the substitution of a more arylated base for dimethylaniline decreases the basicity of the first ion of a diaminotriphenylmethane dye, that a second such substitution still further decreases the basicity, and that the radicals of triphenylamine and carbazole are much more effective than those of diphenylamine and methyldiphenylamine.

Summary

The spectra of the second ions of malachite green and some more highly phenylated diamino triphenylmethane dyes were measured. These spectra have been compared with those of the first ions of the same dyes.

It was found that phenylation has a much greater bathochromic effect on the x-band of a second ion than on the corresponding band of a first ion, and that the frequency differences between the x-bands and y-bands are greater in second ions than in first ions, and that phenylation increases this frequency difference in second ions, but decreases it in first ions.

It was found that the spectra of solutions containing both first and second ions were not linear combinations of the spectra of the first and second ions.

BERKELEY, CALIF.   RECEIVED NOVEMBER 13, 1946

Spectrophotometric and Potentiometric Evaluation of Apparent Acid Dissociation Exponents of Various 4-Aminoquinolines[1]

BY J. LOGAN IRVIN AND ELINOR MOORE IRVIN

Albert and Goldacre[2] have demonstrated that 4-aminoquinoline has the greatest basicity (attraction for protons) of the various mono-amino derivatives of quinoline. They attribute this enhancement of basicity to stabilization of the proton-donor (cation) by resonance of the type shown in Fig. 1. In the present paper, apparent acid dissociation exponents are reported for a series of substituted 4-aminoquinolines. These compounds possess varying degrees of antimalarial activity,[3] and the data reported here will permit an attempt to correlate antimalarial activity and physiological disposition of members of this series with their basicities and, consequently, with the relative proportions of the various ionic species which are present at the pH of body fluids. Such pharmacological and physiological studies have been made by Drs. J. Taggart and J. Shannon and will be reported elsewhere.

As represented in Fig. 1, the aromatic nuclei of the 4-aminoquinolines accept a single proton in dilute aqueous solutions as determined potentio-

(1) This is one of a series of studies in connection with a program in this Department on antimalarial compounds and the biochemistry of malaria. The work reported in this paper was done on a volunteer basis in coöperation with the main program which was conducted under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Johns Hopkins University. A preliminary report on this work has been published (Federation Proc., 5, 139 (1946)).

(2) Albert and Goldacre, Nature, 153, 467 (1944).

(3) Some of these compounds were synthesized in the laboratories of various universities under contracts with the Office of Scientific Research and Development, and others were prepared in the laboratories of several pharmaceutical companies. Sources of the compounds are acknowledged in Table I. Data concerning these and many other compounds have been assembled by the Survey of Antimalarial Drugs and will be published as a monograph, "A Survey of Antimalarial Drugs, 1941-1945," edited by F. Y. Wiselogle. At some points in this paper, these compounds are designated by numbers preceded by the letters, SN. These are the code numbers assigned by the Office of the Survey.

metrically or spectrophotometrically. A second proton is accepted by the aromatic nucleus in very concentrated aqueous solutions of strong mineral acids, as demonstrated spectrophotometrically. Most of the compounds with which this report is concerned have an aliphatic side-chain possessing a diethylamino group which will accept a proton in dilute aqueous solutions, as determined potentiometrically. Therefore, the acid–base reactions involving these compounds in *dilute aqueous solutions* can be formulated essentially as described by Adams[4] for the case of unsymmetrical dibasic acids. However, an extension is necessary in order to show the relationship of the intrinsic constants defined by Adams to the constants which are evaluated by the spectrophotometric method, which was not treated by Adams.

Formulation of Acid–Base Equilibria

Throughout this paper activities are indicated by parentheses and concentrations by brackets. The following symbols are used: $^+$HB—B$'$H$^+$, the dipolar cation or proton-donor in which B represents the resonating aromatic nucleus and B$'$ the diethylamino group of the side-chain.

B—B$'$H$^+$ and $^+$HB—B$'$, monopolar cations (see diagram of acid–base reactions).

B—B$'$, dipolar base or proton acceptor.

[S], molar concentration of the compound. [S] = [$^+$HB—B$'$H$^+$] at the origin of the titration.

K_1 and K_2, experimental acid dissociation constants as determined potentiometrically. K_1' and K_2' are the corresponding apparent constants defined in terms of *concentrations* rather than *activities*. (Definition of the apparent constants is restricted to the specification of *constant ionic strength*).

K_S, acid dissociation constant of the group associated with the aromatic nucleus (determined spectrophotometrically). K_S', the corresponding apparent constant (at constant ionic strength).
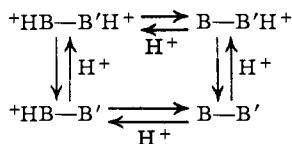
The steps in the acid dissociation of the dipolar proton-donor, $^+$HB—B$'$H$^+$ are

(4) Adams, THIS JOURNAL, 38, 1503 (1916).

$$^+\text{HB—B'H}^+ \rightleftarrows_{\text{H}^+} \text{B—B'H}^+$$

The intrinsic group constants as defined by Adams[4] are

$$K^{\text{I}} = (\text{H}^+)(\text{B—B'H}^+)/(^+\text{HB—B'H}^+)$$
$$K^{\text{II}} = (\text{H}^+)(^+\text{HB—B'})/(^+\text{HB—B'H}^+)$$
$$K^{\text{III}} = (\text{H}^+)(\text{B—B'})/(^+\text{HB—B'})$$
$$K^{\text{IV}} = (\text{H}^+)(\text{B—B'})/(\text{B—B'H}^+)$$

The corresponding apparent intrinsic group constants, as defined in terms of concentrations (at constant ionic strength), are represented as K'^{I}, etc.

Resonance hybrid of the proton acceptor (base)

(3 Kekulé structures)
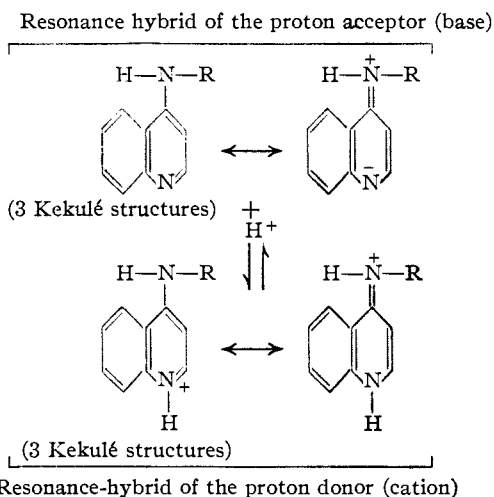
Resonance-hybrid of the proton donor (cation)

Fig. 1.—Formulation of the "first" proton reaction of the 4-aminoquinolines.

The experimental acid dissociation exponents, as determined by potentiometric titration, are defined and are related to the intrinsic group constants by the following equations (Adams[4])

$$K_1 = (\text{H}^+)((^+\text{HB—B'}) + (\text{B—B'H}^+))/(^+\text{HB—B'H}^+) \quad (1)$$
$$= K^{\text{I}} + K^{\text{II}} \quad (2)$$
$$K_2 = (\text{H}^+)(\text{B—B'})/((\text{B—B'H}^+) + (^+\text{HB—B'})) \quad (3)$$
$$= K^{\text{III}} \cdot K^{\text{IV}}/(K^{\text{III}} + K^{\text{IV}}) \quad (4)$$

The apparent acid dissociation exponents, pK'_1 and pK'_2, at constant ionic strength, are defined by logarithmic equations derived from equations 1 and 3

$$pK'_1 = pH - \log\{[[^+\text{HB—B'}] + [\text{B—B'H}^+]]/[^+\text{HB—B'H}^+]\} \quad (5)$$
$$pK'_2 = pH - \log\{[\text{B—B'}]/[[\text{B—B'H}^+] + [^+\text{HB—B'}]]\} \quad (6)$$

An acid–base equilibrium constant for the proton-acceptor group associated with the aromatic nucleus can be determined spectrophotometrically inasmuch as the species B and BH$^+$ differ in their absorption of radiant energy. The spectrophotometric method has been described by Clark[5] and by Flexser, Hammett and Dingwall[6] for cases in which the effect of interaction between groups did not have to be considered. In the formulation for dibasic compounds of the type discussed here, it is necessary to consider that the proton-acceptor form of the aro-

(5) Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, Md., 1928, pp. 152–159.

(6) Flexser, Hammett and Dingwall, THIS JOURNAL, **57**, 2103 (1935).

matic nucleus can exist as B—B'H$^+$ and B—B' and the proton-donor species can exist as $^+$HB—B'H$^+$ and $^+$HB—B'. However, in the region of the spectrum covered in these measurements it seems reasonable to assume that no marked difference in the absorption of radiant energy would exist between the forms B—B' and B—B'H$^+$ or between the species $^+$HB—B'H$^+$ and $^+$HB—B' inasmuch as the saturated aliphatic chain between the groups would exert an insulating effect. Substantial evidence of the validity of this assumption is presented in a subsequent section. With this assumption, K_S as evaluated spectrophotometrically is

$$K_S = (\text{H}^+)((\text{B—B'H}^+) + (\text{B—B'}))/((^+\text{HB—B'H}^+) + (^+\text{HB—B'})) \quad (7)$$

Strictly, K_S cannot be evaluated directly inasmuch as the spectrophotometric method determines *concentrations* rather than *activities* of the species. The corresponding apparent acid dissociation exponent, pK'_S, is defined (at constant ionic strength) as follows

$$pK'_S = pH - \log\{[[\text{B—B'H}^+] + [\text{B—B'}]]/[[^+\text{HB—B'H}^+] + [^+\text{HB—B'}]]\} \quad (8)$$

The value of pK'_S can be determined experimentally by spectrophotometric measurements at a selected wave length as indicated in equation 9

$$pK'_S = pH - \log\{(\epsilon_{\text{BH}^+} - \epsilon)/(\epsilon - \epsilon_\text{B})\} \quad (9)$$

in which ϵ_{BH^+} and ϵ_B are, respectively, the molecular extinction coefficients of the compound at some low pH value at which the aromatic nucleus is transformed completely into the proton-donor, BH$^+$, and at some high pH number at which it is transformed completely into the proton-acceptor, B. ϵ is the extinction coefficient of the compound in a buffer solution of intermediate pH number at which both forms B and BH$^+$ exist. By derivation from equation 7 and the equations for the intrinsic group constants, K_S is related to these constants as follows

$$K_S = (K^{\text{I}}(\text{H}^+) + K^{\text{II}} \cdot K^{\text{III}})/((\text{H}^+) + K^{\text{II}}) \quad (10)$$
$$= K^{\text{I}}((\text{H}^+) + K^{\text{II}} \cdot E)/((\text{H}^+) + K^{\text{II}}) \quad (11)$$

in which E is an electrostatic coefficient,[7] and $K^{\text{III}} = K^{\text{I}} \cdot E$. In the application to the compounds reported here, these general formulations can be separated into two "cases" which comprise restrictions and approximations.

Case a.—The distance separating the two groups is sufficiently short to permit electrostatic interaction, but $K^{\text{II}} \ll K^{\text{I}}$ and $K^{\text{IV}} \ll K^{\text{III}}$: Then equation 2 becomes

$$K_1 \cong K^{\text{I}} \quad (2a)$$

and equation 4 becomes

$$K_2 \cong K^{\text{IV}} \quad (4a)$$

For the conditions of case a, in the range of pH in which K_S is evaluated spectrophotometrically, $(\text{H}^+) \gg K^{\text{II}}$, therefore equation 11 becomes

$$K_S \cong K^{\text{I}} \quad (11a)$$

and from equation 2a

$$K_S \cong K_1 \quad (12a)$$

A majority of the compounds reported in this paper can be placed in the category of case a.

Case b.—The distance separating the two groups is sufficiently short to permit electrostatic interaction: $K^{\text{II}} < K^{\text{I}}$ and $K^{\text{IV}} < K^{\text{III}}$, but the values of K^{II} and K^{IV} are *not* negligible with respect to the values of K^{I} and K^{III}, respectively. In this case the more complex general treatment must be applied. Due to the effect of interaction, $K^{\text{I}} > K^{\text{III}}$ and $K^{\text{II}} > K^{\text{IV}}$. For case b, inasmuch as

(7) From Bjerrum's development[8] of the electrostatic interaction between the two groups of a dibasic acid, it can be shown that the electrostatic coefficient, E, can be approximated closely by the relationship: $E = e^{-\epsilon^2/DakT}$, in which a is the distance between groups, ϵ is the charge on the proton, D is the dielectric constant, k is Boltzmann's constant and T is the absolute temperature.

(8) Bjerrum, Z. physik. Chem., **106**, 219 (1923).

$K^I > K^{III}$, the following relationship exists between the constants

$$K_1 > K^I > K_8 > K^{III} \qquad (2b, 10b)$$

For case b, K_8 is not a true constant, and its value varies with the (H^+). K_8 *tends* to approach the value of K^I at the lower extreme and to approach the value of K^{III} at the upper extreme of the range of pH. The amount of variation of K_8 with pH bears an inverse relationship to the distance separating the groups and a direct relationship to the extent to which the intrinsic basicities of the two groups approach equality. Two of the compounds reported here (SN-3294 and SN-6732) are on the borderline between case a and case b.

Experimental

In the titrations, determinations of pH were made with a glass electrode, all observations being conducted at 30°. At different times in the course of this investigation, potentiometric measurements were made with (a) a Leeds and Northrup type K potentiometer with a quadrant electrometer as null-instrument, and (b) an electron-tube potentiometer. The glass electrode was standardized by means of buffers the pH numbers of which were determined[9] with a hydrogen electrode (the rocking electrode of Clark[10]). The standard of pH was "standard acetate" buffer,[11] a solution 0.1 molar with respect to both acetic acid and sodium acetate; it was assigned a pH number of 4.64 at 30°.

The majority of the titrations were of the continuous type, and in most cases it was possible to conduct the "first step" of the titration upon aqueous solutions of the salts of the compounds. Some of the compounds were sulfate or dihydrochloride salts; these were titrated directly. A few of the compounds were phosphate salts. These could not be titrated directly, therefore the following procedure was adopted: An aqueous solution of the phosphate salt (approximately 0.5 g.) was adjusted to pH 11.5–12 with sodium hydroxide and was extracted three times with 50-ml. portions of pure ethylene dichloride. These extracts were combined and were "washed" twice by extraction with 25 ml. portions of distilled water. Then the compound was extracted into a known amount (excess) of standardized hydrochloric acid. The concentration of the compound in this aqueous solution was calculated by potentiometric titration of the excess hydrochloric acid and independently from the data for the first step of the titration of the compound by application of the mathematical analysis of Reed and Berkson.[12] In some cases, as a test of purity, the extraction procedure was applied to other compounds even though they were already in the form of the hydrochloride salts.

The "first steps" of the titrations of the 4-aminoquinolines were conducted in aqueous solutions[13] the concentrations of which were 0.015 to 0.02 M. In every case the ionic strength of the solution was adjusted to 0.1 by addition of inorganic salt, usually sodium chloride, and it was maintained at this value by suitable additions at frequent intervals throughout the titrations.

From the data for the volumes of standard sodium hydroxide added at progressive intervals in the titration, values of [y] were calculated; [y] $\equiv$ [B—B′H$^+$ + $^+$HB—B′] + [d], in which [d] is the concentration of sodium ions corresponding to the amount of sodium hydroxide added in order to attain the "origin" of the

titration of $^+$HB—B′H$^+$. Values of [y] were calculated from equation 13

$$[y] = [Na^+] - [\overline{OH}] + [H^+] - 2[B-B'] \quad (13)$$

in which [Na$^+$] is the concentration of sodium ions corresponding to the total amount of sodium hydroxide added in the titration up to the interval involved in the calculation; [$\overline{OH}$] and [H$^+$] are the hydroxyl and hydrion concentrations, calculated from the pH and appropriate values of γ_H, $(\gamma_H\gamma_{OH}/a_{H_2O})$, and K_W from data given by Harned and Owen[14] (e. g., for the value of γ_H the mean activity coefficient of hydrochloric acid in 0.01 M concentration in 0.1 M sodium chloride was used). Evaluation of [B—B′] in equation 13 presented a problem which was resolved by a simplification in most cases but which had to be treated more rigorously for a few compounds. In the simple case which applied to most of the compounds, the two apparent acid dissociation exponents differed by 2 or more pH units, therefore [B—B′] could be neglected in calculating values of [y] for the first half of the first step of the titration, i. e., for that portion in which the first step was practically uncomplicated by the second step. Finally, the rectification procedure of Reed and Berkson[12] was applied to the values of [y] and pH (for the first half of the titration of the first step) in order to evaluate pK_1', [d] and [S]. The method of Reed and Berkson[12] has been adapted by Clark and Perkins[15] for rectification of data from oxidation–reduction titrations, and a similar procedure is applicable to acid-base titrations. The treatment applied to the more complicated cases in which [B—B′] could not be neglected is deferred until after the description of the procedure by which pK_2' was evaluated in the uncomplicated cases.

In most cases the second apparent acid dissociation exponent could not be determined by titration in aqueous solution because of the very slight solubility of the bases in water. For the evaluation of this constant, values of pK_2' were determined for aqueous-ethanol solutions of the compound (at constant ionic strength of 0.1), and the value of pK_2' for aqueous solution and $\mu = 0.1$ was estimated by extrapolation to 0 per cent. ethanol. Determinations of the constants for aqueous-ethanol solutions were conducted as follows: Standard solution of sodium hydroxide was added (in an amount which would carry the titration to, or slightly beyond, the midpoint of the second "step") to a 0.01 M solution of the compound in an ethanol–water mixture of known composition (usually 60% ethanol by volume) containing sodium chloride to bring the ionic strength to a value of 0.1. The pH was determined with a glass electrode.[16] Then the solution was diluted *seriatim* with an aqueous solution of sodium chloride ($\mu = 0.1$), the pH number being determined after each dilution. The dilution was continued to the first appearance of turbidity (usually at a concentration of ethanol of 20–25 per cent. by volume). The values of pK_2' corresponding to the various stages in the dilution were calculated by equation 14 derived from equation 6

$$pK_2' = pH - \log[[Na^+] - [d] - [\overline{OH}] - [S] + [^+HB-B'H^+]] + \log[2[S] - [Na^+] + [d] + [\overline{OH}] - 2[^+HB-B'H^+]] \quad (14)$$

The hydroxyl ion concentrations were estimated by "blank" titrations of the various ethanol-water mixtures with sodium hydroxide. The value of [S] was calculated from the known composition of the solution, but independent calculations of [S] and [d] were made in the course of the rectification of the data for the first step of the titration by the method of Reed and Berkson. In the simple

(9) We wish to thank Dr. Curt C. Porter for aid in the hydrogen electrode measurements.

(10) Reference 5, pp. 294–297.

(11) Michaelis, "Die Wasserstoffionenkonzentration," Berlin, 1914; MacInnes, Belcher and Shedlovsky, THIS JOURNAL, **60**, 1094 (1938).

(12) Reed and Berkson, J. Phys. Chem., **33**, 760 (1929).

(13) In most cases, the titrations of the aqueous solutions could not be extended beyond the approximate limit of pH 8.3 to 8.7 because of the insolubility of the un-ionized base. The titrations were terminated at the first appearance of turbidity.

(14) Harned and Owen, "The Physical Chemistry of Electrolytic Solutions," Reinhold Publ. Corp., New York, N. Y., 1943.

(15) Clark and Perkins, THIS JOURNAL, **54**, 1228 (1932).

(16) Potential differences between the glass electrode in *aqueous-ethanol* solutions and the saturated calomel electrode with saturated potassium chloride junction were calculated as "pH numbers" by reference to potentials measured with these electrodes for standardized aqueous buffers

case which applied to the majority of these compounds, $pK_2' - pK_1'$ was equal to, or greater than, 2 pH units, therefore the factor [$^+$HB—B$'$H$^+$] was neglected with an error less than 1 per cent. This procedure was repeated two additional times with separate portions of the solution of the compound in 60 per cent. ethanol-water, the titrations of the second "step" being carried to other points by addition of smaller or larger amounts of sodium hydroxide. Then the averaged values of pK_2' were plotted against the corresponding concentration of ethanol, and the value of pK_2' for $\mu = 0.1$ and ethanol = 0 per cent. was obtained by extrapolation.

For the complicated cases in which $pK_2' - pK_1'$ was less than 2 pH units, these constants were calculated by adapting the mathematical treatment of Simms[17] to the procedure described above. In the treatment of Simms, the data are handled *as if* the titration of a mixture of two *independent monopolar* proton-donors were involved. The constants calculated by means of this assumption were the "apparent titration constants," G_1' and G_2', as defined by Simms. From these constants the apparent acid dissociation constants, K_1' and K_2', were calculated according to Simms by equations 15 and 16:

$$K_1' = G_1' + G_2' \tag{15}$$
$$K_2' = G_1' \cdot G_2'/(G_1' + G_2') \tag{16}$$

In the potentiometric determination of the apparent acid dissociation exponents, the average error of the average was ± 0.02 pH unit for pK_1' and ± 0.05 pH unit for pK_2'. However, the average data are reported to the nearest 0.01 pH unit in order to protect the figure in the tenth-unit position.

The apparent acid dissociation exponent of the first proton-reaction involving the aromatic nucleus, as defined by equations (8) and (9), was determined spectrophotometrically as follows: Measured volumes of an aqueous solution of the salt of the compound were diluted accurately to known final concentrations of 1×10^{-5} to 1×10^{-4} M by means of a series of buffers the pH numbers of which were within the range of the acid–base transformation involving the aromatic nucleus. The ionic strength of the final solution was 0.1 in every case. Each series included one buffer the pH of which was sufficiently low to insure complete transformation of the aromatic nucleus into the proton-donor form, BH$^+$, and another buffer of such high pH that the aromatic nucleus was transformed completely into the proton-acceptor form, B. Complete spectrophotometric absorption curves down to a wave length of 220 mμ in the ultraviolet were obtained on the two latter solutions. All measurements were made with a Beckman Photoelectric Quartz Spectrophotometer, model-DU, the cuvette-compartment of which was equipped with a special plate through which water was circulated from a thermostat, thus maintaining the solutions at 30 $\pm$ 0.5°. The nominal band width was 2 mμ. The buffer solutions were used as "blanks," the transmittancies of these buffers being taken as unity. Absorption data were calculated as molecular extinction coefficients, ϵ, which are defined by the equation, $-\log T = D = \epsilon cl$, in which T is the transmittancy expressed as a fraction of unity, D is the optical density, c is the concentration in moles per liter, and l is the length (cm.) of the light-path through the solution.

At several wave lengths, selected on the basis of marked differences between values of ϵ_{BH^+} and ϵ_B, the extinction coefficients of the various buffered solutions of the compound were determined. The pH numbers of these solutions were measured with a glass electrode, standardized as stated in a preceding section. From the corresponding data for pH, ϵ, ϵ_{BH^+} and ϵ_B at a selected wave length, values of pK_S' could be calculated by equation (9). However, in some cases it was difficult to determine the value of ϵ_B with accuracy because at a pH number at which the aromatic nucleus was completely in the un-ionized form, B, a considerable fraction of the compound

(17) Simms, THIS JOURNAL, **48**, 1239 (1926).

existed as the species, B—B$'$, which was quite insoluble in water and was adsorbed readily upon glass. For this reason, in most cases the value of pK_S' was calculated from the extinction coefficients of the compound at various pH values by rectification of the data by the general method of Reed and Berkson[12] with adaptations from Clark's[18] application of the method to spectrophotometric data for equilibria involving metalloporphyrins. The procedure is as follows: An orienting value of pH is selected from the experimental data, the sign is changed, and the result is termed pH_n. The corresponding value of ϵ is designated ϵ_n. Adding pH_n to both sides of equation (9)

$$pH + pH_n = pK_S' + pH_n + \log \{(\epsilon_{BH^+} - \epsilon)/(\epsilon - \epsilon_B)\} \tag{17}$$

Define

$$pH + pH_n = p \tag{18}$$

and

$$pK_S' + pH_n = \log C \tag{19}$$

From equations (17) − (19)

$$10^p = C(\epsilon_{BH^+} - \epsilon)/(\epsilon - \epsilon_B) \tag{20}$$

Where $p = 0$, $\epsilon = \epsilon_n$, and from equation (20)

$$1 = C(\epsilon_{BH^+} - \epsilon_n)/(\epsilon_n - \epsilon_B) \tag{21}$$

The following relationship is derived from equations (20) and (21)

$$\epsilon = (C + 1)(\epsilon - 10^p \epsilon_n)/(1 - 10^p) - C\epsilon_{BH^+} \tag{22}$$

Equation (22) is an equation of a straight line the properties of which determine pK_S', ϵ_{BH^+} and ϵ_B. The slope of the straight line is $(C + 1)$, from which pK_S' is calculated by equation (19). The intersection with the ϵ axis gives $- C\epsilon_{BH^+}$ from which ϵ_{BH^+} can be calculated.[19] Thence ϵ_B is found from equation (21).

In applying the above method to the calculation of pK_S' for the compounds reported here, particularly those for which $pK_2' - pK_1'$ was less than 2 pH units, only the data for the first half of the transformation were used, i. e., for that region of pH in which the principal species involved were $^+$HB—B$'$H$^+$ and B—B$'$H$^+$ with only negligible amounts of the species, $^+$HB—B$'$ and B—B$'$. Therefore, for the compounds of this series, even those which are in the category of case b, K_S' is practically identical with K'^I, the first apparent intrinsic group constant. By this method of rectification of the data for the first half of the transformation, the value of ϵ_B calculated from equation (21) corresponds to the molecular extinction coefficient of the species B—B$'$H$^+$; thus the necessity for assuming that the extinction coefficients for the species B—B$'$H$^+$ and B—B$'$ are identical (see earlier discussion of the spectrophotometric method) is obviated. Nevertheless, in a number of cases, it was possible *to determine directly* the extinction coefficient of the species B—B$'$ by measurement at a high pH-value, and it was found to be practically identical with the *calculated* value (at a few selected wave lengths) of the extinction coefficient of the species B—B$'$H$^+$. Evidently the saturated aliphatic chain between the groups exerts an insulating effect.

The precision of the spectrophotometric determination of pK_S' was difficult to evaluate inasmuch as the calculations involved fitting a straight line. However, in every case calculations were made from four selections of pH_n (equations 17–22) with each of two independently determined sets of data. The average deviation of the average was ± 0.02 pH unit.

Discussion

Spectrophotometric absorption curves for several derivatives of 4-aminoquinoline in dilute

(18) Clark, Taylor, Davies and Vestling, *J. Biol. Chem.*, **135**, 543 (1940).

(19) In most cases, ϵ_{BH^+} was evaluated more accurately by direct spectrophotometric measurement of an acid solution of the compound.

aqueous solutions at various values of pH are presented in Figs. 2–7. In each case, well-defined isobestic points[20] were maintained throughout the range of pH defined in these figures. This is evidence that the transformation in each case involves only two species, and therefore it is likely that the reaction is a single proton reaction of the type suggested by Albert and Goldacre[2] and formulated in Fig. 1. In each case shown in Figs. 2–7, the curve at the lowest value of pH corresponds to absorption by the proton-donor or monopolar cationic species of the aromatic nucleus; the curve at the highest value of pH is due to the proton-acceptor or base; and the curve at the intermediate pH is due to a mixture of the two species. A majority of the compounds listed in Table I are in the category of case a, and the values of

TABLE I

APPARENT ACID DISSOCIATION EXPONENTS ($\mu = 0.1$; 30°) SERIES OF 4-AMINOQUINOLINES

HNR
(6)   (3)
(7)   N (2)

Source of compound	Survey number[a]	Substituent groups 2	3	6	7	R[b]	pK_S[g]	pK_1'	pK
c	8975	H	H	OCH₃	H	H	8.93	8.95	
d	11283	H	H	H	Cl	H	8.23	8.22	
e	6732	H	H	H	H	R₁	8.82	8.76	10.2₀
c	3294	H	H	OCH₃	H	R₁	8.73	8.68	10.2₀
e,f	7618	H	H	H	Cl	R₁	8.08	8.06	10.1₆
e	7373	H	H	H	Br	R₁	8.12	8.09	10.1₅
e	7135	CH₃	H	H	Cl	R₁	8.54	8.51	10.1₈
e	6911	H	CH₃	H	Cl	R₁	7.28	7.29	10.1₅

[a] Reference 3.   [b] R₁ = —CH(CH₃)·(CH₂)₃N(C₂H₅)₂.   [c] B. Riegel.   [d] R. Elderfield.   [e] Sterling-Winthrop Research Laboratories (A. R. Surrey and H. F. Hammer).   [f] N. Drake.   [g] pK_S' was evaluated by the logistic analysis (equations 17–22) of data for the following ranges of pH: SN-8975, pH 7.8–9.9; SN-11283, pH 7.1–9.2; SN-6732, pH 7.7–8.5; SN-3294, pH 7.5–8.4; SN-7618, pH 7.0–8.3; SN-7373, pH 7.0–8.3; SN-7135, pH 7.3–8.3; SN-6911, pH 6.1–8.0. In each case, pK_S is practically constant in these ranges of pH, and pK_S is essentially pK'_1.

pK_S' determined spectrophotometrically are nearly identical with the corresponding values of pK_1' determined potentiometrically. An example of the agreement in the evaluation of these exponents for a member of case a is presented in Fig. 8. For compounds SN-3294 and SN-6732 the differences between corresponding values of pK_2' and pK_1' are less than 2 pH-units (case b), and the values of pK_S' are greater than the corresponding values of pK_1', as predicted by relationship 2b, 10b, by amounts which are barely significant. These two compounds are on the borderline between case a and case b. A better example of a compound which is more strictly in the category of case b will be presented in a subsequent paper.

In concentrated aqueous solutions of sulfuric acid these 4-aminoquinoline derivatives undergo

(20) Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, Md., 1928, pp. 152–154.

another reversible transformation which probably involves addition of a second proton to the aromatic nucleus, thus forming a dipolar cation. Actually, inasmuch as most of these compounds possess a side chain with a diethylamino group, the molecule as a whole would be a tripolar cation. It seems most likely that this second proton is attached to the secondary amino group. Evidence for this reaction is obtained spectrophotometrically. A typical example is shown in Fig. 9 (SN-7618) in which the curve at the lowest concentration of sulfuric acid is due to the resonating monopolar cationic form of the aromatic nucleus; the curve at the highest concentration of sulfuric acid (17.5 M) is due to the dipolar cationic form of the aromatic nucleus; and the intermediate curve is due to a mixture of the two species. Isobestic points were defined fairly well in most cases although a slight "medium-effect"[6] was observed. For comparison, spectrophotometric absorption curves for solutions of the various compounds in 17.5 M sulfuric acid are presented collectively in Fig. 10. The concentration of sulfuric acid at the *midpoint* of the transformation is recorded for each compound in Table II. The transformation can be reversed in every case by dilution of the solution with water.

TABLE II

MIDPOINTS IN THE TRANSFORMATION OCCURRING IN THE SECOND PROTON REACTION INVOLVING THE AROMATIC NUCLEI OF THE 4-AMINOQUINOLINES

Survey number of cpd.	Concn. of sulfuric acid at midpoint, moles per liter
11283	14.14
6732	14.88
3294	14.09
7618	16.13
7135	14.82
6911	10.93

The absorption curves for these 4-aminoquinoline derivatives in concentrated sulfuric acid (Fig. 10) resemble the curve for unsubstituted quinoline cation[21] in dilute acid aqueous solution. By contrast, the absorption of the 4-aminoquinolines in dilute acid aqueous solution is of a special type (Figs. 2–7), particularly characterized by an intense band at the longer wave lengths which presumably is related to resonance of the type represented in Fig. 1. The curve for SN-3294 in concentrated sulfuric acid is almost identical in shape with the curve for quinine in dilute acid aqueous solution, but the wave lengths of the peaks of the bands are shifted slightly. In concentrated sulfuric acid when excited by daylight or by radiation of the 365 mμ line of the mercury emission spectrum, SN-6732 exhibits pale violet fluorescence; SN-3294 and SN-8975 are powerfully fluorescent (blue color), whereas those 4-aminoquinolines with halogens substituted in the 7-position do not exhibit visible fluoresence in con-
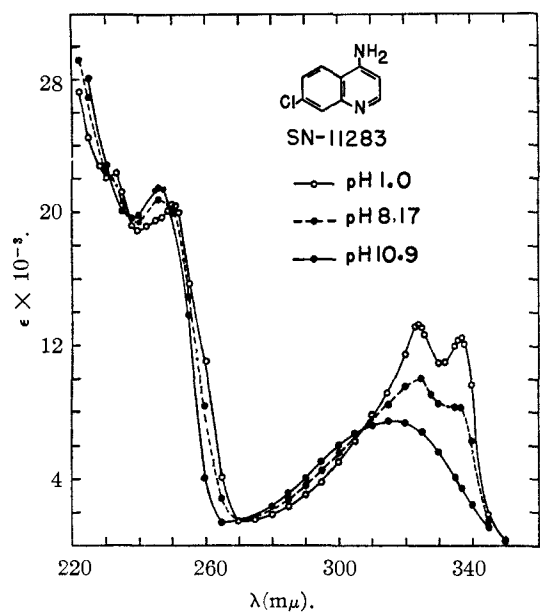
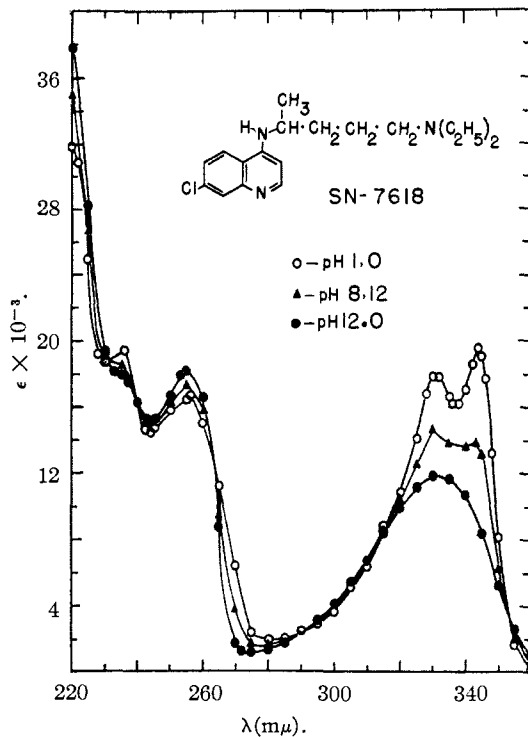(21) Ewing and Steck, THIS JOURNAL, **68**, 2181 (1946).
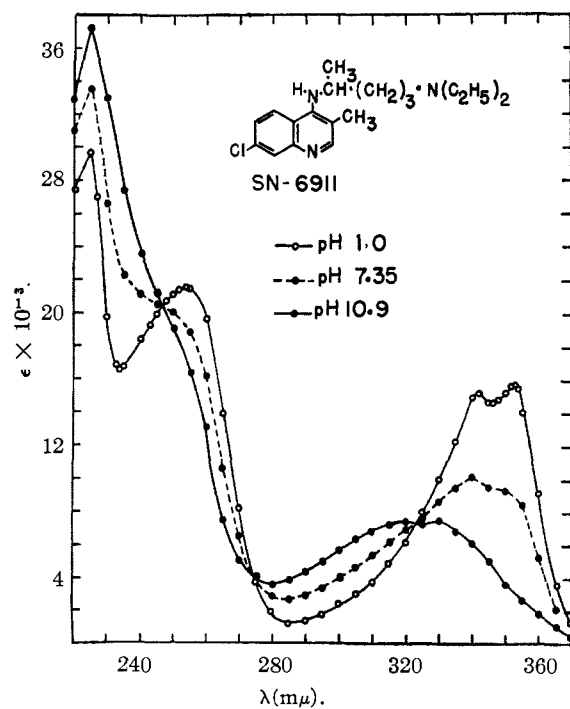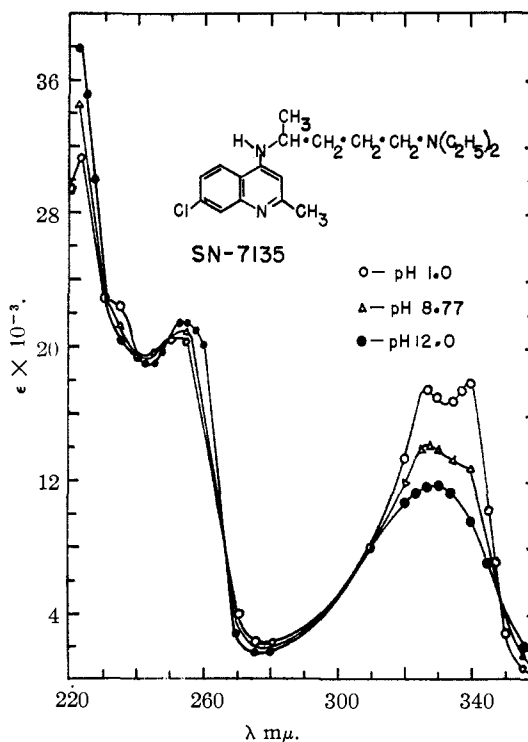
Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Figures 2–7.—Spectrophotometric absorption curves of several derivatives of 4-aminoquinoline in aqueous solutions at various values of pH: Fig. 2, SN-11,283; Fig. 3, SN-7618; Fig. 4, SN-6911; Fig. 5, SN-7135; Fig. 6, SN-3294; Fig. 7, SN-6732.
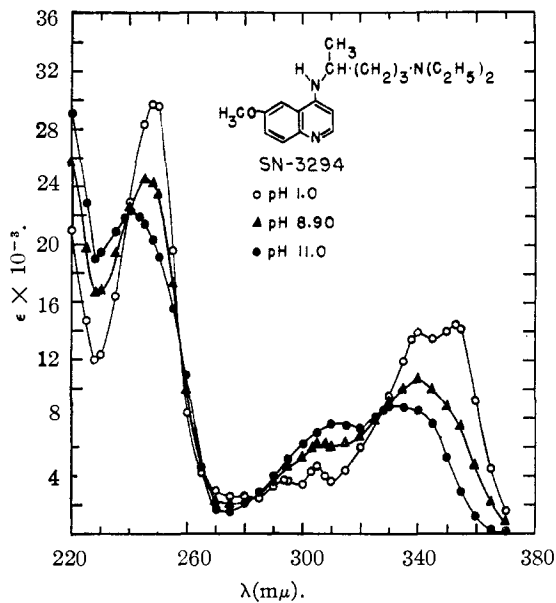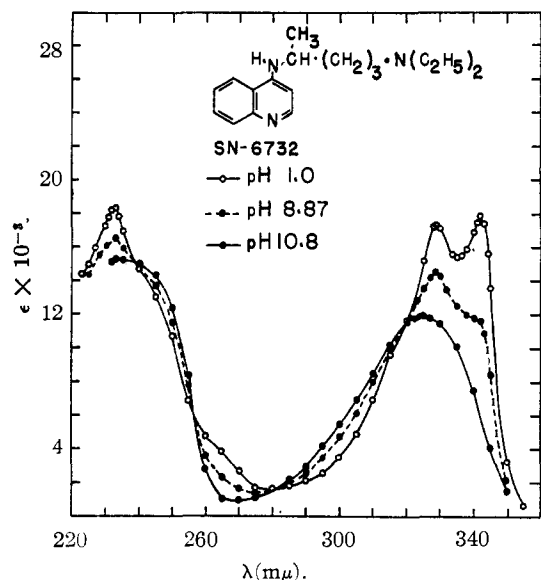
Fig. 6.

Fig. 7.

centrated sulfuric acid. All of the 4-aminoquino-lines examined are practically non-fluorescent in dilute aqueous solutions of sulfuric acid (0.05 M) with the exception of SN-8975 which emits faint violet fluorescence. In the latter case, the pos-sibility that the fluorescence is due to contamina-tion with a trace of a fluorescent impurity has not been excluded conclusively.

Resonance of the monopolar cationic species of the aromatic nucleus of the 4-aminoquinolines (Fig. 1) has a marked effect upon the equilibria involved in the addition of *each* of the two pro-tons to the acceptor-centers of the aromatic nu-cleus. The important resonance of this ionic spe-cies shifts both equilibria in the direction of this

"stabilized" ion. Thus for comparison with the 4-aminoquinolines, pamaquin,[22] the monopolar cation of which is unable to resonate with a quino-noidal "structure" comparable to that of the 4-aminoquinolines, has a value of 3.46 for pK'_S (identical with pK'_1), and the midpoint for the addition of a second proton to the aromatic nu-cleus occurs in 2.8 M sulfuric acid. In both the 8- and the 4-aminoquinolines, the inductive and direct electrostatic effects of the first positive charge on the aromatic nucleus would oppose the acquisition of a second proton, but the very marked opposition in the case of the 4-aminoquin-olines probably is due to the powerful resonance of the monopolar cationic species of the aromatic nucleus.
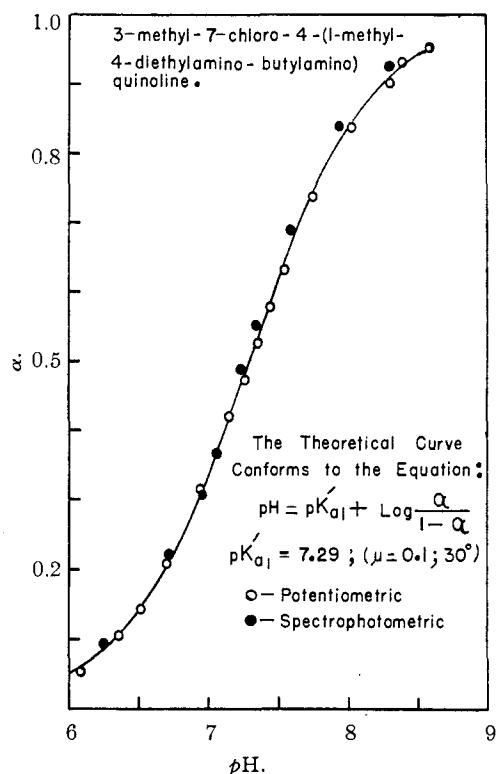

Fig. 8.—Example illustrating agreement between the potentiometric and spectrophotometric methods for the evaluation of the apparent acid dissociation exponent for the first proton-reaction involving the aromatic nucleus of a compound (SN-6911) in the category of case a. For the spectrophotometric method, $pK'a_1 \equiv pK's$. For the potentiometric method, $pK'a_1 \equiv pK'_1$, as defined in this paper. α is the fractional degree of dissociation of the proton-donor (cation) into proton and proton-acceptor.

In Fig. 1 the resonance hybrid of the proton acceptor species of the aromatic nucleus is repre-sented as receiving some contribution from "struc-tures," of which one example is shown, in which there is separation of charge. It is likely that the

(22) Pamaquin (plasmochin) is 8-(4-diethylamino-1-methylbutyl-amino)-6-methoxyquinoline.
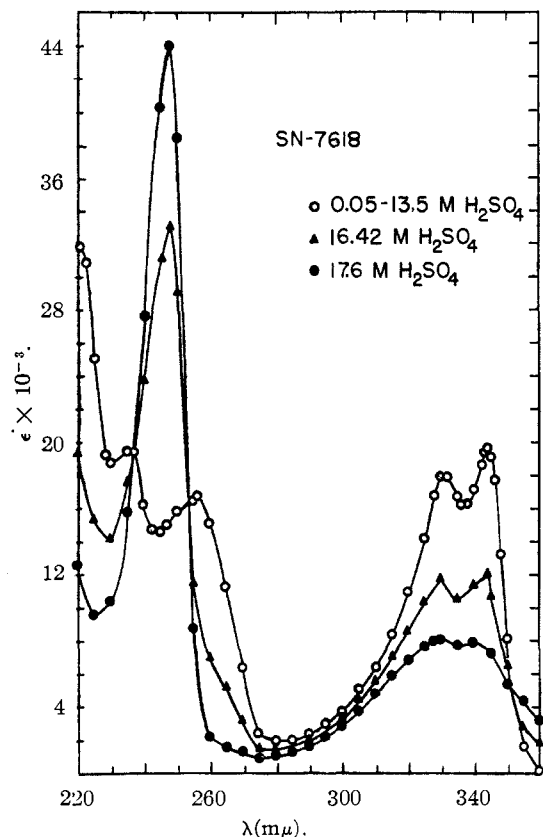
Fig. 9.—Spectrophotometric absorption curves for SN-7618 in various solutions of sulfuric acid, providing evidence of a second proton-reaction involving the aromatic nucleus. The absorption in 0.05 M sulfuric acid (unchanged as the concentration of sulfuric acid is increased to 13.5 M) is due to the monopolar cation of the aromatic nucleus. The absorption in 17.6 M sulfuric acid is due to the dipolar cation.

Fig. 10.—A collection of spectrophotometric absorption curves for various derivatives of 4-aminoquinoline in 17.5 M sulfuric acid.

contribution from such "structures" is small. However, any contribution of this type would tend to increase the attraction of the ring-nitrogen for a proton.

The data in Tables I and II permit a partial analysis of the effects of various substituents upon the reactions of 4-aminoquinolines with protons. Inasmuch as the values of pK_2', which are largely concerned with the diethylamino group of the side-chains, are practically identical for all compounds of this series, the following discussion is confined to an analysis of the effects of substituents on the stepwise addition of protons to the two acceptor-centers of the aromatic nucleus. By comparison of the data for SN-8975 and SN-11283 with the corresponding data for SN-3294 and SN-7618, respectively, it is apparent that substitution of the side-chain on the 4-amino group opposes the acquisition of both protons by the aromatic nucleus probably due to the inductive and direct electrostatic effects of the positive charge which resides upon the diethylamino
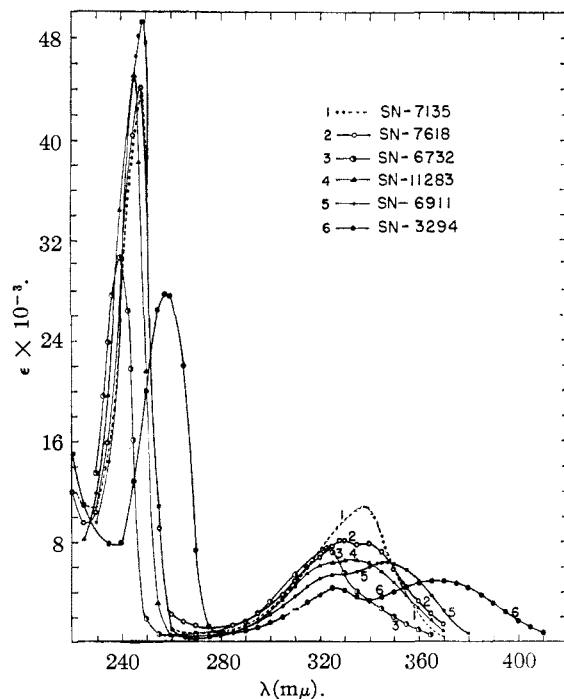
group of the side-chain in the range of acidity in which the proton reactions involving the nucleus are evaluated. The effects of various other substituents can be evaluated most readily by comparison of the individual members of the series with identical side-chains, and especially by comparison of the substituted compounds with SN-6732 which is unsubstituted.

Substituents upon the aromatic nuclei of the 4-aminoquinolines exert polar effects which probably influence the proton reactions in two ways. First, the dipoles produced by such substituents modify electrostatically the "attraction" of the nitrogen atoms for protons. Second, such polar effects may modify the resonance hybrids of the proton acceptor and proton donor species of the compound by changing the relative magnitudes of the contributions from the various resonance "structures." By such modification of the relative contributions from the various "structures" the resonance energy might be either increased or decreased. In this connection, the position of the substituent *relative to the two nitrogen atoms* should be very important. Unsymmetrically placed substituents might be expected to exert the greatest influence upon the resonance hybrids. However, as pointed out by Wheland,[23] from a consideration of the resonance in the various molecules one usually can draw conclusions only in regard to the differences between the corresponding changes in *internal energy* rather than *free energy* with which

(23) Wheland, "The Theory of Resonance," John Wiley and Sons, Inc., New York, N. Y., 1944, pp. 164–167.

chemical equilibria are concerned directly. For this reason, conclusions regarding the effects of substituents are somewhat uncertain.

In SN-3294, substitution of the electron-attracting methoxy group in position-6, which is separated from the two nitrogen atoms by equal numbers of carbon atoms, causes only a slight decrease in pK'_S. This decrease could be due either to the inductive effect of the methoxy group *per se* or to its effect in decreasing the resonance energy of the monopolar cationic species of the aromatic nucleus possibly as a result of further decrease in symmetry of the molecule, or to a combination of both of these effects. Decrease in the resonance energy of the monopolar cationic species could account for the fact that a second proton is added somewhat more readily, the midpoint of the reaction being at 14.09 M sulfuric acid instead of 14.88 M as in the case of SN-6732.

An electron-attracting group in position-7, such as the chloro group in SN-7618, which is closer to the ring nitrogen than to the secondary amino nitrogen, causes a considerable weakening of the reactions with *both* protons. The increased opposition to addition of the *second* proton is somewhat unexpected. It is difficult to explain unless such substitution results in an *increase* in the resonance energy of the monopolar cationic species of the aromatic nucleus by establishment of a resonance hybrid which receives more nearly optimum contributions from Kekulé and quinonoid "structures." However, such an explanation for the weakening of the reaction with the *second* proton introduces difficulty in explaining the weakening of the reaction with the *first* proton unless the inductive or electrostatic effect of the carbon–chlorine dipole diminishes the attraction for the first proton sufficiently to overcome the opposite effect upon the equilibrium which would result from the assumed increase in resonance energy of the monopolar cationic species. The final explanation must await further study, particularly the examination (now in progress) of monochloro derivatives with the chloro group in other positions such as 5, 6 and 8.

In the case of SN-7135, the methyl group (electron-repelling relative to hydrogen) in position-2 tends to oppose the effect of the electron-attracting chloro group in position-7, and the equilibrium constants for the two proton-reactions involving the aromatic nucleus do not differ greatly from the corresponding values for SN-6732 which is unsubstituted. In SN-6911 which has a chloro group in position-7 and a methyl group in posi-

tion-3 ortho to the 4-amino group, there is marked weakening of the reaction of the aromatic nucleus with the first proton (pK'_S, 7.28) and a considerable strengthening of the reaction with the second proton (midpoint at 10.93 M sulfuric acid) as compared with SN-7618 and the other 4-aminoquinolines. These large changes may be due in part to the polar effect of the methyl group, but it seems more reasonable that changes of this magnitude are due principally to a decrease in resonance of the monopolar cationic species by steric hindrance between the 3-methyl group and the side-chain on the amino group. Such steric hindrance would interfere with the coplanarity which is required for the quinonoid "structure" of the resonance hybrid, and the resulting decrease in resonance would shift the equilibria for both proton reactions in the directions of the observed changes.

Summary

Apparent acid dissociation exponents of various derivatives of 4-aminoquinoline were determined potentiometrically and spectrophotometrically. The effect of electrostatic interaction between groups is discussed from the standpoint of the relationship of this effect to the spectrophotometric evaluation of the dissociation exponent of the group associated with the aromatic nucleus.

The aromatic nuclei of the 4-aminoquinolines accept two protons in widely separated steps. The first proton probably is accepted by the ring-nitrogen, and the resulting monopolar cation presumably is a resonance-hybrid receiving contributions from Kekulé and quinonoid "structures." Spectrophotometric and fluorimetric evidence indicates that a second proton reacts reversibly with the aromatic nucleus (probably with the 4-amino group) in concentrated aqueous solutions of sulfuric acid. The marked strengthening of the first proton-reaction and the considerable weakening of the second reaction, in comparison with the 8-aminoquinolines, appears to be due to the important special resonance of the monopolar cation of the 4-aminoquinolines. The effects of various substituent groups on the equilibrium constants for these proton-reactions are described in terms of steric and polar influences.