extracted with 95% ethanol leaving 30 g. of sodium sulfate. The ethanol extract was evaporated to dryness and this residue was washed repeatedly with acetone and ether. This resulted in the isolation of a white crystalline salt, sodium phenylacetaldehyde-α-sulfonate. The yield was 33 g. or 41.3%.

A similar procedure was used for the sulfonation of heptaldehyde and isobutyraldehyde. The aldehydesulfonates failed to form S-benzylthiouronium salts. Attempts to form oximes and phenylhydrazones of the aldehydesulfonates also failed.

Cleavage of Sodium Phenylacetaldehyde-α-sulfonate.—Two grams of sodium phenylacetaldehyde-α-sulfonate was refluxed with 50 cc. of 20% sodium hydroxide for four hours. The alkaline mixture was acidified with sulfuric acid and formic acid was steam distilled from the mixture. Formic acid was identified through its *p*-bromophenacyl ester (m. p. 140°). The acidified mixture was then neutralized with sodium hydroxide solution and evaporated to dryness. The mixture of salts which remained was extracted with 95% ethanol and 1 g. of a sodium sulfonate was obtained by evaporating the ethanol extract to dryness. The S-benzylthiuronium salt of this sulfonate was prepared (m. p. 162°) and it did not depress the melting point of an authentic sample of S-benzylthiuronium ω-toluenesulfonate.

Anal. Calcd. for $C_{15}H_{18}O_3S_2N_2$: N, 8.28. Found: N, 8.12.

Sodium heptaldehyde-α-sulfonate was cleaved in a similar manner to give formic acid and 1 g. of a sodium sulfonate. The S-benzylthiuronium salt of the sodium sulfonate (m. p. 95°) did not depress the melting point of an authentic sample of S-benzylthiuronium 1-hexanesulfonate.

Anal. Calcd. for $C_{14}H_{24}O_3S_2N_2$: N, 8.43. Found: 8.44.

Summary

1. Four ketones, acetophenone, 2-acetothienone, acetomesitylene and β-acetonaphthone, have been sulfonated using dioxane sulfotrioxide. For the first three ketones, the corresponding sodium ω-ketonesulfonates have been isolated as the principal products in approximately 70% yields (recrystallized). β-Acetonaphthone yielded a mixture of sulfonates.

2. Pinacolone, acetone, cyclohexanone, propiophenone, and isobutyrophenone have been sulfonated with dioxane sulfotrioxide to the corresponding sodium ketone-α-sulfonates.

3. Phenylacetaldehyde, isobutyraldehyde, and heptaldehyde have been sulfonated with dioxane sulfotrioxide to the corresponding sodium aldehyde-α-sulfonates.

4. A mechanism has been proposed for the course of this reaction.

LAFAYETTE, INDIANA RECEIVED FEBRUARY 9, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, THE JOHNS HOPKINS UNIVERSITY, SCHOOL OF MEDICINE]

Apparent Ionization Exponents of Homologs of Quinacrine; Electrostatic Effects[1]

BY J. LOGAN IRVIN AND ELINOR MOORE IRVIN

Correlation of the pharmacological properties of the 9-aminoacridine series of antimalarials[2] with their physico chemical characteristics is obviously desirable in attempting to determine their mode of action. For this purpose data concerning the ionization exponents are particularly useful inasmuch as the degree of ionization of these compounds at the *p*H values of body fluids is an important factor in the distribution of these drugs in various tissues,[3,4] in their reversible combination with certain proteins,[3,5] and in their excretion in the urine.[4]

Ionization exponents of quinacrine, 2-methoxy-6-chloro-9-(4′-diethylamino-1′-methylbutylamino)-acridine, have been reported by Christophers.[6] In the present paper data are presented for two homologous series of derivatives of quinacrine with side-chains of varying length but possessing a diethylamino group on the terminal carbon atom in each case. The members of one series of compounds differ from the corresponding members of the other series in lacking the 1′-methyl group of the quinacrine side-chain. The data for these compounds have provided the basis for an evaluation of the effect of electrostatic charges upon proton-equilibria involving a resonating compound. The data are compared with those reported by Schwarzenbach for a series of aliphatic diamines.

Formulation of Equilibria.—By potentiometric titration of aqueous solutions of the 9-aminoacridines reported in this paper two ionization exponents, pK_1' and pK_2', were determined in each case. Another exponent, pK_3', was evaluated spectrophotometrically for each compound by a procedure similar to that described in a study of various 4-aminoquinolines.[7] This exponent is defined by the following equation (with the restriction of constant ionic strength)

(1) The work reported in this paper was aided by a grant from the Penrose Fund of the American Philosophical Society.

(2) Data concerning the pharmacological testing of these and many other compounds have been tabulated in a monograph, "A Survey of Antimalarial Drugs, 1941–1945," edited by Wiselogle, Edwards Bros., Ann Arbor, Mich., 1947. At some points in this paper compounds are designated by numbers preceded by the letters SN. These are the code numbers assigned by the Office of the Survey and recorded in the monograph.

(3) Personal communication from Drs. J. Taggart and J. Shannon.

(4) Jailer, Zubrod, Rosenfeld and Shannon, *J. Pharmacol. Exptl. Therap.*, **92**, 345 (1948); Jailer, Rosenfeld and Shannon, *J. Clin. Invest.*, **26**, 1168 (1947).

(5) Irvin and Irvin, *Federation Proc.*, **8**, 209 (1949).

(6) Christophers, *Ann. Trop. Med. Parasitol.*, **31**, 43 (1937).

(7) Irvin and Irvin, THIS JOURNAL, **69**, 1091 (1947).

$$pK_s' = pH - \log \frac{[B—B'H^+ + B—B']}{[^+HB—B'H^+ + {}^+HB—B']} = pH - \log \frac{(\epsilon_{BH^+} - \epsilon)}{(\epsilon - \epsilon_B)} \quad (1)$$

in which B represents a proton-acceptor associated with the aromatic nucleus and B′ corresponds to the proton-acceptor (diethylamino group) of the aliphatic side-chain. ϵ_{BH^+} and ϵ_B are, respectively, the molecular absorption coefficients, at some selected wave length, of the proton-donor and proton-acceptor species of the aromatic nucleus, and ϵ is the absorption coefficient at an intermediate *p*H value at which both species are present. This exponent is concerned with a proton-exchange involving an acceptor (probably, the ring-nitrogen) associated with the aromatic nucleus. The values of pK_s' were found to differ only slightly from the corresponding values of pK_1' in each case. Thus these compounds can be classified fairly well under the category of *case a* as defined in the previous paper. For compounds in the classification of *case a* there is electrostatic interaction between groups but the intrinsic strengths of the groups differ in magnitude sufficiently that $pK_1' \cong pK_s' \cong pK'^{I}$, in which pK'^{I} is the microscopic ionization exponent concerned with the proton-exchange

$$^+HB—B'H^+ \rightleftarrows H^+ + B—B'H^+ \quad (2)$$

where B and B′ have the significance stated above. At constant ionic strength

$$pK_1' \cong pK_s' \cong pK'^{I} = pH - \log \frac{[B—B'H^+]}{[^+HB—B'H^+]} \quad (3)$$

in which brackets are used to designate concentrations.

Inasmuch as pK_1' for each of these compounds is concerned principally, from the statistical standpoint, with the proton-equilibrium involving the aromatic nucleus, the other potentiometrically determined ionization exponent, pK_2', must refer principally to the proton-equilibrium involving the diethylamino group of the side-chain as proton-acceptor. In other words, $pK_2' \cong pK'^{IV}$, the microscopic exponent which is concerned with the following proton-exchange

$$B—B'H^+ \rightleftarrows H^+ + B—B' \quad (4)$$

Statistically, the ionization steps represented by (5) and (6) are of little importance.

$$^+HB—B'H^+ \rightleftarrows {}^+HB—B' + H^+ \quad (5)$$

$$^+HB—B' \rightleftarrows H^+ + B—B' \quad (6)$$

Experimental

Quinacrine dihydrochloride (SN-390) was obtained from Sterling-Winthrop, and was recrystallized before use. Compounds SN-9626 and 9627 were obtained through the courtesy of Dr. Nathan L. Drake. The other compounds listed in Table I were synthesized in the laboratory of Dr. Charles R. Hauser,[8] and samples were kindly donated by him for this investigation.

The apparent ionization exponents were determined potentiometrically (pK_1' and pK_2') and spectrophotometrically (pKs') as described in detail in previous publications[7,9] on quinoline derivatives. However, in the evaluation of pK_1' the potentiometric titrations had to be conducted upon aqueous solutions which were more dilute than those used in the case of the 4-aminoquinolines[7] due to the smaller solubility of the monopolar cationic species (principally B—B′H⁺) of the 9-aminoacridines reported upon here. Even when the titrations were conducted upon 0.01 molar solutions of the compounds, the monopolar cations appeared, on the basis of the mathematical analysis of the data, to undergo progressive aggregation over a considerable region of the titration before development of turbidity provided definite visible evidence of difficulty. This complication was eliminated by titration of 0.001 molar aqueous solutions of the compounds although the use of such dilute solutions was disadvantageous from the standpoint of the accuracy of the potentiometry. For this reason it was particularly valuable to have the spectrophotometric method for the evaluation of the exponent, pK_s', inasmuch as very dilute solutions (2×10^{-5} to 4×10^{-5} *M*) could be used. But in the case of pK_2' the very low solubility of the non-ionized species of these compounds necessitated, as described previously, potentiometric determination of the values for a series of solutions in water–ethanol mixtures of various proportions. The values of pK_2' for aqueous solution were obtained by extrapolation of these data to 0% ethanol. The ionic strength was maintained at a value of 0.1 in all cases by addition of sodium chloride. All constants reported in Table I are *apparent* ionization exponents determined at a constant ionic strength of 0.1, and the values of these exponents have not been extrapolated to zero ionic strength. All ionization exponents were determined at 30°. In calculating the exponents the spectrophotometric and potentiometric data were rectified by the general method of Reed and Berkson[10] with the adaptation of Clark,[11] applied as described previously.[7,9]

In studying the majority of the compounds reported in Table I, *p*H values were determined with the apparatus described in the previous publication.[7] The compounds SN-9626, SN-12867 and SN-12868 were studied at a different time, and the apparatus employed differed somewhat from that used before in the following respects: a hydrogen electrode of our design was used in the determination of the *p*H of buffers which were used in standardizing the glass electrode. As previously, the hydrogen gas was passed over reduced copper at 600°. Potentials were measured with a Leeds and Northrup students' potentiometer and were recorded in international volts without correction for liquid junction potentials. For measurements with the glass electrode, the null instrument was a number 2420 Leeds and Northrup galvanometer used in conjunction with a Leeds and Northrup thermionic amplifier. This assembly had a sensitivity equivalent to ±0.003 *p*H units, and the over-all accuracy was evaluated as ±0.015 *p*H units in the range *p*H 1 to *p*H 9 and ± 0.03 unit for *p*H 9 to *p*H 12. The primary standard of *p*H was "standard acetate" buffer,[12] a solution 0.1 molar with respect to both acetic acid and sodium acetate. It was assigned a *p*H value of 4.64 at 30°. The value[13] of the factor 2.3026 RT/F was taken as 0.06011 at 30°.

Spectrophotometry was conducted at 30° with a Beckman photoelectric quartz spectrophotometer, model DU, with 1 cm. fused silica cuvettes, by techniques described previously.[7,9]

Although the exponents recorded in Table I are reported to the second decimal place, the exponents were estimated to have been determined with the following

(8) Breslow, Walker, Yost, Shivers and Hauser, THIS JOURNAL, **68**, 100 (1946).

(9) Irvin and Irvin, *J. Biol. Chem.*, **174**, 577 (1948).

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

(11) Clark and Perkins, THIS JOURNAL, **54**, 1228 (1932); Clark, Taylor, Davies and Vestling, *J. Biol. Chem.*, **135**, 543 (1940).

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

(13) Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, Md., 1928, pp. 244–250 and 674.

probable errors: pK'_s, ±0.03 pH unit; pK'_1, ±0.05; pK'_2, ±0.1, the greater error in evaluating pK'_2 being due to uncertainties in the extrapolations which were involved.

Results and Discussion

Spectrophotometric absorption curves for SN-12868 in aqueous solutions at pH 1 and pH 10.9 are presented in Fig. 1. These curves correspond

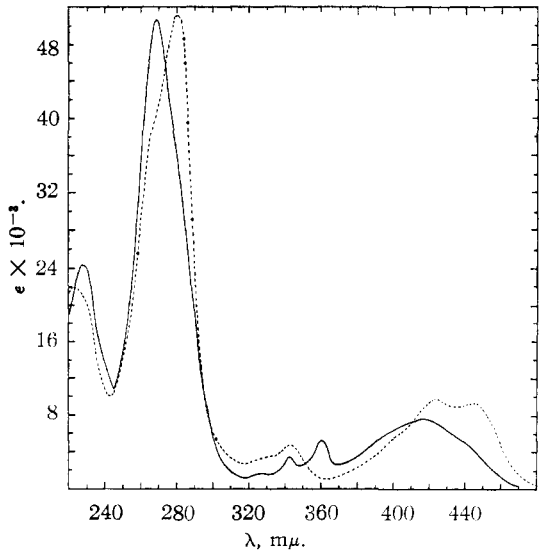
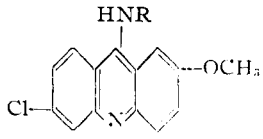


Fig. 1.—Spectrophotometric absorption curves for SN-12868; – – –, pH 1; ——, pH 10.9.

respectively to absorption by the proton-donor (cationic) species and the proton-acceptor species of the aromatic nucleus. Absorption curves of other members of the two series of 9-aminoacridine derivatives were practically identical with those of SN-12868 at these extremes of pH, but the pH range for the transition in absorption curves, corresponding to the proton-exchange involving the ring-nitrogen, varied considerably as shown quantitatively by the values of pK'_s recorded in Table I.

TABLE I

APPARENT IONIZATION EXPONENTS FOR TWO HOMOLOGOUS SERIES OF DERIVATIVES OF 2-METHOXY-6-CHLORO-9-AMINOACRIDINE (ALL VALUES DETERMINED AT 30° AND IONIC STRENGTH, 0.1)



Survey number[2]	n	pK'_s	pK	pK'
		R = $-(CH_2)_nN(C_2H_5)_2$		
5228	2	7.31	7.29	9.68
2033	3	7.79	7.78	10.00
5341	4	7.99	7.96	10.16
8020	5	8.09	8.06	10.29
3870	6	8.19	8.17	10.35
9626	7	8.23	8.21	10.41
9627	8	8.29	8.26	10.45
		R = $-CH(CH_3)(CH_2)_{n-1}N(C_2H_5)_2$		
12867	2	7.10	7.06	9.65
390	4	7.73	7.69	10.18
12868	8	8.03	7.98	10.40

Albert, *et al.*,[14,15] have found that 9-aminoacridine has the greatest proton-accepting strength (basicity) of the various mono-amino derivatives of acridine. They have attributed this enhancement of strength to the special resonance of the proton-donor, the resonance-hybrid of which is considered to receive contributions from Kekulé and quinonoid structures (Fig. 2). As represented in Fig. 2 a single proton is accepted by the ring-nitrogen, resulting in the formation of this resonating proton-donor (cation). This is the proton-exchange for which the microscopic exponent, pK'^{I}, is a measure ($pK'_1 \cong pK'_s \cong pK'^{I}$ as stated above).

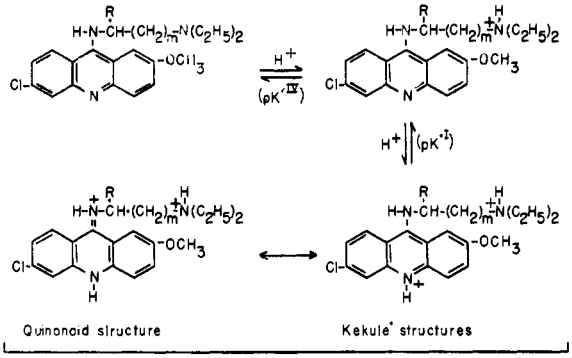


Fig. 2.—Steps in the ionization of a quinacrine homolog, and the structures contributing to the resonance-hybrid of the proton-donor (cationic) species. R is $-CH_3$ in one series of derivatives and H in the other series. $m + 1$ is the number of carbon atoms separating the diethylamino nitrogen and the 9-amino nitrogen.

The secondary amino nitrogen at position-9 theoretically is capable of accepting a proton, but no evidence of this proton-exchange in dilute aqueous solutions was obtained either by potentiometry or spectrophotometry. However, we have obtained spectrophotometric evidence for a second reversible proton-exchange of this type occurring in solutions of these compounds in concentrated sulfuric acid. These observations are being reported in an accompanying paper.[16]

The ionization exponents recorded in Table I demonstrate the effect of electrostatic influences upon the proton-equilibria exhibited by these acridine derivatives. The ratio of the first to the second ionization constant of a dibasic organic acid is always greater than four, and for a symmetrical organic acid the ratio approaches our as the distance separating the two acid groups increases.[17] Bjerrum[18] has proposed that the ratio of the ionization constants can be accounted for by the combination of the statistical factor of four and the electrostatic effect of the charge of one group upon the ionization of the other group.

(14) Albert, Rubbo and Goldacre, *Nature*, **147**, 332 (1941).
(15) Albert and Goldacre, *ibid.*, **153**, 467 (1944).
(16) Irvin, McQuaid and Irvin, THIS JOURNAL, **72**, 2750 (1950).
(17) Adams, *ibid.*, **38**, 1503 (1916).
(18) Bjerrum, *Z. physik. Chem.*, **106**, 219 (1923).

Kirkwood and Westheimer[19-22] have presented an extension of the theory which proposes that the electrostatic effect is dependent not only upon the charges present and the distance between them but also upon the shape of the molecules and upon the positions of the charges. They regard the molecules as forming cavities of low dielectric constant within the solvent of higher dielectric constant. Their method makes possible the calculation of the effective dielectric constant, D_e, and the distance, d, separating the ionizable protons. The Bjerrum equation[18] for a dicarboxylic acid in terms of an effective dielectric constant[23] is

$$\Delta pK = pK_2 - pK_1 - \log 4 = e^2/2.303\ D_e dkT \quad (7)$$

in which e is the charge on the electron, T is the absolute temperature, k is the Boltzmann constant, and 4 is the statistical factor.

It was thought desirable to apply these theories of electrostatic effects to the case of the acridine derivatives which we have studied. However, these compounds conform neither to the spherical model[19] nor the prolate ellipsoid of revolution[20] treated by Kirkwood and Westheimer nor to the oblate ellipsoid treated by Sarmousakis.[24] Nevertheless, it was considered to be profitable to compare our data with those obtained by Schwarzenbach[25] in a study of a series of aliphatic diamines. These aliphatic diamines are structurally of a type which might permit them to be treated as prolate ellipsoids of revolution. Our calculations from the data of Schwarzenbach by the method of Westheimer and Kirkwood[20] are summarized in Table II. The apparent partial molar volumes of these compounds in aqueous solutions were estimated by Traube's rule.[26] In the calculation of $\Delta pK'$ for these compounds, Schwarzenbach's values of pK_1' were used directly, but in view of the apparent inaccuracies in some of the individual values of pK_2' reported by Schwarzenbach we obtained values for pK_2' from the best straight line drawn through a plot of his pK_2' values against $1/n$, in which n is the number of carbon–carbon bonds between the two nitrogen atoms. The feasibility of this procedure was suggested to us during our examination of a similar plot of Schwarzenbach's data in a paper by Hill.[27]

TABLE II

ALIPHATIC DIAMINES $[H_2N(CH_2)_nNH_2]$

			Distance between charges, Å.		
n	$\Delta pK'$ (20°)	D_e	Method of W. and K.[20]	Maximum	Mean square (free rotation)
2	2.47	25.3	3.92	3.72	3.11
3	1.27	38.2	4.76	4.88	3.71
4	0.70	57.9	5.95	6.20	4.23
5	0.41	80.0	7.32	7.41	4.69
8	0.24	94.0	11.3	11.3	5.85

The maximum distances recorded in column 5 of Table II were calculated upon the assumption that the charges of the proton-donor (cationic) species of these aliphatic diamines are located in points corresponding to the centers of the nitrogen atoms. This seemed to be a reasonable simplifying assumption for the purposes of our calculations in view of Pauling's conclusion[28] regarding the ammonium ion, *viz.*, that "each N–H bond has about 20% ionic character and the unit positive charge of the ion is divided about equally among all five atoms, each having a resultant charge of about +0.2." The values for bond distances and bond angles used in these calculations were those given by Pauling (ref. 28, chapters 3 and 5). The mean square distances assuming free rotation were calculated by means of Eyring's equation[29] in the form designated as a special case by Kirkwood and Westheimer.[19]

In Fig. 3 values of $\Delta pK'$ for the aliphatic diamines are plotted on a linear scale against corresponding values of $1/dD_e$, the values of d and D_e being those calculated for this series of compounds by the method of Westheimer and Kirkwood (Table II). The data conform well to the theoretical straight line constructed from equation 7 for a temperature of 20°. In Fig. 3 our data for one series of acridine derivatives are plotted for comparison. The calculation of Westheimer and Kirkwood could not be applied to our series of compounds, therefore, the basis of comparison was the following: A second ordinate was prepared for the *maximum* distances separating the nitrogen atoms of the aliphatic diamines, the scale for this ordinate being arranged in such manner that the values for these maximum distances would lie directly opposite the corresponding values of $1/dD_e$ calculated for these aliphatic diamines by the method of Westheimer and Kirkwood. Our calculated values for the maximum distances between the charges on the acridine compounds were plotted upon this supplementary ordinate scale against corresponding values of $\Delta pK'$. The maximum distances between the charges on the acridine molecules were calculated upon the assumption that one charge was located upon the diethylamino nitrogen of the side-chain and that the other charge was located at one of the following possible positions: (a) upon the secondary amino nitrogen at position-9 (symbol O in Fig. 3), or (b) upon the ring-nitrogen (symbol ▲) in a hybrid receiving no contributions from a quinonoid structure, or (c) that the proton was accepted by the ring-nitrogen and that the resulting cation was a resonance-hybrid (Fig. 2) of Ke-

(19) Kirkwood and Westheimer, *J. Chem. Phys.*, **6**, 506 (1938).
(20) Westheimer and Kirkwood, *ibid.*, **6**, 513 (1938).
(21) Westheimer and Shookhoff, THIS JOURNAL, **61**, 555 (1939).
(22) Westheimer, *ibid.*, **61**, 1977 (1939).
(23) Eucken, *Z. angew. Chem.*, **45**, 203 (1932).
(24) Sarmousakis, *J. Chem. Phys.*, **12**, 277 (1944).
(25) Schwarzenbach, *Helv. Chim. Acta*, **16**, 522 (1933).
(26) Traube, *Samml. chem. chem.-tech., Vort.*, **4**, 255 (1899).
(27) Hill, *J. Phys. Chem.*, **48**, 101 (1944).
(28) Pauling, "The Nature of the Chemical Bond," Cornell University Press, 1945, p. 72.
(29) Eyring, *Phys. Rev.*, **39**, 746 (1932).

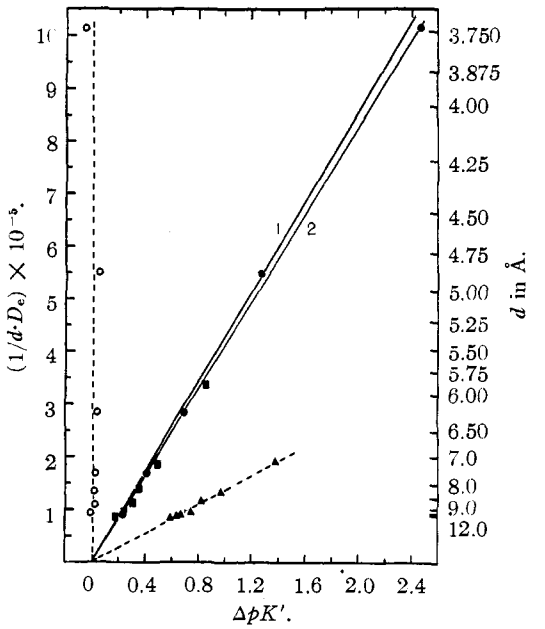


Fig. 3.—Line 1 is the theoretical for equation (7) for 30°, and line 2 is for 20°. In the left-hand ordinate d is expressed in cm. $\Delta pK'$ refers to the change in the ionization exponent of one group resulting solely from the effect of the charge on the second group, as calculated for various assumed configurations. ●, experimental values (from Schwarzenbach) for aliphatic diamines at 20°. For the acridines: O, data plotted on the basis of assumption (a); ▲, assumption (b); ■, assumption (c).

kulé or benzenoid structures with the charge on the ring-nitrogen and a quinonoid structure with the charge on the nitrogen at position-9 and that the "center" of this charge on the hybrid was located at the center of the ring midway between the ring-nitrogen and carbon-9 (symbol ■). The bond distances and bond angles used in these calculations of the maximum distances were obtained from Pauling's book. The nitrogen atom at position-9 was assumed to be coplanar with the ring to which it is attached, an assumption which seems to be reasonable for the average state of the molecule if the quinonoid structure makes an appreciable contribution to the resonance hybrid. The distance between the secondary amino nitrogen and the C-9 carbon atom was assumed to be 1.38 Å., *viz.*, a value between that of the C–N and the C=N bond lengths. The dimensions of the central ring of acridine were calculated from the data of Schomaker and Pauling[30] for pyridine with consideration of the probable modifying influence of the adjacent rings based upon the data of Pauling, *et al.*,[31] for anthracene and benzene. The data for the maximum distances between charges on the basis of the three alternative assumptions are recorded in Table III. Data for the mean square distances assuming free rotation around all of the bonds in the side-chain also are included in Table III.

(30) Schomaker and Pauling, THIS JOURNAL, **61**, 1769 (1939).
(31) Pauling, Brockway and Beach, *ibid.*, **57**, 2705 (1935).

TABLE III

Compound	n	Distance (Å.) between charge on $N(C_2H_5)_2$ and second charge: on ring-nitrogen (assumption b) Maximum	on ring-nitrogen (assumption b) Mean square	at center of ring (assumption c) Maximum	at center of ring (assumption c) Mean square	on nitrogen at C-9 (assumption a) Maximum	on nitrogen at C-9 (assumption a) Mean square
SN-5228	2	7.20	5.58	5.92	4.46	3.72	3.11
SN-2033	3	8.66	5.94	7.34	4.89	4.88	3.71
SN-5341	4	9.70	6.27	8.44	5.28	6.20	4.23
SN-8020	5	11.1	6.58	9.82	5.65	7.41	4.69
SN-3870	6	12.2	6.88	11.0	6.00	8.76	5.10
SN-9626	7	13.6	7.17	12.3	6.33	10.0	5.45
SN-9627	8	14.7	7.45	13.5	6.64	11.3	5.85

Values of $\Delta pK'$, calculated from pK' and $pK'_{\frac{1}{2}}$ by equation (7), for the symmetrical aliphatic diamines represent the effect of the charge on one group on the ionization of the other group. In the case of the acridines reported here, values of $\Delta pK'$ for the pure effect of the charge on the side-chain upon the proton-equilibrium concerned with the aromatic nucleus had to be calculated indirectly inasmuch as these compounds are unsymmetrical proton-donors. As stated previously, pK'_s is practically equal to the microscopic ionization exponent, pK'^{I}, which is a measure of the proton-equilibrium involving the aromatic nucleus. Employing an argument similar to that used by Hill[27] the $\Delta pK'$ corresponding solely to the charge effect can be calculated from the equation

$$\Delta pK' = (pK'_{s(\infty)} - pK'_{s(d)}) - (pK^{\circ\prime}_{\infty} - pK^{\circ\prime}_{d}) \quad (8)$$

in which $pK'_{s(d)}$ is pK'_s for an acridine whose charged groups are separated by the distance d;

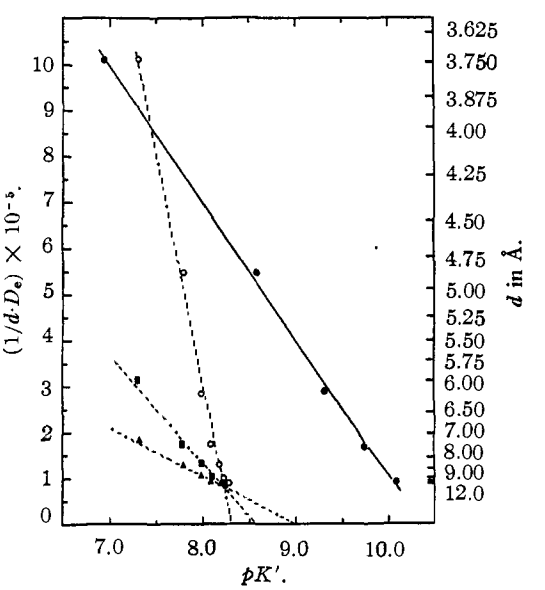


Fig. 4.—In the left-hand ordinate d is expressed in cm. The symbols for the experimental points have the significance of those used in Fig. 3. The abscissa refers to pK'_1 for the aliphatic diamines and to pK'_s for the acridines.

$pK'_{s(\infty)}$ is the value of this exponent when the distance separating the charges is infinite; $(pK^{\circ}_{\infty}' - pK^{\circ}_{d}')$ is the change in the ionization exponent concerned with the aromatic nucleus which would be produced by an *uncharged* diethylamino group on the side-chain at distance d as compared with the effect of the same uncharged group at infinite distance. As shown in Fig. 4 the experimental data for pK'_s can be extrapolated to obtain a value for this exponent for infinite separation of the ionizing groups, $pK'_{s(\infty)}$. However, the value found by such an extrapolation depends to some extent upon the particular assumption made regarding the location of the charge on the aromatic nucleus. In the calculation of $\Delta pK'$ for the acridines, the value of $pK'_{s(\infty)}$ employed was the one, derived from Fig. 4, corresponding to the particular assumption being used in plotting $\Delta pK'$ in Fig. 3. Inasmuch as these acridine derivatives possess two dissimilar proton-acceptors and the intrinsic proton-accepting strength of the diethylamino group is much greater than that of the aromatic nucleus, the value of $(pK^{\circ}_{\infty}' - pK^{\circ}_{d}')$ could not be calculated directly from the data available. However, as a first approximation it was assumed that $(pK^{\circ}_{\infty}' - pK^{\circ}_{d}')$ for the acridines was identical with $(pK^{\circ}_{\infty}' - pK^{\circ}_{d}')$ for the aliphatic diamines for corresponding values of d. This approximation should be close since the effect of an uncharged amino group should be nearly the same as the effect of an uncharged diethylamino group. For the aliphatic diamines pK°_{d}' is identical with the microscopic exponent, pK_{12}, as defined by Hill[27] and as calculated by him for each of the compounds of Schwarzenbach. The value of pK°_{∞}' was estimated by extrapolation of Hill's plot of pK_{12} *versus* the reciprocal of the number of intercarbon distances separating the amino groups. The value of d for the acridines depends upon the particular assumption made concerning the position of the charge on the aromatic nucleus, and therefore, the selected value of $(pK^{\circ}_{\infty}' - pK^{\circ}_{d}')$ also depends upon this assumption.

As demonstrated in Fig. 3 the relationship for the acridines which most closely conforms to the theoretical slope of equation 7 is that which is based upon assumption (c) as stated above. The plot of the data calculated upon this basis passes through the origin as required by the equation. The close approach to the theoretical slope exhibited by the plot of the data for these compounds on the basis of assumption (c) appears to provide evidence for the benzenoid-quinonoid resonance postulated for the cationic species of the 9-aminoacridines. Assumption (c) was drawn upon the basis that the contributions of benzenoid and quinonoid structures to the resonance state were such that the statistical center of the charge was located at the center of the ring. The approximate conformity of the data on this basis with the theoretical slope might be interpreted naïvely as indicating that the ratio of the contributions of the benzenoid and quinonoid structures to the hybrid is equal to, or slightly less than, 2:1. However, although the evaluation of resonance states of molecules through a study of the modifying effects of electrostatic charges in the molecule appears to be a promising approach, the uncertainties of the present study should be emphasized. In particular the assumptions made in the comparison of the acridines with the aliphatic diamines should be restated. The most serious assumption is one that is somewhat hidden in our comparison of the two series of compounds through a scale for the maximum distances separating the charges. This practically demands the assumption that the relationship between the maximum distance and the *effective* distance is the same for the two series of compounds and that the effective dielectric constant corresponding to a certain value of the distance separating the charges is identical for the two series. This may serve for a first approximation, but an accurate and straightforward calculation of the effective dielectric constant for these acridine derivatives is desirable. Another source of difficulty in the comparison of the two series of compounds is that the ionization exponents of the aliphatic diamines were measured at 20° and those of the acridines were determined at 30° However, the effect of this difference in temperature is small as shown in Fig. 3 by the small difference in the slopes of the theoretical lines for these temperatures. Finally, we must admit that the treatment of the acridines according to assumption (c) regarding the placement of charges is somewhat artificial and naïve. A more rigorous and refined analysis of the effect of the positive charge of the side-chain upon the proton-exchange involving the aromatic nucleus should consider separately the two effects: (1) the work involved in bringing a proton from infinity in the solution to the acceptor (the ring-nitrogen) against the repulsion of the positive charge of the side-chain, and (2) the repressive effect of the charge of the side-chain upon the resonance of the cationic species of the aromatic nucleus, involving a probable inhibition of the quinonoid structure in which the charge on the aromatic nucleus is closer to the charged diethylamino group of the side-chain than it would be in the benzenoid structure. However, regardless of the limitations of the present comparison and its analysis, it provides rather definite evidence that the proton which is accepted by a nitrogen atom associated with the aromatic nucleus, in the equilibrium evaluated by pK'_s, is not accepted by the nitrogen atom at C-9. If the proton were accepted by the 9-amino nitrogen, the marked deviation of the slope of the plot of our data upon this basis from the theoretical slope could least reasonably be ascribed to differences in effective dielectric constants due to structural differences in the two series of compounds inasmuch as the 9-amino nitrogen is one of the nitrogens of an aliphatic di-

amine, the side-chain. If an assumption is made that the proton is attached to the secondary amino nitrogen at position-9 (assumption a), the calculated value of $(pK^{\circ\prime}_{\infty} - pK^{\circ}_{d}{}')$ corresponding to the value of d for this assumed configuration is found to be practically equal to the *total* change in the intrinsic ionization exponent $(pK'_{s(\infty)} - pK'_{s(d)})$ in each case, and $\Delta pK'$ due solely to the effect of the charge (calculated by difference according to equation 8) becomes practically equal to zero in each case as shown by the values marked by open circles in Fig. 3. It obviously cannot be correct that the effect of the charge would be zero, therefore, the location of the proton on the secondary amino nitrogen at position-9 is highly improbable and assumption (a) is eliminated. Similarly, the fixed configuration corresponding to assumption (b) also seems to be improbable as indicated in Fig. 3 by the departure of the data, based on this assumption, from the slope of the theoretical line (line 1).

Inasmuch as the preceding method of comparison of the two series of compounds involved the estimation of pK' values for infinite separation of groups by extrapolation, it seemed desirable to make a comparison through directly determined experimental values. For this purpose in Fig. 4 values of pK'_i for the aliphatic diamines are plotted on a linear scale against corresponding values of $1/dD_e$, the values of d and D_e being those calculated for the series of compounds by the method of Westheimer and Kirkwood. The plot is fairly linear although this is not demanded by theory since the change in pK'_i (as contrasted with $\Delta pK'$) caused by a change in the distance of separation of the groups is not due solely to a pure charge effect. In Fig. 4 our data for the pK'_s values for the acridines are plotted for comparison—the basis of plotting again being an indirect one involving intercomparison of the two series of compounds through the common supplementary ordinate of maximum distances between groups. Again the maximum distances were calculated on the basis of three different assumptions regarding the location of the charge on the aromatic nucleus as stated previously. In this comparison, as in the preceding one, the closest agreement between slopes for the two series of compounds is attained on the basis of assumption (c), *viz.*, that the center of the charge on the aromatic nucleus is located statistically at approximately the center of the ring midway between the ring-nitrogen and the carbon at position-9. It should be remarked that comparison of the two series of compounds on the basis of their microscopic ionization exponents would appear to be preferable to the basis used above. However, in the case of the acridine derivatives pK'_s is practically equal to pK'^{I}, the microscopic ionization exponent.[7,17] On the other hand, the aliphatic diamines are symmetrical proton-donors and for this case as stated by Adams[17]

$$K'_i = K'^{\mathrm{I}} + K'^{\mathrm{II}} = 2K'^{\mathrm{I}}$$

and

$$pK'^{\mathrm{I}} = pK'_i + \log 2 \qquad (9)$$

According to equation (9), the slope of the line for the aliphatic diamines in Fig. 4 would not be changed if pK'^{I} instead of pK'_i were plotted *versus* $1/dD_e$, but the position of the line would be shifted 0.301 unit to the right along the abscissa; therefore, the plot as presented is satisfactory for the comparison of slopes.

Summary

Apparent ionization exponents (30° and ionic strength, 0.1) have been determined potentiometrically and spectrophotometrically for two series of homologs of quinacrine. The effect of the positive charge on the diethylamino group of the side-chain upon the ionization of the 9-aminoacridine nucleus and the variation of this effect with the distance separating the charges are interpreted in terms of Bjerrum's theory which is based upon Coulomb's law of electrostatics. For comparison with the 9-aminoacridines, the data of Schwarzenbach for a series of aliphatic diamines have been treated by means of the extension of Bjerrum's theory proposed by Kirkwood and Westheimer. This comparison provides additional evidence for the benzenoid-quinonoid resonance of the cationic species of the 9-aminoacridines originally proposed by Albert and and Goldacre.

BALTIMORE, MD. RECEIVED DECEMBER 16, 1949