanide and aniline bases, it is probable that the absorption in the ion is not solely due to the anilino-group since the acquisition of a whole or partial positive charge by the aniline nitrogen shortens the $\lambda_{max.}$. Structure (X) shows the anilino-group connected by a double bond to the rest of the molecule; here, however, the proton does not appear to be involved in the resonance, and there seems to be no reason why a similar absorption spectrum should not be given by the base. If it is assumed that the $-NH-$ group linking the aryl group to the rest of the molecule does not act as a complete resonance block, and there is evidence from the

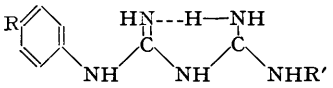

(IX.)


(X.)

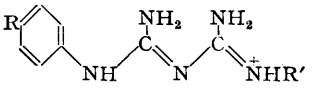
light-absorption of dyes to support this, then alternative formulæ (XI) and (XII) are possible. In these structures is possible a distribution of the cationic charge similar to that in formulæ

(II), (III), and (IV). In none of these, however, is there any obvious reason why the addition of a proton to the base should be accompanied by a longer $\lambda_{max.}$, since the resonance forms of the ion can be paralleled by those deriving from the polarisation of imino-groups in the base; the spectra of the acetamidine ion and base show that dipolar resonance need not be associated with a shorter $\lambda_{max.}$ than positive charge resonance.

Methylation of (IX; R = Cl) in the N^1 and N^2 positions suppresses the maximum at 253 mμ, but methylation in the N^4 position does not. With structure (X), N^1-methylation will involve a molecular rearrangement which may well change the absorption spectrum, but this effect is hardly to be expected with N^2-methylation. There is no obvious explanation of the effect of N-methylation on the basis of structures (XI) and (XII).



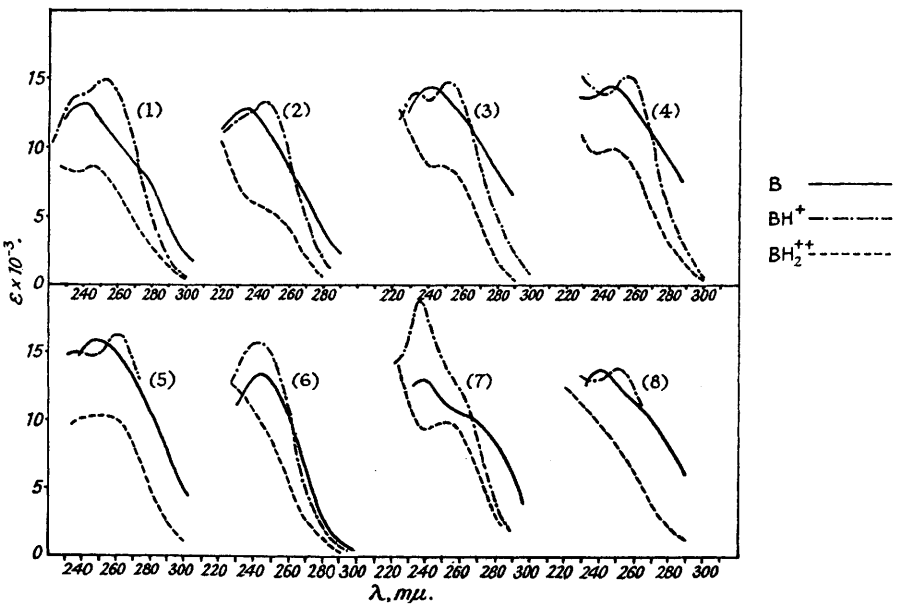
(XI.)

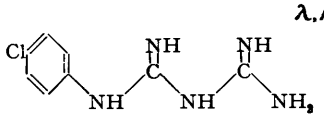


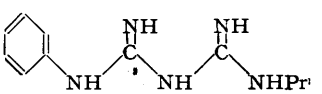
(XII.)

Measurements of the spectrum of (IX; R = Cl) at pH 7·0 over a concentration range from 10^{-3} to 5×10^{-6} molar have revealed no significant change of spectrum with concentration. It is unlikely, therefore, that the characteristic spectrum of the ion is due to molecular association.

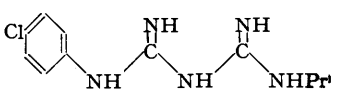
FIG. 4.

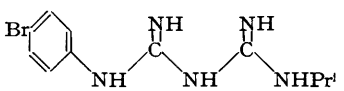


(1) 

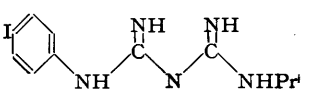


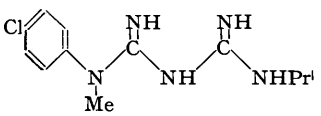
(2)

(3) 



(4)

(5) 



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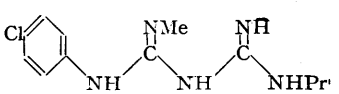
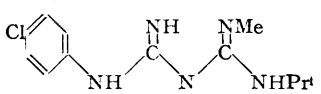
(7) 



(8)

It has been shown that *p*-chlorophenyldiguanide has no antimalarial activity; with increasing length of the alkyl chain at the N^5 position the activity rises to a maximum with the *iso*propyl group ("Paludrine") and then falls (Curd and Rose, *loc. cit.*). No significant difference in absorption spectra or pK value has been noted between *p*-chlorophenyldiguanide and its *iso*propyl homologue; these measurements cannot therefore provide an hypothesis to account for the difference in activity. The substitution of a second alkyl group into the latter produces a decrease in antimalarial activity, but, until the attendant changes in absorption spectra and pK values can be interpreted in terms of molecular structure, no explanation of this dystherapeutic effect is apparent.

Experimental.

The ultra-violet absorption spectra were measured on a Beckman Quartz Spectrophotometer. Solutions of suitable concentrations were prepared to give maximum densities between 0·5 and 1·5 when measured in a 1 cm. quartz cell. Control of hydrogen-ion concentration was obtained by suitable concentrations of hydrochloric acid or sodium hydroxide, or by addition of buffer solution to bring the concentration of buffer to 0·025M. The following buffer solutions were found to give transparent solutions over the range of wave-lengths of importance in this investigation; above pH 8·5, borate; pH 6·0—8·5, phosphate; pH 2·0—3·5, chloride. The range pH 3·5—6·0 was not of great importance; where measurements were required in this range, phosphate buffer with careful addition of hydrochloric acid was used.

When the spectra of the un-ionised and ionised forms of the molecule are known, and are sufficiently widely separated, the dissociation constants can readily be calculated from the spectra of mixtures of the two components at known pH values, provided that in compounds with more than one dissociation constant the pK values are sufficiently far apart, that is, separated by more than about 4 pK units. All the compounds examined here are in this class. The term pK value in this series of papers is applied to the negative logarithm of the constant of the equilibrium $BH^+ \rightleftharpoons B + H^+$. The first and second dissociation constants represent the tendency of the base to accept one or two protons respectively. The pK value is given by the expression

$$pK = pH + \log_{10} \frac{\epsilon_a - \epsilon}{\epsilon - \epsilon_b}$$

where ϵ_a and ϵ_b represent the extinction coefficients of the base and mono-salt, or the mono- and di-salt, all measured at the same wave-length. The precision of the method depends on the magnitude of the difference between ϵ_a and ϵ_b; if the addition of a proton produces only a small change in the spectrum then the method may involve a large error. This error can be minimised by replacing the optical-density values in the above equation by the difference in density of the spectrum at two wave-lengths selected such that the change in this difference, in passing from one form to the other, is a maximum. The figures quoted in the tables are calculated from at least two curves at different pH values; in no case does the variation exceed more than 0·3 pK unit.

Hydrogen-ion concentrations were measured with the glass electrode with a Cambridge valve potentiometer. For pH values above 10·0 the "Alki" glass electrode was used. The experiments were performed at room temperature which varied between 20° and 25°.

Some guide to the selection of suitable pH values for the solutions can be obtained by potentiometric titration. The aryldiguanides have only very slight solubility in water; 0·01M-solutions have been prepared in 2% aqueous acetone and titrated with 0·01N-hydrochloric acid. Reliable values of pK outside the limits 4 and 10 cannot be obtained by titration at this concentration, since the half-neutralisation point has to be corrected by a factor due to the hydrogen- and hydroxyl-ion concentrations; this factor increases rapidly as these limits are exceeded and accuracy diminishes. The experimental results are given in Tables I—IV and Figs. 1—4; in these the formulæ used are those conventionally ascribed to the bases of the compounds examined.

The following compounds were synthesised for the investigation; acknowledgments are made to Mr. S. Birtwell for the first compound.

p-*Chlorophenylacetamidine*.—This was prepared by the method of Hullin, Miller, and Short (*J.*, 1947, 394). *p*-Chloroaniline (12·9 g.) was dissolved in dry ether (30 c.c.), and the solution filtered and added slowly to a solution of ethylmagnesium iodide (55 c.c.) (Braude and Stern, *J.*, 1946, 404). The mixture was refluxed for 15 minutes, cooled, slowly treated with methyl cyanide (5 c.c.) in ether (20 c.c.), refluxed for 30 minutes, and left overnight. Ice (75 g.) and concentrated hydrochloric acid (25 c.c.) were added; the aqueous layer was separated and basified, and then filtered. The chloroform extract of the solid and liquid fractions was dried (Na_2SO_4) and evaporated to dryness. The crystals of p-*chlorophenylacetamidine* obtained were recrystallised twice from *cyclo*hexanone; m. p. 116—117° (Found: C, 57·2; H, 5·0; N, 16·7. $C_8H_9N_2Cl$ requires C, 57·0; H, 5·35; N, 16·2%).

N-p-*Chlorophenyl*-N-*methylguanidine Hydrochloride*.—*N*-Methyl-*p*-chloroaniline (3 g.), cyanamide (0·9 g.), and ethanol (1 c.c.) were heated under reflux for 10 hours. The *product* was recrystallised from ethanol; m. p. 227° (Found: Cl′, 16·9. $C_8H_{10}N_3Cl$,HCl requires Cl′, 16·15%).

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Research Laboratories, Blackley, Manchester, 9. [*Received, March 4th*, 1948.]